TSC1 mutant bladder cancer has a distinct expression signature due to nuclear localization of TFE3

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ABSTRACT

Background: Mutations in the tumor suppressor *TSC1* gene is a frequent event in bladder cancer (BLCA), however whether it functions as a driver for tumor development is uncertain.
Aim: To investigate the functional role of *TSC1* loss in BLCA and to identify novel downstream pathways that are of therapeutic value.
Methods: We performed differential gene expression and pathway analyses using RNA-seq data from the curated TCGA *TSC1* mutant BLCA (*TSC1*mBLCA; n=26) and *TSC1* wild-type BLCA (*TSC1*WTBLCA; n=382) cohort and compared to an internal cohort of putative TSC1/TSC2-driven tumors (n=63). RNA-seq and H3K27ac ChIP-seq were conducted in 3 *TSC1* mutant/WT BLCA cell lines.
Results: Integrative analyses, using DESeq2 and GSEA data, revealed a conserved TSC-associated signature, which implicated mTORC1 hyperactivation, as well as abnormal lysosomal processes as a direct consequence of *TSC1* inactivation in BLCA. We validated our findings by immunohistochemistry in *TSC1*-mutant (n=5) and *TSC1* WT (n=5) BLCA tissues and found that activation of lysosomal processes was likely driven by nuclear localization of TFE3. Mechanistic studies showed increased post-translational modification of TFE3 in *TSC1*-null cells, likely driving activation and subsequent induction of lysosomal gene expression.
Conclusions: *TSC1*mBLCA comprises a distinct subtype of bladder cancer which retains elements of a conserved transcriptional program.

RESULTS

Molecular subtypes and mutation landscape of *TSC1*mBLCA

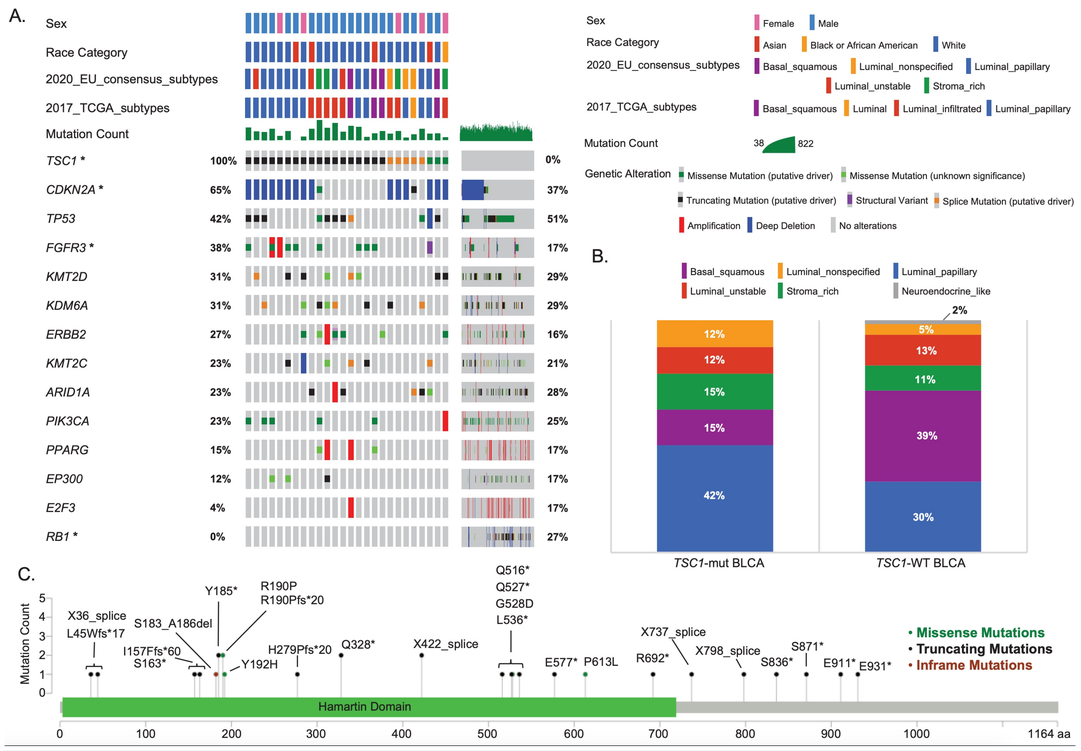

Figure 1: Molecular subtypes and mutation landscape of *TSC1*mBLCA
(A) mRNA expression subtypes, gender, mutation count, survival status and genomic alterations/alteration frequency of thirteen bladder cancer-associated genes in *TSC1*mBLCA (left) in comparison to *TSC1*WTBLCA (right). *Asterisk indicates significant differences in alteration frequencies between *TSC1*-BLCA and *TSC1*WTBLCA. (B) Distribution of the 2020 consensus molecular subtypes in *TSC1*mBLCA and *TSC1*WTBLCA. (C) Map of somatic variants in *TSC1* that we determined to be pathogenic. One patient had two separate somatic variants in *TSC1*, therefore the total number is 27.

Pathway analysis of TSC1mBLCA

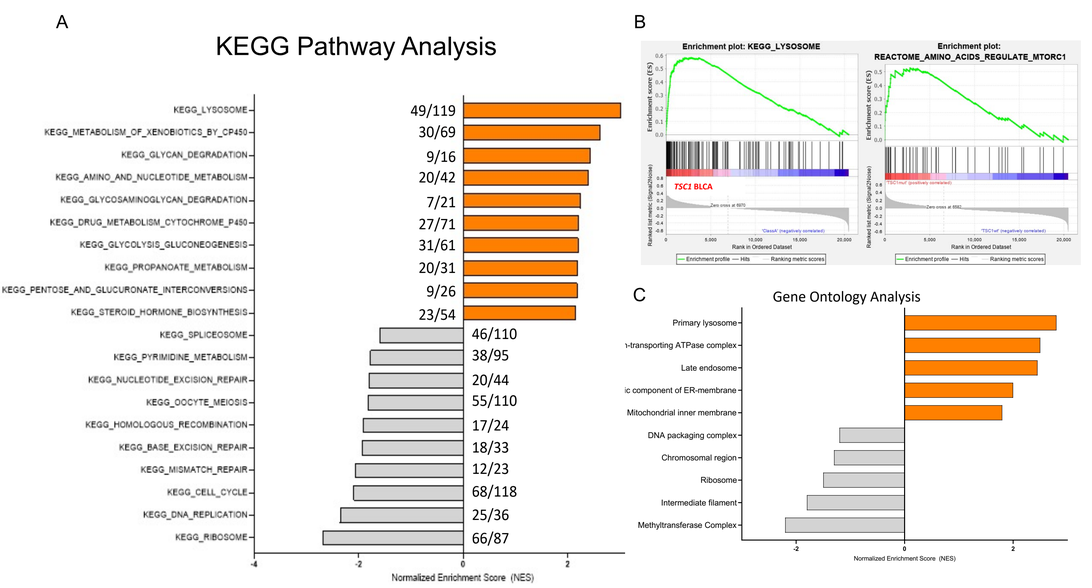

Figure 2: Pathway analysis of *TSC1*mBLCA
(A) Top 10 up- and downregulated KEGG pathways in *TSC1*-BLCA (# of significantly enriched genes/total # of genes in gene set), (B) Enrichment of lysosome and mTORC1 associated pathways in *TSC1*mBLCA compared to *TSC1*WTBLCA(p-value of < 1%; FDR < 0.001 for all gene sets shown). (C) Affinity propagation clustering of Gene Ontology gene sets further implicates the involvement of lysosome associated pathways in TSC1mBLCA.

Conservation of a TSC-associated expression signature in *TSC1*mBLCA

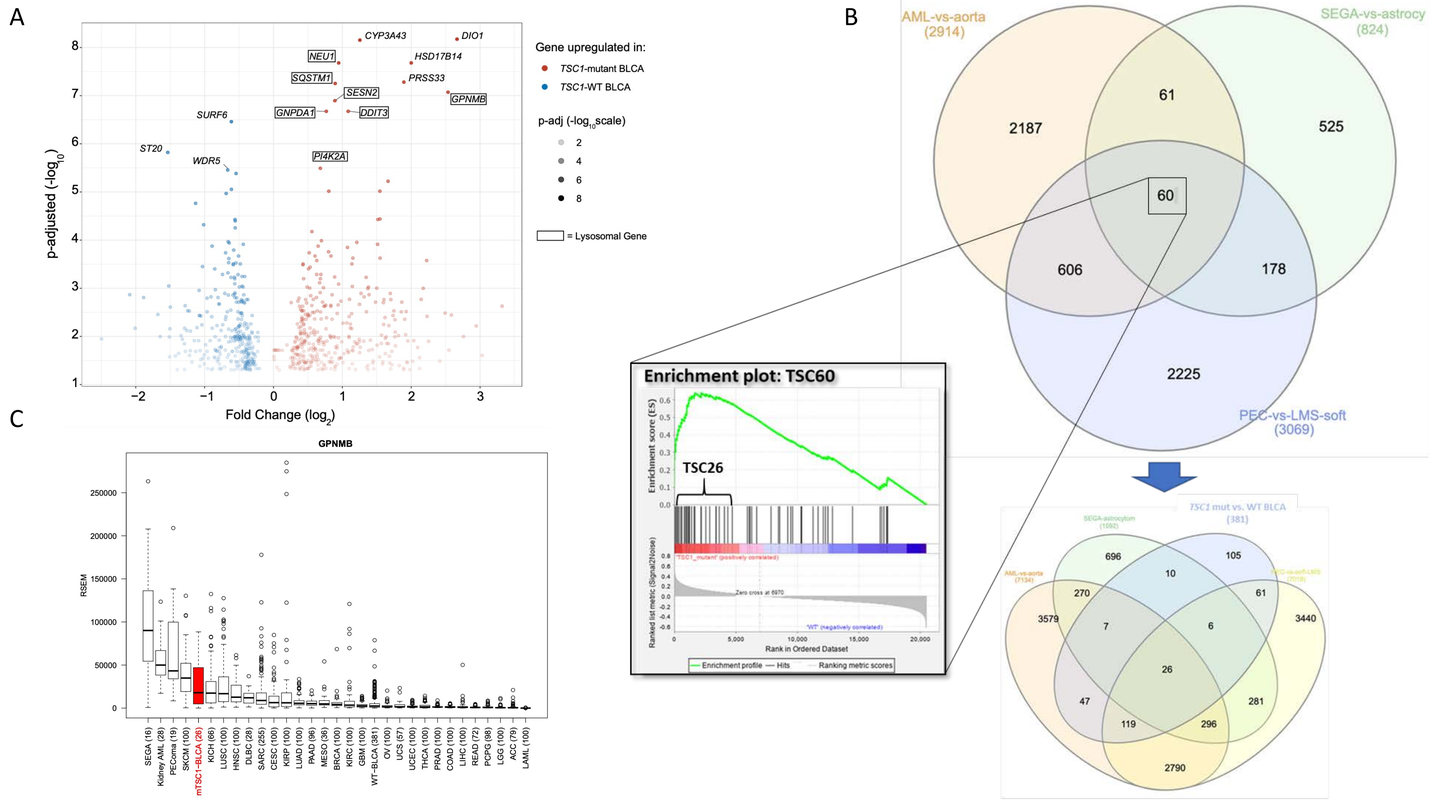

Figure 3: Conservation of a TSC-associated expression signature in *TSC1*mBLCA
(A) Volcano plot of the 381 differentially expressed genes in *TSC1*mBLCA (FDR/q-value<0.05 and log2fold change >2) shows lysosome-associated genes to be the most significantly overexpressed genes compared to *TSC1*WTBLCA. (B) Integrative differential expression analysis of three TSC-associated tumors (1. renal angiomyolipoma(AML), 2. subependymal giant cell astrocytoma (SEGA), 3. perivascular epithelioid cell tumors, (PECOMA), identifies 'TSC60' - a gene set comprised of 60 common differentially expressed genes of TSC-associated tumors. GSEA analysis demonstrates significant enrichment of 26/60 genes from the TSC60 gene set in *TSC1*mBLCA. (C) GPNMB expression is upregulated in TSC-associated tumors, including *TSC1*mBLCA, compared to other TCGA tumors.

GPNMB overexpression in TSC1mBLCA is driven by chromatin remodeling

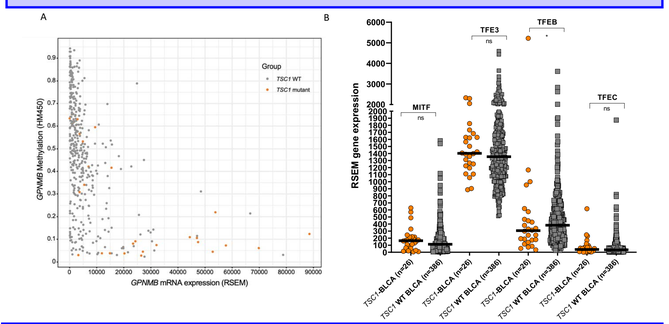

Figure 4. GPNMB overexpression in *TSC1*mBLCA is driven by chromatin remodeling
(A) GPNMB is differentially demethylated in TSC1-BLCA and correlates with increased mRNA expression (Pearson correlation: -0.55; p = 3.47e-34). (B) Expression profile of MiT/TFE family members in *TSC1*-BLCA vs. *TSC1* WT BLCA.

Histological validation of expression differences between TSC1mBLCA and *TSC1*WTBLCA

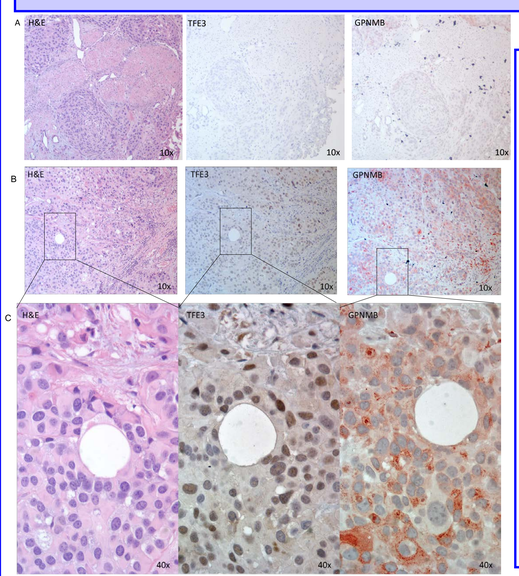

Figure 5.
Representative images from n=5 cancers of each type.
(A) Negative staining for both TFE3 and GPNMB in *TSC1*WTBLCA. (B) Positive nuclear staining for TFE3 and positive staining for GPNMB in *TSC1*mBLCA. (C) High magnification views of IHC for TFE3 and GPNMB reveal a granular/punctate-like cytoplasmic staining pattern for GPNMB, consistent with co-localization with intracellular lysosomes, in cancer cells that also have nuclear TFE3 expression.

Adding back *TSC1* attenuates lysosomal stress in *TSC1*-null bladder cancer cell lines

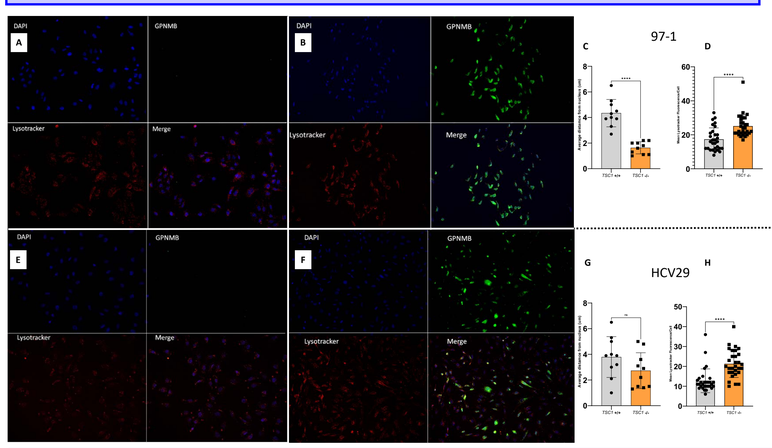

Figure 6. Adding back *TSC1* attenuates lysosomal stress in *TSC1*-null bladder cancer cell lines
(A and E) Negative GPNMB staining and diffuse cytoplasmic distribution of lysotracker-positive clusters in *TSC1* addback bladder cancer cell lines 97-1 (A) and HCV29 (E). Perinuclear accumulation of lysotracker-positive clusters with strong GPNMB signal in the *TSC1*-null bladder cancer cell lines 97-1 (B) and HCV29 (F). Mean lysotracker fluorescence per cell and quantification of distance between lysosomes and nucleus for both 97-1 cells (C, D) and for HCV29 cells (G, H).

Loss of *TSC1* alters the migration and distribution of TFE3 in *TSC1*-null bladder cancer cell lines

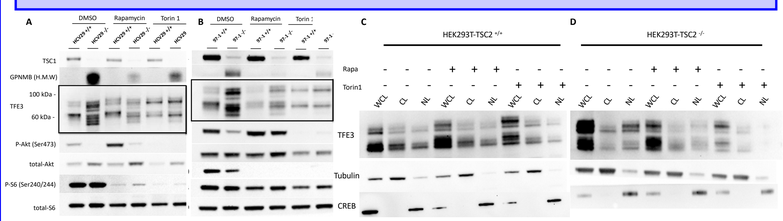

Figure 7. Loss of *TSC1* alters the migration and distribution of TFE3 in *TSC1*-null bladder cancer cell lines
(A and B) *TSC1* inactivation correlated with increased GPNMB expression and increased ratio of post translationally modified TFE3 in both HCV29 (A) and 97-1 (B) bladder cancer cell lines. Inhibition of mTORC1 signaling (20nM Rapamycin or 250nM Torin1 for 24 hours) did not attenuate post-translational modifications, but significantly decreased overall protein levels of TFE3 only in the *TCS1*-null cells and not in the *TSC1* addbacks. (C and D) A similar increase of PTM-TFE3 was also found in whole cell lysates (WCL) from *TSC2* KO HEK293T cells (D) and predominantly localized to the nucleus (nuclear lysates = NL, cytoplasmic lysates = CL), compared to *TSC2* WT HEK293T cells (C). Inhibition of mTORC1 signaling with either 20nM Rapamycin or 250nM Torin1 for 24 hours, resulted in decreased total protein levels of TFE3, but only in the *TSC2* KO cells and not in *TSC2* WT cells.