Supplementary Information for:

# CHAMP1 is an essential regulator for human myoblast fusion and muscle development

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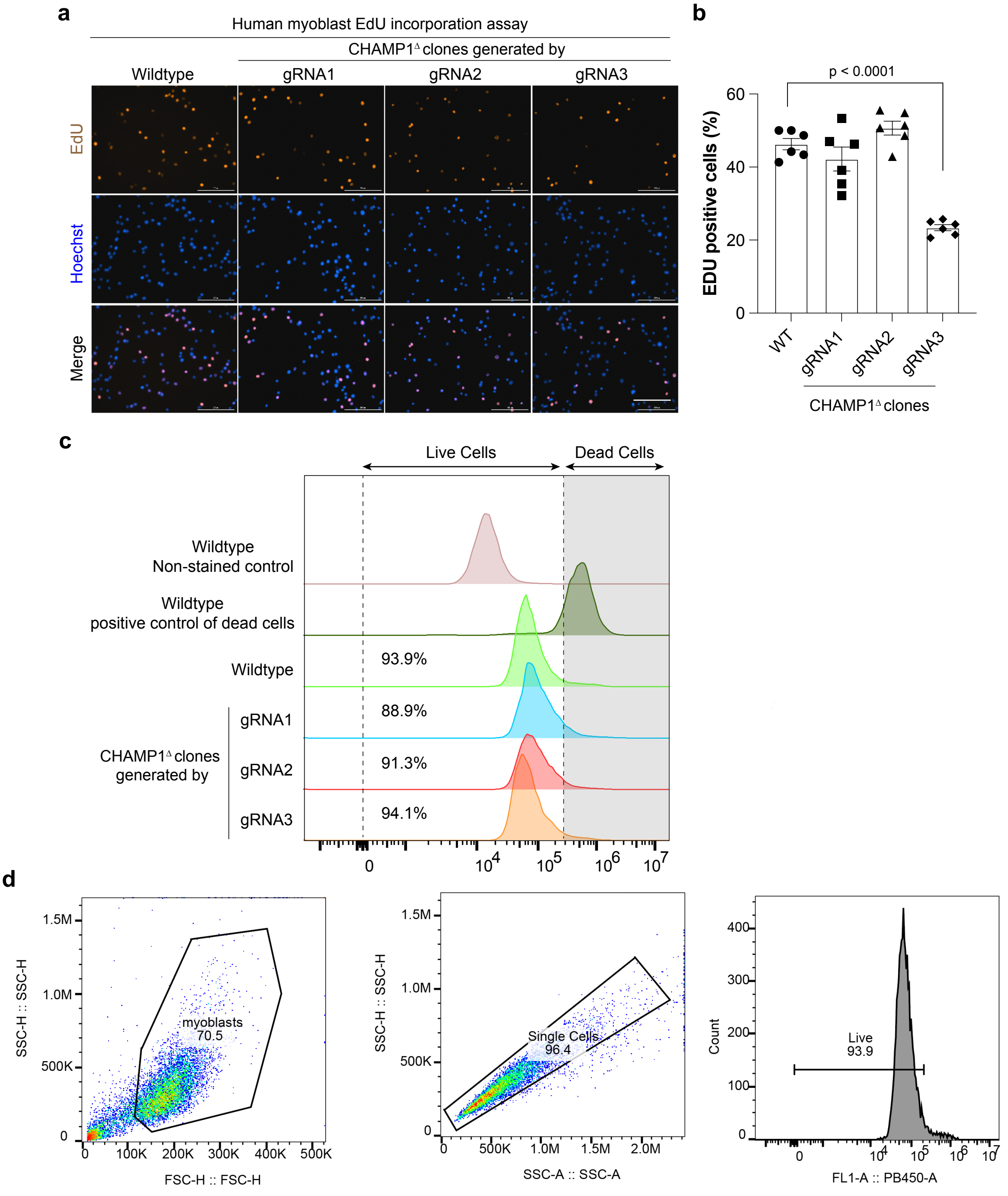
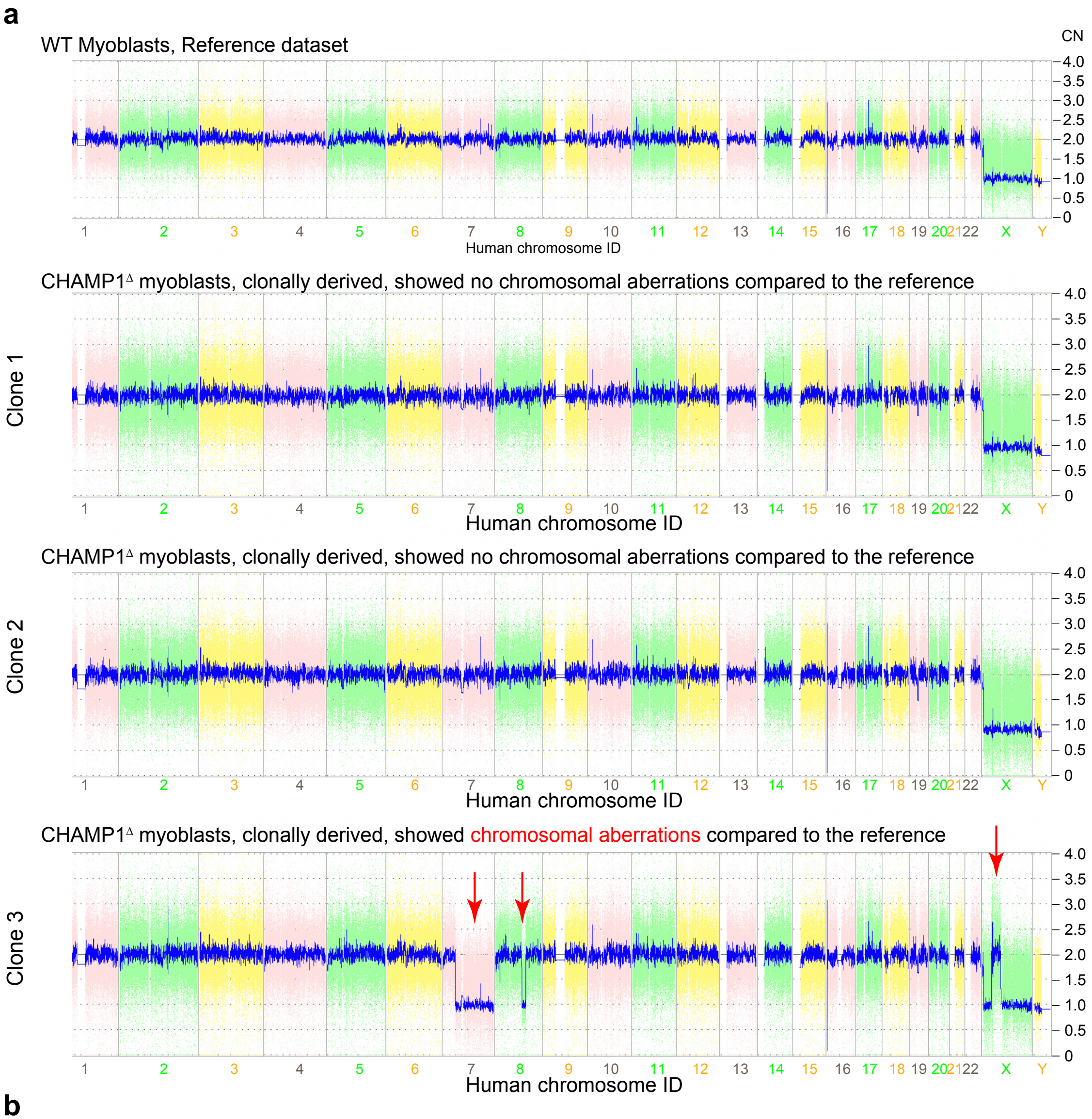
This document contains

Supplementary Figures (1–9)

**Supplementary Figure 1**. Genotype analysis for human CHAMP1$^{\Delta}$ myoblasts.
DNA and protein sequences at three different gRNA targeting regions for the human CHAMP1 gene. Amino acid positions are labeled above the protein sequence. Predicted gRNA cleavage sites are marked by arrows. Sanger sequencing results show biallelic frameshift mutations below the wildtype sequence for each knockout clone, with indels highlighted in green. The protospacer adjacent motif (PAM) is underlined in the wildtype sequence.



**Supplementary Figure 2**. Prolfieration and viability assays for human CHAMP1$^{\Delta}$ myoblasts
**a**, EdU fluorescence images from the EdU incorporation assay to measure the proliferation of human WT and CHAMP1$^{\Delta}$ myoblasts cultured in growth medium. Scale bar: 200 µm. **b**, Quantification of the percentage of EdU positive cells corresponding to panel a. Data are presented as mean ± s.d. P value was calculated using one-way ANOVA followed by Tukey's multiple comparisons test. Source data are provided as a Source Data file. **c**, Histogram from the live/dead cell staining assay that measures viability of human myoblasts in growth medium, with a freeze–thawed sample included as a dead cell control to indicate dye distribution. Note that live cells also are expected to show low-level of staining due to dye binding to cell-surface amines, but the signal is much stronger in dead cells, where membrane permeabilization allows the dye to react with both intracellular and surface amines. **d**, Representative flow cytometry plots showing the gating strategies used for data analysis in panel c.



WT Myoblasts, Reference dataset

CHAMP1$^{\Delta}$ myoblasts, clonally derived, showed no chromosomal aberrations compared to the reference

CHAMP1$^{\Delta}$ myoblasts, clonally derived, showed no chromosomal aberrations compared to the reference

CHAMP1$^{\Delta}$ myoblasts, clonally derived, showed chromosomal aberrations compared to the reference

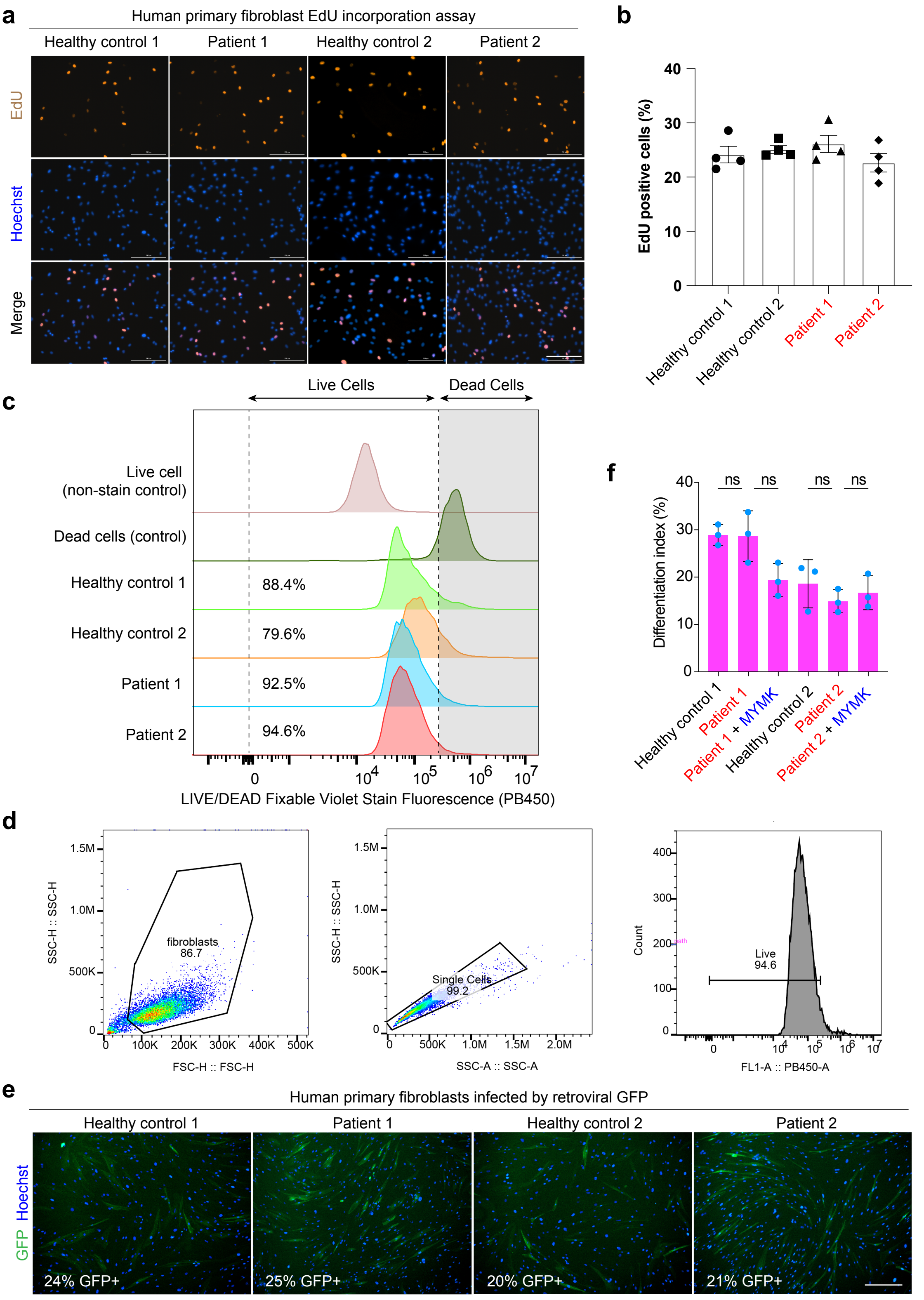
**b**

| Chromosome | Type | Cytoband start | CN state | Size (kilobase pairs) |
|---|---|---|---|---|
| 7 | Loss | p14.1 | 1.00 | 121,051 |
| 8 | Loss | q21.13 | 1.00 | 12,887 |
| X | Gain | p21.3 | 2.00 | 30,326 |

**Supplementary Figure 3**. Karyotype analysis result of WT and clonally derived human CHAMP1$^{\Delta}$ myoblast lines. **a**, Karyotype analysis result of WT and three clonally derived human CHAMP1$^{\Delta}$ myoblast lines revealed no chromosomal aberrations in the first two clones. Regional chromosome loss/gain were detected in the third knockout clone. The value of 2 indicates a normal copy number (CN = 2), while a value of 3 indicates chromosomal gain (CN = 3) and a value of 1 indicates chromosomal loss (CN = 1). The raw signals for individual chromosome probes are shown in pink, green, and yellow, while the normalized probe signal, used for identifying copy number and aberrations, is shown in blue. Aberrations, when present, are marked with red arrows. **b**, Chromosomal aberrations detected in the third human CHAMP1$^{\Delta}$ myoblast clone shown in panel a.

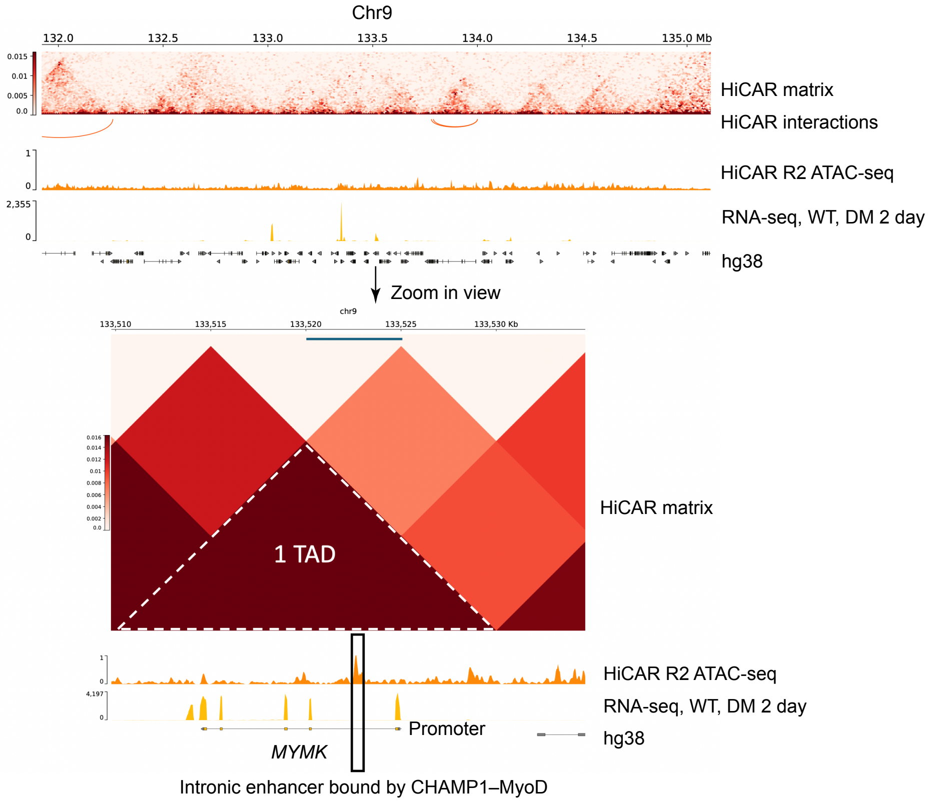
**Supplementary Figure 4**. Heatmap for RNA-seq results.

**a**, **b**, Heatmap of RNA-seq results for selected cell proliferation markers (panel a, KEGG_CELL_CYCLE.v2025) and cell apoptosis markers (panel b, KEGG_APOPTOSIS.v2025) at various time points post-myogenic induction in CHAMP1$^{\Delta}$ cells or isogenic rescued CHAMP1$^{\Delta}$ cell. The TP53 gene appeared in both categories due to its crucial regulatory role in proliferation and cell death. Diff.: differentiation. Heatmap values are based on Z-scores.

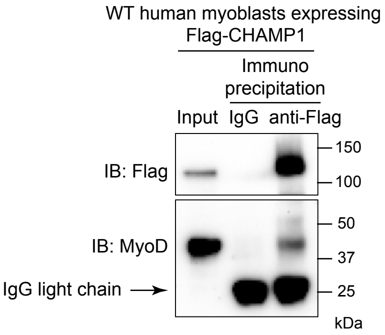


**Supplementary Figure 5**. Proliferation, viability and viral infection assays for human primary fibroblasts.

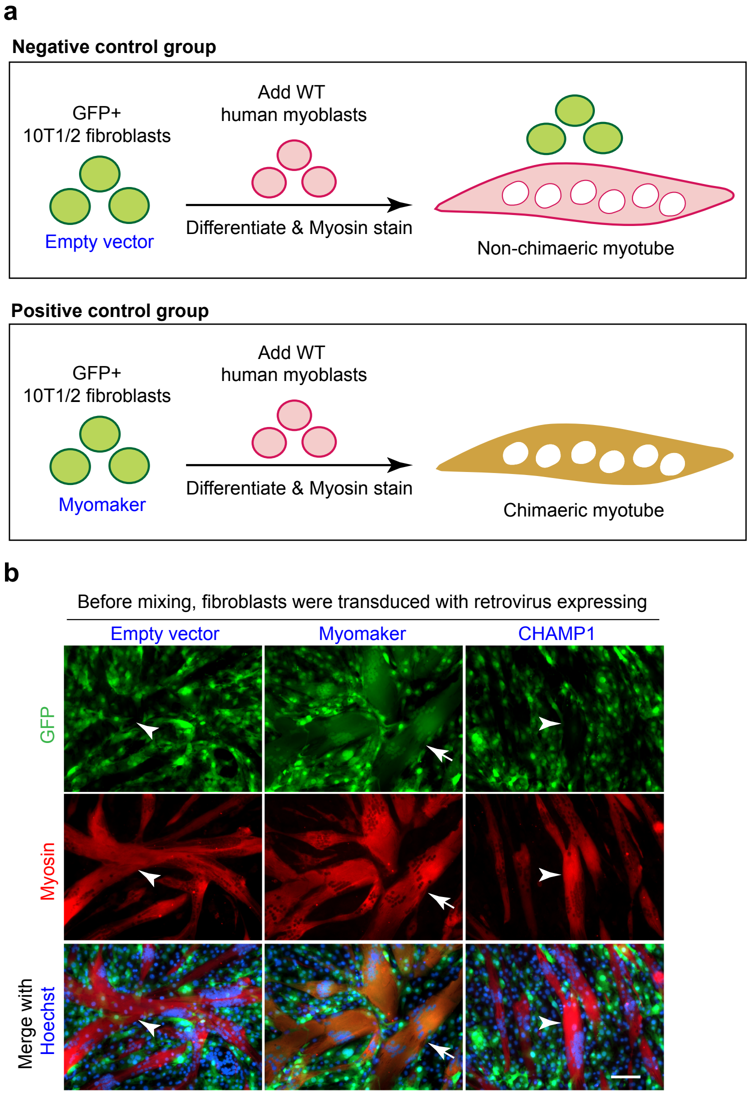
**a**, EdU fluorescence images from the EdU incorporation assay to measure the proliferation of human primary fibroblasts cultured in growth medium. **b**, Quantification of the percentage of EdU positive cells corresponding to panel a. **c**, Histogram from the live/dead cell staining assay that measures viability of human primary fibroblasts in growth medium, with a freeze–thawed sample included as a dead cell control to indicate dye distribution. Note that live cells is expected to be stained due to dye binding to cell-surface amines, but the signal is much stronger in dead cells, where membrane permeabilization allows the dye to react with both intracellular and surface amines. **d**, Representative flow cytometry plots showing the gating strategies used for data analysis in c. **e**, GFP images demonstrating the efficiency of retrovirus transduction in human primary fibroblasts. **f**, Quantification of the differentiation index in human primary fibroblasts transduced with MyoD expressing retrovirus. ns: not significant. Data are presented as mean ± s.d. Statistsics was performed using one-way ANOVA followed by Tukey's multiple comparisons test. Source data are provided as a Source Data file. Scale bars: 200 μm



**Supplementary Figure 6**. HiCAR analysis result of human myoblasts.
HiCAR (High-throughput Chromatin Accessibility and Regulatory interactions) contact matrix, ATAC-seq, RNA-seq, and HiCAR 1D tracks surrounding Myomaker locus in human myoblasts (DM 2 days). Arch tracks: HiCAR interactions (orange).
TAD: topological-associated domain. ATAC-seq: assay for transposase-accessible chromatin sequencing.



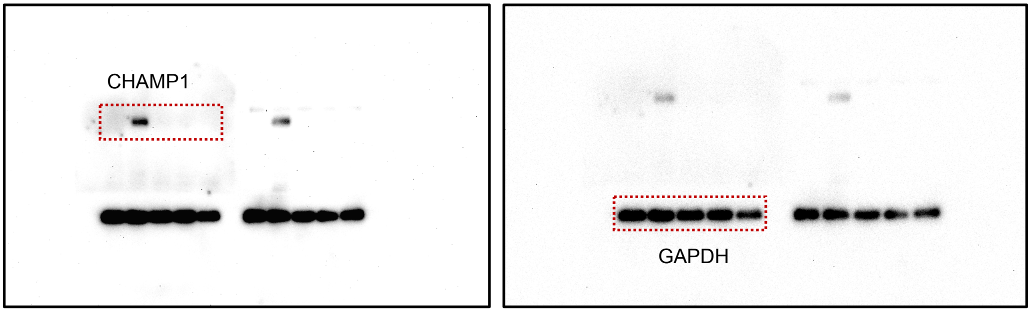
**Supplementary Figure 7**. Co-immunoprecipitation results of CHAMP1 & MyoD interaction in human myoblasts. Western blot analysis of samples from the co-immunoprecipitation assay using Flag antibody, with IgG included as a control to assess antibody specificity. Human myoblasts were differentiated for two days prior to the assay.



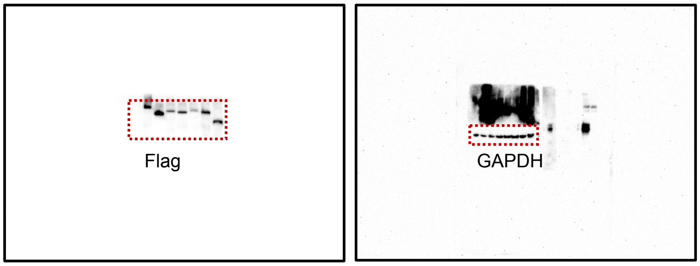
**Supplementary Figure 8**. Fibroblast-myoblast mixing assay result.

**a**, Experiment design of fibroblast fusion assay. GFP labelled 10T1/2 fibroblasts were infected with empty, Myomaker- or CHAMP1-expression retrovirus, then mixed with human myoblasts and differentiated. Myotube formation was monitored by myosin staining, and fusion of fibroblasts was determined by visualization of GFP in myosin+ myotubes. **b**, Fluorescence images to show Myosin+ GFP+ myotubes (arrows) in cultures containing Myomaker-expressing fibroblasts, whereas myosin+ GFP− myotubes (arrowheads) were observed in empty-infected or CHAMP1-infected fibroblast cultures. Scale bar: 100 μm.

uncropped, unprocessed scans of Western blots for **Fig. 2c**

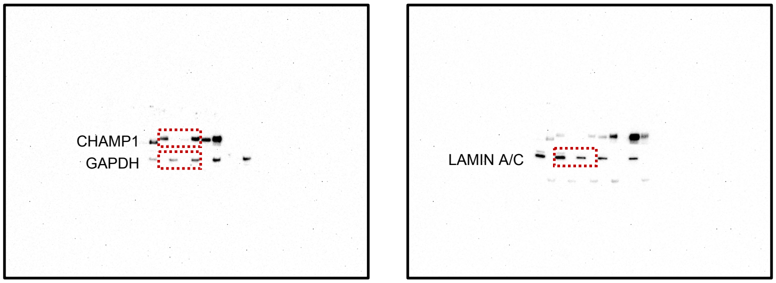


uncropped, unprocessed scans of Western blots for **Fig. 5b**

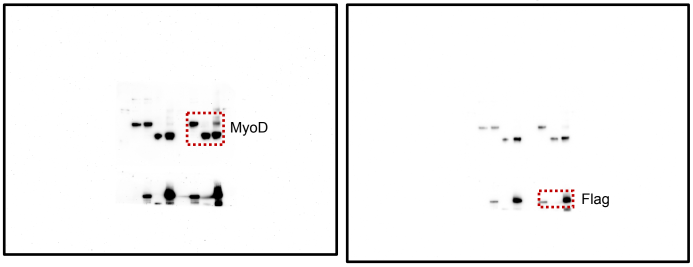


uncropped, unprocessed scans of Western blots for **Fig, 5e**

uncropped, unprocessed scans of Western blots for **Fig. 5i**



uncropped, unprocessed scans of Western blots for **Fig. S7**



**Supplementary Fig. 9**. Uncropped, unprocessed scans of all blots.