SUPPLEMENTARY FIGURE LEGENDS

Figure S1. Experimental Schema. Flow chart outlining the analyses and selection criteria utilized for the identification of inflammatory genes with differential expression in both patient and cell line datasets. For patient and cell line datasets, we performed univariate Student’s t-tests (unpaired with unequal variance) to determine which cytokines/chemokines were more highly expressed in ER-negative versus ER-positive breast cancers in patient tumor datasets and in ER-/Basal-Like versus Luminal-Like cell line datasets. Tests were considered significant at a stringency level of \( p<0.001 \). Next, in vitro and in vivo consensus groups were created from the individual datasets by selecting significant genes that passed these thresholds for \( \geq 2 \) in vitro or in vivo datasets to create consensus union groups of affected genes. Finally, we selected relevant ER-/Basal-Like tumor-specific inflammatory genes at the intersection of these in vitro and in vivo groups.

Figure S2 Verification of inflammatory gene expression differences between triple-negative and ER-positive breast cancer cell lines. A) qRT-PCR was performed in quadruplicate on a series of breast cancer cell lines (representing 6 ER-positive/luminal-like cell lines and 12 triple-negative/basal-like cell lines) to determine the validity of array-based findings for IL-6 (A), IL-8 (B), and CXCL1 (C).

Figure S3. (A) LPAR2 is more highly expressed in basal-like, compared to luminal cancers, and (B) breast cancer cell lines. (C) ELISA determination of IL-6, IL-8, and CXCL1 after treatment with LPAR2 siRNA. (D) LPAR2 contribution to serum stimulation in in serum starved stably transfected cells with shLPAR2. ELISA was used to determine the secretion of IL-6, IL-8, and CXCL1 after treatment with LPAR2 shRNA.
Figure S4. Expression of ERα and its effect on cytokine/chemokine production in SUM159 cells. SUM159 cells were infected with doxycycline inducible ERα lentivirus and selected for stable integration of the construct. A) ERα expression was tested after a 48hr treatment with or without doxycycline (2μg/ml) by qRT-PCR with an ERα-specific expression probe. B-D) Inducible ERα SUM159 cells were treated with or without doxycycline (2μg/ml) for 3 days, with serum starvation performed the last 2 days. Cells were stimulated with LPA (5μM) along with fresh media, and harvested 24hr after media replacement. Harvested media was then assessed for IL-6 (B), IL-8 (C), or CXCL1 (D) concentrations to determine gene suppression in each cell line. In (A-D), n=4, significant inhibition in comparison to Doxy treated counterparts is denoted with # p<0.05 and ## p<0.01, and statistically significant inhibition in comparison to serum-starved control is denoted with * p<0.05 and ** p<0.01. (E) EZH2 expression is reduced after induction of ERα expression. Expression was tested 72 hours by qRT-PCR after treatment with doxycycline (2μg/ml) to induce ERα expression.

Figure S5. Inducible inhibition of IL-6, IL-8, and CXCL1, and tandem inhibition of IL-6, IL-8, and CXCL1 in SUM159, MDA-MB-231, and MDA-MB-468 cells. SUM159 and MDA-MB-231 cells were infected with TRIPZ-shIL6 (A, D), TRIPZ-shIL8 (B, E), and TRIPZ-shCXCL1 (C, F) lentiviral vectors and stably selected using puromycin (2μg/ml). After stable selection, cells were treated 3 days with or without doxycycline (2μg/ml). Media was then replaced, and harvested 24hr post-replacement. Harvested media was assessed for IL-6, IL-8, or CXCL1 concentrations to determine gene suppression in the cell lines (n=3 bars and statistically significant inhibition in comparison to control is denoted with * p<0.05 and ** p<0.01). (G-J) SUM159 cells were infected with combinations of TRIHZ-shIL6, TRIPZ-shIL8, and TRINZ-shCXCL1 lentiviral vectors, and stably selected using puromycin (2μg/ml), hygromycin
(250μg/ml), or neomycin (500μg/ml). After stable selection, cells were treated with or without doxycycline (2μg/ml) for 3 days. Media was then replaced and harvested 24hr post-replacement. Harvested media was assessed for IL-6 (G), IL-8 (H), or CXCL1 (I) concentrations to determine gene suppression in the indicated cell lines. J) MDA-MB-231 and MDA-MB-468 cells were infected with combinations of TRIHZ-shIL6 and TRIPZ-shIL8 lentiviral vectors and stably selected using puromycin (2μg/ml) and hygromycin (250μg/ml). After stable selection, cells were treated with or without doxycycline (2μg/ml) for 3 days. Media was then replaced and harvested 24hr post-replacement. Harvested media was assessed for IL-6 and IL-8 (n =4 and statistically significant inhibition in comparison to control is denoted with * p< 0.05 and ** p<0.01).

**Figure S6.** Inducible inhibition of IL6ST, CXCR1, and CXCR2 in SUM159 and MDA-MB-231 cells, and anchorage-independent growth with specific inhibitors. SUM159 cells were infected with TRIBZ-shIL6ST (A), TRIPZ-shCXCR1 (B), and TRIBZ-shCXCR2 (C) lentiviral vectors and stably selected using puromycin (2μg/ml) or Blasticidin (5μg/ml). After stable selection, cells were treated with or without doxycycline (2μg/ml) for 3 days. RNA was harvested to assess gene expression of IL6ST (A), CXCR1 (B), and CXCR2 (C) by qRT-PCR with gene-specific probes. D) SUM159 and MDA-MB-231 cells were infected with TRIBZ-shIL6ST and TRIPZ-shCXCR1 lentiviral vectors and stably selected. Gene expression was assessed using identical procedures in A-C (n=3 and statistically significant inhibition in comparison to control is denoted with * p< 0.05 and ** p<0.01). E-F) SUM159 cells were plated in an anchorage-independent growth (soft agar) assay with the indicated concentrations of JAK1/2 (Ruxolitinib-downstream of IL6ST) or CXCR1/2 (SB225002) inhibitor. Every 2 days, cells were fed with media containing fresh inhibitor or equal concentration of DMSO solution for
controls. After 2 weeks, colony counts were assessed using a GelCount (Oxford Optronix, Oxford, UK) and results plotted (n=4 and statistically significant inhibition in comparison to control is denoted with ** \( p<0.01 \)). \textbf{G-H}) MCF-7 cells were plated in an anchorage-independent growth (soft agar) assay with the indicated concentrations of JAK1/2 (Ruxolitinib-downstream of IL6ST) or CXCR1/2 (SB225002) inhibitor. Every 2 days, cells were fed with media containing fresh inhibitor or equal concentration of DMSO solution for controls. After 2 weeks, colony counts were assessed using a GelCount (Oxford Optronix, Oxford, UK) and results plotted.