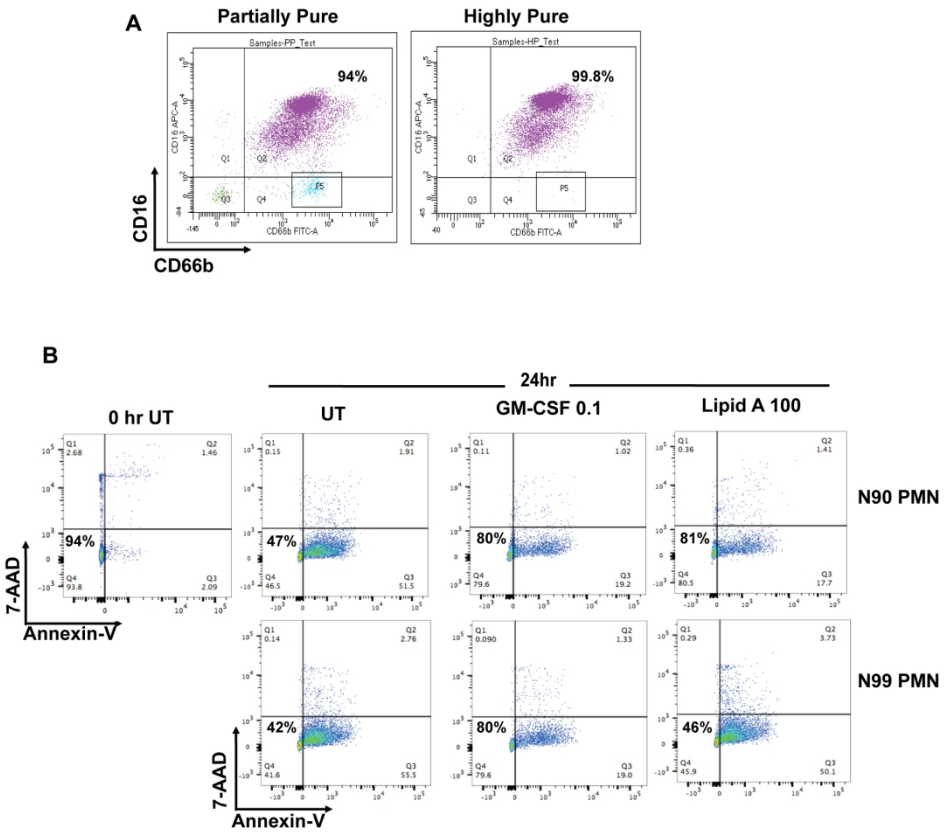
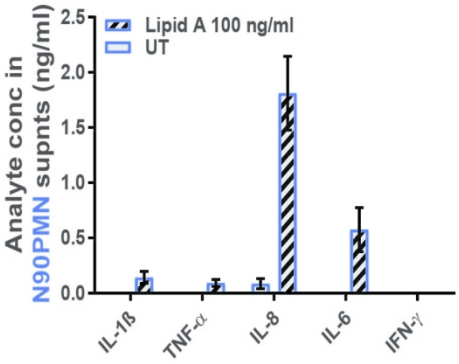


Supplemental Figure 1. Measurement of TLR4 survival effects on N90 vs N99 PMNs.
A) Representative dot plots of partially purified (>90% pure, N90, blue) or highly purified (>99% pure, N99, red) neutrophils detected as positive for both CD16 and CD66b.
B) Dot plots from a representative experiment showing the percent of cells that were viable (negative for both Annexin-V and 7-AAD) at 0 or 24 h after the indicated treatments.

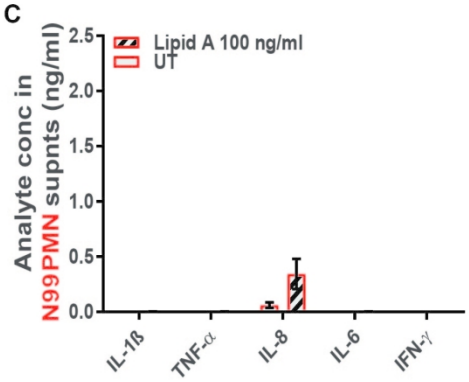
A



B

N90 PMN Supnt donor	Lipid A (ng/ml)	IL-8	IL-6	IL-1β	TNF-α
1	0	308.9	0.5	3.8	0.7
	100	2553.6	538.4	57.1	68.8
2	0	95.9	1.7	0.5	1.6
	100	2799.9	750.3	136.7	204.3
3	0	34.6	0.3	0.1	0.3
	100	1473.0	73.9	18.9	26.3
4	0	35.1	0.1	0.2	0.2
	100	606.5	57.5	33.2	12.5
5	0	23.0	0.5	0.3	0.5
	100	2052.0	1363.7	325.9	159.1
6	0	22.6	1.2	0.3	1.4
	100	1377.7	668.4	283.9	84.6

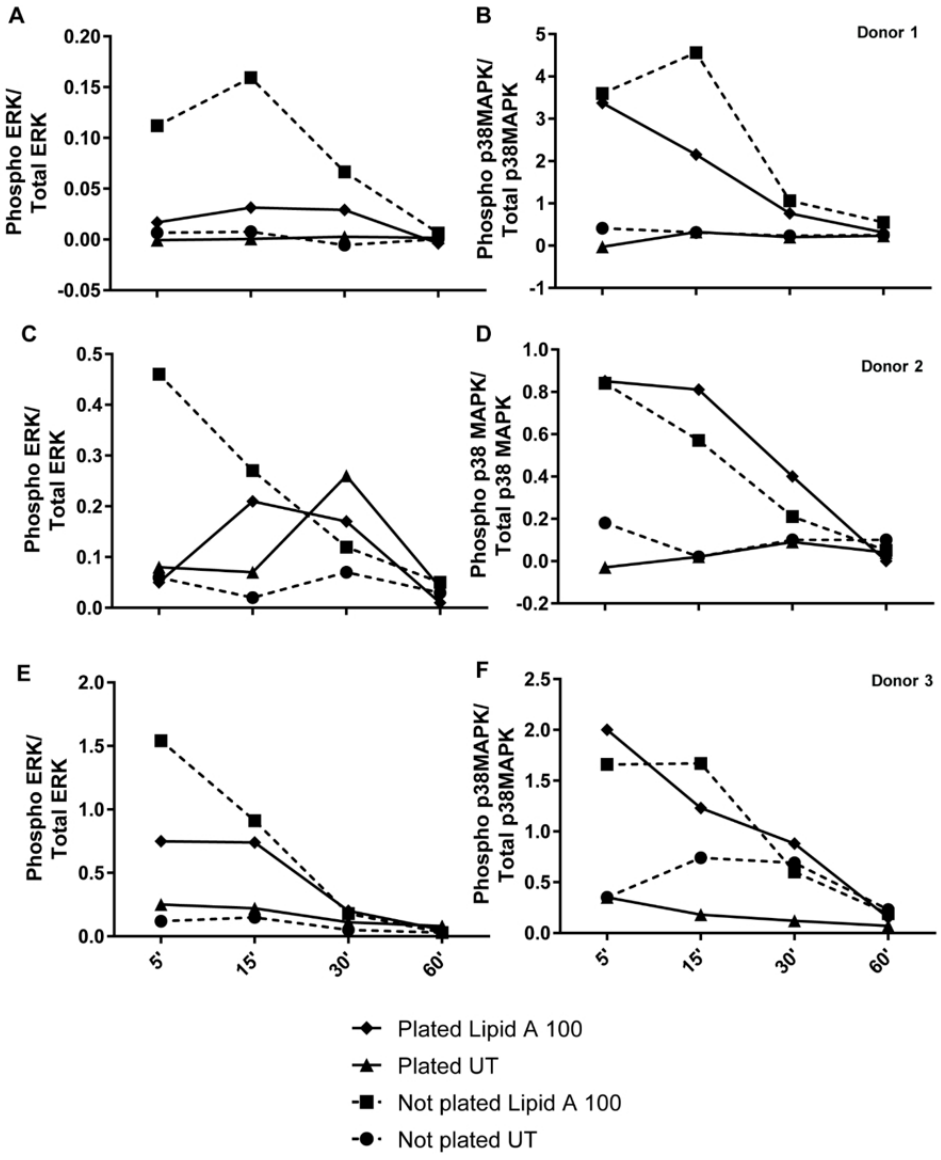
C



D

N99 PMN Supnt donor	Lipid A (ng/ml)	IL-8	IL-6	IL-1β	TNF-α
1	0	175.3	BDL	3.3	0.5
	100	379.8	1.6	5.0	1.4
2	0	73.5	0.1	0.3	0.4
	100	981.5	5.8	1.5	7.5
3	0	20.2	0.0	0.1	0.0
	100	295.8	5.3	0.8	2.4
4	0	75.9	0.1	0.1	0.1
	100	259.8	0.7	0.7	0.8
5	0	14.9	BDL	0.1	0.1
	100	78.3	1.9	0.7	0.9
6	0	11.4	0.0	0.0	0.1
	100	69.2	0.5	0.3	1.2

Supplemental Figure 2. Measurement of candidate survival factors induced by TLR4 activation. Culture supernatants were collected from N90 (A) or N99 (B) neutrophils after culture with 0 or 100 ng/ml lipid A for 20 hr and evaluated for the presence or IFNγ, IL-8, IL-6, IL-β and TNF-α by electrochemiluminescent multiplex assay (MesoScale Discovery). Values were measured in culture supernatants from each of n=6 blood donors; IFNγ was not detected (not shown). Corresponding values in pg/ml for each analyte and donor are tabulated in (B) and (D).



Supplemental Figure 3. Differential stimulation of ERK phosphorylation by lipid A in N99 PMN. Plated or unplated N99 PMNs were stimulated with 100 ng/ml lipid A for the indicated times and then cell lysates were analyzed by immunoblot using antibodies specific for phosphorylated or total ERK1/2 and p38 MAPK. The extent of kinase activation in each of n=3 blood donors was quantified as the ratio of chemiluminescent signals corresponding to phosphorylated versus total ERK1 and ERK2 (A, C, E) or p38 MAPK (B, D, F).