# Expanded View Figures

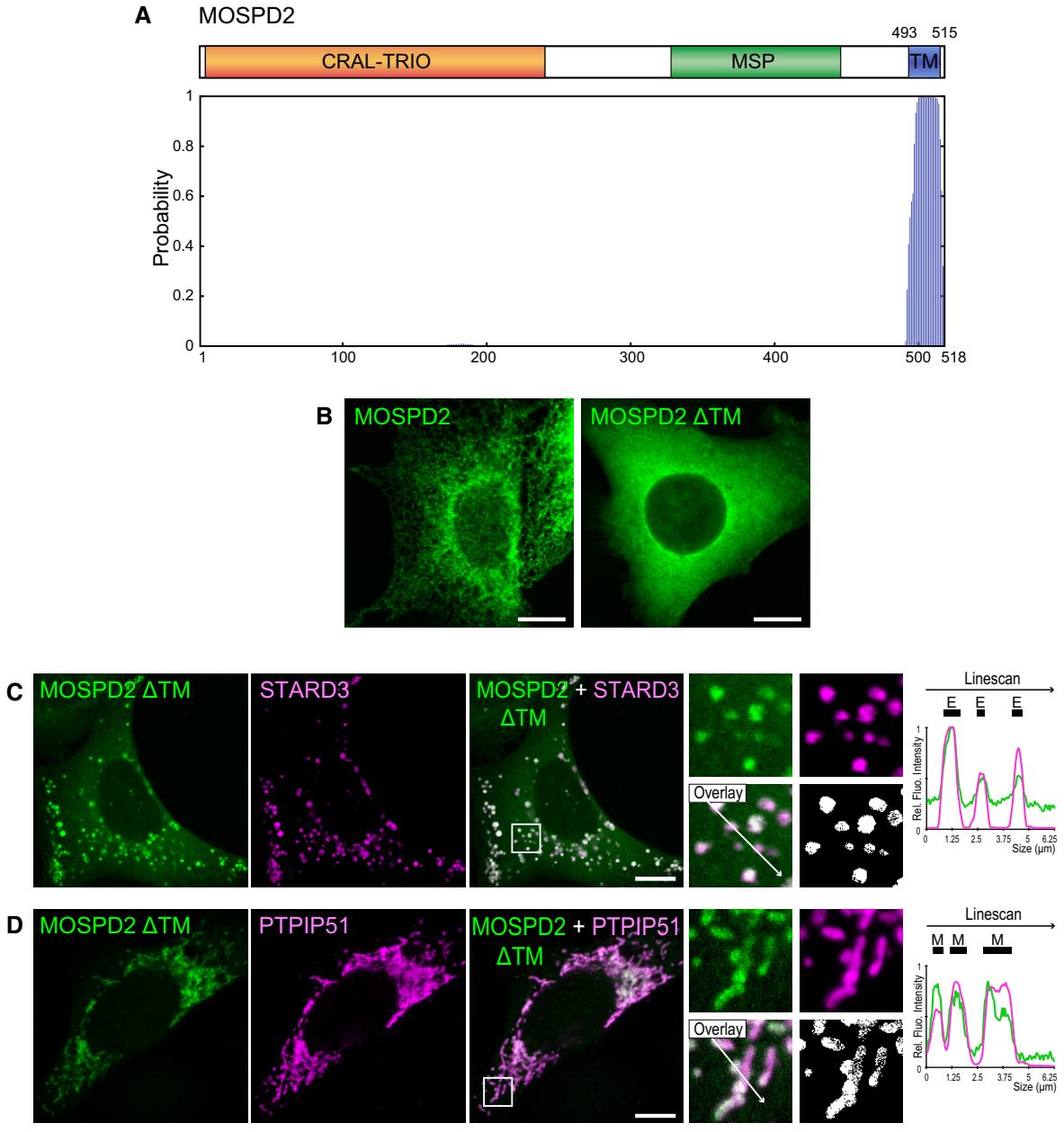


**Figure EV1. MOSPD2 is a putative tail-anchored protein.**

A Top: Schematic representation of MOSPD2. Numbers indicate the positions of the first and the last residue of the putative transmembrane domain (blue box). Bottom: Determination of the number and position of transmembrane helices using TMHMM software [73]. Vertical bars of the diagram represent the probability for a given amino acid to be included in a transmembrane helix. MOSPD2 is predicted to possess a single carboxyl-terminus transmembrane helix.

B Localization of GFP-MOSPD2 (left, green) and GFP-MOSPD2 ΔTM (right, green) expressed in HeLa cells. The GFP-MOSPD2 ΔTM protein is lacking the last 26 amino acids of MOSPD2, which include the transmembrane domain. Scale bars: 10 μm.

C, D Localization of GFP-MOSPD2 ΔTM (green) and Flag-STARD3 (C, magenta), or HA-PTPIP51 (D, magenta), expressed in HeLa cells. The subpanels on the right are higher magnification (3.5×) images of the area outlined in white. The Overlay panel shows merged green and magenta images. The Coloc panel displays a colocalization mask on which pixels where the green and the magenta channels co-localize are shown in white. Right: Linescan analyses with fluorescence intensities of the green and magenta channels along the white arrow shown on the subpanel Overlay. Black rectangles indicate the positions of late endosomes (E) and mitochondria (M). Scale bars: 10 μm.

© 2018 The Authors

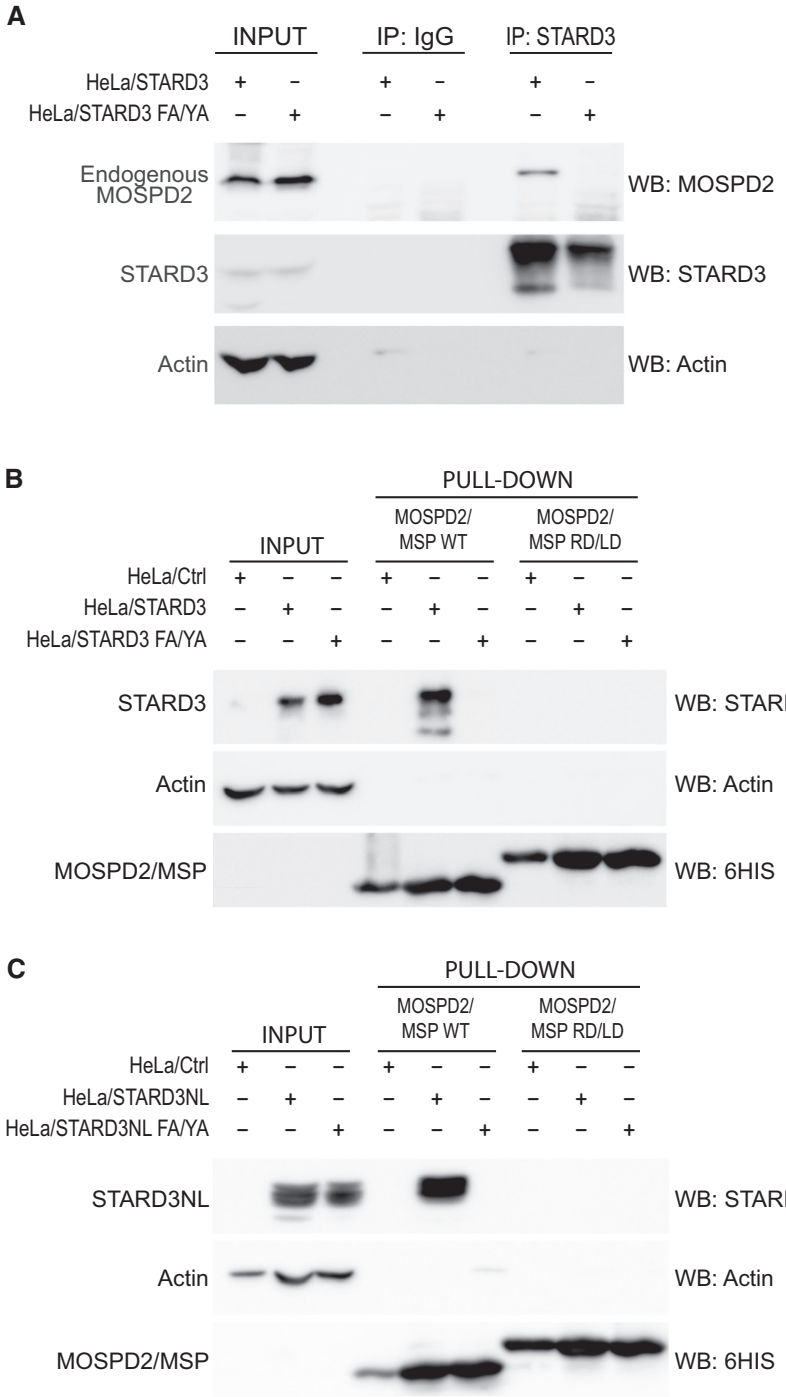


**Figure EV2. The MSP domain of MOSPD2 is sufficient to interact with STARD3 and STARD3NL.**

A Immunoprecipitation experiment between STARD3 (WT and FFAT-deficient) and endogenous MOSPD2. Proteins were immunoprecipitated using anti-STARD3 antibodies or control IgG. Protein extracts and immunoprecipitates were analyzed by Western blot using anti-MOSPD2, anti-STARD3, and anti-actin antibodies.

B, C Interaction of STARD3 (B), or STARD3NL (C), with the recombinant MSP domain of MOSPD2. The WT and the RD/LD mutant MSP domain of MOSPD2 were bound to a $Ni^{2+}$-NTA resin and used in a pull-down assay with protein extracts of control (HeLa/Ctrl), STARD3, and STARD3 FA/YA FFAT-defective mutant (B), and STARD3NL and STARD3NL FA/YA FFAT-defective mutant (C) expressing cells. Total and bound proteins were analyzed by Western blot using anti-STARD3 (B), anti-STARD3NL (C), anti-HIS tag (B and C), and anti-actin (B and C).

Source data are available online for this figure.

 © 2018 The Authors

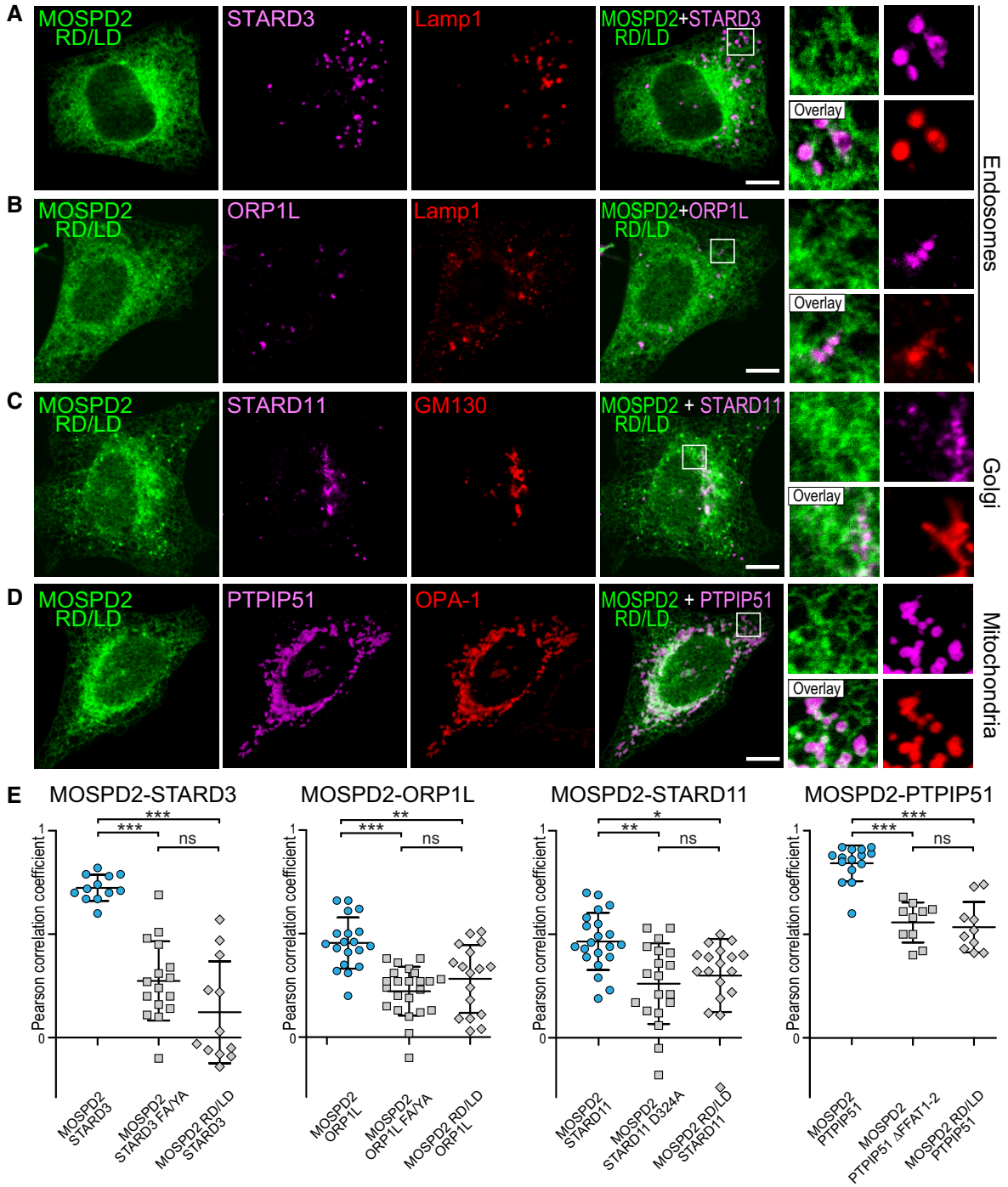


**Figure EV3. MOSPD2 recruitment to interorganelle contact sites depends on the interaction between the FFAT motif and the MSP domain.**

A–D GFP-MOSPD2 RD/LD (green)-expressing cells were transfected with Flag-STARD3 (A), Flag-ORP1L (B), Flag-STARD11 (C), or HA-PTPIP51 (D), and labeled using anti-Flag (A–C; magenta), anti-HA (D: magenta), and anti-Lamp1 (A and B; red), GM130 (C; red), or OPA-1 (D; red), as markers of late endosomes/lysosomes, Golgi, and mitochondria, respectively. The subpanels on the right are higher magnification (3.5×) images of the area outlined in white. The Overlay panel shows merged green and magenta images. Scale bars: 10 μm.

E Pearson correlation coefficients between MOSPD2 (WT or RD/LD mutant) and STARD3 (WT or FA/YA), ORP1L (WT or FA/YA), STARD11 (WT or D324A), and PTPIP51 (WT or ΔFFAT 1–2) staining are shown. Each dot represents a single cell from three independent experiments (number of cells: MOSPD2–STARD3: 12; MOSPD2–STARD3 FA/YA: 16; MOSPD2 RD/LD–STARD3: 12; MOSPD2–ORP1L: 19; MOSPD2–ORP1L FA/YA: 23; MOSPD2 RD/LD–ORP1L: 17; MOSPD2–STARD11: 21; MOSPD2–STARD11 D324A: 19; MOSPD2 RD/LD–STARD11: 18; MOSPD2–PTPIP51: 15; MOSPD2–PTPIP51 ΔFFAT 1–2: 10; MOSPD2 RD/LD–PTPIP51: 10). Means and error bars (SD) are shown. Kruskal–Wallis with Dunn's multiple comparison test (*$P$-values < 0.05; **$P$ < 0.01; ***$P$ < 0.001).

© 2018 The Authors

**Figure EV4. Endogenous MOSPD2 is recruited to interorganelle contact sites by FFAT-containing proteins.**

A–H Endogenous MOSPD2 (green) staining in HeLa cells expressing Flag-STARD3 (A), Flag-STARD3 FA/YA (B), GFP-ORP1L (C), GFP-ORP1L FA/YA (D), Flag-STARD11 (E), Flag-STARD11 D324A (F), HA-PTPIP51 (G), or HA-PTPIP51 ΔFFAT (H) (magenta). Endogenous MOSPD2 was recruited around endosomes, Golgi, and mitochondria by STARD3 and ORP1L, STARD11 and PTPIP51, respectively. FFAT-deficient STARD3, ORP1L, STARD11, and PTPIP51 mutants did not recruit endogenous MOSPD2. Note that in agreement with immunoprecipitation assays (Fig 5F), PTPIP51 ΔFFAT retained a partial ability to recruit MOSPD2. The subpanels on the right are higher magnification (3.5×) images of the area outlined in white. The Overlay panel shows merged green and magenta images. The Coloc panel displays a co-localization mask on which pixels where the green and the magenta channels co-localize are shown in white. Right: Linescan analyses with fluorescence intensities of the green and magenta channels along the white arrow shown on the subpanel Overlay. Black rectangles indicate the positions of late endosomes (E), Golgi stacks (G), and mitochondria (M). Scale bars: 10 μm.


© 2018 The Authors

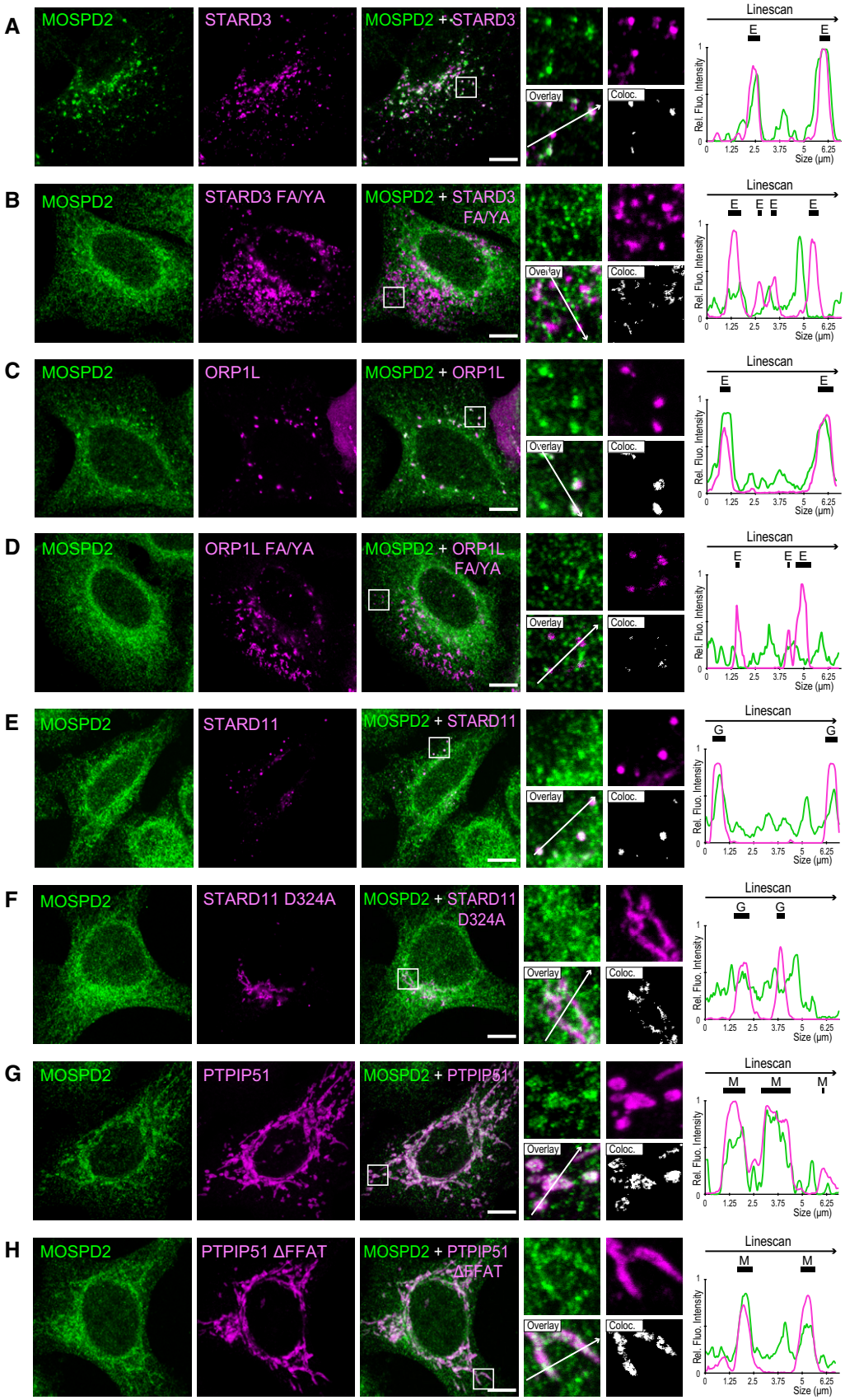
A
MOSPD2
STARD3
MOSPD2 + STARD3
Overlay
Coloc.
Linescan
E
E
Rel. Fluo. Intensity
Size (µm)
B
MOSPD2
STARD3 FA/YA
MOSPD2 + STARD3 FA/YA
C
MOSPD2
ORP1L
MOSPD2 + ORP1L
D
MOSPD2
ORP1L FA/YA
MOSPD2 + ORP1L FA/YA
E
MOSPD2
STARD11
MOSPD2 + STARD11
G
F
MOSPD2
STARD11 D324A
MOSPD2 + STARD11 D324A
G
MOSPD2
PTPIP51
MOSPD2 + PTPIP51
M
H
MOSPD2
PTPIP51 ΔFFAT
MOSPD2 + PTPIP51 ΔFFAT

**Figure EV4.**

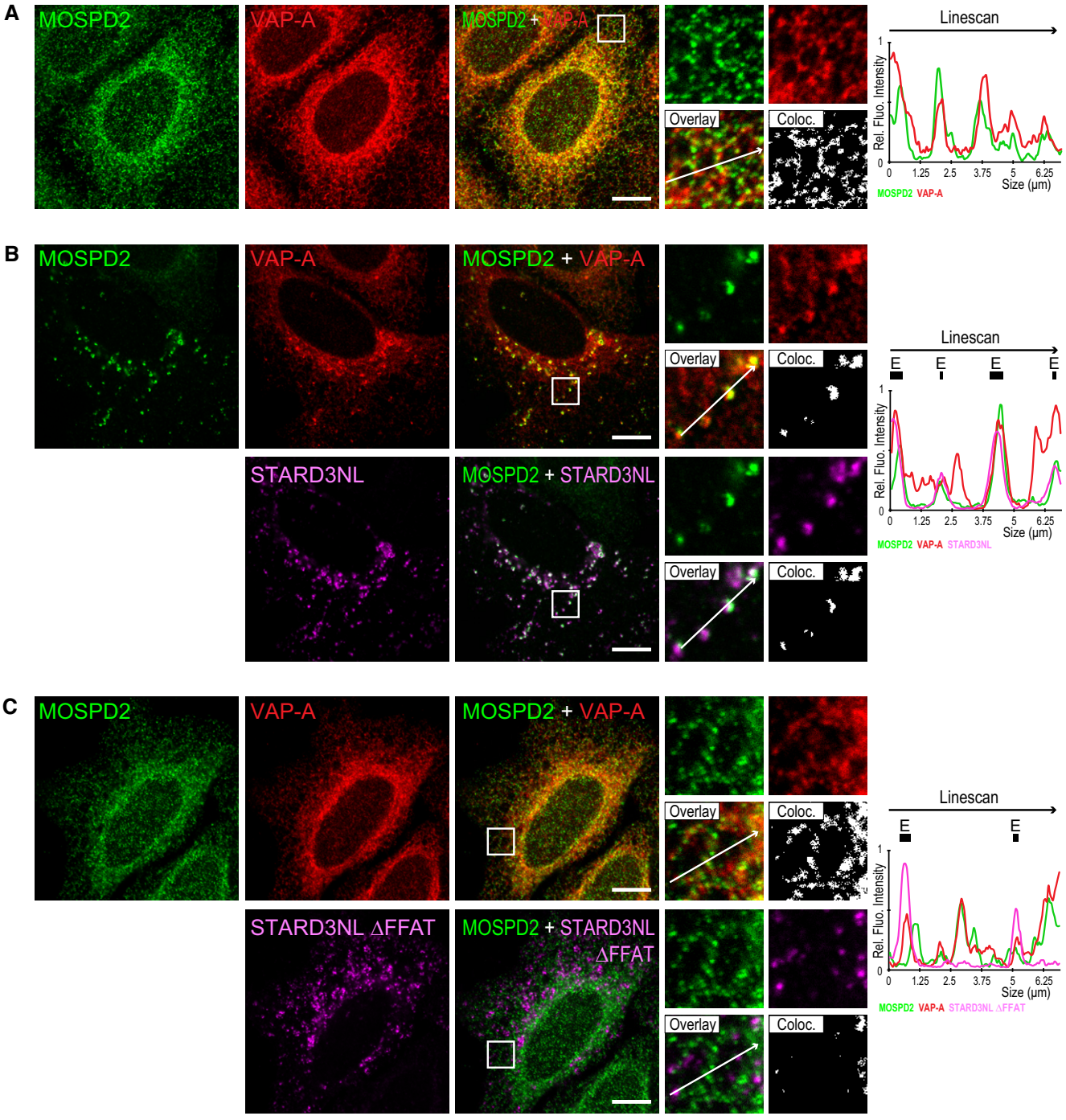


**Figure EV5. VAP-A is recruited to interorganelle contact sites together with MOSPD2.**

A HeLa cells were labeled with anti-MOSPD2 (green) and anti-VAP-A (red) antibodies.

B, C HeLa cells expressing Flag-STARD3NL (B) or Flag-STARD3NL ΔFFAT (C) were labeled with anti-MOSPD2 (green), anti-VAP-A (red), and anti-Flag (magenta) antibodies.

Data information: The subpanels on the right are higher magnification (3.5×) images of the area outlined in white. The Overlay panel shows merged green and magenta images. The Coloc panel displays a colocalization mask on which pixels where green and red (up), or green and magenta (bottom) channels co-localize are shown in white. Right: Linescan analyses with fluorescence intensities of the green, red, and magenta channels along the white arrow shown on the subpanel Overlay. Black rectangles indicate the positions of late endosomes (E). Scale bars: 10 µm.


© 2018 The Authors