



Supplementary Figure 3. Localization of bestrophin-1 (green, Alexa Fluor 488 goat anti-mouse) expressed either in canine retina (**A**) or transfected MDCK cells (**B, C**). Cell nuclei are indicated with DAPI (blue), while the cell membranes are highlighted with wheat germ agglutinin (WGA), a plasma membrane marker (red, Alexa Fluor 594 conjugate, 5 μ g/ml; Invitrogen). **A.** Endogenous bestrophin-1 expression in the normal canine retina. Bestrophin-1 is found exclusively in the basolateral membrane of the RPE cells, co-localizing with WGA staining (merge, *arrow*). Bright field photography was used to confirm the intact retinal structure. **B.** *cBEST1* transiently transfected MDCK cells expressing the wild type canine bestrophin-1 (green), co-stained with the WGA (red). Merge: wild type Best1 is associated with the cell membrane (*arrow*). **C.** Overexpression of cBest1 *in vitro*. MDCK cells transiently transfected with the wild type *cBEST1* construct (10 x 1:6 DNA to Lipofectamine TM 2000) showing overabundance of the bestrophin-1 (green). Best1 is observed to saturate the cell membrane at first, and the excess traffics to the cytosol. Best1 saturated membrane co-localizes with the WGA staining (merge, *arrow*). WGA: wheat germ agglutinin; RPE: retinal pigment epithelium; OS: photoreceptor outer segment; IS: photoreceptor inner segment; ONL: outer nuclear layer; Scale bar: 10 μ m.