Zoudilova et al. Supplemental Figure Legends:

Supplementary Fig. 1. Cytospin images of bone marrow leukocytes prepared from wild type (wt), β-arrestin-1 knockout (βarr1$^{-/-}$), or β-arrestin-2 knockout (βarr2$^{-/-}$) mice. Cytospins of bone marrow leukocytes were prepared as described in the materials and methods and stained with the Hema 3 System and visualized with a Nikon Eclipse E600 upright microscope with a 40X objective.

Supplementary Fig. 2. Phospho-cofilin levels are increased in bone marrow leukocytes from β-arrestin or PAR-2 knockout mice compared to wild type. Bone marrow leukocytes from wild type mice and either βarrestin-1$^{-/-}$ and β-arrestin-2$^{-/-}$ mice **(A)** or PAR-2$^{-/-}$ mice **(B)** were prepared as described in the main text, and lysed in PBS supplemented with 1% TX100. Cleared lysates were analyzed by 15% SDS-PAGE followed by western blotting with anti-phospho-cofilin (pcofilin) or anti-total-cofilin (tcofilin). The data from these western blots is presented in Table 1.

Supplementary Fig. 3. PAR-2 induced cell migration of peritoneal neutrophils from β-arrestin knockout mice is impaired. A. Diagram of under agarose migration assay. Agar is poured into a 35mm cell culture dish, pre-coated with matrigel. Two wells, 2mm apart are punched out of the agarose and cells are placed in one well, while agonist is placed in the other. Cells are allowed to migrate under the agarose towards the agonist for 90 minutes, then imaged using a Nikon inverted microscope, with a 25X objective. **B.** Low magnification images of neutrophils from wild type (wt), β-arrestin-1 knockout (barr1KO) or β-arrestin-2 knockout (barr2KO) mice after addition of 2fAP to the agonist well. **C.** Magnified image (40X) of cells found in the region in between the two wells (left panels) and that have migrated to the agonist well (right panels) after addition of PBS (negative control, upper panels) or 2fAP (lower panels). **D.** Quantification of cells that migrated to the agonist well in response to 2fAP. **E.** Average distance of 2fAP induced migration in wild type, β-arrestin-1 or β-arrestin-2 knockout neutrophils was determined.

SupplementarySupplemental Fig. 4. Stokes radii of size exclusion column standards (•) were graphed as a function of their partition coefficients on this column. Positions corresponding to the Stokes radii reported for cofilin and β-arrestin, the predicted Stoke's radius of CIN and the apparent Stoke's radius of the cofilin/CIN/β-arrestin complexes), based on their elution profiles, are also indicated on the graph.

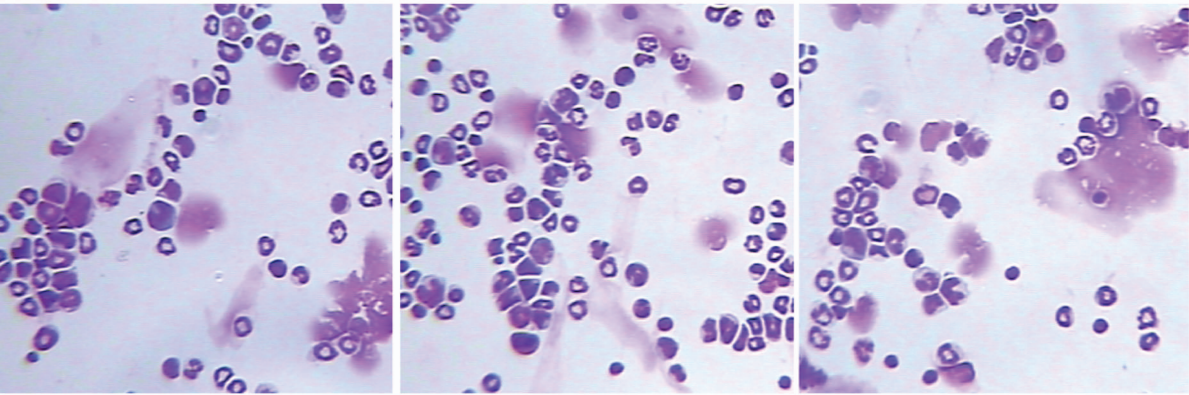
Supplementary Fig. 5. Formation of free actin barbed ends at the cell requires β-arrestins (whole cell images). Images (100X magnification, no zoom) of MEFwt (upper 2 panels) and MEFβarrDKO (lower 2 panels), treated with or without 2fAP for 1 minute, in the presence of 300nM labeled actin monomers (red). Cells were fixed and cross-stained with phalloidin (green); merged images are shown in the third panel. Scale bar =20microns.

Supplementary Fig. 6. Formation of free actin barbed ends at the cell edge is partially restored by transfection of β-arrestin-1 or β-arrestin-2. A-B. Images (100X magnification) of MEFDKO+βarr1 (**A**) and MEFDKO+βarr2 (**B**), treated with or without

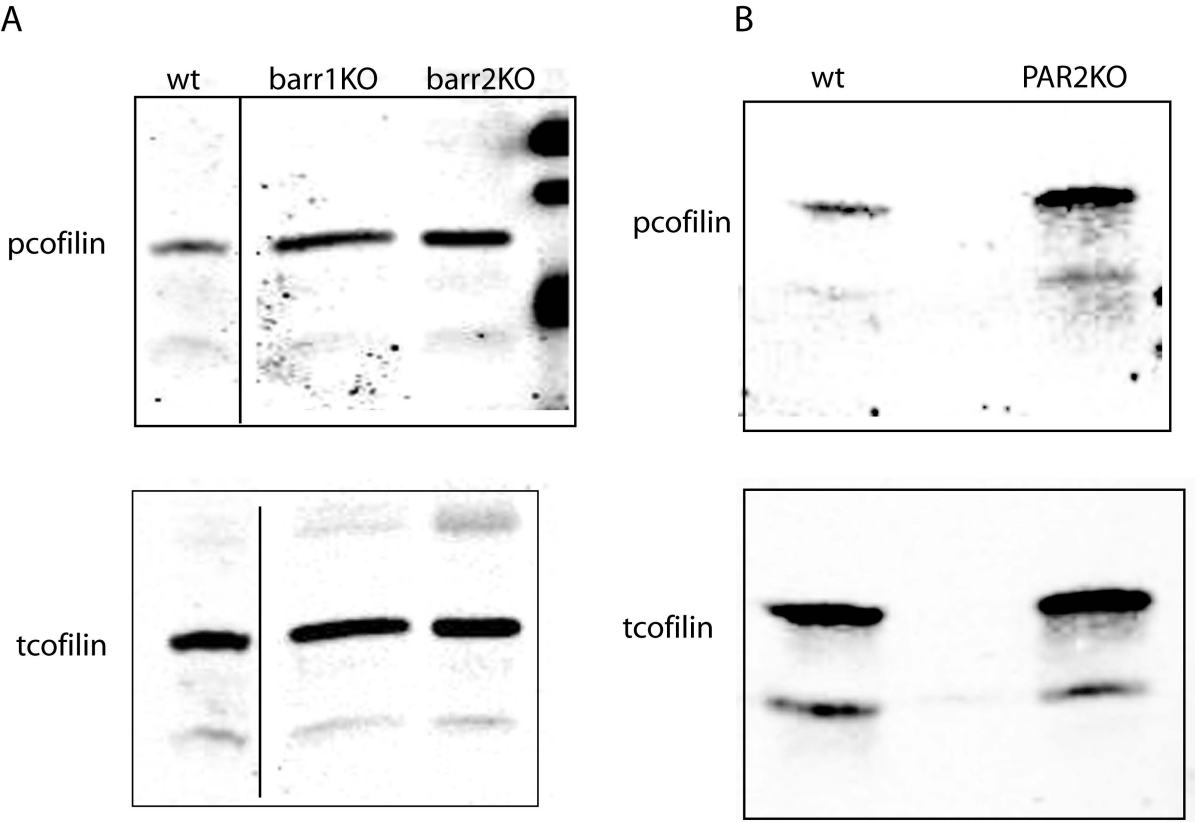
2fAP for 1 minute, in the presence of 300nM labeled actin monomers (red). Cells were fixed and cross-stained with phalloidin (green). Lower panels represent enlargement (3X zoom) of merged images of membrane boxed regions. **C.** Fluorescence Intensity in arbitrary units (AU) was determined for each condition and is graphed as a function of distance from the cell edge. In gray, the values for MEFwt and MEFβarrDKO, treated with 2fAP, are shown to demonstrate the partial rescue of barbed end formation by each β-arrestin. **D.** The distance from the cell edge at which maximal actin monomer incorporation was observed is shown for 2fAP-treated cells in each group.

Supplementary Fig. 7. Transfection of β-arrestin-1 and 2 rescues CIN translocation to the membrane. MEFβarrDKO cells were transfected with either β-arrestin-1 (DKO+βarr1), βarrestin-2 (DKO+βarr2) or both β-arrestins (DKO+βarr1/2) and Myc-tagged-CIN, treated with or without 2fAP for 1 minute, fixed and imaged by confocal microscopy.

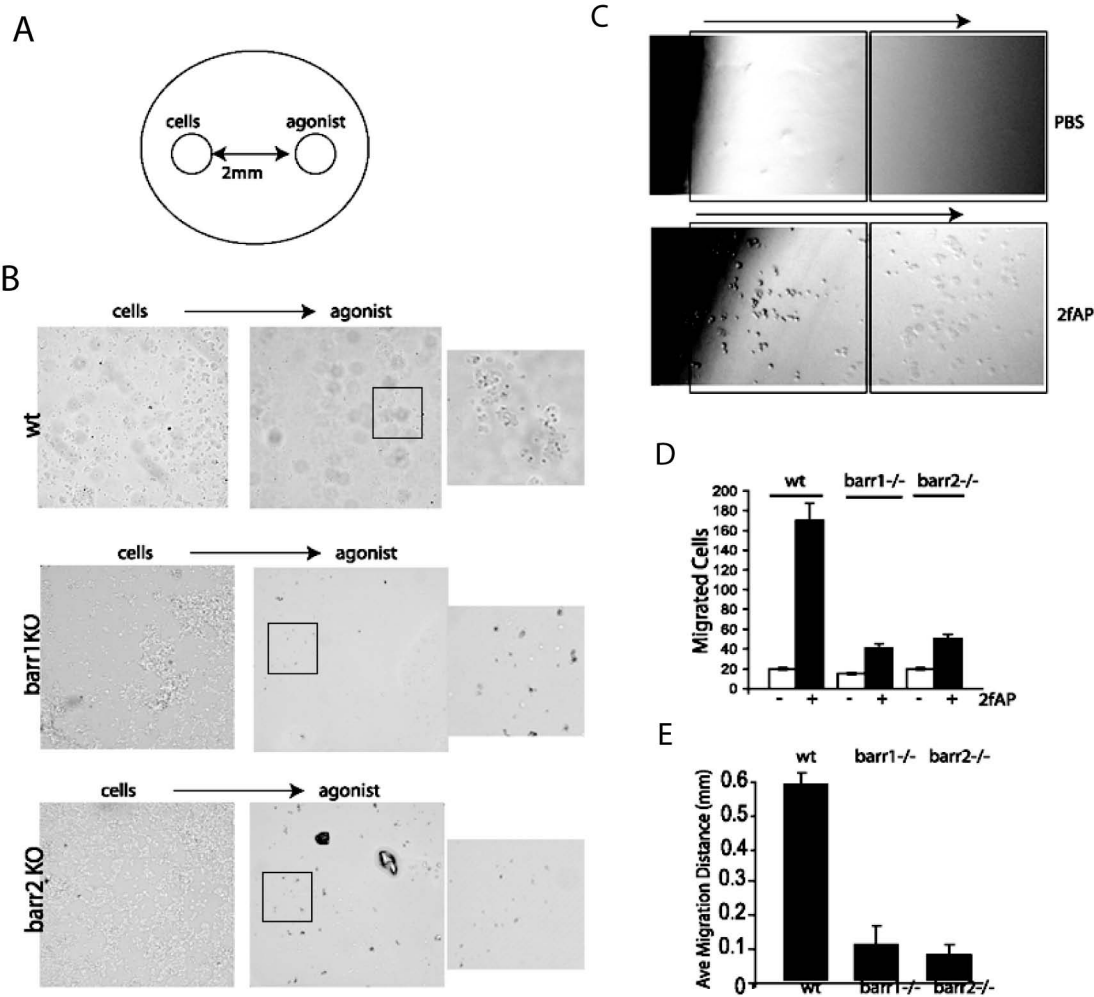
Supplementary Fig. 8. PAR-2 stimulates translocation of cofilin to the membrane in a β-arrestin-dependent manner. MEFwt, MEFβarrDKO, DKO+βarr1 or DKO+βarr2 cells were treated with or without 2fAP for 5 minutes, lysed in hypotonic buffer (20mM Tris-Cl pH 7, 250mM sucrose, 5mM KCl, 1mM EGTA, 1mM PMSF) using a Teflon dounce (25 strokes) and nuclear/insoluble fractions removed by low speed centrifugation. Supernatants were then centrifuged at 100,000g for 1 hour and pellets (membrane fractions) were resuspended in TBS supplemented with 1% TX100 and 0.1% SDS. Cytosolic (high speed supernatant) and membrane fractions were analyzed by 12.5% SDS-PAGE, transferred to PVDF membrane and western blots probed with anti-cofilin.



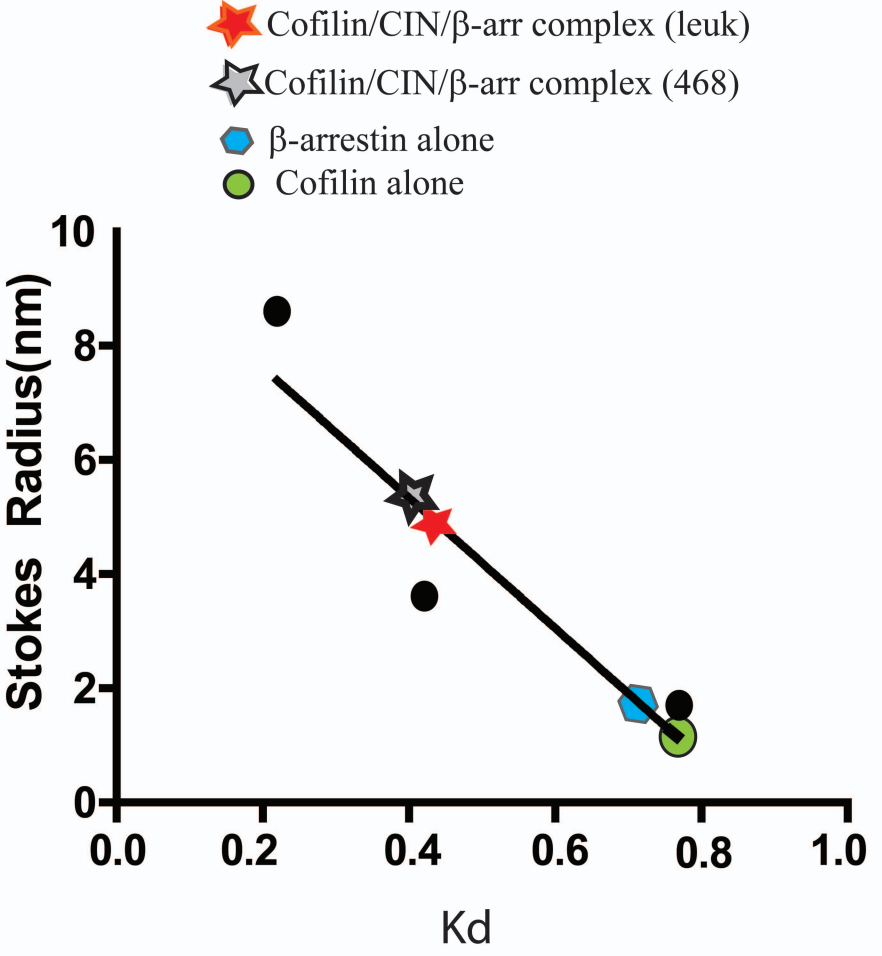
Zoudilova Supplementary Fig. 1



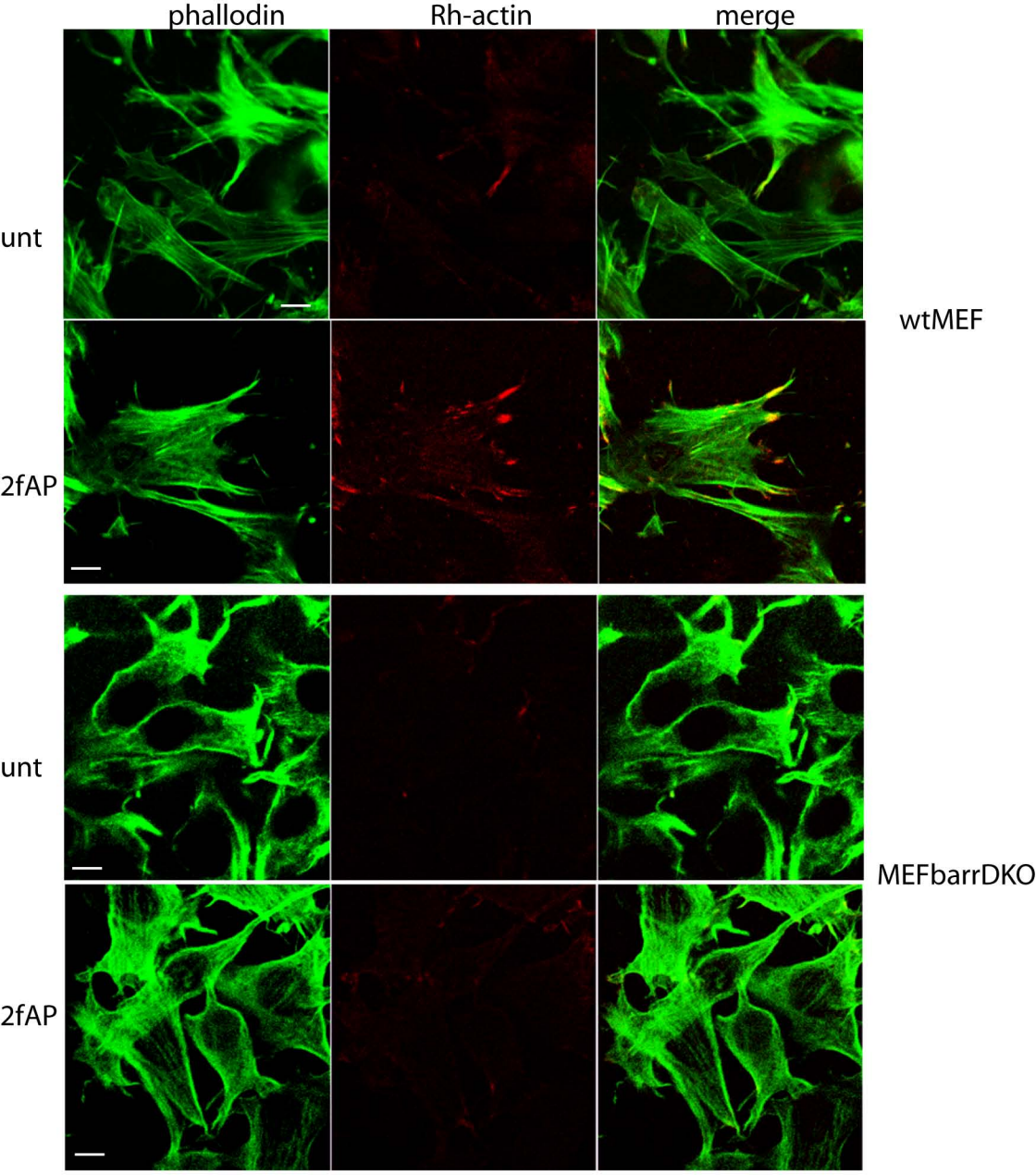
Zoudilova, Supplementary Fig 2



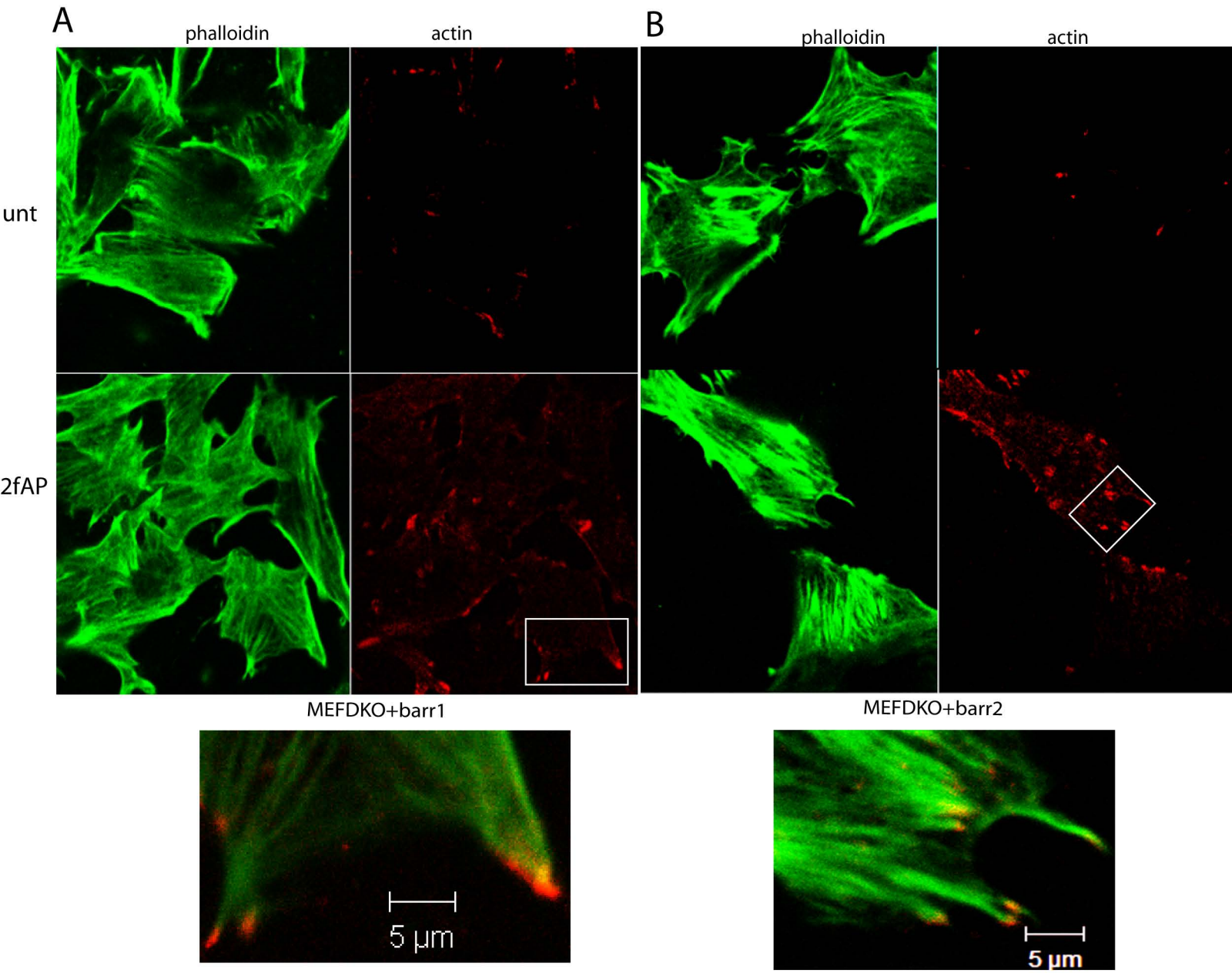
Zoudilova et al., Supplementary Fig. 3

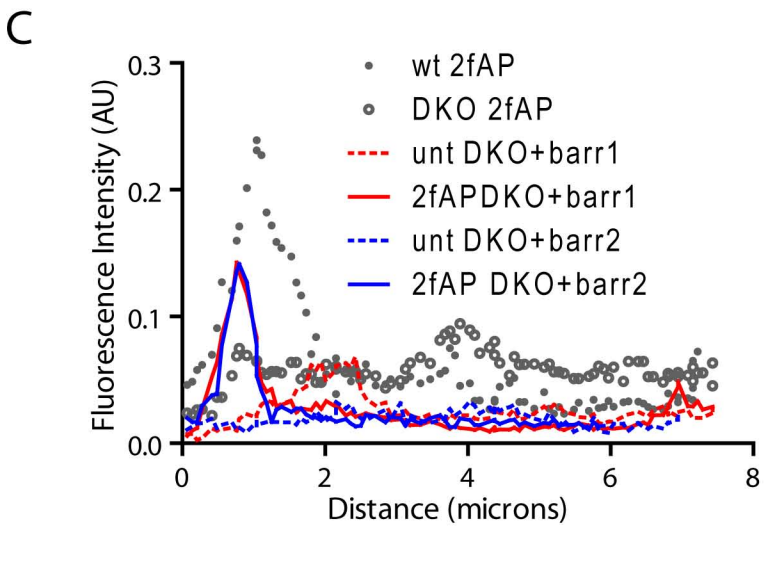


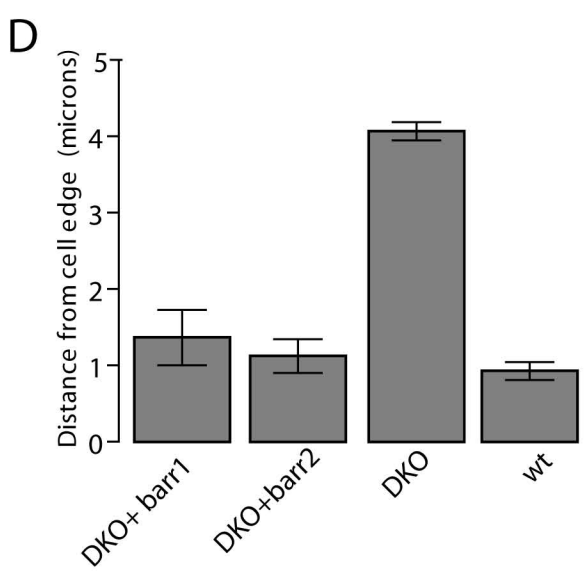
Zoudilova et al., Supplementary Fig. 4



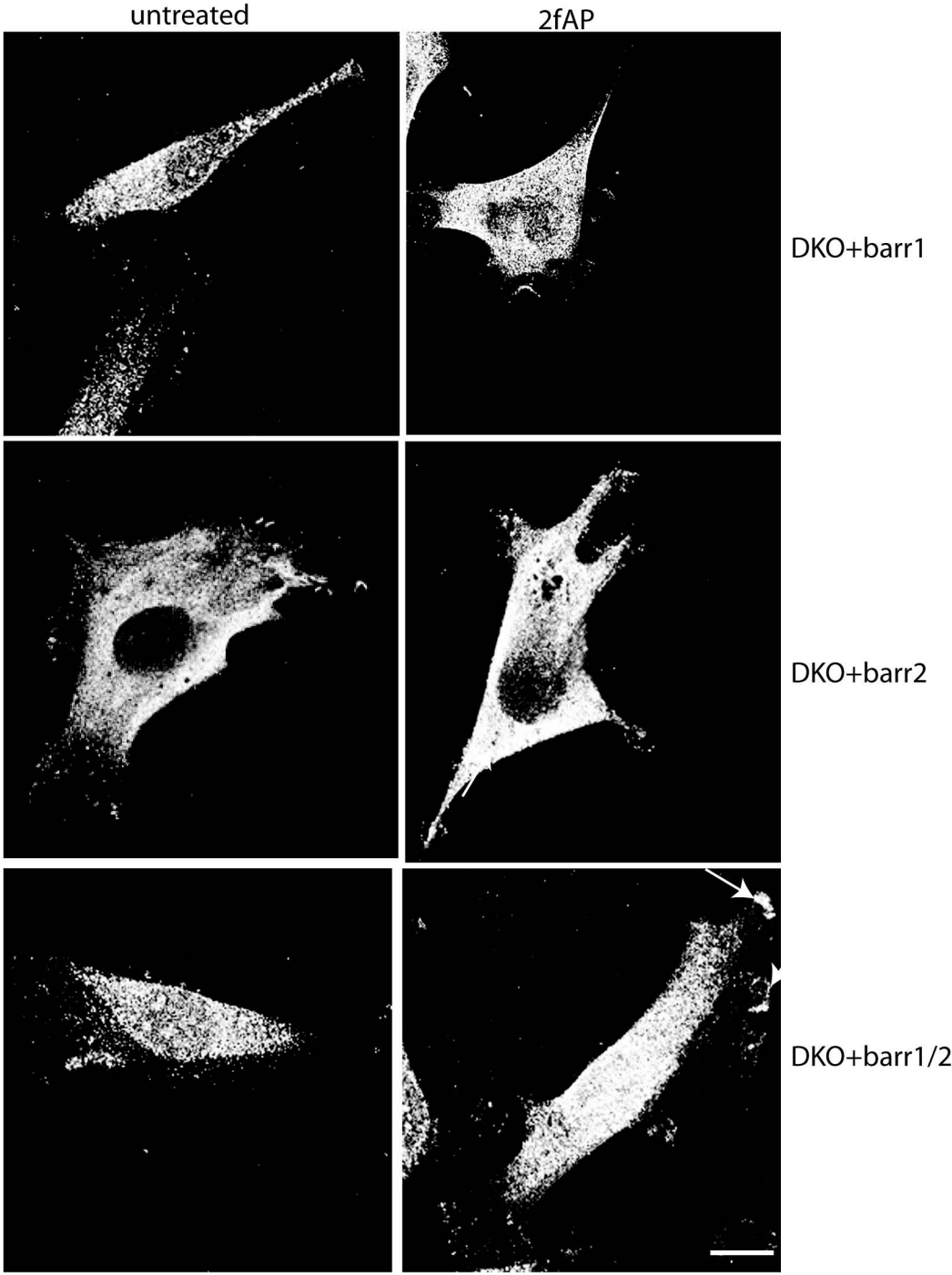
Zoudilova et al., Supplementary Fig 5







Zoudilova et al. Supplementary Fig. 6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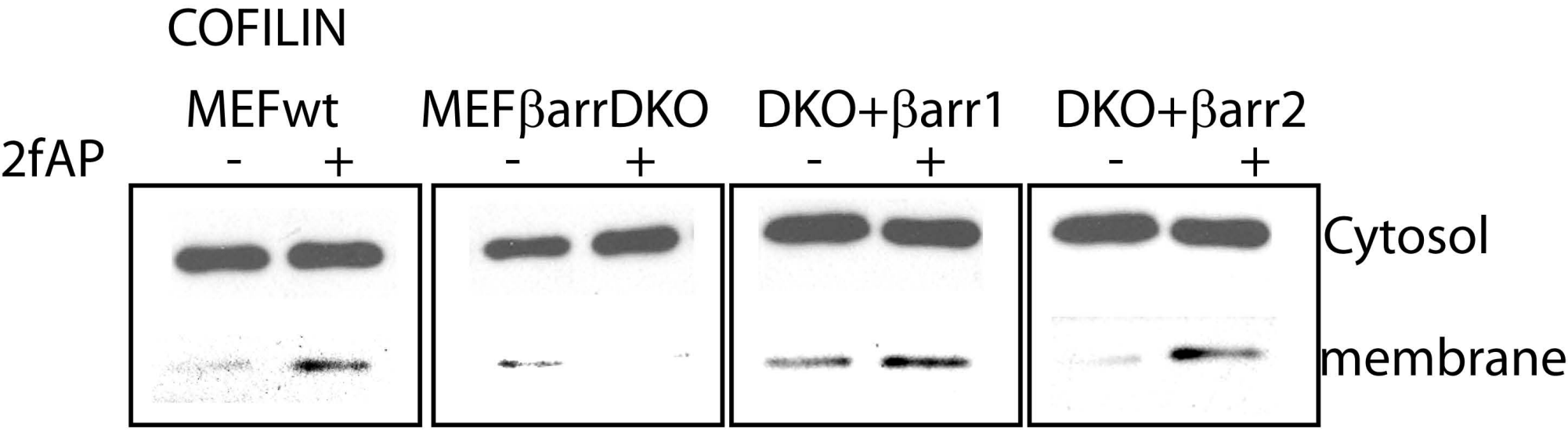
DKO+barr1
DKO+barr2
DKO+barr1/2



Zoudilova et al., Supplementary Fig. 8