Figure S4. Control experiments supporting a role for FGFs but not Wnt8 or BMP4 in Stat3 activation. (A) Stat3 is not phosphorylated by Wnt8 or BMP4. Western blot analysis of animal caps derived from embryos injected with Stat3 (500 pg) alone or together with Wnt8 (50 pg) or BMP4 (100 pg) mRNA using anti-phosphorylated Tyr705-Stat3 (top) and anti-Stat3 (bottom) antibodies. Neither Wnt8 nor BMP4 induce pTyr705-Stat3 phosphorylation. (B-G) Whole-mount immunostaining of bissected embryos with pTyr705-Stat3 antibody at the indicated stages. eFGF (25 pg) and caFGFR4 (500 pg) mRNA injection increase the number of cells with strong pTyr705 Stat3 staining in embryos. Cross sections of gastrula embryos (B-D) and of neurula embryos at the level of the neural crest region (E-G) are shown. Higher magnification views of the ectoderm of embryos in B-D (B’-D’). Nuclear DAPI staining (B’’-D’’). (H) Luciferase assay using animal caps derived from embryos injected with a Stat reporter plasmid alone or together with FGF8a, eFGF, Wnt8 or BMP4 mRNA (50 pg each). FGF8a and eFGF, but not Wnt8 or BMP4, increases luciferase activity. (I,J) Stat3 is not required for NC downstream of Wnt8. Whole-mount in situ hybridization of embryos injected with Wnt8 (50 pg) together with Stat3 MO7mis (15 ng) or Stat3 MO (15 ng). Neither the of Stat3 MO nor Stat3 MO7mis did block the ability of Wnt8 to expand Snail2 (C, 73% n = 33; D, 54% n = 37). Neurula embryos are shown in dorso-anterior views, with the injected side to the right.