

Supplementary information

Title

The CXCL5/CXCR2 axis is sufficient to promote breast cancer colonization during bone metastasis

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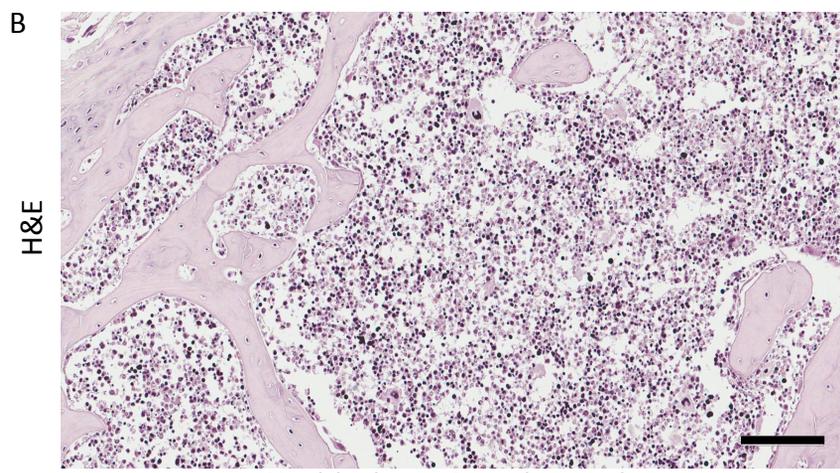
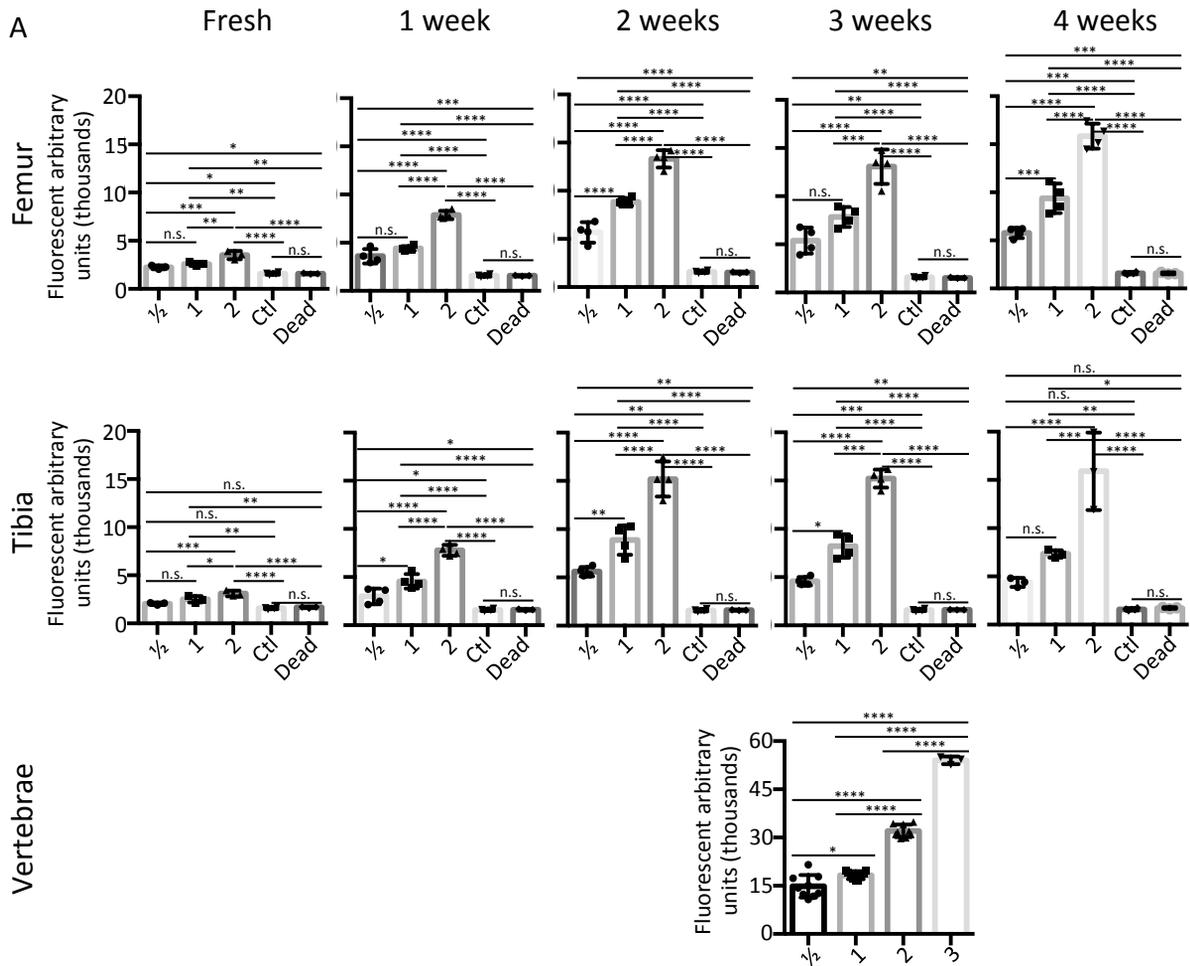
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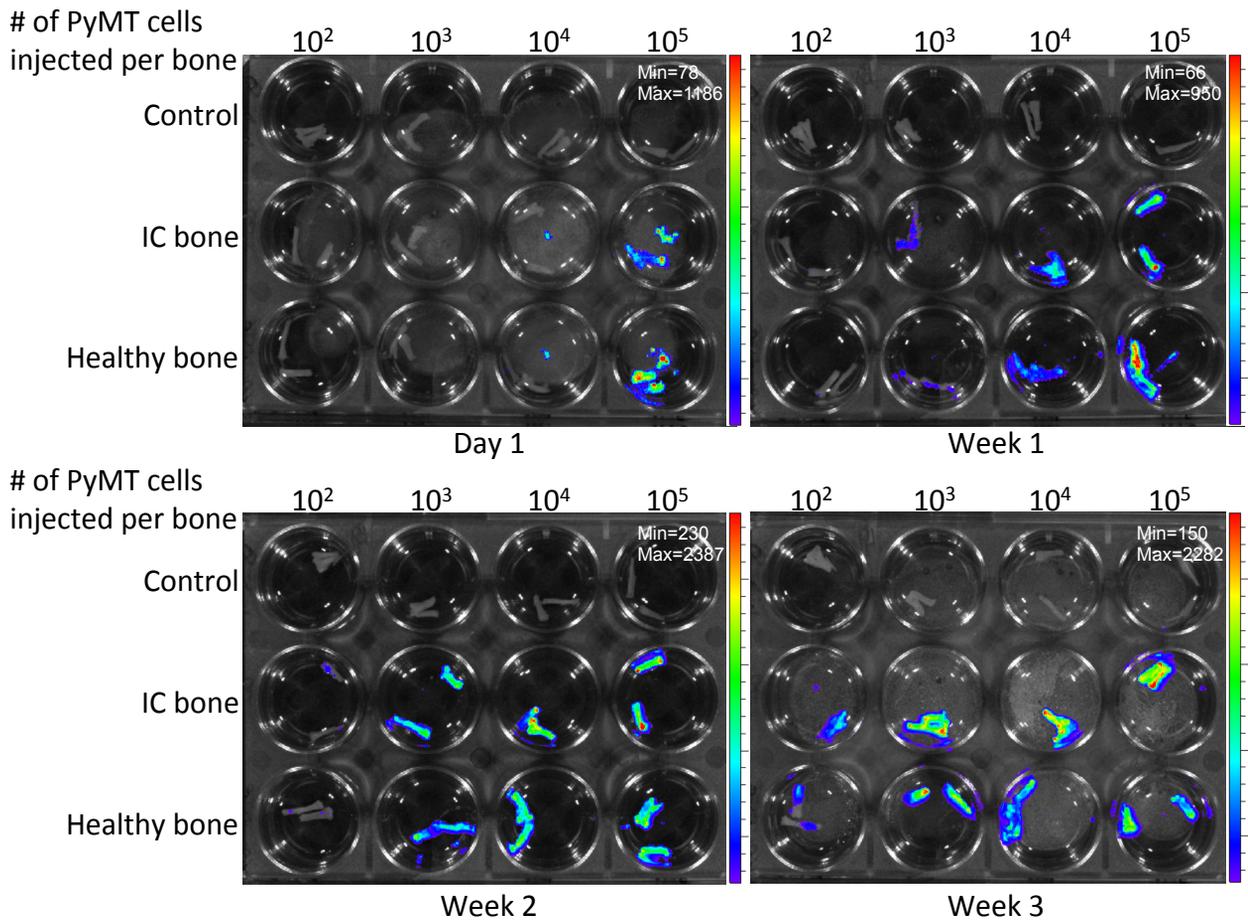
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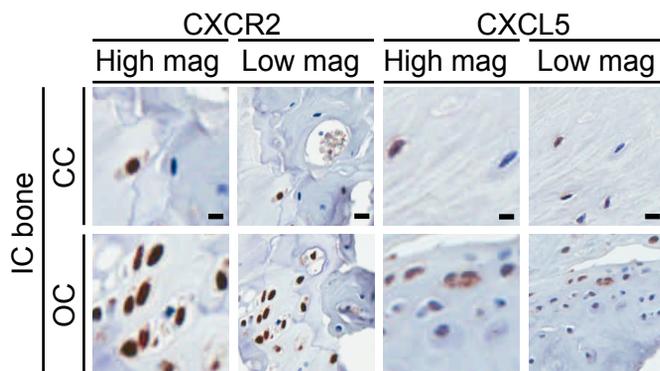
Healthy bone 4 weeks in culture

Supplementary Figure 1. (A) 4-week timeline of the metabolic activity of *ex vivo* bone cultures compared to the metabolic activity of a dead bone and media controls. CellTiter Blue (cell viability) assay to test metabolic activity of an increasing number of mouse bone explants in *ex vivo* culture up to four weeks in culture (p values obtained by student's t test, multiple comparisons). Lines show the mean and standard deviation. Please note that the different time points of the bone cultures were set up as staggered experiments so that the assay occurred on the same day for all of the timepoints using the same bottle of CellTiter Blue reagent and the same instrument settings. (B) H&E of a cultured mouse bone cultured for four weeks at a low magnification to show a wide area within the cultured bone (scale bar: 100 μ m). * significance at $p < 0.05$; ** significance at $p < 0.01$; *** significance at $p < 0.001$; **** significance at $p < 0.0001$; n.s. not significant.

A



B



OC = Osteocyte
CC = Chondrocyte

Supplementary Figure 2. (A) Luciferase assay on healthy or IC mouse bone cultures colonized by a luciferase-expressing PyMT cell line. The intensity bars (rainbow) and scale information (Min/max) for BLI signal are provided. Bioluminescent PyMT cancer cells co-localize with the bone in culture. (B) IHC staining for CXCR2 (left) and CXCL5 (right) of IC bone co-cultures injected with PyMT cells. We are showing that chondrocytes (CC) and osteocytes (OC) are capable of expressing both CXCR2 and CXCL5 and these are not restricted to cells in the bone marrow or to cancer cells (scale bars: High mag = 5 μm ; Low mag = 10 μm).