Supplementary Materials and Methods

Sex as a biological variable. This study exclusively examined female mice. It is unknown whether the findings are relevant for male mice.

Animals. BALB/c-nu female mice were purchased from Shanghai Model Organisms, Shanghai, China. Mice aged 8-10 week and weighed 20-22 g were used.

Perigastric lymphatic drainage network mapping. Mice were anesthetized with 0.1% pentobarbital sodium (60 mg/kg) through intraperitoneal injection. The mice were then secured on a platform using surgical tape (1530-1, 3M, USA). An incision was made along the abdominal linea alba, and intestine were moved to the side until the stomach and omentum were completely exposed. Carbon nanoparticle suspension (Lummy Pharmaceutical Co. Ltd, Chongqing, China) was injected into the subserosa of the stomach at multiple points under 7X magnification. The injection site should bleb lightly. Allow 5 to 30 minutes for the carbon nanoparticle to travel through the lymphatic vessels and lymph nodes. Then the mice were euthanized using an overdose of anesthesia, and dissect to locate the lymphatic vessels and lymph nodes of interest.

Cell culture and luciferase label. The human gastric cancer cell line MKN-45 was obtained from Cell Bank, Shanghai Institute of Biochemistry and Cell Biology (SIBCB). Cell line was cultured in RPMI-1640 medium (Gibco) in a humidified incubator at 5% CO2 and 37℃. Culture media were supplemented with 10% fetal bovine serum (Gibco) and 1% penicillin-streptomycin (Gibco). Lentivirus with luciferase fluorescence and resistance gene of purine were purchased from Genechem (Shanghai, China). Stable cell clones were selected by 2μg/mL puromycin (M3637, Abmole, USA).

Orthotopic xenograft gastric cancer model. The Luciferase-labeled MKN-45 cell were harvested and subcutaneously transplanted into the dorsal flanks of BALB/c-nu mice. Once the tumor volume reached 300 mm^3, mice were sacrificed using overdose anesthesia and tumors were collected. Under sterile condition, the fresh tumor tissues were sliced into pieces about 1×0.5×0.6 mm^3 in phosphate-

buffered saline. Mice were anesthetized with 0.1% pentobarbital sodium. An incision was made through the upper abdomen. Under 7x magnification, the gastric wall was carefully exposed. We made an incision of 2 mm in diameter on the glandular stomach, cutting through the serosal and muscular layers, thereby exposing the mucosal layer. Minced tumor pieces were then implanted into the submucosa through the incision using an inoculation needle. The gastric wall incision was sealed with Vetbond Tissue Adhesives (3M, USA). Then, the stomach was repositioned into the abdominal cavity, and the abdominal wall was sutured in layers.

In vivo bioluminescence imaging. Tumor growth of orthotopic xenograft mouse model were tracked by IVIS Spectrum Xenogen machine (Perkin Elmer, Waltham, USA). The mice were intraperitoneally injected with D-luciferin working solution (15 mg/mL) at 10 μL/g body-weight concentration, and fluorescence imaging was performed 15 min later. Tumor growth was validated by the *in vivo* bioluminescence imaging.

In vivo MR Imaging. In vivo MRI was conducted on a 9.4T animal MRI system (Bruker BioSpin MRI GmbH, Germany). The imaging was performed using a cylindrical volume RF coil in small animals. Anesthesia was induced with 3.0% isoflurane in oxygen and maintained at 1.0-2.0% isoflurane during the imaging process. Electrocardiogram and respiratory data were monitored throughout the scan. The MRI parameters for different sequences were as follows: For the localizer image, TR was 50 msec, TE was 1.9 msec, the number of averages was 4, FOV was 50 x 50 mm, the acquisition matrix was 256 x 256, spatial resolution was 195 x 195 x 1000 μm, acquisition time was 12 sec, and there were 3 images in an acquisition. For the axial T2-weighted image, TR was 2000 msec, TE was 13.5 msec, the number of averages was 4, FOV was 40 x 40 mm, the acquisition matrix was 256 x 192, spatial resolution was 208 x 156 x 500 μm, acquisition time was 6 min 24 sec, and there were 16 images in an acquisition.

Immunohistochemistry and Hematoxylin-Eosin Staining. Samples were fixed in 10% formalin, dehydrated and embedded in paraffin. Sample slides with 3-μm-thick sections were stained with

hematoxylin and eosin (H&E), and examined under microscope. For immunohistochemistry (IHC) staining, sample slides were deparaffinized in xylene, rehydrated in descending concentrations of ethanol and PBS, and subjected to antigen retrieval by heated citrate buffer (pH 6.0). After antigen retrieval, slides were incubated with CK-Pan (ZM-0069, ZSGB-BIO, Beijing, China) at 4℃ overnight. Immunocomplexes were visualized by DAB method, and sections were counterstained with hematoxylin. H&E and IHC images were obtained using a digital pathology slide scanner (KFBIO, China).

Evaluation of the size of lymph nodes. In the MR imaging analysis, we selected the slice that displayed the maximum length of the lymph nodes in cross-section and measured the diameters. In the ex vivo lymph node specimen analysis, we measured the length and width of the lymph nodes, and calculated the volume using the following formula: $V = (W^2 \times L)/2$, where W is the width and L is the length of lymph node.

Statistics. Data analyses were performed using GraphPad Prism (version 9, GraphPad Prism Software, USA). Comparisons between the two groups were performed by the multiple unpaired t tests (2-way). A P value less than 0.05 was considered significant.

Study approval. The study was approved by the Institutional Animal Care and Use Committee at The First Affiliated Hospital, Sun Yat-sen University.

Data availability. All data are available in the Supporting data values file. Request for materials or other information will be addressed by the corresponding authors.

Author Contributions

H.F., Q.C. and Z.Z. participated in the manuscript preparation and formal analysis and performed the experiments. Y.H., M.L. and D.Y. conceived and directed the project.

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Conflict of interest:

The authors have declared that no conflict of interest exists.