

Gloerich et al., <http://www.jcb.org/cgi/content/full/jcb.201011126/DC1>

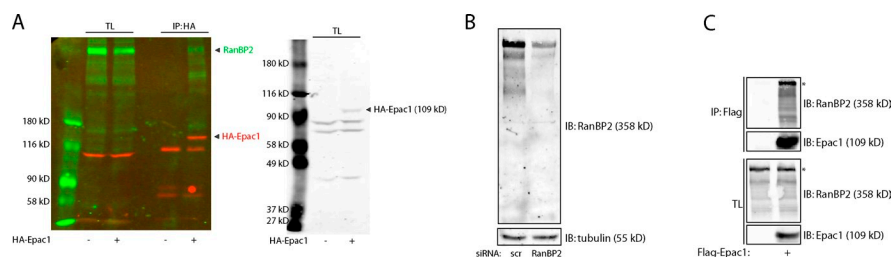


Figure S1. **Coimmunoprecipitation of RanBP2 with Epac1.** (A) Coimmunoprecipitation of endogenous RanBP2 with Flag-tagged Epac1 in HEK293T cells as shown in Fig. 1 B showing the uncropped Western blots of this experiment. (B) Western blot showing protein expression of RanBP2 in scrambled (scr) siRNA- and RanBP2 siRNA-transfected HEK293T cells with tubulin as a loading control. (C) Coimmunoprecipitation of endogenous RanBP2 with Flag-tagged Epac1 in U2OS cells. Multiple bands for RanBP2 are visible on the 6% gel; the 358-kD RanBP2 band is indicated with an asterisk. IB, immunoblot; IP, immunoprecipitation; TL, total lysate.

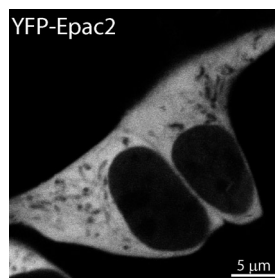


Figure S2. **Localization of YFP-Epac2 in HEK293T cells.** Confocal live imaging of HEK293T cells transfected with YFP-Epac2, which is absent from the nuclear envelope.

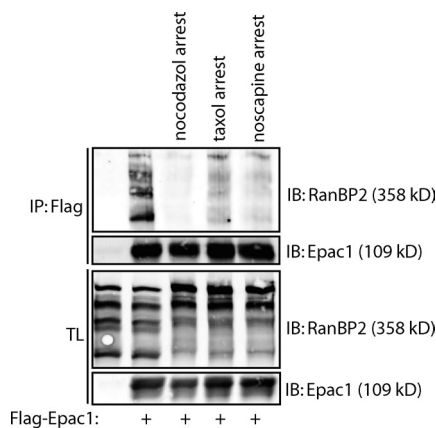


Figure S3. **Coimmunoprecipitation of RanBP2 with Epac1 in cells arrested in mitosis by incubation with nocodazole, taxol, or nocapine.** Coimmunoprecipitation of endogenous RanBP2 with Flag-tagged Epac1 in U2OS cells that were arrested in mitosis by 24-h incubation with 2.5 μM thymidine, washed three times with PBS, and incubated for 16 h with 250 ng/ml nocodazole, 2 μM taxol, or 25 μM nocapine. Mitotic cells were subsequently collected by mitotic shake off and subjected to coimmunoprecipitation. IB, immunoblot; IP, immunoprecipitation; TL, total lysate.

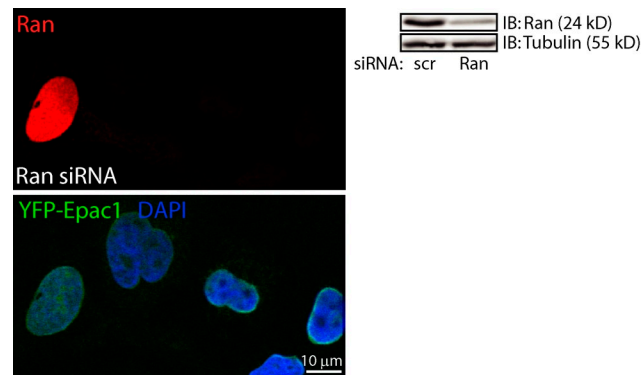


Figure S4. **Localization of YFP-Epac1 and endogenous Ran in U2OS cells.** Cells were transfected with Ran siRNAs and, subsequently, with YFP-Epac1. 60 h after siRNA transfection, cells were fixed and stained for endogenous Ran. This shows that the presence of Epac1 at the nuclear envelope is not affected by depletion of Ran (as compared with an adjacent cell that has not been hit by the siRNAs). IB, immunoblot; scr, scrambled.

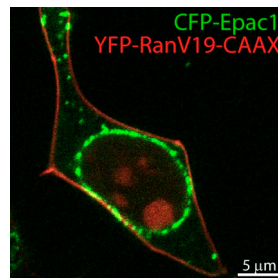


Figure S5. **Localization of CFP-Epac1 in cells expressing YFP-RanV19-CAAX.** Live imaging of CFP-Epac1 together with YFP-RanV19-CAAX in HEK293T cells showing that RanV19-CAAX is not able to target Epac1 to the plasma membrane.