

Supporting information

Microplastics Interact with SARS-CoV-2 and Facilitate Host Cell Infection

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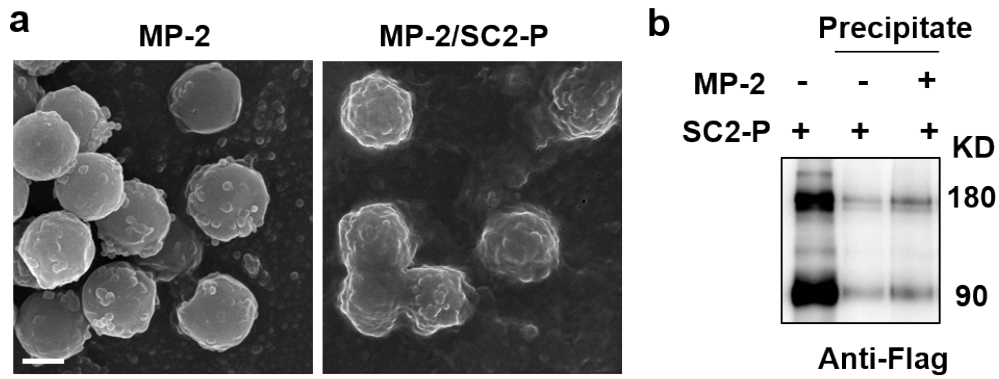
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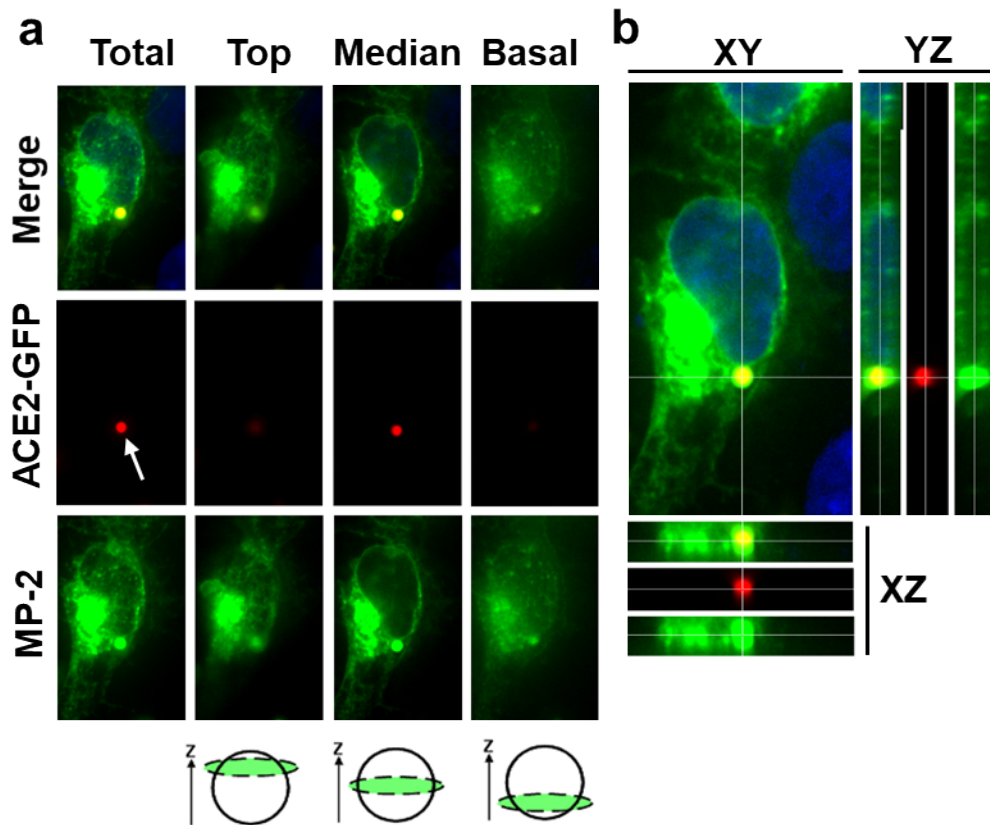
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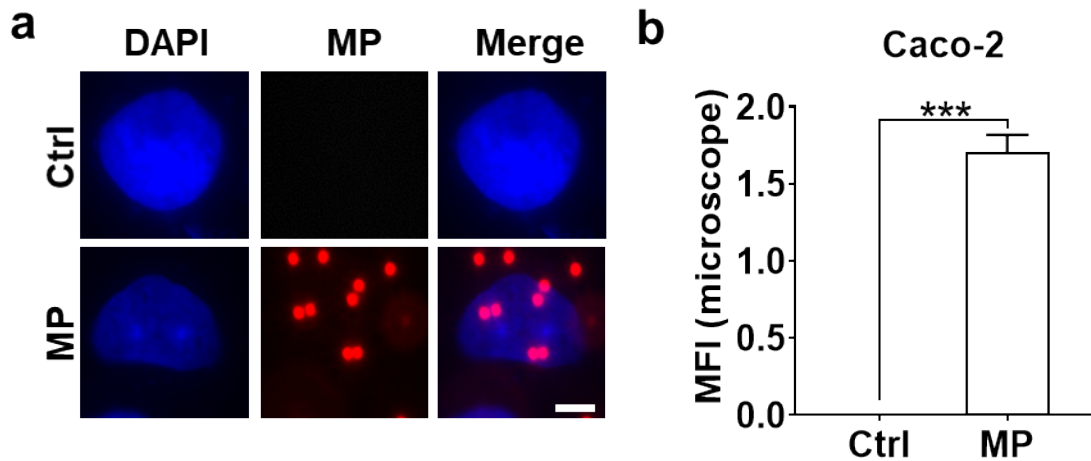
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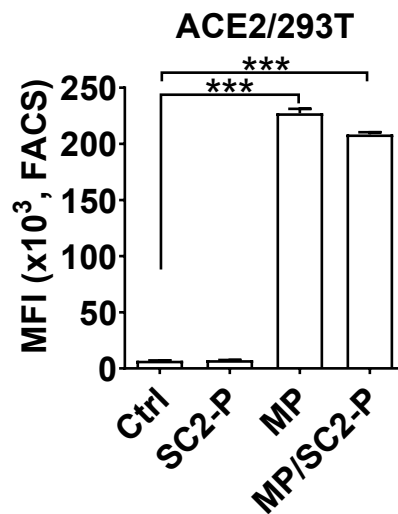
Supporting Information Figure S1. Surface adsorption of SARS-CoV-2 pseudovirus on microplastics. Red fluorescence-labeled MP (MP-2) were incubated with the SARS-CoV-2 pseudovirus (SC2-P) for 2 h. The MP-2/SC2-P complex was collected and characterized by SEM (a), and Western blotting (b).



Supporting Information Figure S2. Intracellular localization of microplastics. 293T/ACE2 cells were incubated with red fluorescence-labeled MP-2 for 24 h. MP-2 uptake was observed by confocal microscopy. A Z-axis scan of the MP-2 in 293T/ACE2 cells shows that MP-2 (white arrow) can only be observed in the median cell section (a). The 3D images of MP-2 in cell (b).

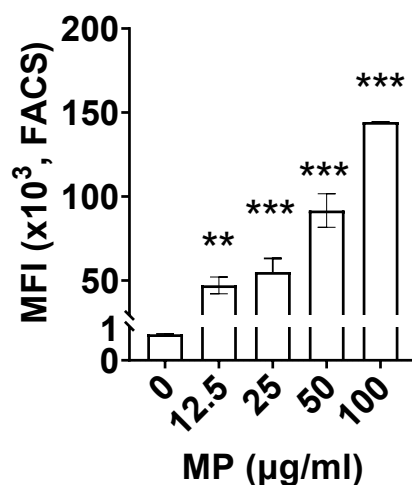


Supporting Information Figure S3. Association of microplastics with Caco-2 cells. Caco-2 cells were incubated with red fluorescence-labeled MP-2 for 24 h. MP-2 association to cells was observed by fluorescence microscopy (a). Mean fluorescence intensity (MFI) was analyzed by ImageJ (b). Bar = 5 μ m in panel a. Data are presented as mean \pm SEM (n=3). ***, p < 0.001 by Student's *t*-test.

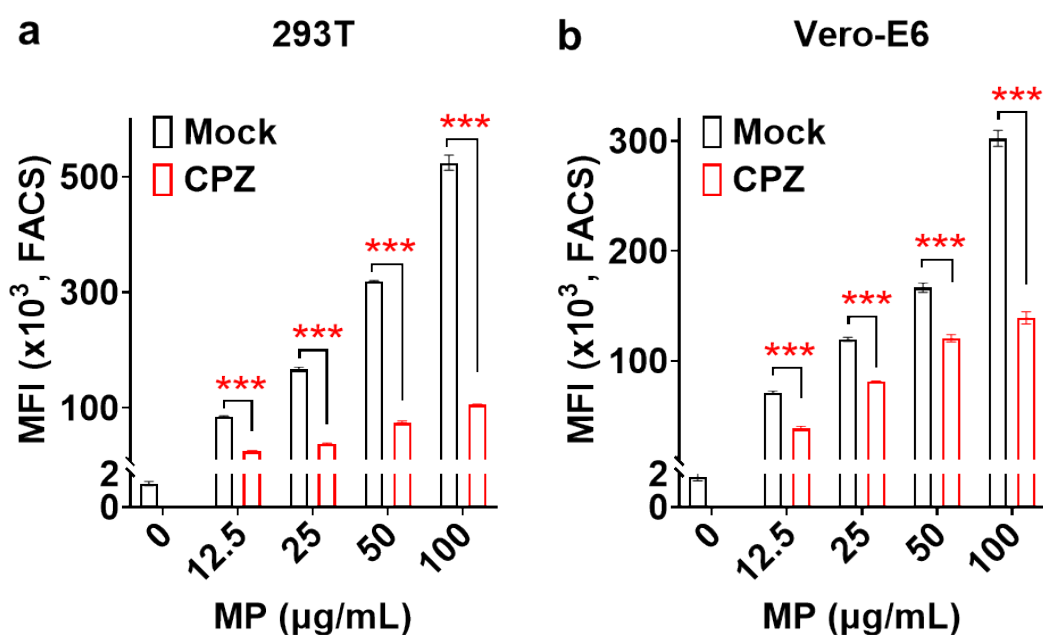


Supporting Information Figure S4. Association and internalization of the MP/pseudovirus complex in cells. ACE2/293T cells were incubated with SC2-P, MP-2 (red fluorescence-labeled) or MP/SC2-P complex for 24 h. Particle association/internalization was analyzed by FACS. Data are presented as mean \pm SEM (n=3). ***, p < 0.001 by Student's *t*-test.

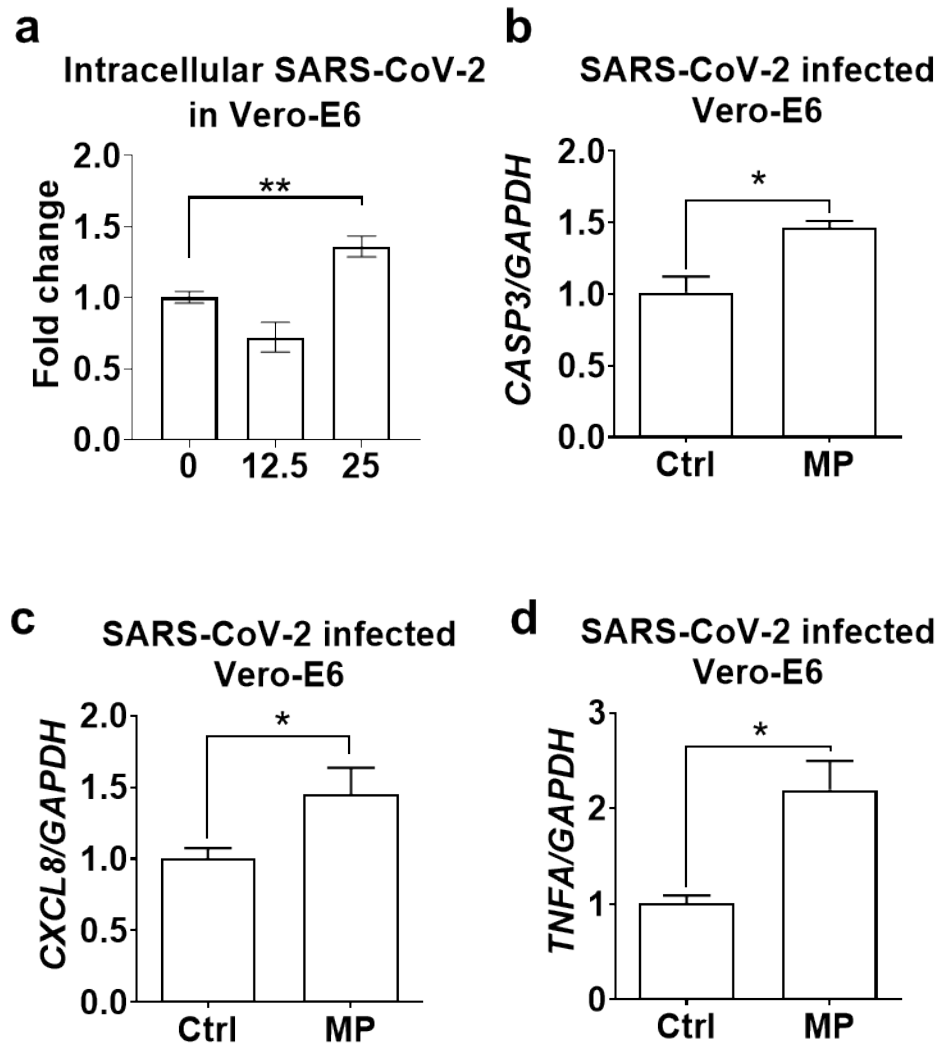
293T



Supporting Information Figure S5. Association and internalization of microplastics in 293T cells. 293T cells were incubated with red fluorescence-labelled MP-2 for 24 h. Particle association/internalization was analyzed by FACS. Data are presented as mean \pm SEM (n=3). **, p<0.01***, p<0.001 by ANOVA.



Supporting Information Figure S6. Microplastics uptake is inhibited by CPZ. 293T (a) and Vero-E6 (b) cells were firstly treated with/without chlorpromazine hydrochloride (CPZ) for 2 h and then incubated with red fluorescence-labelled MP-2 for another 24 h in the presence or absence of CPZ. Particle uptake was analyzed by FACS. Data are presented as mean \pm SEM (n=3). ***, p<0.001 by ANOVA.



Supporting Information Figure S7. Co-exposure to microplastics and SARS-CoV-2 enhances intracellular viral levels and triggers inflammation. Vero-E6 cells were incubated with authentic SARS-CoV-2 in the presence or absence of MP for 2 h, then cultured in virus-free medium for 48 h. Cells were collected, the intracellular SARS-CoV-2 was detected by qPCR, and the fold change of intracellular virus was calculated by normalizing to the virus control (without MP). a) Fold change of intracellular virus in Vero-E6 cells. b-d) Expression of *CASP3*, *CXCL8* (IL-8) and *TNFA* in the SARS-CoV-2-infected Vero-E6 cells in the absence or presence of MP (25 μg/mL). *GAPDH* was used as housekeeping gene. Data are presented as mean ± SEM (n=3). *, p<0.05; **, p<0.01; ***, p<0.001 by Student's *t*-test.

Supporting Information Table S1. Primers used in this work

| Primers | Forward (5'-3') | Reverse (5'-3') |
|---------------|----------------------------|----------------------------|
| N-1 | 5'ATGCATTTGCATCAGAGGCT3' | 5'TTGTTATAGCGGCCTTCTGT3' |
| N-2 | 5'GGGGAACCTTCCTGCTAGAAT3' | 5'CAGACATTTTGCTCTCAAGCTG3' |
| Probe | TTGCTGCTGCTTGACAGATT | |
| GAPDH | 5'TCACCATCTTCCAGGAACGAGA3' | 5'ACCCATGACAAACATAGGGGC3' |
| TNF- α | 5'CTAAGAGCGCAGGTCAGACA3' | 5'GCTTGAGGGTTTGCTACAACA3' |
| CXCL8 | 5'ACATGACTTCCAAGCTGGCG3' | 5'TACCTTGGGGTCCAGACAGAG3' |
| Caspase-3 | 5'TTCATTATTCAGGCCTGCCG3' | 5'AGCTTGTCGGCGTACTGTTT3' |