Onodera et al., http://www.jcb.org/cgi/content/full/jcb.201201065/DC1

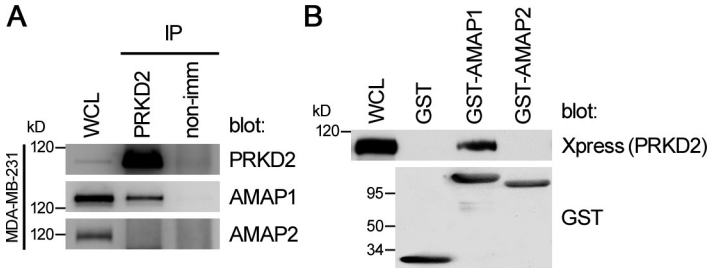

Figure S1. **Supplemental assays of AMAP1–PRKD2 binding.** (A) MDA-MB-231 cell lysates were immunoprecipitated with an anti-PRKD2 antibody, then subjected to blotting with the indicated antibodies, after being separated by electrophoresis. Nonimmune rabbit IgG was used as a control (non-imm). WCL, whole cell lysate (5 µg). (B) GST-tagged full-length AMAP1 or AMAP2 were incubated in vitro with lysates of Cos7 cells expressing the full-length Xpress-PRKD2, and coprecipitation of PRKD2 with GST fusion proteins, bound to glutathione-beads, was analyzed by immunoblotting using an anti-Xpress antibody (B and C). WCL, whole cell lysate (10 µg); GST, GST alone used as a control.

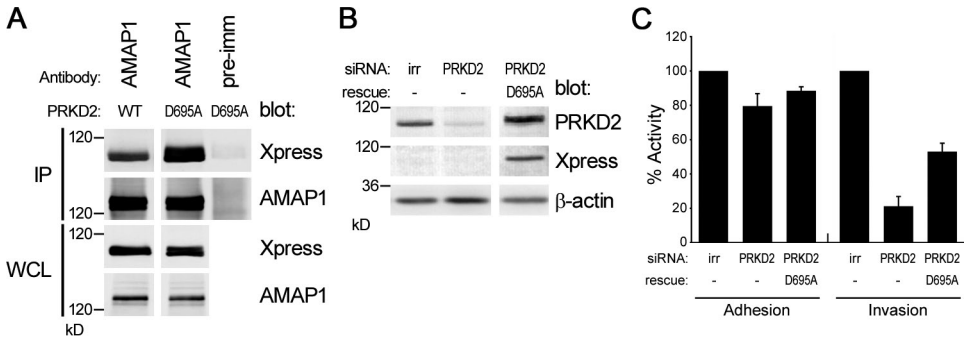

Figure S2. **Partial rescue of invasive activities by the kinase-dead mutant of PRKD2.** (A) Endogenous AMAP1 was immunoprecipitated from lysates of MDA-MB-231 cells expressing Xpress-tagged wild-type PRKD2 (WT) or its kinase-dead mutant (D695A), and coprecipitation of PRKD2 proteins was analyzed by immunoblotting using an anti-Xpress antibody. Preimmune serum of the anti-AMAP1 antibody was used as a control (Pre-imm). WCL, whole cell lysate (10 µg). (B and C) MDA-MB-231 cells were transfected with PRKD2 siRNA oligonucleotides or with a control irrelevant sequence (irr), together with pcDNA3.1 HisC-resPRKD2 D695A (rescue construct of the kinase-dead mutant of PRKD2) or control pcDNA3 empty vector (−), as indicated (B). Matrigel chemoinvasion activities and adhesion (to collagen) were then measured, in which data are presented as percentages calculated by normalizing the values obtained for the control cells as 100% (C). 4,213 ± 516 (~4.21%) control cells were calculated to have transmigrated per 6.4-mm-diam Matrigel-coated Boyden chamber filter under these conditions. Data are shown as mean ± SEM of triplicate experiments (error bars).

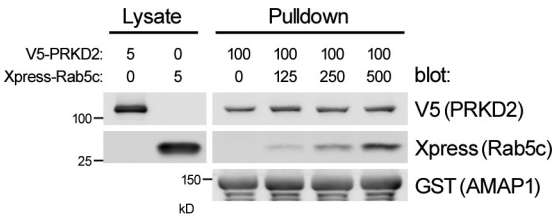

Figure S3. **Rab5c is not likely to mediate the physical association of AMAP1 with PRKD2.** Lysates from 293T cells expressing V5-PRKD2 or Xpress-Rab5c (Q86L) were mixed in different ratios, as indicated, and incubated in vitro with GST-tagged AMAP1 bound to glutathione beads. Precipitates were analyzed by immunoblotting using anti-V5, Xpress, and GST antibodies. GST alone was used as a control.

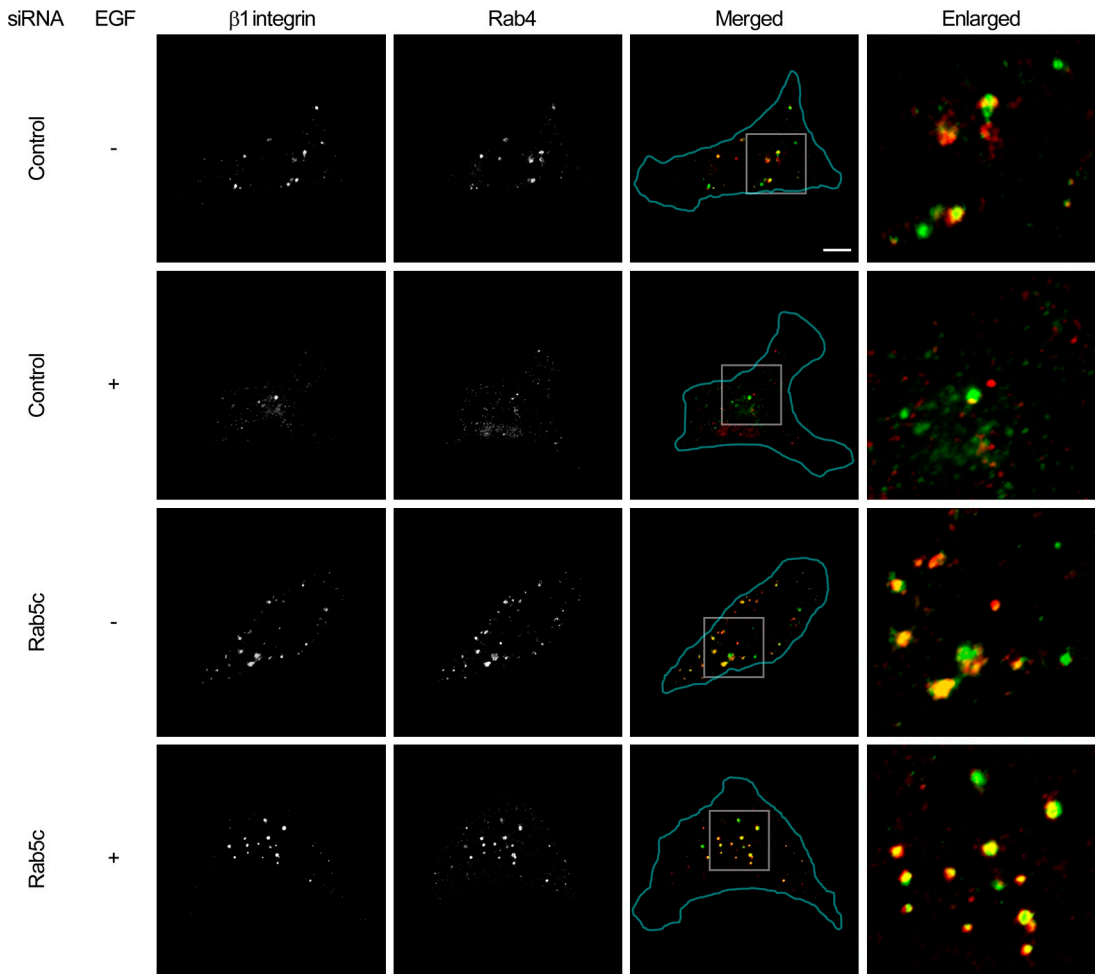

Figure S4. **Rab5c knockdown confines β1 integrin within Rab4-positive large vesicles.** MDA-MB-231 cells were cultured on collagen I–coated dishes and transfected with pEF-BOS myc-Rab4 together with either a nontargeting control or Rab5c siRNAs. Cells were labeled with an anti–β1 integrin antibody on ice, and internalization of β1 integrin was induced by incubation in serum- and EGF-free medium at 37°C. After removal of the antibody remaining on the cell surface by acid wash, cells were fixed directly or treated with 10 ng/ml EGF (1 h) and acid-washed before fixation. Samples were labeled with an anti-myc epitope antibody and stained with secondary antibodies conjugated with a fluorophore. In the merged picture, β1 integrin and myc-Rab4 are shown as green and red, respectively. Gray and blue lines in the merged picture indicate the enlarged area and cell outline based on the heavily contrasted image of myc-Rab4 staining, respectively. Bar, 10 μm.