Supporting Information

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PNAS PNAS

Α			С			
505		KOR	EGF	<u>E15 E16 E17 E</u>	18 E19 E20 P0	P1 P2 P3
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EGFR	- boa		EGFR			
KOR			KOR			
GAP43			TAG-1		Press, Company of Company of	
TAG-1		to im	GAP43			
Grb7 Actin			Grb7			
	P0 newb	orn mice	Actin			

В													
	Control					Gefitinib			Erlotinib				
	E18	E19	E20	PO		E18	E19	E20	P 0	E18	E19	E20	P0
EGF													
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TAG-1													,1°4
GAP43										·····			
Grb7													
Actin					-								

Fig. S1. Corresponding Western blots for Figs. 1 and 2. (A) Fig. 1. (B and C) Fig. 2.



Fig. S2. The axon extension marker proteins, GAP43 and TAG-1, were expressed in similar level in KOR-null ($KOR^{-/-}$) and WT cerebellums. Western blot of WT and KOR-null ($KOR^{-/-}$) newborn cerebellums. Quantification of GAP43 and TAG-1 are shown on the right. The data represent the mean \pm SEM of the quadruple samples. #, P > 0.05.



Fig. S3. The efficiency of siRNAs against Grb7 or KOR in primary rat DRG neurons.



Fig. S4. Anti-dynorphin A antibody preincubated with dynorphin A peptide failed to block EGF-induced axon extension. (*Bottom*) Two immunohistochemical images from primary rat DRG neurons treated with EGF and anti-dynorphin A antibody preincubated with dynorphin A peptide. (*Top* and *Middle*) EGF-treated neurons or neurons treated with anti-dynorphin antibody and EGF serve as controls. (*Right*) Quantitative analysis of axon length from 50 neurons of each experiment. *, *P* < 0.05.



Fig. S5. EGF does not alter KOR mRNA level. Real-time RT-qPCR was performed to detect KOR and Actin mRNA from P19 cells treated with EGF.



Fig. S6. Expression of functional KOR from both KOR- and 5'-UTR-KOR-IRES-GFP constructs in P19 cells. (*A*) Plasmid constructs used in this study. (*B*) Western blots of KOR, GFP, and Actin from control, KOR-IRES-EGFP, or 5'-UTR-KOR-IRES-GFP transfected P19 cells. Longer exposure of KOR signal is shown on the bottom. (*C*) Quantitative ligand-binding assay using 3H-U69,593 in control, KOR-IRES-GFP or 5'-UTR-KOR-IRES-GFP transfected P19 cells. *, *P* < 0.05.



Fig. 57. Silencing Grb7 does not alter KOR mRNA stability. Real-time RT-qPCR was performed to detect KOR and Actin mRNA from P19 cells treated with transcription inhibitor actinomycin-D for 0, 3, 6, and 9 h. The efficiency of Grb7 silencing is shown at the bottom.