Wade et al., Supplementary Figures

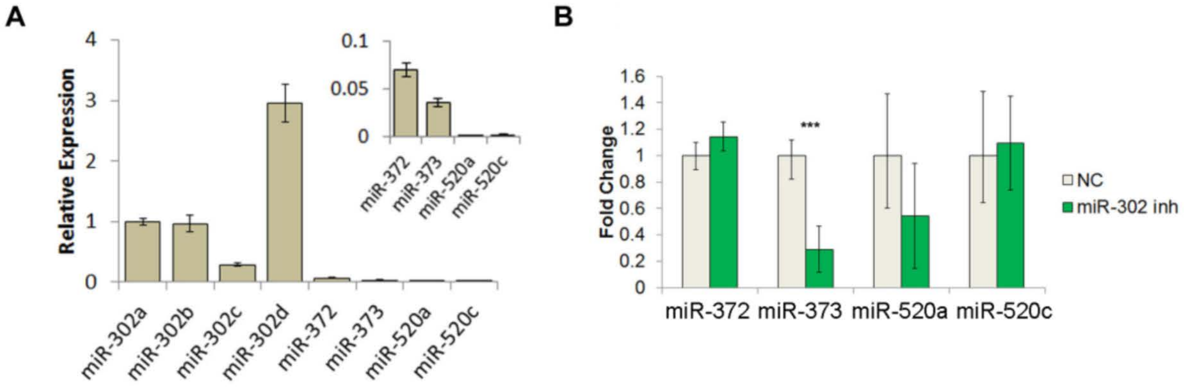

Figure S1: Levels of miR-302-related miRNAs in H1 cells and effect of miR-302 inhibitors.

(A) Relative expression levels of all AAGUGCU seed-containing miRNAs in H1 hESCs compared to miR-302a expression. Inset – adjusted y-axis scale for relatively lowly expressed miRs miR-372, miR- 373, miR-520a and miR-520c.

(B) Levels of other AAGUGCU seed-containing miRNAs in H1 cells treated with miR-302 inhibitors. Error bars represent standard deviation of at least three independent replicates. *** $p < 0.05$

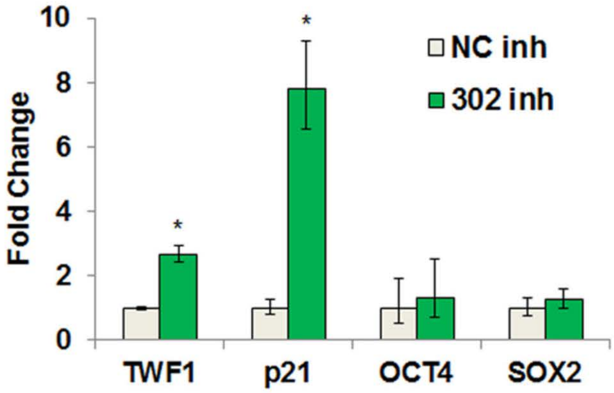

Figure S2: Upregulation of miR-302 targets upon miR-302 inhibition.

mRNA levels of known miR-302 targets, TWF1 and p21, and pluripotency markers, Oct4 and Sox2, in hESCs following inhibition of the miR-302 family compared to negative control. Error bars represent standard deviation of three independent replicates. * $p < 0.005$

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                                 agugguuuuguaccUUCGUGAAu  miR-302a
BAF170                                         ||||||||
WT 3’UTR    GGATGTGAAGTTTTAAAACCTGAAATTGTTTTTTAAAGCACTTAAGCACCTCCATATTATG

BAF170
mutant      GGATGTGAAGTTTTAAAACCTGAAATTGTTTTTTAgctgcaggAAGCACCTCCATATTATG


                            agugguuuuguaccuuCGUGAAu  miR-302a
BAF53a                                  | ||||||
WT 3’UTR    TCTATAAATTAAGTTTGTGCTTTCCTTGAAATGCACTTATTCTTATTACAAGCATTTTATAAT

BAF53a
mutant      TCTATAAATTAAGTTTGTGCTTTCCTTGAAggatcaggATTCTTATTACAAGCATTTTATAAT
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Figure S3: Sequence of 3’UTR fragments from the BAF170 and BAF53a genes cloned into pMIR- Report. The miR-302 seed site complementary region is highlighted in red. This region was changed to eliminate complementarity and miR-302 binding in the mutant constructs (bases in red lower case).

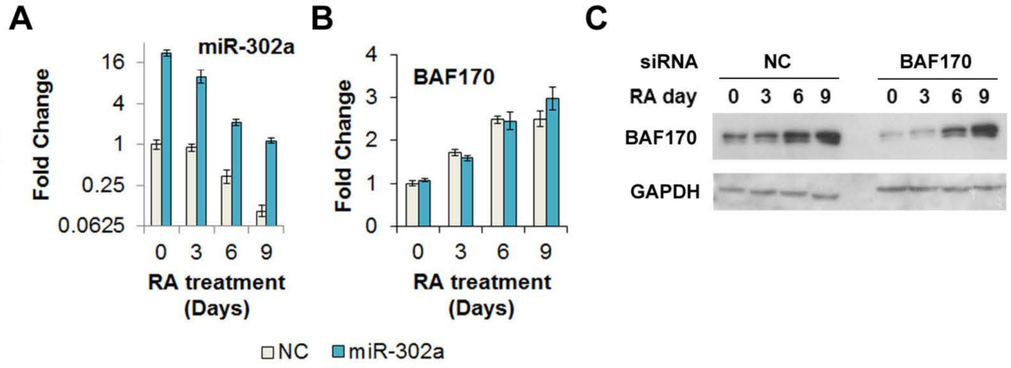

Figure S4: miR-302 overexpression represses BAF170 induction during RA-induced differentiation. (A-B) miR-302a and BAF170 mRNA levels during RA-induced differentiation of H1 cells transfected with miR-302a or negative control miRNA mimic. (C) Western blotting of BAF170 in miR-302a overexpressing H1 cells during RA-induced differentiation.

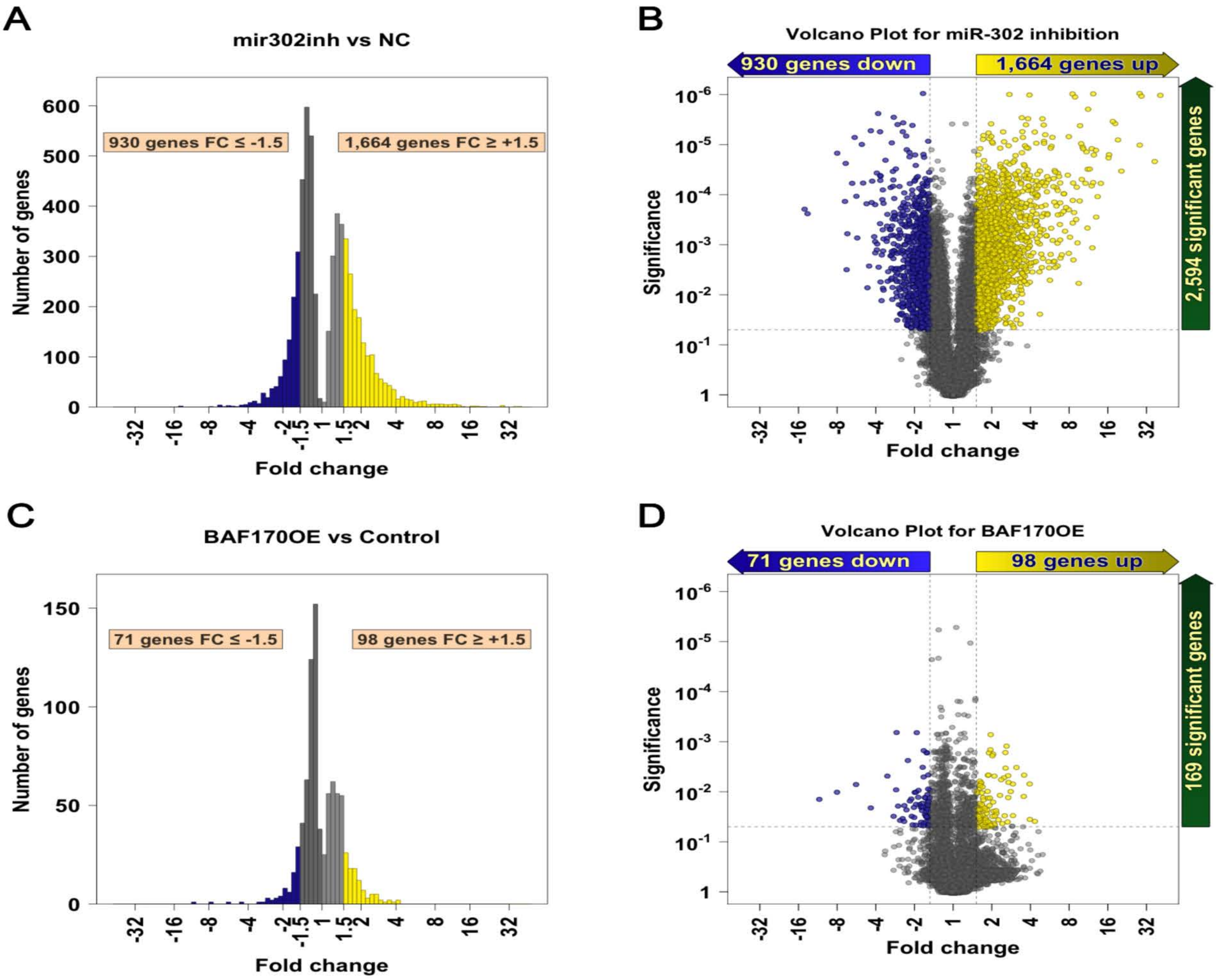

Figure S5: BAF170 overexpression and miR-302 inhibition affect gene expression in hESCs. (A and C) Distribution of significantly affected genes (FDR < 0.05) 48 hours after miR-302 inhibition (A) and in BAF170 overexpressing cell line (C). X-axes are fold change compared to negative controls. Y-axes represent the number of genes. Yellow and blue bars represent the portion of the data with greater than 1.5 fold increase and decrease, respectively. The numbers of genes meeting the statistical significance fold change cut-offs are given at the top of each figure. Gray bars represent significant genes (FDR < 0.05) with a fold change smaller than 1.5.

(C and D) Volcano plots of microarray data from miR-302 inhibition (C) and BAF170 overexpressing cell lines (D) compared with negative controls. Genes significantly upregulated greater than 1.5 fold are highlighted in yellow, and genes significantly downregulated greater than 1.5 fold are highlighted in blue.

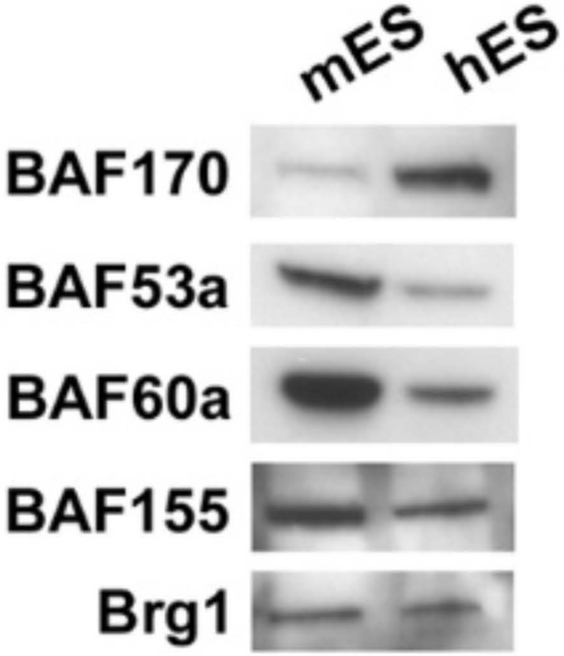

Figure S6: Protein expression of Brg1 complex subunits in H1 hESCs and E14-derived ESOct4GiP mESCs, which were a generous gift from Dr. Guang Hu's lab.