Supplemental Table 1: Characteristics of Adeno-iPS clones

Line	Source	Oct4^{IND}	GFP	Ploidy	Viral Integrations	ESM	TF	PC	GL
FL-1	Fetal liver	Yes	No	2n	No[2]	Yes	ND	ND	ND
FL-2	Fetal liver	Yes	No	2n	ND	Yes	ND	ND	ND
FL-3	Fetal liver	Yes	No	2n	No[2]	Yes	ND	ND	ND
FL-4	Fetal liver	Yes	No	2n	No[2]	Yes	ND	ND	ND
FL-5	Fetal liver	Yes	No	2n	No[2]	Yes	Yes	ND	ND
FL-6	Fetal liver	Yes	No	2n	ND	Yes	Yes	ND	ND
FL-7	Fetal liver	Yes	Sox2	2n	No[1,2]	Yes	Yes	Yes	ND
FL-8	Fetal liver	Yes	Sox2	4n	ND	ND	ND	ND	ND
FL-9	Fetal liver	Yes	Sox2	2n	No[1,2]	Yes	Yes	Yes	Yes
TTF-1	Fibroblast	Yes	Oct4	2n	No[1,2]	Yes	Yes	Yes	Yes
HEP-1	Hepatocyte	No	Oct4	4n	No[1]	ND	ND	ND	ND
HEP-2	Hepatocyte	No	Oct4	2n	No[1,2]	Yes	Yes	Yes	ND
HEP-3	Hepatocyte	No	Oct4	4n	No[1]	ND	ND	ND	ND

Oct4^{IND} = Oct4^{ind} allele present
GFP = GFP reporter gene present
No[1] = tested by PCR
No[2] = tested by Southern
ESM = expression of ES cell markers (SSEA1 and Oct4 or Sox2)
TF = teratoma formation
PC = postnatal chimeras
GL = germline transmission

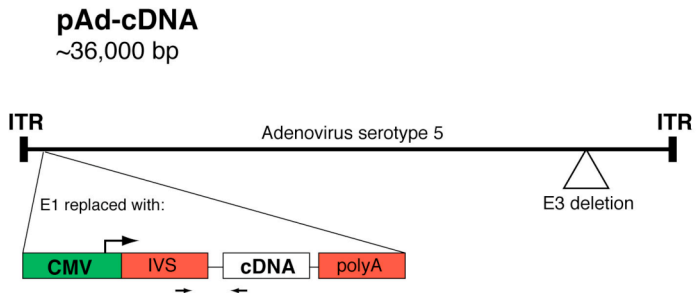
Supplemental Table 2: PCR primers to test for adenoviral integration

Gene	**Forward primer** (5' to 3')	**Reverse primer** (5' to 3')
Myc	ATCTTCCTCCCACAGCTCCT	CACCGAGTCGTAGTCGAGGT
Klf4	ATCTTCCTCCCACAGCTCCT	TCGTTGAACTCCTCGGTCTC
Oct4	ATCTTCCTCCCACAGCTCCT	CCAAGGTGATCCTCTTCTGC
Sox2	ATCTTCCTCCCACAGCTCCT	CTCCGGGAAGCGTGTACTTA

Supplemental Table 3: Primers used for qPCR analysis

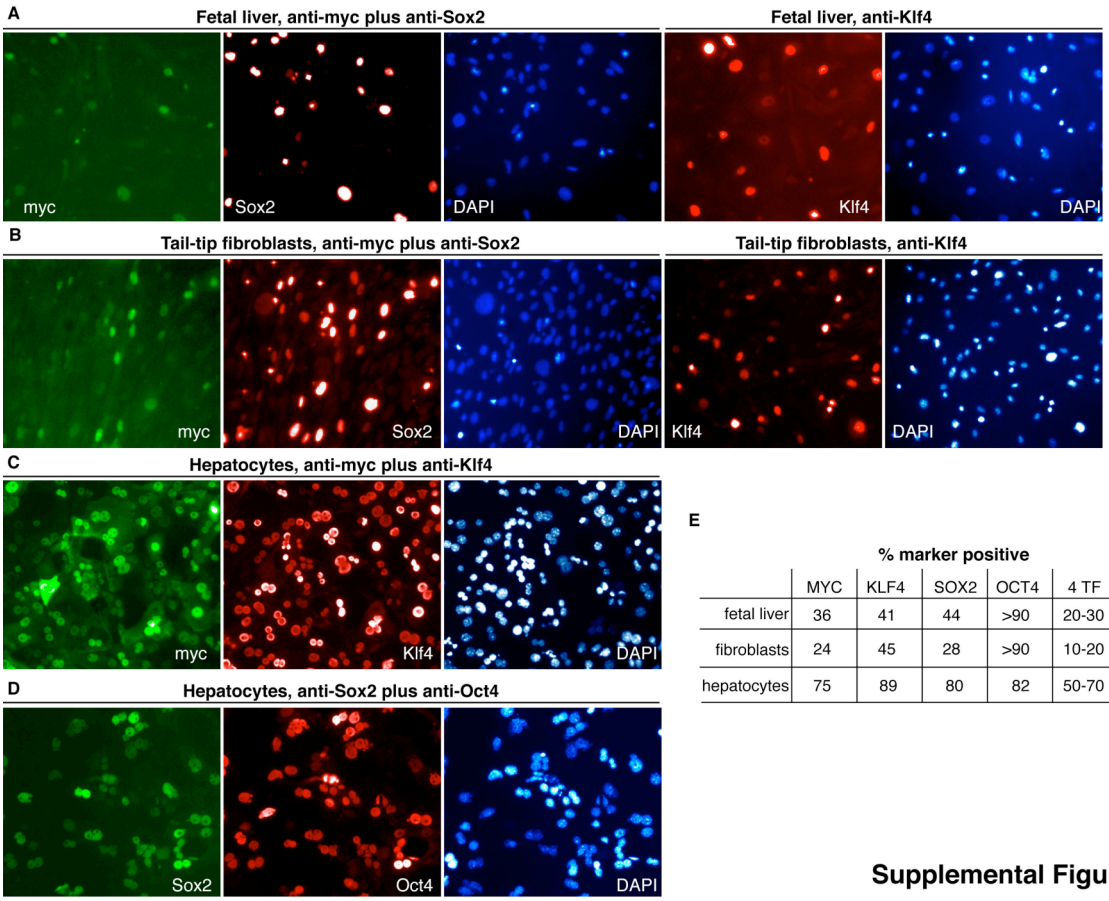
Gene	**Forward primer** (5' to 3')	**Reverse primer** (5' to 3')
Myc	TAACTCGAGGAGGAGCTGGA	GCCAAGGTTGTGAGGTTAGG
Klf4	AACATGCCCGGACTTACAAA	TTCAAGGGAATCCTGGTCTTC
Oct4	TAGGTGAGCCGTCTTTCCAC	GCTTAGCCAGGTTCGAGGAT
Sox2	AGGGCTGGGAGAAAGAAGAG	CCGCGATTGTTGTGATTAGT
nanog	TTGCTTACAAGGGTCTGCTACT	ACTGGTAGAAGAATCAGGGCT
gapdh	AGGTCGGTGTGAACGGATTTG	TGTAGACCATGTAGTTGAGGTCA
Myc (viral)	CGGCCAGCGACTAGTAGAA	GAAGCTAACGTTGAGGGGTCT
Klf4 (viral)	CACCATGGACTACAAAGACGA	ACGCAGTGTCTTCTCCCTTC
Oct4 (viral)	CAACGTGCTGGTCTGTGTG	CGAAGTCTGAAGCCAGGTGT
Sox2 (viral)	ACGTGCTGGTCTGTGTGCT	TTCAGCTCCGTCTCCATCAT

Supplemental Figure 1



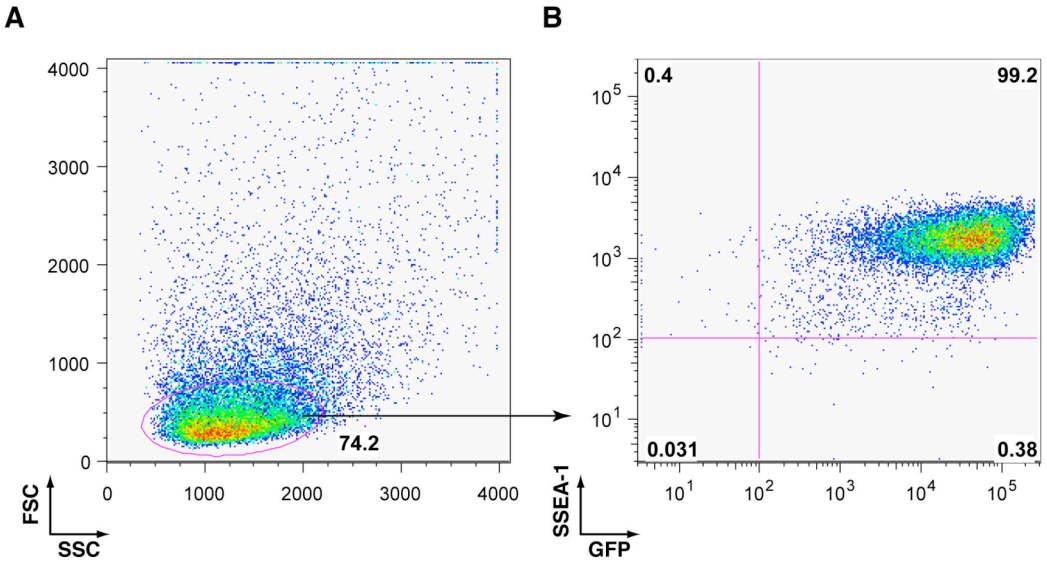
Supplemental Figure 1: Structure of the adenoviral vector

Schematic drawing of the basic elements of the adenoviral vector showing the viral Inverted Terminal Repeats (ITR) as well as the E3 deletion. The viral E1 region was replaced with a transgenic expression cassette consisting of a cytomegalovirus (CMV) promoter, the intervening sequence (IVS) and the polyadenylation signal (polyA) of the human beta globin gene. The position where the cDNA of the individual reprogramming genes was integrated is shown. Small arrows indicate the location of the primers used to test for adenoviral integration into the genome (see Figure 2B).



Supplemental Figure 2: Adenoviral infection efficiency of different cell types

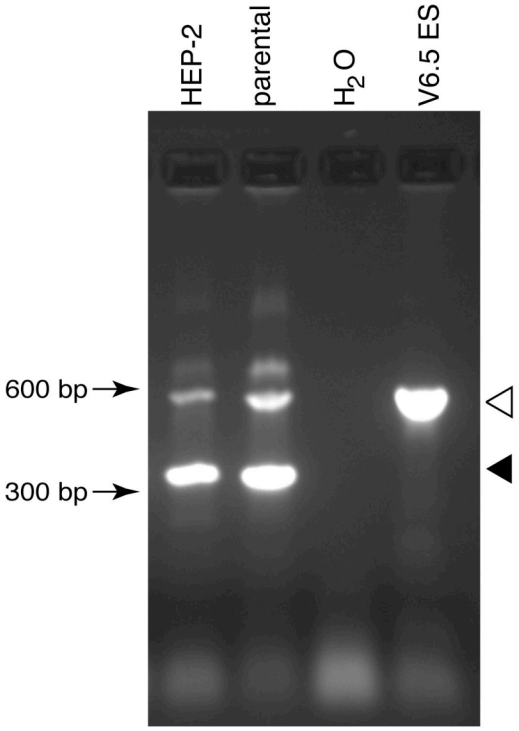
(A, B) Immunofluorescence labeling of fetal liver cells (A) or tail-tip fibroblasts (B), either co-infected with adenoviruses expressing c-myc and Sox2 or with a Klf4 expressing adenovirus alone. Nuclei were counterstained with DAPI. (C, D) Immunofluorescence labeling of adult hepatocytes after co-infection with adenoviruses expressing either c-myc and Klf4 (C) or Sox2 and Oct4 (D). DAPI staining is shown in blue. Note that in all three cell types the majority of cells that got infected expressed more than one viral gene. (E) Table summarizing the infection efficiency for the different cell types as well as the percentages of cells assumed to express all four reprogramming factors, estimated based on the frequency of double-infected cells. In cells derived from Oct4^{IND} mice, a high percentage (~90%) of cells has been shown to express the transgene (*8*).



Supplemental Figure 3: Marker gene expression in Adeno-iPS clone HEP-2

(A, B) FACS diagram of HEP-2 Adeno-iPS cells showing forward (FSC) and side (SSC) scatter (A) or expression of SSEA-1 and GFP (from the endogenous Oct4 locus), respectively. In (B) only the cells gated on in (A) are shown. Numbers indicate percentages of cells in the respective quadrants.

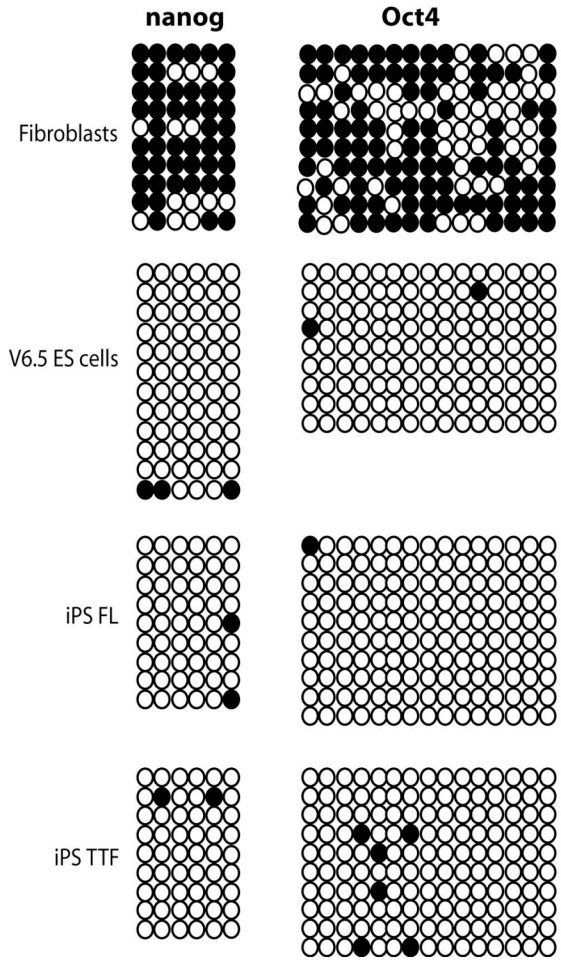
Supplemental Figure 4



Supplemental Figure 4: "Fingerprinting" of Adeno-iPS clone HEP-2

PCR analysis of the ROSA26 locus using genomic DNA from the Adeno-iPS clone HEP-2, from a tail-tip biopsy of the parental mouse originally used for hepatocyte isolation and from V6.5 ES cells. An open arrowhead indicates the position of the band representing a wildtype *ROSA26* allele (present in all DNA samples) and a solid arrowhead the position of the modified *ROSA26* allele containing the rtTA sequence (only present in the HEP-2 and parental samples).

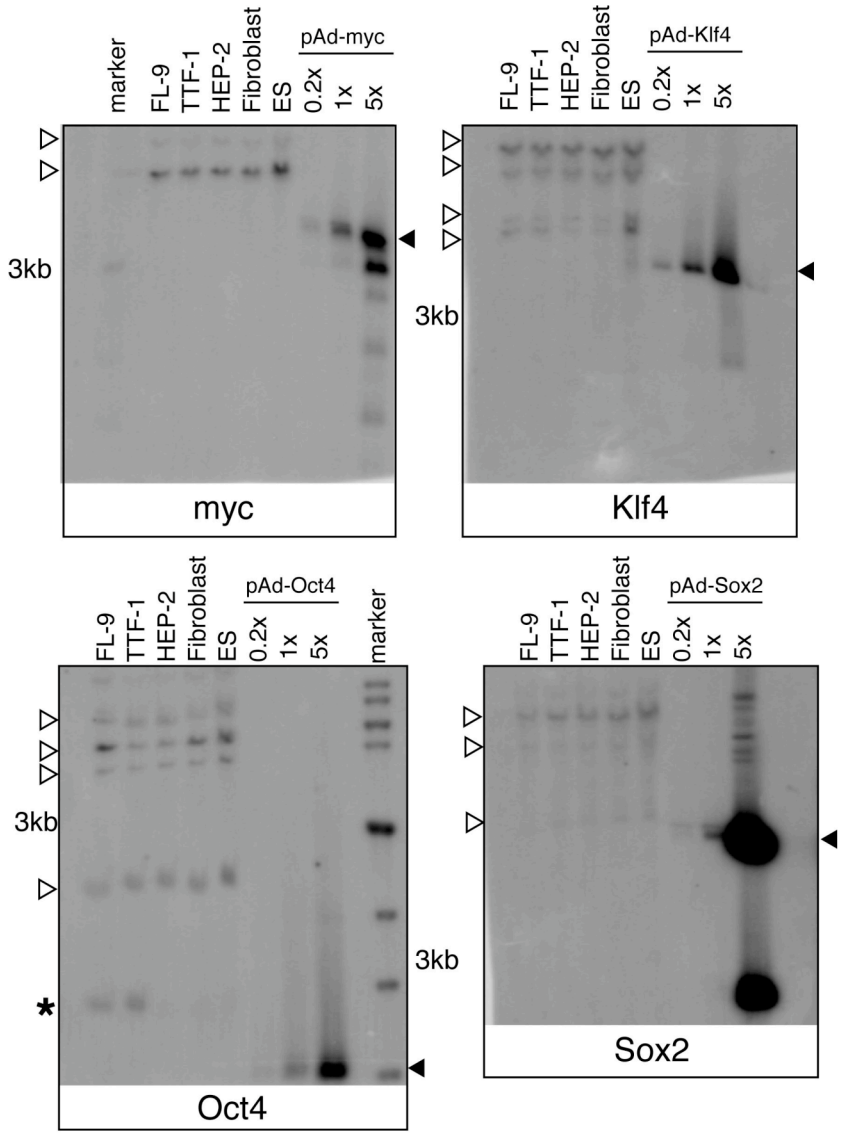
Supplemental Figure 5



Supplemental Figure 5: Promoter methylation of Adeno-iPS cells

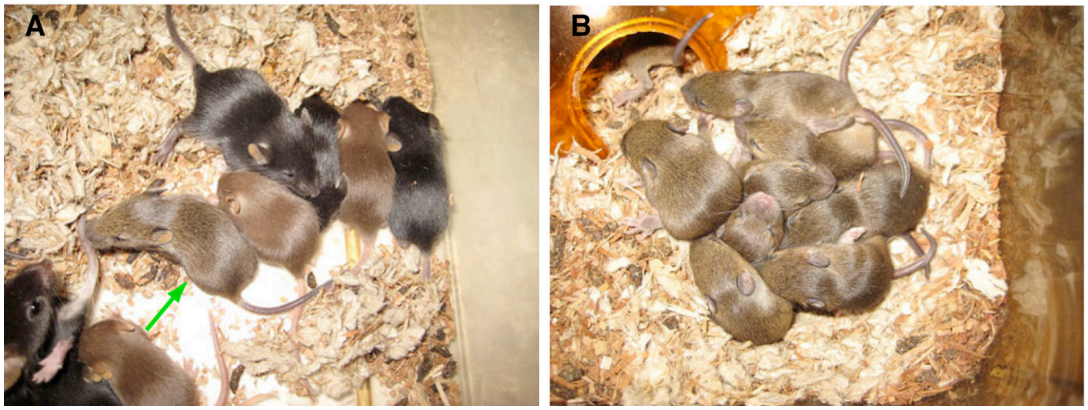
Bisulfite sequencing of the *Oct4* and *Nanog* promotors in tail-tip fibroblasts, V6.5 ES cells and iPS cells derived from fetal liver (FL) or fibroblasts (TFF). Open circles represent unmethylated CpGs; closed circles denote methylated CpGs.

Supplemental Figure 6



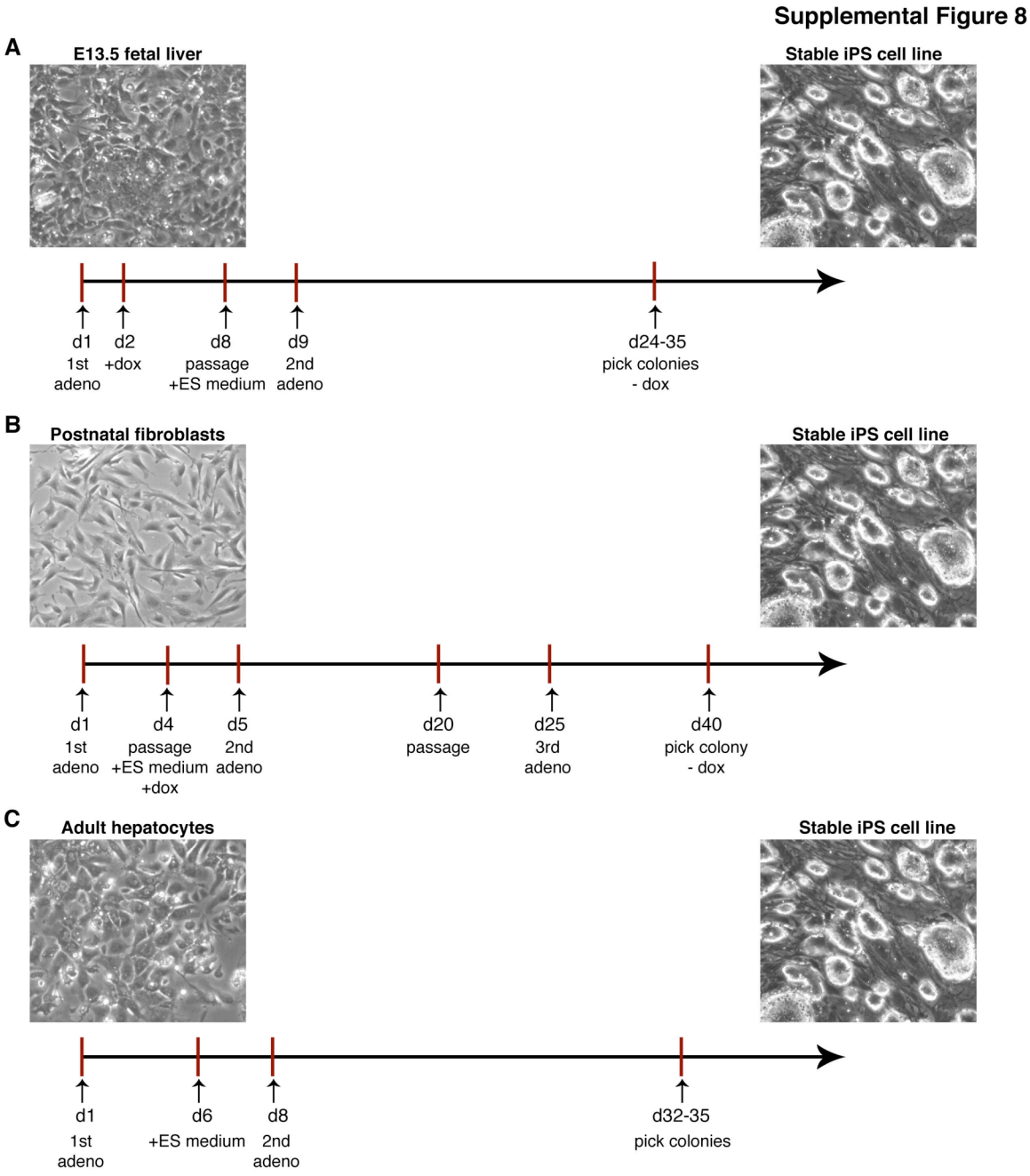
Supplemental Figure 6: Southern blot analysis for adenoviral cDNA integration

Southern blot analysis using cDNA fragments of the four reprogramming genes as probes. Plasmid DNA diluted to the equivalent of 0.2, 1 or 5 integrations per genome was used as positive controls (the positions of bands expected in the presence of viral transgenes in the DNA samples are indicated by closed arrowheads). The positions of endogenous genes and pseudogenes also recognized by the cDNA probes are highlighted by open arrowheads. An asterisk in the Oct4 blot indicates the position of a band corresponding to the *Oct4* cDNA integrated in the Col1A locus in Oct4^{IND} cells.



Supplemental Figure 7: Germline contribution of Adeno-iPS cells

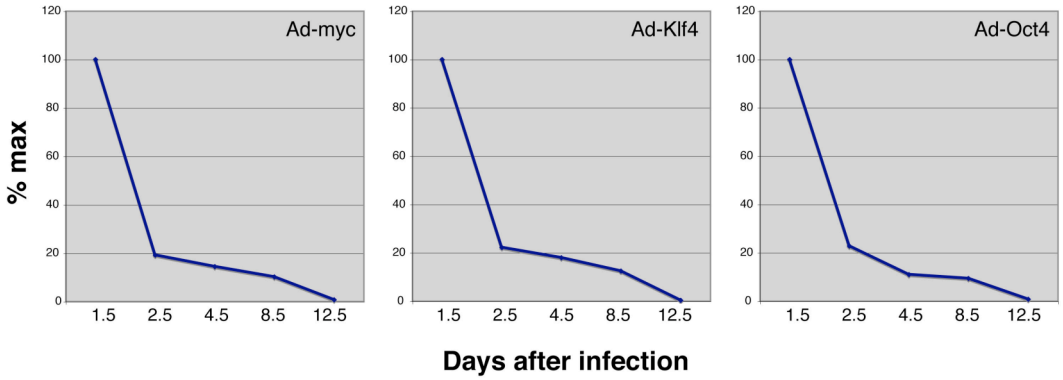
(A, B) Images of pups derived from matings between BDF1 females and male chimeras generated with FL-9 Adeno-iPS cells (A) or TTF-1 Adeno-iPS cells (B), respectively. Agouti coat color is indicative of pups of germline transmission. Only one iPS-derived pup is present in the FL-9 litter (green arrow) while the entire TTF-1 litter is iPS-derived.



Supplemental Figure 8: Timeline of adenoviral reprogramming experiments

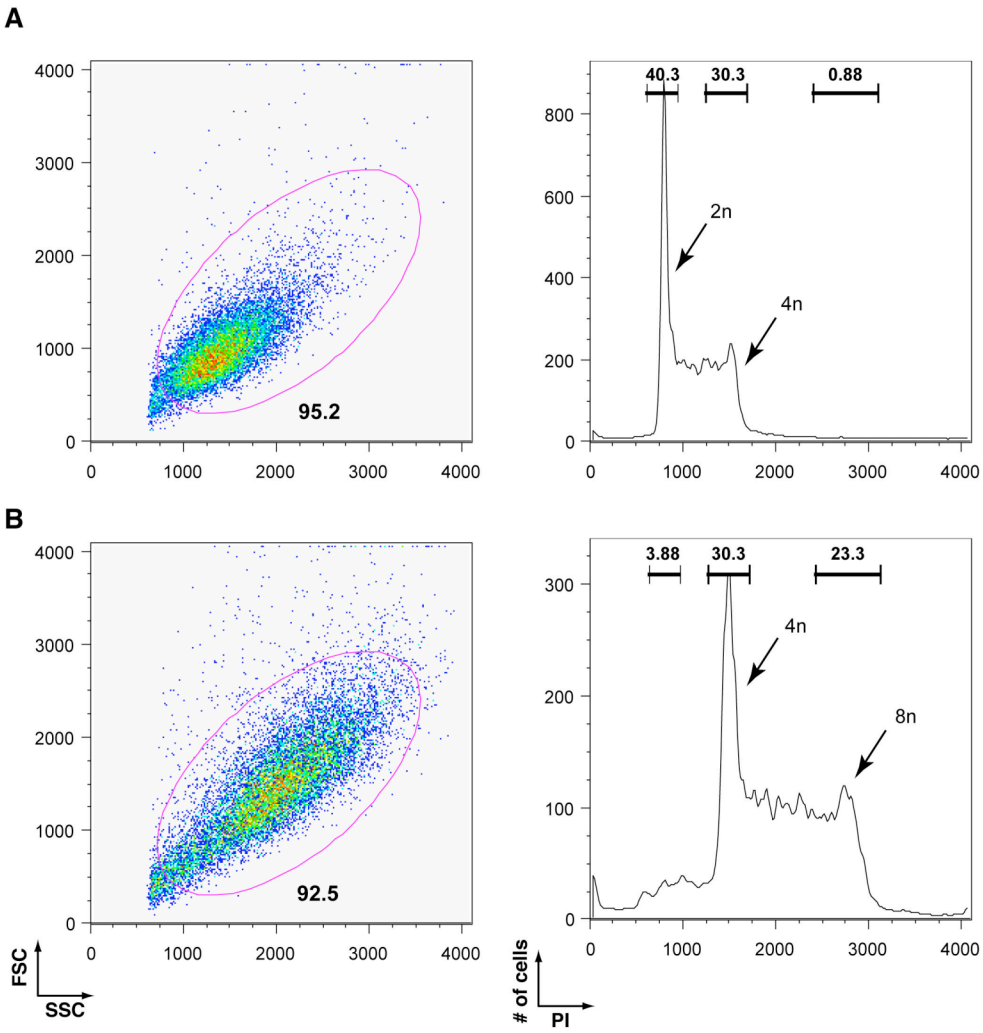
(A-C) Experimental timelines for the derivation of Adeno-iPS cells from fetal liver (A), postnatal fibroblasts (B) and adult hepatocytes (C). The images to the left show the respective starting populations at the time of adenoviral infection. On the right, an image of an established Adeno-iPS cell line is shown. Arrows indicate important experimental steps at the respective days (d).

Supplemental Figure 9



Supplemental Figure 9: Kinetics of adenoviral gene expression

Diagrams showing the decrease in expression of adenoviral myc, Klf4 and Oct4 in infected wildtype tail-tip fibroblasts as measured by qPCR. The cells were kept subconfluent to allow dilution of the adenoviral vectors by continuous cell divisions. The significant drop in viral gene expression between days 1.5 and 2.5 is possibly caused by the death of cells expressing the highest levels of viral transgenes.



Supplemental Figure 10: Ploidy analysis of Adeno-iPS cells

(A, B) FACS diagrams showing forward (FSC) and side (SSC) scatter (left panel) and PI labeling (right panel) of diploid (A) and tetraploid (B) Adeno-iPS cells. In the right panel only the cells gated on in the left panel are shown. Note that tetraploid cells are larger than diploid cells, as evidenced by the increased FSC and SSC values (right panel).

Supplemental References

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