

# Supporting Information

Tan et al. 10.1073/pnas.1420463111

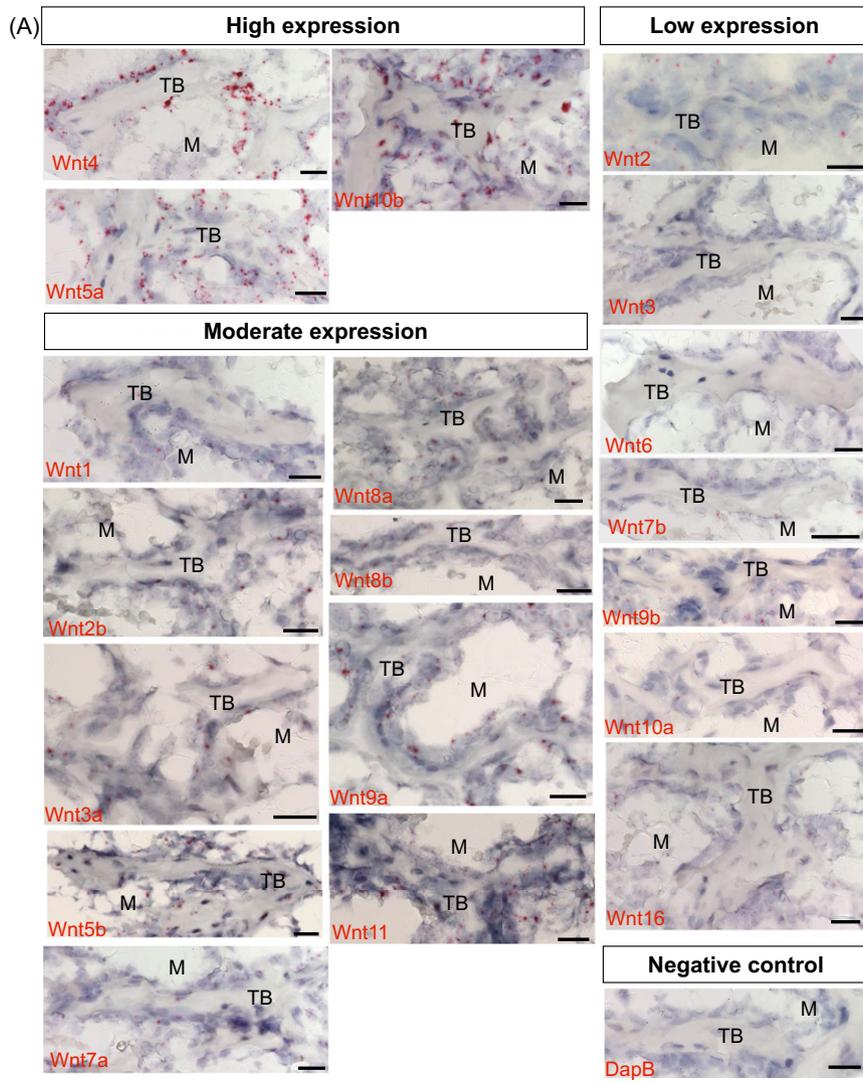
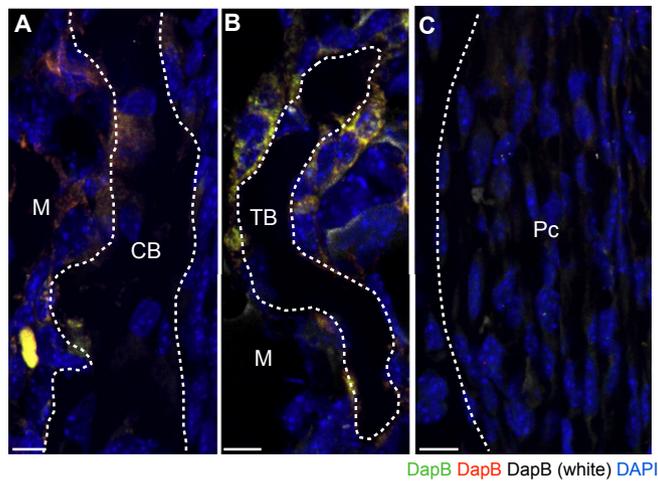


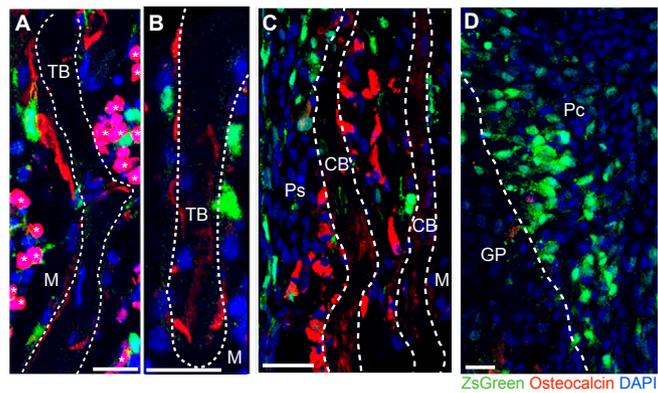
Fig. S1. (Continued)



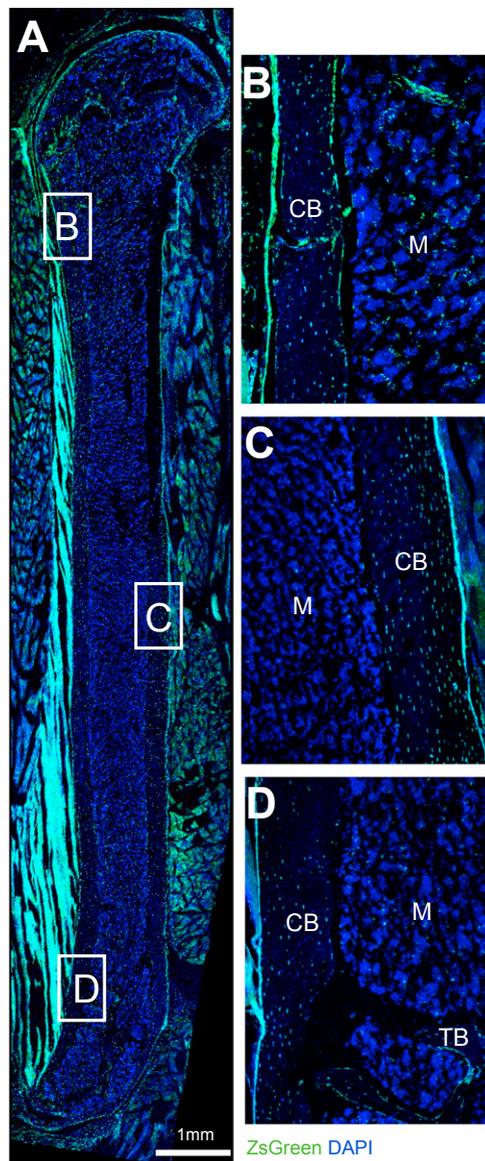




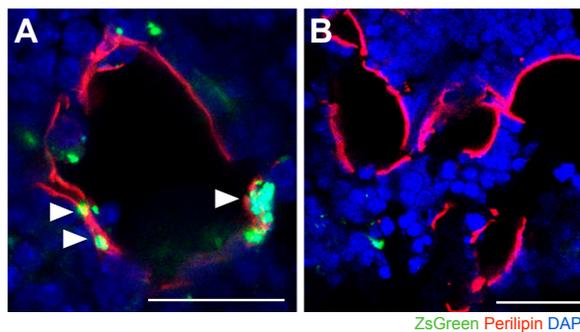
**Fig. 52.** Negative controls for multiplex fluorescent ISH. Negative controls in the green, red and white channels using bacterial gene DapB in the cortical bone (A), trabecular bone (B), and perichondrium (C). The autofluorescence is because of erythrocytes and cell cytoplasm. CB, cortical bone; GP, growth plate; M, marrow; Pc, perichondrium; TB, trabecular bone. (Scale bars: 10  $\mu$ m.)



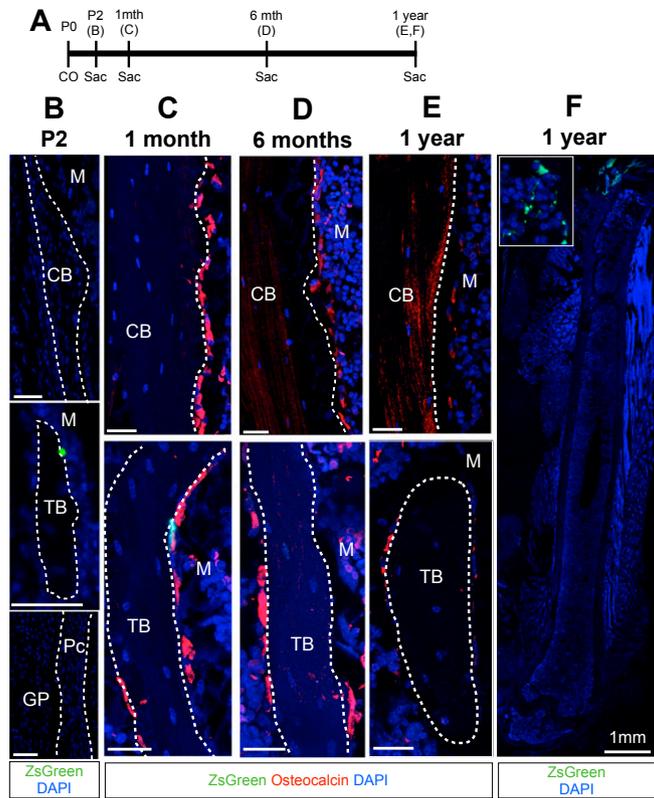
**Fig. 53.** Initial AXCT2-labeled population does not contain Osteocalcin-expressing mature osteoblasts or osteocytes. Ocn-expressing mature osteoblasts (red) and osteocytes (embedded in the bone) are not labeled by AXCT2;Ai6-labeled cells (green) after 48 h of trace. Representative images from the trabecular bone (A and B), cortical bone (C), and perichondrium (D) are shown. The perichondrium does not contain osteocytes or Ocn-expressing osteoblasts. The antibody against Ocn routinely stains for a hematopoietic population (marked by asterisks), as suggested by their rounded morphology. CB, cortical bone; GP, growth plate; M, marrow; Pc, perichondrium; Ps, periosteum; TB, trabecular bone. (Scale bars: 25  $\mu$ m.)



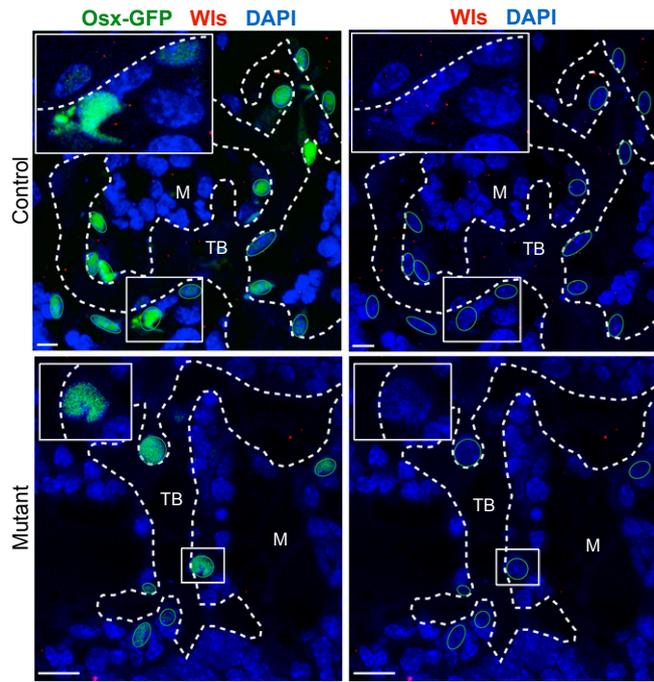
**Fig. 54.** Progeny of AXCT2-labeled cells are ubiquitously found throughout the 1-y traced femur, and do not display regional biases. (A) Montage of a whole femur from a 1-y traced AXCT2;Ai6 mouse (*Left*), with magnified views of various parts of the bone (B–D). Note the large number of labeled osteocytes and cells lining the endosteum throughout the femur. The muscle fibers surrounding the bone also contain ZsGreen-labeled cells. (Magnification: A, 4.5 $\times$ .)



**Fig. 55.** Some adipocytes are labeled with ZsGreen after 6 mo of trace. After 6 mo of trace, most adipocytes (marked by Perilipin expression in red) do not express ZsGreen (B), but some occasional adipocytes that carry the ZsGreen label can still be observed (A). (Scale bar: 25  $\mu$ m.)



**Fig. 56.** Spontaneous labeling is very rare in the bones of AXCT2;Ai6 mice. (A) Pulse-chase scheme for corn oil controls. (B–F) At all of the time points surveyed, there was a very low level of ectopic labeling in the trabecular bone (*Upper*) and cortical bone (*Lower*) regions. (F) Montage of a whole femur 1 y after corn oil injection at P0. Vast majority of the ectopically labeled cells have a dendritic morphology and reside in the marrow (F, *Inset*), distinct from osteolineage cells. (Magnification: F, *Inset*, 65 $\times$ .) CO, corn oil; CB, cortical bone; GP, growth plate; M, marrow; Pc, perichondrium; TB, trabecular bone. (Scale bars: 25  $\mu$ m in B–E.)



**Fig. 57.** Immunostaining against Wls. Control Osx-GFP::Cre;Wls<sup>fl/+</sup> (*Upper*) and mutant Osx-GFP::Cre;Wls<sup>fl/fl</sup> (*OW, Lower*) samples stained with antibody against Wntless, which appear as punctae. Trabecular bones are outlined with white dotted lines, Osx-GFP<sup>+</sup> nuclei are outlined in green solid lines. Left and right images within each panel are identical, except that the image on the right lacks GFP staining to better visualize the red punctate Wntless staining. There are fewer red dots associated with Osterix-GFP<sup>+</sup> nuclei in the mutant than in the control. Insets show magnified views of boxed regions. M, marrow; TB, trabecular bone. (Scale bars: 25  $\mu$ m.) (Magnification: *Insets*, 2 $\times$ .)



**Table S2. Comparison of relative Wnt expression levels in ISH survey with published transcriptome profiling studies**

Neonatal ISH present study		E18.5 and 1-wk-old qPCR, Andrade et al. (1)				Adult RNASeq, Ayturk et al. (2)	
Gene	ISH score	Gene	Pc	CB	Total	Gene	Score
<b>Wnt10b</b>	<b>22</b>	<b>Wnt5a</b>	<b>72.5</b>	<b>22.5</b>	<b>95.0</b>	<b>Wnt10b</b>	<b>8.442</b>
<b>Wnt5a</b>	<b>21</b>	<b>Wnt5b</b>	<b>60.8</b>	<b>33.9</b>	<b>94.7</b>	<b>Wnt4</b>	<b>6.004</b>
<b>Wnt5b</b>	<b>15</b>	<b>Wnt4</b>	<b>14.7</b>	<b>39.2</b>	<b>53.9</b>	<b>Wnt16</b>	<b>4.273</b>
<b>Wnt9a</b>	<b>12</b>	<b>Wnt16</b>	<b>31.6</b>	<b>7.1</b>	<b>38.7</b>	<b>Wnt5b</b>	<b>4.235</b>
<b>Wnt11</b>	<b>12</b>	<b>Wnt10b</b>	<b>20.2</b>	<b>4.0</b>	<b>24.2</b>	<b>Wnt5a</b>	<b>1.535</b>
<b>Wnt4</b>	<b>11</b>	<b>Wnt11</b>	<b>7.5</b>	<b>1.2</b>	<b>8.7</b>	<i>Wnt2b</i>	1.239
<b>Wnt16</b>	<b>10</b>	<b>Wnt9a</b>	<b>2.7</b>	<b>0.3</b>	<b>3.0</b>	<b>Wnt9a</b>	<b>0.840</b>
<i>Wnt8a</i>	9	<i>Wnt1</i>	ND	2.7	2.7	<i>Wnt1</i>	0.666
<i>Wnt2b</i>	8	<i>Wnt2b</i>	0.9	1.0	1.9	<b>Wnt11</b>	<b>0.187</b>
<i>Wnt7b</i>	8	<i>Wnt2</i>	0.58	ND	0.58	<i>Wnt7b</i>	0.170
<i>Wnt1</i>	7	<i>Wnt3</i>	ND	ND	ND	<i>Wnt6</i>	0.129
<i>Wnt3a</i>	7	<i>Wnt3a</i>	ND	ND	ND	<i>Wnt10a</i>	0.092
<i>Wnt7a</i>	6	<i>Wnt6</i>	ND	ND	ND	<i>Wnt2</i>	0.081
<i>Wnt8b</i>	6	<i>Wnt7a</i>	ND	ND	ND	<i>Wnt9b</i>	0.036
<i>Wnt10a</i>	6	<i>Wnt7b</i>	ND	ND	ND	<i>Wnt3a</i>	0.012
<i>Wnt2</i>	5	<i>Wnt8a</i>	ND	ND	ND	<i>Wnt8b</i>	0.011
<i>Wnt3</i>	4	<i>Wnt8b</i>	ND	ND	ND	<i>Wnt8a</i>	0.008
<i>Wnt9b</i>	4	<i>Wnt9b</i>	ND	ND	ND	<i>Wnt3</i>	0.002
<i>Wnt6</i>	3	<i>Wnt10a</i>	ND	ND	ND	<i>Wnt7a</i>	0.000

The Wnts are ranked in descending order of expression level in each of the three studies. Left columns, current study; center columns, adapted from Andrade et al. (1); right columns, adapted from Ayturk et al. (2). The seven most highly expressed Wnts from our ISH survey are the top ranked genes in the published studies (in boldface), thus validating our approach. CB, cortical bone; Pc, perichondrium; ND, not determined.

1. Andrade AC, Nilsson O, Barnes KM, Baron J (2007) Wnt gene expression in the post-natal growth plate: regulation with chondrocyte differentiation. *Bone* 40(5):1361–1369.
2. Ayturk UM, et al. (2013) An RNA-seq protocol to identify mRNA expression changes in mouse diaphyseal bone: Applications in mice with bone property altering *Lrp5* mutations. *J Bone Miner Res* 28(10):2081–2093.