



Figure S1. Secretion of the CLCA1 proteins examined in the experiments shown in Figs. 1 (A), 2 (B) and 3 (C). HEK293T cells were cultured and transfected as described in the Experimental Procedures. Medium from cells transfected with CLCA1 protein constructs, or from untransfected cells cultured side-by-side, was diluted 1:1 in SDS containing 2-mercaptoethanol. Samples (~30 μ g total protein, as determined by the BCA Protein Assay; Thermo Fisher Scientific, Rockford, IL) were boiled for 5 min, and then loaded on a 4–12% Bis-Tris Nupage gel (Life Technologies). The proteins were transferred to nitrocellulose membranes using an iBlot Gel Transfer Device (Life Technologies). Membranes were blocked by 0.5% nonfat milk in PBS with 0.1% TWEEN, and incubated in rabbit anti-6-His antibody HRP-conjugate (1:5000, Bethyl Laboratories) in blocking buffer for 10 min. Signal was detected using Pierce ECL Western Blotting Substrate (Thermo Fisher Scientific), and the membranes were exposed to an autoradiographic film. Arrows (A, B) or box (C) indicate bands of the predicted molecular weight: proteolytically processed full-length CLCA1 (His-tagged C-terminus), ~35-40 kDa; N-CLCA1, 75 kDa; CAT + VWA, 55 kDa; CAT, 32 kDa; and WT and mutant VWA, 22 kDa.