

1 **Title:**

2 Genipin Crosslinks the Extracellular Matrix to Rescue Developmental and Degenerative
3 Defects, and Accelerates Regeneration of Peripheral Neurons

4

5 **Authors:**

6 Kenyi Saito-Diaz¹, Paula Dietrich², Hsueh-Fu Wu^{1,3}, Xin Sun⁴, Archie Jayesh Patel¹,
7 Camryn Gale Wzientek⁵, Anthony Robert Prudden⁵, Geert-Jan Boons^{5,6,7}, Shuibing
8 Chen^{8,9}, Lorenz Studer^{9,10}, Bingqian Xu⁴, Ioannis Dragatsis², and Nadja Zeltner^{1,3,11,*}

9

10 **Affiliations:**

11 ¹Center for Molecular Medicine, University of Georgia, Athens GA, USA

12 ²Department of Physiology, The University of Tennessee, Health Science Center,
13 Memphis, TN, USA

14 ³Department of Biochemistry and Molecular Biology, University of Georgia, Athens GA,
15 USA

16 ⁴College of Engineering, University of Georgia, Athens GA, USA

17 ⁵Complex Carbohydrate Research Center, University of Georgia, Athens, GA, USA,

18 ⁶Department of Chemistry, University of Georgia, Athens, GA, USA

19 ⁷Department of Chemical Biology and Drug Discovery, Utrecht Institute for
20 Pharmaceutical Sciences, and Bijvoet Center for Biomolecular Research, Utrecht
21 University, Utrecht, The Netherlands

22 ⁸Department of Surgery and Department of Biochemistry at Weill Cornell Medical College,
23 New York, NY, USA

24 ⁹Center for Stem Cell Biology, Sloan Kettering Institute, New York, NY, USA

25 ¹⁰Department of Developmental Biology, Sloan Kettering Institute, New York, NY, USA

26 ¹¹Department of Cellular Biology, University of Georgia, Athens GA, USA

27 *Corresponding author: Nadja Zeltner, University of Georgia, Center for Molecular
28 Medicine, 325 Riverbend Road, Athens, GA, 30602 USA. Phone: +1-706-542-2650.
29 Email: nadja.zeltner@uga.edu

30

31 **Abstract:**

32 The peripheral nervous system (PNS) is essential for proper body function. A high
33 percentage of the population suffer nerve degeneration or peripheral damage. For
34 example, over 40% of patients with diabetes or undergoing chemotherapy develop
35 peripheral neuropathies. Despite this, there are major gaps in the knowledge of human
36 PNS development and therefore, there are no available treatments. Familial
37 Dysautonomia (FD) is a devastating disorder that specifically affects the PNS making it
38 an ideal model to study PNS dysfunction. FD is caused by a homozygous point mutation
39 in *ELP1* leading to developmental and degenerative defects in the sensory and autonomic
40 lineages. We previously employed human pluripotent stem cells (hPSCs) to show that
41 peripheral sensory neurons (SNs) are not generated efficiently and degenerate over time
42 in FD. Here, we conducted a chemical screen to identify compounds able to rescue this
43 SN differentiation inefficiency. We identified that genipin, a compound prescribed in
44 Traditional Chinese Medicine for neurodegenerative disorders, restores neural crest and
45 SN development in FD, both in the hPSC model and in a FD mouse model. Additionally,
46 genipin prevented FD neuronal degeneration, suggesting that it could be offered to

47 patients suffering from PNS neurodegenerative disorders. We found that genipin
48 crosslinks the extracellular matrix, increases the stiffness of the ECM, reorganizes the
49 actin cytoskeleton, and promotes transcription of YAP-dependent genes. Finally, we show
50 that genipin enhances axon regeneration in an *in vitro* axotomy model in healthy sensory
51 and sympathetic neurons (part of the PNS) and in prefrontal cortical neurons (part of the
52 central nervous system, CNS). Our results suggest genipin can be used as a promising
53 drug candidate for treatment of neurodevelopmental and neurodegenerative diseases,
54 and as a enhancer of neuronal regeneration.

55

56 **One sentence summary:**

57 Genipin rescues the developmental and degenerative phenotypes of the peripheral
58 neuropathy familial dysautonomia and enhances neuron regeneration after injury.

59

60 **Main text:**

61 **Introduction**

62 The peripheral nervous system (PNS) consists of sensory, sympathetic,
63 parasympathetic, and enteric neurons, all of which develop from the neural crest (NC).
64 The PNS innervates all organs of the body and is essential for its homeostasis. Loss, as
65 well as hyper-function of the PNS lead to an array of human disorders caused by genetic
66 mutations, metabolic problems, traumatic injury, inflammation, toxins, and infections.
67 While lots of research is invested in finding treatments¹, to date, there is no FDA approved
68 treatment available. For example, peripheral nerve degeneration (peripheral neuropathy)
69 and peripheral nerve damage are common, i.e. 20 million people and 20-30% of the
70 population, respectively are sufferers. Together, they cause a large impact on life and are
71 a major healthcare cost burden^{2,3}.

72 To improve our general understanding of PNS function and missregulation, it is
73 useful to study a model disease; ideally one that is well defined, specific and causes a
74 strong phenotype⁴. The autosomal recessive childhood disorder Familial Dysautonomia
75 (FD) serves as such a model disorder. FD is caused by a homozygous point mutation in
76 the gene *ELP1* (formerly *IKBKAP*), the scaffolding component of the transcriptional
77 elongator complex⁵. The mutation leads to aberrant splicing and tissue-specific reduction
78 of functional ELP1 protein⁶. This reduction is particularly prominent in the neural crest
79 (NC) progenitor lineage and its progeny: the peripheral nervous system (PNS), especially
80 in sensory and autonomic neurons⁶. These neurons develop at a reduced rate and they
81 degenerate rapidly over time⁷. As a result, patients experience loss of pain perception,
82 ataxic gait, trouble regulating heart rate and blood pressure and debilitating dysautonomic

83 crisis, characterized by tachycardia, blood pressure spikes and extensive vomiting^{2,8}.
84 Although FD mouse models show the same phenotypes: reduced size and neuron
85 numbers in the dorsal root ganglia (DRG)⁹, the mechanism of how SNs fail to develop
86 and quickly degenerate remains elusive. Thus, none of the interventions available to FD
87 patients target SN symptoms¹⁰. However, this problem is not limited to FD: other patients
88 with SN loss or defects stemming, for example from peripheral neuropathies or cancer
89 chemotherapy treatment have limited pharmacological options as well¹¹. Thus, a drug
90 that targets SNs is necessary to treat a wide range of diseases.

91 Human pluripotent stem cell (hPSC) technology¹² is ideal to gain cellular and
92 mechanistic insight into genetic, early-onset human disorders; as it allows the retrieval of
93 patient specific cells, their reprogramming into hPSCs, followed by their differentiation
94 into SNs¹³, and parallel comparison between healthy control and disease cells for disease
95 mechanistic studies¹⁴. Additionally, it provides the possibility to generate large numbers
96 of neurons that can be employed for high-throughput drug screening approaches. In fact,
97 FD was one of the first diseases that was modeled using the hPSCs technology¹⁵ and
98 provided one of the first proof-of-principle drug screens employing hPSCs¹⁶. We
99 previously showed that this disease modeling technique is highly sensitive in that it allows
100 the recapitulation of specific phenotypes accounting for patient's varying severity of
101 symptoms¹⁷. We showed that in FD, both NC cells and SNs are not developing efficiently
102 and that the SNs degenerate/die over time *in vitro*. These findings are consistent with
103 patients being born with less SNs in the DRG⁷, equivalent findings in the FD mouse DRG⁹
104 and the fact that patient's pain/temperature perception decreases with age¹⁸.

105 Here, we conducted a high-throughput chemical screen and identified genipin as
106 a hit compound that targets SN defects in FD. Genipin is derived from the fruit of *Gardenia*
107 *Jasminoides* and is the active ingredient of the Traditional Chinese Medicine (TCM) Zhi
108 Zi¹⁹, which is being prescribed for neurodegenerative disorders, including Alzheimer's^{20,21}
109 and Parkinson's disease^{22,23}. In this context genipin has shown a safe profile in humans.
110 Genipin has been implicated as an inhibitor of UCP2, preventing UCP2-mediated proton
111 leak in mitochondria and increasing mitochondrial membrane potential²⁴. Furthermore,
112 genipin has been shown to have anti-inflammatory effects by inhibiting nitric oxide
113 synthesis *in vitro* and *in vivo*²⁵. Lastly, genipin is being used by bioengineers to crosslink
114 extracellular matrices (ECM), due to its low toxicity^{26,27}. We show that genipin reverses
115 the differentiation inefficiency observed in FD NC and SNs and prevent degeneration of
116 SNs *in vitro*. We also demonstrate that genipin can be fed to pregnant FD mice to rescue
117 the neurodevelopmental phenotypes in embryos. We provide evidence that genipin acts
118 via ECM crosslinking and reverses FD phenotypes by activating the transcription of YAP-
119 dependent genes. Lastly, we show that genipin promotes rapid regeneration of neurons
120 from the peripheral (sensory and sympathetic) and central (prefrontal cortex) nervous
121 systems. Together, our data provides support that genipin is a novel drug candidate to
122 treat neurodevelopmental diseases, prevent neurodegeneration, and promote neuronal
123 regeneration upon injury. Thus, genipin has the potential to be used as a treatment for a
124 broad range of neurological diseases.

125

126 **Results**

127 Chemical screen to rescue sensory neuron phenotypes in Familial Dysautonomia

128 We first reproduced our previous findings that hPSC-derived SNs from FD patients
129 cannot be generated efficiently *in vitro*¹⁷. The differentiation protocol defined by
130 Chambers et al.²⁸ (**Fig. 1A**) was employed to differentiate hPSCs, derived from healthy
131 control embryonic stem cells (H9/WA09, hPSC-ctr-H9), healthy control iPSCs (iPSC-ctr-
132 C1) and two iPSC lines from FD patients (iPSC-FD-S2 and iPSC-FD-S3)¹⁷. While the
133 undifferentiated colonies looked normal in all four lines, once SNs were generated by
134 day 13 of the differentiation protocol, the efficiency/number of neurons dropped
135 dramatically in the FD lines compared to the control lines (**Fig. 1B**). Based on this
136 phenotype, we set out to conduct a chemical drug screen to identify compounds that
137 could reverse this phenotype and allow FD-iPSCs to efficiently generate SNs. We first
138 adapted the SN differentiation protocol to high-throughput screening conditions. We
139 tested the following adaptation parameters: 96 and 384 well plate formats, feeding
140 frequency of twice (day 2, 5) or three times (day 2, 5, 8; normal, non-screening feeding
141 is done every other day), shortening the protocol to 10 days total and thus removing the
142 replating step at day 12, and replacing the media gradient with a 1:1 mixture of the KSR
143 and N2 media, respectively instead of the gradual change of media (**Fig. 1C**). The
144 simplifications of the protocol lead to an overall decrease in SN numbers from ~60% (in
145 the regular protocol²⁸ to ~30% (in our screening protocol) in hPSC-ctr-H9 cells. Within
146 these conditions, the 96-well format and 3 times feeding produced the most robust SNs
147 in the healthy control (**Fig. 1D**) and the largest difference between control and FD (**Sup.**
148 **Fig. 1A, B**). We used these conditions to conduct a pilot screening using only positive (
149 hPSC-ctr-H9) and negative (iPSC-FD-S2) controls. In these conditions, the calculated Z'
150 factor for the screen was 0.14, which denotes a marginal assay. However, thanks to the

151 design and read-out of our screening, positive and negative controls were clearly
152 distinguishable. We planned for a relatively small screen and thus proceeded with
153 confidence. We reasoned that treatment starting on differentiation day 2 would allow
154 initial specification into ectoderm but influence differentiation into NC and SN fates. We
155 then screened 640 compounds, i.e. half of the LOPAC chemical library of
156 pharmacological active compounds from Sigma, that contains a mixture of compounds
157 from the fields of cell signaling and neuroscience (**Fig. 1E**). Each compound was
158 screened at 1 mM and 10 mM in DMSO. All wells were then stained for the pan-SN
159 marker *BRN3A* and DAPI and hit compounds were called if the fold change (FC) over
160 the average of all DMSO wells was above the average of all compounds plus 3 SDs
161 (**Fig. 1F, Sup. Table 1, 2**). Our screen resulted in 3 hits: Fluphenazine dihydrochloride
162 (Flu, at 10 mM, a dopamine receptor antagonist), AC-93253 iodide (AC, at 1 mM, a
163 retinoic acid receptor agonist) and genipin (at 1 mM and 10 mM, **Sup. Fig. 1C**). The
164 controls on the screen were: Healthy hPSC-ctr-H9 and iPSC-FD-S2, both in DMSO, and
165 DMSO alone. The Z-score for genipin was 15 at 1 mM and 17 at 10 mM.

166

167 Hit validation

168 Next, we performed several validation assays to further assess the hit compounds. The
169 3 hit compounds were tested in a repeat of the same conditions as performed in the
170 screen itself (**Sup. Fig. 1D**) and then repeated in a different well format (48-wells) using
171 the non-screening differentiation protocol (**Sup. Fig. 1E**). This analysis revealed that
172 indeed genipin rescues the SN defect in FD (**Sup. Fig. 1E**, red rectangle), whereas Flu
173 did not have much of a rescue effect and was auto-fluorescent (**Sup. Fig. 1E**, blue

174 rectangle), and AC did not rescue the FD phenotype and showed high cytotoxicity
175 (DAPI staining, **Sup. Fig. 1E**, green rectangle). We also tested the three hits on
176 additional cell lines, including healthy iPSC-ctr-C1 and a FD line from patients with
177 milder symptoms (iPSC-FD-M2)¹⁷. We observed the same phenotypes in iPSC-ctr-C1
178 and iPSC-FD-M2 cells (**Sup. Fig. 1F**). Thus, we focused on genipin. We titrated the
179 genipin concentration (**Fig. 2A, B**) and found that, while genipin increased the number
180 of SNs in a dose dependent manner in control hPSC-ctr-H9 and iPSC-ctr-C1 cells (about
181 7-fold), the increase of SNs in genipin treated iPSC-FD-S2 cells was dramatically larger
182 (over 30-fold). To further confirm genipin's effect, we tested it in our newer SN
183 differentiation protocol that is fully chemically defined (E8/E6)¹³ (**Sup. Fig. 1G**). Indeed,
184 we found that genipin robustly rescued both the NC and the SN defect in iPSC-FD-S2
185 cells (**Fig. 2C**). However, in this protocol the cells were more sensitive, showing mild
186 cytotoxic effects at 20 μ M (likely due to the lack of the buffering KSR), thus the ideal
187 concentration of genipin was lower (10 μ M) (**Fig. 2C-E**). All data from here on was done
188 in the chemically defined SN protocol¹³. Lastly, we tested genipin from different
189 commercial sources (Sigma and Biomaterials) and found that, while both compounds
190 worked, genipin from Biomaterials showed a more robust phenotype at 10 μ M. Further
191 experiments were done using genipin from this source (**Fig. 2F**). Together, we show
192 that our small molecule screen resulted in the identification of genipin as a rescue
193 compound in our FD model.

194

195 Effects of genipin on neurodevelopment of neural crest cells and sensory neurons in FD
196 iPSCs

197 We sought to carefully characterize genipin's effects throughout development. In
198 human embryonic development, NC cells (marked by *SOX10*) give rise to SN-specified
199 NC cells (marked by *NGN1/2*), followed by *BRN3A*⁺ SNs that also express *ISL1*, *PRPH*,
200 *TRKA*, *TRKB*, and *TRKC*. Thus, we first assessed the effects of genipin on NC formation.
201 We found an increase of typical dark NC ridges that correlate with *SOX10* expression (on
202 mRNA and protein level) in all the cell lines tested (**Fig. 3A-C**, arrows and **Sup. Fig. 2A**).
203 Additional NC genes (*FOXS1* and *P75NTR*) were restored by genipin (**Sup. Fig. 2B**). We
204 found that genipin increased *TFAP2A*, however non-significant, probably because
205 *TFAP2A* is also expressed in non-neural ectoderm (**Sup. Fig. 2B**). We then confirmed
206 that genipin further is capable of rescuing SN development by increasing the number of
207 SNs in FD via staining for *BRN3A*, *TUJ1*, *ISL1*, and *PRPH* (**Fig. 3D**, **Sup. Fig 2C**) and
208 quantification of the number of *BRN3A*⁺ positive neurons via intracellular FACS analysis
209 (**Fig. 3E**, **Sup. Fig. 2D**). Furthermore, the expression of various SN genes (*BRN3A*, *ISL1*,
210 *PRPH*, *TRKA*, *TRKB*, and *TRKC*) was rescued in FD by genipin, shown by RT-qPCR
211 (**Fig. 3F**, **Sup. Fig. 2E**) and immunoblotting (**Fig. 3G**). Finally, we show that the firing rate
212 in FD SNs also increases upon treatment with genipin compared to DMSO (**Fig. 3H**),
213 likely because of the increased number of differentiated SNs. From here on forward, we
214 pooled iPSC-FD-S2 and iPSC-FD-S3 data, also indicated in the figure legends. In sum,
215 our results show that genipin rescues FD neurodevelopmental defects at the NC and SN
216 stage.

217

218 Genipin rescues sensory deficits in FD mice

219 We next assessed whether genipin can also rescue the FD neurodevelopmental
220 defects *in vivo*. Since FD mutant mouse embryos (*Elp1*^{Δ20/flox}) have significant deficits
221 already at late gestation⁹, we assessed whether genipin has the potential to rescue their
222 developmental defects. Pregnant dams were treated with genipin-supplemented chow
223 from mid-gestation. At E18.5 embryos were harvested and analyzed (**Fig. 4A**). Genipin
224 was well tolerated during gestation and no significant effect was observed in female
225 gestational weight gain, litter size, or embryonic development (**Sup. Fig. 2F** and **Sup.**
226 **Table 3**).

227 At E18.5, FD embryos treated with genipin did not differ significantly in weight and
228 size compared to untreated FD embryos (779.33 mg ± 94.00 versus 783.6 mg ± 137.62,
229 respectively) but remained significantly smaller than control embryos⁹. On the other hand,
230 genipin treatment during gestation significantly rescued FD DRG volume and neuronal
231 cell numbers (**Fig. 4B-D**). Since sensory nociceptive neurons are particularly depleted in
232 FD at late gestation^{9,29,30}, we assessed the paucity of nociceptive neurons by
233 immunohistochemistry for calcitonin gene related protein (CGRP), a specific marker for
234 nociceptive neurons³¹. As shown in **Fig. 4E** and **F**, while in FD embryos CGRP-positive
235 neurons are reduced to 20% of total lumbar L1 DRG neurons, in genipin-treated FD
236 embryos the number of CGRP-positive neurons increased significantly to about 40% of
237 total neurons. Interestingly, we found that genipin treatment also rescued sensory skin
238 innervation in FD mouse embryos (**Fig. 4G**), which is severely compromised in FD
239 patients and FD mouse models^{29,32}. Unexpectedly, we found that genipin also rescued
240 neurodevelopmental phenotypes in sympathetic neurons, assessed by an increase in the
241 size of the stellate ganglion (**Fig. 4H, I**).

242 These results suggest that genipin is well tolerated and rescues FD sensory and
243 sympathetic phenotypes *in vivo*. Furthermore, they confirm the results we obtained in the
244 hPSC-based system and strengthen our hypothesis that genipin is a suitable candidate
245 for as a therapeutic to treat neurodevelopmental diseases such as FD.

246

247 Effects of genipin on neurodegeneration in sensory neurons in FD

248 We have previously shown that SNs in FD degenerate and die over time *in vitro* in
249 our PSC model¹⁷. This correlates with reports in FD patients of deteriorating symptoms
250 with age, including pain perception and autonomic regulation¹⁸. Since FD is both a
251 neurodevelopmental and a neurodegenerative disorder and thus patients are born with
252 reduced numbers of SNs, it would be imperative for patient care to develop a novel drug
253 that halts or slows degeneration of existing SNs. Therefore, we set out to assess genipin's
254 capability to halt SN degeneration. We first had to adapt our *in vitro* degeneration assay¹⁷
255 to the new SN differentiation protocol¹³. Titration of NGF revealed that 1 ng/ml of NGF
256 leads to degeneration of iPSC-FD-S2 SNs over 20 days while being able to maintain
257 healthy SNs alive (**Sup. Fig. 3A**, red rectangles). Also, exclusion experiments showed
258 that LM, but not FN or PO could be omitted in the surface coating for SNs (**Sup. Fig. 3B**).
259 Thus, survival assays were done in 1 ng/ml NGF on dishes coated with PO and FN. SNs
260 were differentiated from hPSC lines without genipin until day 12, when they were replated
261 and treatment with genipin began (**Fig. 5A**). We found that, healthy control SNs survive
262 well *in vitro* until day 34 and beyond, and they show the classical clustering/ganglia-like
263 structure formation over time (**Fig. 5B**, arrows). Severe FD SNs, however, die starting
264 from day 13 (**Fig. 5B, C**). When the SNs are treated with genipin from day 13, this

265 degeneration is prevented (**Fig. 5B, C**). We confirmed this effect in the KSR-based
266 protocol (**Fig. 1A, Sup. Fig. 3C**). Furthermore, we show that neurite length from FD SNs
267 increases compared to untreated SNs (**Fig. 5D, E**). We also found that genipin increased
268 the number of dendrites of FD SNs and renders a distribution more similar to healthy SNs
269 (**Sup. Fig. 3D**). It is of note that in a 2D *in vitro* system SNs show more neurites compared
270 to *in vivo*³³. To make sure that there are not substantial numbers of NC cells remaining at
271 day 13, that could be induced by genipin to make more SNs, we stained day 13 and day
272 34 cultures for *SOX10*, showing the largely absence of NC cells (**Sup. Fig. 3E**). Thus,
273 genipin truly supports survival of SNs in FD. Together, these results suggest that genipin
274 is a promising candidate drug to halt the progressive SNs degeneration in FD and further
275 might become an interesting candidate drug for the treatment of other peripheral
276 neuropathies.

277

278 ELP1 targets ECM gene expression

279 We next addressed the mode of action through which genipin exerts these rescue
280 effects. FD is caused by a homozygous mutation in *ELP1*, leading to a splicing defect and
281 eventually causing patients to have reduced levels of wild type ELP1 protein, with
282 particularly low levels in PNS tissues, including SNs⁶. Several reports have shown
283 compounds that reverse this splicing defect³⁴⁻³⁷, thus we first assessed if genipin directly
284 affects ELP1. We found that genipin does not change splicing of *ELP1*, assessed on the
285 mRNA and on the protein level (**Sup. Fig. 4A, B**). Thus, in FD at the SN level genipin
286 must exert its action in a different way.

287 Genipin has been implicated in several contexts, including inhibition of
288 mitochondrial protein UCP2, activation of neuronal nitric oxide synthetase (NOS)²⁰ and
289 its ability to crosslink ECM proteins³⁸. Our previous work ¹⁷ has shown that severe FD
290 patients have a modifier mutation in *LAMB4*, which is an ECM protein. Furthermore,
291 proper composition and availability of ECM proteins have been shown to be essential for
292 SN development^{39–41}. Additionally, Goffena et al. showed that loss of Elp1 particularly
293 affects long transcripts and AA- and AG-ending transcripts⁴². We analyzed their
294 sequencing data and found that ECM proteins fall within this highly affected group (**Sup.**
295 **Fig. 4C, D**). Furthermore, we conducted RNA sequencing analysis comparing SNs from
296 healthy control and iPSC-FD-S2 cells. Gene ontology analysis revealed that ECM
297 proteins significantly downregulated in FD SNs (**Sup. Fig. 4E**). Further analysis of gene
298 differential expression showed that the ECM-related proteins *FBLN1*, *COL1A2*, *HAPLN1*,
299 *TNC*, and *HSPG2*, among others are downregulated (**Sup. Fig. 4F**). Thus, loss of *ELP1*
300 is predicted to lead to ECM defects and we hypothesized that genipin's ability to crosslink
301 ECM proteins might alleviate these issues in FD.

302

303 Genipin acts via crosslinking of extracellular matrix proteins in Familial Dysautonomia

304 Genipin forms inter- and intramolecular crosslinks (**Fig. 6A**), which cause the cells
305 to be stained blue due to formation of genipin-methylamine monomers during crosslinking
306 reactions (**Fig. 6B**). Thus, we reasoned that if the rescue in FD functions via ECM
307 crosslinking, then other crosslinking agents should have the same rescue effect as
308 genipin in our FD model. We first tried dimethyl pimelimidate (DMP), a membrane-
309 permeable compound that crosslinks crosslink primary amines of proteins (due to

310 imidoester groups present on each end of the molecule) both within the ECM and inside
311 the cell (**Sup. Fig. 5A**). We found that DMP rescued both the NC deficit (**Sup. Fig. 5B**,
312 top) as well as the SN deficit (**Sup. Fig. 5B**, bottom) in FD cells in a dose dependent
313 manner (**Sup. Fig. 5C**). This result suggests that protein crosslinking is sufficient to
314 rescue the neurodevelopmental FD phenotype. However, it remains unclear whether
315 crosslinking of extracellular or intracellular proteins or both rescue the FD phenotype.
316 Thus, we used bis-(sulfosuccinimidyl) suberate (BS3), a membrane-impermeable
317 crosslinker which only crosslinks proteins in the ECM (**Fig. 6C**). We found that BS3
318 rescues both the NC (**Fig. 6D**, top) as well as the SN phenotypes (**Fig. 6D**, bottom) in a
319 dose dependent manner (**Sup. Fig. 5D**). Additionally, when we used N-
320 hydroxysulfosuccinimide (Sulfo-NHS), a form of BS3 with no crosslinking capabilities, the
321 rescue effect was abolished in both the NC and SN phenotypes (**Sup. Fig. 5E**). Together,
322 our data suggests that crosslinking of only extracellular proteins (i.e. the ECM) is
323 necessary and sufficient to rescue the developmental phenotype in FD. Moreover, these
324 results strongly support a model where genipin exerts its action in FD via crosslinking
325 proteins in the ECM. To confirm that the FD phenotype is rescued only by genipin's
326 crosslinking activity and not its other predicted function (i.e. UCP2 inhibition), we used
327 1,10-antigenipin (AG, **Fig. 6E**). AG has been shown to specifically remove genipin's
328 ability to crosslink proteins, while keeping its other actions intact²⁴. Confirming that AG
329 does not have crosslink activity, we found that AG did not stain the FD cells blue (**Fig.**
330 **6F**). Furthermore, in contrast to genipin, AG was not able to rescue the NC (**Sup. Fig. 5F**,
331 **G**) nor the SN phenotype in FD cells (**Fig. 6G,H, Sup. Fig. 5H, I**). Together, our results
332 show that genipin's crosslinking action is responsible for the FD phenotype rescue.

333

334 Genipin increases stiffness of the ECM of FD SNs

335 Atomic force microscopy (AFM) can be used to study how the morphology and
336 mechanical properties of live or fixed cells are affected under different conditions (for
337 example, changes in the ECM)^{43–45}. In AFM, a tip is roughly aligned on the surface of the
338 cell using an optical microscope, then contact AFM mode is applied where the tip scans
339 the surface (**Fig. 6I**). A laser beam, aimed at the cantilever holding the tip, is detected by
340 a photodetector. Vertical changes in the position of the tip (due to different height in the
341 surface) are measured, resulting in high-resolution topographic images of the samples
342 (**Fig. 6I, Sup. Fig. 6A**). Interestingly, we found that genipin re-orientates the ECM as
343 shown by the presence of ordered “strips” (possibly due to the presence of crosslinking
344 bundles), in stark contrast to DMSO controls where the ordered “strips” are missing (**Sup.**
345 **Fig 6A**). Also, the height of the samples increased with genipin (from to 2.09 μm to 3.5
346 μm for SNs, from 0.8 μm to 1.2 μm for ECM) (**Sup. Fig 6A**) due to formation of crosslinked
347 bundles. From these acquired cell images, we selected different points on the surface
348 (**Fig. 6I**, black crosses) and measured their force spectroscopy. The deflection of the
349 cantilever tip was recorded and converted to force-distance curves (**Sup. Fig. 6B**, solid
350 lines). Force-distance curves were further fitted with the Hertz model (suitable for fitting
351 under small deformation in nanoindentation measurements⁴⁶) to calculate the Young’s
352 modulus (a measurement of stiffness) of individual cells (**Sup. Fig. 6B**, dotted lines). We
353 found that FD SNs treated with genipin have a higher Young’s modulus ($\sim 8,000$ Pa)
354 compared to DMSO control ($\sim 2,000$ Pa) (**Fig. 6J, Sup. Fig 6B, C**), agreeing with previous
355 reports^{45,47,48}. Furthermore, genipin-treated SNs show narrower distributions, suggesting

356 a stiffer and more uniform surface due to ECM crosslinking (**Sup. Fig 6D**). To confirm our
357 results, we removed the SNs and measured only the remaining ECM. Agreeing with our
358 previous results, we found that the ECM deposited by genipin-treated SNs has a Young's
359 Modulus ten times higher compared to untreated controls (~13,300 Pa vs ~1,300 Pa)
360 (**Fig. 6J, Sup. Fig 6B, C**). These changes could only be attributed to the direct
361 crosslinking of genipin with ECM, further strengthening our hypothesis that ECM
362 crosslinking by genipin is necessary to rescue the FD neurodevelopmental and
363 neurodegenerative phenotypes.

364

365 ECM crosslinking causes actin reorganization and transcription of YAP-dependent genes

366 Based on our previous results, we asked whether a stiffer ECM (due to genipin-
367 dependent crosslinking) has any intracellular effects. The ECM is necessary for proper
368 development and distribution of the cytoskeleton⁴⁹, and previous reports showed that the
369 actin cytoskeleton is missregulated in FD⁵⁰. Thus, we first assessed whether the actin
370 expression pattern is different between healthy and FD SNs. We measured the signal
371 from the cell bodies (**Sup. Fig. 6E**, black line) or the axons (**Sup. Fig. 6E**, blue lines). We
372 found that the actin signal in healthy SNs is the highest in the cell body, whereas FD SNs
373 show stronger actin signal in the axon (**Fig. 6K, Sup. Fig. 6F, G**). Treatment with genipin
374 was able to partially restore the actin expression pattern in FD SNs (**Fig. 6K**). Changes
375 in actin cytoskeleton organization due to ECM stiffness activate the Hippo pathway⁵¹. In
376 a stiff ECM, the transcriptional coactivator YAP (the most downstream effector of the
377 Hippo pathway), translocates from the cytoplasm to the nucleus to activate a
378 transcriptional program⁵¹. We sought to assess YAP localization in SNs by

379 immunofluorescence. We found that in both healthy and FD SNs, YAP is present in the
380 cytoplasm as assessed by the presence of YAP signal outside of the nucleus (stained
381 with DAPI) (**Fig. 6L, M**, black arrows). However, upon genipin treatment, YAP is
382 transported to the nucleus as shown by detection of YAP fluorescent signal only within
383 the boundaries of the nucleus (stained with DAPI) (**Fig. 6L, M**). This suggests that the
384 increase in ECM stiffness due to genipin-mediated crosslinking activates the Hippo
385 pathway in SNs. We next assessed whether YAP nuclear translocation results in
386 transcription of YAP-dependent genes. Indeed, we found that *YAP1* and *CYR61*
387 expression is significantly higher in FD SNs treated with genipin (**Fig. 6N**). *RUNX1* is a
388 transcription factor necessary for maturation of nociceptors (a subtype of SNs).
389 Interestingly, in cancer YAP interacts with *RUNX1* to regulate transcription^{52,53}, thus we
390 asked whether these genes are also activated in FD SNs in the presence of genipin. We
391 found that genipin increased the expression of all the tested genes that are dependent on
392 YAP-*RUNX1* interaction: *SULF1*, *S100A8*, *S100A14*, *COL12A1*, and *TMEM2* (**Fig. 6M**).
393 Together, our data suggest that genipin-dependent ECM crosslinking causes actin
394 reorganization and activation of the Hippo pathway in SNs, which rescue development
395 and survival in FD phenotypes.

396

397 Genipin enhances axon regeneration of neurons from the peripheral and central nervous
398 system.

399 Our results show that genipin rescues both developmental and degenerative FD
400 phenotypes in SNs, suggesting its beneficial support of neurons in this disease. We
401 wondered whether genipin might be beneficial in more common disorders of the PNS. To

402 test this hypothesis, we inquired if genipin is beneficial for axon regeneration. We first
403 assessed whether we can use our *in vitro* system as an axotomy model. We have
404 previously shown that in our system, hPSC-ctr-H9-derived SNs can be replated at late
405 stage¹³, which is known to enzymatically cut neurites and thus serving as an axotomy
406 model (**Fig. 7A**). Employing this approach, in combination with genipin treatment during
407 the regeneration phase, we found that this process causes expression of *ATF3* and
408 *SPRR1A*, two critical genes involved in neuron regeneration⁵⁴, in DMSO treated SNs
409 differentiated from hPSC-ctr-H9 cells (**Fig. 7B**). Interestingly, we found that SNs replated
410 in the presence of genipin express higher levels of both genes (**Fig. 7B**). Furthermore,
411 high *ATF3* expression is maintained for a longer period of time. We next assessed
412 whether genipin affects the speed of axon regeneration. We found that SNs treated with
413 genipin showed longer axons 18 hours and 6 days post replating (**Fig. 7C, D**). To expand
414 genipin's application in the PNS further, we tested peripheral sympathetic neuron
415 regeneration. To do so, we employed a microfluidic device, which maintains cell bodies
416 and axons in two different compartments, allowing us to break the axons without
417 disturbing the cell bodies (**Fig. 7E**). An NGF gradient guides the direction of axonal growth
418 and neurites can be cut and removed with a pipet tip from the left chamber without hurting
419 the cell bodies in the right chamber (**Fig. 7E**). We found that genipin increases the number
420 and length of regenerated axons from hPSC-ctr-H9-derived sympathetic neurons 2 days
421 post injury, measured by an increase in PRPH staining by immunofluorescence (**Fig. 7F,**
422 **G**). These results suggest that our system can be used to model axonal injury in the PNS
423 and that genipin increases the regeneration speed in peripheral neurons. Finally, we
424 asked if genipin might be beneficial for neurite regeneration in CNS neurons, possibly

425 extending its usefulness further to spinal cord injury in a broader context. To do that, we
426 tested prefrontal cortical (PFC) neurons differentiated from hPSC-ctr-H9 cells⁵⁵. Using
427 our replating method (**Fig. 7H**), we found that PFC neurons treated with genipin
428 regenerate their axons faster than DMSO-treated neurons by IF, measured by the
429 increase in the number of pixels positive for TUJ1 (**Fig. 7I-J**). Taken together, our results
430 suggest that genipin can be used to promote neuronal regeneration in both PNS and CNS
431 neurons applicable to a broad range of degenerative diseases.

432

433 **Discussion**

434 Here, we discovered that genipin rescues both neurodevelopmental and
435 neurodegenerative phenotypes in a hPSC-based disease model of FD. We further
436 showed that genipin rescues sensory ganglia phenotypes *in vivo*. Additionally, we
437 demonstrate that these phenotypes are due to crosslinking of ECM proteins causing
438 rearrangement of the cytoskeleton and activates transcription of YAP-dependent genes.
439 Finally, we showed that genipin promotes regeneration of neurons from the peripheral
440 and central nervous system after injury. Thus, genipin has a broad application potential
441 in a wide range of neurological disorders.

442 To date there is a lack of treatments that specifically target either the
443 neurodevelopmental or the neurodegenerative aspects of FD. This void leads to patient's
444 symptoms being managed both surgically and pharmacologically^{2,10} without clearly
445 targeting the molecular cause of the disease. Over the past 20 years, multiple compounds
446 have been identified that target the ELP1 splicing defect in FD have been identified,
447 however they have failed in clinical trials or there is no evidence that they target the PNS.

448 These include the plant hormone kinetin, which, despite its promising results in FD
449 animals³⁴, worsened the nausea and vomiting episodes in patients². Phosphatidylserine
450 (PS) which increased *ELP1* expression in patient-derived fibroblasts⁵⁶ and animal
451 models⁵⁷ but has not successfully undergone clinical trials². Finally, tocotrienol (vitamin
452 E) and the antioxidant epigallocatechin gallate (EGCG) were increase *ELP1* expression
453 in cells lines^{58,59} but did not increase *ELP1* expression in patients, nor improved clinical
454 outcomes^{16,60,61}. A recent study showed that the small molecule RECTAS restores normal
455 *ELP1* splicing in SNs differentiated from FD iPSCs and *in vivo*, however it is unclear
456 whether it rescues any FD phenotype³⁷. Finally, gene therapy approaches, such as
457 antisense oligonucleotides (ASO), have been shown to restore *ELP1* expression in
458 patient fibroblasts and FD animals⁶²⁻⁶⁴. Importantly, however, they did not show a rescue
459 in PNS tissues and human clinical trials are not initiated yet. In sum, the field lacks
460 specific drugs to treat FD and the available candidates have not been tested or are not
461 effective in human tissues affected in patients, such as PNS neurons.

462 Similar to FD, more common neurodegenerative disorders affecting the PNS
463 experience a dramatic absence of available treatments. These include diabetes (in up to
464 50% of patients) or chemotherapy-induced (in up to 40% of patients) peripheral
465 neuropathy, as well as neuropathies caused by injury, infections, medications,
466 alcoholism, and even vitamin deficiencies. In fact, there are very few FDA-approved drugs
467 available for peripheral neuropathy and PNS injury and the ones available mainly focus
468 on managing symptoms^{11,65}. Furthermore, although there is extensive research aimed to
469 identify treatment that regenerate peripheral neurons after injury, very few of them are or
470 have been in clinical trials. They include gene therapy, electrical stimulation, and nerve

471 transfer of an expendable, uninjured donor nerve⁶⁶. Due to these difficulties, genipin could
472 be a cost-effective treatment for nerve regeneration. Our results show that genipin is a
473 highly promising compound with potential to be further developed as a treatment option
474 for neurodevelopmental and neurodegenerative diseases (including peripheral
475 neuropathies). Additionally, its lack of toxicity on cells²⁶ and *in vivo*^{24,25}, and the fact that
476 it can transverse through the placenta, makes genipin a safe drug. Further studies will be
477 needed to address this.

478 Our results showing genipin's ability to rescue neurodevelopmental defects in
479 hPSC-derived FD SNs and in SNs in mice highlight the importance of ECM proteins
480 during development. This data lays the groundwork to further investigate the ECM
481 composition necessary for normal PNS development, as well as how defects in ECM
482 proteins, such as laminin¹⁷, contribute to PNS disorders⁶⁷. The rescue on
483 neurodevelopment that we see in FD mice is interesting and urges the investigation of
484 the effects of genipin on neurodegeneration further. As we report, genipin reverses the
485 progressive loss of SNs in the FD hPSC-based disease model and it enhances neurite
486 growth and density (**Fig. 5B-E, Sup. Fig. 3D**). This is consistent with the literature, where
487 it has been shown that the extracellular environment (presence of growth factors, ECM
488 composition, and 2D vs 3D cultures) affect neurite length and arborization^{33,40,68,69}. This
489 is in agreement with the role of genipin as an ECM crosslinker. It will be crucial to assess
490 this finding further, as this may provide real promise for patients with neurodegenerative
491 diseases. For instance, in chemotherapy-derived neuropathies, patients who are more
492 susceptible to axonal degeneration are affected by paclitaxel-induced neuropathy⁷⁴.
493 Genipin could be used as a supplement to prevent neuronal degeneration.

494 Most research on the molecular implications of FD focus primarily on *ELP1*. We
495 show that genipin exerts its effects via crosslinking of ECM proteins and that its rescue
496 effect is removed upon removal of its crosslinking activity (**Fig. 6E-H, Sup. Fig. 5F-I**).
497 Agreeing with this, we show that genipin increases surface stiffness of SNs and isolated,
498 deposited ECM proteins (**Fig. 6J, Sup. Fig. 6B-D**). Previous reports, showed that loss of
499 *ELP1* results in defective actin and microtubule organization in fibroblasts or brain
500 tissues^{50,70}. Here, we report that these phenotypes are conserved in SNs differentiated
501 from human FD iPSCs (**Fig. 6K, Sup. Fig. 6F, G**). Furthermore, we found that increasing
502 stiffness of the ECM by crosslinking rescues this phenotype, agreeing with studies
503 showing that biomechanical cues (such as stiffness of the ECM) affect actin cytoskeleton
504 organization⁷¹. Our RNAseq results show that upon loss of *ELP1* a myriad of ECM-related
505 proteins are downregulated causing an aberrant cellular environment. Therefore,
506 signaling multiple pathways are missregulated. One of them is the Hippo pathway, which
507 translates external mechanical cues into biochemical signals that affect gene
508 expression⁵¹. Interestingly, we show that YAP (the most downstream effector of the Hippo
509 pathway) is mainly present in the cytoplasm of SNs, suggesting that it is inactive, agreeing
510 with previous reports⁷². However, upon genipin treatment, YAP translocates to the
511 nucleus and promotes transcription of genes associated with cell proliferation (*CYR61*,
512 *S100A14*), cell cycle progression (*S100A8*), ECM remodeling (*SULF1*, *COL12A2*,
513 *TMEM2*) (**Fig. 6L-N**). We hypothesized that YAP activation initiates a transcriptional
514 program that allows the proliferation of progenitor cells, thus increasing the number and
515 survival of SNs. Very little is known of the role of the Hippo pathway and YAP function in
516 the PNS, although mechanotransduction (including ECM-cell interaction) is important in

517 NC migration and differentiation⁶⁷. Furthermore, it has been proposed that mechanical
518 modulation of the Hippo pathway could potentially be used as a treatment for peripheral
519 neuropathies⁷³. Genipin, by its ECM-crosslinking activity, fit in this category. Our results
520 show that potential treatment options for FD lay beyond ELP1 splicing correction and that
521 modifying the ECM may be key. Furthermore, our findings suggest that looking at basic
522 cellular mechanisms is necessary to identify new potential therapeutic targets.

523 In sum, genipin shows great promise as a future treatment option for FD as well
524 as other more common neuropathies. Its action via ECM crosslinking and our exciting
525 results about its ability to increase axon regeneration make it an interesting compound
526 for the future application to nerve regeneration in the PNS and possibly prevention of
527 peripheral neuropathies.

528

529 **Materials and methods**

530 Chemical screen

531 For the chemical screen hPSC-ctr-H9 control and iPSC-FD-S2 cells were cultured in KSR
532 conditions (see below). 96-well plates were coated with Matrigel. hPSCs were detached
533 from MEF cultures using Trypsin (52) and cells were seeded at 80,000 cells/well for iPSC-
534 FD-S2 and 40,000 cells/well for hPSC-ctr-H9 in order to start the differentiation at equal
535 cell numbers the following day (=day 0). The SN differentiation was performed as
536 described in Fig. 1c. The compounds of the LOPAC chemical library (Sigma) were added
537 on differentiation days 2, 5 and 8 and the plates were fixed and stained for BRN3A and
538 DAPI on day 10. 16 tiles/well were imaged using the MetaXPress software: Cell Scoring
539 by Molecular Devices. The percentage of BRN3A+ cells over DAPI of all DMSO control

540 wells was averaged. Each compound-treated well number was divided by this DMSO
541 average to calculate the fold change/fold increase of compound-treated wells over
542 controls. To call a hit the average + 3 SD was used. The Z' score (53) ($Z'=0.25$) was
543 calculated before conducting the screen, using a pilot screen with only hPSC-ctr-H9 and
544 iPSC-FD-S2 lines in a small format, but in the final screening conditions. The Z-score for
545 genipin at 1 mM was 15 and at 10mM it was 17.

546

547 hPSC maintenance

548 In KSR conditions, hPSCs were maintained on MEFs as described¹⁷. In E8 conditions,
549 human embryonic stem cells (WA-09, WiCell) and all iPSC lines were grown on dishes
550 coated with vitronectin (5 μ g/mL, 1h at RT) at 37°C with 5% CO₂ and fed daily with
551 Essential 8 Medium + Supplement. For splitting, hPSC-ctr-H9 colonies were washed with
552 PBS and incubated with 0.5mM EDTA, 3.08M NaCl in PBS with for 2 min at 37°C. Cells
553 were then resuspended in E8 + Supplement and split at a ratio of 1:10. iPSC-ctr-C1,
554 iPSC-FD-M2, iPSC-FD-S2, and iPSC-FD-S3 cells were previously characterized¹⁷ and
555 maintained under the same conditions.

556

557 Neural crest and sensory neuron differentiation

558 In KSR conditions: hPSCs were differentiated into SNs as described previously¹⁷. In E8/6
559 conditions: Differentiations were done as previously described¹³. Briefly, prior to
560 differentiation, plates were coated with vitronectin (5 μ g/mL) and incubated for 1h at RT.
561 hPSCs were harvested using 0.5 mM EDTA, 3.08 M NaCl in PBS for 20 min and plated
562 at a density of 200,000 cells/cm². On day of plating (day 0) and day 1, cells were fed with

563 NC differentiation media (day 0-1) containing: Essential 6 Medium, 10 μ M SB431542, 1
564 ng/mL BMP4, 300 nM CHIR99021, and 10 μ M Y-27632. BMP4 concentration is very
565 sensitive at this stage and was titrated for each line. Accordingly, BMP4 was not used
566 with iPSC-FD-S3 cells. From day 2 to 12, cells were fed every other day with NC
567 differentiation media (day 2-12): Essential 6 Medium, 10 μ M SB431542 0.75 μ M
568 CHIR99021, 2.5 μ M SU5402, and 2.5 μ M DAPT.

569 On day 12, cells were replated at a density of 250,000 cells/cm² onto plates coated with
570 15 μ g/ml poly-L-ornithine, 2 μ g/ml laminin-1, and 2 μ g/ml human fibronectin (PO/LM/FN).
571 Cells were incubated with Accutase for 20 min, washed with PBS, and resuspended in
572 SN Media: Neurobasal media containing N2, B-27, 2 mM L-glutamine, 20 ng/ml GDNF,
573 20 ng/ml BDNF, 25 ng/ml NGF, 600 ng/ml of laminin-1, 600 ng/ml fibronectin, 1 μ M DAPT
574 and 0.125 μ M retinoic acid. Cells were fed every 2-3 days until day 20. On day 20, DAPT
575 was removed. Differentiation progress was followed using a brightfield microscope
576 (Leica). Genipin (Sigma or Biomaterials), 1,10 anhydrogenipin (AG), dimethyl
577 pimelimidate (DMP, ThermoFisher), bis-(Sulfosuccinimidyl) suberate (BS3, CovaChem),
578 N-hydroxysulfosuccinimide (Sulfo-NHS, ThermoFisher) were added on day 2 of the
579 differentiation and included every media change. Genipin and AG were resuspended in
580 DMSO and aliquoted. DMP, BS3, and sulfo-NHS were resuspended in DMSO (DMP and
581 BS3) or PBS (sulfo-NHS) immediately prior to use.

582

583 Sympathetic neuron (symN) differentiation

584 The detailed differentiation protocol was described in previous publications^{17,75}. Briefly,
585 hPSCs were dissociated using EDTA and replated on Geltrex (Invitrogen, A1413202)-

586 coated plates at $125 \times 10^3/\text{cm}^2$ density in Essential 6 medium+0.4 ng/ml BMP4
587 (PeproTech, 314-BP)+10 μM SB431542 (R&D Systems, 1614)+300 nM CHIR99021
588 (R&D Systems, 4423). On day two, cells were fed with Essential 6 medium+10 μM
589 SB431542+0.75 μM CHIR99021 every two days until day 10 for neural crest induction.
590 Day 10 neural crests were dissociated using Accutase and replated on ultra-low
591 attachment plates (Corning, 07 200) in Neurobasal medium+B27+L-Glutamine+3 μM
592 CHIR99021+ 10 ng/ml FGF2 to induce sympathoblast spheroids. On day 14, spheroids
593 were dissociated using Accutase and replated on PO/LM/FN-coated plates at $1 \times 10^5/\text{cm}^2$
594 density in Neurobasal medium+B27+L-Glutamine+25 ng/ml GDNF+25 ng/ml BDNF+25
595 ng/ml NGF+200 μM ascorbic acid (Sigma, A8960)+0.2 mM dbcAMP (Sigma,
596 D0627)+0.125 μM retinoic acid. Medium was changed every three days from day 14 to
597 20. After day 20, neurons were fed weekly until desired timepoints.

598

599 Prefrontal cortical neuron (PFC) differentiation

600 The detailed differentiation protocol was described in previous publications^{55,76}. Briefly,
601 hPSCs were dissociated using EDTA and replated on Matrigel (Corning, 356234)-coated
602 plates at 260,000 cells/ cm^2 density in Essential 8 medium supplied with Y-27632. Next
603 day (defined as day 0), cells were fed with E6+100nM LDN193189 (Selleck Chemicals,
604 S2618)+10 μM SB431542 +2 μM XAV939 (TOCRIS, 3748). On day 2, cells were fed with
605 E6 + 100 nM LDN193189 + 10 μM SB every two days until day 8. On day 8, cells were
606 dissociated using Accutase and replated as droplets on PO/LM/FN-coated plates (cells
607 from each well of the 6-well plate were replated into 10 droplets, 10 μl per droplet) in
608 Neurobasal+N2+B27 (1:1000)+FGF8 (50 ng/mL, R&D)+SHH (25 ng/mL, R&D). On day

609 16, droplets were dissociated using Accutase and replated on PO/LM/FN-coated plates
610 at 1×10^6 cells/cm² density in Neurobasal+N2+B27 (1:1000)+FGF8 (50 ng/mL). On day
611 22, neurons were dissociated using Accutase and replated on PO/LM/FN-coated plates
612 at 100,000 cells/cm² density in Neurobasal+N2+B27 (1:50)+FGF8 (50 ng/mL). Neurons
613 were fed without FGF8 after day 40.

614

615

616 Electrophysiology

617 Experiments were performed using a Maestro Pro (Axion Biosystems) MEA system.
618 BioCircuit MEA 96 plates containing 8 embedded electrodes/well were coated with Poly-
619 L-ornithine/laminin/fibronectin (as previously described), seeded with day 12 NCCs
620 ($250,000$ cell/cm²) and allowed to continue differentiating. Repeated recordings were
621 made every 2-3 days at 37°C with a sampling frequency of 12.5 kHz for 5 min. Recordings
622 from at least 6 wells per reading were averaged. Firing frequency was normalized to the
623 number of active electrodes.

624

625 Survival and neurite growth assays

626 Day 12 NCCs, cells were replated on 4-well plates, at $250,000$ cells/cm² (for survival
627 assay) or $25,000$ cells/cm² (neurite growth assay), coated with PO/FN in SN media with
628 1 ng/ml NGF. Media was changed every 2-3 days. DAPT was removed after day 20. Cells
629 were fixed on day 13, 20, 27, and 34 and stained for BRN3A and TUJ1 (survival assay)
630 or on day 25 (neurite growth assay) and stained for TUJ1.

631

632 Axotomy models

633 *For SN regeneration*, day 25 SNs were dissociated with Accutase for 45min. Cells were
634 then collected in a conical tube and filled 1X with PBS. Cells were then centrifuged and
635 resuspended in SN media with DMSO or genipin (10 μ M). SNs were seed in PO/LM/FN-
636 coated plates at 250,000 cells/cm² and incubated for 6 days. RNA was collected on the
637 indicated days. Alternatively, SNs were fixed as previously described at the indicated
638 times.

639 *For PFC regeneration*, day 40 PFCs were dissociated using Accutase and replated on
640 PO/LM/FN-coated plates at 25x10³/cm² density with DMSO or genipin (10 μ M). Replated
641 neurons were fed every two days until day 45 and fixed for evaluation.

642 *For symN regeneration*, day 14 sympathoblasts were replated on the PO/LM/FN-coated
643 microfluidic culture devices (eNUVIO, OMEGA4) on one side at 100,000 cells/cm²
644 density. The replating side was defined as the cell body chamber, while another was
645 defined as the axon chamber. NGF containing medium was given to both chambers. On
646 day 20, NGF was given only to the axon chamber to induce axon outgrowth for 10 days.
647 On day 30, axons in the axon chamber were removed using the suction, and DMSO or
648 genipin containing media were added to both chambers for 48 hours. On day 32,
649 regenerating axons were fixed for evaluation.

650

651 Antibodies

652 The following primary antibodies were used: SOX10 (Santa Cruz, cat# sc-365692),
653 TFAP2A (Abcam, cat# ab108311), BRN3A (Millipore, cat# MAB1585), TUJ1 (Biolegend,
654 cat# 801201), ISL1 (DSHB, cat# 39.4D5-c), PRPH (Santa Cruz, cat# sc-377093), Actin

655 (BD Biosciences, cat# 612656), YAP1 (Proteintech, cat# 13584-1-AP), Phalloidin-iFluor
656 488 (abcam, cat# ab176753). The following secondary antibodies were used: From
657 ThermoFisher: goat anti-mouse IgG1 AF488 (cat# A21121), goat anti-mouse IgG2a (cat#
658 A-21131), goat anti-mouse IgG2b (cat# A21242), donkey anti-rabbit AF647 (cat#
659 A31573), donkey anti-mouse AF488 (cat# A21202), goat anti-mouse HRP (cat# 62-
660 6520), and goat anti-rabbit HRP (cat# 65-6120). The dilutions used are indicated in each
661 section.

662

663 Flow cytometry

664 Cells were dissociated with Accutase for 30 min and then washed in Flow buffer (DMEM,
665 2% FBS, and 1mM L-glutamine). Cells were centrifuged at 200 g for 4 min, resuspended
666 in cold PBS, counted, and diluted to a concentration of 1×10^6 cells/100 μ L. Cells were then
667 centrifuged and resuspended in 300 μ L BD Cytofix/Cytoperm (BD Biosciences) buffer
668 and incubated on ice for 30 min. Cells were centrifuged for 4 min at 2,000 RPM and
669 resuspended in 600 μ L of cold BD Permeabilization/Wash buffer (BD Biosciences). 30 μ L
670 goat serum was added followed by incubation on ice for 30 min. Cells were divided in 3:
671 unstained control, secondary antibody control, and sample to stain (200 μ L each). All
672 tubes were centrifuged for 4 min at 2,000 RPM and the cells were resuspended in 200 μ L
673 of Antibody buffer: BD perm/wash buffer + 10 μ L goat serum with or without BRN3A
674 antibody (1:100). Cells were incubated o/n at 4°C. Cells were then washed twice with 300
675 μ L BD Permeabilization/Wash buffer, resuspended in Antibody buffer with or without
676 AF488 goat-anti-mouse (1:500), and incubated on ice for 30 min. Cells were then washed

677 3X with BD perm/wash buffer. Cells were filtered and analyzed using a Cytoflex S
678 (Beckman). Analysis was done using FlowJo.

679

680 Immunoblotting

681 Whole cell lysates were obtained from day 12 NCCs or day 20 SNs. Cells were washed
682 once with PBS and incubated with RIPA buffer (ThermoFisher) with 1 mM PMSF and
683 Phospho-STOP (Roche) for 15min on ice. Cells were then vortexed for 10 s and
684 centrifuged at 12,000 RPM for 10 min at 4°C. Protein concentration from supernatants
685 was measured and ran in 7.5% polyacrylamide gels using MOPS buffer (GenScript) at
686 130 V. Proteins were transferred to a nitrocellulose membrane and blocked for 30 min in
687 5% non-fat dry milk in 0.1% Tween-20 in TBS (TBS-T, 50 mM Tris-HCl, 150 mM NaCl,
688 pH7.6). Primary antibodies were added to blocking buffer (SOX10 - 1:1000, BRN3A -
689 1:1000, and Actin - 1:5000) and membranes were incubated overnight at 4°C. Blots were
690 then washed 3X with 0.1% TBS-T and incubated with goat anti-mouse HRP antibody
691 (1:5000) for 1 h at room temperature. Blots were washed 3X with 0.1% TBS-T followed
692 by incubation with Clarity Western ECL Substrate (BioRad). Chemiluminescence signal
693 was detected using UVP ChemStudio (Analytic Jena).

694

695 Immunofluorescence

696 NCCs and SNs (day 12 and day 20, respectively, unless indicated otherwise) from either
697 24- or 4-well plates were fixed with 4% paraformaldehyde for 20 min at RT. Cells were
698 washed with PBS and incubated for 20 min with Permeabilization buffer containing 1%
699 BSA, 0.3% Triton-X, 3% goat or donkey serum and 0.01% sodium azide in PBS. Cells

700 were then incubated with the indicated primary antibodies (SOX10 – 1:100, TFAP2A –
701 1:500, BRN3A – 1:100, TUJ1 – 1:1500, ISL1 – 1:200, PRPH – 1:100) in Antibody buffer
702 containing 1% BSA, 3% goat or donkey serum and 0.01% sodium azide overnight at 4°C.
703 The cells were washed 3X in PBS and incubated for 1h with Secondary antibodies in
704 Antibody buffer. Cells were washed with PBS and incubated with DAPI (1:1,000) for 5min,
705 washed with PBS and stored at 4°C. All imaging was done using a Lionheart FX
706 fluorescence microscope (BioTek). All image analysis and quantifications were done in
707 Fiji. For quantifications, 5 different fields were imaged and quantified. To measure the
708 area of NCCs, day 12 NCCs images were analyzed using Gen5. A mask measuring DAPI
709 signal intensity was used. Threshold was established as the 25% signal intensity from the
710 average signal of each field. The areas of objects between 100 µm to 1000 µm where
711 DAPI signal above background were measured. To measure neurite length, images were
712 transformed to 8 bit grayscale images and neurites were measured using NeuronJ⁷⁷. For
713 confocal microscopy, on day 12, 50,000 NCCs were seeded in iBidi dishes (cat# 80426)
714 in the presence or absence of genipin. On day 20, SNs were fixed and steined as
715 previously described. Primary antibodies used: TUJ1 – 1:1500, YAP1 – 1:100. Phalloidin-
716 iFluor 488 (1:1000) was incubated with secondary antibodies for 1 h. Imaging was done
717 in an Olympus FV1200 Confocal Laser Scanning Microscope using Argon and Helium-
718 Neon lasers. Images were taken as Z-stacks of 3 µm of height. ImageJ was used to obtain
719 maximum intensity projections and to measure the signal intensity profiles.

720

721 RT-qPCR

722 RNA was isolated using Trizol (ambion) according to manufacturer's conditions,
723 resuspended in 20 μ L RNase-free water and concentration was measured using
724 NanoDrop One (Thermo Scientific). 1 μ g of RNA was converted to cDNA using iScript
725 cDNA Synthesis kit (BioRad) according to manufacturer's instructions and diluted 1:100
726 in water. RT-qPCR reactions were run with 1 ul of cDNA and SYBR Green Supermix
727 (BioRad) according to the manufacturer's conditions. Reactions were run in a C1000
728 Touch Thermal Cycler CFX96 (BioRad) using the following cycling parameters: 95°C for
729 5 min, 40 cycles of 95°C for 5s and 60°C for 10 s. Results were analyzed using the
730 comparative CT method. GAPDH was used as a housekeeping gene.

731

732 Primers

733 The following primers were used in this study:

734 SOX10f-5'CCAGGCCCACTACAAGAGC, SOX10r-5'CTCTGGCCTGAGGGGTGC,
735 TFAP2Af-5'GACCTCTCGATCCACTCCTTAC, TFAP2Ar-
736 5'GAGACGGCATTGCTGTTGGACT, FOXS1f-5'ATCCGCCACAACCTGTCACTCA,
737 FOXS1r-5'GTAGGAAGCTGCCGTGCTCAAA, P75f-
738 5'CCTCATCCCTGTCTATTGCTCC, P75r-5'GTTGGCTCCTTGCTTGTCTGC, NGN1f-
739 5'GCCTCCGAAGACTTCACCTACC, NGN1r-5'GGAAAGTAACAGTGTCTACAAAGG
740 NGN2f-5'CAAGCTCACCAAGATCGAGACC, NGN2r
741 5'AGCAAACTGCCTCGGAGAAGA, BRN3Af-5'AGTACCCGTCGCTGCACTCCA,
742 BRN3Ar- 5'TTGCCCTGGGACACGGCGATG, ISL1f-
743 5'GCAGAGTGACATAGATCAGCCTG, ISL1r-5'GCCTCAATAGGACTGGCTACCA,
744 PRPHf-5'GTGCCCGTCCATTCTTTTGC, PRPHr-5'GTGCCCGTCCATTCTTTTGC,

745 TRKAf-5'CACTAACAGCACATCTGGAGACC, TRKAr-
746 5'TGAGCACAAGGAGCAGCGTAGA, TRKBf-5'ACAGTCAGCTCAAGCCAGACAC,
747 TRKBr-5'GTCCTGCTCAGGACAGAGGTTA, TRKCf-
748 5'CCGACACTGTGGTCATTGGCAT, TRKCr-5'CAGTTCTCGCTTCAGCACGATG,
749 YAP1f-5' TGTCCCAGATGAACGTCACAGC, YAP1r-5'
750 TGGTGGCTGTTTCACTGGAGCA, CYR61f-5' GGAAAAGGCAGCTCACTGAAGC,
751 CYR61r-5' GGAGATACCAGTTCCACAGGTC, SULF1f-5'
752 GGTCCAAGTGTAGAACCAGGATC, SULF1r-5' GACAGACTTGCCGTCCACATCA,
753 S100A8f-5' ATGCCGTCTACAGGGATGACCT, S100A8r-5'
754 AGAATGAGGAACTCCTGGAAGTTA, S100A14f-5'
755 CCTCATCAAGAACTTTCACCAGTA, S100A14r-5' GGTTGGCAATTTTCTCTTCCAGG,
756 COL12A1f-5' CAGTGCCTGTAGTCAGCCTGAA, COL12A1r-5'
757 GGTCTTGTTGGCTCTGTGTCCT, TMEM2f-5' GGAATAGGACTGACCTTTGCCAG,
758 TMEM2r-5' TTCTGACCACCCTGAAAGCCGT, ATF3f-5'
759 CGCTGGAATCAGTCACTGTCAG, ATF3r-5' CTTGTTTCGGCACTTTGCAGCTG,
760 SPRR1Af-5' GTGAAACAACCTTGCCAGCCTC, SPRR1Ar-5'
761 TGGCAGGGCTCTGGAACCTTG

762

763 RNAseq

764 *Global gene clustering.* For the first round, paired-end RNA sequencing was performed
765 on hPSC-ctr-H9, iPSC-ctr-C1, and iPSC-FD-S2 day 15 SNs (differentiated as previously
766 described ¹⁷) with no replicates. Reads were mapped to the human genome build hg19
767 using TopHat software ⁷⁸. Reads that aligned with no more than 2-bp mismatches were

768 accepted for downstream analysis. After mapping, we computed the expression count
769 matrix from the mapped reads using HTSeq (<https://htseq.readthedocs.io/en/master/>)
770 and one of several possible gene model databases. The generated count matrix was then
771 processed using the R Bioconductor package DESeq
772 (<http://www.huber.embl.de/users/anders/DESeq/>) to normalize the full data set and
773 analyze differential expression between samples.

774 *GOterm analysis.* Genes that showed significant downregulation in iPSC-FD-S2 SNs vs
775 hPSC-ctr-H9 and iPSC-ctr-C1 SNs were subjected to GO term analysis using the DAVID
776 functional annotation tool (<https://david.ncifcrf.gov/tools.jsp>). The occurrence of GO terms
777 with a FDR score <0.05 were graphed.

778

779 Synthesis of 1,10-anhydrogenipin

780 Genipin (1.52 g, 6.7 mmol) and triphenylphosphine (1.85 g, 7.1 mmol) were dissolved in
781 33.5 mL of distilled methylene chloride, brought to 0°C, and stirred for 15 minutes. To this
782 solution was added diisopropyl azodicarboxylate (DIAD, 1.38 ml, 7.1 mmol) dropwise over
783 10 min and the mixture was stirred on ice for one hour. The reaction was removed from
784 the ice bath and allowed to be stirred overnight at room temperature. TLC (R_f=0.55 in 5:1
785 hexane/EtOAc) analysis revealed the presence of the product which was purified by flash
786 chromatography (5:1 hexane/EtOAc) to yield a white solid (0.45g, 33%). Mass
787 spectrometry and NMR analysis matched previously published data ²⁴.

788

789 Atomic Force Microscopy measurements

790 To prepare the samples for AFM imaging and Young's Modulus measurements, Day 25
791 sensory neurons (SNs) were differentiated in the presence of DMSO or genipin in
792 Nunclon Delta surface dishes (ThermoFisher, cat# 150460 vendor). Cells were washed
793 with PBS and fixed with 4% paraformaldehyde for 20 min at room temperature, followed
794 by 3X washes with PBS. Alternatively, SN-derived extracellular matrix (ECM) was isolated
795 as previously described⁷⁹. SNs were washed with PBS and stripped by incubation with
796 20mM Ammonium Hydroxide with agitation for 5min. The dishes were then washed 5X
797 with water and the ECM was fixed with 4% paraformaldehyde for 20 min at room
798 temperature followed by 3 washes with PBS. AFM measurements were done using an
799 Agilent 5500 SPM system. Before the AFM experiments, the spring constant of the
800 conical AFM tip on a rectangular cantilever (NANOSENSORS qp-BioAC) was calibrated
801 to be 6pN/nm. The sensitivity of the cantilever was obtained using a very hard glass
802 substrate to be 34.15nm/V. The AFM tip was roughly aligned on the surface of the cell by
803 using an optical microscope, then contact AFM mode was applied to obtain the high-
804 resolution topographic images of the samples. Large area (~80 μ m²) AFM images were
805 obtained . For each sample, we chose multiple spots on the sample to measure the force-
806 distance curves and calculate the YMs so that a reliable YM can be statically determined.
807 Force-distance curves were further fitted with Hertz model ⁴⁶ (suitable for fitting under
808 small deformation in nanoindentation measurements) and calculate the YM of individual
809 cell by a commercial software PUNIAS ^{80,81}. We carefully control the applied force
810 (setpoint) of 60pN to minimize the deformation. ECM was isolated as previously described
811 ⁷⁹. Briefly, on day 20, SNs were washed with PBS and incubated for 5 min with 20 mM

812 Ammonium Hydroxide (Sigma, cat# 221228-100ML-A) with constant shaking. Dishes
813 were washed with 5 mL of de-ionized H₂O and fixed with 4% PFA for 20 min. Cells were
814 then washed 3 times with PBS prior to processing for AFM.

815

816 Animal experiments

817 *Mice.* *Elp1^{flox/+}* and *Elp1^{Δ20/+}* mice (previously named *lkbkap⁹*) were housed and bred at the
818 University of Tennessee Health Science Center Comparative Medicine Department
819 animal core facility.

820 *Timed-pregnancies and Genipin treatment.* Female *Elp1^{flox/+}* mice were crossed with
821 *Elp1^{Δ20/+}* males ⁹ for timed-pregnancies. At E10.5 a fresh mouse cage was provided to
822 pregnant female mice for acclimatization and shortly after breeder mouse chow was
823 replaced with genipin-containing moist breeder chow (500 mg/Kg of chow), corresponding
824 to daily consumption of 75 mg of genipin per Kg of body weight. Genipin-containing moist
825 chow was supplied fresh daily. Females were weighed daily and sacrificed at late
826 gestation (E18.5).

827 *Genotyping of embryos.* Genomic DNA was prepared from embryo tail biopsies, and
828 embryos were genotyped by PCR amplification as described previously ⁹.

829 *Histological analyses.* Embryos were fixed in 4% paraformaldehyde in phosphate-
830 buffered saline (PBS) for at least one week. Embryos and placentas were weighed and
831 then incubated for 24 hours at 4°C in PBS containing 0.25 M sucrose, 0.2 M glycine;
832 dehydrated; cleared with toluene; and embedded in paraffin. Paraffin blocks were serially

833 sectioned at 7 μm , mounted in superfrost slides (Fisher) and stained with haematoxylin
834 and eosin (H&E).

835 *Volumetric determination and neuronal counts.* Volumes of sensory ganglia were
836 determined as described previously^{9,29}. In brief, H&E-stained serial paraffin sections (7
837 μm) spanning the whole ganglia were analyzed under a Zeiss stereomicroscope, and
838 width and length were measured every 5th section. Volumetric measurements were
839 performed by calculating and adding the volumes between every section analyzed. For
840 neuronal counts, neurons with clearly visible nucleoli were counted from
841 photomicrographs of H&E-stained paraffin sections. Total neuronal numbers were
842 estimated based on the total volume of the ganglia.

843 *Immunohistochemistry on paraffin sections.* For immunohistochemistry on paraffin
844 sections (IHC-P), slides were deparaffinized, rehydrated, and incubated with 0.3% H_2O_2
845 in methanol for 20 min, to quench endogenous peroxidase. Sections were then washed
846 with PBS, blocked for 1 hr with 4% BSA, 0.2% Triton X-100 in PBS, and incubated at 4°C
847 for 24hrs with primary rabbit monoclonal anti-CGRP antibody (Calbiochem PC205)
848 diluted 1:200 in 0.4% BSA; 0.2% Triton X-100 in PBS. After several washes in PBS,
849 primary antibody detection was carried out using the Vector ABC Elite kit according to
850 manufacturers' instructions, followed by incubation with DAB brown substrate (BD
851 Biosciences).

852 *Quantification of CGRP+ sensory nociceptive neurons.* Nociceptive neurons were
853 quantified at E18.5 by counting CGRP-positive staining of neurons in cross sections of
854 lumbar L1 DRG. For each embryo, four images per DRG from at least two independently-
855 immunostained sections were captured at 20X and divided into quadrants. Total number

856 of cells and number of CGRP-positive cells per quadrant were determined using ImageJ.
857 Positive signal was established as 30% above background.

858

859 Statistical analysis

860 All analysis and graphs were done using PRISM (GraphPad). Number of independent
861 experiments (biological replicates, n) and statistical analysis are indicated on each figure
862 legends. Biological replicates are defined as independent differentiations started at least
863 3 days apart or from a freshly thawed vial of cells. Statistics for animal data, see below.
864 Data presented are shown as mean \pm SD. For experiments *in vivo*: data were derived
865 from multiple independent experiments from distinct mice. Animal studies were performed
866 without blinding of the investigator. Histological analyses were performed in at least 3
867 embryos per genotype and treatment. No statistical method was used to predetermine
868 sample size, but sample size was based on preliminary data and previous publications.
869 In all experiments the differences were considered significant when $p < 0.05$. The
870 differences between groups were assessed using one-way ANOVA followed by Tuckey
871 HSD post hoc test.

872

873 Study approval:

874 Animal experiments were carried out in strict accordance with the Guide for the Care and
875 Use of Laboratory Animals of the National Institutes of Health. The protocol was approved
876 by the Animal Care and Use Committee of the University of Tennessee Health Science
877 Center.

878

879 **List of Supplementary Materials:**

880 Sup Fig. 1 to Sup. Fig. 6

881 Sup. Table 1 to Sup. Table 3

882

883 **References and notes:**

884 1. Meng, J., Zhang, Q., Yang, C., Xiao, L., Xue, Z., and Zhu, J. (2019). Duloxetine,
885 a Balanced Serotonin-Norepinephrine Reuptake Inhibitor, Improves Painful
886 Chemotherapy-Induced Peripheral Neuropathy by Inhibiting Activation of p38
887 MAPK and NF- κ B. *Front Pharmacol* 10, 365. 10.3389/fphar.2019.00365.

888 2. Norcliffe-Kaufmann, L., Slaugenhaupt, S.A., and Kaufmann, H. (2017). Familial
889 dysautonomia: History, genotype, phenotype and translational research. *Prog*
890 *Neurobiol* 152, 131–148. 10.1016/j.pneurobio.2016.06.003.

891 3. O'Connor, A.B. (2009). Neuropathic pain: quality-of-life impact, costs and cost
892 effectiveness of therapy. *Pharmacoeconomics* 27, 95–112. 10.2165/00019053-
893 200927020-00002.

894 4. Benam, K.H., Dauth, S., Hassell, B., Herland, A., Jain, A., Jang, K.-J., Karalis, K.,
895 Kim, H.J., MacQueen, L., Mahmoodian, R., et al. (2015). Engineered in vitro
896 disease models. *Annu Rev Pathol* 10, 195–262. 10.1146/annurev-pathol-012414-
897 040418.

898 5. Blumenfeld, A., Slaugenhaupt, S.A., Liebert, C.B., Temper, V., Maayan, C., Gill,
899 S., Lucente, D.E., Idelson, M., MacCormack, K., Monahan, M.A., et al. (1999).
900 Precise genetic mapping and haplotype analysis of the familial dysautonomia
901 gene on human chromosome 9q31. *Am J Hum Genet* 64, 1110–1118.
902 10.1086/302339.

903 6. Slaugenhaupt, S.A., Blumenfeld, A., Gill, S.P., Leyne, M., Mull, J., Cuajungco,
904 M.P., Liebert, C.B., Chadwick, B., Idelson, M., Reznik, L., et al. (2001). Tissue-
905 Specific Expression of a Splicing Mutation in the IKBKAP Gene Causes Familial
906 Dysautonomia. *The American Journal of Human Genetics* 68, 598–605.
907 10.1086/318810.

908 7. Pearson, J., Pytel, B.A., Grover-Johnson, N., Axelrod, F., and Dancis, J. (1978).
909 Quantitative studies of dorsal root ganglia and neuropathologic observations on
910 spinal cords in familial dysautonomia. *J Neurol Sci* 35, 77–92. 10.1016/0022-
911 510x(78)90103-x.

912 8. Axelrod, F.B. (2006). A world without pain or tears. *Clin. Auton. Res.* 16, 90–97.
913 10.1007/s10286-006-0326-7.

- 914 9. Dietrich, P., Alli, S., Shanmugasundaram, R., and Dragatsis, I. (2012). IKAP
915 expression levels modulate disease severity in a mouse model of familial
916 dysautonomia. *Hum Mol Genet* 21, 5078–5090. 10.1093/hmg/dds354.
- 917 10. Palma, J.-A., Norcliffe-Kaufmann, L., Fuente-Mora, C., Percival, L., Mendoza-
918 Santiesteban, C., and Kaufmann, H. (2014). Current treatments in familial
919 dysautonomia. *Expert Opin Pharmacother* 15, 2653–2671.
920 10.1517/14656566.2014.970530.
- 921 11. Barrell, K., and Smith, A.G. (2019). Peripheral Neuropathy. *Med Clin North Am*
922 103, 383–397. 10.1016/j.mcna.2018.10.006.
- 923 12. Zeltner, N., and Studer, L. (2015). Pluripotent stem cell-based disease modeling:
924 current hurdles and future promise. *Curr. Opin. Cell Biol.* 37, 102–110.
925 10.1016/j.ceb.2015.10.008.
- 926 13. Saito-Diaz, K., Street, J.R., Ulrichs, H., and Zeltner, N. (2021). Derivation of
927 Peripheral Nociceptive, Mechanoreceptive, and Proprioceptive Sensory Neurons
928 from the same Culture of Human Pluripotent Stem Cells. *Stem Cell Reports* 16,
929 446–457. 10.1016/j.stemcr.2021.01.001.
- 930 14. Saito-Diaz, K., and Zeltner, N. (2019). Induced pluripotent stem cells for disease
931 modeling, cell therapy and drug discovery in genetic autonomic disorders: a
932 review. *Clin Auton Res* 29, 367–384. 10.1007/s10286-018-00587-4.
- 933 15. Lee, G., Papapetrou, E.P., Kim, H., Chambers, S.M., Tomishima, M.J., Fasano,
934 C.A., Ganat, Y.M., Menon, J., Shimizu, F., Viale, A., et al. (2009). Modeling
935 Pathogenesis and Treatment of Familial Dysautonomia using Patient Specific
936 iPSCs. *Nature* 461, 402–406. 10.1038/nature08320.
- 937 16. Lee, G., Ramirez, C.N., Kim, H., Zeltner, N., Liu, B., Radu, C., Bhinder, B., Kim,
938 Y.J., Choi, I.Y., Mukherjee-Clavin, B., et al. (2012). Large-scale screening using
939 familial dysautonomia induced pluripotent stem cells identifies compounds that
940 rescue IKBKAP expression. *Nat. Biotechnol.* 30, 1244–1248. 10.1038/nbt.2435.
- 941 17. Zeltner, N., Fattahi, F., Dubois, N.C., Saurat, N., Lafaille, F., Shang, L., Zimmer,
942 B., Tchieu, J., Soliman, M.A., Lee, G., et al. (2016). Capturing the biology of
943 disease severity in a PSC-based model of familial dysautonomia. *Nat. Med.* 22,
944 1421–1427. 10.1038/nm.4220.
- 945 18. Axelrod, F.B., Iyer, K., Fish, I., Pearson, J., Sein, M.E., and Spielholz, N. (1981).
946 Progressive sensory loss in familial dysautonomia. *Pediatrics* 67, 517–522.
- 947 19. Xiao, W., Li, S., Wang, S., and Ho, C.-T. (2017). Chemistry and bioactivity of
948 *Gardenia jasminoides*. *J Food Drug Anal* 25, 43–61. 10.1016/j.jfda.2016.11.005.

- 949 20. Li, Y., Li, L., and Hölscher, C. (2016). Therapeutic Potential of Genipin in Central
950 Neurodegenerative Diseases. *CNS Drugs* 30, 889–897. 10.1007/s40263-016-
951 0369-9.
- 952 21. Li, M., Cai, N., Gu, L., Yao, L., Bi, D., Fang, W., Lin, Z., Wu, Y., Xu, H., Li, H., et
953 al. (2021). Genipin Attenuates Tau Phosphorylation and A β Levels in Cellular
954 Models of Alzheimer’s Disease. *Mol Neurobiol* 58, 4134–4144. 10.1007/s12035-
955 021-02389-8.
- 956 22. Chen, Y., Zhang, Y., Li, L., and Hölscher, C. (2015). Neuroprotective effects of
957 geniposide in the MPTP mouse model of Parkinson’s disease. *Eur J Pharmacol*
958 768, 21–27. 10.1016/j.ejphar.2015.09.029.
- 959 23. Su, C., Yang, X., and Lou, J. (2016). Geniposide reduces α -synuclein by blocking
960 microRNA-21/lysosome-associated membrane protein 2A interaction in
961 Parkinson disease models. *Brain Res* 1644, 98–106.
962 10.1016/j.brainres.2016.05.011.
- 963 24. Zhang, C.-Y., Parton, L.E., Ye, C.P., Krauss, S., Shen, R., Lin, C.-T., Porco, J.A.,
964 and Lowell, B.B. (2006). Genipin inhibits UCP2-mediated proton leak and acutely
965 reverses obesity- and high glucose-induced beta cell dysfunction in isolated
966 pancreatic islets. *Cell Metab.* 3, 417–427. 10.1016/j.cmet.2006.04.010.
- 967 25. Koo, H.-J., Song, Y.S., Kim, H.-J., Lee, Y.-H., Hong, S.-M., Kim, S.-J., Kim, B.-C.,
968 Jin, C., Lim, C.-J., and Park, E.-H. (2004). Antiinflammatory effects of genipin, an
969 active principle of gardenia. *European Journal of Pharmacology* 495, 201–208.
970 10.1016/j.ejphar.2004.05.031.
- 971 26. Xi-xun, Y., Fei, L., Yuan-ting, X., and Chang-xiu, W. (2010). In vitro study in the
972 endothelial cell compatibility and endothelialization of genipin-crosslinked
973 biological tissues for tissue-engineered vascular scaffolds. *J Mater Sci Mater*
974 *Med* 21, 777–785. 10.1007/s10856-009-3933-8.
- 975 27. Hobbs, C.A., Koyanagi, M., Swartz, C., Davis, J., Maronpot, R., Recio, L., and
976 Hayashi, S.-M. (2018). Genotoxicity evaluation of the naturally-derived food
977 colorant, gardenia blue, and its precursor, genipin. *Food Chem Toxicol* 118, 695–
978 708. 10.1016/j.fct.2018.06.001.
- 979 28. Chambers, S.M., Qi, Y., Mica, Y., Lee, G., Zhang, X.-J., Niu, L., Bilsland, J., Cao,
980 L., Stevens, E., Whiting, P., et al. (2012). Combined small-molecule inhibition
981 accelerates developmental timing and converts human pluripotent stem cells
982 into nociceptors. *Nat. Biotechnol.* 30, 715–720. 10.1038/nbt.2249.
- 983 29. Morini, E., Dietrich, P., Salani, M., Downs, H.M., Wojtkiewicz, G.R., Alli, S.,
984 Brenner, A., Nilbratt, M., LeClair, J.W., Oaklander, A.L., et al. (2016). Sensory
985 and autonomic deficits in a new humanized mouse model of familial
986 dysautonomia. *Hum. Mol. Genet.* 25, 1116–1128. 10.1093/hmg/ddv634.

- 987 30. Dietrich, P., and Dragatsis, I. (2016). Familial Dysautonomia: Mechanisms and
988 Models. *Genet Mol Biol* 39, 497–514. 10.1590/1678-4685-GMB-2015-0335.
- 989 31. Ernsberger, U. (2009). Role of neurotrophin signalling in the differentiation of
990 neurons from dorsal root ganglia and sympathetic ganglia. *Cell Tissue Res* 336,
991 349–384. 10.1007/s00441-009-0784-z.
- 992 32. Hilz, M.J., Axelrod, F.B., Bickel, A., Stemper, B., Brys, M., Wendelschafer-Crabb,
993 G., and Kennedy, W.R. (2004). Assessing function and pathology in familial
994 dysautonomia: assessment of temperature perception, sweating and cutaneous
995 innervation. *Brain* 127, 2090–2098. 10.1093/brain/awh235.
- 996 33. Ribeiro, A., Vargo, S., Powell, E.M., and Leach, J.B. (2012). Substrate three-
997 dimensionality induces elemental morphological transformation of sensory
998 neurons on a physiologic timescale. *Tissue Eng Part A* 18, 93–102.
999 10.1089/ten.tea.2011.0221.
- 1000 34. Morini, E., Gao, D., Montgomery, C.M., Salani, M., Mazzasette, C., Krussig, T.A.,
1001 Swain, B., Dietrich, P., Narasimhan, J., Gabbeta, V., et al. (2019). ELP1 Splicing
1002 Correction Reverses Proprioceptive Sensory Loss in Familial Dysautonomia. *Am*
1003 *J Hum Genet* 104, 638–650. 10.1016/j.ajhg.2019.02.009.
- 1004 35. Yannai, S., Zonszain, J., Donyo, M., and Ast, G. (2019). Combinatorial treatment
1005 increases IKAP levels in human cells generated from Familial Dysautonomia
1006 patients. *PLoS One* 14, e0211602. 10.1371/journal.pone.0211602.
- 1007 36. Slaugenhaupt, S.A., Mull, J., Leyne, M., Cuajungco, M.P., Gill, S.P., Hims, M.M.,
1008 Quintero, F., Axelrod, F.B., and Gusella, J.F. (2004). Rescue of a human mRNA
1009 splicing defect by the plant cytokinin kinetin. *Hum Mol Genet* 13, 429–436.
1010 10.1093/hmg/ddh046.
- 1011 37. Ajiro, M., Awaya, T., Kim, Y.J., Iida, K., Denawa, M., Tanaka, N., Kurosawa, R.,
1012 Matsushima, S., Shibata, S., Sakamoto, T., et al. (2021). Therapeutic
1013 manipulation of IKBKAP mis-splicing with a small molecule to cure familial
1014 dysautonomia. *Nat Commun* 12, 4507. 10.1038/s41467-021-24705-5.
- 1015 38. Muzzarelli, R.A.A., El Mehtedi, M., Bottegoni, C., and Gigante, A. (2016).
1016 Physical properties imparted by genipin to chitosan for tissue regeneration with
1017 human stem cells: A review. *Int J Biol Macromol* 93, 1366–1381.
1018 10.1016/j.ijbiomac.2016.03.075.
- 1019 39. Chen, Z.-L., and Strickland, S. (2003). Laminin γ 1 is critical for Schwann cell
1020 differentiation, axon myelination, and regeneration in the peripheral nerve. *J Cell*
1021 *Biol* 163, 889–899. 10.1083/jcb.200307068.
- 1022 40. Gardiner, N.J. (2011). Integrins and the extracellular matrix: key mediators of
1023 development and regeneration of the sensory nervous system. *Dev Neurobiol* 71,
1024 1054–1072. 10.1002/dneu.20950.

- 1025 41. Yap, L., Tay, H.G., Nguyen, M.T.X., Tjin, M.S., and Tryggvason, K. (2019).
1026 Laminins in Cellular Differentiation. *Trends Cell Biol.* 29, 987–1000.
1027 10.1016/j.tcb.2019.10.001.
- 1028 42. Goffena, J., Lefcort, F., Zhang, Y., Lehrmann, E., Chaverra, M., Felig, J.,
1029 Walters, J., Buksch, R., Becker, K.G., and George, L. (2018). Elongator and
1030 codon bias regulate protein levels in mammalian peripheral neurons. *Nat*
1031 *Commun* 9, 889. 10.1038/s41467-018-03221-z.
- 1032 43. Park, S., Koch, D., Cardenas, R., Käs, J., and Shih, C.K. (2005). Cell motility and
1033 local viscoelasticity of fibroblasts. *Biophys J* 89, 4330–4342.
1034 10.1529/biophysj.104.053462.
- 1035 44. Ungureanu, A.-A., Benilova, I., Krylychkina, O., Braeken, D., De Strooper, B.,
1036 Van Haesendonck, C., Dotti, C.G., and Bartic, C. (2016). Amyloid beta oligomers
1037 induce neuronal elasticity changes in age-dependent manner: a force
1038 spectroscopy study on living hippocampal neurons. *Sci Rep* 6, 25841.
1039 10.1038/srep25841.
- 1040 45. Xiong, Y., Lee, A.C., Suter, D.M., and Lee, G.U. (2009). Topography and
1041 nanomechanics of live neuronal growth cones analyzed by atomic force
1042 microscopy. *Biophys J* 96, 5060–5072. 10.1016/j.bpj.2009.03.032.
- 1043 46. Harris, A.R., and Charras, G.T. (2011). Experimental validation of atomic force
1044 microscopy-based cell elasticity measurements. *Nanotechnology* 22, 345102.
1045 10.1088/0957-4484/22/34/345102.
- 1046 47. Spedden, E., and Staii, C. (2013). Neuron biomechanics probed by atomic force
1047 microscopy. *Int J Mol Sci* 14, 16124–16140. 10.3390/ijms140816124.
- 1048 48. Spedden, E., White, J.D., Naumova, E.N., Kaplan, D.L., and Staii, C. (2012).
1049 Elasticity maps of living neurons measured by combined fluorescence and
1050 atomic force microscopy. *Biophys J* 103, 868–877. 10.1016/j.bpj.2012.08.005.
- 1051 49. Geiger, B., Bershadsky, A., Pankov, R., and Yamada, K.M. (2001).
1052 Transmembrane crosstalk between the extracellular matrix--cytoskeleton
1053 crosstalk. *Nat Rev Mol Cell Biol* 2, 793–805. 10.1038/35099066.
- 1054 50. Johansen, L.D., Naumanen, T., Knudsen, A., Westerlund, N., Gromova, I.,
1055 Junntila, M., Nielsen, C., Bøttzauw, T., Tolkovsky, A., Westermarck, J., et al.
1056 (2008). IKAP localizes to membrane ruffles with filamin A and regulates actin
1057 cytoskeleton organization and cell migration. *J Cell Sci* 121, 854–864.
1058 10.1242/jcs.013722.
- 1059 51. Totaro, A., Panciera, T., and Piccolo, S. (2018). YAP/TAZ upstream signals and
1060 downstream responses. *Nat Cell Biol* 20, 888–899. 10.1038/s41556-018-0142-z.

- 1061 52. Kulkarni, M., Tan, T.Z., Syed Sulaiman, N.B., Lamar, J.M., Bansal, P., Cui, J.,
1062 Qiao, Y., and Ito, Y. (2018). RUNX1 and RUNX3 protect against YAP-mediated
1063 EMT, stem-ness and shorter survival outcomes in breast cancer. *Oncotarget* 9,
1064 14175–14192. [10.18632/oncotarget.24419](https://doi.org/10.18632/oncotarget.24419).
- 1065 53. Hong, D., Fritz, A.J., Gordon, J.A., Tye, C.E., Boyd, J.R., Tracy, K.M., Fietze,
1066 S.E., Carr, F.E., Nickerson, J.A., Van Wijnen, A.J., et al. (2019). RUNX1-
1067 dependent mechanisms in biological control and dysregulation in cancer. *J Cell*
1068 *Physiol* 234, 8597–8609. [10.1002/jcp.27841](https://doi.org/10.1002/jcp.27841).
- 1069 54. Renthall, W., Tochitsky, I., Yang, L., Cheng, Y.-C., Li, E., Kawaguchi, R.,
1070 Geschwind, D.H., and Woolf, C.J. (2020). Transcriptional Reprogramming of
1071 Distinct Peripheral Sensory Neuron Subtypes after Axonal Injury. *Neuron* 108,
1072 128-144.e9. [10.1016/j.neuron.2020.07.026](https://doi.org/10.1016/j.neuron.2020.07.026).
- 1073 55. Cederquist, G.Y., Tchieu, J., Callahan, S.J., Ramnarine, K., Ryan, S., Zhang, C.,
1074 Rittenhouse, C., Zeltner, N., Chung, S.Y., Zhou, T., et al. (2020). A Multiplex
1075 Human Pluripotent Stem Cell Platform Defines Molecular and Functional
1076 Subclasses of Autism-Related Genes. *Cell Stem Cell* 27, 35-49.e6.
1077 [10.1016/j.stem.2020.06.004](https://doi.org/10.1016/j.stem.2020.06.004).
- 1078 56. Keren, H., Donyo, M., Zeevi, D., Maayan, C., Pupko, T., and Ast, G. (2010).
1079 Phosphatidylserine Increases IKBKAP Levels in Familial Dysautonomia Cells.
1080 *PLOS ONE* 5, e15884. [10.1371/journal.pone.0015884](https://doi.org/10.1371/journal.pone.0015884).
- 1081 57. Bochner, R., Ziv, Y., Zeevi, D., Donyo, M., Abraham, L., Ashery-Padan, R., and
1082 Ast, G. (2013). Phosphatidylserine increases IKBKAP levels in a humanized
1083 knock-in IKBKAP mouse model. *Human Molecular Genetics* 22, 2785–2794.
1084 [10.1093/hmg/ddt126](https://doi.org/10.1093/hmg/ddt126).
- 1085 58. Anderson, S.L., Qiu, J., and Rubin, B.Y. (2003). Tocotrienols induce IKBKAP
1086 expression: a possible therapy for familial dysautonomia. *Biochemical and*
1087 *Biophysical Research Communications* 306, 303–309. [10.1016/S0006-](https://doi.org/10.1016/S0006-291X(03)00971-9)
1088 [291X\(03\)00971-9](https://doi.org/10.1016/S0006-291X(03)00971-9).
- 1089 59. Anderson, S.L., Qiu, J., and Rubin, B.Y. (2003). EGCG corrects aberrant splicing
1090 of IKAP mRNA in cells from patients with familial dysautonomia. *Biochemical and*
1091 *Biophysical Research Communications* 310, 627–633.
1092 [10.1016/j.bbrc.2003.09.019](https://doi.org/10.1016/j.bbrc.2003.09.019).
- 1093 60. Gold-von Simson, G., Leyne, M., Mull, J., Rolnitzky, L.M., Goldberg, J.D., Berlin,
1094 D., Axelrod, F.B., and Slaugenhaupt, S.A. (2008). IKBKAP mRNA in Peripheral
1095 Blood Leukocytes: A Molecular Marker of Gene Expression and Splicing in
1096 Familial Dysautonomia. *Pediatr Res* 63, 186–190.
1097 [10.1203/PDR.0b013e31815ef74b](https://doi.org/10.1203/PDR.0b013e31815ef74b).

- 1098 61. Cheishvili, D., Maayan, C., Holzer, N., Tsenter, J., Lax, E., Petropoulos, S., and
1099 Razin, A. (2016). Tocotrienol Treatment in Familial Dysautonomia: Open-Label
1100 Pilot Study. *J Mol Neurosci* 59, 382–391. 10.1007/s12031-016-0760-5.
- 1101 62. Bruun, G.H., Bang, J.M.V., Christensen, L.L., Brøner, S., Petersen, U.S.S.,
1102 Guerra, B., Grønning, A.G.B., Doktor, T.K., and Andresen, B.S. (2018). Blocking
1103 of an intronic splicing silencer completely rescues IKBKAP exon 20 splicing in
1104 familial dysautonomia patient cells. *Nucleic Acids Res* 46, 7938–7952.
1105 10.1093/nar/gky395.
- 1106 63. Sinha, R., Kim, Y.J., Nomakuchi, T., Sahashi, K., Hua, Y., Rigo, F., Bennett,
1107 C.F., and Krainer, A.R. (2018). Antisense oligonucleotides correct the familial
1108 dysautonomia splicing defect in IKBKAP transgenic mice. *Nucleic Acids*
1109 *Research* 46, 4833–4844. 10.1093/nar/gky249.
- 1110 64. Donadon, I., Pinotti, M., Rajkowska, K., Pianigiani, G., Barbon, E., Morini, E.,
1111 Motaln, H., Rogelj, B., Mingozzi, F., Slauchhaupt, S.A., et al. (2018). Exon-
1112 specific U1 snRNAs improve ELP1 exon 20 definition and rescue ELP1 protein
1113 expression in a familial dysautonomia mouse model. *Hum Mol Genet* 27, 2466–
1114 2476. 10.1093/hmg/ddy151.
- 1115 65. Pop-Busui, R., Boulton, A.J.M., Feldman, E.L., Bril, V., Freeman, R., Malik, R.A.,
1116 Sosenko, J.M., and Ziegler, D. (2017). Diabetic Neuropathy: A Position
1117 Statement by the American Diabetes Association. *Diabetes Care* 40, 136–154.
1118 10.2337/dc16-2042.
- 1119 66. O'Brien, A.L., West, J.M., Saffari, T.M., Nguyen, M., and Moore, A.M. (2022).
1120 Promoting Nerve Regeneration: Electrical Stimulation, Gene Therapy, and
1121 Beyond. *Physiology* 37, 302–310. 10.1152/physiol.00008.2022.
- 1122 67. Coutiño, B.C., and Mayor, R. (2022). Neural crest mechanosensors: Seeing old
1123 proteins in a new light. *Developmental Cell* 57, 1792–1801.
1124 10.1016/j.devcel.2022.07.005.
- 1125 68. Jiang, Z.G., Smith, R.A., and Neilson, M.M. (1995). The effects of nerve growth
1126 factor on neurite outgrowth from cultured adult and aged mouse sensory
1127 neurons. *Brain Res Dev Brain Res* 85, 212–219. 10.1016/0165-3806(94)00214-
1128 k.
- 1129 69. McGuire, P.G., and Seeds, N.W. (1990). Degradation of underlying extracellular
1130 matrix by sensory neurons during neurite outgrowth. *Neuron* 4, 633–642.
1131 10.1016/0896-6273(90)90121-u.
- 1132 70. Cheishvili, D., Maayan, C., Cohen-Kupiec, R., Lefler, S., Weil, M., Ast, G., and
1133 Razin, A. (2011). IKAP/Elp1 involvement in cytoskeleton regulation and
1134 implication for familial dysautonomia. *Hum. Mol. Genet.* 20, 1585–1594.
1135 10.1093/hmg/ddr036.

- 1136 71. Ciobanasu, C., Faivre, B., and Le Clainche, C. (2013). Integrating actin
1137 dynamics, mechanotransduction and integrin activation: the multiple functions of
1138 actin binding proteins in focal adhesions. *Eur J Cell Biol* 92, 339–348.
1139 10.1016/j.ejcb.2013.10.009.
- 1140 72. Li, N., Lim, G., Chen, L., McCabe, M.F., Kim, H., Zhang, S., and Mao, J. (2013).
1141 Spinal expression of Hippo signaling components YAP and TAZ following
1142 peripheral nerve injury in rats. *Brain Res* 1535, 137–147.
1143 10.1016/j.brainres.2013.08.049.
- 1144 73. Feltri, M.L., Weaver, M.R., Belin, S., and Poitelon, Y. (2021). The Hippo pathway:
1145 Horizons for innovative treatments of peripheral nerve diseases. *J Peripher Nerv*
1146 *Syst* 26, 4–16. 10.1111/jns.12431.
- 1147 74. Richter, M.N., and Fattahi, F. (2022). Stem Cell-Based Models for Studying the
1148 Effects of Cancer and Cancer Therapies on the Peripheral Nervous System.
1149 *Advanced Biology* 6, 2200009. 10.1002/adbi.202200009.
- 1150 75. Wu, H.F., and Zeltner, N. (2020). Efficient Differentiation of Postganglionic
1151 Sympathetic Neurons using Human Pluripotent Stem Cells under Feeder-free
1152 and Chemically Defined Culture Conditions. *J Vis Exp*. 10.3791/60843.
- 1153 76. Latchoumane, C.-F.V., Chopra, P., Sun, L., Ahmed, A., Palmieri, F., Wu, H.-F.,
1154 Guerreso, R., Thorne, K., Zeltner, N., Boons, G.-J., et al. (2022). Synthetic
1155 Heparan Sulfate Hydrogels Regulate Neurotrophic Factor Signaling and
1156 Neuronal Network Activity. *ACS Appl Mater Interfaces* 14, 28476–28488.
1157 10.1021/acscami.2c01575.
- 1158 77. Meijering, E., Jacob, M., Sarria, J.-C.F., Steiner, P., Hirling, H., and Unser, M.
1159 (2004). Design and validation of a tool for neurite tracing and analysis in
1160 fluorescence microscopy images. *Cytometry A* 58, 167–176.
1161 10.1002/cyto.a.20022.
- 1162 78. Trapnell, C., Pachter, L., and Salzberg, S.L. (2009). TopHat: discovering splice
1163 junctions with RNA-Seq. *Bioinformatics* 25, 1105–1111.
1164 10.1093/bioinformatics/btp120.
- 1165 79. Hellewell, A.L., Rosini, S., and Adams, J.C. (2017). A Rapid, Scalable Method for
1166 the Isolation, Functional Study, and Analysis of Cell-derived Extracellular Matrix.
1167 *J Vis Exp*. 10.3791/55051.
- 1168 80. Carl, P., and Schillers, H. (2008). Elasticity measurement of living cells with an
1169 atomic force microscope: data acquisition and processing. *Pflugers Arch* 457,
1170 551–559. 10.1007/s00424-008-0524-3.
- 1171 81. Gavan, K.B., Drift, E.W.J.M. van der, Venstra, W.J., Zuiddam, M.R., and Zant,
1172 H.S.J. van der (2009). Effect of undercut on the resonant behaviour of silicon

1173 nitride cantilevers. *J. Micromech. Microeng.* 19, 035003. 10.1088/0960-
1174 1317/19/3/035003.

1175

1176 **Acknowledgements:** We thank Michael Tiemeyer and Yao Yao for critical discussions.
1177 Harold ‘Skip’ Ralph at the Weill Cornell Microscopy and Image Analysis Core facility for
1178 image analysis. Julie Nelson from the UGA Cytometry Shared Resource Laboratory for
1179 her help with flow cytometry experiments.

1180

1181 **Funding:** This work was funded by faculty start-up funds from the University of Georgia
1182 to N.Z. and NIH/NINDS 1R01NS114567-01A1 to N.Z., a core grant from the US National
1183 Institutes of Health (P30CA08748), the New York State Stem Cell Science (NYSTEM)
1184 (C026446 and C026447) and the Tri-institutional Stem Cell Initiative (Starr Foundation)
1185 to L.S. Schematics were done using Biorender.com.

1186

1187 **Author Contributions.** KS-D conceived, conducted and analyzed experiments, and
1188 wrote manuscript. PD and ID conducted animal experiments and tissue analysis. AJP
1189 conducted experiments. CW, ARP, and GB synthesized 1,10-anhydrogenipin, SC
1190 provided the chemical library, LS provided support, advice, and funds for the chemical
1191 screen. NZ conceived, designed, led the study, conducted experiments, wrote the
1192 manuscript and provided funds.

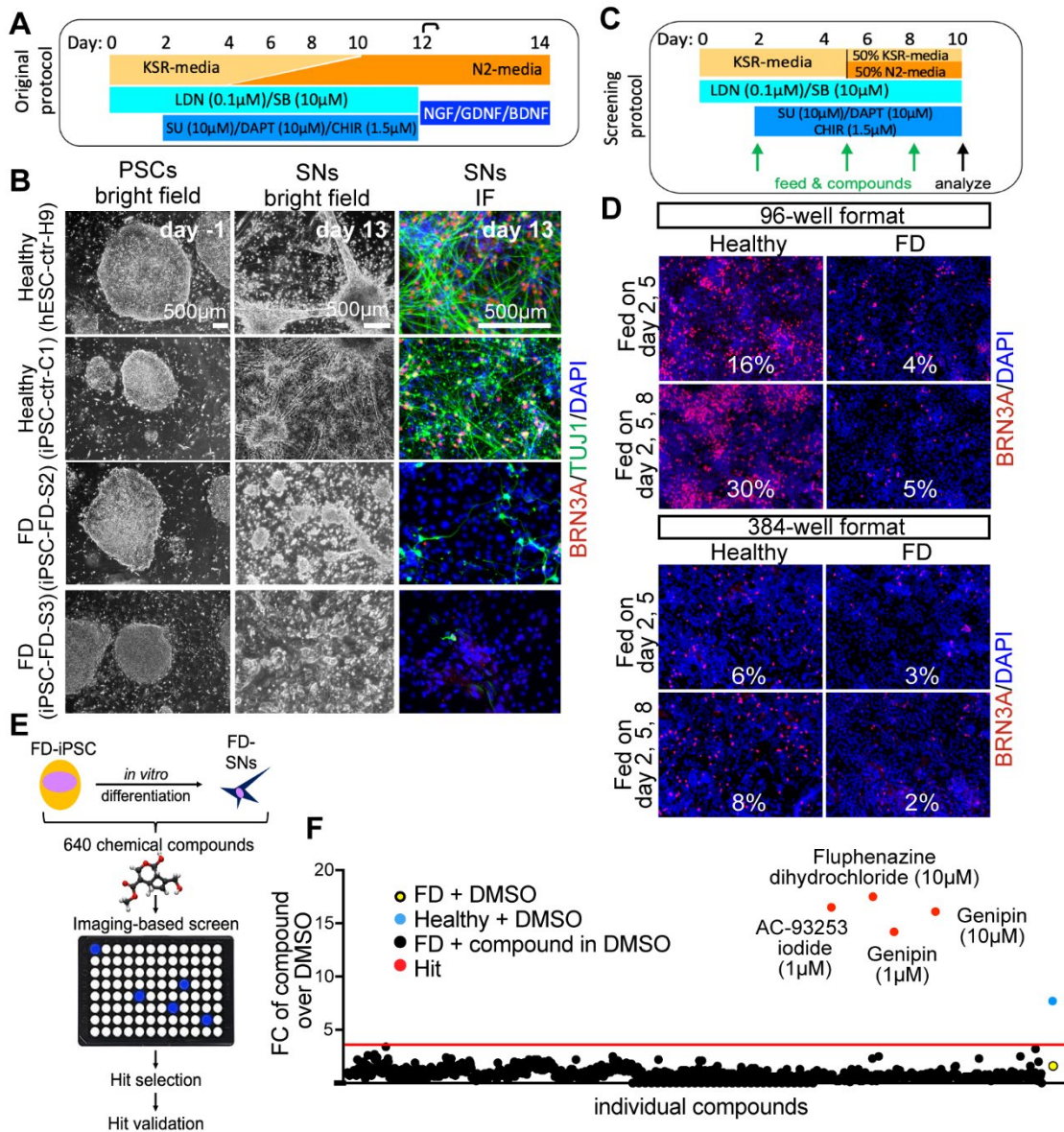
1193

1194 **Competing interests:** L.S. is a scientific co-founder and consultant and has received
1195 research support from BlueRock Therapeutics.

1196

1197 **Data and materials availability:** All data needed to evaluate the conclusions in the paper
1198 are present in the paper or the Supplementary Materials. Requests for reagents should
1199 be directed to the corresponding author, Nadja Zeltner (nadja.zeltner@uga.edu)
1200

1201 **Figures:**



1202

1203 **Figure 1. Chemical screen on FD sensory neurons. A)** Differentiation protocol adapted

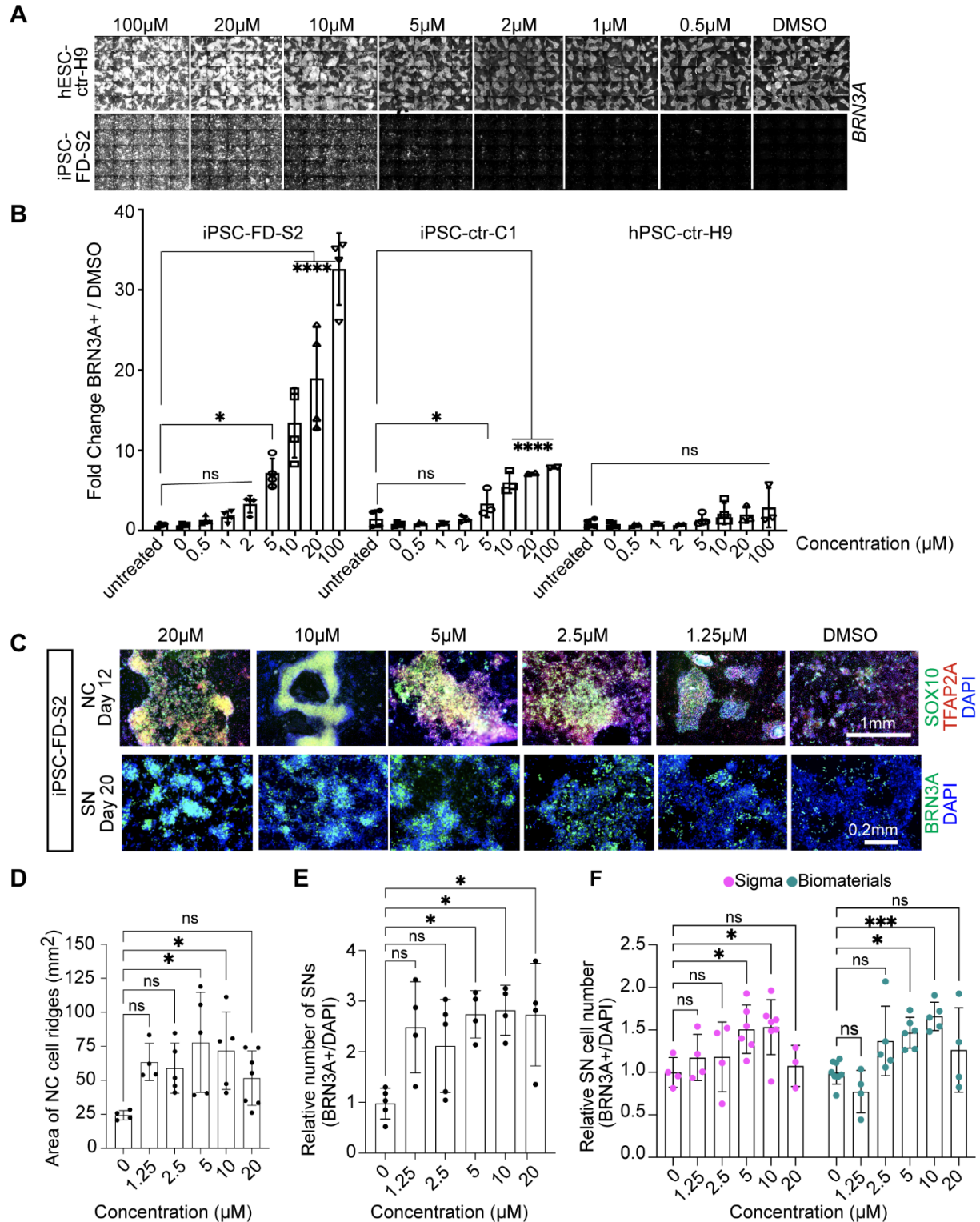
1204 from Chambers et al., 2012. **B)** Bright filed imaging shows normal morphology of

1205 undifferentiated hPSCs in all lines (left column). SN differentiation is impaired in both FD

1206 lines, but normal in healthy control lines (right two columns). **C)** Differentiation protocol

1207 adapted to high-throughput screening conditions. **D)** The SN differentiation protocol is

1208 most efficient in 96 wells when the cells are fed three times. **E)** Cartoon of the screen set
1209 up. **F)** 640 compounds from the LOPAC chemical library (the first half) were screened.
1210 Controls included DMSO only wells, healthy hPSC-ctr-H9 with DMSO (blue dot = positive
1211 control), iPSC-FD-S2 iPSCs with DMSO (yellow dot=negative control). Hit compounds
1212 were called when the fold change (FC) over the average of all DMSO wells was above
1213 the average of all compounds plus 3 SDs (here 3.8, red dots). Every compound was
1214 screened at 1 mM and 10 mM. 16 images were taken for each well, all wells were imaged
1215 for the ratio of BRN3A⁺/DAPI⁺ staining.
1216



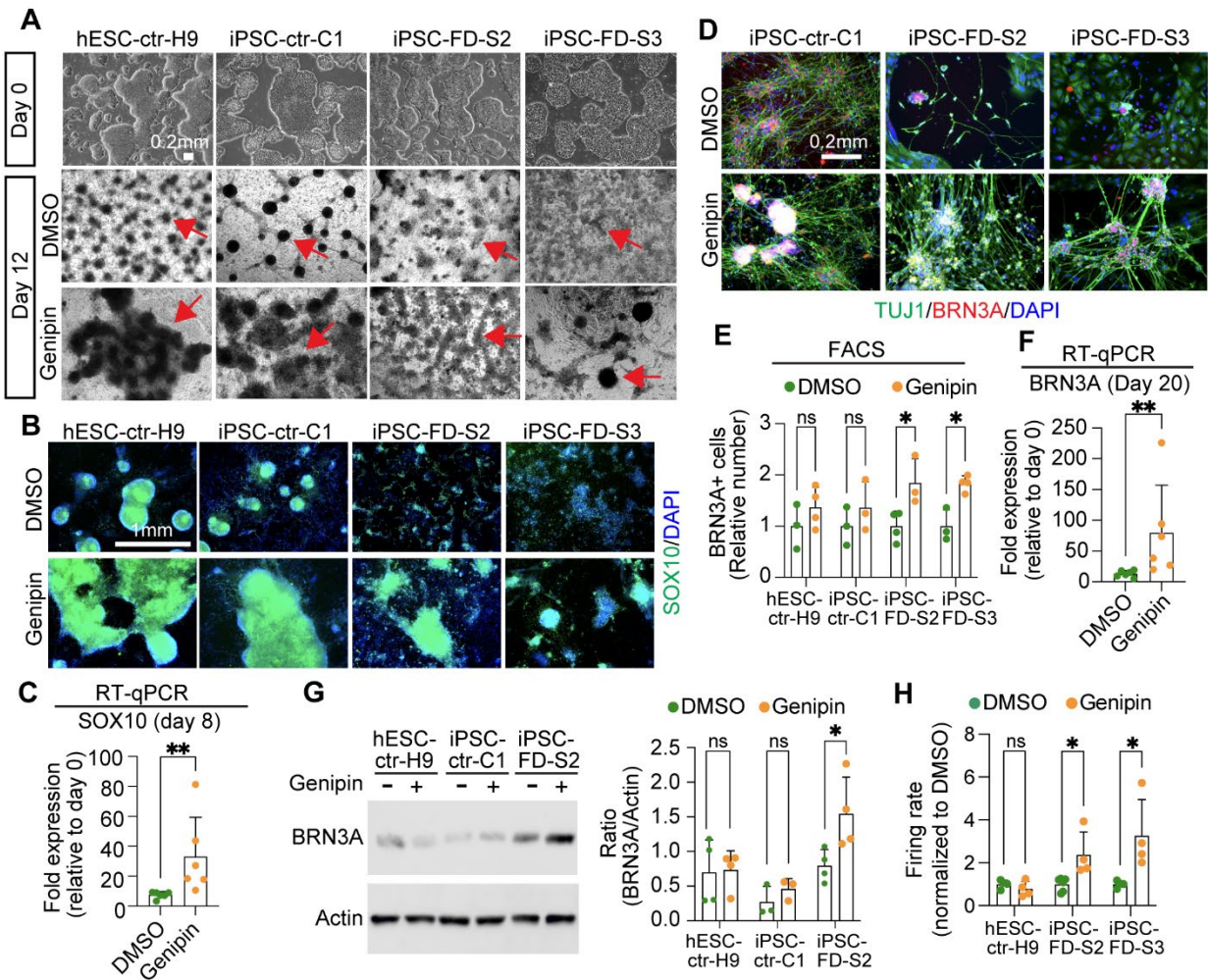
1217

1218 **Figure 2. Validation of the hit compound genipin. A)** Titration of genipin during the SN

1219 differentiation in healthy hPSC-ctr-H9 and iPSC-FD-S2 iPSCs. Differentiation protocol

1220 depicted in Fig. 1a was used. **B)** Quantification of genipin titration based on IF of BRN3A⁺
1221 SNs. n=3-4 biological replicates. **C)** Titration of genipin in iPSC-FD-S2 cells during
1222 differentiation into SN-biased neural crest cells (top row) and SNs (bottom row). Cells
1223 were treated with indicated concentrations of genipin starting on day 2. Cells were then
1224 fixed on the indicated days and stained for SOX10 and TFAP2A (top) or BRN3A (bottom)
1225 and DAPI. **D and E)** Quantification of size of NC ridges and number of SNs upon genipin
1226 treatment. **D)** Area of ridges in **c** marked by DAPI staining (n=4-7 biological replicates)
1227 and **E)** number of BRN3A⁺ SNs in **c** were quantified (n=3-5 biological replicates). **F)**
1228 Genipin commercially obtained from both Sigma and Biomaterials rescues SN
1229 differentiation in iPSC-FD-S2. Cells were differentiated in the presence of genipin from
1230 either source starting on day 2. Cells were fixed on day 20 and stained for BRN3A and
1231 DAPI. n=3-7 biological replicates. All graphs show mean \pm s.d. For **B**, **D**, **E**, and **F**, one-
1232 way ANOVA, *p<0.05, **p<0.005.

1233



1234

1235 **Figure 3. Genipin rescues neural crest and sensory neuron-related phenotypes in**

1236 **FD. A)** hPSC lines look normal at the pluripotent stage (day 0), but differentiation into NC

1237 cells is diminished in the FD lines (DMSO, dark ridges/red arrow indicate NC cells); this

1238 is rescued by genipin (10 μ M) treatment. **B and C)** SOX10 expression is restored in NC

1239 cells upon genipin treatment. Cells were differentiated in the presence of genipin (10 μ M)

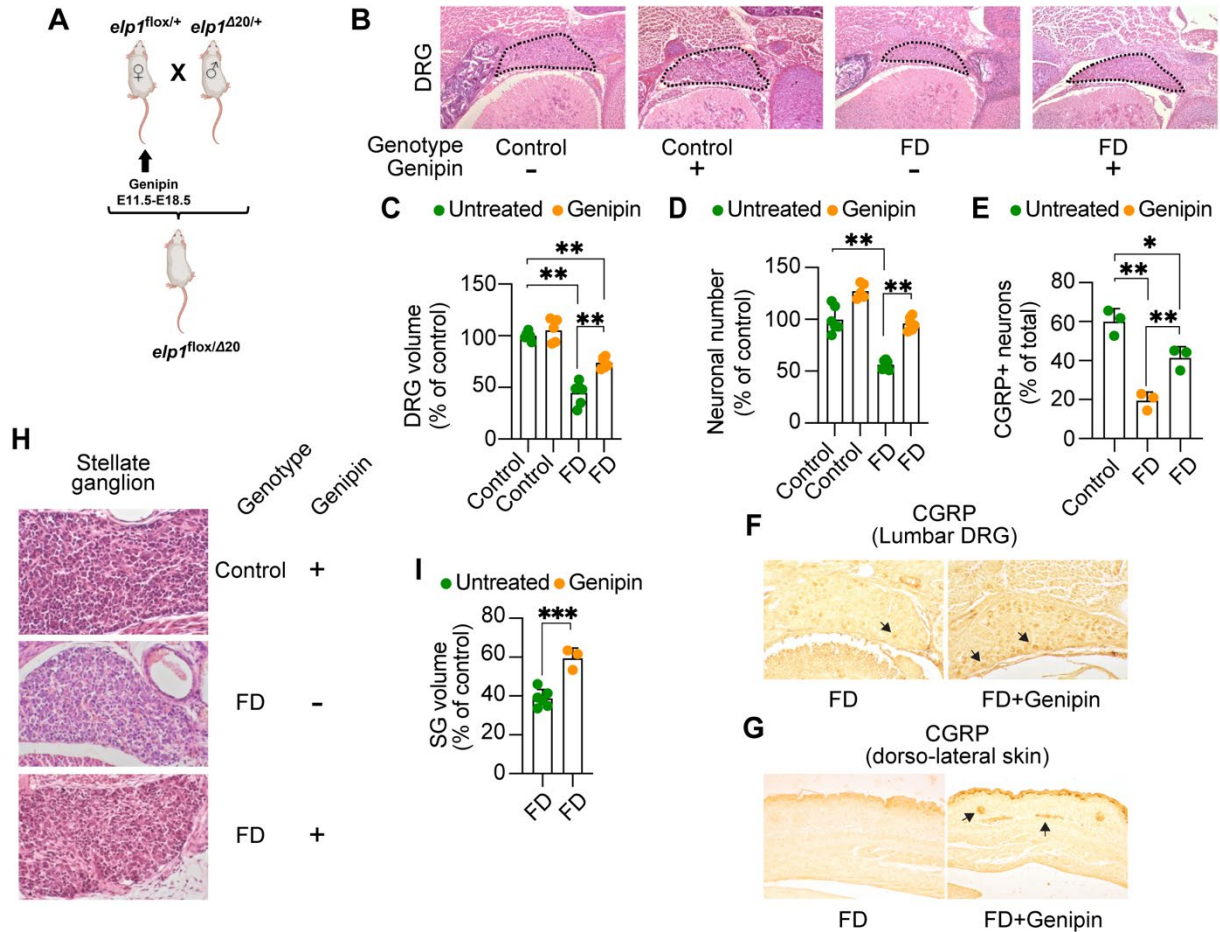
1240 and fixed on day 12. **B)** Cells were stained for SOX10 and DAPI and analyzed by IF. n=5

1241 biological replicates. **C)** Genipin increases SOX10 expression. RNA isolated from FD

1242 cells differentiated in the presence of genipin (10 μ M) was analyzed by RT-qPCR. n=6

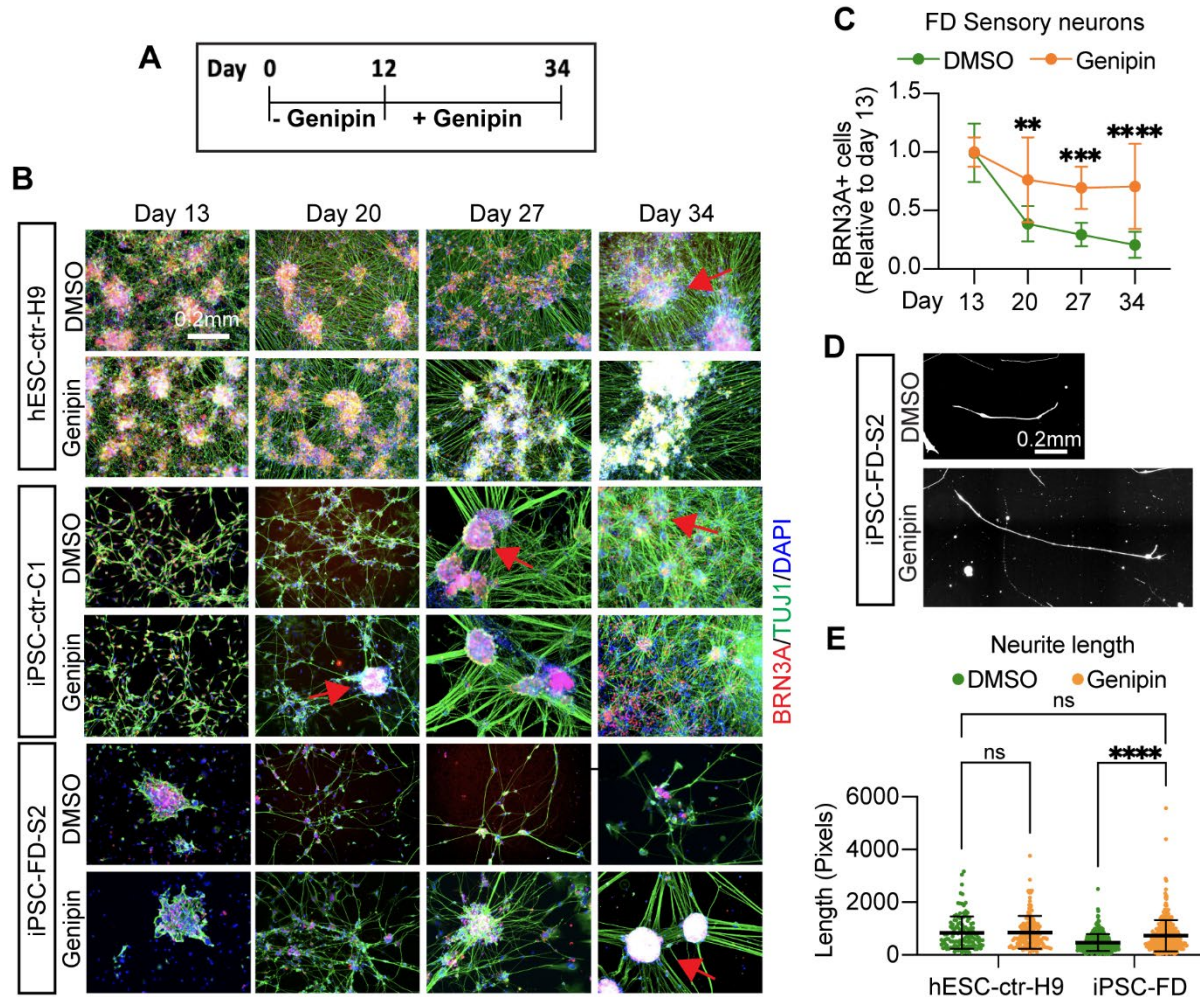
1243 biological replicates. **D-F)** Genipin restores SN differentiation. iPSC-FD-S2 and iPSC-FD-

1244 S3 cells were treated with genipin (10 μ M) and differentiated into SNs. RNA was isolated
1245 on day 20 or cells were fixed and stained using the indicated antibodies and analyzed by
1246 **D)** IF (n=5 biological replicates), **E)** intracellular flow cytometry (n=3-4 biological
1247 replicates), and **F)** RT-qPCR (n=6 biological replicates). **G)** Western blot analysis
1248 confirms the increase in SN production upon genipin treatment. Cells differentiated with
1249 genipin (10 μ M) were lysed on day 20 and immunoblotted with the indicated antibodies
1250 (left) and quantified (right). n=3-4 biological replicates. **H)** Genipin increases firing rate of
1251 FD SNs. Cells were differentiated in the presence of genipin (10 μ M) and firing rate was
1252 analyzed by MEA. n=4-6 biological replicates. In **C**, **E**, **F**, **G**, **H**, Two-tailed Student's t-
1253 test. ns, non-significant, *p<0.05, **p<0.005. All graphs show mean \pm s.d. Data from iPSC-
1254 FD-S2 and iPSC-FD-S3 are pooled as FD in **C** and **F**.
1255



1256
1257 **Figure 4.** Genipin rescues FD peripheral deficits *in vivo*. **A)** Breeding and treatment
1258 schematic. **B)** Representative H&E-stained transverse sections through lumbar (L1)
1259 dorsal root ganglia (DRG) of untreated control, genipin-treated control, untreated FD, and
1260 genipin-treated FD E18.5 embryos, at their largest dimensions. **C)** Volumes of lumbar
1261 (L1) DRGs of untreated (n=6) and genipin-treated (n=5) controls, and untreated (n=6) and
1262 genipin-treated (n=6) FD E18.5 embryos, displayed as percentage of control. **D)** Neuronal
1263 counts of lumbar (L1) DRGs of untreated (n=6) and genipin-treated (n=5) controls, and
1264 untreated (n=6) and genipin-treated (n=6) FD E18.5 embryos, displayed as percentage
1265 of control. **E)** Percentage of CGRP-positive neurons in lumbar (L1) DRGs of control
1266 untreated (n=3), FD untreated (n=3), and genipin-treated FD (n=3). **F)** Representative
1267 images of transverse sections through lumbar (L1) DRGs of untreated FD and genipin-

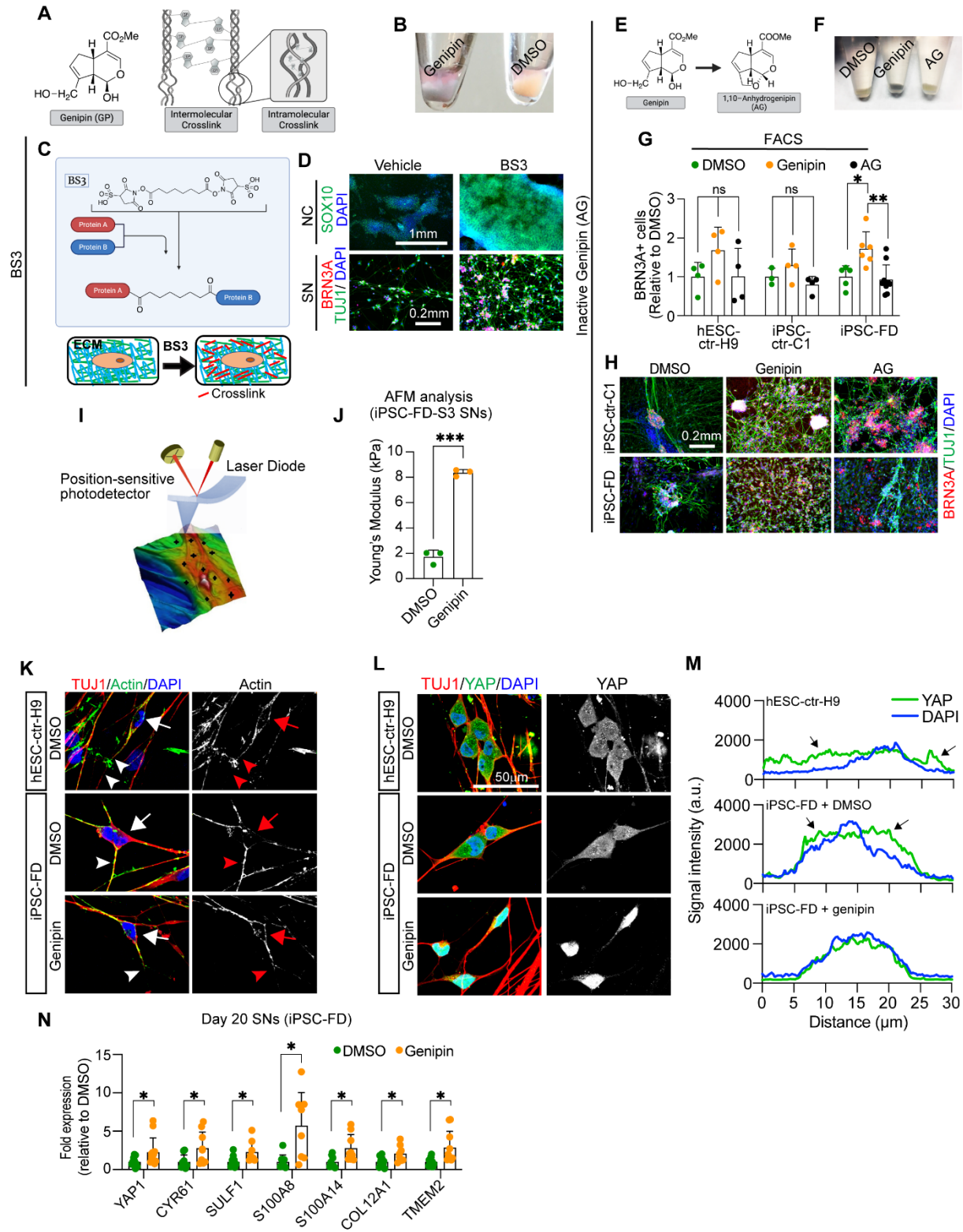
1268 treated FD E18.5 embryos immunostained with CGRP. **G)** Representative images of
1269 transverse sections through dorso-lateral skin of untreated FD and genipin-treated FD
1270 E18.5 embryos immunostained with CGRP. Arrows show positive signal. **H)**
1271 Representative H&E-stained sections of stellate ganglia (SG) of genipin-treated control,
1272 untreated FD, and genipin-treated FD E18.5 embryos. **I)** Volumes of SG of untreated
1273 (n=6) and genipin-treated (n=3) FD E18.5 embryos, plotted as percentage of control. All
1274 graphs show mean \pm s.d.. For **C, D, E**, one-way ANOVA followed by Tuckey HSD post
1275 hoc test. For **I**, two-tailed t-test. *p<0.05, **p<0.01, ***p<0.001
1276



1277

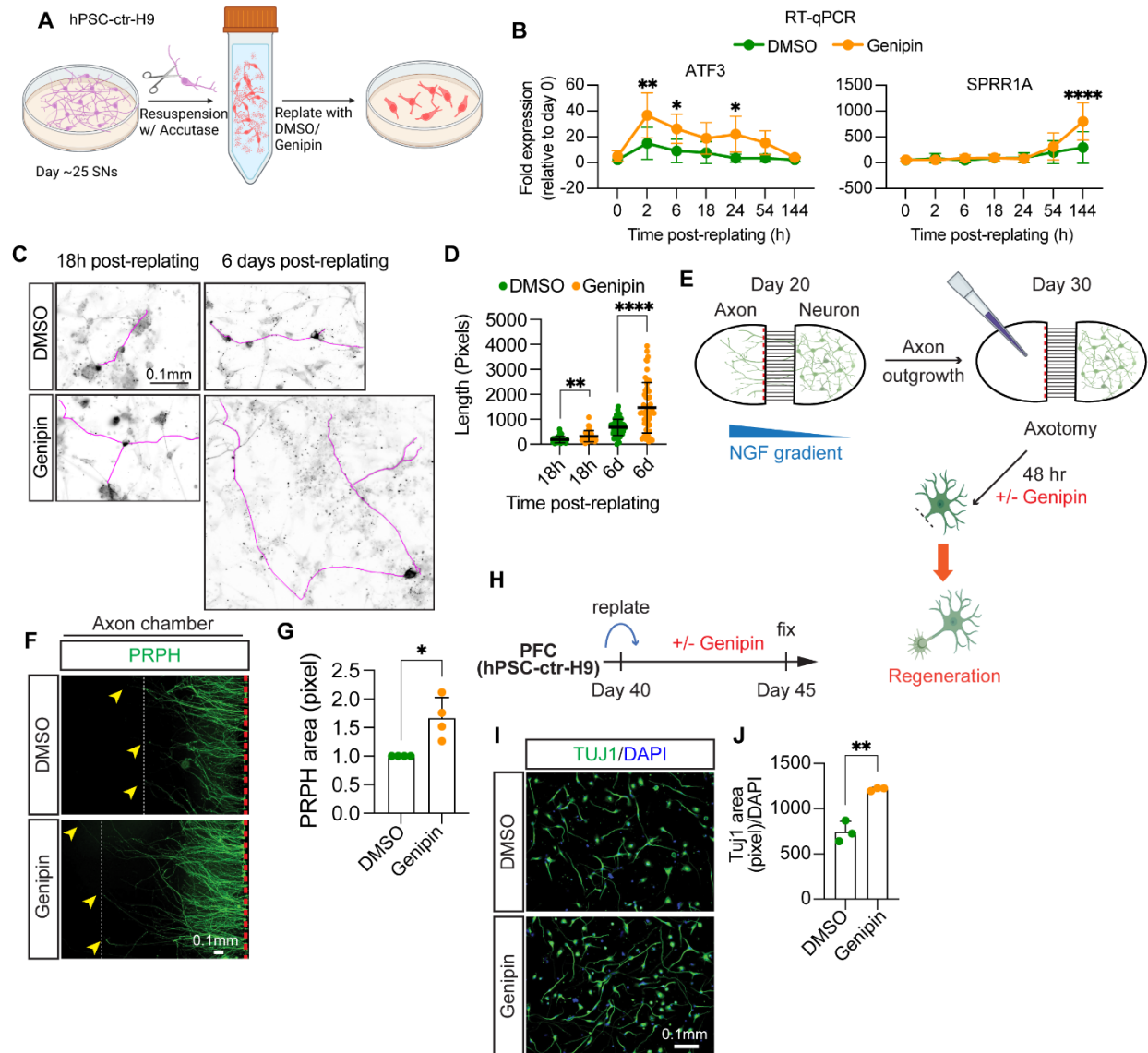
1278 **Figure 5. Genipin rescues sensory neuron degeneration in FD.** **A)** Schematic of
 1279 survival assay. **B)** Healthy or FD cells were treated with genipin from day 12 on (when
 1280 neurons are born) and monitored for survival for 21 days. Cells were fixed on the indicated
 1281 days and stained for BRN3A, TUJ1 and DAPI. Arrows indicate healthy, ganglia-like SN
 1282 clusters. **C)** Image quantification of **B**. n=8, Two-way ANOVA followed by Šídák multiple
 1283 comparisons. **p<0.005, ***p<0.001, ****p<0.0001. **D,E**, Genipin increases neurite length
 1284 and number in FD cells. **D)** Representative images of neurite length. **E)** Measurement of
 1285 neurite length (number of pixels) of **D**. n=6-8 biological replicates, one-way ANOVA

1286 followed by Tukey's multiple comparisons. ns, non-significant, **** $p < 0.0001$. All graphs
1287 show mean \pm s.d. Data from iPSC-FD-S2 and iPSC-FD-S3 are pooled as FD in **C**, and
1288 **E**.
1289



1291 **Figure 6. Genipin rescues FD phenotypes via crosslinking of extracellular matrix**
1292 **proteins and activates. A)** Genipin chemical structure and schematic of intermolecular
1293 and intramolecular crosslinking. **B)** Treatment with genipin turns cells blue. **C)** Schematic
1294 of BS3 crosslinking action and its extracellular location. **D)** BS3 rescues the NC and SN
1295 differentiation defect in FD. FD cells were differentiated in the presence of DMP and fixed
1296 on day 12 (NC) and day 20 (SN). Following staining using the indicated antibodies. n=3-
1297 5 biological replicates. **E-H)** 1,10-anhydrogenipin (AG) does not rescue the SN defect in
1298 FD. **E)** AG chemical structure. **F)** AG does not turn cells blue. **G, H)** AG does not promote
1299 FD SN differentiation. Cells were differentiated into SNs in the presence of genipin or AG.
1300 SNs were fixed on day 20 and stained for BRN3A for intracellular flow cytometry analysis
1301 (**G**, n=6-8 biological replicates), or stained for BRN3A, TUJ1, and DAPI for IF (**H**, n=6-8
1302 biological replicates). **I, J)** Genipin-mediated crosslinking increases ECM stiffness. **I)** AFM
1303 Experiment schematics. **J)** Genipin increases the Young's modulus of SNs. iPSC-FD-S3
1304 SNs were fixed on day 25 and analyzed by AFM (n=3 biological replicates). **K-M)** Genipin
1305 reorganizes the actin cytoskeleton and induces transcription of YAP-dependent genes.
1306 **K)** Genipin partially rescues the differences of actin expression pattern in healthy and FD
1307 SNs. Day 20 SNs were fixed and stained for the indicated antibodies. Images were
1308 obtained by confocal microscopy. Actin signal in the cell body (arrows) or the axons
1309 (arrowheads) are highlighted. **L, M)** YAP localization changes in the presence of genipin.
1310 Day 20 SNs were fixed and stained for the indicated antibodies. **M)** YAP and DAPI signal
1311 intensity from images on **L)** was measured and plotted (n=6-7 biological replicates).
1312 Arrows indicate YAP signal outside of the nucleus (stained with DAPI). **N)** Expression of
1313 YAP-dependent genes. RNA from FD SNs treated with DMSO or Genipin was extracted

1314 on day 20 and gene expression was analyzed by RT-qPCR (n=7-9 biological replicates).
1315 All graphs show mean \pm s.d.. For **G**, one-way ANOVA followed by Tukey's multiple
1316 comparisons. For **J**, two-tailed t test with Welch's correction. For **N**, two-tailed t test. ns,
1317 non-significant, *p<0.05, **p<0.005, ***p<0.001. In **G**, **M**, and **L** data from iPSC-FD-S2
1318 and iPSC-FD-S3 are pooled as FD.
1319



1320
1321

1322 **Figure 7.** Genipin accelerates axon regeneration after injury in different neuronal types.

1323 **A-D)** Genipin enhances reneeration of healthy SNs. **A)** Experiment schematics. **B)**

1324 Genipin increases expresision of injury-related genes. RNA from hPSC-ctr-H9 SNs was

1325 isolated at indicated times and gene expression was measured by RT-qPCR (n=4-7

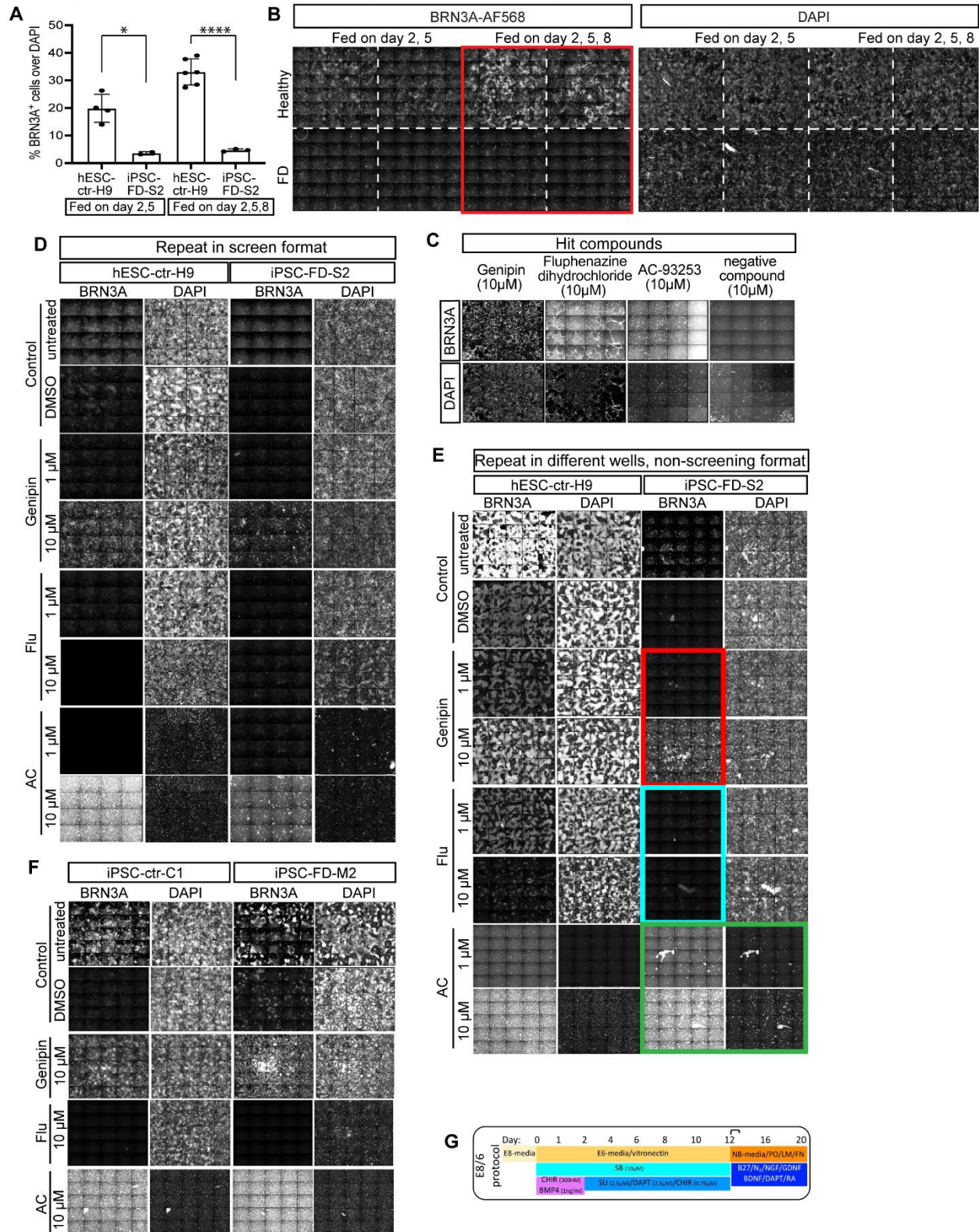
1326 biological replicates). **C,D)** Genipin increases axon length after injury. Day 25 SNs from

1327 hPSC-ctr-H9 cells were fixed at indicated times after replating in the presence of DMSO

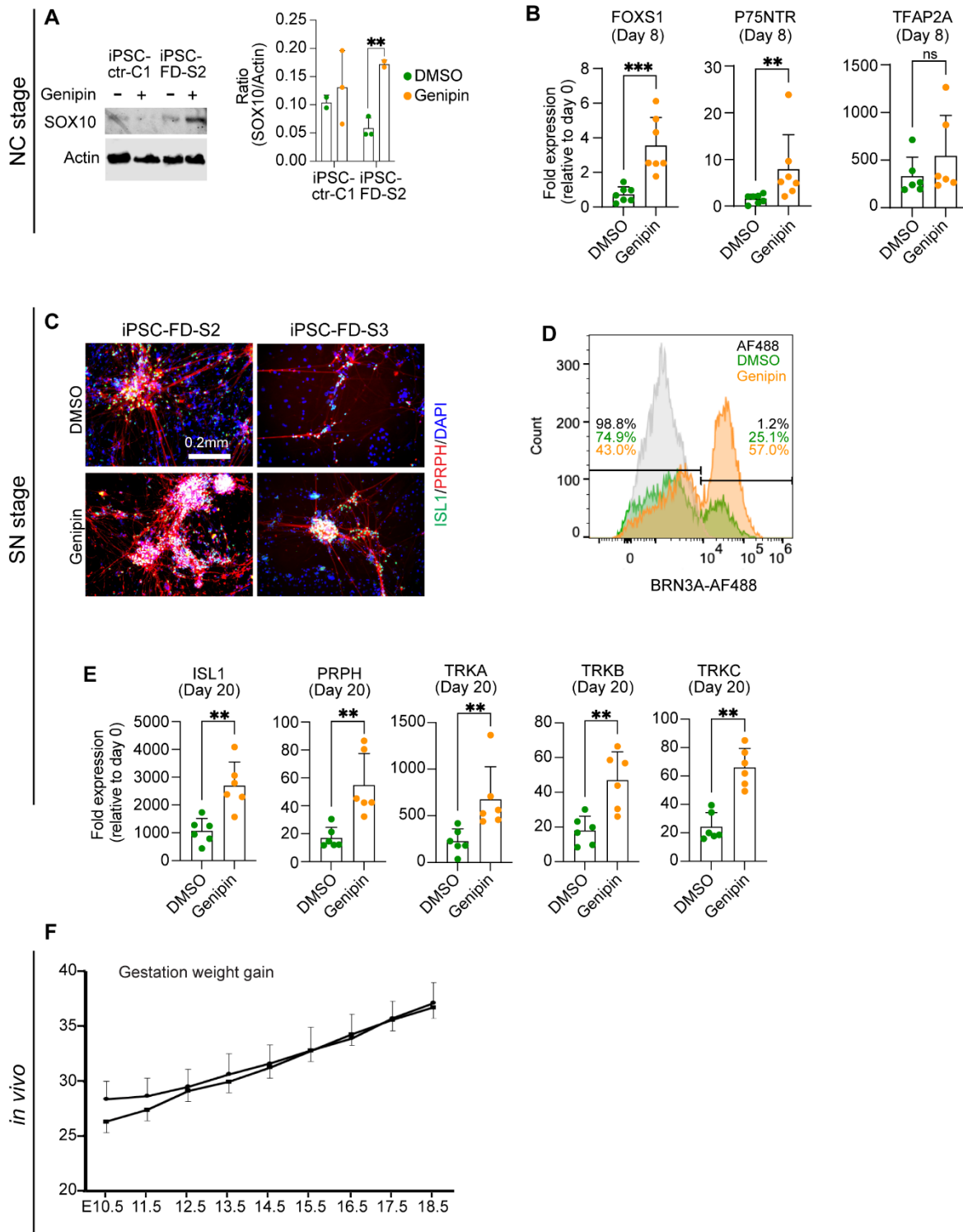
1328 or Genipin, and stained for TUJ1. Axons were traced (magenta) and measurement were

1329 plotted in **D** ($n > 20$ cells from 5 biological replicates). **E-G**) Genipin increases length and
1330 complexity of axons from sympathetic neurons. **E**) Experiment schematics. For details,
1331 see Methods. **F,G**) Axons from sympathetic neurons were removed. After 48 h treatment
1332 with Genipin, neurons were fixed and stained for PRPH. Axons were measured
1333 (arrowheads indicate length of axons) and the pixels with high PRPH signal were
1334 measured and graphed in **J** ($n = 4$ biological replicates). **H-J**) Genipin promotes axon
1335 regeneration in prefrontal cortical (PFC) neurons. **H**) Experiment schematics. For details,
1336 see Methods. **I,J**) hPSC-ctr-H9 PFC neurons replated and incubated with Genipin for 5
1337 days. Neurons were then fixed and stained for TUJ1 as shown in **I**. Measurement of the
1338 pixels with high TUJ1 signal were normalized to DAPI and plotted in **J** ($n = 3$ biological
1339 replicates). All graphs show mean \pm s.d. For **B**, two-way ANOVA followed by Šídák
1340 multiple comparisons. For **D**, **G**, and **H**, two-tailed t test. * $p < 0.05$, ** $p < 0.005$, *** $p < 0.001$,
1341 **** $p < 0.0001$.

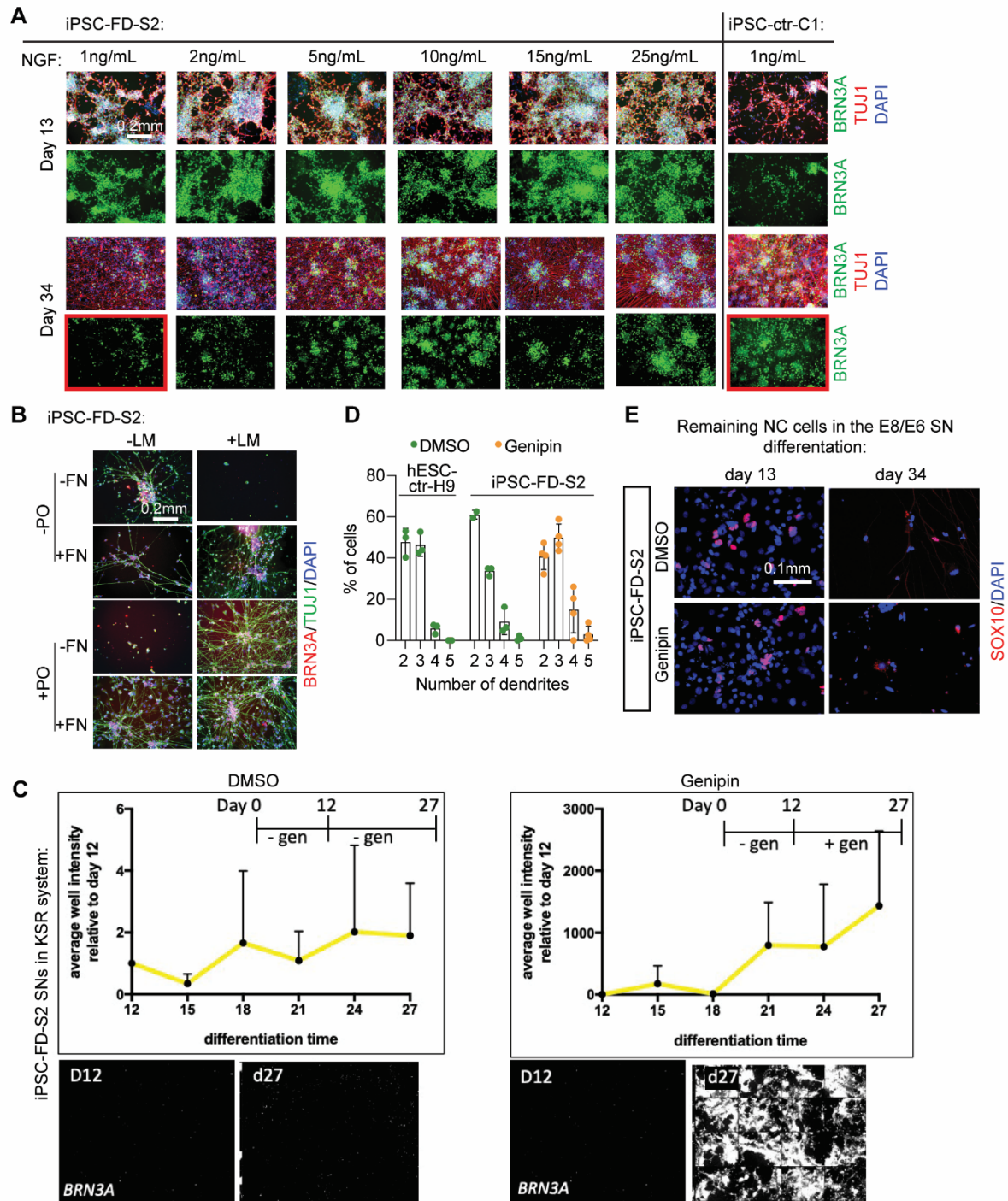
Supplementary Figures



Supplementary Figure 1. Adaptation of the SN differentiation protocol to high-throughput screening conditions and hit validation. **A)** Quantification of the number of BRN3A⁺ SNs over total DAPI⁺ cells. Two-tailed Student's t-test, *p<0.01. **B)** Imaging of 30 fields per 96 well for each condition. **C)** Example wells are shown from the screen. **D)** Repeat of the SN differentiation in the presence of the hit compounds in 96 wells in screening conditions outlined in Fig. 1C. Whole wells are shown, imaged in 16 tiles. **E)** Repeat of SN differentiation in the presence of the hit compounds in a different well format, i.e. 48 wells and using non-screening conditions outlined in Fig. 1A. Whole wells are shown, imaged in 25 tiles. **F)** Effect of hit compounds tested on additional cell lines, i.e. healthy iPSCs and FD iPSCs from patients with mild FD symptoms (iPSC-FD-M2). Non-screening differentiation conditions were used in a 48 well format. All differentiations were performed in KSR conditions. **G)** Schematic representation of the SN differentiation in E8/E6 conditions (as previously reported¹).



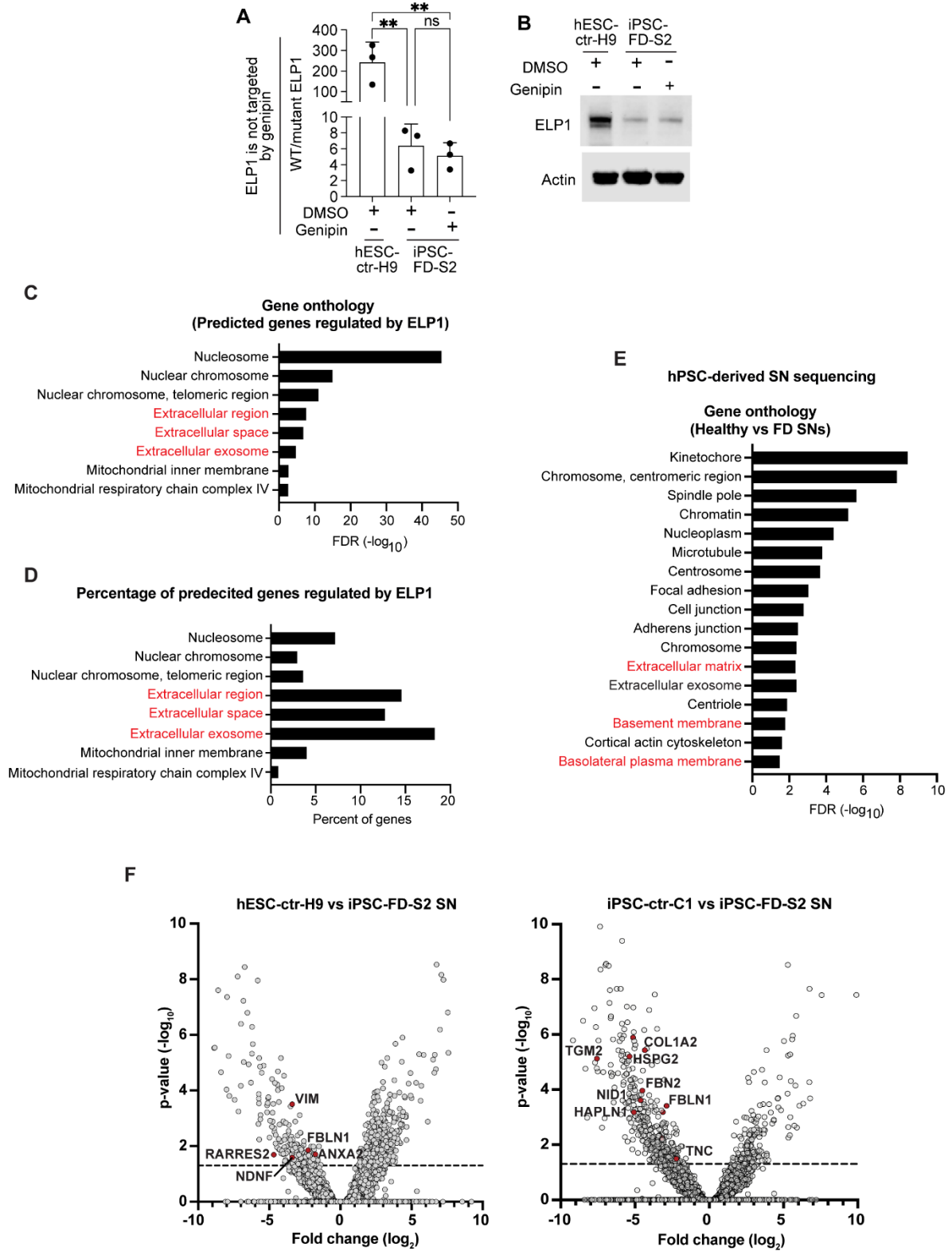
Supplementary Figure 2. *in vitro* and *in vivo* developmental phenotypes in FD are rescued by genipin (related to Fig.3 and Fig. 4). **A)** Immunoblotting of SOX10 from day 12 NC cells. Representative blot is shown (left) and quantification (right, n=2-3 biological replicates). **B)** RT-qPCR-based gene expression analysis of SN-primed NC at day 8 (n=6-7 biological replicates). **C)** Genipin restores SN differentiation. Day 20 SNs were treated with genipin and fixed and stained using the indicated antibodies (n=5 biological replicates). **D)** Representative histogram of BRN3A signal measured by flow cytometry. **E)** RT-qPCR-based gene expression analysis of SNs at day 20 (n=6 biological replicates). **F)** Gestational female weight gain in genipin-treated (squares, n=6) and untreated (circles, n=3) dams. For **A**, **B** and **D**, Two-tailed Student's t-test. ns, non-significant, **p<0.005, ***p<0.001. All graphs show mean \pm s.d. For **B** and **D**, iPSC-FD-S2 and iPSC-FD-S3 data are pooled as FD.



Supplementary Figure 3. SN survival assay adaptation and characterization

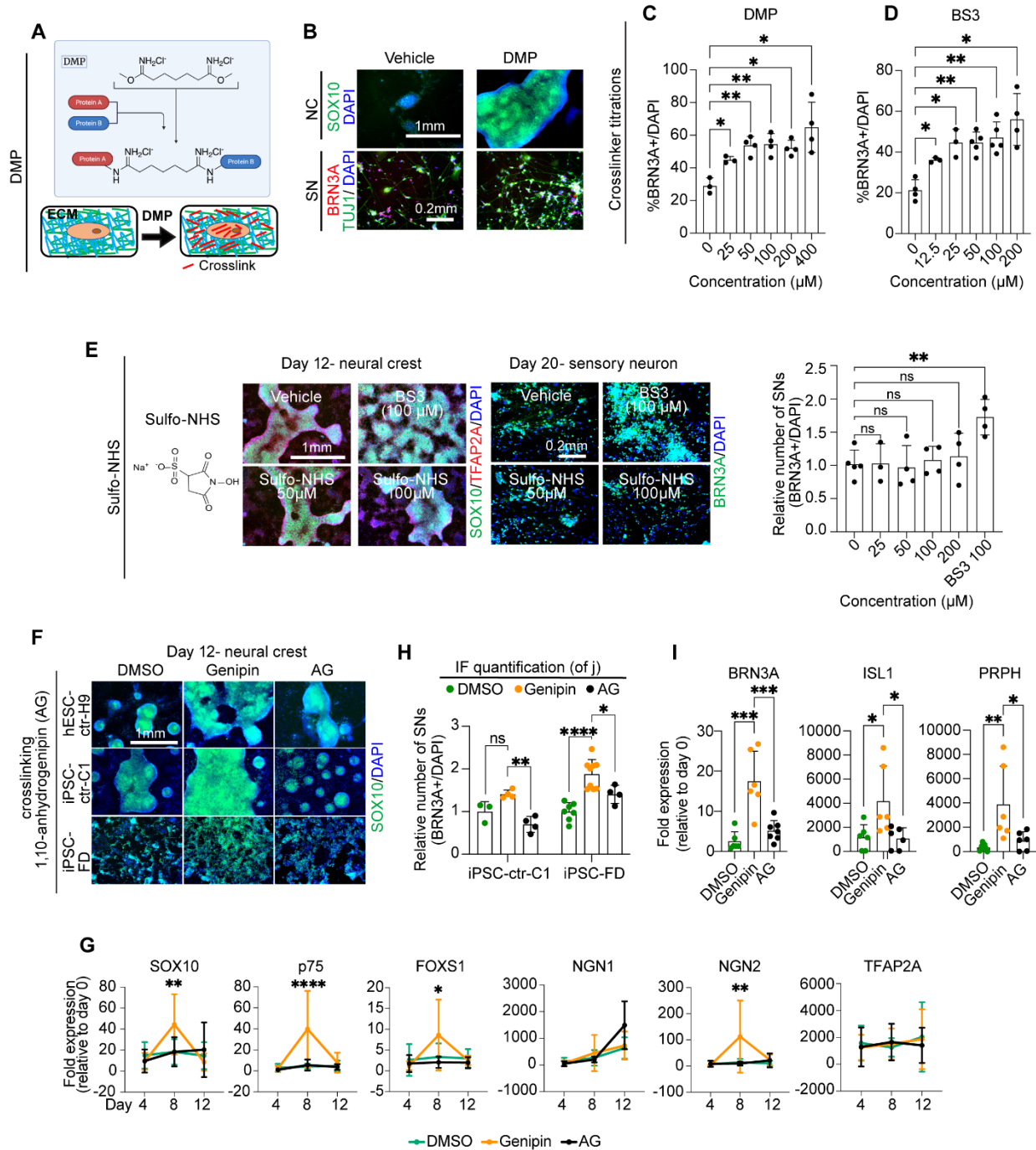
(related to Fig 5). **A**) NGF titration to assess SN survival over 21 days by IF in iPSC-

FD-S2 and healthy iPSC-ctr-C1 cells. **B)** Exclusion experiment to assess which of the surface coating proteins LM, PO, FN are required in iPSC-FD-S2 SNs. **C)** Survival assay performed in KSR conditions (Fig 1A). 16-tile images are shown of SNs at day 12 and day 27, with or without genipin treatment (bottom). Quantification of BRN3A signal intensity is plotted relative to day 12 (top). **D)** Number of neurites of hPSC-ctr-H9 and iPSC-FD-S2 SNs differentiated in the presence of genipin (10 μ M) were quantified. n=2-4 biological replicates. **E)** Few SOX10+ NC cells are present in the SN differentiation at day 13 and 20. All graphs show mean \pm s.d.



Supplementary Figure 4. Effects of genipin in ELP1 and expression of ECM-

related genes in FD mouse and iPSC-derived FD SNs. A) Treatment with genipin of NC cells up to day 8 does not alter *ELP1* splicing inefficiency (RT-qPCR). n=3 biological replicates. one-way ANOVA followed by Tukey's multiple comparisons. ns, non-significant, **p<0.005. **B)** Genipin does not change ELP1 protein levels in FD (immunoblot). n=3 biological replicates. Representative blot is shown. **C,D)** Analysis of genes predicted to be controlled by Elp1 in FD mouse. **C)** Gene ontology of predicted genes that are regulated by Elp1. **D)** Percentage of genes predicted to be regulated by Elp1. **E, F)** RNA sequencing in hPSC-derived SNs comparing healthy (hPSC-ctr-H9 and iPSC-ctr-C1) versus FD (iPSC-FD-S2). **E)** Gene ontology analysis of significantly downregulated genes in FD vs healthy SNs. **F)** Differential gene expression of hPSC-ctr-H9 vs FD SNs (left) and iPSC-ctr-C1 vs FD SNs (right). ECM-related genes downregulated in FD are highlighted. Dotted line indicates significance threshold (p<0.05).



Supplementary Figure 5. Genipin's mode of action is through crosslinking of ECM proteins (related to Fig 6). A) Schematic of DMP crosslinking action and its intracellular/extracellular location. **D)** DMP rescues the NC and SN differentiation defect in FD. iPSC-FD-S2 cells were differentiated in the presence of DMP and fixed on day 12

(NC) and day 20 (SN). Following staining using the indicated antibodies. n=3-4 biological replicates.

C) DMP titration on iPSC-FD-S2-derived SNs on day 20. n=3-4 biological replicates. one-way ANOVA followed by Tukey's multiple comparisons. * $p < 0.05$, ** $p < 0.005$.

D) BS3 titration on iPSC-FD-S2-derived SNs on day 20. n=3-5 biological replicates. one-way ANOVA followed by Tukey's multiple comparisons. * $p < 0.05$, ** $p < 0.005$. **E)**

Sulfo-NHS (inactive BS3) does not rescue NC formation, nor SN formation in iPSC-FD-S2 cells. n=3-5 biological replicates. one-way ANOVA followed by Tukey's multiple

comparisons. ns, non-significant, ** $p < 0.005$. **F)** Genipin that has been chemically altered to delete its crosslinking effects (1,10-anhydrogenipin) is not capable to rescue

NC formation in FD cells. **G-I)** 1,10-anhydrogenipin does not properly restore NC cells

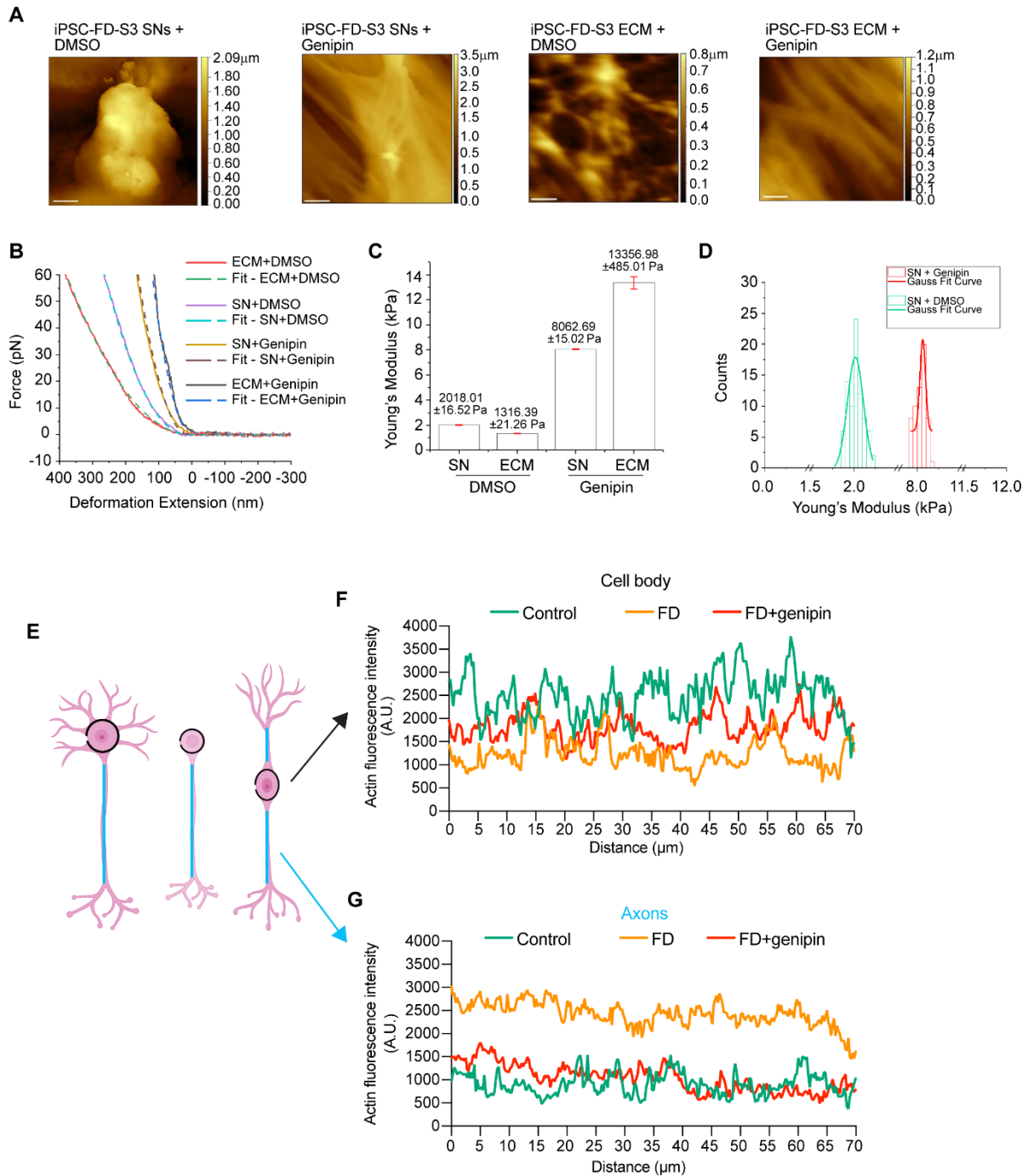
(G) or SNs **(H and I)** compared to genipin. Assessed by RT-qPCR (**G and I**) and IF quantification (**H**, related to **Fig. 6H**). For **G**, n=7-8 biological replicates. Two-way

ANOVA followed by Šídák multiple comparisons. For **H and I**, n=6-8 biological

replicates. one-way ANOVA followed by Tukey's multiple comparisons. * $p < 0.05$,

** $p < 0.005$, *** $p < 0.001$, **** $p < 0.0001$. All graphs show mean \pm s.d. For **F, G, H, and I**,

iPSC-FD-S2 and iPSC-FD-S3 data are pooled as FD.



Supplemental Figure 6. Effects of Genipin crosslinking of ECM (related to Fig 6).

A) AFM topographic images (Scale bar: 10 μm). **B)** Force-distance curves (solid)

recorded by force spectroscopy, along with Hertz model fitted curves (dash) which was used to calculate the Young's Modulus. **C)** Comparison of Young's Modulus for four different samples from a representative experiment. **D)** Histogram of Young's Modulus for SNs in DMSO (green) and genipin (red) with Gaussian distribution. A Gaussian distribution fit curve is overlaid to highlight sample variability. **E)** Schematics for actin signal measurement in cell bodies (black) and axons (blue) in SNs with different *in vitro* morphology. **F, G)** Quantification of actin signal intensity from images in **Fig.6K** (n=7-10 cells from 3 biological replicates).

Supplementary tables

Category	Parameter	Description
Assay	Type of assay	IF staining
	Target	Increase in sensory neuron differentiation
	Primary measurement	IF staining of BRN3A+ cells
	Key reagents	Differentiated sensory neurons
	Assay protocol	Adapted from Chambers et al., 2012
	Additional comments	
Library	Library size	640 compounds, i.e. half of the LOPAC library
	Library composition	Cell signaling and neuroscience
	Source	Sigma
	Additional comments	
Screen	Format	96 well plates
	Concentration(s) tested	1mM and 10mM
	Plate controls	DMSO treated wells, healthy hPSCs (+ control) and disease cells without compounds (- control)
	Reagent/ compound dispensing system	Manual multi-pipettor
	Detection instrument and software	MetaXPress software: Cell scoring Module from Molecular Devices
	Assay validation/QC	+ and – controls and DMSO only wells (see above)
	Correction factors	n/a
	Normalization	DAPI+ cells and DMSO only treated wells
	Additional comments	
Post-HTS analysis	Hit criteria	Phenotype reproducible in screening and non-screening format
	Hit rate	1
	Additional assay(s)	Phenotype reproducible in different well format Phenotype reproducible in additional control and disease hPSC lines
	Confirmation of hit purity and structure	n/a
	Additional comments	

Supplementary Table 1. Small molecule screening data

Compound name	Screen [c]	Fold change over DMSO (average)
DMSO only	10 μ M	4.5
hPSC-ctr-H9 in DMSO	10 μ M	7.7
iPSC-FD in DMSO	10 μ M	1.6
Fluphenazine dihydrochloride	10 μ M	17.5
AC-93253 iodide	1 μ M	16.5
genipin	1 μ M	14.2
genipin	10 μ M	16.1

Supplementary Table 2. Hit compounds

	Total embryos	Live embryos	Resorptions	Number of litters
No treatment	46	40	6	6
+ Genipin	44	41	3	6

Supplementary Table 3. Genipin does not affect normal embryonic development