Expanded View Figures

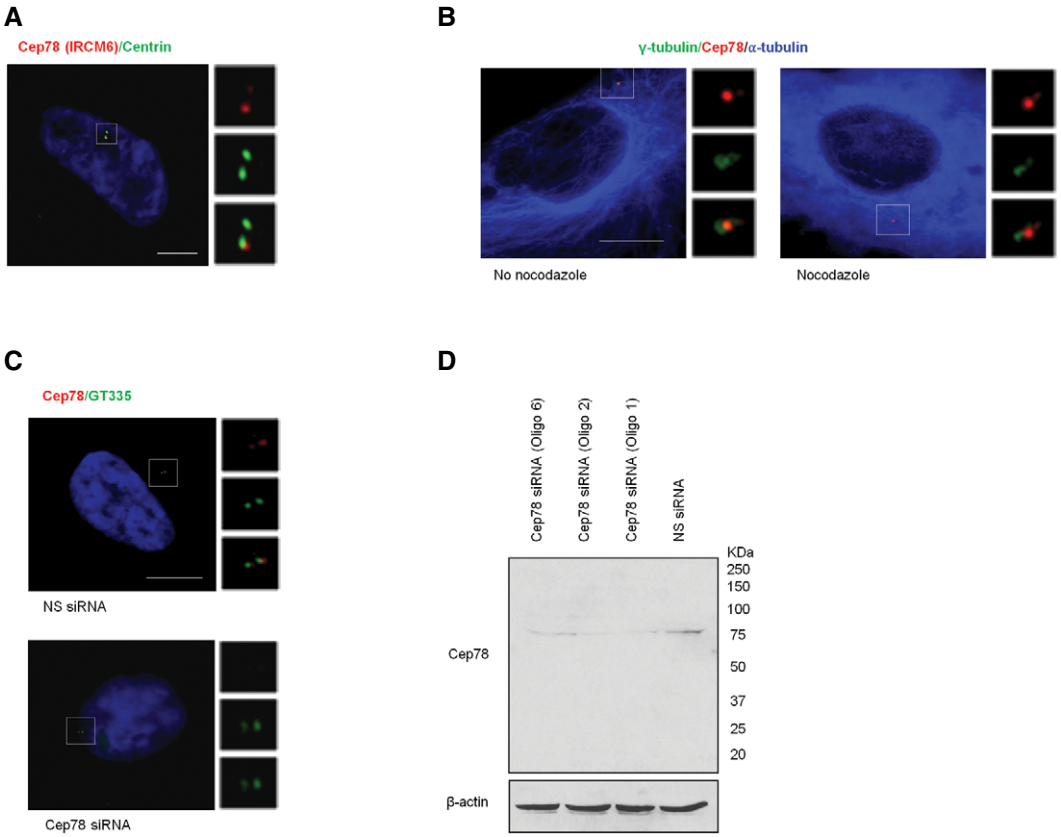

Figure EV1. Characterization of Cep78.

A RPE-1 cells were stained with DAPI (blue) and antibodies against centrin (green) and Cep78 raised against the C-terminal region of the protein (IRCM6, red). Scale bar, 1 μm.

B RPE-1 cells untreated or treated with 10 μM nocodazole for 1 h to induce microtubule depolymerization were stained with antibodies against γ-tubulin (green), α-tubulin (blue), and Cep78 (red). Scale bar, 1 μm.

C RPE-1 cells transfected with NS siRNA or Cep78 siRNA were stained with DAPI (blue) and antibodies against Cep78 (red) and polyglutamylated tubulin (GT335, green). Scale bar, 1 μm.

D RPE-1 cells were transfected with NS siRNA or Cep78 siRNA (oligo 1, 2 or 6). Lysates were Western blotted with antibody against Cep78. β-actin was used as a loading control.

Source data are available online for this figure.

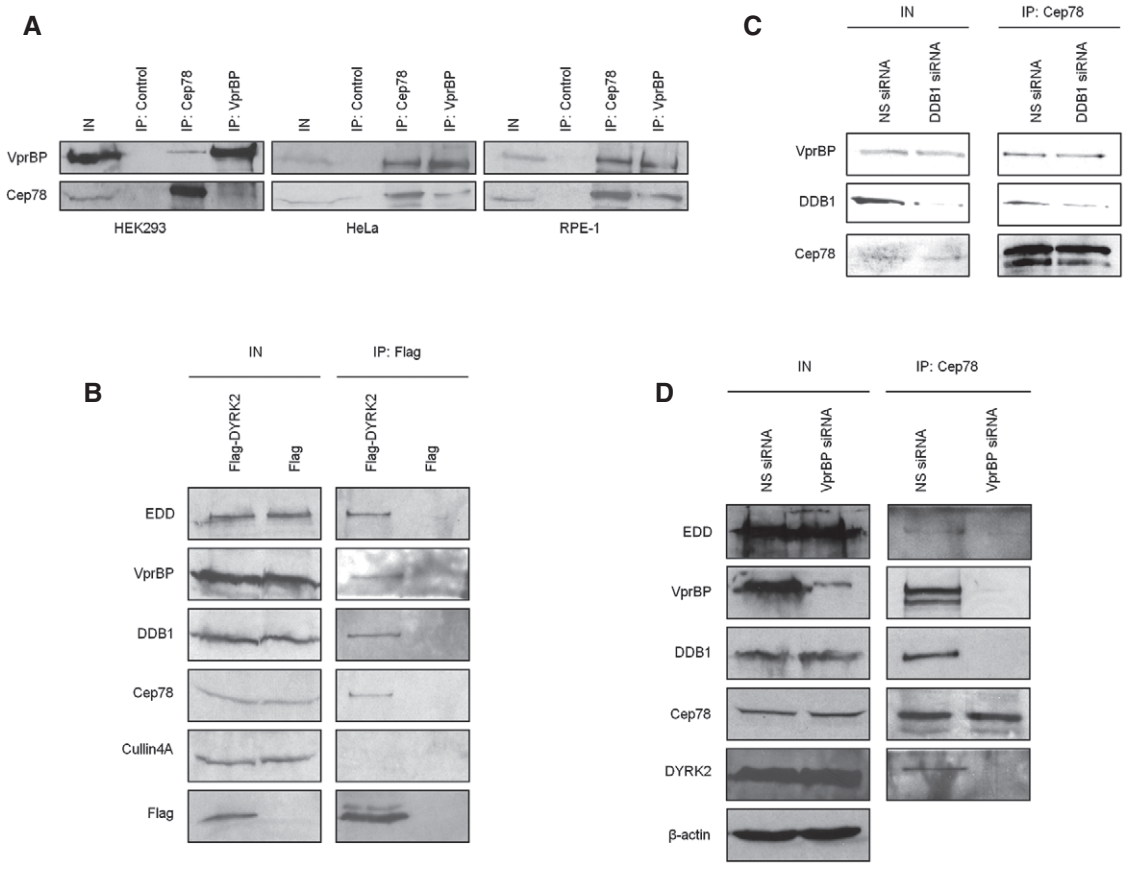

Figure EV2. Cep78 binds to EDD-DYRK2-DDB1^{VprBP} through VprBP.

A HEK293, HeLa, or RPE-1 lysates were immunoprecipitated with an anti-Flag (control), anti-Cep78 or anti-VprBP antibody, and Western blotted with the indicated antibodies. IN, input.

B Flag or Flag-DYRK2 was expressed in HEK293 cells. Lysates were immunoprecipitated with an anti-Flag antibody and Western blotted with the indicated antibodies. IN, input.

C, D HEK293 cells were transfected with NS, DDB1, or VprBP siRNA. Lysates were immunoprecipitated with an anti-Cep78 antibody and Western blotted with the indicated antibodies. IN, input. β-actin was used as a loading control.

Source data are available online for this figure.

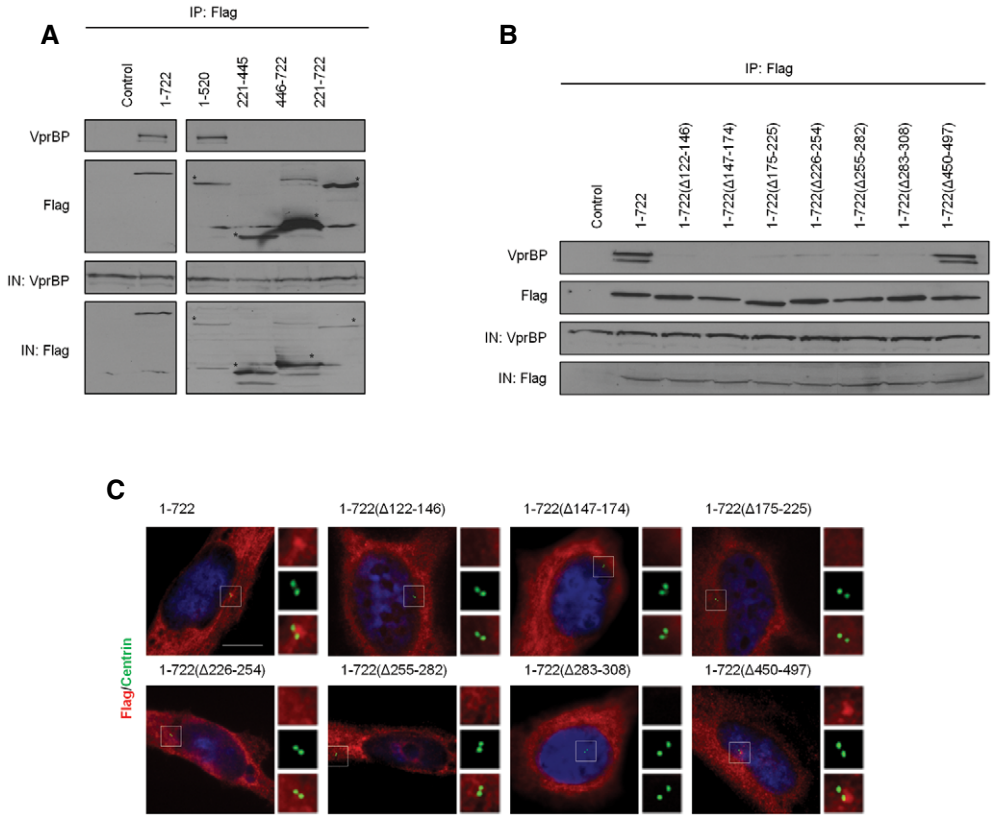

Figure EV3. Mapping of the centrosomal localization and VprBP-binding domains of Cep78.

A, B Flag (control), Flag-Cep78 full-length (1–722), or Flag-Cep78 truncated/deletion mutants were expressed in HEK293 cells. Lysates were immunoprecipitated with an anti-Flag antibody and Western blotted with the indicated antibodies. IN, input. *denotes bands corresponding to the expected proteins.

C RPE-1 cells expressing Flag-Cep78 full-length or truncated/deletion mutants were stained with DAPI (blue) and antibodies against Flag (red) and centrin (green). Scale bar, 1 μm.

Source data are available online for this figure.

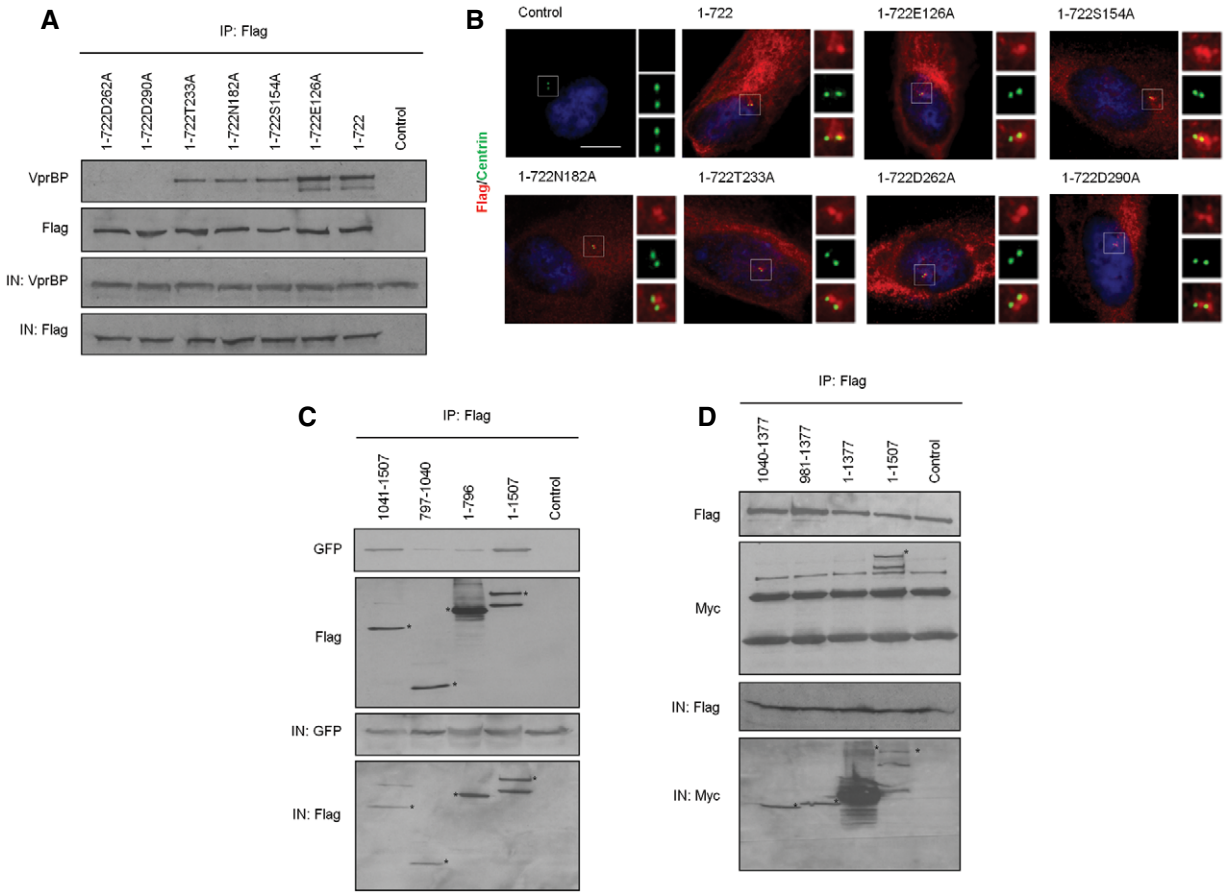

Figure EV4. Mapping of interaction domains of Cep78 and EDD-DYRK2-DDB1^{VprBP}.

A Flag (control), Flag-Cep78 full-length (1–722), or Flag-Cep78 point mutants were expressed in HEK293 cells. Lysates were immunoprecipitated with an anti-Flag antibody and Western blotted with the indicated antibodies. IN, input.

B RPE-1 cells expressing Flag (control), Flag-Cep78 full-length, or point mutants were stained with DAPI (blue) and antibodies against Flag (red) and centrin (green). Scale bar, 1 μm.

C Flag (control), Flag-VprBP full-length (1–1,507), or Flag-VprBP truncated mutants were co-expressed with GFP-Cep78 in HEK293 cells. Lysates were immunoprecipitated with an anti-Flag antibody and Western blotted with the indicated antibodies. IN, input.

D Myc, Myc-VprBP full-length, or Myc-VprBP truncated mutants were co-expressed with Flag-Cep78 in HEK293 cells. Lysates were immunoprecipitated with an anti-Flag antibody and Western blotted with the indicated antibodies. IN, input. *denotes bands corresponding to the expected proteins.

Source data are available online for this figure.

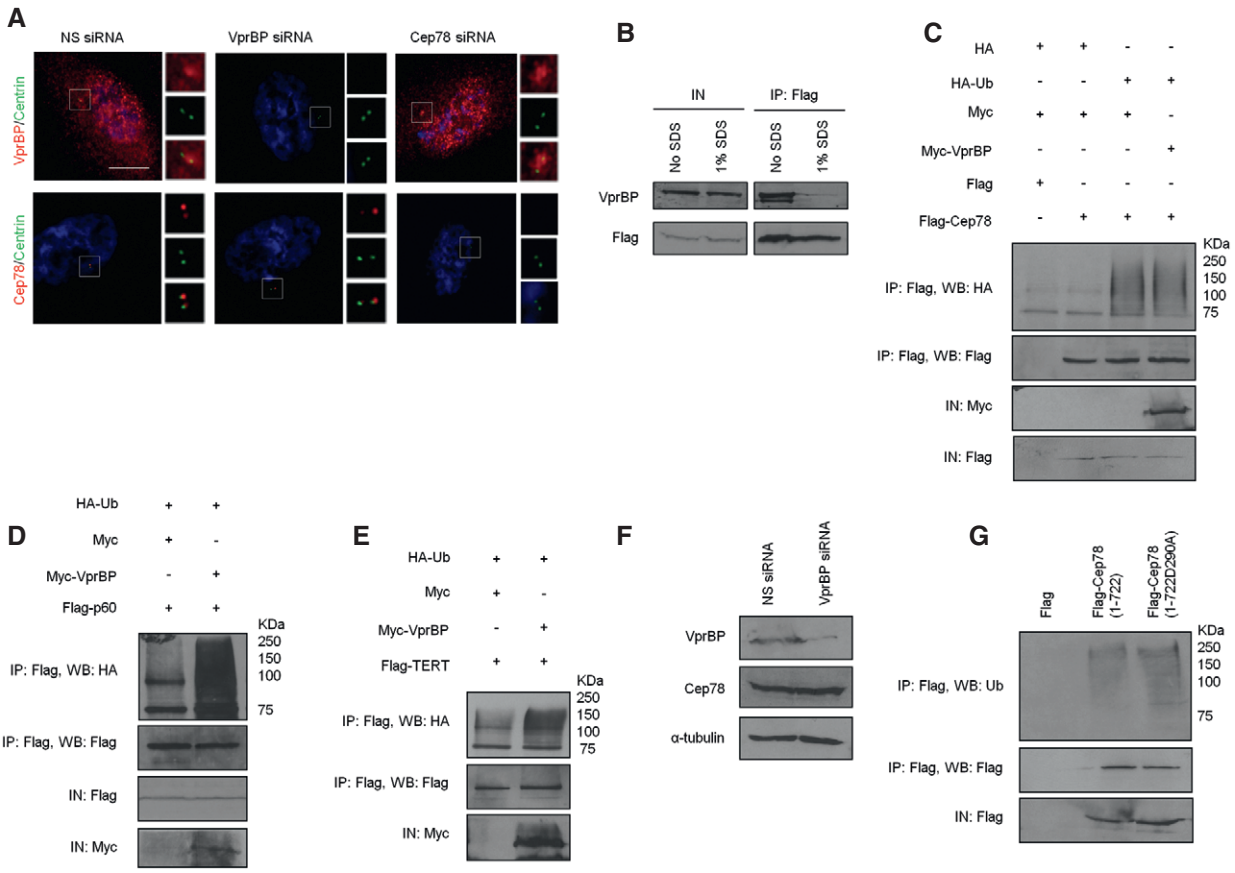

Figure EV5. Cep78 and VprBP are independently recruited to the centrosome and Cep78 is not an EDD-DYRK2-DDB1^{VprBP} substrate.

A RPE-1 cells transfected with NS siRNA, Cep78 siRNA, or VprBP siRNA were stained with DAPI (blue) and antibodies against centrin (green) and Cep78 or VprBP (red). Scale bar, 1 μm.

B Flag-Cep78 was expressed in HEK293 cells. Lysates were immunoprecipitated with an anti-Flag antibody in the presence or absence of 1% SDS and Western blotted with the indicated antibodies. IN, input.

C HEK293 cells expressing Flag or Flag-Cep78, HA or HA-Ub, and Myc or Myc-VprBP were synchronized in mitosis with nocodazole. Lysates were immunoprecipitated with an anti-Flag antibody in 1% SDS and Western blotted with the indicated antibodies. IN, input.

D, E HA-Ub, Flag-katanin p60 or Flag-TERT, and Myc or Myc-VprBP were co-expressed in HEK293 cells. Lysates were immunoprecipitated with an anti-Flag antibody in 1% SDS and Western blotted with the indicated antibodies. IN, input.

F HEK293 cells transfected with NS siRNA or VprBP siRNA were synchronized in mitosis. Lysates were Western blotted with the indicated antibodies. α-tubulin was used as a loading control.

G HEK293 cells expressing Flag, Flag-Cep78 wild type (1–722), or mutant (1-722D290A) were synchronized in mitosis. Lysates were immunoprecipitated with an anti-Flag antibody in 1% SDS and Western blotted with the indicated antibodies. IN, input.

Source data are available online for this figure.