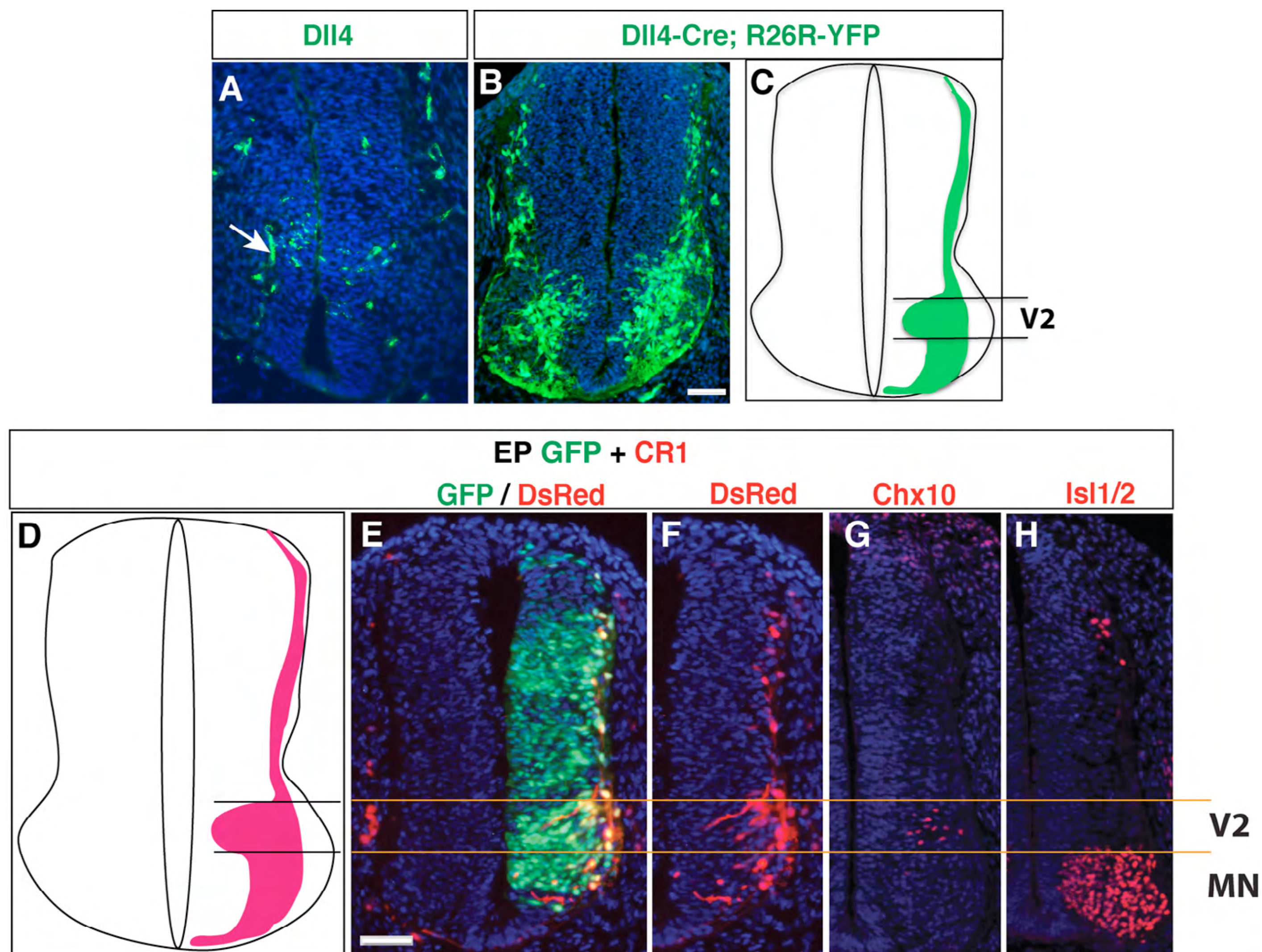

Fig. S1. CR1 drives reporter gene expression that mimics the endogenous Dll4 expression pattern. (A) Immunofluorescence with an anti-Dll4 antibody resulted in sparse cytoplasmic staining in V2 precursors and blood vessels (indicated by arrows) in E11.5 mouse developing spinal cord. (B,C) Lineage tracing in Dll4-Cre; R26R-YFP mice at the same stage however revealed a distinct expression pattern that encompasses not only the V2 domain, but also a larger subgroup of differentiating neurons in the mantle zone along the dorsoventral axis. (D-F) In chick spinal cords co-electroporated with the CR1 reporter construct and a GFP expression plasmid, CR1 drove DsRed expression in a pattern mimicing that of the mouse Dll4. (G,H) Immunostaining of adjacent spinal cord sections with Chx10 and Isl1/2 antibodies revealed DsRed expression predominantly in the V2 domain. Scale bar in B: 20 μm in A,B. Scale bar in H: 30 μm in E-H.

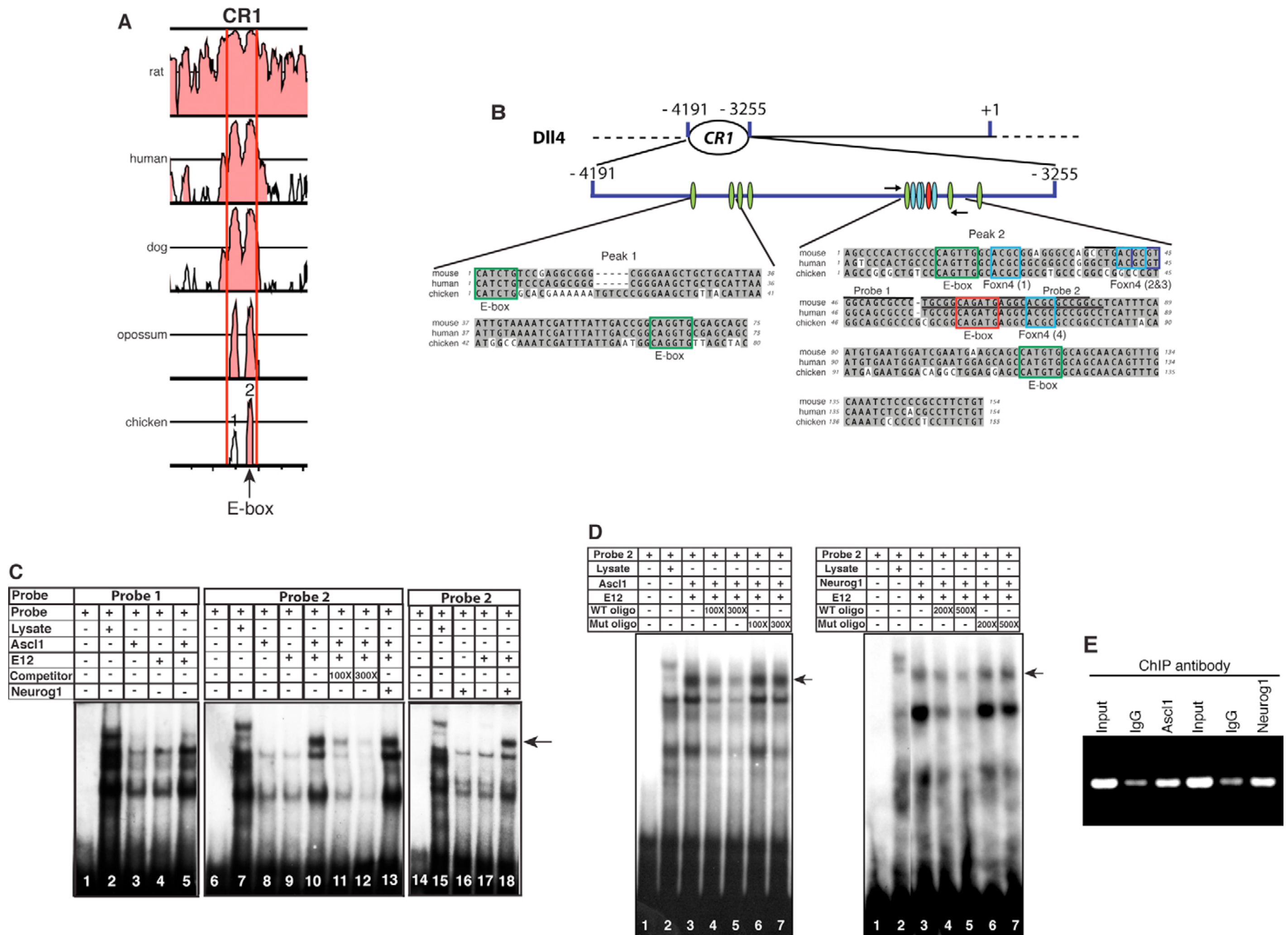

Fig. S2. Specific binding of Ascl1 and Neurog1 to the critical E-box in the *Dll4* CR1 enhancer. (A) VISTA analysis revealed two peaks of conserved sequences in CR1 among several vertebrate species and the critical E-box is located within the more conserved peak 2. (B) Alignment of mouse, human and chicken DNA sequences for the conserved peaks 1 and 2 identified by VISTA analysis in the *Dll4* CR1 enhancer. Identical bases are shaded. Overlined are the mouse Probe 1 and Probe 2 DNA sequences used for EMSA, and underlined is the 26-bp region critical for Ascl1 activation. All conserved E-boxes are boxed in green or red (the critical E-box) and the Foxn4 binding motifs are boxed in cyan and blue. Indicated also are primers flanking the critical E-box used for ChIP assay. (C) EMSA was carried out using *in vitro* translated Ascl1, Neurog1 and E12. No specific binding was observed with Probe1 (lanes 1-5). With Probe 2, Ascl1 and Neurog1 could not bind alone (lanes 8 and 16) but formed specific heterodimers (indicated by the arrow) in the presence of E12 (lanes 10, 13 and 18), which could be abrogated with excess cold oligonucleotides (lanes 11 and 12). (**D**) EMSA was carried out using *in vitro* translated Ascl1, Neurog1 and E12 with Probe 2. Ascl1 or Neurog1 formed specific heterodimers (indicated by the arrow) in the presence of E12 (lane 3), which could be diminished with excess wild-type (WT) cold oligonucleotides (lanes 4 and 5) but not by excess mutant (Mut) cold oligonucleotides (lanes 6 and 7). (**E**) ChIP assay showing enrichment of the critical E-box region by anti-Ascl1 and anti-Neurog1 antibodies. Chromatin DNA was prepared from 293T cells co-transfected with the CR1 reporter construct and Ascl1, Neurog1 and E12 expression plasmids. Input lane represents 2% of chromatin DNA used for ChIP assay.

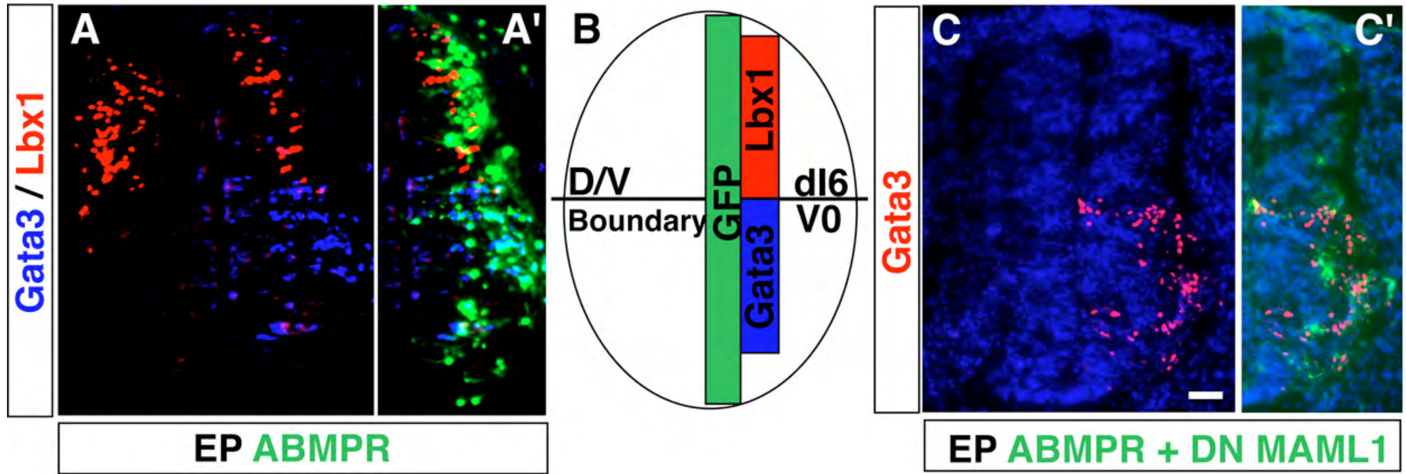

Fig. S3. Ventrally restricted expression of Gata3 ectopically induced by ABMPR. (A,A') Despite GFP expression at all dorso-ventral aspects of the neural tube, ABMPR misexpression ectopically induced Gata3 expression in the ventral region but failed to induce Gata3 expression in the dorsal Lbx1-positive region. (B) Schematic depicting this ventral restriction of Gata3 induction. (C,C') Co-expression of ABMPR with dominant-negative MAML1 still caused upregulated Gata3 expression. Scale bar: 9 µm.