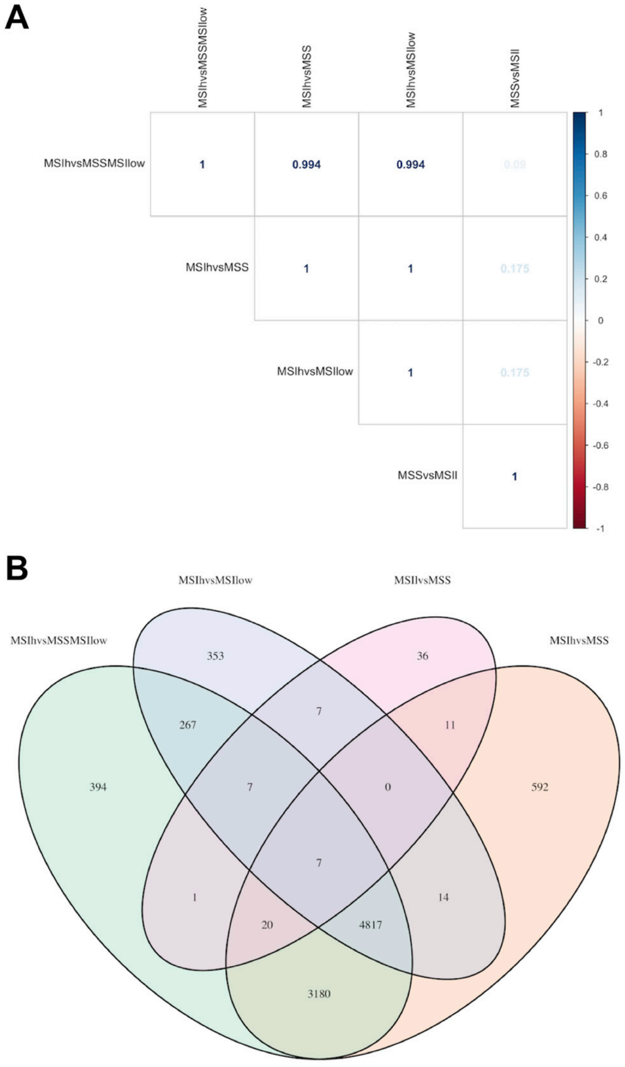
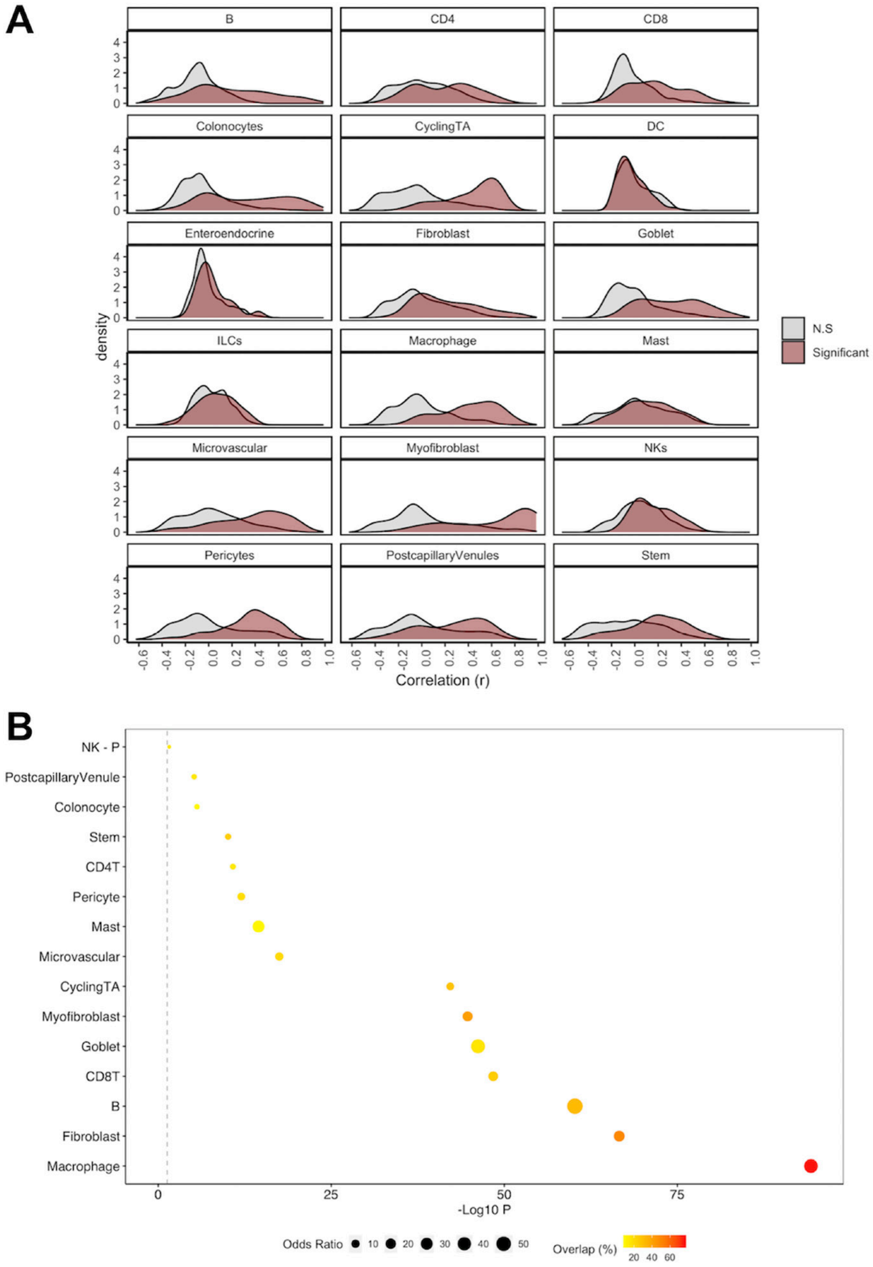
Controlling for cellular heterogeneity using single-cell deconvolution of gene expression reveals novel markers of colorectal tumors exhibiting microsatellite instability

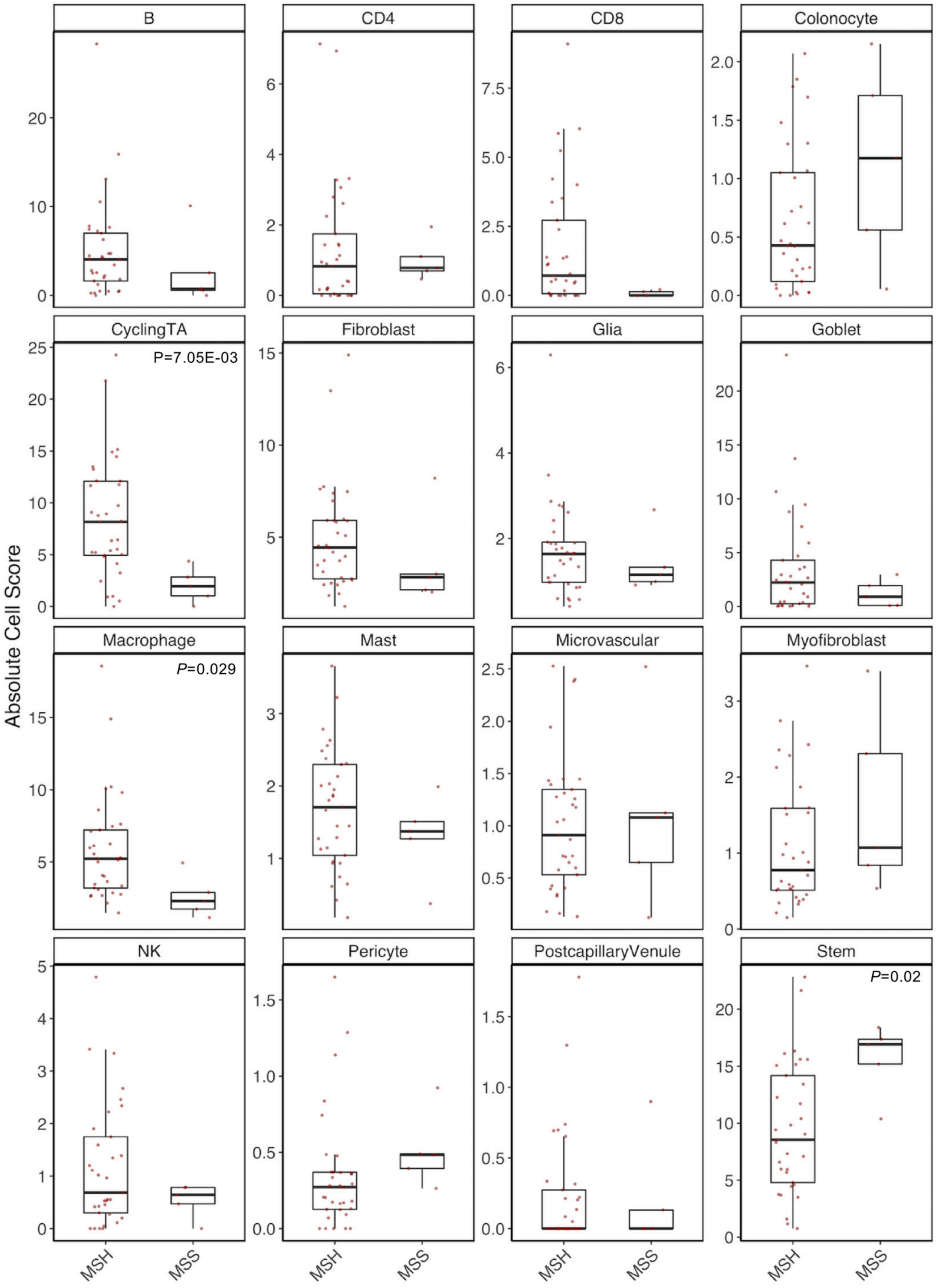
SUPPLEMENTARY MATERIALS



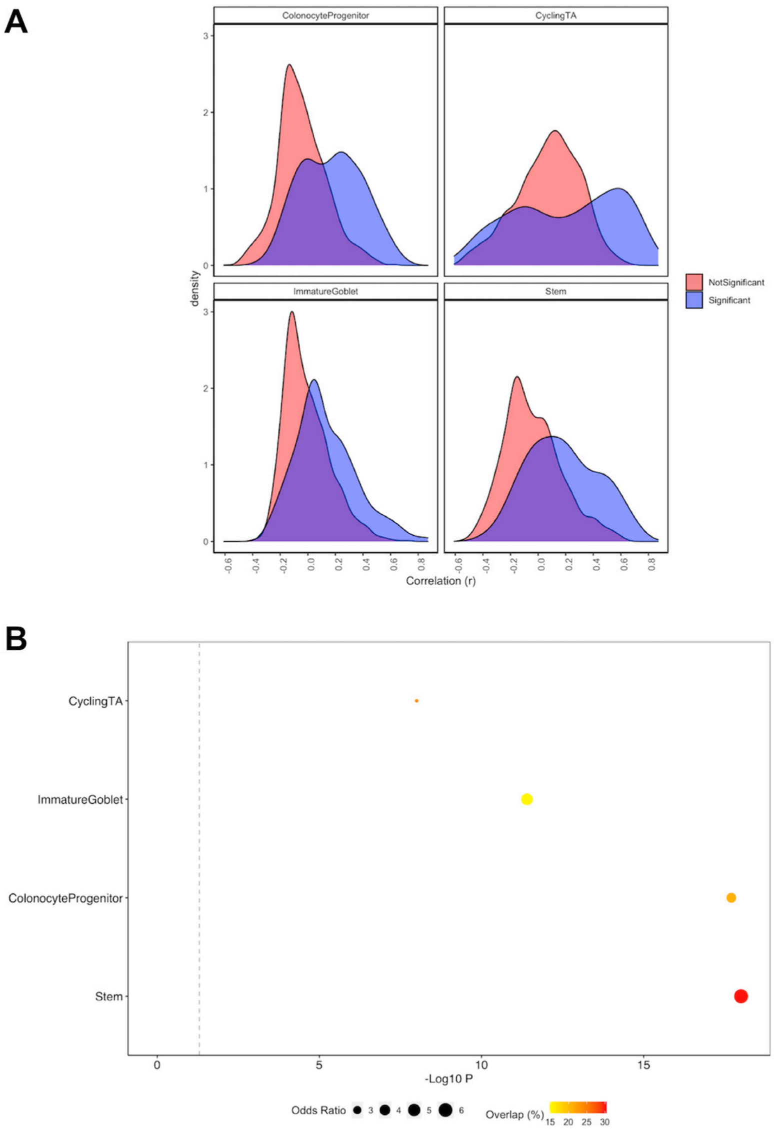
Supplementary Figure 1: Determination of TCGA-COAD comparisons. (**A**) Test-statistics for each regression were correlated to determine the similarity of global transcriptomic response between analyses. (**B**) Significant DEGs ($q = 0.05$) identified in each regression were overlaid using a Venn diagram to determine the extent of overlap.



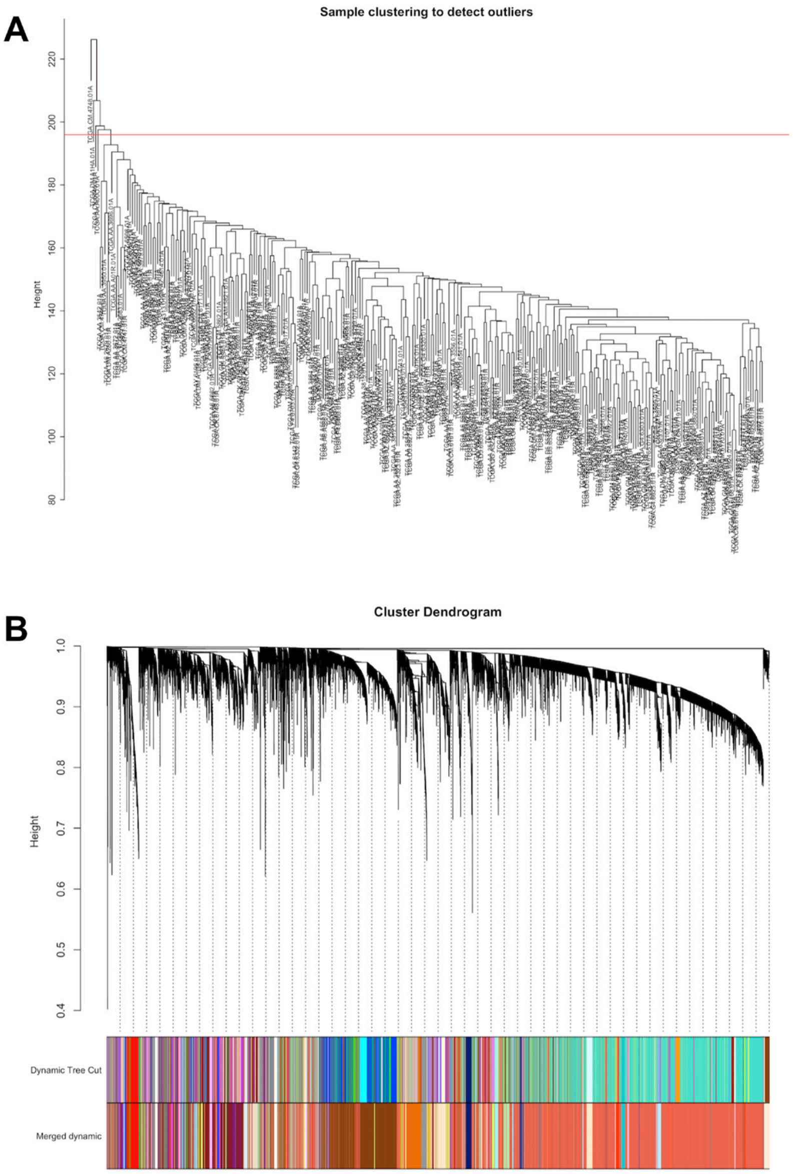
Supplementary Figure 2: Single-cell deconvolution of GSE146889 CRC tumors. (**A**) Cell scores were correlated to markers of cell types. Correlations between cell scores and significant markers of that cell type are shown in red. For background, correlations between a cell score and markers of other cell types were displayed in grey. (**B**) Summary of enrichment analysis (one-way Fisher's exact test) for cell type markers in differential expression analysis of cell scores. Grey line represents $\log_{10}(0.05)$. Percentage overlap reflects percentage of cell type markers for a given cell type that were significant within regression of cell score. For NK cells, enrichment was only identified using nominally significant DEGs ($P = 0.05$).



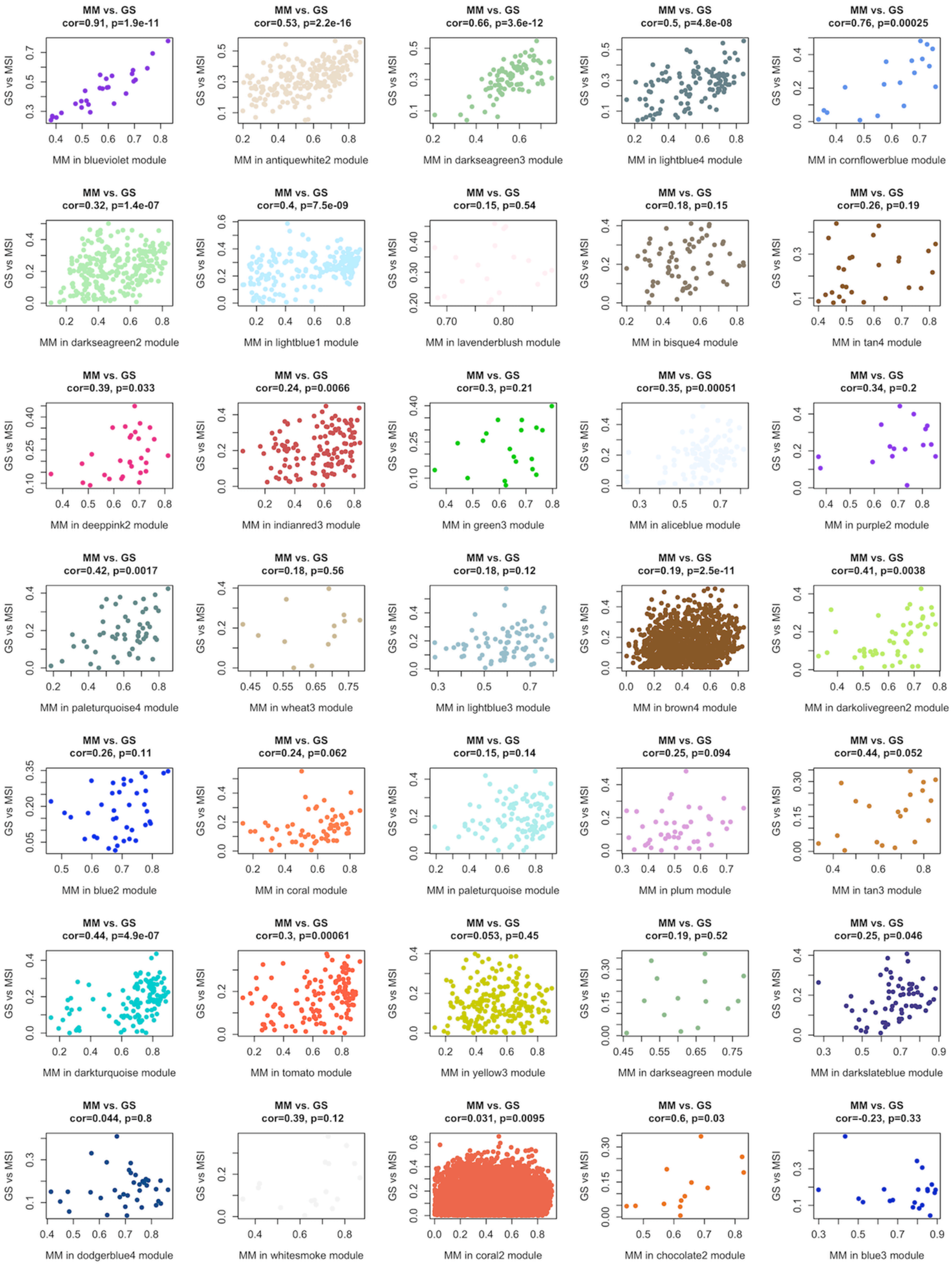
Supplementary Figure 3: Summary of cell score regressions on MSI status in GSE146889 CRC tumor dataset.



Supplementary Figure 4: Single-cell deconvolution of CCLE colon cancer cell lines. (**A**) Cell scores were correlated to markers of cell types. Correlations between cell scores and significant markers of that cell type are shown in blue. For background, correlations between a cell score and markers of other cell types were displayed in red. (**B**) Summary of enrichment analysis (one-way Fisher's exact test) for cell type markers in differential expression analysis of cell scores. Grey line represents $\log_{10}(0.05)$. Percentage overlap reflects percentage of cell type markers for a given cell type that were significant within regression of cell score.



Supplementary Figure 5: Overview of WGCNA performed in TCGA-COAD dataset. (**A**) Hierarchical clustering identified four samples as potential outliers based on their dissimilarity to other samples. These four samples were removed. (**B**) Adjacency matrix was raised to the power of 4 and transformed into a topological overlap matrix. Hierarchical clustering was performed on this matrix. Genes with high levels of co-expression are grouped. Module colors were assigned (dynamic tree cut) and modules that are highly co-expressed (*r* = 0.7) were merged (merged dynamic). These merged modules were used for downstream association testing.



Supplementary Figure 6: Summary of correlation results between gene significance and module membership for each of the 35 modules significantly associated with MSI status (q = 0.05).

Supplementary Table 1: Overlap of cell type markers with DEGs identified in MSI-H vs MSS/MSI-L analysis of TCGA-COAD cohort

Cell Type	No. DEGs overexpressed in MSI-H	No. DEGs underexpressed in MSI-H
B cell	37 [3]	16 [4]
CD4T	42 [4]	7 [0]
CD8T	60 [1]	3 [1]
Colonocyte	45 [2]	101 [6]
CyclingTA	91 [9]	28 [4]
DC	59 [4]	13 [1]
Enteroendocrine	16 [1]	17 [2]
Fibroblast	49 [3]	107 [10]
Glia	NA$^+$	NA
Goblet	52 [6]	29 [2]
ILCs	18 [1]	13 [2]
Macrophages	80 [2]	9 [1]
Mast	29 [6]	17 [1]
Microvascular	22 [3]	13 [7]
Myofibroblasts	10 [2]	20 [1]
NKs	16 [0]	1 [0]
Pericytes	8 [0]	26 [1]
Postcapillary Venules	18 [3]	15 [1]
Stem	9 [7]	41 [2]

Values without brackets represent FDR corrected DEGs ($q = 0.05$). Values in brackets represent the number of additional DEGs that were nominally significant ($P = 0.05$) that did not reach FDR correction. $^+$Cell markers not available.

Supplementary Table 2: Correlation of cell-type expression markers to cell scores generated in each approach. See Supplementary Table 2

Supplementary Table 3: Cell type agnostic DEGs found to be significant (*q* = 0.05) in regression analysis of MSI status in TCGA-COAD data that were replicated in similar analysis of CCLE (*P* = 0.05). See Supplementary Table 3

Supplementary Table 4: Overview of modules identified in WGCNA. See Supplementay Table 4

Supplementary Table 5: Novel significant DEGs (*q* = 0.05) identified in TCGA-COAD regression of MSI-H vs MSS/MSI-L tumors following adjustment for cell composition that were replicated in a similar analysis of colon cancer cell lines (*P* = 0.05). See Supplementary Table 5