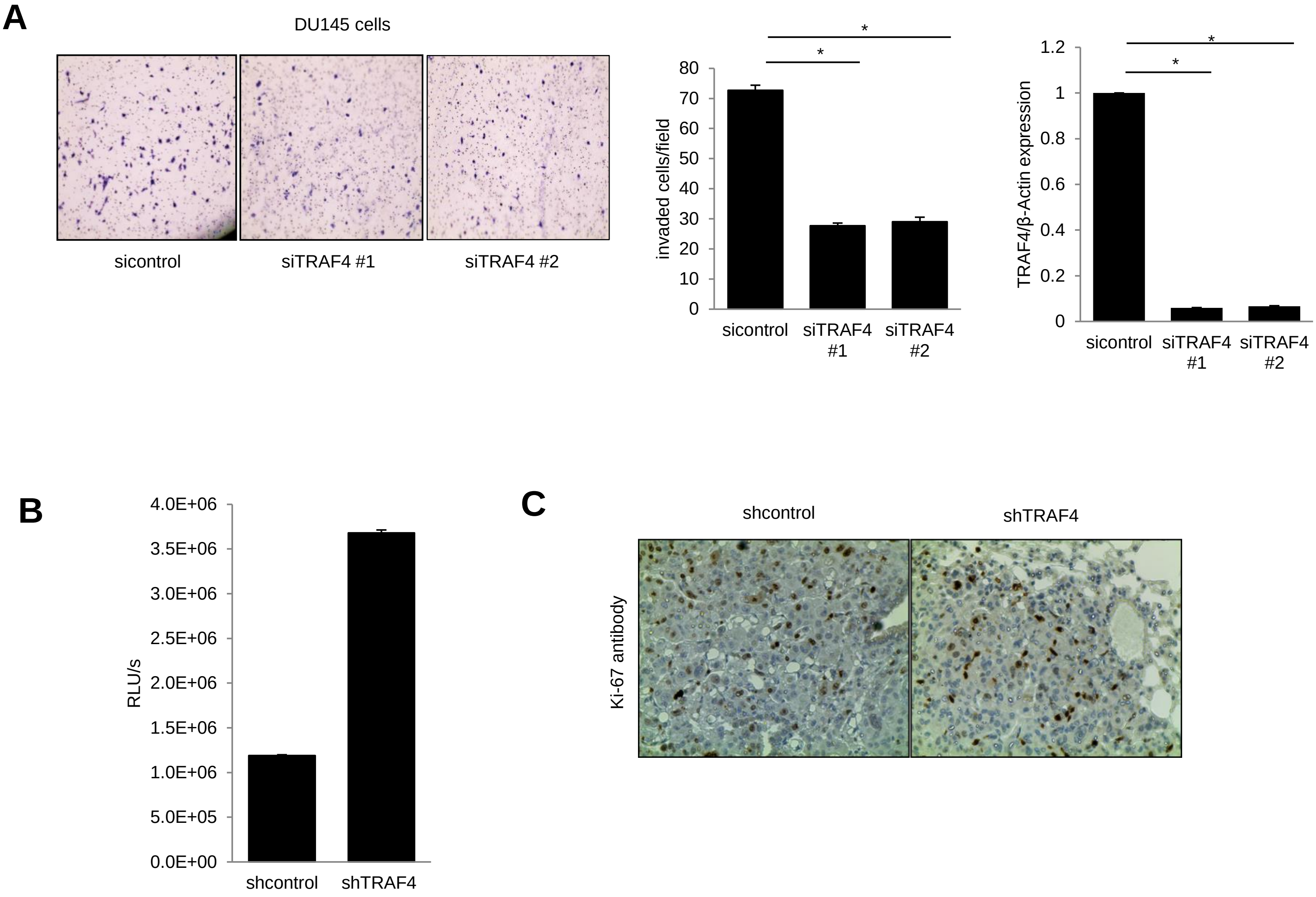
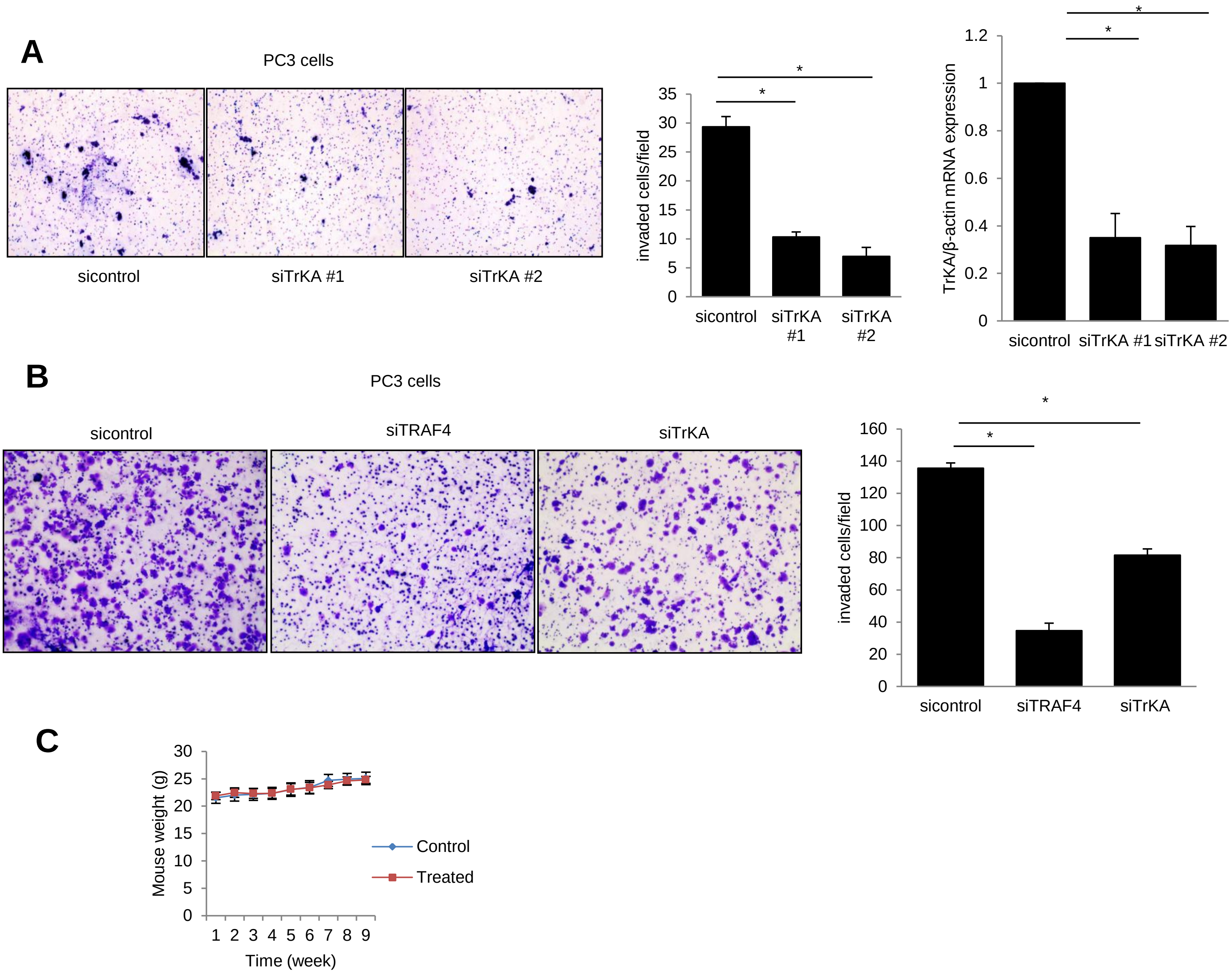


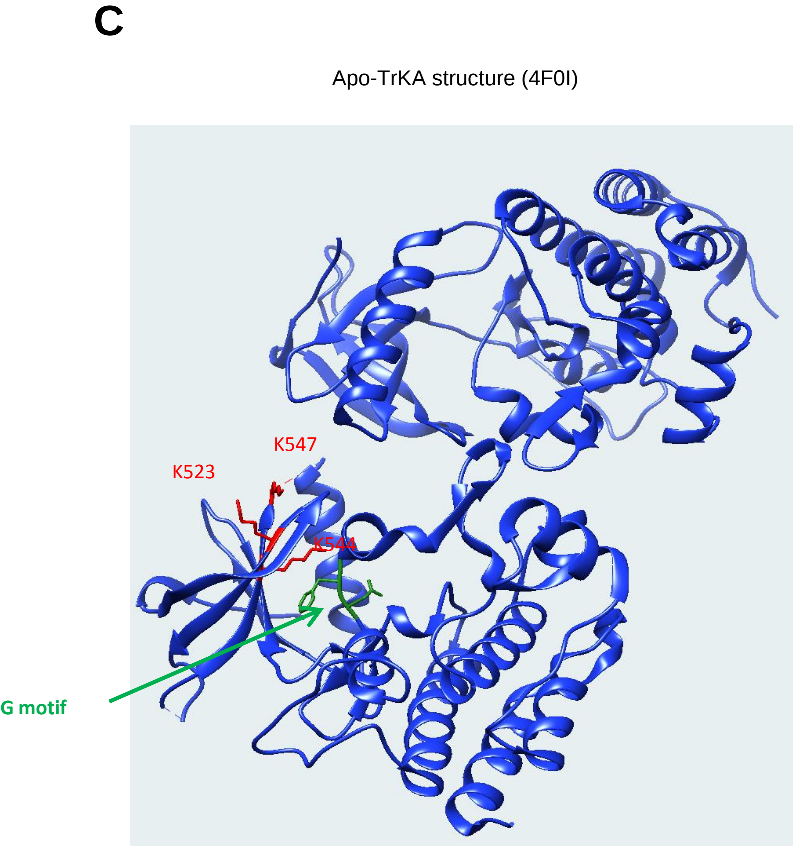
Supplemental Figure 1. (A) Knockdown of TRAF4 using specific siRNAs decreased DU145 cell invasion. Left panel, crystal violet staining of invaded cells (n=3). Images were obtained at 100X magnification. Middle panel, quantitation of invaded cells per field. Right panel, the levels of TRAF4 in control or TRAF4 knockdown cells as assessed by qRT-PCR. *P < 0.05 by one way ANOVA with Dunnett's multiple comparisons test. (n=3) (B) PC3 shTRAF4 cells have higher luminescence intensity than shcontrol cells. (C) Comparable levels of Ki-67 staining in shctrl and shTARF4 tumor samples as determined by IHC analysis. Images were obtained at 100X magnification. Data are represented as the mean ± SEM.



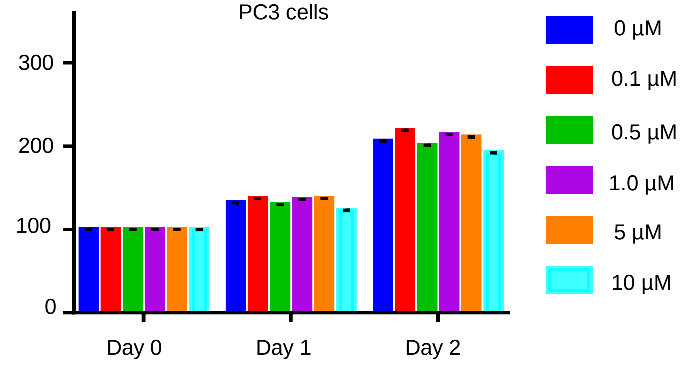
Supplemental Figure 2. (A) Knockdown of TrkA using specific siRNAs decreased PC3 cell invasion (n=3). Images were obtained at 100X magnification. Right panel, the levels of TrkA in control or TrkA knockdown cells as assessed by qRT-PCR. (n=3). *P < 0.05 by one way ANOVA with Dunnett's multiple comparisons test. (B) TrkA knockdown has an inhibitory effect on PC3 cell invasion similar to the effect of TRAF4 knockdown (n=3). Images were obtained at 100X magnification. Right panel, quantitation of TRAF4 or TrKA siRNA knockdown invaded cells per field after invasion. *P < 0.05 by one way ANOVA with Dunnett's multiple comparisons test. (C) TrkA inhibitor treatment did not change mouse weights. Data are represented as the mean ± SEM. (n=3). *P <0.05 by two tailed Student's t test.

A

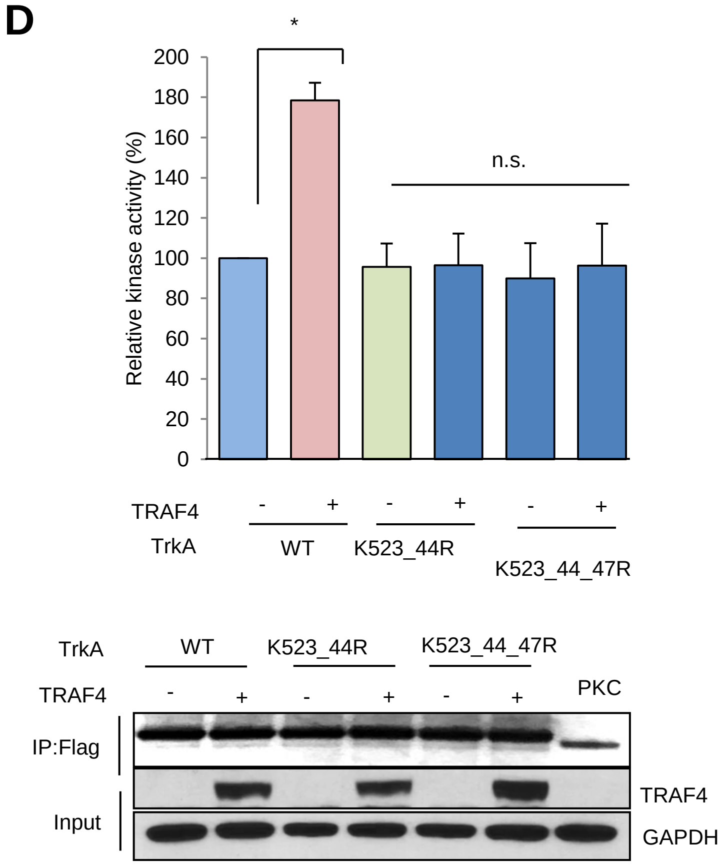
C



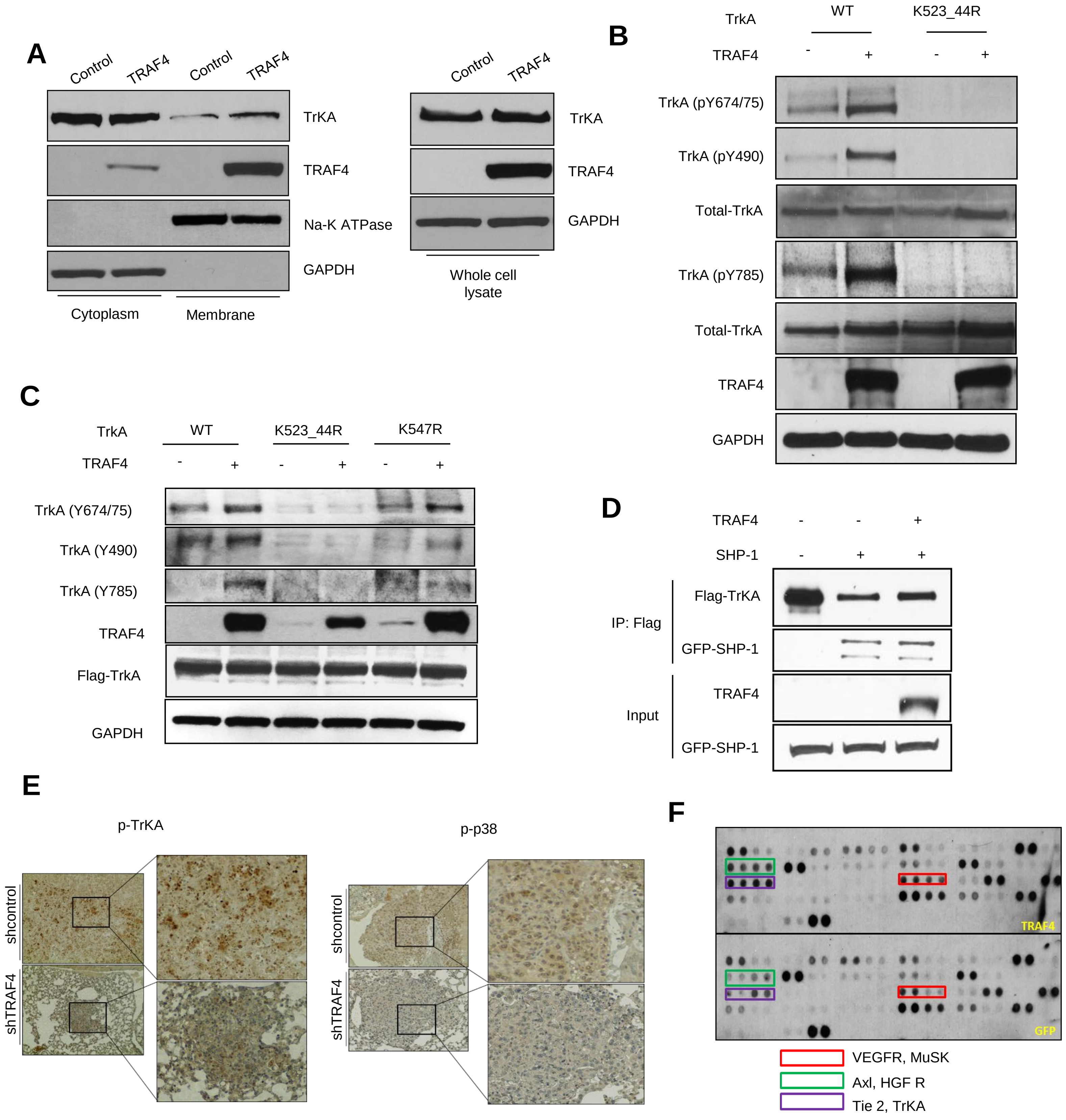
B



D



Supplemental Figure 3. (A) TRAF4 promoted TrkA ubiquitination in vitro. Flag-TrkA was transiently tranfected into 293T cells and purified using an anti-flag antibody. The purified protein was then incubated with HA-ubiquitin, UBE1, UbcH5a in the absence or presence of purified TRAF4. The levels of TrkA ubiquitination was determined by immunoprecipitation using an anti-flag antibody followed by Westernb blot analysis using an anti-HA antibody. (B) TrkA inhibitor, GW441756, did not significantly affect cell growth in PC3 cells for specified dose and time treatment in PC3 cells. (n=3). (C) Lysine residues K523, K544 and K547 (shown in red) are located close proximity to the DFG motif (shown in green) in the kinase activation loop. Show is crystal structure of apo-TrkA kinase domain (PDB ID: 4F0I (44)). (D) Mutation of K523 and K544 to R abolished TRAF4-mediated TrkA activation in vitro. The upper panel shows the in vitro kinase activity of TrkA and its mutants in the absence or presence of TRAF4 overexpression. The bottom panel shows that comparable amount of TrkA proteins were used in the in vitro kinase assay as assessed by the Western blot analysis. Data are represented as the mean ± SEM. (n=3). *P <0.05 by one way ANOVA.



Supplemental Figure 4. (A) TRAF4 overexpression did not have a major effect on TrkA cellular distribution. DU145 cells were serum starved and transfected with or without TRAF4 followed by NGF stimulation. Cytosolic and membrane fraction were then isolated and the levels of TrKA and TRAF4 in each fraction were visualized by Western blot analysis using specific antibodies. Right panel, total TrKA level in DU145 whole cell lysates with NGF treatment. Na-K ATPase was used as an internal control for membrane fraction and GAPDH for cytoplasmic and total protein. (B) K523_44R mutation abolished TrkA tyrosine phosphorylation. (C) K547R single mutation did not abolish TrkA tyrosine phosphorylation. (D) TRAF4 overexpression did not alter the interaction between TrkA and SHP-1 phosphatase. Flag-TrkA was transfected into 293T cells with or without TRAF4 co-transfection. Shown is a co-IP experiment using a flag-specific antibody. (E) The levels of p-p38 (pT180/Y182) and p-TrKA (pY785) were significantly reduced in TRAF4 knockdown metastatic tumors compared tumors derived from control cells. Shown are representative immunohistochemistry images at 40X and 100X (inset) magnification from lung tumors derived from PC3 cell tail-vein injection (n=3 per group). (F) TRAF4 overexpression increased ubiquitination of several receptor tyrosine kinases. Equal amount of cell lysates from GFP (control) or TRAF4 expressing adenovirus infected PC3 cells were incubated with a RTK array. Each duplicate dot represents the ubiquitination level of a target protein recognized by an anti-ubiquitin antibody.

Supplemental Table T1

	Gene	Primer sequence
1	TrKA_5.1	5'-agtcagccacggtgatgaa-3'
2	TrKA_3.1	5'-cgttcttcctgttgaggtcac-3'
3	SLUG_5.1	5'-tggttgcttcaaggacacat-3'
4	SLUG_3.1	5'-gcaaatgctctgttgcagtg-3'
5	IL-6_5.1	5'-gatgagtacaaaagtcctgatcca-3'
6	IL-6_3.1	5'-ctgcagccactggttctgt-3'
7	Cox-2_5.1	5'cttcacgcatcagtttttcaag-3'
8	Cox-2_3.1	5'tcaccgtaaatatgatttaagtccac-3'
9	Integrin_α4_5.1	5'-gatgaaaatgagcctgaaacg-3'
10	Integrin_α4_3.1	5'-gccatactattgccagtgttga-3'
11	Integrin_β4_5.1	5'-tcagcctctctgggacctt-3'
12	Integrin_β4_3.1	5'-tccttatccacacggacaca-3'
13	Integrin_β1_5.1	5'-cgatgccatcatgcaagt-3'
14	Integrin_β1_3.1	5'-acaccagcagccgtgtaac-3'
15	ACTB_5.1	5'-ccaaccgcgagaagatga-3'
16	ACTB_3.1	5'-ccagaggcgtacagggatag-3'

Supplemental Table T2

	Gene	Primer sequence
1	TrKA-ΔCT–domain-3-XhoI	5’-ccattctcgagggcttgcagccgggcgtgca-3’
2	TrKA-ΔTK–domain-3-XhoI	5’-ccattctcgaggtgaacacaggcatcactgaa-3’
3	TrKA-ΔJM–domain-3-XhoI	5’-ccattctcgaggagcacaaggagcagcgtaga-3’
4	TrKA-ΔTM–domain-3-XhoI	5’-ccattctcgaggcccacagccaccgagacccca-3’
5	TrKA-IC–5-EcoRI	5’-ccattgaattcctggccgtctttgcctgcctct-3’
6	TrKA-ΔTK1–domain-3-XhoI	5’-ccattctcgaggaagaagcgcacgatgtgctggt-3’
7	TrKA_TK_EcoRI _5.1	5’-ccattgaattccacatcaagcgccgggacatc-3’
8	TrKA_K775R_5.1	5'-cacagcatcagggatgtgcacgcc-3'
9	TrKA_K775R_3.1	5'-gcgttgctggggctcccg-3'
10	TrKA_K725R_5.1	5'-acctacggcaggcagccctgg-3'
11	TrKA_K725R_3.1	5'-gaagatctcccagagcaccacg-3'
12	TrKA_K703R_5.1	5'-ctgtaccgtaggttcaccaccg-3'
13	TrKA_K703R_3.1	5'-gatgctctcgggcggcat-3'
14	TrKA_K665R_5.1	5'-ctggtggtcaggattggtgattttg-3'
15	TrKA_K665R_3.1	5'-tccctggcccactagaca-3'
16	TrKA_K609R_5.1	5'-cctgatgccaggctgctggct-3'
17	TrKA_K609R_3.1	5'-tccatgggatcggaggaagc-3'
18	TrKA_K544_47R_5.1	5'-gtggctgtcagggcactgagggagg-3'
19	TrKA_K544_47R_3.1	5'-cagcatcttgtcctgctc-3'
20	TrKA_K523R_5.1	5'-gcctttgggagggtcttccttgctgag-3'
21	TrKA_K523R_3.1	5'-gccctcccccagctccca-3'
22	TrKA_K547R_5.1	5'-aaggcactgagggaggcgtcc-3'
23	TrKA_K547R_3.1	5'-gacagccaccagcatcttg-3'
24	TrKA_K523R_5.2	5'-gcctttgggagggtcttccttg-3'
25	TrKA_K523R_3.2	5'-gccctcccccagctcccactt-3'

Supplemental Table 1, 2. RT-qPCR primer sequence and cloning sequence used in the study. 5.1 denotes forward and 3.1 denotes reverse primer.

Supplemental Table T3

S. no	Antibody
1	14-3-3zeta,gamma,eta_R_V
2	Akt_R_V
3	p-Akt(S473)_R_V
4	p-Akt(T308)_R_V
5	ALK(D5F3)XP_R_V
6	p-ALK(3B4)(Y1586)_R_V
7	p-ALK(Y1604)_R_V
8	p-AMPKa(40H9)(T172)_R_V
9	p-AMPKa1(S485)_R_V
10	p-AMPKb1(S108)_R_V
11	Annexin1_R_V
12	ATM_R_V
13	ATR_R_V
14	p-ATR(S428)_R_V
15	p-AuroraA(T288)/B(T232)/C(T198)_R_V
16	AuroraA/AIK_R_V
17	p-Axl(D12B2)(Y702)_R_V
18	Bad_R_V
19	p-Bad(S112)_R_V
20	p-Bad(S136)_R_V
21	p-Bad(S155)_R_V
22	Bak_R_V
23	Bax_R_V
24	p-Bcl2(5H2)(S70)_R_V
25	p-Bcl2(T56)_R_V
26	Bcl-xL_R_V
27	BRCA1_R_V
28	BRCA2_R_V
29	E-Cadherin(24E10)_R_V
30	N-Cadherin_R_V
31	Caspase-3_R_V
32	p-Beta-Catenin(S33/37/T41)_R_V
33	Caveolin-1(D46G3)XP_R_V
34	p-Caveolin-1(EPR2288Y)(Y14)_R_V
35	CD24(FL-80)_R_V
36	p-Chk1(S345)_R_V
37	p-Chk2(S33/35)_R_V
38	Claudin-1_R_V
39	c-Met_R_V
40	p-c-Myc(T58)_R_V
41	DKK1_R_V
42	EGFR(D38B1)XP_R_V
43	EGFR(L858R)_R_V
44	p-EGFR(S1046/1047)_R_V
45	p-EGFR(Y1045)_R_V
46	p-EGFR(Y1148)_R_V
47	p-EGFR(Y1173)_R_V
48	p-EGFR(Y1173)(53A5)_R_V
49	p-EGFR(Y1068)(D7A5)XP_R_V
50	Ezh2(D2C9)XP_R_V

51	FGFR1(D8E4)XP_R_V
52	FoxO1(C29H4)_R_V
53	FSP1/S100A4_R_V
54	p-HER2/ErbB2(Y1248)_R_V
55	p-HER2/ErbB2(Y877)_R_V
56	HER3/ErbB3_R_V
57	p-HER3/ErbB3(Y1197)_R_V
58	HDAC1_R_V
59	HDAC3_R_V
60	HDAC4_R_V
61	HDAC6_R_V
62	ILK1_R_V
63	p-Jak1(Y1022/1023)_R_V
64	Kit-c_R_V
65	Lipocalin-1_R_V
66	LRP6_R_V
67	MEK1_R_V
68	p-MEK1/2(S217/221)_R_V
69	p-Met(Y1234/1235)_R_V
70	MMP-9_R_V
71	mTOR_R_V
72	p-mTOR(D9C2)XP(S2448)_R_V
73	Notch1(C44H11)_R_V
74	p-p27(T187)_R_V
75	p-p27/KIP1(T198)_R_V
76	p27/KIP1(C-term)_R_V
77	p38/MAPK_R_V
78	p-p38(D3F9) XP(T180/Y182)_R_V
79	p44/42MAPK(Erk1/2)_R_V
80	p-p44/42MAPK(Erk1/2)(T202/Y204)(197G2)_R_V
81	p53_R_V
82	p-p53(S15)_R_V
83	p70S6K_R_V
84	p-p70S6K(S371)_R_V
85	p-p70S6K(T389)_R_V
86	p-p70S6K(T412)_R_V
87	p-PDGFRa(23B2)(Y754)_R_V
88	PDGFRb(28E1)_R_V
89	p-PDGFRb(Y751)_R_V
90	p-PDK1(S241)_R_V
91	PIAS1_R_V
92	PTEN(D4.3)XP_R_V
93	p-PTEN(S380)_R_V
94	p-RafB(S445)_R_V
95	RANKL_R_V
96	p-Ret(Y905)_R_V
97	SerpinA1_R_V
98	Slug(C19G7)_R_V
99	p-Smad2(S465/467)_R_V
100	SOCS1(A156)_R_V
101	SOCS3_R_V
102	Sox2(D6D9) XP_R_V
103	p-Src(Y527)_R_V

104	Stat1_R_V
105	p-Stat1(Y701)(D4A7)_R_V
106	p-Stat2(Y690)_R_V
107	p-Stat3(S727)_R_V
108	p-Stat4(Y693)_R_V
109	p-Stat5(Y694)_R_V
110	Stat6_R_V
111	p-Stat6(Y641)_R_V
112	p-Tuberin/TSC2(T1462)_R_V
113	Vimentin(D21H3)XP_R_V
114	Wnt5a/b(C27E8)_R_V
115	YAP(H125)_R_V
116	ZO-1_R_V
117	cleaved Caspase-7_R_V
118	VEGFR2(55B11)_R_V
119	Stat5a(L-20)_R_V
120	SOX9_R_V
121	p-SAPK/JNK(T183/Y185)_R_V
122	Zeb1_R_V
123	PHF8(pAb)_R_V
124	ASH2_R_V
125	CBP(A-22)_R_V
126	c-Fos(9F6)_R_V
127	CHAF1A(D77D5)XP_R_V
128	c-Jun(60A8)_R_V
129	CRSP1-TRAP220_R_V
130	CtBP2_R_V
131	Cyclin C_R_V
132	DRIP130_R_V
133	FBXO11_R_V
134	FoxK2_R_V
135	KLF4_R_V
136	MED12-Abcam_R_V
137	p-c-Fos(S32)_R_V
138	PPP1R10(EPR11706)_R_V
139	Stat3(D3Z2G)_R_V
140	p-TRAP220-MED1(T1457)_R_V
141	HIF-2A(D9E3)_R_V
142	p-c-Jun(S63)_R_V
143	Sin3b_R_V
144	p-Rb(S807/811)_R_V
145	p-FAK(Y576/577)_R_V
146	Ki67_R_V
147	Integrina4(D2E1)XP_R_V
148	Integrina5_R_V
149	IntegrinaV_R_V
150	Integrinb1(D2E5)_R_V
151	Integrinb3(D7X3P)XP_R_V
152	Integrinb4_R_V
153	PI3Kp85_R_V
154	AMPKa(23A3)_R_V
155	PI3Kp110a(C73F8)_R_V
156	ERa(SP1)_R_V

157	MEK6_R_V
158	p21_R_V
159	Atg12(D88H11)_R_V
160	Beclin-1(D40C5)_R_V
161	Caspase-3(Asp175)_R_V
162	Beta-Catenin(CT)_R_V
163	Atg3_R_V
164	Atg7_R_V
165	LC3A(D50G8)XP_R_V
166	LC3B(D11)XP_R_V
167	p-EGFR(Y845)_R_V
168	p-EGFR(Y992)_R_V
169	p-ErbB2/HER2(Y1248)_R_V
170	HER2/c-ErbB2_R_V
171	ALDH_M_V
172	ALDH2_M_V
173	p-ATM(S1981)_M_V
174	Axl_M_V
175	Chk2(1C12)_M_V
176	Cox-2_M_V
177	GATA3_M_V
178	GSK-3a/b_M_V
179	HER2/c-ErbB2-P185_M_V
180	HIF-1a_M_V
181	p-IkappaB-a(S32/36)_M_V
182	S100A7/CBP_M_V
183	Twist(Twist2C1a)_M_V
184	SRC-3(clone 1208/D1)_M_V
185	c-Myc(clone 1123)_M_V
186	SRC-2(TIF2)_M_V
187	SRC-1(clone 1135/H4,1136/H4H6)_M_V
188	PR(1294)_M_V
189	Rb(4H1)_M_V
190	p-FAK(Y397)_M_V
191	AR-441_M_V
192	Bcl2(Clone-124)_M_V
193	c-Src(B-12)_M_V
194	IGF-IR(3B7)_M_V
195	Laminin5_M_V
196	Aromatase-A(Clone 677H7F10)_M_V
197	AOX1(cloneAO15)_M_V
198	IDH2_M_V
199	SCD1 (CD.E10)_M_V
200	p-SHC(2431)(Y317)_R_V
201	SRD5A1_R_V
202	FAS_R_V
203	HexokinaseII(C64G5)_R_V
204	PFKFB3 (C-terminal)_R_V
205	RRM2 (EPR11820)_R_V
206	GLDC_R_V
207	GLS1_R_V
208	LDHA_R_V
209	PHGDH_R_V

210	PKM2_R_V
211	PKM1/2_R_V
212	p-Rb(S780)(C84F6)_R_V
213	p300(C-20)_R_V