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Table S1. Scoring of immunofluorescent staining for ABL pathway targets in human primary lung adenocarcinoma and metastases specimens

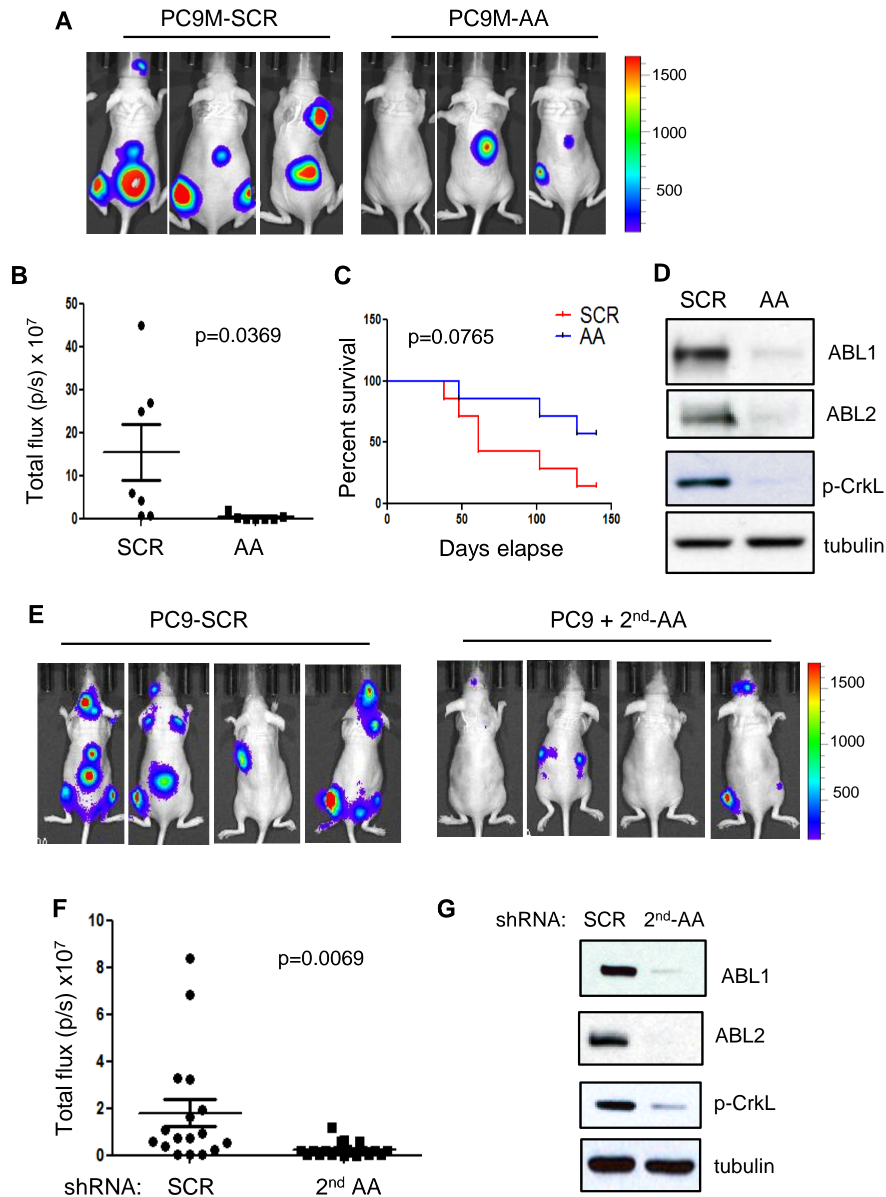
Tissue specimen	Biomarkers	Sample ID	Survivin	β-catenin	S100A4	TAZ
Metastases (lymph node)	ALK-/ROS1-/Met-/EGFR-/KRAS-	1909	+++++++	+++++	++++	++++
	ALK-/KRAS-	4099	++++++++	++++++++	++++	+/-
Primary tumor	ALK-/ROS1-/Met-/EGFR-/KRAS+	1593	++++	++++	++++	+
		1594 (normal)	+/-	+/-	+	-
	ALK-/ROS1-/Met-/EGFR-/KRAS+	4060	++++	+++++	++	+++
		4061 (normal)	+/-	+	++	-
	ALK-/ROS1-/EGFR-/KRAS-	892	+++	++++	++++	++
		894 (normal)	+	+/-	+	-
	ALK-/ROS1-/EGFR-/KRAS-	3278	+++++	++	++++	++++
		3279 (normal)	+/-	+	+	+++

Table S2. List of Antibodies

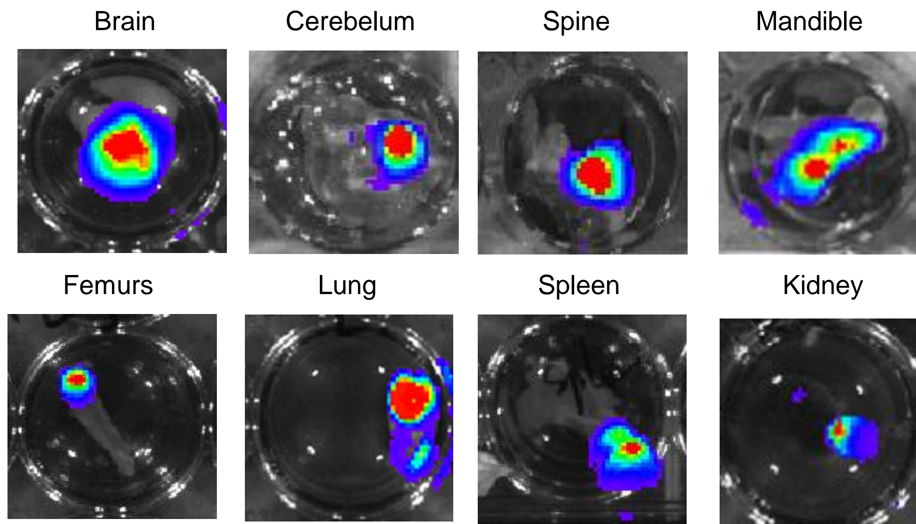
Antibody	Source	Catalog number	Clone
ABL1	BD Biosciences	554148	8E9
ABL2	Santa Cruz Biotech.	Sc-134268	9H5
CyR61	Santa Cruz Biotech.	Sc-13100	H-78
Lamin A	Santa Cruz Biotech.	Sc-20680	H-102
p-TAZ (S89)	Santa Cruz Biotech.	Sc-17610-R	
p-CrkL (Y207)	Cell Signaling Tech	3181	
p-CrkL (Y221)	Cell Signaling Tech	3491	
Yap/TAZ	Cell Signaling Tech	8418	
TAZ	Cell Signaling Tech	4883	
TAZ	BD Biosciences	560235	M2-616
TAZ	abcam	110239	
p-YAP (S127)	Cell Signaling Tech	13008	D9W2I
β-catenin	Cell Signaling Tech	9582	
β-catenin	BD Biosciences	610154	14/Beta-Catenin
p-β-catenin (S45)	Cell Signaling Tech	9564	
p-β-catenin (S33/37/T41)	Cell Signaling Tech	9561	
p-β-catenin (S675)	Cell Signaling Tech	4176	
Survivin	Cell Signaling Tech	2808	
Bcl-XL	Cell Signaling Tech	2764	
AXIN2	Cell Signaling Tech	2151	
p-GSK3β (S9)	Cell Signaling Tech	9323	
p-Akt (S473)	Cell Signaling Tech	9271	
β-TrCP	Cell Signaling Tech	11984	
14-3-3	Cell Signaling Tech	8312	
MYC	Cell Signaling Tech	5605	
MCM4	Cell Signaling Tech	12973	
Cyclin D1	Cell Signaling Tech	2978	
Cyclin D3	Cell Signaling Tech	2936	
p-Erk (T202/Y204)	Cell Signaling Tech	4376	
S100A4	abcam	Ab27957	
EDN1	abcam	Ab117757	
TNC	abcam	Ab108930	
CD31	BD Biosciences	553370	
actin	Sigma	A4700	AC-40
tubulin	Sigma	T0198	D66

Table S3. Primers used for quantitative RT-PCR

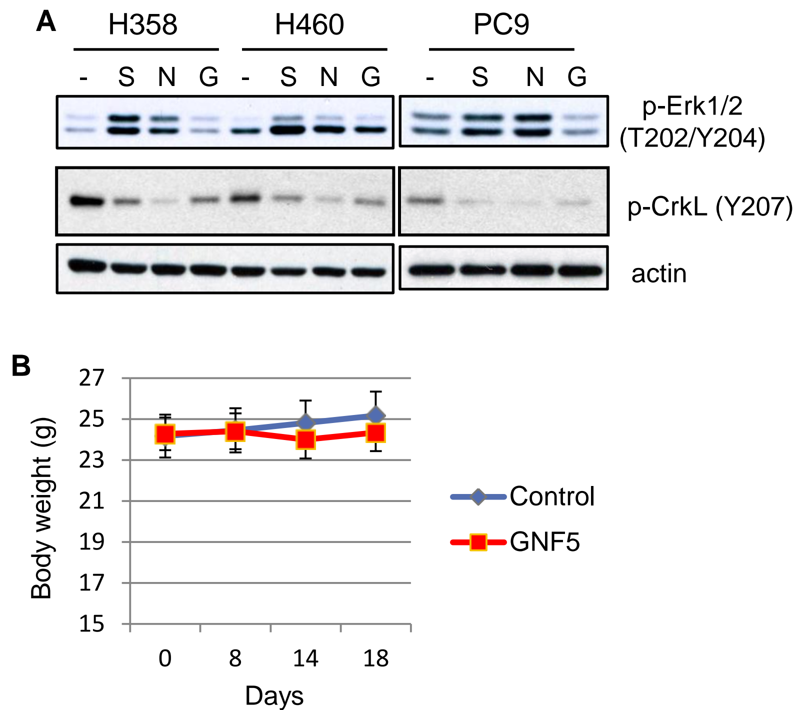
GENE	Forward 5'->3'	Reverse 5'->3'
TCF4	GCAGAGTCTCCTTGGAGGTG	GTGCTTGCTGATGGAGCATA
EDN1	CCAAGGAGCTCCAGAAACAG	GATGTCCAGGTGGCAGAAGT
CTGF	CCGTACTCCCAAAATCTCCA	GTAATGGCAGGCACAGGTCT
S100A4	GGTGTCCACCTTCCACAAGT	GCTGTCCAAGTTGCTCATCA
BIRC5	GGACCACCGCATCTCTACAT	TCCGCAGTTTCCTCAAATTC
BCL2L1	GGACTGAATCGGAGATGGAG	TGGGATGTCAGGTCACTGAA
TAZ	GGCTGGGAGATGACCTTCAC	AGGCACTGGTGTGGAACTGAC
CTNNB1	GAAACGGCTTTCAGTTGAGC	CTGGCCATATCCACCAGAGT
GAPDH	GGCTCTCCAGAACATCATCCCTGC	GGGTGTCGCTGTTGAAGTCAGAGG



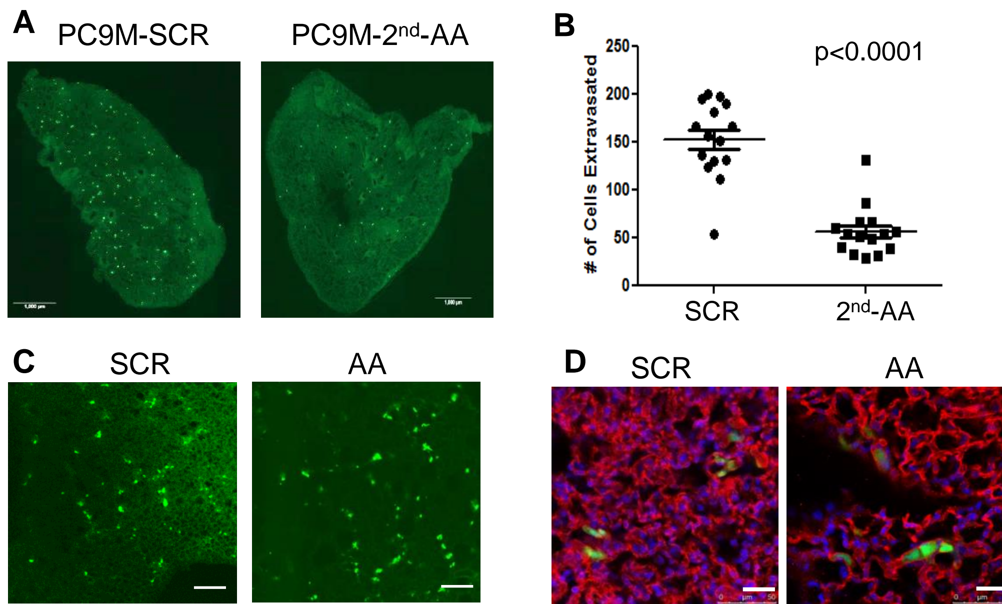
Supplemental Figure 1. Depletion of ABL1 and ABL2 impairs NSCLC metastasis. (**A-D**) PC9M cells labeled with luciferase were transduced with lentiviruses encoding either scrambled (SCR) or the first set of ABL1/ABL2-specific shRNAs (AA); cells were then delivered by intracardiac injection into nude mice. (**A**) Representative mice with bioluminescent imaging (BLI). (**B**) BLI counts of each individual mouse for the indicated groups (n=7 for each group). (**C**) Percentage of mice surviving over the indicated times. (**D**) ABL1/ABL2-knockdown was analyzed by western blotting. (**E-G**) PC9 cells labeled with luciferase were transduced with either SCR or a second set of ABL1/ABL2-specific shRNAs (2nd-AA), and the cells were delivered by intracardiac injection. (**E**) Representative BLI images. (**F**) BLI counts of each individual mouse for the indicated groups (SCR n=17, AA n=20). (**G**) ABL1/ABL2-knockdown was analyzed by western blotting. ABL kinase activity was analyzed by blotting for p-CrkL. Tubulin is used as loading controls. Data are represented as mean ± SEM. All *P*-values were determined by Student's *t*-test or Log-rank test.



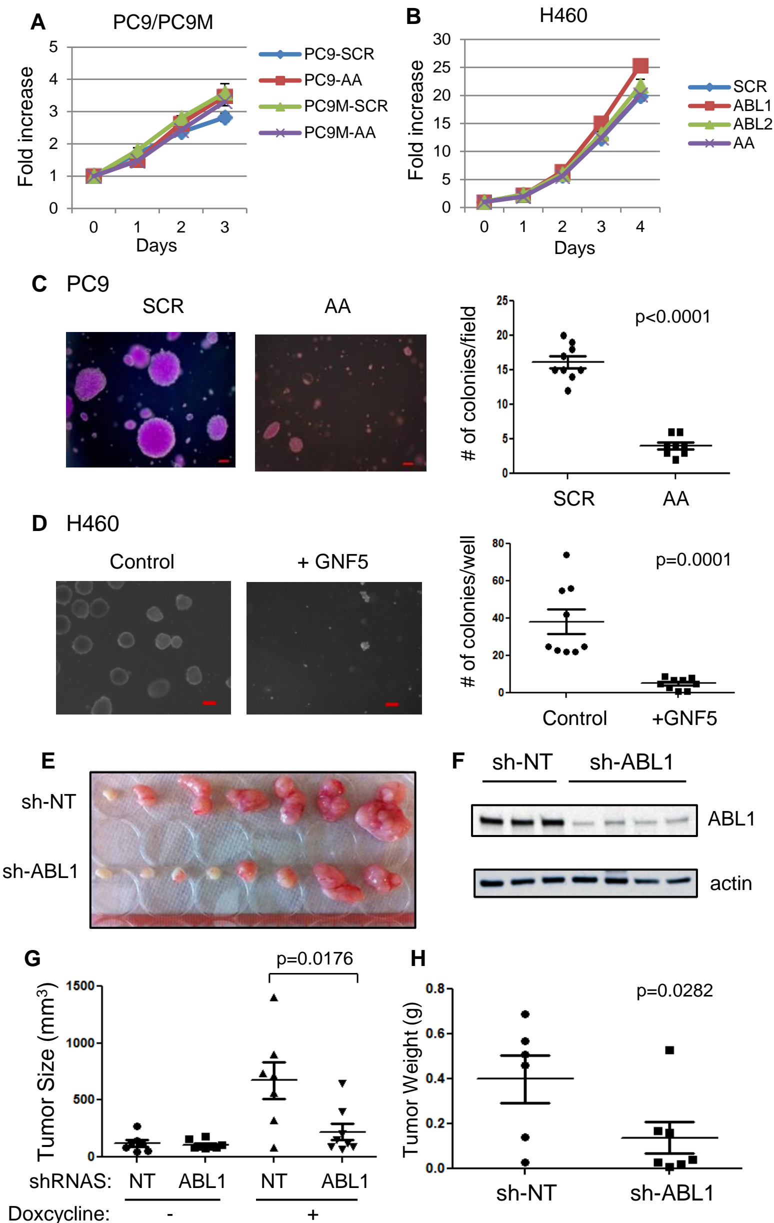
Supplemental Figure 2. NSCLC cells metastasize to multiple organs. Mice were injected by intracardiac route with luciferase-labeled PC9 or H460 lung cancer cells. After approximately 20 days, mice were injected with luciferin before euthanizing. The indicated organs and tissues were isolated, placed in tissue culture dishes followed by bioluminescent imaging.



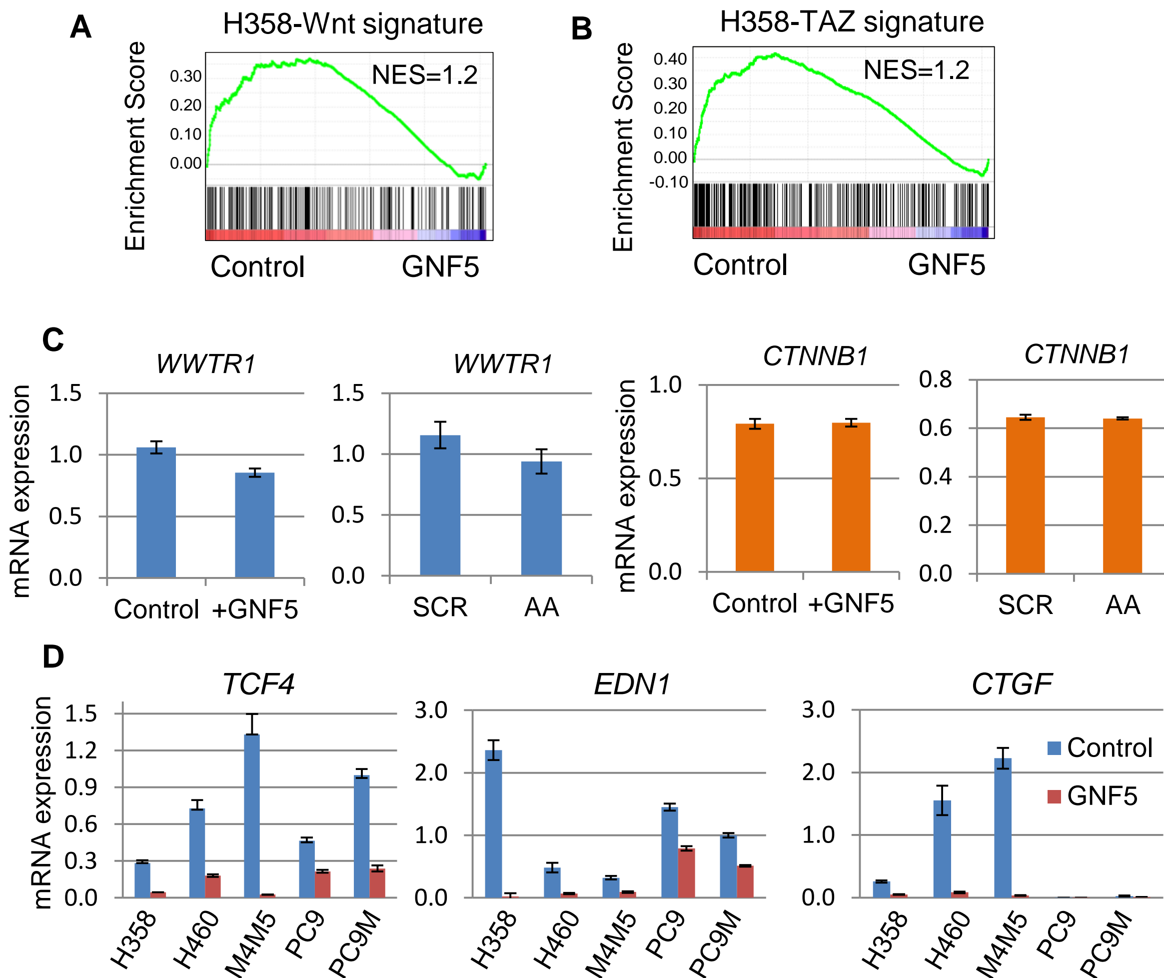
Supplemental Figure 3. The GNF5 allosteric inhibitor exhibits signaling specificity and is well tolerated in mice. (**A**) Three ABL kinase inhibitors STI571 (S, 10 μM), Nilotinib (N, 2 μM) and GNF5 (G, 20 μM) were used to treat the indicated NSCLC cell lines for 3 hrs. ERK1/2 activation was detected by phosphorylation of ERK1/2 on T202/Y204, and ABL kinase activity was detected by p-CrKL antibody specific for phospho-Y207. Total cell lysates were blotted for the indicated proteins; actin was used for loading control. (**B**) PC9M cells labeled with luciferase were intracardiac injected into nude mice, and the mice were treated with GNF5 one day after injection by oral gavage. Mice were weighed on the indicated days (n=6 each group). Data presented as Mean ± SEM.



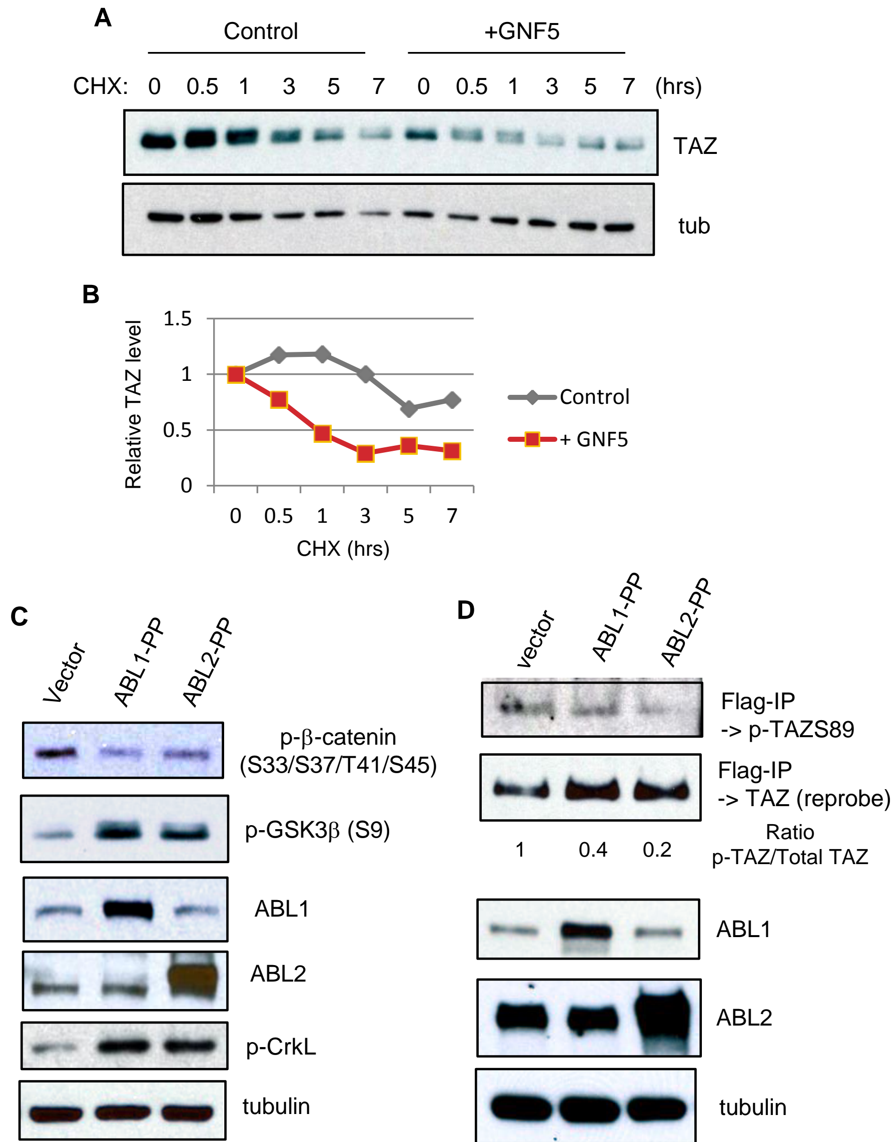
Supplemental Figure 4. Depletion of ABL kinases impairs NSCLC dissemination to the lung parenchyma but does not decrease NSCLC cell numbers in pulmonary blood vessels prior to extravasation. PC9M cells transduced with lentiviruses encoding either scrambled (SCR) or the 2nd-set of ABL1/ABL2-specific shRNAs (AA) were labeled with Cell-Tracker Green followed by tail vein injection into nude mice. (**A**) Lungs were isolated at 24 h after tail vein injection. Representative images of whole lung section are shown (scale bar: 1000 μm). (**B**) Quantification of extravasated PC9M cells in the lungs at 24 h post-injection (100 micron sections, n=5 for each mouse) for the indicated mice (SCR n=3, 2nd-AA n=3). Data are represented as mean ± SEM. The *P*-values were determined by Student's *t*-test. (**C**) PC9 cells were transduced with either SCR or AA shRNAs followed by tail vein injection into nude mice. Lungs were collected at 2 h post-injection, representative images are shown (scale bar: 20 μm). (**D**) The same lung sections as in (**C**) were immune-stained with anti-CD31 to visualize blood vessels, representative images are shown (scale bar: 25 μm).



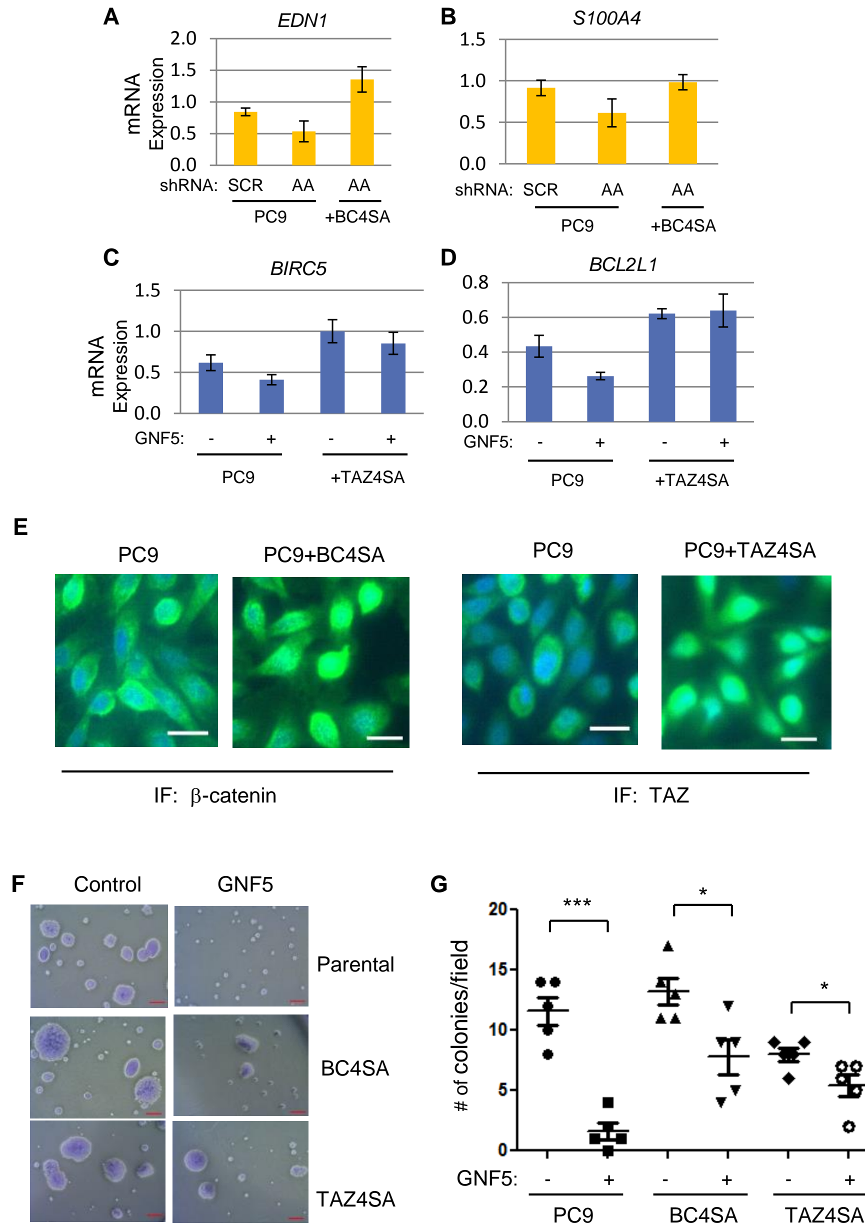
Supplemental Figure 5. ABL kinases are required for anchorage-independent NSCLC colony formation and tumor xenograft growth. (A and B) PC9 or PC9M cells were transduced with either scrambled (SCR) or ABL1/ABL2-specific (AA) shRNAs (**A**); H460 cells were transduced with SCR, ABL1, ABL2 and double ABL1/ABL2 (AA) shRNAs (**B**); cells were plated in 96 well-plates in triplicates and assayed for cell growth and viability each day using Cell Titer-glo; data were plotted as fold increase compared to day 0. (**C and D**) PC9 cells were transduced with either SCR or AA shRNAs (**C**) and H460 cells were treated with or without ABL allosteric inhibitor GNF5 (**D**). Cells were assayed for anchorage-independent colony formation; representative images are shown (**C** and **D** left side; scale bar: 200 μm) with quantification of colony numbers (**C** and **D** right side; PC9 n=9 each; H460 n=9 each). (**E-H**) H460 cells harboring doxycycline-inducible ABL1-specific shRNA were injected subcutaneously into nude mice. Once tumor reached ~100 mm^3 mice were treated with doxycycline. Cells harboring non-targeting shRNA (sh-NT) were used as controls. Tumors were isolated after 13 days of induction (**E**), and representative tumor lysates were used to evaluate depletion of ABL1 protein (**F**). Tumors were measured before and after doxycycline induction (**G**), and tumors were weighted at the end of the experiment (**H**). Data are represented as mean ± SEM (sh-NT: n=7; sh-ABL1:n=8). All *P*-values were determined Student's *t*-test.



Supplemental Figure 6. ABL kinases regulate the expression of WNT and TAZ downstream target genes in lung cancer cells. (**A** and **B**) GSEA analysis of WNT (**A**) and TAZ (**B**) signature genes using RNAseq data from H358 cells treated with or without GNF5. (**C**) PC9 cells were treated with or without GNF5, or transduced with either scrambled (SCR) or ABL1/ABL2-specific (AA) shRNAs. Expression of *WWTR1* (TAZ) or *CTNNB1* (β-catenin) mRNAs was detected by quantitative RT-PCR in triplicates. Results were normalized to the expression of *GAPDH*. Data are represented as mean ± SEM. (**D**) Expression of *TCF4*, *EDN1* (endothelin 1)and *CTGF* (connective tissue growth factor) in the indicated NSCLC cell lines treated with (red) or without (blue) GNF5 were measured by quantitative RT-PCR in triplicates. Results were normalized to the expression of *GAPDH*. Data are represented as mean ± SEM.



Supplemental Figure 7. ABL kinases regulate β-catenin and TAZ protein stability. (**A**) PC9 cells were treated with cycloheximide (100 μg/ml) for the indicated times in the absence or presence of GNF5. TAZ and tubulin (loading control) protein levels were analyzed by Western blotting. (**B**) Relative TAZ protein levels were calculated after normalization to tubulin protein. (**C**) PC9 cells were transduced with lentiviruses encoding vector control or active forms of ABL1 (ABL1-PP) or ABL2 (ABL2-PP). Total cell lysates were analyzed by Western blotting with the indicated antibodies. (**D**) PC9 cells carrying Flag-tagged wild type TAZ were transduced with vector control or active forms of ABL1 (ABL1-PP) or ABL2 (ABL2-PP). Immunoprecipitated (IP)-TAZ was performed using anti-Flag-gel followed by Western blotting with anti-TAZ (S89) and re-probed with anti-TAZ antibodies. Band intensity was quantify using Fiji imageJ software and ratios p-TAZ/total TAZ were indicated with cells containing vector control set as 1. Total cell lysates were used for blotting with the indicated antibodies; tubulin was used as loading control. Same samples were run contemporaneously on several parallel gels to blot with indicated antibodies.



Supplemental Figure 8. Active β-catenin and TAZ rescue expression of downstream target genes, localize predominantly to the nucleus, and partially rescue colony growth in PC9 cells lacking active ABL kinases. (**A** and **B**) PC9 parental or PC9 cells expressing active β-catenin (BC4SA) were transduced with either scrambled (SCR) or ABL1/ABL2-specific (AA) shRNAs; (**C** and **D**) PC9 parental or PC9 cells expressing active TAZ (TAZ4SA) were treated with or without GNF5. Expression of the indicated genes was analyzed by quantitative RT-PCR in triplicates; results were normalized to the expression of *GAPDH*. Data are represented as mean ± SEM. (**E**) Immunofluorescence (IF) staining was performed on PC9 parental or PC9 cells expressing BC4SA or TAZ4SA using anti-β-catenin (green) or anti-TAZ (green) antibodies as indicated; cells were co-stained with Hoechst (blue) to visualize the nucleus. (Scale bar: 20 μm). (**F** and **G**) PC9 parental and PC9-expressing BC4SA or TAZ4SA cells were evaluated for in vitro colony formation in the absence or presence of GNF5 (5 μM). Representative colony images (**F**) and quantification (**G**) are shown (n=5 each). Data are represented as mean ± SEM. (Scale bar: 200 μm). All *P*-values were determined by Student's *t*-test. (* $p<0.05$; *** $p<0.0001$).