Figure 7: Working model of *etv5a* AND *etv4* function in MCC patterning during nephrogenesis. Interplay between retinoic acid (RA), *etv5a,* and Notch signaling in the renal progenitor field mediates multiciliated cell (MCC) formation during nephrogenesis. *etv5a* responds downstream of RA signaling to promote MCC fate, although it is likely that *etv5a* is not the only target of RA in this pathway. Conversely, Notch signaling inhibits *etv5a* activity to restrict MCC formation and favor transportive cell identity. In addition, *etv4* promotes MCC fate, although it is not resolved if *etv4* acts within renal progenitors or neighboring tissues, and whether other known MCC specification factors impact *etv4* in other embryonic locales to affect pronephros development. Abbreviations: P (podocyte), N (neck), PCT (proximal convoluted tubule), PST (proximal straight tubule), DE (distal early), CS (corpuscle of Stannius), DL (distal late), PD (pronephric duct), C (cloaca).

SUPPLEMENTARY FIGURE LEGENDS

Supplemental Figure 1: Comparison of *etv5a* knockdown phenotypes and blood pooling in *etv5a* morphants. (A) WT embryos were injected with either *etv5a* MO2 or *etv5a*$^{\Delta acidic}$ at the one-cell stage, and then monitored over time for any physical changes. In this live time course, both *etv5a* MO2 and *etv5a*$^{\Delta acidic}$ morphants have some head darkening at the 24 hpf stage (black arrow), which is not seen in the uninjected siblings. **(B)** *etv5a* MO injected embryos stained with o-dianisidine show some blood pooling in the head region (black asterisk) when compared to uninjected siblings. **(C)** Quantification of o-dianisidine experiments demonstrate approximately 30% of *etv5a* morphants have abnormal blood pooling in the head, where the uninjected siblings do not display a circulation problem.

Supplemental Figure 2: *etv5a* MO2 assays. (A) Embryos injected with *etv5a* MO2 have more cell death, as shown by AO staining, in the head region compared to control embryos at the same time points. However, the amount of cell death is reduced in *etv5a* MO2 injected embryos from the 24 ss to the 30 ss. **(B)** WISH analysis on 6 ss embryos shows reduced *scl* (purple) expression in embryos injected with *etv5a* MO2. Embryo counts for each observed phenotype are as indicated in the lower left corner of the corresponding panel. For control 30 ss, 2/45 embryos had elevated AO cell numbers compared to the majority phenotype that is shown, and for *etv5a* MO2 24 ss, 3/50 embryos had further elevated AO cell numbers compared to the majority phenotype that is shown.

Supplemental Figure 3: *etv5a* splice MO validation. (A) Schematic of *etv5a* cDNA, where black boxes depict exons. The arrow on exon 2 represents the ATG site. The region between exons 6 and 7 is magnified, showing the *etv5a* MO binding site (red). Arrows mark the forward and reverse primer binding sites. **(B)** Image of DNA product after RT-PCR. The control band is about 400 bp in size, as expected when adding exon 6 to exon 7. *etv5a* MO amplified DNA makes three bands: two bands around the control size of 400 bp and a larger morphant band at around 500 bp, denoted by the red asterisk. **(C)** Schematic of *etv5a* protein sequences after sequencing of the three conditions: control, *etv5a* MO* and *etv5a* MO$^{\#}$. The control protein sequence is 525 amino acids (aa) long, where *etv5a* MO* is truncated at 128 aa as a result to the inclusion of the intron between exons 6 and 7. The second *etv5a* MO$^{\#}$ protein sequence is missing 6 aa translated from exon 6.

Supplemental Figure 4: MCC counting method. (A) Representative images of *odf3b* expression in the zebrafish pronephros at 24 hpf of traditional WISH (purple) and fluorescent WISH (red). Black and white arrowheads denote individual *odf3b*$^{+}$ cells. **(B)** Quantification of average MCC number as marked by *odf3b* comparing control and *etv5a* MO embryos analyzed by WISH (n = 33 each) and fluorescent WISH (n = 10 each). There is no significant difference between the two controls or the two groups of *etv5a* morphants determined by two-tailed student's t-test. P-value *****p<0.001, N.S. (non-significant).

Supplemental Figure 5: *etv4* splice MO validation. (A) Schematic of *etv4* DNA sequence with exons as black boxes, where the arrow represents the ATG start site in exon 1. The region between exons 8 -11 is magnified below, with the *etv4* MO binding site highlighted in red. Directional arrows above exon 8 and below exon 11 denote the forward and reverse primers. **(B)** Image of DNA products after RT-PCR. The control DNA produce is around 400 bp in size, as expected when adding exons 8, 9, 10, and 11. *etv4* MO DNA produced two bands at 400 bp and 200 bp (red). **(C)** Schematic of the predicted translated *etv4* protein products after sequencing RT-PCR DNA products. *etv4* missplicing cause excision of exon 10, resulting in a predicted frameshift within the coding region of exon 11 that would result in a premature truncation in the protein. Therefore, while the control protein is 495 amino acids (aa) in size, *etv4* MO* is only 344 aa in length.

Supplemental Figure 6: *etv4* expression in renal progenitors is unaffected by alterations in RA or Notch expression levels. In WT embryos, *etv4* transcript expression localizes to the central nervous system, as well as Rohon-Beard neurons and the tailbud, while showing low-level ubiquitous expression throughout the axial mesoderm at 24 hpf. Embryos treated with RA, DEAB or DAPT exhibited no change in *etv4* expression in the pronephros.

Dev Biol 2015_49 Manuscript Highlights:

- zebrafish renal progenitors show regionalized *etv5a* expression in multiciliated cell precursors