Cation channel conductance and pH gating of the innate immunity factor APOL1 is governed by pore lining residues within the C-terminal domain

Charles Schaub[1, 2], Joseph Verdi[1, 3], Penny Lee[1], Nada Terra[1], Gina Limon[1, 4], Jayne Raper[1] and Russell Thomson[1*]

[1]Department of Biological sciences, Hunter College, CUNY, New York, USA.

Now at [2]Vanderbilt University, Nashville, Tennessee, USA; [3]German Cancer Research Center, Heidelberg, Germany; [4]NYU school of Medicine, New York, USA

*-Corresponding author

E-mail: rthomson@genectr.hunter.cuny.edu

Supplementary Figures S1-S9

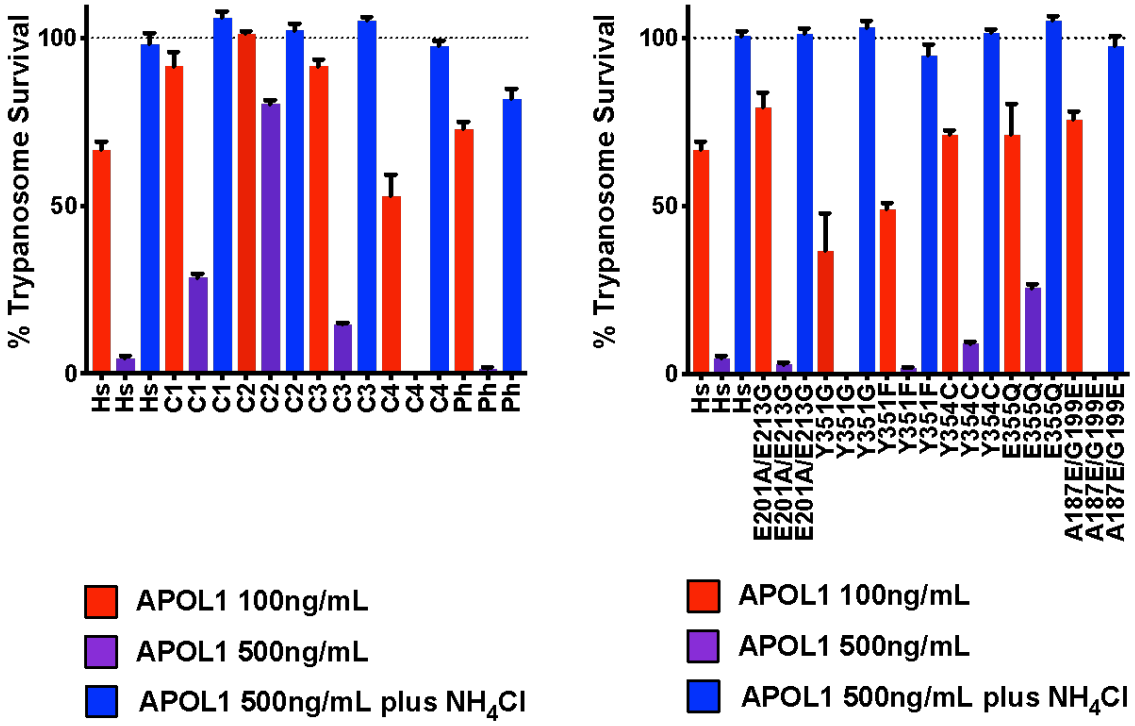

Figure S1. Killing of *T. brucei brucei* by human, baboon and chimeric rAPOL1 proteins. *T. b. brucei* 427 trypanosomes were incubated with either 100, or 500 ng/ml rAPOL1 proteins (see Figures 2 and 4) at 37°C, and parasite viability was determined after 24 h by the alamarBlue assay. Plotted are the mean survival values of triplicate measurements +/- S.D., relative to untreated controls. As a control for specificity of lysis, trypanosomes were also treated with 10 mM ammonium chloride as indicated.

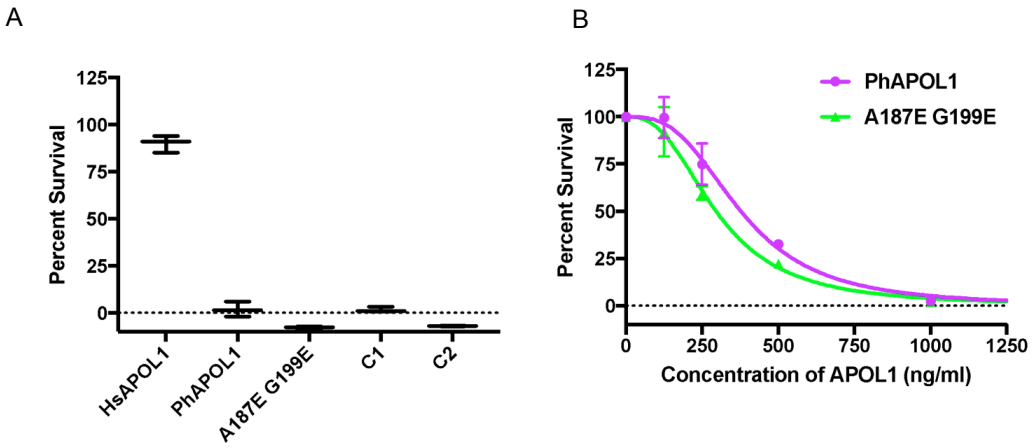

Figure S2. Killing of Human-infective trypanosomes by human, baboon and chimeric rAPOL1 proteins. Trypanosomes were incubated with the indicated rAPOL1 proteins (See Figures 2 and 5) for 24 hours at 37°C, and parasite viability was determined by the alamar blue assay. Plotted are the mean survival values of triplicate measurements +/- S.D., relative to untreated controls. (A) Killing of *T. brucei* 427-SRA trypanosomes after incubation with 800 ng/ml of human, baboon, and chimeric rAPOL1. (B) Killing of *T. b. gambiense* ELIANE by serially diluted PhAPOL1, or PhAPOL1 A187E G199E.

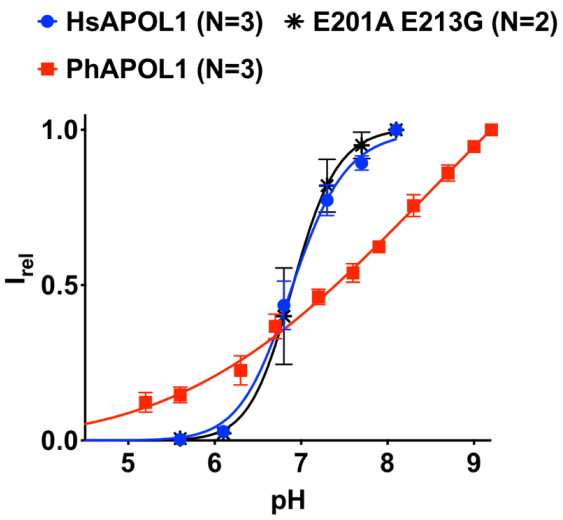

Figure S3. Substitution of HsAPOL1 H-L-H glutamate residues (E201A E213G) does not affect pH gating. A conductance was obtained with the indicated proteins and then the pH of both the cis and trans sides were titrated with KOH and HCl as detailed in the legend to Figure 4. The current at each value of pH was normalized to the maximal current to obtain the relative current (I_{rel}). Plotted is the average I_{rel} of N independent experiments +/-S.D. The data was fit to the Hill equation. In some cases error bars are smaller than the symbols used to represent data points.

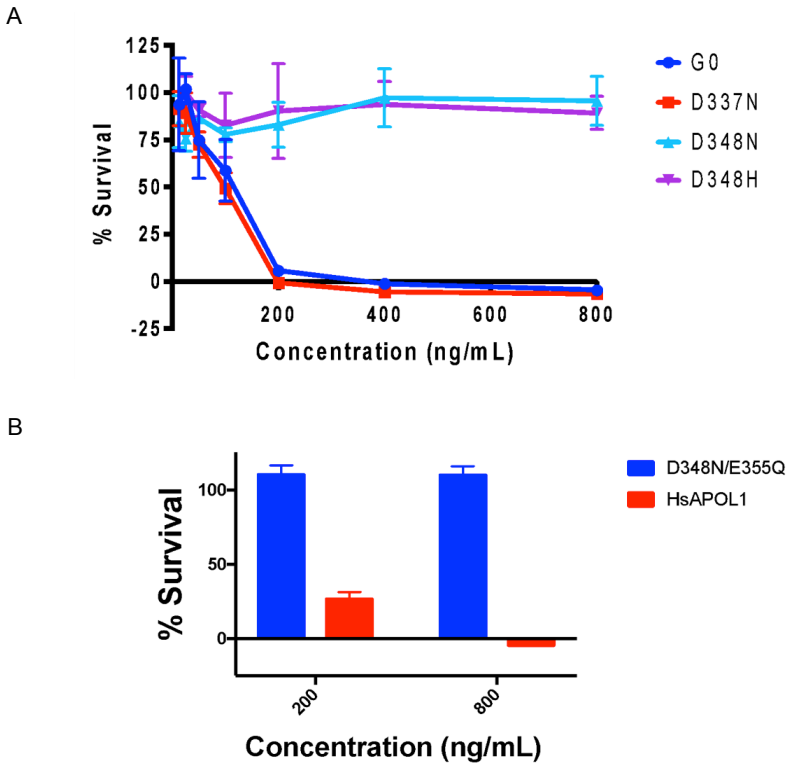

Figure S4. Substitution of HsAPOL1 D348, but not D337 abrogates trypanolytic activity. (A) *T. b. brucei* 427 trypanosomes were incubated with various concentrations of G0, D337N, D348N and D348H rAPOL1 proteins for 24 hours at 37°C, and parasite viability was determined by the alamar blue assay. Survival of APOL1-treated cells is plotted relative to untreated cells (all alive) and detergent treated (all dead) wells. Plotted are the mean survival values of triplicate measurements +/- S.D., relative to untreated controls. Notably, the D337N substitution had no effect on lytic activity, whereas D348N and D348H resulted in loss of APOL1 lytic activity. (B) Survival of *T. b. brucei* 427, after incubation with 200 and 800 ng/mL APOL1 D348N E355Q. Plotted are the mean survival values of triplicate measurements +/- S.D., relative to untreated controls. As expected the double mutant was inactive compared to HsAPOL1.

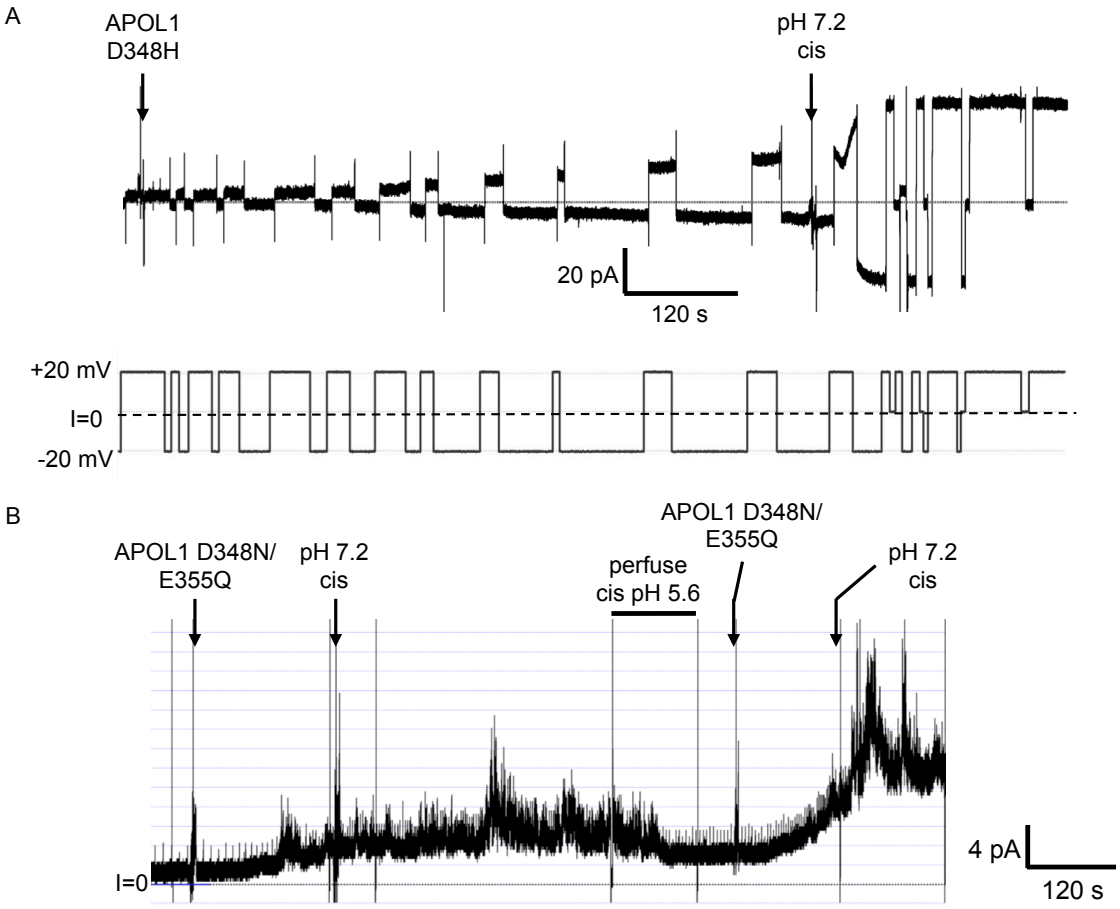

Figure S5. Activity of APOL1 D348H and APOL1 D348N/E355Q in planar lipid bilayers. (A) The voltage was alternated between + and - 20 mV (lower trace) as the current was recorded (upper trace). Before the start of the record the cis chamber was adjusted to pH 6.0 with HCl, with the trans side held at pH 7.2. At the start of the record, 200 ng of D348H rAPOL1 was added to the cis side, which resulted in a gradual increase in bilayer conductance. Upon neutralization of the cis pH there was a small, ~2-fold increase in conductance at +20 mV. (B) The starting conditions were cis pH 5.6 and trans pH 7.2. The voltage was held at +20 mV throughout. After addition of 300 ng of the double mutant APOL1 D348N/E355Q there was a small increase in bilayer conductance. However, when the cis side was then neutralized with 1 M KOH to pH 7.2, there was no observable increase in conductance due to opening of pH gated channels. This minor conductance was then maintained after cis perfusion at pH 5.6. The addition of more protein at cis pH 5.6 resulted in a further increase in conductance, but again, there was no further increase upon subsequent pH neutralization.

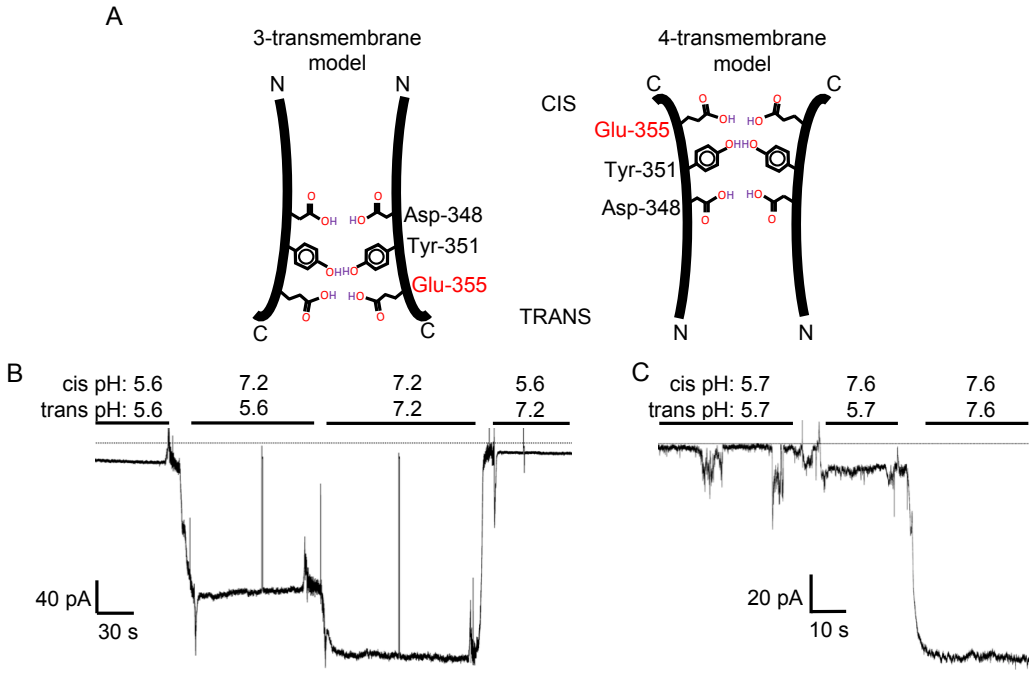

Figure S6. APOL1 E355Q substitution decreases the cis pH dependence of channel conductance. (A) In the 3-transmembrane model Glu-355 localizes to the trans side of the channel, whereas in a 4-transmembrane model it would localize to the cis side (see also Figure 8). (B and C) Before the start of the record a full cis/trans pH titration was performed with HsAPOL1 (B), or APOL1 E355Q (C), and then the pH was lowered to the values indicated. Except for brief excursions to 0 mV, the voltage was held at -20 mV, such that a downward deflection in the current reflects an increase in bilayer conductance. The cis and then the trans pH were raised and lowered with KOH and HCl as indicated. Note that the E355Q substitution decreased the relative effect of cis pH on conductance.

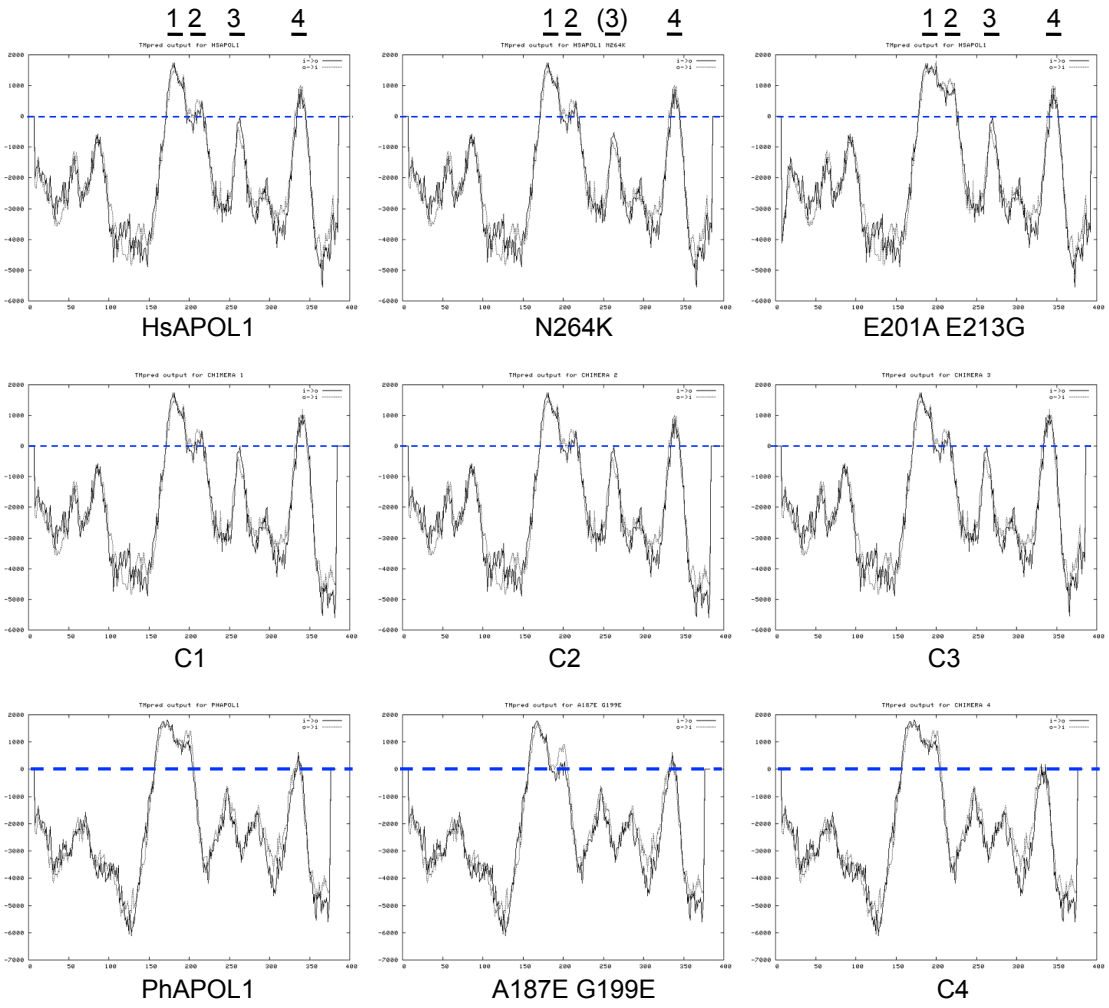

Figure S7. TMPred analysis of APOL1 proteins used in this study. Transmembrane propensity (y-axis) as calculated by TMPred server (https://embnet.vital-it.ch/software/TMPRED_form.html) is plotted from N to C-terminus (left to right). Putative positions of HsAPOL1 transmembrane helices (TM 1-4) are shown above the graphs. For purpose of comparison, a dashed blue line is shown at y=0 on each graph. Note that TM 3 of APOL1 N264K has a substantially lower transmembrane propensity than TM 3 of HsAPOL1

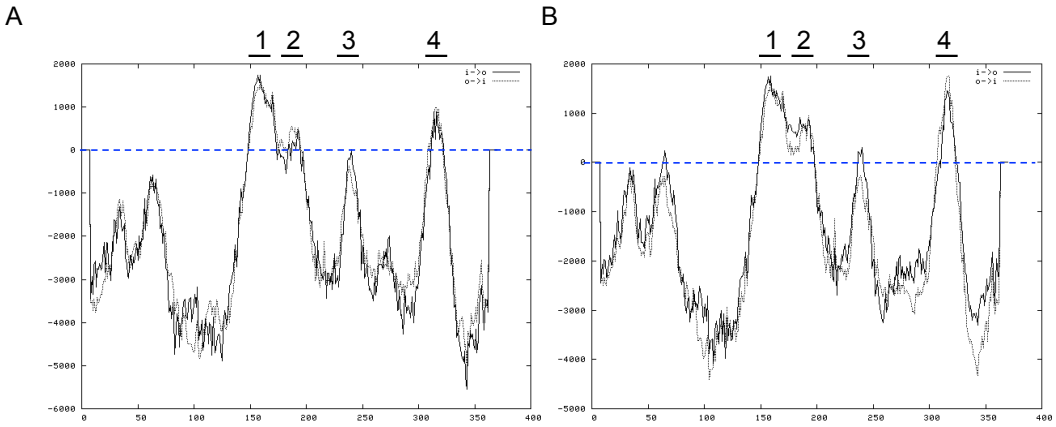

Figure S8. TMPred analysis. Transmembrane propensity (y-axis) as calculated by TMPred server (https://embnet.vital-it.ch/software/TMPRED_form.html) is plotted from N to C-terminus (left to right). Putative positions of HsAPOL1 transmembrane helices (TM 1-4) are shown above the graphs. (A) HsAPOL1. (B) Derivatized HsAPOL1, wherein all glutamates were replaced by glutamines and all aspartates were replaced by asparagines to simulate protonation of negatively charged residues at acidic pH. For purpose of comparison, a dashed blue line is shown at y=0 on each graph. Note that the transmembrane propensities of TM2, 3 and 4 are increased by the elimination of negatively charged residues.

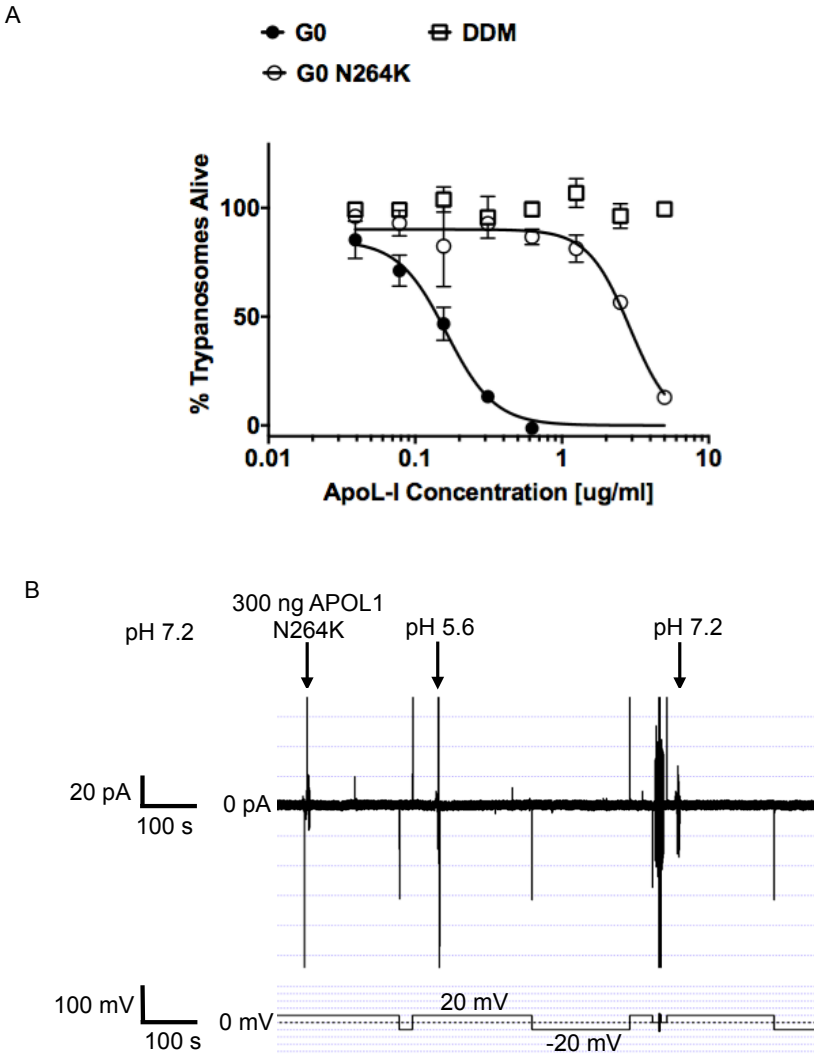

Figure S9. The N264K substitution results in reduced trypanosome killing and reduced channel forming activity. (A) Trypanosome viability is plotted after 24-hour exposure to increasing concentrations of HsAPOL1 (G0), APOL1 N264K (G0 N264K), or an equivalent concentration of vehicle (dodecylmaltoside, DDM). Plotted are the mean survival values of triplicate measurements +/- S.D., relative to untreated controls. The N264K mutation resulted in a ~20-fold increase in the IC50 relative to HsAPOL1. (B) A planar lipid bilayer was formed between symmetric solutions of chamber buffer, pH 7.2. The current was recorded (upper trace, pA), as the voltage was manipulated by the experimenter (lower trace, mV). After adding APOL1 N264K at cis pH 7.2 the cis side was adjusted to pH 5.6 with HCl, and then back to pH 7.2 with KOH. There was no detectable change in bilayer conductance.