

[illegible]

CD46	99.9 (90.6 100.0)	99.2 (97.6 100.0)	99.9 (99.6 100.0)	99.9 (99.7 100.0)	100.0 (99.8 100.0)	100.0 (100.0 100.0)	100.0 (99.9 100.0)	100.0 (99.8 100.0)	100.0 (99.9 100.0)	100.0 (99.8 100.0)	100.0 (99.9 100.0)	100.0 (99.9 100.0)
CD55	94.6 (90.1 98.6)	98.5 (97.9 99.9)	99.1 (98.7 99.4)	96.6 (94.3 98.7)	94.2 (92.1 99.3)	97.4 (95.5 98.9)	99.0 (96.1 99.5)	99.3 (97.9 99.5)	96.1 (92.9 99.1)	98.5 (94.4 99.5)	98.1 (95.8 99.5)	99.1 (97.4 99.4)

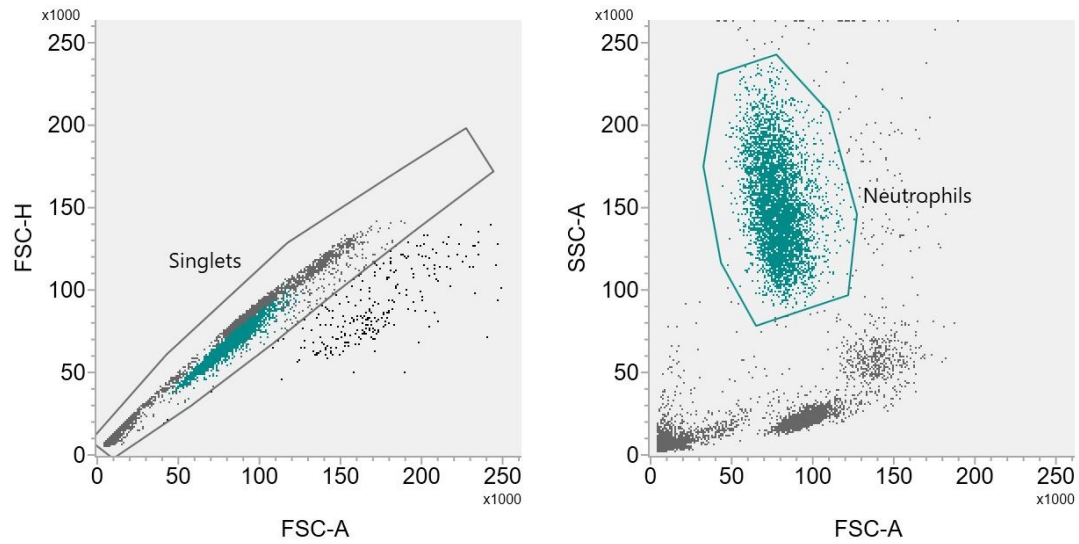
Supplementary Table S1. Summary of the neutrophils positive for the used surface markers in relationship to a respective isotype. $n = 6 - 8$, median (first quartile | third quartile).

PC	CD10	CD15	CD16	CD62L	CD35	CD11b	C3aR	C5aR1	C5aR2	CD46	CD55	explained variance [%]	explained variance cumulative [%]
1	0.36	0.35	0.35	-0.31	0.30	0.36	0.02	-0.29	-0.12	0.32	0.33	62.29	62.29
2	-0.06	-0.03	0.01	0.01	-0.10	-0.11	0.81	0.39	-0.08	0.30	0.27	13.09	75.38
3	0.03	0.06	-0.02	-0.08	-0.09	0.07	-0.03	0.07	0.97	0.15	0.10	8.64	84.03
4	0.09	0.24	0.34	0.65	0.41	0.19	0.10	0.28	0.10	-0.25	-0.17	4.67	88.70
5	0.10	0.39	0.31	0.16	-0.72	0.00	0.14	-0.25	-0.03	0.04	-0.32	4.32	93.02
6	-0.05	-0.21	0.01	0.53	0.14	-0.35	-0.03	-0.49	0.04	0.52	0.11	2.40	95.43
7	0.62	-0.45	0.01	-0.15	0.12	-0.02	0.25	-0.06	0.07	0.04	-0.54	1.58	97.01
8	0.14	-0.01	0.30	-0.04	-0.10	-0.24	-0.48	0.59	-0.11	0.47	-0.08	1.16	98.17
9	-0.24	0.32	-0.49	0.00	0.16	0.34	0.03	0.06	-0.05	0.46	-0.49	0.92	99.09
10	0.51	-0.06	-0.47	0.37	-0.31	0.34	-0.15	0.13	-0.10	0.02	0.34	0.61	99.71
11	0.34	0.55	-0.34	-0.07	0.17	-0.63	0.03	0.01	0.02	-0.14	0.02	0.29	100.00

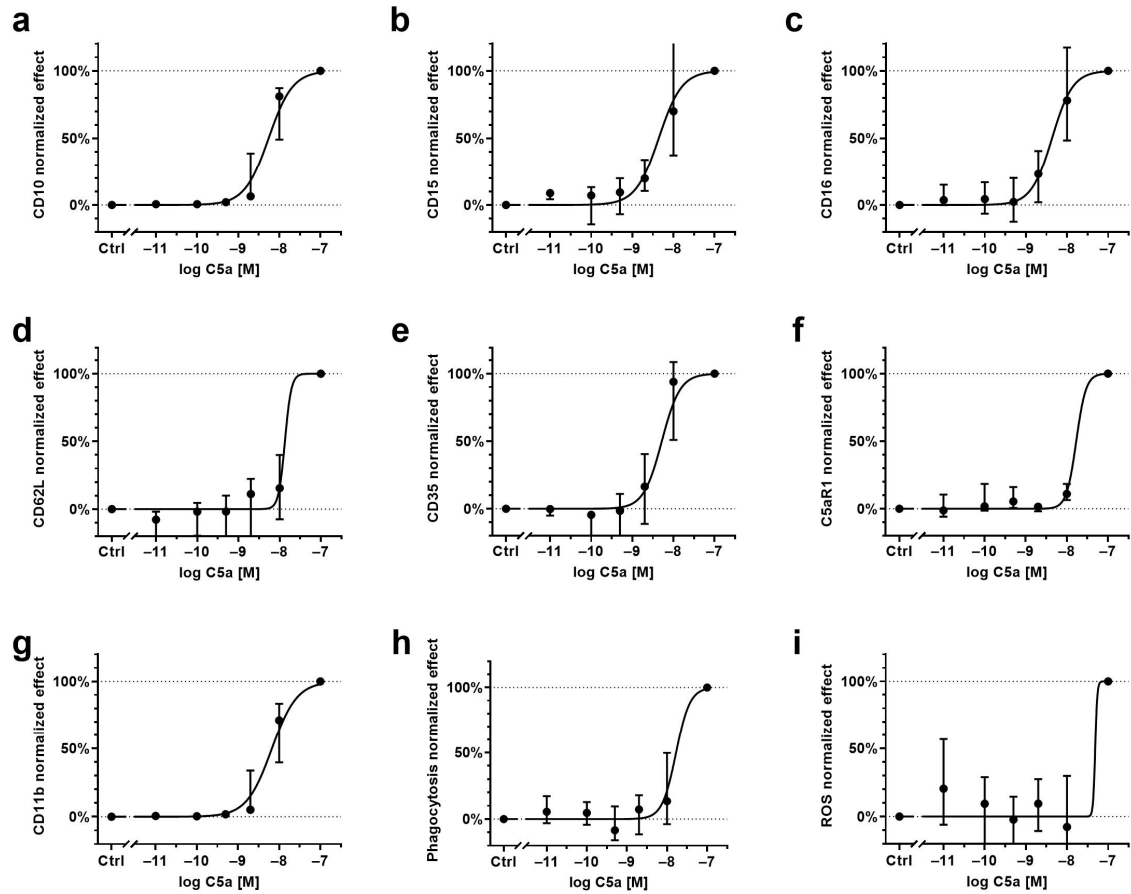
Supplementary Table S2. Principal component analysis (PCA) as well as its related first five principal components (PC) and loading matrix analyzing the change of indicated markers in stimulated neutrophils as described in the methods section.

	p-value screening	p-value confirmation	Ctrl	C5a
CD10	0.0003	0.0079	569.6 (549.3 725.6)	4524 (3976 5164)
CD15	0.1953	0.0159	788.2 (582 872.2)	1313 (982.5 1486)
CD62L	0.0134	0.5476	2583 (1645 3420)	2014 (1488 2724)
ROS	0.2759	0.9999	959.6 (693.7 2021)	1102 (660.3 1853)
Phagocytosis	0.0229	0.1255	18.89 (16.26 23.20)	24.64 (20.61 41.72)
CD16	0.2112	0.0411	4360 (2985 6877)	8415 (4813 10177)
CD35	0.1451	0.0649	1727 (1450 2268)	12368 (5577 18881)
CD11b	0.0018	0.0079	915.4 (738.9 1310)	7807 (7255 12919)
C5aR1	0.0275	0.2086	3782 (3696 5094)	2937 (2630 4566)
C5aR2	0.0439	0.0043	1829 (1729 2013)	1604 (1485 1656)
CD46	0.0657	0.0175	1548 (1526 1646)	1844 (1758 1934)
CD55	0.0382	0.0087	5236 (4512 6834)	8124 (6877 11556)
FSC	0.0006	0.0079	79420 (75756 83090)	127612 (123216 145483)
MP	0.0165	0.0262	4437 (3980 5727)	6365 (6054 8046)
pHi	0.0001	0.0079	7.50 (7.43 7.58)	7.91 (7.89 7.97)
GlcU	0.0039	0.0079	3668 (3191 4072)	6088 (5581 7191)

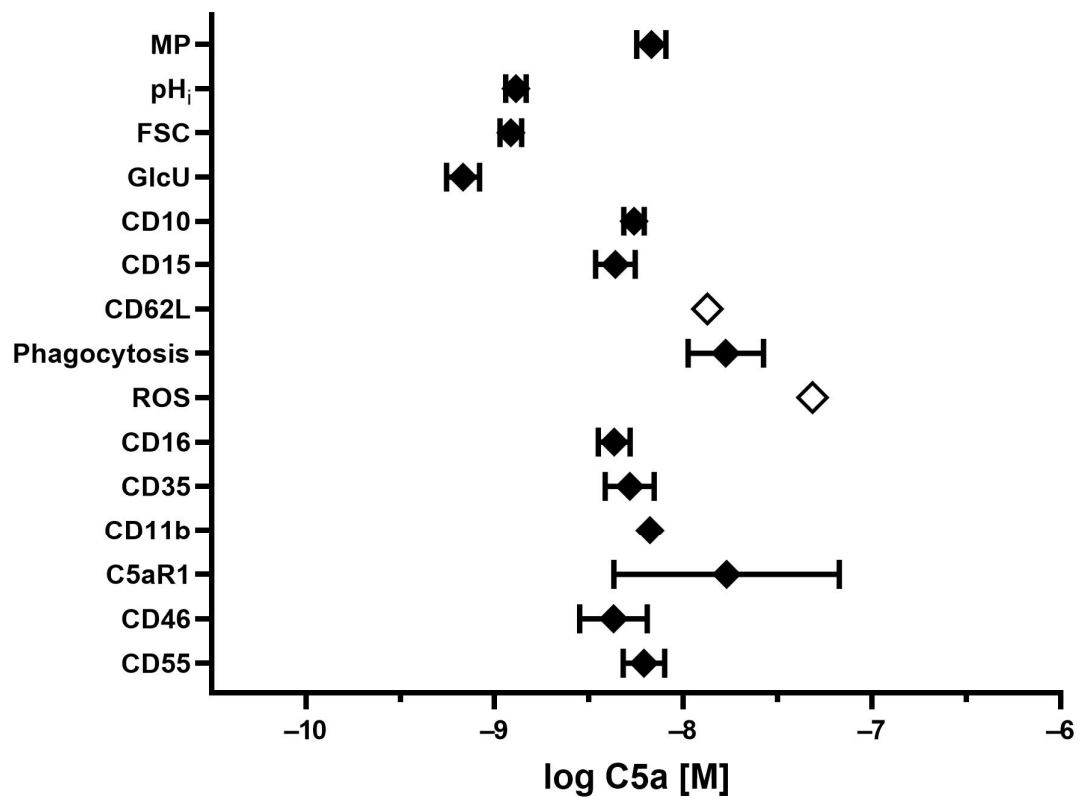
Supplementary Table S3. Comparison of resulting p-values of the screening process (corresponding results are reported in Figures 2 and 3) and the confirmation approach including the original data. MP is reported as DiBAC₄(3) fluorescence, pHi determined by SNARF fluorescence, and GlcU is quantified by 2NBDG fluorescence. The statistical approach is reported in detail in the method section using Kruskal-Wallis test with uncorrected Dunn's test for the screening process and Mann Whitney to confirm the findings. $n = 6 - 8$.



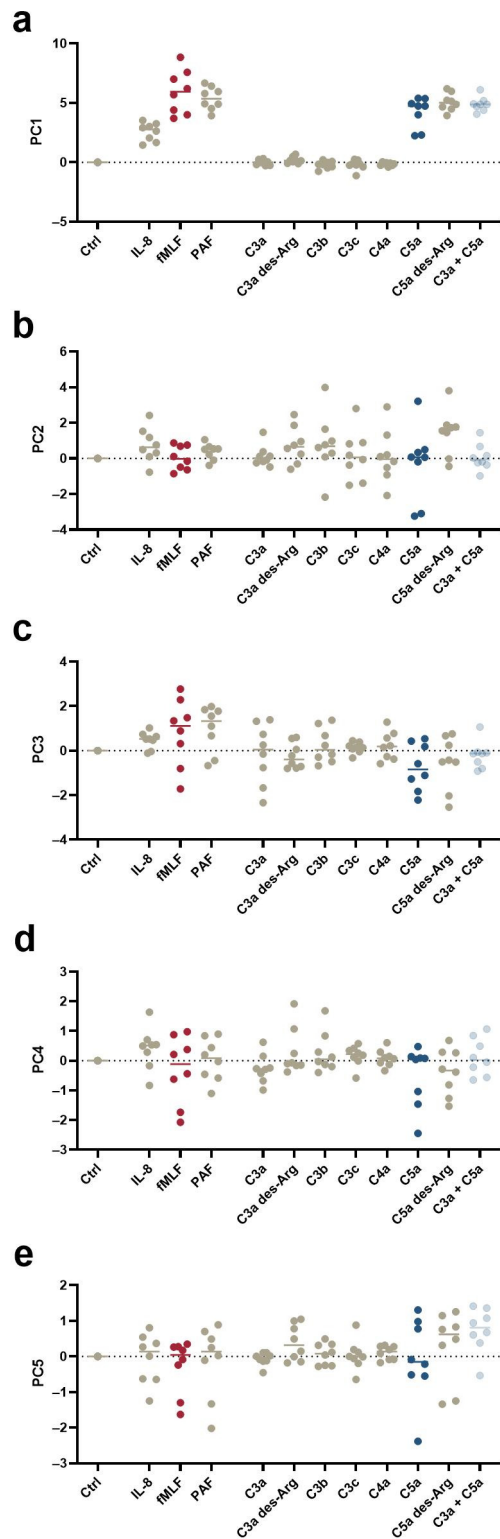
Supplementary Figure S1. Representative result of the leukocyte isolation procedure and summary of the applied gating strategy. Following identification of singlets (here: 98.1% of parent population) by checking linearity of FSC-A vs. FSC-H polymorphonuclear granulocytes (here referred to as neutrophils: 41.6% of parent population) mainly consisting of neutrophils were identified by FSC-A and SSC-A.



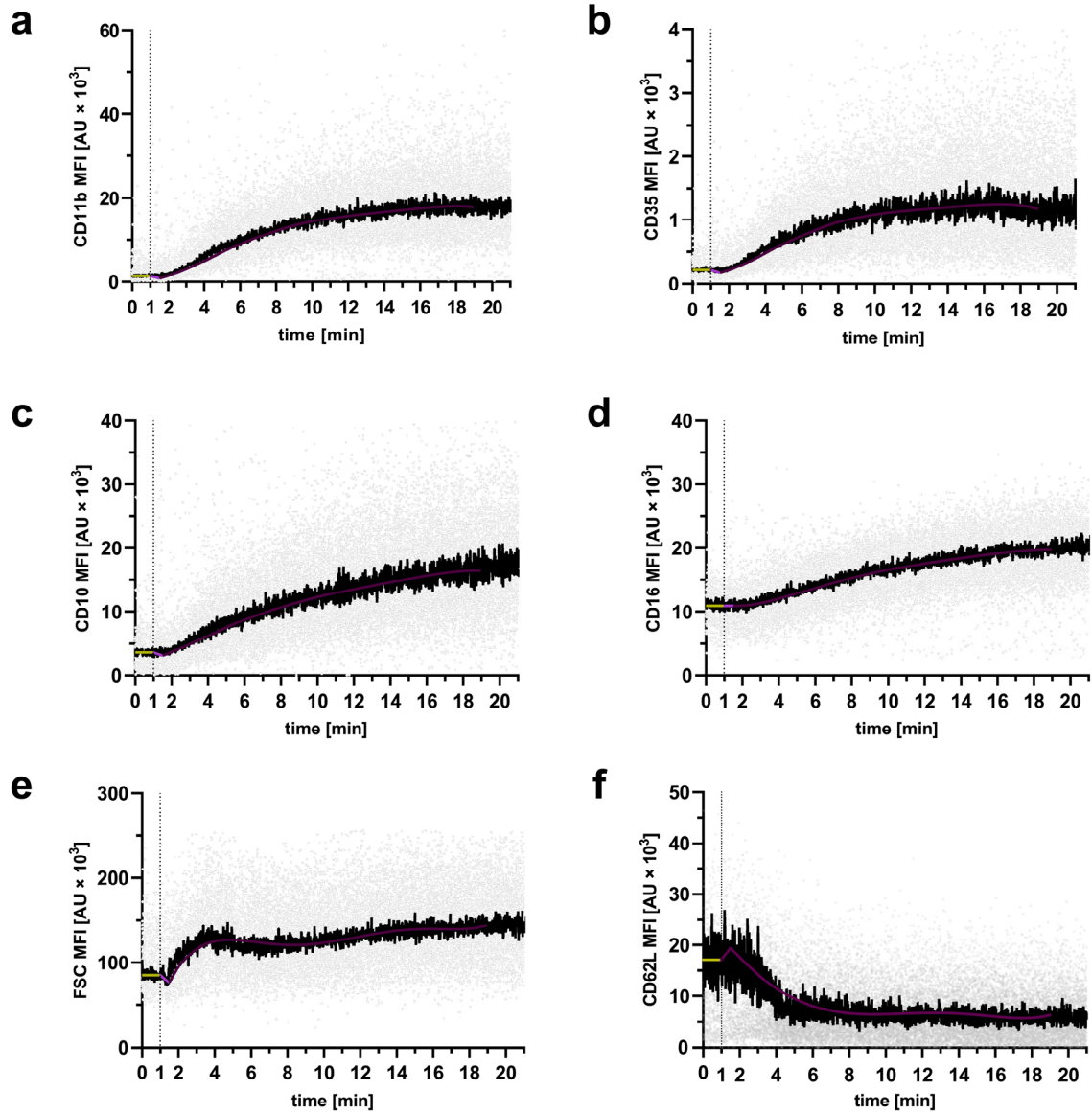
Supplementary Figure S2. Concentration-response curves of cellular function and neutrophil phenotype after stimulation with C5a. (a) CD10, (b) CD15, (c) CD16, (d) CD62L, (e) CD35, (f) C5aR1, (g) CD11b, (h) Phagocytosis and (i) ROS. Curves were normalized to PBS^{+/+} as control. $n = 6 - 7$, median \pm interquartile range.



Supplementary Figure S3. Calculated EC₅₀ for the activation of neutrophils elicited by C5a. $n = 6 - 7$, mean \pm standard error of the mean of the EC₅₀. For empty diamonds, confidence intervals could not be calculated.



Supplementary Figure S4. Result of the principal component analysis (PCA) evaluating the first principal components (PC). The PCA was conducted by analyzing the change in neutrophil phenotype elicited by the respective stimulus as listed on the Y-axis. (a–e) Unstimulated neutrophils were set to 0. The corresponding loading matrix is reported in Supplementary Table S2. n = 8.



Supplementary Figure S5. Curve fitting for (a) CD11b, (b) CD35, (c) CD10, (d) CD16, (e) FSC, and (f) CD62L from a representative experiment. Gray dots indicate single cells. The black line shows a moving median analyzing nine consecutive neutrophils. The yellow line indicates the baseline, pink indicates the first 30 seconds as a linear approximation, and violet displays the used polynomial as described in the methods section.