Supplementary Information

Metabolic synthetic lethality by targeting NOP56 and mTOR signaling in *KRAS*-mutant lung cancer

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   Table S2. Inhibitors used for synthetic lethal chemical screens
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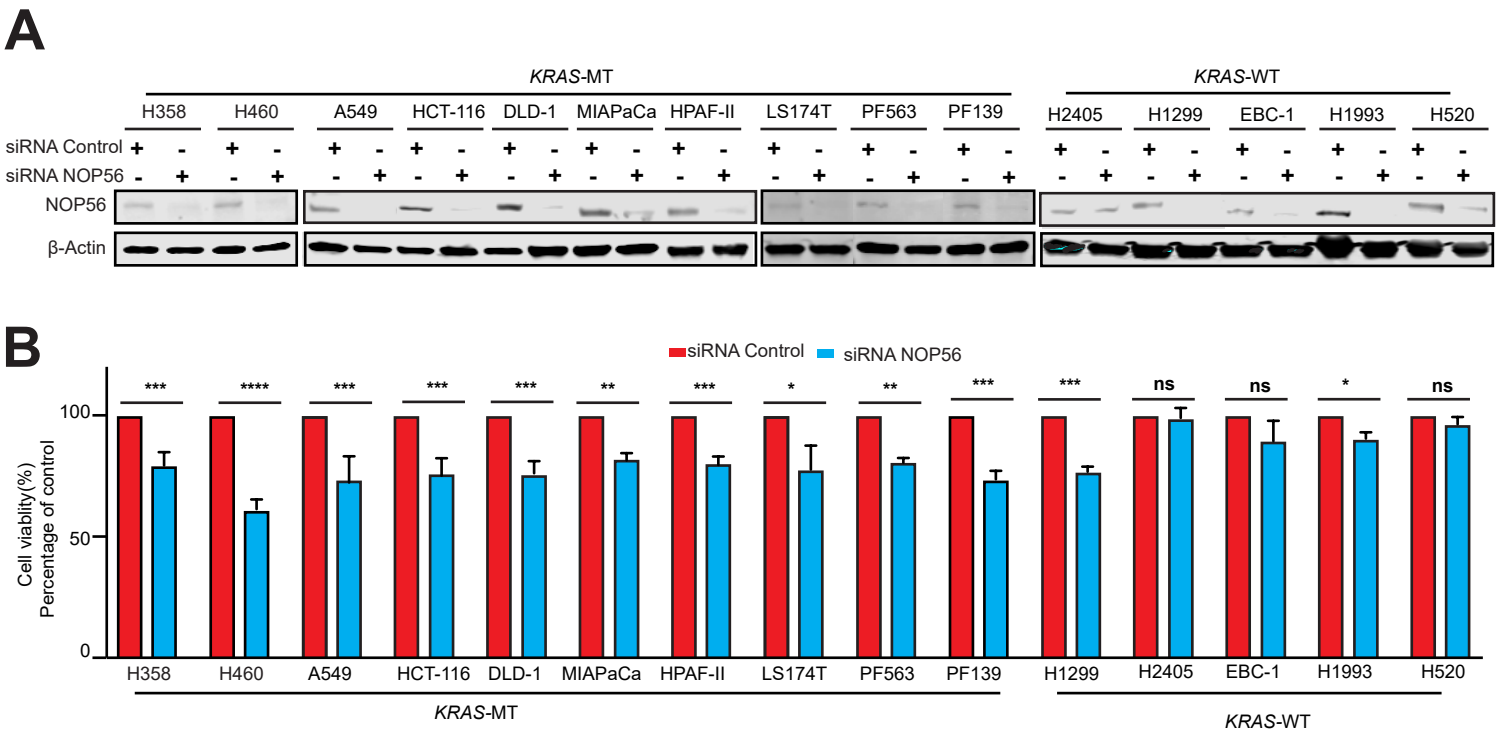
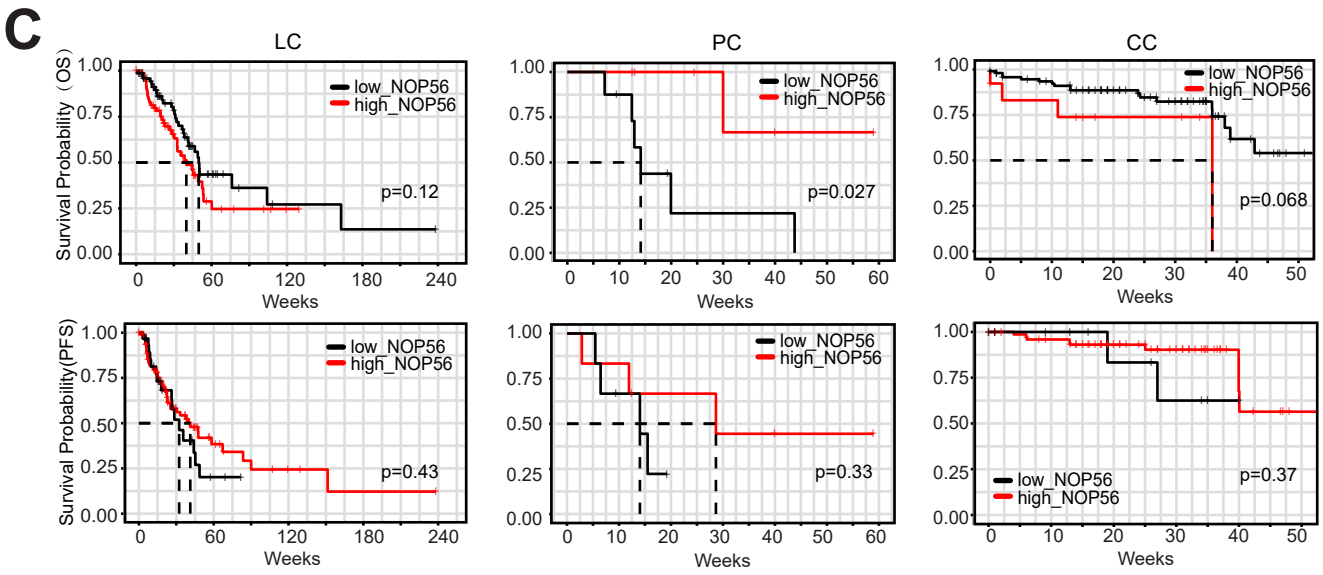
Figure S1


A
KRAS-MT
KRAS-WT
H358
H460
A549
HCT-116
DLD-1
MIAPaCa
HPAF-II
LS174T
PF563
PF139
H2405
H1299
EBC-1
H1993
H520
siRNA Control
siRNA NOP56
NOP56
β-Actin

B
siRNA Control
siRNA NOP56
Cell viability(%)
Percentage of control
100
50
0
H358
H460
A549
HCT-116
DLD-1
MIAPaCa
HPAF-II
LS174T
PF563
PF139
H1299
H2405
EBC-1
H1993
H520
KRAS-MT
KRAS-WT


C
LC
PC
CC
Survival Probability（OS）
Survival Probability(PFS)
low_NOP56
high_NOP56
p=0.12
p=0.027
p=0.068
p=0.43
p=0.33
p=0.37
Weeks

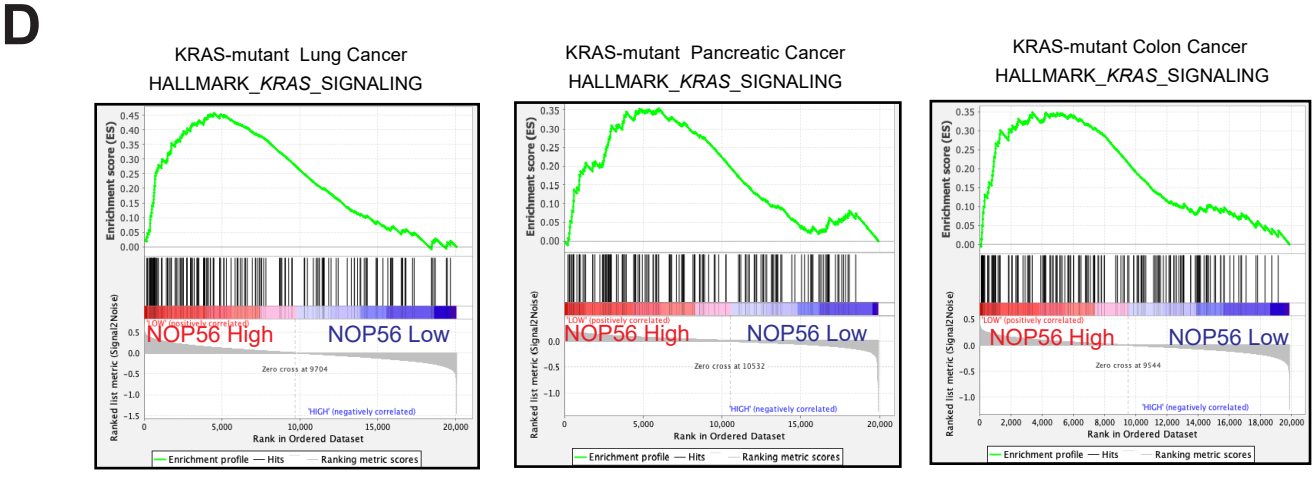

D
KRAS-mutant Lung Cancer
HALLMARK_KRAS_SIGNALING
KRAS-mutant Pancreatic Cancer
HALLMARK_KRAS_SIGNALING
KRAS-mutant Colon Cancer
HALLMARK_KRAS_SIGNALING
Enrichment score (ES)
Ranked list metric (Signal2Noise)
NOP56 High
NOP56 Low
Rank in Ordered Dataset
Enrichment profile
Hits
Ranking metric scores

Figure S1: NOP56 knockdown inhibits proliferation of *KRAS*-mutant cancer cells.

A, Immunoblots of *KRAS*-mutant and *KRAS*-wild type cancer cells that were transfected with *NOP56*-specific siRNAs (si-*NOP56*) or scramble control siRNAs (si-Control).

B, KRAS mutant and KRAS wild type cancer cells were transfected with control siRNAs or *NOP56*-specific siRNAs. Cell viability was determined 72 hours post transfection. Data are presented as mean ± SD (n=3). **p*<0.05, ***p*<0.01, ****P*<0.001, *****P*<0.0001 and ns *P* > 0.05 by two-way ANOVA with Tukey's multiple comparisons test.

C, *NOP56* is not a biomarker of survival in patients with *KRAS*-wild-type lung adenocarcinoma (LC), pancreatic cancer (PC) and colon cancer (CC). Kaplan–Meier survival analyses of patient cohorts in TCGA were stratified by the optimal cut-off value of the mRNA level of *NOP56*.

D, Gene set enrichment analysis (GSEA) of a TCGA cohort of patients with *KRAS*-mutant lung (n=141), pancreatic (n=133) and colon cancer (n=170).

Figure S2

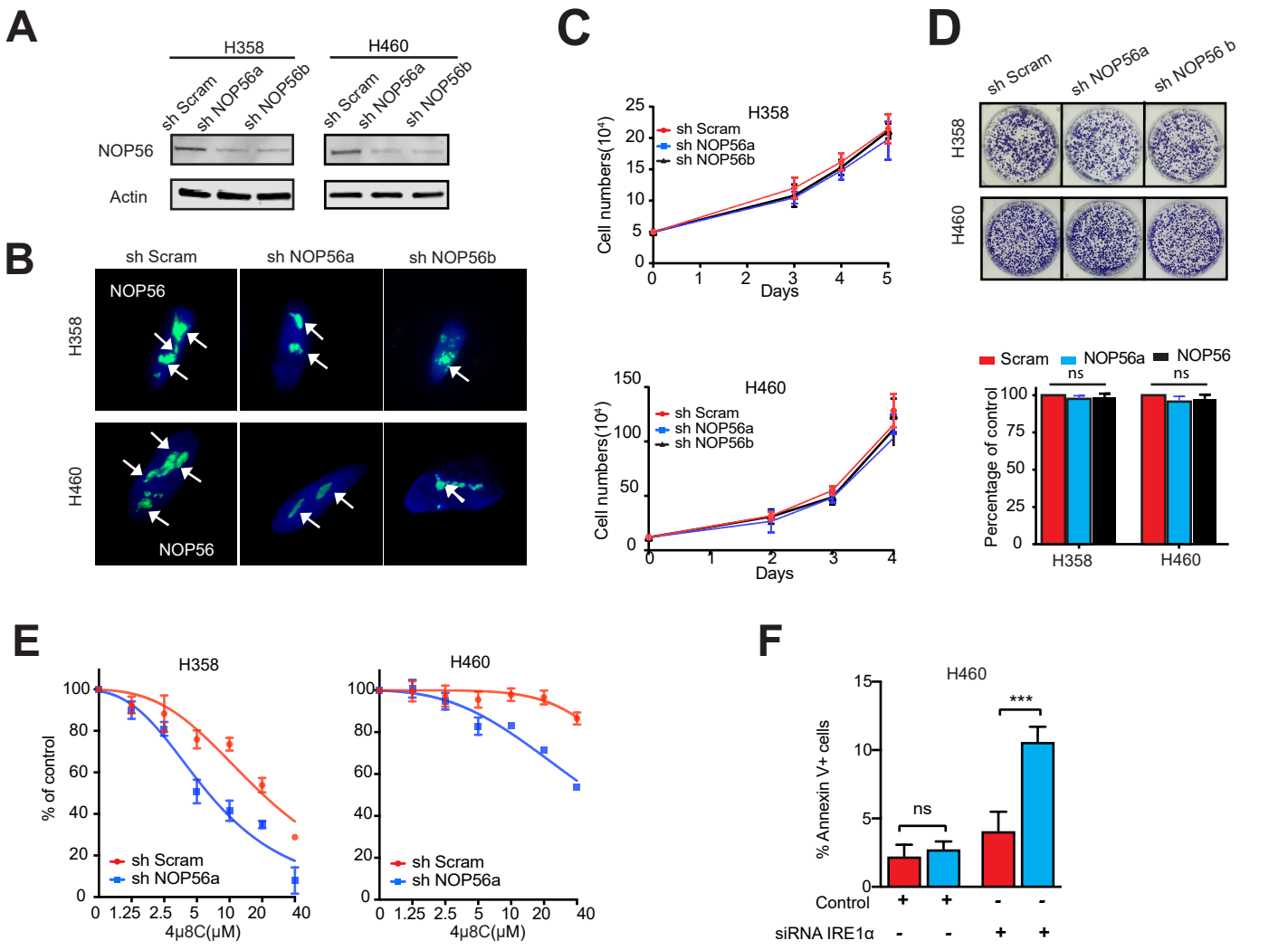

A
H358
H460
sh Scram
sh NOP56a
sh NOP56b
NOP56
Actin
B
sh Scram
sh NOP56a
sh NOP56b
H358
H460
NOP56
C
H358
sh Scram
sh NOP56a
sh NOP56b
Cell numbers(10⁴)
Days
H460
D
sh Scram
sh NOP56a
sh NOP56 b
Scram
NOP56a
NOP56
ns
Percentage of control
E
H358
H460
% of control
4μ8C(μM)
F
H460
% Annexin V+ cells
***
Control
siRNA IRE1α

Figure S2: Stable expression of *NOP56*-specific shRNAs activates IRE1α-mediated UPR

A, Immunoblots of H358 and H460 cells expressing scrambled control or *NOP56* shRNAs.

B, Immunofluorescence of H358 and H460 cells that express scrambled control or *NOP56* shRNAs. The NOP56 signal is indicated by arrowheads.

C, The cell viability curve of H358 and H460 cells expressing scramble control shRNA or the *NOP56*-targeted shRNAs was measured at the indicated time points.

D, Clongenic assay of H358 and H460 cells expressing scramble control or *NOP56*-targeted shRNAs. Quantification of clongenic assay were shown underneath. Data are presented as mean ± SD (n=3).

E, Growth inhibition of H358 and H460 cells expressing control shRNA or *NOP56*-targeted shRNA (3000 cells/well) treated for 72h with the indicated doses of an IRE1α inhibitor (4μ8C). Data are presented as mean ± SD (n=3).

F, Apoptosis assay of H460 cells expressing scrambled control or *NOP56*-targeted shRNAs after transfection with *IRE1α*-specific or control siRNAs for 72 h. Data are presented as mean ± SD (n=3). ***$P<0.001$ and ns $P > 0.05$ by two-way ANOVA with Tukey's multiple comparisons test.

Figure S3

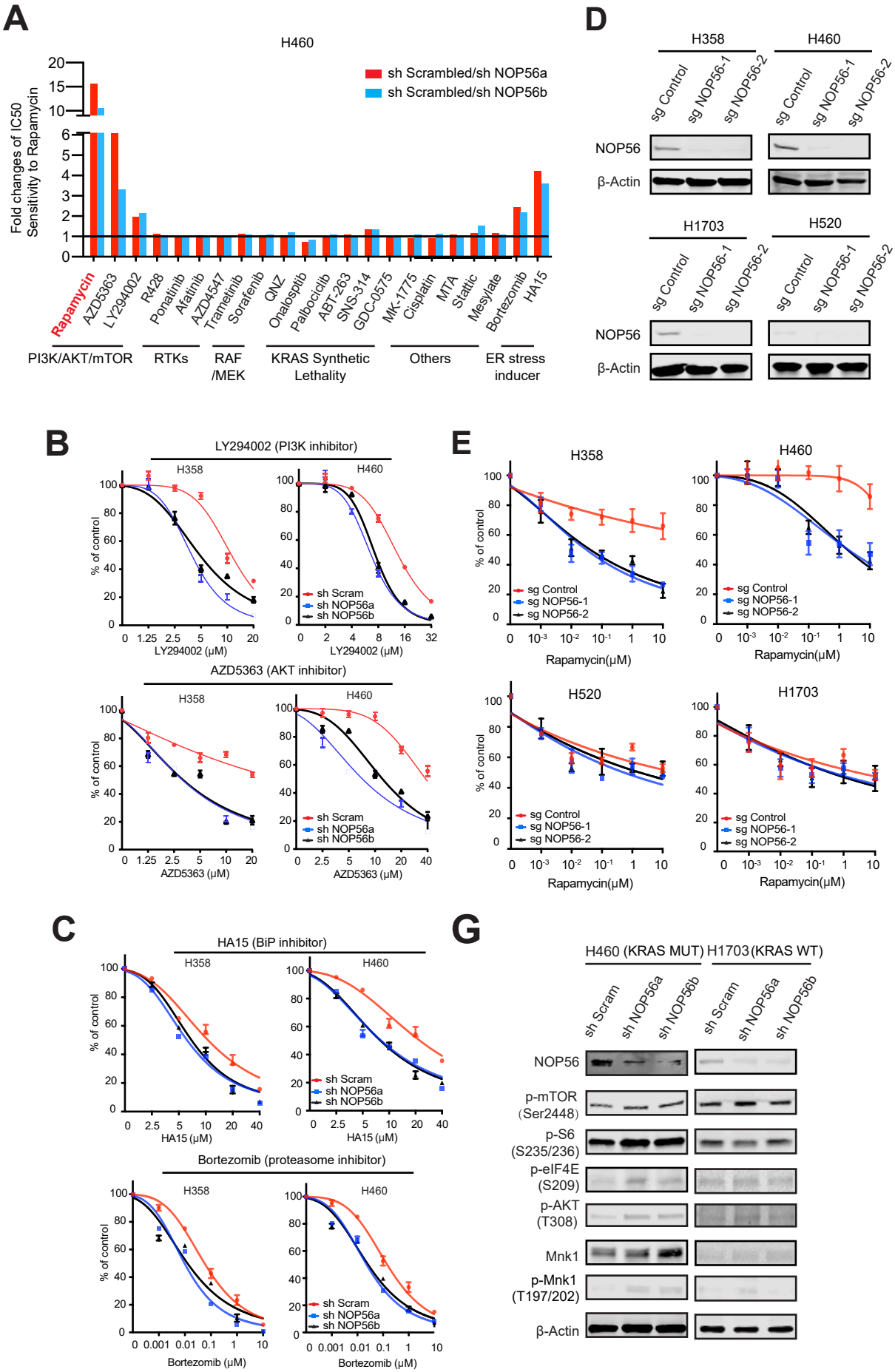

A
H460
sh Scrambled/sh NOP56a
sh Scrambled/sh NOP56b
Fold changes of IC50 Sensitivity to Rapamycin
Rapamycin
AZD5363
LY294002
R428
Ponatinib
Afatinib
AZD4547
Trametinib
Sorafenib
QNZ
Omalosptib
Palbocicdib
ABT-263
SNS-314
GDC-0575
MK-1775
Cisplatin
MTA
Stattic
Mesylate
Bortezomib
HA15
PI3K/AKT/mTOR
RTKs
RAF /MEK
KRAS Synthetic Lethality
Others
ER stress inducer
D
H358
H460
H1703
H520
sg Control
sg NOP56-1
sg NOP56-2
NOP56
β-Actin
B
LY294002 (PI3K inhibitor)
AZD5363 (AKT inhibitor)
% of control
sh Scram
sh NOP56a
sh NOP56b
LY294002 (μM)
AZD5363 (μM)
E
Rapamycin(μM)
C
HA15 (BiP inhibitor)
Bortezomib (proteasome inhibitor)
HA15 (μM)
Bortezomib (μM)
G
H460 (KRAS MUT)
H1703 (KRAS WT)
NOP56
p-mTOR (Ser2448)
p-S6 (S235/236)
p-eIF4E (S209)
p-AKT (T308)
Mnk1
p-Mnk1 (T197/202)
β-Actin

Figure S3

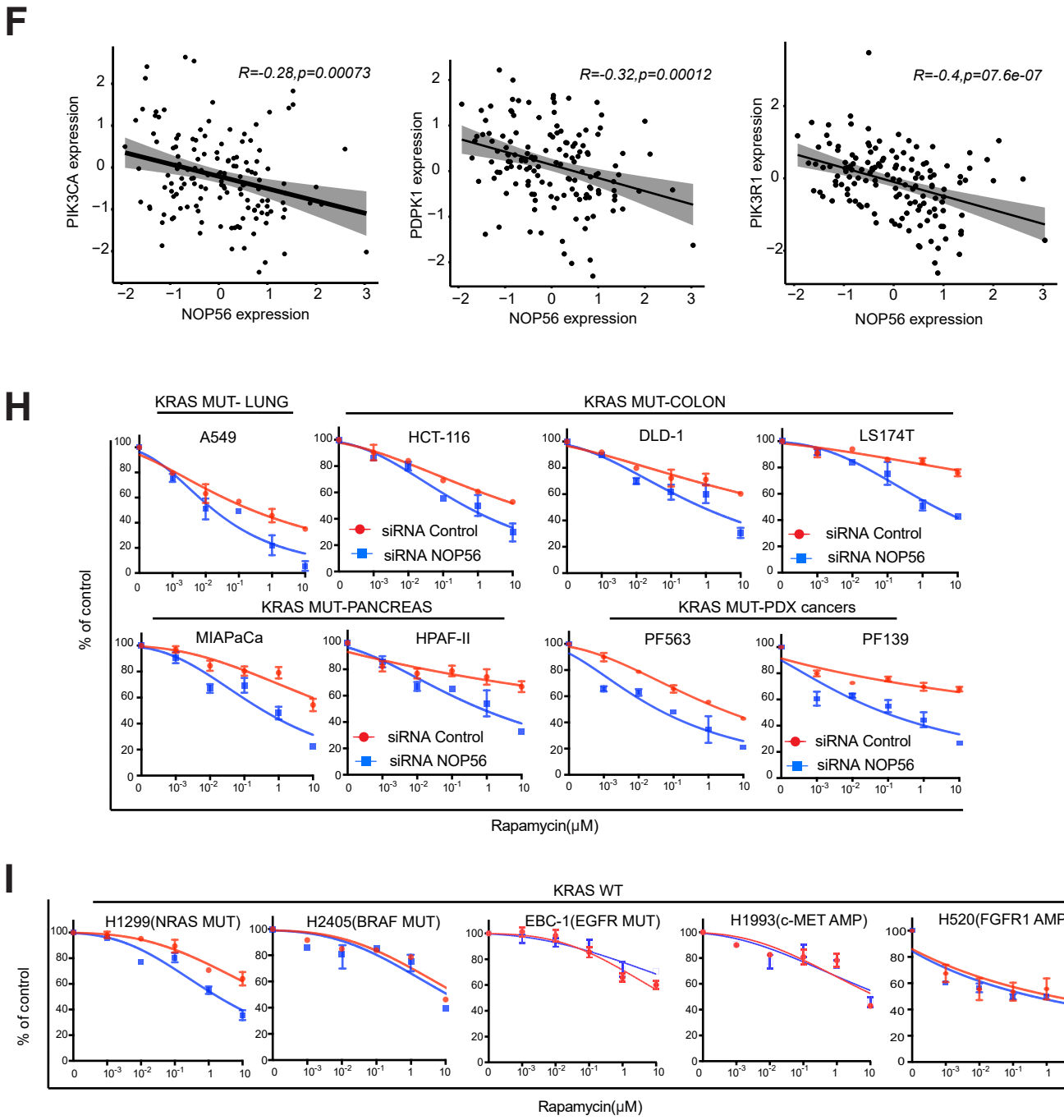

F
R=-0.28,p=0.00073
PIK3CA expression
NOP56 expression
R=-0.32,p=0.00012
PDPK1 expression
NOP56 expression
R=-0.4,p=07.6e-07
PIK3R1 expression
NOP56 expression
H
KRAS MUT- LUNG
KRAS MUT-COLON
A549
HCT-116
DLD-1
LS174T
siRNA Control
siRNA NOP56
KRAS MUT-PANCREAS
KRAS MUT-PDX cancers
MIAPaCa
HPAF-II
PF563
PF139
% of control
Rapamycin(μM)
I
KRAS WT
H1299(NRAS MUT)
H2405(BRAF MUT)
EBC-1(EGFR MUT)
H1993(c-MET AMP)
H520(FGFR1 AMP)
% of control
Rapamycin(μM)

Figure S3: NOP56 KD renders *KRAS*-mutant lung cancer cells susceptible to mTOR inhibition.

A, Bar graphs illustrating the change of sensitivity to different inhibitors in H460 cells after *NOP56* knockdown. Data are presented as IC_{50} values of the indicated inhibitors in H460 cells expressing scramble control shRNAs compared to IC_{50} in H460 cells expressing *NOP56*-targeted shRNAs. Data are shown as mean (n=2).

B, Viability assay of H460 and H358 cells expressing control shRNA or NOP56-targeted shRNA (3000 cells/well) after treated for 72 h with the indicated doses of PI3K inhibitor (LY294002) and AKT inhibitor (AZD5363). Data are presented as mean ± SD (n=3).

C, Viability assay of H460 and H358 cells expressing control shRNA or NOP56-targeted shRNA (3000 cells/well) after treated for 72 h with the indicated doses of BiP inhibitor (HA15) and ER stress inducer (bortezomib). Data are presented as mean ± SD (n=3).

D, Immunoblots of *KRAS*-mutant (H358, H460) and wild-type (H1703, H520) cells expressing *NOP56*-specific sgRNAs.

E, Viability assay of the cells expressing control or *NOP56*-specific sgRNAs after treated with rapamycin for 72 h. Data are shown as mean ± SD (n=3).

F, *NOP56* is negatively correlated with PI3K/AKT/mTOR pathway genes (*PI3KCA*, *PDPK1*, *PIK3R1*) in *KRAS*-mutant lung cancer patients. Pearson and Spearman coefficient and significance (p-value) are analyzed using R software (Cor.test function).

G, Immunoblots of H460 and H1703 cells expressing control or *NOP56*-targeted shRNAs.

H, I, Viability assay of *KRAS*-mutant (**H**) and wildtype (**I**) cancer cells expressing control or *NOP56*-specific siRNAs after treated with rapamycin. The assay was performed 72 h after drug treatment (96h after siRNA transfection).

Figure S4

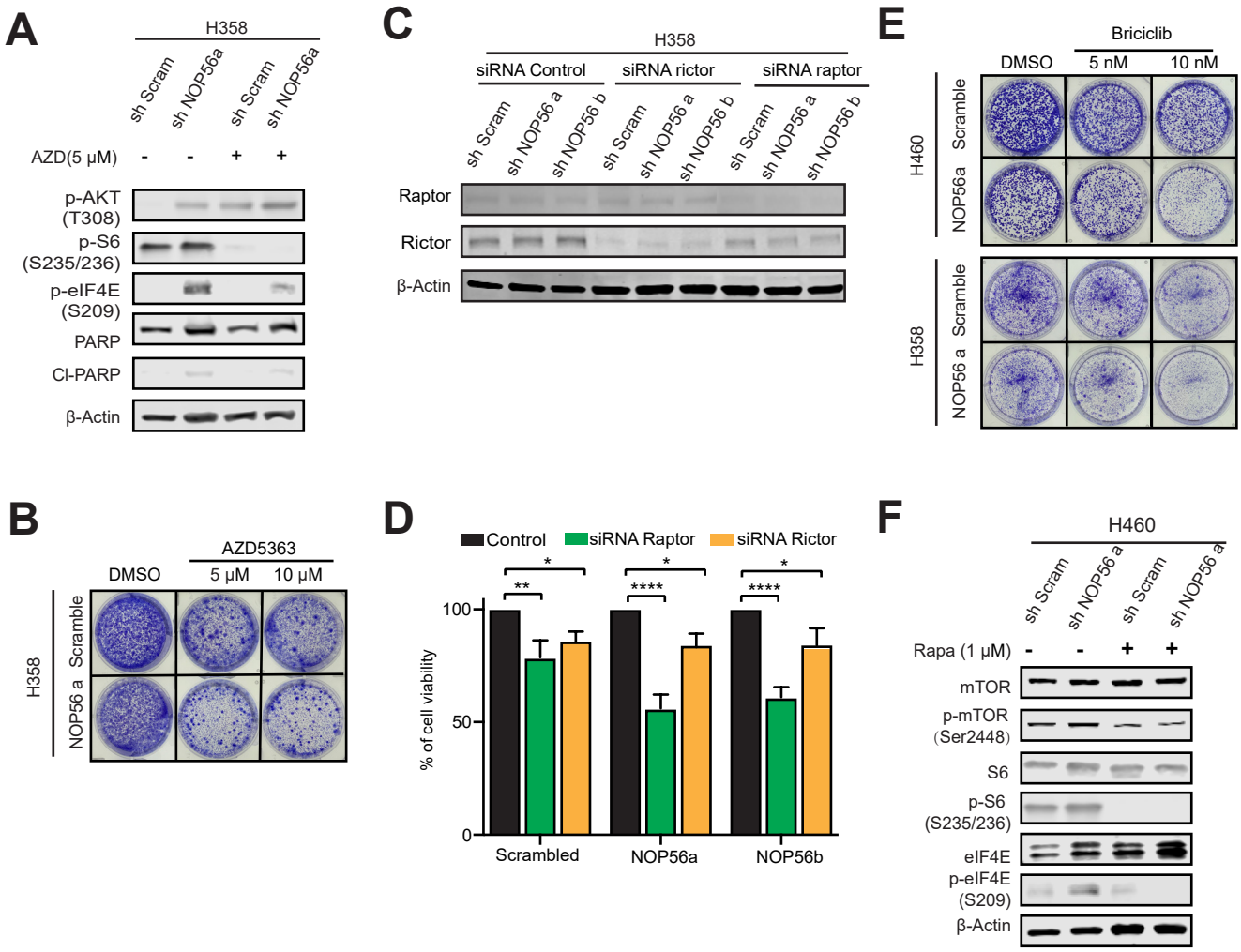

A
H358
sh Scram
sh NOP56a
sh Scram
sh NOP56a
AZD(5 μM) - - + +
p-AKT (T308)
p-S6 (S235/236)
p-eIF4E (S209)
PARP
Cl-PARP
β-Actin
B
AZD5363
DMSO
5 μM
10 μM
H358
Scramble
NOP56 a
C
H358
siRNA Control
siRNA rictor
siRNA raptor
sh Scram
sh NOP56 a
sh NOP56 b
Raptor
Rictor
β-Actin
D
Control
siRNA Raptor
siRNA Rictor
% of cell viability
100
50
0
Scrambled
NOP56a
NOP56b
E
Briciclib
DMSO
5 nM
10 nM
H460
Scramble
NOP56a
H358
Scramble
NOP56 a
F
H460
sh Scram
sh NOP56 a
Rapa (1 μM) - - + +
mTOR
p-mTOR (Ser2448)
S6
p-S6 (S235/236)
eIF4E
p-eIF4E (S209)
β-Actin

Figure S4: NOP56 KD activates and induces dependence on the mTOR pathway in *KRAS*-mutant cancer cells.

A, Immunoblots of H358 cells expressing control or *NOP56*-targeted shRNAs after treated with the AKT inhibitor (AZD5363) for 24 h.

B, Clongenic assay of H358 cells expressing control or *NOP56*-specific shRNAs after treated with indicated doses of AZD5363. Representative images are shown.

C, D, Immunoblots (C) and viability assay (D) of H358 cells expressing control or *NOP56*-specific shRNAs after transfected with *raptor*- or *rictor*-specific or control siRNAs for 72 h. Data are presented as mean ± SD (n=3).

E, Clongenic assay of H358 and H460 cells expressing control shRNA or *NOP56*-specific shRNAs after treatment with indicated doses of eIF4E inhibitor (Briciclib). Representative images are shown.

F, Immunoblots of H460 cells expressing control or *NOP56*-target shRNAs after treated with rapamycin (1 μM) for 24 h.

Figure S5

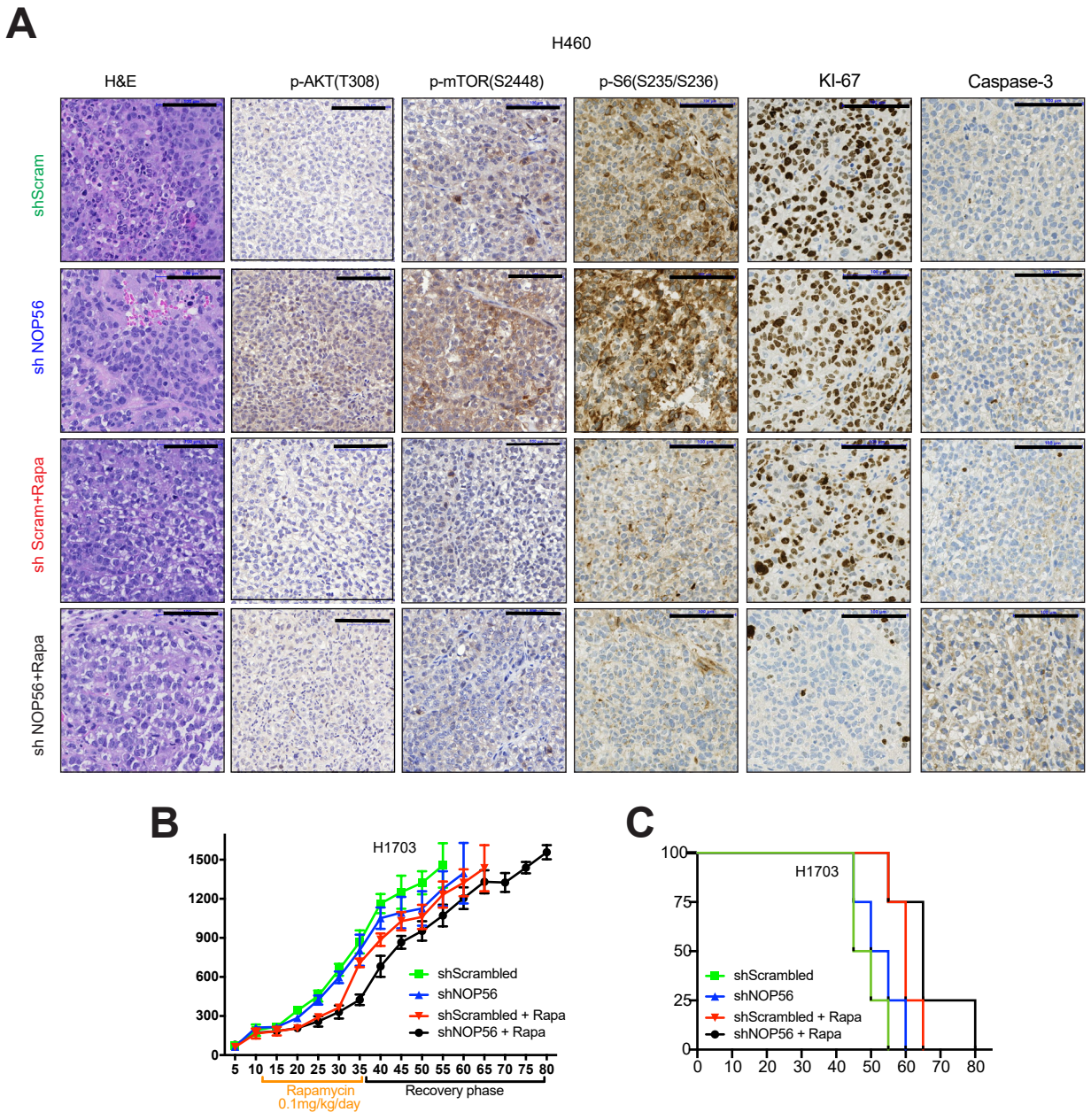

A
H460
H&E
p-AKT(T308)
p-mTOR(S2448)
p-S6(S235/S236)
KI-67
Caspase-3
shScram
sh NOP56
sh Scram+Rapa
sh NOP56+Rapa
B
H1703
1500
1200
900
600
300
0
5 10 15 20 25 30 35 40 45 50 55 60 65 70 75 80
shScrambled
shNOP56
shScrambled + Rapa
shNOP56 + Rapa
Rapamycin
0.1mg/kg/day
Recovery phase
C
H1703
100
75
50
25
0
0 10 20 30 40 50 60 70 80
shScrambled
shNOP56
shScrambled + Rapa
shNOP56 + Rapa

Figure S5: *In vivo* activity and selectivity of co-targeting NOP56 and mTOR in *KRAS*-mutant lung cancer

A, H&E and IHC analysis of p-AKT(T308), p-mTOR(S2448),p-S6(S235/236), Ki67 and Caspase-3) in residual H460 xenograft tumors after the indicated treatment. Scale bars 100 μm

B, Tumor volume of H1703 xenografts in immunocompromised (NSG) mice. H1703 cells were transduced with either a control or an shRNA against NOP56 (shNOP56a). Tumors were measured every 5 days with a caliper.

C, Kaplan-Meier survival curve of mice harboring H1703 xenografts from the experiment shown in B