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Supplemental Data

Sequential Expression of Pluripotency Markers during Direct Reprogramming of Mouse Somatic Cells

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Figure S1

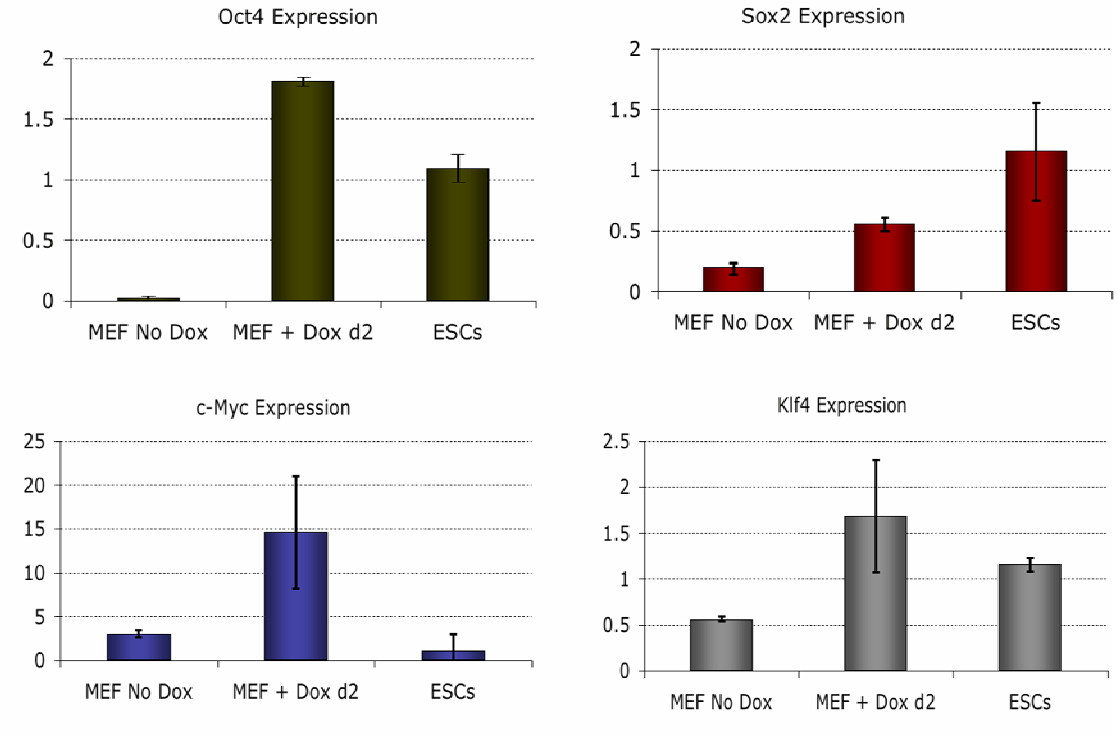

Figure S1: Quantitative RT-PCR assays of viral trangene expression in infected MEFs.

MEFs were infected and grown in the absence or the presence of dox. Average relative expression levels and standard deviations from two RT-PCR reactions are shown for all four factors. ES cell values are given as a positive control.

Figure S2

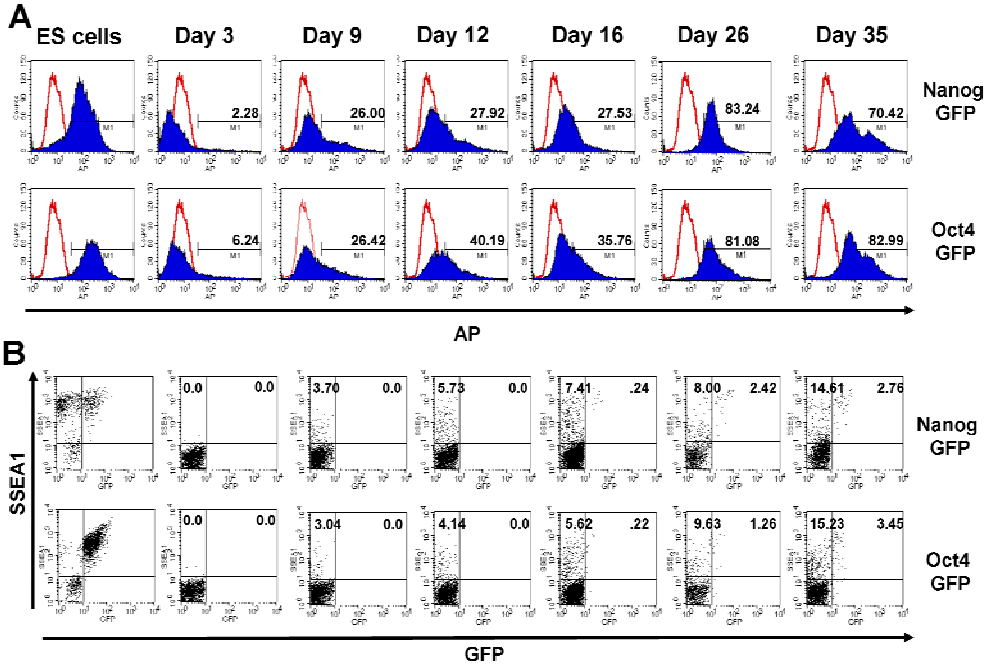

Figure S2: FACS analyses of AP, SSEA1 and GFP expression in infected MEFs and control ES cells.

FACS analysis of AP, SSEA1 and GFP reactivation was performed on NanogGFP/M2rtTA MEFs and Oct4GFP/M2rtTA MEFs at different times after the induction of reprogramming. Cells were harvested at various time points after the addition of dox to the medium and stained with an APC labeled anti-SSEA1 antibody and a fluorescent substrate detecting AP activity. Representative FACS plots of three independent experiments are shown. The fraction of AP positive cells increases with time after transgene induction. In the histogram plots of AP staining, the red line represents the negative control (infected MEFs cultured without dox) and the solid purple represents induced MEFs analyzed at the specified time (A). The numbers displayed for each plot are the number of cells in the M1 gate which was set so that less than 1% of cells in the negative control were in the M1 population. Analysis of SSEA1 and GFP expression (B). Dot plots of GFP and SSEA1 signals are displayed and the percentages of cells for the SSEA1+/GFP- and the SSEA1+/GFP+ populations are shown. AP positive cells were first observed on day 3, while SSEA1 positive cells appeared on Day 9. Cells positive for Nanog-GFP or Oct4-GFP were first detected on day 16 and most of these cells also stained positive for SSEA1.

Figure S3

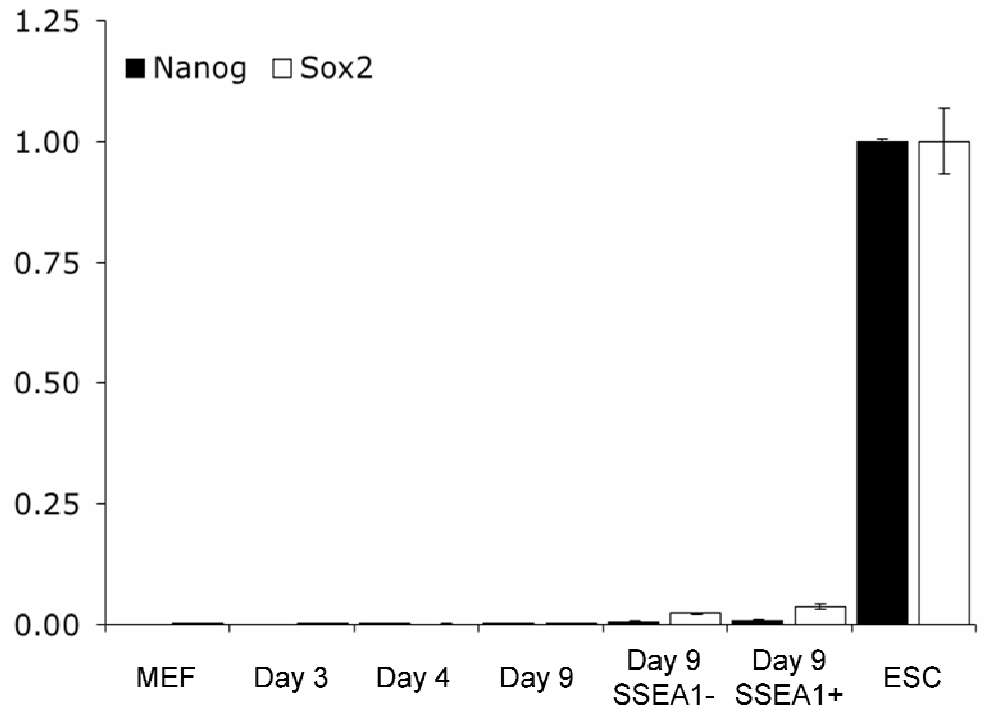

Figure S3: Quantitative RT-PCR analysis of endogenous Nanog and Sox2 expression.

Expression levels of endogenous Nanog and Sox2 transcripts in whole cell populations and in SSEA1 sorted cells at various time points prior to the activation of GFP are displayed. ES cells were used as a comparison. Expression in the cells undergoing reprogramming was similar to that seen in non-infected MEFs and substantially lower than that in ES cells. Average relative expression levels and standard deviations from two RT-PCR reactions for each sample are shown.

Figure S4

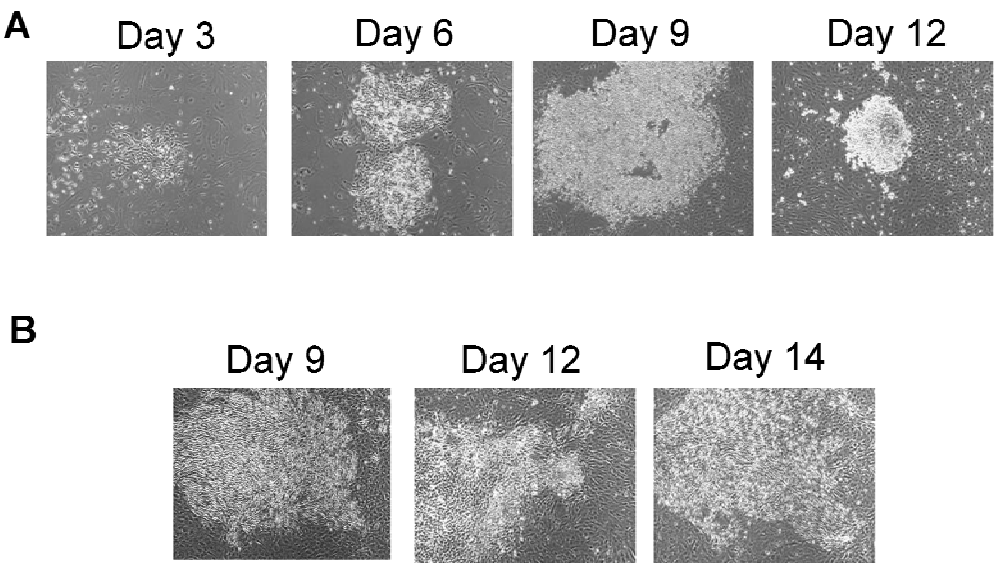

Figure S4: Morphology of non-reprogrammed cell colonies.

Pictures of cell colonies on induced plates at the time specified (A). Plates were re-assessed at day 35 of the experiment and pictures of cell colonies that were detectable are shown (B).