

Figure S1: Antibody cross-linking of CD18 induces release of primary, secondary, and tertiary granules as well as secretory vesicles. Freshly isolated PMN were incubated with primary anti-CD18 mAb IB4 (ctrl) or the primary antibody plus secondary cross-linking antibody (PMN-*sec*). Supernatants were analyzed for the presence of granule marker proteins by Western blot (Albumin, MMP-9, lactoferrin) or by analysis of enzymatic activity (MPO). Bands and bars are representative of three independent experiments.

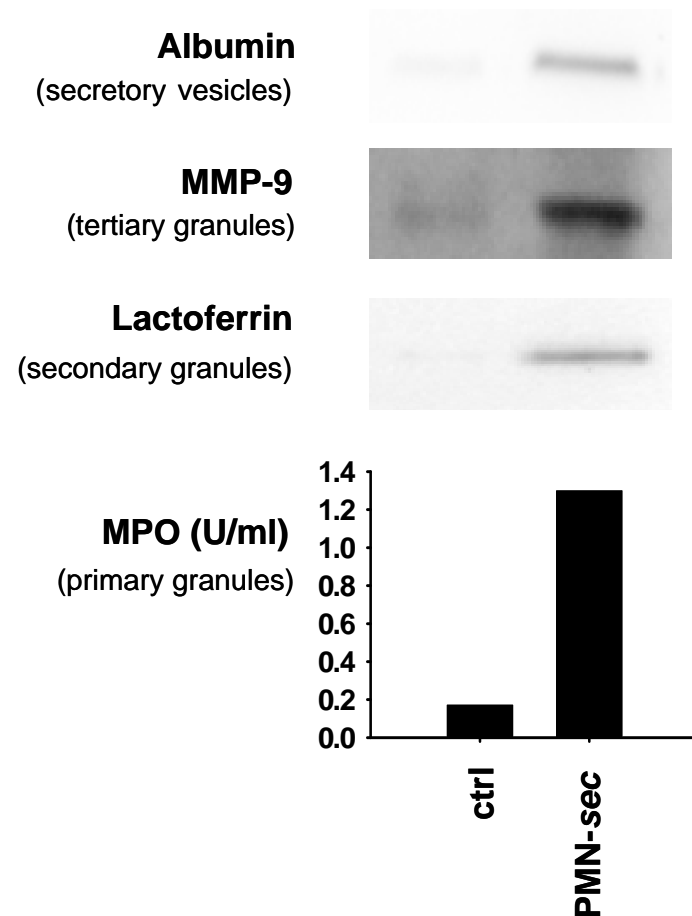


Figure S2: PMN secretion enhances bacterial killing by Mø. Human Mø were treated with PMN-sec for 24 hours. Thereafter they were allowed to phagocytose a) *S. aureus* (Newman strain) or b) *E. coli* (D21). At time 0 bacteria were washed off and the intracellular killing was quantified at various time points after hypotonic lysis of Mø and culture of bacteria on LB agar. Data are expressed as percent of CFU at time 0. Comparisons are made between Mø treated with medium (ctrl) or with PMN secretion. n=3 for each bar. * indicates significant difference between treatment with PMN-sec and control at respective time point.

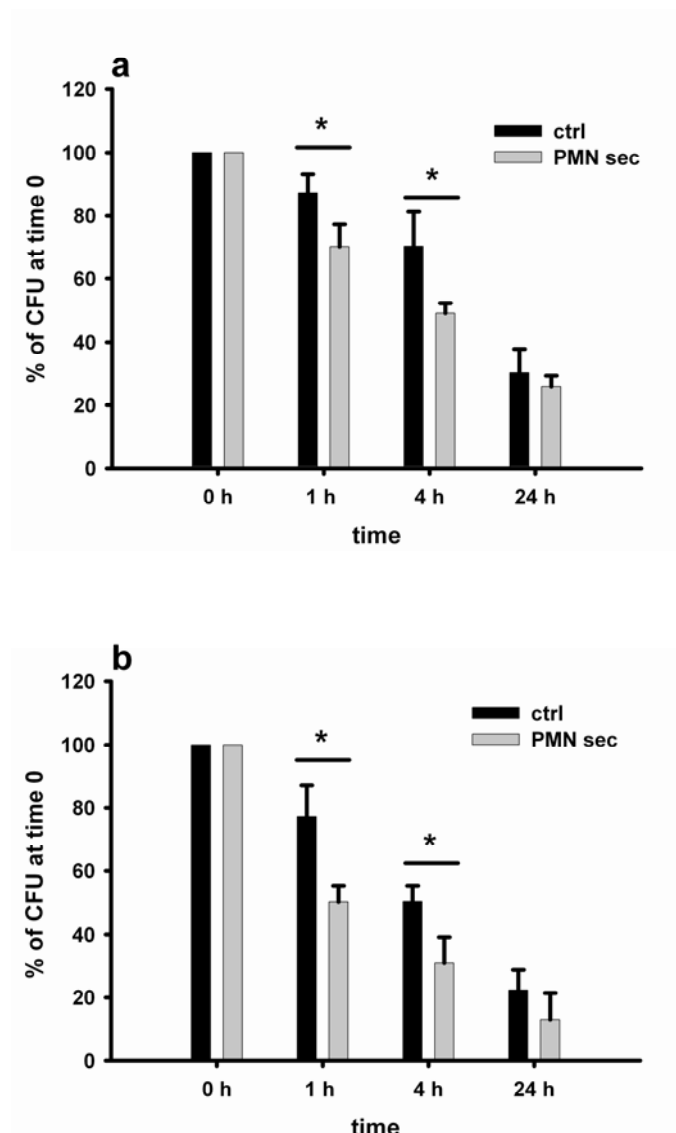


Figure S3: PMN secretion enhances activation of Mø and expression of FcγRs.

Expression of (a) activation markers and (b) phagocytic receptors in Mø in response to treatment with PMN-*sec* for 24 h. The expression is adjusted for the number of cells as assessed by nuclear staining with DAPI. Expression is given as percent change compared to basal expression per DAPI fluorescence. All values are isotype corrected. n = 3 for each bar.

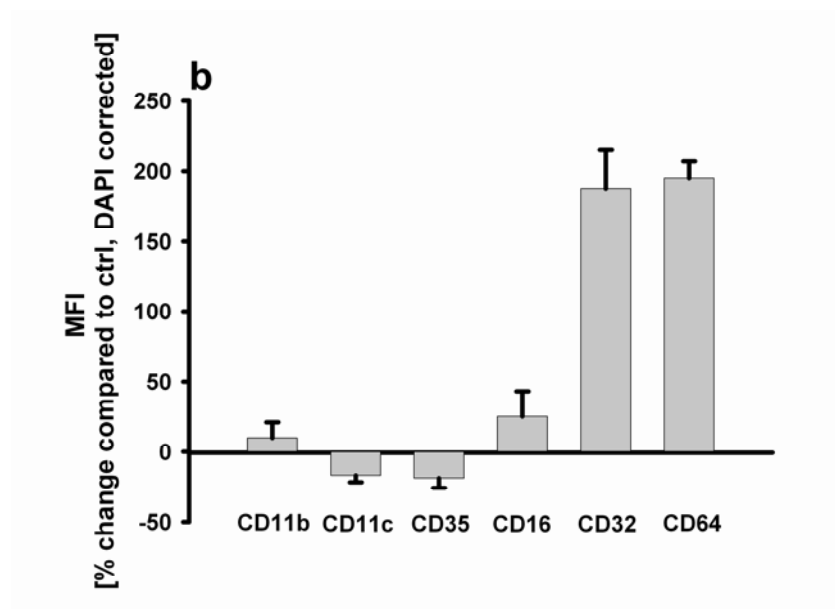
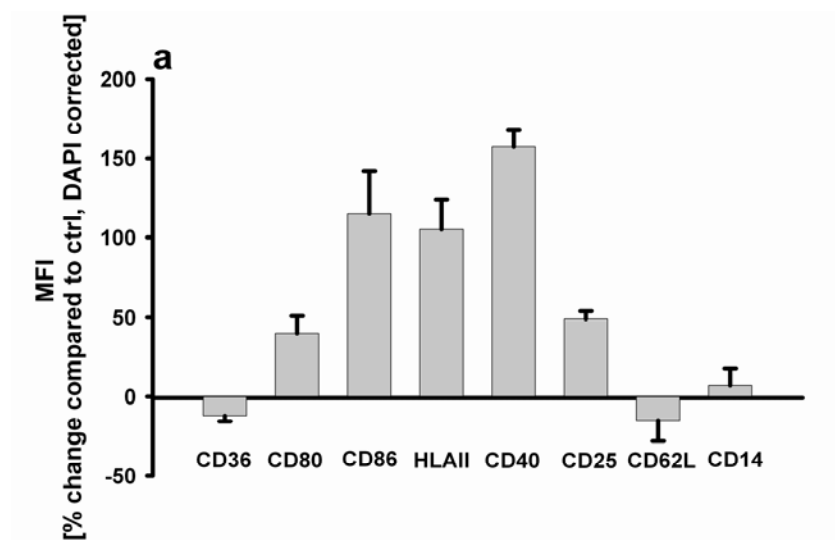


Figure S4: HBP and HNP1-3 enhance the phagocytic capacity of peritoneal macrophages in neutropenic rats. Comparison of phagocytic activity of peritoneal macrophages from rats with intact WBC and neutropenic rats. In addition, neutropenic rats were injected intraperitoneally with HBP (100 μ g/rat) or HNP1-3 (20 μ g/rat). Mean fluorescence intensity (MFI) as a measure of phagocytosed bacteria was read in a plate reader. Data are expressed as mean \pm SD. n = 2 for each bar.

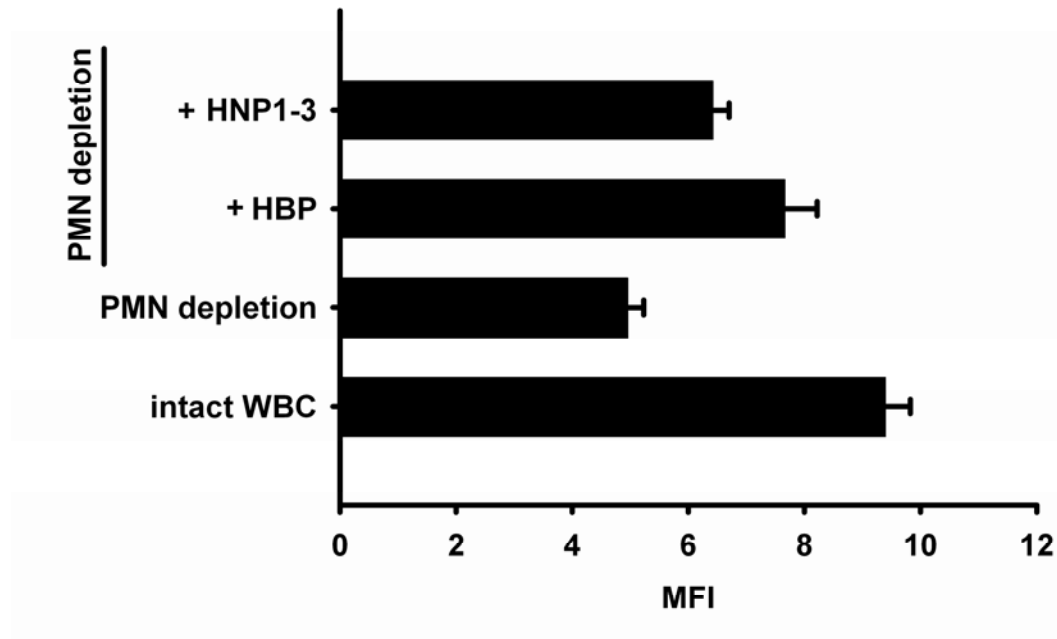


Figure S5: Neutralization of TNF α and IFN γ reduces upregulation of CD32 and CD64 in response to HBP and HNP1-3. Importance of TNF α and IFN for the up-regulation of CD32 and CD64 on human M ϕ in response to a) HBP or b) HNP1-3 evaluated through use of neutralizing antibodies. Receptor expression is assessed by immunofluorescence staining and displayed as % change compared to control. To control for the number of cells in each well, M ϕ were counterstained with DAPI and the fluorescence values obtained for CD32 and CD64 are corrected by the DAPI fluorescence. n = 3 for each data point. * indicates significant difference compared to HBP or HNP1-3 treatment.

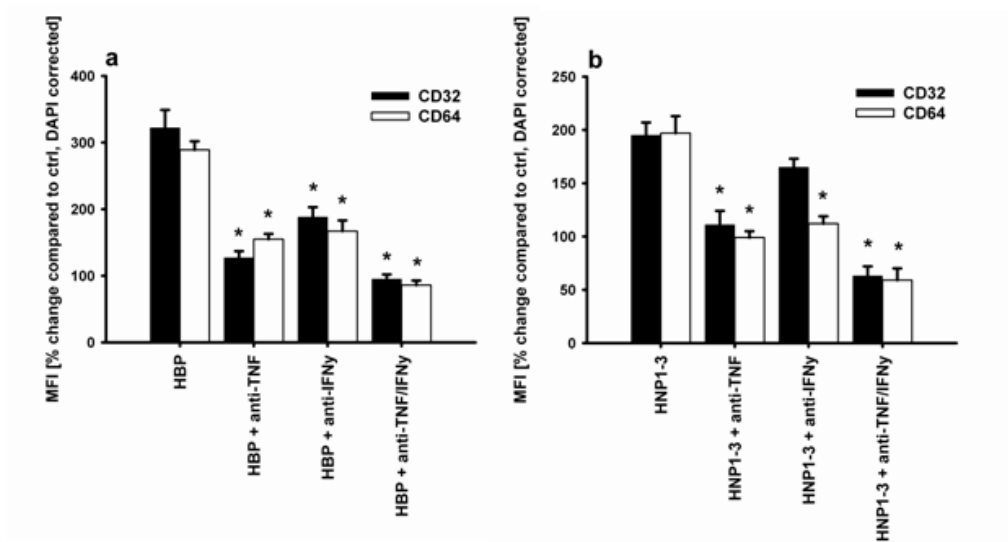


Figure S6: Effect of cytokine neutralization on HBP and HNP1-3 mediated expression of HLA II, CD40, and CD86. The influence of neutralizing antibodies to TNF or IFN γ on enhanced expression of HLAII, CD40, and CD86 in human macrophages in response to stimulation with a) HBP and b) HNP1-3 was assessed by immunofluorescence. Data are expressed as isotype corrected MFI. n = 6 for each bar. * indicates significant difference compared to treatment with HBP or HNP1-3.

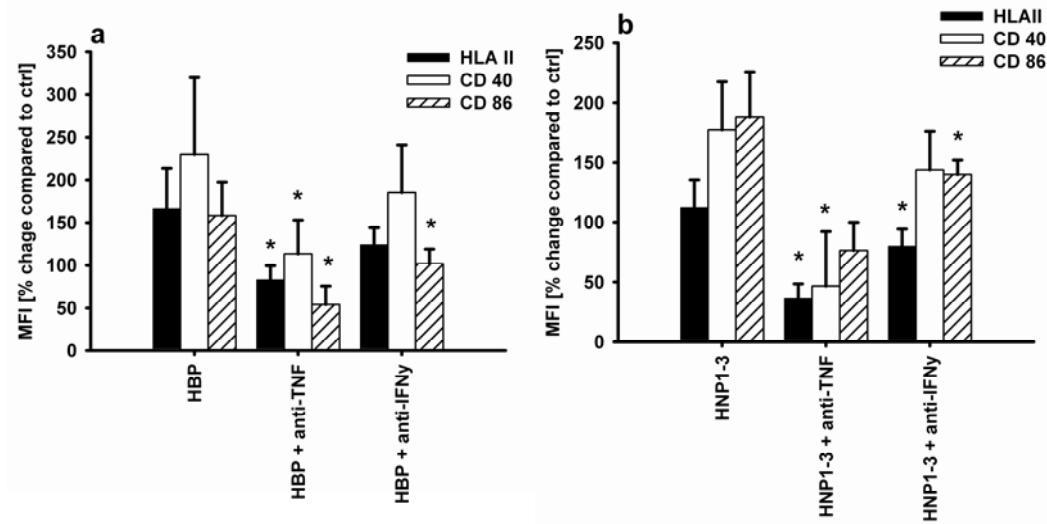


Table S1: Quantification of PMN granule proteins MPO and MMP-9 in the PMN secretion and the peritoneal lavage fluid. PMN secretion was obtained by antibody cross-linking of CD18. The peritoneum of mice with intact WBC was lavaged four days after thioglycollate injection and the number of PMN was quantified by FACS. The MPO and MMP-9 activity was analysed in either specimen by spectrophotometry using enzyme specific substrates. Values are given as mean of three independent experiments.

	PMN-sec			Peritoneal lavage fluid		
	U/ml	PMN/ml	U/10 ⁶ PMN	U/ml	PMN/ml	U/10 ⁶ PMN
MPO (primary granule)	1.3	10x10 ⁶	0.13	0.55	2.1x10 ⁶	0.26
MMP-9 (secondary and tertiary granule)	1.7	10x10 ⁶	0.17	0.43	2.1x10 ⁶	0.2

Table S2: Effect of β_2 -integrin antibodies and signaling inhibitors on HBP-mediated expression of CD32 and CD64, release of TNF and IFN γ , and enhanced phagocytosis. Human macrophages were treated with HBP and the expression of CD32 and CD64 was assessed in the presence or absence of various signaling inhibitors. Data are expressed in percent of ctrl. Similarly, concentrations of TNF (in ng/ml) and IFN (in pg/ml) were analysed by ELISA after treatment of human macrophages with HBP in the presence of various inhibitors. Data for phagocytic capacity of human macrophages in response to different treatment are displayed as number of phagocytosed *S. aureus* per cell.

<i>Treatment</i>	<i>Target</i>	<i>Expression of</i>		<i>Release of</i>		<i>Phagocytosis</i>
		<i>CD32</i>	<i>CD64</i>	<i>TNF</i>	<i>IFNγ</i>	
Ctrl		100	100	17,5 \pm 6,45	75 \pm 28,86	1,83 \pm 0,56
HBP		362 \pm 54	305 \pm 62	303,3 \pm 17,56	130 \pm 34,64	5,72 \pm 0,54
HBP + emetine	Protein synthesis	127 \pm 9	170 \pm 72	72,5 \pm 34,27	55 \pm 78,52	1,95 \pm 0,62
HBP + anisomycin	Protein synthesis	124 \pm 16	970 \pm 68			
HBP + IB4	CD18	103 \pm 42	160 \pm 76	13,75 \pm 17,97	90 \pm 35,59	2,15 \pm 0,62
HBP + PTx	Gi protein	313 \pm 39	264 \pm 66	326,25 \pm 30,92	125 \pm 113,87	5,05 \pm 0,41
HBP + genistein	Tyrosine kinases	85 \pm 41	152 \pm 84	113,25 \pm 49,89	77,5 \pm 6,96	
HBP + wortmannin	Phosphatidylinositol-3kinase	207 \pm 115				
HBP + GF109203	Protein Kinase C	269 \pm 27	255 \pm 62			
HBP + PD98059	MAPK pathway	102 \pm 35	177 \pm 37	103,01 \pm 38,47	59,4 \pm 7,91	
HBP + U73122	Phospholipase C	335 \pm 122	389 \pm 137	257,51 \pm 43,77	157 \pm 12,16	
HBP + Herbimycin	Protein tyrosine kinase	90 \pm 37	205 \pm 114			

Table S3: Effect of β_2 -integrin antibodies and signaling inhibitors on HNP1-3-mediated expression of CD32 and CD64, release of TNF and IFN γ , and enhanced phagocytosis. Human macrophages were treated with HNP1-3 and the expression of CD32 and CD64 was assessed in the presence or absence of various signaling inhibitors. Data are expressed in percent of ctrl. Similarly, concentrations of TNF (in ng/ml) and IFN (in pg/ml) were analysed by ELISA after treatment of human macrophages with HNP1-3 in the presence of various inhibitors. Data for phagocytic capacity of human macrophages in response to different treatment are displayed as number of phagocytosed *S. aureus* per cell.

<i>Treatment</i>	<i>Target</i>	<i>Expression of</i>		<i>Release of</i>		<i>Phagocytosis</i>
		<i>CD32</i>	<i>CD64</i>	<i>TNF</i>	<i>IFNγ</i>	
Ctrl		100	100	17,5 \pm 6,45	75 \pm 28,86	1,83 \pm 0,56
HNP1-3		295 \pm 56	225 \pm 19	180 \pm 36,51	140 \pm 40,82	4,21 \pm 1,19
HNP1-3 + emetine	Protein synthesis	52 \pm 47	45 \pm 39	93,75 \pm 34,24	67,5 \pm 73,2	1,32 \pm 0,076
HNP1-3 + anisomycin	Protein synthesis	49 \pm 45	101 \pm 36			
HNP1-3 + IB4	CD18	298 \pm 46	247 \pm 15	172,5 \pm 39,26	87,5 \pm 9,57	4,05 \pm 0,53
HNP1-3 + PTx	Gi protein	325 \pm 50	231 \pm 14	291,25 \pm 17,96	110 \pm 34,64	3,95 \pm 0,78
HNP1-3 + genistein	Tyrosine kinases	180 \pm 56	168 \pm 43	251,25 \pm 13,76	85 \pm 12,91	
HNP1-3 + wortmannin	Phosphatidylinositol-3kinase	241 \pm 82	183 \pm 50			
HNP1-3 + GF109203	Protein Kinase C	225 \pm 21	209 \pm 34			
HNP1-3 + PD98059	MAPK pathway	202 \pm 85	171 \pm 32	205 \pm 12,05	137,05 \pm 41,75	
HNP1-3 + U73122	Phospholipase C	302 \pm 27	247 \pm 50	155,3 \pm 13,88	115,9 \pm 12,59	
HNP1-3 + Herbimycin	Protein tyrosine kinase	220 \pm 17	225 \pm 22			

Table S4: Accession number and sequences of siRNA as provided by producer.

Gene name	Accession number	Sequence
FCGR1	NM_000566	<i>Sense1:</i> GAGAAGACUCUGGGUUAUAUU <i>Antisense1:</i> 5'- PUAUAACCCAGAGUCUUCUCUU
		<i>Sense2:</i> GGAACACACAUCCUCUGAAUAUU <i>Antisense2:</i> 5'- PUAUUCAGAGGAUGUGUCCUU
		<i>Sense3:</i> GGAAAUGUCCUUAAGCGCAUU <i>Antisense3:</i> 5'- PUGCGCUUAAGGACAUUCCUU
		<i>Sense4:</i> AAACAAAGUUGCUCUUGCAUU <i>Antisense4:</i> 5'- PUGCAAGAGCAACUUUGUUUUU