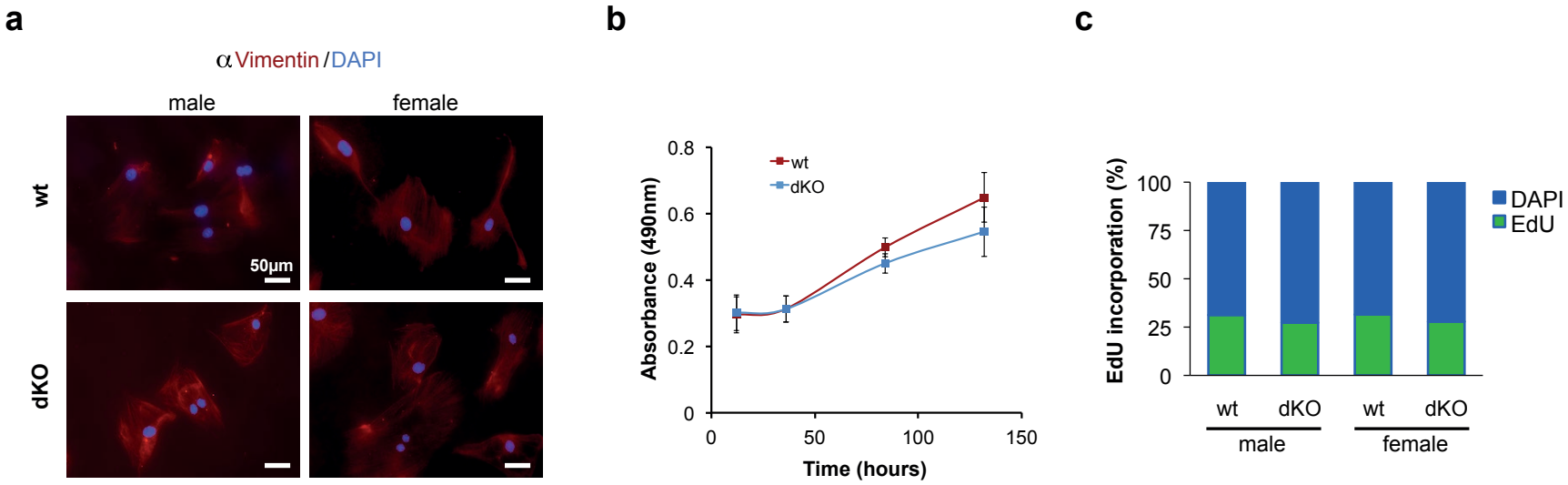
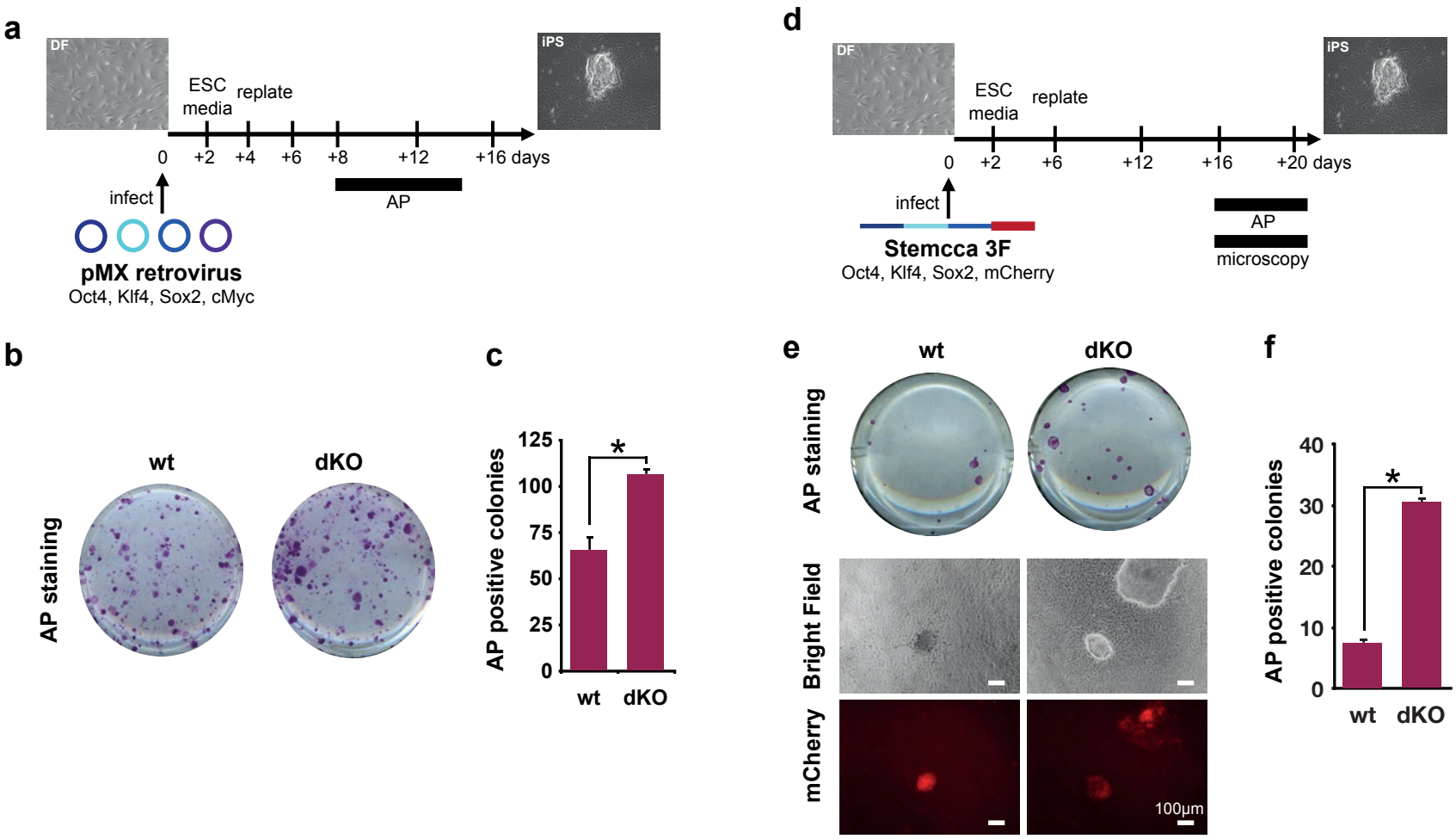
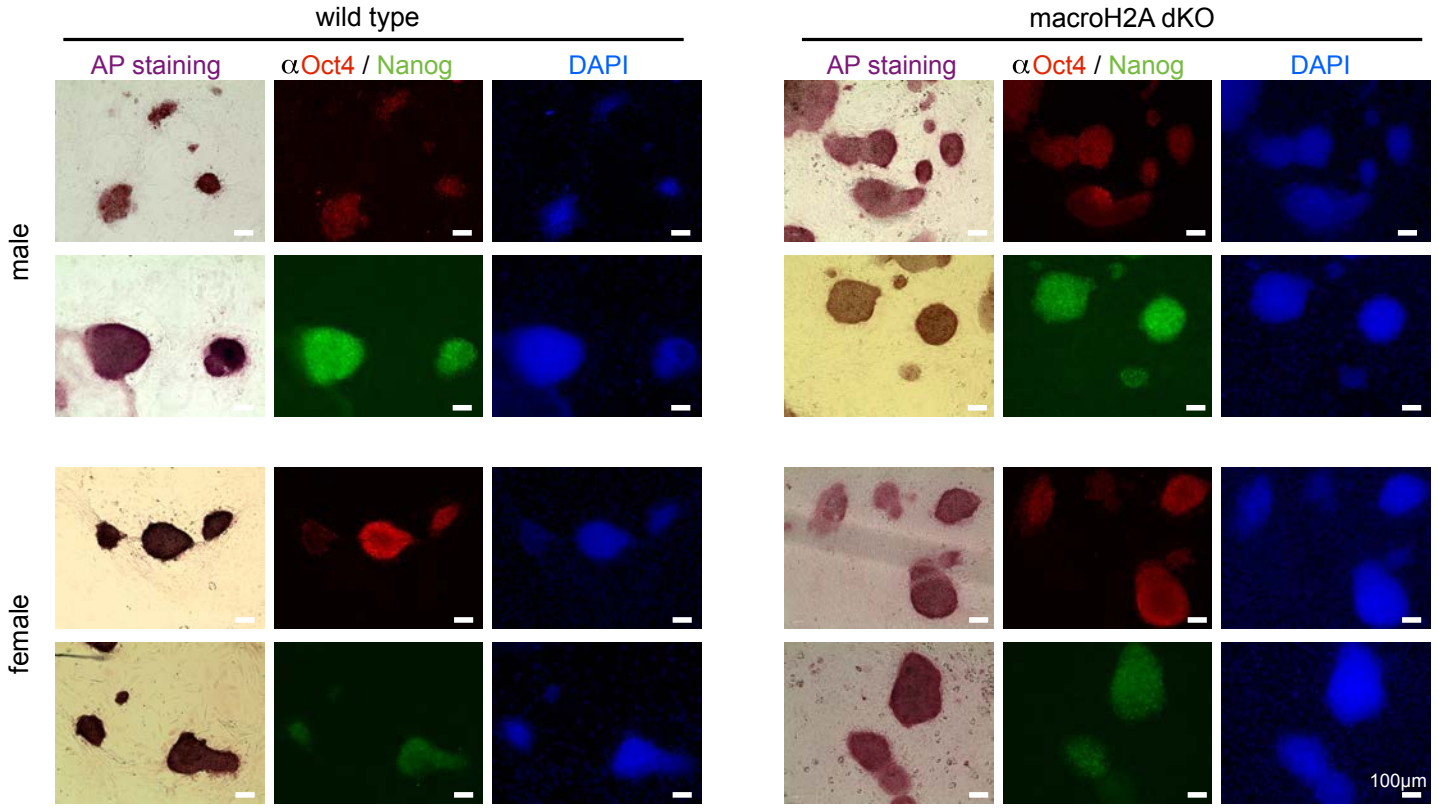


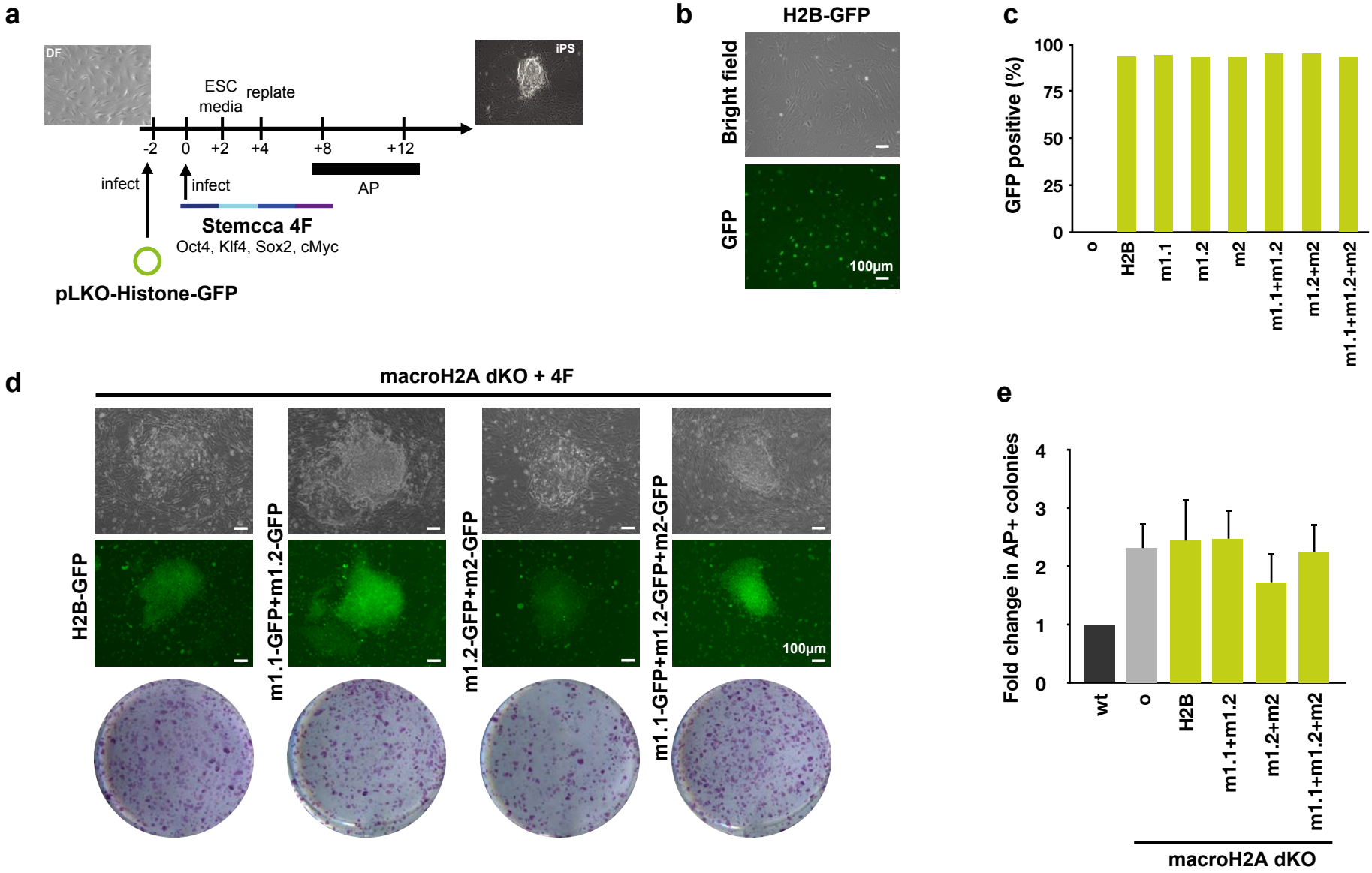
Supplementary Figure S 1. Characterization of dermal fibroblasts isolated from wild type and macroH2A double knockout (macroH2A1 -/-; macroH2A2 -/-) mice. (a) Immunofluorescence (IF) of Vimentin in both wt and dKO DFs. DAPI used to stain DNA; Scale bar, 50µm (b) MTS assay of wt and dKO DF showing proliferation using four time points (12, 36, 84 and 132 hours); mean ± s.d. (n=6), unpaired Student's test (two tailed) $p = 0.24$; male and female results combined. (c) EdU incorporation in wt and dKO DFs; EdU positive nuclei shown as percentage of the total DAPI-stained nuclei, mean (n=4).



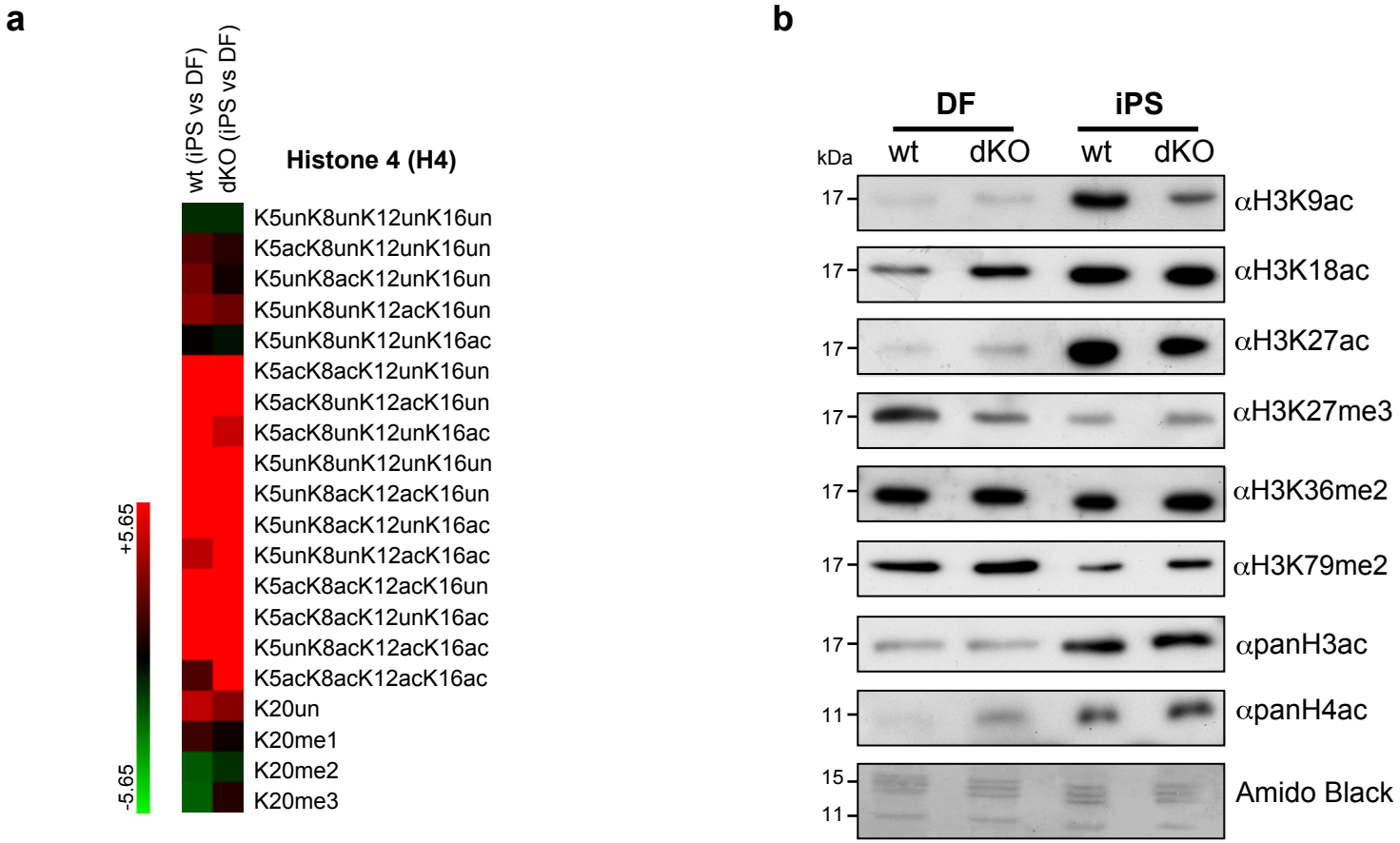
Supplementary Figure S 2. macroH2A deficiency improves iPS reprogramming efficiency using different iPS methodologies. (a) Experimental scheme of iPS reprogramming of DF using four individual pMX retroviral vectors encoding OSKM. (b) Representative wells of AP positive iPS colonies, indicating increased reprogramming efficiency in dKO DFs 14 days post-infection. (c) Number of AP positive colonies at day 14 post-infection; mean ± s.d. (n=3); unpaired Student's test (two tailed) *p*=0.02 (asterisk). (d) Experimental scheme of iPS reprogramming of DF using a polycistronic lentiviral vector (Stemcca) encoding three factors (3F) and a reporter gene (mCherry). (e) Representative wells of AP positive iPS colonies, indicating increased reprogramming efficiency in dKO DFs 20 days post-infection, bright field and mCherry images of iPS colonies; Scale bar, 100µm. (f) Number of AP positive colonies at day 20 post-infection; mean ± s.d. (n=3); unpaired Student's test (two tailed) *p*=0.0001 (asterisk).



Supplementary Figure S 3. Characterization of macroH2A wild type and dKO iPS colonies. IF of AP positive iPS colonies from both wt and dKO, male and female DFs, expressing Oct4 and endogenous Nanog, at 14 days post-infection. DAPI used to stain DNA; Scale bar, 100μm.



Supplementary Figure S 4. Expression of multiple macroH2A isoforms simultaneously during reprogramming . (a) Experimental scheme of iPS reprogramming from DFs using pLKO-based lentiviral vector for over-expression of macroH2A isoforms, and a polycistronic lentiviral vector (Stemcca) encoding four factors (4F). (b) Bright field and GFP image of infected DFs; Scale bar, 100µm. (c) Percentage of cells infected with GFP-tagged histones, as analyzed by FACS. (d) Representative wells of AP positive iPS colonies at 12 days post-infection, bright field and GFP image of single colonies shown; Scale bar, 100µm. (e) Quantitation of fold-change in AP positive colonies over wt; [o] represents uninfected; mean ± s.d. (n=3).

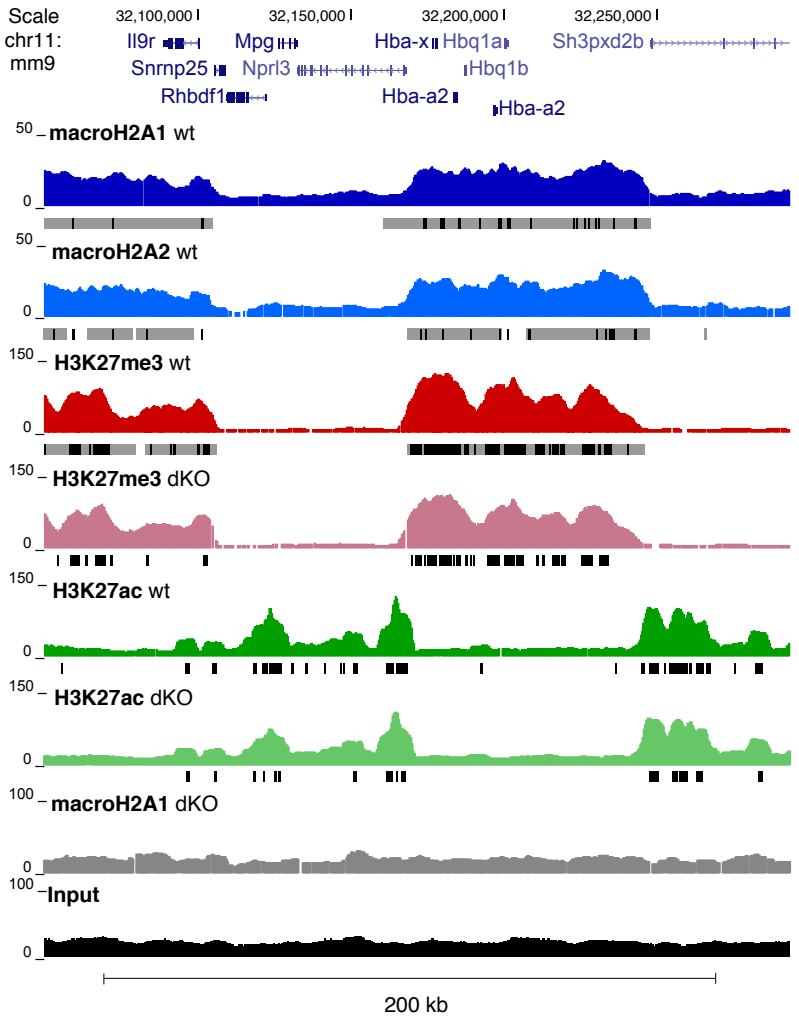


Supplementary Figure S5. qMS heatmap of Histone 4 (H4) PTMs and validation of Histone PTM changes by immunoblot. (a) qMS heatmap of H4 PTMs comparing iPS cells with DFs, in wt and macroH2A dKO cells. Note increased levels of acetylation in both wt and dKO cells after reprogramming. (b) Chromatin extracts from wt and macroH2A dKO DFs and iPS cells probed for Histone PTMs; Amido Black of core histones used for loading.

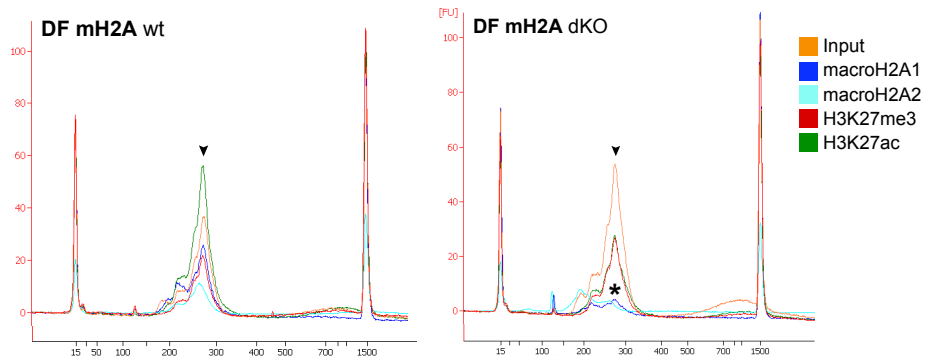
a

		mH2A1		mH2A2	H3K27me3		H3K27ac	
	Input	WT	DKO	WT	WT	DKO	WT	DKO
Raw reads	134989009	136521456	52818348	80960398	71365535	56322898	39631148	58286340
Bowtie alignments (wiggle)	84038942	76593240	22298153	62803448	51817491	42454756	29580671	43093454
Alignments analyzed (MACS)	72501485	47995347	11390081	46205381	44238865	36191678	26549590	36908463
Total Peak count (MACS)	-	156296	90656	165617	132437	110575	67873	65882
Total peak length (bp) (MACS)	-	65385906	30293877	112170432	85980203	63377672	79925863	74414216
Total domains (Sicer)	-	32339	-	55751	31517	-	-	-

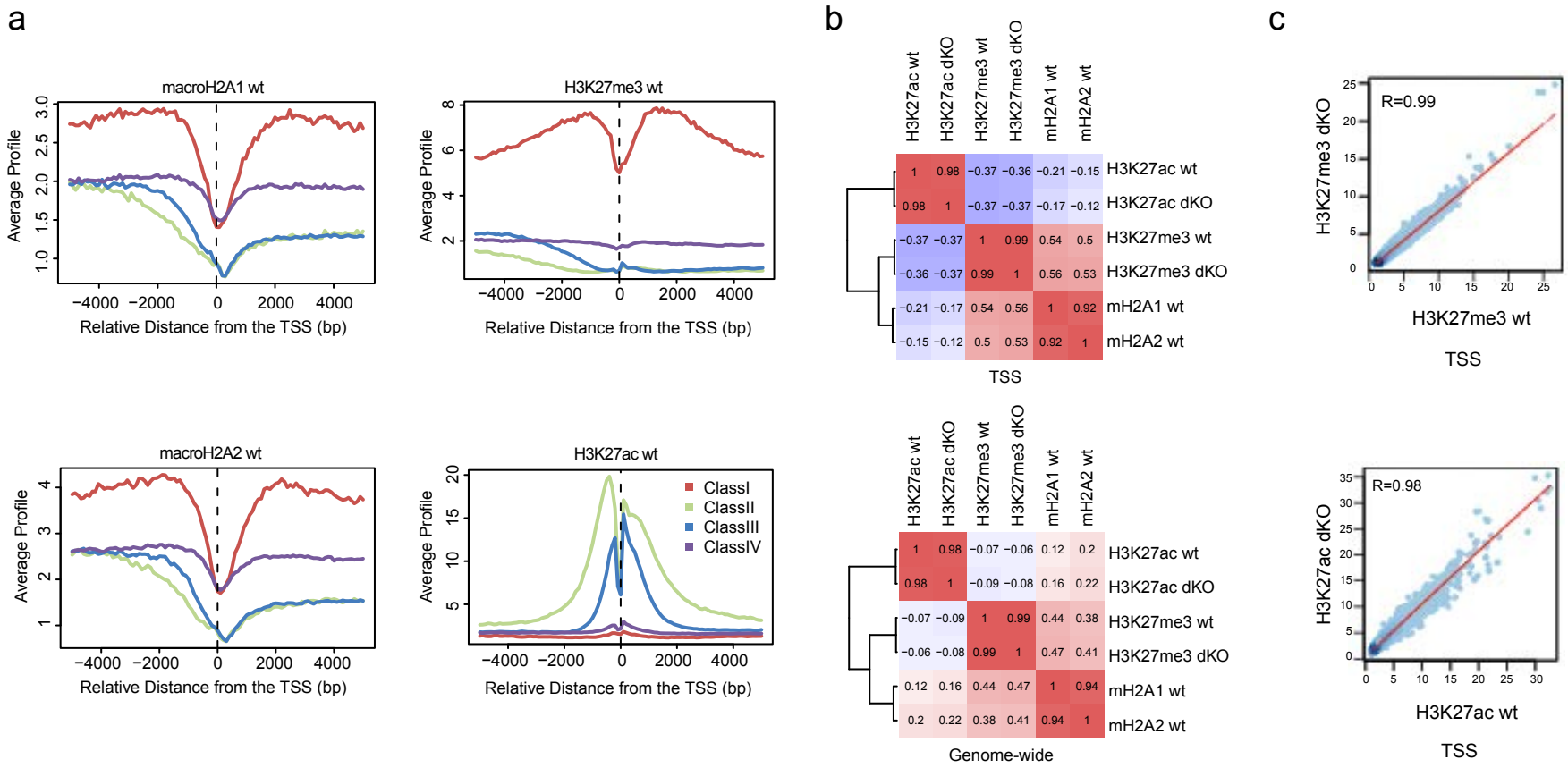
b



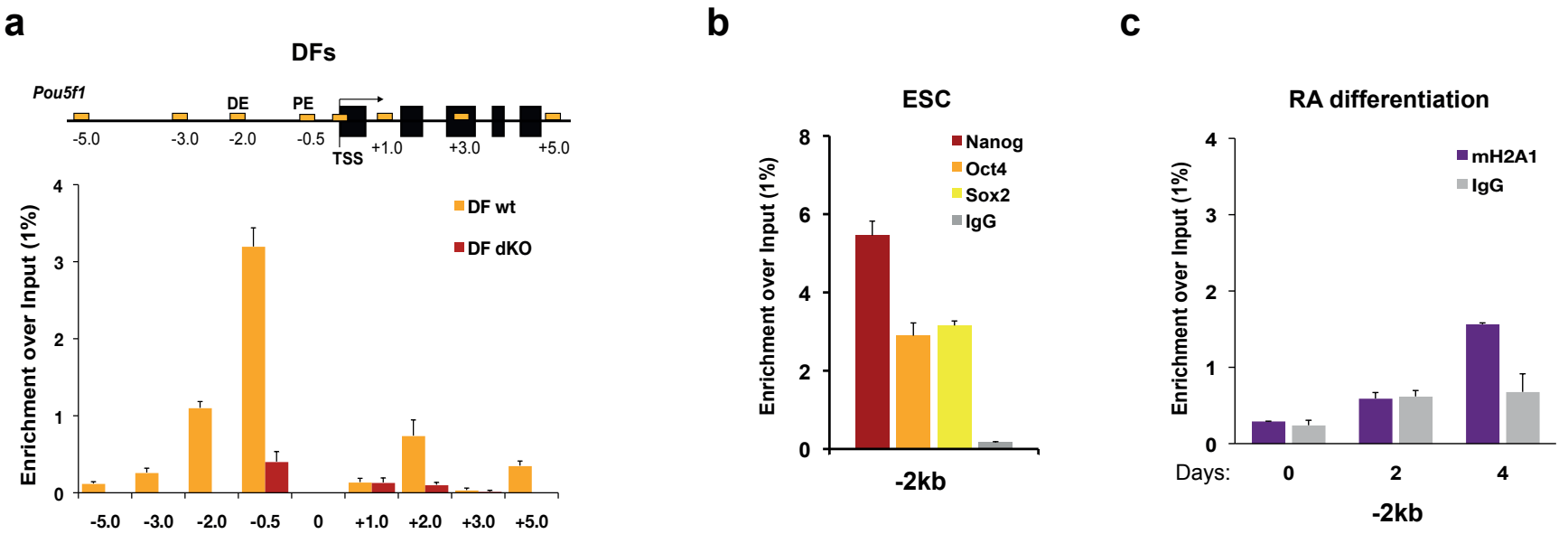
c



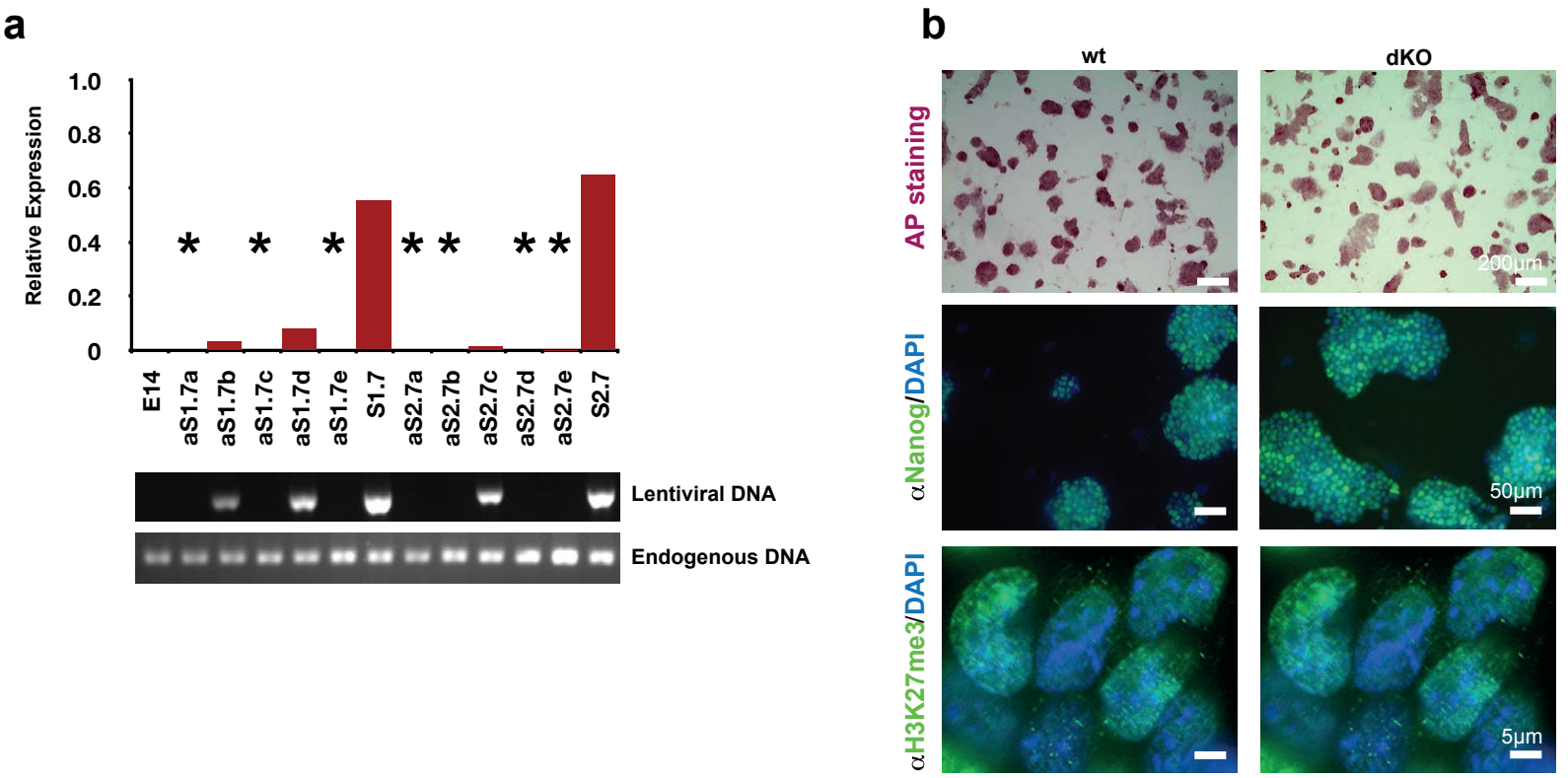
Supplementary Figure S6. ChIP-seq in macroH2A wild type and dKO DF cells. (a) Raw number of reads obtained by Illumina Hi-Seq, total number of alignments and alignments used for peak calling for macroH2A1, macroH2A2 H3K27me3 and H3K27ac. (b) UCSC genome browser snapshot for ChIP-seq profiles of macroH2A1, macroH2A2, H3K27me3 and H3K27ac at the alpha globin cluster (Hba genes). MACS peaks (black bars) and Sicer domains (grey bars) are annotated under each profile. ChIP-seq performed with macroH2A1 in macroH2A dKO cells (dKO) and Input were used to confirm specificity of ChIP-seq signals. (c) Bioanalyzer traces of Input, macroH2A1, macroH2A2, H3K27me3 and H3K27ac ChIP-seq library DNA from wild type and dKO dermal fibroblasts; arrow represents mononucleosomes. Note low levels of DNA in macroH2A1 and macroH2A2 ChIP in dKO cells (asterisk).



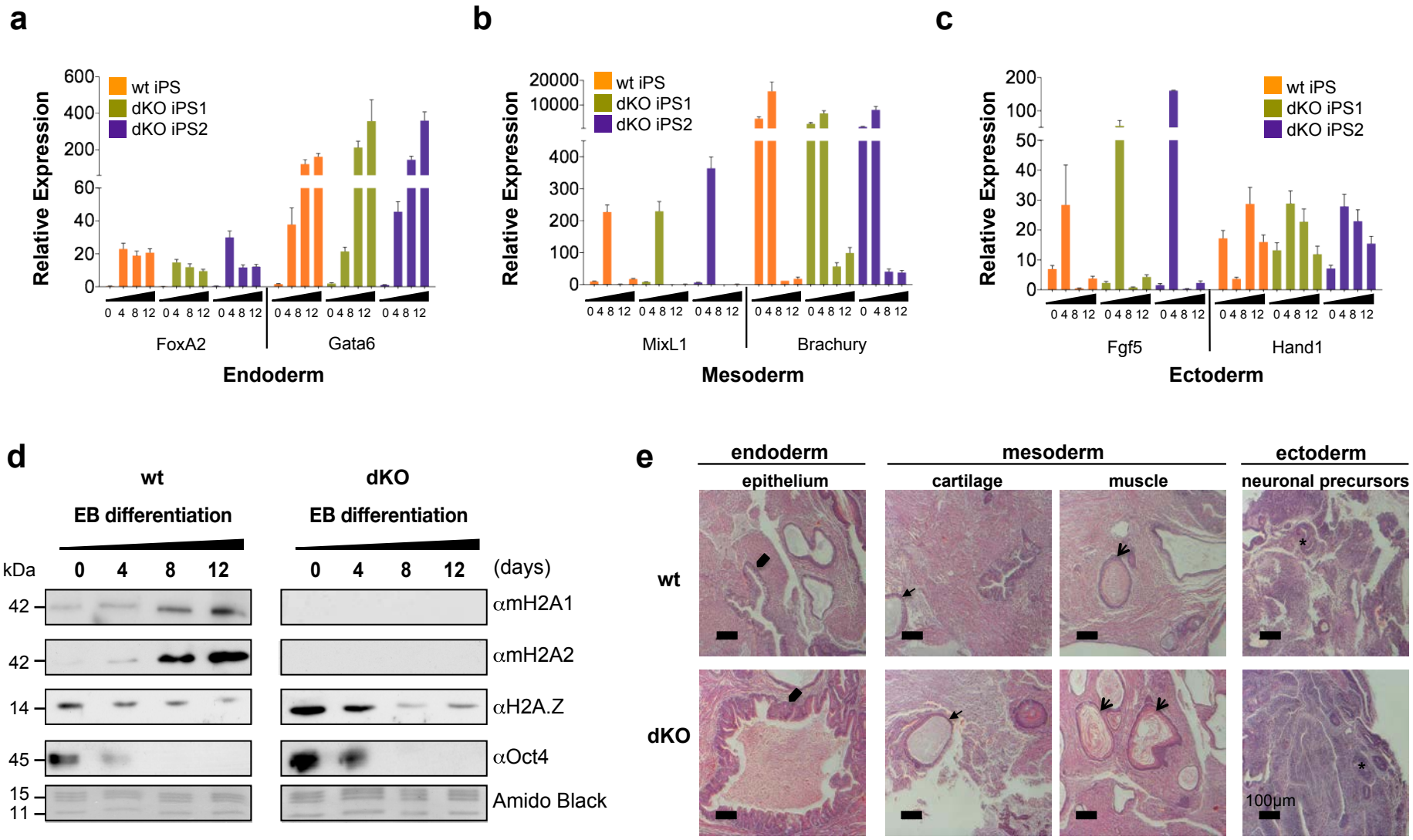
Supplementary Figure S7. ChIP-seq analysis in macroH2A wt and dKO DFs. (a) Average signal of ChIP-seq reads around the transcriptional start site (TSS) of the four classes of genes identified by K-means clustering, for each chromatin mark. (b) Correlation heatmap of H3K27 modifications and histone variants macroH2A1 and macroH2A2 performed with ChIP-seq reads from macroH2A wt and dKO DFs, 5Kb around the TSS and genome-wide, using autosomes. Hierarchical clustering shows that macroH2A variants correlate with H3K27me3. (c) Correlation plot of H3K27me3 and H3K27ac in TSS analysis of autosomal genes of ChIP-seq reads from macroH2A wt and dKO DFs. Pearson correlation value top left.



Supplementary Figure S8. ChIP analysis of macroH2A1 at the *Oct4* locus.(a) Native ChIP for macroH2A1 followed by qPCR demonstrates deposition of macroH2A1 upstream of the transcription start site (TSS) of the Oct4 gene in mouse DFs (*Pou5f1* , chr17: 35642982-35647721); mean ± s.d. (n=3). Map of the Oct4 locus with primer positions shown above; DE (distal enhancer) and PE (proximal enhancer). (b) Cross-linked ChIP in ESCs for Oct4, Nanog and Sox2 occupancy at the -2kb position of Oct4, mean ± s.d. (n=3). (c) Cross-linked ChIP-qPCR analysis of macroH2A1 at the -2kb position shows enrichment upon ESC differentiation by RA.



Supplementary Figure S 9. Characterization of macroH2A wild type and dKO iPS cells. (a) Excision of Stemcca lentiviral vector integration was performed by qPCR of DNA isolated from iPS clones after adenoviral transduction of Cre-recombinase. Lentiviral-free clones (marked by an asterisk) were then expanded and further analyzed. Primers for endogenous DNA were used as a control for the qPCR. (b) Bright field and IF of iPS cells derived from wt and dKO DFs displaying AP and Nanog positive colonies; H3K27me3 IF showing no evidence of Xi and similar levels of H3K27me3; DAPI used to stain DNA; Scale bars: 200μm (AP staining), 50μm (Nanog), 5μm (H3K27me3).



Supplementary Figure S10. Characterization of the differentiation potential of macroH2A dKO iPS cells. (a-c) qRT-PCR analysis of EB differentiation of wt and dKO iPS cells as described in Fig. 6D. Lineage markers from all three germ layers shown; relative expression is plotted using ribosomal L7 as a house-keeping gene, and normalized to ESC basal expression, mean ± s.d. (n=3). (d) Immunoblots of H2A variants using the chromatin fraction of cells from an EB differentiation time course (12 days). Increased macroH2A deposition is observed in wt but not dKO cells; Amido Black stain of histones is used for loading. Blots represent 2 independent EB differentiations. (e) Hematoxylin and eosin staining of representative wt and dKO teratomas derived from iPSCs, exhibiting ectoderm, mesoderm, and endodermal differentiation. Arrows and asterisks depict respective structures; scale bar, 100μm. One of two independent experiments shown (see also Fig. 6d).

Supplementary Methods

Cell culture and viral production. Dermal fibroblasts (DFs) and mouse embryonic fibroblasts (MEFs) were grown in DMEM (CellGro) with 10% FBS and 1% Pen/Strep. ESCs and iPSCs were maintained on 0.1% gelatin-coated plates or on a feeder layer of irradiated MEFs, and grown in DMEM supplemented with 15% FBS (Benchmark), 1 mM L-glutamine, 0.1 mM nonessential amino acids, 1% Pen/Strep, 1 mM sodium pyruvate, 0.1 mM 2-mercaptoethanol and recombinant LIF. HEK293T cells used for viral production were grown in DMEM with 10% FBS, 1% Pen/Strep. Cells were plated at a density of 2×107 cells per 150mm dish. The next day, cells were transfected with 60µg of DNA using Lipofectamine 2000 (Invitrogen). Supernatant was collected 48h, 60h and 72 h post-transfection, combined and filtered through 45µm filters. Viral supernatants were concentrated by ultracentrifugation (50,000g for 3h at 4ºC) and resuspended in PBS (Stemcca or pMX) or using Amicon (Millipore) column centrifugation (3,000g for 30 min at 4ºC) for GFP-tagged histones.

Plasmids. The iPS reprogramming 4F (Oct4, Sox2, Klf4 and Myc) are encoded individually in the pMX retroviral vector, or together in a 3 Factor (with reporter gene mCherry) polycystronic lentiviral vector (Stemcca, kindly provided by Gustavo Mostoslavsky, Boston University). GFP-tagged rat macroH2A1.1, rat macroH2A1.2, and human macroH2A2 constructs were cloned into lentiviral pLKO.1 backbone and human H2B-GFP is encoded in pLKO.1.

Proliferation and EdU incorporation. For the MTS proliferation assay, DFs were plated in 96-well plates (1,000 or 2,000 cells per well, one plate per time point) using six technical replicates and four biological samples per condition (wt and dKO). Time points were taken using the MTS tetrazolium compound (3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium) according to the manufacturer's instructions (Promega). Absorbance values (490 nm) were recorded using a BIOTEK Microplate Reader. P value was

calculated between wt and dKO using an unpaired Student's t-test (two tailed). For EdU incorporation (Invitrogen), dermal fibroblasts were plated in 8 chamber slides (20,000 cells per chamber) using two technical replicates and four biological samples per condition (wt and dKO). 5-ethynyl-2'-deoxyuridine (EdU) was added to the media and washed out after 12h. Detection of EdU incorporation was performed as described in the manufacture's instructions, and EdU/DAPI signals were detected using a fluorescent microscope, Number of cells were counted using Image J software.

Lentivirus excision. Excision of the lentiviral vector was performed using an adenovirus expressing Cre-recombinase. iPS clones were trypsinized and 10,000 cells incubated for three hours with $1x10^8$ PFU of adeno-Cre-GFP (Vector Labs) at 37ºC and 0.5ml of ESC media. Cells were then plated onto 100mm dish plates and colonies were picked after 10-15 days. Lentiviral -free clones were then expanded and tested for the presence of the lentiviral vector using primers for the DNA of the Stemcca construct (cMYC F 5' -GGA ACT CTT GTG CGT AAG TCG ATA G-3'; WPRE R 5' -GGA GGC GGC CCA AAG GGA GAT CCG-3') and endogenous DNA was used as qPCR control, using the promoter region of the Cbx7 gene, 5Kb upstream of the TSS (F 5'-CTC CAG CCC CAT AAT TTG AA- 3'; 5' - AAC CAA GCC CTC ACT GCT TA- 3').

ChIP and qRT-PCR Primers

Native ChIP primers

Pou5f1 gene locus (Chromosome 17: 35,642,982-35,647,721)

-5.0Kb	CACTGGTGGTGTGAGCAAGT	TGGCTGGCTTAGAGTGTGAA
-3.0Kb	ACCTTTTCATGCTGGTGGAC	CTTGCCACAAACCACCTGTA
-2.0Kb	GACGGCAGATGCATAACAAA	AGGAAGGGCTAGGACGAGAG
-0.5Kb	CTGGGGACATATCTGGTTGG	CCCAGTATTTCAGCCCATGT
0Kb (TSS)	TAGGTGAGCCGTCTTTCCAC	CGAAGTCTGAAGCCAGGTGT
+1Kb	TGTGGAGTAGGGCTCACCTT	AGATCTGCACCTCAGGCACT
+3Kb	GTCCCAGCTGGTGTGACTCT	CCAAGGTGATCCTCTTCTGC
+5Kb	AGGAAGGCATTGCATATTCG	TGCCAGACAATGGCTATGAG

Formaldehyde cross-linked ChIP

Pou5f1 gene (region A)

GGAACTGGGTGTGGGGAGGTTGTA	AGCAGATTAAGGAAGGGCTAGGACGAGAG

qRT-PCR - ESC and lineage markers

Gene	**Forward primer**	**Reverse primer**
L7 (control)	AGCGGATTGCCTTGACAGAT	AACTTGAAGGGCCACAGGAA
Brachyury	CCGGTGCTGAAGGTAAATGT	CCTCCATTGAGCTTGTTGGT
Fgf5	CTGTATGGACCCACAGGGAGTAAC	GTAGAGGAGGATAACACGCACTG
Flk1	TTTGGCAAATACAACCCTTCAGA	GCAGAAGATACTGTCACCACC
FoxA2	TAGCGGAGGCAAGAAGACC	CTTAGGCCACCTCGCTTGT
Gata4	CCCTACCCAGCCTACATGG	ACATATCGAGATTGGGGTGTCT
Gata6	ACAGCCCACTTCTGTGTTCCC	CTTCTGTTTCCGATCAGCTCCCTTG
Hand1	GCGTCAGTACCCTGATGCCTTC	GCCCATGTGATCTGACACCCTGAG
MixL1	TTGAATTGAACCCTGTTGTCCC	ACTCTAGGTATCCGTCAGGGAAG
Nanog	AGGGTCTGCTACTGAGATGCTCTG	CAACCACTGGTTTTTCTGCCACCG
Nestin	CCCTGAAGTCGAGGAGCTG	CTGCTGCACCTCTAAGCGA
Oct4	CTGTAGGGAGGGCTTCGGGCACTT	CTGAGGGCCAGGCAGGAGCACGAG
Pax3	ATAAGCCCAGGACACAGAGTTGTG	CTCGGTCAAGGATGGAAGC
Rex1	CAGTCCAGAATACCAGAGTGGAA	GGTCTTCATGGATTCCTCCAGCTTC

qRT-PCR - Time course Reprogramming

Gene	**Forward primer**	**Reverse primer**
Fgf4	CAAGCTCTTCGGTGTGCCTTTC	CGTAGGCGTTGTAGTTGTTGGG
Sall1	AGCCCTTTGCTTGCACTATCTGTG	ATATGGGTGCCCATGTGGACCTTC
Sall4	TCACCACGAAAGGCAACCTGAAGG	CATTCAGGACGCTGGTGTACTGGTT

Antibody	Company	Cat #	Application
Alpha-SMA	Abcam	ab5694	IHC
FoxA2	Cell Signaling	8186S	IHC
GFP	Roche	11814460001	WB
H2A.Z	Millipore	07-594	WB
H3K18ac	Millipore	07-354	WB
H3K27ac	Abcam	ab4729	WB, nChIP
H3K27me3	Millipore	07-449	WB, nChIP, IF
H3K36me2	Millipore	07-369	WB
H3K79me2	Abcam	ab3594	WB
H3K9ac	Millipore	07-352	WB
IgG	Millipore	12-370	ChIP
macroH2A1	Abcam	ab37264	nChIP, IF
macroH2A1	Millipore	07-219	WB
macroH2A2	Pehrson and Bernstein labs		WB, nChIP
Nanog	CosmoBio	RCAB0002P-F	IF
Nestin	Stem Cell Technologies	01418	IHC
Oct4	Santa Cruz	sc8628	WB, IF
panH3ac	Millipore	06-599	WB
panH4ac	Millipore	06-866	WB
Sox2	Santa Cruz	sc17320	ChIP
Vimentin	Santa Cruz	sc7557	IF
Alexa-488 (anti-goat IgG)	Molecular Probes	A11055	IF (Secondary)
Alexa-488 (anti-rabbit IgG)	Molecular Probes	A21206	IF (Secondary)
Alexa-488 (anti-mouse IgG)	Molecular Probes	A21202	IF (Secondary)
Alexa-594 (anti-goat IgG)	Molecular Probes	A11058	IF (Secondary)
Alexa-594 (anti-rabbit IgG)	Molecular Probes	A21207	IF (Secondary)
Alexa-594 (anti-mouse IgG)	Molecular Probes	A21203	IF (Secondary)
IgG-HRP (anti-rabbit)	Millipore	12348	WB (secondary)
IgG-HRP (anti-mouse)	Invitrogen	616520	WB (secondary)