Supporting Information

Tsai et al. 10.1073/pnas.0912367107

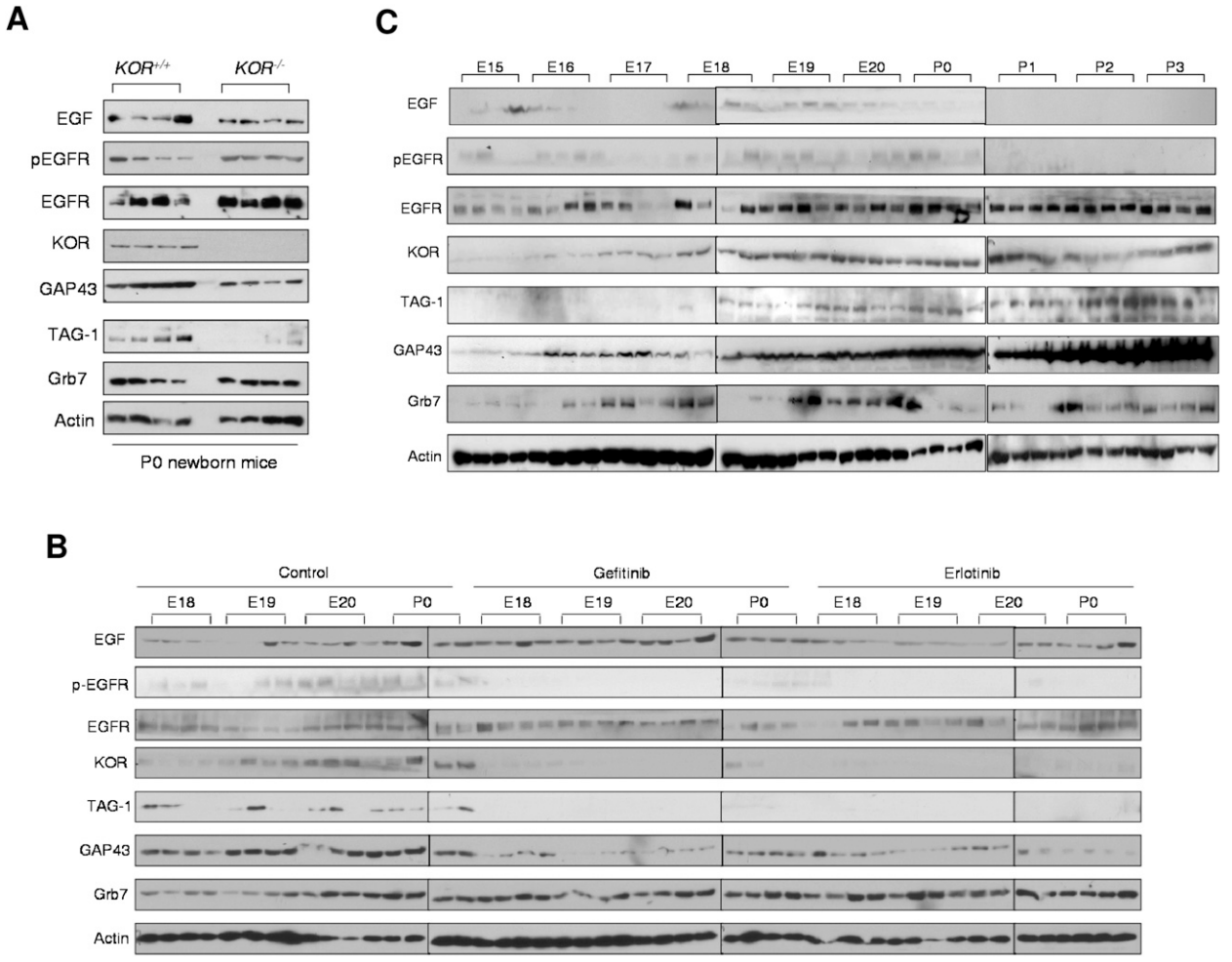

Fig. S1. Corresponding Western blots for Figs. 1 and 2. (*A*) Fig. 1. (*B* and *C*) Fig. 2.

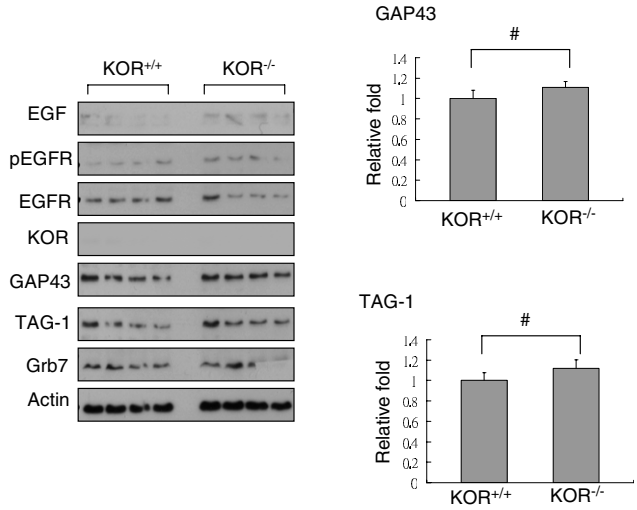

Fig. S2. The axon extension marker proteins, GAP43 and TAG-1, were expressed in similar level in KOR-null (*KOR*$^{-/-}$) and WT cerebellums. Western blot of WT and KOR-null (*KOR*$^{-/-}$) newborn cerebellums. Quantification of GAP43 and TAG-1 are shown on the right. The data represent the mean ± SEM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

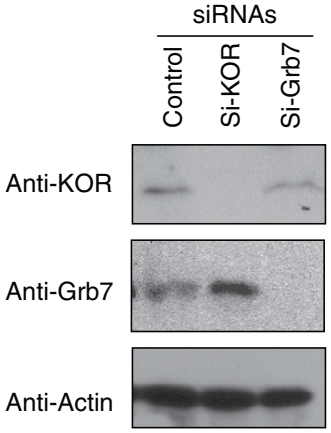

Fig. S3. The efficiency of siRNAs against Grb7 or KOR in primary rat DRG neurons.

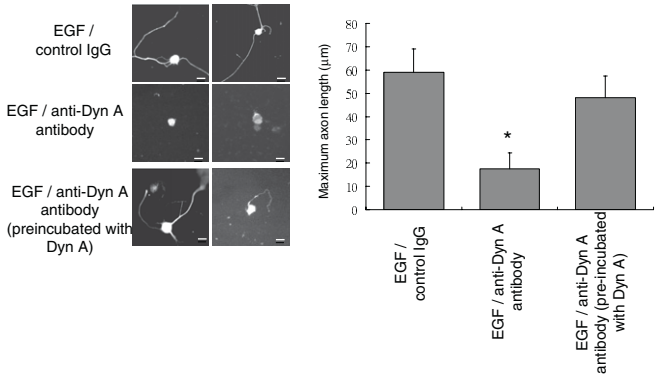

Fig. S4. Anti-dynorphin A antibody preincubated with dynorphin A peptide failed to block EGF-induced axon extension. (*Bottom*) Two immunohistochemical images from primary rat DRG neurons treated with EGF and anti-dynorphin A antibody preincubated with dynorphin A peptide. (*Top* and *Middle*) EGF-treated 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. *, $P < 0.05$.

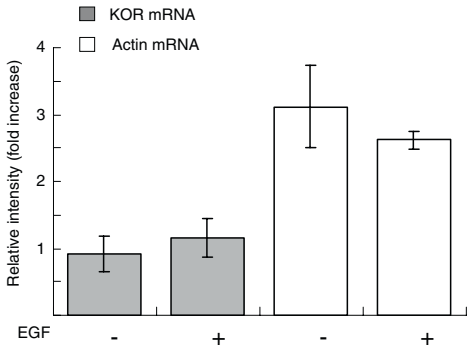

Fig. S5. EGF does not alter KOR mRNA level. Real-time RT-qPCR was performed to detect KOR and Actin mRNA from P19 cells treated with EGF.

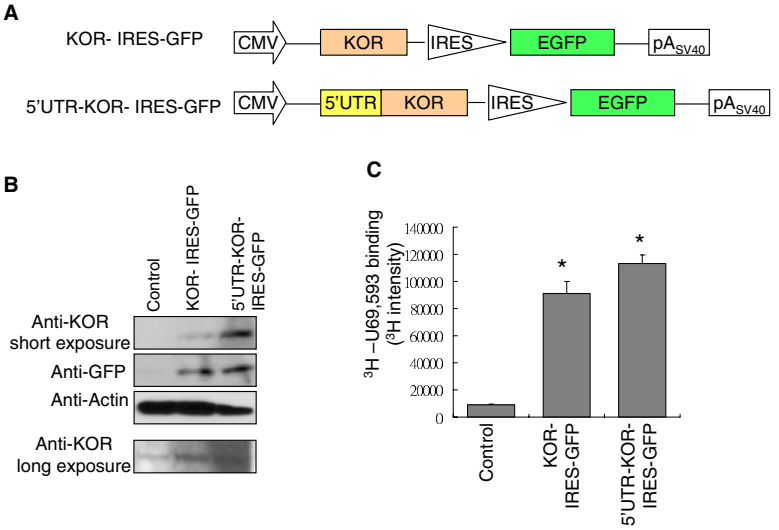

Fig. S6. Expression of functional KOR from both KOR- and 5′-UTR-KOR-IRES-GFP constructs in P19 cells. (*A*) Plasmid constructs used in this study. (*B*) Western blots of KOR, GFP, and Actin from control, KOR-IRES-EGFP, or 5′-UTR-KOR-IRES-GFP transfected P19 cells. Longer exposure of KOR signal is shown 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. *, $P < 0.05$.

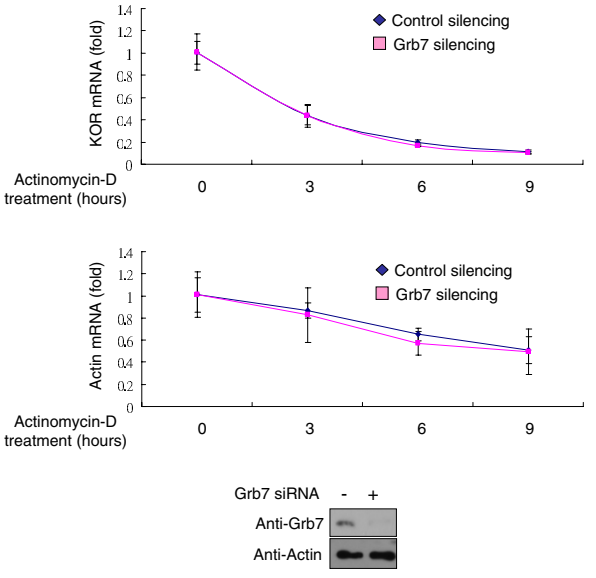

Fig. S7. Silencing Grb7 does not alter KOR mRNA stability. Real-time RT-qPCR was performed to detect KOR and Actin mRNA from P19 cells treated with transcription inhibitor actinomycin-D for 0, 3, 6, and 9 h. The efficiency of Grb7 silencing is shown at the bottom.