Supporting Information

Lead-start isothermal polymerase amplification controlled by DNAzymatic switches

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Supplementary Figures and Tables

Table S1. Oligonucleotides in this study

Name	DNA Sequence (5' → 3')
OFF → ON system	
OFF→ON switch (optimal locking)	GAGTCCAATrAGGAAGAGTCCAATGTACAGTATTGGA CTCTGAAGTAGCGCCGCCGTATTGGACTCTTCC
OFF→OFF switch (too strong locking)	GCGGCGTCACTATrAGGAAGATCCAATGTACAGTATTG GATCTGAAGTAGCGCCGCCGTATAGTGACGCCGC
ON→ON switch (too weak locking)	AAGACAATrAGGAAGACAATGTACAGTATTGTCTGAA GTAGCGCCGCCGTATTGTCTTC
ON → OFF system	
ON→OFF switch (optimal unlocking)	CTGTACATTGTrAGGAAGAGCTTTGCTCTGAAGTAGCG CCGCCGTACAATGTACAGTATTGTACG
OFF→OFF switch (too weak unlocking)	CTGTACATTACTrAGGAAGAGCTTTGCTCTGAAGTAGC GCCGCCGTAGTAATGTACAGTATTACTACG
ON→ON switch (too strong unlocking)	CGTAATACTGTACATTrAGGAAGAGCTTTGCTCTGAAG TAGCGCCGCCGTAATGTACAGTATTACG
DNA templates and primers	
Template	CAGAAATCTCAGGGACTCTAAAGCTCAACTTGCATAA ACTTCTGAGGA
FAM-Primer	FAM-TCCTCAGAAGTTTATGCA
COVID-19 Template	CACATTGGCACCCGCAATCCTGCTAACAATGCTGCAA TCGTGCTACAACT

COVID-19 Primer	AGTTGTAGCACGATTGCAGC
HPV-16 Template	CACTATTTTGGAGGACTGGAATTTTGGTTTACAACCTC CTCCAGGAGGCA
HPV-16 Primer	TGCCTCCTGGAGGAGGTTGT

The RNA nucleotides were colored in red (rA).

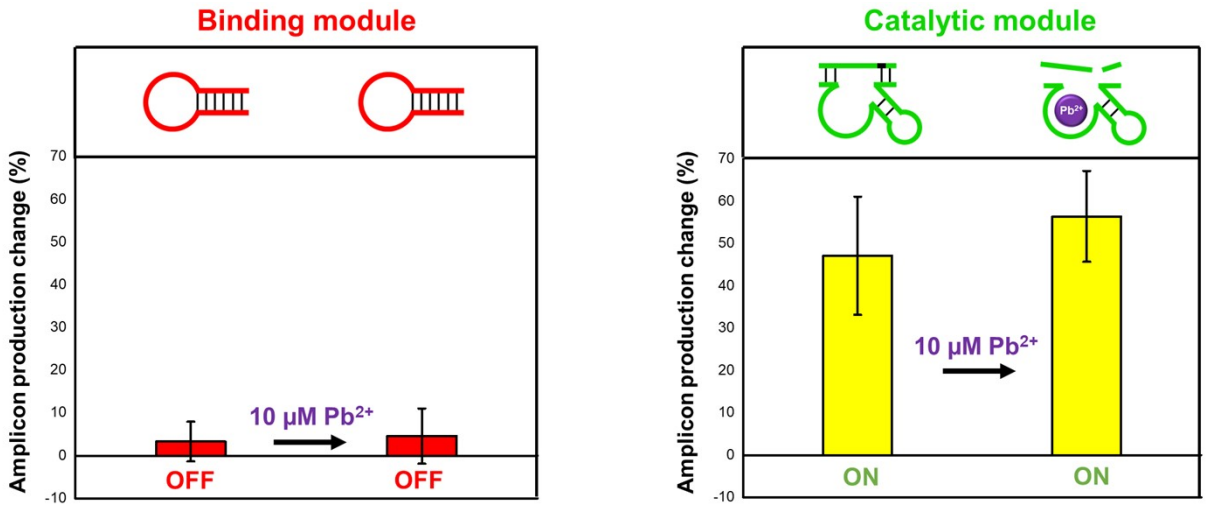

Fig. S1. Effects of individual binding and catalytic modules on the activity of DNA polymerase. Regardless of Pb^{2+} addition, TQ30 aptamer completely inhibited the DNA polymerase activity. After Pb^{2+} addition, GR5 DNAzyme did not change the activity of DNA polymerase significantly.

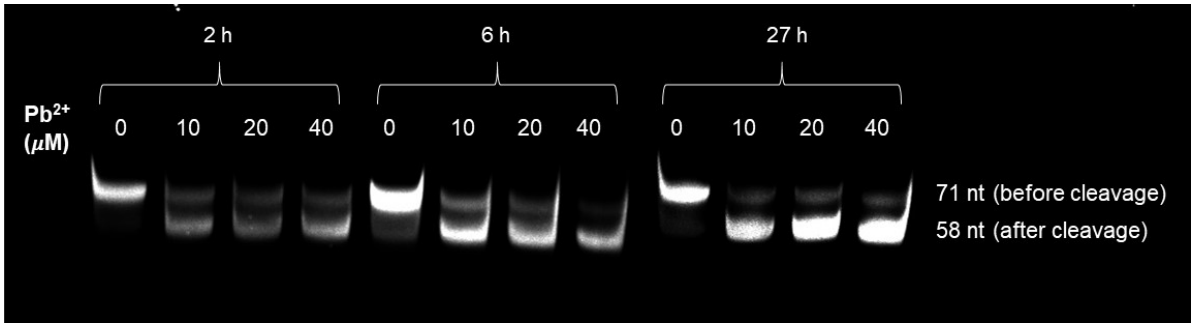

Fig. S2. Time-dependent cleavage of GR5 DNAzyme by varying concentrations of Pb^{2+}. Original GR5 DNAzyme module with 71-nt length was treated with diverse concentrations of Pb^{2+} (0, 10, 20, 40 μM) and incubated for 2 h, 6 h, and 27 h. When RNA cleavage occured, the GR5 DNAzyme module became split to be 58-nt and 13-nt which can be clearly distinguished through the PAGE. The GR5 DNAzyme module showed a fast cleavage rate, as 71.6 % of cleavage was carried out within only 2 h upon the addition of 10 μM Pb^{2+}.

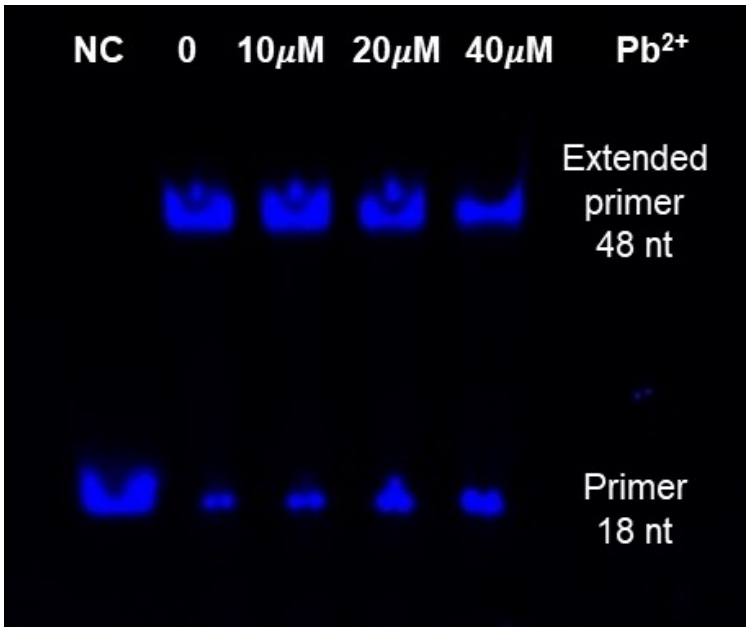

Fig. S3. Effect of Pb^{2+} ions on Taq DNA polymerase activity. To test if Pb^{2+} by itself can hamper the intrinsic enzymatic activity of Taq DNA polymerase, we added diverse concentrations of Pb^{2+} to polymerase and quantified the amount of extended primers.

Compared to the activity of polymerase without Pb^{2+}, the addition of 10 μM Pb^{2+} gave no change in the ability of the polymerase to extend the primers. However, the addition of 20 μM and 40 μM of Pb^{2+} slightly reduced the total amount of the extended primers, which means that there is somehow weak suppression of polymerase activity. For this reason, we decided to use 10 μM (final concentration) of Pb^{2+} to trigger the reaction of our molecular switches.

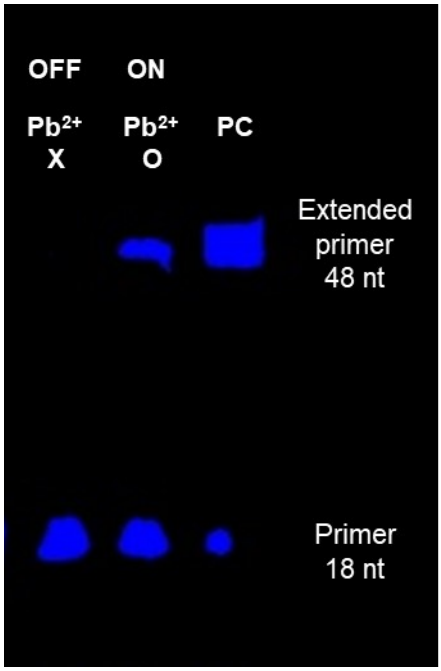

Fig. S4. Operation of the OFF→ON switch upon Pb^{2+} addition. We tested the performance of our OFF→ON switch by quantitatively comparing the amount of extended FAM-primers before and after the addition of Pb^{2+}. Establishing positive control (PC) as the percentage of the extended FAM-primer in the absence of our OFF→ON switch, we calculated the relative band intensity at each condition to derive the amplicon production change (%). In presence of our OFF→ON switch, the signal change greatly increased from 2.45 % (OFF) to 48.74 % (ON) upon 10 μM Pb^{2+} addition, which means the proper operation of our switch to recover the DNA polymerase activity.

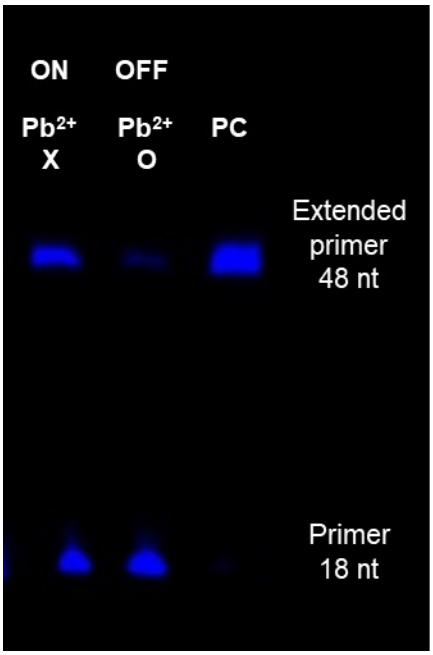

Fig. S5. Operation of the ON→OFF switch upon Pb^{2+} addition. In the same way as Figure S3, we calculated the amplicon production change (%) before and after adding 10 μM of Pb^{2+}. In presence of our ON→OFF switch, the signal change decreased from 54.84 % (ON) to 16.45 % (OFF) upon 10 μM Pb^{2+} addition, confirming the proper function of our switch.

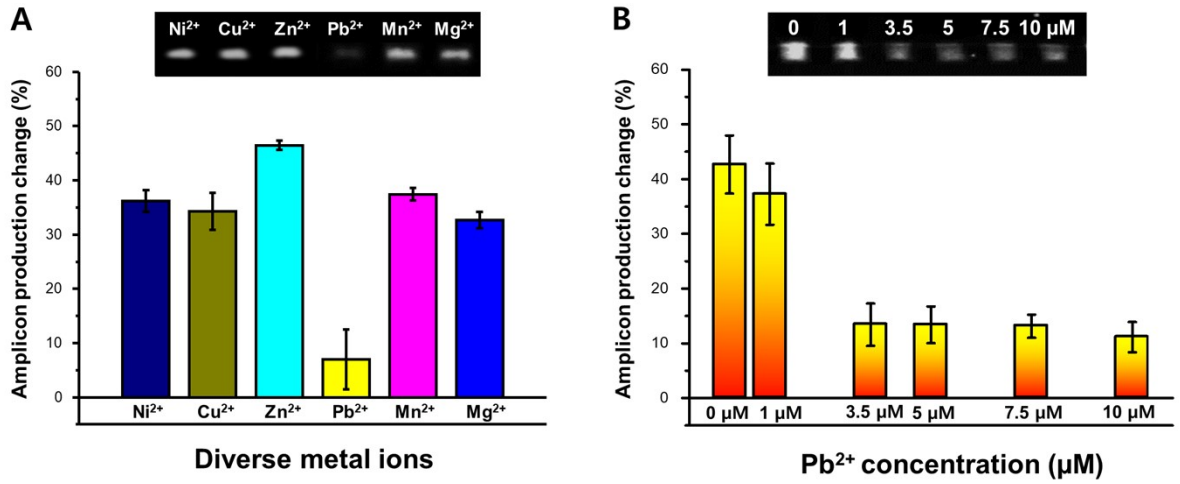

Fig. S6. Pb^{2+} specificity and concentration dependence for the ON→OFF switch. **A)** High Pb^{2+} specificity in operating the ON→OFF switch. Among diverse metal ions (Ni^{2+}, Cu^{2+}, Zn^{2+}, Pb^{2+}, Mn^{2+}, and Mg^{2+}), only Pb^{2+} could induce the deactivation of DNA polymerase. **B)** By varying Pb^{2+} concentrations, the ON→OFF switch could control the degree of isothermal amplification by DNA polymerases.

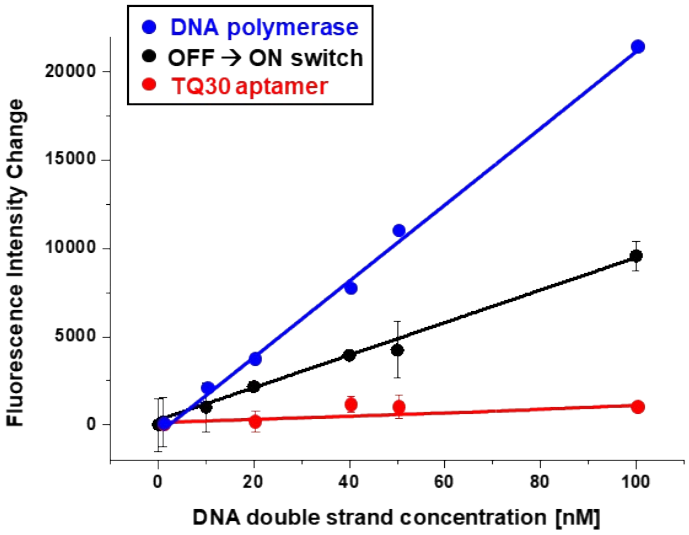

Fig. S7. Positive (blue) and negative (red) control experiments for quantification of HPV type 16 DNA in 5 % human serum. For the positive control experiment, only DNA polymerase was applied without the OFF→ON switch. There was a strong linear relationship between the

HPV16 DNA concentration and the fluorescence intensity change, but the slope of the positive control (217.5/nM) was steeper than that of the OFF→ON switch (92.1/nM), indicating the lowered activity of DNA polymerase in the presence of the DNAzymatic switch. For the negative control experiment, the TQ30 aptamer was applied instead of the OFF→ON switch, and there was almost no increment of fluorescence intensity over varied DNA double strand concentrations.

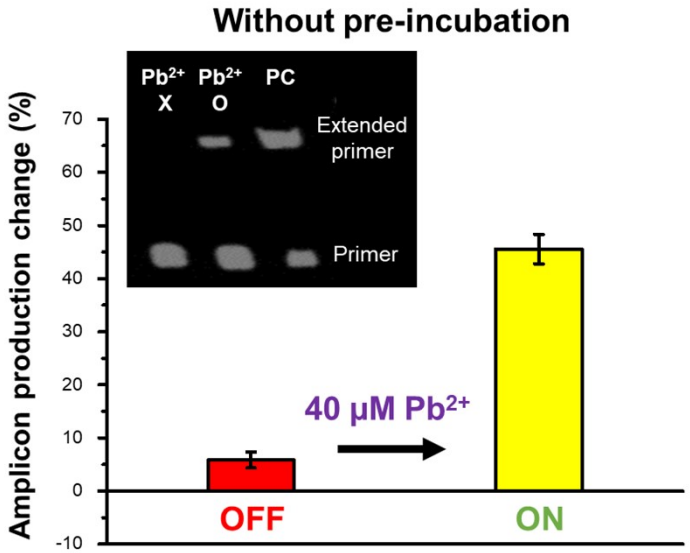

Fig. S8. The OFF→ON switch works well without Pb^{2+}-induced pre-cleavage. In allowing Pb^{2+} to immediately initiate the isothermal amplification reaction, 300 nM OFF→ON switch was mixed with Taq DNA polymerase, Taq buffer, dNTPs, template, and primer all at once, and 40 μM Pb^{2+} was added at last. As a result, a similar level of amplicon production change was observed by Pb^{2+} addition compared to that by pre-incubation (see Fig. 2A, middle). However, the background level was slightly higher, and a relatively large amount of Pb^{2+} was needed.

References

[1] C. Dang, S.D. Jayasena, Oligonucleotide inhibitors ofTaqDNA polymerase facilitate detection of low copy number targets by PCR, J. Mol. Biol. 264 (1996) 268-278.

[2] R. Saran, J. Liu, A comparison of two classic Pb^{2+}-dependent RNA-cleaving DNAzymes, Inorg. Chem. Front. 3 (2016) 494-501.

[3] K.S. Park, C.Y. Lee, H.G. Park, Target DNA induced switches of DNA polymerase activity, Chem. Commun. 51 (2015) 9942-9945.

[4] K.S. Park, C.Y. Lee, H.G. Park, Metal ion triggers for reversible switching of DNA polymerase, Chem. Commun. 52 (2016) 4868-4871.