

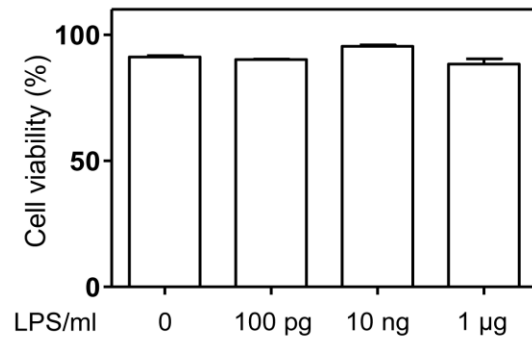
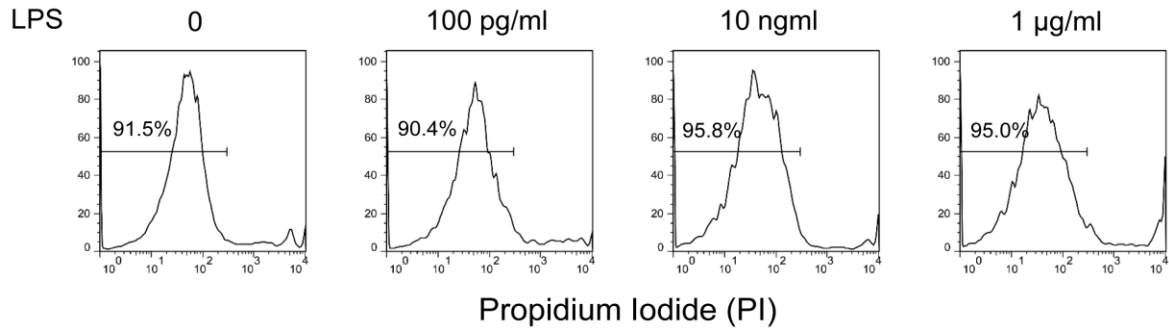
Supplementary Figures

**Molecular mechanisms that underlie the dynamic adaptation of innate monocyte memory to varying stimulant strength of TLR ligands**

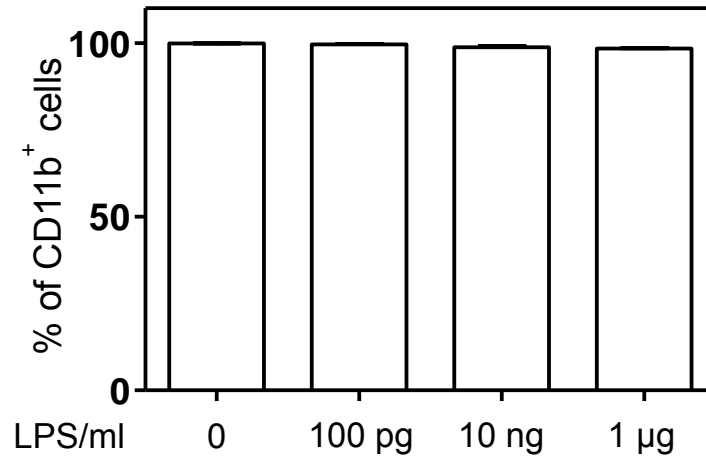
Ruoxi Yuan,<sup>1,2</sup>, Shuo Geng<sup>1</sup>, Liwu Li,<sup>1,2,\*</sup>

<sup>1</sup> Department of Biological Sciences, Virginia Polytechnic Institute and State University,  
Blacksburg, VA 24061, USA

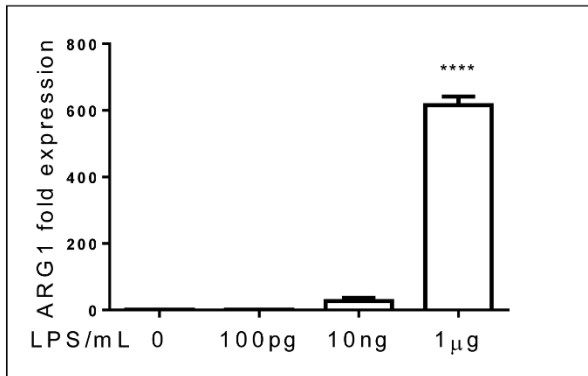
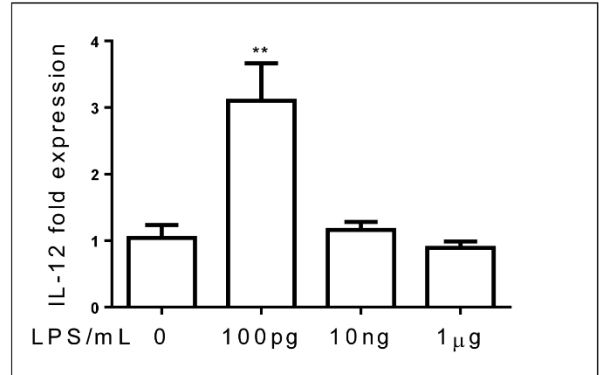
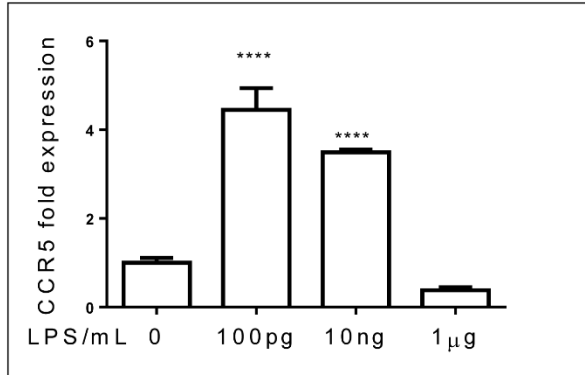
<sup>2</sup> Department of Biomedical Sciences & Pathobiology, Virginia-Maryland College of Veterinary  
Medicine, Virginia Polytechnic Institute and State University, Blacksburg, VA 24061, USA



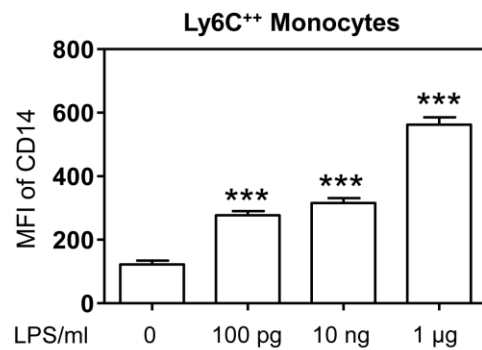
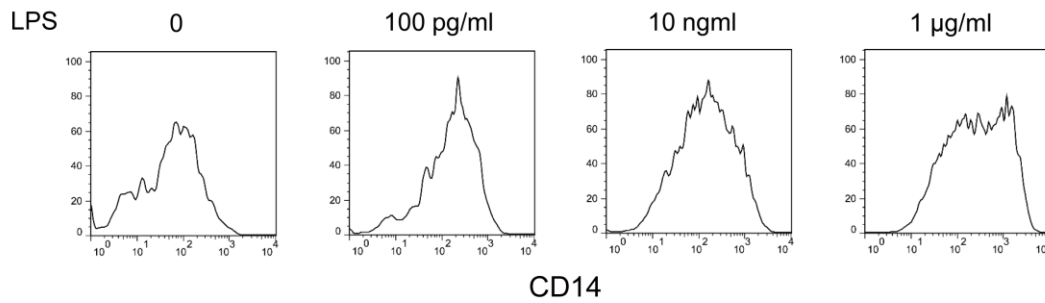
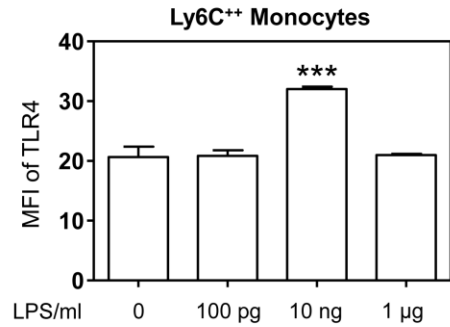
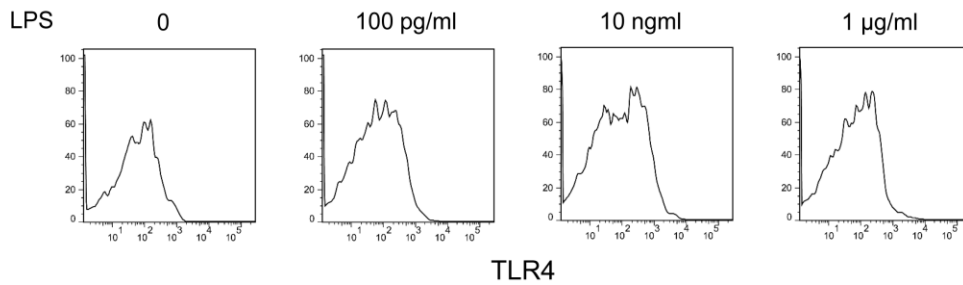
**Supplementary Figure S1. Cell viability measurement of co-cultured monocytes with varying dosages of LPS and M-CSF.** BM cells were cultured with M-CSF (10 ng/ml) and different doses of LPS for 5 days, and then stained with PI. Cell viability was determined by flow cytometry. Error bar represents s.e.m. of three experiments.



**Supplementary Figure S2. Cell surface levels of CD11b measurement of cultured monocytes with M-CSF.** BM cells were cultured with M-CSF (10 ng/ml) and different doses of LPS for 5 days, and then stained with anti-CD11b antibody and PI. Percentages of CD11b<sup>+</sup> cells within viable cells was analyzed by flow cytometry. Data represents three experiments.



**Supplementary Figure S3. Additional experiments demonstrating the differential modulation of selected genes by varying dosages of LPS.** Total RNA was isolated from monocytes treated with different dosages of LPS for 5 days. Real-time PCR was performed to determine the expression levels of CCR5 (A), IL-12 (B), ARG1 (C) and iNOS (D). Data are representative of three separate experiments (error bar represent s.e.m., \*\*,  $p < 0.05$ , \*\*\*\*,  $p < 0.0005$ ; as compared to non-treated control group, student t test).



**Supplementary Figure S4. Cell surface levels of TLR4 and CD14 measurement of co-cultured monocytes with varying dosages of LPS and M-CSF.** BM cells were cultured with M-CSF (10 ng/ml) and different doses of LPS for 5 days. Cell surface expression of TLR4 and CD14 within monocytes was detected by flow cytometry. Error bar represents s.e.m. of three experiments. (\*\*\*,  $p < 0.001$ ; as compared to non-treated control group, student t test).