

Welsh_INVENTORY OF SUPPLEMENTARY INFORMATION

1. Supplementary Figure 1: Transgenic analysis of e926 sequence and conserved Pitx2 binding motifs. Relates to Figure 1 of main text.

2. Supplementary Figure 2: GRO-seq analysis of chicken Pitx2 locus from different developmental stages and tissue samples. Relates to Figure 2 of main text.

3. Supplementary Figure 3: dREG analysis of chicken Pitx2 locus from different developmental stages and tissue samples. Relates to Figure 2 of main text.

4. Supplementary Figure 4: Reproducibility of FISH data in biological replicates of chicken and mouse embryos, box plots of Playrr-Pitx2 FISH data in E10.5 WT and Pitx2^{-/-} embryos, and TaqMan QRT of WT vs Pitx2^{-/-} expression. Relates to Figures 4 and 5 of main text.

5. Supplementary Figure 5: Cumulative frequency curves of chicken and mouse FISH data

6. Supplementary Figure 6: Divergent *C4orf32* and *Enpep* expression in chicken vs. mouse DM, summary of *Pitx2* locus topology, and conservation of Pitx2 locus in human, mouse, chicken, and zebrafish. Relates to Figure 6 and discussion of main text.

7. Supplementary Figure 7: ChIP-seq data from mouse ES cells. Relates to Figure 6 of main text.

8. Supplementary Figure Legends