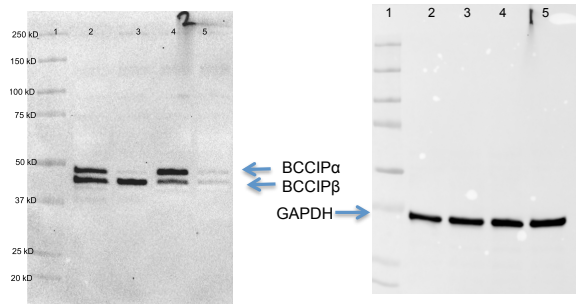
Supplement S1, Validations of BCCIP localization to centrosomes.

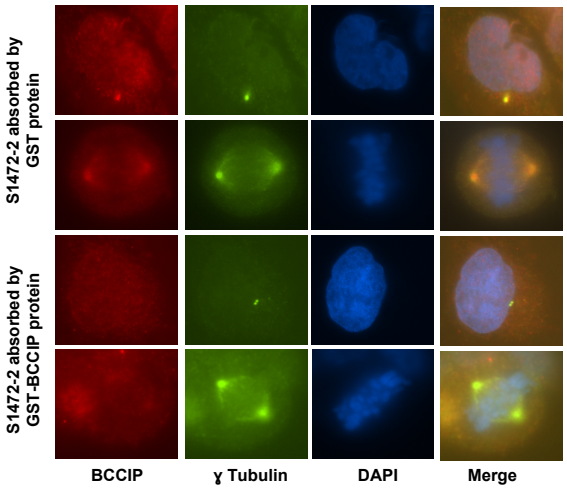
a



1. Protein markers
2. U2OS cells
3. U2OS infected with lenti-virus expressing shRNA against BCCIPα
4. U2OS infected with lenti-virus expressing shRNA against BCCIPβ
5. U2OS infected with lenti-virus expressing shRNA against BCCIPα/β

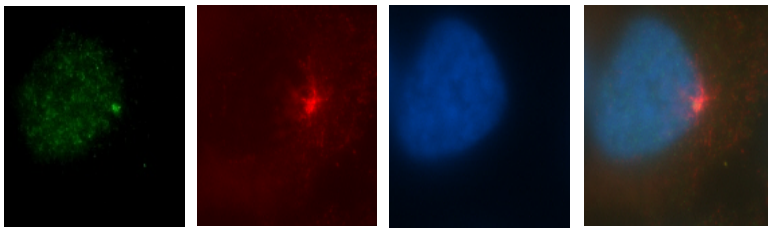
A. BCCIP antibody validation by Western blot. Whole cell extract of human U2OS cells with isoform specific knockdown of BCCIPα (lane 3), BCCIPβ (lane 4), and both isoform (lane 5) were used in Western blot using rabbit antibodies BR5 against human BCCIP. As seen here the antibody is able to detect both isoforms. Blotting with GAPDH (right panel) was used as a loading control.

b



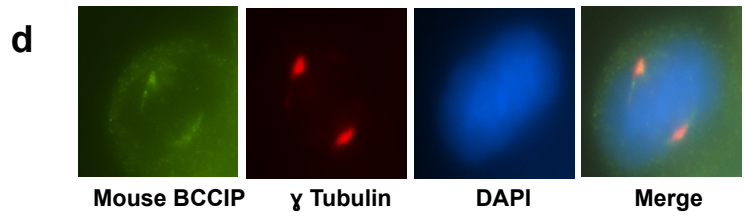
B: BCCIP antibody validation by immunofluorescent staining. To validate the specificity of the antibodies on endogenous centrosome localization of BCCIP during the procedures of immune-fluorescent staining, BCCIP antibody was diluted to a final concentration of 1:100 in IF block buffer. The diluted antibody was divided into equal aliquots and incubated with 500ng GST-BCCIPα and 500ng GST-BCCIPβ or 1μg GST-vector. Then, 50μl of glutathione resin (Novagen) was added to each mixture and the resin was incubated overnight. The next day the resin was pelleted and the supernatant from each group was carefully collected and utilized for BCCIP immunofluorescent staining as above. Slides were stained in parallel with either GST-BCCIP or GST, and images were acquired with equal exposure times. As shown here, the incubation of the antibodies with the GST-BCCIPα/β mixture reduced the nuclear, centrosomal, and spindle staining of BCCIP, while the incubation with GST alone did not reduce the staining intensity of these structures.

C

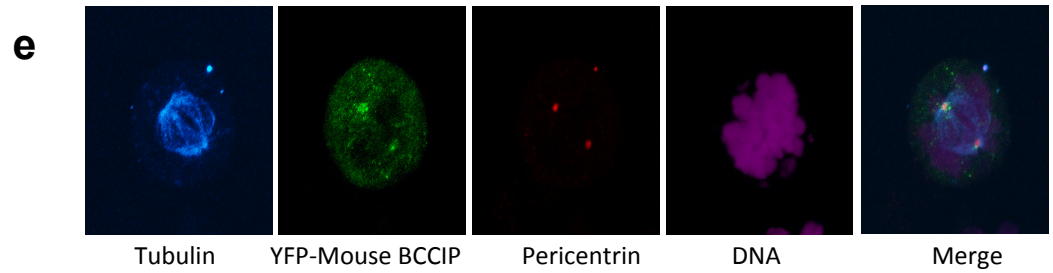


C: Visualization of centrosomal BCCIP using alternative cell lines. HeLa cells were probed with a previously characterized anti-BCCIP BR5 antibody and were co-stained with α-

Tubulin following nocodazole treatment. This establishes that BCCIP can be visualized within centrosomes using different secondary antibodies and centrosome markers. Likewise, a similar centrosomal localization of BCCIP was observed in U2OS, MCF10A, and HeLa cells (not shown).



D: The Centrosome association of BCCIP is conserved in mice. NIH3T3 cells were seeded onto coverslips and co-stained with anti-mouse BCCIP and γ-Tubulin antibodies.



E: Transiently expressed mouse BCCIP displays a spindle pole localization in human cells. YFP-Mouse BCCIP was transiently expressed in 293T cells, processed for immunofluorescence, and stained with γ-Tubulin. YFP-Mouse BCCIP expressing cells were then assessed for co-localization with γ-Tubulin.