Cell, Volume *133*

Supplemental Data

Direct Reprogramming of Terminally Differentiated Mature B Lymphocytes to Pluripotency

Jacob Hanna, Styliani Markoulaki, Patrick Schorderet, Bryce W. Carey, Caroline Beard, Marius Wernig, Menno P. Creyghton, Eveline J. Steine, John P. Cassady, Ruth Foreman, Christopher J. Lengner, Jessica A. Dausman,and Rudolf Jaenisch

Supplemental Experimental Procedures

Cell culture and viral infections. ES and established iPS cells were cultured on irradiated MEFs in DME containing 15% FCS, leukemia inhibiting factor (LIF), penicillin/streptomycin, L-glutamine, beta-mercaptoethanol and nonessential amino acids. MEFs used to derived primary iPS lines by infections with inducible lentiviruses were harvested at 13.5dpc from F1 matings between ROSA26-M2rtTA mice (Beard et al., 2006) and Nanog-GFP mice (Brambrink et al., 2008). Mouse C/EBPα cDNA was cloned into EcoRI cloning site of pLib, MSCV-Neo and pMig retroviral vectors. Primers for cloning: (i)5' mC/EBPα: 5' - GAA TTC ATG GAG TCG GCC GAC TTC TAC - 3'. (ii) 3' mC/EBPα: 5' - GAA TTC TCA CGC GCA GTT GCC CAT GG - 3'.

pMXs vectors encoding ES pluripotency genes were previously described (Takahashi and Yamanaka, 2006). Lentiviral preparation and infection with Dox inducible lentiviruses encoding Oct4, Klf4, c-Myc and Sox2 cDNA driven by the TetO/CMV promoter, were previously described (Brambrink et al., 2008). Retrovirus stocks were prepared by transient transfection of Phoenix-Eco cells using Fugene (Roche), and supernatants were harvested 48 hr later. For infection, purified B cell subsets were resuspended in IMDM with 15% FCS as well as IL-4, IL-7, Flt-3L, SCF (10ng/ml each, Peprotech), anti-CD40 (0.1μg/ml, BD-Biosciences), LPS (10ng/ml, Sigma-Aldrich) and Dox (4μg/ml). Then, 2 ml aliquots were plated onto a 24-well plate precoated with retronectin (Takara) followed by 2 ml of retrovirus supernatant to

which polybrene (Sigma) was added (8 μg/ml). The plates were incubated at 37°C for 2 hours, and afterwards 1ml of viral supernatant was replaced with B cells resuspended in the cytokine conditioned media described above. Plates were centrifuged for 90 min at 900RPM and then incubated 24 hours at 37°C 5%CO2. Infected cells were then transferred onto OP9 bone marrow stromal cells line (ATCC) in fresh cytokine and Dox supplemented media. After 14 days on Dox, colonies were picked and cultured on MEF feeder cells in ES media (without hematopoietic cytokines or Dox) and in the presence of puromycin (2 μg/ml) to eliminate any remaining OP9 cells. As a note, it appeared possible that the transgenic non-infected B cells were able to proliferate in conditioned media with Dox for a relatively extended period (up to 6 weeks) due to induction of c-Myc, which is known to promote B cell growth and transformation (Zhu et al., 2005).

V(D)J rearrangement analysis. IgH, Igκ and Igλ rearrangements were amplified by PCR using degenerate primer sets as previously described (Chang et al., 1992; Cobaleda et al., 2007; Schlissel et al., 1991) (Table S2). To characterize individual V-DJ rearrangements, the PCR fragments were cloned in TOPO vector, and at least 5 clones corresponding to the same PCR fragment were sequenced. Sequences were analyzed with DNAPLOT search engine (http://www.dnaplot.de). V-DJ and D-J rearrangements at the *Igh* locus were detected by Southern blot analysis on genomic DNA of the indicated iPS lines digested with EcoRI and using a 3'JH4 probe (1.6-kb HindIII-EcoRI fragment of plasmid JH4.3) (Alt et al., 1981). Vκ-Jκ rearrangements at the *Igk* locus were determined by Southern blot analysis of BamHI-digested genomic DNA using a 3'Jκ5 probe (1-kb XbaI-EcoRV fragment of plasmid pBS-JκMAR) (Lewis et al., 1982). Notably, B-iPS#4 cell line likely arose from a contaminating IgM+IgD- cells during the sorting process because IgM+IgD+ B cells had been selected for reprogramming and this selection would be expected to yield only naïve mature B (Matthias and Rolink, 2005).

DNA methylation and histone marks analysis. For the methylation status of Oct4 and Nanog promoters, bisulphite sequencing analysis was performed as described previously (Wernig et al., 2007). A total of 10–20 clones of each sample was

sequenced in both directions. The status of H3K4 and H3K27 bivalent domains was determined by chromatin immunopercipitation followed by quantitative PCR analysis, as previously described (Bernstein et al., 2006).

Blastocyst injections and teratoma formation. Diploid or tetraploid blastocysts (94–98 h after HCG injection) were placed in a drop of DMEM with 15% FCS under mineral oil. A flat-tip microinjection pipette with an internal diameter of 1.2–1.5 mm was used for iPS cell injection (using a Piezo micromanipulator 34). A controlled number of cells was injected into the blastocyst cavity. After injection, blastocysts were returned to KSOM media and placed at 37 °C until transferred to recipient females. Ten to fifteen injected blastocysts were transferred to each uterine horn of 2.5 days post coitum pseudo-pregnant B6D2F1 females. To recover full-term pups, recipient mothers were killed at 19.5 days post coitum. Surviving pups were fostered to lactating BALB/c mothers. For teratoma generation, 2*10^6 cells were injected subcutaneously into both flanks of recipient SCID mice, and tumors were harvested for sectioning 3-6 weeks after initial injection.

Immunofluorescence staining. Cells were fixed in 4% paraformaldehyde for 20 minutes at 25 °C, washed 3 times with PBS and blocked for 15 min with 5% FBS in PBS containing 0.1% Triton-X. After incubation with primary antibodies against Nanog (polyclonal rabbit, Bethyl) and SSEA1 (monoclonal mouse, Developmental Studies Hybridoma Bank) for 1 h in 1% FBS in PBS containing 0.1% Triton-X, cells were washed 3 times with PBS and incubated with fluorophore-labeled appropriate secondary antibodies purchased from Jackson Immunoresearch. Specimens were analyzed on an Olympus Fluorescence microscope and images were acquired with a Zeiss Axiocam camera.

Quantitative RT-PCR. Bone marrow B cells were grown on OP-9 cells in media supplemented with IL-7, SCF, Flt3L, while spleen B cells were grown with IL-4, anti-CD40 and LPS. OP9 cells were depleted by pre-plating on gelatin coated plates before the cells were harvested for mRNA preparation. A functional puromycin resistance gene had been inserted into the ROSA26 locus as part of the targeting

strategy of M2rtTA and allowed elimination of host derived non-transgenic cells by puromycin selection. Puromycin was added to fibroblast (2μg/ml) and B cell (0.3μg/ml) cultures to eliminate non-transgenic cells. Total RNA was isolated using Rneasy Kit (Qiagen). Three micrograms of total RNA was treated with DNase I to remove potential contamination of genomic DNA using a DNA Free RNA kit (Zymo Research, Orange, CA). One microgram of DNase I-treated RNA was reverse transcribed using a First Strand Synthesis kit (Invitrogen) and ultimately resuspended in 100 ul of water. Quantitative PCR analysis was performed in triplicate using 1/50 of the reverse transcription reaction in an ABI Prism 7000 (Applied Biosystems, Foster City, CA) with Platinum SYBR green qPCR SuperMix-UDG with ROX (Invitrogen). Primers used for amplification were as follows: c-Myc: F, 5'-ACCTAACTCGAGGAGGAGCTGG-3' and R, 5'-TCCACATAGCGTAAAAGGAGC-3'; Klf4: F, 5'-ACACTGTCTTCCCACGAGGG-3'and R, 5'-GGCATTAAAGCAGCGTATCCA-3'; Sox2: F, 5'-CATTAACGGCACACTGCCC-3' and R, 5'-GGCATTAAAGCAGCGTATCCA-3'; Oct4: F, 5'-AGCCTGGCCTGTCTGTCACTC-3' and R, 5'-GGCATTAAAGCAGCGTATCCA-3'. To ensure equal loading of cDNA into RT reactions, GAPDH mRNA was amplified using the following primers: F, 5'-TTCACCACCATGGAGAAGGC-3'; and R, 5'-CCCTTTTGGCTCCACCCT-3'. Data were extracted from the linear range of amplification. All graphs of qRT-PCR data shown represent samples of RNA that were DNase treated, reverse transcribed, and amplified in parallel to avoid variation inherent in these procedures. Gene expression analysis for ES markers was performed by PCR using previously published primers (Takahashi and Yamanaka, 2006).

Cloning of mouse Pax5 knockdown hairpins:

shRNAs were designed using the pSicoOligomaker 1.5 (developed by A. Ventura, Jacks Lab), which is freely available at http://web.mit.edu/ccr/labs/jacks/protocols/pSico.html. Cloning into pSicoR vector (Ventura et al., 2004) was done as described on the website. Oligo sequence chosen for Pax5 knockdown were:

Senses:TGGCTCCTCATACTCCATCATTCAAGAGATGATGGAGTATGAGGAG CCTTTTTTC. Antisense: TCGAGAAAAAAGGCTCCTCATACTCCATCATCTCT TGAATGATGGAGTATGAGGAGCCA. 5 µg of lentiviral vector and 2.5 µg of each packaging vector were cotransfected in 293T cells by using the FuGENE 6 reagent (Roche Diagnostics). Supernatants were collected 36–48 h after transfection, filtered through a 0.4-µm filter, and used directly to infect transgenic B cells as described earlier for C/EBPα retroviral infections. Control hairpins were previously described (Meissner and Jaenisch, 2006). PCR primers for mouse Pax5 were previously described (Delogu et al., 2006; Schebesta et al., 2007).

Flow cytometry analysis and cell sorting. The following fluorescently conjugated antibodies (PE, FITC, Cy-Chrome or APC labeled) were used for FACS analysis and cell sorting: anti-SSEA1 (RnD systems), anti-Igκ, anti-Igλ1,2,3, anti-CD19, anti-B220, anti-c-Kit, anti-CD25, anti-sIgM, anti-sIgD (all obtained from BD-Biosciences). Cell sorting was performed by using FACS-Aria (BD-Biosciences), and consistently achieved cell sorting purity of >97%. For isolation of mature IgM+ IgD+ B cells from spleen and lymph nodes, cells were depleted of Lin+ non-B cells by MACS sorting after staining with lineage markers antibodies (CD3ε, CD4, CD8, CD11c, Gr1, c-Kit, Mac1 and Ter119) prior to sorting.

Supplemental References

Alt, F., Rosenberg, N., Lewis, S., Thomas, E., and Baltimore, D. (1981). Organization and reorganization of immunoglobulin genes in A-MULV-transformed cells: rearrangement of heavy but not light chain genes. Cell *27*, 381-390.

Bernstein, B.E., Mikkelsen, T.S., Xie, X., Kamal, M., Huebert, D.J., Cuff, J., Fry, B., Meissner, A., Wernig, M., Plath, K., *et al.* (2006). A bivalent chromatin structure marks key developmental genes in embryonic stem cells. Cell *125*, 315-326.

Brambrink, T., Ruth Foreman, G. Grant Welstead, Christopher J. Lengner, Marius Wernig, Heikyung Suh, and Rudolf Jaenisch (2008). Sequential Expression of Pluripotency Markers during Direct Reprogramming of Mouse Somatic Cells. Cell Stem Cell *2*, 151-159.

Chang, Y., Paige, C.J., and Wu, G.E. (1992). Enumeration and characterization of DJH structures in mouse fetal liver. The EMBO journal *11*, 1891-1899.

Cobaleda, C., Jochum, W., and Busslinger, M. (2007). Conversion of mature B cells into T cells by dedifferentiation to uncommitted progenitors. Nature *449*, 473-477.
Delogu, A., Schebesta, A., Sun, Q., Aschenbrenner, K., Perlot, T., and Busslinger, M. (2006). Gene repression by Pax5 in B cells is essential for blood cell homeostasis and is reversed in plasma cells. Immunity *24*, 269-281.

Lewis, S., Rosenberg, N., Alt, F., and Baltimore, D. (1982). Continuing kappa-gene rearrangement in a cell line transformed by Abelson murine leukemia virus. Cell *30*, 807-816.

Matthias, P., and Rolink, A.G. (2005). Transcriptional networks in developing and mature B cells. Nature reviews *5*, 497-508.

Meissner, A., and Jaenisch, R. (2006). Generation of nuclear transfer-derived pluripotent ES cells from cloned Cdx2-deficient blastocysts. Nature *439*, 212-215.

Schebesta, A., McManus, S., Salvagiotto, G., Delogu, A., Busslinger, G.A., and Busslinger, M. (2007). Transcription factor Pax5 activates the chromatin of key genes involved in B cell signaling, adhesion, migration, and immune function. Immunity *27*, 49-63.

Schlissel, M.S., Corcoran, L.M., and Baltimore, D. (1991). Virus-transformed pre-B cells show ordered activation but not inactivation of immunoglobulin gene rearrangement and transcription. The Journal of experimental medicine *173*, 711-720.

Takahashi, K., and Yamanaka, S. (2006). Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. Cell *126*, 663-676.

Ventura, A., Meissner, A., Dillon, C.P., McManus, M., Sharp, P.A., Van Parijs, L., Jaenisch, R., and Jacks, T. (2004). Cre-lox-regulated conditional RNA interference

from transgenes. Proceedings of the National Academy of Sciences of the United States of America *101*, 10380-10385.

Wernig, M., Meissner, A., Foreman, R., Brambrink, T., Ku, M., Hochedlinger, K., Bernstein, B.E., and Jaenisch, R. (2007). In vitro reprogramming of fibroblasts into a pluripotent ES-cell-like state. Nature *448*, 318-324.

Zhu, D., Qi, C.F., Morse, H.C., 3rd, Janz, S., and Stevenson, F.K. (2005). Deregulated expression of the Myc cellular oncogene drives development of mouse "Burkitt-like" lymphomas from naive B cells. Blood *105*, 2135-2137.

Supplemental Figures and Tables

Figure S1. Characterization of MEF-iPS line #1.

Cell line was stained for AP, Oct4 and SSEA1 pluripotency markers.

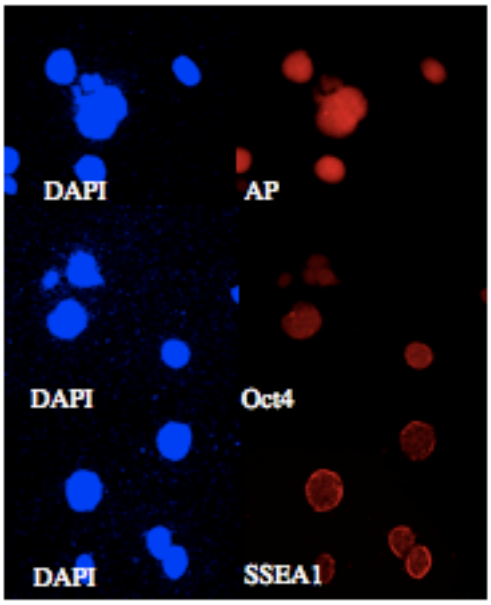

Figure S1.

Figure S2. Establishing culture conditions supporting ES, iPs, and B cell growth.

Cells were grown on OP9 bone marrow stromal cell line as indicated in Experimental Procedures section. (A) Images representing Nanog-GFP iPS and ES cells passaged on OP9 for 5 passages, while maintaining their ES-like morphology and Nanog-GFP reporter expression. (B) Typical immature loosely adherent hematopoietic B cell colonies observed after 8 days of growth. (C) Mature B cells after 21 days in culture, displaying typical small round cell morphology. (D) FACS analysis on B cells after 21 days in culture, which remained positive for IgM, IgK and B220 B cells markers and did not show any expression of SSEA1 ES marker under continuous Dox treatment.

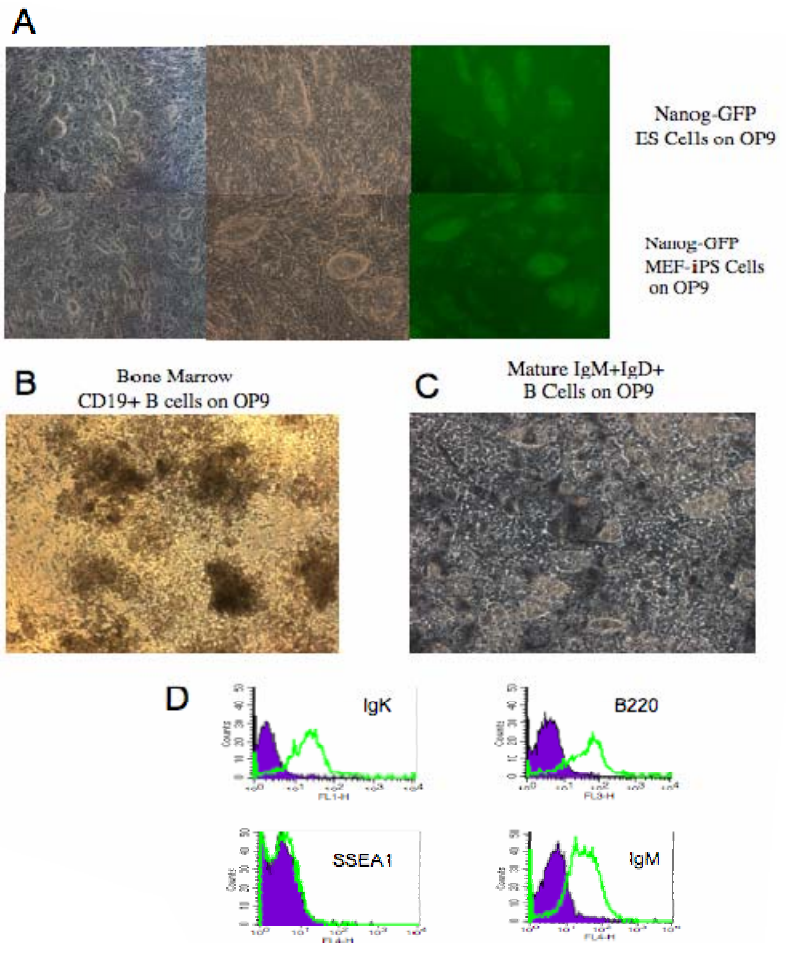

Figure S2.

Figure S3. Southern blot analysis for heavy and light chain rearrangements on iB-iPS lines.

Genomic DNA samples made from iB-iPS lines grown in ES media on irradiated MEF feeder were digested with (A) EcoRI and analyzed for V(D)J rearrangements at the *Igh* locus by Southern blotting using a 3' J_H4 probe. (B) V_κ-J_κ rearrangements at the *Igκ* locus were determined by Southern blot analysis of BamHI digested genomic DNA using a 3'$J_{\kappa5}$ probe. (A-B) Any rearrangement occurring in these loci abolishes the asterik marked restriction sites of the digesting enzyme used. After rearrangements, the size of the genomic fragment bound by the probe depends on the next available 5' restriction site in relation to the abolished asterik marked sites. MEFs served as negative control, where as CD19+ splenocytes were used as positive controls. GL denotes the position of germline DNA fragment. Red arrows indicate rearranged alleles. It is important to note, that all lines tested were grown MEF feeder cells, thus a band corresponding to a germline allele of IgH the locus was detected in all cell lines, including those carrying two IgH rearranged alleles (e.g. i-Bips #5 and #8).

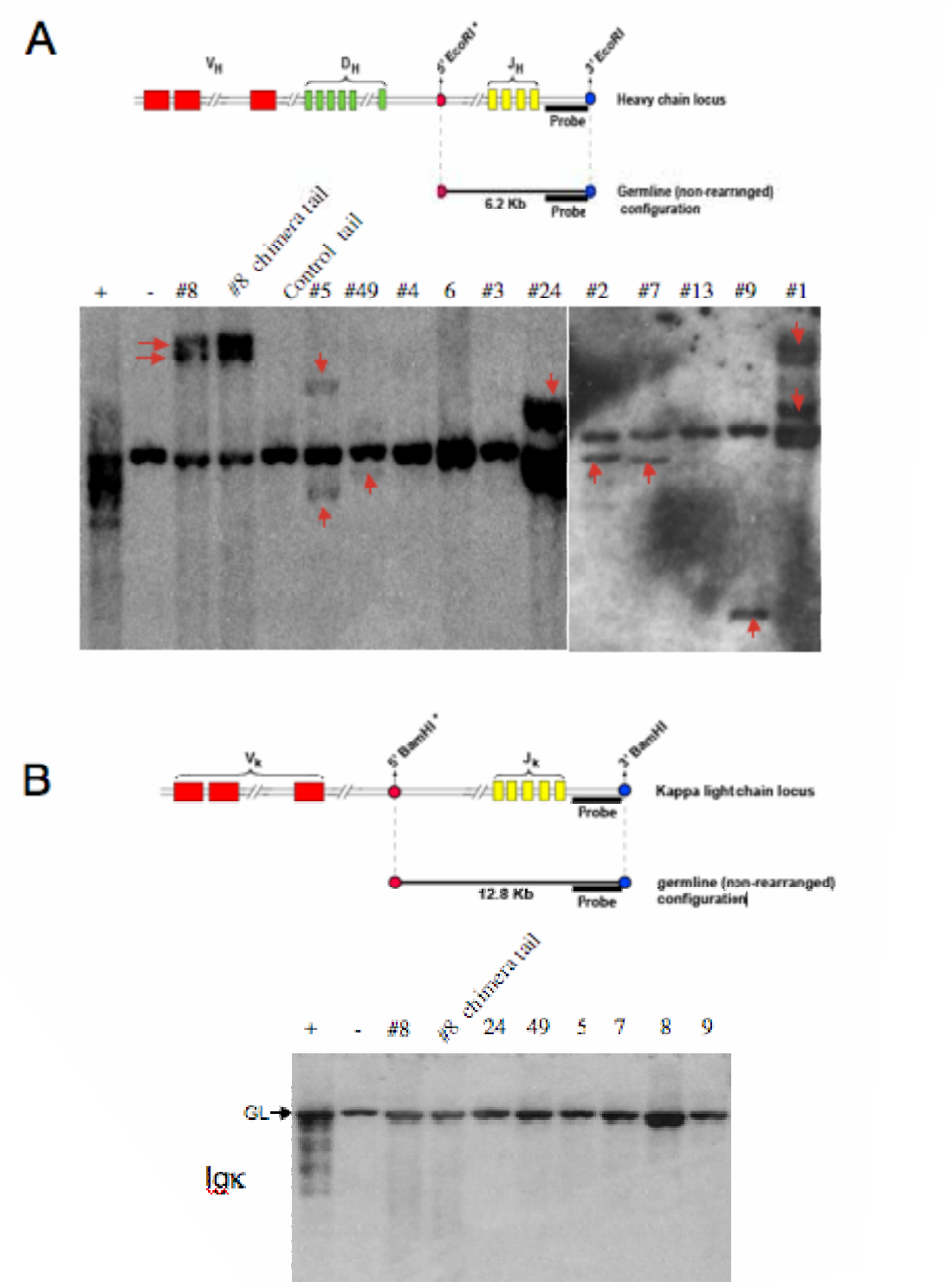
A
V_H
D_H
5' EcoRI*
J_H
3' EcoRI
Heavy chain locus
Probe
6.2 Kb
Probe
Germline (non-rearranged) configuration
+ - #8 #8 chimera tail Control tail #5 #49 #4 6 #3 #24 #2 #7 #13 #9 #1
B
V_K
5' BamHI*
J_K
3' BamHI
Kappa light chain locus
Probe
12.8 Kb
Probe
germline (non-rearranged) configuration
+ - #8 #8 chimera tail 24 49 5 7 8 9
GL
Igκ

Figure S3.

Figure S4. Teratoma formation by iB-iPS cells.

Hematoxylin and eosin staining of a representative teratoma derived from iB-iPS line #5.

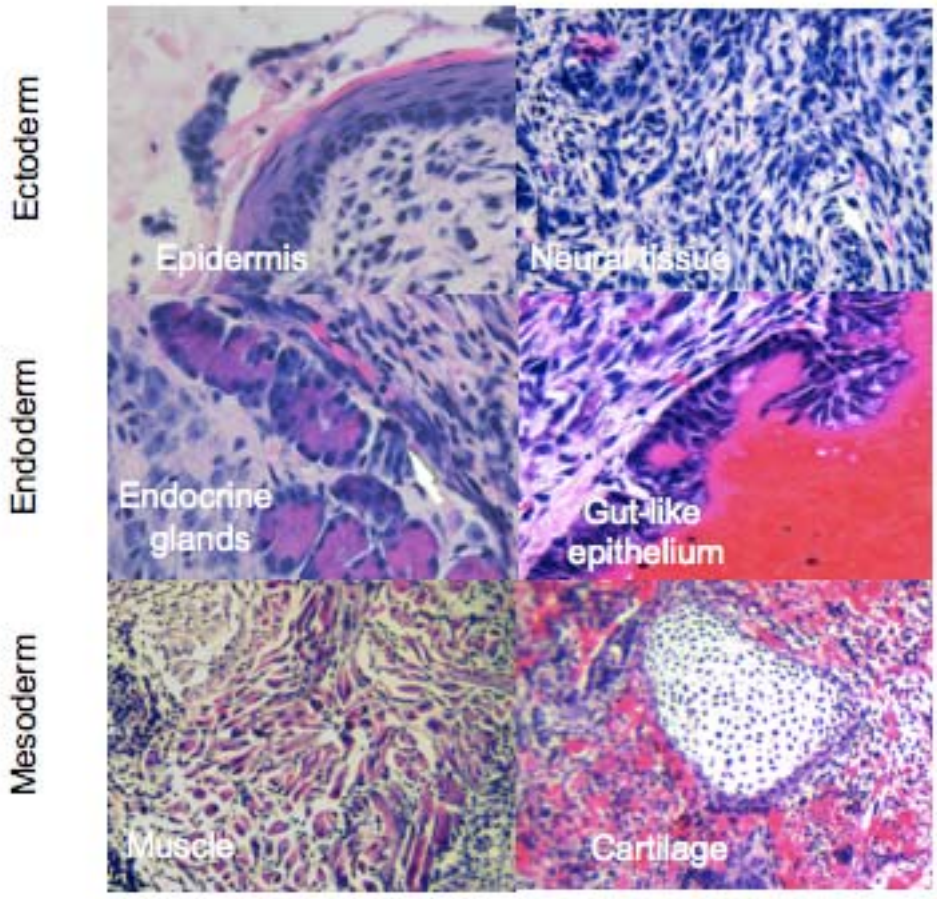

Figure S4.

Figure S5. Segregation of iB-iPS IgH rearranged allele via germline transmission.

Genomic DNA from mouse tail samples was digested with EcoRI and analyzed for V(D)J rearrangements at the *Igh* locus by Southern blotting using a 3' J_H4 probe. Red arrows indicate rearranged IgH alleles. The analysis showed detection of the same rearranged heavy chain allele present in iB-iPS line #9 (compare to Figure S3) and its derived chimera, in 2 out of 7 mice analyzed. This proves germline transmission of a rearranged heavy chain allele.

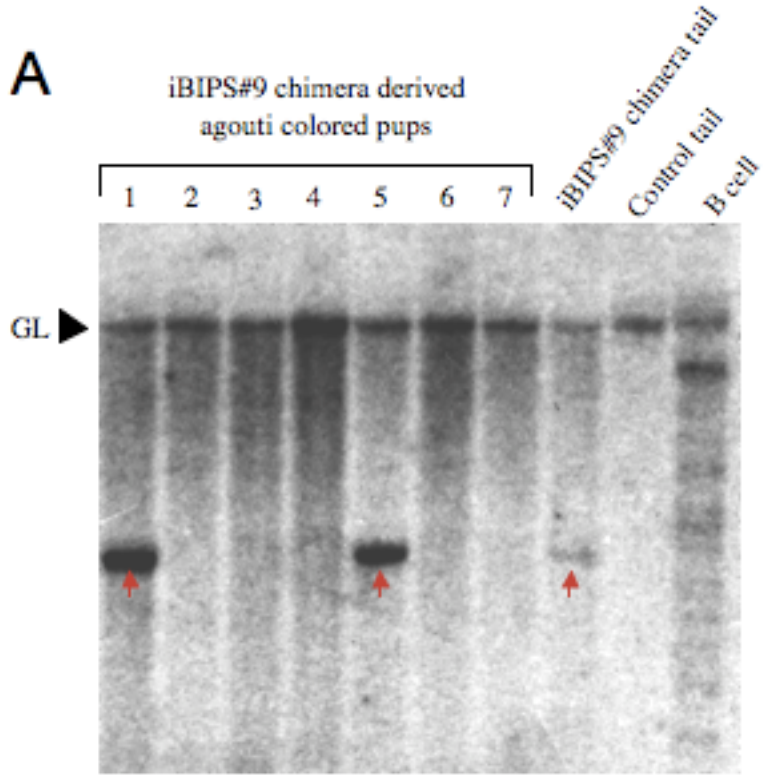

Figure S5.

Figure S6. Induction levels of Oct4, Sox2, Klf4 and c-Myc in C/EBPα infected transgenic B cells.

C/EBPα transcription factor was cloned in MSCV-IRES-GFP (pMIG) retroviral vector. CD19+ adult spleen B cells from iPS chimeras were infected with C/EBPα-pMIG retrovirus and grown in cytokine conditioned media and Dox for 5 days. RNA was prepared from sorted C/EBPα+ (GFP+) and C/EBPα- (GFP-) B cells. RT-PCR measurement for transgene induction between the two populations did not show any influence for C/EBPα expression on Oct4, Sxo2, Klf4 or c-MYC expression levels in response to Dox.

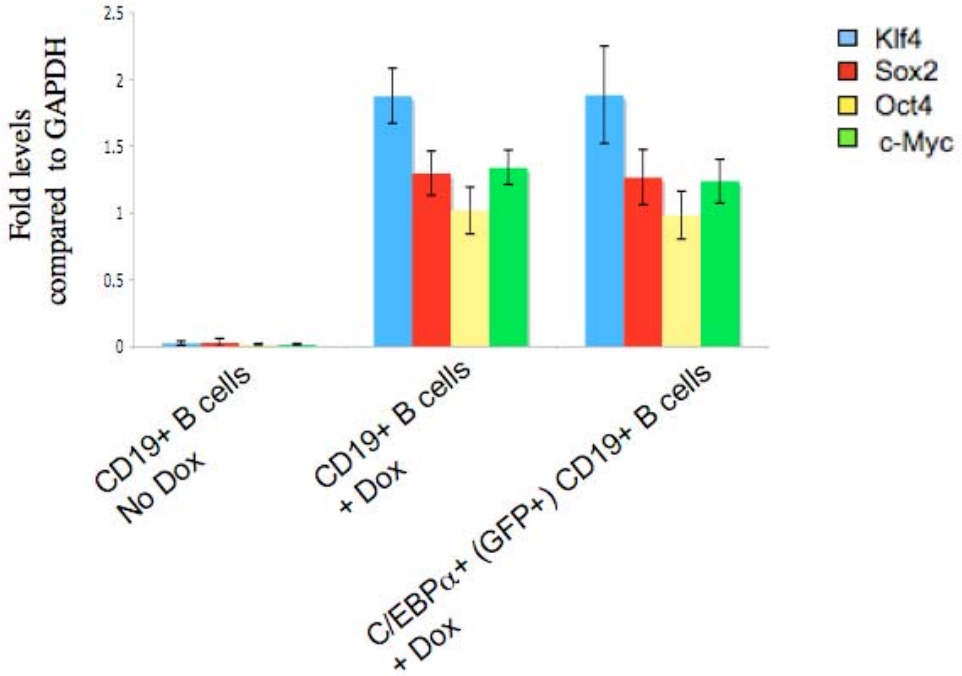

Figure S6.

Figure S7. Kinetics of pluripotency markers on induced B cell cultures.

Timing of pluripotency marker reactivation as analyzed by FACS staining for SSEA1 and Nanog-GFP. Top: Non fully differentiated bone marrow derived B cells induced with Dox on OP9 bone marrow stroma. Bottom: Fully differentiated IgM+IgD+ adult spleen B cells infected with C/EBPα and induced with Dox on OP9 cells. We performed FACS analysis to measure kinetics of SSEA1 and Nanog pluripotency marker activation in Dox induced bone marrow B220+ B cell populations and mature spleen IgM+IgD+ B cells infected with C/EBPα retrovirus. This assay showed similar reprogramming kinetics in which SSEA1+ cells were initially detected at day 7 and became abundant at the day 11 after Dox addition. Nanog expression was detected at ~ day 15 similar to the sequential appearance of pluripotency markers during reprogramming of MEFs (Brambrink et al., 2008).

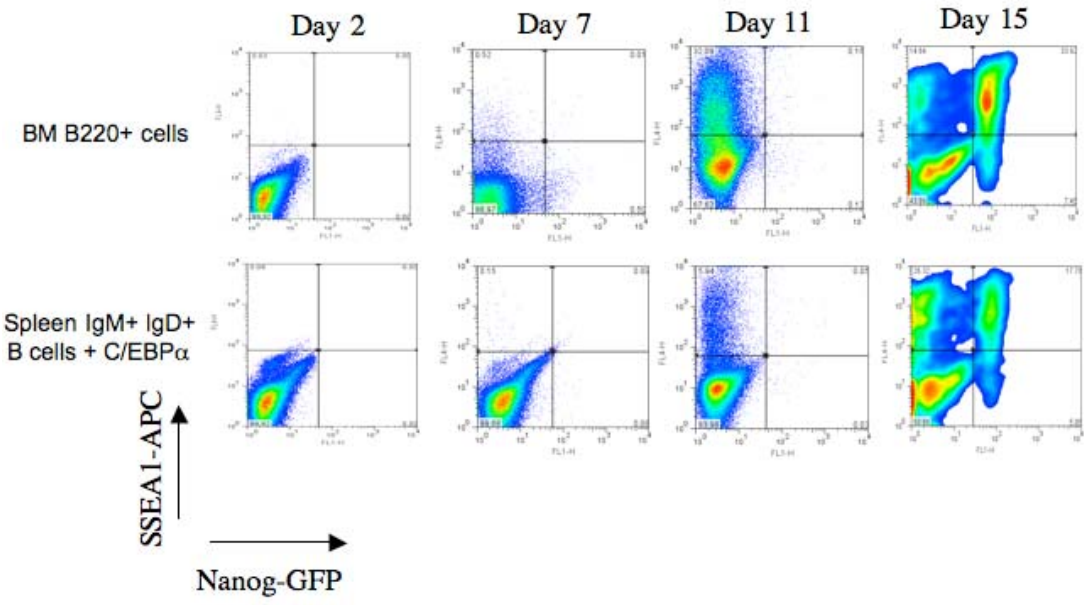

Figure S7.

Figure S8. Genomic detection of IgK rearrangements in somatic tissues in B-iPS derived chimeras.

Southern blot analysis for detection of IgK light chain rearrangements on BamH1 digested genomic DNA (as detailed in Figure 5A) obtained from different tissues obtained from MEF-iPS #1 derived chimeras (used as negative control) and B-iPS #1 cell line. Rearranged fragments observed in B-iPS derived chimeras match exactly those found in the injected iPS line thus proving monoclonality of the cell line and the origin of donor nuclei that were reprogrammed to pluripotency.

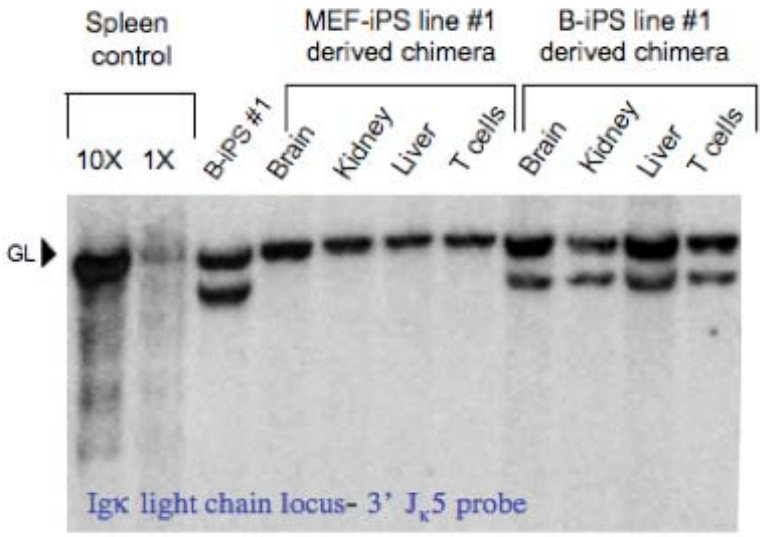

Figure S8.

Figure S9. Segregation of B-iPS Igκ rearranged allele via germline transmission.

Genomic DNA from mouse tail samples was digested with BamH1 and analyzed for VJ rearrangements at the Igκ locus by Southern blotting using a 3' Jκ5 probe. Red arrows indicate rearranged Igκ alleles. The analysis showed detection of the same rearranged heavy chain allele present in B-iPS line and it`s derived chimera, in 2 out of 3 GFP+ mice analyzed. This proves germline transmission of a rearranged light chain allele.

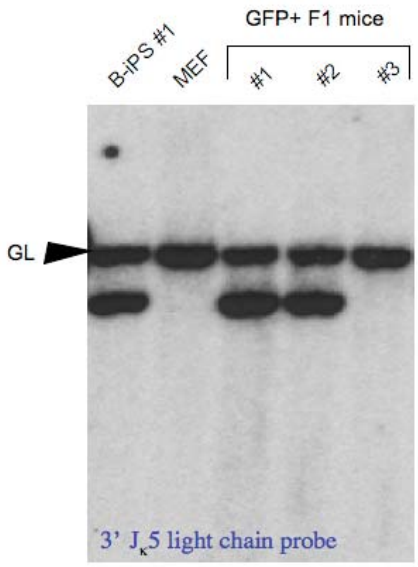

Figure S9.

Figure S10. “All B-iPS embryos”.

“All B-iPS cell embryos” were generated by injection of B-iPS cell into fused 4N blastocysts. Live day E12.5 embryos generated from B-iPS#9 cell line. GFP was uniformly detected in the obtained embryos as donor B-iPS was constitutively labeled with GFP prior to blastocysts injections (marked with lentiviral ubiquitin-EGFP vector).

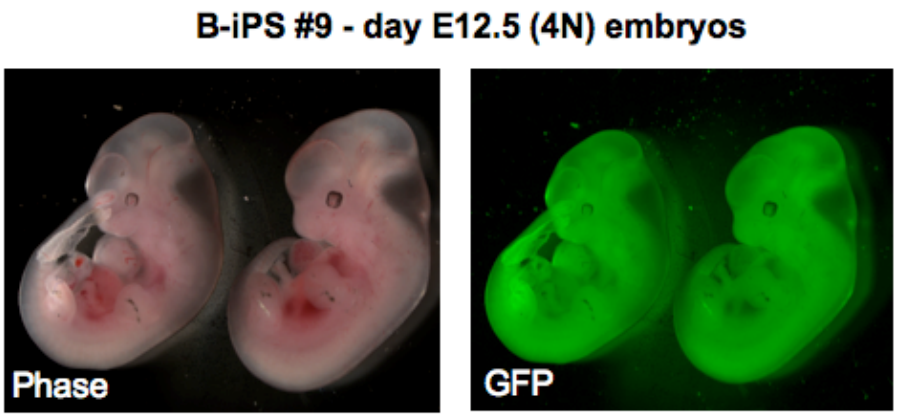

Figure S10.

Figure S11. Interruption with B cell transcriptional state via Pax5 knockdown sensitizes mature B cells for reprogramming.

(A) Adult spleen CD19 B cells were infected with Pax5 or Ecat1 shRNA hairpins cloned into pSicoR lentiviral vectors or empty pSicoR lentiviral backbone. Infected GFP+ cells were grown in cytokine conditioned media and RNA was harvested 60 hours later to verify specific knockdown of Pax5 transcript by the matching hairpin (~70% transcript knockdown, $p<0.01$). (B) IgM+IgD+ spleen cells were obtained from 4 week old adult C57Bl6/129SJ F1 carrying the R26M2rtTa allele male donor. Cells were infected with Pax5 KD-HP or control KD-HP (hairpins against Ecat1 or CD8) and were afterwards plated on irradiated OP9 stromal cell line in medium containing Dox. Cell colonies were picked at day 14 for further expansion. Original wells were stained for AP. One out of 2 independent experiments is shown. (C) Representative images for colonies at day 11 of Dox induction. (KD- Knock down; HP – Hairpin). (D) After subsequent culturing for ~30 days eventually acquired ES-like morphology and were Dox independent. (E) PCR analysis of Vκ-Jκ and Vλ1-Jλ light chain rearrangements and of V_H7183-DJ_H, andV_HJ558-DJ_H rearrangements on two different B-iPS lines obtained. Rearrangements to different J segments are numbered on the left side of panels. Detailed characterization of the different immunoglobulin gene rearrangements by cloning and sequencing was performed to confirm the presence of productive heavy and light chain rearrangements in the cell lines obtained. Blue arrows indicate “productive rearrangements” and red arrows indicate” non-productive rearrangements. This proved that both cell lines had unique productive heavy and light chain rearrangements. (F) Live newborn 2N chimera

obtained by injecting B-iPS #123 in host blastocysts, as detected by the agouti coat color.

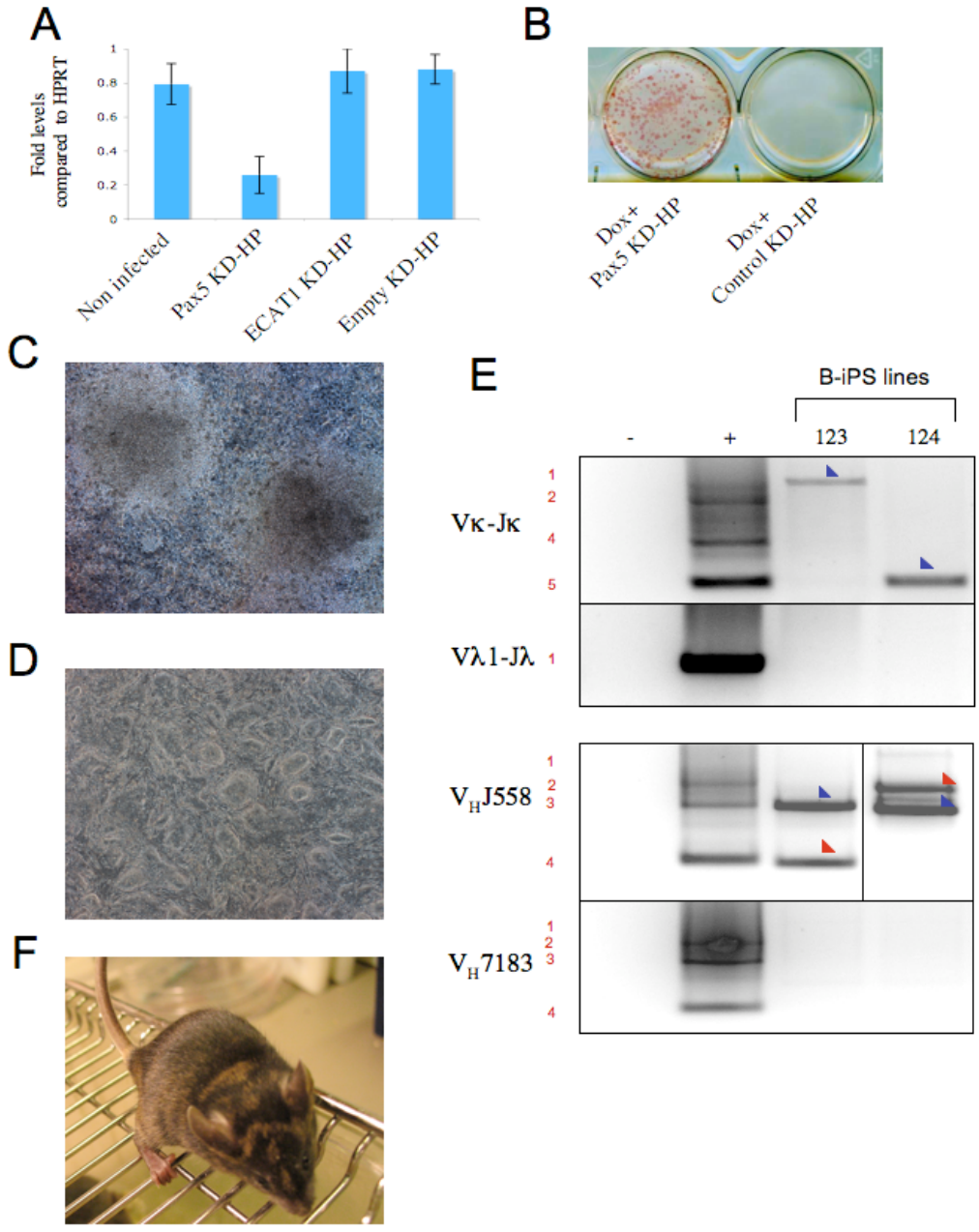

Figure S11.

Figure S12. Direct reprogramming of genetically unmodified adult terminally differentiated B cells.

IgM+IgD+ spleen cells were obtained from 22 week old adult C57Bl6/129SJ F1 carrying the R26M2rtTa allele male donor. Cells were subjected to 2 rounds of infection with Dox-inducible lentiviruses during the first 48 hours of culturing, then were plated on irradiated OP9 stromal cell line in medium containing Dox. Cells were subjected to another round of infection at day 10.(A) We were able to derive 2 cell lines that after subsequent culturing for ~25 days eventually acquired ES-like morphology and were Dox independent. Representative images of deriving B-iPS #121 cell line. (B) PCR analysis of Vκ-Jκ and Vλ1-Jλ light chain rearrangements and of V_H7183-DJ_H, andV_HJ558-DJ_H rearrangements. Rearrangements to different J segments are numbered on the left side of panels. Detailed characterization of the different immunoglobulin gene rearrangements by cloning and sequencing was performed to confirm the presence of productive heavy and light chain rearrangements in the cell lines obtained. Blue arrows indicate “productive rearrangements” and red arrows indicate” non-productive rearrangements. This proved that both cell lines had unique productive heavy and light chain rearrangements. (C) Representative staining for pluripotency markers on B-iPS #122 cell line. (D) Live newborn 2N chimeras obtained by injecting B-iPS #121 in host blastocysts, as detected by constitutive GFP marker that was introduced to the iPS cells prior to injection. Arrows indicate chimeric mice.

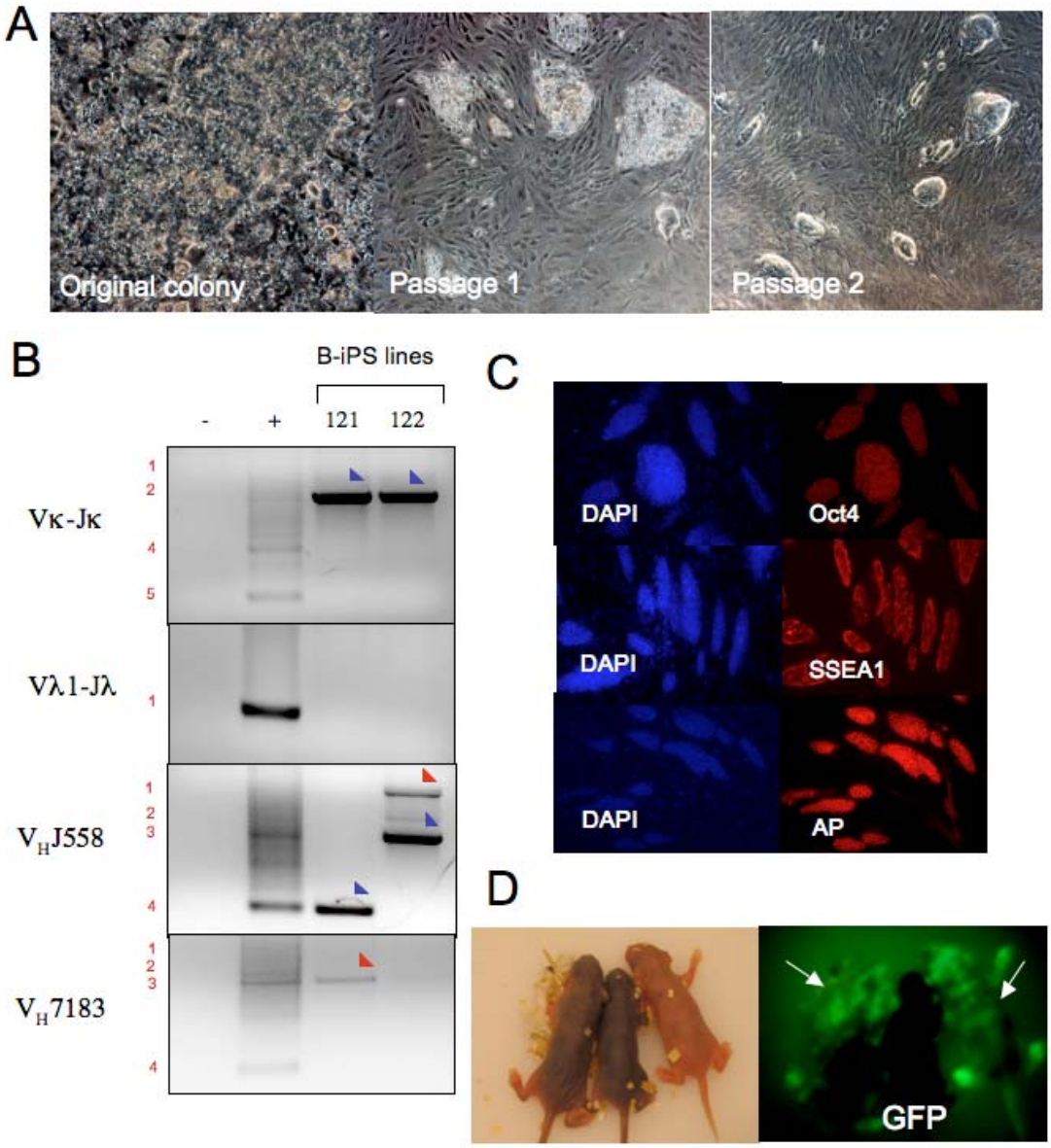
A
Original colony
Passage 1
Passage 2
B
B-iPS lines
-
+
121
122
Vκ-Jκ
Vλ1-Jλ
V_H J558
V_H 7183
C
DAPI
Oct4
DAPI
SSEA1
DAPI
AP
D
GFP

Figure S12.

Figure S13. Screening for successfully reprogrammed monoclonal mature B cells.

FACS examples of Nanog-GFP- (Top) and GFP+ wells (Bottom). PE channel was used to detect auto-florescence and confirm the specificity of the positive signal detected on the GFP channel.

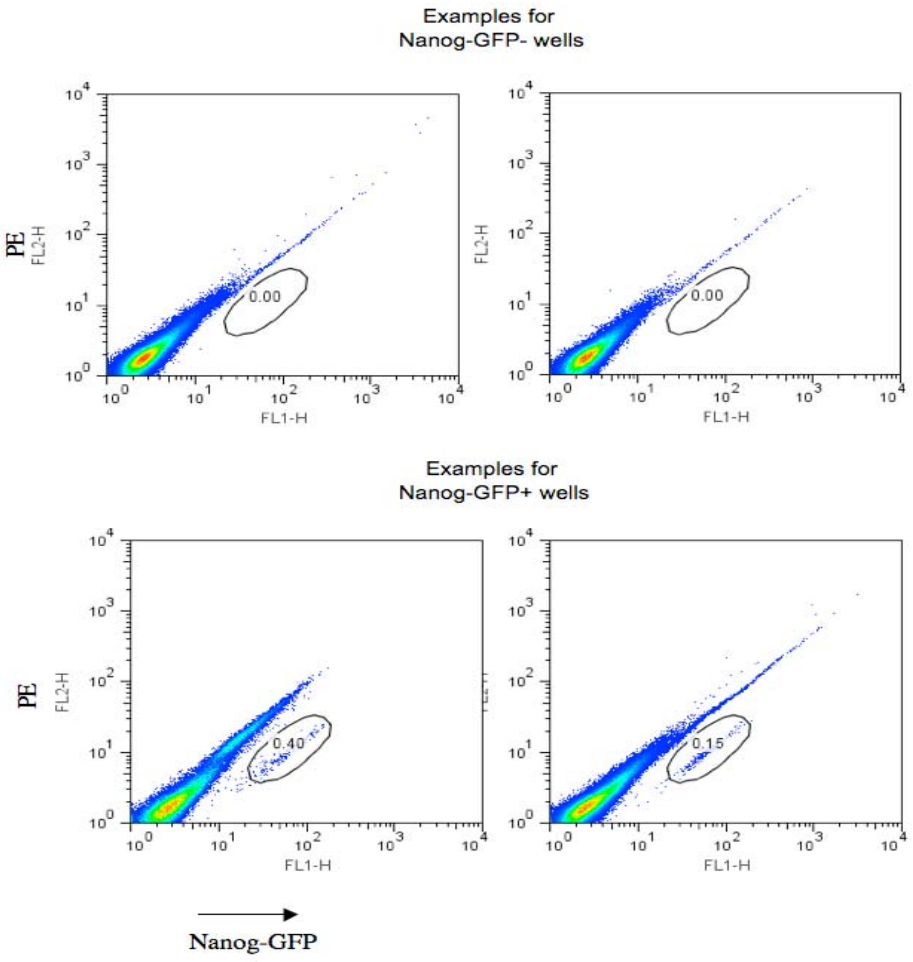

Figure S13.

Table S1. Summary of blastocyst injections.

The extent of chimerism was estimated on the basis of coat color or EGFP expression. ND, not determined. 4N injected blastocysts were analyzed between day E10.5 and E14.5. 'Analyzed' indicates the day of embryonic development analyzed; 'arrested' indicates the estimated stage of development of dead embryos.

	2N injections				4N injections		
Cell line	Injected blastocysts	Live chimeras	chimerism	germline	Injected blastocysts	Dead embryos (arrested)	Live embryos (analyzed)
iB-iPS #1	36	1	10-30	ND	ND	ND	ND
iB-iPS #4	95	5	40--70	Yes	ND	ND	ND
iB-iPS #8	20	2	50-70	No	ND	ND	ND
B-iPS #1	40	3	20-60	Yes	ND	ND	ND
B-iPS #2	24	2	30-50	No	ND	ND	ND
B-iPS #4	135	6	30-80	ND	115	7 (E10-14.5)	3 (E12.5) 2 (E14.5)
B-iPS #9	95	8	30-80	ND	90	5 (E9-12.5)	5 (E12.5)
B-iPS #123	48	1	30-50	ND	ND	ND	ND
B-iPS #121	46	3	30-60	ND	ND	ND	ND

Table S2. Primers used for PCR analysis of *Ig* rearrangements

K: G or T, M: A or C, S: C or G, R: A or G, W: A or T, Y: C or T.

Sense Oligonucleotides		
Igh locus	**V_HJ558**	CGAGCTCTCCARCACAGCCTWCATGCARCTCARC
	V_H7183	CGGTACCAAGAASAMCCTGTWCCTGCAAATGASC
	V_HQ52	CGGTACCAGACTGARCATCASCAAGGACAAYTCC
	V_HGam3.8	CAAGGGACGGTTTGCCTTCTCTTTGGAA
	DSF	AGGGATCCTTGTGAAGGGATCTACTACTGTG
IgL loci	**Vλ1**	GCCATTTCCCCAGGCTGTTGTGACTCAGG
	Vκ	GGCTGCAGSTTCAGTGGCAGTGGRTCWGGRAC
Antisense Oligonucleotides		
Igh locus	**J_H4**	TCTCAGCCGGCTCCCTCAGGG
	J_H4 (used with DSF primer)	AAAGACCTGCAGAGGCCATTCTTACC
IgL loci	**Jλ1,3**	ACTCACCTAGGACAGTCAGCTTGGTTCC
	Jκ5	ATGCGACGTCAACTGATAATGAGCCCTCTCC