

Supplementary Data

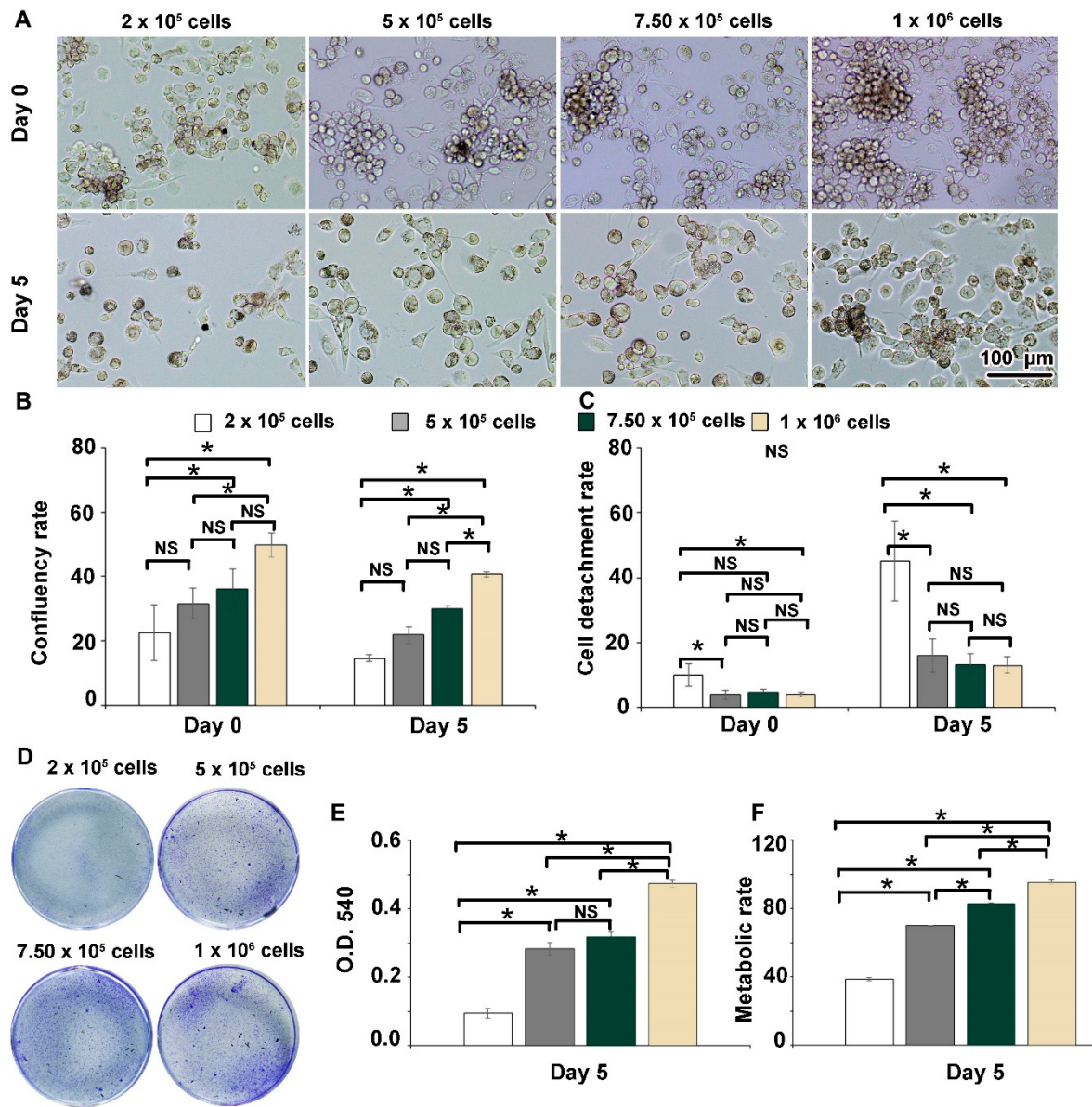


Figure S1. Increased THP-1 initial cell seeding density increased the number of THP-1 macrophages (THP-1m) in a 5-day culture experiment in RPMI-1640. (A) Microscopic images showing cell morphology. (B) Percentage of cell confluency of THP-1m. (C) Percentage of detached cells (D) Representative image of crystal violet staining on day 5, classified by cell number. (E) Quantitative results of crystal violet staining on day 5. (F) Metabolic activity rate of THP-1m by resazurin assay. Values are presented as mean $\pm$ standard deviation. *Statistically significant ($p < 0.05$).

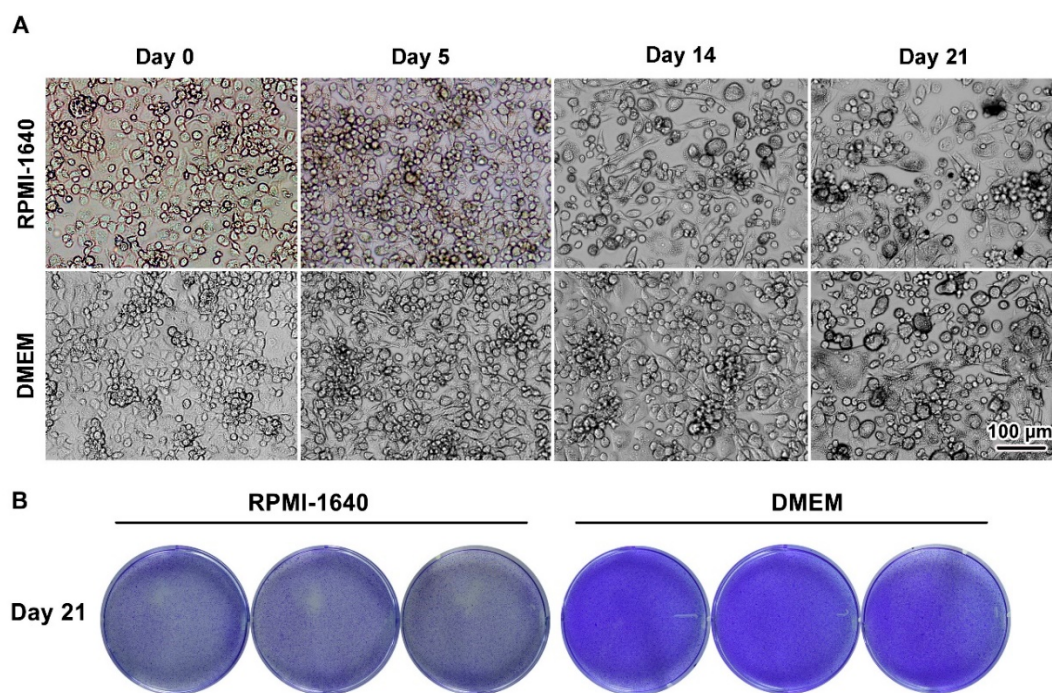


Figure S2. Long-term culture of THP-1m in RPMI-1640 and DMEM. **(A)** Live images of cell morphology. **(B)** Whole-well images of crystal violet staining on day 21.

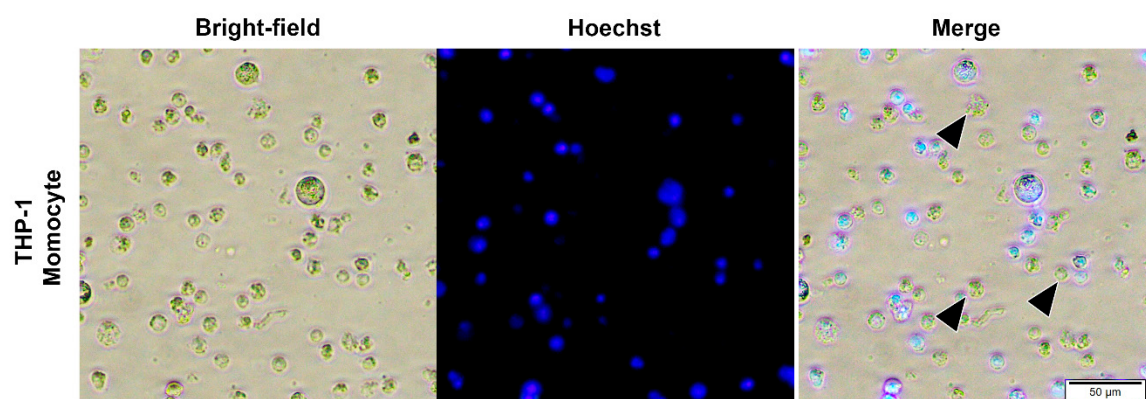


Figure S3. Hoechst staining of nuclei of THP-1 monocyte cells. Arrowheads indicate THP-1 monocytes that cannot be stained with Hoechst dye.

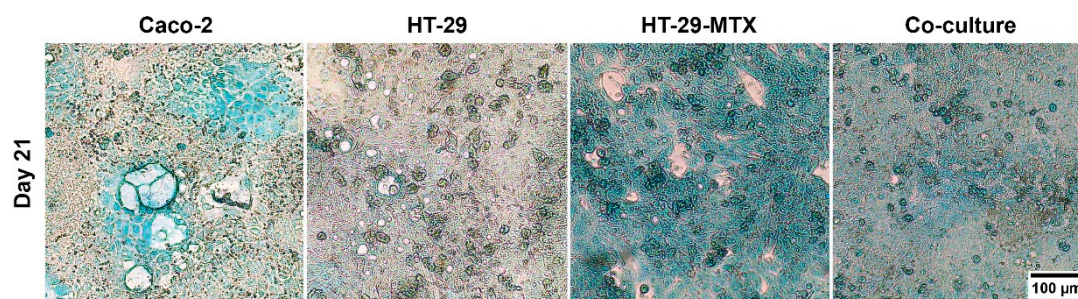


Figure S4. Mucus staining with Alcian blue of the Caco-2, HT-29, and HT-29-MTX monolayer and the Caco-2/HT-29-MTX co-culture at a ratio of 9:1 after 21 days of culture.

Table S1. Morphological parameters and size of the nucleus prepared in THP-1 monocytes and THP-1m. Data are presented as average $\pm$ standard deviation (n = 89).

Type	Mean $\pm$ S.D.			
	Max Feret (μm)	Min Feret (μm)	Ratio	Nucleus Size (μm)
THP-1 monocyte	11.3 $\pm$ 1.6	10.0 $\pm$ 1.5	1.1 $\pm$ 0.1	10.6 $\pm$ 1.5
Round	17.4 $\pm$ 4.0	14.7 $\pm$ 3.7	1.2 $\pm$ 0.1	24.8 $\pm$ 1.5
Fried egg	33.1 $\pm$ 6.2	27.8 $\pm$ 5.1	1.2 $\pm$ 0.1	46.8 $\pm$ 8.7
Spindle-like	28.4 $\pm$ 5.1	21.9 $\pm$ 5.1	1.3 $\pm$ 0.1	39.4 $\pm$ 7.0