Supplemental Tables

Supplemental Table S1 – Table summarizing the known clinical information for NBM/AML/NSM samples.

Supplemental Table S2 – List of differentially expressed genes for all annotated cell types. A Wilcoxon rank-sum test was used comparing normalized gene expression in each cell type against all other cell types, and the default 0.25 threshold of log2-fold change was applied.

Supplemental Table S3 – Results of the CellChat analysis in Figure 3. Each significant ligand-receptor pair is included with information including the source, target, significance, and the pathway the interaction falls into.

Supplemental Table S4 – Summary of the cell types detected using CODEX multiplexed imaging and what markers used to annotate the different cell types.

Supplemental Table S5 – List of antibodies used in the study, the vendors, clone information, and other relevant information about antibodies to allow for replication of our panel.

Supplemental Table S6 – Imaging parameters including the exposure times and cycle / channel assignment for each antibody in the panel.

Supplemental Table S7 – Comprehensive metrics for the structural analysis. Coefficient 1 is β_0 and correlated with population size, and coefficient 2 is β_1 and corresponds to the distance to the structure. P-value is derived from permutation testing on a per-sample basis. Both rank and normalized rank (1 is not proximal, 0 is the most proximal) are included. We computed the variability in distance as the interquartile range (75^{th} minus 25^{th} percentile of distance across cells measured in microns).

Supplemental Figures

Supplemental Figure 1 – Characteristics of our updated scRNA-Seq human bone marrow atlas.

A) A representative image of femoral head samples received as surgical waste from total hip arthroplasty surgeries prior to enzymatic digestion, showing that there is ample visible red marrow in many of the samples. The area immediately next to the areas collected for sequencing and histology analysis was subjected to microCT analysis at a 6-µm isotropic voxel size to show the normal trabecular bone structure (circle). **B)** Diagram showing femoral head enzymatic digestion protocol. **C)** Violin plots showing the final atlas distribution of unique expressed genes, sequencing depth (UMI counts, y-axis cut at 99th percentile), and percentage of reads mapping to the mitochondrial genome after filtering out low quality cells. **D)** UMAP showing the inflammatory response signature score of cells in the atlas. AUCell was used to calculate the gene set enrichment score for the GSEA Hallmark Inflammatory Response term. **E)** Normalized gene expression is projected onto UMAPs to visualize expression of key marker genes highlighting hematopoietic and non-hematopoietic cell types in the data – *CXCL12* – stromal, *NCAM1* – osteolineage, *CDH5* – endothelial, *PTPRC*- hematopoietic, *MZB1* – plasma cell, *CSF3R* – granulocyte. **F)** Comparison of stromal cell content in a major published bone marrow atlas compared to our study. The annotated bone marrow Seurat object from the Azimuth database was downloaded and the existing cluster annotations were visualized in UMAP space. The circled regions represent the non-hematopoietic bone marrow fractions captured in published data compared to our updated atlas designed to capture non-hematopoietic cells.

Supplemental Figure S2 – **Quality control, selected expression profiles, and frequencies of non-hematopoietic cell subsets.**
A) Violin plots showing the non-hematopoietic cell distribution of unique expressed genes, sequencing depth (UMI counts), and percentage of reads mapped to the mitochondrial genome. RNAlo MSCs were removed from subsequent analysis based on these metrics. **B)** Dotplot showing gene expression of additional canonical marker genes in bone marrow non-hematopoietic cells **C)** Day 28 micrographs of adipogenic, osteogenic and chondrogenic differentiation. Adipogenic differentiation was assessed by Oil Red O staining, osteogenic by Alizarin Red staining, and chondrogenic by Alcian blue staining. **D)** Day 14 qPCR comparing RNA expression following differentiation of cultured Fibro-MSCs using adipogenic or osteogenic medium. Control samples were taken from Day 0 of the differentiation. **E)** Stacked bar plot showing the per-sample frequency of MSC subpopulations. **F)** UMAP plots computed on just endothelial cells with overlaid expression of *PDPN, LYVE1,* and *PROX1*, which are markers of lymphatic endothelial cells. **G)** Stacked bar plots showing the per-sample frequency of endothelial cell types.

Supplemental Figure S3 – Signaling pathways comprising intercellular communication modules.
A) Gating strategy used to sort MSC subpopulations in Figure 2E-F. Sorting markers were selected based on scRNA-Seq. **B)** Violin plot showing normalized expression of genes coding for markers used in cell sorting to distinguish MSC subsets. **C)** UMAP plot of the entire scRNA-Seq atlas with overlaid normalized *CSF3* expression across all cell types in the atlas. **D)** Heatmap showing the contribution of each specific signaling pathway to the inferred cellular communication modules from Figure 3. Contribution scores were calculated as previously described in Jin et al., *Nature Communications*, 2021.

Supplemental Figure S4 – CODEX cell typing validation and cell phenotype maps.
A) CODEX images with selected markers and corresponding cell phenotype maps (CPMs) showing appropriate co-labeling of certain markers and associated cell phenotype maps with registered H&E images. Grey cell masks refer to all other segmented cells in the final analysis (imaging artifacts and Mast Cell Tryptase (MCT)+ autofluorescent cells removed). MCT is used

as a marker of autofluorescence in these images (See Methods). Further details on marker combinations used to define each cell type can be found in Supplemental Table S4. Registered H&E images of the exact imaged region is also provided. **B)** CODEX image of Podoplanin+ CXCL12+ Fibro-MSC corresponding to our scRNA-Seq data demonstrating that the rare cells are found in the region where the bone detached from the slide (boundary manually illustrated in grey, drawn based on H&E imagesand morphology). MCT is included as it labeled highly autofluorescent cells. **C)** CODEX image showing an Osteo-MSC and osteoblast at the bone boundary, where the bone detached from the slide (boundary manually illustrated in grey, drawn based on H&E staining and morphology). **D)** CODEX image of macrophages showing that CD163 better labels cytoplasmic projections but bone marrow macrophages label for both CD68 and CD163. MCT is included to label autofluorescent cells.

Supplemental Figure S5 – CODEX reveals a relatively hyperoxygenated peri-arteriolar/peri-endosteal niche.
A) Boxplot showing each neighborhood ranked by its proximity to manually annotated bone in each sample, and the normalized rank was computed for each comparison, such that a value of 0 means most proximal and 1 means least proximal. **B)** CODEX images shown with selected markers and paired neighborhood masks demonstrating the capturing of spatial organization of the tissue with respect to both hematopoietic and non-hematopoietic cell types. Neighborhoods with the same annotation (e.g. Erythroid 1 (CN13) and Erythroid 2 (CN15)) were combined for visualization purposes. **C)** Violin plots showing centered log ratio (CLR) HIF1a expression across all cell types.

Supplemental Figure S6 – Structural analysis annotations and result summary.
A) Paired CODEX images and overlaid masks with representative examples of the structural masks compared to the actual fluorescent images. The sinusoid and adipocyte annotations are shown in the same region of interest. **B)** Chord diagram showing only significant interactions ($p<0.05$, Stouffer's method for meta-analysis of permutation test p-values across all 12 samples), chord thickness is proportional to the negative $\log_{10}$ of the median normalized rank + 0.000000000001.

Supplemental Figure S7 – CODEX enables classification and downstream analysis of *NPM1* mutant blasts.
A) Violin plot of CODEX CLR-normalized protein expression showing the non-specific expression of the mutant-specific NPM1C antibody in negative staging marrow samples. **B)** CODEX images showing that CD141, but not early myeloid or erythroid markers (MPO and GATA1) almost completely co-stains with mutant-specific NPM1C in a representative negative staging marrow sample, showing that exclusion of these CD141+ cells enables use of the antibody to identify true mutant cells. **C)** Chart showing the detected NPM1c mutant percentage in each sample compared to the clinical variant allele frequency for NPM1c. **D)** CODEX fluorescent image of a diagnostic AML sample showing MSCs and *NPM1* mutant blasts. White arrows point to FOXC1+ CD271+ MSCs. **E)** Boxplot showing the relative proximity to bone (normalized rank by AML/NSM neighborhood, where 0 is the closest in a sample and 1 is the furthest), with the leukemic neighborhoods highlighted in magenta and CN7, the neighborhood corresponding to the healthy early myeloid progenitor niche, highlighted in blue. All other neighborhoods are green. **F)** Violin plots of myeloid cell types in AML and negative staging marrow samples showing that *NPM1* mutant blasts have low HIF1a levels consistent with an early myeloid progenitor phenotype. **G-H)** BCL2 and Complex IV expression levels in *NPM1* mutant blasts. Wilcox Rank Sum test was performed in Seurat to compare between diagnostic samples and post-therapy samples.