Supplementary Information

A hammerhead ribozyme selects mechanically stable conformations for catalysis against viral RNA

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Supplementary Table 1. Sequences of DNA and RNA

Name	Sequence （5′-3′）
ODN 1	TCAGCCATGACATTAACCTATAAAAATAGGCGTAT
ODN 2	AGGCACCGTGTATGAAATCTAACAATGCGCTCATCGTCATCGG
ODN 3	CCTCGTGATACGCCTATTTTTATAGGTTAATGTCATGGC
ODN 4	GACCCGATGACGATGAGCGCATTGTTAGATTTCATACACGGTGCCTGACTGCG
Mini ribozyme	UUUCCCACUGAUGAGUCCGAGGAGGACGAAAGACAUGUAC
Inactive mini ribozyme	UUUCCCACUGAU**dG**AGUCCGAGGAGGACGAAAGACAUGUAC
Tiny ribozyme	UUCCCACUGAUGAGACUGGACGAAAGACAUGUAU
Substrate	ACAUGUCUCUGGGA
biotin-F	GACCGAGATAGGGTTGAGTG
biotin-R	GCATCGGCTGAGG AAAGGGAACAAAAGCTGG
dig-F	ATCGTAGGGTCCTGACCGAGATAGGGTTGAGTG
dig-R	AAAGGGAACAAAAGCTGG

Note: The bold '**dG**' in the 'Inactive mini ribozyme' sequence represents deoxyguanosine, which is distinct from the other RNA bases.

Supplementary Table 2. Statistics of single-molecule experiments

Data set	N	Mean	Standard deviation	95% confidence interval
Fig 2b	462	13.4 nm	7.2 nm	13.4 ± 0.7 nm
Fig 2c (left)	180	38.6 pN	11.6 pN	38.6 ± 1.7 pN
Fig 2c (middle)	187	35.0 pN	12.2 pN	35.0 ± 1.8 pN
Fig 2c (right)	95	36.8 pN	12.9 pN	36.8 ± 2.6 pN
Fig 3b	339	13.1 nm	6.5 nm	13.1 ± 0.7 nm
Fig 3c (left)	128	38.9 pN	9.8 pN	38.9 ± 1.7 pN
Fig 3c (middle)	159	36.5 pN	13.3 pN	36.5 ± 2.1 pN
Fig 3c (right)	52	37.2 pN	14.3 pN	37.2 ± 4.0 pN
Fig 4b	235	9.4 nm	5.0 nm	9.4 ± 0.7 nm
Fig 4c (left)	99	36.3 pN	11.1 pN	36.3 ± 2.2 pN
Fig 4c (right)	136	34.7 pN	11.6 pN	34.7 ± 2.0 pN
Fig 4d	133	9.2 nm	4.8 nm	9.2 ± 0.8 nm
Fig 4e (left)	78	40.3 pN	10.2 pN	40.3 ± 2.3 pN
Fig 4e (right)	55	38.2 pN	10.9 pN	38.2 ± 3.0 pN
Supplementary Fig 6b	88	11.3 nm	6.4 nm	11.3 ± 1.4 nm
Supplementary Fig 6c (left)	52	41.8 pN	10.3 pN	41.8 ± 2.9 pN
Supplementary Fig 6c (right)	36	37.4 pN	11.5 pN	37.4 ± 3.8 pN
Supplementary Fig 6d	141	9.9 nm	4.5 nm	9.9 ± 0.8 nm

Supplementary Fig 6e (left)	111	37.8 pN	12.0 pN	37.8 ± 2.3 pN
Supplementary Fig 6e (right)	30	37.1 pN	11.6 pN	37.1 ± 4.2 pN
Supplementary Fig 7b	62	10.6 nm	6.7 nm	10.6 ± 1.7 nm
Supplementary Fig 7c (left)	38	20.1 pN	6.8 pN	20.1 ± 2.2 pN
Supplementary Fig 7c (right)	24	40.4 pN	9.1 pN	40.4 ± 3.7 pN
Supplementary Fig 7d	49	9.6 nm	6.6 nm	9.6 ± 1.7 nm
Supplementary Fig 7e (left)	25	29.6 pN	9.8 pN	29.6 ± 3.9 pN
Supplementary Fig 7e (right)	24	39.4 pN	10.8 pN	39.4 ± 4.4 pN
Supplementary Fig 7f	60	8.6 nm	3.6 nm	8.6 ± 0.9 nm
Supplementary Fig 7g (left)	27	39.8 pN	8.8 pN	39.8 ± 3.4 pN
Supplementary Fig 7g (right)	33	41.5 pN	10.5 pN	41.5 ± 3.7 pN
Supplementary Fig 7h	50	6.2 nm	2.3 nm	6.2 ± 0.6 nm
Supplementary Fig 7i (left)	29	42.0 pN	8.1 pN	42.0 ± 3.0 pN
Supplementary Fig 7i (right)	21	38.5 pN	10.0 pN	38.5 ± 4.4 pN

Supplementary Table 3. Stability of conformational states revealed by single-molecule experiments

Data set	ΔG (k_BT) of α state	ΔG (k_BT) of β1 state	ΔG (k_BT) of β2 state	ΔG (k_BT) of γ1 state	ΔG (k_BT) of γ2 state
Fig 2c (left)	-62.87	---	---	---	---
Fig 2c (middle)	---	-73.33	-151.84	---	---
Fig 2c (right)	---	---	---	-140.29	-295.46
Fig 3c (left)	-65.20	---	---	---	---
Fig 3c (middle)	---	---	-127.98	---	---
Fig 3c (right)	---	---	---	-106.73	-260.88
Fig 4c (left)	-53.72	---	---	---	---
Fig 4c (right)	---	---	-91.10	---	---
Fig 4e (left)	-59.15	---	---	---	---
Fig 4e (right)	---	-82.08	-166.28	---	---

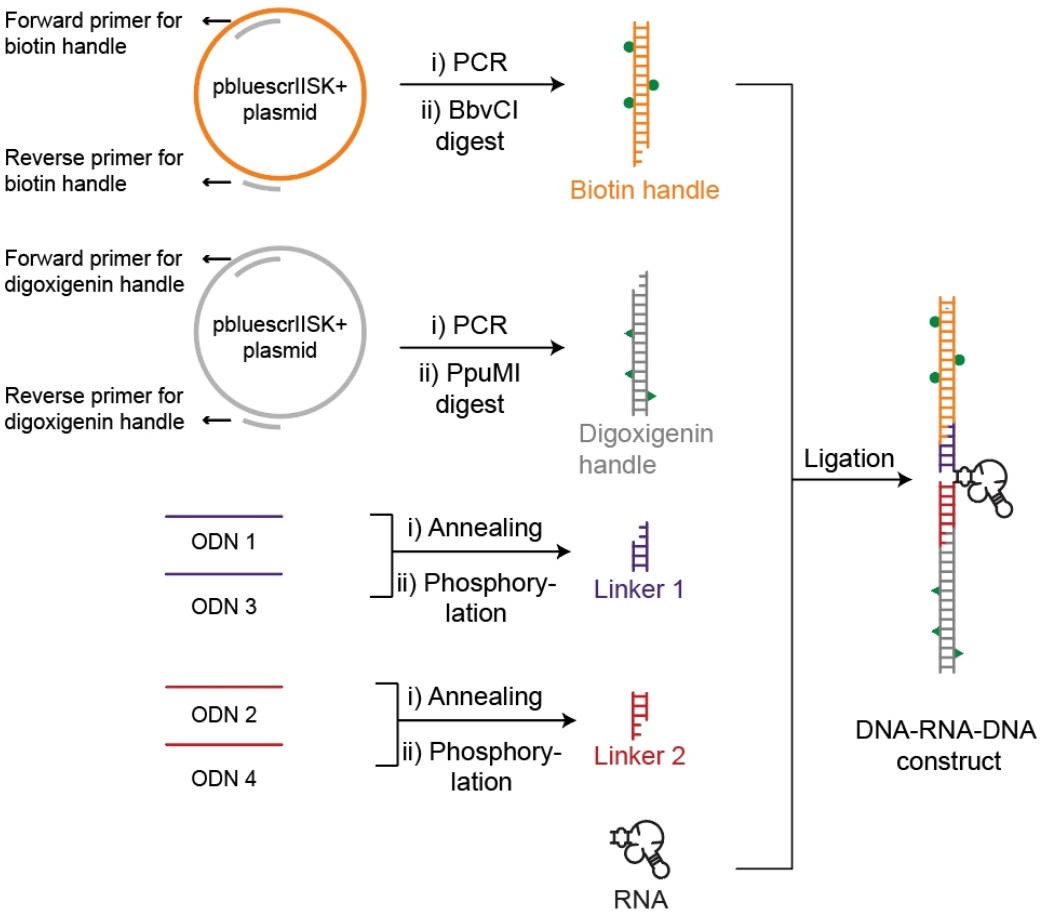
Note: The experimental free energies of conformations were estimated using Jarzynski's equation [1,2].

$$\exp[-\beta \Delta G(z)] = lim_{N\to\infty} < \exp\left[-\beta w_i(z,r)\right] >_N$$

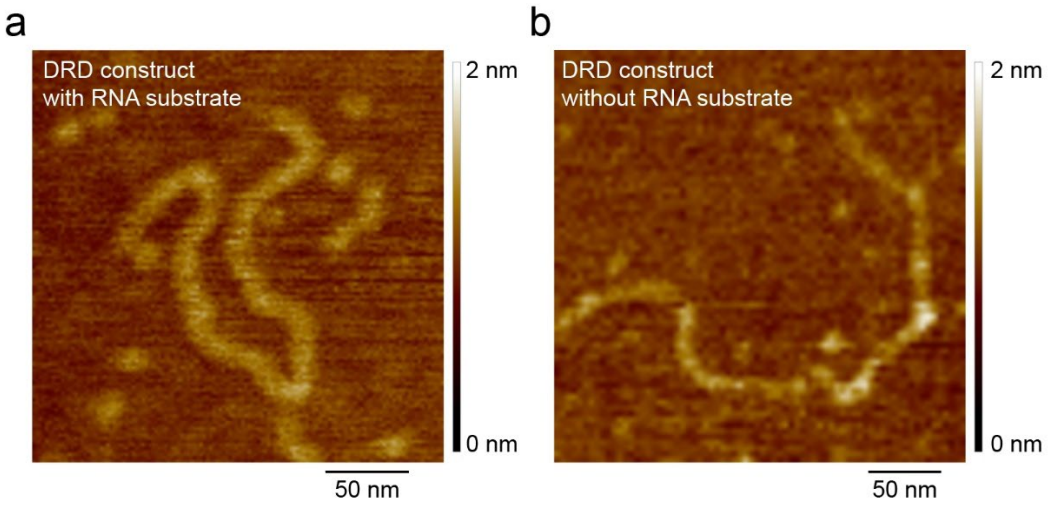
where $< >_N$ denotes averaging over N work traces, $w_i(z,r)$ represents the work done in the $\mathrm{i}\mathrm{th}$ trace, and r is the switching rate. The mechanical work $w_i(z,r)$ required to switch the system between positions 0 and z under an external force F is given by:

$$w_i(z,r) = \int_0^z F_i(z',r)dz'$$

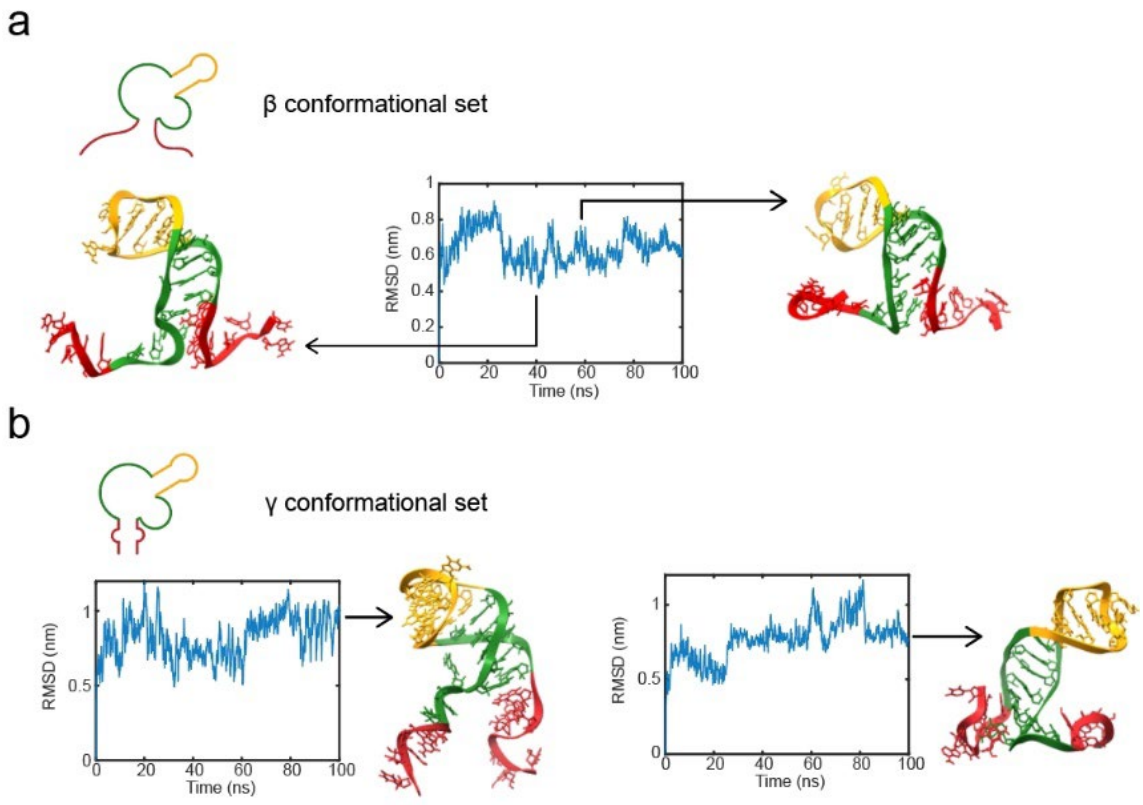
where $F_i(z',r)$ is the external force applied at position z' with switching rate r.



Supplementary Figure 1. Flowchart for preparation of the DNA-RNA-DNA construct



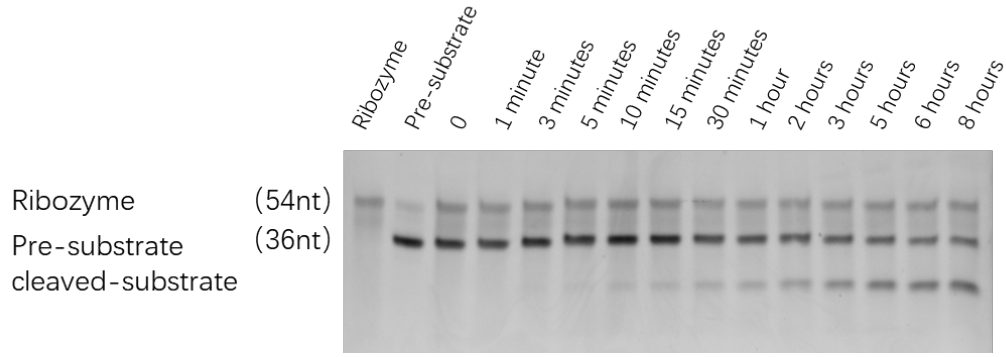
Supplementary Figure 2. AFM imaging of the DNA-RNA-DNA construct of the mini ribozyme with and without its substrate



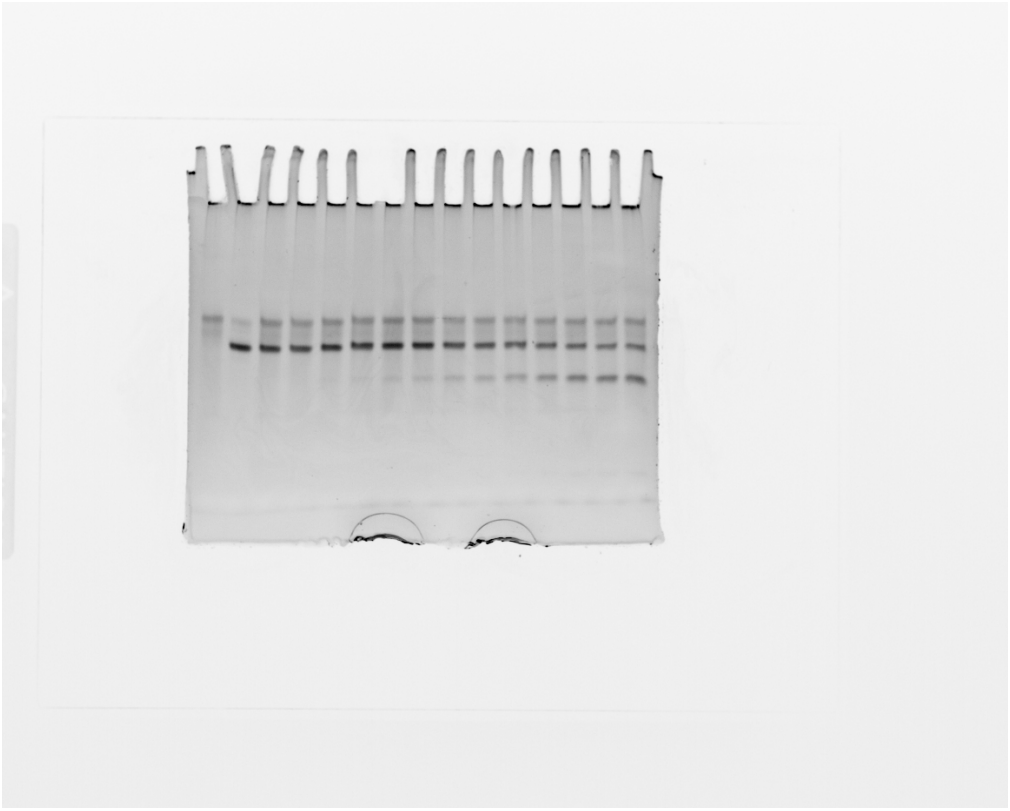
Supplementary Figure 3. Conformational sets of the mini ribozyme revealed by MD simulations

Snapshots from MD simulations without force loading are shown (Supplementary Data 2-5), with the corresponding times indicated by arrows.

a



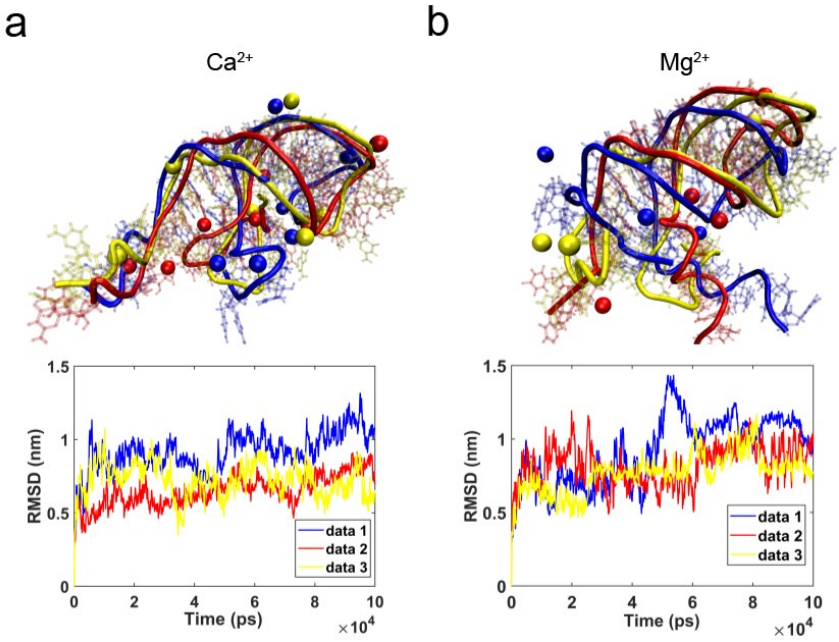
b



Supplementary Figure 4. Representative gel demonstrating the activity of the mini hammerhead ribozyme

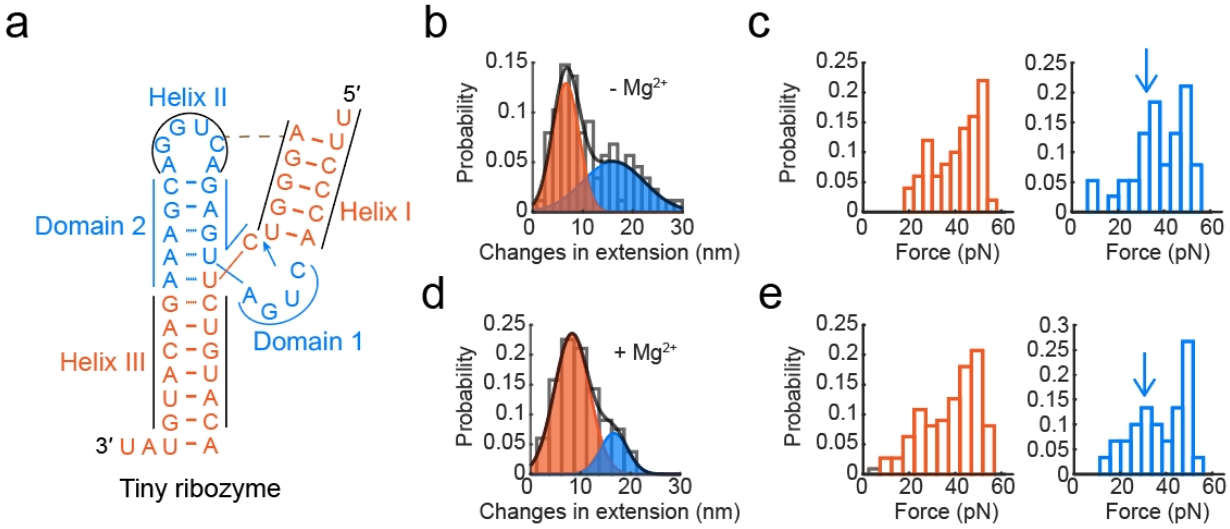
a) The mini ribozyme consists of RNA only, without the DNA handles used in single-molecule mechanical experiments. The substrate is extended to 36 nucleotides at the 3' end to form a stem-loop motif for gel staining purpose. The k_{obs} of the observed cleavage rate is 0.0059 min^{-1}.

b) Unedited gel image for that used in a).



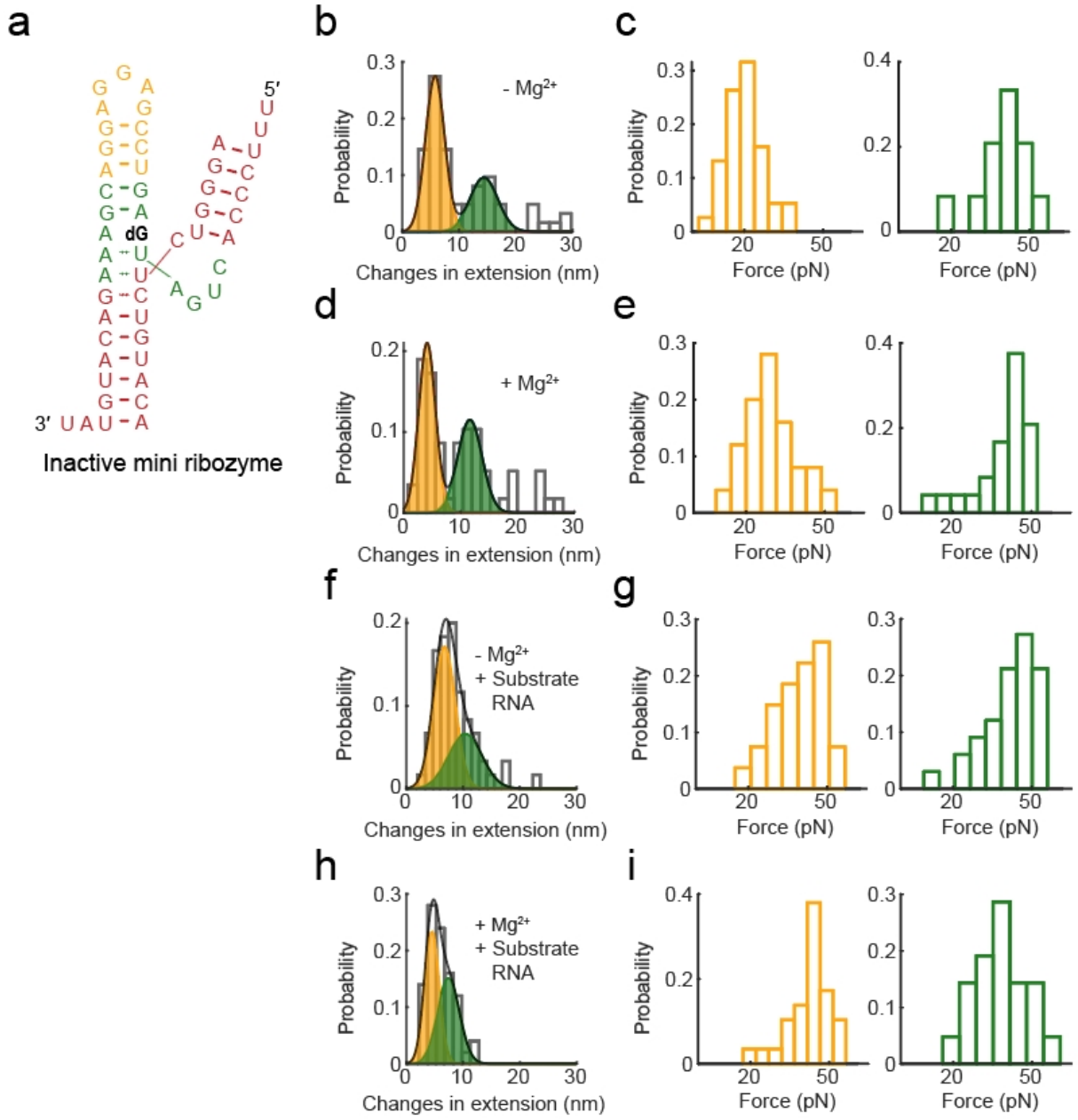
Supplementary Figure 5. Molecular dynamics simulations of the mini ribozyme with divalent ions

Three data sets are presented for conditions with calcium ions (a) and magnesium ions (b) (Supplementary Data 6-11). The red and yellow data sets for magnesium ions are the same as those in Supplementary Figure 3b.



Supplementary Figure 6. Single-molecule mechanical assays of tiny ribozyme

Blue arrows indicate the mechanical states that respond to magnesium ions. Statistical data are provided in Supplementary Table 2.



Supplementary Figure 7. Single-molecule mechanical assays of inactive mini ribozyme

Statistical data are provided in Supplementary Table 2.

Supplementary References

1. Jarzynski, C. Nonequilibrium Equality for Free Energy Differences. *Physical Review Letters* **78**, 2690-2693 (1997).
2. Liphardt, J., Dumont, S., Smith, S.B., Tinoco, I., Jr. & Bustamante, C. Equilibrium information from nonequilibrium measurements in an experimental test of Jarzynski's equality. *Science* **296**, 1832-5 (2002).