

Supporting Information for

Apoptosome formation through disruption of the K192-D616 salt bridge in the Apaf-1 closed form.

Fatemeh Sahebazzamani¹, Saman Hosseinkhani^{1}, Leif A. Eriksson², Howard O. Fearnhead³*

AUTHOR ADDRESS

1: Department of Biochemistry, Faculty of Biological Sciences, Tarbiat Modares University.

Tehran, Iran.

2: Department of Chemistry and Molecular Biology, University of Gothenburg, 405 30

Göteborg, Sweden.

3: Pharmacology and Therapeutics, School of Medicine, NUI Galway. Galway, Ireland.

This PDF file includes:

Figures S1 to S2

Table S1

SI References

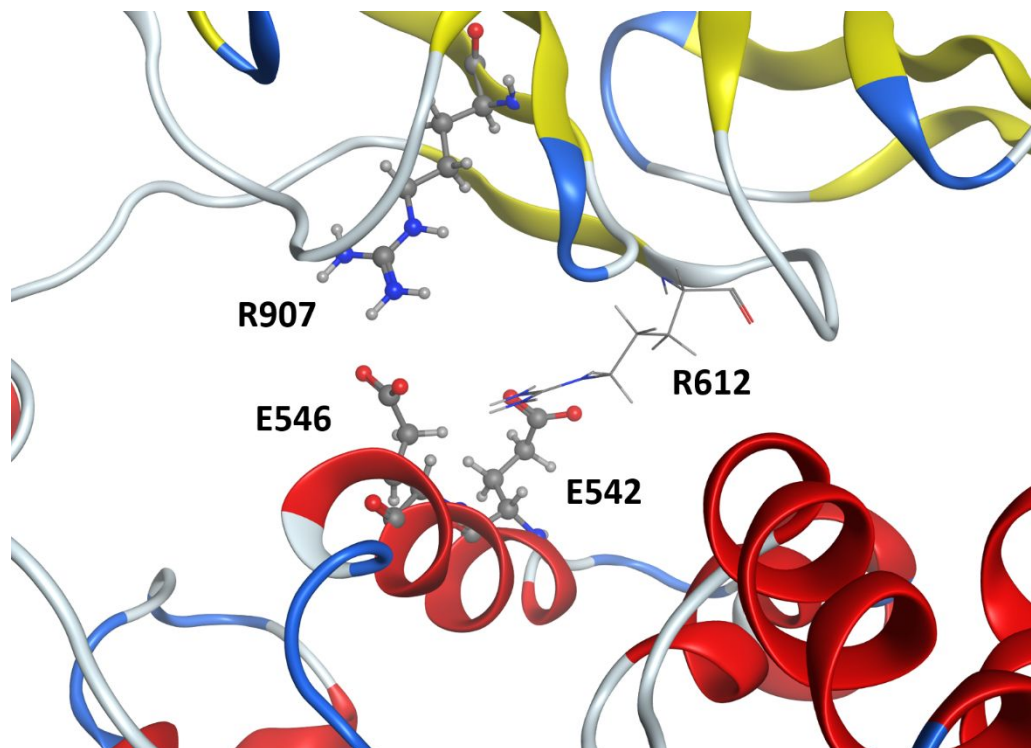


Figure S1. Interaction between E564 and E542 of the NBD-HD1 region and R612 and R907 in WD1 of the inactive (closed) form of Apaf-1₁. R612 shown in stick model; other residues as ball-and-stick.

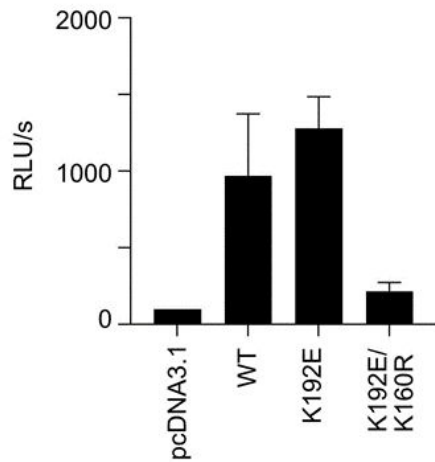


Figure S2. Determination of apoptosome formation by live cell luminescence assay. Transfected cells (equal numbers of cells per well) were incubated with a cell permeant luciferase substrate and luminescence assessed. (N=3, mean \pm SD).

Table S1. (A) The used primers for mutagenesis and (B) the converted codons.

Primers List	Primers Sequence
Forward. E41K Ap	ACAATATCAGAAGAGAAAAAAGTAAGAAATGAGCCCAC
Reverse. E41K Ap	GTGGGCTCATTTCTTACTTTTTTCTCTTCTGATATTGT
Forward. K81G+D82R Ap	CTACATGAAGGATATGGAAGGCTTGCTGCCCTTCTC
Reverse. K81G+D82R Ap	GAGAAGGGCAGCAAGCCTTCCATATCCTTCATGTAG
Forward. K160R AP*	GAATGGCAGGCTGTGGGAGGTCTGTATTAGCTGCAG
Reverse. K160R Ap*	CTGCAGCTAATACAGACCTCCCACAGCCTGCCATTC

Forward.K192E.Ap	GTTGGGAAACAAGACGAATCTGGGCTTCTGATG
Reverse.K192E.Ap	CATCAGAAGCCCAGATTCGTCTTGTTTCCCAAC
Forward.D616A.Ap	GTCCGCCCCCACACAGCTGCTGTTTACCATGC
Reverse.D616A.Ap	GCATGGTAAACAGCAGCTGTGTGGGGGCGGAC
Forward D616K Ap	GTCCGCCCCCACACAAAGGCTGTTTACCATGC
Reverse D616K Ap	GCATGGTAAACAGCCTTTGTGTGGGGGCGGAC

**Previously designed for another study 2.*

B. Mutation	Converted codon(s)
E41K	GAA to AAA
K81G-D82R	AAA-GAT to GGA-AGG
K160R	AAG to AGG
K192E	AAA to GAA
D616A	GAT to GCT
D616K	GAT to AAG

SI REFERENCES

1. Reubold, T. F.; Wohlgemuth, S.; Eschenburg, S. Crystal structure of full-length Apaf-1: how the death signal is relayed in the mitochondrial pathway of apoptosis. *Structure*. **2011**, *19*, 1074-1083, DOI: 10.1016/j.str.2011.05.013.
2. Noori, A.-R.; Tashakor, A.; Nikkhah, M.; Eriksson, L. A.; Hosseinkhani, S.; Fearnhead, H. O. Loss of WD2 subdomain of Apaf-1 forms an apoptosome structure which blocks activation of caspase-3 and caspase-9. *Biochimie*. **2020**, *180*, 23-29, DOI: 10.1016/j.biochi.2020.10.013.