Supplemental Materials

Molecular Biology of the Cell

Narayanan et al.

Supplementary Figure Legends

Supplementary Figure 1. Loss of Bcr and/or Abr does not affect cell proliferation in primary astrocytes.

No change in cell proliferation was observed when WT, *Abr*$^{-/-}$, *Bcr*$^{-/-}$, and *Bcr*$^{-/-}$*Abr*$^{-/-}$ astrocytes were subjected to a BrdU assay. N=3.

Supplementary Figure 2. Bcr mutant astrocytes show defects in polarized morphology.

(A) Schematic representation of WT, *Abr*$^{-/-}$, *Bcr*$^{-/-}$ and *Bcr*$^{-/-}$*Abr*$^{-/-}$ astrocytes in a wound-healing assay. Astrocytes were stained with antibodies against acetylated tubulin to visualize microtubules **(B)** and phalloidin to visualize F-actin **(C)**. WT and *Abr*$^{-/-}$ astrocytes displayed more organized microtubules and F-actin when compared to *Bcr*$^{-/-}$ and *Bcr*$^{-/-}$*Abr*$^{-/-}$ astrocytes. Dashed lines represent scratch location. Bar-10μm. N=3.

Supplementary Figure 3. Characterization of Par complex interactions

(A) Par6 interacts with PKCζ but not Tiam1 or Bcr. Myc-Par6 was expressed alone or in the presence of one of the following Flag-tagged proteins: PKCζ, Tiam1, or Bcr. The Flag-tagged proteins were then immunoprecipitated with α-Flag antibodies, and then immunoblotted with α-myc antibodies to assess Par6 association. Lysates were also immunoblotted with α-Flag and α-myc antibodies to confirm protein expression. N=3. **(B)** Abr does not interact with Par3 in cortical astrocytes. Lysates from cultured rat cortical astrocytes (DIV21) were immunoprecipitated with control IgG (NI) or α-Abr antibodies, and then immbunoblotted with α-Par3 and α-Abr antibodies. **(C)** Abr does not interact with PKCζ in cortical astrocytes. Lysates from cultured rat cortical astrocytes (DIV21) were immunoprecipitated with control (NI) or α-Abr antibodies, and then immbunoblotted with α-PKCζ and α-Abr antibodies. N=3. **(D)** Bcr interacts with Par3 in astrocytes in the presence or absence of a migratory stimulus. Cultured rat astrocyte monolayers (DIV21) were left alone or scratched multiple times and then lysed 4 hrs later. Lysates were immunoprecipitated with control IgG (nonimmune=NI) or α-Bcr antibodies, and then immbunoblotted with α-Par3 or α-Bcr antibodies. N=3. **(E)** Bcr interacts with PKCζ in cortical astrocytes plus or minus scratch stimulation. Lysates from cultured rat cortical astrocytes (DIV21) plus or minus scratch stimulation were immunoprecipitated with control IgG (NI) or α-Bcr antibodies, and then immbunoblotted with α-PKCζ or α-Bcr antibodies. N=3. **(F)** Lysates from control and scratched cultured rat cortical astrocytes (DIV21) were immunoblotted for β-catenin as a positive control for scratch stimulation. Lysates were also blotted with an α-GAPDH antibody for a loading control. N=3.

Supplementary Figure 4. Loss of Abr does not affect PKCζ signaling.

(A) Representative images demonstrate Par3 localization in WT and *Bcr*$^{-/-}$ polarized astrocytes migrating in a scratch assay. Par3 is located at the leading edge (white arrows) regardless of the presence or absence of Bcr. Black dashed line shows scratch location. N=3. **(B)** Representative images also show that Tiam1 localizes to the leading edge in both WT and *Bcr*$^{-/-}$ polarized astrocytes migrating in a scratch assay. N=3. **(C)** Western blot analysis of lysates from WT and *Bcr*$^{-/-}$ cells treated overnight with PBS (control) or 10μM of PKCζ pseudosubstrate inhibitor. Bcr-deficient astrocytes showed an increase in p-GSK-3β levels, which was inhibited when *Bcr*$^{-/-}$ cells were treated with the PKCζ pseudosubstrate inhibitor. Lysates were also immunoblotted for total GSK-3βas a loading control. N=3. **(D)** Quantification of p-GSK-3β levels. N=3. **(E)** Western blot analysis of lysates obtained from WT and *Abr*$^{-/-}$ mouse cortical astrocytes. Abr-deficient astrocytes showed no change in PKCζ or p-GSK-3β levels. Lysates were also probed with α-GAPDH antibodies for a loading control. N=3. **(F)** Quantification of PKCζ levels. N=3. **(H)** Quantification of p-GSK-3β levels. N=3. **(H)** Western blot analysis of Cdc42 activation assay. Activated Cdc42 was affinity-purified from lysates of cultured WT and *Bcr*$^{-/-}$ cortical astrocytes using GST-PBD beads and then blotted for Cdc42. Total lysates were also blotted for Cdc42 to demonstrate equal protein loading. Activated Cdc42 levels were not altered in the absence of Bcr. N=3. **(I)** Quantification of the Cdc42 activation assay. N=3.

Supplementary Figure 5. ΔN-Bcr does not reduce PKCζ levels or rescue polarity defects in *Bcr*$^{-/-}$ astrocytes despite having Rac-GAP activity.

(A) ΔN-Bcr overexpression reduces Pak phosphorylation but not PKCζ levels in COS7 cells. Western blot analysis of lysates from COS7 cells expressing control (myc), myc-Bcr, or myc-ΔN-Bcr, immunoblotted with α-pPAK or α-PKCζ antibodies. Lysates were blotted with α-PAK antibodies for a loading control and α-myc antibodies to confirm protein expression. N=3. **(B)** Quantification of pPAK levels from (A). N=3. **(C)** Quantification of PKCζ levels from (A). N=3. **(D)** Expression of Bcr, but not ΔN-Bcr, rescues protrusion defects in *Bcr*$^{-/-}$ cortical mouse astrocytes. *Bcr*$^{-/-}$ astrocytes were transfected with expression plasmids encoding eGFP, eGFP and Bcr, or GFP and ΔN-Bcr, and then the astrocytes were subjected to a scratch assay. Cells were fixed 18 hrs post-scratch and immunostained for acetylated tubulin to visualize microtubules (red). Yellow dashed lines indicate scratch locations. Bar-10μm. n= ~100, N=3. **(E)** Expression of Bcr, but not ΔN-Bcr, rescues centrosome re-orientation defects in *Bcr*$^{-/-}$ cortical mouse astrocytes. Quantification of polarized centrosomes in *Bcr*$^{-/-}$ cortical mouse astrocytes transfected with expression plasmids encoding eGFP, eGFP and Bcr, or GFP and ΔN-Bcr. n= ~100, N=3. Data are shown ± SEM. All the experiments were performed at least three times from independent cultures, ~ 100 cells were counted per experiment.

Supplementary Movie Legends.

Movie S1. Scratch assay of WT astrocytes. Frames are taken every 30 min for a total of 48 hrs. N=3.

Movie S2. Scratch assay of *Abr*$^{-/-}$ astrocytes. Frames are taken every 30 min for a total of 48 hrs. N=3.

Movie S3. Scratch assay of *Bcr*$^{-/-}$ astrocytes. Frames are taken every 30 min for a total of 48 hrs. N=3.

Movie S4. Scratch assay of *Bcr*$^{-/-}$*Abr*$^{-/-}$ astrocytes. Frames are taken every 30 min for a total of 48 hrs. N=3.

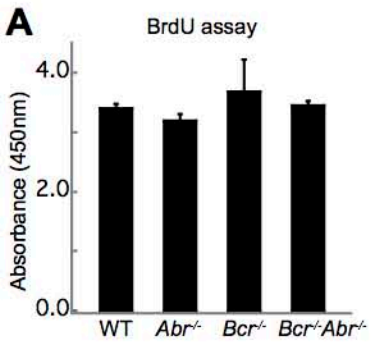
Movie S5. Random migration of WT astrocytes. Frames are taken every 30 min for a total of 24 hrs. N=3.

Movie S6. Random migration of *Abr*$^{-/-}$ astrocytes. Frames are taken every 30 min for a total of 24 hrs. N=3.

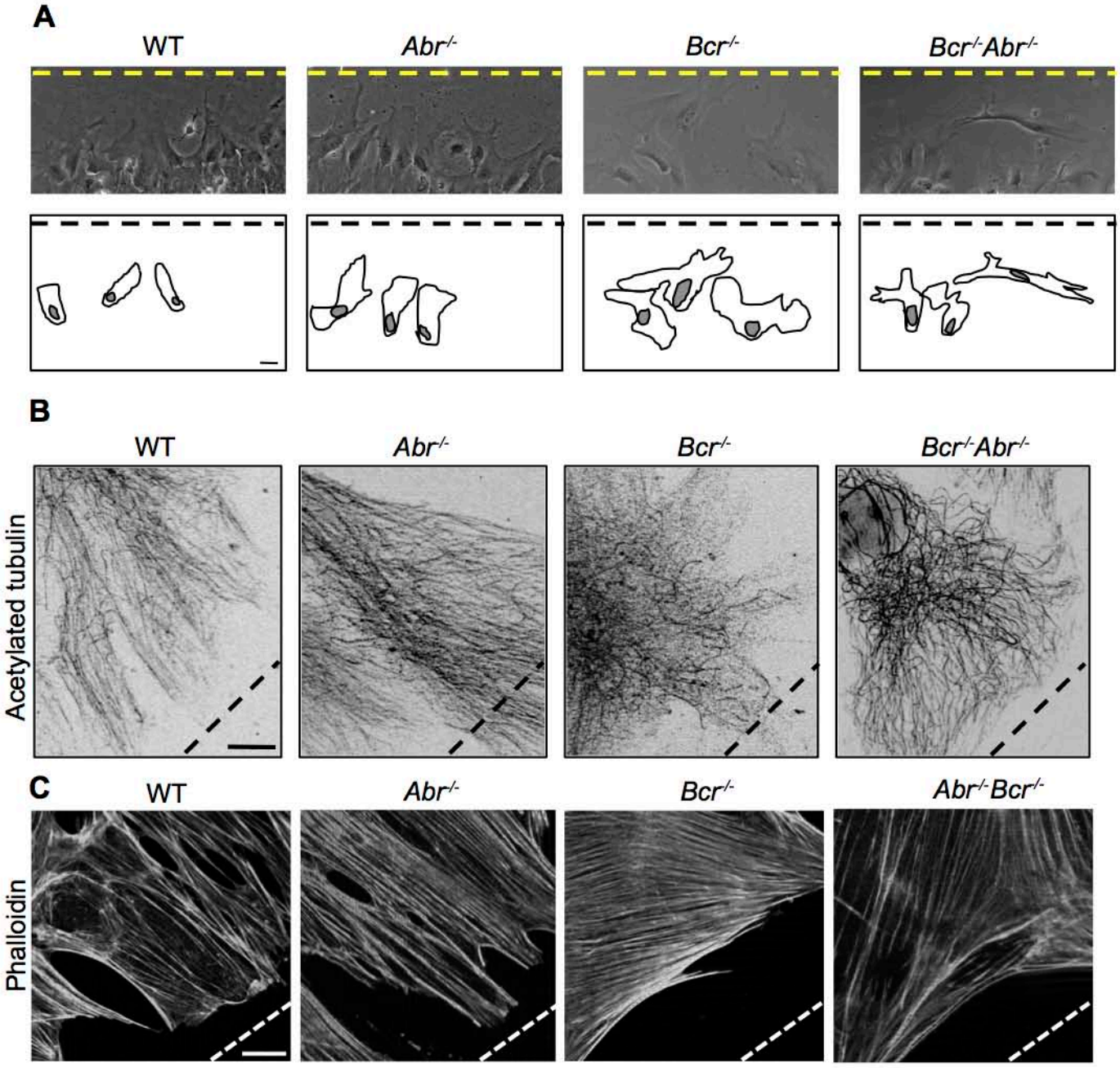
Movie S7. Random migration of *Bcr*$^{-/-}$ astrocytes. Frames are taken every 30 min for a total of 24 hrs. N=3.

Movie S8. Random migration of *Bcr*$^{-/-}$*Abr*$^{-/-}$ astrocytes. Frames are taken every 30 min for a total of 24 hrs. N=3.

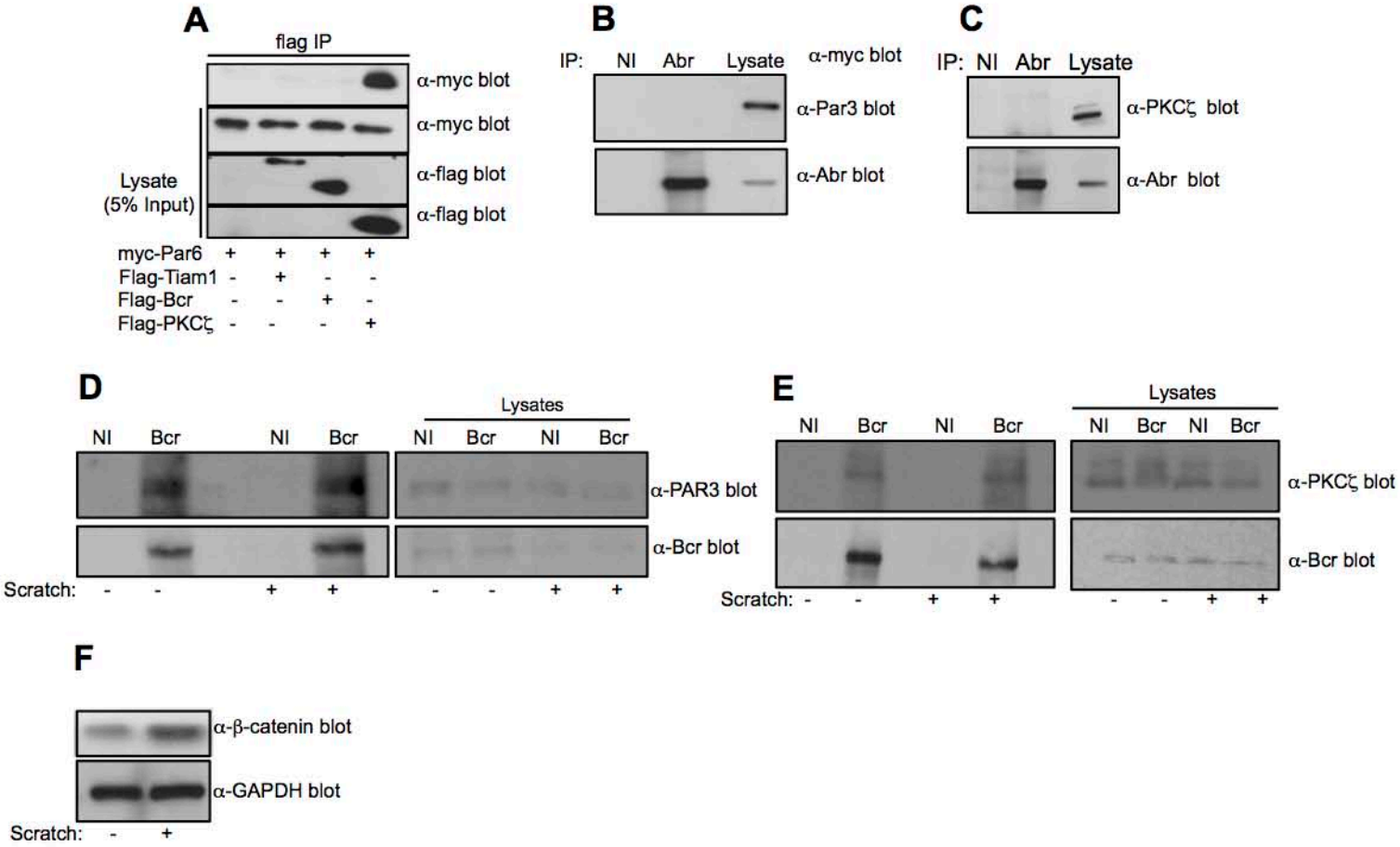
Supplementary Figure. S1 (Related to Figure.1)

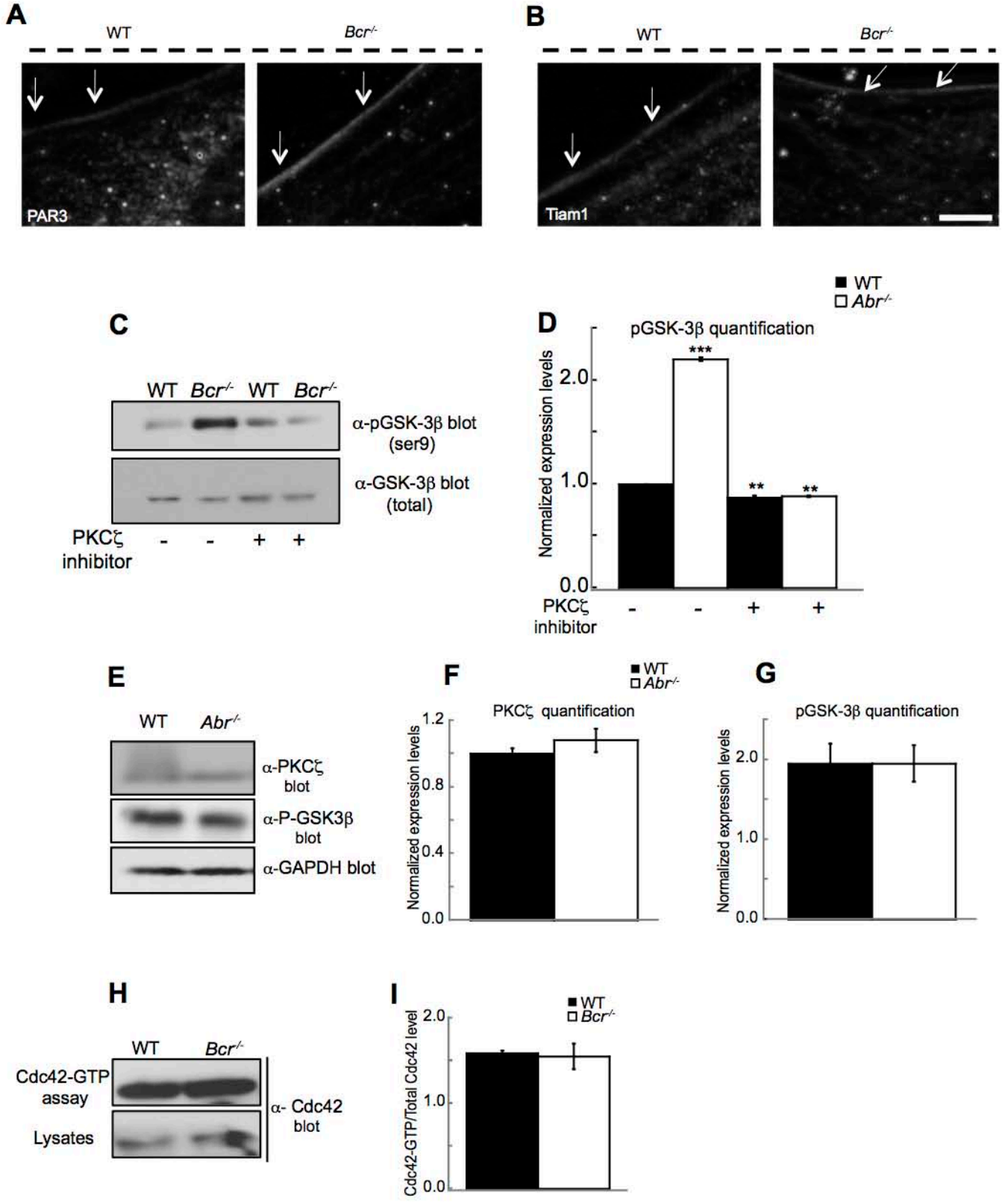


Supplementary Figure. S2 (Related to Figure.2)



Supplementary Figure. S3 (Related to Figure.3)




A
WT
Bcr-/-
PAR3
B
WT
Bcr-/-
Tiam1
C
WT Bcr-/- WT Bcr-/-
α-pGSK-3β blot (ser9)
α-GSK-3β blot (total)
PKCζ inhibitor - - + +
D
WT
Abr-/-
pGSK-3β quantification
Normalized expression levels
2.0
1.0
0.0
***
**
**
PKCζ inhibitor - - + +
E
WT Abr-/-
α-PKCζ blot
α-P-GSK3β blot
α-GAPDH blot
F
WT
Abr-/-
PKCζ quantification
Normalized expression levels
1.2
0.8
0.4
0.0
G
pGSK-3β quantification
Normalized expression levels
2.0
1.0
0.0
H
WT Bcr-/-
Cdc42-GTP assay
Lysates
α- Cdc42 blot
I
WT
Bcr-/-
Cdc42-GTP/Total Cdc42 level
2.0
1.0
0.0

Supplementary Figure. S5 (Related to Figure.5)