



**Figure S1. Quantitation and localization of Tbx6 across various genotypes and background strains.** (a) Protein isolated from pooled e10.5 tailbuds from *Tbx6<sup>rv/rv</sup>*, *Tbx6<sup>+/-</sup>* and *Tbx6<sup>Tg46/Tg46</sup>* (*Tg46/Tg46*) lines and their respective background strains as indicated was subjected to Western blot analysis using anti-Tbx6 and anti-actin antibodies. (b) Relative Tbx6 protein levels quantified using Kodak Image Station software and normalized to total actin levels. Increasing amounts of Tbx6 protein were found in the *Tbx6<sup>rv/rv</sup>*, *Tbx6<sup>+/-</sup>*, *Tg46/Tg46* lines as well as their respective background strains. (c) Tailbuds from e10.5 embryos analyzed by immunohistochemistry for Tbx6 protein expression with the tailbud of each embryo shown. Tailbud morphology was

affected across the various genotypes. Compared to their wild-type littermates, an enlarged tailbud was noted for the *Tbx6<sup>rv/rv</sup>* embryo, while a smaller tailbud was observed for the *Tg46/Tg46* embryo. Absence of Tbx6 staining within the *Tbx6<sup>rv/rv</sup>* tailbud (arrowhead and red dashed outline) is neural tissue. **(d)** Whole mount *in situ* hybridization of *T* expression in e10.5 embryos dissected from matings of *Tbx6<sup>Tg46</sup>* heterozygotes (side and dorsal views). The small tail phenotype of *Tg46/Tg46* embryos is apparent by e10.5 as is the reduced expression of *T* compared to the wild type (+/+ control).