Supplementary Figure Legends:

Fig. S1. Pertussis toxin treatment inhibits, but fails to eliminate, C5a Ca^{2+} responses. BMDM were treated with 5-100 ng/ml Ptx for 18 hr prior to Fura-2-based population Ca^{2+} assays. Cells were stimulated with C5a (10 nM), UDP (10 μM) or HBSS (control) and the peak-offset of the responses was measured. Shown is a summary of 3 experiments with 4 replicate samples per condition per experiment. Values are mean+/-SEM (n=12). Comparison of C5a without Ptx to C5a + 100ng/ml Ptx and C5a + 100 ng/ml Ptx to HBSS (no stimulation) were significant by t-test with Bonferroni correction (*$p<0.001$).

Fig S2. Synergy does not require PI3-kinase or PKC activity. Intracellular Ca^{2+} responses were measured in Fura-2-loaded BMDMs. BMDM were treated with (A) LY294002 (50 μM) or (B) Calphostin C (500 nM) for 30 min prior to assay. Cells were stimulated with C5a (0.75 nM), UDP (500 nM), or simultaneous C5a and UDP. (A,B) Results of representative experiments showing the mean +/- SEM of 3-4 individual wells per condition quantified by integration of Ca^{2+} responses above baseline over the first 20 seconds after ligand addition. (C) Summary of data from n=7 (LY294002 (LY)), n=5 (calphostin C (Cal C)), and n=3 (staurosporine (Stauro), 150nM) experiments of identical design.

Fig. S3. Efficacy of pharmacologic inhibitors of PI3-K and PKC in BMDMs. BMDM were treated with (A) LY294002 (50 μM) and (B) Calphostin C (500 nM) or staurosporine (0.15-3 μM) for 30 min prior to stimulation for phosphoprotein assay. A: Cells were stimulated with C5a (0.75 nM) or FcγR cross-linking for 1 or 3 min, respectively, before lysis in SDS sample buffer. B: Cells were stimulated with PMA (100 nM) or DMSO (control) for 3 min before lysis in SDS sample buffer. Non-stimulated (NS) samples were prepared in parallel. Samples were blotted for P-Akt or P-MARCKS in parallel with Rho-GDI for normalization of loading. Each inhibitor robustly reduced the phosphorylation state of the target protein.

Fig. S4. Knockdown of PLCβ in RAW264.7 cells. RNAi against PLCβ isoforms in RAW264.7 cells was performed by lentiviral-mediated RNAi using shRNA encoding constructs. Control lines lacking only shRNA were prepared and analyzed in parallel with each RNAi line. Target depletion was determined by quantitative RT-PCR for each isoform and by Western blotting for PLCβ3. Results of KD efficiency in independently derived replicate lines are shown in the table at left and Western blot results for the first replicate of PLCβ3 are shown in the image at right. The blot shows samples of the cell lines taken at day 18 (d18) and day 24 (d24) after infection with lentivirus.

Supplemental data:

Figure S1

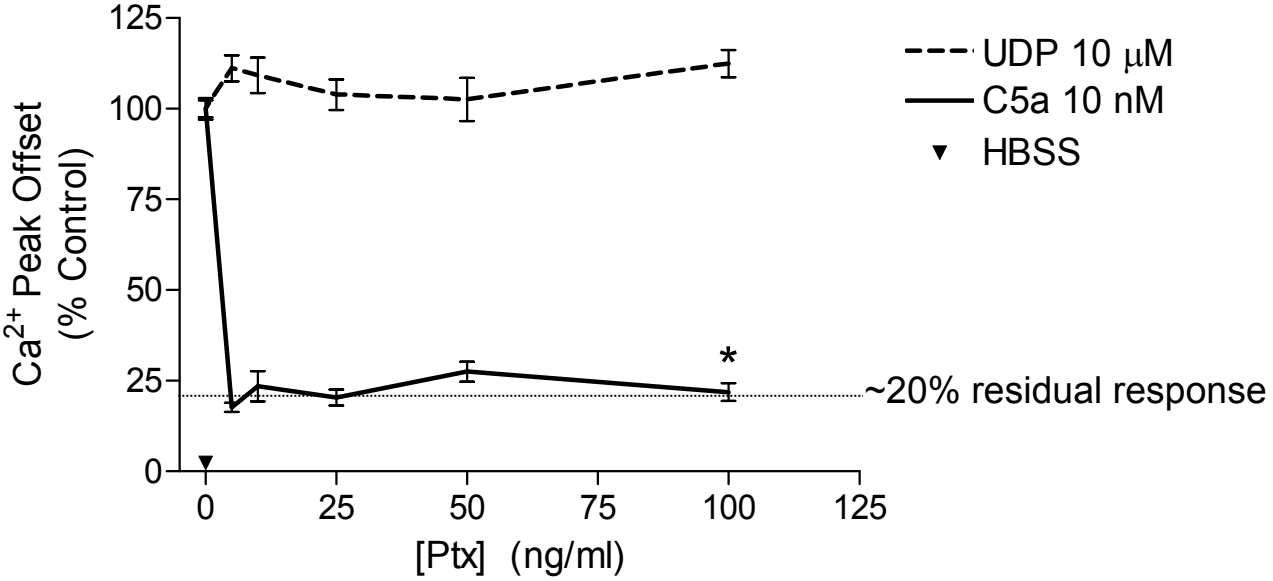


Figure S2

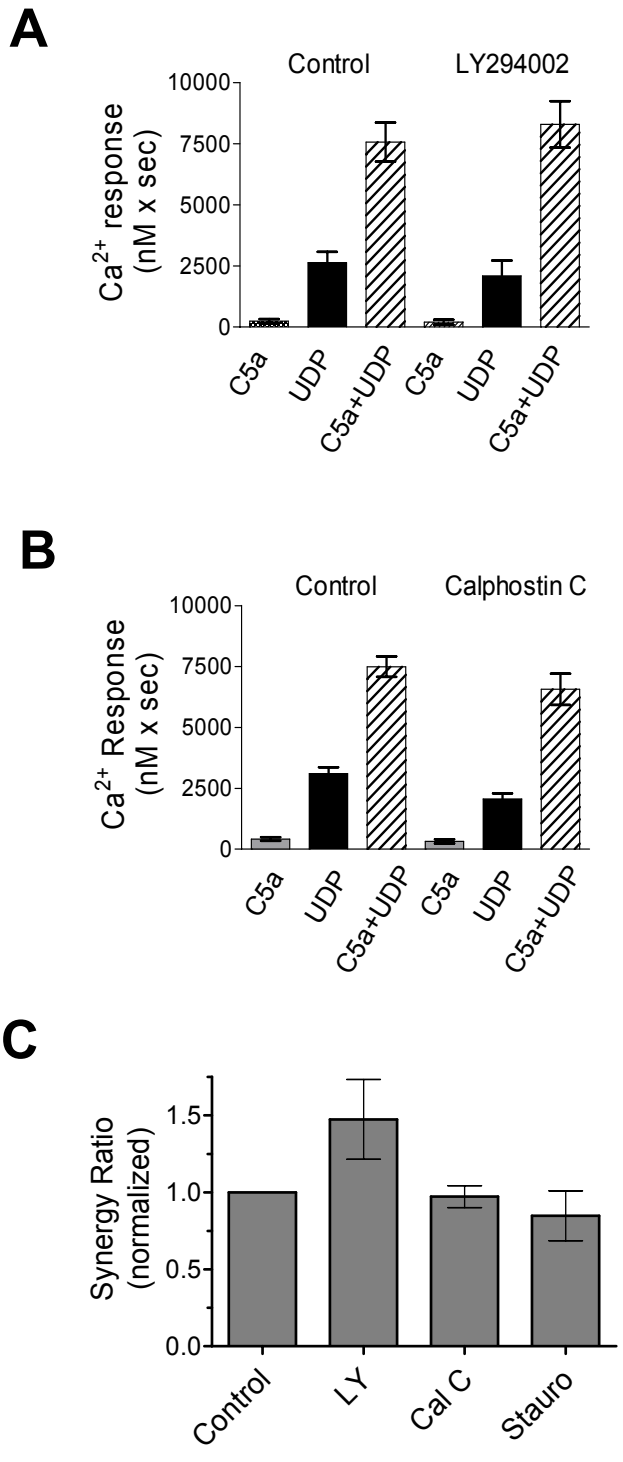
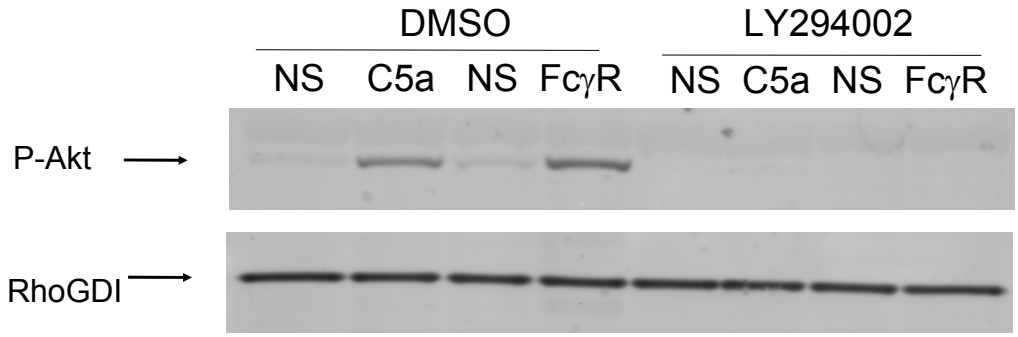

A
Control
LY294002
10000
7500
5000
2500
0
Ca2+ response
(nM x sec)
C5a
UDP
C5a+UDP
C5a
UDP
C5a+UDP
B
Control
Calphostin C
10000
7500
5000
2500
0
Ca2+ Response
(nM x sec)
C5a
UDP
C5a+UDP
C5a
UDP
C5a+UDP
C
Synergy Ratio
(normalized)
1.5
1.0
0.5
0.0
Control
LY
Cal C
Stauro

Figure S3

A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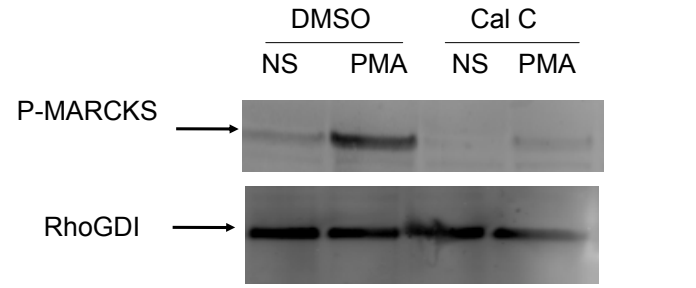
LY294002
NS
C5a
NS
FcγR
NS
C5a
NS
FcγR
P-Akt
RhoGDI

B


DMSO
Cal C
NS
PMA
NS
PMA
P-MARCKS
RhoGDI

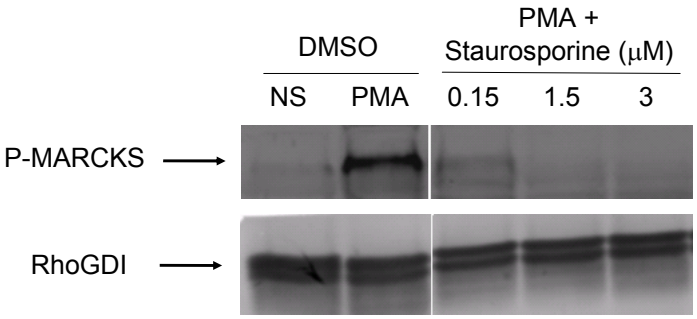
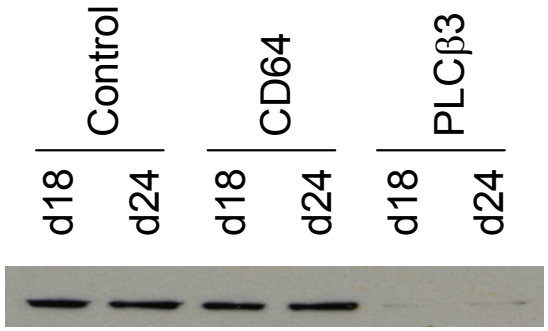

DMSO
PMA +
Staurosporine (μM)
NS
PMA
0.15
1.5
3
P-MARCKS
RhoGDI

Figure S4

mRNA levels

PLCβ isoform	Replicate	KD (% Loss) qRT-PCR	KD (% Loss) Western
2	1	90	
	2	80	
3	1	ND	83
	2	60	48
4	1	87	
	2	84	
	3	64	
	4	59	
	5	51	

Protein levels



PLCβ3