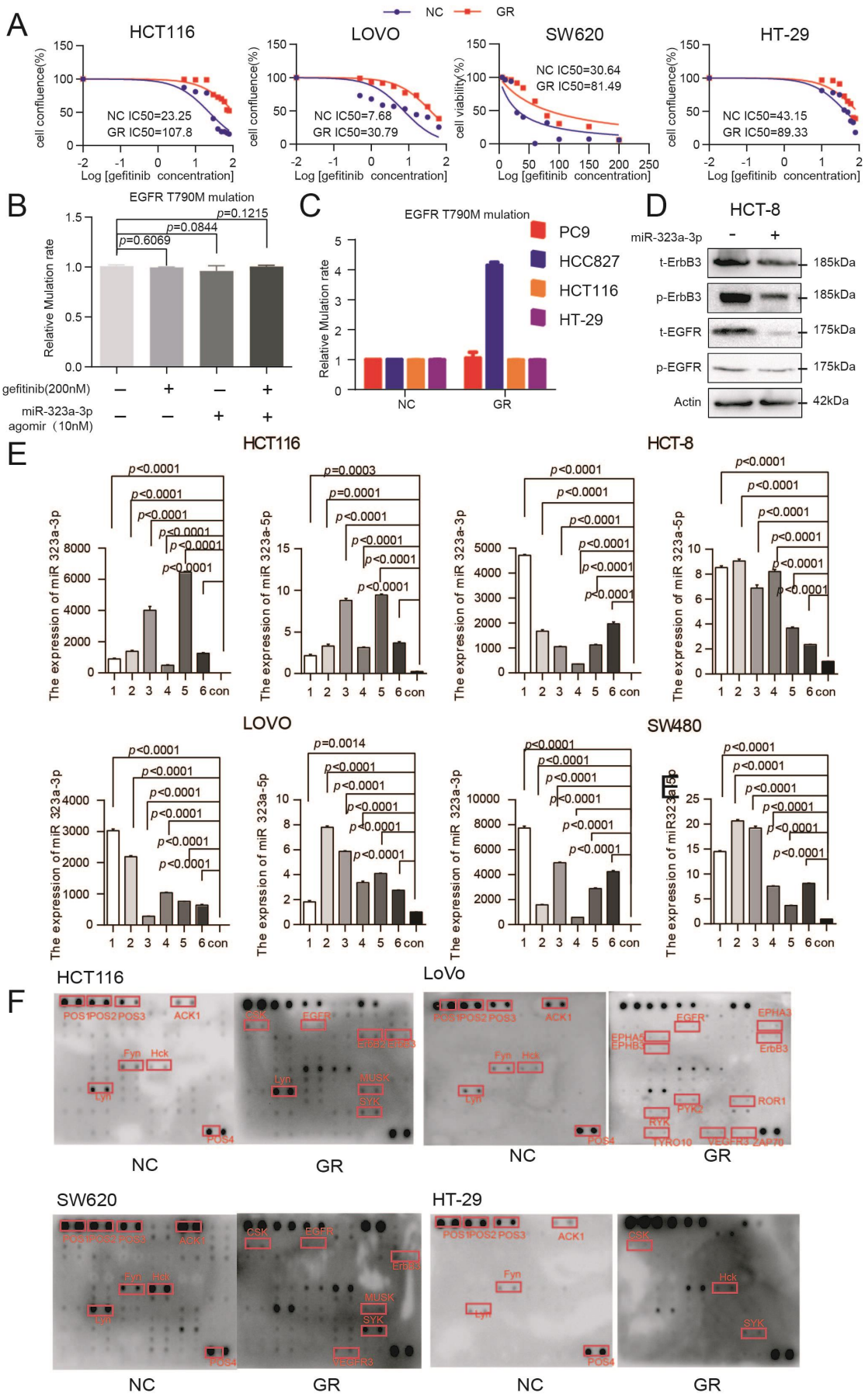
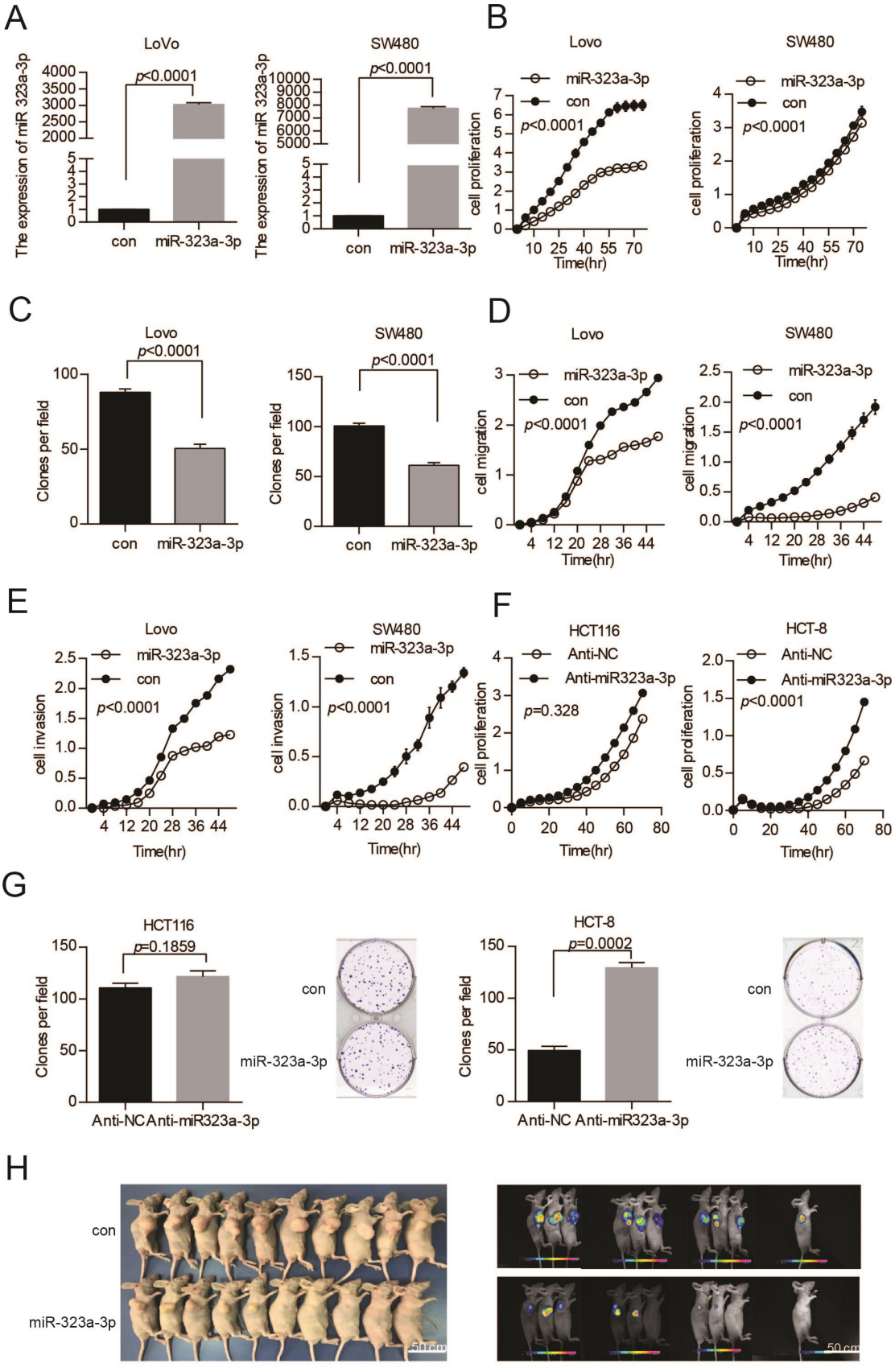

A
NC
GR
HCT116
LOVO
SW620
HT-29
cell confluence(%)
cell viability(%)
Log [gefitinib concentration]
NC IC50=23.25
GR IC50=107.8
NC IC50=7.68
GR IC50=30.79
NC IC50=30.64
GR IC50=81.49
NC IC50=43.15
GR IC50=89.33
B
EGFR T790M mulation
Relative Mulation rate
p=0.6069
p=0.0844
p=0.1215
gefitinib(200nM)
miR-323a-3p agomir（10nM）
C
EGFR T790M mulation
PC9
HCC827
HCT116
HT-29
NC
GR
D
HCT-8
miR-323a-3p
t-ErbB3
p-ErbB3
t-EGFR
p-EGFR
Actin
185kDa
185kDa
175kDa
175kDa
42kDa
E
HCT116
HCT-8
LOVO
SW480
The expression of miR 323a-3p
The expression of miR 323a-5p
p<0.0001
p=0.0003
p=0.0001
p=0.0014
1 2 3 4 5 6 con
F
HCT116
LoVo
SW620
HT-29
NC
GR
POS1 POS2 POS3
ACK1
CSK
EGFR
ErbB2
ErbB3
Fyn
Hck
Lyn
MUSK
SYK
POS4
EPHA3
EPHA5
EPHB3
PYK2
ROR1
RYK
TYRO10
VEGFR3
ZAP70

S Figure1 Construction of gefitinib-resistant cell lines, establishment of miR-323a-3p overexpressing cell lines and detection of drug-resistant cell line RTK phosphorylation.

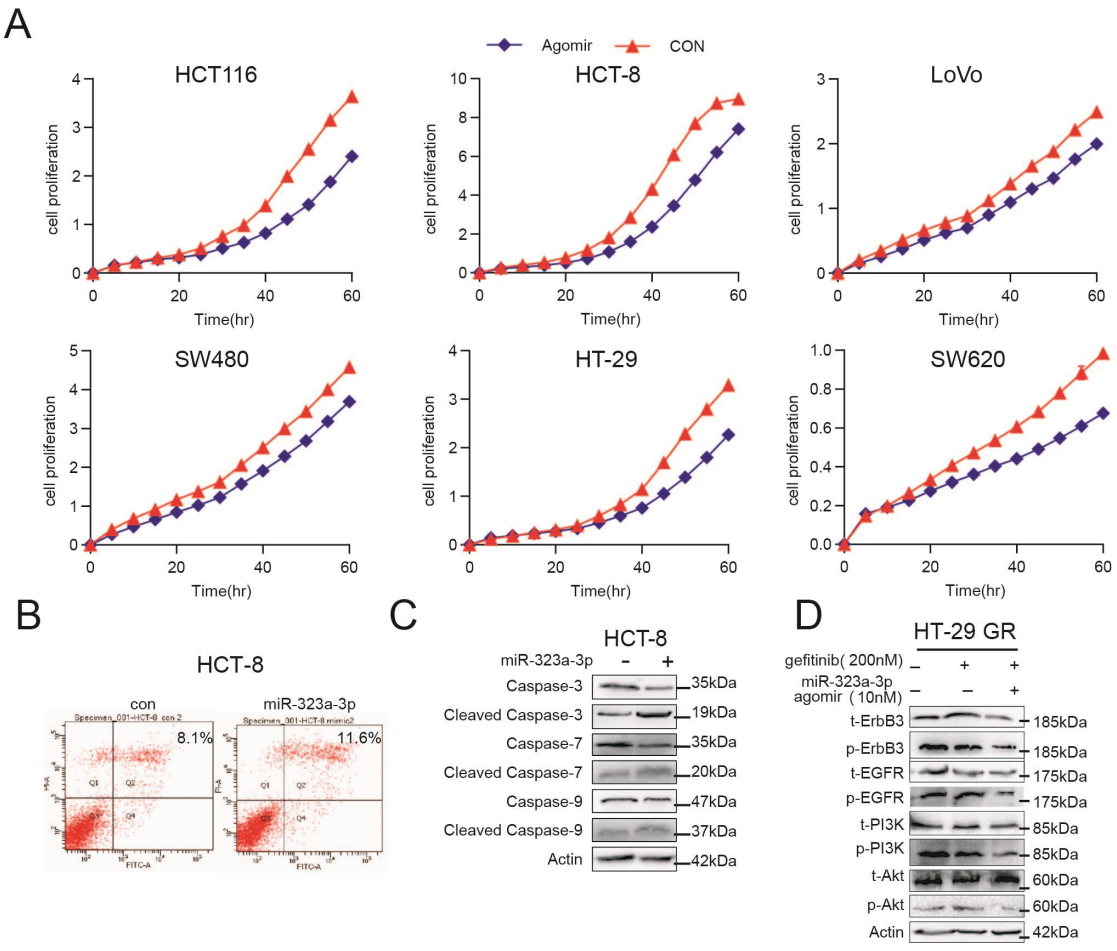
A. Construction of HCT116, LoVo, SW620 and HT-29 gefitinib resistant cell lines and their relative IC50. B. MiR-323a-3p combined with gefitinib showed no mutation in EGFR T790M in subcutaneous tumor tissues. C. There was no EGFR T790M mutation in gefitinib-resistant CRC cell lines, and HCC827 was A positive control. D. Mir-323a-3p inhibited the expression of EGFR/ErbB3 in HCT-8 cell line. E. Construction of four stable overexpression 323 cell lines (HCT116, HCT-8, LoVo and SW480). F. Raw data of RTK phosphorylation in HCT116, LoVo, SW620 and HT-29.

S-Figure 2


A
LoVo
p<0.0001
The expression of miR 323a-3p
con
miR-323a-3p
SW480
p<0.0001
B
Lovo
miR-323a-3p
con
p<0.0001
cell proliferation
Time(hr)
SW480
C
Lovo
p<0.0001
Clones per field
SW480
D
Lovo
cell migration
SW480
E
Lovo
cell invasion
SW480
F
HCT116
Anti-NC
Anti-miR323a-3p
p=0.328
HCT-8
p<0.0001
G
HCT116
p=0.1859
Anti-NC Anti-miR323a-3p
HCT-8
p=0.0002
H

S Figure2　MiR-323a-3p inhibits tumor biology by targeting EGFR/ERBB3.

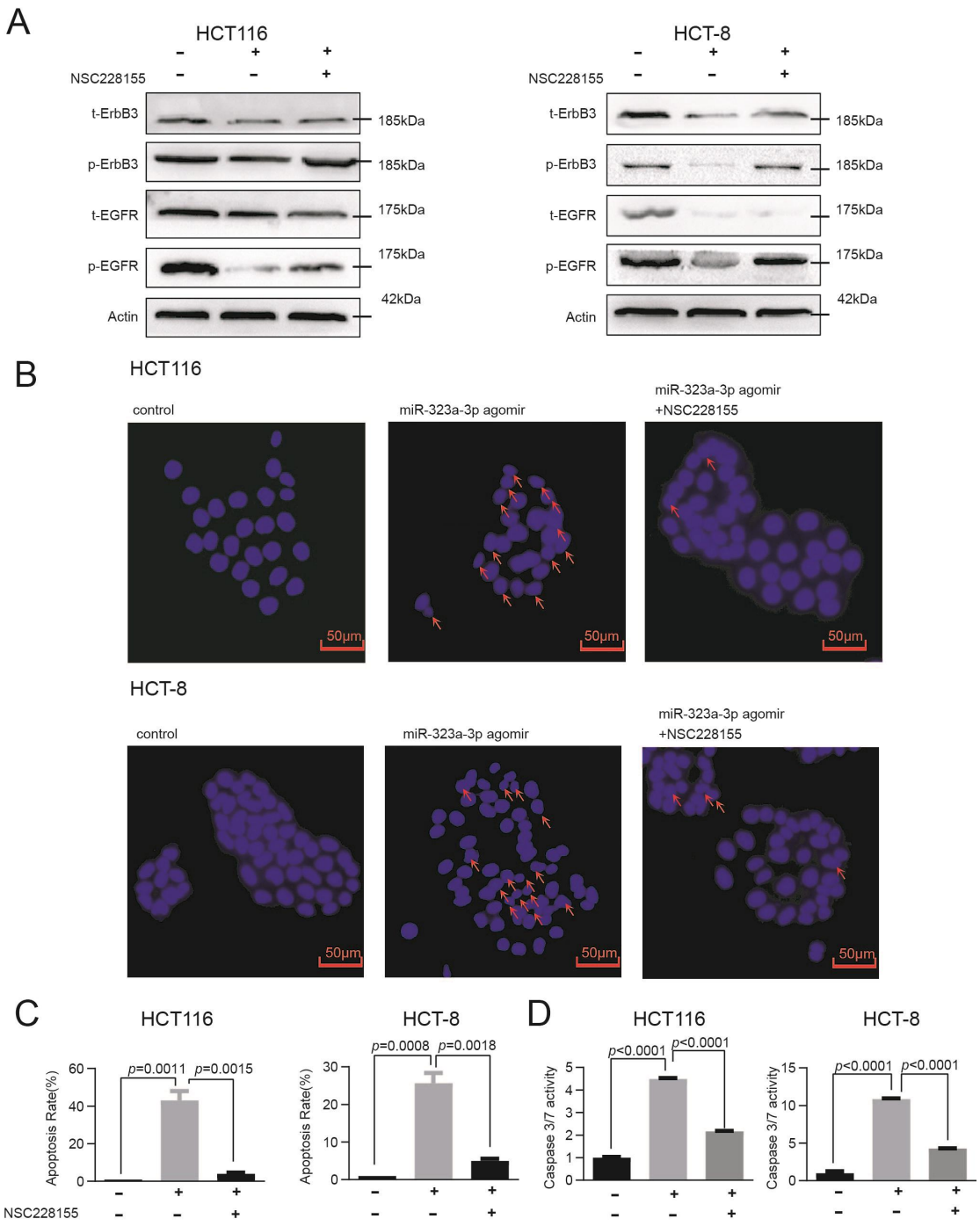
A. miR-323a-3p inhibited RNA levels of EGFR/ ErbB3 in HCT116 and HCT-8. B. miR-323a-3p inhibited the proliferation of SW480 and HCT-8 cells. C. Verification of miR-323a-3p overexpression in HCT116, HCT-8, LOVO and SW480 cell lines. D. miR-323a-3p inhibited the migration of HCT116, HCT-8, LOVO and SW480 cell lines. E. miR-323a-3p inhibited the invasion of HCT116, HCT-8, LOVO and SW480 cell lines. F. Inhibition of the expression of miR-323a-3p promoted the formation in HCT-8 and HCT116 cell lines. G. miR-323a-3p inhibited the growth of subcutaneous tumor of HCT116.



S Figure3 MiR-323a-3p promotes apoptosis by inhibiting activation of EGFR/ ErbB3-PI3K /Akt.

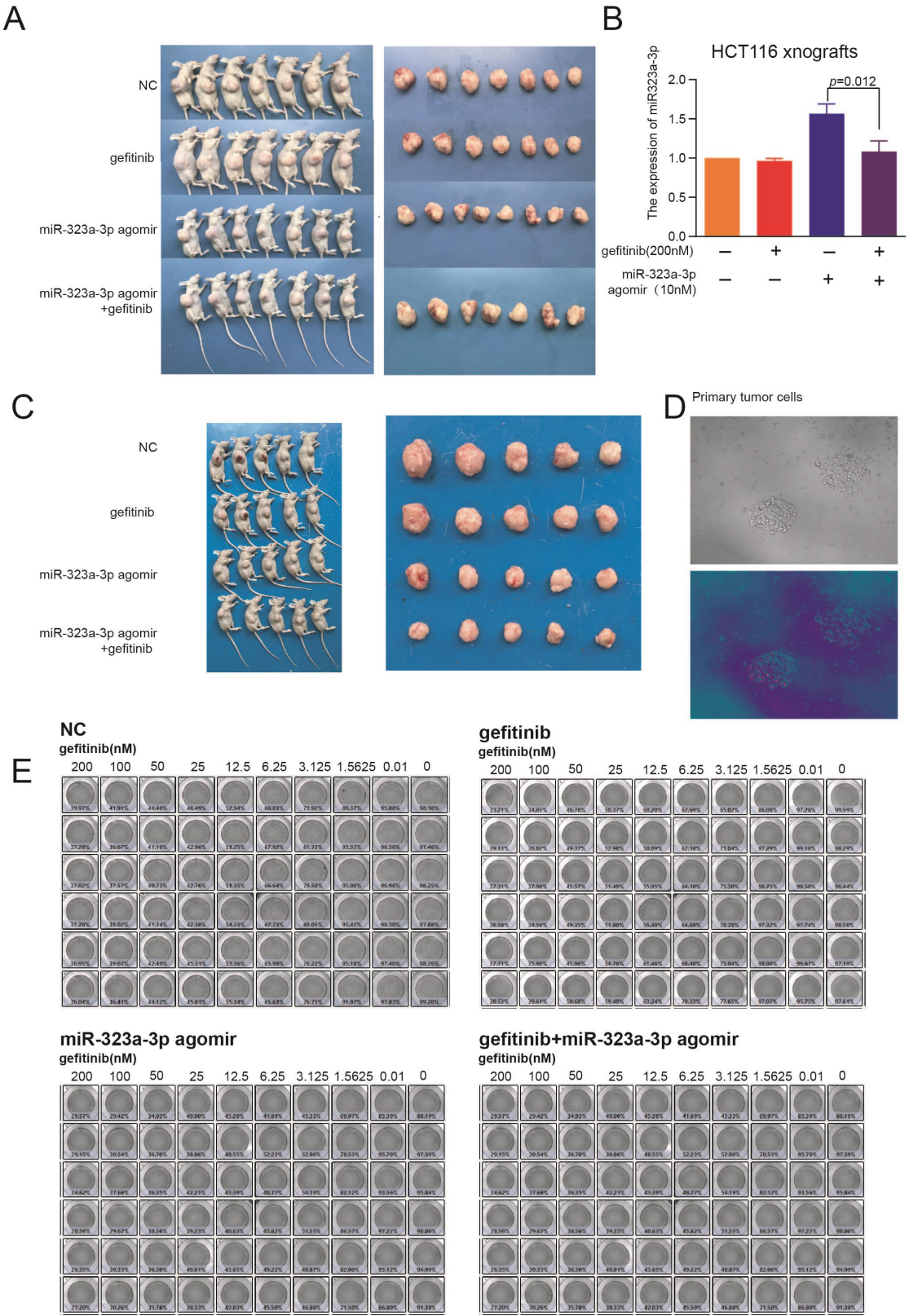
A. miR-323a-3p inhibits cell proliferation in HCT116, HCT-8, LOVO, HT-29,

SW620 and SW480. B. miR-323a-3p promoted the apoptosis rate of HCT-8 cell line. C. miR-323a-3p promotes apoptosis marker in HCT-8 cell line. D. Phosphorylated EGFR, ErbB3, PI3K and Akt proteins in HT-29 GR and LoVo GR cells did not change after gefitinib administration, but they were significantly reduced after the addition of agomir (n=3 per group).



S Figure4 Rescue experiment of miR-323a-3p targeting EGFR/ErbB3.

A. Phosphorylated protein expression levels of EGFR and ErbB3 in HCT116 and HCT-8 were significantly increased by NSC228155 (EGFR agonist). B. Hoechst 33258 fluorescent dye in HCT116 and HCT-8 were decreased by NSC228155. C. Analysis of the Hoechst 33258 fluorescent dye apoptosis rate. D. Caspase3/7 activity in HCT116 and HCT-8 were decreased by NSC228155.



S Figure5 MiR-323a-3p and gefitinib synergistically inhibit tumor growth, and miR-323a-3p blocks acquired gefitinib resistance formation in a xenograft model.

A. The volume of subcutaneous tumors decreased more significantly with the combination of gefitinib and agomir administration than with single administration. B.

miR-323a-3p was metabolized over a period of time in nude mice. C. Subcutaneous tumors grew more slowly and survived longer with the combination of gefitinib and agomir than with single dosing of either agent. D. Construction of primary tumor cells. E. The IC50 of gefitinib in tumor progenitor cells in the xenograft model was much lower in the coadministered group than in the single agent-administered group.