Supplemental Figure legends:

Supplemental Movie 1: (A-B) Loss of Myo1c binding reduces turnover rate of Neph1 at the podocyte cell membrane. The cultured podocytes stably transfected with mCherry Neph1-wt (A) and mCherry Neph1-K761A (B) were analyzed by fluorescence recovery after photobleaching (FRAP). A region of interest at the cell junction was selected and photobleached and recovery of Neph1 at the photobleached region was recorded over a period of 5 minutes for both the mCherry-Neph1-wt and mCherry-Neph1-K761A. The FRAP analysis suggests a high turnover rate for Neph1-wt that is significantly higher than the mutant Neph1-K761A.

Supplemental Movie 2: (A-B) mCherry Neph1-wt and K761A mutant co-localization was captured with late endosome using time lapse live microscopy. Images were collected for 2 minutes for every 10 seconds.

Supplemental Movie 3: (A-B) Loss of Myo1c binding attenuates the movement of Neph1 late endosome vesicles. Podocytes stably expressing mCherry-Neph1-wt (A) and mCherry-Neph1-K761A (B) were analyzed for the movement of Neph1 containing vesicles using live imaging microscopy. Images were collected for 2 minutes for every 10 seconds. mCherry-Neph1-wt containing vesicles showed significantly higher displacement and velocity when compared to mCherry-Neph1-K761A containing vesicles.