Supplementary Information

One-step isothermal RNA detection with LNA-modified MNAzymes chaperoned by cationic copolymer

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Table S1 Oligonucleotide sequences and molecular weight.

Name	**Sequences (5'-3')**[a]	**Molecular weight (Da)**[b]
miR-21 (RNA)	UAGCUUAUCAGACUGAUGUUGA	7005.797
substrate	AAGGTXTCCTC**GU**CCCTGGGCA-BHQ-1	7789.990
Mz(10,11) partzyme I	TCAACATCAGT**ACAACGA***GAGGAAACCTT*	8888.528
Mz(10,11) partzyme II	*TGCCCAGGGA***GGCTAGCT**CTGATAAGCTA	8943.244
Lz3(10,11) partzyme I	TCAACATCAGT**ACAACGA***GAGGAAACCTT*	8986.882
Lz3(10,11) partzyme II	*TGCCCAGGGA***GGCTAGCT**CTGATAAGCTA	9041.671
Lz4(10,11) partzyme I	TCAACATCAGT**ACAACGA***GAGGAAACCTT*	9014.446
Lz4(10,11) partzyme II	*TGCCCAGGGA***GGCTAGCT**CTGATAAGCTA	9069.982
Mz(7,8) partzyme I	TCAACATCAGT**ACAACGA***GAGGAAAC*	7991.414
Mz(7,8) partzyme II	*CCAGGGA***GGCTAGCT**CTGATAAGCTA	8021.714
Lz3(7,8) partzyme I	TCAACATCAGT**ACAACGA***GAGGAAAC*	8090.126
Lz3(7,8) partzyme II	*CCAGGGA***GGCTAGCT**CTGATAAGCTA	8119.304
Lz4(7,8) partzyme I	TCAACATCAGT**ACAACGA***GAGGAAAC*	8117.033
Lz4(7,8) partzyme II	*CCAGGGA***GGCTAGCT**CTGATAAGCTA	8147.374

[a] Boldface type indicates catalytic core residues in partzymes and RNA cleavage site in substrate; underlining indicates sites of LNA modification; italic type indicates substrate-binding arms; X in the substrate represents Fluorescein-dT.

[b]Molecular weight analyzed with MALDI-TOF mass spectrometer.

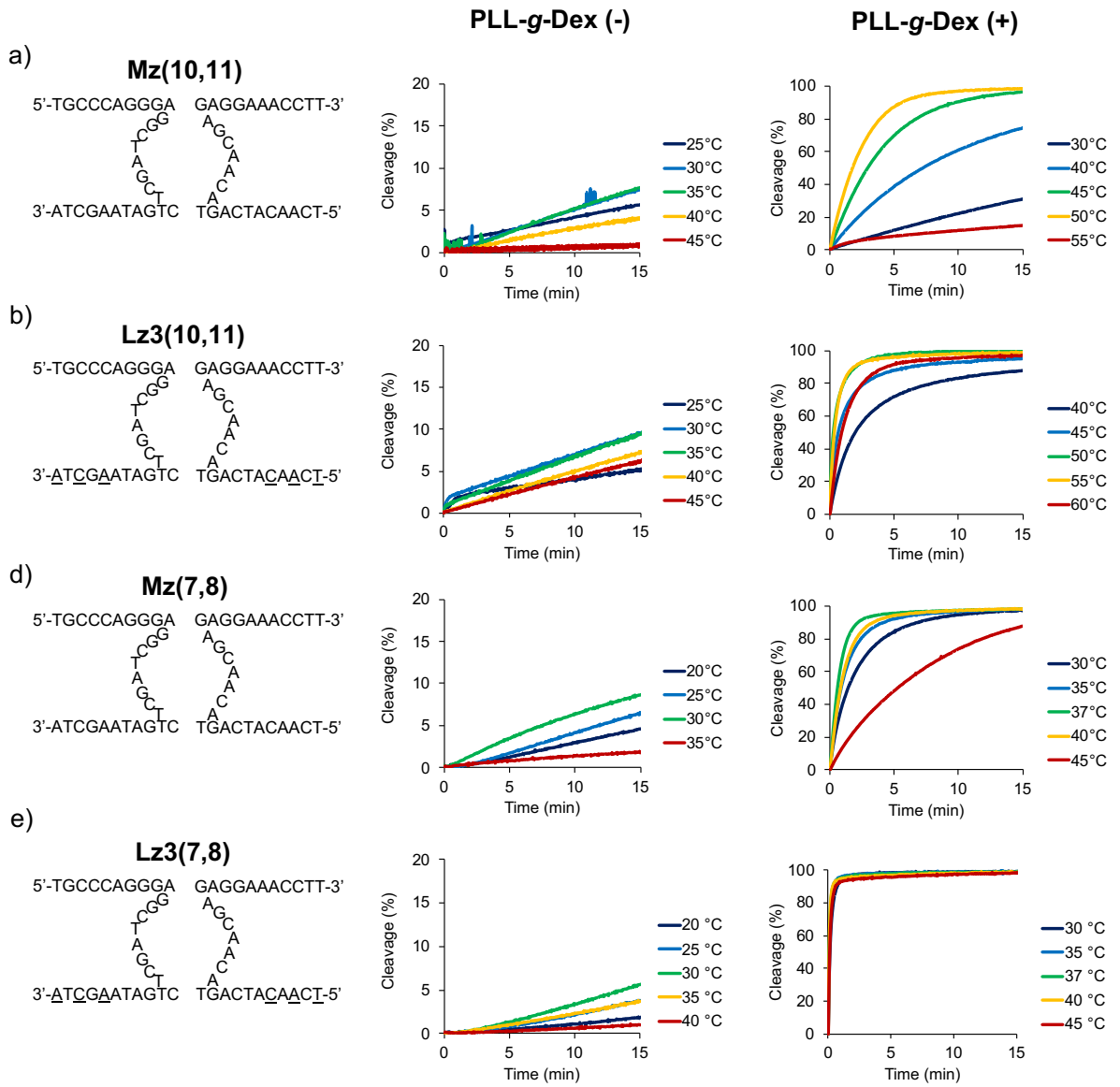

Fig. S1. Percentage of substrate cleavage over time by (a) Mz(10,11), (b) Lz3(10,11), (c) Mz(7,8), (d) Lz3(7,8) at different reaction temperatures. Reaction solutions contained 100 nM substrate, 20 nM each partzymes, and 20 nM miR-21 in the presence (N/P = 2) or absence of copolymer. Underlining indicates sites of LNA modification

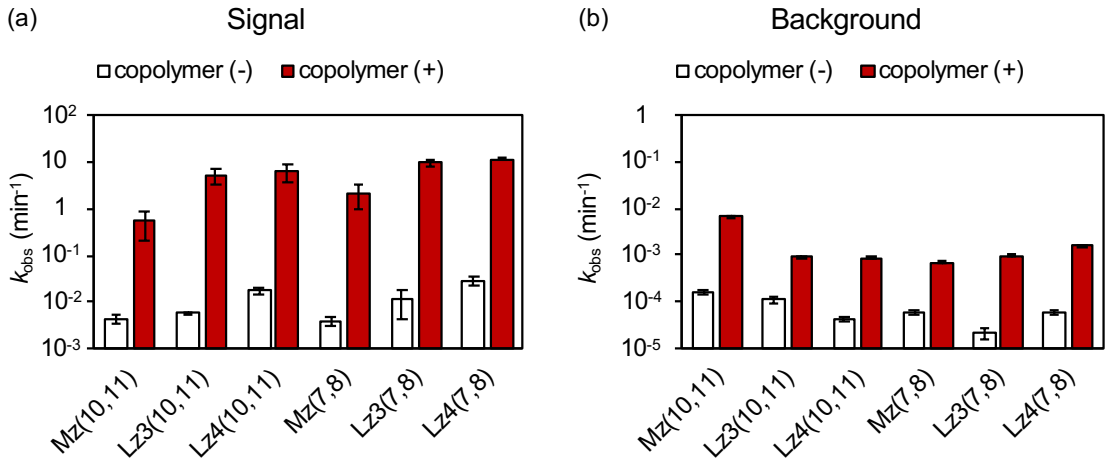

Fig. S2. Reaction rates of MNAzymes and LNA-modified MNAzymes (a) in the presence of 20 nM miR-21 target (signal) and (b) in the absence of miR-21 target (background). Reactions were performed with 100 nM substrate, 20 nM each partzyme, and 0 or 20 nM miR-21 at the optimal temperature for each condition. Experiments were repeated three times.

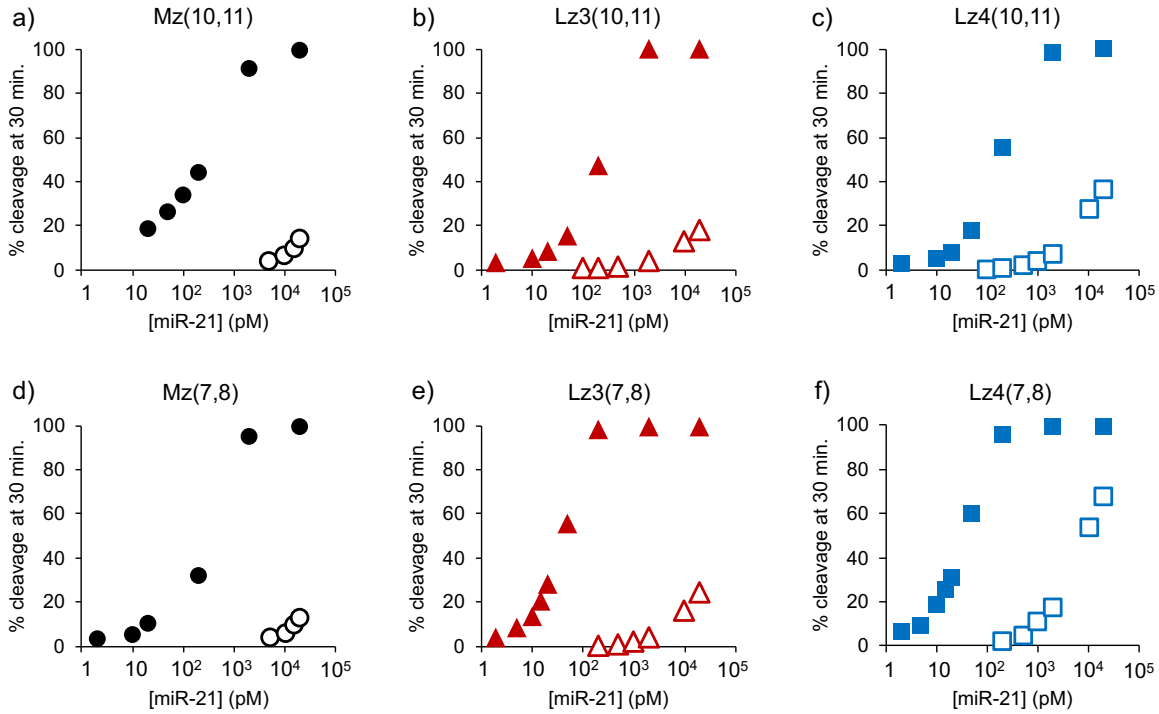

Fig. S3. Target concentration dependence of MNAzymes and LNA-modified MNAzymes at optimal temperatures. Reaction solutions contained 100 nM substrate, 20 nM each partzyme, and various miR-21 concentration as indicated, in the presence (closed symbols) or absence (open symbols) of PLL-*g*-Dex.

Table S2 Comparison table for RNA detection by isothermal amplification methods.

method	protein enzymes	primers	reverse transcription (RT)	additional amplification	temperature	reaction time	sensitivity	ref.
RCA	DNA ligase and DNA polymerase	-	-	strand displacement	37 ºC (85 ºC for heat inactivation)	6.5 h	3.2 pM	(Wang et al., 2016)
HCR	Horseradish peroxidase	-	-	-	Room temp.	3 h	32 fM	(Ying et al., 2017)
CHA	-	-	-	-	40 ºC	2 h	3.5 pM	(Liu et al., 2017)
RT-LAMP	Reverse transcriptase, DNA polymerase	required	required	-	63 °C (95 °C for heat inactivation)	1.5 h	100 copies	(Teoh et al., 2013)
RT-RPA (TwistAmp RT exo kit)	Reverse transcriptase, recombinase, DNA polymerase	required	required	-	40 ºC	20 min. (not include RT)	10 copies	(Teoh et al., 2015)
CRISPR-Cas	Cas13a, reverse transcriptase, recombinase, DNA polymerase	required	required	RT-RPA	37 ºC	3 h	aM	(Gootenberg et al., 2017)
Our method	-	-	-	-	37 ºC	30 min.	73 fM	

References

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