

**Supplemental Figure 1.** *DLKI-DIO3* miRNA/mRNA complementarity. Complementarity between the indicated *DLKI-DIO3* cluster miRNAs and the 3' UTR of *SOX2*, *SOX9*, *HIF1A*, *ZEB1*, *ZEB2*, *STAT3* and *CDH1* with mirSVR and PhastCons scoring.

**Supplemental Figure 2.** Effects of *DLKI-DIO3* miRNA gain and loss-of-function on expression of EMT-associated targets in TGF- $\beta$ 1 treated MCF-10A cells. MCF-10A cells were transfected with the indicated miRNA mimic or antagomir, treated with TGF- $\beta$ 1, and 5 days later expressed levels of the indicated mRNA targets were quantified by RT-PCR. Data are expressed as mean + SEM of three separate experiments. \*,  $p < 0.05$ .

**Supplemental Figure 3.** Effects of *DLKI-DIO3* miRNA gain and loss-of-function on expression of EMT-associated targets in MDA-MB-231 cells. MDA-MB-231 cells were transfected with the indicated miRNA antagomirs and at 5 days post-transfection expressed levels of the indicated mRNA targets were quantified by real-time PCR. Data are expressed as mean + SEM of three separate experiments. \*,  $p < 0.05$ .

**Supplemental Figure 4.** Effects of *DLKI-DIO3* miRNA gain and loss-of-function on expression of EMT-associated targets in MCF-10A cells. MCF-10A cells were transfected with the indicated miRNA antagomirs and at 5 days post-transfection expressed levels of the indicated mRNA targets were quantified by RT-PCR. Data are expressed as mean + SEM of three separate experiments. \*,  $p < 0.05$ .

**Supplemental Figure 5.** *DLKI-DIO3* miRNAs affect changes in E-cadherin expression in the MCF-10A cell line. Phase contrast microscopy, DAPI (blue), and E-cadherin (green) staining of the MCF-10A cell line following 20 days of repeated transfection with the indicated miRNA mimics (Upper) or antagomirs (Lower).

**Supplemental Figure 6.** *DLKI-DIO3* miRNAs fail to upregulate E-cadherin in cells that have already undergone EMT. Phase contrast microscopy, DAPI (blue), and E-cadherin (green) staining of the MDA-MB-231 cell line following 20 days of repeated transfection with the indicated miRNA mimics (Upper) or antagomirs (Lower).

**Supplemental Figure 7.** *DLKI-DIO3* miRNAs are sufficient to block TGF- $\beta$ 1 induced EMT and E-cadherin down regulation in the MCF-10A cell line. Phase contrast microscopy, DAPI (blue), and E-cadherin (green) staining of the TGF- $\beta$ 1 treated MCF-10A cell line following 20 days of repeated transfection with the indicated miRNA mimics (Upper) or antagomirs (Lower).

**Supplemental Table 1.** Microarray analysis. MDA-MB-231 cells transfected with miR-544 or control-miR were analyzed by micro-array and mRNAs that exhibited 2, 5, or 10-fold changes in expression were determined by comparative analysis.

**Supplementary Table 2.** Microarray data. MDA-MB-231 cells transfected with miR-544 or control-miR were analyzed by micro-array and raw values from the array data are provided.

**Supplementary Table 3.** 3' UTR primer sequences. Primer sequences used to amplify and clone the 3'UTR of each indicated gene are listed.

**Supplementary Table 4.** Real time RT-PCR primer sequences. The primer sequences used to quantify expressed levels of each miRNA or mRNA target are listed.

**Supplementary Table 5.** CpG island methylation assay primer sequences. The primer sequences used to determine the methylation status of CpG islands upstream of the *DLKI-DIO3* miRNA cluster are listed.

Figure S1.

3' ucUCUCUCAGACGGGAACAUU 5' hsa-miR-300 : :	mirSVR: -0.3618 PhastCons: 0.5823	3' cuccaaAGGGCACAUAACAAAGu 5' hsa-miR-494     :	mirSVR: -0.9865 PhastCons: 0.7131
300:5' cqGGGGAAU-GGACCUUGUAUA 3' SOX2		722:5' qaqaauUCAUCUG-AUGUUUCu 3' HIF1A	
3' ucucucucagacgggAACAUU 5' hsa-miR-300 	mirSVR: -0.2788 PhastCons: 0.7507	3' uuucucacguggcgUUACAA 5' hsa-miR-543 	mirSVR: -0.1967 PhastCons: 0.7345
941:5' cuuuuuuuuccgugUGUUAU 3' SOX2		928:5' auuugcuaaaauacAAUGUUu 3' HIF1A	
3' gcUUAGGUGUGCUUGUUGAAg 5' hsa-miR-382       : : :     :	mirSVR: -0.2155 PhastCons: 0.7754	3' cuccaaagggcacauACAAGU 5' hsa-miR-494 	mirSVR: -0.1026 PhastCons: 0.7026
452:5' uuAAUUAU-UUGCAAGCAACUU 3' SOX2		1165:5' uuuuuggcuaaaucUGUUUCc 3' ZEB1	
3' gcuuagugugucuuGUUGAAg 5' hsa-miR-382 	mirSVR: -0.6280 PhastCons: 0.8471	3' ugugugUUUCUUAUAAAGAGg 5' hsa-miR-539 :       :	mirSVR: -0.6979 PhastCons: 0.7703
1040:5' uaacagguacuuuuCAACUUa 3' SOX2		872:5' gugugUAAGUCCAAUUUCUc 3' ZEB1	
3' uuucucacguggcgUUACAA 5' hsa-miR-543 	mirSVR: -0.2117 PhastCons: 0.7293	3' uguguguuuccUAUUAAGAGg 5' hsa-miR-539 	mirSVR: -0.9944 PhastCons: 0.6932
431:5' ccaagcgcaaaaaAAUGUUu 3' SOX2		1070:5' gauuguuuuAGAAUUUCUCu 3' ZEB1	
3' ucucucucAGACGGGAACAUU 5' hsa-miR-300       : :	mirSVR: -0.1284 PhastCons: 0.6255	3' uuucucACGGGUACAACAAA 5' hsa-miR-495     :	mirSVR: -0.3155 PhastCons: 0.7710
1409:5' aacuuuuUUUUUUUGUAU 3' SOX9		866:5' auuuucUGUACU-UUUUUUUUc 3' ZEB2	
3' ucucucucAGACGGGAACAUU 5' hsa-miR-300 :     : :	mirSVR: -0.4809 PhastCons: 0.6517	3' uuucUCACGGGUACAACAAA 5' hsa-miR-495     : :	mirSVR: -0.6735 PhastCons: 0.8506
1973:5' uuucuuuuUUUUUUUGUAU 3' SOX9		1166:5' uuuuAUUUUUUUUUUUUUU 3' ZEB2	
3' cuccAAAGGGCACAUAACAAg 5' hsa-miR-494     :	mirSVR: -0.3831 PhastCons: 0.5542	3' uuucucacGUGGUACAACAAA 5' hsa-miR-495     :	mirSVR: -0.4304 PhastCons: 0.5973
961:5' aaucUGUUCUGGGAAUGUUUc 3' SOX9		1316:5' agguccuCAUCCUGUUUGUU 3' STAT3	
3' uuucucacGUGGUACAACAAA 5' hsa-miR-495     :	mirSVR: -0.7565 PhastCons: 0.6139	3' uuucucacCGUGGUACAACAAA 5' hsa-miR-495       : :	mirSVR: -0.6347 PhastCons: 0.5559
1886:5' uguguucGUGUUUGUUUGUU 3' SOX9		1783:5' uuucGUGAAUUCUGUUUGUU 3' STAT3	
3' ugUGUGGUUCCUAUUAAGAGg 5' hsa-miR-539 : : :	mirSVR: -0.2711 PhastCons: 0.5304	3' cuugaacGAUUUUUACGUCUU 5' hsa-miR-544   :     :	mirSVR: -0.4626 PhastCons: 0.7336
1559:5' auGUGUCAUCCAU-AUUUCUCu 3' SOX9		5:5' cugagaaCGGAAGCUGCAGAAA 3' STAT3	
3' uguguguuuccuuuAAAGAGg 5' hsa-miR-539 	mirSVR: -0.2059 PhastCons: 0.6328	3' uuucucacgugguacAAACAAA 5' hsa-miR-495 	mirSVR: -0.3982 PhastCons: 0.6466
1957:5' accuuuccuuuuuuUUUCUCu 3' SOX9		2015:5' auuuuuuuuuuuuuUUUGUU 3' CDH1	
		3' gcUUAGGUGUGCUUGUUGAAg 5' hsa-miR-382 	mirSVR: -0.5225 PhastCons: 0.5342
		271:5' auAUUCCA-GAAGAACAACUU 3' CDH1	

Figure S2.

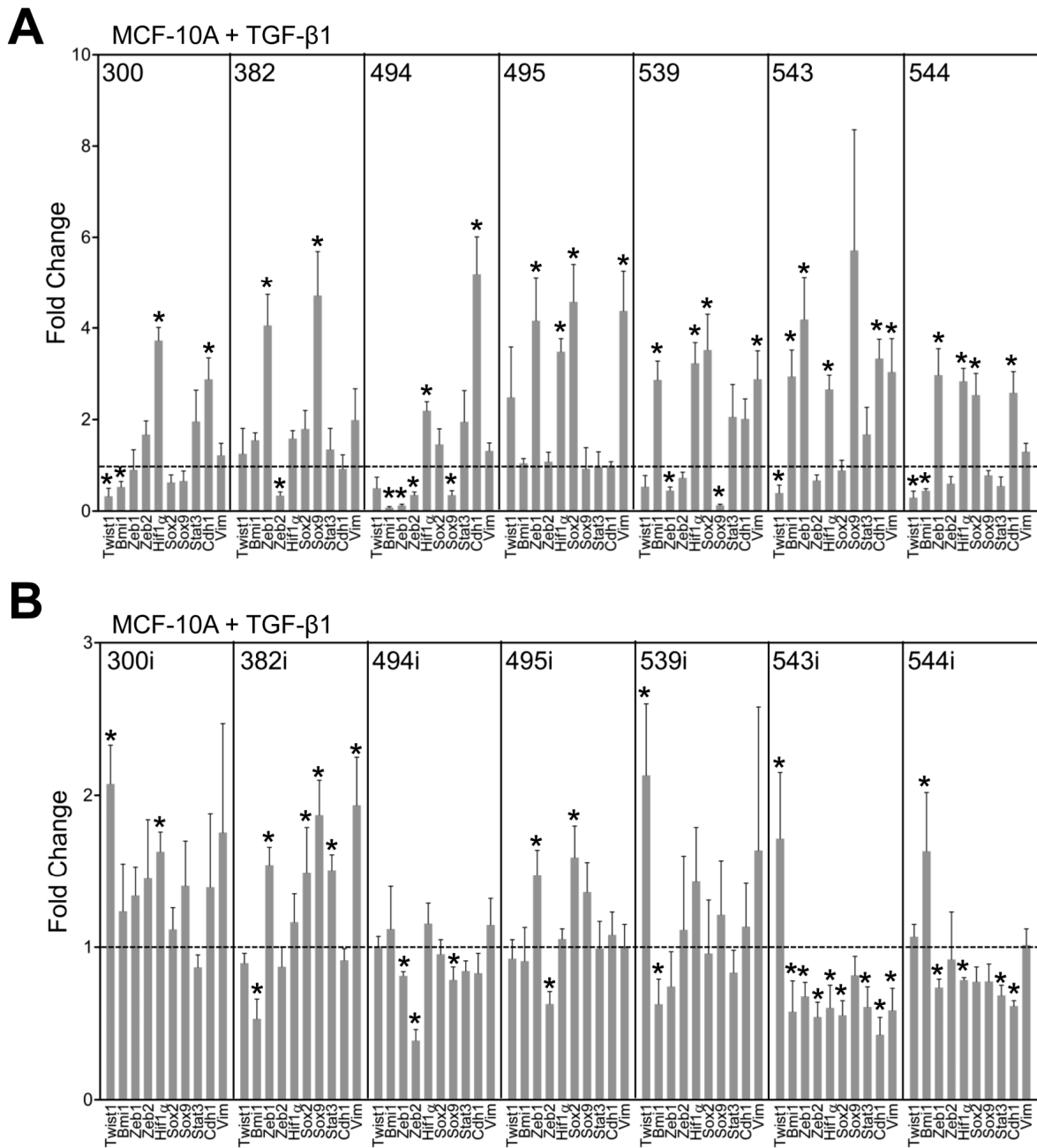




Figure S4.

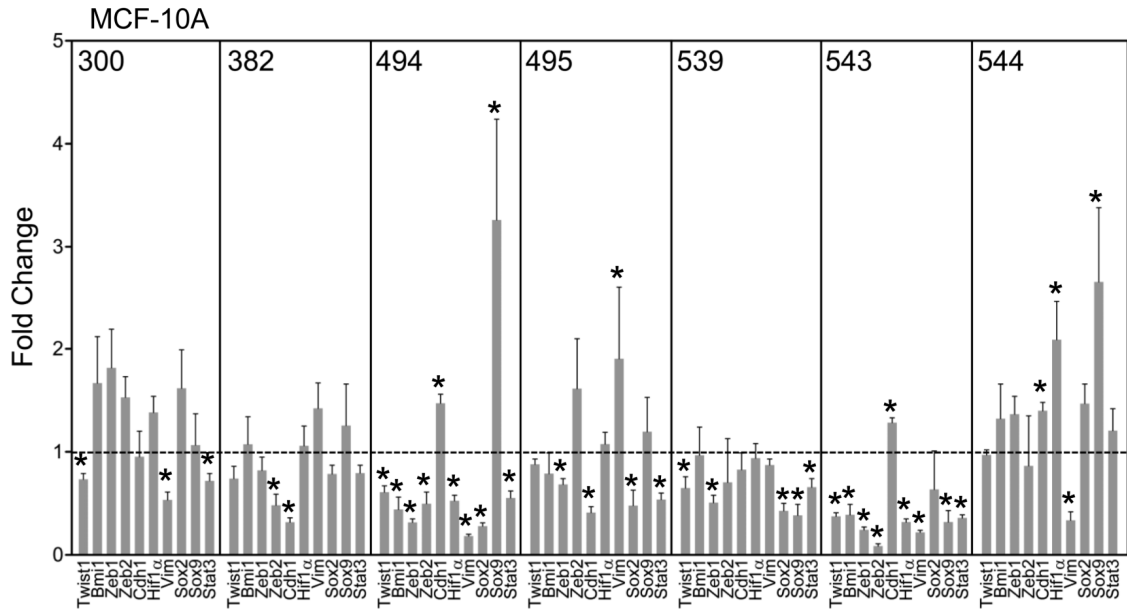


Figure S5.

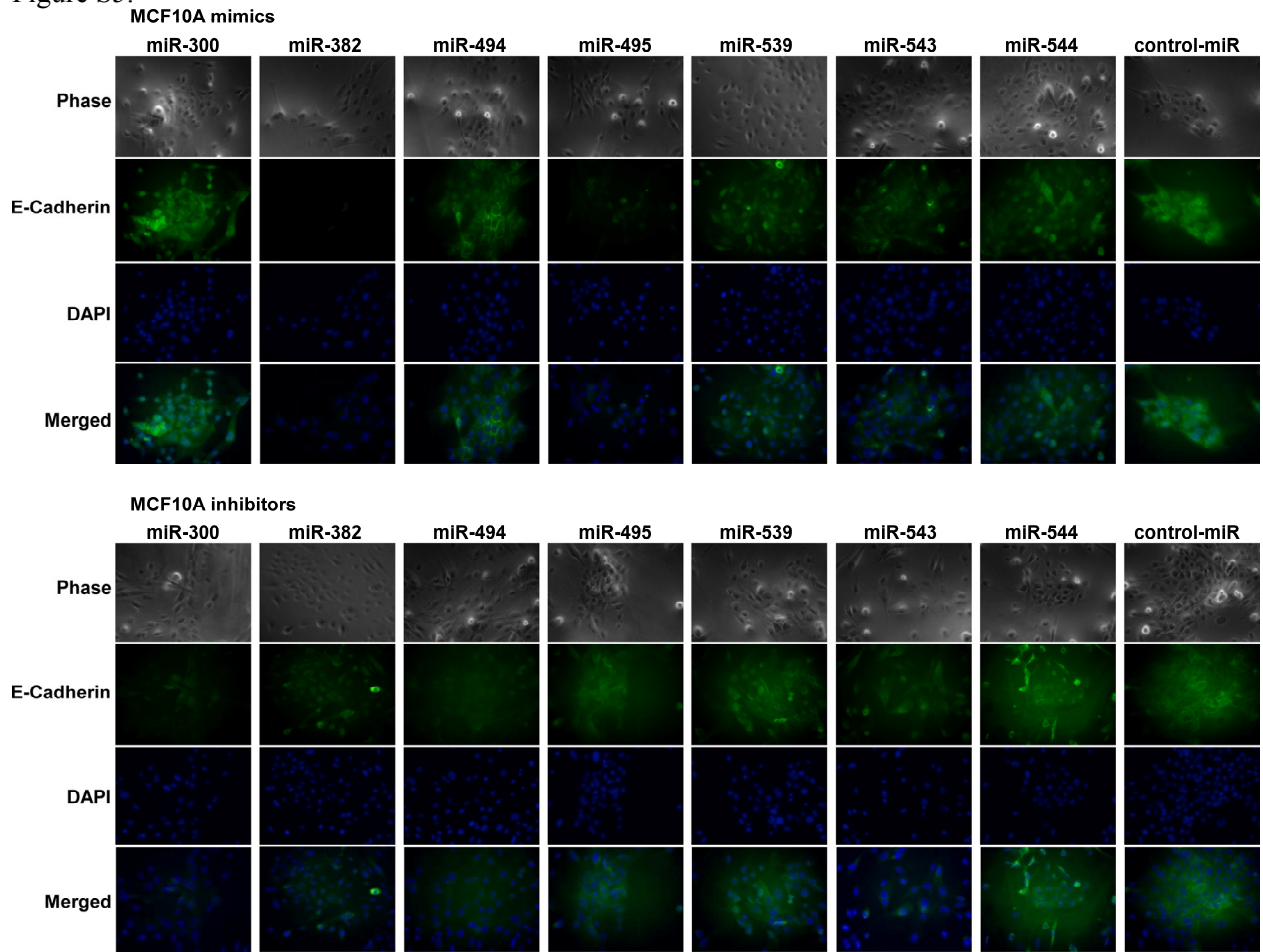


Figure S6.

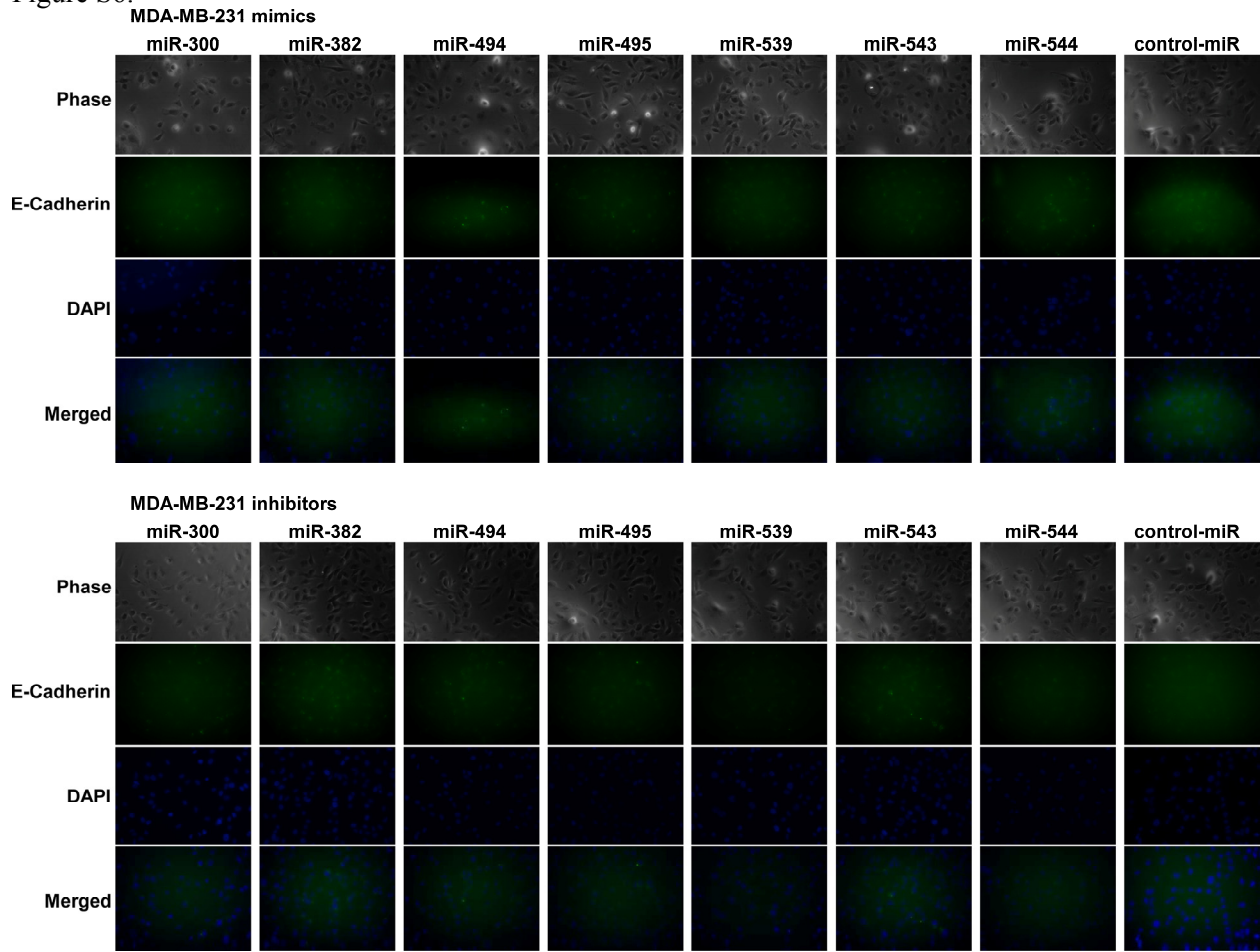
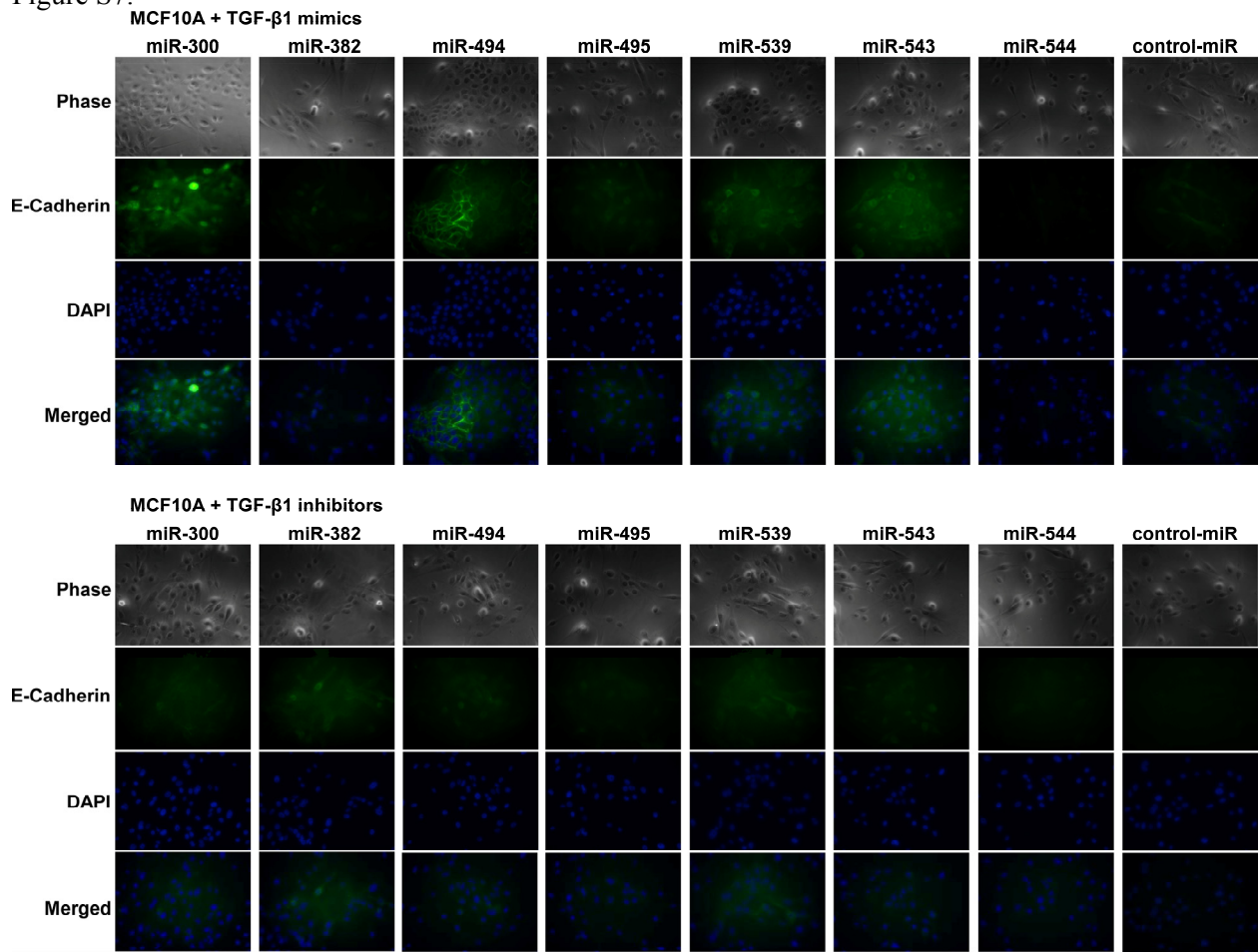


Figure S7.





**Supplemental Table 3. 3' UTR primer sequences.**

<b>Gene</b>	<b>Forward</b>	<b>Reverse</b>
MYC	5' CAA ATG CAA CCT CAC AAC CTT GGC 3'	5' TGG CTC AAT GAT ATA TTT GCC AGT 3'
FOXO1	5' TGC CCA ATG TGT GCA GGT TAT GTG 3'	5' TAA TGG CAC GGG AGG AAA GTG ACA 3'
SOX9	5' TGA CCT ATC CAA GCG CAT TAC CCA 3'	5' GGG AAA GGT AAG TTT CAC GGA GAG 3'
OSTERIX	5' TCA CCA TGA GAC TGG CTT TCC ACA 3'	5' ACT TGA GGT TTC ACA GCT TCT GGC 3'
SOX2	5' TCA CCA TGA GAC TGG CTT TCC ACA 3'	5' ACT TGA GGT TTC ACA GCT TCT GGC 3'
TAZ1	5' ATA GAA GGC AGG TGA GCA ACC AGT 3'	5' CAG GCC CTA GGA TTC AAG TGA TGT 3'
TWIST2	5' CCG AGC CTC TGC ATG ATT GTT TCA 3'	5' ATG GCT GCG CGG ACG TCT TTA TTT 3'
SIX1	5' CGC CTG TTT CAG CAG CCT TTC TTT 3'	5' TTT CTA GAC ACC ATC CGG CAG CA 3'
ID2	5' GCC CTT TCT GCA GTT GGA AGG TTT 3'	5' CTG GTC CTC TGA AAT AAA GCA GGC 3'
TWIST1	5' ACC ATC CTC ACA CCT CTG CAT TCT 3'	5' CTT GTG CCT GTC AGT AGC TGC TTT 3'
HIF1 $\alpha$	5' GTA CGT GGT AGC CAC AAT TGC ACA 3'	5' CCA ACA GGG TAG GCA GAA CAT TTA GG 3'
CDH1	5' CAC TCT TTA CAT GGT GGT GAT GTC C 3'	5' ACC TAG TCA AGA TGT GGC CAG ACA 3'
BMI1	5' TGG ACT ACA TGT GAT ACT CCT ATG 3'	5' GCA TGT ACT TCT TAG CCA CAT CAC 3'
ZEB1	5' AAT AAA TCC GGG TGT GCC TGA ACC 3'	5' TTG TGA GAT GGG AGT CTG GT 3'
ZEB2	5' CGA AGT CTT AAA GTG ACC TGG ACG 3'	5' ACA TTG TAT TTA ACA GTC CCT CTT T 3'
OCLN	5' CTG GGC TGA ACA CTC CAA TTA AGG 3'	5' AGG GAG GCT GGT AGA TCA TCA CAT 3'
STAT3	5' ACA GCT GAG AAC GGA AGC TGC AGA 3'	5' TGA TCA GCT GAG GCA AGG TGG TTT 3'

Supplementary Table 4. Real time RT-PCR primer sequences.

Gene	Forward	Reverse
SOX9	5' GTA CCC GCA CTT GCA CAA C 3'	5' GTG GTC CTT CTT GTG CTG C 3'
ATM	5' ATC TGC TGC CGT CAA CTA GAA 3'	5' GAT CTC GAA TCA GGC GCT TAA A 3'
SOX2	5' AAC CAA GAC GCT CAT GAA GAA G 3'	5' CTGCGAGTAGGACATGCTGTAG 3'
TWIST1	5' GTC CGC GTC CCA CTA GC 3'	5' TCC ATT TTC TCC TTC TCT GGA A 3'
HIF1 $\alpha$	5' CGC GAA CGA CAA GAA AAA G 3'	5' AAG TGG CAA CTG ATG AGC AA 3'
CDH1	5' TTG ACG CCG AGA GCT ACA C 3'	5' GAC CGG TGC AAT CTT CAA A 3'
BMI1	5' CCA CCT GAT GTG TGT GCT TTG 3'	5' TTC AGT AGT GGT CTG GTC TTG T 3'
ZEB1	5' TGT TAC CAG GGA GGA GCA GT 3'	5' GCT TCA TCT GCC TGA GCT TC 3'
ZEB2	5' CGA TCC AGA CCG CAA TTA AC 3'	5' TGC TGA CTG CAT GAC CAT C 3'
STAT3	5' CCC TTG GAT TGA GAG TCA AGA 3'	5' AAG CGG CTA TAC TGC TGG TC 3'
VIM	5' AGG AAA TGG CTC GTC ACC TTC GTG 3'	5' GGA GTG TCG GTT GTT AAG AAC TAG 3'
miR-200a	5' TAA CAC TGT CTG GTA ACG ATG T 3'	Qiagen Universal Reverse
miR-200b	5' TAA TAC TGC CTG GTA ATG ATG A 3'	Qiagen Universal Reverse
miR-200c	5' TAA TAC TGC CGG GTA ATG ATG GA 3'	Qiagen Universal Reverse
miR-429	5' TAA TAC TGT CTG GTA AAA CCG T 3'	Qiagen Universal Reverse
miR-141	5' TAA CAC TGT CTG GTA AAG ATG G 3'	Qiagen Universal Reverse
miR-300	5' TAT ACA AGG GCA GAC TCT CT 3'	Qiagen Universal Reverse
miR-382	5' GAA GTT GTT CGT GGT GGA TT 3'	Qiagen Universal Reverse
miR-494	5' TGA AAC ATA CAC GGG AAA CC 3'	Qiagen Universal Reverse
miR-495	5' AAA CAA ACA TGG TGC ACT TC 3'	Qiagen Universal Reverse
miR-539	5' GGA GAA ATT ATC CTT GGT GT 3'	Qiagen Universal Reverse
miR-543	5' AAA CAT TCG CGG TGC ACT TC 3'	Qiagen Universal Reverse
miR-544	5' ATT CTG CAT TTT TAG CAA GTT C 3'	Qiagen Universal Reverse
GAPDH	5' TGT GTC CGT CGT GGA TCT GA 3'	5' CCT GCT TCA CCA CCT TCT TGA 3'
RNU6b	Qiagen RNU6b Forward	Qiagen Universal Reverse

**Supplementary Table 5. CpG island methylation assay primer sequences.**

<b>CpG Island</b>	<b>Forward</b>	<b>Reverse</b>
CpG 1	5' GTT TAG GGT AGG GAG TTA GAG G 3'	5' CCT CCT ACC TAC TAA CCC TCT C 3'
CpG 2	5' ATT TTA GGG GAA GGG ATT T 3'	5' TAA AAT CCA AAC ATA CAC TAT CC 3'
CpG 3	5' AGA AGG AAA AGA ATT TGT TTA TGG 3'	5' ATC CCC AAT ATA ATT TCC TCA A 3'
CpG 4	5' AGA GTG AAT GGG TTT TTG AAA T 3'	5' TCC CTT CCT AAA AAC TCC TTA A 3'
CpG 5	5' TTG TTA TAG AAT TTG GGG GGT 3'	5' ACT TCC CCC CAA AAA AAT ATA C 3'
CpG 6	5' TTG GTT TTT TTT TGT ATT TTG G 3'	5' CCA CTC TCC CTA CTA CCT AAA AA 3'
CpG 7	5' GTT TTT GGA GAG TGT GAG GTT T 3'	5' TCC CTT CAA ATA CCA AAA AAA A 3'