

1 **Microbial metabolite butyrate facilitates M2 macrophage polarization and**
2 **function**

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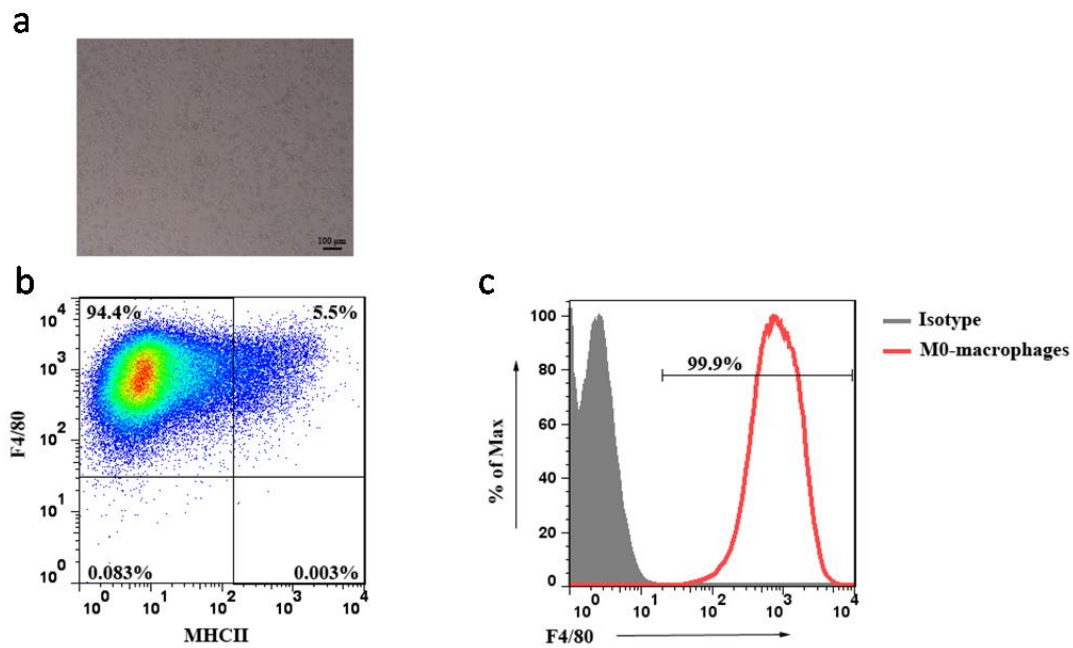
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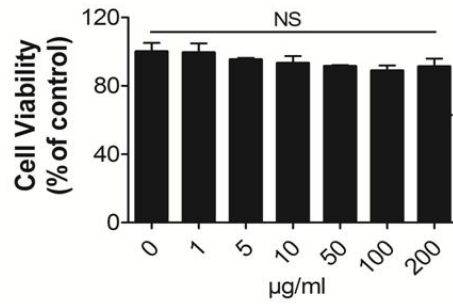
16 **Supplementary Figure 1: Generation of bone marrow-derived macrophages. (a)**

17 Microscope image of 6 day-cultured BMDMs. **(b–c)** F4/80 surface expression was

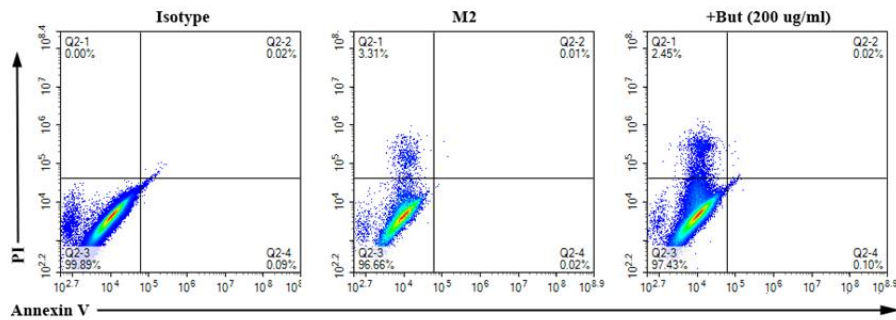
18 analyzed by flow cytometry.

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a



b



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21 **Supplementary Figure 2: The effect of butyrate treatment on the viability of**

22 **M2-BMDMs.** Differentiated M2-BMDMs were treated with butyrate for 24 h.

23 Cellular viability was determined by the Cell Counting Kit-8 (CCK-8), and analysis

24 of annexin V and propidium iodide (PI) staining by flow cytometry. (a) Cell viability

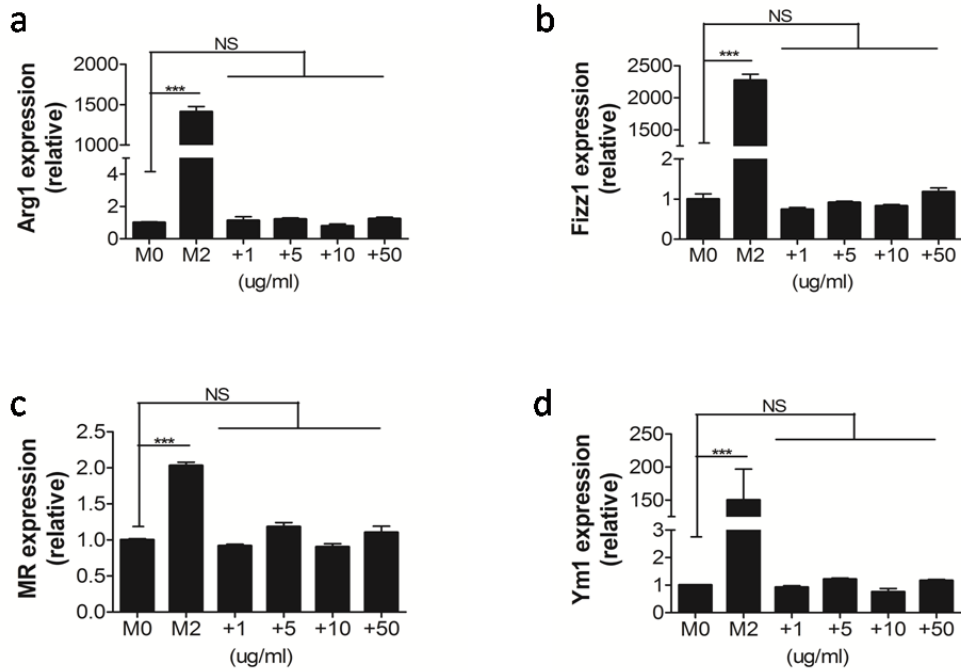
25 was determined by the relative absorbance, untreated cells were used as controls. (b)

26 Cells were double stained with annexin-V-FITC and PI and analyzed by flow

27 cytometry. Data are shown as means \pm SD for at least three

28 independent experiments. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ (t test).

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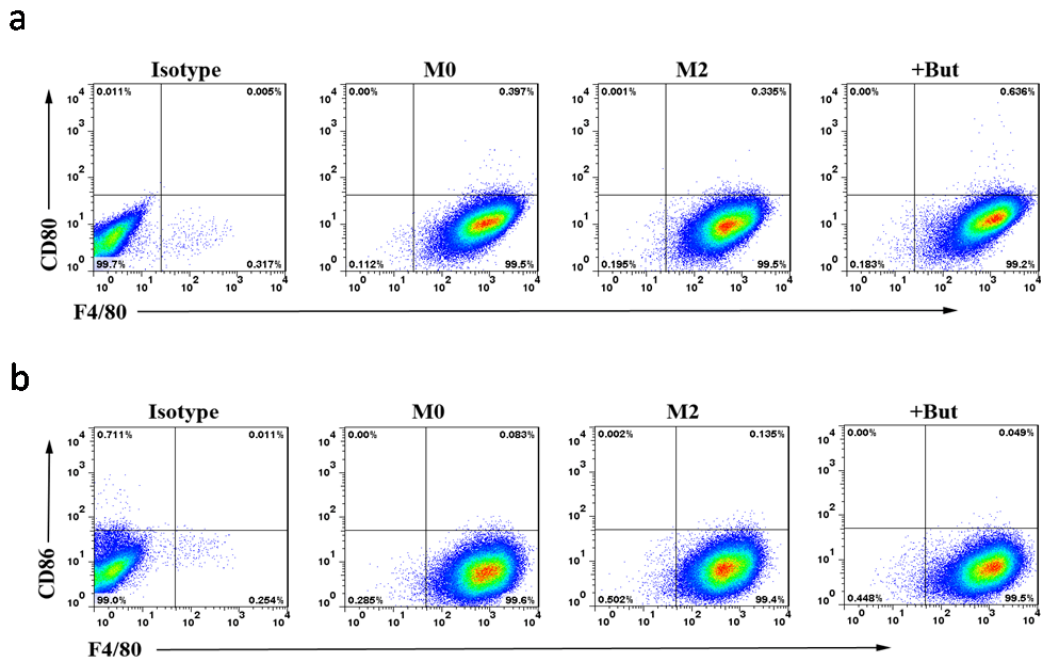
31 **Supplementary Figure 3: Effect of butyrate on gene expression in M2-BMDMs.**

32 (a–d) Expression of *Arg1*, *Fizz1*, *Ym1*, and *MR* by M2-BMDMs after treatment with

33 various concentrations of butyrate was measured by quantitative PCR. Data are shown

34 as mean \pm SD for at least three independent experiments. * $P < 0.05$, ** $P < 0.01$, *** P

35 < 0.001 (t test).



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37 **Supplementary Figure 4: The activation profiles of M2-BMDMs treated with**
 38 **butyrate.** (a–c) M2-BMDMs were treated with butyrate for 12 h. Cells were
 39 harvested, washed, and the expression of CD80 and CD86 was analyzed by flow
 40 cytometry. Results are representative of three independent experiments.