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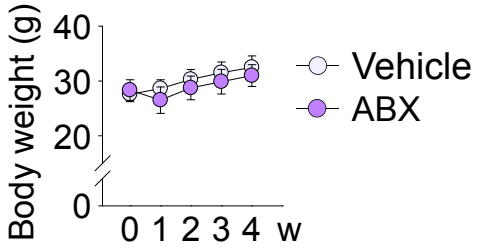
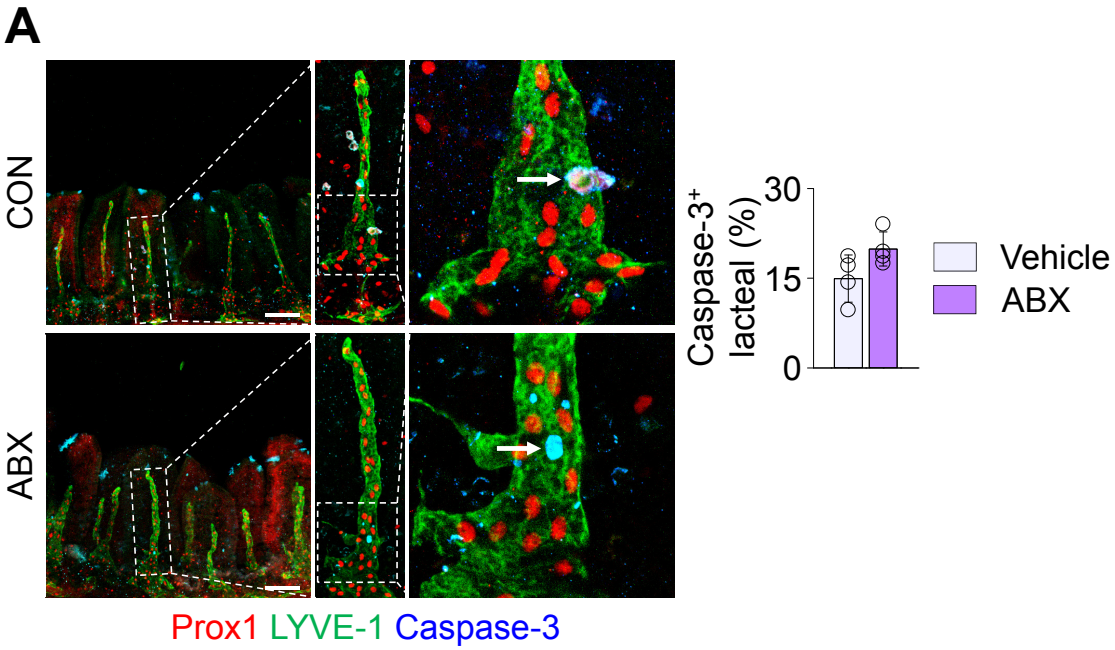
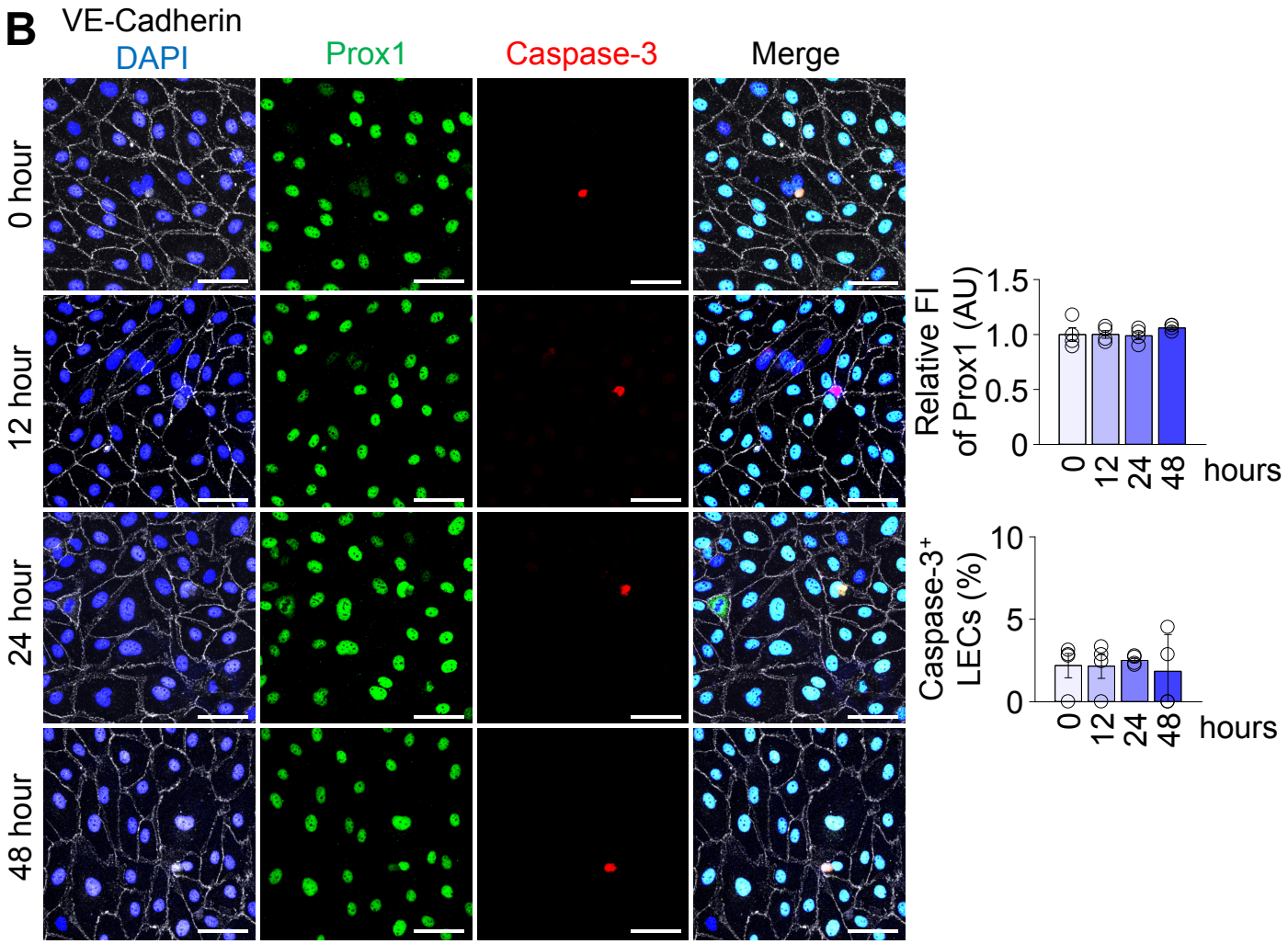


Appendix Figure S1. The effect of gut microbiota depletion is insignificant on the body weight during regular chow feeding.
Body weight curve of vehicle- and ABX-treated mice. w, weeks after vehicle or ABX treatment (n = 8 mice/group).
Data information: Data are represented as means ± SD.

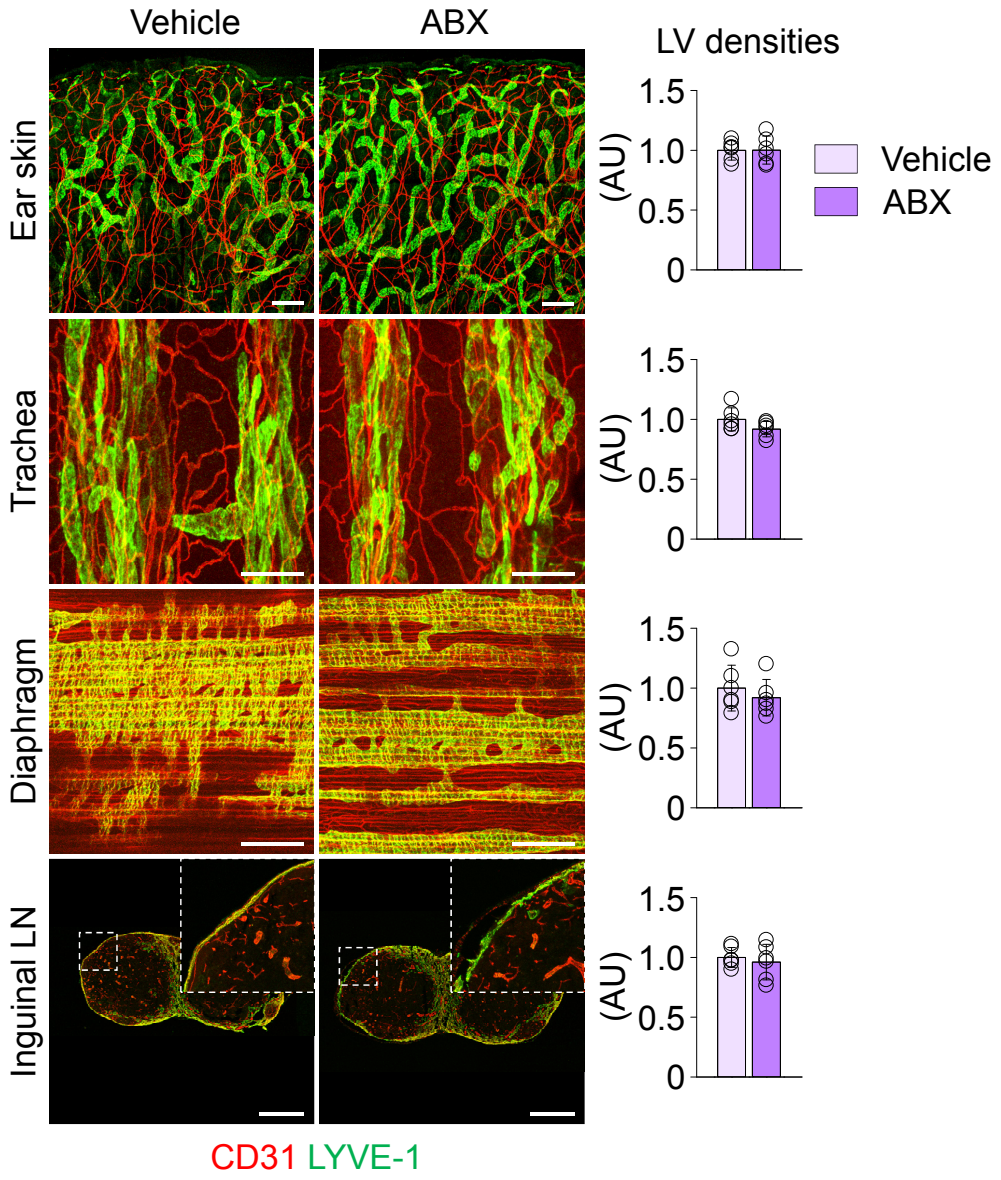

A
CON
ABX
Prox1 LYVE-1 Caspase-3
Caspase-3+ lacteal (%)
30
15
0
Vehicle
ABX


B
VE-Cadherin
DAPI
Prox1
Caspase-3
Merge
0 hour
12 hour
24 hour
48 hour
Relative FI of Prox1 (AU)
1.5
1.0
0.5
0
0
12
24
48
hours
Caspase-3+ LECs (%)
10
5
0
0
12
24
48
hours

Appendix Figure S2. Depletion of gut microbiota does not induce the apoptosis of LECs of lacteals.

(A) Images and comparisons of the percentage for caspase-3$^+$ signals on LYVE-1$^+$ lacteals in jejunum from vehicle- or ABX-treated mice. Mice were sacrificed at 1-week post treatment of vehicle or ABX. Each boxed portion is highly magnified in right. Note that caspase-3 signal (arrows) is not merged with Prox1 signal. Each dot indicates mean value of 5-10 villi in a mouse (n = 4 mice/group). Scale bars, 100 μm. (B) Images and comparisons of Prox1 and caspase-3 expression in human dermal LEC at indicated time points after antibiotics combination treatment. The relative expression levels of Prox1 presented as fold (vehicle as 1) and the percentage of caspase-3$^+$ cells were quantified (n = 4). The concentrations of each antibiotics are vancomyin, 50 ng/ml; amipicillin, 100 ng/ml; metronidazole, 100 ng/ml; neomycin, 100 ng/ml. Scale bars, 50 μm.

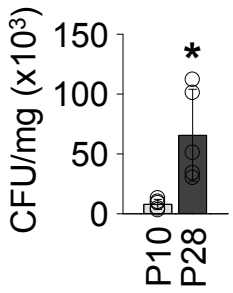
Data information: Data are represented as means ± SD (A) and means ± SEM (B).



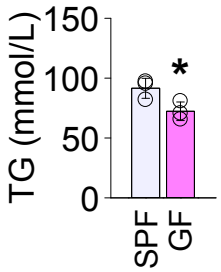
Appendix Figure S3. Depletion of gut microbiota does not affect lymphatic vessel densities of extra-intestinal organs.

Images and comparisons of LYVE-1$^+$ lymphatic vessel (LV) densities in the ear skin, trachea, diaphragm, and inguinal LN from vehicle- and ABX-treated mice. AU, arbitrary unit. All scale bars, 200 μm. Each dot indicates mean value of 5 sites in a mouse (n = 6 mice/group).

Data information: Data are represented as means ± SD.



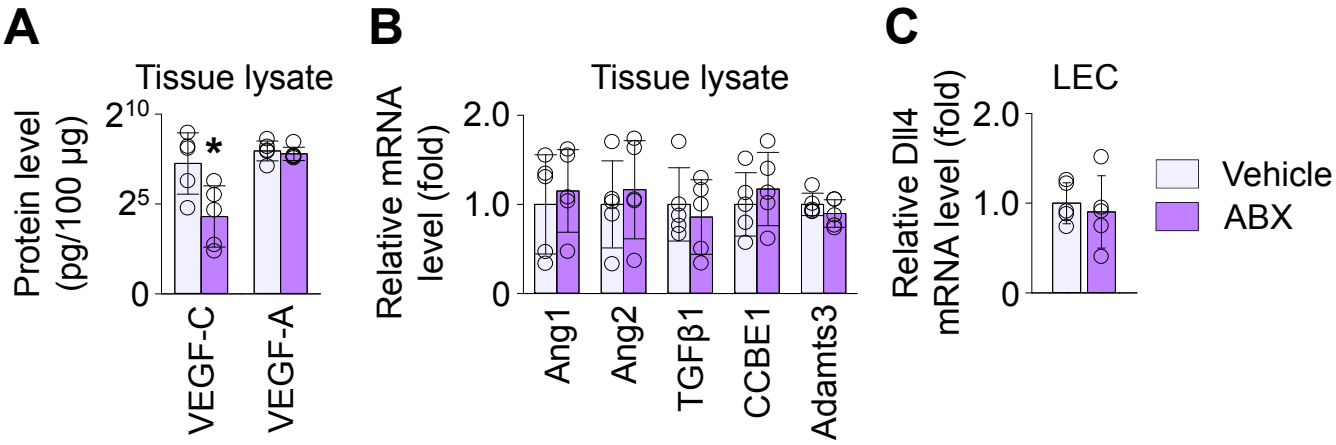
Appendix Figure S4. Gut microbiota quantitatively expands during weaning from lactation.
Comparison of bacterial colony forming unit (CFU) in feces from P10 and P28 mice (n = 5 mice/group).
Data information: Data are represented as means ± SD. *P < 0.05 vs. P10 mice by two-tailed unpaired Student's t-test.



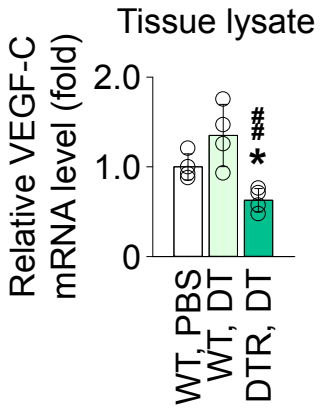
Appendix Figure S5. Triglyceride level in the lymph from thoracic duct is reduced in GF mice.

Comparisons of triglyceride (TG) in in the lymph from thoracic duct from SPF and GF mice (n = 3 mice/group). The lymph was collected 1 hour after oral lipid loading.

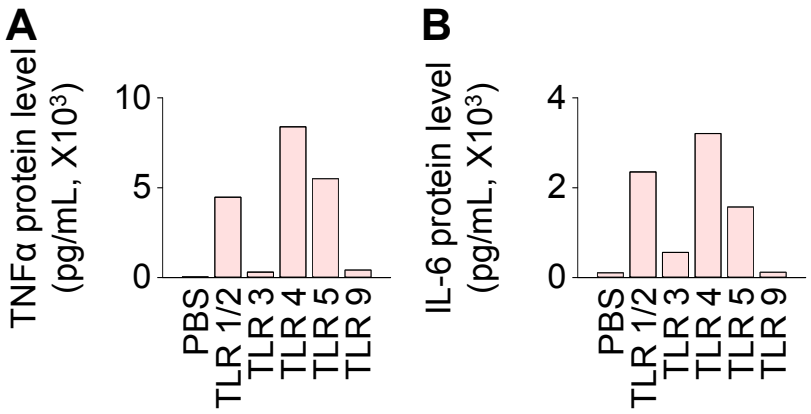
Data information: Data are represented as means ± SD. *P < 0.05 vs. SPF mice by two-tailed unpaired Student's t-test.



Appendix Figure S6. Tissue VEGF-C level depends on gut microbiota.
(A) Comparison of protein levels of VEGF-C and VEGF-A in jejunum and ileum of vehicle- and ABX-treated mice (n = 5 mice/group). (B) Comparison of mRNA levels of angiopoietin 1 (Ang1), Ang2, transforming growth factor β1 (TGFβ1), collagen- and calcium-binding EGF domains 1 (CCBE1) and a disintegrin and metalloproteinase with thrombospondin motifs 3 (Adamts3) in the whole tissue of jejunum and ileum from vehicle- and ABX-treated mice (n = 5 mice/group). (C) Comparison of mRNA levels of Dll4 in LECs of jejunum and ileum from vehicle- and ABX-treated mice (n = 5 mice/group).
Data information: Data are represented as means ± SD. *P < 0.05 vs. vehicle-treated mice by two-tailed unpaired Student's t-test.



Appendix Figure S7. Intraperitoneal administration of diphtheria toxin does not increase tissue VEGF-C level in the intestine of wild type mice.
Comparison of VEGF-C mRNA levels in the jejunum and ileum of PBS-treated WT (WT, PBS), diphtheria toxin (DT)-treated WT (WT, DT), and DT-treated CX3CR1-DTR (DTR, DT) mice (n = 4 mice/group).
Data information: Data are represented as means ± SD. *P < 0.05 vs WT, PBS; ## P < 0.01 vs. WT, DT by one-way ANOVA with Bonferroni's multiple comparison test.



Appendix Figure S8. The stimulation of TLRs with specific agonists induces the production of inflammatory cytokines by macrophages.

(A, B)Measurement of TNFα (A) and IL-6 (B) protein level in the culture media after stimulation of primary intestinal macrophages with specific TLR agonists (n = 3). The following TLR agonists at indicated concentrations were treated: palmitoyl-3-cysteineserine-lysine-4 (TLR1/2 agonist, 1 μg/ml), poly(I-C) (TLR3 agonist, 10 μg/ml), lipopolysaccharide (TLR4 agonist, 5 μg/ml), flagellin (TLR5 agonist, 0.1 mg/ml), or bacterial DNA (TLR9 agonist, 10 μg/ml).

Data information: Data are representative of three independent experiments.

Genes		Sequence
mVegf-C	Forward	CGTTCTCTGCCAGCAACATTACCAC
	Reverse	CTTGTTGGGTCCACAGACATCATGG
mAngpt1	Forward	CACATAGGGTGCAGCAACCA
	Reverse	CGTCGTGTTCTGGAAGAATGA
mAngpt2	Forward	CCTCGACTACGACGACTCAGT
	Reverse	TCTGCACCACATTCTGTTGGA
mTgfb1	Forward	CTCCCGTGGCTTCTAGTGC
	Reverse	GCCTTAGTTTGGACAGGATCTG
mCcbe1	Forward	AAACAAGATCACCACGACCAAA
	Reverse	CTCGCGGTCATATCGGTATCC
mAdamts3	Forward	ATTACCTCCTCACCCTGATGAAC
	Reverse	ATATGCACTCCGAGGGACTCATC
mDll4	Forward	GGAACCTTCTCACTCAACATCC
	Reverse	CTCGTCTGTTCGCCAAATCT
mGapdh	Forward	AATGTGTCCGTCGTGGATCT
	Reverse	CATCGAAGGTGGAAGAGTGG

Appendix Table S1. Primer sequences for quantitative real-time PCR.