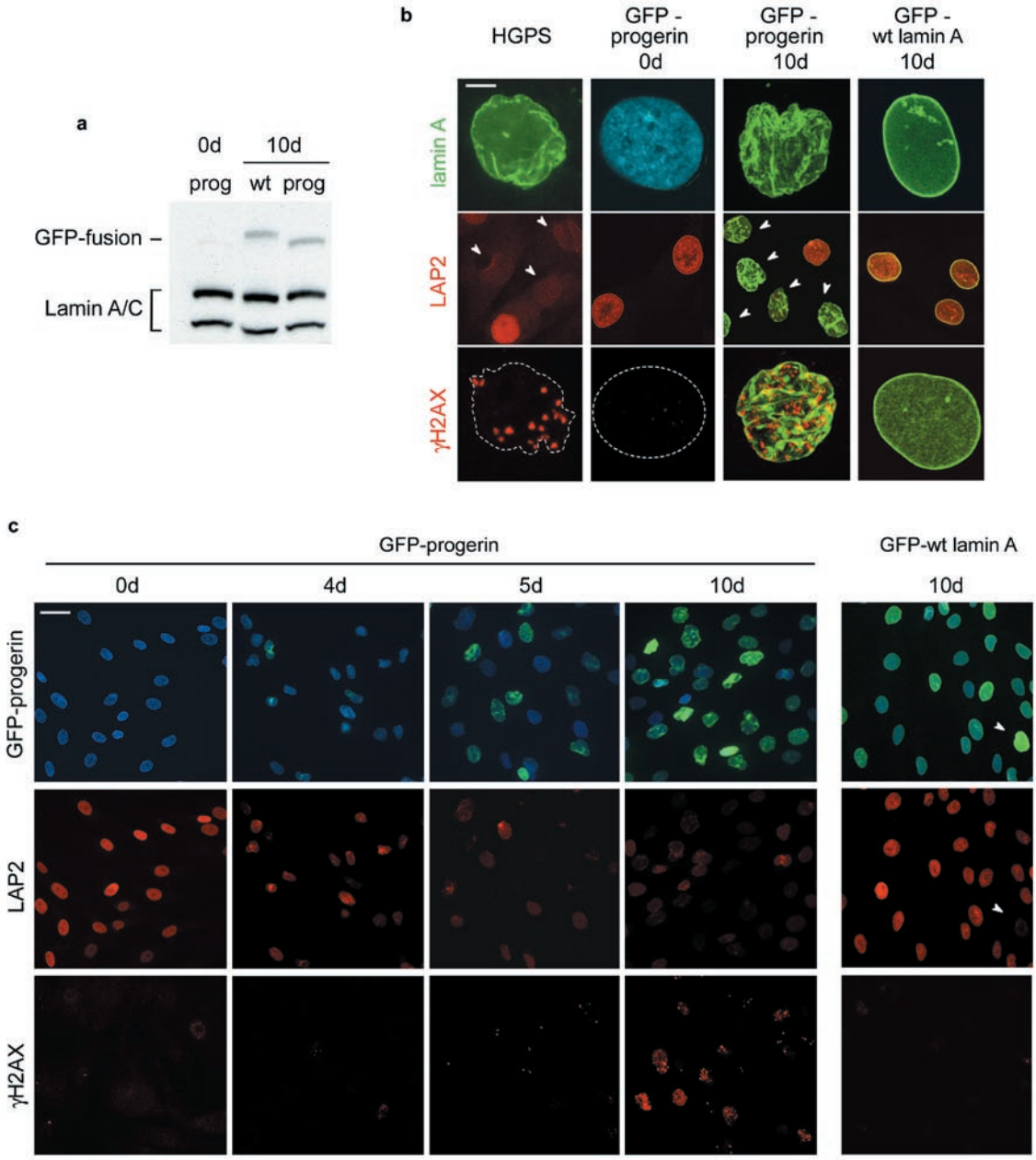

Figure S1 Characterization of tet-off inducible cell lines expressing GFP-progerin and GFP-wt lamin A. **a**, Western blot analysis of GFP-progerin- or GFP-wt lamin A- expressing cells before induction (0d) and after 10d using an anti-lamin A/C antibody. Expression of exogenous progerin was adjusted to physiological levels observed in HGPS cells by maintaining the cells in the presence of 0.02 ng/ml doxycycline. **b**, Immunofluorescence microscopy of HGPS cells, GFP-progerin- and GFP-wt lamin A-expressing cells, before induction (0d) and after 10d using the indicated antibodies. The green signal in HGPS cells corresponds to endogenous lamin A detected with an anti-lamin A/C antibody and in the inducible cell lines to the GFP-fused exogenous proteins. DAPI staining (blue) is shown to visualize nuclear shape before induction of GFP-progerin. Merge between LAP2 and γH2AX signals (red) and GFP signal (green) in the inducible cell lines is shown. Reduction of LAP2 as observed in HGPS cells correlates with high levels of GFP-progerin (arrows). Dashed lines indicate nuclear edge. γH2AX foci as observed in HGPS cells accumulate in GFP-progerin-expressing cells upon induction but not in GFP-wt lamin A-expressing cells. Scale bar: 5 μm. **c**, Immunofluorescence microscopy of GFP-progerin- and GFP-wt lamin A-expressing cells. Time course showing progressive accumulation of GFP-progerin in the population and appearance of nuclear defects starting 4d after doxycycline removal (final concentration 0.2 ng/ml). Levels of GFP-progerin reach a plateau at 10d. GFP-wt lamin A has no effect on the majority of the cells, although excessive accumulation of lamin A induces mild HGPS-like abnormalities in nuclear shape and LAP2 levels (arrow). Scale bar: 20 μm.

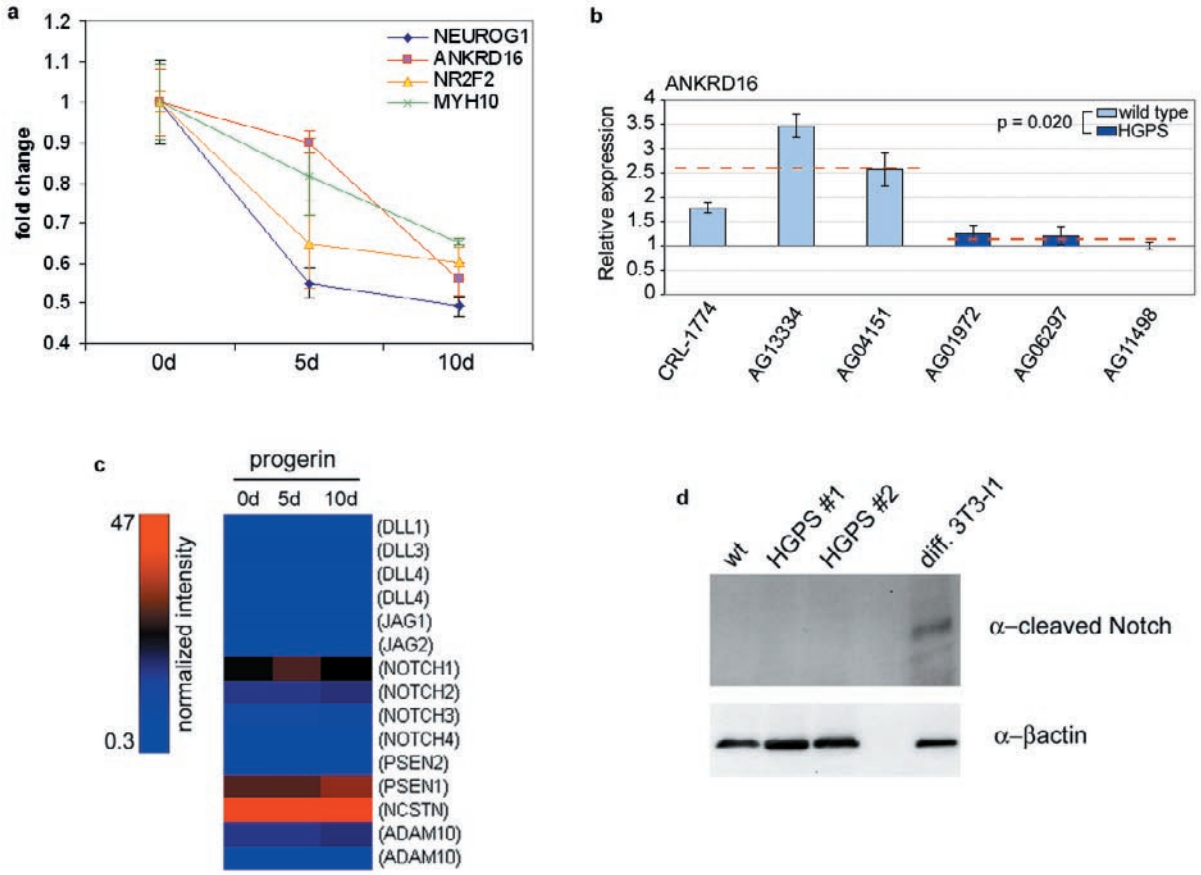

Figure S2 HES/HEY target genes are downregulated in response to progerin while upstream components of Notch signalling pathway are not affected. **a,** Time-dependent changes in expression levels of the indicated genes in progerin-expressing cells as detected by microarray analysis. Values represent average ± s.d. from two biological replicates. *NEUROG1*: neurogenin 1[1], *ANKRD16*: ankyrin repeat domain 16[2], *NR2F2*: transcription factor *COUP2*[3], *MYH10*: myosin, heavy chain 10[4]. **b,** qRT-PCR analysis of HEY1 target gene *ANKRD16* expression levels in 3 different HGPS cells lines and 3 wild type control cell lines. Values are normalized to the housekeeping gene cyclophillin A. Statistical significance of the differences between the two groups of cell lines is indicated. Values represent averages ± s.d from at least 2 experiments. **c**, Heatmap representing normalized microarray signal for genes encoding Notch receptors (*NOTCH1-4*), ligands (*DLL1, 3, 4* and *JAG1,2*) and components of the proteolytic complexes involved in NICD release (*PSEN1,2, NCSTN, ADAM10*) in progerin-expressing cells. No statistically significant difference ($p > 0.1$) in any of these genes was observed upon induction of progerin. Values corresponding to multiple probes are shown for some of the genes. **d**, Western blot analysis of two HGPS cell lines and one wild type control using an anti-cleaved Notch antibody. 3T3-L1 cells differentiated into adipocytes for 10 days are used as positive control. No specific band is detected in either wild type or HGPS cell lines, suggesting that activation of downstream Notch effectors by progerin is independent of NICD. β-actin is used as loading control.

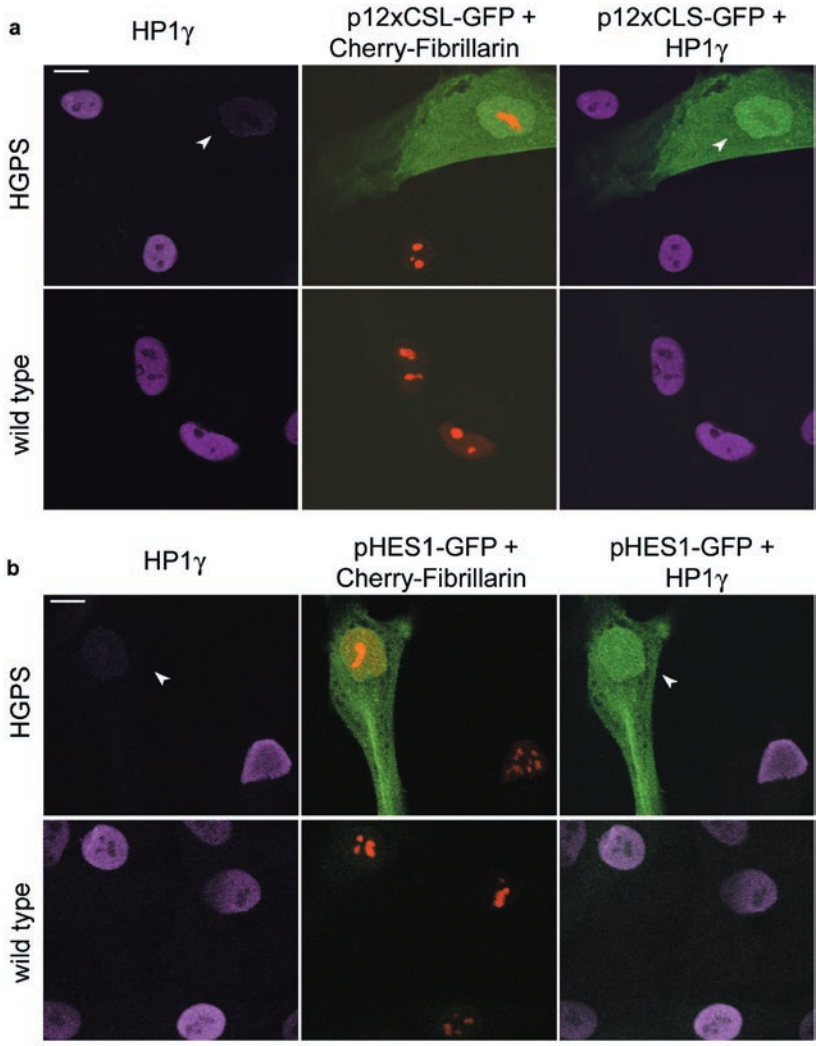

Figure S3 Activation of Notch reporters in HGPS cells. **a, b,** Immunofluorescence microscopy of HGPS and wild type cells transfected with (**a**) the general Notch reporter p12xCSL-GFP containing 12 copies of the CBF1 DNA binding site or (**b**) a HES1-promoter reporter. Cherry-fibrillarin was cotransfected with the reporters and used as transfection marker. Both Notch reporters are activated in HGPS cells displaying progerin-induced nuclear defects as indicated by loss of HP1γ. Scale bar: 10 μm.

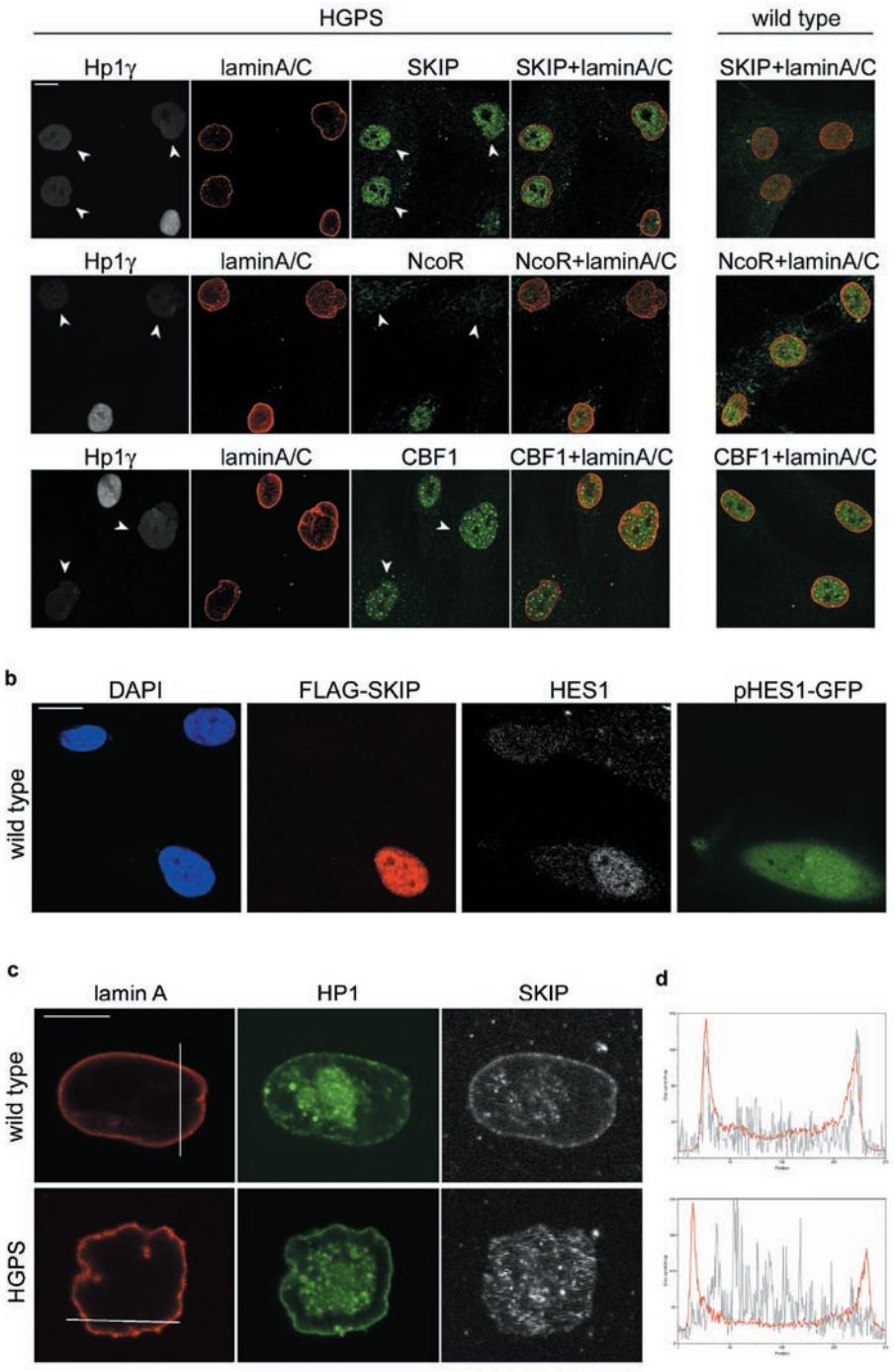

Figure S4 Altered levels and subnuclear distribution of transcriptional regulators of Notch effectors. **a,** Immunofluorescence microscopy of HGPS and wild type cells using the indicated antibody. HGPS cells displaying progerin-induced nuclear defects as indicated by loss of HP1γ show increased levels of the transcriptional coactivator SKIP, reduced levels of the corepressor NcoR and unaltered levels of the NICD-binding protein CBF1 compared to wild type cells. Scale bar: 10 μm. **b,** Immunofluorescence microscopy of wild type cells transfected with FLAG-tagged SKIP. Cells overexpressing SKIP display high levels of endogenous HES1 and show activation of a *HES1* reporter. Scale bar: 10 μm. **c,** Immunofluorescence microscopy of HGPS and wild type cells after *in situ* nuclear matrix preparation using the indicated antibodies. SKIP localizes at the nuclear periphery in wild type cells like HP1γ, used as control for matrix preparation quality. In contrast, in HGPS cells the association of SKIP with the nuclear lamina is disrupted. Scale bar: 5 μm. **d,** Line scan showing colocalization of SKIP with the nuclear lamina in wild type cells (upper panel) and loss of association in HGPS cells (lower panel). Red: lamin A, gray: SKIP.

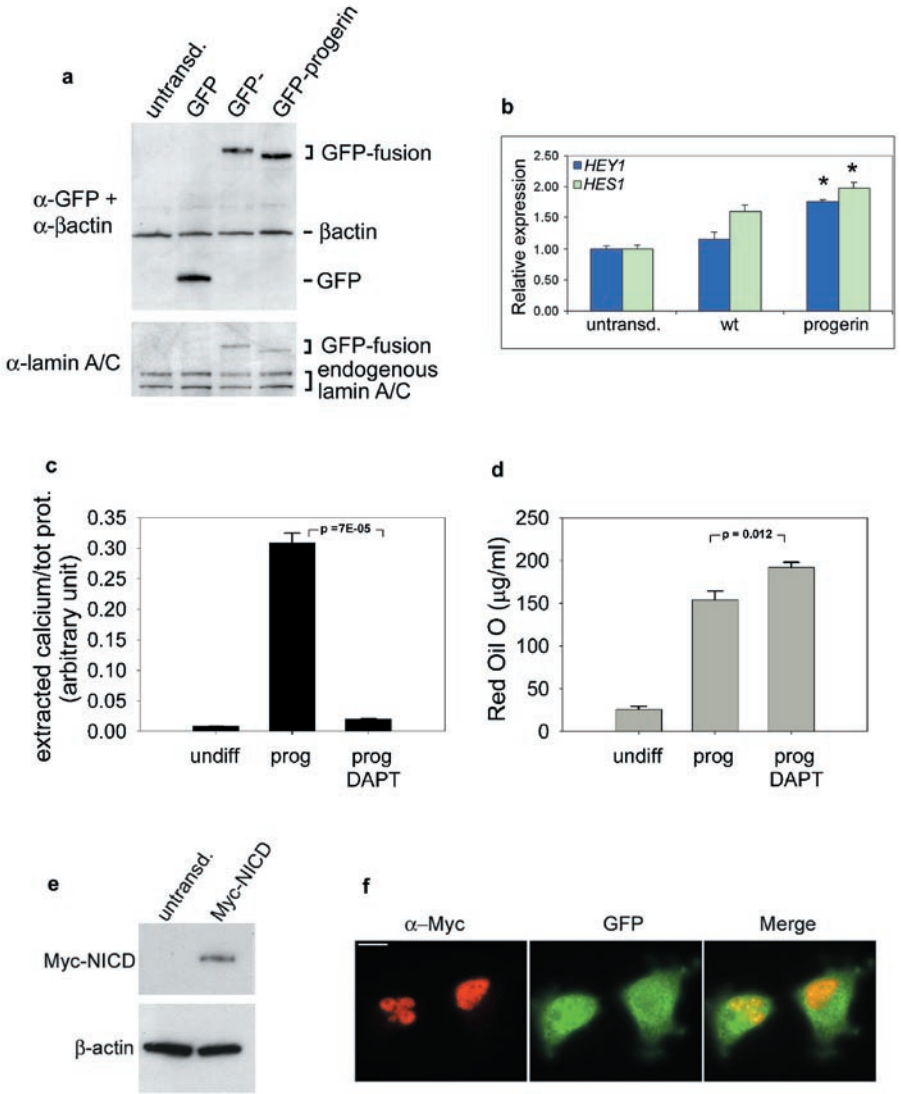

Figure S5 Characterization of hMSCs cell lines constitutively expressing GFP-progerin, GFP-wt lamin A, or Myc-NICD. **a**, Western blot analysis of untransduced hMSCs and hMSCs expressing GFP, GFP-progerin or GFP-wt lamin A using anti-GFP and anti-lamin A/C antibodies. β-actin is used as loading control. Similar levels of exogenous proteins are expressed in the three transduced cell lines. After three sequential infections transduced cells express progerin at levels similar to those observed in HGPS cells. **b**, qRT-PCR analysis of *HES1* and *HEY1* expression levels in hMSCs expressing GFP-progerin or GFP-wt lamin A or in untransduced control cells. Since *HES1* mRNA levels oscillate with a period of 2h [5], RNA was extracted from cells collected at 45 min intervals and average expression levels over time were measured to minimize variations due to *HES1* mRNA fast turnover. Values represent mean and standard deviation from 3 sequential RNA preparations. Statistical significance of the difference between untransduced and progerin-expressing cells is indicated (asterisk: $p < 0.05$). **c,** Quantitative analysis of calcium deposition by progerin-expressing hMSCs treated with the Notch signalling inhibitor DAPT or DMSO during osteogenic differentiation. Values represent average ± s.d. from three biological replicates. Statistical significance of the differences is indicated. **d,** Quantitative analysis of incorporated Oil Red O by progerin-expressing hMSCs treated with the Notch signalling inhibitor DAPT or DMSO during adipogenic differentiation. Values represent average ± s.d. deviation from three biological replicates. Statistical significance of the differences is indicated. **e,** Western blot analysis of untransduced hMSCs and hMSCs expressing Myc-NICD using an anti-Myc antibody. β-actin is used as loading control. **f,** Immunofluorescence microscopy of hMSCs expressing Myc-NICD. GFP is expressed by the Myc-NICD containing plasmid and serves as transfection marker. Scale bar: 10 μm.

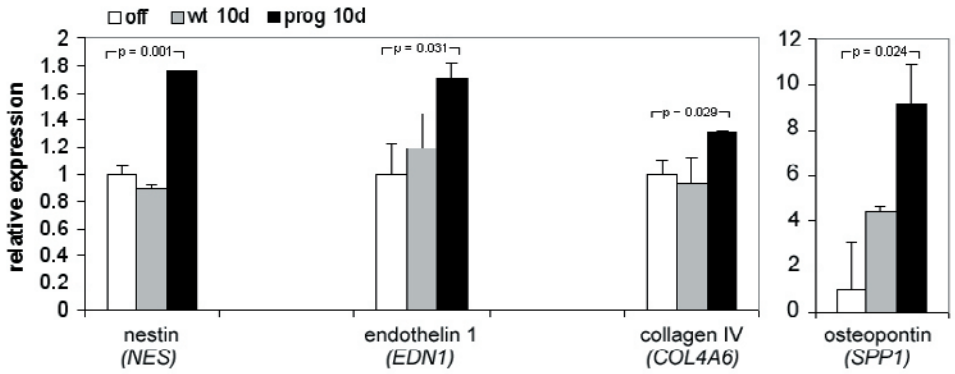

Figure S6 Expression of differentiation markers in progerin expressing fibroblasts. Relative expression levels of the indicated differentiation markers in the inducible fibroblast cell lines expressing either wild type lamin A or progerin after 10d of induction compared to the uninduced state of the wild type cell line (off) as detected by microarray analysis. Statistical significance in a student t-test between uninduced and progerin-expressing cells is indicated.

hes1F 5’- CAAGCTGGAGAAGGCGGAC - 3’
hes1R 5’- CACACTTGGGTCTGTGCTCA - 3’
hes5F 5’- CGACATCCTGGAGATGGC - 3’
hes5R 5’- GAGTAGCCTTCGCTGTAGTCC - 3’
hey1bisF 5’- AGATCCTGCAGATGACCGTG - 3’
hey1bisR 5’- CAAACTCCGATAGTCCATAGC - 3’
ankrdF 5’- GCTGCTCCTCGATGAACATG - 3’
ankrdR 5’- GAATCGGATGGCTTCGTCCT - 3’
et1F 5’- CAAGGAGCTCCAGAAACAGC - 3’
et1R 5’- CCGCCAGGGTGGACTGGG - 3’
vegfr1F 5’- AAGAAGGAAACAGAATCTGCAA - 3’
vegfr1R 5’- TCCCTTCCTTCAGTCATGTGT - 3’
runxF 5’- CAAGTAGCAAGGTTCAACGATC - 3’
runxR 5’- GGGAGGATTTGTGAAGACGG - 3’
opnFbis 5’- GTGATTTGCTTTTGCCTCCT - 3’
opnRbis 5’- GCCACAGCATCTGGGTATTT - 3’
pparF 5’- TGTCAGTACTGTCGGTTTCAG - 3’
pparR 5’- CTGGAGATCTCCGCCAACAG - 3’
srebfp1F 5’- GAGCCGTGCGATCTGGA - 3’
srebfp1R 5’- CAGCATAGGGTGGGTCAAAT - 3’
cebpaF 5’- ACGATCAGTCCATCCCAGAG - 3’
cebpaR 5’- TTCACATTGCACAAGGCACT - 3’
sox9F 5’- CTTGCACAACGCCGAGCTC - 3’
sox9R 5’- CCGCTCCGCCTCCTCCAC - 3’
Col2a1_F 5’- CCTGAAGGATGGCTGCACG - 3’
Col2a1_R 5’- TGGGTGCAATGTCAATGATGG - 3’
Coll10F 5’- CCACTACCCAACACCAAGAC - 3’
Coll10R 5’- TGGACCAGGAGTACCTTGCT - 3’

Figure S7 Primer sequences used to amplify Notch effectors and differentiation markers by qRT-PCR

Supplementary References

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