Supplemental Data

Reactive myelopoiesis and FX-expressing macrophages triggered by chemotherapy promote cancer lung metastasis

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Supplemental Table 1. Antibodies used in Flow cytometry and Western blotting

Antibodies	**Company and catalog number**
PerCP/Cyanine5.5 anti-mouse CD45	BioLegend No. 157208
PE/Cyanine7 anti-mouse CD45	BioLegend No. 103114
APC anti-mouse/human CD11b	BioLegend No. 101212
PE anti-mouse Ly6G	BioLegend No. 127608
PerCP anti-mouse Ly6C	BioLegend No. 128028
PE anti-mouse F4/80	BioLegend No. 123110
FITC anti-mouse CD11c	BioLegend No. 117306
APC anti-mouse CD3	BioLegend No. 100236
APC anti-mouse CD4	BioLegend No. 100412
FITC anti-mouse CD8a	BioLegend No. 100706
APC anti-mouse NK-1.1	BioLegend No. 108710
APC anti-mouse CD19	BioLegend No. 115512
APC anti-mouse Ly-6G/Ly-6C	BioLegend No. 108412
APC anti-mouse TER-119	BioLegend No. 116212
Anti-mouse Ly6A/E (Sca-1)	BD Bioscience No. 553108
PE/Cyanine7 anti-mouse CD117 (c-kit)	BioLegend No. 135112
FITC anti-mouse CD48	BioLegend No. 103404
PE/Cyanine5 anti-mouse CD150 (SLAM)	BioLegend No. 115912
PE anti-mouse IFN-γ	BioLegend No. 505808
PE anti-human/mouse Granzyme B	BioLegend No. 372208
PE anti-mouse FoxP3	BioLegend No. 126404
APC-conjugated anti-mouse CCR2	R&D Systems No. FAB5538A-100
Fixable Viability Dye eFluor™ 780	Thermo Fisher Scientific No. 65-0865-14
Polyclonal Rabbit anti-Human F10 / Factor X Antibody	LifeSpan BioSciences No. LS-C331476

Supplemental Table 2. Antibodies used in CyTOF

	Antibodies	**Company and catalog number**
1	Anti-Mouse CD45 (30-F11)-89Y	Fluidigm, No. 3089005B
2	Anti-Mouse Ly-6G (1A8)-141Pr	Fluidigm, No. 3141008B
3	Anti-Mouse CD11c (N418)-142Nd	Fluidigm, No. 3142003B
4	Anti-Mouse CD69 (H1.2F3)-143Nd	Fluidigm, No. 3143004B
5	Anti-Mouse CD4 (RM4-5)-145Nd	Fluidigm, No. 3145002B
6	Anti-Mouse F4/80 (BM8)-146Nd	Fluidigm, No. 3146008B
7	Anti-Mouse CD103 (2E7)/148Nd	Biolegend, No. 121402
8	Anti-Mouse CD19 (6D5)-149Sm	Fluidigm, No. 3149002B
9	Anti-Mouse Ly-6C (HK1.4)-150Nd	Fluidigm, No. 3150010B
10	Anti-Mouse CD25 (3C7)-151Eu	Fluidigm, No. 3151007B
11	Anti-Mouse CD3e (145-2C11)-152Sm	Fluidigm, No. 3152004B
12	Anti-Mouse CD274/PD-L1-153Eu (10F.9G2)	Fluidigm, No. 3153016B
13	Anti-Mouse PD-1 (29F.1A12)-159Tb	Fluidigm, No. 3159024B
14	Anti-Mouse CD62L (MEL-14)-160Gd	Fluidigm, No. 3160008B
15	Anti-Human/Mouse CD44 (IM7)-162Dy	Fluidigm, No. 3162030B
16	Anti-Mouse CX3CR1 (SA011F11)-164Dy	Fluidigm, No. 3164023B
17	Anti-Mouse CD8a (53-6.7)-168Er	Fluidigm, No. 3168003B
18	Anti-Mouse CD206/MMR (C068C2)-169Tm	Fluidigm, No. 3169021B
19	Anti-Mouse NK1.1 (PK136)-170Er	Fluidigm, No. 3170002B
20	Anti-Mouse CD11b (M1/70)-172Yb	Fluidigm, No. 3172012B
21	Anti-Mouse CD223/LAG3 (C9B7W)-174Yb	Fluidigm, No. 3174019B
22	Anti-Human/Mouse CD45R/B220 (RA3-6B2)-176Yb	Fluidigm, No. 3176002B
23	Anti-Mouse I-A/I-E (M5/114.15.2)-209Bi	Fluidigm, No. 3209006B
24	Anti-Mouse CD127/IL7Ra (A7R34)-175Lu	Fluidigm, No. 3175006B

Supplemental Figure 1

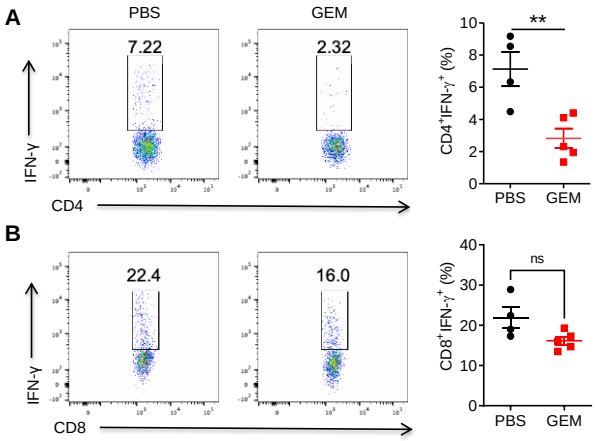

Figure S1. Mice bearing subcutaneous E0771 tumor cells were treated with 4 times of GEM in two weeks. Lung tissues were collected 2 days later after last GEM treatment. Cells were stimulated with PMA/Ionomycin in the presence of protein transport inhibitor brefeldin A for 4 hours. IFN-γ producing CD4 T^+ cells (**A**) and $CD8^+$ T cells (**B**) were determined by intracellular cytokine staining and Flow cytometry. Each dot represents one mouse (n=4-5). ***p <0.01* by unpaired 2-sided *t* test.

Supplemental Figure 2

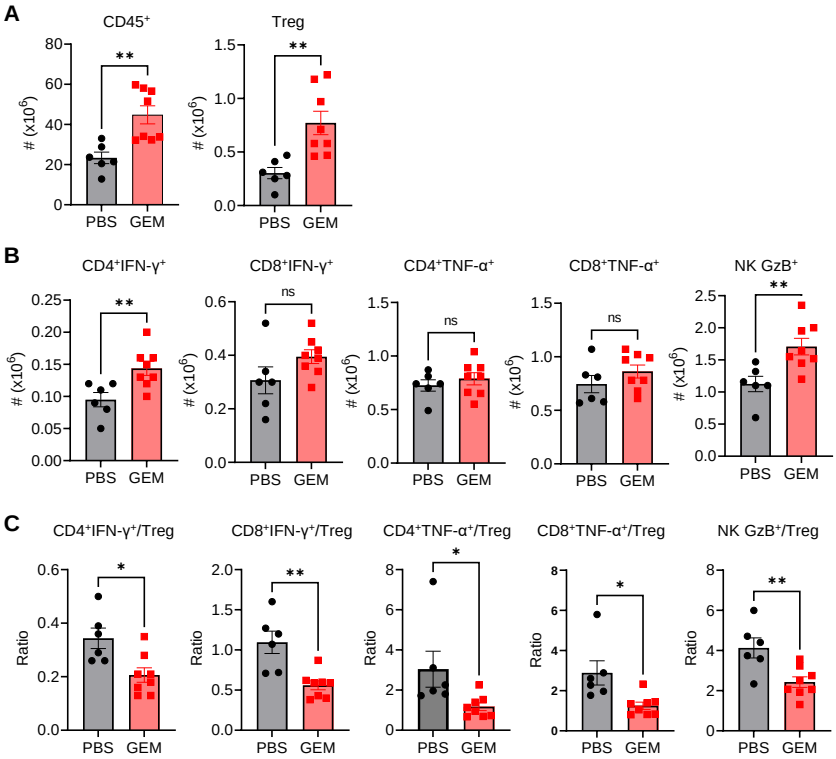

Figure S2. Tumor free mice were treated with 4 times of GEM or PBS followed by intravenous injection of $4x10^5$ E0771-GFP cells. Lung tissues were collected at day 14 after tumor cell injection. (**A**) Total numbers of $CD45^+$ and Treg cells in each lung. (**B**) Total numbers of effective T cells and NK cells. (**C**) Ratios of effective T cells and NK cells to Treg cells. Each dot represents one mouse (n=6-8). **p* <0.05, ***p* <0.01 by unpaired 2-sided *t* test.

Supplemental Figure 3

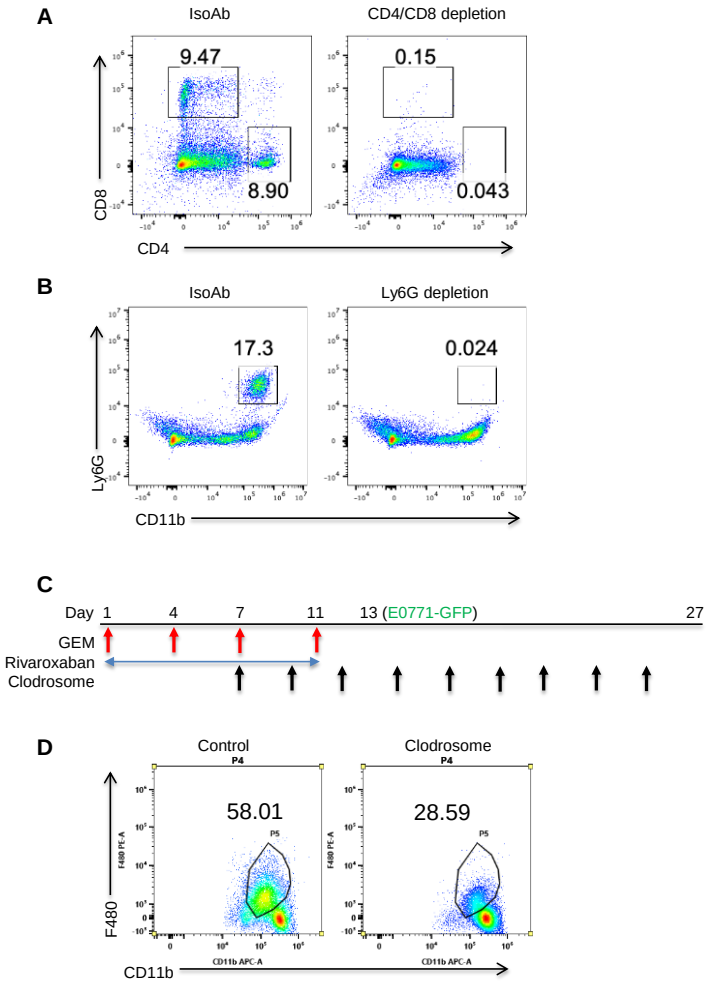

Figure S3. (**A**) Depletion efficiency of $CD4^+$ and $CD8^+$ T cells in the lungs. (**B**) Depletion efficiency of $Ly6G^+$ cells in the lungs. (**C**) Schema for in vivo GEM and Rivaroxaban treatment, macrophage depletion, and E0771-GFP tumor cell intravenous injection. (**D**) Lung macrophage depletion efficacy after intravenous injection of 3 times of Clodrosome was determined by staining cells with CD45, CD11b, F4/80, and viability dye. Cells were gated on $CD45^+CD11b^+$ population.

Supplemental Figure 4

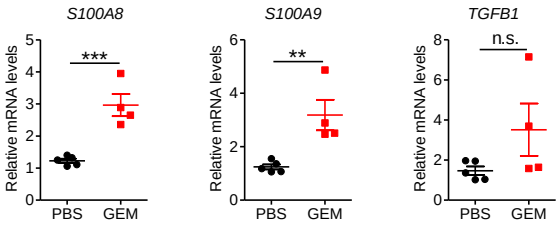

Figure S4. Naïve mice were treated with 4 times of GEM in two weeks. Lung tissues were collected 48 hours later after last treatment. Gene expression of *S100A8*, *S100A9*, and *TGFB1* in lung tissues from GEM and PBS treated mice was determined with qRT-PCR. Each dot represents one mouse (n=4-5). ***p* <0.01, ****p* <0.001 by unpaired 2-sided *t* test.

Supplemental Figure 5

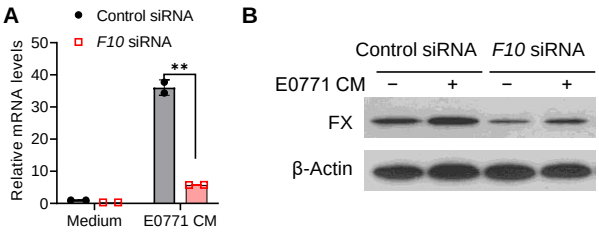

Figure S5. BM-derived macrophages were transfected with control siRNA and *F10* siRNA. 24 hours later cells were treated with 20% E0771 conditioned medium (CM) for 24 hours. *F10* gene knock down efficiency was determined by using qRT-PCR (**A**) and Western blotting (**B**). **p <0.01 by unpaired 2-sided t test.

Supplemental Figure 6

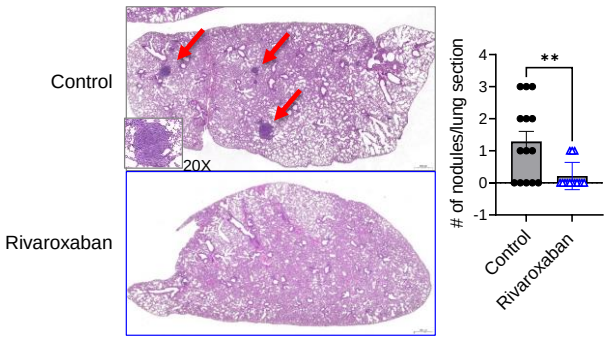

Figure S6. E0771-GFP subcutaneous tumor-bearing mice (primary tumor size between 6-8 mm in diameter) were treated with Rivaroxaban (oral, 20 mg/kg, daily) or solvent control for two weeks. Lung tissues were collected at day 31 after tumor cell injection. The metastasis in lungs was determined by evaluation of tumor nodules using H&E staining. One section represents one mouse (n=14). Numbers of nodule per lung section were counted and summarized. ***p* <0.01 by unpaired 2-sided *t* test.

Supplemental Figure 7

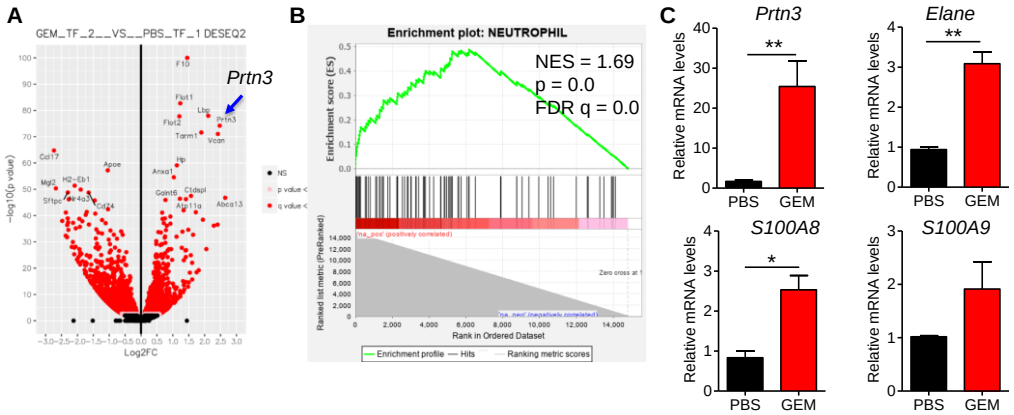

Figure S7. (**A**) Differential gene expression in sorted macrophages from GEM-treated tumor-free mice as compared to macrophages from PBS-treated mice. Gene *Prtn3* is one of DEGs in lung macrophages. (**B**) Analysis of lung macrophage RNA-seq data revealed the enrichment plot with upregulation of neutrophil signature genes in lung macrophages from GEM-treated versus PBS-treated tumor-free mice. (**C**) Lung monocytes ($CD45^{+}CD11b^{+}Ly6C^{high}$) were sorted for gene expression analysis using qRT-PCR. *p <0.05, **p <0.01 by unpaired 2-sided t test.

Supplemental Figure 8

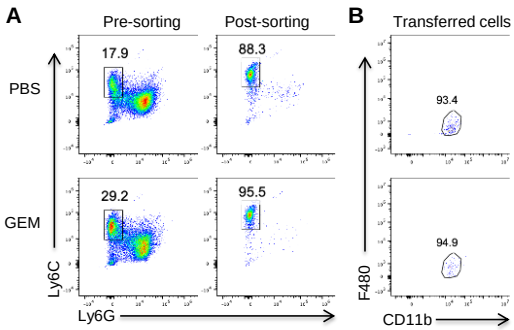

Figure S8. (**A**) Percentages of BM monocytes from PBS and GEM treated mice before and after purification. (**B**) CFSE labeled purified monocytes ($1.5x10^6$) were intravenously injected into CCR2 KO mice. Expression of CD11b and F480 in transferred cells ($CFSE^+$) in the lungs analyzed 48 hours later.

Supplemental Figure 9

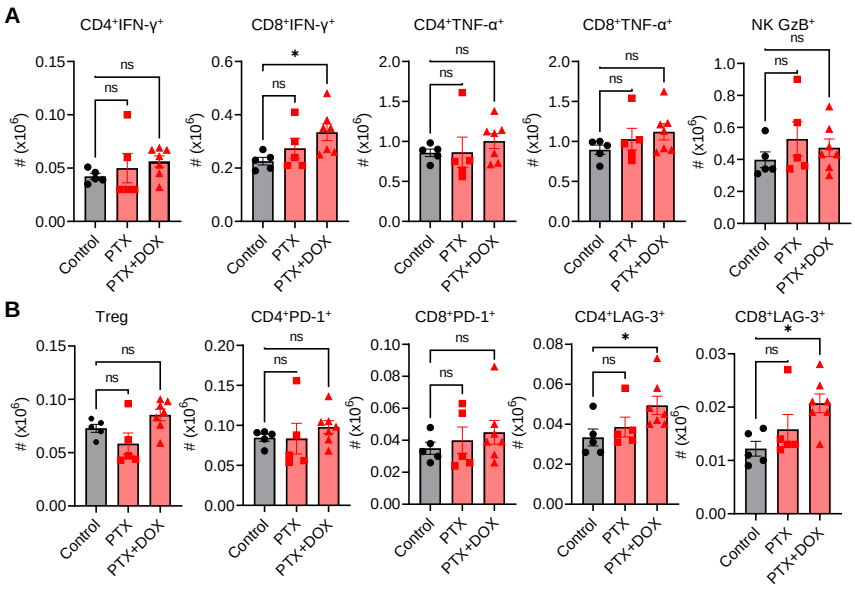

Figure S9. Tumor free mice were treated with PTX or PTX plus DOX. Lung tissues were collected 48 hours later. (**A**) Total numbers of effective $CD4^+$, $CD8^+$ T cells and granzyme B $(GzB)^+$ NK cells. (**B**) Total cell number of immunosuppressive Treg and PD-1, LAG-3 expressing $CD4^+$, $CD8^+$ T cells. *p <0.05 by ordinary one-way ANOVA.