

Table 1. Description of the mouse strains in this study.

Strain	Description	Reference
<i>Csf1^{mut}</i>	Mice have a single base pair insertion in <i>Csf1</i> that results in the formation of a truncated protein. Homozygous mutant mice have a defect in the development of macrophages and osteoclasts.	Yoshida et al., 1990.
<i>LSL-Pik3ca^{H1047R}</i>	The strain carries a floxed transcriptional stop cassette upstream of an active form of <i>Pik3ca</i> in the <i>Rosa26</i> locus.	Adams et al., 2011.
<i>Lyve1-Cre</i>	The <i>Lyve1-Cre</i> allele labels lymphatic endothelial cells, macrophages, a subset of hematopoietic stem cells, and blood vessels in the lung, liver and yolk sac.	Pham et al., 2010.
<i>mT/mG</i>	This strain has a Cre-inducible GFP reporter at the <i>Rosa26</i> locus.	Muzumdar et al., 2007.
<i>Prox1-CreER^{T2}</i>	These mice express a CreER ^{T2} fusion protein from the endogenous <i>Prox1</i> locus. CreERT2 activity is present in lymphatic endothelial cells, cardiomyocytes, hepatocytes, pancreatic ducts, islets, and neurons.	Rodriguez-Laguna et al., 2019. Srinivasan et al., 2007.
<i>Osx-tTA-TetO-Cre</i>	Transgenic mice that express a tetracycline transactivator protein and Cre recombinase in osteoblasts, osteocytes and chondrocytes. This is a Tet-Off strain.	Rodda and McMahon, 2006.
<i>TetO-Vegfc</i>	The expression of VEGFC can be controlled by doxycycline when bred with either a tTA (Tet-Off) or rtTA (Tet-On) driver.	Lohela et al., 2008.

Table 2. Lymphatic vessel index values for *TetO-Vegfc* and *Osx-tTA;TetO-Vegfc* mice.

Age	Muscle		Cortical Bone		Bone Marrow	
	<i>TetO-Vegfc</i>	<i>Osx-tTA-TetO-Vegfc</i>	<i>TetO-Vegfc</i>	<i>Osx-tTA-TetO-Vegfc</i>	<i>TetO-Vegfc</i>	<i>Osx-tTA-TetO-Vegfc</i>
P20	12.5 ± 2.754, n=3	25.83 ± 4.604, n=3	0 ± 0, n = 3	0 ± 0, n = 3	0 ± 0, n = 3	0 ± 0, n = 3
P24	14.6 ± 4.581, n=5	80.8 ± 6.176, n=5****	0 ± 0, n = 5	48.2 ± 7.96, n = 5****	0 ± 0, n = 5	0 ± 0, n = 5
P28	22.8 ± 2.177, n=5	131.3 ± 13.23, n=6****	0 ± 0, n = 5	61.5 ± 8.172, n = 5****	0 ± 0, n = 5	6.9 ± 5.84, n = 5
P32	13.79 ± 1.786, n=7	192.1 ± 14.86, n=4****	0 ± 0, n = 7	84 ± 7.72, n = 4****	0 ± 0, n = 7	52.25 ± 22.01, n = 4**

**** P < 0.0001, ** P < 0.01

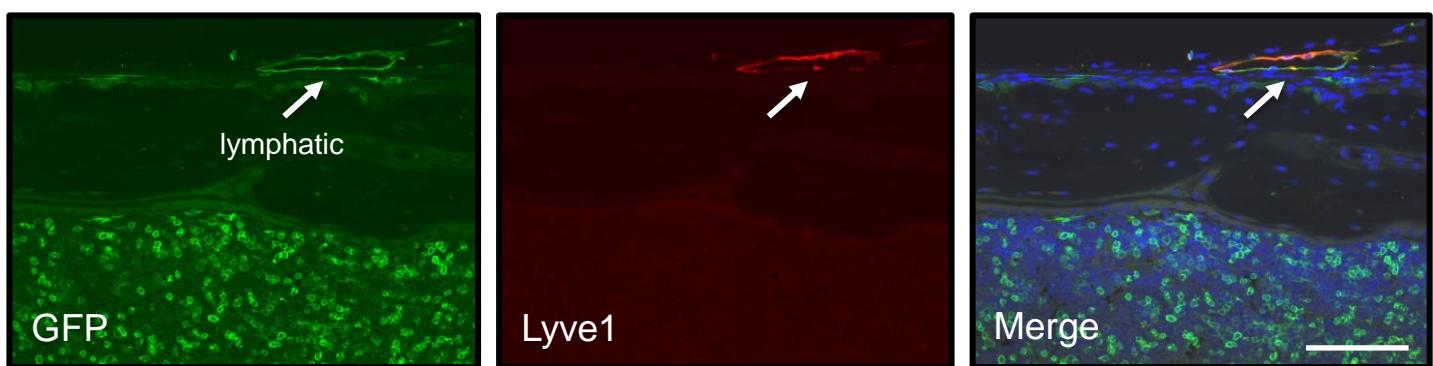


Fig S1. Cells labeled by *Lyve1-Cre* do not express *Lyve1*. Femurs from *Lyve1-Cre;mT/mG* were stained with antibodies against GFP (green) and Lyve1 (red) and counterstained with DAPI (blue). The GFP positive cells in the marrow cavity do not express Lyve1. However, the lymphatic in the periosseous connective tissue (arrow) expresses GFP and Lyve1. Scale bar: 100 μm .

Osx-tTA-TetO-Cre;mT/mG;TetO-Vegfc

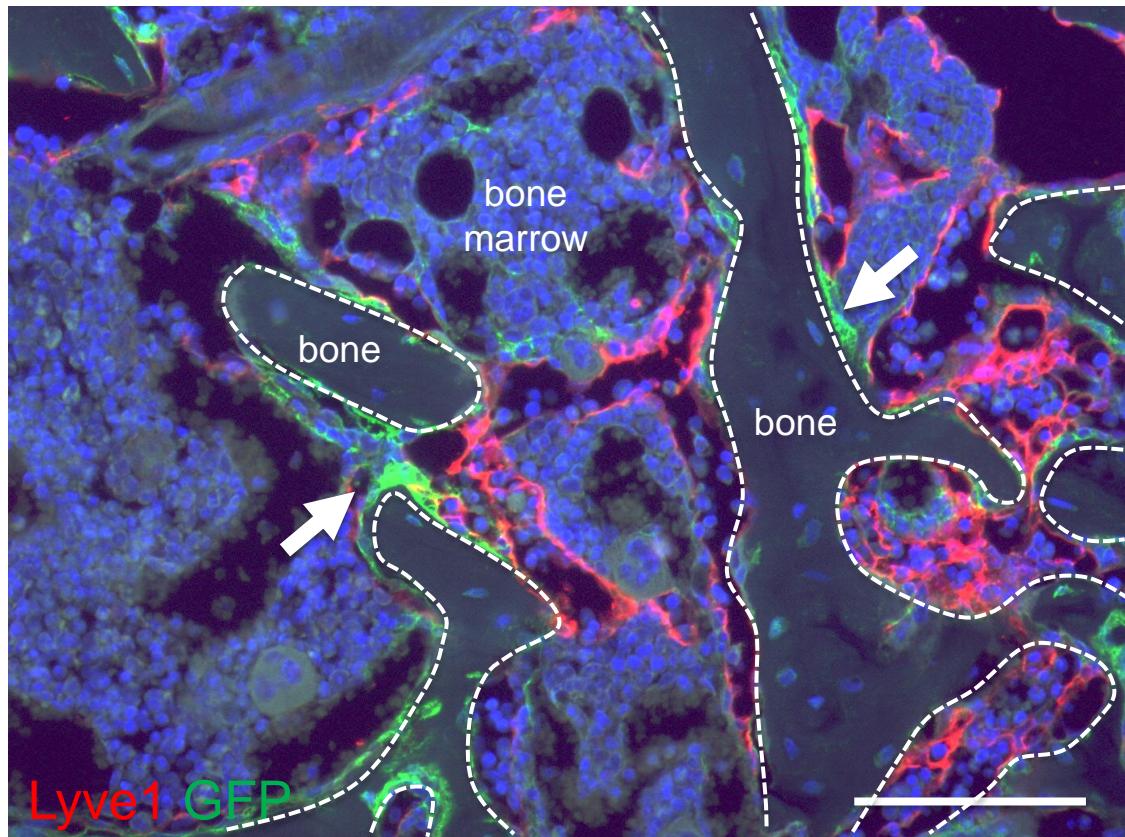


Fig S2. Bone cells in *Osx-tTA-TetO-Cre;TetO-Vegfc;mT/mG* mice express GFP. The arrows point to GFP-positive cells on the surface of bone in an *Osx-tTA-TetO-Cre;TetO-Vegfc;mT/mG* mouse. The Lyve1-positive lymphatics do not express GFP. Scale bar: 100 μm .

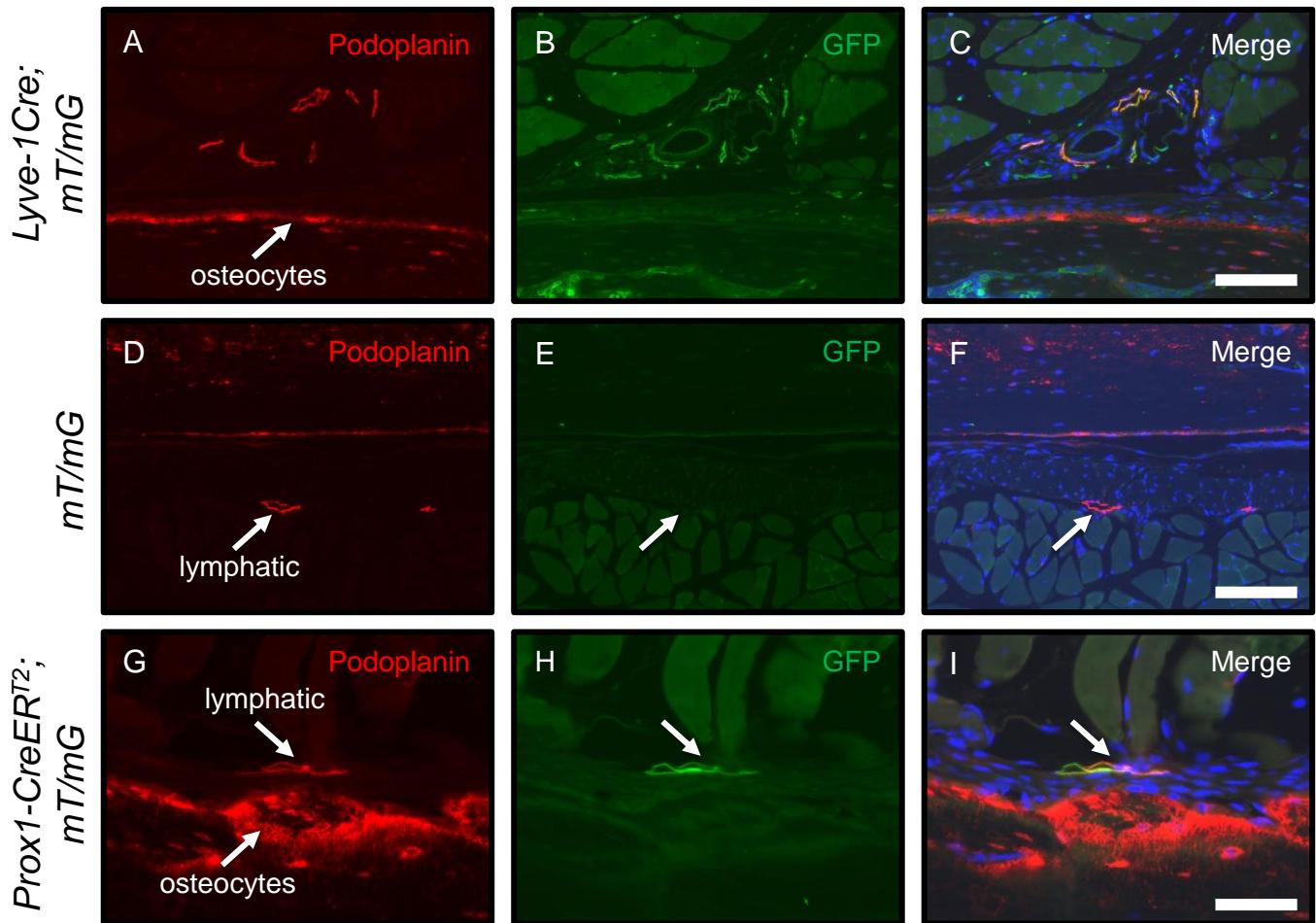


Fig S3. Periosseous lymphatics in *Prox1-CreER*^{T2};*mT/mG* mice express GFP. Femurs from *Lyve1-Cre;mT/mG* mice (positive control for immunostaining for GFP; A-C; n = 3 mice), *mT/mG* mice (negative control for immunostaining for GFP; D-F; n = 3 mice) and *Prox1-CreER*^{T2};*mT/mG* mice (G-I; n = 3 mice) were stained with antibodies against podoplanin and GFP. Periosseous lymphatics and osteocytes expressed podoplanin. Periosseous lymphatics in *Lyve1-Cre;mT/mG* mice and *Prox1-CreER*^{T2};*mT/mG* mice expressed GFP. Scale bar in panels C and F: 100 μm. Scale bar in panel I: 50 μm.

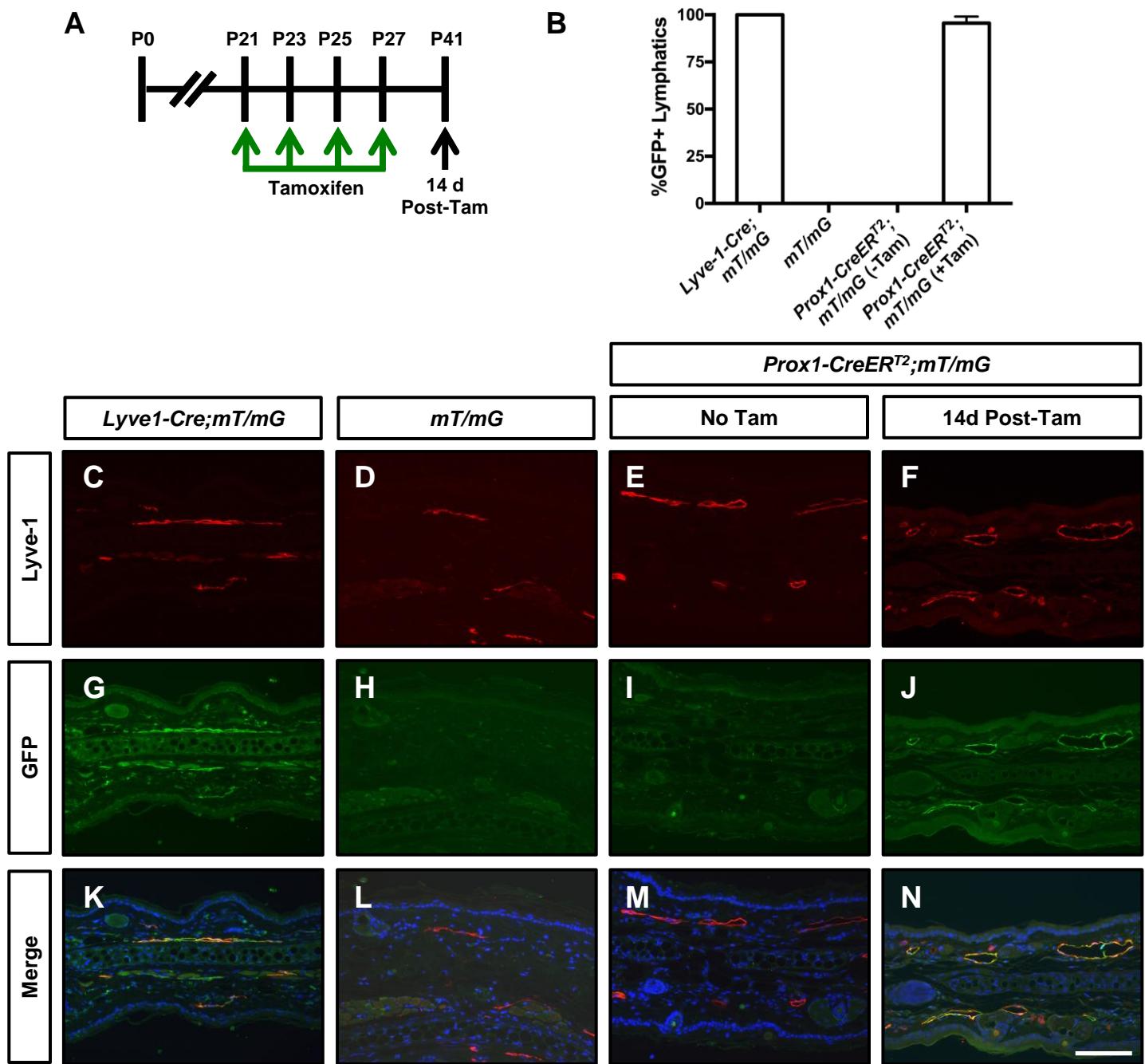


Fig S4. The *Prox1-CreER^{T2}* allele is not active in the absence of tamoxifen. **A.** Schematic showing when *Prox1-CreER^{T2}; mT/mG* mice were injected with tamoxifen (2 mg; i.p.). **B.** Graph showing the percent of GFP-positive lymphatics in the skin of *Lyve-1Cre; mT/mG* mice (100 ± 0, n = 3), *mT/mG* mice (0 ± 0, n = 2), *Prox1-CreER^{T2}; mT/mG* mice that did not receive tamoxifen (0 ± 0, n = 3), and *Prox1-CreER^{T2}; mT/mG* mice that received tamoxifen (95.6 ± 3.4, n = 3). **C-N.** Representative images of ear skin sections stained with antibodies against Lyve-1 and GFP. Data are presented as mean ± SEM. Scale bar: 100 μm.

Osx-tTA; TetO-Vegfc

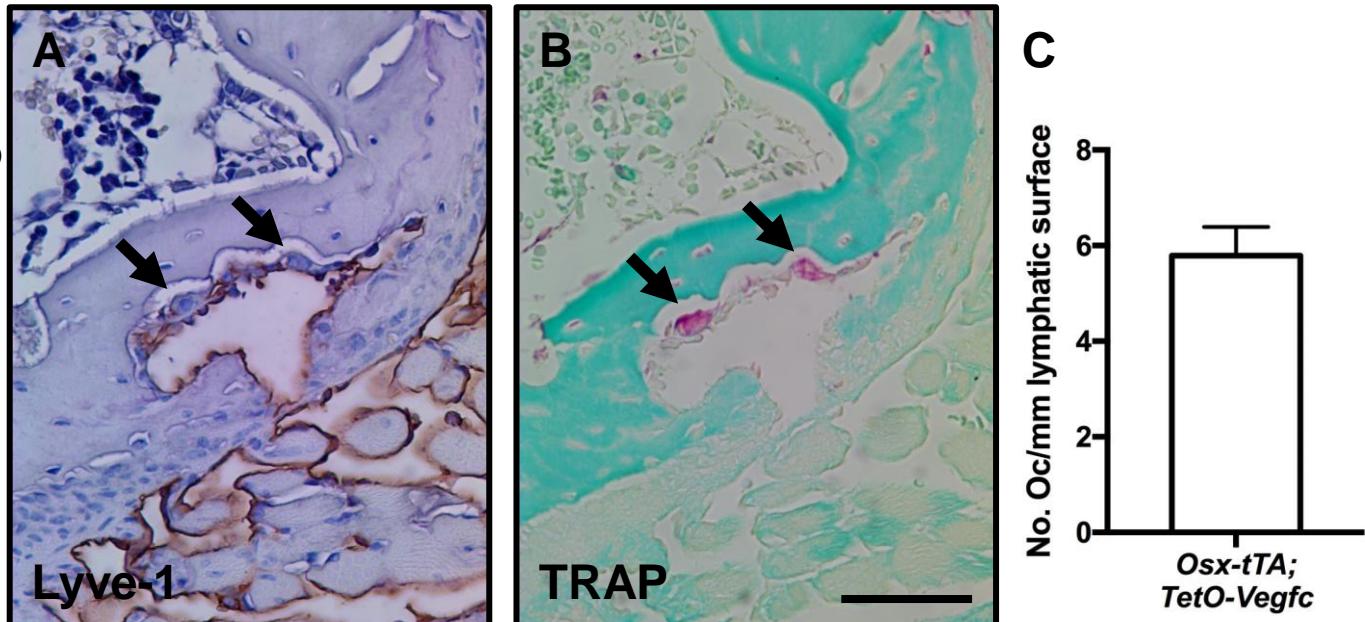


Fig S5. Osteoclasts are closely associated with lymphatics invading bone in *Osx-tTA;TetO-Vegfc* mice.
A,B. Representative images of rib serial sections stained for Lyve1 (A) and TRAP activity (B). The arrows in panels A and B point to osteoclasts. **C.** Graph showing the number of osteoclasts per millimeter of lymphatic surface (5.8 ± 0.61 ; $n = 3$). Scale bar: 50 μm .