Supplementary Table 1

Primary data for Figure 3a, *left panel*

Assay #	MEF isolate (ID #)	Genotype	Initial number of cells seeded	% of infected cells (GFP+)	Number of infected cells	Number of AP+ colonies	Reprogramming efficiency (% of AP+ colonies relative to infected cells)	Relative efficiency with respect to wt
1	D1	wt	250000	40,18	100450	744	**0,74**	average
		wt	duplicated			804	**0,80**	of the 3 wt:
	D3	wt	250000	38,36	95900	688	**0,72**	**1,0**
	C102	*Ink4a/Arf*-null	250000	43,36	108400	6040	**5,57**	**7,4**
	C101	*Ink4a/Arf*-null	250000	40,9	102250	6795	**6,65**	**8,8**
	F206	*p53*-null	250000	66,72	166800	2266	**1,36**	**1,8**
	A10	*Arf*-null	250000	36,01	90025	5010	**5,57**	**7,4**
	A9	*Arf*-null	250000	35,7	89250	5800	**6,50**	**8,6**
	K3	*p21*-null	250000	47,34	118350	1880	**1,59**	**2,1**
	K2	*p21*-null	250000	36,26	90650	1890	**2,08**	**2,8**
2	D5	wt	250000	64,09	160225	428	**0,27**	**1,0**
	C102	*Ink4a/Arf*-null	250000	60,59	151475	6528	**4,31**	**16,1**
	A10	*Arf*-null	250000	77,24	193100	3224	**1,67**	**6,3**
	A9	*Arf*-null	250000	62,81	157025	2990	**1,90**	**7,1**
3	D5	wt	250000	63,32	158300	544	**0,34**	**1,0**
	C102	*Ink4a/Arf*-null	250000	53,07	132675	6775	**5,11**	**14,9**
	A10	*Arf*-null	250000	60,52	151300	3021	**2,00**	**5,8**
4	D2	wt	250000	9,79	24475	240	**0,98**	**1,0**
	C40	*Ink4a/Arf*-null	250000	13,55	33875	5320	**15,70**	**16,0**
	C79	*Ink4a/Arf*-null	250000	13,76	34400	4608	**13,40**	**13,7**
	C81	*Ink4a/Arf*-null	250000	13,4	33500	7742	**23,11**	**23,6**
	F201	*p53*-null	250000	18,85	47125	2352	**4,99**	**5,1**
	F205	*p53*-null	250000	16,69	41725	2824	**6,77**	**6,9**
	F203	*p53*-null	250000	11,9	29750	2178	**7,32**	**7,5**
	F192	*p53*-null	250000	12,3	30750	1835	**5,97**	**6,1**
	K1	*p21*-null	250000	13,39	33475	1864	**5,57**	**5,7**
		p21-null	duplicated			1380	**4,10**	**4,2**
	K2	*p21*-null	250000	13,64	34100	1006	**2,95**	**3,0**
		p21-null	duplicated			1506	**4,40**	**4,5**
	K3	*p21*-null	250000	10,02	25050	1298	**5,18**	**5,3**
		p21-null	duplicated			894	**3,60**	**3,6**
5	D5	wt	250000	64,09	160225	528	**0,33**	**1,0**
	C102	*Ink4a/Arf*-null	250000	60,51	151275	6771	**4,48**	**13,6**
	A9	*Arf*-null	250000	59,81	149525	4135	**2,77**	**8,4**
	A10	*Arf*-null	250000	57,24	143100	4539	**3,17**	**9,6**
6	D4	wt	250000	50,04	125100	224	**0,18**	**1,0**
	C81	*Ink4a/Arf*-null	250000	58,96	147400	6308	**4,28**	**23,9**
	C79	*Ink4a/Arf*-null	250000	67,43	168575	8068	**4,79**	**26,7**
	A5	*Arf*-null	250000	60,56	151400	2808	**1,85**	**10,4**
	F301	*p53*-null	250000	64,89	162225	1026	**0,63**	**3,5**
	F304	*p53*-null	250000	70,7	176750	1656	**0,94**	**5,2**

Primary data for Figure 3a, *right panel*

MEF isolate (ID #)	Genotype	Reprogramming factors	Initial number of cells seeded	% of infected cells (GFP+)	Number of infected cells	Number of AP+ colonies	Reprogramming efficiency (% of AP+ colonies relative to infected cells)	Relative efficiency with respect to wt-3F
D4	wt	3F	250000	56,52	141300	844	**0,60**	average wt-3F:
D5	wt	3F	250000	53,20	133000	630	**0,47**	**1,0**
D4	wt	4F	250000	34,76	86900	1692	**1,95**	**3,6**
D5	wt	4F	250000	30,50	76250	1350	**1,77**	**3,3**
C102	*Ink4a/Arf*-null	3F	250000	71,89	179725	5290	**2,94**	**5,5**
C101	*Ink4a/Arf*-null	3F	250000	60,50	151250	6000	**3,97**	**4,4**
C102	*Ink4a/Arf*-null	4F	250000	56,58	141450	4736	**3,35**	**6,3**
C101	*Ink4a/Arf*-null	4F	250000	45,23	113075	5200	**4,60**	**5,1**

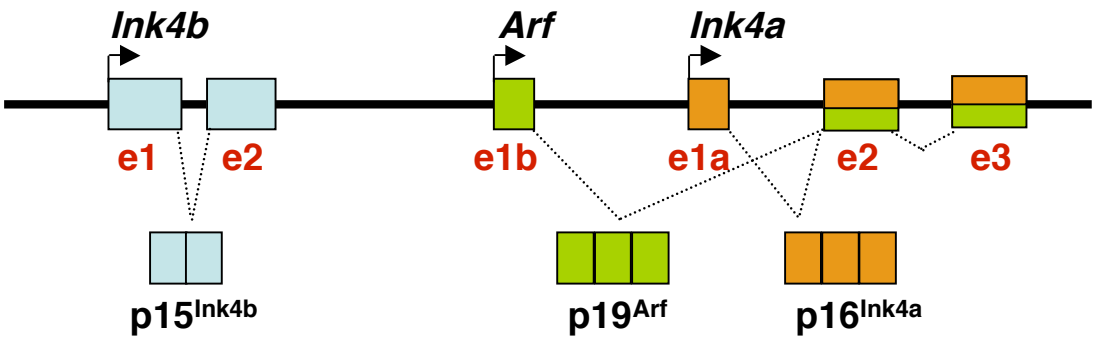
(continuation of Supplementary Table 1)

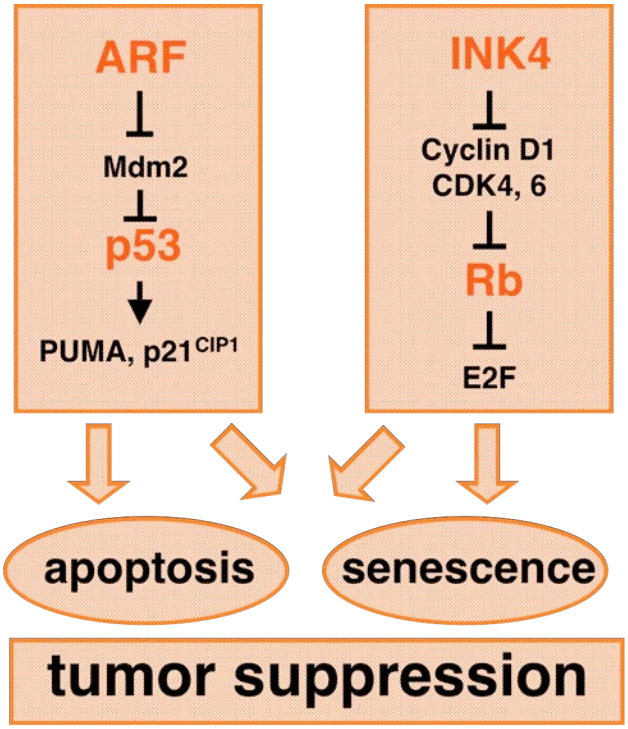
Primary data for Figure 3b

Assay #	Genotype	shRNA (retroviral)	Initial number of cells seeded	Number of infected cells	Number of AP+ colonies	Reprogramming efficiency (% of AP+ colonies relative to infected cells)	Relative efficiency with respect to wt of each assay
1	wt	empty vector	250000	44,77	152	**0,14**	**1,0**
		shInk4a	250000	45,00	312	**0,28**	**2,0**
		shArf	250000	40,00	800	**0,80**	**5,9**
		sh(Ink4a/Arf)	250000	48,00	1590	**1,33**	**9,8**
2	wt	empty vector	250000	53,41	200	**0,15**	average wt:
		empty vector	duplicated		279	**0,21**	**1,0**
		shInk4a	250000	50,01	505	**0,40**	**2,3**
		shInk4a	duplicated		476	**0,38**	**2,1**
		shArf	250000	55,27	1200	**0,87**	**4,8**
		shArf	duplicated		1432	**1,04**	**5,8**
		sh(Ink4a/Arf)	250000	51,32	2192	**1,71**	**9,5**
		sh(Ink4a/Arf)	duplicated		2314	**1,80**	**10,1**

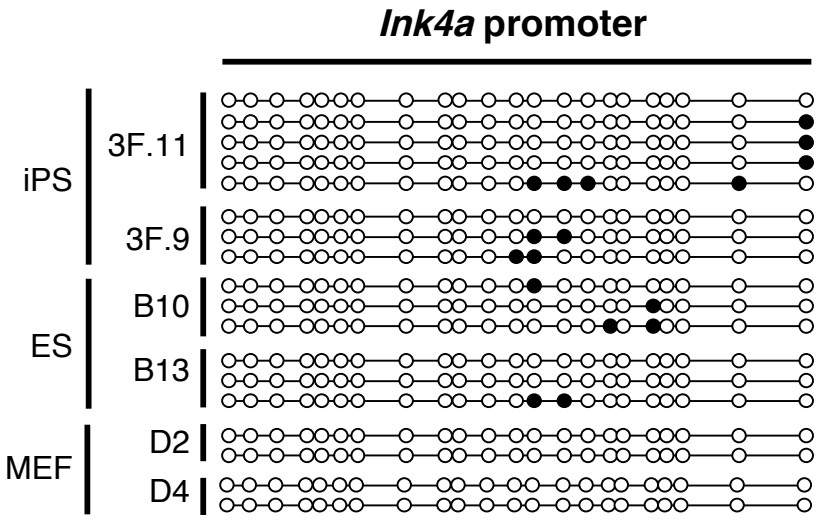
Primary data for Figure 3g

Human diploid fibroblasts	Reprogramming factors	shRNA (retroviral)	Initial number of cells seeded	% of infected cells (GFP+)	Number of infected cells	Number of AP+ colonies	Reprogramming efficiency (% of AP+ colonies relative to infected cells)	Relative efficiency with respect to wt
IMR90/TERT	3F	none	200000	14,00	28000	239	**0,85**	average no-sh:
		none	duplicated			188	**0,67**	**1,0**
		shINK4a	200000	15,00	30000	465	**1,55**	**2,0**
		shINK4a	duplicated			500	**1,67**	**2,2**
		shARF	200000	13,00	26000	190	**0,73**	**1,0**
		shARF	duplicated			169	**0,65**	**0,9**
	4F	none	200000	14,00	28000	130	**0,46**	average no-sh:
		none	duplicated			145	**0,52**	**1,0**
		shINK4a	200000	4,26	8520	240	**2,82**	**5,6**
		shINK4a	duplicated			270	**3,17**	**6,5**
		shARF	200000	9,26	18520	113	**0,61**	**1,2**
		shARF	duplicated			140	**0,76**	**1,5**

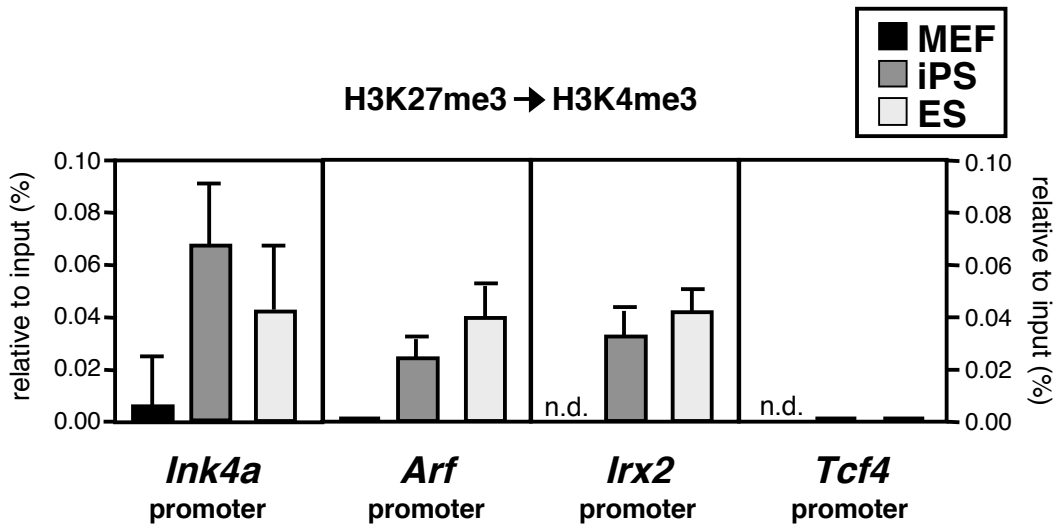




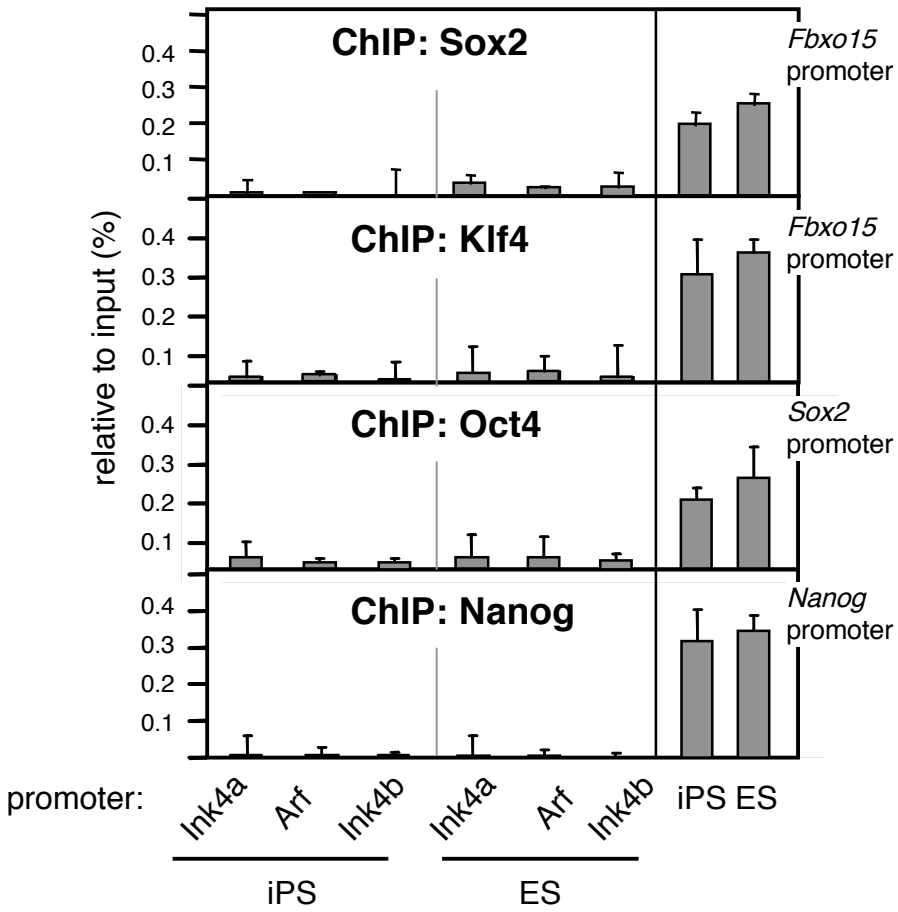
Supplementary Figure 1. Structure of the *Ink4/Arf* locus and its role in the regulation of the p53 and Rb pathways.



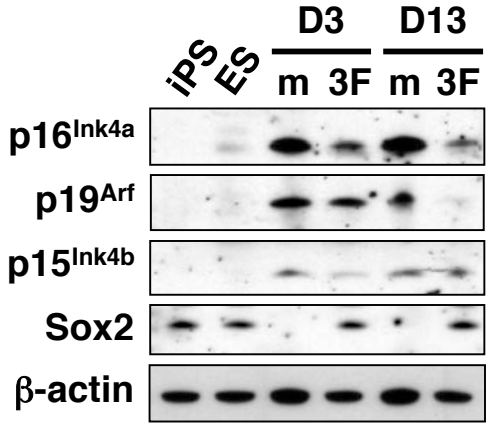
Supplementary Figure 2. Absence of DNA methylation at the *Ink4a* promoter in iPS cells.
DNA methylation analysis of the *Ink4a* promoter in the indicated cells. Methylation was analyzed using the standard bisulfite sequencing method. Each line represents a single clone. Dark dots indicate methylation.



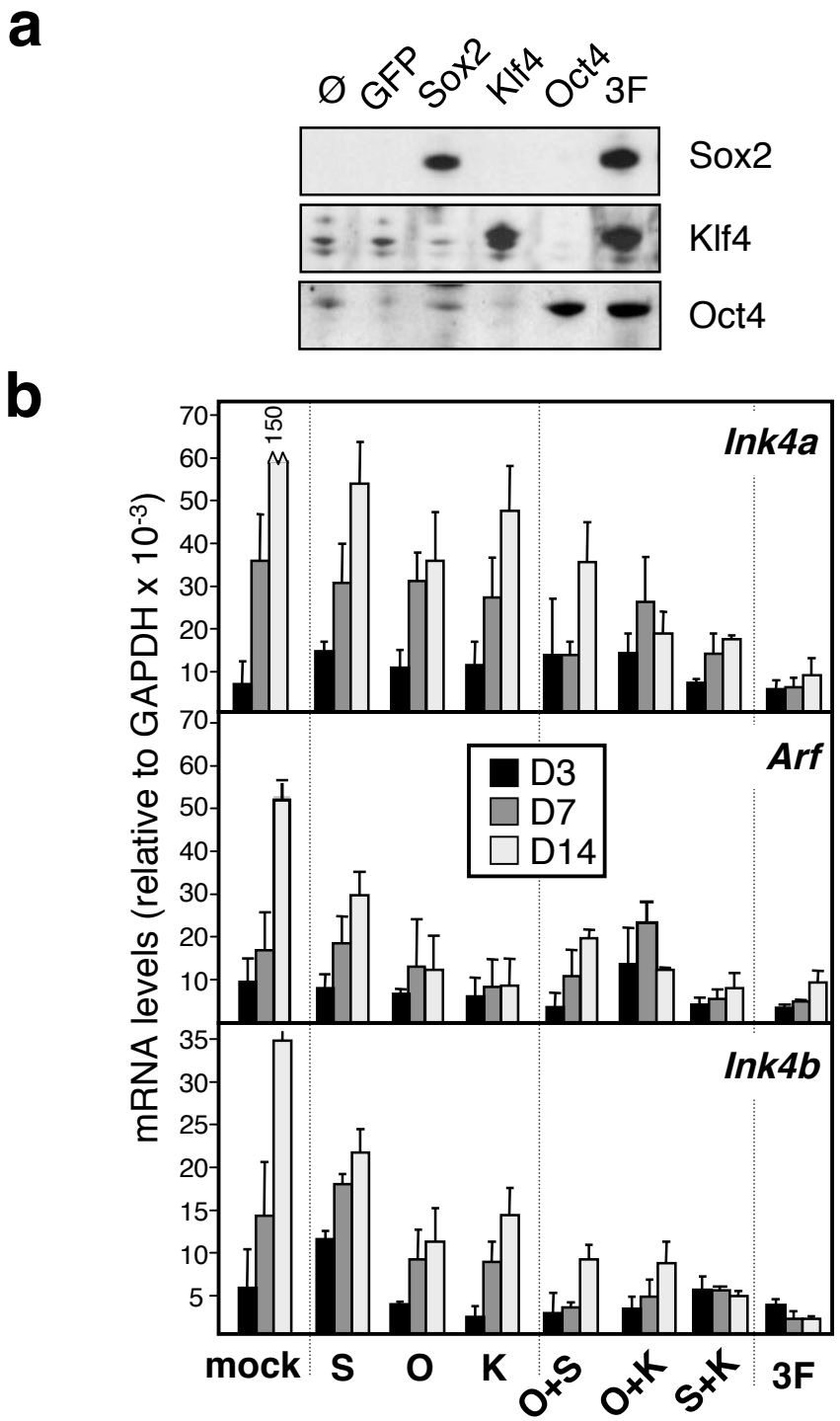
Supplementary Figure 3. Sequential ChIP to detect bivalent chromatin.
Sequential ChIP, first of H3K27me3 and then of H3K4me3, of the indicated promoters. The data for the *Ink4a* and *Arf* promoters are the same as in Fig. 1b of the main paper. The *Irx2* promoter is shown as a positive control for bivalent chromatin, and the *Tcf4* promoter as a negative control. Not determined, n.d.



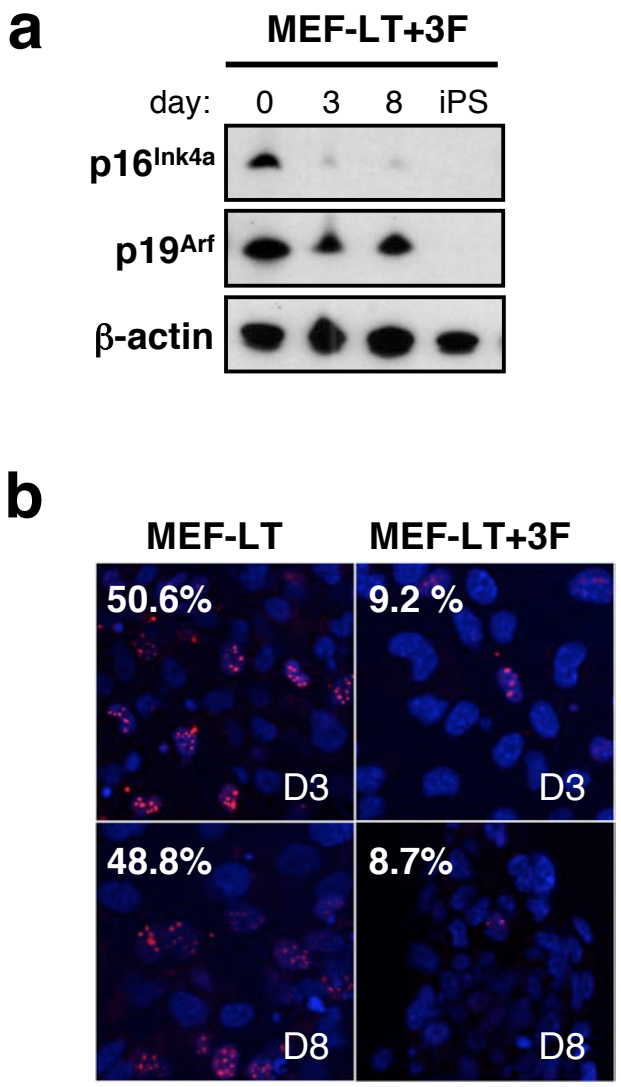
Supplementary Figure 4. Lack of binding of the reprogramming factors to the promoters of the *Ink4/Arf* locus.
Chromatin immunoprecipitation assays using specific antibodies against Sox2, Klf4, Oct4 and Nanog were performed on iPS or ES cells followed by qRT-PCR with specific primers to detect the promoter regions of *Ink4a*, *Arf* and *Ink4b*. Primers amplifying the promoter regions of *Fbxo15*, *Sox2*, and *Nanog* genes were used as positive controls.



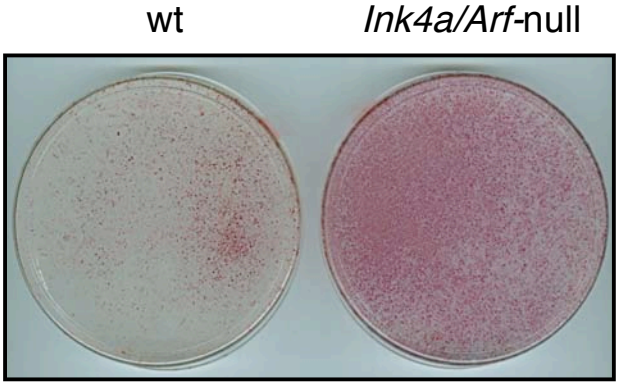
Supplementary Figure 5. Silencing of the *Ink4/Arf* locus during reprogramming.
Western-blot analysis of the indicated proteins at days 3 (D3) and 13 (D13) during 3F-reprogramming compared to mock-infected cells (m).



Supplementary Figure 6. Repression of the *Ink4/Arf* locus requires the 3F.
a. Confirmation of expression of Sox2, Klf4 and Oct4 in primary wild-type MEFs retrovirally-infected with the indicated reprogramming factors and analyzed 2 days after. **b.** Expression of the *Ink4/Arf* locus, at the indicated days, in MEF cultures retrovirally infected as follows: mock; S, Sox2; O, Oct4; K, Klf4; or 3F.



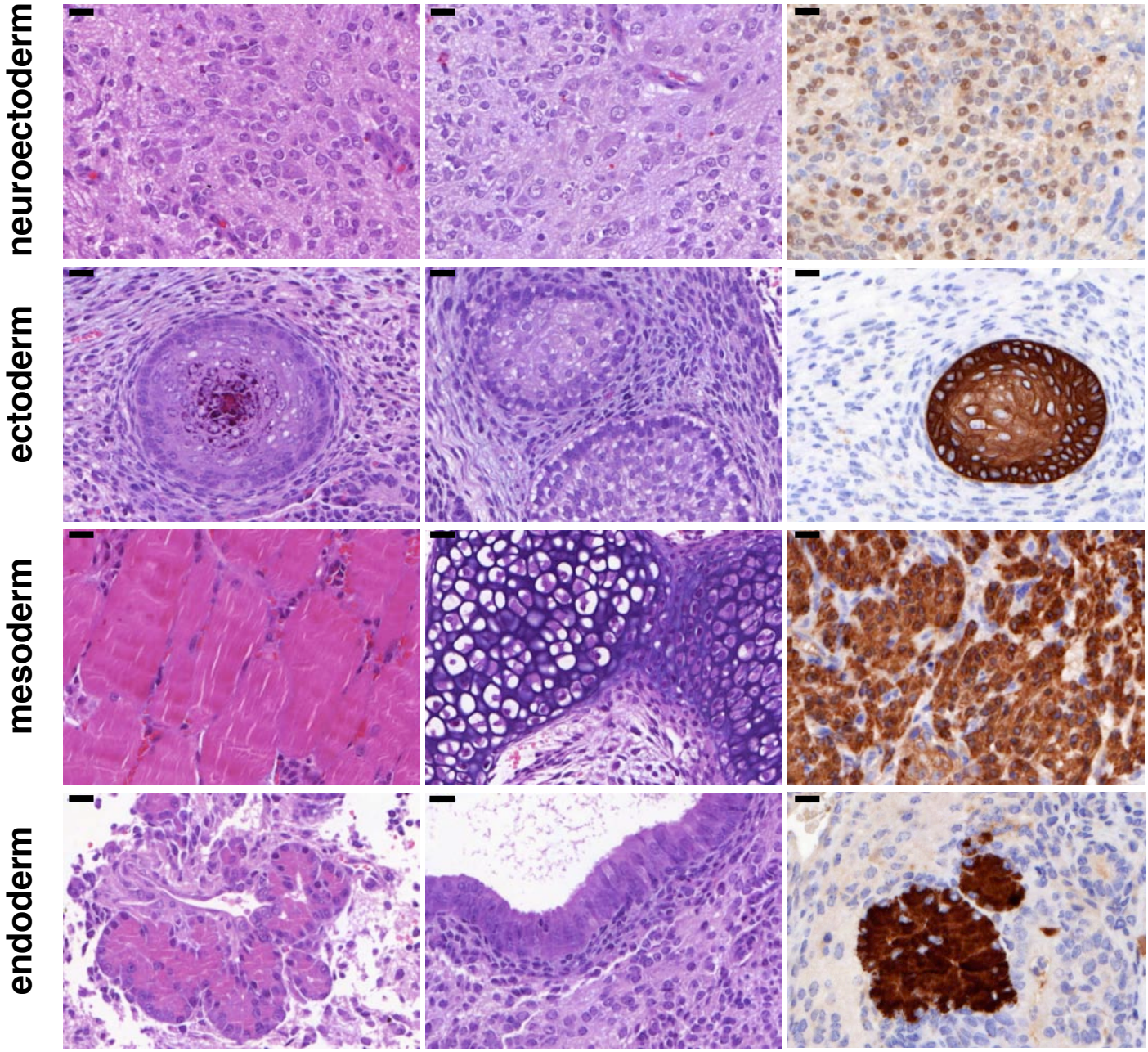
Supplementary Figure 7. Silencing of the *Ink4a/Arf* locus in MEFs expressing large-T (MEF-LT) during reprogramming.
a. Western-blot analysis. **b.** Immunofluorescence analysis of $p19^{Arf}$ at days 3 (D3) and 8 (D8). The percentage of $p19^{Arf}$ positive cells is shown in the upper left corner of each panel.



Supplementary Figure 8. Representative photograph of reprogramming plates. MEFs ($2.5x10^5$ cells) of the indicated genotype were subjected to the reprogramming protocol and plates were stained at day 14 (see Fig. 2a) with alkaline phosphatase.

clone	genotype	metaphases analyzed	euploid metaphases (% with 40 chrom.)
3F-7	wt	30	90%
3F-9	wt	10	100%
F206-21	*p53*-null	10	100%
F206-22	*p53*-null	30	90%
K2-21	*p21*-null	10	100%
C101.2-4	*Ink4a/Arf*-null	20	75%
C101.2-5	*Ink4a/Arf*-null	20	80%
C101.2-6	*Ink4a/Arf*-null	20	70%
C101.2-7	*Ink4a/Arf*-null	13	77%
C102.2-4	*Ink4a/Arf*-null	10	100%
C102.2-5	*Ink4a/Arf*-null	20	80%

Supplementary Figure 9. Analysis of euploidy in iPS clones.

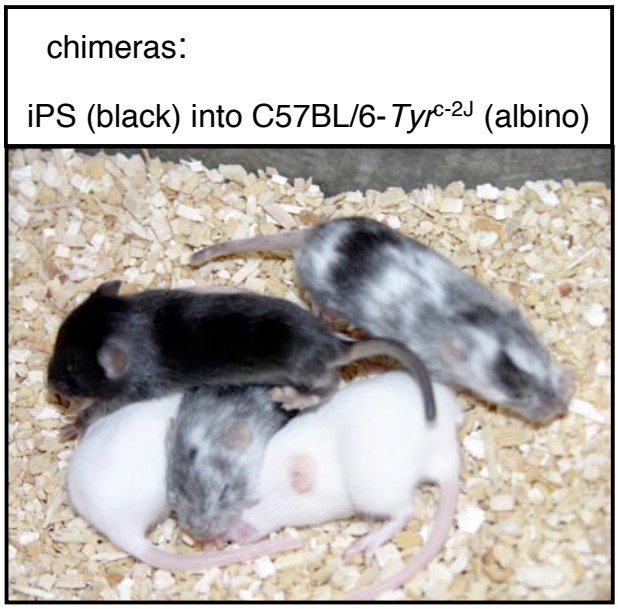


Supplementary Figure 10. Teratoma formation by *Ink4/Arf*-null iPS.
A total of 4 *Ink4a/Arf*-null (3F) iPS injections produced correspondingly 4 teratomas each (see Methods). Histological analysis of haematoxylin and eosin stained sections revealed features of (from top to bottom): neuroectoderm (neural tissues, left and middle panels); ectoderm (hair follicle and epidermal tissue, left and middle panels respectively); mesoderm (striated muscle and cartilage, left and middle panels respectively); endoderm (pancreatic acini and duct-like structures and pseudostratified ciliated epithelium, left and middle panels respectively). Immunohistochemical stainings confirmed the pathological analyses, in particular (right column, from top to bottom) anti-neuronal nuclei (NeuN, MAB377, Chemicon), cytokeratin-19 (CK-19, Dev. Stu. Hybridoma Bank), common-muscle actin (HHF-35, M0635, Dak o), and chymotrypsin (2100-0657, Serotec). Scale bar shown on the upper left corner: 20 μm.

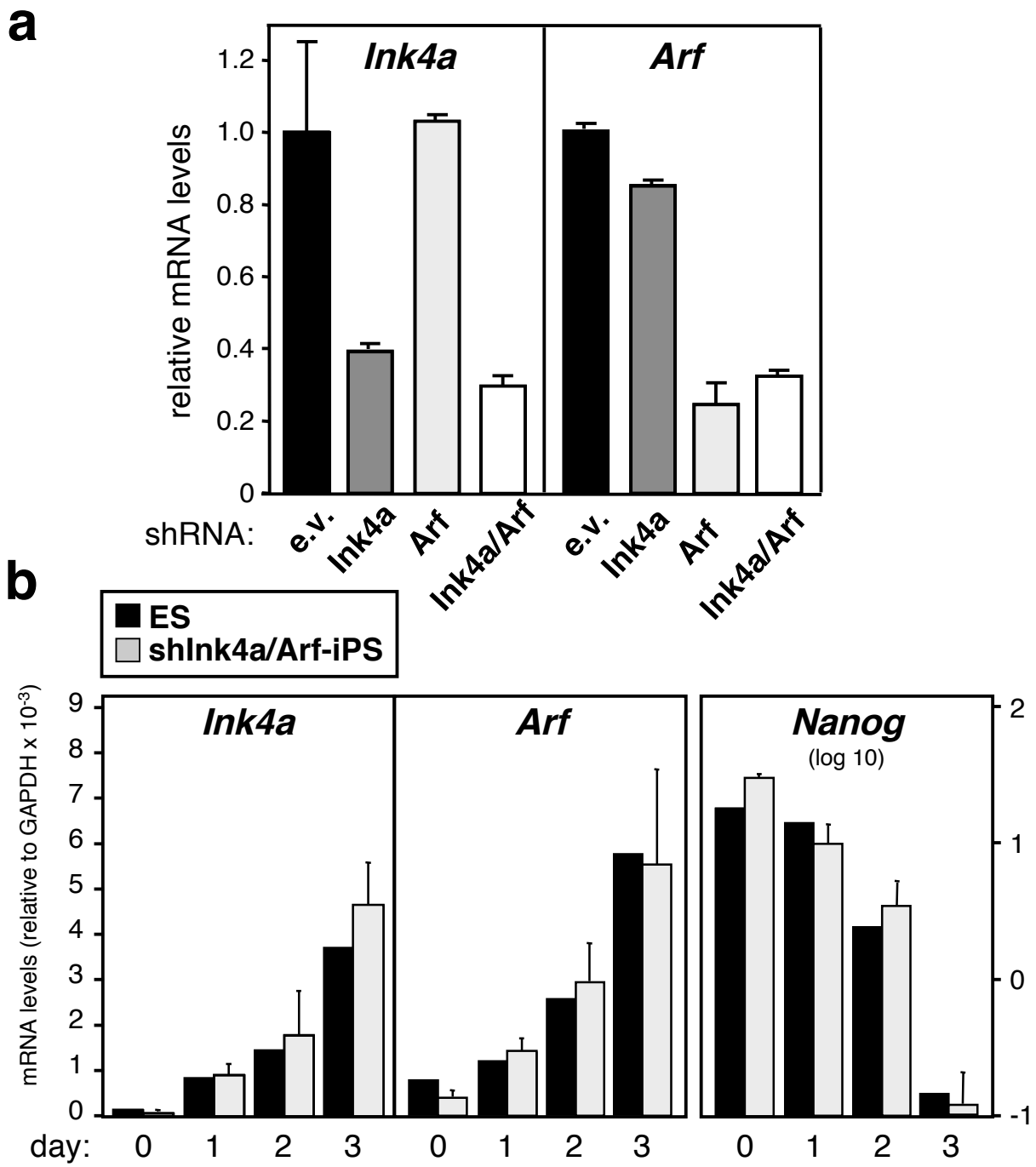
Microinjection in B6-*Tyr*$^{c-2J}$ blastocysts					
iPS clone	Genotype	Blastocysts injected	Cells injected	Pups born	Chimeras Sex (% pigmentation)*
3F-7	WT	52	5-8	5	1M(100%)
3F-9	WT	43	5-8	4	1F(60%)
4F-4	WT	58	5-6	8	2M (80%), 1F(100%)
F206.21	*p53*-null	52	5-6	7	1M(60%), 1M(30%)
C101.2-4	*Ink4a/Arf*-null	33	5-7	5	1F(70%)
C101.2-7	*Ink4a/Arf*-null	22	5-7	5	0

* red indicates that there was germline transmission

Aggregation with CD1 morulae					
iPS clone	Genotype	Morulae aggregated	Cells aggregated	Pups born	Chimeras Sex (% pigmentation)
4F-3	WT	171	4-8	15	1M(30%), 1M(5%) 1F (50%), 2F(30%)
old-MSF.1385	WT	235	4-12	13	1M(40%), 1F(60%)
C101.2-4	*Ink4a/Arf*-null	102	4-15	12	0
C101.2-7	*Ink4a/Arf*-null	72	4-15	17	0
C102.2-4	*Ink4a/Arf*-null	94	4-15	22	1M(100%), 1M(50%), 1F(50%)

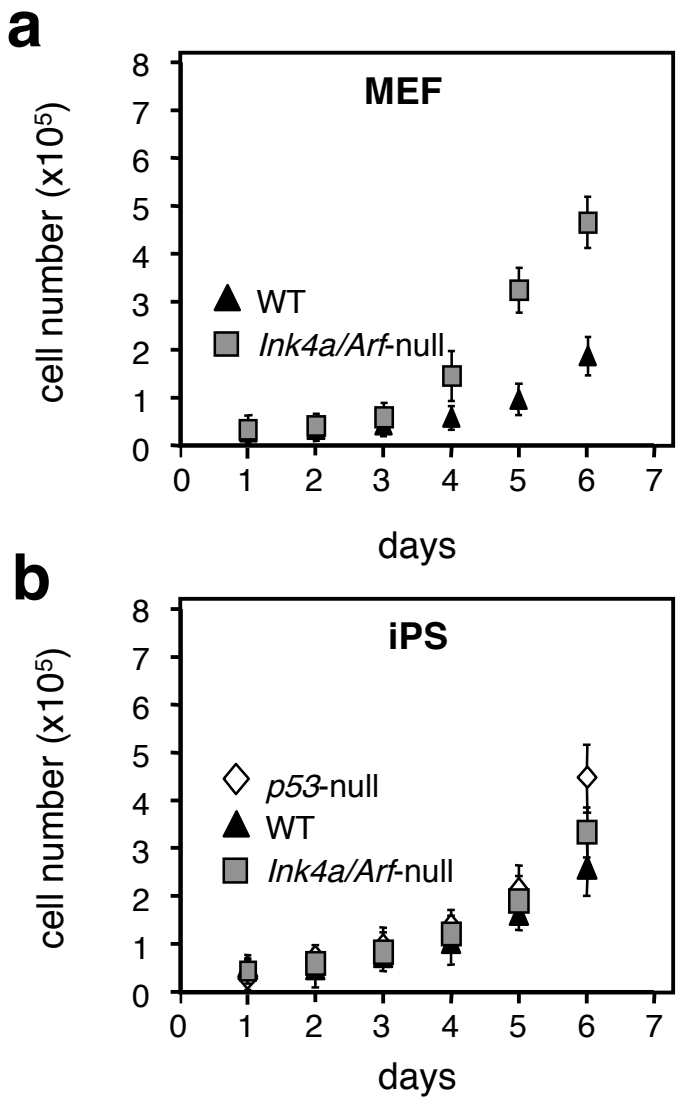


Supplementary Figure 11. Generation of chimeras from *Ink4a/Arf*-null iPS.

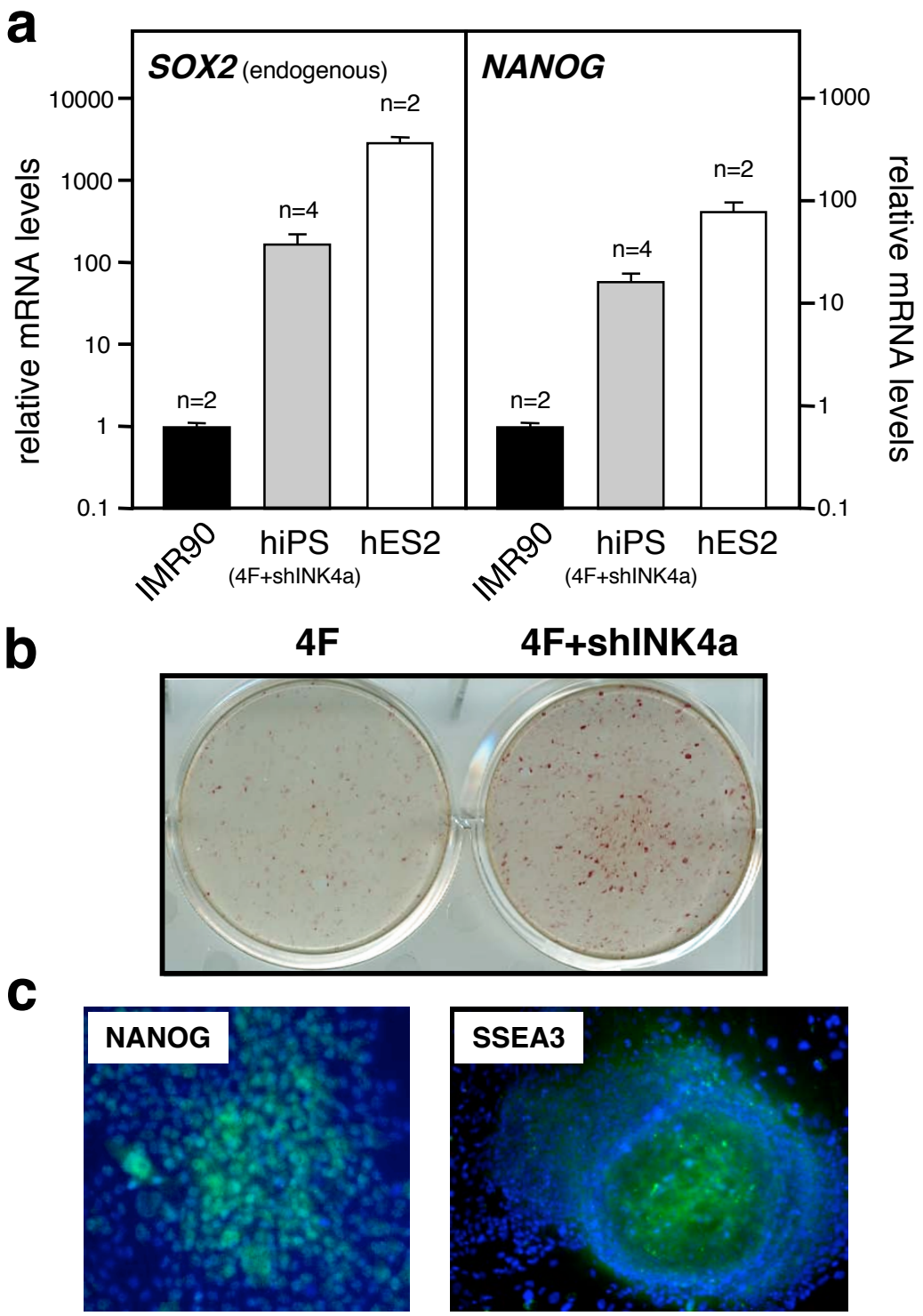


Supplementary Figure 12. Analysis of the *Ink4*/*Arf* locus activity on iPS after transient knockdown of the locus.

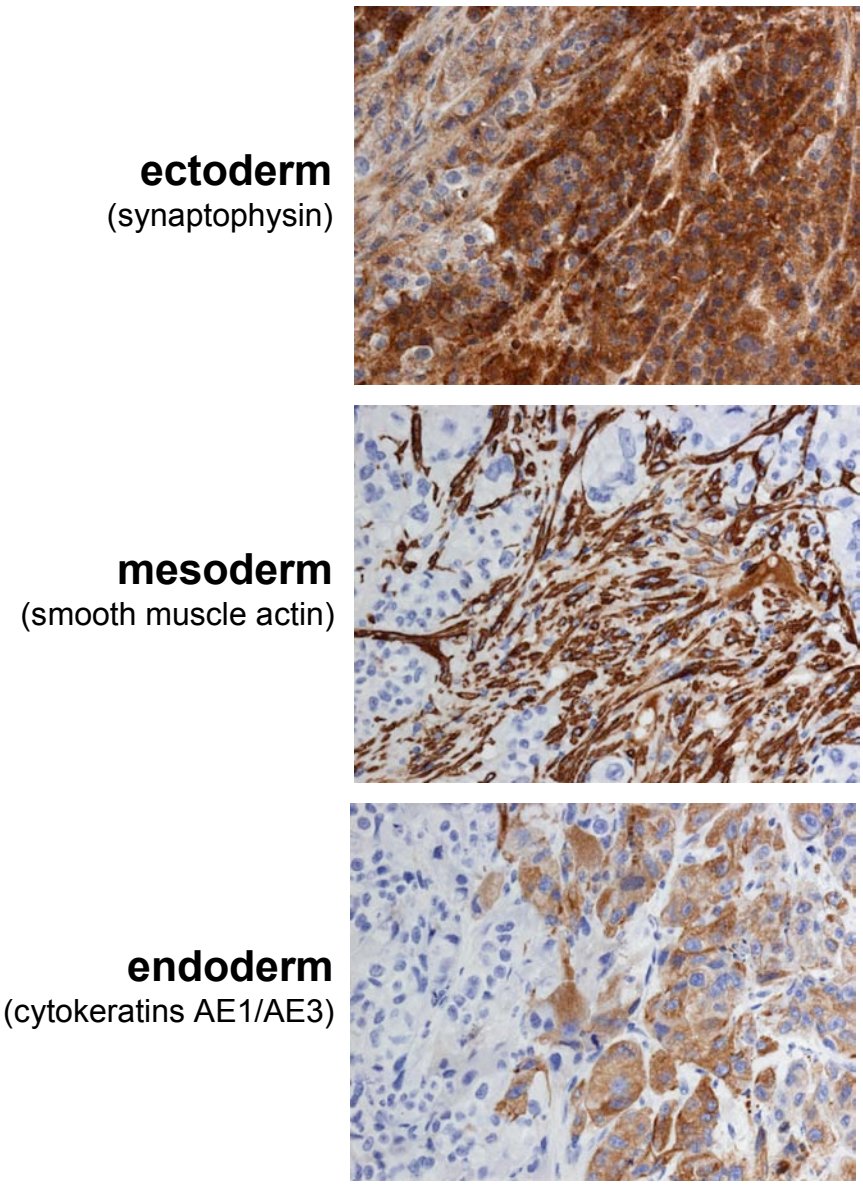
a. Expression levels of *Ink4a* and *Arf* in MEFs, 48 h after infection with retroviruses expressing shRNAs targeted to *Ink4a*, *Arf* or both *Ink4a*/*Arf* as measured by quantitative RT-PCR. Empty vector is shown as reference. **b.** Retinoic acid differentiation of iPS cells generated after knockdown of *Ink4a*/*Arf* showing the increase in expression of *Ink4a* and *Arf* at the indicated days post retinoic acid withdrawal. *Nanog* expression in the same experimental setting is shown as a control of differentiation. Data correspond to the average ± s.d. of 3 independent assays, using different isolates of iPS clones.



Supplementary Figure 13. Proliferation rate of MEFs and iPS cells.
a. MEFs ($2.5x10^{4)}$ of the indicated genotypes were seeded and counted at the indicated days using trypan blue exclusion to identify live cells. **b.** iPS cells (10^4) of the indicated genotype were seeded on 24 well plate coated with gelatin. Cell number was counted daily two days after seeding for 6 days and live cells were determined by trypan blue exclusion. Data correspond to the average ± s.d. of 2 independent assays using 2 different MEF or iPS isolates each.



Supplementary Figure 14. Characterization of human shINK4a-iPS.
a. Expression of endogenous *SOX2* and *NANOG* in human shINK4a-iPS (4F) compared with the parental IMR90/TERT fibroblasts and with human ES cell line hES2 (NIH code ES02) measured by quantitative RT-PCR. Data correspond to the average ± s.d. of 3 independent assays, using different hiPS clones. **b.** Reprogramming plates of IMR90/TERT cells stained with alkaline phosphatase. **c.** Immunostaining of human shINK4a-iPS (4F) iPS colonies with antibodies for NANOG and SSEA3.



Supplementary Figure 15. Teratoma formation by human shINK4a-iPS
Human iPS cells ($2x10^6$ cells) derived from IMR90/TERT fibroblasts reprogrammed with 4F+shINK4a were subcutaneously injected into irradiated (400-rad) nude mice. Tumors were formed in all cases. After 9 weeks, tumors were surgically removed, fixed in formalin at 4ºC, embedded in paraffin wax, and sectioned at a thickness of 5 μm. Sections were stained with hematoxylin and eosin for pathological examination or processed for immunohistochemical analysis using antibodies against synaptophysin (SY38, Dako, as ectoderm marker), smooth muscle actin (SMA, 1A4, Dako, as mesoderm marker) and cytokeratins (AE1/AE3, Dako, as endoderm marker).