

# Supplementary Material

## A Microwell-Based Intestinal Organoid-Macrophage Co-Culture System to Study Intestinal Inflammation

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### Supplementary table

Table S1. Description of the culture conditions used for the experiments

Name	Culture Conditions
MIO	Mouse intestinal organoids cultured in microwells
RAW	RAW cells cultured in microwells
TNF- $\alpha$ untreated	MIO treated with TNF- $\alpha$ for 5 days
TNF- $\alpha$ neutralizing antibody	MIO treated with TNF- $\alpha$ for 5 days and TNF- $\alpha$ neutralizing antibody from day 3 onwards
TNF- $\alpha$ removal	MIO treated with TNF- $\alpha$ until day 3 and then remove TNF- $\alpha$ from the culture medium
dMIO+RAW untreated	Direct co-culture of MIO with RAW for 5 days
indMIO+RAW untreated	Indirect co-culture of MIO with RAW for 5 days
indMIO+RAW removal	Indirect co-culture of MIO with RAW and on day 3 removal of RAW cells

### Supplementary figures

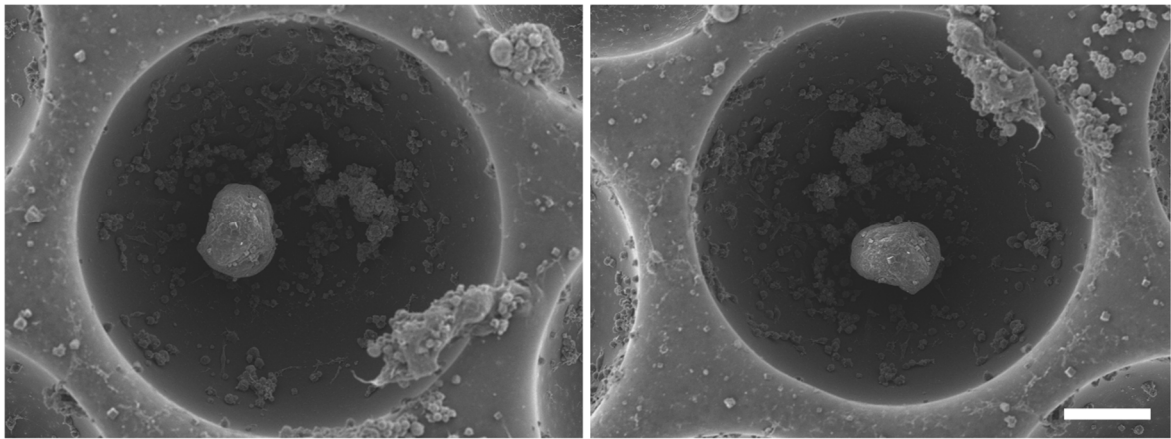
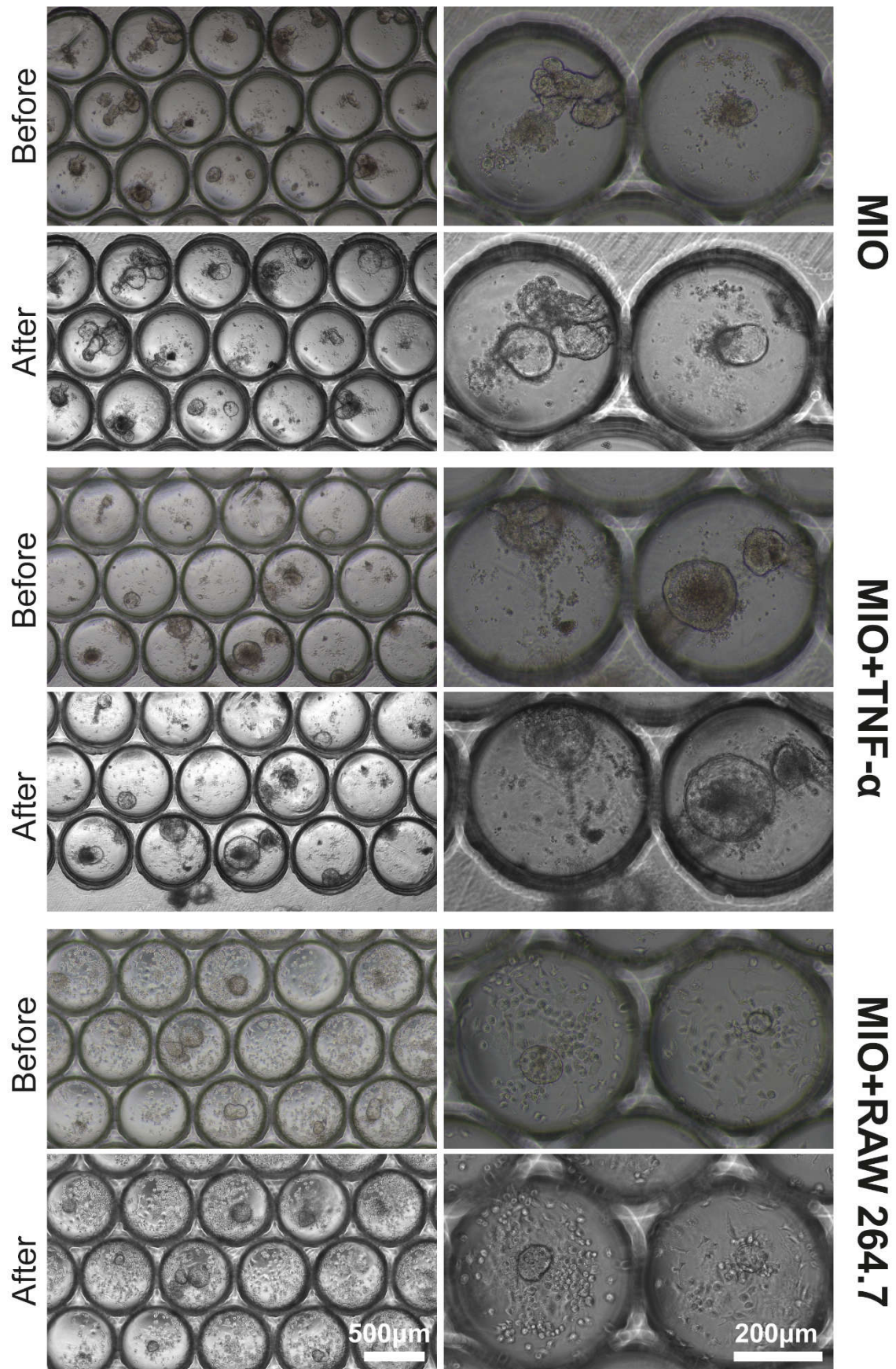


Figure S1. SEM images demonstrating the direct co-culture of intestinal organoids with RAW 264.7 cells. Scale bar represents 100  $\mu$ m.



**Figure S2.** Bright-field images demonstrating the swelling of organoids upon treatment with 5  $\mu$ M Forskolin for 3 hours.

### **Supplementary video**

**Video S1.** Time-lapse imaging of the direct co-culture of intestinal organoids with 50,000 RAW 264.7 cells. Single plane images were taken every 1 h for 3 days.