Supplementary Material

Title: Angiotensin II Enhances Bacterial Clearance via Myeloid Signaling in a Murine Sepsis Model

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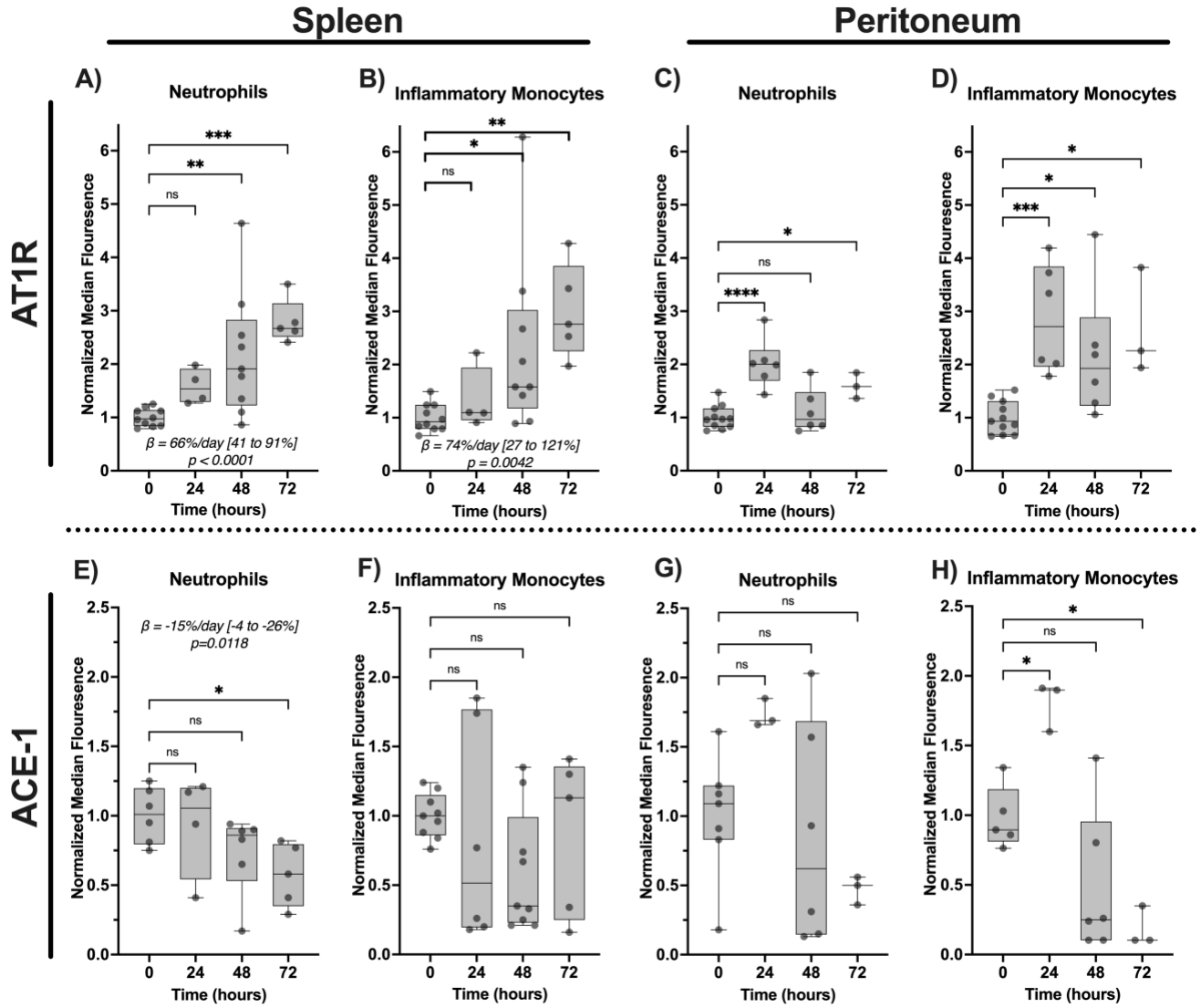
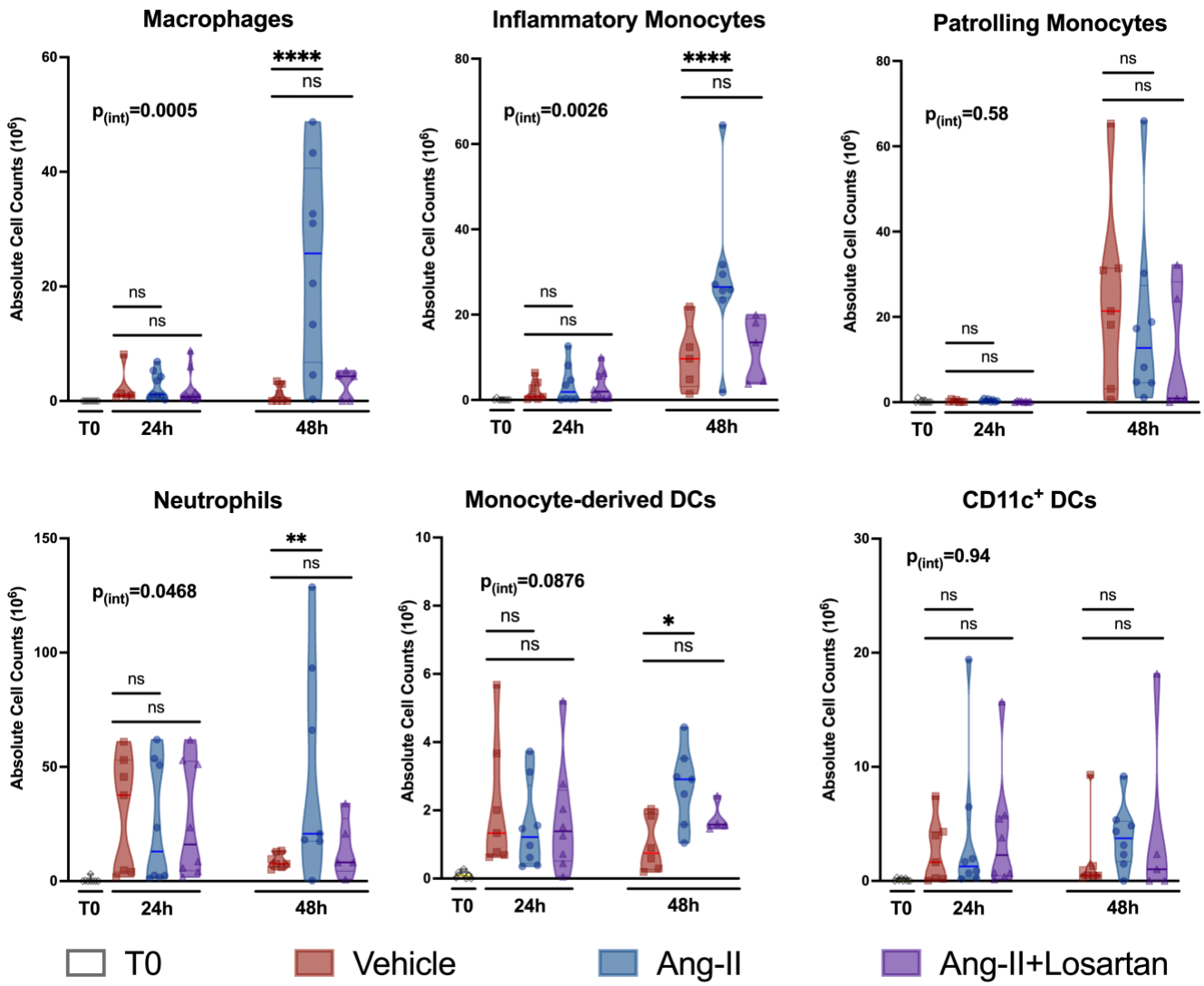


eFIGURE 1 – Splenic and Peritoneal Innate Cells Alter Surface AT1R and ACE-1 Expression After CLP

Legend:

Displays AT1R (top row) and ACE-1 (bottom row) expressed on the surface of splenic (left columns) and peritoneal (right columns) neutrophils ($CD11b^{+}/Ly6G^{+}$) and inflammatory monocytes ($CD11b^{+}/Ly6G^{-}/MHCII^{-}/Ly6C^{Hi}$). The x axis displays time from T0 and the y-axis displays the normalized median fluorescence intensity. Results were normalized across experiments by standardizing each measurement to the mean T0 group fluorescence intensity from that experiment. Dots represents individual mice. Horizontal bars represent group median, boxes interquartile range, error bars range. Asterisks indicate p values for the bracketed comparison as follows: ns – $p > 0.05$, * – $p<0.05$, ** – $p<0.005$, *** – $p<0.0005$, **** – $p <0.0001$. Where a linear effect of time was calculated, the coefficient (β) and 95% confidence interval are displayed.

Abbreviations: AT1R – angiotensin-II type-1 receptor; ACE-1 – Angiotensin Converting Enzyme-1; Ly6C – Lymphocyte Antigen 6, locus C1; T0 – time zero.

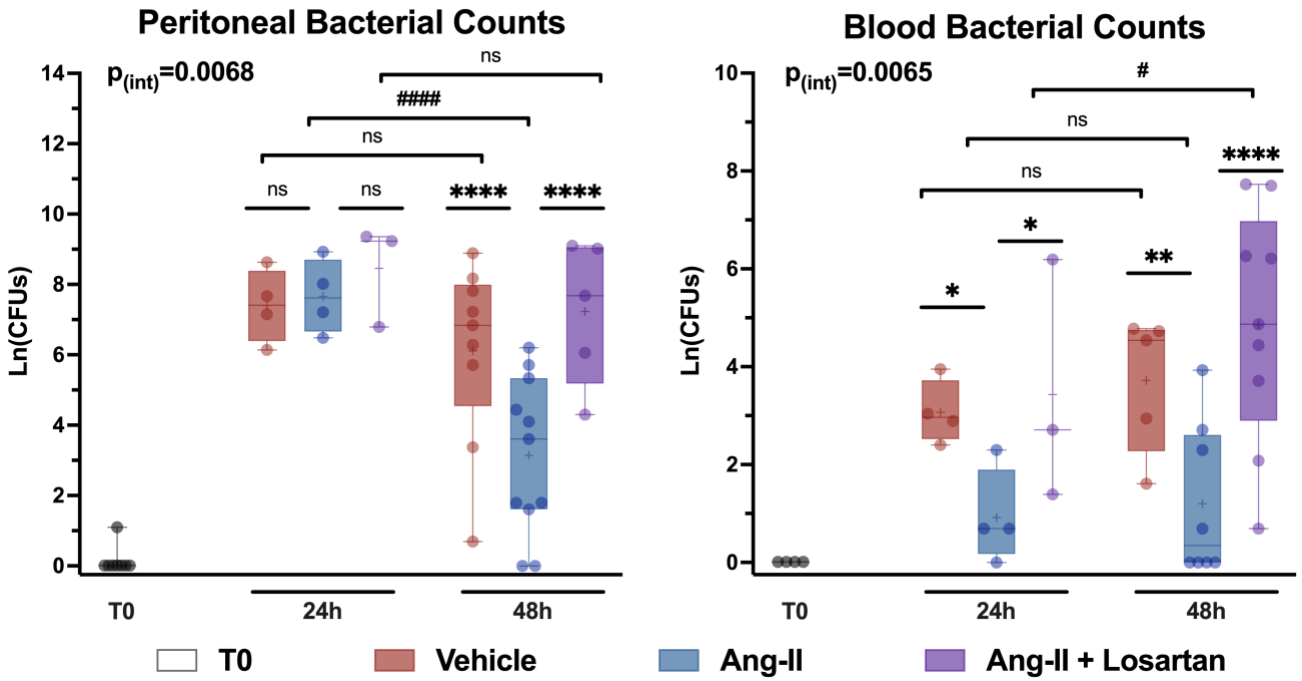


eFIGURE 2 – Angiotensin-II Treatment Increases Some Innate Cell Populations in the Peritoneum in an AT1R-Dependent Manner by 48-Hours After CLP

Legend:

Violin plots of peritoneal cell counts at T0 and at 24h and 48h post-CLP. The y-axis displays cell counts. Dots represents individual mice. Violin height reflects the full range of the data while width reflects that distribution density at that y-value quantity. The p value for the overall interaction effect of time with treatment group, reflecting whether differences between time points differed by treatment group, is displayed below each graph. Asterisks above horizontal bars above boxes indicate the (multiple comparison adjusted) p-values vs. the control (vehicle) group at the indicated time point as follows: ns – p >0.05, * – p<0.05, ** – p<0.005, *** – p<0.0005, **** – p <0.0001. Cells were identified as follows: Neutrophils ($CD11b^+$ / $Ly6G^+$), Macrophages ($CD11b^+$ / $Ly6G^-$ /$MHCII^-$ / $CD64^+$ / $Ly6C^+$), Inflammatory Monocytes ($CD11b^+$ / $Ly6G^-$ / $MHCII^-$ / $CD64^-$ / $Ly6C^{Hi}$), Patrolling Monocytes ($CD11b^+$ / $Ly6G^-$ / $MHCII^-$ / $CD64^-$ / $Ly6C^{Lo}$), Monocyte-derived DCs ($CD11b^+$ / $Ly6G^-$ / $MHCI^+$ / $MHCII^+$), and CD11c+ DCs ($CD11b^+$ / $CD11c^+$).

Abbreviations: DCs – dendritic cells; T0 – time zero.

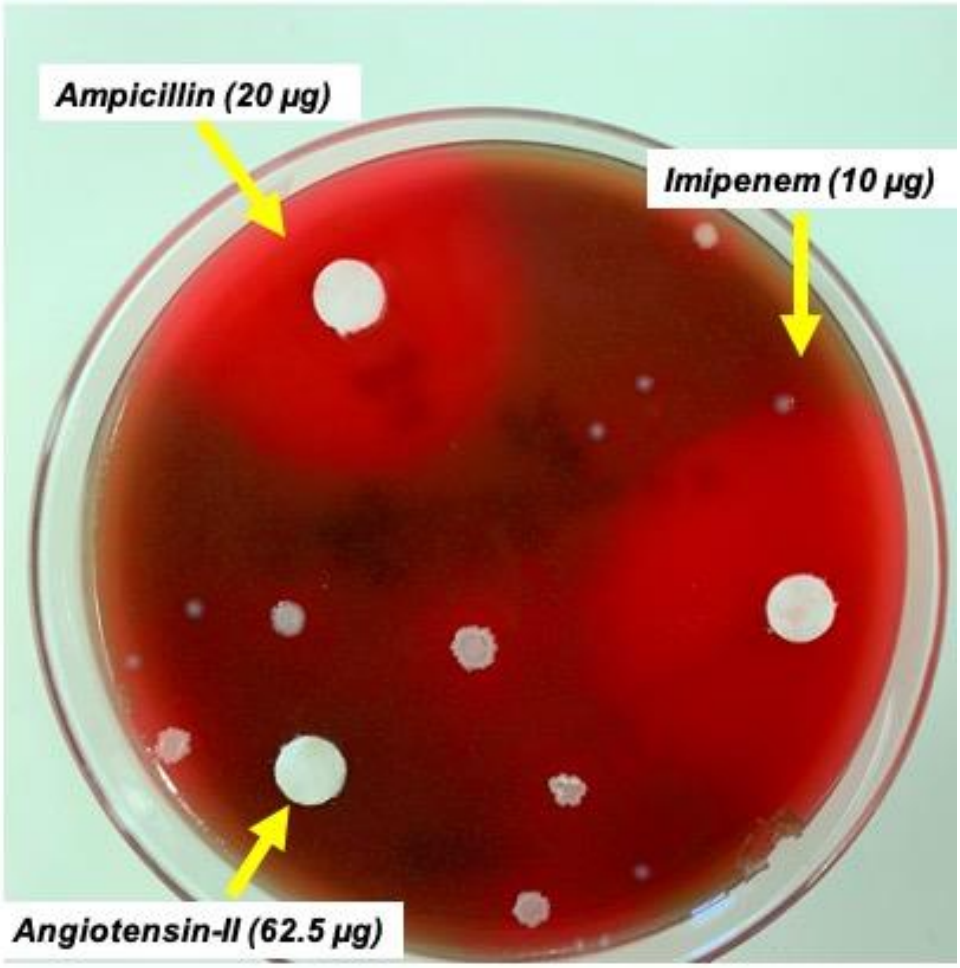


eFIGURE 3 – Peritoneal and Blood Bacterial Counts at 24 vs. 48-hours post-CLP by Treatment

Legend:

Bacterial counts in treatment groups at 24 and 48-hours post-CLP. The y-axis displays bacterial counts on a natural log scale. Dots represents individual mice. Boxes height represents interquartile range, error bars range. Asterisks over black bars indicate the (multiple comparison adjusted) p-values vs. the Ang-II group at the indicated time point as follows: ns – p >0.05, * – p<0.05, ** – p<0.005, *** – p<0.0005, **** – p <0.0001. Hash symbols over down brackets indicate the (multiple comparison adjusted) p-values for 24 vs. 48-hours post-CLP within the indicated treatment group as follows: ns – p >0.05, # – p<0.05, ## – p<0.005, ### – p<0.0005, #### – p <0.0001. The p(int) indicates the overall interaction p-value for time and treatment group.

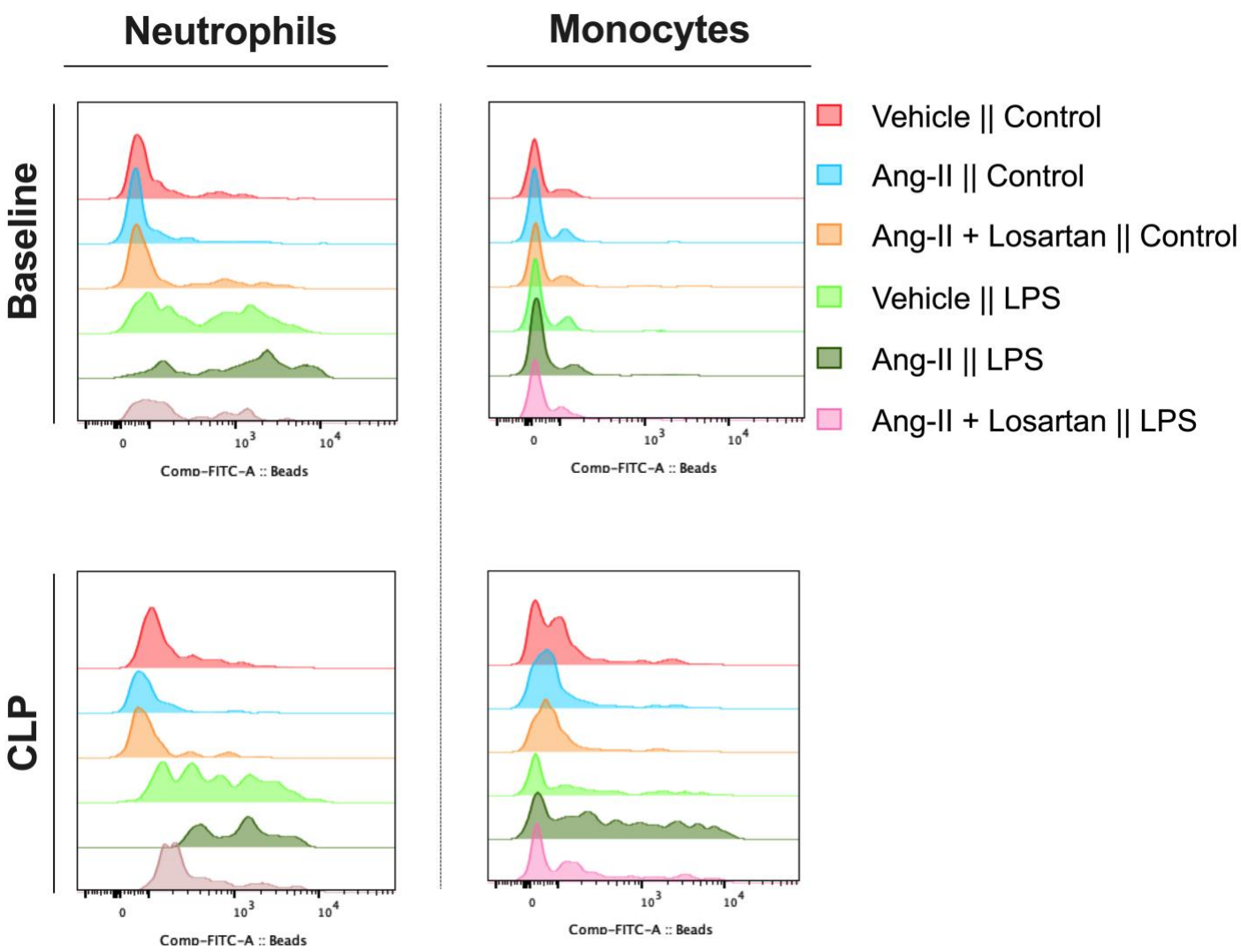
Abbreviations: Ang-II – angiotensin-II; Ln(CFUs) – Natural log of Colony Forming Units; T0 – time-zero.



eFIGURE 4 – Representative Kirby Bauer Disk Diffusion Plate Demonstrating No Direct Antibacterial Effect of Angiotensin-II *in vitro*

Legend:

Representative image of culture plate flooded with cecal aspirate and incubated overnight. The light red area surrounding the imipenem and ampicillin disks indicates inhibition of bacterial growth in contrast to the angiotensin-II disk, around which a bacterial lawn formed unimpeded.

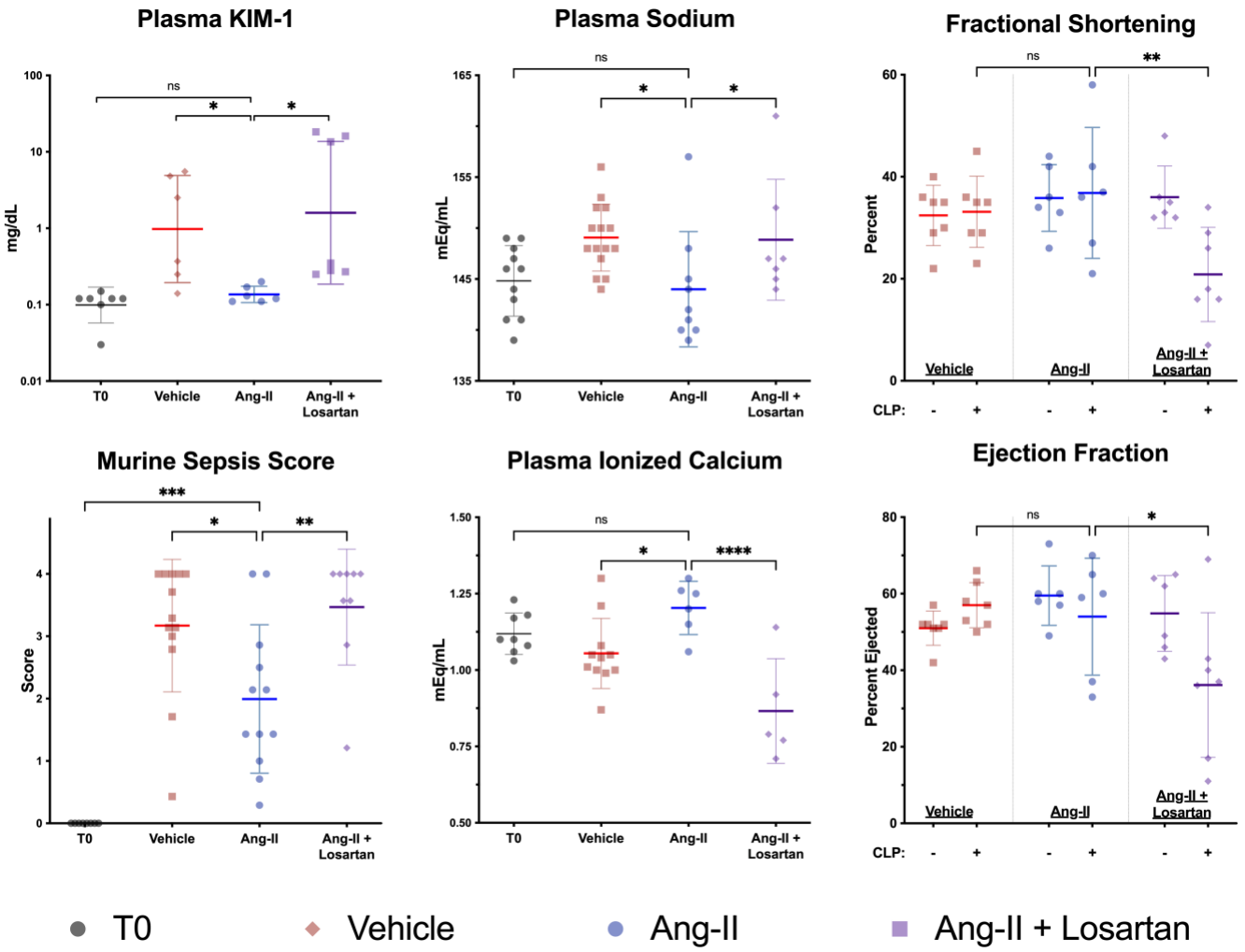


eFIGURE 5 – Angiotensin-II Increases Neutrophil and Monocyte *ex vivo* Phagocytosis After CLP

Legend:

Displays representative frequency distributions of fluorescent signal after ex vivo incubation with or without LPS stimulation. The x-axis shows the fluorescence intensity (proportional to the number of phagocytosed beads), and the y-axis shows the frequency of cells with the indicated fluorescence intensity. Results are shown for neutrophils in the left column and monocytes on the right. The top shows cells isolated from unoperated mice and the bottom shows cells obtained post-CLP. Colors indicate the treatment and LPS conditions as indicated by the legend.

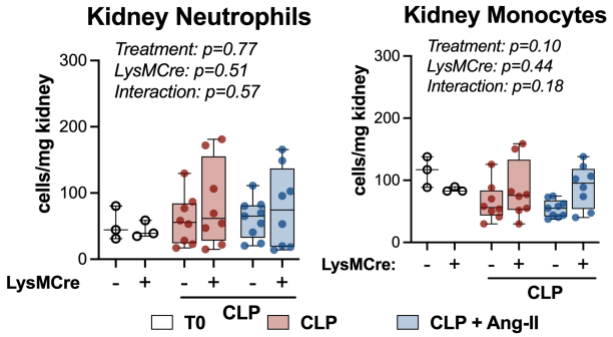
Abbreviations: CLP – cecal ligation and puncture; Ang-II – angiotensin-II; T0 – time-zero; FITC – fluorescein isothiocyanate.



eFIGURE 6 – Measures of Organ Dysfunction After CLP

Measures of organ dysfunction after CLP. Dots represents individual mice. Horizontal bars represent means, error bars standard deviations. Colors denote treatment group accordingly the legend. For the echocardiographic assessments in the right column, measurements were taken pre-operatively (denoted CLP -) and post-CLP (denoted CLP +) to account for changes from baseline. Asterisks indicate the (multiple comparison adjusted) p-values vs. the Ang-II group at the indicated time point as follows: ns – p >0.05, * – p<0.05, ** – p<0.005, *** – p<0.0005, **** – p <0.0001.

Abbreviations: Ang-II – angiotensin-II; KIM-1 – Kidney Injury Molecule-1; T0 – time zero.

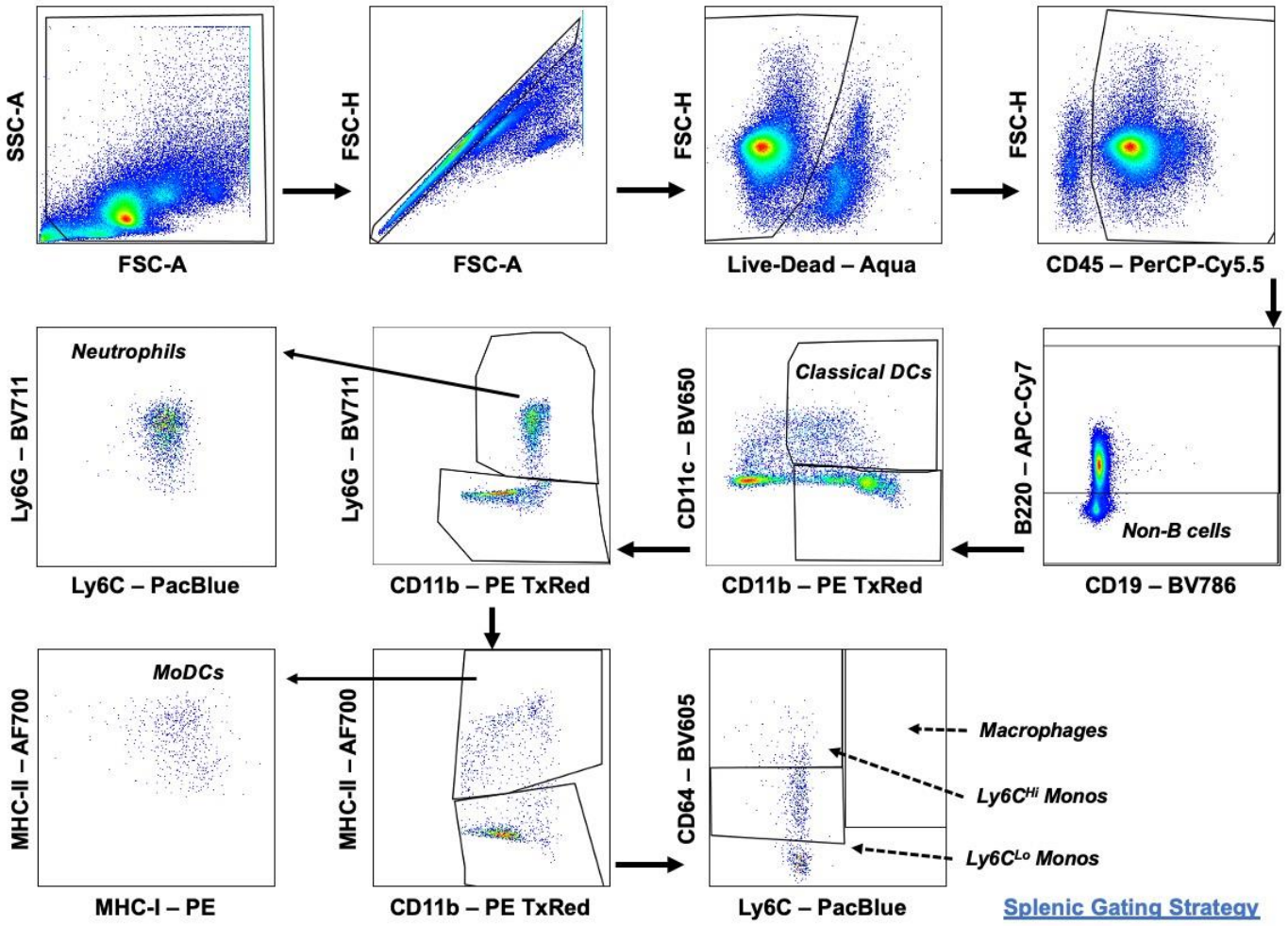


eFIGURE 7 – Angiotensin-II Treatment Does Not Affect Innate Cell Trafficking to the Kidney After CLP

Legend:

Displays the number of neutrophils and monocytes isolated from whole kidney digestion. Dots represent the average of the technical replicates from each individual mouse. Boxes height represents interquartile range, error bars the range. Genotype is indicated below the graph. Color coding indicates the treatment group.

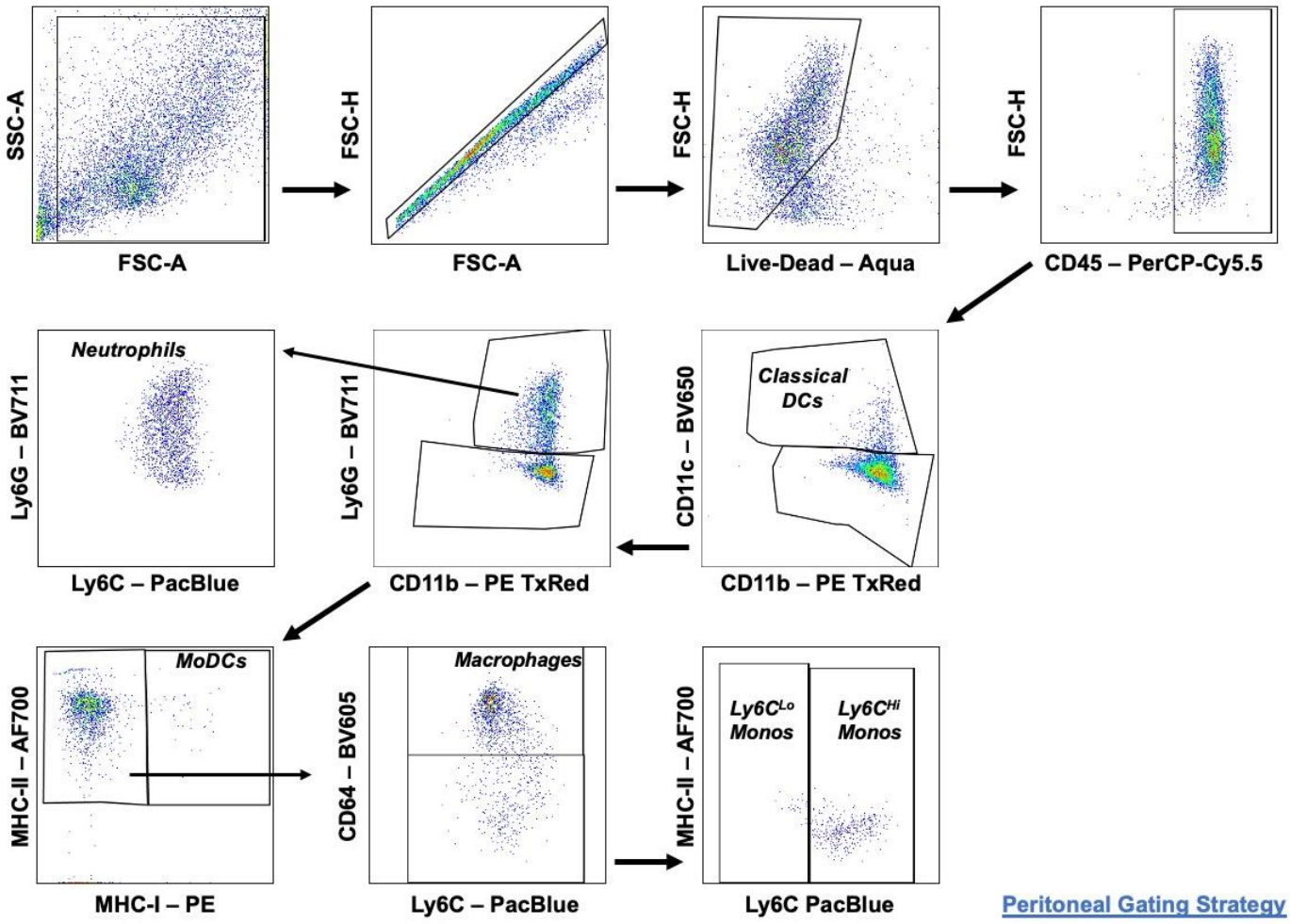
Abbreviations: CLP – cecal ligation and puncture; Ang-II – angiotensin-II; T0 – time-zero.



eFIGURE 8 – Gating Strategy For Flow Cytometric Analysis of Splenic Cells

Legend:

Displays representative sample gating for splenic cell identification. Axes labelled in the format 'cell surface marker targeted by antibody – conjugated flourophore'. Abbreviations: SSC-A – side scatter area; FSC-A – forward scatter area; FSC-H – forward scatter height; DCs – dendritic cells; Monos – monocytes; CD – cluster of differentiation; MHC – major histocompatibility complex



eFIGURE 9 – Gating Strategy For Flow Cytometric Analysis of Peritoneal Cells

Legend:

Displays representative sample gating for splenic cell identification. Axes labelled in the format 'cell surface marker targeted by antibody – conjugated flourophore'.

Abbreviations: SSC-A – side scatter area; FSC-A – forward scatter area; FSC-H – forward scatter height; DCs – dendritic cells; Monos – monocytes; CD – cluster of differentiation; MHC – major histocompatibility complex

SUPPLEMENTAL TABLES

eTable-1 Antibodies Used in Flow Cytometry Experiments

Target	Clone	Cat. No.	Company	Fluorophore	Host	Target Species
Ly6C	HK1.4	128014	Biolegend	PacBlue	Rat	Mouse
CD64	X54-5/7.1	139323	Biolegend	BV605	Mouse	Mouse
CD11c	HL3	564079	BD Bioscience	BV650	Hamster	Mouse
Ly6G	1A8	127643	Biolegend	BV711	Rat	Mouse
CD19	6D5	115543	Biolegend	BV785	Rat	Mouse
MHC-I	SF1-1.1	116608	Biolegend	PE	Mouse	Mouse
CD11b	M1/70	101256	Biolegend	PE/Dazzle594	Rat	Mouse/Human
CD45	30-F11	103132	Biolegend	PerCP/Cy5.5	Rat	Mouse
CD90	53-2.1	140310	Biolegend	PE/Cy7	Rat	Mouse
MHC-II	M5/114.15.2	107622	Biolegend	AF700	Rat	Mouse
B220	RA3-6B2	103224	Biolegend	APC-Cy7	Rat	Mouse/Human
AT1R	(Polyclonal)	AAR-011-F	Alomone	FITC	Rabbit	Mouse/Human
ACE-1	230214	FAB15131R	R&D Systems	AF647	Mouse	Mouse
Live/Dead Fixable Stain	--	L34957	ThermoFisher	Aqua	--	--
CellRox Green Flourogenic Reagent	--	C10492	ThermoFisher	--	--	--