SUPPLEMENTARY FIGURE LEGENDS

Supplementary Figure S1. Further characterization of *OIP5-AS1* levels, miR-7 levels, and myoblast morphology. (A) Number of *OIP5-AS1* and miR-7 copies per myoblast (AB678) and per nucleus-equivalent in AB678 myotubes, as calculated by standard curve analysis, proportional Ct value relative to an RNA of known abundance, and (only for *OIP5-AS1*) ddPCR analysis (Materials and Methods). **(B)** Human AB678 myoblasts were transfected with Ctrl siRNA or with ZSWIM8 siRNA #1 or siRNA #2; 24 h later, the efficiency of *ZSWIM8* mRNA silencing was determined by RT-qPCR analysis. Data were normalized to *GAPDH* mRNA levels in the same RNA samples. **(C)** AB678 myoblasts were transfected with Ctrl siRNA or *OIP5-AS1*-directed siRNA #2; 24 h later, myoblasts were placed in differentiation medium for indicated times. The efficiency of silencing *OIP5-AS1* was determined by RT-qPCR analysis (*graph*), and the impact on differentiation (Diff) was monitored by assessing MYH levels by immunofluorescence (*micrographs*). **(D)** Human AB678 myoblasts were transfected with Ctrl miR or with miR-7 mimic at the indicated concentration; 24 h later, myoblasts were placed in differentiation medium, and differentiation was monitored 72 h later by myotube formation using phase-contrast microscopy. **(E)** Human AB678 myoblasts were transfected with Ctrl miR, miR-7 mimic, or miR-7 inhibitor; 24 h later, myoblasts were placed in differentiation media, and differentiation was monitored 72 h later by assessing myotube formation using phase-contrast microscopy. Data in (B,C) represent the means ± SEM from three or more independent experiments. Significance was established using Student's *t*-test. **, $p<0.01$; ***, $p<0.001$. Other data are representative of three or more biological replicates.

Supplementary Figure S2. Expression levels and impact of MYMX on myogenesis. (A) Volcano plot representation of RNAs differentially abundant when comparing proliferating and differentiated myoblasts. *MYMX* mRNA is highlighted as one of the most robustly increased transcripts in the differentiated population. **(B)** Number of *MYMX* mRNA copies per myoblast (AB678) and per nucleus-equivalent in AB678 myotubes, as calculated by standard curve analysis and proportional Ct value relative to an RNA of known abundance (Materials and Methods). **(C, D)** AB678 myoblasts were transfected with Ctrl siRNA or one of two different MYMX-directed siRNAs; 24 h later, they were placed in differentiation medium for 72 h, and the presence of myotubes was monitored to evaluate the extent of differentiation after transfecting MYMX siRNA #1 (C) or MYMX siRNA #2 (D). **(E)** AB678 myoblasts were transfected with Ctrl siRNA or MYMX-directed siRNA #2; 24 h later, myoblasts were placed in differentiation medium for the indicated times (Diff), and the silencing efficiency and presence

of myotubes was monitored by Western blots. Representative images from three or more biological replicates are shown.

Supplementary Figure S3. Effect of miR-7 overexpression on polysome profiles and reporter construct analysis. (A) Twenty-four hours after transfecting plasmids psiCHECK2-MYMX(3'WT) or psiCHECK2-MYMX(3'mut), AB678 myoblasts were further incubated with fresh proliferation medium for 24 h, and luciferase activity (RL/FL) was calculated. **(B)** Twenty-four hours after co-transfecting psiCHECK2 or test reporter psiCHECK2-MYMX(3'WT) with either Ctrl siRNA or OIP5-AS1 siRNA, AB678 myoblasts were further incubated with fresh proliferation medium for 24 h, whereupon luciferase (RL/FL) activity was calculated. **(C)** Twenty-four hours after transfecting Ctrl miR or miR-7 mimic, AB678 myoblasts were placed in differentiation medium for an additional 24 h, and then harvested. Cytoplasmic extracts were fractionated by centrifugation through sucrose density gradients. Global polysome profiles depicting ribosomal subunits (40S, 60S), monosomes (80S), as well as low- and high-molecular weight polysomes (LMWP, HMWP) were prepared. Representative polysomes from three or more biological replicates are shown. **(D-F)** Twelve hours after co-transfecting the parent reporter (psiCHECK2) or test reporter psiCHECK2-MYMX(3'WT) along with either Ctrl miR or miR-7, into AB678 myoblasts and inducing differentiation, *RL* mRNA and *FL* mRNA levels were quantified by RT-qPCR analysis, and *RL* mRNA/*FL* mRNA ratios were calculated (D). Twenty-four hours after co-transfecting the plasmids psiCHECK2-MYMX(3'WT) and psiCHECK2-MYMX(3'mut), AB678 myoblasts were induced to differentiate for 24 h; *RL* mRNA and *FL* mRNA levels were then quantified by RT-qPCR analysis, and the relative ratios of *RL* mRNA to *FL* mRNA were calculated (E). Twenty-four hours after co-transfecting psiCHECK2 or test reporter psiCHECK2-MYMX(3'WT) and control siRNA or *OIP5-AS1*-directed siRNA, AB678 myoblasts were induced to differentiate for 24 h, *RL* mRNA and *FL* mRNA levels were quantified by RT-qPCR analysis, and the ratios of *RL* mRNA to *FL* mRNA were calculated (F). Data in (A,B,D-F) represent the means ± SEM from three or more independent experiments. Significance was established using Student's *t*-test. *, $p<0.05$.

Supplementary Figure S4. Further analysis of myoblast fusion using fluorescent reporter cells. (A) Individual fluorescence fields from the merged images in main Figure 6B and 6C are shown. EGFP-labeled AB678 cells were transfected with Ctrl miR or miR-7 mimic and further mixed with mCherry-labeled AB678. Representative micrographs of individual fluorescence (*left*, *center*) and merged fluorescence (*right*) are shown; homologous fusion (EGFP+ only or mCherry+ only) as well as heterologous syncytia (both EGFP+ and mCherry+) can be observed. **(B)** Individual fluorescence fields

from the merged images in main Figure 6B and 6C are shown. EGFP-labeled AB678 cells were transfected with Ctrl siRNA or OIP5-AS1-directed siRNA and further mixed with mCherry-labeled AB678 cells. Representative micrographs of individual fluorescence (*left*, *center*) and merged fluorescence (*right*) are shown; homologous fusion (EGFP+ only or mCherry+ only) and heterologous syncytia (both EGFP+ only and mCherry+ only) can be observed. Representative images from three or more biological replicates are shown.

Supplementary Figure S5. Additional characterization of OIP5-AS1:miR-7 TSB. (A) AB678 myoblasts were transfected with Ctrl TSB or OIP5-AS1:miR-7 TSB; 24 h later, they were placed in differentiation medium, and differentiation was determined 72 h later by monitoring myotube formation using phase-contrast microscopy. **(B)** As described in the main Figure 7H, EGFP-labeled AB678 cells were transfected with Ctrl TSB (*top*) or OIP5-AS1:miR-7 TSB (*bottom*) and further mixed with mCherry-labeled AB678. The individual fluorescence signals (*left*, *center*) and merged fluorescence signals (*right*) are shown. Fusion ability was assessed by monitoring homologous fusion (EGFP+ only or mCherry+ only) as well as heterologous syncytia (both EGFP+ and mCherry+). Representative images from three or more biological replicates are shown.

Supplementary Figure S6. Additional characterization of miR-7 on myogenic gene expression, as assessed by the presence of OIP5-AS1:miR-7 TSB. Expression levels of 37 mRNAs that adhered to three criteria: (1) predicted to be miR-7 targets as determined by using the miRDB database, (2) significantly downregulated in myoblasts transfected with OIP5-AS1:miR-7 TSB (at either 0, 24 or 48 h of differentiation), and upregulated during myogenesis (at 24 h). Significance was established using padj < 0.05, and log2FC >1.

Supplementary Figure S7. Additional characterization of OIP5-AS1:MEF2C TSB. (A) AB678 myoblasts were transfected with Ctrl TSB or OIP5-AS1:MEF2C TSB; 24 h later, they were placed in differentiation medium for 24 h. Cell lysates were then collected, incubated with *OIP5-AS1*-directed biotinylated ASOs, and RNA complexes pulled down using streptavidin beads (Materials and Methods). The presence of *OIP5-AS1* and *MEF2C* mRNAs in the pulldown material was assessed by RT-qPCR analysis and normalized to the levels of *GAPDH* mRNA. **(B)** AB678 myoblasts were transfected with Ctrl TSB or OIP5-AS1:MEF2C TSB; 24 h later, they were placed in differentiation medium for 24 h, and the levels of *MEF2C* mRNAs were assessed by RT-qPCR analysis. **(C)** AB1167 myoblasts were transfected with Ctrl TSB (50 nM), OIP5-AS1:miR-7 TSB (50 nM), OIP5-AS1:MEF2C TSB (50 nM)

or a cocktail of OIP5-AS1:miR-7 TSB (25 nM) plus OIP5-AS1:MEF2C TSB (25 nM); 24 h later, they were placed in differentiation medium for 72 h, and differentiation was monitored by assessing MYH signals by immunofluorescence. Data in (A,B) represent the means ± SEM from three or more independent experiments. Significance was established using Student's *t*-test. *, $p<0.05$; **, $p<0.01$; ***, $p<0.001$. Other data are representative of three or more biological replicates.

Supplementary Table S1. Oligomers used in this study.

Oligomer name	Oligomer sequence
MEF2C-F	GCAACAGCAACACCTACATAAC
MEF2C-R	GTACGGTCTCTAGGAGGAGAAA
MYOG-F	GCCCTGAATTGAGAGAGAAGAA
MYOG-R	TGGCAGCTTTACAAACAACAC
OIP5-AS1-F	TTGAGAAGCTGCGAAGATGG
OIP5-AS1-R	GGGAGGAAGAATGTTCGGTTAG
MYH7-F	AAAGCTGCTGGAACGTAGAG
MYH7-R	GCCATCTCCTTCTCTCTTTCTG
18S rRNA-F	CGAACGTCTGCCCTATCAACTT
18S rRNA-R	ACCCGTGGTCACCATGGTA
ACTB-F	CATGTACGTTGCTATCCAGGC
ACTB-R	CTCCTTAATGTCACGCACGAT
GAPDH-F	GGACGCAGATCCCTCCAAAAT
GAPDH-R	GGCTGTTGTCATACTTCTCATGG
MYMX-F	CTCCCAAAGACCACTCCTAATC
MYMX-R	TGTCACTTGTCCCTCAGTTAAG
ZSWIM8-F	CCCAGTCAACAGCCATCAATA
ZSWIM8-R	GAGTGTCGATTCATCCAGATACC
Renilla Luciferase-F	TCGTCCATGCTGAGAGTGTC
Renilla Luciferase-R	CTAACCTCGCCCTTCTCCTT
Firefly Luciferase-F	TGCAGAAGATCCTGAACGTG
Firefly Luciferase-R	CGGTAGACCCAGAGCTGTTC
Pre-miR-7 primer	GATGTTGGCCTAGTTCTGTG
miR-7-5p primer	TGGAAGACTAGTGATTTTGTTGTT
miR-7-3p primer	CAACAAATCACAGTCTGCCATA
OIP5-AS1 siRNA #1 sense	rGrGrCrUrGrArGrUrUrUrCrArUrUrUrGrArArArCrArGrGTG
OIP5-AS1 siRNA #1 antisense	rCrArCrCrUrGrUrUrUrCrArArArUrGrArArArCrUrCrArGrCrCrUrU
OIP5-AS1 siRNA #2 sense	rGrArArArUrGrUrGrGrCrUrArUrCrArCrUrUrCrUrCrUrACA
OIP5-AS1 siRNA #2 antisense	rUrGrUrArGrArGrArArGrUrGrArUrArGrCrCrArCrArUrUrUrCrArA
MYMX siRNA#1 sense	rGrGrArCrCrUrArCrCrUrUrArArArArUrArArUrArUrCrUGA
MYMX siRNA#1 antisense	rUrCrArGrArUrArUrUrUrUrArArGrGrUrArGrGrUrCrCrUrG
MYMX siRNA#2 sense	rArArCrArGrArArArUrGrUrCrUrUrUrCrUrGrGrArArGrAAT
MYMX siRNA#2 antisense	rArUrUrCrUrUrCrCrArGrArArArGrArCrArUrUrUrCrUrGrUrUrGrG
ZSWIM8 siRNA#1 sense	rCrUrUrGrGrUrArUrCrUrGrGrArUrGrArArUrCrGrArCrACT
ZSWIM8 siRNA#1 antisense	rArGrUrGrUrCrGrArUrUrCrArUrCrCrArGrArUrArCrCrArArGrUrA
ZSWIM8 siRNA#2 sense	rCrCrUrUrUrUrUrUrArCrUrCrUrArGrUrCrGrArArArArAAA
ZSWIM8 siRNA#2 antisense	rUrUrUrUrUrUrUrCrGrArCrUrArGrArGrUrArArArArArArGrGrArG
hsa-miR-7-5p (for standard curve)	UGGAAGACUAGUGAUUUUGUUGUU

Supplementary Table S2. Antisense Oligomers (ASOs) used in this study.

ASO name	3'-Biotin ASO sequence
OIP5-AS1 _1	GGGAGGAAGAATGTTCGGTT
OIP5-AS1 _2	ATTTTCTTCCTGTGATAAGG
OIP5-AS1 _3	AAAGAAGCAGGACTACCCAC
OIP5-AS1 _4	AGCAGGATAACTGGAATCCT
OIP5-AS1 _5	TTCACAAATACCACCACCTA
OIP5-AS1 _6	CTGTGCTTATCATGGTAGTG
OIP5-AS1 _7	GGAAAATTCTCTCATCCTCC
OIP5-AS1 _8	TTGGGTTGCAGGAAGAGTTA
OIP5-AS1 _9	AGCCTTTTCAGCCTAGAAAT
OIP5-AS1 _10	GGTTTCTTTTCCACGATGAC
OIP5-AS1 _11	AATACATACAATGGTCCTCT
OIP5-AS1 _12	ATGGTGCCAAAAGTACAGGT
OIP5-AS1 _13	TGGTTTCCAGATACCTTATG
OIP5-AS1 _14	CAAGGCAGCTTTTAGAGGTA
OIP5-AS1 _15	GCAGGGTCTTTAACCTTTAA
OIP5-AS1 _16	AGCAGCTAGTTTTATTCAGC
OIP5-AS1 _17	ACTGCTGACATCATTGTACT
OIP5-AS1 _18	TCAAGCTATCAATACCCTGA
OIP5-AS1 _19	AGAAGCTCCACATCTATCAC
OIP5-AS1 _20	GTCCTGACAGTTTGAATGTC
OIP5-AS1 _21	GCAGGGGTACTTTATAGTTG
OIP5-AS1 _22	CGTCATTTGTCACAATCACT
OIP5-AS1 _23	GTTCTTAATCTTTACTGGCT
OIP5-AS1 _24	CAGAGCTTCCAACTTTTTTA
(control) LacZ_1	CAGTTGGTCTGGTGTCAAAA
(control) LacZ_2	TGGCTGAATATCGACGGTTT
(control) LacZ_3	GGGACGCGCGAATTGAATTA
(control) LacZ_4	GGATTAGGGCCGCAAGAAAA
(control) LacZ_5	GATGGTAGTGGTCAAATGGC
(control) LacZ_6	AGTGCTCGGCAGATACACTT
(control) LacZ_7	CTGGATAACGACATTGGCGT

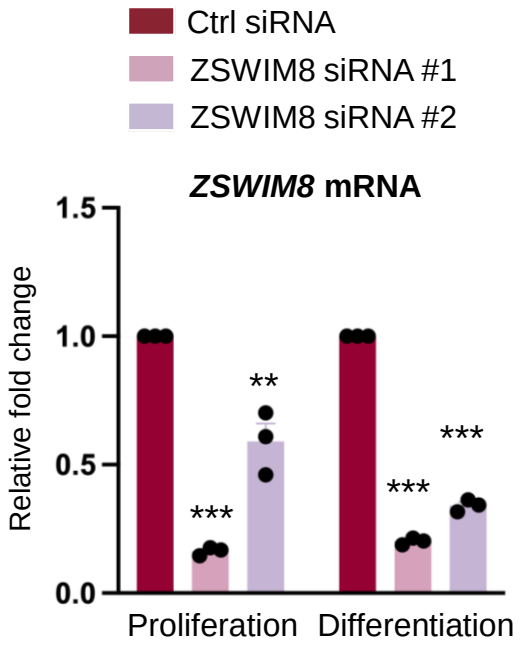
Yang et al. Supplementary Figure S1

A

Stage	Copies of *OIP5-AS1*
Proliferating	~25-41 per myoblast
Differentiated	~79-126 per nucleus-equivalent in myotubes

Stage	Copies of miR-7
Proliferating	~70-84 per myoblast
Differentiated	~14-17 per nucleus-equivalent in myotubes

B



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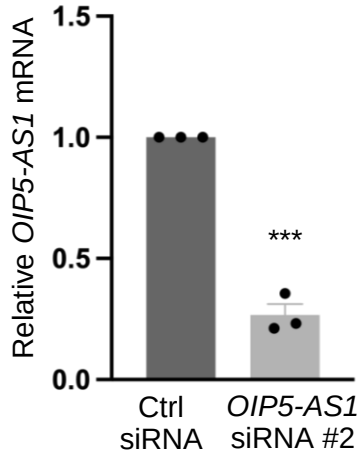

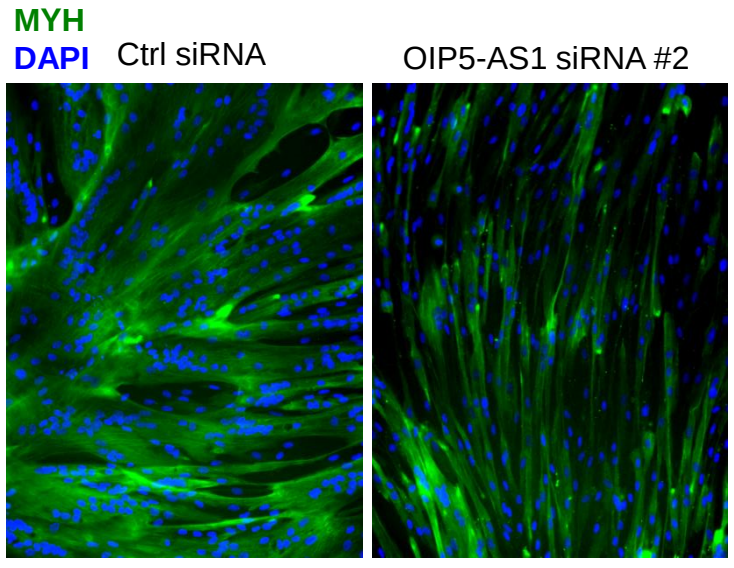

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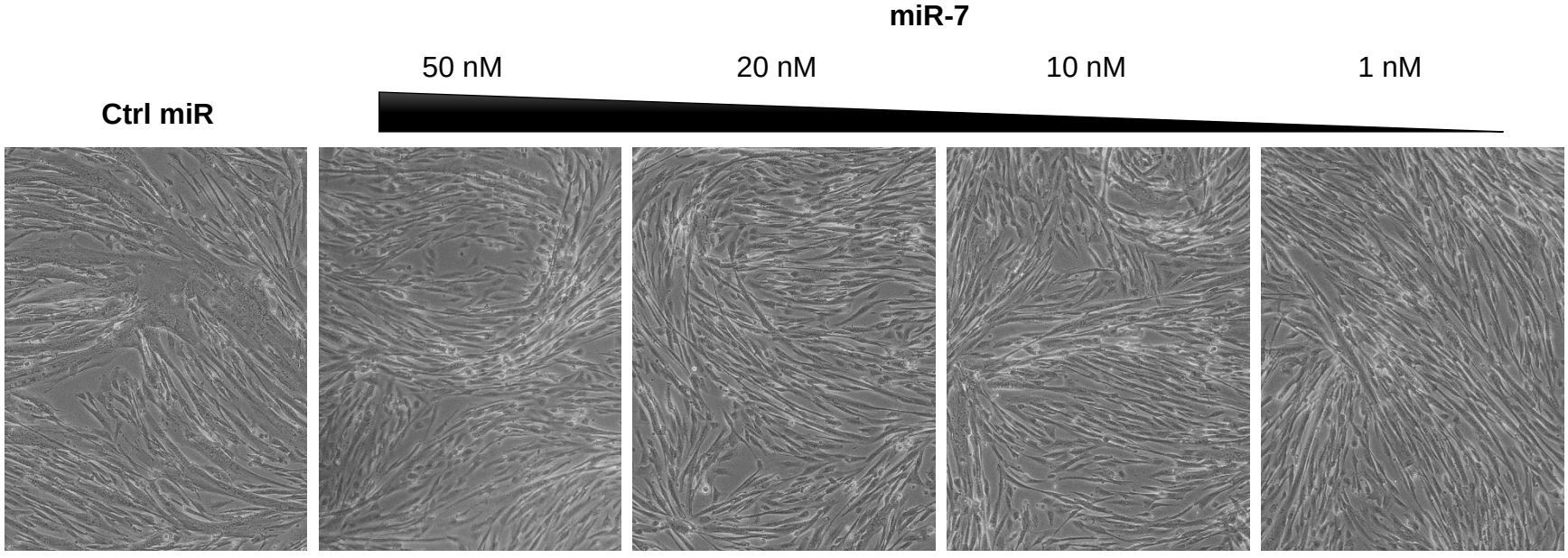

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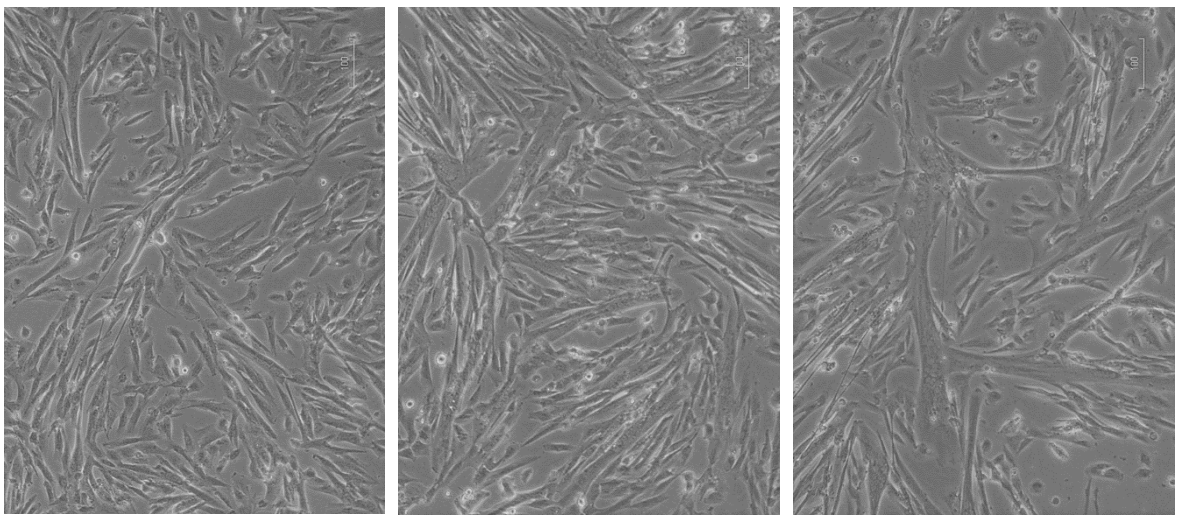
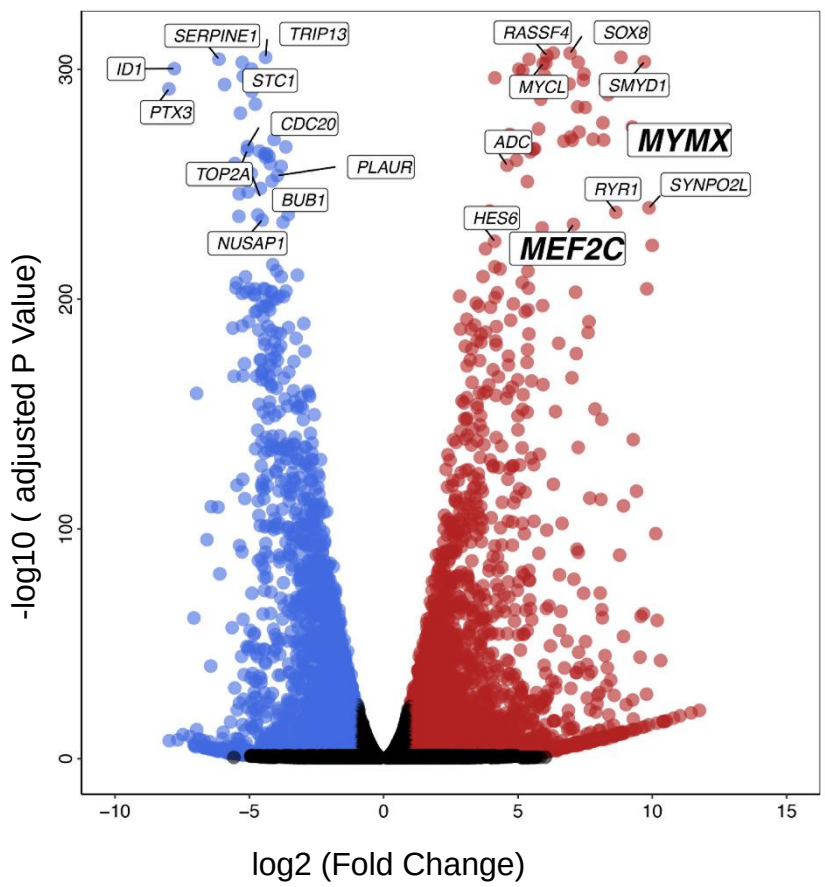


Yang et al. Supplementary Figure S2

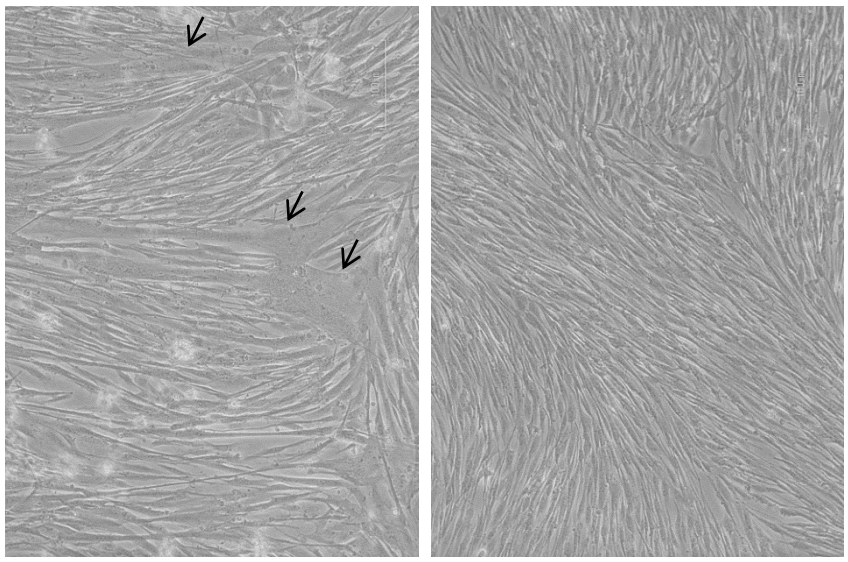
A



B

Stage	Copies of *MYMX mRNA*
Proliferating	~8 per myoblast
Differentiated	~137-111 per nucleus-equivalent at peak levels

C



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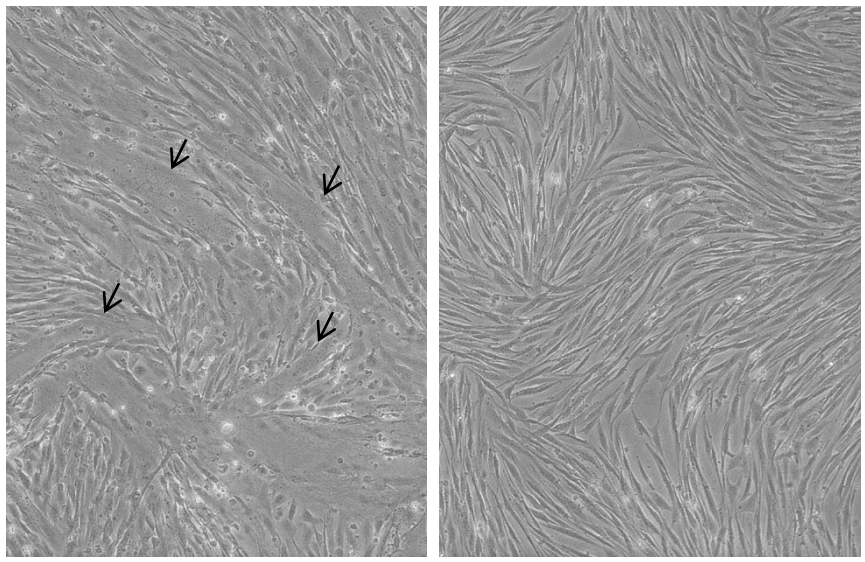

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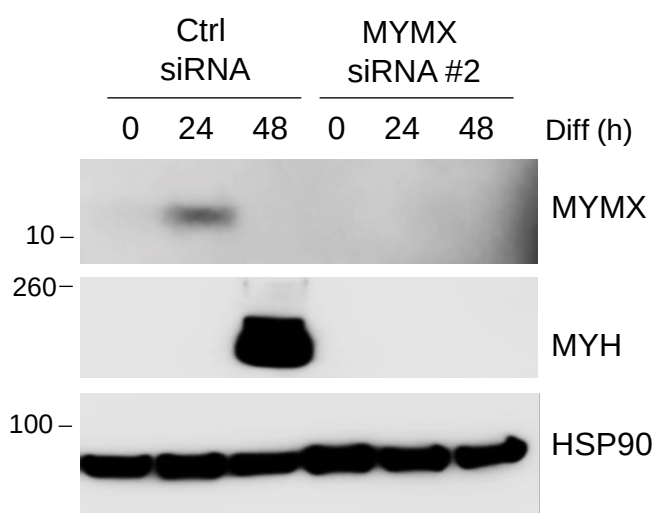
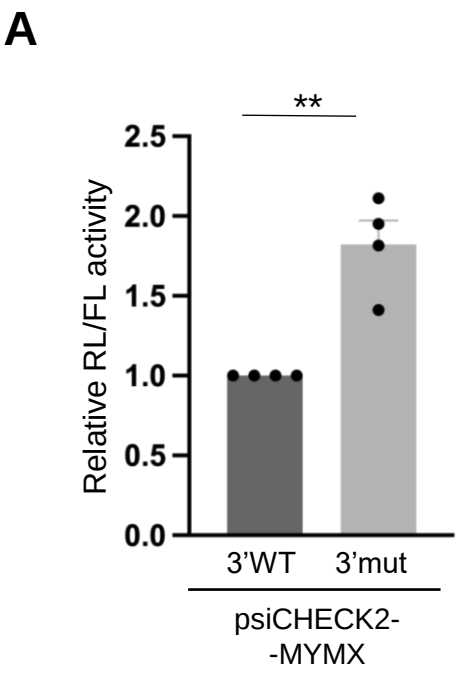
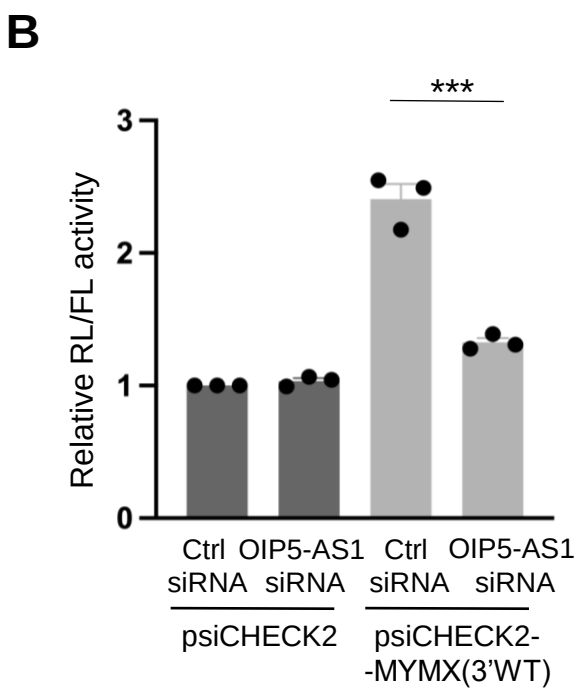
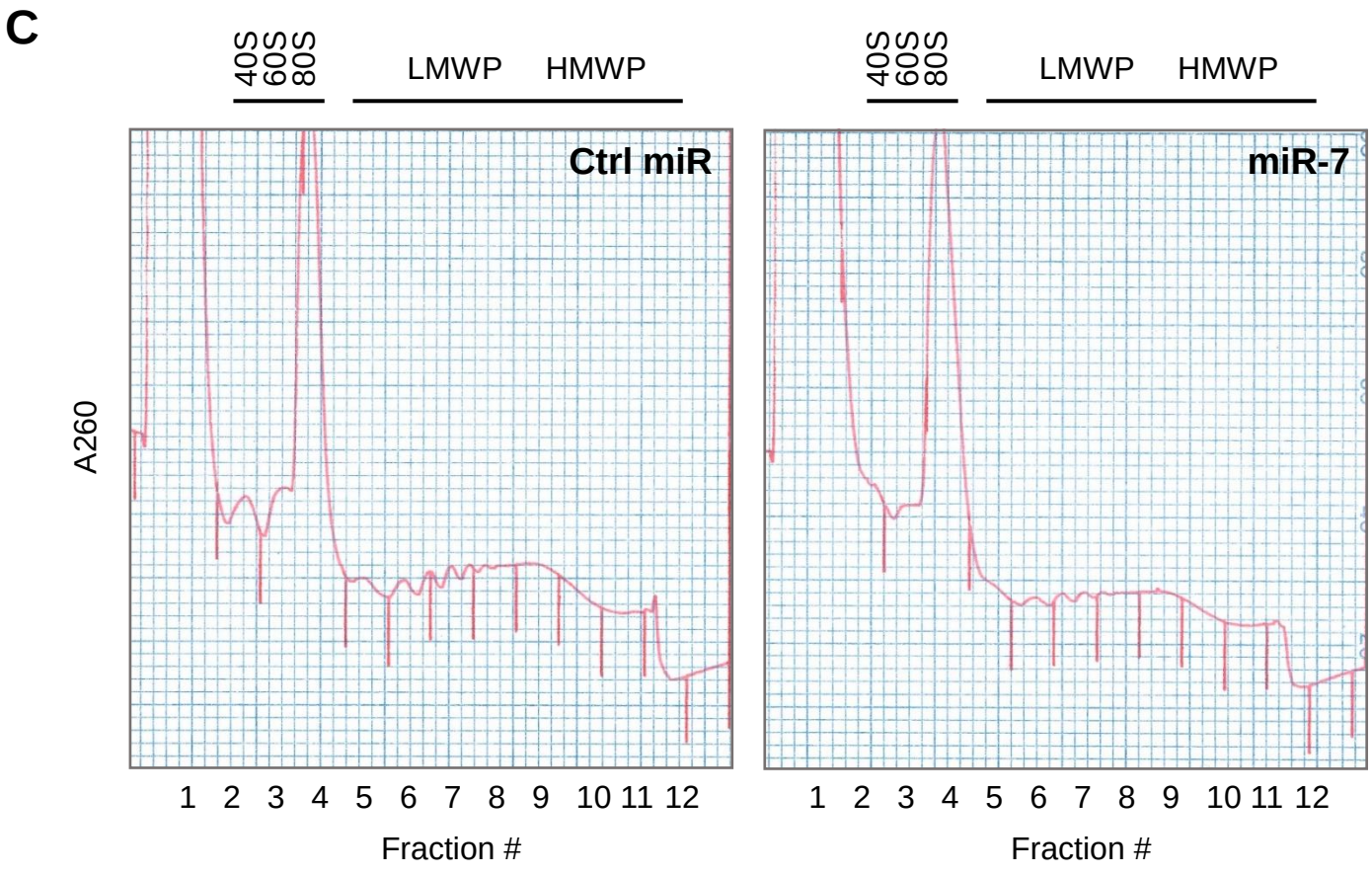
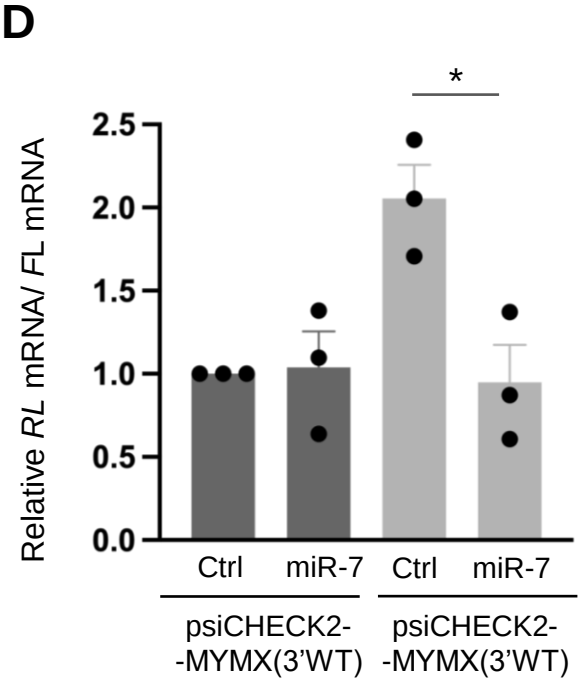


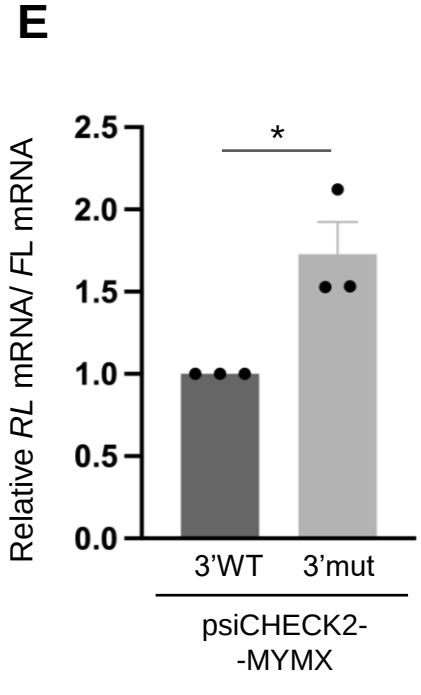
Yang et al. Supplementary Figure S3

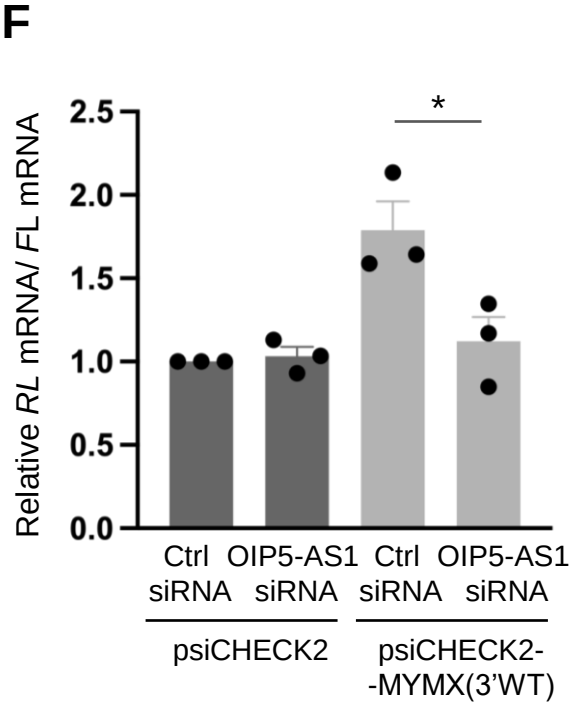










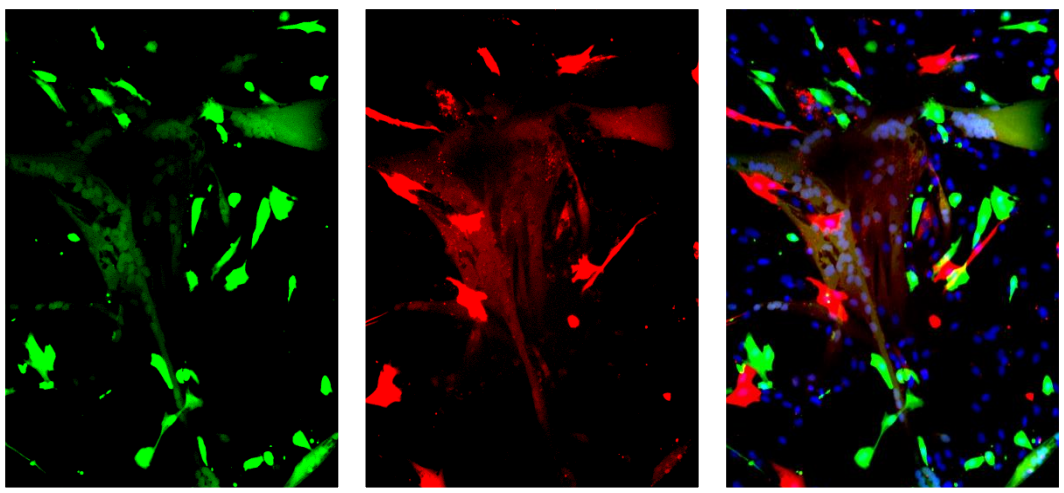


Yang et al. Supplementary Figure S4

A

EGFP
mCherry
DAPI

Ctrl miR



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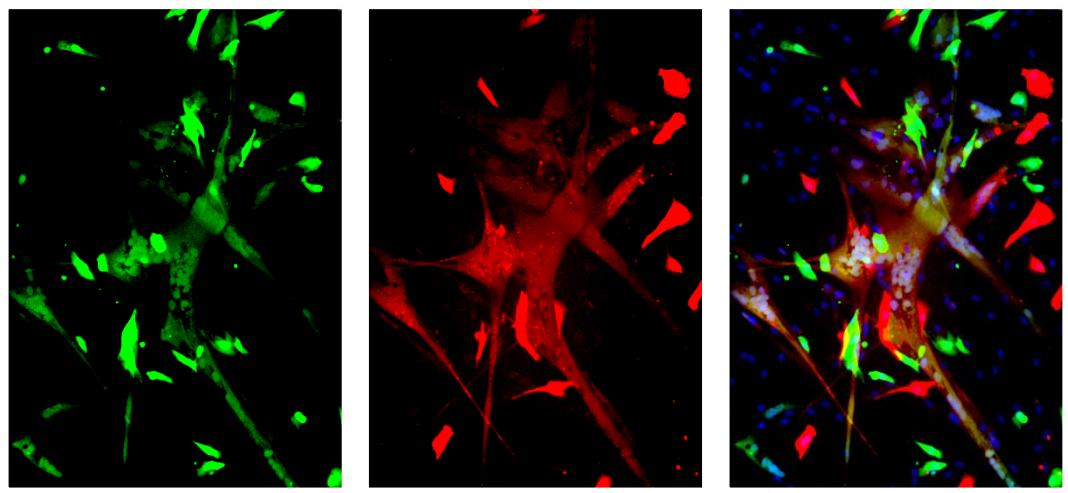

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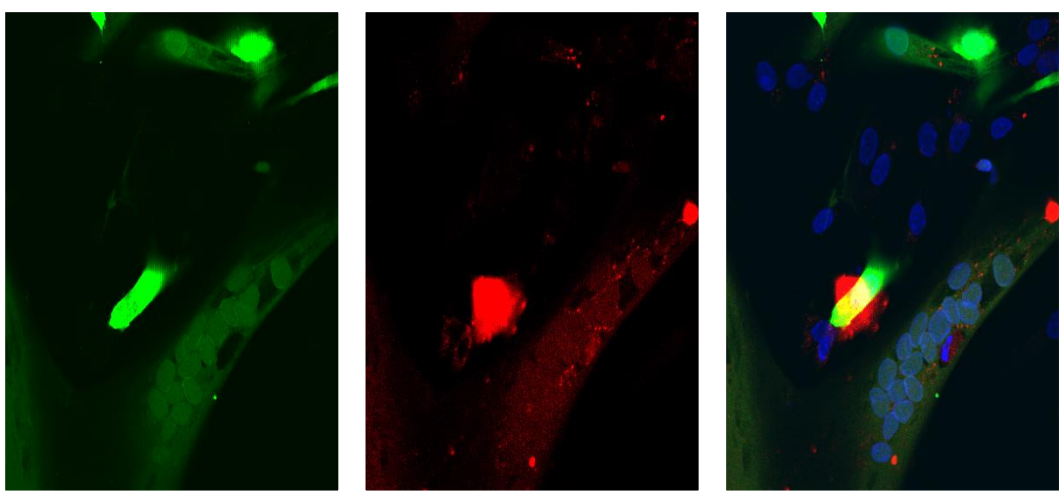

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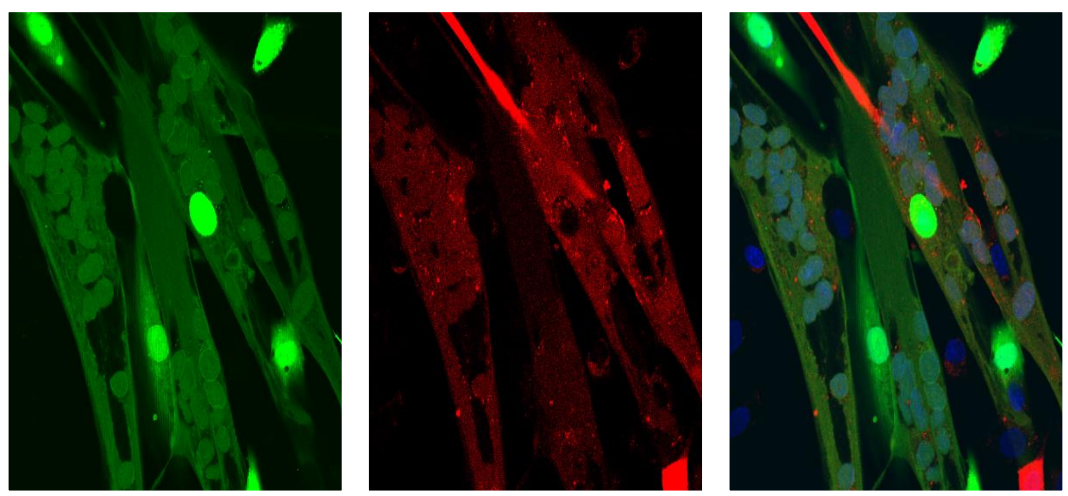
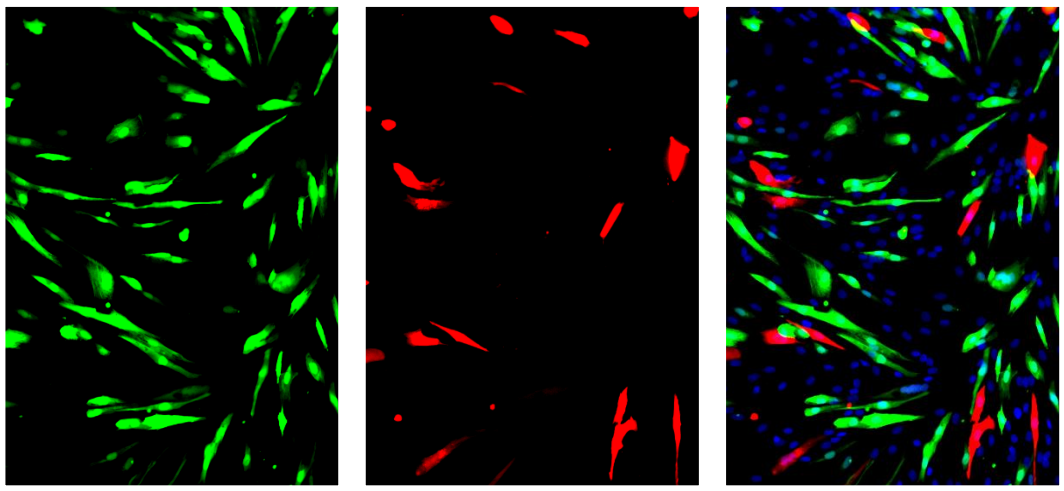


B

miR-7



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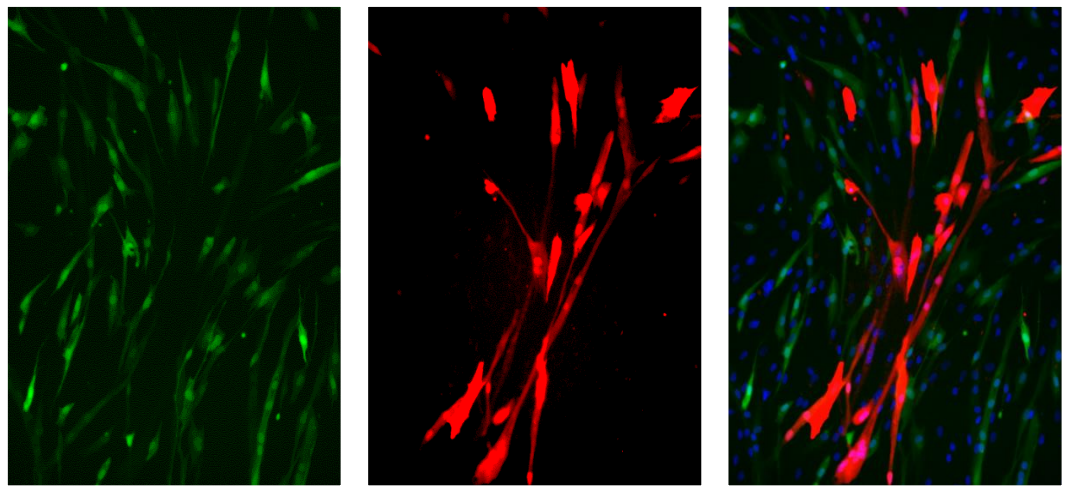

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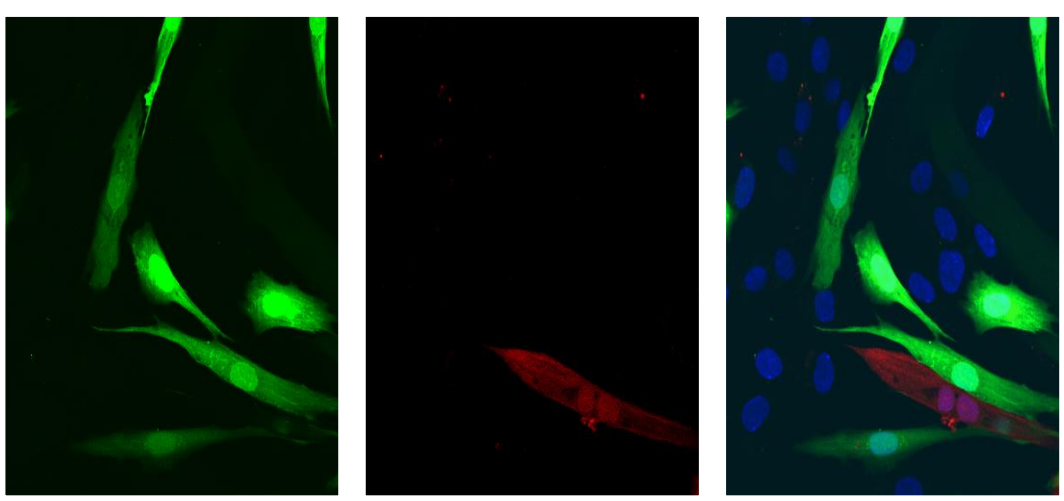

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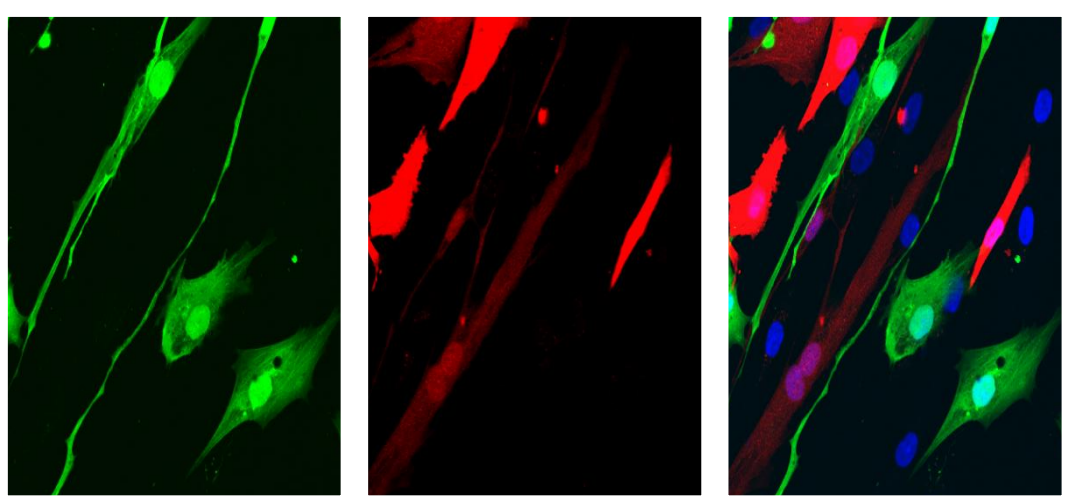
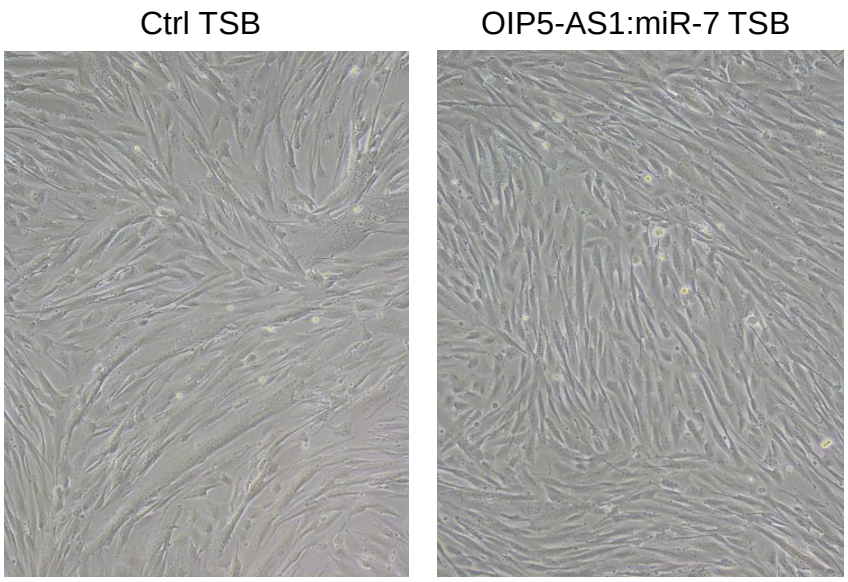


Yang et al. Supplementary Figure S5

A



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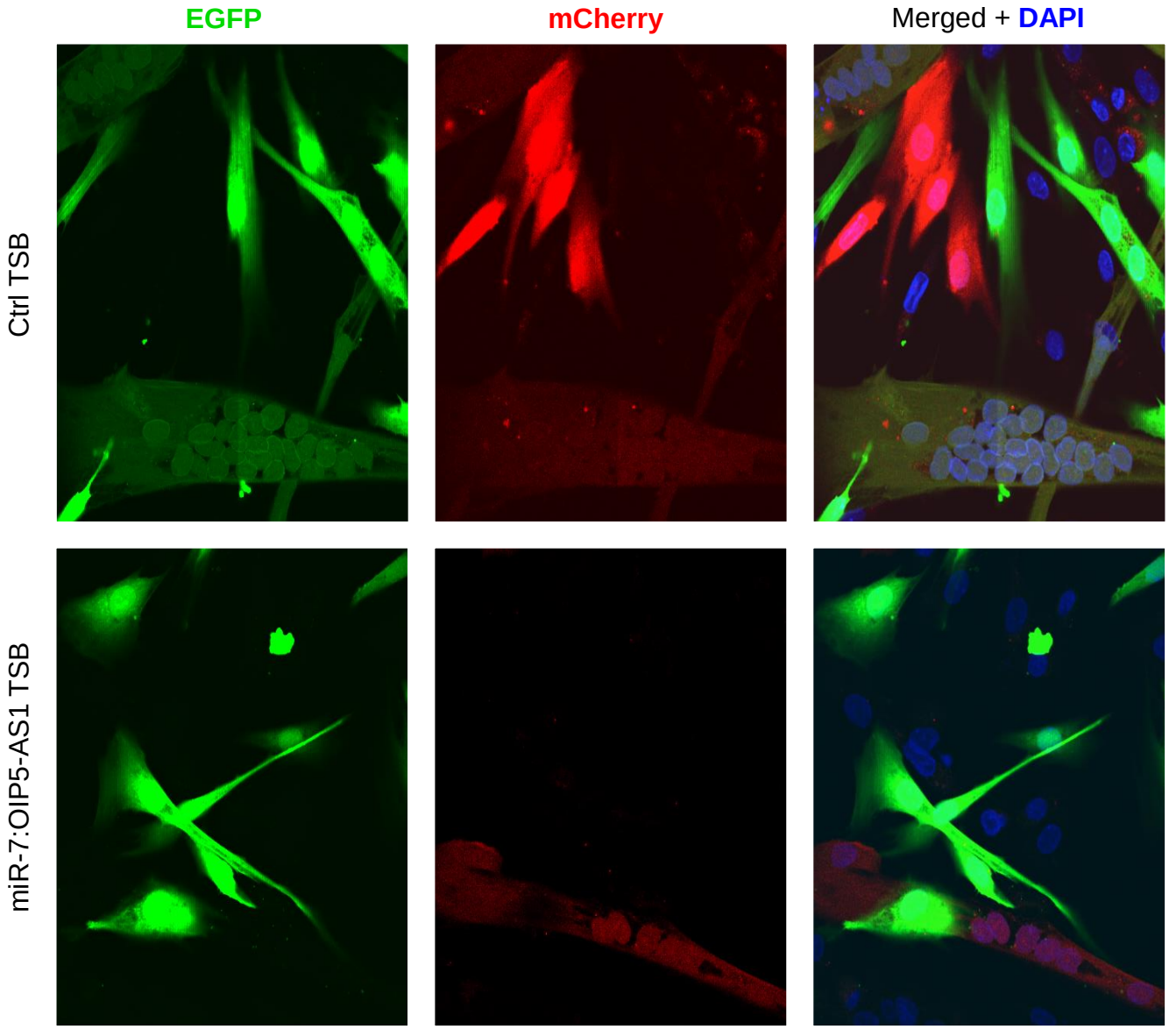
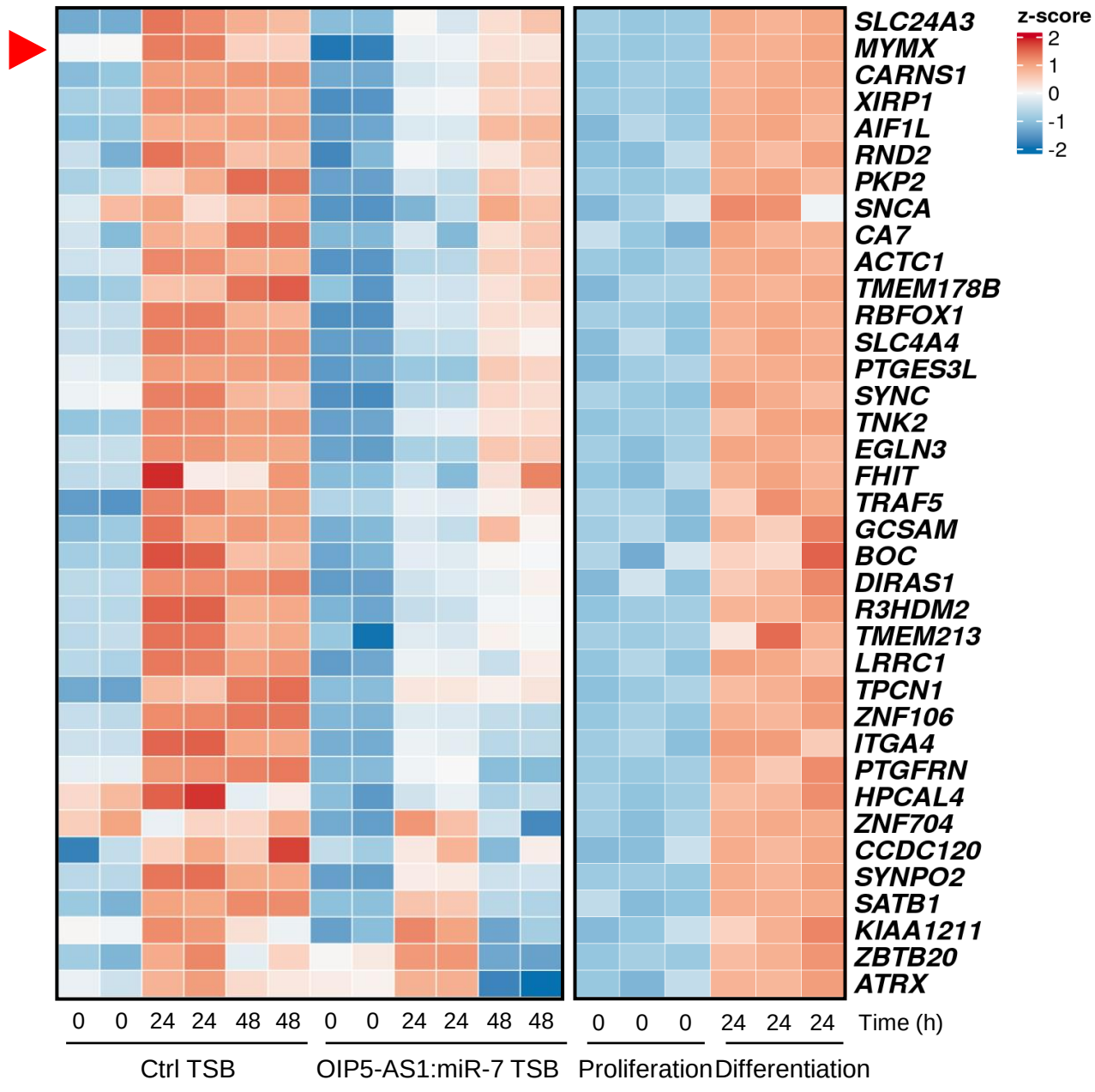
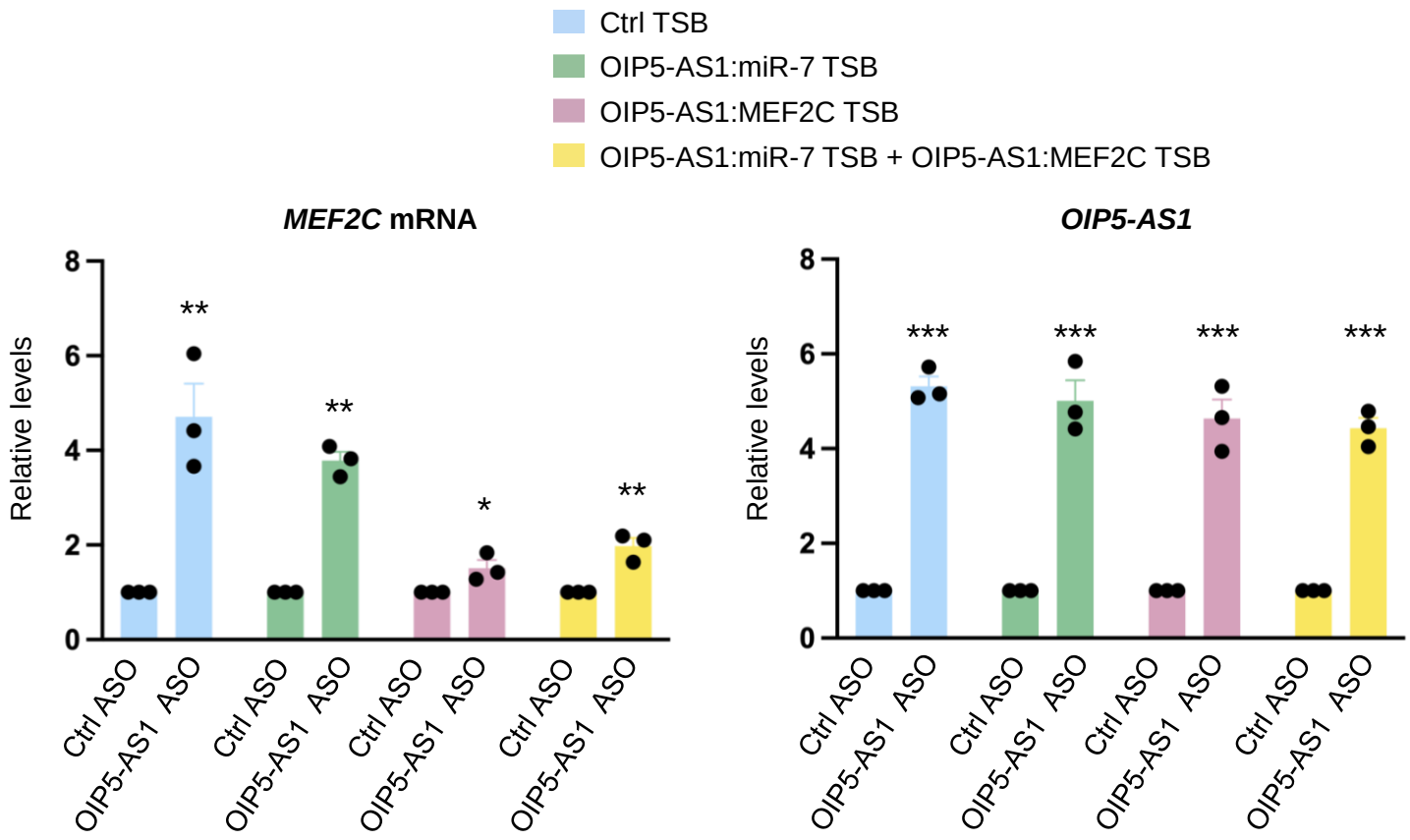


Yang et al. Supplementary Figure S6



Yang et al. Supplementary Figure S7

A



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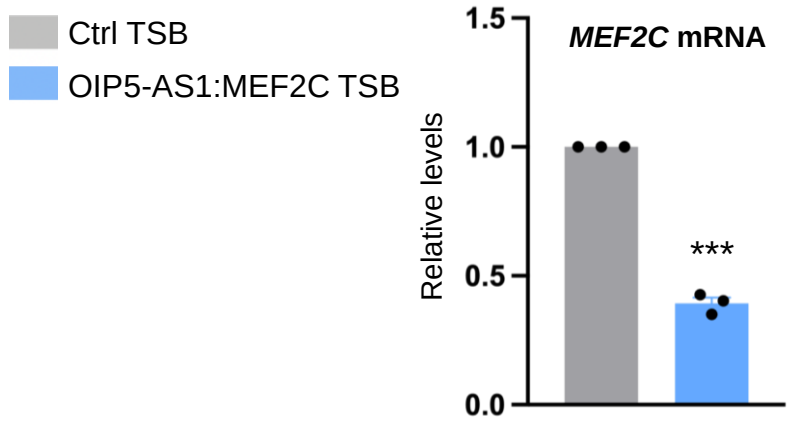

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