Supporting Information

Carey *et al.* 10.1073/pnas.0811426106

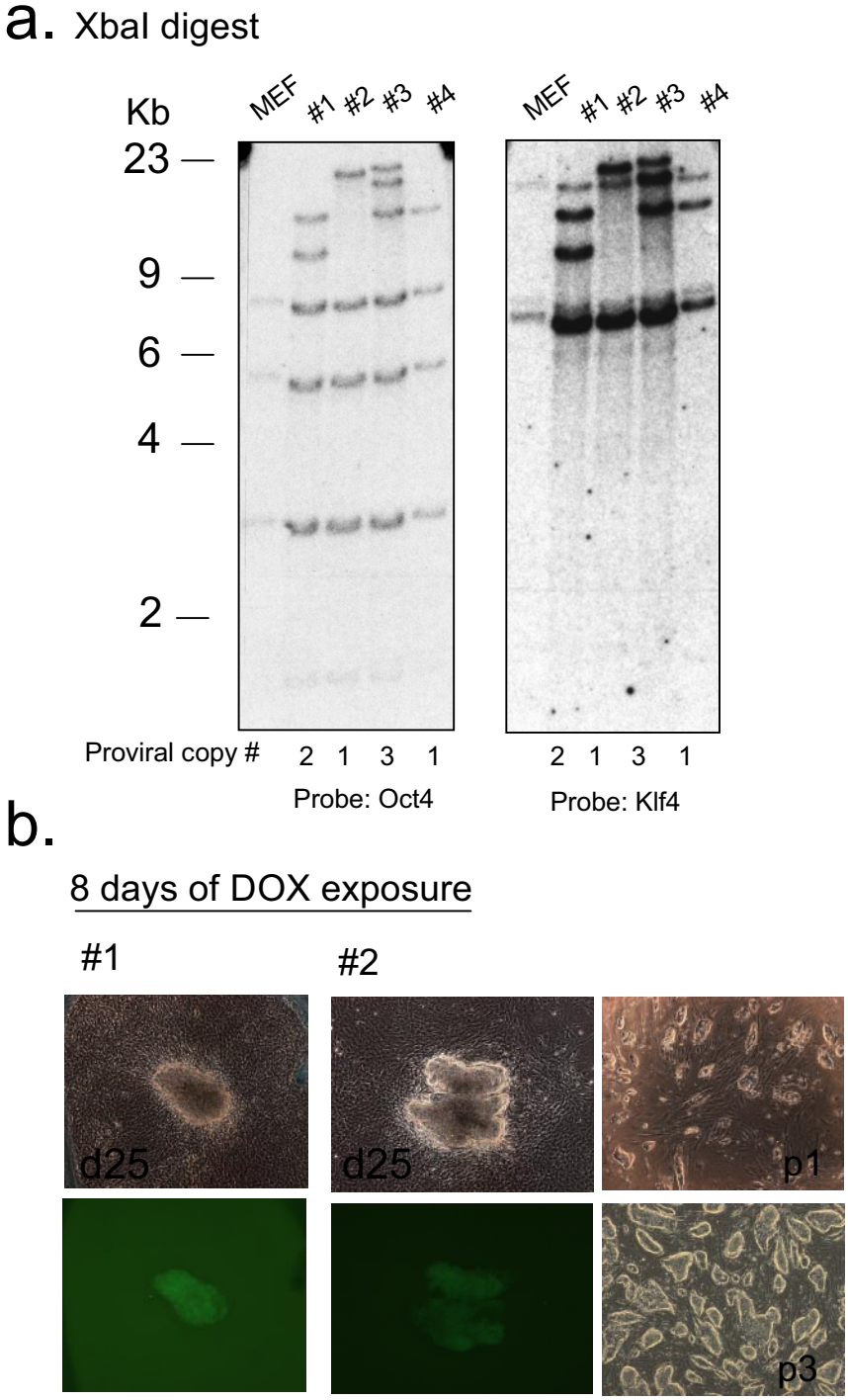

Fig. S1. Southern blot of MEF-derived iPS lines and DOX-withdrawal indicating 8 days is sufficient to generate iPS lines. (*A*) Southern blot analysis of 4F2A MEF iPS lines. A second digest was performed (XbaI) to confirm the proviral copy number. In this digest iPS line nos.2 and 4 show one proviral copy; however, only no.4 had one proviral copy in both digests. (*B*) DOX-withdrawl after 8 days postinfection of Nanog-GFP MEFs with rtTA + OSKM generated two iPS lines. Both generated stable iPS lines after one to two passages.

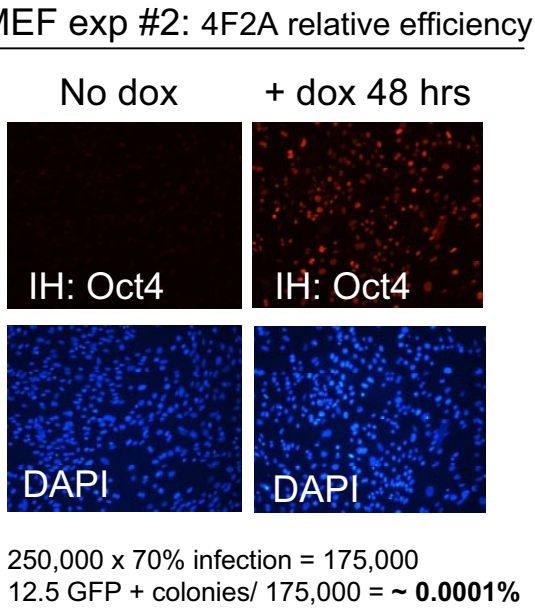

Fig. S2. Relative efficiencies of reprogramming using 4F2A. (*A*) Relative reprogramming efficiency of 4F2A in MEFs. Nanog-GFP MEFs were infected with 4F2A + rtTA and cultured in ES media (+/− DOX) for 48 h. Cells were fixed and stained for Oct4 protein. An estimated infection efficiency was ≈70%. The same virus was also used to infect 0.25×10^6 Nanog-GFP MEFs and cells were cultured on DOX for 20 days. After withdrawal of DOX at day 20, GFP+ colonies were counted at day 25, and three plates 10, 10, and 17 GFP+ colonies were observed.

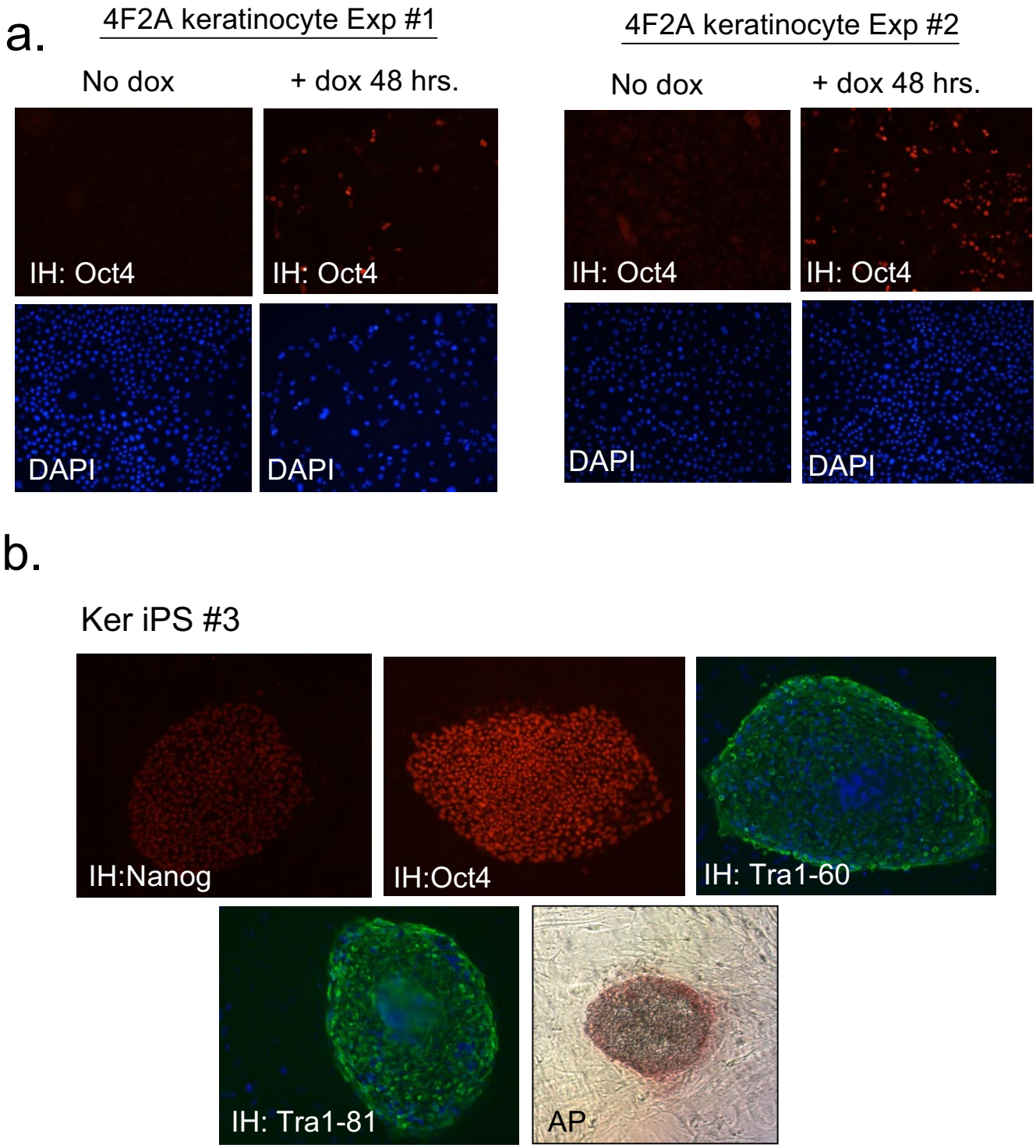

Fig. S3. Infection efficiency and pluripotency analysis of keratinocyte-derived human iPS lines. (*A*) Infection efficiency from two experiments as detected by Oct4 immunostaining in keratinocytes infected with 4F2A + rtTA and cultured in hES media + DOX for 48 h. Efficiency of infection was ≈10−20%, based on fraction of cells positive for Oct4 protein. (*B*) Human iPS lines stain positive for pluripotency markers expressed in hES cells (Ker-iPS no. 3 is shown).

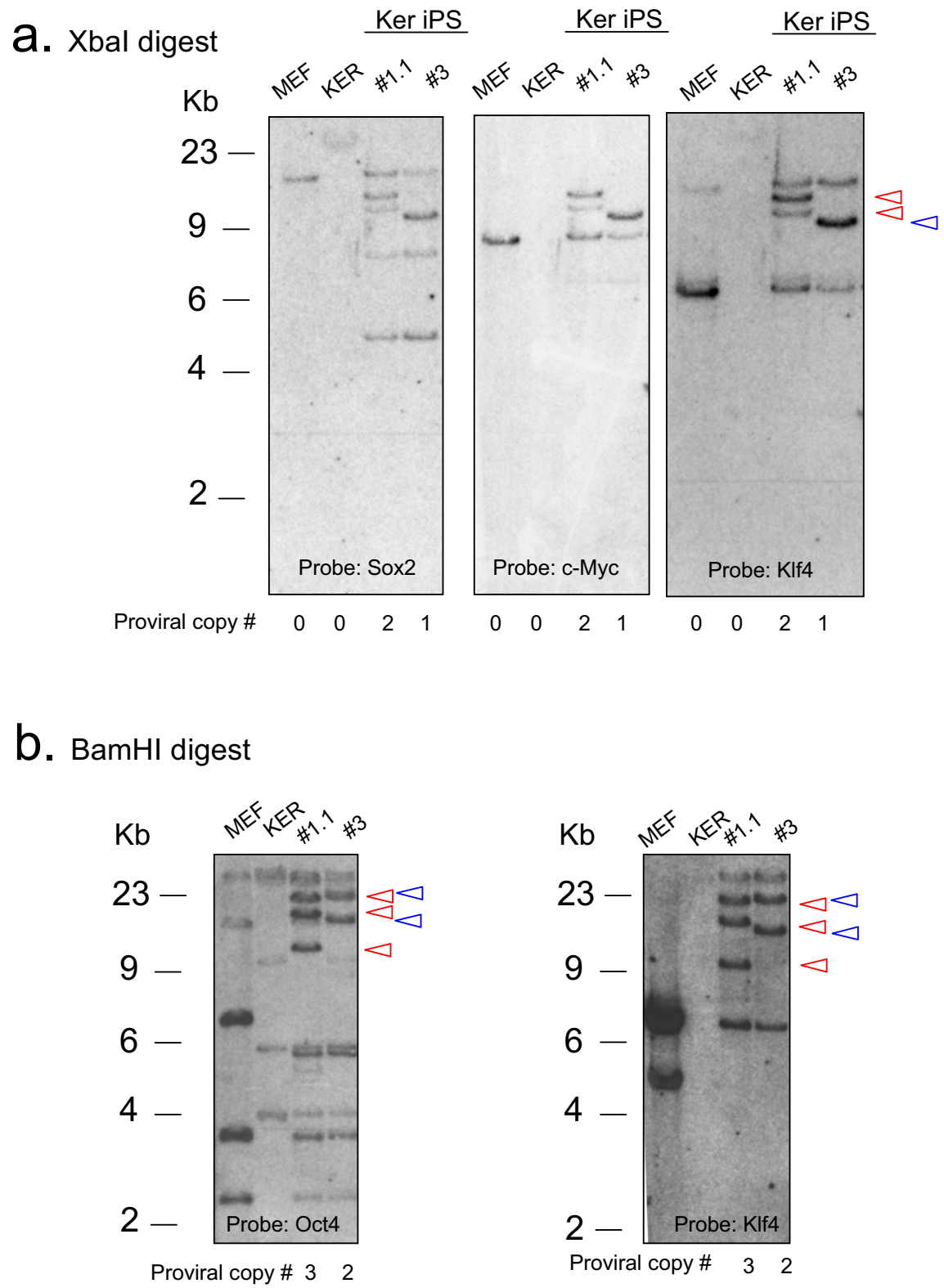

Fig. S4. Proviral copy number of keratinocyte-derived human iPS lines. (*A*) Southern blot analysis of Ker-iPS lines. Ten micrograms of genomic DNA were harvested and digested with XbaI. Hybridization of the same molecular-weight fragment indicates presence of 4F2A provirus. Probes for Sox2, Klf4, and c-Myc suggested two (no. 1.1) and one (no. 3) proviral copies. Common bands observed between the two iPS lines are not viral integration as these were derived from independent infections. (*B*) Southern blot analysis of Ker-iPS lines. Ten milligrams of genomic DNA were harvested and digested with BamHI. Hybridization of the same molecular-weight fragment indicates presence of 4F2A provirus. Probes for Oct4 and c-Myc indicate three (no. 1.1) and two (no. 3) proviral copies.

Table S1. Summary of pluripotency tests and relative efficiencies for all iPS lines generated

Source of cells	GFP	iPS lines	Efficiency (iPS/input, %)	ES	TF	PC
Embryonic fib (m)	Nanog	4	0.0001	Yes	Yes	Yes
Adult fib (m)	No	4	ND	Yes	No	No
Keratinocytes (h)	No	2	0.00001	Yes	Yes	No

Cell line	Blast injected	Live pups	Chimeric (no.)	Chimerism (%)
MEF iPS no. 4	60	30	2	30–50
MEF iPS no. 2	20	14	1	10

GFP, GFP reporter gene present; ES, expression of ES cell markers (AP, SSEA1, Oct4, or Sox2); TF, teratoma formation; PC, postnatal chimeras; m, mouse; h, human. Mouse chimerism was estimated by agouti coat color.