Supplemental Material to

H3K9 histone acetylation predicts pluripotency and reprogramming capacity of ES cells

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Supplementary Figures

Figure S1. Characterization of the MEF/ESC hybrid cells. (*A*) Hybrid production strategy: HPRT$^-$ ESC and Oct4/GFP/neo^R /ROSA26- β-geo; Pgk-Neo MEFs at a ratio of 5:1 were fused in the presence of Baculovirus in low pH. GFP positive hybrids were selected in the ESC medium supplemented with G-418 and HAT. (*B*) Top: Phase contrast of colony morphologies of ESC (left) and MEF (right) parental fusion partner cell lines. Bottom: Fluorescence images of GFP-positive ESC/MEF hybrid colonies. A single representative MEF/ESC hybrid cell colony and hybrids at passage 10 that express high GFP levels (bottom). (*C-D*) MEFs fused with ESC were stained with fluorescent lipids PKH26-red, and are labeled with white dots (white arrows, *C*) and MEF morphology under phase contrast (black arrow, *D*). (*E*) Fluorescence image of PKH26-red-stained MEF (black arrow). (*F*) MEF/ESC lines stained blue in the presence of 5-bromo-4-chloro-3-indolyl-β-D-galactoside demonstrating expression of the ROSA26-β-geo transgene. (*G*) Genotype assays for MEFs, R1 and MEF/ESC hybrids for neo, LacZ and GFP transgenes. (*H*) Karyotype analysis of MEF/ESC hybrid lines. Histogram of the number of chromosomes detected by metaphase chromosome spreads. (*I*) Representative karyotypes of 2n MEFs (left) and 2n ESCs (middle) and nearly tetraploid MEF/ESC hybrid cells (right). (*J*) Expression of ES (left) and somatic (right) cell markers. In each panel: lane 1: MEF; lane 2: R1 ES cells; lane 3 and 4: MEF/ESC hybrid lines. (*K*) EBs express early markers of the endoderm, mesoderm and ectoderm

germ layers. Lane 1: MEF/ESC hybrids prior to differentiation; lane 2: R1 ES cells; lane 3 and 4: 10-day-old EBs from R1 and MEF/ES hybrids, respectively.

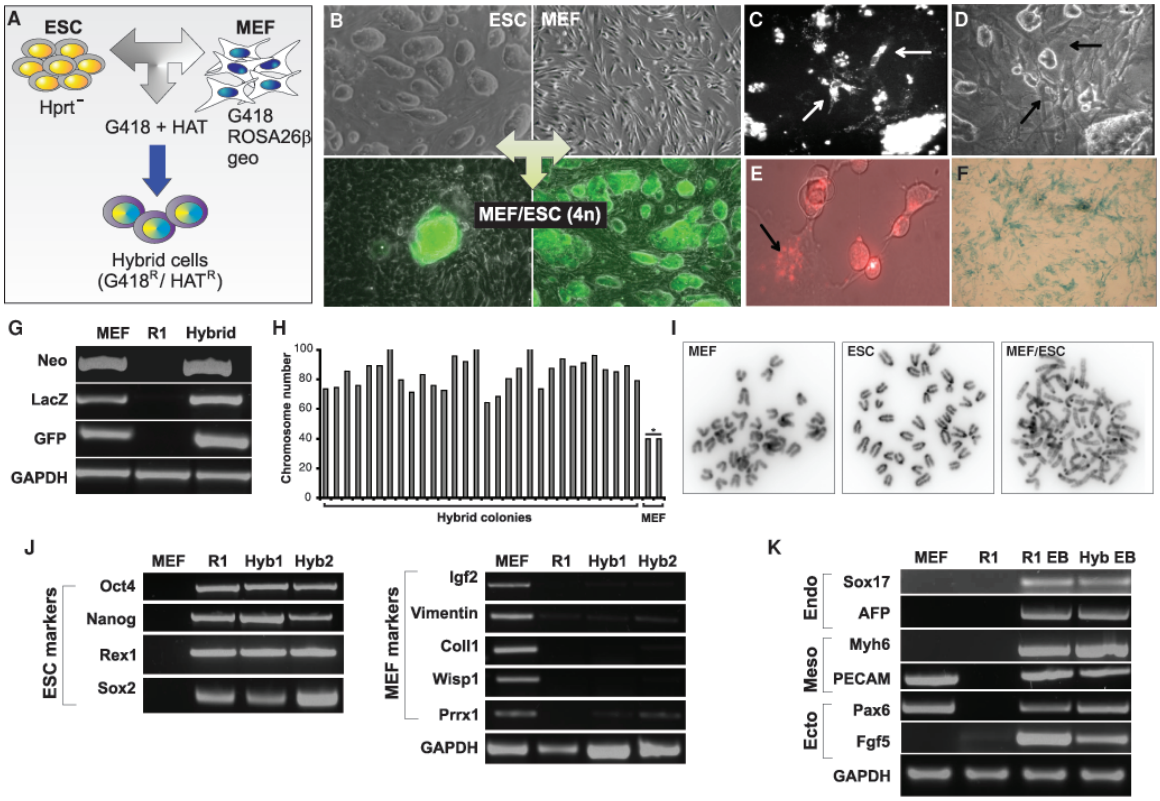


Figure S2. ESC-like properties of chromatin in MEF/ESC hybrid cells. (*A*) Chromatin morphology. DAPI images of ESC (top left), MEF/ESC hybrid cells fusion (top right) and MEFs (bottom left). ESC and MEF/ESC hybrid cells are characterized by diffuse chromatin structure while MEFs show compact heterochromatin structures. Scale bar = 10 μm. (*B*) Fluorescent recovery after photobleaching (FRAP) of HP1 in ESC (empty circles), Rogo2 MEFs (black circles) and in the Rogo2/ESC fusion products (gray circles). Values represent at least 20 cells from 2 independent experiments.

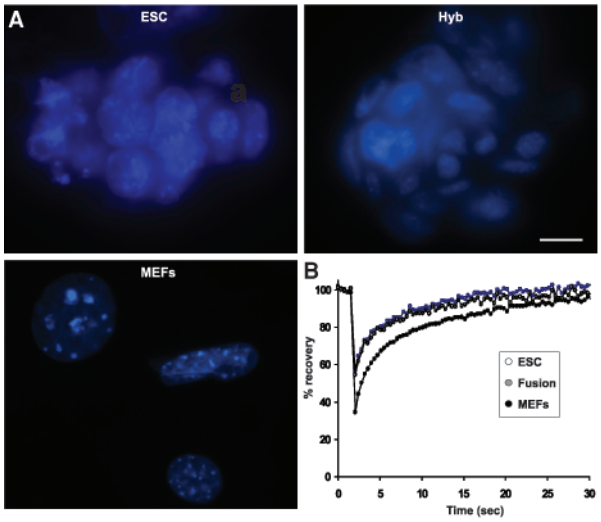


Figure S3. Microarray analysis of E14 ESCs treated with low levels VPA for 16 hrs. (**a**) Scatter plot of average values of 2 independent experiment of Ct Vs VPA-treated E14 ESCs for 16 hrs. Red dots represent upregulated gens and blue dots represent down-regulated genes. (**b**) Unsupervised clustering of microarray results together with previously published data. (**c**) Gene ontology (GO) analysis of the upregulated genes in response to 16 hrs of VPA treatment in E14 ESCs. GO analysis of down-regulated genes displayed no significantly altered categories. (**d**) Microarray validation of several up-regulated genes using real-time PCR.

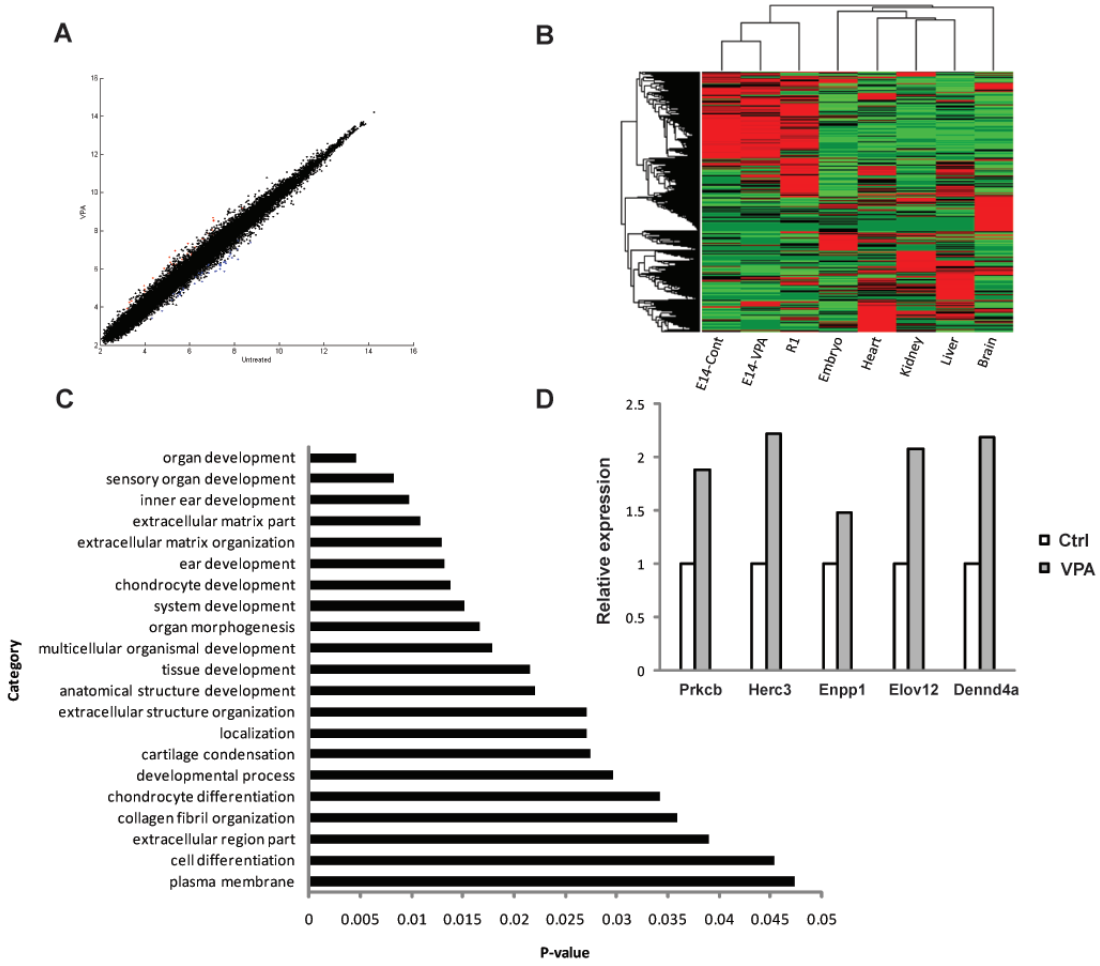


Figure S4. COL11a1 protein distribution in ESCs and MEFs and cytoplasmic translocation following VPA treatment. E14 cells plated on primary mouse fibroblasts were either untreated or treated with VPA (0.5 mM) for 4 or 16 hrs, fixed and stained with Col11a1 antibodies. Arrowheads point at MEF nuclei; double arrowheads point at MEF cytoplasms; arrow points at an small ESC colony. Untreated cells show mostly nuclear staining and some cytoplasmic staining (left); after 4 hrs both nuclear staining and cytoplasmic staining is observed, and after 16 hrs of VPA treatment, COL11a1 staining is mostly cytoplamic and some nuclear. Similar translocation was observed in ESCs but to a lesser extent.

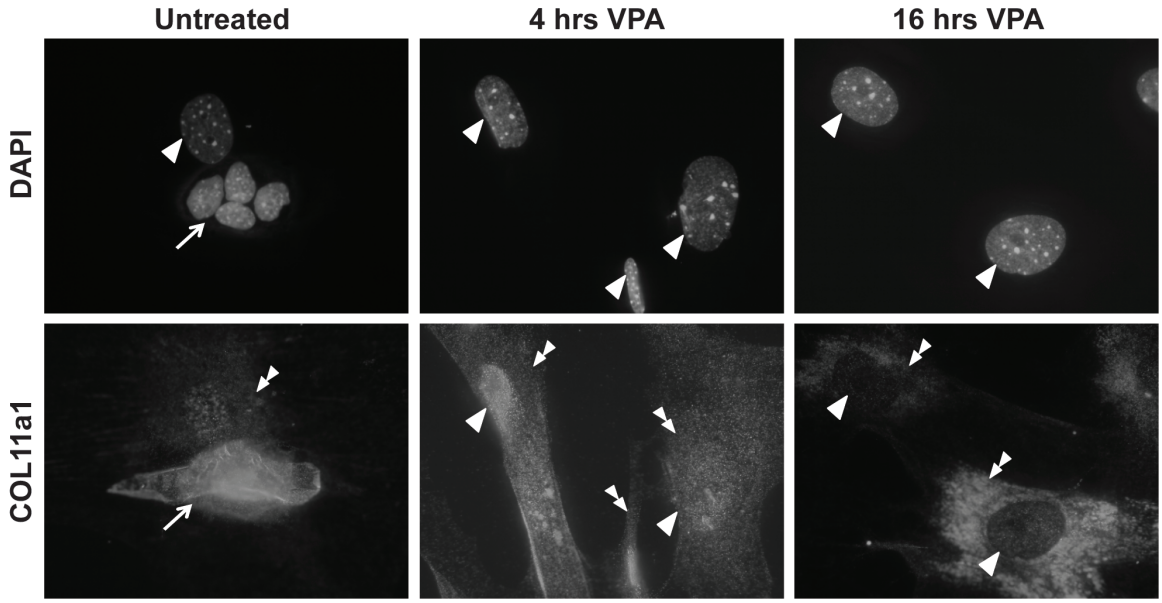
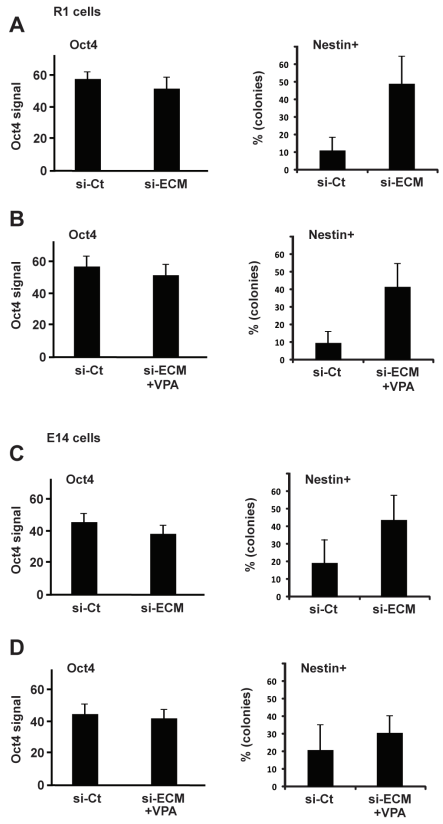


Figure S5. ECM factors are required to maintain the pluripotent state. (**a**) R1 ESCs were treated with two different siRNAs (Smartpool, Dharmacon) targeting the ECM-related genes *Acan* and *Coll11a1* for 54 hrs, fixed, and stained with anti-Oct4 (left) and anti-Nestin (right) antibodies. Control E14 ESCs were treated with non-targeting control siRNAs (Dharmacon). In ~50% of the colonies, a significant increase in Nestin levels were observed ($P < 0.01$). A significant reduction was also observed in OCT4 levels ($P = 0.014$). (**b**) The same experiment was performed as in (**a**) but in the presence of VPA. In R1 cells, the spontaneous differentiation observed following knockdown of Acan and Col11a1 was slightly inhibited by VPA. (**c**) The same experiment as in (a) using E14 ESCs. Similar results were observed. (**d**) The same experiment was performed as in (**c**) but in the presence of VPA. In E14 cells, the spontaneous differentiation observed following knockdown of ACAN and COL11a1 was almost completely prevented by VPA.



Supplementary Table 1

Gene of interest	Forward primer sequence	Reverse primer sequence	Annealing temperature/^{0}C
GFP	AACCACTACCTGAGCACCC	ACCTCTACAAATGTGG TATG	54
LacZ	ACAACGTCGTGACTGGGAAA	CGGATTGACCGTAATTGGGATA	55
neo	GATCCCCTCAGAAGAACT CGT	CTGTGCTCGACGTTGTCACTG	62
Oct4	TTGGGCTCCCTTCTTGCT	AATGGGAACAGGGAAACAT	56
Nanog	AGGGTCTGCTACTGAGATGCTC	CAACCACTGGTTTTTCTGCCAC	56
Rex1	AAAGTGAGATTAGCCCCGAG	TCCCCATCCCCTTCAATAGCA	56
Sox2	GGCAGCTACAGCATGATGCAGGAGC	CTGGTCATGGAGTTGTACTGCAGG	55
Sox17	GGAGGGTCACCACTGCTTTA	AGATGTCTGGAGGTGCTGCT	57
α-fetoprotein	AGTGCGTGACGGAGAAGAAT	TGTCTGGAAGCACTCCTCCT	58
Myh 6	CAGAGGAGAAGGCTGGTGTC	CTGCCCCTTGGTGACATACT	57
PECAM	GTCATGGCCATGGTCGAGTA	CTCCTCGGCATCTTGCTGAA	58
Pax 6	AGACTTTAACCAAGGGCGGT	TAGCCAGGTTGCGAAGAACT	58
Fgf5	GCTGTGTCTCAGGGGATTGT	CACTCTCGGCCTGTCTTTTC	56
Igf2	CCATCAATCTGTGACCTCCTCTTG	TGTTGTTCTCAGCCAGCTTTACAC	58
H19	GCACTAAGTCGATTGCACTGG	GCCTCAAGCACACGGCCACA	56
Prrx1	GCGGAGAAACAGGACAACAT	ACTTGGCTCTTCGGTTCTGA	56
Wisp1	GTC CAG GAC TTC ACA ATT GAG C	CCA GGC TTT GCT TCC ATT	55
Coll1α	CTTTGCTTCCCAGATGTCCT	CCCCATCATCTCCATTCTTG	53
Vimentin	AAAGCACCCTGCAGTCATTC	AGCCACGCTTTCATACTGCT	56