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SUPPLEMENTARY INFORMATION

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# VacA generates a protective intracellular reservoir for *Helicobacter pylori* that is eliminated by activation of the lysosomal calcium channel TRPML1

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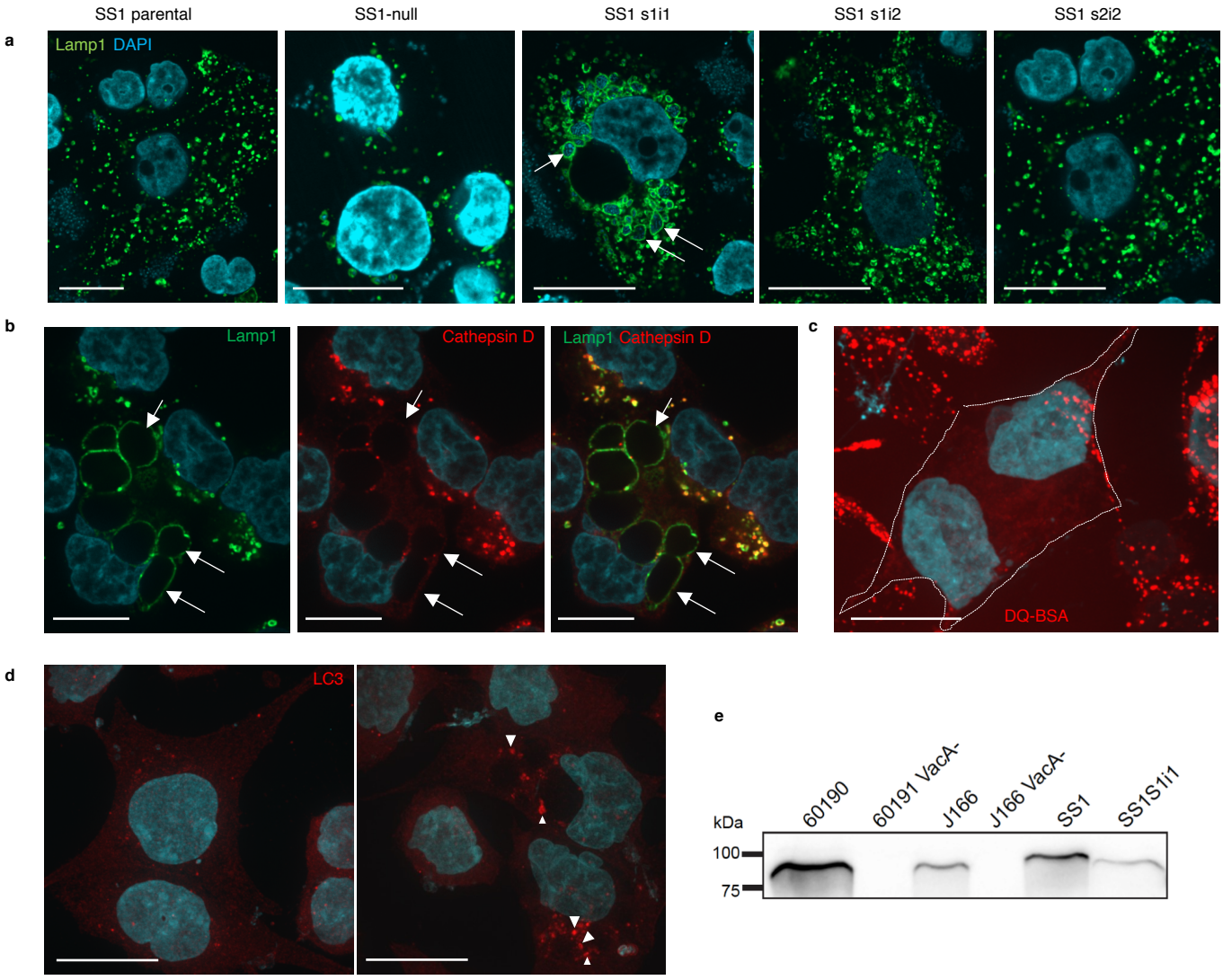
**VacA generates a protective intracellular reservoir for *Helicobacter pylori* which is eliminated by activation of the lysosomal calcium channel TRPML1**

**This file contains:**

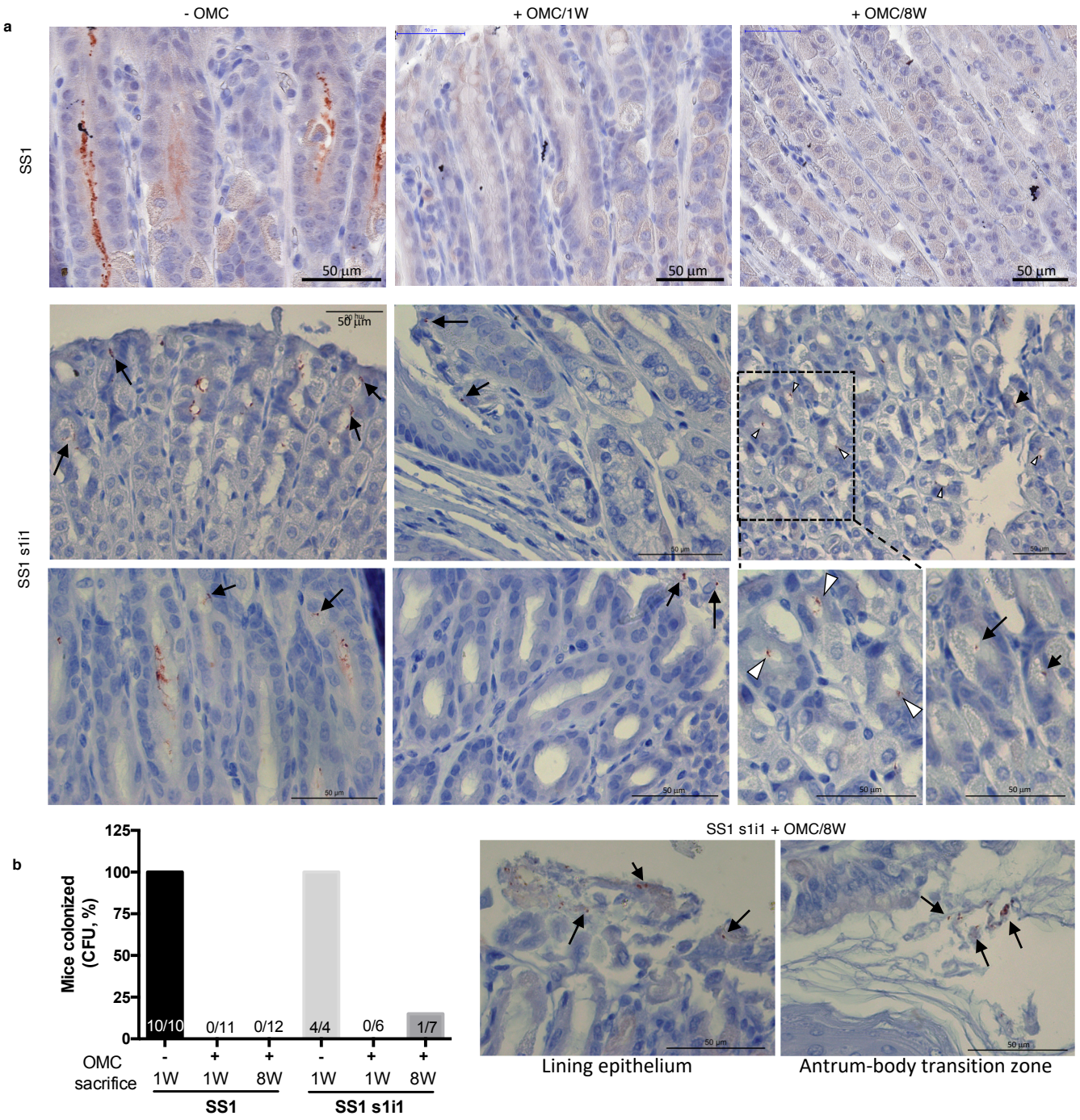
Supplementary Table 1, Supplementary Figures 1 – 19, uncropped whole blots of figures used in this study and Legends for the Supplementary Movies 1 and 2.

| Strain | VacA | | | CagA | Vacuolation |
|---|---|---|---|---|---|
| | s region | i region | m region | | |
| **60190** | s1 | i1 | m1 | Functional | ++ |
| **60190 VacA mutant** | - | - | - | Functional | - |
| **SS1** | s2 | i2 | m2 | Non-functional | - |
| **SS1 S1i1** | s1 | i1 | m2 | Non-functional | + |
| **J166** | s1 | i1 | m1 | Functional | + |
| **J166 VacA mutant** | - | - | - | Functional | - |

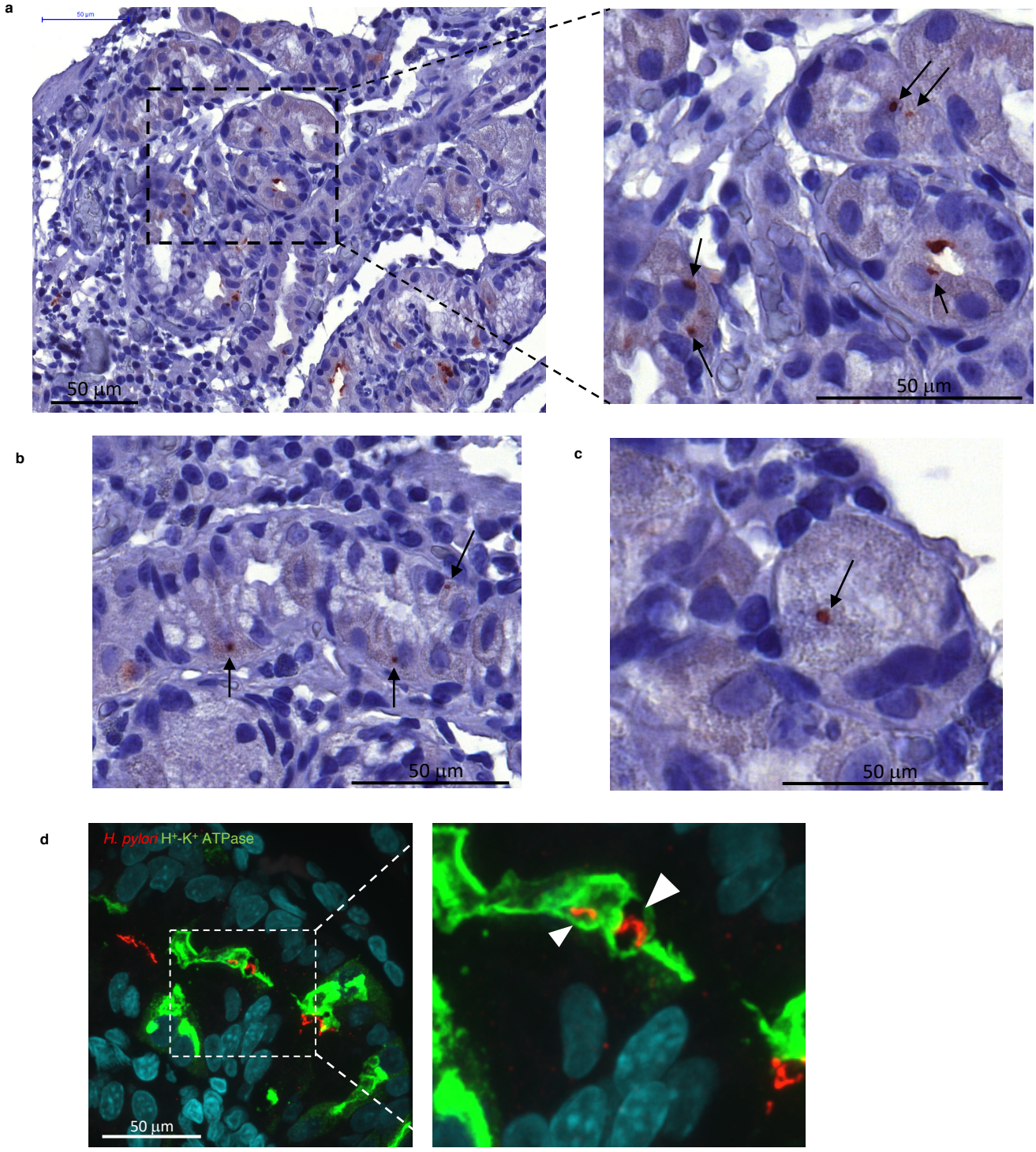
**Supplementary Table 1:** VacA genotype, cagA functionality and vacuolation capability information for the *H. pylori* strains used in this study.



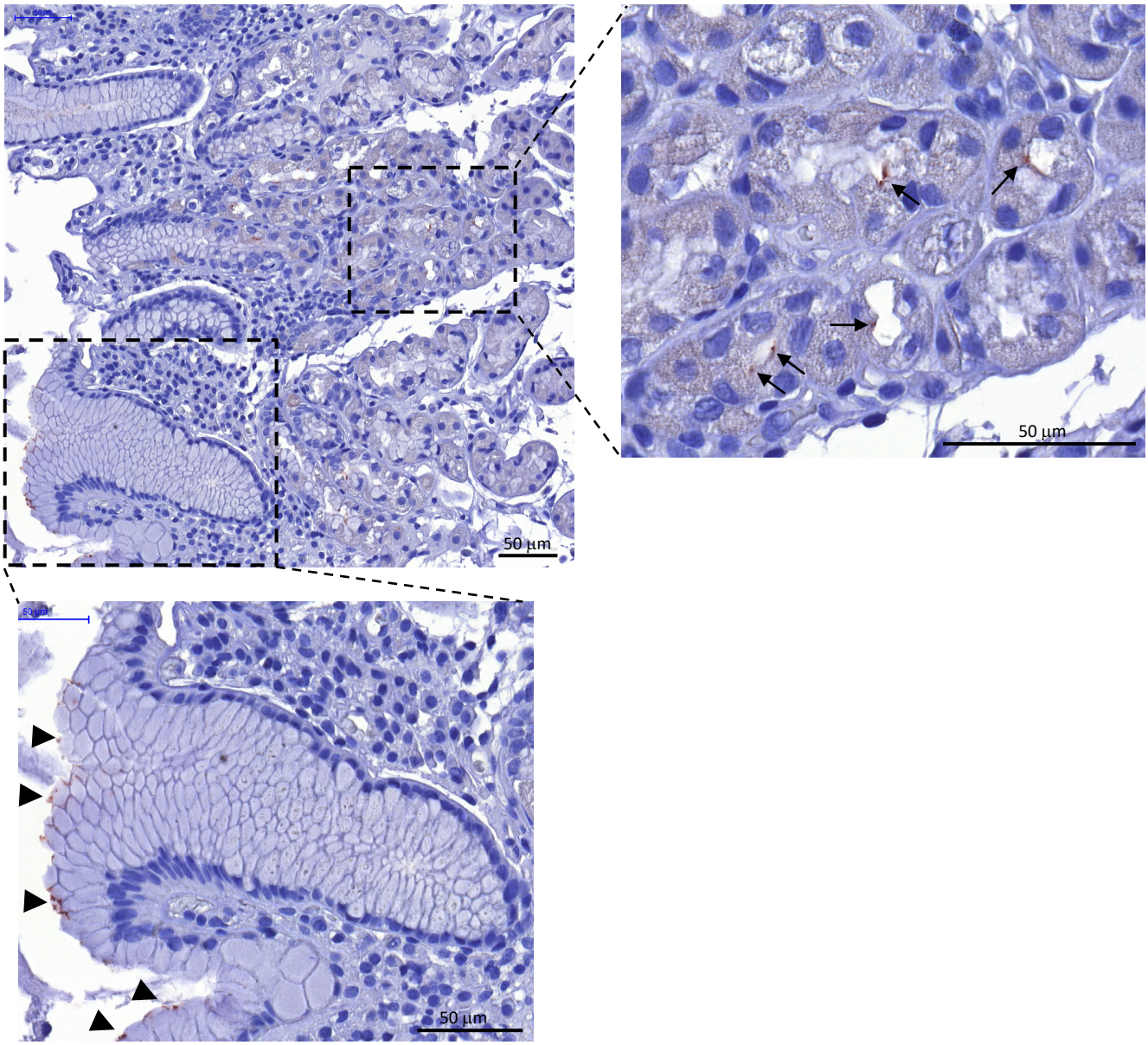

**Supplementary Figure 1: Validation for the presence of toxigenic VacA in VacA+ SS1 *H. pylori*. (a)** AGS cells infected with parental SS1 (s2i2) or genetically engineered SS1 expressing s1i1, s1i2, s2i2 or null for VacA *H. pylori* strains were fixed and subjected to Lamp1 and DAPI staining. Note the presence of big vacuoles containing bacteria in SS1 s1i1 infected cells only (arrows). **b-d** Lamp1 and cathepsin D staining (**b**), chromogenic protease substrate DQ-red-BSA (DQ-BSA) (**c**), and LC3 staining (**d**) of AGS cells after overnight incubation with culture supernatants from *H. pylori* SS1s1i1 strain. Note that SS1 s1i1 supernatant affects around 30% of AGS cells. The intoxicated cells display big vacuoles deprived of cathepsin D (**b, arrow**), non-degradative lysosomes (**c**) and autophagosome accumulation (**d, arrowhead**) as compared with unaffected neighbour cells. (**e**) VacA expression levels in culture supernatants from the indicated *H. pylori* strains. SS1 s1i1 toxigenic effect cannot be attributed to higher levels of VacA expression. Bars = 15 μm. Experiments were repeated a minimum of 3 times with similar results.



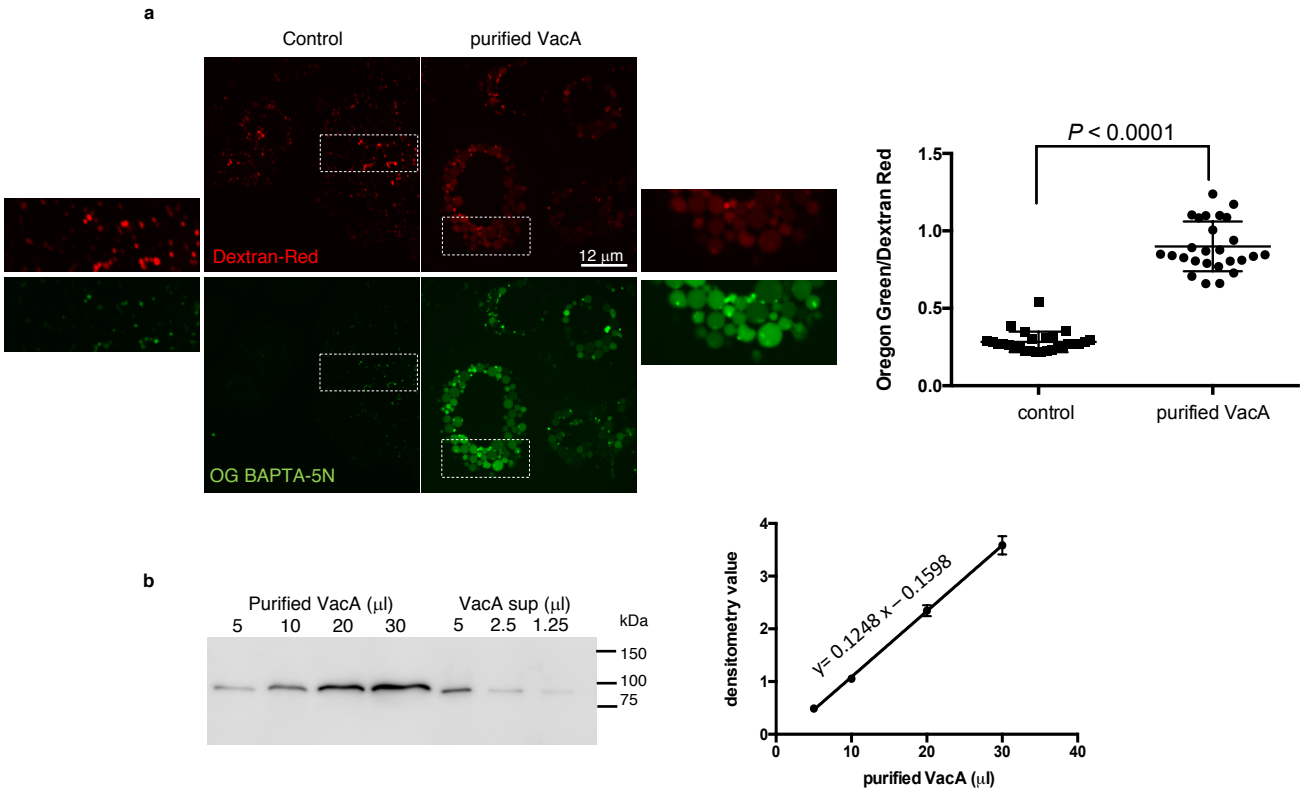

**Supplementary Figure 2: SS1 s1i1 *H. pylori* persist after eradication therapy.** Mice were infected with parental SS1 *H. pylori* (SS1) or isogenic SS1 s1i1 for 8 weeks, followed by 7-day standard eradication therapy consisting of omeprazole, clarithromycin and metronidazole (OMC). Mice were sacrificed 1 week (+OMC/1W) or 8 weeks (+OMC/8W) after OMC. Infected, no OMC treated control mice (-OMC) were sacrificed along with +OMC/1W. (**a**) Representative *H. pylori* immunohistochemistry of gastric mucosa dissected from mouse from the indicated groups. Arrows point to *H. pylori*. Higher magnification of the dashed area shows bacteria in the glandular lumens (white arrowhead). Immunohistochemistry was performed for all the animals with similar results. (**b**) Proportion of mice from which *H. pylori* was recovered. Numbers within the bars denote the ratio of *H. pylori* positive/total mice per group.



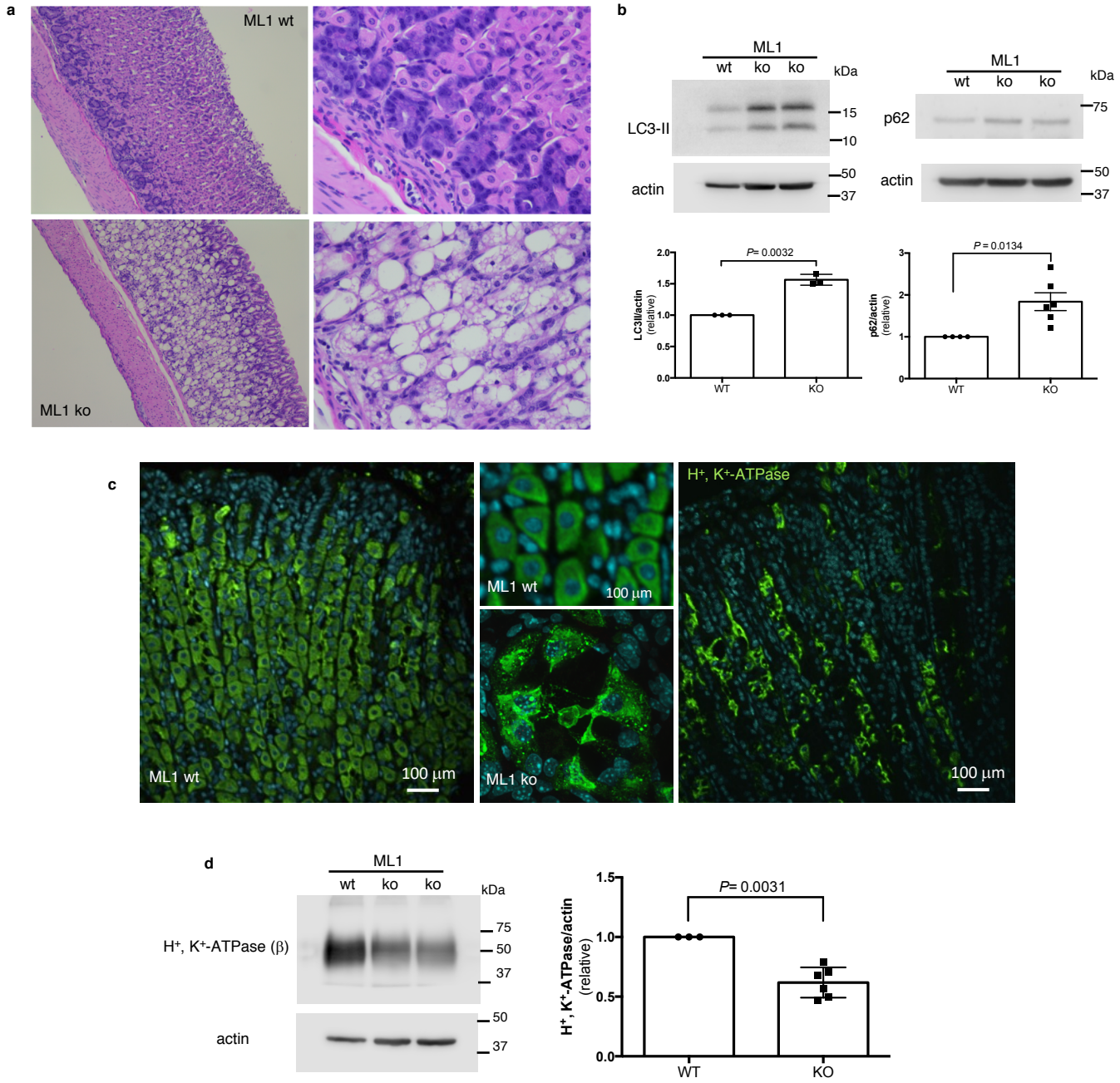

**Supplementary Figure 3: *H. pylori* strain expressing toxigenic VacA s1m1 colonize parietal cells.** *H. pylori* immunohistochemistry **(a-c)** or double *H. pylori*/$H^+$-$K^+$ ATPase immunofluorescence (**d**) on human corpus gastric biopsies from patients infected with VacA s1m1 strains of *H. pylori*. Higher magnification of the dashed areas are shown on the right. Arrows indicate intracellular bacteria in parietal cells. White arrowheads point to intravacuolar bacteria. Six biopsies were stained with similar results.



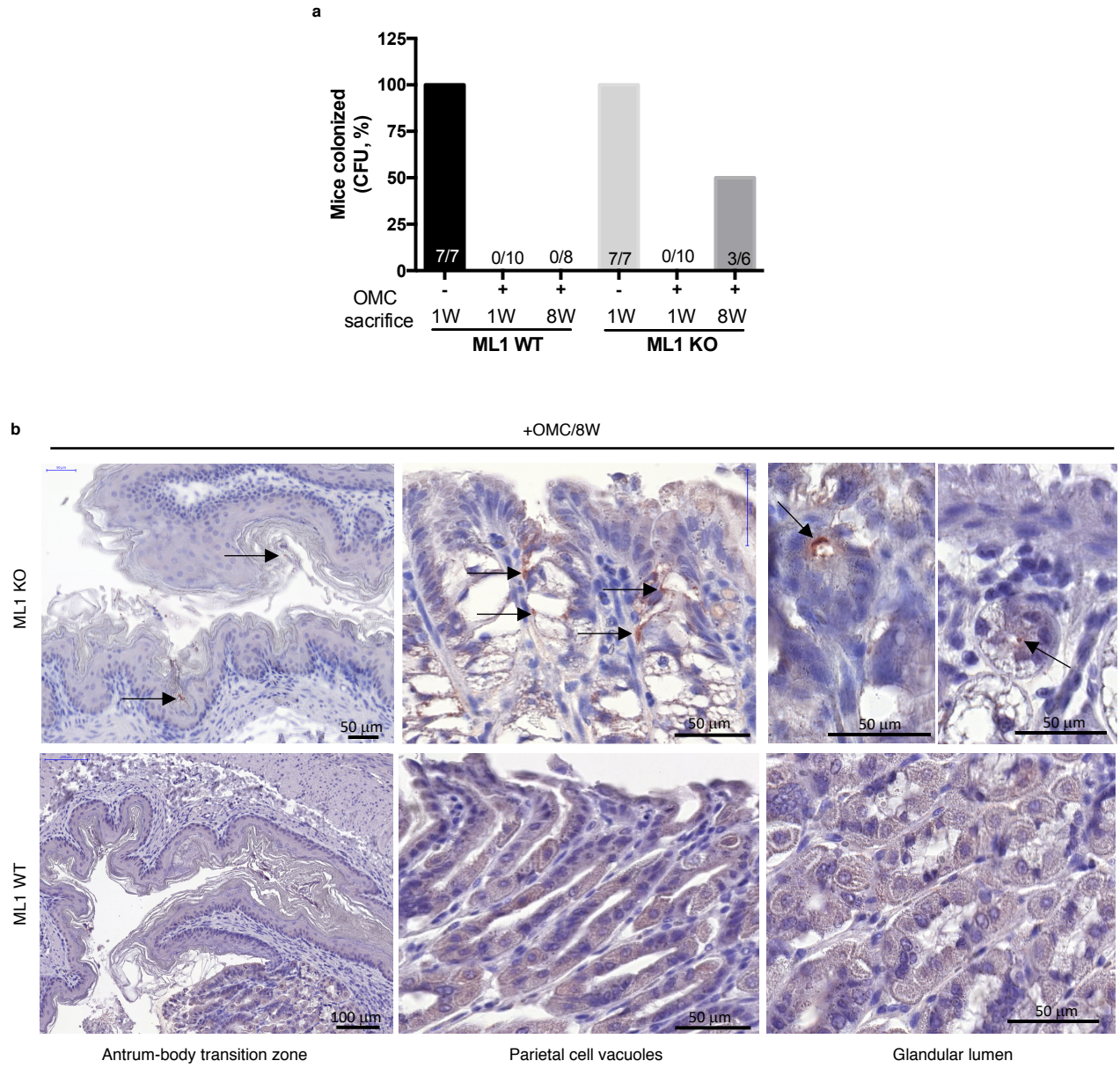

**Supplementary Figure 4: *H. pylori* immunohistochemistry on corpus gastric biopsies from patients infected with VacA s2m2 strains.** Higher magnification of the dashed areas show *H. pylori* within glandular lumen, attached to, but not invading parietal cells (arrows, right) or in the lining gastric epithelium (arrowheads, bottom). Six biopsies were stained with similar results.



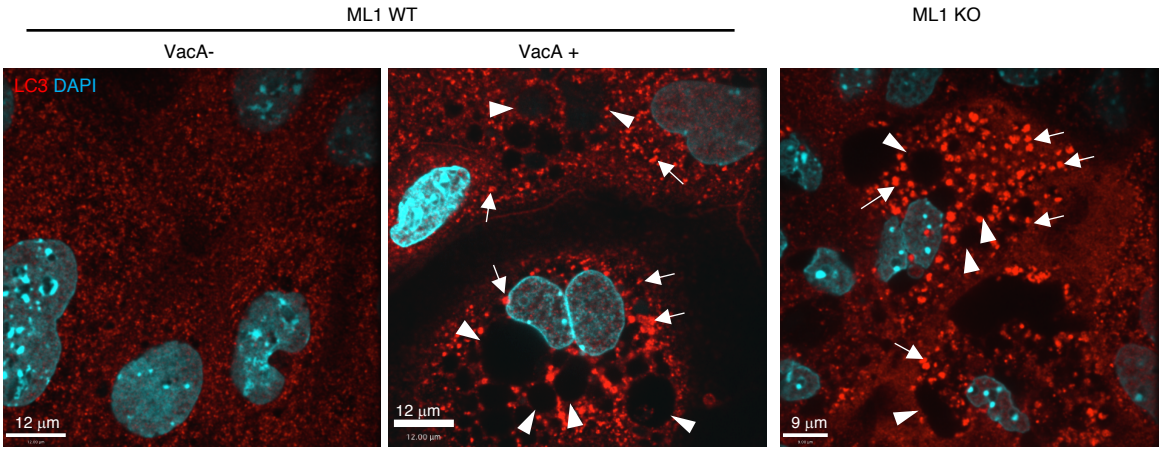

**Supplementary Figure 5: Purified VacA toxin is sufficient to induce lysosomal calcium accumulation. (a)** Intraluminal lysosomal $Ca^{2+}$ levels in AGS cells treated with purified VacA toxin or non-treated (control) cells. AGS cells were co-loaded with the calcium indicator Oregon Green 488 BAPTA-5N (OG BAPTA-5N), and Alexa Fluo586-conjugated dextran (Dextran-Red, as a Calcium-insensitive probe) by endocytosis. Representative images are shown with higher magnification of the dashed areas on each side. The fluorescence ratio between green and red signals was compared. Representative scatter plot (mean +/- SEM) of Oregon Green/Dextran Red intensity ratio of 23 fields containing 164 control cells and 26 fields containing 136 purified VacA treated cells is displayed on the right. Two-tailed unpaired t-test was utilized for data analysis. Experiment was repeated 3 times with similar result. **(b)** VacA western blotting comparing VacA levels present in purified VacA and VacA culture supernatant (VacA sup) containing media. Experiment was repeated twice with similar result. Bands were scanned and standard curve built based on densitometry values (mean +/- SD). Culture supernatant contains ~ 2 times more VacA.



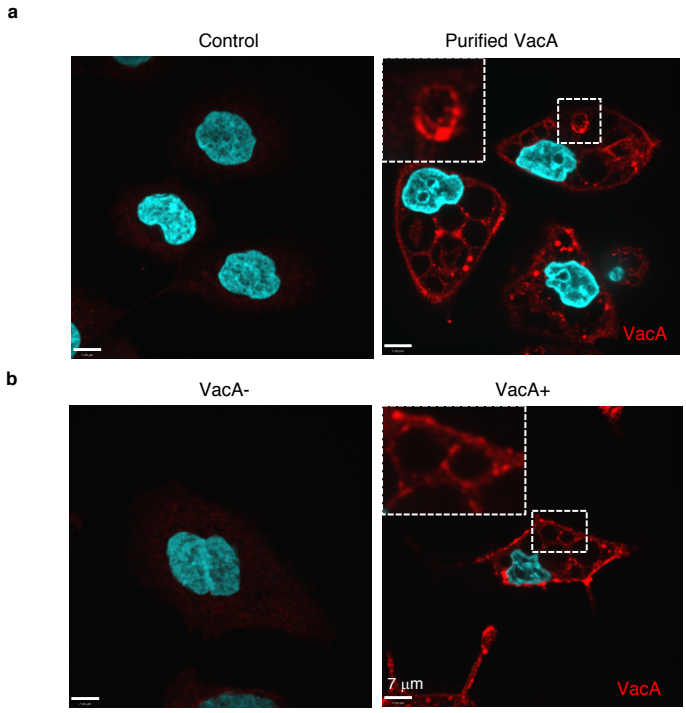

**Supplementary Figure 6: Characterization of the *trpml1*-/- gastric mucosa. (a)** H&E staining of gastric mucosa from 9-week-old trpml1 wild-type (ML1 wt) or *trpml1*-deficient (ML1 ko) mice. Higher magnification is shown on the right. (**b**) Western blotting of LC3 or p62 using actin as loading control in stomachs from 9-week-old mice. Graph show relative LC3-II or p62 normalized to actin (mean +/- SEM, n=3 wt, 3ko littermates for LC3; 4 wt, 6 ko for p62). (**c**) $H^+$, $K^+$-ATPase staining of gastric mucosa from 15-week-old ML1 wt or ML1 ko mice. Higher magnification images are shown in the middle panels. Note that vacuoles are restricted to parietal cells and that $H^+$, $K^+$-ATPase display miss-localization and reduced levels in ML1 ko gastric mucosa. **(d)** Western blotting of $H^+$, $K^+$-ATPase, β–subunit using actin as loading control in stomachs from 9-week-old mice. Graph shows relative quantification of $H^+$,$K^+$-ATPase normalized to actin (mean +/- SEM of n=4 wt, 6 ko littermates). All experiments were independently repeated 3 times with similar results. Two-tailed unpaired t test was used for data analysis.



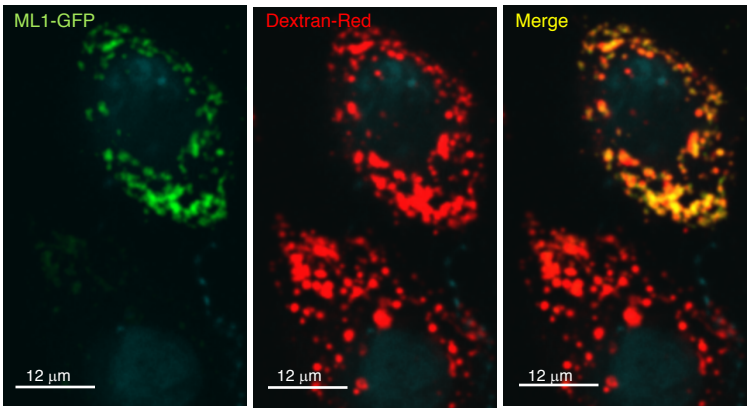

**Supplementary Figure 7: SS1 *H. pylori* persist after eradication therapy in TRPML1 KO stomach.** Wild-type (ML1 WT) or *trpml1*-knockout (ML1 KO) littermate mice were infected with SS1 *H. pylori* for 8 weeks, followed by 7-day eradication therapy (OMC) and sacrificed 1 week (+OMC/1W) or 8 weeks (+OMC/8W) after OMC. Infected, no OMC treated control mice (-OMC) were sacrificed along with +OMC/1W. (**a**) Proportion of mice from which *H. pylori* was recovered. Numbers within the bars denote the ratio of *H. pylori* positive/total mice per group. (**b**) Representative *H. pylori* immunohistochemistry of gastric mucosa dissected from ML1 WT and KO mouse of the +OMC/8W groups. Arrows point to the presence of *H. pylori* in the antrum-body transition zone, in parietal cell vacuoles, and in glandular lumen of the KO mice. Immunohistochemistry was performed for all the animals with similar results.



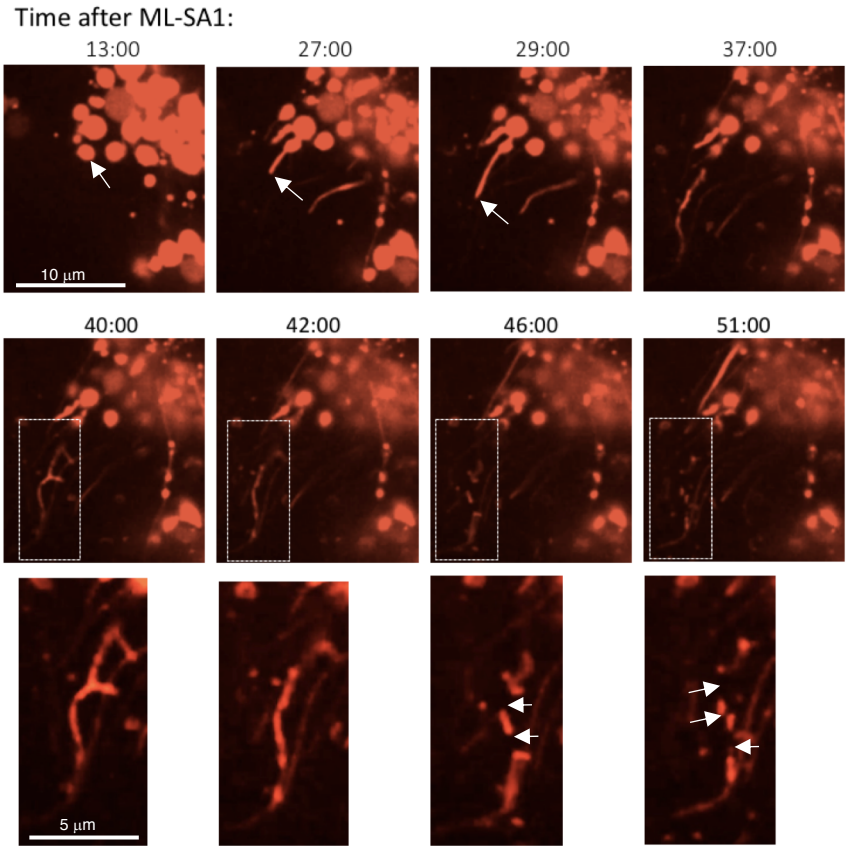

**Supplementary Figure 8: ML1 KO-derived gastric organoids phenocopy VacA-toxigenic effects.** LC3 and DAPI staining of gastric organoids monolayers derived from wild-type (ML1 WT) or *trpml1*-null (ML1 KO) mice. ML1 WT-derived organoids were treated overnight with VacA- or VacA+ culture supernatant prior fixation and staining. Note that ML1 KO monolayers display vacuolation (arrowheads) and accumulation of autophagosomes (arrows) similarly to VacA+ treated wild type organoids. Experiment was repeated 3 times with organoids isolated from independent ML1 WT and ML1 KO littermates.



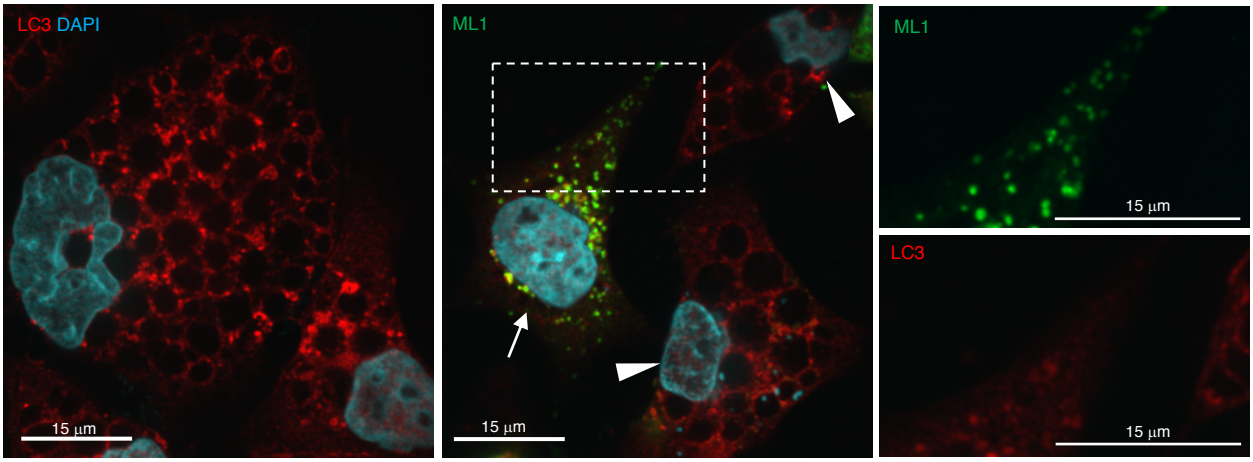

**Supplementary Figure 9: VacA treatment generates VacA-containing vacuoles.** VacA staining of AGS cells after overnight treatment with purified VacA (a) or VacA- or VacA+ culture supernatant (b). Higher magnification of the dashed area shows VacA-induced vacuoles delineated by VacA. Experiment was independently repeated 3 times with similar results.



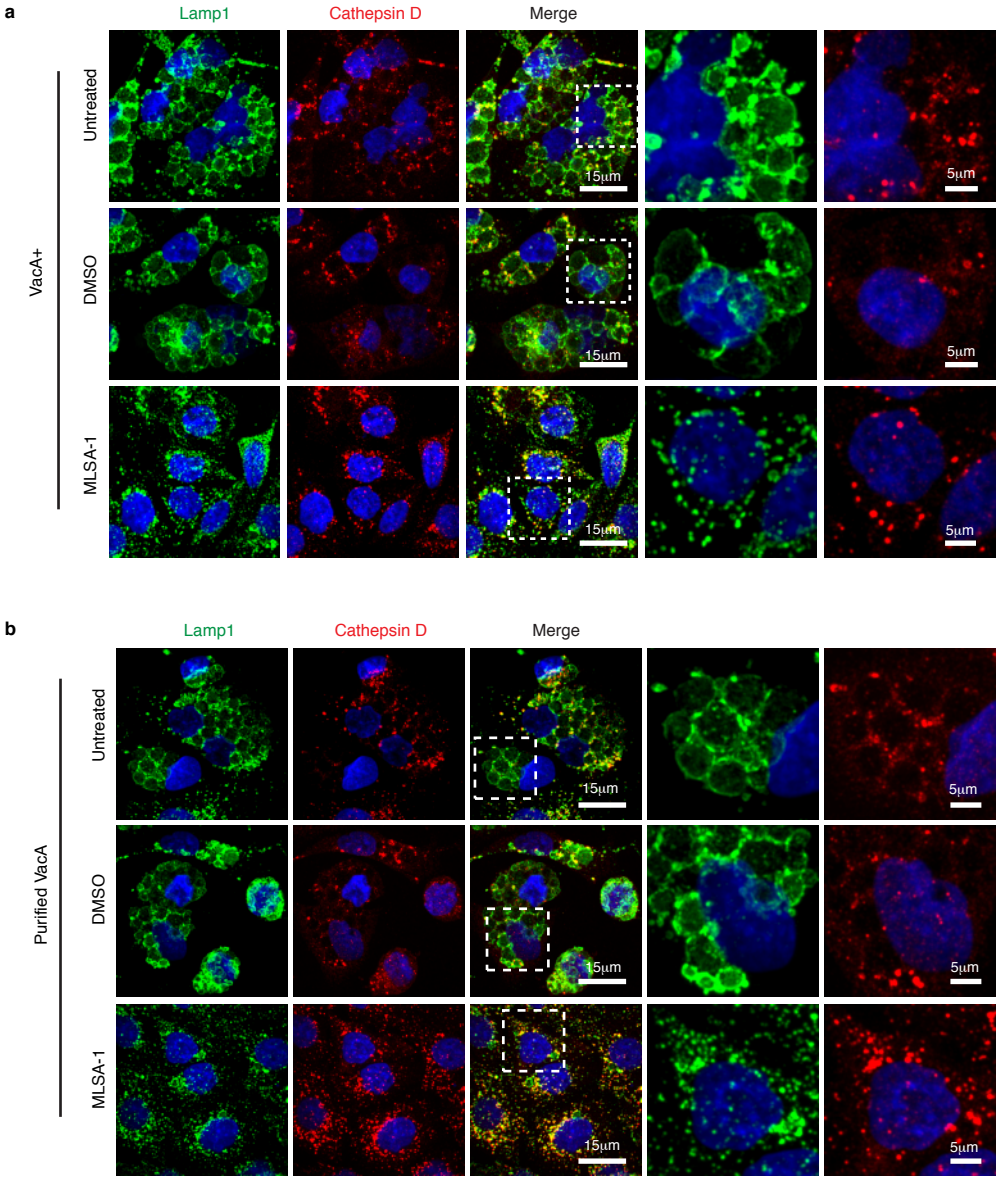

**Supplementary Figure 10: Exogenous ML1-GFP localizes to lysosomes.** To confirm the proper localization of exogenously expressed TPMRL1-GFP (ML1-GFP) in the lysosomes, ML1-GFP transfected AGS cells were loaded with Alexa Fluo586-conjugated 10 kDa Dextran (Dextran-Red) for 3 h and chased overnight before fixation and imaging. Yellow in merge panel indicates co-localization. Representative images are shown. Experiment was repeated 3 times with similar result.



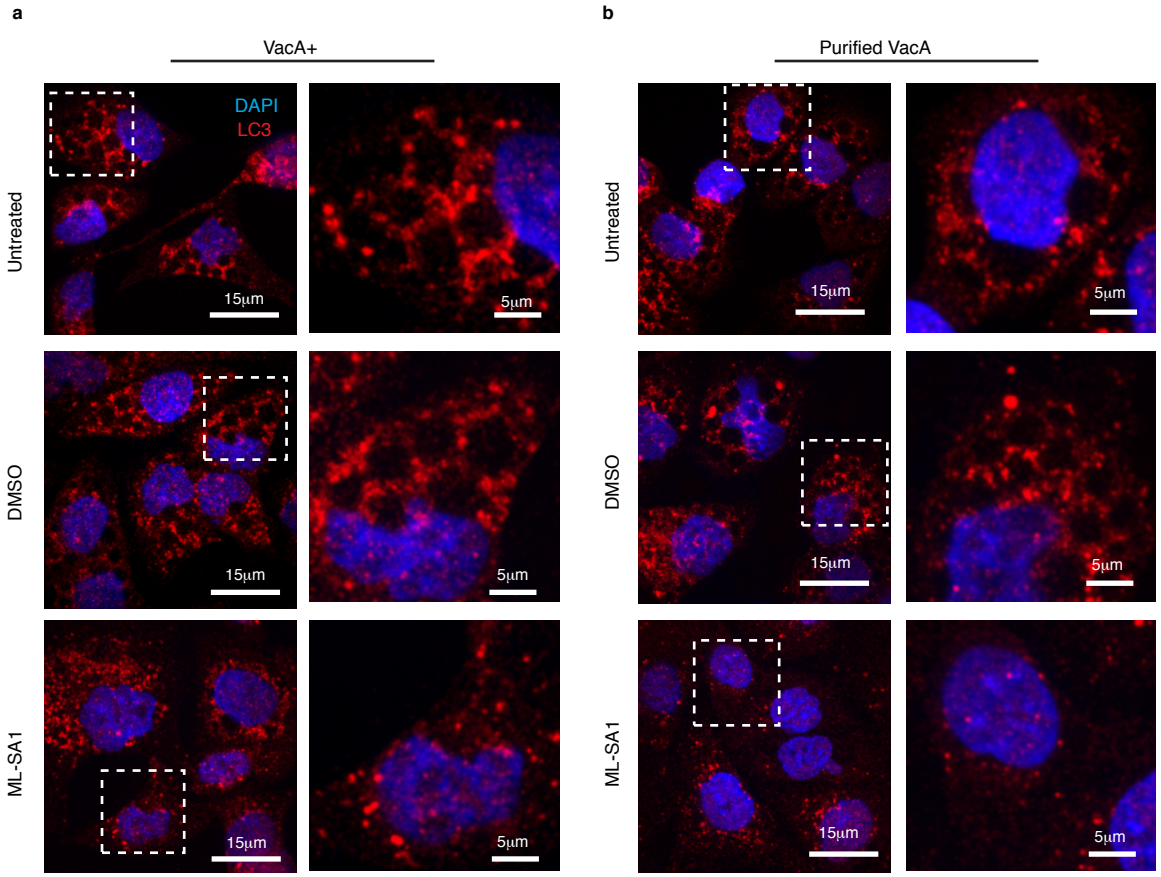

**Supplementary Figure 11: ML-SA1 administration to VacA+ treated AGS cells triggers vacuole tubulation and tubule fragmentation.** AGS cells were loaded with Alexa Fluo586-conjugated Dextran (10 kDa) for 2 h and chased in the presence of VacA$^{+}$ culture supernatant for an additional 3 h period. Growth media was replaced with live imaging buffer containing ML-SA1 (20 μM). Live imaging (8 frames/minute) was performed after the addition of ML-SA1. Images corresponding to the indicated times (in minutes) after ML-SA1 addition are shown. Arrows indicate vacuole tubulation and fragmentation. DMSO (vehicle) administration did not induce any change in the vacuolar size (data not shown). Similar movies were obtained from 3 independent experiments.



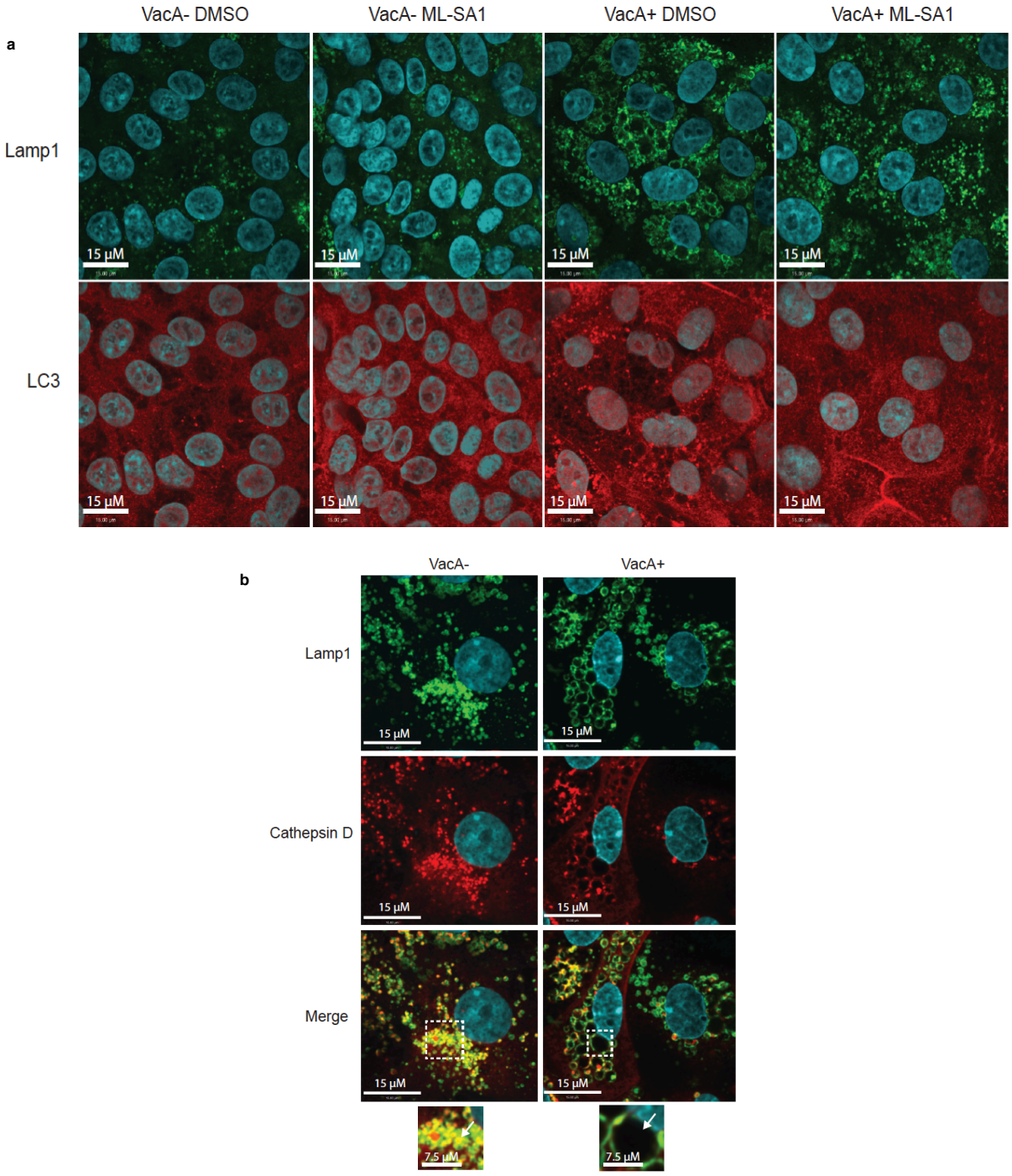

**Supplementary Figure 12: TRPML1 overexpression restores autophagosome maturation.** LC3 staining of control (mock transfected, left panel) or TRPML1-GFP (ML1) transfected AGS cells, after overnight VacA+ culture supernatant treatment. Higher magnification of the dashed area showing the separate channels on the right. Arrow points to ML1-transfected cell. Note that cell overexpressing TRPML1 does not form large vacuoles or accumulates autophagosomes in response of toxigenic VacA, an effect not observed in the untransfected neighbour cells (arrowheads). Experiment was performed 2 independent times with similar results.



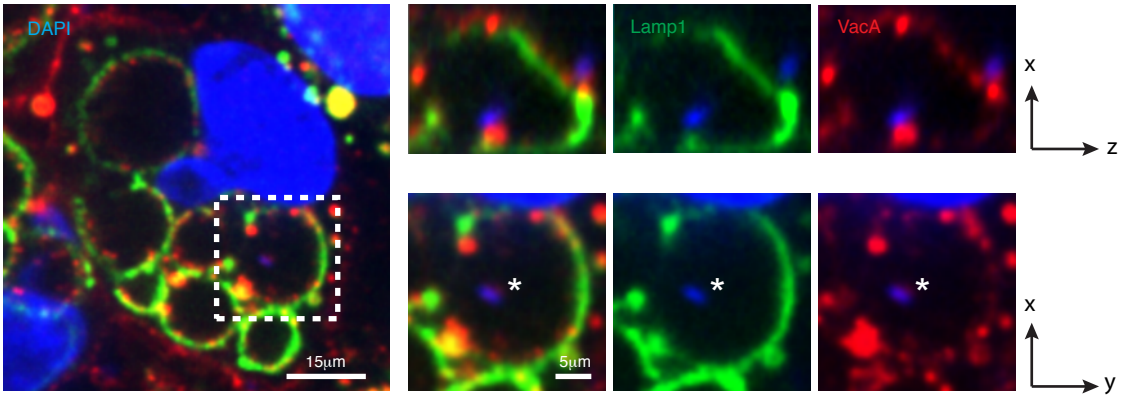

**Supplementary Figure 13: Purified VacA toxin is sufficient to disrupt endolysosomal trafficking.** Lamp1 and Cathepsin D staining of AGS cells after overnight treatment with VacA- or VacA+ culture supernatant (a) or purified VacA (b), followed by 4 h ML-SA1 (20μM) or vehicle (DMSO) treatment. Higher magnification of the dashed area, showing the separate channels on the right. Experiment was performed 3 times with similar results.



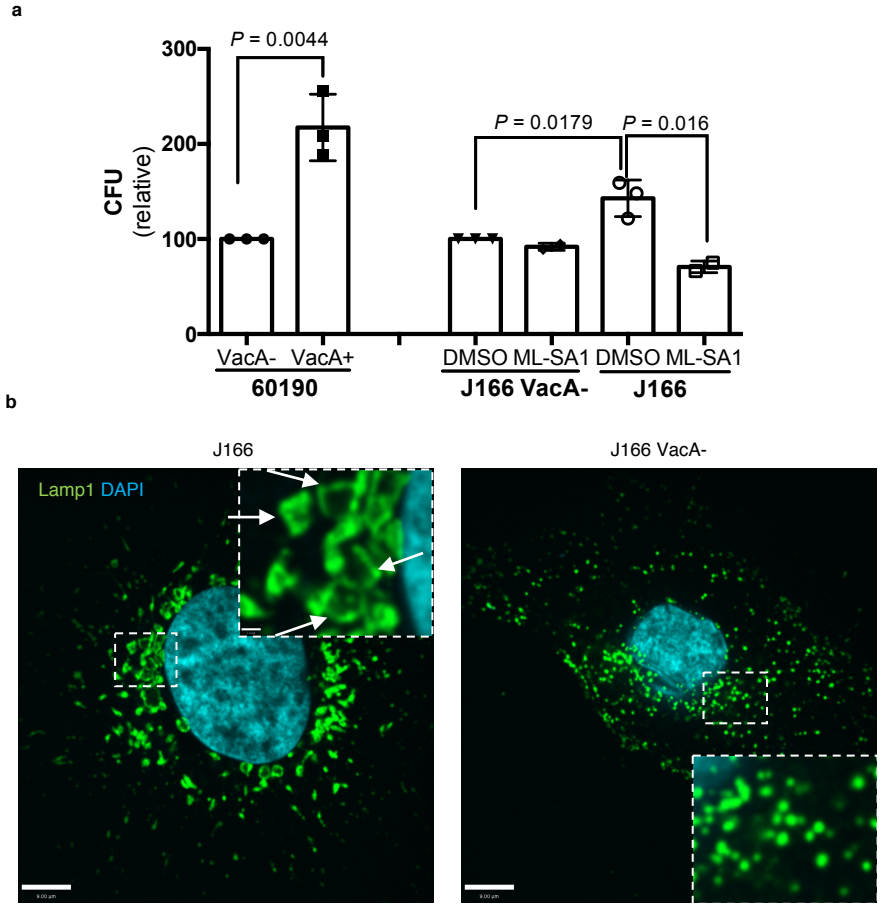

**Supplementary Figure 14: Purified VacA toxin is sufficient to disrupt autophagosome maturation.** LC3 staining of AGS cells after overnight treatment with VacA- or VacA+ culture supernatant (a) or purified VacA (b), followed by 4 h ML-SA1 (20μM) or vehicle (DMSO) treatment. Higher magnification of the dashed area on the right. Experiment was performed 3 times with similar results.



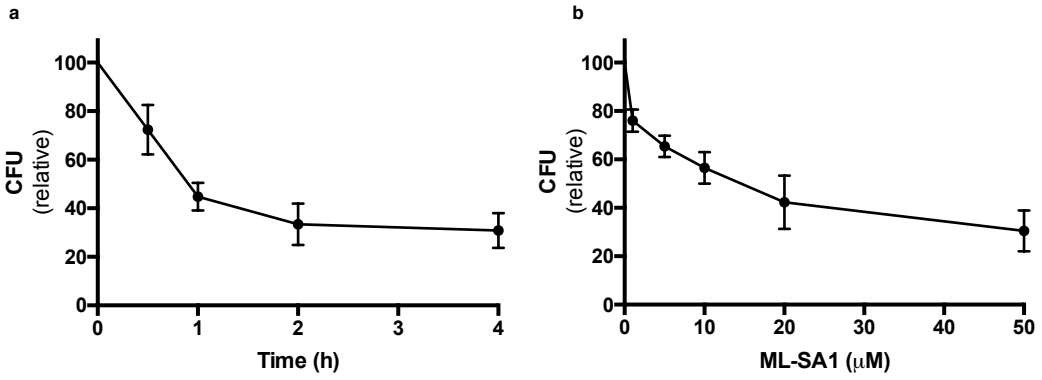

**Supplementary Figure 15: Validation of the VacA-TRPML1 axis in human gastric organoids.** Lamp1 and LC3 staining in gastric organoid monolayers treated with VacA- or VacA+ supernatants, followed by 4h ML-SA1 (20 µM) or DMSO (vehicle) administration. Note the presence of large vacuoles and LC3 puncta in cells treated with VacA+. (**b**) Cathepsin D and Lamp1 staining in organoid monolayers treated as in (a). Note large vacuoles devoid of cathepsin D in cells treated with VacA+ (arrow, inset). Experiment was performed with organoids isolated from 3 different patients with similar results.



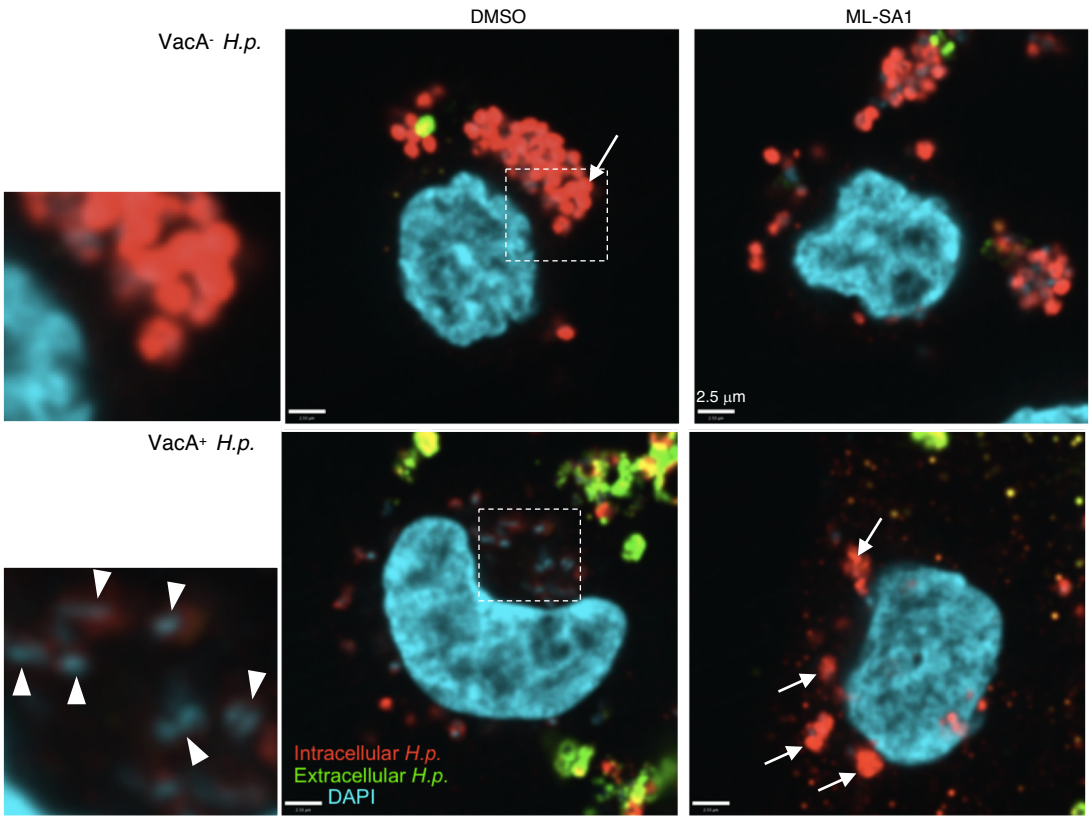

**Supplementary Figure 16: VacA+ 60190 *H. pylori* resides within VacA-containing vacuoles in infected AGS cells.** Lamp1 and VacA staining of AGS cells infected with *H. pylori* 60190 strain, that produces high levels of the toxigenic VacA. Higher magnification of the dashed area in the xz and xy planes showing the separate channels on the right. Note the presence of bacteria (*) inside VacA and Lamp1 delineated vacuoles. Experiment was performed 3 times with similar results.




**Supplementary Figure 17: TRPML1 activation eliminates survival advantage of VacA+ *H. pylori* J166 strain.** AGS cells infected with wild-type (VacA+) or isogenic VacA-null mutant (VacA-) 60190 and J166 strains were incubated with gentamycin to kill extracellular bacteria and treated with ML-SA1 (20 μM) or vehicle (DMSO) for additional for 4 h. (**a**) Intracellular *H. pylori* were retrieved and CFU quantified. Graph shows relative percentage of CFU (mean +/- SEM of 3 different experiments), considering the bacteria retrieved from VacA- infected, DMSO-treated AGS cells as 100%. Two-tailed unpaired t test was used for data analysis. (**b**) Lamp1 staining of cells infected with wild-type J166 or VacA- isogenic mutant (J166 VacA-). Higher magnification of the dashed area show the intracellular niche (vacuoles) generated by J166 infection (arrows). Experiment was repeated 3 times with similar results.




**Supplementary Figure 18: Time course and dose-dependence ML-SA1 effect on VacA+ *H. pylori* intracellular survival.** AGS cells infected overnight with wild-type (VacA+) 60190 strain were incubated with gentamycin to kill extracellular bacteria and treated with ML-SA1 (20 μM) for the indicated time points (**a**) or different ML-SA1 concentrations for 4 h (**b**). Intracellular *H. pylori* were retrieved and CFU quantified. Graph shows relative percentage of CFU (mean +/- SEM of 4 (**a**) or 3 (**b**) independent experiments), considering the bacteria retrieved from non-treated control AGS cells as 100% .




**Supplementary Figure 19: TRPML1 activation eliminates VacA+ *H. pylori* generated intracellular niche.** AGS cells infected with wild-type (VacA+ *H.p.*) or isogenic VacA-null mutant (VacA- *H.p.*) 60190 strain were incubated with gentamycin to kill extracellular bacteria and treated with ML-SA1 (20 µM) or vehicle (DMSO) for additional for 4 h. Intracellular (red) and extracellular (green-yellow) *H. pylori* staining. Higher magnification of the selected dashed areas are included on the left. Note the lysosomal-like intracellular staining pattern obtained with VacA- *H. pylori* infection that is being degraded by the host cell (arrows) in contrast to the discrete viable bacteria detected inside the vacuoles in VacA+ *H. pylori* infection (arrowhead). ML-SA1 treatment of VacA+ *H. pylori* infected cells produce intracellular staining that resembles the one obtained for the VacA- bacteria (arrows). Experiment was repeated 3 times with similar result.

Figure 4C

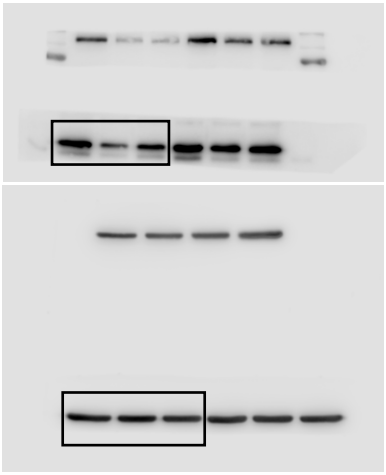


Figure 4i

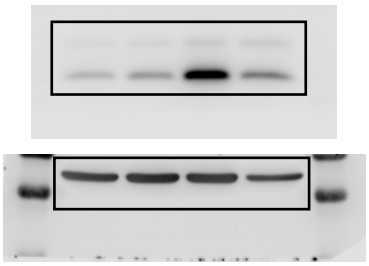


Figure 5c

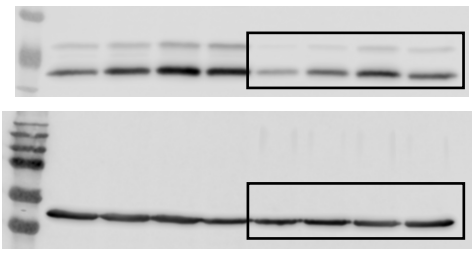


Figure 6c

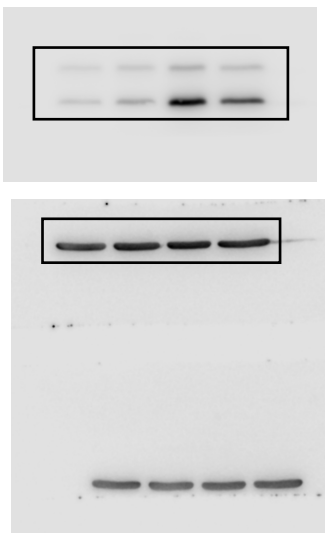


Figure S1e

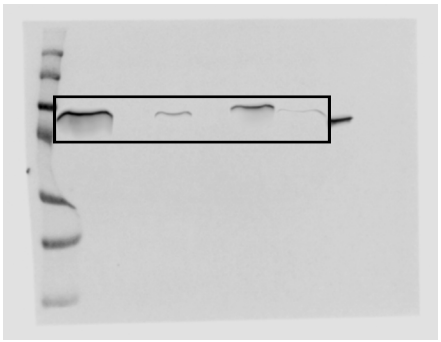


Figure S5b

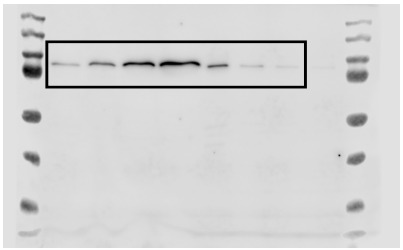


Figure S6b

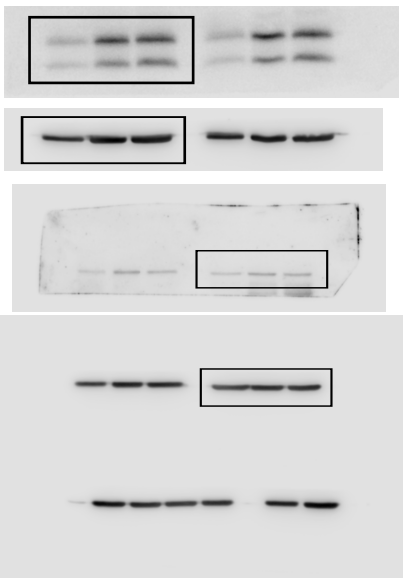
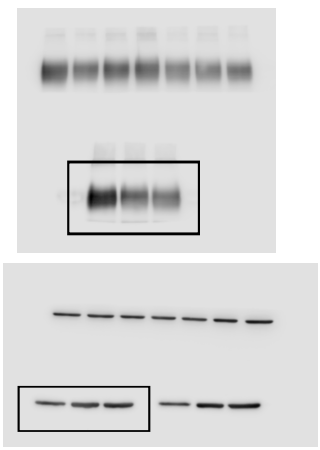


Figure S6d



# Supplementary Movies

**Supplementary Movie 1**: SS1 *H. pylori* is restricted to glandular lumen in wild-type mice. *H. pylori* staining of gastric tissue obtained from wild-type infected mice. The movie was created by a 3-D reconstruction of confocal z-sections acquired each 0.20 μm using a 40X water objective and deconvolved utilizing Volocity software. Similar staining was obtained for all the wild-type mice (n=12).

**Supplementary Movie 2**: SS1 *H pylori* colonize vacuolar compartments of parietal cells in *trpml1-/-* mice. *H. pylori* staining of gastric tissue obtained from *trmpl1*-deficient infected mice. The movie was created by a 3-D reconstruction of confocal z-sections acquired each 0.20 μm using a 40X water objective and deconvolved utilizing Volocity software. *H. pylori* within vacuolar compartments in parietal cells were identified in all the *trmpl1*-deficient mice analyzed (n=8).