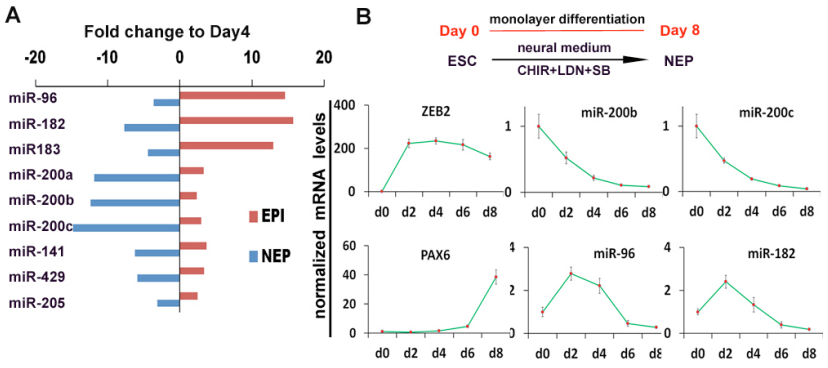

Fig. S1. The expression pattern of miR-200 and miR-96 families during neural induction from hESCs. (**A**) The differential expression of nine members of miR-200 and miR-96 families in day 10 NEP and EPI cells differentiated from hESC line H1. (**B**) The expression of ZEB2/miR-200 and PAX6/miR-96 was quantified by qPCR during hESC differentiation using the dual Nodal/BMP inhibition method. The relative gene expression levels were normalized to day 0 hESCs.

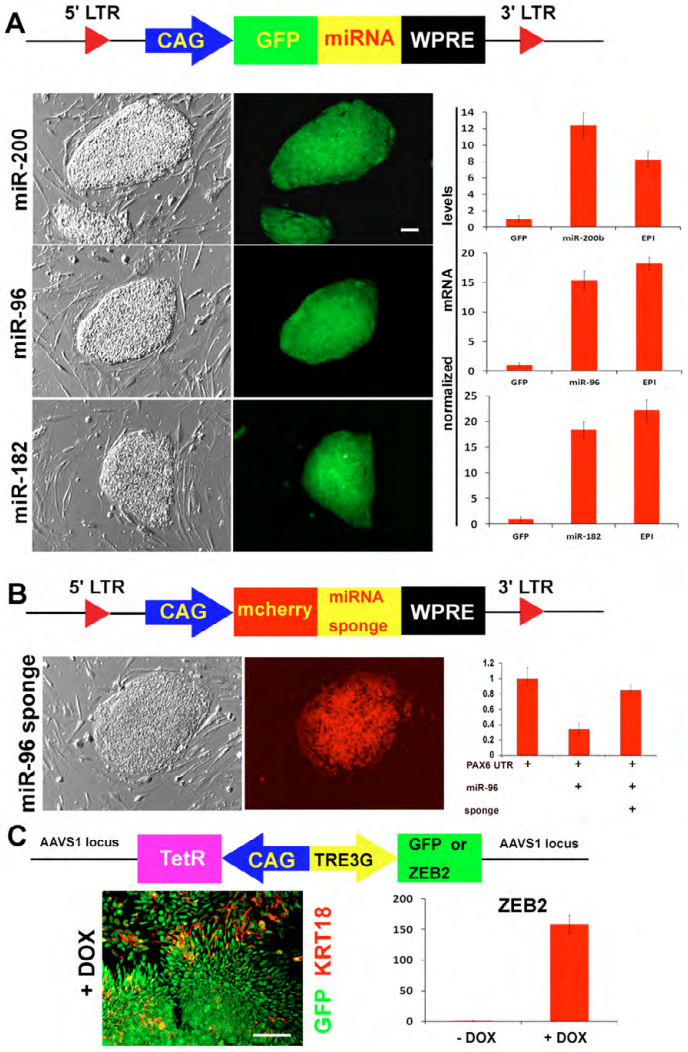

Fig. S2. Establishment of miRNA overexpressing and miRNA sponge hESC lines. (**A**) The structure of lentiviral vector for overexpressing miRNAs. The miR-200, miR-96 and miR-182 hESCs were visualized under phase-contrast and fluorescent microscopy. Scale bars: 50 µm. The right column shows the miRNA levels of corresponding cell lines at day 10 of differentiation when compared with the control GFP hESCs and EPI cells. (**B**) The structure of lentiviral vector for expressing miRNA sponge. The miR-96-182 sponge hESCs were visualized under phase-contrast and fluorescent microscopy. Scale bars: 50 µm. The right column showed the inhibiting activity of miR-96-182 sponge in the reporter assay. (**C**) Shown is the structure of the inducible expression system in the AAVS1 locus. Induced GFP expression was visualized under fluorescent microscopy in EPI cells (red). Scale bars: 50 µm. Induced ZEB2 expression was quantified by qPCR.v

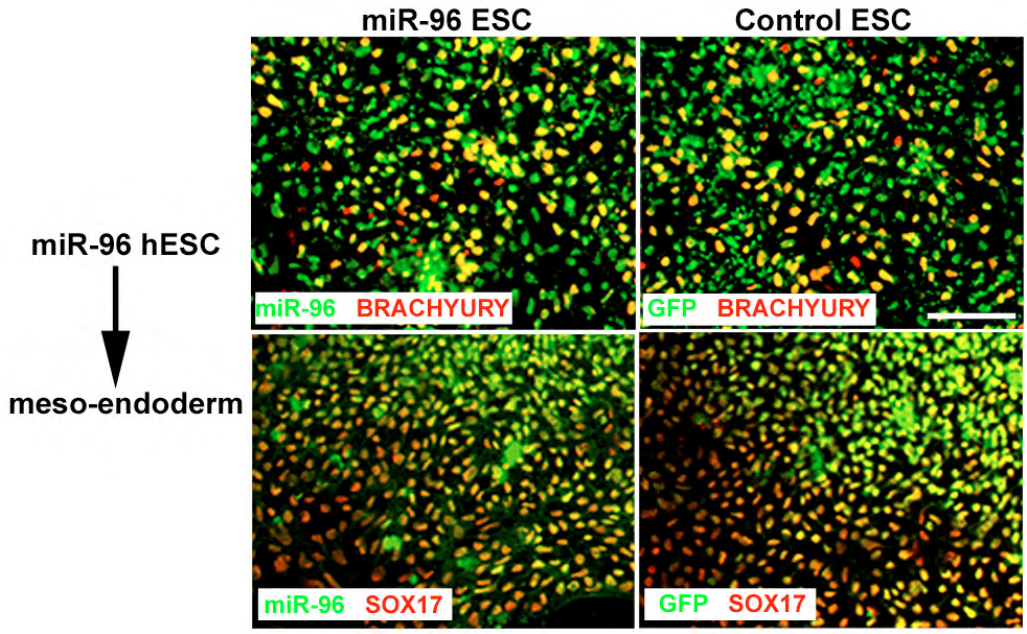

Fig. S3. Overexpression of miR-96 family does not interfere with meso-endoderm differentiation from hESCs. The miR-96 hESCs (left) and GFP control hESCs (right) were differentiated toward meso-endorderm lineages, and stained for mesoderm marker brachyury and endoderm marker SOX17. Scale bars: 50 μm.

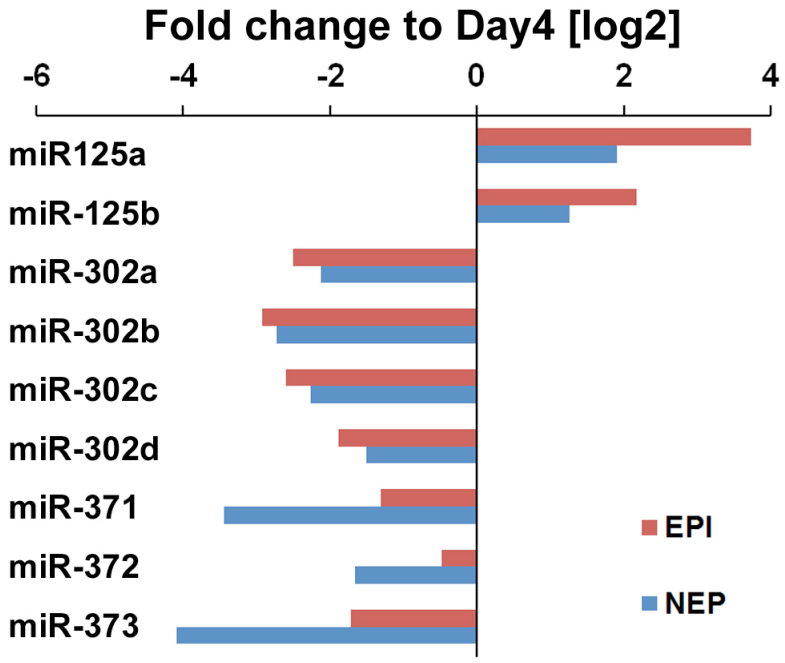

Fig. S4. The expression pattern of miR-125, miR-302 and miR-371 families. The expression of members of miR-125, miR-302 and miR-371 families was compared during hESC differentiation into the neuroectoderm (NEP) with that of the epidermis (EPI) at day 10.

Table S1. Fold change of miRNAs in day 10 NEP and EPI cells when compared with day 4 cells (shown as log_2)

miRNA	NEP	EPI
let-7b	–2.781158	–2.904227
miR-1	7.388859	5.373822
miR-100	2.096294	2.858324
miR-122a	–3.659820	–2.049011
miR-125a	1.912681	3.732400
miR-125b	1.267455	2.168344
miR-149	1.896574	2.391545
miR-181a	2.154590	3.481077
miR-181b	2.646930	3.764541
miR-181c	2.558172	3.934785
miR-181d	2.683006	3.770286
miR-199a	1.629753	1.917602
miR-199b	2.173490	2.128746
miR-216a	1.995136	2.034965
miR-216b	2.899187	2.256661
miR-217	4.393095	3.552109
miR-26b	2.269018	2.055425
miR-27a	3.883983	2.003635
miR-28	1.790957	2.519526
miR-29c	2.180244	3.367905
miR-301a	2.303150	2.459690
miR-301b	1.937745	2.334515
miR-302a	–2.125153	–2.505480
miR-302b	–2.726693	–2.925864
miR-302c	–2.268908	–2.596383
miR-302d	–1.500470	–1.887767
miR-31	3.382716	3.718355
miR-371	–3.444514	–1.302560
miR-373	–4.091098	–1.720301
miR-432	2.704942	1.688595
miR-495	–15.18792	–18.46438
miR-515	–4.297706	–1.994740
miR-516b	–2.706411	–3.153307
miR-517a	–3.769689	–3.043031
miR-517b	–3.351724	–1.884623
miR-517c	–3.944521	–2.659739
miR-518b	–2.996644	–3.478819
miR-518c	–3.444238	–4.019979
miR-518d	–3.712232	–1.994429
miR-518e	–3.602468	–3.060808

miR-518f	–4.159568	–2.816399
miR-519a	–3.662864	–2.817062
miR-519d	–3.187913	–2.696241
miR-520a	–3.172559	–2.756888
miR-520b	–2.427431	–3.271626
miR-520g	–4.083886	–4.088064
miR-522	–4.611940	–2.272161
miR-523	–3.541162	–2.967402
miR-524	–2.629966	–1.921285
miR-525	–3.885591	–3.721121
miR-526b	–2.376941	–2.360448
miR-614	–2.086076	–2.298528
miR-626	2.352647	2.446251
miR-663	–2.171612	–2.190557
miR-9	3.360588	3.145603
miR-92a	4.278383	2.939442
miR-99a	2.892189	3.902149

Table S2. Oligonucleotides used in qPCR, cloning and mutagenesis studies

Primer	Sequence
qPCR	
ZEB1 forward	CTACAACAACAAGACACTGCTGT
ZEB1 reverse	TGTTCTTTCAGAGAGGTAAAGCG
ZEB2 forward	CAA GAG GCG CAA ACA AGC
ZEB2 reverse	GGT TGG CAA TAC CGT CAT CC
CDH2 forward	CAGGGTGGACGTCATTGTAG
CDH2 reverse	AGGGTCTCCACCACTGATTC
EOMES forward	ATCATTACGAAACAGGGCAGGC
EOMES reverse	CGGGGTTGGTATTTGTGTAAGG
SOX7 forward	ACGCCGAGCTCAGCAAGAT
SOX7 reverse	TCCACGTACGGCCTCTTCTG
Cloning	
miR-200 forward	GTCGACCCACTCCGACCTAGTCCTC
miR-200 reverse	GCGGCCGCCTCCGGGTATCTGTGACTGTGAC
miR-96 forward	CTCGAGTCCTTGAAGGTCATCTTGGGCT
miR-96 reverse	GCGGCCGCAGGCAGTGTAAGGCGATCT
miR-182 forward	CTCGAGATGCCTGCCCACAGGAACT
miR-182 reverse	GCGGCCGCTGCAGGGAAACACAGAGTGTCA
PAX6 3'UTR forward	GTTTAAACGGGACACAACAGTTGAGCTTTC
PAX6 3'UTR reverse	GTCGACAGGCTGACAATGGAAATCTGCC
ZEB2 3'UTR forward	GTTTAAACATACTAGTGGAGTTGGAGCTGGGTATTG

ZEB2 3'UTR reverse	GTCGACACTAGTTGGAATCAGGATCAGTTGAGAA
TS1 site forward	AAACAGATATAAATTCAAGGAAGAAAAAAAGTTGA TAGCTAAAAGGTAGAGTGTC
TS1 site reverse	TCGAGACACTCTACCTTTTAGCTATCAACTTTTTTTCT TCCTTGAATTTATATCTGTTT
TS2 site forward	AAACCAAAATTAACCATTGTTGATTGTAAAAAACCA TGCCAAAGCCTTTGTATTC
TS2 site reverse	TCGAGAATACAAAGGCTTTGGCATGGTTTTTTACAAT CAACAATGGTTAATTTTGGTTT
Mutagenesis	
TS1 site	ATTCAAGGAAGAAAAAAAGTTGATAGGGAAAAGGT AGAGTGTGTCT
TS2 site	AAAATTAACCATTGTTGATTGTAAAAAACCATGGGA AAGCCTTTGTATTTC