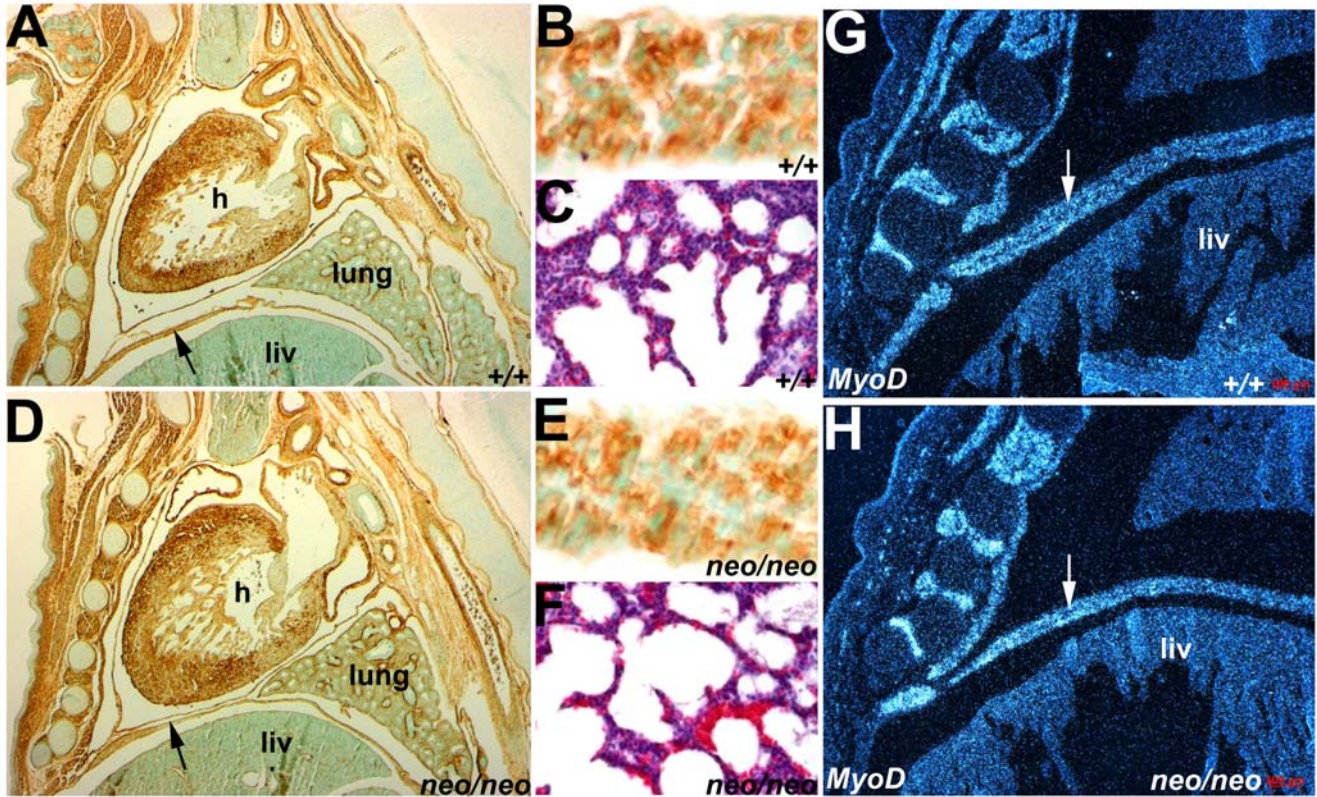


Supplemental Figure 1

Supplemental Figure 1. Reduced Pax3 expression levels do not affect Pax3^{neo/neo} neural crest morphogenesis or heart development. (A-D) H&E stained transverse sections of E14.5 wildtype (+/+, A,C) and Pax3^{neo/neo} (neo/neo, B,D) hearts revealed the Pax3^{neo/neo} OFT is appropriately septated (B) and the interventricular septum is intact (D). **(E,F)** Wholemout non-radioactive *Crabp1 in situ* hybridization revealed that appropriate cardiac NC migration (arrowhead) occurs within Pax3^{neo/neo} E10.5 embryos, whilst radioactive *in situ* hybridization analysis for both *Periostin* (*Peri*) **(G,H)** and *Sox10* **(I,J)** revealed that NC-derived Pax3^{neo/neo} dorsal root ganglia (arrows) are unaffected. Similar patterns of *Periostin* expression are also present within the wildtype (G) and Pax3^{neo/neo} (H) OFT mesenchymal cushions (arrowheads), indicating that OFT morphogenesis occurs normally despite the reduced Pax3 expression levels. Abbreviations: h, heart; nt, neural tube; Ao, aorta; P, pulmonary artery; lv, left ventricle; rv, right ventricle.



Supplemental Figure 2

Supplemental Figure 2. Diaphragmatic muscle morphogenesis tolerates reduced Pax3 levels. Immunohistochemistry (for α smooth muscle actin; α SMA), H&E and radioactive *in situ* detection of *MyoD* mRNA muscle marker expression were used to assess development of the diaphragm. **(A,B)** Wildtype (+/+) and **(D,E)** *Pax3*^{neo/neo} E15.5 sagittal sections stained for α SMA expression. B & E are enlarged views of α SMA-stained diaphragm indicated via arrows in A&D. Note α SMA expression and thickness is comparable between the two genotypes. **(C,F)** H&E staining of newborn lung, showing inflated alveoli with similar sized air spaces and alveoli thickness in wildtype (C) and *Pax3*^{neo/neo} (F). **(G,H)** *In situ* hybridization of *MyoD* on serial E15.5 sagittal sections. Note *MyoD* is robustly expressed in both wildtype (G) and hypomorphic (H) diaphragmatic (arrows), intercostal and abdominal muscle masses.