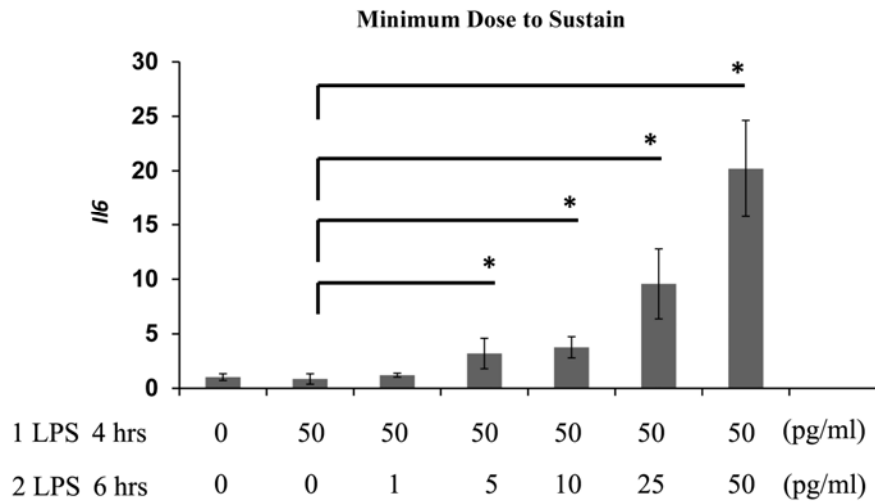
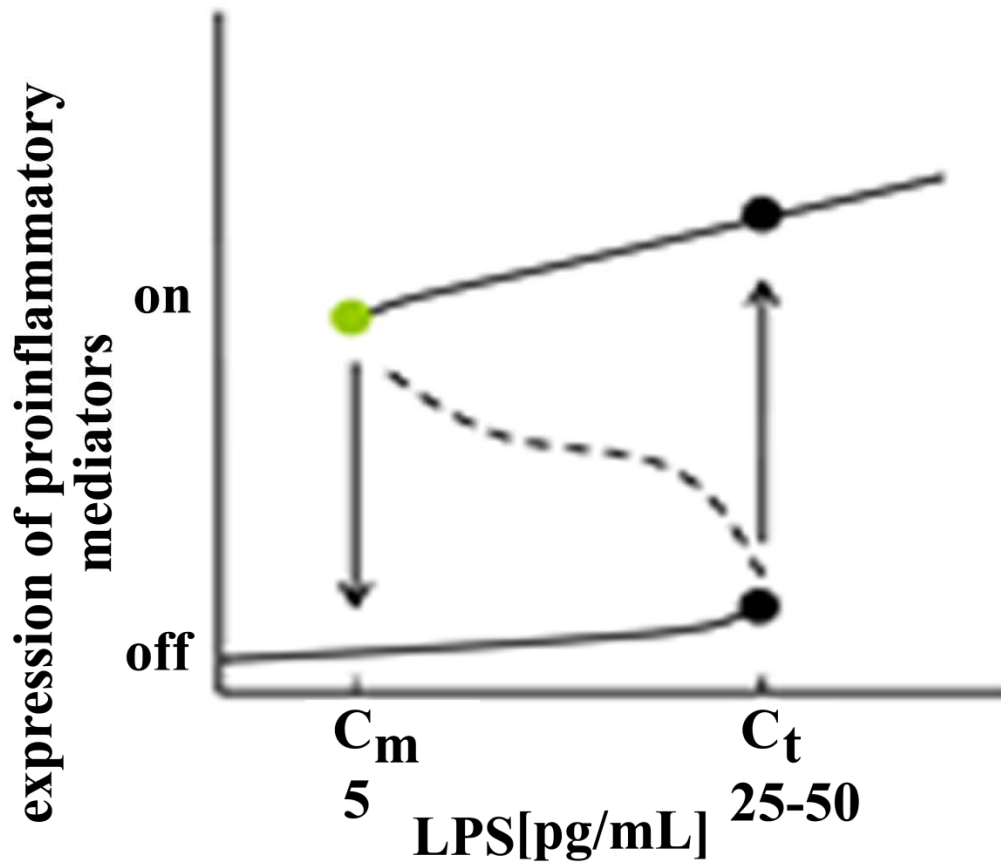


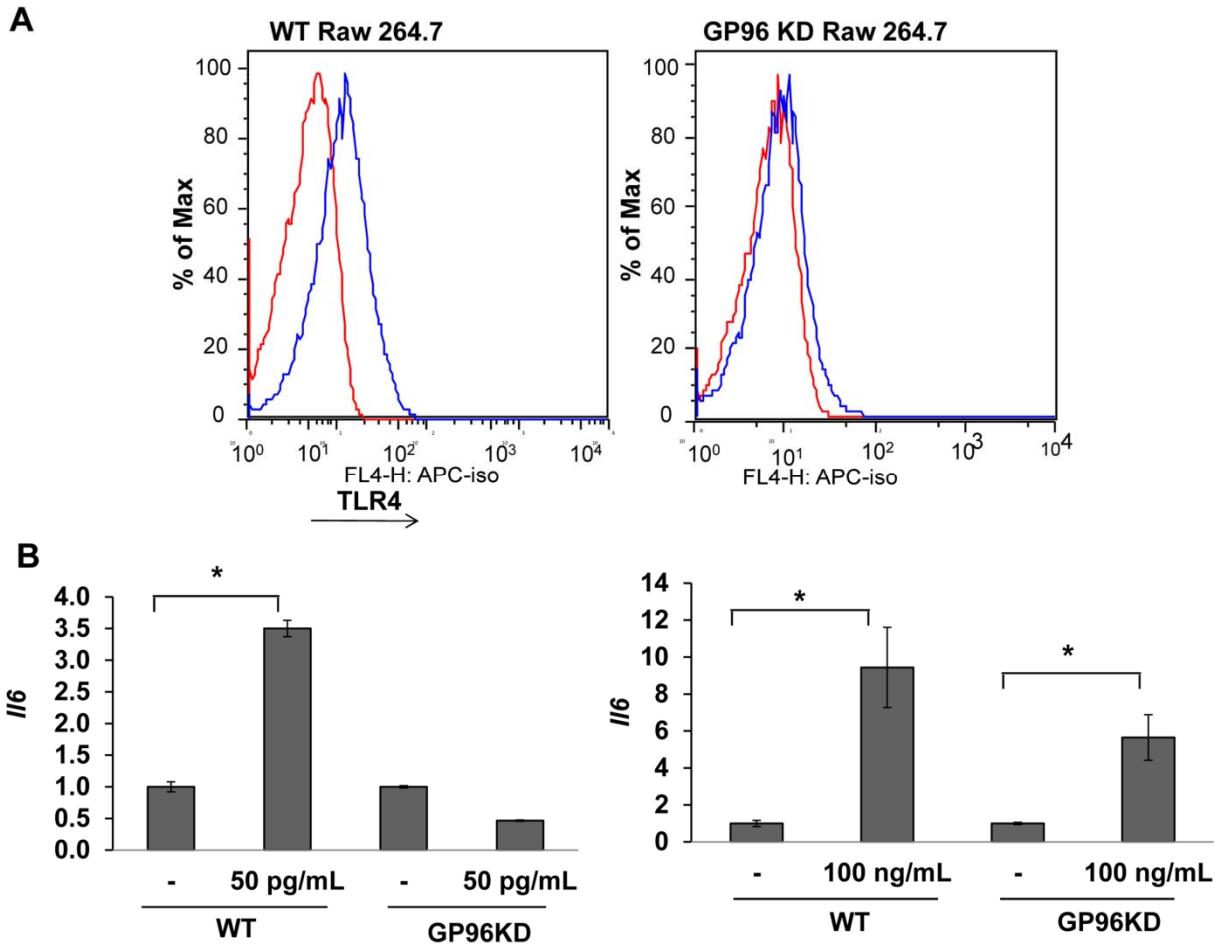
Supplementary Figure S1. Minimum dosage of LPS required for the induction of *Il-6* in macrophages. WT BMDM were treated with varying dosages of LPS for 6 hrs. The levels of expressed *Il-6* mRNA were measured by real-time RT-PCR. n=3. * p<0.05.



Supplementary Figure S2. Minimum dosage of LPS required to sustain the expression of *IL-6*. WT BMDM were treated with 50 pg/ml LPS for 4 hrs to induce the expression of *IL-6*. The cells were then washed twice with PBS, and re-incubated with fresh medium supplemented with varying dosages of LPS for an additional 6 hrs. The levels of *IL-6* were measured by real-time RT-PCR.



Supplementary FigureS3. An illustration of the bi-stable “digital” activation of macrophages by low dose LPS. Macrophages have to be challenged with a critical threshold amount of LPS (C_t) in order to be turned on and express pro-inflammatory mediators. Once the cells are turned on, a significantly lower LPS concentration (C_m) can still maintain its activation and expression of proinflammatory mediators.



Supplementary Figure S4. Surface TLR4 is required for low-grade induction of pro-inflammatory mediators

LPS has been shown to signal both through cellular surface and endosomal TLR4 (64). Thus, we examined whether cell surface or endosomal TLR4 is required for the effect of low dose LPS. To test this, we employed both wild type Raw264.7 murine macrophages (expressing cell surface TLR4), as well as Raw264.7 cells with GP96 knocked-down (GP96KD) (52). GP96 is required for the assembly and trafficking of TLR4 from the endosome to the cell membrane. GP96KD cells only have endosomal TLR4 and lack cell surface TLR4 (52). Indeed, as determined by flow cytometry, cell surface TLR4 was only detectable in WT Raw264.7 cells but not GP96KD cells (Fig. 7A). Following treatment with a low dose LPS, wild type Raw264.7 cells expressed *Il-6* (Fig. 7B). In contrast, the induction of *Il-6* was completely ablated in GP96KD cells. In agreement with previous findings that show high dose LPS can signal through intra-cellular TLR4, we observed that a high dose LPS was still capable of inducing *Il-6* expression in GP96KD cells. This observation indicates that cell surface TLR4 is critically required for the effect of low dose LPS.