Legends for supplementary figures

Supplementary Fig. 1. Illustration for measuring the length of protrusion.

To determine the "length" of membrane protrusion, cells were fixed followed by staining with phalloidin. Background was subtracted, then phalloidin positive peripheral region was measured by Image J. Scoring was done blind to which cell was being analyzed.

Supplementary Fig. 2. Specificity of anti-WAVE2 antibody.

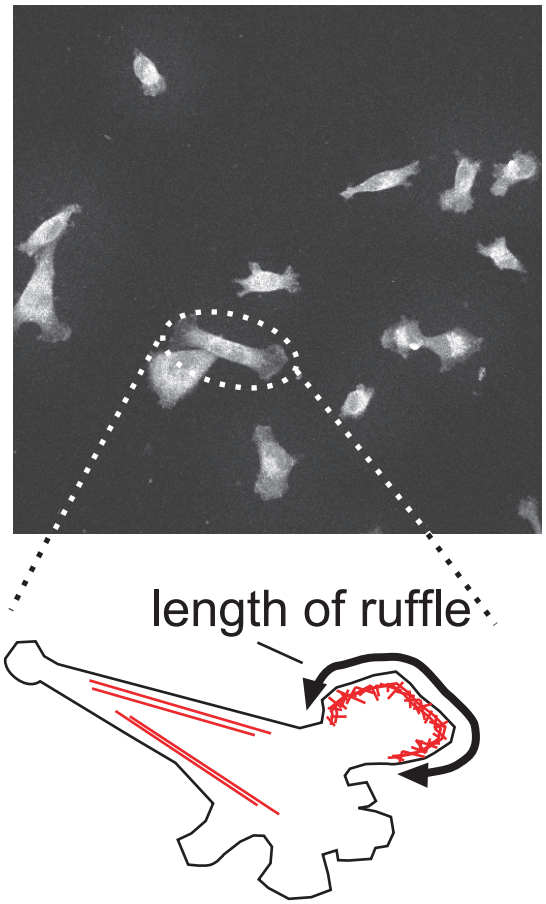
Cells were immunostained using pre-incubated anti-WAVE2 antibody with purified GST or GST-WAVE2 for 1h at 4 °C. Pre-incubation with GST-WAVE2 reduced the WAVE2 staining at the leading edge of cells, but the staining of center region was not disappeared.

Supplementary Fig. 3. WAVE2 knockdown inhibits membrane protrusions induced by PKA activation.

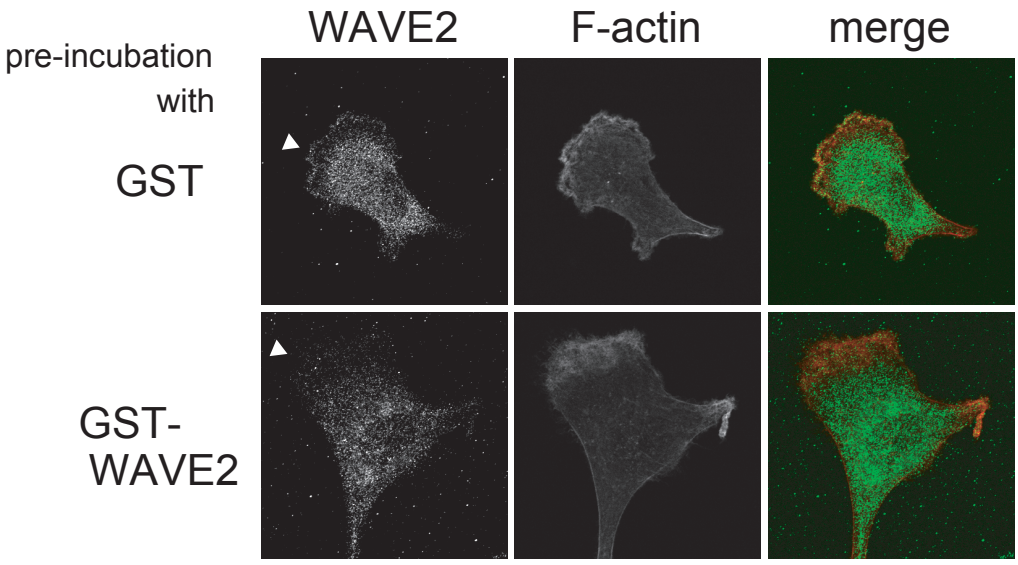
siRNAs (siRNA#1, #2) against WAVE2 purchased from Invitrogen were transfected into MDA-MB-231 cells. **A:** The expression level of WAVE2 was examined by immunoblotting. **B, C:** The length of ruffles were measured as in Fig.7. PKA activation induced ruffle elongation in control cells. However, it did not affect the length of ruffles in both siRNA #1- and #2-transfected cells.

Supplementary Fig. 4. PKA changes their binding affinity to WAVE-family proteins in cell type-dependent manner.

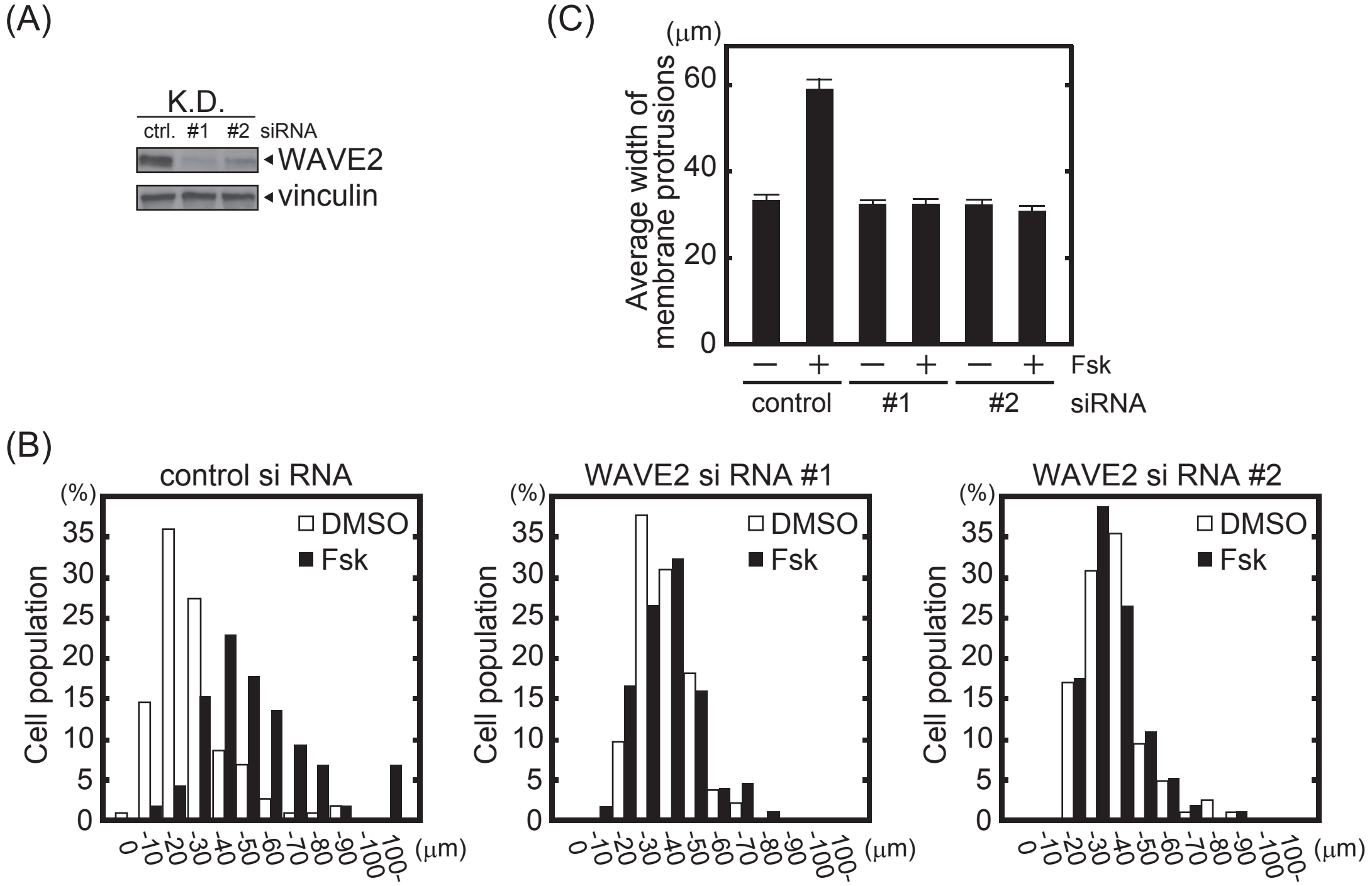
A,B: FLAG-WAVE1 or -WAVE2 was co-transfected with PKA Cα-HA into either COS-7 cells (A) or NIH3T3 cells (B). Cells were lysed and FLAG-WAVE1 or FLAG-WAVE2 was immunoprecipitated with anti-FLAG M2 antibody. Co-precipitated PKA Cα-HA was visualized by immunoblotting using anti-HA antibody. Vinculin was used as a loading control.



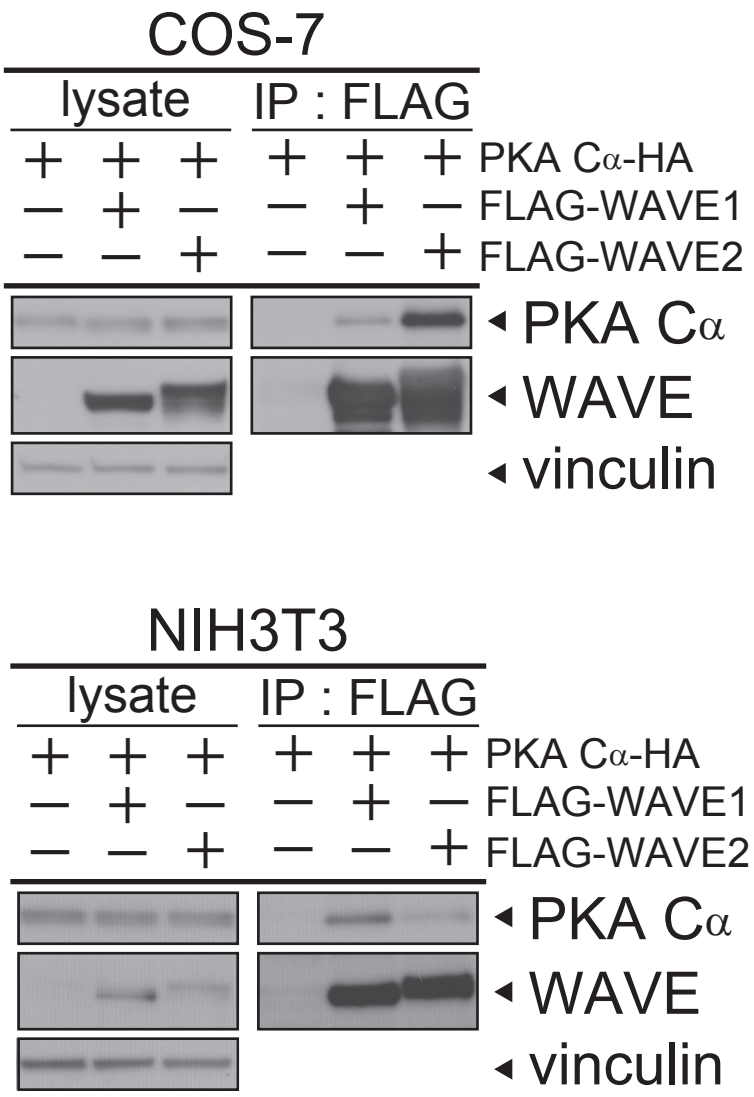
Supplementary Fig. 1 Yamashita el al.



Yamashita et al. Supplementary Fig.2



Supplementary Fig. 3 Yamashita et al.



Supplementary Fig. 4 Yamashita et al.