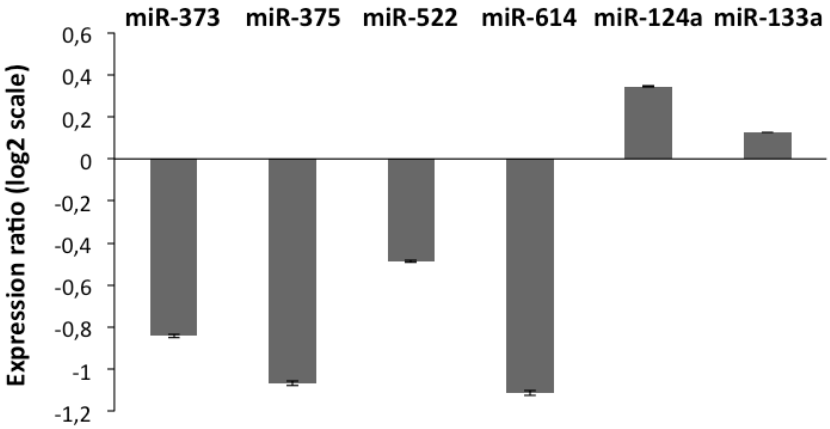
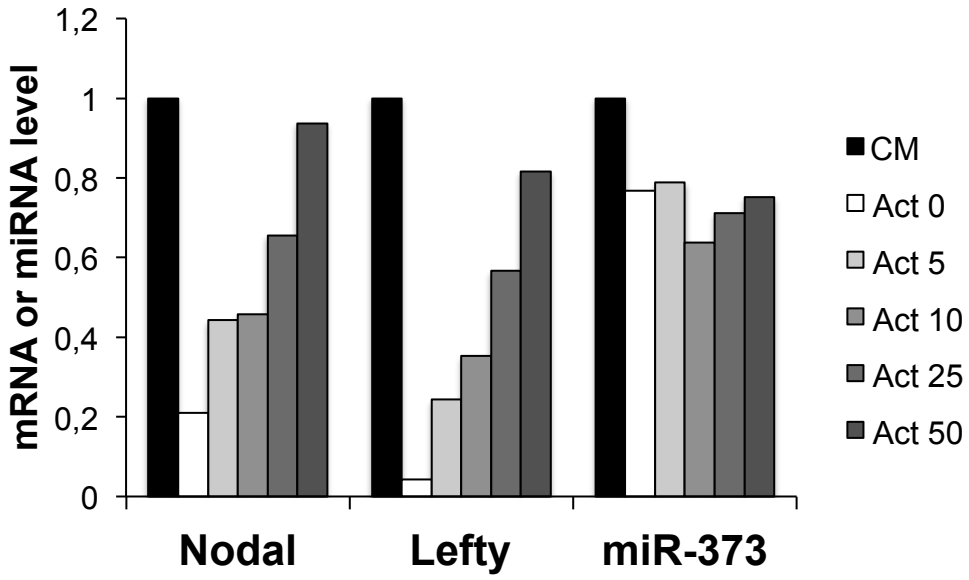
Rosa et al., 2013

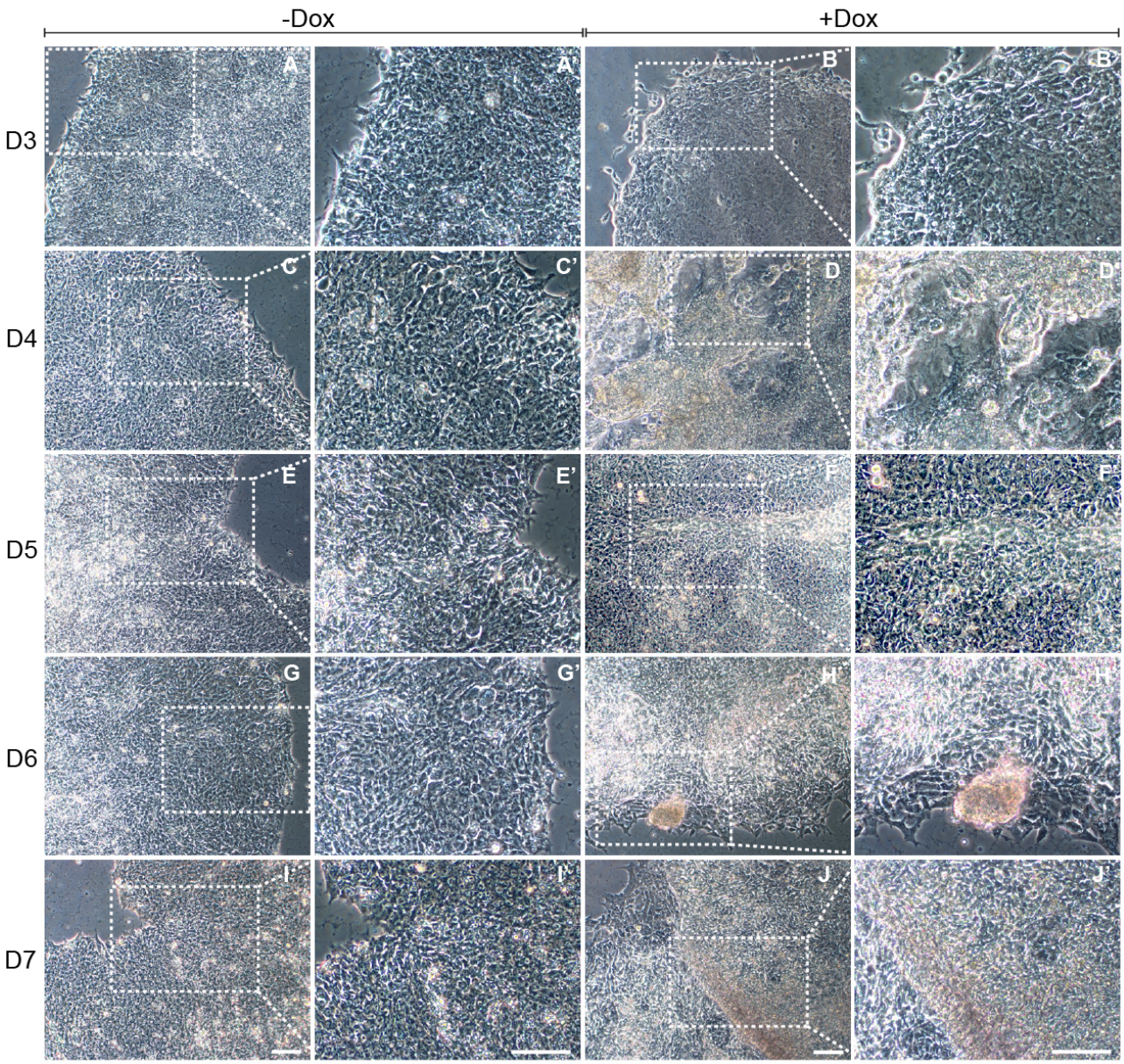
Supplementary figures and tables



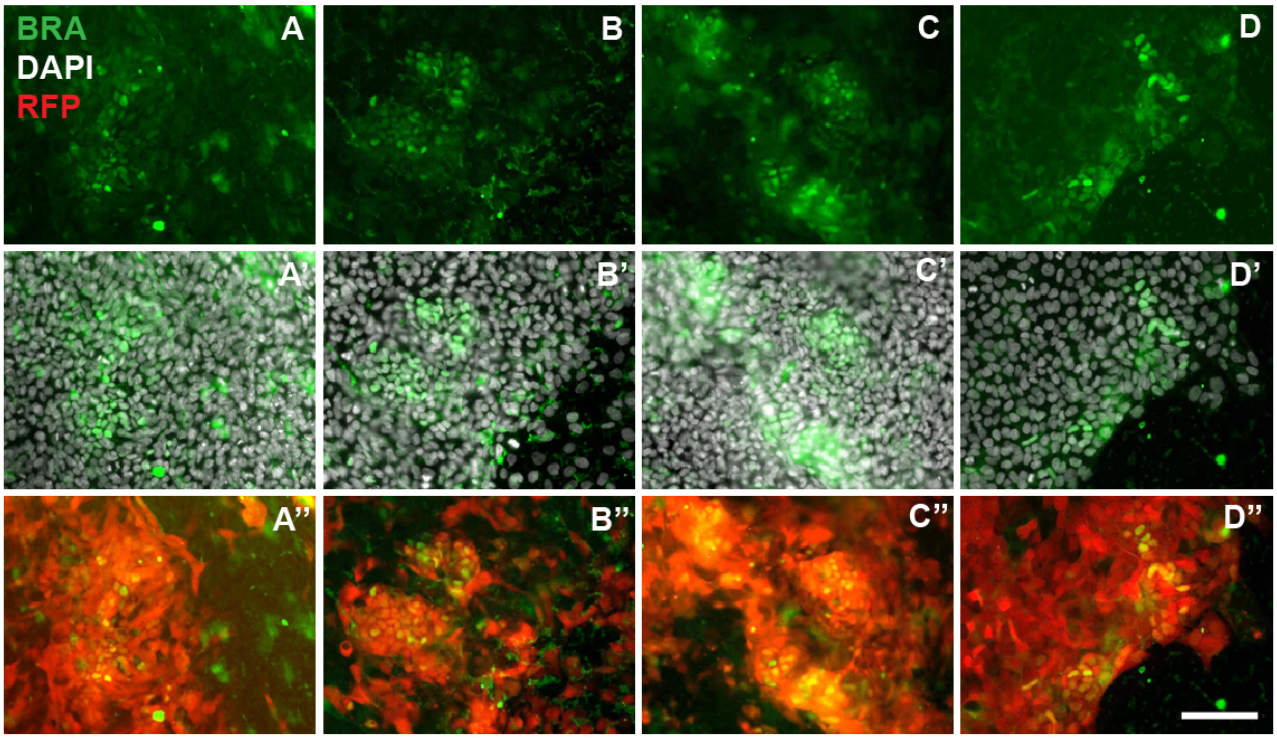
Supplementary Figure 1. Real-time qPCR confirms the Illumina microarray results. qPCR was performed on SB- or DMSO-treated RUES2 cells for 24 hours in order to validate the results obtained through Illumina analysis. Data are presented as a fold-change of expression compared to DMSO-treated cells, on a log2 scale.



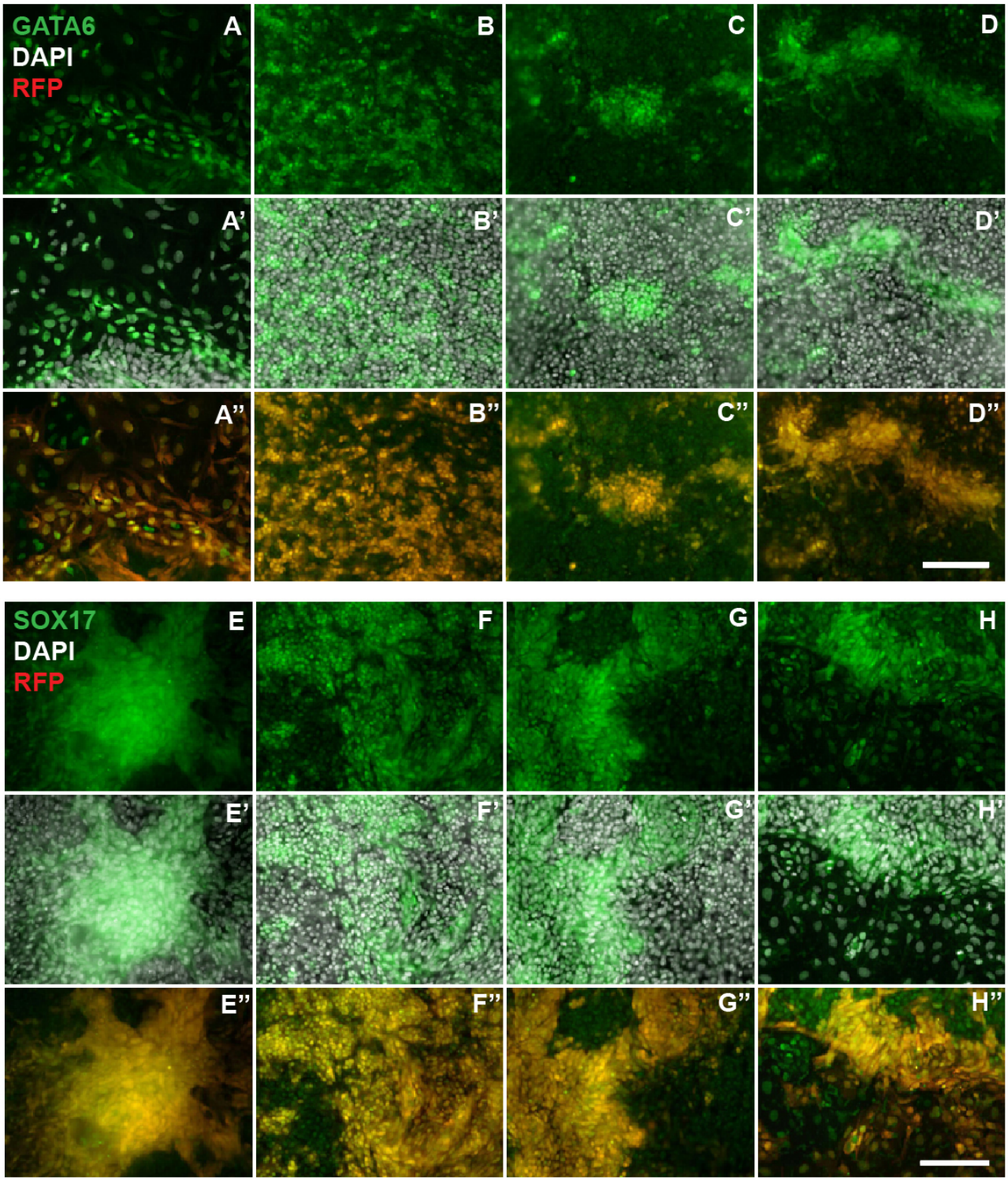
Supplementary Figure 2. Real-time qPCR analysis of the levels of Nodal and Lefty mRNAs and miR-373 upon induction with recombinant ActivinA in non MEF-Conditioned hESC Medium (nCM). RUES2 cells were maintained in MEF-Conditioned Medium (CM, black bars) or switched to nCM for 24 hours in the absence (white bars) or presence (grey bars) of increasing amounts of ActivinA (5-10-25-50 ng/ml). While Nodal/Activin signaling is necessary and sufficient for proper Nodal and Lefty expression, it is necessary (see Figure 1) but not sufficient for miR-373 expression, as other components of the CM may be required for miR-373 activation.



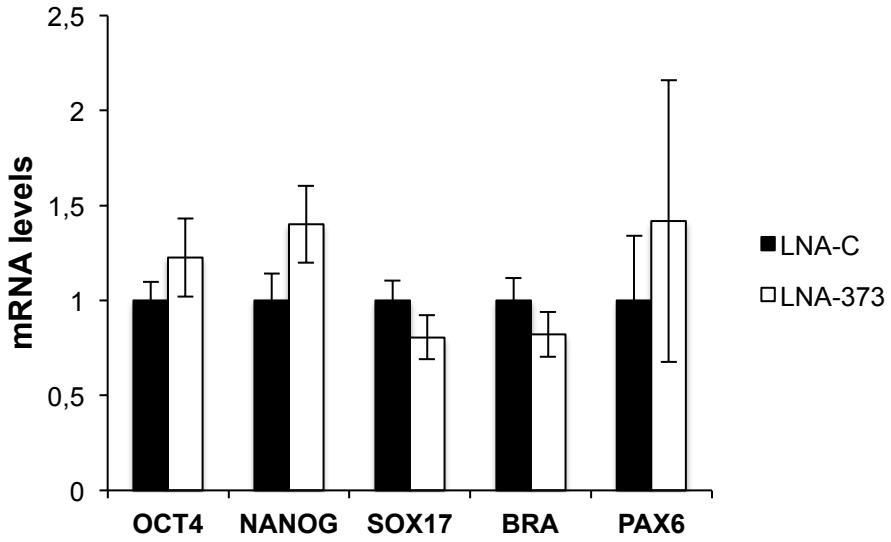
Supplementary Figure 3. Upon miR-373 overexpression, hESCs differentiate morphologically as early as day 3. Shown here are representative images of TT-miR-373 cells that were induced (+Dox) or not (-Dox) over a period of seven days. Areas highlighted with white dashed boxes in the first and third columns are shown in higher magnification in their adjacent images. Scale bars: 100μm.



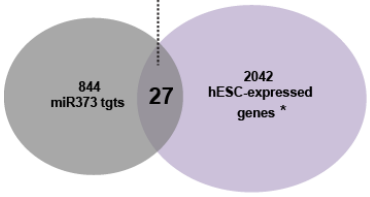
Supplementary Figure 4. Numerous BRA-positive cells appeared dispersed within miR-373-overexpressing colonies. Shown here are representative images of areas harboring BRA+ cells in TT-miR-373 induced colonies. BRA+ cells were mostly found dispersed within a colony, either in (A, C) or close to the borders (B, D) of a colony. DAPI was used for nuclear staining; reporter RFP expression was also visualized. Scale bars: 100μm.



Supplementary Figure 5. GATA6 and SOX17-positive cells appear in clusters upon miR-373 overexpression. Shown here are representative images of GATA6 (A-D'') or SOX17 (E-F'') positive cells appearing within TT-miR-373-induced colonies after seven days of doxycycline treatment. GATA6+ and SOX17+ cells appeared in clusters, mostly within the center of colonies (B, C, D for GATA 6, E, F, G for SOX17), but also close to their edges (A for GATA6, H for SOX17). DAPI was used for nuclear staining; reporter RFP expression was also visualized. Scale bars: 100μm.



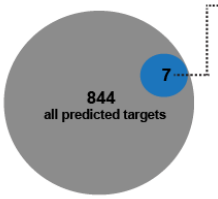
Supplementary Figure 6. Real-time qPCR analysis of the levels of the indicated mRNAs in RUES2 cells transfected with control (LNA-C, black bars) or miR-373 antisense LNAs (LNA-373, white bars), both purchased from Exiqon. RUES2 cells, maintained in MEF-Conditioned Medium, were transfected with Lipofectamine 2000 with 75nM LNA on day 0 and again on day 2 and collected for RNA extraction at day 4. Data points represent average from technical triplicates.



** Ismailoglu and Brivanlou, 2013, unpublished data*

miR-373 targets expressed in hESCs:

MTCH2	NM_014342	mitochondrial carrier 2
LEFTY1	NM_020997	left-right determination factor 1
TARDBP	NM_007375	TAR DNA binding protein
MYCN	NM_005378	v-myc myelocytomatosis viral related oncogene, neuroblastoma derived (avian)
PFN2	NM_002628	profilin 2
DNAJA2	NM_005880	DnaJ (Hsp40) homolog, subfamily A, member 2
KPNA2	NM_002266	karyopherin alpha 2 (RAG cohort 1, importin alpha 1)
DNAJC9	NM_015190	DnaJ (Hsp40) homolog, subfamily C, member 9
IPO7	NM_006391	importin 7
PPP3R1	NM_000945	protein phosphatase 3, regulatory subunit B, alpha
ECT2	NM_018098	epithelial cell transforming sequence 2 oncogene
TCF7L1	NM_031283	transcription factor 7-like 1 (T-cell specific, HMG-box)
POLR3G	NM_006467	polymerase (RNA) III (DNA directed) polypeptide G (32kD)
METAP1	NM_015143	methionyl aminopeptidase 1
CHAF1A	NM_005483	chromatin assembly factor 1, subunit A (p150)
SNX5	NM_014426	sorting nexin 5
APP	NM_000484	amyloid beta (A4) precursor protein
RBM25	NM_021239	RNA binding motif protein 25
TMEM167	NM_174909	transmembrane protein 167A
RAB5C	NM_004583	RAB5C, member RAS oncogene family
MCM3	NM_002388	minichromosome maintenance complex component 3
C19orf43	NM_024038	chromosome 19 open reading frame 43
HK1	NM_000188	hexokinase 1
SLC38A1	NM_001077484	solute carrier family 38, member 1
EIF5	NM_001969	eukaryotic translation initiation factor 5
EIF4B	NM_001417	eukaryotic translation initiation factor 4B
UBFD1	NM_019116	ubiquitin family domain containing 1
LEFTY2	NM_001172425	left-right determination factor 2

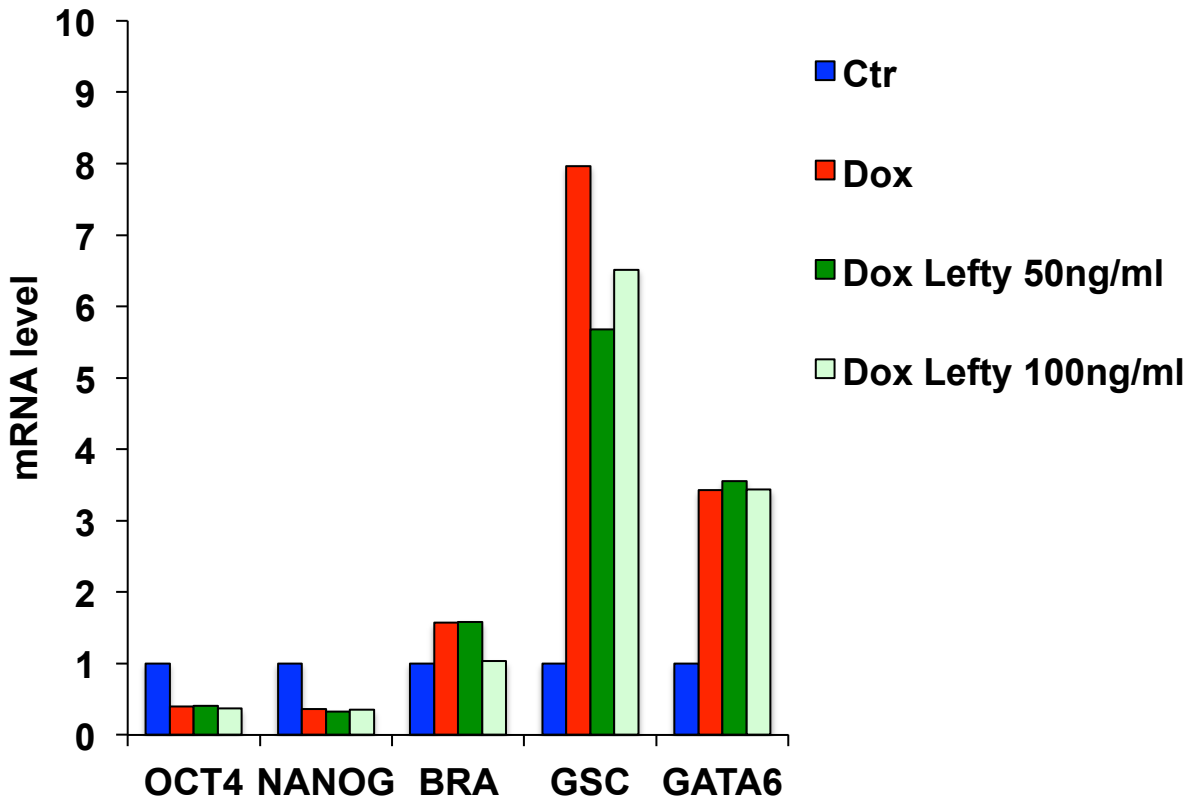


miR-373 targets that are members of TGF-β signaling pathways:

Gene Symbol	RefSeq mRNA	Gene Full Name
TGFBR2	NM_001024847	transforming growth factor, beta receptor II (70/80kDa)
LEFTY1	NM_020997	left-right determination factor 1
LEFTY2	NM_001172425	left-right determination factor 2
ACVR1C	NM_001111031	activin A receptor, type IC
SMAD2	NM_001003652	SMAD family member 2
INHBB	NM_002193	inhibin, beta B
BMPR2	NM_001204	bone morphogenetic protein receptor, type II (serine/threonine kinase)

miR-373 targets expressed in hESCs and TGF-β members:
Lefty1, Lefty2

Supplementary figure 7. Bioinformatics analysis using the TargetScan software (Lewis et al, 2005) predicts 844 targets for miR-373 in humans. The top panel shows the overlap of this set of genes with genes that are expressed in undifferentiated hESCs (Ismailoglu and Brivanlou, unpublished data); 27 genes are in common. The middle panel shows that 7 genes, among the predicted miR-373 targets, encode for factors involved in the TGF-β signaling pathway. Two of them, *Lefty1* and *Lefty2*, are also expressed in hESCs.



Supplementary figure 8. Real-time qPCR of the mRNA levels of the indicated marker genes in TT-miR-373 cells that were induced for 5 days with doxycycline in absence (Dox) or in presence of recombinant LeftyA (R&D, rhLEFTY-A cat#746-LF) at the indicated concentrations. Ctr: uninduced TT-miR-373 cells. BRA: Brachyury. GSC: Goosecoid.

Probe	SB_6h	DMSO_6h	SB_24h	DMSO_24h	p.val_6h	p.val_24h	MATURE_MIRNA_SEQ
hsa-miR-124a	7.84	7.14	8.66	7.3	0.0449	0.0011	TTAAGGCACGCGGTGAATGCCA
hsa-miR-133a	9.49	8.92	9.8	8.98	0.0185	0.0024	TTGGTCCCCTTCAACCAGCTGT
hsa-miR-582	8.8	8.5	9.02	8.42	0.048	0.0009	TTACAGTTGTTCAACCAGTTACT
hsa-miR-371	11.6	11.97	11.19	12.13	0.0754	0.0005	GTGCCGCCATCTTTTGAGTGT
hsa-miR-373	12.11	12.08	11.68	12.34	0.8009	0.0003	GAAGTGCTTCGATTTTGGGGTGT
hsa-miR-373*	7.89	8.2	7.55	8.6	0.2123	0.0009	ACTCAAAATGGGGGCGCTTTCC
hsa-miR-375	10.88	11.36	10.49	11.5	0.0228	0.0002	TTTGTTCGTTCGGCTCGCGTGA
hsa-miR-522	10.48	10.97	10.5	11.12	0.0169	0.0047	AAAATGGTTCCCTTTAGAGTGTT
hsa-miR-614	7.64	7.58	7.15	7.76	0.7345	0.0064	GAACGCCTGTTCTTGCCAGGTGG

Supplementary Table 1.
Shown here are the nine miRNAs whose expression was significantly up- or down- regulated in RUES2 cells after 24 hours of SB treatment. The raw data values for miRNA expression in SB and control-DMSO samples at both 6 and 24 hours of treatment are given here, along with the p value (p.val) of the DMSO/SB ratio, and the mature miRNA sequence.

Gene	F Primer (5'-3')	R Primer (5'-3')
Nodal	AGACATCATCCGCAGCCTACA	GACCTGGGACAAAGTGACAGTGAA
Lefty2	ACCTCAGGGACTATGGAGCTCAGG	AGAAATGGCCAATTGAAGGCCAGG
Lefty1	TGCTACAGGTGTCGGTGCAGAGG	AGAAACGGCCACTTGAAGGCCAGG
hOct4	CAAGCTCCTGAAGCAGAAGAGGAT	CTCACTCGGTTCTCGATACTGGTT
hNanog	CCGGTCAAGAAACAGAAGACCAGA	CCATTGCTATTCTTCGGCCAGTTG
hSox17	GGCGCAGCAGAATCCAGA	CCACGACTTGCCCAGCAT
hGata6	TTTCCGGCAGAGCAGTAAGAGG	CCGTCAGTCAAGGCCATCCA
hBra	ATGACAATTGGTCCAGCCTT	CGTTGCTCACAGACCACA
hGoosecoid	GAGGAGAAAGTGGAGGTCTGGTT	CTCTGATGAGGACCGCTTCTG
hMixl1	GGTACCCCGACATCCACTT	GCCTGTTCTGGAACCATACCT
hSox1	CACAACTCGGAGATCAGCAA	GTCCTTCTTGAGCAGCGTCT
hPax6	TCACCATGGCAAATAACCTG	CAGCATGCAGGAGTATGAGG
hAtp5o	ACTCGGGTTTGACCTACAGC	GGTACTGAAGCATCGCACCT
miR-373 SBE1	ACCTTACCAGCCCACTCTTA	CCAAGGCCTCCTACATCAAAG
miR-373 SBE2	GGTTTCAGGGTGAGGATTCA	ATCAGAAGTTCTTCCTGCTGAG
ChIP Ctr	TTCTGATTCTTAAAGGAGTGAC	TTCCTAACATCCACAAGATAAC

Supplementary Table 2
Primers for Real-Time qPCR analysis