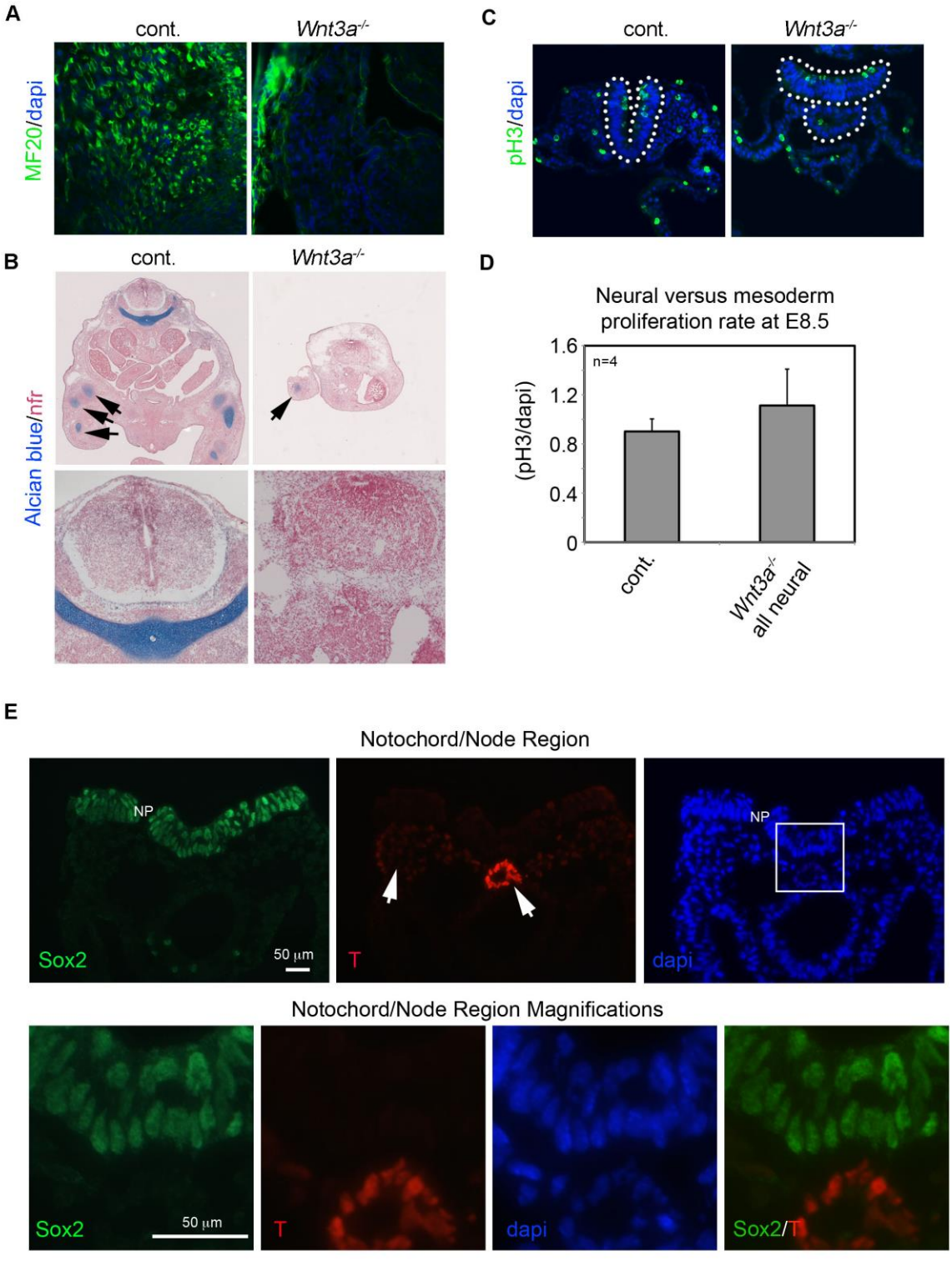
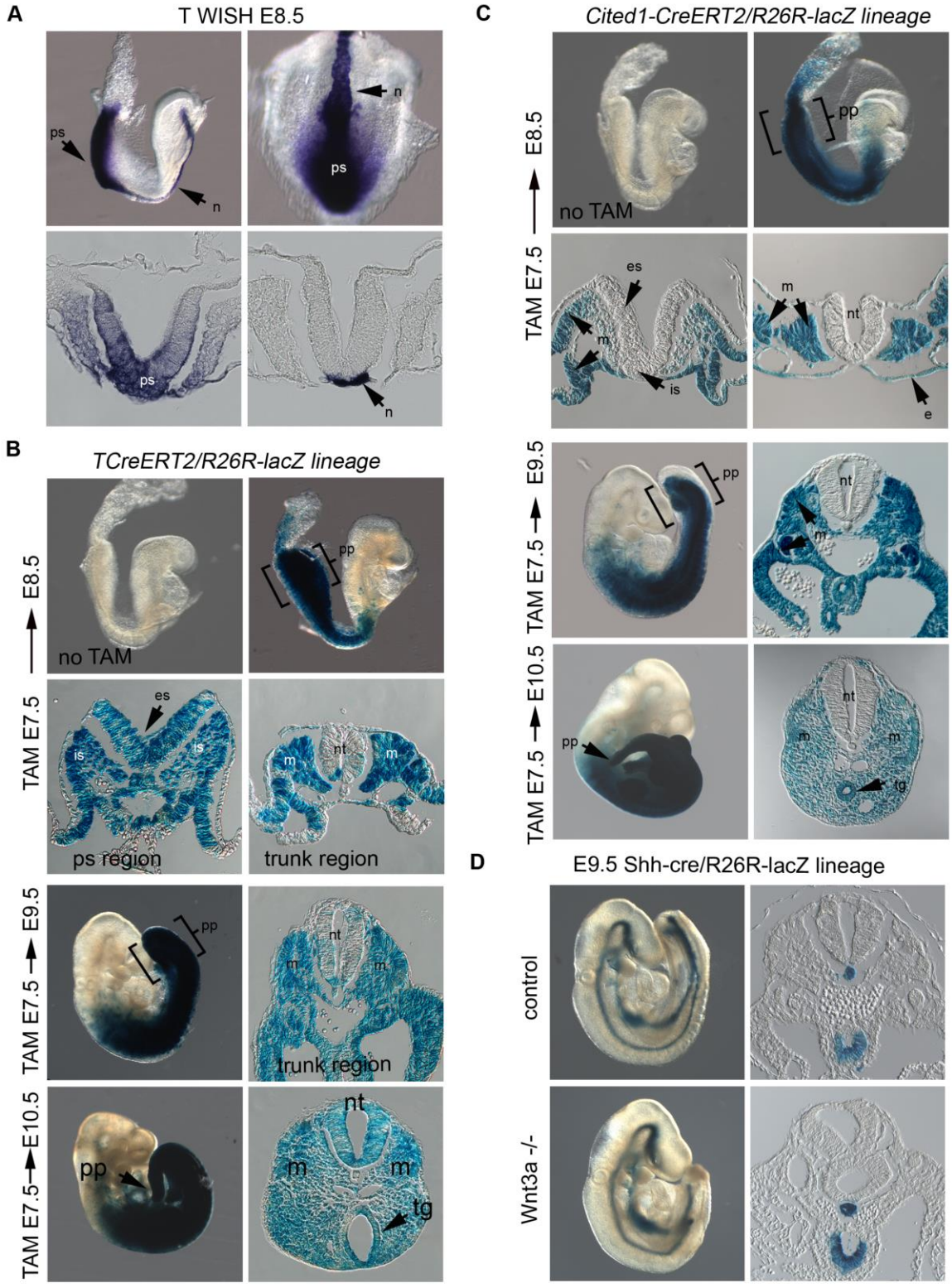
Supplemental data

Figure S1 (related to Figure 1)



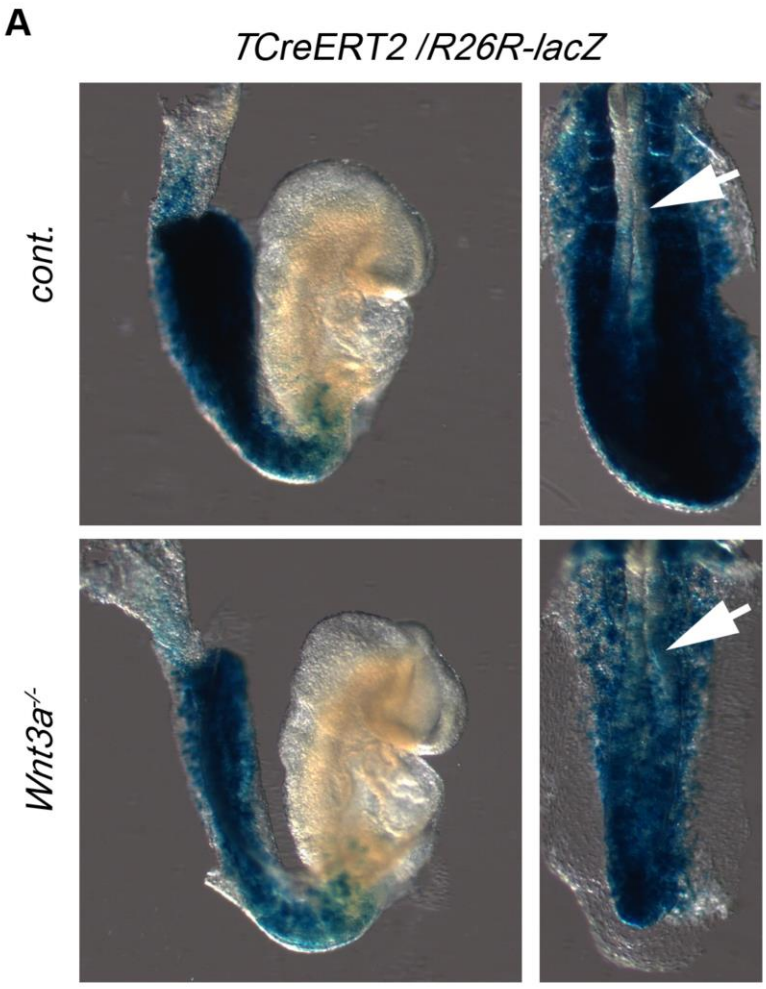
Supplemental Figure 1. Reduction in differentiated musculoskeletal cell types in *Wnt3a*$^{-/-}$ mutants. (A) Magnified view of paraxial region of E13.5 embryo containing differentiated skeletal myocytes stained with MF20 (myosin) antibody in a cross-section through the posterior trunk (frozen section). Control embryos show distinct cortical distribution of MF20 indicating differentiating myocytes while *Wnt3a*$^{-/-}$ mutant embryos do not show cortical MF20 staining. MF20 staining in *Wnt3a*$^{-/-}$ mutants is only observed in subdermal regions. (B) Alcian blue staining of cartilage with nuclear fast red (nfr) counter staining of cross sections through the posterior trunk at the level of the hindlimb (paraffin section). Control embryos show alcian blue staining under the neural tube, indicating axial skeletal development, and in the forming hindlimb (arrows). *Wnt3a*$^{-/-}$ mutants did not show alcian blue staining of the developing axial skeleton but did in the hindlimbs. Note that limb skeletal elements are derived from lateral plate mesoderm while the axial skeleton arises from the paraxial trunk mesoderm. (C) Detection of proliferating cells by phospho-Histone H3 detection in E8.5 trunk region (around the position of the 10th somite) of control and *Wnt3a*$^{-/-}$ embryo. Dotted line indicates the neural tube(s). (D) Graph of neural verses mesodermal proliferation rate in control and *Wnt3a*$^{-/-}$ embryo at E8.5 where intermediate/lateral mesoderm proliferation rate was used to normalize data (see Materials and Methods) (n=4 control and n=4 *Wnt3a*$^{-/-}$ embryos). (E) Detection of Sox2 and T in frozen sections of the notochord/node region of an E8.5 embryo, taken anterior to the primitive streak (PS). At this level of the A-P axis, Sox2 and T were detected in separate progenitor populations. Sox2 was detected in the neural plate while T was detected in the paraxial mesoderm and notochord/node (arrows). Abbreviations: NP, neural plate.

Figure S2 (related to Figure 2)



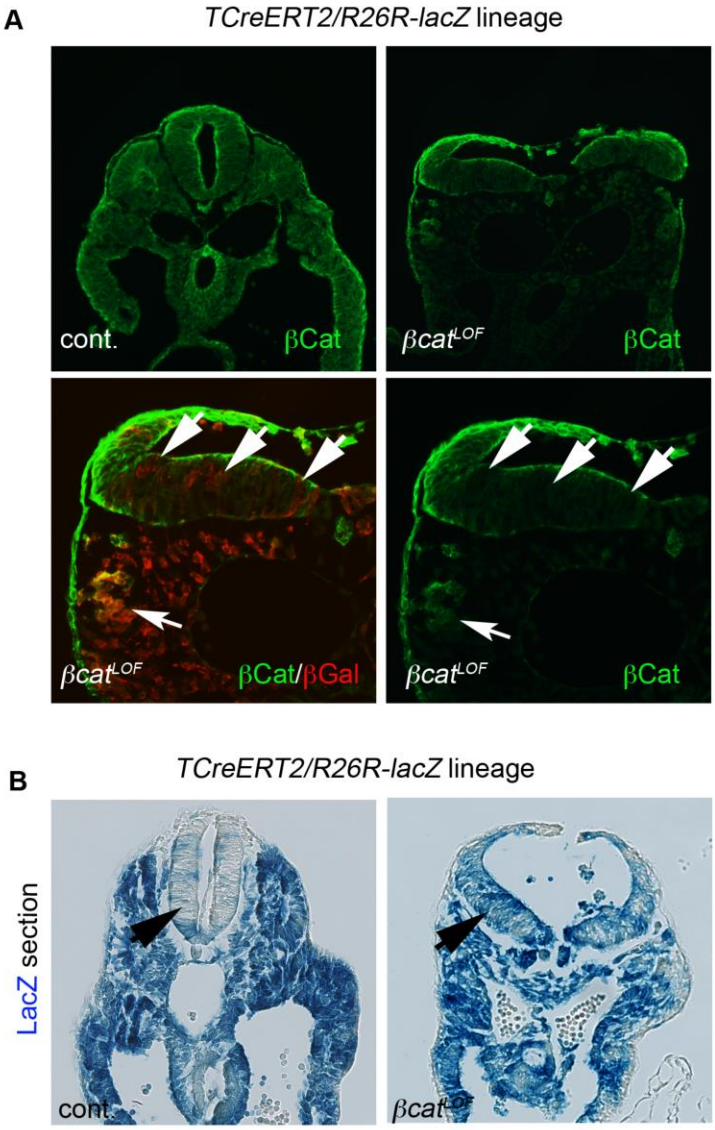
Supplemental Figure 2. Tracing posterior progenitor cells in the PS region using tissue specific Cre recombinases. (A) Endogenous *T* expression at E8.5 by WISH showing *T* expression in the primitive streak (ps) and notochord. *T* expression occurred throughout the PS while in anterior regions of the embryo *T* was only observed in the notochord. (B) *TCreERT2* lineage traced by lacZ-staining (*R26R-lacZ*) after initiation at E7.5 through injection of tamoxifen (TAM) observed at stages E8.5 through E10.5. Note that the *TCreERT2* lineage was observed in both the epithelial PS (es) and ingressed PS (is). This lineage tracing resulted in the labeling of all mesodermal lineages of the trunk axis and increasing contributions of neural progenitors at posterior regions of the embryo through development. The tracing was maintained in the posterior progenitors (pp) indicating that self-renewing populations were labeled. *TCreERT2* lineage also included gut lineages. (C) *Cited1-CreERT2* lineage traced by lacZ-staining (*R26R-lacZ*) after initiation at E7.5 through TAM injection, observed at stages E8.5 through E10.5. The *Cited1CreERT2* transgene traced cells of the ingressed PS but not the epithelial PS. Note that *the Cited1-CreERT2* lineage included all mesodermal and hindgut lineages of the trunk axis but not the neural lineage. The tracing was maintained in the posterior progenitors (pp) suggesting that a self-renewing ingressed PS population was labeled. (D) Tracing notochord and gut lineages with *Shh-Cre* traced by LacZ-staining (*R26R-LacZ*). *Wnt3a*$^{-/-}$ mutants do show normal distribution of *Shh-Cre* lineage in notochord and gut with no detectable transfated cells in the neural tubes. Note that photographs of embryos at different stages are not to scale between stages. Abbreviations: ps, primitive streak; pp, posterior progenitors; es, epithelial primitive streak; is, ingressed primitive streak; m, mesoderm; nt, neural tube; n, notochord.

Figure S3 (related to Figure 2)



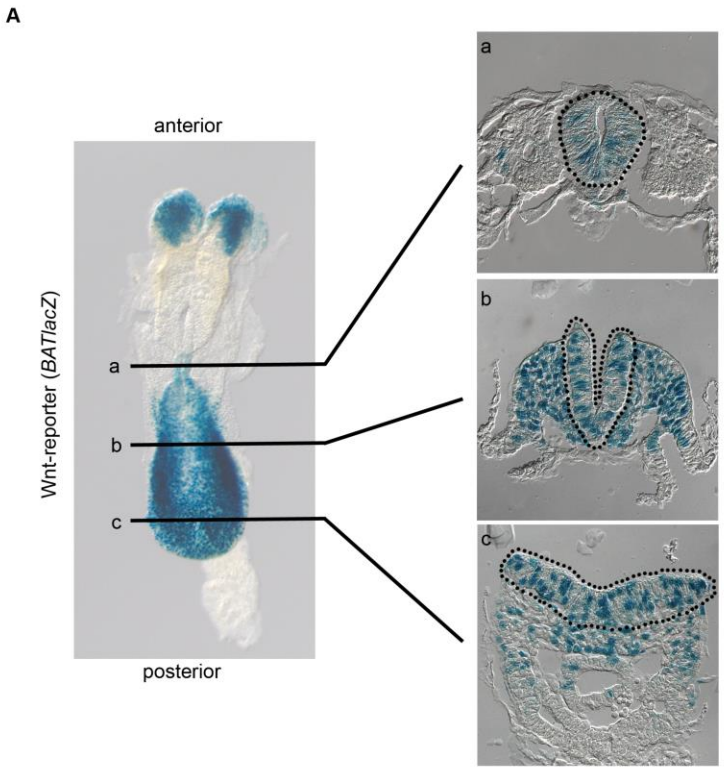
Supplemental Figure 3. *TCreERT2* lineage traced by βGal-staining (*R26R-lacZ*) after TAM injection at E7.5 and observed at E8.5 in control and *Wnt3a*$^{-/-}$ mutants. (A) Lateral view of control and *Wnt3a*$^{-/-}$ mutant embryo and magnified dorsal view of the PS region and somites. Note that *TCreERT2* predominantly labeled mesodermal cells and that relatively few cells were labeled in the midline neural region in control embryos at the position of the seventh somite (arrow). In *Wnt3a*$^{-/-}$ mutant embryos fewer traced cells were observed in the PS region, however, midline neural regions showed more traced cells at an equivalent position (arrow). This data suggests that *TCreERT2* traced cells are accumulating in neural tissues in *Wnt3a*$^{-/-}$ mutants during early trunk development.

Figure S4 (related to Figure 3)



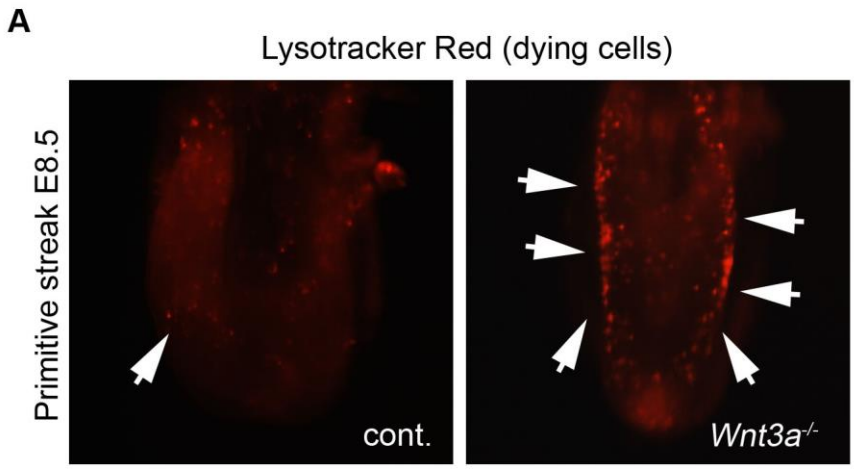
Supplemental Figure 4. Conditional *TCreERT2* deletion of *β-catenin* and lineage tracing of *TCreERT2; βcat^{LOF}* cells at E9.5. (A) Antibody detection of β-catenin in *TCreERT2; βcat^{LOF}* embryos showed *R26R-lacZ* traced cells corresponding to areas of the embryo devoid of β-catenin (Arrows). However, β-catenin was detected in the mesoderm aggregated into structures similar to somite fragments. To note, rudimentary somites were detected by *Uncx4.1* WISH providing an explanation for the detection of any somite tissue in *βcat^{LOF}* embryos (Figure 3A). Some of the β-catenin expressing cells were also *R26R-lacZ* traced (small arrows) indicating unequal excision of both *R26R-lacZ* and *β-catenin* (*Ctnnb1$^{flox/flox}$*) in our experiments. (B) Tracing of *βcat^{LOF}* cells by *R26R-lacZ* using βgal-staining showed increased numbers of traced cells in the neural tube of βcat^{LOF} embryos compared to controls (black arrows). This data allows for a comparison to *Wnt3a$^{-/-}$* mutant tracings in Figure 2.

Figure S5 (related to Figure 4)



Supplemental Figure 5: (A) E8.5 whole-mount *BATlacZ* detection by βGal-staining shown in dorsal view and in representative sections along the anterior to posterior axis. Note that βGal-staining is observed in the posterior PS through to more differentiated cells of the anterior epithelial PS and neural tube. The epithelial primitive streak and neural tube is indicated in sections by a black dotted line.

Figure S6 (related to Figure 6)



Supplemental Figure 6: (A) Dorsal view of Lysotracker staining of E8.5 PS showing few dying cells (arrows) in controls but many dying cells (arrows) along the lateral edge of the PS in *Wnt3a*$^{-/-}$ mutant embryos.