Cell Reports, Volume *30*

Supplemental Information

Transcriptional Programming of Human Mechanosensory Neuron Subtypes from Pluripotent Stem Cells

Alec R. Nickolls, Michelle M. Lee, David F. Espinoza, Marcin Szczot, Ruby M. Lam, Qi Wang, Jeanette Beers, Jizhong Zou, Minh Q. Nguyen, Hans J. Solinski, Aisha A. AlJanahi, Kory R. Johnson, Michael E. Ward, Alexander T. Chesler, and Carsten G. Bönnemann

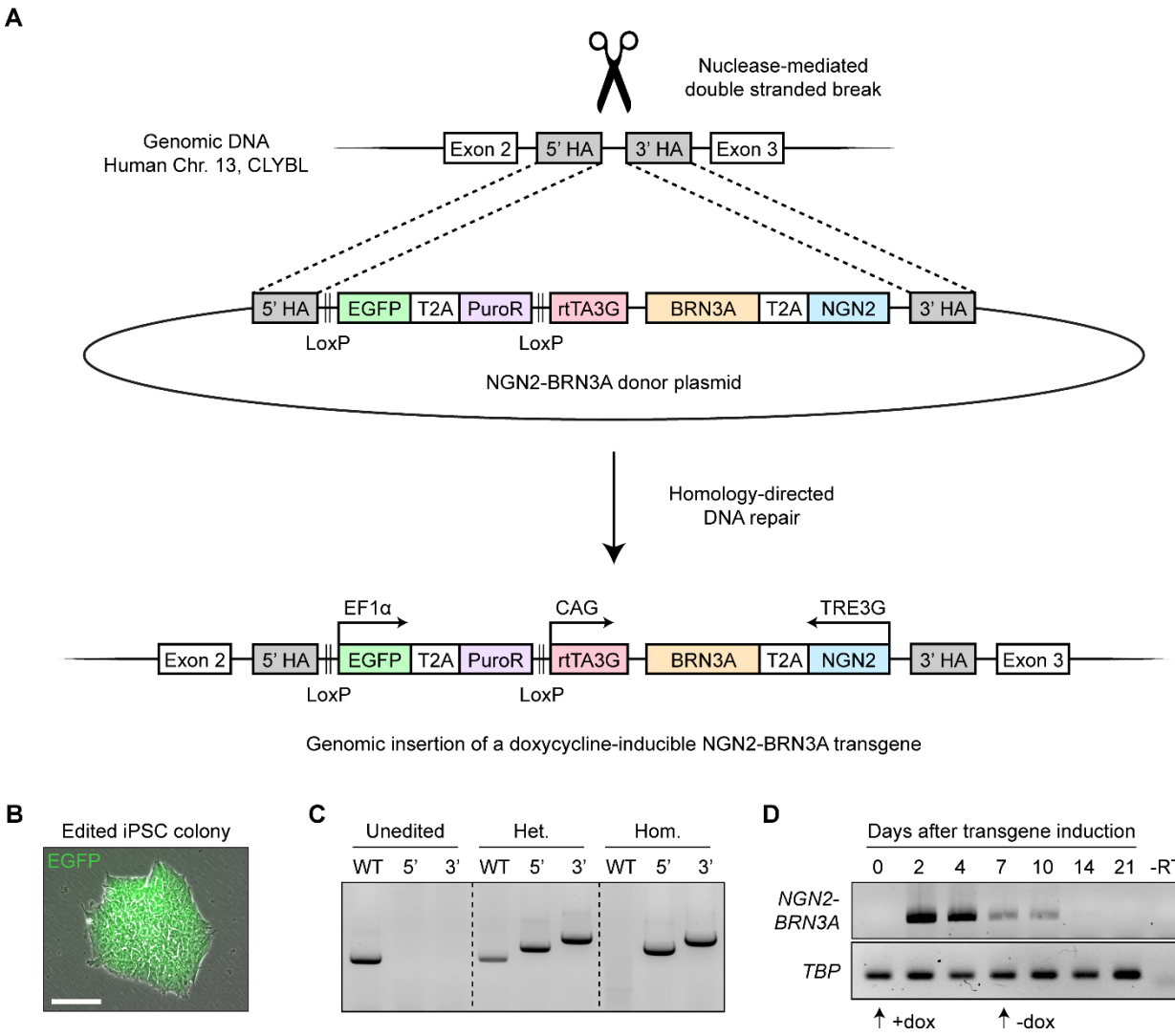

Figure S1. Genomic Insertion of a Doxycycline-Inducible *NGN2-BRN3A* Transgene Cassette in Human iPSCs, Related to Figure 1

(A) Schematic of homology-directed repair at the CLYBL genomic safe harbor site using a donor plasmid containing *NGN2* and *BRN3A*.

(B) Example of an EGFP+ stably engineered iPSC colony before Cre recombinase-mediated excision of the reporter. Scale bar, 100 μm.

(C) PCR genotyping gel illustrating the three possible outcomes of the genome engineering. Primers targeted either the unedited CLYBL locus (WT), the 5' junction where the insert homology arm meets the genomic DNA (5'), or the 3' junction where the insert homology arm meets the genomic DNA (3'). Het., heterozygous. Hom., homozygous.

(D) RT-PCR validation of the doxycycline (dox)-inducible transgene expression over time.

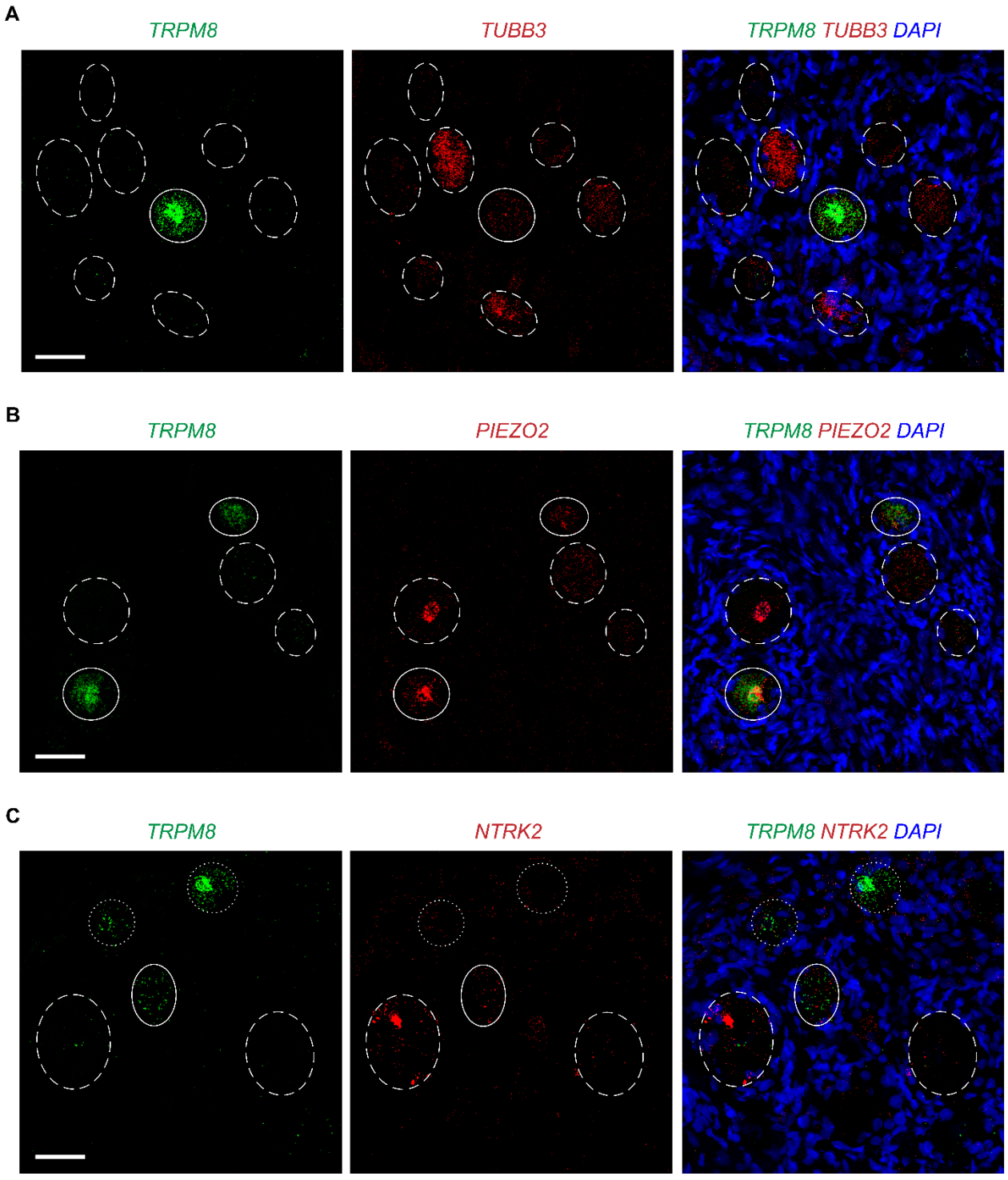

Figure S2. *In Situ* Hybridization of Human DRG Neurons, Related to Figure 2

(A) RNA *in situ* hybridization of human DRG. Neurons are identified by the pan-neuronal marker *TUBB3* (dotted lines), with a subset also expressing *TRPM8* (solid lines).
(B) Representative image of *PIEZO2*+ neurons (dashed lines) and *TRPM8*+/*PIEZO2*+ neurons (solid lines).
(C) Representative image of *TRPM8*+ (dotted lines), *NTRK2*+ (dashed lines), and *TRPM8*+/*NTRK2*+ (solid lines) neurons. Scale bars, 50 μm.

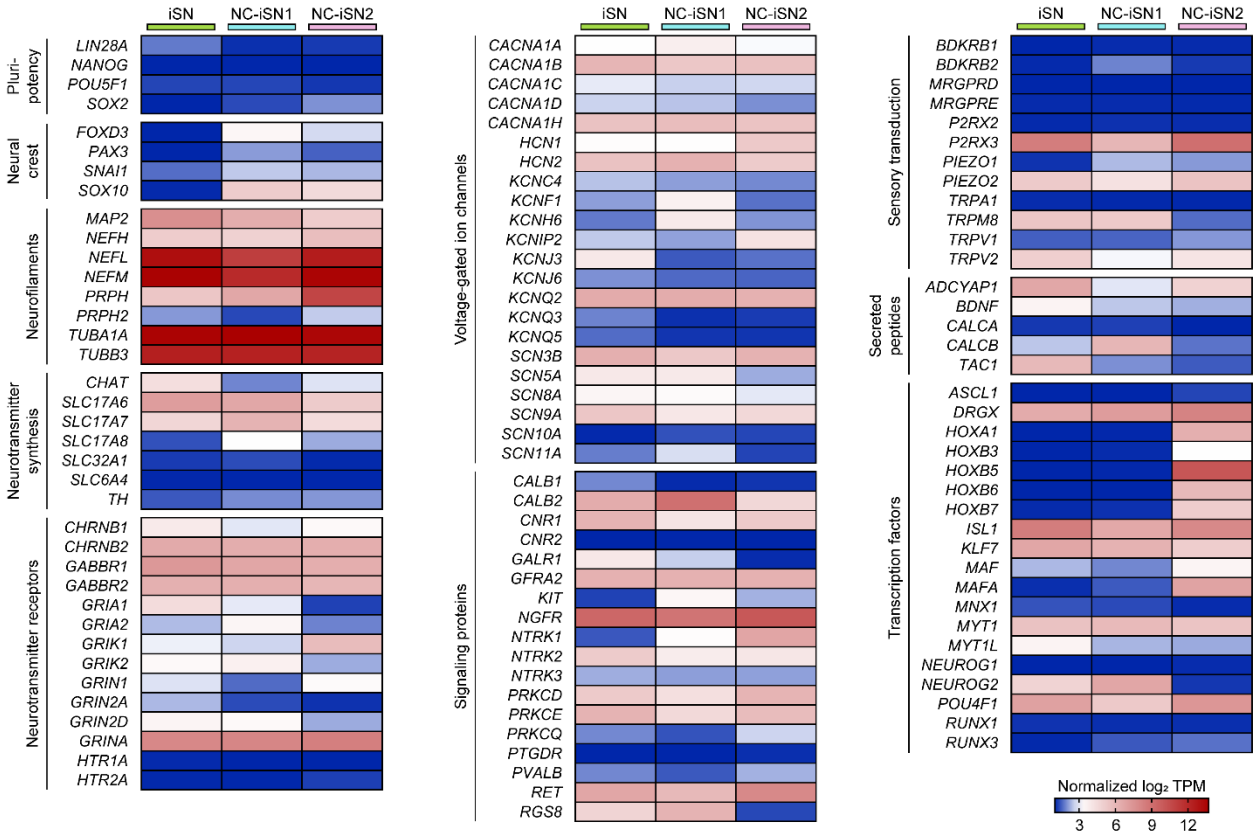

Figure S3. Gene Expression Profile of iSN Subtypes, Related to Figure 4

RNA sequencing heatmap expression data of genes grouped by function for the three iSN subtypes. Every column indicates the average $\log_2$ TPM expression of three independent culture replicates.

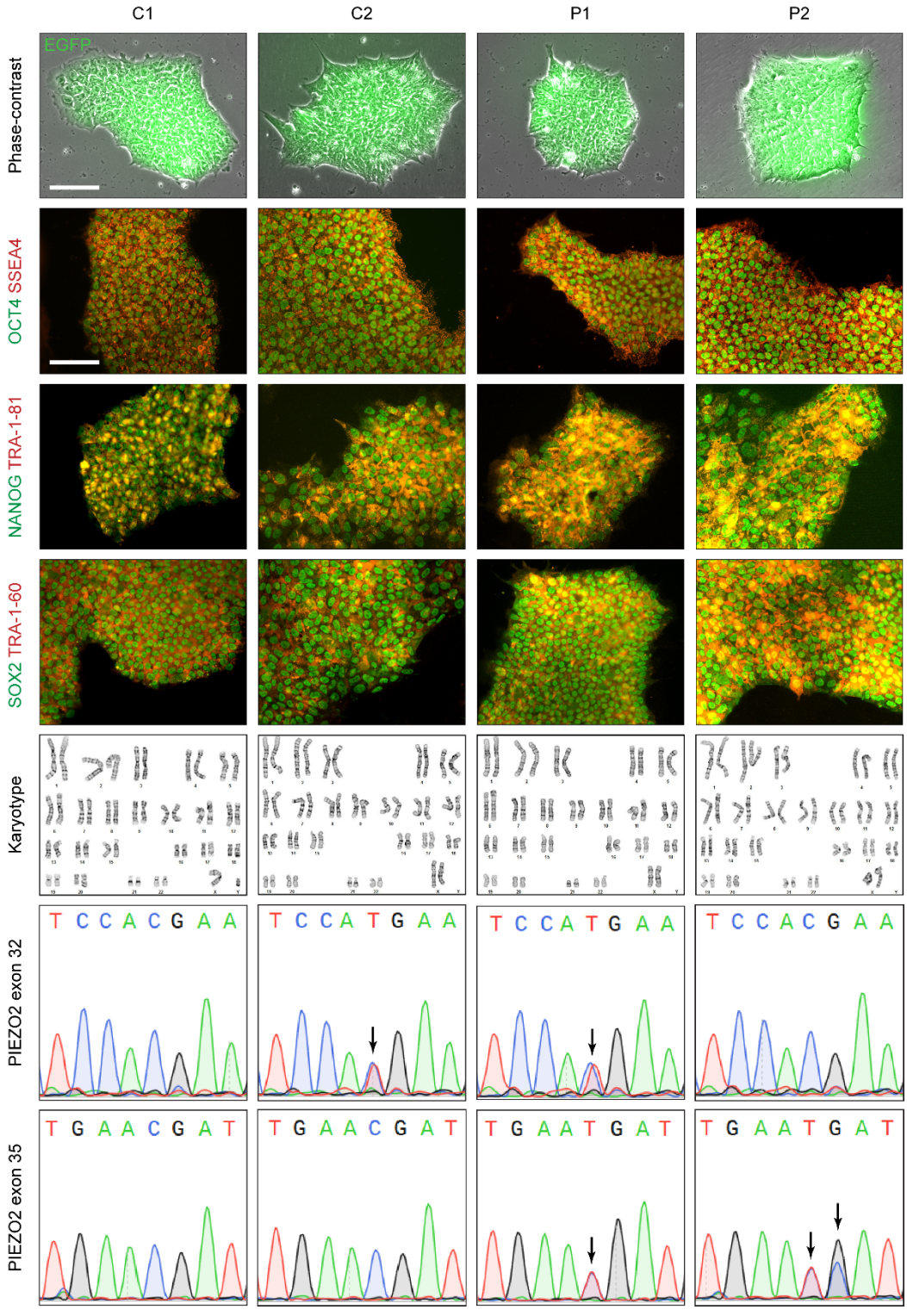

Figure S4. Generation of Control and PIEZO2^{LOF} *NGN2-BRN3A* iPSC Lines, Related to Figure 6
Quality control experiments demonstrating EGFP expression after isolation and genotyping (data not shown) of selected *NGN2-BRN3A*-engineered clones, before Cre recombinase excision of the reporter. Representative images are shown of pluripotency marker staining, karyotype analysis, and Sanger sequencing of *PIEZO2* on genomic DNA. Arrows indicate location of mutations. Scale bars, 100 μm.

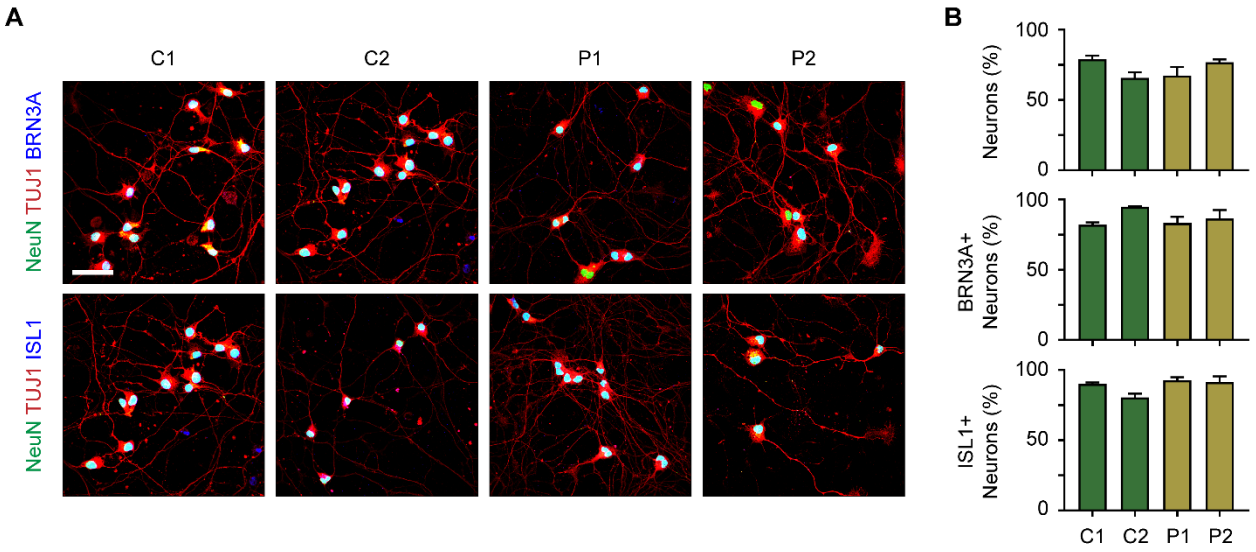

Figure S5. Characterization of Control and PIEZO2^{LOF} iSN Conversion, Related to Figure 6

(A) Immunocytochemical analysis of day 21 iSNs from control and patient cell lines. Images are representative of at least three independent differentiation experiments per cell line. Scale bar, 50 μm.

(B) Quantification of neuronal and sensory markers. For every cell line, *n* = 3 coverslips were stained each for BRN3A or for ISL1 and co-stained for NeuN. At least 200 cells were counted per cell line per stain. Values expressed as mean ± SEM.

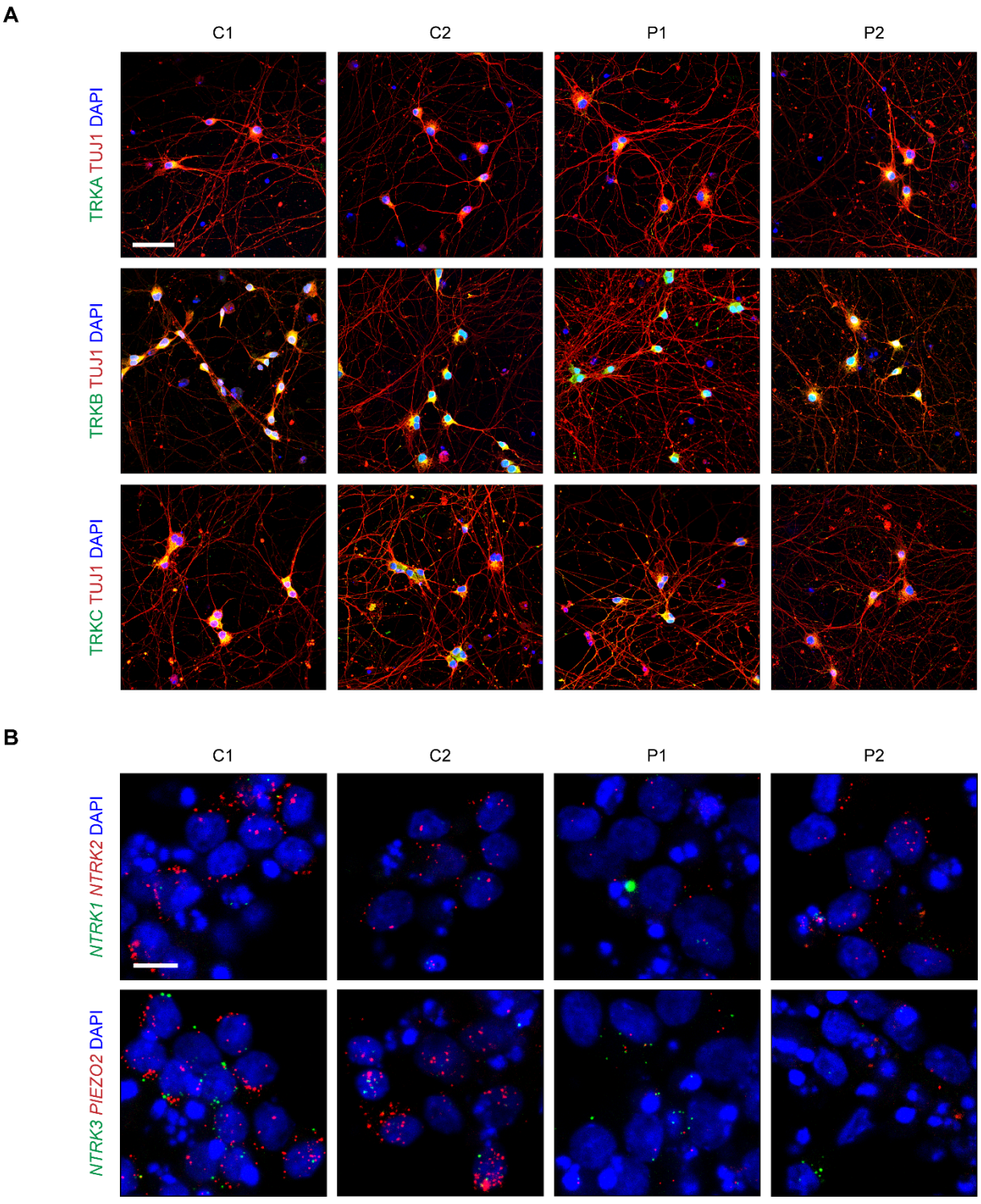

Figure S6. Analysis of Neurotrophin Receptors and *PIEZO2* Expression in Control and PIEZO2^{LOF} iSNs, Related to Figure 6

(A) Immunocytochemistry on day 21 control and patient iSNs for neurotrophin receptors. Images are examples from at least three differentiations per cell line. Scale bar, 50 μm.

(B) Representative images of RNA *in situ* hybridization from at least three differentiations per cell line for neurotrophin receptor gene transcripts and *PIEZO2*. Scale bar, 10 μm.

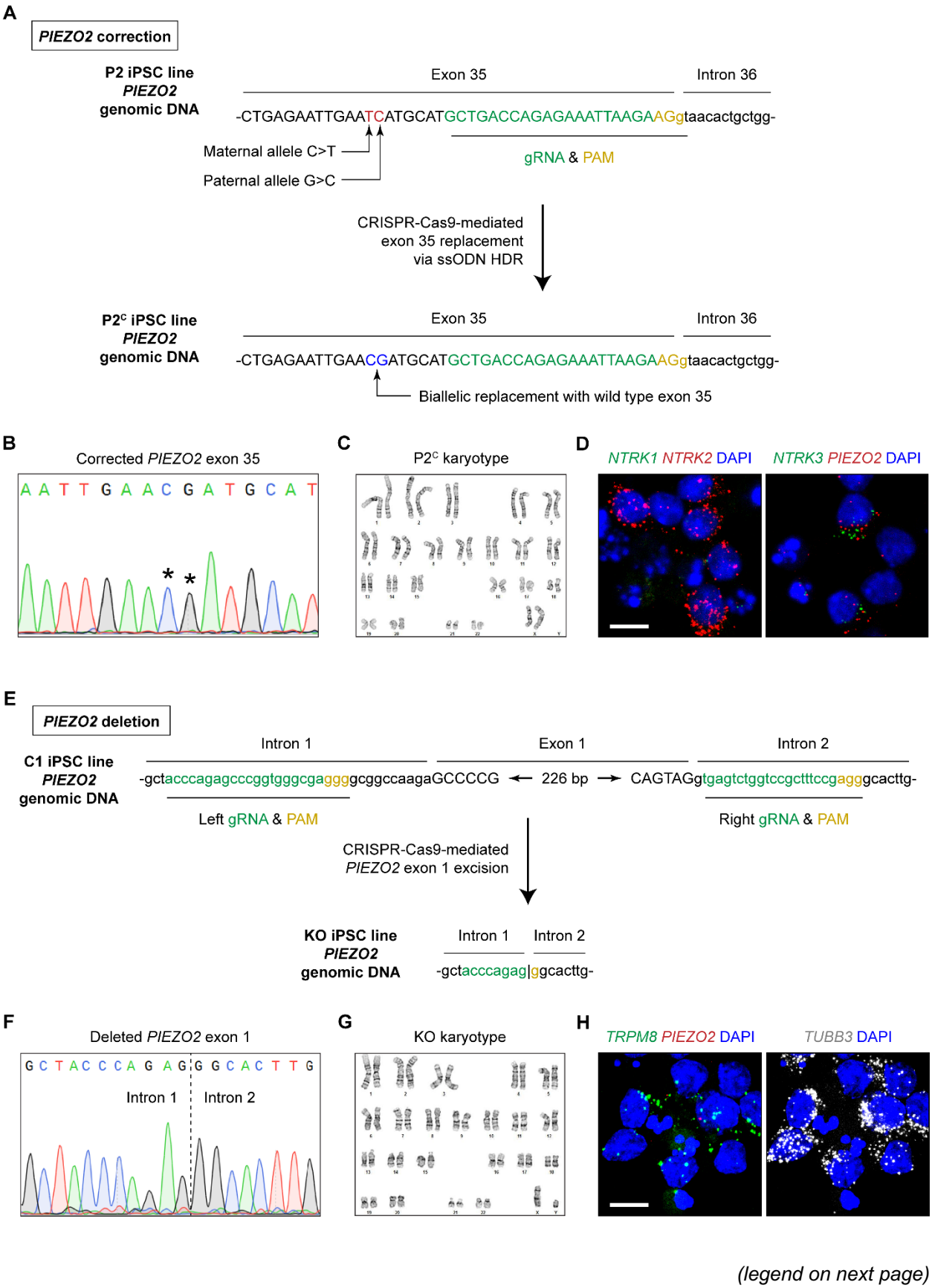

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Figure S7. Generation of a Corrected PIEZO2^{LOF} Patient iPSC Line and a PIEZO2 Knockout iPSC Line, Related to Figure 6

(A) Schematic of *PIEZO2* genetic correction strategy in P2 iPSCs using CRISPR-Cas9-mediated insertion of a single-stranded donor oligonucleotide (ssODN) by homology-directed repair (HDR). The patient harbors compound heterozygous mutations on *PIEZO2* exon 35, and the ssODN encoded the wild type sequence of exon 35.

(B, C) Example verification of wild type genomic DNA sequence by Sanger sequencing and normal karyotype in selected iPSC clones after editing. Asterisks indicate the corrected DNA bases.

(D) Representative images of RNA *in situ* hybridization for neurotrophin receptors and *PIEZO2* in iSNs from two corrected iPSC clones.

(E) Schematic of PIEZO2 genetic deletion strategy in C1 iPSCs using CRISPR-Cas9-mediated excision of exon 1 using two flanking gRNAs.

(F) Example Sanger sequencing on genomic DNA to verify absence of exon 1 in selected clones.

(G) Normal karyotype was identified in a single knockout clone.

(H) Representative RNA *in situ* hybridization from three coverslips showing absence of *PIEZO2* expression in the knockout iPSCs. The cells retained expression of *TRPM8* and *TUBB3* that are characteristic of iSNs. Scale bars, 10 μm.

Table S1. Summary of DRG Donors, Related to Figure 2

Donor	Age (yr.)	Gender	Ancestry	Cause of death
1	42	Female	European	Chronic inflammatory demyelinating polyneuropathy
2	48	Male	African	Congestive heart failure
3	41	Male	European	Atherosclerosis
4	57	Female	Asian	Stroke

Supplemental Table S2. STR Analysis of P2 and $P2^C$ iPSCs, Related to Figure 6

STR locus	P2	$P2^C$ clone 1	PS^C clone 2
FGA	18, 21	18, 21	18, 21
TPOX	8, 11	8, 11	8, 11
D8S1179	11, 15	11, 15	11, 15
vWA	16, 17	16, 17	16, 17
Amelogenin	X, X	X, X	X, X
Penta_D	9, 13	9, 13	9, 13
CSF1PO	10, 11	10, 11	10, 11
D16S539	11, 12	11, 12	11, 12
D7S820	10, 12	10, 12	10, 12
D13S317	8, 13	8, 13	8, 13
D5S818	12, 13	12, 13	12, 13
Penta_E	5, 12	5, 12	5, 12
D18S51	16, 22	16, 16	16, 16
D21S11	27, 32.2	27, 32.2	27, 32.2
TH01	7, 9.3	7, 9.3	7, 9.3
D3S1358	16, 16	16, 16	16, 16

D18S51, duplicated STR on chromosome 18.