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Supplemental information

Multiomics profiling of urothelial carcinoma *in situ* reveals CIS-specific gene signature and immune characteristics

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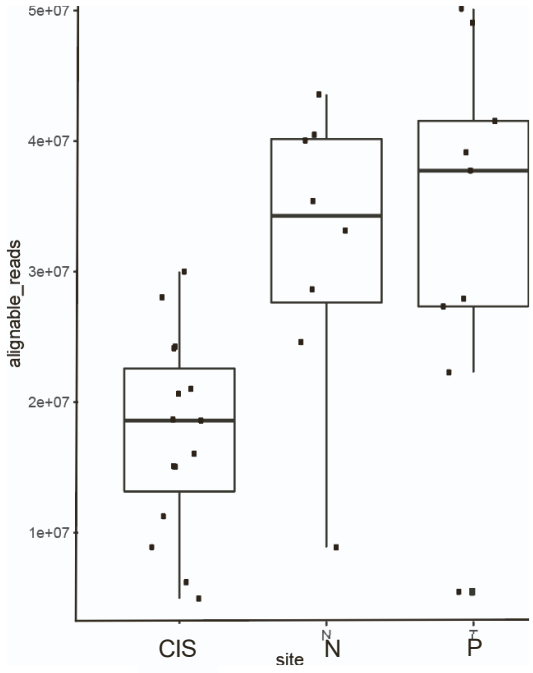
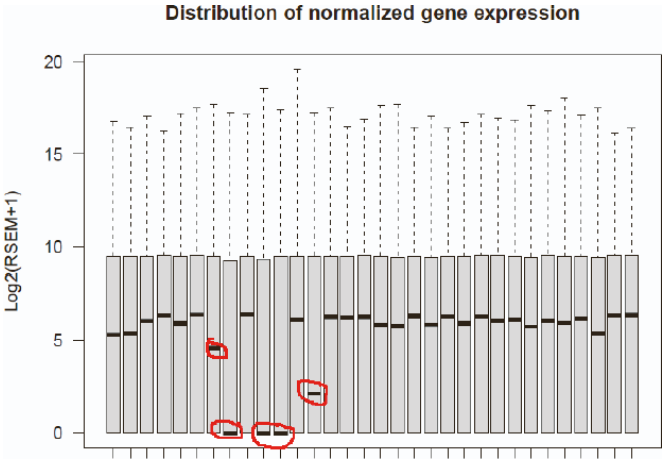
A)


5e+07
4e+07
3e+07
2e+07
1e+07
alignable_reads
CIS
N
P
site

B)


Distribution of normalized gene expression
20
15
10
5
0
Log2(RSEM+1)

C)

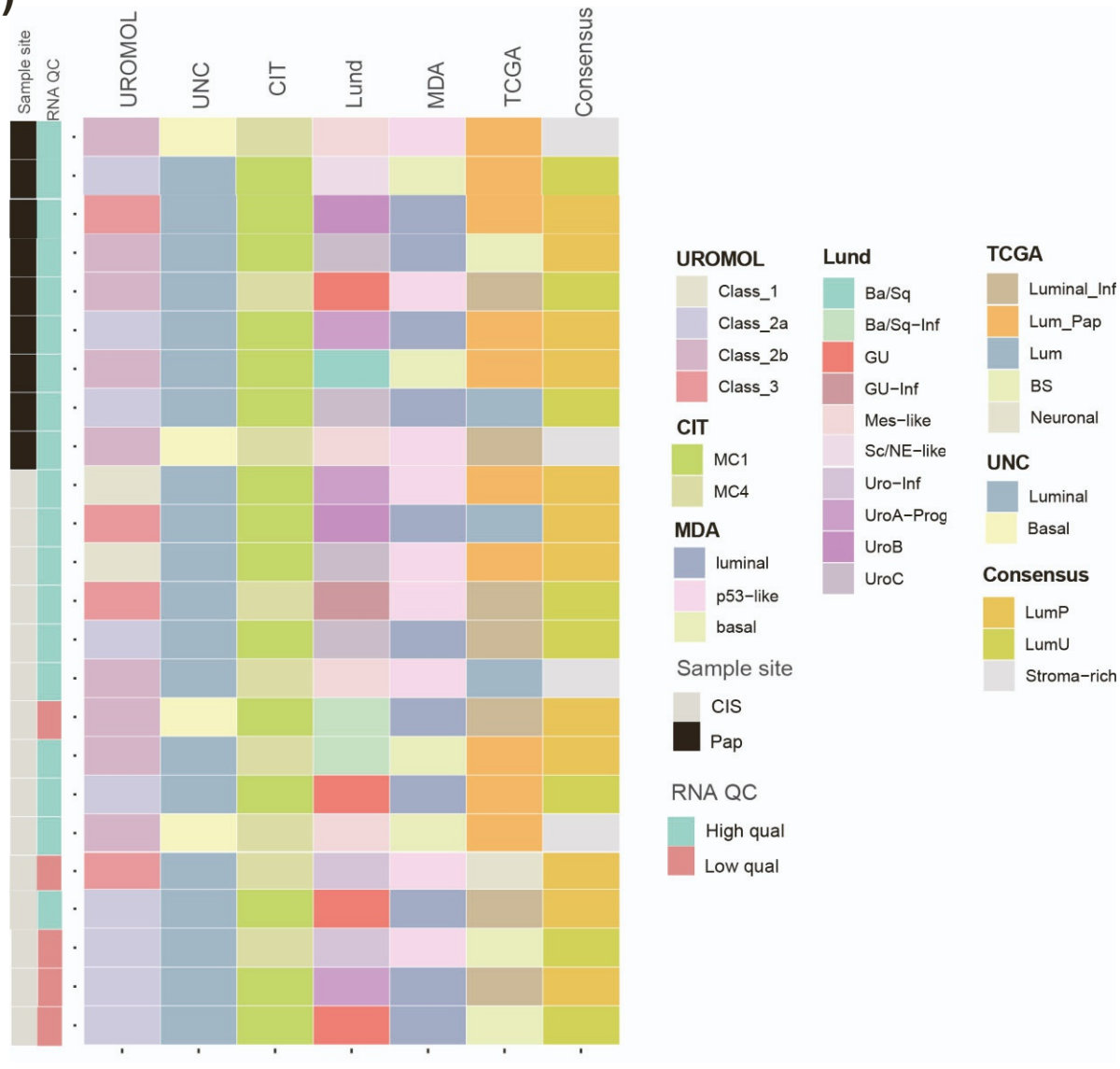
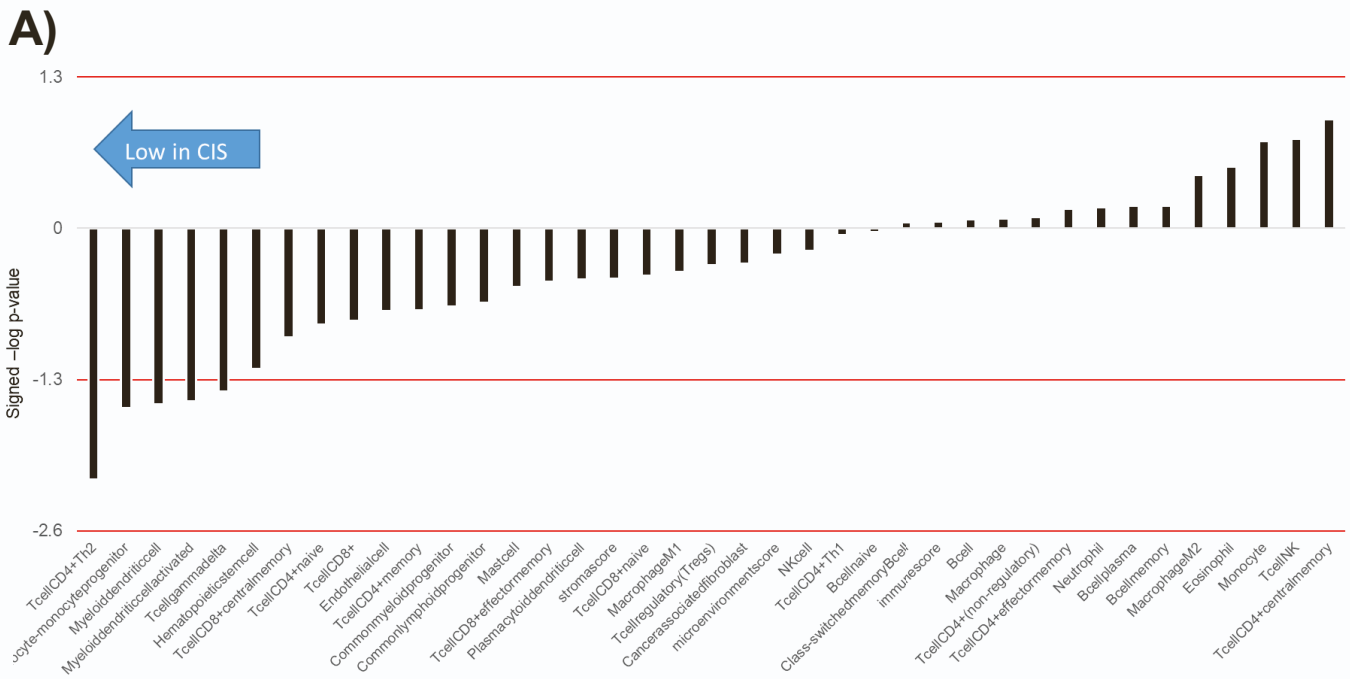
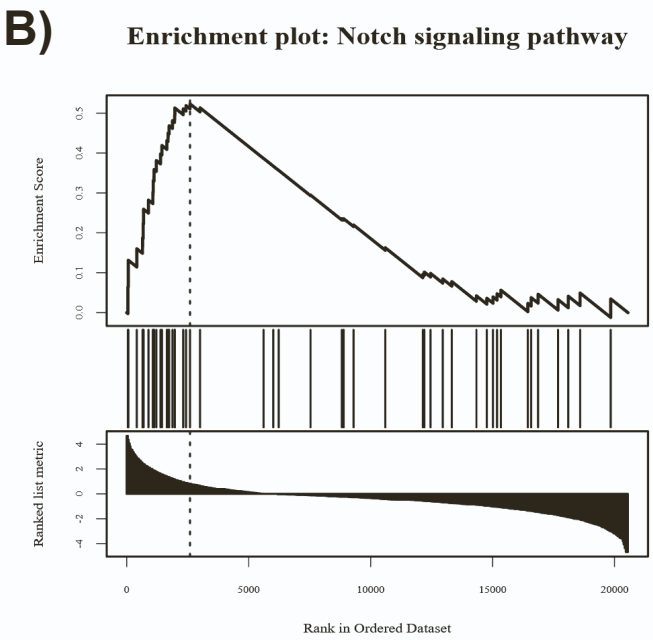
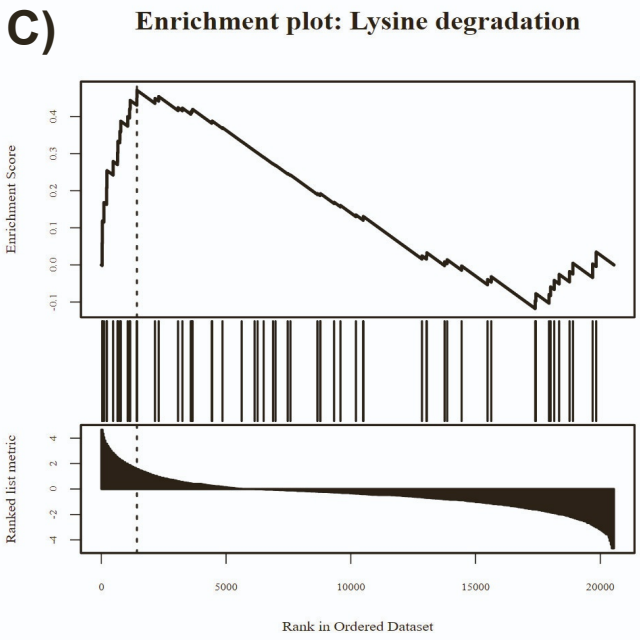

Sample site
RNA QC
UROMOL
UNC
CIT
Lund
MDA
TCGA
Consensus
UROMOL
Class_1
Class_2a
Class_2b
Class_3
CIT
MC1
MC4
MDA
luminal
p53−like
basal
Sample site
CIS
Pap
RNA QC
High qual
Low qual
Lund
Ba/Sq
Ba/Sq−Inf
GU
GU−Inf
Mes−like
Sc/NE−like
Uro−Inf
UroA−Prog
UroB
UroC
TCGA
Luminal_Inf
Lum_Pap
Lum
BS
Neuronal
UNC
Luminal
Basal
Consensus
LumP
LumU
Stroma−rich

Fig S2.

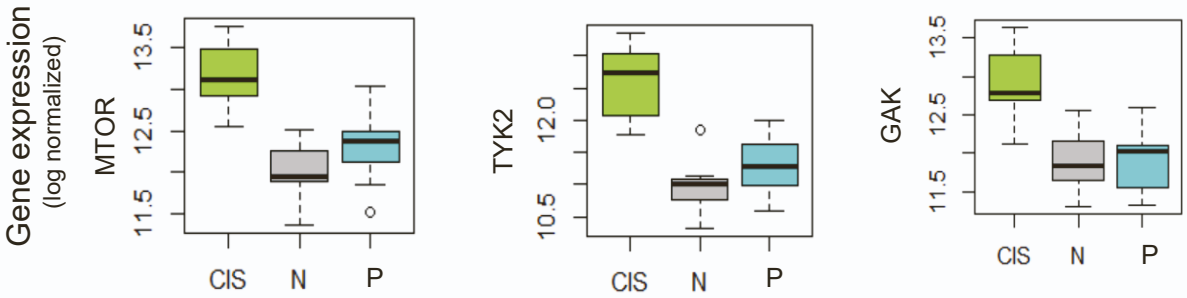

A)
1.3
Low in CIS
0
-1.3
-2.6
Signed –log p-value
TcellCD4+Th2
ocyte-monocyteprogenitor
Myeloiddendriticcell
Myeloiddendriticcellactivated
Tcellgammadelta
Hematopoieticstemcell
TcellCD8+centralmemory
TcellCD4+naive
TcellCD8+
Endothelialcell
TcellCD4+memory
Commonmyeloidprogenitor
Commonlymphoidprogenitor
Mastcell
TcellCD8+effectormemory
Plasmacytoiddendriticcell
stromascore
TcellCD8+naive
MacrophageM1
Tcellregulatory(Tregs)
Cancerassociatedfibroblast
microenvironmentscore
NKcell
TcellCD4+Th1
Bcellnaive
Class-switchedmemoryBcell
immunescore
Bcell
Macrophage
TcellCD4+(non-regulatory)
TcellCD4+effectormemory
Neutrophil
Bcellplasma
Bcellmemory
MacrophageM2
Eosinophil
Monocyte
TcellNK
TcellCD4+centralmemory


B)
Enrichment plot: Notch signaling pathway
Enrichment Score
Ranked list metric
Rank in Ordered Dataset

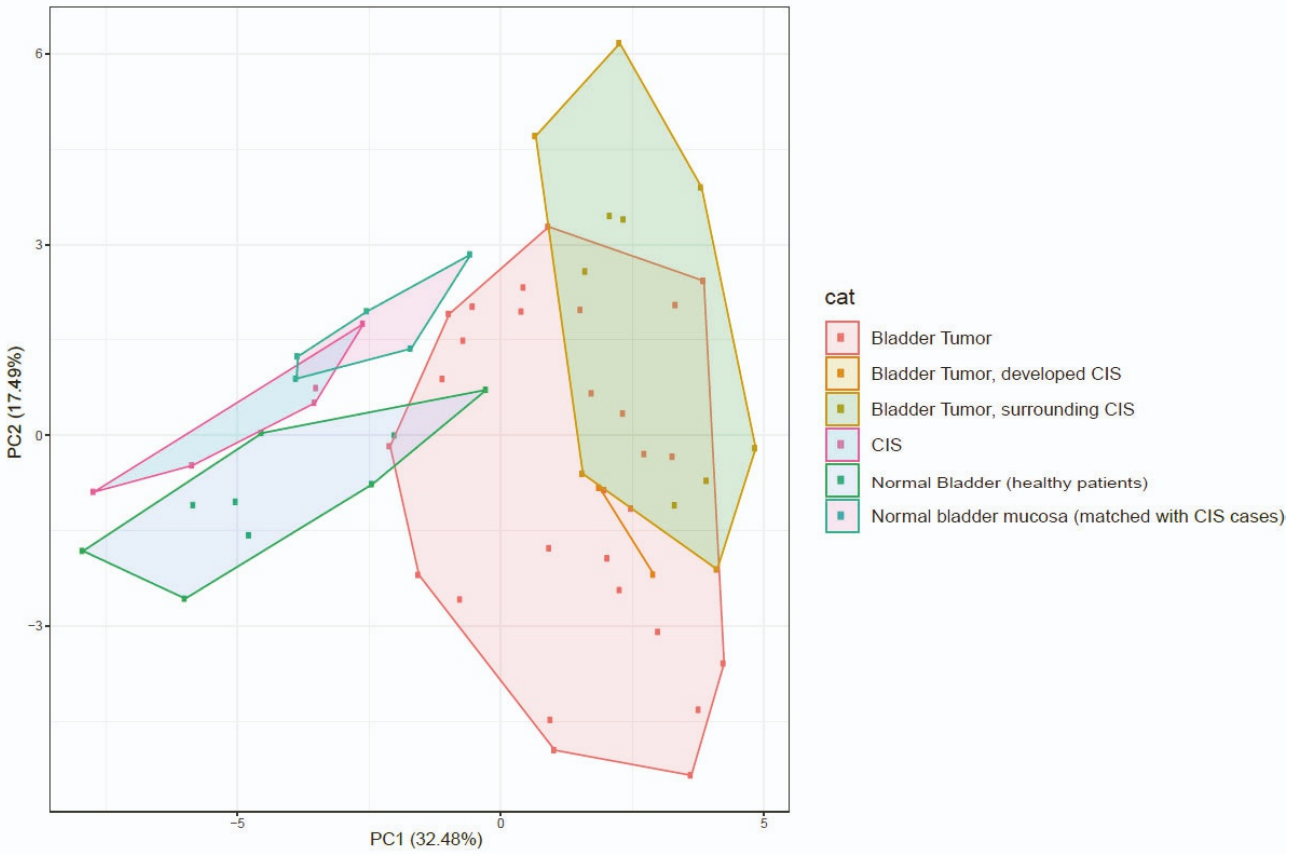

C)
Enrichment plot: Lysine degradation
Enrichment Score
Ranked list metric
Rank in Ordered Dataset

A)

Fig S3


Gene expression
(log normalized)
MTOR
TYK2
GAK
CIS
N
P

B)


PC2 (17.49%)
PC1 (32.48%)
cat
Bladder Tumor
Bladder Tumor, developed CIS
Bladder Tumor, surrounding CIS
CIS
Normal Bladder (healthy patients)
Normal bladder mucosa (matched with CIS cases)

C)

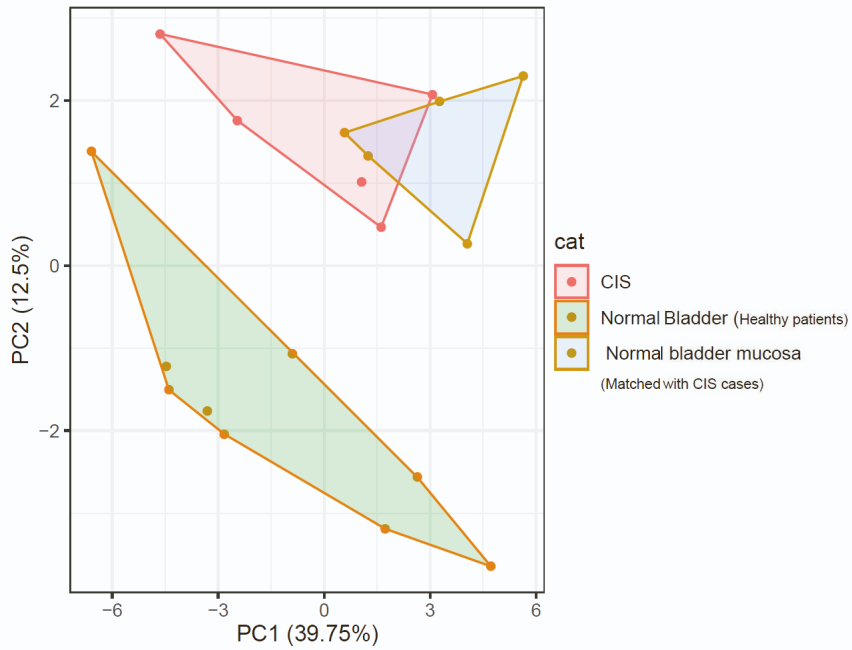

PC2 (12.5%)
PC1 (39.75%)
cat
CIS
Normal Bladder (Healthy patients)
Normal bladder mucosa
(Matched with CIS cases)

Fig S4.

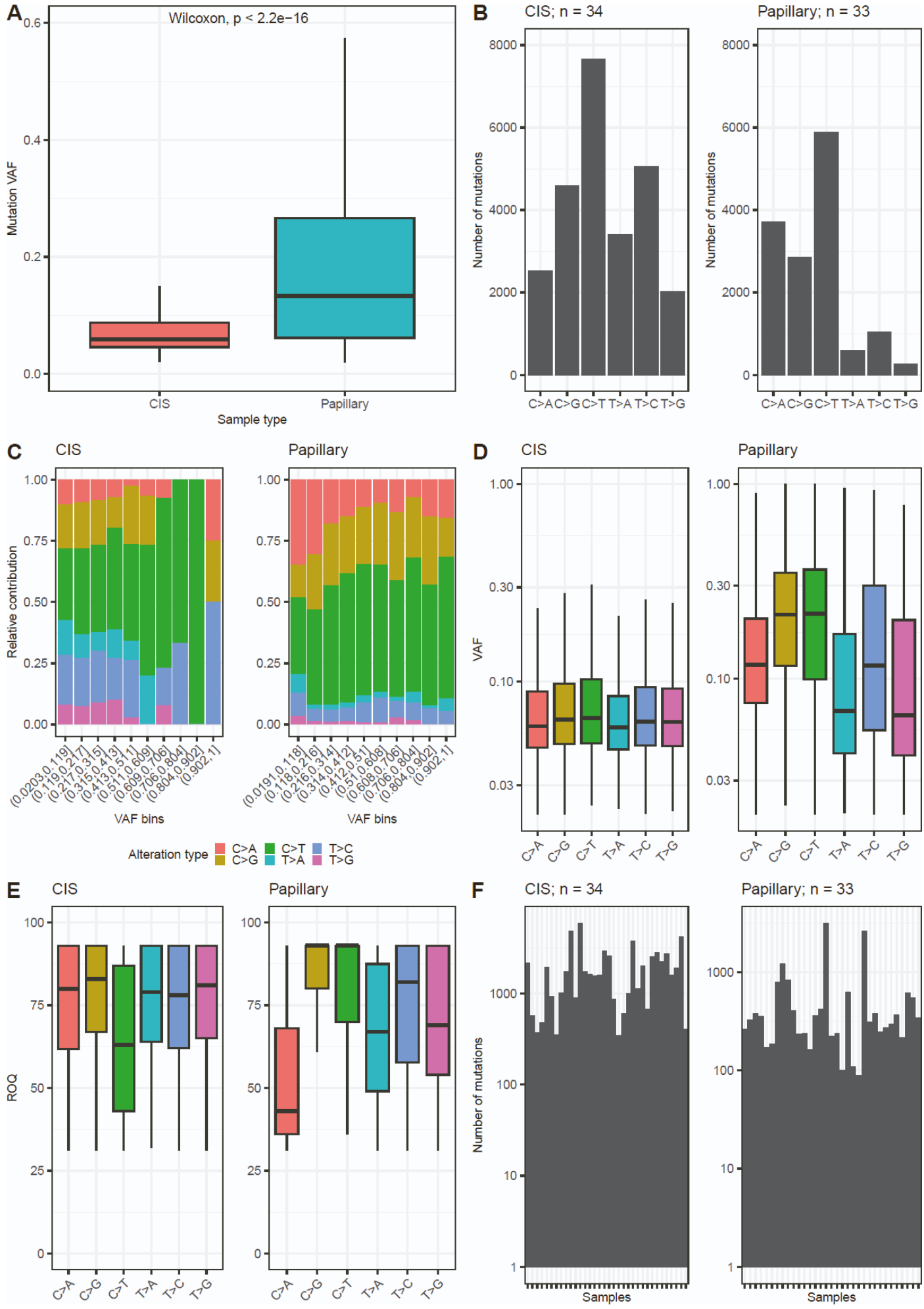

A
Wilcoxon, p < 2.2e−16
Mutation VAF
Sample type
CIS
Papillary
B
CIS; n = 34
Papillary; n = 33
Number of mutations
C>A C>G C>T T>A T>C T>G
C
CIS
Papillary
Relative contribution
VAF bins
Alteration type
C>A
C>G
C>T
T>A
T>C
T>G
D
CIS
Papillary
VAF
E
CIS
Papillary
ROQ
F
CIS; n = 34
Papillary; n = 33
Number of mutations
Samples

Fig S5

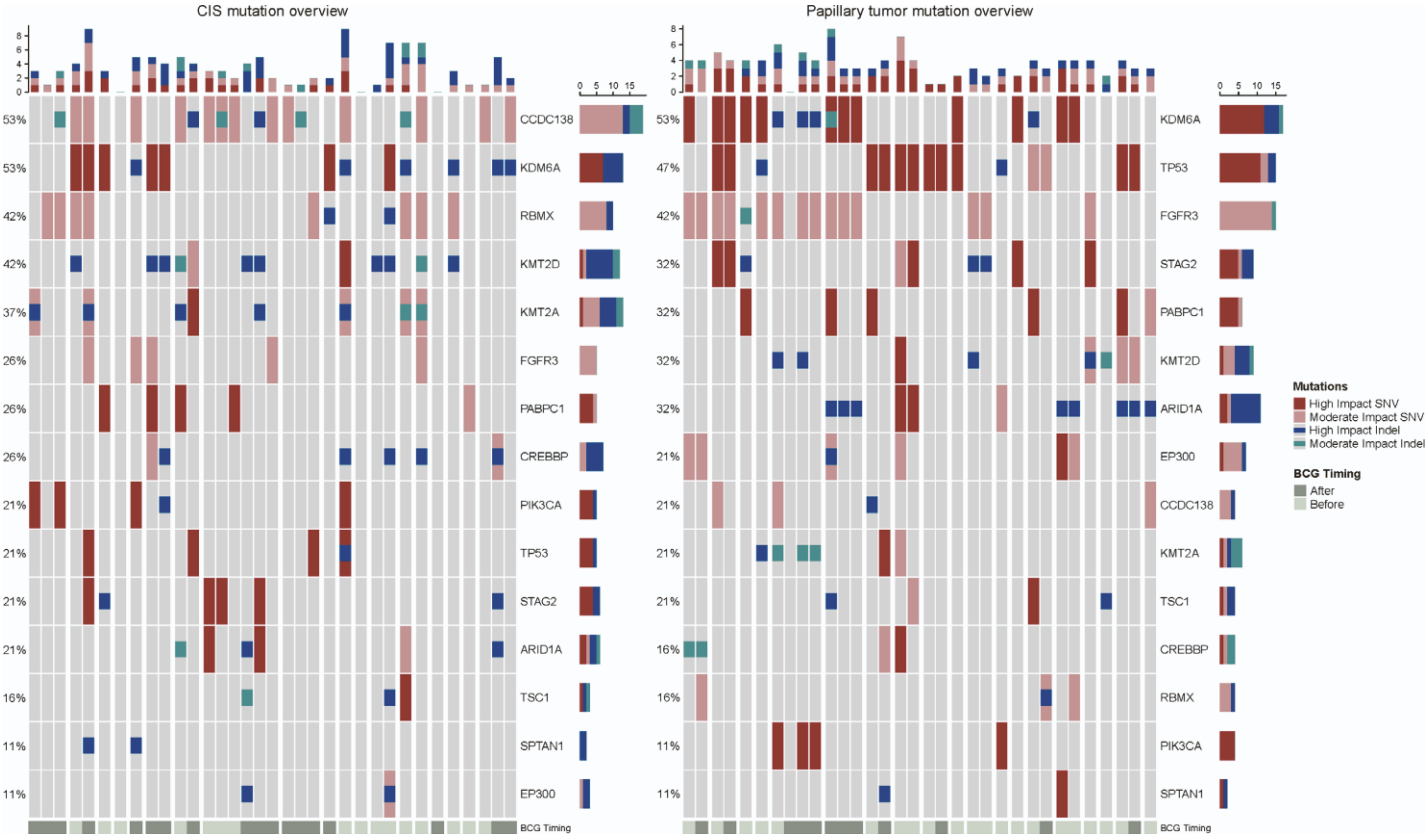
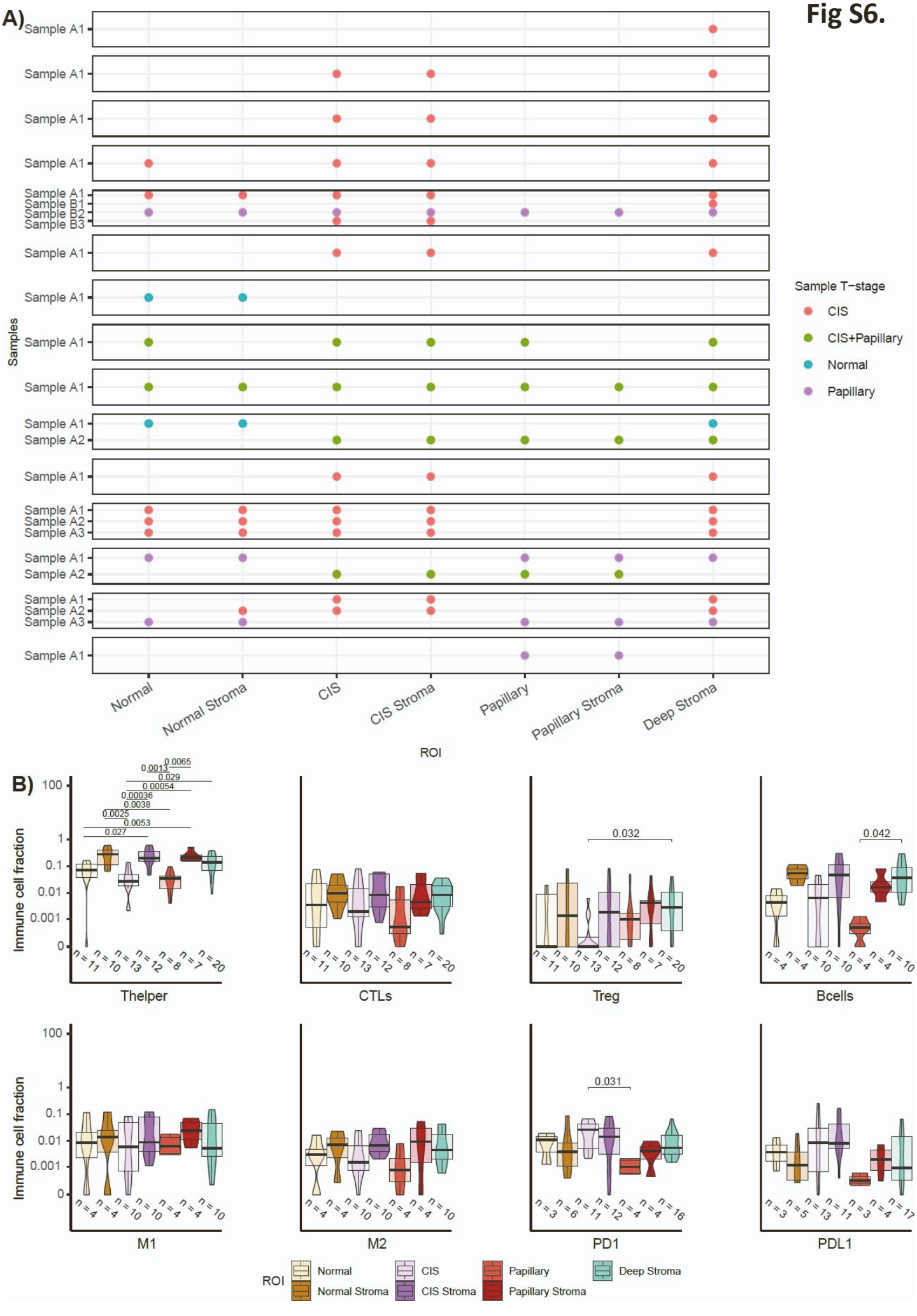

CIS mutation overview
Papillary tumor mutation overview
53% CCDC138
53% KDM6A
42% RBMX
42% KMT2D
37% KMT2A
26% FGFR3
26% PABPC1
26% CREBBP
21% PIK3CA
21% TP53
21% STAG2
21% ARID1A
16% TSC1
11% SPTAN1
11% EP300
BCG Timing
53% KDM6A
47% TP53
42% FGFR3
32% STAG2
32% PABPC1
32% KMT2D
32% ARID1A
21% EP300
21% CCDC138
21% KMT2A
21% TSC1
16% CREBBP
16% RBMX
11% PIK3CA
11% SPTAN1
BCG Timing
Mutations
High Impact SNV
Moderate Impact SNV
High Impact Indel
Moderate Impact Indel
BCG Timing
After
Before

Fig S6.


A)
Samples
Sample A1
Sample B1
Sample B2
Sample B3
Sample A2
Sample A3
Normal
Normal Stroma
CIS
CIS Stroma
Papillary
Papillary Stroma
Deep Stroma
ROI
Sample T−stage
CIS
CIS+Papillary
Normal
Papillary
B)
Immune cell fraction
Thelper
CTLs
Treg
Bcells
M1
M2
PD1
PDL1
0.0065
0.0013
0.029
0.00054
0.00036
0.0038
0.0025
0.0053
0.027
0.032
0.042
0.031
ROI
Normal
Normal Stroma
CIS
CIS Stroma
Papillary
Papillary Stroma
Deep Stroma

Supplementary table and figure legends.

Table S1. Gene expression matrix of 24 samples from Cohort A. This table contains upper quartile normalized RSEM values median centered by gene.

Figure S1. RNAseq data quality and sample subtypes. A) Boxplot showing alignable number of reads by sample site- CIS, Normal (N) and Papillary tumors (P). B) Plot showing distribution of normalized gene expression across all samples included in the study. C) Heatmap showing different subtype classifications for the CIS and tumor samples. The sample site and RNA-based sample quality is shown as rows.

Figure S2. Cell type and gene enrichment score. A) Cell type (xCell) enrichment score for cohort A. Y-axis represents signed log p-value of comparison of CIS and papillary tumors. B-C) GSEA enrichment plot for Notch signaling and Lysine degradation pathways.

Figure S3. **CIS genes signature** A) Boxplot showing MTOR, TYK2 and GAK RNA levels across CIS, papillary (P) and Normal (N) samples. B) PCA plot of 36 (out of 46) CIS signature genes from GSE3167 including all samples. C) PCA plot of CIS signature genes across CIS, match normal bladder mucosa samples and normal bladder samples from healthy patients.

Figure S4: **CIS and papillary mutation characteristics**. A) Mutation VAF for alterations in CIS and papillary tumors (Wilcoxon rank sum test). B) The number of mutations of six alteration types in all CIS (left) or all papillary (right) samples. C) The relative distribution of the six alteration types in VAF bins for CIS samples (left) and papillary tumor samples (right). D) The VAF of mutations of the six alteration types measured in CIS samples (left) and papillary tumor samples (right). E) The ROQ of called mutations of the six alteration types measured in CIS samples (left) and papillary tumor samples (right). F) The total number of alterations in all samples of CIS (left) or papillary tumor (right) origin.

Figure S5: **Mutational landscape of CIS and papillary tumor samples separated** (moderate and high impact SNVs and indels). Columns correspond to unique samples. Groups of columns correspond to all analyzed samples from a single patient. Top: The number of the four mutation types in every sample. Middle: Oncoplot showing the four mutation types in selected genes, sorted by mutation frequency across patients. Frequencies of patients with mutations in the given gene are indicated to the left. Bars to the right indicate the numbers of the different mutation types within each gene across the samples in the cohort. Bottom: sample information. BCG Timing: sample timing in relation to BCG treatment.

Figure S6: **mIF and IHC sample overview and stromal compartments**. A) Overview of tissue types and ROIs. From each patient, samples are noted. Letters indicate different clinical visits followed by the

sample number from the visit in question. Colored circles indicate the sample T-stage. The different ROIs annotated in every sample are shown. B) Immune cell fractions. The fractions of immune cell types in all epithelial and stromal regions of interest. Statistically significant adjusted *p*-values are indicated (Wilcoxon rank sum test).