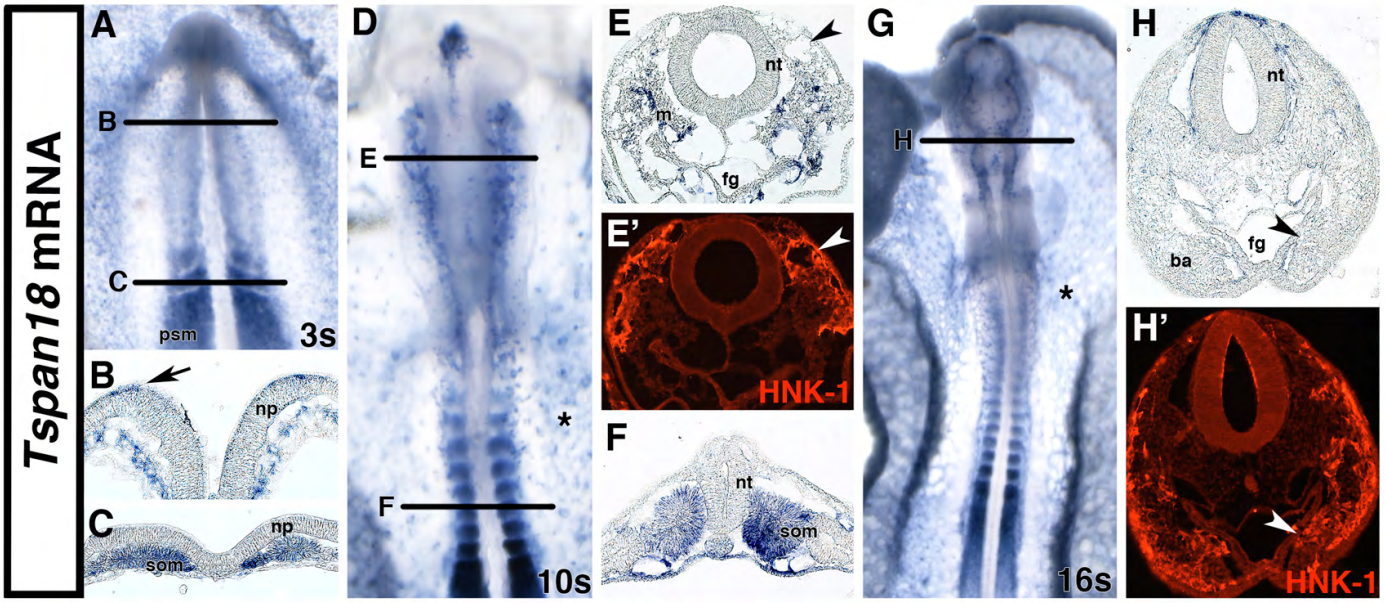

Fig. S1. *Tspan18* is expressed during early chick development, but is absent from cranial migratory neural crest cells. Whole mount in situ hybridization for *Tspan18* in 3 somite (s; A-C), 10s (D-F) and 16s (G,H) chick embryos (dorsal view with corresponding transverse sections at the levels indicated in A,D,G). In 3s embryos *Tspan18* is expressed in the dorsal neural plate (np; arrow in B), in the presomitic mesoderm (psm), and the newly formed, epithelial somites (som). At 10s, *Tspan18* is expressed in the developing vasculature (asterisk in D), the head mesenchyme (m) and epithelial somites (F). However, *Tspan18* is absent in migrating neural crest cells that express HNK-1 (arrowheads in E,E') and rostral, dissociated somites. At 16s, *Tspan18* mRNA persists in the vasculature (asterisk in G) and epithelial somites, but remains absent from HNK-1-positive migratory neural crest cells in the branchial arch (ba; arrowheads in H,H'). nt, neural tube; fg, foregut.

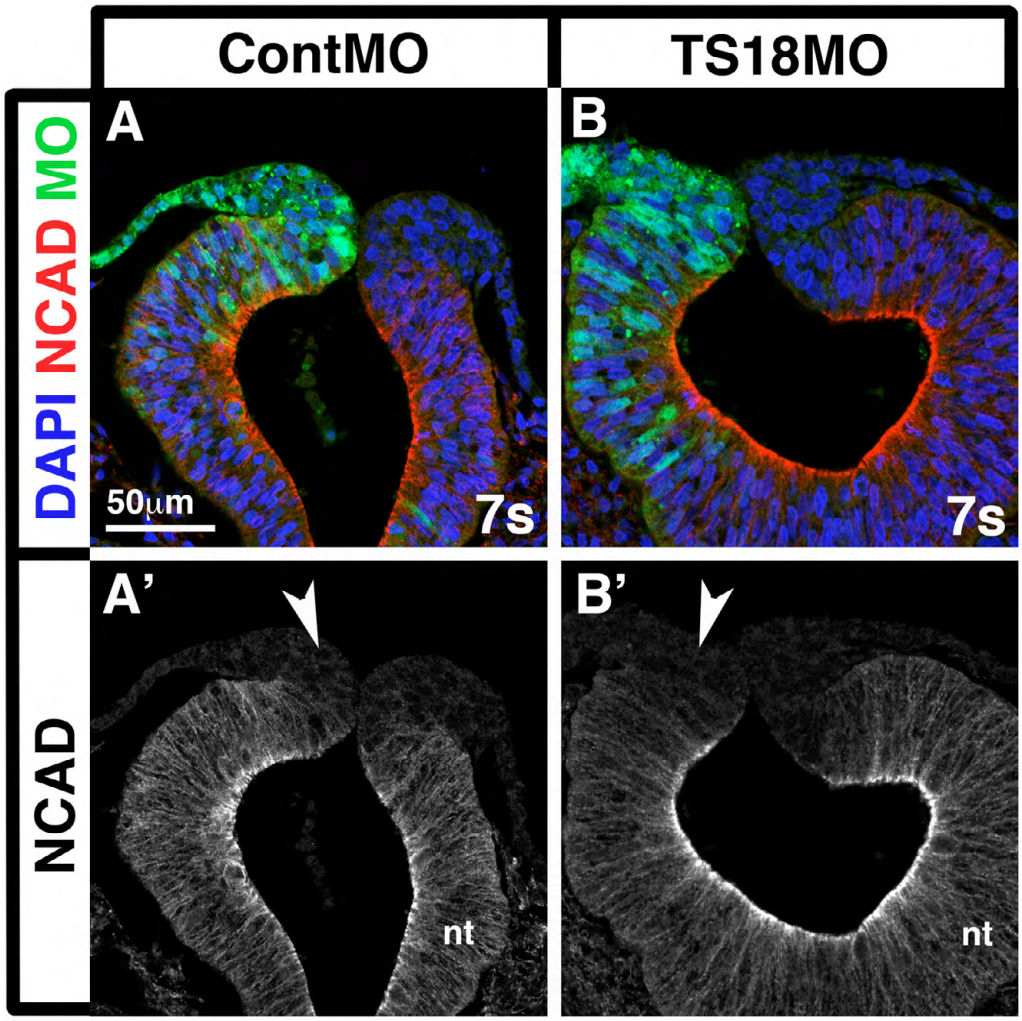

Fig. S2. Tspan18 knock down does not affect N-cadherin protein levels. Embryos were unilaterally electroporated at HH4+ with ContMO (A, green) or TS18MO (B, green) and subsequently sectioned and immunostained for N-cad (A, B, red; A', B'). N-cad expression in the cranial neural tube at 7s, including its absence from cranial neural folds (Dady et al., 2012), is unaffected by ContMO (arrowhead in A') or TS18MO electroporation (arrowhead in B'). nt, neural tube; Scale bar in A= 50 μm.

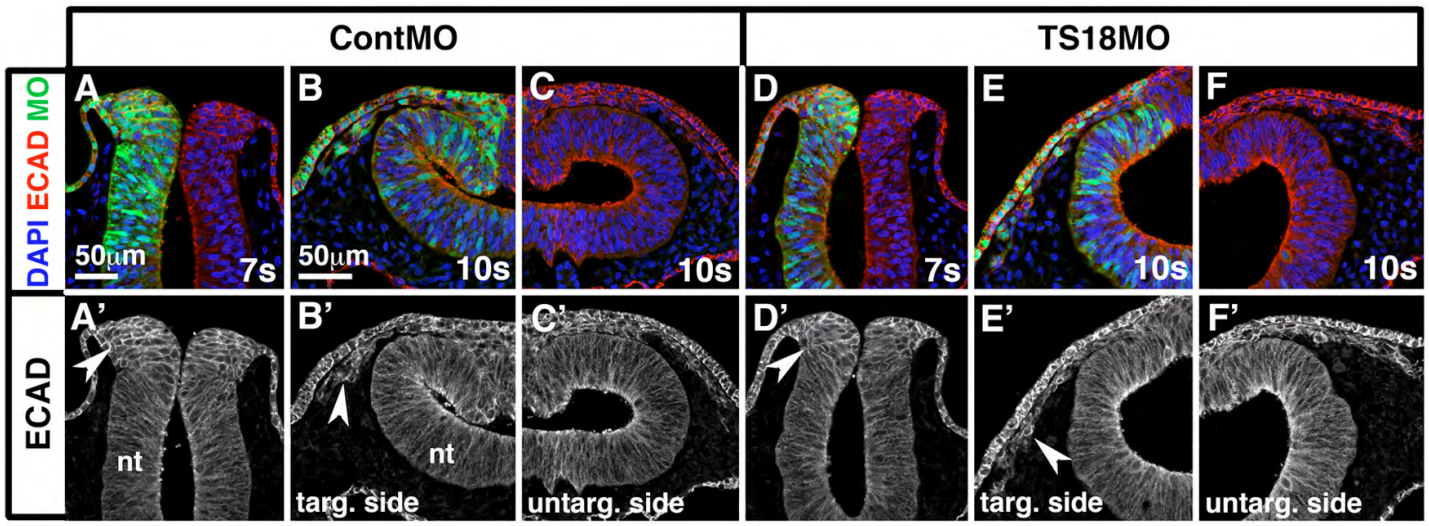

Fig. S3. Tspan18 knock down does not affect E-cadherin protein levels. Embryos were unilaterally electroporated at HH4+ with ContMO (A, B, green) or TS18MO (D, E, green) and subsequently sectioned and immunostained for E-Cad (A-F, red; A'-F'). E-cad expression is unaffected by ContMO (arrowheads in A'-C') or TS18MO (arrowheads in D'-F') electroporation both in the cranial neural tube at 7s (A, D) and in migrating neural crest cells at 10s (B,C,E,F; Dady et.al., 2012). C and F are the unelectroporated halves of the embryos shown in B and E. nt, neural tube; Scale bars in A,B= 50μm.

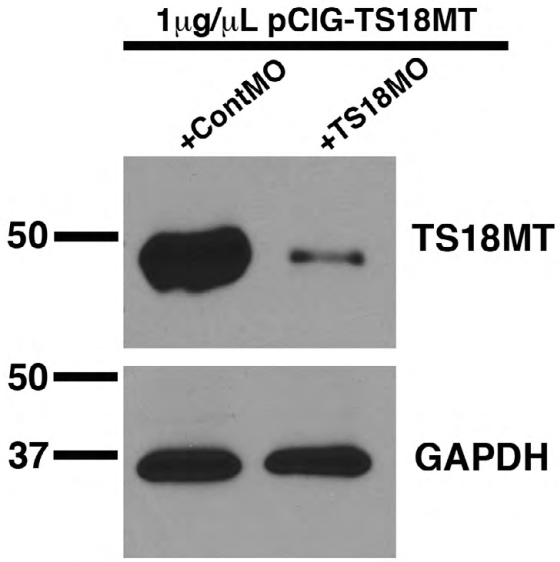

Fig. S4. TS18MO efficiently prevents Tspan18 protein synthesis. Embryos were bilaterally co-electroporated with 1 μg/μl pCIG-TS18MT (to express Tspan18 with a C-terminal 6x myc tag fusion) and either ContMO or TS18MO. Embryos were incubated to 7-10s and embryo heads with bright, equivalently targeted GFP fluorescence (indicating expression of the bicistronic message) were dissected. Embryo heads from 12 embryos were pooled, lysed, and analyzed by western blot with antibodies against myc tag (to detect TS18MT; 9E10; Developmental Studies Hybridoma Bank) or GAPDH (6C5; Life Technologies) as a loading control. Abundant TS18MT was detected following co-electroporation with ContMO. However, TS18MO drastically reduced the amount of TS18MT expressed from the pCIG-TS18MT plasmid, although some residual TS18MT was translated.

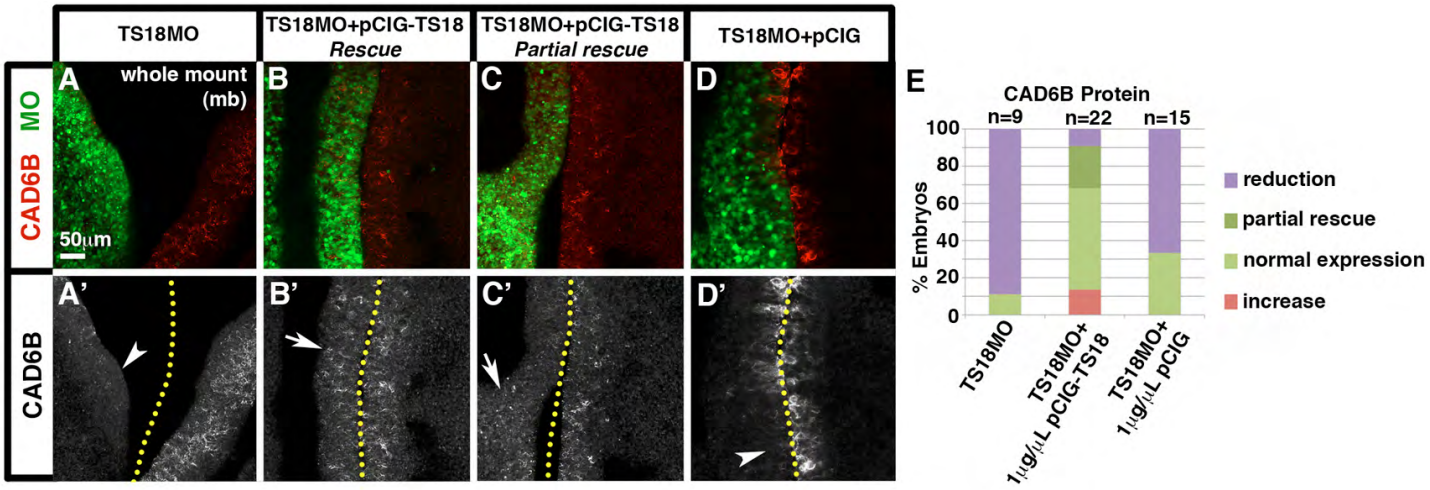

Fig. S5. Co-electroporation of TS18MO with pCIG-TS18 rescues Cad6B protein levels. Embryos were unilaterally electroporated with TS18MO alone (A, green) or TS18MO mixed with 1μg/μl of pCIG-TS18 (B,C, green) or empty pCIG (D, green) and immunostained for Cad6B protein (A-D, red; A'-D'). As previously observed, Cad6B protein was largely absent in midbrain cells targeted with TS18MO (arrowhead in A'). Following co-electroporation of TS18MO and pCIG-TS18, Cad6B protein levels were normal or increased in 68% of embryos (arrow in B'), while Cad6B protein staining was speckled and partially rescued in another 23% (arrowhead in C'; $p=1.7x10^{-4}$, n=22). Co-electroporation of TS18MO with empty pCIG did not rescue Cad6B protein (arrowhead in D'; p=0.35, n=15). (E) A bar graph of the number of electroporated embryos showing a reduction, rescue, or increase in Cad6B protein levels. mb, midbrain. Scale bar in A= 50 μm. Dotted lines in A'-D' indicate embryo midline.

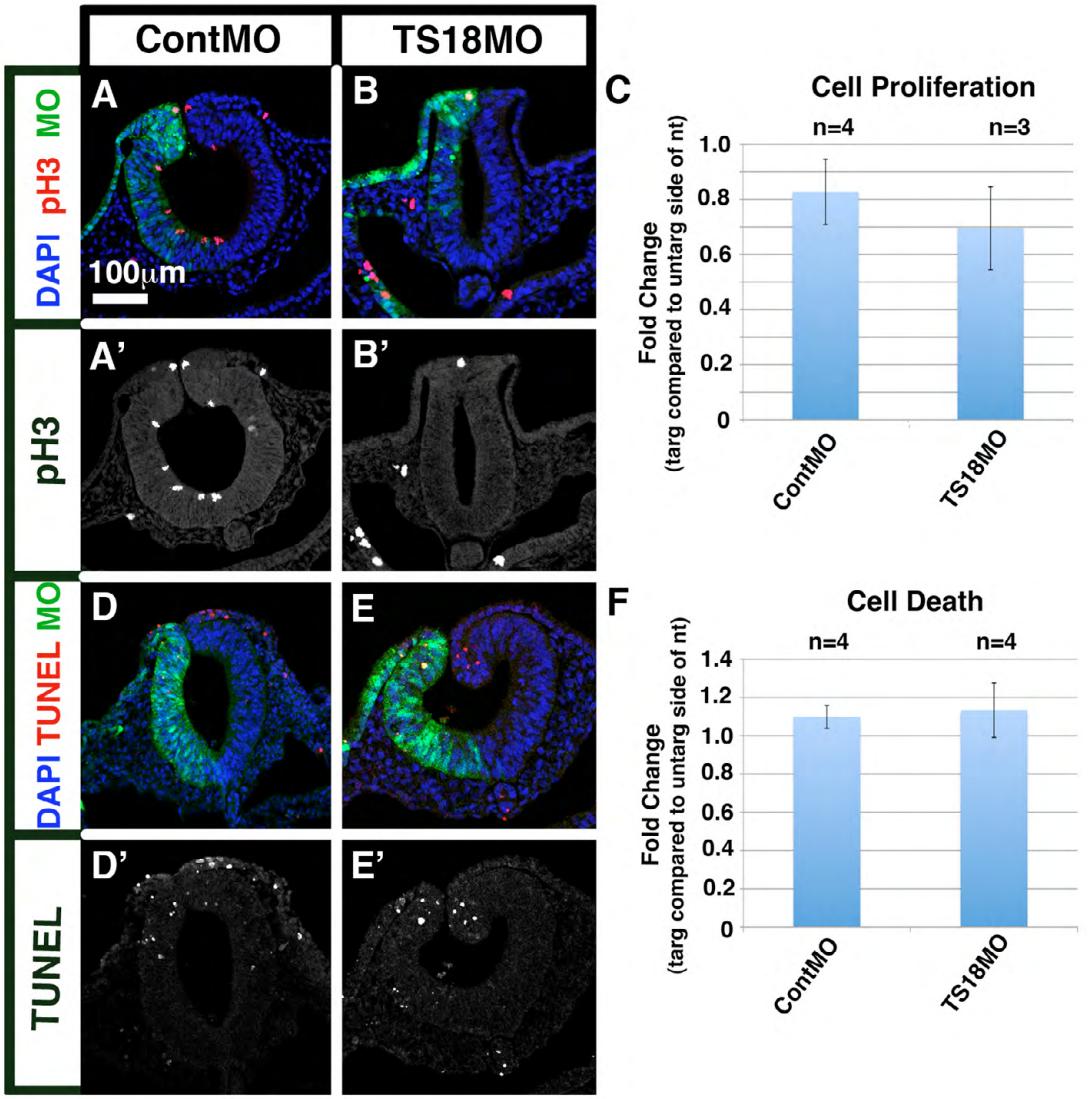

Fig. S6. Tspan18 knockdown does not affect cell proliferation or death. Embryos unilaterally electroporated with ContMO (A,D) or TS18MO (B,E) were sectioned and immunostained for phosphohistone H3 (pH3; A,B, red; A', B') or labeled by TUNEL staining (D,E, red; D',E'; Roche Applied Science; Indianapolis, IN). There was no apparent difference in the number of pH3-positive cells on the targeted side of the neural tube, as compared to the untargeted side, in ContMO (A') or TS18MO (B') electroporated embryos. (C) Effects on proliferation were quantitated by counting the number of pH3-positive cells divided by the total number of cells in the dorsal quarter of the neural tube on the targeted and untargeted sides in 5 sections of 3 or 4 electroporated embryos ($p=0.270$). Likewise, there was no apparent difference in the relative number of TUNEL-positive cells on the targeted side of the neural tube in ContMO (D') or TS18MO (E') electroporated embryos. (F) Effects on cell death were quantitated by counting the number of TUNEL-positive cells divided by the total number of cells in the dorsal quarter of the neural tube on the targeted and untargeted sides in 5 sections of 4 electroporated embryos ($p=0.415$). Statistics were performed using the paired Student's *t* test in Excel (Microscoft). Error bars indicate +/- the standard error of the mean. nt, neural tube. Scale bar in A= 100 μm.

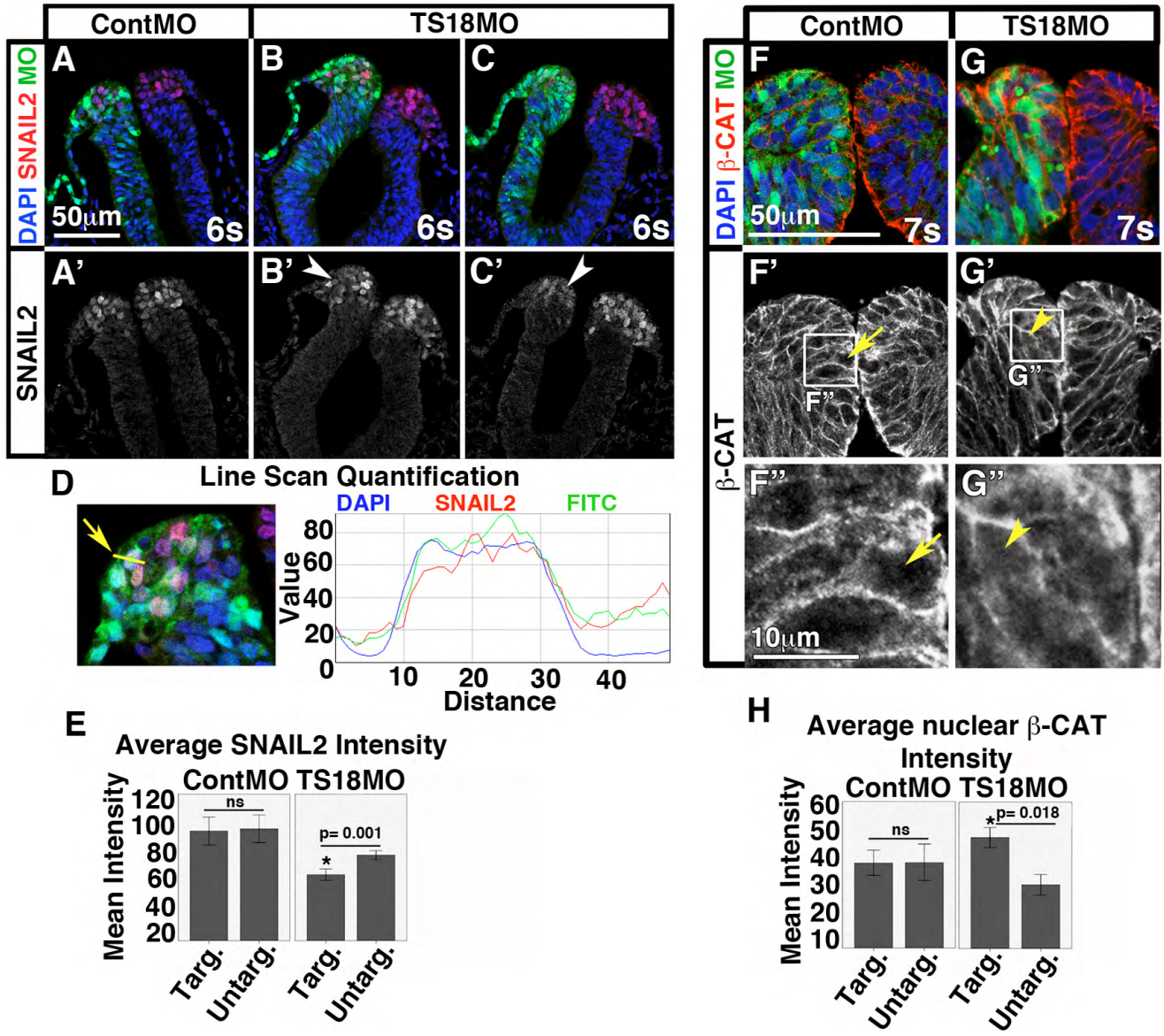

Fig. S7. Downregulation of *Cad6B* mRNA following Tspan18 knock down does not correlate with Snail2 but may be the consequence of increased nuclear β-catenin. Embryos unilaterally electroporated with ContMO (A,F) or TS18MO (B,C,G) were immunostained for Snail2 (A-C) or β-catenin (F, G). (A-C) Transverse midbrain sections of 6s embryos. Snail2 protein levels (red; A'-C') are unaffected by ContMO electroporation (A, green) but reduced on the targeted side in embryos electroporated with TS18MO (B',C', green; arrowheads). (D) A representative line scan used to measure the average immunofluorescence intensity in a nucleus (yellow arrow; same procedure used for Snail2 and β-catenin). (E) Nuclear Snail2 immunofluorescence was quantified by line scan, comparing cells of the targeted (targ) and untargeted (untarg) sides of the neural tube within individual sections (horizontal lines above the graphs) to control for experimental variations in staining between sections and embryos. These measurements verified that ContMO had no effect, while TS18MO electroporation led to reduced Snail2 levels (n=4; p=0.001). (F-G) Transverse midbrain sections of 6-7s embryos reveal abundant membrane-localized β-catenin in embryos electroporated with both ContMO (F') and TS18MO (G'). However, higher magnification images (box in F',G') suggest increased nuclear-localized β-catenin on the targeted (green) side of the neural tube in TS18MO-electroporated embryos (arrowheads in G") compared to ContMO-electroporated embryos (arrow in F"). (H) Nuclear β-catenin was quantified by line scan, comparing cells of the targeted (targ) and untargeted (untarg) sides of the neural tube within individual sections (horizontal lines above the graphs) to control for experimental variations in staining between sections and embryos. These measurements documented a significant increase in nuclear-localized β-catenin on the targeted side of the neural tube in TS18MO-electroporated embryos (n=3; p=0.018). To quantify Snail2 and β-catenin, 10 nuclei were chosen from 3 sections per embryo (Snail2 n=4 embryos; β-catenin n=3 embryos). Nuclei were selected based on the following criteria: 1) in the dorsal-most quarter of the neural tube, 2) non-overlapping with other nuclei, 3) from cells containing detectable levels of MO. The RGB profiler plug-in in ImageJ was used to perform line scans (with 5 pixel lines) to measure the average intensity of the red channel within each DAPI-positive nucleus. Statistical evaluation of intensity measurements was performed using SPSS 16.0 for Windows (Chicago, IL) using paired comparisons in a MANOVA. A p value < 0.05 was considered statistically significant. Error bars indicate +/- the standard error of the mean. Scale bars in A, F= 50 μm; scale bar in F"=10 μm.

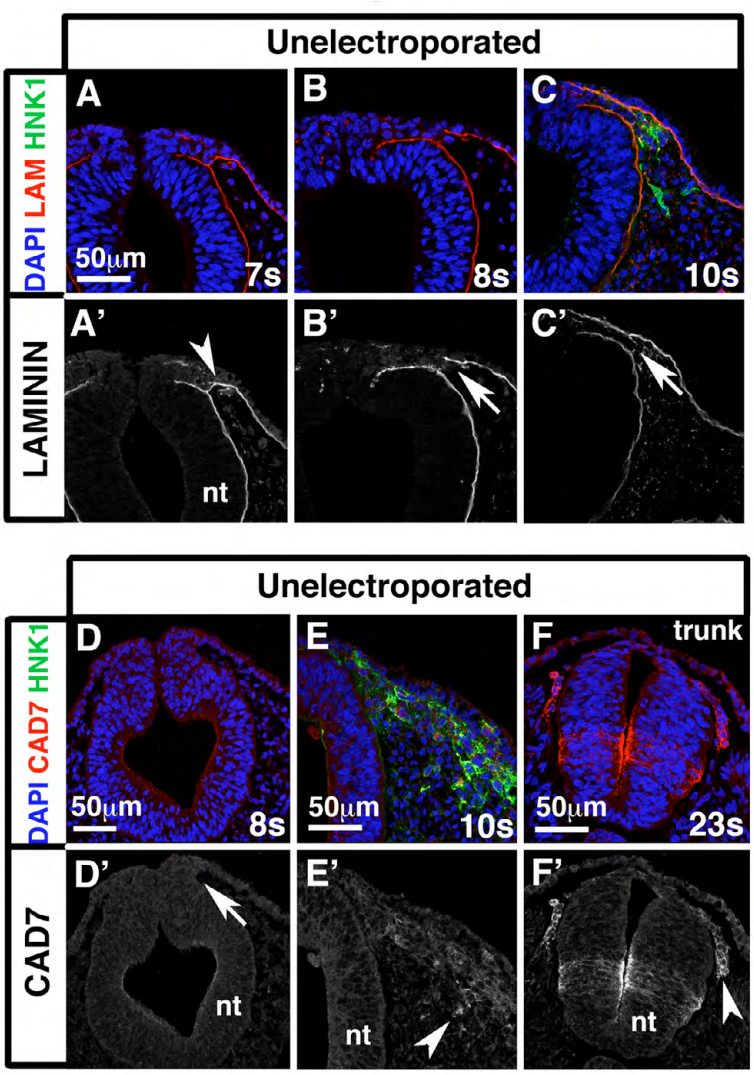

Fig. S8. Normal expression patterns of Laminin and Cad7 during EMT. Transverse midbrain sections of unelectroporated embryos immunostained for Laminin (A-C, red; A'-C') or Cad7 (D-F, red; D'-F'). (A-C) The intact basal lamina connecting the basal surface of the neural tube with the non-neural ectoderm at 7s (arrowhead in A') becomes discontinuous at 8s (arrow in B') in the region that HNK-1-positive cranial neural crest cells (green in C) exit the neural tube at 10s (arrow in C'). (D-F) Cad7 protein is undetectable in early migrating neural crest cells at 8s (arrow in D') but accumulates by 10s in HNK-1-positive migratory cranial neural crest cells (arrowhead in E'), similar to the pattern in 23s trunk migratory neural crest cells (arrowhead in F'; (Nakagawa and Takeichi, 1998)). nt, neural tube. Scale bars= 50 μm.

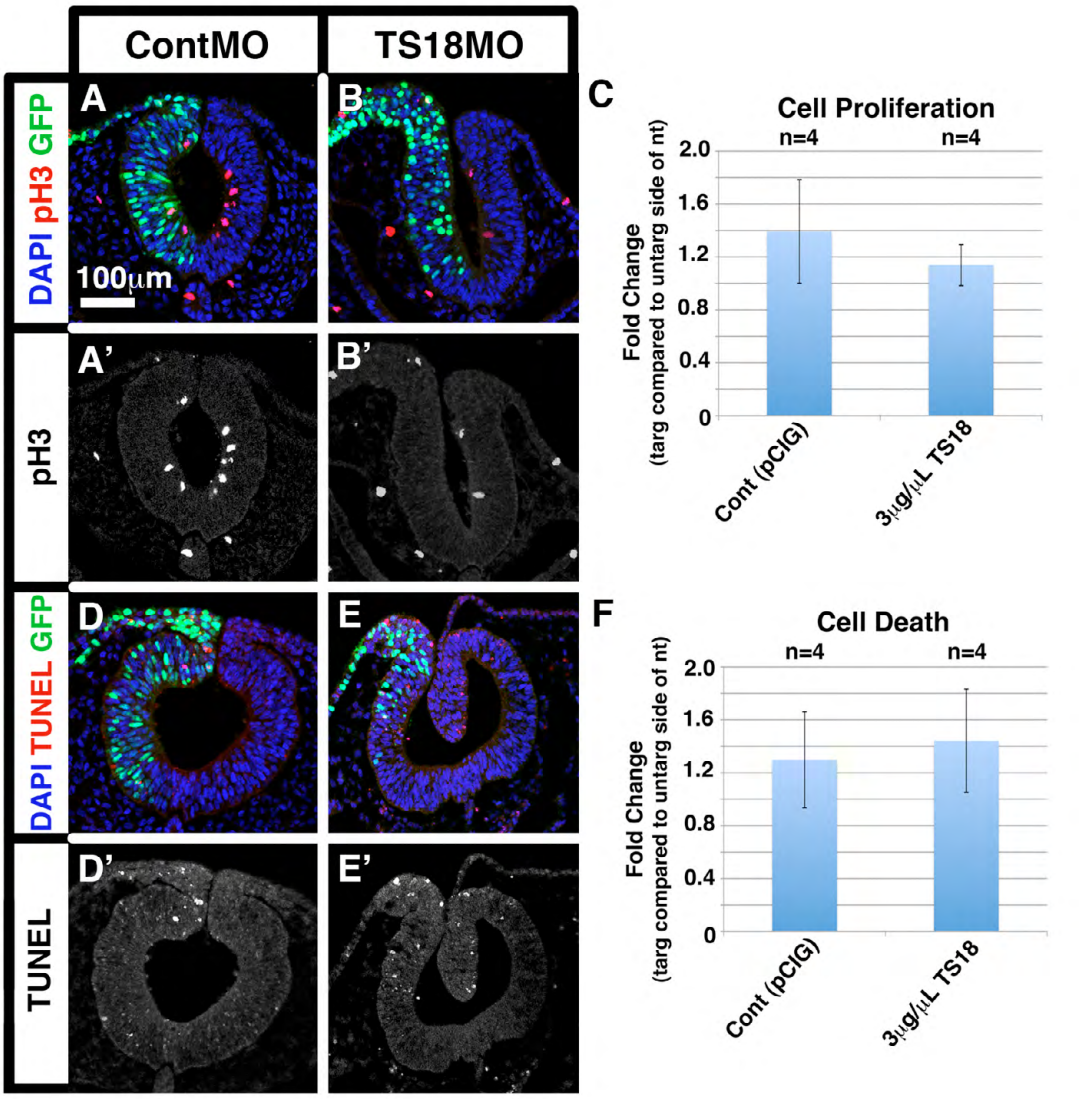

Fig. S9. Tspan18 overexpression does not affect cell proliferation or death. Embryos unilaterally electroporated with empty pCIG (A,D) or pCIG-TS18 (B,E) were sectioned and immunostained for phosphohistone H3 (pH3; A,B) or labeled by TUNEL staining (D,E). (C) There was no noticeable difference in the number of pH3-positive cells on the targeted side of the dorsal neural tube, as compared to the untargeted side, in pCIG (A') or pCIG-TS18 (B') electroporated embryos. Effects on proliferation were quantitated by counting the number of pH3-positive cells divided by the total number of cells in the dorsal quarter of the neural tube on the targeted and untargeted sides in 5 sections of 4 electroporated embryos (p=0.285). (F) Likewise, there was no noticeable difference in the number of TUNEL-positive cells on the targeted side of the neural tube in embryos electroporated with either empty pCIG (D') or pCIG-TS18 (E'). Effects on cell death were quantitated by counting the number of TUNEL-positive cells divided by the total number of cells in the dorsal quarter of the neural tube on the targeted and untargeted sides in 5 sections of 4 electroporated embryos (p=0.398). Statistics were performed using the paired Student's *t* test in Excel (Microsoft). Error bars indicate +/- the standard error of the mean. nt; neural tube. Scale bar in A= 100 μm.