

Figure S1. False-positive droplets threshold determination for: (A) TERT promoter mutation C228T; and (B) TERT promoter mutation C250T. TERT, telomerase reverse transcriptase.

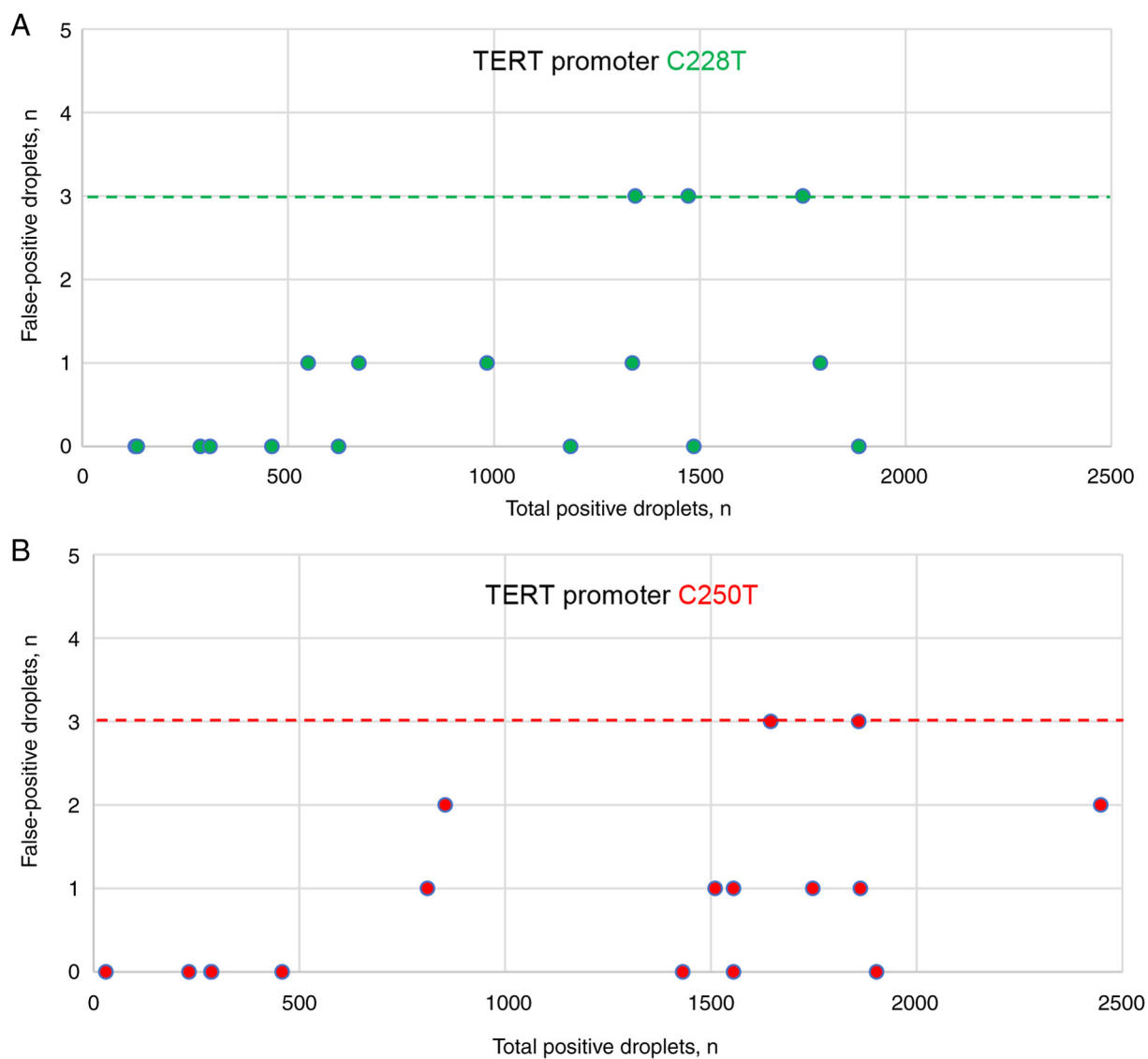


Figure S2. Determination of the linearity of the TERT promoter mutation quantitative analysis. (A) TERT promoter mutation C228T assay; and (B) TERT promoter mutation C250T assay. MAF, minor allele frequency; TERT, telomerase reverse transcriptase. \*Linear correlation coefficient value.

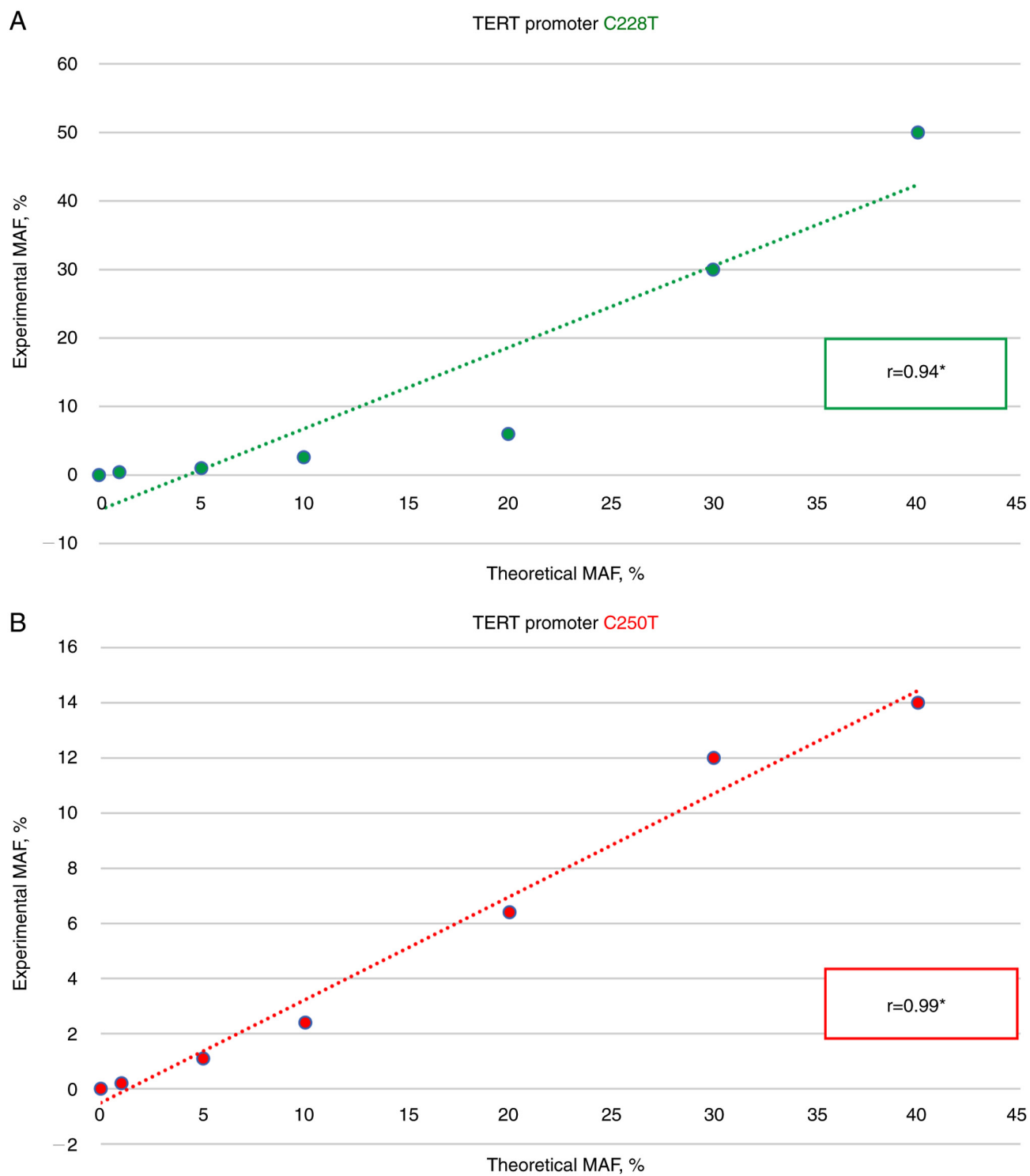


Figure S3. Examples of digital droplet PCR outcome: (A) Patient with low levels of urine DNA; (B) patient with high levels of urine DNA.

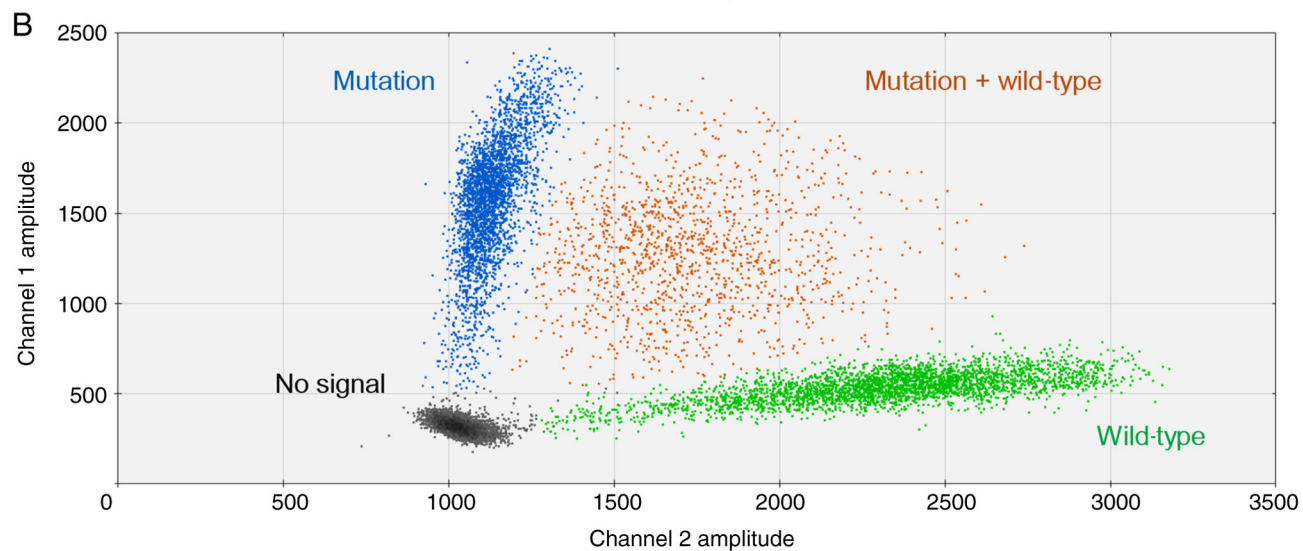
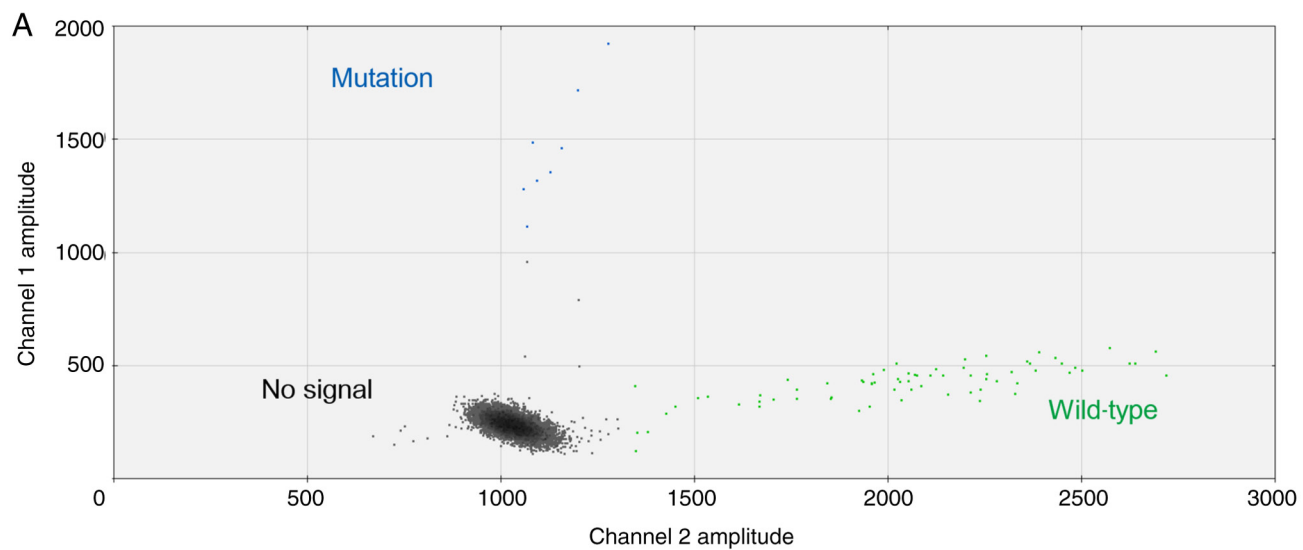


Table SI. Results of urine TERT promoter C228T and C250T detection in each clinical sample.

Sample ID	Bladder cancer status	C228T fraction <sup>a</sup> , %	C250T fraction <sup>a</sup> , %
1T	Positive	33.10	0
3T	Positive	3.55	1.03
4T	Positive	0	0
5T	Positive	0	0
7T	Positive	8.41	0
8T	Positive	48.77	0
11T	Positive	49.76	0
13T	Positive	0	31.39
14T	Positive	0	0
16T	Positive	29.46	1.93
19T	Positive	0	0
20T	Positive	27.76	0.02 <sup>b</sup>
24T	Positive	30.79	0
25T	Positive	10.77	0
26T	Positive	0	0
29T	Positive	0	0
31T	Positive	0	0
37T	Positive	25.27	0
38T	Positive	19.64	0
42T	Positive	0	0
43T	Positive	5.41	0
44T	Positive	0.45	0.59
51T	Positive	0	0
52T	Positive	2.29	0
53T	Positive	0	0
54T	Positive	0	0
59T	Positive	0	0
1K	Negative	0	0
2K	Negative	0	0
3K	Negative	0	0
4K	Negative	0	0
5K	Negative	0	0
6K	Negative	0	0
7K	Negative	0	0
8K	Negative	0	0
9K	Negative	0	0
10K	Negative	0	0
11K	Negative	0	0
12K	Negative	0	0
2S	Negative	0	0
6S	Negative	0	0
10S	Negative	0	0.08 <sup>b</sup>
17S	Negative	0	0
22S	Negative	0	0
23S	Negative	0	0
30S	Negative	0	0
33S	Negative	0	0
39S	Negative	0	0
55S	Negative	0	0
58S	Negative	0	0
15L	Post-resection	0	0
21L	Post-resection	0	0
28L	Post-resection	0	0
32L	Post-resection	0	0
34L	Post-resection	6.61	0
35L	Post-resection	2.48	0

Table SI. Continued.

Sample ID	Bladder cancerstatus	C228T fraction <sup>a</sup> , %	C250T fraction <sup>a</sup> , %
35L	Post-resection	2.48	0
40L	Post-resection	0	1.94
41L	Post-resection	0	0
56L	Post-resection	0.9	0
57L	Post-resection	0	0

<sup>a</sup>Fraction of urine tumor DNA determined by means of TERT promoter mutations C228T and C250T analysis.<sup>b</sup>Tumor DNA fraction did not exceed the calculated cut-off value of 0.3%, thus the sample was considered as mutation-negative. TERT, telomerase reverse transcriptase.