

Stem Cell Reports, Volume 2

Supplemental Information

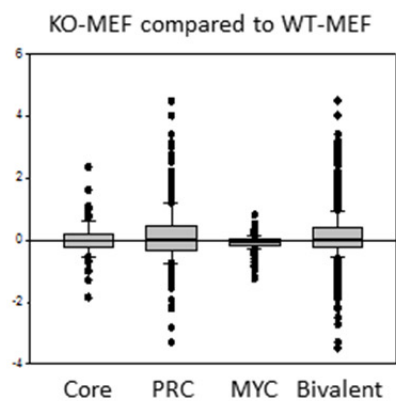
***Bright/Arid3A* Acts as a Barrier to Somatic**

Cell Reprogramming through Direct

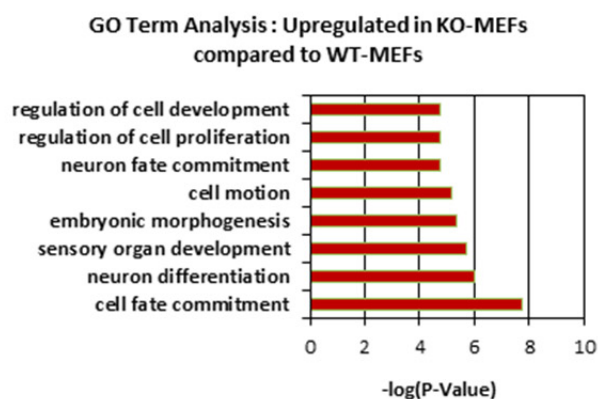
Regulation of *Oct4*, *Sox2*, and *Nanog*

Melissa Popowski, Troy D. Templeton, Bum-Kyu Lee, Catherine Rhee, He Li, Cathrine Miner, Joseph D. Dekker, Shari Orlanski, Yehudit Bergman, Vishwanath R. Iyer, Carol F. Webb, and Haley Tucker

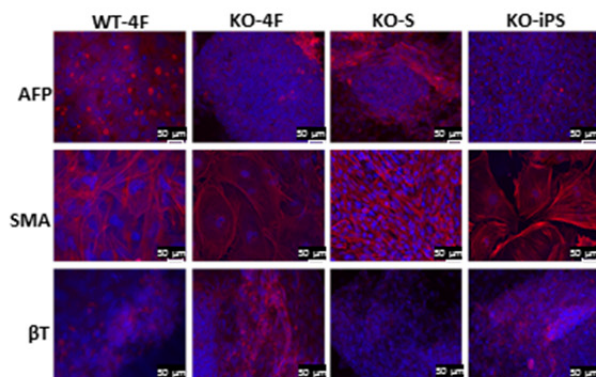
Supplemental Figure 1A



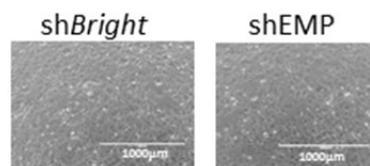
Supplemental Figure 1B



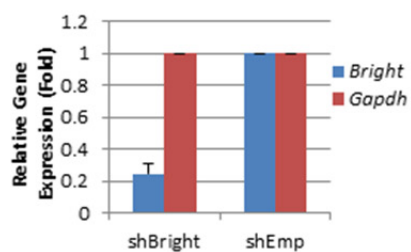
Supplemental Figure 1C



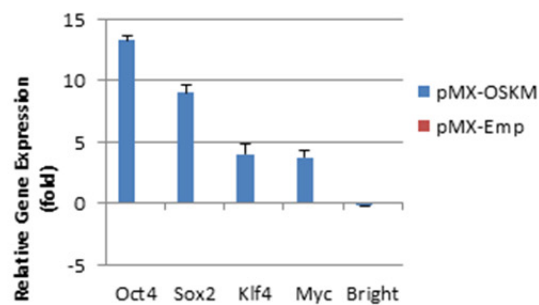
Supplemental Figure 1E



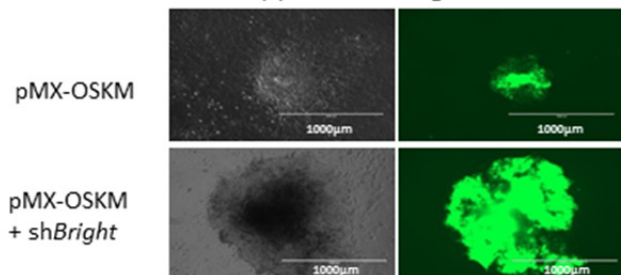
Supplemental Figure 1D



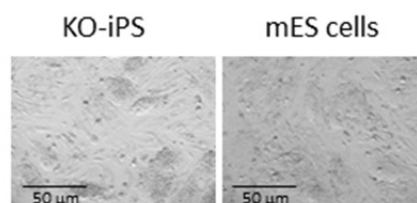
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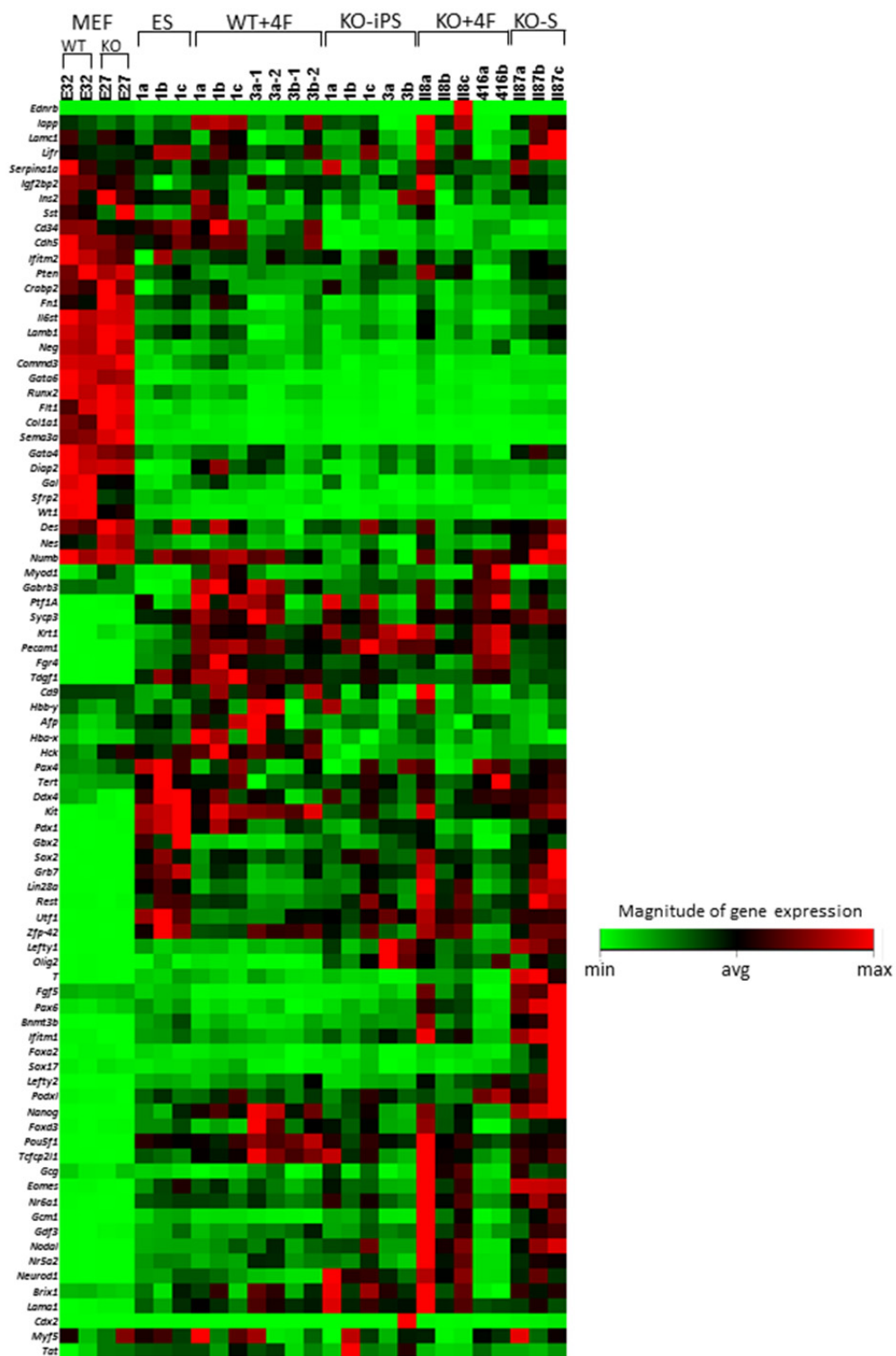
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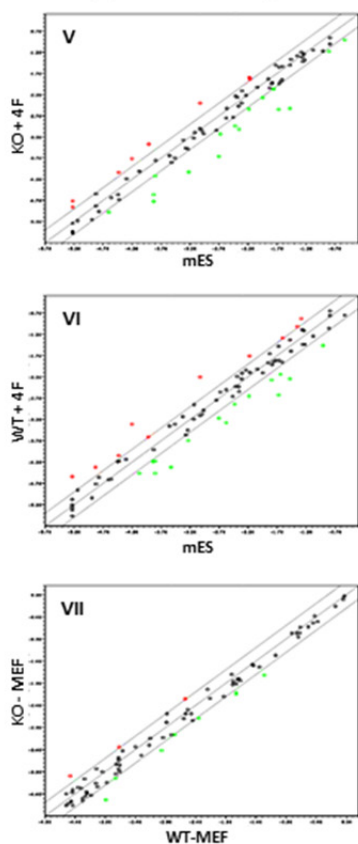
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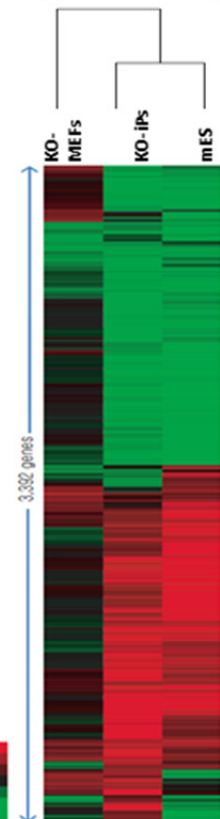
Supplemental Figure 2



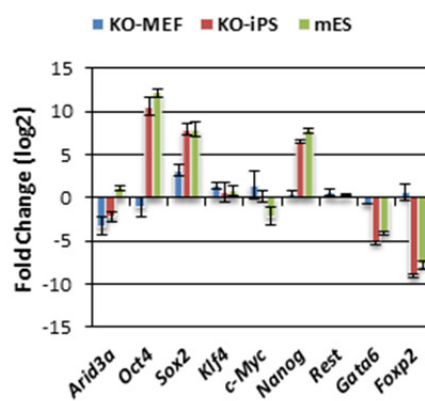
Supplemental Figure 3A



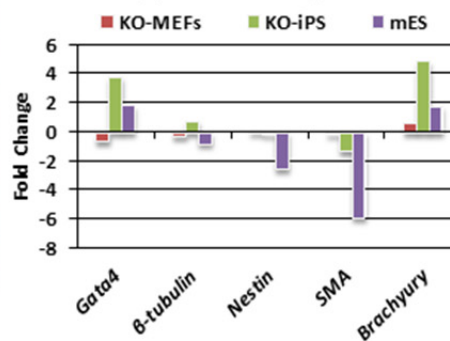
Supplemental Figure 3B



Supplemental Figure 3C

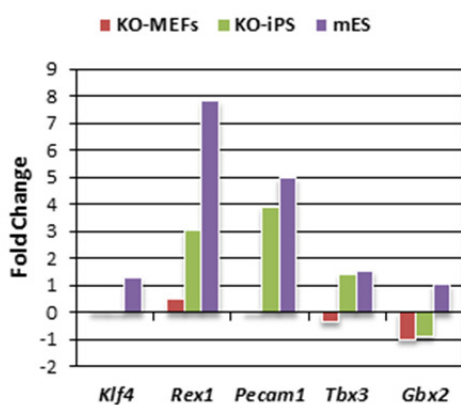


Supplemental Figure 3D

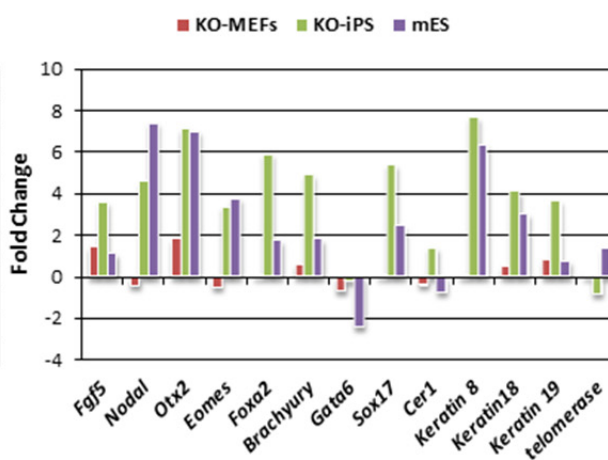


Supplemental Figure 3E

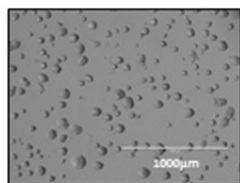
Upregulated in mES



Upregulated in EpiSC

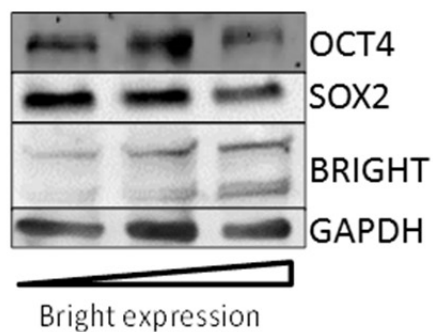


Supplemental Figure 4A

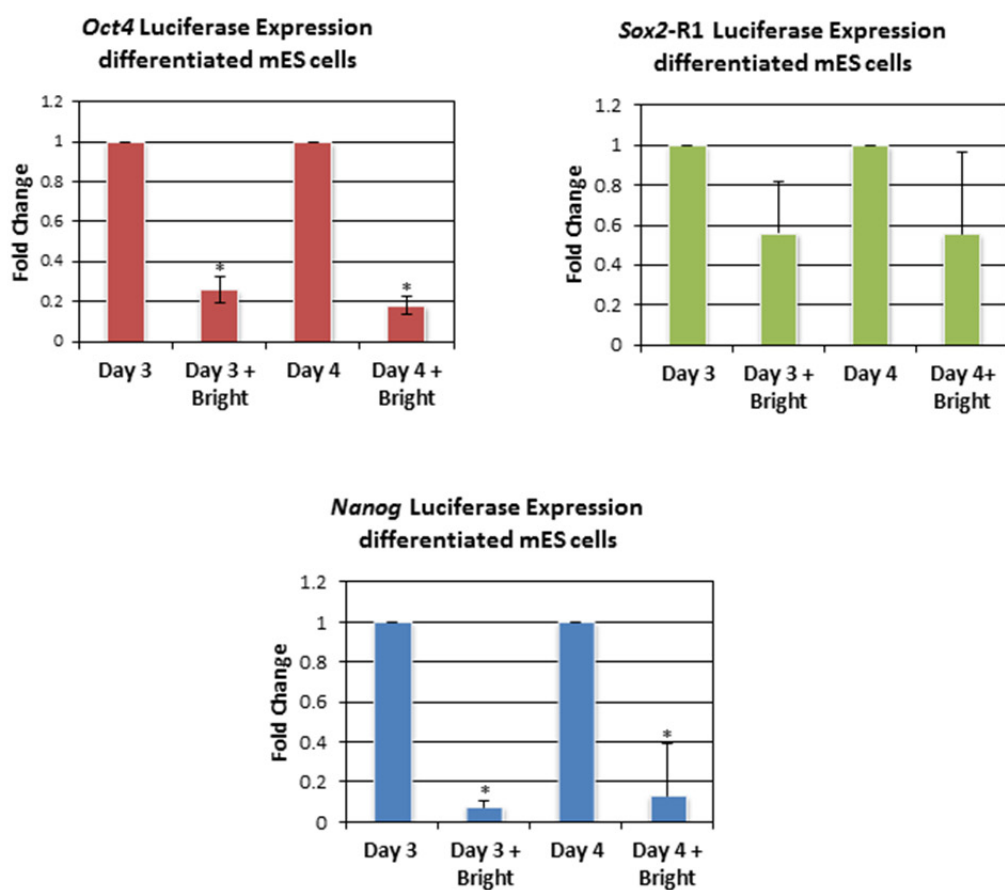


BRIGHT overexpressing mES

Supplemental Figure 4B



Supplemental Figure 4C



Supplemental Figure Legends

Supplemental Figure S1. *Bright*^{-/-} MEFs maintain a more plastic genetic state, Related to

Figure 1. (A) Cluster analysis of KO-MEF and WT-MEF microarray data. Expression of gene modules upregulated in pluripotent cells (Kim et al., 2010) shows little differences between KO-MEFs and WT-MEFs. Core, genes which regulate the pluripotent state; PRC, Polycomb complexes associated with the chromatin mark H3K27me3; MYC, Myc-centered regulatory network in mES cells, and Bivalent, mES gene promoters with both H3K4m3 and H3K27m3 chromatin marks. N=1. **(B)** Left, GO term analyses (Ashburner et al., 2000) of genes upregulated in KO-MEFs compared to WT-MEFs; the x-axis denotes p-values. Right, a heatmap display of microarray data of individual genes comprising the 8 most highly upregulated GO categories. **(C)** Reprogrammed clones differentiate into multiple tissues that express proteins representative of all three germ layers. Clones were differentiated in suspension into embryoid bodies, plated on glass cover slips and after 5 days were stained (red) for markers of endoderm, α -fetoprotein (AFP); mesoderm, smooth muscle actin (SMA); and ectoderm, β III-tubulin (β T). DAPI (blue) was used for nuclear staining. **(D)** *Bright* is reduced ~75% by shRNA. qPCR analysis of the relative gene expression of MEFs infected either with lentiviral shRNA specific for *Bright* (shBright) or the empty vector (shEMP) as a control. N=3, biological replicas, each with 3 technical replicates; error bars are standard deviations. **(E)** Reduction of BRIGHT by shRNA does not induce morphological changes. Brightfield images of MEFs infected either with shRNA against BRIGHT (shBright) or an empty vector (shEMP) as a control; image at 10x. **(F)** Conventional 4F-reprogramming does not induce *Bright* expression. qPCR analysis of endogenous *Bright* following lentiviral infection of *Oct4*, *Sox2*, *Klf4*, and *Myc* (pMX-OSKM) or the empty vector (pMX-Emp). N=3, biological replicates; error bars are standard deviations. **(G)**

Brightfield (left) and fluorescent (right) images of GFP-*Oct4* expressing MEFs reprogramed in the presence (pMX-OSKM) or absence (pMX-OSKM+shBright) of *Bright*; images are at 10x

(H) Brightfield images of stable KO-iPS clone and mES cells; image at 10x.

Supplemental Figure S2. Reprogrammed KO clones are molecularly similar to mES cells,

Related to Figure 2. Gene expression patterns of KO-iPS closely resembled mES cells.

SABiosciences mouse stem cell qPCR arrays were used to assess individual and replicate clones of WT and KO-MEFs, mES, WT+4F, KO-iPS, KO+4F, and KO-S. Data are shown as a heatmap adjusted to reflect the variation from minimum to maximum values (generated using software from SABiosciences) for each individual gene indicated below

Supplemental Figure S3. Gene expression pattern of reprogrammed KO clones is highly

similar to mES cells, Related to Figure 2. (A) Scatter plot comparisons of SABiosciences qPCR array data are shown for (V) KO+4F versus mES, (VI) WT+4F versus mES, (VII) KO-MEF versus WT-MEF. Genes differentially expressed with $p > 0.05$ indicated by red (upregulated) or green (downregulated) circles (see also Fig. 2C). Individual genes and relative expression differences composing each category are: **(I)** *Gabrb3*, 3.72; *Krt1*, 4.14; *Myod1*, 3.97; *CD34*, -5.39; *Cdh5*, -13.6; *Coll1a1*, -9.39; *Flt1*, -2.83; *Fn1*, -2.38; *Hck*, -2.27; *Kit*, -2.10; *Pdx1*, -2.62; *Runx2*, -8.17; *Sema3a*, -12.52; and *Sfrp2*, -3.91; **(II)** *Lefty*, 2.22; *Nog*, 2.03; *Tat*, -2.94; **(III)** *CD34*, 3.50; *Cdh5*, 11.47; *Commd3*, 2.10; *Diap2*, 3.12; *Hba-x*, 3.31; *Hck*, 3.02; *Nog*, 2.33; *Dnmt3b*, -2.63; *FGF5*, -3.66; *Gbx2*, -4.53; *Ifitm1*, -2.36; *Lefty*, -3.77; *Lin28a*, -2.08; *Olig2*, -21.9; *Pax6*, -2.23; **(IV)** *Olig2*, 12.64; *Afp*, -2.27; *CD34*, -3.00; *CDh5*, -4.63; *Commd3*, -2.48; *Diap2*, -2.52; *Hba-x*, -3.19; *Hck*, -3.85; *Tat*, -2.34; *Tdgf1*, -2.22; **(V)** *Gabrb3*, 4.2; *Pecam1*, 2.36; *CD34*, -4.62; *Cdh5*, -5.49; *Coll1a1*, -11.29; *Hck*, -2.89; *Pdx1*, -7.81; *Runx2*, -6.35; *Sema3a*, -9.08; *Sfrp2*, -3.25; *Tat*, -2.37; **(VI)** *Diap2*, 7.77; *Gabrb3*, 6.72; *Pecam1*, 2.94; *Cdx2*, -4.54; *Coll1a1*, -5.88;

Dnmt3b, -3.06; Fgf5, -6.17; Gbx2, -8.99; Gcm1, -2.42; Grb7, -2.94; Lin28a, -3.14; Pax6, -2.39; Pdx1, -2.26; Sfrp2, -2.58; Utf1, -3.58; (VII) CD34, -2.42; Eomes, -2.06; Ifitm1, -2.67. Three replicates each were used for the scatter plot data in (VII). All other scatter plots were graphed based on data for all replicates shown in the array except the WT-4F samples. In that case, only the first five replicates were used to avoid skewing towards one clone and to maintain equivalent numbers of replicates for all comparisons. (B) Heatmap of microarray data showing that of 3392 genes whose expression in spontaneous KO-MEF colonies (KO-iPS) and mES differed >2 fold from WT-MEFs, only ~200 genes differed between KO-iPS and mES. (C) qPCR confirmation of selected genes from microarrays. Primer sequences are shown in Suppl. Methods. Key pluripotency (eg, *Oct4*, *Sox2*, *Klf4*, *c-Myc*, and *Nanog*) and early differentiation (eg, *Rest*, *Gata6*, *Foxp2*) mRNA expression is consistent with microarray data. N=3, biological replicates analyzed with at least three technical replicates each. (D) Graphic representation of microarray data of (C) indicate that early differentiation factors are comparably expressed in KO-iPS and mES but not in KO-MEFs. (E) Microarray analysis of genes selectively upregulated in mES (upper panel) or in epiblast stem cells (EpiSC) (lower panel). Of the few genes that have been previously observed to be differentially expressed between mES and EpiSC, KO-iPS have an intermediate gene expression pattern.

Supplemental Figure S4. Overexpression of Bright decreases endogenous *Oct4*, *Sox2*, and *Nanog* expression, Related to Figure 4. (A) Brightfield image of stable *Bright* overexpressing mES used in Fig 4D and E. Image shows ES morphology was maintained. (B) Increasing levels of BRIGHT down-regulate core factor proteins in p19 embryonic carcinoma cells. Cells were transfected with increasing amounts of a CMV promoter-driven *Bright* expression vector, and harvested two days after transfection. A representative SDS-PAGE analysis is shown.

Quantitation using ImageQuant software (not shown) of western blots from 4 independent experiments indicated ~20-60% down-regulation was achieved with ~2-3 -fold increases in BRIGHT levels; p-value < .02 for OCT4 and <.001 for SOX2. (C) *Bright* overexpression represses *Oct4* (left), *Nanog* (below) and *Sox2* (right) promoter/enhancer-driven reporter expression during differentiation of mES cells. Firefly *Luciferase* constructs containing regions indicated in Figure 3B were co-transfected into mES with *Renilla* controls and either *Myc-Bright* or empty vector control. Cells were grown in the absence of LIF or feeder cells to allow differentiation and were harvested 3 to 4 days post-transfection. Three biological replicates performed with three technical replicates per experiment which vector-only values were set to 1. Error bars are standard deviations. Asterisks denote statistical significance, p-value $\leq .1$, as determined by student's T-test.

Supplemental Table S1. Bright-deficient mouse embryonic fibroblasts undergo both enhanced conventional and spontaneous reprogramming, Related to Figure 1.

Clone type ^a	Efficiency of initial colony formation ^b	Total number of independently isolated clones	Number of stably reprogrammed clones ^c
WT+4F	0.025	56	8
KO+4F	0.37	119	40
KO-S	0.13	99	4
KO-O	0.027	19	0
KO-K	0.045	82	2
KO-M	0.07	42	0
KO-iPS	0.05	5	3

^a KO-MEFs and WT-MEFs reprogrammed with either all four (KO+4F, WT+4F) standard factors (*Oct4*, *Sox2*, *KLF4*, and *c-Myc*) or in the absence of *Oct4* (KO-O), *Sox2* (KO-S), *KLF4* (KO-K), or *c-Myc* (KO-M) or formed spontaneously from KO-MEFs (KO-iPS).

^b Average number of colonies observed at day 17 divided by number of initial MEFs plated from 3-5 independent experiments. Data for the KO-iPS were derived from a single experiment.

^c Number of isolated clones defined to have undergone stable reprogramming based on survival for > 4 passages, expression of endogenous pluripotency markers, formation of embryoid bodies, and differentiation into cells expressing markers of all three germ line tissues.

Supplemental Table S2. KO-iPS are pluripotent *in vivo*, Related to Figure 1.

Cell Type	No. of injections	No. of teratomas	Efficiency
mES	7	3	.43
WT+4F	2	2	1.0
KO+4F	2	2	1.0
KO-iPS	22	7	.32
KO-MEFs	5	0	0.0

Approximately 3.5×10^5 mES, WT+4F, KO+4F, KO-iPS or $\sim 7 \times 10^5$ KO-MEFs were injected subcutaneously into the flank of NSG mice. Tumor development was monitored over 4-6 weeks, and tumors were harvested, fixed, sectioned and stained with hemotoxylin and eosin. Slides were read by trained pathologists. Efficiency of teratoma formation was determined as the number of tumors divided by the number of injections performed.

Supplemental Experimental Procedures

Cell culture: All cells were cultured in 5% CO₂ at 37°C in media containing 100U/ml Penicillin G, and 100ug/ml Streptomycin Sulfate (Invitrogen). MEFs were cultured in Dulbecco's Modified Eagle Media (DMEM) supplemented with 10% fetal bovine serum (FBS). Clones from *Bright* KO-MEFs were picked under sterile conditions. ES cells and reprogrammed clones were grown on STO feeder cells or wild type MEFs that were mitotically inactivated with Mitomycin C (10ug/ml) in Dulbecco's Modified Eagle Media (DMEM) supplemented with 20% ES-grade fetal bovine serum (Hyclone), 100U/ml Penicillin G, 100ug/ml Streptomycin Sulfate, and 10ng/ml leukemia-inhibitory factor (LIF).

MEF generation: Embryos from E10.5 *Bright*^{-/+} x129/B6 mice were used to generate WT and KO MEFs. Embryos were removed and washed in PBS. Brain and organs were microdissected out and remaining material was chopped into small pieces using a scalpel and digested in collagenase at 37°C for 20 minutes. MEF lines used in reprogramming experiments were derived using paws from E11-12 embryos. The resulting cell suspension was filtered through a 45µm cell strainer and plated onto cell culture plates in DMEM supplemented with 10% FBS, 100U/ml Penicillin G, and 100ug/ml Streptomycin Sulfate and incubated at 37°C, 5% CO₂. Genotypes were confirmed as previously reported (Webb et al., 2011).

Growth curve: Actively growing WT-MEFs and KO-MEFs (~3.5 X 10⁵) were plated onto a 10cm² cell culture dish for each indicated time-point. Cells were trypsinized and counted every 3 days. Media was changed every second day. Experiments were performed a minimum of 4 times for each cell line. Statistics performed using Microsoft Excel.

Senescence staining: Sub-confluent plates of WT-MEFs (<15 doublings) and KO-MEFs (>50 doubling) were fixed on cell culture plates for 20 minutes with 2% formaldehyde, washed in PBS, and incubated overnight at 37°C in 5mg β -galactosidase/40mM citric acid/40mM sodium phosphate pH 6.0/5mM potassium ferricyanide/5mM potassium ferrocyanide/150mM NaCl/3mM MgCl₂ (Dimri et al., 1995)

Immunocytochemistry (ICC): Indicated clones were plated on chamber slides or cover slips. In some cases, cells were grown under MEF cell culture conditions for two days and alkaline phosphatase presence was detected using the Vector kit (SK-5100). For pluripotency marker staining, individual clones were grown under ES cell culture conditions. Fixing, permeabilization, and immunostaining was performed with anti-NANOG, SOX2, OCT4, and SSEA-1 specific antibodies and appropriate fluorescently tagged secondary antibodies as previously described (An et al., 2010).

Reprogramming: Stable KO-iPS clones were derived from KO-MEFs grown to confluency under ES cell culture conditions, inclusive of LIF. Individual colonies were picked from plates, briefly trypsinized (5% trypsin/1% EDTA) and replated on mitotically inactive, WT-MEF feeder cell lines in ES media. For standard reprogramming, lentiviruses were generated from 293T cells transfected using LipoD293 (SigmaGen) with the Tet-inducible plasmids TetO-FUW-*Oct4*, -*Sox2*, -*Klf4*, -*c-Myc* or empty vector obtained from Addgene repository (www.addgene.com, plasmids 20323, 20326, 20322, 20324, and 12260, respectively). Confluent WT- and KO-MEFs were infected on day 1, and fed with tetracycline for the next two weeks. Individual clones were counted, isolated, expanded by consecutive passage >4x, and aliquots were frozen. Independent experiments were performed 3-5 times and averages used to calculate the reprogramming efficiency for all variations of the standard reprogramming methodology.

Teratoma generation and assessment: Cells nearing confluency were lightly trypsinized (5% trypsin/1% EDTA) and washed twice in PBS. Approximately 3.5×10^5 cells in 100ul were injected subcutaneously into the flanks of adult NSG (*NOD.Cg-Prkdcscid Il2rgtm1Wjl/SzJ*) mice. Mice were palpated for tumor growth and sacrificed when visible tumors were 1-2 cm across the longest diameter. Tumors were embedded, sectioned and stained with Hematoxylin and Eosin (H&E) at the core facility and analyzed by a trained pathologist at M.D. Anderson – Science Park Histology and Tissue Processing Facility Core located at The Virginia Harris Cockrell Cancer Research Center at The University of Texas MD Anderson Cancer Center, Science Park facility or at the University of Oklahoma Health Sciences Center.

Chimera generation: Chimeras were generated in the Mouse Genetic Engineering Facility at the University of Texas at Austin. Pre-implantation embryos from C57BL/6-cBrd/cBrd/Cr (C57BL/6 albino) mice were injected at the 8-16 cell stage with either KO-iPS1 or KO-iPS2 cell lines. Chimeric embryos were transplanted into recipient mothers. Due to fostering issues, chimeric pups were harvest at E18.5 for analysis. Chimerism was determined by the presence of the *Bright* transgenic gene either by PCR amplification or X-gal staining. PCR of mouse tail DNA was performed using the following primers: KO F 5'- GTCTGCAGGTGCTTGAATGA-3' R 5'- GCCTGAAGAACGAGATCAGC-3'; GAPDH F 5'- GTGGACCTGACCTGCCGTCT-3' R 3'- GGAGGAGTGGGTGTCGCTGT-3'. X-gal staining of whole embryos was performed as previously described (Fire, 1992).

Microarray: Cells were harvested by trypsin digestion. Total RNA was isolated (Qiagen RNeasy). On-column DNase digestion was performed (Qiagen) to remove genomic DNA contamination. RNA was reversed transcribed (Invitrogen). Labeling with cy3 random nonamers, array hybridizations, and data normalization were performed as described in the

Nimblegen expression array protocol. Heatmaps were created using Java Treeview software (Saldanha, 2004). GO analysis was performed using the DAVID online software (Huang et al., 2008, 2009)

qPCR Array: cDNA was generated from total mRNA isolated from WT- and KO-MEFs, ES cells and reprogrammed clones as indicated. qPCR array analyses were performed using the mouse embryonic stem cell RT² Profiler™ PCR Array according to the manufacturer's directions (SABiosciences PAMM-081A). Array results were analyzed using the online software provided by the manufacturer (<http://www.sabiosciences.com/pcr/arrayanalysis.php>). These data were normalized to *Gapdh*, and fold change was determined by comparing iPS values to those of WT-MEFs using GraphPad Prism® software.

DNA Methylation Assay: Bisulfite analysis was conducted over a 175bp region, incorporating 9 CpG sites, centered approximately 125bp upstream of the *Oct4* transcription start site, as previously described (Fraenkel et al., 2007).

qPCR: Total RNA was isolated using the Qiagen RNeasy kit, and cDNA was generated using Superscript III (Invitrogen). All qPCR assays were performed on an Applied Biosystems ViiA7 thermocycler using SYBR green chemistry (Applied Biosystem SYBRGreen or Quanta BioSciences SYBR Green). qPCR primers were as follows: *Bright* F 5'-

GAGGTTATCAACAAGAACTGT-3' R 5'-GATACTTCATGTACTGTGTCCG-3'; *GAPDH* F 5'-TTCACCACCATGGAGAAGGC-3'R 5'-CCCTTTTGGCTCCACCCT-3'; *Oct4* F 5'-ACATCGCCAATCAGCTTGG-3' R, 5'-AGAACCATACTCGAACCACATCC-3'; *Sox2* F 5'-ACAGATGCAACCGATGCACC -3' R 5'-TGGAGTTGTACTGCAGGGCG-3'; *Nanog* F 5'-TCTTCCTGGTCCCCACAGTTT -3' R 5'-GCAAGAATAGTTCTCGGGATGAA -3'; *KLF4* F

5'-GTAAGGTTTCTCGCCCTGTG-3' R 5'-CAGGCTGTGGCAAACCTAT-3'; *REST* F 5'-ACCTGCAGCAAGTGCAACTA-3' R 5'-GCGTTCTCCCTGTGTGAGTT-3'; *Gata6* F 5'-TCCTTCTACACAAGCGACCA-3' R 5'-TCTCCCACTGCAGACATCAC-3'; *Foxp2* F 5'-ACATCGACAGCAATGGGAAC-3' F 5'-CACGGGTTCTTCCTTGACAT-3'. Statistics were performed using Microsoft Excel.

Chromatin immunoprecipitation (ChIP): MEF cells were first crosslinked with DTBP (Pierce) for 30 minutes, quenched with 100 mM Tris-HCl, pH 8.0, 150 mM NaCl, washed with PBS, then crosslinked with 1% formaldehyde for 30 minutes, and quenched with 125mM glycine for 10 minutes. mES cells were crosslinked with formaldehyde only. Cells were then washed with PBS+PMSF and lysed in SDS lysis buffer (1% SDS, 10mM EDTA, and 50 mM Tris-Cl pH 8.1). Samples were sonicated using the Bioruptor (Diagenode) at medium speed for 20 minutes, diluted 1:5 with dilution buffer (0.1% SDS, 10% Triton-X, 0.5M EDTA, 1M Tris pH 8.1, 5M NaCl), and pre-cleared with Protein G Agarose beads at 4°C > 4 hours. 10 µg of α-BRIGHT rabbit polyclonal antibody was added overnight at 4°C. Samples were incubated with Protein G Agarose beads at 4°C > 4 hours and then washed twice in a low salt buffer (1% Deoxycholate, 1% Triton X-100, 1mM EDTA, 50mM HEPES pH 7.5, 150mM NaCl), once in a high salt buffer (1% Deoxycholate, 1% Triton X-100, 1mM EDTA, 50mM HEPES pH 7.5, 500mM NaCl), once in a LiCl buffer (250mM LiCl, 0.5% NP-40, 0.5% Deoxycholate, 1mM EDTA, 10mM Tris pH 8.1), and twice in TE. Samples were eluted at 65°C in elution buffer (1% SDS, 10mM EDTA, 50mM Tris pH 8.1). Crosslinking was reversed overnight at 70°C. Samples were incubated in RNase A (15µg) at 37°C for 30 minutes, then in 40µg of glycogen and 120µg of proteinase K at 37°C for 2 hours. DNA was Phenol/Chloroform extracted, ethanol precipitated, and resuspended in TE buffer. ChIP primers used were: *Oct4* F 5'-AAAGTTTCTGTGGGGGACCT-3', R 5'-

AAAACCGGGAGACACAACCTG-3'; *Sox2* F 5'- TCATTTTCAGGTGTAGAGTTGG-3', R 5'- CCTATGTGTGAGCAAGAACCTG-3'; *Nanog* (MEF ChIP) F 5'- GTTGGAAACGTGGTGAACCT-3', R 5'- GAAAACCGAGCAACAGAACC-3'; Negative control 5'- GGAGTCCCCTAGGAAGGCATTAATAGTTT-3', R 5'- GGATTCTCTCGGCTTCAGACAGACTTT-3'.

ChIP-Sequencing: ChIP was performed as described above. The NEB ChIP-seq library preparation kit (cat #: E6240) was used for ChIP-seq library generation following manufacturer's instructions. ChIP DNA was sequenced using Illumina sequencing technology. Paired-sequence reads were ~2 X 50bp, and read numbers ranged from ~22-32M. Sequenced reads were mapped to the genome using the BWA aligner. Peak calling was performed using a Parzen window based algorithm. Binding sites were visualized with a fixed step wiggle file in the UCSC genome browser.

Electrophoretic mobility shift assay (EMSA): *In vitro* translated BRIGHT was prepared per manufacturer's instruction (Pierce). Nuclear extracts were prepared by iso-osmotic/NP-40 lysis. Protein concentrations were quantified with Bradford reagents (Bio-Rad), and BRIGHT binding complexes were resolved on 4% nondenaturing acrylamide gels following incubation for 1 hour at 37°C with γ -³²P-labeled probe as previously described (Nixon et al., 2004). The *Nanog* probe was a 120 bp *AccI*-*SspI* fragment positioned ~350bp upstream of the *Nanog* start site. The *Oct4* distal enhancer probe (DE, 289bp) and *Bright* binding probe (Bb, 353 bp) were PCR amplified from the *Oct4* promoter region. The *Sox2* probe was a *MslI*-*ApaI* fragment positioned ~3500bp upstream of the *Sox2* start site. In some instances, antibodies were added after 20 minutes at RT and then incubated for 30 minutes on ice. α -BRIGHT rabbit polyclonal antibody was employed for supershifts. Cold competitions were performed using unlabeled probes as well as a previously

established BRIGHT-binding site (a 150 bp *Bam*HI–*Fok*I fragment, IgV_H) from the S107 V1 5' flanking region (Webb et al., 1991). Competitors were pre-incubated in binding reaction at ~ 1X and 100X molar excess to labeled probes (~80,000 dpm per reaction). Complexes were identified by phosphoimaging.

Immunoprecipitation (IP): Cells were harvested, and lysates were pre-cleared with 100µl Protein G Agarose beads at 4°C > 4 hours. Lysates were incubated with 2µg of α-BRIGHT rabbit polyclonal antibody overnight at 4°C. Protein A Sepharose beads were incubated with the protein-antibody complex at 4°C > 4 hours, and beads were washed three times in TBS-T and once in TBS. 2X Sample buffer (125mM Tris pH 6.8, 2% SDS, 16% glycerol, 3% 2-Mercaptoethanol, and Bromophenol blue) was added and the samples boiled for 5 minutes prior to resolution on SDS-PAGE. Blots were probed using the indicated primary antibodies as for western blots.

Cell fractionation: mES were grown in the presence (undifferentiated) or absence (differentiated) of LIF for 3 days. Cells were separated into cytoplasmic (Cy), soluble nuclear protein (NP), chromatin (CH), and nuclear matrix (NM) fractions as follows. Approximately 1×10^8 cells were washed twice in PBS and the pellet was resuspended in 2ml HNB buffer (500mM sucrose/15mM Tris-HCL pH 7.5/60mM KCL/.25mM EDTA/.125mM EGTA/.5mM spermidine). Then 1ml HNB buffer was added dropwise (HNB buffer+ 1% NP-40) and samples were incubated at 4°C for 5 minutes and centrifuged at 6,000g for 3 min at 4°C; the supernatant contained the Cy fraction. The pellet was resuspended in 1ml CSKT buffer (CSK buffer + 1% Triton-X), incubated at 4°C for 5 minutes before centrifugation at 3,000g for 3 min at 4°C; the supernatant contained the NP fraction. The pellet was resuspended in 720µl CSK buffer (10mM Pipes pH 6.8/300mM sucrose/3mM MgCl₂/2mM EGTA) and 30µl RNase-free

DNase, incubated at 37°C for 15 minutes, then 250µl 1M AmSO₄/CSK was added and incubated at 4°C for 5 minutes, before centrifugation at 3,000g for 3 min at 4°C; the supernatant included the CH fraction. The pellet was resuspended in 1ml 8M Urea, centrifuged at 13,000g for 5 minutes, and the supernatant included the NM fraction. Equal amounts of protein for each fraction was determined by Bradford assay, separated on SDS-PAGE and probed with anti-BRIGHT polyclonal antibody.

Stable Bright overexpression: Stable BRIGHT overexpressing mES cells were created using an *in vivo* biotinylation system as previously described (Kim et al., 2008). Briefly, *BirA* expressing mES were created by electroporating mES cells with the pEF1α*BirAV5*-neo vector and selecting cells with 300µg ml⁻¹ G418. BRIGHT overexpressing cells were created by electroporating the pEF1αFLBIO-puro-*Bright* vector into *BirA*-mES and selecting stable clones using 1µg ml⁻¹ puromycin. Overexpression within individual clones, estimated by western analysis with anti-BRIGHT, was ~3 to 4-fold above endogenous BRIGHT levels.

Luciferase reporter assays: DNA (~2µg) of the indicated luciferase vector, 0.7 µg Renilla luciferase, and ~3µg pCEM-*Bright* or pCME-EV (empty vector) were electroporated into ~5 X 10⁵ single cell suspensions of sub-confluent mES cells (Lonza, VPH-1001). Cells were seeded in 6 well plates either under standard ES conditions (undifferentiated) or in the absence of either feeder cells or LIF (differentiated). *Oct4* and *Nanog* luciferase constructs were obtained from Addgene repository (www.addgene.com): *Oct4*-luciferase (Addgene plasmid 17221) (Takahashi et al., 2007), *Nanog*-luciferase (Addgene plasmid 16337)(Gu et al., 2005). *Sox2* luciferase constructs were a kind gift from Dr. Angel Martin, Fundación Inbiomed, San Sebastian, Spain. At least 2 independent experiments were performed in triplicate. Statistics were performed using Microsoft Excel.

Western blots: Cells were harvested using trypsin/EDTA and lysed in RIPA buffer containing 1x protease inhibitors (Roche, 11-836-170-001) and PMSF (1mM). Cells were briefly sonicated and centrifuged at >13,000g for 10 minutes at 4°C. Protein concentrations of lysate were measured by Bradford assay (Bio-Rad), and ~ 40 ug total protein was resolved by 8% SDS-PAGE. Blots were probed as indicated with anti-BRIGHT, anti-OCT4 (Santa Cruz sc-5279), anti-SOX2 (Santa Cruz sc-365964), anti-NANOG (Santa Cruz sc-374001), and anti-GAPDH (Santa Cruz sc-166545). Signal was detected after secondary incubation with appropriate anti-IgG by chemiluminescence using an ECL+ (GE) on a STORM phosphoimager.

Supplemental References

- An, G., Miner, C.A., Nixon, J.C., Kincade, P.W., Bryant, J., Tucker, P.W., and Webb, C.F. (2010). Loss of Bright/ARID3a function promotes developmental plasticity. *Stem Cells Dayt. Ohio* 28, 1560–1567.
- Ashburner, M., Ball, C.A., Blake, J.A., Botstein, D., Butler, H., Cherry, J.M., Davis, A.P., Dolinski, K., Dwight, S.S., Eppig, J.T., et al. (2000). Gene ontology: tool for the unification of biology. The Gene Ontology Consortium. *Nat. Genet.* 25, 25–29.
- Dimri, G.P., Lee, X., Basile, G., Acosta, M., Scott, G., Roskelley, C., Medrano, E.E., Linskens, M., Rubelj, I., and Pereira-Smith, O. (1995). A biomarker that identifies senescent human cells in culture and in aging skin in vivo. *Proc. Natl. Acad. Sci. U. S. A.* 92, 9363–9367.
- Fire, A. (1992). Histochemical techniques for locating *Escherichia coli* β -galactosidase activity in transgenic organisms. *Genet. Anal. Biomol. Eng.* 9, 151–158.
- Fraenkel, S., Mostoslavsky, R., Novobrantseva, T.I., Pelanda, R., Chaudhuri, J., Esposito, G., Jung, S., Alt, F.W., Rajewsky, K., Cedar, H., et al. (2007). Allelic “choice” governs somatic hypermutation in vivo at the immunoglobulin κ -chain locus. *Nat. Immunol.* 8, 715–722.
- Gu, P., LeMenuet, D., Chung, A.C.-K., Mancini, M., Wheeler, D.A., and Cooney, A.J. (2005). Orphan Nuclear Receptor GCNF Is Required for the Repression of Pluripotency Genes during Retinoic Acid-Induced Embryonic Stem Cell Differentiation. *Mol. Cell. Biol.* 25, 8507–8519.
- Huang, D.W., Sherman, B.T., and Lempicki, R.A. (2008). Systematic and integrative analysis of large gene lists using DAVID bioinformatics resources. *Nat. Protoc.* 4, 44–57.
- Huang, D.W., Sherman, B.T., and Lempicki, R.A. (2009). Bioinformatics enrichment tools: paths toward the comprehensive functional analysis of large gene lists. *Nucleic Acids Res.* 37, 1–13.
- Kim, J., Chu, J., Shen, X., Wang, J., and Orkin, S.H. (2008). An Extended Transcriptional Network for Pluripotency of Embryonic Stem Cells. *Cell* 132, 1049–1061.
- Kim, J., Woo, A.J., Chu, J., Snow, J.W., Fujiwara, Y., Kim, C.G., Cantor, A.B., and Orkin, S.H. (2010). A Myc network accounts for similarities between embryonic stem and cancer cell transcription programs. *Cell* 143, 313–324.
- Nixon, J.C., Rajaiya, J., and Webb, C.F. (2004). Mutations in the DNA-binding domain of the transcription factor Bright act as dominant negative proteins and interfere with immunoglobulin transactivation. *J. Biol. Chem.* 279, 52465–52472.
- Saldanha, A.J. (2004). Java Treeview—extensible visualization of microarray data. *Bioinformatics* 20, 3246–3248.
- Takahashi, K., Tanabe, K., Ohnuki, M., Narita, M., Ichisaka, T., Tomoda, K., and Yamanaka, S. (2007). Induction of Pluripotent Stem Cells from Adult Human Fibroblasts by Defined Factors. *Cell* 131, 861–872.

Webb, C.F., Das, C., Eaton, S., Calame, K., and Tucker, P.W. (1991). Novel Protein-DNA Interactions Associated with Increased Immunoglobulin Transcription in Response to Antigen Plus Interleukin-5. *Mol. Cell. Biol.* *11*, 5197–5205.

Webb, C.F., Bryant, J., Popowski, M., Allred, L., Kim, D., Harriss, J., Schmidt, C., Miner, C.A., Rose, K., Cheng, H.-L., et al. (2011). The ARID family transcription factor bright is required for both hematopoietic stem cell and B lineage development. *Mol. Cell. Biol.* *31*, 1041–1053.