Supplemental Figure 1. **BV2 and BV2-hEP2 microglial growth.** Cell growth was determined daily by measuring the absorbance at 450 nm in untransfected BV2 and BV2-hEP2 cultures in a 96-well plate in the presence or absence of G418 (800 µg/ml). Data are the mean ± SEM (n = 3 independent experiments). *p < 0.05, **p < 0.01 by Two-way ANOVA and post hoc Tukey’s multiple comparisons test (BV2 vs. BV2-hEP2).
Supplemental Figure 2. BV2 vs. BV2-pcDNA3.1(+) . Phase contrast images were taken of untransfected BV2 (A) and BV2-pcDNA3.1(+) (B) microglia with a Zeiss AxioObserver A1 fluorescence microscope. Representative images are shown. Scale bar = 30 µm. C, ONO-AE1-259-1 and CP544326 (selective and potent EP2 agonists) were added to untransfected BV2 and BV2-pcDNA3.1(+) cultures at different concentrations for 40 minutes. The EP2 agonists did not alter intracellular cAMP in untransfected BV2 and BV2-pcDNA3.1(+) cultures. D, CP544326 increased cellular cAMP in BV2-hEP2 microglia in a concentration-dependent manner (open red circles). The EP2 antagonist TG4-155 (300 nM) caused a parallel shift to the right in the agonist concentration-response curve (open blue squares). Untransfected BV2 (parent) microglia did not respond to increasing concentrations of CP544326 (open black
triangles) up to at least 1 μM. Data points represent mean and SEM (error bars) from a single experiment run in technical replicates of 8.
Supplemental Figure 3. Prolonged exposure to ONO-AE1-259 and TG4-155 does not modify the phagocytosis of fluorescent latex microspheres in activated BV2 and BV2-hEP2 microglia. Combined fluorescence and phase microscopy imaging of lipopolysaccharide (LPS) activated BV2 and BV2-hEP2. A, BV2 microglia following incubation with the vehicle. BV2-hEP2 microglia following incubation with vehicle (B), ONO-AE1-259-1 (30 nM) (C) or TG4-155 (1 µM) (D) for 16 hours followed by a 45-minute incubation with fluorescent latex microspheres. The scale bar = 50 µm. The live fluorescence activated cell sorting of untransfected BV2 (E) and BV2-hEP2 (F) microglia treated with the vehicle for 16 hours revealed multiple populations. Twenty-thousand events were counted. The graph on the left is
the live cell gating strategy of untransfected BV2 and BV2-hEP2 microglia based on forward scatter (FSC) and fluorescence using the phycoerythrin (PE-A) filter. The graph on the right is a gray histogram of fluorescence observed in untransfected BV2 and BV2-hEP2 microglia. Three populations were identified by the histogram (cells void of microspheres, cells containing a single microsphere and cells containing multiple microspheres). The live fluorescence activated cell sorting of untransfected BV2-hEP2 microglia in the presence of ONO-AE1-259-1 (30nM) (G) and TG4-155 (1 μM) for 16 hours. Incubation in cytochalasin D (10 μM) for 3 hours (I) revealed a reduction in the multiple populations of cells that phagocytosed fluorescent microspheres. There was no change in the multiple populations of cells by the EP2 receptor modulators.