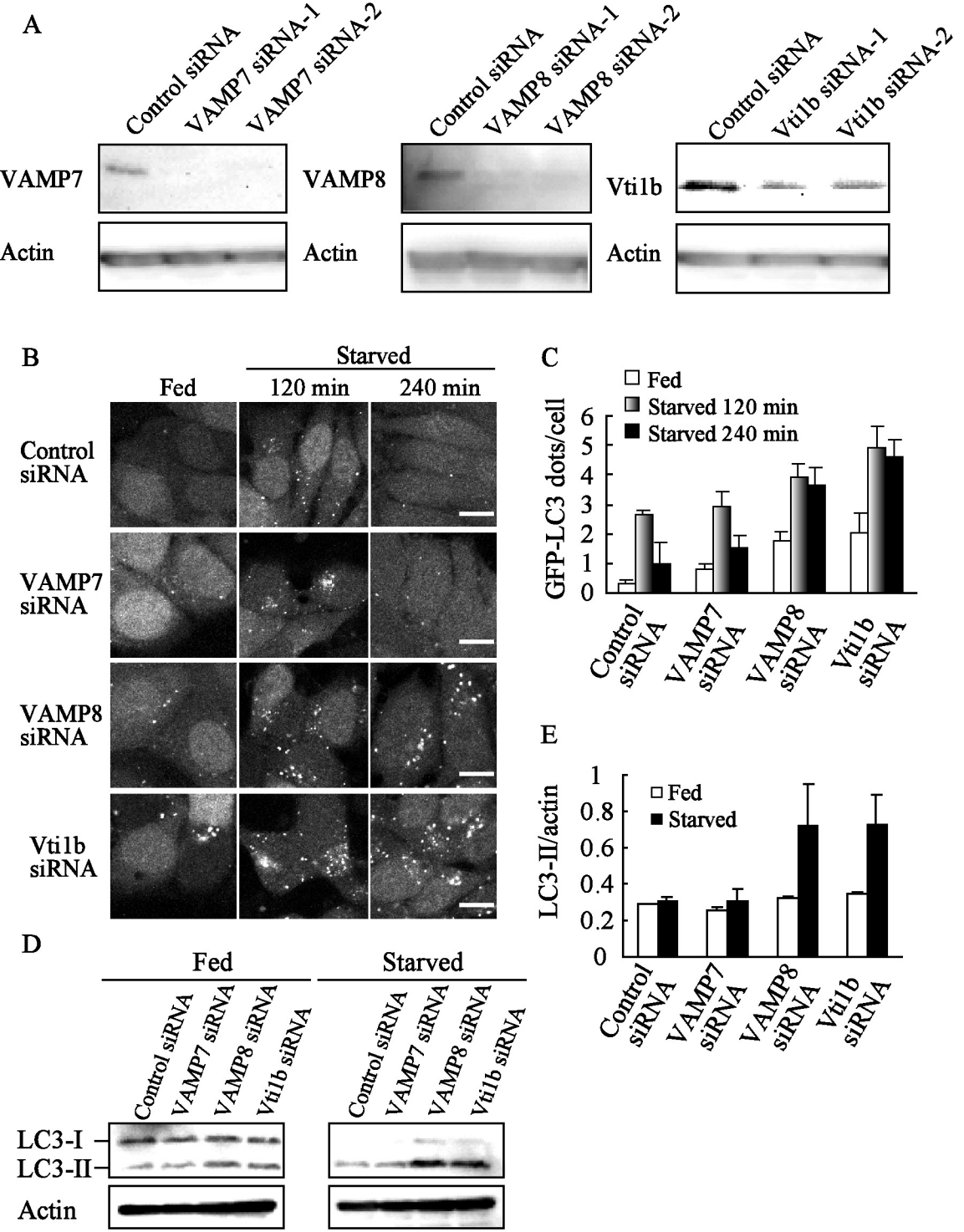
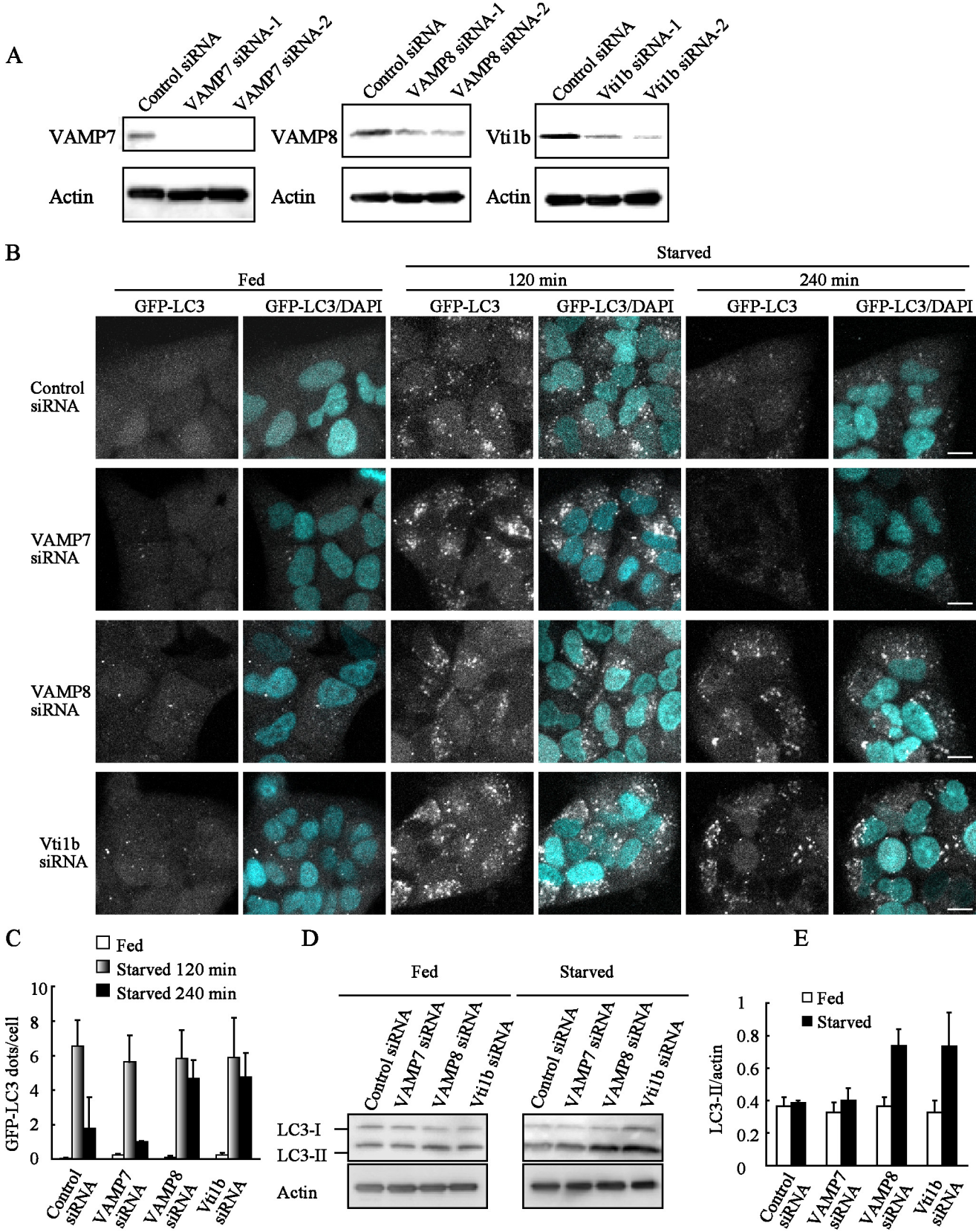
Supplemental Figure Legends

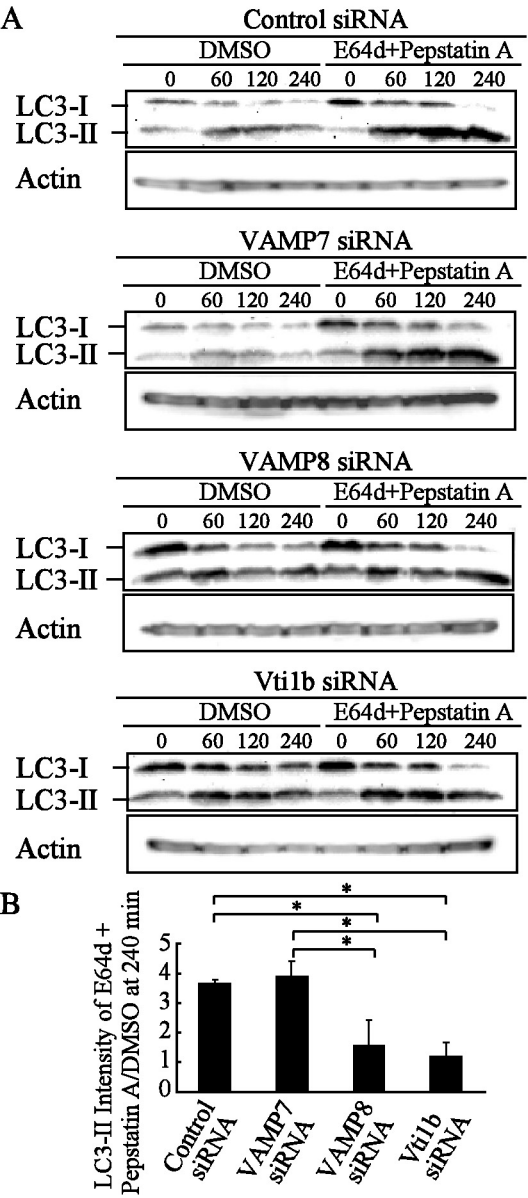
Supplemental Figure 1. Antimicrobial effects of xenophagosomes on Syntaxin7 and Syntaxin8-depleted cells. (A) HeLa cells were transfected with siRNA for the control, Syntaxin7, and Syntaxin8. At 48 hours after transfection, the cells were lysed and examined by western blotting using anti-Syntaxin7, -Syntaxin8, and actin antibodies. (B) HeLa cells stably expressing GFP-LC3 were transfected with siRNA for the control, Syntaxin7, and Syntaxin8. After 48 hours, the cells were infected with GAS for 180 minutes at an MOI of 100 as described in Materials and Methods. Following fixation, the cells were incubated with anti-LAMP1 antibodies and observed with a confocal microscope. Cellular and bacterial DNA were stained with DAPI. The boxed regions in the upper panels are enlarged in the lower panels. Bars indicate 10 μm (upper panels) or 5 μm (lower panels). (C) The co-localization frequencies of GcAVs with LAMP1 signals were manually measured and are presented as the percentage of total number of GcAVs. Data shown represent results of more than 30 cells.

Supplemental Figure 2. Maturation of canonical autophagosomes in Syntaxin7 and Syntaxin8-depleted cells. (A) MCF7 cells were transfected with siRNA for the control, Syntaxin7, and Syntaxin8. At 48 hours after transfection, the cells were lysed, then examined by western blotting using anti-Syntaxin7, -Syntaxin8, and actin antibodies. (B) MCF7 cells stably expressing GFP-LC3 were treated with siRNA in the same manner as described in Supplemental Figure 2A. The cells were further cultured in growth medium (Fed) or EBS solution (Starved) for the indicated times, then fixed and observed with a confocal microscope. Cellular DNA was stained with DAPI (blue). Bars indicate 10 μm. (C) siRNA-treated MCF7 cells were cultured in Fed and Starved conditions for 240 minutes, then the cellular lysates were subjected to western blotting using anti-LC3 and actin antibodies.

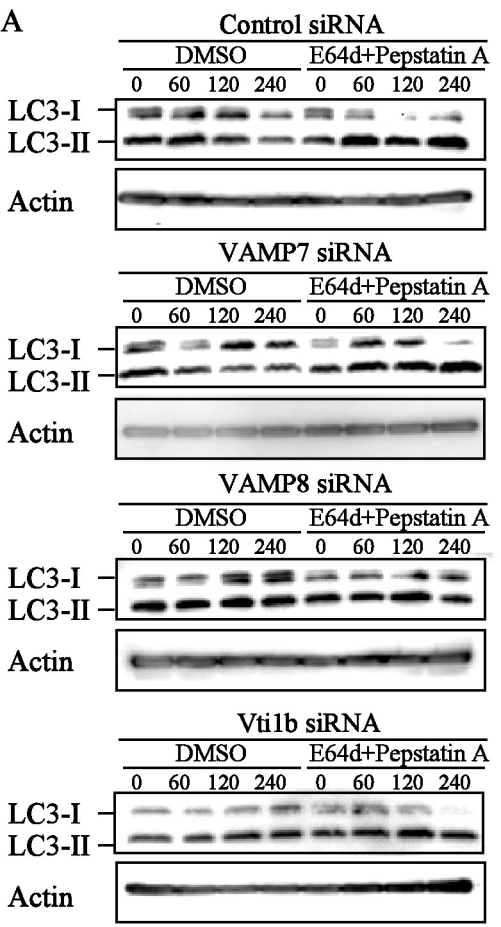

A
Control siRNA
VAMP7 siRNA-1
VAMP7 siRNA-2
VAMP7
Actin
Control siRNA
VAMP8 siRNA-1
VAMP8 siRNA-2
VAMP8
Actin
Control siRNA
Vti1b siRNA-1
Vti1b siRNA-2
Vti1b
Actin
B
Fed
Starved
120 min
240 min
Control siRNA
VAMP7 siRNA
VAMP8 siRNA
Vti1b siRNA
C
Fed
Starved 120 min
Starved 240 min
GFP-LC3 dots/cell
0
1
2
3
4
5
6
Control siRNA
VAMP7 siRNA
VAMP8 siRNA
Vti1b siRNA
D
Fed
Starved
Control siRNA
VAMP7 siRNA
VAMP8 siRNA
Vti1b siRNA
LC3-I
LC3-II
Actin
E
Fed
Starved
LC3-II/actin
0
0.2
0.4
0.6
0.8
1
Control siRNA
VAMP7 siRNA
VAMP8 siRNA
Vti1b siRNA

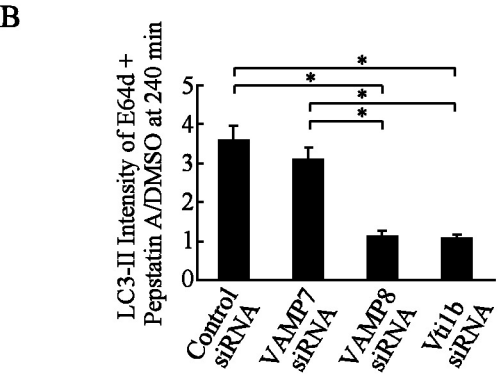

A
Control siRNA
VAMP7 siRNA-1
VAMP7 siRNA-2
VAMP7
Actin
Control siRNA
VAMP8 siRNA-1
VAMP8 siRNA-2
VAMP8
Actin
Control siRNA
Vti1b siRNA-1
Vti1b siRNA-2
Vti1b
Actin
B
Fed
Starved
120 min
240 min
GFP-LC3
GFP-LC3/DAPI
Control siRNA
VAMP7 siRNA
VAMP8 siRNA
Vti1b siRNA
C
Fed
Starved 120 min
Starved 240 min
GFP-LC3 dots/cell
Control siRNA
VAMP7 siRNA
VAMP8 siRNA
Vti1b siRNA
D
Fed
Starved
Control siRNA
VAMP7 siRNA
VAMP8 siRNA
Vti1b siRNA
LC3-I
LC3-II
Actin
E
Fed
Starved
LC3-II/actin
Control siRNA
VAMP7 siRNA
VAMP8 siRNA
Vti1b siRNA

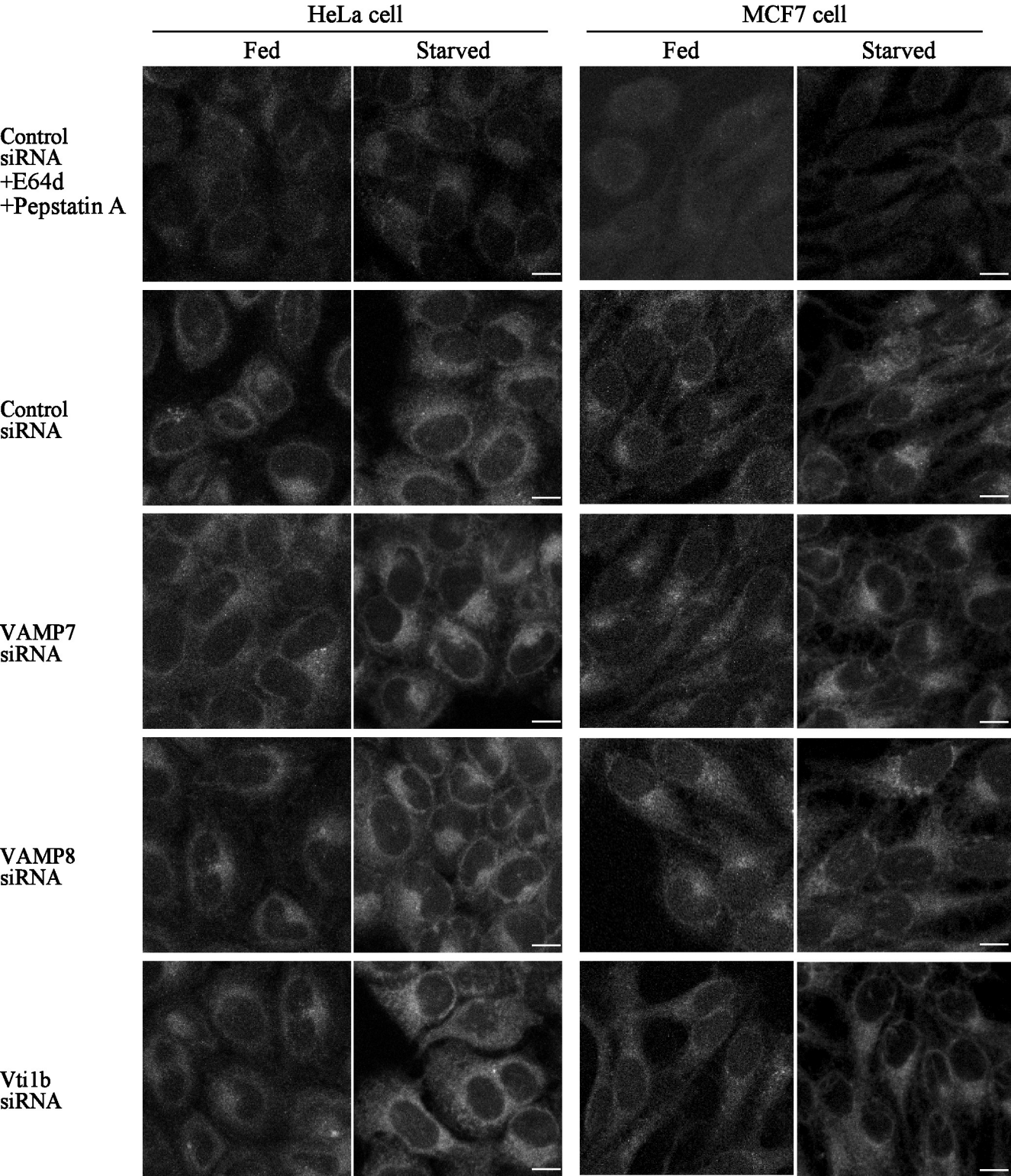
Furuta *et al.*, Supplemental Figure 3

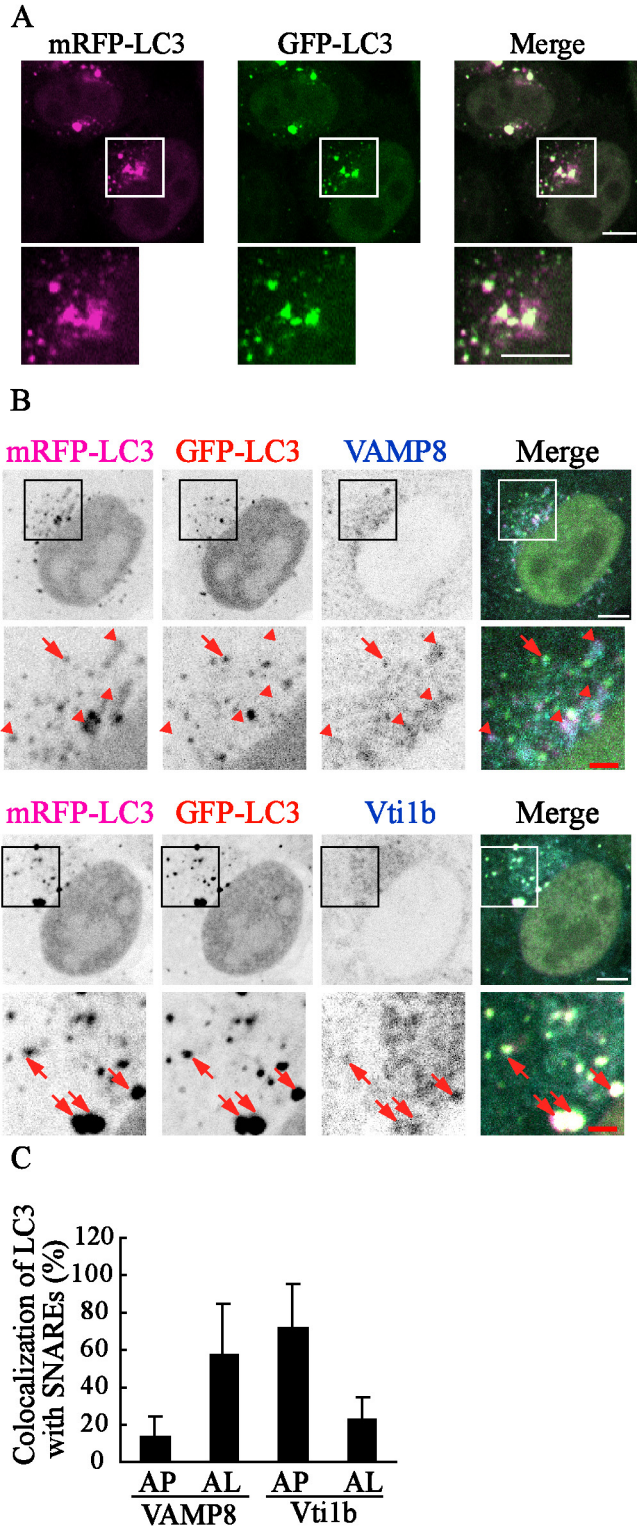


Furuta *et al.*, Supplemental Figure 4






HeLa cell
MCF7 cell
Fed
Starved
Fed
Starved
Control siRNA +E64d +Pepstatin A
Control siRNA
VAMP7 siRNA
VAMP8 siRNA
Vti1b siRNA


A
mRFP-LC3
GFP-LC3
Merge
B
mRFP-LC3
GFP-LC3
VAMP8
Merge
mRFP-LC3
GFP-LC3
Vti1b
Merge
C
Colocalization of LC3 with SNAREs (%)
120
100
80
60
40
20
0
AP
AL
AP
AL
VAMP8
Vti1b

Furuta *et al.*, Supplemental Figure 7