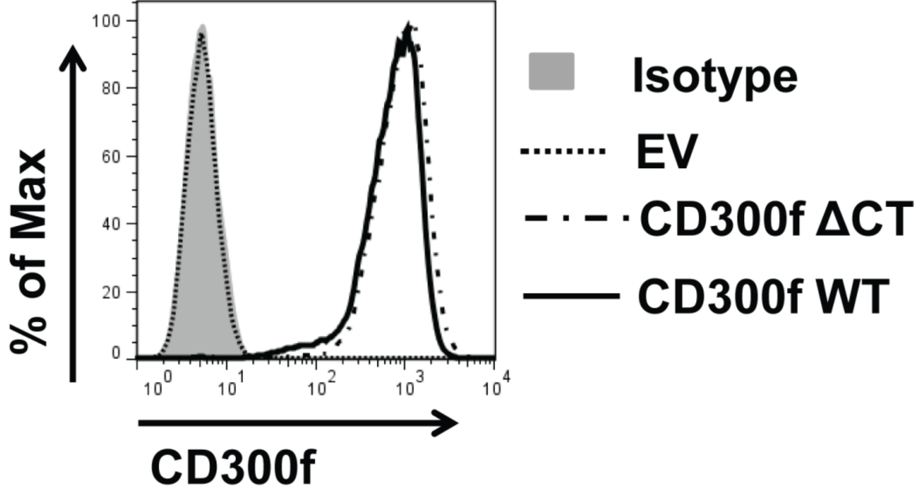
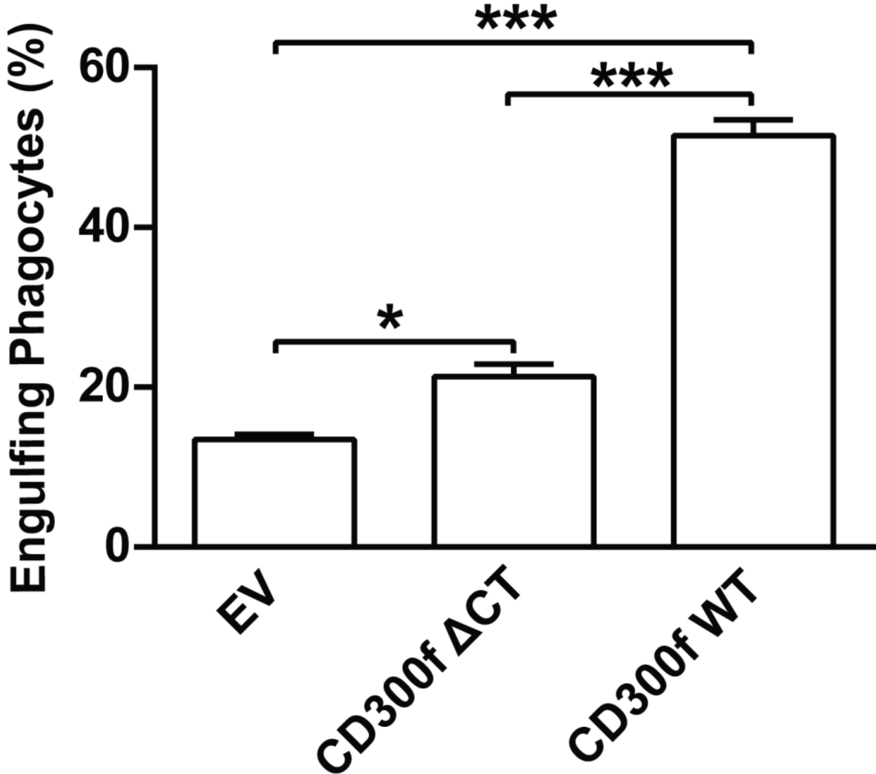
Supplementary Information

Supplementary Figure 1



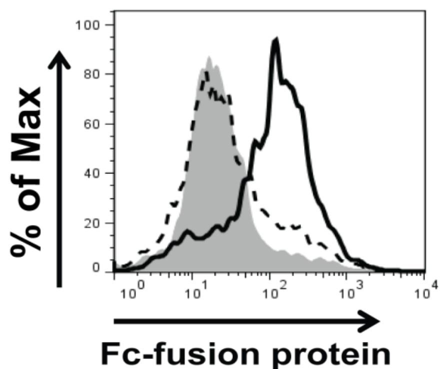


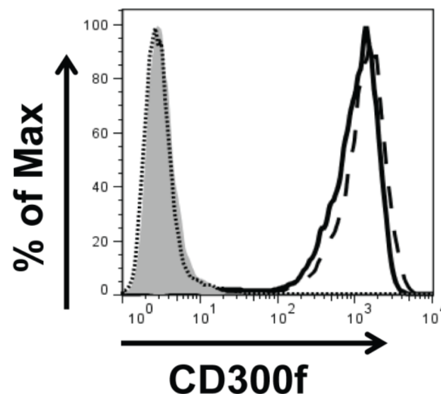
Supplementary Figure 1: Over-expression of CD300f in NIH3T3 cells enhances their

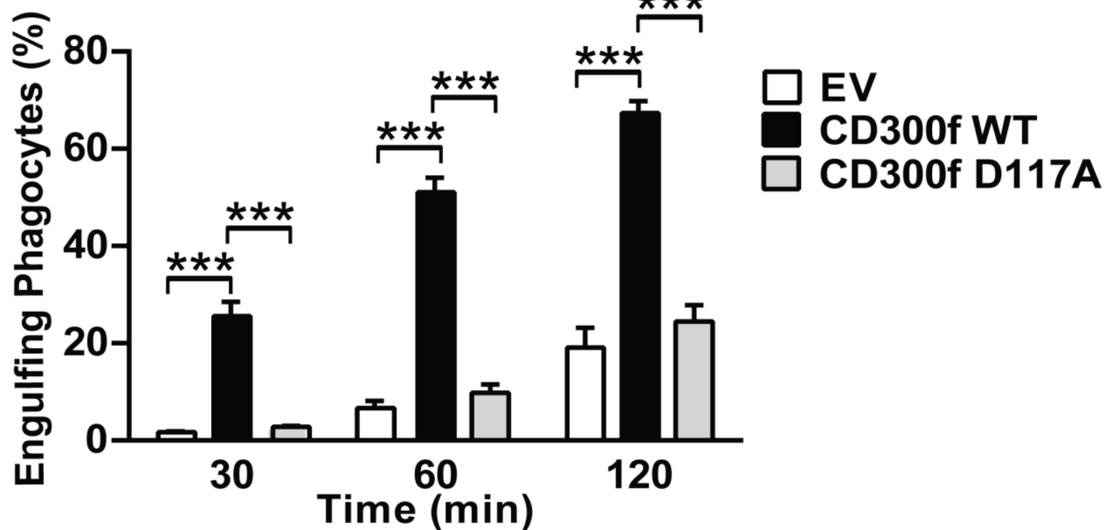
capacity to phagocytize AC.

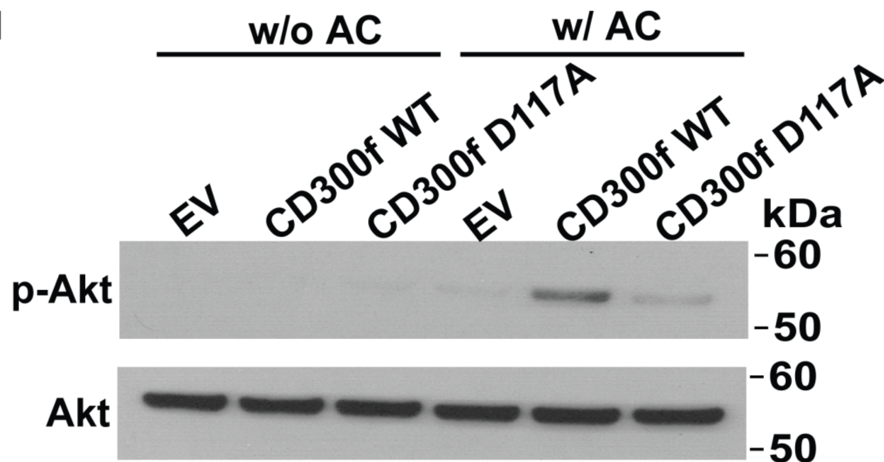
(**a**) NIH3T3 cells were stably transduced by EV, CD300f WT or CD300f ΔCT constructs. Cells were stained with Alexa Fluor 488-conjugated anti-CD300f or isotype control Ab, and the analysis of CD300f cell surface expression levels was determined by flow cytometry. The isotype control Ab staining is indicated by the grey-filled peak. Staining of the EV-transduced cells is represented by the dotted line, while the solid line and dot-dash line illustrates CD300f level on cells transduced by CD300f WT and CD300f ΔCT respectively. (**b**) NIH3T3 cells transduced with EV or the indicated CD300f constructs were mixed with pHrodo-labelled AC at a 1:3 ratio for 1h. Following the incubation, the cells were analysed by flow cytometry as in Fig. 1. The graph shows the mean values + SEM from 3 experiments. Asterisks indicate statistical significance (∗ $p<0.05$, ∗∗∗$p<0.001$, Student *t*-test).

Supplementary Figure 2





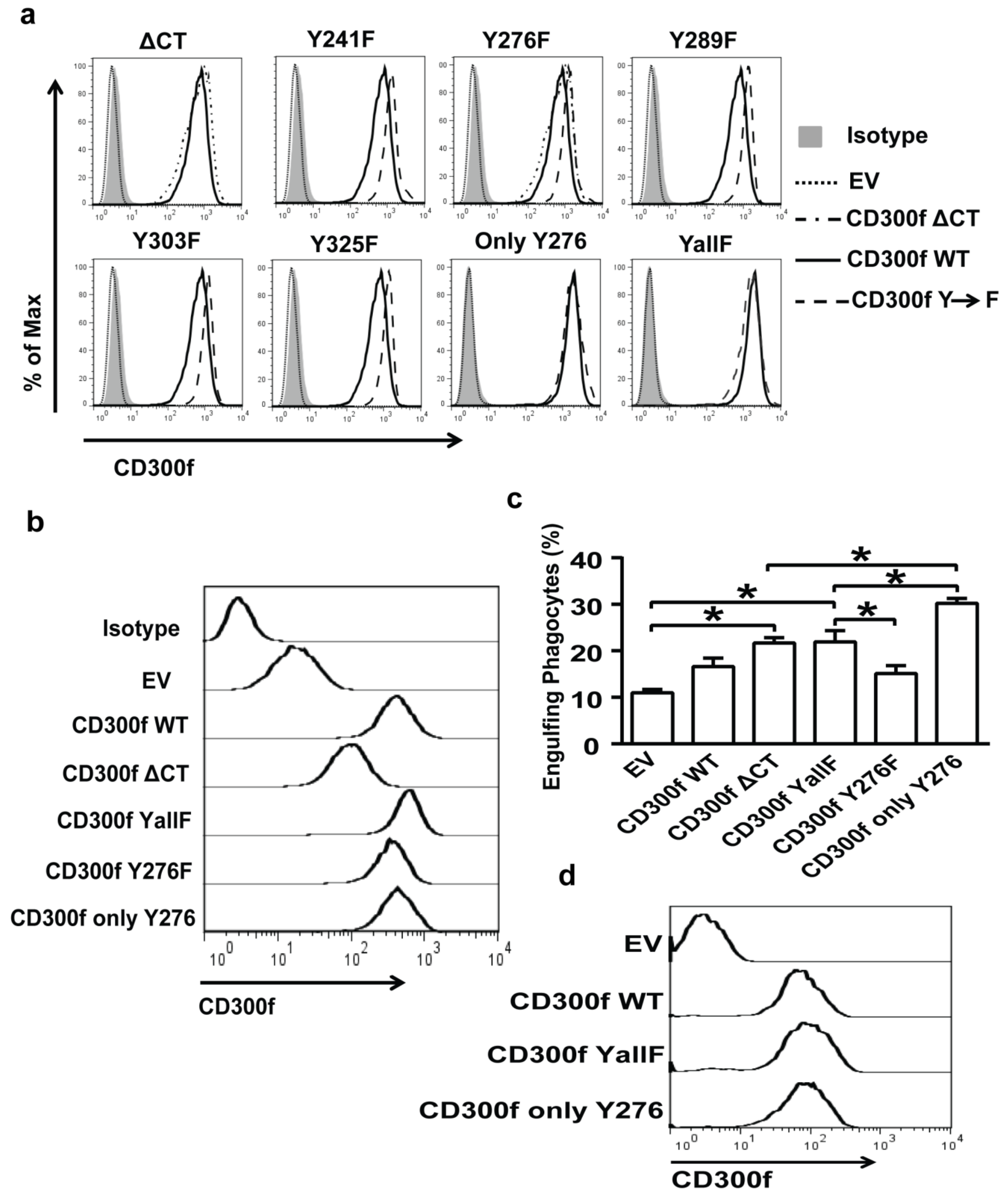




Supplementary Figure 2: Aspartic acid at position 117 is important for PS recognition and enhanced phagocytosis.

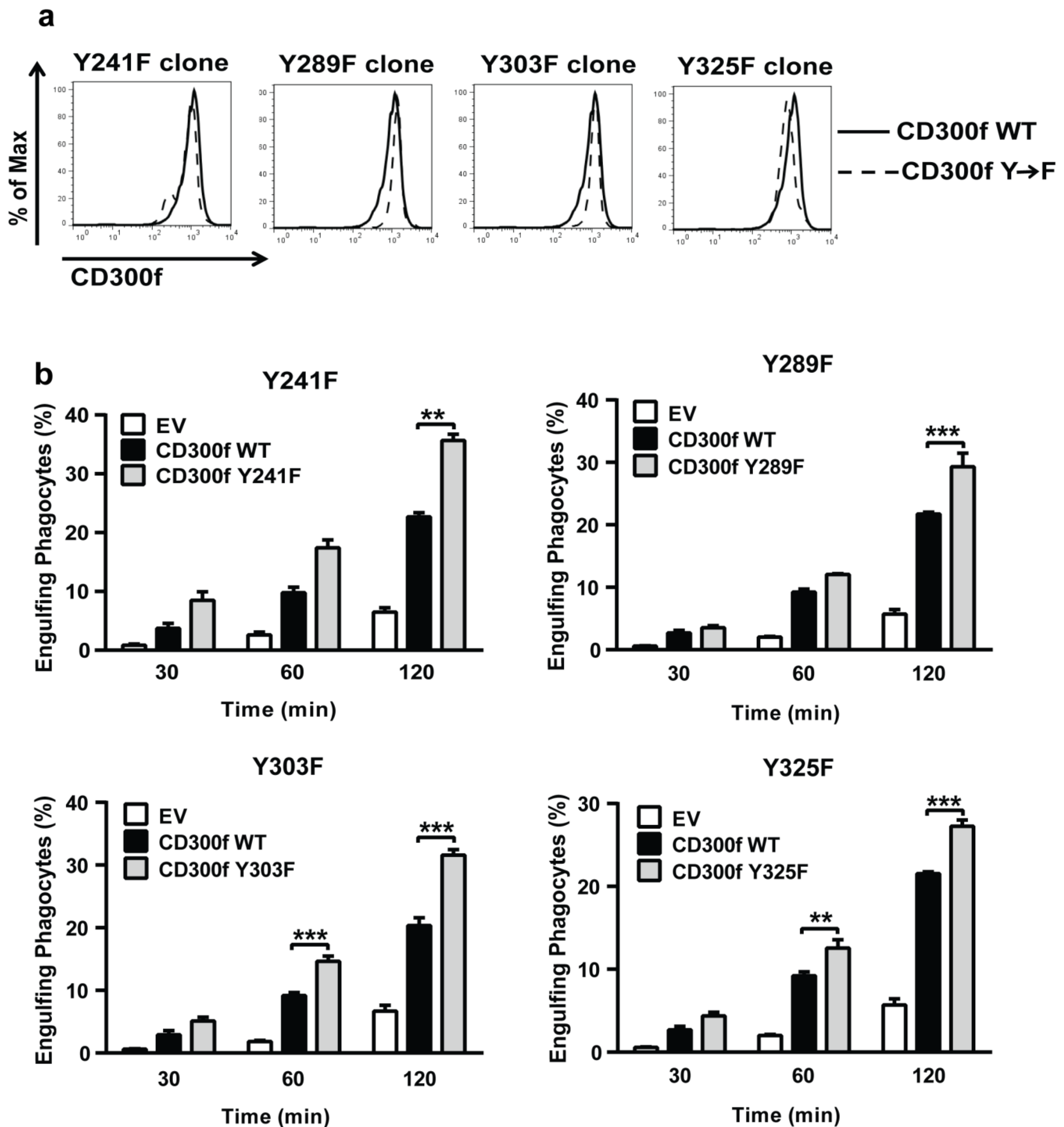
(**a**) 1×10^6 AC were incubated at RT for 45 min with the following Fc fusion proteins: CD300f WT, CD300f D117A mutant and LAIR-1 R65K mutant (a collagen receptor negative for binding, negative control). Next, the Fc fusion proteins bound to AC were detected by staining with FITC-conjugated anti-human IgG Fc-gamma fragment specific antibody for 15 min, and flow cytometric analysis. The histogram shows a representative result from 3 experiments. (**b**) L929 cells were stably transduced with EV, CD300f WT or CD300f D117A mutant. The histograms show the cell surface level of CD300f, as determined by anti-CD300f staining. The isotype control Ab (gray-filled histograms) serves as the negative control. Staining of the EV-transduced cells is represented by the dotted line, while the solid line and dashed line illustrates CD300f level on cells transduced by CD300f WT and CD300f D117A, respectively. (**c**) EV-, CD300f WT- and CD300f D117A-transduced L929 cells were incubated with pHrodo-labelled AC at a 1:5 ratio for the indicated times. Next, the cells were suspended in pH 8.8 buffer, and the percentage of cells with engulfed AC was determined by flow cytometry. The graph shows the means with SEM (error bars) from 3 experiments. Asterisks indicate statistical significance (***$p<0.001$, Student *t*-test). (d) L929 cells transduced with the indicated constructs were incubated with (w/AC) or without (w/o AC) AC at 4°C for 10 min, then transferred to 37°C for 15 min before lysis. Anti-phospho-Akt or anti-Akt (loading control) blots were performed on whole lysates.

Supplementary Figure 3



Supplementary Figure 3: CD300f cell surface expression levels on the transduced cells, and phagocytosis of AC by J774 cells.

(**a**) L929 cells were stably transduced with EV, CD300f WT, or the indicated CD300f mutants. The histograms show the cell surface level of CD300f, as determined by anti-CD300f staining. The isotype control Ab (grey-filled histograms) served as the negative control. The level of CD300f on the cells transduced with EV, CD300f WT or CD300f ΔCT is shown in each panel for the comparison of protein cell surface levels among different CD300f mutants. (**b**) J774 cells transduced with EV, CD300f WT and the indicated CD300f mutants were analysed for CD300f cell surface expression using flow cytometry. (**c**) J774 cells, transduced with the indicated constructs, were mixed with pHrodo-labelled AC at a 1:3 ratio, suspended in the pH 8.8 buffer and analysed by flow cytometry as in Fig. 1. The graph shows the means with SEM (error bars) from 3 independent experiments; asterisks indicate statistical significance (* $p < 0.05$, Student *t*-test). (**d**) BMDM cells from *Cd300f* $^{-/-}$ mice were transduced with EV, CD300f WT and the indicated CD300f mutants. Cell surface level of CD300f was analysed by flow cytometry.

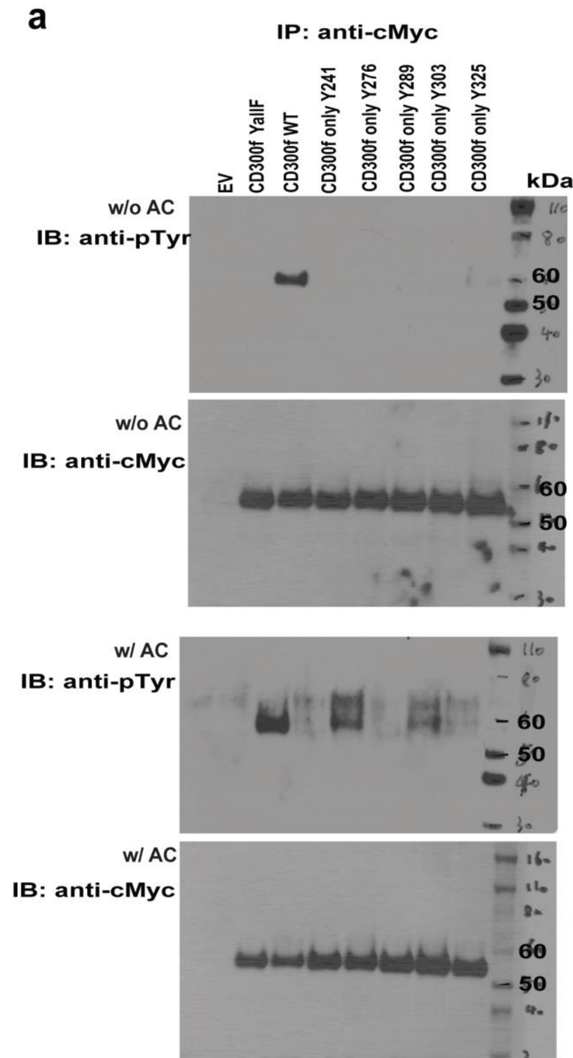


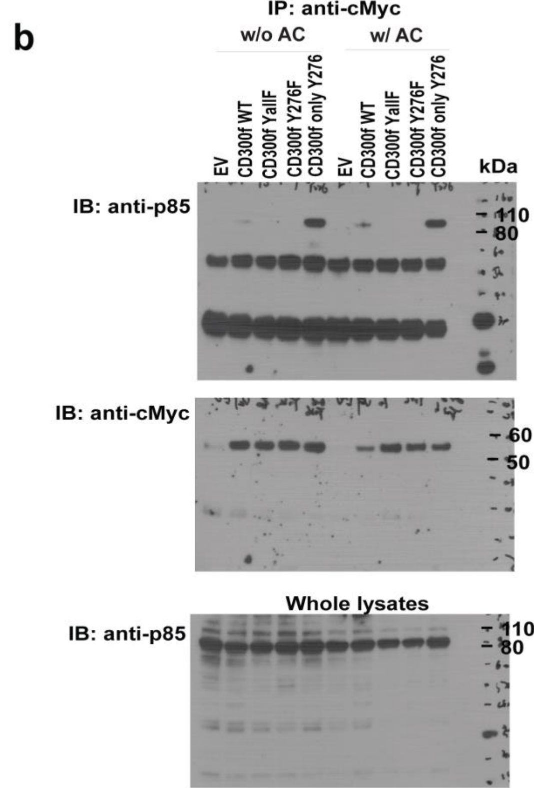
Supplementary Figure 4: Phagocytosis of AC by L929 cell clones, expressing equivalent levels of different CD300f forms.

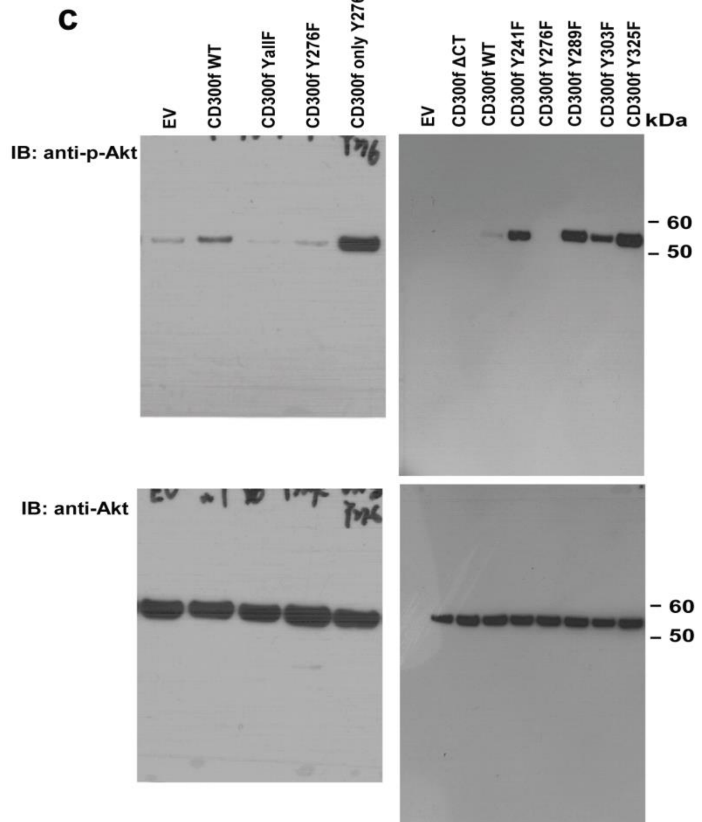
(**a**) L929 cells were stably transduced with the indicated CD300f constructs. Clones of L929 cells expressing CD300f mutants or CD300f WT were analysed by flow cytometry for CD300f cell surface expression level, using Alexa Fluor 488-conjugated anti-CD300f antibody. Solid lines indicate the expression of CD300f WT, dashed lines show the expression levels of CD300f

mutants. (**b**) L929 cell clones stably transduced with EV, CD300f WT or the indicated CD300f mutants were mixed with TFL-4-labeled AC for indicated times. The ability of cells to phagocytose AC was analysed by flow cytometry. The graphs show the means with SEM (error bars) from 3 experiments. Asterisks indicate statistical significance (**$p<0.01$, ***$p<0.001$; two-way ANOVA).

Supplementary Figure 5





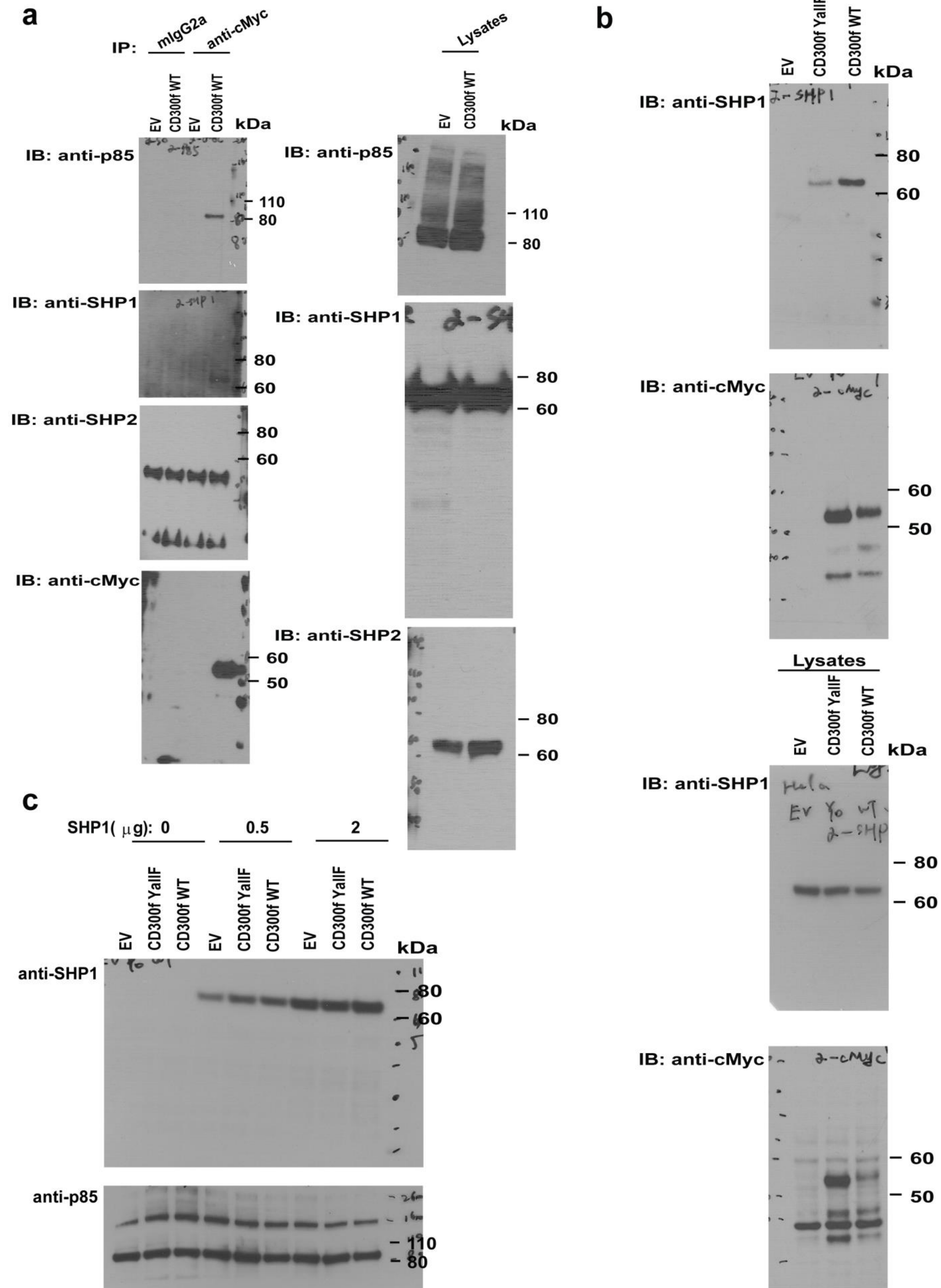


Supplementary Figure 5: CD300f associates with p85α subunit of PI3K to regulate the AC

phagocytosis

L929 cells transduced with EV or cMyc-tagged CD300f constructs were incubated with (a,b,c) or without (a,b) AC at 4°C for 10 min, then transferred to 37°C for 15 min before lysis. Immunoprecipitation was performed with anti-cMyc Ab (a,b), and blotting with antibodies against phosphotyrosine (a), p85 (b) or cMyc (a,b) (loading control). The presence of PI3K subunit was verified by anti-p85 immunoblotting of whole lysates (b); anti-phospho-Akt or anti-Akt (loading control) blots were performed on whole lysates (c).

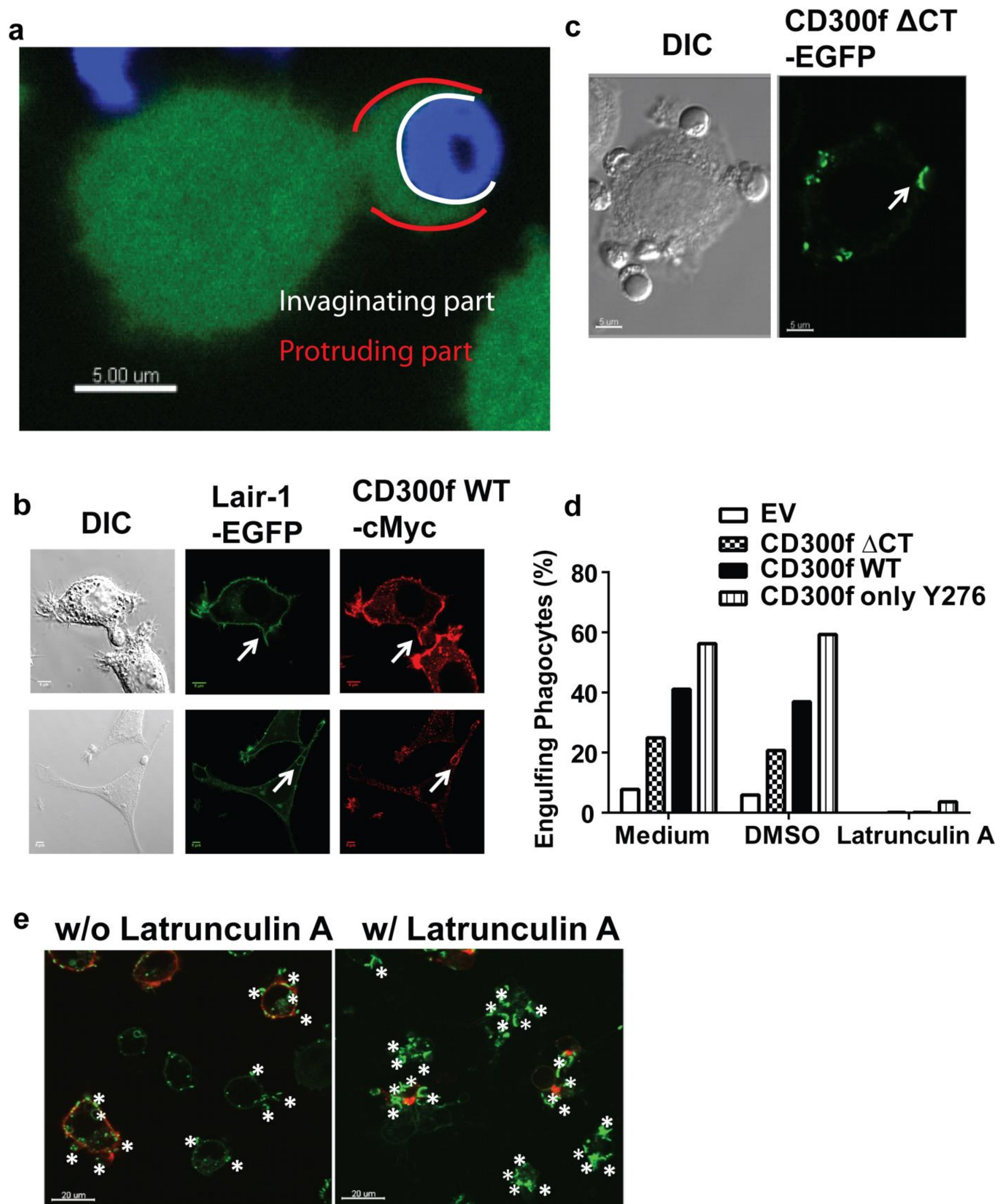
Supplementary Figure 6



Supplementary Figure 6: CD300f associates with SHP1 to regulate the AC phagocytosis

(**a**) J774 cells transduced with EV or cMyc-tagged CD300f WT were treated with pervanadate for 15 min at 37°C, lysed, immunoprecipitated with anti-cMyc or isotype control antibodies, and then blotted with anti-p85, anti-SHP1, anti-SHP2 or anti-cMyc antibodies. Lysates blotted with anti-p85, anti-SHP1 or anti-SHP2 antibodies served as controls. (**b**) The indicated cMyc-tagged CD300f constructs were co-transfected with SHP1 into HeLa cells. Cells, treated with pervanadate, were lysed, immunoprecipitated with anti-cMyc Ab, and blotted with anti-SHP1 or anti-cMyc antibodies. Cell lysates were blotted with anti-SHP1 or anti-cMyc for the presence of SHP1 or CD300f. (c) The indicated cMyc-tagged CD300f constructs were co-transfected with 0, 0.5 or 2 μg of SHP1-encoding vector into HeLa cells. Cell lysates were blotted with anti-SHP1 or anti-p85 as the sample loading control.

Supplementary Figure 7



Supplementary Figure 7: CD300f location during AC phagocytosis.

(**a**) CFSE-stained L929 cells (green) stably expressing CD300f WT were mixed with TFL-4-labeled AC (blue) for 30 min, fixed and imaged by confocal microscopy. White line illustrates the invaginating part of the phagocytic cup, red lines indicate the protruding parts of the phagocytic cup; Scale bar, 5μm. (**b**) NIH3T3 cells stably expressing CD300f WT tagged with cMyc were transiently transfected with LAIR-1-EGFP (green). Cells were mixed with AC for 30 min, fixed, and permeabilized. Next, the cells were stained with anti-cMyc Ab, followed by Alexa Fluor 647-conjugated secondary antibody, and imaged by confocal microscopy. The upper panels show a typical example of the distribution of CD300f and LAIR-1 in the phagocytic cup formed around the AC, indicated by the arrow. The lower panels show the distribution of CD300f and LAIR-1 on the phagosomes indicated by the arrow. Scale bars, 5μm. (**c**) L929 cells stably transduced with CD300f ΔCT-EGFP were mixed with AC for 30 min, fixed, and analysed by confocal microscopy. The distribution of CD300f ΔCT on the phagocytic cup is indicated by the arrow. Scale bars, 5μm. (d) L929 cells transduced with the indicated constructs were pre-incubated with medium or DMSO (controls), or 2μM latrunculin A for 10 min at 37°C, followed by 2 h incubation with pHrodo-labelled AC. The percentage of cells containing AC was determined as in Fig. 1. The graph shown is representative of 2 experiments. (**e**) L929 cells co-expressing cMyc-tagged CD300f Only-Y276 and LifeAct-RFP were pre-treated with or without 2μM latrunculin A at 37°C for 10 min, and then mixed with unlabelled AC for 1h. Following the incubation, the cells were fixed, permeabilized, and stained with anti-cMyc Ab, followed Alexa Fluor 647-conjugated secondary antibody (green). F-actin was visualized with LifeAct (red). Asterisks indicate the position of AC. Scale bars, 20 μm.

Supplementary Table 1: The DNA sequence of the primers used for plasmid constructs

CD300f in PCDH-puro	
Forward primer	5'-GGAATTCGCCACCATGCATTTGTCATTGCTGGTC-3'
Reverse primer	5'-AAGGAAAAAAGCGGCCGCAGGCATGGCTGCAGGCAA-3'
EGFP at the 3' terminus of CD300f in PCDH-puro	
Forward primer	5'-AAGGAAAAAAGCGGCCGCTATGGTGAGCAAGGG-3'
Reverse primer	5'-AAGGAAAAAAGCGGCCGCCTTGTACAGCTCGTCC-3'
CD300f full length fused with c-Myc at the 3' terminus in PCDH-puro	
Forward primer	5'-GGAATTCGCCACCATGCATTTGTCATTGCTGGTC-3'
Reverse primer 1 (designed on c-Myc)	5'-CAGATCCTCTTCTGAGATGAGTTTTTGTTCAGGCATGGCTGCAGGCAA-3'
Reverse primer 2 (designed on c-Myc with Not I)	5'-AAGGAAAAAAGCGGCCGCCAGATCCTCTTCTGAGATGAG-3'
CD300f fused with c-Myc at the 3' terminus in pMX-puro	
Forward primer	5'-CTGCAGAACCAGTGTGCTGGGCCACCATGCATTTGTCATTGCTGGTC-3'
Reverse primer	5'-ATGCATCCAAAGCTGTGGTTACAGATCCTCTTCTGAGATGAG-3'
Cytoplasmic tail deleted CD300f fused with c-Myc at the 3' terminus in PCDH-puro	
Forward primer	5'-GGAATTCGCCACCATGCATTTGTCATTGCTGGTC-3'
Reverse primer 1(designed on c-Myc)	5'-CAGATCCTCTTCTGAGATGAGTTTTTGTTCAGCAAAGAGCGAGGCCAC-3'
Reverse primer 2(designed on c-Myc with Not I)	5'-AAGGAAAAAAGCGGCCGCCAGATCCTCTTCTGAGATGAG-3'
CD300f with mutation of Y241F	
Forward primer	5'-GGGTGATCTCTGTTTTGCAGACCTGTCCC-3'
Reverse primer	5'-GGGACAGGTCTGCAAAACAGAGATCACCC-3'
CD300f with mutation of Y276F	
Forward primer	5'-GGAAGTGGAATTTGTCACCATGGCTCCC -3'
Reverse primer	5'-GGGAGCCATGGTGACAAATTCCACTTCC-3'
CD300f with mutation of Y289F	
Forward primer	5'-GGGAGGAGGTTTCATTTGCCGCTCTG-3'
Reverse primer	5'-CAGAGCGGCAAATGAAACCTCCTCCC-3'
CD300f with mutation of Y303F	
Forward primer	5'-GGTCAGGAGCCTACTTTTGGCAATACTGGC-3'
Reverse primer	5'-GCCAGTATTGCCAAAAGTAGGCTCCTGACC-3'
CD300f with mutation of Y325F	
Forward primer	5'-GAGACCACAGAGTTCAGCAGCATCAGG-3'
Reverse primer	5'-CCTGATGCTGCTGAACTCTGTGGTCTC-3'
Life-Act-RFP in PCDH-puro	
Forward primer	5'-GGAATTCGCCACCATGGGTGTCGCAGATTTG-3'
Reverse primer	5'-AAGGAAAAAAGCGGCCGCATTAAGTTTGTGCCCC-3'
PLCδ1-PH-YFP or Btk-PH-YFP in PCDH-puro	
Forward primer(PLCδ1)	5'-AAGGAAAAAAGCGGCCGCGCCACCATGGACTCGGGCCGGGACTT-3'
Forward primer(Btk)	5'-AAGGAAAAAAGCGGCCGCGCCACCATGGCCGCAGTGATTCTG-3'
Reverse primer	5'-AAGGAAAAAAGCGGCCGCGTCAGTTAGATCGTTTTCC-3'