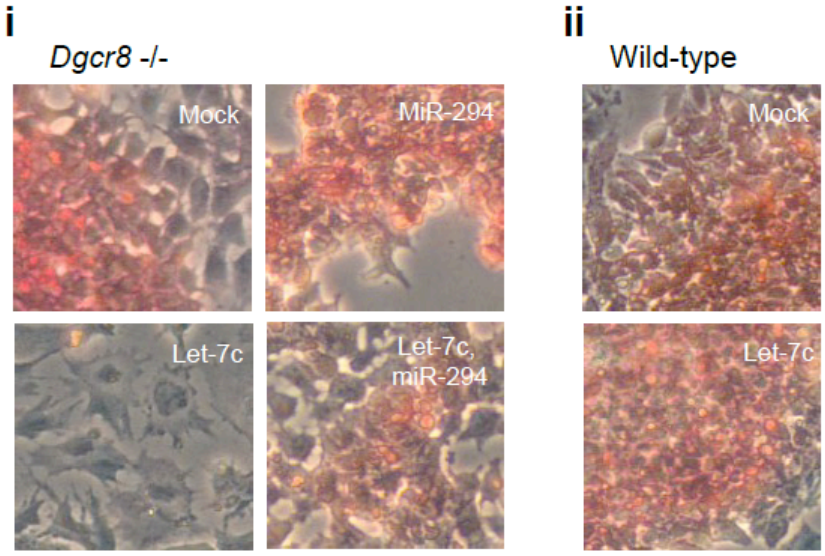
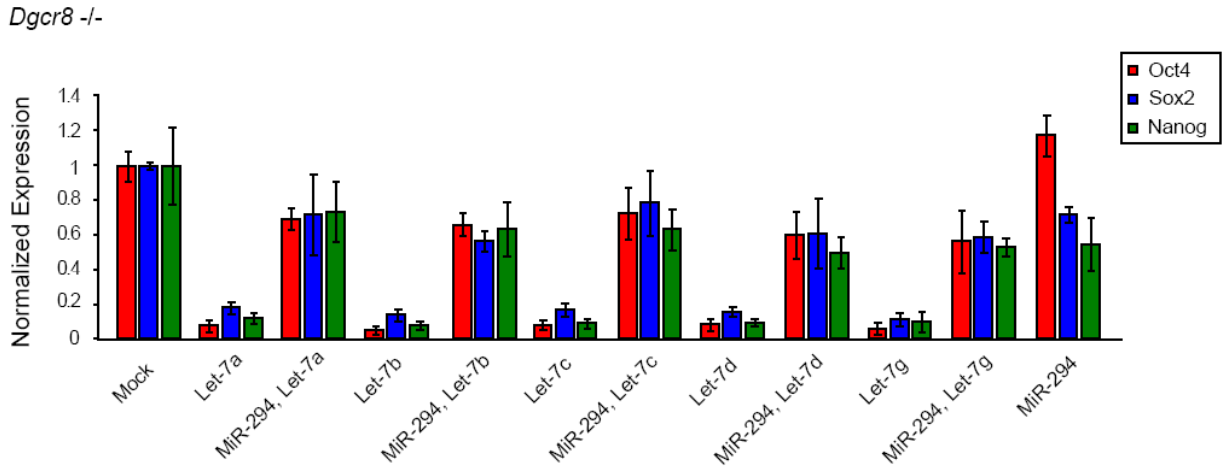
**Figure S1**



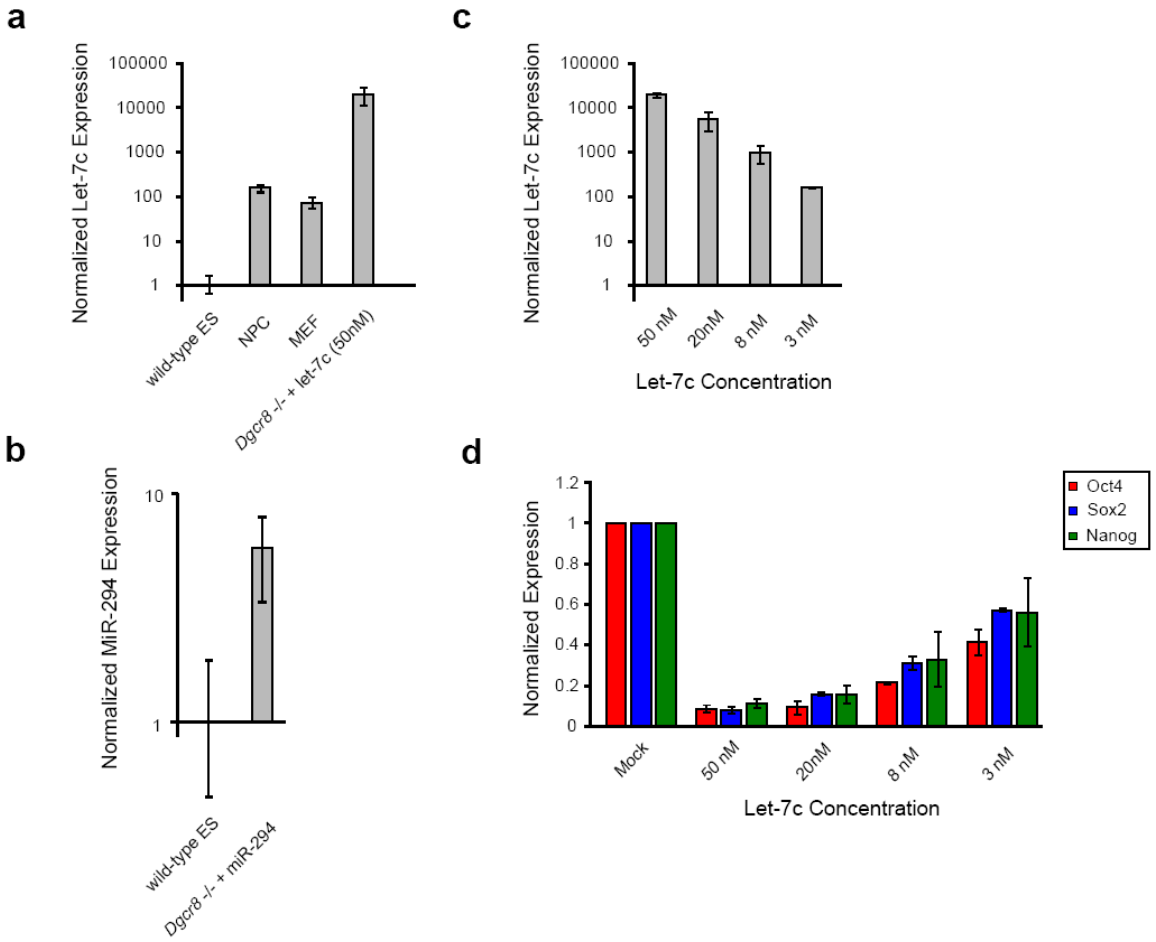

The let-7 and ESCC families of miRNAs have opposing roles in regulating ESC self-renewal. Alkaline phosphatase staining 3 days after transfection of miRNAs into *Dgcr8* -/- (i) and wild-type (ii) ESCs. Representative images, n = 3.

**Figure S2**



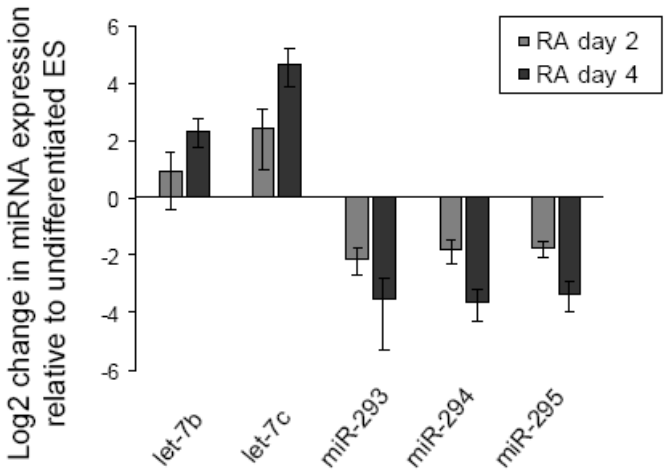

The let-7 family of miRNAs function to suppress self-renewal in *Dgcr8 -/-* ESCs. qRT-PCR for Pou5f1/Oct4, Sox2, and Nanog normalized to beta-actin after transfection with different let-7 family members either alone or in combination with miR-294. n = 3, error bars represent standard deviation.

**Figure S3**



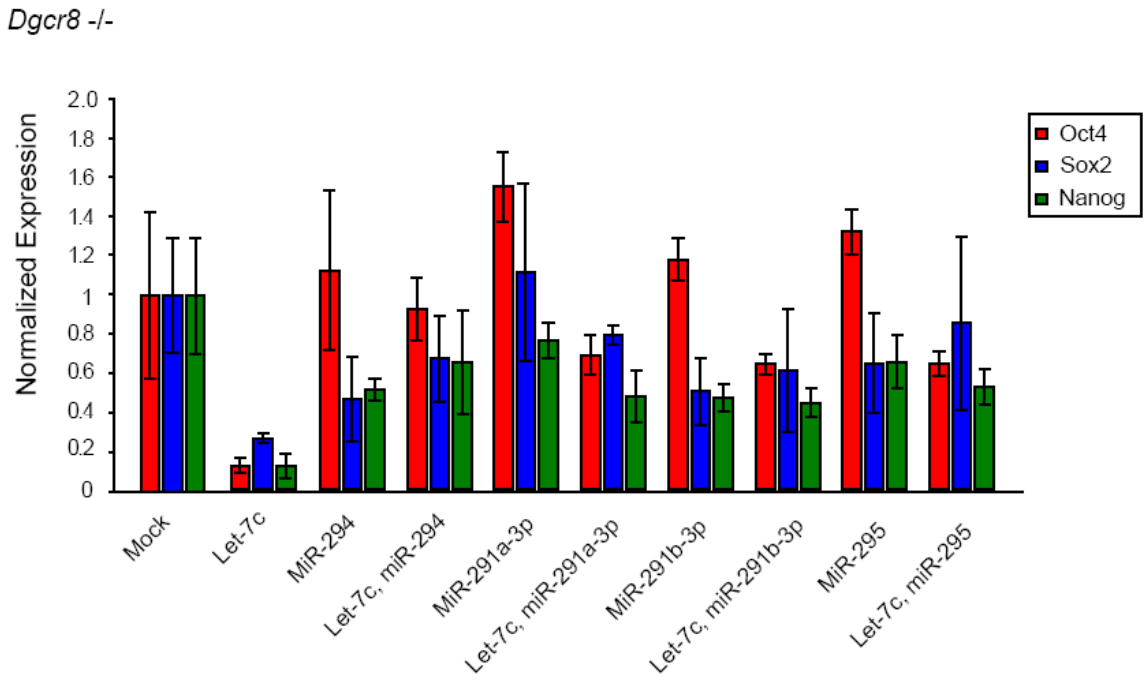

Let-7 and ESCC miRNAs exert their effects at physiologically relevant concentrations. (a) TaqMan miRNA qPCR day 3 after transfection for let-7c normalized to sno202 in wild-type ESCs, neural progenitor cells (NPCs), mouse embryonic fibroblasts (MEFs), and *Dgcr8*-/- ESCs transfected with let-7c at 50nM. Error represents range of n = 3. (b) TaqMan miRNA qPCR for miR-294 in wild-type ESCs and *Dgcr8*-/- ESCs transfected with miR-294 at 50nM day 3. Error represents range of n = 3. (c) TaqMan qRT-PCR for let-7c normalized to sno202 on day 3 of a dilutional series reducing the concentration of let-7c from 50nM to 3nM reduces the final concentration of let-7c to near NPC and MEF levels. Error represents range of n = 2. (d) qRT-PCR for Pou5f1/Oct4, Sox2, and Nanog demonstrates that let-7c at reduced concentrations still silences self-renewal in *Dgcr8* -/- ESCs. Note, both NPCs and MEFs express many members of the let-7 family thus the physiologic levels of let-7 are achieved somewhere between 8 and 50nM.

**Figure S4**



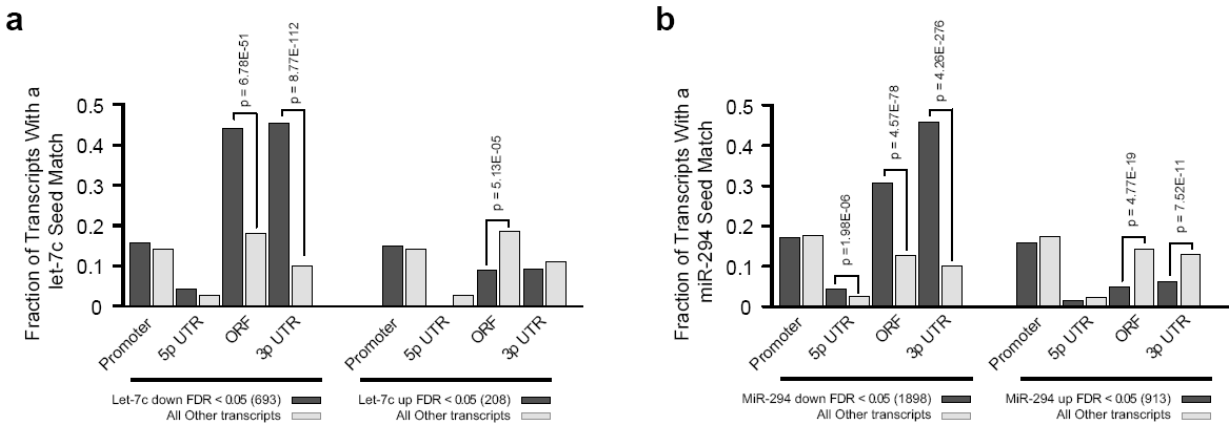

Let-7 and ESCC miRNAs show inverse expression during ESC differentiation. polyA miRNA qPCR time course during retinoic acid induced differentiation of wild-type ESCs. Data are normalized to U6. Error represents standard deviation of n = 3.

**Figure S5**



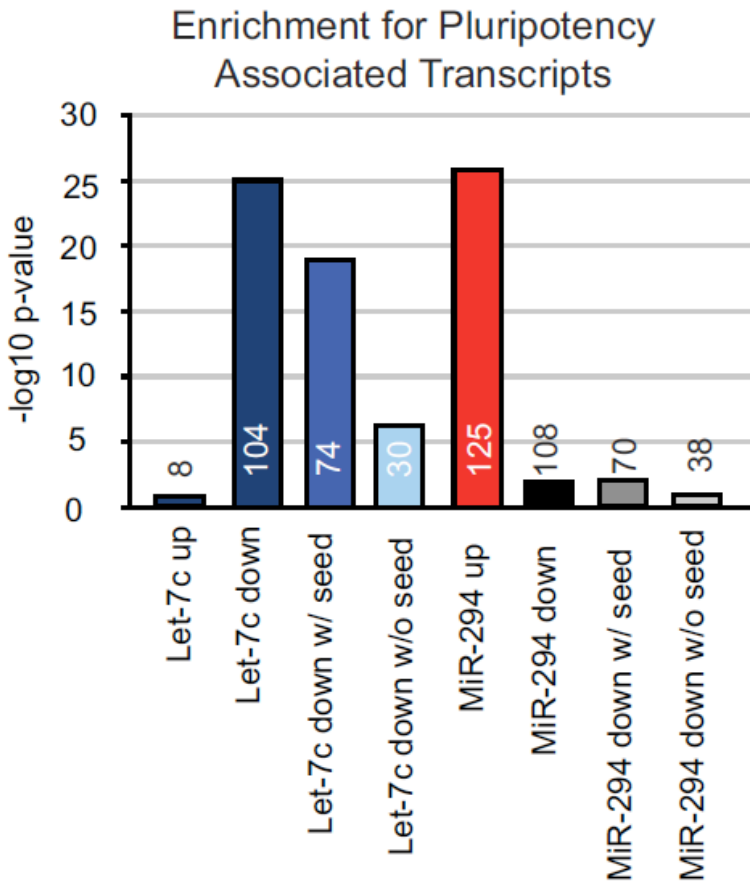

miR-290 cluster ESCC family members function to suppress let-7c induced silencing of ESC self-renewal in *Dgcr8 -/-* ESCs. qRT-PCR for Pou5f1/Oct4, Sox2, and Nanog normalized to beta-actin after transfection with different ESCC family members either alone or in combination with let-7c. n = 3, error bars represent standard deviation.

**Figure S6**



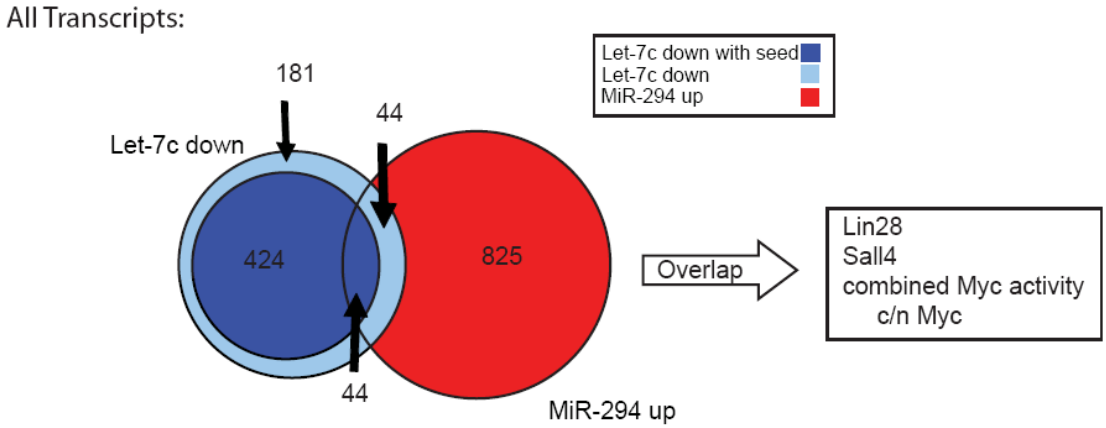

Let-7c and miR-294 suppress direct targets through binding of ORF and 3´UTRs. This analysis is similar to the analysis presented in **Fig. 2** except that there is no correction for sequence length. (a) Analysis of seed matches in the promoter, 5´UTR, ORF, and 3´UTR of let-7c downregulated and upregulated transcripts. Presented are the fraction of transcripts with a seed match in different regions (promoter, 5´UTR, ORF, and 3´UTR) for the listed groups of altered genes described in **Fig. 2a**. (b) An identical analysis to (a) but for miR-294 seed matches in miR-294 altered gene sets. Indicated p-values are calculated by Fischer's Exact Test and are presented only for $p < 0.001$

**Figure S7**



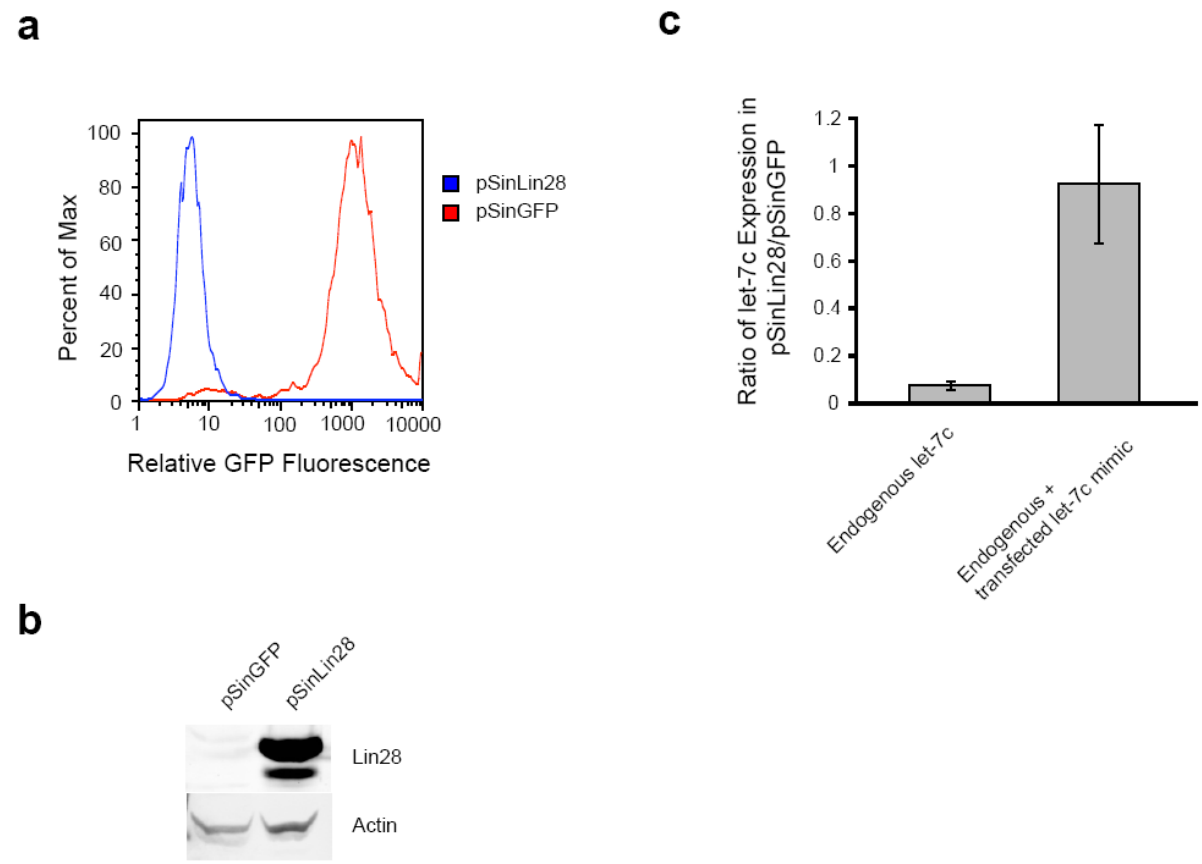

Let-7c downregulates and miR-294 upregulates transcripts highly enriched for pluripotency genes. Enrichment analysis for pluripotency associated transcripts among the different sets of miRNA up and downregulated transcripts. –log10 p-value is plotted and calculated by Fischer's exact test. In each column is the number of transcripts observed in each category.

**Figure S8**



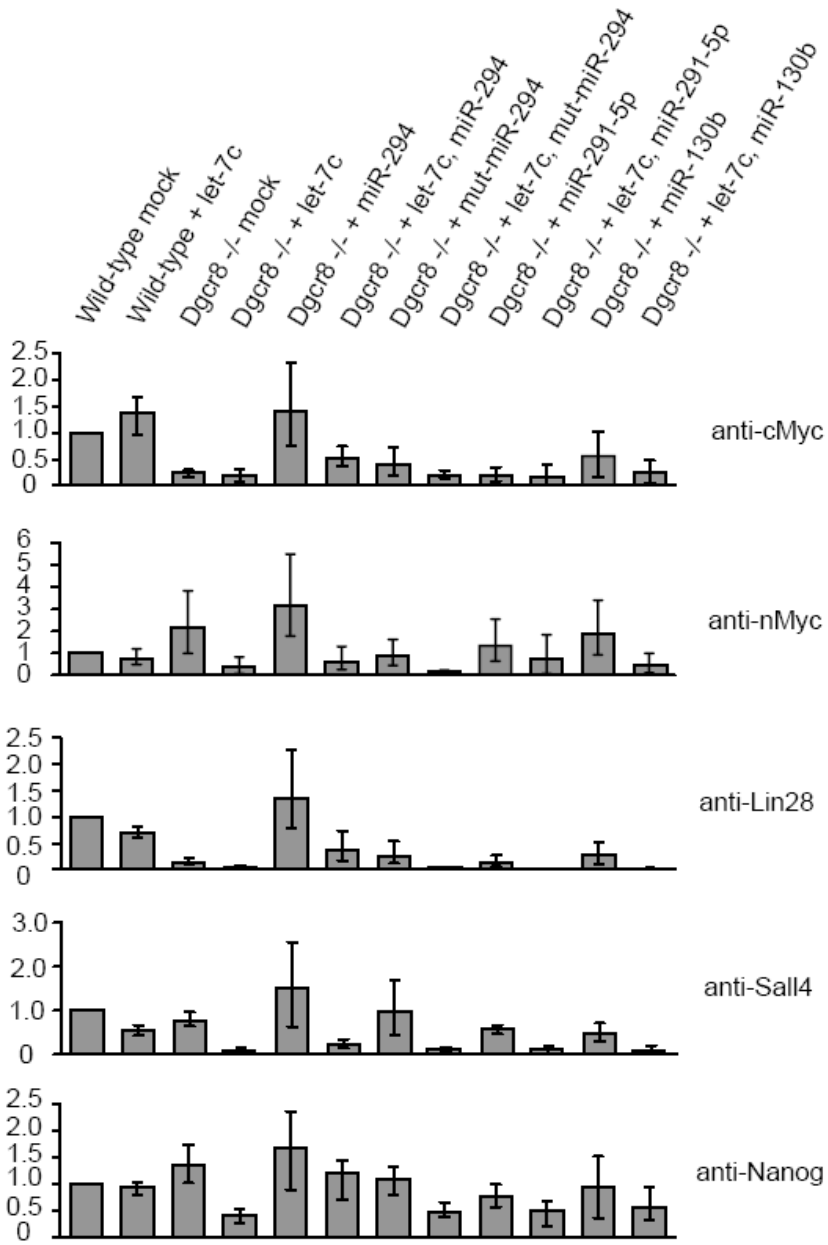

Overlap of transcripts downregulated by let-7c and upregulated by miR-294. A Venn diagram displaying the overlap between miR-294 upregulated and let-7c downregulated transcripts. Lin28, Sall4, and the combination of cMyc and nMyc are found in this overlap. Gene list can be found in **Table S3**.

**Figure S9**



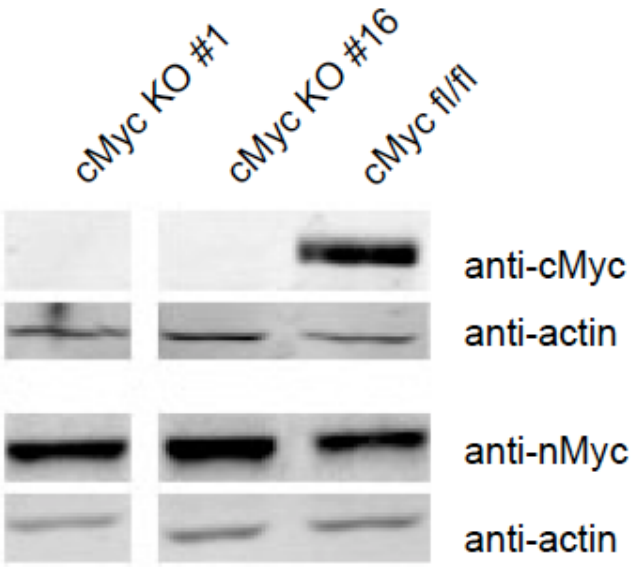

Lin28 does not promote degradation of let-7c mimic. (a) 293T cells were transfected with pSinLin28 and pSinGFP constructs and selected for puromycin resistance. Flow cytometry for GFP in GFP control cells demonstrates that following selection in puromycin most cell express the exogenous gene. (b) Western analysis shows that the 293T cells transfected with Lin28 overexpresses Lin28 protein. (c) Taqman miRNA qPCR for mature let-7c normalized to sno202 shows that Lin28 represses maturation of endogenous let-7c but not transfected let-7c mimic. Error represents standard deviation of n = 3.

**Figure S10**



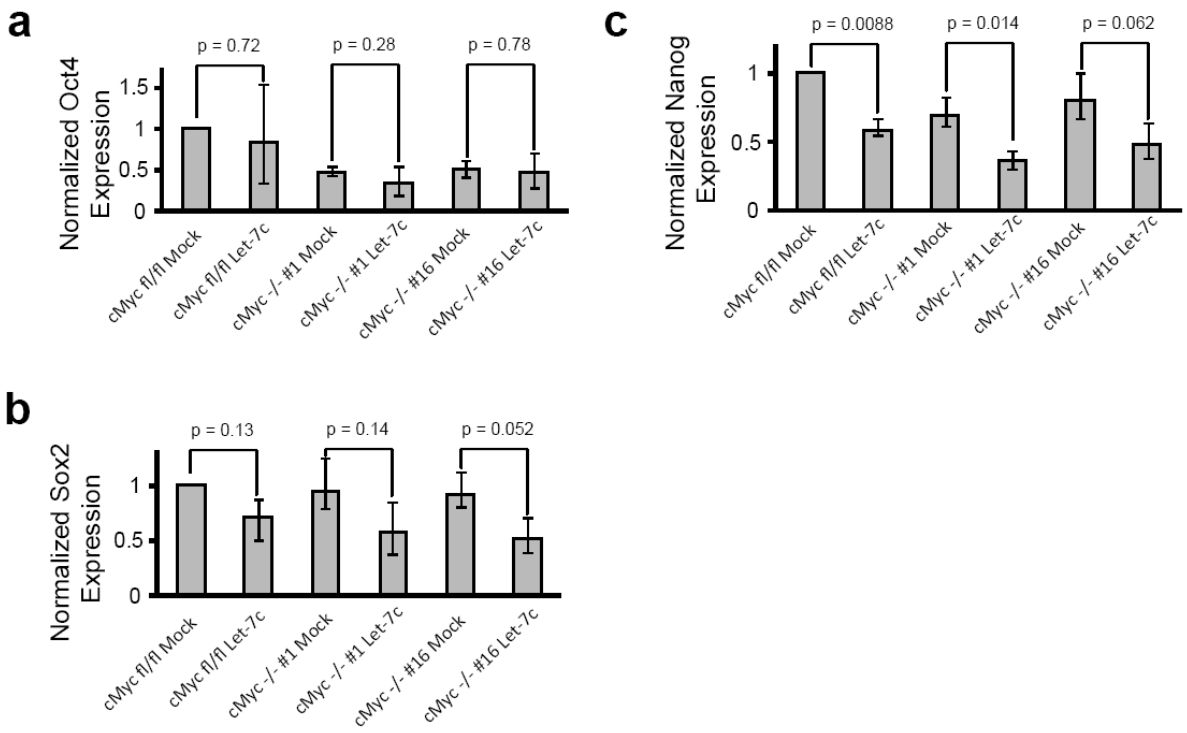

Opposing regulation of Myc, Lin28, and Sall4 protein levels by let-7c and miR-294. Quantification of Western analysis from **Fig. 4b**. To compare between replicates samples were normalized to set wild-type mock to one. Error represents standard deviation of n = 3 for these normalized samples.

**Figure S11**



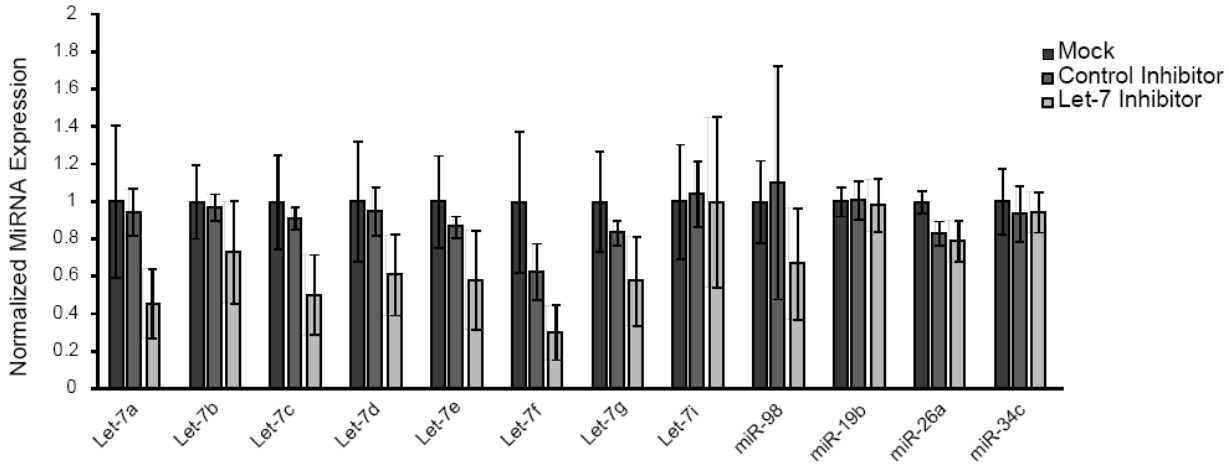

Loss of cMyc in cMyc -/- ESCs. Western analysis of cMyc and nMyc in two separate cMyc -/- ESC lines and their parental cMyc fl/fl ESC line. For Western analysis, cells were taken off the irradiated MEF feeder layer for approximately 3-4 passages.

**Figure S12**



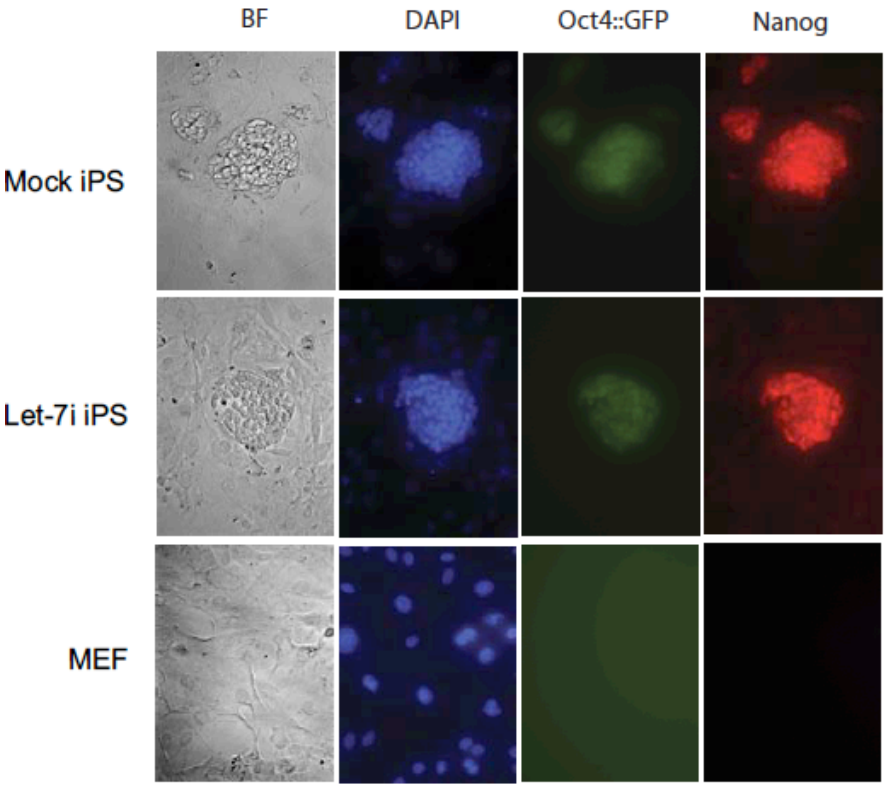

cMyc -/- ESCs show partial susceptibility to let-7c induced silencing of ESC self-renewal. (a) qRT-PCR for Pou5f1/Oct4 in cMyc fl/fl and two derived cMyc -/- lines either mock or let-7c treated. Oct-4 levels are reduced in cMyc -/- cells and do not significantly change upon let-7c treatment. (b) qRT-PCR for Sox2 and (c) Nanog as in a. Let-7c decreases expression of Sox2 and Nanog in cMyc -/- ESCs. Note, the decrease in cMyc fl/fl control differs from V6.5 ESCs used in **Fig. 1**. This suggests slight differences between wild-type lines in their response to let-7. Uncorrected p-values were generated by t-test. Error bars represent range of n = 3.

**Figure S13**



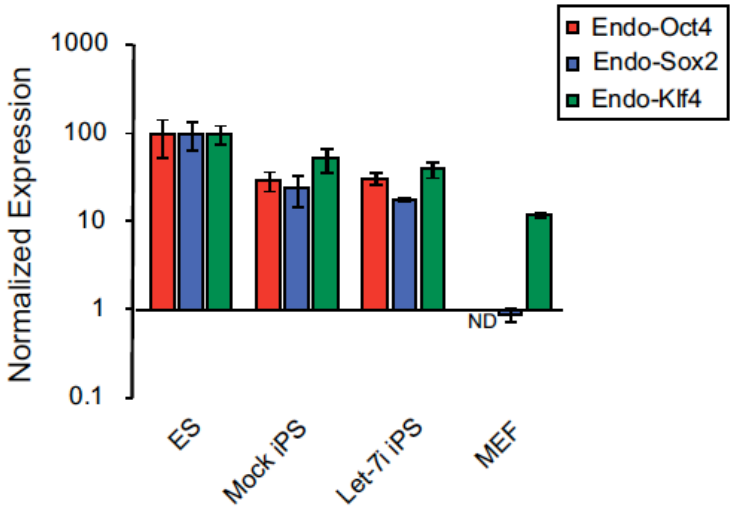

The let-7 inhibitor reduces levels of the majority of mature let-7 family miRNAs. polyA miRNA qPCR was performed in mock, control inhibitor, and let-7 inhibitor treated MEFs. Let-7 family miRNAs but not other highy expressed miRNAs (miR-19b, miR-26a, and miR-34c) have reduced levels in the presence of the let-7 inhibitor.

**Figure S14**



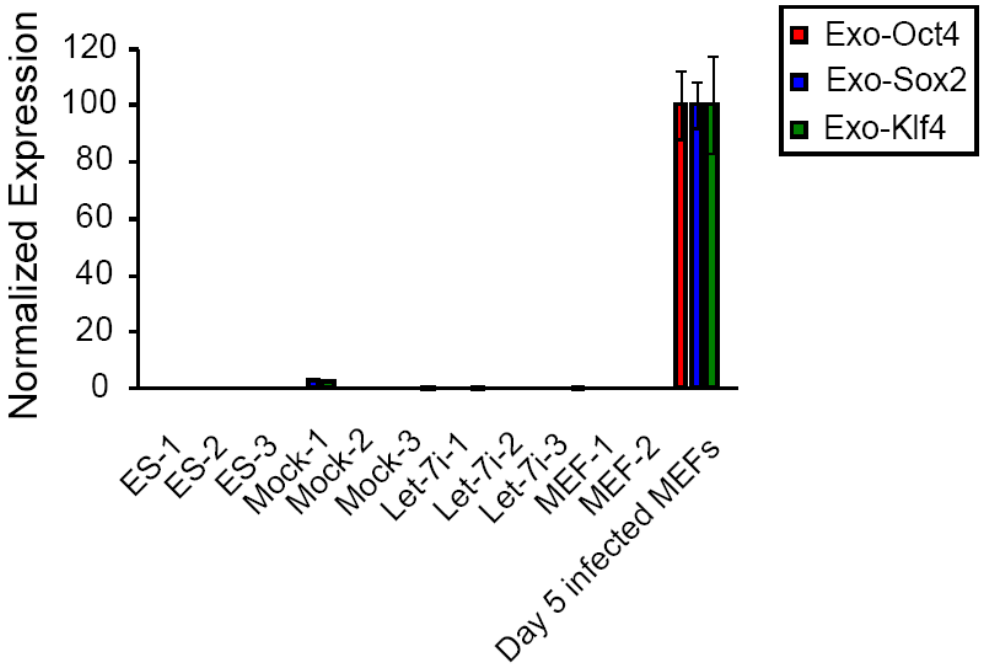

Oct4::GFP positive colonies co-express endogenous Nanog protein. Microscopy of 3TF mock and let-7 inhibitor reprogrammed cell lines for brightfield (BF), DAPI, Oct4::GFP, and Nanog immunostaining.

**Figure S15**



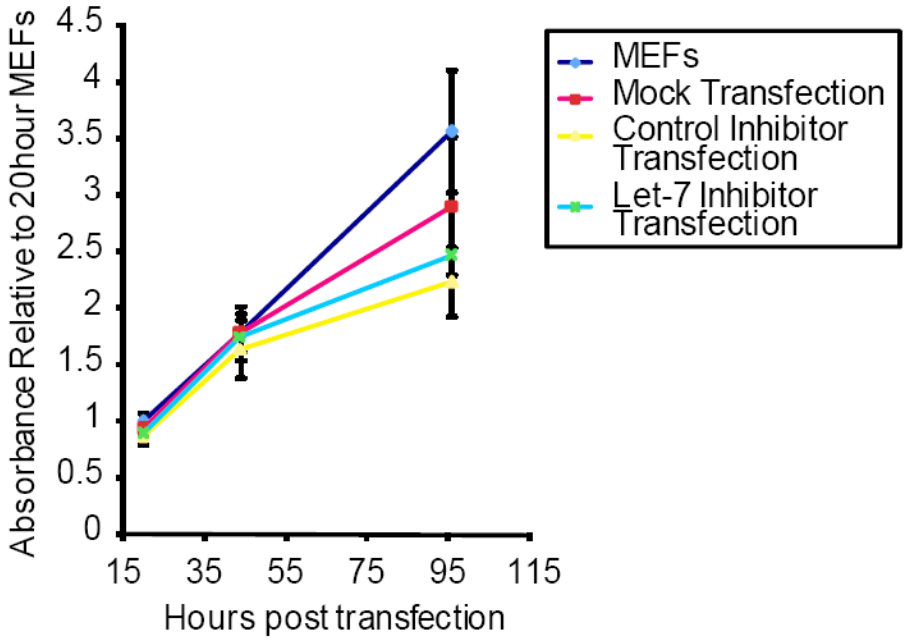

Oct4::GFP positive colonies express ESC-like levels of endogenous Pou5f1/Oct4, Sox2, and Klf4 mRNA. qRT-PCR for endogenous Pou5f1/Oct4, Sox2, and Klf4 in the indicated reprogrammed cell lines. Data are represented as mean +/- standard deviation for n=3. ND = not determined.

**Figure S16**



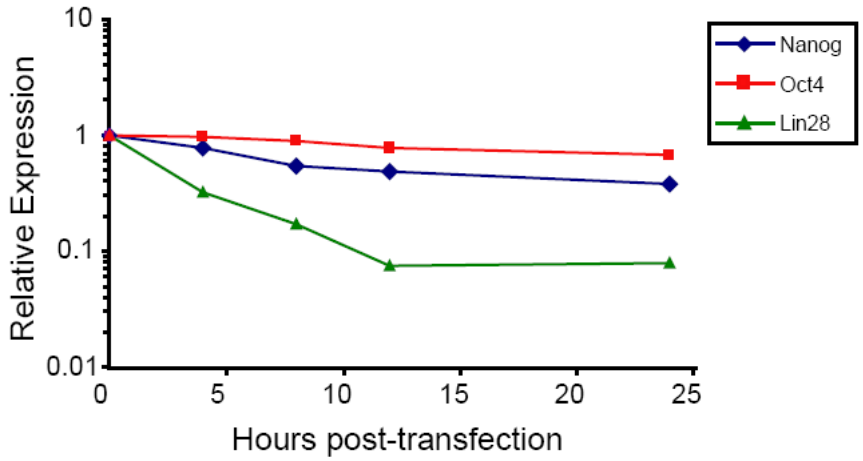

Oct4::GFP positive colonies have silenced exogenously introduced factors. qRT-PCR for exogenous Pou5f1/Oct4, Sox2, and Klf4 in the indicated reprogrammed cell lines. Data are represented as mean +/- standard deviation for n=3.

**Figure S17**




Treatment with the let-7 inhibitor does not increase proliferation in MEFs. MEFs untreated or after mock, control inhibitor, or let-7 inhibitor transfection were subjected to MTT assay as a surrogate marker of proliferation.

**Figure S18**




Let-7c induces maximal degradation of its target Lin28 12 hours after transfection without significant evidence of differentiation (as measured by Nanog and Pou5f1/Oct4 expression). qRT-PCR time course for Pou5f1/Oct4, Nanog, and Lin28 normalized to beta-actin is plotted after transfection of *Dgcr8* -/- ESCs. n = 1.