## **Supporting Information**

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**Fig. S1.** Construction of human cis-telomerase RNAs. In vitro transcribed telomerase RNA pseudoknot domain, either acceptor RNA A [sequence shown in (*B*)] or acceptor RNA B [sequence shown in (*C*)], 5'-monophosphorylated DNA primer (Table S1), and a DNA splint oligo (Table S1) were mixed at 1:3:2.5 molar ratio. The mixture was annealed and then ligated by T4 RNA ligase 2 (from NEB). The only difference between acceptor RNA A and B is the sequence at the very 3' end (colored in *Green*) that is used as the annealing region in the splinted ligation process and forms part of the linker in cis-telomerase.



**Fig. 52.** Trans primer challenge experiment of cis-telomerases with 2, 4, and 13 nt linkers. Increasing amount of the trans primer was added to the cis-telomerase reactions to compete for the conjugated primer of the cis-telomerase. The concentrations of the trans primer are indicated above each lane. The effect of trans primer concentration on the inhibition of cis-telomerase activity is very similar for cis-telomerases with 2, 4, and 13 nt linkers, indicating that the 2 and 4 nt-linker cis-telomerases are also true cis-systems acting on their own conjugated primers. LC, loading control. Here, the 13 nt linker is 13 nt #2 shown in Table S1.



**Fig. S3.** A 3D molecular model of the human telomerase RNA core region. Model was adapted from Nucleic Acid Database Entry ID 2INA with the distance between the 3' end of the pseudoknot region and the RNA template region indicated. The secondary structure model of the human telomerase RNA core region is shown in the *Upper Right* with the same colors for the corresponding regions.

## Table S1. Oligonucleotides used for engineering human cis-telomerases RNAs

Linker Length	Acceptor RNA	Donor DNA (Linker $+$ telomerase primer)	Splint DNA
2 nt	А	p <u>GTTAGG</u>	CCTAACCAGCTGACATTTTTTGTTTGC
4 nt	А	pCT <u>GTTAGG</u>	CCTAACAGCAGCTGACATTTTTTGTTTGC
5 nt	А	pCTT <u>GTTAGG</u>	CCTAACAAGCAGCTGACATTTTTTGTTTGC
6 nt	А	pCTTC <u>GTTAGG</u>	CCTAACGAAGCAGCTGACATTTTTTGTTTGC
7 nt	В	p <u>GTTAGG</u>	CCTAACGCAAGCAGCTG
9 nt	В	pCT <u>GTTAGG</u>	CCTAACAGGCAAGCAGCTG
13 nt <sup>#1</sup>	В	pCCTTTT <u>GTTAGG</u>	CCTAACAAAAGGGCAAGCAGCTG
13 nt <sup>#2</sup>	В	pCCAAAA <u>GTTAGG</u>	CCTAACTTTTGGGCAAGCAGCTG
17 nt	В	pCCAAAAAAA <u>GTTAGG</u>	CCTAACTTTTTTTGG GCAAGCAGCTG
20 nt	В	pCCAAAAAAAAAAAA <u>GTTAGG</u>	CTAACTTTTTTTTTGG GCAAGCAGCTG
23 nt	В	pCCAAAAAAAAAAAAAAAAAA	CCTAACTTTTTTTTTTTTTGGGCAAGCAGCTG
26 nt	В	pCCAAAAAAAAAAAAAAAAAAAAAA	CTAACTTTTTTTTTTTTTTTTGGGCAAGCAGCTG

All nucleotide sequences are listed in 5' to 3' direction and the telomerase primer regions of the donor DNAs are underlined. Please refer to Fig. S1B and C for the sequences of Acceptor RNA A and B, resp.

ONT A C