Supplemental Information

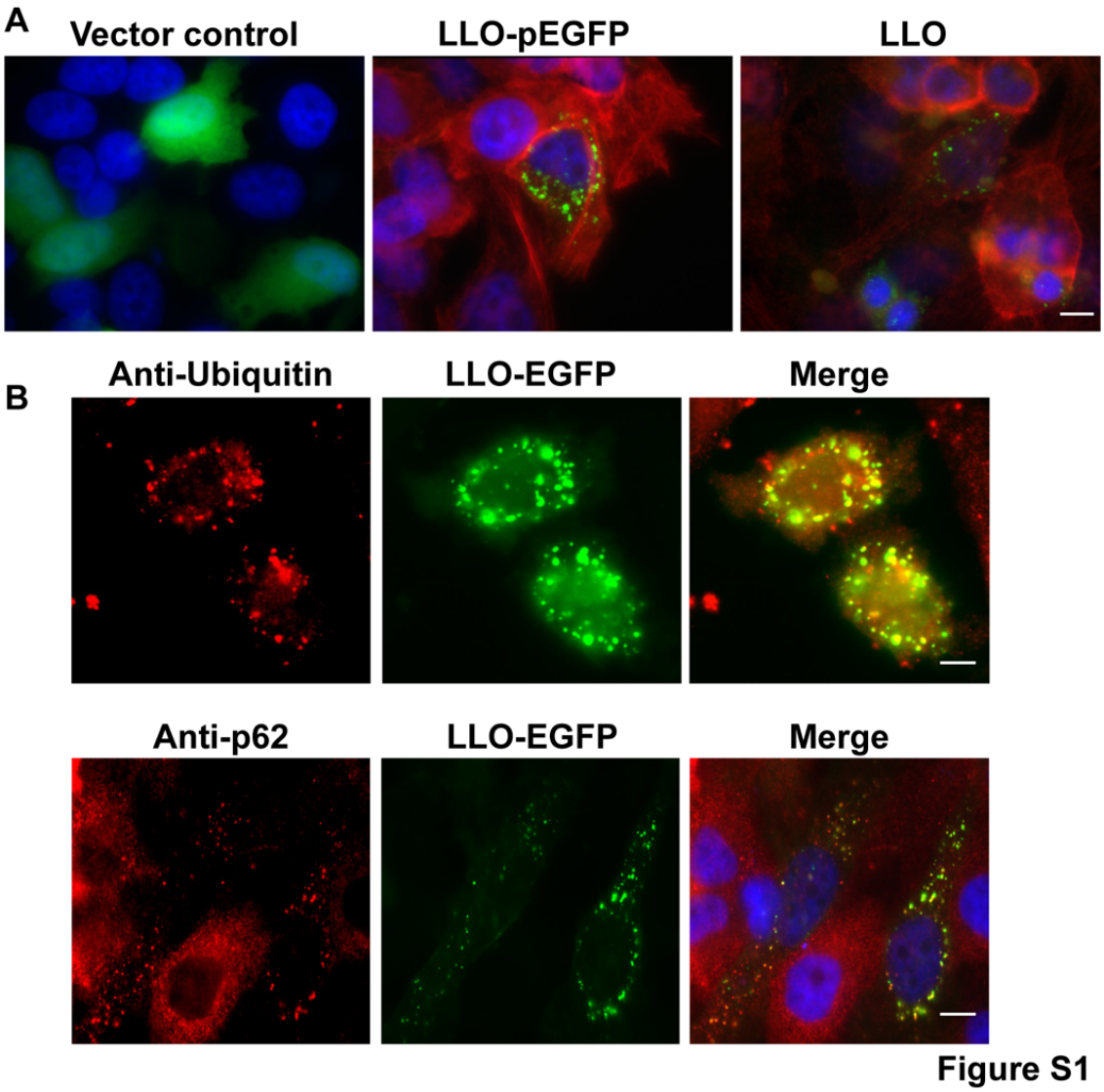

Figure S1. Related to Figure 1. Transient expression of LLO in HeLa cell formed punctate structure and colocalized with ubiquitin and p62 in HeLa cells.
(A) HeLa cells were transfected with LLO-pEGFP-N1 (LLO-EGFP) or LLO-pcDNA3 (LLO-no tag) plasmids for 24 hours. HeLa cells transfected with empty pEGFP-N1 plasmid were used as controls (left panel). Cells were fixed and stained with nuclear dye DAPI (blue), anti-LLO antibody (green) and rhodamine-labeled phalloidin for actin (red). (B) HeLa cells were transfected with LLO-pEGFP-N1 (LLO-EGFP) plasmids for 24 hours and stained with nuclear dye DAPI (blue), anti-Ubiquitin antibody (green) or anti-p62 antibody (green). Scale bars are 10 μm.

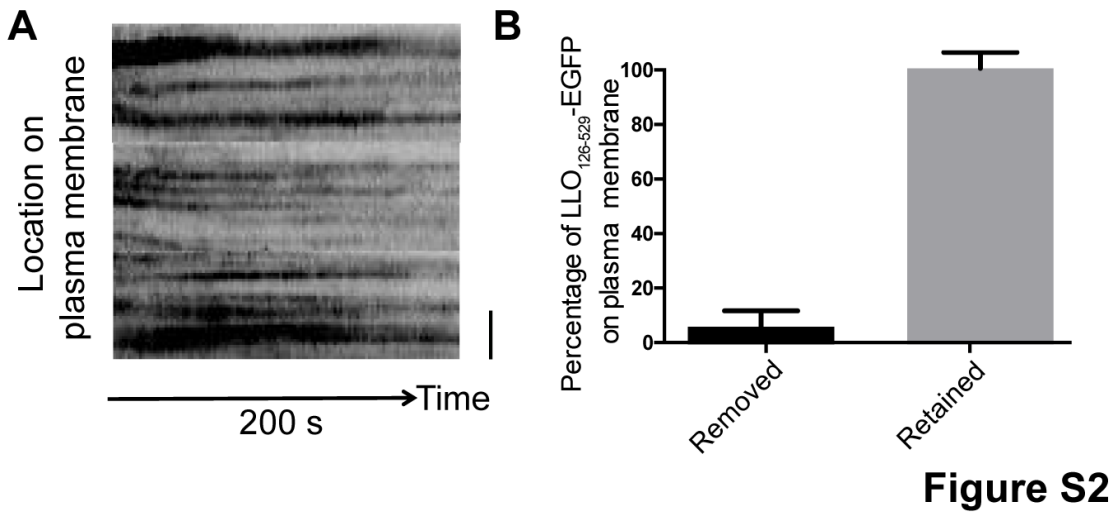

Figure S2. Related to Figure 2. N-terminal truncated $LLO_{126-529}$ cannot be efficiently removed from the plasma membrane in transfected cells.

(A) Representative kymograph from a time-series TIRF imaging of plasma membrane-associated $LLO_{126-529}$-EGFP. Bar, 1 μm. (B) The percentage of $LLO_{126-529}$-EGFP signal removed from plasma membrane within 540 seconds of time-series obtained using 100-ms exposures. (N=100 EGFP sites in 4 cells).

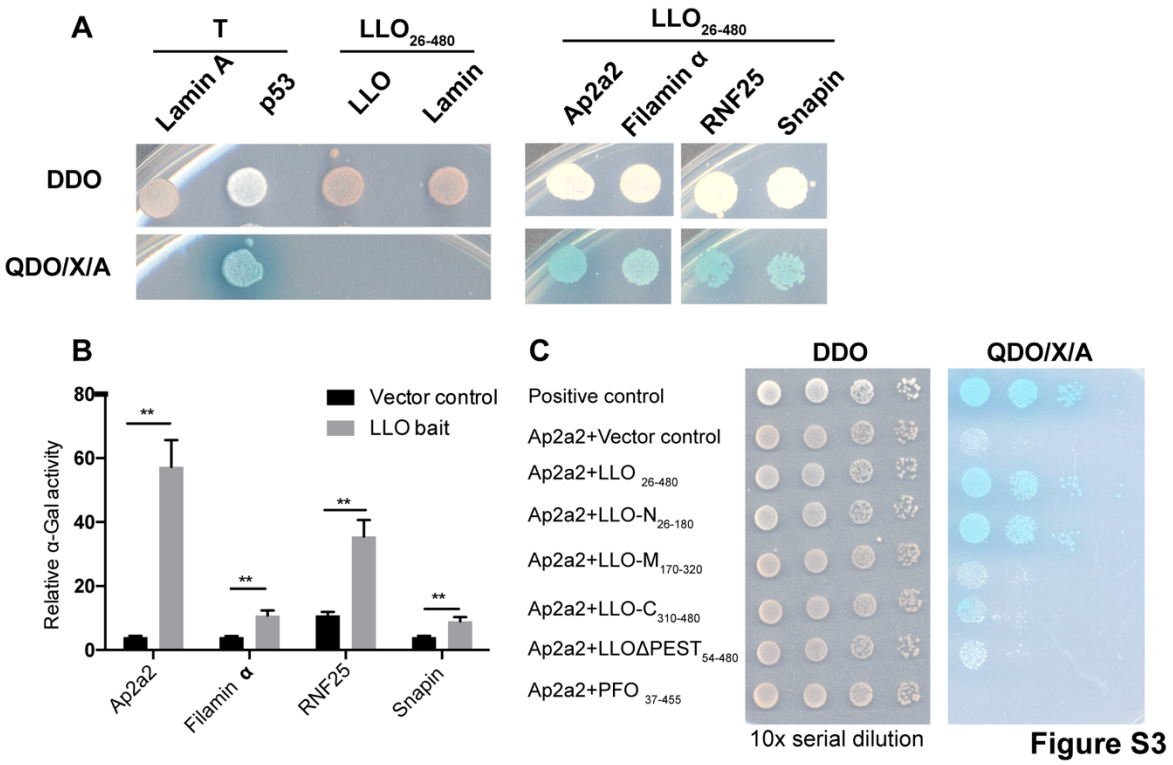

Figure S3. Related to Figure 3. Identification of Ap2a2 as the LLO PEST-like region interacting protein using yeast two-hybrid assay.

(A) Representative images on auxotrophic selecting plate following a GAL4-based two-hybrid interaction in *S. cerevisiae*. (B) Quantification of secreted alpha-galactosidase activity from the culture supernatant of Y2HGold yeast colonies. The negative control experiment was performed using pGBKT7-Lam (which encodes the Gal4 BD fused with lamin) as bait plasmid. The results represented the percentage of alpha-galactosidase activity of positive control (p53+ SV40 large T antigen). (C) Representative image on auxotrophic selecting plate following a GAL4-based two-hybrid interaction in *S. cerevisiae* that expressed Ap2a2 and different fragments of LLO or PFO.

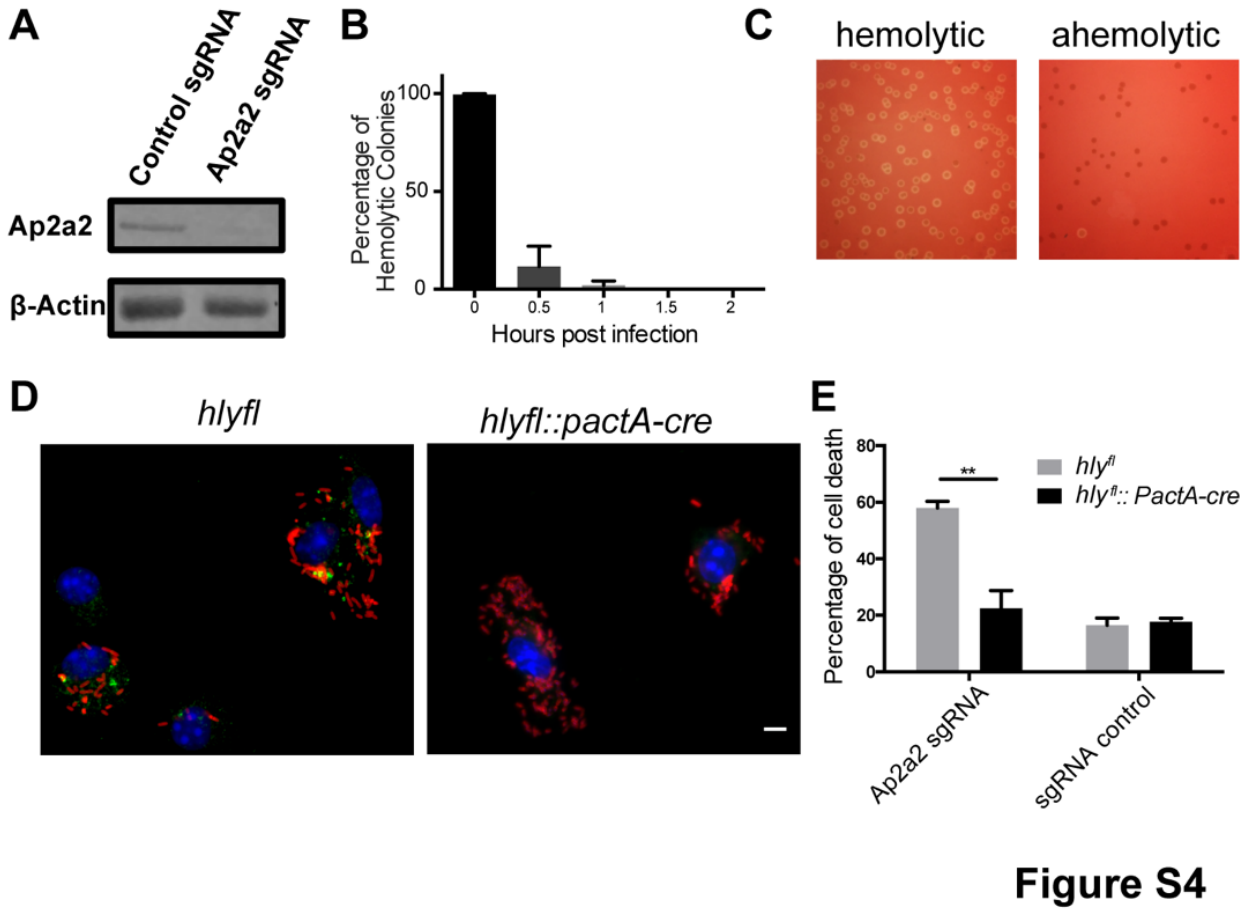

Figure S4. Related to Figure 4. Cytosolic deletion of *hly* gene rescued the cytotoxicity of *L. monocytogenes* in Ap2a2 knockdown BMMs.

(A) Western blot analysis of the efficiency of CRISPR/Cas9-mediated Ap2a2 knockout in $Cas9^{+}$ BMMs. (B) To quantify the excision efficiency of hly^{fl} during infection, BMMs were infected with hly^{fl} and lysed in water at different times post-infection. Both hemolytic and ahemolytic colonies recovered from BMMs were enumerated by plating bacteria on blood agar plates as excision of *hly* results in loss of hemolytic activity. (C) Representative images of hemolytic and ahemolytic colonies on blood agar plates. (D) Representative images of BMMs infected with hly^{fl} strain and hly^{fl}*::pactA-cre* strains for 5 hours. Cells were fixed and stained with nuclear dye DAPI (blue), anti-LLO antibody (green), and anti-*L. monocytogenes* antibody (red). Scale bar is 10 μm. (E) LDH release from BMMs infected with hly^{fl} control bacteria or hly^{fl} expressing the Cre recombinase at 8 hours post infection. Bars and error represent mean ± SD of replicate measurements. ** $p<0.01$ (Student's t test).

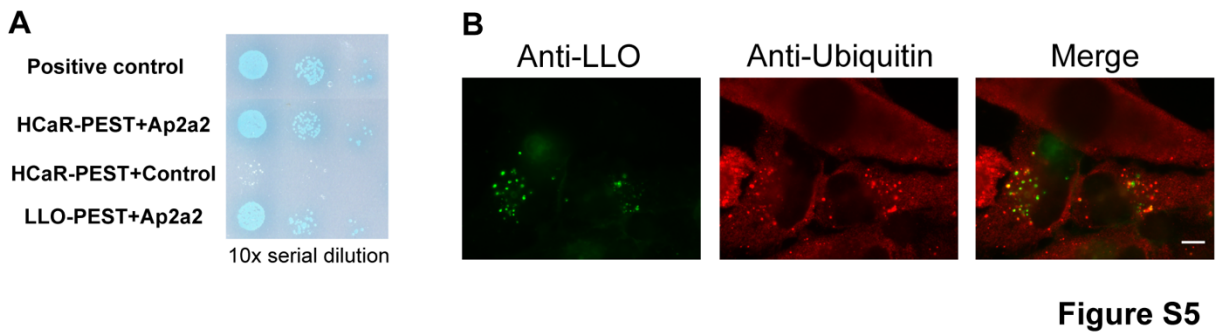

Figure S5. Related to Figure 5. The PEST-like sequence from human calcium receptor (HCaR-PEST) is sufficient to interact with Ap2a2 and promotes the formation of intracellular punctates in transfected HeLa cells.
(A) Representative Image of spotting assay on auxotrophic selecting plate following a GAL4-based two-hybrid interaction in *S. cerevisiae*. (B) HeLa cells were transfected with HCaR-LLO-pcDNA3 plasmids for 24 hours. Cells were fixed and stained with anti-LLO antibody (green) and anti-Ubiquitin antibody (red). Scale bars is 10 µm.

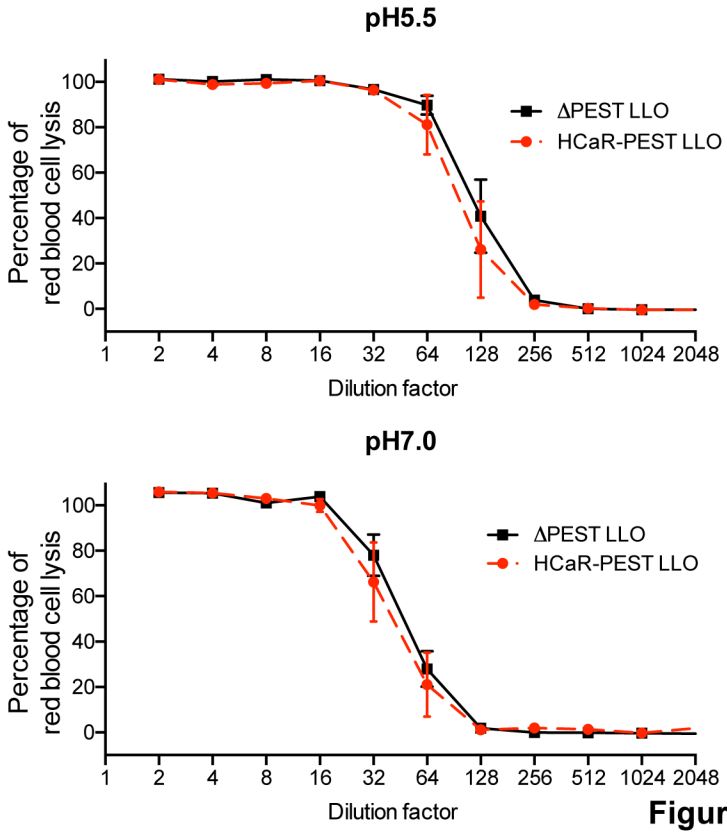

Figure S6. Related to Figure 5. Comparison the hemolytic activity of secreted PEST LLO and HCaR-PEST LLO.

Logarithmic phase bacterial culture supernatants were normalized to 1 at OD_{600nm}. Hemolytic activities of two-fold serial diluted supernatants were quantified at pH 5.5 and pH 7.0.