Supplemental figure legends

The uncropped blots of the western blotting experiments are exhibited (online supplemental figure S9–S12).

Figure S1 Ccl3 is significantly induced in macrophages by DTX.

(A) Experimental design scheme of macrophage clearance for DTX chemotherapy.

(B) IHC staining of CD68 (a pan-macrophage marker) with paraffin-embedded tumor sections. The representative pictures are shown, and the bar graph represents the fold change of $CD68^+$ cell percentage in six random fields under 40× objective. Scale bar: 100 μm.

(C) Tumor image for figure 1A.

(D) qRT-PCR was performed to quantify Ccl3 expression after DTX (10 nM for iBMMs and 30 nM for BMDMs) treatment for 2 and 3 days in iBMMs and BMDMs.

(E) qRT-PCR was performed to quantify Ccl3 expression after DTX treatment at indicated concentrations in iBMMs and BMDMs.

(F) Tumors of 4T1 orthotopic allografts were digested and sorted by fluorescence-activated cell sorting assay. Different sub-populations, including total cells (cancer and immune cells), $CD45^+$ cells (whole immune cells), $CD45^+CD11b^+F4/80^+$ cells (Mac), $CD45^+CD19^+$ cells (B cells) and $CD45^+CD3^+$ cells (T cells) were obtained to analyze the expression levels of Ccl3, Ccr1 and

Ccr5 by qRT-PCR.

Tbp was used as the internal control. Data are presented as mean±SEM. *P<0.05, **p<0.01, ***p<0.001, ****p<0.0001. BMDMs, bone marrow-derived macrophages; Ccl3, C–C motif chemokine ligand 3; Ccr, C–C motif chemokine receptor; CLD-Lp, Clodronate liposomes; DTX, docetaxel; IHC, immunohistochemistry; iBMMs, immortalized bone marrow-derived macrophages; Mac, macrophage; PBS-Lp, PBS liposomes; qRT-PCR, quantitative real-time PCR; Tbp, TATA-binding protein; Veh, vehicle.

Figure S2 Ccl3 enhances the chemotherapeutic efficacy of DTX in breast cancer.

(A) Knockout effect was determined in all three mouse strains, including C57BL/6, FVB and BALB/c, using BM cells stimulated with LPS (1 μg/ml) for 4 hours via western blotting.

(B) Expression levels of C–C chemokines were quantified in WT and *Ccl3*$^{-/-}$ BMDMs using qRT-PCR.

(C-E) At the end of the experiment in figure 1G, mice were sacrificed. The harvested tumors were photographed (left) and weighed (right).

(F) HE staining of paraffin-embedded tumor sections obtained in figure 1G. Areas of necrosis were separated from tumor mass by black dotted lines and indicated by a red star. Scale bar: 100 μm.

(G-H) At the end of the experiment in figure 1H, mice were sacrificed. The harvested tumors were photographed (left) and weighed (right).

(I) Knockdown efficiency of Ccl3 in iBMMs was measured via western blotting. Gapdh was used as the loading control. Tbp was used as the internal control. Data are presented as mean±SEM. *P<0.05, **p<0.01, ***p<0.001, ****p<0.0001; NS, no significance. BM, bone marrow; BMDMs, bone marrow-derived macrophages; Ccl3, C–C motif chemokine ligand 3; DTX, docetaxel; Gapdh, glyceraldehyde-3-phosphate dehydrogenase; HE, hematoxylin and eosin; iBMM, immortalized bone marrow-derived macrophages; LPS, lipopolysaccharide; qRT-PCR, quantitative real-time PCR; sh, short hairpin RNA; shNT, short hairpin RNA of non-target; Tbp, TATA-binding protein; Veh, vehicle; WT, wild type.

Figure S3 DTX-induced proinflammatory macrophage polarization is abolished by Ccl3-knockout.

(A) Flow cytometry gating strategy of proinflammatory macrophages using cells from digested tumor of 4T1 allografts.

(B) Paraffin-embedded tumor sections obtained in figure 1G were used for IHC staining with antibody against CD68 and Cox2 (proinflammatory macrophage marker). The representative pictures are shown, and the bar graphs represent the fold change of $CD68^{+}Cox2^{+}$ cell percentage in six random fields under 40×

objective. Scale bar: 100 μm.

(C) Knockdown efficiencies of Ccr1 and Ccr5 in iBMMs were measured via qRT-PCR.

Tbp was used as the internal control. Data are presented as mean±SEM. **P<0.01, ****p<0.0001. Ccl3, C–C motif chemokine ligand 3; Ccr, C–C motif chemokine receptor; Cox2, cyclooxygenase; DTX, docetaxel; IHC, immunohistochemistry; iBMMs, immortalized bone marrow-derived macrophages; qRT-PCR, quantitative real-time PCR; Tbp, TATA-binding protein; Veh, vehicle; WT, wild type.

Figure S4 Ccl3 is indispensable for DTX-enhanced macrophage phagocytosis of cancer cells, including CSCs.

(A) DTX-pretreated iBMMs were cocultured with total Py8119 cells at both 37°C (upper panel) and 4°C (lower panel) for in vitro phagocytosis assay and analyzed via flow cytometry.

(B) DTX-pretreated iBMMs (GFP-labeled) were cocultured with total Py8119 cells (mCherry-labeled) at both 37°C (upper panel) and 4°C (lower panel) for in vitro phagocytosis assay and analyzed by directly visualized confocal microscope photography.

(C) Representative images for figure S4B. The different stages of phagocytosis showing macrophages from contacting to internalizing cancer

cells were observed at 37°C. Scale bar: 30 μm.

(D-E) In vitro phagocytosis assay was performed using DTX-pretreated iBMMs cocultured with sorted $ALDH^-$ or $ALDH^+$ Py8119 cells and analyzed by flow cytometry analysis (D) and directly visualized confocal microscope photography (E).

Data are presented as mean±SEM. *P<0.05, **p<0.01, ***p<0.001, ****p<0.0001. ALDH, aldehyde dehydrogenase; Ccl3, C–C motif chemokine ligand 3; CSCs, cancer stem cells; DTX, docetaxel; iBMMs, immortalized bone marrow-derived macrophages; sh, short hairpin RNA; shNT, short hairpin RNA of non-target; Veh, vehicle.

Figure S5 Ccl3 enhances DTX chemotherapeutic efficacy and Ccl3 overexpression suppresses tumor progression in vivo.

(A) Ccl3 knockdown efficiency was measured by qRT-PCR in 4T1.

(B) At the end of the experiment in figure 4E, mice were sacrificed. The harvested tumors were photographed (left) and weighed (right).

(C) Ccl3 overexpression effect was determined via western blotting in 4T1 and Mvt1.

(D) At the end of the experiment in figure 4F, mice were sacrificed. The harvested tumors were photographed (left) and weighed (right).

(E) IHC staining of paraffin-embedded sections obtained in figure 4F using

antibodies against CD68 and Cox2. The representative pictures are shown, and the bar graphs represent the fold change of $CD68^{+}Cox2^{+}$ cell percentage in six random fields under 40× objective. Scale bar: 100 μm.

Gapdh was used as the loading control. Tbp was used as the internal control. Data are presented as mean±SEM. *P<0.05, **p<0.01, ***p<0.001, ****p<0.0001; NS, no significance. Ccl3, C–C motif chemokine ligand 3; Cox2, cyclooxygenase; DAPI, 4',6-diamidino-2-phenylindole; DTX, docetaxel; IHC, immunohistochemistry; qRT-PCR, quantitative real-time PCR; sh, short hairpin RNA; shNT, short hairpin RNA of non-target; Tbp, TATA-binding protein; Veh, vehicle.

Figure S6 DTX-induced Ccl3 creates a positive feedback effect loop between macrophages and cancer cells.

(A) 4T1 and Mvt1 were pretreated with DTX (5 nM) for 7 days, and then the drug was washed out and cells were cultured in serum-deprived medium for 2 days to collect CM. Thereafter, iBMMs were cultured with the CM for 2 days and the cells were collected and lysed for western blotting to detect the expression of Ccl3, iNos and Cox2.

(B) 4T1 and Mvt1 overexpressing Ccl3 were cultured in serum-deprived medium for 2 days to collect CM. Thereafter, iBMMs were cultured with the CM for 2 days and the cells were collected and lysed for western blotting to detect

the expression of Ccl3, iNos and Cox2.

(C) iBMMs were pretreated with DTX (10 nM) for 7 days, and then the drug was washed out and cells were cultured in serum-deprived medium for 2 days to collect CM. Thereafter, 4T1 and Mvt1 were cultured with the CM for 2 days and the cells were collected and lysed for qRT-PCR to quantify Ccl3 expression.

(D) Ccl3 overexpression was determined by western blotting in iBMMs.

(E) iBMMs with Ccl3 overexpression were cultured in serum-deprived medium for 2 days to collect CM, and then 4T1 and Mvt1 were cultured with the CM for 2 days. The cells were collected and lysed for RNA isolation to quantify Ccl3 expression by qRT-PCR.

Gapdh was used as the loading control. Tbp was used as the internal control. Data are presented as mean±SEM. **P<0.01, ***p<0.001. ****p<0.0001. Ccl3, C–C motif chemokine ligand 3; Cox2, cyclooxygenase; CM, conditioned medium; DTX, docetaxel; iBMM, immortalized bone marrow-derived macrophages; iNos, inducible nitric oxide synthase; qRT-PCR, quantitative real-time PCR; Tbp, TATA-binding protein; Veh, vehicle.

Figure S7 DTX induces Ccl3 by relieving the inhibitory effect of Creb via ROS accumulation.

(A) ROS levels in BMDMs and Py8119 cells treated with DTX (30 nM) for 2

days were determined using flow cytometry.

(B) Western blotting was performed to detect the expression of Ccl3, Creb and p-Creb in BMDMs treated with different concentrations of DTX (0, 10, 30 and 50 nM) for 1 day.

(C) Western blotting was used to detect the expression of Creb and p-Creb, whereas qRT-PCR was utilized to quantify Ccl3 expression in Py8119 treated with different concentrations of DTX (0, 15, 30 and 60 nM) for 1 day.

(D) iBMMs and BMDMs, as well as breast cancer cell lines 4T1 and Py8119, were treated with indicated concentrations of DTX for 1 day. qRT-PCR was performed to quantify Creb expression.

(E) Various concentrations of H_2O_2 (0, 50, 100 and 250 μM) were used to induce ROS in BMDMs for 16 hours. Ccl3, Creb, and p-Creb expression levels were determined via western blotting.

(F) Various concentrations of H_2O_2 (0, 100, 250, 500 and 1000 μM) were used to induce ROS in Py8119 for 16 hours. Western blotting was utilized to detect the expression of Creb and p-Creb, whereas qRT-PCR was used to quantify Ccl3 expression.

(G) NAC (10 mM) was used to neutralize ROS in combination with DTX treatment (30 nM) for 1 day in BMDMs. Ccl3 and Creb expression levels were determined via western blotting.

(H) NAC (10 mM) was used to neutralize ROS in combination with DTX

169 treatment (30 nM) for 1 day in Py8119. Creb and p-Creb expression levels
170 were determined via western blotting, whereas Ccl3 expression was quantified
171 by qRT-PCR.
172 (I) Creb inhibitor 666-15 (200 nM) was administered to BMDMs for 12 hours.
173 The cells were collected for western blotting and qRT-PCR analysis to detect
174 Ccl3.
175 (J) Creb inhibitor 666-15 (200 nM) was administered to Py8119 for 12 hours.
176 qRT-PCR was performed to quantify Ccl3 expression.
177 (K) Ccl3 expression was quantified via qRT-PCR in Py8119 knocking down
178 Creb.
179 (L) Py8119 stable cell line overexpressing Creb was subjected to DTX (30 nM)
180 and DOC (1 μg/ml) treatment for 2 days. Creb was cloned into an inducible
181 pTRIPZ overexpression vector induced with DOC. The cells were collected for
182 qRT-PCR to quantify Ccl3 expression.
183 Gapdh was used as the loading control. Tbp was used as the internal control.
184 Data are presented as mean±SEM. *P<0.05, **p<0.01, ***p<0.001,
185 ****p<0.0001; NS, no significance. BMDMs, bone marrow-derived
186 macrophages; Ccl3, C–C motif chemokine ligand 3; Creb, cAMP-response
187 element binding protein; DOC, doxycycline; DTX, docetaxel; Gapdh,
188 glyceraldehyde-3-phosphate dehydrogenase; H_2O_2, hydrogen dioxide; iBMM,
189 immortalized bone marrow-derived macrophages; NAC, N-acetyl-L-cysteine;

190 NES, normalized enrichment score; qRT-PCR, quantitative real-time PCR;
191 RNA-Seq, RNA sequencing; ROS, reactive oxygen species; sh, short hairpin
192 RNA; shNT, short hairpin RNA of non-target; Tbp, TATA-binding protein; Veh,
193 vehicle.
194
195 **Figure S8 High CCL3 expression predicts better prognosis and Creb**
196 **inhibitor or rmCcl3 increases DTX chemosensitivity in breast cancer.**
197 (A) IHC staining of CCL3 was performed using paired tumor sections of
198 patients before or after TNC (n=100). CCL3 expression levels of cancer cells
199 and TME cells were separately calculated by H-score.
200 (B-C) Representative pictures of IHC staining for figure 6C and figure 6D are
201 shown. Scale bar: 100 μm.
202 (D) Indicated concentrations of 666-15 and DTX were combined to treat
203 Py8119 for 20 hours, and qRT-PCR was performed to quantify Ccl3
204 expression.
205 (E) At the end of the experiment in figure 6G, mice were sacrificed. The
206 harvested tumors were photographed (left) and weighed (right).
207 (F) At the end of the experiment in figure 6I, mice were sacrificed. The
208 harvested tumors were photographed (left) and weighed (right).
209 Tbp was used as the internal control. Data are presented as mean±SEM.
210 *P<0.05, **p<0.01, ***p<0.001, ****p<0.0001; NS, no significance. Ccl3, C–C

motif chemokine ligand 3; Cox2, cyclooxygenase 2; Creb, cAMP-response element binding protein; TNC, Taxane-containing neoadjuvant chemotherapy; DTX, docetaxel; H-score, histo-score; IHC, immunohistochemistry; qRT-PCR, quantitative real-time PCR; rmCcl3, recombinant mouse Ccl3; Tbp, TATA-binding protein; TME, tumor microenvironment; Veh, vehicle.

Figure S9 Uncropped blots of Figure 1 and 2.

Figure S10 Uncropped blots of Figure 5 and 6.

Figure S11 Uncropped blots of Figure S2, S5 and S6.

Figure S12 Uncropped blots of Figure S7.