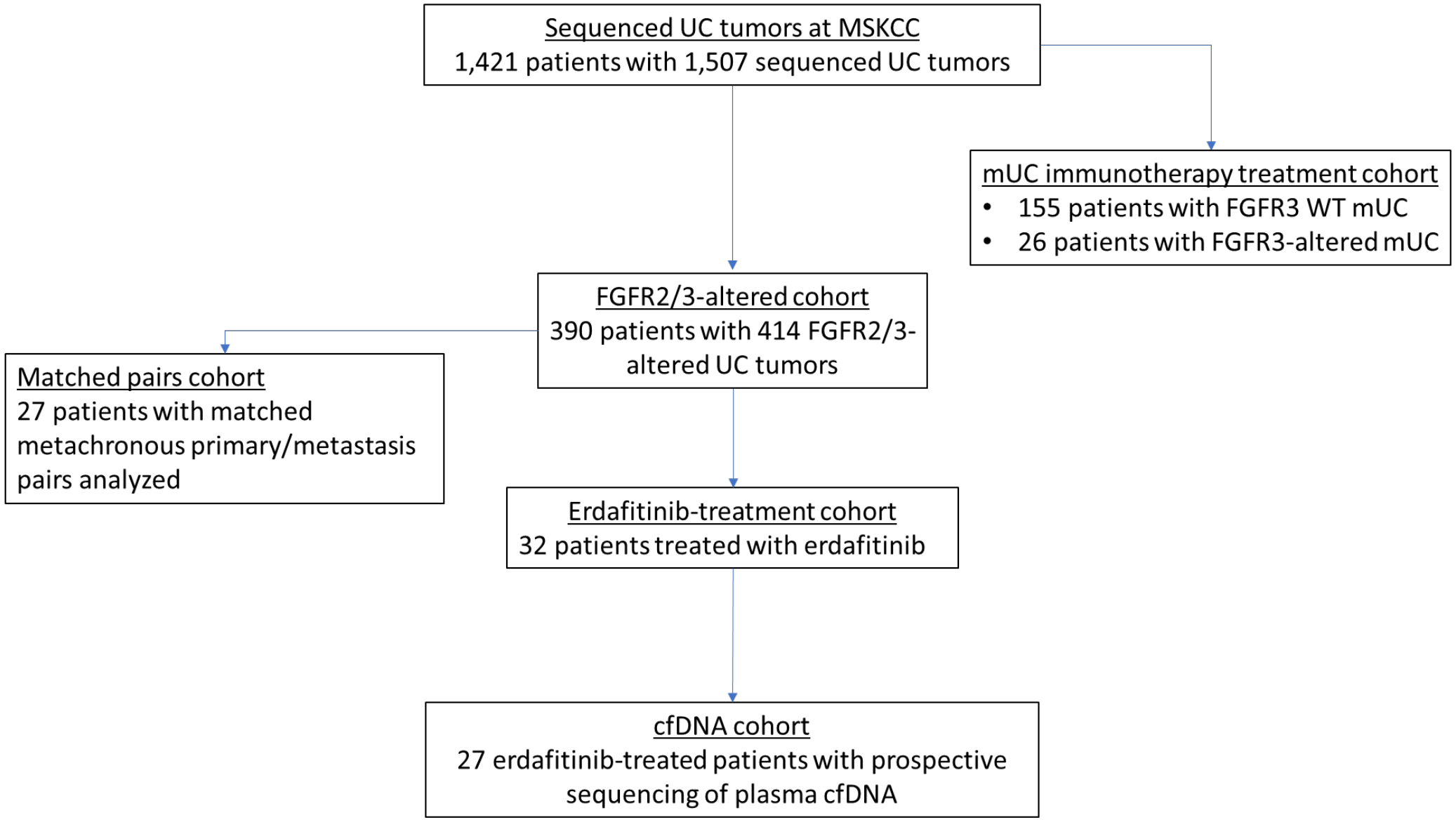
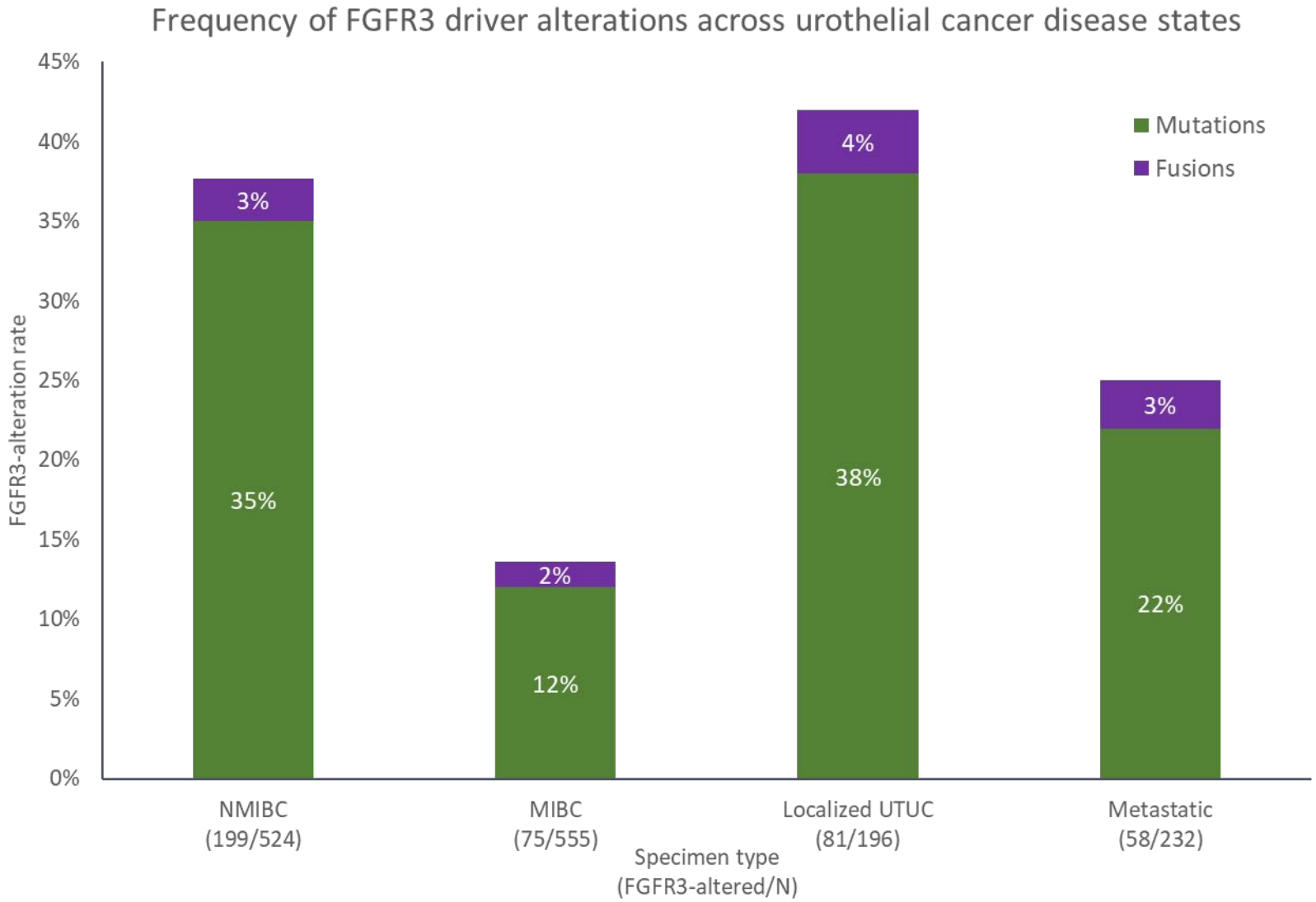
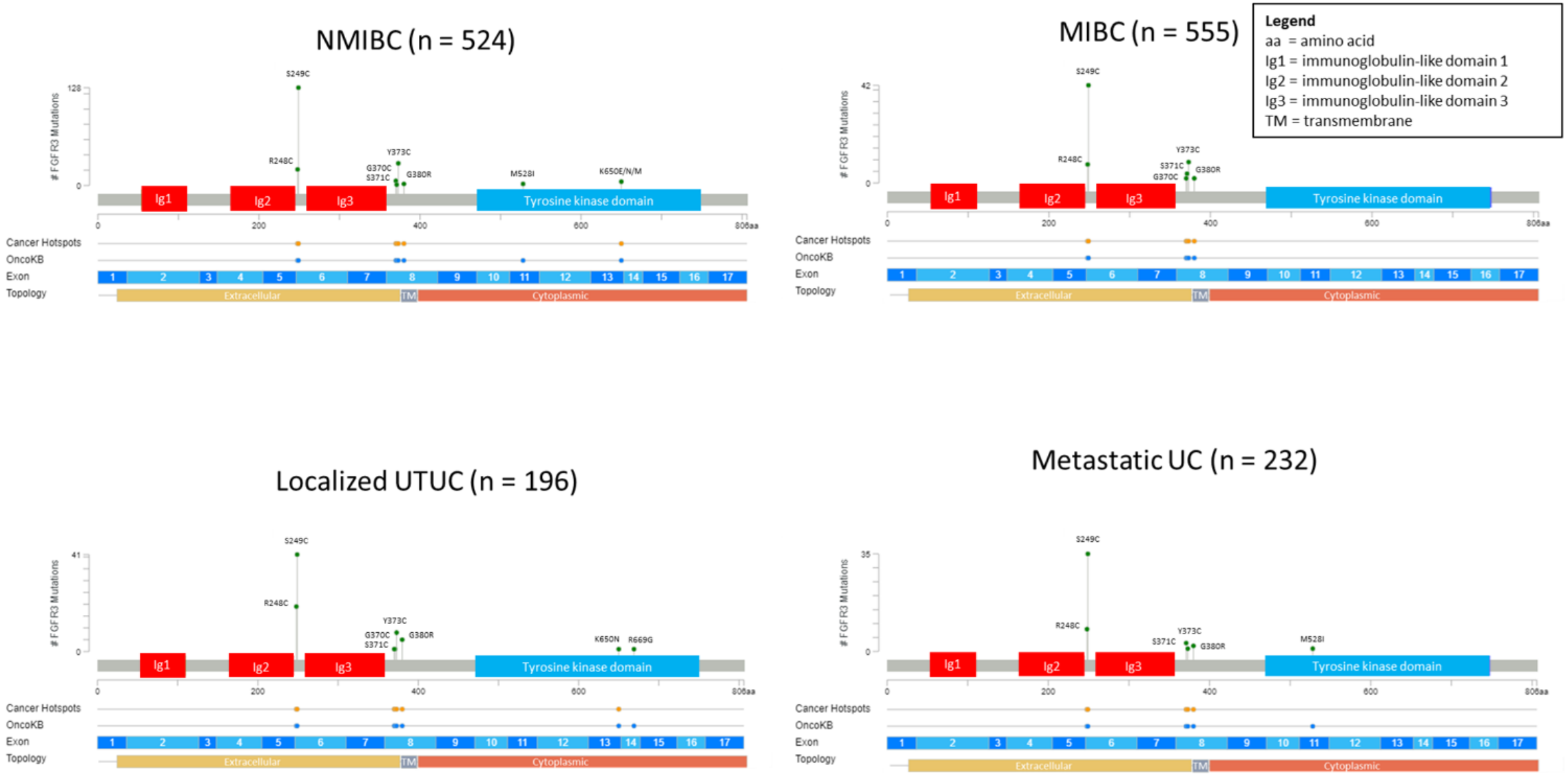


Supplemental Figure 1. Consort diagram.
cfDNA, cell-free DNA; UC, urothelial carcinoma; MSKCC, Memorial Sloan Kettering Cancer Center; mUC, metastatic urothelial carcinoma; WT, wildtype



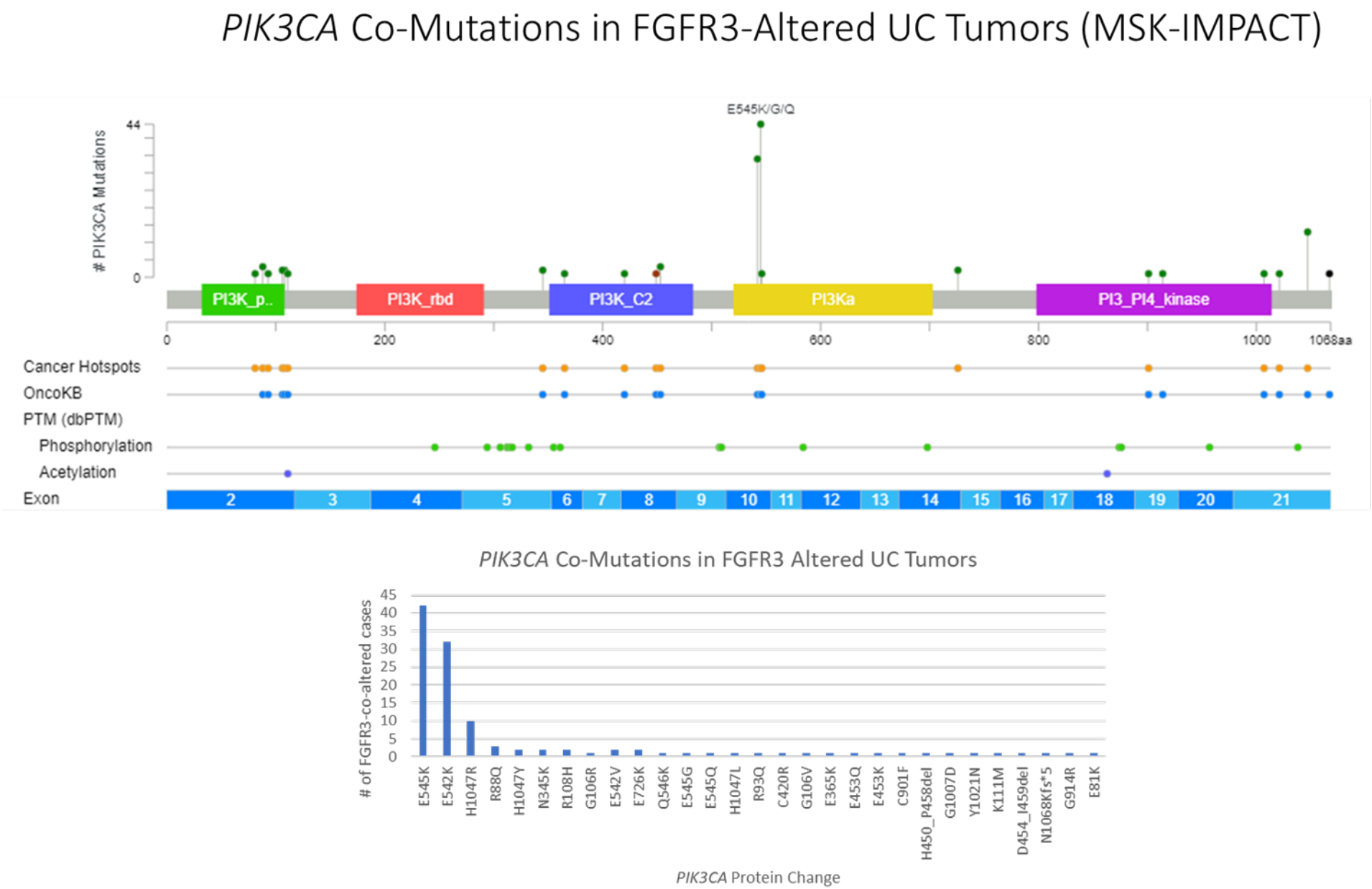
Supplemental Figure 2. Histogram depicting the frequency of oncogenic FGFR3 alterations in urothelial carcinoma tumors across various disease states.

MIBC, muscle-invasive bladder cancer; NMIBC, non-muscle invasive bladder cancer; UTUC, upper tract urothelial cancer



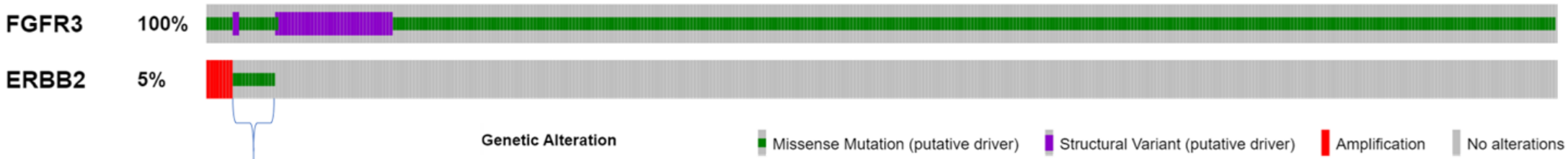
Supplemental Figure 3. Lollipop plots displaying the spectrum of oncogenic FGFR3 mutations observed across various disease states of urothelial carcinoma (N = 1,507).

MIBC, muscle-invasive bladder cancer; NMIBC, non-muscle invasive bladder cancer; UC, urothelial carcinoma; UTUC, upper tract urothelial carcinoma

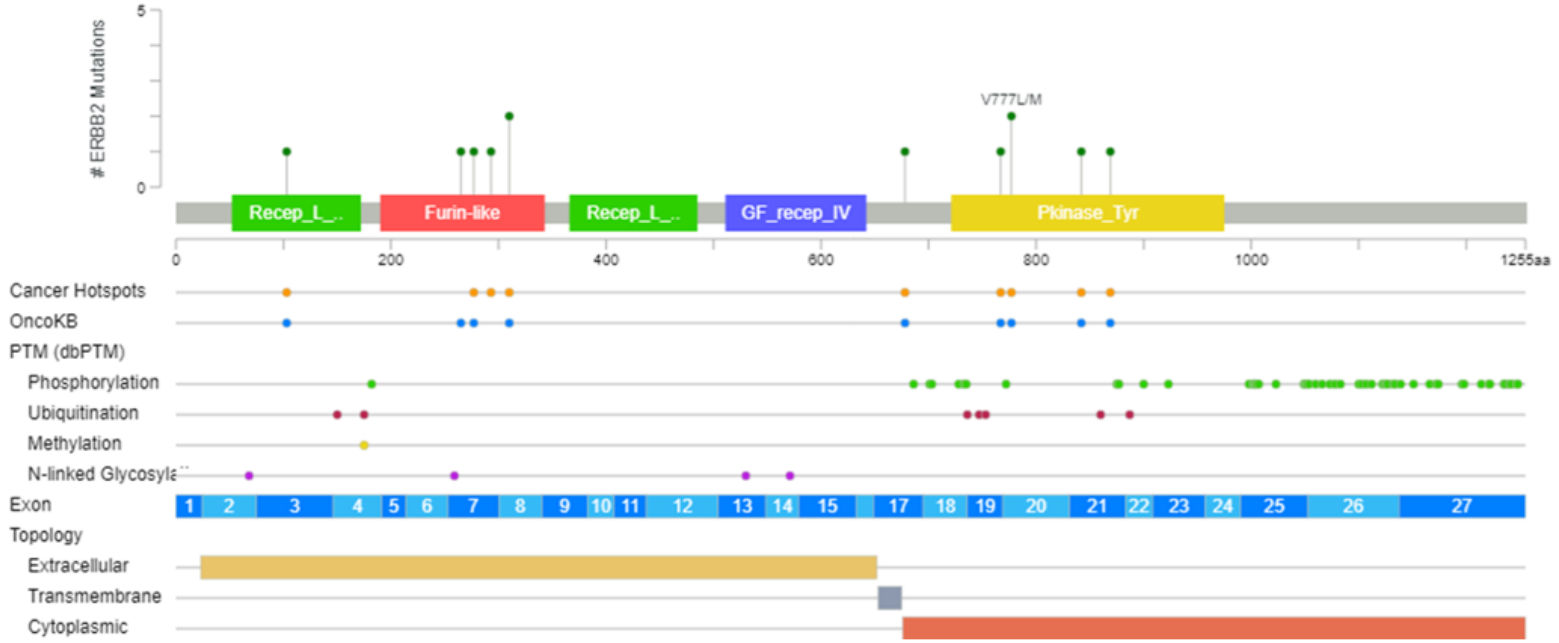


Supplemental Figure 4A. Lollipop plot and histogram displaying the spectrum of oncogenic *PIK3CA* co-mutations observed across all 414 FGFR3-altered urothelial carcinoma tumors of the entire 1,507 tumor cohort.
aa, amino acid; PTM, post-translational modification; UC, urothelial carcinoma

ERBB2 Co-Mutations in FGFR3-Altered UC Tumors (MSK-IMPACT)

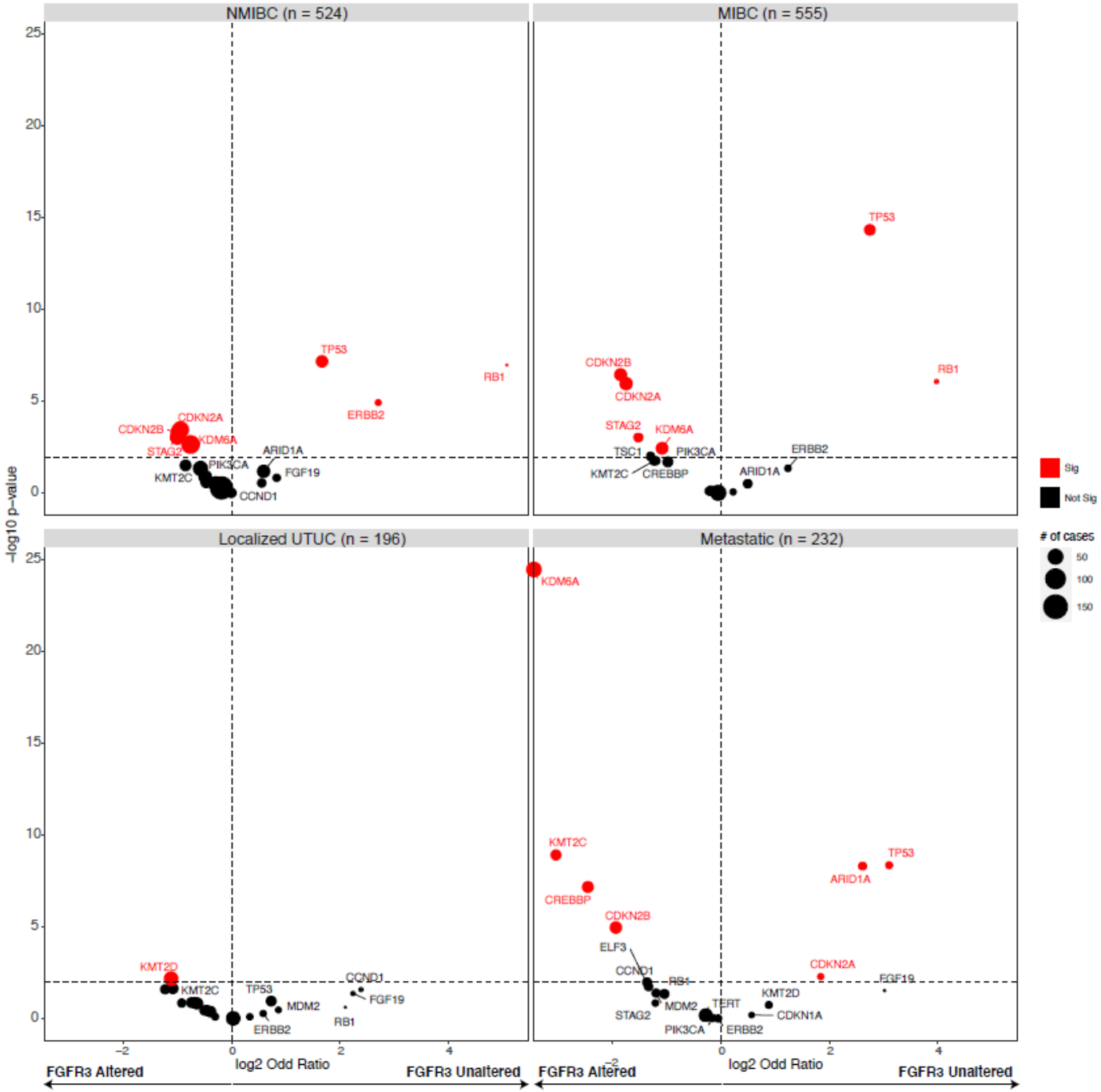


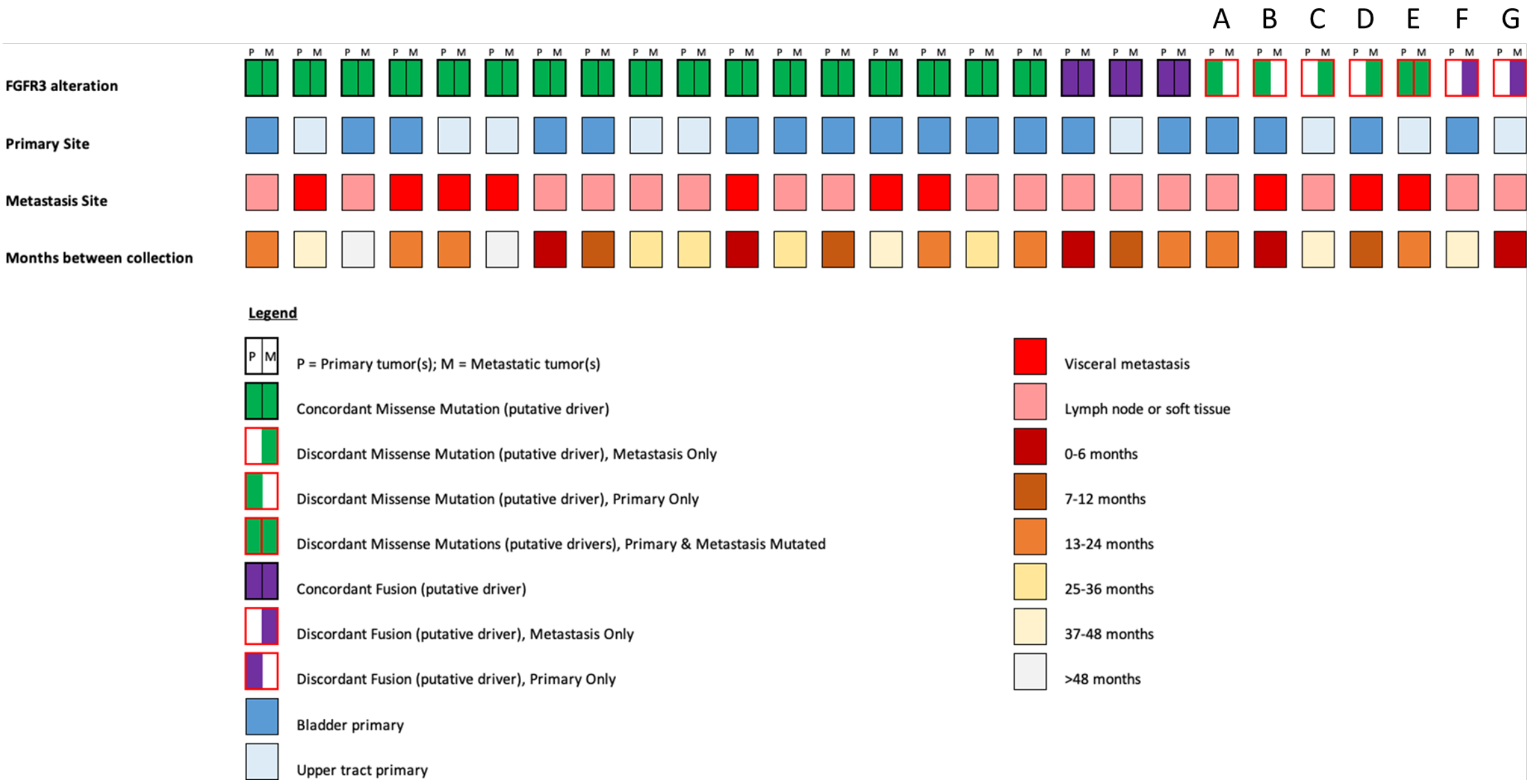
Sample ID	Primary Site	HER2 Protein change	FGFR3 Protein Change
P-0044415-T01-IM6	Bladder	A293T	R248C
P-0044415-T02-IM6	Bladder	A293T	R248C
P-0031860-T01-IM6	Bladder	E265K	S249C
P-0050195-T01-IM6	Upper Tract	I767M	S249C
P-0003279-T02-IM6	Bladder	L869R	TACC3-FGFR3 fusion
P-0050435-T01-IM6	Bladder	R103Q	Y373C
P-0048716-T01-IM6	Bladder	R678Q	K650N
P-0048716-T02-IM6	Upper Tract	R678Q	K650N
P-0011345-T01-IM5	Upper Tract	S310F	R248C
P-0023261-T01-IM6	Bladder	S310F	FGFR3-TACC3 fusion
P-0034651-T01-IM6	Bladder	V777L	R248C
P-0047715-T01-IM6	Bladder	V777M	S249C
P-0036882-T01-IM6	Upper Tract	V842I	R248C



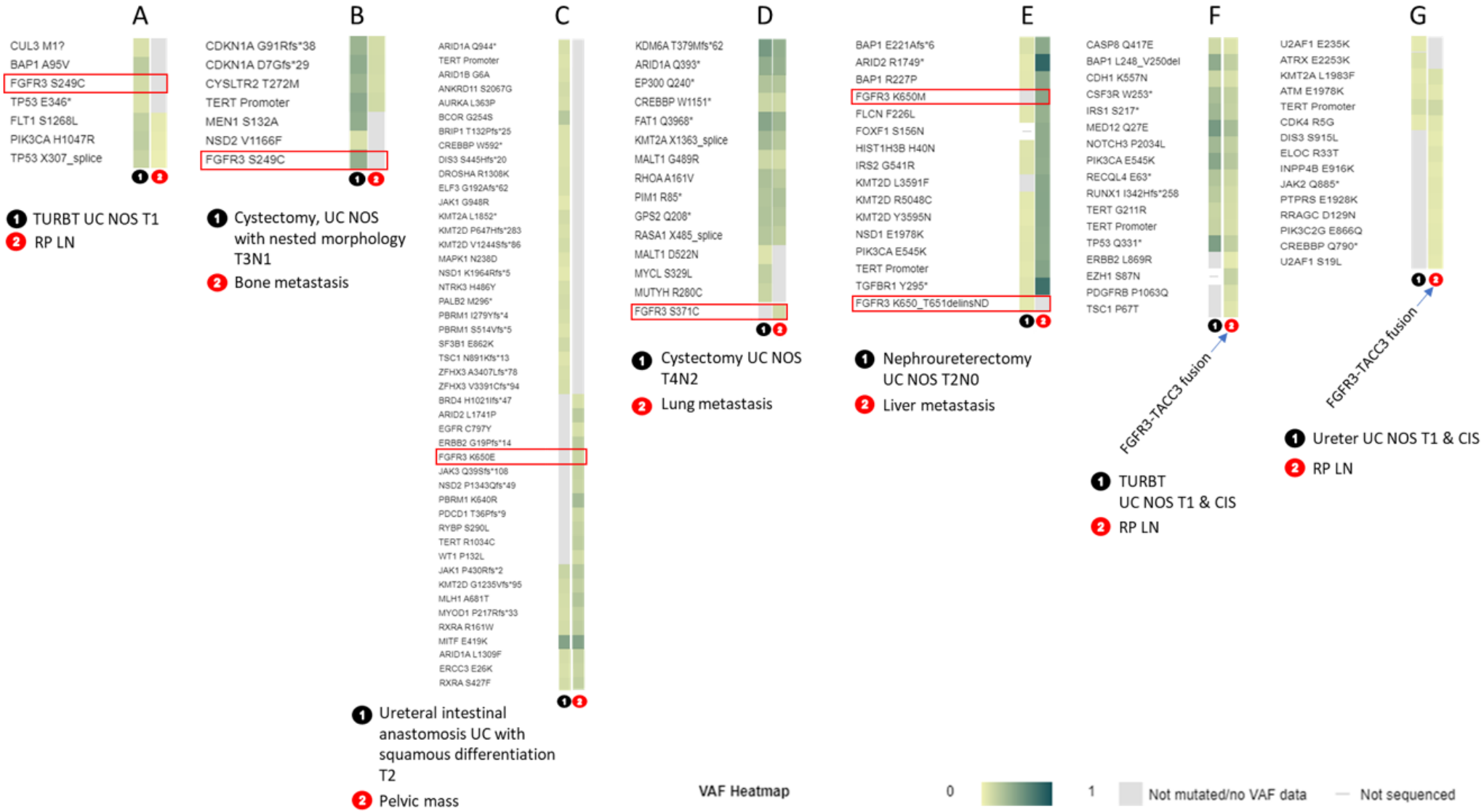
Supplemental Figure 4B. Oncoprint and lollipop plot displaying the spectrum of oncogenic HER2 co-mutations observed across all 414 FGFR3-altered urothelial carcinoma tumors of the entire 1,507 tumor cohort.
Aa, amino acid; PTM, post-translational modification; UC, urothelial carcinoma

Supplemental Figure 5. Volcano plot depicting associations of genomic alterations with FGFR3 oncogenic alterations across 1,507 urothelial carcinoma tumors, categorized by disease state. The dotted line represents a p-value cutoff of 0.01; the circle size represents the number of cases that have FGFR3 co-alterations. Variants of unknown significance were excluded. P-values were not corrected for multiple comparisons.
MIBC, muscle-invasive bladder cancer; NMIBC, non-muscle invasive bladder cancer; Sig, significant; UTUC, upper tract urothelial carcinoma.



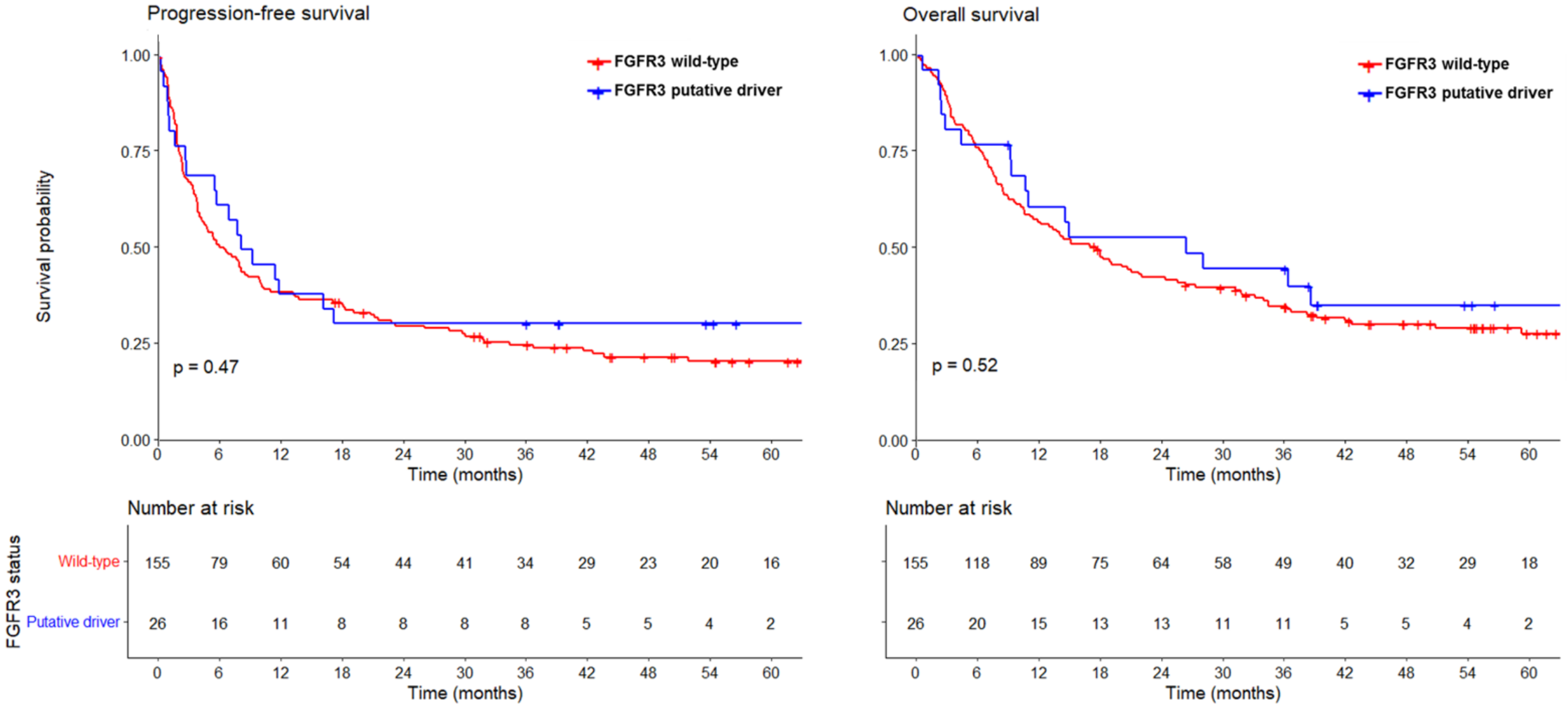


Supplemental Figure 6A. Oncoprint depicting concordance/discordance of FGFR3 alteration status between matched primary and metastatic tumors. Letters for discordant cases correspond to genomic heatmaps in Supplementary Figure 6B.

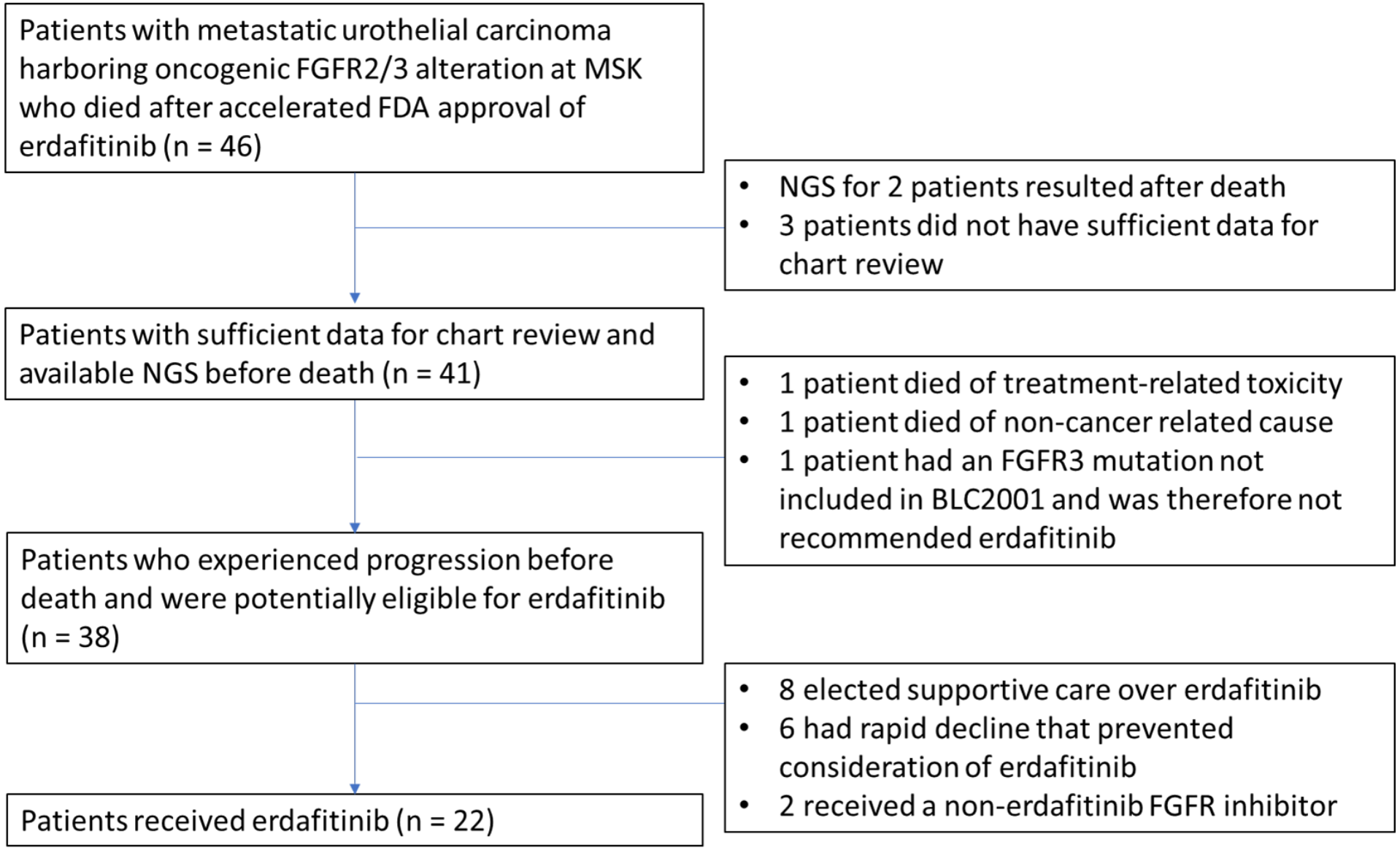


Supplemental Figure 6B. Heatmaps of mutations detected by next-generation sequencing of all available tumors for patients with discordant FGFR3 alteration status between matched primary and metastatic tumors in Supplemental Figure 6A. Each case is labeled with a letter (A-G) corresponding to a discordant case in Supplemental Figure 6A. Primary tumors are labeled with black numbers, metastases with red numbers.

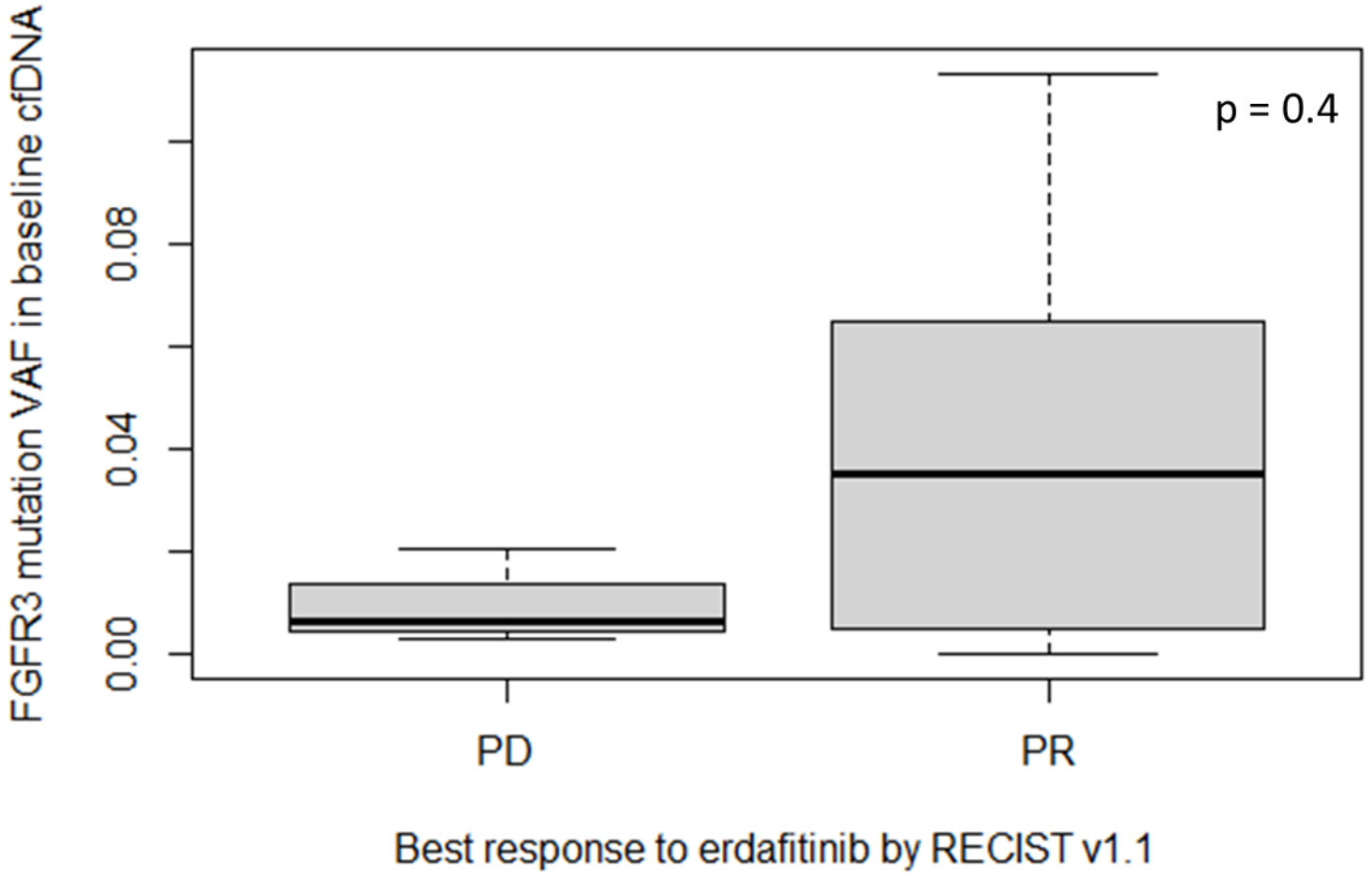
CIS, carcinoma in situ; N0, regional lymph node negative; N1, 1 involved regional lymph node; RP LN, retroperitoneal lymph node; UC NOS, urothelial carcinoma not otherwise specified; T1, invading lamina propria; T2, invading muscularis propria; T3, tumor with microscopic extravesical extension into surrounding fat; T4, tumor invading adjacent organs/structures; TURBT, transurethral resection of bladder tumor; VAF, variant allele frequency



Supplemental Figure 7. Progression-free and overall survival of patients with advanced/metastatic urothelial cancer treated with immune checkpoint blockade, stratified by FGFR3 alteration status.

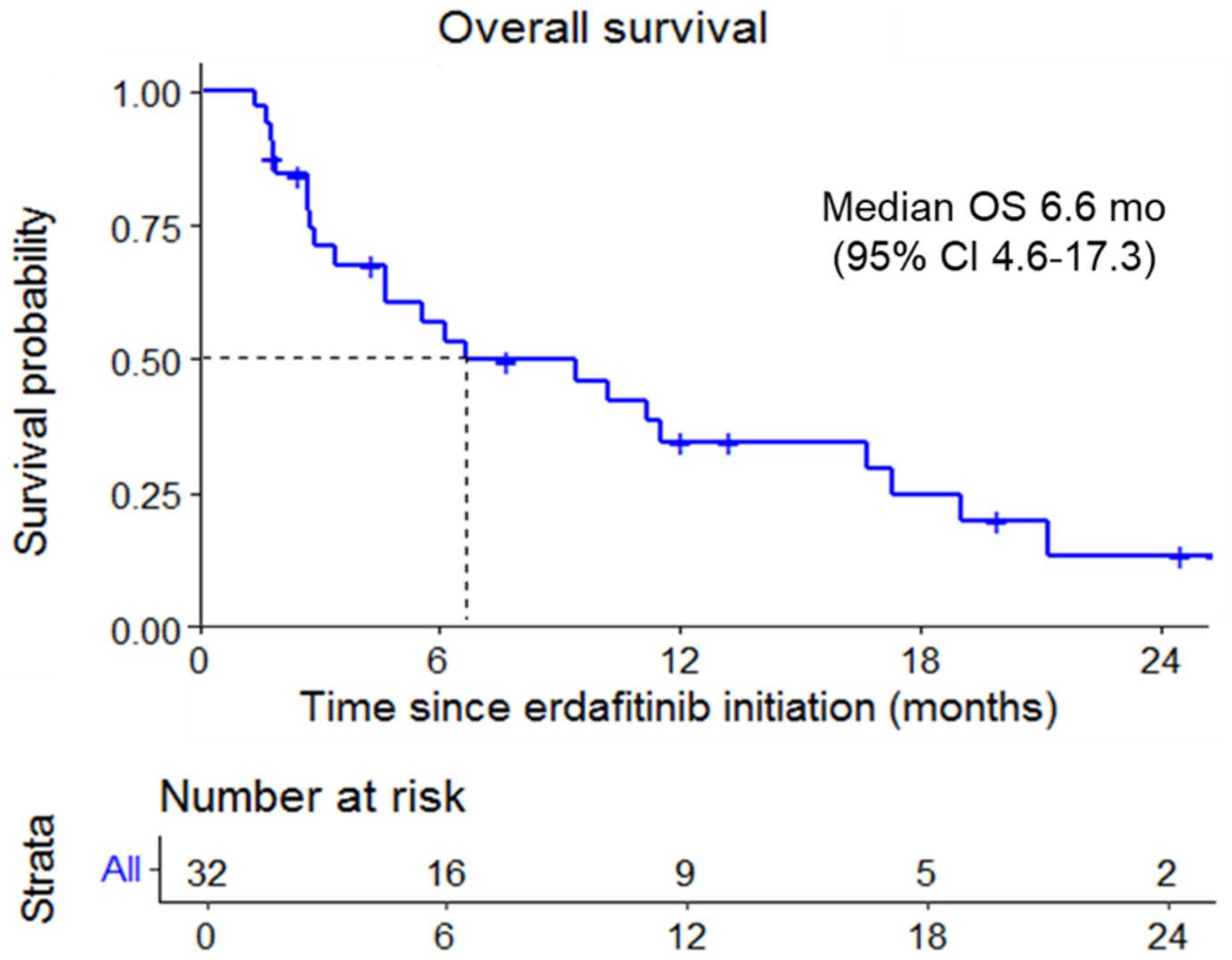


Supplemental Figure 8. Consort diagram depicting the disposition of patients at our institution with advanced/metastatic urothelial carcinoma and FGFR2/3 alterations eligible for treatment with erdafitinib.
FDA, Food and Drug Administration; MSK, Memorial Sloan Kettering Cancer Center; NGS, next-generation sequencing

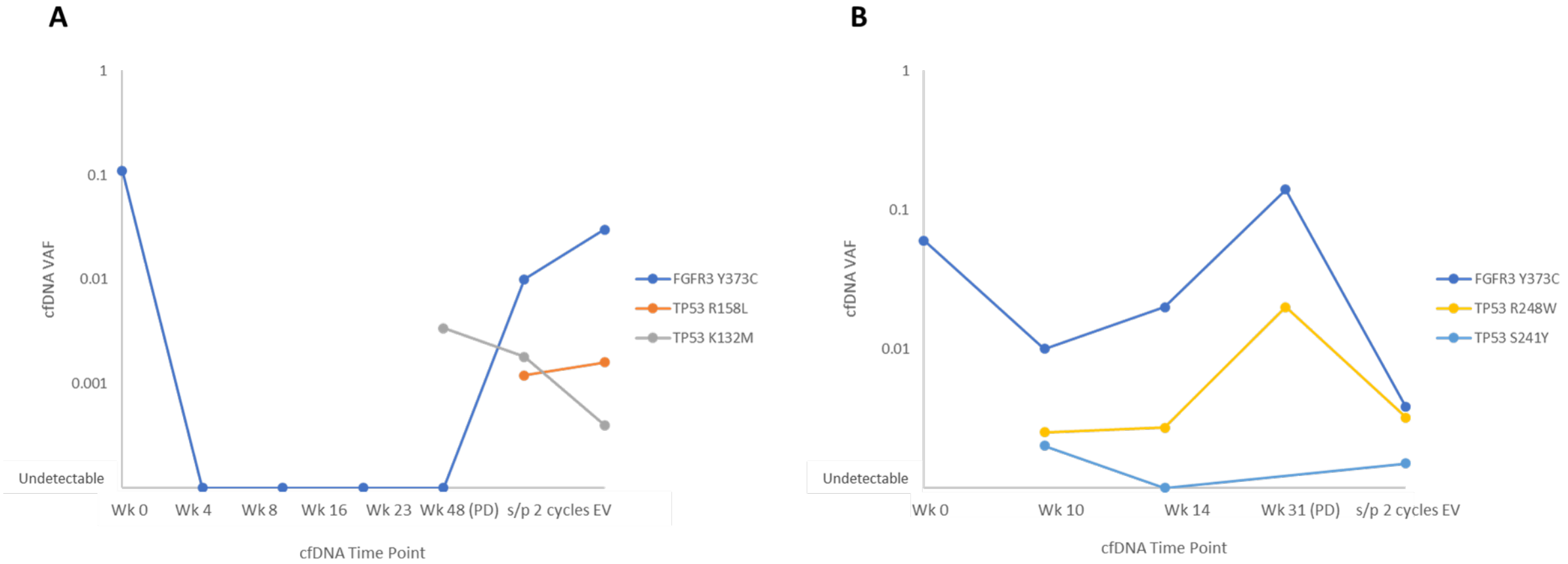


Supplemental Figure 9. Box and whisker plot depicting the FGFR3 mutation VAF in baseline cfDNA stratified by best radiographic response to erdafitinib. These analyses exclude patients who did not have cfDNA sequenced or had FGFR3 fusions for which VAF could not be calculated.

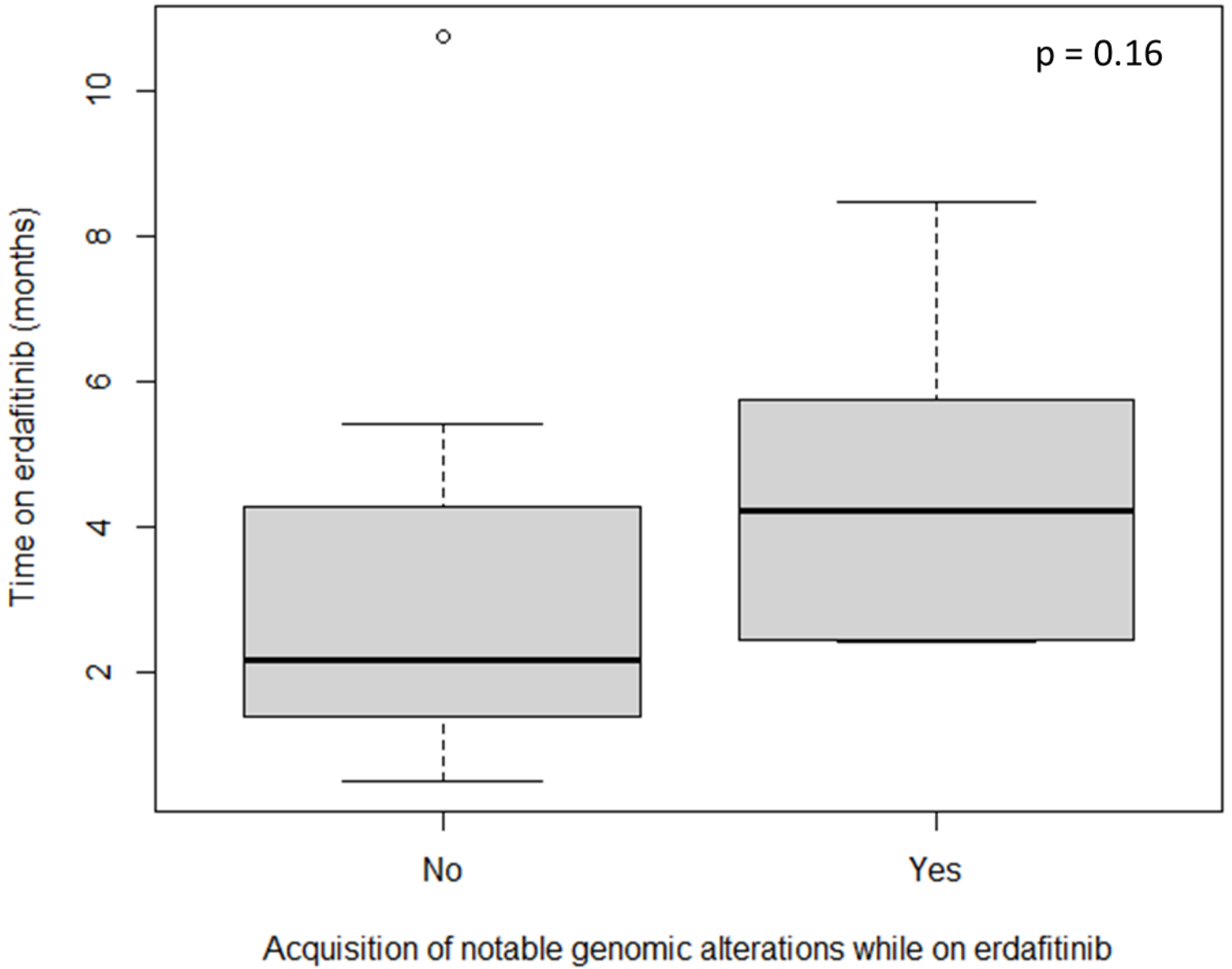
cfDNA, cell-free DNA; PD, progression of disease; PR, partial response; VAF, variant allele frequency



Supplemental Figure 10. Overall survival of patients treated with erdafitinib therapy.
CI, confidence interval; OS, overall survival; mo, months.



Supplemental Figure 11. Spaghetti plots of cfDNA VAF for selected patients who acquired new alterations of TP53. Plots include changes in the baseline cfDNA FGFR3 VAF as well as VAF of the newly acquired TP53 alterations.
cfDNA, cell-free DNA; EV, enfortumab vedotin; PD, progression of disease; s/p, status post; VAF, variant allele frequency; Wk, week

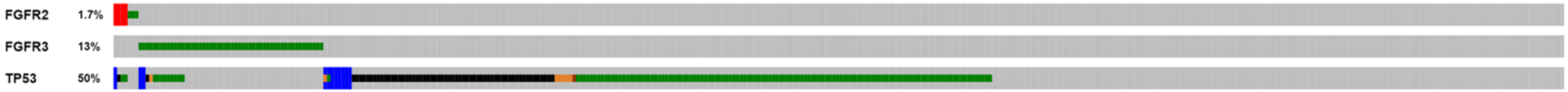


Supplemental Figure 12. Box and whisker plot depicting the amount of time on treatment with erdafitinib, comparing patients who demonstrated on-treatment acquisition of cell-free DNA alterations involved in FGFR3 signaling or TP53, versus patients who did not. These analyses exclude patients who did not have cell-free DNA sequenced.

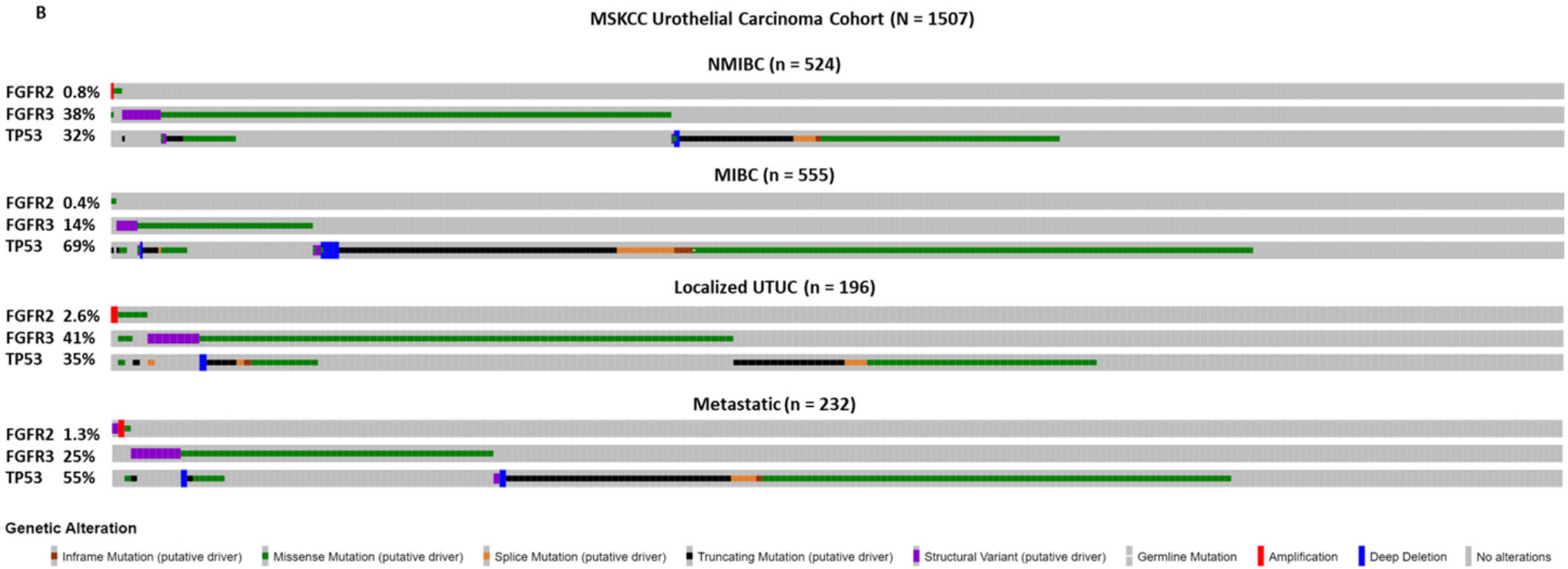
A

Urothelial TCGA (N = 408)

Gene Status	*FGFR3* WT	*FGFR* Oncodriver
***TP53* WT**	164 (40%)	39 (10%)
***TP53* Oncodriver**	192 (47%)	13 (3%)



B



Supplemental Figure 13. Oncoprints displaying alterations of FGFR2, FGFR3, and TP53 among (A) patients with muscle-invasive bladder cancer in the TCGA (Robertson et al. Cell 2017;171(3):540-556.e25) and (B) patients with urothelial carcinoma at Memorial Sloan Kettering Cancer Center, stratified by disease state (N = 1,507). Variants of unknown significance excluded.

NMIBC, non-muscle invasive bladder cancer; MIBC, muscle-invasive bladder cancer; MSKCC, Memorial Sloan Kettering Cancer Center; UTUC, upper tract urothelial carcinoma; WT, wild-type.