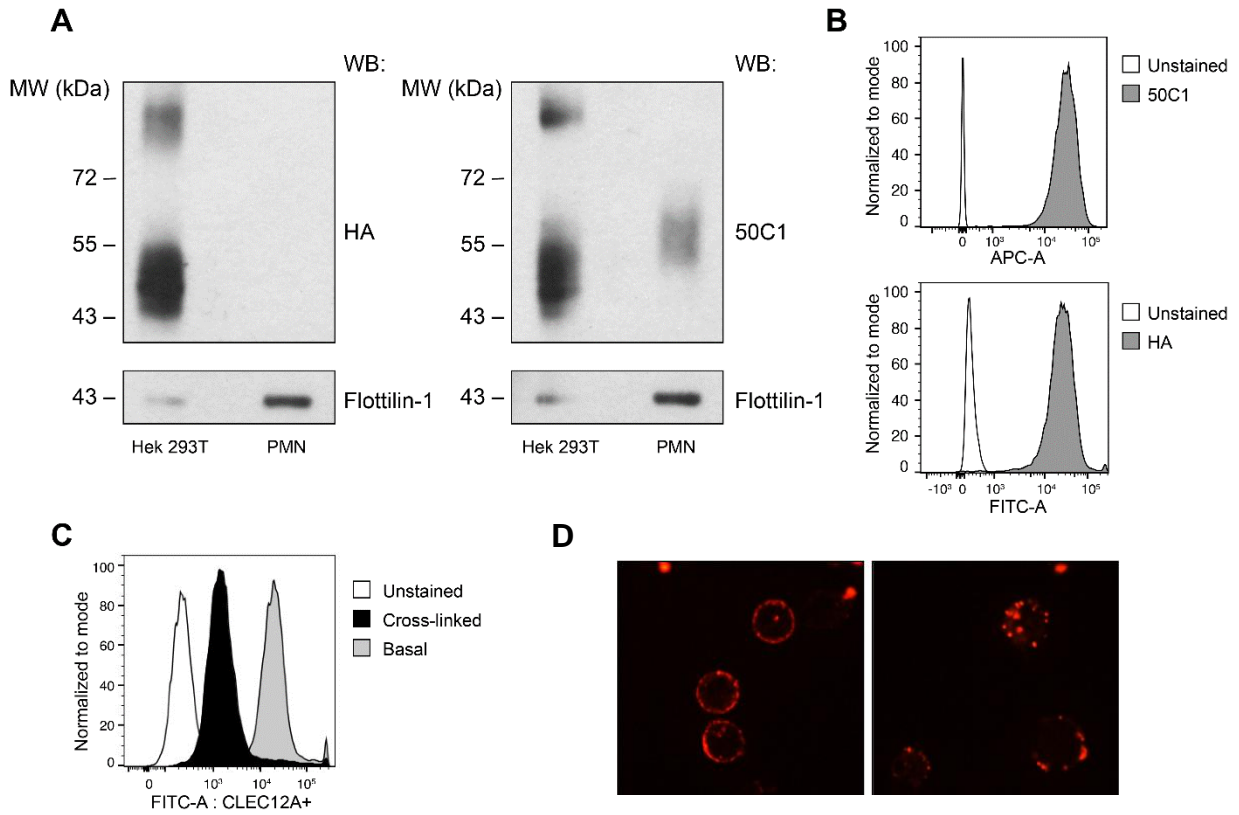
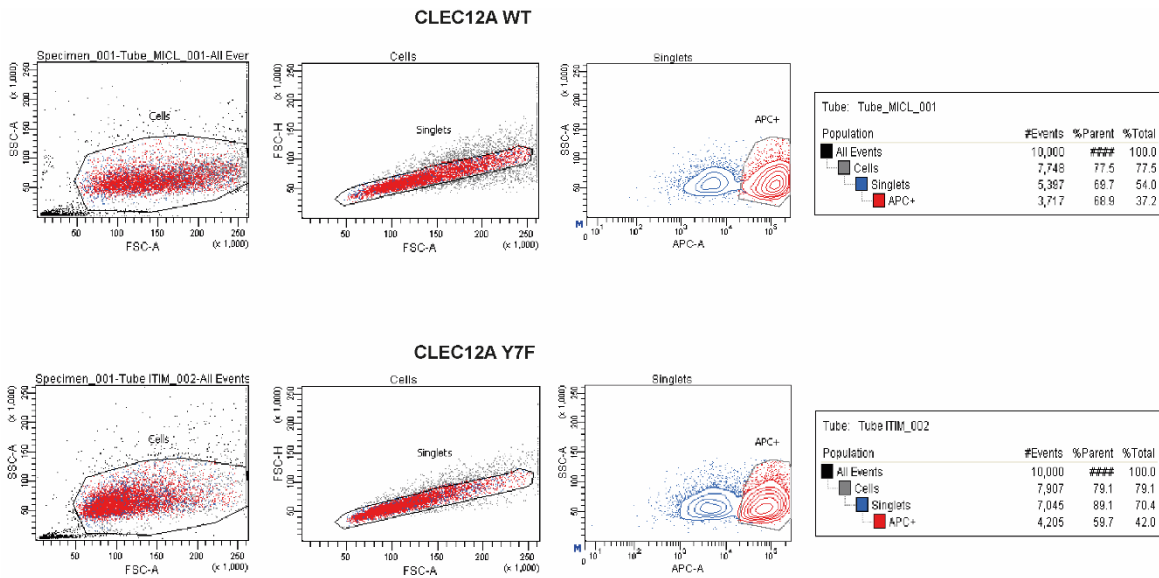


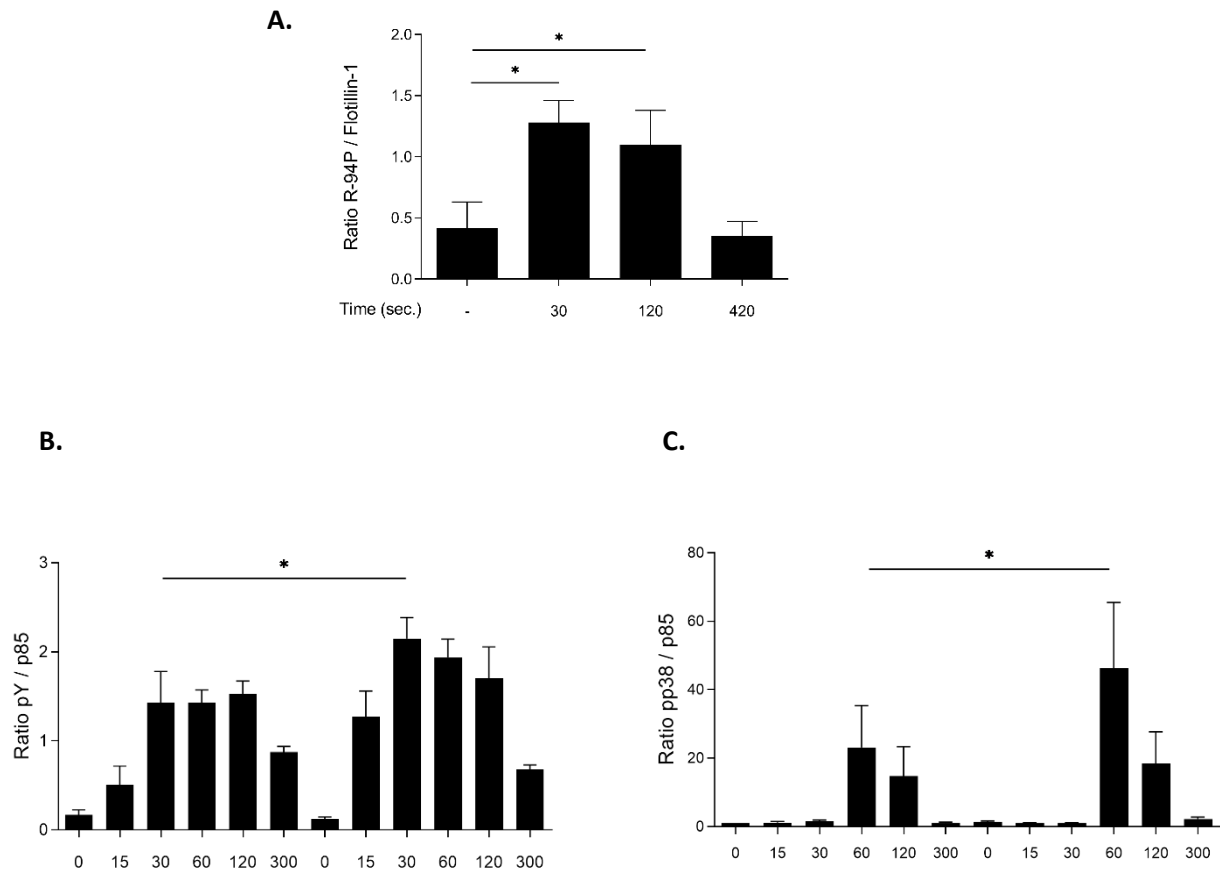
Supplementary Material



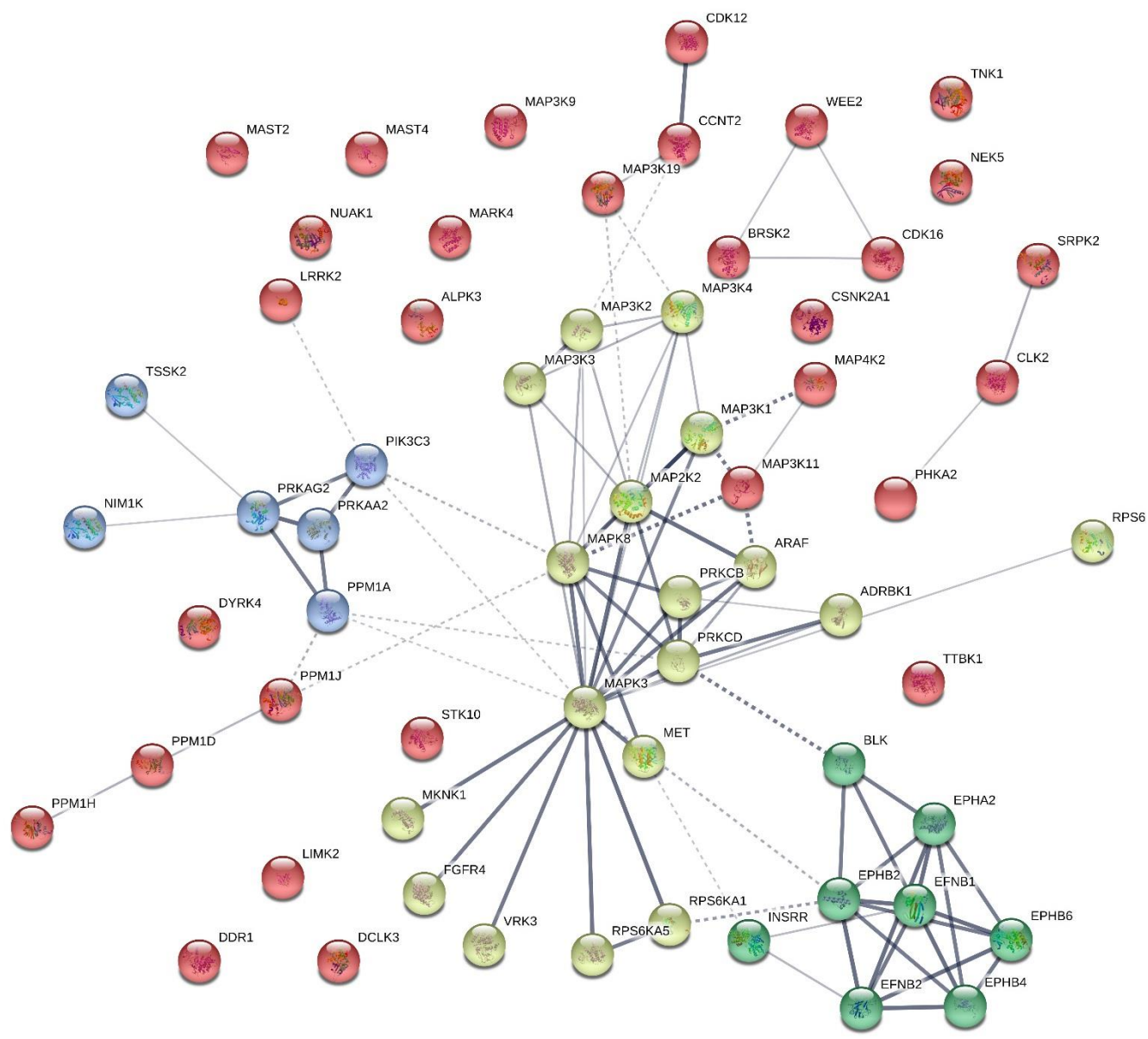
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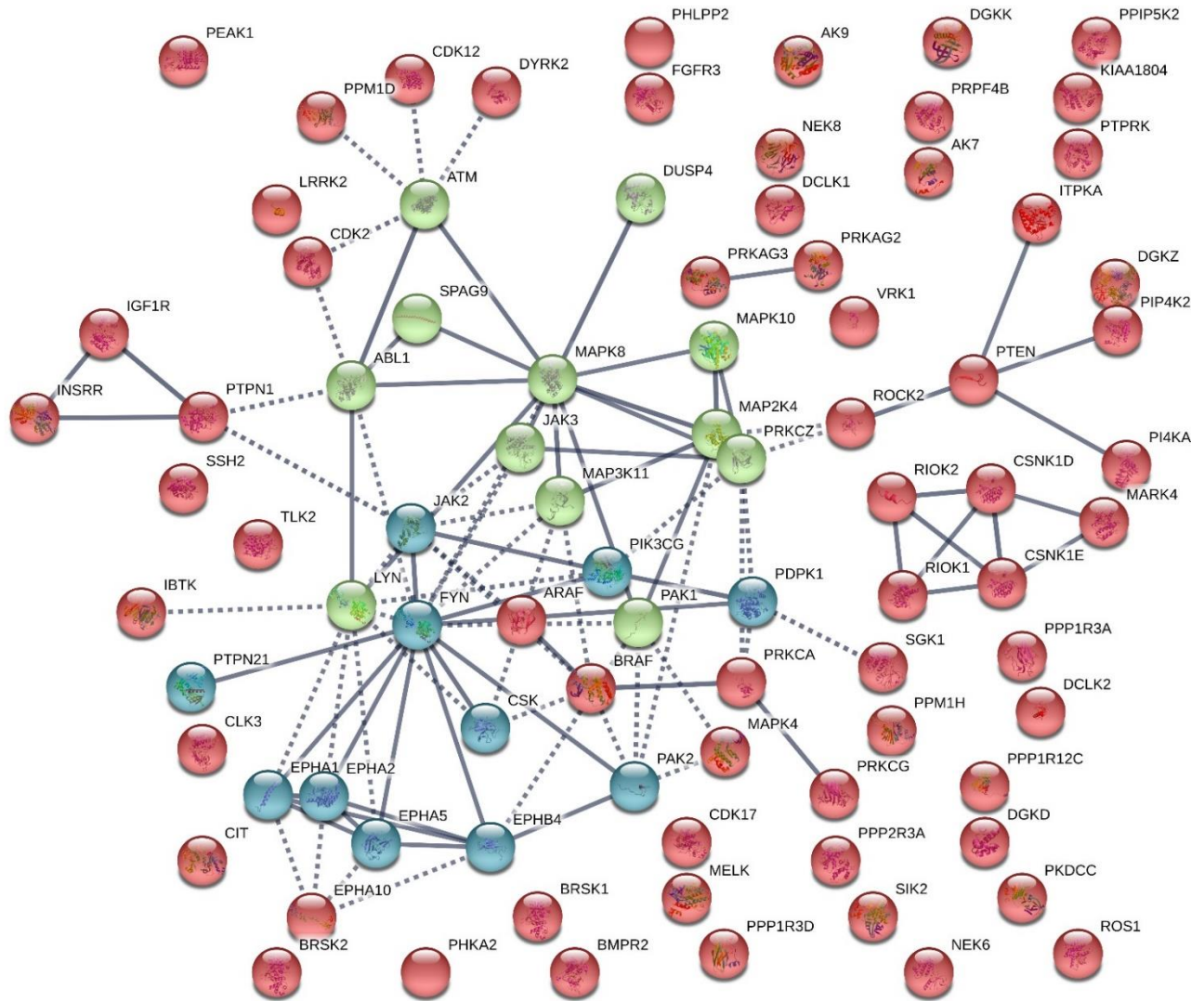
Supplementary Figure 1. HEK-293T cells express CLEC12A-HA-wt on their surface that can be internalised by antibody-induced cross-linking as in human neutrophils. (A) Total cell lysates were prepared from HEK-293T cells transiently transfected with CLEC12A-HA-wt or from human neutrophils isolated from healthy donors as described in *Materials and Methods* prior to immunoblotting with an anti-HA antibody or 50C1 antibodies, *left* and *right* gels, respectively. The anti-flotillin-1 antibody was used as a loading control. (B) Cell-surface expression of CLEC12Awt-HA was confirmed by flow cytometry with the same antibodies conjugated to the indicated fluorochromes. (C) The internalisation of CLEC12Awt-HA expressed on transiently transfected HEK-293T was determined by flow cytometry after cross-linking with an anti-HA and an anti-F(ab')₂ secondary antibody as described in *Materials and Methods*. These data are representative of 3 experiments. Similar observations were made in HeLa cells (*data not shown*). (D) The internalisation of CLEC12A in human neutrophils induced by fluorescently-labelled 50C1 after incubation at 37⁰C (*right image*) for 10 minutes. Cell-surface CLEC12A staining without internalisation is shown in the *left image*. Images were acquired at 63X with the Quorum WAVFX spinning disc system (Quorum Technologies, Guelph, Ontario, Canada) and images analyzed with the Volocity quantitation module as previously described in Gagné(9). (E) Gating strategy for the sorting of cells stably transfected with CLEC12A by flow cytometry using an APC-labeled CLEC12A antibody.



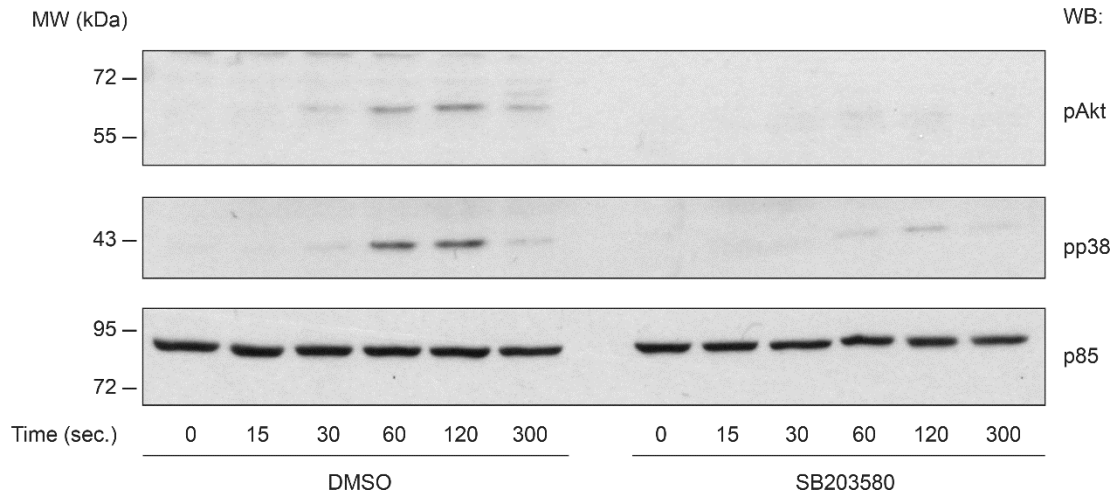
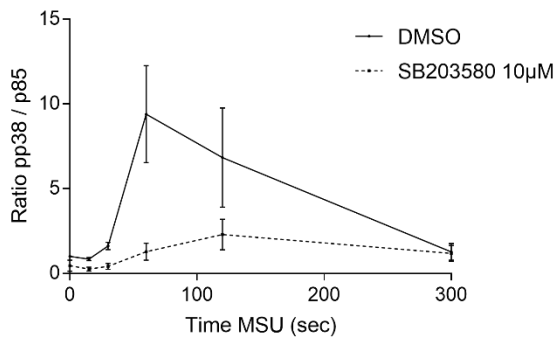
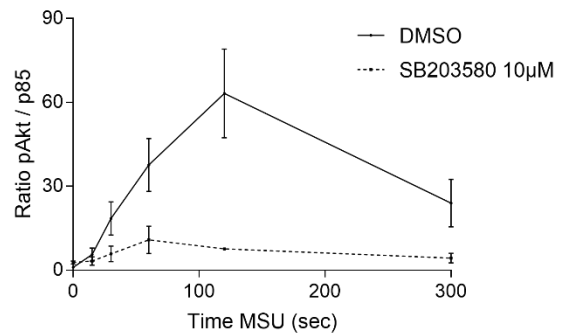
Supplementary Figure 2. Quantification of Western blots by densitometry- Densitometry analysis was performed with Image Lab software (Bio-Rad) on blots in Figure 4a (A) and Figure 6b ((B), *anti-tyrosine phosphorylation bands >72 kd*; (C), *p38 blot*) of the main manuscript. Statistical analysis: Two way ANOVA and the Dunnett's multiple comparisons test was performed to compare the treated cells to the control cells in (A) and the paired t-test was performed in (B) and (C). * P <0.05.



Supplementary Figure 3. STRING analysis of proteins with increased phosphorylation after CLEC12A cross-linking compared to isotype cross-linking.

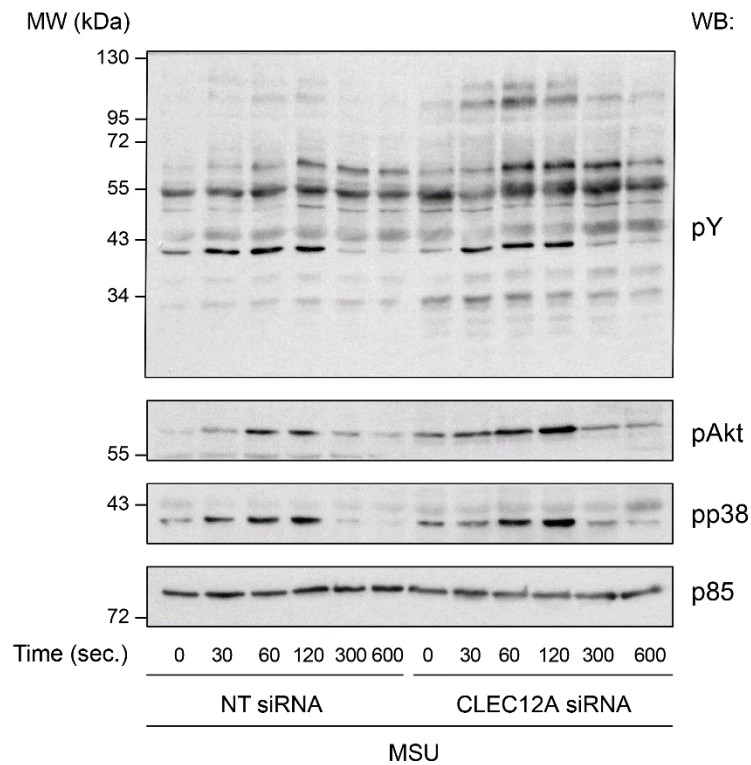


Supplementary Figure 4. STRING analysis of kinases with increased phosphorylation in human neutrophils stimulated with TNF- α compared to neutrophils stimulated with TNF- α and cross-linked for CLEC12A cross-linking.

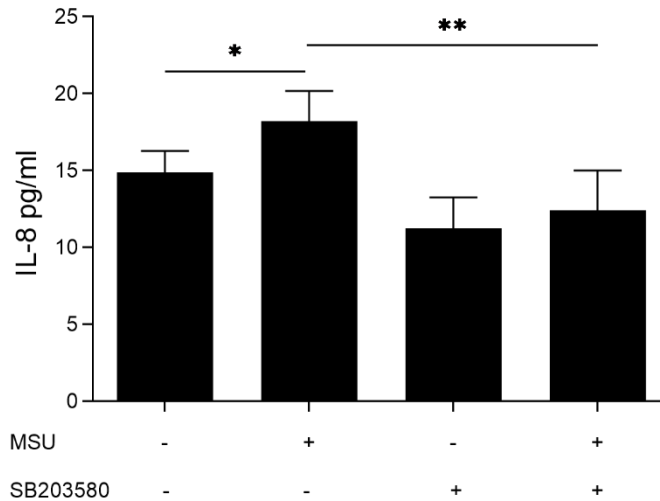
A**B****C**

Supplementary Figure 5. MSU induce the phosphorylation of Akt via p38 in human

neutrophils. (A) Neutrophils were stimulated with MSU in the presence or absence of SB203580 as described in *Materials and Methods*. Cell lysates were then immunoblotted with an anti-phospho Akt (*pAkt*) or anti-phospho-p38 (*pp38*) antibody as well as an anti-PI3K p85 subunit antibody as a loading control. (B & C) Densitometry analysis of the immunoblots in (A). These data are representative of 3 independent experiments.



Supplementary Figure 6. CLEC12A knock-down in THP-1 cells enhances MSU-induced global tyrosine phosphorylation and the phosphorylation of Akt and p38. The monocytic cell line, THP-1, was cultured in RPMI supplemented with 10% heat-inactivated fetal calf or bovine serum. THP-1 cells were transfected with CLEC12A siRNA prior to stimulation with MSU as described in *Materials and Methods* followed by immunoblotting with an anti-phosphotyrosine (pY), anti-phospho Akt and a PI3K p85 subunit (loading control) antibody. These data are representative of 3 experiments.



Supplementary Figure 7. MSU-induced IL-8 production in human neutrophils is p38-

dependent. Human neutrophils were stimulated with 0.3 mg/ml MSU in the presence or absence of SB203580 or diluant ((-); DMSO) and released CXCL8/IL-8 determined by ELISA. Data are representative of 3 independent experiments. Statistical analysis: Two way ANOVA and the Turkey's multiple comparisons test was performed to compare the treated cells to the control cells. * P < 0.05, ** P < 0.01.

Table S1. GO molecular functions of CLEC12A-Induced Phosphoproteins							
Enrichment of phosphoproteins CLEC12A>isotype GO molecular function							
	Homo sapiens (REF)	CLEC12A- induced					
GO molecular function	#	#	Expected	Fold Enrichment	+/-	Raw P value	FDR
MAP kinase kinase kinase activity	28	9	1.74	5.18	+	2.18E- 04	2.90E- 02
protein kinase activity	614	64	38.13	1.68	+	1.65E- 04	2.40E- 02
guanyl-nucleotide exchange factor activity	218	30	13.54	2.22	+	1.72E- 04	2.43E- 02
GTPase binding	545	62	33.85	1.83	+	2.10E- 05	3.75E- 03
GTPase regulator activity	303	37	18.82	1.97	+	2.63E- 04	3.30E- 02
phospholipid binding	426	49	26.46	1.85	+	1.45E- 04	2.26E- 02
cell adhesion molecule binding	483	55	30	1.83	+	5.46E- 05	9.42E- 03
actin binding	435	49	27.02	1.81	+	1.76E- 04	2.41E- 02
calcium ion binding	715	73	44.41	1.64	+	1.01E- 04	1.62E- 02

Table S2. Protein Kinases and Phosphatases Phosphorylated upon CLEC12A Cross-linking

Protein	Gene	Protein	Gene
Beta-adrenergic receptor kinase 1	ADRBK1	Mitogen-activated protein kinase kinase kinase 2	MAP4K2
Alpha-protein kinase 3	ALPK3	Mitogen-activated protein kinase 3	MAPK3
Serine/threonine-protein kinase A-Raf	ARAF	Mitogen-activated protein kinase 8	MAPK8
Tyrosine-protein kinase Blk	BLK	MAP/microtubule affinity-regulating kinase 4	MARK4
Serine/threonine-protein kinase BRSK2	BRSK2	Microtubule-associated serine/threonine-protein kinase 2	MAST2
Cyclin-T2	CCNT2	Microtubule-associated serine/threonine-protein kinase 4	MAST4
Cyclin-dependent kinase 12	CDK12	Hepatocyte growth factor receptor	MET
Cyclin-dependent kinase 16	CDK16	MAP kinase-interacting serine/threonine-protein kinase 1	MKNK1
Dual-specificity protein kinase CLK2	CLK2	Serine/threonine-protein kinase Nek5	NEK5
Casein kinase II subunit alpha	CSNK2A1	Serine/threonine-protein kinase NIM1	NIM1K
Serine/threonine-protein kinase DCLK3	DCLK3	AMPK-related protein kinase 5	NUAK1
Epithelial discoidin domain-containing receptor 1	DDR1	Phosphorylase b kinase regulatory subunit alpha	PHKA2
Dual-specificity tyrosine-phosphorylation-regulated kinase 4	DYRK4	Phosphatidylinositol 3-kinase catalytic subunit type 3	PIK3C3
Ephrin-B1	EFNB1	Protein phosphatase 1A	PPM1A

Ephrin-B2	EFNB2	Protein phosphatase 1D	PPM1D
Ephrin type-A receptor 2	EPHA2	Protein phosphatase 1H	PPM1H
Ephrin type-B receptor 2	EPHB2	Protein phosphatase 1J	PPM1J
Ephrin type-B receptor 4	EPHB4	5'-AMP-activated protein kinase subunit alpha-2	PRKAA2
Ephrin type-B receptor 6	EPHB6	5'-AMP-activated protein kinase subunit gamma-2	PRKAG2
Fibroblast growth factor receptor 4	FGFR4	Protein kinase C beta	PRKCB
Insulin receptor-related protein	INSRR	Protein kinase C delta	PRKCD
LIM domain kinase 2	LIMK2	Ribosomal protein S6 kinase alpha-1	RPS6KA1
Leucine-rich repeat serine/threonine-protein kinase 2	LRRK2	Ribosomal protein S6 kinase alpha-5	RPS6KA5
Mitogen-activated protein kinase kinase 2	MAP2K2	Ribosomal protein S6 kinase delta-1	RPS6KC1
Mitogen-activated protein kinase kinase kinase 1	MAP3K1	SRSF protein kinase 2	SRPK2
Mitogen-activated protein kinase kinase kinase 11	MAP3K11	Serine/threonine-protein kinase 10	STK10
Mitogen-activated protein kinase kinase kinase 19	MAP3K19	Non-receptor tyrosine kinase TNK1	TNK1
Mitogen-activated protein kinase kinase kinase 2	MAP3K2	Testis-specific serine/threonine-protein kinase 2	TSSK2
Mitogen-activated protein kinase kinase kinase 3	MAP3K3	Tau-tubulin kinase 1	TTBK1

Mitogen-activated protein kinase kinase kinase 4	MAP3K4	Inactive serine/threonine-protein kinase VRK3	VRK3
Mitogen-activated protein kinase kinase kinase 9	MAP3K9	Wee1-like protein kinase 2	WEE2

Table S3. Molecular Functions of TNF-α Phosphoproteins Reduced by CLEC12A Cross-linking						
	Homo sapiens	Current List				
GO molecular function	#	#	Expected	Fold Enrichment	P value	FDR
microtubule motor activity	86	18	5.27	3.41	2.64E-05	2.02E-03
tau-protein kinase activity	22	8	1.35	5.93	2.26E-04	1.42E-02
protein kinase activity	614	71	37.64	1.89	1.68E-06	1.73E-04
dynein complex binding	23	8	1.41	5.67	2.89E-04	1.66E-02
dynein intermediate chain binding	29	9	1.78	5.06	2.47E-04	1.53E-02
ionotropic glutamate receptor binding	34	10	2.08	4.8	1.63E-04	1.07E-02
glutamate receptor binding	50	13	3.06	4.24	5.24E-05	3.69E-03
alpha-actinin binding	32	9	1.96	4.59	4.54E-04	2.55E-02
histone-lysine N-methyltransferase activity	44	10	2.7	3.71	9.30E-04	4.65E-02
structural constituent of cytoskeleton	99	21	6.07	3.46	4.85E-06	4.51E-04
Rac GTPase binding	69	14	4.23	3.31	2.67E-04	1.60E-02
Rho GTPase binding	180	29	11.03	2.63	1.45E-05	1.19E-03
Ras GTPase binding	431	56	26.42	2.12	9.72E-07	1.05E-04
methylated histone binding	66	13	4.05	3.21	5.60E-04	3.03E-02

Rho guanyl-nucleotide exchange factor activity	82	16	5.03	3.18	1.49E-04	9.91E-03
Ras guanyl-nucleotide exchange factor activity	140	23	8.58	2.68	6.52E-05	4.53E-03
microtubule binding	241	47	14.77	3.18	1.03E-10	2.00E-08
actin filament binding	194	34	11.89	2.86	3.60E-07	4.41E-05
thiol-dependent ubiquitin-specific protease activity	107	17	6.56	2.59	7.82E-04	4.05E-02
calmodulin binding	198	31	12.14	2.55	1.02E-05	8.76E-04
ion channel binding	128	20	7.85	2.55	4.87E-04	2.70E-02
protein tyrosine kinase activity	136	20	8.34	2.4	7.70E-04	4.03E-02
cadherin binding	315	44	19.31	2.28	3.02E-06	2.99E-04
protein kinase binding	658	78	40.33	1.93	2.01E-07	2.59E-05
protein serine/threonine kinase activity	446	52	27.34	1.9	3.69E-05	2.73E-03
GTPase regulator activity	303	35	18.57	1.88	8.68E-04	4.44E-02
phospholipid binding	426	48	26.11	1.84	1.96E-04	1.25E-02
RNA binding	1661	152	101.81	1.49	2.67E-06	2.70E-04

Table S4. Protein Kinases and Phosphatases with Reduced Phosphorylation following CLEC12A Cross-linking			
Protein	Gene	Protein	Gene
Abelson murine leukemia viral oncogene homolog 1	ABL1	maternal embryonic leucine zipper kinase	MELK
Adenylate kinase 7	AK7	Mitogen-activated protein kinase kinase kinase MLK4	MLK4
Adenylate kinase 9	AK9	Serine/threonine-protein kinase Nek6	NEK6
Serine/threonine-protein kinase A-Raf	ARAF	Serine/threonine-protein kinase Nek8	NEK8
Serine-protein kinase ATM	ATM	Serine/threonine-protein kinase PAK 1	PAK1
Bone morphogenetic protein receptor type-2	BMPR2	Serine/threonine-protein kinase PAK 2	PAK2
Serine/threonine-protein kinase B-raf	BRAF	Putative 3-phosphoinositide-dependent protein kinase 1	PDPK1
Serine/threonine-protein kinase BRSK1	BRSK1	Pseudopodium-enriched atypical kinase 1	PEAK1
Serine/threonine-protein kinase BRSK2	BRSK2	Phosphorylase b kinase regulatory subunit alpha	PHKA2
Cyclin-dependent kinase 12	CDK12	PH domain leucine-rich repeat-containing protein phosphatase 2	PHLPP2
Cyclin-dependent kinase 17	CDK17	Phosphatidylinositol 4-kinase alpha	PI4KA
Cyclin-dependent kinase 2	CDK2	Phosphatidylinositol 4,5-bisphosphate 3-kinase catalytic subunit gamma	PIK3CG

citron rho-interacting serine/threonine kinase	CIT	Phosphatidylinositol 5-phosphate 4-kinase type-2 alpha	PIP4K2A
CDC-like kinase 3	CLK3	Protein kinase domain-containing protein, cytoplasmic	PKDCC
Tyrosine-protein kinase CSK	CSK	Inositol hexakisphosphate and diphosphoinositol-pentakisphosphate kinase 2	PPIP5K2
Casein kinase I isoform delta	CSNK1D	Protein phosphatase 1D	PPM1D
Casein kinase I isoform epsilon	CSNK1E	Protein phosphatase 1H	PPM1H
Doublecortin and CaM kinase-like 1	DCLK1	Protein phosphatase 1 regulatory subunit 12C	PPP1R12C
Doublecortin and CaM kinase-like 2	DCLK2	Protein phosphatase 1 regulatory subunit 3A	PPP1R3A
Diacylglycerol kinase delta	DGKD	Protein phosphatase 1 regulatory subunit 3D	PPP1R3D
Diacylglycerol kinase kappa	DGKK	Phosphatase 2A regulatory subunit B subunit alpha	PPP2R3A
Diacylglycerol kinase zeta	DGKZ	5'-AMP-activated protein kinase subunit gamma-2	PRKAG2
Dual specificity protein phosphatase 4	DUSP4	5'-AMP-activated protein kinase subunit gamma-2	PRKAG2
Dual specificity tyrosine-phosphorylation-regulated kinase 2	DYRK2	5'-AMP-activated protein kinase subunit gamma-3	PRKAG3
Ephrin type-A receptor 1	EPHA1	5'-AMP-activated protein kinase subunit gamma-3	PRKAG3
Ephrin type-A receptor 1	EPHA1	Protein kinase C alpha type	PRKCA

Ephrin type-A receptor 10	EPHA10	Protein kinase C gamma type	PRKCG
Ephrin type-A receptor 2	EPHA2	Protein kinase C gamma type	PRKCG
Ephrin type-A receptor 5	EPHA5	Protein kinase C zeta type	PRKCZ
Ephrin type-B receptor 4	EPHB4	Putative serine/threonine-protein kinase PRKY	PRKY
Fibroblast growth factor receptor	FGFR3	Serine/threonine-protein kinase PRP4	PRPF4B
Tyrosine-protein kinase Fyn	FYN	Phosphatidylinositol 3,4,5-trisphosphate 3-phosphatase and dual-specificity protein phosphatase PTEN	PTEN
Inhibitor of Bruton tyrosine kinase	IBTK	Tyrosine-protein phosphatase non-receptor type	PTPN1
Tyrosine-protein kinase receptor	IGF1R	Tyrosine-protein phosphatase non-receptor type 21	PTPN21
Insulin receptor-related protein	INSRR	Receptor-type tyrosine-protein phosphatase kappa	PTPRK
Inositol-trisphosphate 3-kinase A	ITPKA	Serine/threonine-protein kinase RIO1	RIOK1
Tyrosine-protein kinase	JAK2	Serine/threonine-protein kinase RIO2	RIOK2
Tyrosine-protein kinase JAK3	JAK3	Rho-associated protein kinase 2	ROCK2
Leucine-rich repeat serine/threonine-protein kinase 2	LRRK2	Tyrosine-protein kinase receptor	ROS1
LYN tyrosine kinase	LYN	Serine/threonine-protein kinase Sgk1	SGK1
Dual-specificity mitogen-activated protein kinase kinase 4	MAP2K4	Serine/threonine-protein kinase SIK2	SIK2

Mitogen-activated protein kinase kinase 11	MAP3K11	C-Jun-amino-terminal kinase-interacting protein 4	SPAG9
mitogen-activated protein kinase 10	MAPK10	Protein phosphatase Slingshot homolog 2	SSH2
Mitogen-activated protein kinase 4	MAPK4	Serine/threonine-protein kinase tousel-like 2	TLK2
Mitogen-activated protein kinase 8	MAPK8	Serine/threonine-protein kinase VRK1	VRK1
MAP/microtubule affinity-regulating kinase 4	MARK4		