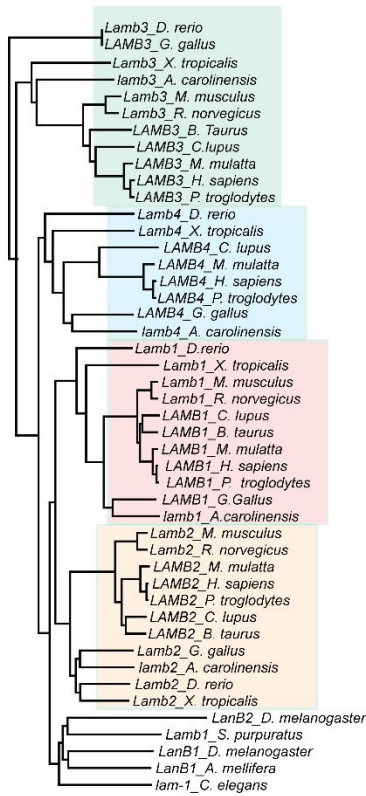
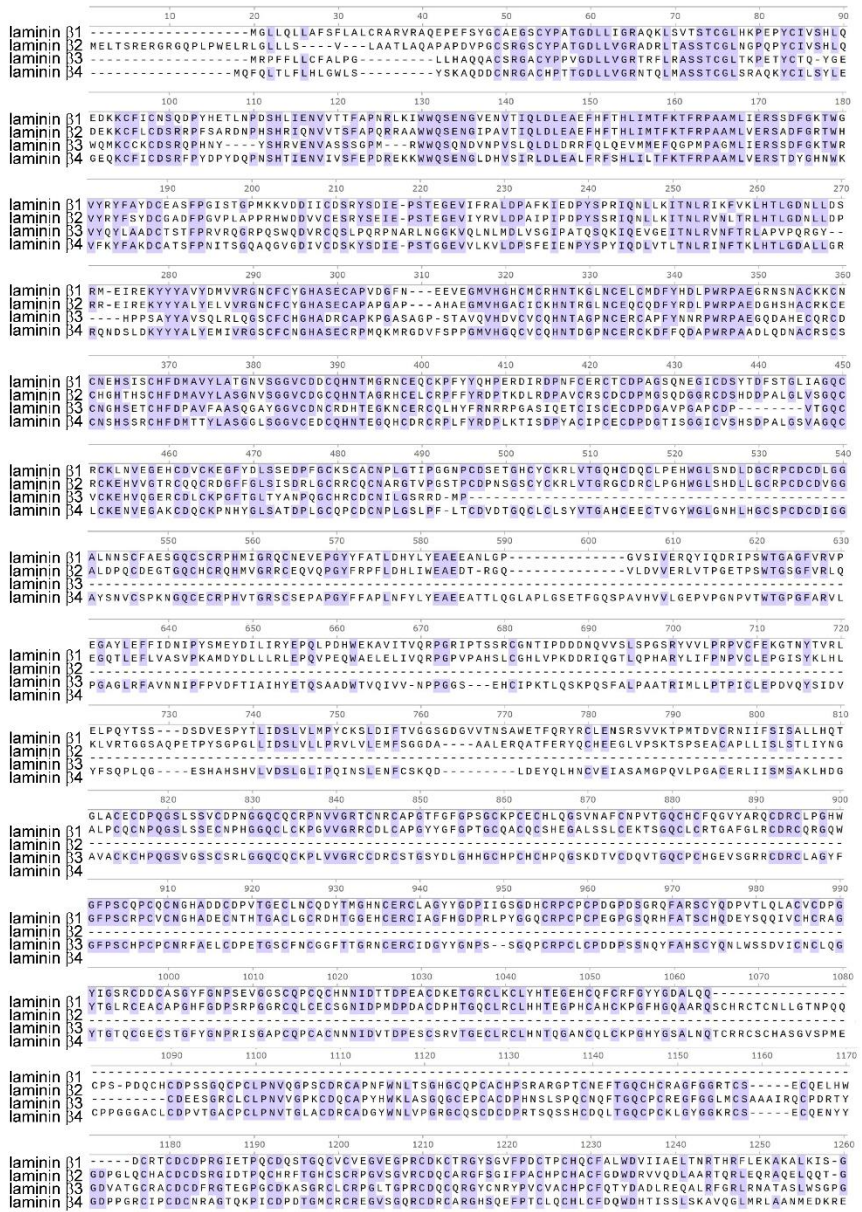


1 Supplementary Figures

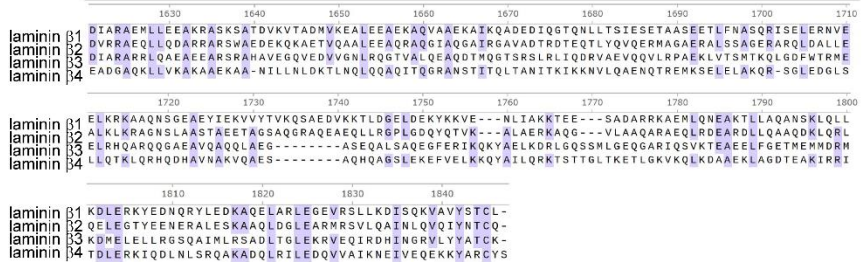
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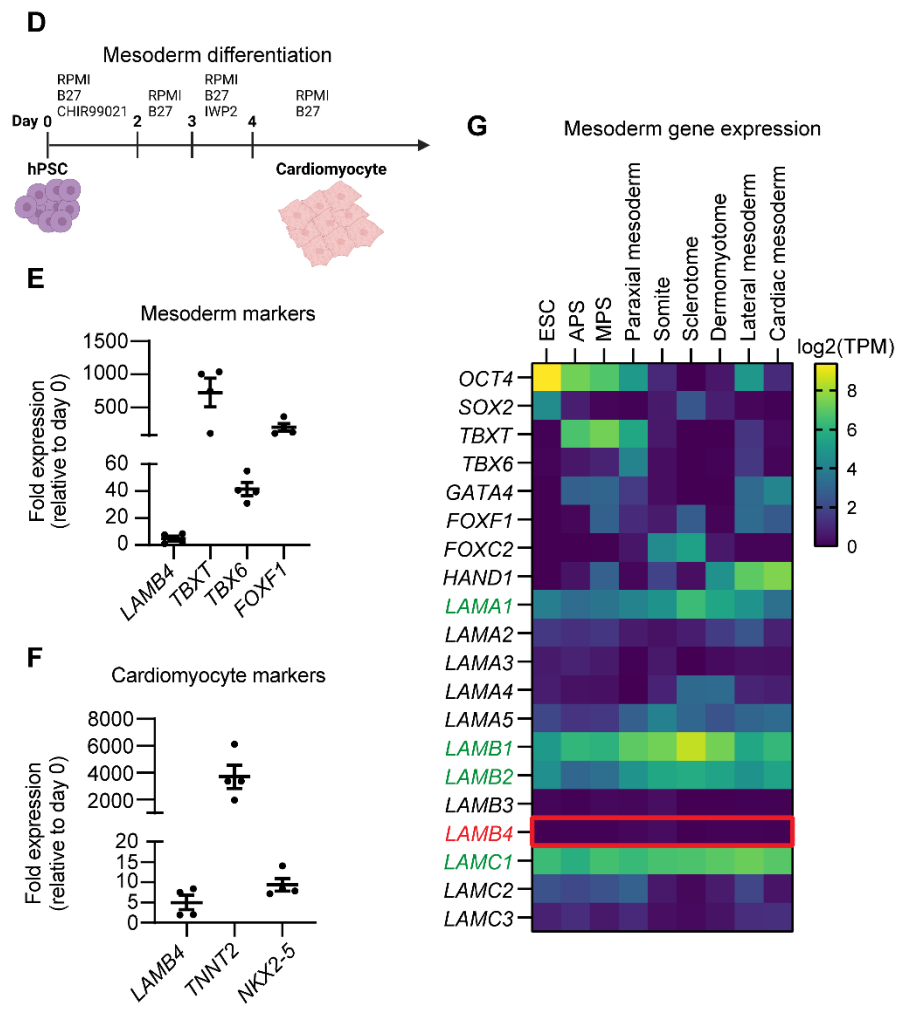
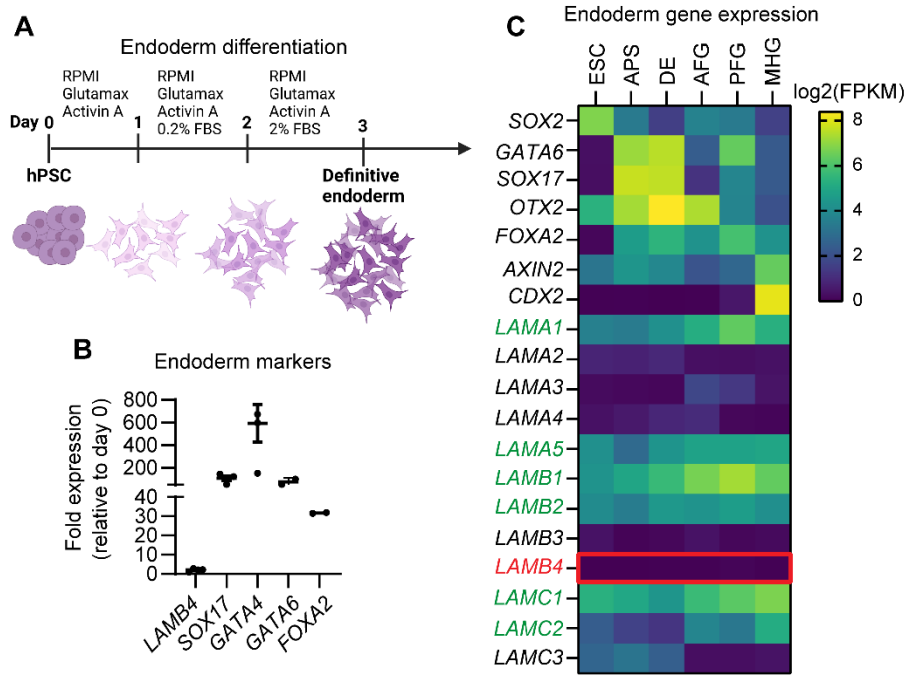
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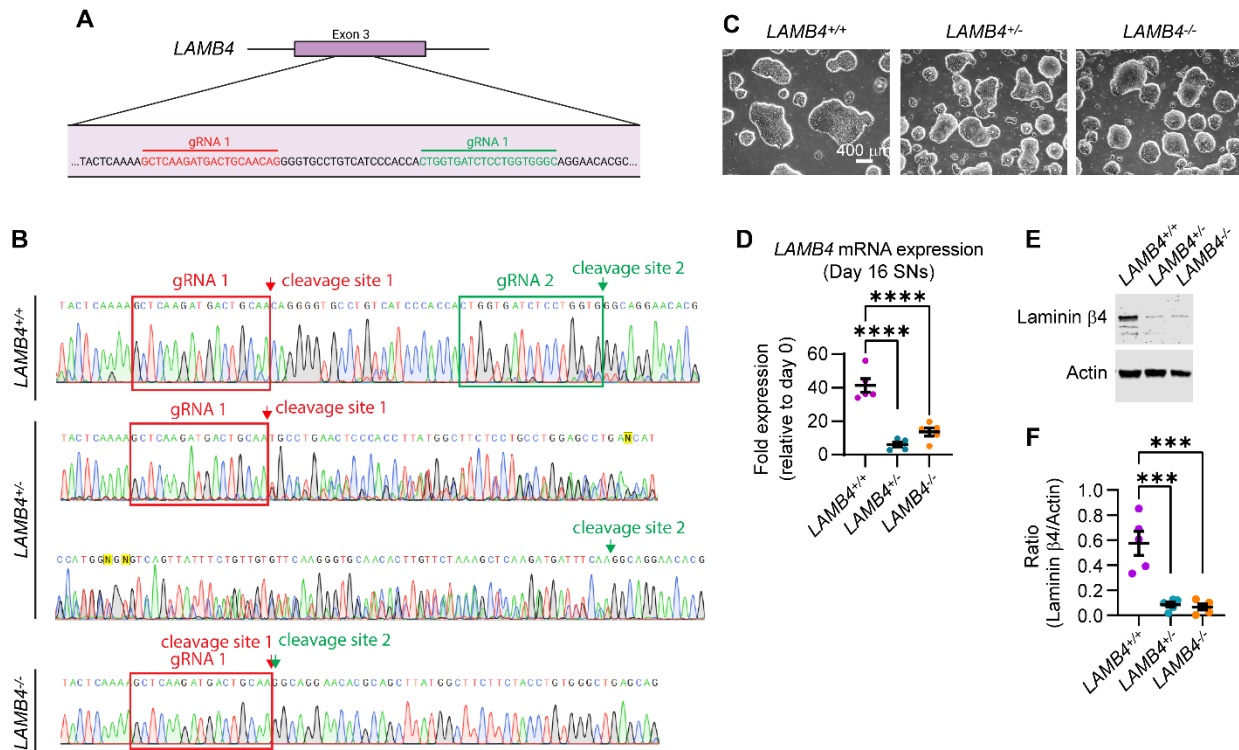
C



3 **Figure S1. Similarities between *LAMB* genes.** **A)** Dendrogram of the *LAMB* genes among
4 species. **B and C)** Alignment of regions in the **B)** N-terminal and **C)** C-terminal domains of the
5 amino acid sequences of the human laminin β chains.

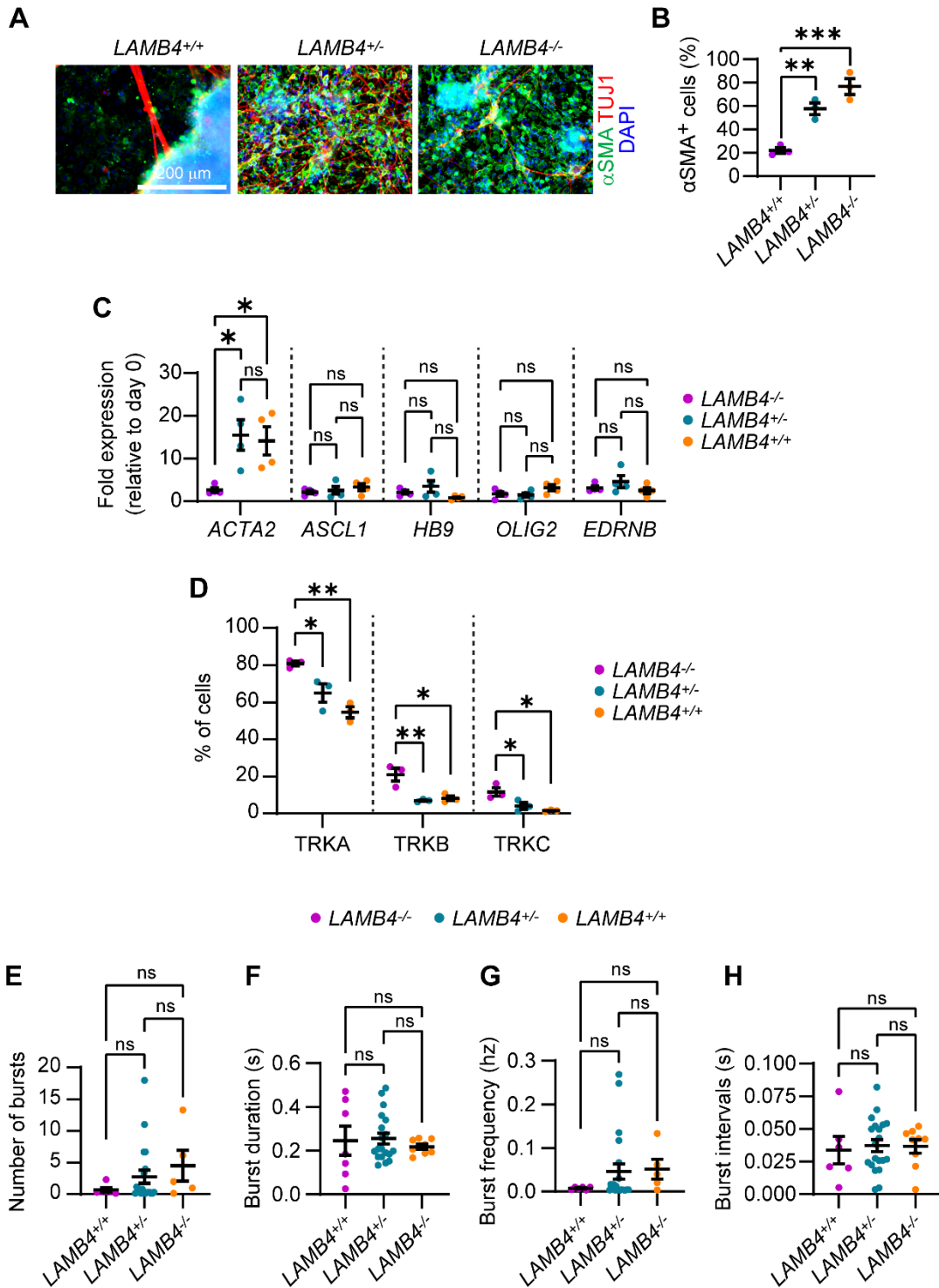


7 **Figure S2. *LAMB4* is not expressed in mesoderm and endoderm.** **A)** Schematic of the
8 endoderm differentiation protocol. **B)** Gene expression of endoderm markers. hPSC-ctr-H9 cells
9 were differentiated into endoderm and RNA was isolated on day 3. mRNA levels were measured
10 using RT-qPCR (n=2-4 biological replicates). **C)** *LAMB4* expression during endoderm
11 differentiated from hPSCs. **D)** Schematics of the mesoderm differentiation protocol. **E)** Expression
12 of early mesoderm marker. **F)** Expression of cardiomyocyte-related genes. Previously published
13 RNAseq data was analyzed to assess the expression of laminin chains. **G)** *LAMB4* expression
14 from mesoderm and cardiomyocytes differentiated from hPSCs. Highly expressed laminin genes
15 are shown in green. *LAMB4* shown in red.



16

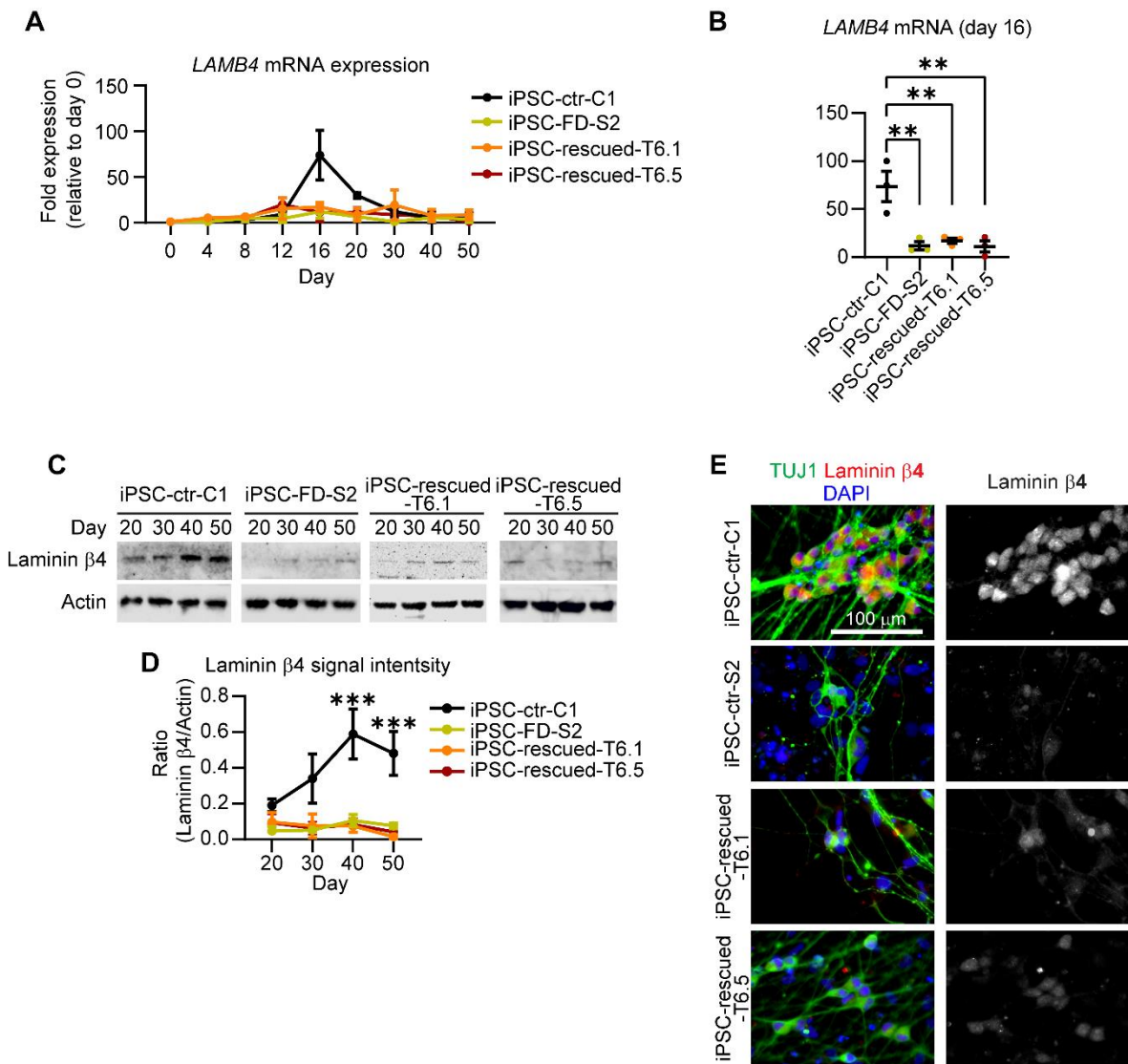
17 **Figure S3. *LAMB4* editing strategy and characterization. A)** Schematics of the strategy used
 18 to edit *LAMB4* by CRISPR/Cas9. **B)** Sequencing results of *LAMB4*^{+/+}, *LAMB4*^{+/-}, and *LAMB4*^{-/-}
 19 hPSCs. **C)** Characterization of *LAMB4*^{+/+}, *LAMB4*^{+/-}, and *LAMB4*^{-/-} hPSCs by brightfield
 20 microscopy. **D)** *LAMB4* expression in SNs differentiated from *LAMB4*^{+/+}, *LAMB4*^{+/-}, and *LAMB4*^{-/-}
 21 hPSCs. RNA was isolated on day 16 and *LAMB4* expression was measured by RT-qPCR (n=5
 22 biological replicates). **E)** Laminin β 4 levels in *LAMB4*^{+/+}, *LAMB4*^{+/-}, and *LAMB4*^{-/-} SNs. Total protein
 23 of day 20 SNs was immunoblotted for laminin β 4 and actin. **F)** Measuring of signal intensity of the
 24 immunoblots from **F)** (n=5 biological replicates). For **D)** and **F)**, one-way ANOVA followed by
 25 Tukey's multiple comparisons test. ***p<0.001, ****p<0.0001. Graphs show mean \pm SEM.



26

27 **Figure S4. Characterization of *LAMB4* mutant SNs.** **A)** Expression of markers upon loss of
 28 *LAMB4*. *LAMB4^{+/+}*, *LAMB4^{+/-}*, and *LAMB4^{-/-}* SNs were fixed on day 20 and stained for the non-
 29 neural ectoderm marker α -Smooth Muscle Actin (α SMA). Nuclei were stained with DAPI. **B)**

30 Percentage of α SMA⁺ cells from **A)** over DAPI was plotted. **C)** Expression of genes not expressed
31 in SNs. RNA of day 20 SNs differentiated from *LAMB4*^{+/+}, *LAMB4*^{+/-}, and *LAMB4*^{-/-} hPSCs was
32 isolated. Gene expression was measured by RT-qPCR (n=4 biological replicates). **D)** Percentage
33 of cells expressing TRK proteins. Day 20 SNs were stained with TRKA, TRKB, and TRKC
34 fluorescently-tagged antibodies and analyzed by flow cytometry (n=3 biological replicates). **E-H)**
35 Measurement of electrical activity of *LAMB4*^{+/+}, *LAMB4*^{+/-}, and *LAMB4*^{-/-} SNs. **E)** Number, **F)**
36 duration, **G)** Frequency, and **H)** intervals of bursts were measured. Each dot represents the mean
37 firing rate of 6 wells measured over 40 days (n=4 biological replicates). For **C)**, one-way ANOVA
38 followed by Dunnett's multiple comparisons test. For **B)**, **D)**, **E)**, **F)**, **G)**, and **H)** one-way ANOVA
39 followed by Tukey's multiple comparisons test. ns, non-significant, *p<0.05, **p<0.005. Graphs
40 show mean \pm SEM.



41

42 **Figure S5. *LAMB4* expression is not dependent on *ELP1*.** **A)** *LAMB4* expression in SNs

43 differentiated from *ELP1*-rescued iPSCs of severe FD patients. *ELP1*^{+/-} severe FD iPSCs (iPSC-

44 rescued-T6.1 and iPSC-rescued-T6.5), one severe FD iPSC line (S2), and one healthy iPSC

45 control line (C1) were differentiated into SNs. Total RNA was isolated at the indicated times and

46 gene expression was measured by RT-qPCR (n=3 biological replicates). **B)** *LAMB4* expression

47 by SNs on day 16 shown in **A)** is shown (n=3 biological replicates). **C)** Laminin β4 expression

48 during SN development. iPSC-rescued-T6.1, iPSC-rescued-T6.5, iPSC-FD-S2, and iPSC-ctr-C1

49 cells were differentiated into SNs. Lysates were collected on the indicated days and were

50 immunoblotted for laminin β 4 and actin. **D)** Quantification of signal intensity of immunoblots shown
51 in **C)** (n=3 biological replicates). **E)** Laminin β 4 in *ELP1*-rescued severe FD SNs. iPSC-rescued-
52 T6.1, iPSC-rescued-T6.5, iPSC-FD-S2, and iPSC-ctr-C1 hPSCs were differentiated into SNs.
53 Cells were fixed on day 20 and stained for laminin β 4, TUJ1, and DAPI. For **B)**, one-way ANOVA
54 followed by Tukey's multiple comparisons test. For **D)**, two-way ANOVA followed by Šídák's
55 multiple comparisons test. **p<0.005, ***p<0.001. Graphs show mean \pm SEM.

Supplementary Table 1 - List of primers used in this study

Gene	Forward sequence	Reverse sequence
<i>SOX10</i>	CCAGGCCCACTACAAGAGC	CTCTGGCCTGAGGGGTGC
<i>P75NTR</i>	CCTCATCCCTGTCTATTGCTCC	GTTGGCTCCTTGCTTGTTCTGC
<i>NGN1</i>	GCCTCCGAAGACTTCACCTACC	GGAAAGTAACAGTGTCTACAAAGG
<i>NGN2</i>	CAAGCTCACCAAGATCGAGACC	AGCAACACTGCCTCGGAGAAGA
<i>BRN3A</i>	AGTACCCGTCGCTGCACTCCA	TTGCCCTGGGACACGGCGATG
<i>RUNX1</i>	CCACCTACCACAGAGCCATCAA	TTCACTGAGCCGCTCGGAAAAG
<i>RUNX3</i>	GGCAATGACGAGAACTACTCCG	GATGGTCAGGGTGAAACTCTTCC
<i>TRKA</i>	CACTAACAGCACATCTGGAGACC	ACAGTCAGCTCAAGCCAGACAC
<i>TRKB</i>	ACAGTCAGCTCAAGCCAGACAC	GTCCTGCTCAGGACAGAGGTTA
<i>TRKC</i>	CCGACACTGTGGTCATTGGCAT	CAGTTCTCGCTTCAGCACGATG
<i>TBXT</i>	CCTTCAGCAAAGTCAAGCTCACC	TGAACTGGGTCTCAGGGAAGCA
<i>TBX6</i>	TCATCTCCGTGACAGCCTACCA	CCGCAGTTTCCTCTTCACACGG
<i>FOXF1</i>	CAGCCTCACATCACGCAAGG	AGCCGAGCTGCAAGGCATC
<i>TNNT2</i>	AAGAGGCAGACTGAGCGGGAAA	AGATGCTCTGCCACAGCTCCTT
<i>NKK2-5</i>	AAGTGTGCGTCTGCCTTTCCCG	TTGTCCGCCTCTGTCTTCTCCA
<i>GAPDH</i>	GTCTCCTCTGACTTCAACAGCG	ACCACCCTGTTGCTGTAGCCAA
<i>LAMA1</i>	GAAGGTGACTGGCTCAGCAAGT	AGGCGTCACAACGGAAATCGTG
<i>LAMA2</i>	GGCAATCTGAATACACTCGTGAC	TGTGTTGGTCCTCTCAGCATCC
<i>LAMA3</i>	TAGAGGAAGCCTCTGACACAGG	CCGATAGTATCCAGGGCTACAAC
<i>LAMA4</i>	GAGATGACTCTCTGCTGGACCT	AGTTCCAGGCAGCCAACAAAGC
<i>LAMA5</i>	AACCAGATGAGCATCACATTCTG	ACAGTGTTGCGCGTCTCCGTAT
<i>LAMB1</i>	GAGGTGTCTCAAGTGCCTGTAC	ACTGGCAGTCAGAGCCGTTACA
<i>LAMB2</i>	GCGGACTTGTTCTGAGTGCCAA	ACCTGTGAAGCGGTGACACTGA
<i>LAMB3</i>	GTCACAGAGCAGGAGGTGGCT	GCTTCTGTCAAGACTCTCCAGG
<i>LAMB4</i>	GTGGAGGCTTTACAACCTGGCAG	GGATCATCTGGACACAGGCAAG
<i>LAMC1</i>	CTGTGAGGTCAACCACTTTGGG	AGCCTTCTCTGCATTACAGCG
<i>LAMC2</i>	TACAGAGCTGGAAGGCAGGATG	GTTCTCTTGGCTCCTCACCTTG
<i>LAMC3</i>	CTGTAACCAGCATGGCACCTGT	ACCTGGCAAACAGCGTTCACAG