Optical activation of TrkA signaling

Supplementary Information

Figure S1

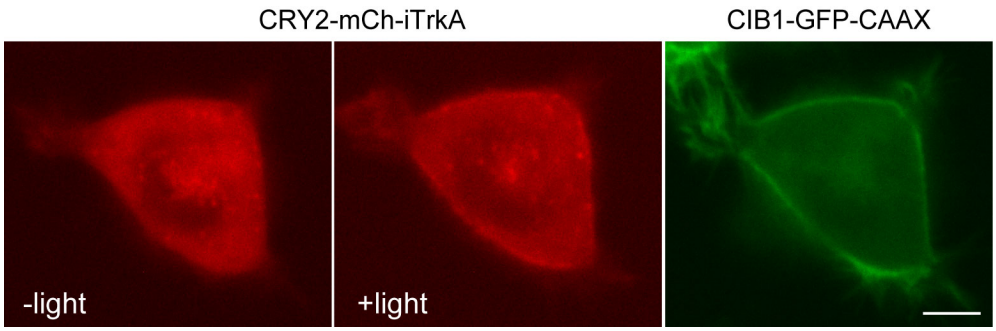

Figure S1: CRY2-mCh-iTrkA (opto-iTrkA) can be successfully recruited to plasma membrane by binding with CIB1-GFP-CAAX. PC12 cells were co-transfected with CRY2-mCh-iTrkA and CIB1-GFP-CAAX and subject to one pulse of 200 ms blue light exposure. Scale bar, 5μm.

Figure S2

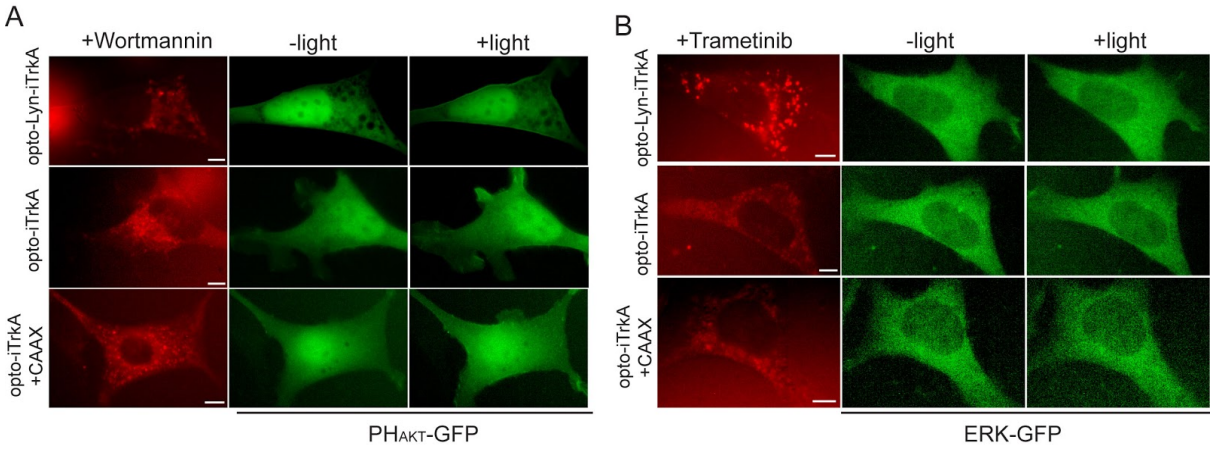

Figure S2: PI3K inhibitor wortmannin or MEK inhibitor trametinib suppressed the translocation of PH_{AKT}-GFP or ERK-GFP in cells expressing TrkA/CRY2 systems. 3T3 cells were transfected with opto-Lyn-iTrkA, opto-iTrA or opto-iTrA+CAAX as well as PH_{AKT}-GFP (A) or ERK-GFP (B). In cells expressing opto-iTrkA+CAAX, CIB1-CAAX was used without fluorescence reporter. The redistribution of PH_{AKT}-GFP or ERK-GFP was suppressed compared with cells without the addition of inhibitors. Scale bars, 5μm.

Figure S3

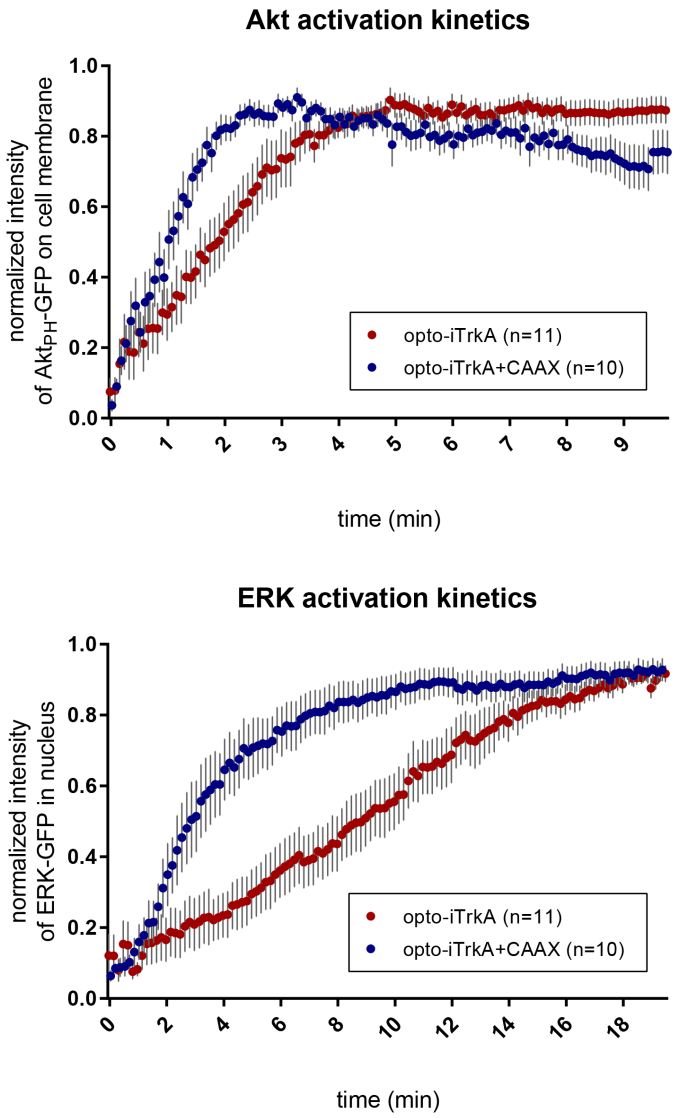

Figure S3: Kinetics of downstream activation of ERK and AKT after blue light illumination for opto-iTrkA (n=11 cells) and opto-iTrkA+CAAX (n=10 cells) systems. AKT activation was monitored by translocation of PH_{AKT}-GFP to the plasma membrane after blue light stimulation (200 ms, 9.7 W/cm^2, blue light pulses delivered at 5 s intervals for 10 min). ERK activation was monitored by translocation of ERK-GFP into the nucleus after blue light stimulation (200 ms, 9.7 W/cm^2, blue light pulses delivered at 10 s intervals for 20 min). Kinetics of activation of downstream signaling cascades are delayed for opto-iTrkA (cytosolic) compared to opto-iTrkA+CAAX. The quantification method is described in Methods: Data Analysis. The time dependent change of the ratio was normalized for each cell and then averaged to obtain the kinetic plots.

Figure S4

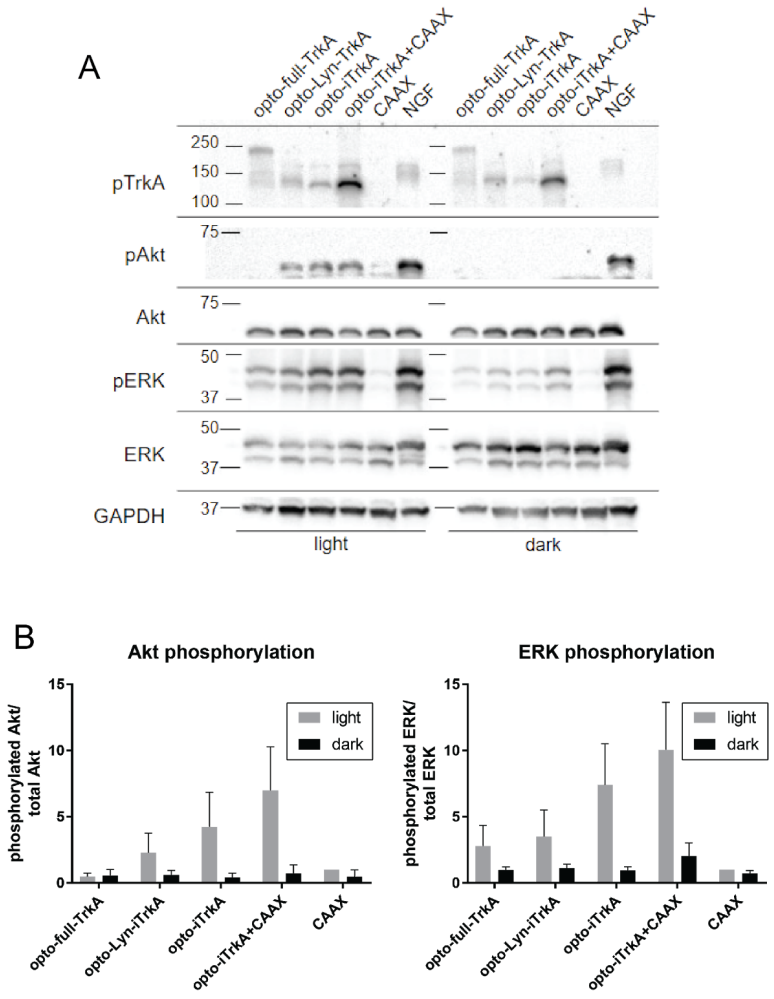

Figure S4: (A) Western blot analysis of optoTrkA systems for phosphorylated TrkA (Tyr490), phosphorylated ERK (Thr202 and Tyr204) and phosphorylated AKT (Ser473). Cells were transfected with CIBN CAAX alone as a negative control. Untransfected cells were treated with NGF for 20 minutes as a positive control. Opto-iTrkA and opto-iTrkA+CAAX exhibit TrkA, AKT, and ERK phosphorylation upon blue light stimulation. (200 μW/cm^2 continuous blue light illumination for 20 minutes) (B) Blots from three independent biological replicates were quantified by densitometry. Plots show mean + SEM, normalized to negative control CAAX with light illumination.

Figure S5

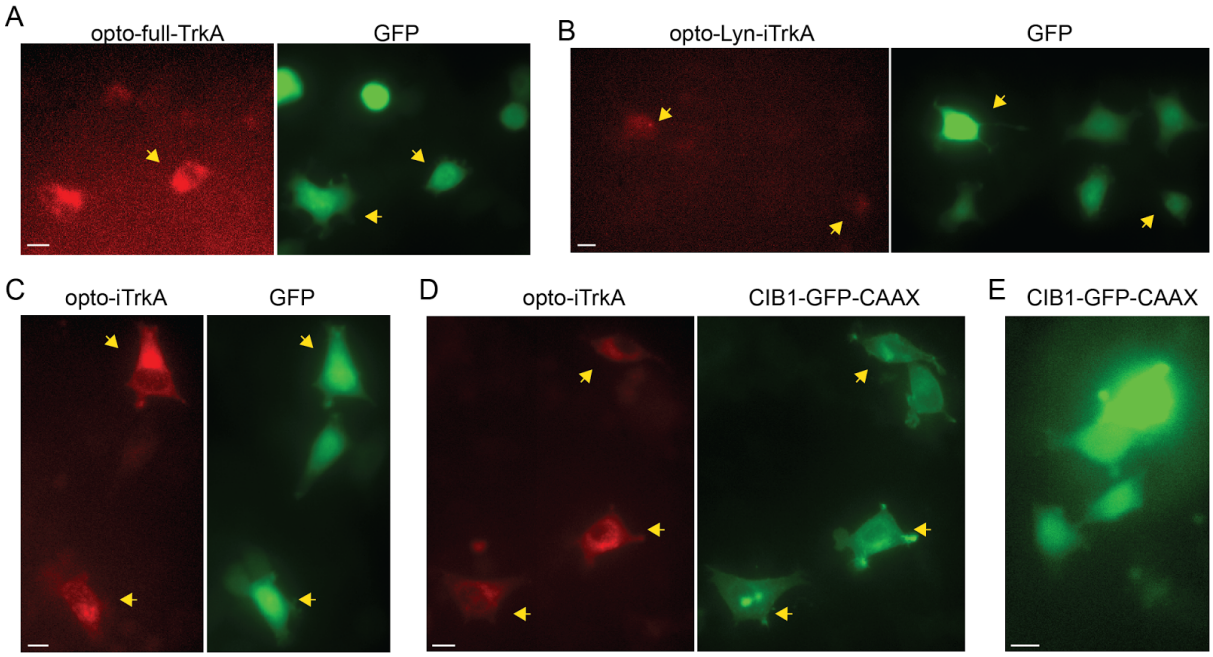

Figure S5: In dark, PC12 cells expressing each of the four TrkA/CRY2 systems(A-D) or CIB1-GFP-CAAX (E) showed no obvious neurite growth. Scale bars, 10μm.

Table S1

		opto-full-TrkA		opto-Lyn-iTrkA		opto-iTrkA		opto-iTrkA+CAX		CAAX only	
	#of cells	Total #	+ neurite	Total #	+ neurite	Total #	+ neurite	Total #	+ neurite	Total #	+ neurite
Set 1	dark	286	4	290	14	306	12	289	2	278	6
	light	243	9	199	53	292	140	194	142	293	6
Set 2	dark	262	13	300	40	306	21	289	8	255	1
	light	272	22	285	55	238	52	181	113	265	3
Set 3	dark	312	28	301	60	341	27	289	4	261	7
	light	231	21	275	53	230	76	208	98	292	2

Table S1: number of PC12 cells for quantification in Fig. 3

Table S2

		opto-full-TrkA		opto-Lyn-iTrkA		opto-iTrkA		opto-iTrkA+CAX	
	#of cells	Total #	surived	Total #	surived	Total #	surived	Total #	surived
Set 1	dark	122	1	179	2	96	0	194	8
	light	366	33	197	79	422	333	701	615
Set 2	dark	119	5	94	8	194	8	99	1
	light	76	6	223	11	88	47	226	119
Set 3	dark	136	1	75	4	132	3	70	1
	light	77	5	103	41	145	58	120	96

Table S2: number of DRG cells for quantification in Fig. 4