Expanded View Figures

Figure EV1. ORP5 and ORP8 localization at ER–PM and ER–mitochondria contact sites.

A, B IEM micrographs of HeLa cells transfected with EGFP-ORP5 (A) or EGFP-ORP8 (B) and immunogold labeled with anti-GFP (15 nm gold) and anti-PDI (10 nm gold), showing ORP5 and ORP8 localization at both ER–PM (black arrows) and ER–mitochondria contact sites (red arrows). Note that the bulk of ORP5 localizes to ER–PM contacts while the bulk of ORP8 localizes to non-cER membranes (arrowheads). Scale bar, 200 nm.

C Representative electron micrographs of HeLa cells transfected with HRP-KDEL (Ctrl) or co-transfected with EGFP-ORP5. Arrows indicate ER–PM contact sites. Scale bar, 2 μm.

D Quantification of the percentage of ER–PM contact sites per cell (upper panel), the average number (middle panel) or length (lower panel) of ER–PM contact sites per cell of HeLa cells transfected with HRP-KDEL (Ctrl) or co-transfected with EGFP-ORP5 (n = 20 cells). % ER–PM contact sites ± SEM. *$P < 0.001$ compared to Ctrl.

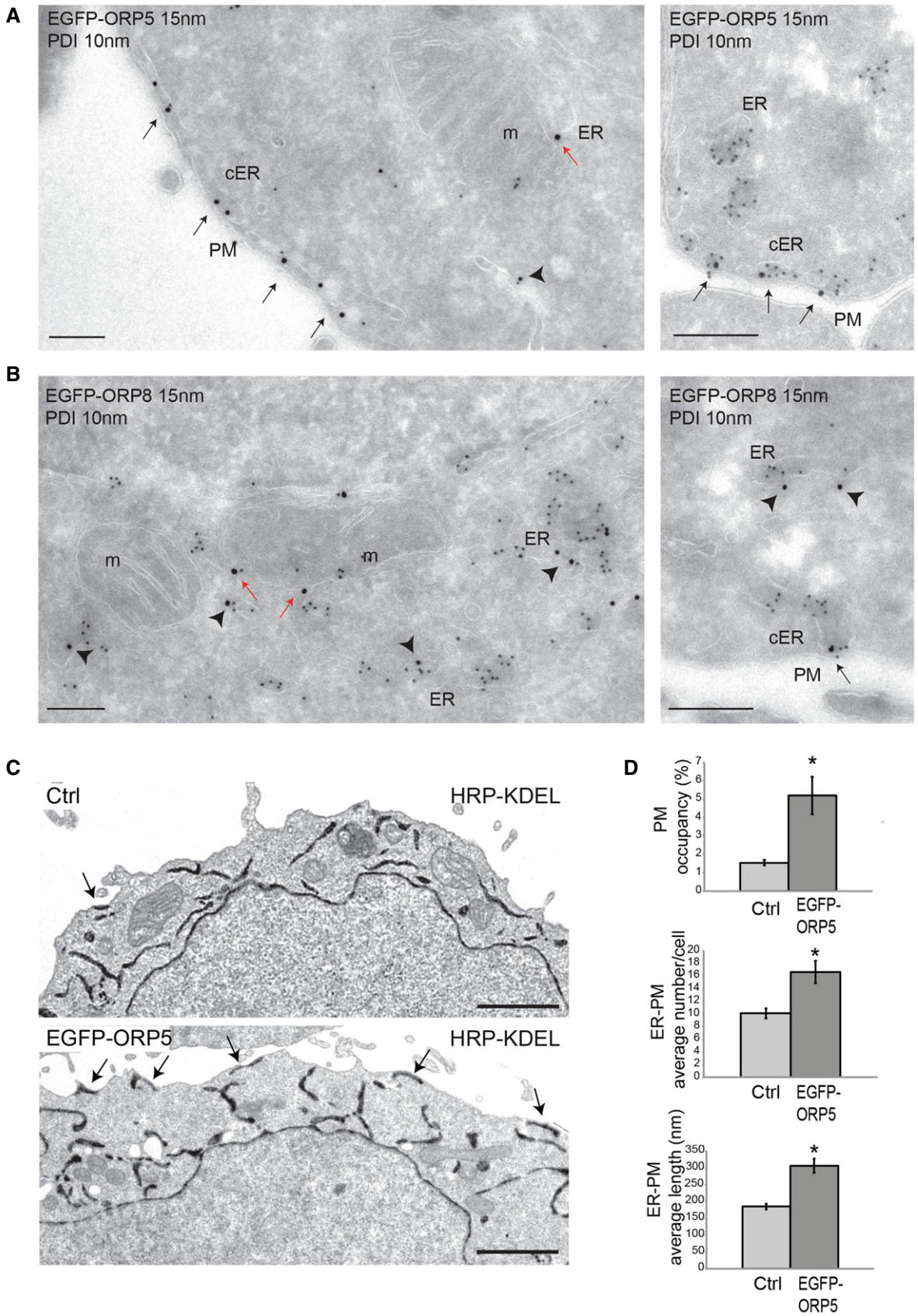
A
EGFP-ORP5 15nm
PDI 10nm
m
ER
cER
PM
EGFP-ORP5 15nm
PDI 10nm
ER
cER
PM
B
EGFP-ORP8 15nm
PDI 10nm
m
m
ER
ER
EGFP-ORP8 15nm
PDI 10nm
ER
cER
PM
C
Ctrl
HRP-KDEL
EGFP-ORP5
HRP-KDEL
D
PM occupancy (%)
Ctrl
EGFP-ORP5
ER-PM average number/cell
Ctrl
EGFP-ORP5
ER-PM average length (nm)
Ctrl
EGFP-ORP5

Figure EV1.

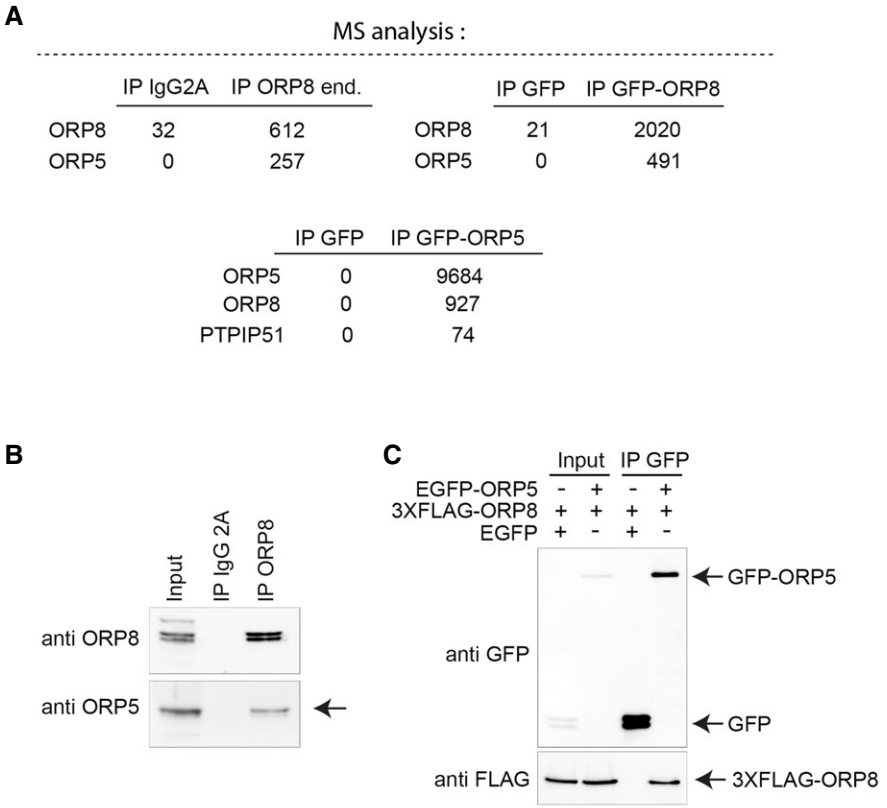

Figure EV2. ORP5 and ORP8 interaction.

A Mass spectrometry (MS) analysis on immunoprecipitated endogenous ORP8 or overexpressed EGFP-ORP8 or EGFP-ORP5 indicates ORP5 or ORP8 as the major interacting partners. Note the presence of PTPIP51 in the MS of EGFP-ORP5 immunoprecipitates.

B Immunoprecipitation of endogenous ORP8 reveals interaction with endogenous ORP5 (arrow).

C EGFP-ORP5 or EGFP alone were immunoprecipitated from lysates of HeLa cells co-expressing EGFP or EGFP-ORP5 with 3XFLAG-ORP8 and analyzed by Western blot using antibodies against GFP (for ORP5) or FLAG (for ORP8).

D Fluorescent images of HeLa cells co-transfected with EGFP-ORP5 and 3XFLAG-ORP8 and stained with anti-FLAG. Cortical layer (upper panel) and medial layer (lower panel) of the same cell are shown. Scale bar, 10 μm.

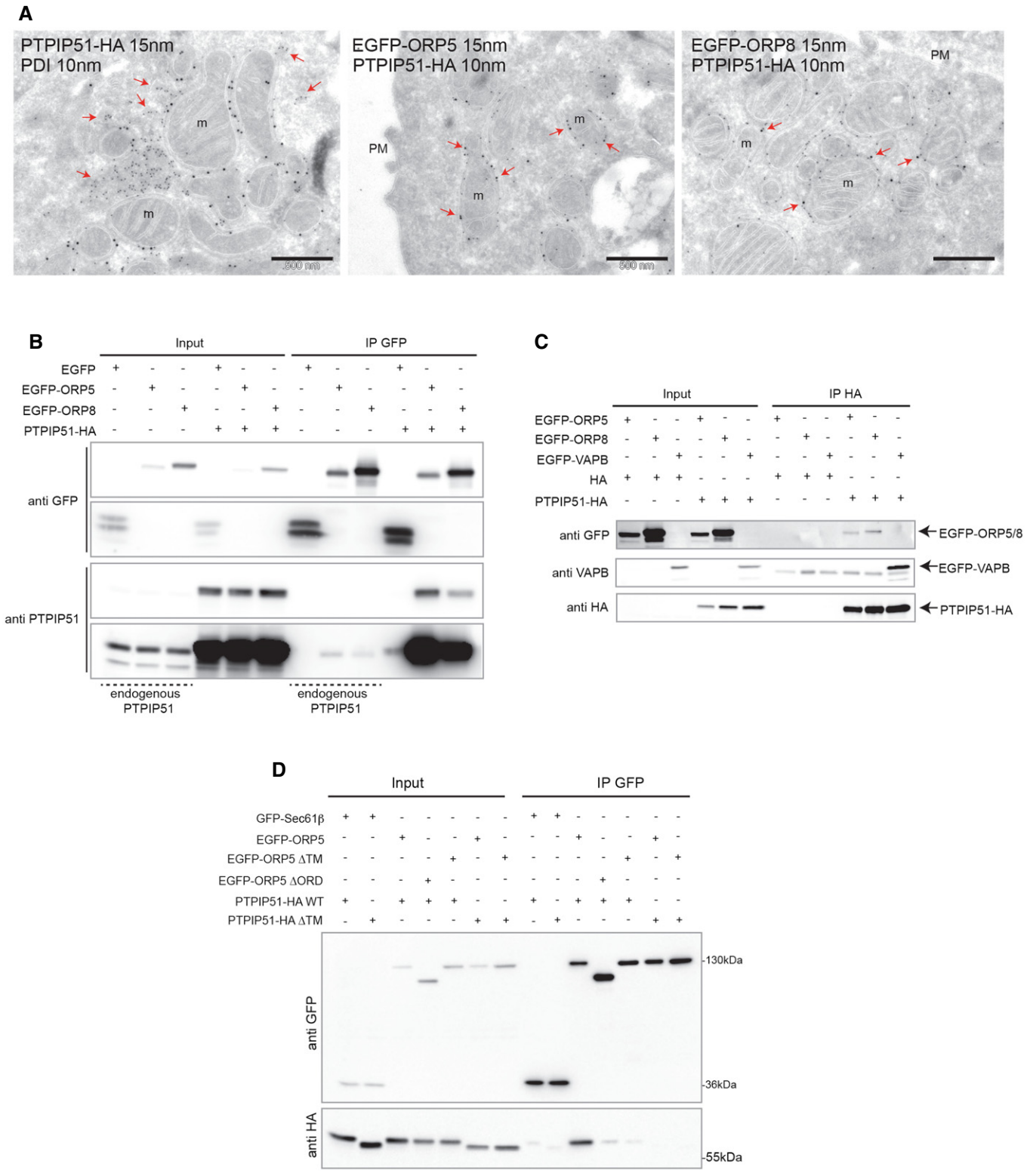

Figure EV3.

◀ **Figure EV3. ORP5 and ORP8 localization at ER–mitochondria contacts is promoted by the mitochondrial protein PTPIP51.**

A Electron micrographs of ultrathin cryosections of HeLa cells transfected with PTPIP51-HA alone, or with EGFP-ORP5 and EGFP-ORP8 together with PTPIP51-HA and double-immunogold stained with anti-PDI to detect the ER (10 nm gold) and anti-HA (15 nm gold) to detect PTPIP51 or with anti-GFP (15 nm gold) to detect ORP5 or ORP8 and anti-HA (10 nm gold) to detect PTPIP51. Red arrows indicate PDI in the lumen of ER structures not in contact with mitochondria, and ORP5 or ORP8 at ER–mitochondria contact sites. Scale bar, 500 nm.

B EGFP, EGFP-ORP5, or EGFP-ORP8 were immunoprecipitated from lysates of HeLa cells co-expressing or not PTPIP51-HA and analyzed by Western blot using antibodies against GFP and PTPIP51 to detect endogenous and overexpressed proteins.

C PTPIP51-HA was immunoprecipitated from lysates of HeLa cells co-expressing EGFP, EGFP-ORP5, or EGFP-ORP8 and analyzed by Western blot using antibodies against GFP and HA.

D GFP-Sec61β, EGFP-ORP5, EGFP-ORP5ΔTM, or EGFP-ORP5ΔORD were immunoprecipitated from lysates of HeLa cells co-expressing PTPIP51-HA or PTPIP51-HA ΔTM and analyzed by Western blot using antibodies against GFP and HA.

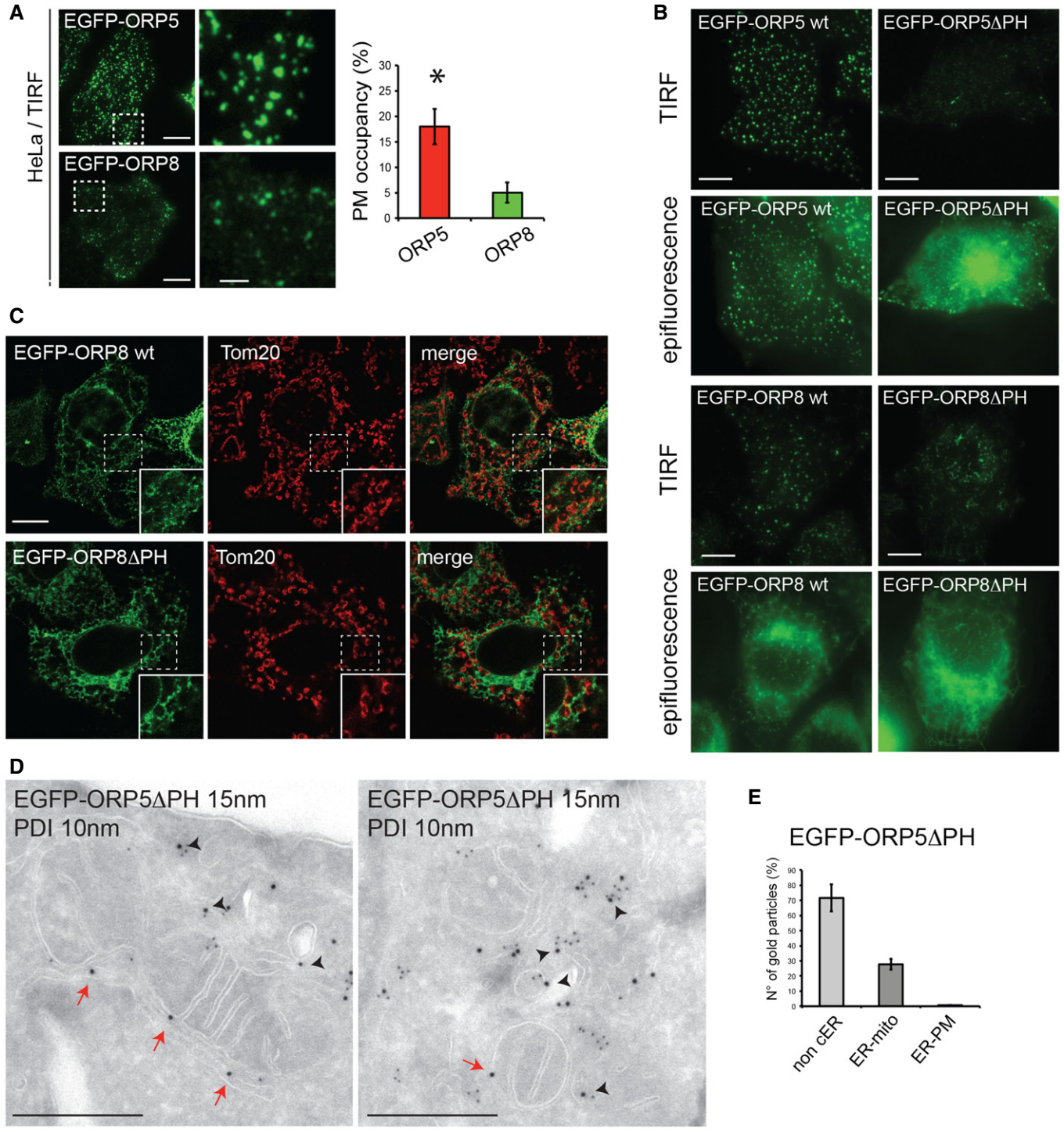

Figure EV4.

◀ **Figure EV4. Recruitment of ORP5 and ORP8 to ER–mitochondria contact sites.**

A TIRF microscopy of HeLa cells overexpressing EGFP-ORP5 or EGFP-ORP8 showing the localization of these proteins within the evanescent field (100 nm from the PM). Scale bar, 10 μm. Quantifications of the TIRF microscopy showing the percentage of PM occupied by cER (% ± SEM, $n = 8$ for each of 3 biological replicates, $^*P < 0.001$).

B Representative TIRF microscopy images (upper panel) and corresponding epifluorescence images (lower panel) of HeLa cells expressing EGFP-ORP5, EGFP-ORP5ΔPH, EGFP-ORP8, or EGFP-ORP8ΔPH. Scale bar, 10 μm.

C Confocal images of HeLa cells transfected with EGFP-ORP8 or EGFP-ORP8ΔPH and immunostained using anti-TOM20 antibody to visualize mitochondria. Scale bar, 10 μm.

D Electron micrographs of ultrathin cryosections of HeLa cells transfected with EGFP-ORP5ΔPH and immunogold stained with anti-GFP (15 nm gold) to detect ORP5ΔPH and anti-PDI (10 nm gold) to detect the ER. Red arrows indicate EGFP-ORP5ΔPH at ER–mitochondria contact sites. Black arrowheads indicate EGFP-ORP5ΔPH in the reticular ER. Scale bar, 200 nm.

E Quantification of the IEM labeling for EGFP-ORP5ΔPH in transfected HeLa cells. Results are presented as the percentage of the total number of gold particles (800 per conditions) for ORP5ΔPH in the indicated compartments (non-cER, non-cortical ER; ER-mito, ER–mitochondria contact sites; ER-PM, ER–PM contact sites). % gold particles ± SEM, $n = 32$ cells, $^*P < 0.001$ compared to non-cER and ER-mito and to ER-PM.