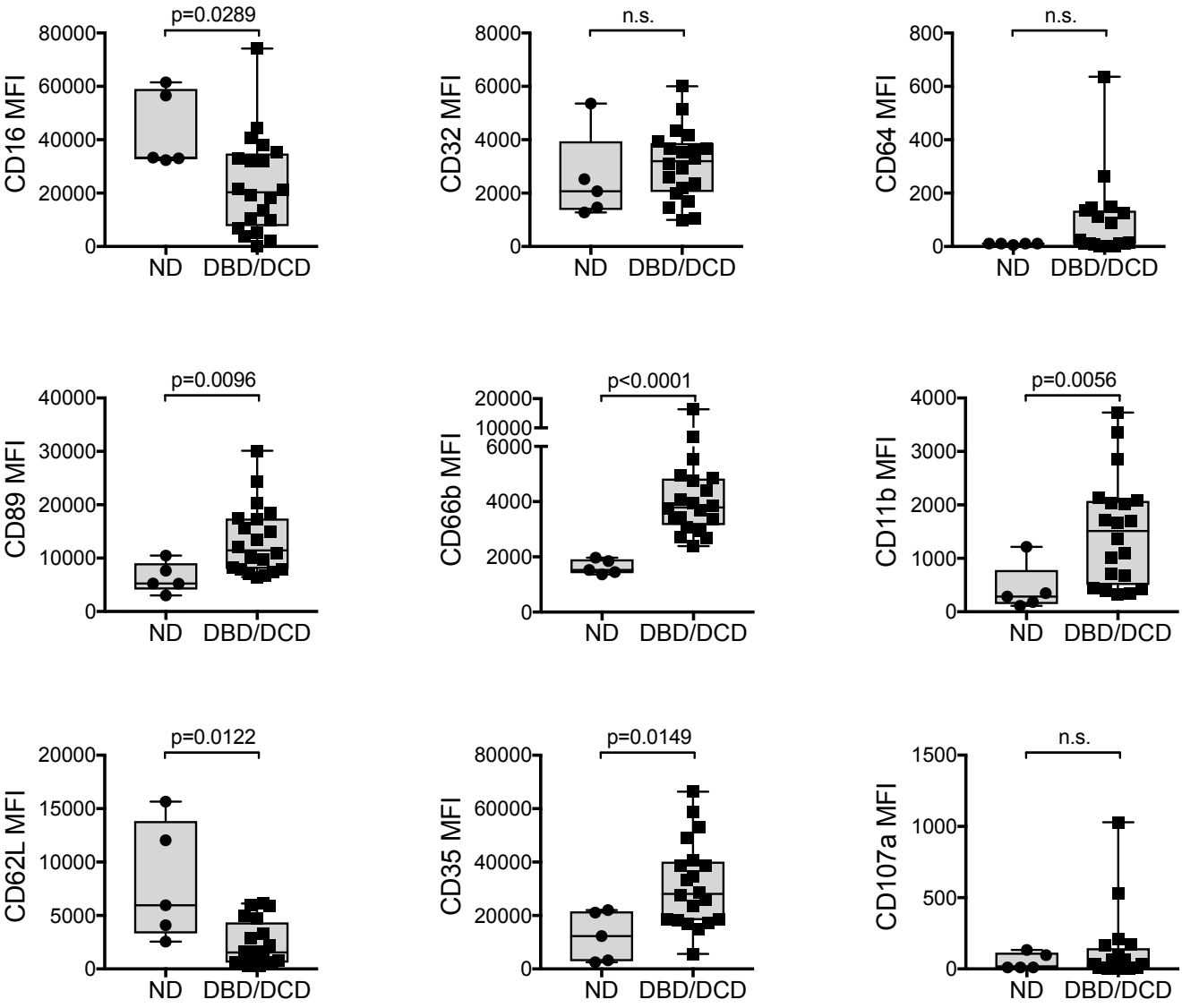
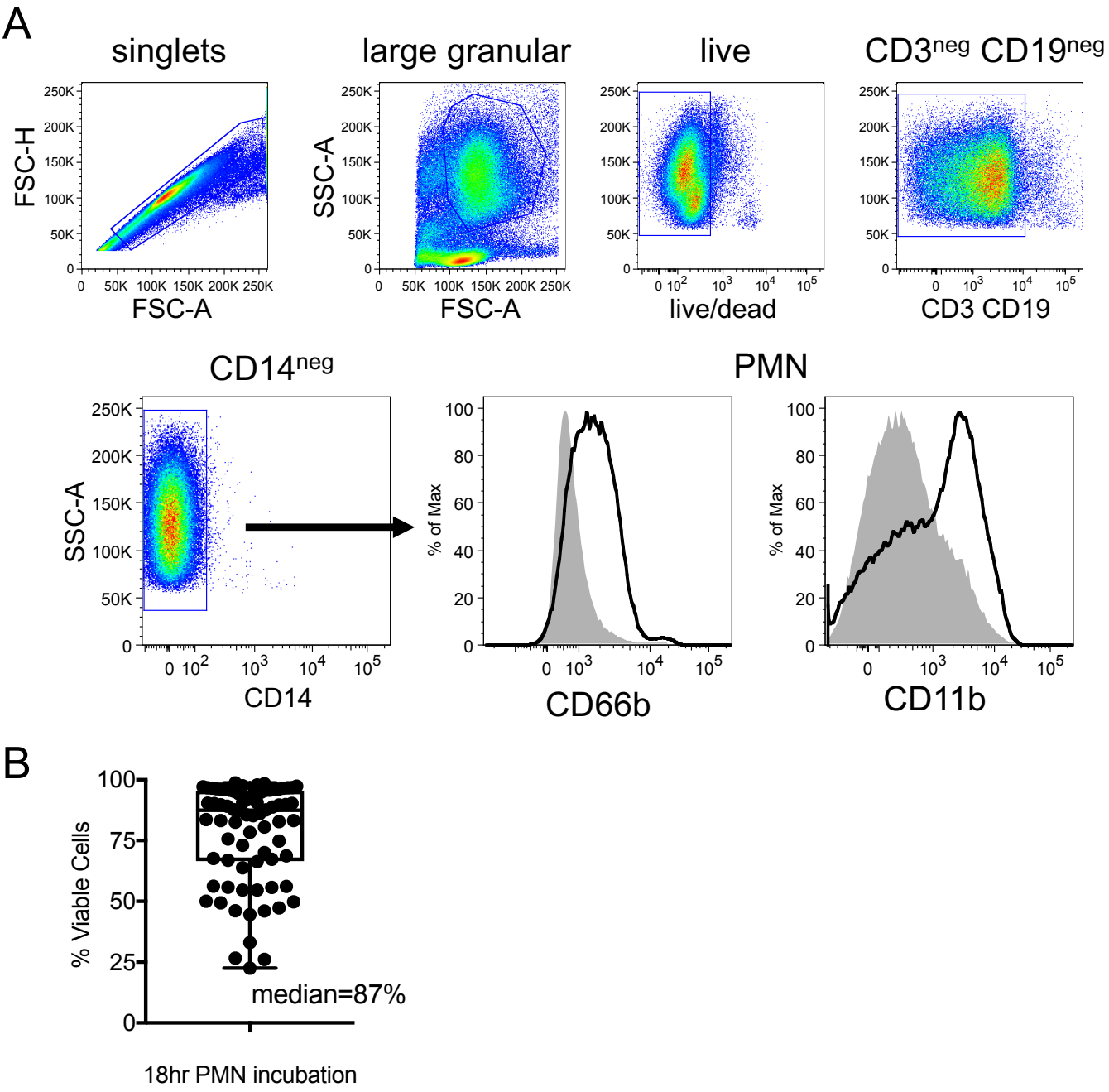
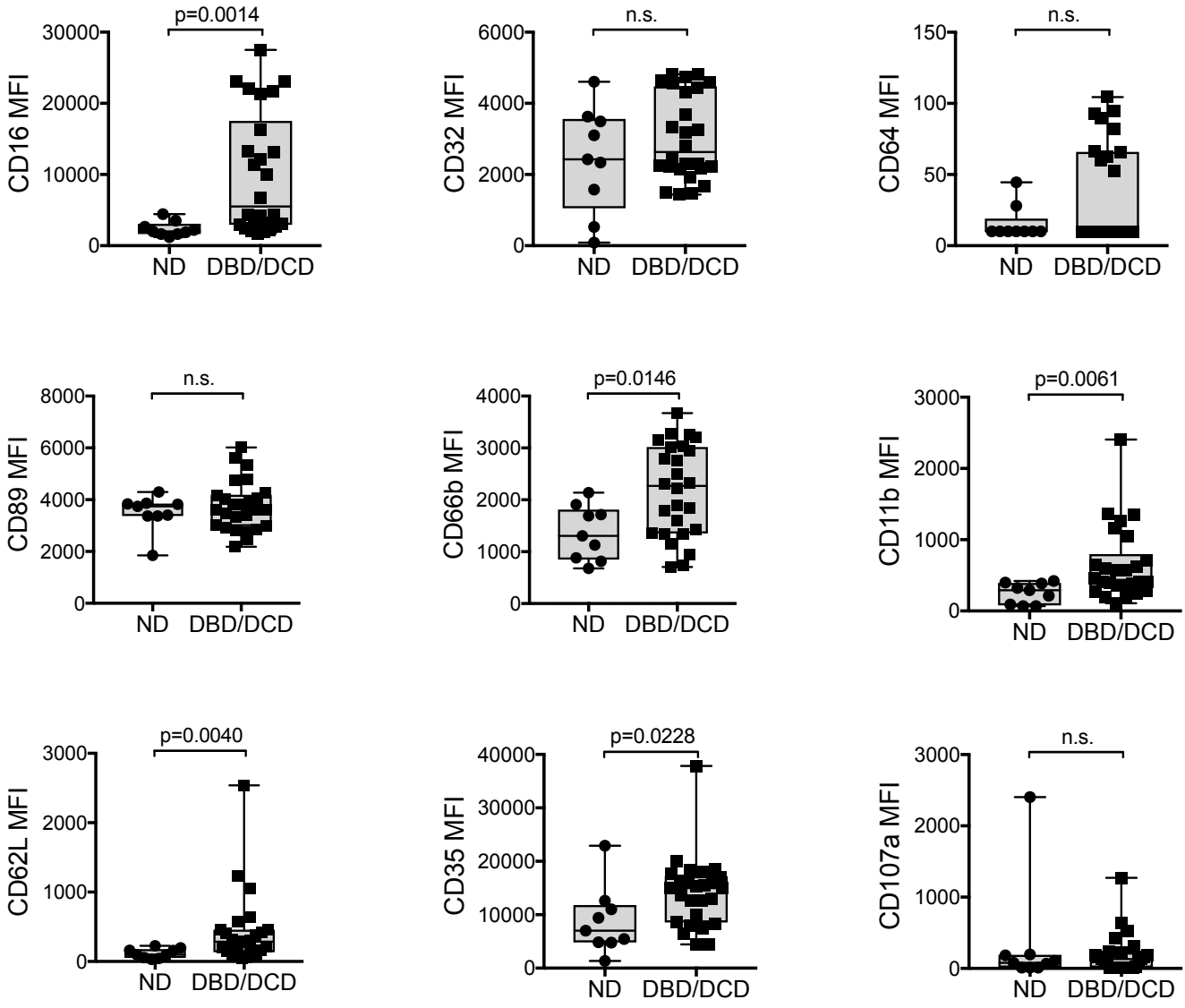


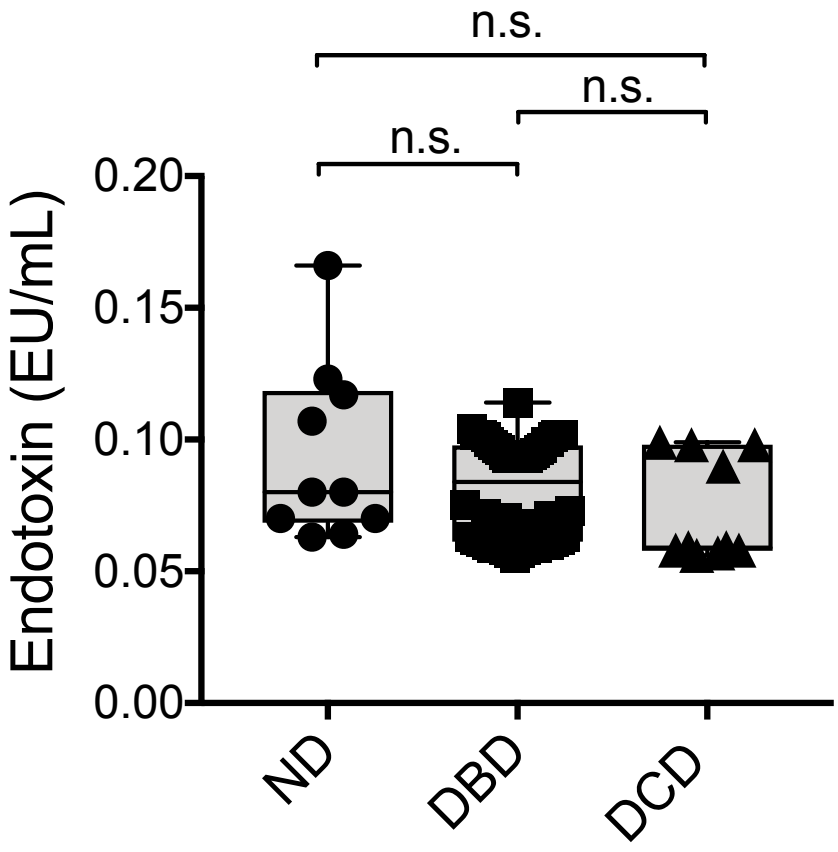
Supplemental Figure 1. Median fluorescent intensities (MFIs) of cell-surface antigens on PMN in whole blood of deceased organ donors and healthy normal donors (ND). MFIs of the indicated cell surface antigens (y-axis labels) on the surface of PMN from deceased organ donors (DBD/DCD, n=20) and healthy living ND (n=5) were determined by flow cytometry analysis after staining of whole blood. The gray boxes represent the interquartile range, the line represents the median, and whiskers indicate the range of observed responses. Comparisons were performed using a Mann-Whitney test. A p-value <0.05 was considered significant; n.s., not significant.



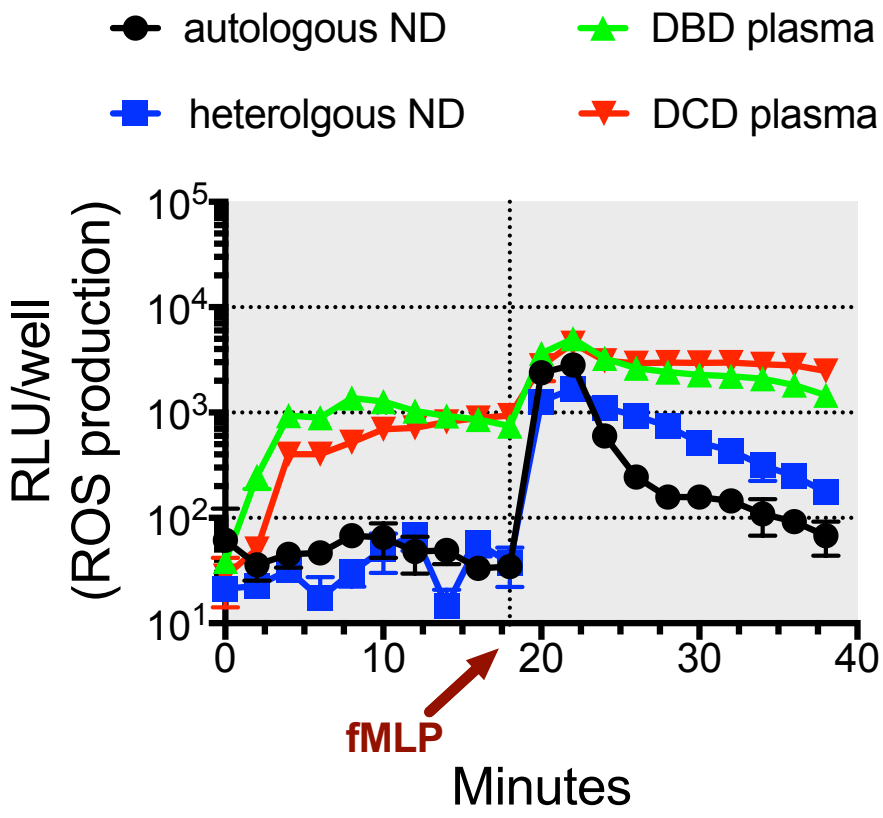
Supplemental Figure 2. Gating strategy and viability of healthy PMN stimulated ex-vivo with deceased donor plasma samples. A) Gating strategy used for analysis of healthy donor PMNs after overnight incubation with deceased donor plasma. Histograms represent example comparisons of MFIs for selected activation markers on the surface of healthy donor PMNs after overnight incubation with autologous plasma (gray filled histograms) or plasma from a deceased donor (black line). B) Viability of PMN stimulated ex-vivo with deceased donor plasma. Each data point represents the viability measured by flow cytometry for each tested plasma samples and health donor PMN combination (minimum of 2 independent experiments per plasma sample, total n=76). The gray boxes represent the interquartile range, the line represents the median, and whiskers indicate the range of observed responses.



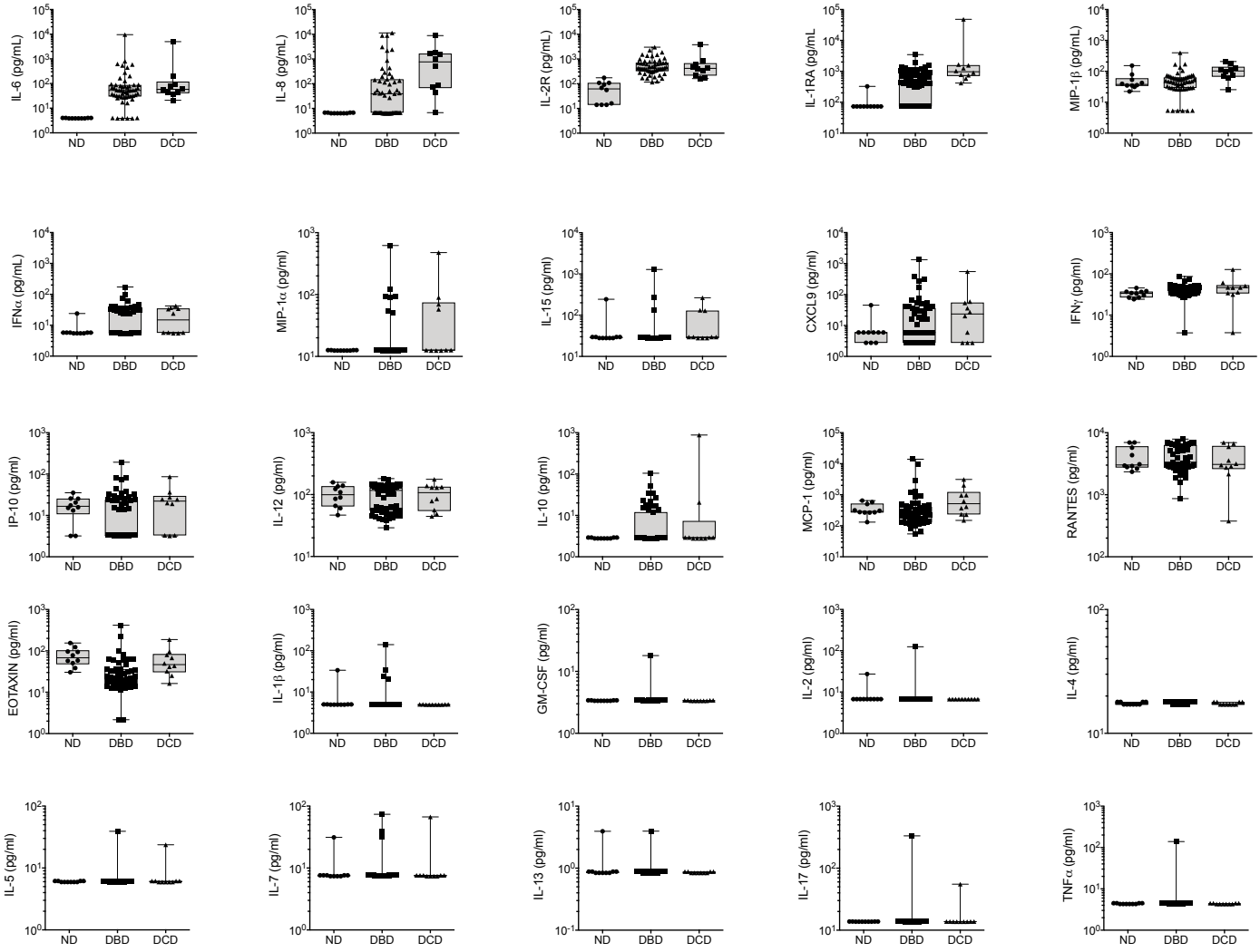
Supplemental Figure 3. Median fluorescent intensities (MFIs) of cell-surface antigens on PMN incubated with plasma deceased organ donors or healthy normal donors (ND) ex vivo. MFIs of the indicated cell surface antigens (y-axis labels) on the surface of PMN incubated overnight with plasma from deceased organ donors (DBD/DCD, n=26) or healthy living ND (n=9) were determined by flow cytometry analysis. The gray boxes represent the interquartile range, the line represents the median, and whiskers indicate the range of observed responses. Comparisons were performed using a Mann-Whitney test. A p-value <0.05 was considered significant; n.s., not significant.



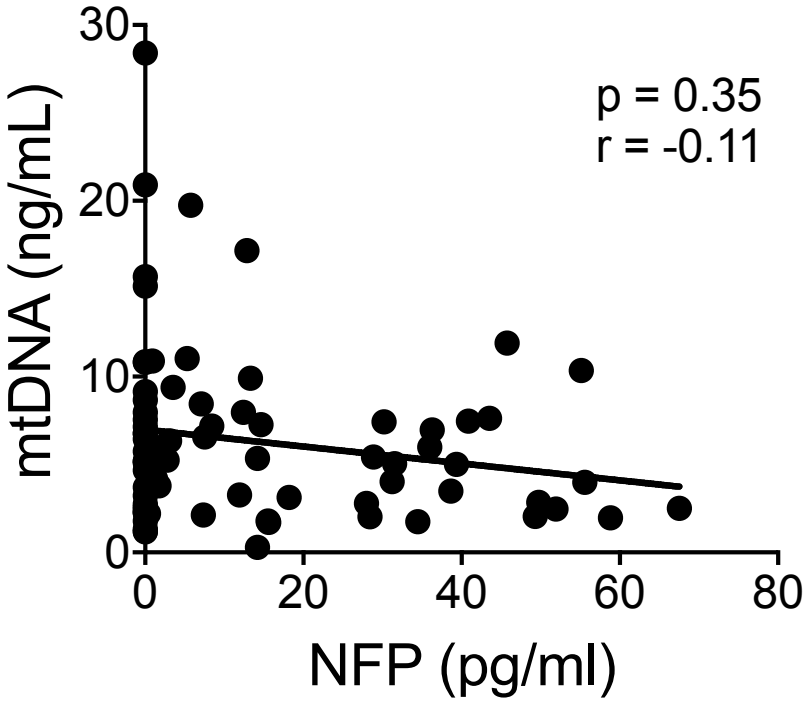
Supplemental Figure 4. Absence of endotoxin contamination in deceased donor serum. Endotoxin (lipopolysaccharide, LPS) in normal donor (ND), donation after brain death (DBD), and donation after cardiac death (DCD) donor serum samples was quantified by Limulus assay. The gray boxes represent the interquartile range, the line represents the median, and whiskers indicate the range of observed responses. Comparisons were performed using a Mann-Whitney test. A p-value <0.05 was considered significant; n.s., not significant.



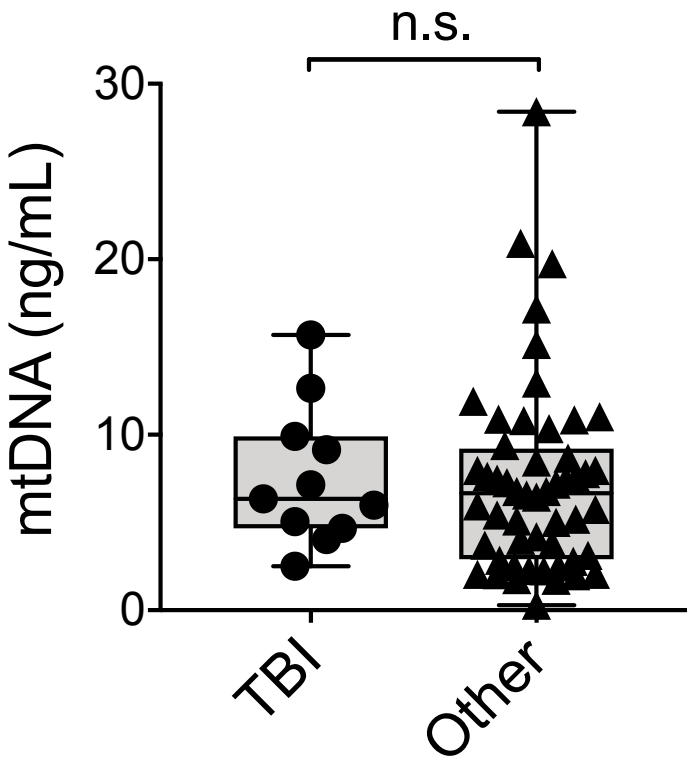
Supplemental Figure 5. Plasma from deceased organ donors is able to induce production of ROS by purified PMN. Lymphocytes were removed from whole peripheral blood collected from a heathy normal donors (ND) by density dependent centrifugation over Ficoll (Ficoll Paque Plus, GE Healthcare, Marlborough, MA) . The red blood cell and granulocyte containing fraction was recovered and red blood cells were depleted with HetaSep according to the manufacturers recommended protocol (STEMCELL Technologies Inc., Vancouver, BC). Granulocytes were recovered and PMN purity was determined by flow cytometry (83% of cells were live, $CD3^{neg}$, $CD19^{neg}$, $CD14^{neg}$ $CD66b^{pos}$). The cells were then stimulated with ND plasma (autologous, black line; heterologous, blue line) or plasma from deceased donors (donation after brain death (DBD), green line; and donation after cardiac death (DCD), purple line) and ROS production was measured by chemiluminescent detection over time. Stimulation with N-formylmethionyl-leucyl-phenylalanine (fMLP) is indicated. Data represents the mean and standard deviation of RLU resulting from oxidation of luminol by ROS measured in triplicate.



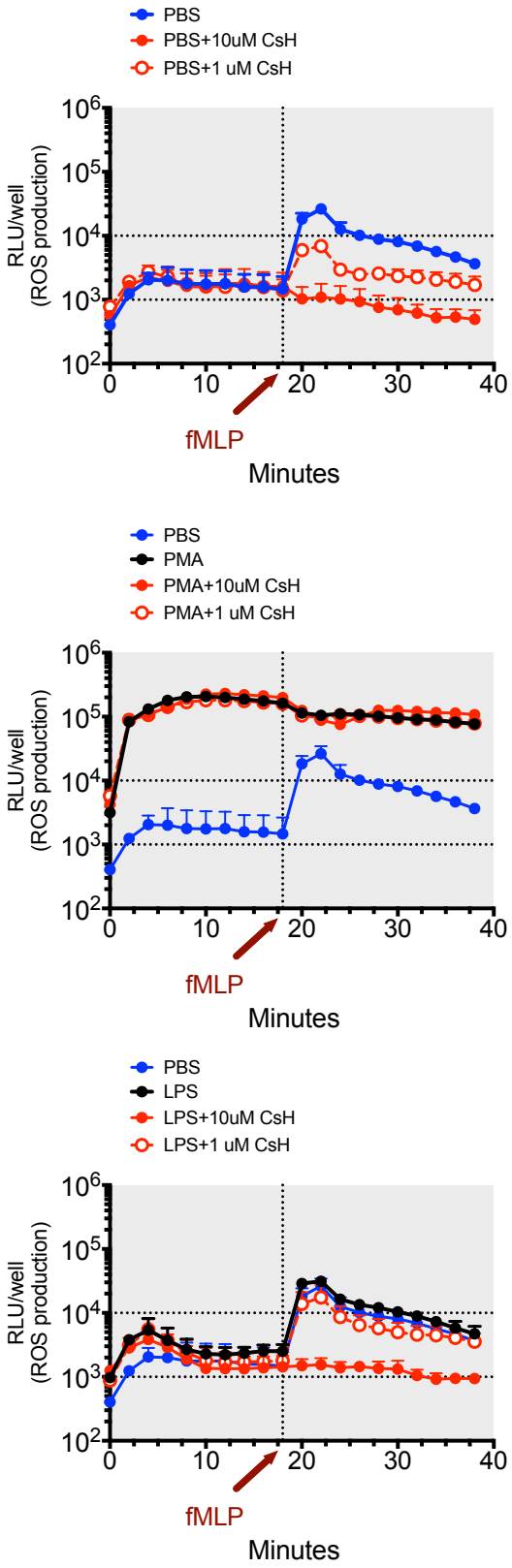
Supplemental Figure 6. Circulating levels of cytokines and chemokines in deceased and healthy living donors. Circulating levels of chemokines and cytokines in serum from healthy living normal donors (ND, n=10), and deceased donors (donation after brain death, DBD, n=55; donation after cardiac death, DCD, n=10) were evaluated by multiplex cytokine assay. The gray boxes represent the interquartile range, the line represents the median, and whiskers indicate the range of observed responses.



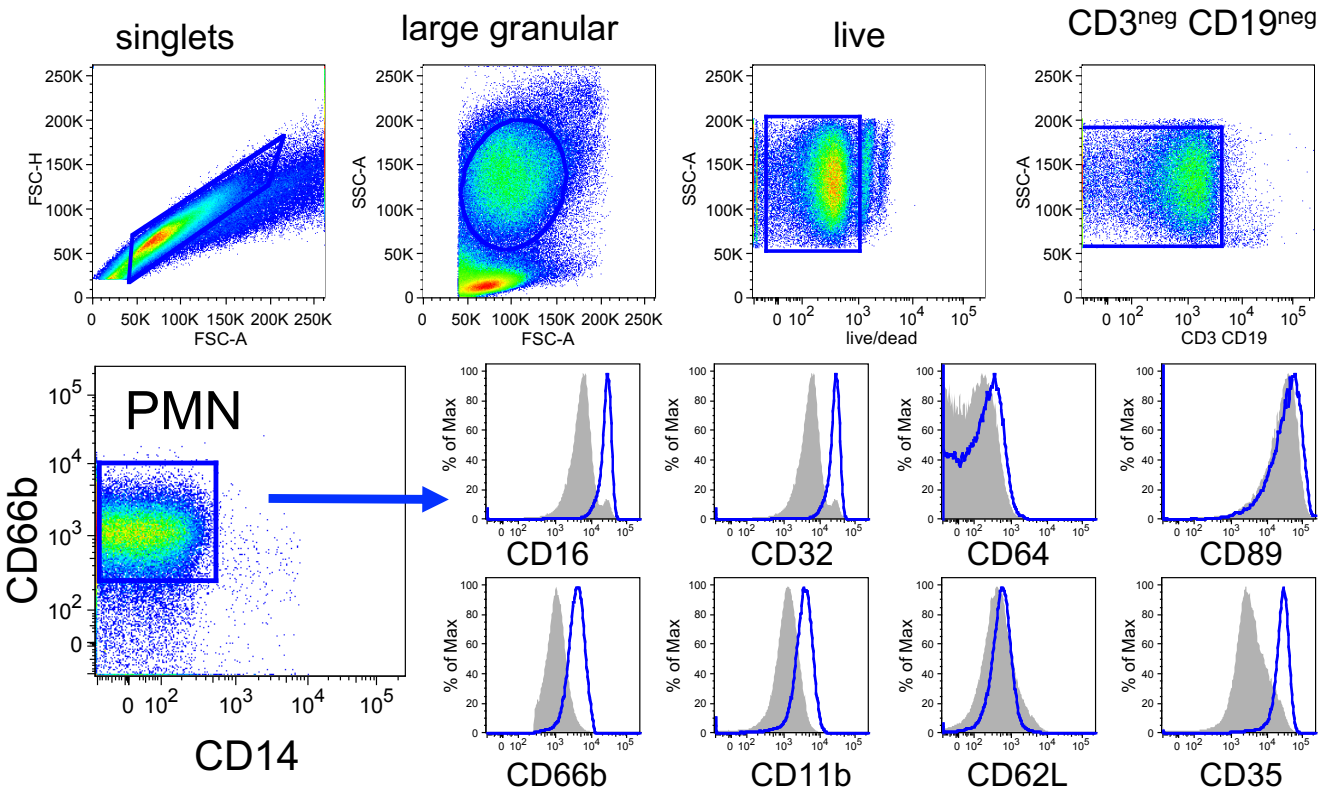
Supplemental Figure 7. Comparison of donor plasma mtDNA and NFP levels. Donor plasma samples tested for both NFP by ELISA and mtDNA by qPCR are plotted (n=55). Spearman's rank correlation test was used to examine for correlations between the variables.



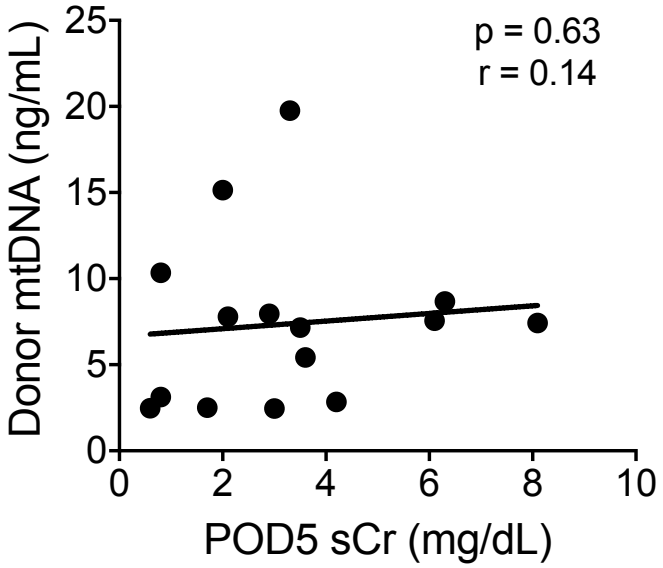
Supplemental Figure 8. Plasma levels of mtDNA were not associated with traumatic brain injury (TBI). Levels of mtDNA was compared between patients that died from TBI (n=11) or by other mechanism (n=52) using a Mann-Whitney test. The gray boxes represent the interquartile range, the line represents the median, and whiskers indicate the range of observed responses. A p-value <0.05 was considered significant; n.s., not significant.



Supplemental Figure 9. Inhibition of ROS release by cyclosporin H (CsH) is specific for N-formyl peptide signaling. Control ROS release experiments were performed using leukocytes from 2 healthy living normal donors (NDs) to determine if CsH treatment impacted ROS production after incubation with A) PBS, B) PMA, or C) LPS. N-formylmethionyl-leucyl-phenylalanine (fMLP) was added to each assay at the time indicated. Data are mean and error from 2 independent experiments.



Supplemental Figure 10. Immunoprofile of PMNs from healthy donors after stimulation with N-formylmethionyl-leucyl-phenylalanine (fMLP). Whole leukocyte preparations were collected from a normal healthy donor and stimulated with autologous plasma or autologous plasma containing fMLP (16 ng/uL) for 2 hr at 37ºC, 5% CO_2 and then analyzed by direct immunofluorescence staining and flow cytometry. Gating strategy is shown. Histograms represent comparisons of median fluorescence intensity (MFI) of the cellular antigens indicated on the surface of PMNs incubated with autologous plasma (gray filled histograms), or PMNs incubated with fMLP-spiked autologous plasma (blue lines).



Supplemental Figure 11. Donor circulation mitochondrial DNA (mtDNA) levels do no correlate with serum creatinine (sCr) levels of kidney allograft recipients in the early post-operative period. Donor mtDNA quantified by qPCR compared with recipient sCr at 5 days post-transplantation (n=15). Spearman's rank correlation test was used to examine for correlations between the variables.

Supplemental Table 1. Antibody panel used for flow cytometry analysis of PMNs.

Cell Surface Antigen	Fluorophore	Vendor	Clone	Catalog number	Final dilution[A]
CD3	PE-TR	Thermo Fisher Scientific, Waltham, MA	S4.1/7D6	MHCD0317	1:20
CD19	PE-TR	Thermo Fisher Scientific	SJ25-C1	MHCD1917	1:20
CD14	APC-Cy7	BD Biosciences, San Jose, CA	MφP9	557831	1:80
CD16	PacBlue	BD Biosciences	3G8	558122	1:80
CD32	APC	Thermo Fisher Scientific	6C4	17-0329-42	1:20
CD64	Alexa Fluor 700	BD Biosciences	10.1	561188	1:40
CD89	PE	BD Biosciences	A59	555686	1:10
CD66b	PerCP-Cy5.5	BD Biosciences	G10F5	562254	1:20
CD11b	PE-Cy7	Biolegend, San Diego, CA	CBRM1/5	301412	1:160
CD62L	BV650	BD Biosciences	DREG56	563808	1:40
CD35	BB515	BD Biosciences	E11	565330	1:40
CD107a	PE-Cy5	BD Biosciences	H4A3	555802	1:80

[A]Final dilution determined by titration of each antibody for optimal detection of target antigen.