Expanded View Figures

Figure EV1. Analysis of genes whose expression is altered during cellular reprogramming.

A Comparison of the transcriptomic profiles between mESCs and miPSCs. The two black diagonal lines enclose 95% of expressed genes with high correlation, that is, with similar expression profiles in both cell types. Spearman's correlation coefficient = 0.97; normalization with RMA.

B Line plot depicting the number of differentially expressed genes (DEGs), which either change their expression transiently or permanently at least once during reprogramming.

C Bar graph showing the number of up- and downregulated DEGs, transiently or permanently, during reprogramming.

D Hierarchical clustering of the gene expression profiles of MEFs undergoing reprogramming from day 0 to day 18. Clustering revealed four distinct phases, of which day 6 is characterized by Nanog activation.

E PCA of the gene expression profiles described in (*D*).

F Line plot depicting the number of upregulated (blue line) and downregulated DEGs (green line) during MEF reprogramming. The bars shown below the line plot represent the enrichment of selected gene ontology categories at each time point.

G Gene Ontology (GO) analysis categorization of up- and downregulated DEGs until day 6 of reprogramming (*i, iii* for MEFs *and ii, iv* for mHEPs). Transcriptional regulators are highlighted in blue color.

H Heat map depicting the pattern of expression of well-characterized mesenchymal, epithelial, and pluripotent markers during MEF reprogramming (data derived from qPCR assays).

I Heat map showing the expression pattern of the nine TRs at selected time points during reprogramming of MEFs, mHeps (data derived from DNA microarray assay), and hFBs (data derived from qPCR assays).

Source data are available online for this figure.

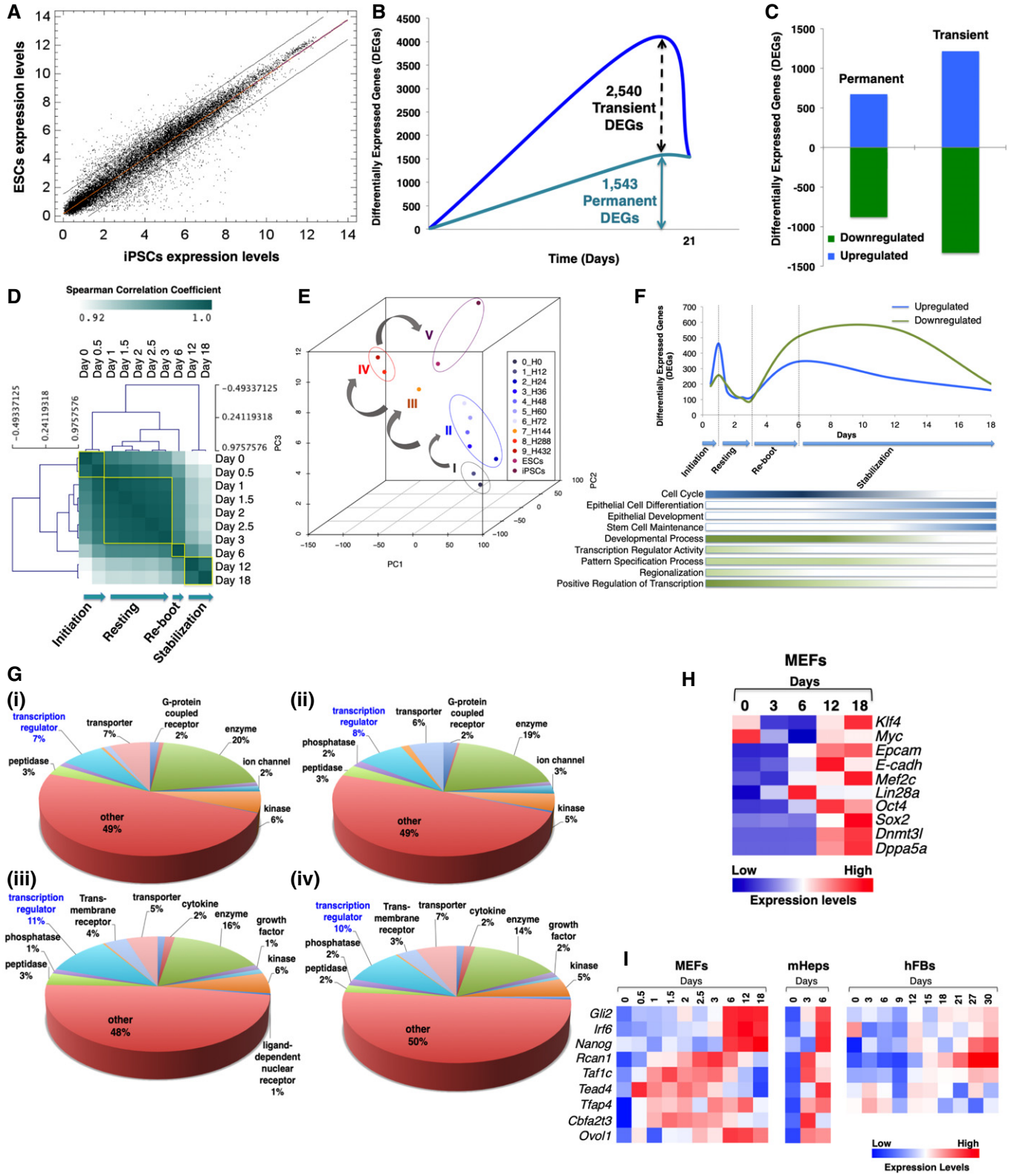


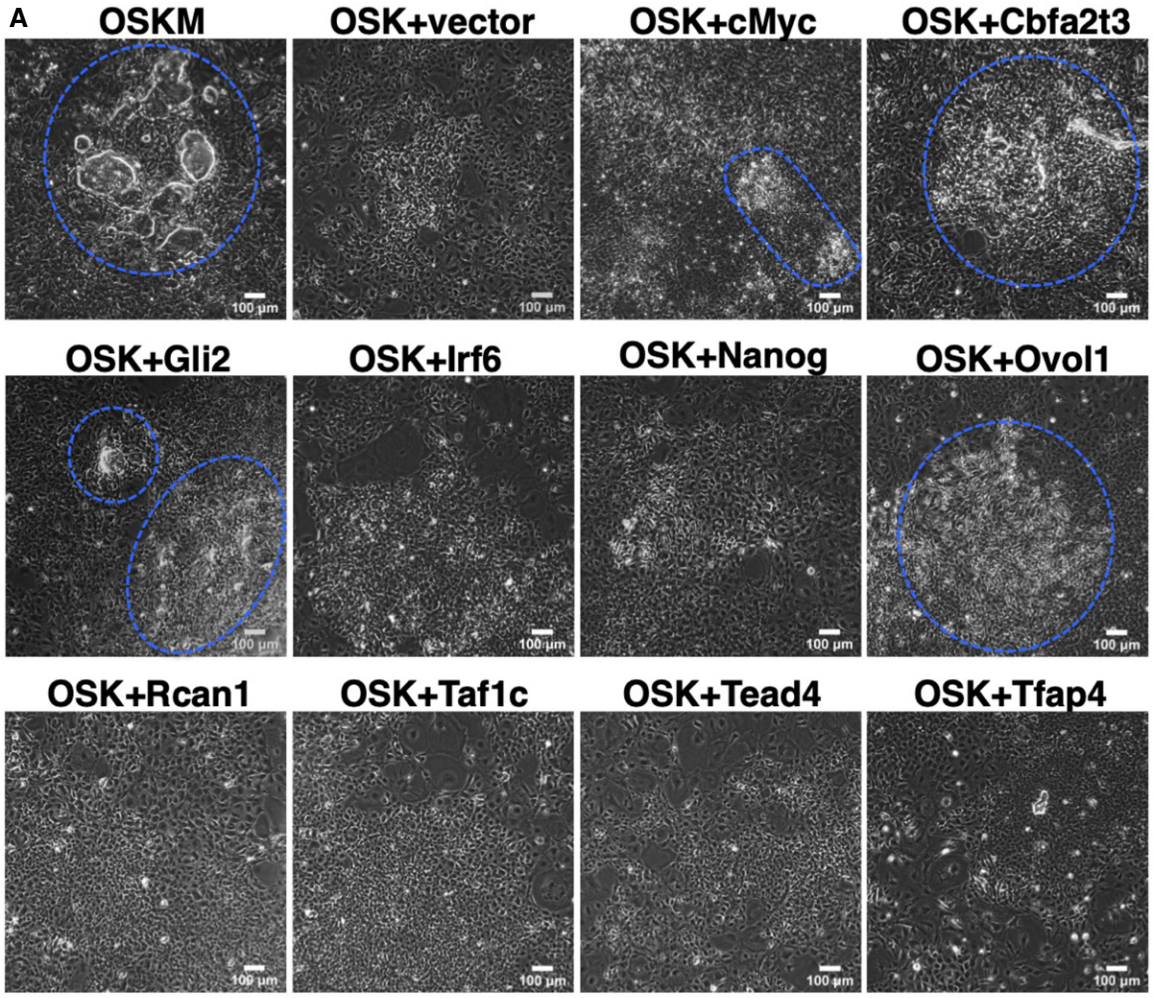
Figure EV1.

Figure EV2. Substitution of c-Myc by nine TRs.

A MEFs expressing OSK were transduced with lentiviruses overexpressing each of the nine TRs or c-Myc as a control. The transduced cultures were examined on day 6 for the formation of early-iPSC colonies. The boundaries of the early-iPSC formations are indicated by a blue dashed line.

B Western blot depicting the expression of O/S/K/M during MEF reprogramming. Gapdh was used as loading control. O: Oct4, S: Sox2, K: Klf4, and M: c-Myc.

Source data are available online for this figure.



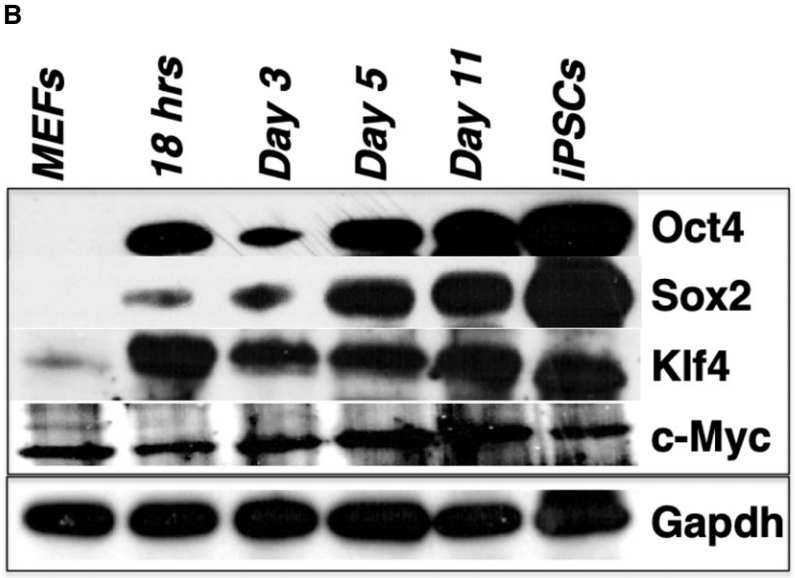


Figure EV2.

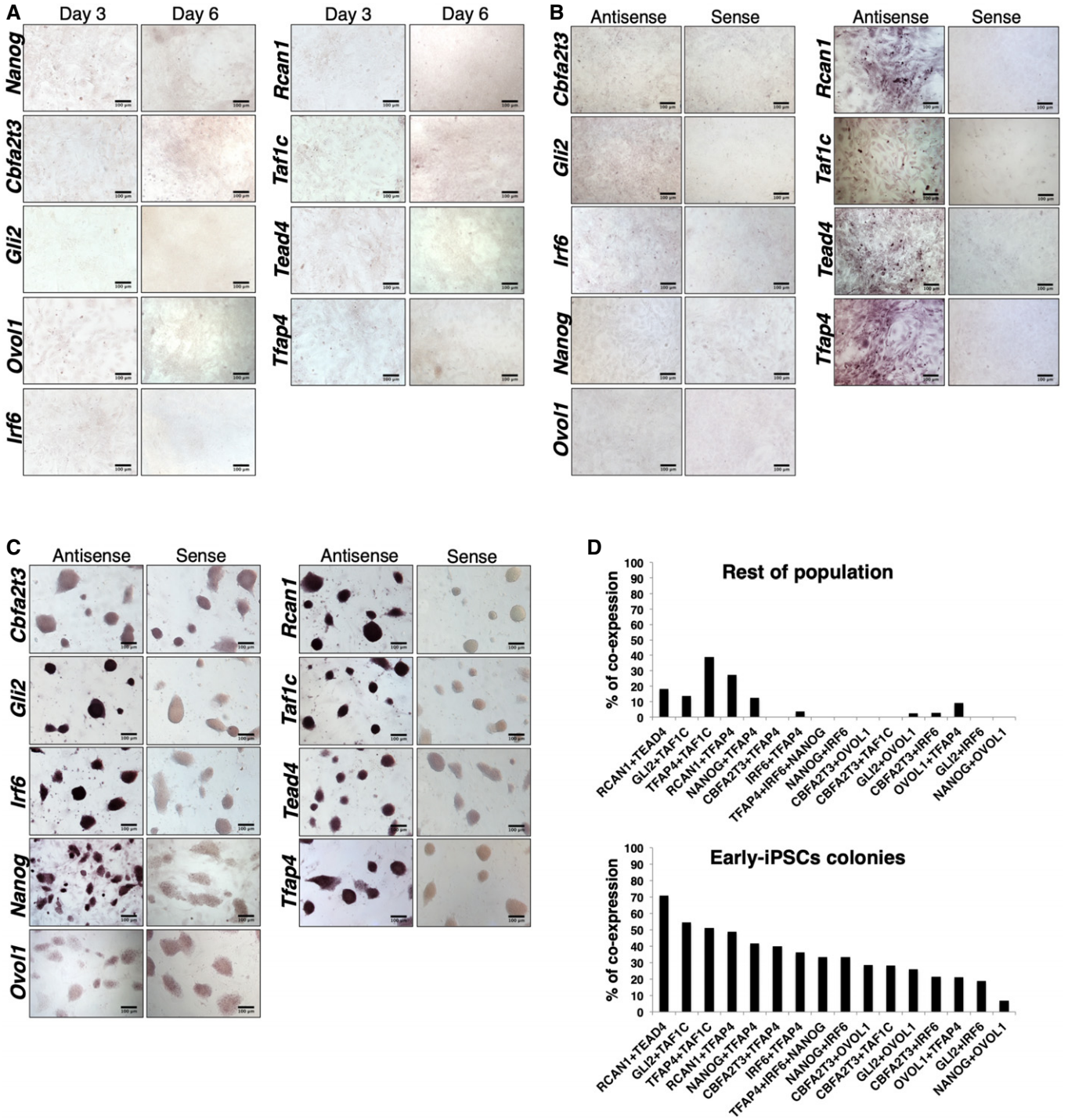


Figure EV3. The spatiotemporal (co)-expression pattern of nine TRs.

A RNA *in situ* hybridization (ISH) experiments in MEFs undergoing reprogramming (day 3 and day 6), depicting the hybridization pattern using the sense RNA strand of each of the nine TRs as probes. Scale bars, 100 μm.

B RNA *in situ* hybridization experiments using naïve MEFs hybridized with the sense or the antisense RNA strand of each of the nine TRs, as indicated at the top and at the left side of each panel, respectively. Scale bars, 100 μm.

C Same as in (B) except that mESCs were used.

D Bar graph depicting the co-expression frequencies of the indicated combinations of nine TRs within the early-iPSC colonies and in the rest of the population of MEFs, based on double and triple single-cell experiments.

Source data are available online for this figure.

Figure EV4. Binding of OSKM to the O/S/K/M genes in MEFs and to nine TR genes in human fibroblasts.

A Shown are ChIP-seq big wig files in IGV browser depicting binding of Oct4 (red), Sox2 (blue), Klf4 (green), and c-Myc (black) to regulatory regions of the O/S/K/M/ genes in MEFs undergoing reprogramming at the 18 h, day 3, and day 5, as well as in control MEFs, mESCs, and miPSCs. The relative sizes of the represented genomic loci and the corresponding TSSs are also indicated. The side color bar depicts the expression levels of each TR at the indicated time points. All peaks have been normalized against input DNA

B Shown are ChIP-seq big wig files in IGV browser depicting OSKM binding to regulatory regions of the indicated TRs on day 2 of human fibroblasts undergoing reprogramming. The results shown were derived from meta-analyses of the published ChIP-seq data from K.S Zaret Lab (Soufi *et al*, 2012).

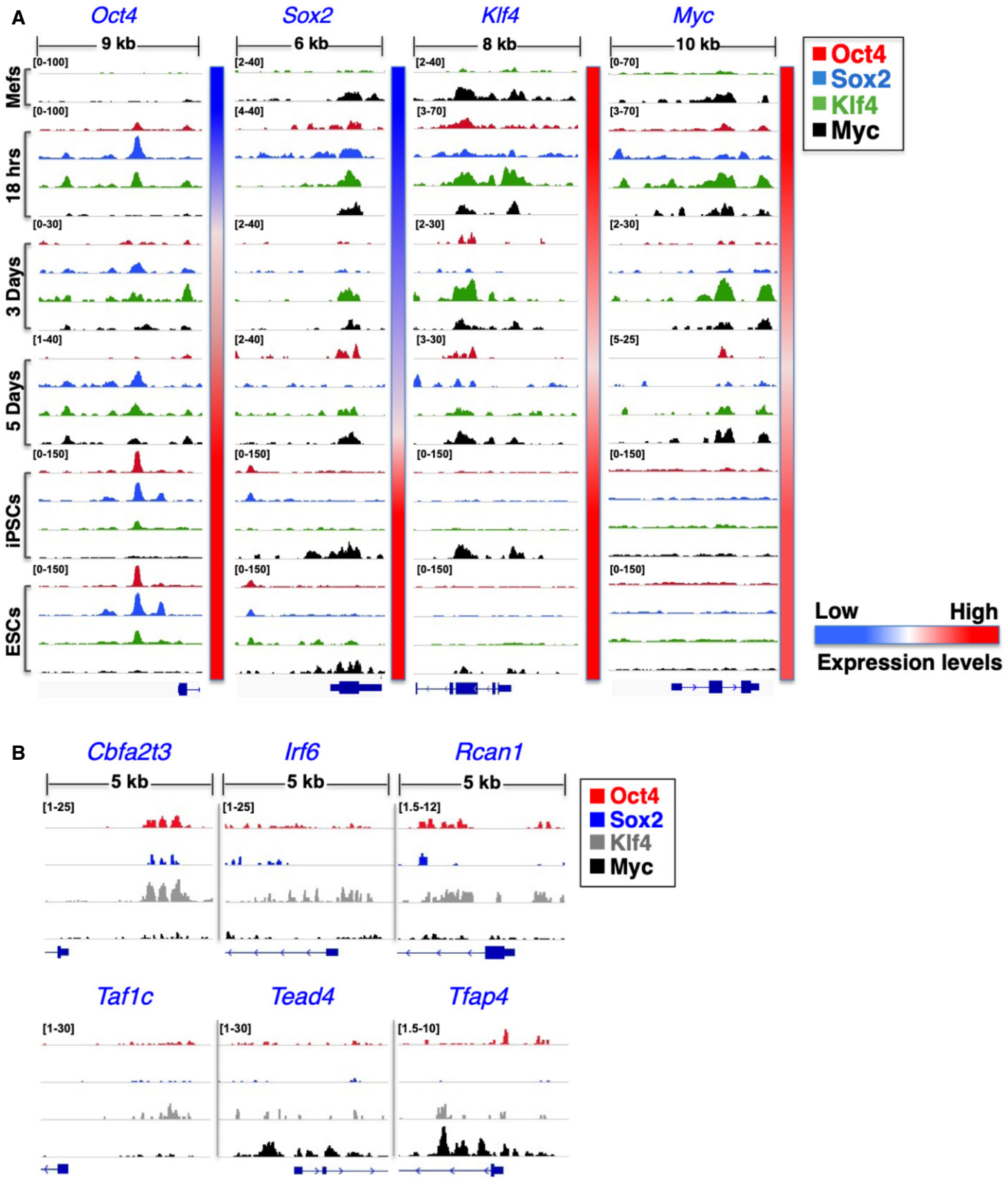


Figure EV4.

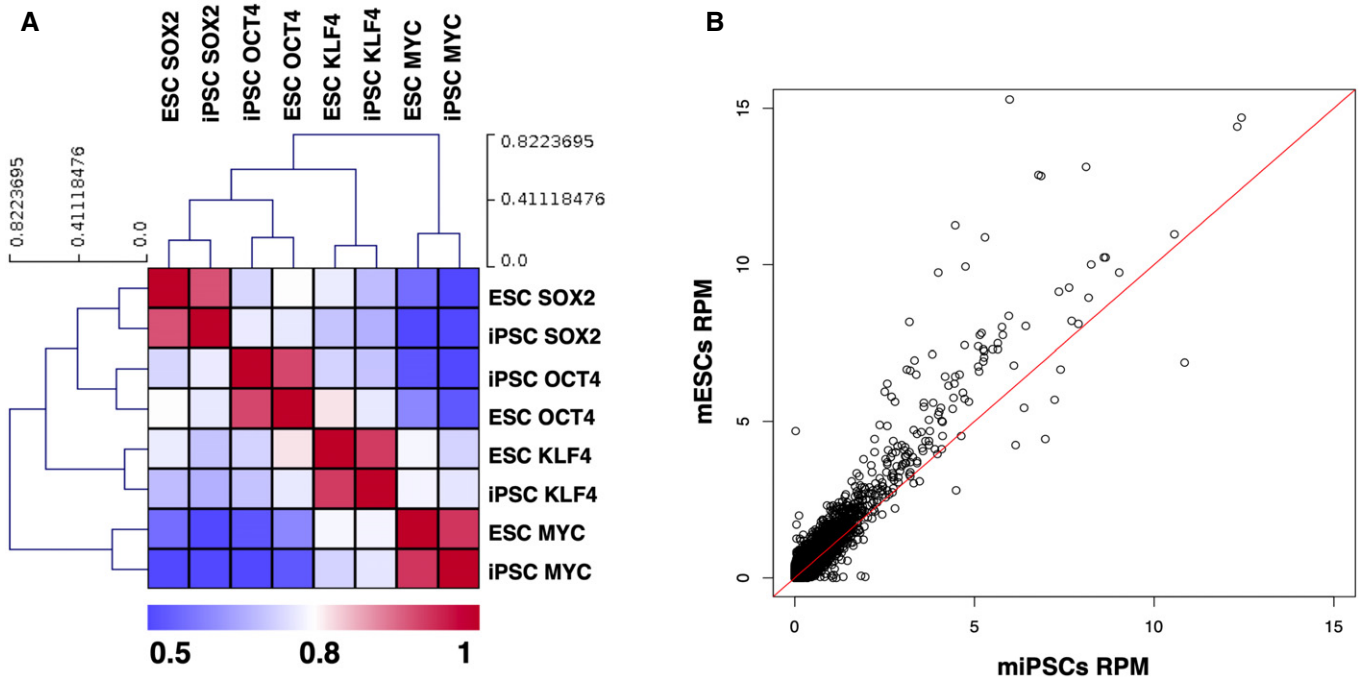


Figure EV5. Genome-wide correlation of OSKM binding between mESCs and miPSCs.

A Shown is a heat map depicting the similarity of O/S/K/M binding between mESCs and iPSCs. All ChIP-seq peaks were merged and the total number of reads corresponding in each peak was counted, the signal was normalized for sequencing depth (RPM—reads per million), and the Pearson's correlation coefficient was estimated for all ESC-iPSC and O/S/K/M pairs.

B Scatter plot depicting the extensive similarity of the Sox2 genome-wide DNA-binding pattern between iPSCs and ESCs. The red line represents the correlation equal to 1.

Figure EV6. The effect of nine TR knockdown on network integrity.

A Shown is a schematic representation of the knockdown (KD) effects of each of the indicated TRs on the expression of the other factors and on network integrity, as determined by RT–qPCR analyses. Dark blue color denotes TRs upregulated more than twofold. Reduction by more than 2 fold in the expression is depicted by the absence of the corresponding TR. The light red and blue colored circles indicate downregulation or upregulation in the range of 1.3 to twofold, respectively, while gray circles indicate no change in gene expression. O: Oct4, S: Sox2, K: Klf4, and M: c-Myc. The light gray-green and red boxes depict the upstream and downstream layers of the 9TR GRN. The first panel on the left denotes the native 9TR GRN as shown in Fig 4B (phase III).

B Same as in (A) except that double KDs of the indicated TRs were carried out.

Source data are available online for this figure.

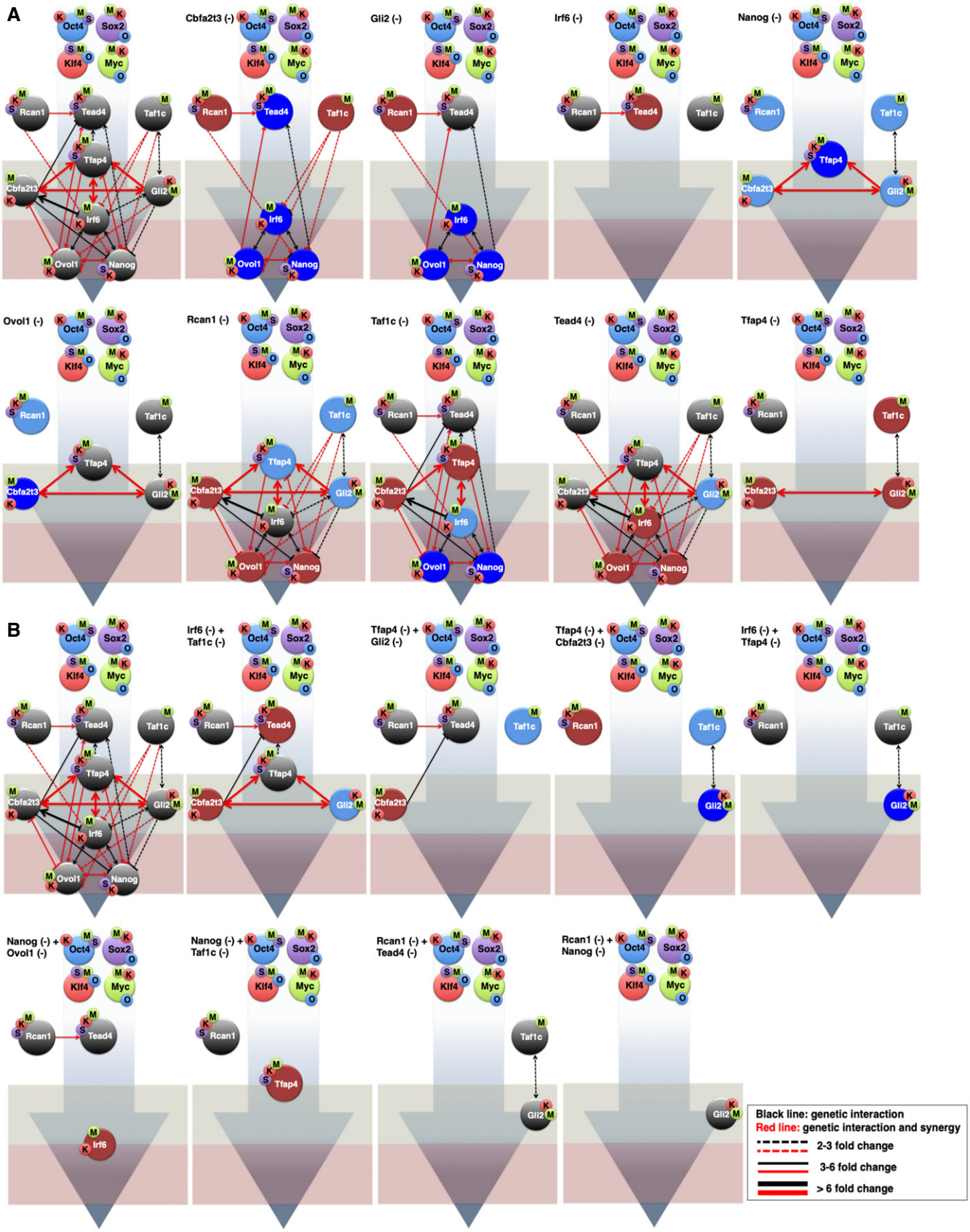

A
Cbfa2t3 (-)
Gli2 (-)
Irf6 (-)
Nanog (-)
Ovol1 (-)
Rcan1 (-)
Taf1c (-)
Tead4 (-)
Tfap4 (-)
B
Irf6 (-) + Taf1c (-)
Tfap4 (-) + Gli2 (-)
Tfap4 (-) + Cbfa2t3 (-)
Irf6 (-) + Tfap4 (-)
Nanog (-) + Ovol1 (-)
Nanog (-) + Taf1c (-)
Rcan1 (-) + Tead4 (-)
Rcan1 (-) + Nanog (-)
Oct4
Sox2
Klf4
Myc
Rcan1
Tead4
Taf1c
Tfap4
Cbfa2t3
Gli2
Irf6
Ovol1
Nanog
Black line: genetic interaction
Red line: genetic interaction and synergy
2-3 fold change
3-6 fold change
> 6 fold change

Figure EV6.