

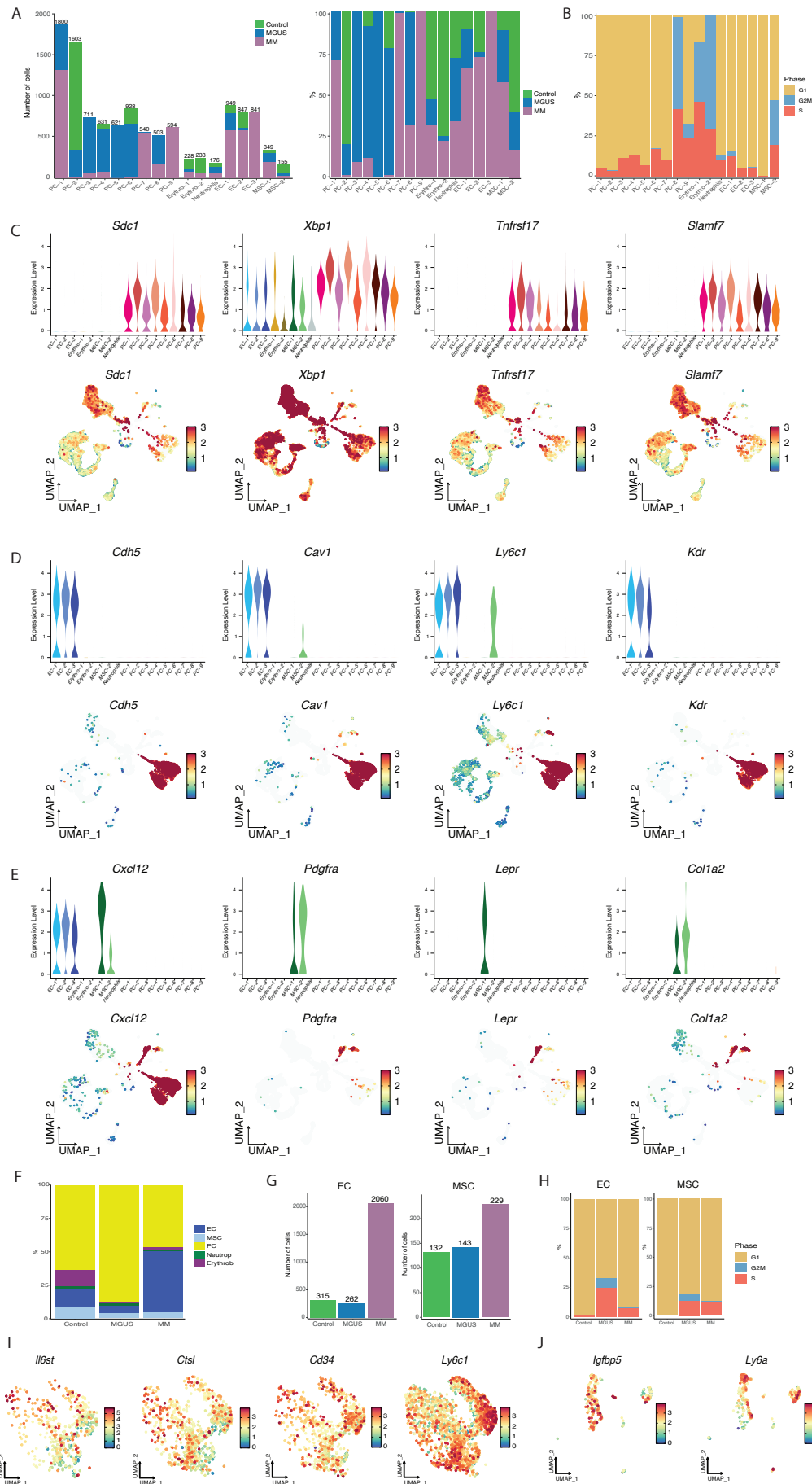
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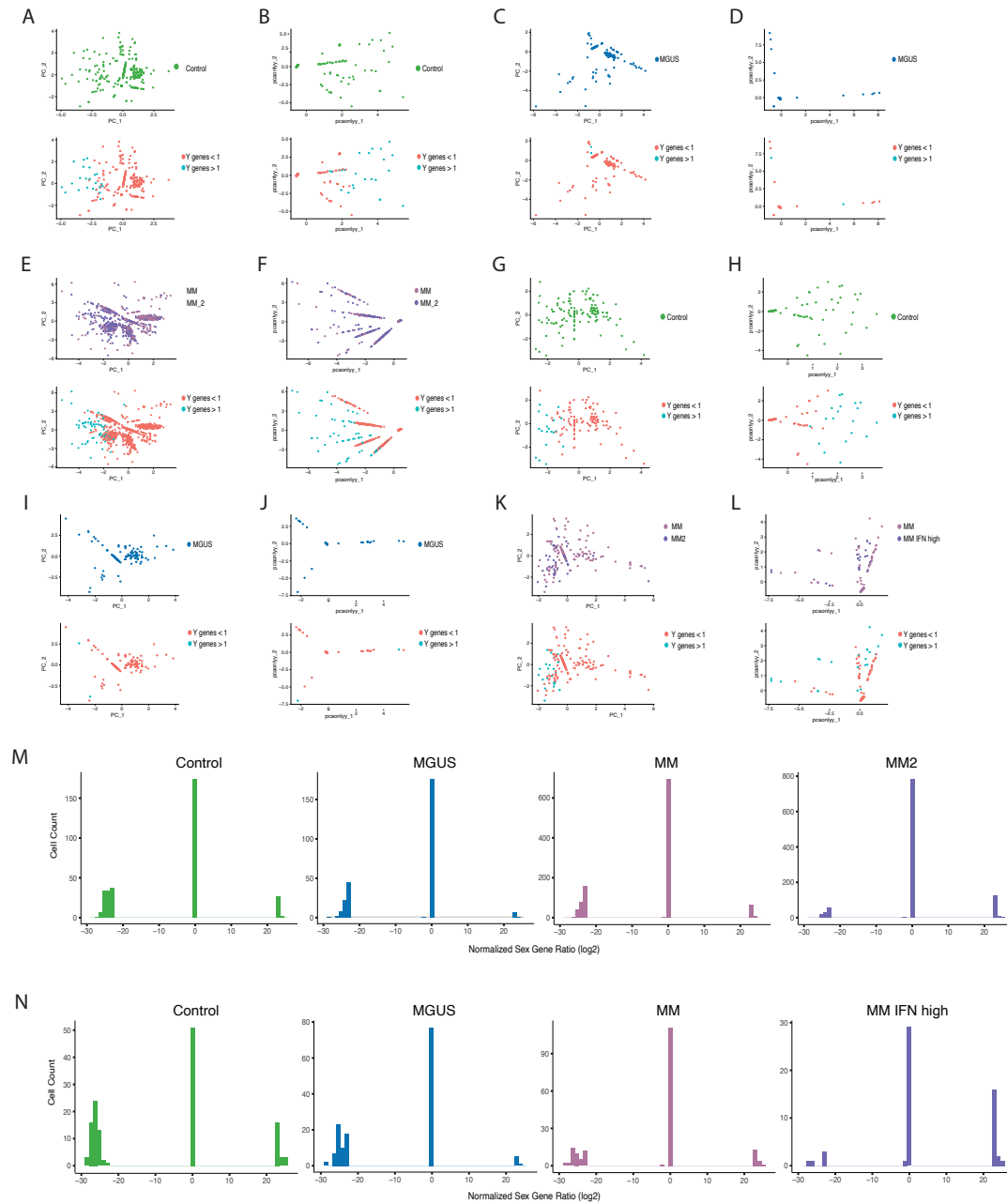
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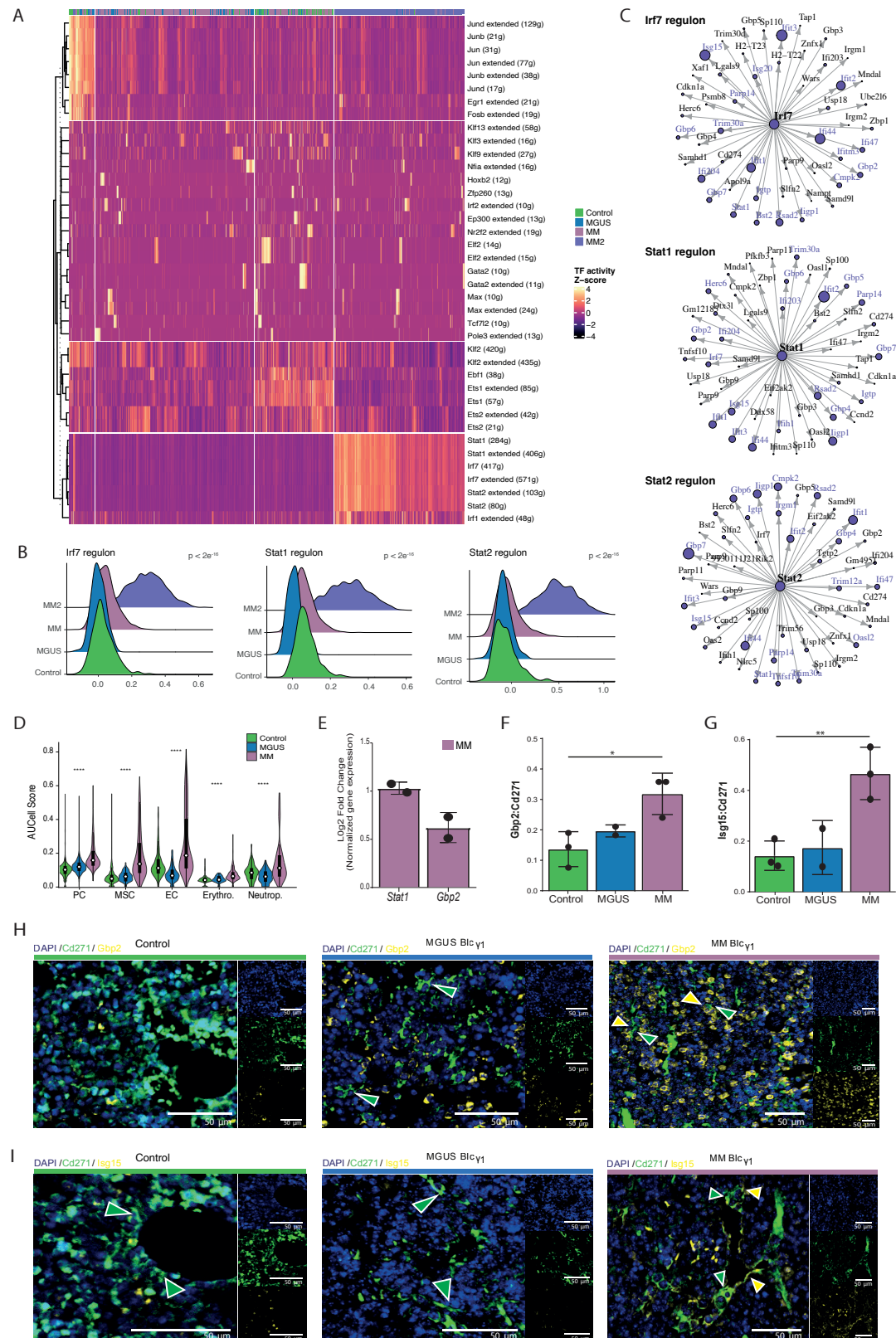


Supplementary Figure 2: scRNA-seq analysis of murine BM cells. (A) Left panel: bar plots showing the number of cells per cluster and dataset; Right panel: stacked bar plot representing the proportion of cells per cluster and dataset. (B) Stacked bar plot of the proportion of cells per cycle stage in each cluster. (C) Upper panel: Violin plot of gene expression of well-known markers for PC. Bottom panel: UMAP visualization of representative canonical markers for PC. (D-E) Similar to C for EC (D) and MSC (E). (F) Stacked bar plot of the proportion of BM cell populations per disease dataset. (G) Bar plot depicting the total number of cells per stage for EC (left) and MSC (right) after final filtering (see methods). (H) Stacked bar plot of the proportion of cells per cycle stage in each stage for EC (left) and MSC (right). (I) UMAP visualization of markers for sinusoids (*Il6st*, *Ctsl*) and arteries (*Cd34*, *Ly6c1*) in EC. (J) UMAP visualization of MSC canonical markers in MSC.

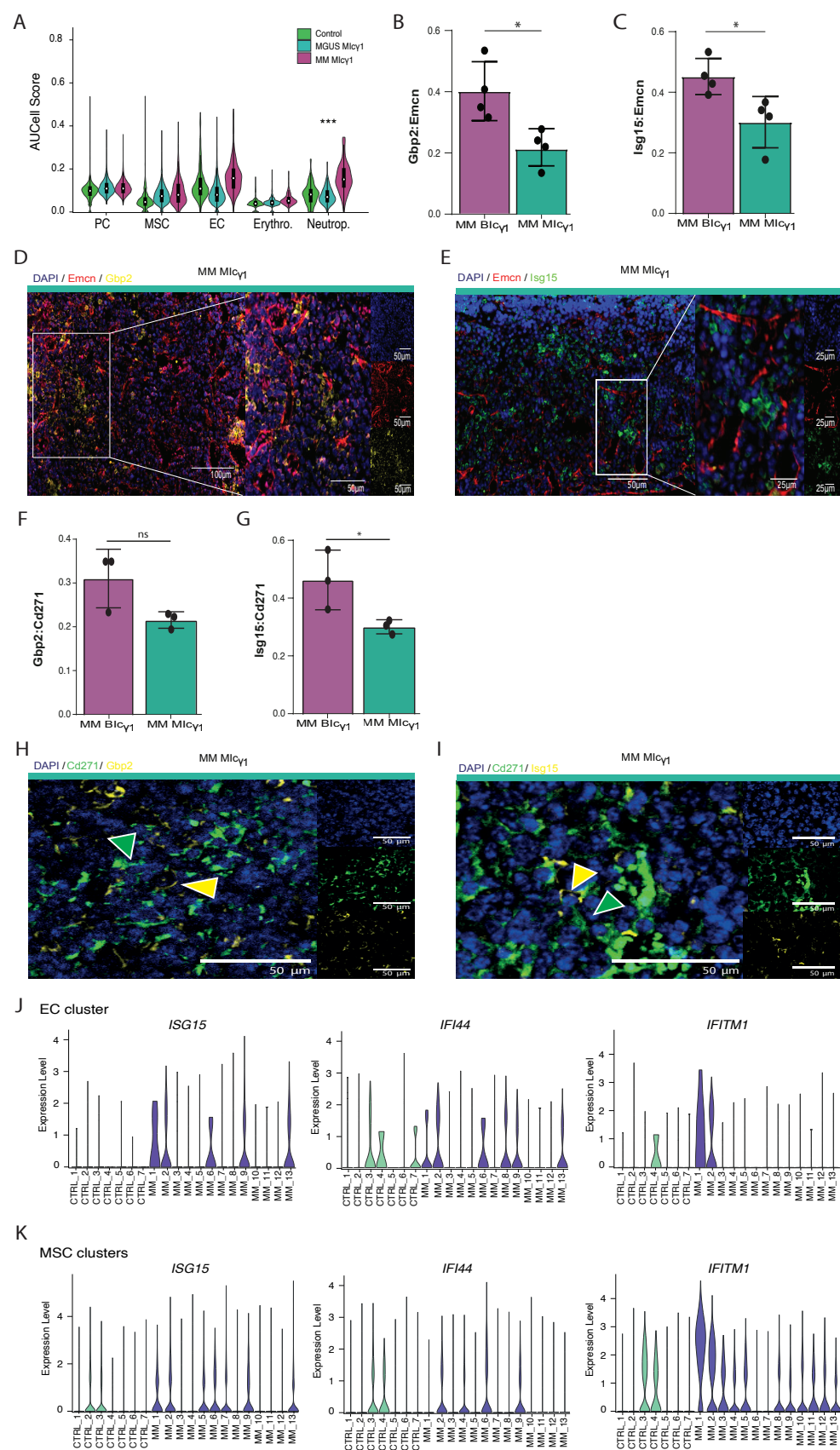


Supplementary Figure 3: Analysis of mouse contribution to control, MGUS, and MM BL_{c71} EC and MSC using sex-specific genes. (A) PCA plot showing separation of control EC based only on the expression of sex-specific genes colored by disease (upper panel) and Y-chromosome gene expression, where blue denotes cells with more than one count in Y-chromosome genes (bottom panel). (B) PCA plot showing separation of control EC based only on the expression of Y-chromosome genes colored by disease (upper panel) and Y-chromosome gene expression, where blue denotes cells with more than one count in Y-chromosome genes (bottom panel). (C,D) Similar to A,B for MGUS EC. (E,F) Similar to A,B for MM EC. MM EC cells were split into

27 MM and MM2 clusters. (G,H) Similar to A,B for Control MSC. (I,J) Similar to A,B for MGUS
28 MSC. (K,L) Similar to A,B for MM MSC. MM MSC cells were split into MM IFN low and high
29 score. (M) Histogram of number of EC plotted by male to female gene ratios (scored continuously
30 along the x-axis) in control, MGUS, and MM BI_{cyl} EC. MM EC cells were split into MM and
31 MM2 clusters. Three discrete peaks emerge, representing cells likely originating from either sex
32 or those with indeterminate ratios due to technical drop-off in scRNA-seq reads. (N) Similar to
33 M for MSC.

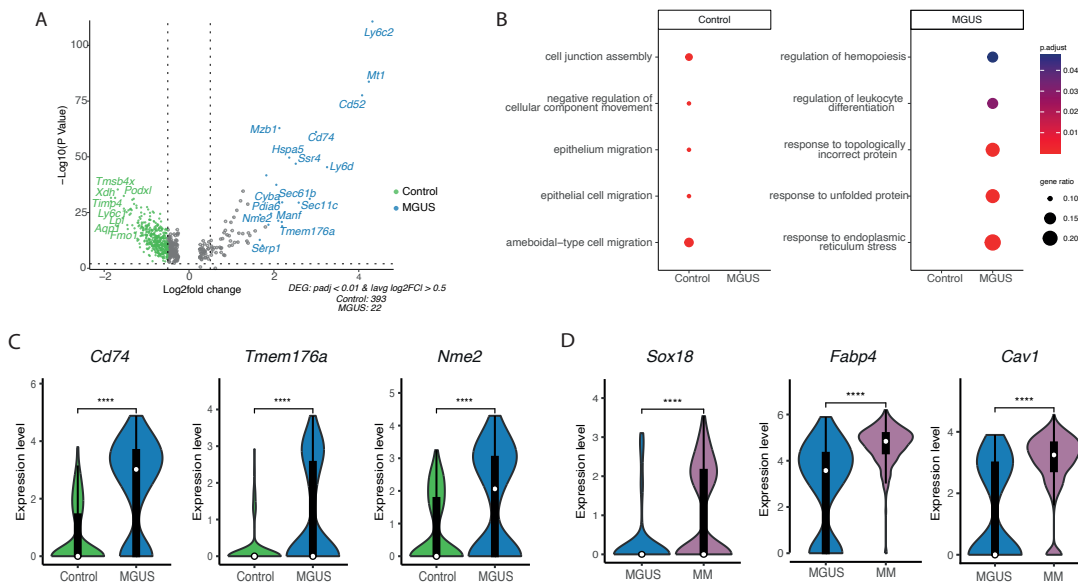


Supplementary Figure 4: Additional information on the MM IFN-related signature in the $BI_{c\gamma 1}$ model. (A) Heatmap showing the activities of the enriched regulons in EC (Z-score of regulon activity). (B) Density plots showing the score of Irf7, Stat1, and Stat2 regulons in EC (p. = adjusted p-value). (C) Regulon network of Irf7, Stat1, and Stat2 representing the top 50 target genes. (D) Violin plots showing AUC-score for MM IFN-related signature within BM populations in the $BI_{c\gamma 1}$ model. (E) Quantitative PCR (qPCR) analysis of the IFN regulon (*Stat1*) and the associated gene (*Gbp2*) in MSC derived from mesenspheres of MM $BI_{c\gamma 1}$ mice. Gene expression is presented as $\log_2$ fold change relative to MSC derived from mesenspheres of control mice. Data were normalized to the reference gene *Gapdh*. n=2 independent biological replicates per group. (F-G) Quantification of the percentage of mesenchymal stromal cells IFN-enriched cells, based on the coexpression of Cd271⁺ with IFN⁺ cells (*Gbp2*, (F); *Isg15*, (G)) in femurs from Control, MGUS $BI_{c\gamma 1}$ and MM $BI_{c\gamma 1}$ mice. n=3 independent biological replicates per control and MM group, and n=2 independent biological replicates for MGUS group. (H-I) IF staining of MSC (Cd271, green), and IFN response genes markers (*Gbp2* (yellow, (H)); *Isg15* (yellow, (I))), and nucleus (DAPI) (blue) in FFPE femurs from Control, MGUS $BI_{c\gamma 1}$, and MM $BI_{c\gamma 1}$ mice. Green arrows point to MSC (Cd271⁺), and yellow arrows point to IFN⁺ (*Gbp2*/*Isg15*). n=3 independent biological replicates per control and MM group, and n=2 independent biological replicates for MGUS group. Scale bars, 50 μ m.



Supplementary Figure 5: Validation in the MI_{cy1} model and scRNA-seq human data.

(A) Violin plots showing AUC-score for MM IFN-related signature within BM populations in the MI_{cy1} model. (B-C) Quantification of the percentage of endothelial IFN-enriched cells, based on the coexpression of *Emcn* with IFN⁺ cells (*Gbp2*, (B); *Isg15*, (C)) in femurs from MM BI_{cy1} and MM MI_{cy1} mice. n = 4 mice per group. (D-E) IF staining of EC (*Emcn*, red), and IFN response genes markers (*Gbp2* (yellow, (D)); *Isg15* (green, (E))), and nucleus (DAPI) (blue) in FFPE femurs from MM MI_{cy1} mice. Representative images from n = 4 mice with similar results. Scale bars, 50 μ m. (F-G) Quantification of the percentage of mesenchymal stromal cells IFN-enriched cells, based on the coexpression of *Cd271*⁺ with IFN⁺ cells (*Gbp2*, (F); *Isg15*, (G)) in femurs from MM BI_{cy1} and MM MI_{cy1} mice. n = 3 mice per group. (H-I) IF staining of MSC (*Cd271*, green), and IFN response genes markers (*Gbp2* (yellow, (H)); *Isg15* (yellow, (I))), and nucleus (DAPI) (blue) in FFPE femurs from MM MI_{cy1} mice. Green arrows point to MSC (*Cd271*⁺), and yellow arrows point to IFN⁺ (*Gbp2*/*Isg15*). Representative images from n = 3 mice with similar results. Scale bars, 50 μ m. (J) Violin plots comparing the distribution of the gene expression of *ISG15*, *IFI44*, and *IFITM1* in EC from MM patients and healthy controls from scRNA-seq published data¹. (K) Similar to J for MSC from the same healthy controls and MM human patients from scRNA-seq published data¹.

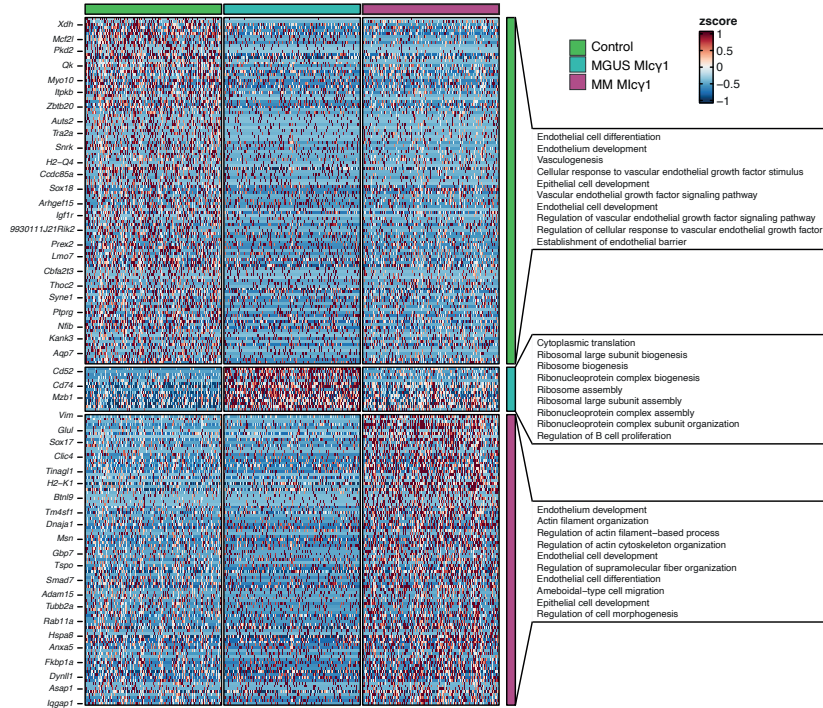


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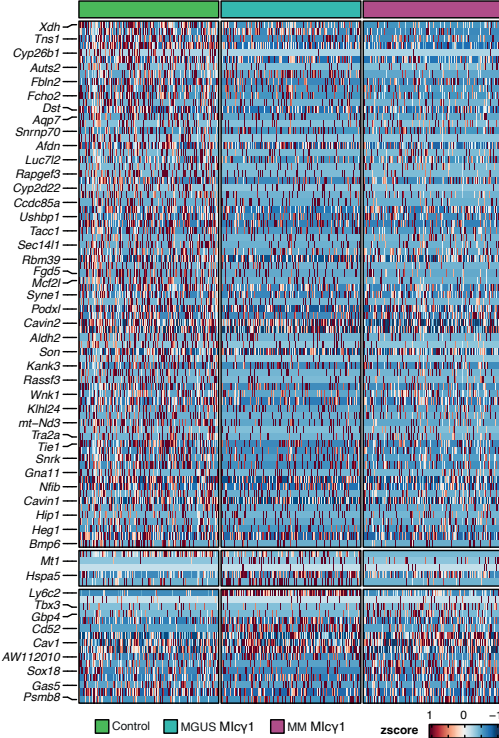
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74 **Supplementary Figure 6: Additional visualizations of transcriptional differences detected**75 **between EC from control, MGUS, and MM excluding MM IFN cells. (A) Volcano plots for**76 **the differential expression analysis between EC from control and MGUS. The y-axis represents**77 **the $-\log_{10}(\text{p-value})$, and the x-axis represents the $\log_2\text{FC}$ of the gene. The dot's color denotes the**78 **disease stage for which that DEG was detected, with grey dots representing non-significant genes.**79 **(B) GO ORA analysis between EC from control and MGUS. (C) Violin plots showing the**80 **expression of relevant upregulated genes in MGUS vs control EC. (D) Violin plots showing the**81 **expression of upregulated genes in EC from MM compared to MGUS.**

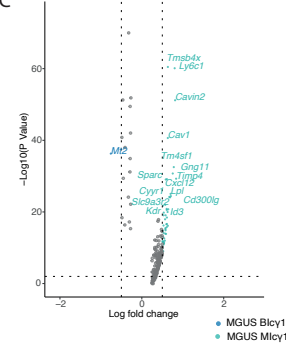
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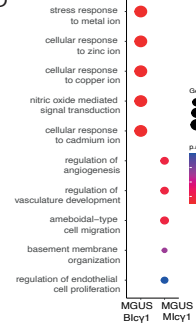
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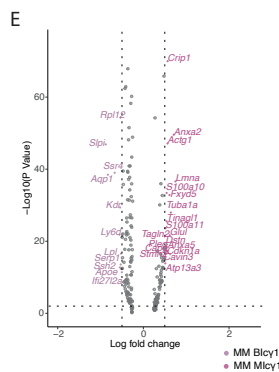
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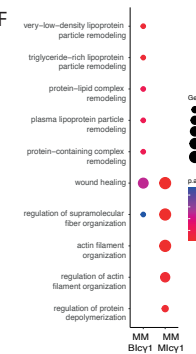
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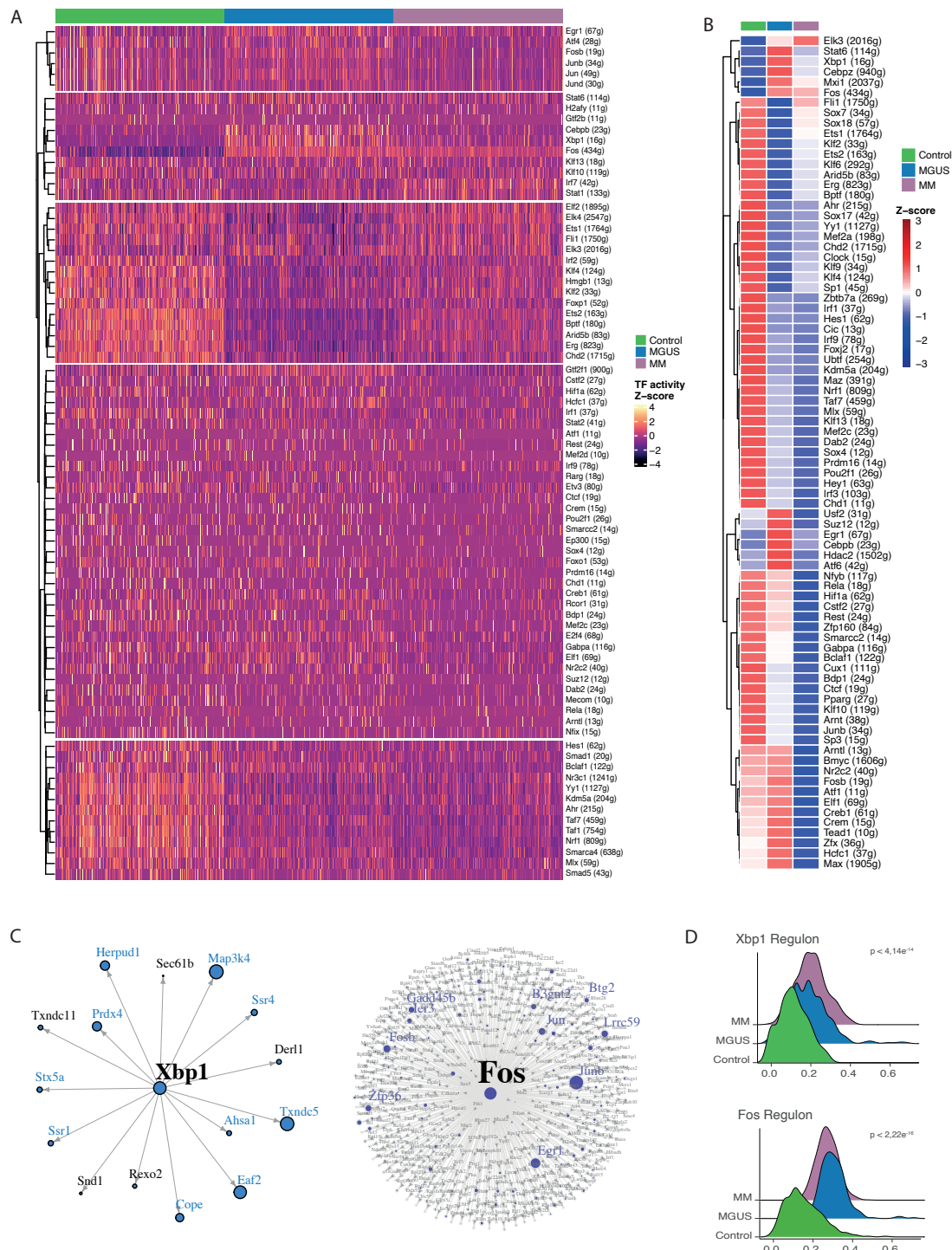


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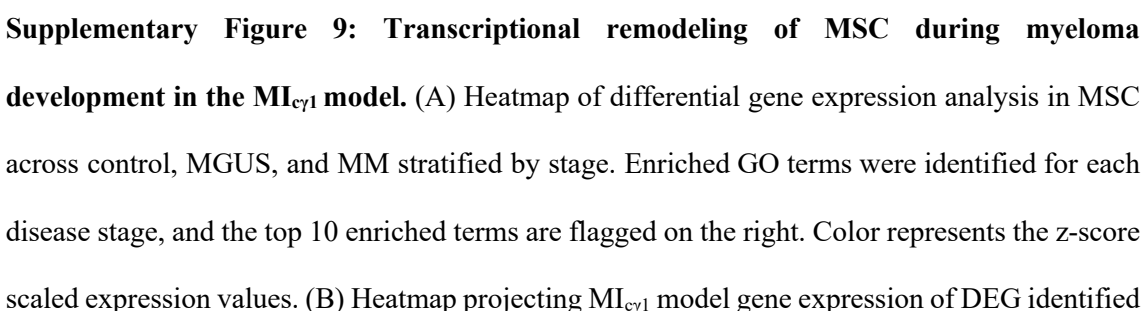


Supplementary Figure 7: Transcriptional changes in EC during myeloma progression in the Mlc₁ model. (A) Heatmap of differential gene expression analysis in EC across control, MGUS, and MM. Enriched GO terms were identified for each disease stage, and the top 10 enriched terms are flagged on the right. Color represents the z-score scaled expression values. (B)

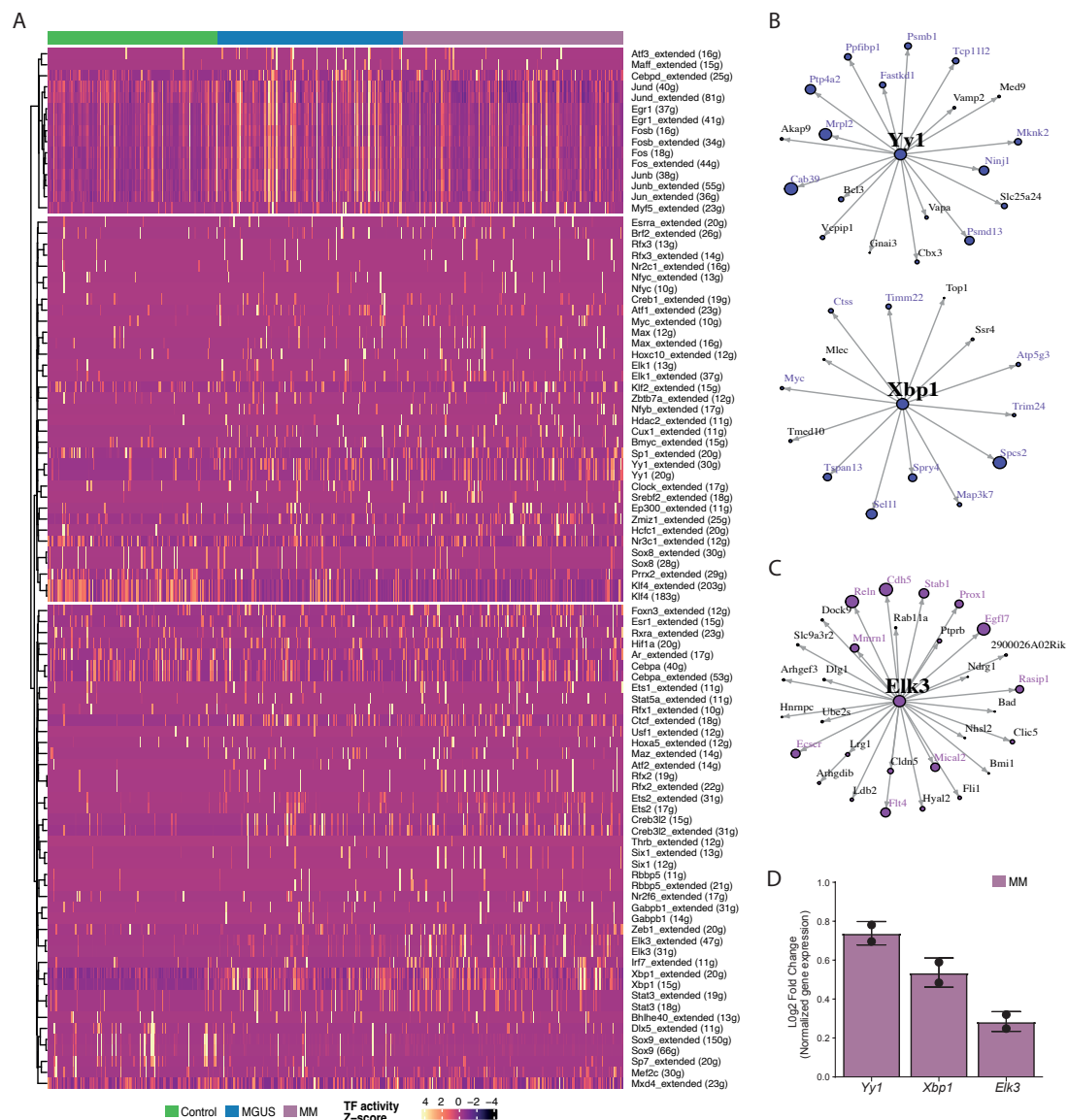
88 Heatmap projecting MI_{cy1} model gene expression of DEGs identified in EC from BI_{cy1} at different
89 stages of the disease. Color represents the z-score scaled expression values of EC from the MI_{cy1}
90 model. (C) Volcano plot of the DEGs between EC from MGUS in BI_{cy1} and MI_{cy1} model. The y-
91 axis represents the $-\log_{10}(\text{p-value})$, and the x-axis represents the $\log_2\text{FC}$ of the gene. The dot's
92 color denotes the model for which that DEG was detected, with grey dots representing non-
93 significant genes. (D) GO ORA analysis between EC from BI_{cy1} and MI_{cy1} model at the MGUS
94 stage. (E-F) Similar to (C-D) for the comparison between BI_{cy1} and MI_{cy1} model in the MM stage.



Supplementary Figure 8: Gene regulatory network of EC during MM progression excluding MM IFN signal. (A) Heatmap showing the activities of the enriched regulons in EC from control, MGUS and MM (Z-score of regulon activity). (B) Heatmap of mean regulon activity score of significantly enriched regulons in each stage. The color scale represents the z-score of the activity scaled by row. (C) Regulon network of Xbp1 and Fos representing the top target genes. (D) Density plots showing the score of Xbp1 and Fos regulons in EC.



108 from BI_{cy1} model in control, MGUS and MM. Color represents the z-score scaled expression
109 values of MSC from the MI_{cy1} model. (C) Volcano plot of the DEGs between MSC in MGUS
110 from BI_{cy1} and MI_{cy1} model. The y-axis represents the $-\log_{10}(p\text{-value})$, and the x-axis represents
111 the $\log_2FC$ of the gene. The dot's color denotes the model from which that DEG was detected,
112 with grey dots representing non-significant genes. (D) GO ORA between MSC from BI_{cy1} and
113 MI_{cy1} model at the MGUS stage. (E-F) Similar to (C-D) for the comparison between MSC from
114 BI_{cy1} and MI_{cy1} model in the MM stage.



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117 **Supplementary Figure 10: Gene regulatory network of MSC during MM progression**

118 **excluding MM IFN signal.** (A) Heatmap showing the activities of the enriched regulons in MSC

119 from control, MGUS, and MM (Z-score of regulon activity). (B) Regulon network of Yy1 and

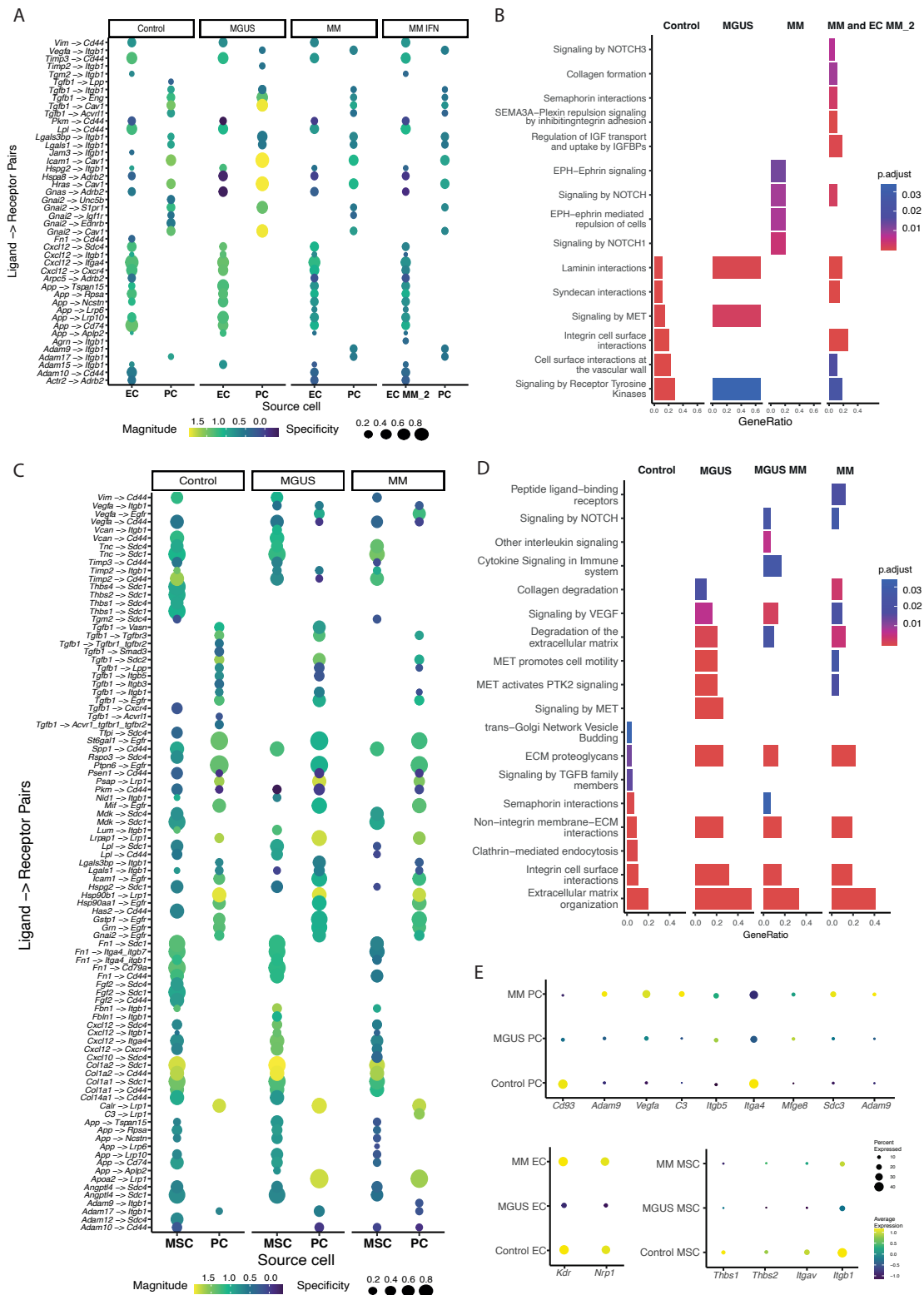
120 Xbp1, representing the top associated genes (C) Regulon network of Elk3 representing the top

121 associated genes. (D) Quantitative PCR (qPCR) analysis of the enriched regulons (*Yy1*, *Xbp1*, and

122 *Elk3*) in MSC derived from mesospheres of MM B1cγ1 mice. Gene expression is presented as

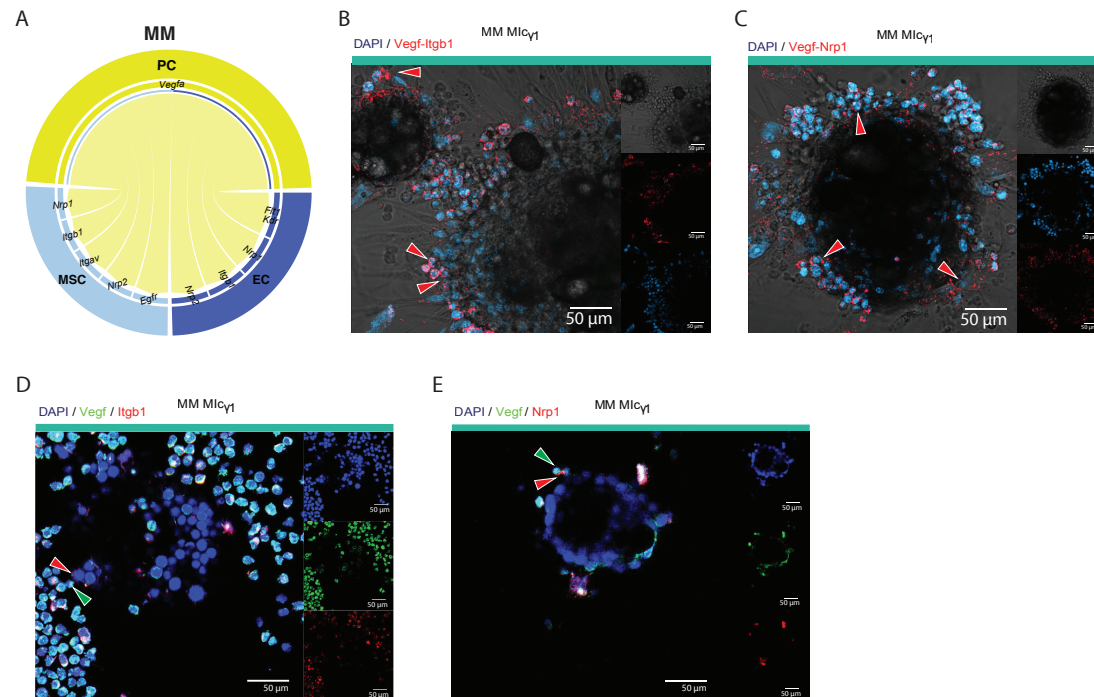
123 log₂ fold change relative to MSC derived from mesospheres of control mice. Data were

124 normalized to the reference gene *Gapdh*. qPCR data are mean $\pm$ s.e.m.; n = 2 independent
 125 biological replicates per group.

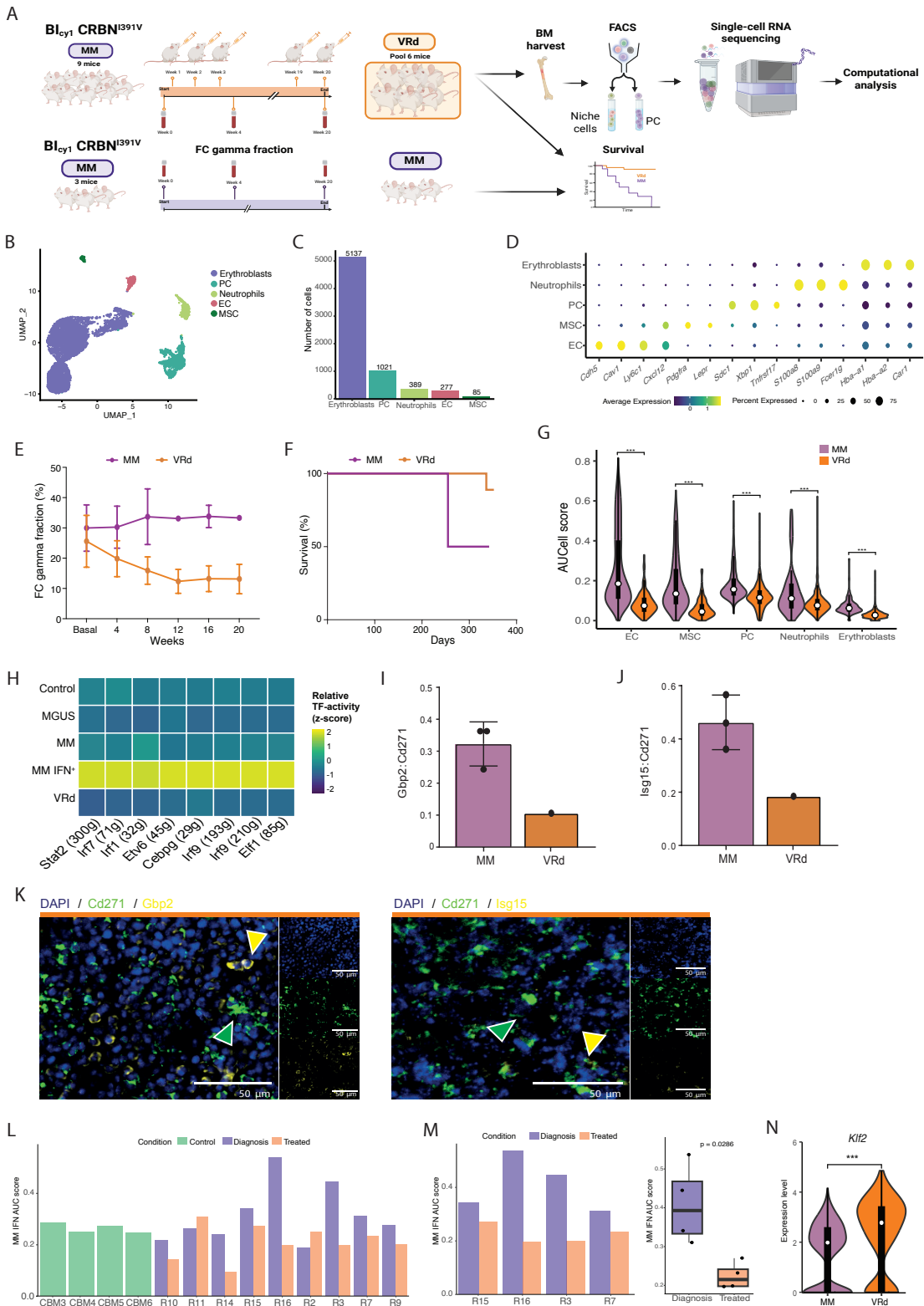


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 127 **Supplementary Figure 11: Cell-cell communication analysis between EC, MSC and PC. (A)**

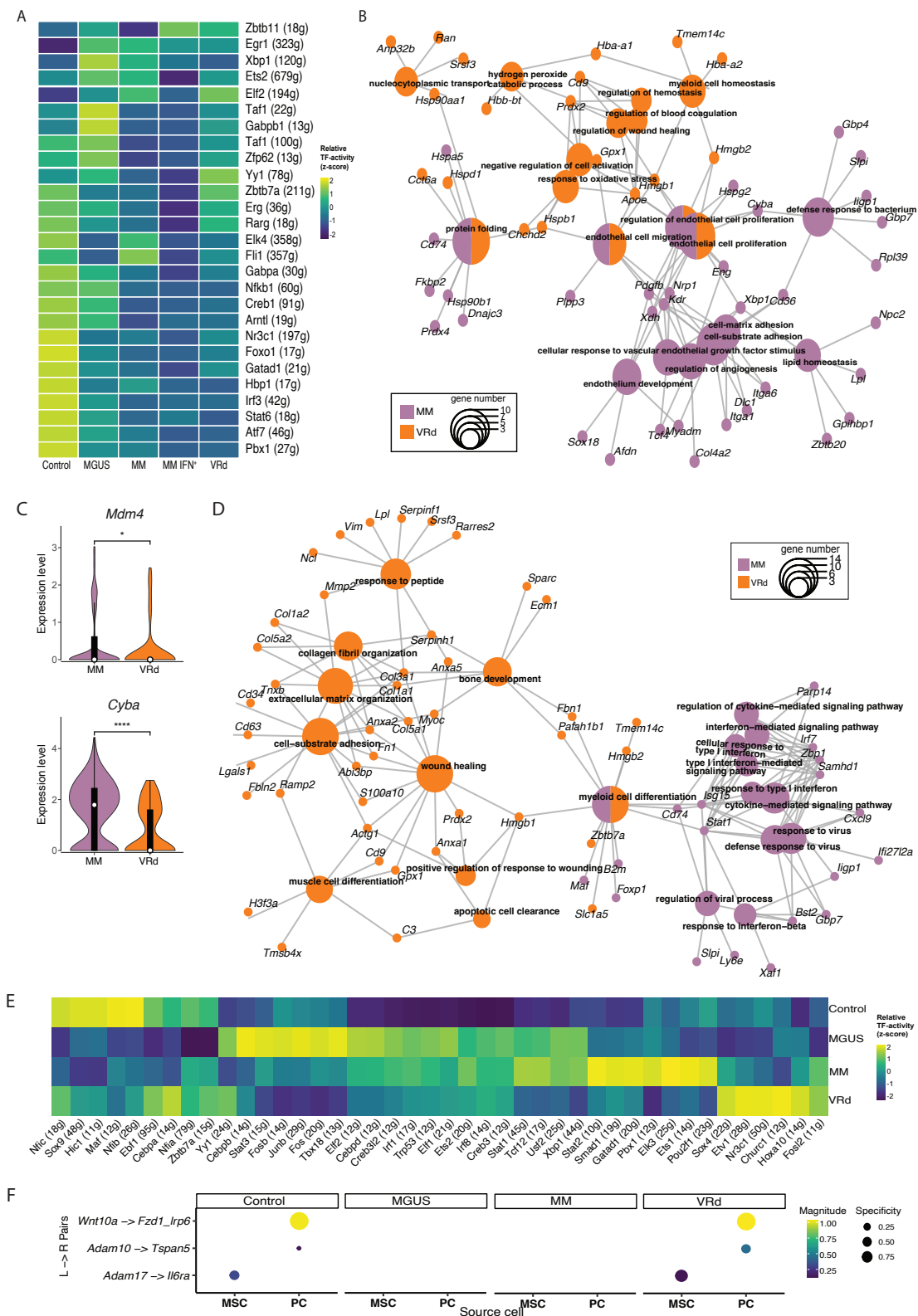
128 Dot plots of ligand-receptor interactions between PC and EC that are maintained along the disease
129 stages. Dot size indicates specificity score; color indicates interaction strength. (B) Representative
130 Reactome pathways enriched in EC-PC L-R pairs specific for each stage. (C-D) Similar to (A-B)
131 for MSC-PC communication. (E) Dot plot showing the expression of relevant ligands and
132 receptors in PC, EC and MSC across disease stages. The dot size reflects the percentage of cells
133 within the cluster expressing each gene, and the color represents the average expression level.



Supplementary Figure 12. MM-specific interactions among EC, MSC, and PC in MM Mlcγ1 mice and functional validation assays. (A) Chord diagrams illustrating L-R pairs shared between PC-EC and PC-MSC communication within the MM stage from the MM Mlcγ1 mouse model. Colors and width represent signal senders and strength, respectively. PC-derived *Vegfa* engages multiple receptors in both cell types. (B-C) Proximity ligation assay (Navinci Kit) analysis of MM Mlcγ1 interactions (Vegf-Itgb1 (B) and Vegf-Nrp1 (C)) in coculture of PC (cell line MM5080) with MSC derived from mesenspheres of MM Mlcγ1 mice. Representative confocal images show PLA-positive puncta (red arrows), indicating protein-protein interactions, with nuclei counterstained with DAPI (blue). (D-E) IF staining of PC ligands (Vegf (green)), with MSC-receptors (Itgb1 (red (D)) and Nrp1 (red (E))), and nucleus (DAPI) (blue) in coculture of PC (cell line MM5080) with MSC derived from mesenspheres of MM Mlcγ1 mice. Green arrows point to PC-Vegf⁺, and red arrows point to MSC-Itgb1 (D) or Nrp1 (E). (B-E) Representative images from n = 2 independent coculture experiments with similar results. Scale bars, 50 μm.

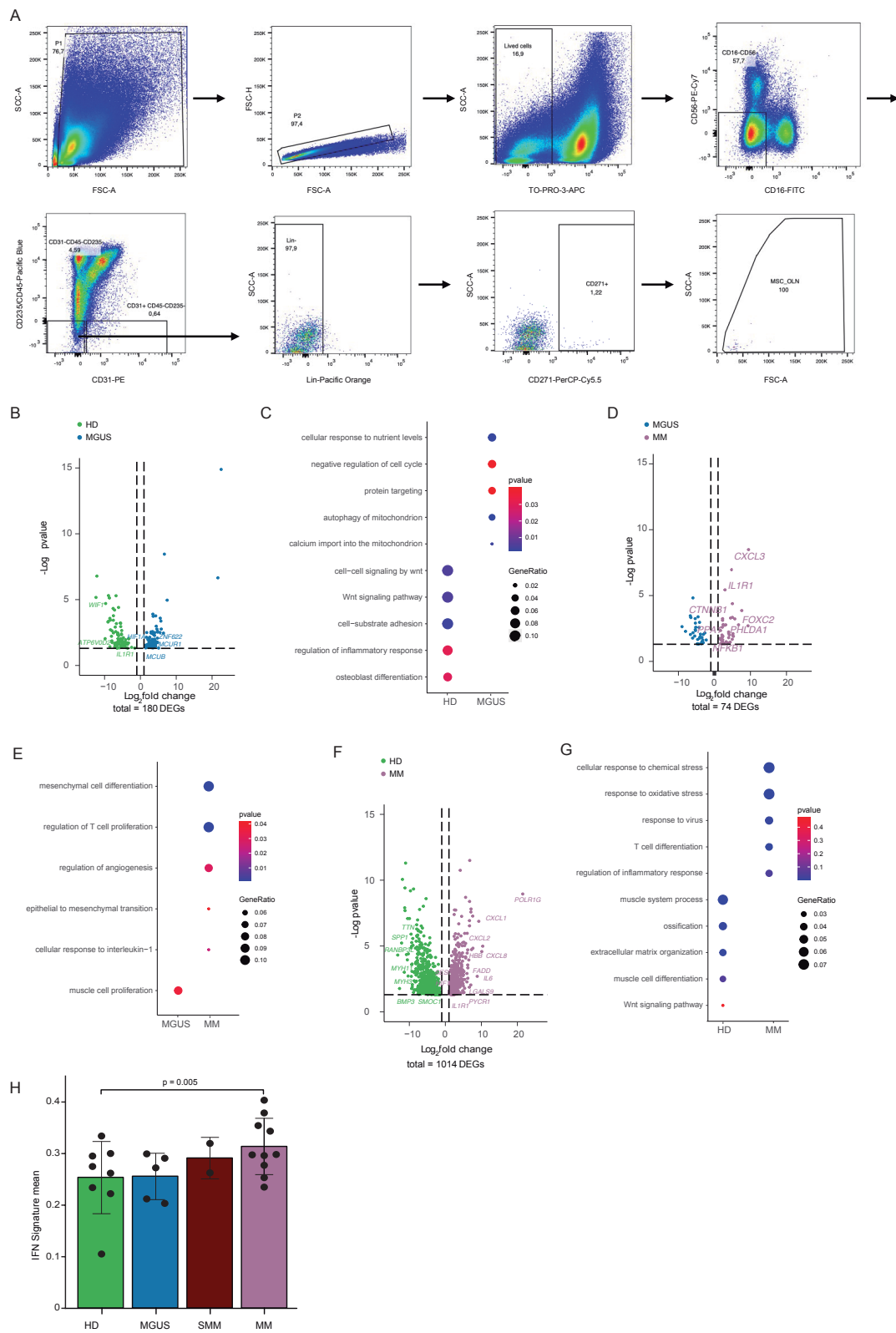


treatment administration, serum collection for FC gamma fraction, and scRNA-seq isolation of
 PC, MSC, and EC from MM BI_{cy1} *Crbn*^{I391V} (pool of six mice). MM BI_{cy1} *Crbn*^{I391V} were used as
 controls for FC gamma fraction and survival. Created with BioRender.com. (B) UMAP
 representation of BM microenvironment cells from BI_{cy1}-*Crbn*^{I391V} VRd-treated mice, colored by
 cell clustering. (C) Bar plots showing the number of cells per BM population. (D) Dot plot of
 canonical markers used to define PC, EC, MSC, erythroblasts, and neutrophils. The dot size
 reflects the percentage of cells within the cluster expressing each gene, and the color represents
 the average expression level. (E) Representation of the percentage of serum FC gamma fraction
 in VRd-treated versus the MM group. (F) Kaplan–Meier survival curves of treated and non-
 treated MM BI_{cy1} mice. (G) Violin plots showing AUC-score for MM IFN-related signature
 within BM populations in VRd-treated and MM BI_{cy1} mice. (H) Heatmap representing z-scaled
 mean regulon activity score of IFN-related regulons in EC across each condition. (I-J)
 Quantification of the percentage of mesenchymal IFN⁺ cells, determined by the coexpression of
 Cd271 with IFN response genes (Gbp2 (I) and Isg15 (J) in femurs from n=3 MM BI_{cy1} (MM) and
 n=1 MM BI_{cy1}-*Crbn*^{I391V} VRd-treated (VRd) mice. (K) IF staining of MSC (Cd271, green), IFN
 response (Gbp2, yellow (left plot) and Isg15, yellow (right plot)), and nucleus (DAPI) (blue) in
 FFPE femurs from VRd-treated mice. Green arrows point to MSC (Cd271⁺), and yellow arrows
 point to IFN⁺ (Gbp2/Isg15). n = 1 mice per group. Scale bars, 50 µm. (L) Bar plots showing the
 AUC-score for MM IFN-related signature in MSC from healthy controls and MM human patients
 before and post-induction treatment from bulk RNA-seq published data¹. (M) Bar plots showing
 the AUC-score for MM IFN-related in MSC from the subset of high IFN MM human patients
 before and after induction treatment¹ by individuals (left panel) and grouped by disease (right
 panel). (N) Violin plot displaying the upregulation of *Klf2* in VRd-treated EC vs non-treated MM
 EC.



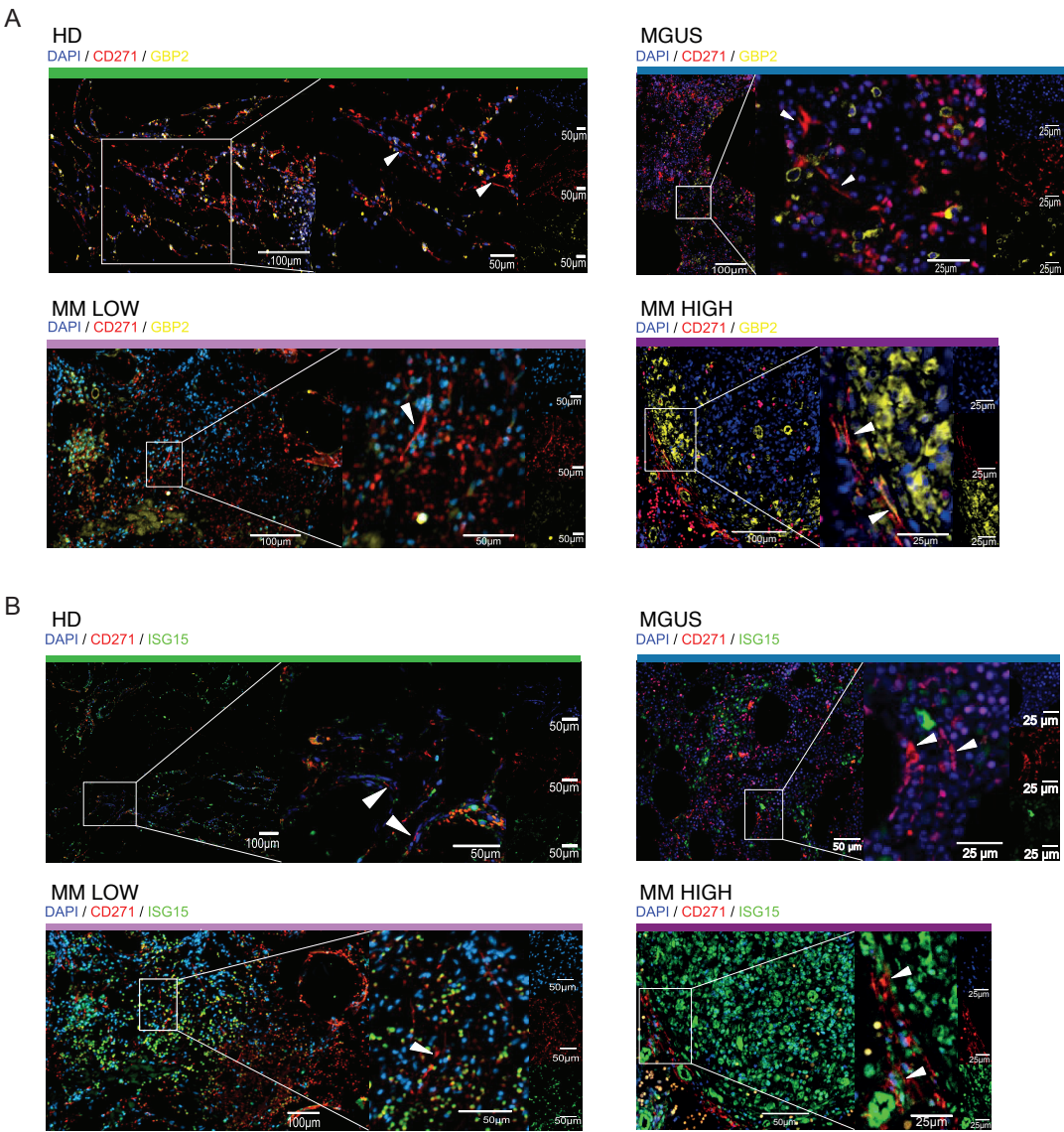
Supplementary Figure 14: Additional information of the transcriptional remodeling of EC and MSC following VRd treatment. (A) Heatmap representing z-scaled mean regulon activity score of inferred top specific regulons for EC in each condition. (B) Cnetplot showing the links

between genes and biological processes derived from the comparison between VRd-treated and non-IFN MM EC. Node size reflects the number of significantly enriched genes in the node and colors the group condition. (C) Violin plots displaying the expression of *Mdm4* and *Cyba* in VRd-treated and MM BI_{cy1} MSC. (D) Cnetplot showing the links between genes and biological processes derived from the comparison between VRd-treated and MM MSC. Node size reflects the number of significantly enriched genes in the node and colors the group condition (E) Heatmap representing z-scaled mean regulon activity score of inferred top specific regulons for MSC in each condition. (F) Dot plots representing L-R pairs common to control and VRd-treated mice involved in the communication between MSC and PC. Dot color represent the strength and size indicate the specificity of the interaction.



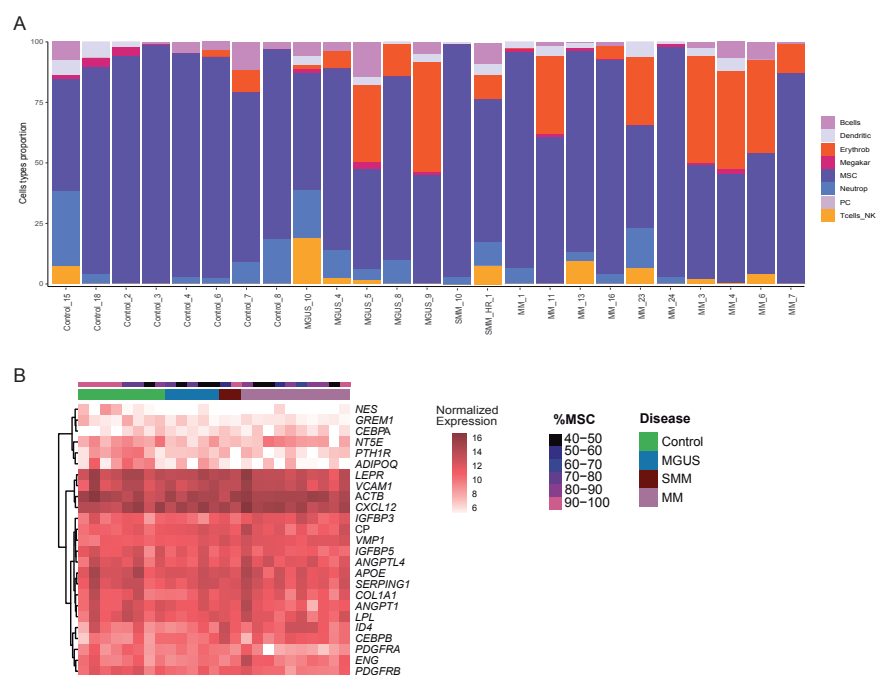
Supplementary Figure 15: Cell sorting strategy and additional information on the transcriptional alterations of human MSC during myeloma progression. (A) Sorting gating strategy for isolation of human BM MSC (TO-PRO-3⁻, CD16⁻, CD56⁻, CD45⁻, CD235⁻, CD31⁻,

Lin⁻, CD271⁺). (B) Volcano plot of the DEGs between MGUS-MSC and HD-MSC. The y-axis represents the $-\log_{10}(\text{p-value})$, and the x-axis represents the $\log_2\text{FC}$ of the gene. The dot's color represents the patient's disease condition. (C) GO ORA between MSC from HD and MGUS patients. (D-E) Similar to (B-C) for the comparison between MSC from MGUS and MM patients. (F-G) Similar to (B-C) for the comparison between HD-MSC and MM-MSC. (H) Bar plot depicting the mean IFN signature score across different disease conditions. Points represent individual human samples; HD n = 8, MGUS n = 5, SMM n = 2 and MM n = 10. Data are presented as mean $\pm$ SD. Statistical significance was determined using an unpaired two-sided Student's t-test (assuming equal variances), exact p values provided.



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205 **Supplementary Figure 16: Expanded immunofluorescence analysis of human MSC during**
206 **myeloma progression.** (A-B) IF staining of MSC (CD271, red), IFN response genes (*GBP2*,
207 yellow (A); *ISG15*, green (B)), and nucleus (DAPI) (blue) in FFPE biopsies from HD and archived
208 paraffined human BM biopsies of MGUS and MM patients with high (MM HIGH) and low IFN
209 signal (MM LOW) in stromal cells (see Supplementary Data 13). White arrows point MSC
210 CD271+ in HD, MGUS and MM LOW. In MM HIGH white arrows point MSC CD271+ IFN+
211 (GBP2/ISG15). Representative images from HD (n = 3), MGUS (n = 4), MM low IFN (n = 3),
212 MM high IFN (n = 3) samples. Scale bars, 50 μm.



Supplementary Figure 17: Evaluation of MSC proportion in bulk RNA-seq human samples.

(A) Bar plot showing the percentage of different cell types present on each sample estimated by CibersortX. Each bar represents one human sample. (B) Heatmap of MSC canonical markers gene expression correlated with MSC percentage estimated by CibersortX.

SUPPLEMENTARY TABLES

Supplementary Data 1. Differentially expressed genes between MGUS and control EC.

Differential expression was assessed using two-sided MAST with Bonferroni correction.

Supplementary Data 2. Differentially expressed genes between MM and MGUS EC. Differential

expression was assessed using two-sided MAST with Bonferroni correction.

Supplementary Data 3. Gene Ontology over-representation analysis across MSC stages. Adjusted

P values were calculated using the Benjamini–Hochberg method.

Supplementary Data 4. Differentially expressed genes between MGUS and control MSC.

Differential expression was assessed using two-sided MAST with Bonferroni correction.

Supplementary Data 5. Differentially expressed genes between MM and MGUS MSC.

Differential expression was assessed using two-sided MAST with Bonferroni correction.

Supplementary Data 6. Full list of significant ligand–receptor interactions between EC and PC,

and between MSC and PC. Interactions were identified using LIANA aggregate ranking.

Supplementary Data 7. Differentially expressed genes between VRd-treated and untreated MM

EC. Differential expression was assessed using two-sided MAST with Bonferroni correction.

Supplementary Data 8. Differentially expressed genes between VRd-treated and untreated MM

MSC. Differential expression was assessed using two-sided MAST with Bonferroni correction.

Supplementary Data 9. Differential expression analysis comparing human MGUS-MSC and

healthy donor MSC. Differential expression was performed using the two-sided Wald test in

DESeq2 with Benjamini–Hochberg correction.

Supplementary Data 10. Differential expression analysis comparing human MM-MSC and

MGUS-MSC. Differential expression was performed using the two-sided Wald test in DESeq2

with Benjamini–Hochberg correction.

241 Supplementary Data 11. Differential expression analysis comparing human MM-MSC and
242 healthy donor MSC. Differential expression was performed using the two-sided Wald test in
243 DESeq2 with Benjamini–Hochberg correction.

244 Supplementary Data 12. Description of mice used in the study for scRNA-seq and
245 immunofluorescence analyses.

246 Supplementary Data 13. Clinical characteristics of human individuals used for bulk RNA-seq and
247 immunofluorescence analyses.

248 **REFERENCES**

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