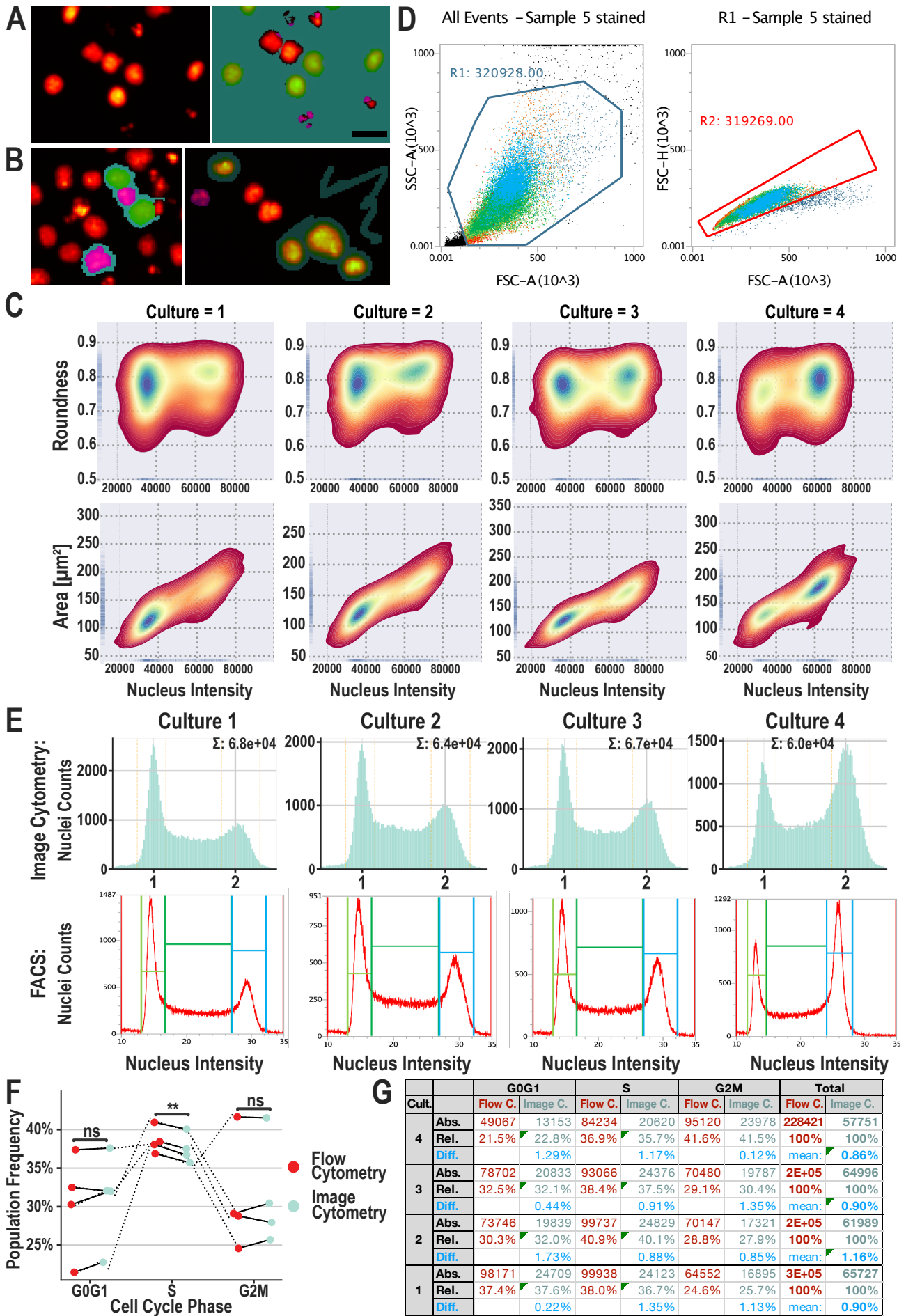
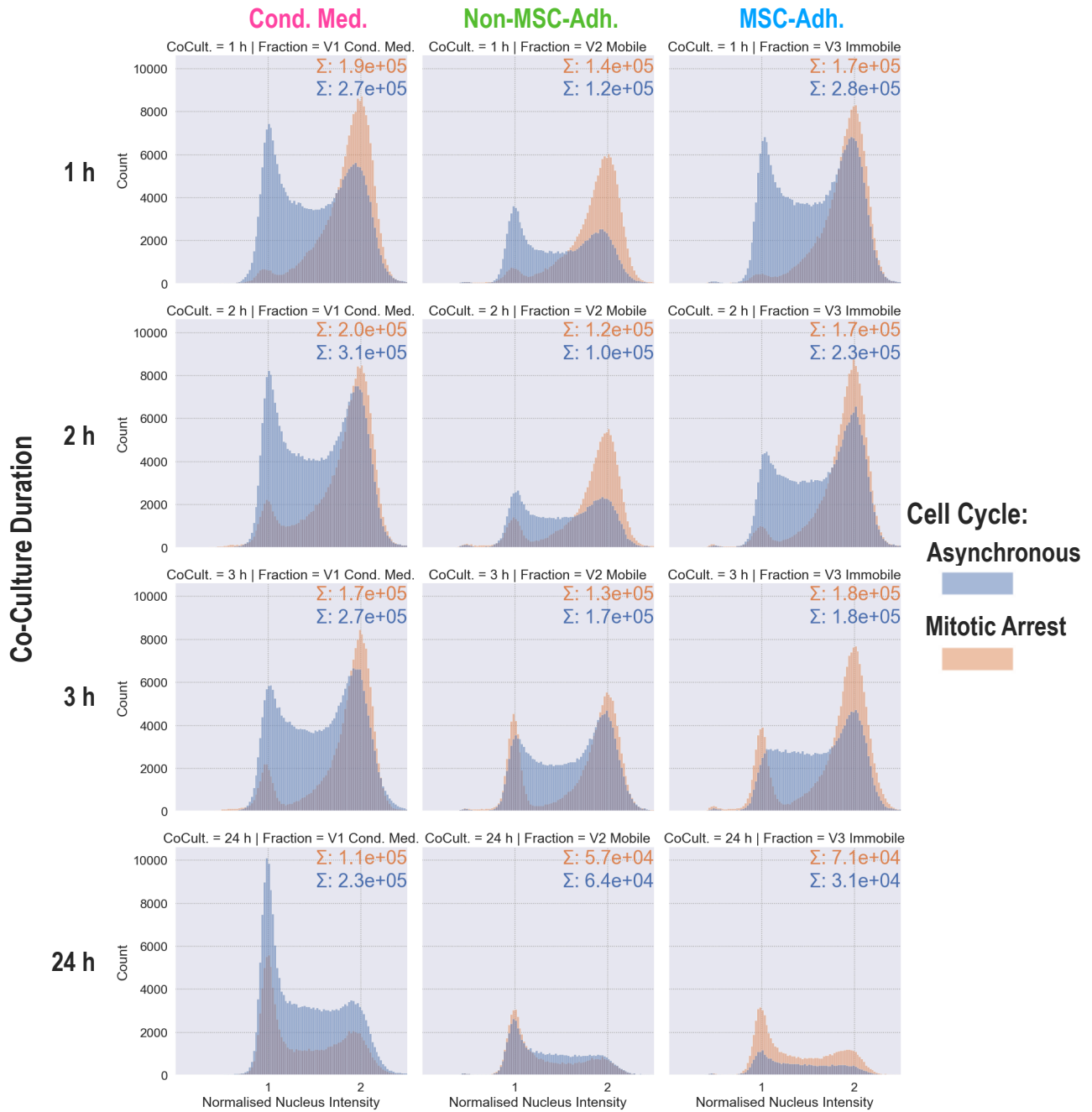


**Supplementary Figure 1:** Principle and quantification of the V-well adhesion assay of fluorescently labeled myeloma cells adapted by Weetall et al. 2001. **A:** Sample: Subsequent rounds of centrifugation and removal of cell pellet yielded the size of adhesive subpopulations. Fluorescently stained INA-6 cells were added to an hMSC monolayer. Non-adherent INA-6 cells (V2) were pelleted in the well-tip. Pellets were quantified by fluorescence brightness and isolated by pipetting. Immobile INA-6 cells (V3) were manually detached by forceful pipetting. Reference: Omitting adhesive hMSC-layer yielded ~100% non-adherent cells (V1) after the first centrifugation step; Background: hMSC monolayer was used as background signal. **B:** Calculation of the population size relative to total cells starting with pellet intensity. The shown example is the pellet gained by centrifuging mobile subpopulation (V2) after 1 h of co-culture. (see Fig. 2 for context): Intensity values from pellet images were summarized. After subtracting the unlabeled hMSC signal and normalization by a full-size pellet (reference), the resulting values represented the fraction of the adhesive subpopulation. **C:** One of three biological replicates summarized in Fig. 2. Line range shows the standard deviation of four technical replicates. Non.Adh. Rem.: Fluorescence signal after removal of V2. **D:** Example images of myeloma cell lines (INA-6, U266, MM.1S) pelleted in the tip of V-wells. The leftmost image shows the recorded area in a complete V-well. Scale bar = 200  $\mu$ m. **E:** Results from (D) comparing adhesion strength of three myeloma cell lines to hMSC. Error bars represent technical deviation. MM=Multiple Myeloma.

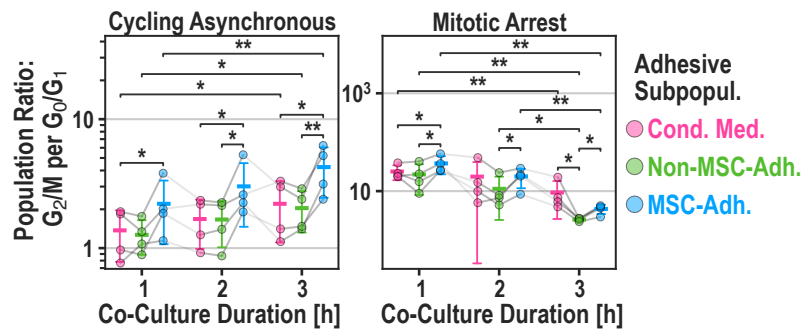


**Supplementary Figure 2:** Validation of image cytometric analysis of cell cycle in four INA-6 cultures **A:** Left: Example image cytometric scan: INA-6 cells were stained with Hoechst33342 and scanned by automated fluorescence microscopy. Right: The image was segmented using a convolutional neural network (ZEISS ZEN intellesis) trained to discern healthy nuclei (green) from fragmented ones (magenta). Doublets are excluded by setting an area- and roundness threshold. Scale bar: 20  $\mu\text{m}$ . **B:** Two example images from the training set. **C:** Quality of image cytometric data was ensured by plotting the distribution of nuclei brightnesses vs. the distribution of both nuclei-roundnesses and nuclei-areas. Nuclei with double fluorescence intensity have the same roundness while their area increases, as expected from a cell in G2 phase. **D:** The same samples from (C) were also measured with flow cytometry. Representative example of gating strategy: Left: Dead cells were excluded by setting a minimum threshold for side-scattering (SSC-A). Right: Doublets were excluded by setting a maximum threshold for forward scatter area (FSC-A) (sample “5” represents culture “4” in this figure). **E:** Cell cycle profiles of four independent INA-6 cultures were measured by both image cytometry (top) and flow cytometry (bottom). For both methods, frequencies of G0/G1, S, and G2M were summed up by setting fluorescence intensity thresholds. **F:** Image cytometry yields the same frequencies for G0/G1, S, and G2M when compared to flow cytometry. RM-ANOVA showed that the method has no significant effect on the frequencies of cell cycle populations [ $F(1,3)=1.421$ ,  $p\text{-unc}=.32$ ]. **G:** Results from (F) in tabular form. On average, frequencies for G0/G1, S, and G2M measured by Image cytometry differ by 0.95 percent points compared to flow cytometry measurement. Cult.: Culture; C.: Image cytometry; Abs.: Absolute cell count; Rel.: Relative cell count; Diff.: Difference between relative cell counts determined by flow cytometry and image cytometry.

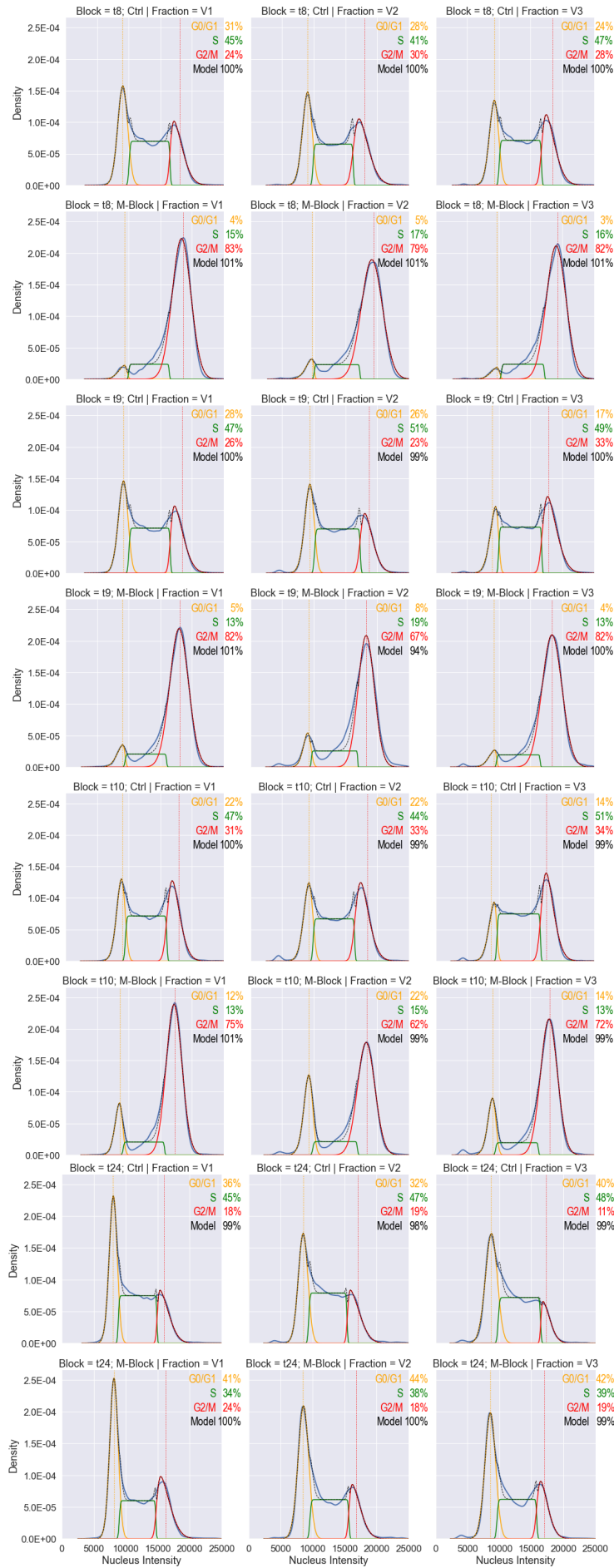
A



B

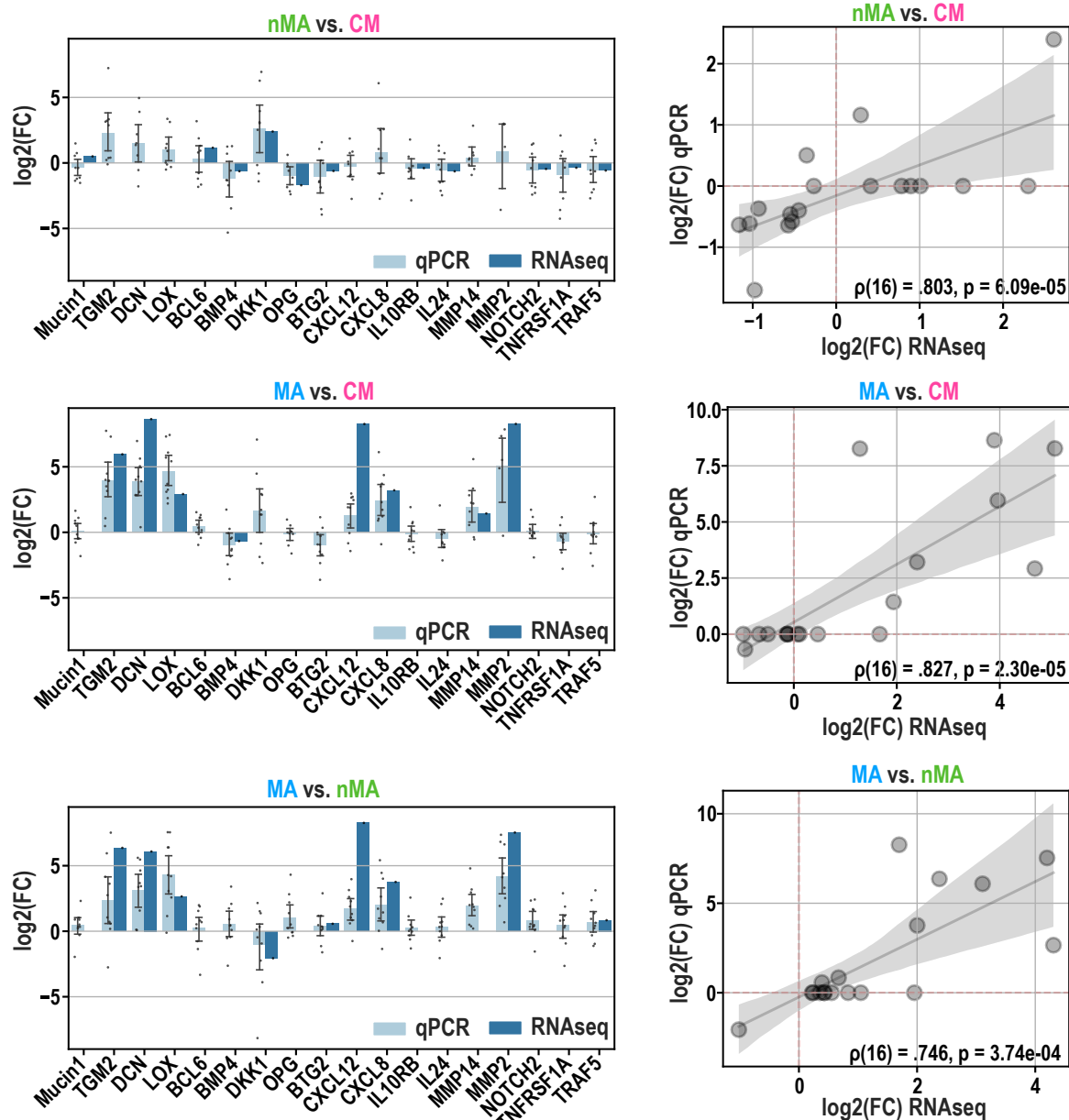


**Supplementary Figure 3:** Cell cycle analysis of INA-6 pellets gained from V-Well Adhesion assay (Fig. 3). **A:** Cell cycle profiles of MSC-adhering subpopulations. INA-6 cells were synchronized by double thymidine block followed by nocodazole. Cell cycle was released directly before addition to hMSCs. Histograms were normalized and summed up across all biological replicates (n=4). Technical replicates (3) were pooled prior to cell cycle profiling. CoCult. = Co-culture duration. Fraction = Adhesion subpopulations. **B:** Similar figure to Fig. 3C displaying ratio of INA-6 populations (G2/M to G0/G1). **Statistics:** Paired t-test (B).

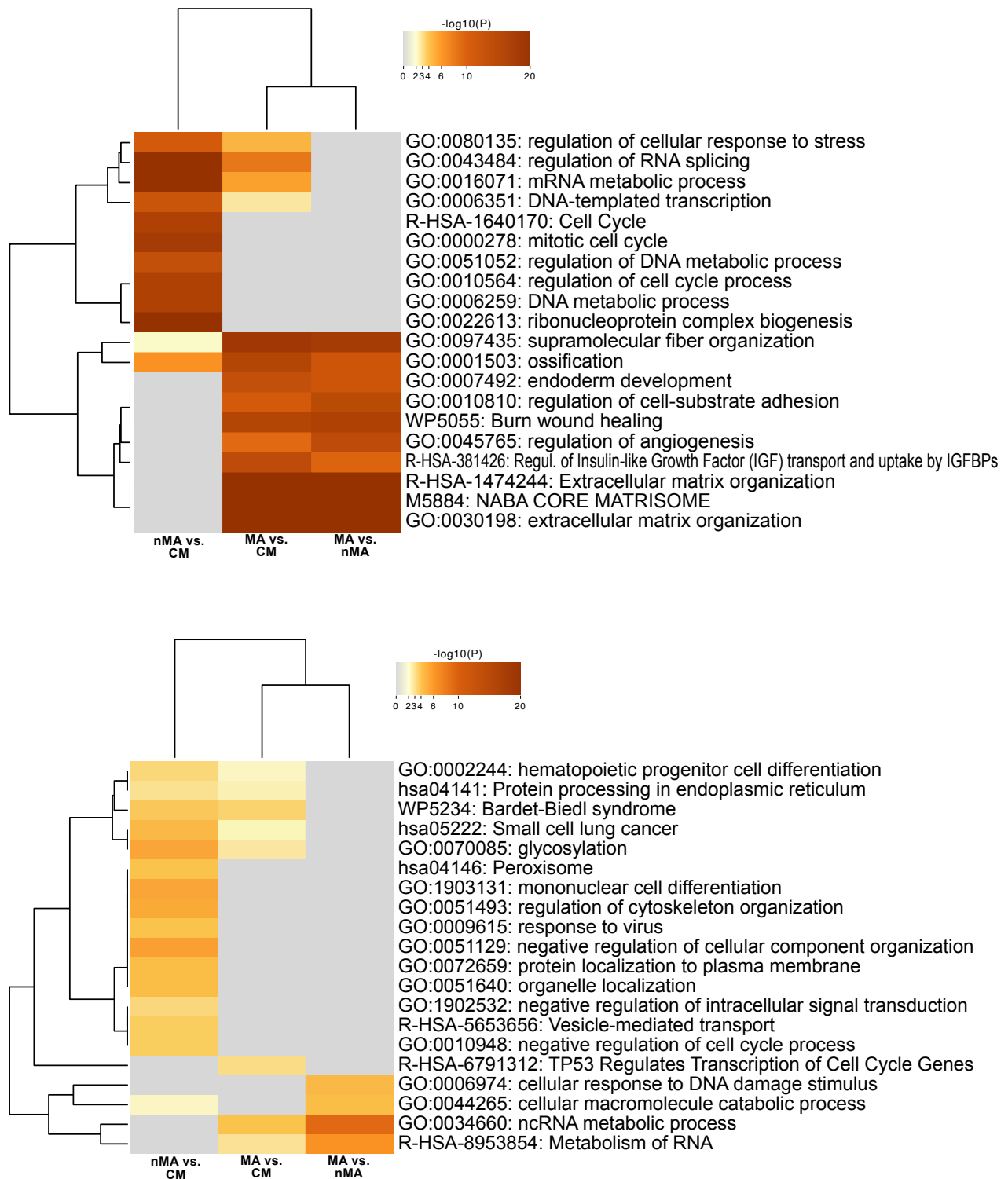


**Supplementary Figure 4:** Representative (one of the four independent sample sets as seen in Supplementary Figure 3) curve fitting analysis of cell cycle profiles generated by Image Cytometry. t8, t9, t10, and t24 refer to 1, 2, 3, and 24 hours after the addition of INA-6 cells to hMSCs.



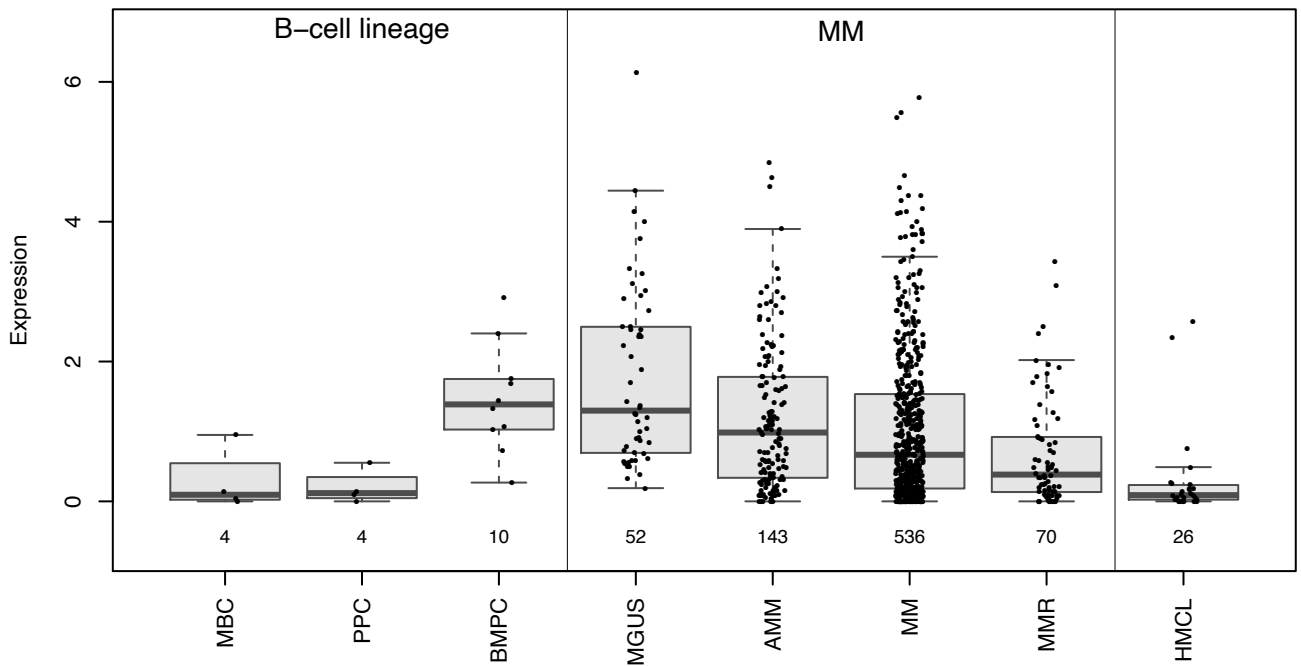


**Supplementary Figure 5:** Correlation of RNAseq with qPCR **Left:** Validation of RNAseq results (Fig. 3) with qPCR showing the log<sub>2</sub>(foldchange expression) of 18 genes. For qPCR, Datapoints each represent one biological replicate (n=10), which is the mean of technical replicates (n=3). Bar height represents mean of biological replicates, error bars show standard deviation of biological replicates. **Right:** Correlation between qPCR and RNAseq in terms of log<sub>2</sub>(mean foldchange expression per gene). Each dot represents one gene shown in the barplot to the left. Genes measured with qPCR that showed no differential expression in RNAseq were set to have a log<sub>2</sub>(FC) = 0. Shaded area shows the confidence interval of linear regression. Correlation coefficient (ρ) was calculated using Spearman's rank. N = 18 genes. FC = fold change expression.

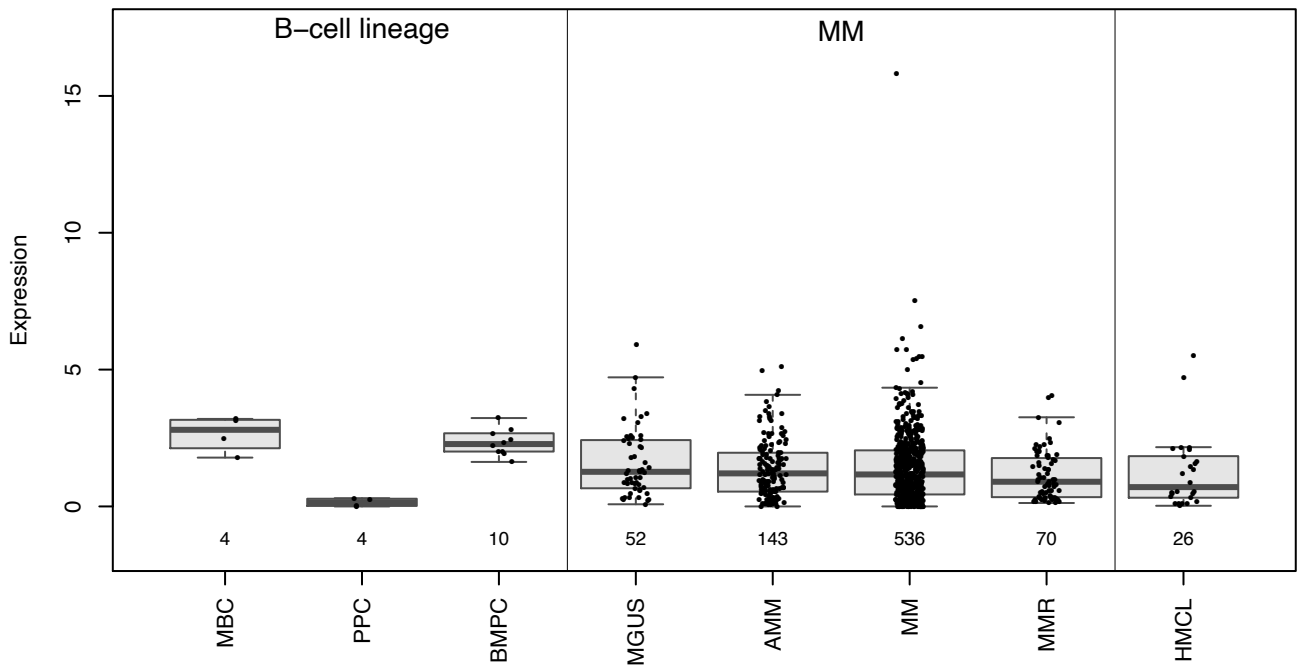


**Supplementary Figure 6:** Functional enrichment analysis by Metascape using genes that are differentially expressed between MSC-interacting subpopulations. **Top:** Upregulated genes. **Bottom:** Downregulated genes.

**log2 RNA-Seq CYP1B1 ( ENSG00000138061 )**

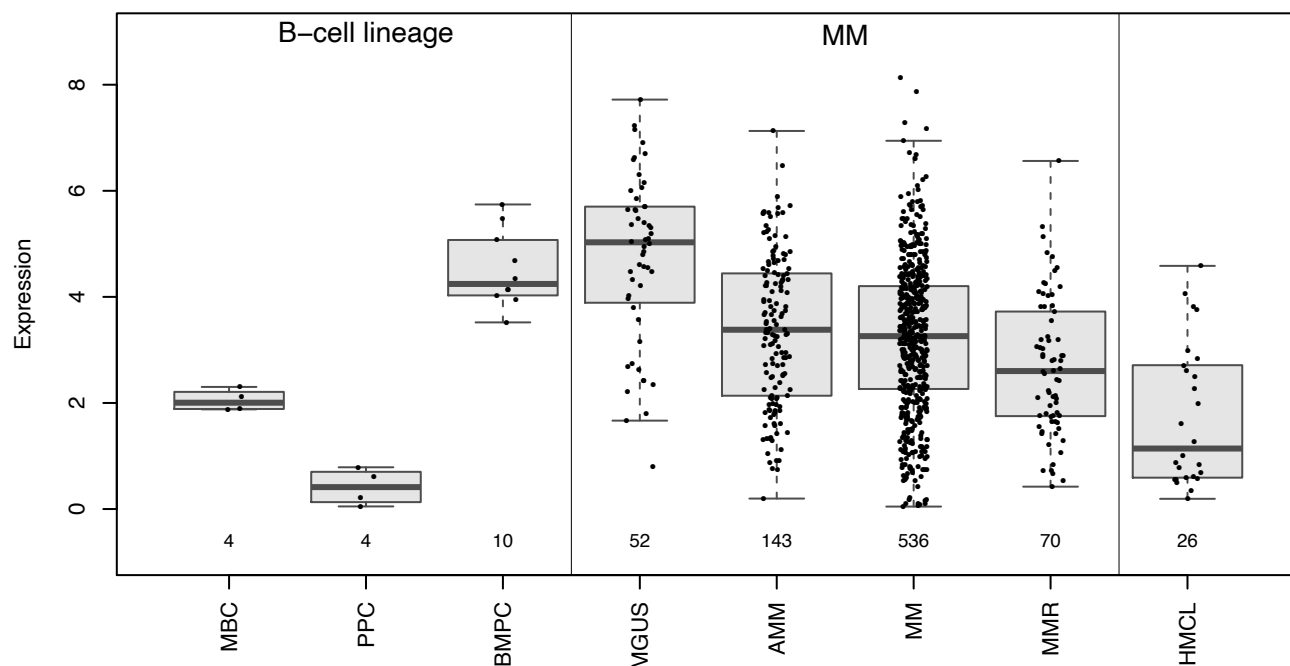


**log2 RNA-Seq COL1A1 ( ENSG00000108821 )**

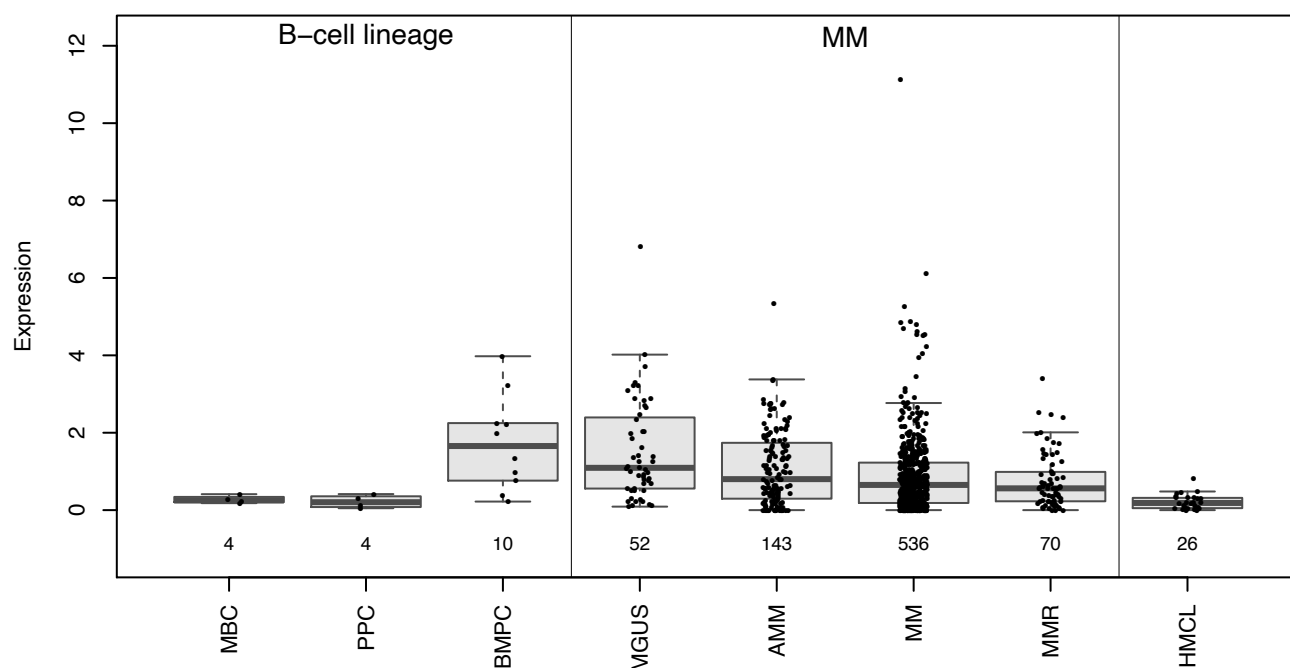


**Supplementary Figure 7:** Expression levels of adhesion genes that are downregulated and associated with survival ( $p < 0.01$ ). Bone Marrow Plasma Cell (BMPC), Monoclonal Gammopathy of Undetermined Significance (MGUS), Smoldering Multiple Myeloma (sMM), Multiple Myeloma (MM), Multiple Myeloma Relapse (MMR).

**log2 RNA-Seq LRP1 ( ENSG00000123384 )**

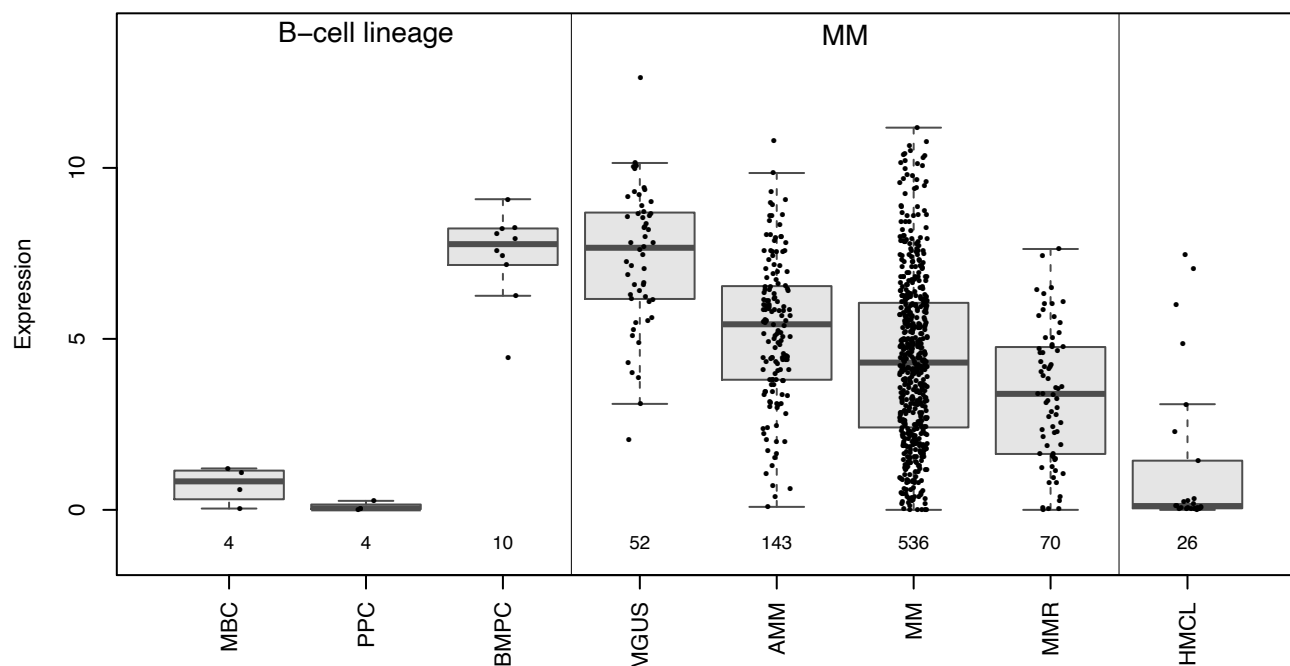


**log2 RNA-Seq DCN ( ENSG00000011465 )**

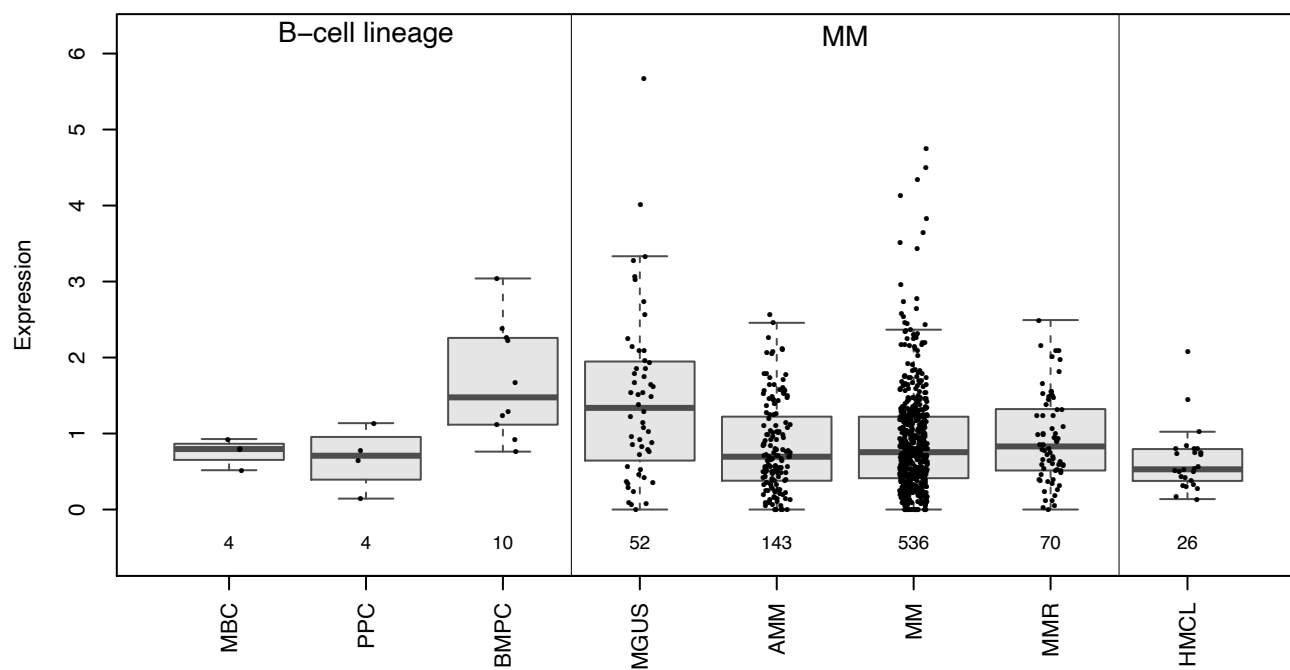


**Supplementary Figure 7 (continued)**

**log2 RNA-Seq CXCL12 ( ENSG00000107562 )**

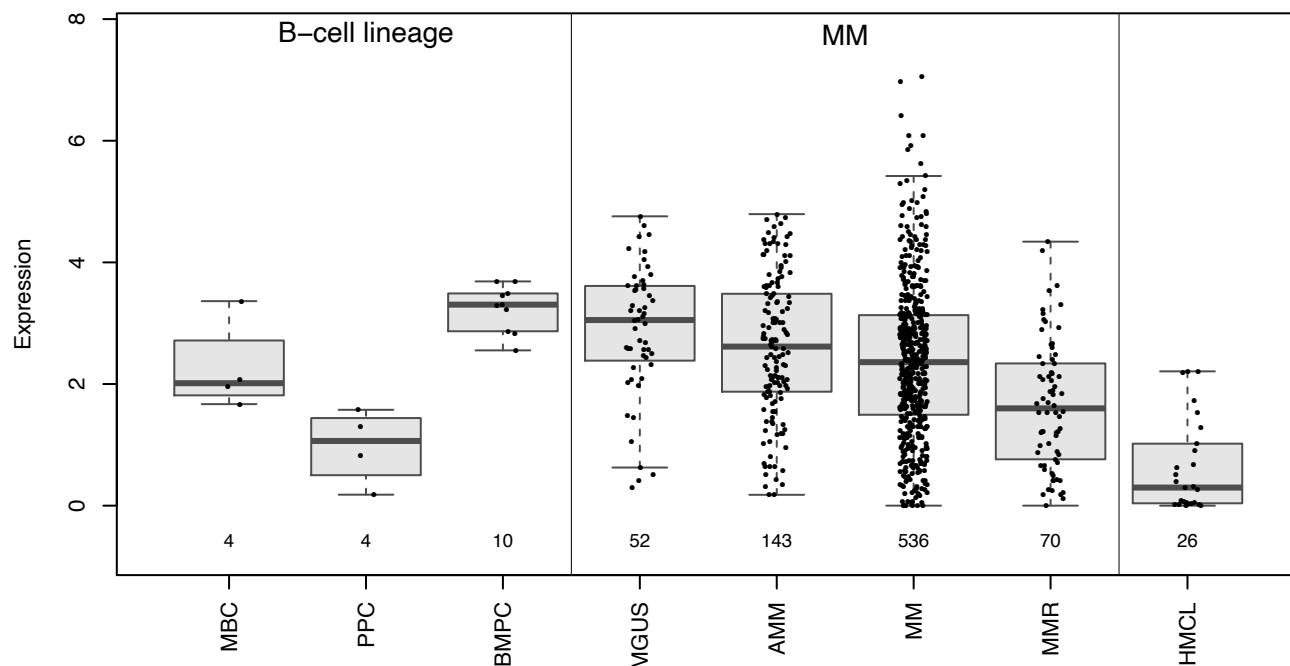


**log2 RNA-Seq LTBP2 ( ENSG00000119681 )**

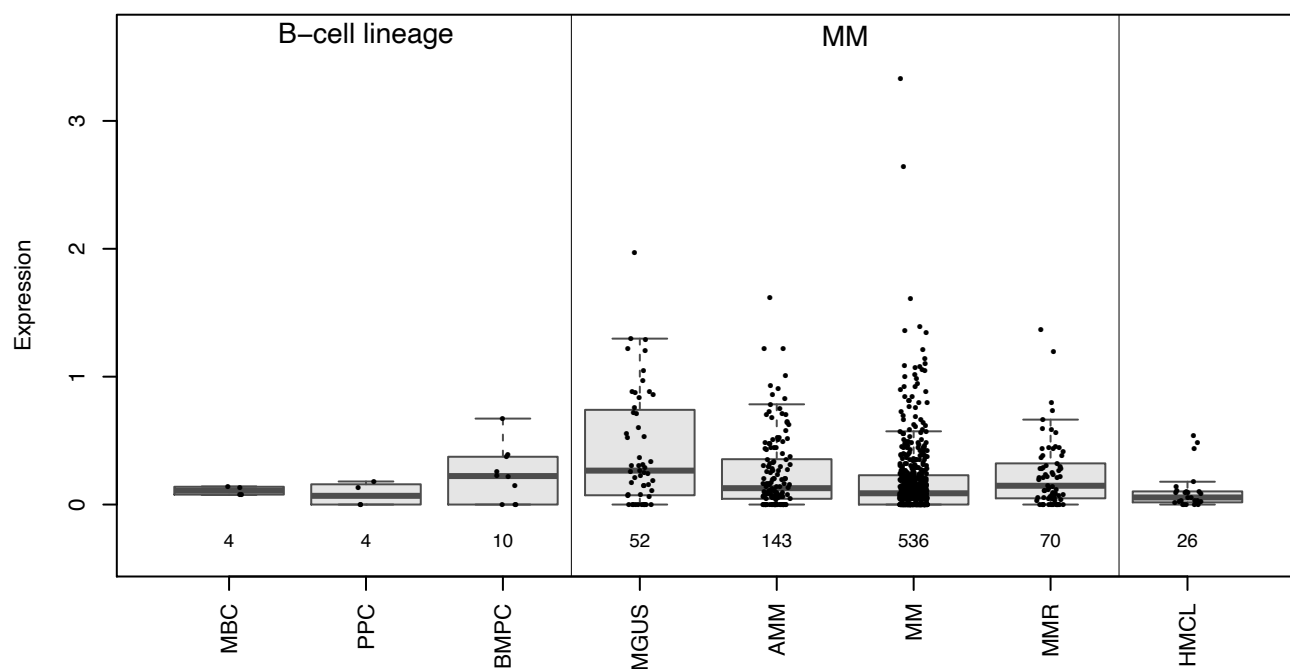


**Supplementary Figure 7 (continued)**

**log2 RNA-Seq MYL9 ( ENSG00000101335 )**

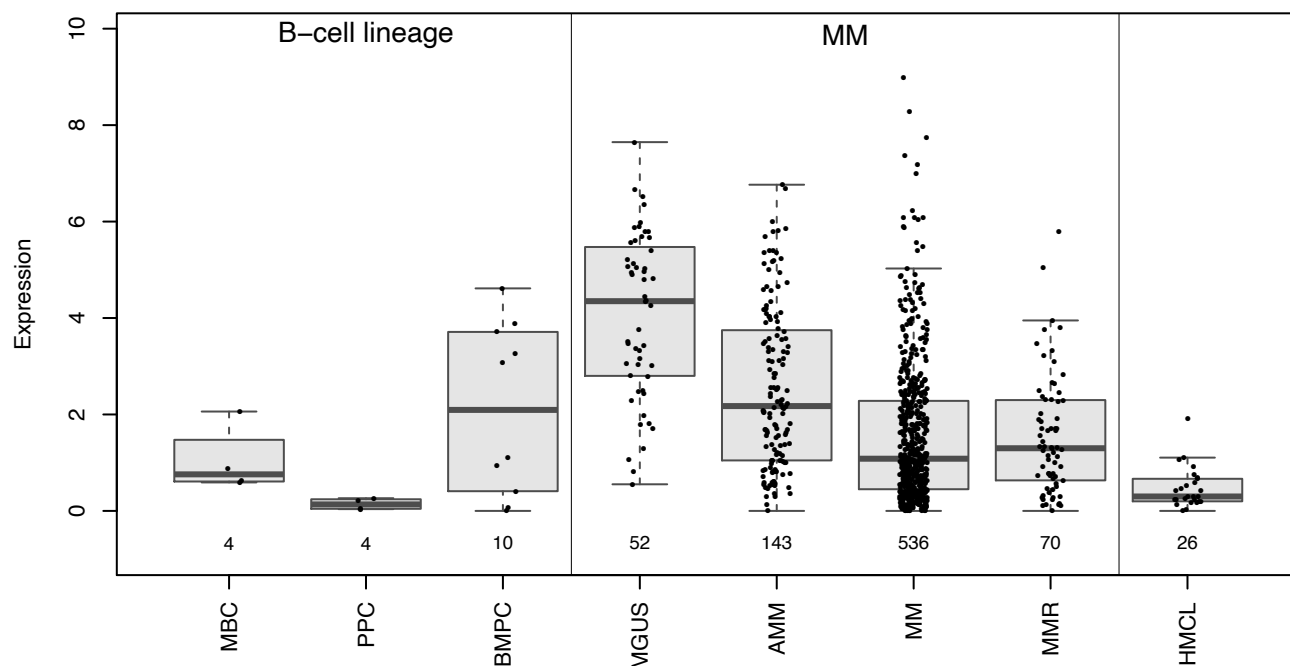


**log2 RNA-Seq MFAP5 ( ENSG00000197614 )**

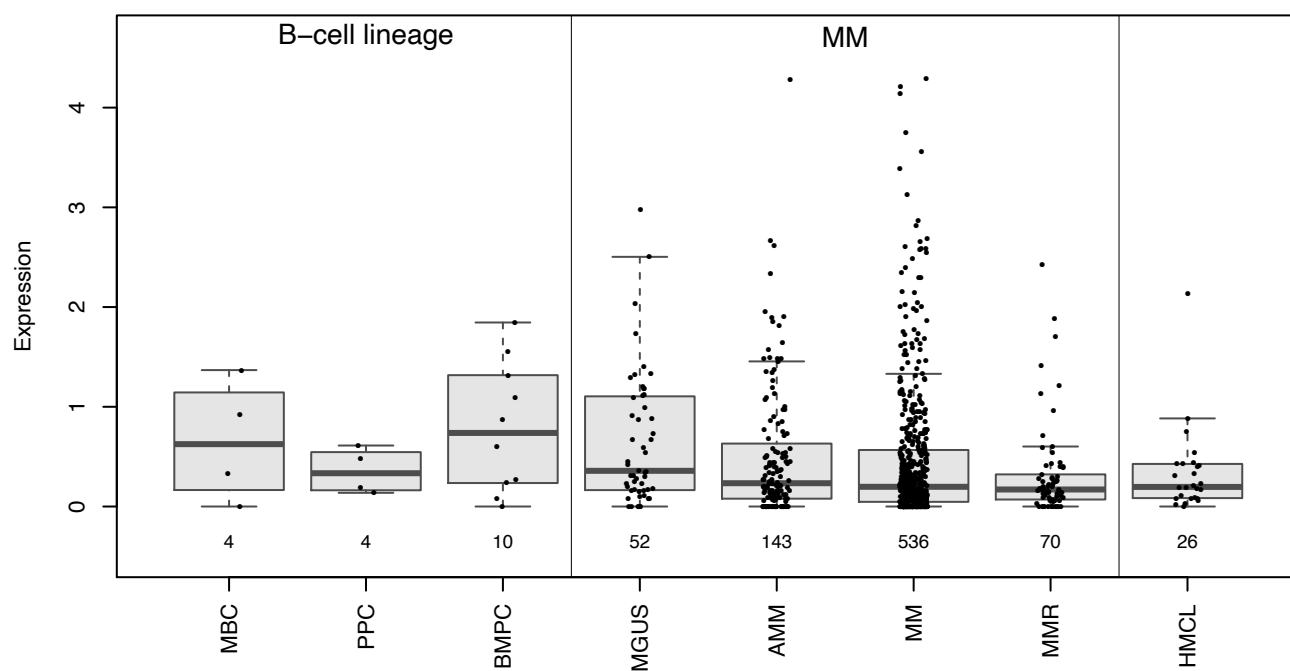


**Supplementary Figure 7 (continued)**

**log2 RNA-Seq AXL ( ENSG00000167601 )**

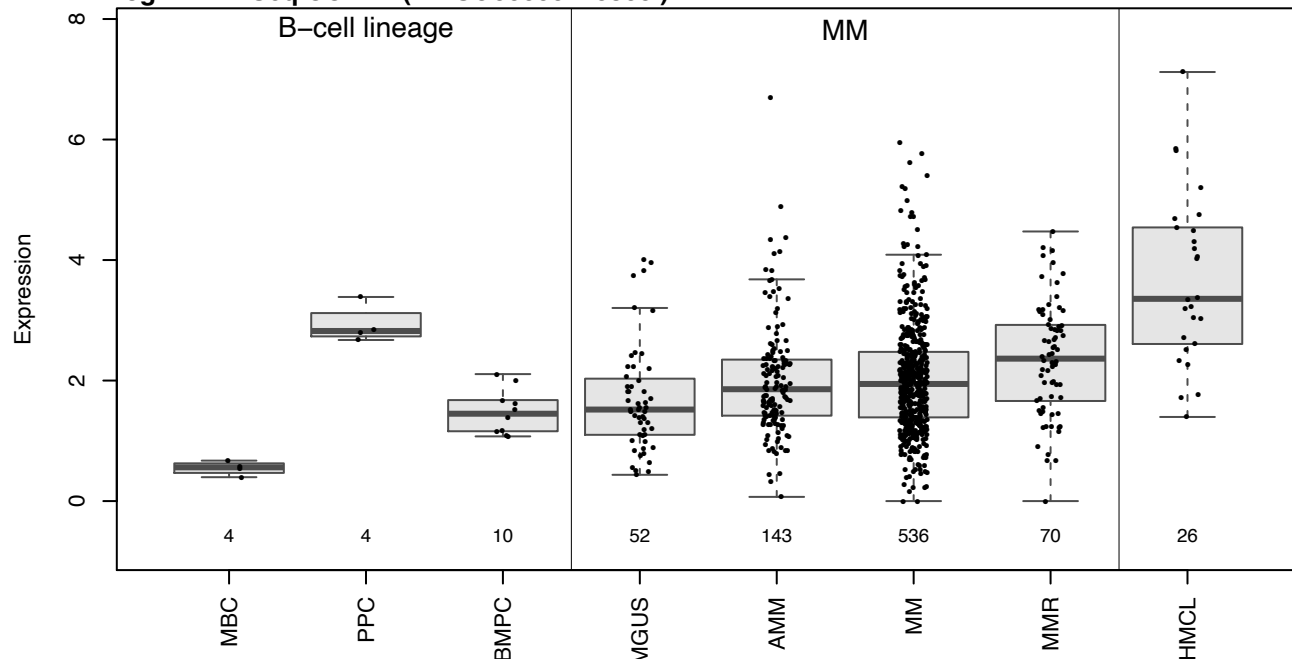


**log2 RNA-Seq MMP14 ( ENSG00000157227 )**

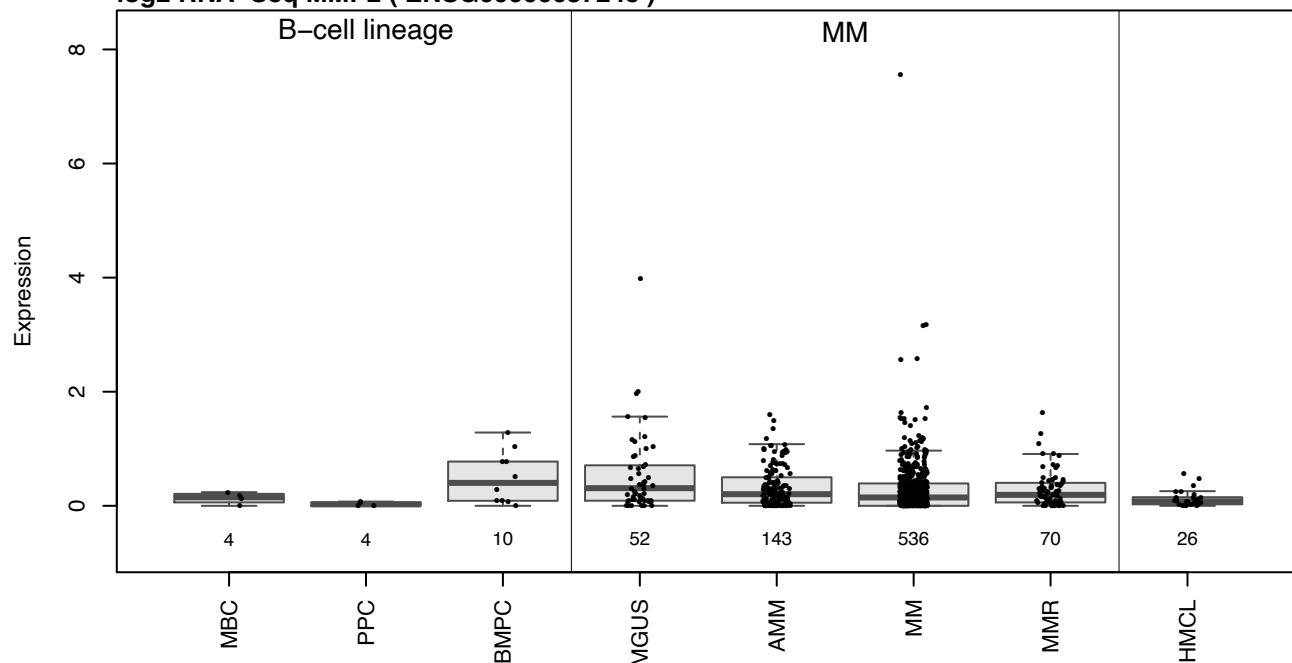


**Supplementary Figure 7 (continued)**

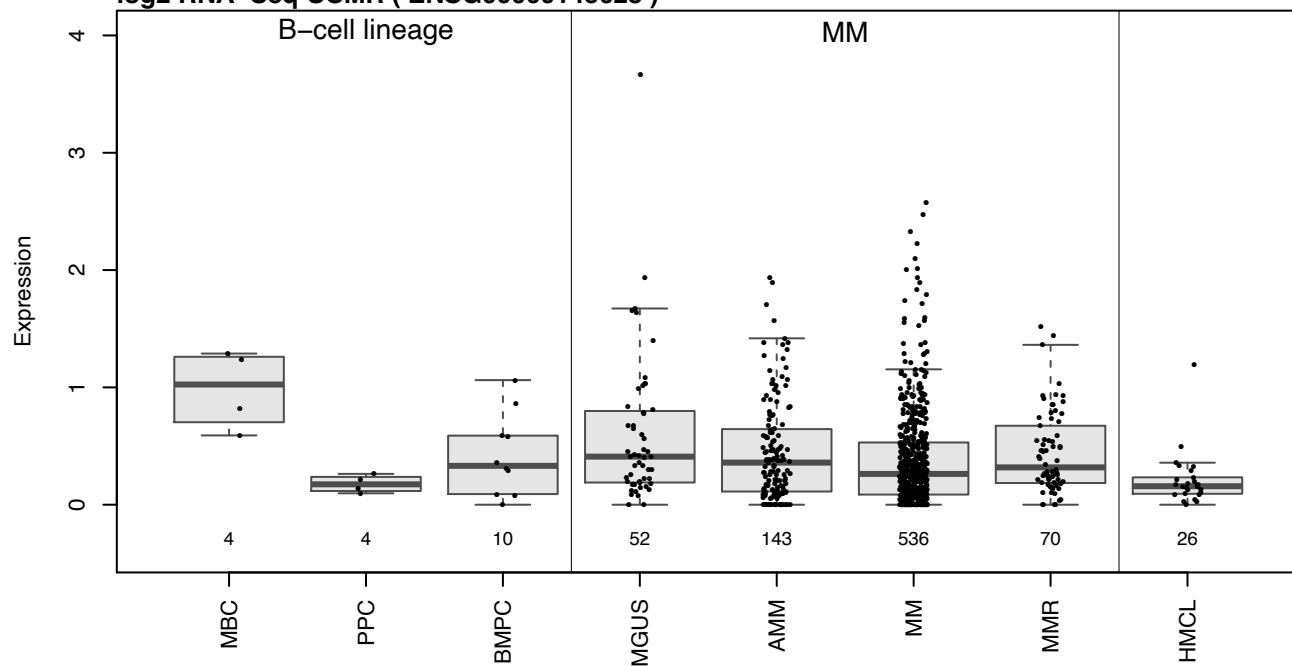
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log2 RNA-Seq MMP2 ( ENSG00000087245 )



log2 RNA-Seq OSMR ( ENSG00000145623 )





**Supplementary Figure 8:** Expression levels of adhesion genes that are not downregulated and associated with survival ( $p < 0.01$ ). Bone Marrow Plasma Cell (BMPC), Monoclonal Gammopathy of Undetermined Significance (MGUS), Smoldering Multiple Myeloma (sMM), Multiple Myeloma (MM), Multiple Myeloma Relapse (MMR).