MPGES-1-derived PGE2 suppresses CD80 expression on tumor-associated phagocytes to inhibit anti-tumor immune responses in breast cancer

Supplementary Material
**Figure S1:** (A) Gating strategy for discrimination of T cell subsets and intracellular detection of their respective Granzyme B expression. PBMCs were isolated out of PBMC MCF-7 spheroid co-cultures and prepared for intracellular staining as described in Methods. First, viable single cells were identified. Single cells were classified as T cells (CD45+CD3+), Th cells (CD45+CD3+CD4+), and cytotoxic T cells (CD45+CD3+CD8+). Granzyme B was stained in T cell subsets to determine the functionality of respective T cell subsets.

(B) Gating strategy for discrimination of CD14+ CD11c+ phagocytes in PBMC MCF-7 co-cultures and detection of their respective polarization markers. PBMCs were isolated from PBMC MCF-7 spheroid co-cultures as described in Methods. First, viable single cells were identified. Single cells were classified as leukocytes (CD45+), CD45+CD14+CD11c+ phagocytes, and CD45+CD14−CD11c− lymphocytes. Extracellular CD80, CD86, and CD206 were stained to characterize polarization of CD14+ CD11c+ phagocytes.

(C) Gating strategy for the discrimination of phagocyte subsets in PyMT breast tumors. PyMT mice were sacrificed 20 weeks after birth and tumor suspension cells were isolated from PyMT breast tumors as described in Methods. First, single immune cells were identified by gating on SSC-A<sub>low</sub> CD45+ and FSC-W<sub>low</sub>, FSC-H<sub>int</sub> cells. These were subgated as CD11c<sup>-</sup>CD11b<sup>low</sup>F4/80<sup>+</sup> tumor-associated macrophages (TAMs), F4/80<sup>-</sup>CD11c<sup>+</sup>MHCII<sup>+</sup> DCs and F4/80<sup>-</sup>CD11c<sup>-</sup>CD11b<sup>high</sup> resident macrophages (M<sup>+</sup>).
Figure S2: (A-B) MCF-7 cells were detached and seeded on agarose to generate tumor spheroids. (A) mRNA expression of PGE\(_2\) metabolizing enzymes in monolayer cells or five days old tumor spheroids was analyzed using qPCR and (B) PGE\(_2\) levels in MCF-7 culture supernatants were determined by PGE\(_2\) EIA. Data are means ± SD of four independent experiments. Data were analyzed using GraphPad Prism 5.0. \(p\)-values were calculated using one-way ANOVA (A) or two-way ANOVA (B-C) with Bonferroni’s correction. Asterisks indicate significant differences between experimental groups (*, \(p < .05\), **, \(p < .01\), ***, \(p < .001\))
Figure A: COX-2 mRNA levels in macrophages and tumor cells. 

Figure B: COX-1 mRNA levels in macrophages and tumor cells. 

Figure C: Mean fluorescence index of CD80, CD86, and CD206 in BMDMs with and without mPGES-1. 

Figure D: Comparison of concentrations of PGD$_2$, PGE$_2$, PGF$_2$, and TxB$_2$ in BMDMs with and without mPGES-1.
**Figure S3:** (A-B) PyMT mice were sacrificed 20 weeks after birth. mRNA expression of (A) COX-2 and (B) COX-1 in PyMT tumors and unstimulated BMDM macrophages was analyzed using qPCR. (C-D) Bone marrow-derived macrophages were isolated using standard protocols and stimulated with LPS/IFN-γ for 8h. (C) Prostanoids PGE$_2$, PGD$_2$, PGF$_2\alpha$ and TxB$_2$ in supernatants of PBMC MCF-7 spheroid co-cultures were measured by LC-MS/MS and (D) Polarization markers of CD14$^+$ CD11c$^+$ phagocytes is displayed. Data are means ± SD of at least four independent donors. Data were analyzed using GraphPad Prism 5.0. p-values were calculated using one-way ANOVA (A,B) or two-way ANOVA (C,D) with Bonferroni’s correction. Asterisks indicate significant differences between experimental groups (*, p < .05, **, p < .01, ***, p < .001)