Supporting Online Material

Material and Methods:

Chemicals

Buffers were purchased from AppliChem (Darmstadt, Germany), Merck (Darmstadt, Germany). All other chemicals were purchased from Sigma (Munich, Germany) and of the highest purity available.

PCR/primer/cloning/protein expression conditions

PCR reactions were performed using the following primer combinations:

Primer combination	Vector	Restriction sites	Fragment
FW: 5'-GGGCGCATATGAACAACAACTCGCAGGAGC-3'			
RW: 5'-CCGGGAATTCGGATCGAATGTCGGGACG-3'	pET21b	NDE1/EcoR1	SpHtp1$^{69-198}$
FW: 5'-GGGCGCATATGCGCATTCACCACCCGTTGACC-3'			
RW: 5'-CCGGGAATTCGGATCGAATGTCGGGACG-3'	pET21b	NDE1/EcoR1	SpHtp1$^{24-198}$
FW: 5'-GGGCGCATATGCGCATTCACCACCCGTTGACC-3'			
RW: 5'-CGAGAATTCGGCTTGTCGATAACTTGTCC-3'	pET21b	NDE1/EcoR1	SpHtp1$^{24-68}$
FW: 5'-CTCCGTCGACATGGCCTCCTCCGAGGA-3'			
RW: 5'-CACTCCACCGGCGCCAAAGCGGCCGCACT-3'	pET21b	Sal1/Not1	mRFP

with KOD-Hot start DNA polymerase (Novagen; Lot# M00057770). The PCR-products were blunt end cloned into pETblue-2 or pUC19, digested using the *Nde*I and *Eco*RI restriction sites embedded in the primers and subcloned in pre-cut pET21b. The resulting fragments were in frame with the $(His)_6$ tag encoded by pET21b. Monomeric red fluorescent protein was cloned into pET21b using the *SalI/Not*I cleavage sites. These are located 3' from the *Nde*I and *Eco*RI and in frame with the $(His)_6$-tag of the vector. The DNA template for the mutant SpHtp1 construct, SpHtp1$^{24-198}$mRFP$(His)_6$ KRHLR/GGHLG was purchased from Genscript.

The sequenced constructs in pET21b were transformed in *Escherichia coli* Rosetta gami B (DE3, pLys; Novagen) cells and over expressed under the control of the T7 promoter. For protein expression, cells where grown in LB-media to an OD_{600} between 0.6 and 0.8 and induced with 1mM

IPTG for 6h at 37°C. Aliquots of 12 g cells were re-suspended in 40 ml 50 mM sodium-phosphate pH 7.1 and incubated for 20 min with 250 U of Benzonase (Sigma), two tablets of protease inhibitor (Roche, #11873580001) and 0.1 g lysozyme (Fluka, #62971). After the incubation, the solution was French-pressed, diluted 1:5 in the respective buffer and the soluble fraction was separated from the non-soluble by centrifugation at 50,000*x*g for 1 h.

Column specifications:

NTA column: 15 ml NTA Agarose (Invitrogen, #60-0441), column dimensions: 1 cm Ø, 20 cm height

SO_3^- column: 40 ml Fractogel-EMD-SO^{3-} (M) (Merck, #1.16882.0100), column dimensions: 2 cm Ø, 20 cm height

QAE column: 35 ml QAE-sephadex A25 (GE Healthcare, #17-0190-01), column dimensions: 2 cm Ø, 20 cm height

Purification strategies for SpHtp1$^{24-198}$(His)$_6$ and SpHtp1$^{68-198}$(His)$_6$ can be found in the according reference (1).

Purification of SpHtp1$^{24-68}$mRFP(His)$_6$

The French press supernatant was adjusted to pH 7.8 and passed onto the SO_3^- column adjusted with 10 mM Tris buffer pH 7.8 and was washed with 5 volumes of the same buffer containing 300 mM NaCl. From this column, the protein was eluted with 10 mM Tris buffer (pH 7.8) containing 2 M NaCl. The eluted fractions containing the protein were passed through the NTA agarose column. Subsequently, the column was washed with 100 ml 50 mM phosphate buffer containing 30 mM imidazole (pH 7.8) and the protein was eluted with 50 mM phosphate buffer containing 300 mM imidazole, adjusted to pH 7.8 and fractions were analysed by SDS-PAGE.

Purification of SpHtp1$^{24-198}$mRFP(His)$_6$GGHLG

The French press supernatant was adjusted to pH 7.8 and passed onto the SO_3^- column adjusted with 10 mM Tris buffer pH 7.8. Additionally, the flow through of this column, containing the protein, was passed onto the NTA agarose column. Subsequently, the column was washed with 100 ml 50 mM phosphate buffer containing 30 mM imidazole (pH 7.8) and the protein was eluted with 50 mM phosphate buffer containing 300 mM imidazole adjusted to pH 7.8. The fractions containing the protein were then incubated for 20 min at 55°C and centrifuged for 20 min at 15,000 *x*g (4°C) and the supernatant was diluted 5x in 25 mM phosphate buffer pH 7.5. Finally the protein was subjected onto a QAE agarose column, washed with buffer containing 100 mM NaCl, eluted with 200 mM NaCl and fractions were analysed by SDS-PAGE.

Purification of mRFP(His)$_6$

The French press supernatant was adjusted to pH 7.5 and passed onto the SO_3^- column adjusted with 25 mM HEPES buffer pH 7.5. Additionally, the flow through of this column, containing the protein, was

heated for 30 min at 75°C and centrifuged for 30 min at 8,000 *x*g (4°C). The heat treatment was performed to precipitate non-heat stable proteins since mRFP is extremely heat stable and heat treatment does not affect the fluorescence of the protein (2). The supernatant was subsequently passed onto the NTA agarose column, washed with 100 ml 50 mM phosphate buffer containing 30 mM imidazole (pH 7.5) and the protein was eluted with 50 mM phosphate buffer containing 300 mM imidazole adjusted to pH 7.5. Finally the protein was subjected onto a QAE agarose column, washed with buffer containing 100 mM NaCl, eluted with 200 mM NaCl and fractions were analysed by SDS-PAGE.

Generally, after purification all fractions of pure protein were dialysed 2x 3 h and 1x overnight against 50 mM sodium phosphate buffer pH 7.5.

Isothermal titration calorimety (ITC)

Titration experiments were performed with a MicroCal ITC_{200} at 20°C. Before the experiment, the instrument was heat-pulse-calibrated and the protein samples were extensively dialysed against the respective buffers. Titrant stock solutions were prepared with the same batch of buffer as used for dialysis. All solutions used were degassed before filling the sample cell and syringe. Titration steps, compound concentrations and volumes are given in the figure legends for the individual experiments. The ITC stirring speed was set to 1000 rpm; the feedback gain mode was set to medium. Since the initial injection generally delivers inaccurate data, the first step was omitted from the analysis. The collected data were analysed using the program “Origin” (MicroCal) and binding isotherms were fitted using the binding models provided by the supplier. Errors correspond to the standard deviation (SD) of the nonlinear least-squares fit of the data points of the titration curve.

Phospholipid binding assays

The lipid spot membranes (Echelon Bioscience; #S-6000, #P-6001, #P6002) were equilibrated for 10 min with PBS containing 0.1 % Tween 20 and 5 % lipid free bovine serum albumin (BSA). The solution was then changed and the protein was added to a final concentration of 3 µM and incubated with the membranes for 20 min. Subsequently, the membranes were washed 3x with PBS/Tween/BSA. Antibody detection was performed with a horse radish peroxidase (HRP) coupled anti-His-antibody (Qiagen penta His) in phosphate buffer saline (PBS)/Tween/BSA at a titre of 1:10,000. Detection was carried out using ECL (Amersham).

Live cell imaging

Cell culture maintenance and procedure of confluent layer growth on cover slips have been described previously (1). HEK293 cells were also handled according to this protocol with the exception that DMEM medium was used and the cells were grown at 37°C in an atmosphere of 5 % CO_2. The

washed cover slips were transferred to optical Petri-dishes (Ø 3cm) and covered with the respective media containing 10 % foetal calf serum (FCS) and the mRFP tagged proteins at the indicated concentrations and temperatures. Before imaging the cells were washed 3x 5 min in PBS, 2x 5 min with PBS adjusted to pH 5.5, 2x 5 min in PBS adjusted to pH 8.5 and finally 2x 1 min with L15 medium containing 10 % FCS (DMEM medium for HEK293 cells). Images were recorded using a Zeiss LSM510 confocal microscope equipped with a water dipping lens. All images were obtained using the same microscope settings for all treatments. The microscope settings were: optical slice = 2 µm, Red channel: Excitation: 543 nm; Detector gain: 750; Filter setting: LP 560 nm

Onion cell translocation assay

Onion skin epidermal cells were washed 3x for 5 min with 25 mM sodium phosphate buffer pH 7.5 before applying 3 µM SpHtp1$^{24-68}$mRFP(His)$_6$ in 25 mM sodium phosphate buffer containing 10% BSA (to block unspecific protein binding sites). Incubations were carried out for 1 h. After incubation the epidermal skin was washed 5x for 10 min with PBS and imaged on a Zeiss LSM 510 confocal microscope equipped with a water-dipping lens. Settings: Optical slice = 2 µm; Red channel: Excitation: 543 nm; Detector gain: 750; Filter setting: LP 560 nm

Viability controls

To evaluate if incubation of cells with SpHtp1$^{24-68}$mRFP(His)$_6$ impairs the trafficking or endocytosis of the used cell types co-translocation experiments were performed. To check onion cells the cells were incubated as described above in presence of 16 µM FM1-43 (Invitrogen). For HEK293 and RTG-2 cells Alexa Flour 488 labelled wheat germ agglutinin (Invitrogen) was used at a final concentration of 10 µg/ml.

Flow cytometry (FACS)

Equalised RTG-2 cell suspensions (~1•10^5 cells in 10 ml medium; 25 cm^2 flasks) were grown to confluent layers. For sulfotransferase inhibition $NaClO_3$ was added at a final concentration of 70 mM to the medium. After 2 days of growth the cells were washed 5x with fresh medium and subsequently incubated with the respective protein for 1 h. To minimise signals from protein molecules that were eventually stuck on the cell surfaces, the cell samples were washed 3x 5 min with PBS, 1x 10 min with PBS containing the DNA stain TO-PRO-3 iodide (Invitrogen 1:1000 diluted), 2x 5 min with PBS adjusted to pH 5.5, 2x 5 min with PBS at pH 8.5 and 2x for 1 min with L15 medium. Additionally, the cells were dislodged with 1 ml of a trypsin solution (0.5 mg/ml, + 1mM EDTA) filtrated through a cell strainer and counted. On average a cell concentration of ~2•10^6 cells/ml was obtained. The mRFP fluorescence present in the cells was then quantified on an LSR II flow cytometer (BD Bioscience). From 10,000 counted events, a homogenous population of cells that were TO-PRO-3 iodide negative was selected (on average ~80 % of all events) and further analysed for mRFP fluorescence.

Excitation for mRFP fluorescence was 488 nm and the detector filter setting was 610/20 BP (600-620 nm). Excitation for the TO-PRO-3 iodide fluorescence was 633 nm and the detector filter setting was 660/20 BP (650-670 nm). Data analysis was carried out using FlowJo v 7.6.

Sample preparation for western blotting

Samples were prepared as described for the FACS analysis, but lacking the TO-PRO-3 iodide incubation step. After trypsination the cells were harvested by centrifugation and resuspended 2x in PBS to remove the excess of trypsin. The wet cell pellets containing ~$2 \cdot 10^6$ cells (~ 20 µl) were lysed at 95°C in 100 µl of Laemmli sample buffer containing 8 M urea, 2 % β-mercaptoethanol and 1 mM PMSF. Sample volume loaded on the gel was 15 µl.

General information

Sulphatase type VI from *Aerobacter aerogenes*, $NaClO_3$, lipid free BSA and inositol-1,4-diphosphate were obtained from Sigma (#S1629; 403016; A7030; I0510), H-Tyr(PO_3)-OH (F4180) and H-Tyr(SO_3)-OH (E-3645) were purchased from Bachem (Germany). Fmoc-Tyr(SO_3)-OH was obtained from Novabiochem (04-12-11-5). The anti-tyrosine-sulphate antibody was from Millipore (05-1100), the anti-tyrosine-phosphate antibody was obtained from CellSignaling (9410). Inositol-1,3-diphosphate and the lipid spot membranes were obtained from Echelon Bioscience.

SDS-PAGE

SDS-PAGE was essentially performed according to the manufacturer's instructions (Invitrogen). Gradient 4-12% Bis-Tris NuPage gels were used with NuPage MES-SDS running buffer (Invitrogen). Protein samples were dissolved in Laemmli SDS buffer (Invitrogen) containing 8M urea and 2% ß-mercaptoethanol.

Supplementary Notes

Note 1:

We have to note that SpHtp1$^{24-68}$mRFP$(His)_6$ was able to attach to the surface of HEK293 cells only when very densely grown cultures were used. However, even in these circumstances we could not detect any uptake of SpHtp1$^{24-68}$mRFP$(His)_6$ into human cells by employing confocal microscopy.

We also have to note that the experimental conditions of the translocation assay presented here are not identical to these reported by **Kale** *et al*. (3) since we used 15x less protein and 12x shorter incubation times. Also a different human cell line was used.

Note 2:

Some batches of arylsulfatase VI were less active and required longer cell pre-treatments. The batch that was used to obtain the data shown here was #090M8619.

Note 3:

In general the experiments were repeated at least three times apart from the ITC and lipid binding experiments which were only repeated twice (since no interaction/relevant signals could be detected) to reduce costs. All experiments shown were consistent when repeated.

Supplemental Figures

Figure S1

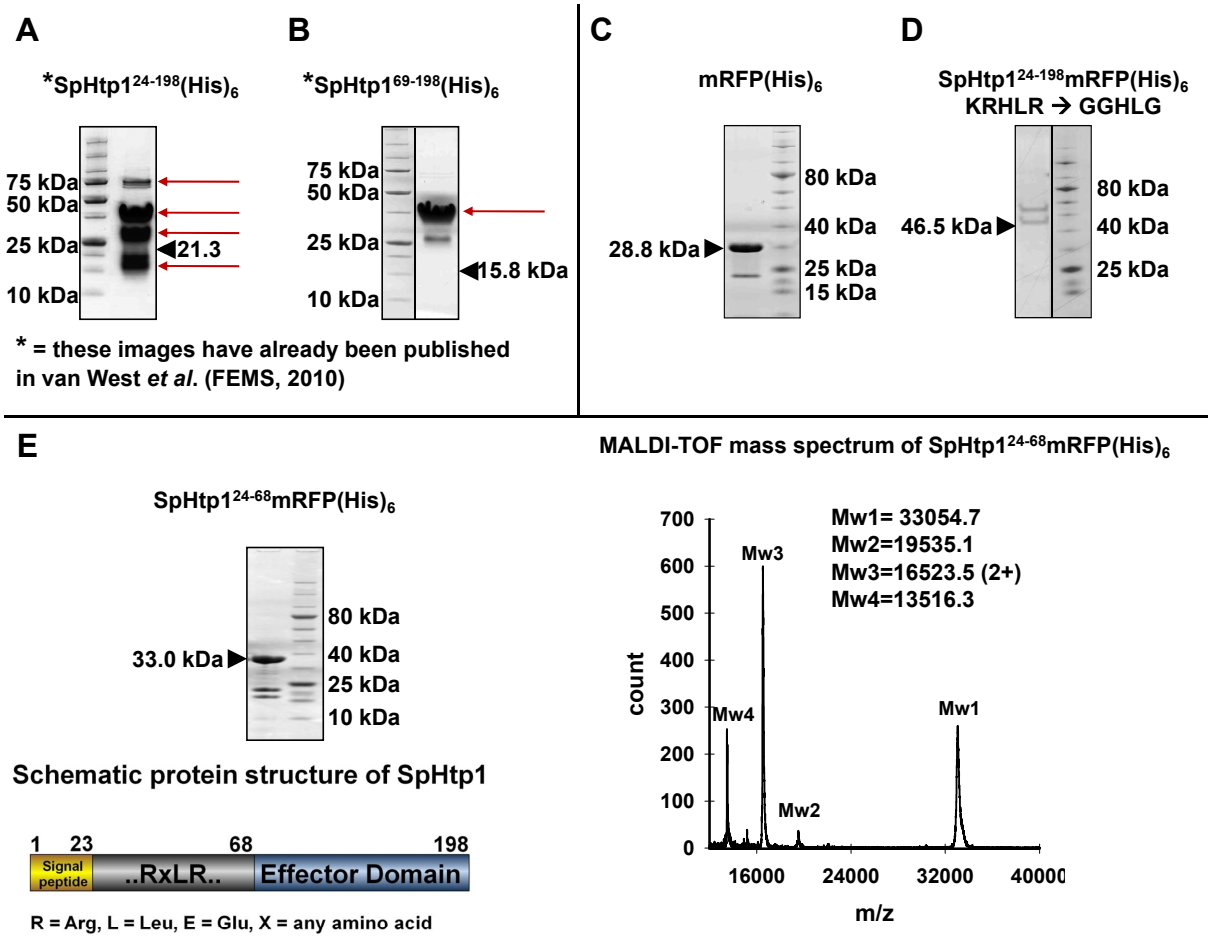


Fig. S1:

Coomassie SDS-PAGE gels of the indicated purified recombinant proteins. The theoretical retention according to the respective size of the protein constructs are indicated with black arrows. **A**,**B**) SpHtp1$^{24\text{-}198}$(His)$_6$ and SpHtp1$^{69\text{-}198}$(His)$_6$ do not run according to their molecular weight on SDS-PAGES because the C-terminally domain of SpHtp1 is partially SDS-resistant (a more detailed analysis of this phenomenon can be found in van West *et al*. 2010 (1)). All bands indicated by red arrows have been subjected to a MS-MS analysis that proved that all bands correspond to the respective protein constructs. **C**) SDS-PAGE of the purified mRFP(His)$_6$. **D**) SDS-PAGE of the mutated full length SpHtp1-mRFP fusion protein, SpHtp1$^{24\text{-}198}$mRFP(His)$_6$ GGHLG, where the amino acids of the KRHLR were changed to GGHLG. Two bands were found after purification on SDS-PAGEs presumably caused by the SDS-resistant property of SpHtp1, since both bands were identified as SpHtp1$^{24\text{-}198}$mRFP(His)$_6$GGHLG by MS-MS analysis. **E**) SDS-PAGE of SpHtp1$^{24\text{-}68}$mRFP(His)$_6$. After purification two additional bands were found at an apparent molecular weight of 20-25 kDa. The upper band of these is recognised by the anti-His-antibody, the lower one

not. The MALDI-TOF spectrum showed that both bands are the result of a proteolytic cleavage within the mRFP domain of the fusion protein. These protein fragments could not be separated from the uncleaved protein by size exclusion chromatography, which indicates that, despite the cleavage both fragments are still interacting with one another. These non-covalent linked polypeptides seem to complement to a functional protein since the band recognised by the anti-His-antibody was found inside RTG-2 cells after incubation with the protein solution (**Fig. 3S** lane 5). Another possibility for the presence of the anti-His antibody recognized band inside RTG-2 cells is that cleavage occurs after translocation into the host cell.

Figure S2

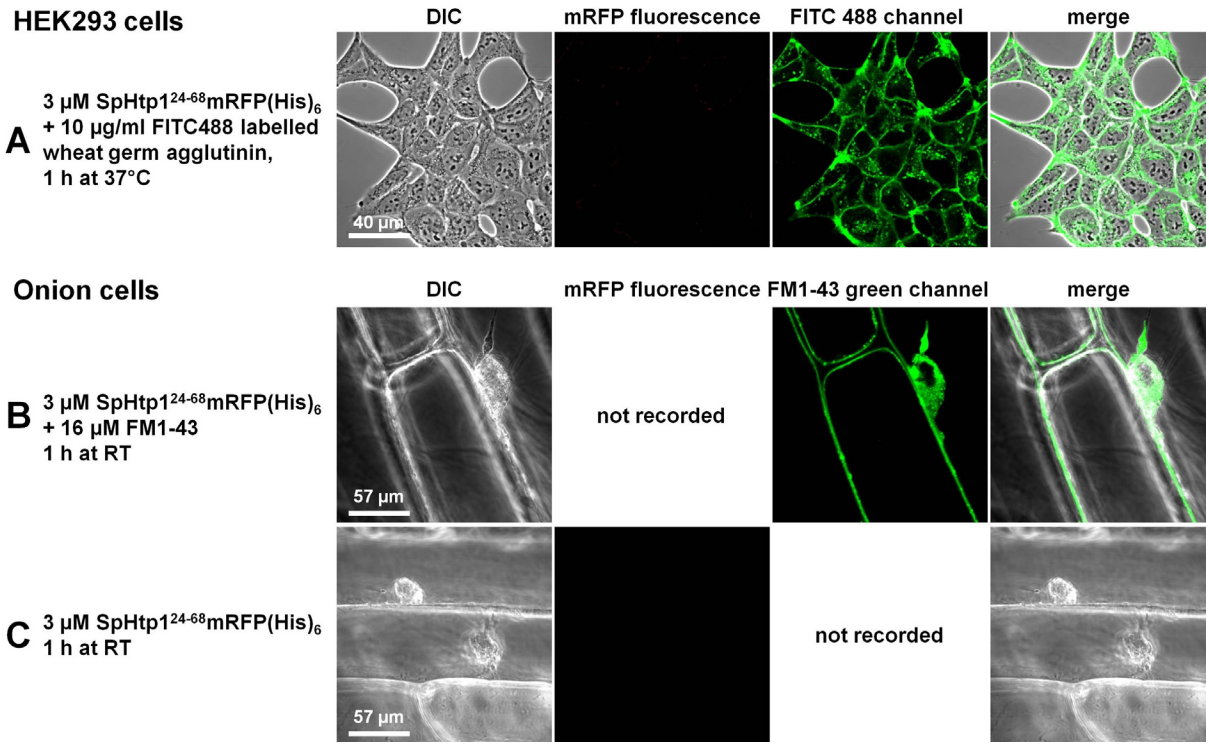
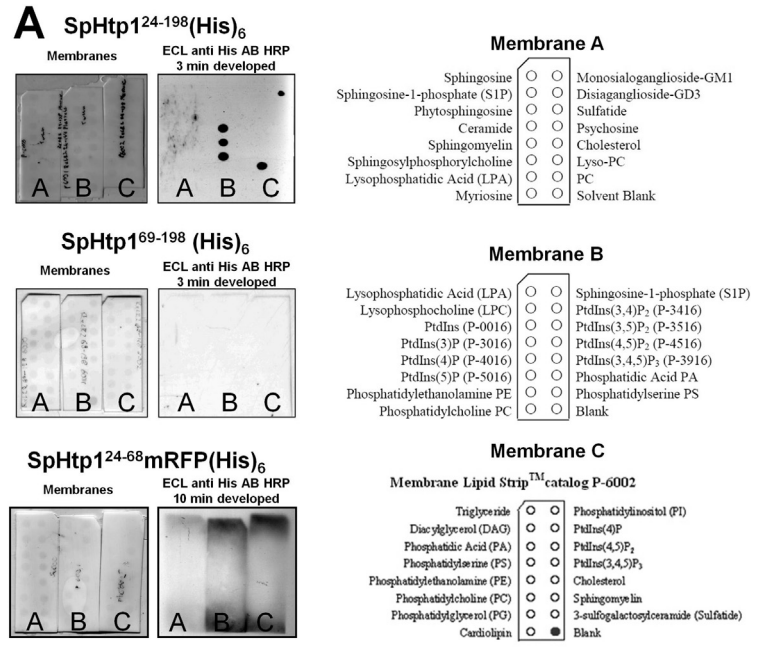


Fig. S2.

Viability controls for HEK293 and onion epidermis cells. **A**) After co-incubation of HEK293 with SpHtp1$^{24-68}$mRFP(His)$_6$ and Alexa Flour 488 labelled wheat germ agglutinin (WGA) only green fluorescence could be detected inside the cells. This showed that these cells were alive and not impaired in their protein uptake ability by the treatment. Cells were incubated for 1 h with 3 µM SpHtp1$^{24-68}$mRFP(His)$_6$ and 10 µg/ml WGA in DMEM medium containing 10 % foetal calf serum (FCS). **B/C**) In order to show that the onion cells were alive, co-incubation with 16 µM FM1-43 and 3 µM SpHtp1$^{24-68}$mRFP(His)$_6$ were performed (B) since the lectin WGA is not able to enter plant cells. Because FM1-43 is known to have emissions when incubated in biological membranes that overlap

with that of mRFP under the given conditions (4) we omitted the recording of the red channel. In parallel, as a control we imaged onion cells from the same tissue that were only incubated with 3 µM SpHtp1$^{24-68}$mRFP(His)$_6$ (C). These images show that the uptake of the membrane stain is not impaired by SpHtp1$^{24-68}$mRFP(His)$_6$. The onion cells were incubated as described. Red channel: mRFP fluorescence; green channel: Alexa Flour 488 fluorescence; white channel: DIC

Figure S3



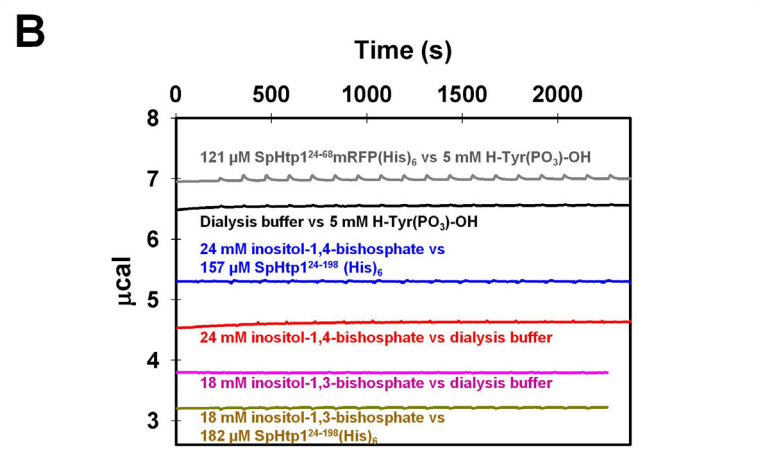


Fig. S3.

A) Lipid binding profiles of SpHtp1$^{24\text{-}198}$(His)$_6$, SpHtp1$^{69\text{-}198}$(His)$_6$ and SpHtp1$^{24\text{-}68}$mRFP(His)$_6$. The lipid membranes were incubated with 3 µM protein. Only SpHtp1$^{24\text{-}198}$(His)$_6$ showed signals after antibody detection. The lipid recognised on membrane A was sulfatide (weak), those on membrane B are phosphatidylinositol(3)-, phosphatidylinositol(4)- and phosphatidylinositol(5)-phosphate and those on membrane C are cardiolipin and phosphatidylinositol(4)-phosphate. Please note that the membranes of the lipid blots shown for SpHtp1$^{24\text{-}68}$mRFP(His)$_6$ are inverted (cut on the top right corners).

B) The N-terminal-leader of SpHtp1 does not bind to either inositol-1,4- or inositol-1,3-bisphosphate (I1,4P2 and I1,3P2), the polar head groups of phosphatidylinositol(4)- and phosphatidylinositol(3)-phosphate. No difference between a titration of 24 mM I1,4P2 into the dialysis buffer (red) and buffer

containing 157 µM SpHtp1$^{24-68}$mRFP(His)$_6$ (blue) was found. Similar observations were made for I1,3P2 (purple and dark yellow).

Titration of 5 mM H-Tyr(PO_3)-OH into the dialysis buffer showed that the dilution heat for this compound was minimal (black). The thermogram obtained for a titration of 5 mM H-Tyr(PO_3)-OH to 121 µM SpHtp1$^{24-68}$mRFP(His)$_6$ showed only a very weak signals (grey). For all titration the dialysis buffer was 50 mM sodium phosphate pH 7.5.

Figure S4

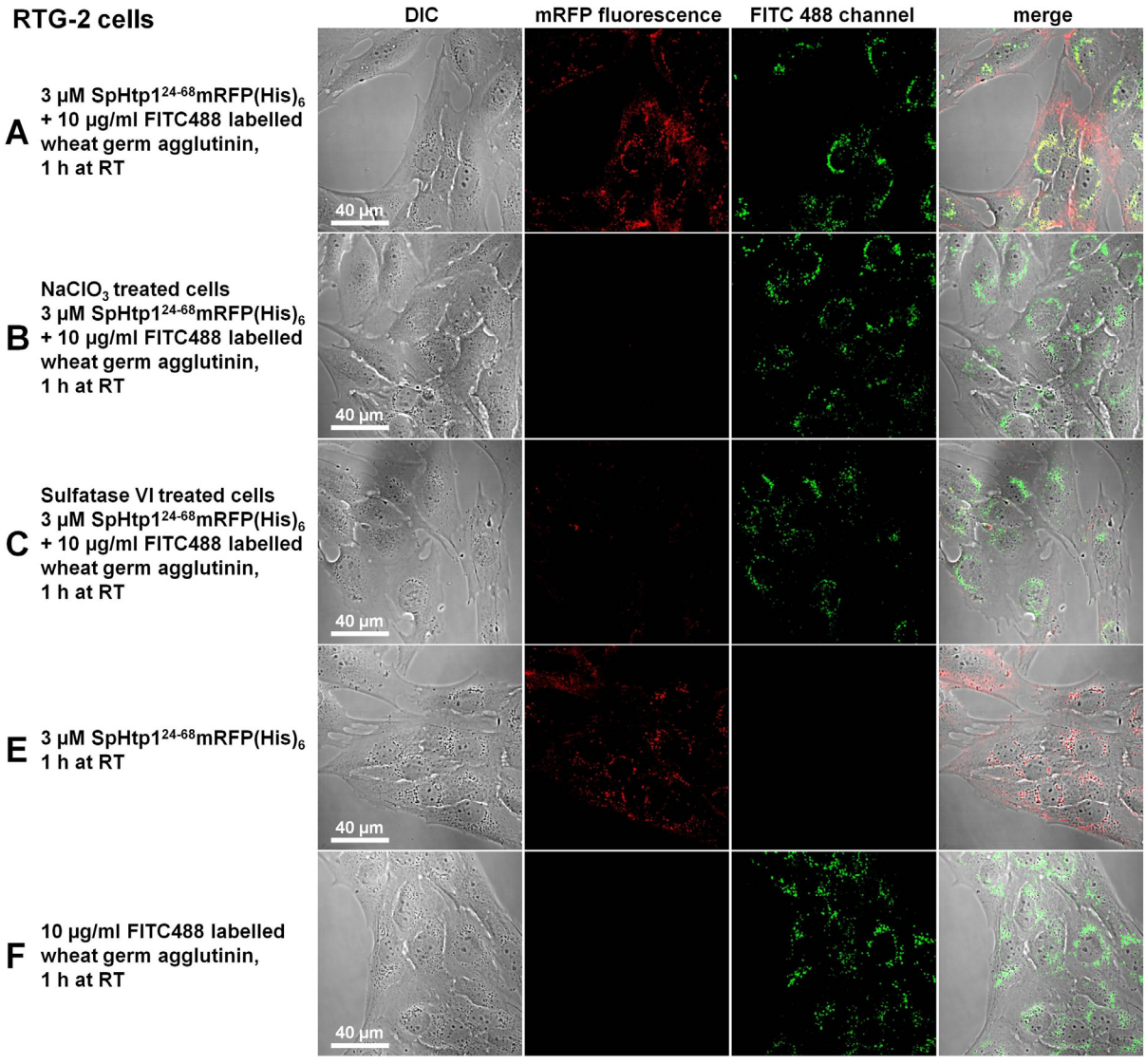
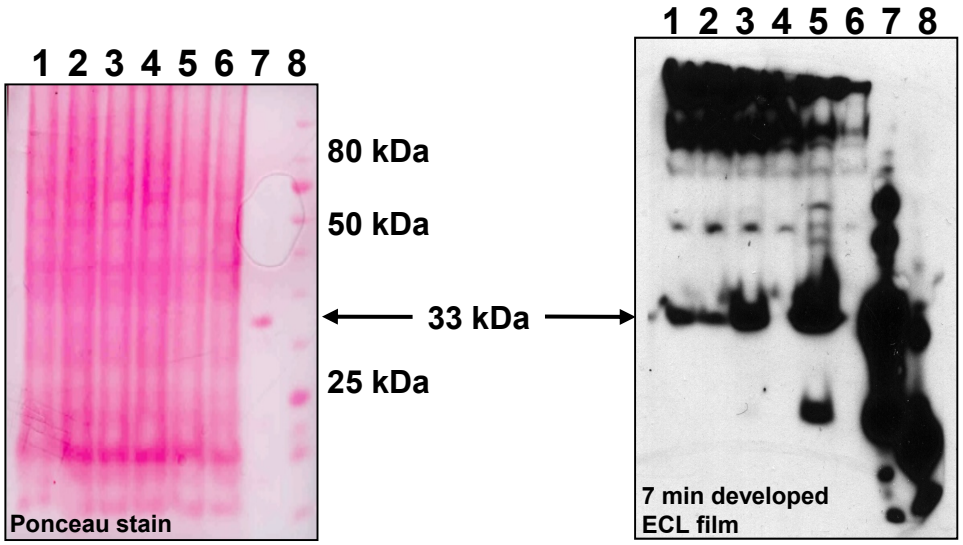


Fig. S4.

Viability controls for $NaClO_3$ and aryl-sulfatase treated RTG-2 cells. After co-incubation of RTG-2 cells with 3 µM SpHtp1$^{24-68}$mRFP(His)$_6$ and 10 µg/ml Alexa Flour 488 labelled wheat germ agglutinin (WGA) a co-localisation of green and red fluorescence in vesicles around the nucleus was observed

(**A**). Whilst pre-treatment of the RTG-2 cells with 70 mM $NaClO_3$ (48 h) (**B**) or 1 U aryl-sulfatase (3 h) (**C**) strongly reduced the uptake of SpHtp1$^{24-68}$mRFP(His)$_6$ the translocation of WGA remained unaffected. For comparison RTG-2 cells only treated with SpHtp1$^{24-68}$mRFP(His)$_6$ (**E**) or WGA (**F**) are shown. The cells were incubated for 1 h with the respective proteins in L15-medium containing 10 % FCS. Red channel: mRFP fluorescence; green channel: Alexa Flour 488 fluorescence; white channel: DIC

Figure S5



1 = RTG-2 cells + 3 µM SpHtp1$^{24-68}$mRFP(His)$_6$ + 2 mM FMOC-Tyr(SO_3)-OH

2 = RTG-2 cells + 3 µM SpHtp1$^{24-68}$mRFP(His)$_6$ + 70 mM $NaClO_3$

3 = RTG-2 cells + 3 µM SpHtp1$^{24-68}$mRFP(His)$_6$ + 2 mM H-Tyr(SO_3)-OH

4 = RTG-2 cells + 15 µM mRFP(His)$_6$

5 = RTG-2 cells + 3 µM SpHtp1$^{24-68}$mRFP(His)$_6$

6 = RTG-2 cells

7 = 60 pmol SpHtp1$^{24-68}$mRFP(His)$_6$

8 = protein ladder

Fig. S5

Translocation of SpHtp1$^{24-68}$mRFP(His)$_6$ into RTG-2 cells under the indicated conditions analysed by western blot. Left shows the Ponceau stained nitrocellulose membrane that corresponds to the developed ECL film. A concentration of 2 mM H-Tyr(SO_3)-OH and FMOC-Tyr(SO_3)-OH, respectively, was used to show the different effects on the SpHtp1$^{24-68}$mRFP(His)$_6$ translocation for both compounds more clearly. We have to note that this is 2.5x less compared to the experiments shown in **Fig. 2.** 2 mM of FMOC-Tyr(SO_3)-OH blocked the uptake of SpHtp1$^{24-68}$mRFP(His)$_6$ into the cells (lane 1) nearly as efficient as a $NaClO_3$ treatment (lane 2, 70 mM 48 h). H-Tyr(SO_3)-OH proved to be less effective under the same conditions (lane 3) but still was able to inhibit the SpHtp1$^{24-68}$mRFP(His)$_6$ translocation by ~45 % (compare to lane 5). No uptake of mRFP(His)$_6$ into

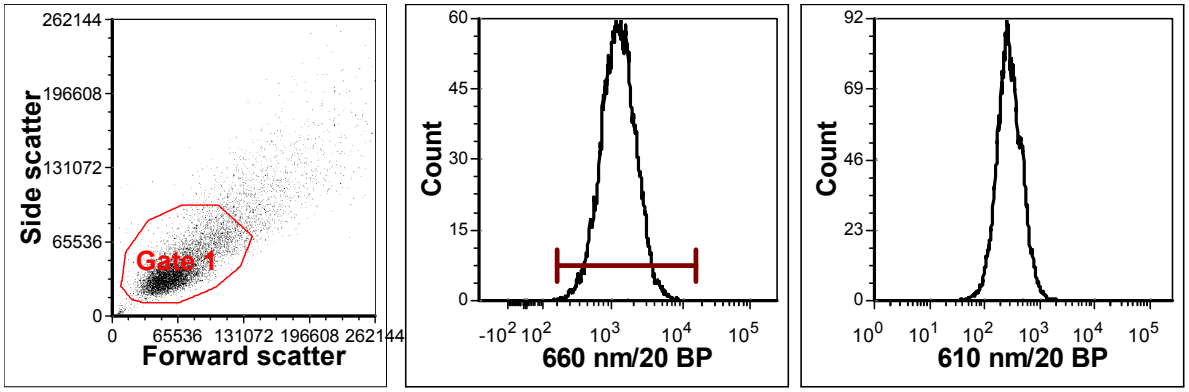
RTG-2 cells was detected. Lane 6 contained a sample of non-treated RTG-2 cells; lane 7 contained 60 pmol of purified SpHtp1$^{24-68}$mRFP(His)$_6$.

Supplementary References

1. van West P*, et al.* (2010) The putative RxLR effector protein SpHtp1 from the fish pathogenic oomycete Saprolegnia parasitica is translocated into fish cells. (Translated from eng) *FEMS Microbiol Lett* 310(2):127-137 (in eng).
2. Verkhusha VV*, et al.* (2003) High stability of Discosoma DsRed as compared to Aequorea EGFP. (Translated from eng) *Biochemistry* 42(26):7879-7884 (in eng).
3. Kale SD*, et al.* (2010) External lipid PI3P mediates entry of eukaryotic pathogen effectors into plant and animal host cells. (Translated from eng) *Cell* 142(2):284-295 (in eng).
4. Johnson JM & Betz WJ (2008) The Color of Lactotroph Secretory Granules Stained with FM1-43 Depends on Dye Concentration. *Biophysical Journal* 94(8):3167-3177.

Appendix (raw FACS data, one example run of Fig. 2K)

3 µM $mRFP(His)_6$

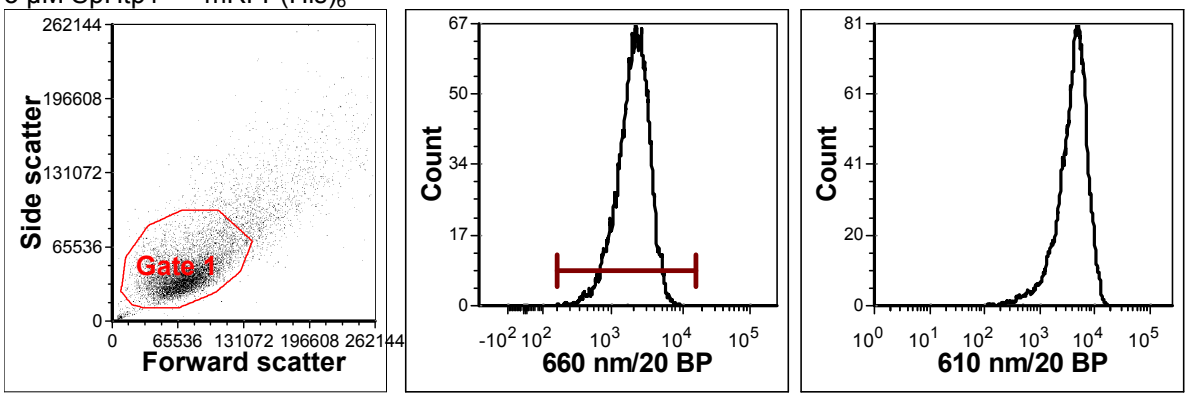


Gate	# of Events	% of All Cells
None	10000	100.0
Gate 1	8595	85.95

# of Events	% of gated cells
8588	99.92

# of Events	Median
8588	278.64

3 µM $SpHtp1^{24-68}mRFP(His)_6$

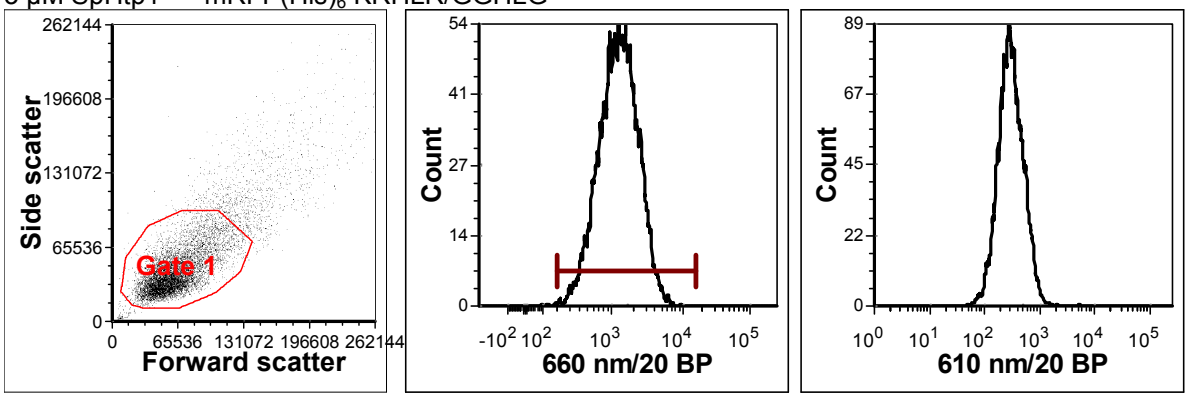


Gate	# of Events	% of All Cells
None	10000	100.0
Gate 1	8258	82.58

# of Events	% of gated cells
8254	99.95

# of Events	Median
8254	4356.76

3 µM SpHtp1$^{24-68}$mRFP(His)$_6$ KRHLR/GGHLG

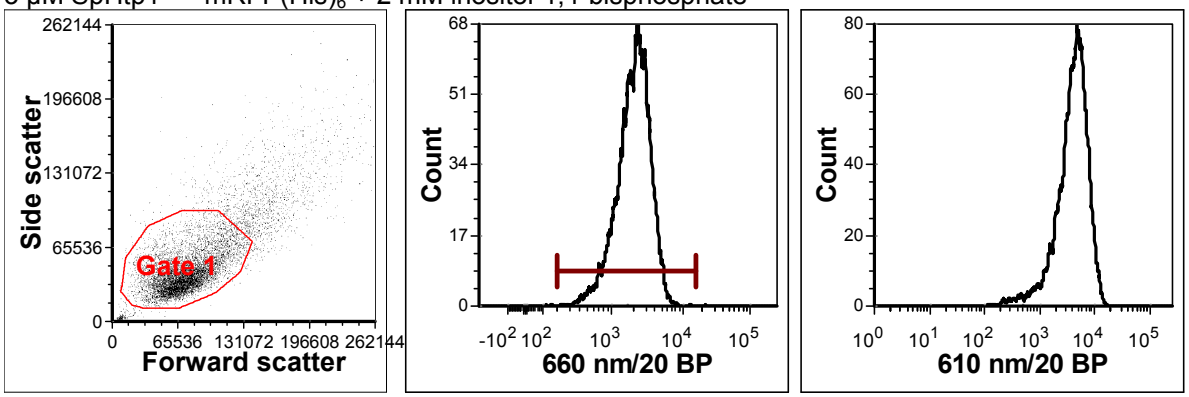


Gate	# of Events	% of All Cells
None	10000	100.0
Gate 1	8729	87.29

# of Events	% of gated cells
8714	99.83

# of Events	Median
8714	296.7

3 µM SpHtp1$^{24-68}$mRFP(His)$_6$ + 2 mM inositol-1,4-bisphosphate

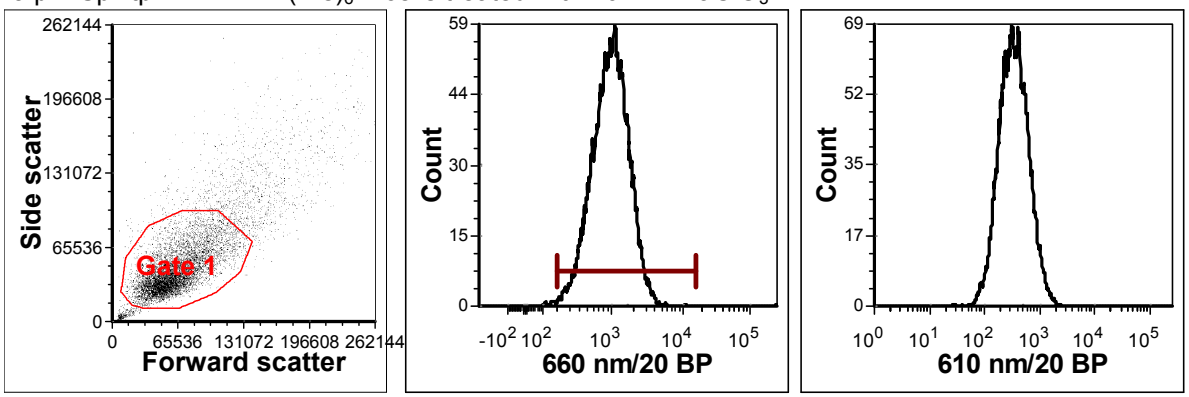


Gate	# of Events	% of All Cells
None	10000	100.0
Gate 1	8273	82.73

# of Events	% of gated cells
8264	99.89

# of Events	Median
8264	4327.09

3 µM SpHtp1$^{24-68}$mRFP(His)$_6$ + cells treated with 70 mM $NaClO_3$

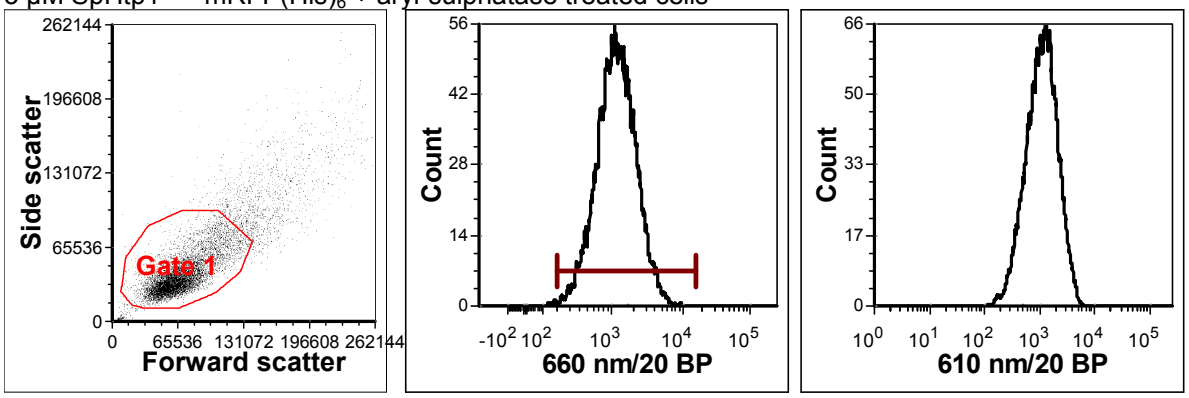


Gate	# of Events	% of All Cells
None	10000	100.0
Gate 1	8421	84.21

# of Events	% of gated cells
8389	99.62

# of Events	Median
8389	337.98

3 µM SpHtp1$^{24-68}$mRFP(His)$_6$ + aryl-sulphatase treated cells

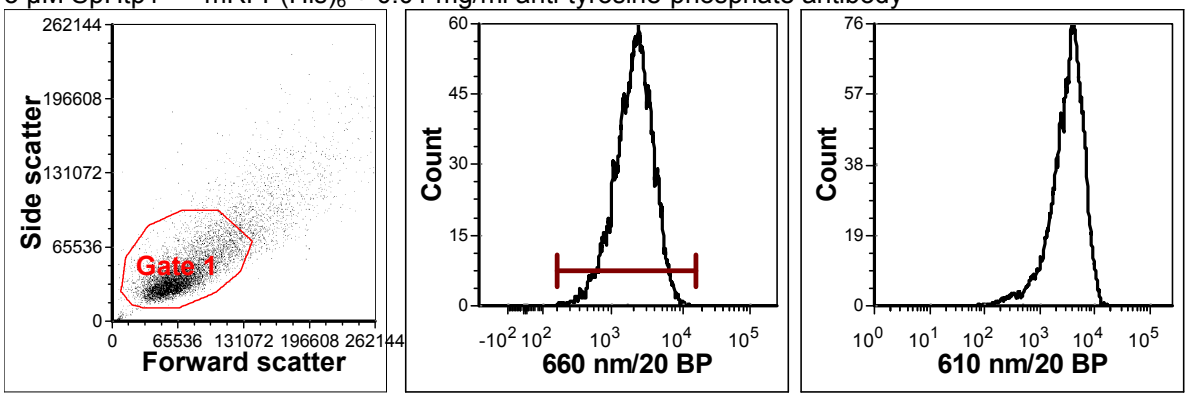


Gate	# of Events	% of All Cells
None	10000	100.0
Gate 1	8531	85.31

# of Events	% of gated cells
8500	99.64

# of Events	Median
8500	1128.32

3 µM SpHtp1$^{24-68}$mRFP(His)$_6$ + 0.01 mg/ml anti-tyrosine-phosphate antibody

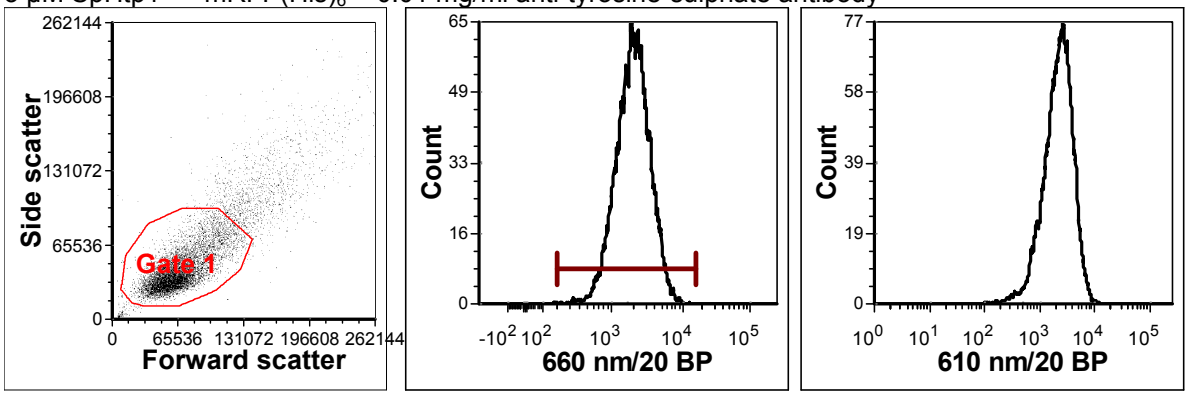


Gate	# of Events	% of All Cells
None	10000	100.0
Gate 1	8581	85.81

# of Events	% of gated cells
8575	99.93

# of Events	Median
8575	3556.1

3 µM SpHtp1$^{24-68}$mRFP(His)$_6$ + 0.01 mg/ml anti-tyrosine-sulphate antibody

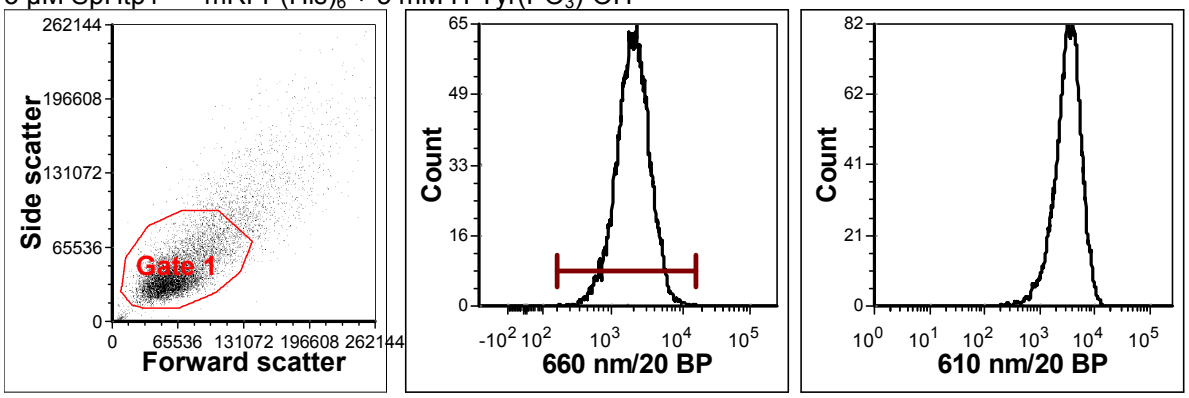


Gate	# of Events	% of All Cells
None	10000	100.0
Gate 1	8500	85.0

# of Events	% of gated cells
8491	99.89

# of Events	Median
8491	2310.82

3 µM SpHtp1$^{24-68}$mRFP(His)$_6$ + 5 mM H-Tyr(PO$_3$)-OH

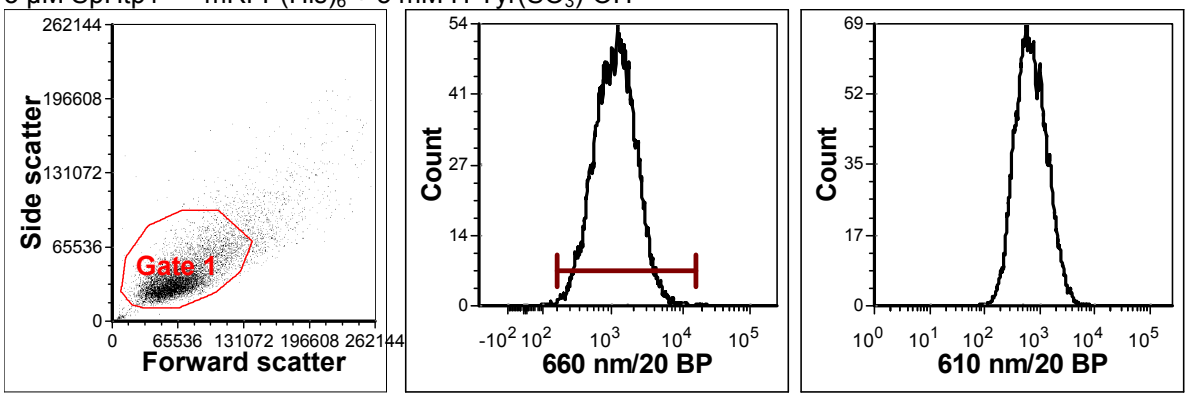


Gate	# of Events	% of All Cells
None	10000	100.0
Gate 1	8491	84.91

# of Events	% of gated cells
8489	99.98

# of Events	Median
8490	3401.73

3 µM SpHtp1$^{24-68}$mRFP(His)$_6$ + 5 mM H-Tyr(SO$_3$)-OH

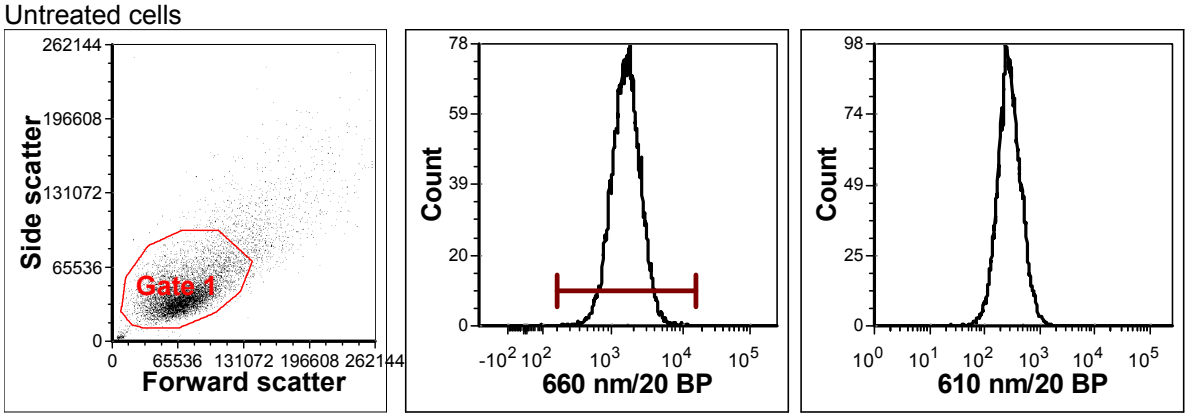


Gate	# of Events	% of All Cells
None	10000	100.0
Gate 1	8988	89.88

# of Events	% of gated cells
8952	99.6

# of Events	Median
8953	690.58

Untreated cells



Gate	# of Events	% of All Cells
None	10000	100.0
Gate 1	8739	87.39

# of Events	% of gated cells
8733	99.93

# of Events	Median
8733	280.36