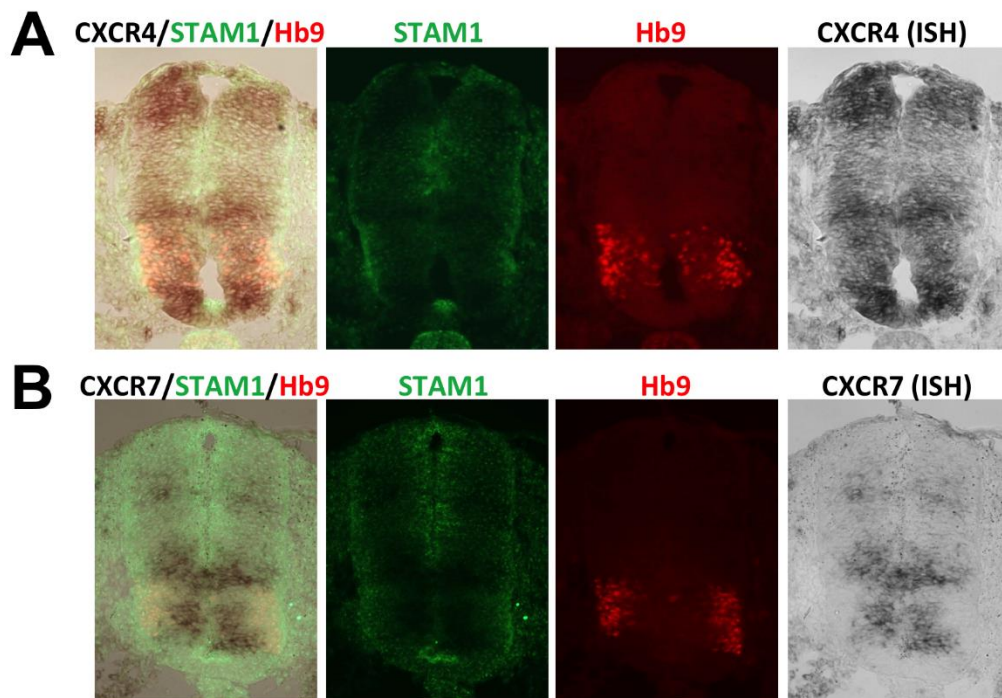
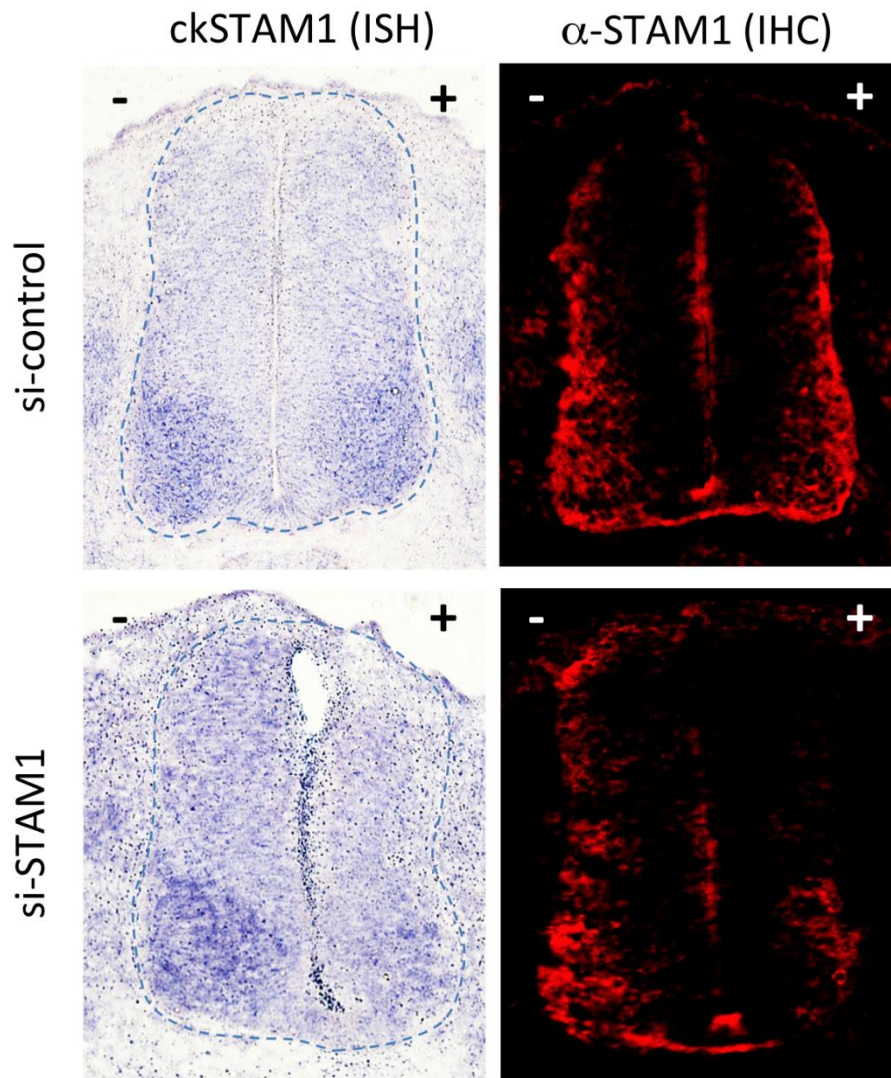


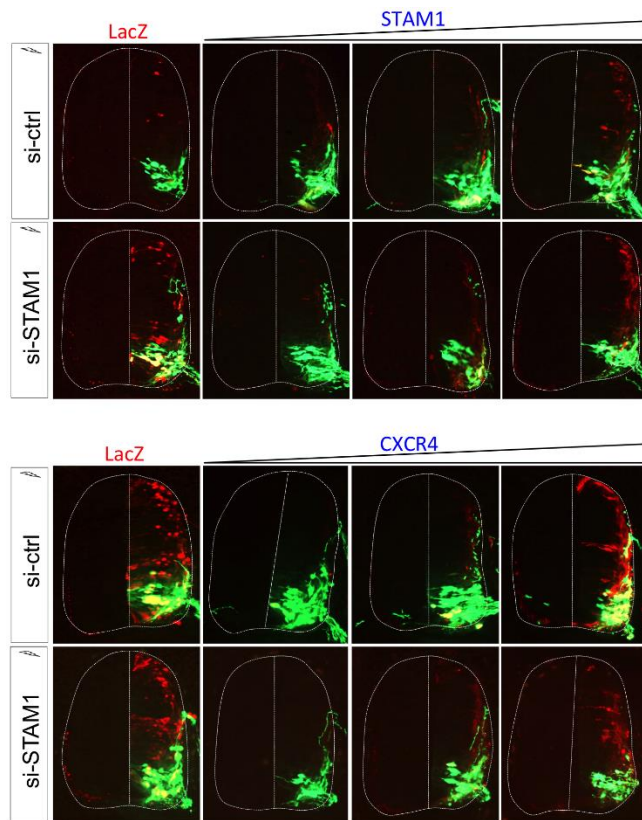
S-Fig. 1. Induction of STAM1 in developing MNs. (A) Expression of higher levels of STAM1 in MNs derived from mouse embryonic stem cells (ESCs) in comparison to proliferating ESCs as determined by RT-PCR. The ESC line was maintained in an undifferentiated state on 0.1% gelatin-coated dishes in the ESC growth media that consist of knockout DMEM, 10% FBS, 0.1 mM nonessential amino acids, 2 mM L-glutamine, 0.1 mM β -mercaptoethanol, and recombinant leukemia inhibitory factor (LIF) (1,000 units/ml, Chemicon). For MN differentiation assays, ESCs were trypsinized and grown in the ESC growth medium without LIF in suspension as cell aggregates for two days. The ESC aggregates (embryoid bodies, EBs) were treated with all-trans RA (0.5 μ M) and a Shh agonist Purmorphamine (1 μ M, Calbiochem) for 4 days. (B) Quantification of the RT-PCR results is as shown.



S-Fig. 2. Expression of CXCR4 and CXCR7 in developing chick spinal cord. (A) CXCR4 is expressed highly at early stage of chick embryonic spinal cord (HH18) and some of the signals overlap with Hb9 and STAM1 in motor neuron area. In situ hybridization (ISH) is used for CXCR4 expression followed by immunohistochemistry for Hb9 and STAM1 and the merged image is as shown. (B) ISH shows the expression of CXCR7 in the ventricular zone of chick spinal cord and this CXCR7 expression is excluded from the motor neuron area.



S-Fig. 3. Validation of si-STAM1. To test the knock-down efficiency of si-STAM1, chick neural tubes were electroporated with si-control and si-STAM1, followed by in situ hybridization (ISH) against chick STAM1 and immunohistochemistry with anti-STAM1 antibody at two days post-electroporation (2dpe). +, electroporated side.



S-Fig. 4. Varying amount of STAM1 and CXCR4 affects motor axon projections. Increasing the expression of STAM1 or CXCR4 alone results in *SE1*-GFP labeled motor axons to project dorsally. This phenotype is rescued when the expression of STAM1 or CXCR4 is increased maximally, suggesting that a specific dose is required for both STAM1 and CXCR4 for proper ventral motor axon outgrowth.