SUPPLEMENTAL FIGURE LEGENDS

Supplemental Figure S1. The anti-VDAC antibody recognizes VDAC1 and VDAC3 but not VDAC2

(A) Lysates from wild-type MEFs and VDAC1/3$^{-/-}$ MEFs were analyzed by western blotting with anti-VDAC (top panel) or anti-Tom20 antibodies (bottom panel). The anti-VDAC antibody does not recognize VDAC2 in VDAC1/3$^{-/-}$ MEFs.

(B) HA-tagged VDAC1, 2 or 3 were expressed in VDAC1/3$^{-/-}$ MEFs, immunoprecipitated with anti-HA antibodies and analyzed by western blotting with anti-HA antibodies (top panel) or anti-VDAC antibodies (bottom panel). The anti-VDAC antibodies recognize HA-VDAC1 and HA-VDAC3, but not HA-VDAC2. Note that HA-VDAC3 migrates at a lower molecular weight than HA-VDAC1 and HA-VDAC2.

Molecular weights are indicated in kDa.

Supplemental Figure S2. CCCP disrupts the inner mitochondrial membrane potential

(A) VDAC1/3$^{-/-}$ MEFs were incubated with 20 μM CCCP or DMSO as a control for 20 min, and then with 50 nM tetramethylrhodamine methyl ester (TMRM) or no TMRM as a negative control (blue line) for 20 min to label mitochondria with an intact inner membrane potential. Subsequently, fluorescence of 10,000 cells was measured with a flow cytometer (BD-LSR, BD Biosciences) using excitation from a 488nm laser and a 575/26 nm emission filter. CCCP treatment efficiently blocks TMRM labeling.

(B) VDAC1/3$^{-/-}$ MEFs were transfected with control or VDAC2 siRNAs. 48 hrs post-transfection, cells were treated and analyzed as in (A). CCCP treatment efficiently prevents TMRM labeling of cells under these conditions.

(C) Mean fluorescence values from (A) and (B) are shown. CCCP treatment reduces TMRM fluorescence of cells two- to threefold.

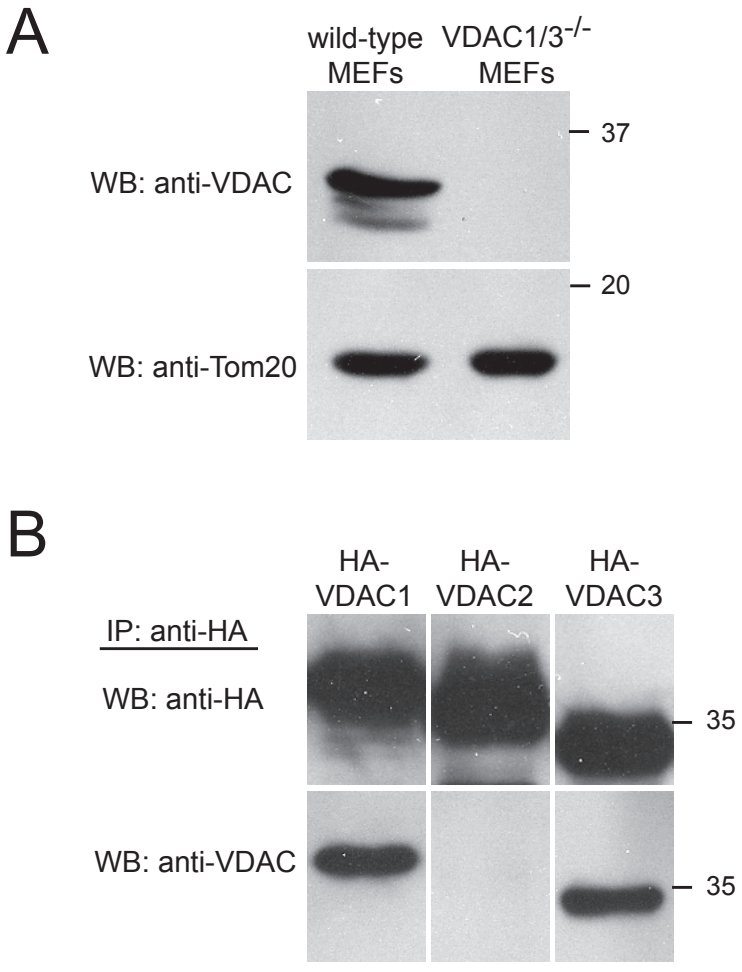
Supplemental Figure S3: Parkin localization in VDAC1/3$^{-/-}$ MEFs expressing VDAC1 or VDAC3

(A) VDAC1/3$^{-/-}$ MEFs expressing Flag-Parkin and HA-VDAC1 were transfected with the indicated siRNAs. 48hrs post-transfection, cells were immunostained for Parkin and the mitochondrial marker Tom20. Parkin is diffusely localized throughout the cell.

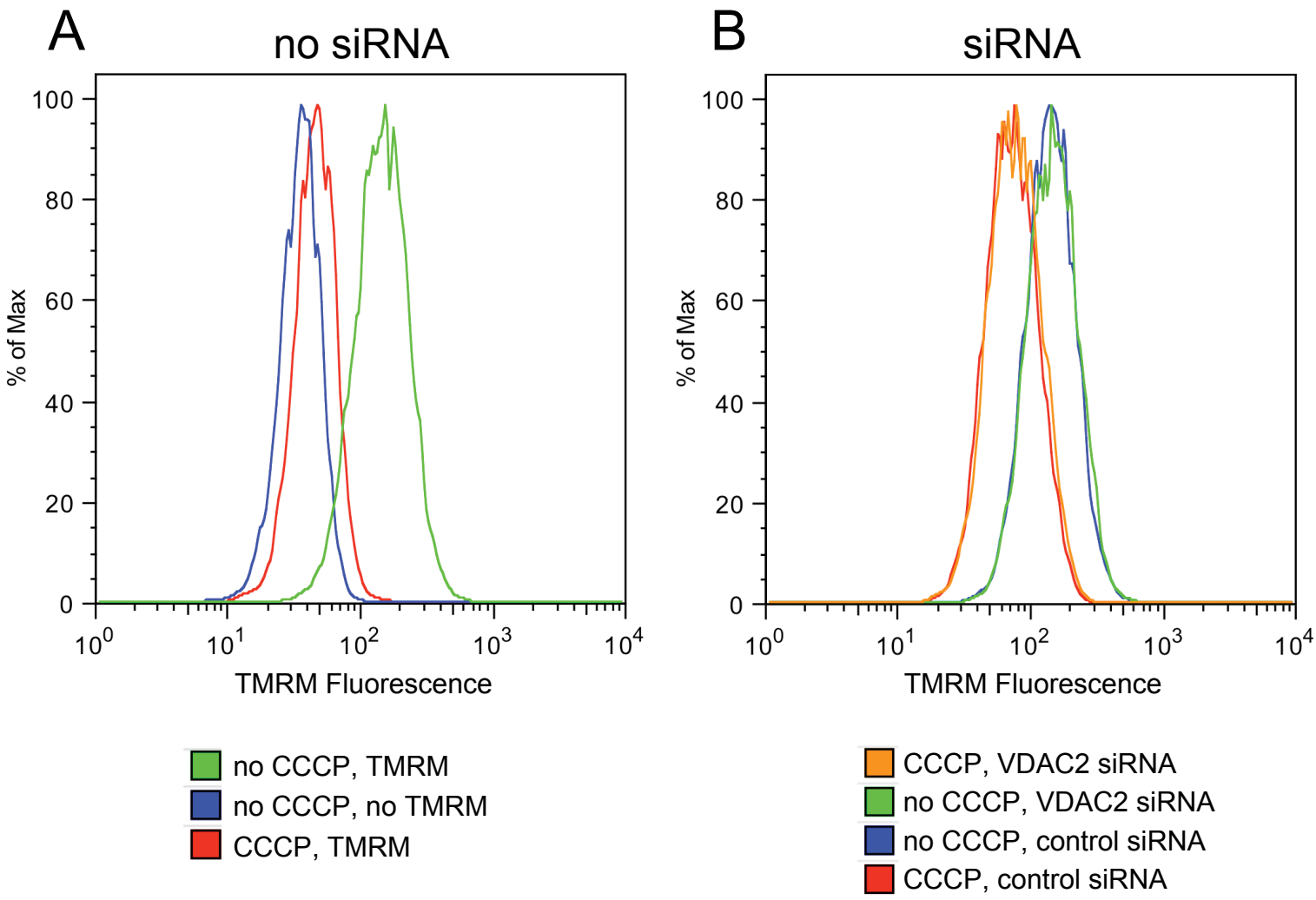
(B) As in (A), except that VDAC1/3$^{-/-}$ MEFs expressed Flag-Parkin and HA-VDAC3, and were immunostained for HA-VDAC3 instead of Tom20.

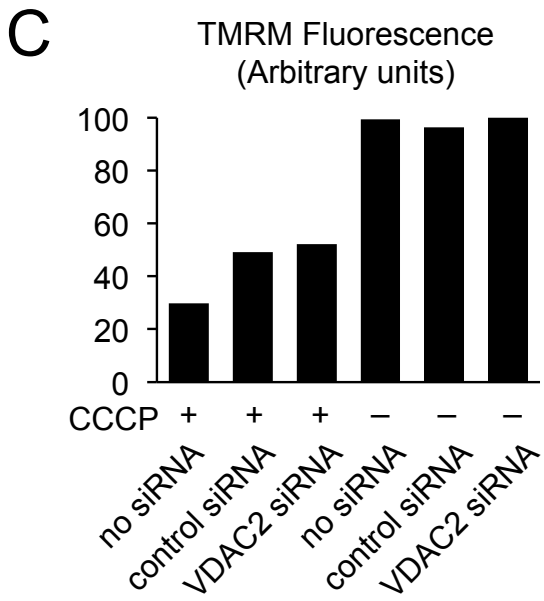
Scale bar, 20μm.

Supplemental Figure S1



Supplemental Figure S2





Supplemental Figure S3