SUPPLEMENTARY MATERIALS

Prognostic power of a tumor differentiation gene signature for bladder urothelial carcinomas

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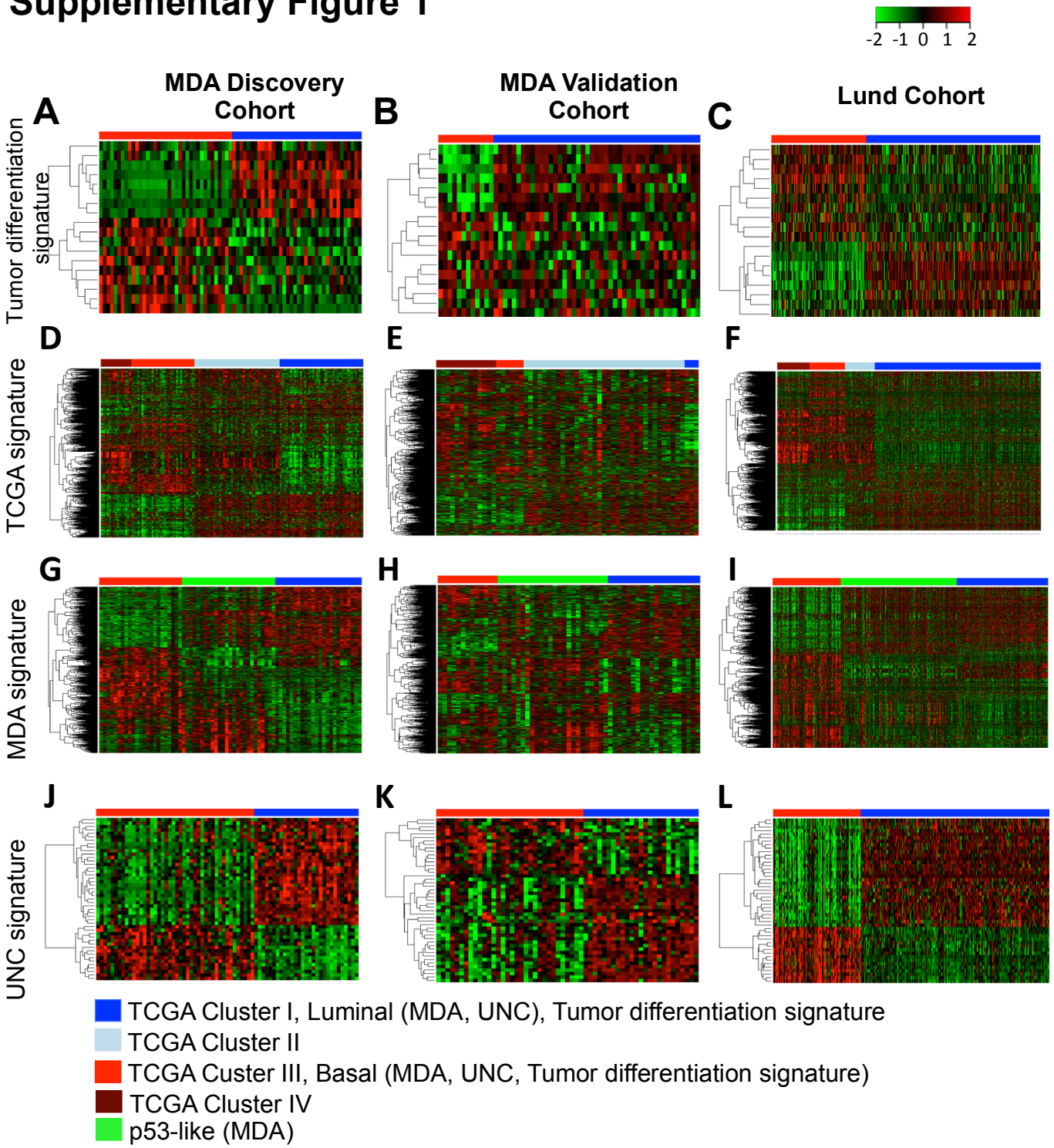
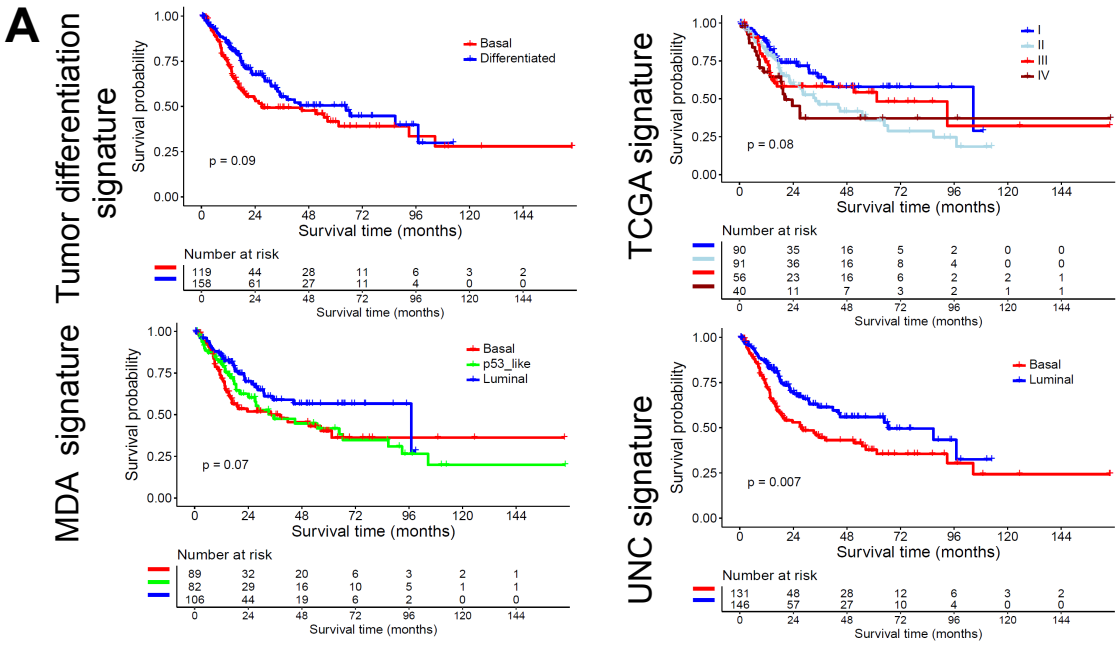
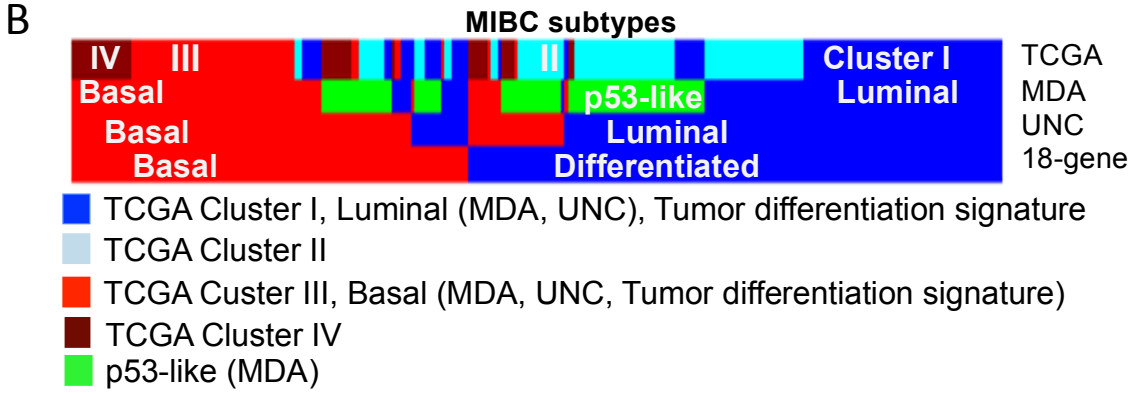
Supplementary Figure 1

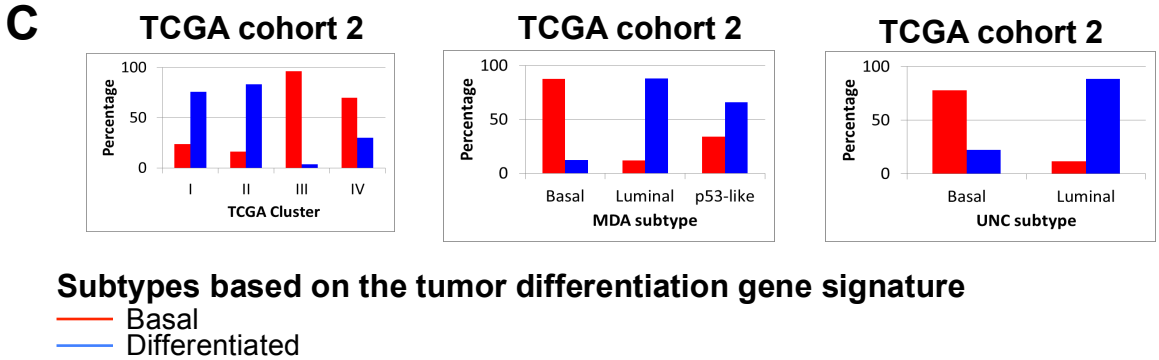


Supplementary Figure 1. Classification of MIBCs into various subtypes by four different gene signatures. Classification of MDA discovery and MDA validation cohorts of MIBC patients using the 1-nearest neighbor algorithm and the tumor differentiation (**A-B**), TCGA (**D-E**), MDA (**G-H**) and UNC (**J-K**) signatures respectively. The tumor differentiation signature stratifies MIBC patients into basal (red) and differentiated (blue) subtypes. The TCGA signature classifies MIBC patients into four clusters: I, II, III and IV as indicated by colorimetric legends. The MDA signature classifies MIBC patients into three subtypes: basal (red), p53-like (green), and luminal (blue). The UNC signature classifies MIBC patients into basal (red) and luminal (blue) subtypes. **(C,F,I,L)** Classification of Lund cohort of patients, which comprises of both muscle-invasive and non-muscle invasive bladder cancers using the same methodology as in MIBC pure cohorts. Although non-invasive and muscle-invasive bladder cancers were reported to develop through independent pathogenesis with certain overlap, the signatures were able to stratify them into similar subtypes as those in MIBC pure cohorts.

Supplementary Figure 2

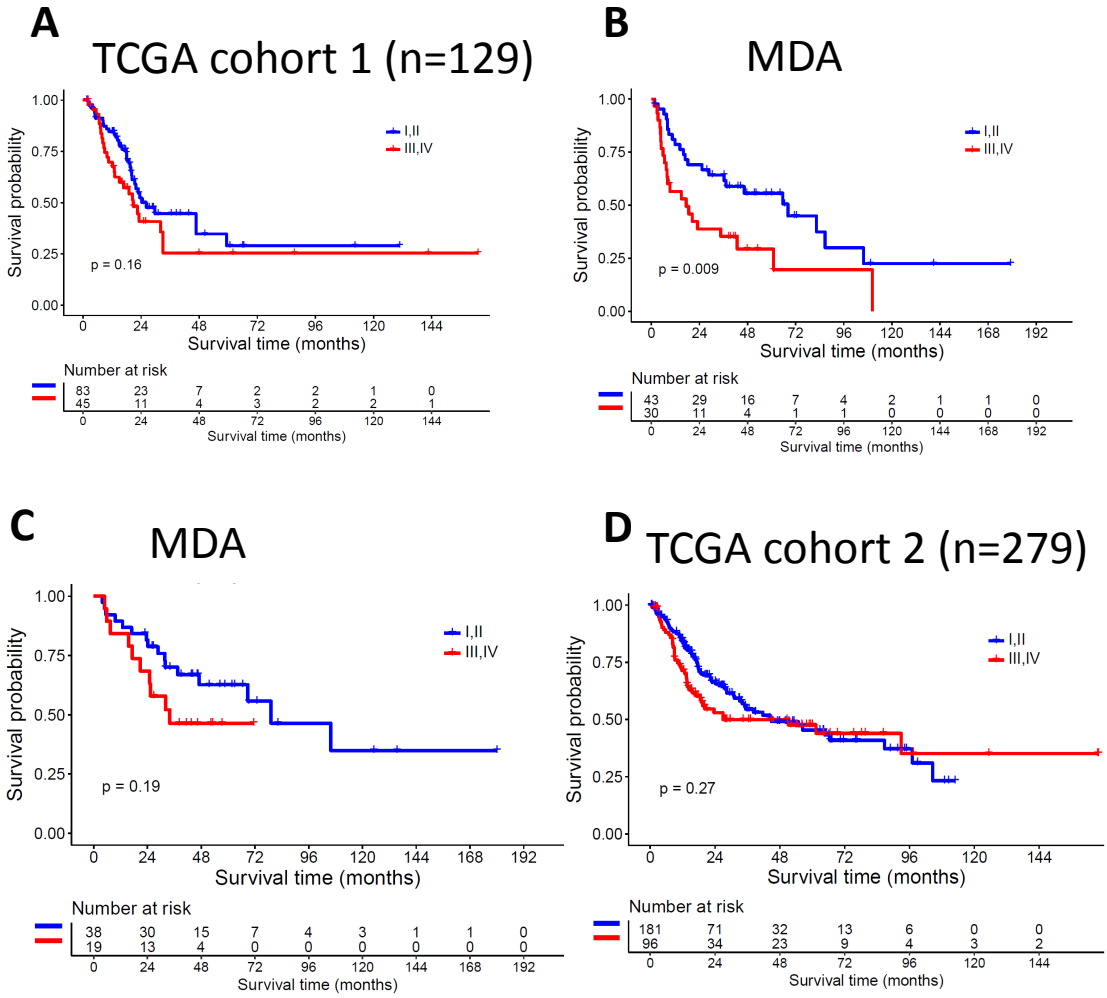






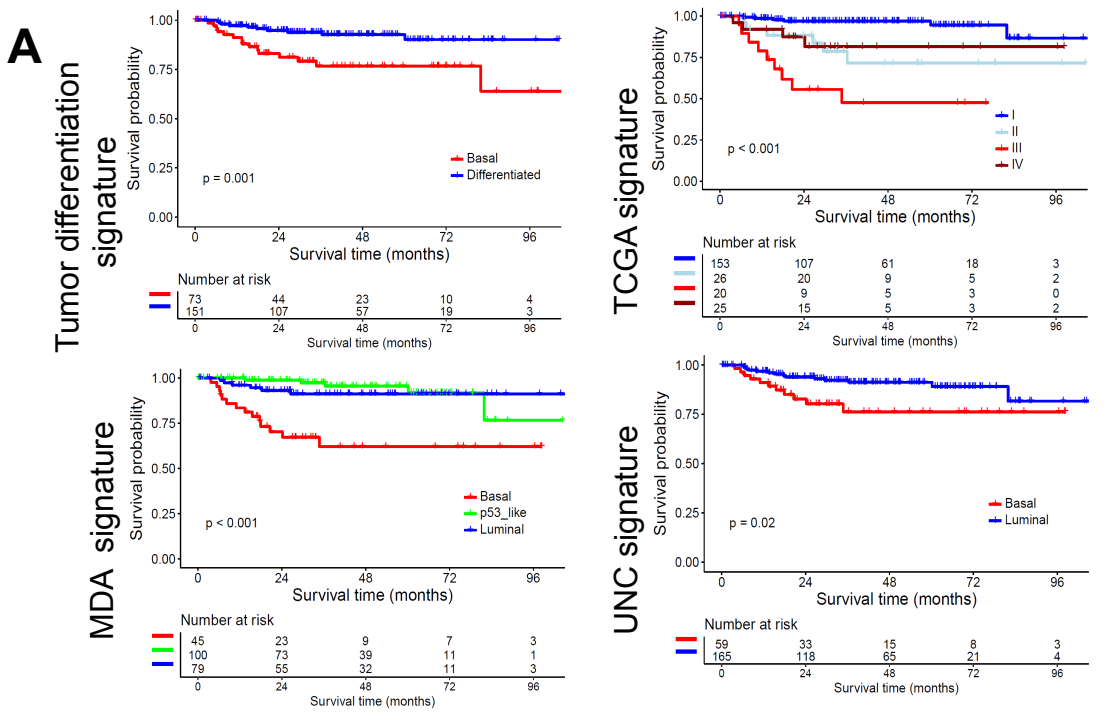
Supplementary Figure 2. Analysis of the unpublished TCGA cohort by four different molecular signatures. (A) Application of the tumor differentiation signature in the unpublished TCGA cohort (n=279) in direct comparison with the TCGA/MDA/UNC signatures in stratifying overall survival of MIBC patients. (B**)** Colorimetric chart demonstrating the distribution of unpublished TCGA cohort patients assigned by TGCA/MDA/UNC and the tumor differentiation signatures and their relation to other signatures. **(C)** Bar graph representation demonstrating the percentage of basal and differentiated tumors assigned by the tumor differentiation signature, in direct comparison to subtype assignments by the TCGA/MDA/UNC signatures in the unpublished TCGA cohort of patients. The P values (Log-rank test) are two-sided.

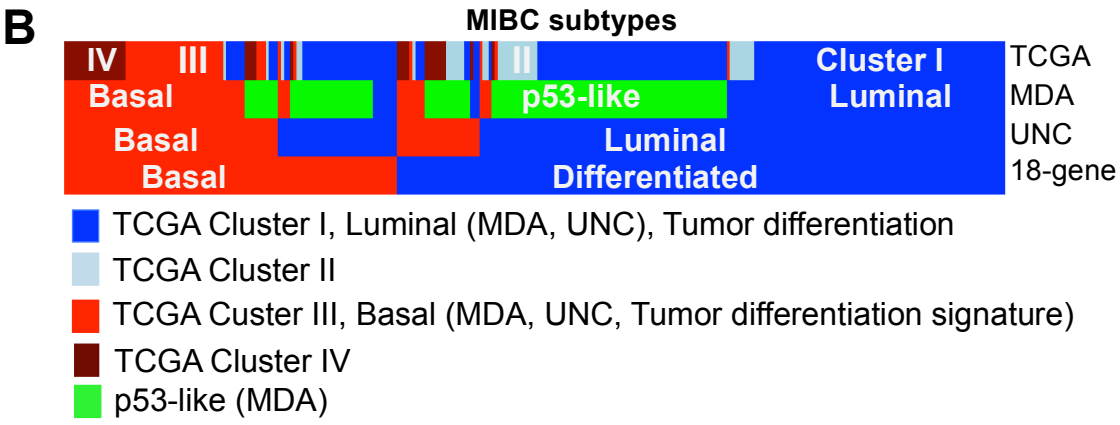
Supplementary Figure 3

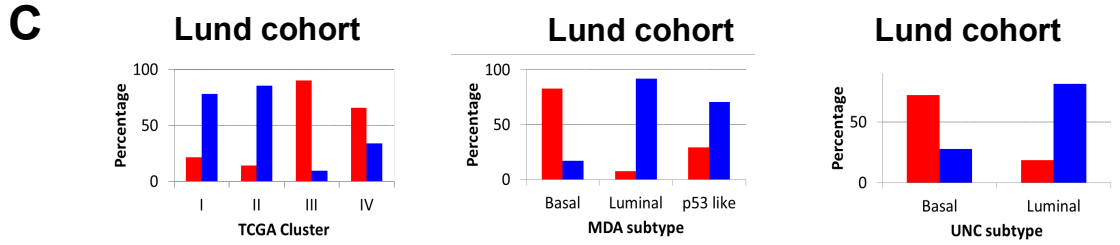


Supplementary Figure 3. Analysis of MIBC cohorts by combining TCGA molecular clusters. Analysis of the overall survival probability in the published TCGA cohort 1 (n=129) **(A)**, unpublished TCGA cohort 2 (n=279) **(B),** MDA Discovery **(C)**, and MDA Validation **(D)** MIBC cohorts by combining TCGA cluster I+II and cluster III+IV. The P values (Log-rank test) are two-sided.

Supplementary Figure 4

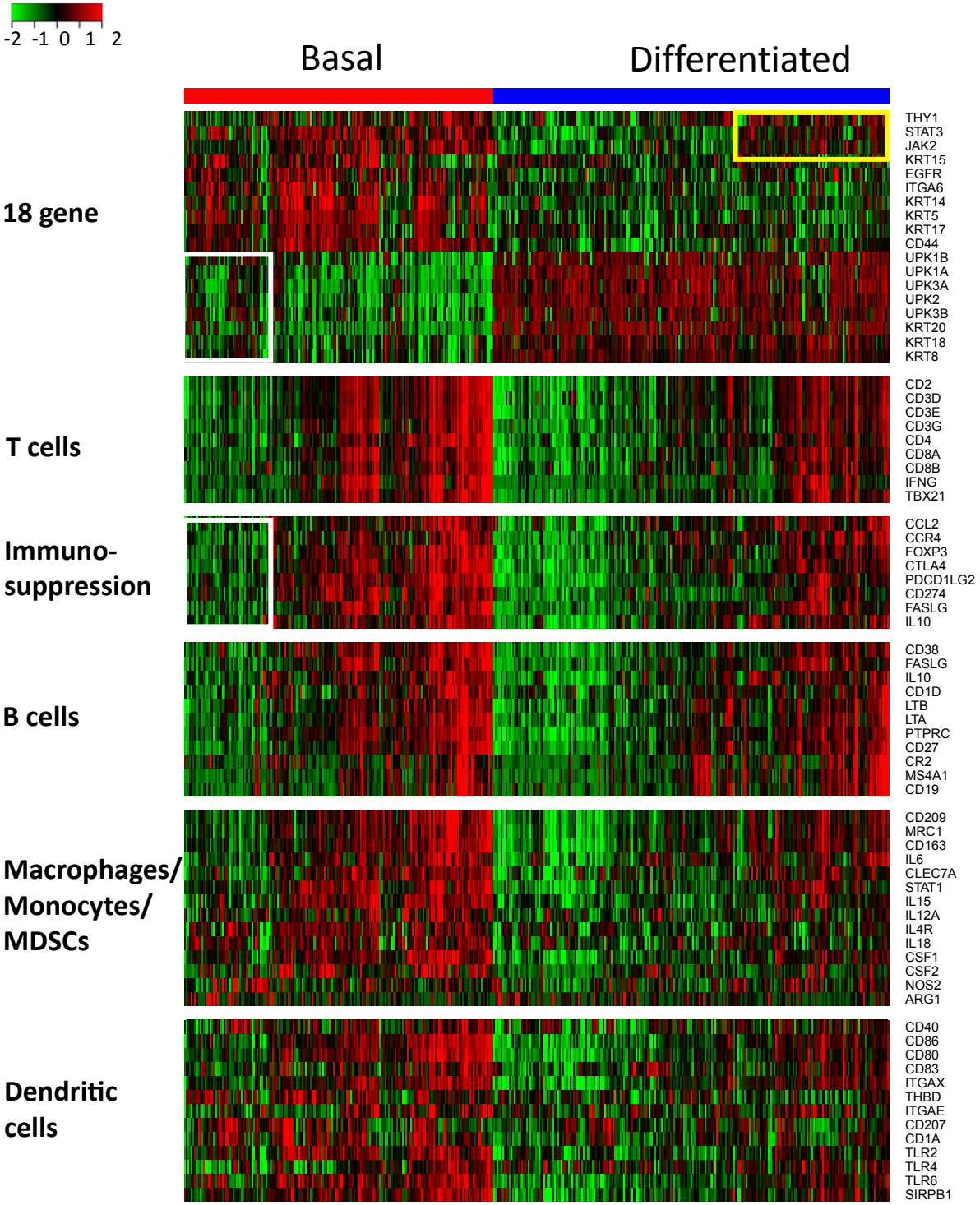






Supplementary Figure 4. Analysis of the Lund cohort with both muscle-invasive and non-invasive bladder cancers. (A) Application of the tumor differentiation signature in the Lund cohorts in direct comparison with the TCGA/MDA/UNC signatures in stratifying overall survival of MIBC patients. (B**)** Colorimetric chart demonstrating the distribution of Lund cohort patients assigned by TGCA/MDA/UNC and the tumor differentiation signatures and their relation to other signatures. **(C)** The percentage of basal and differentiated tumors assigned by the tumor differentiation signature, in the subtype assignments by the TCGA/MDA/UNC signatures in the Lund cohort of patients. The P values (Log-rank test) are two-sided.

Supplementary Figure 5



Supplementary Figure 5. Highlighted portions of the immune cell gene expression analysis in basal and differentiated MIBCs of the combined TCGA cohort 1 and 2. Replica of heat map from figure 6F with highlighted areas of differential expression in differentiated (yellow boxes) and basal (white boxes) subgroups.