# ABC transporters control ATP release through cholesterol-dependent volume-regulated anion channel activity

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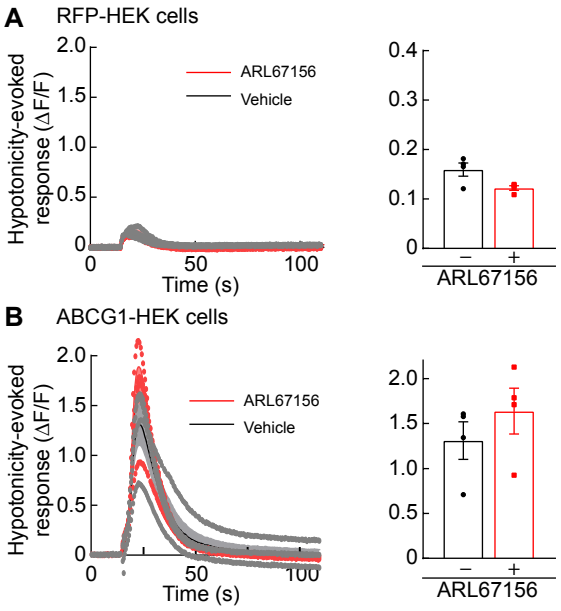


**Figure S1. The exonuclease inhibitor did not alter hypotonicity-induced calcium responses**
*A* and *B*, HEK cells transfected transiently with RFP (*A*) or ABCG1 (*B*) were preincubated with an exonuclease inhibitor, 100 µM ARL67156, and calcium responses were measured after hypotonic stimulation (final, 250 mmol/kg). The exonuclease inhibitor did not alter hypotonicity-induced calcium responses. Traces and quantifications of peak calcium responses (ΔF/F) with error bars are shown (n = 4). Data are mean ± standard deviation.

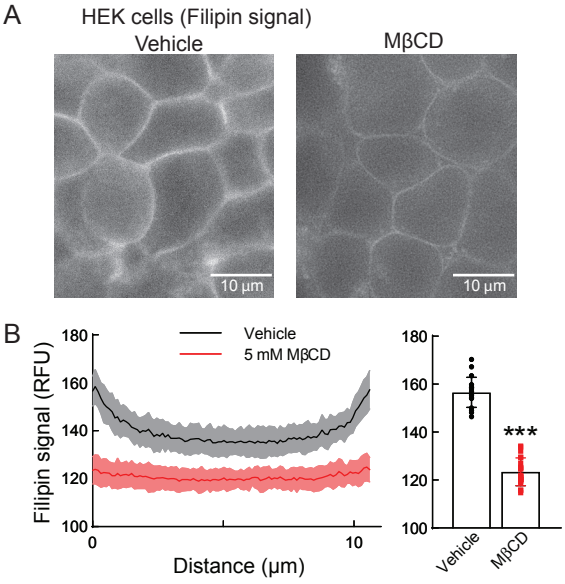


**Figure S2. MβCD treatment reduces filipin fluorescence intensity**
HEK293 cells were treated with 5 mM methyl-beta cyclodextrin (MβCD) for one hour at 37°C for cholesterol depletion. Filipin staining was performed using the cell-based cholesterol detection assay kit (Cayman Chemical) before images were taken using a DeltaVision microscope equipped with an oil Plan Apo N 60x/1.42 NA objective (Olympus). *A*, representative images of filipin fluorscence in HEK cells treated with vehicle or MβCD *B*, filipin fluorescence intensity was quantified by line scanning of individual HEK cells (18 cells from 3 experiments each). MβCD treatment reduced filipin signal. Data are mean ± standard deviation; unpaired t test (*B*); ***p < 0.001.

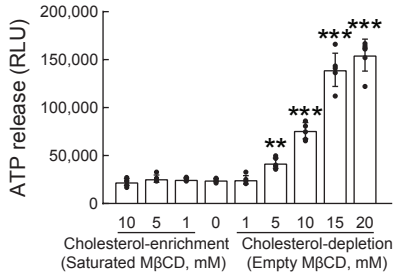


**Figure S3. The cellular cholesterol level controls ATP release**
ATP release induced by hypotonicity (final, 250 mmol/kg) from HEK cells incubated with various concentrations of methyl-beta-cyclodextrin (MβCD) for cholesterol depletion or cholesterol mixed with MβCD for cholesterol repletion (n=6). ). One-way ANOVA followed by Tukey's test with pair-wise comparisons made to vehicle alone (0 mM); $F(8,45) = 204.3$, $p < 0.001$. Data are mean ± standard deviation; *p < 0.05, ***p < 0.001

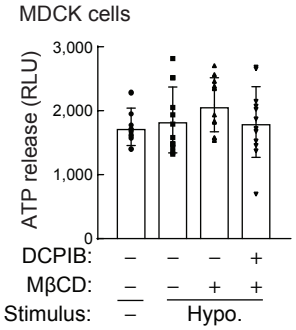


**Figure S4. MDCK cells did not release ATP upon hypotonic stimulation**
Extracellular ATP in the medium was measured from MDCK cells treated with hypotonic solution (final, 185 mmol/kg), 5 mM MβCD and 5 mM MβCD preincubated with a VRAC inhibitor (final, 20 μM DCPIB)(n=12). One-way ANOVA did not detect siginificant difference. Data are mean ± standard deviation.