Spatiotemporal control of neutrophil fate to tune inflammation and repair for myocardial infarction therapy

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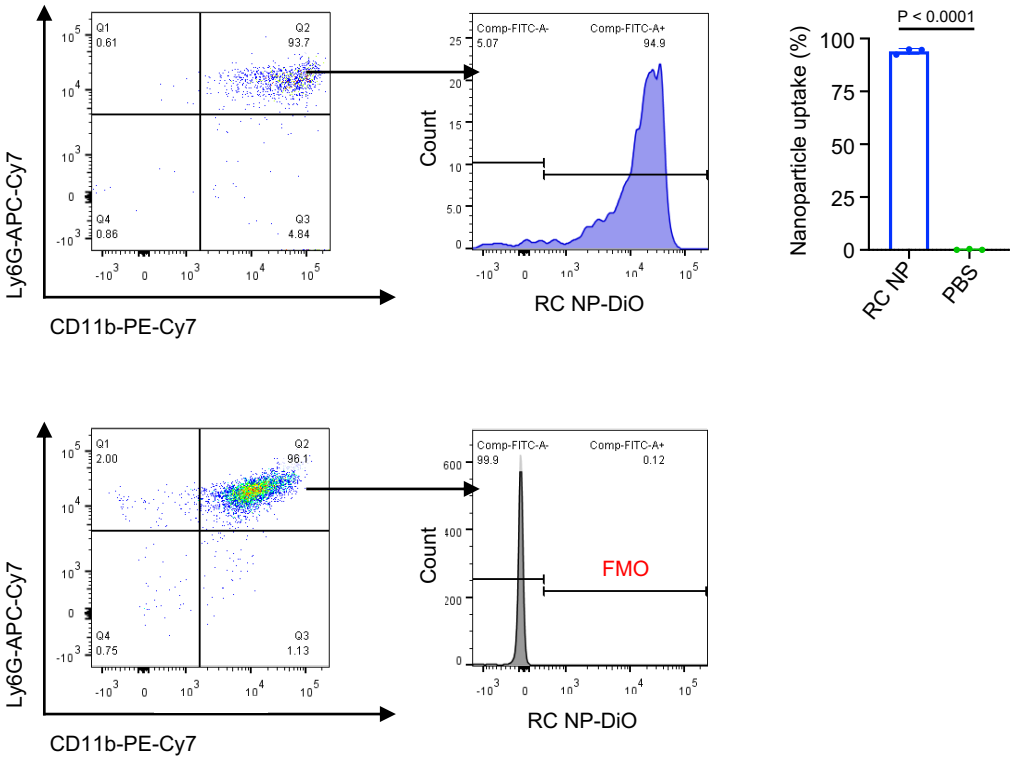
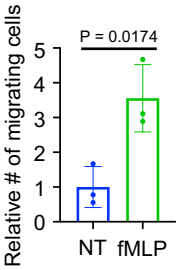
†These authors contributed equally.

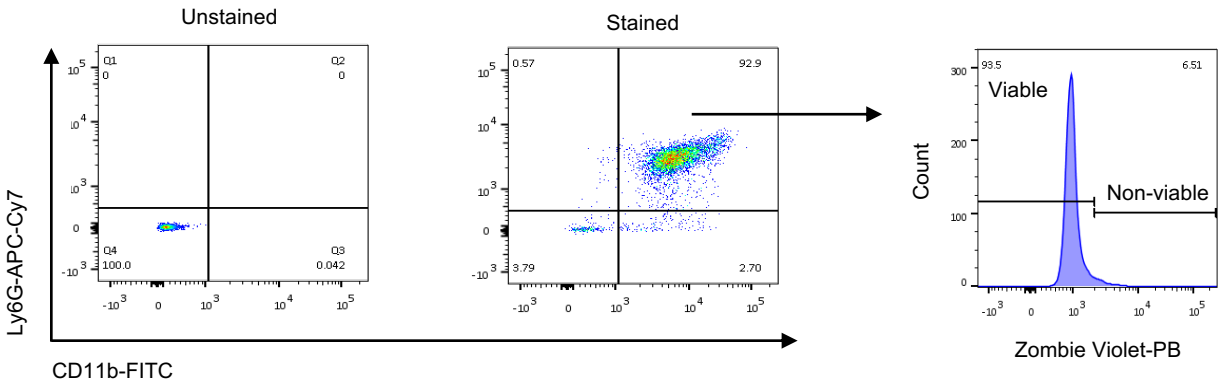
Contents:



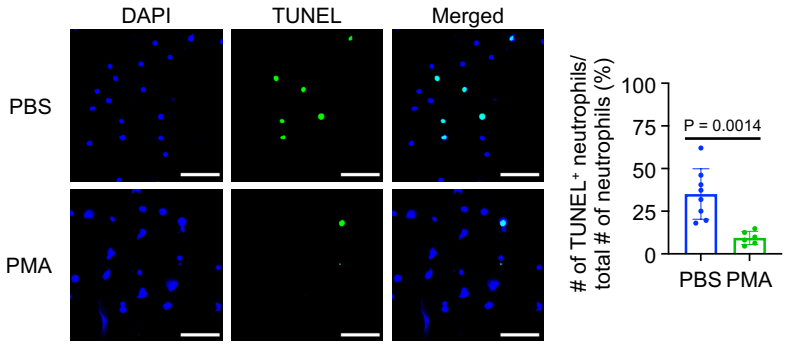
Supplementary Fig. 1. Neutrophil uptake of RC NPs in vitro. Neutrophil uptake of DiO-labelled RC NPs was determined by flow cytometry (n = 3 biological replicates). RC NP$^+$ neutrophils were defined as Ly6G$^+$CD11b$^+$DiO$^+$ cells. Unpaired two-sided *t*-tests were used for comparisons. Data presented as mean ± SD.



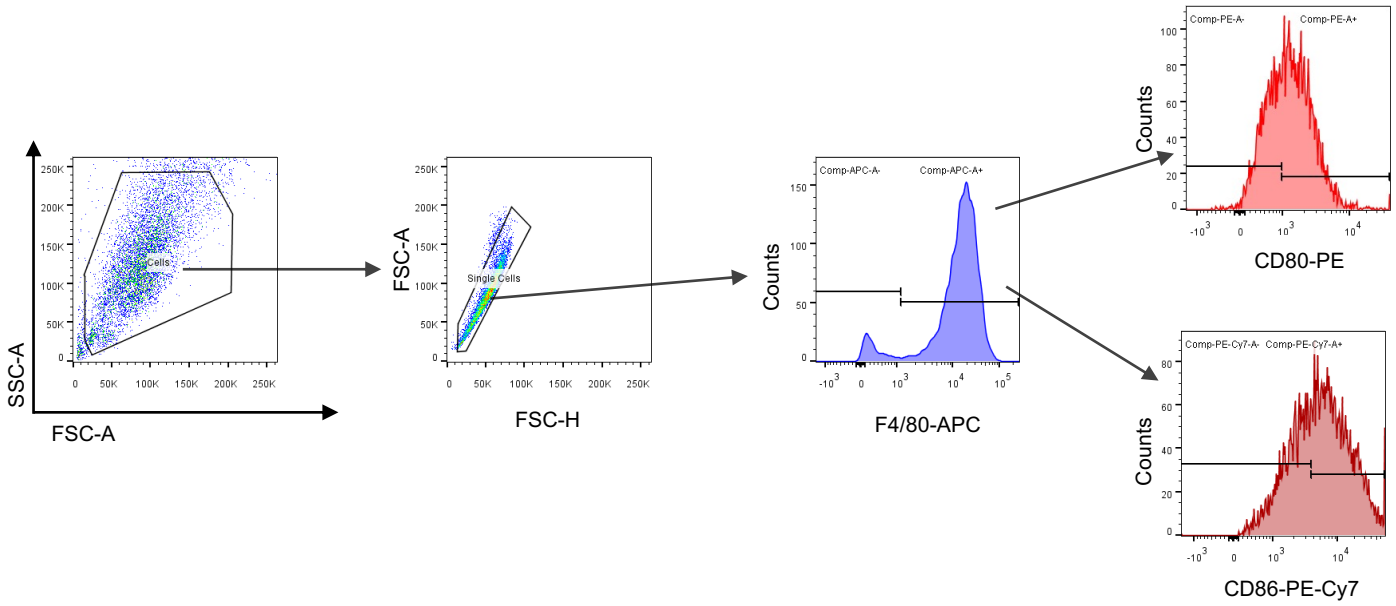
Supplementary Fig. 2. fMLP induces neutrophil chemotaxis. The relative number of neutrophils migrating through transwells was significantly increased upon fMLP (1 μM) treatment. (n = 3 biological replicates). Unpaired two-sided *t*-tests were used for comparison. Data presented as mean ± SD.



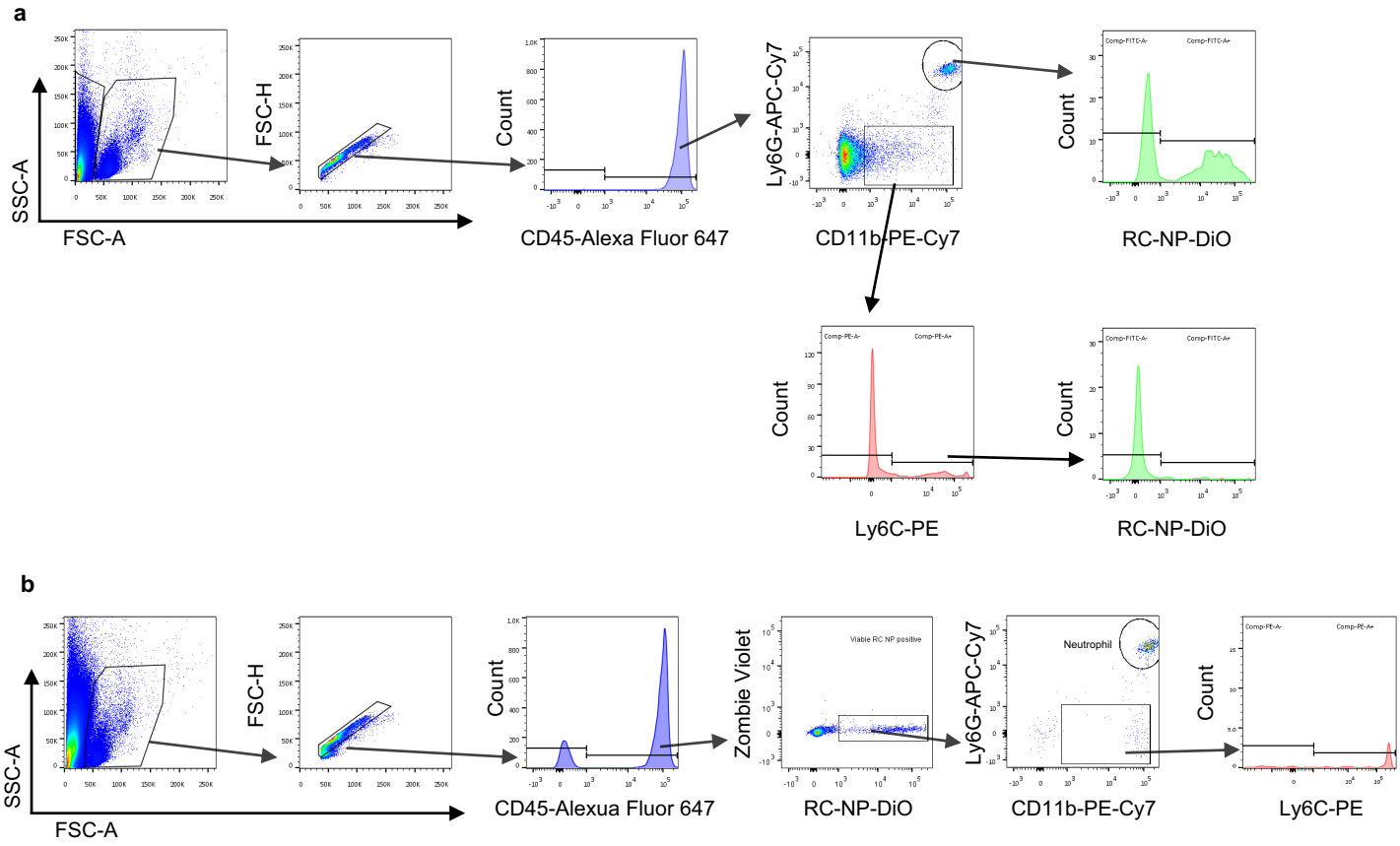
Supplementary Fig. 3. Neutrophil viability in vitro. Representative scatter plots after the in vitro treatments described in Fig. 2k. Neutrophils were isolated by density centrifugation with over 90% purity as determined by flow cytometry. Nonviable neutrophils were defined as $Ly6G^{+}CD11b^{+}$Zombie $Violet^{+}$ cells.



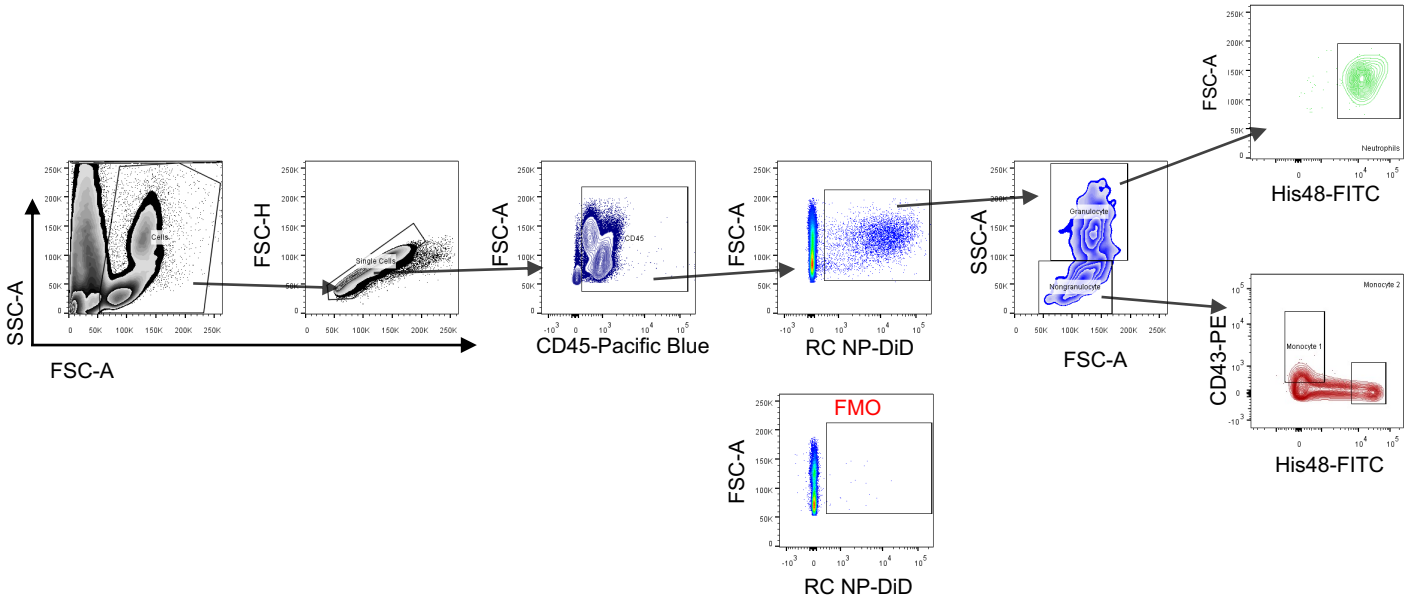
Supplementary Fig. 4. PMA treatment significantly reduces neutrophil apoptosis. Representative immunofluorescence images (left) and quantitative analysis (right) of TUNEL-stained neutrophils 15 hours after PBS or PMA (750 nM) treatment. (n = 6 for PMA, 8 for PBS). Scale bars, 50 μm. Unpaired two-sided *t*-tests were used for comparison. Data presented as mean ± SD.



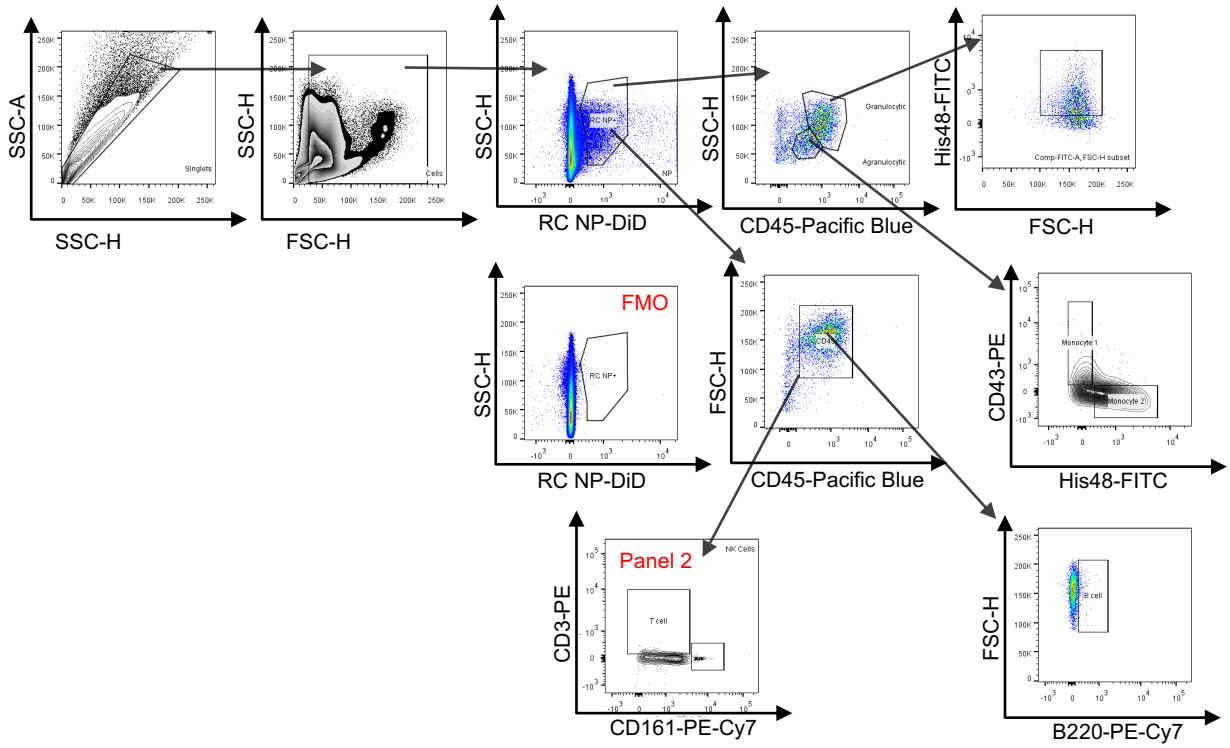
Supplementary Fig. 5. Analysis of macrophage phenotypes after coculture. Representative scatter plots for Fig. 4c, d. Macrophages were defined as F4/80$^+$ cells.



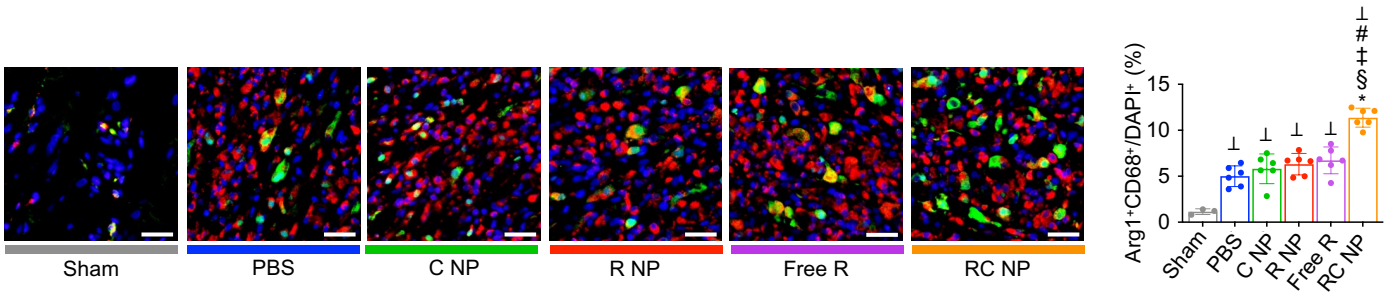
Supplementary Fig. 6. Analysis of nanoparticle uptake by circulating neutrophils and monocytes in vivo. Representative scatter plots for Fig. 5a, b. Neutrophils were defined as $CD45^+Ly6G^+CD11b^+$ cells and monocytes were defined as $CD45^+Ly6g^-CD11b^+Ly6C^+$ cells. RC NPs were labelled with DiO. **a** The percentage of RC NP^+ neutrophils and monocytes was determined by analyzing the percentage of neutrophils or monocytes positive for the DiO signal while **b** the percentage of circulating phagocytes responsible for RC NP uptake amongst all circulating leukocytes was calculated by analyzing the percentage of $CD45^+DiO^+$ cells which were monocytes and neutrophils.



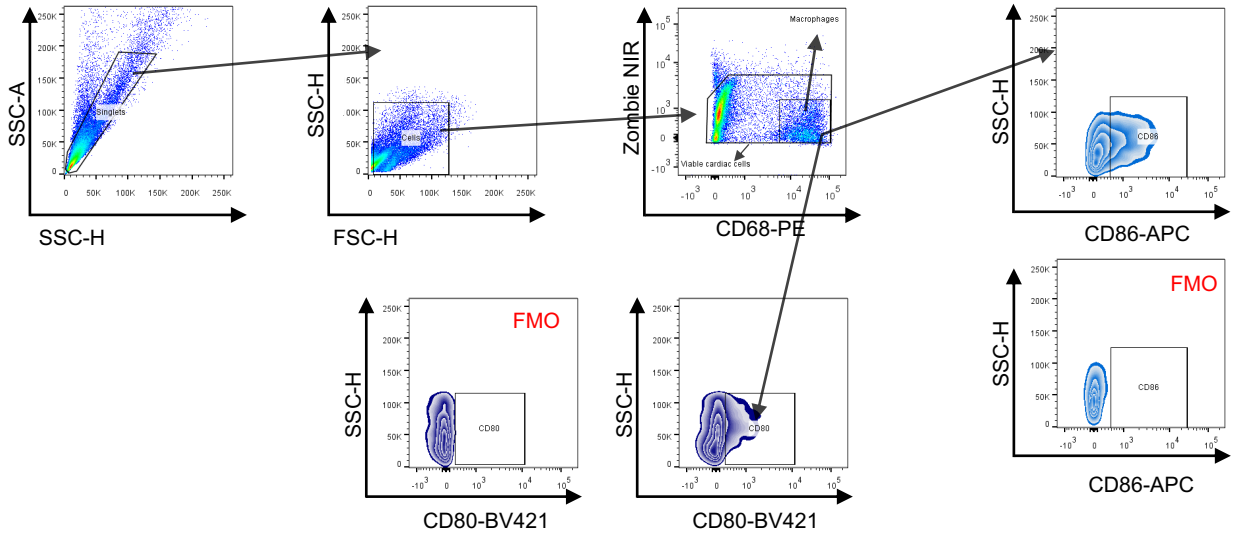
Supplementary Fig. 7. Analysis of nanoparticle uptake by neutrophils in rat blood. Representative scatter plots for Fig. 5b. Neutrophils were defined as $CD45^{+}His48^{+}SSC^{high}$ cells while monocytes were defined as $CD45^{+}CD43^{high}His48^{low}$ (nonclassical) and $CD45^{+}CD43^{low}His48^{high}$ (classical) cells. RC NP uptake was determined by analyzing the percentage of cells positive for the DiD signal.



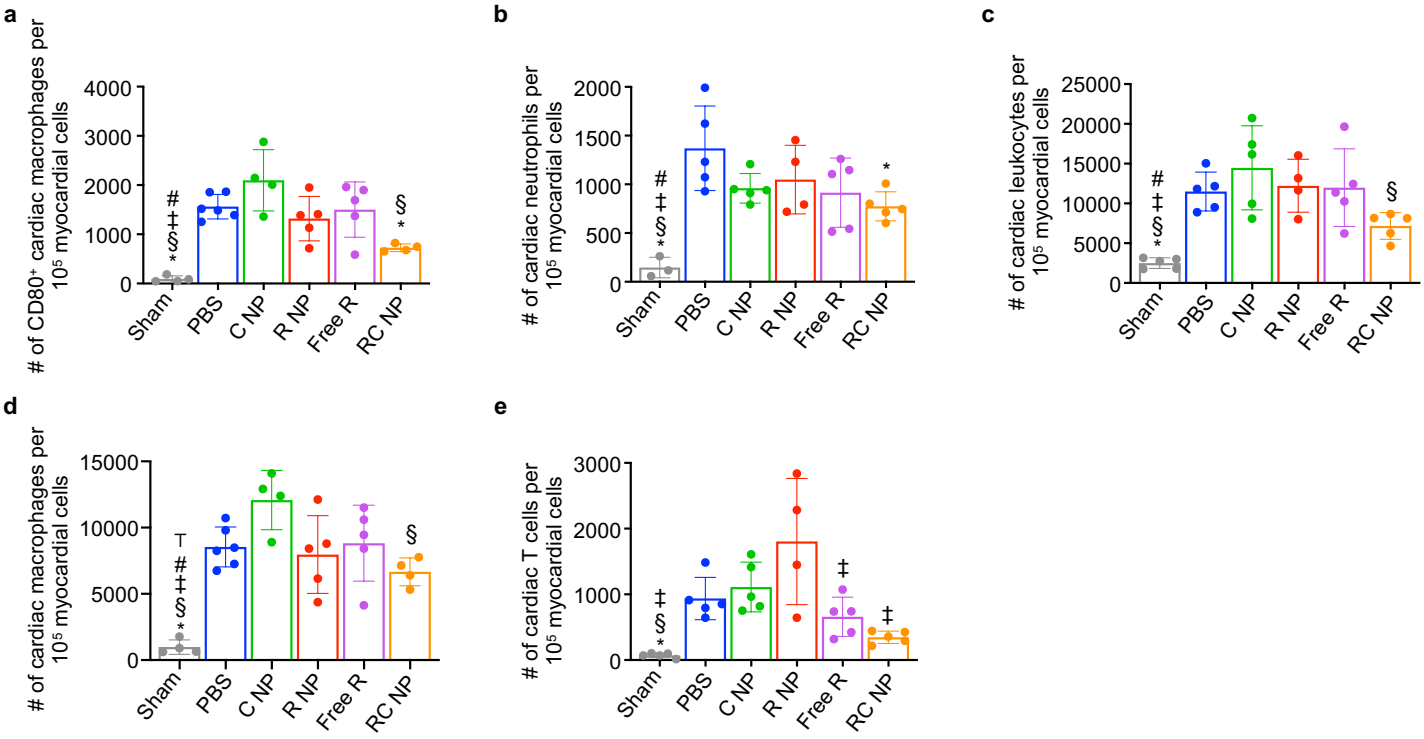
Supplementary Fig. 8. Analysis of nanoparticle uptake in rat hearts. Representative scatter plots for Fig. 5f. Neutrophils were defined as $CD45^{+}His48^{+}SSC^{high}$ cells, monocytes as $CD45^{+}CD43^{high}His48^{low}$ (nonclassical) and $CD45^{+}CD43^{low}His48^{high}$ (classical) cells, B cells as $CD45^{+}B220^{+}$ cells, T cells as $CD45^{+}CD3^{+}$ cells, and NK cells as $CD45^{+}CD161^{+}$ cells. RC NP uptake was determined by analyzing the percentage of cells positive for the DiD signal.



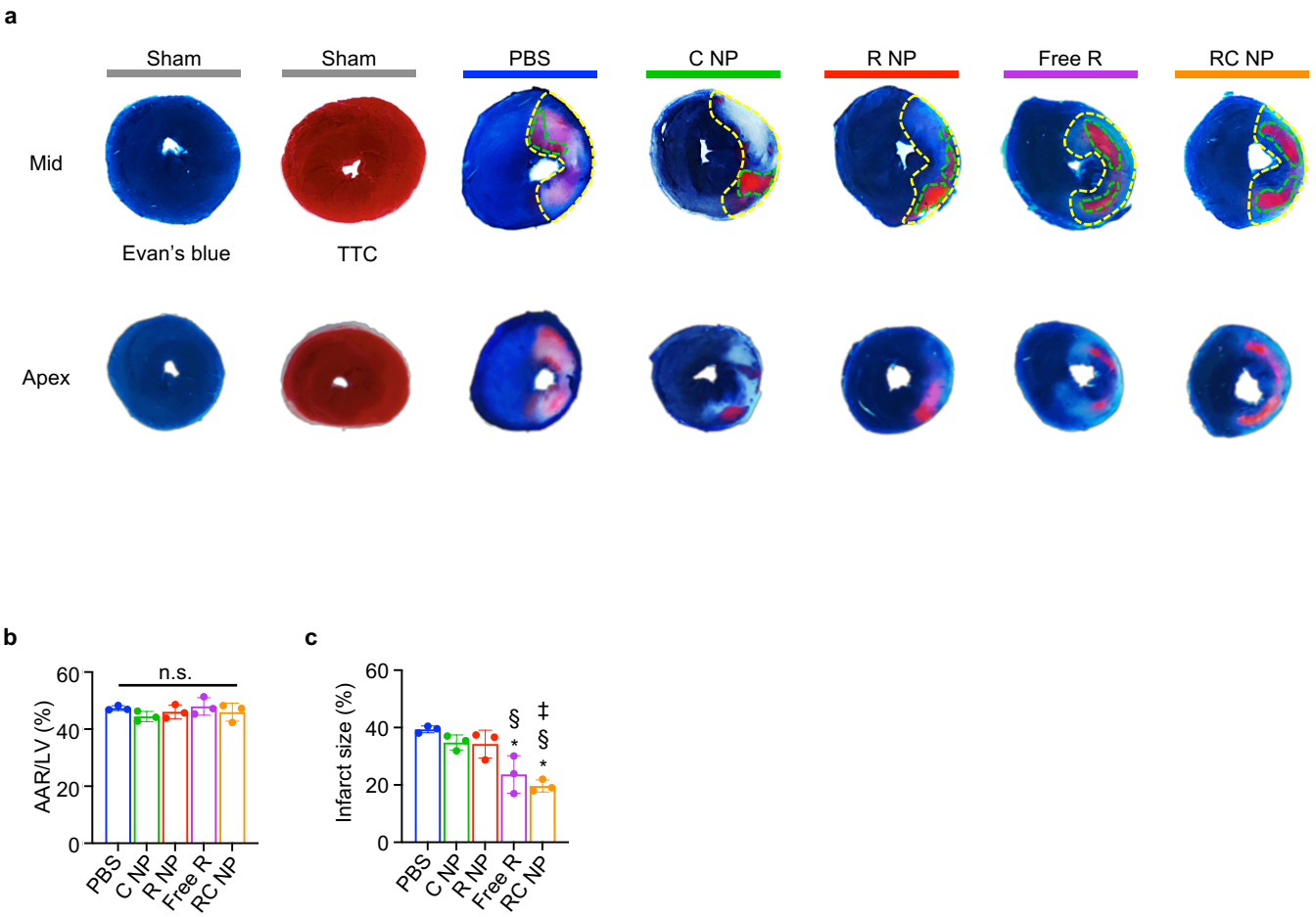
Supplementary Fig. 9. Arg1 expression in cardiac macrophages. Representative IHC images and quantitative analysis of Arg1$^+$ (green) macrophages (CD68$^+$, red) in infarcted hearts 3 days post-MI (n = 3 for sham, 6 for other groups). Scale bars, 25 μm. *p < 0.05 vs PBS; §p < 0.05 vs C NP; ‡p < 0.05 vs R NP; #p < 0.05 vs Free R; ⊥ p < 0.05 vs Sham. All data presented as mean ± SD. One-way ANOVA was used for all comparisons.



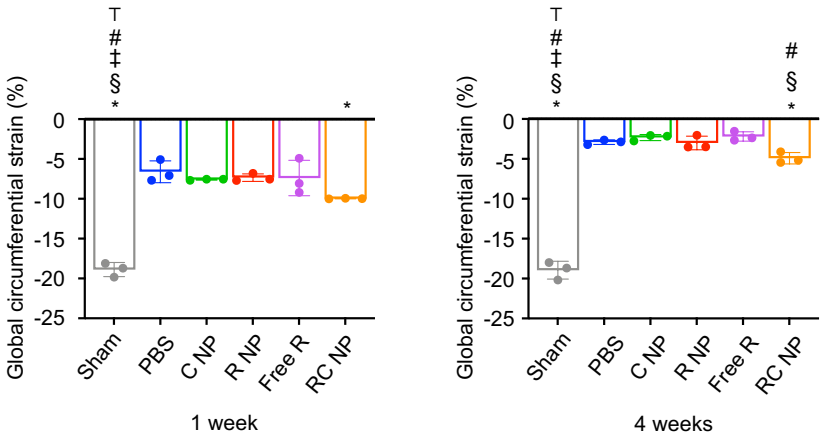
Supplementary Fig. 10. Analysis of macrophage phenotypes in the hearts of MI rats. Representative scatter plots for Fig. 6e. Macrophages were defined as $CD68^+$ cells.



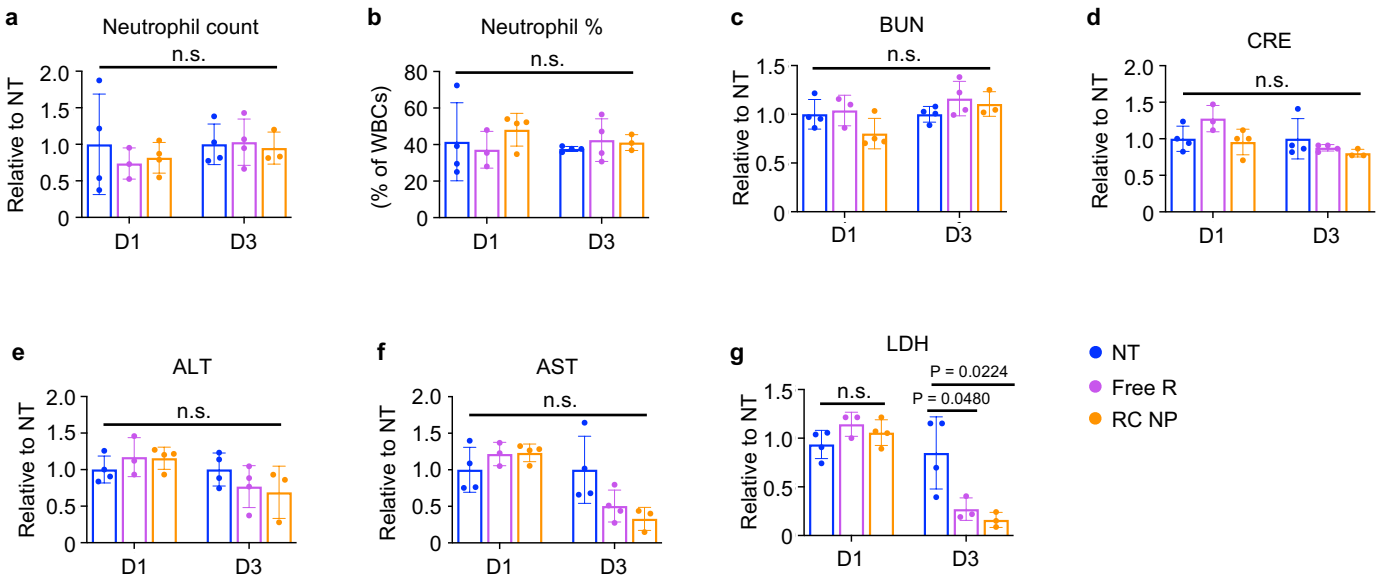
Supplementary Fig. 11. Number of various leukocyte subsets per 10^5 myocardial cells in the hearts of MI rats. Numbers of **a** M1 ($C80^+$) cardiac macrophages and **b** cardiac neutrophils, **c** cardiac leukocytes, **d** cardiac macrophages, and **e** cardiac T cells per 10^5 myocardial cells 5 days after MI as determined by flow cytometry. For **a** and **d**, n = 4 for sham and RC NP, 5 for R NP and Free R, and 6 for PBS. For **b**, n = 3 for sham, 4 for R NP, and 5 for other groups. For **c** and **e**, n = 4 for R NP and 5 for other groups. *p < 0.05 vs PBS; §p < 0.05 vs C NP; ‡p < 0.05 vs R NP; #p < 0.05 vs Free R; ⊤ p < 0.05 vs RC NP. All data presented as mean ± SD. One-way ANOVA was used for all comparisons.



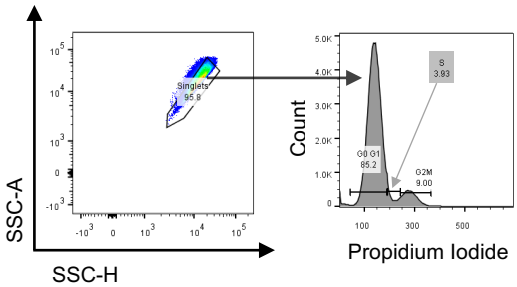
Supplementary Fig. 12. Infarct sizes 24 hours after MI. a Evan's blue and TTC staining of infarcted hearts 24 hours after MI. Yellow dotted lines indicate area at risk (AAR); green lines indicate viable myocardium area within the area at risk; white area (unstained area) indicates infarct size. Quantification of (**b**) AAR and (**c**) infarct sizes (n = 3). *p < 0.05 vs PBS; §p < 0.05 vs C NP; ‡p < 0.05 vs R NP; n.s., not significant. One-way ANOVA was used for all comparisons. All data presented as mean ± SD.



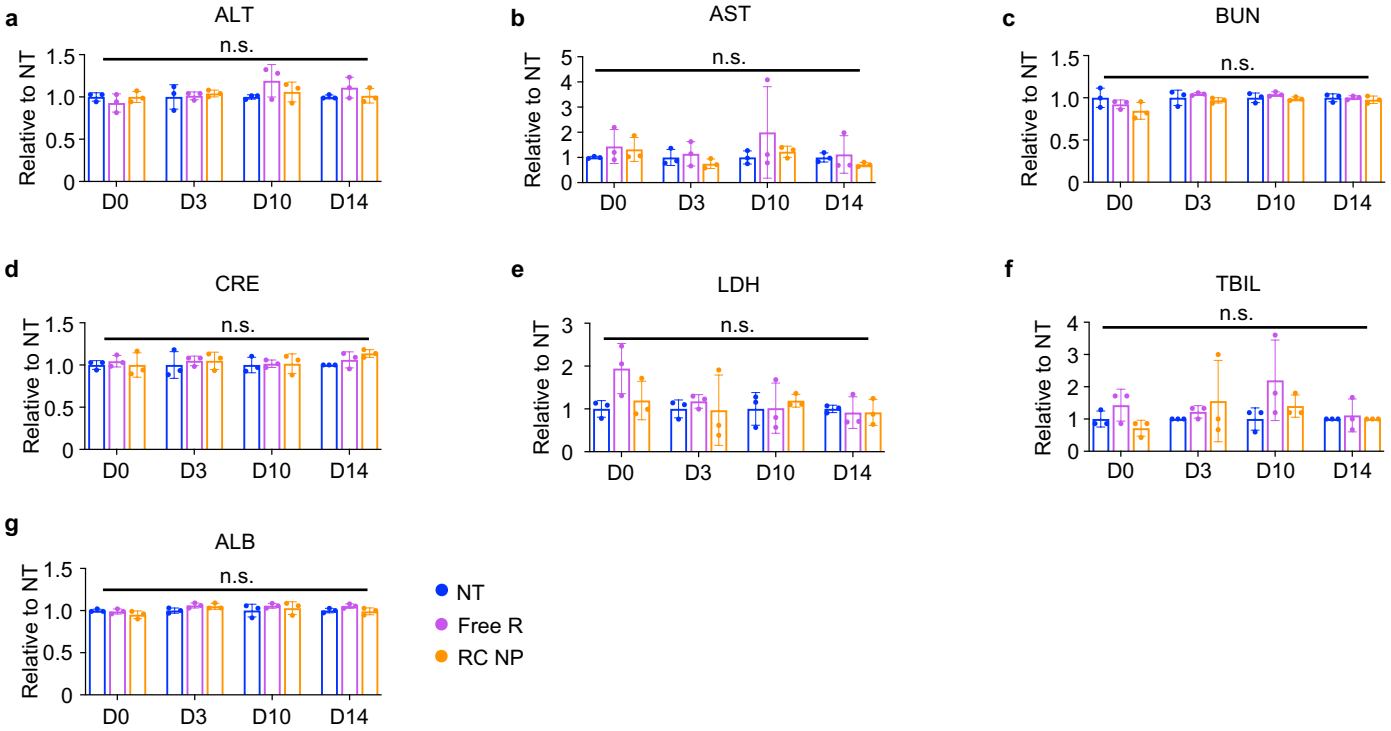
Supplementary Fig. 13. Cardiac strain analysis 4 weeks after MI. Global circumferential strain measurements 1 and 4 weeks after MI (n = 3). *p < 0.05 vs PBS; §p < 0.05 vs C NP; ‡p < 0.05 vs R NP; #p < 0.05 vs Free R; ⊤p <0.05 vs RC NP. All data presented as mean ± SD. One-way ANOVA was used for comparisons.



Supplementary Fig. 14. RC NP biocompatibility in MI rats. Blood profiles of MI rats receiving no injection, Free R injection, or RC NP injection 1 and 3 days after injection. (n =3 for D1 Free R, D3 RC NP, and D3 Free R LDH, 4 for other groups); n.s, not significant). **a** Relative neutrophil counts, **b** neutrophil percentage, **c** relative BUN blood urea nitrogen levels, **d** relative CRE creatinine levels, **e** Relative ALT alanine aminotransferase levels, **f** relative AST aspartate aminotransferase levels, and **g** relative LDH lactate dehydrogenase levels. All data presented as mean ± SD. One-way ANOVA was used for comparisons.



Supplementary Fig. 15. Analysis of cell-cycle arrest in rats. Representative scatter plots for Fig 10c.



Supplementary Fig. 16. RC NP biocompatibility in uninjured rats. Blood serum chemistry of uninjured rats receiving no injection, Free R injection, or RC NP injection 3, 10, and 14 days after injection. Day 0 indicates blood serum profiles immediately before intravenous injection **a** Relative ALT alanine aminotransferase levels, **b** relative AST aspartate aminotransferase levels, **c** relative BUN blood urea nitrogen levels, **d** relative CRE creatinine levels, **e** relative LDH lactate dehydrogenase levels, **f** relative TBIL total bilirubin levels, and **g** relative ALB albumin levels. (n =3; n.s, not significant). All data presented as mean ± SD. Two-way ANOVA was used for comparisons.

Gene	Forward	Backward
Gapdh (mouse)	ATGTGTCCGTCGTGGATCTGA	TGCCTGCTTCACCTTCT
Nos2 (mouse)	CTTTGCCACGGACGAGAC	TCATTGTACTCTGAGGGCTGAC
Mertk (mouse)	GTGGCAGTGAAGACCATGAAGTTG	GAACTCCGGGATAGGGAGTCAT
Gapdh (rat)	CCTGGCCAAGGTCATCCATGACAACTTTGG	GCCATGAGGTCCACCACCCTGTTGCTGTAG
Bax (rat)	CTGCAGAGGATGATTGCTGA	GATCAGCTCGGGCACTTTAG
Bcl2 (rat)	GCTACGAGTGGGATACTG	GTGTGCAGATGCCGGTTC
Bclxl (rat)	ATGTCTCAGAGAACCGGC	TCACTTCCGACTGAAGAGTG

Supplementary Table 1. Primers used for qRT-PCR analyses