Figure 4

The kinase activity of EphA4 is not necessary for cell death induction. Two mutants were compared to the wild type form: a kinase dead mutant (K653M) and a mutant with two mutated juxtamembrane tyrosine residues (Y596/602F) that prevent full intracellular domain activation. (a) Over-expression of EphA4 in HEK 293T cells leads to phosphorylation of the receptor, whereas the two mutants do not show tyrosine phosphorylation. (b) Despite their lack of tyrosine phosphorylation, the two EphA4 mutants are still able to induce cell death like the wild type form when over-expressed. (c) The D773/774N mutant EphA4 receptor could be phosphorylated when incubated with ephrinB3. (d) Phosphorylation of EphA4 D773/774N is increased after ephrinB3 addition. Lanes 1, 2: pcDNA 3; lanes 3, 4: EphA4-V5; lanes 5, 6: EphA4 D773/774N-V5. Lanes 2, 4 and 6: stimulation with ephrinB3. *$p<0.05$; **$p<0.01$.

Figure 5

EphA4$^{-/-}$ animals have an enlarged SVZ and RMS. (a) EphA4 is expressed in the SVZ tissue and SVZ progenitor cells grown as neurospheres *in vitro*. (b) EphA4 protein is expressed in progenitor cells grown as neurospheres, but is absent in neurospheres derived from EphA4$^{-/-}$ mice. The EphA4$^{-/-}$ mice show an accumulation of PSA-NCAM-positive (red) neuroblasts (d) as compared to wild type littermates (c) The green staining corresponds to GFAP. The RMS of EphA4$^{-/-}$ animals is also enlarged and contains more neuroblasts (f) compared to wild type littermates (e). (g) Proliferation in the SVZ of EphA4$^{-/-}$, analyzed by BrdU incorporation or Ki67 staining, was found to be unaffected in the EphA4$^{-/-}$ mice. (h) EphA4$^{-/-}$ mice have a similar number of dying cells in the SVZ (labeled by TUNEL), whereas ephrinB3$^{-/-}$ show a significant increase in cell death in the SVZ. However, compared to the total cell population or the neuroblasts population of the SVZ, less dying cells are present in the EphA4$^{-/-}$ SVZ. (j) Infusion of soluble ephrinB3-Fc into the lateral ventricle of ephrinB3$^{-/-}$ and wild type mice reduces the amount of TUNEL-positive cells, showing that it can act as an anti-apoptotic factor *in vivo*.

Supplementary figure 1

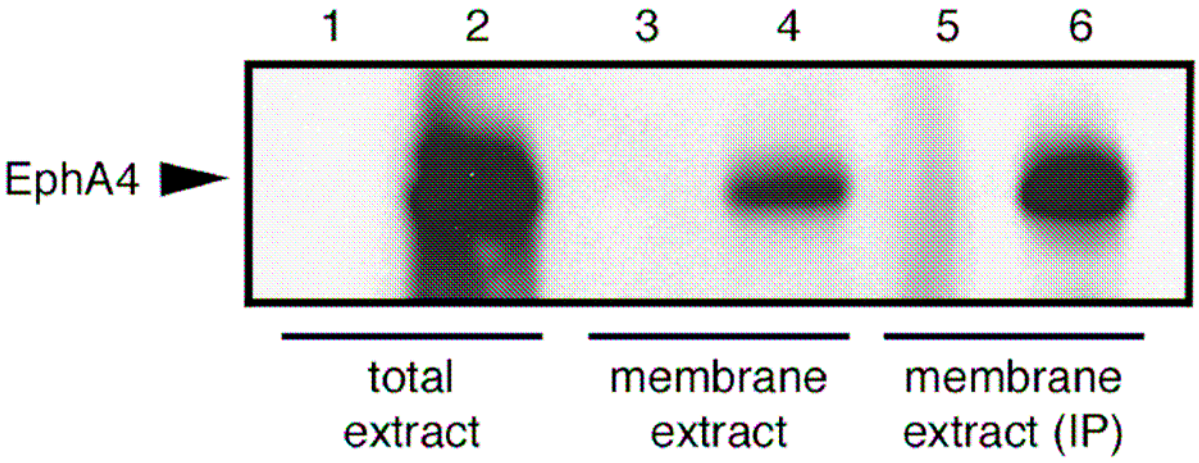
EphA4 is expressed in EphA4-transfected HEK 293 cells. a) Cells were transfected with pcDNA3.1-V5 (controls, lanes 1, 3 and 5) or with pcDNA 3.1-EphA4 (lanes 2, 4 and 6), and EphA4 was visualized with an anti-EphA4 antibody (Santa Cruz). Lanes 1 and 2 represents 20 µg total extract (RIPA buffer), lanes 3 and 4 represent 4 µg membrane extract (MEM-PER kit, Pierce), and lanes 5 and 6 represent 150 µg membrane extract immunoprecipitated with 1.5 µg anti-EphA4 antibody.

Supplementary Figure 2

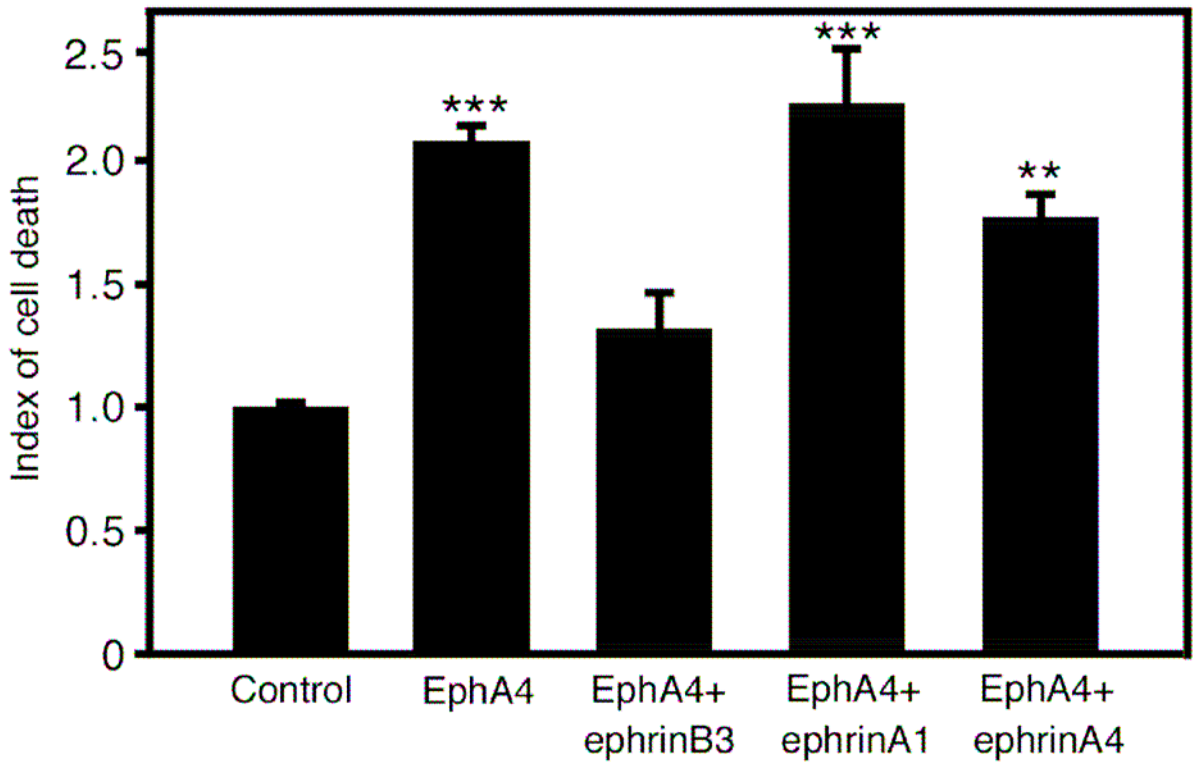
The dependence receptor function is not widespread among Eph receptors and ephrin ligands. EphrinA ligands known to bind EphA4, ephrinA1 and ephrinA4 (used at 1µg/ml) were unable to block EphA4-mediated cell death as shown for ephrinB3. **$p<0.01$; ***$p<0.001$.

Supplementary Figure 3

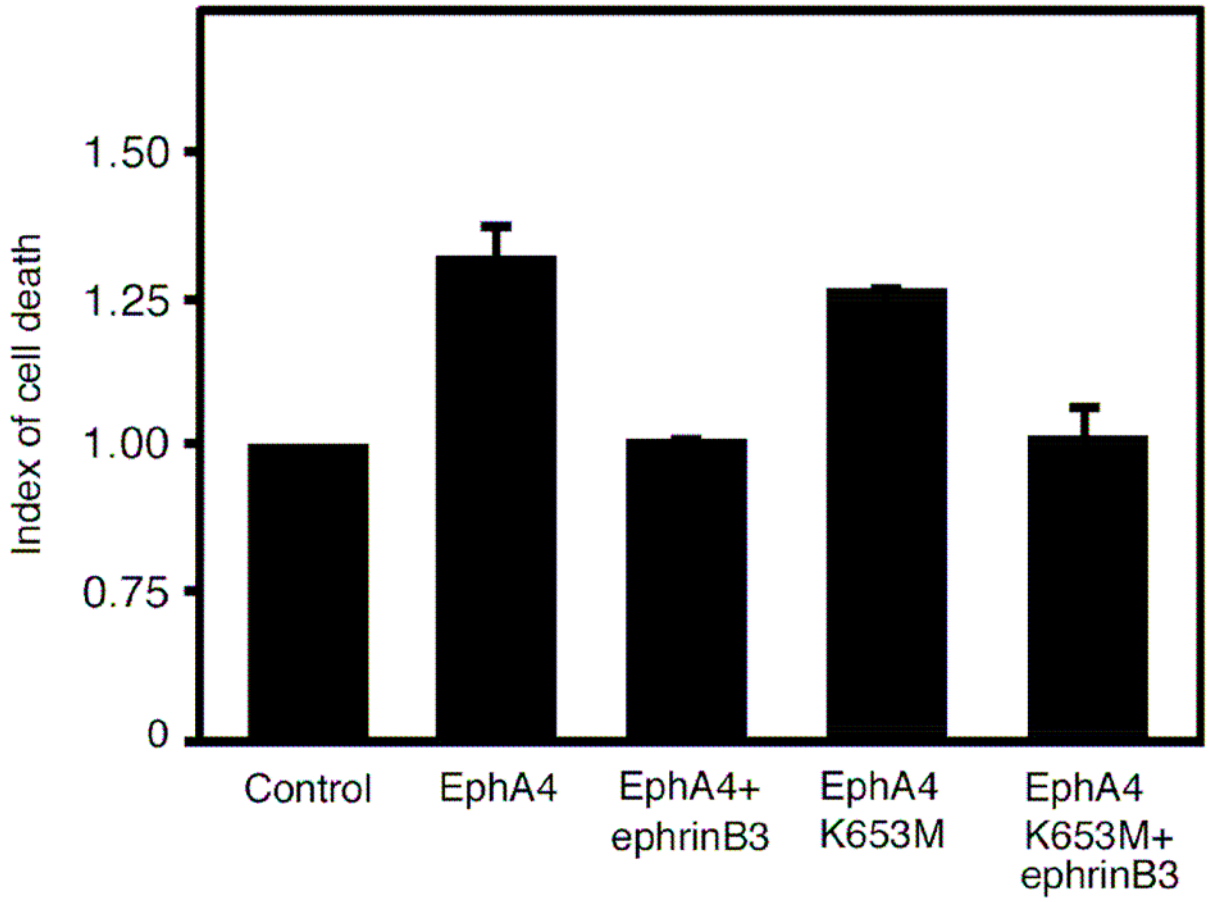
EphA4 kinase activity does not regulate cell death or survival. Cells were transfected with EphA4 or the kinase-dead EphA4K653M, and were left untreated or were treated with ephrinB3. EphrinB3 was able to inhibit EphA4-induced cell death, despite the lack of kinase activity, demonstrating that ephrinB3-induced inhibition does not function through EphA4 phosphorylation.



Supplementary figure 1



Supplementary figure 2



Supplementary figure 3