Supplementary Information

Molecular locker probe enrichment of gene fusion variants from matched patient liquid biopsy specimens for magneto-bioelectrocatalytic nanosensing

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Table S1. Oligonucleotide sequences used in experiments. Locker probe sequences are <u>underlined</u> to indicate key fusion breakpoints in mutant variants and **highlighted** to indicate locked nucleic acid basemodifications. Key: Bio-C6-, Biotin modification with 6-carbon spacer.

Oligos	5'-Sequence-3'
TMPRSS2 exons 1-2 locker probe	<u>CAATAT</u>**G*****A**C***CT***GCCG
ERG exons 3-4 locker probe	<u>GGCTT</u>**C*****CT***T**G***ATATG
ERG exons 4-5 locker probe	<u>GGAGAGTT</u>**C*****CT***T**G***AG
ETV1 exons 3-4 locker probe	<u>ACCTGA</u>**G*****CT***T**C***TGCA
T1E4 RPA Forward Primer	Bio-C6-CGGCAGGAAGCCTTATCA
T1E4 RPA Reverse Primer	GTTACATTCCATTTTGAT
T1E5 RPA Forward Primer	Bio-C6-CGGCAGGAAGCCTTATGA
T1E5 RPA Reverse Primer	CTGCTGGCACGATAACTC
T1ETV1 RPA Forward Primer	Bio-C6-CGGCAGGAAGCCTTATCT
T1ETV1 RPA Reverse Primer	TTTCTGAACATGGACTGT
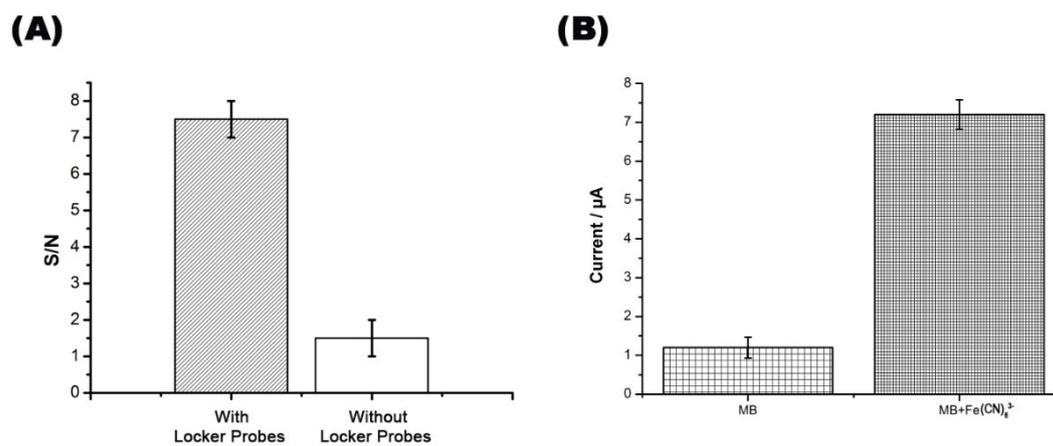
Fig. S1 Evaluation of the effects of locker probe enrichment and magneto-bioelectrocatalytic cycling on endpoint current measurements. (A) Current measurements in presence and absence of locker probe enrichment. (B) Current measurements using only DNA-intercalating MB or dual DNA-intercalating MB/freely diffusive $Fe(CN)_6^{3-}$ bioelectrocatalytic cycling. Error bars represent standard deviation of three independent experiments ($n = 3$).
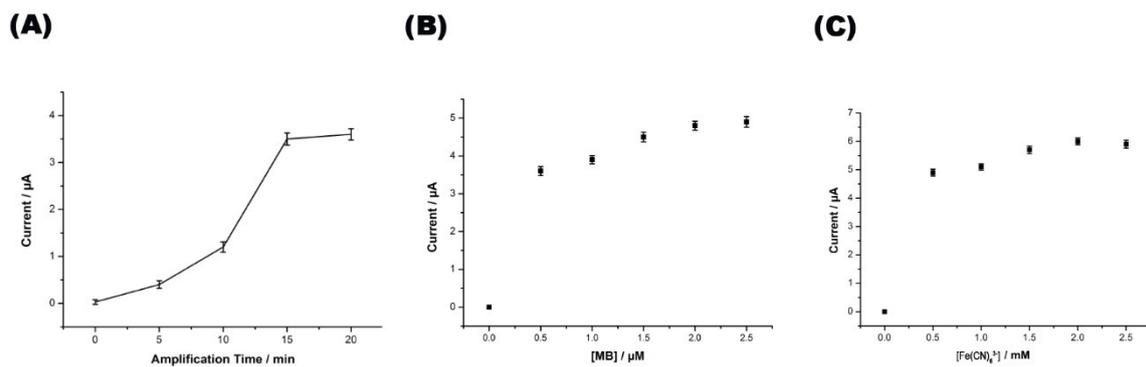
Fig. S2 Optimization of endpoint current signal. Current measurements at different (A) biotaget amplification times (B) MB concentrations and (C) $Fe(CN)_6^{3-}$ concentrations. Error bars represent standard deviation of three independent experiments ($n = 3$).
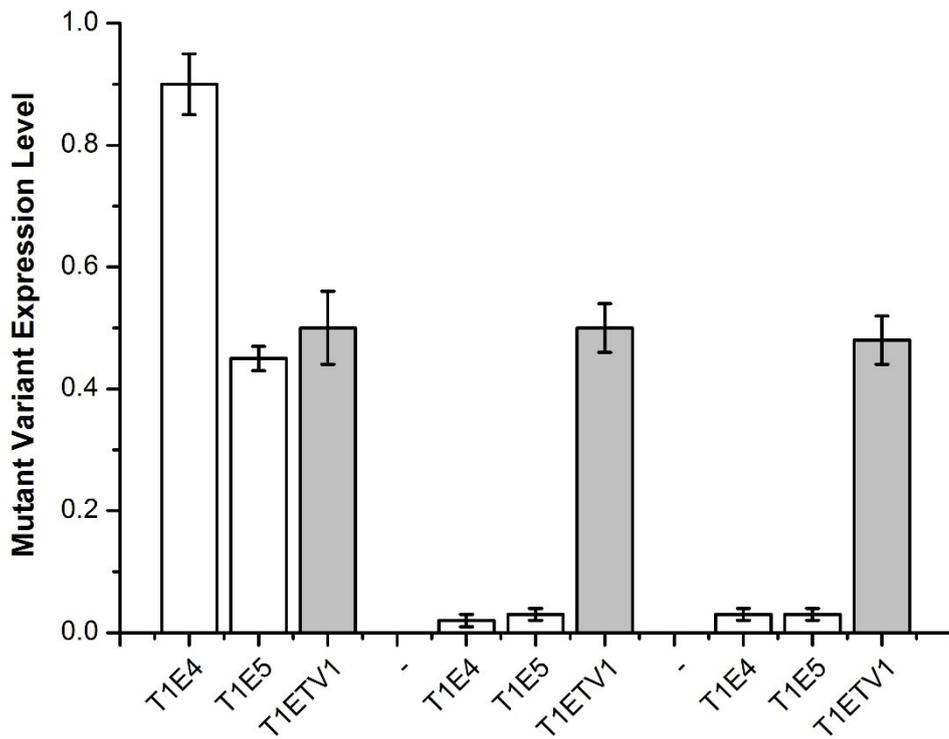
Fig. S3 Detection specificity for gene fusion mutant variants in cancer cell lines. specific T1ETV1 detection was shown by addition of T1ETV1 variant sequences (in grey) into all three cell lines. Error bars represent standard deviation of three independent experiments ($n = 3$).
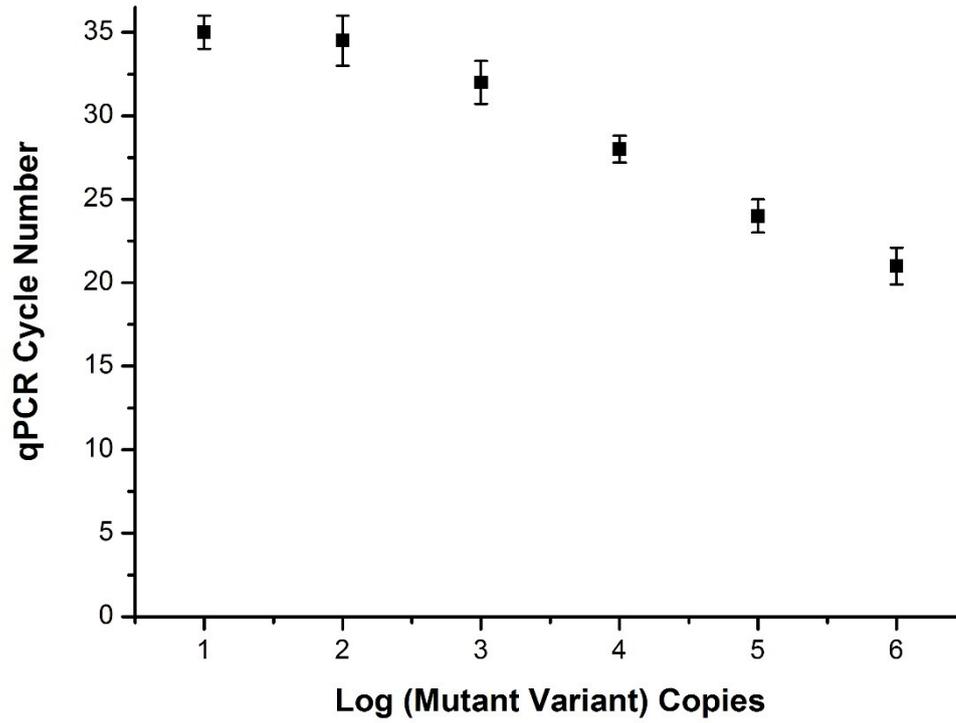
Fig. S4 Representative qPCR detection sensitivity limit and dynamic range for gene fusion mutant variants. Error bars represent standard deviations of triplicate independent measurements ($n = 3$).

Patient Urine Specimens

	P1	P2	P3	P4	P5	P6	P7	P8	P9	P10	P11	P12	P13	P14	P15	P16	P17	P18	P19	P20	P21	P22	P23	P24	P25	P26	P27	P28	P29	P30	H1	H2	H3	H4	H5	H6	H7	H8	H9	H10
T1E4	0	0.9	0.6	0.3	0	0	0.9	0	0.8	0	0.2	0.8	0.7	0.8	0.8	0	0.3	0.9	0.9	0	0.8	0	0.7	0.9	0.8	0	0.8	0	0	0.7	0	0	0	0	0	0	0	0	0	0
T1E5	0	0.7	0.6	0.2	0	0.2	0	0	0.7	0	0.3	0.2	0	0.2	1	0	0.6	0.7	0.8	0	0.6	0	0.7	0.9	0.4	0	0.9	0	0.4	0.7	0	0	0	0	0	0	0	0	0	0
T1ETV1	0	0	0	0	0	0	0.7	0	0.2	0	0	0.3	0	0.3	0	0.5	0	0	0.2	0	0.4	0.7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Matched Patient Plasma Specimens

	P1	P2	P3	P4	P5	P6	P7	P8	P9	P10	P11	P12	P13	P14	P15	P16	P17	P18	P19	P20	P21	P22	P23	P24	P25	P26	P27	P28	P29	P30	H1	H2	H3	H4	H5	H6	H7	H8	H9	H10
T1E4	0	0.8	0.6	0.1	0	0	0.8	0	0.7	0	0	0.5	0.7	0.9	0.8	0	0	0.9	0.8	0	0.7	0	0.7	0.9	0.7	0	0.8	0	0	0.5	0	0	0	0	0	0	0	0	0	0
T1E5	0	0.5	0.6	0.2	0	0.5	0	0	0.5	0	0.2	0	0	0	1	0	0.4	0.7	0.9	0	0.8	0	0.7	0.8	0.5	0	0.8	0	0.4	0.8	0	0	0	0	0	0	0	0	0	0
T1ETV1	0	0	0	0	0	0	0.7	0	0	0	0	0.3	0	0.3	0	0.3	0	0	0	0	0.6	0	0	0.5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Fig. S5 Heat maps representing numerical data of mutant variant expression levels of T1E4, T1E5 and T1ETV1 in matched (top) urine and (bottom) plasma specimens. "P" denotes "Prostate Cancer Patient" and "H" denotes "Healthy Donor". In bold are patients whose liquid biopsy specimens showed high expression for two or more of the gene fusion variant biotargets, and with poorer tumor grading outcomes.

Table S2. Tumor Gleason scores (GS) for study cohort of prostate cancer patiens. In **bold** are patients whose liquid biopsy specimens showed high expression for two or more of the gene fusion variant biotargets. Key: GS<7, low-grade cancer; GS≥7, high-grade aggressive cancer.

	Gleason scoring
P1	GS<7
P2	**GS≥7**
P3	**GS≥7**
P4	GS<7
P5	GS<7
P6	GS<7
P7	**GS≥7**
P8	GS<7
P9	**GS≥7**
P10	GS<7
P11	GS<7
P12	GS<7
P13	GS≥7
P14	**GS≥7**
P15	**GS≥7**
P16	GS<7
P17	GS<7
P18	GS<7
P19	**GS≥7**
P20	GS≥7
P21	**GS≥7**
P22	GS≥7
P23	**GS≥7**
P24	**GS≥7**
P25	**GS≥7**
P26	GS<7
P27	**GS≥7**
P28	GS<7
P29	GS≥7
P30	**GS≥7**