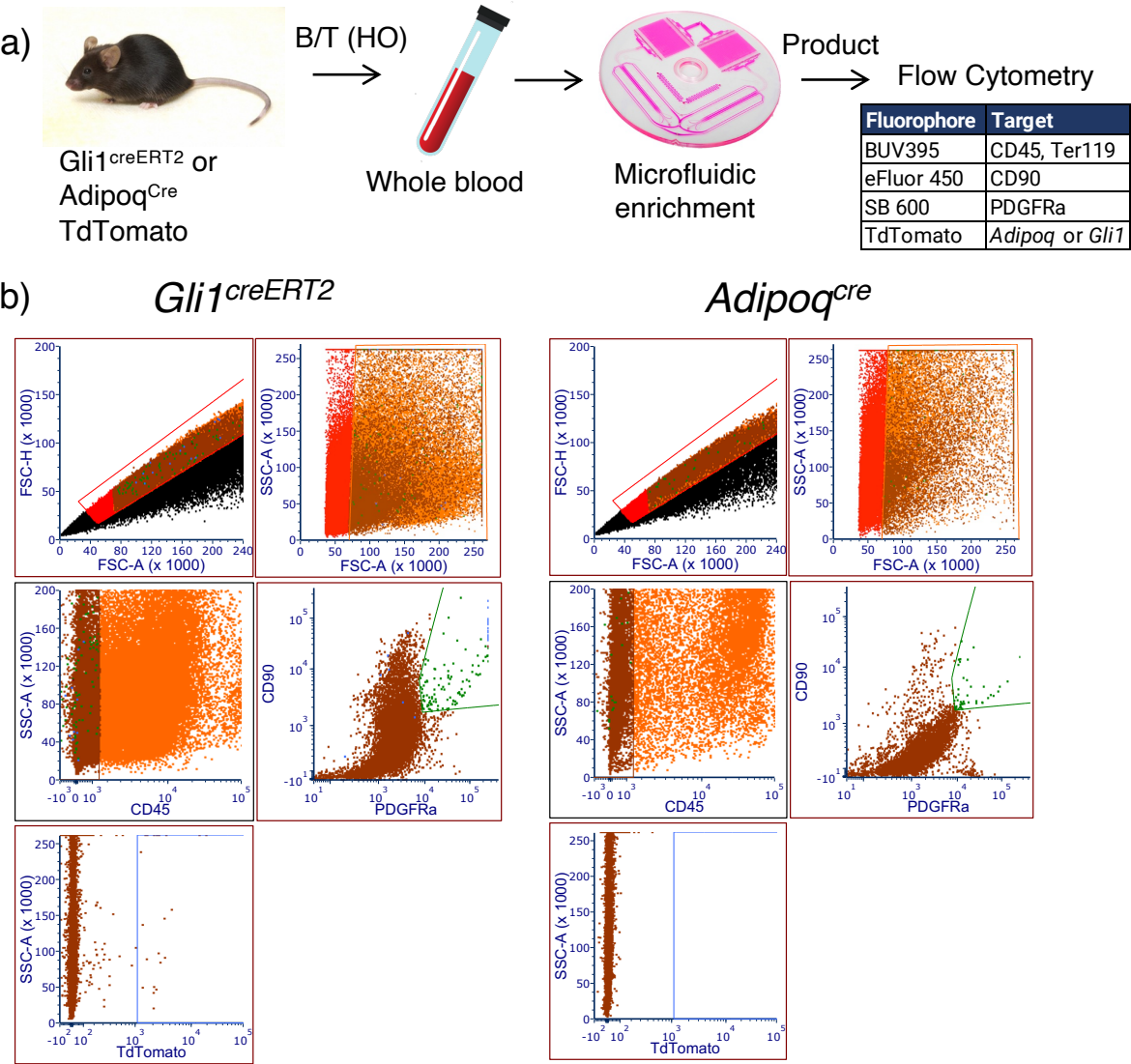


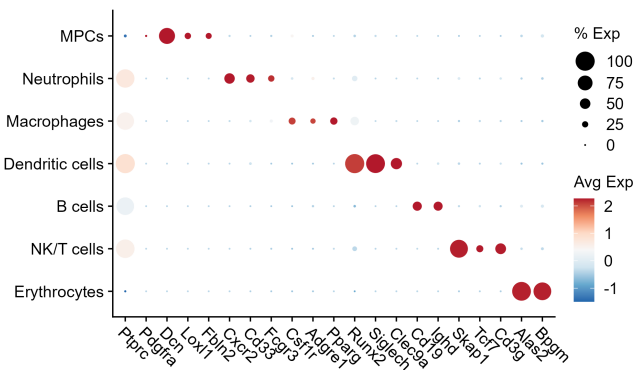
SUPPLEMENTARY FIGURE 1



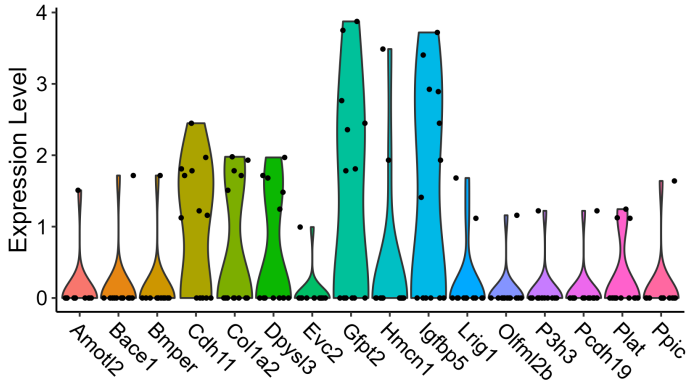
Supplementary Figure 1: Experimental approach to test the lineage of circulating PDGFRa/CD90+ cells. a) Experimental design, b) Gating strategy.

SUPPLEMENTARY FIGURE 2

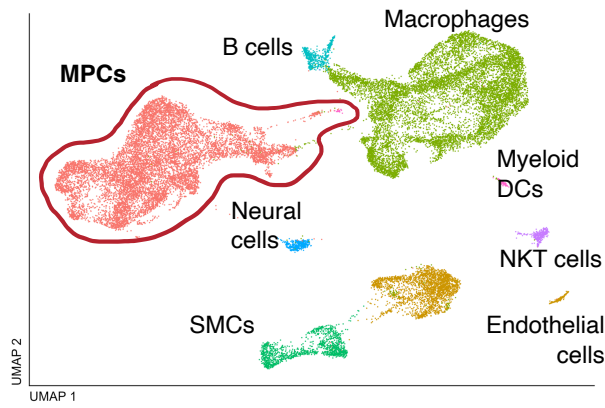
a) Enriched blood cell clusters



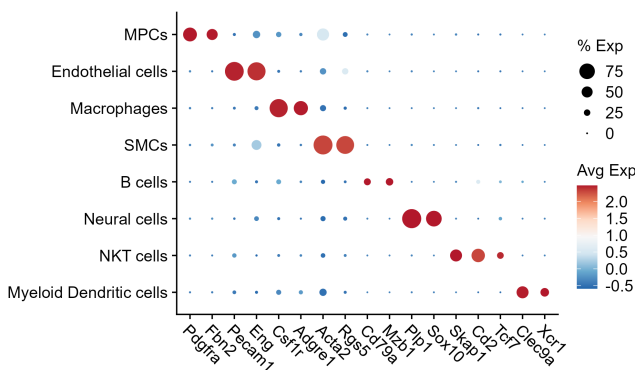
b) Circulating MPC cluster



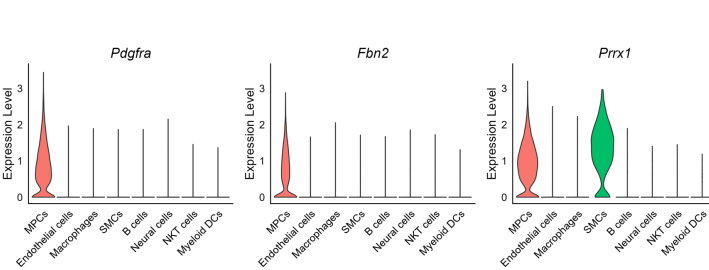
c) HO site UMAP



d) HO anlagen cell clusters



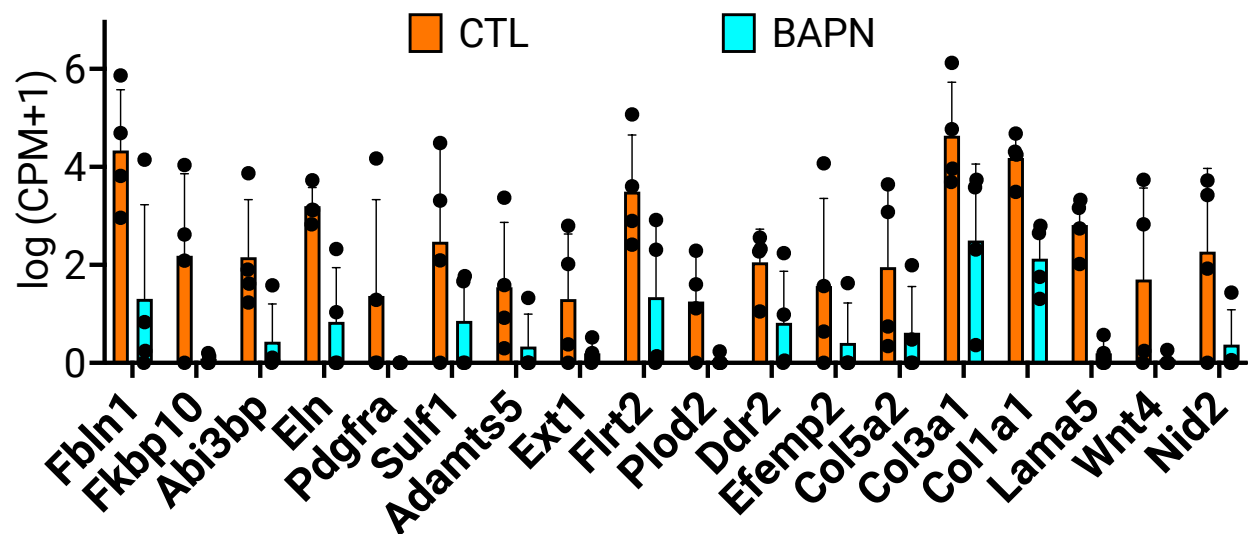
e) HO-resident MPC cluster-specific genes



Supplementary Figure 2: Single cell RNAseq detects cMPCs expressing HO gene expression signature in B/T blood samples.

a) UMAP representation of cells detected in iChip-enriched blood product from B/T samples (n=2). b) Exhibit shows genes from HO signature that are detected in cMPCs. c) UMAP representation of cells in HO anlagen post-B/T (GSE126060). d) Top differentially expressed genes in cell clusters in HO anlagen cell clusters. e) *Pdgfra*, *Fbn2* and *Prrx1* as MPC-specific genes in the HO anlagen.

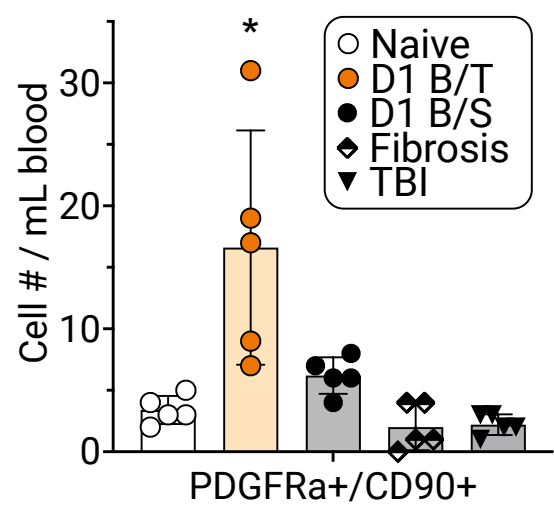
SUPPLEMENTARY FIGURE 3



Supplementary Figure 3: LOX inhibition decreases many ECM associated cMPC genes.

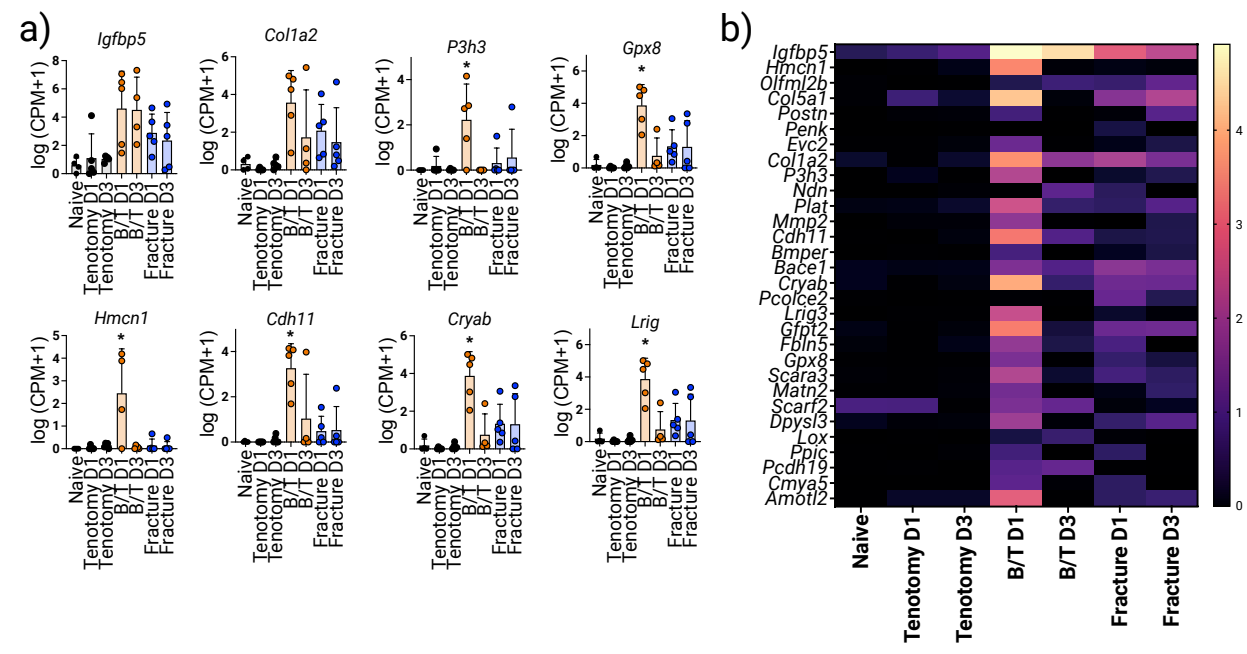
All genes are part of ECM associated function (by Gene Ontology) and are expressed by HO-associated MPCs (GSE126060). All genes $q < 0.10$ FDR, $n = 4$, vehicle and BAPN treated mice 3 days post B/T. These genes are in addition to ECM genes part of cMPC HO signature (Col1a2, Fbln5, Matn2, Olfml2b) that were significantly decreased upon BAPN treatment

SUPPLEMENTARY FIGURE 4



Supplementary Figure 4: PDGFRα+CD90+ MPCs' release is unique to HO. iChip-flow cytometry identifies significantly increased CD45-CD11b-PDGFRα+CD90+ cMPCs at 24 hours post-B/T, when compared to naïve, sham or non-HO inducing traumatic conditions. This data validates that PDGFRα/CD90 MPCs released into circulation specifically in B/T (HO forming injury), but not in healthy or other non-HO forming traumatic injuries (n=5 mice per condition, *p<0.05 by one-way-ANOVA). TBI: Traumatic brain injury model, Fibr: Muscle fibrosis model.

SUPPLEMENTARY FIGURE 5



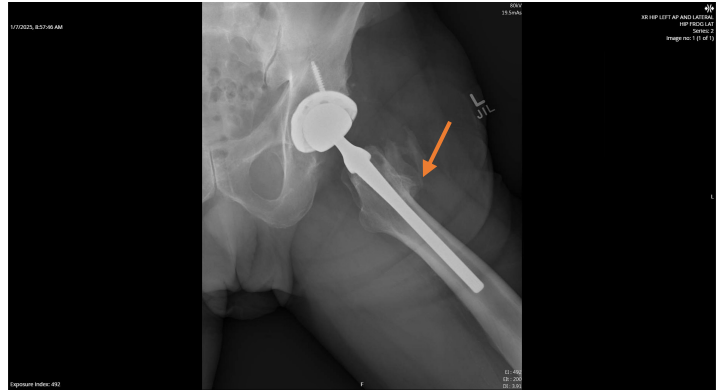
Supplementary Figure 5: Fracture that does not lead to HO results in detection of a portion of cMPC genes from blood but can be differentiated from post-B/T cMPCs. Left panel exhibits differentially expressed genes that enable differentiating HO^{pos} B/T and HO^{neg} fracture in mouse models. Right panel exhibits mean of cMPC HO signature genes across controls (naïve, Achilles tenotomy) and injury models (B/T, fracture). The mean level of HO cMPC signature genes was highest on day 1 after B/T, significantly higher than day 1 after fracture (adjusted p*=0.0014 via ANOVA adjusted for multiple comparisons by Tukey method).

SUPPLEMENTARY FIGURE 6

HO^{neg} patient



HO^{pos} patient



Supplementary Figure 6: Representative x-ray radiographic images from hip arthroplasty patients enrolled in the study. Left panel: HO^{neg} patient, right panel: HO^{pos} patient. Arrow indicates location of heterotopic ossification.

SUPPLEMENTARY TABLE 1

Gene	Function/Process	Relevant publication
AMOTL2	Wnt, Hippo signaling	PMID: 24106267, 38126343
BACE1	Regulatory Hydrolase, Protease	doi.org/10.1007/s00223-020-00796-y
BMPER	Controls BMP signaling	PMID: 30125619, 37311809, 38800044
CRYAB	Chondrogenic differentiation	PMID:16219039, 24239963, 38787373
DPYSL3	Filament organization, BMP target	PMID: 24583261
EVC2	Hedgehog signaling	PMID: 28950429, 28027321
FBLN5	Cell adhesion	PMID: 37608663
GFPT2	Osteogenic regulation	PMID: 36195245, 18405367
HMCN1	ECM component	PMID: 34504132, 30193837
IGFBP5	Igf signaling, ECM production	PMID: 17317255, 39193188, 16269403
LOX	Collagen crosslinking	PMID: 38472175
LRIG3	Regulate BMP signaling	PMID: 33469151, 35969315
MATN2	ECM organization	PMID: 12009334, 31963938, 17502381
MMP2	Osteocytic network formation	PMID: 16959767, 39257420, 35508470
NDN	Growth, transcription regulation	PMID: 28507020
OLFML2B	ECM organization	PMID: 35005449
P3H3	Collagen crosslinking	PMID: 38409111
PCOLCE2	Collagen synthesis pathway	PMID: 38049428
PENK	IL-10 modulator, bone modeling	PMID: 24496384, 34389038, 29122676
PLAT	Osteogenic regulation	PMID: 38407813, 38082497
POSTN	Cell adhesion	PMID: 29472541, 37362382, 39377227
SCARA3	Bone formation, stress response	PMID: 34254370
SCARF2	Cell adhesion	PMID: 20887961, 29882479
TSPAN10	Notch signaling	PMID: 24935633

Supplementary Table 1: Functional relevance of cMPC HO signature genes to bone formation.