# Table S1-metadata

| samples | scATAC | visiumHD | scRNA-seq | K27ac | RNA-seq | stage | material | histology |
|---|---|---|---|---|---|---|---|---|
| HGT1_1 | yes | | | | | | FF | URO |
| HGT1_2 | yes | | | | | | FF | URO |
| mpbc1 | | yes | | | | MIBC | FFPE | URO+MP |
| mpbc2 | | yes | | | | MIBC | FFPE | URO+MP |
| vh176 | | | yes | yes | yes | NMIBC-HGT | FFPE | URO |
| vh61 | | | yes | yes | yes | NMIBC-HGT | FFPE | URO |
| vh12 | | | yes | | yes | NMIBC-HGT | FFPE | URO |
| vh122 | | | yes | | yes | NMIBC-HGT | FFPE | URO |
| vh125 | | | yes | | yes | NMIBC-HGT | FFPE | URO |
| vh15 | | | yes | | yes | NMIBC-HGT | FFPE | URO |
| vh24 | | | yes | | yes | NMIBC-HGT | FFPE | URO |
| vh1 | | | yes | yes | | NMIBC-HGT | FFPE | URO |
| vh75 | | | yes | | | NMIBC-HGT | FFPE | URO |
| vh100 | | | | yes | yes | NMIBC-HGT | FFPE | URO |
| vh126 | | | | yes | yes | NMIBC-HGT | FFPE | URO |
| vh21 | | | | yes | yes | NMIBC-HGT | FFPE | URO |
| vh81 | | | | yes | yes | NMIBC-HGT | FFPE | URO |
| vh93 | | | | yes | yes | NMIBC-HGT | FFPE | URO |
| vh96 | | | | yes | yes | NMIBC-HGT | FFPE | URO |
| vh101 | | | | | yes | NMIBC-HGT | FFPE | URO |
| vh102 | | | | | yes | NMIBC-HGT | FFPE | URO |
| vh103 | | | | | yes | NMIBC-HGT | FFPE | URO |
| vh105 | | | | | yes | NMIBC-HGT | FFPE | URO |
| vh112 | | | | | yes | NMIBC-HGT | FFPE | URO |
| vh113 | | | | | yes | NMIBC-HGT | FFPE | URO |
| vh116 | | | | | yes | NMIBC-HGT | FFPE | URO |
| vh121 | | | | | yes | NMIBC-HGT | FFPE | URO |
| vh123 | | | | | yes | NMIBC-HGT | FFPE | URO |
| vh134 | | | | | yes | NMIBC-HGT | FFPE | URO |
| vh135 | | | | | yes | NMIBC-HGT | FFPE | URO |
| vh14 | | | | | yes | NMIBC-HGT | FFPE | URO |
| vh140 | | | | | yes | NMIBC-HGT | FFPE | URO |
| vh144 | | | | | yes | NMIBC-HGT | FFPE | URO |
| vh145 | | | | | yes | NMIBC-HGT | FFPE | URO |
| vh146 | | | | | yes | NMIBC-HGT | FFPE | URO |
| vh150 | | | | | yes | NMIBC-HGT | FFPE | URO |
| vh151 | | | | | yes | NMIBC-HGT | FFPE | URO |
| vh159 | | | | | yes | NMIBC-HGT | FFPE | URO |
| vh16 | | | | | yes | NMIBC-HGT | FFPE | URO |
| vh163 | | | | | yes | NMIBC-HGT | FFPE | URO |
| vh165 | | | | | yes | NMIBC-HGT | FFPE | URO |
| vh171 | | | | | yes | NMIBC-HGT | FFPE | URO |
| vh180 | | | | | yes | NMIBC-HGT | FFPE | URO |
| vh184.c | | | | | yes | NMIBC-HGT | FFPE | URO |
| vh19 | | | | | yes | NMIBC-HGT | FFPE | URO |
| vh29 | | | | | yes | NMIBC-HGT | FFPE | URO |
| vh30 | | | | | yes | NMIBC-HGT | FFPE | URO |
| vh31 | | | | | yes | NMIBC-HGT | FFPE | URO |
| vh32 | | | | | yes | NMIBC-HGT | FFPE | URO |
| vh34 | | | | | yes | NMIBC-HGT | FFPE | URO |
| vh42 | | | | | yes | NMIBC-HGT | FFPE | URO |
| vh45 | | | | | yes | NMIBC-HGT | FFPE | URO |
| vh46 | | | | | yes | NMIBC-HGT | FFPE | URO |
| vh49 | | | | | yes | NMIBC-HGT | FFPE | URO |
| vh5 | | | | | yes | NMIBC-HGT | FFPE | URO |
| vh52 | | | | | yes | NMIBC-HGT | FFPE | URO |
| vh54 | | | | | yes | NMIBC-HGT | FFPE | URO |
| vh55 | | | | | yes | NMIBC-HGT | FFPE | URO |
| vh56 | | | | | yes | NMIBC-HGT | FFPE | URO |
| vh62 | | | | | yes | NMIBC-HGT | FFPE | URO |
| vh66 | | | | | yes | NMIBC-HGT | FFPE | URO |
| vh68 | | | | | yes | NMIBC-HGT | FFPE | URO |
| vh7 | | | | | yes | NMIBC-HGT | FFPE | URO |
| vh73 | | | | | yes | NMIBC-HGT | FFPE | URO |
| vh78 | | | | | yes | NMIBC-HGT | FFPE | URO |
| vh79 | | | | | yes | NMIBC-HGT | FFPE | URO |
| vh91 | | | | | yes | NMIBC-HGT | FFPE | URO |
| vh97 | | | | | yes | NMIBC-HGT | FFPE | URO |
| MP1 | | | | yes | | | FFPE | MP |
| MP3 | | | | yes | | | FFPE | MP |
| MP5 | | | | yes | | | FFPE | MP |
| MP6 | | | | yes | | | FFPE | MP |
| MP8 | | | | yes | | | FFPE | MP |
| MP9 | | | | yes | | | FFPE | MP |
| vh127 | | | | yes | | | FFPE | MP |
| vh77 | | | | yes | | | FFPE | MP |

Table S2. FiTAc-seq information

| Sample | UniqMappedReads(M) | TotalPeaks | FRiP | DHS_% |
|---|---|---|---|---|
| MP1 | 57.6 | 15503 | 4.4 | 85.34 |
| MP3 | 47 | 40979 | 7.9 | 94.1 |
| MP5 | 50.7 | 38726 | 10.7 | 96.7 |
| MP6 | 64 | 56555 | 36.6 | 99.3 |
| MP8 | 56.5 | 21048 | 6.4 | 96.12 |
| MP9 | 96.2 | 52180 | 23.5 | 99.28 |
| UCC1 | 36.3 | 41461 | 23.8 | 99.34 |
| UCC10 | 154.9 | 29937 | 13.7 | 95.32 |
| UCC11 | 74.4 | 31915 | 16 | 95.82 |
| UCC12 | 75.5 | 29122 | 17.4 | 97.4 |
| UCC13 | 152.5 | 40480 | 15.9 | 95.7 |
| UCC3 | 73.2 | 10606 | 3.1 | 90.42 |
| UCC4 | 78.3 | 39672 | 20.5 | 98.78 |
| UCC5 | 80.2 | 44241 | 25.6 | 99.5 |
| UCC7 | 79.8 | 53933 | 29.7 | 99.66 |
| UCC8 | 57 | 53594 | 33.3 | 99.36 |
| UCC9 | 89.7 | 33869 | 19.1 | 96.86 |

# Table S3. Motif analysis

| MP | | | | | | | | vs | URO | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Name | PValue | log(PValue) | # Target Sequ | % of Targets S | # Backgrounc | % of Background | Sequences with Motif | | Name | PValue | log(PValue) | # Target Seq | % of Targets | # Backgroun | % of Background | Sequences with Motif |
| Jun-AP1(bZIP)/K562-cJun-ChIP-Seq( | 1.00E-07 | -17.703283 | 278 | 9.75% | 3205 | 6.98% | | | p73(p53)/p6 | 1.00E-173 | -399.82086 | 675 | 11.15% | 1373.7 | 3.11% | |
| GRHL2(CP2)/HBE-GRHL2-ChIP-Sec | 1.00E-03 | -8.608498 | 442 | 15.51% | 6055.5 | 13.18% | | | IRF2(IRF)/Er | 1.00E-26 | -60.973941 | 700 | 11.56% | 3372.9 | 7.64% | |
| Unknown3/Drosophila-Promoters/H | 1.00E-02 | -6.194808 | 92 | 3.23% | 1082.9 | 2.36% | | | Ets1-distal(E | 1.00E-22 | -51.614331 | 1529 | 25.25% | 8848.5 | 20.03% | |
| TFE3(bHLH)/MEF-TFE3-ChIP-Seq(G | 1.00E-02 | -5.241718 | 79 | 2.77% | 940.1 | 2.05% | | | Bach1(bZIP)/ | 1.00E-16 | -38.368215 | 346 | 5.71% | 1564.1 | 3.54% | |
| RFX(HTH)/K562-RFX3-ChIP-Seq(SR | 1.00E-02 | -6.567761 | 69 | 2.42% | 757.2 | 1.65% | | | Tcfcp2l1(CP2 | 1.00E-15 | -35.744305 | 743 | 12.27% | 4033 | 9.13% | |
| MP + URO-LLI | | | | | | | | | URO-BL | | | | | | | |
| Name | PValue | log(PValue) | # Target Sequ | % of Targets S | # Backgrounc | % of Background | Sequences with Motif | | Name | PValue | log(PValue) | # Target Seq | % of Targets | # Backgroun | % of Background | Sequences with Motif |
| Hnf1(Homeobox)/Liver-Foxa2-Chip- | 1.00E-07 | -16.192816 | 261 | 11.36% | 3818 | 8.21% | | | p73(p53)/p6 | 1.00E-211 | -487.82993 | 901 | 10.24% | 1265.9 | 3.05% | |
| GRHL2(CP2)/HBE-GRHL2-ChIP-Sec | 1.00E-04 | -10.903541 | 497 | 21.64% | 8477.4 | 18.22% | | | Jun-AP1(bZIF | 1.00E-141 | -326.93713 | 2290 | 26.03% | 6429 | 15.48% | |
| FAR1(FAR1)/col-FAR1-DAP-Seq(GSI | 1.00E-03 | -7.455878 | 51 | 2.22% | 629.9 | 1.35% | | | Tcfcp2l1(CP2 | 1.00E-32 | -75.316065 | 1052 | 11.96% | 3417.7 | 8.23% | |
| GT1(Trihelix)/col-GT1-DAP-Seq(GSE | 1.00E-03 | -8.266723 | 13 | 0.57% | 8.6 | 0.19% | | | GFY-Staf(?,Z | 1.00E-13 | -31.923202 | 309 | 3.51% | 920.8 | 2.22% | |
| GATA3(Zf),DR8/iTreg-Gata3-ChIP-Se | 1.00E-03 | -7.484742 | 106 | 4.61% | 153.5 | 3.32% | | | RORg(NR)/L | 1.00E-13 | -30.50067 | 839 | 9.54% | 3065.9 | 7.38% | |
| MP + URO-BL | | | | | | | | | URO-LLI | | | | | | | |
| Name | PValue | log(PValue) | # Target Seq | % of Targets | # Backgrounc | % of Background | Sequences with Motif | | Name | PValue | log(PValue) | # Target Seq | % of Targets | # Backgroun | % of Background | Sequences with Motif |
| Jun-AP1(bZIP)/K562-cJun-ChIP-Seq( | 1.00E-71 | -164.14494 | 564 | 39.14% | 9219.6 | 18.80% | | | IRF2(IRF)/Er | 1.00E-18 | -43.435107 | 202 | 16.37% | 4112.1 | 8.42% | |
| p73(p53)/p63/Trachea-p73-ChIP-Se | 1.00E-18 | -43.563735 | 128 | 8.88% | 1786 | 3.64% | | | Ets1-distal(E | 1.00E-16 | -37.704382 | 376 | 30.47% | 9958.7 | 20.39% | |
| Ets1-distal(ETS)/CD4+-PolII-ChIP-Se | 1.00E-07 | -17.527748 | 436 | 30.26% | 11736.6 | 23.93% | | | ETS:RUNX(ET | 1.00E-07 | -16.576669 | 143 | 11.59% | 3582.7 | 7.34% | |
| Tcfcp2l1(CP2)/mES-Tcfcp2l1-ChIP-S | 1.00E-05 | -12.255455 | 197 | 13.67% | 4893.1 | 9.98% | | | T1ISRE(IRF)/ | 1.00E-04 | -10.597738 | 27 | 2.19% | 431.4 | 0.88% | |
| RAR:RXR(NR),DR5/ES-RAR-ChIP-Se | 1.00E-04 | -10.040651 | 56 | 3.89% | 1074.5 | 2.19% | | | GATA:SCL(Zf | 1.00E-04 | -10.48091 | 156 | 12.64% | 4462.6 | 9.14% | |
| URO-BL | | | | | | | | | URO-LLI | | | | | | | |
| Name | PValue | log(PValue) | # Target Sequ | % of Targets S | # Backgrounc | % of Background | Sequences with Motif | | Name | PValue | log(PValue) | # Target Seq | % of Targets | # Backgroun | % of Background | Sequences with Motif |
| Jun-AP1(bZIP)/K562-cJun-ChIP-Seq( | 1.00E-241 | -555.97164 | 2673 | 28.20% | 6073.2 | 14.87% | | | Hnf1(Homeo | 1.00E-10 | -23.251818 | 425 | 11.95% | 4068.5 | 8.76% | |
| p73(p53)/p63/Trachea-p73-ChIP-Se | 1.00E-161 | -371.82482 | 832 | 8.78% | 1213.3 | 2.97% | | | IRF2(IRF)/Er | 1.00E-09 | -22.953174 | 295 | 8.30% | 2631.1 | 5.66% | |
| Tcfcp2l1(CP2)/mES-Tcfcp2l1-ChIP-S | 1.00E-27 | -63.065982 | 1067 | 11.26% | 3281.9 | 8.03% | | | GRHL2(CP2)/ | 1.00E-08 | -19.147771 | 857 | 24.10% | 9356.9 | 20.14% | |
| ETS(ETS)/Promoter/Homer | 1.00E-18 | -43.739891 | 1818 | 19.18% | 6418 | 15.71% | | | GATA(Zf),IR4 | 1.00E-05 | -12.969869 | 210 | 5.91% | 1978.9 | 4.26% | |
| RAR:RXR(NR),DR5/ES-RAR-ChIP-Se | 1.00E-08 | -18.982154 | 256 | 2.70% | 757.3 | 1.85% | | | Ets1-distal(E | 1.00E-04 | -9.461224 | 574 | 16.14% | 6452.7 | 13.89% | |

Table S4. LLI and BL signatures

| **LLI** | **BL** |
|---|---|
| FBN2 | BMP7 |
| SLC4A4 | ERN2 |
| CTTNBP2 | SRPX2 |
| KALRN | UGT1A10 |
| SELL | CLCA2 |
| PMP22 | SPOCD1 |
| UGT2B7 | SERPINB5 |
| COL12A1 | SEMA4B |
| PTH2R | CLCA4 |
| ANKRD36 | CLU |
| DCDC2 | UGT1A7 |
| PRTG | SH3PXD2A |
| BPGM | AQP3 |
| PDE9A | ANXA10 |
| ANXA3 | SLITRK6 |

Table S5. MP signature

| **MP** |
| --- |
| MUC4 |
| ANPEP |
| MAL |
| MMP7 |
| COL4A4 |
| PLCXD3 |
| SPNS2 |
| AREG |
| FAT3 |
| ASS1 |
| FBN2 |
| BPGM |
| TMC5 |
| LIPC |
| ELOVL7 |

**Supplementary Figure 1**

A) Heatmap representation of the differential H3K27ac regions distinguishing MP, URO2 and URO1 clusters. B) Results of motif enrichment analysis performed on MP-specific differential peaks. C) Signal distribution of H3K27ac marked enhancers in representative URO1 and URO2 samples. Representative super-enhancers identified using the ROSE algorithm are indicated. D) Integrated UMAP visualization of the two scATAC-seq datasets (HGT1_1 and HGT1_2), colored by sample origin. E) Copy-number variation (CNV) analysis of tumor cells from the two scATAC-seq samples (HGT1_1 and HGT1_2). Rows represent individual cells clustered using K-means. Green bars indicate normal cells, whereas purple bars indicate tumor cells. The color scale represents relative CNV signal centered around neutral copy number. Red indicates copy-number gains and blue indicates copy-number losses. F) Annotation of normal cell populations identified in the scATAC-seq datasets, as described in Methods. G) Integrated UMAP visualization of HGT1_1 and HGT1_2 showing enrichment of the basal-like (BL, red, top) and luminal-like inflammatory (LLI, blue, bottom) chromatin signature scores. Color scales indicate relative enrichment values. H) Integrated UMAP visualization showing accessibility at the KRT5 and KRT20 promoter regions. I) Integrated UMAP visualization showing enrichment of TP63 and GATA3 transcription factor motifs. J) Integrated UMAP analysis of HGT1_1 and HGT1_2 showing transcription factor motif enrichment across clusters defined using the Louvain graph-based clustering algorithm, as described in Methods.

**Supplementary Figure 2**

A) Boxplots showing expression levels of representative marker genes in tumors classified as basal-like (BL, red) or luminal-like inflammatory (LLI, blue) in the HGT1 cohort. Groups were defined using the upper and lower quartiles of the corresponding subtype signature scores. P-values were calculated using two-sided t-tests. B) Boxplots showing expression levels of representative marker genes in tumors classified as basal-like (BL, red) or luminal-like inflammatory (LLI, blue) in the UROMOL cohort. Groups were defined using the upper and lower quartiles of the corresponding subtype signature scores. P-values were calculated using two-sided t-tests.

**Supplementary Figure 3**

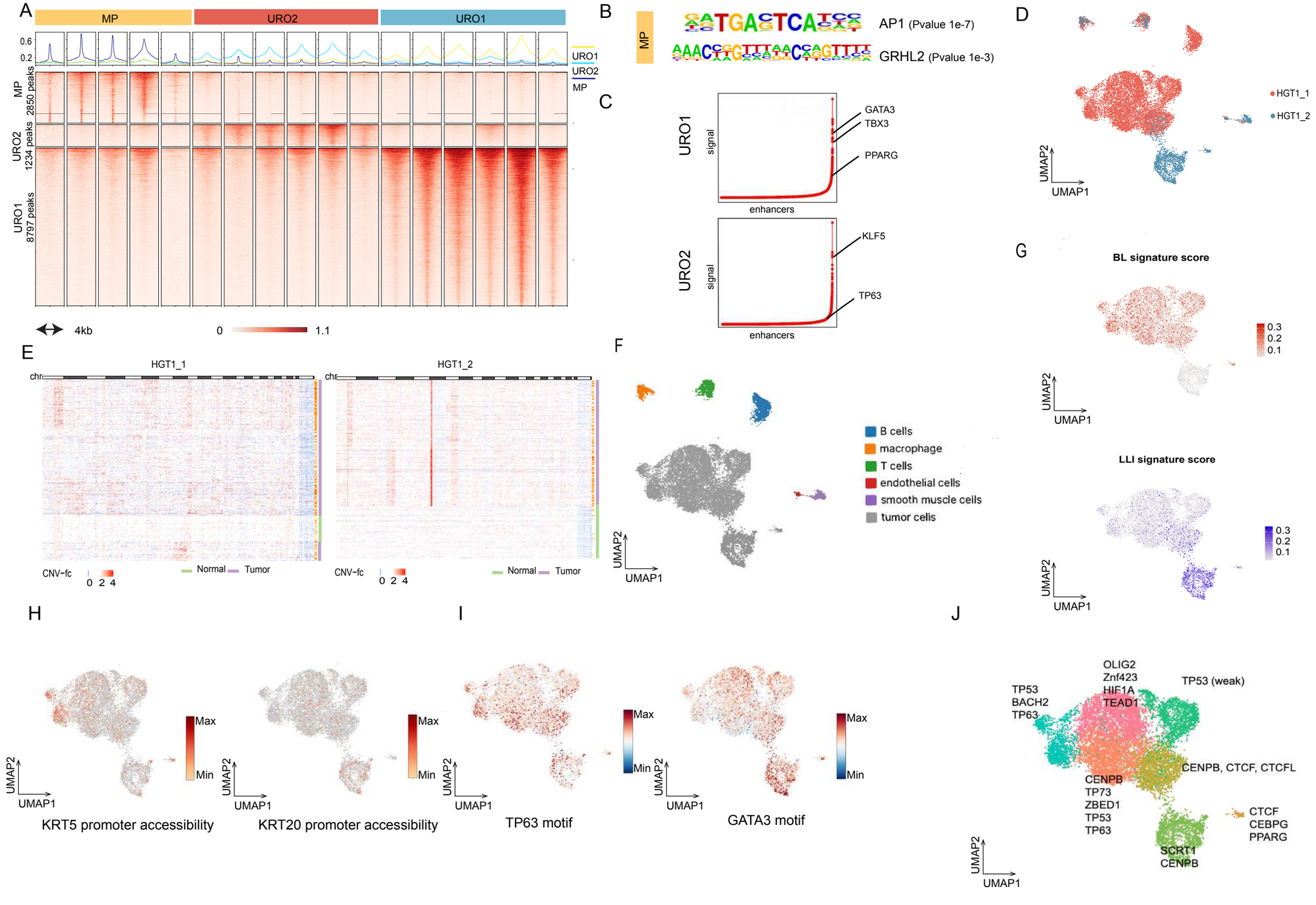
A) UMAP visualization of all eleven snRNA-seq datasets comprising 37,879 nuclei, colored by sample ID. B) UMAP visualization showing log-normalized NECTIN4 expression levels (color scale). C) CNV analysis of two representative tumors analyzed by snRNA-seq (vh75, left; vh125, right). The color scale represents relative copy-number variation (CNV) signal centered around neutral copy number. Red indicates copy-number gains, whereas blue indicates copy-number losses. D) UMAP visualization of all eleven snRNA-seq datasets annotated by tumor and normal cell populations, including classification of normal cell types. E) Proportional bar plot showing the relative abundance of each cell type across tumor samples. F) Association between differential H3K27ac regions and differential gene expression in MP samples. The volcano plot depicts RNA-seq log2 fold-change (x-axis) versus adjusted p-value calculated by DESeq2 (y-axis). Each dot represents one gene. Yellow dots indicate significantly differentially expressed genes associated with nearby differential H3K27ac regions in MP samples, whereas gray dots indicate differentially expressed genes without nearby significant differential H3K27ac regions. G) UMAP visualization of all eleven snRNA-seq datasets showing log-normalized expression of KRT20 (left), KRT5 (middle), and TP63 (right) (color scales). H) UMAP visualization of cancer

cells from nine HGT1 tumors annotated according to the UROMOL2021 classification system. Cells were assigned to one of four UROMOL2021 classes: class 1 (orange), class 2a (light blue), class 2b (dark blue), and class 3 (red), while unclassified cells are shown in gray. No cells classified as class 2b were detected at the single-cell level. I) Summary of immunohistochemistry (IHC) scoring for KRT5 and KRT20 staining in the tissue microarray (TMA). Tumors were classified as KRT5-positive, KRT20-positive, KRT5/KRT20 double-positive, or double-negative (DN). J) Representative KRT5 and KRT20 staining demonstrating their distinct spatial distributions. Expanded views illustrate the anticorrelation between KRT5 and KRT20 staining and show the relative proximity of KRT5-positive cells (red arrows) and KRT20-positive cells (blue arrows) to blood vessels. K) Comparison of the mean distance to the nearest blood vessel for KRT5-positive and KRT20-positive cells in tumors positive for both markers (N = 16). KRT5-positive cells were significantly closer to blood vessels. L) Representative staining for KRT5, KRT20, and TP63 showing that TP63-high cells exhibit greater overlap with KRT5 expression than with KRT20.

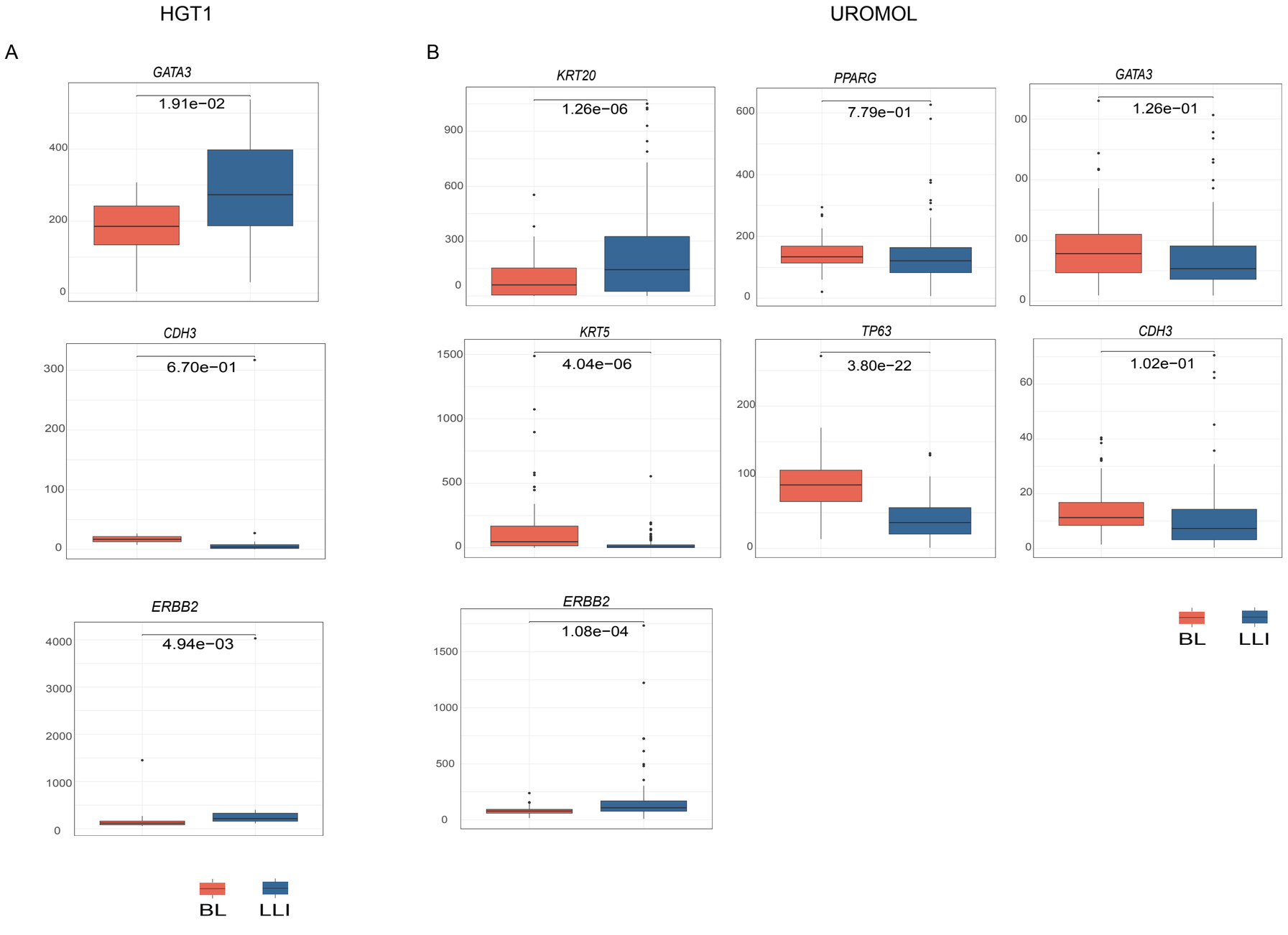
**Supplementary Figure 4**

A) H&E staining of the two micropapillary bladder cancer (MPBC) cases analyzed by spatial transcriptomics. B) UMAP visualization showing cell type clustering for MPBC1 (left) and MPBC2 (right), as described in Methods. C) Spatial representation of CDS subtype scoring in MPBC1, showing enrichment of basal-like (BL, left), micropapillary (MP, middle), and luminal-like inflammatory (LLI, right) transcriptional programs. Color scales indicate relative enrichment values. D) Spatial representation of CDS subtype scoring in MPBC2, showing enrichment of basal-like (BL, left), micropapillary (MP, middle), and luminal-like inflammatory (LLI, right) transcriptional programs. Color scales indicate relative enrichment values. E) Spatial mapping of cancer-associated fibroblasts (CAFs) in MPBC1 reveals distinct stromal organization between the MP and URO components. The MP region (dashed area, left) contains a high density of CAFs interspersed with tumor cells, whereas the URO region (dashed area, right) shows minimal CAF infiltration. F) Spatial representation of canonical CAF marker expression in the MP and URO regions. These analyses demonstrate reduced CAF-associated programs in the URO component relative to the MP component. G) Top: UMAP visualization showing log-normalized EGLN3 expression across cancer cells from the eleven snRNA-seq datasets. Color scales indicate relative enrichment values. Bottom: violin plot showing EGLN3 expression in snRNA-seq cells classified as BL, LLI, or MP. EGLN3 expression is significantly enriched in BL cells relative to the other groups ($p < 0.001$, two-sided t-test).

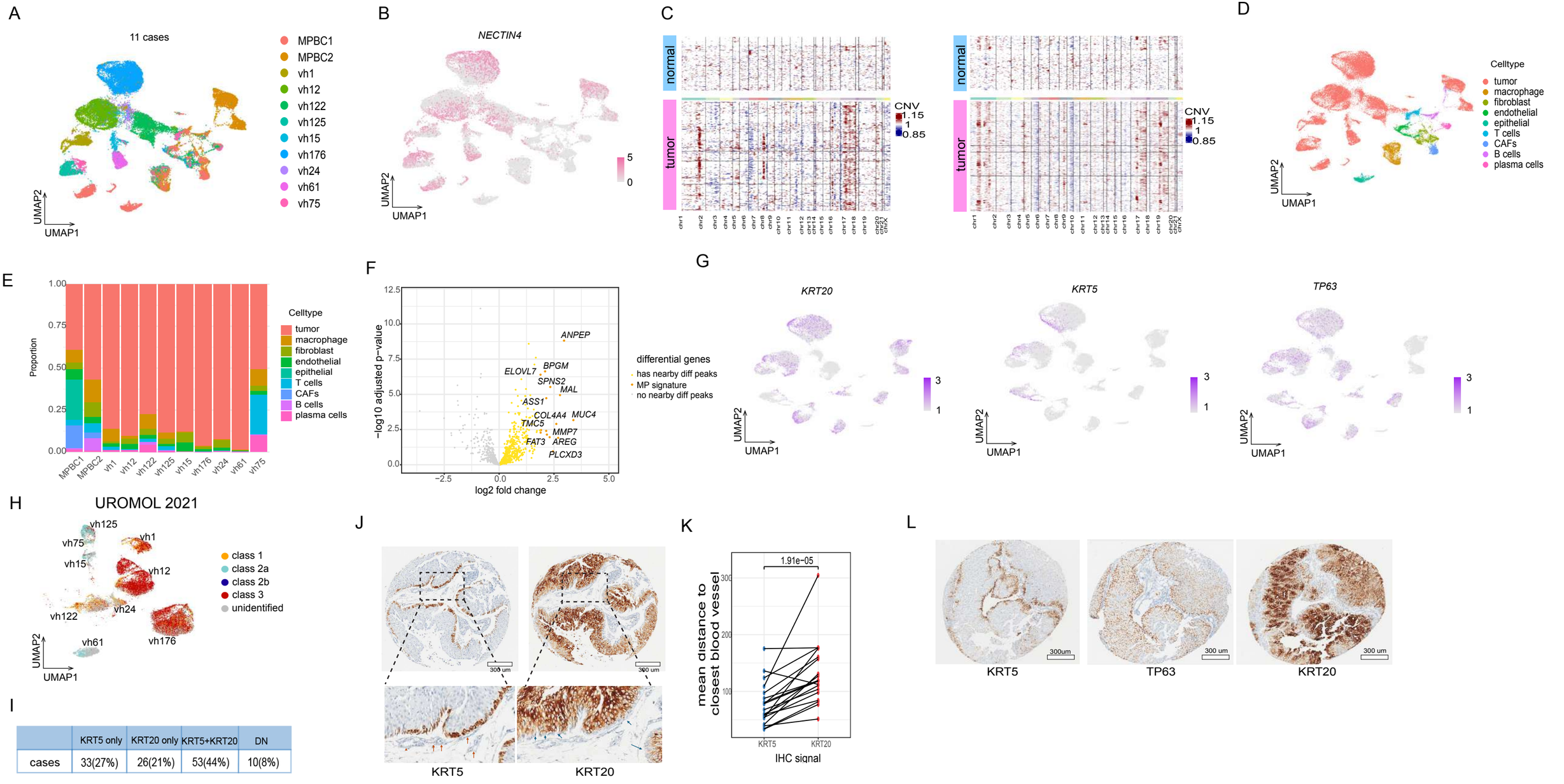
Supplementary Figure 1



Supplementary Figure 2



Supplementary Figure 3




| | KRT5 only | KRT20 only | KRT5+KRT20 | DN |
|---|---|---|---|---|
| cases | 33(27%) | 26(21%) | 53(44%) | 10(8%) |

Supplementary Figure 4