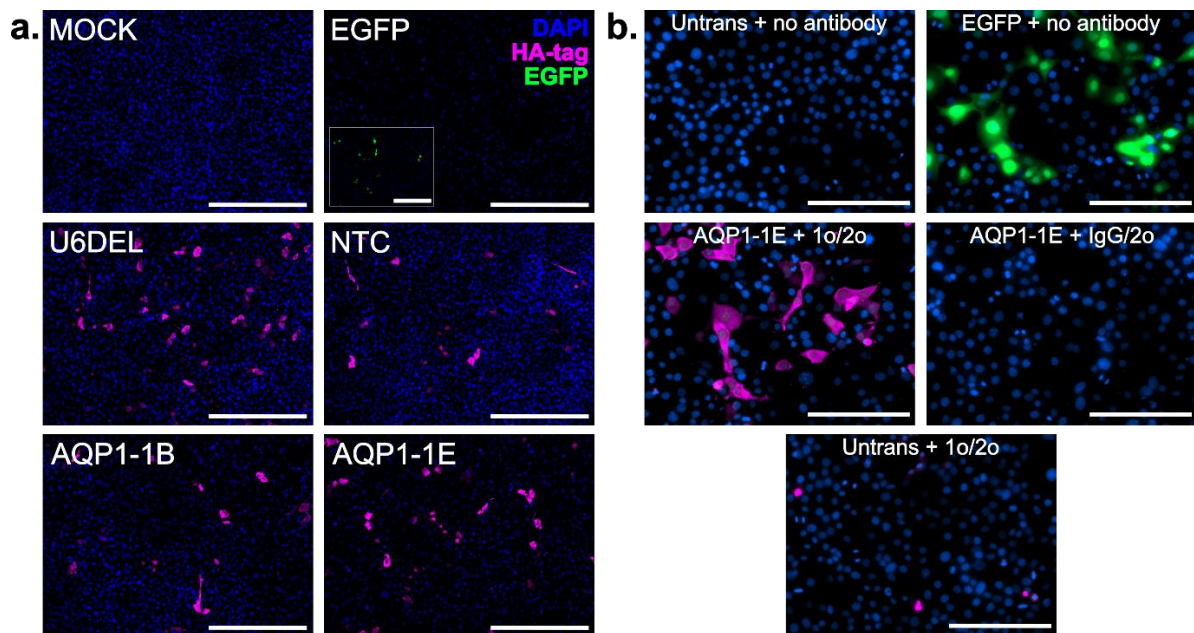


Additional File 4



Supplemental Figure S3. Fluorescent immunocytochemistry of B6-RPE07 cells transiently transfected with the constructs indicated or MOCK (no DNA). (a) Negative control constructs U6DEL and NTC express SaCas9 (anti-HA tag staining) even though they fail to cut genomic DNA at the Aqp1 locus. Targeting guide sequences 1B and 1E result in delivery of the expressed SaCas9 to the Aqp1 locus for cutting of genomic DNA. SaCas9 was not observed as expected when a EGFP containing construct was transfected instead, with native EGFP fluorescence observable instead in the appropriate channel (inset). Scale bars all 400 μm . (b) Validation of the anti-HA primary antibody used to detect SaCas9 through a C-terminal HA tagging. Signal is detected only when primary antibody (1o), secondary antibody (2o) and cells transfected with SaCas9-expressing constructs (AQP1-1E) are present. No signal is observed is cells are untransfected (Untrans), transfected with an EGFP expressing construct instead (EGFP), or the primary antibody is replaced with a non-immune IgG (IgG). Scale bars all 200 μm .