SUPPLEMENTAL FILE

Supplemental Figure 1: Specificity of Myo1c 5 antibody was determined by pre-incubating the antibody with Myo1c full length protein prior to the staining of zebrafish embryos. Pre-incubation with Myo1c protein completely blocked Myo1c 5 antibody staining confirming the specificity of this antibody.

Supplemental Figure 2: Construction and characterization of DIG labeled RNA probes includes PCR of Nephrin and Myo1c homologs, purification of PCR products, qualitative analysis of the RNA generated from cDNA and dot blot assay to determine RNA labeling with DIG.

Supplemental Figure 3: (A) The whole mount *in-situ* hybridization of zebrafish embryos showed that both Myo1c homologs are expressed in and around the glomerular region. **(B)** *In situ* hybridization of zebrafish sections using anti-sense RNA probes (experimental) and sense probes (control) against the Myo1c homologs reveals a widespread expression of both Myo1c homologs in various tissues including glomerulus.

Supplemental Figure 4: (A) The two categories of edema, moderate (prominent pericardial edema) and severe (whole body edema) used in our experimental analysis are shown. (B) The capped mRNA from mouse Myo1c ortholog was prepared and co-injected with Myo1c morpholinos in one cell stage embryos and the phenotypic rescue was determined at 48-72hpf. Representative images from multiple independent experiments show that in addition to edema, the physical deformities are significantly reduced in rescued (Myo1c_Chr5/15_MO + GFP-Myo1c RNA) fish.

Supplemental Figure 5: Histological comparison of 72hpf control and Myo1c morphant embryos shows renal deformities, where prominent tubular dilation and enlarged bowman's space was noted in the Myo1c knockdown embryos. Presence of edema in the vicinity of gut region was also observed in the mutant fish.

Supplemental Figure 6: The Myo1c knockdown and control embryos (96hpf) were injected with 500kD Rhodamine labeled dextran though common cardinal vein. The presence of red

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dextran in the tubules of cross sectioned embryos is indicated by the arrows. The images were stained and analyzed by fluorescence microscopy.

Supplemental Table 1: Sequences of primers used in our experiments to construct DIG labeled RNA probes (anti-sense and sense) against the two Myo1c homologs and Nephrin are listed.



PCR from specific primers



Purified PCR Prodcuct

Myo1c_Chro_5



DIG	dot	blot	RNA	Labe	ling

1	2	3	4	5	6	7
8	9	10	11	12	13	14
15	16	17	18	19	20	21
22	23	24	25	26	27	



1-9 = Control Labelled RNA
10-12 = Nephrin Experimental RNA
13-15 = Nephrin Control RNA
16-18 = Myo1c Chr_5 Experimental RNA

19-21 = Myo1c Chr_5 Control RNA

22-24 = Myo1c Chr_15 Experimental RNA

25-27 = Myo1c Chr_15 Control RNA

RNA Qulaity on gel



(A)



(B)

Anti-sense DIG labelled RNA Sense DIG labelled RNA (Control)



Notochord; 2. Gut; 3. Ear; 4. Pre-chordal plate; 5. Eye;
 Pectoral fin ; The Bar represents 20µm





Tubules; 2. Glomerulus; 3. Notochord; 4. Somitic Muscle;
 Neural tube; 6. Common Cardinal Vein; 7. Gut; 8. Yolk



G = Gut

SUPPLEMENTAL TABLE 1

Primers used for the construction of RNA probe

Myo1c Chromosome 5

Experimental /Antisense

Myo1c_FP_ISHE: GAAAACCTGCGCCGGCGGTAC Myo1c_RP_ISHE: TAATACGACTCACTATAGGGTCTCCCAGCCTTCGGCCTCA

Control/Sense

Myo1c_FP_ISHC: TAATACGACTCACTATAGGGGAAAACCTGCGCCGGCGGTAC Myo1c_RP_ISHC: TCTCCCAGCCTTCGGCCTCA

Myo1c Chromosome 15

Experimental /Antisense

Myo1c15_FP_ISHE: TGAGAACCTCCGCAAACGC Myo1c15_ RP_ISHE: TAATACGACTCACTATAGGGGTGCACGACGCGGGATTTCT

Control/Sense

Myo1c15_FP_ISHC: TAATACGACTCACTATAGGGTGAGAACCTCCGCAAACGC Myo1c15_ RP_ISHC: GTGCACGACGCGGGGATTTCT

Nephrin

Experimental /Antisense

Nephrin_FP_ISHE: GTGAACGTGTCTCGGCGGGAC Nephrin RP_ISHE: TAATACGACTCACTATAGGGTTCGCATCAGCAGTGGTGACCA

Control/Sense

Nephrin_FP_ISHC: TAATACGACTCACTATAGGGGTGAACGTGTCTCGGCGGGAC Nephrin_RP_ISHC: TTCGCATCAGCAGTGGTGACCA