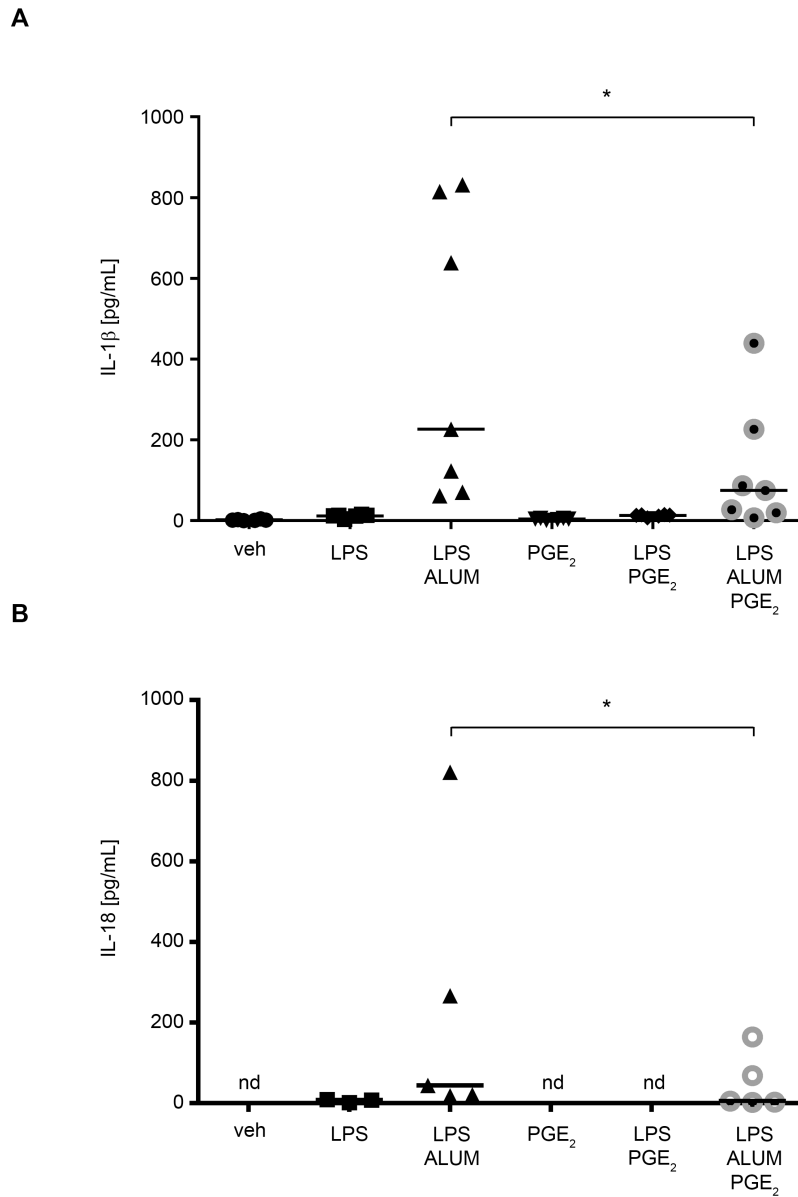
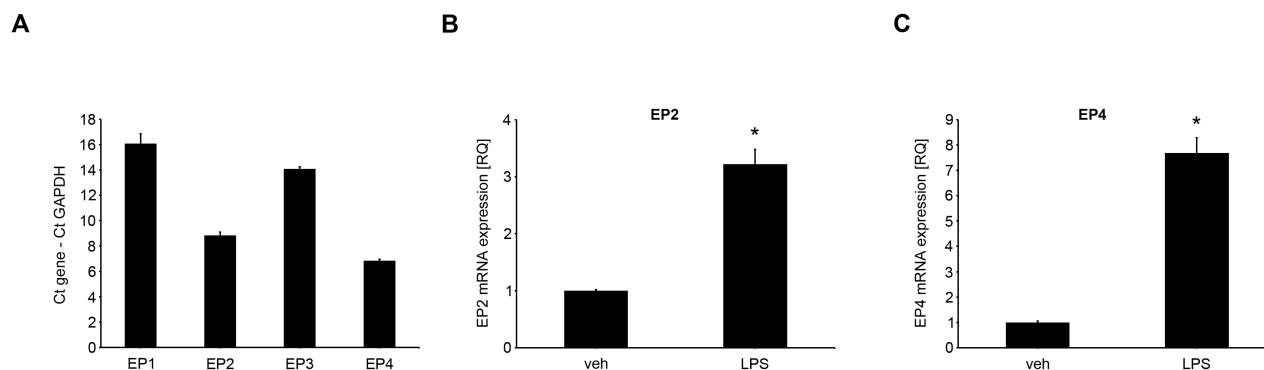


Supplementary figure 1



Supplementary Figure 1. PGE₂ inhibits IL-1 β and IL-18 production. (A, B) Human primary monocyte-derived macrophages (MDM) were treated with/without LPS (100 ng/ml) for 4 h in complete medium, followed by 30 min treatment with PGE₂ (0.1 μ M) or vehicle (EtOH), then stimulated with alum (400 μ g/ml for 5 h) in serum-free medium. Supernatants were collected for IL-1 β (A) or IL-18 (B) ELISA. The dots are the average values from independent experiments performed in duplicate using MDM from 7 (A) or 5 (B) healthy donors. The line presents the median. * $P < .05$ as indicated, as assessed by RM ANOVA, followed by Sidak post hoc test. As depicted, there were substantial differences in the IL-1 β or IL-18 release between donors. In order to combine similar experiments and calculate statistical significance the values in all experiments are presented as the percentage of the response after LPS/alum treatment. The range of the raw values from all experiments is included in the figure legend.

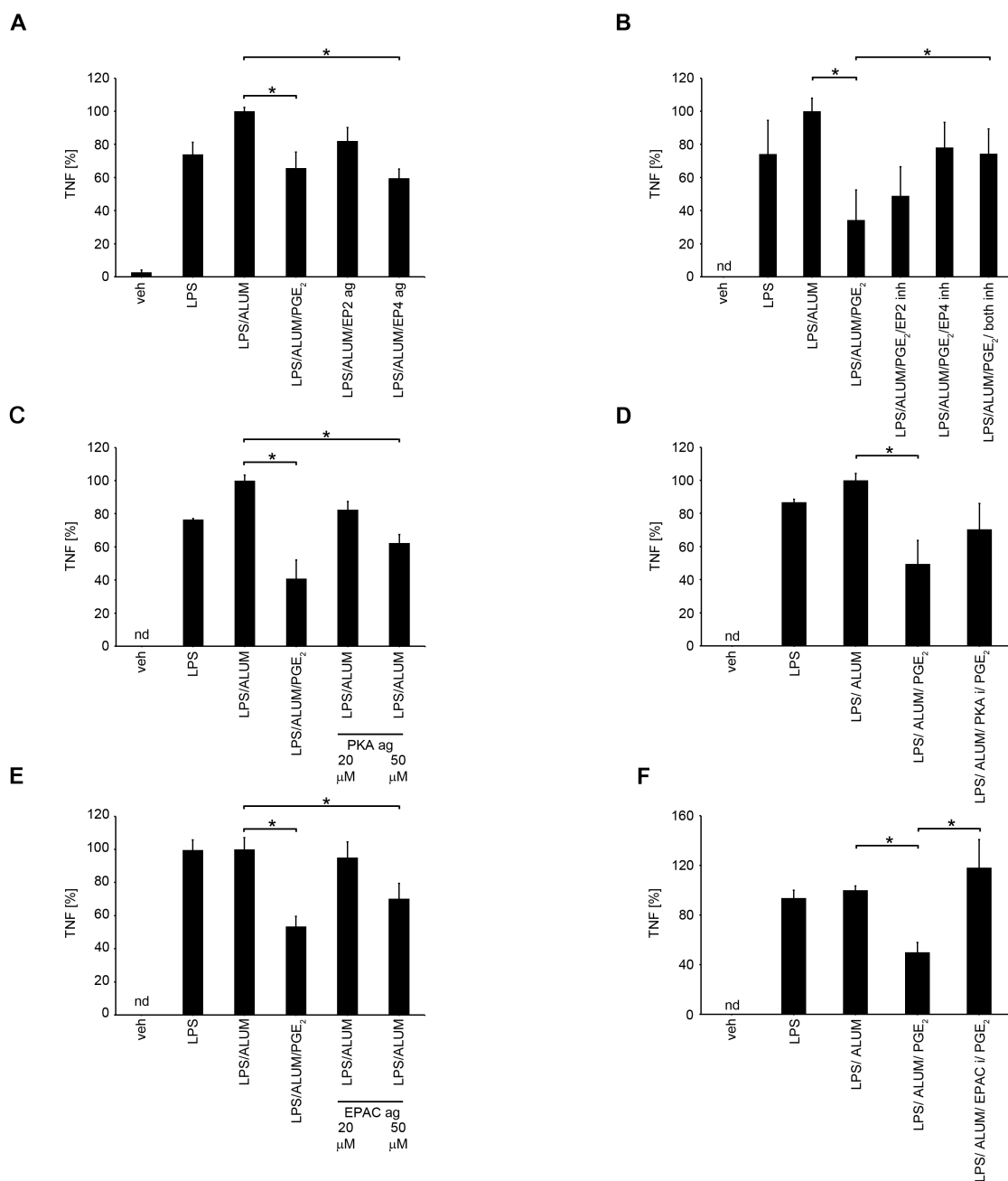
Supplementary figure 2



Supplementary Figure 2. EP1-4 receptor expression in primary human monocyte-derived macrophages.

(A-C) Primary MDM were treated with/without LPS (100 ng/ml) for 4 h in complete medium. Cells were lysed and total RNA was extracted. Gene expression was assessed using RT PCR. (A) Baseline expression of each receptor is presented as the difference between the gene Ct value and GAPDH. (B and C) gene expression was normalized to GAPDH transcripts and represented as a relative quantification (RQ) compared with the vehicle-treated cells. Data represent the mean \pm SEM from 4 independent experiments from 4 healthy donors, performed in duplicate. * $P < .05$ as compared with vehicle, as assessed by Student t-test.

Supplementary figure 3



Supplementary Figure 3. PGE₂ inhibits TNF production via EP4/PKA/Epac-dependent pathway (A) primary MDM were treated with/without LPS (100 ng/ml) for 4 h in complete medium, followed by 30 min treatment with EP2 agonist (butaprost, free acid, 0.5 μM), EP4 agonist (CAY10598, 0.1 μM), PGE₂ (0.1 μM) or vehicle (EtOH), then stimulated with alum (400 μg/ml for 5 h) in serum-free medium. (B) primary MDM were treated with/without LPS (100 ng/ml) for 4 h in complete culture medium, then 30 min with EP2 inhibitor (PF-04418948, 0.5 μM), EP4 inhibitor (GW627368X, 2 μM), both inhibitors or vehicle (DMSO), followed by 30 min treatment with PGE₂ (0.1 μM) or vehicle (EtOH), and stimulation with alum (400 μg/ml for 5 h) in serum-free medium. (C, E) primary MDM were treated with/without LPS (100 ng/ml) for 4 h in complete medium, followed by 30 min treatment with PKA selective agonist (6-Bnz-8-PIP-cAMP, 20 or 50 μM) (C), EPAC selective agonist (8-(4-Chlorophenylthio)-2'-O-methyladenosine 3',5'-cyclic Monophosphate . sodium salt, 20 or 50 μM) (E), PGE₂ (0.1 μM) or vehicle, then stimulated with alum (400 μg/ml for 5 h) in serum-free medium. (D, F) primary MDM were treated with/without LPS (100 ng/ml) for 4 h in complete culture medium, then 30 min with PKA inhibitor (8-Bromoadenosine 3',5'-cyclic Monophosphothioate, Rp-Isomer . sodium salt, 50 μM) (D), Epac inhibitor (4- Cyclopentyl-2- (2, 5- dimethylbenzylsulfanyl)- 6- oxo- 1, 6- dihydropyrimidine- 5- carbonitrile, 10 μM) (F) or vehicle (DMSO), followed by 30 min treatment with PGE₂ (0.1 μM) or vehicle (EtOH), and stimulation with alum (400 μg/ml for 5 h) in serum-free medium. TNF data are presented as the percentage of the response after LPS/alum treatment, which ranged from 67.4 to 4285.4 pg/ml. TNF release data represent the mean ± SEM from 2-3 independent experiments from 2-3 healthy donors, each performed in duplicate. * P < .05 as indicated, as assessed by RM ANOVA, followed by Sidak's post hoc test.