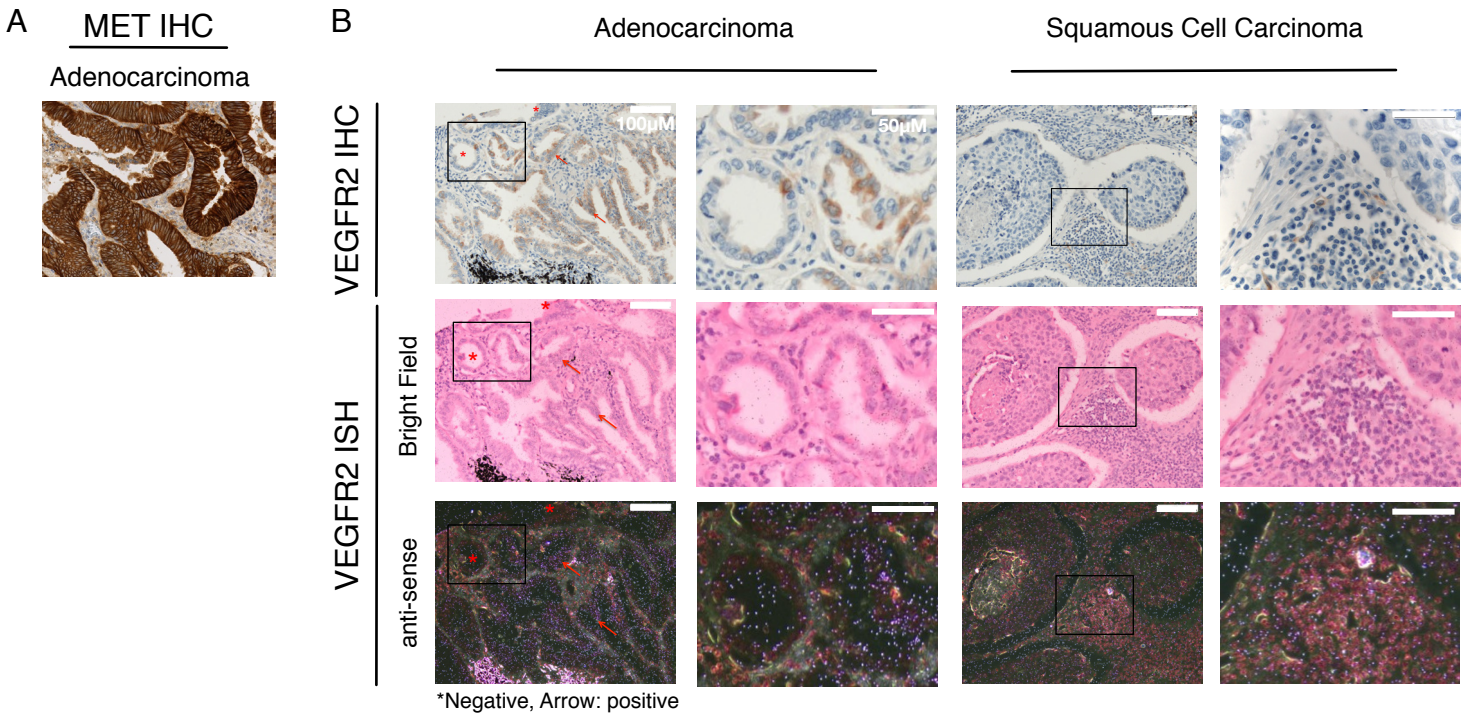
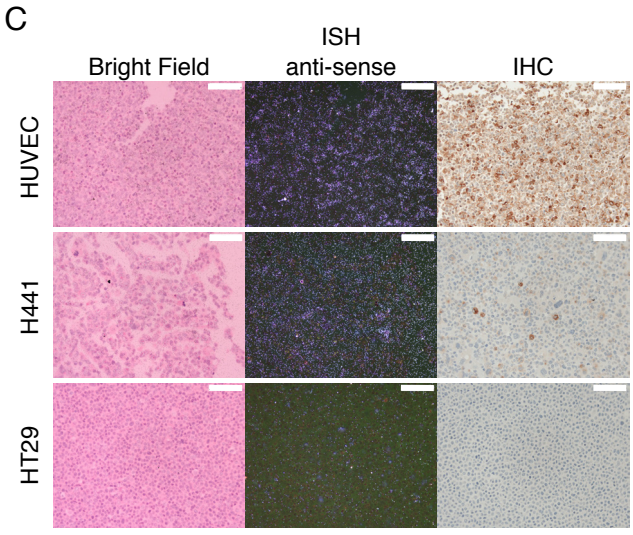
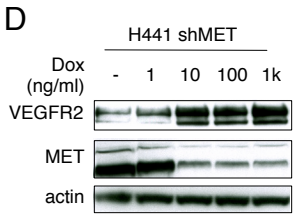
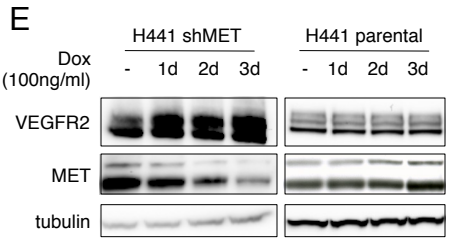
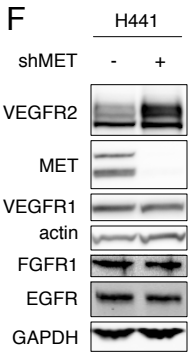
Sup Figure 1.


A
MET IHC
Adenocarcinoma
B
Adenocarcinoma
Squamous Cell Carcinoma
VEGFR2 IHC
VEGFR2 ISH
Bright Field
anti-sense
100μM
50μM
*Negative, Arrow: positive


C
ISH
Bright Field
anti-sense
IHC
HUVEC
H441
HT29


D
H441 shMET
Dox (ng/ml)
- 1 10 100 1k
VEGFR2
MET
actin


E
H441 shMET
H441 parental
Dox (100ng/ml)
- 1d 2d 3d
- 1d 2d 3d
VEGFR2
MET
tubulin


F
H441
shMET
- +
VEGFR2
MET
VEGFR1
actin
FGFR1
EGFR
GAPDH

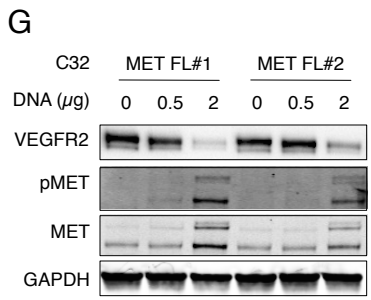

G
C32
MET FL#1
MET FL#2
DNA (μg)
0 0.5 2 0 0.5 2
VEGFR2
pMET
MET
GAPDH

Figure S1. Expression of MET and VEGFR2 in tumors and effect of MET knockdown on VEGFR2 levels. IHC analysis of MET expression in epithelial cells in NSCLC adenocarcinoma (A) and VEGFR2 expression in endothelial cells in NSCLC squamous cell carcinoma. Brown chromogen indicates signal (B, top panels). (B, middle and bottom panels) ISH analysis of the same region shows mRNA expression of MET and VEGFR2 in NSCLC adenocarcinoma, but not squamous cell carcinoma. Silver Grains (White dots) indicate signal. * denotes negative staining, arrow indicates positive staining. Higher magnifications of the boxed areas are shown on the right. (C) ISH and IHC analysis of HUVEC, H441 and HT29 cells. Scale bar is 100μm. (D) H441 shMET cells were treated with doxycyclin (dox) at the indicated concentration over 48 hr and VEGFR2 and MET levels were analyzed by immunoblot. (E) H441 parental and shMET clones were treated with 100 ng/ml dox for the indicated duration. VEGFR2 and MET were analyzed by immunoblot. (F) Effect of MET knockdown on the indicated cell surface receptor levels in H441 cells. (G) C32 cells were transfected with full-length MET cDNA expression constructs driven by CMV or TK-Neo at the indicated amount over 48 hr and VEGFR2, pMET and MET levels were analyzed by IB.

Sup Figure 2.

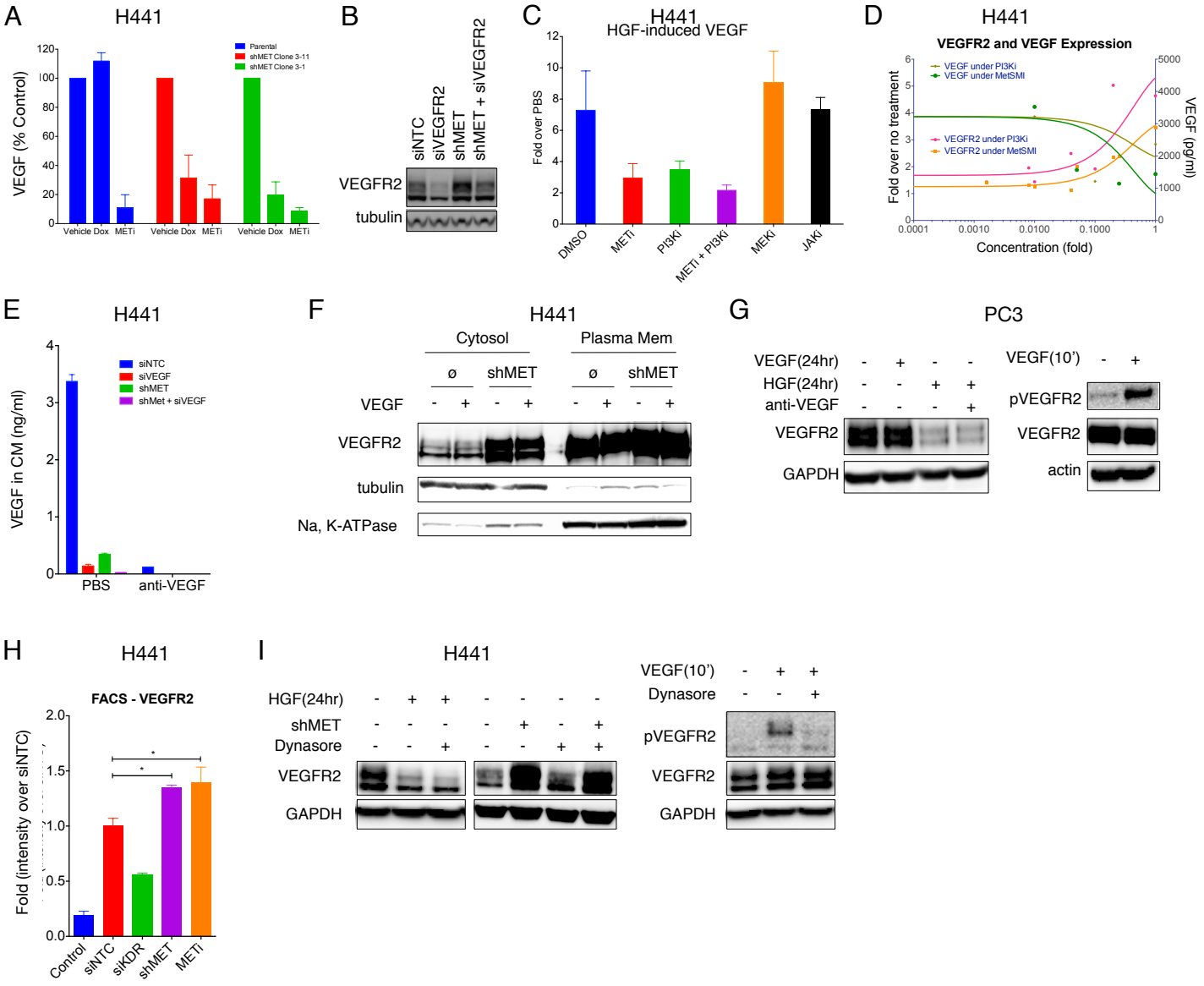

A
H441
VEGF (% Control)
Parental
shMET Clone 3-11
shMET Clone 3-1
Vehicle Dox
METi
B
siNTC
siVEGFR2
shMET
shMET + siVEGFR2
VEGFR2
tubulin
C
H441
HGF-induced VEGF
Fold over PBS
DMSO
METi
PI3Ki
METi + PI3Ki
MEKi
JAKi
D
H441
VEGFR2 and VEGF Expression
VEGF under PI3Ki
VEGF under MetSMI
VEGFR2 under PI3Ki
VEGFR2 under MetSMI
Fold over no treatment
VEGF (pg/ml)
Concentration (fold)
E
H441
VEGF in CM (ng/ml)
siNTC
siVEGF
shMET
shMet + siVEGF
PBS
anti-VEGF
F
H441
Cytosol
Plasma Mem
ø
shMET
VEGF
VEGFR2
tubulin
Na, K-ATPase
G
PC3
VEGF(24hr)
HGF(24hr)
anti-VEGF
VEGFR2
GAPDH
VEGF(10')
pVEGFR2
actin
H
H441
FACS - VEGFR2
Fold (intensity over siNTC)
Control
siNTC
siKDR
shMET
METi
I
H441
HGF(24hr)
shMET
Dynasore
VEGFR2
GAPDH
VEGF(10')
pVEGFR2

Figure S2. MET suppresses VEGFR2 via PI3K-induced intracrine VEGF action. (A) Effect of MET knockdown (shMET) or inhibition (GDC-0712, 1 μM) on VEGF levels in wildtype H441 cells or shMET-expressing clones. (B) VEGFR2 IB verifying knockdown with VEGFR2 siRNA as shown in Figure 2B. (C) VEGF levels based on VEGF ELISA readings in Figure 2D. The numbers indicate fold change for HGF- over vehicle-treated cells. (D) Relationship of VEGFR2 levels and secreted VEGF levels as a function of MET and PI3K SMI concentration. Results are based on densitometric analyses of VEGFR2 and on VEGF ELISA results of cell supernatants from the same study (data are normalized in pair-wise fashion to untreated controls). The lines are based on a non-linear fit for each data set. (E) VEGF ELISA results for cells subjected to siRNA or shRNA knockdown as indicated, in the presence or absence of anti-VEGF. (F) Subcellular fractionation of H441 shMET clones grown in the absence or presence of doxycylin and treated with VEGF (100 ng/ml) for 15 min. VEGFR2 as well as the respective markers against each compartment were then measured by immunoblot. (G, left) Effect of VEGF (100 ng/ml) or HGF (100 ng/ml) or anti-VEGF (10 μg/ml) on VEGFR2 expression in PC3 cells. Phosphorylation assay of PC3 cells subjected to a 10 min treatment with VEGF (right). (H) FACS analysis of VEGFR2 positive cells under indicated condition. Bar graph depicts the mean data of three individual experiments, with normalization to control siNTC. Error bars indicate S.E.M., *$p<0.05$ for indicated comparison. (I) Immunoblot analysis of VEGFR2 for H441 cells with or without MET knockdown (shMET) treated with HGF (100 ng/ml) for 24 hr in the absence or presence of dynasore (80 μM). Right panel, H441 shMET clones incubated with or without VEGF for 10 min in absence or presence of dynasore were analyzed by immunoblot.

Sup Figure 3.

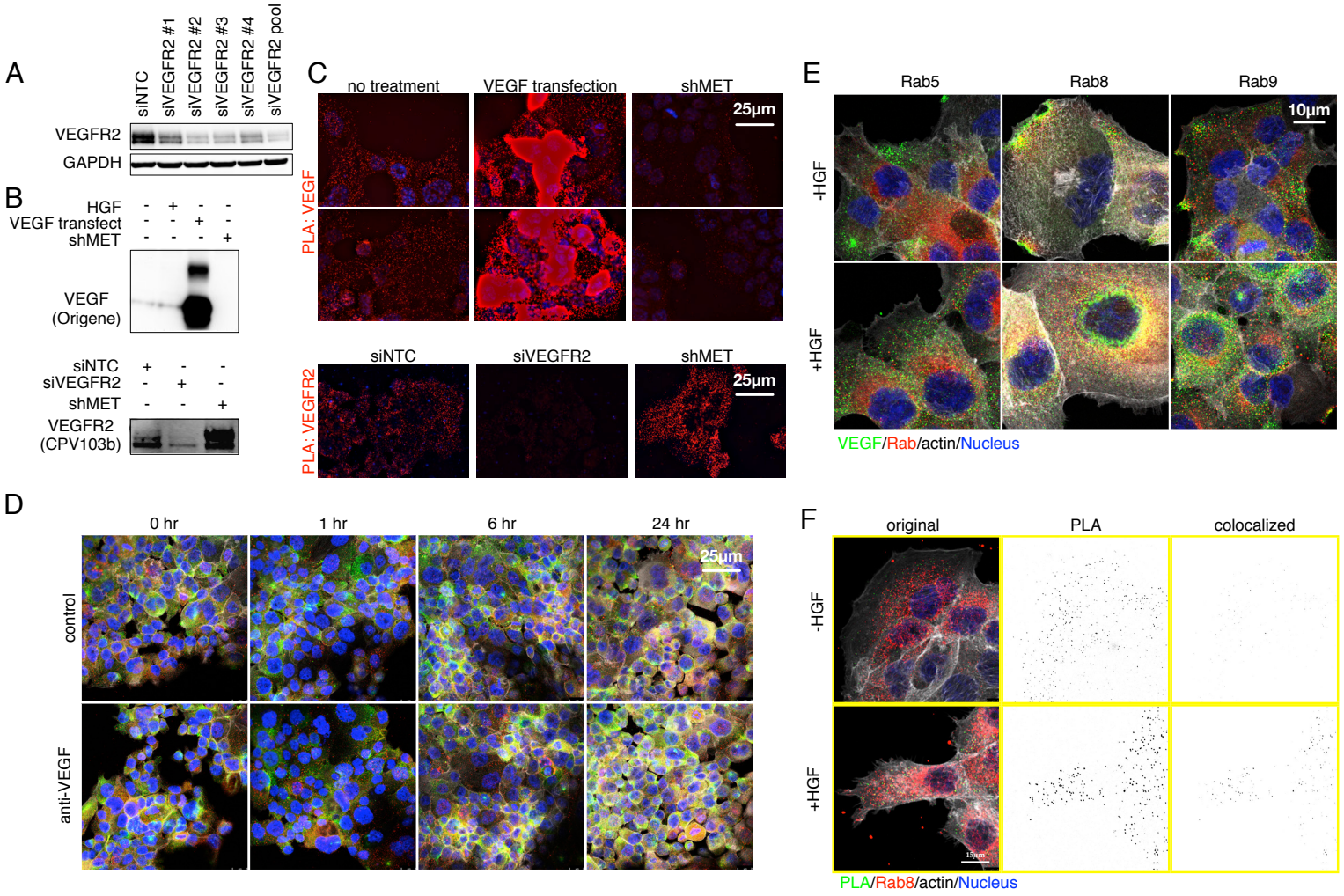

A
siNTC
siVEGFR2 #1
siVEGFR2 #2
siVEGFR2 #3
siVEGFR2 #4
siVEGFR2 pool
VEGFR2
GAPDH
B
HGF
VEGF transfect
shMET
VEGF (Origene)
siNTC
siVEGFR2
shMET
VEGFR2 (CPV103b)
C
no treatment
VEGF transfection
shMET
25μm
PLA: VEGF
siNTC
siVEGFR2
shMET
25μm
PLA: VEGFR2
D
0 hr
1 hr
6 hr
24 hr
25μm
control
anti-VEGF
E
Rab5
Rab8
Rab9
10μm
-HGF
+HGF
VEGF/Rab/actin/Nucleus
F
original
PLA
colocalized
-HGF
+HGF
PLA/Rab8/actin/Nucleus

Figure S3. HGF induces close intracellular association of VEGF and VEGFR2. (A) Immunoblot of VEGFR2 verifying knockdown with individual or pooled VEGFR2 siRNAs. (B) Immunoblot detection of VEGF (top) or VEGFR2 (bottom) under each indicated condition in H441 cells. (C) PLA analysis of VEGF or VEGFR2 alone in the context of each indicated treatment in H441 cells. (D) Immunofluorescence analysis of H441 cells treated for indicated time with 100 ng/ml HGF, in the absence or presence of anti-VEGF, and stained for VEGF and VEGFR2 protein (VEGF in pseudo-green, VEGFR2 in pseudo-red, nucleus with DAPI in blue). (E) Immunocytochemical staining of H441 cells incubated for 24 hr in the absence or presence of HGF (100 ng/ml): actin with phalloidin (white), VEGF (green) and Rab (red) with specific antibodies, nuclei with DAPI (blue). (F) Computer-generated images for PLA and Rab8 colocalization depicted in Figure 3E were used for quantitation of total PLA signal (middle, VEGF:VEGFR2 complex) and to identify colocalized signals (right, VEGF:VEGFR2:Rab8 complex).

Sup Figure 4.

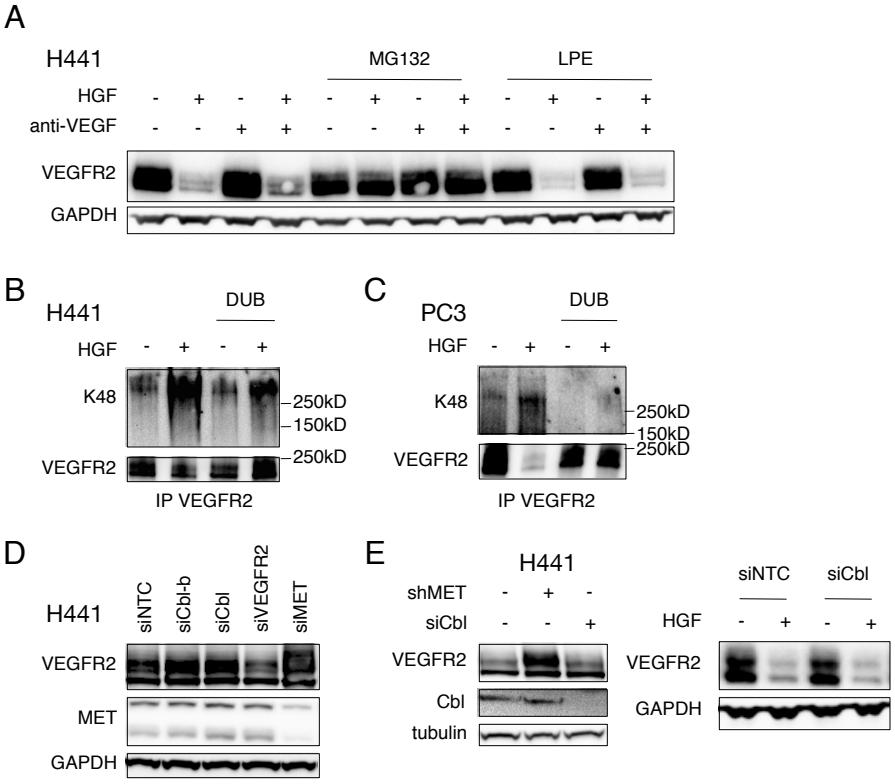

A
H441
MG132
LPE
HGF
anti-VEGF
VEGFR2
GAPDH
B
H441
DUB
HGF
K48
250kD
150kD
VEGFR2
250kD
IP VEGFR2
C
PC3
DUB
HGF
K48
250kD
150kD
VEGFR2
250kD
IP VEGFR2
D
H441
siNTC
siCbl-b
siCbl
siVEGFR2
siMET
VEGFR2
MET
GAPDH
E
H441
shMET
siCbl
VEGFR2
Cbl
tubulin
siNTC
siCbl
HGF
VEGFR2
GAPDH

Figure S4. Cbl is dispensable for suppression of VEGFR2 by MET. (A) Effect of proteasome or lysosome inhibitors on HGF modulation of VEGFR2. H441 cells were treated with HGF (100 ng/ml) with or without anti-VEGF (10 μg/ml) for 24 hr in absence or presence of MG132 (10 μM) or LPE (10 μM) and VEGFR2 levels were analyzed by IB. (B and C) H441 (B) or PC3 cells (C) were treated with or without HGF (100 ng/ml). Cell lysates were subjected to VEGFR2 IP under denaturing conditions followed by in vitro deubiquitinaion by a cocktail of human recombinant deubiquitinases (see Supplemental Materials and Methods). Samples were then analyzed by IB with K48-ubiquitin and VEGFR2 antibodies. (D) Effect of siRNA knockdown of Cbl-b, Cbl, VEGFR2 and MET on VEGFR2 and MET levels in H441 cells. (E, left) Effect of MET shRNA knockdown (shMET) or Cbl siRNA knockdown (siCbl) on VEGFR2 levels in H441 cells. (F, right) H441 cells were treated with or without HGF (100 ng/ml) for 24 hr in the context of scrambled (siNTC) or Cbl siRNA knockdown (siCbl). VEGFR2 level were analyzed by immunoblot.

Sup Figure 5.

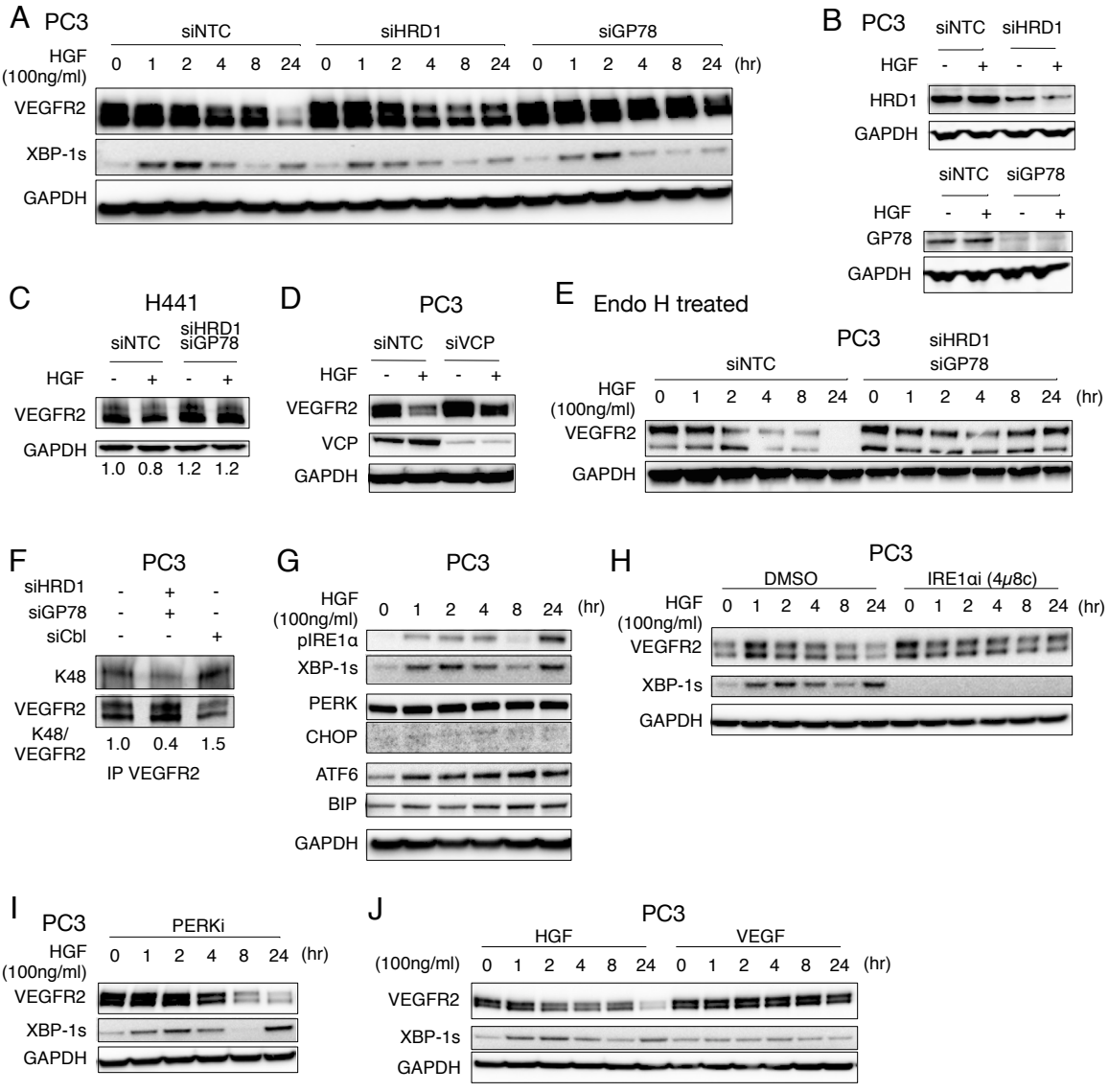

A PC3
siNTC
siHRD1
siGP78
HGF (100ng/ml)
0 1 2 4 8 24 0 1 2 4 8 24 0 1 2 4 8 24 (hr)
VEGFR2
XBP-1s
GAPDH
B PC3
siNTC siHRD1
HGF - + - +
HRD1
GAPDH
siNTC siGP78
HGF - + - +
GP78
GAPDH
C H441
siNTC siHRD1 siGP78
HGF - + - +
VEGFR2
GAPDH
1.0 0.8 1.2 1.2
D PC3
siNTC siVCP
HGF - + - +
VEGFR2
VCP
GAPDH
E Endo H treated
PC3
siNTC siHRD1 siGP78
HGF (100ng/ml)
0 1 2 4 8 24 0 1 2 4 8 24 (hr)
VEGFR2
GAPDH
F PC3
siHRD1 - + -
siGP78 - + -
siCbl - - +
K48
VEGFR2
K48/ VEGFR2
1.0 0.4 1.5
IP VEGFR2
G PC3
HGF (100ng/ml)
0 1 2 4 8 24 (hr)
pIRE1α
XBP-1s
PERK
CHOP
ATF6
BIP
GAPDH
H PC3
DMSO
IRE1αi (4μ8c)
HGF (100ng/ml)
0 1 2 4 8 24 0 1 2 4 8 24 (hr)
VEGFR2
XBP-1s
GAPDH
I PC3
PERKi
HGF (100ng/ml)
0 1 2 4 8 24 (hr)
VEGFR2
XBP-1s
GAPDH
J PC3
HGF
VEGF
(100ng/ml)
0 1 2 4 8 24 0 1 2 4 8 24 (hr)
VEGFR2
XBP-1s
GAPDH

Figure S5. ERAD-linked E3 ligases and IRE1α mediate VEGFR2 disposal. (A) PC3 cells were transfected with non-targeted control siRNA (siNTC), or siRNA targeted against gp78 (siGP78) or HRD1 (siHRD1) and treated with 100 ng/ml HGF for the indicated time period. Samples were then analyzed by IB for VEGFR2 and XBP-1s expression. (B) Same as (A) for the presence of HRD1 (top) or gp78 (bottom). (C) Effect of combined HRD1 and gp78 combo knockdown on VEGFR2 modulation by HGF (100 ng/ml, 24 hr) in H441 cells. Numbers at the bottom of the Immunoblot indicate densitometric quantification. (D) PC3 cells were transfected with siNTC or siRNA against p97/VCP (siVCP) and treated with or without 100 ng/ml HGF for 24 hr. Samples were analyzed by IB for VEGFR2 and VCP. (E) Same samples as in Figure 5C but treated with Endo H and analyzed by IB. (F) Effect of Cbl, or combined gp78 and HRD1 siRNA knockdown on levels of K48-linked VEGFR2 in PC3 cells. Numbers at the bottom indicate densitometric quantification. (G) PC3 cells were exposed to HGF for the indicated period of time. Samples were then analyzed by IB as indicated. (H) PC3 cells were exposed to HGF for the indicated time in the presence of either vehicle (DMSO) or 1 μM IRE1α SMI 4μ8c. VEGFR2 and XBP-1s were analyzed by immnuoblot. (I) PC3 cells were treated as in (G) but under PERK inhibition (PERKi, 1 μM). (J) PC3 cells were treated with HGF or VEGF for the indicated time period. VEGFR2 and XBP-1s were then analyzed by IB.

Sup Figure 6.

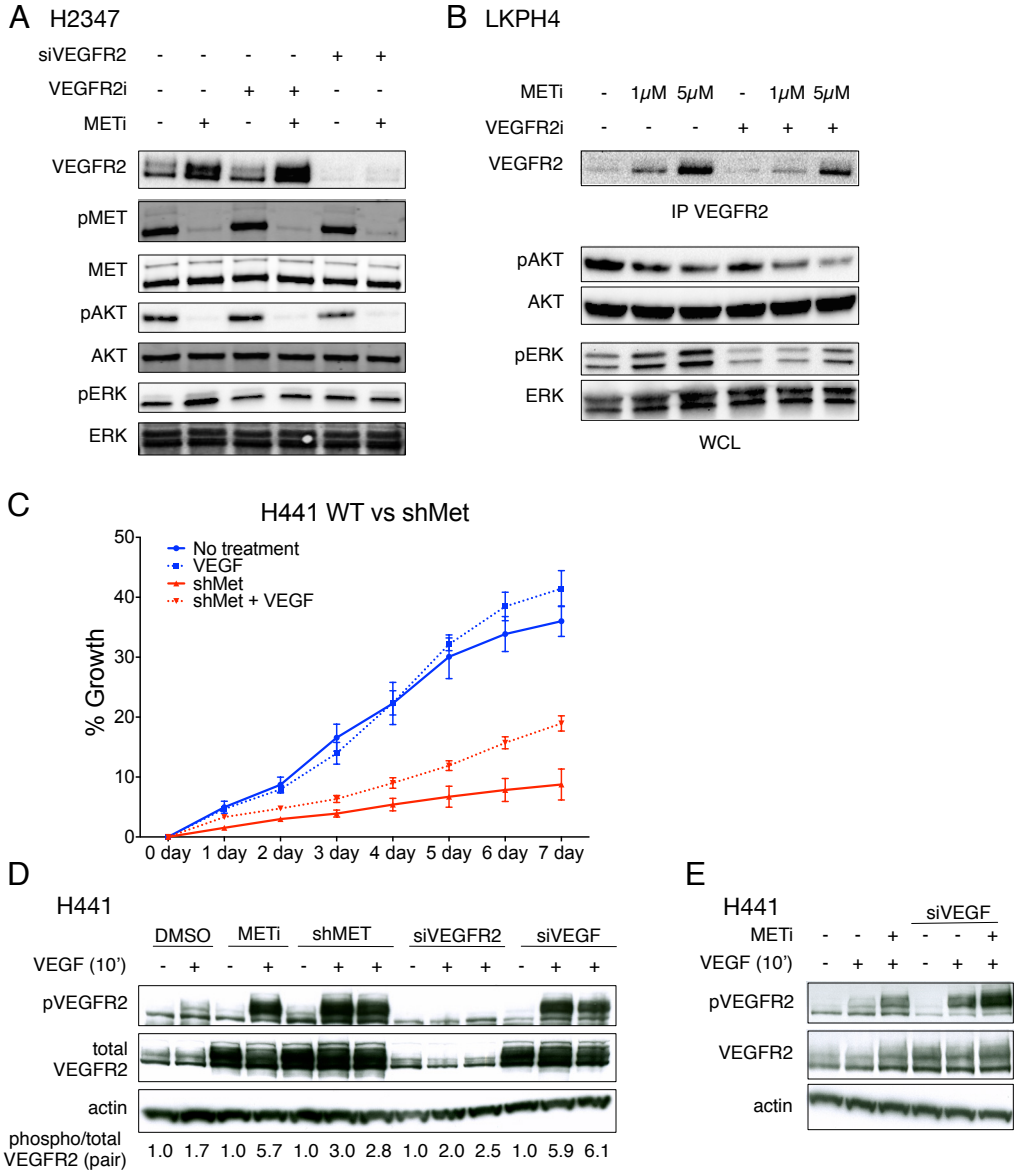

A H2347
siVEGFR2 - - - - + +
VEGFR2i - - + + - -
METi - + - + - +
VEGFR2
pMET
MET
pAKT
AKT
pERK
ERK
B LKPH4
METi - 1μM 5μM - 1μM 5μM
VEGFR2i - - - + + +
VEGFR2
IP VEGFR2
pAKT
AKT
pERK
ERK
WCL
C
H441 WT vs shMet
No treatment
VEGF
shMet
shMet + VEGF
% Growth
0 10 20 30 40 50
0 day 1 day 2 day 3 day 4 day 5 day 6 day 7 day
D
H441
DMSO METi shMET siVEGFR2 siVEGF
VEGF (10') - + - + - + + - + + - + +
pVEGFR2
total VEGFR2
actin
phospho/total VEGFR2 (pair)
1.0 1.7 1.0 5.7 1.0 3.0 2.8 1.0 2.0 2.5 1.0 5.9 6.1
E
H441
siVEGF
METi - - + - - +
VEGF (10') - + + - + +
pVEGFR2
VEGFR2
actin

Figure S6. MET disruption enhances responsiveness to exogenous VEGF. (A) Effect of VEGFR2 kinase inhibitor (VEGFR2i, 4 μM) or VEGFR2 siRNA knockdown (siVEGFR2) on VEGFR2 and on phosphorylation of downstream markers upon MET inhibition (GDC-0712, 1 μM) in H2347 cells. (B) Effect of VEGFR2 kinase inhibitor (VEGFR2i, 4 μM) on VEGFR2 and on phosphorylation of downstream markers upon MET inhibition (METi) in LKPH4 cells. (C) Analysis of H441 cell growth over 7 days in serum-free medium using an incucyte instrument. Cells were incubated and monitored without treatment or in the presence of exogenous VEGF (100 ng/ml). Cells were also subjected to MET knockdown as indicated (shMET). (D) Same as 6G but with comparison to siRNA knockdown of VEGFR2 or VEGF and the corresponding effects on phospho-VEGFR2 and total VEGFR2 levels by immunoblot. (E) H441 cells were treated with vehicle or VEGF (100 ng/ml) for 10 min under the indicated condition and phospho-VEGFR2 and total VEGFR2 levels were analyzed by immunoblot.

Sup Figure 7.

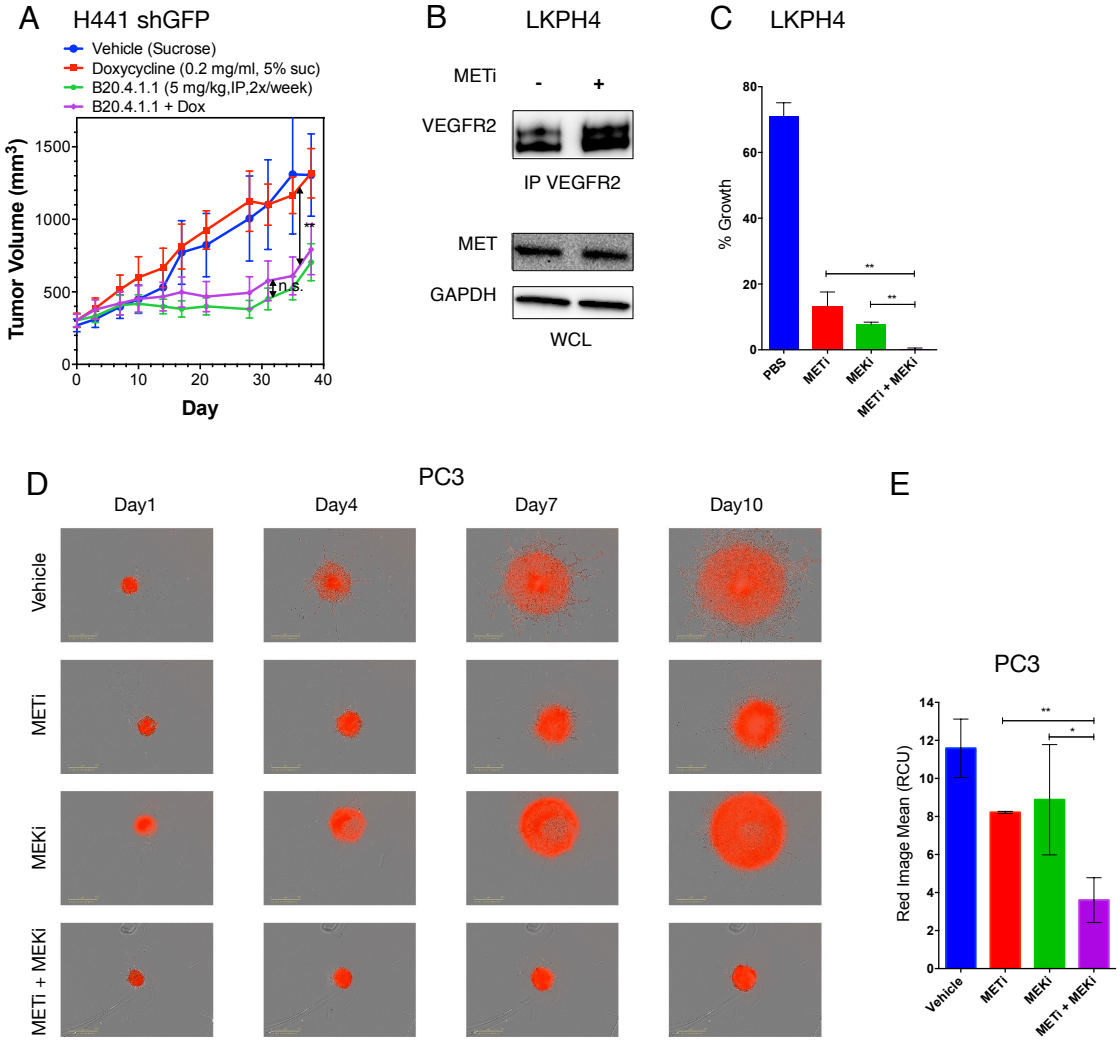

A
H441 shGFP
Vehicle (Sucrose)
Doxycycline (0.2 mg/ml, 5% suc)
B20.4.1.1 (5 mg/kg,IP,2x/week)
B20.4.1.1 + Dox
Tumor Volume (mm3)
Day
n.s.
**
B
LKPH4
METi
VEGFR2
IP VEGFR2
MET
GAPDH
WCL
C
LKPH4
% Growth
PBS
METi
MEKi
METi + MEKi
D
PC3
Day1
Day4
Day7
Day10
Vehicle
METi
MEKi
METi + MEKi
E
PC3
Red Image Mean (RCU)
Vehicle
METi
MEKi
METi + MEKi

Figure S7. Combined inhibition of MET and MEK blocks cell growth. (A) Effect of Doxycycline treatment, VEGF neutralization, or both on in vivo tumor growth of shGFP-harboring H441 cells as a control for shMET-harboring H441 cells in Figure 7A. (B) LKPH4 cells were treated with or without GDC-0712 (1 μM) and VEGFR2 and MET were analyzed by IB. (C) LKPH4 cells were subjected to SMI blockade of MET (GDC-0712, 1 μM) or MEK (cobimetinib, 1 μM) or both and analyzed for growth at 72 hr. Error bars indicate S.E.M., n = 5; **p < 0.01 for indicated comparison. (D) Images for NucLight Red-labeled PC3 cells grown in 3D culture under treatment with vehicle, GDC-0712 (1 μM), cobimetinib (1 μM) or both inhibitors. (E) Quantitative analysis of Day-10 cell growth for (D). Error bars indicate S.E.M., n = 3; *p < 0.05, **p < 0.01 for indicated comparison.