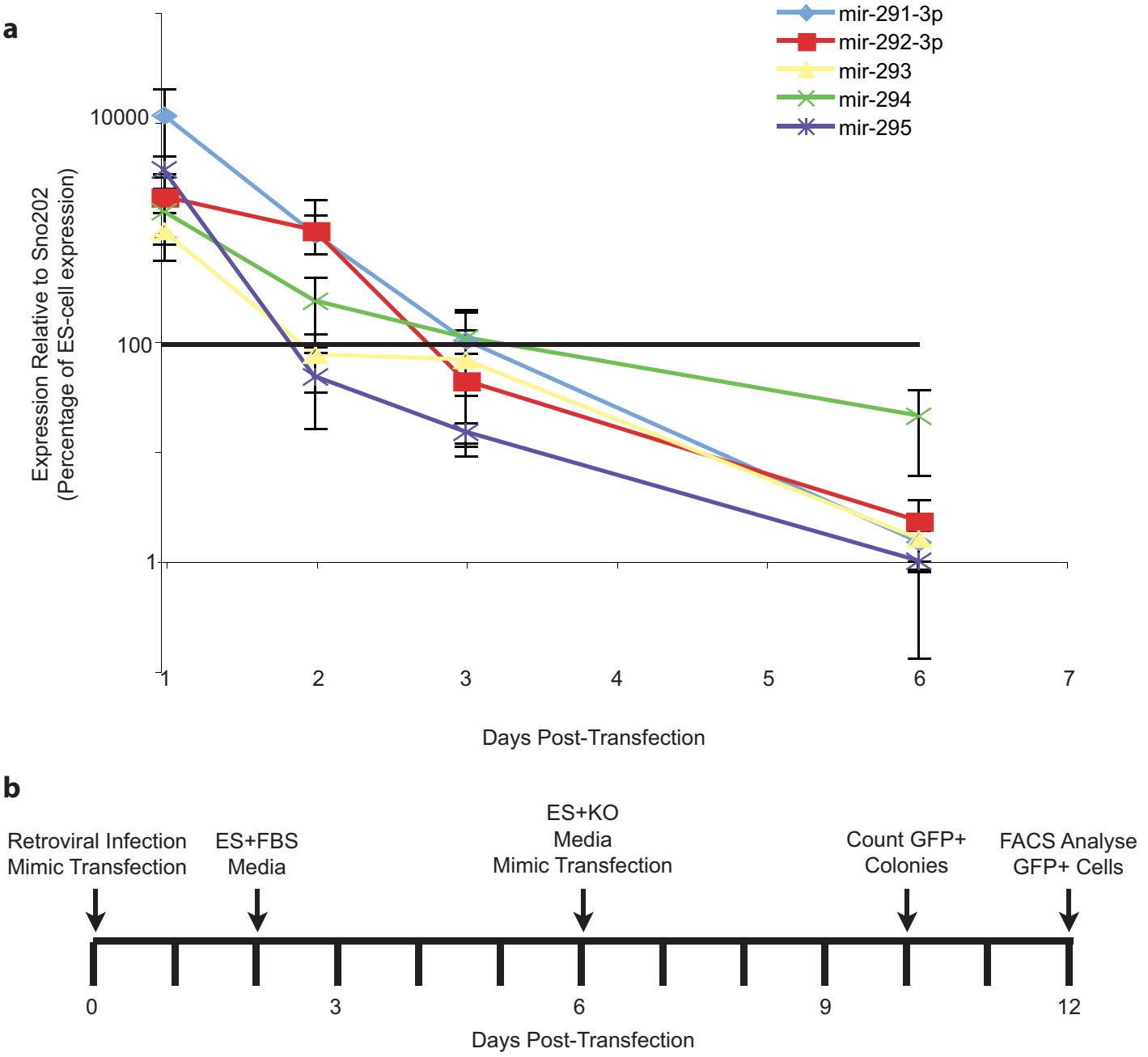
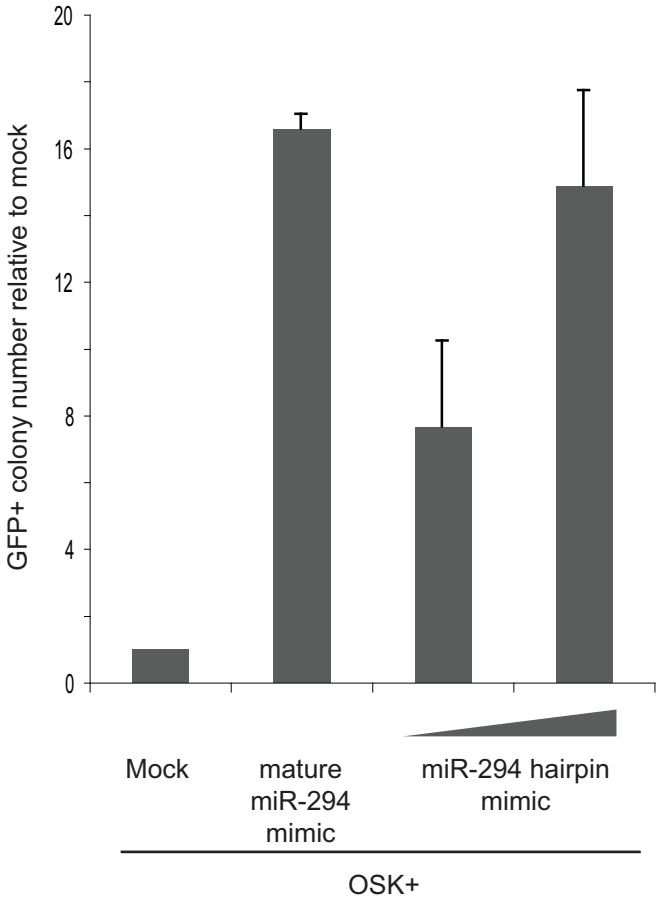
Supplementary Figure 1



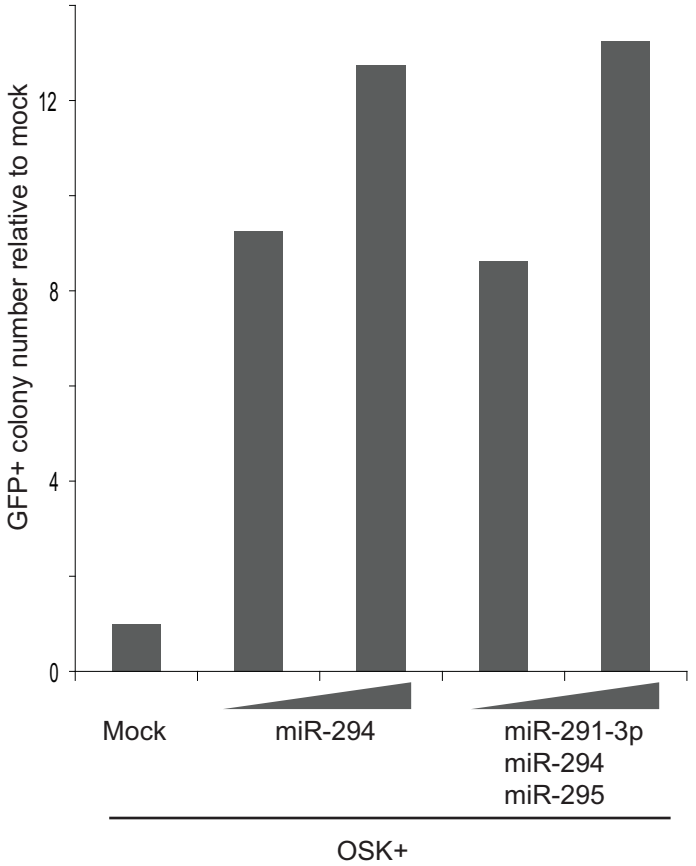
Supplementary Figure 1: Characterization and application of miR-290 cluster mimics. (**a**) miR-290 cluster expression levels in mimic-transfected MEFs. MiRNA mimic (16nM) was transfected into MEFs, and RNA was collected on days 1, 2, 3, and 6. Relative miRNA levels were compared via RT-qPCR to control (v6.5) ES cells (black horizontal bar). Mimic levels were found to be well above ES expression levels one day after transfection, but close to physiological levels between days 2 and 3. (**b**) Reprogramming assay timeline. In reprogramming assays, MEFs were transfected on days 0 and 6 in order to retain ES-like levels of the mimics.

Supplementary Figure 2



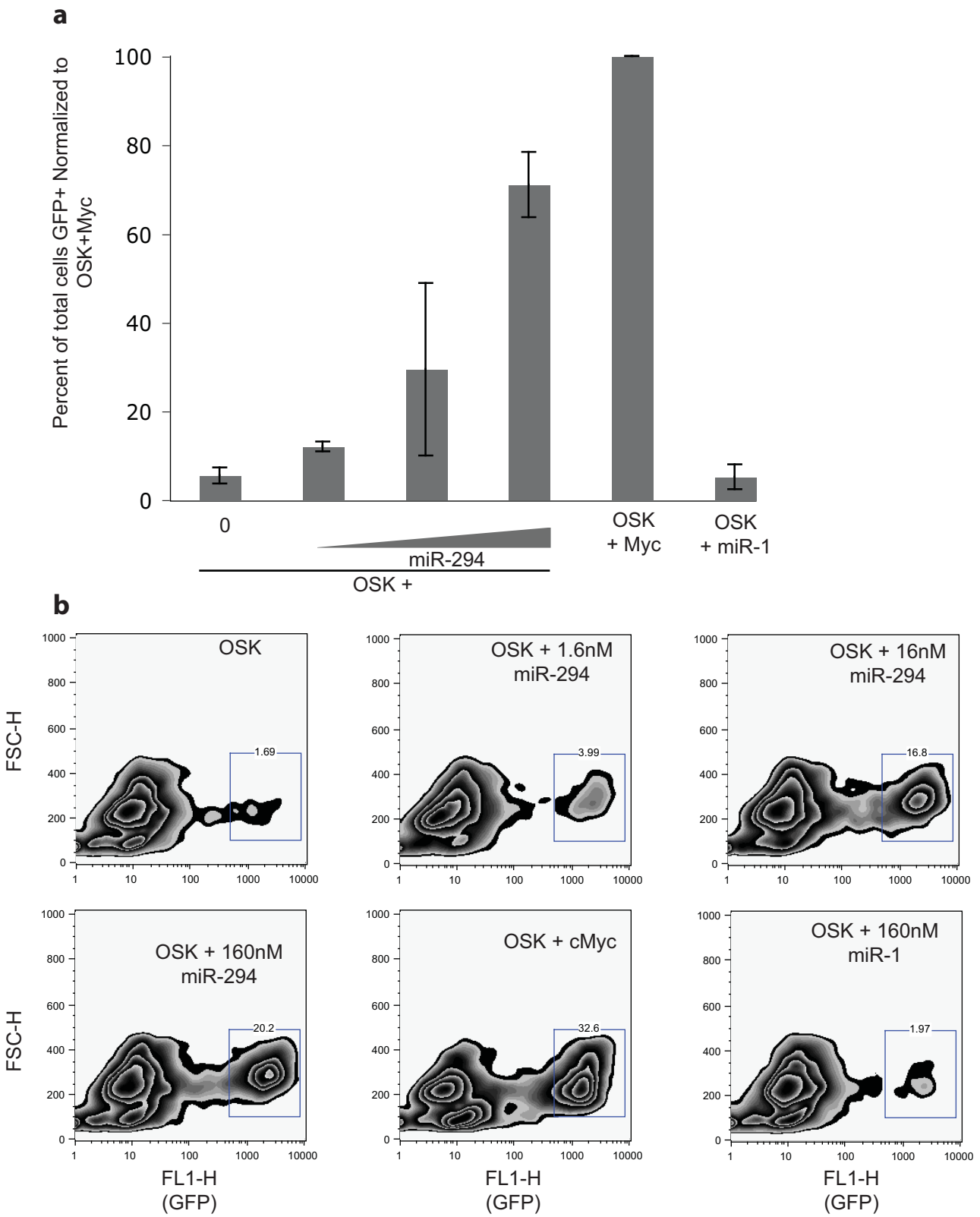
Supplementary Figure 2. Generation of GFP+ colonies with retroviruses expressing Oct4, Sox2, and Klf4 together with either duplex miR-294 mimic (16nM), hairpin miR-294 mimic (16nM and 160nM) or transfection reagent only (mock). Error bars indicate standard deviation of N=3.

Supplementary Figure 3



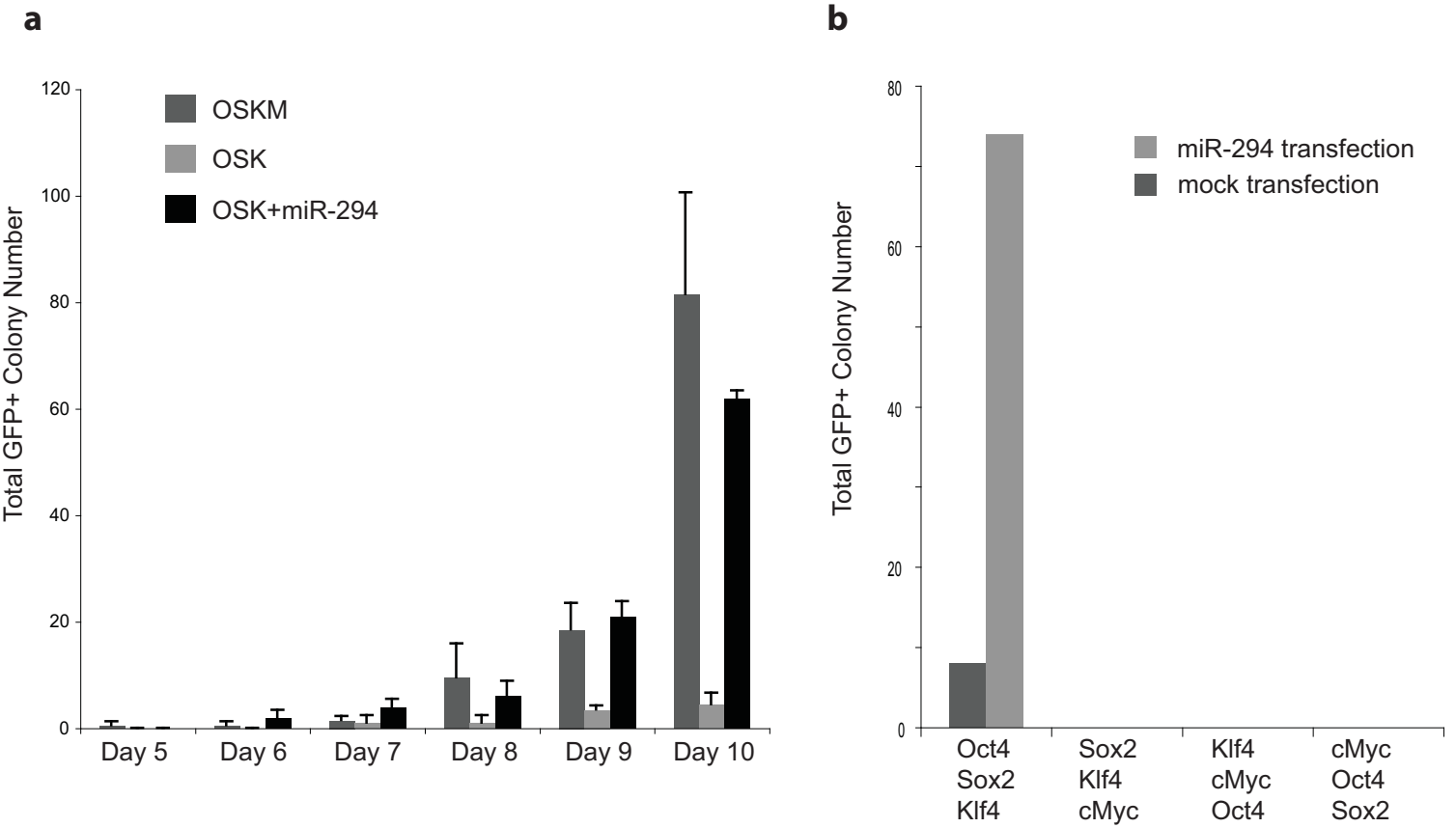
Supplementary Figure 3 Effect of combining ESCC miRNAs on reprogramming. Generation of GFP+ colonies with retroviruses expressing Oct4, Sox2, and Klf4 (OSK) together with either miR-294 (16nM or 48nM) or a mixture of miR-291-3p, miR-294 and miR-295 (5.3nM each or 16nM each) or transfection reagent only (mock).

Supplementary Figure 4



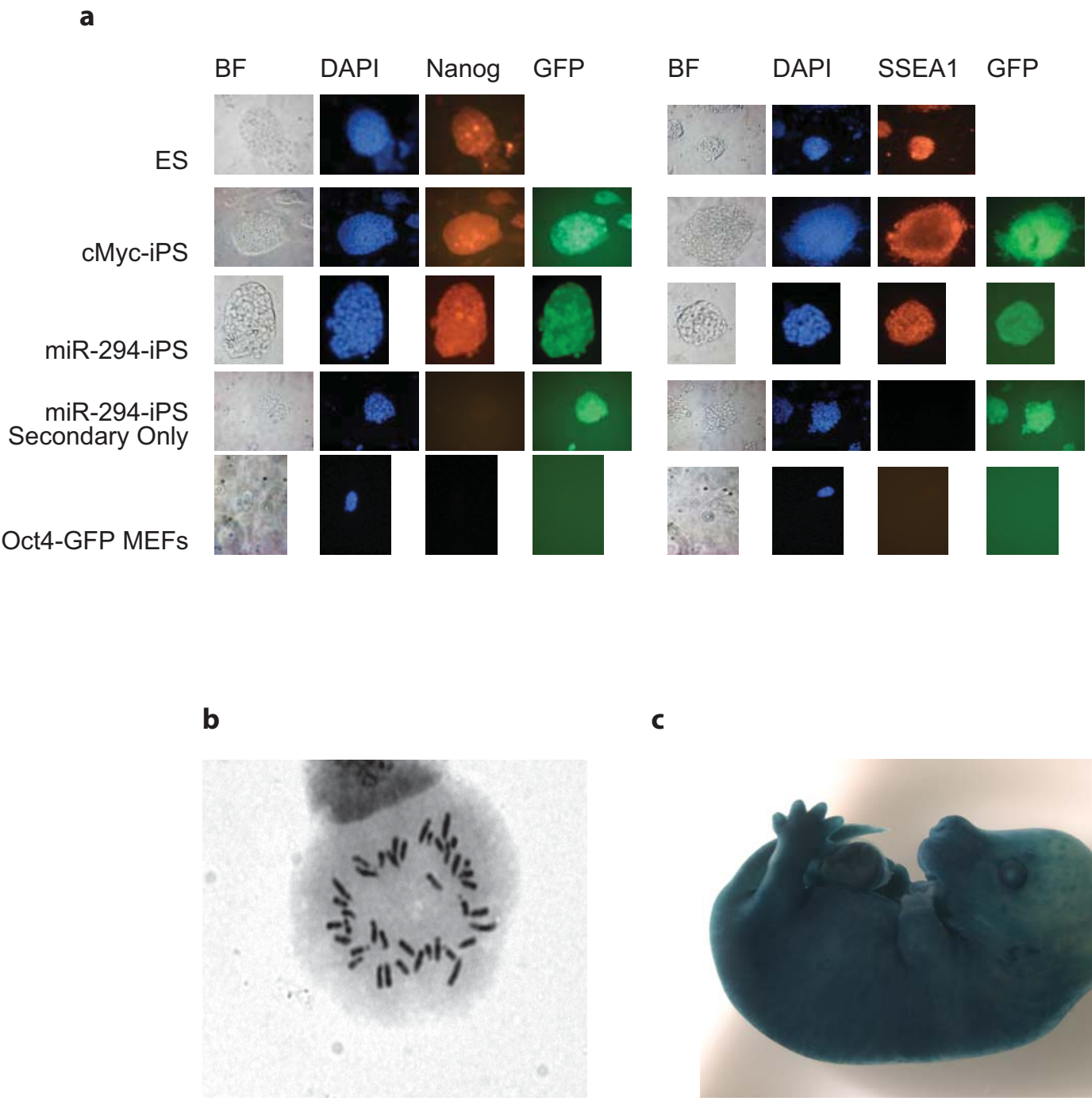
Supplementary Figure 4. FACS analysis of GFP+ cells from MEFs infected with Oct4, Sox2, and Klf4 (OSK) and transfected with miRNA mimics. (**a**) Graphic display of FACS analysis for GFP+ sorted cells on day 12. Wedge indicates increasing concentrations (1.6, 16 and 160nM) of mimic. N=3. Error bars indicate standard deviation. (**b**) FACS plots of representative samples.

Supplementary Figure 5



Supplementary Figure 5: Kinetics of reprogramming and effects of miR-294 on other combinations of transcription factors. (**a**) Generation of GFP+ colonies with retroviral expression of Oct4, Sox2, and Klf4 alone (OSK), with the addition of cMyc (OSKM), or with transfection of 16nM miR-294 mimic (OSK+miR-294). GFP+ colonies were counted on days 5-10. GFP+ microcolonies were reliably first seen in OSKM and OSK+miR-294 by day 7 with identifiable GFP+ES-like colonies seen by day 9. GFP+ microcolonies and ES-like colonies were generally seen in OSK on days 8 and 10, respectively. Error bars represent standard deviation for N=3. (**b**) Generation of GFP+ colonies with combinations of retroviruses expressing Oct4, Sox2, Klf4 or cMyc with and without transfection of miR-294 (16nM). Lane 1 depicts a single experiment representative of many (**Supplementary Table 2**). Lanes 2, 3 and 4 depict N=1, 1, and 3, respectively.

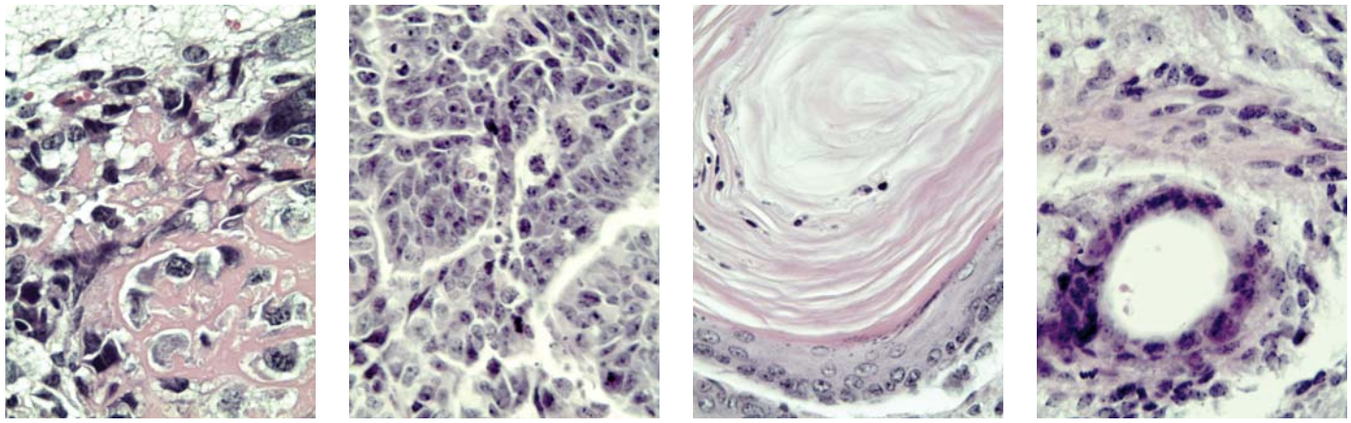
Supplementary Figure 6



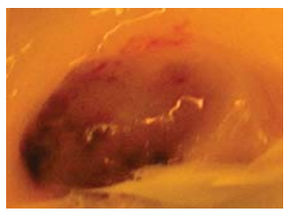
Supplementary Figure 6: Verification of miR-294-iPS pluripotency. (**a**) Brightfield and immunofluorescent photographs of miR-294-iPS colonies. Images are representative of six independent iPS lines. Staining controls include ES (V6.5) cells, cMyc-iPS colonies, miR-294-iPS colonies with secondary antibody only, and MEFs (**b**) Representative karyotype of iPS cells induced with Oct4, Sox2, Klf4 and miR-294 mimic. (**c**) An E15 chimera derived from blastocyst injection of miR-294-iPS cells carrying a ubiquitously expressed β-galactosidase reporter.

Supplementary Figure 7

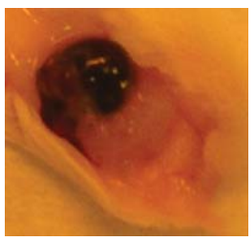
a



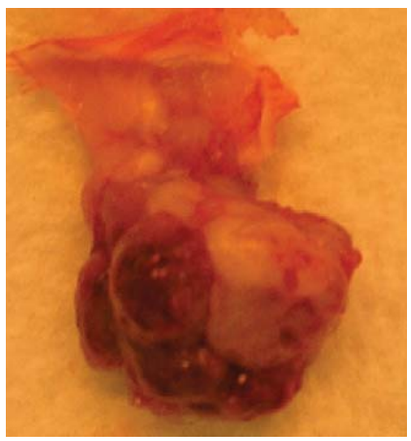
b



V6.5



miR-294 iPS



Myc iPS

c

Cell Lines	Mice Injected	Teratomas Formed	Teratomas Invasive	Day Post-injected Collected	Percent Teratoma	Percent Invasive
V6.5	6	10	0	1x15, 4x31, 1x55	**100**	**0**
OSK+Myc						
10-4	1	2	0	24		
10-5	1	2	1	15		
10-6	1	2	1	24		
12-2	2	3	0	31	**78**	**43**
12-3	3	4	1	31		
12-4	1	0	0	31		
12-5	1	0	0	31		
14-1	1	1	0	31		
14-2	1	1	0	31		
OSK+294						
10-1	1	2	0	31		
10-2	1	2	0	31		
10-3	1	2	0	31		
12-19	2	3	0	31		
12-20	2	1	0	31	**100**	**0**
12-21	2	2	0	31		
12-22	2	3	0	15		
12-23	1	1	0	15		

Supplementary Figure 7 Teratoma generation from V6.5, miR-294-iPS, and cMyc-iPS cell lines. (**a**) H&E staining of miR-294-iPS derived teratomas. Images depict, left to right, bone, neural tissue, keratinizing squamous epithelial tissue, and glandular tissue. (**b**) Left and Middle, images of representative control ES (V6.5) and miR-294-iPS noninvasive teratomas. Right, image of representative cMyc-iPS invasive teratoma. (**c**) Number of invasive and non-invasive tumors with different cell lines injected. Columns display from left to right, independent cell lines, number of mice injected, number of total teratomas isolated, number of total teratomas found to be invasive, and the number of days after injection teratomas were harvested. Percent teratoma refers to the percentage of cell lines that formed teratomas. Percent invasive refers to the percentage of teratomas found to be invasive, defined as migration through the underlying body wall.

Supplementary Table 1

	GFP+ Colonies		
OSK+	**Exp. 1**	**Exp. 3**	**Exp. 4**
mock	19	6	4
miR-291-3p	61	14	35
miR-292-3p	26	4	4
miR-293	32	7	3
miR-294	106	44	35
miR-295	80	27	19

Supplementary Table 1 Total numbers of GFP+ colonies on day 10 after retroviral induction of Oct4, Sox2, and Klf4 (OSK) plus transfection of either 16nM miRNA mimics or just transfection reagent (mock). Data depicted graphically in **Figure 1a**.

Supplementary Table 2

		OSK+			OSKM+		
	Viral Prep	**mock**	**miR-294**	**Fold Change**	**mock**	**miR-294**	**Fold Change**
Exp. 1	*1*	19	106	5.6			
Exp. 2	*1*	16	260	16.3			
Exp. 3	*2*	6	44	7.3			
Exp. 4	*2*	4	35	8.8	58	33	0.6
Exp. 5	*2*	1	9	9	40	30	0.8
Exp. 6	*2*	2	17	8.5	56	35	0.6
Exp. 7	*3*	1	28	28	130	150	1.2
Exp. 8	*3*	1	37	37	258	255	1
Exp. 9	*3*	0	21	NA	220	95	0.4
Exp. 10	*3*	0	17	NA			
Exp. 11	*4*	8	74	9.3			
Exp. 12	*4*	6	61	10.2			
Exp. 13	*4*	6	84	14			
Exp. 14	*4*	6	63	10.5			
Exp. 15	*4*	4	79	19.8			
Exp. 16	*4*	6	78	13			
Exp. 17	*4*	4	75	18.8			
Exp. 18	*4*	4	36	9			
Exp. 19	*4*	1	18	18			
Exp. 20	*4*	2	21	10.5			
Exp. 21	*4*	3	26	8.7			
Exp. 22	*4*	0	14	NA			
Exp. 23	*4*	2	19	9.5			
Exp. 24	*4*	2	14	7			
Exp. 25	*4*	2	19	9.5			
Exp. 26	*4*	4	19	4.8			

Supplementary Table 2 Total numbers of GFP+ colonies on day 10 after retroviral induction of Oct4, Sox2, and Klf4 (OSK) or Oct4, Sox2, Klf4, and cMyc (OSKM) plus transfection of either 16nM miR-294 mimic or just transfection reagent control (mock). Data depicted graphically in **Figure 1c**.

Methods:

All animal experiments described in this article have been approved by UCSF's Institutional Animal Care and Use Committee.

Cell Culture: MEF isolation: E13.5 embryos were washed in HBSS. Heads and visceral tissues were removed, washed in fresh HBSS, briefly rinsed with 70% ethanol, then submerged in 0.05mM trypsin / 1mM EDTA HBSS solution and incubated at 37°C for 10 minutes. Embryos were pipetted repeatedly to aid in tissue dissociation, then added to MEF media containing 10% FBS and plated (passage 0). iPS lines were maintained in ES media + 15% knock-out serum on irradiated MEF feeders or gelatin.

Retrovirus infection: The retroviral packaging vector pCL-ECO was transfected into 293T cells simultaneously with pMXs vectors containing either Oct4, Sox2, Klf4, or cMyc cDNA using Fugene 6 (Roche) [1]. At 24 hours, the media was changed, and at 48 hours, the media was collected, filtered (0.45μM), and frozen in aliquots at -80°C. Retrovirus was never thawed more than once. Data from four independent viral preparations is presented (**Supplementary Table 2**). To induce reprogramming, passage 3 Oct4-GFP, Rosa26-β-galactosidase/neo MEFs[2] were plated on gelatin at 3,000 cells per cm^2. Retrovirus-containing media was added 24 hours later (Day 0). Cells were transfected with 1.6, 16 or 160nM microRNA mimics (Dharmacon) with Dharmafect 1(Dharmacon) as according to manufacturers' protocol. As transfection of miRNA mimics is transient, demonstrating an ~10,000 fold drop in expression from day 1 to day 6 post-transfection (**Supplementary Fig. 1**), cells in reprogramming assays were

transfected on days 0 and 6 post-retroviral-infection. Media was changed daily. Media was replaced with ES media + 15% FBS + LIF on day 2, and ES media + 15% knock-out serum replacement (Invitrogen) + LIF on day 6. To determine total cell number (**Fig. 2e)**, cells were trypsinized on day 7, counted, and passaged onto irradiated MEF feeders. GFP+ colonies were counted on days indicated in figures (day 10 except for **Supplementary Figure 5a**) and FACS sorted for GFP (BD FACSCalibur) on day 12. Reprogramming efficiency was calculated as the total number of day 10 GFP+ colonies divided by the number of day 0 plated MEFs. P-value of colony number (**Fig. 1c**) was calculated using standard t-test. Individual iPS colonies were picked between days 10 and 15.

Immunohistochemistry: iPS lines were grown in a 24-well plate, fixed with 4% paraformaldehyde and washed in 1xPBS with 0.1% Triton x-100 (PBT). PBT with 2% BSA and 1% goat-serum was used to block for one hour before addition of primary anti-bodies against SSEA1 (DSHB: MC-480) and Nanog (Abcam: ab21603), which were incubated overnight at 4°C. Cells were washed with PBT, and blocked with PBT with 2% BSA and 10% goat-serum for 1 hour before addition of secondary antibodies (Invitrogen: Alexa Fluor 594 goat anti-rabbit IgG and Biolegend: PE anti-mouse IgM).

Quantitative real-time PCR: Total RNA was isolated using TRizol (Invitrogen), and DNase treated (Invitrogen), according to manufacturers' protocols. For mRNA, reverse transcription was performed using random hexamers and the Superscript III kit (Invitrogen). Real-time quantitative PCR for mRNA was conduced with SYBR Green

PCR master mix (Applied Biosystems) according to the manufacturers' protocol using the following primer sets: Rex1, (gattgtggagccatacattgca, tgccgtagcctcgcttgt), Nanog (gctcagcaccagtggagtatcc, tccagatgcgttcaccagatag), Endogenous Oct4 (tctttccaccaggcccccggctc, tgcgggcggacatggggagatcc)[1], Endogenous Sox2 (tagagatagactccgggcgatga, ttgccttaaacaagaccacgaaa)[1], Endogenous Klf4 (gaattgtgtttcgatgatgc, tcgcttcctcttcctccgacaca), Lin28 (agtctgccaagggtctggaa, cgctcactcccaatacagaaca), Exogenous Oct4 (tctcccatgcattcaaactg, cttttattttatcgtcgacc), Exogenous Klf4 (ccttacacatgaagaggcac, cttttattttatcgtcgacc), Exogenous Sox2 (ctgcccctgtcgcacatgtg, cttttattttatcgtcgacc), cMyc (cagaggaggaacgagctgaagcgc, ttatgcaccagagtttcgaagctgttcg) and RPL7 (gattgtggagccatacattgca, tgccgtagcctcgcttgt). Real-time quantitative PCR for microRNAs was conducted using the Taqman approach, as previously described[3].

Teratoma formation: iPS lines were grown on irradiated MEFs or gelatin, trypsinized, and resuspended in PBS. One million iPS cells were injected subcutaneously per side in severe combined immunodeficient (SCID) mice (NCI-Frederick). Tumors were removed when they reached a size of 1-1.5cm in long diameter, fixed in 10% formalin, embedded in paraffin, sectioned, and H&E stained.

Blastocyst injection and chimera formation: Super-ovulation of B6D2F1/Cr females (NCI-Frederick) was induced via PMSG (Calbiochem) injection (day 0) and hCG (Calbiochem) injection (day 2). On day 2, females were crossed to B6D2F1/Cr males, oocytes were isolated on day 3, washed in M2 media (Specialty Media) and grown in

KSOM media (Specialty Media) for three days. IPS cells were karyotyped as previously described[2]. On day 6, 5-10 iPS cells were injected into cultured blastocysts, which were then transplanted into the uteri of pseudo-pregnant Swiss-Webster females. For analysis of tissue contribution, embryos were collected on E15 and stained as previously described[2]. For analysis of germ line contribution, embryos were collected on E12.5 and gonads were isolated and imaged under fluorescence. 45+/-7% of implanted blastocysts demonstrated high-grade chimeric contribution of iPS lines.

Mir-290 promoter analysis

Previously published ChIP-seq data for c-Myc, n-Myc[4], H3K4me3, and H3K27me3[5] were downloaded as fastq files and aligned to the mm9 (NCBI Build 37.1) assembly of the mouse genome using Eland (GA Pipeline 1.0, Illumina). The mm9 assembly contains the mir-290 locus[6], which was missing from previous assemblies. Following alignment, peak scores were assigned using the Findpeaks 3.1.9.2 algorithm[7]. The peak scores were normalized to the number of genome-mapping sequence reads.

1. Takahashi, K. & Yamanaka, S. Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. *Cell* **126**, 663-676 (2006).
2. Blelloch, R., Venere, M., Yen, J. & Ramalho-Santos, M. Generation of induced pluripotent stem cells in the absence of drug selection. *Cell Stem Cell* **1**, 245-247 (2007).
3. Tang, F., Hajkova, P., Barton, S.C., Lao, K. & Surani, M.A. MicroRNA expression profiling of single whole embryonic stem cells. *Nucleic Acids Res* **34**, e9 (2006).
4. Chen, X. et al. Integration of external signaling pathways with the core transcriptional network in embryonic stem cells. *Cell* **133**, 1106-1117 (2008).
5. Mikkelsen, T.S. et al. Genome-wide maps of chromatin state in pluripotent and lineage-committed cells. *Nature* **448**, 553-560 (2007).
6. Marson, A. et al. Connecting microRNA genes to the core transcriptional regulatory circuitry of embryonic stem cells. *Cell* **134**, 521-533 (2008).

7. Fejes, A.P. et al. FindPeaks 3.1: a tool for identifying areas of enrichment from massively parallel short-read sequencing technology. *Bioinformatics* **24**, 1729-1730 (2008).