Supplementary Figure Legends

Supplementary Figure 1. EPHA2 expression and activity in NSCLC. (**A**) EPHA2 expression was assessed in the lung tissue of a mutant *Kras* model of NSCLC by western blot analysis of paired tumor and normal lung tissue. Two representative mice were used per condition ($Kras^{G12D}EphA2^{+/+}$, A and B, and $Kras^{G12D}EphA2^{-/-}$, C and D). EPHA2 expression levels in mouse tumors were compared with those in human NSCLC lines. (**B**) Cells were serum starved overnight and stimulated with EPHRIN-A1 (EFNA1,100ng/mL) for 15 minutes before lysis. EPHA2 was immunoprecipitated, followed by immunoblotting for phospho-tyrosine (pY). (**C**) Total cell lysates of cells starved and stimulated with 10% FBS were probed for phosphorylated EPHA2 (S897). Relative levels of EPHA2 expression across the various cell lines are shown. β-TUBULIN expression was used as a loading control.

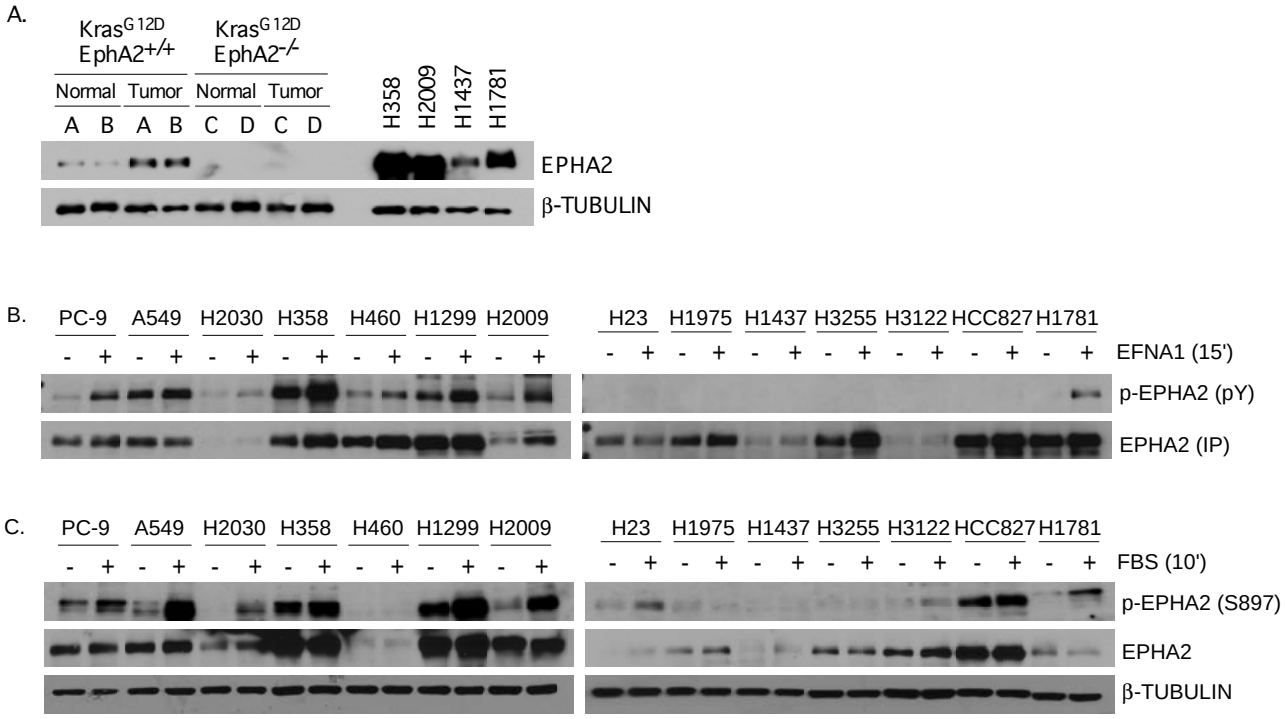
Supplementary Figure 2. ALW-II-41-27 inhibits cell viability and promotes apoptosis. (**A**) H358 cells were treated with 1μM NG-25 or ALW-II-41-27 over a time course and cell viability was determined via the MTT assay. Experiments were repeated three times and data are presented as the percentage of viable cells after NG-25 or ALW-II-41-27 treatment relative to DMSO treatment $\pm$ SEM. (**B**) Cells were treated with 1μM of indicated drug for 6 hours, and apoptosis was assessed by quantifying the histone associated DNA fragments via a cell death ELISA Kit (Roche). Data was normalized to respective DMSO controls. * $p<0.05$, ** $p<0.01$.

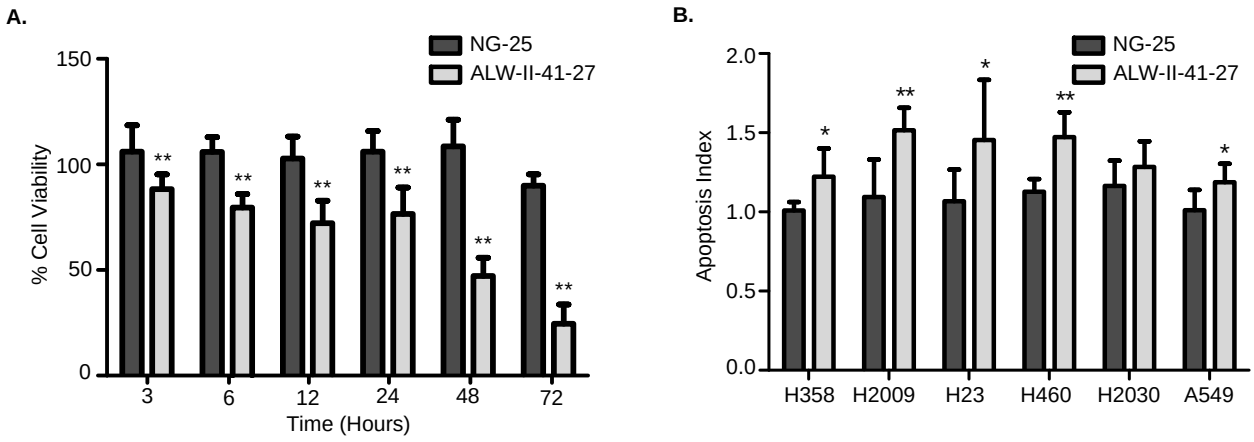
Supplementary Figure 3. Histopathologic analysis of vital organs in mice treated with ALW-II-41-27, NG-25, or the vehicle. H358 cells were engrafted in nude mice and treated with 15mg/kg of ALW-II-41-27, NG-25, or the vehicle twice daily for 14 days, as in Figure 8A. Heart, liver, and kidney were harvested at the end of studies and tissue sections were stained with H&E. Scale bars indicate 100 μm.

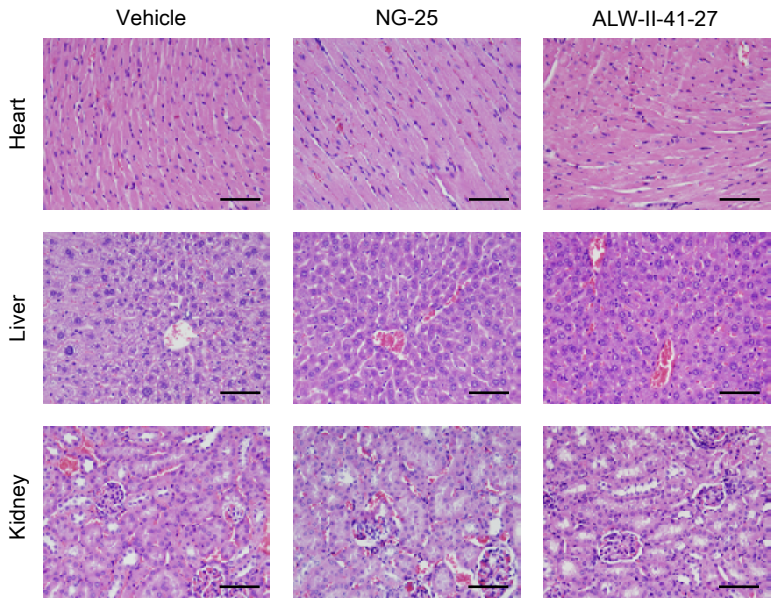
Supplementary Figure 4. Comparison of the efficacy of tyrosine kinase inhibitors relative to ALW-II-41-27 in NSCLC cell lines. Cells were treated for 72 hours with the indicated TKIs before measuring viability by the MTT assay. H2009 and H358 cells contain a mutation in *KRAS*. PC-9 and H1437 cells carry a mutation in *EGFR* and a mutation in *MEK-1*, respectively.

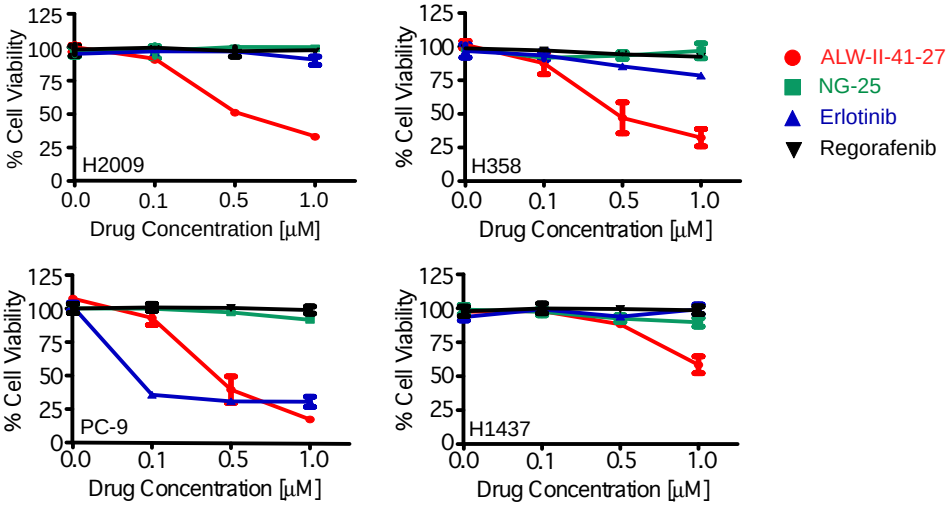
Supplementary Table 1. Lung cancer cell lines used in the study with their respective driver mutations.

Supplementary Table 2. Drug-target interaction in xenograft tumors in situ. *H358 xenograft tumors treated with 30mg/kg ALW-II-41-27 for five days (n=5/group) (as in Figure 8I) were analyzed for drug-target interaction by chemical proteomics platform, KiNativ. Tumor lysate was incubated with ATP-biotin labeled probes to assess which kinases received protection from the drug binding. Percent inhibition of kinase labeling by the ATP-biotin probes is shown with the larger numbers representing a stronger interaction between the inhibitor and the kinase. Additionally, A375 cells were tested in a similar manner in vitro by treatment with 5μM and 0.5μM ALW-II-41-27 or NG-25 (control compound). Colors indicate the level of inhibition of ATP binding to the respective kinase: Brown (90% inhibition), Red (90-75% inhibition), Orange (75-50% inhibition), Yellow (50-35% inhibition), and Green (no change). ACT = Activation loop; Lys1 = Conserved Lysine 1; Lys2 = Conserved Lysine 2; Other = Labeling of residue outside of the protein kinase domain; N.D. = Not detected. ** The in vitro IC_{50} of ALW-II-41-27 and NG-25 was determined by in vitro kinase assay using the SelectScreenTM Kinase Profiling Service (Life Technologies).


A.
Kras G12D EphA2 +/+
Kras G12D EphA2 -/-
Normal
Tumor
Normal
Tumor
A B A B C D C D
H358
H2009
H1437
H1781
EPHA2
β-TUBULIN
B.
PC-9 A549 H2030 H358 H460 H1299 H2009
H23 H1975 H1437 H3255 H3122 HCC827 H1781
- + - + - + - + - + - + - +
EFNA1 (15')
p-EPHA2 (pY)
EPHA2 (IP)
C.
PC-9 A549 H2030 H358 H460 H1299 H2009
H23 H1975 H1437 H3255 H3122 HCC827 H1781
- + - + - + - + - + - + - +
FBS (10')
p-EPHA2 (S897)
EPHA2
β-TUBULIN


A.
NG-25
ALW-II-41-27
% Cell Viability
150
100
50
0
**
3
6
12
24
48
72
Time (Hours)
B.
NG-25
ALW-II-41-27
Apoptosis Index
2.0
1.5
1.0
0.5
0.0
*
H358
H2009
H23
H460
H2030
A549


Vehicle
NG-25
ALW-II-41-27
Heart
Liver
Kidney


% Cell Viability
125
100
75
50
25
0
H2009
0.0
0.1
0.5
1.0
Drug Concentration [μM]
H358
PC-9
H1437
ALW-II-41-27
NG-25
Erlotinib
Regorafenib

Cell Line	KRAS	EGFR	Other Mutations
A549	G12S		
H2009	G12A		
H2030	G12C		
H23	G12C		
H358	G12C		
H460	Q61H		
H1975		L858R; T790M	
H3255		L858R	
HCC827		Δ19	
PC-9		Δ19	
H1299			NRAS
H1437			MEK1
H1781			HER2
H3122			EML4-ALK

Kinases	Sequence	Labeling Site	KiNativ Inhibition Percentage					** Enzymatic IC_{50} (nM)	
				ALW-II-41-27		NG-25			
			* H358 Xenograft	A375					
				5.0 µM	0.5 µM	5.0 µM	0.5 µM	ALW-II-41-27	NG-25
ABL,ARG	LMTGDTYTAHAGAKFPIK	ACT	>98.2	>95	>95	> 98	> 98	7.7	75.2
ABL,ARG	YSLTVAVKTLKEDTMEVEEFLK	Lys1	>95.9	>90	>90	86.8	85.5		
GAK	DLKVENLLLSNQGTIK	Lys2	>95.2	N.D	N.D	N.D	N.D		
LYN	VAVKTLKPGTMSVQAFLEEANLMK	Lys1	>94.8	N.D	N.D	> 95	90.5	0.8	12.9
ZAK	WISQDKEVAVKK	Lys1	>93.6	>98	>98	98.1	93.0	40.2	698
p38a	QELNKTIWEVPER	Other	98.5	97.7	98.1	53.3	51.9	55.6	102
EPHA2	VLEDDPEATYTTSGGKIPIR	ACT	95.1	98.3	97.5	57.7	27.9	11	773
p38b	QELNKTVWEVPQR	Other	91.6	38.7	44.2	-241.6	-228.7	138	194
FRK	HEIKLPVK	ACT	91.6	N.D	N.D	N.D	N.D	17.3	144
CSK	VSDFGLTKEASSTQDTGKLPVK	ACT	87.7	99.4	95.0	78.2	36.5	13.9	56.4
FYN,SRC,YES	QGAKFPIKWTAPEAALYGR	ACT	87.2	93.4	85.4	89.6	36.5	14.4	113
p38a	DLKPSNLAVNEDCELK	Lys2	62.9	65.3	71.7	21.9	20.2	55.6	102
EPHB4	FLEENSSDPTYTSSLGGKIPIR	ACT	61.4	N.D	N.D	-4.2	39.2	13.1	999
HER3/ErbB3	GVWIPEGESIKIPVCIKVIEDK	Lys1	56.7	-43.1	-14.3	27.3	22.8		
EPHB2	FLEDDTSDPTYTSALGGKIPIR	ACT	52.2	>85	12.2	21.6	-2.2	14.4	672
EPHB3	FLEDDPSDPTYTSSLGGKIPIR	ACT	45.1	N.D	N.D	N.D	N.D	119	3710
BRAF	DLKSNNIFLHEDLTVK	Lys2	44.0	>80	>80	66.8	22.5	1280	>10000
BTK	YVLDDEYTSSVGSKFPVR	ACT	40.3	N.D	N.D	N.D	N.D		
JAK1 domain2	IGDFGLTKAIETDKEYYTVK	ACT	37.9	83.7	48.7	95.4	20.8		
PRP4	CNILHADIKPDNILVNESK	Lys2	37.8	-15.4	-4.3	-33.5	-34		
YSK1	EVVAIKIIDLEEAEDEIEDIQQEITVLSQCDSPYITR	Lys1	37.5	N.D	N.D	N.D	N.D		
EPHA1	LLDDFDGTYETQGGKIPIR	ACT	31.4	N.D	N.D	N.D	N.D		
EPHA7	VIEDDPEAVYTTTGGKIPVR	ACT	28.3	N.D	N.D	N.D	N.D		
EGFR	LLGAEEKEYHAEGGKVPIK	ACT	24	84.7	31.3	0.7	4.8		
PI3KCA	RPLWLNWENPDIMSELLFQNNEIIFKNGDDLRQDMLTLQIIR	ATP	-0.8	N.D	N.D	28.3	21.9		
ERK1	DLKPSNLLINTTCDLK	Lys2	-11.5	0.3	7.4	-49.9	-31.9		
HER2/ERBB2	GIWIPDGENVKIPVAIKVLR	Lus1	-22.5	97.1	70.9	-19.4	-60.4		