Electronic Supplementary Materials

Parallel G-quadruplex-mediated protein dimerization and activation

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Construction of plasmids: RHAU-CFP, RHAU-YFP, RHAU-casp9 and casp9

RHAU-CFP, RHAU-YFP and RHAU-Casp9 were generated by incorporating an engineered RHAU peptide (consisting of 30 aa with high binding affinity to parallel G4, unpublished) with the CFP, YFP and casp9, respectively. DNA encoding for the RHAU peptide was amplified by PCR using the *RHAU53* gene[1] as the template and a pair of primer ON1/ON2. the PCR product was cloned into treated pETDuet1 (Merck Millipore, Germany) at NcoI and BglII, resulting in plasmid pRHAU.

DNAs encoding for the CFP and YFP were amplified by PCR using the pHT582[2] containing the *CFP* gene and pHT584[2] containing the *YFP* gene as the templates and a pair of primer ON3/ON4. These PCR products were cloned into treated pRHAU at BglII and XhoI, resulting in plasmid pRHAU-CFP and pRHAU-YFP, respectively.

DNA encoding for the casp9 was amplified by PCR using the *casp9* gene[3] as the template and a pair of primer ON5/ON6. The PCR products was cloned into treated pRHAU at BglII and XhoI, resulting in plasmid pRHAU-casp9 (Supporting Figure 1).

Plasmid for expression of a normal reference casp9 (without RHAU) was generated by directly cloning *casp9* gene[3] into pETDuet1. DNA encoding for reference casp9 was amplified by PCR using *casp9* gene as the template and a pair of primer ON7/ON6. The PCR products was clone into treated pRHAU at BglII and XhoI, resulting in plasmid pcasp9.

ON1: 5'-tataccatgggcagccatcaccatcatcaccacagccgtacaactgctggccgcccac-3'

ON 2: 5'-attgagatctcttgttcttctgcccctgttttttc-3'

ON 3: 5'-tgcgagatctggcggcggcagcatggtgagc-3'

ON 4: 5'-cagactcgagttacttgtacagctcgtccatg-3'

ON 5: 5'-caagagatctagcggaggcggttccggtggcggtgctcttgagagtttg-3'

ON 6: 5'-cagactcgagtcattcgagtgcggccgctgatg-3'

ON 7: 5'-gccaggatccggctcttgagagtttgaggggaaatg-3'

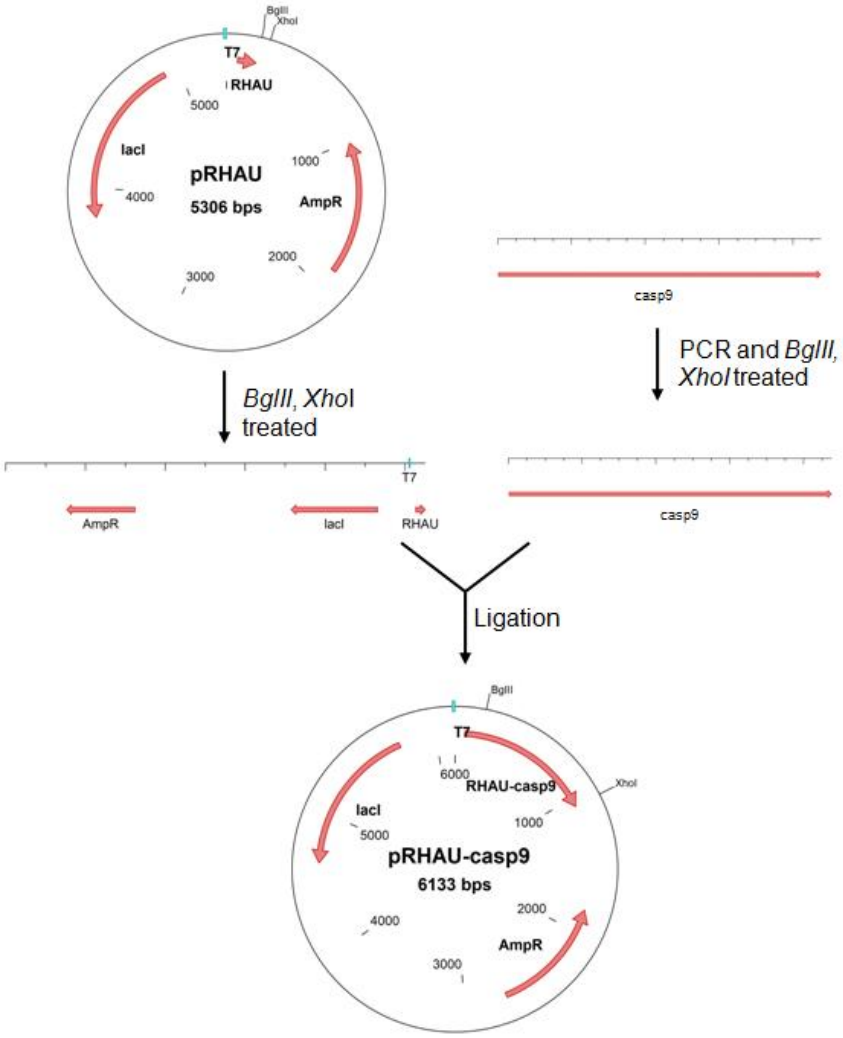

Fig. S1 Construction of plasmid pRHAU-casp9

Protein expression and purification

The plasmids pRHAU-CFP (coding RHAU-CFP), pRHAU-YFP (coding RHAU-YFP), pRHAU-cas9 (coding RHAU-casp9), pcasp9 (coding casp9) and pcasp3[3] were transformed into *E.coli* BL21(DE3). The bacteria were cultured in Luria-Bertani medium containing 100 mg/L of ampicillin and the cells were grown at 37 ˚C, shaking 220 rpm to an OD600 of 0.8, then IPTG (Sigma Aldrich, St. Louis, MO, USA) was added to a final concentration of 0.3 mM. The cells were continuously incubated overnight at 16 ˚C, shaking 180 rpm before being harvested. The pellet was resuspended into the bugBuster protein extraction reagent (EMD Millipore, Burlington, MA, USA) plus bezonase nuclease (to degrade DNA and RNA) and the insoluble cell debris was removed by centrifugation at 20000 rpm for 40 min at 4 ˚C. The soluble fraction was applied to the His-tag column (ThermoFisher Scientifi, Waltham, MA, USA). Following that, the column was washed with 20 column volumes of 20 mM Tris-HCl, 100 mM NaCl and 10 mM imidazole buffer, pH 6.5. The

proteins were eluted with 20 mM Tris-HCl, 100 mM NaCl and 200 mM imidazole buffer. The imidazole reagent in protein solution was then removed by using the Amicon Ultra-15 centrifugal filter (EMD Milipore). The purified proteins were collected and analyzed by SDS-PAGE.

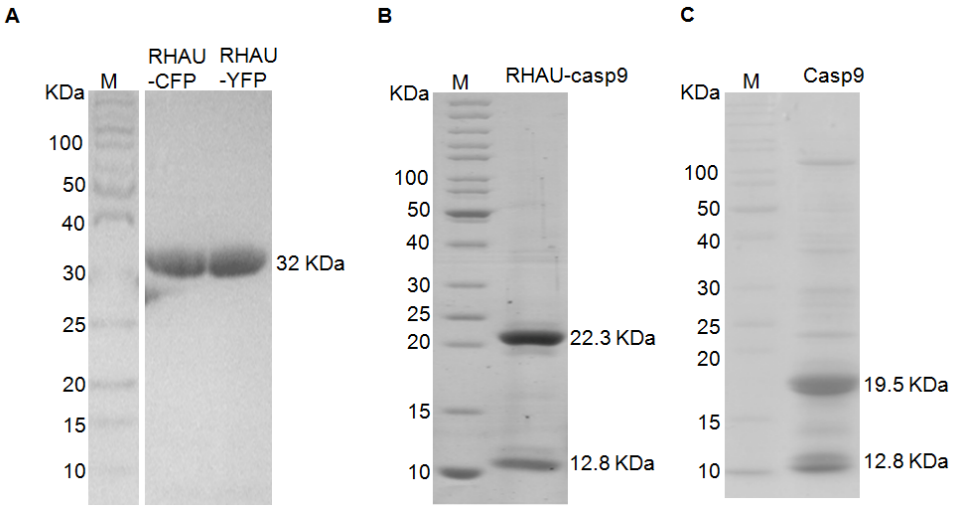

Fig. S2 SDS-PAGE analysis of proteins A) RHAU-CFP and RHAU-YFP, B) RHAU-casp9, C) casp9.

FRET measurements

All samples for FRET measurements were measured in a buffer containing 20 mM potassium phosphate, pH 6.5 using the quartz cuvettes of 10 mm path length (Hellma). The protein samples for measurements were used at concentration of 1 µM. All of the FRET measurements were carried out with a Cary Eclipse fluorescence spectrophotometer (Agilent Inc.) and all fluorescence data were recorded at 25 ˚C with an excitation wavelength of 410 nm. The parameters of measurements were kept constant over all measurements to enable data comparison.

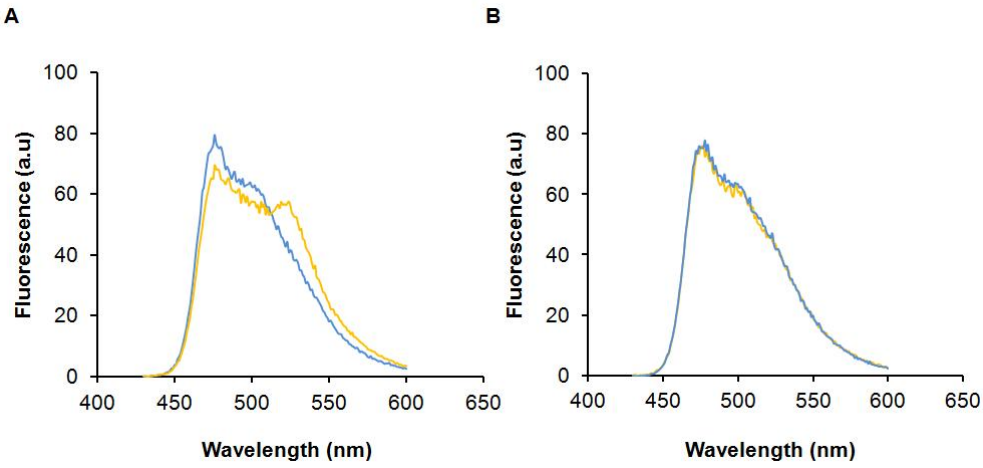

Fig. S3 FRET studies with RHAU-CFP/RHAU-YFP protein pair under the G4*s*. Representative spectra of a mixture of RHAU-CFP and RHAU-YFP (both at 1 µM) in the absence (blue) and presence (yellow) of T95-2T (1 µM, parallel DNA G4) (A) and Htelo2 (1 µM, non parallel DNA G4) (B).

Casp-9 activity screening on casp3 substrate[3]

The activity of the casp9 variants for casp3 cleavage was determined in a solution based assay with casp9 (150 nM) and substrate, casp3 (12 µM), in the absence or presence of TERRA (100 nM) diluted in assay buffer (25 mM HEPES, 100 mM NaCl, 4 mM MgCl2, 2 mM DTT, pH 6.5). Reactions were incubated at 37˚C and samples were collected in time intervals and reactions were stopped by addition of SDS-loading buffer. All samples were separated on 8 to 15% SDS-PAGE and proteins detected with blue staining. The extent of casp3 cleavage was determined by densitometry using the AlphaEaseFC software (Alpha Innotech).

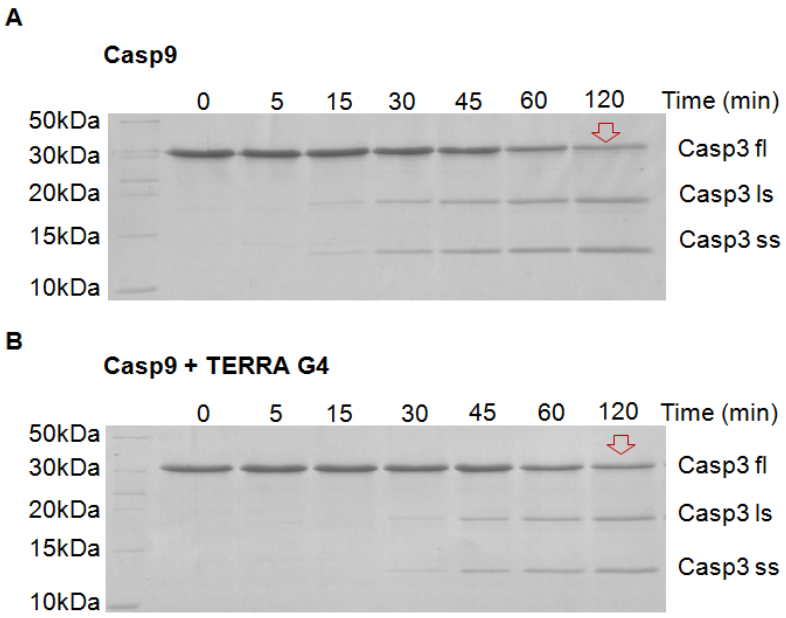

Fig. S4 Catalytic activity of casp-9 (0.15 µM) for natural substrate, casp3 (12 µM): A) in the absence of TERRA, B) in the presence of TERRA (1 µM). Caspase-3 fl (full length); ls (large subunit); ss (small subunit). Red arrow shows cleavage of a half of casp3 substrate by casp9.

Proteins	$t_{1/2}$ casp3 cleavage (minute)		
	-TERRA	+TERRA	FE
casp9	120	120	1
RHAU-casp9	120	2	60

$t_{1/2}$: time for cleavage of a half of casp3 substrate by casp9; FE: fold enhancement

References

[1] D. T. Dang and A. T. Phan, *Chembiochem*, 2016, **17**, 42-45.

[2] H. D. Nguyen, D. T. Dang, J. L. van Dongen and L. Brunsveld, *Angew Chem Int Ed Engl*, 2010, **49**, 895-898.

[3] D. T. Dang, H. D. Nguyen, M. Merkx and L. Brunsveld, *Angew Chem Int Ed Engl*, 2013, **52**, 2915-2919.