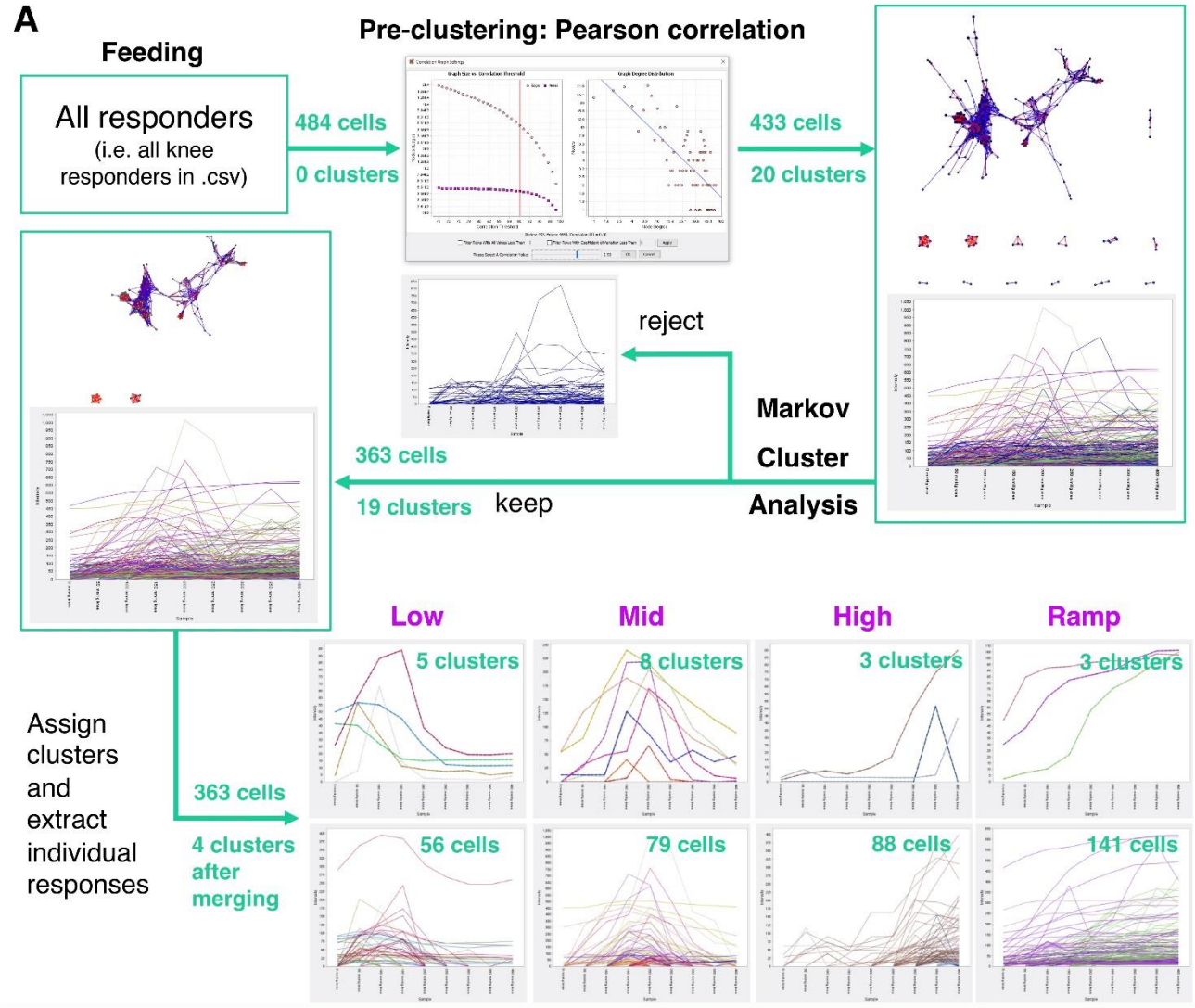
Supplementary data



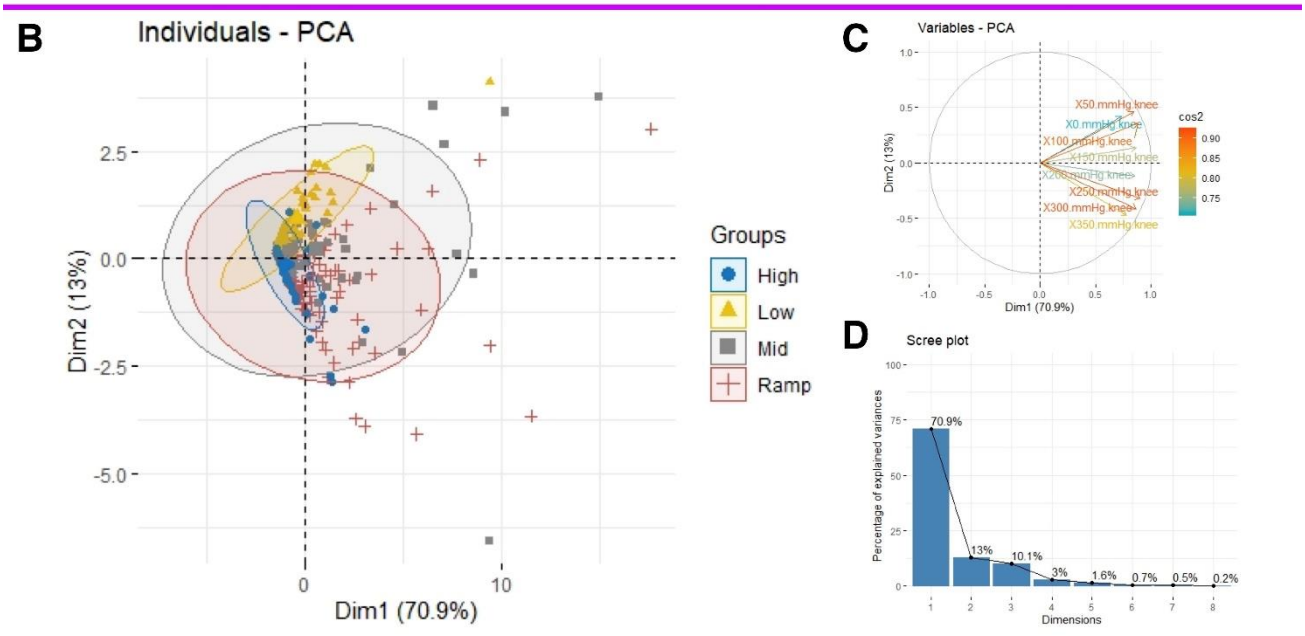
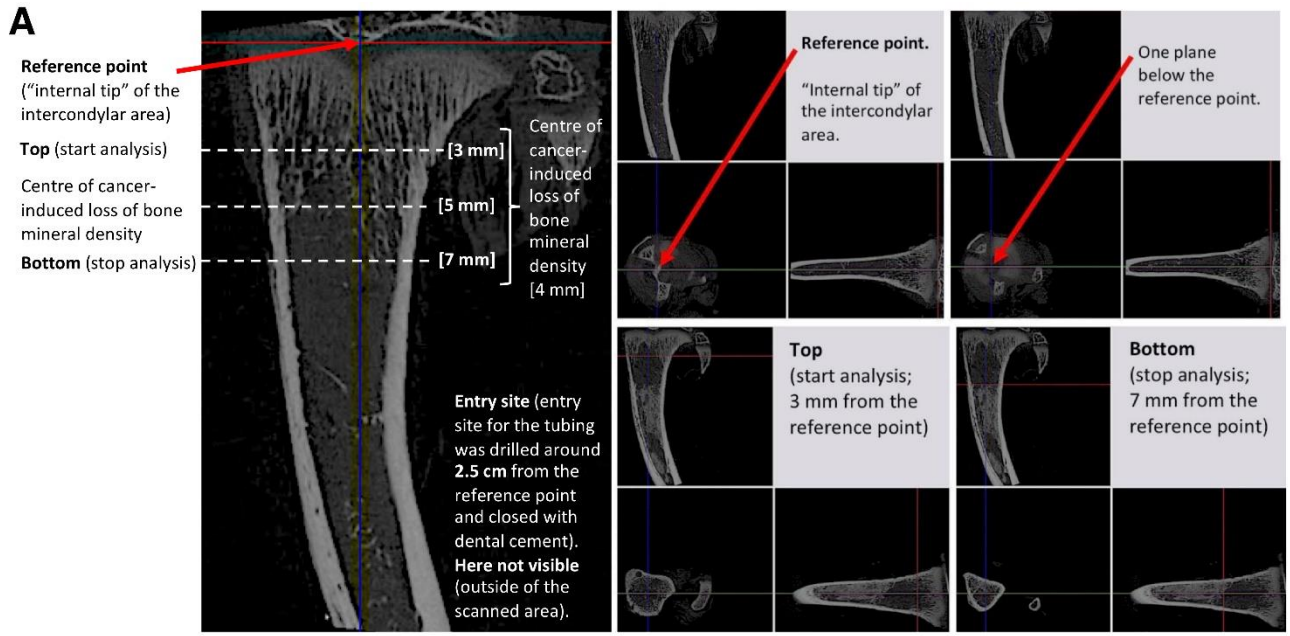


Figure S1. Leg compression and position are differentially coded by DRG sensory neurons. A pipeline of the largely unsupervised Markov Clustering Analysis (MCA) of primary afferents responses. See the full description in the Material and Methods section **(A)**. Principal component analysis (PCA) was used to compare neuronal responses in pre-defined clusters. 363 knee responders fluorescence values after MCA clustering in (A) each with a cluster's tag were used for PCA. Note that only the two most distinctive clusters ('low' vs. 'high') could be revealed with this approach (blue and yellow groups have opposite directions of the eigenvectors). The longitudinal nature of the gathered data (pressure ramp) requires advanced clustering **(B)**. Eigenvectors in the performed PCA showing the directions of the variables **(C)**. Percentage of the explained variability by each principal component after the PCA performed on the fluorescence intensity values of the knee responders **(D)**.



Area chosen for analysis: **4 mm** between "top" and "bottom" encompassing 119 virtual planes (each 34 µm thick).

B

Parameter	Sham Early		Cancer Early		Sham Late		Cancer Late	
	Average	SEM	Average	SEM	Average	SEM	Average	SEM
Surface area [µm^2]	4.56E+07	4.27E+06	2.42E+07 ##, *	3.37E+06	3.90E+07	4.06E+06	2.02E+07 ###, **	2.87E+06
Bone volume [µm^3]	7.27E+08	5.23E+07	3.94E+08 ##, *	5.66E+07	6.37E+08	6.69E+07	3.41E+08 ###, **	4.78E+07
Total volume [µm^3]	1.10E+10	5.09E+08	9.23E+09	3.89E+08	1.17E+10	2.50E+08	1.48E+10 #, $$$	1.40E+09
Volume ratio	0.067	0.008	0.043	0.006	0.055	0.006	0.025 ###, *	0.005
Tb Th Mean [µm]	55.14	1.56	53.83	2.25	55.60	0.60	58.57	2.97
Tb Th StDev [µm]	15.35	1.43	14.75	2.37	16.19	1.45	18.20	2.19
Tb Th Max [µm]	122.16	8.85	119.48	20.23	121.89	9.73	121.79	11.90
Tb Sp Mean [µm]	1212.58	90.69	1124.56	44.71	1257.19	63.72	1351.83	65.16
Tb Sp StDev [µm]	812.25	49.57	697.01	22.98	823.03	24.85	731.95	54.00
Tb Sp Max [µm]	2277.69	132.75	2158.01	66.99	2455.47	101.64	2457.98	148.78
Euler characteristic (χ)	-1730.20	492.24	-548.57 #	129.17	-1070.80	159.23	-340.17 ##	96.87
Corr Euler (χ + Δχ)	-1777.00	492.24	-587.64 #	132.08	-1127.70	161.21	-355.08 ##	100.29
Connectivity	1778.00	492.24	588.64 #	132.08	1128.70	161.21	356.08 ##	100.29
Connectivity density [µm^3]	3.83	1.12	1.47 #	0.32	2.23	0.35	0.60 ##	0.17

Figure S2. The impact of cancer progression on bone microarchitecture. Example micro-computer tomography reconstructions of rat tibiae. The figure outlines the method used to obtain the reference point for bone mineral density quantification **(A).** A table summarising other parameters obtained from the micro-computer tomography analysis. Data represent the mean ± SEM. One-Way

ANOVA with Tukey post-hoc test: *p < 0.05, **p < 0.01, ***p < 0.001. # vs. Early Sham, * vs. Late Sham, $ vs. Early Cancer. N = number of animals: Sham Early (5), Cancer Early (7), Sham Late (5), Cancer Late (6) **(B).**

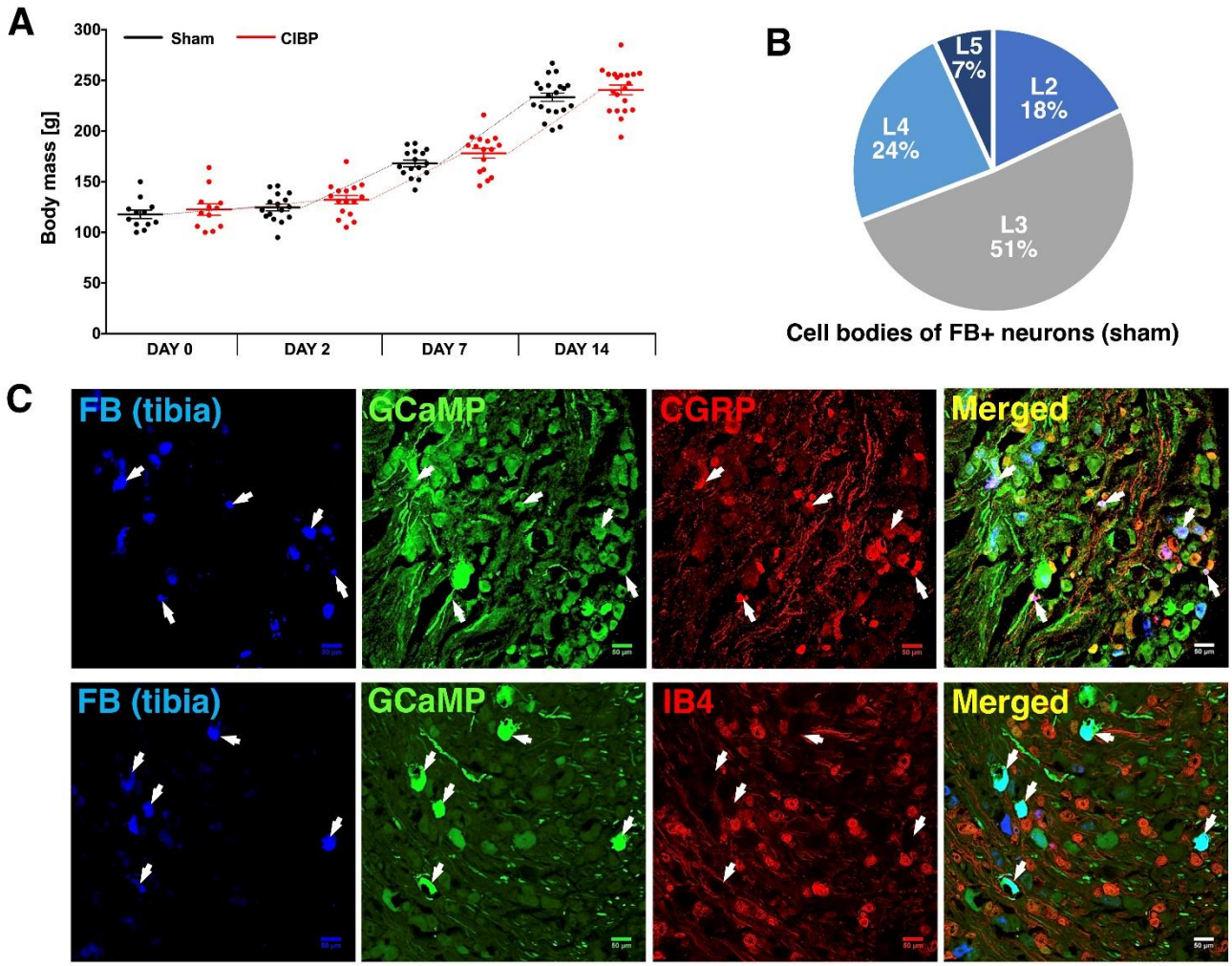


Figure S3. The impact of cancer progression on bone innervation. Body mass gain in rats after cancer implantation to tibia is shown. Within a timepoint, each dot represents a single animal (n = 13-20 per group). Data represent the mean ± SEM. Kruskal-Wallis H for independent samples: all non-significant **(A).** Distribution of all Fast Blue positive (FB) intratibial afferents within analysed ipsilateral lumbar DRG (L2-L5) from 6 sham rats. No FB positivity was noticed in the contralateral lumbar DRG (not shown) **(B).** Representative images of lumbar 3 (L3) DRG expressing GCaMP immunostained for peptidergic neurons marker – CGRP (top panel) and for non-peptidergic neurons marker – IB4 (bottom panel) in Fast Blue (FB) traced tibial afferents. Arrows indicate FB neurons. Scale bars, 50 µm **(C).**