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Supplemental information

Genomic heterogeneity as a barrier to precision oncology in urothelial cancer

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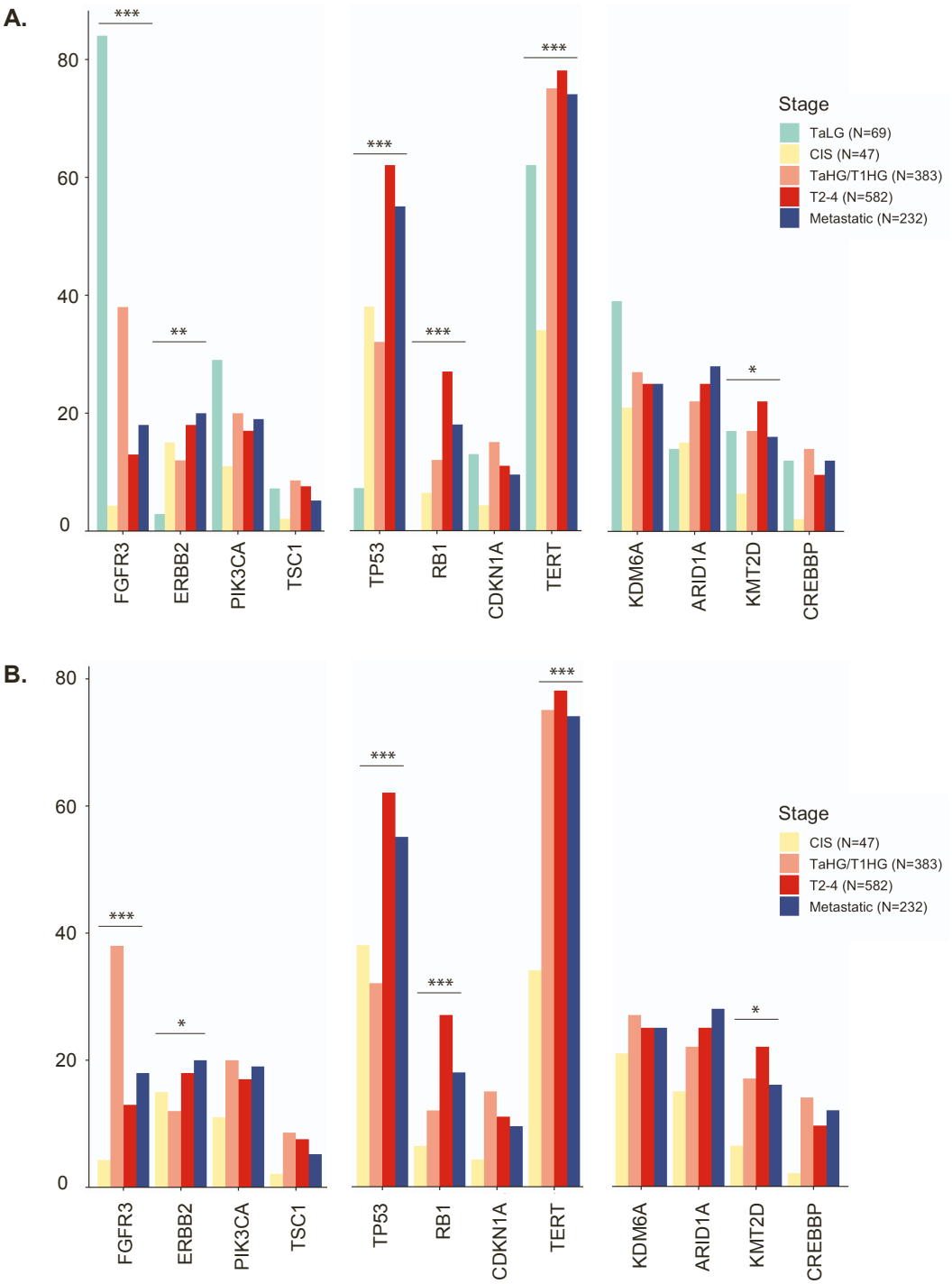

Figure S1. Alteration frequency in oncogenes in urothelial carcinoma stratified by disease state. (a) Frequency of alterations in frequently mutated oncogenes in the MSK urothelial cancer cohort stratified by disease state (Ta low-grade primary tumors, carcinoma in-situ (CIS), Ta/T1 high grade non-muscle invasive primary tumors, muscle invasive primary tumors and metastatic sites). (b) Frequency of alterations in frequently mutated oncogenes in high grade cancers in the MSK urothelial cancer cohort stratified by disease state (CIS, Ta/T1 high grade non-muscle invasive primary tumors, muscle invasive primary tumors and metastatic sites). Significant values are labeled in adjusted p-value (q-value): * q < 0.005, ** q < 0.01, *** q < 0.001. Related to Figure 1 and Table S1.

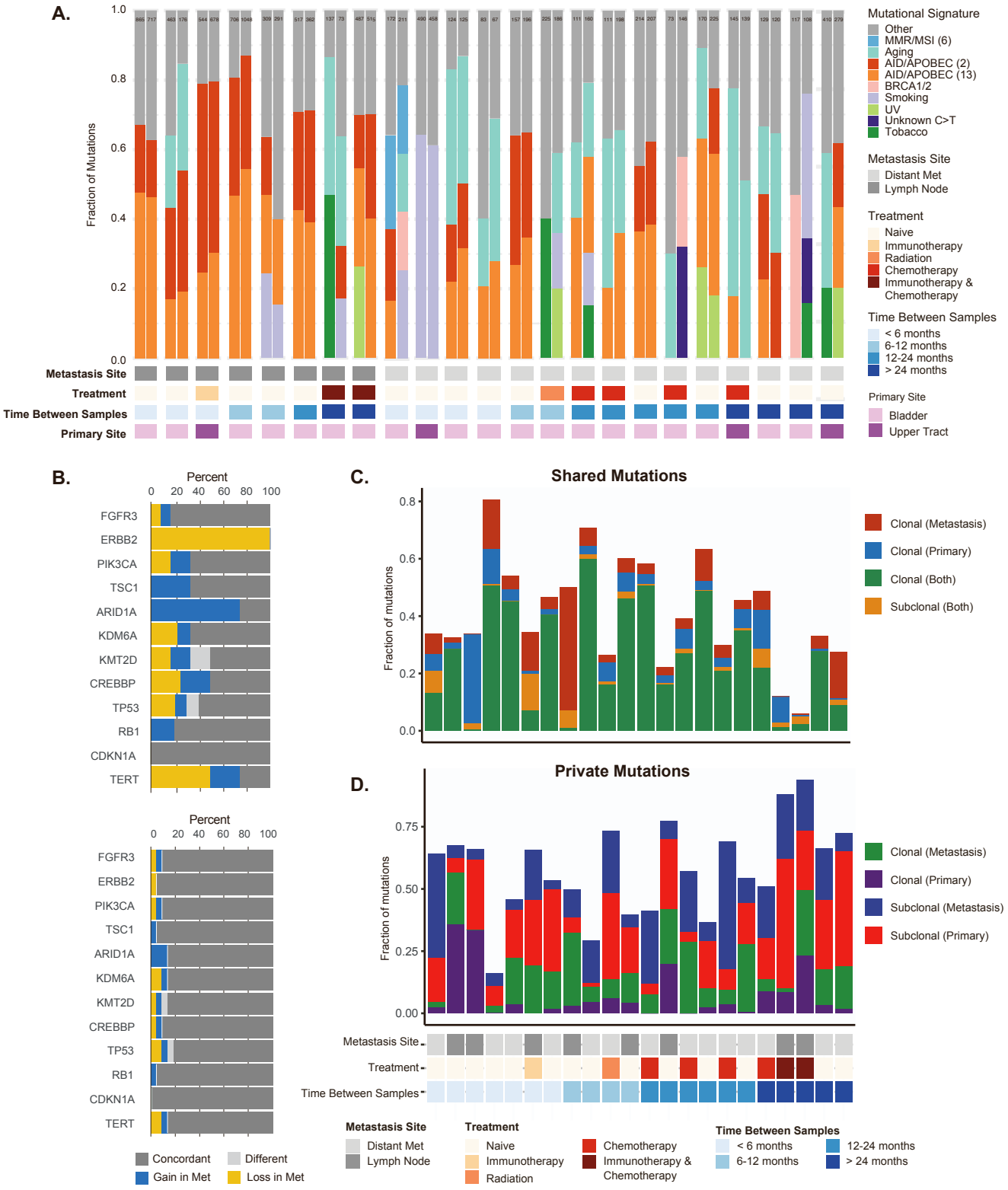

Figure S2. Whole exome sequencing of paired primary and metastatic specimens. (a) Mutation signature deconvolution of patient-matched primary and metastatic specimens. (b - top) includes only patients with a mutation in the designated gene in either the primary or metastasis or both; (b - bottom) includes all WES pairs including those not mutated. Clonal composition of shared mutations across each pair (c) and private mutations across each pair (d). Related to Figure 2.

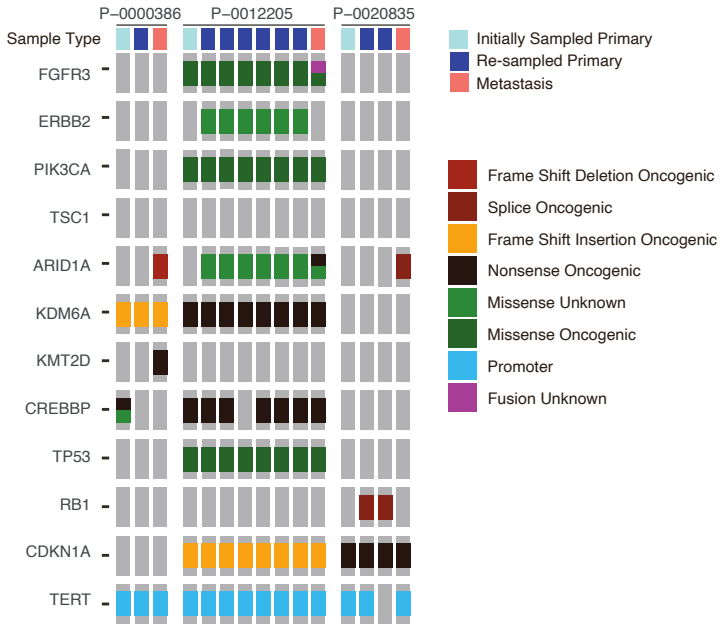

Figure S3. Resampling of bladder primary tumors from patients with discordant *ARID1A* mutational status. Analysis of spatially distinct regions of the primary tumors of 3 patients who had *ARID1A* mutations in the metastasis but not in the initially profiled region of the primary tumor. Manual review of the raw BAM files did not detect any mutant reads demonstrating the oncogenic *ARID1A* mutations identified in the metastasis in the resampled regions of the primary bladder tumor. Related to Figure 3.

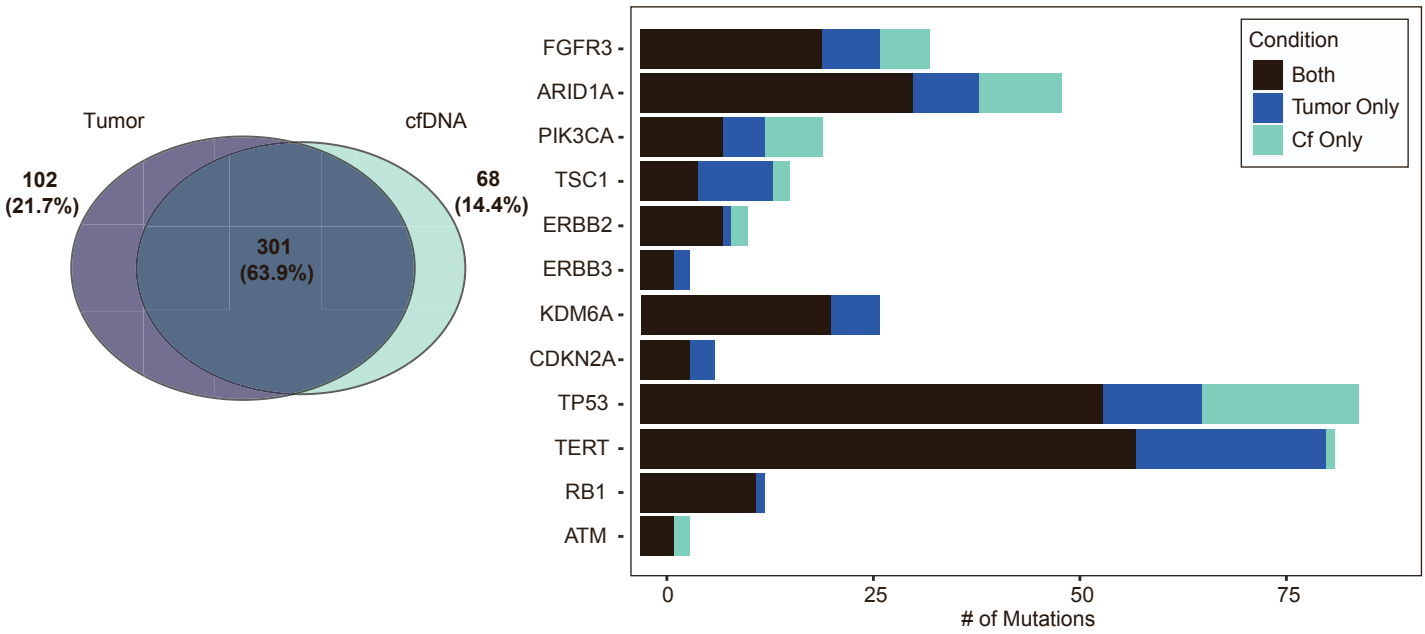

Figure S4. Concordance of oncogenic mutations between tumor (primary or metastatic) and cell free DNA (cfDNA).
Mutational concordance of select cancer-associated genes between patient-matched tumor and cfDNA samples (N = 123, oncogenic and likely oncogenic mutations only). Related to Figure 4.

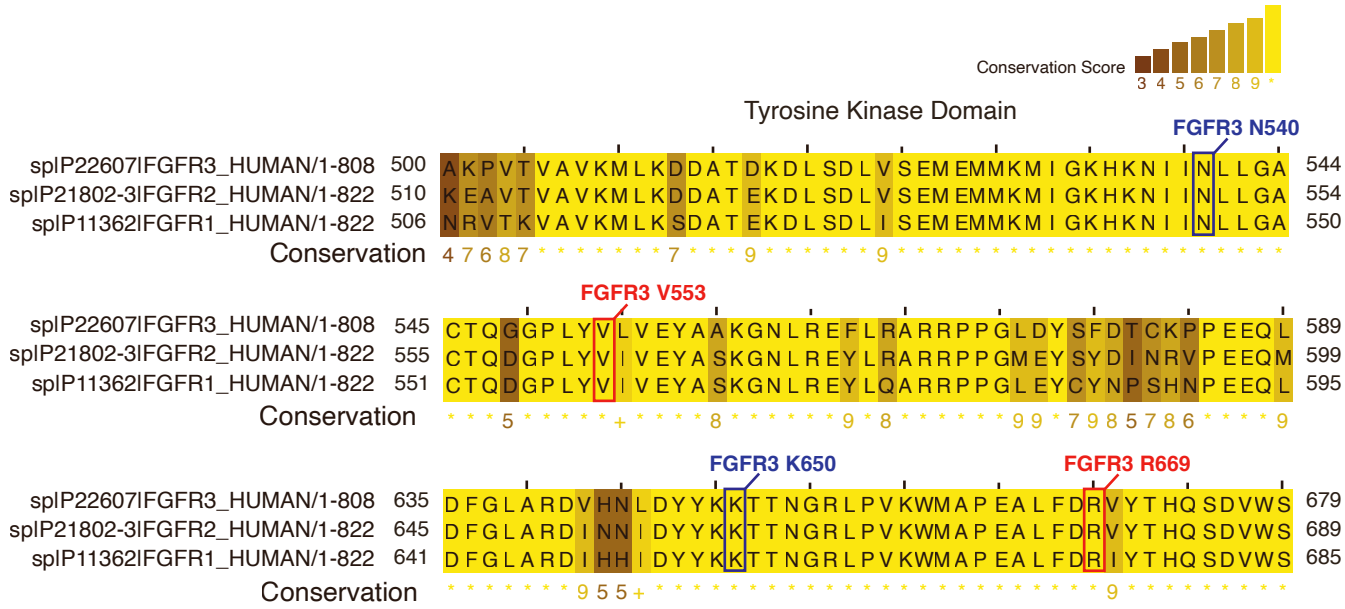

Figure S5. Paralogy analysis between FGFR1/FGFR2/FGFR3. Paralogy analysis was performed using Jalview. Mutations found in cfDNA collected post-treatment are highlighted. Related to Figure 4.

Table S1. Genomic differences between lower tract urothelial carcinoma stratified by grade and stage. Related to Figure 1 and S1.

Characteristic[1]	Low-Grade, n = 69	HG Non-Invasive, n = 235	HG Invasive, n = 777	Metastatic, n = 232	p-value[2]	**Adj. p-value (q-value)** [3]
KDM6A	27 (39%)	71 (30%)	190 (24%)	59 (25%)	0.028	0.06
ARID1A	10 (14%)	44 (19%)	193 (25%)	64 (28%)	0.03	0.06
KMT2D	12 (17%)	40 (17%)	156 (20%)	36 (16%)	0.39	0.59
KMT2C	8 (12%)	19 (8.1%)	52 (6.7%)	11 (4.7%)	0.19	0.31
EP300	6 (8.7%)	13 (5.5%)	63 (8.1%)	15 (6.5%)	0.53	0.68
CREBBP	8 (12%)	29 (12%)	80 (10%)	27 (12%)	0.81	0.86
TP53	5 (7.2%)	55 (23%)	448 (58%)	127 (55%)	<0.001	**<0.001**
RB1	0 (0%)	9 (3.8%)	200 (26%)	41 (18%)	<0.001	**<0.001**
CDKN1A	9 (13%)	30 (13%)	92 (12%)	22 (9.5%)	0.68	0.81
FGFR3	58 (84%)	104 (44%)	121 (16%)	42 (18%)	<0.001	**<0.001**
ERBB2	2 (2.9%)	23 (9.8%)	133 (17%)	46 (20%)	<0.001	**0.001**
PIK3CA	20 (29%)	55 (23%)	128 (16%)	43 (19%)	0.013	**0.04**
TSC1	5 (7.2%)	16 (6.8%)	62 (8.0%)	12 (5.2%)	0.56	0.69
TERT	43 (62%)	153 (65%)	605 (78%)	172 (74%)	<0.001	**<0.001**
ELF3	5 (12%)	14 (8.1%)	70 (11%)	27 (16%)	0.18	0.31
FAT1	1 (1.4%)	10 (4.3%)	54 (6.9%)	14 (6.0%)	0.18	0.31
STAG2	20 (29%)	29 (12%)	68 (8.8%)	11 (4.7%)	<0.001	**<0.001**
KMT2A	4 (5.8%)	14 (6.0%)	52 (6.7%)	14 (6.0%)	0.98	0.98
ERCC2	1 (1.4%)	29 (12%)	61 (7.9%)	8 (3.4%)	<0.001	**0.003**
ERBB3	2 (2.9%)	12 (5.1%)	51 (6.6%)	10 (4.3%)	0.47	0.64
RBM10	3 (4.3%)	18 (7.7%)	52 (6.7%)	14 (6.0%)	0.83	0.86
FBXW7	2 (2.9%)	15 (6.4%)	59 (7.6%)	6 (2.6%)	0.022	0.06
CDKN2A	11 (16%)	49 (21%)	182 (23%)	56 (24%)	0.43	0.62
CCND1	0 (0%)	27 (11%)	90 (12%)	30 (13%)	0.023	0.06
MDM2	1 (1.4%)	17 (7.2%)	79 (10%)	33 (14%)	0.006	**0.02**
CDKN2B	9 (13%)	42 (18%)	138 (18%)	42 (18%)	0.79	0.86

[1] Statistics presented: n (%). [2] Statistical tests performed: Chi-square test of independence. [3] Adjustment method: False Discovery Rate