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Supplemental Information

comBO: A combined human bone and lympho-myeloid bone marrow organoid for preclinical modeling of hematopoietic disorders

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Figure S1

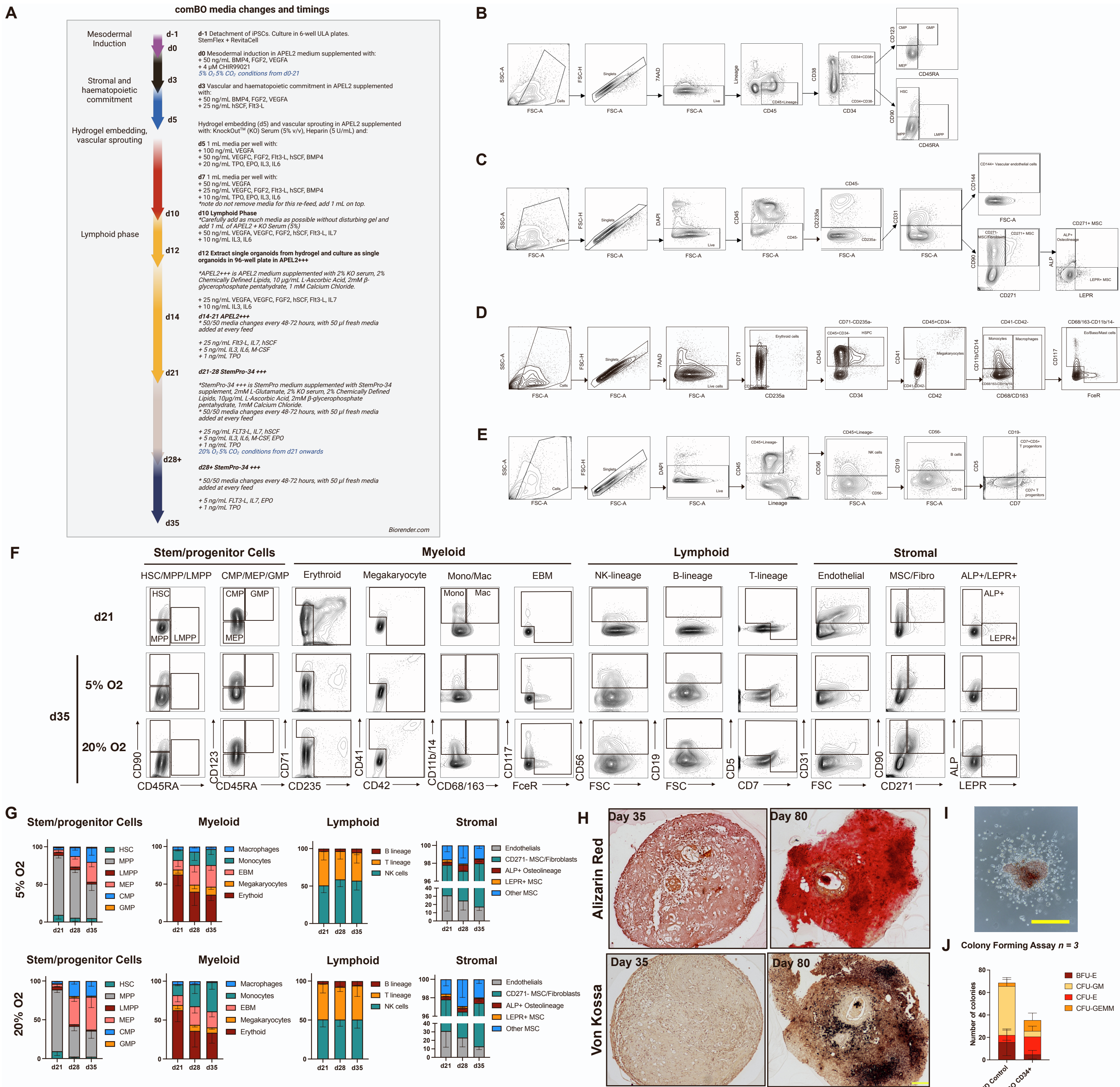

Figure S1. Complete flow cytometry data and supplementary validation for Figure 1, related to STAR Methods.

(A) Schematic of differentiation workflow and timeline to generate comBO.

(B) Gating strategy for HSPC panel. CD45+ lineage- haematopoietic cells are gated from live singlets. Then CD34+ CD38- cells are classified as HSCs (CD90+ CD45RA-), MPPs (CD90- CD45RA-), or LMPPs (CD90- CD45RA+) based on their expression of CD90 and CD45RA. CD34+ CD38+ cells are classified as CMPs (CD123+ CD45RA-), MEPs (CD123- CD45RA-), or GMPs (CD123+ CD45RA+) based on their expression of CD123 and CD45RA.

(C) Gating strategy for myeloid panel. After gating live singlets, erythroid cells are identified as CD71hi CD235hi. Then CD45+ CD34+ cells are classified as HSPCs. From CD45+ CD34- haematopoietic cells, megakaryocytes are identified as CD41+ CD42+ cells; macrophages as CD41- CD42- CD68/CD163+ cells; myelomonocytic cells as CD41- CD42- CD68/CD163- CD11b/CD14+ cells. The remaining negative population is further characterised to identify CD117/FceR+ cells as Eo/Baso/Mast cells.

(D) Gating strategy for lymphoid panel. CD45+ lineage- haematopoietic cells are gated from live singlets. Then CD56+ cells are identified as NK cells, and CD56- cells are further analysed to identify B cells based on the expression of

CD19. The CD19- cells are then used to identify T lineage cells. CD7+ CD5- cells as CD7+ T progenitors and CD7+ CD5+ cells as CD7+ CD5+ T progenitors.
(E) Gating strategy for stromal panel. From live singlets, stromal cells are identified from non-erythroid (CD235a-) and non-haematopoietic (CD45-) cells. Endothelial cells are identified as CD31+, with a subset further defined as CD144+ vascular endothelial cells. MSCs/fibroblasts are identified as CD31- CD90+ CD271-, while CD31- CD90+ CD271+ cells as classified as MSCs. From the CD271+ MSCs, osteolineage cells are identified as ALP+ and LEPR+ MSCs are identified as LEPR+.
(F) Representative flow cytometry plots at dat 21 and day 35 of comBO cultures maintained at either 5% or 20% oxygen from day 21-day 35.
(G) Quantification of cell fractions at day 35 in either 5% or 20% oxygen between days 21 and day 35. No significant differences in populations are identified across conditions.
(H) Assessment of mineralization of organoids at day 35 and 80 of cultures. Both alizarin red and von kossa staining confirm extensive mineralisation at day 80 of culture. Scale bar = 200 μm.
(I) Representative image of a colony forming unit. Scale bar = 400 μm.
(J) Quantification of the number of colonies detected at day 14 of the methocult assay. While colonies were produced from comBO cells, they were notably fewer in number when compared to primary CD34+ healthy donors.

Figure S2

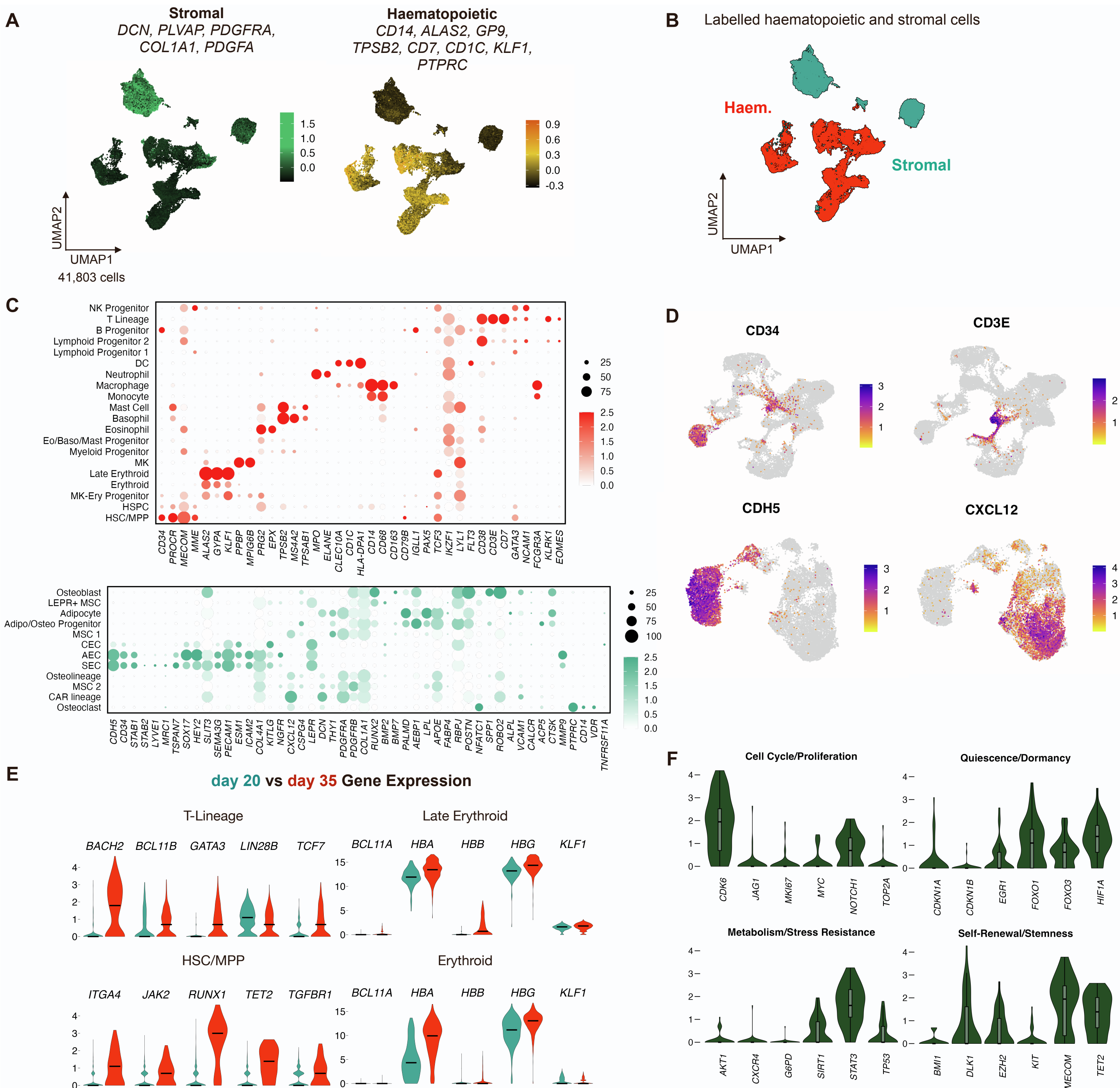

Figure S2. scRNA-seq analyses of comBO, related to Figure 1.
(A) Total integrated day 20 single cell RNA sequencing data was first annotated into stromal and haematopoietic cells on the basis of canonical gene scoring (*DCN, PLVAP, PDGFRA, COL1A1, PDGFA)* for stromal cells and *CD14, ALAS2, GP9, TPSB2, CD7, CD1C, KLF1, PTPRC* for haematopoietic cells).

(B) Objects were then subsetted on the basis of either stromal or haematopoietic lineages before further sub clustering and analysis.

(C) Haematopoietic cells included HSC/MPP (*CD34, PROCR, MECOM, MME*), HSPC/Lympho-Myeloid progenitors (low *CD34, PROCR, MECOM, CD79B, PAX5, GATA3, PRG2, TPSB2*), Erythroid lineage (*ALAS2, GYPA, KLF1, GFI1B*), MK lineage (*GP9, PF4, PPBP, MPIG6B*), Eo/Baso/Mast (*GATA2*, *HDC*, *PRG2, TPSB2*), DC (*CD1C, HLA-DPA1*), monocyte/macrophage (*CD14, CD68, CD163*), B-progenitor (*CD79B, IGLL1*), neutrophil (*MPO, ELANE*) and T-lineage (*CD3E, CD7*) clusters were identified. Stromal cells included: Pan-endothelial genes *CDH5* and *PECAM1*, sinusoidal endothelial cells were identified by a cluster specific expression of *STAB2, LYVE1, TSPAN7* and *GGT5*. Arterial endothelial cells were separated by the lack of sinusoidal markers, and a high expression of *SOX17*. Capillary like endothelium were identified by high *KITLG*, *ESM1* expression and a lack of sinusoidal endothelial markers. MSCs

were labelled with a canonical expression of MSC markers (e.g. *NGFR, CXCL12*), fibroblasts were separated by high *DCN* expression. Adipocytes were separated by a high expression of *AEBP1, PALMD, LPL, and APOE*. Osteoblasts were identified by a high expression of *RUNX2, POSTN, NFATC1, SPP1, ROBO2, MSX2 and VCAM1.*

(D) Feature plots of key haematopoietic gene sets *CD3E* (T progenitor) and*CD34* (HSC/MPP). Feature plots of key stromal gene sets *CDH5* (Endothelial), *CXCL12* (CAR and other MSC) compartment is comprised of much more extensive range of stromal cells, a heterogenous vasculature, and a comBO specific osteoblast population.

(E) A comparison of adult gene expression in key populations in day 20 and day 35 comBO. A notable increase in T-lineage markers of adult transcriptomic profiles is observed (*BACH2, BCL11B, GATA3, TCF7*) as welll as a reduction in the fetal marker *LIN28B.* HSC/MPPs demonstrated an increase in *ITGA4, JAK2, RUNX1, TET2, TGFBR1*, and erythroid cells demonstrate an increase in adult haemoglobin (*HBB)* at the late erythroid stage. The mix of *HBA* and *HBG* expression indicates a mixed fetal and adult transcriptome.

(F) Further analysis of the HSC/MPP compartment indicates a decreased expression of cell cycle/proliferation markers (e.g, *TOP2A, MKI67*), the expression of markers of quiescence (*e.g. FOXO1/3, HIF1A*), and markers of self-renewal/stemness (e.g. *MECOM, TET2, DLK1*). Gene set enrichment analysis comparing day 20 and day 30 data against gene sets characterising adult, fetal, and diseased HSC/MPPs reported a significant up regulation in adult HSC profiles (*e.g.* Eppert et al. Drissen *et al.*) and hallmark quiescence gene profiles. Together this data indicates a trend towards adult gene expression in the comBO system. A methocult assay was performed to assess the stem cell potential of CD34+ cells produced from the comBO. Cells were sorted on day 21 of differentiation (Live + CD34+ CD45+) and seeded in MethoCult at a density of 1,500 cells per well. In parallel, healthy donor CD34+ cells were similarly sorted and plated at an identical density. In total, 3 independent differentiations and healthy donors were used for this assay, with data summarised here.

Figure S3

Figure S3. Comparison of comBO single cell data with reference data sets and existing bone marrow organoid systems, related to Figure 1.
(A) (i) UMAP of a recently published adult bone marrow atlas (Bandyopadhay *et al.*), and projection mapping of comBO similarity scores after integration (analysis detailed in STAR Methods). (ii) UMAP of a fetal bone marrow atlas (Jardine *et al.*), and projection mapping of comBO similarity scores after integration. comBO similarity scores are higher when compared to the adult atlas than when compared to fetal data.
(B-C) Comparison of projection and projection scores for comBO, the Khan *et al.* (abbreviated to BMO), and the Frenz-Weissner *et al.* (Weiss) vs. adult and fetal bone marrow. comBO shows a higher degree of similarity (higher projection

scores) in comparisons to adult bone marrow, suggesting a trend towards adult-like gene expression and indicating that comBO data much more closely resembles native haematopoietic tissue, with an increased and quantifiable similarity to adult bone marrow.

(D) The expression of key (i) haematopoietic (ii) stromal and endothelial markers and hallmark bone marrow (iii) key ligand-receptor pairs and chemokines across data sets. Compared to Khan *et al.* (BMO) and Frenz-Weissner *et al.* (Weiss), comBO features a broader range of hallmark bone marrow haematopoeitic lineages. When integrated and compared to a bone marrow atlas dataset, comBO demonstrates improved expression of key stromal markers (e.g. *LEPR, CXCL12, RUNX2, POSTN, CTSK)* and ligand-receptor pairs/chemokines (e.g. *IL7*, *CXCR4, KLL1, TGFB1, VCAM1*) facilitating the improved, adult like haematopoiesis reported.

Figure S4

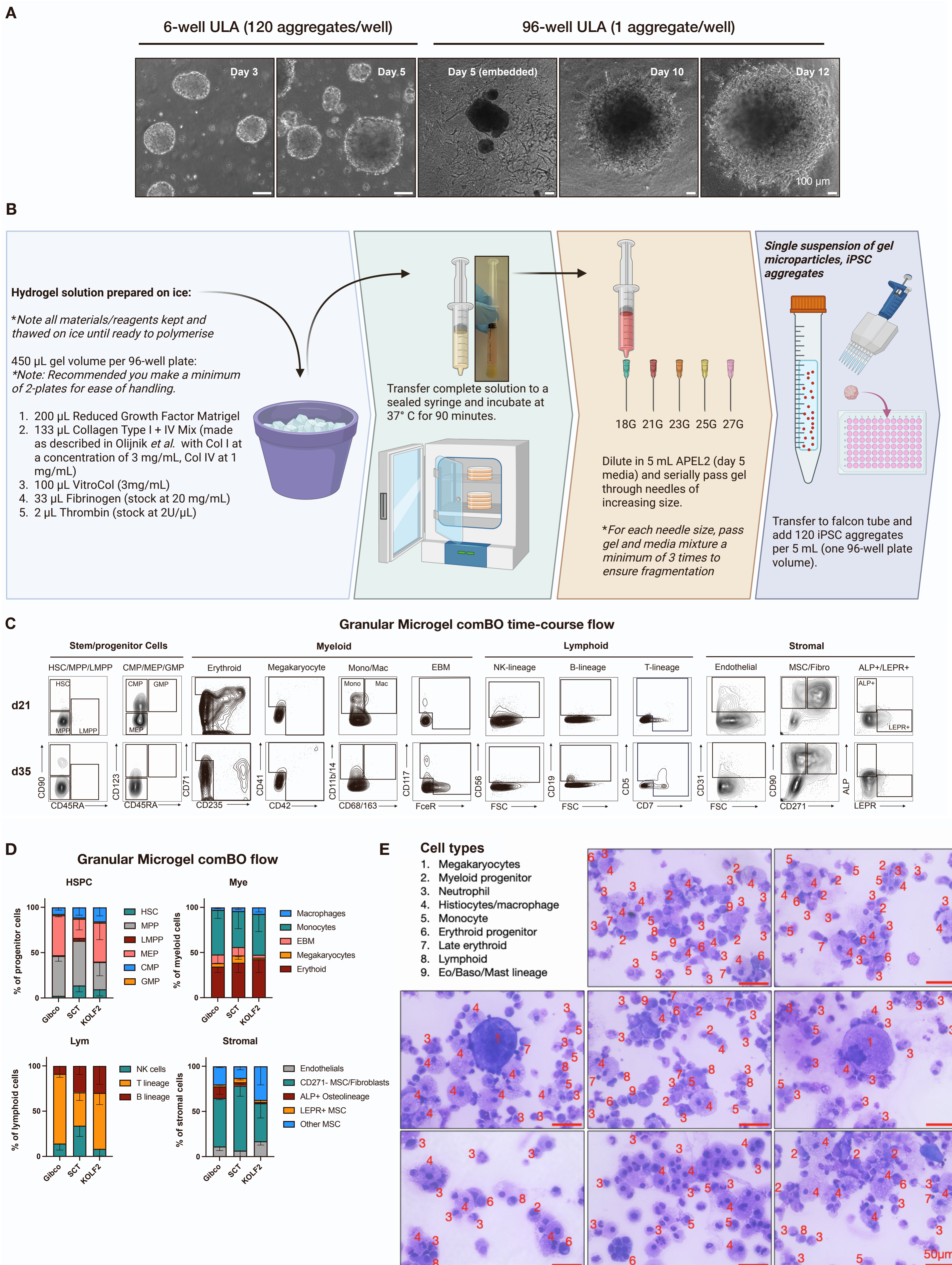

Figure S4. Details of comBO generation using a granular microgel method, related to Figure 2 and STAR Methods.

(A) Images of hiPSC aggregates in a 6-well ULA at day 3 and day 5 before embedding in a microgel, and images of single well at day 5, day 10 and day 12 post embedding.

(B) A step wise process to generate microgel on day 5. First a hydrogel solution is prepared on ice (as detailed in the diagram), before this solution is transferred to a sterile, sealed syringe. After incubation for 90 minutes, the fully polymerised hydrogel is diluted in 5 mL of media (APEL2 supplemented with cytokines, knock out serum) and then passed serially through needles of increasing gauges to fragment the bulk gel and produce a suspension. Finally, the granular micro gel is transferred to a falcon tube, day 5 hiPSC aggregates are added to said tube, and then redistributed to a 96-well plate (50 µL volume per well). Plates are centrifuged (300G, 3min, room temperature) to compact the micro gels, and organoids returned to routine cell culture incubation. Schematic created in Biorender.com.

(C) Representative flow plots at day 21 and day 35 of comBO differentiation, demonstrating the differentiation of distinct lineages. B-lineage cells notably acquire an increase in CD19 between day 21 and day 35, while osteolineage cells for example, demonstrate an increase in ALP expression.

(D) The comBO approach was applied across 3x distinct hiPSC lines showing a comparable generation of all lineages across each of the lines tested. Data is collated from 3x separate differentiations as 3x distinct biological repeats.

(E) Cytospins of comBO-derived cells. comBOs were cultured under gentle agitation to encourage the egress of haematopoietic cells. Released haematopoietic cells were collected and prepared using cytospin and Wright's staining. Cells were then imaged and annotated as shown here. A range of cell types at different stages of maturation are observed, including myeloid and lymphoid cells as indicated.

Figure S5

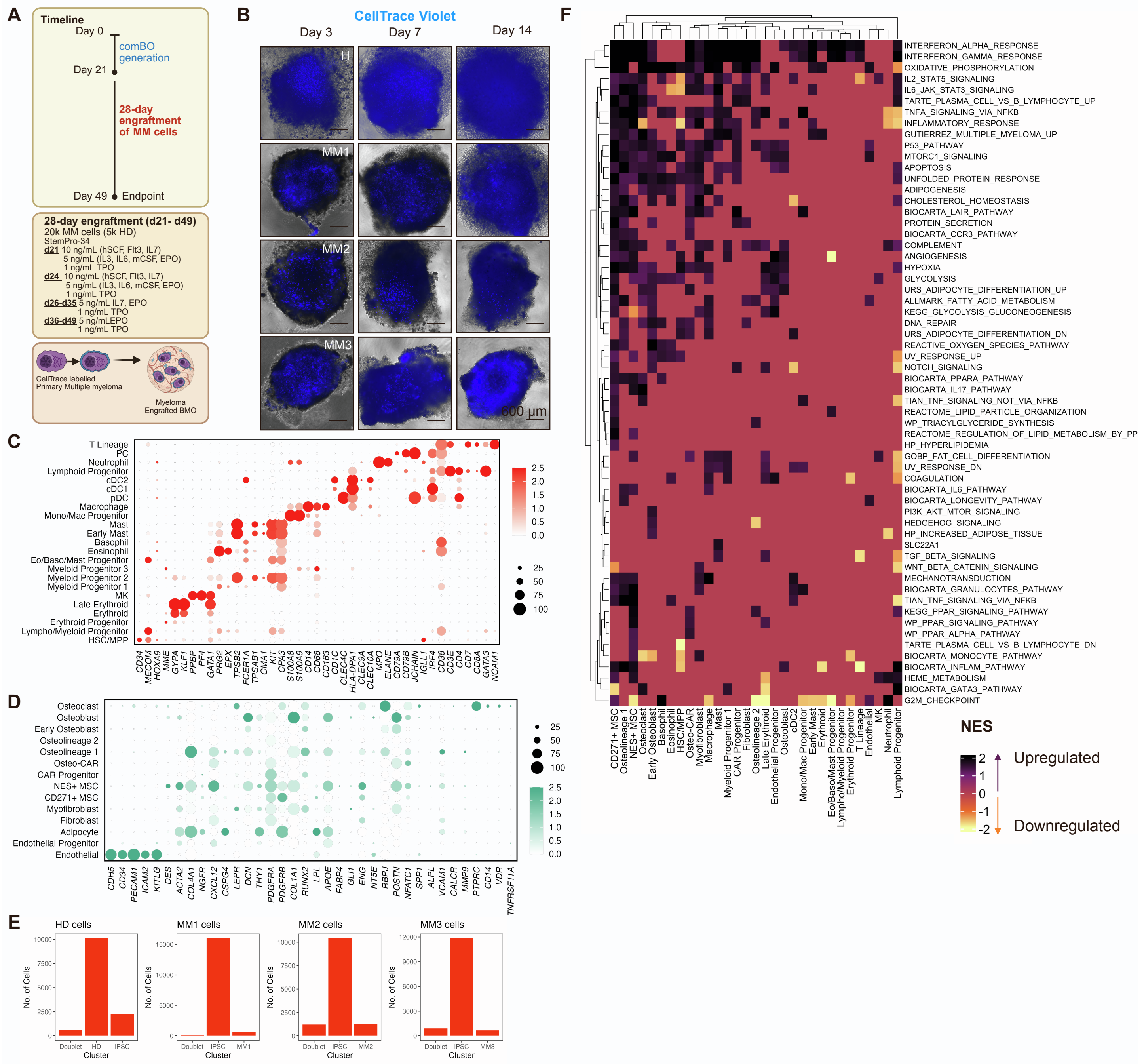

Figure S5. Details of engrafted primary multiple myeloma comBO (MM-comBO) samples, related to Figure 4.
(A) A workflow was devised whereby comBOs were generated using the microgel approach described and engrafted with CD138+ multiple myeloma cells (patients detailed in Supplementary Table 2). Patient cells were engrafted at 20,000 cells per well at day 21 and cultured for a further 2-weeks under cytokine conditions as detailed.
(B) Myeloma cells were engrafted in parallel to healthy donor CD34+ cells and CellTrace labelled to track engraftment and proliferation over time. As expected, between days 3 and 14, healthy donor cells demonstrate a marked dilution of CellTrace consistent with proliferation and differentiation. Myeloma cells across all patients (MM1-MM3) demonstrate effective engraftment, but varying degrees of CellTrace dilution. Summary single cell data of unengrafted, control CD34+ healthy donor cells, and multiple myeloma engrafted comBOs (*n* = 3).
(**C)** In total 45,706 cells were analysed by single cell RNA sequencing, and annotated as haematopoietic and defined by canonical gene expression.
(D) 16,706 stromal cells were collected post-processing, and annotated by canonical gene expression.
(E) Specific genotypes for engrafted samples were identified using souporcell.
(F) A clustered heatmap of all significantly enriched gene sets in MM-comBO single cell RNA sequencing data (Normalised Enrichment Scores (NES)). Differential gene expression and gene set enrichment analysis were performed on an integrated control and multiple myeloma patient engrafted samples. CD271+ MSC, Osteolineage1, NES+MSC, osteoblasts, and osteoclasts cluster distinctly demonstrating dysregulation in interferon, *IL2, IL6, TNF*, and adipogenesis/fatty acid metabolism gene sets.

Figure S6

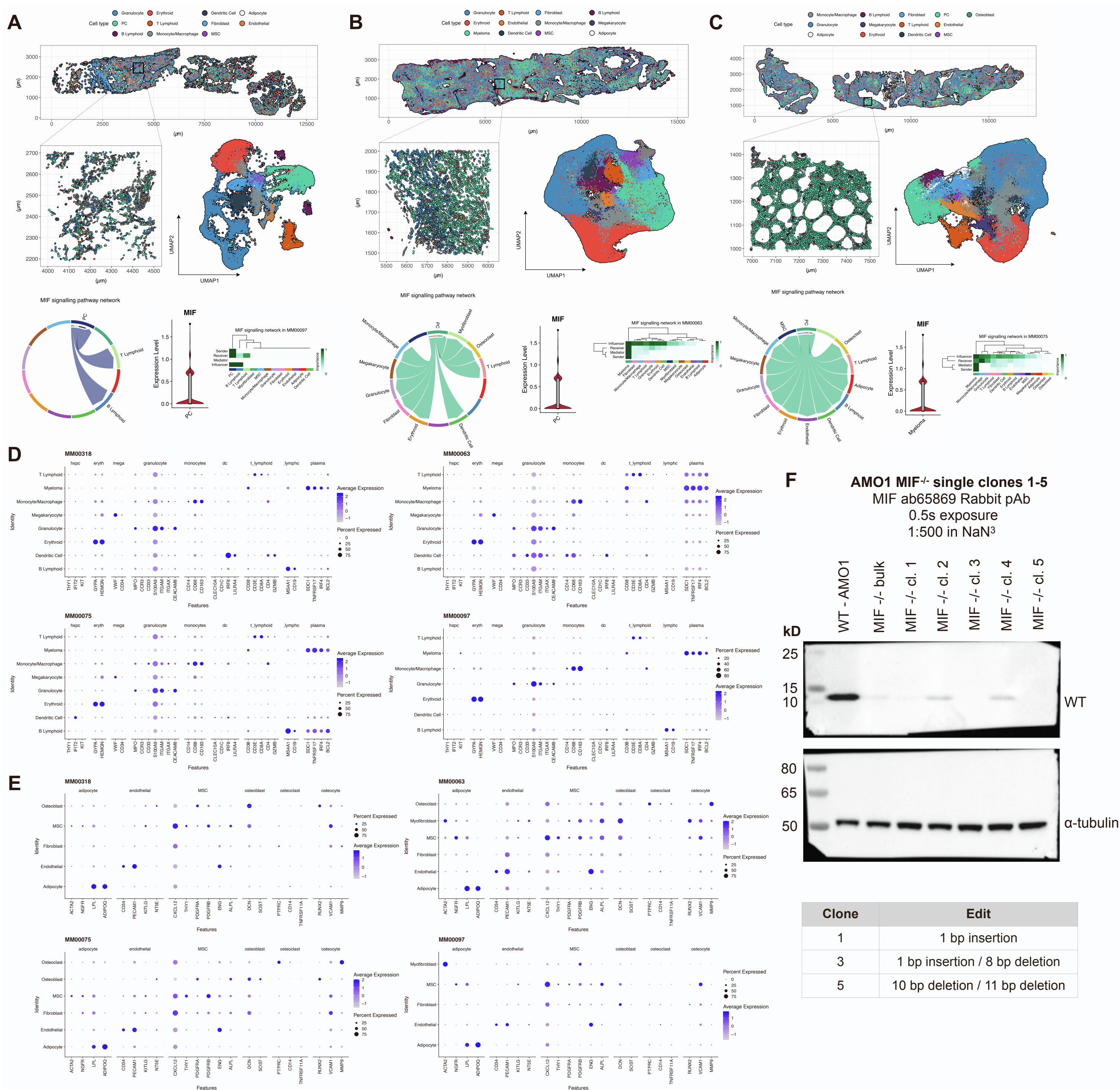

Figure S6. Xenium spatial transcriptomic assessment of 3 additional multiple myeloma patients (patient details in Table S2) and generation of MIF KO lines, related to Figure 6.

(A-C) Images, UMAP, and MIF signalling pathways shown per patient, with canonical gene expression.

(D-E) highlighting basis of cluster annotation.

(F) (Top) Validation of MIF KO in AMO-1 cell lines using western blotting. (Bottom) Interpretation of MIF KO clone genotypes by TIDE analysis of sequencing data.

Table S1. Flow cytometry panels, related to Figures 1-3, Figure S1, Figure S4, and STAR Methods.

HSPC panel

Antibody	Color	Clone	Manufacturer	Catalog #
CD45	APC-ef780	2D1	BioLegend	368516
CD34	BV650	561	BioLegend	343624
CD38	PE-Dazzle 594	HIT2	BioLegend	303538
CD45RA	BV570	HI100	BioLegend	304132
CD90	BV711	5E+10	BD Biosciences	740786
CD123	PE-Cy7	6H6	eBioscience	25-1239-42
Lineage (CD3, CD11b, CD14, CD19, CD20, CD56, CD235)				
CD3	PerCP-Cy5.5	UCHT1	Biolegend	300430
CD11b	PerCP-Cy5.5	ICRF44	Biolegend	301328
CD14	PerCP-Cy5.5	M5E2	Biolegend	301824
CD19	PerCP-Cy5.5	HIB19	Biolegend	302230
CD20	PerCP-Cy5.5	2H7	Biolegend	302326
CD56	PerCP-Cy5.5	5.1H11	Biolegend	362506
CD235ab	PerCP-Cy5.5	HIR2	Biolegend	306614
7AAD			Cayman chemical	11397

Myeloid panel

Antibody	Color	Clone	Manufacturer	Catalog #
CD45	APC-ef780	2D1	BioLegend	368516
CD34	BV650	561	BioLegend	343624
CD11b	AF700	ICRF44	BioLegend	301355
CD14	AF700	63D3	BioLegend	367113
CD117 (c-kit)	BV711	104D2	BioLegend	313230
FcεRIα	APC	AER-37 (CRA-1)	BioLegend	334612
CD41	FITC	HIP8	BioLegend	303704
CD42a	PE	ALMA.16	BD Biosciences	558819
CD71	BV510	L01.1	BD Biosciences	744926
CD235ab	PE-Cy7	HIR2	BioLegend	306620
Lineage (CD3, 10, 19, 56)				
CD3	PerCP-Cy5.5	UCHT1	Biolegend	300430
CD10	PerCP-Cy5.5	HI10a	Biolegend	312216
CD19	PerCP-Cy5.5	HIB19	Biolegend	302230
CD56	PerCP-Cy5.5	5.1H11	Biolegend	362506
7AAD			Cayman chemical	11397

Lymphoid panel

Antibody	Color	Clone	Manufacturer	Catalog #
CD45	APC-ef780	2D1	BioLegend	368516
CD34	BV650	561	BioLegend	343624
CD5	PE	UCHT2	Biolegend	300608
CD7	PE-Cy5	CD7-6B7	Biolegend	343110
CD19	APC	HIB19	Biolegend	302212
CD56	BV605	HCD56	Biolegend	318334
Lineage (CD11b, CD14, CD235)				
CD11b	PerCP-Cy5.5	ICRF44	Biolegend	301328
CD14	PerCP-Cy5.5	M5E2	Biolegend	301824
CD235ab	PerCP-Cy5.5	HIR2	Biolegend	306614
DAPI			Life Technologies	D3571

Stromal panel

Antibody	Color	Clone	Manufacturer	Catalog #
CD271	BUV395	C40-1457	BD Biosciences	743362
CD235	PE-Cy7	HIR2	BioLegend	306620
CD45	FITC	2D1	BioLegend	368508
CD31	APC-Cy7	WM59	BioLegend	303120
CD144	PerCP-Cy5.5	BV9	BioLegend	348510
CD90	PE-Cy5	5E+10	BioLegend	328112
ALP	BUV737	B4-78	BD Biosciences	748692
LEPR (CD295)	AF647	52263	BD Biosciences	564376
DAPI			Life Technologies	D3571

ATO readout panel

Antibody	Color	Clone	Manufacturer	Catalog #
CD3	BUV395	UCHT1	BD Biosciences	563546
CD4	BUV805	SK3	BD Horizon	612937
CD5	PE	UCHT2	Biolegend	300608
CD7	FITC	CD7-6B7	Biolegend	343103
CD8	PE-Cy7	SK1	Biolegend	344748
TCR α/β	Pacific Blue	IP26	Biolegend	306715
Lineage (CD14, CD19, CD56)				
CD14	PerCP-Cy5.5	M5E2	Biolegend	301824
CD19	PerCP-Cy5.5	HIB19	Biolegend	302230
CD56	PerCP-Cy5.5	5.1H11	Biolegend	362506
Propidium iodide			Sigma-Aldrich	P4864

Flow panel for myeloma cell characterisation in the engrafted organoids

Antibody	Color	Clone	Manufacturer	Catalog #
mScarlet organoids				
Cell Trace Violet myeloma cells				
CD38	BV605	HIT2	BioLegend	303538
CD138	BV711	MI15	BD Biosciences	563184
CD56	FITC	5.1H11	BioLegend	362546
7AAD			Cayman chemical	11397

Table S2

ID	Sex, Age	Clonal Ig Type	ISS	FISH-determined chromosomal aberrations	ND/RRMM	Prior Lines of treatment		Expt. Use
MM1	M, 68	IgAk	NK	1q gain	RRMM (1st relapse)	1	VTd	scRNAseq
MM2	F, 75	IgAl	II	1q gain t(11;14)	RRMM (1st relapse)	1	VCd	
MM3	M, 66	IgAk	III	1q gain loss of *IGH* near-tetraploidy	ND	-	NA	
MM4	M, 68	IgAk	NK	1q gain	RRMM (1st relapse)	1	VTd	Inhibitor experiments (Luminex assays)
MM5	M, 75	IgGk	NK	1q gain 1p loss 17p loss (sub clonal @14%)	RRMM (4th relapse)	1 2 3 4	CTd + auto + R-Vorinostat (Myeloma XI trial) KCd (MUK 5 trial) VCd Pomd	
MM6	M, 77	LLC	NK	1q gain 17p loss Loss of *IGH*	RRMM (5th relapse)	1 2 3 4 5	CTd + auto VCd R Id Dd	
MM7	M, 59	IgGk	NK	1q gain 17p loss *IGH* rearrangement with unknown partner	RRMM	1 2 3 4 5 6 7	VCd + auto IRd Cp D Pomd CC92480 (trial) DTPACE	Xenium
MM8	M, 62	LLC	I	Unknown	RRMM	1 2 3 4 5 6	VCd CTd + auto IRd D Pomd CC92480	
MM9	M, 71	IgAk	II	1q gain	ND	-	NA	
MM10	M, 78	IgGk	I	Failed	ND	-	NA	
MM11	M, 68	IgAk	2	1q21 gain 11q13 gain	RRMM	1 2 3 4 5 6 7	4xKCD and ASCT VTD DVD IRD Vel/Pano/Dex Modakafusp trial CA057-003 trial; arm C dose level 3 ;oral Mezigdomide and Trametinib	Inhibitor experiments (MIF cell cycling and viability)
MM12	M, 42	KLC	1	t(11:14) near tetraploidy	ND	-	NA	
MM13	F, 81	IgGk	2	1q21 gain 11q13 gain	RRMM§	1 2	VCD DVD	

Table S2. Clinical details of patients who donated cells or tissues used in experiments reported, related to Figures 4-6, Figures S5-S6, and STAR Methods.

Ig: immunoglobulin. ISS: International staging system for myeloma. FISH: Fluorescence in situ hybridisation. ND: newly diagnosed myeloma. RRMM: relapsed/refractory myeloma.

Myeloma clonal Ig types: IgA/G, k/l: myeloma expresses immunoglobulin A or G respectively, kappa or lambda light chain. LLC: lambda light chain myeloma.

NK: Not known. NA: Not applicable. *IGH:* immunoglobulin heavy chain gene.

Treatment regimens: VTd: velcade thalidomide dexamethasone; VCd: valcade cyclophosphamide dexamethasone; CTd: cyclophosphamide, thalidomide dexamethasone; auto: high dose melphalan autologous stem cell transplantation; R-vorinostat: lenalidomide, vorinostat; KCd: Carfilzomib, cyclophosphamide, dexamethasone; Pomd: pomalidomide, dexamethasone; R: lenalidomide; Id: Ixazomib, dexamethasone; Dd: Daratumumab, dexamethasone; IRd: Ixazomib, lenalidomide, dexamethasone; Cp: cyclophosphamide, prednisolone; D: daratumumab; DTPACE: dexamethasone, thalidomide, cisplatin, doxorubicin, cyclophosphamide, etoposide.