



Supplementary Figure 2. Netrin-1 mediates growth cone elaboration through sAC. **(a)** DCC expression in DRG neurons increases during development. Upper panel, the lysates of DIV 1, 3, and 5 E15 DRG neurons as well as DIV4 DSC neurons and HEK293T cells were immunoblotted with anti-DCC (clone G97-449, 1:1000, BD Biosciences Pharmingen). Lower panel, the same blot was probed with anti-Actin to confirm equal loading in all lanes. **(b-j)** E15 DRG explant cultures (DIV 3) were pretreated with the indicated drug for 30 min and the morphology of individual growth cones were measured at 0 and 60 min following netrin-1 (300 ng ml⁻¹) or vehicle treatment. The percent of growth cones that displayed an increase in filopodia number and the relative increase in growth cone size were quantified. **(b,c)** Phase-contrast images of DRG growth cones at 0 min **(b)** and 60 min **(c)** after the addition of netrin-1. **(d)** Netrin-1-induced growth cone elaboration is blocked by pretreatment with a DCC-blocking antibody (1 mg ml⁻¹). **(e,f)** Netrin-1-induced growth cone elaboration is blocked by pretreatment with KH7 (3 μM) **(e)**, but not by KH7.15 (3 μM) **(f)**. **(g,h)** Netrin-1-induced growth cone elaboration is blocked by pretreatment with 2-hydroxy-estradiol (OH-E, 50 μM) **(g)** but not by 2-methoxy-estradiol (Me-E, 50 μM) **(h)**. Scale bar 10 μm. **(i)** Quantification of percent of growth cones with increased filopodia after 60 min for the conditions in a-h. *n* = 50 growth cones from 3 different experiments. **(j)** Quantification of fold-increase in growth cone size after 60 min for the conditions in a-h. *n* = 27 growth cones from 3 different experiments. * *P* < 0.01 and ** *P* < 0.001. Error bars represent s.e.m.