

Electronic supplementary material for:

**MiR-218 affects hypertrophic differentiation of human mesenchymal stromal cells during chondrogenesis *via* targeting *RUNX2*, *MEF2C*, and *COL10A1***

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Supplementary Tables S1 – S2

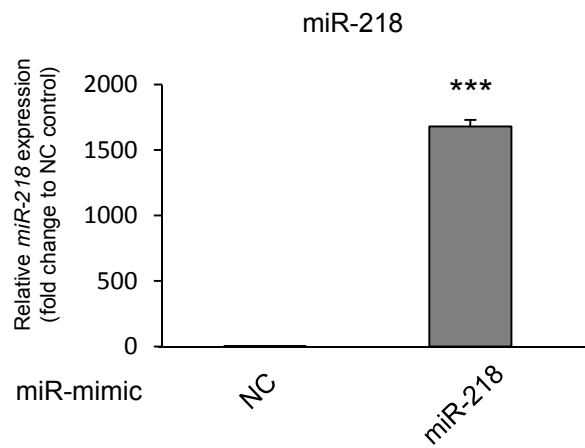
Supplementary Fig. S1 – S3

**Supplementary Table S1** List of qRT-PCR primers used in this study

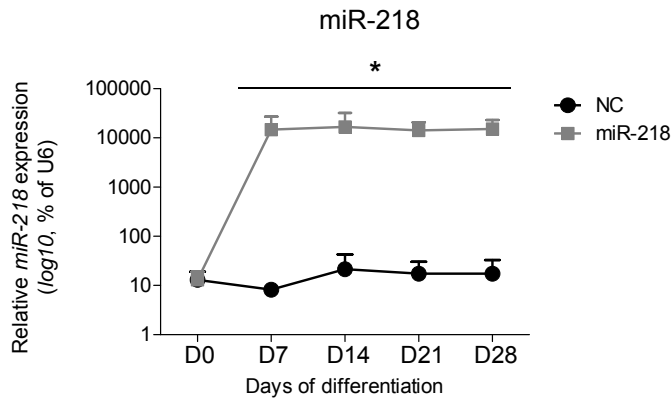
<b>Gene Name</b>	<b>Sequence</b>
<i>ACTB</i>	CTCTTCCAGCCTTCCTTCCT CGATCCACACGGAGTACTTG
<i>ALPL</i>	CACCAACGTGGCTAAGAATG ATCTCCAGCCTGGTCTCCTC
<i>AXIN2</i>	TAGCTGAGGTGTCGAAGCC CCTTCAGCATCCTCCGGTAT
<i>COL10A1</i>	TTGCTCTCCTCTTACTGCTAT TTACGCTGAACGATACCAA
<i>COL2A1</i>	TGGCCTGAGACAGCATGAC AGTGTTGGGAGCCAGATTGT
<i>HPRT</i>	AAGGGTGTTTATTCCTCATGGA CCTCCATCTCCTTCATCAC
<i>CPSF6</i>	AAGATTGCCTTCATGGAATTGAG TCGTGATCTACTATGGTCCCTCTCT
<i>MEF2C</i>	GTATGGCAATCCCCGAAACT ATCGTATTCTTGCTGCCTGG
<i>RUNX2</i>	ACTCTACCACCCCGCTGTC CAGAGGTGGCAGTGTCATCA
<i>RLP13</i>	CATTTCTGGCAATTTCTACAG CAGGCAACGCATGAGGAAT
<i>ROBO1</i>	AGTGACGCTGGCAAATATGT CTGCCAAGTTACTGGGTCTC

**Supplementary Table S2** List of cloning primers used in this study

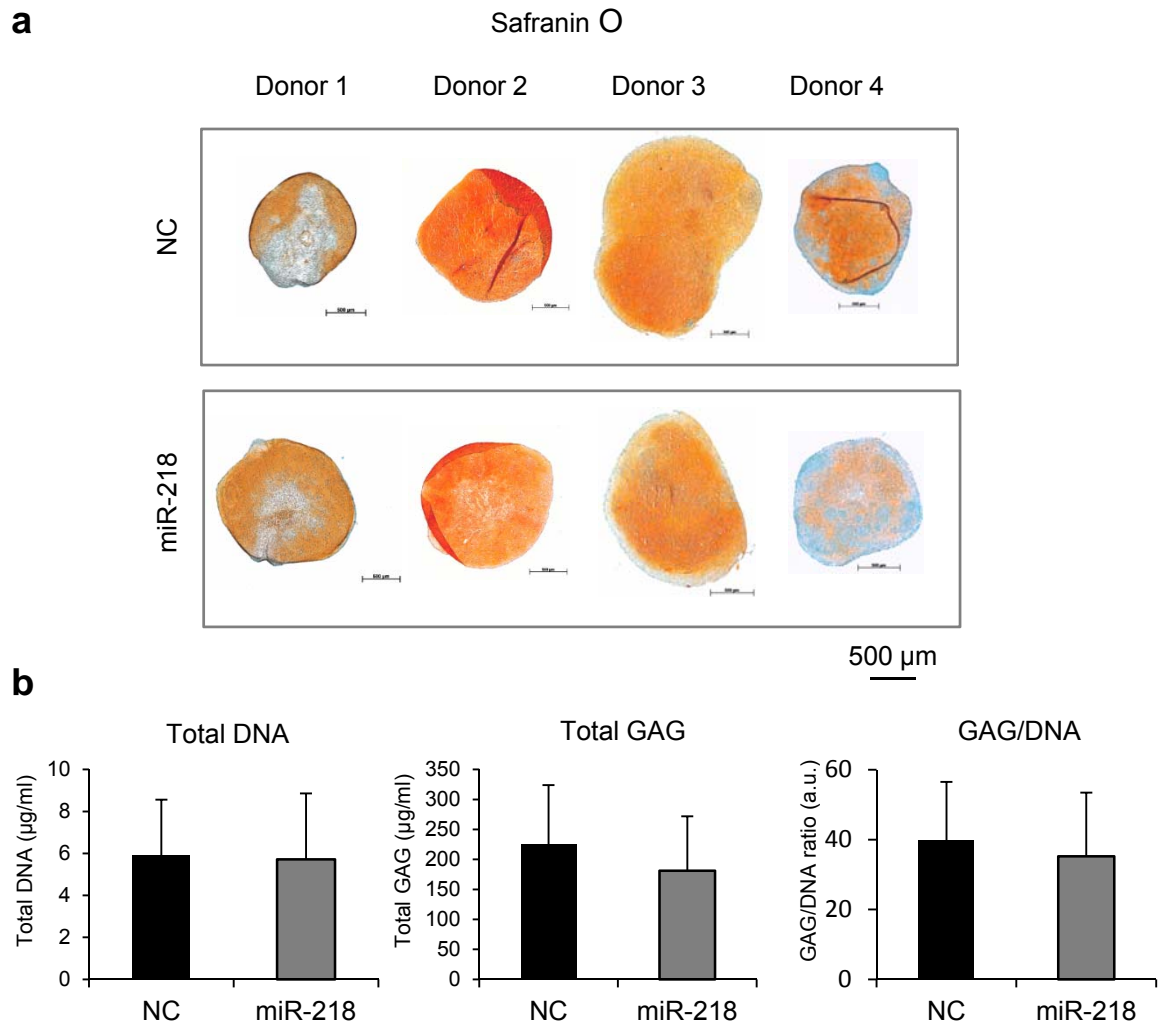
<b>Gene Name</b>	<b>Sequence</b>
<i>MEF2C</i>	AAACTAGTCCCATCCTTACAACCCATCCT CGCAAGCTTTGTTACTCTAGGACGGGTTATGT
<i>COL10A1</i>	AAACTAGTGCTGAGCCTTGTGCCTCCCATTCAAGT GCGCGCAAGCTTAGCCTTGAAAGAATGGTTGAGA



**Fig. S1** miR-218 transfection efficiency in SaOS-2 cells. SaOS-2 cells were transiently transfected with miR-218 (grey), or with non-targeting control (NC, black) mimic, and transfection efficiency was controlled by qRT-PCR. Mean values  $\pm$  SD are shown,  $n = 2$ ; \*\*\* $p \leq 0.001$ , two-tailed  $t$ -test.



**Fig. S2** miR-218 transfection efficiency in human MSC. MSC cells (N = 3 donors) were transiently transfected with miR-218 (grey), or with non-targeting control (NC, black) mimic before cells were subjected to chondrogenic differentiation for 28 days, and transfection efficiency was controlled by qRT-PCR at indicated time points. Mean values  $\pm$  SD are shown; \* $p \leq 0.05$ , NC vs miR-218; expression kinetic curves were compared using two-tailed paired  $t$ -test.



**Fig. S3** Gain of function of miR-218 at the start of chondrogenic differentiation does not additionally boost MSC chondrogenesis. MSC (N = 4 - 6) were transfected with miR-218 (grey), or with a control non-targeting miR (NC, black) mimics at day 0, and subjected to chondrogenic differentiation for 28 days. **a**, Safranin O staining of 3D pellet cultures at day 28 chondrogenesis. Scale bar, 500  $\mu$ m. **b**, Total GAG and DNA content of 3D pellet cultures was assessed by DMMB and PicoGreen assays at day 28 of chondrogenesis; Mean values  $\pm$  SD are shown; N = 6 donors.