Landes Bioscience www.landesbioscience.com

Supplemental Material to:

Shubhra Majumder, Mark Slabodnick, Amanda Pike, Joseph Marquardt and Harold A. Fisk

VDAC3 regulates centriole assembly by targeting Mps1 to centrosomes

2012; 11(19)
http://dx.doi.org/10.4161/cc.21927

http://www.landesbioscience.com/journals/cc/article/21927

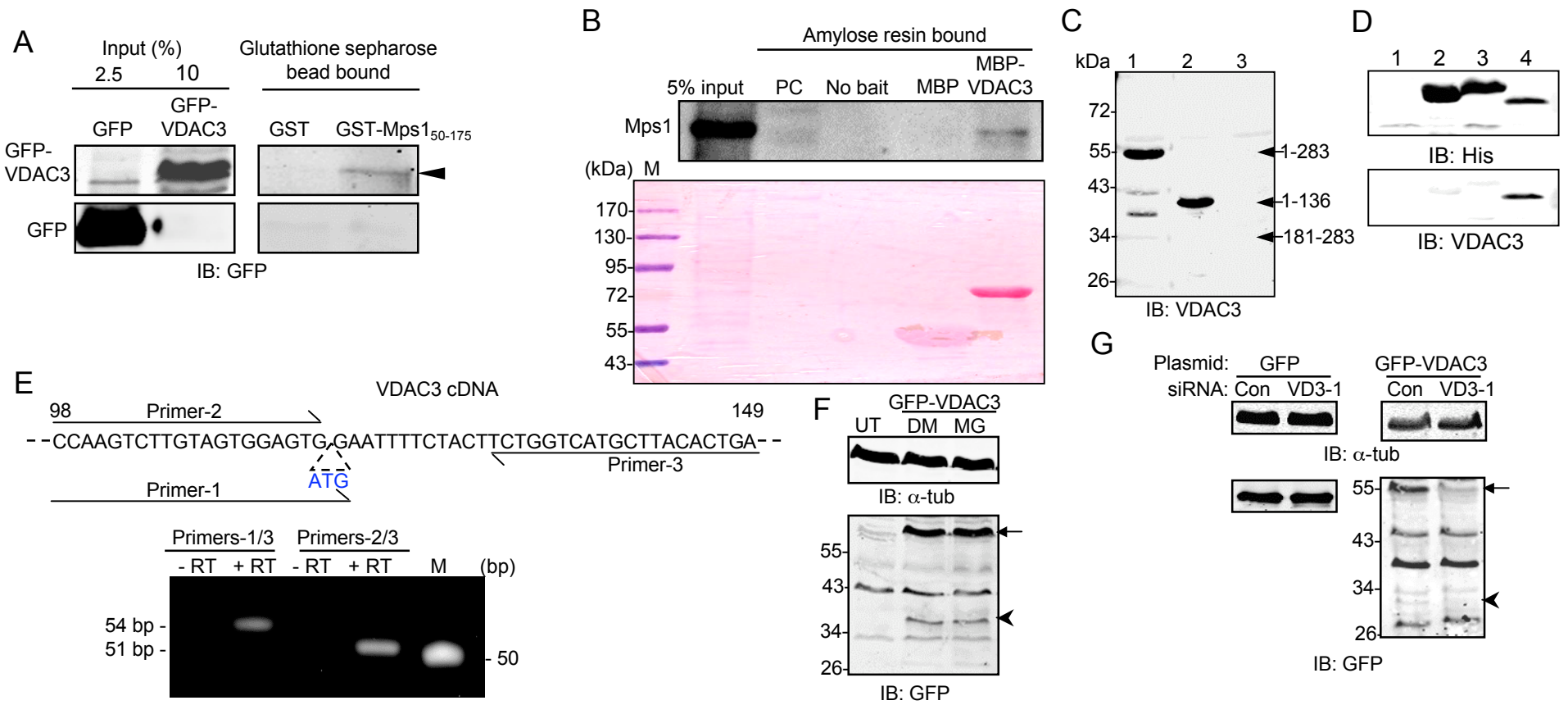


Fig. S1. [A] *GST-Mps1$_{50-175}$ interacts with VDAC3.* Glutathione sepharose beads bound to GST or GST-Mps1$_{50-175}$ were incubated with lysates from HEK293 cells expressing GFP or GFP-VDAC3, washed and analyzed by immunoblotting using anti-GFP antibody. **[B]** *MBP-VDAC3 interacts with Mps1.* After pre-clearing (PC), the cellular lysate (roughly 750 µg of total protein) from HeLa cells were incubated with MBP or MBP-VDAC3 immobilized on amyloze resin or the resin alone (no bait) for 4 h. Bead-bound proteins were immunoblotted (ponceau S stained membrane is shown at the bottom where M is the marker) with anti-Mps1 antibody. **[C]** *The VDAC3 antibody is specific to the N-terminus of VDAC3.* Crude lysates of bacterial cells expressing GST-VDAC3$_{1-283}$ (1), GST-VDAC3$_{1-136}$ (2) and GST-VDAC3$_{181-283}$ (3) were immunoblotted using anti-VDAC3 antibody. The positions of the recombinant proteins on the immunoblots are shown. **[D]** *The anti-VDAC3 antibody barely detects VDAC1 or VDAC2 on immunoblots*. Crude lysates of uninduced (1) and induced bacterial cells expressing His-VDAC1 (2), His-VDAC2 (3), His-VDAC3 (4) were immunoblotted with anti-His and anti-VDAC3 antibodies. At least ten-fold excess of His-VDAC1 or His-VDAC2 compared to His-VDAC3, as analyzed by coomassie staining were used for immunoblotting. **[E]** *The most abundant isoform of VDAC3 mRNA lacks the ^{118}ATG*. Analysis of the RT-PCR products (+/- indicates with or without the RT enzyme) on total RNA isolated from RPE1 cells. The position and nucleotide compositions of the primers used for RT-PCR are aligned with the VDAC3 cDNA sequence (nucleotides 98-149). The presence of the additional ATG (^{118}ATG; shown in blue) in VDAC3 cDNA would allow efficient binding of the 3'-end of the primer1. **[F]** *A truncated form of GFP-VDAC3 that did not arose via degradation was detected in cells expressing GFP-VDAC3.* Lysates of untransfected (UT) and transfected HeLa cells with a GFP-VDAC3 construct were immunoblotted using anti-GFP antibody. Transfected cells were treated with proteasome inhibitor MG115 or DMSO as solvent control. [G] GFP-VDAC3 expression was downregulated in cells treated with VDAC3 siRNA. Lysates of control and VDAC3- siRNA treated HeLa cells transfected with GFP or GFP-VDAC3 construct were analyzed by immunoblotting. In both [F] and [G], arrow shows the band of expected size (roughly 55 kDa), arrowhead shows the truncated form of GFP-VDAC3 and numbers at the right indicates the molecular weight standards (kDa) while α-tubulin was the loading control.

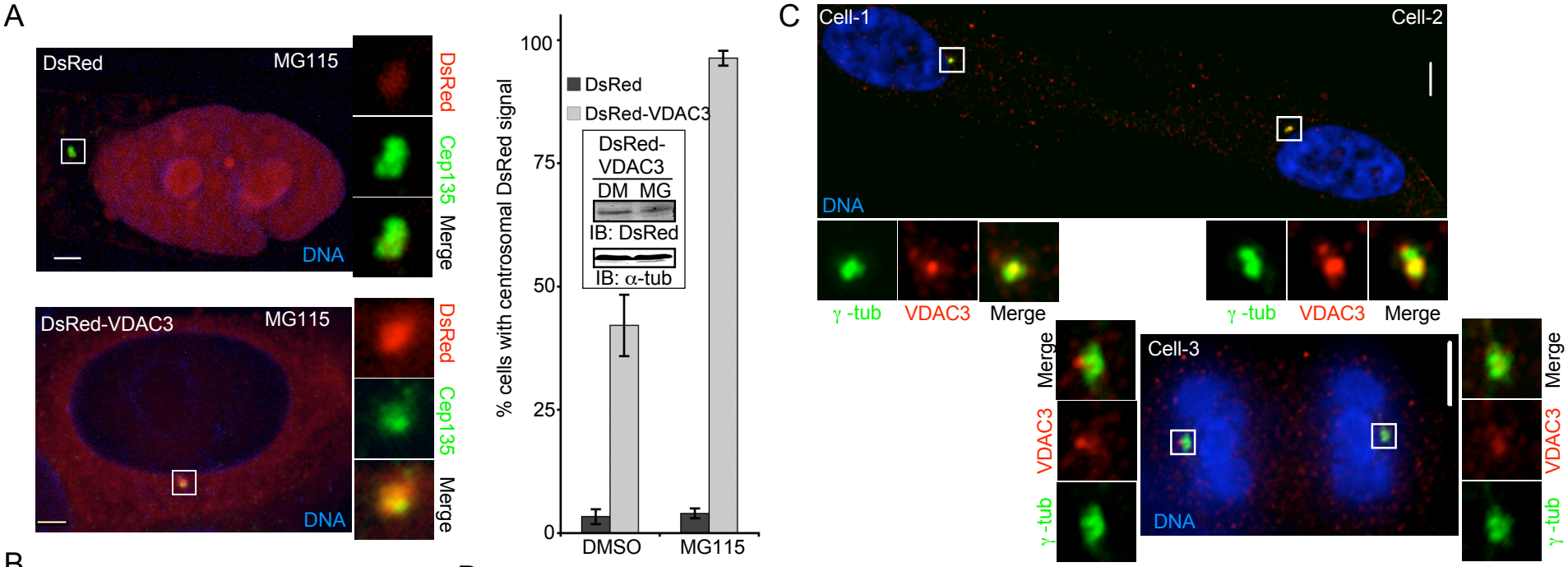


Fig. S2. [A] *Centrosomal localization of an ectopically expressed VDAC3 was enhanced by proteasome inhibition*. HeLa cells expressing either DsRed alone or DsRed-VDAC3 were arrested in S-phase treated with either MG115 or DMSO for the last four hour of HU treatment, fixed and stained for Cep135 (green). Shown are representative images of MG115-treated cells. DNA is blue and bar is 5 μm. In the graph, values represent the mean ± S.D. of three independent experiments where 50-100 cells were analyzed for the presence of DsRed signal (red) at the centrosomes, per replicate. Immunoblots show the level of DsRed-VDAC3 in DMSO or MG115 treated cells where α-tubulin was the loading control. **[B]** *The centrosomal signal detected by VDAC3 antibody was blocked by the purified VDAC3.* Representative images of HeLa cells stained for DNA (blue), γ-tubulin (γ-tub, green) and VDAC3 (red) using a VDAC3 antibody that was pre-soaked on purified GST alone or GST-VDAC3$_{1-136}$, immobilized on glutathione sepharose beads. Here and in subsequent supplemental figures, panels show magnified images of the box surrounding the centrosomes. DNA is blue and bar is 5 μm. **[C]** The centrosomal VDAC3 signal varies with cell cycle. Shown are representative images of the RPE1 cells from the same coverslip that are stained for γ-tubulin (γ-tub, green) and VDAC3 (red), and were imaged under identical conditions. Based on centrosome number of these cells that were asynchronously growing, we considered cell-1 was at G1, cell-2 at S and cell-3 at M-phase of the cell cycle. **[D]** Representative image of asynchronously growing RPE1 cells expressing GFP-Mps1$_{53-175}$ (GFP-CLD) that were treated with MG115 and stained for γ-tubulin (γ-tub; red). DNA is blue and bar is 5 μm in [C] and [D].

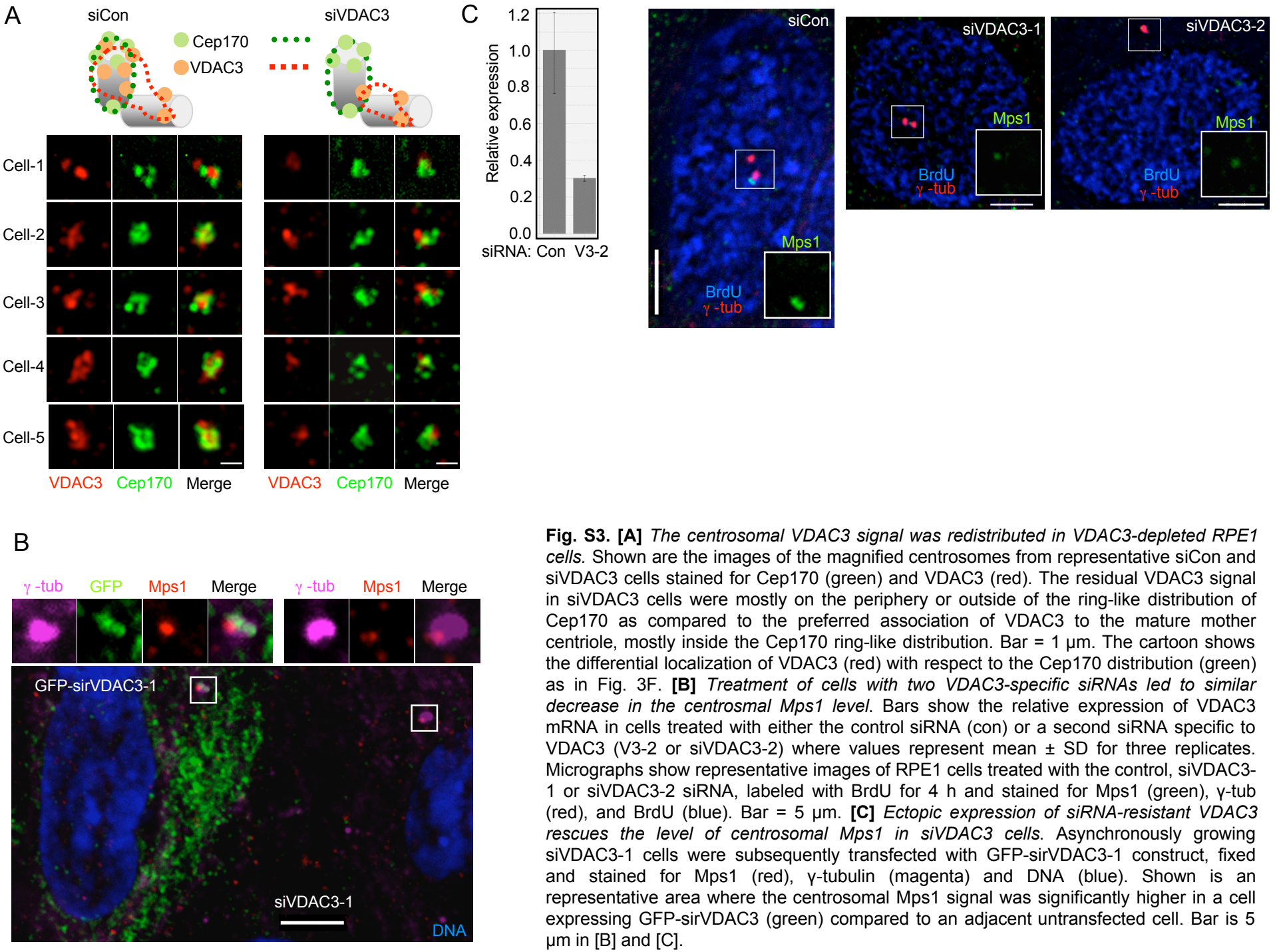


Fig. S3. [A] *The centrosomal VDAC3 signal was redistributed in VDAC3-depleted RPE1 cells.* Shown are the images of the magnified centrosomes from representative siCon and siVDAC3 cells stained for Cep170 (green) and VDAC3 (red). The residual VDAC3 signal in siVDAC3 cells were mostly on the periphery or outside of the ring-like distribution of Cep170 as compared to the preferred association of VDAC3 to the mature mother centriole, mostly inside the Cep170 ring-like distribution. Bar = 1 μm. The cartoon shows the differential localization of VDAC3 (red) with respect to the Cep170 distribution (green) as in Fig. 3F. **[B]** *Treatment of cells with two VDAC3-specific siRNAs led to similar decrease in the centrosmal Mps1 level*. Bars show the relative expression of VDAC3 mRNA in cells treated with either the control siRNA (con) or a second siRNA specific to VDAC3 (V3-2 or siVDAC3-2) where values represent mean ± SD for three replicates. Micrographs show representative images of RPE1 cells treated with the control, siVDAC3-1 or siVDAC3-2 siRNA, labeled with BrdU for 4 h and stained for Mps1 (green), γ-tub (red), and BrdU (blue). Bar = 5 μm. **[C]** *Ectopic expression of siRNA-resistant VDAC3 rescues the level of centrosomal Mps1 in siVDAC3 cells.* Asynchronously growing siVDAC3-1 cells were subsequently transfected with GFP-sirVDAC3-1 construct, fixed and stained for Mps1 (red), γ-tubulin (magenta) and DNA (blue). Shown is an representative area where the centrosomal Mps1 signal was significantly higher in a cell expressing GFP-sirVDAC3 (green) compared to an adjacent untransfected cell. Bar is 5 μm in [B] and [C].

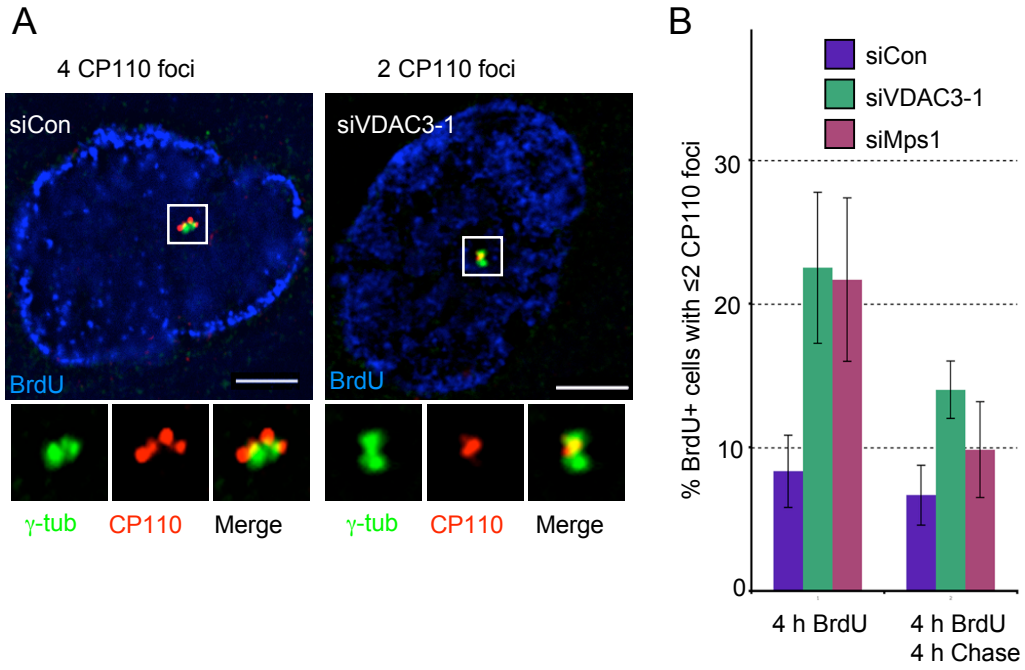
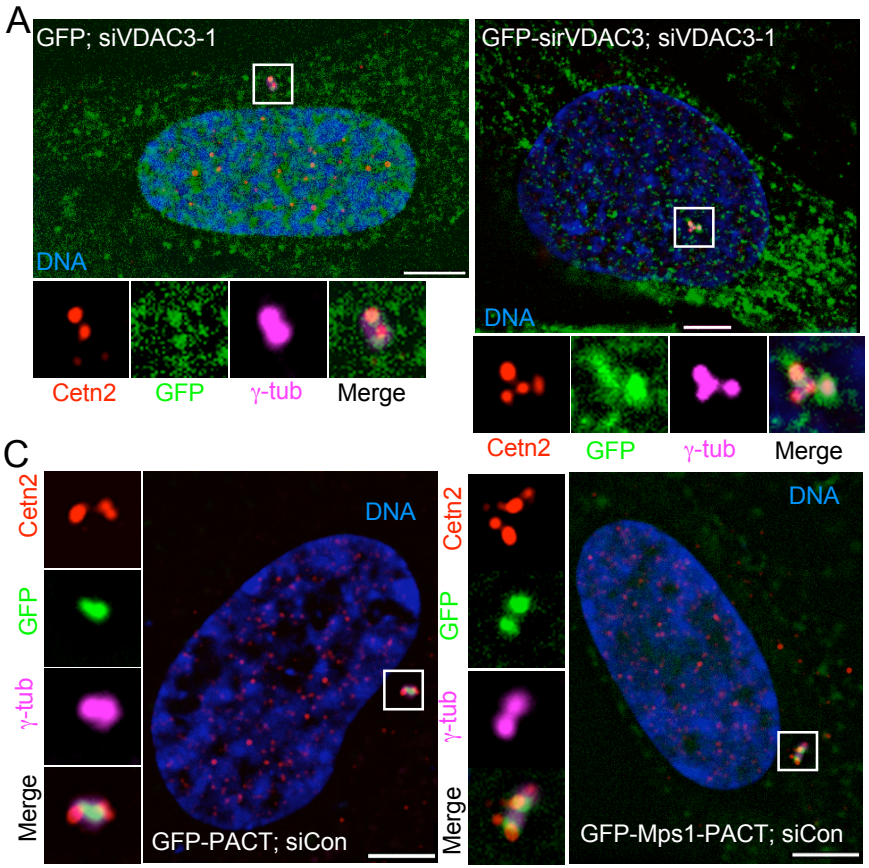
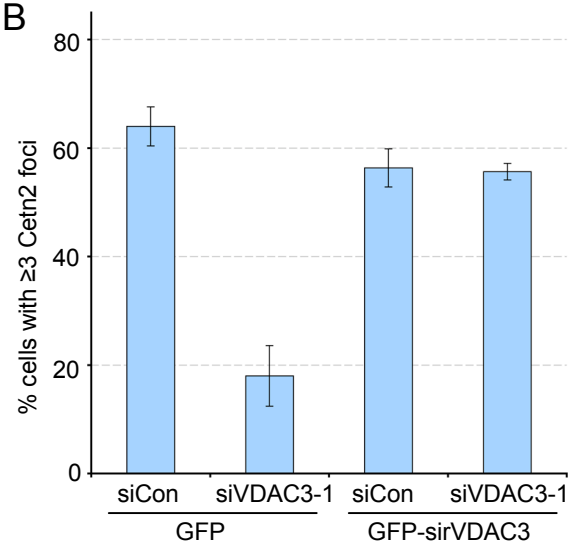


Fig. S4. **[A-B]** *Depletion of VDAC3 and Mps1 led to a delay in CP110 recruitment to the daughter centrioles.* Asynchronously growing siCon, siVDAC3-1, or siMps1 cells were labeled with a 4 h pulse of BrdU as in Fig. 4 A-B and the cells were examined for CP110 foci at the centrosomes. Representative images of cells stained for CP110 (red), γ-tub (green) and BrdU (blue). Bar = 5 μm. [B] Percentage of BrdU-positive cells with ≤ 2 CP110 foci was plotted as bars. Values represent mean ± SD for three independent experiments, 75-100 cells counted per replicate. **[C]** Cellular viability of RPE1 cells treated with Erastin (Era) or FCCP at indicated concentrations are plotted as the percentage of DMSO (D) treated cells. Values represent mean ± SD for triplicate of each treatment group from a representative experiment. **[D]** Shown are representative cells treated with 0.1 mM FCCP or DMSO for 4 h and stained for Cetn2 (red), α-tub (green) and BrdU (blue). Panels show magnified centrosomes. Bar = 5 μm.





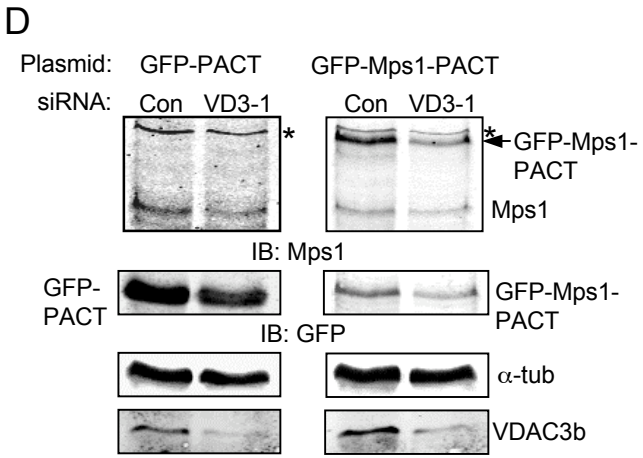


Fig. S5. [A-B] *Expression of a siRNA-resistant VDAC3 suppressed the centriole assembly defect during S-phase arrest*. siCon or siVDAC3-1 treated RPE1 cells expressing either GFP alone or GFP-sirVDAC3-1 were arrested in S-phase by a 24 h HU treatment and were examined for Cetn2 foci. [A] Shown are the representative images of siVDAC3-1 cells expressing either GFP (green) or GFP-sirVDAC3-1 (green) stained for Cetn2 (red), γ-tub (magenta) and DNA (blue). Bar = 5 μm. [B] Percentage of GFP-positive cells with ≥3 Cetn2 foci, where values represent the mean ± SD for three independent experiments, where roughly 50 cells were counted per replicate. **[C]** Shown are the representative images of siCon treated RPE1 cells expressing either GFP-PACT (green) or GFP-Mps1-PACT (green), arrested in S-phase by a 24 h HU treatment and stained for Cetn2 (red), γ-tub (magenta) and DNA (blue). Bar = 5 μm. Panels are magnified centrosomes. **[D]** Immunoblots show the expression of GFP-Mps1-PACT and endogenous Mps1 (using Mps1 antibody), GFP-PACT and GFP-Mps1-PACT (using GFP antibody) in RPE1 cells transfected with either control siRNA (Con) or siVDAC-1 (VD3-1). Downregulation of endogenous VDAC3 was verified by immunoblotting using anti-VDAC3 antibody where α-tubulin (α-tub) was the loading control. Since VDAC3b was predominantly downregulated in siVDAC3-1 cells (as in Fig. 3D, 5F), only the level of VDAC3b in these cells were shown for convenience. (*) indicates the presence of a non-specific band just above the GFP-Mps1-PACT.