Dreesen et al., http://www.jcb.org/cgi/content/full/jcb.201206121/DC1

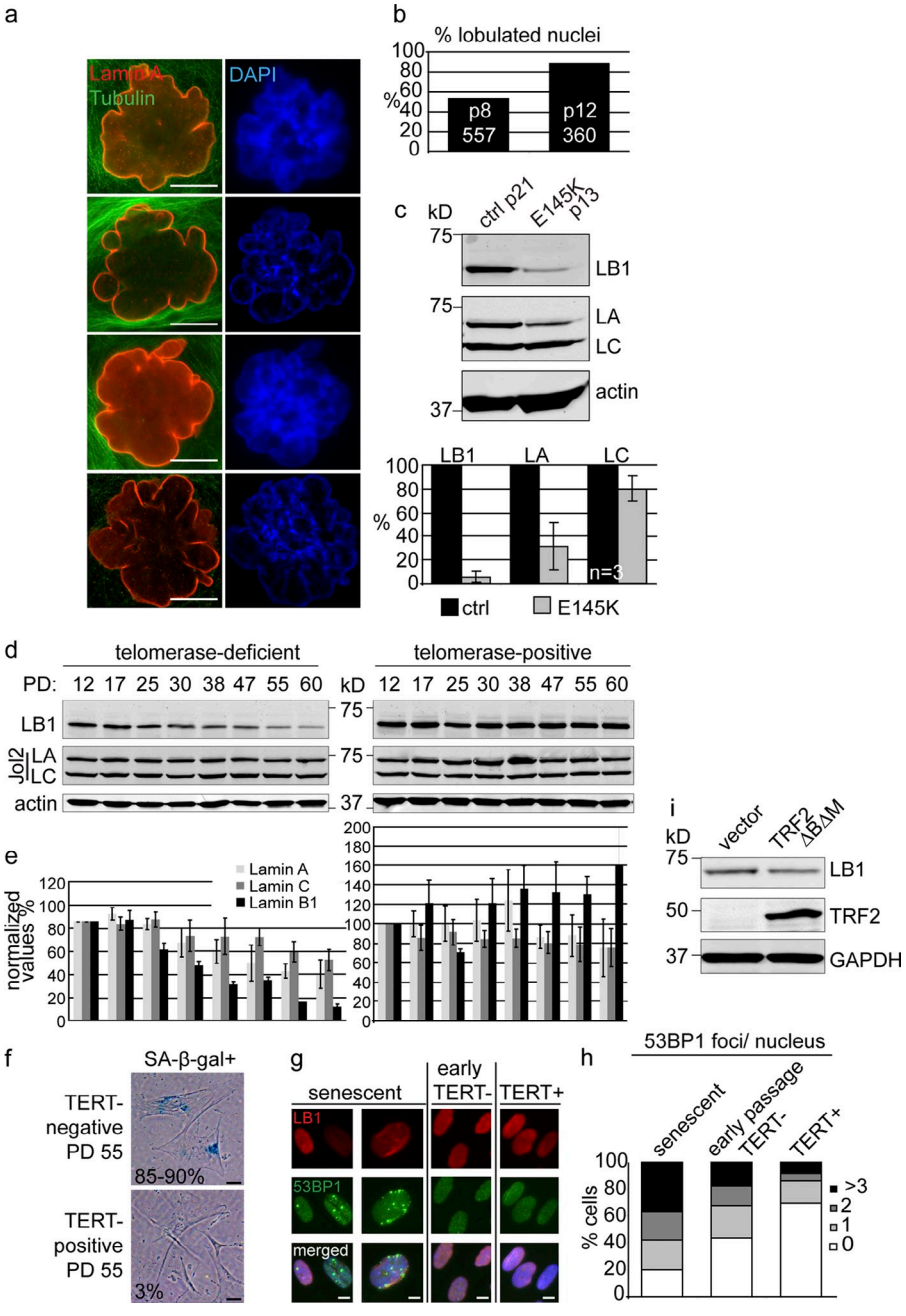


Figure S1. **Loss of LMNB1 in patient-derived fibroblasts carrying the *LMNA* E145K mutation, senescent human dermal fibroblasts, and fibroblasts expressing TRF2ΔBΔM.** (a) Immunofluorescence microscopy shows severe nuclear abnormalities in fibroblasts from patients carrying the *LMNA* E145K mutation. Bars, 10 µm. (b) Quantification of nuclear abnormalities in passage 8 (p8) and passage 12 (p12) E145K patient fibroblasts (number of cells counted is indicated within the bars). (c, top) Western blot showing LMNB1, LMNA (LA), and LMNC (LC) levels in control (ctrl; passage 21) and *LMNA* E145K (passage 13) fibroblasts. (bottom) Quantification of LMNB1 and LMNA/C levels normalized to actin (d) 60 PD time course of hTERT-negative (left) and hTERT-positive (right) fibroblasts. Western blot shows LMNB1 (LB1), LMNA/C (Jol2 antibody), and actin levels during the time course (PDs are indicated on top). (e) Quantification of normalized LMNB1 and LMNA/C levels of the Western blot above. (f) SA-β-gal staining of hTERT-negative and hTERT-positive fibroblasts after 55 PD of the time course. Percentages of SA-β-gal–positive cells are indicated. Bars, 20 µm. (g) Immunofluorescence microscopy of senescent, early passage primary, and hTERT-positive dermal fibroblasts. LMNB1 and 53BP1 antibodies were used; DNA was visualized by DAPI staining. Bars, 10 µm. (h) Quantification of DNA damage foci by 53BP1 staining in senescent, early passage primary, and hTERT-positive fibroblasts. At least 350 cells were counted for each condition. (i) Western blot showing LMNB1 levels in cells expressing either vector control or TRF2ΔBΔM. Antibodies are LMNB1, TRF2, and GAPDH. Data are presented as means ± SD (n = 3).

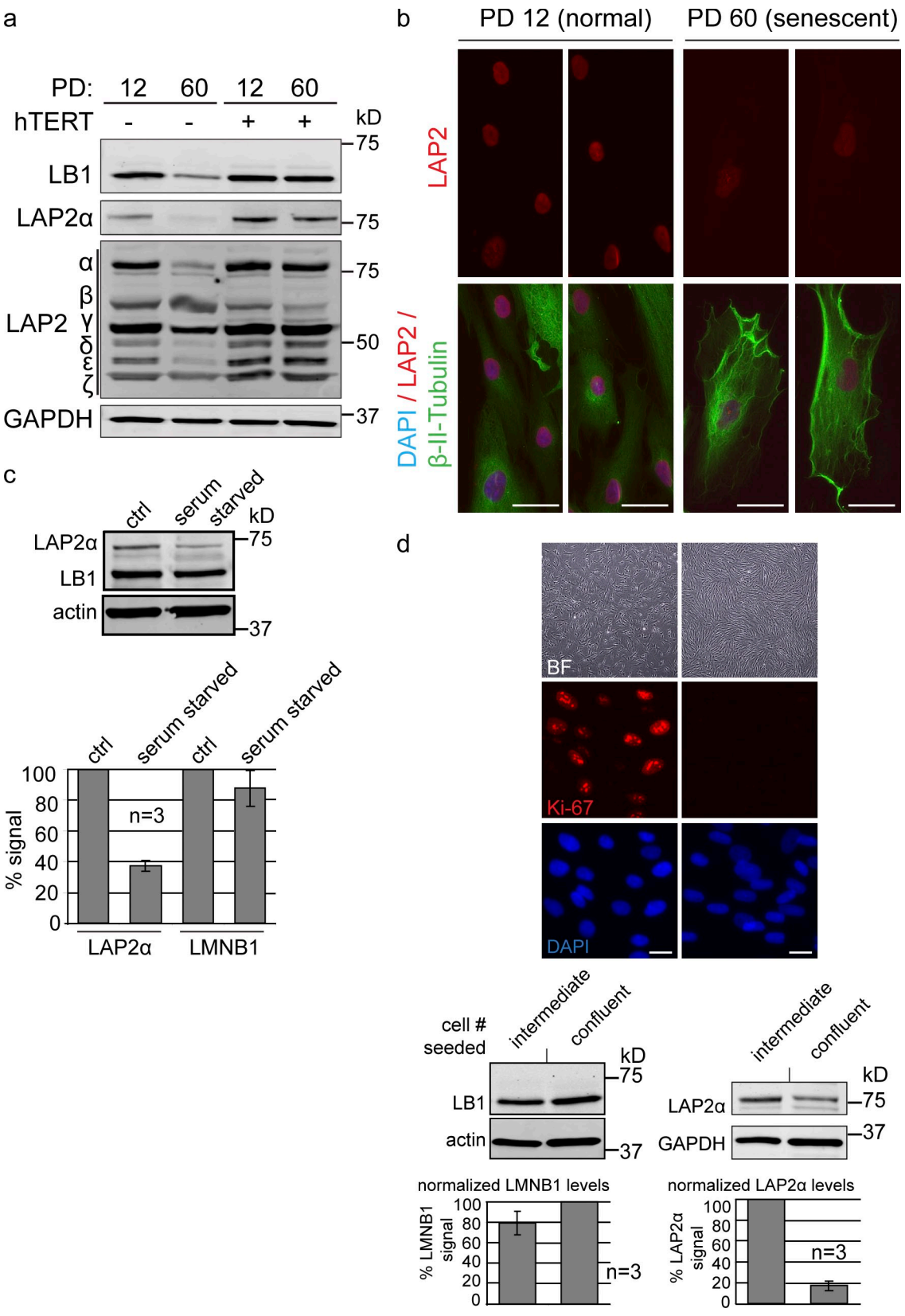
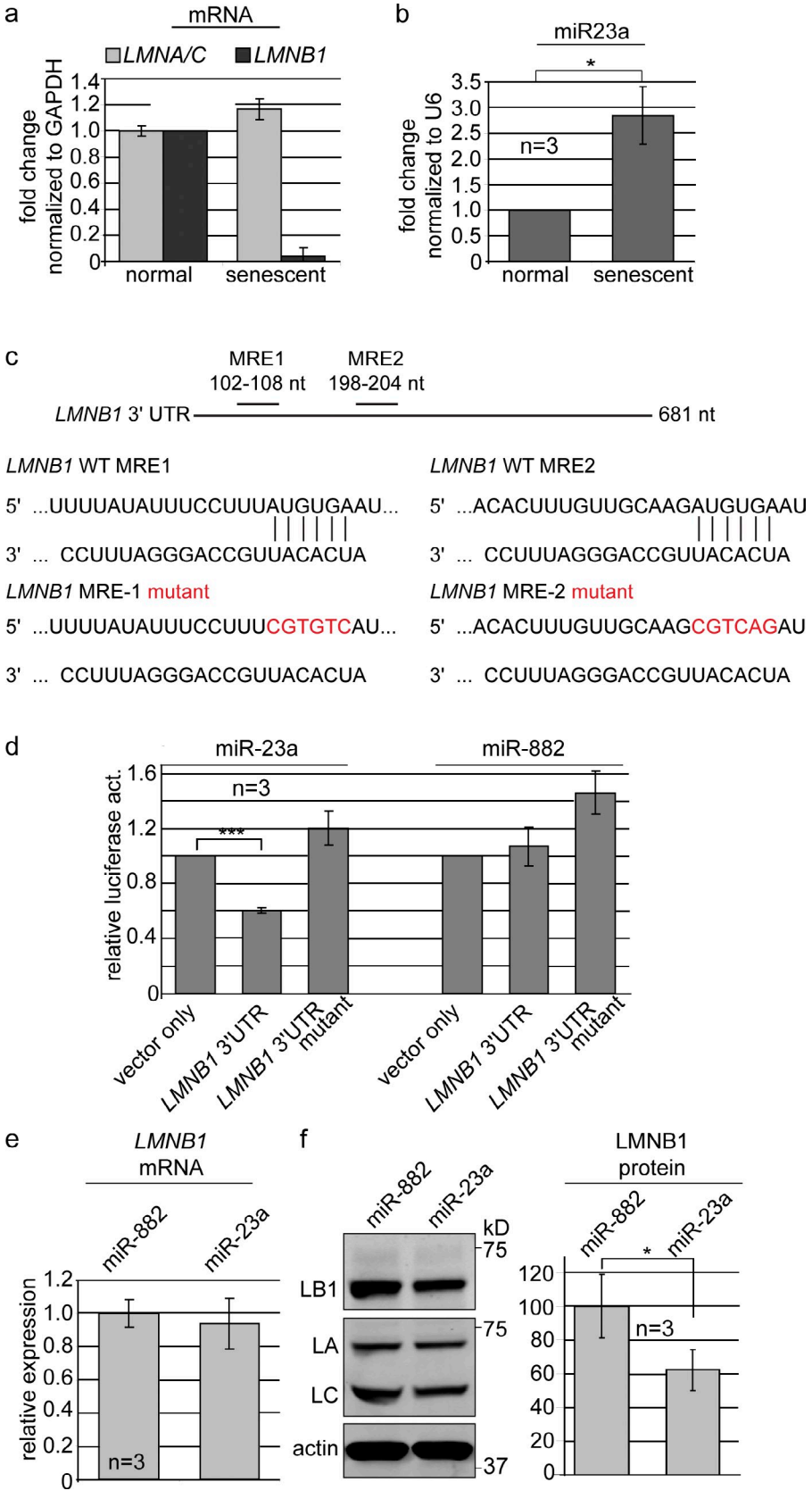


Figure S2. **LAP2 levels decline in senescent, serum-starved, and confluent primary dermal fibroblasts.** (a) Western blot of primary and TERT-positive fibroblasts after 12 and 60 PDs. Antibodies recognizing LMNB1 (LB1), LAP2-α, and different isoforms of LAP2 are indicated. Loading control is GAPDH. (b) Immunofluorescence staining of LAP2 and β-II-tubulin in early (PD 12) versus senescent (PD 60) fibroblasts. Bars, 50 µm. (c, top) Western blot indicating LAP2-α, LMNB1, and actin levels in control (ctrl) versus serum-starved cells. (bottom) Quantification of LMNB1 and LAP2-α levels normalized to actin. (d, images, top) Bright field (BF) image of intermediate versus confluent cells. (middle) Immunofluorescence staining of intermediate and confluent cells with Ki-67 and DAPI. Bars, 20 µm. (bottom) Western blot showing LMNB1 (left) or LAP2-α (right) levels in intermediate versus confluent cells. Quantified values normalized to actin or GAPDH are shown. Means ± SD are shown (n = 3).

Figure S3. **Regulation of LMNB1 by miR-23a in senescent primary human fibroblasts.** (a) qRT-PCR of *LMNA/C* and *LMNB1* mRNA in normal (early passage) and senescent dermal fibroblasts. Values were normalized to GAPDH, and fold changes are indicated on the y axis. (b) qRT-PCR analysis of miR-23a levels in normal versus senescent fibroblasts. Values were normalized to U6. *, P < 0.05. (c) Predicted miRNA recognition elements (MRE) for miR-23a in the *LMNB1* 3′UTR. Mutated sites are shown in red. (d) Relative luciferase activity of the reporter gene only (vector) fused to the *LMNB1* 3′UTR or *LMNB1* 3′UTR harboring mutations in the miR-23 binding site, upon addition of 500 ng miR-23a or control miR-882. ***, P < 0.001. (e) Effects of miR23a or miR-882 expression on *LMNB1* mRNA levels. Both miRNAs were transfected into fibroblasts and *LMNB1* levels assessed by qRT-PCR. (f) Effects of miR23a/miR-882 expression on LMNB1 protein levels. (left) Western blot; LMNB1 (LB1), LMNA/C (LA and LC), and actin antibodies were used. (right) Quantification of LMNB1 signal from Western blots of three different experiments. *, P < 0.05. WT, wild type. Means ± SD are shown (*n* = 3).



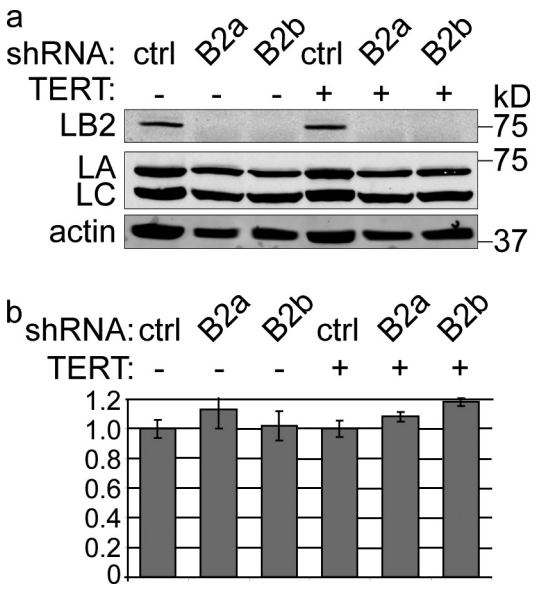


Figure S4. **Lamin B2 deficiency does not affect proliferation of human fibroblasts.** (a) Western blot of cells expressing control or two different shRNA against *LMNB2* (B2a and B2b). Antibodies are indicated on the left. (b) Proliferation assay of primary and TERT-positive fibroblasts expressing control or *LMNB* shRNA. 5,000 cells were plated at day 0 and counted on day 4. Cell numbers of scrambled controls were normalized to 1. Growth assays were performed in triplicates. ctrl, control; LB2, LMNB2; LA, LMNA; LC, LMNC. Means ± SD are shown (n = 3).

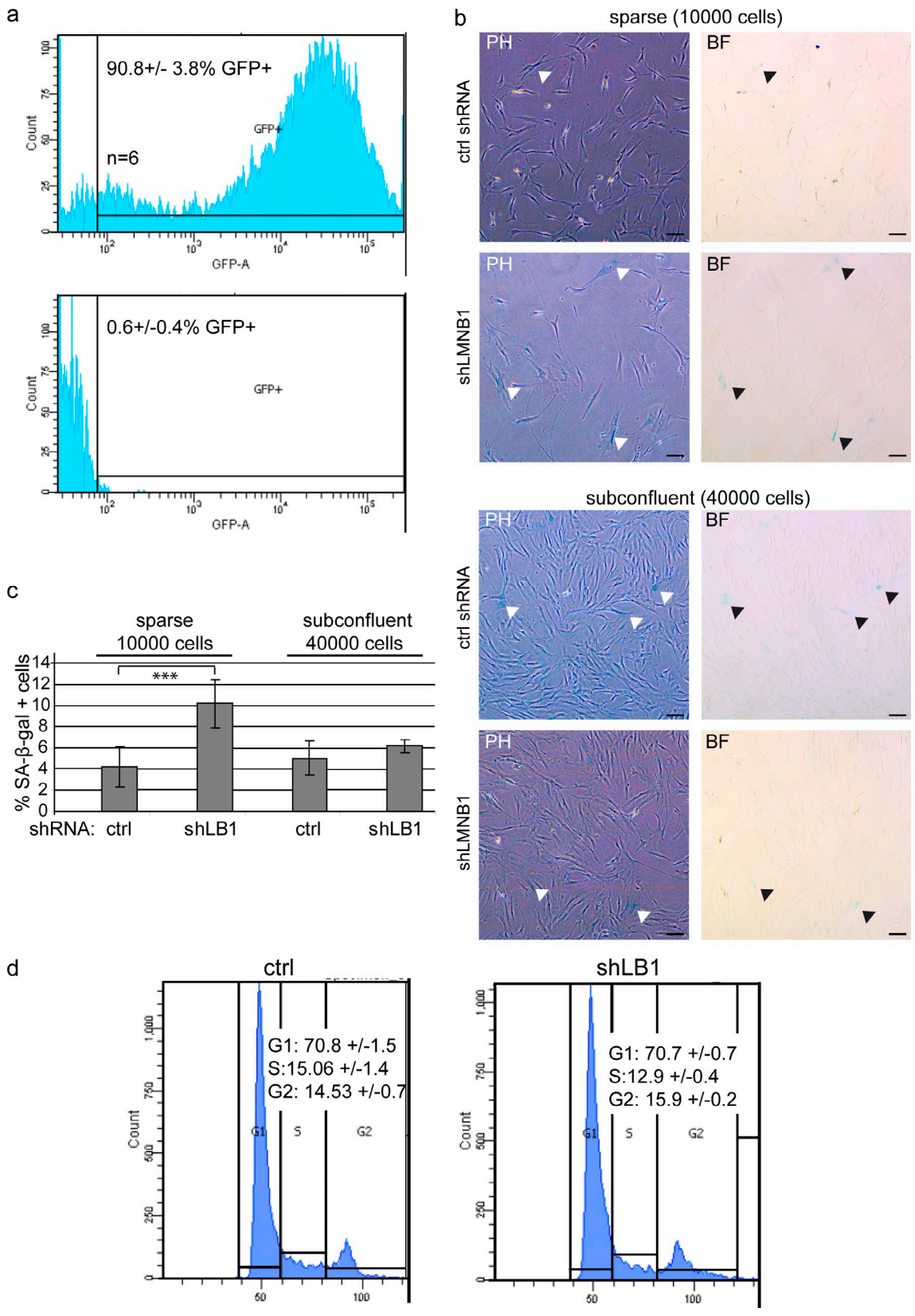


Figure S5. **Consequences of growing normal or LMNB1-depleted fibroblasts under different cell culture conditions.** (a) Lentiviral transduction efficiency, assessed by FACS sorting (constitutive pGIPZ LMNB1 shRNA constructs contain a GFP marker). Transduced cells (top) versus nontransduced controls (bottom). Percentage of GFP$^+$ cells are indicated (n = 6). The horizontal line indicates GFP$^+$ gated cells, while the vertical line marks the left side of the GFP gate. (b, top) SA-β-gal staining of control and LMNB1-depleted (shLMNB1) cells grown under sparse conditions (seeding density of 10,000 cells). (bottom) Staining of the same cells grown under subconfluent conditions (seeding density of 40,000). Arrowheads indicate SA-β-gal–positive cells. PH, phase contrast; BF, bright field. Bars, 50 µm. (c) Quantification of SA-β-gal–positive cells from three independent experiments. ***, $P < 0.001$. (d) Cell cycle analysis of control versus LMNB1-depleted cells (n = 8). Percentages of cells in different cell cycle stages are indicated. Means ± SD are shown. ctrl, control.