Supplemental Figure 1. TARC and CD11b/c expression in lungs of 4T1 challenged mice. Confocal imaging picture of mouse tumor-bearing lung tissues stained for TARC Ab (green) and CD11b/c (red). The data indicate that TARC is not expressed by CD11b/c stained cells. Panels B and C show staining for TARC alone and CD11b/c alone, respectively. Panel D is light microscopy image, Panel E is overlay of B, C and D (same as panel A, in higher magnification). Panel F is for control antibody staining (red blood cells show some low level auto fluorescence). **Results of confocal study:** TARC was predominantly expressed in metastatic lungs and possibly by tumor stromal cells. There was a significant infiltration of tumor with CD11b/c positive cells which did not express significant amounts of TARC. A few infiltrating T cells ($CD3^+$) and B cells ($CD45R^+$) were also negative for TARC (data not shown).

Antibodies and reagents and experimental procedure used for confocal staining of mouse lungs: Anti-TARC Ab (N20**,** sc-12271) was from Santa Cruz Biotechnology Inc., Santa Cruz, CA; anti-CD11b/c (ab53187), anti-CD3ε (ab49943), and anti-CD45R (ab64100) Abs were from Abcam Inc., Cambridge, MA. Antigen unmasking solution (H-3300), normal horse serum (S-2000), Avidin-Biotin blocking kit (SP2001), goat IgG (I-5000), fluorescein Avidin DCS (A2011), biotinilated anti-goat IgG (BA-9500), and vectashield Mounting medium (H-1400) were from Vector Laboratories, Inc., Burlingame, CA. Alexa 555 conjugated anti-rat and anti-rabbit IgGs were from Invitrogen Corp., Carlsbad, CA.

Formalin fixed and paraffin embedded lung tissue sections were deparafffinazed and rehydrated. Antigen retrieval was performed by boiling for 25 min in antigen

unmasking solution. Sections were blocked with with avidin-biotin blocking kit followed by 5% normal horse serum and were incubated with goat anti-Tarc Ab (2 ug/ml) diluted in 5% normal horse serum o/n at 4C. Biotinilated anti-goat IgG produced in horse was used at 3 ug/ml for 1 hours at 37C followed by fluorescein avidin DCS (5 ug/ml) for 30 min at RT. After washings, sections were blocked with 5% normal donkey serum for 1 hour at 37C and incubated with either anti-CD11b/c (1:100), CD3ε (1:200), or CD45R (1:100) Abs for 1 hour at 37C. Thereafter, section were incubated with corresponding donkey secondary Abs conjugated to Alexa 555 for 30 min at 37C and mounted using Vectashield mounting medium. Images were acquired with a x40 objective on an Axiovert 200 microscope (Carl Zeiss Vision) and using Axiovison software (Carl Zeiss Vision).

Supplemental Figure 2. (A) $CD11b^-$ cells, but not $CD11b^+$ immune cells, constitutively produce MDC. It can be further augmented by in vivo treatment with tumor CM (tumor CM, mice # 1-3), but not with control medium (Medium, mice # 4 and 5) or PBS (Naïve, mouse # 6). Mice were i.p. injected with media as in Fig.1B and lung $CD11b^+$ and $CD11b^-$ cells were separated by magnetic sorting and overnight ex vivo cultured before testing for production of MDC by ELISA. Note, TARC levels were below sensitivity of the assay, but only mouse #2 $CD11b^-$ expressed about 80 pg/ml TARC. **(B)** Cultured mouse primary fibroblasts (C57, MLE12) produce MDC upon treatment with tumor CM. In contrast, immortalized fibroblast cell line NIH3T3 failed to produce MDC. Shown, mean ± SD (pg/ml) of triplicates of secreted MDC from cells after overnight treatment with tumor CM (Tumor CM) or control medium (Medium).

Supplemental Figure 3. CCR4 is expressed in murine breast 4T1 tumor cells. (A) Schema of CCR4 – specific primer locations on cDNA of murine CCR4 (NM_009916) and (**B**) results of RT/PCR are shown. Several sets of specific primers were tested to circumvent non-specific amplification. The coding region of CCR4 is in a single exon (rectangle). Total RNA was isolated combining Trizol reagent (Invitrogen) and a RNeasy kit (Qiagen Inc., Valencia, CA); and cDNA was made using Superscript II RT with a hexamer random primer at 37°C for 30 min and amplified using 2U *Taq* DNA polymerase (New England Biolabs Inc., Beverly, MA): 35 cycles of 94°C for 1 min, 60°C for 1 min, and 72°C for 1 min. Murine CCR4 cDNA fragments were PCR amplified using any combination of pairs of primers (see Suppl. Fig.1), such as forward primers: PRmCCR4-6 (ATTCTGTTGTGGTTCTGGTCCT), or PRmCCR4-R1 (AGGTCTGTGCAAGATCGTTTCATGG), or PRmCCR4-2 (GGCCTCTTGTTCAGCACTTG); and reverse primers: PRmCCR4-R6 (GCAGTGTTGCAGAGTCCTAATG), or PRmCCR4-R4 (CCGTACAACGTGGTGCTTTT), or PRmCCR4-1 (ATCCTGAAGGACTTCAAGCTCCA), or PRmCCR4-R8 (CACCTGCAGGGTGAGAAGCCATCTTGCCAT). Control RT/PCR was performed using murine glyceraldehyde-3-phosphate dehydrogenase, PRuGAPDH-1, 5'-TGTGGAAGGGCTCATGACCACAGTCCAT-3' and PRuGAPDH-R1, 5'-GCCTGCTTCACCACCTTCTTGATG-3'.

(**C**) CCR4 is also expressed on a **s**mall but significant proportion of human breast cancer MCF-7, but not MDA-231. The cells were stained with anti-CCR4 Ab or control isotype-matched Ab (not shown) and tested by FACS. Numbers indicate percentage of cells in the corresponding quadrant.

Supplemental Figure 4. (A) TARC-PE38 specifically kills purified CCR4-expressing CCRF-CEM (CEM), but not CCR4-negative MOLT-4 cells. The viability of cells treated with TARC-Ag or PBS was not affected (not shown). Cell death was evaluated using WST assay after 2 days of treatment. Shown, triplicate mean percentage of viable cells compared with untreated cells ± SD from three independent experiments. X-axis, treatment dose of TARC-PE38 (μg/ml). *$P<0.01$ is for comparisons with control treatments. **(B)** TARC-PE38 induces death of 4T1 and 4T1.2 tumor cells. In contrast, toxins that target CCR7 and CCR10 (SLC-PE38 and CCL27, respectively) or control TARC-Ag do not induce cell death of 4T1 tumor cells. Shown as percentage of viable cells to untreated cells evaluated by WST assay after 2 days of treatment. X-axis, treatment dose of TARC-PE38 (μg/ml). Representative data (mean ± SD of triplicates) from four independent experiments. X-axis, treatment dose (μg/ml). **(C)** 4T1-PE and 4T1.2-PE cells have stable phenotype, as they retain TARC-PE38 resistance even after several months of cultivation. Viability was assessed after treatment with titrated amounts of TARC-PE38 (μg/ml, X-axis). Control TARC-Ag or irrelevant chemotoxins did not kill any of the cells (data not shown).

Supplemental Figure 5. CCR4 – expressing tumors (4T1 and 4T1.2 tumors), but not TARC-PE38 -resistant tumors (4T1wt-PE and 4T1.2-PE), metastasize to lung. Metastatic nodules (arrows) are readily visible in lungs of BALB/C mice after challenge with 4T1 and 4T1.2 cells. Mice were euthanized at day 28 after tumor challenge with 4T1, 4T1.2, 4T1wt-PE and 4T1.2-PE tumors.

Supplemental Figure 6. TARC and MDC are produced in lungs of 4T1 tumor – bearing NOD/SCID mice. BAL of individual tumor free naïve mice (Naïve) and 4T1.2 tumor – bearing mice (4T1.2 bearer) was assessed for TARC and MDC expression by ELISA. Average amount of secreted chemokines (pg/ml) of three individual lungs ± SEM is shown on Y-axis.

Supplemental Figure 7. The majority of cells that migrated to TARC are FoxP3$^+$. The cells from lower wells of the chemotaxis chamber (migrated cells in Fig.3D) were stained for CD4 (Y-axis) and FoxP3 (X-axis) and analyzed by by FACS. Numbers indicate percentage of cells in the corresponding quadrant. Data are reproduced two times.

Supplemental Figure 8. CCR4$^+$ Tregs, but not non-Tregs facilitate lung metastasis of 4T1 tumors. Splenic Tregs and non Tregs, which were isolated from naïve BALB/C mice, were pretreated with TARC-PE38 for 1 hour. Then, the cells were washed and adoptively transferred into NOD/SCID mice challenged with 4T1.2 cells. Control tumor

– bearing NOD/SCID mice that did not receive cells (PBS) did not metastasize to lung. Mean of lung metastasis ±SEM of four per group experiment, which was reproduced twice.

Supplemental Figure 9. Effector non-Treg T cells do not affect lung metastasis. 4T1.2 tumor-bearing four per group BALB/C mice were i.p. treated with 400 μg anti-CD4 mAb (FGK 1.5), or anti-CD8 mAb (FGK 2.43), or control Ab at 3, 7, 12 and 15 days post tumor challenge. The depletion of $CD4^+$ and $CD8^+$ cells was >98% at the time of sacrifice (day 28) as assessed by FACS. Shown, mean lung metastasis ±SEM at day 28 post tumor challenge of four mice per group experiment. Experiments were reproduced two times.

Supplemental Figure 10. Lung metastasis can be controlled by targeting $CCR4^+$ cells. 4T1.2 tumor-bearing mice were i.v. treated daily 5μg/ml for 5 days with TARC-PE38, or CCL27-PE38, or mock (PBS). Shown, mean lung metastasis ±SEM at day 28 post tumor challenge of four mice per group experiment. Experiments were reproduced two times. *P <0.05.