E2F1-miR-20a-5p/20b-5p auto-regulatory feedback loop involved in myoblast proliferation and differentiation

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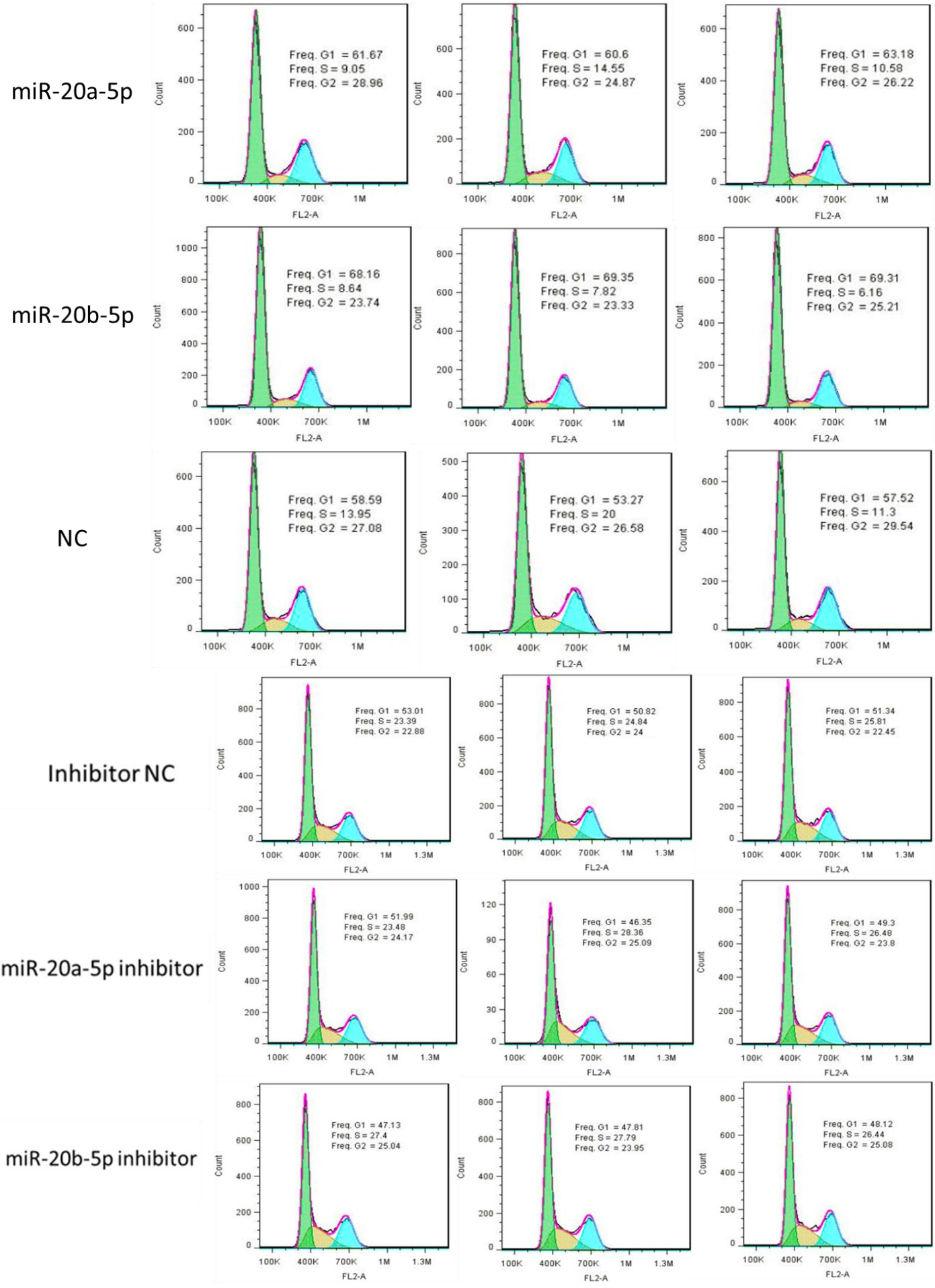
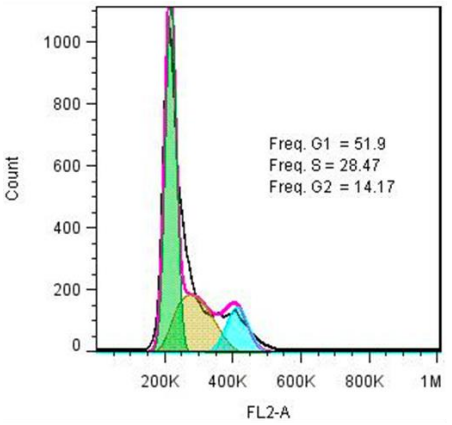
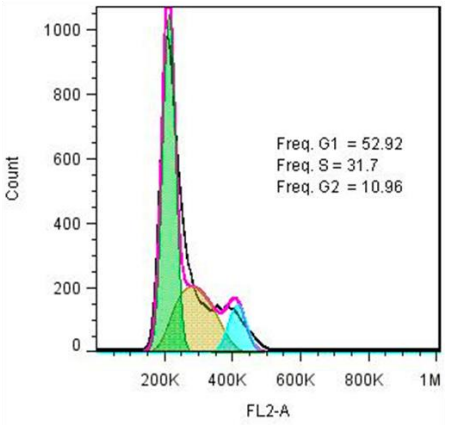
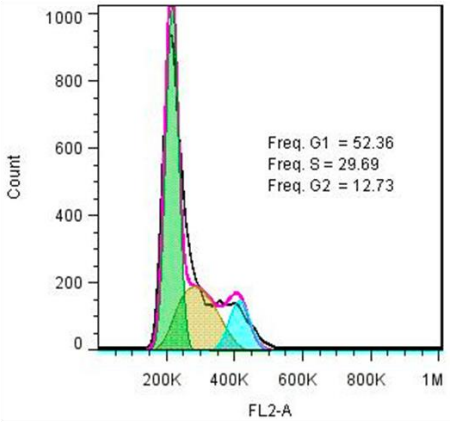
Supplementary file 1. The histograms of cell cycle analyzed by flow cytometry.



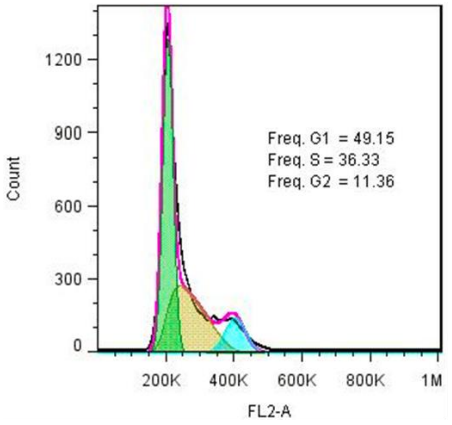
miR-20a-5p/20b-5p and pcDNA3.1-EGFP

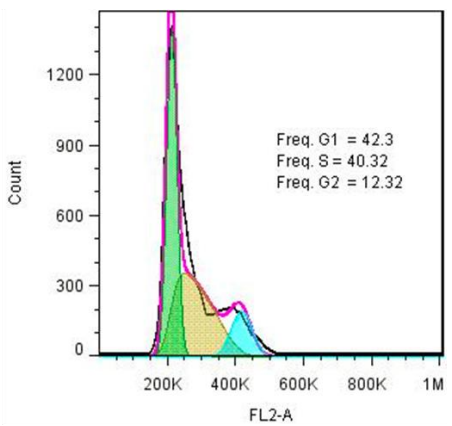


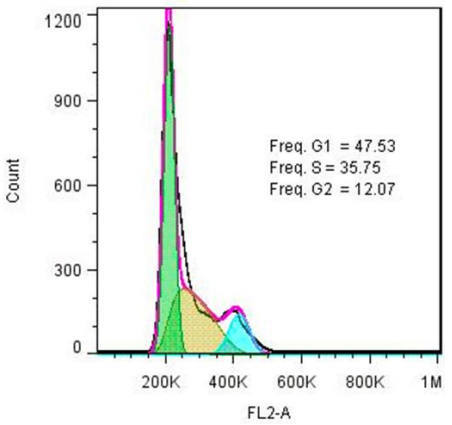




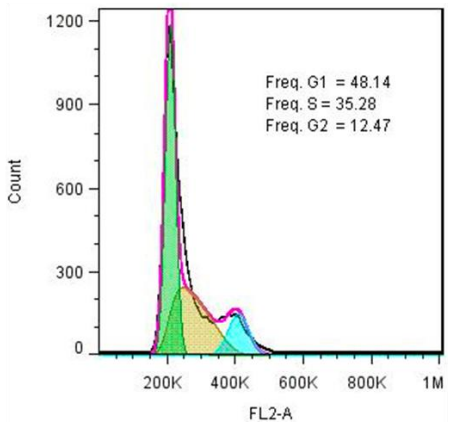
miR-20a-5p/20b-5p and pcDNA3.1-E2F1

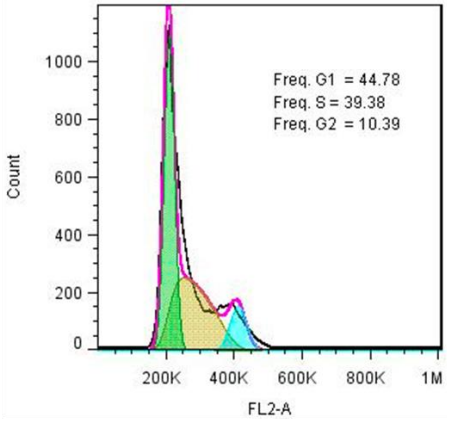


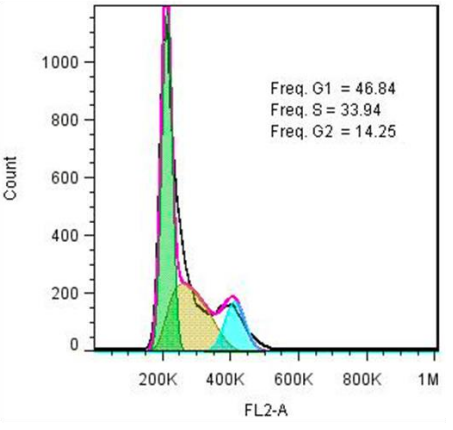




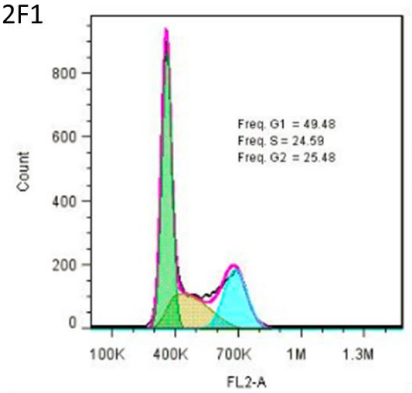
NC and pcDNA3.1-EGFP

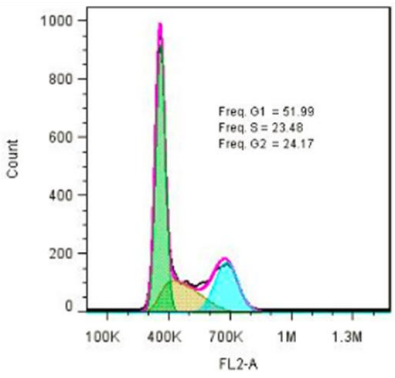


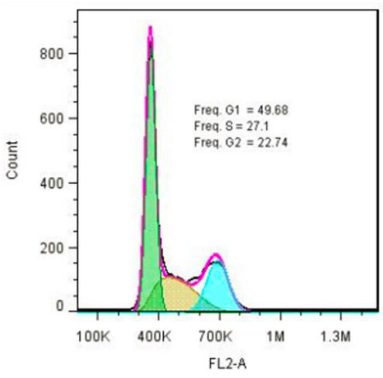




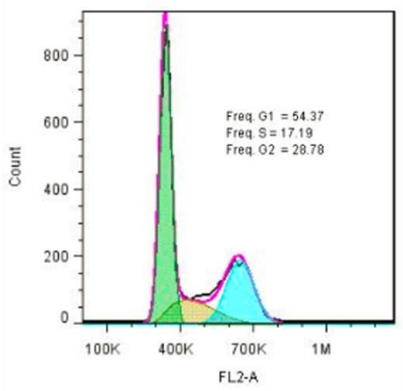
pcDNA3.1-E2F1

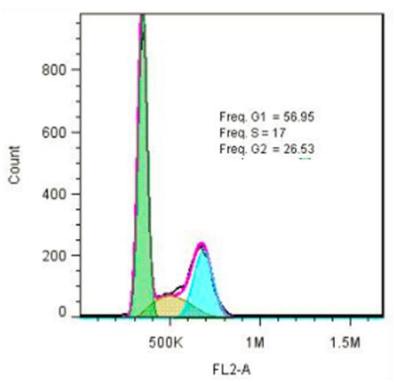


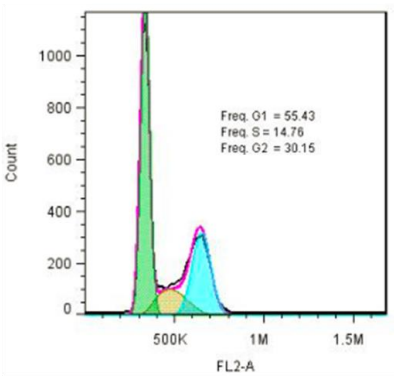




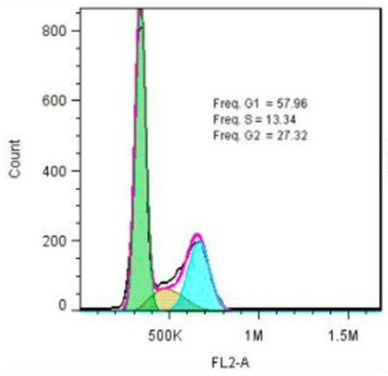
pcDNA3.1-EGFP

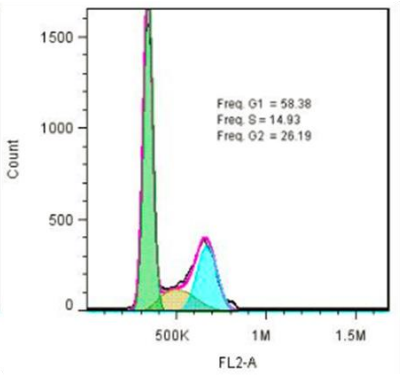


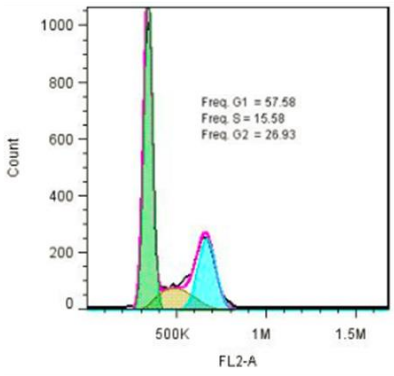




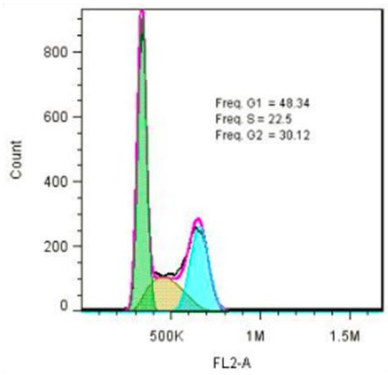
si-E2F1

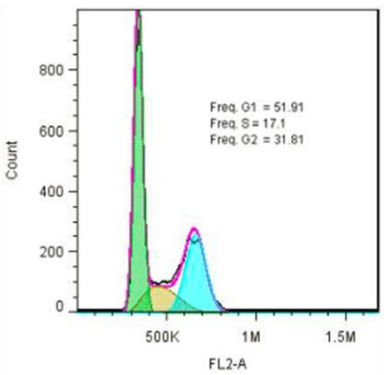


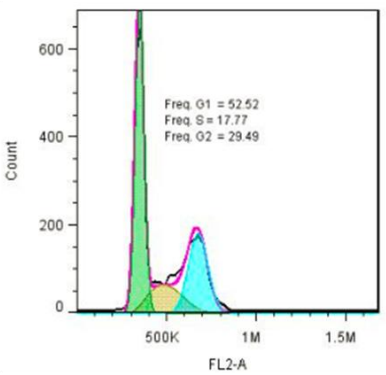




si-NC







Supplementary File 2.

Oligonucleotides:
For ChIP-qPCR or PCR analysis the following primers were used:
Amplico A:
s: 5’- GCACTTGTCCGAGCGGGAATC
as: 5’- CAGTCGCAGCTCATGGTGGC
Amplico B:
s: 5’ - TACTCGCTTTGGGTTGCTG
as: 5’ - CGGACGGCTGCTGGTTT
Amplico C:
s: 5’ - AAACAGTTGTATGGGTGA
as: 5’ - GACAGGGTCTTATTATGC
Amplico D:
s: 5’ - CCGGTCCTGCCATGTTT
as: 5’ - ACGCTTCCACTGCTCCTG
Amplico E:
s: 5’ - GAGAATGGAAAGATAAACACGG
as: 5’ - TTTTGGCTGGTCGGTGC

For cloning the following primers were used for PCR:
E2F1 CDS (reference sequence: NM_205219.1) cloning for pcDNA3.1:
s: 5’- GGGGTACCCCCGTCACCTGAGCCATG
as: 5’- CCGCTCGAGGAGGAGGAAACAAACCCAAAA
Reporter-1 cloning: s: 5’- cggGGTACCGGGAAAGGGAGGTCTCG
as: 5’- tccCCCGGGGGCTGTAAGTTACCCAAGC
Reporter-2 cloning: s: 5’- cggGGTACCAACCACCACAACACGCAATG
as: 5’- ccgCTCGAGATGTTGACCTCTCGCCATCG
Reporter-3 cloning: s: 5’- cGAGCTCTTTGGCTGGTCGGTGCG
as: 5’- tccCCCGGGGCGAAAGAAAAGAAAGAAAGG
Reporter-4 cloning: s: 5’- cggGGTACCAGAAGGGAAGAGTGAGCCG
as: 5’- ccgCTCGAGTCGCAGGAATGCCAAAA
E2F1 3’UTR Fragment (reference sequence: NM_205219.1):
s: 5’- CCGCTCGAGTGCCTCACTGCCAAGGG
as: 5’- ACGCGTCGACCGAAGCTCAGCGGAACAC

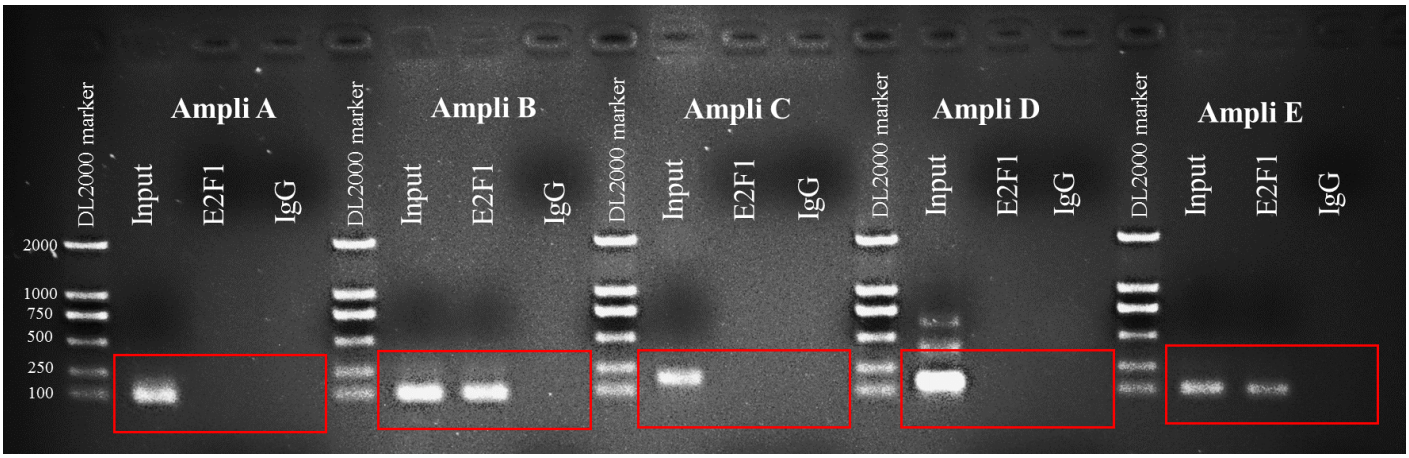
For mutagenesis the following primers were used for PCR:
E2F1-3’UTR-mut:
s: 5’- AGGCCCCGGGGCCAGGCTGGAGCCGAGAGCGCACTTACTGCCTTTAACAAGCTTGT
as: 5’- ACAAGCTTGTTAAAGGCAGTAAGTGCGCTCTCGGCTCCAGCCTGGCCCCGGGGCCT

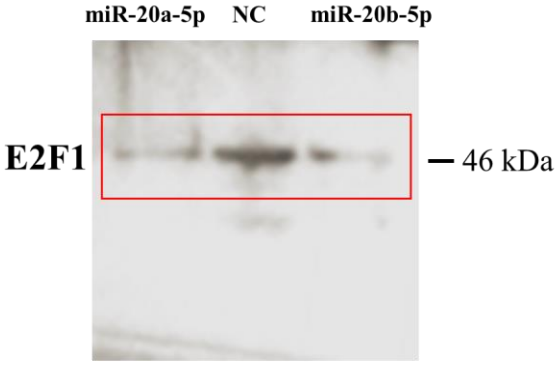
For quantitative real time RT-PCR, the following primers were used:
Myomaker-qPCR (reference sequence: KP230536.1): s: 5’- TGGGTGTCCCTGATGGC
as: 5’- CCCGATGGGTCCTGAGTAG
MYOD-qPCR (reference sequence: NM_204214.2): s: 5’- GCTACTACACGGAATCACCAAAT
as: 5’- CTGGGCTCCACTGTCACTCA
MYOG-qPCR (reference sequence: NM_204184.1): s: 5’- CGGAGGCTGAAGAAGGTGAA
as: 5’- CGGTCCTCTGCCTGGTCAT
MyHC-qPCR (reference sequence: ENSGALT00000001427):

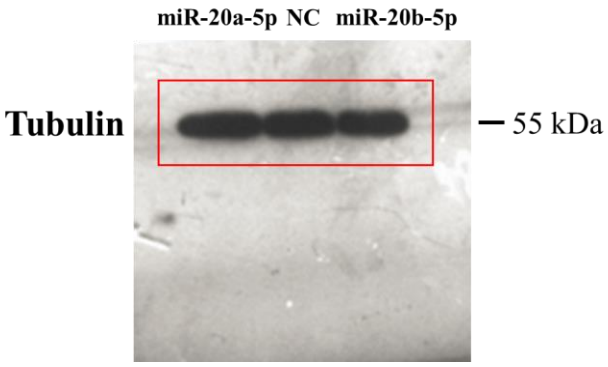
s: 5’- CTCCTCACGCTTTGGTAA
as: 5’- TGATAGTCGTATGGGTTGGT
E2F1-qPCR (reference sequence: NM_205219.1): s: 5’- AGACGGATCACCAGTACATAGCC
as: 5’- GTTCAGCGAGGTTTCATAGCG
β-actin-qPCR (reference sequence: NM_205518.1): s: 5’- TTGTTGACAATGGCTCCGGT
as: 5’- AACCATCACACCCTGATGTCT
pri-miR-17~92-qPCR (reference sequence: chicken genome, chr1: 147252628-147254423):
s: 5’- CCTGAGGCAGCTGTCAACAT
as: 5’- AAAGCTGGTTGCGTCAGAGT
pri-miR-106a~363-qPCR (reference sequence: chicken genome, chr4: 3945818-3947210):
s: 5’- TGTGGAGGAAAGGAGGAC
as: 5’- TTGCGGTTTACAGATGGA

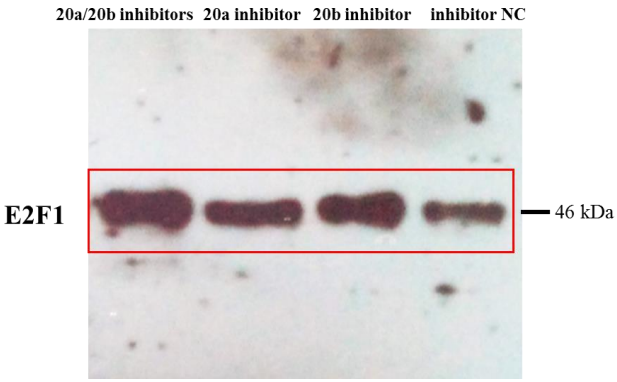
Supplementary File 3.

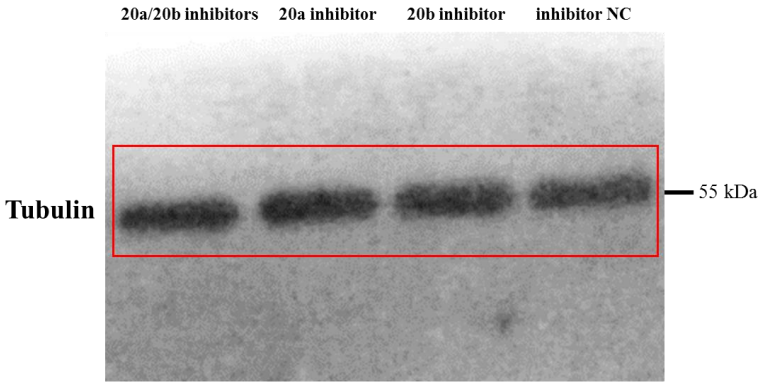
The used in the main figures of full-gels are shown. All cropped gels have been run under the same experimental condition.

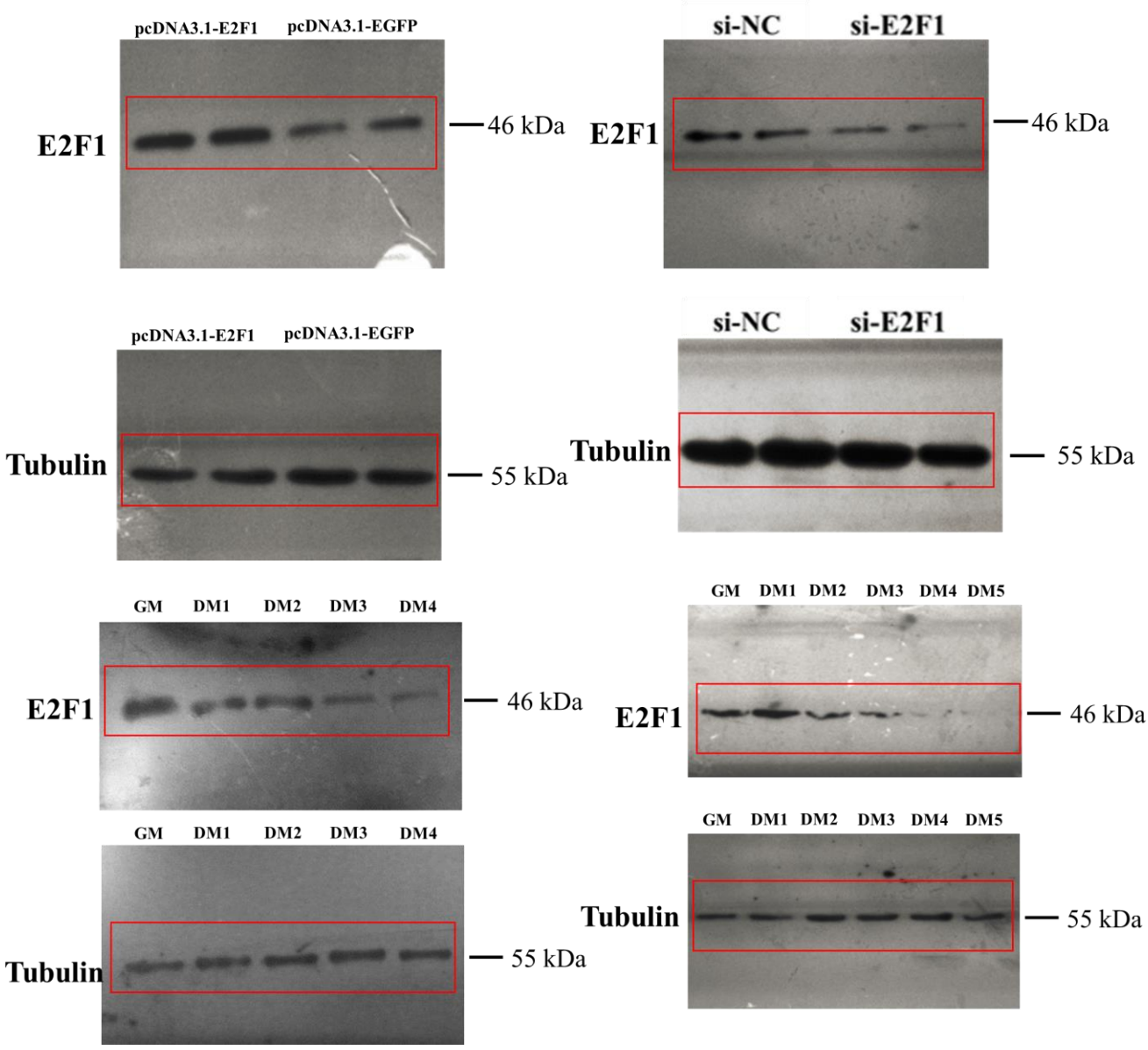








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pcDNA3.1-EGFP
E2F1
46 kDa
si-NC
si-E2F1
E2F1
46 kDa
pcDNA3.1-E2F1
pcDNA3.1-EGFP
Tubulin
55 kDa
si-NC
si-E2F1
Tubulin
55 kDa
GM
DM1
DM2
DM3
DM4
E2F1
46 kDa
GM
DM1
DM2
DM3
DM4
DM5
E2F1
46 kDa
GM
DM1
DM2
DM3
DM4
Tubulin
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GM
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DM2
DM3
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DM5
Tubulin
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