Supplementary Figure 1: Expression of claudin subtypes and ZO-1(red) in mouse kidney by immunohistochemistry in low (Claudin-15, ZO-1) and high magnification (the others). Nuclei were stained with DAPI (blue). (Scale bars: Claudin15, ZO1:100 µm, the others: 50 µm)
Supplementary Figure 2; To assess the cytotoxicity of 10.3 mM ouabain on B4G12 cells, cell viability was examined using a live/dead viability/cytotoxicity kit (Molecular Probes, Eugene, OR). Staining was performed according to the manufacturer’s instructions. Ouabain was added at the same concentration (10.3 mM) and the same period of time (5 minutes) with Ussing chamber experiments. Cell viability was analyzed by fluorescence microscopy (Axio Imager). (A) Live/dead assay image of B4G12 cells with or without ouabain treatment in each siRNA treated group. Viable cells express green fluorescence and dead cells express red fluorescence (scale bars; 100 µm). (B) Green and red cells were counted per four fields at 400-fold magnification, and the percentage of cells with green fluorescence / all cells was then calculated. There was no difference in percentage of viable cells with or without ouabain treatment.