

## Supplemental Information

**Supplemental Table 1:** Sequence information of the mRNAs tested in the manuscript.

**Supplemental Figure S1:** Gene expression analysis of miR-301a in EBs at day 4, day 8 and day 12 demonstrated an ~5-fold increase in miR-301a levels in EBs at day 8 and day 12 compared to day 4. A quantitative real-time PCR method was applied. Data are presented as the mean  $\pm$  SEM in triplicate (n=3). \*\*p<0.01.

**Supplemental Figure S2:** A: Alkaline phosphatase staining of mES clones with or without overexpression of miR-301a to determine the effects of miR-301a on the properties of mES cells. B: Quantitative analysis of alkaline phosphatase-positive clone numbers in the NC and miR-301a groups.

**Supplemental Figure S3:** Gene expression analysis of cardiac cell markers including MEF2C, TBX5, GATA4, MHC and MLC in mES cells with or without overexpression of miR-301a. EBs at day 8 in the NC group were used as a positive control. Fold changes for gene expression were calculated by values in mES cells vs. EBs at day 8. The results indicated very low or undetectable expression of these cardiac markers in mES cells, which was not affected by miR-301a. Data are presented as the mean  $\pm$  SEM in triplicate (n=3).

Supplemental video 1: Video recording of beating EBs at day 10 in the NC group.

Supplemental video 2: Video recording of beating EBs at day 12 in the NC group.

Supplemental video 3: Video recording of beating EBs at day 9 in the miR-301a group.

Supplemental video 4: Video recording of beating EBs at day 10 in the miR-301a group.

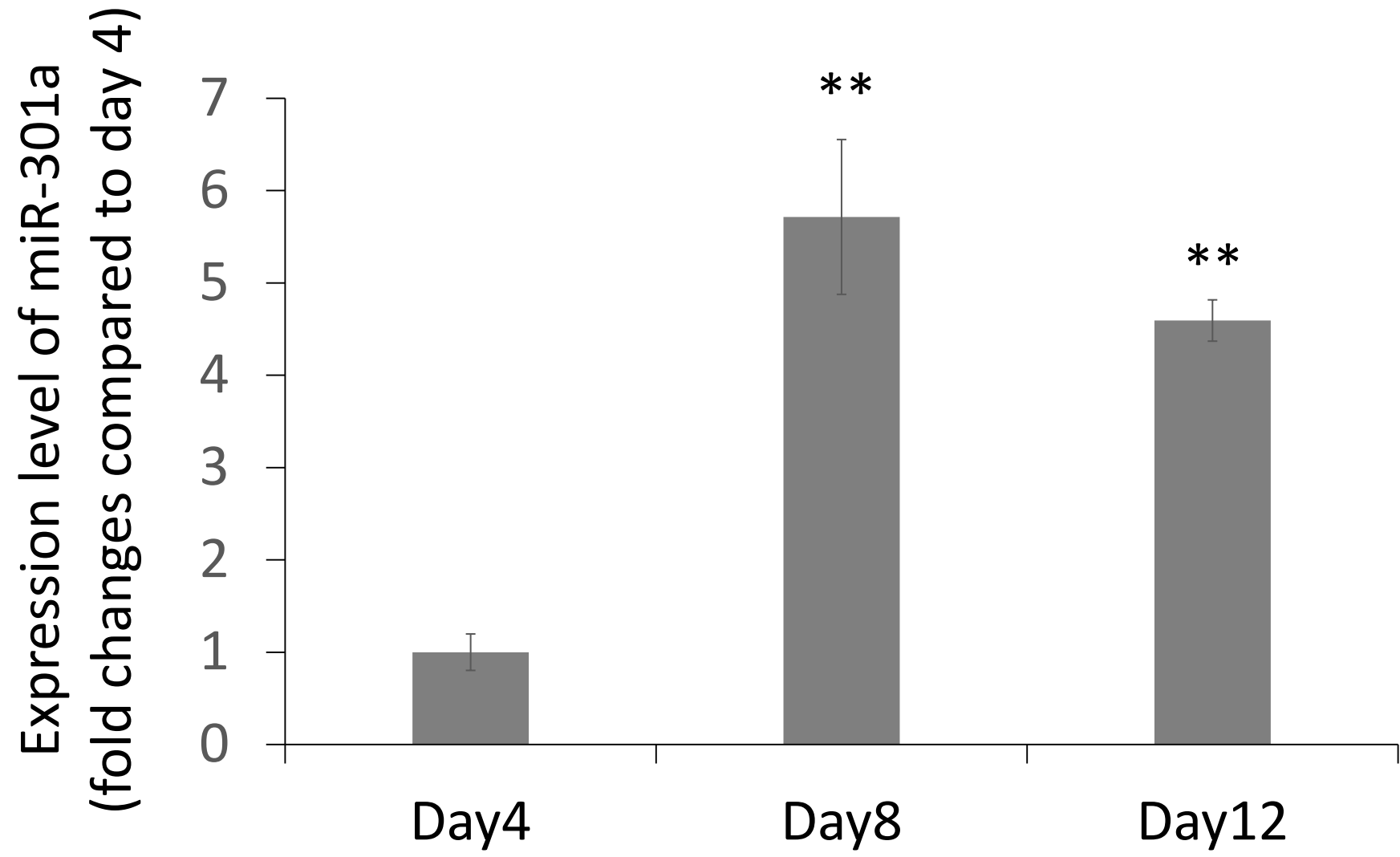
Supplemental video 5: Video recording of beating EBs at day 12 in the miR-301a group.

Supplemental Table 1

Primer information of mRNAs

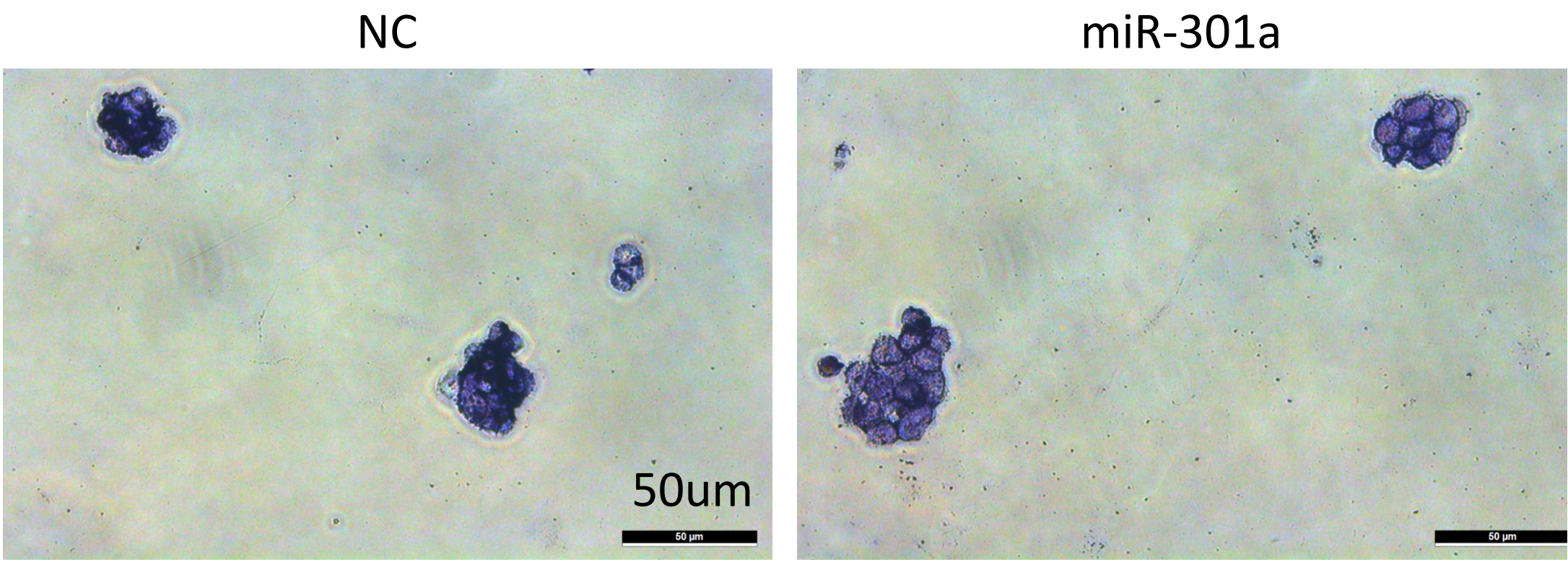
Gene Symbol	GeneBank #	Forward primer (5'-3')	Reverse primer (5'-3')	Length of the PCR product
OCT4	NM_001252452.1	ATTTTGTCTCAGTGGGGCG	TTCCAAAGAGAACGCCCAGG	182 bp
NANOG	NM_028016.3	AGCCGTTGGCCTTCAGATAG	ATGGCGAGGGAAGGGATTTC	194 bp
SOX2	NM_011443.4	CAAAAACCGTGATGCCGACT	CGCCCTCAGGTTTTCTCTGT	92 bp
KLF4	NM_010637.3	TACCCCTACACTGAGTCCCG	GGAAAGGAGGGTAGTTGGGC	110 bp
α-MHC	NM_010856.4	GATTTCTCCAACCCAGCTGCGCC	CGGATGTCAAAGGGCCGGGTC	201 bp
MLC	NM_010861.4	TGTGGGTTCACCTGAGGCTGTGGTTCAG	GAAGGCTGACTATGTCCGGGAGATGC	190 bp
MEF2C	NM_001347564.1	GATACCCACAACACACCACGCGCC	ATCCTTCAGAGAGTCGCATGCGCTT	195 bp
TBX5	NM_011537.3	ATCCCCAGCACAAACTCCAG	CGCGAGGTTCTATTCTCGCTC	125 bp
GATA4	NM_001310610.1	CCCTGGAAGACACCCCAATC	TTTGAATCCCCTCCTTCCGC	359 bp
α-actinin	NM_033268.4	TGGCACCCAGATCGAGAAC	GTGGAACCGCATTTTTCCCC	121 bp
GAPDH	NM_001289726.1	AGGTCGGTGTGAACGGATTTG	TGTAGACCATGTAGTTGAGGTCA	123 bp

Supplemental Figure S1

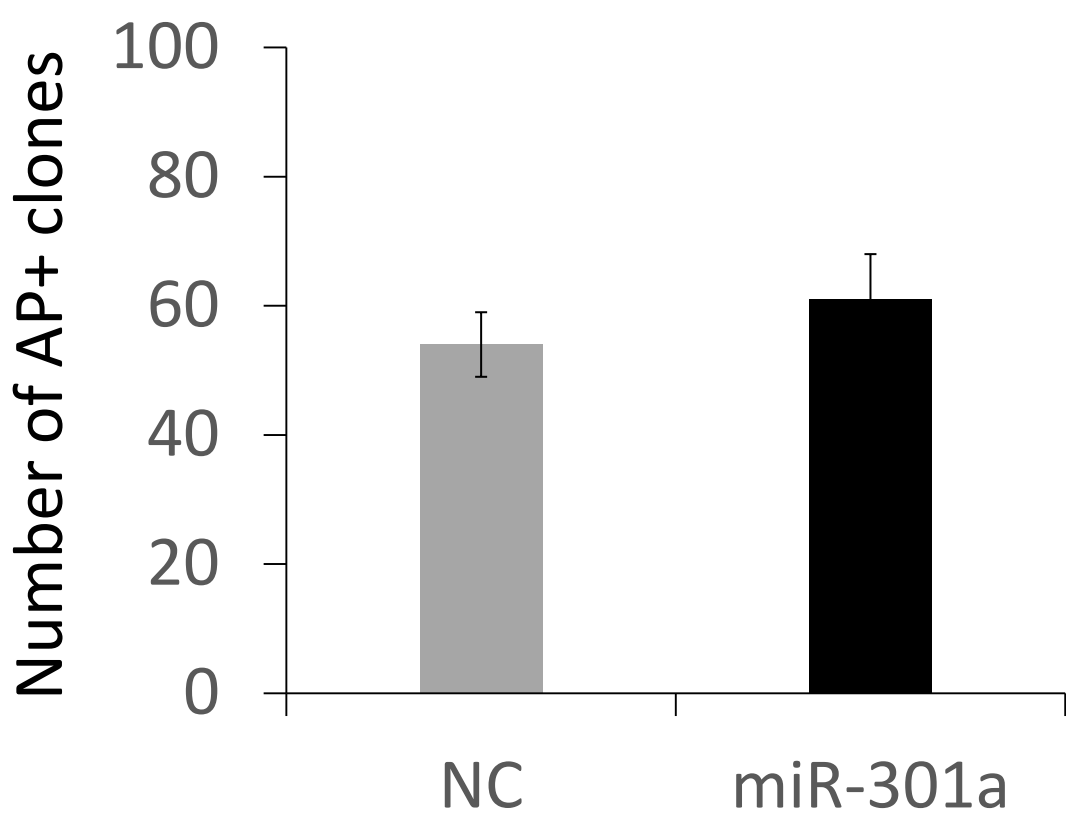


Supplemental Figure S2

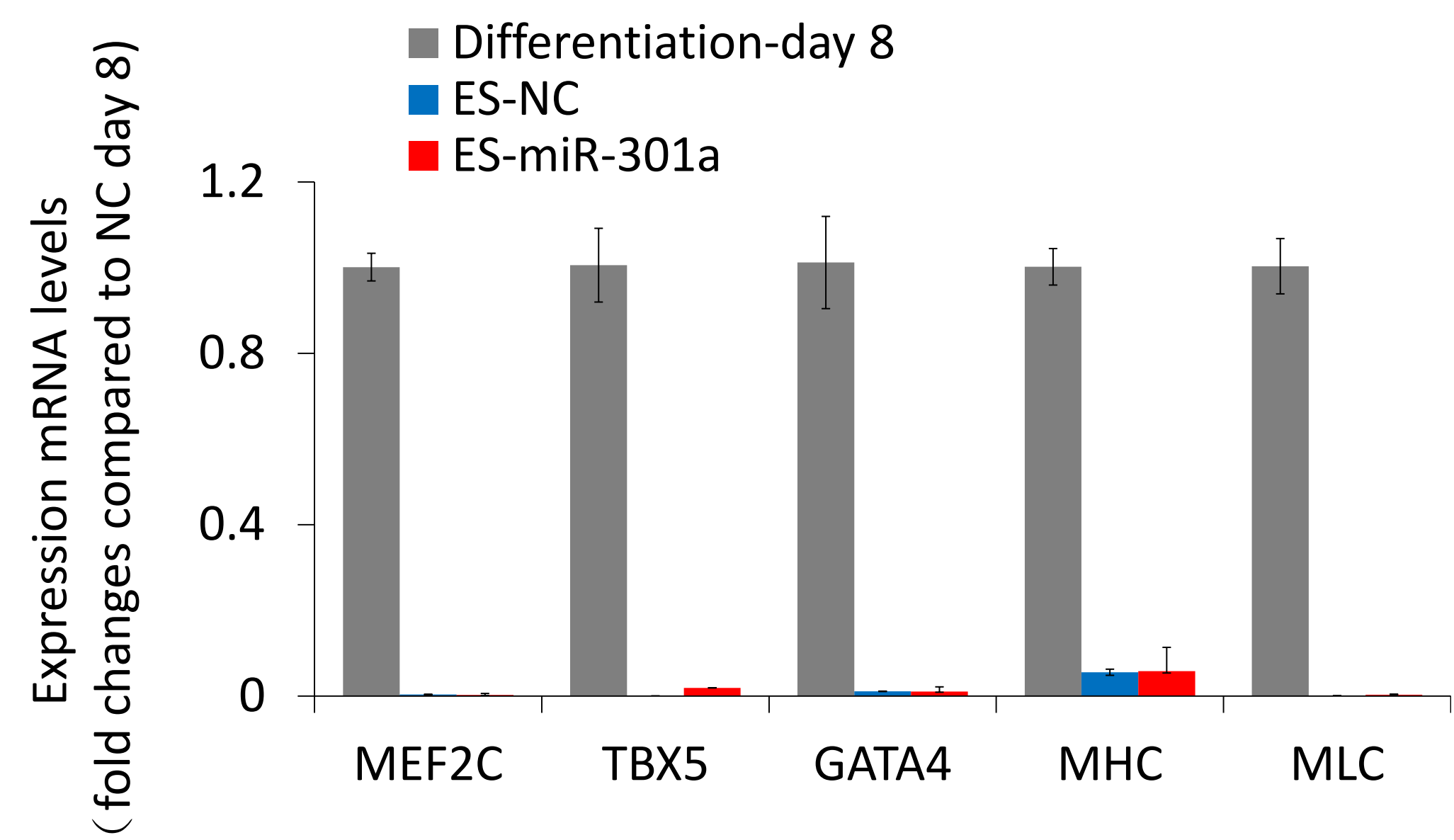
A



B



Supplemental Figure S3



Supplemental videos

- V1: miR-NC day 10
- V2: miR-NC day 12
- V3: miR-301a day 9
- V4: miR-301a day 10
- V5: miR-301a day 12