# Supplementary Figures and Tables

# miR-124-5p regulates phagocytosis of human macrophages by targeting the actin cytoskeleton via the ARP2/3 complex

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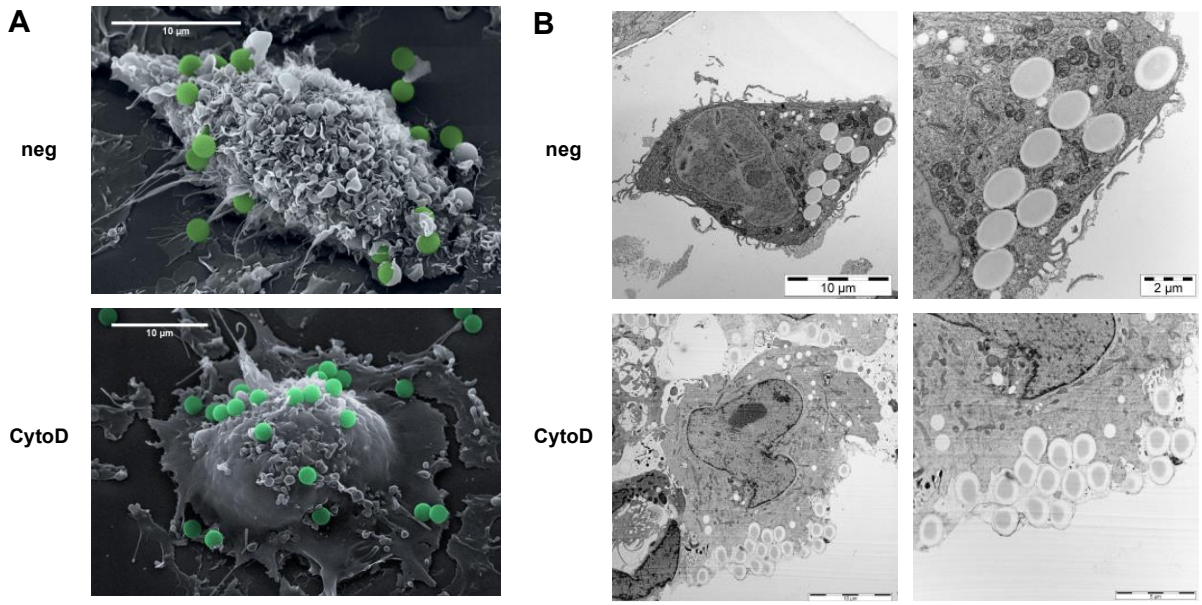
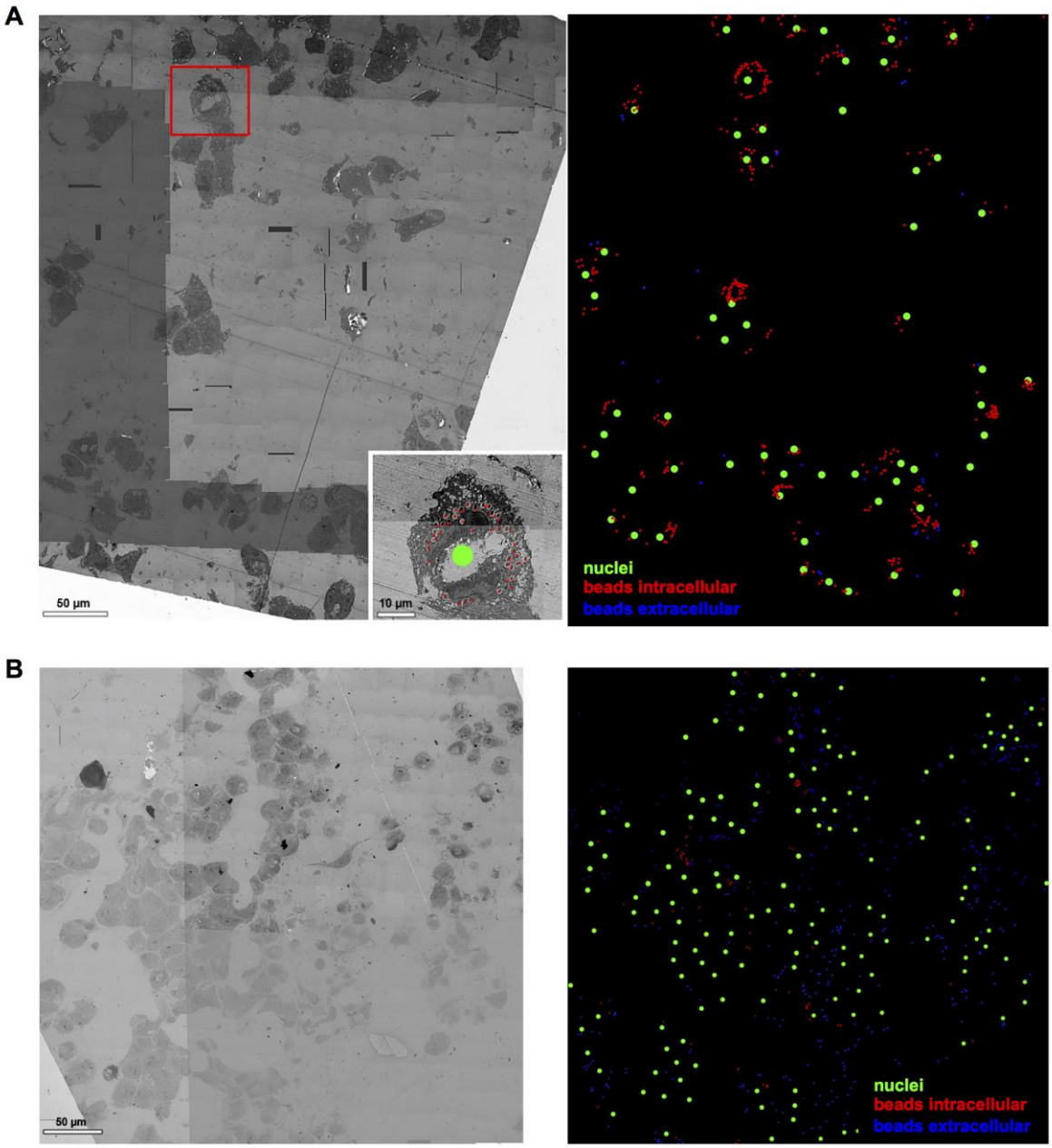
## Supplementary Figures




**<u>Supplementary Figure S1:</u> Electron microscopy to confirm reduced levels of intracellular latex beads in PMA-activated THP-1 MΦ treated with cytochalasin D.** (A) SEM and (B) TEM images of untreated (neg) or cytochalasin D-treated PMA-activated (CytoD) THP-1 MΦ.



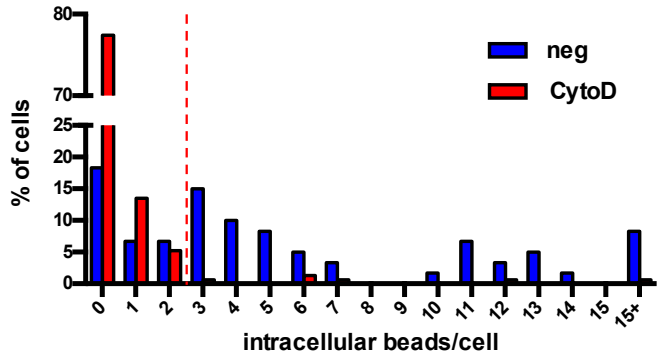

**<u>Supplementary Figure S2:</u> Electron microscopy of PMA-activated THP-1 cells phagocytosing latex beads.** TEM panorama images of untreated (A) or cytochalasin D-treated, PMA-activated THP-1 Mq (B). Individual, high resolution images were aquired on a Zeiss 912 transmission electron with 14 nm per pixel and collated together to a panoramic overview across the entire specimen (left panels). These images were imported into Adobe Photoshop CC 2017 and processed by labeling of nuclei with green dots, intracellular beads by red dots and extracellular beads by blue dots as shown in the inset in (A). Subsequently, the image

background was replaced by a uniform black background (right panels), and green, red, and blue dots were automatically to provide the numbers of cells, intra- and extracellular beads.

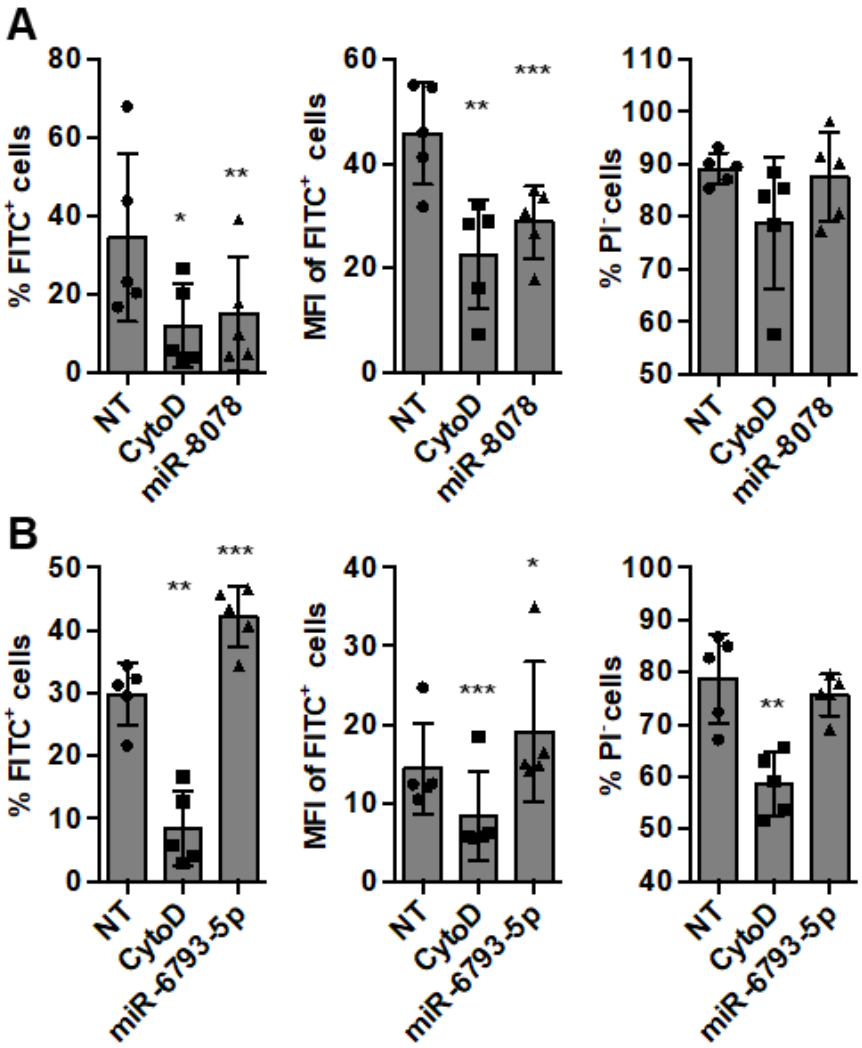
A

| | neg | CytoD |
|---|---|---|
| Total cells | 60 | 155 |
| Intracellular beads | 394 | 84 |
| Extracellular beads | 37 | 482 |
| % intracellular beads | 91.4 % | 14.8 % |
| Intracellular beads/cell | 6.6 | 0.5 |
| Extracellular beads/cell | 0.6 | 3.1 |

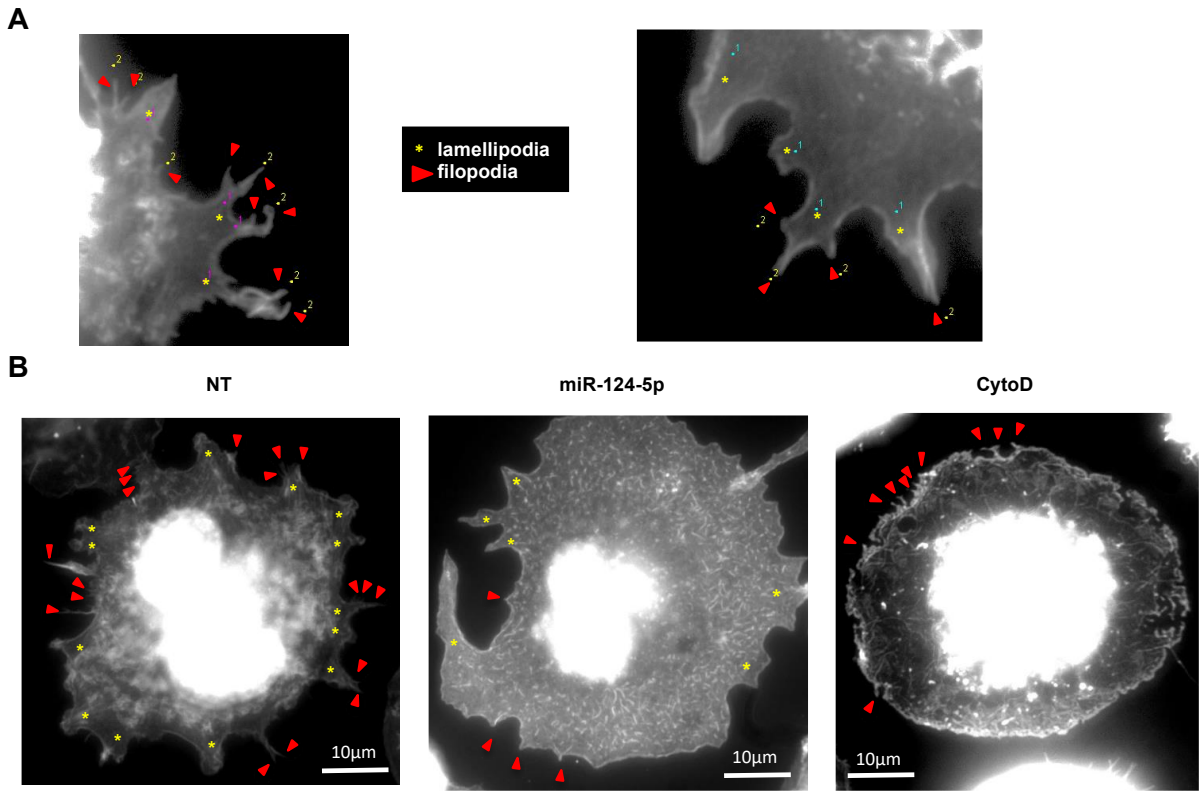
B




**<u>Supplementary Figure S3:</u> Quantitative image analysis on intra- and extracellular beads counted in cells of panorama TEM images shown in Supplementary Figure S2).** Values in (A) are cumulative numbers of cells and beads counted in all cells analyzed whereas values in (B) are percentage of cells with the indicated number of intracellular beads.




**<u>Supplementary Figure S4:</u> Validation of the results obtained in the screens for miR-8078 and miR-6793-5p.** Phagocytosis of FITC-labeled, opsonized latex beads by PMA-activated THP-1 MΦ treated with an NT siRNA (NT), cytochalasin D (CytoD) or mir-8078 (A) or miR-6793-5p (B) as percentage of phagocytic cells (% $FITC^+$ cells, left), phagocytic activity (MFI of $FITC^+$ cells, middle) and viability (% $PI^-$ cells) across n = 5 independent experiments. Statistical analysis was performed using repeated measures, one-way ANOVA with Geisser-Greenhouse correction. Dunnett's post analysis was used to calculate *p*-values adjusted for multiple comparisons with the NT set as control condition (*: $p < 0.05$; **: $p < 0.01$; ***: $p < 0.001$)




**<u>Supplementary Figure S5:</u> Fluorescence microscopic analysis of the effect of miR-124-5p on formation of filopodia and lamellipodia.** Fluorescence microscopy of PMA-activated THP-1 MΦ following the indicated treatments. Actin cytoskeleton was stained with Alexa Fluor 488-phalloidin and nuclei with Hoechst 33342. Images were aquired with a Zeiss AxioObserver Z1 using a 100× objective (scale bars: 10 µm) and fluorescence intensity of phalloidin-stained actin is shown. (A) Digital magnification on a parts of two NT-treated control cells to illustarte how lamellipodia (asterisks) and filopodia (red triangles) were manually labeled for subsequent image analysis. (B) Representative cells for each condition. Labeled lamellipodia and filopodia were automatically counted using the ImageJ software. Quantitative analysis of 47-57 individual cells across n = 5 independent experiments are shown in Figure 5B.

## Supplementary Tables

**Supplementary Table S1:** Predicted binding sites of miR-124-5p in potential target gene mRNAs cloned into a pmirGLO® Dual Luciferase miRNA target expression vector.

| Gene | Target sequence | Location[1] | ΔG [°C][2] | Database |
|---|---|---|---|---|
| *ARPC1A* | CAACAUGUCUGCCAUGGAACGCU | 1009-1031 (CDS) | -22.4 | RNA22 v2.0 |
| *ARPC1B* | AACAAGUUUGCUGUGGGCAGCG | 634-655 (CDS) | -28.1 | RNA22 v2.0 |
| *ARPC2* | AAGCGGCUGGCAACUGAAGGCUGGAACACUUGCUACUG | 993-1025 (3'-UTR) | -23.5 | miRmap |
| *ARPC3* | UGUGAAGAGACAGUUCAUGCGUAA | 583-604 (CDS) | -13.8 | STarMir |
| *ARPC4* | GAAAAGGUUCUGAUUGAGGGCU | 237-258 (CDS) | -19.4 | RNA22 v2.0 |
| *ACTR2* | GGUGAUGAGGCAAGUGAAUUACG | 260-283 (CDS) | -16.5 | miRWalk2.0 |

**[1] Position of predicted binding motifs with nucleotides numbers and its location in the coding sequences (CDS) or 3' untranslated region (3'UTR) of target mRNA.**
**[2] Binding energy of the interaction with miR-124-5p and the respective target sequence predicted by the STarMir algorithm.**

**Supplementary Table S2:** Oligonucleotide primers used for qRT-PCR quantification of target gene expression levels.

| oligonucleotide | Sequence (5' → 3') | Reference[1] |
|---|---|---|
| | **qRT-PCR primers** | |
| ARPC1A_fwd<br>ARPC1A_rev | ATTGCCCTCAGTCCCAATAATCA<br>CAAGTGACAATGCGGTCGC | 300360514c1 |
| ARPC1B_fwd<br>ARPC1B_rev | CAAGGACCGCACCCAGATT<br>TGCCGCAGGTCACAATACG | 325197176c1 |
| ARPC2_fwd<br>ARPC2_rev | GCAGATTTCGATGGGGTCCTC<br>ACTCCCGTACACCCTCTTTAAT | 23238210c2 |
| ARPC3_fwd<br>ARPC3_rev | GTGCAATTCCAAAAGCCAAGG<br>GGCTCTCATCACTTCATCTTCC | 23397667c1 |
| ARPC4_fwd<br>ARPC4_rev | AACGACACAACAAGCCGGAA<br>GGCACAAAATCTTCTCGATCTCA | 5031595a3 |
| ARPC5_fwd<br>ARPC5_rev | TGGTGTGGATCTCCTAATGAAGT<br>CACGAACAATGGACCCTACTC | 23238212c1 |
| ARPC5L_fwd<br>ARPC5L_rev | ATCGACGAATTTGACGAGAACA<br>GAATGCCCGAAGCATGTCC | 13569955c1 |
| ACTR2_fwd<br>ACTR2_rev | GGCAGTTCTGACTTTGTACGC<br>CCAGTCTCCTGGTAAGATGAGG | 205361120c2 |
| ACTR3_fwd<br>ACTR3_rev | GGAGAACGGACGTTGACCG<br>TCCTGCGATTGGAATGTGTTT | 34452698c1 |
| GAPDH_fwd<br>GAPDH_rev | GAGTCAACGGATTTGGTCGT<br>TTGATTTTGGAGGGATCTCG | NCBI Primer BLAST |

**1: numbers indicate IDs in PrimerBank (https://pga.mgh.harvard.edu/primerbank/).**

**Supplementary Table S3:** Oligonucleotide primers used cloning of predicted wildtype or mutated miR-124-5p binding sites for dual luciferase assays to probe miRNA-mRNA interactions.

| oligonucleotide | Sequence (5' → 3') | Reference |
|---|---|---|
| Right_universal_rev | CTTTCCGCCTCAGAAGGTAC | this study |
| Left_universal_fwd | CGTCCGGCGTAGAGGATCGA | this study |
| ARPC1A_WT_right_fwd | CAACATGTCTGCCATGGAACGCTCCTCGAGTCTAGAGTCGACCTG | this study |
| ARPC1A_WT_left_rev | AGCGTTCCATGGCAGACATGTTGCTAGCGAGCTCGTTTAAACA | this study |
| ARPC1B_WT_right_fwd | AACAAGTTTGCTGTGGGCAGCGCCTCGAGTCTAGAGTCGACCTG | this study |
| ARPC1B_WT_left_rev | CGCTGCCCACAGCAAACTTGTTCTAGCGAGCTCGTTTAAACA | this study |
| ARPC2_WT_right_fwd | GGCAACTGAAGGCTGGAACACTCCTCGAGTCTAGAGTCGACCTG | this study |
| ARPC2_WT_left_rev | AGTGTTCCAGCCTTCAGTTGCCCTAGCGAGCTCGTTTAAACA | this study |
| ARPC3_WT_right_fwd | GAAGAGACAGTTCATGAACAAGACCTCGAGTCTAGAGTCGACCTG | this study |
| ARPC3_WT_left_rev | TCTTGTTCATGAACTGTCTCTTCCTAGCGAGCTCGTTTAAACA | this study |
| ARPC3_MUT_right_fwd | GAAGAGACAGTTCATGCGTAAGACCTCGAGTCTAGAGTCGACCTG | this study |
| ARPC3_MUT_left_rev | TCTTACGCATGAACTGTCTCTTCCTAGCGAGCTCGTTTAAACA | this study |
| ARPC4_WT_right_fwd | GAAAAGGTTCTGATTGAGGGCTCCTCGAGTCTAGAGTCGACCTG | this study |
| ARPC4_WT_left_rev | AGCCCTCAATCAGAACCTTTTCCTAGCGAGCTCGTTTAAACA | this study |
| ARPC4_MUT_right_fwd | GAAAAGGTTCTGATAATTAAATCCTCGAGTCTAGAGTCGACCTG | this study |
| ARPC4_MUT_left_rev | ATTTAATTATCAGAACCTTTTC CTAGCGAGCTCGTTTAAACA | this study |
| ACTR2_WT_right_fwd | GGTGATGAGGCAAGTGAATTACGCCTCGAGTCTAGAGTCGACCTG | this study |
| ACTR2_WT_left_rev | CGTAATTCACTTGCCTCATCACCCTAGCGAGCTCGTTTAAACA | this study |