Adoptive transfer
Naïve lymphocytes were isolated from spleen and lymph nodes of donor Balb/c mice. Total splenocytes were used or T cells were enriched using an R&D Systems Mouse CD3+ T cell enrichment column, according to manufacturer's instructions. Splenocytes or enriched T cells were labeled with CFSE (Carboxyfluorescein succinimidyl ester at a 2.5 μM final concentration) (ThermoFisher, cat# 65-0850-84). Purified T cells were labeled with CellTracker™ Green CMFDA (5-chloromethylfluorescein diacetate at a 1 μM final concentration) (Molecular Probes, cat# C7025) according to manufacturer's instructions. After rinsing, 10, 14, or 20 million splenocytes or T cells were suspended in a 100μL volume of PBS and injected intravenously into each animal with a 30G 1/2-inch needle. T cells were allowed to circulate for 4 hours before tissue collection.

Lectin perfusion
Biotinylated lectin (5-20 µg Vector Labs, Inc., CA) was injected retro-orbitally and allowed to circulate for 10 minutes. Lymph nodes were excised and fixed for 1 hour at room temperature in 4% formaldehyde followed by dehydration overnight in 30% sucrose.

Detection of metastases
For determining the incidence of LN metastasis, tumor-draining lymph nodes were pooled and sectioned serially until the frozen block was depleted. Slides from serial sectioning were then stained with cytokeratin to detect metastasis. For determining the number of pulmonary metastases, visible tumor nodules on the surface of the lung were manually counted.

Immunofluorescence staining
Murine
Murine lymph nodes were harvested and embedded with optimal cutting temperature compound prior to freezing at -80°C and 7-10-μm frozen tissue sections were cut using a cryostat. Slides from fresh frozen samples were fixed in -20°C acetone for 10 minutes and allowed to air-dry. Tissue on slides was then blocked in 5% normal donkey serum for 30 minutes at room temperature to minimize non-specific antibody staining. Macrophages were stained with anti-F4/80 (anti-mouse, Biorad cat # MCA497) and anti-CD68 (anti-human, DAKO cat # M0718). For high endothelial venule visualization, we used a purified anti-mouse/human PNAd antibody (Biolegend, cat # 120801). To identify CD3+ T cells, APC conjugated anti-mouse CD3 antibody (Biolegend cat # 100236) or purified anti-CD3 antibody reactive with several species, including mouse, (Abcam cat # ab16669) was used. CD8+ T cells were stained with PE-conjugated anti-mouse CD8 antibody (Biolegend cat # 100708). For B cells, PE- conjugated anti-mouse B220 antibody (BD Bioscience, cat # 553390) was used. To visualize murine tumor cells, purified anti-cytokeratin or FITC-conjugated anti-cytokeratin antibodies (Sigma-Aldrich cat #s C2931, F3418, respectively) were used. Cells expressing Granzyme B were stained with an anti-Granzyme B antibody (Abcam cat # ab4059). Cells expressing PD-1 were stained with a biotinylated anti-PD-1 antibody (eBioscience cat #13-9985-82). Tissue was also stained with ICAM-1 (R&D cat# AF796,) Ki67 (abcam cat# ab15580), HABP (Millipore cat# 385911), collagen I (cat# ab21286) and collagen I LF-68 clone (provided by Dr. Larry Fisher, NIDCR). Secondary antibodies coupled with non-overlapping fluorophores and specific to the primary antibody isotype were added after incubation of non-conjugated primary antibodies, where appropriate. To visualize vessels perfused with biotinylated lectin, Streptavidin, Alexa Fluor® 647 was used. All secondary antibodies were from Jackson Immunoresearch Laboratories.

Human
Lymph nodes from patients with colon cancer or breast cancer were obtained from archival specimens in the MGH Department of Pathology. Lymph nodes from head and neck cancer patients were obtained from Samsung Medical Center. The study was approved by the MGH Institutional Review Board. Tissue sections were deparaffinized, rehydrated, and heat-induced epitope retrieval was performed in a 97°C waterbath with slides immersed in a citrate-based antigen unmasking solution (Vector Laboratories cat# H3300, 1mM EDTA, or Dako Target Retrieval Solution). After epitope retrieval, tissue on slides was blocked in 5% normal donkey serum for 60 minutes at room temperature followed by immunofluorescent staining. CD8+ T cells were stained with a purified anti-human CD8 antibody (Biorad, MCA1817T). For B cells, a purified anti-human CD20 (Santa Cruz, cat # sc-7735) antibody was used. To visualize human cancer cells, purified anti-cytokeratin or FITC-conjugated anti-cytokeratin antibodies (Sigma-Aldrich cat #s C2931, F3418, respectively) were used. All slides were mounted with ProLong™ Gold Antifade with DAPI (Thermo Fisher Scientific).

Stained lymph node sections were imaged with a confocal microscope (Olympus IX81) using 10x air and 20x or 60x oil UPlanSApo objectives (Olympus). Images were analyzed using custom MATLAB scripts.
For Figure 7c (bottom row), equivalent regions of interest (ROI) were outlined for lymph node from vehicle or losartan-treated animal and the integrated density of the collagen I staining was calculated using Image J.

Flow cytometry
Naïve axillary lymph nodes or tumor draining axillary lymph nodes two weeks after primary tumor resection were isolated and harvested from anesthetized mice. After mincing the lymph nodes, single-cell suspensions were obtained by flowing minced cells through a 40-μm cell strainer. Cells were resuspended in flow cytometry buffer (1% BSA, 0.1% sodium azide in PBS) and incubated with an anti-mouse CD16/CD32 antibody (Biolegend, cat # 101302) for 10 minutes at 4°C, followed by staining with indicated fluorochrome-conjugated antibodies. Acquisition of flow cytometry data was performed using an LSRII flow cytometer (Becton Dickinson) and analyzed using FlowJO or FACSDiva software. The following non-overlapping fluorophore-conjugated anti-mouse antibodies were used for flow cytometry staining: CD45 (30-F11, BioLegend), CD3 (17A2, BioLegend), CD4 (RM4-5, BioLegend), CD8a (53-6.7, BioLegend), CD25 (PC61, BioLegend), FoxP3 (FJK-16s, eBioscience), CD19 (6D5, BioLegend), and Granzyme B (GZ11, BioLegend).

Intervention Studies
14 days after implantation (before primary tumors reached a maximal size of 10 mm in diameter), primary 4T1 tumors were resected. Mice were randomly separated into groups for control or targeted treatment. For survival studies, mice were humanely sacrificed when they became moribund. For studies quantifying the incidence of lymph node metastasis, mice were sacrificed 2 weeks after starting treatment.

Anti-PD-1
Mice were treated with an intraperitoneal administration of 200 µg of an anti-PD-1 antibody (BioXcell clone RMP1-14, cat# BE0146) or rat IgG (Jackson Immunoresearch Laboratories, cat. # 012-000-003) every 3 days.

1-Methyl-DL-tryptophan

1 gram of the IDO inhibitor, 1-Methyl-DL-tryptophan (Sigma, cat. # 860646) was dissolved in 1 mL of DMSO and 1 mL of ethanol. The final solution consisted of 10% DMSO and ethanol in water. The solution was administered daily by oral gavage at a concentration of 400 mg/kg.

Cyclophosphamide
The chemotherapeutic agent, cyclophosphamide (Sigma Aldrich, cat # C3250000), was dissolved in water and administered intraperitoneally every 7 days at a concentration of 100 mg/kg.

Losartan
Losartan Potassium (TCI America) was administered intraperitoneally once daily for 14 days at a concentration of 50 mg/kg.

qPCR
Mice were anesthetized and axillary lymph nodes (single LN per sample) of naïve Balb/C mice and tumor–draining lymph nodes of Balb/C mice 4 weeks after implantation with 4T1 cells were collected. For RNA isolation from whole lymph nodes, nodes were snap frozen in liquid nitrogen and homogenized in RLT lysis buffer (Qiagen). To separate tumor and non-tumor regions of lymph nodes for RNA isolation, frozen tissue sections were obtained from tumor-draining lymph nodes under RNAase-free conditions. Tumor macrometastases and non-tumor tissue were distinguished based on the visible variation in tissue architecture between metastatic lesion and lymph node tissue. Briefly, sterile blades were used to scrape tumor or non-tumor regions from serial 10-μm thick metastatic lymph node slide sections; 3-5 slides were used for each sample. All RNA was isolated with an RNeasy mini kit with QIAshredder to homogenize cells and on-column DNase treatment to eliminate genomic DNA contamination according to the manufacturer's instructions (Qiagen). The quality and quantity of RNA were determined spectrophotometrically at 260 nm and 280 nm using a NanoDrop ND-1000. Purified mRNA was reverse transcribed to cDNA using a RT^2 PreAMP cDNA synthesis kit (for tumor/nontumor areas) and an RT^2First Strand kit (Qiagen) according to the manufacturer's instructions. Next, real-time PCR for was performed according to the manufacturer's instructions using SYBR green (ThemoFisher Scientific, cat# 4367659) on a Stratagene Mx3000P system to amplify the following genes: *Chst4*, (forward) 5'-AGAGGCACTCCCCCAAAA, (reverse) 5'-CTGCCACAAGCAGGAATGAGA; *Fut7* (forward) 5'- GGACCTCCTCGGGCCACCTACG, (reverse) 5'- CGCCAAGCAAAGAAGCCACGATAA; *Madcam1*, (forward) 5'-GTCCTGCACGGCCCACAACAT, (reverse) 5'-CCAGTAGCAGGGCAAAGGAGAG ; *Glycam1*, (forward) 5'- CCTGCCTGGGTCCAAAGATGAAC, (reverse) 5'-CTGGTGTAGCTGGTGGGAGTGGAC; *Icam1*, (forward) 5'-GTGATGCTCAGGTATCCATCCA, (reverse) 5'- CACAGTTCTCAAAGCACAGCG; *Vcam1*, (forward) 5'- GTTCCAGCGAGGGTCTACC, (reverse) 5'-AACTCTTGGCAAACATTAGGTGT; *SELP*, (forward) 5' CATCTGGTTCAGTGCTTTGATCT, (reverse) 5'- ACCCGTGAGTTATTCCATGAGT; *Ccl19*, (forward) 5'-GGGGTGCTAATGATGCGGAA, (reverse) 5'-CCTTAGTGTGGTGAACACAACA; *Ccl21*,(forward) 5'-GTGATGGAGGGGGTCAGGA, (reverse) 5'-GGGATGGGACAGCCTAAACT ;
Cd274,(forward) 5'- GCTCCAAAGGACTTGTACGTG, (reverse) 5'-TGATCTGAAGGGCAGCATTTC;
Pdcd1,(forward) 5'-ACCCTGGTCATTCACTTGGG, (reverse) 5'-CATTTGCTCCCTCTGACACTG;

Ido1,(forward) 5'-TGGGGAGATACCACATTTCTGA, (reverse) 5'-TGAGGAAGTCTGAGGGCAATTT ;
Krt18, (forward) 5'- ACTCCGCAAGGTGGTAGATGA, (reverse) 5'-TCCACTTCCACAGTCAATCCA; CD31,(forward) 5'-CTGCCAGTCCGAAAATGGAAC, (reverse) 5'-CTTCATCCACCGGGGCTATC; *Gapdh*, (forward) 5'-CATGGCCTTCCGTCTTCCTA-, (reverse) 5'- GCGGCACGTCAGATCCA.