Table S1
Quantification of oligomerization state of SWAP-70 red labeled molecules and SWAP-70 green labeled molecules in solution by FCCS

Number (N) of monomers in SWAP-70 red and SWAP-70 green molecules as calculated from molecular weight of SWAP-70 obtained from its diffusion time in corresponding spectral channel (red or green) (Supplementary figure 1, equation (S6)). Molecular weight and the diffusion times of the following standards were used for calculations: green or red labeled 42 kDa monomeric SWAP-70 fragment or fluorescent dyes Alexa 488 and Atto 655.

<table>
<thead>
<tr>
<th>N of monomers SWAP-70 green</th>
<th>Standard used</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.1 ± 0.3</td>
<td>42 kDa fragment green</td>
</tr>
<tr>
<td>2.8 ± 0.6</td>
<td>Alexa 488</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>N of monomers SWAP-70 red</th>
<th>Standard used</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.7 ± 0.5</td>
<td>42 kDa fragment red</td>
</tr>
<tr>
<td>2.5 ± 0.4</td>
<td>Atto 655</td>
</tr>
</tbody>
</table>
**Movie 1**

**SWAP-70 translocates to the membrane after vanadate stimulation of the cells**

Translocation of SWAP-70 to the membrane upon vanadate stimulation was observed by confocal microscopy. SWAP-70-GFP is shown in green. Minutes after vanadate stimulation of the cells are presented in the upper left corner.

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**Movie 2**

**SWAP-70 translocates back to the cytoplasm after vanadate removal**

Translocation of SWAP-70 to the cytoplasm after vanadate removal was observed by confocal microscopy. SWAP-70-GFP is shown in green. Minutes after replacement of vanadate with the imaging buffer are presented in the upper left corner.
Movie 3

Actin and SWAP-70 translocate to membrane ruffles upon vanadate stimulation of the cells

SWAP-70 and actin are enriched in membrane ruffles after vanadate stimulation. Data was obtained by confocal microscopy, SWAP-70-GFP is shown in green, actin lifeact RFP is shown in red. Minutes after vanadate stimulation of the cells are presented in the upper left corner.
Figure S1

Fluorescence cross-correlation spectroscopy to study SWAP-70 oligomerization

(A) FCCS setup: Confocal inverted microscope equipped with a high-numerical aperture objective and appropriate dichroic mirrors and emission filters to split the fluorescence for red and green spectral channels. For FCCS measurement the laser is focused in the solution of labeled molecules and fluorophores diffusing in and out of the detection volume induce fluctuations in the fluorescence intensity signal, which is collected by the microscope objective and detected by means of avalanche photo-diode (APD) giving rise to a fluctuating intensity trace $F(t)$. By temporally correlating this trace in time one can obtain the autocorrelation curves (red and green dashed lines). (B) CC% for the mixture of red and green single-labeled SWAP-70 in different experimental conditions: 22 °C (PBS); 37 °C (PBS); 22 °C (PBS + 0.5 M NaCl); 22 °C (PBS + 2 mM MgCl$_2$); 22 °C (PBS + 1mM DTT); 22 °C (PBS + 0.5 mM ATP); 22 °C (PBS + 0.5 M ATP + 2 mM CaCl$_2$) (C) Diffusion time of SWAP-70 in the red spectral channel under experimental conditions as listed in (C). (D) Diffusion time of SWAP-70 full length protein and its 42 kDa monomeric fragment in green and red spectral channel. (E) CC% for the mixture of red and green single-labeled SWAP-70 after addition and subsequent removal of Triton X-100 (0.1 %), Tween-20 (0.1 %), SDS (2 %), Cholate (2 %), or CHAPS (2 %). (F) Diffusion time in of SWAP-70 in the red spectral channel under experimental conditions as listed in (E).
Figure S2

SWAP-70 translocates to the membrane after stimulation of the cells with tyrosine phosphatase inhibitor vanadate

Representative confocal fluorescence microscopy images of untreated (left) and vanadate-treated (right) B16 melanoma cells expressing SWAP-70 GFP. (A) Wt SWAP-70 in cells treated with wortmannin. The protein fails to translocate to the cytoplasmic membrane. (B-E) Mutants of SWAP-70 GFP translocate to the membrane after stimulation: (B) Δ1-197 mutant (C) Y216F SWAP-70 mutant. (D) Y241,242F SWAP-70 mutant. (E) Δ353-585 SWAP-70 mutant. (F) Y302F SWAP-70 mutant. Scale bars: 5 µM.
Figure S3
Visualization of F-actin patches in cells treated with cytochalasin D; 3D surface intensity plots.

(A-C) Representative confocal fluorescence microscopy images of untreated cells expressing wt or mutant SWAP-70 GFP (green) and LifeAct-RFP (red), visualising actin. (A) wt SWAP-70 GFP, (B) AB mutant, (C) Y426F mutant. (D) average intensity values for wt SWAP-70 GFP, the Y426F mutant and actin at the cytoplasmic membrane and in the cytoplasm measured in the red and green fluorescent channels. The intensity for Y426F mutant in the positions of accumulated actin at the membrane is higher then in the cytoplasm, which is not the case for the wt protein. (E-F) Left images: SWAP-70-GFP and its mutants. Middle images: actin visualised with Life-Act RFP. Right images: SWAP-70 GFP image after subtraction of brightest pixels in RFP channel. One can see the disappearance of the brightest intensity spots from GFP image if they were colocalized with actin patches. (E) wt SWAP-70. (F) SWAP-70 AB mutant. (G) Y426F mutant. (H-J) Quantification of SWAP-70 and actin patches in the cells treated with Cytochalasins-D. Left images: merge of SWAP-70-GFP (green) and Actin Lifeact-RFP (red) fluorescent images. Right images: particle analysis for green and red fluorescent patches performed in FIJI. The thresholds of green and red images were adjusted to similar levels to predominantly visualise fluorescent patches. Patches in size of 10-500 pixels were selected for the analysis. In the images the merge of green (SWAP-70) and red (actin) patches is shown. (H) wt SWAP-70. (I) SWAP-70 AB mutant. (J) Y426F mutant. (K) Number of actin patches in cells transfected with wt SWAP-70, SWAP-70 AB mutant, Y426F mutant. (L) Number of SWAP-70 patches in cells transfected with wt SWAP-70, SWAP-70 AB mutant, Y426F mutant. Almost no patches were detected for AB mutant. (M) Percentage of actin patches found in SWAP-70 patch position for cells transfected with wt SWAP-70 and Y426F mutant. (N) Percentage of actin patches localised at the membrane in the cells transfected with wt SWAP-70, SWAP-70 AB mutant and Y426F mutant. There were more actin patches in the membrane in the cells transfected with Y426F mutant. (O) Number of SWAP-70 containing actin patches at the cell bottom in the untreated cells expressing Y426F mutant and in treated cells expressing wt and AB mutant.
Figure S4

Representative decay curves for FLIM-FRET experiments

(A) vanadate treated cells over-expressing Cerulean. (B) cells over-expressing FRET positive standard Cerulean-Venus fused. (C) Untreated cells over-expressing SWAP-70 Cerulean and SWAP-70 Venus. (D) Vanadate treated cells over-expressing SWAP-70 Cerulean and SWAP-70 Venus.
Figure S5

Double exponential fit of FLIM-FRET data

From top to bottom, first row: mean lifetime $t_m$, second row: lifetime of the first component $t_1$, third row: percentage of the first component ($a_1$) obtained in untreated and vanadate treated cells for: (A-C) wt SWAP-70 and its mutants R546A and K553A, (D-F) wt SWAP-70 and its mutants Y426F and Y426E.
Figure S6
Dimerization of SWAP-70 in cytochalasin-D treated cells

(A) From left to right, GFP lifetime for untreated and vanadate treated cells: wt SWAP-70, AB mutant (Δ564-585), wt SWAP-70 after cytochalasin D addition. In cytochalasin D treated cells interaction with actin is inhibited. (B) Representative FLIM-FRET images of cells co-transfected with wt SWAP-70 Cerulean and SWAP-70-Venus. Cells were untreated or treated with vanadate after incubation with cytochalasin D. Mean Cerulean fluorescence lifetime (ps) in the cell is presented in colour code according to the scale on the left. (C) Mean Cerulean fluorescence lifetime of wt and Y426F mutant SWAP-70 after incubation of the cells with cytochalasin D in vanadate untreated cells in the cytoplasm (white), in vanadate untreated cells in the protein clusters (grey), in vanadate treated cells at the membrane (dark grey).
Figure S7 SWAP-70 dimerisation upon 12(S)-HETE stimulation

(A) Representative confocal fluorescence microscopy images of unstimulated (left) and stimulated with (12S)-HETE (right) cells expressing SWAP-70 GFP (green). (B) Percentage of cells showing ruffle-like membrane protrusions in stimulated and unstimulated sample. (C) SWAP-70 localizes at the ruffle-like structures and spots at the cell edge. (D) Representative FLIM-FRET images in cells co-transfected with wt SWAP-70 Cerulean and SWAP-70-Venus in unstimulated cells, cells treated with vanadate and cells stimulated with (12S)-HETE. Mean Cerulean fluorescence lifetime (ps) in the cell is presented in colour code according to the scale on the left. Lifetime decreases in membrane protrusions at the cell edges (white arrow). (E) Cerulean wt SWAP-70 fluorescence lifetime (ps) calculated from FLIM-FRET images in the cytoplasm/membrane of untreated cells, in the membrane of vanadate treated cells (grey), in cell ruffle-like membrane structures of cells stimulated with (12S)-HETE.