Supplemental Materials

Molecular Biology of the Cell

Battaglia *et al*.

Supplemental Tables

Table S1: Summary of off-target Sanger sequencing from CRISPR/Cas9 editing of Patient 7

Gene name	Gene ID	Parental	Clone B3	Clone G5	Clone 2D1	Clone 2D3
ATXN2L	11273	Homozygous: T>A (chr.16, 28832748)	Same as parental	Same as parental	Same as parental	Same as parental
BNIP3L	665	N.M.	N.M.	N.M.	N.M.	N.M.
CLRN1-AS1	116933	N.M.	N.M.	N.M.	N.M.	N.M.
URI1	8725	N.M.	N.M.	N.M.	N.M.	N.M.
SCN9A	6335	Heterozygous: A>G, G>A (chr.2, 166303519, 166303436)	Same as parental	Same as parental	Same as parental	Same as parental
COL19A1	1310	N.M.	N.M.	N.M.	N.M.	N.M.
RFLNB	359845	N.M.	N.M.	N.M.	N.M.	N.M.
CSN1S2AP	286828	N.M.	N.M.	N.M.	N.M.	N.M.
PDZD2	23037	N.M.	N.M.	N.M.	N.M.	N.M.
PNLIPRP2	5408	Heterozygous: G>C (chr. 10, 116624046)	Same as parental	Same as parental	Same as parental	Same as parental
DIP2A	23181	N.M.	N.M.	N.M.	N.M.	N.M.
EGFEM1P	93556	N.M.	N.M.	N.M.	N.M.	N.M.
GINS2	51659	N.M.	N.M.	N.M.	N.M.	N.M.
SCN3A	6328	N.M.	N.M.	N.M.	N.M.	N.M.
SGMS1	259230	N.M.	N.M.	N.M.	N.M.	N.M.
RUBCNL	80183	N.M.	N.M.	N.M.	N.M.	N.M.
c11orf58	10944	N.M.	N.M.	N.M.	N.M.	N.M.
BTN1A1	696	N.M.	N.M.	N.M.	N.M.	N.M.
USP50	373509	N.M.	N.M.	N.M.	N.M.	N.M.
IRF4	3662	N.M.	N.M.	N.M.	N.M.	N.M.

*N.M. = no mutations

Table S2: GAN (*KLHL16*) qRT-PCR primers

Primer Name	Target	Sequence (5' → 3')
GAN_exon2-3_F	*GAN*	TCGGTAATGGTTATGAGAGAGATCC
GAN_exon2-3_R	*GAN*	TAAGGTCCGTCAGTAGCAGC
GAN_exon10-11_F	*GAN*	CCGCCAGTTCCTCTTTTGTT
GAN_exon10-11_R	*GAN*	GTCGGATGGAAGGAGTGGTTT

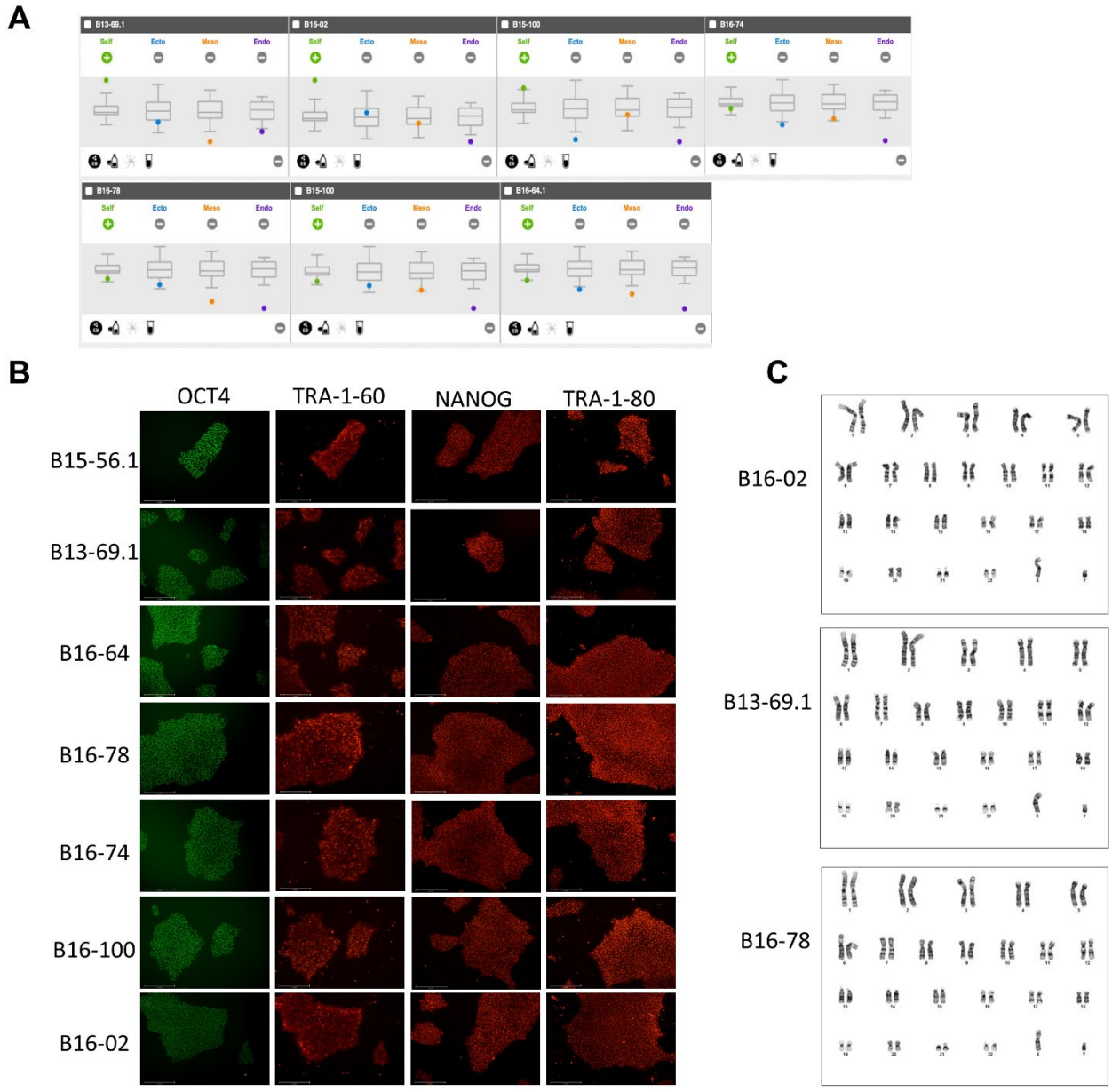
Table S3: *KLHL* gene family primers

Primer Name	5' --> 3' Sequence
hsKLHL1_F	AAACTCTTCAGCCACCCGTC
hsKLHL1_R	AGAAAGTGCTCACACCGCTT
hsKLHL2_F	CTGCCAACAGAGCAGCGTAT
hsKLHL2_R	CCGGATAGCCTTTGGTGCTT
hsKLHL3_F	GGAGGGTGAAAGTGTCAAGC
hsKLHL3_R	AACATCGCACAGAAGTAGGGG
hsKLHL4_F	TGCCCTCTTGGAGACAAACTC
hsKLHL4_R	AGGGTAGCTTCACCACTACA
hsKLHL5_F	GTGGCTGTACTGGAAGGTCC
hsKLHL5_R	GCCTGAGGGTCCCATCTTTC
hsKLHL6_F	CTCTTCCAGTTCCTGCGGAT
hsKLHL6_R	ACGGGCAGGTCAAGAAACTC
hsKLHL7_F	GAAACTGGGTCCTCCGACAC
hsKLHL7_R	CATTGGCTTCCACCATCCCA
hsKLHL8_F	CTGAAGCCAAGCAAACGCT
hsKLHL8_R	CAACAAGCTCTAGCCACCAGT
hsKLHL9_F	GCAGGAACCACACGCTTTTT
hsKLHL9_R	TTCCACCTGTGAACATGGCT
hsKLHL10_F	GTGCCTATCACACCGGACAA
hsKLHL10_R	ACCCCTGACGATACCCATGA
hsKLHL11_F	GCCTACCGATATTGTGCGGA
hsKLHL11_R	CGTGACAAGCAGTGGCTCTA
hsKLHL12_F	GTGGAGCTTTTTGCCAAGCA
hsKLHL12_R	ACTGAACTAAGGCGGGAACG
hsKLHL13_F	ATGGTGTGAGCAAAGTCGGT
hsKLHL13_R	TAGGTGTTGGCAATCCGTCC
hsKLHL14_F	TTGGGTGGAGAGGACCAGTG
hsKLHL14_R	TTTCCTGCATGGGTGGAAGTT
hsKLHL15_F	CGTGTTTGACCCCAGCAAAG
hsKLHL15_R	CACAGACACCACCGAAGACA
hsKLHL17_F	CCTCCAACTGCCTGGGTATC
hsKLHL17_R	TCCAGAACCTGTTTCAGGGGC
hsKLHL18_F	ATGTGTGCTGTGCTGTACGA
hsKLHL18_R	CAGGGCCAGGAACTCTTCTG
hsKeap1_F	TCTTCAACCTGTCCCACTGC

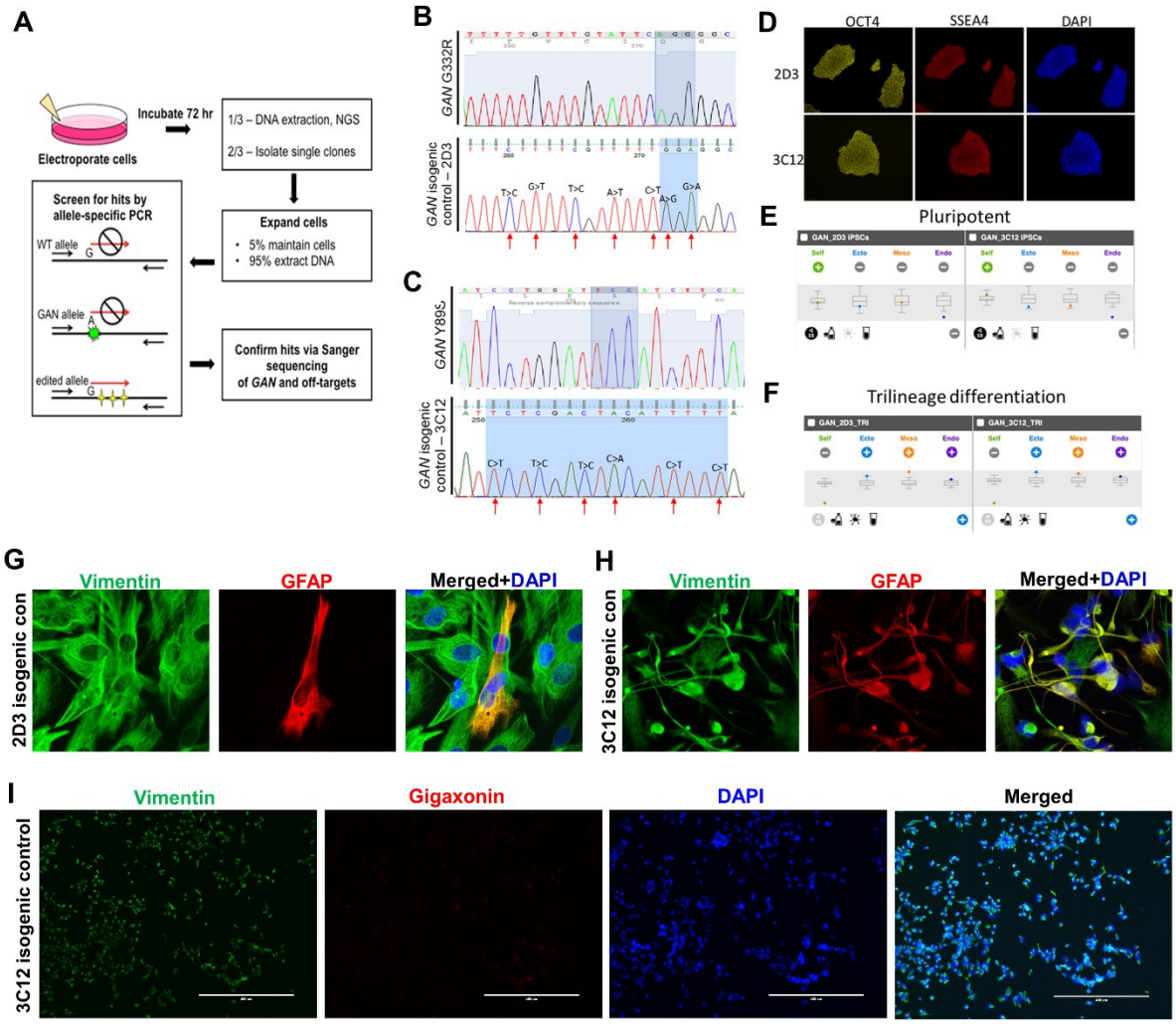
hsKeap1_R	TTCGCAGTCGTACTTGACCC
hsKLHL20_F	AAGCACCCTCGACAAACCTT
hsKLHL20_R	GACGGCTCTCTGCCAATTCT
hsKLHL21_F	CTATGACTGCGTGTGGAGGT
hsKLHL21_R	GGAGTCAGTGGTGTGGTCAT
hsKLHL22_F	TTTGTGGCCTTCTCTCGGAC
hsKLHL22_R	AACCGCACTGTCTCAAGGAG
hsKLHL23_F	GGGGCTACAGGACGGATAAC
hsKLHL23_R	CTTCTGCTGGAGCCCCTTTT
hsKLHL24_F	CTATGTTGCCGGTGGACTGA
hsKLHL24_R	CATTGCAGCTACCCCTGTGA
hsKLHL25_F	TTGCCTACTCCTCACGCATC
hsKLHL25_R	TGCGCCAGGAGAACTCATAC
hsKLHL26_F	CTCCTCGATGTTGTGCTGAC
hsKLHL26_R	CTGGGGAACAGTAGCCTGAAG
hsIPP_F	CTTTGGGTGGATGGGTTGGA
hsIPP_R	CCTTGCATTTCACAGCACCC
hsKLHL28_F	TTCCTACCCTGCTCTGTACC
hsKLHL28_R	CAGAGTTCGTGATGTTGGCG
hsKLHL29_F	TGGAGTTTGTCTACACGGGC
hsKLHL29_R	CGAGAAAGGACACGCAGACT
hsKLHL30_F	CCATGTTTGCGGGTGACTTC
hsKLHL30_R	ACGAAGTCCACCAGTTGTCC
hsKLHL31_F	AGGCGAACAAGAATCCGAGG
hsKLHL31_R	CCGTAAGCTTGCTCCATCCA
hsKLHL32_F	AAGTGGATAAGCCGTAGCCC
hsKLHL32_R	CATTTTGGCCAGTCAGCAGG
hsKLHL33_F	TTTGGAGGGCCAGTTGTACG
hsKLHL33_R	TCAGAAACGTCCCTGGCTTC
hsKLHL34_F	GGAGCAGGTTTGGAGCAAGA
hsKLHL34_R	CAGAGAAGGGCTCGTATCGC
hsKLHL35_F	GGCAAGCTCTTCGTGATTGG
hsKLHL35_R	TCCTTGGGGTCAAAGCACTG
hsKLHL36_F	TGCGGCCTCCAATCTTCTTT
hsKLHL36_R	AGGTAGAAATCCACACGGCG
hsENC1_F	GTGGACCAAGGTGGGAGATG
hsENC1_R	GCATCGCTGAATGCCAAAGT
hsKLHL38_F	AGAACCCTGTGCGCCTTATC

hsKLHL38_R	ATGACAATCCGCTCCCCAAG
hsIVNS1ABP_F	AATCAACTGGGTGCAGCGTA
hsIVNS1ABP_R	AACACCTCAGCCTGTCCATC
hsKLHL40_F	GAGAGATCAAGGACGGCGAG
hsKLHL40_R	GGGTCCGATTCACCCCATTT
hsKLHL41_F	CTTCTTGACTGCCCGAGACT
hsKLHL41_R	CGCACCCATTTCATCACTGC
hsKLHL42_F	TTACAACCCCGAGCAGGATG
hsKLHL42_R	GTTCATGTTCCGGTCTCTGGT

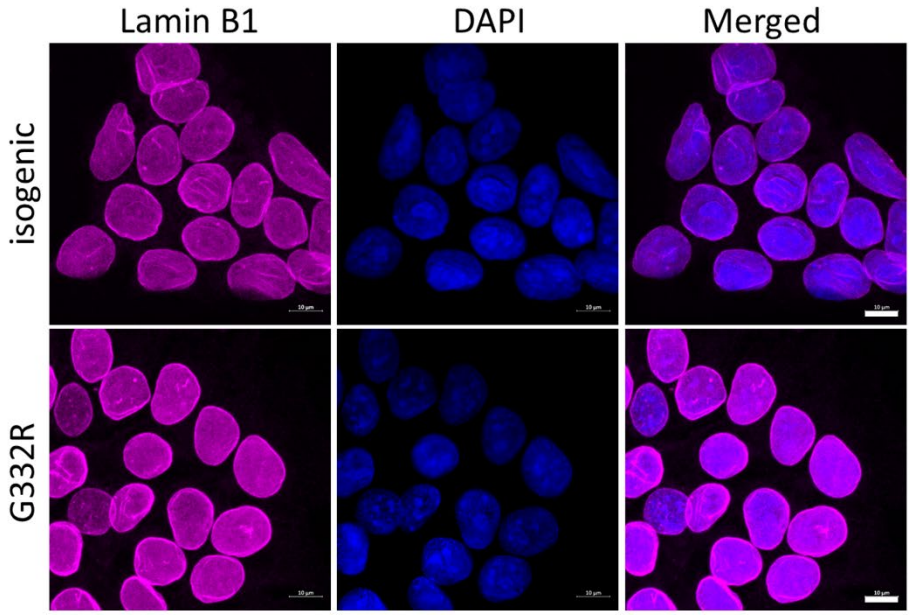
Supplemental Figures



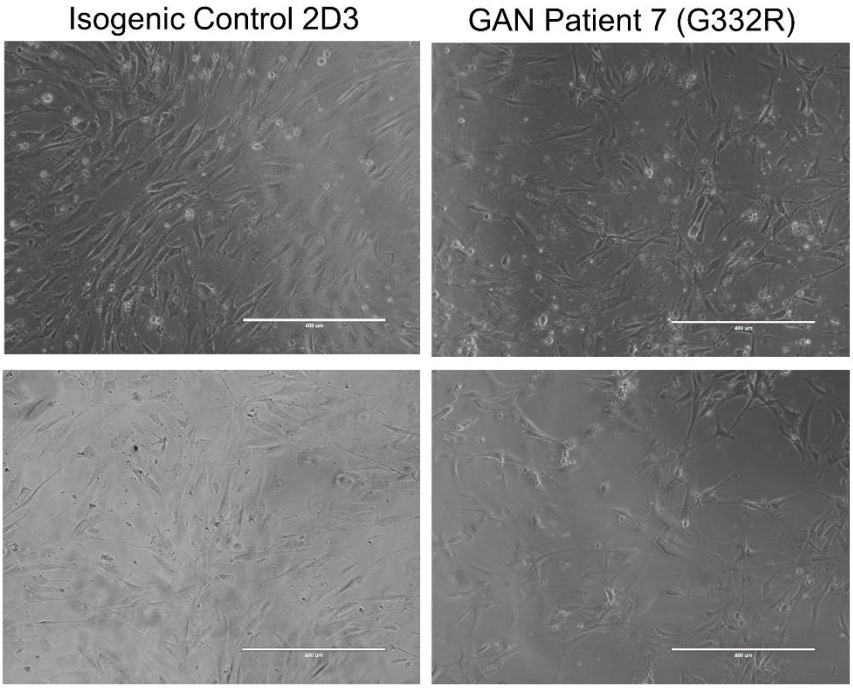
Supplemental Figure 1. Generation of *GAN* iPSCs from patient fibroblasts. **(A)** Pluripotency was assessed via ThermoFisher Taqman hPSC scorecard analysis, which assesses self-renewal and trilineage differentiation potential using real-time qPCR expression analysis. **B)** Immunofluorescence staining for OCT4 (green), TRA-1-60, NANOG, and TRA-1-80 (red) pluripotency markers. **(C)** GAN iPSCs were characterized for genomic stability via karyotyping as shown by representative images from GAN patients 2, 4, and 7 (please also see Figure 1C).



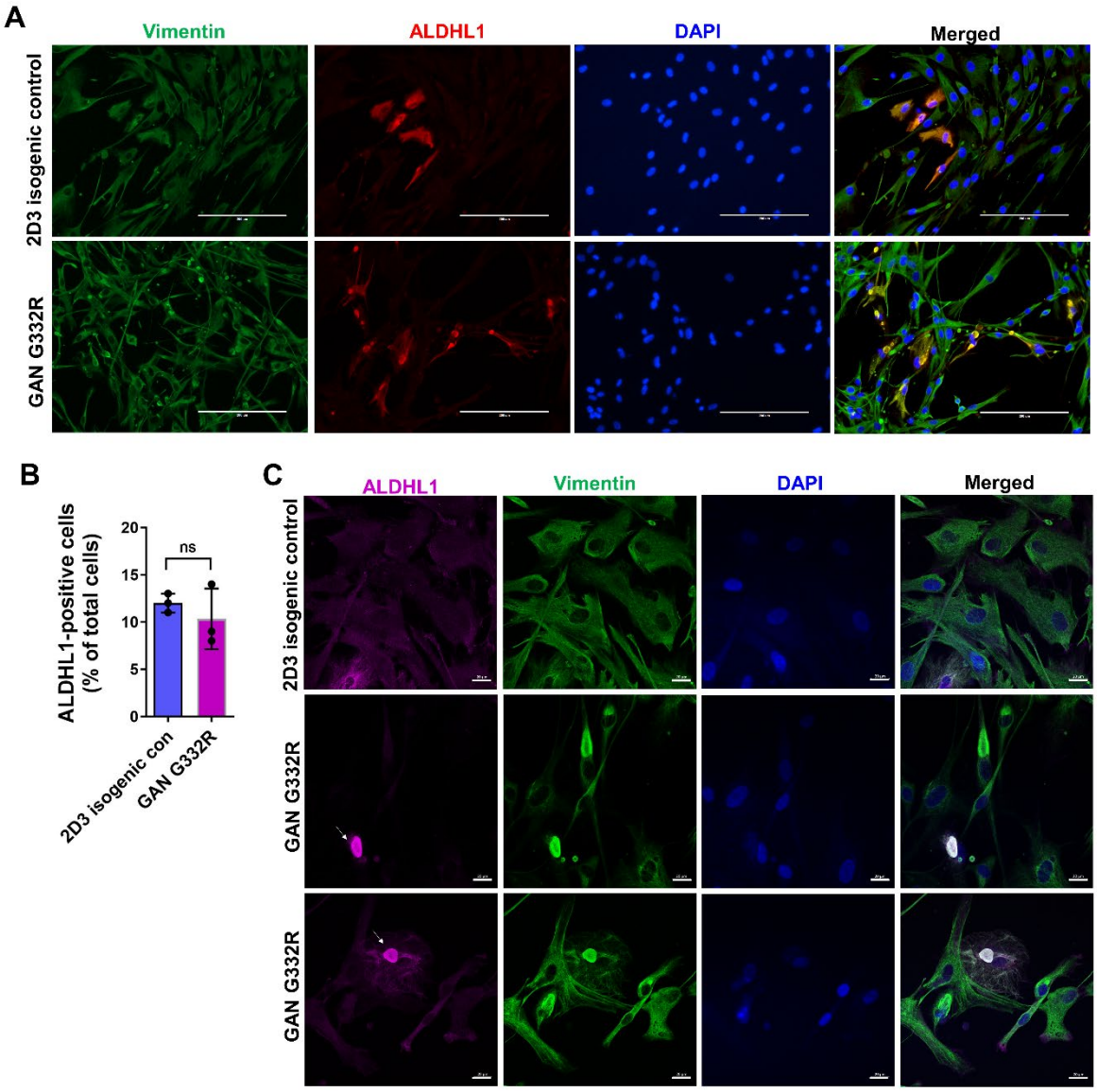
Supplemental Figure 2. Correction of *KLHL16* mutations via CRISPR/Cas9 gene editing. **(A)** Gene editing strategy for GAN Patient 7 iPSCs. The G is the wild-type allele, the green shape represents the GAN mutation (G>A), and the yellow stars represent the silent mutations introduced by the repair construct. Black arrows depict universal primers, whereas the red arrow is an allele specific primer that exclusively binds to the corrected sequence. **(B-C)** Chromatograms display the original GAN mutant sequence (G332R, GGG>AGG; Y89S, TAC>TCC) and the sequence from a corrected clone where silent mutations are indicated by red arrows. **(D)** Immunofluorescence staining for pluripotency markers OCT4 and SSEA4 expression in isogenic control lines 2D3 and 3C12. Pluripotency **(E)** and trilineage differentiation **(F)** were assessed in clone 2D3 via ThermoFisher Taqman scorecard analysis. **(G)** Immunofluorescence analysis of IF proteins vimentin (green) and GFAP (red) in isogenic clone 2D3 iPSC-astrocytes from patient 7 (correction of G332R mutation). **(H)** Immunofluorescence analysis of IF proteins vimentin (green) and GFAP (red) in isogenic clone 3C12 iPSC-astrocytes from patient 2 (correction of the Y89S mutation). **(I)** Immunofluorescence analysis of vimentin (green), gigaxonin (red) and DAPI (blue) in isogenic clone 3C12 iPSC-astrocytes from patient 2.



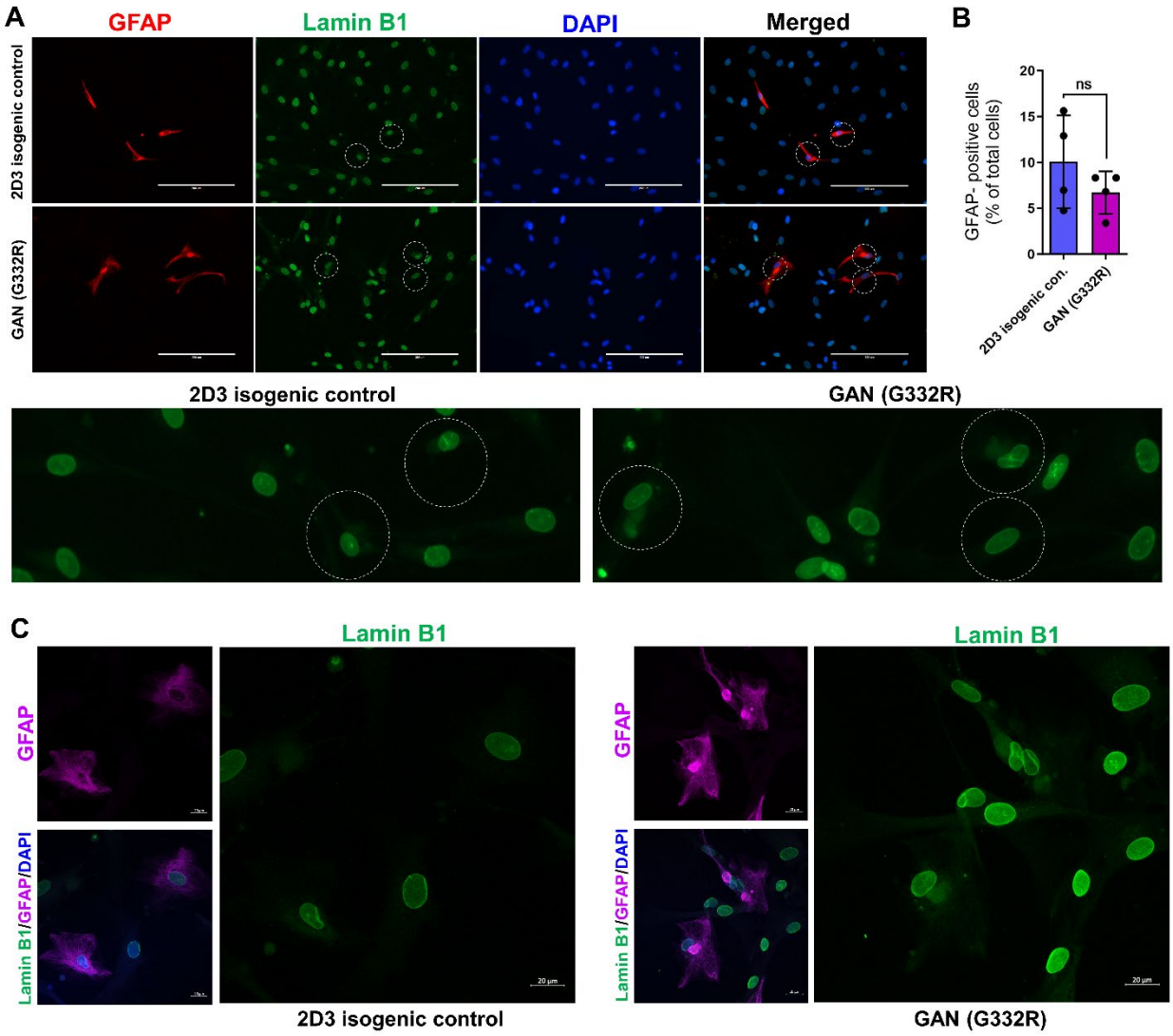
Supplemental Figure 3. Normal lamin B1 organization in GAN iPSCs. Confocal imaging of lamin B1 (magenta) and DAPI (blue) in GAN patient 7 (G332R) and corresponding isogenic control iPSCs (clone 2D3). Scale bars=10μm.



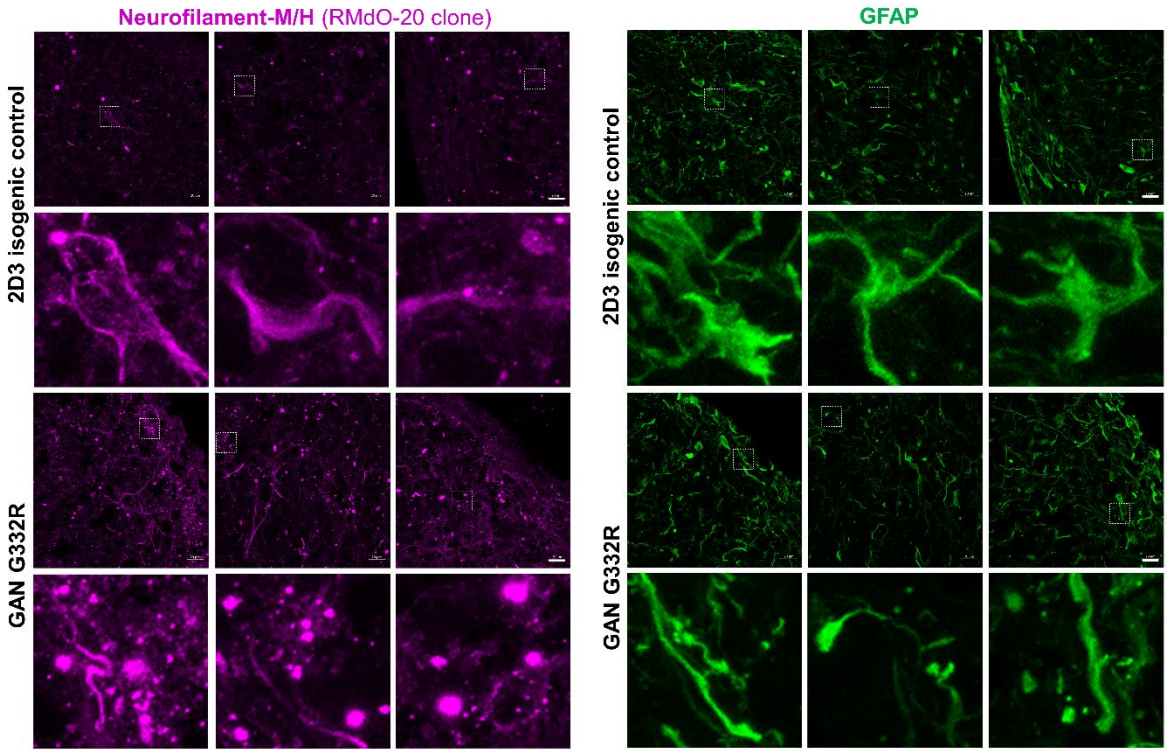
Supplemental Figure 4. Morphology of isogenic control and GAN iPSC-astrocytes in culture. Bright field images of isogenic control 2D3 (left panels) and unedited line from GAN patient 7 (G332R) iPSC-astrocytes at day 60 of the differentiation protocol outlined in Fig.4A.



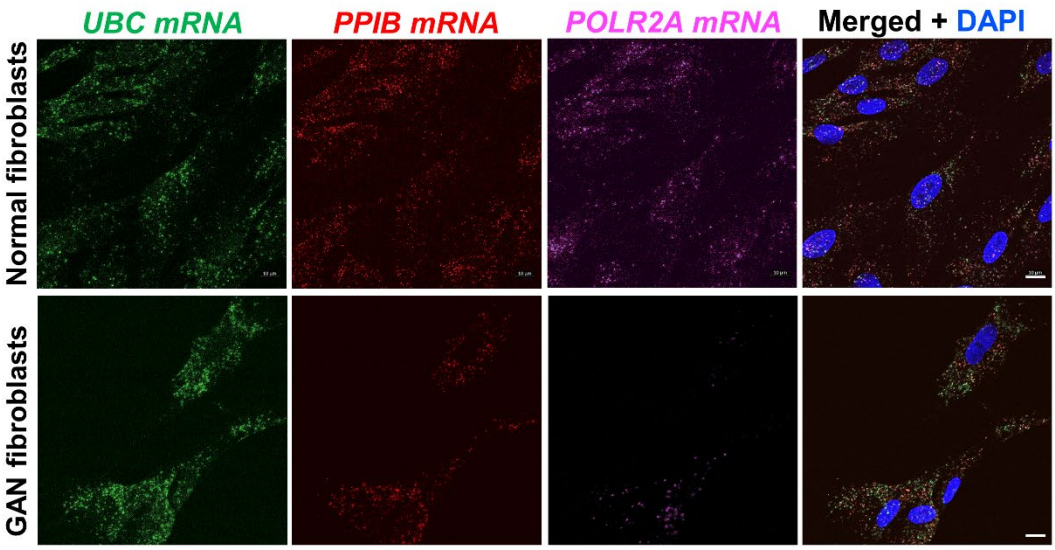
Supplemental Figure 5. ALDHL1 expression in GAN iPSC-astrocytes. **(A)** GAN patient 7 (G332R) and corresponding isogenic control (clone 2D3) iPSC-astrocytes were stained for ALDHL1 (red), vimentin (green), and DAPI (blue) Scale bars=200 µm. **(B)** Quantification of ALDHL1-positive cells as a percentage of total cells from 3 representative fields of view (n=136 WT; n=216 G332R total cells counted). n.s.=not significant; unpaired t-test. **(C)** GAN patient 7 (G332R) and isogenic control iPSC-astrocytes (clone 2D3) were stained for ALDHL1 (magenta), vimentin (green) and DAPI (blue). Scale bars=10 µm. Arrows highlight ALDHL1-positive aggregates in some GAN astrocytes.



Supplemental Figure 6. GFAP and Lamin B1 in GAN and 2D3 isogenic control iPSC-astrocytes. **(A)** 2D3 isogenic iPSC-astrocytes stained for GFAP, Lamin B1 and DAPI. Scale bars=200µm. Lower panels show magnified areas of Lamin B1 staining in GFAP positive cells (circles). **(B)** Quantification of GFAP-positive cells as a percentage of total cells from 4 representative fields of view (n=148 WT; n=199 G332R; total number of cells counted). **(C)** Confocal images of 2D3 isogenic control (left) and GAN G332R iPSC-astrocytes (right) stained for GFAP (magenta), Lamin B1 (green), and DAPI (blue).



Supplemental Figure 7. Neurofilament and GFAP aggregation in GAN brain organoids. Confocal images of immunofluorescence staining for neurofilaments-M/H (magenta) and GFAP (green) in GAN patient 7 (G332R) and corresponding isogenic control brain organoid clone 2D3. Scale bars=20µm. Images in the second and fourth rows show selected magnified areas denoted by the dashed squares from the top and third row images.



Supplemental Figure 8. RNAScope imaging analysis of control mRNAs in normal and GAN fibroblasts. Co-staining of the mRNAs for three control genes: *UBC* (green), *PPIB* (red), *POLR2A* (magenta) and DAPI (blue) in control unaffected human fibroblasts (top) GAN patient fibroblasts (bottom). Scale bars=10μm.