are reduced to lower steady-state levels due to inhibition of canonical Wnt signaling activity by DKK2.

Supplemental Figure 1: Rapid extinction of PITX2 protein expression following tamoxifen-induced temporal knockout of the *Pitx2* gene. (A) Experimental timeline for generation of temporal *Pitx2* knockout (*Pitx2-tko*) mice. Tamoxifen was injected into timed pregnant dams at e10.5 to activate the dormant Cre-ER fusion protein and embryos were harvested for analysis either at e11.5 (B-G) or e14.5 (Fig. 4). (B-G) PITX2 immunofluorescence (red), DAPI nuclear stain (blue) and merge on eyes taken from control (B-D) and e11.5 *Pitx2-tko* littermates harvested from a timed pregnant dam injected with tamoxifen at e10.5. PITX2 protein is completely lost from *Pitx2-tko* eyes within 24 hours post-injection.

Supplemental Figure 2: LEF1 and b-catenin do not bind the *Pitx2c* promoter or 3' pituitary enhancer in ocular neural crest. (A) Schematic of the *Pitx2c* promoter and 3' pituitary enhancer is diagramed. Locations of the primer pairs for amplifying *Pitx2c* promoter (P2c) and 3' pituitary enhancer (En) sequences are indicated. (B) ChIP analysis was performed on mouse embryonic fibroblasts cultured from microdissected e12.5 wild type eyes using rabbit anti-β-catenin antibody, normal rabbit serum (NRS), mouse anti-LEF1 antibody, and normal mouse serum (NMS). Precipitated and control samples were amplified by PCR using primers directed against the *Pitx2c* promoter sequences (P2c) that contain no predicted TCF/LEF sites. (C) ChIP analysis was performed as in (B) except that precipitated and control samples were amplified by PCR using primers

directed against the 3’ pituitary enhancer sequences (En) that contain a single TCF/LEF site. Apart from input, no enrichment of PCR product was reproducibly observed in any sample using either primer set.