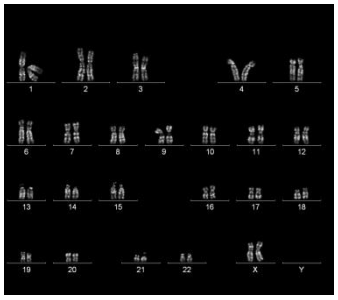
Epigenome rejuvenation: HP1β mobility as a measure of pluripotent and senescent chromatin ground states

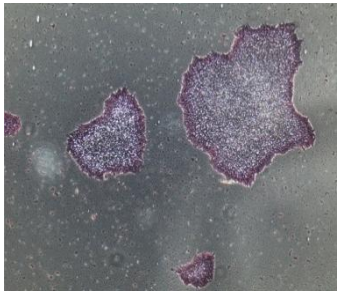
By

Maria Manukyan[1*] and Prim B. Singh[2*]

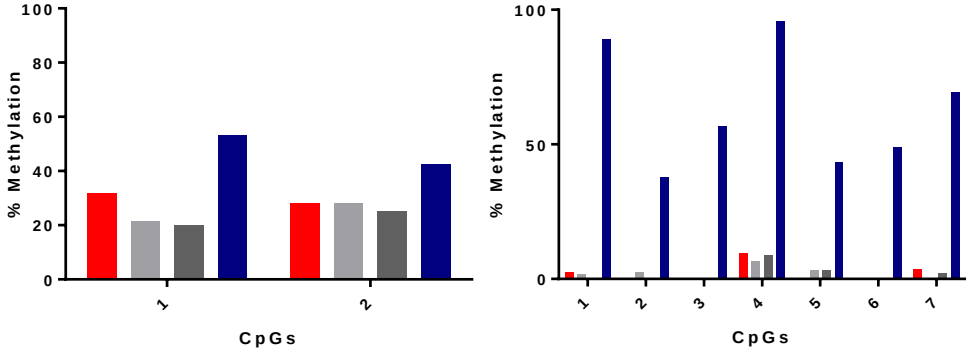
a.



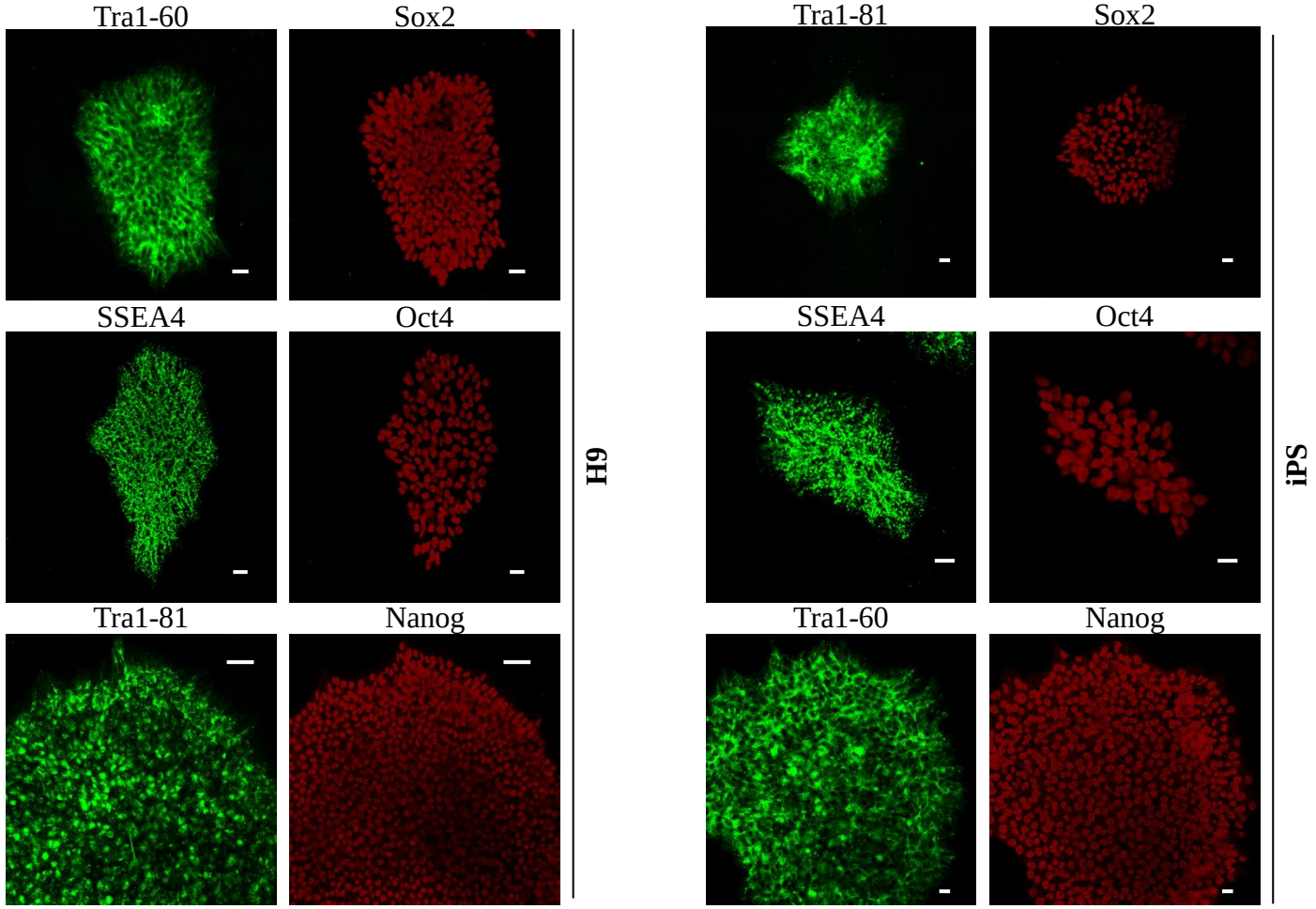
b.

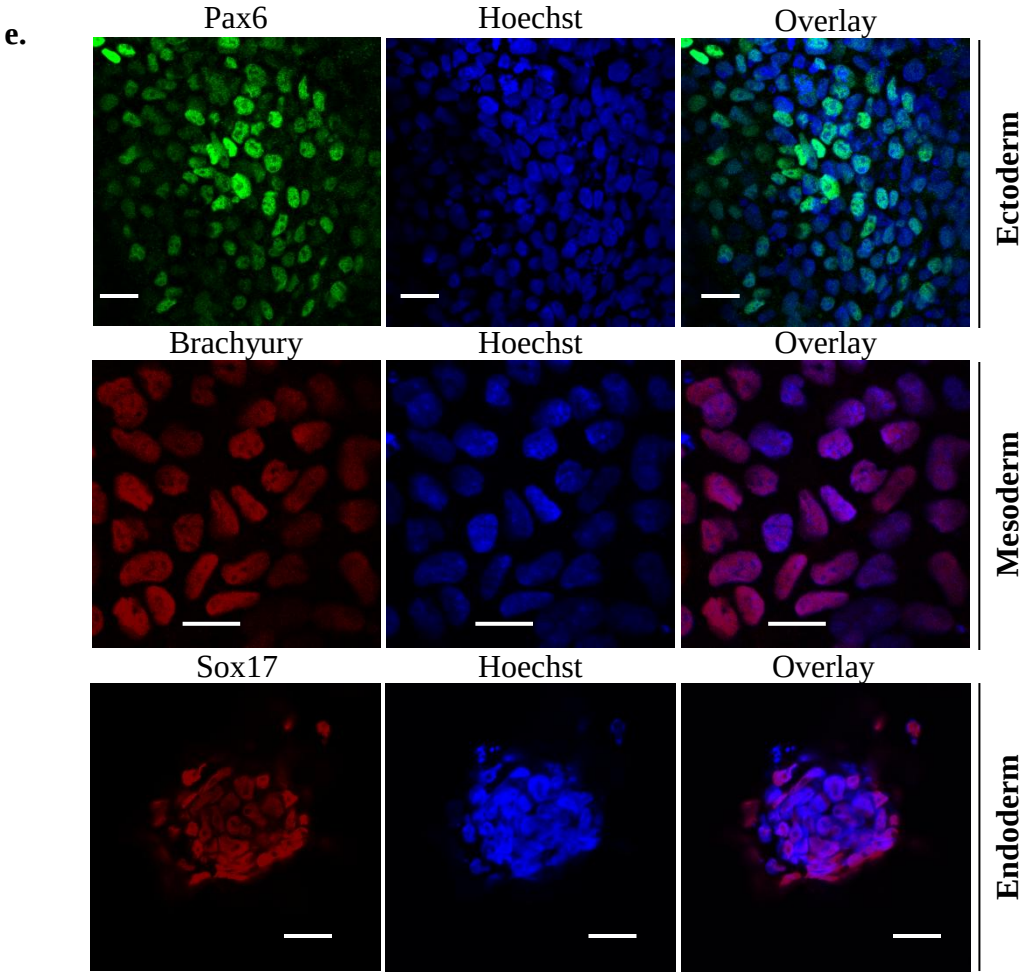


c.

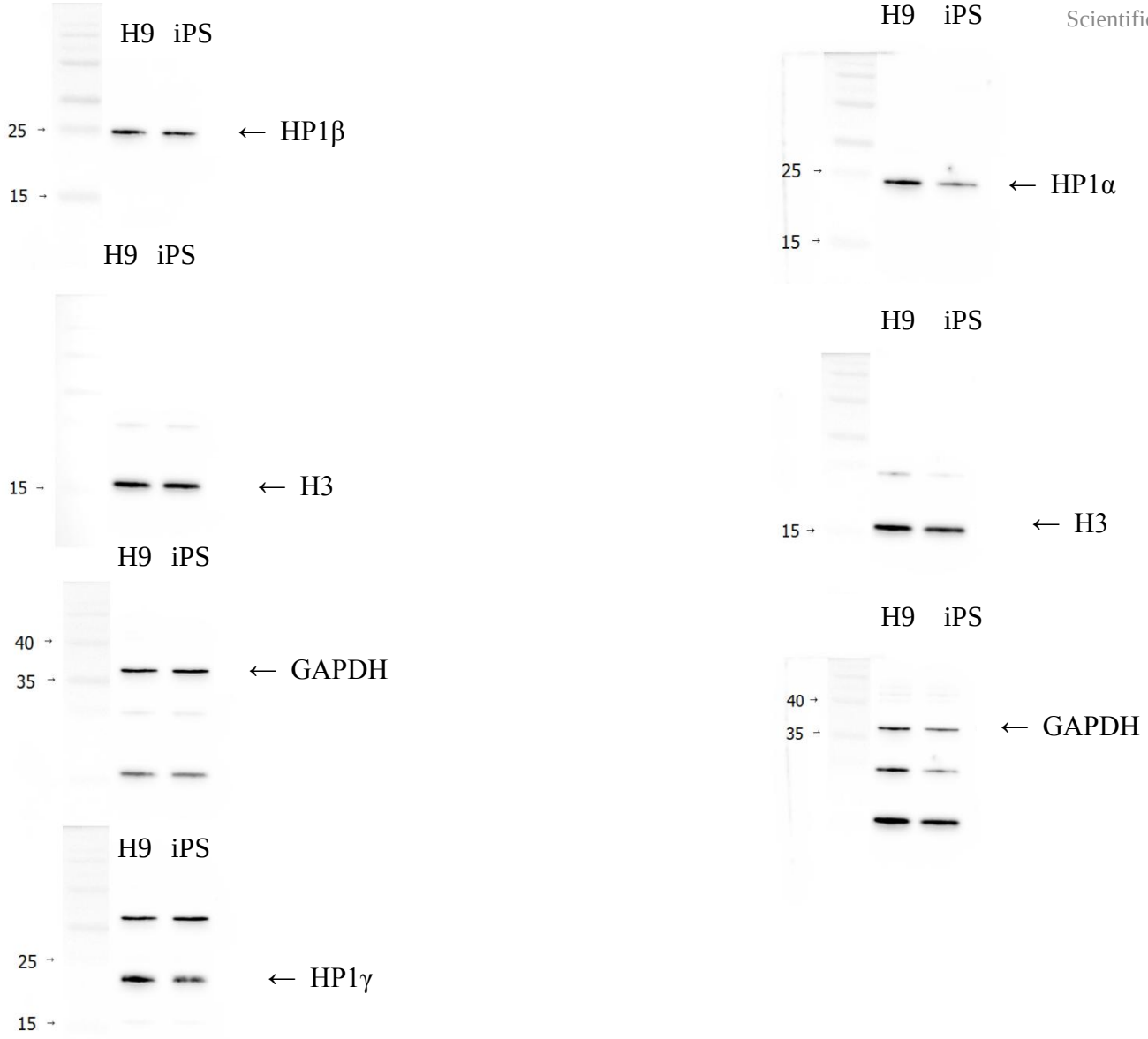

100
80
60
40
20
0
% Methylation
1
2
CpGs
100
50
0
% Methylation
1
2
3
4
5
6
7
CpGs
H9
iPS #2.4
iPS #2.2
LF1

d.

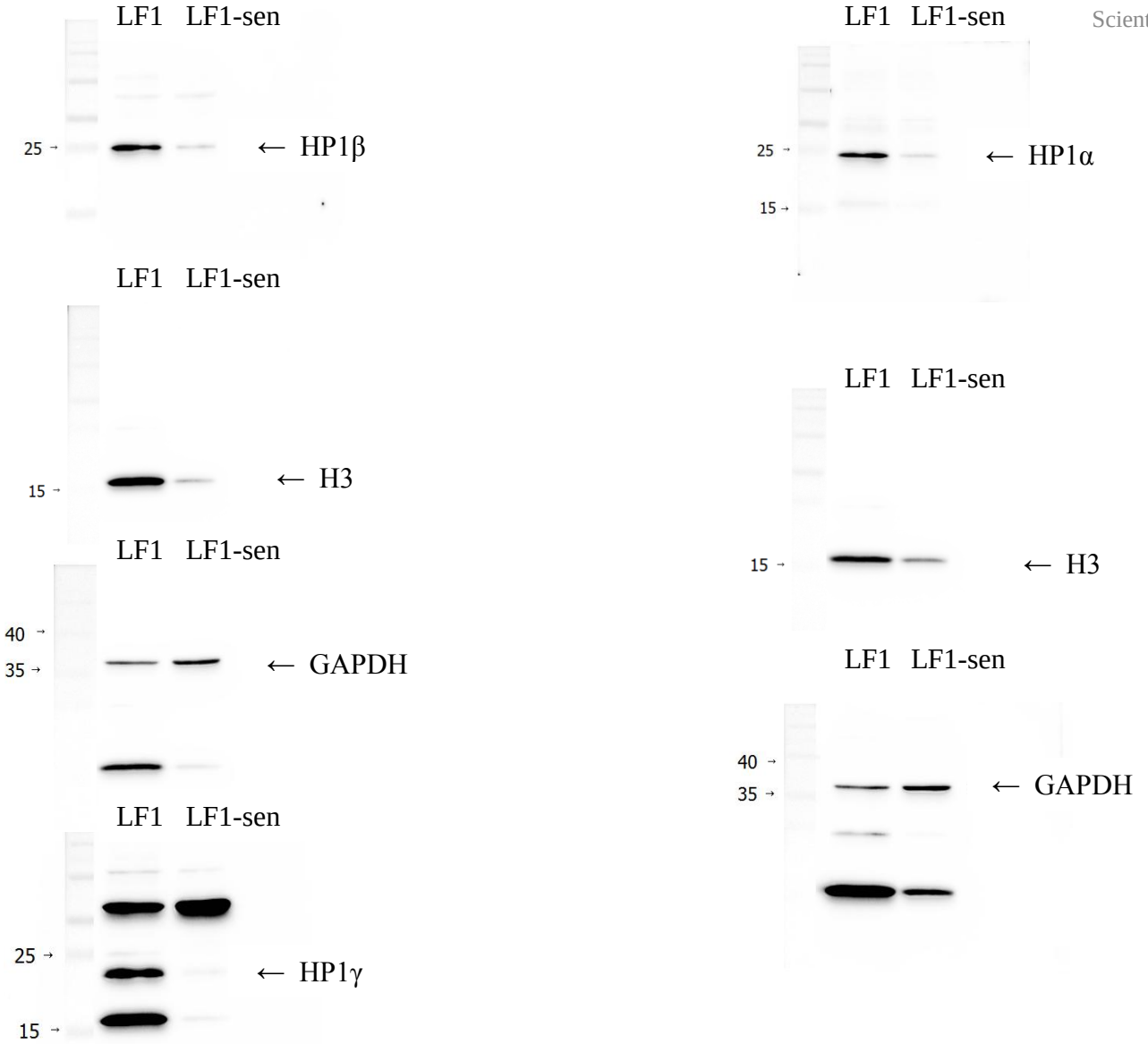

Tra1-60
Sox2
Tra1-81
Sox2
SSEA4
Oct4
SSEA4
Oct4
Tra1-81
Nanog
Tra1-60
Nanog
H9
iPS



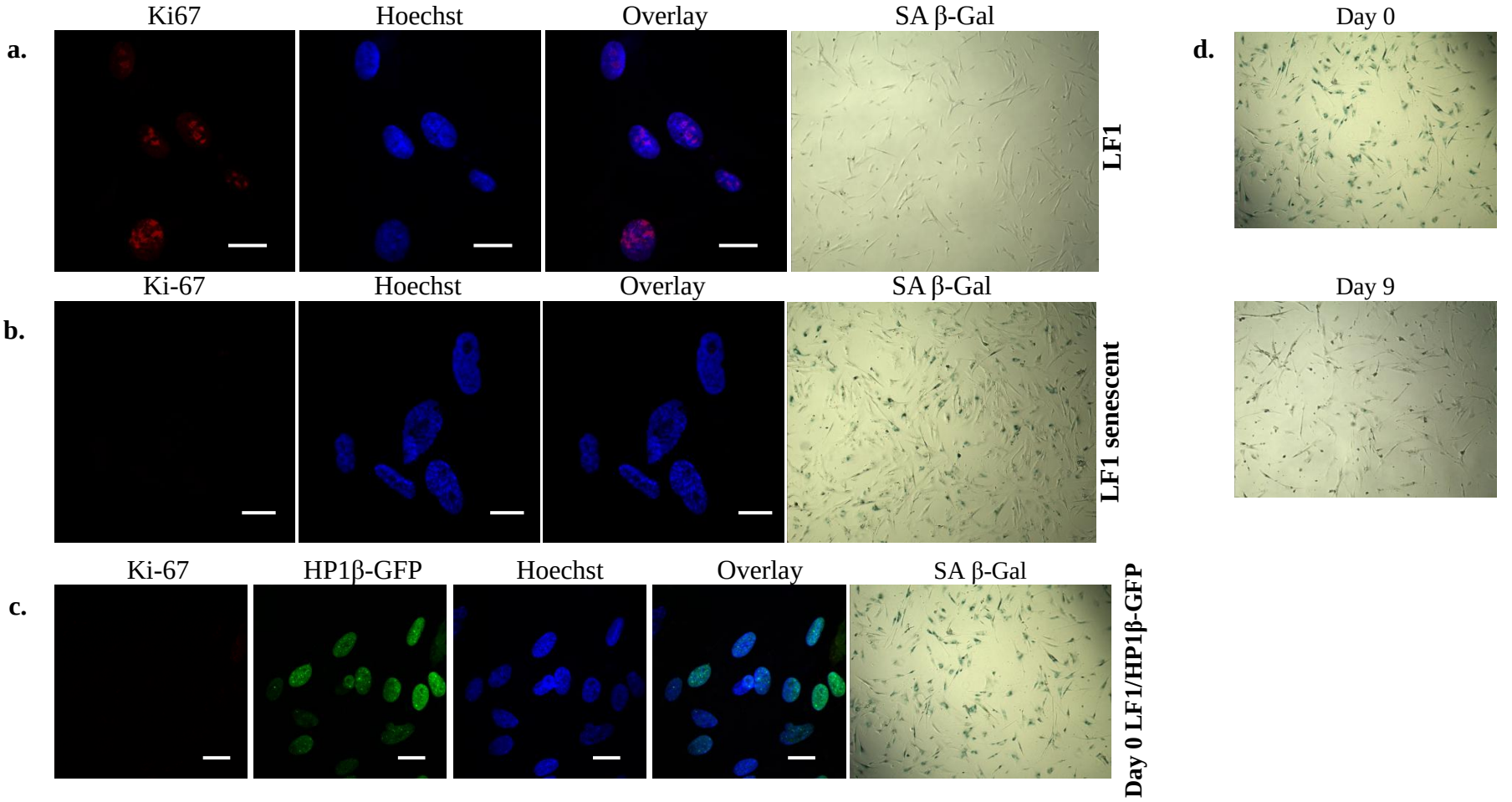
Supplementary Figure S1: Characterisation of the iPS line #2.4. **(a)** iPS line #2.4 is female (XX) and possesses a normal (46) chromosome complement. **(b)** iPS line #2.4 stains positively for the embryonic stem cell marker alkaline phosphatase. **(c)** Bisulphite sequencing of the *Oct4* and *Nanog* genes was undertaken in H9 hES cells, the iPS lines #2.4 and #2.2 and the LF1 fibroblast cell line from which iPS cell lines were derived. Analysis of the methylation levels was performed at 7 methylated CpGs in the Oct4 gene and at 2 methylated CpGs in the Nanog gene. **(d)** Control H9 hES cells and iPS cell line #2.4 are both positive for six stem cell markers, Tra1-60 (green), Sox2 (red) SSEA4 (green), Oct4 (red) Tra1-81 (green) and Nanog (red). **(e)** The iPS line #2.4 can differentiate into all three germ layers. Line #2.4 can differentiate into Pax6-positive ectoderm, Brachyury-positive mesoderm and Sox17-positive endoderm. The scale bar in all panels is 20 μm.



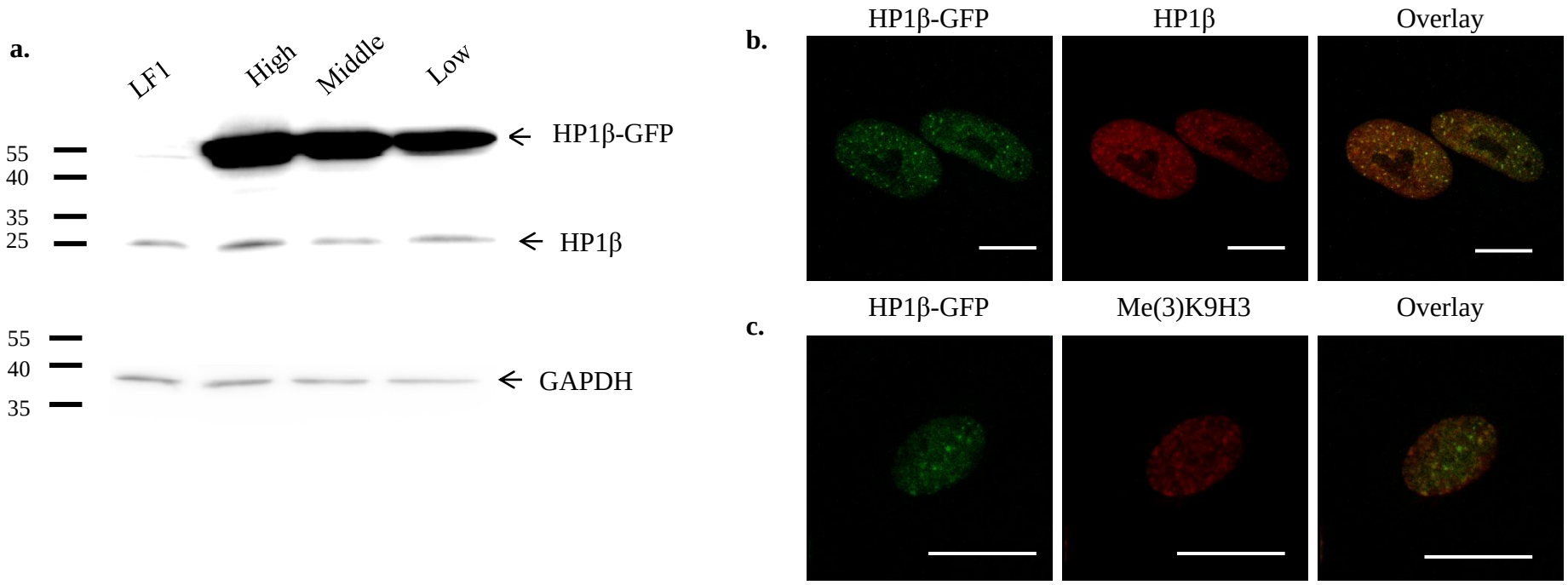
Supplementary Figure S2a: Full size blots presented in Fig 1b. Each column represents a single blot that was probed multiple times. In the left hand column the blot was probed first with anti-HP1β then stripped and re-probed with anti-H3, followed by anti-GAPDH and finally anti-HP1γ. The anti-HP1β and anti-HP1γ antibodies are of different species (see Materials and Methods). In the right hand column the blot was first probed with anti-HP1α then re-probed with anti-H3 and finally with anti-GAPDH. H3 and GAPDH are the loading controls (protein loading amount 20 µg). The original marker lanes of each blot are pasted on the left side of the each corresponding blot using Fusion-Capt Software for Fusion Chemiluminescence Imaging System .



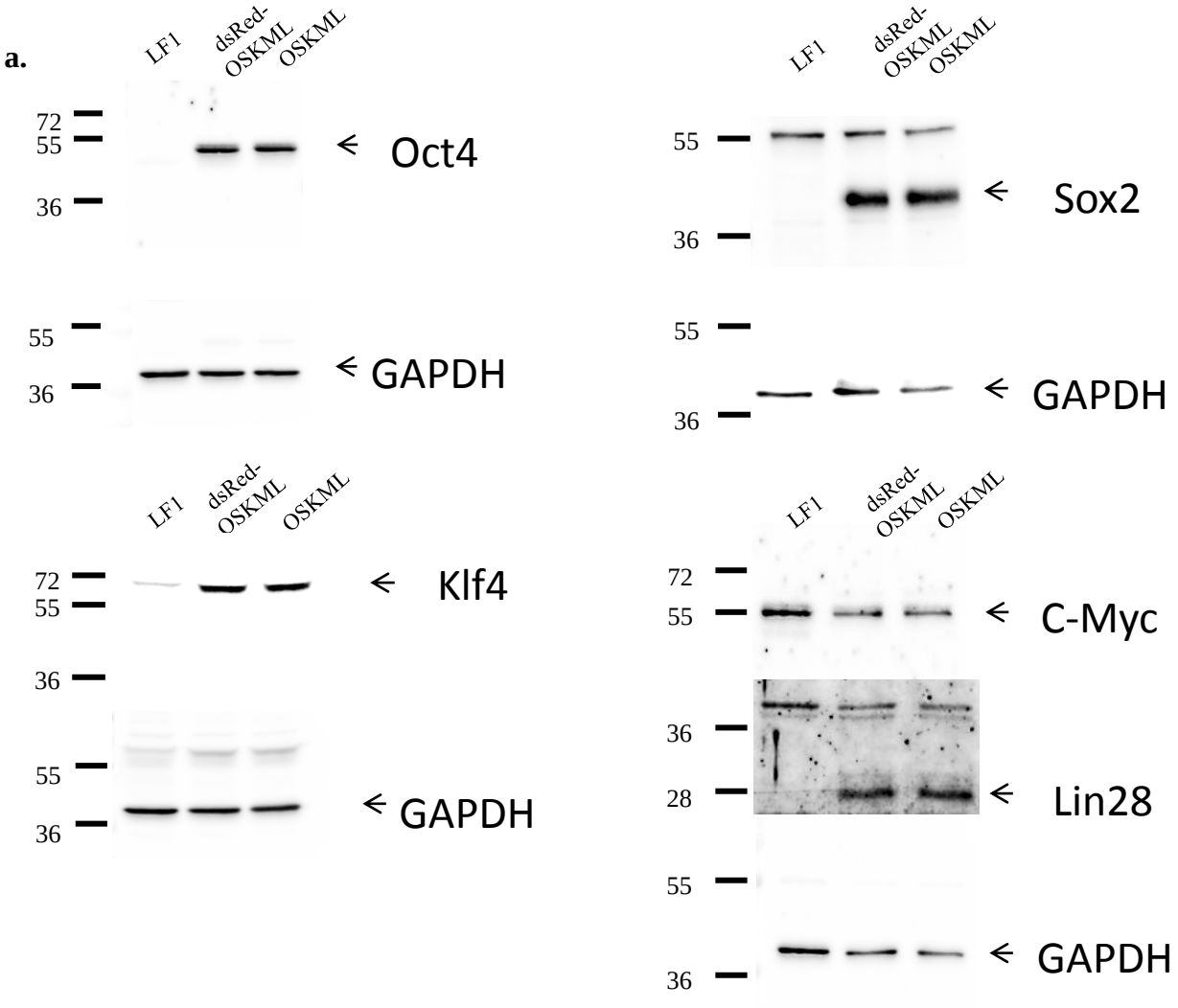
Supplementary Figure S2b: Full size blots presented in Fig 1b. Each column represents a single blot that was probed multiple times. In the left hand column the blot was probed first with anti-HP1β then stripped and re-probed with anti-H3, followed by anti-GAPDH and finally anti-HP1γ. The anti-HP1β and anti-HP1γ antibodies are of different species (see Materials and Methods). In the right hand column the blot was first probed with anti-HP1α then re-probed with anti-H3 and finally with anti-GAPDH. H3 and GAPDH are the loading controls (protein loading amount 30 µg). The original marker lanes of each blot are pasted on the left side of the each corresponding blot using Fusion-Capt Software for Fusion Chemiluminescence Imaging System .



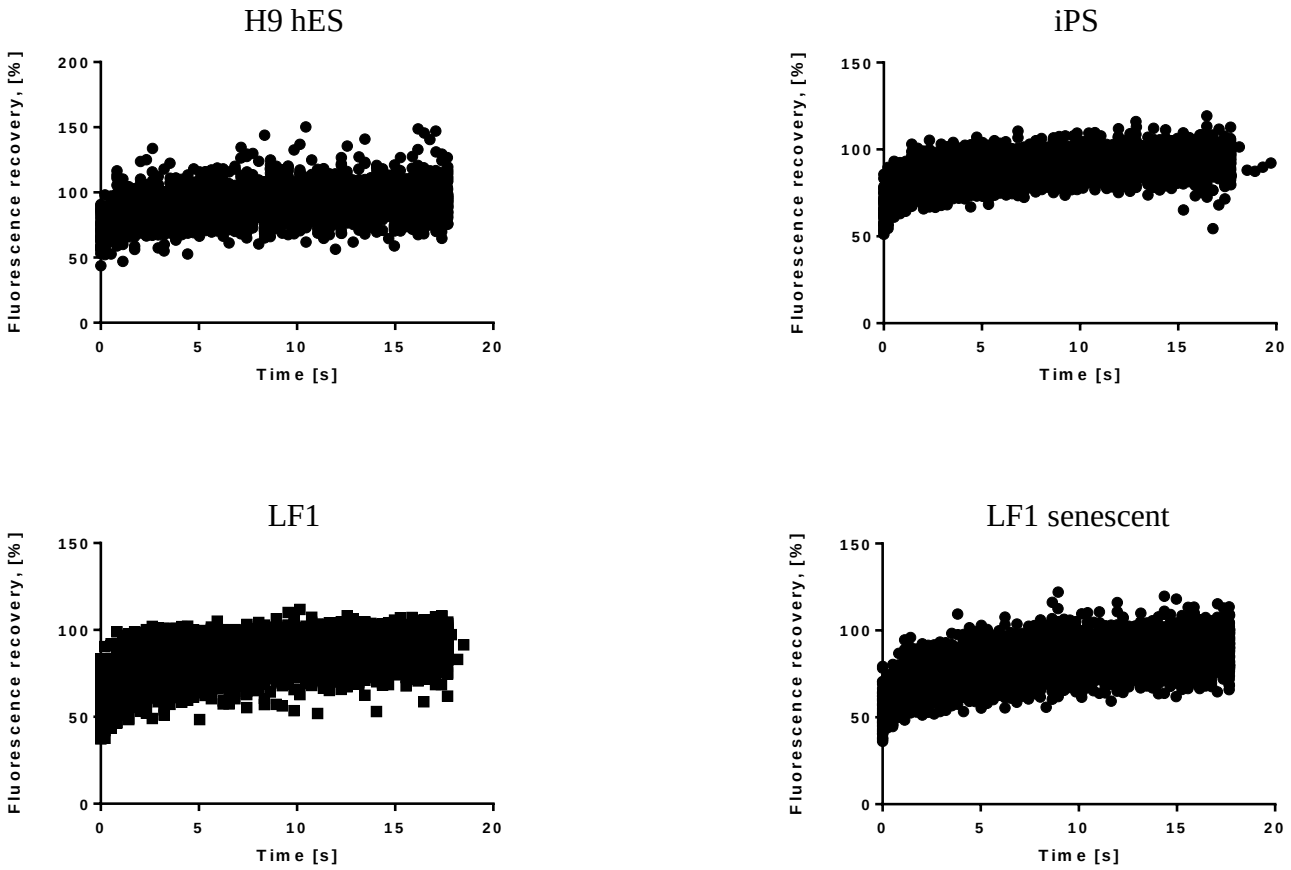
Supplementary Figure S3:Characterisation of young and senescent LF1 fibroblasts and Day 0 senescent LF1/HP1β-GFP fibroblasts. (a) Young, dividing, LF1 fibroblasts are positive for Ki-67 and negative for SA β-galactosidase staining. **(b)** Senescent LF1 fibroblasts are negative for Ki-67 staining and positive for SA β-galactosidase. **(c)** Day 0 senescent LF1/HP1β-GFP fibroblasts, before lipofection with dsRed-OSKML, are negative for Ki-67 and are positive for SA β-galactosidase staining. **(d) S**enescent LF1/HP1β-GFP fibroblasts are positive for SA β-galactosidase at Day 0 (90 ± 3) % and Day 9 (49 ± 7) %. The scale bar in all panels is 20 µm.



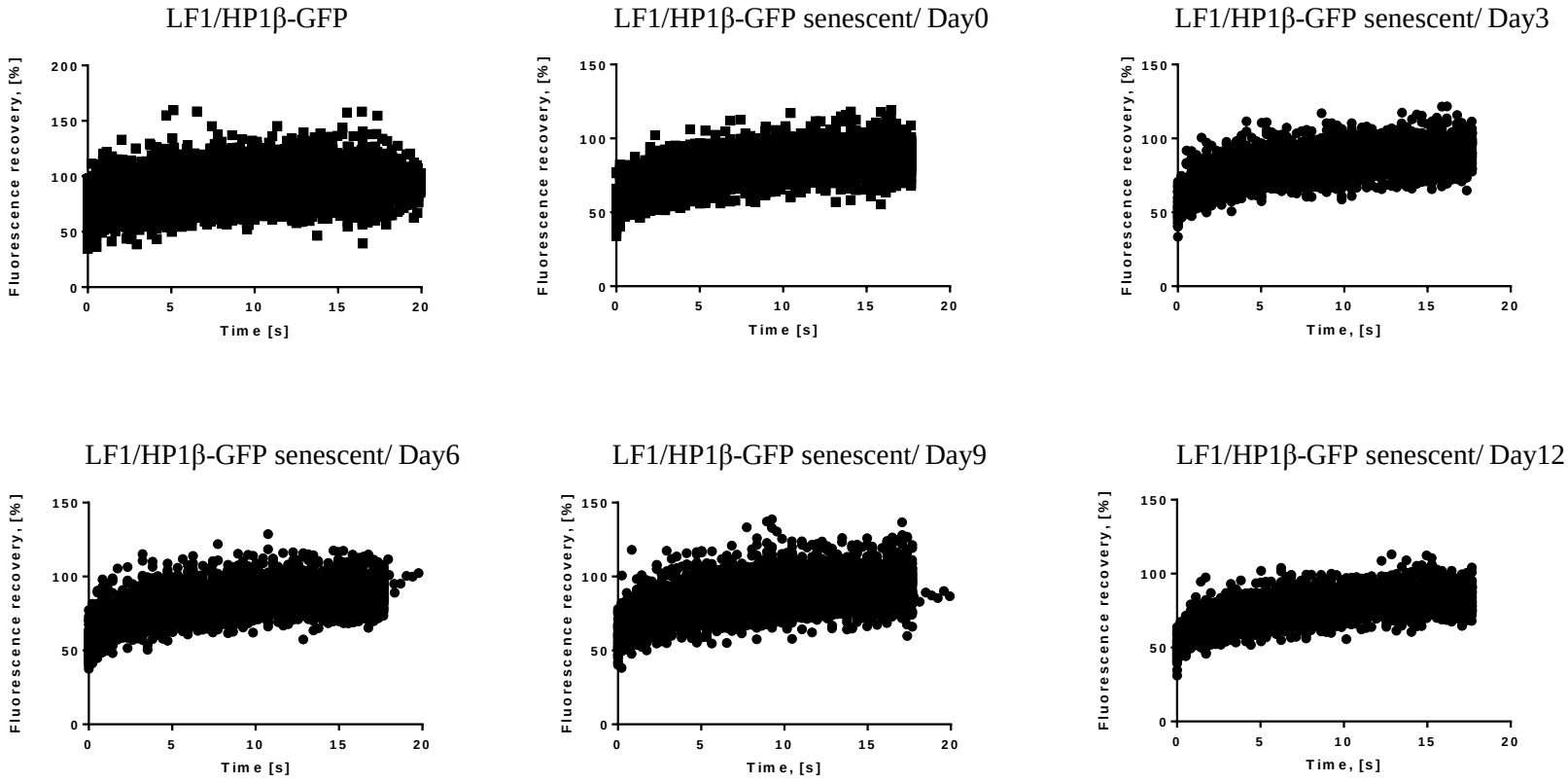
Supplementary Figure S4: HP1β-GFP expression in LF1/HP1β-GFP fibroblasts. (a) High, Medium and Low expressing LF1/HP1β-GFP fibroblasts express higher levels (at least 20 times higher) of HP1β-GFP compared to endogenous levels of HP1β. The "Low" LF1/HP1β-GFP fibroblasts fraction was used for all experiments in this paper. **(b)** HP1β-GFP and endogenous HP1β co-localise in LF1/HP1β-GFP fibroblasts. **(c)** HP1β-GFP and Me(3)K9H3 co-localise in LF1/HP1β-GFP fibroblasts. The scale bar in all panels is 20 μm.



Supplementary Figure S5: Expression of the five reprogramming factors from dsRed-OSKML after lipofection into LF1 cells. **(a)** The protein products of the reprogramming factors, *Oct4*, *Sox2*, *Klf4*, *cMyc* and *Lin28* can be detected in cellular extracts from LF1 cells lipofected with the newly developed construct dsRed-OSKML. The expression levels of the five factors in extracts from dsRed-OSKML lipofected LF1 fibroblasts is the same as that from LF1 cells lipofected with the parental OSKML vector. The blots were partially cropped for conciseness. **(b)** dsRed expression after lipofection of dsRed-OSKML /hyPBase constructs into LF1/HP1β-GFP cells; dsRed (red), Hoechst (blue). Estimated transfection efficiency is 44 ± 11 %. The scale bar in magnified upper panel is 100 µm, in the lower panel it is 1000 µm.



Supplementary Figure S6: HP1β FRAP measurements for H9 hES, iPS, young LF1 and LF1 senescent cells. The diagrams depict the individual HP1β FRAP measurements for H9 hES (n= 60), iPS (n= 55), young LF1 (n= 80) and LF1 senescent cells (n= 94). The non-linear regression analyses of these data are shown as pairwise comparisons in Figure 2.



Supplementary Figure S7: HP1β FRAP measurements for LF1/HP1β -GFP fibroblasts. The diagrams depict the individual HP1β FRAP measurements for young LF1/HP1β -GFP fibroblasts (n= 149), senescent LF1/HP1β-GFP fibroblasts at day 0 (just before lipofection with dsRed-OSKML), (n= 60); at day 3 (3 days after lipofection with dsRed-OSKML), (n= 55); at day 6 (6 days after lipofection with dsRed-OSKML), (n= 63); at day 9 (9 days after lipofection with dsRed-OSKML), (n= 60); and at day 12 (12 days after lipofection with dsRed-OSKML), (n= 47). In Fig. **3a-e** the non-linear regression analyses of these data are shown as pairwise comparisons of the young LF1/HP1β-GFP fibroblasts against the senescent LF1/HP1β-GFP fibroblasts at days 0, 3, 6, 9 and 12.