Supplemental Materials

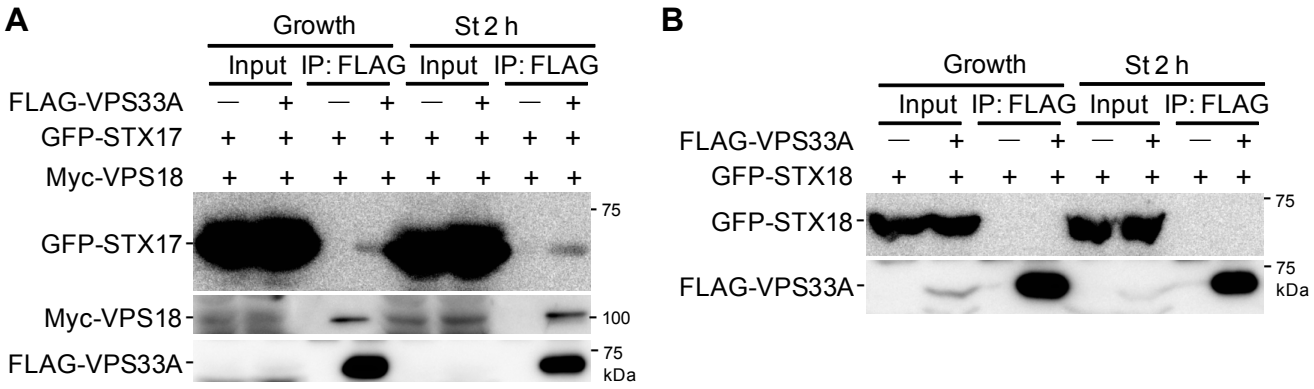
Molecular Biology of the Cell

Jiang et al.

Supplemental Figure 1: VPS33A interacts with STX17 but not with STX18
(A and B) HEK293T cells were transfected with indicated constructs. Three days later, cells were cultured in regular or starvation medium for 2 h, followed by immunoprecipitation using anti-FLAG antibody (M2 affinity gel). Input corresponds to 6% of the cell lysate used for each immunoprecipitation. The immunoprecipitates were then immunoblotted with anti-GFP, anti-Myc, and anti-FLAG antibodies.

Supplemental Figure 2: Knockdown of VPS33A and VPS39 causes accumulation of STX17 and LC3 double-positive structures under starvation conditions.
HeLa cells stably expressing GFP-STX17 were treated with siRNA against luciferase, VPS33A, VPS39, UVRAG, or VPS33A+UVRAG. Then, cells were cultured in starvation medium for 2 h and analyzed by immunofluorescence microscopy using anti-LC3 and anti-LAMP1 antibodies. Structures positive for STX17 and LC3 but not for LAMP1 are indicated by arrows. Scale bars: 10 μm.

Supplemental Figure 3: Knockdown of VPS33A reduces the amount of STX17-VAMP8 complex.
HeLa cells were treated with siRNA oligonucleotides against luciferase or VPS33A. After 72 h, cells were starved for 2 h, followed by immunoprecipitation using anti-STX17 antibody. Input corresponds to 6% of the cell lysate used for each immunoprecipitation. The immunoprecipitates were then immunoblotted with the indicated antibodies.



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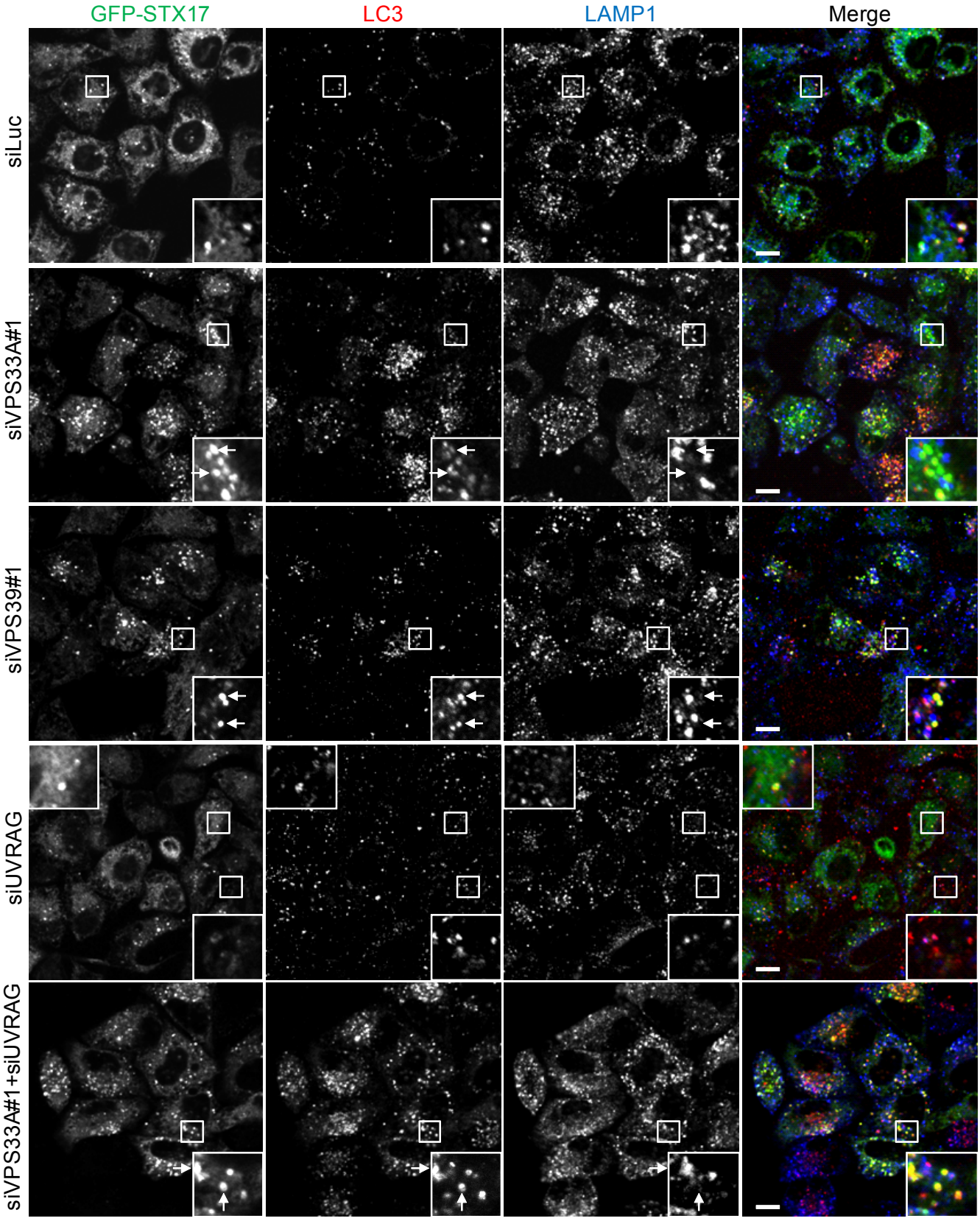

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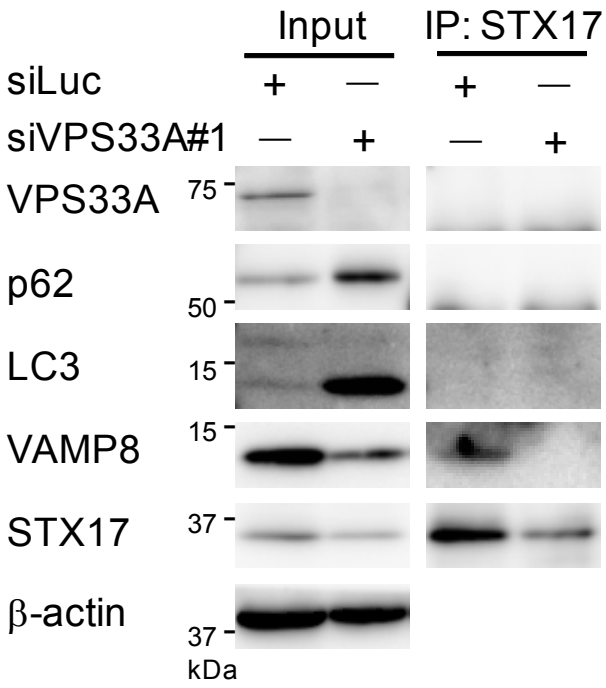

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