

# Supplementary Information

## **Subcutaneous nanotherapy repurposes the immunosuppressive mechanism of rapamycin to enhance allogeneic islet graft viability**

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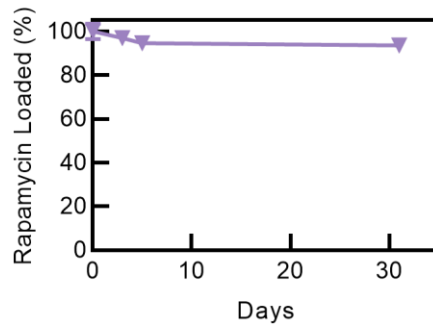
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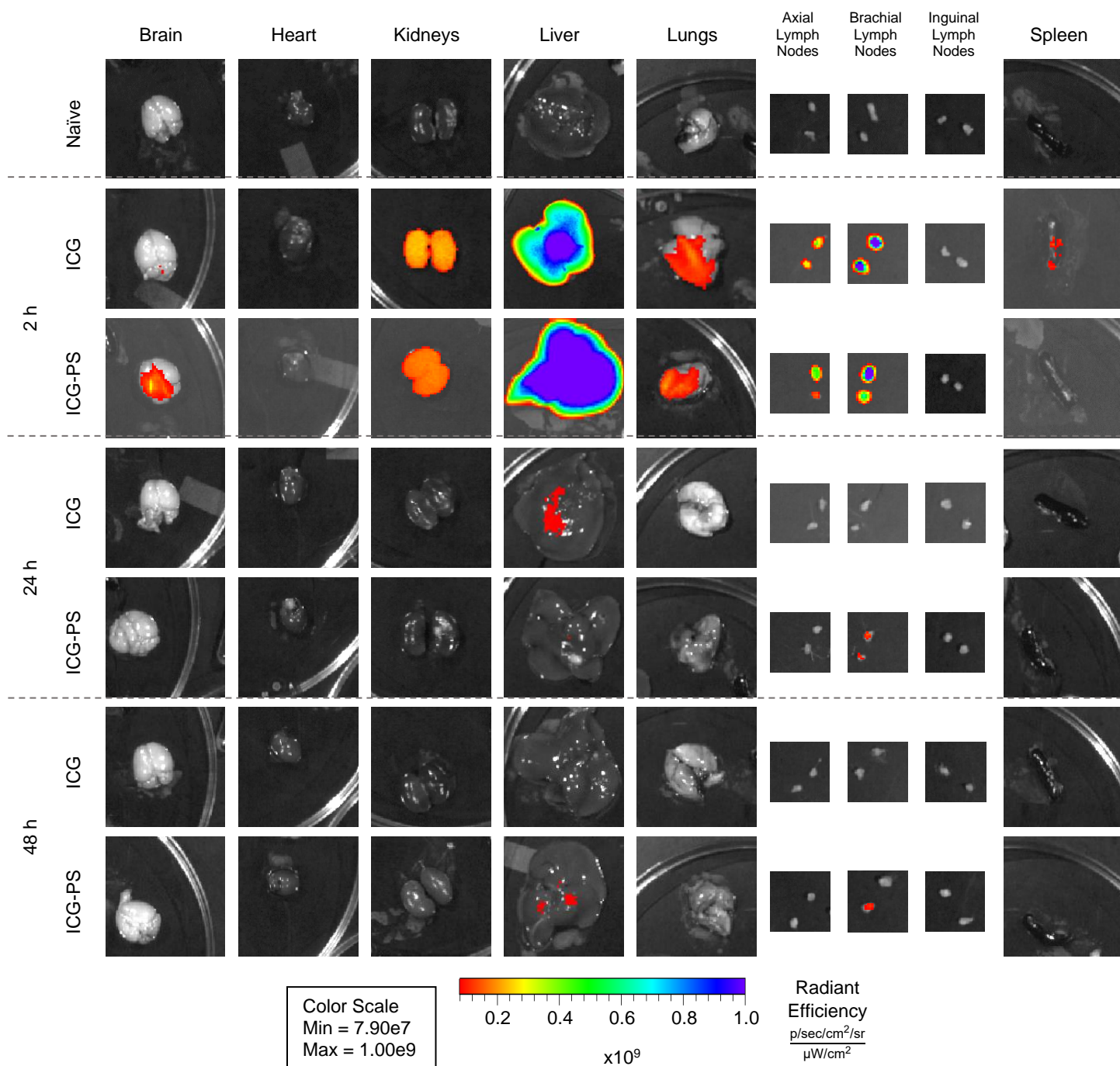
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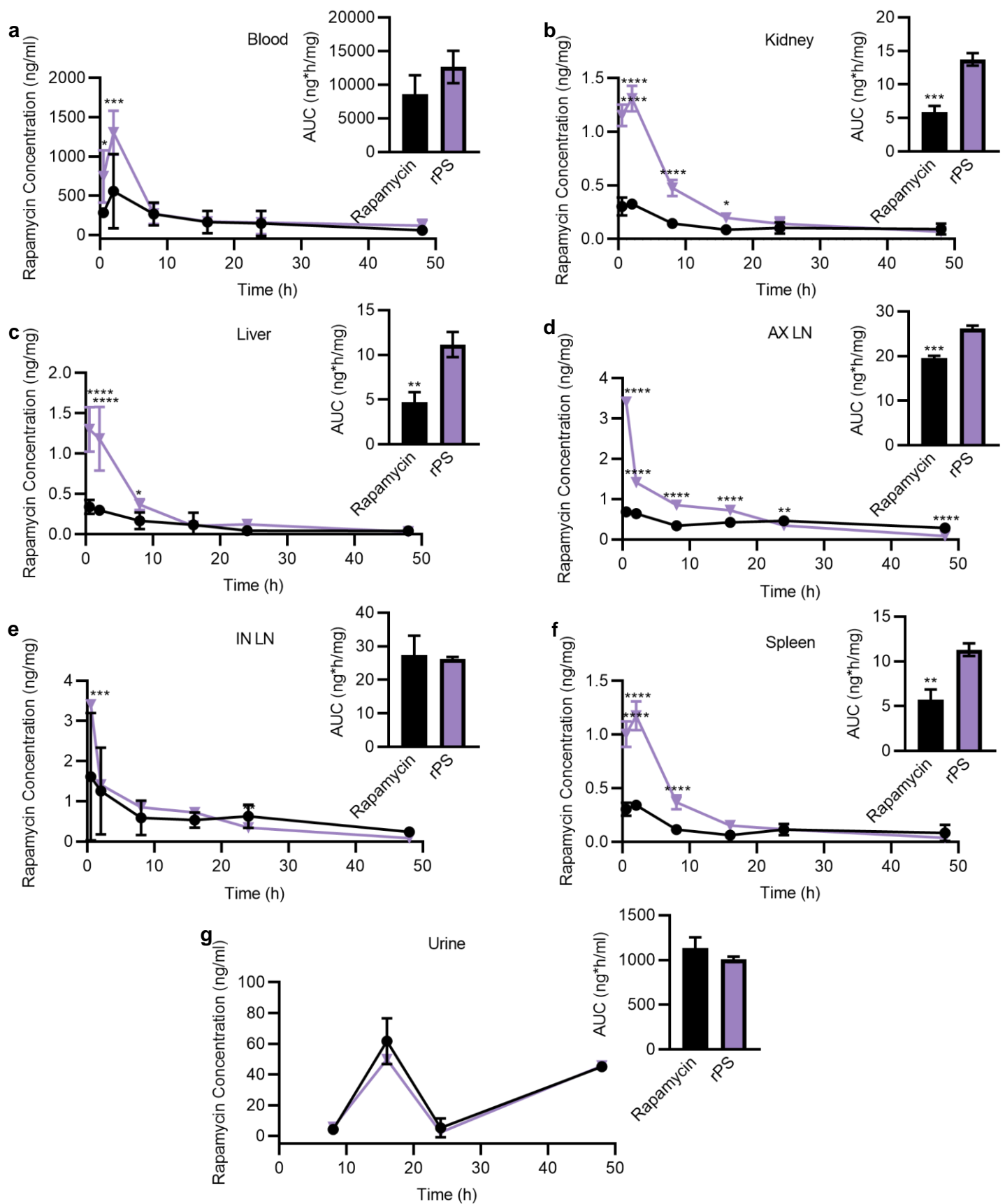
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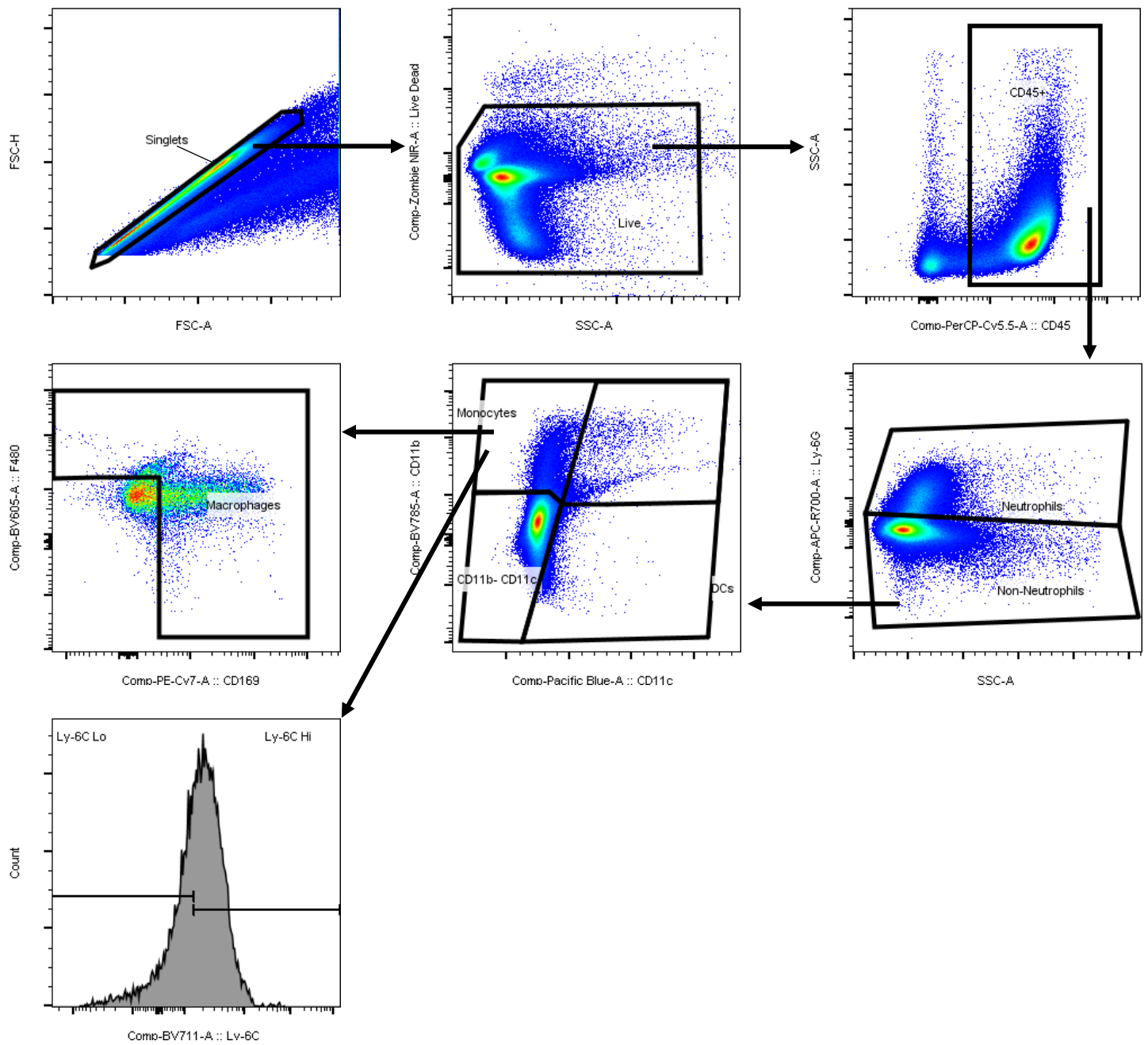
**Fig. S1 | Characterization of rapamycin-loaded polymersomes (rPS) encapsulation stability.** rPS were fabricated, unencapsulated rapamycin was removed and rPS samples were stored at 4 °C in phosphate buffered saline (PBS) at a concentration of 0.125 mg rapamycin/ml. At various time points, released rapamycin was removed and loaded rapamycin concentration was assessed via HPLC-UV. (n = 3-5).



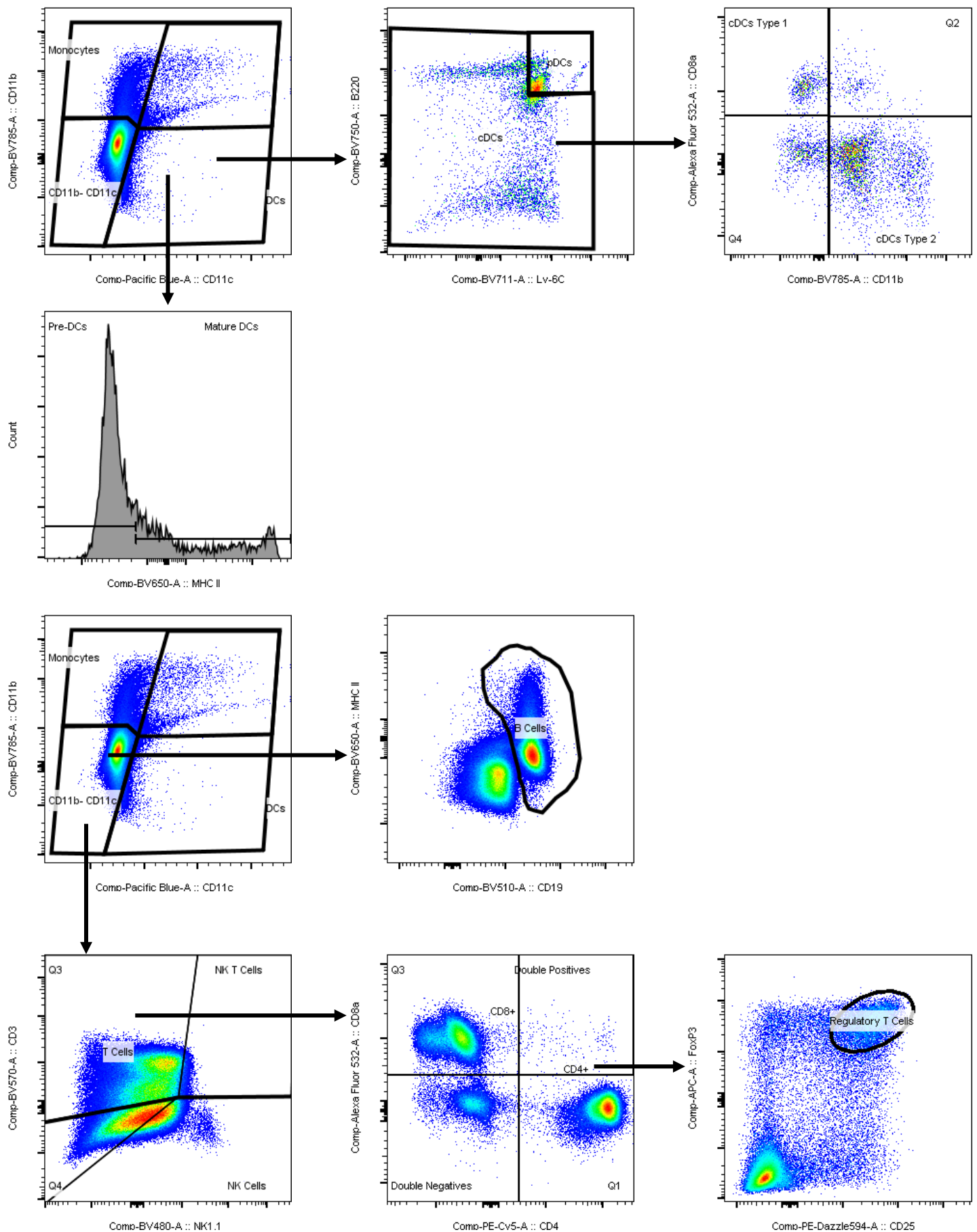
**Fig. S2 | Biodistribution of indocyanine green (ICG) dye and ICG loaded in to polymersomes (ICG-PS).** Mice were subcutaneously injected with ICG-PS or ICG and sacrificed at 2, 24 and 48 hours after injection (n = 5 mice). IVIS was performed on extracted organs to quantify ICG dye.



**Fig. S3 | Biodistribution of rapamycin by formulation.** Rapamycin concentration in the **a**, blood, **b**, kidneys, **c**, liver, **d**, axillary lymphocenter (deep axillary/axillary/axial and superficial axillary/brachial lymph nodes; AX LN), **e**, subiliac lymphocenter (subiliac/inguinal lymph nodes; IN LN) **f**, spleen and **g**, urine 0.5 h, 2 h, 8 h, 16 h, 24 h and 48 h after a single subcutaneous injection of rapamycin (in 0.2% carboxymethyl cellulose (CMC)) (—■—) or rPS (—▼—). Rapamycin concentration was also analyzed in the lungs, brain, heart, and fat; concentrations were below 1 ng/mg for both rapamycin and rPS at all timepoints. Analysis was performed using LC-MS/MS. (n = 3 mice/group).

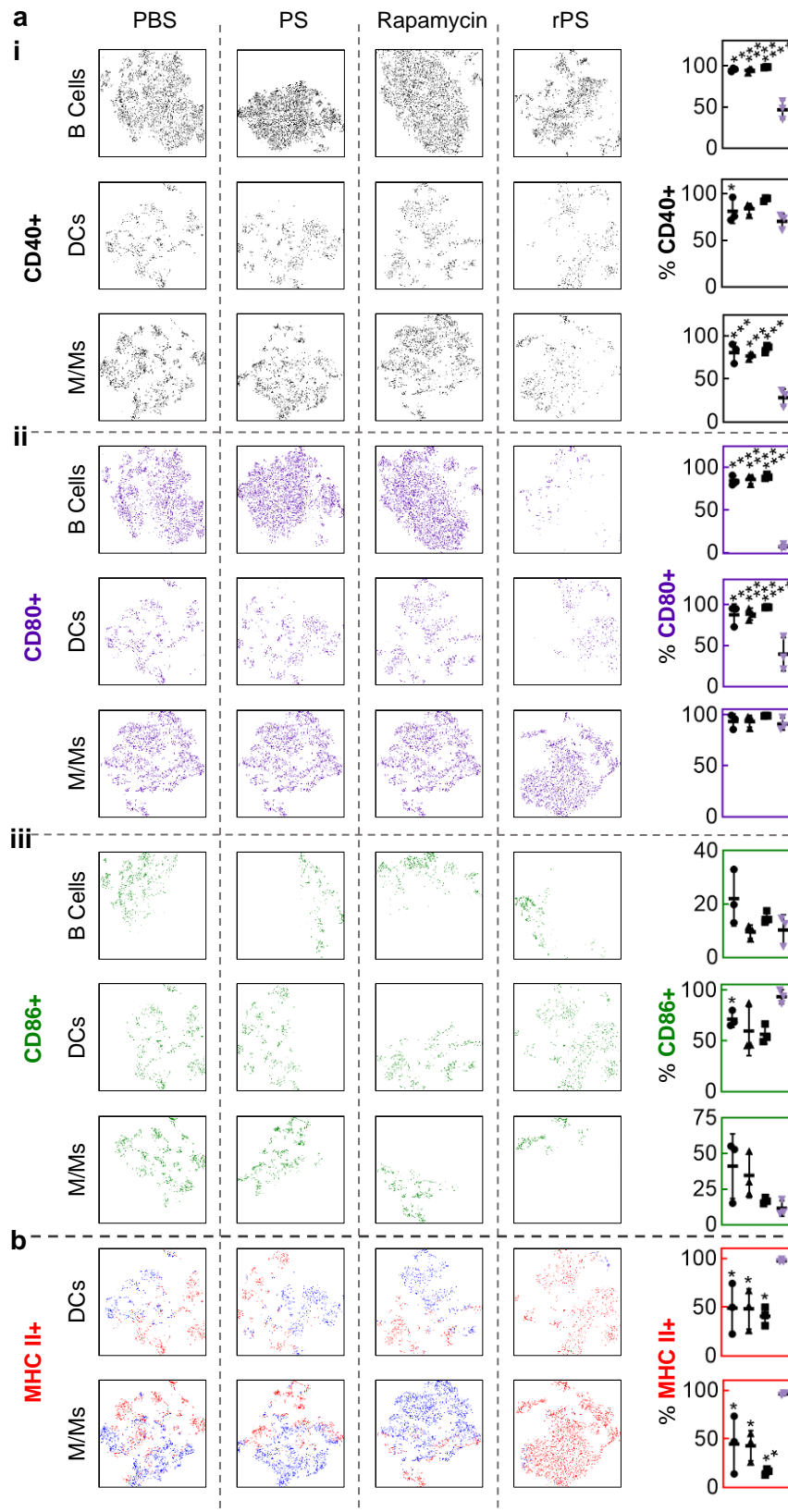


**Fig. S4 | Gating strategy for cell populations in flow cytometry studies.** Representative pseudocolor plots and histograms are displayed from an example mouse lymph node.

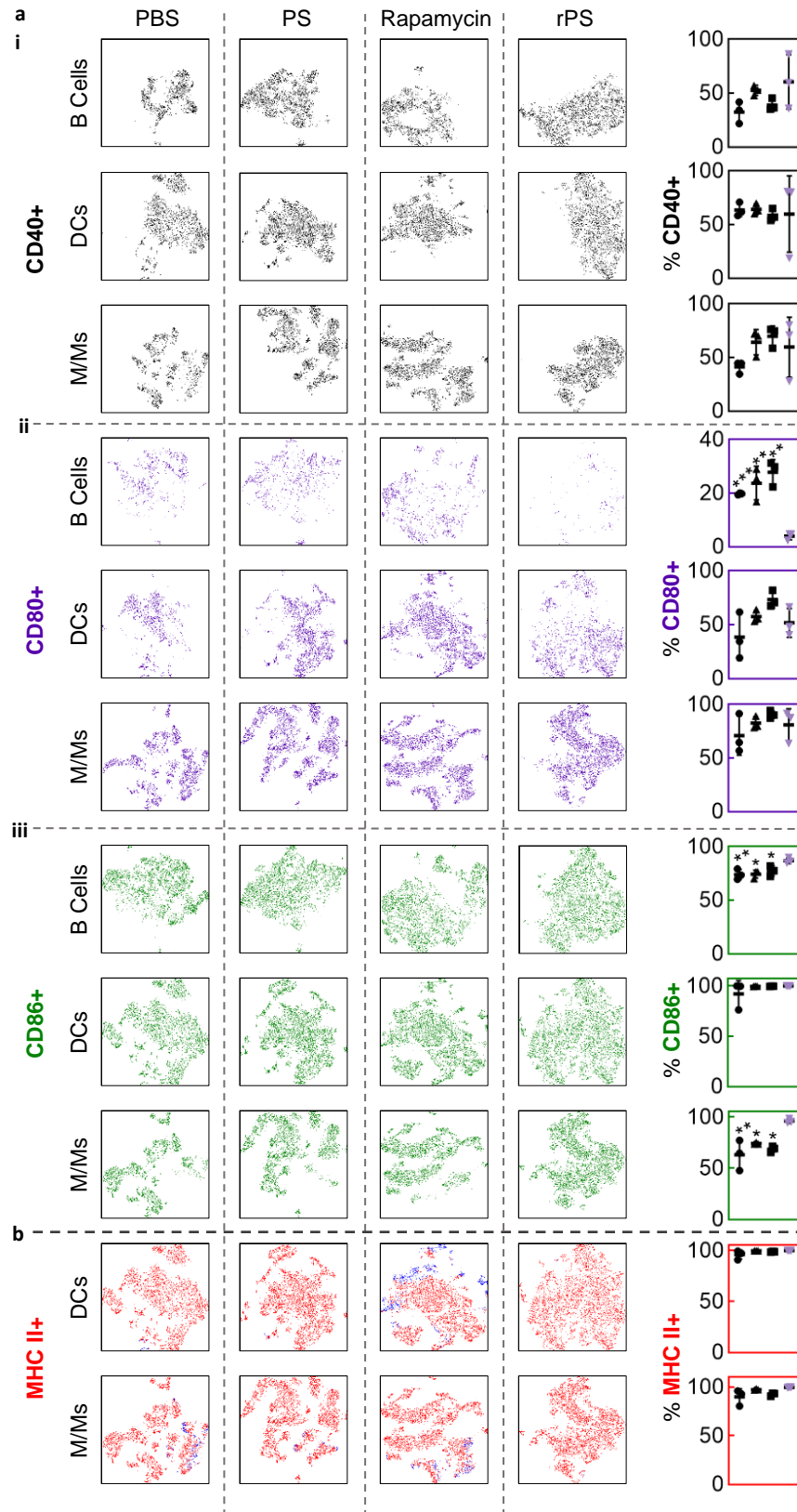


**Fig. S4 (continued) | Gating strategy for cell populations in flow cytometry studies.** Representative pseudocolor plots and histograms are displayed from an example mouse lymph node.



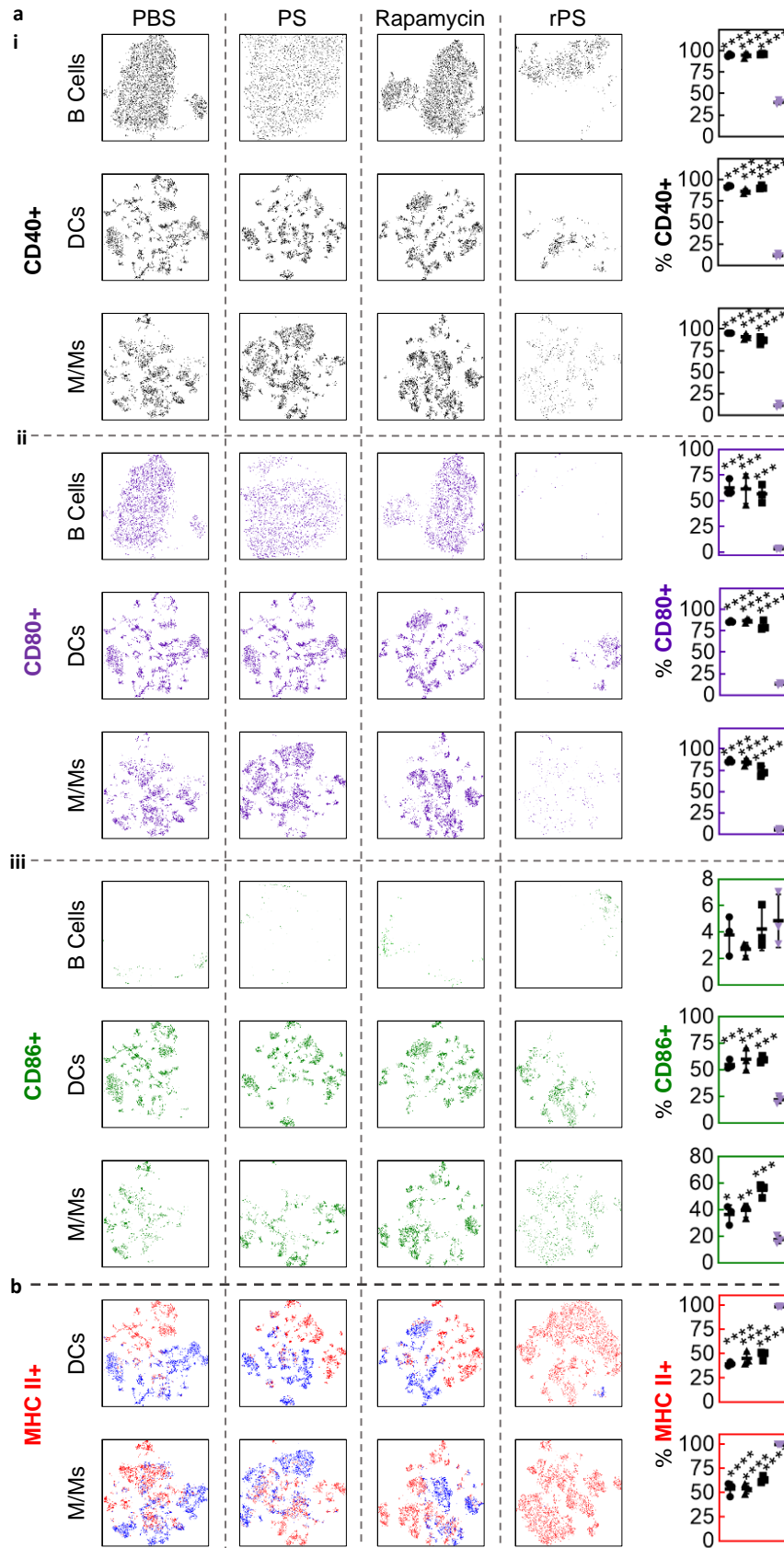


**Fig. S5 | Coreceptor and MHC II expression in blood.** Flow cytometry analysis of CD45<sup>+</sup> cell populations from mice subcutaneously injected with phosphate buffered saline (PBS; ●), polymersomes (PS; ▲), rapamycin (■) or rapamycin-loaded polymersomes (rPS; ▼) using the standard dosage protocol (11 injections, 1 mg/kg rapamycin or equivalent). **a**, tSNE visualization of coreceptor CD40 (**a i**; black), CD80 (**a ii**; purple) and CD86 (**a iii**; forest green) positive B cells, dendritic cells (DCs) and monocyte-and-macrophage-lineage cells (M/Ms). **b**, tSNE visualization of major-histocompatibility complex (MHC) II presentation (MHC II<sup>+</sup> red; MHC II<sup>-</sup> blue) on DCs and M/Ms. All data are presented as mean percentage (of DCs or M/Ms)  $\pm$  SD with \* $p < 0.05$ ; \*\*  $p < 0.01$ ; \*\*\*  $p < 0.001$ ; \*\*\*\*  $p < 0.0001$  relative to rPS treatment. Statistical significance was determined by one-way ANOVA with Tukey's multiple comparisons test.

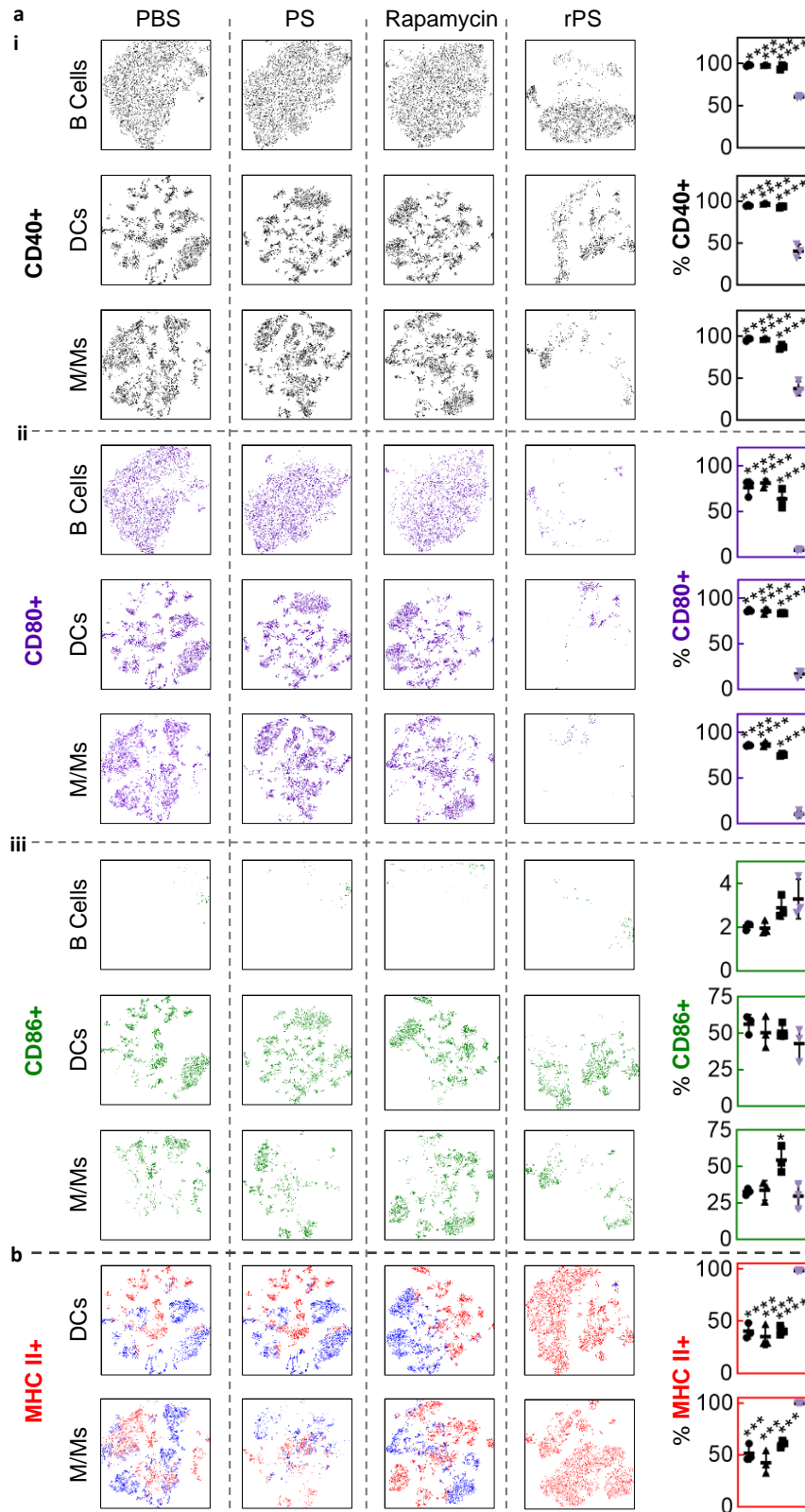


**Fig. S6 | Coreceptor and MHC II expression in liver.** Flow cytometry analysis of CD45<sup>+</sup> cell populations from mice subcutaneously injected with phosphate buffered saline (PBS; ●), polymersomes (PS; ▲), rapamycin (■) or rapamycin-loaded polymersomes (rPS; ▼) using the standard dosage protocol (11 injections, 1 mg/kg rapamycin or equivalent). **a**, tSNE visualization of coreceptor CD40 (**a i**; black), CD80 (**a ii**; purple) and CD86 (**a iii**; forest green) positive B cells, dendritic cells (DCs) and monocyte-and-macrophage-lineage cells (M/Ms). **b**, tSNE visualization of major-histocompatibility complex (MHC) II presentation (MHC II<sup>+</sup> red; MHC II<sup>-</sup> blue) on DCs and M/Ms. All data are presented as mean percentage (of DCs or M/Ms) ± SD with \*p<0.05; \*\* p<0.01; \*\*\* p<0.001; \*\*\*\* p<0.0001 relative to rPS treatment. Statistical significance was determined by one-way ANOVA with Tukey's multiple comparisons test.

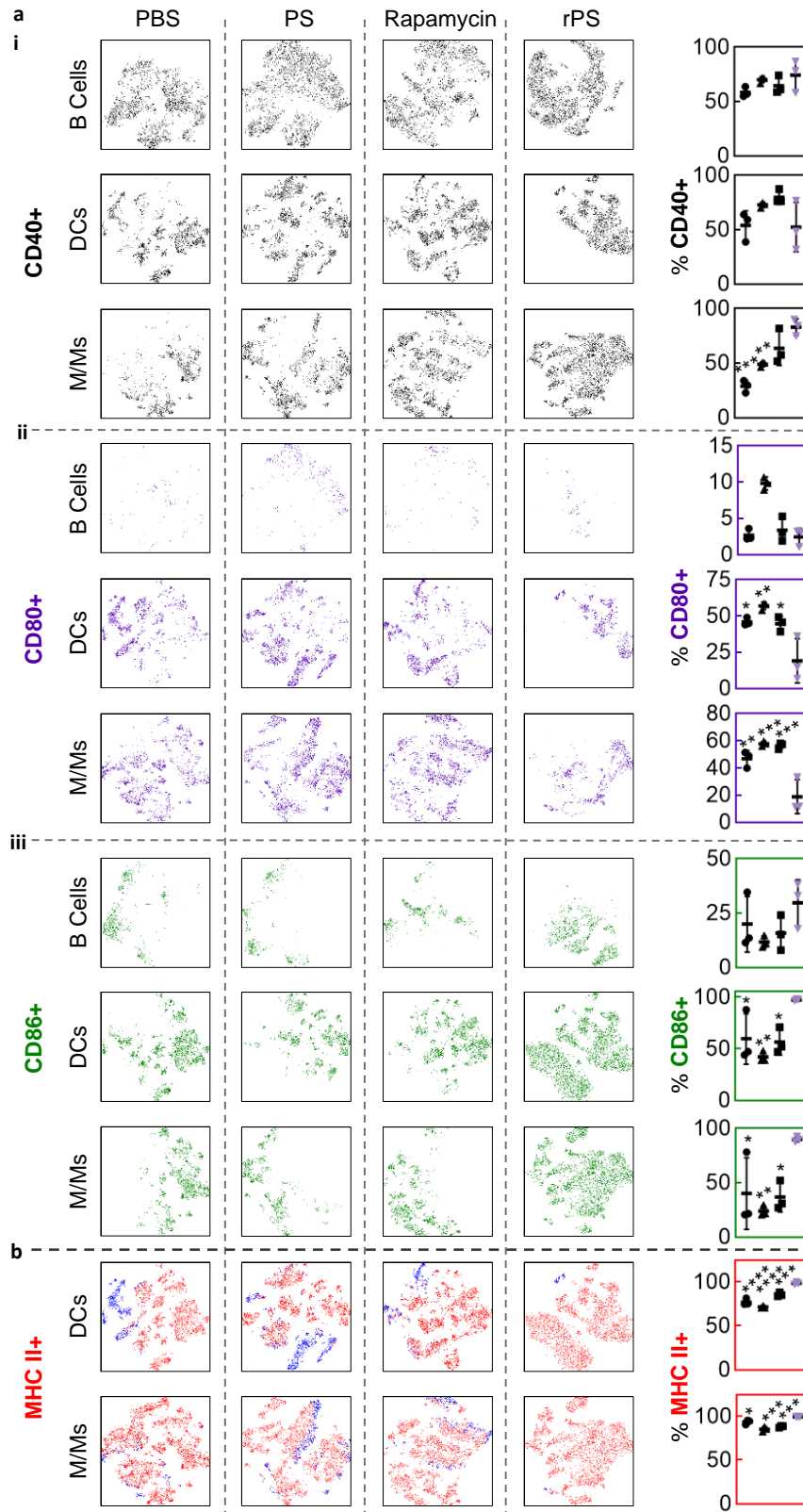




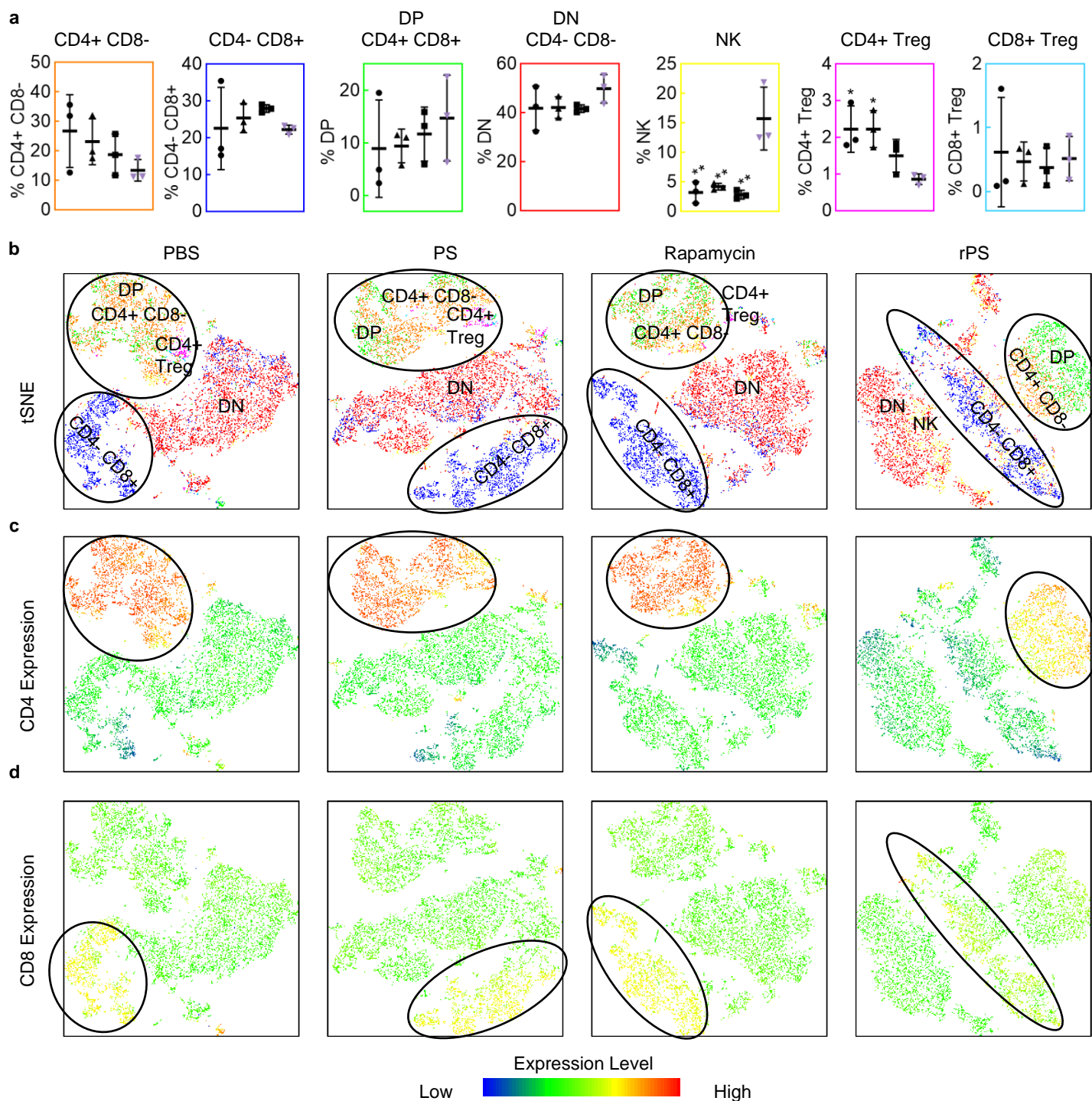
**Fig. S7 | Coreceptor and MHC II expression in AX LN.** Flow cytometry analysis of CD45+ cell populations from mice subcutaneously injected with phosphate buffered saline (PBS; ●), polymersomes (PS; ▲), rapamycin (■) or rapamycin-loaded polymersomes (rPS; ▼) using the standard dosage protocol (11 injections, 1 mg/kg rapamycin or equivalent). **a**, tSNE visualization of coreceptor CD40 (**a i**; black), CD80 (**a ii**; purple) and CD86 (**a iii**; forest green) positive B cells, dendritic cells (DCs) and monocyte-and-macrophage-lineage cells (M/Ms). **b**, tSNE visualization of major-histocompatibility complex (MHC) II presentation (MHC II+ red; MHC II- blue) on DCs and M/Ms. All data are presented as mean percentage (of DCs or M/Ms)  $\pm$  SD with \* $p < 0.05$ ; \*\*  $p < 0.01$ ; \*\*\*  $p < 0.001$ ; \*\*\*\*  $p < 0.0001$  relative to rPS treatment. Statistical significance was determined by one-way ANOVA with Tukey's multiple comparisons test. AX LN: axillary lymphocenter (deep axillary/axillary/axial and superficial axillary/brachial lymph nodes).



**Fig. S8 | Coreceptor and MHC II expression in IN LN.** Flow cytometry analysis of CD45<sup>+</sup> cell populations from mice subcutaneously injected with phosphate buffered saline (PBS; ●), polymersomes (PS; ▲), rapamycin (■) or rapamycin-loaded polymersomes (rPS; ▼) using the standard dosage protocol (11 injections, 1 mg/kg rapamycin or equivalent). **a**, tSNE visualization of coreceptor CD40 (**a i**; black), CD80 (**a ii**; purple) and CD86 (**a iii**; forest green) positive B cells, dendritic cells (DCs) and monocyte-and-macrophage-lineage cells (M/Ms). **b**, tSNE visualization of major-histocompatibility complex (MHC) II presentation (MHC II<sup>+</sup> red; MHC II<sup>-</sup> blue) on DCs and M/Ms. All data are presented as mean percentage (of DCs or M/Ms)  $\pm$  SD with \* $p < 0.05$ ; \*\* $p < 0.01$ ; \*\*\* $p < 0.001$ ; \*\*\*\* $p < 0.0001$  relative to rPS treatment. Statistical significance was determined by one-way ANOVA with Tukey's multiple comparisons test. IN LN: subiliac lymphocenter (subiliac/inguinal lymph nodes).

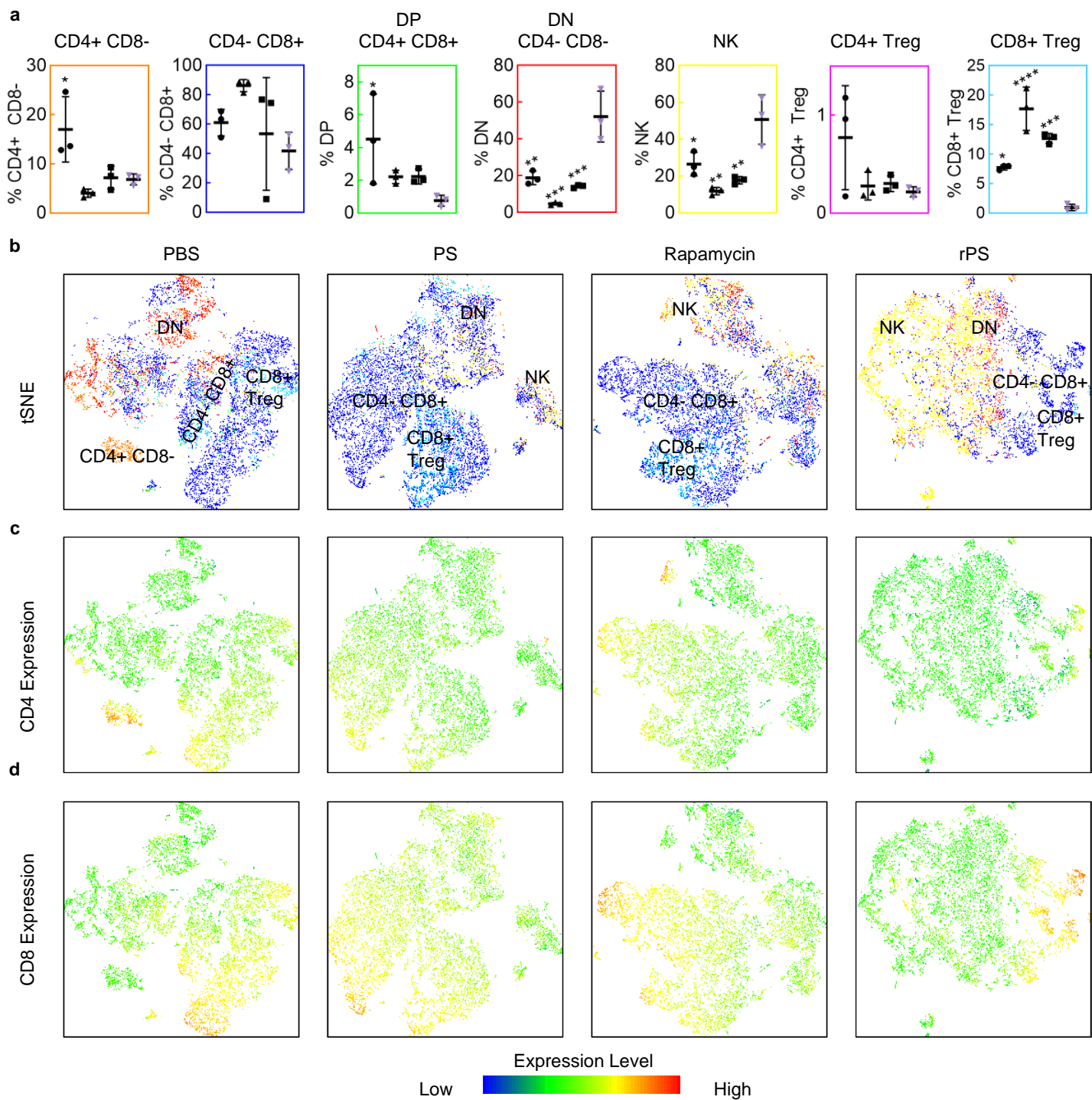


**Fig. S9 | Coreceptor and MHC II expression in the spleen.** Flow cytometry analysis of CD45<sup>+</sup> cell populations from mice subcutaneously injected with phosphate buffered saline (PBS; ●), polymersomes (PS; ▲), rapamycin (■) or rapamycin-loaded polymersomes (rPS; ▼) using the standard dosage protocol (11 injections, 1 mg/kg rapamycin or equivalent). **a**, tSNE visualization of coreceptor CD40 (**a i**; black), CD80 (**a ii**; purple) and CD86 (**a iii**; forest green) positive B cells, dendritic cells (DCs) and monocyte-and-macrophage-lineage cells (M/Ms). **b**, tSNE visualization of major-histocompatibility complex (MHC) II presentation (MHC II+ red; MHC II- blue) on DCs and M/Ms. All data are presented as mean percentage (of DCs or M/Ms)  $\pm$  SD with \* $p < 0.05$ ; \*\*  $p < 0.01$ ; \*\*\*  $p < 0.001$ ; \*\*\*\*  $p < 0.0001$  relative to rPS treatment. Statistical significance was determined by one-way ANOVA with Tukey's multiple comparisons test.

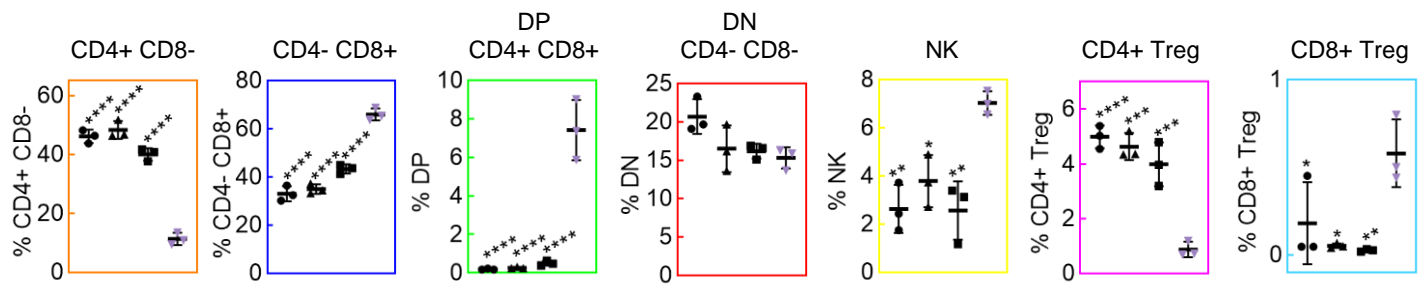


**Fig. S10 | T cell populations in blood.** Flow cytometry analysis of CD3+ T cell populations from mice subcutaneously injected with phosphate buffered saline (PBS; ●), polymersomes (PS; ▲), rapamycin (■) or rapamycin-loaded polymersomes (rPS; ▼) using the standard dosage protocol (11 injections, 1 mg/kg rapamycin or equivalent). **a**, Analysis of CD3+ T cell populations: Percentage of CD3+ T cells (from left to right): CD4+ CD8- (orange), CD4- CD8+ (blue), CD4+ CD8+ double positive (DP; green), CD4- CD8- double negative (DN; red), and natural killer (NK; yellow), CD4+ regulatory (CD4+ Treg; magenta) and CD8+ regulatory (CD8+ Treg; light blue) in blood. All data are presented as mean percentage (of CD3+ cells)  $\pm$  SD with \* $p$ <0.05; \*\* $p$ <0.01; \*\*\* $p$ <0.001; \*\*\*\* $p$ <0.0001 relative to rPS treatment. Statistical significance was determined by one-way ANOVA with Tukey's multiple comparisons test. **b**, tSNE visualization of CD3+ immune cell populations from the liver with color-coded gated overlays of the previously described cell populations: CD4+ CD8- (orange), CD4- CD8+ (blue), CD4+ CD8+ double positive (DP; green), CD4- CD8- double negative (DN; red), and natural killer (NK; yellow), CD4+ regulatory (CD4+ Treg; magenta) and CD8+ regulatory (CD8+ Treg; light blue). **c,d**, tSNE heatmap statistic of CD4 (**c**) and CD8 (**d**) expression. (n=3 mice/group).



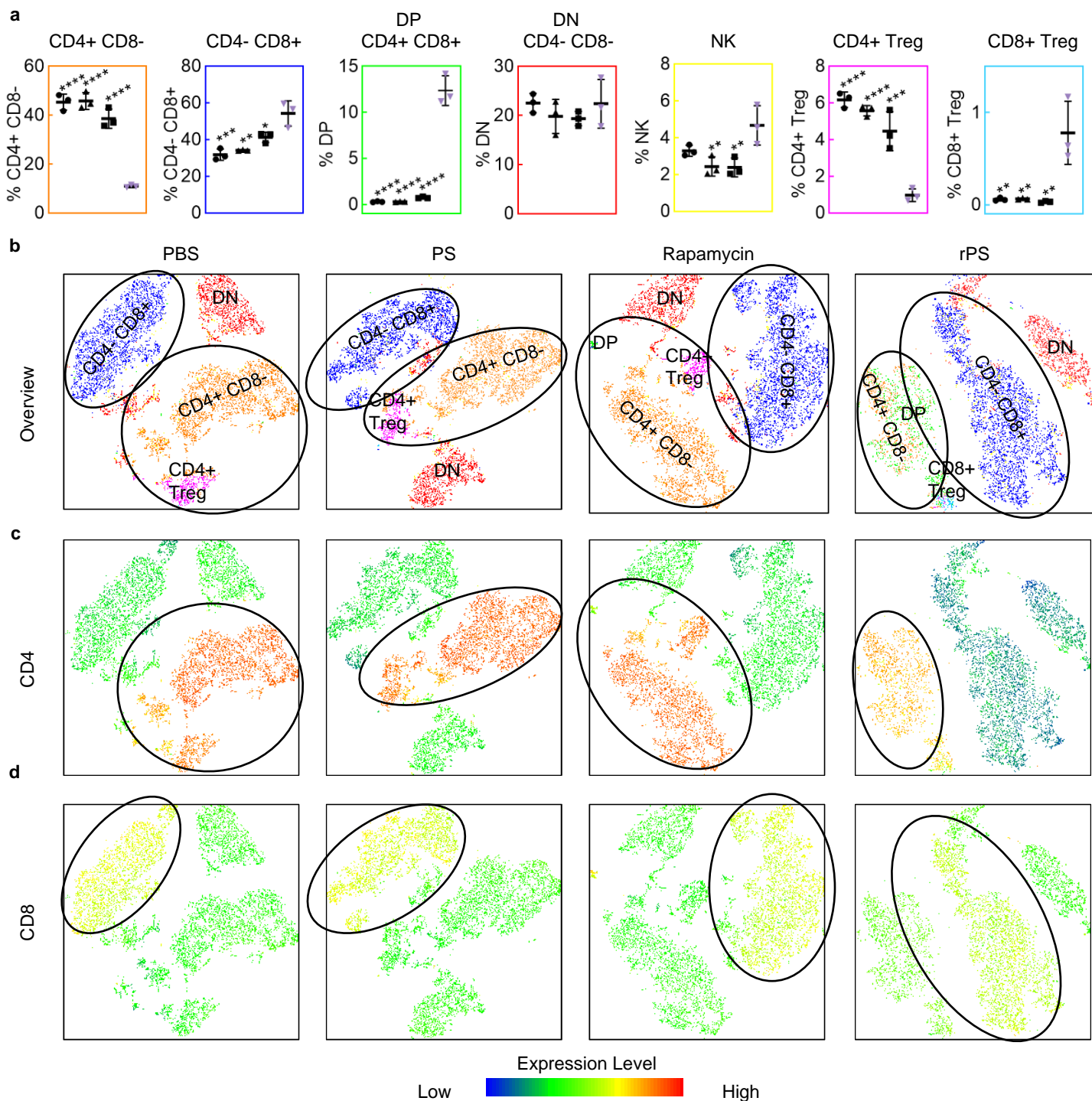


**Fig. S11 | T cell populations in the liver.** Flow cytometry analysis of CD3+ T cell populations from mice subcutaneously injected with phosphate buffered saline (PBS; ●), polymersomes (PS; ▲), rapamycin (■) or rapamycin-loaded polymersomes (rPS; ▼) using the standard dosage protocol (11 injections, 1 mg/kg rapamycin or equivalent). **a**, Analysis of CD3+ T cell populations: Percentage of CD3+ T cells (from left to right): CD4+ CD8- (orange), CD4- CD8+ (blue), CD4+ CD8+ double positive (DP; green), CD4- CD8- double negative (DN; red), and natural killer (NK; yellow), CD4+ regulatory (CD4+ Treg; magenta) and CD8+ regulatory (CD8+ Treg; light blue) in the liver. All data are presented as mean percentage (of CD3+ cells)  $\pm$  SD with \* $p$ <0.05; \*\* $p$ <0.01; \*\*\* $p$ <0.001; \*\*\*\* $p$ <0.0001 relative to rPS treatment. Statistical significance was determined by one-way ANOVA with Tukey's multiple comparisons test. **b**, tSNE visualization of CD3+ immune cell populations from the liver with color-coded gated overlays of the previously described cell populations: CD4+ CD8- (orange), CD4- CD8+ (blue), CD4+ CD8+ double positive (DP; green), CD4- CD8- double negative (DN; red), and natural killer (NK; yellow), CD4+ regulatory (CD4+ Treg; magenta) and CD8+ regulatory (CD8+ Treg; light blue). **c,d**, tSNE heatmap statistic of CD4 (**c**) and CD8 (**d**) expression. (n=3 mice/group).

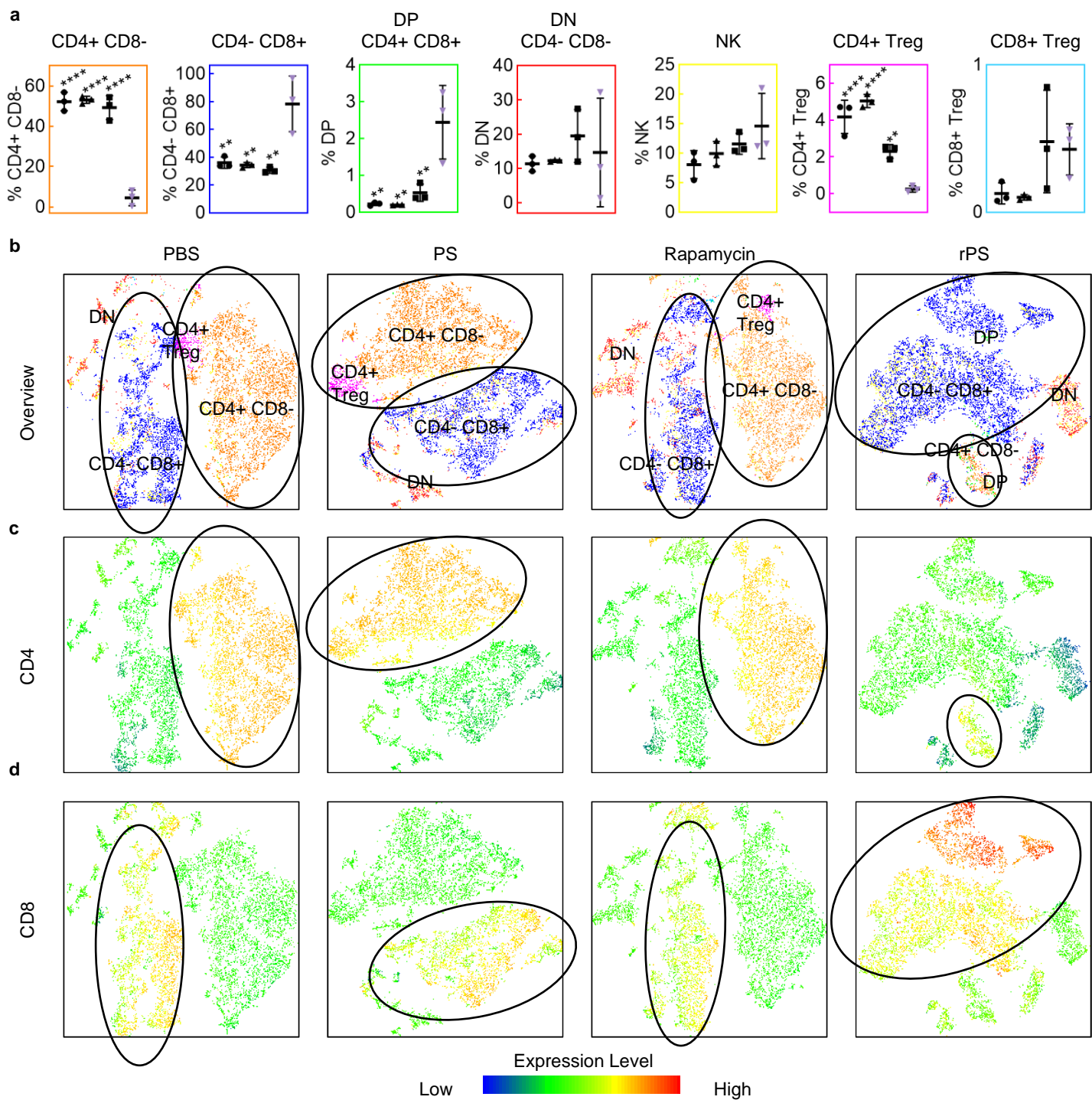


**Fig. S12 | T cell populations in the AX LN.** Flow cytometry analysis of CD3+ T cell populations from mice subcutaneously injected with phosphate buffered saline (PBS; ●), polymersomes (PS; ▲), rapamycin (■) or rapamycin-loaded polymersomes (rPS; ▼) using the standard dosage protocol (11 injections, 1 mg/kg rapamycin or equivalent). **a**, Analysis of CD3+ T cell populations: Percentage of CD3+ T cells (from left to right): CD4+ CD8- (orange), CD4- CD8+ (blue), CD4+ CD8+ double positive (DP; green), CD4- CD8- double negative (DN; red), and natural killer (NK; yellow), CD4+ regulatory (CD4+ Treg; magenta) and CD8+ regulatory (CD8+ Treg; light blue) in the liver. All data are presented as mean percentage (of CD3+ cells)  $\pm$  SD with \* $p<0.05$ ; \*\* $p<0.01$ ; \*\*\* $p<0.001$ ; \*\*\*\* $p<0.0001$  relative to rPS treatment. Statistical significance was determined by one-way ANOVA with Tukey's multiple comparisons test. (n=3 mice/group). AX LN: axillary lymphocenter (deep axillary/axillary/axial and superficial axillary/brachial lymph nodes).

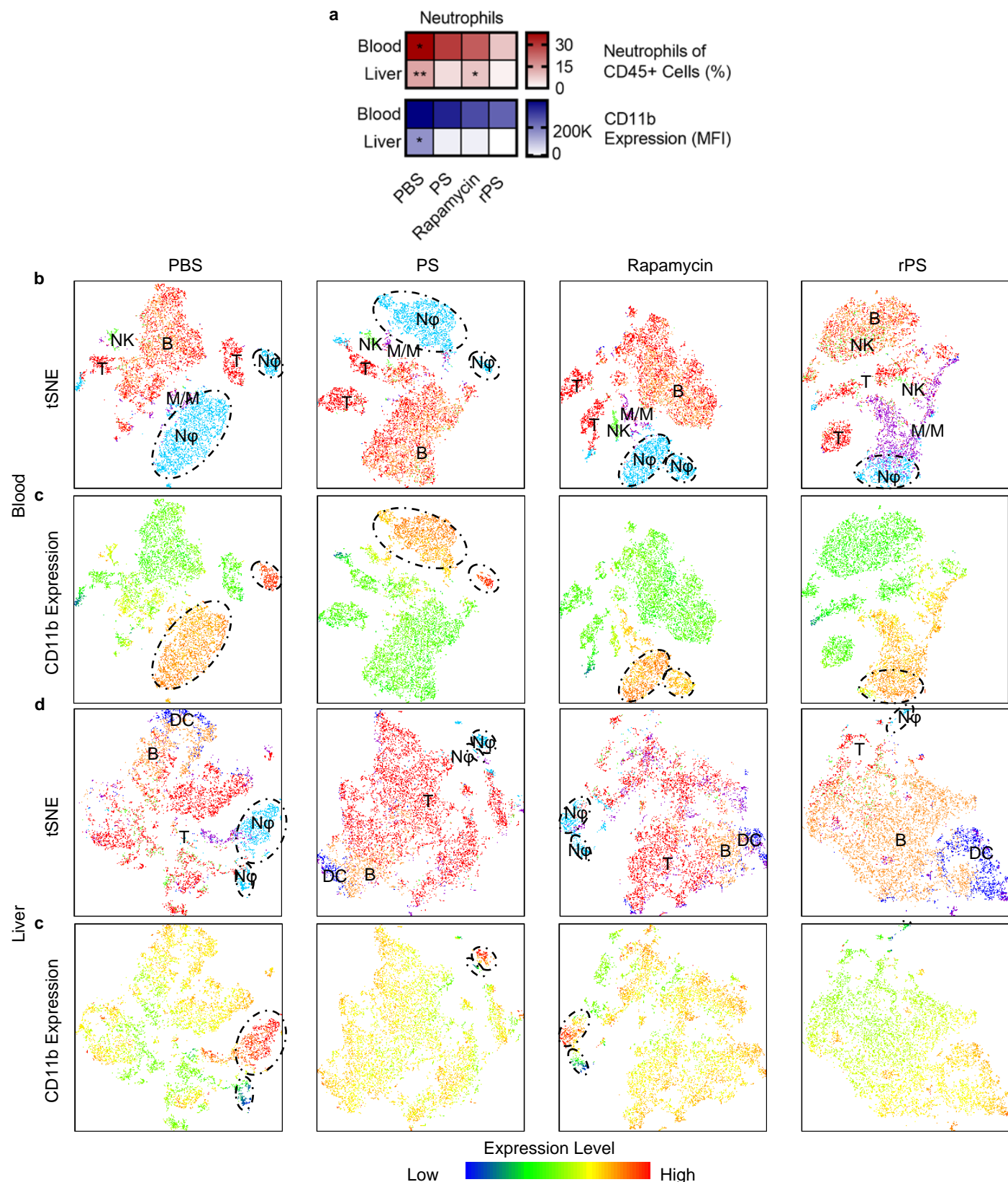




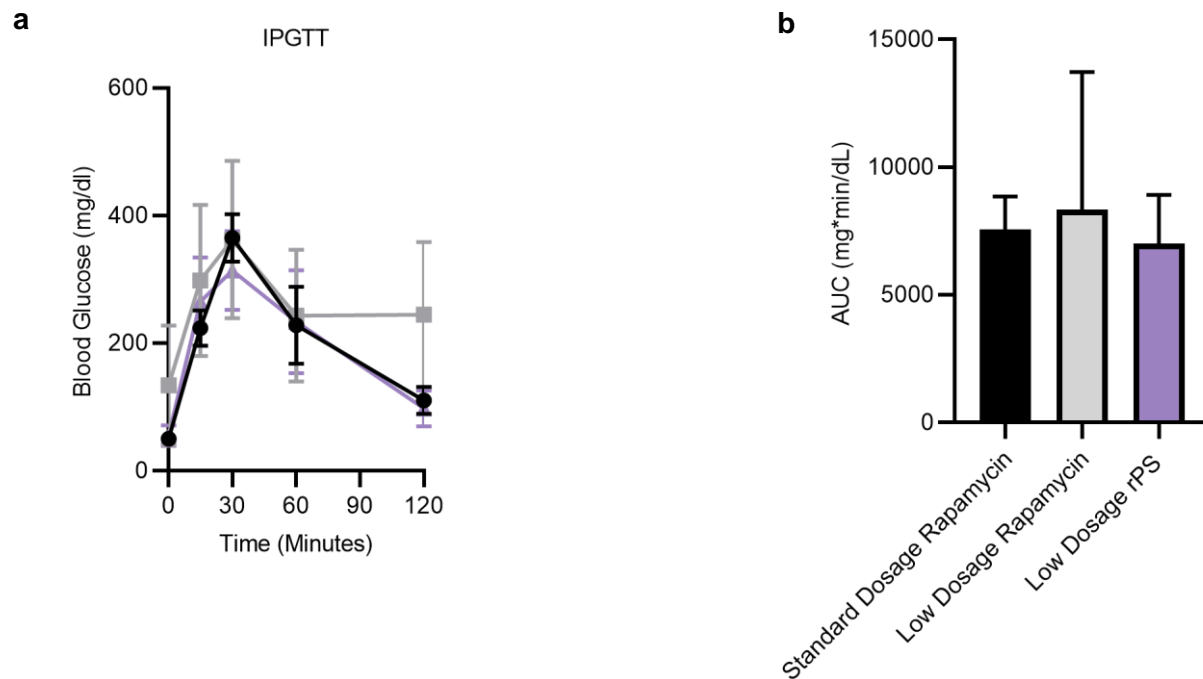
**Fig. S13 | T cell populations in the IN LN.** Flow cytometry analysis of CD3+ T cell populations from mice subcutaneously injected with phosphate buffered saline (PBS; ●), polymersomes (PS; ▲), rapamycin (■) or rapamycin-loaded polymersomes (rPS; ▼) using the standard dosage protocol (11 injections, 1 mg/kg rapamycin or equivalent). **a**, Analysis of CD3+ T cell populations: Percentage of CD3+ T cells (from left to right): CD4+ CD8- (orange), CD4- CD8+ (blue), CD4+ CD8+ double positive (DP; green), CD4- CD8- double negative (DN; red), and natural killer (NK; yellow), CD4+ regulatory (CD4+ Treg; magenta) and CD8+ regulatory (CD8+ Treg; light blue) in the liver. All data are presented as mean percentage (of CD3+ cells)  $\pm$  SD with \* $p < 0.05$ ; \*\*  $p < 0.01$ ; \*\*\*  $p < 0.001$ ; \*\*\*\*  $p < 0.0001$  relative to rPS treatment. Statistical significance was determined by one-way ANOVA with Tukey's multiple comparisons test. **b**, tSNE visualization of CD3+ immune cell populations from the subiliac lymphocenter (subiliac/inguinal lymph nodes; IN LN) with color-coded gated overlays of the previously described cell populations: CD4+ CD8- (orange), CD4- CD8+ (blue), CD4+ CD8+ double positive (DP; green), CD4- CD8- double negative (DN; red), and natural killer (NK; yellow), CD4+ regulatory (CD4+ Treg; magenta) and CD8+ regulatory (CD8+ Treg; light blue). **c,d**, tSNE heatmap statistic of CD4 (**c**) and CD8 (**d**) expression. (n=3 mice/group).



**Fig. S14 | T cell populations in the spleen.** Flow cytometry analysis of CD3+ T cell populations from mice subcutaneously injected with phosphate buffered saline (PBS; ●), polymersomes (PS; ▲), rapamycin (■) or rapamycin-loaded polymersomes (rPS; ▼) using the standard dosage protocol (11 injections, 1 mg/kg rapamycin or equivalent). **a**, Analysis of CD3+ T cell populations: Percentage of CD3+ T cells (from left to right): CD4+ CD8- (orange), CD4- CD8+ (blue), CD4+ CD8+ double positive (DP; green), CD4- CD8- double negative (DN; red), and natural killer (NK; yellow), CD4+ regulatory (CD4+ Treg; magenta) and CD8+ regulatory (CD8+ Treg; light blue) in the liver. All data are presented as mean percentage (of CD3+ cells)  $\pm$  SD with \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$ , \*\*\*\* $p < 0.0001$  relative to rPS treatment. Statistical significance was determined by one-way ANOVA with Tukey's multiple comparisons test. **b**, tSNE visualization of CD3+ immune cell populations from the spleen with color-coded gated overlays of the previously described cell populations: CD4+ CD8- (orange), CD4- CD8+ (blue), CD4+ CD8+ double positive (DP; green), CD4- CD8- double negative (DN; red), and natural killer (NK; yellow), CD4+ regulatory (CD4+ Treg; magenta) and CD8+ regulatory (CD8+ Treg; light blue). **c,d**, tSNE heatmap statistic of CD4 (**c**) and CD8 (**d**) expression. (n=3 mice/group).

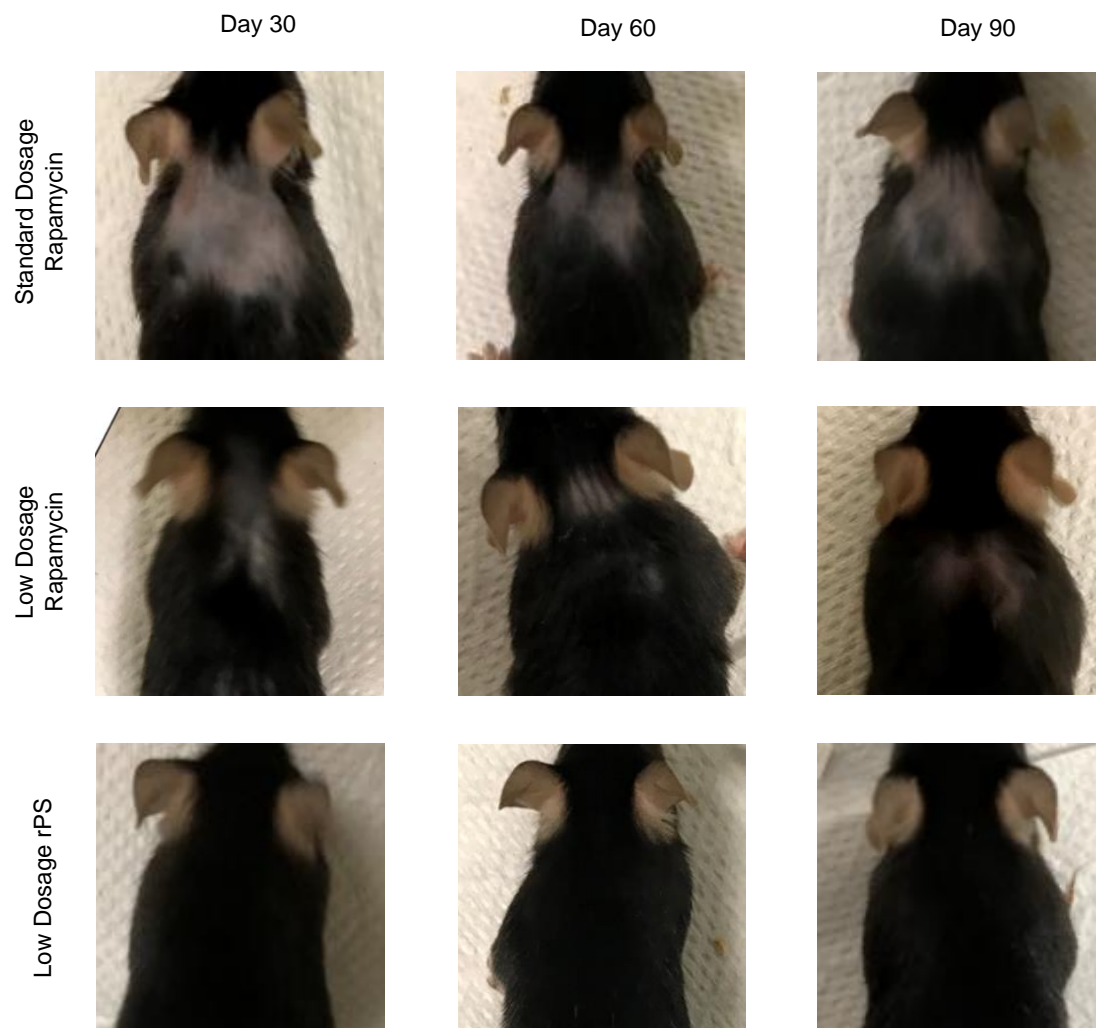


**Fig. S15 | Neutrophil populations in the blood and liver.** Flow cytometry analysis of CD45+ cells from mice subcutaneously injected with phosphate buffered saline (PBS), polymersomes (PS), rapamycin or rapamycin-loaded polymersomes (rPS) using the standard dosage protocol (11 injections, 1 mg/kg rapamycin or equivalent). **a**, Top: Percentage of neutrophils of CD45+ cells in blood and liver. All data are presented as mean percentage (of CD45+ cells)  $\pm$  SD with \* $p$ <0.05; \*\*  $p$ <0.01 relative to rPS treatment. Bottom: CD11b expression by neutrophils in the blood and liver. All data are presented as mean median fluorescent intensity (MFI)  $\pm$  SD with \* $p$ <0.05 relative to rPS treatment. **b,d**, tSNE visualization of CD45+ cells from blood (**b**) and liver (**d**) with color-coded gated overlays of specific cell populations: B cells (B; orange), dendritic cells (DC; blue), monocyte-and-macrophage-lineage cells (M/M; purple), neutrophils (N $\phi$ ; light blue), and T cells (T; red). **c,e**, tSNE heatmap statistic of CD11b for blood (**c**) and liver (**e**). Dashed-line ovals indicate N $\phi$  populations.



**Fig. S16 | Intraperitoneal glucose tolerance test (IPGTT).** **a**, Blood glucose concentration over time after intraperitoneal glucose challenge. **b**, Area under the curve from IPGTT. (n = 5-7 mice/group). All data is presented as mean  $\pm$  SD.





**Fig. S17 | rPS reduce injection site alopecia associated with rapamycin.** (n = 5-7 mice/group).

**Table S1 | Single-cell RNA sequencing analysis workflow**

Workflow	Program	Command Line
Quality Control	FastQC v0.11.5	fastqc <input_path_to/untrimmed.fq.gz>
Trimming and Filtering	Trimmomatic v0.39	java -jar ./Trimmomatic-0.39/trimmomatic-0.39.jar SE -threads 16 -phred33 <input_path_to/untrimmed.fq.gz> <output_path_to/trimmed.fq.gz> ILLUMINACLIP:TruSeq3-SE.fa:2:30:10 LEADING:30 TRAILING:30 MINLEN:36
Alignment	STAR v2.6.0a	STAR --genomeDir ../star_index/ --readFilesCommand zcat --readFilesIn <input_path_to/trimmed.fq.gz> --outFilterType BySJout --runThreadN 16 --outFilterMultimapNmax 100 --alignSJoverhangMin 8 --alignSJDBoverhangMin 1 --outFilterMismatchNoverLmax 0.05 --alignIntronMin 20 --alignIntronMax 1000000 --alignMatesGapMax 1000000 --outSAMattributes NH HI NM MD --outSAMstrandField intronMotif --outSAMmapqUnique 60 --outSAMtype BAM SortedByCoordinate --outReadsUnmapped Fastx --limitBAMsortRAM 30000000000 --outFileNamePrefix <output_path_to/sorted_bam>
Quantification and Differential Expression Analysis	Cufflinks v2.2.1	cuffdiff -L PBS,PS,R,RPS -o &lt;path_to/output_dir/> -p 16 -b ../genome_fasta/GRCm38.primary_assembly.genome.fa -u ../GTF/genecode.vM24.primary_assembly.annotation.gtf treatment_1_sample_1.bam,treatment_1_sample_2.bam,treatment_1_sample_3.bam treatment_2_sample_1.bam,treatment_2_sample_2.bam,treatment_2_sample_3.bam treatment_3_sample_1.bam,treatment_3_sample_2.bam,treatment_3_sample_3.bam treatment_4_sample_1.bam,treatment_4_sample_2.bam,treatment_4_sample_3.bam

**Table S2 | Single-cell RNA sequencing raw data**

Spleen Macrophages			PS		Rapamycin		rPS		Rapamycin vs rPS	
	Ensembl Code	Geneset Name	Fold Change	P Value	Fold Change	P Value	Fold Change	P Value	Fold Change	P Value
2279	ENSMUSG00000020053	IGF1	-6.39305	0.01815	-9.10778	0.00425	-6.03822	0.02255	3.06956	0.02915
10384	ENSMUSG00000036561	PPP6R2	-5.69947	0.011	-24	0.00005	-5.5058	0.01425	24	0.00005
5190	ENSMUSG00000025786	ZDHHC3	-5.15342	0.02015	-8.14259	0.03895	-4.23154	0.04065	3.91105	0.0462
2460	ENSMUSG00000020346	MGAT1	-2.54064	0.0472	5.0747	0.0171	-3.04016	0.01425	-8.11486	0.001
Spleen CD4+ Regulatory T Cells			PS		Rapamycin		rPS		Rapamycin vs rPS	
	Ensembl Code	Geneset Name	Fold Change	P Value	Fold Change	P Value	Fold Change	P Value	Fold Change	P Value
573	ENSMUSG00000003379	CD79A	-2.52198	0.0419	-24	0.00005	-4.38266	0.00455	24	0.00005
4444	ENSMUSG00000024353	MZB1	-2.06991	0.0297	-24	0.00005	-2.50151	0.0115	24	0.00005
947	ENSMUSG00000006134	CRKL	-0.689499	0.4598	7.38707	0.02155	-1.97122	0.04935	-9.3583	0.00525
12235	ENSMUSG00000041417	PIK3R1	1.96275	0.0626	-12	0.00005	3.12528	0.07685	12	0.00005
Liver Macrophages			PS		Rapamycin		rPS		Rapamycin vs rPS	
	Ensembl Code	Geneset Name	Fold Change	P Value	Fold Change	P Value	Fold Change	P Value	Fold Change	P Value
1331	ENSMUSG00000010651	ACAA1	-2.44129	0.0089	-3.83338	0.0077	-1.23789	0.03675	2.5955	0.0395