Supplementary Results

Macrophage Engineered Vesicles for Therapeutic Delivery and Bidirectional Reprogramming of Immune Cell Polarization

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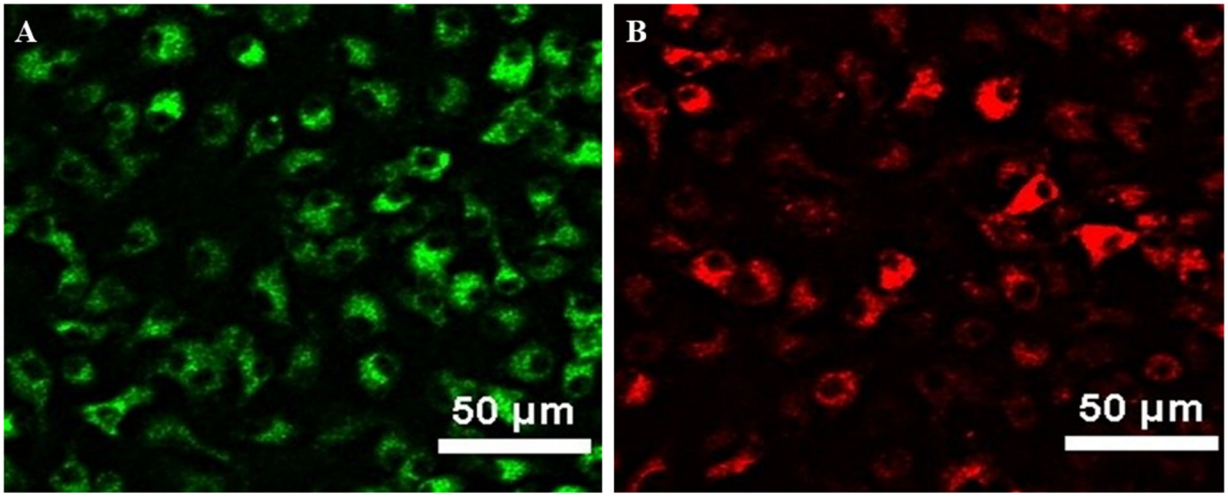

Figure S1. M1EVs as drug delivery systems to deliver cargo to the interior of cells. A. Confocal image showing M0 macrophages exposed to fluorescein (soluble dye) loaded vesicles exhibit fluorescence after 2 hours of interaction with fluorescein loaded vesicles. B. Confocal image of DiI-labelled M1EVs delivered to M0 macrophages after 2 hours of interaction showing clear uptake of vesicles by macrophages.

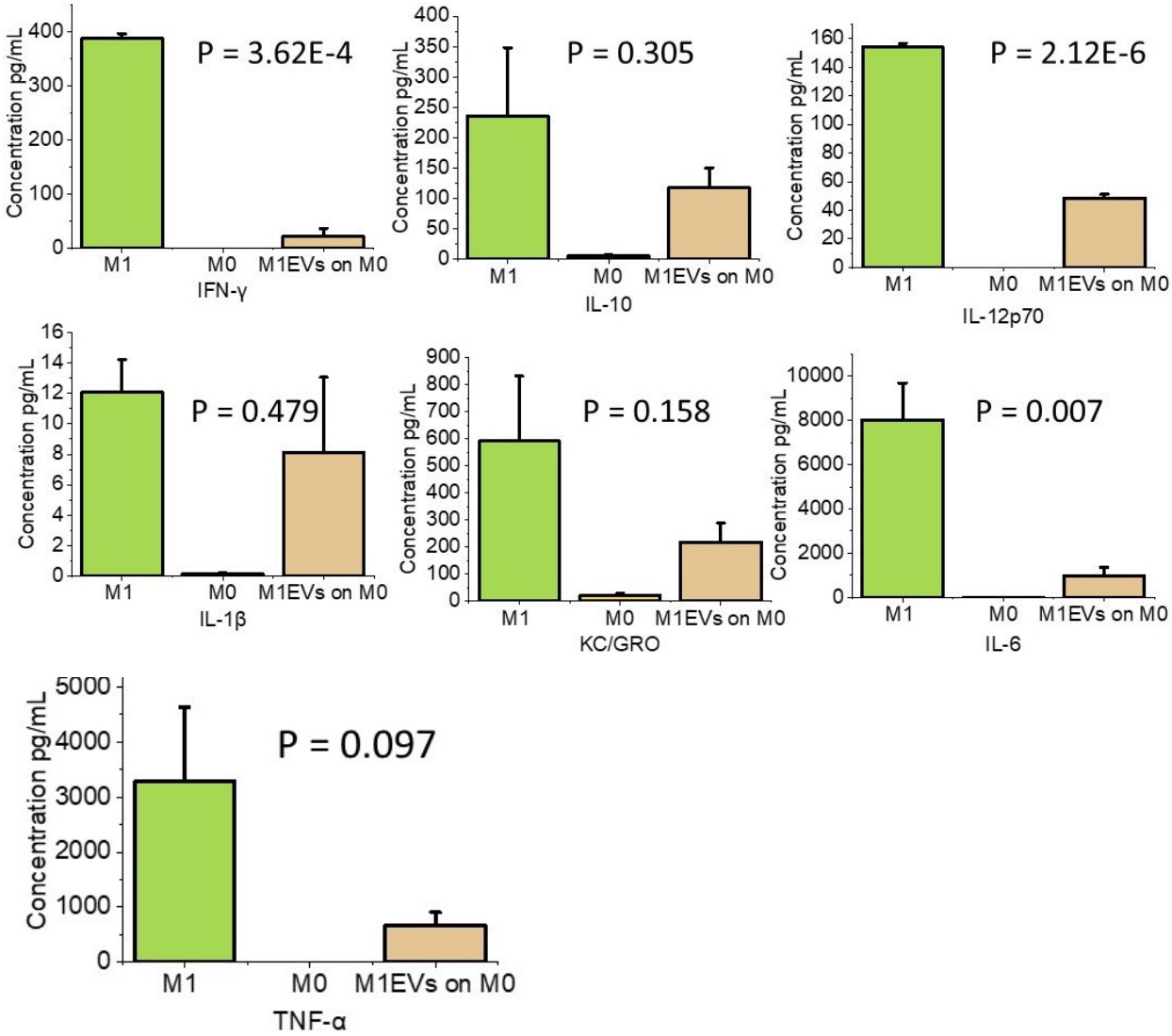

Figure S2. Quantification of cytokine expression my M0 macrophages when left to incubate the with M1EVs *in vitro*. Each data point is the average of at least 3 experiments. One-Way ANOVA was done to test the statistical significance of the results. The data are presented as the mean ± standard error of mean (SEM).

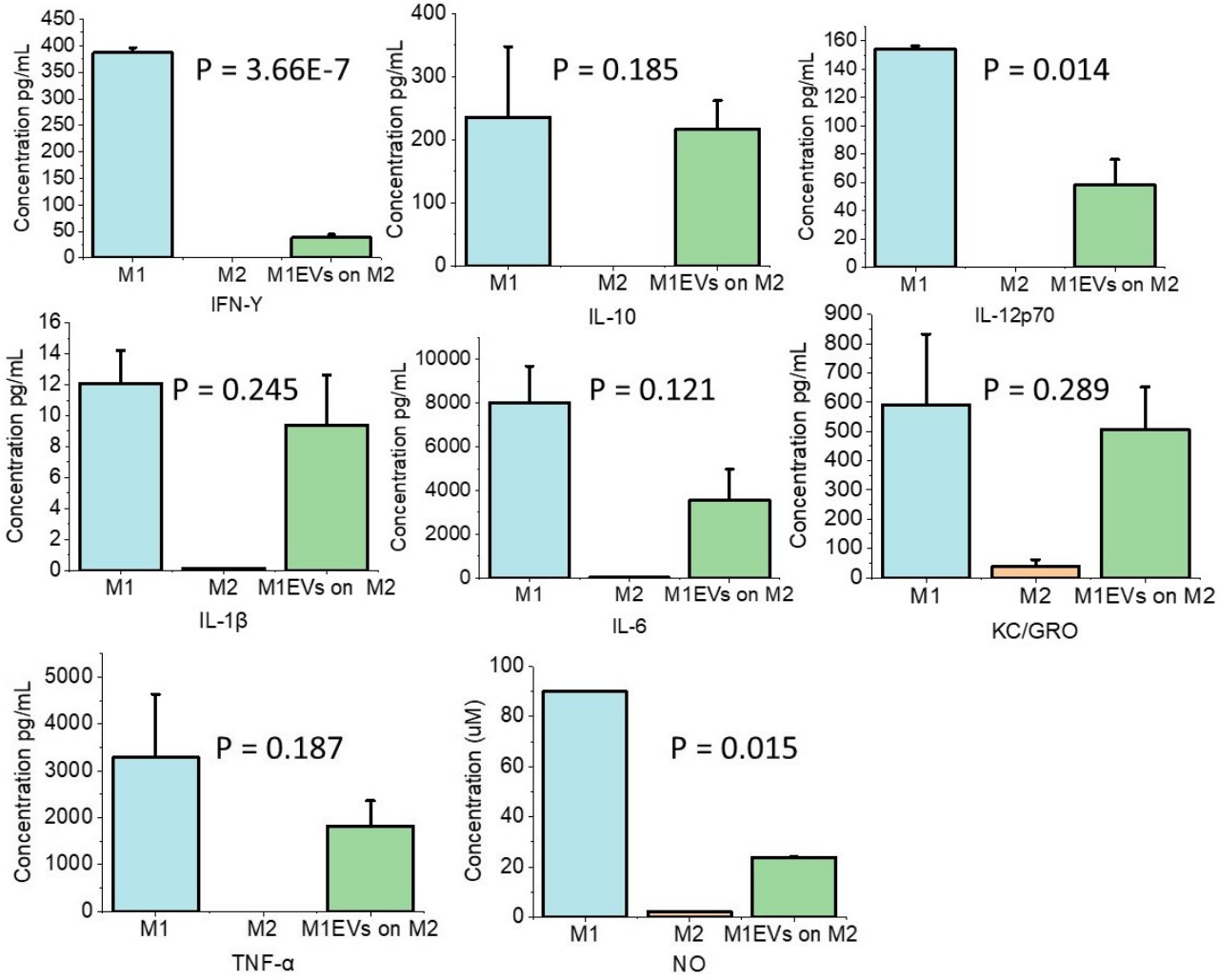

Figure S3. Polarization of M2 macrophages towards M1 macrophages *in vitro* when treated with M1EVs. Each data point is the average of at least 3 experiments. One-Way ANOVA was done to test the statistical significance of the results. Data are presented as the mean ± standard error of mean (SEM).

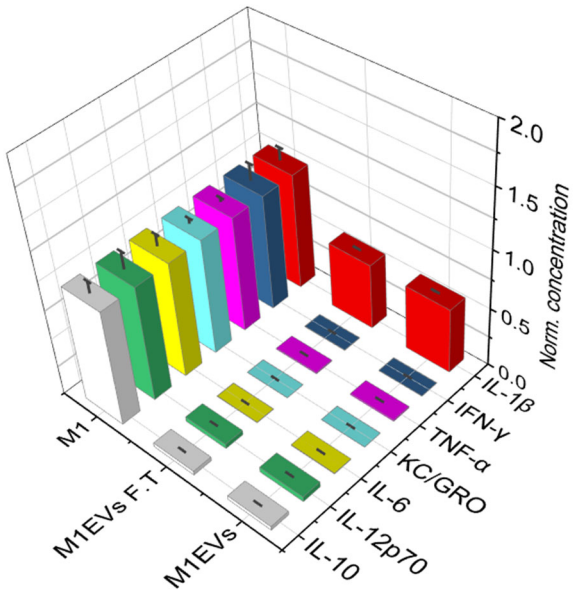

Figure S4. Quantification of pro-inflammatory cytokines present on M1EVs. M1EVs were freeze-thawed (M1EVs F.T) to break them and release the encapsulated cargo if present inside vesicles.

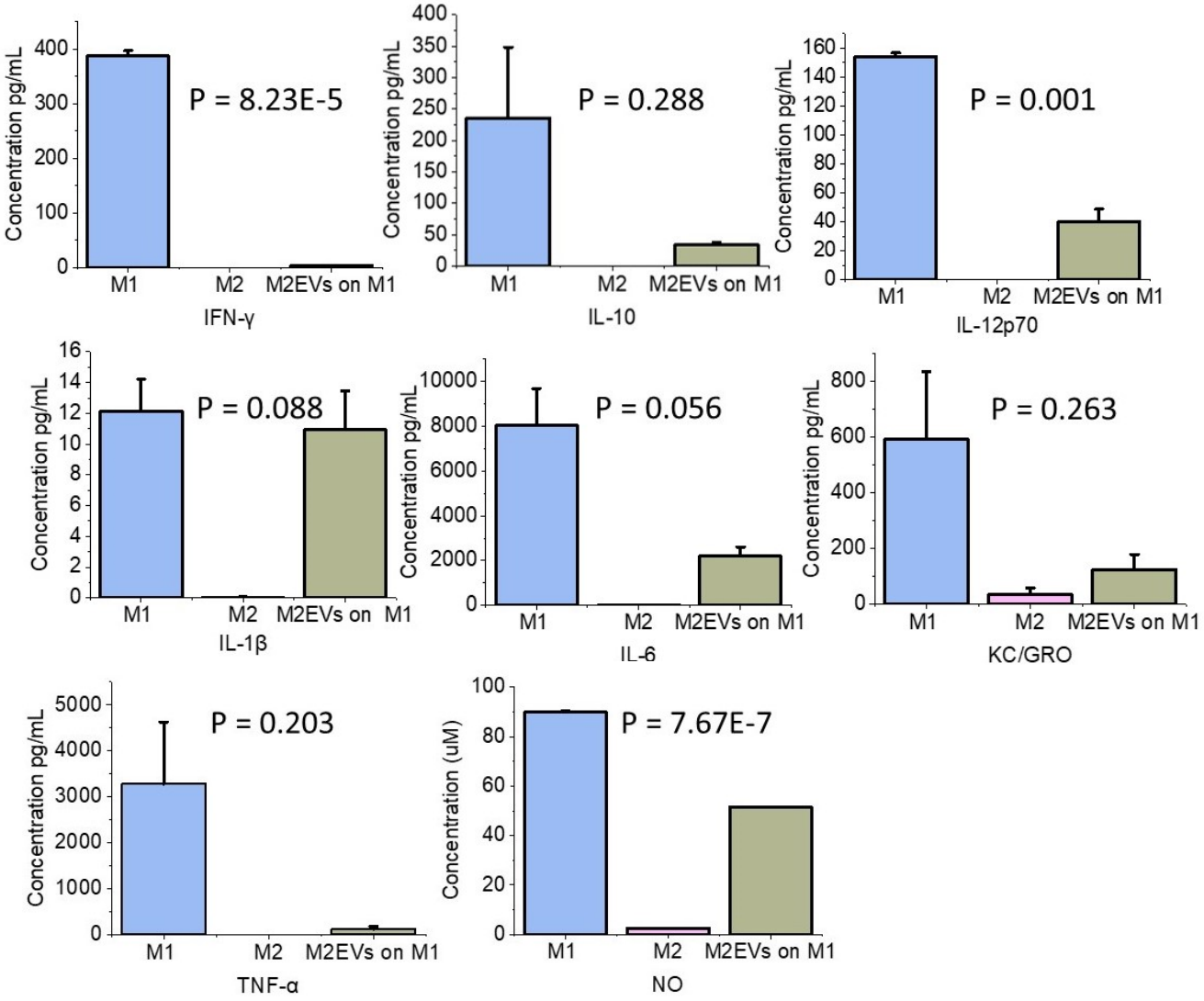

Figure S5. Polarization of M1 macrophages towards M2 macrophages *in vitro* when treated with M2EVs. Each data point is the average of at least 3 experiments. One-Way ANOVA was done to test the statistical significance of the results. The data are presented as the mean ± standard error of mean (SEM).

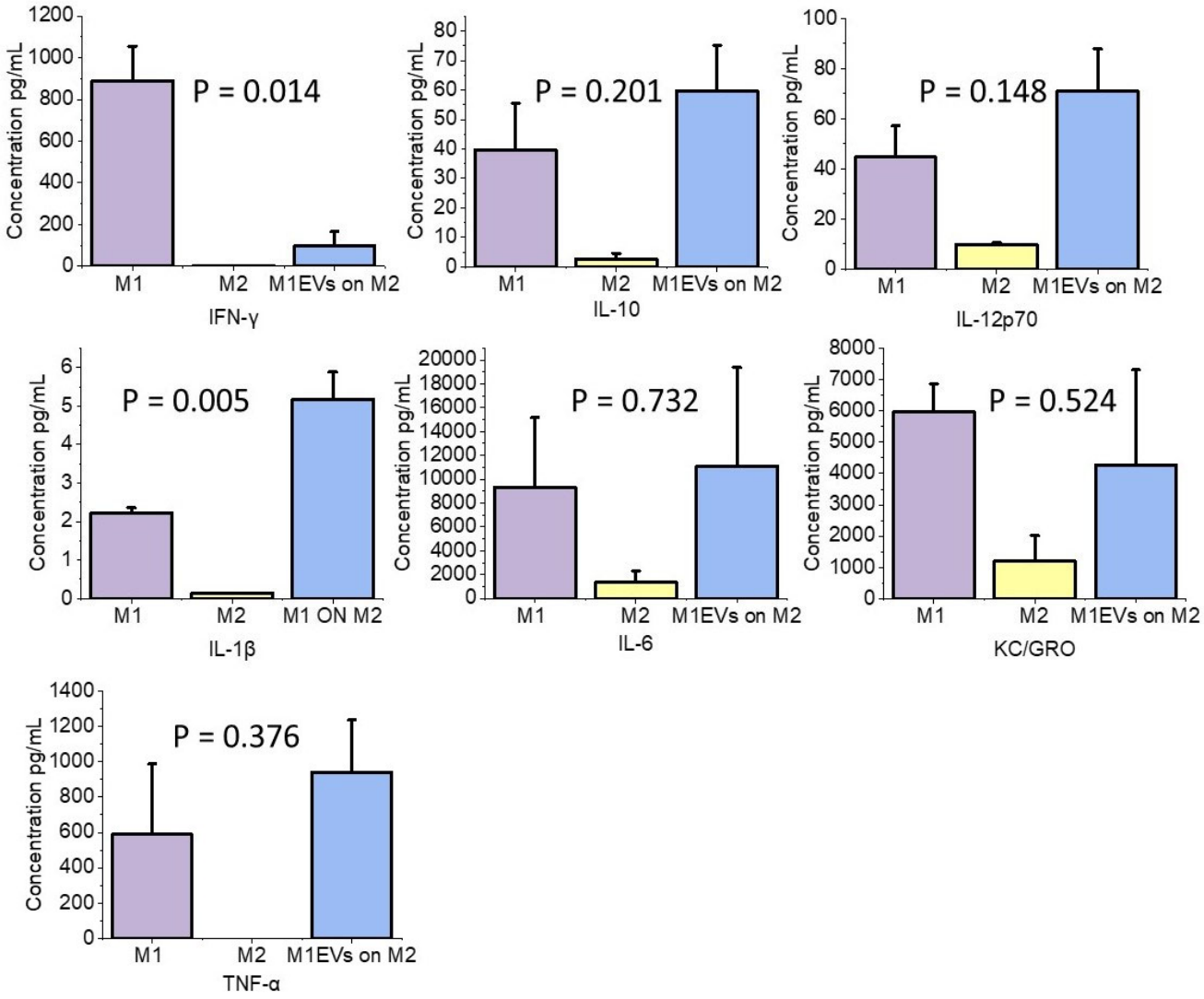

Figure S6. Quantification of cytokine released by M1 microglia, M2 microglia and M2 microglia that were incubated with M1EVs. Each data point is the average of at least 3 experiments. The data are presented as the mean ± SEM. One-Way ANOVA was done to test the statistical significance of the results.

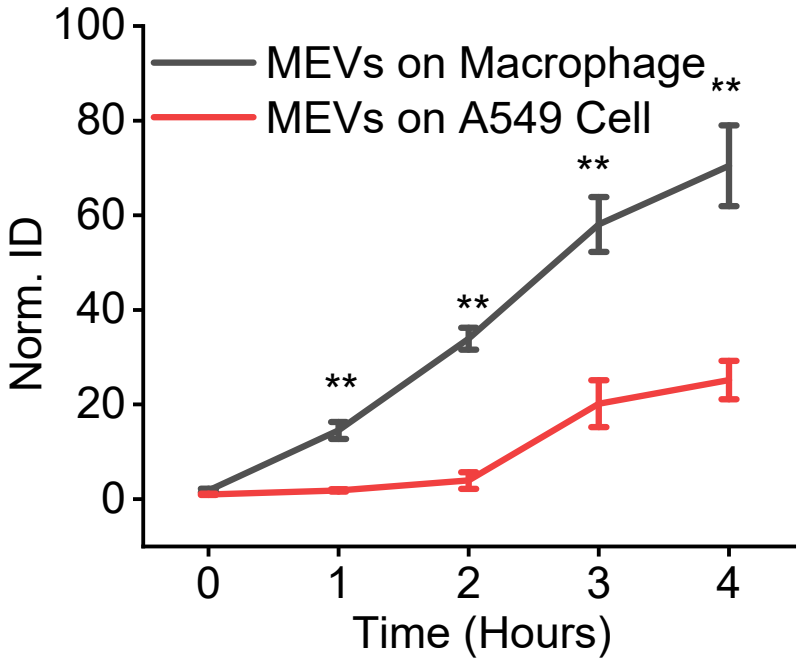

Figure S7. Specificity of targeting of MEVs on macrophages and A549 cells. Each datapoint is the average of five independent replicates. Norm. ID is the mean integrated density of the image normalized to the mean integrated density value of macrophages prior to vesicles addition. The data are presented as the mean ± SEM (n=5). **P < 0.01 indicates a significant difference in the vesicle uptake by macrophages and A549 cells at respective time points.