Supplemental figure legends

FIGURE. S1. Localization of intracellular GAS and Rab proteins in infected cells. HeLa cells expressing fluorescently labeled Rab5 (upper panels) or Rab7 (lower panels) were infected with JRS4 for the indicated period. Rab proteins are shown in green. Bacterial and host DNA were stained red. The merged images are also shown in Fig. 1A. Bars: 10 μm.

FIGURE. S2. Localization of endosomal structures and autophagosomes in GAS-infected cells. (A, B) Cells expressing fluorescently labeled Rab5 (A), Rab7 (B), and LC3 (A, B) were infected with JRS4 for the indicated period. Rab proteins and autophagosomes are shown in green and red, respectively. Bacterial and host DNA were stained blue. Merged images are also shown in Figs. 2A and B. Bars: 10 μm.

FIGURE. S3. Influence of DA mutants for Rab expression on the formation and maturation of early endosomes in GAS-infected cells. Cells expressing fluorescently labeled Rab5Q79L (upper panels) or Rab7Q67L (lower panels) were infected with JRS4. Rab proteins are shown in green. Bacterial and host DNA were stained red. Cells expressing Rab7Q67L were also stained with an anti-LAMP-1 antibody to indicate lysosomes (blue). Merged images are also shown in Fig. 3A. Bars: 10 μm.

FIGURE. S4. Influence of DN mutants for Rab expression on the bacterial invasion into cells and fusion of Rab7-positive compartments with lysosomes. (A, B) Cells expressing Rab5S34N (A) or Rab7T22N (B) were infected with JRS4 for the indicated periods. Rab5S34N (A) or Rab7T22N (B) was visualized with EGFP (green). Bacterial and cellular DNA was stained with PI (red). In (B), cells were also stained with an anti-LAMP-1 antibody to indicate lysosomes (blue). Bars: 10 μm.

FIGURE. S5. Localization of early endosomes, autophagosomes, lysosomes, and a lysosomal enzyme in GAS-infected cells. (A-C) Cells expressing fluorescently labeled LC3, a marker of autophagosomes (A, C) or Rab5, a marker of early endosomes (B) were infected with JRS4 for 3 or 4 h. Autophagosomes or early endosomes are shown in green. Bacterial and host DNA were stained red. To label lysosomes, an anti-LAMP-1 (A, B) and an anti-cathepsin D antibody (C) were used (blue). (A, B) Merged images are also shown in Figs. 4A and B. Bars: 10 μm. (C) Arrows and insets show bacteria sequestered by autophagosomes, where cathepsin D

was also localized. Bars: 10 μm.

FIGURE. S6.

Confirmation of *slo*-complemented strain of JRS4Δ*slo*. (A) SDS-PAGE and Western blot analyses of *E. coli* strains. DH10B was transformed with pOGW-SLO, and the transformants were incubated with (+) or without (-) 1 mM IPTG. Coomassie brilliant blue (CBB) staining and western blot using an anti-SLO peptide antibody are shown. 'M' indicates a molecular marker at 62 kDa. (B) PCR and western blot analysis of JRS4, JRS4Δ*slo* (Δ*slo*), and JRS4Δ*slo*-comp (comp). The *slo* gene in each GAS strain was assessed by PCR, and the expression of SLO protein in culture supernatants with (+) or without (-) 1 mM IPTG was examined by western blotting using a specific antibody.

FIGURE. S7. Influence of SLO on the induction of autophagy in GAS-infected cells. (A, B) Cells expressing fluorescently labeled LC3 to indicate autophagosomes were infected with each GAS strain, JRS4, JRS4Δ*slo* (Δ*slo*), or JRS4Δ*slo*-comp (comp) for 2 (B), 3, or 4 h (A). Autophagosomes are shown in green. Bacterial and host DNA were stained red. Anti-LAMP-1, to indicate lysosomes (A) and anti-EEA-1, to indicate early endosomes (B) antibodies were used (blue). Merged images are also shown in Figs. 5A and B. Bars: 10 μm.

FIGURE. S8. The lack of SLO accumulation in endosomes influences the behavior of intracellular GAS. (A, B) Cells expressing Rab5 (A) or Rab5Q79L (B) were infected with JRS4 (B) or JRS4Δ*slo* (Δ*slo*) (A, B). Rab proteins are shown in green. Bacterial and host DNA were stained red. In (B), an anti-SLO antibody was used to indicate lysosomes (blue). Merged images are also shown in Figs. 7A and B. Bars: 10 μm.

FIGURE. S9. Persistence of the *slo*-deficient mutant JRS4 strain in endosomal structures of cells. (A, B) Cells expressing Rab5Q79L (A) or wild-type Rab7 (B) were infected with JRS4Δ*slo*. Endosomal structures were visualized with EGFP (green). Bacterial and host DNA were stained with PI (red). Insets show bacteria located in early endosomal structures (A) or Rab7-positive compartments (B). Bars: 10 μm. (C) JRS4Δ*slo*-infected cells were observed by electron microscopy. Arrows indicate endosomes surrounding bacteria. Bars: 1 μm.