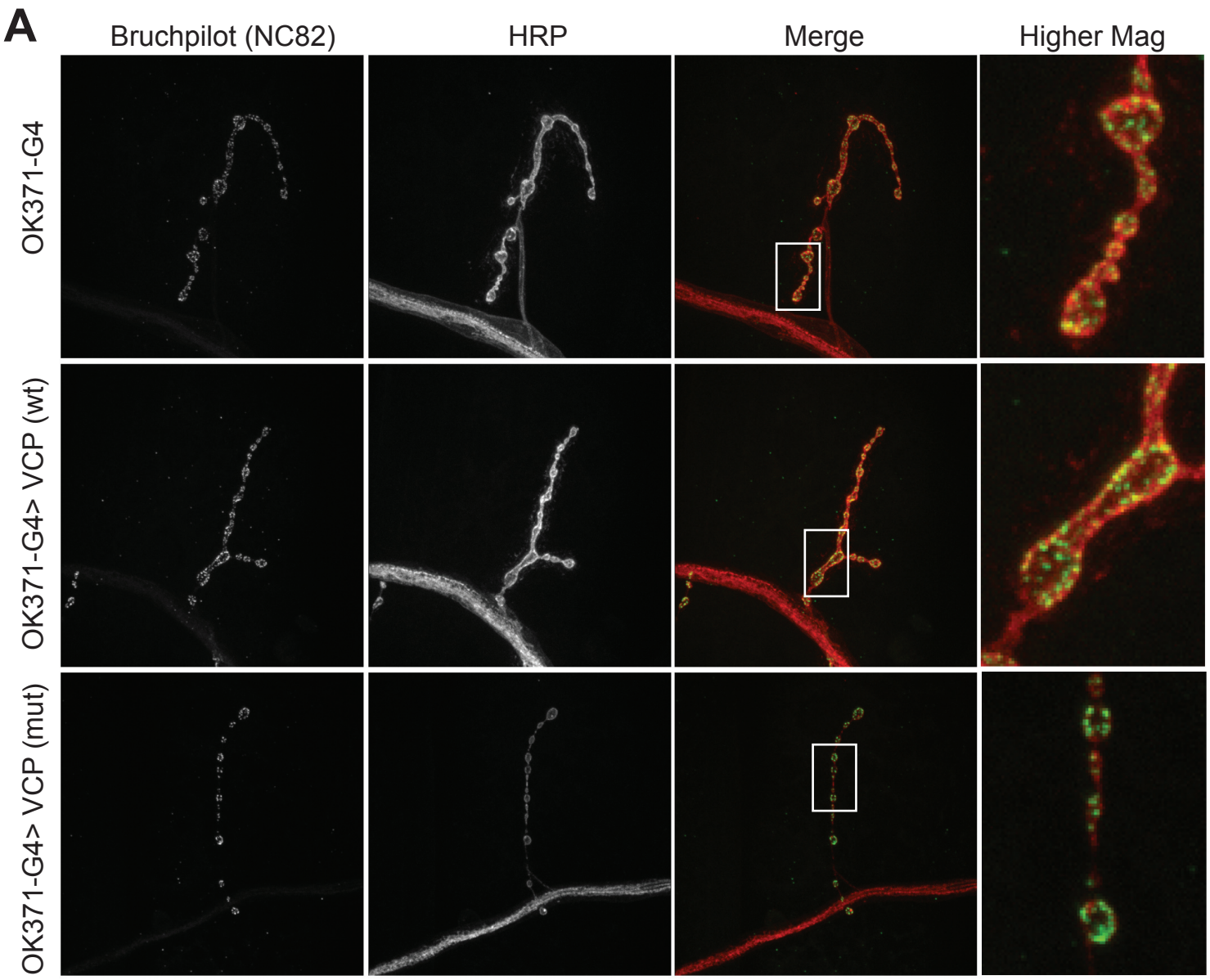
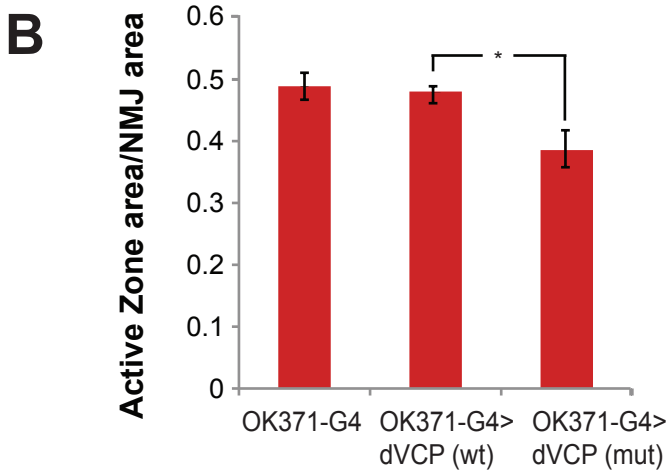
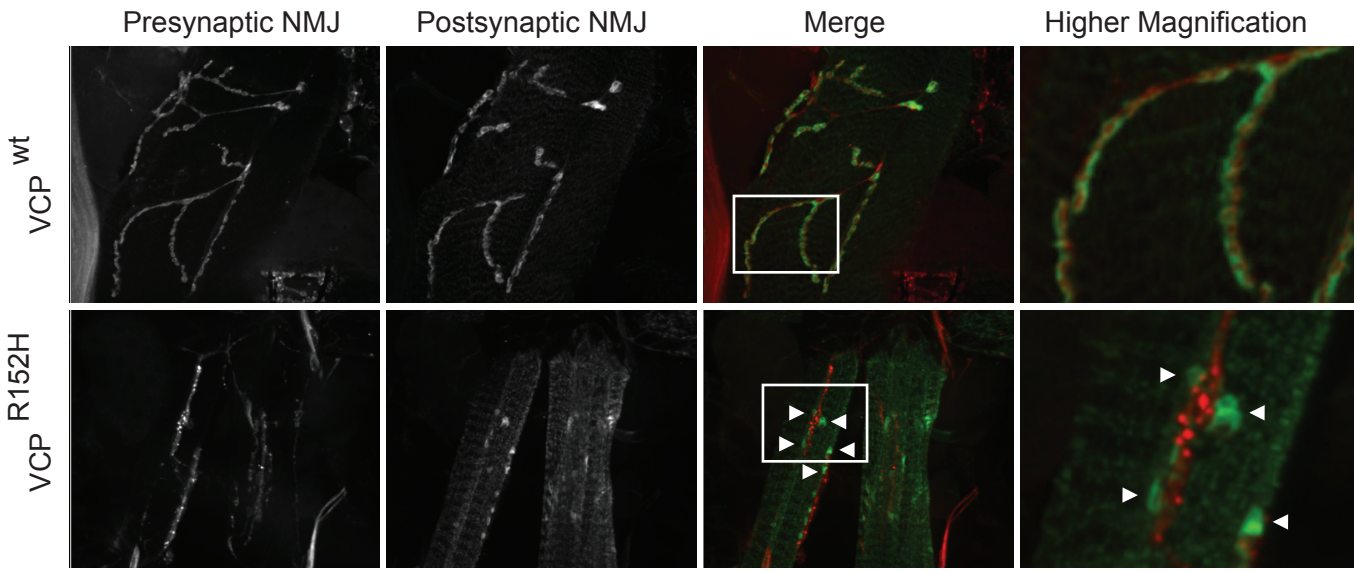
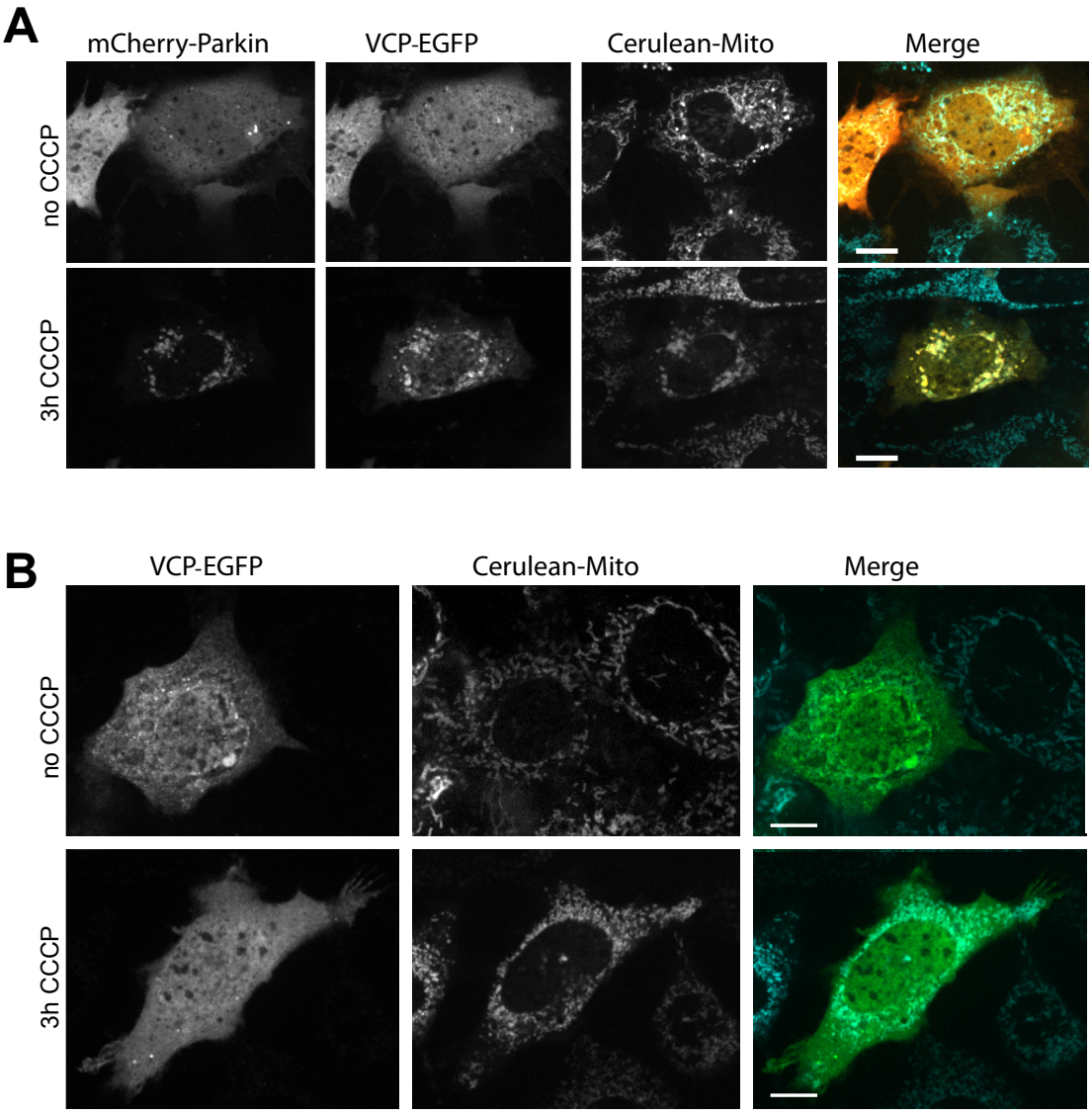




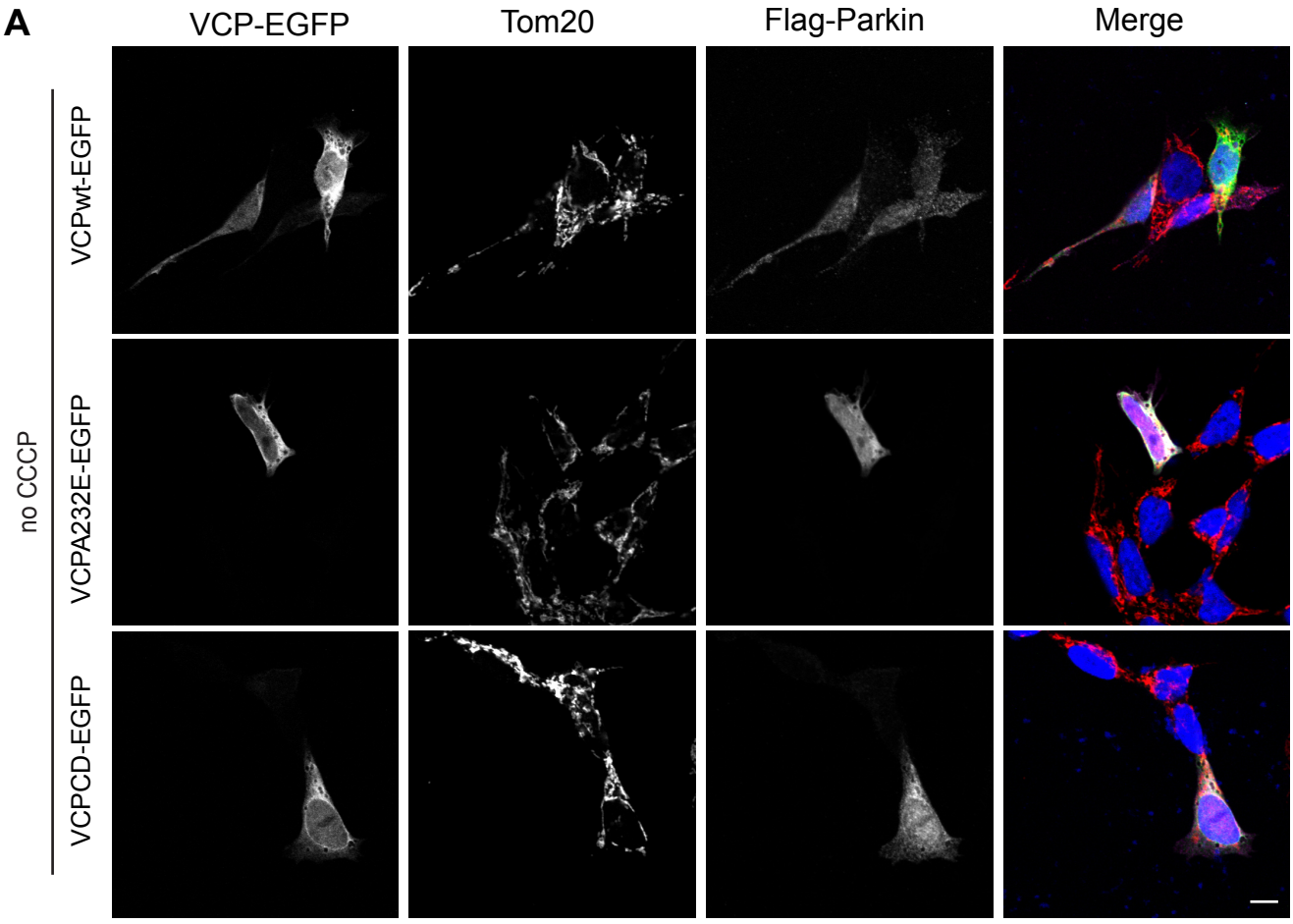
Kim et al. Supplemental Figure 1

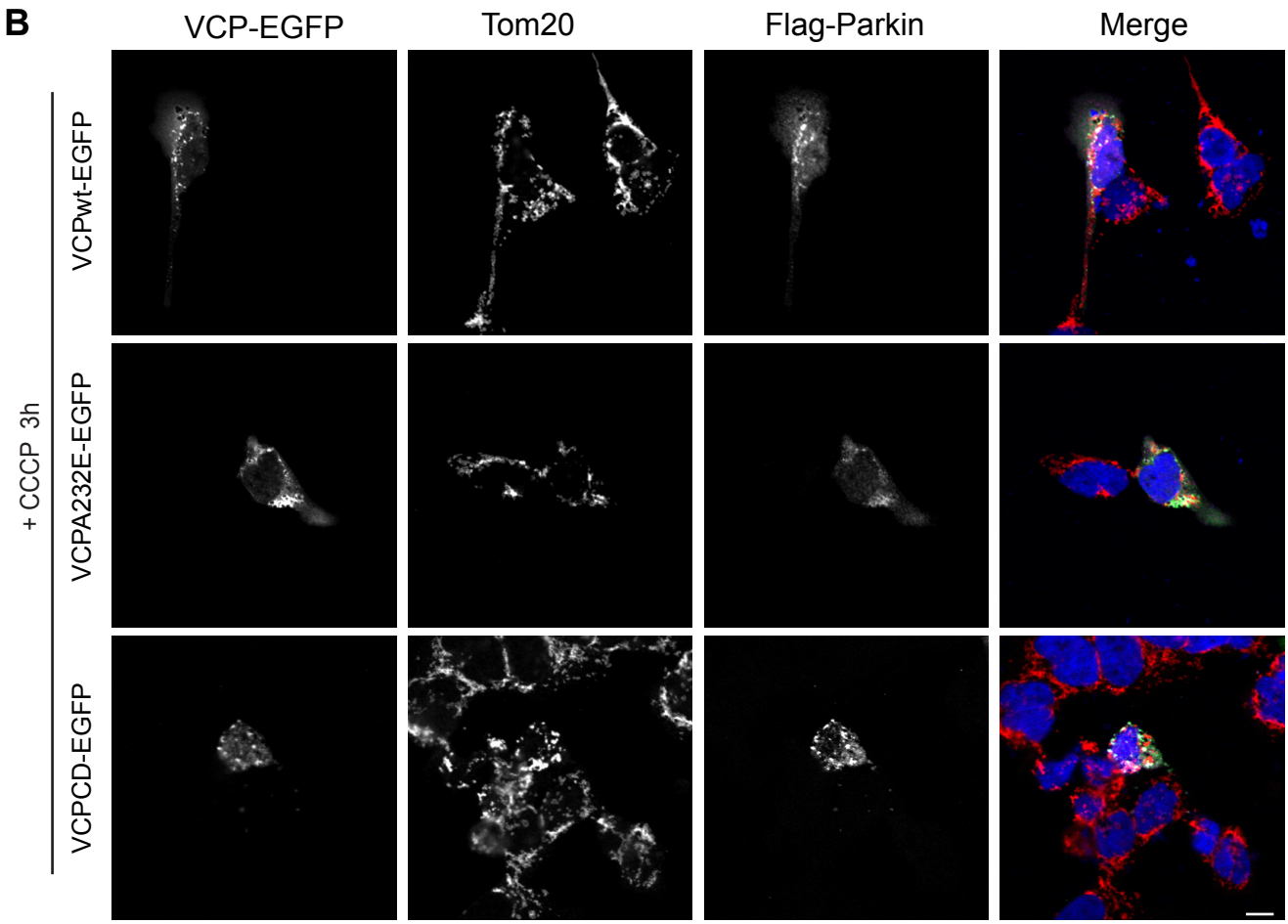


Kim et al. Supplemental Figure 2



Kim et al. Supplemental Figure 3





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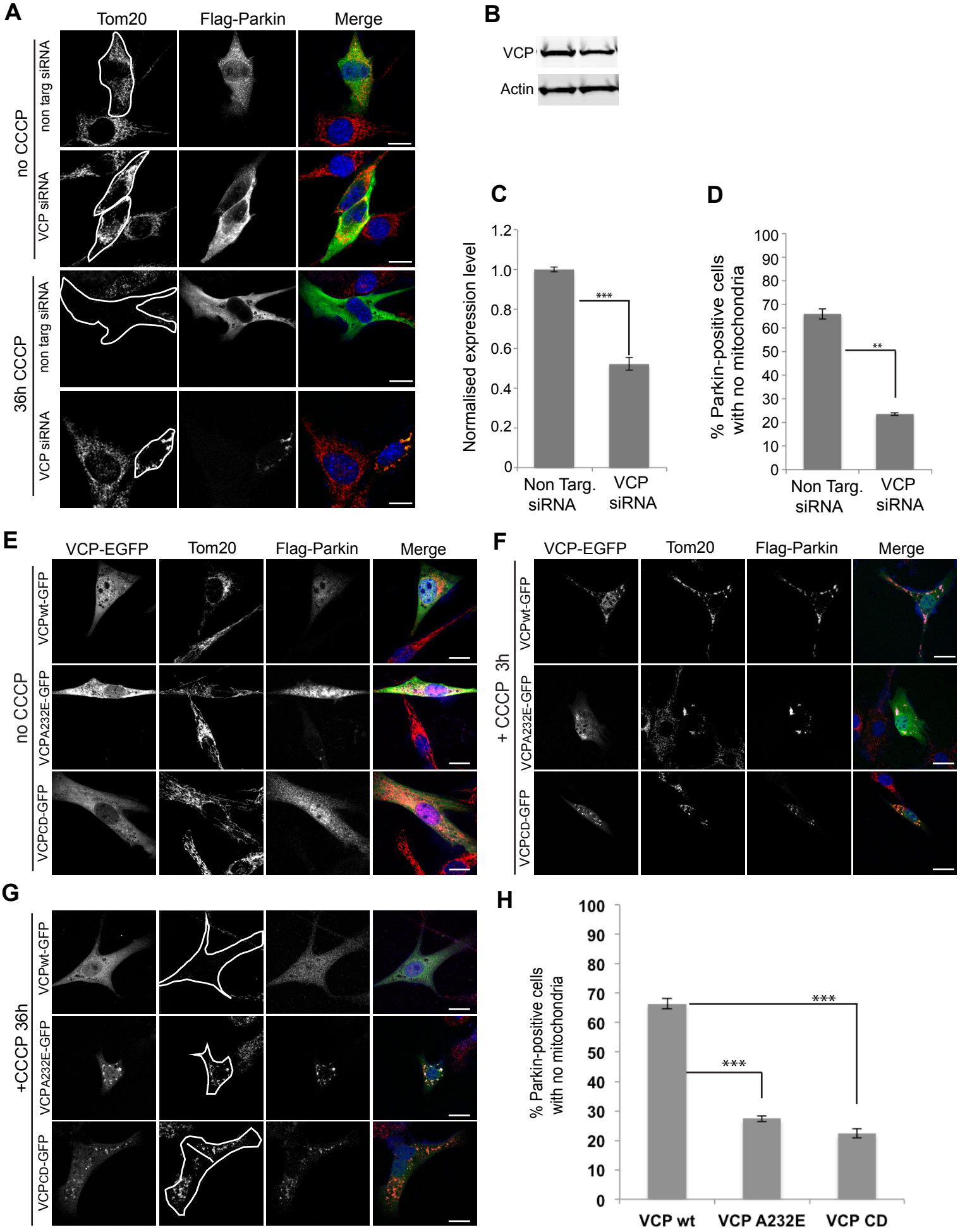

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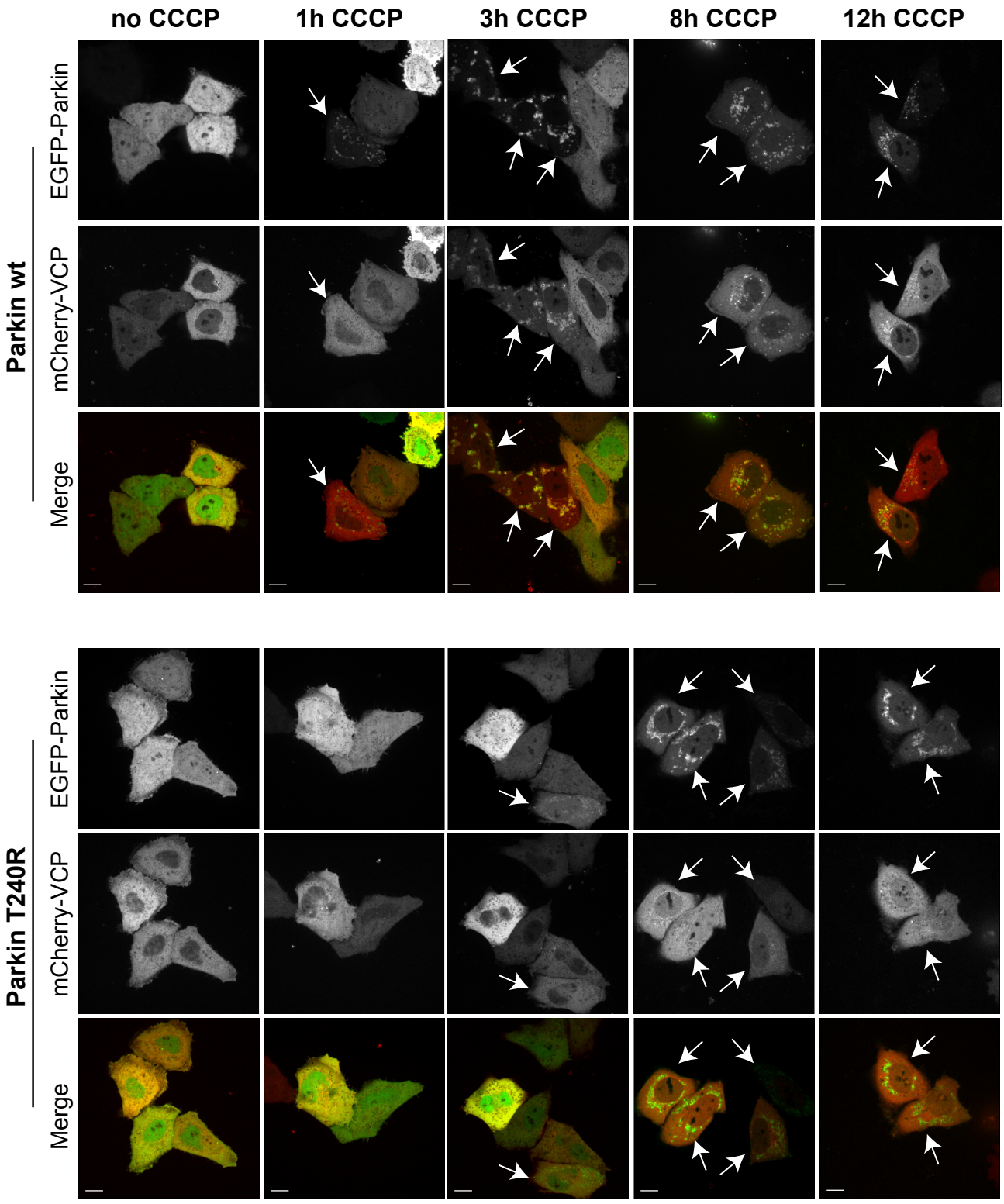

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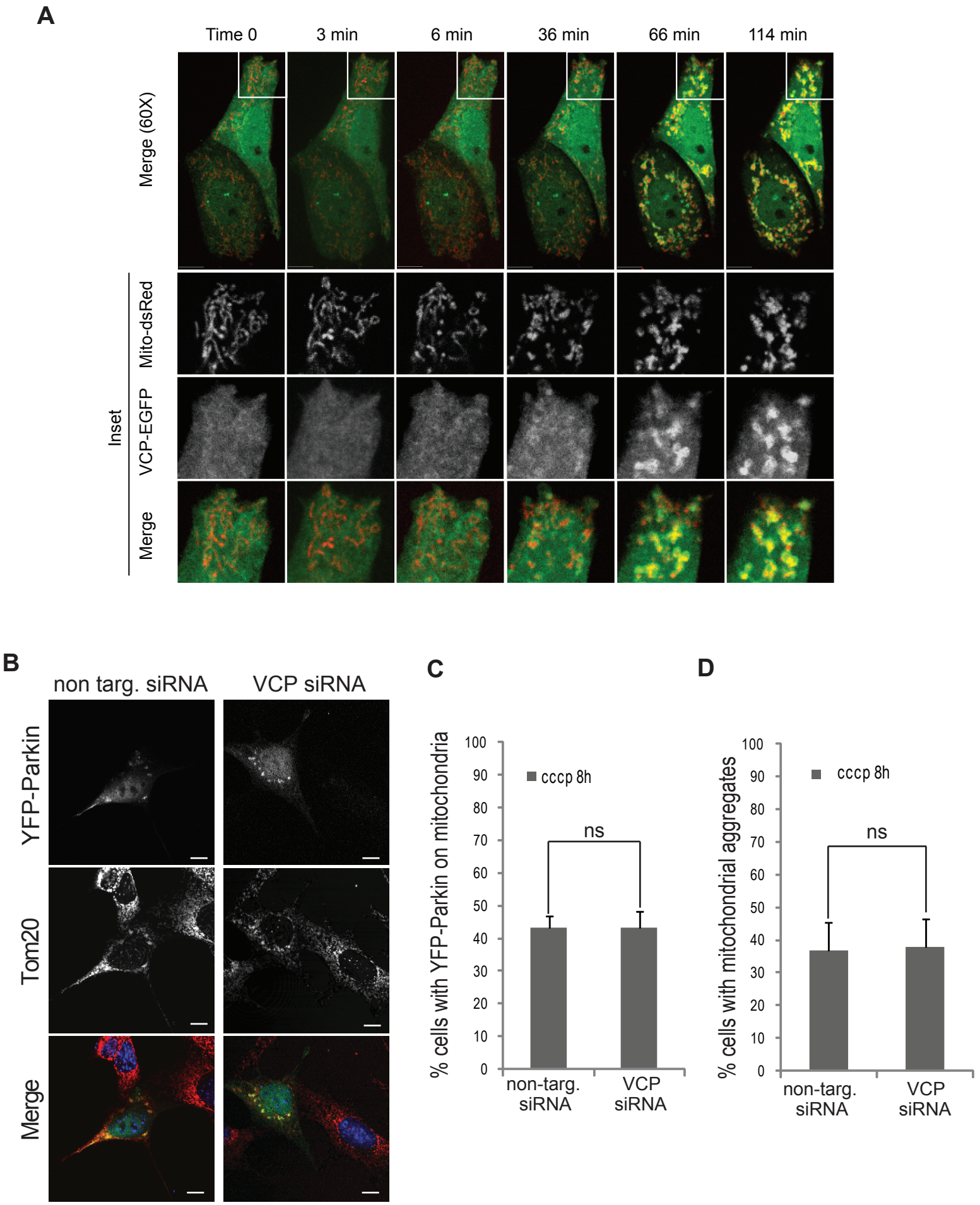
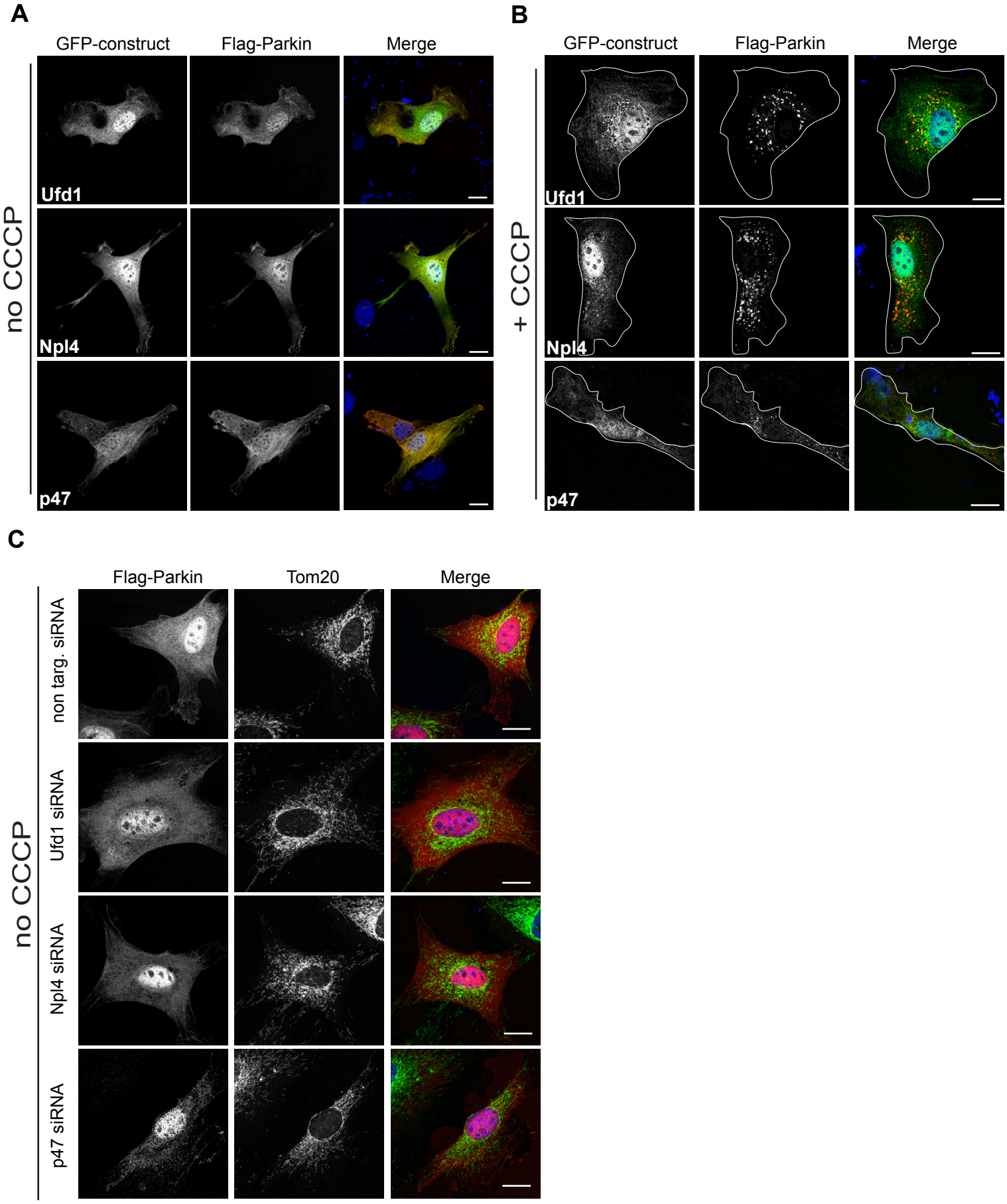


Kim et al. Supplemental Figure 7



Kim et al. Supplemental Figure 8

Supplemental Figure Legends

Supplemental Figure 1. dVCP mutation-dependent reduction in active zones in NMJ of *Drosophila*, related to Figure 1 **A.** Expression of mutant dVCP (but not wild type) in motor neurons resulted in abnormal NMJ morphology with reduced numbers of active zones. The presynaptic area was stained with anti-HRP antibody and an active zone marker Bruchpilot was stained with antibody NC82. NMJs at muscle 4 were used for all analyses. Panel **A** shows representative images from control flies (OK371-GAL4) or flies expressing wild type dVCP or mutant dVCP under control of the motor neuron driver OK371-GAL4 and Panel B shows quantitative analysis from N = 12 NMJs from 3 animals per genotype. Error bars indicate standard error.

Supplemental Figure 2. dVCP mutation-dependent NMJ defect and denervation in adult *Drosophila* escapers, related to Figure 2. Expression of mutant dVCP (but not wild type) in motor neurons resulted in abnormal NMJ morphology with accumulation of 'footprints' representing areas where postsynaptic membrane lacks appositional presynaptic membrane, consistent with denervation. Presynaptic membrane is stained with anti-HRP antibody and postsynaptic membrane is stained with anti-discs large antibody.

Supplemental Figure 3. VCP and Parkin relocalize to mitochondria in MEFs after mitochondrial depolarization with CCCP, and VCP recruitment requires exogenous Parkin, related to Figure 3. **A.** VCP-EGFP and mCherry-Parkin in Mito-Cerulean stable MEFs at 0 and 3h after addition of CCCP. Scale bars equal 10 μm. **B.**VCP-EGFP in Mito-Cerulean stable MEFs at 0 and 3h after addition of CCCP. Scale bars equal 10 μm.

Supplemental Figure 4. Parkin and wild type or mutant forms of VCP relocalize to mitochondria in Sy5y cells after mitochondrial depolarization with CCCP, related to Figure 3. **A.** VCP-EGFP and FLAG-Parkin are diffuse in Sy5y cells pretreatment. Mitochondria were stained with TOM20. Scale bars equal 10 μm. **B.**VCP-EGFP and

FLAG-Parkin relocalized to mitochondria 3h after addition of CCCP. Scale bars equal 10 μm.

Supplemental Figure 5. VCP is essential for clearance of damaged mitochondria in C2C12 cells and clearance of damaged mitochondria is impaired by VCP mutations, related to Figure 3 and Figure 8. **A.** Immunostaining against TOM20 (red) in C2C12 cells transfected with FLAG-Parkin (green) and non-targeting or VCP-targeting siRNA. Cells were treated with DMSO or CCCP for 36 h. **B.** Immunoblot of VCP and Actin in C2C12 cells transfected with non-targeting or VCP-targeting siRNA. **C.** Quantification of VCP normalized against Actin in cells transfected with non-targeting or VCP-targeting siRNA. Error bars indicate standard deviation from triplicates. **D.** Quantification of cells with mitochondrial clearance. Cells were treated for 36 h with DMSO or CCCP. At least 30 cells were counted for each sample. Errors bars indicate standard error from 3 independent replicates. **E.** Imaging VCP-EGFP and immunostaining for FLAG-Parkin and TOM20 in C2C12 cells prior to with CCCP. Scale bars equal 10μm. **F.** FLAG-Parkin and wild type and mutant forms of VCP-EGFP relocalized to mitochondria 3h after addition of CCCP. **G.** 36h after treatment with CCCP, mitochondria were cleared in cells expressing wild type but not mutant forms of VCP. **H.** Quantification of cells with mitochondrial clearance. At least 30 cells were counted for each sample. Errors bars indicate standard error from 3 independent replicates.

Supplemental Figure 6. Mitochondrial ubiquitination by Parkin is essential for VCP recruitment, related to Figure 5. Cells were transfected with VCP-mCherry-and EGFP-Parkin wt or -Parkin ^{T240R} and VCP recruitment was monitored for the times indicated following treatment with CCCP. Scale bars equal 10 μm. Arrows indicate cells relocalization of Parkin to mitochondria. VCP relocalize to mitochondria in cells expressing GFP-Parkin wt but not GFP-Parkin^{T240R}.

Supplemental Figure 7. Recruitment of VCP to mitochondria coincides with the beginning of mitochondrial fragmentation and mitochondrial clearance requires VCP, related to a paragraph "VCP contributes to regulation of mitochondrial dynamics"

and Figure 7. **A.** Time lapse imaging of mitochondria (Mito-dsRed) and VCP (VCP-EGFP) following treatment with CCCP. Recruitment of VCP to mitochondria immediately precedes mitochondrial fission. These still images were extracted from Supplemental Movie 3. **B.** Immunostaining against TOM20 (red) in MEFs transfected with YFP-Parkin (green) and non-targeting or VCP-targeting siRNA. Cells were treated with CCCP for 8 hours. Scale bars equal 10 μm. **C.** Quantification of cells with Parkin on mitochondria. Cells were treated for 8 hours with CCCP. At least 30 cells were counted for each sample. Errors bars indicate standard error from 3 independent replicates. **D.** Quantification of cells with aggregation of mitochondria. Cells were treated for 8 hours with CCCP. At least 30 cells were counted for each sample. Errors bars mean standard error from 3 independent replicates.

Supplemental Figure 8. Parkin and the VCP adaptors Ufd1 and Npl4 (but not p47) relocalize to mitochondria after mitochondrial depolarization with CCCP, related to Figure 7. **A.** GFP-tagged Ufd1, Npl4, or p47 together with FLAG-Parkin are diffuse in MEFs pretreatment. Scale bars equal 10 μm. **B.** GFP-tagged Ufd1, Npl4, but not p47 together relocalized to mitochondria in concert with FLAG-Parkin 3h after addition of CCCP. Scale bars equal 10 μm. **C.** Immunostaining against TOM20 (red) in MEFs transfected with FLAG-Parkin (green) and non-targeting siRNA or siRNA targeting Ufd1, Npl4, or p47. Cells were treated with DMSO or CCCP for 24 h. (Cells treated with CCCP shown in Figure 7F).

Supplemental Movie Files

Supplementary Movie 1. VCP-EGFP and Parkin-mCherry observation following addition of CCCP in HeLa cells. Movie starts 15 min after addition of CCCP, related to Figure 3.

Supplementary Movie 2. Magnification of Supplementary Movie 1.

Supplementary Movie 3. VCP-EGFP and Mito-DsRed observation following addition of CCCP in HeLa cells expressing Flag-Parkin, related to Supplemental Figure 7A.