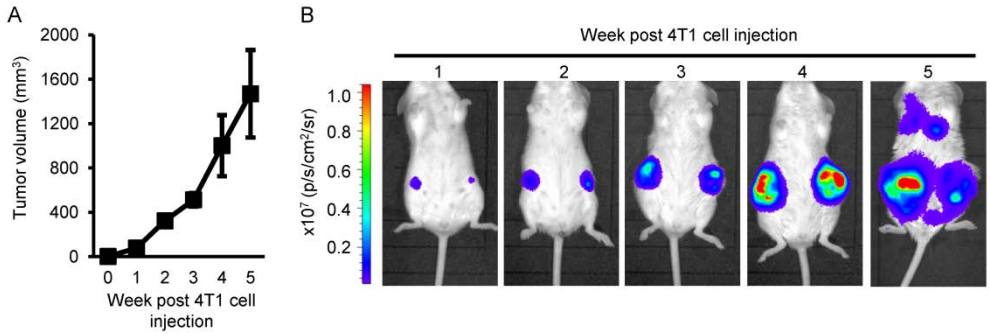
Appendix

Table of content

Appendix Figure S1



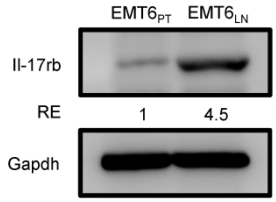
Appendix Figure S1. Kinetics of distant organ metastasis in 4T1 tumor-bearing mice.

(A) Tumor growth cruve in BALB/c mice injected with 4T1 cells. $1x10^6$ 4T1 cells were injected into the fourth mammary fat pad on the both side and the tumor volumes were evaluated weekly.

(B) Representative IVIS images of the 4T1 tumor-bearing mice. The bioluminescent signal (pseudocolor) was recorded as photons per second per centimeter squared per steradian ($p/s/cm^2/sr$). The luminescent image was overlaid on the photographic image.

Data information: In (A), data are presented as mean ± SD (n = 3 mice per time point).

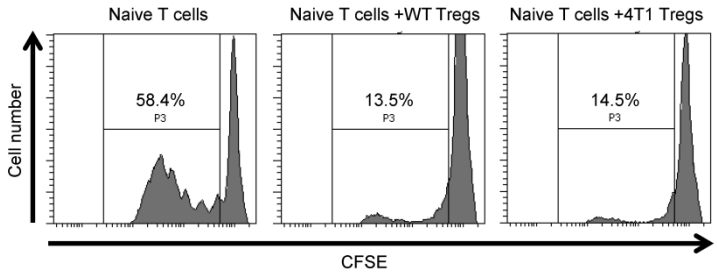
Appendix Figure S2



Appendix Figure S2. Il-17rb expression in $EMT6_{PT}$ and $EMT6_{LN}$ cells.

Il-17rb expression in $EMT6_{PT}$ and $EMT6_{LN}$ cells were examined by Western blotting analysis. The intensity of each band was quantified using the Image J software, and Gapdh was used as a loading control. Relative expression (RE) of Il-17rb levels in $EMT6_{LN}$ cells to $EMT6_{PT}$ cells is indicated.

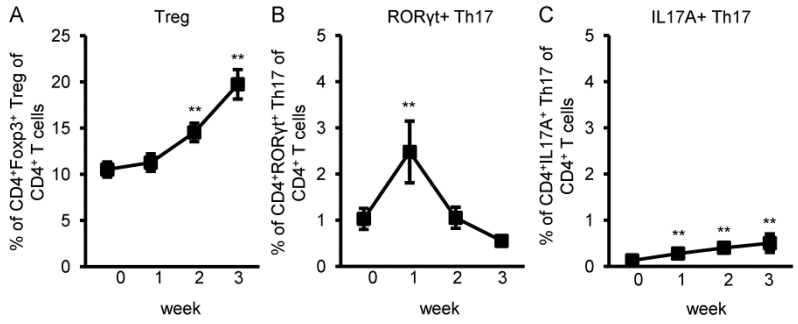
Appendix Figure S3



Appendix Figure S3. Tregs isolated from inguinal LNs in 4T1 tumor-bearing mice exhibit similar T cell suppressive activity to Tregs isolated from WT mice.

Tregs ($CD4^+CD25^+GITR^+$cells) were isolated from the inguinal LNs of WT mice (WT Tregs) and from the 4T1 tumor-bearing mice at the third week after initial injection (4T1 Tregs) by FACS sorting. $5x10^4$ Tregs were then co-cultured with $1x10^5$ CFSE-labeled $CD3^+$ T cells (naive T cells) from WT mice in the 96-well U bottom plate pre-coated with anti-mouse CD3 Abs and anti-mouse CD28 Abs. Five days later, the percentage of proliferating CFSE-labeled $CD3^+$ T cells were analyzed by FACS.

Appendix Figure S4

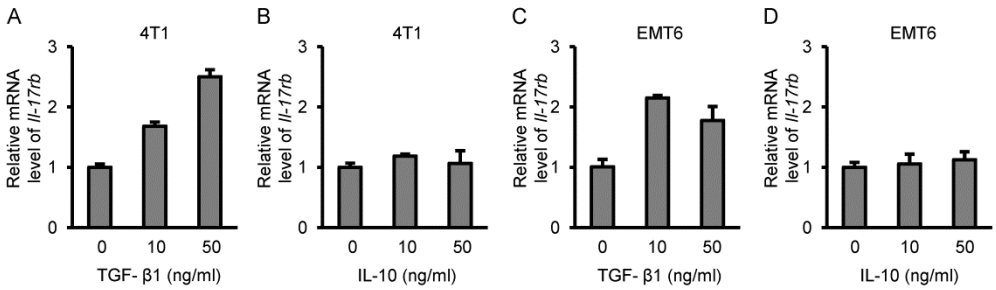


Appendix Figure S4. Percentage of Tregs and Th17 cells in inguinal LN of 4T1 tumor-bearing mice.

Percentage of $CD4^+Foxp3^+$ Tregs (A), $CD4^+ROR\gamma t^+$ Th17 cells (B), or $CD4^+IL\text{-}17A^+$ Th17 cells (C) of total $CD4^+$ cells in inguinal LN of BALB/c mice injected with 4T1 cells was analyzed by FACS.

Data information: All values are presented as mean ± SD (n = 4 mice per group). In (A), **P = 0.0017 and **P = 0.0001 for the percentage of $CD4^+Foxp3^+$ Tregs at week 2 and week 3, respectively. In (B), **P = 0.0012 for the percentage of $CD4^+ROR\gamma t^+$ Th17 cells at week 1. In (C), **P = 0.0054, **P = 0.0012, and **P = 0.0109 for the percentage of $CD4^+IL\text{-}17A^+$ Th17 cells at week 1, 2, and 3, respectively. Level of significance was determined using two-tailed unpaired t-test.

Appendix Figure S5

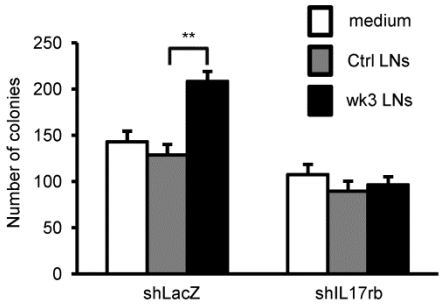


Appendix Figure S5. TGF-β1, but not IL-10, induces Il-17rb expression in 4T1 and EMT6 breast cancer cells.

RT-qPCR analysis of *Il-17rb* in 4T1 cells (A and B) or EMT6 cells (C and D) treated with recombinant TGF-β1 (A and C) or IL-10 (B and D) for 4 hours. Gapdh was used as internal control.

Data information: All experimental data were verified in at least two independent experiments. Data were presented as means ± SD (triplicate measurement).

Appendix Figure S6

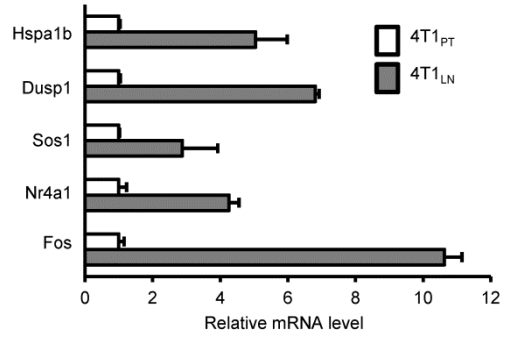


Appendix Figure S6. Depletion of Il-17rb abolishes the colony forming ability induced by the tumor-draining LNs.

4T1-injected BALB/c mice were sacrificed at the third week after initial injection. Total cells isolated from inguinal lymph node tissues were co-cultured with shLacZ or shIl-17rb 4T1 cells in a transwell plate ($5x10^2$ cells/well, n = 6 wells per group). Inguinal lymph node tissues from un-injected BALB/c mice were used as a control. After 5-day co-culture, the colony forming ability of 4T1 cells at lower wells was examined in soft agar.

Data information: Data were presented as means ± SD. (n = 6 wells per group, **P = 0.0000012). Level of significance was determined using two-tailed unpaired t-test.

Appendix Figure S7



Appendix Figure S7. Gene expression of *Fos, Nr4a1, Sos1, Dusp1,* and *Hspa1b* involved in MAPK signaling pathway is increased in $4T1_{LN}$ cells.

mRNA expression of each candidate gene in $4T1_{PT}$ and $4T1_{LN}$ cells was determined by RT-qPCR. Gapdh was used as an internal control.

Data information: Data were presented as means ± SD (triplicate measurement).

Appendix Table S1. KEGG pathway enrichment analysis of the up-regulated genes in $4T1_{LN}$ cells.

Term	*P* value	Genes
MAPK signaling pathway	0.033	Fgf16,Fos,Nr4a1,Sos1,Dusp1, Hspa1b
PI3K-Akt signaling pathway	0.038	Fgf16,Nr4a1,Tnxb,Sos1,Cdc37, Itga10,Thbs2
Prolactin signaling pathway	0.095	Fos, Sos1, Socs3

Appendix Table S2. KEGG pathway enrichment analysis of the down-regulated genes in shIL-17RB MDA-MB-361 cells.

Term	*P* value	Genes
Transcriptional misregulation in cancer	0.073	DDIT3,ETV4,SUPT3H,ARNT2, MMP9
Pathways in cancer	0.075	MECOM,WNT3,ARNT2,FGF1, LAMA2,MMP1, MMP9,PDGFRB
MAPK signaling pathway	0.091	DDOT3,MECOM,DUSP4,FGF1, PDGFRB,PTPRR
Staphylococcus aureus infection	0.091	C1R,C5,HLA-DRA
Legionellosis	0.091	CXCL2,CXCL3,EEF1A2

Appendix Table S3. Primers used in this study.

a. Primer sequence for real-time PCR.

Gene	Forward	Reverse
Il-17rb	5'-tggtctatcttgggggagca-3'	5'-aaagctgtggcgtccttcat-3'
Cd300Ig	5'-gaccgtgatcatgagggacc-3'	5'-accaggagaagctggaaagac-3'
Gpr56	5'-ctgcctgggctctatctact-3'	5'-ctgggtctctgggtaagagtg-3'
Scara5	5'-gttccatgatcgtcgttggg-3'	5'-ccaaatcctccctgtgcctt-3'
Oprk1	5'-caagtgccaccttctcgctt-3'	5'-ctgtcggattctgcccagtt-3'
GAPDH	5'-ctttggcattgtggaagggc-3'	5'-cagggatgatgttctgggca-3'
Hspa1b	5'-caccatcgaggaggtggattag-3'	5'-ttgacagtaatcggtgcccaa-3'
Dusp1	5'-gcttgacacacccaccagta-3'	5'-cagaccaccgacctacacaa-3'
Sos1	5'-agccagtgcagcaaaacttg-3'	5'-ggaactccctttgtgagcca-3'
Nr4a1	5'-gagttcggcaagcctaccat-3'	5'-ggtgtcaaactctccggtgt-3'
Fos	5'-tactaccattccccagccga-3'	5'-gctgtcaccgtggggataaa-3'

b. Primers used for the cloning of Il-17rb into pLAS5w.Pbsd-L-tRFP-C vector.

Gene	Forward	Reverse
Il-17rb	5'-gatcttcgaaatgttgctagtgttgctgat-3'	5'-aagcgctagcctacaagggtgaacagctat-3'

c. Primers used for the detection of chromosomal deletions of Il-17rb.

	Forward	Reverse
Primer	5'-actctgactgcttgtgtttgt-3'	5'-tgggccggaaacaggagtat-3'

d. Primer used for the sequencing analysis of chromosomal deletions of Il-17rb.

	Forward	Reverse
Primer	5'-gattccttttgctcttgggc-3'	5'-tgaacaggtgatggaacaggag-3'