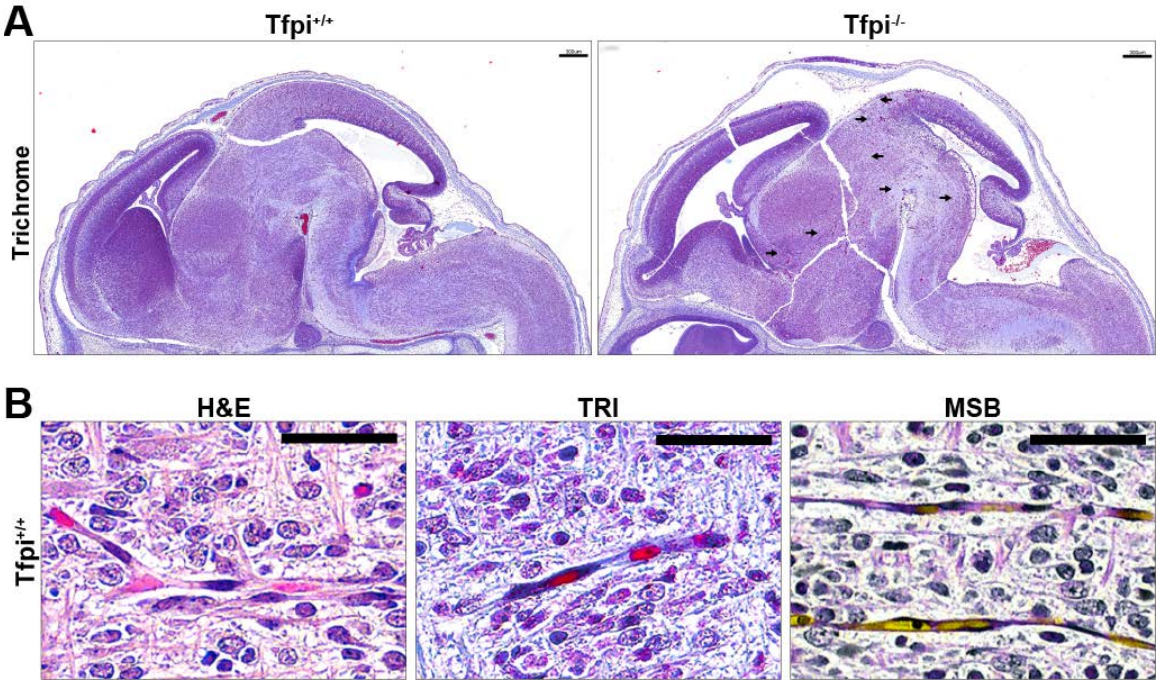
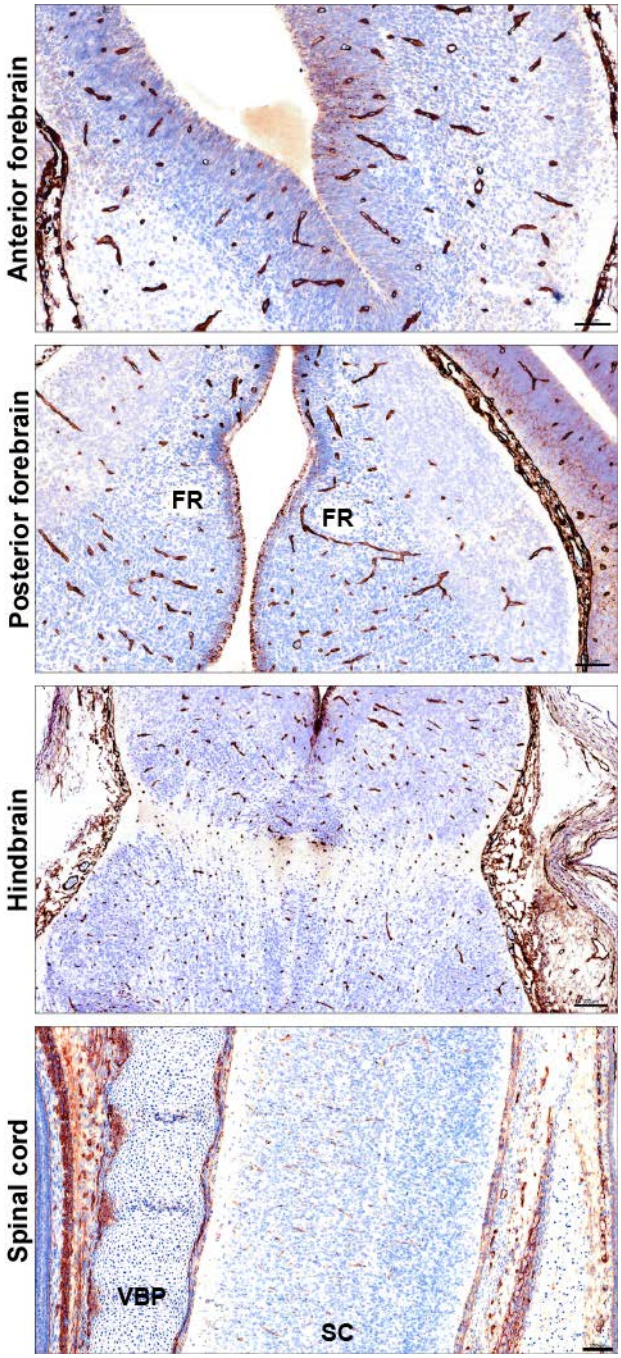
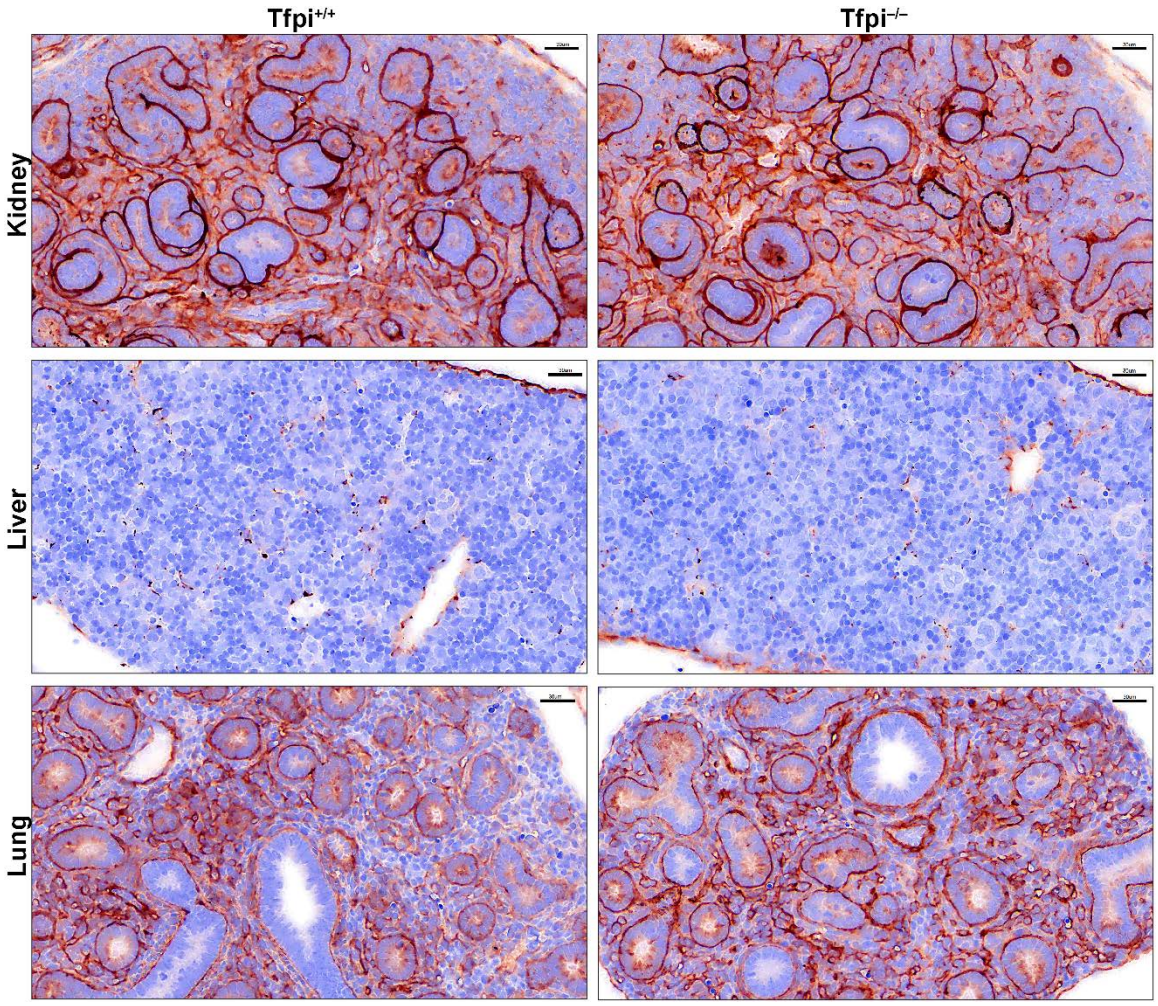


Supplemental Figure 1. (A) Trichrome stained brain sections from E15.5 *Tfpi*$^{+/+}$ and *Tfpi*$^{-/-}$ littermates had features of delayed brain growth and maturation in *Tfpi*$^{-/-}$ embryos (bar=300µm). Brain development in *Tfpi*$^{+/+}$ embryos was appropriate for age with the brain filling the calvarium, appropriate growth of the choroid plexus, and normal brain architecture. In contrast, *Tfpi*$^{-/-}$ embryos had smaller brains that did not fill the calvarium, the choroid plexus was underdeveloped, and there were numerous glomeruloid bodies (arrows) throughout the brain parenchyma, often associated with hemorrhage and cellular death. (B) Normal embryonic vascular brain development stained with H&E, Trichrome (TRI), and Martius Scarlet Blue (MSB). Vessels with parallel walls lined with endothelial cells and containing red blood cells are depicted. In the H&E: red blood cells are pink and endothelial cell nuclei are dark purple; TRI: red blood cells are red and endothelial cell nuclei are deep blue-purple; MSB: red blood cells are yellow and endothelial cell nuclei are dark blue-black. (bar=30µm).

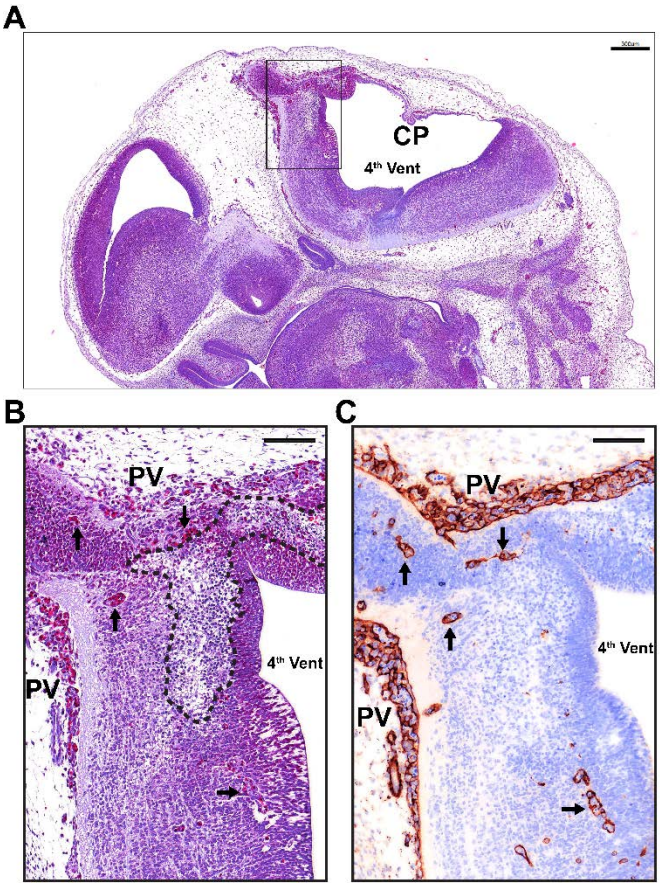


Supplemental Figure 2: Coronal sections of *Tfpi*$^{+/+}$ mouse embryo brains stained for laminin and demonstrating normal vascular formation in the anterior forebrain (bar=60μm), the posterior forebrain (bar=80μm), the hindbrain (bar=100μm) and spinal cord (SC) (bar=100μm, VBP=vertebral body primordium). The axonal tracts of the Fasciculus Retroflexus (FR) were noted in the posterior forebrain as a part of the Habenula tract system relaying information between the forebrain, midbrain and

hindbrain. In the hindbrain, the penetrating parallel-walled vasculature from the smooth pia-brain interface were well organized.



Supplemental Figure 3. Kidney, liver, and lung from E15.5 *Tfpi*$^{+/+}$ and *Tfpi*$^{–/–}$ littermates stained for laminin and counterstained with hematoxylin had normal tissue and vascular architecture in both genotypes (bar=30μm).



Supplemental Figure 4. Brain of a E12.5 *Tfpi*$^{-/-}$ embryo. (A) Trichrome stain of a sagittal brain section with glomeruloid bodies, hemorrhage, and cell death characterized by pyknotic nuclei and loss of brain parenchyma. The choroid plexus (CP) was beginning to grow into the fourth ventricle (bar=300μm). (B) Higher magnification of the boxed area in (A). The dotted line outlines cellular death at the pons-midbrain junction. There were numerous glomeruloid bodies (arrows) surrounding the area of cellular death. Increased density of the pial vessels (PV) suggested a decreased penetration of the endothelial cells into the brain parenchyma (bar=100μm). (C) Brain stained with an anti-laminin polyclonal antibody. Glomeruloid bodies were seen within the brain parenchyma at the pons-midbrain junction. The increased density of the pial vasculature can be seen with the staining of the laminin basement membrane (bar=100μm).