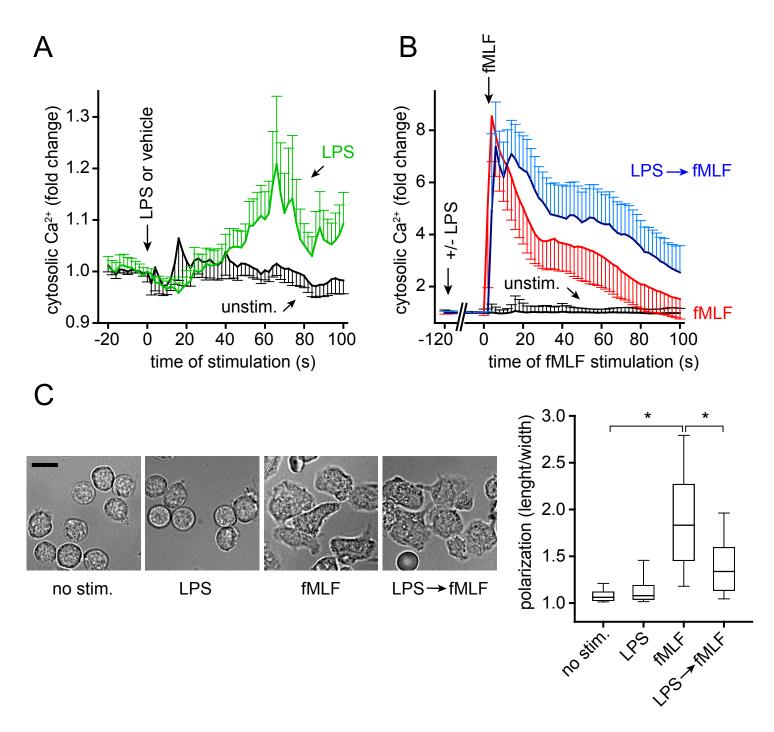
## **Supplemental Figure S2**



## Figure S2. LPS primes fMLF-induced Ca<sup>2+</sup> signaling and impairs PMN polarization.

(A) Human PMNs were labeled with Fluo-4 and changes in cytosolic Ca<sup>2+</sup> levels after the addition of LPS (100 pg/ml) or vehicle control were recorded by fluorescence microscopy. (B) PMNs were stimulated with LPS (100 pg/ml) or not, followed by fMLF (10 nM) 2 min later, and changes in cytosolic Ca<sup>2+</sup> were recorded. Data in (A) and (B) are averaged results of 3 independent experiments (mean + SD) and represent >50 cells per condition. (C) Human PMNs were allowed to attach to fibronectin-coated glass bottom dishes.Cells were stimulated with LPS (10 ng/ml), fMLF (10 nM), or with LPS followed by fMLF 10 min later. Cell polarization was assessed as the ratio of cell length to cell width. Representative images and boxplots representing 112-190 cells from at least 3 independent experiments are shown (\*p<0.05, one-way ANOVA). Scale bar: 10 µm.