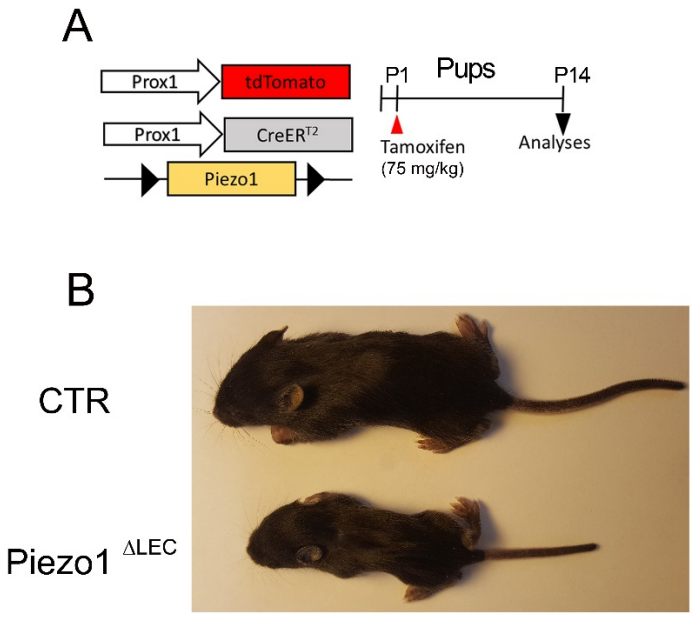
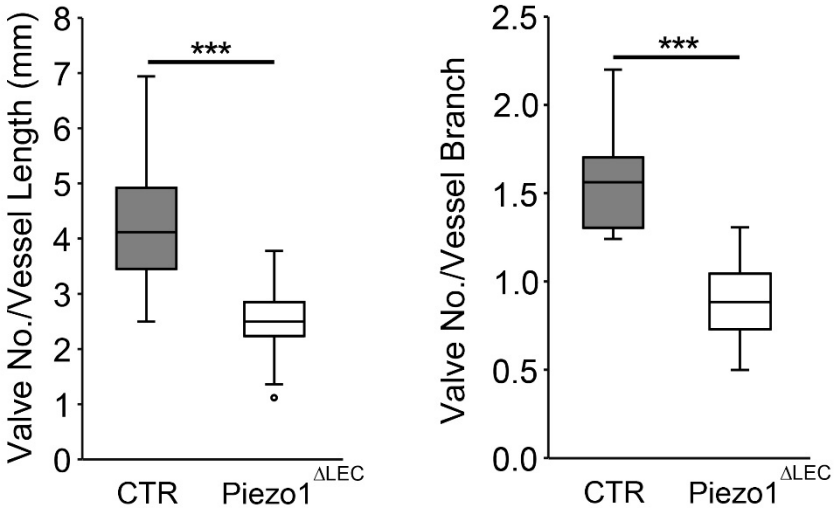
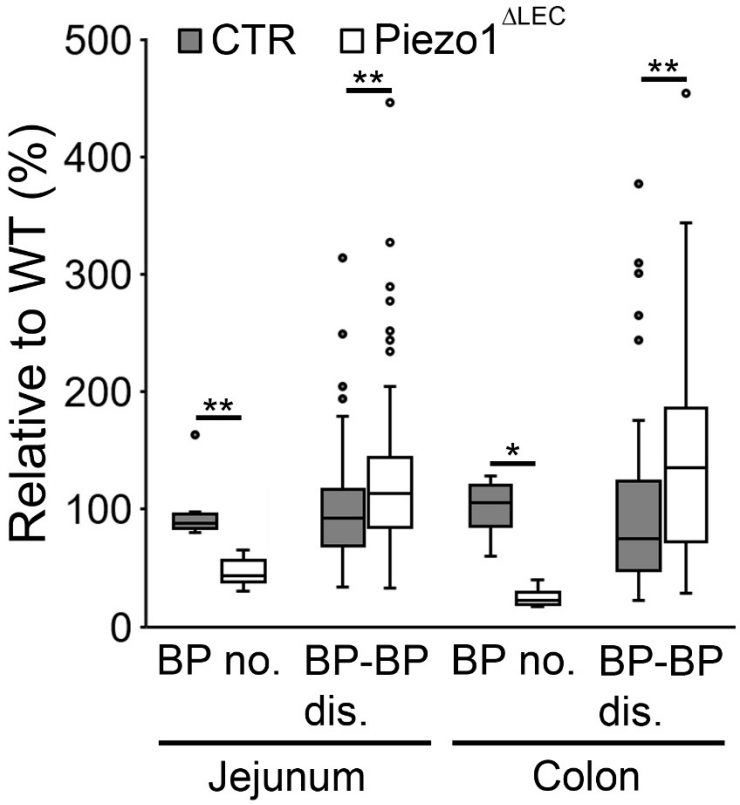
SUPPLEMENTAL INFORMATION



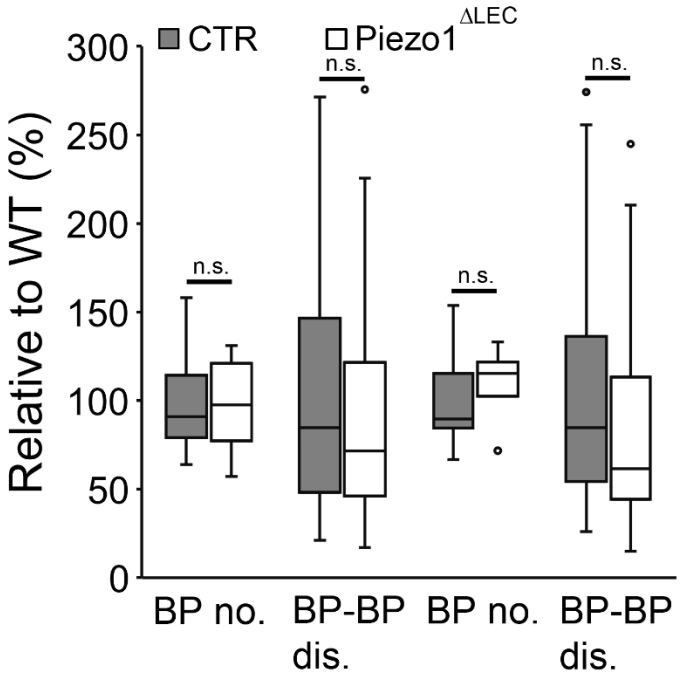
Supplemental Figure 1. Effect of Lymphatic *Piezo1* Deletion on Body Growth. (A) Experimental design: Control pups (CTR) harbor *Prox1-tdTomato* and *Prox1-CreER^{T2}* alleles without floxed *Piezo1*. Lymphatic *Piezo1* KO (Piezo1 $^{\Delta LEC}$) pups have *Prox1-tdTomato*, *Prox1-CreER^{T2}*, and *Piezo1 $^{fl/fl}$*. Tamoxifen (75 mg/kg) was injected into pups at P1, and whole mouse images were taken at P14. (**B**) Lymphatic *Piezo1* KO inhibited the body growth of the mutant pups, compared to the control littermate.



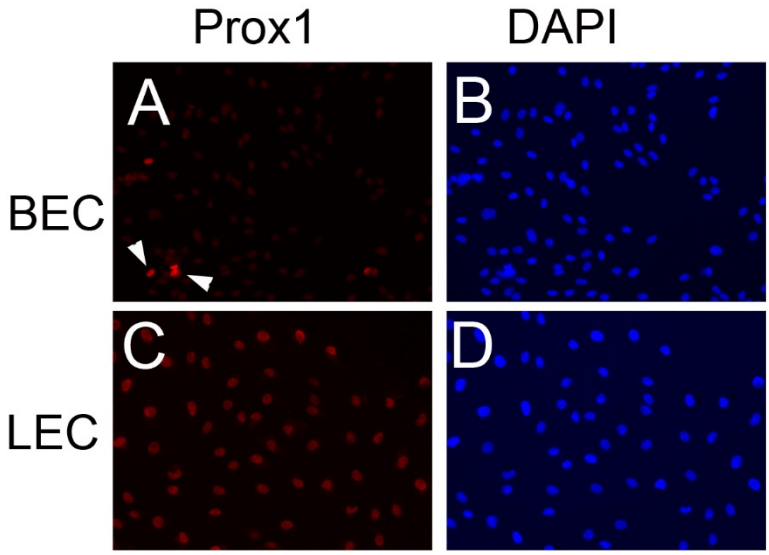
Supplemental Figure 2. *Piezo1* Deletion by Cdh5 (PAC)-CreER^{T2} Allele Reduced Mesenteric Lymphatic Valve Formation in the Jejunum. Number of lymphatic valves shown in Figure 1 V and W was quantified in the jejunum of the control or *Piezo1* KO pups (*Cdh5 (PAC)-CreER^{T2}; Prox1-tdTomato; Piezo1 $^{fl/fl}$*). Statistics: ***, $p < 0.001$, unpaired, two-tailed, *t*-test. > 5 pups (males and females) were used for each group.



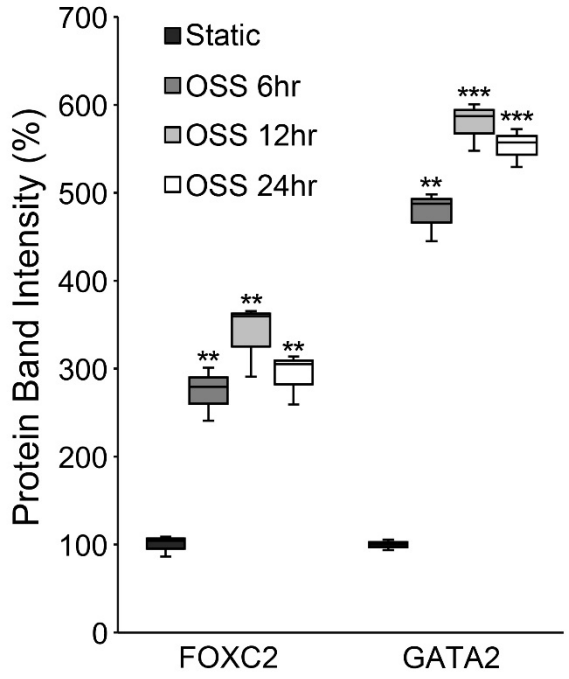
Supplemental Figure 3. *Piezo1* Deletion Reduced Mesenteric Lymphatic Density in the Intestine. Lymphatic vessel density of *Piezo1* KO pups (*Prox1-CreER^{T2}; Prox1-tdTomato; Piezo1 $^{fl/fl}$*), shown in Figure 1 B-E (Jejunum) and F-I (Colon), was quantified and expressed against that of wild type control pups. BP, branching point of lymphatic vessels; BP-BP dis., distance between two branching points. Statistics: *, $p < 0.05$; **, $p < 0.01$, unpaired, two-tailed, *t*-test. > 5 pups (males and females) were used for each group.



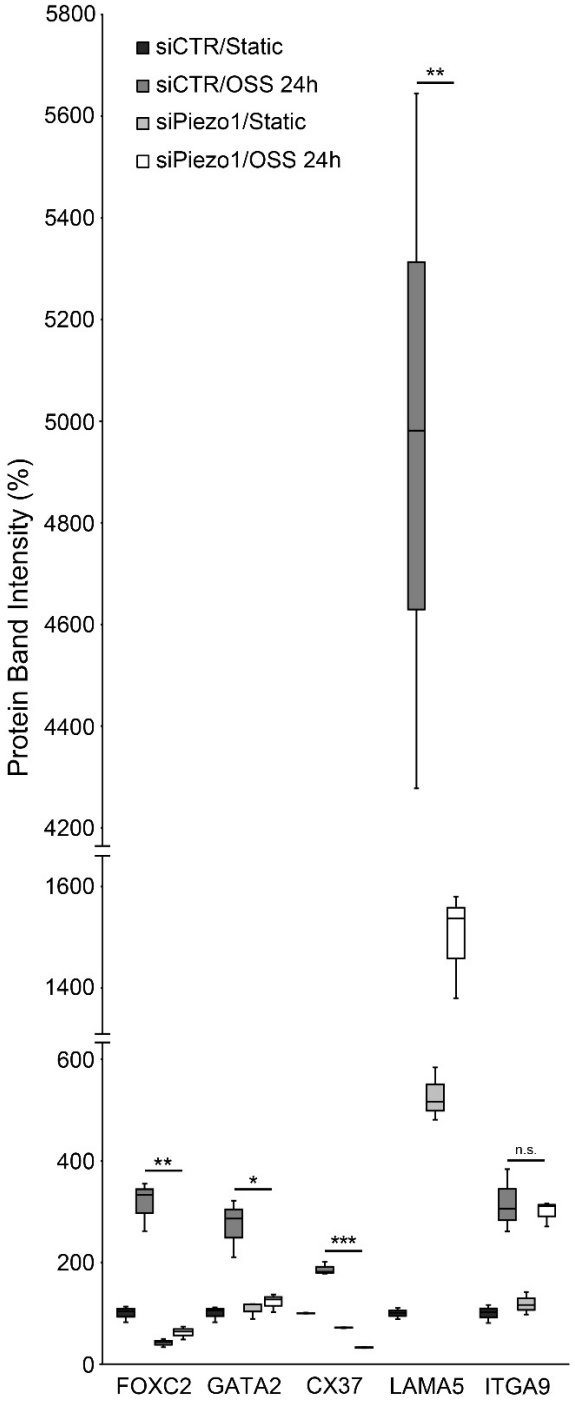
Supplemental Figure 4. *Piezo1* Deletion by *Prox1-CreER^{T2}* Allele Activated with Low-dose Tamoxifen Did Not Affect Lymphatic Vessel Density. Lymphatic vessel density was quantified in the control or Piezo1 KO pups, as shown in Figure2 F-I (Jejunum) and J-M (Colon). No significant difference was found in the lymphatic density, expressed by the number of branching point (BP no), and distance between two branching points (BP-BP dis.). n.s., not significant. Unpaired, two-tailed, *t*-test. Three pups were used for each group.



Supplemental Figure 5. Validation of Purified Primary Human Lymphatic Endothelial Cells. Immunofluorescence staining for Prox1 in purified dermal blood vessel endothelial cells (BEC;A,B) and lymphatic endothelial cells (LEC;C,D). Note that LECs are all Prox1-positive (C). Prox1 staining images (A,C) were acquired with the same capture condition (exposure time, gain, etc). White arrows in panel A point 1~2% cells of BEC population that are Prox1-positive, which are presumably contaminated LEC from the cell purification process, and serve as positive controls for Prox1 staining.



Supplemental Figure 6. Upregulation of FOXC2 and GATA2 in LECs by OSS. Relative protein band intensity for FOXC2 and GATA2 shown in Figure 4C. Statistics: **, $p < 0.01$, ***, $p < 0.001$, unpaired, two-tailed, *t*-test.



Supplemental Figure 7. Piezo1-Dependent Expression of Lymphatic Valve-Signature Genes by Oscillatory Shear Stress. Relative intensity of the western protein band for FOXC2, GATA2, CX37, LAMA5, and ITGA9 shown in Figure 4F. Statistics: *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$; n.s., not significant; unpaired, two-tailed, *t*-test.