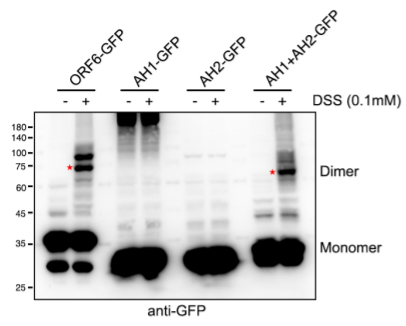
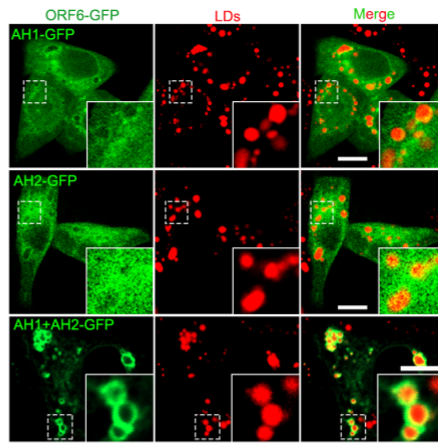


# Appendix Fig S1

**A**



**B**



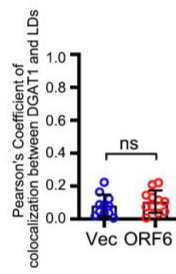
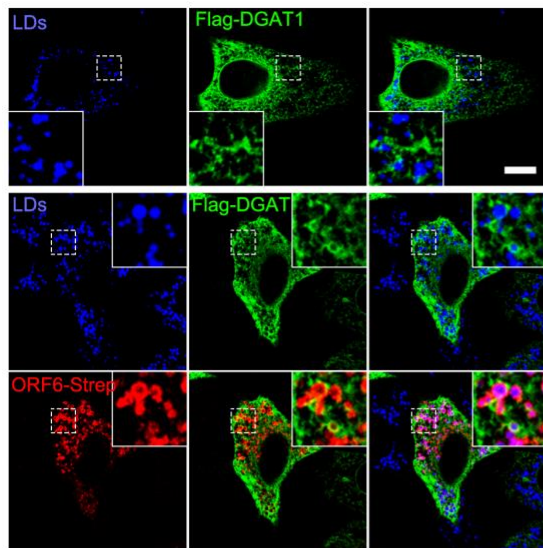
2 **Appendix Figure S1. ORF6 Homodimerization is Important for LD Targeting.**

3 **(A)** HEK293T cells were transfected with ORF6-GFP or indicated mutants and were treated  
4 with 0.1 mM DSS for 30 min. Cell lysates were analyzed via WB.

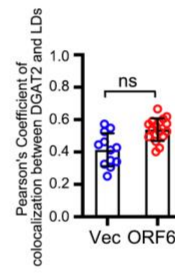
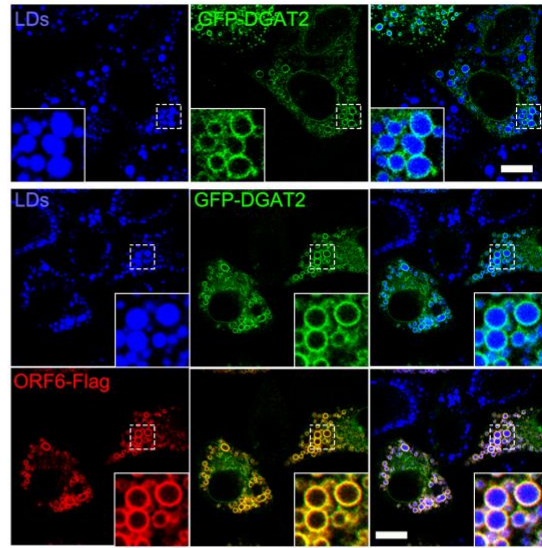
5 **(B)** HeLa cells were transfected with ORF6-GFP or indicated mutants and were treated  
6 with 200  $\mu$ M OA for 12 h, and then fixed. LDs were labeled with LipidTOX Red. Cells were  
7 imaged by confocal microscopy. Scar bar represents 10  $\mu$ m.

# Appendix Fig S2

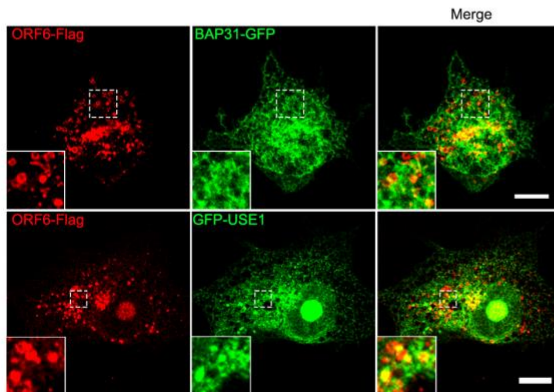
A



B



C



9 **Appendix Figure S2. ORF6 promotes LD biogenesis by linking the ER to LDs.**

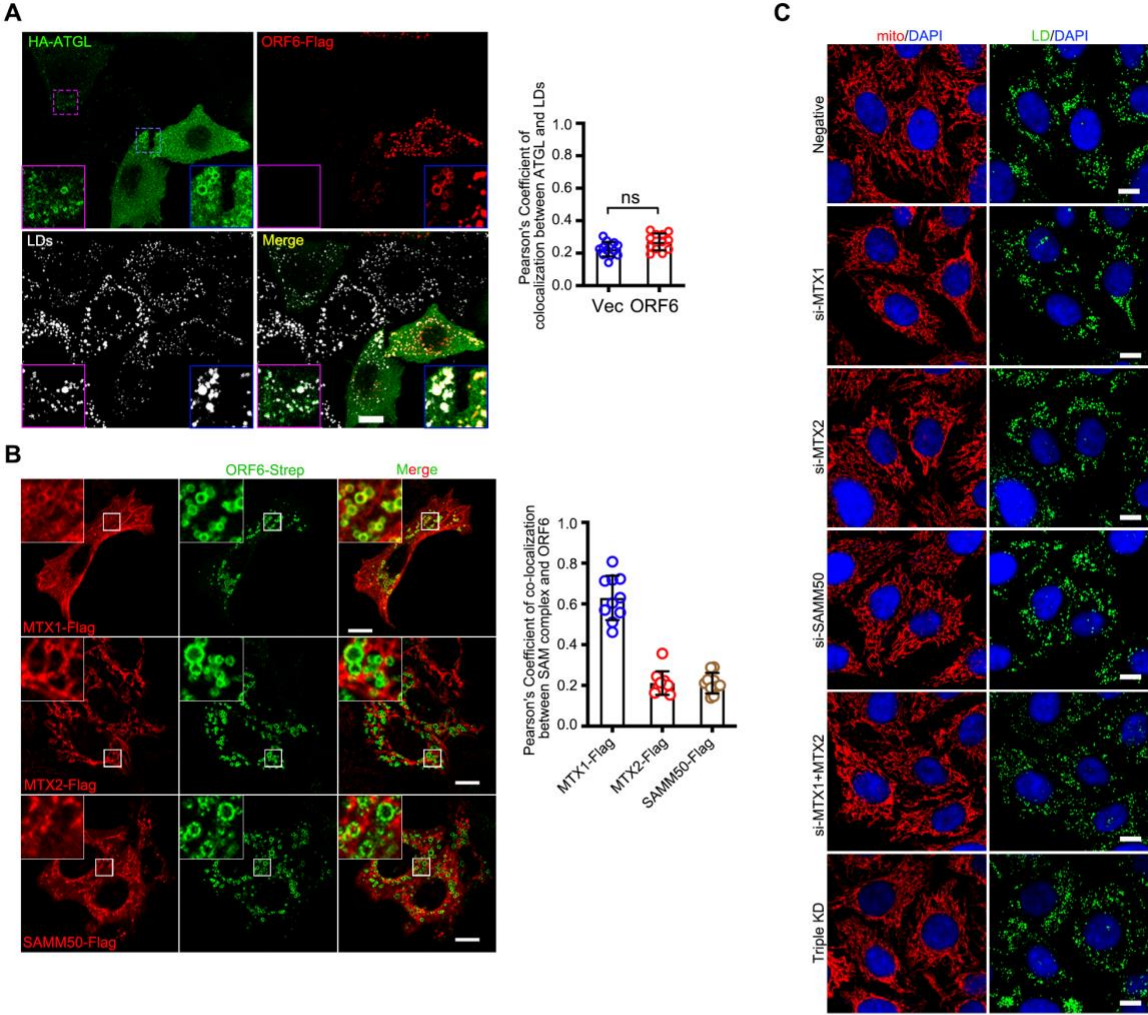
10 **(A)** Cells were transfected with Flag-DGAT1 with or without ORF6-Strep for 24 h and further  
11 were treated with 200  $\mu$ M OA for 12 h. LDs were labeled with LipidTOX Deep Red (blue).  
12 Cells were fixed and then stained with anti-Strep (red) and anti-Flag (green). Cells were  
13 imaged by confocal microscopy. Scar bar represents 10  $\mu$ m. Colocalization of DGAT1 and  
14 LDs (Pearson's Coefficient), n=11 cells, two independent experiments. Two-tailed Unpaired  
15 Student's t-test, ns means no significance. Error bars represent as the mean  $\pm$  SD.

16 **(B)** Cells were transfected with with GFP-DGAT2 with or without ORF6-Flag for 24 h and  
17 further were treated with 200  $\mu$ M OA for 12 h. LDs were labeled with LipidTOX Deep Red  
18 (blue). Cells were fixed and then stained with anti-Flag (red). Cells were imaged by confocal  
19 microscopy. Scar bar represents 10  $\mu$ m. Colocalization of DGAT2 and LDs (Pearson's  
20 Coefficient), n=12 cells (Vec) and n=17 cells (ORF6-Flag), two independent experiments.  
21 Two-tailed Unpaired Student's t-test, ns means no significance. Error bars represent as the  
22 mean  $\pm$  SD.

23 **(C)** HeLa cells were transfected with ORF6-Flag and BAP31-GFP or GFP-USE1 for 12 h  
24 and treated with 200  $\mu$ M OA for 12 h, then fixed and stained with anti-Flag (red). Cells were  
25 imaged by confocal microscopy. Scar bar represents 10  $\mu$ m.

26

# Appendix Fig S3



28 **Appendix Figure S3. ORF6 Interacts with SAM complexes.**

29 **(A)** HeLa cells were transfected with vector or ORF6-Flag and HA-ATGL for 12 h and  
30 treated with 200  $\mu$ M OA for 12 h, then fixed and stained with anti-Flag (red) and anti-HA  
31 (green). LDs were labeled with LipidTOX Deep Red (white). Cells were imaged by confocal  
32 microscopy. Scar bar represents 10  $\mu$ m. Blue ROIs indicate cells expressing ORF6 and  
33 purple ROIs indicate the cells without ORF6 expression. Colocalization of ATGL and LDs  
34 (Pearson's Coefficient), n=12 cells, two independent experiments. Two-tailed Unpaired  
35 Student's t-test, ns means no significance. Error bars represent as the mean  $\pm$  SD.

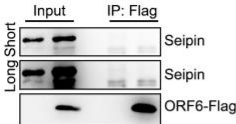
36 **(B)** HeLa cells were transfected with ORF6-Strep and Flag-MTX1, or MTX2-Flag, or  
37 SAMM50-Flag for 12 h and treated with 200  $\mu$ M OA for 12 h, then fixed and stained with  
38 anti-Strep (green) and anti-Flag (red). Cells were imaged by confocal microscopy. Scar bar  
39 represents 10  $\mu$ m. Colocalization of ORF6 and MTX1 or MTX2 or SAMM50 (Pearson's  
40 Coefficient), n=10 cells, two independent experiments. Error bars represent as the mean  $\pm$   
41 SD.

42 **(C)** HeLa cells were transfected with negative or indicated siRNAs for 36 h and treated with  
43 200  $\mu$ M OA for another 12 h. Cells were fixed and stained with anti-Tom20 (red). Tom20  
44 represents mitochondria marker. LDs were labeled with BODIPY-493/503 (green). Cells  
45 were imaged by confocal microscopy. Scar bar represents 10  $\mu$ m.

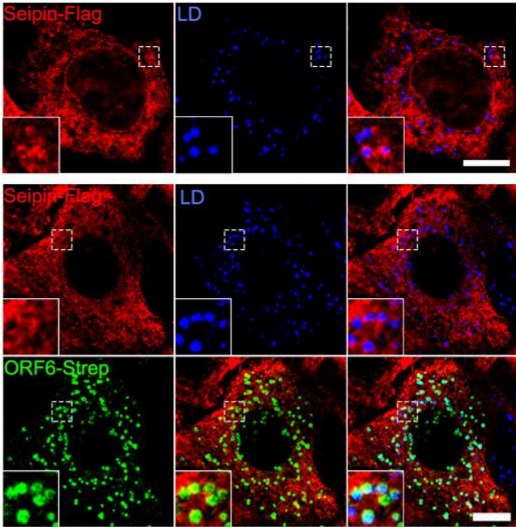
46

# Appendix Fig S4

A



B



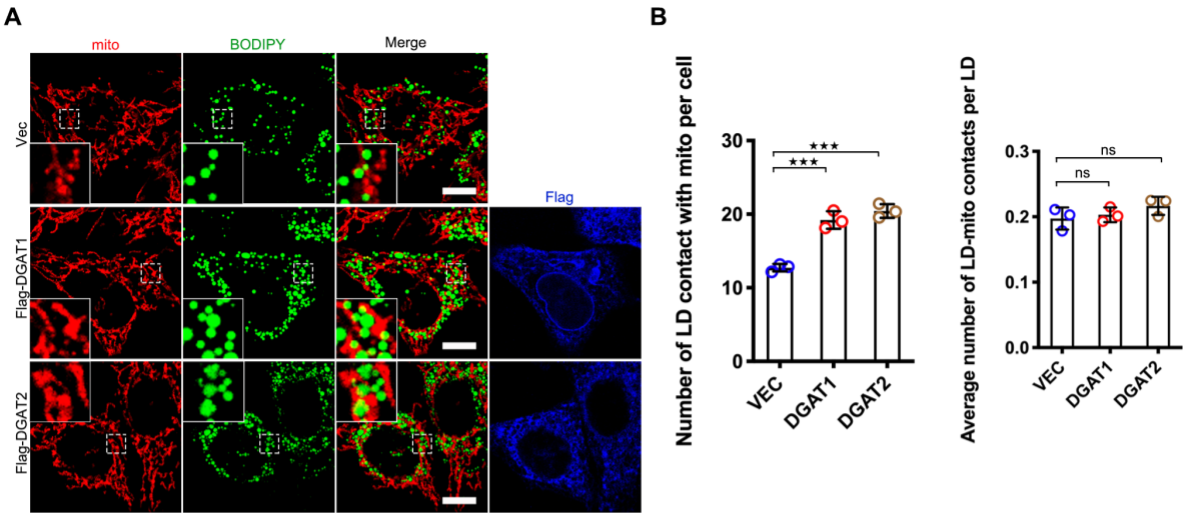
48 **Appendix Figure S4. ORF6 failed to bind and colocalize with Seipin.**

49 **(A)** HEK293T cells were transfected with ORF6-Flag. Protein interactions were detected  
50 by immunoprecipitation with anti-Flag beads and immunoblotting analysis with Seipin  
51 antibody.

52 **(B)** HeLa cells were transfected with ORF6-Strep and Seipin-Flag for 22 h and treated  
53 with 200  $\mu$ M OA for 2 h, then fixed and stained with anti-Flag (red) and anti-Strep  
54 (green). LDs were labeled with LipidTOX Deep Red (blue). Cells were imaged by  
55 confocal microscopy. Scar bar represents 10  $\mu$ m.



# Appendix Fig S5



57 **Appendix Figure S5. overexpression of DGAT1 or DGAT2 has no effect on**  
58 **percentage of LD contact with mitochondria.**

59 **(A)** HeLa cells were transfected with vector, or Flag-DGAT1, or Flag-DGAT2 for 12 h and  
60 further treated with 200  $\mu$ M OA for 12 h, and then were fixed and stained with anti-Flag  
61 (blue) and anti-Tom20 (red). LDs were labeled with BODIPY-493/503 (green).

62 **(B)** Number of LDs in each cell and average number of LD-mitochondria contacts per LD  
63 in **(A)** were counted from 20 cells of three independent experiments. Two-tailed Unpaired  
64 Student's t-test, \*\*\* $p < 0.001$ , ns means no significance. Error bars represent as the mean  
65  $\pm$  SD.