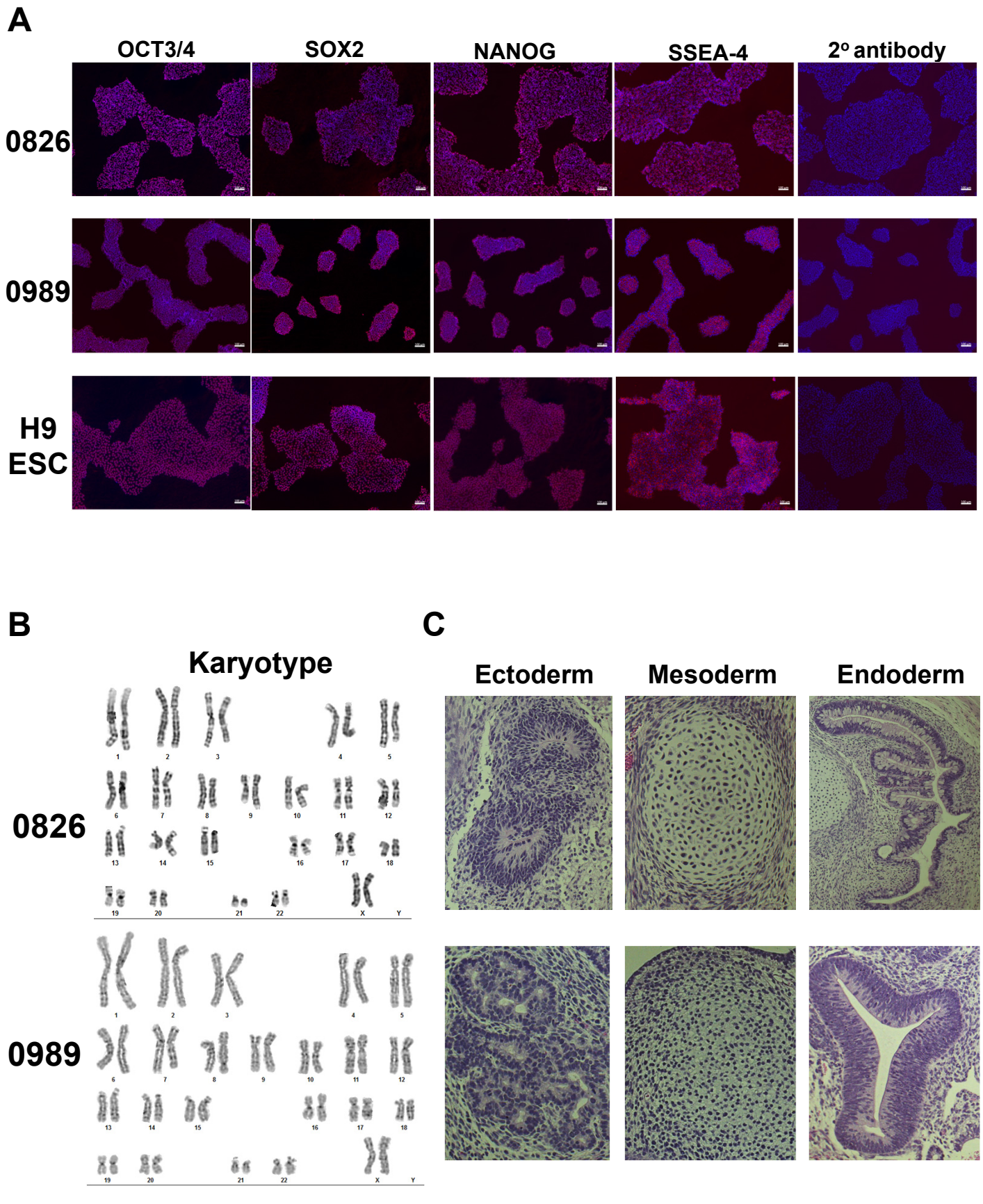
YMTHE, Volume *27*

## Supplemental Information

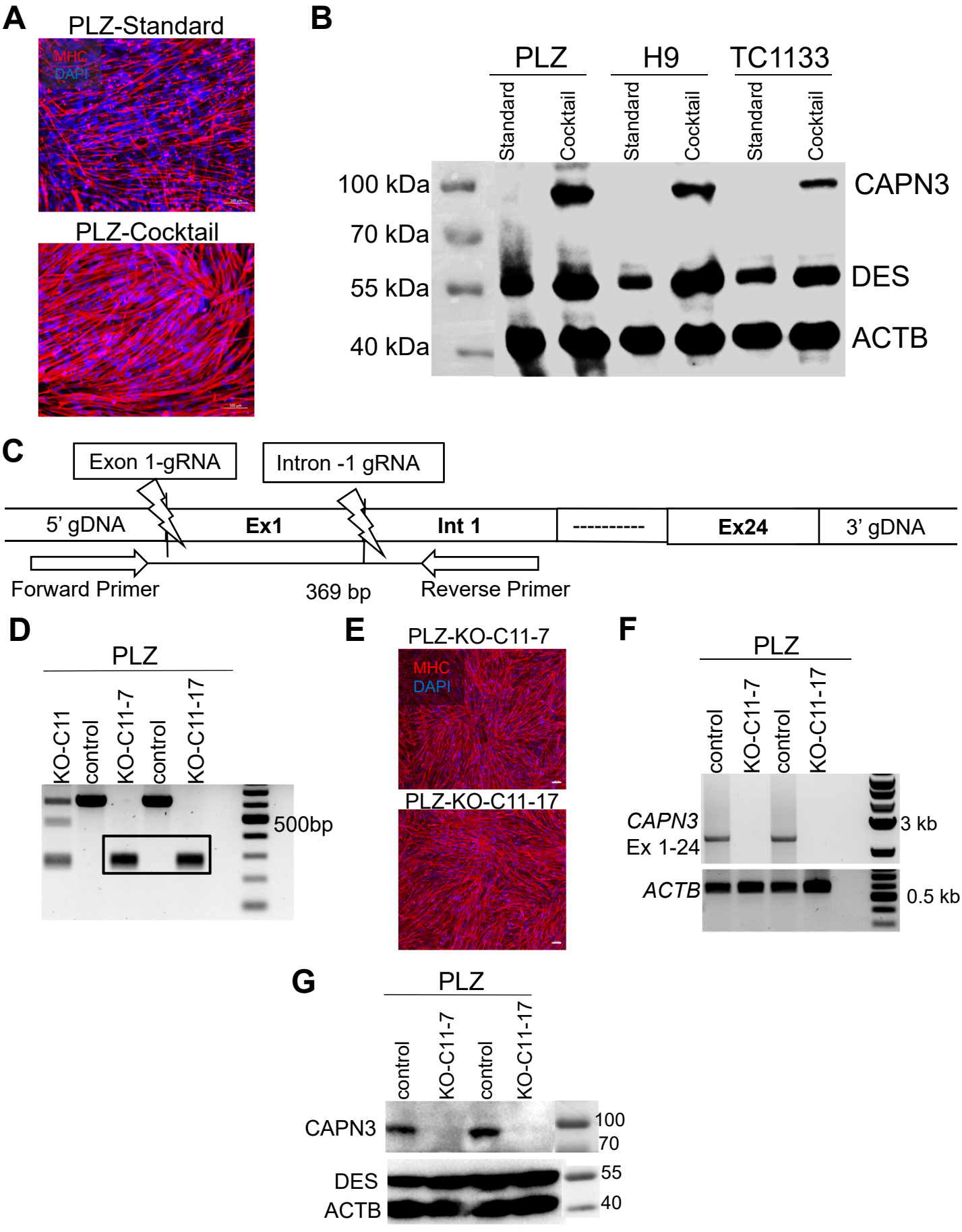
# Gene Correction of LGMD2A Patient-Specific iPSCs for the Development of Targeted Autologous Cell Therapy

**Sridhar Selvaraj, Neha R. Dhoke, James Kiley, Alba Judith Mateos-Aierdi, Sudheer Tungtur, Ricardo Mondragon-Gonzalez, Grace Killeen, Vanessa K.P. Oliveira, Adolfo López de Munain, and Rita C.R. Perlingeiro**

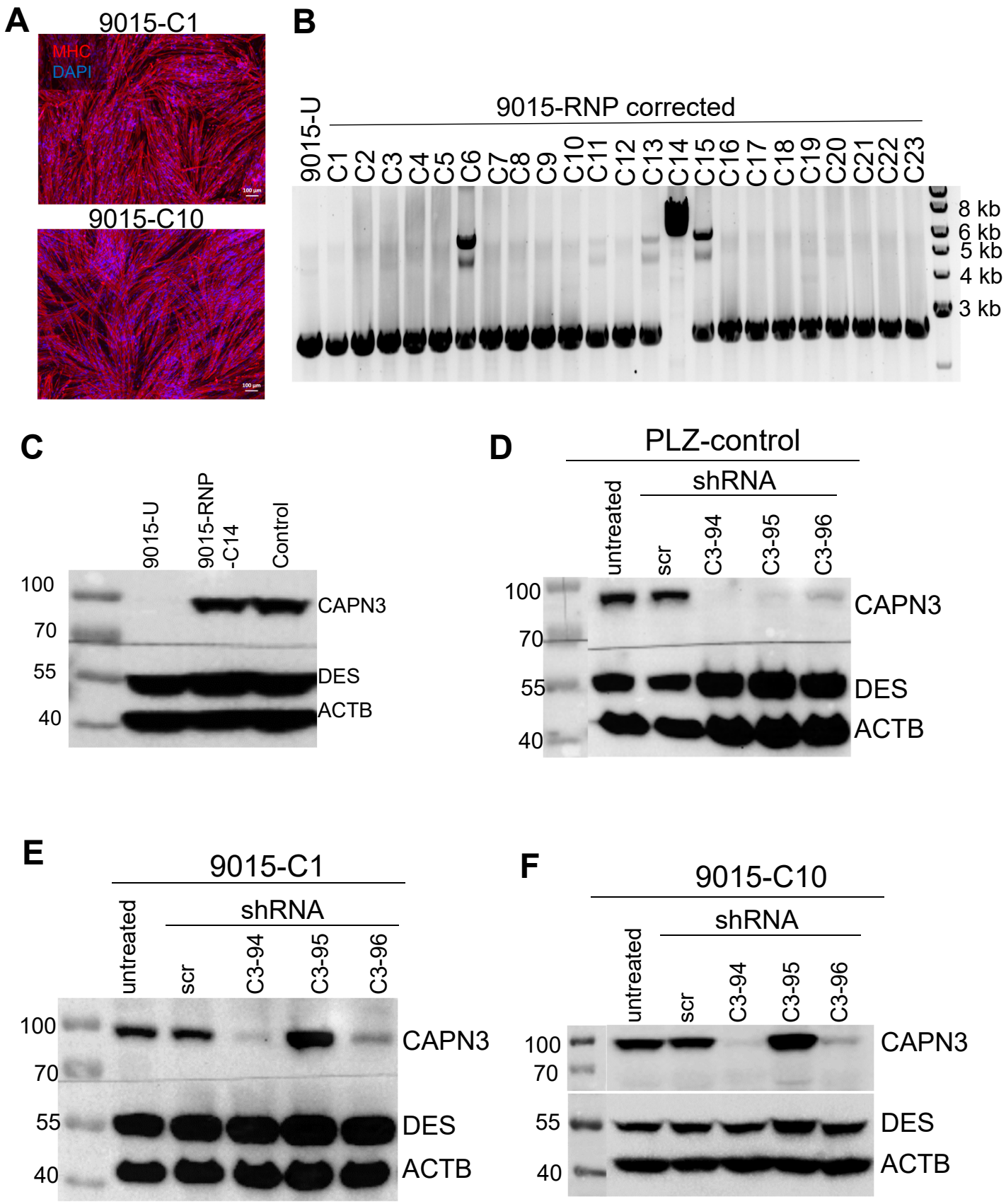
# Supplementary Figure 1



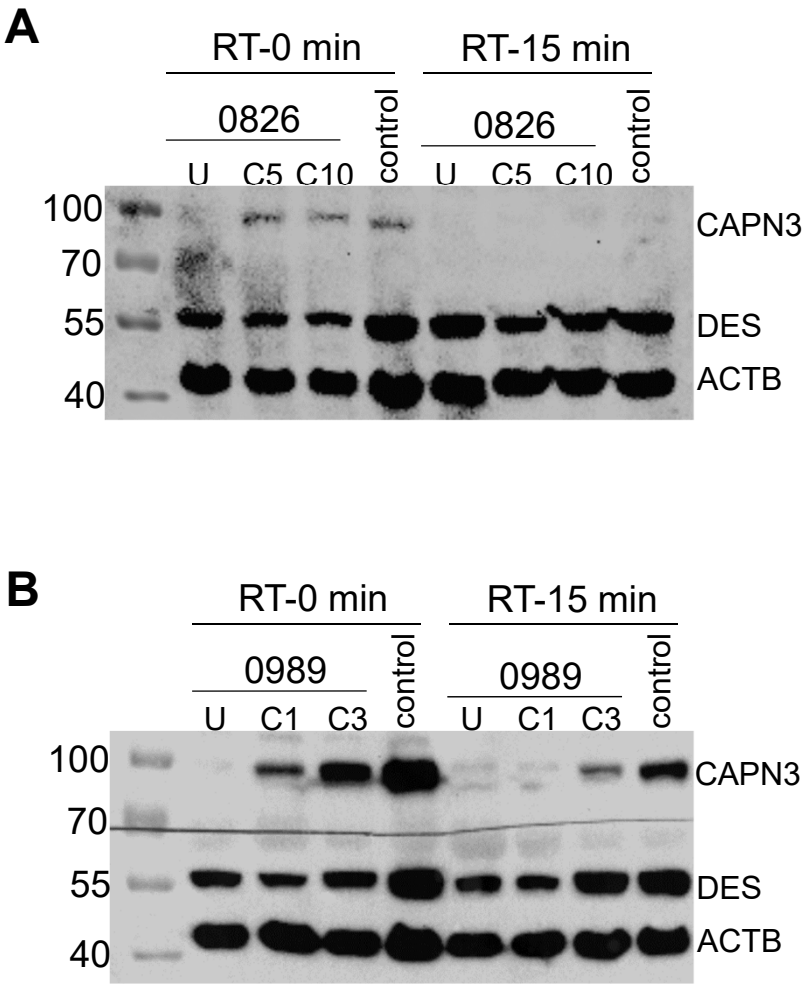
# Supplementary Figure 2



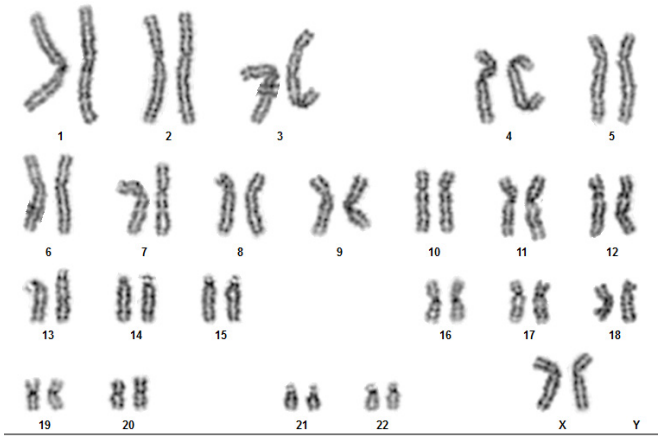
# Supplementary Figure 3



# Supplementary Figure 4



# Supplementary Figure 5



# Supplementary Figure 6

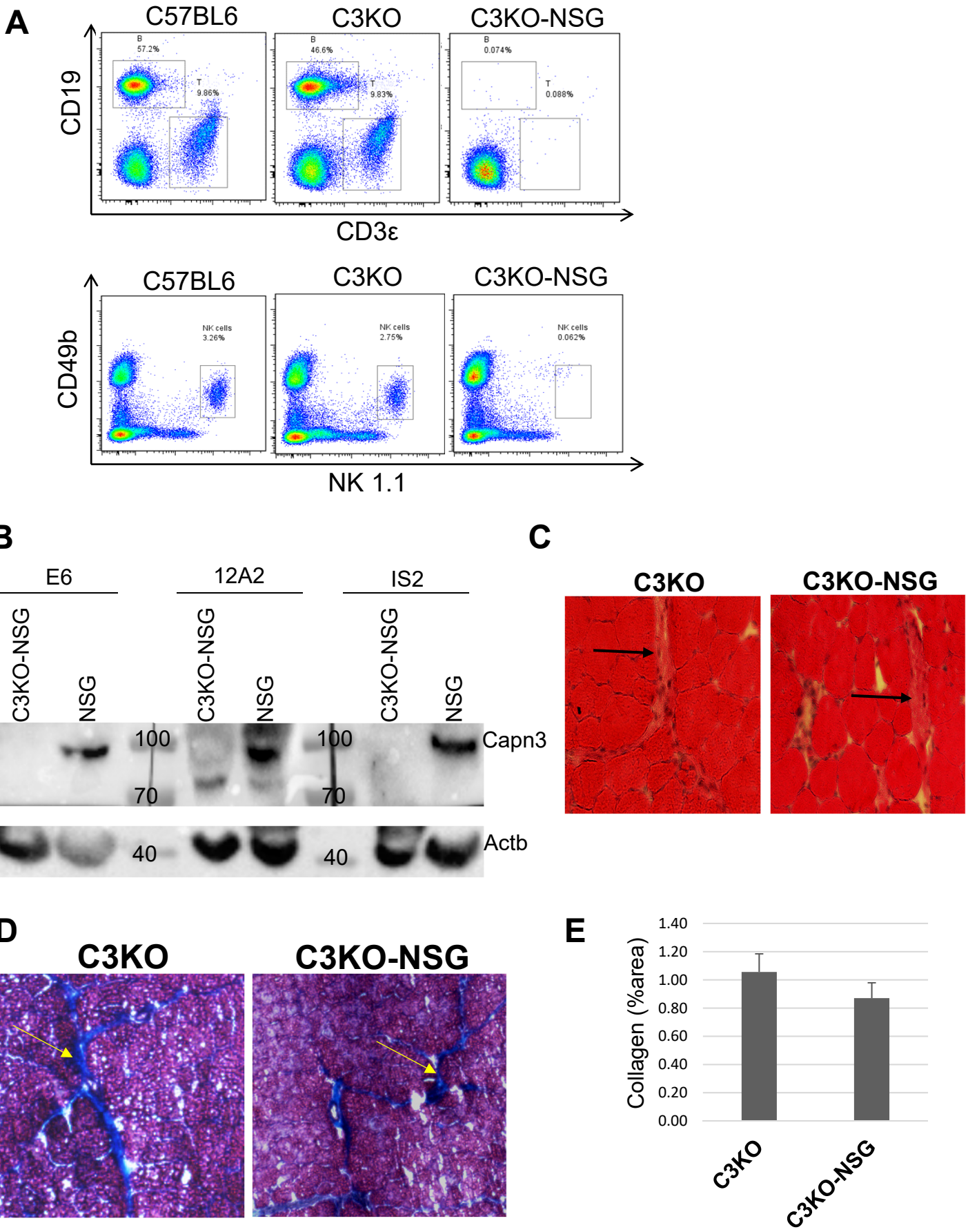
## A

| gRNA-PAM sequence | Gene (chr) | Predicted off-target percentage based on TIDE analysis | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | **9015 -C1** | **9015 -C10** | **0826 -C5** | **0826 -C10** | **0989 -C1** | **0989 -C3** | **0989 -bulk** |
| tATCTgCtTGGATCt GCCAG-AGG | ACRV1 (chr 11) | 0.5% | 4.4% | 2.7% | 0.9% | 1.8% | 2% | 2.4% |
| CcTCTaCGTGGATg tGCCAG-AGG | N/A (chr 2) | 0.5% | 0.3% | 0.3% | 3.5% | 0.7% | 2.5% | 2.9% |
| CcTCTCtGctGATC GGCCAG-TGG | GLIS1 (chr 1) | 3.3% | 2.3% | 1.1% | 0.5% | 1.7% | 0.3% | 1.8% |
| CtTCTCCcTGGcTg GGCCAG-TGG | N/A (chr 3) | 3.7% | 1.5% | 1.2% | 2.2% | 4.5% | 4.4% | 1.5% |
| CAaCTCCagcGATC GGCCAG-AGG | CTNNBIP1 (chr 1) | 2.3% | 1.7% | 1.8% | 1.2% | 2.6% | 2.0% | 5.6% |

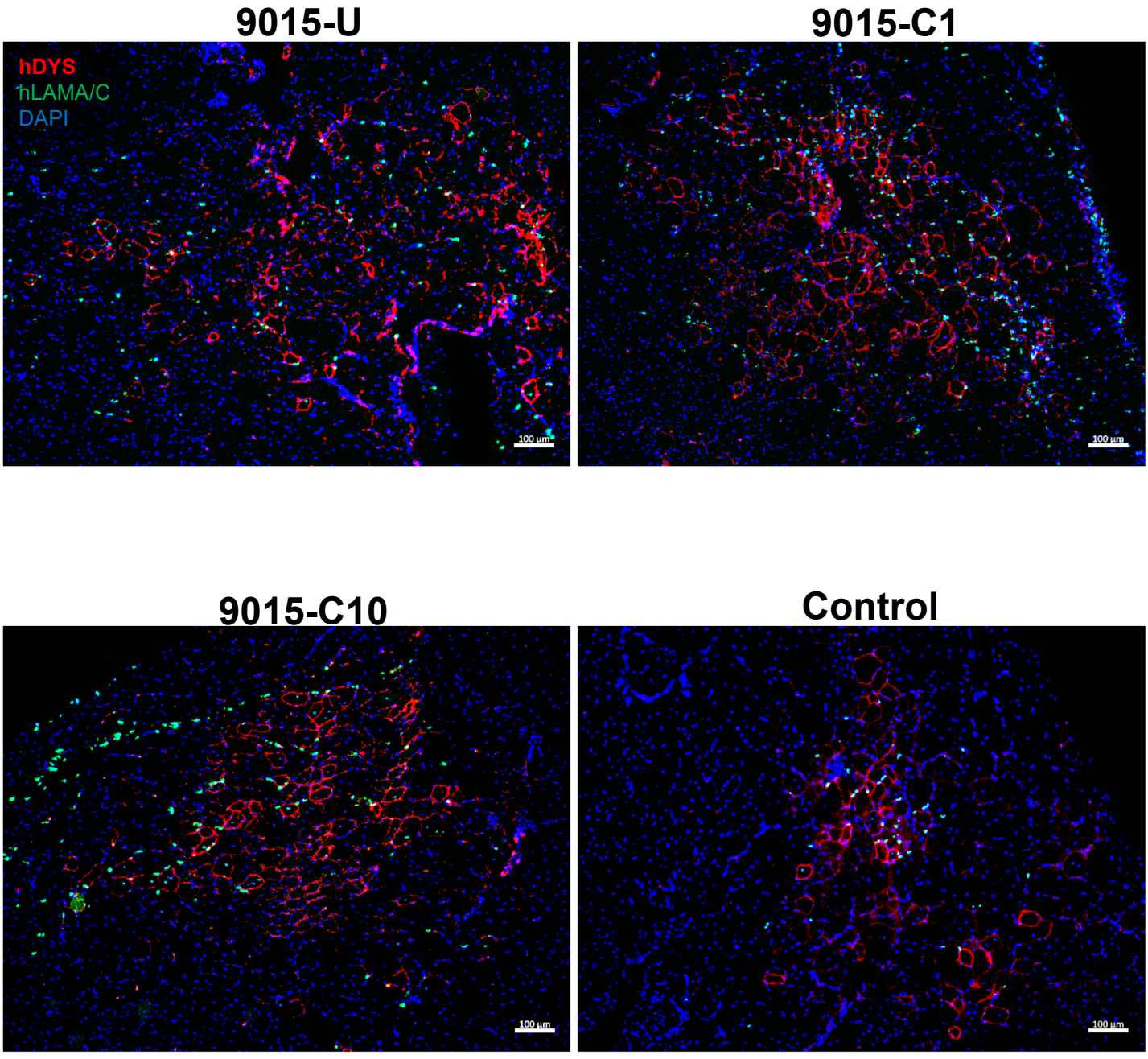
## B

| gRNA-PAM sequence | Gene (chr) | Predicted off-target percentage based on ICE analysis | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | **9015 -C1** | **9015 -C10** | **0826 -C5** | **0826 -C10** | **0989 -C1** | **0989 -C3** | **0989 -bulk** |
| tATCTgCtTGGATCt GCCAG-AGG | ACRV1 (chr 11) | 0% | 3% | 1% | 1% | 0% | 1% | 1% |
| CcTCTaCGTGGATg tGCCAG-AGG | N/A (chr 2) | 0% | 0% | 1% | 1% | 0% | 0% | 0% |
| CcTCTCtGctGATC GGCCAG-TGG | GLIS1 (chr 1) | 1% | 2% | 0% | 0% | 1% | 0% | 0% |
| CtTCTCCcTGGcTg GGCCAG-TGG | N/A (chr 3) | 3% | 1% | 1% | 3% | 5% | 3% | 0% |
| CAaCTCCagcGATC GGCCAG-AGG | CTNNBIP1 (chr 1) | 0% | 1% | 0% | 1% | 0% | 1% | 0% |

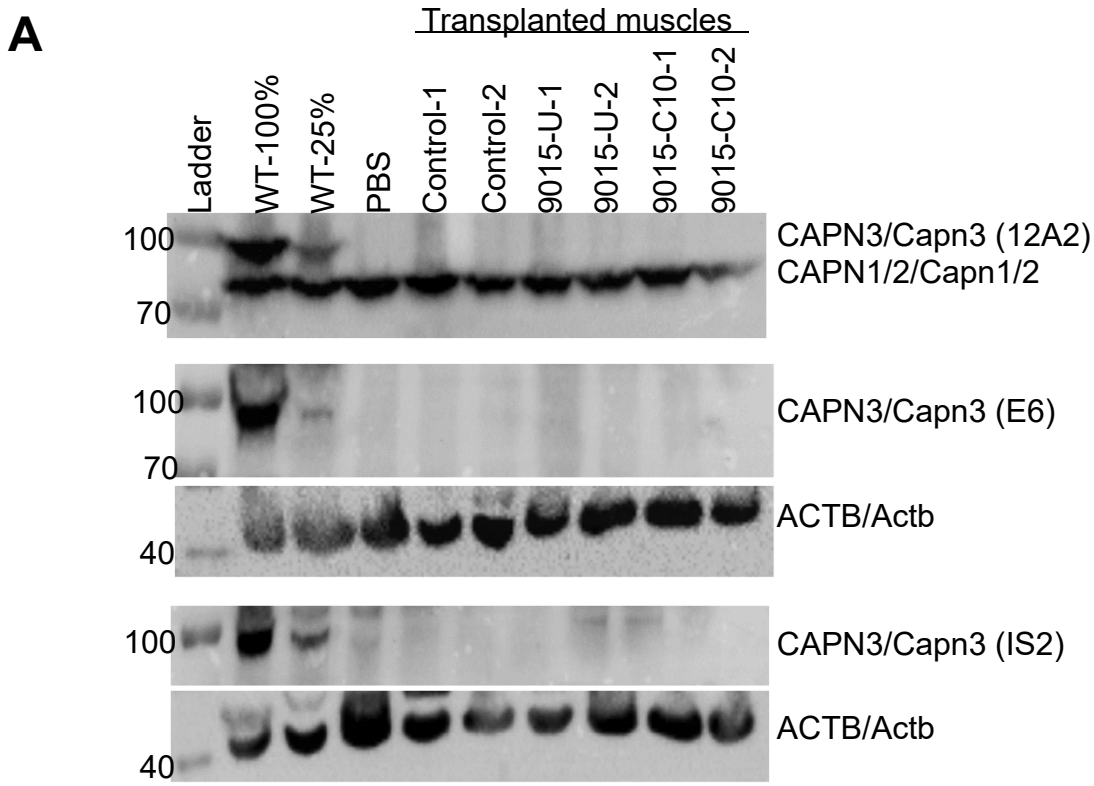
# Supplementary Figure 7

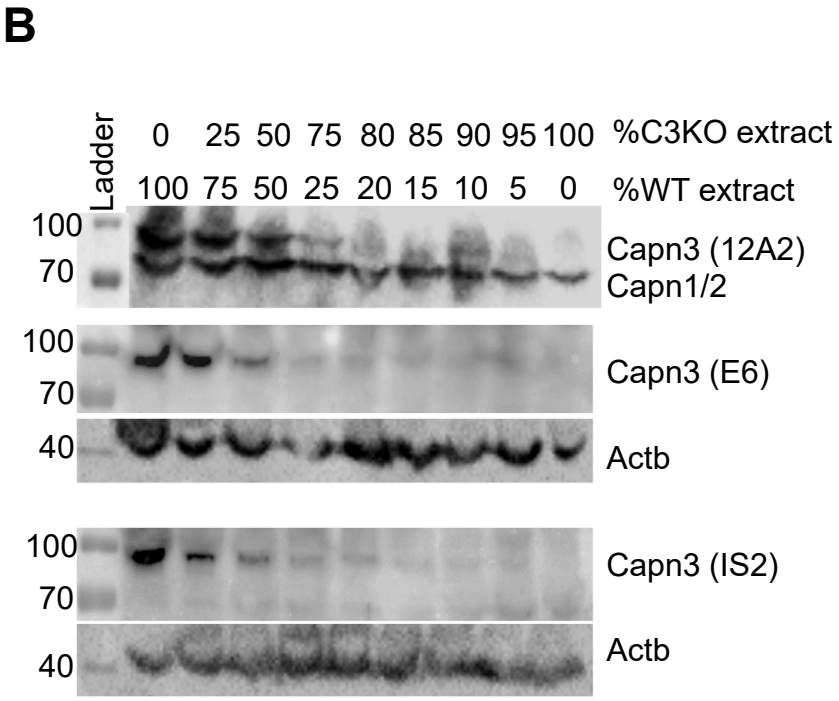


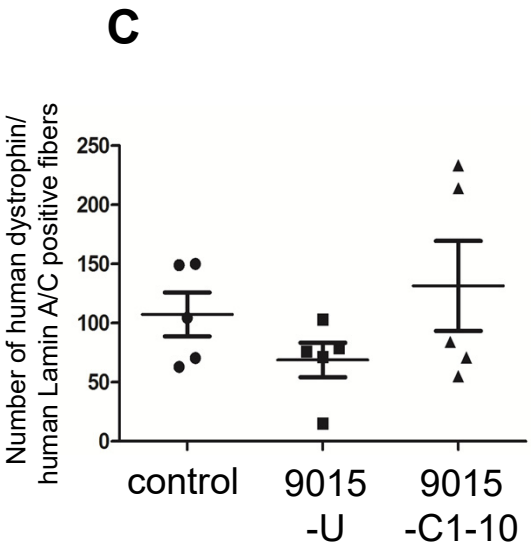
# Supplementary Figure 8



# Supplementary Figure 9



## SUPPLEMENTARY FIGURE LEGENDS

**Supplementary Figure 1. Characterization of pluripotency.** (A) Representative images show staining of LGMD2A patient-specific 0826, 0989 iPS and H9 ES cells for OCT3/4, SOX2, NANOG and SSEA4 (all in red). DAPI stains nuclei (in blue). Bar: 100 μm. Negative control consisted of staining with secondary antibody only. (B) Cytogenetic analyses show normal karyotypes of reprogrammed cell lines. (C) Injection of LGMD2A iPS cells into NSG mice results in teratoma formation. Images show H&E staining of teratomas generated by reprogrammed 0826 and 0989 iPS cell lines showing the presence of tissues derived from all three germ layers.

**Supplementary Figure 2. *In vitro* detection of CAPN3 and generation of CAPN3 knockout iPS cell line.** (A) Staining for MHC (in red) in iPS cell-derived myotubes shows superior differentiation and fusion upon cocktail treatment. DAPI stains nuclei (in blue). Bar: 100 μm. (B) Western blot shows evident detection of CAPN3 only in cocktail-treated samples in myotubes from control iPS cells (PLZ and TC-1133) and H9 embryonic stem cells. (C) Schematic representation of strategy used to generate a CAPN3 knockout, in which guide RNA pairs were designed to target Exon 1 and Intron 1, resulting in a loss of 369 bp region. (D) PCR amplification of region spanning the deletion in clone 11 and respective subclones C11-7 and 11-17. (E) Representative images show staining for MHC (in red) in CAPN3 knockout iPS cell-derived myotubes (C11-7 and 11-17). DAPI stains nuclei (in blue). Bar: 100 μm. (F-G) Lack of CAPN3 expression was confirmed by RT-PCR (F) and western blot (G) in myotubes derived from CAPN3 knockout iPS cell lines (C11-7 and 11-17).

**Supplementary Figure 3. CRISPR-Cas9 RNP-based gene correction and shRNA CAPN3 knockdown.** (A) Representative images showing MHC (in red) staining in gene corrected 9015 (C1 and10) iPS cell-derived myotubes. DAPI stains nuclei (in blue). Bar: 100 μm. (B) PCR shows amplification of the region spanning knock-in in 9015 iPS cells that had been gene corrected using CRISPR-Cas9 RNP based genome editing. (C) Western blot shows rescue of CAPN3 protein in RNP gene corrected 9015 iPS cell-derived myotubes (C14). DES and ACTB were used as the differentiation and loading controls, respectively. (D-F) shRNA knockdown and western blot analysis for CAPN3 in control (PLZ, D), 9015 corrected C1 (E) and C10 (F) iPS cell-derived myotubes. Untreated denotes no treatment, Scr denotes scrambled shRNA, C3-94, 95, 96 denote the CAPN3 shRNA (clone id: TRCN0000003494-exon 1, TRCN0000003495-3'UTR and TRCN0000003496-exon 22 respectively).

**Supplementary Figure 4. Autocatalytic assay for CAPN3.** (A-B) Autocatalytic activity of CAPN3 is shown by western blot analysis in 0826 (A) and 0989 (B) iPS cell-derived myotubes. DES and ACTB were used as the differentiation and loading controls respectively.

**Supplementary Figure 5. Karyotype analysis.** Karyotype of 9015 gene corrected (C10) iPS cells.

**Supplementary Figure 6. CRISPR-Cas9 off-target percentage as determined by sequencing followed by TIDE and ICE analysis.** The off-target percentages for gene corrected iPS clones from 9015 (C1, C10), 0826 (C5, C10), 0989 (C1 and C3) and the bulk gene corrected 0989 iPS cells are shown, following TIDE (A) and ICE (B) analyses. The off-target gRNA sequence,

chromosome location (in brackets), and the gene names are listed along with corresponding off-target percentage. The lowercase in the gRNA sequence denotes the mismatches in comparison with the on-target gRNA sequence.

**Supplementary Figure 7. Characterization of immunodeficient CAPN3 knockout mouse model.** (A) FACS profile of circulating NK (CD49b/NK1.1), T (CD3e) and B (CD19) cells from C57BL6, CAPN3 knockout (C3KO) and immunodeficient CAPN3 knockout (C3KO-NSG). (B) Western blot for CAPN3 on TA muscles from C3KO-NSG and NSG mice. The E6 and IS2 antibodies detect CAPN3 only, whereas the 12A2 antibody detects CAPN1/2 and CAPN3 proteins. Actb was used as loading control. (C-D) Representative images show H&E (C) and Masson's trichrome (D) staining of TA muscles from C3KO and C3KO-NSG mice. Muscles show some signs of fibrosis, as indicated by the black arrows in (C) and by the collagen deposition in blue (D; yellow arrows). (E) Quantification of the % area of collagen staining in C3KO and C3KO-NSG mice at 8-12 weeks of age. Data are shown as mean of seven independent replicates ±S.E.M.

**Supplementary Figure 8. Engraftment of human iPS cell-derived myogenic progenitors in C3KO-NSG mice.** Representative images show immunofluorescence staining for human DYSTROPHIN (DYS in red) and LAMIN A/C (LAM A/C in green) in TA muscles that had been transplanted myogenic progenitors from corrected (C1, C10), uncorrected (U) 9015 iPS cells, or control iPS cells. DAPI stains nuclei (in blue). Bar: 100 μm. Lower magnification images of transplanted muscles show the area of engraftment as well as surrounding non-engrafted areas.

**Supplementary Figure 9. Western blot for CAPN3 in transplanted muscles and engraftment quantification.** (A) Western blot shows lack of CAPN3 protein expression in C3KO-NSG muscles transplanted with control or corrected (9015-C10) iPS cell-derived myogenic progenitors. Positive control consisted of NSG and NSG/C3KO-NSG muscle lysates combined at a ratio of 25% and 75%, respectively. Negative controls consisted of C3KO-NSG muscles injected with PBS or transplanted with uncorrected (9015-U) iPS cell-derived myogenic progenitors. ACTB/Actb was used as the loading control. (B) Assessment of sensitivity for the detection of CAPN3 by western blot. Total protein extracts from skeletal muscles of WT NSG and C3KO-NSG mice were mixed at different percentages, and assessed by western blot using three different antibodies (12A2, IS2 and E6). Western blot shows that the limit of detection for CAPN3 is approximately 20% of WT-extract. ACTB/Actb was used as the loading control. (C) Column scatter plot shows the total number of donor-derived myofibers in TA muscles that had been transplanted with control (PLZ), uncorrected (9015-U) and corrected (9015-C1-10) iPS cell-derived myogenic progenitors, quantified based on double expression for human DYSTROPHIN and human LAMIN A/C. Data are shown as mean of five independent replicates ±S.E.M.