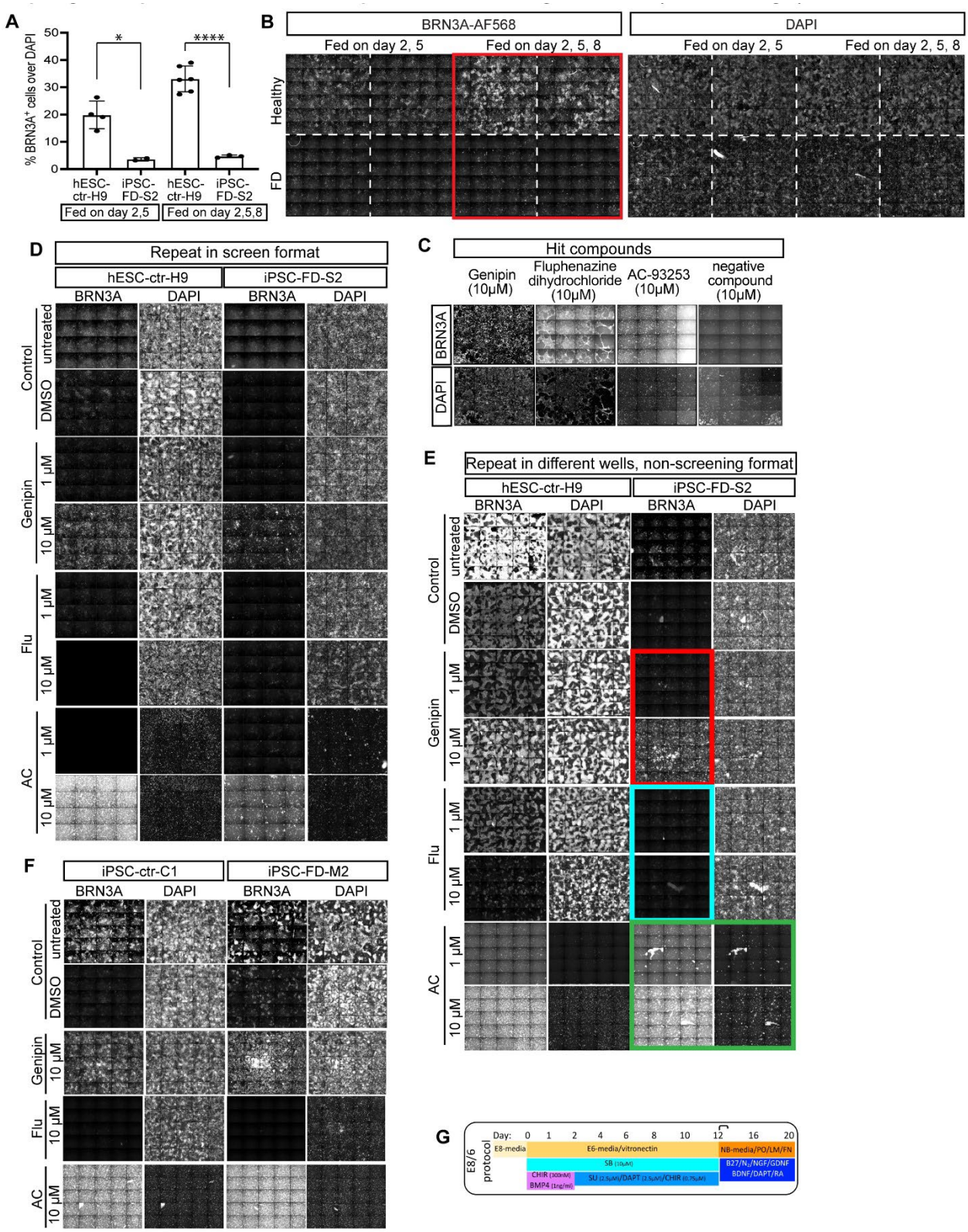
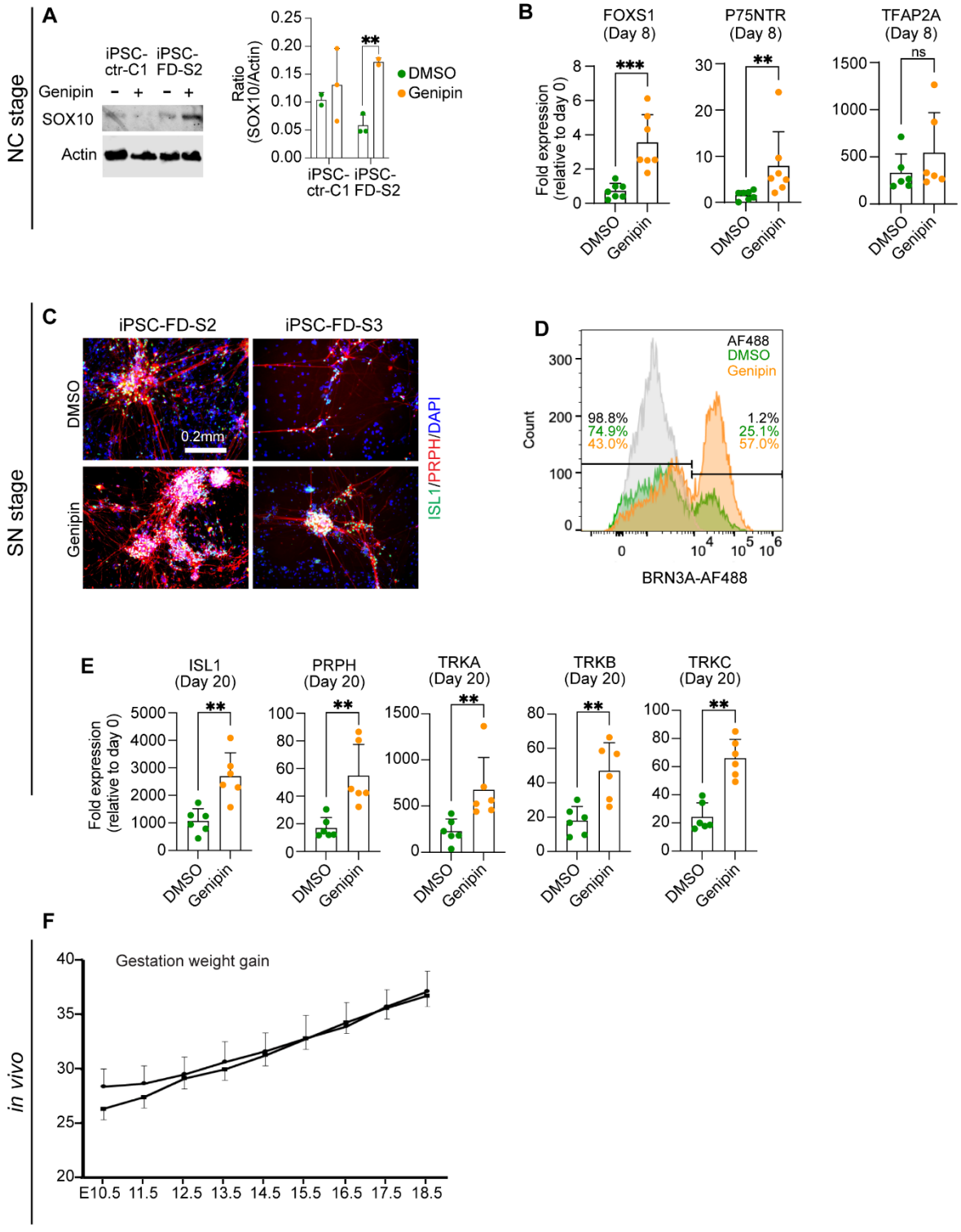
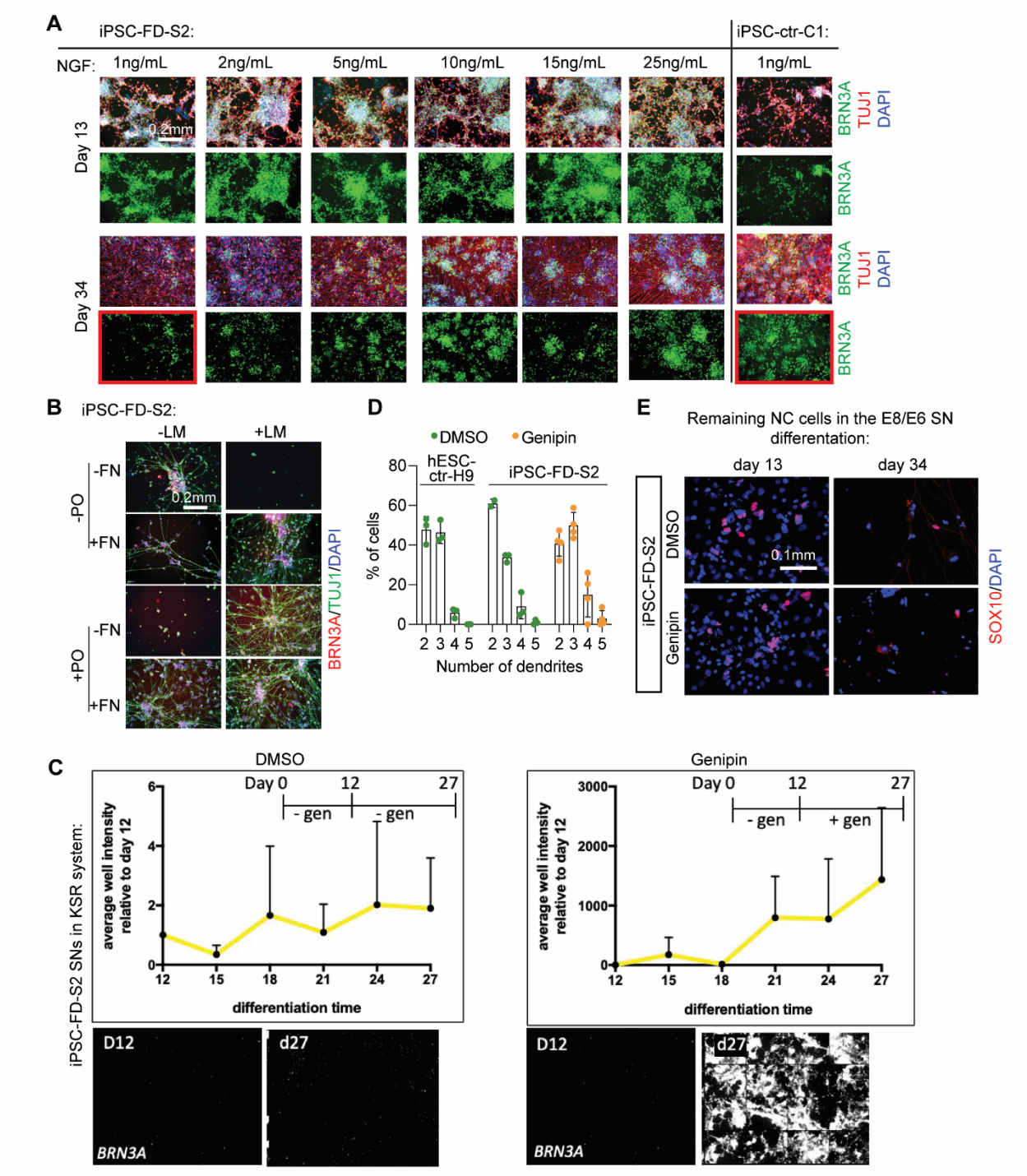
# Supplementary Figures



**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 reported[1]).

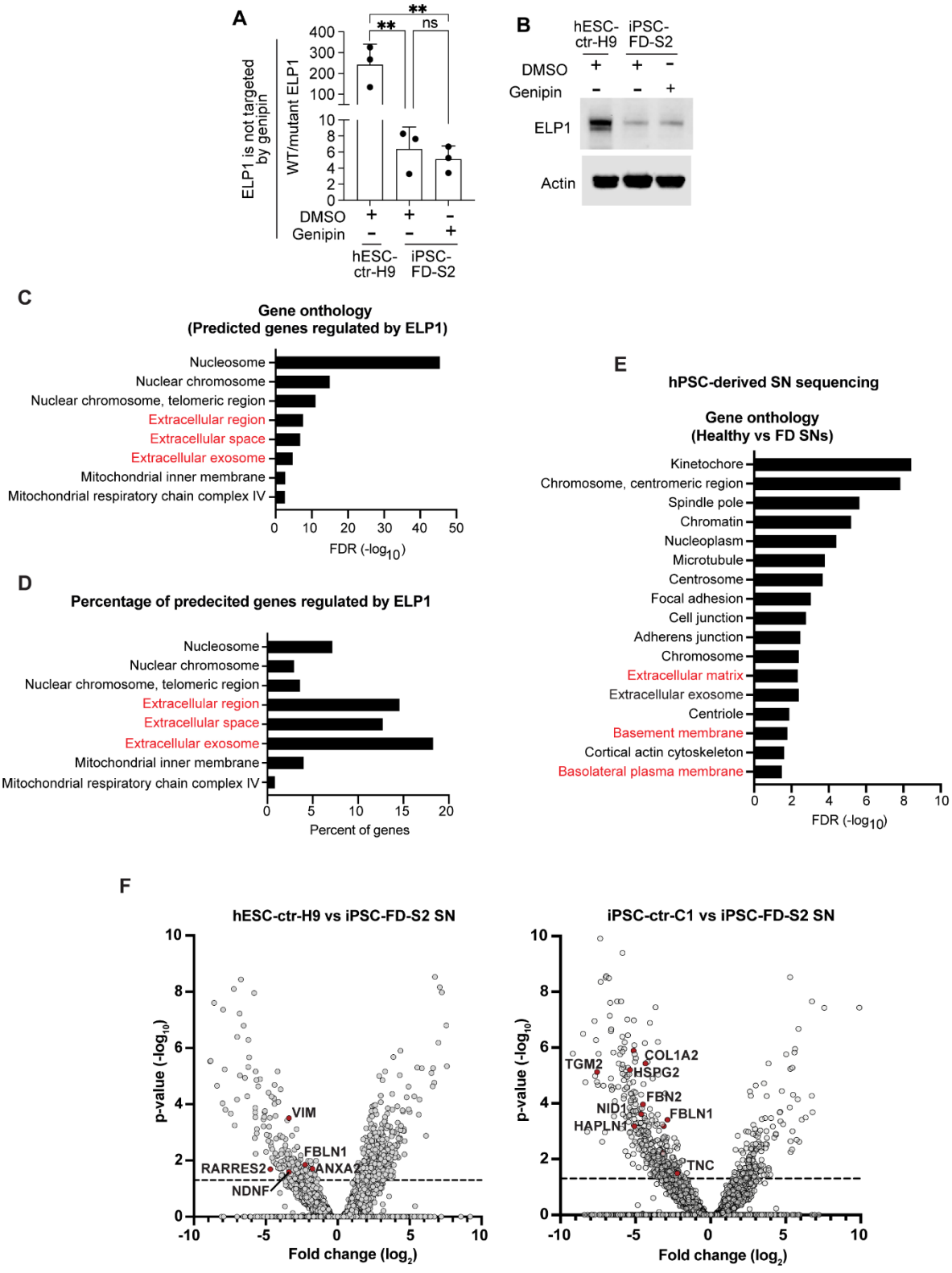


**Supplementary Figure 2**. ***in vitro*** **and** ***in vivo*** **developmental phenotypes in FD are rescued by genipin (related to Fig.3 and Fig. 4). A)** Immunoblotting of SOX10 from day 12 NC cells. Representative blot is shown (left) and quantification (right, n=2-3 biological replicates). **B)** 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 ± s.d. For **B** and **D**, iPSC-FD-S2 and iPSC-FD-S3 data are pooled as FD.

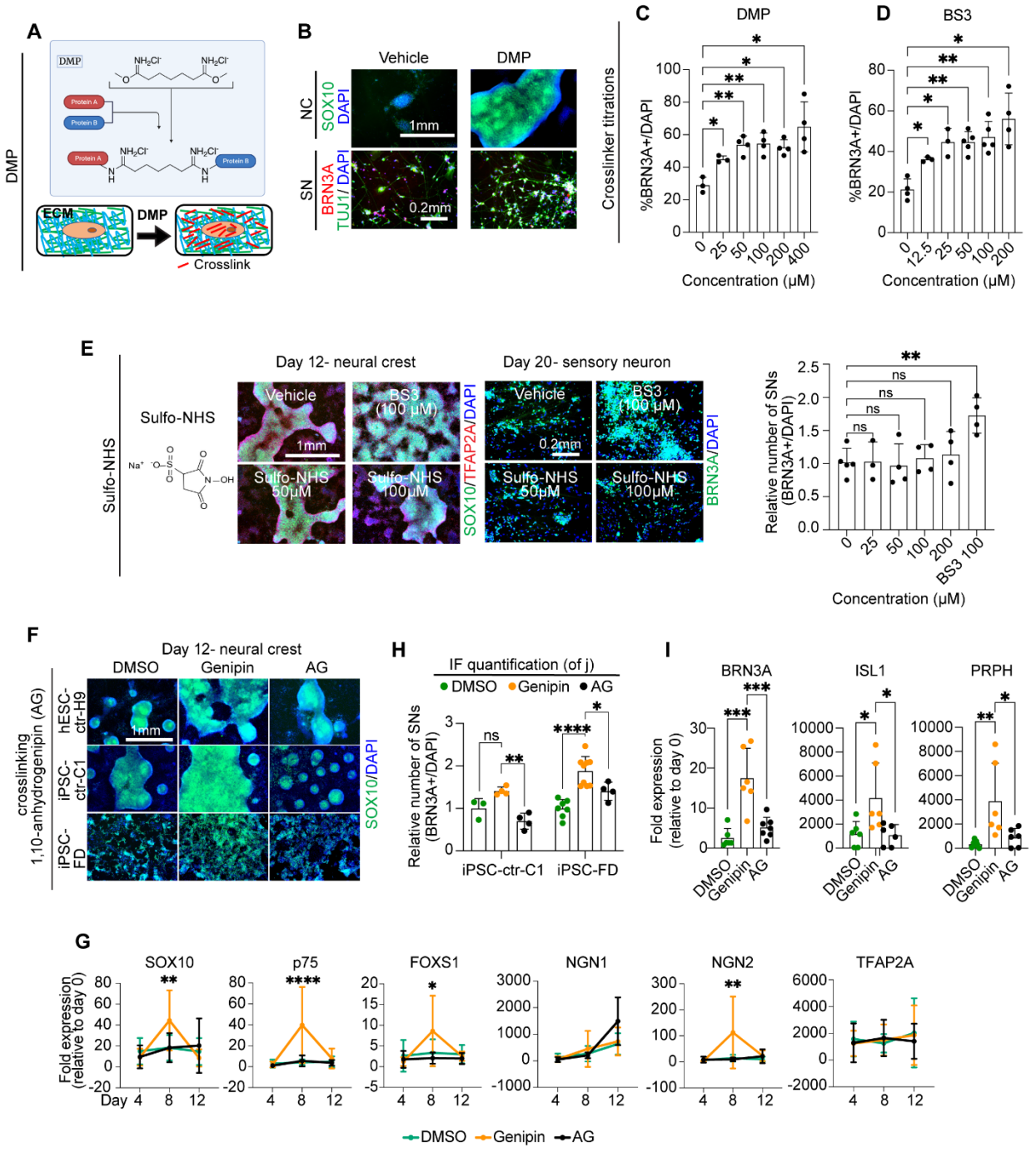



**Supplementary Figure 3. SN survival assay adaptation and characterization (related to Fig 5). A)** NGF titration to assess SN survival over 21 days by IF in iPSC-

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 μM) 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 ± 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).

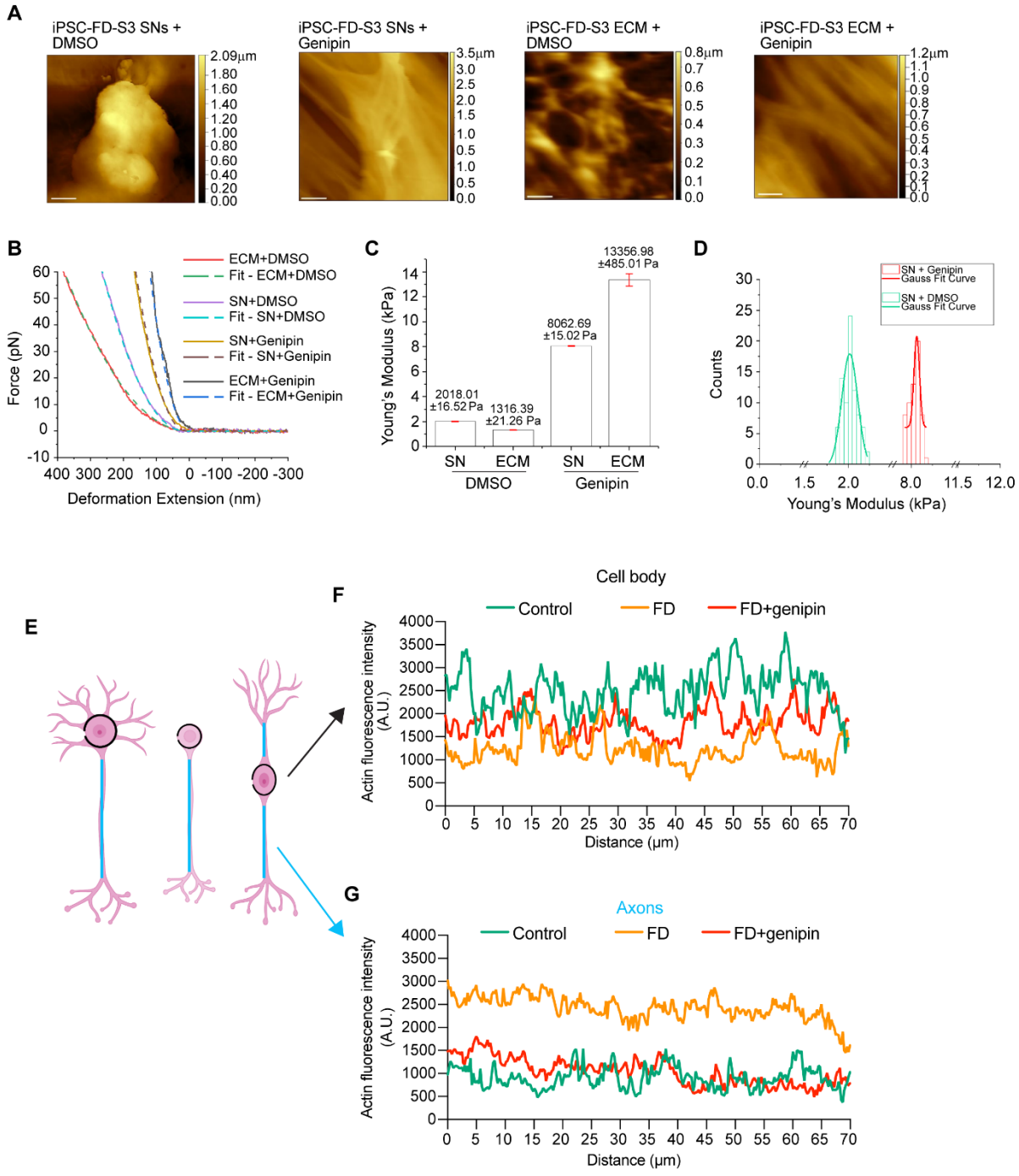



**Supplementary Figure 5**. **Genipin's mode of action is through crosslinking of ECM proteins (related to Fig 6). A)** Schematic of DMP crosslinking action and its intracellular/extracellular location. **D)** DMP rescues the NC and SN differentiation defect in FD. iPSC-FD-S2 cells were differentiated 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 ± 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: 10μm). **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 overlayed 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<br>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 | 10μM | 4.5 |
| hPSC-ctr-H9 in DMSO | 10μM | 7.7 |
| iPSC-FD in DMSO | 10μM | 1.6 |
| Fluphenazine dihydrochloride | 10μM | 17.5 |
| AC-93253 iodide | 1μM | 16.5 |
| genipin | 1μM | 14.2 |
| genipin | 10μM | 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