

Unraveling IRAK4 extremely damaging variants and their potential implications on IRAK4 inhibitors efficacy

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Supporting materials

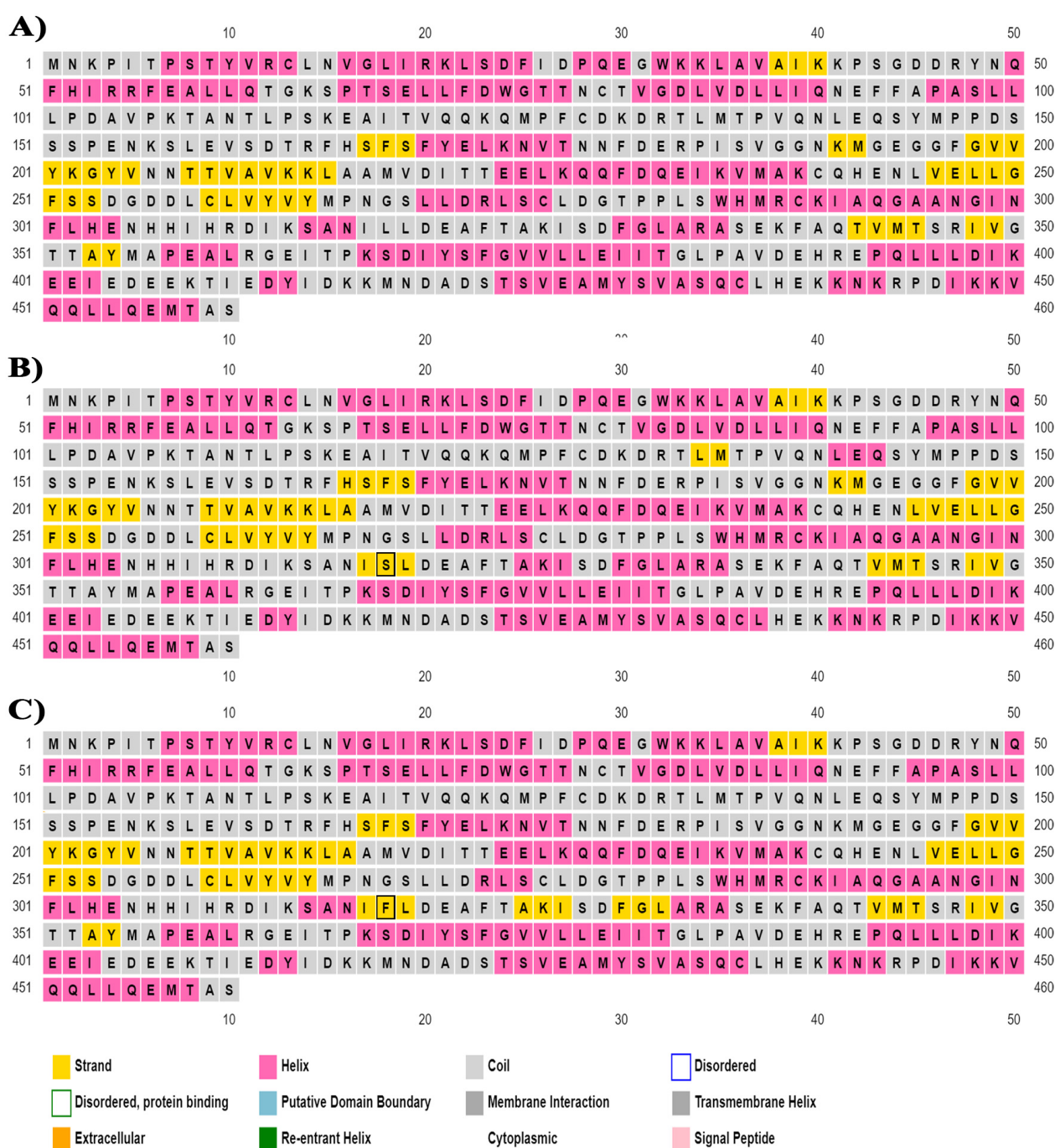


Figure S1. Investigation of IRAK4's secondary structure produced by PSIPRED server: (A) secondary structure of the wild form, (B) secondary structure with L318S mutation, and (C) secondary structure with L318F mutation.

ConSurf Results for job:IRAK4 date:13/05/2023



The conservation scale:



- e** - An exposed residue according to the neural network algorithm.
- b** - A buried residue according to the neural network algorithm.
- f** - A predicted functional residue (highly conserved and exposed).
- s** - A predicted structural residue (highly conserved and buried).
- X** - Insufficient data - the calculation for this site was performed on less than 10% of the sequences.

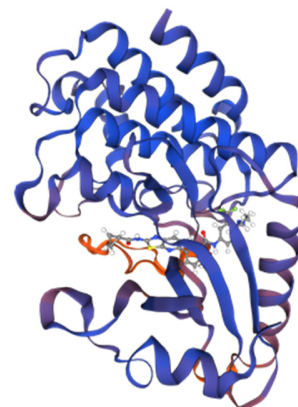
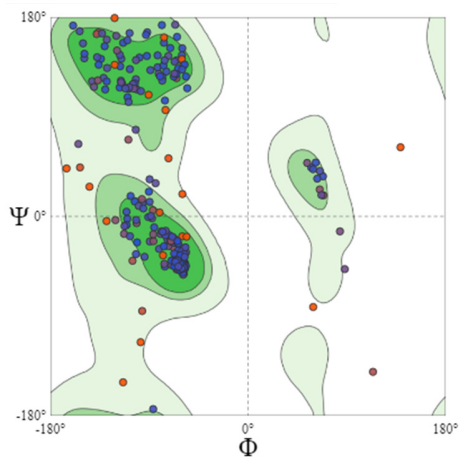
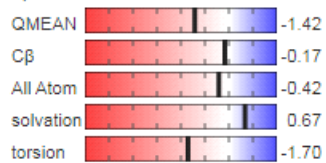
Figure S2. Investigation of IRAK4's phylogenetic conservation produced by ConSurf.

(A)

QMEANDisCo Global: ⓘ

0.79 ± 0.05

QMEAN Z-Scores



MolProbity Score = 2.23

Clash Score = 8.20

Ramachandran Favoured = 92.81%

Ramachandran Outliers = 2.05%

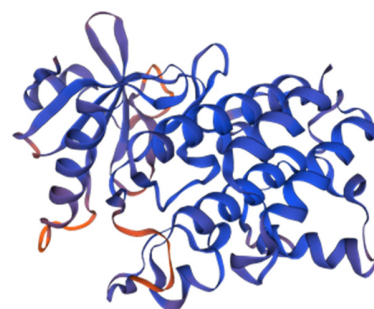
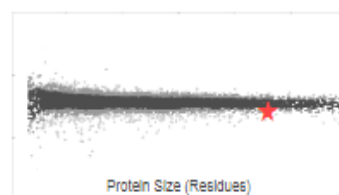
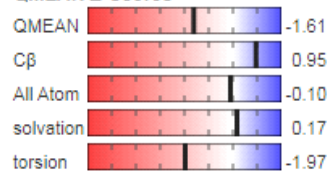
Rotamer Outliers = 2.75%

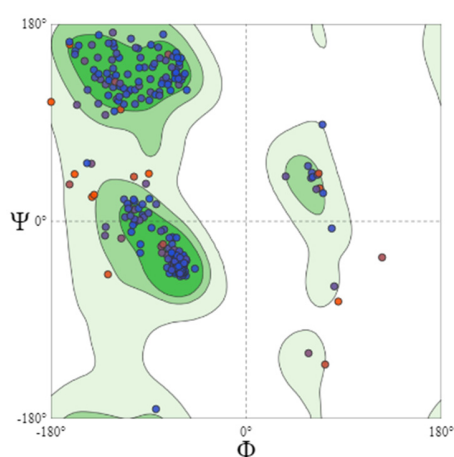
(B)

QMEANDisCo Global:

0.83 ± 0.05

QMEAN Z-Scores





MolProbity Score = 1.18
 Clash Score = 0.22
 Ramachandran Favoured = 92.86%
 Ramachandran Outliers = 2.38%
 Rotamer Outliers = 1.56%

Figure S3. Overall structural quality assessment of IRAK4 Holo (A) and Apo (B) modelled missing loops at the respective deposited PDF.file, using SWISS-MODEL MolProbity validation parameters

Table S1. Monitored Δ RMSF for HG-12-6 bounded IRAK4 protein across the whole molecular dynamics runs

IRAK4 important structural domains	Residues	D311H	F330V	G195R	G198E	G198R	L318S	L318F	Native
N-lobe Schellman loop	Pro184	-0.83	-0.85	-0.16	-0.61	-0.12	-0.62	-0.22	-0.10
	Ile185	-0.09	-0.16	0.44	0.03	0.46	-0.06	0.31	0.49
	Ser186	0.92	0.18	0.73	0.15	1.17	0.78	1.12	0.83
	Val187	1.46	1.20	1.62	1.05	1.78	1.19	1.64	1.80
	Gly188	1.76	2.03	2.34	1.83	2.32	1.64	2.10	2.56
	Gly189	1.63	2.02	2.42	2.13	2.07	1.49	1.94	2.63
N-lobe G-rich loop	Gly193	-0.58	-1.42	-0.88	-1.19	-0.28	-0.59	-0.14	-0.73
	Glu194	-0.90	-1.00	-0.35	-1.21	-0.46	-1.50	-0.57	-0.71
	Gly/x195	0.22	0.23	-0.25	0.13	0.24	0.85	0.28	0.95
	Gly196	-0.14	0.70	1.26	-0.36	0.08	-0.40	0.64	0.54
	Phe197	0.63	1.50	1.96	0.27	0.33	0.41	1.43	1.53
	Gly/x198	1.30	1.07	0.45	0.22	0.87	1.19	0.95	1.39
N-lobe α C-helix	ILle221	-1.58	-2.13	-0.04	-2.57	-1.82	-1.46	0.49	0.59
	Thr222	0.24	-2.50	1.45	-0.16	-0.88	0.28	1.50	1.86
	Thr223	0.73	-1.58	2.00	0.36	-1.36	0.33	1.72	2.04
	Glu224	0.52	-1.19	1.32	0.47	-0.67	0.06	1.37	1.27
	Glu225	0.70	0.89	1.47	0.45	-1.11	-0.08	1.26	1.30
	Leu226	-0.17	0.59	0.94	-0.54	-0.95	-0.90	0.49	0.77
	Lys227	0.27	0.88	1.11	0.26	0.32	-0.23	0.94	1.03
	Gln228	-0.70	-0.44	-0.31	-1.16	-2.03	-1.36	-0.34	-0.50
	Gln229	-0.93	-0.44	-0.26	-1.77	-2.41	-1.71	-0.58	-0.56
	Phe230	-0.85	-0.10	0.03	-1.16	-1.26	-1.44	-0.36	-0.14
	Asp231	-0.50	-0.28	-0.20	-0.82	-0.56	-1.04	-0.20	-0.32
	Gln232	-0.62	-0.46	-0.39	-1.75	-0.77	-1.35	-0.49	-0.65
	Glu233	-0.78	-0.18	-0.08	-1.57	-0.63	-1.39	-0.45	-0.35
	Ile234	-0.57	-0.17	-0.05	-0.63	-0.42	-1.04	-0.26	-0.22
	Lys235	-0.47	-0.49	-0.37	-1.33	-0.33	-1.01	-0.33	-0.65
	Val236	-0.79	-0.54	-0.38	-1.70	-0.44	-1.42	-0.48	-0.74

	Met237	-0.83	-0.39	-0.08	-0.69	-0.55	-1.32	-0.36	-0.37
	Ala238	-0.68	-0.77	-0.36	-0.99	-0.48	-0.98	-0.34	-0.79
	Lys239	-0.89	-1.10	-0.63	-1.78	-1.17	-1.56	-0.51	-1.39
	Cys240	-1.12	-1.02	-0.47	-1.27	-1.78	-1.87	-0.41	-1.11
Hinge region	Tyr262 (GK)	-0.14	-0.33	-0.12	-0.35	-0.18	-0.20	-0.08	-0.01
	Val263	-0.17	-0.50	-0.31	-0.48	-0.21	-0.23	-0.14	-0.13
	Tyr264	-0.32	-0.64	-0.38	-0.64	-0.29	-0.42	-0.25	-0.18
	Met265	-0.23	-0.23	-0.20	-0.55	-0.39	-0.28	-0.11	-0.11
	Pro266	-0.40	-0.29	-0.55	-0.83	-0.32	-0.21	-0.23	-0.05
	Asn267	-0.59	-0.20	-0.15	-0.53	-0.59	-0.42	-0.17	-0.15
	Gly268	-0.27	-0.13	0.06	-0.42	-0.13	-0.14	0.08	-0.01
	Ser269	-0.41	-0.21	-0.04	-0.40	-0.25	-0.27	-0.06	-0.10
	Leu270	-0.25	-0.03	0.11	-0.18	-0.05	-0.11	0.01	0.35
α -DE loop	Cys276	-0.41	-0.38	-0.26	-0.30	-0.38	-0.26	-0.36	-0.267
	Leu277	-0.27	-0.37	-0.22	-0.29	-0.21	-0.11	-0.38	-0.044
	Asp278	-0.44	-0.59	-0.40	-0.55	-0.34	-0.27	-0.58	-0.18
	Gly279	-0.59	-0.53	-0.35	-0.46	-0.50	-0.44	-0.50	-0.275
	Thr280	-0.35	-0.03	0.12	0.00	-0.28	-0.21	-0.07	0.04
	Pro281	-0.02	-0.08	-0.15	-0.09	-0.03	-0.10	-0.22	0.19
	Pro282	-0.26	-0.11	0.00	-0.05	-0.21	-0.12	-0.06	0.05
Leu318 with vicinal residues	Asn316	-0.25	-0.35	-0.30	-0.34	-0.28	-0.41	-0.60	-0.34
	Ile317	-0.20	-0.06	0.00	-0.33	-0.30	-0.38	-0.40	0.00
	Leu/x318	-0.09	-0.12	0.03	-0.09	-0.04	-0.22	-0.49	0.00
	Leu319	-0.24	-0.17	-0.09	-0.11	-0.12	-0.39	-0.56	-0.08
	Ap320	-0.25	-0.27	-0.24	-0.11	-0.13	-0.38	-0.79	-0.21
Activation Segment	Asp329	-0.41	-0.27	-0.31	-1.19	-0.17	-0.24	-0.24	-0.09
	Phe/x330	-0.51	-0.98	-0.58	-1.33	-0.44	-0.42	-0.34	-0.261
	Gly331	-0.78	-0.57	-0.71	-2.37	-0.89	-0.94	-0.56	-0.545
	Leu332	-0.93	-1.79	-0.94	-2.35	-1.10	-0.86	-1.24	-0.63
	Ala333	-1.01	-2.13	-1.15	-3.11	-1.07	-0.81	-1.12	-0.67
	Arg334	-1.34	-2.01	-1.90	-4.32	-1.45	-1.19	-2.85	-0.56
	Ala335	-1.77	-3.84	-4.14	-5.33	-1.67	-2.29	-2.45	-0.55
	Ser336	-2.93	-3.61	-4.32	-5.18	-1.62	-3.05	-2.63	-0.48
	Glu337	-3.57	-3.50	-4.19	-5.62	-2.08	-4.38	-2.52	-0.64
	Lys338	-4.36	-4.42	-5.32	-5.82	-2.04	-5.30	-2.79	-0.93
	Phe339	-2.95	-3.77	-4.19	-4.38	-1.05	-4.75	-2.30	0.13
	Ala340	-1.36	-3.20	-2.57	-4.88	-0.58	-2.15	0.09	0.84
	Gln341	-1.47	-1.18	-2.78	-5.92	-2.33	-1.45	0.17	0.89
	Thr342	-1.92	-1.54	-2.92	-6.08	-4.71	-1.75	-0.69	0.45
	Val343	-2.35	-2.10	-1.91	-6.08	-4.41	-0.86	-0.40	0.14
	Met344	-2.13	-2.40	-2.07	-5.88	-4.65	0.04	-0.44	-0.18
	Thr345	-4.07	-3.77	-3.00	-6.82	-5.01	-0.55	-1.84	-0.70
	Ser346	-4.53	-3.88	-2.49	-5.73	-3.92	-1.34	-2.40	-0.81
	Arg347	-3.91	-4.06	-1.47	-4.50	-3.72	-0.52	-2.82	-0.74
	Ile348	-4.31	-3.99	-1.03	-3.30	-2.53	0.16	-1.82	-0.34
	Val349	-5.09	-1.67	0.13	-1.86	-1.51	-0.28	-0.32	-0.05
	Gly350	-3.70	-1.92	0.54	-1.00	-0.43	-0.59	-0.73	0.47

	Thr351	-1.93	-0.15	1.17	0.37	-1.20	0.43	-0.36	1.09
	Thr352	0.00	1.76	1.92	1.26	0.91	1.05	0.67	1.34
	Ala353	-0.29	0.76	1.10	0.42	0.74	0.16	0.63	0.16
	Tyr354	-0.45	0.08	0.07	-0.31	0.12	-0.18	-0.18	-0.36
	Met355	-0.12	-0.07	-0.41	-0.65	0.14	0.02	0.13	-0.28
	Ala356	-0.61	-0.26	-0.68	-0.79	-0.19	-0.38	-0.25	-0.40
	Pro357	-0.49	-0.43	-1.00	-1.02	-0.20	-0.40	-0.21	-0.58
	Glu358	-0.73	-0.49	-1.28	-1.14	-0.50	-0.72	-0.51	-0.74

^a $\Delta\text{RMSF} \geq 0.30 \text{ \AA}$ cut-off are in bold red numbers inferring residues showing significant immobility/inflexibility.