The N-terminal loop of IRAK-4 death domain regulates ordered assembly of the Myddosome signalling scaffold

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SUPPLEMENTARY METHODS - PHOSPHOSITE MAPPING.

Phosphosite mapping analysis was then carried out on the isolated IRAK-4 death domain. The band corresponding to IRAK-4 DD extracted from the reducing SDS-PAGE gel was digested using Trypsin, Chymotrypsin and AspN endoproteinases. The analysis was carried out using MALDI-TOF MS on an Ultraflex mass spectrometer and by LC-ESI MS on an Agilent HPLC coupled to a Bruker Amazon ion-trap and an Orbitrap mass spectrophotometer where appropriate. One hundred and twenty nine peptides were identified by LC/MS-MS and 44 peptides were identified by MALDI-TOF MS covering 100 % of the IRAK-4 DD (-7) to 107 (Supplementary data tables 1 to 6). The stoichiometry of both species was calculated from the relevant ion intensities of corresponding ion chromatograms. The stoichiometry of the singly phosphorylated form was determined to be equal at 53 % with an error as high as ± 10 % (as it depends on the relative ionisation efficiency of each form; phosphorylated and non-phosphorylated). In total 16 peptide string masses identified confirmed single phosphorylation of Serine 8 in IRAK-4 DD (Supplementary data tables 1 to 6). Phosphorylation at Serine 8 was further confirmed by the fragmentation of the peptide GAMDPEFMNKPITPSTYVR by MS/MS as shown in supplementary tables 7 and 8.

Table 1: a) List 1 of 2 of peptides identified by LC / MS – MS after digestion of IRAK-4 DD using Chymotrypsin. b) List 2 of 2 of peptides identified by LC / MS – MS after digestion of IRAK-4 DD (Section 3.2.4) using Chymotrypsin.

X

904.97 0.0054

A	cc. #	Protei	n ID	Pe	p. #	% C	ov. Se	core	
9947		IRAK4 DEATH DOMAIN			57	9	4%	1182	
G A M D G D D R F A P A	PEFMNK YNQFHI SLLLPD	C PITPSTYVRC L RRFEALLQTG K D AVPK	NVGLIRKLS DF SPTSELLFD WO	ID ITT	P Q E N C T	G WK V G D	K L A L V D	V A I K	K P S N E F
Red =	MS-MS Un	iderline = PMF							
Comme	ents: Indivi	dual ions scores > 23 indi	cate identity or extensi	ve ho	omolo	gy (p	<0.05).		
Score		Peptide	Modifications		Obs.	m/z	Delta	MS-MS	? PMF?
66	GAMDPEF	MNKPITPSTYVRCL.N	(M)	011	84	47.04	0.0022	×	
40	GAMDPEF	MNKPITPSTY.V	2 Oxidation (M)		90	55.93	0.0031	×	
33	GAMDPEF	MNKPITPSTYVRCLNVGL.I	Phospho (ST); 2 Oxida (M)	tion	97	74.78	0.0029	×	
(23)	GAMDPEF	MNKPITPSTY.V	Oxidation (M)		9	57.94	0.0031	×	
(30)	GAMDPEF	MNKPITPSTY.V	2 Oxidation (M)		96	55.93	0.0000	×	
(30)	GAMDPEF	MNKPITPSTYVRCL.N	Phospho (ST); Oxidatio (M)	on	1,26	52.06	0.0040	×	
(32)	GAMDPEF	MNKPITPSTY.V	Phospho (Y); 2 Oxidati (M)	on	1,00	05.92	0.0006	×	
(34)	GAMDPEF	MNKPITPSTY.V	Oxidation (M)		9	57.93	-0.0002	×	
(35)	GAMDPEF	MNKPITPSTY.V			94	49.94	0.0001	×	
(49)	GAMDPEF	MNKPITPSTYVRCL.N	Phospho (ST); Oxidatio (M)	on	84	41.71	0.0019	×	
(56)	GAMDPEF	MNKPITPSTYVRCL.N	Phospho (ST)		83	36.38	0.0029	×	
56	F.MNKPITP	STY.V	Oxidation (M)		58	34.29	0.0002	×	
(32)	F.MNKPITP	STY.V	Phospho (ST); Oxidatio (M)	on	63	24.27	0.0012	×	
(50)	F.MNKPITP	STY.V			57	76.29	0.0030	×	
42	Y.VRCLNV	GL.I			4	65.76	-0.0006	×	
46	L.NVGLIRK	LSDFIDPQEGW.K			6	96.37	0.0009	×	
43	L.NVGLIRK	LSDF.I			6	31.37	0.0004	×	
30	L.NVGLIRK	L.S			4	56.80	-0.0005	X	
(30)	L.NVGLIRK	LSDFIDPQEGW.K			6	96.37	0.0016	×	
(32)	L.NVGLIRK	LSDFIDPQEGW.K			6	96.37	0.0000	×	
(34)	L.NVGLIRK	LSDFIDPQEGW.K			6	96.37	-0.0011	×	
34	L.IRKLSDF	IDPQEGW.K			5	68.63	-0.0002	X	
28	L.IRKLSDF	IDPQEGWKKL.A			5	19.04	-0.0017	×	
(23)	L.IRKLSDF	IDPQEGW.K			8	52.44	-0.0006	x	
35	L.SDFIDPQ	EGWKKL.A			7	81.90	0.0000	×	
31	L.SDFIDPQ	EGW.K			5	97.26	-0.0010	X	
(27)	L.SDFIDPQ	EGWKKL.A			5	21.60	-0.0012	×	
33	W.KKLAVA	IKKPSGDDRY.N			4	48.01	-0.0005	X	
(26)	W.KKLAVA	IKKPSGDDRY.N			4	48.01	0.0001	×	
67	L.AVAIKKP	SGDDRY.N			7	10.38	0.0005	X	

37 L.AVAIKKPSGDDRYNQF.H

(b)

A	сс. #	Prot	ein ID	Pe	p. #	% C	ov.	So	ore		
9947		IRAK4 DEATH DOMAIN			57	9	4%	1	1182		
G A M D G D D R F A P A	PEFMNK YNQFHI SLLLPD	C PITPSTYVRC RRFEALLQTG AVPK	LNVGLIRKLS D KSPTSELLFD W	FID GTT	P Q E N C T	G WH TVG D	K L	L A V D	V A I L L I	K K Q N	PS EF
Red =	MS-MS Un	nderline = PMF									
Comme	ents: Indivi	idual ions scores > 23 ir	dicate identity or extensi	ive ho	omolo	gy (p	< 0.0	5).		_	
Score		Peptide	Modifications		Obs.	m/z	De	lta	MS-M	S?	PMF?
37	L.AVAIKKP	SGDDRYNQF.H			9	04.97	0.0	054	X	_	
(24)	L.AVAIKKP	SGDDRYNQF.H			6	03.65	0.0	035	×		
(24)	L.AVAIKKP	SGDDRYNQF.H	Phospho (ST)		6	30.30	0.0	002	×		
(31)	L.AVAIKKP	SGDDRY.N			4	73.92	-0.0	008	×		
48	F.EALLQTG	KSPTSELL.F			7	93.94	0.0	005	×		
43	F.EALLQTG	KSPTSEL.L			7	37.39	-0.0	007	×		
(42)	F.EALLQTG	KSPTSEL.L			7	37.39	0.0	012	×		
47	L.LQTGKSP	PTSELLF.D			7	10.89	0.0	001	×		
24	L.LQTGKSP	PTSEL.L			5	80.81	0.0	006	×		
36	L.QTGKSPT	TSEL.L			5	24.27	-0.0	004	×		
34	L.QTGKSPT	TSELLF.D			6	54.35	0.0	001	×		
29	L.QTGKSPT	TSELL.F			5	80.81	0.0	003	×		
30	W.GTTNCT	VGDLVDLLIQNEF.F			1,0	55.01	0.0	029	×		
41	L.LIQNEFFA	APASL.L			6	75.36	0.0	002	×		
27	L.IQNEFFA	PASLL.L			6	75.36	0.0	007	X		
25	L.IQNEFFAF	PASL.L			6	18.82	0.0	001	×		
71	F.FAPASLLI	LPDAVPK			7	19.92	0.0	007	×		
(25)	F.FAPASLLI	LPDAVPK			7	19.92	0.0	005	X		
(27)	F.FAPASLLI	LPDAVPK			7	19.92	0.0	008	X	_	
(29)	F.FAPASLLI	LPDAVPK			7	19.92	0.0	001	×		
(33)	F.FAPASLLI	LPDAVPK			7	19.92	0.0	012	×		
(38)	F.FAPASLLI	LPDAVPK			7	19.92	0.0	006	×		
(54)	F.FAPASLLI	LPDAVPK			7	19.92	0.0	011	×		
(60)	F.FAPASLLI	LPDAVPK			7	19.92	0.0	014	×		
(66)	F.FAPASLLI	LPDAVPK			7	19.92	-0.0	003	×		
57	F.APASLLLF	PDAVPK			6	46.38	-0.0	003	×		
49	L.LPDAVPK	(3	/0.22	0.0	001	×		

Table 2: a) List 1 of 2 of peptides identified by LC / MS – MS after digestion of IRAK-4 using Trypsin.b) Part 2 of 2 of peptides identified by LC / MS – MS after digestion of IRAK-4 DD using Trypsin.

(a)

1	Acc. #	F	Pep. #	% Cov.	Score		
9947		IRAK4 DEATH DOM	59	62%	645		
A M D D D F A P A	O P E F M N K R Y N Q F H I A S L L L P C	RRFEALLQT AVPK	C LNVGLIRKLS DF G KSPTSELLFD WG	I DPQE TTNCT	GWK K VGD L	VDLLI	Q N E
Red =	MS-MS Un	iderline = PMF					
Comm	ents: Indivi	dual ions scores > 2	20 indicate identity or extensiv	e homolog	y (p<0.05).	
Score		Peptide	Modifications	Obs. m/z	Delta	MS-MS?	PMF?
47	GAMDPEF	MNKPITPSTYVR.C	Phospho (ST)	745.34	-0.0003	×	0
(20)	GAMDPEF	MNKPITPSTYVR.C	Phospho (ST)	1,117.51	0.0008	×	-
(22)	GAMDPEF	MNKPITPSTYVR.C	Phospho (ST); Oxidation (M)	1,125.50	-0.0007		0
(24)	GAMDPEF	MNKPITPSTYVR.C	Phospho (ST); Oxidation (M)	750.67	-0.0006		
(26)	GAMDPEF	MNKPITPSTYVR.C	Oxidation (M)	1,085.52	0.0037	×	
(29)	GAMDPEF	MNKPITPSTYVR.C	2 Oxidation (M)	729.35	5 0.0020	×	
(32)	GAMDPEF	MNKPITPSTYVR.C	Oxidation (M)	724.02	0.0009	×	
(33)	GAMDPEF	MNKPITPSTYVR.C		718.68	8 -0.0009	×	
(33)	GAMDPEF	MNKPITPSTYVR.C		1,077.52	0.0014	×	0
(33)	GAMDPEF	MNKPITPSTYVR.C	2 Oxidation (M)	1,093.52	0.0066	×	
(41)	GAMDPEF	MNKPITPSTYVR.C	Phospho (ST); 2 Oxidation (M) 756.00	-0.0005	×	
59	R.CLNVGLI	R.K		472.77	-0.0006	×	
(38)	R.CLNVGLI	R.K		472.77	0.0001	×	
(44)	R.CLNVGLI	R.K		472.77	-0.0005	×	
(49)	R.CLNVGLI	R.K		472.77	0.0010	×	
(50)	R.CLNVGLI	R.K		472.77	0.0042	×	
69	R.KLSDFIDF	PQEGWK.K		781.90	0.0014	×	
22	R.KLSDFIDF	PQEGWKK.L		564.30	-0.0003	×	
(21)	R.KLSDFIDF	PQEGWK.K		521.60	-0.0027	×	
(67)	R.KLSDFIDF	PQEGWK.K		781.90	-0.0008	×	
44	K.LSDFIDPO	QEGWK.K		717.85	5 -0.0012	×	
34	K.LSDFIDPO	QEGWKK.L		781.90	0.0004	×	
(25)	K.LSDFIDPO	QEGWKK.L		521.60	-0.0014	×	
(38)	K.LSDFIDPO	QEGWK.K		717.85	5 0.0011	×	
50	K.LAVAIKK	PSGDDR.Y		457.26	0.0002	×	
39	K.LAVAIK.K			307.72	-0.0006	×	
(26)	K.LAVAIK.K	(307.72	0.0005	×	
40	K.KPSGDDR	LΥ		387.69	0.0002	×	
30	K.KPSGDDR	YNQFHIR.R		866.93	0.0014	×	
(20)	K.KPSGDDR	YNQFHIR.R		433.97	-0.0014	×	
(20)	K.KPSGDDR	YNQFHIR.R		578.29	-0.0011	×	
(36)	K.KPSGDDR	LY		387.69	-0.0002	×	
(36)	K.KPSGDDR	L.Y		387.69	0.0000	×	
(36)	K.KPSGDDR	LΥ		387.69	0.0002	×	
(40)	K.KPSGDDR	LY		387.69	0.0000	×	

(b)

	Acc. #	F	Protein ID	Pep. #	% Cov.	Score	
9947		IRAK4 DEATH DOM	IAIN	59	62%	645	
GAM			C LNVGLIRKLS DE	FIDPQE	GWK K		KKPS
FAP	ASLLLPC	AVPK	G KSPISELEPD W	arriver	VGD L	VDEEI	QNEP
Red =	= MS-MS <u>Un</u>	derline = PMF					
Comm	nents: Indivi	dual ions scores > 2	20 indicate identity or extensi	ve homolog	ly (p<0.05	5).	
Score		Peptide	Modifications	Obs. m/z	z Delta	MS-MS?	PMF?
(36)	K.KPSGDDR	.γ 		387.69	9 0.0002	2 💌	
(40)	K.KPSGDDR	.γ		387.65	9 0.0000		
(40)	K.KPSGDDR	.γ 		387.69	9 0.000		
(40)	K.KPSGDDR			387.65	9 0.0001		
(40)	K.KPSGDDR			387.65	0.000		
45	R.YNQFHIR	.к		489.2	5-0.0003		
(20)	R.YNQFHIR	.к Р		326.50	-0.0002		
(23)	R.YNQFHIR	.к р		489.2	-0.0003		
(28)	R.YNQFHIR	.к Р		489.2	-0.0012		
(28)	R.YNQFHIR	.к р		489.2	-0.0009		
(28)		.к. Р		489.2	-0.0002		
(30)		P		405.2	5 -0.0011		
(++)	P PEEALLO			501.0	2 -0.0012		
(20)	P PEEALLO	TGKIS		501.0	2 -0.00012		
(20)	R REEALLO	TGKIS		388.2	2 0 0004		
(37)	R REEALLO	TGKIS		581.8	3 -0.0003		
(42)	R.REEALLO	TGKIS		581.8	3 0.0001	X	
(44)	R.REEALLO	TGKIS		581.8	3 0.000		
(46)	R.REEALLO	TGK.S		581.8	3 -0.0006		
(46)	R.RFEALLO	TGK.S		581.8	3 -0.0002	×	
(54)	R.RFEALLO	TGK.S		581.83	3 0.0006	5 🗙	
55	R.FEALLOT	GK.S		503.7	8 -0.0009		
44	K.TANTLPS	K.E		416.23	3 0.0002	2 🗙	
27	K.EAITVOO	K.Q		458.7	5 0.0000	×	
47	K.SLEVSDT	R.F		453.73	3 0.000	×	

(c)

9947	IRAK4 DEATH DOMAIN		88	62%	645	
	EFMNK PITPSTYVRC L	NVGLIRKLS SPTSELLED	DFIDPQE	GWK K		K P S
FAPAS	LLLPD AVPK	0,0,0,0,0,0,0				211 21
Red = M	IS-MS Underline = PMF					
Commer	its: Individual ions scores > 20 indi	cate identity or exte	nsive homolog	gy (p<0.05	5).	
Score 47	Peptide K.LAVAIK.K	Modifications	Obs. m/z 307.72	Delta -0.0001	MS-MS?	PMF?
(29)	K.LAVAIK.K		307.72	-0.0002	×	
(33)	K.LAVAIK.K		307.72	0.0000	×	
(43)	K.LAVAIK.K		307.72	0.0005	×	
(45)	K.LAVAIK.K		307.72	0.0005	×	
40	K.KPSGDDR.Y		387.69	0.0000	×	
34	K.KPSGDDRYNQFHIR.R		866.93	0.0030	×	
(25)	K.KPSGDDRYNQFHIR.R		578.29	0.0014	×	
(37)	K.KPSGDDR.Y		387.69	0.0002	×	
(39)	K.KPSGDDR.Y		387.69	0.0002	×	
(40)	K.KPSGDDR.Y		387.69	0.0001	×	
(40)	K.KPSGDDR.Y		387.69	0.0001	×	
(40)	K.KPSGDDR.Y		387.69	0.0002	×	
50	R.YNQFHIR.R		489.25	-0.0001	×	
(25)	R.YNQFHIR.R		489.25	-0.0007	×	
(29)	R.YNQFHIR.R		489.25	0.0005	×	
(31)	R.YNQFHIR.R		489.25	-0.0003	×	
(34)	R.YNQFHIR.R		489.25	-0.0003	×	
(36)	R.YNQFHIR.R		489.25	0.0010	×	
(38)	R.YNQFHIR.R		489.25	0.0001	×	
(38)	R.YNQFHIR.R		489.25	0.0005	×	
(41)	R.YNQFHIR.R		489.25	-0.0007	×	
(41)	R.YNQFHIR.R		489.25	-0.0002	×	
(41)	R.YNQFHIR.R		489.25	-0.0002	×	
(45)	R.YNQFHIR.R		489.25	-0.0005	×	

(d)

9947	IRAK4 DEATH DOMAIN		88	62%	645	
GAMDE	EFMNK PITPSTYVRC L	NVGLIRKLS SPTSELLED	DFIDPQE WGTTNCT	GWK K	LAVAI K	N E F
FAPAS	LLLPD AVPK					
Red = №	IS-MS <u>Underline = PMF</u>					
Commer	its: Individual ions scores > 20 indi	cate identity or exte	nsive homolog	gy (p<0.05	5). 	
(46)	R.YNOFHIR.R	Modifications	489.25	-0.0005	MS-MS?	PMF?
(47)	R.YNQFHIR.R		489.25	-0.0010	×	
67	R.RFEALLQTGK.S		581.83	-0.0010	×	
(20)	R.RFEALLQTGK.S		388.22	0.0011	×	
(31)	R.RFEALLQTGK.S		581.83	0.0010	×	
(36)	R.RFEALLQTGK.S		388.22	-0.0005	×	
(50)	R.RFEALLQTGK.S		581.83	-0.0003	×	
(51)	R.RFEALLQTGK.S		581.83	0.0005	×	
(55)	R.RFEALLQTGK.S		581.83	-0.0002	×	
(55)	R.RFEALLQTGK.S		581.83	-0.0002	×	
(55)	R.RFEALLQTGK.S		581.83	-0.0001	×	
(55)	R.RFEALLQTGK.S		581.83	0.0003	×	
(55)	R.RFEALLQTGK.S		581.83	0.0003	×	
(55)	R.RFEALLQTGK.S		581.83	0.0003	×	
(55)	R.RFEALLQTGK.S		581.83	0.0004	×	
(56)	R.RFEALLQTGK.S		581.83	0.0004	×	
(56)	R.RFEALLQTGK.S		581.83	0.0004	×	
(61)	R.RFEALLQTGK.S		581.83	0.0003	×	
(61)	R.RFEALLQTGK.S		581.83	0.0004	×	
(65)	R.RFEALLQTGK.S		581.83	-0.0001	×	
55	R.FEALLQTGK.S		503.78	-0.0019	×	
(20)	R.FEALLQTGK.S		503.78	-0.0001	×	
(27)	R.FEALLQTGK.S		503.78	0.0000	×	
(42)	R.FEALLQTGK.S		503.78	-0.0006	×	

9947	IRAK4 DEATH DOMA	IN	19 6	52%	427	
GAMD GDDR FAPA	PEFMNK PITPSTYVRG YNQFHI RRFEALLQTG SLLLPD AVPK	L <u>NVGLIRKLS</u> D <u>FI</u> Ksptsellfd wgt	D P Q E G WI T N C T V G I		VAIK LLIQ	(<u>PS</u> EF
Red =	MS-MS <u>Underline = PMF</u>					
Comme	ents: Individual ions scores > 28	indicate identity or extensive h	nomology (p	<0.05).		
Score	Peptide	Modifications	Obs. m/z	Delta	MS-MS?	PMF?
//	GAMDPEFMNKPITPSTYVR.C		2,154.14	0.10/1	<u> </u>	
0	GAMDPEFMNKPITPSTYVR.C	Oxidation (M)	2,170.15	0.1183		×
0	GAMDPEFMNKPITPSTYVR.C	2 Oxidation (M)	2,186.16	0.1288		×
(48)	GAMDPEFMNKPITPSTYVR.C	Phospho (ST)	2,234.13	0.1295	×	
(57)	GAMDPEFMNKPITPSTYVR.C	Oxidation (M); Phospho (ST)	2,250.12	0.1276	×	
(74)	GAMDPEFMNKPITPSTYVR.C	2 Oxidation (M); Phospho (ST)	2,266.13	0.1421	×	
0	R.CLNVGLIR.K		944.58	0.0449		×
92	R.KLSDFIDPQEGWKK.L		1,690.96	0.0765	×	
73	R.KLSDFIDPQEGWK.K		1,562.86	0.0785	×	
0	R.KLSDFIDPQEGWKK.L	Phospho (ST)	1,770.89	0.0462		X
0	K.LSDFIDPQEGWK.K		1,434.77	0.0769		×
0	K.LAVAIKKPSGDDRYNQFHIR.R		2,328.40	0.1441		×
0	K.LAVAIKKPSGDDRYNQFHIRR.F	Phospho (ST)	2,564.29	-0.0364		×
89	K.KPSGDDRYNQFHIR.R		1,732.96	0.1135	×	
0	K.KPSGDDR.Y	Phospho (ST)	854.32	-0.0173		×
0	K.KPSGDDRYNQFHIRR.F		1,889.05	0.0922		×
44	R.YNQFHIR.R		977.54	0.0472	×	
0	R.YNQFHIRR.F		1,133.64	0.0386		×
52	R.RFEALLQTGK.S		1,162.72	0.0650	×	

9947	IRAK4 DEATH DOMA	AIN	20 8	30%	357	
G A M D G D D R F A P A	PEFMNK PITPSIYVR YNQFHI RRFEALLQI SLLLPD AVPK	C L <mark>NVGLIRKLS DFI</mark> G KSPTSELLED W <mark>GT</mark>	PQEGW INCTVGI	K KLA D L V D	VAIK LLIQI	(<u>PS</u> EF
Red =	MS-MS Underline = PMF) indicate identity or extensive h	omology (n	<0.05)		
Score	Peptide	Modifications	Obs. m/z	Delta	MS-MS?	PMF?
34	GAMDPEFMNKPITPSTY.V	2 Oxidation (M); Phospho (ST)	2,010.89	0.0626	×	
0	GAMDPEF.M		766.38	0.0711		X
0	GAMDPEFMNKPITPSTY.V		1,898.92	0.0535		X
0	GAMDPEFMNKPITPSTY.V	Oxidation (M)	1,914.91	0.0525		X
0	GAMDPEFMNKPITPSTY.V	2 Oxidation (M)	1,930.91	0.0573		×
79	L.NVGLIRKLSDFIDPQEGW.K		2,087.17	0.0762	×	
0	L.NVGLIRKL.S		912.62	0.0219		×
69	L.IRKLSDFIDPQEGW.K		1,703.92	0.0454	×	
0	L.IRKLSDFIDPQEGWKKL.A		2,073.20	0.0546		×
0	L.SDFIDPQEGWKKL.A		1,562.82	0.0359		×
0	W.KKLAVAIKKPSGDDRY.N		1,789.07	0.0377		X
0	W.KKLAVAIKKPSGDDRYNQF.H		2,178.27	0.0699		X
78	L.AVAIKKPSGDDRYNQF.H		1,808.98	0.0558	×	
55	L.AVAIKKPSGDDRY.N		1,419.80	0.0398	×	
0	Y.NQFHIRRF.E		1,117.63	0.0318		×
0	F.HIRRF.E		728.43	-0.0031		X
0	F.HIRRFEAL.L		1,041.62	0.0269		X
0	F.HIRRFEALL.Q		1,154.72	0.0354		×
0	L.QTGKSPTSELLFDW.G	Phospho (ST)	1,688.90	0.1433		×
42	F.FAPASLLLPDAVPK		1,438.87	0.0371	×	

 Table 4: Peptides identified by MALDI-TOF MS after digestion of IRAK-4 DD using Chymotrypsin.

9947	IRAK4 DEATH DOMAIN		11	82%	280									
G A M D G D D R F A P A	PEFMNK PITPSTYVRC LNVG YNQFHI RRFEALLQTG KSPT SLLLPD AVPK	LIRKLS DF Sellfd wg	I D P Q E G I T T N C T V (WK KL GD LV	AVAIK DLLIQ	K P S N E F								
Red =	MS-MS Underline = PMF													
Comments: Individual ions scores > 19 indicate identity or extensive homology (p<0.05).														
Score	Peptide	Modifications	Obs. m/z	Delta	MS-MS?	PMF?								
19	M.DPEFMNKPITPSTYVRCLNVGLIRKLS.D	Oxidation (M)	791.92	0.0028	×									
25	S.DFIDPQEGWKKLAVAIKKPSG.D		776.43	0.0013	×									
31	I.DPQEGWKKLAVAIKKPSG.D		651.37	0.0031	×									
29	I.DPQEGWKKLAVAIKKPSGD.D		689.71	-0.0010	×									
(28)	I.DPQEGWKKLAVAIKKPSG.D		976.56	0.0066	×									
23	Q.EGWKKLAVAIKKPSG.D		403.75	0.0006	×									
31	G.DDRYNQFHIRRF.E		417.46	-0.0004	×									
(24)	G.DDRYNQFHIRRF.E		417.46	0.0006	×									
67	F.EALLQTGKSPTS.E		616.33	-0.0008	×									
22	V.DLLIQNEFFAPASLLLPDAVPK		804.45	0.0013	×									
33	N.EFFAPASLLLPDAVPK		857.98	0.0015	×									

Table 5: Peptides identified by LC / MS - MS after digestion of IRAK-4 DD using Asp-N.

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Table 6: Peptides identified by MALDI-TOF MS after digestion of IRAK-4 DD using Asp-N.

947 IRAK4 DEATH DOMAIN															5 63%						%	249										
GAMDPEEMNK PITPSTYVRC LNVGLIRKLS DFIDPQEGWK KLAVAIKK GDDRYNQFHI RRFEALLQIG KSPISELLFD WGTTNCTVGD LVDLLIQN FAPASLLLPD AVPK Red = MS-MS Underline = PMF Comments: Individual ions scores > 23 indicate identity or extensive homology (p<0.05).															(P NE	F																
Score Peptide											Modifications (Obs	5.	m	/z		De	lta	9	М	s-	M	5?	PI	1F?				
M.DPEFMN	KPITP	STY	VRCL	.NV	/GL	IR	KLS	S.D		C	Dxi	da	tio	n (M)			3,	,1(54.	86	5	0.:	198	39		[[X	
S.DFIDPQ	EGWK		AIK	KP:	SG	.D												2,	3	27	43	3	0.:	153	34			×		[
I.DPQEGW		VAIK	(KPS(G.C	D												Γ	1	,9	52.	22	2	0.:	128	30			×		[
G.DDRYNQFHIRRF.E																		1	6	56.	92	2	0.:	100	08		[[X	
0 D.DRYNQFHIRRFEALLQTGKSPTS.E										F	ho	sp	ho	(5	T)		Γ	2	84	44.	.30) -	0.0	097	75		[[×	
	PEFMN YNQFHI SLLLPD MS-MSUI MS-MSUI M.DPEFMN S.DFIDPQI I.DPQEGW G.DDRYNQF	IRAK	IRAK4 DE P E E MN K P I T P Y N Q E H I R E E S L L L P D A V P K MS-MS Underline = nts: Individual ions s Peg M.DPEFMNKPITPSTY S.DFIDPQEGWKKLAV I.DPQEGWKKLAVAIK G.DDRYNQFHIRRF.E D.DRYNQFHIRRFEAL	IRAK4 DEATH PEEMNK PITPST NOFHI REFEAL SLLLPDAVPK MS-MS Underline = PM nts: Individual ions score Peptide M.DPEFMNKPITPSTYVRCL S.DFIDPQEGWKKLAVAIK I.DPQEGWKKLAVAIKKPS G.DDRYNQFHIRRF.E D.DRYNQFHIRRFEALLQTG	IRAK4 DEATH DO PEEMNK PIIPSIY NOFHI REEALLO SLLLPDAVPK MS-MS Underline = PMF nts: Individual ions scores : Peptide M.DPEFMNKPITPSTYVRCLNV S.DFIDPQEGWKKLAVAIKKPSG.I G.DDRYNQFHIRRF.E D.DRYNQFHIRRFEALLQTGKS	IRAK4 DEATH DOM PEFMNK PITPSIYVR YNOFHI REFEALLOI SLLLPD AVPK MS-MS Underline = PMF nts: Individual ions scores > 2 Peptide M.DPEFMNKPITPSTYVRCLNVGL S.DFIDPQEGWKKLAVAIKKPSG.D I.DPQEGWKKLAVAIKKPSG.D G.DDRYNQFHIRRF.E D.DRYNQFHIRRFEALLQTGKSPT	IRAK4 DEATH DOMAIN PEFMNK PITPSTYVRC YNOFHI REFEALLOTG SLLLPD AVPK MS-MS Underline = PMF nts: Individual ions scores > 23 ir Peptide M.DPEFMNKPITPSTYVRCLNVGLIRI S.DFIDPQEGWKKLAVAIKKPSG.D I.DPQEGWKKLAVAIKKPSG.D G.DDRYNQFHIRRF.E D.DRYNQFHIRRFEALLQTGKSPTS.E	IRAK4 DEATH DOMAIN PEEMNK PIIPSIYVRC L YNQEHI RRFEALLQIG K SLLLPD AVPK MS-MS Underline = PMF nts: Individual ions scores > 23 india MDPEFMNKPITPSTYVRCLNVGLIRKLS S.DFIDPQEGWKKLAVAIKKPSG.D I.DPQEGWKKLAVAIKKPSG.D G.DDRYNQFHIRRF.E D.DRYNOFHIRRFEALLOTGKSPTS.E	IRAK4 DEATH DOMAIN PEEMNK PIIPSIYVRC LNY YNQEHI RREEALLQIG KSE SLLLPD AVPK MS-MS Underline = PMF nts: Individual ions scores > 23 indicate Peptide M.DPEFMNKPITPSTYVRCLNVGLIRKLS.D S.DFIDPQEGWKKLAVAIKKPSG.D I.DPQEGWKKLAVAIKKPSG.D G.DDRYNQFHIRRF.E D.DRYNOFHIRRFEALLOTGKSPTS.E	IRAK4 DEATH DOMAIN PEEMNK PIIPSIYVRC LNVG YNQEHI RREEALLQIG KSPI SLLLPD AVPK MS-MS Underline = PMF nts: Individual ions scores > 23 indicate ic Peptide M.DPEFMNKPITPSTYVRCLNVGLIRKLS.D S.DFIDPQEGWKKLAVAIKKPSG.D I.DPQEGWKKLAVAIKKPSG.D G.DDRYNQFHIRRF.E D.DRYNOFHIRRFEALLOTGKSPTS.E	IRAK4 DEATH DOMAIN PEEMNK PIIPSIYVRC NYRC NYGL NYGL NYGL NYGL NYGL SLLLPD AVPK MS-MS Underline = PMF nts: Individual ions scores > 23 indicate ider Peptide M.DPEFMNKPITPSTYVRCLNVGLIRKLS.D S.DFIDPQEGWKKLAVAIKKPSG.D I.DPQEGWKKLAVAIKKPSG.D G.DDRYNQFHIRRF.E D.DRYNOFHIRRFEALLOTGKSPTS.E	IRAK4 DEATH DOMAIN PEEMNK PIIPSIYVRC LNVGLIN YNQEHI RREEALLQIG KSPISE SLLLPD AVPK MS-MS Underline = PMF nts: Individual ions scores > 23 indicate identit Peptide M.DPEFMNKPITPSTYVRCLNVGLIRKLS.D Oxi S.DFIDPQEGWKKLAVAIKKPSG.D I I.DPQEGWKKLAVAIKKPSG.D G G.DDRYNQFHIRRF.E D D.DRYNOFHIRRFEALLOTGKSPTS.E Pho	IRAK4 DEATH DOMAIN PEEMNK PIIPSIYVRC LNYGLIRE YNQEHI REFEALL QIG KSPISELL SLLLPD AVPK MS-MS Underline = PMF nts: Individual ions scores > 23 indicate identity of Modi M.DPEFMNKPITPSTYVRCLNVGLIRKLS.D Oxida S.DFIDPQEGWKKLAVAIKKPSG.D I I.DPQEGWKKLAVAIKKPSG.D Oxida G.DDRYNQFHIRRF.E D.DRYNOFHIRRFEALLOTGKSPTS.E Phosp	IRAK4 DEATH DOMAIN PEEMNK PITPSTYVRC LNVGLIRKL YNQEHI RRFEALLQIG KSPISELLF SLLLPD AVPK MS-MS Underline = PMF nts: Individual ions scores > 23 indicate identity or peptide Modific M.DPEFMNKPITPSTYVRCLNVGLIRKLS.D Oxidatio S.DFIDPQEGWKKLAVAIKKPSG.D I.DPQEGWKKLAVAIKKPSG.D I.DPQEGWKKLAVAIKKPSG.D O.DDRYNQFHIRRF.E D.DRYNOFHIRRFEALLOTGKSPTS.E Phospho	IRAK4 DEATH DOMAIN PEEMNK PIIPSIYVRC LNVGLIRKLS YNQEHI REFEALLQIG KSPISELLFD SLLLPD AVPK MS-MS Underline = PMF nts: Individual ions scores > 23 indicate identity or ext Peptide Modificat M.DPEFMNKPITPSTYVRCLNVGLIRKLS.D Oxidation (S.DFIDPQEGWKKLAVAIKKPSG.D I.DPQEGWKKLAVAIKKPSG.D I.DPQEGWKKLAVAIKKPSG.D G.DDRYNQFHIRRF.E D.DRYNOFHIRRFEALLOTGKSPTS.E Phospho (S	IRAK4 DEATH DOMAIN PEEMNK PIIPSIYVRC LNVGLIRKLS NYGLIRKLS NYGLIRKLS NYGLIRKLS NYGLIRKLS NYGLIRKLS Modification NO NYGLIRKLS.D Oxidation (M) S.DFIDPQEGWKKLAVAIKKPSG.D I.DPQEGWKKLAVAIKKPSG.D G.DDRYNQFHIRRF.E D.DRYNOFHIRRFEALLOTGKSPTS.E	IRAK4 DEATH DOMAIN PEEMNK PIIPSIYVRC LNVGLIRKLS DF NOFHI REFEALLOIG KSPISELLFD WG SLLLPD AVPK MS-MS Underline = PMF nts: Individual ions scores > 23 indicate identity or extensiv Peptide Modifications M.DPEFMNKPITPSTYVRCLNVGLIRKLS.D Oxidation (M) S.DFIDPQEGWKKLAVAIKKPSG.D I.DPQEGWKKLAVAIKKPSG.D I.DPQEGWKKLAVAIKKPSG.D Oxidation (M) S.DFINOPHIRRF.E Phospho (ST)	IRAK4 DEATH DOMAIN PEEMNK PIIPSIYVRC LNVGLIRKLS DFI YNQEHI REFEALL QIG KSPISELLED WGT SLLLPD AVPK MS-MS Underline = PMF nts: Individual ions scores > 23 indicate identity or extensive Peptide Modifications O M.DPEFMNKPITPSTYVRCLNVGLIRKLS.D Oxidation (M) I S.DFIDPQEGWKKLAVAIKKPSG.D I.DPQEGWKKLAVAIKKPSG.D I I.DPQEGWKKLAVAIKKPSG.D Oxidation (M) I G.DDRYNQFHIRRF.E D.DRYNOFHIRRFEALLOTGKSPTS.E Phospho (ST)	IRAK4 DEATH DOMAIN PEEMNK PIIPSIYVRC LNVGLIRKLS DFIDE NOFHI REFEALLOIG KSPISELLFD DFIDE SLLLPD AVPK MS-MS Underline = PMF nts: Individual ions scores > 23 indicate identity or extensive hor Peptide Modifications Obs M.DPEFMNKPITPSTYVRCLNVGLIRKLS.D Oxidation (M) 3, S.DFIDPQEGWKKLAVAIKKPSG.D 2, I.DPQEGWKKLAVAIKKPSG.D 1, G.DDRYNQFHIRRF.E 1, D.DRYNOFHIRRFEALLOTGKSPTS.E Phospho (ST) 2,	IRAK4 DEATH DOMAIN PEEMNK PIIPSIYVRC LNVGLIRKLS DFIDPO YNQEHI RRFEALLQIG KSPISELLFD DFIDPO SLLLPD AVPK MS-MS Underline = PMF Modifications Obs. MS-MS Underline scores > 23 indicate identity or extensive homo Modifications Obs. M.DPEFMNKPITPSTYVRCLNVGLIRKLS.D Oxidation (M) 3,16 S.DFIDPQEGWKKLAVAIKKPSG.D 2,33 I.DPQEGWKKLAVAIKKPSG.D 1,99 G.DDRYNQFHIRRF.E 1,66 D.DRYNOFHIRRFEALLOTGKSPTS.E Phospho (ST) 2,84	IRAK4 DEATH DOMAIN SILL POINT PEEMNK PIIPSIYVRC LNVGLIRKLS DFIDPOE NOEHI REFEALLOIG KSPISELLFD WGTTNCT SILL PD AVPK MS-MS Underline = PMF nts: Individual ions scores > 23 indicate identity or extensive homolo Peptide Modifications Obs. m. M.DPEFMNKPITPSTYVRCLNVGLIRKLS.D Oxidation (M) 3,164. S.DFIDPQEGWKKLAVAIKKPSG.D 2,327. 1,092. G.DDRYNQFHIRRF.E 1,666. 1,666. D.DRYNOFHIRRFEALLOTGKSPTS.E Phospho (ST) 2,844.	IRAK4 DEATH DOMAIN S PEEMNK PIIPSIYVRC LNYGLIRKLS DFIDPOEG NOEHI REEALLOIG KSPISELLFD WGTTNCTV SLLLPD AVPK MS-MS Underline = PMF nts: Individual ions scores > 23 indicate identity or extensive homology Peptide Modifications Obs. m/z M.DPEFMNKPITPSTYVRCLNVGLIRKLS.D Oxidation (M) 3,164.86 S.DFIDPQEGWKKLAVAIKKPSG.D 2,327.43 1,952.22 G.DDRYNQFHIRRF.E 1,666.92 1,666.92 D.DRYNOFHIRRFEALLOTGKSPTS.E Phospho (ST) 2,844.30	IRAK4 DEATH DOMAIN SILL PO AKANA PEEMNK PIIPSIYVRC LNVGLIRKLS DFIDPOEGW NOT	IRAK4 DEATH DOMAIN 5 633 PEEMNK PIIPSIYVRC LNVGLIRKLS DFIDPQEGWK NOT	IRAK4 DEATH DOMAIN 5 63% PEEMNK PIIPSIYVRC LNVGLIRKLS DFIDPQEGWK K NYGLIRKLS DFIDPQEGWK K YNQEHI REFEALLQIG KSPISELLFD DFIDPQEGWK K SLLLPD AVPK MS-MS Underline = PMF nts: Individual ions scores > 23 indicate identity or extensive homology (p<0.0) Peptide Modifications Obs. m/z Delta M.DPEFMNKPITPSTYVRCLNVGLIRKLS.D Oxidation (M) 3,164.86 0.198 S.DFIDPQEGWKKLAVAIKKPSG.D Q.327.43 0.153 0.153 I.DPQEGWKKLAVAIKKPSG.D 1,952.22 0.100 G.DDRYNQFHIRRF.E Phospho (ST) 2,844.30 -0.093	IRAK4 DEATH DOMAIN 5 63% PEEEMNK PIIPSIYYRC LNYGLIRKLS DFIDPQEGWKKL YNQEHI REFEALLQIG KSPISELLFD WGTTNCTVGDLV SLLLPD AVPK MS-MS Underline = PMF nts: Individual ions scores > 23 indicate identity or extensive homology (p<0.05).	IRAK4 DEATH DOMAIN 5 63% PEEEMNK PIIPSIYYRC LNYGLIRKLS DFIDPQEGWK KLAY YNQEHI REFEALLQIG KSPISELLFD WGTTNCTVGD VOL SLLLPD AVPK WGTTNCTVGD VOL VOL MS-MS Underline = PMF Modifications Obs. m/z Delta M M.DPEFMNKPITPSTYVRCLNVGLIRKLS.D Oxidation (M) 3,164.86 0.1989 S.DFIDPQEGWKKLAVAIKKPSG.D 0xidation (M) 3,164.86 0.1989 I.DPQEGWKKLAVAIKKPSG.D 1,952.22 0.1280 G.DDRYNQFHIRRF.E Phospho (ST) 2,844.30 -0.0975	IRAK4 DEATH DOMAIN 5 63% 24 PEEEMNK PIIPSIYYRC LNYGLIRKLS DFIDPQEGWK KLAYA NOFHI REFEALLOIG KLAY NOFHI POREGWK KLAY NOFHI POREGWK KLAY MS-MS DFIDPQEGWK KLAY MS-MS Underline = PME nts: Individual ions scores > 23 indicate identity or extensive homology (p<0.05). Peptide Modifications Obs. m/z Delta MS-MS M.DPEFMNKPITPSTYVRCLNVGLIRKLS.D Oxidation (M) 3,164.86 0.1989 I S.DFIDPQEGWKKLAVAIKKPSG.D 1,952.22 0.1280 I G.DDRYNQFHIRRF.E Phospho (ST) 2,844.30 -0.0975 I	IRAK4 DEATH DOMAIN 5 63% 249 PEEEMNK PITPSTYVRC LNYGLIRKLS DFIDPQEGWK KLAVAI NOFHI RFEALLOIG LNYGLIRKLS DFIDPQEGWK KLAVAI NOFHI RFEALLOIG KLAVAI NOFHI RFEALLOIG KLAVAI SULL P D A VPK MS-MS Underline = PMF Ints: Individual ions scores > 23 indicate identity or extensive homology (p<0.05). Peptide Modifications Obs. m/z Delta MS-MS MS-MS Onlog MS-MS Modifications Obs. m/z Delta MS-MS MS-MS Onlog O.05). Peptide Modifications Obs. m/z Delta MS-MS S.DFIDPQEGWKKLAVAIKKPSG.D O.1280 X	IRAK4 DEATH DOMAIN 5 63% 249 PEEEMNK PITPSTYVRC LNVGLIRKLS DFIDPQEGWK KLAVAIKP N OF I DPQEG WK KLAVAIKP MS-MS Underline = PMF nts: Individual ions scores > 23 indicate identity or extensive homology (p<0.05). Peptide Modifications Obs. m/z Delta MS-MS? M.DPEFMNKPITPSTYVRCLNVGLIRKLS.D Oxidation (M) 3,164.86 0.1989 Image: Colspan="2">Image: Colspan="2" Image: Colspan="2" <td>IRAK4 DEATH DOMAIN 5 63% 249 PEEEMNK PITPSTYVRC LNVGLIRKLS DFIDPQEGWK KLAVAIKKP NOFIDPQEGWK KLAVAIKKP NOFIDPQEGWK KLAVAIKKP MS-MS Underline = PMF nts: Individual ions scores > 23 indicate identity or extensive homology (p<0.05). Peptide Modifications Obs. m/z Delta MS-MS-MS-MS-MS-MS M.DPEFMNKPITPSTYVRCLNVGLIRKLS.D Oxidation (M) 3,164.86 0.1989 Image: Colspan="2">Image: Colspan="2">Image: Colspan="2">Image: Colspan="2">Image: Colspan="2">Image: Colspan="2">Image: Colspan="2">Image: Colspan="2">Image: Colspan="2">Colspan="2">Image: Colspan="2">Colspan="2">Image: Colspan="2">Image: Colspan="2" Image: Colspan="2" Image: Colspan="2" Image: Colspan="2" <th c<="" td=""></th></td>	IRAK4 DEATH DOMAIN 5 63% 249 PEEEMNK PITPSTYVRC LNVGLIRKLS DFIDPQEGWK KLAVAIKKP NOFIDPQEGWK KLAVAIKKP NOFIDPQEGWK KLAVAIKKP MS-MS Underline = PMF nts: Individual ions scores > 23 indicate identity or extensive homology (p<0.05). Peptide Modifications Obs. m/z Delta MS-MS-MS-MS-MS-MS M.DPEFMNKPITPSTYVRCLNVGLIRKLS.D Oxidation (M) 3,164.86 0.1989 Image: Colspan="2">Image: Colspan="2">Image: Colspan="2">Image: Colspan="2">Image: Colspan="2">Image: Colspan="2">Image: Colspan="2">Image: Colspan="2">Image: Colspan="2">Colspan="2">Image: Colspan="2">Colspan="2">Image: Colspan="2">Image: Colspan="2" Image: Colspan="2" Image: Colspan="2" Image: Colspan="2" <th c<="" td=""></th>	

Table 7: MS/MS Fragmentation of GAMDPEFMNKPITPSTYVR

(a) Peptide fragment spectrum of b- and y- ion series corresponding to the cleavage of the amide bond at the N-terminus and C-terminus respectively. (b) Table of the corresponding observed fragmentation masses showing matching peptide hits in Bold Red: 45/272 fragment ions using 125 most intense peaks used to give an ion score of 20 in the MASCOT database. (c) Zoomed area of peptide fragment spectrum from 1600 to 1750 m/z showing the ion b*(15) corresponding to the loss of 98 Da at Ser8. (d) Zoomed area of peptide fragment spectrum from 300 to 400 m/z showing the ion y⁺⁺(5) corresponding to the loss of 98 Da at Ser8.

(a)





(d)



(c)

Table 8: MS/MS Fragmentation of GAMDPEFMNKPITPSTYVR

(a) Peptide fragment spectrum of b- and y- ion series corresponding to the cleavage of the amide bond at the N-terminus and C-terminus respectively. (b) Table of the corresponding observed fragmentation masses showing matching peptide hits in 56/272 fragment ions using 129 most intense peaks used to give an ion score of 48 in the MASCOT database. (c) Zoomed area of peptide fragment spectrum from 1550 to 1650 m/z showing the ion b(15) corresponding to the loss of 98 Da at Ser8. (d) Zoomed area of peptide fragment spectrum from 550 to 650 m/z showing the ions y(5) and y*(5) corresponding to the loss of 98 Da at Ser8.



(a)

#	b	b++	b*	b***	Seq.	у	y**	y*	y***	#
1	58.0287	29.5180			G					19
2	129.0659	65.0366			Α	2079.0038	1040.0055	2061.9772	1031.4923	18
3	260.1063	130.5568			M	2007.9667	1004.4870	1990.9401	995.9737	17
4	375.1333	188.0703			D	1876.9262	938.9667	1859.8996	930.4535	16
5	472.1860	236.5967			P	1761.8992	881.4533	1744.8727	872.9400	15
6	601.2286	301.1180			E	1664.8465	832.9269	1647.8199	824.4136	14
7	748.2971	374.6522			F	1535.8039	768.4056	1518.7773	759.8923	13
8	879.3375	440.1724			M	1388.7355	694.8714	1371.7089	686.3581	12
9	993.3805	497.1939	976.3539	488.6806	N	1257.6950	629.3511	1240.6684	620.8379	11
10	1121.4754	561.2414	1104.4489	552.7281	K	1143.6521	572.3297	1126.6255	563.8164	10
11	1218.5282	609.7677	1201.5016	601.2545	P	1015.5571	508.2822	998.5306	499.7689	9
12	1331.6123	666.3098	1314.5857	657.7965	Ι	918.5043	459.7558	901.4778	451.2425	8
13	1432.6599	716.8336	1415.6334	708.3203	Τ	805.4203	403.2138	788.3937	394.7005	7
14	1 529.712 7	765.3600	1512.6861	756.8467	P	704.3726	352.6899	687.3460	344.1767	6
15	1598.7342	799.8707	1581.7076	791.3574	S	607.3198	304.1636	590.2933	295.6503	5
16	1699.7818	850.3946	1682.7553	841.8813	Τ	538.2984	269.6528	521.2718	261.1396	4
17	1862.8452	931.9262	1845.8186	923.4129	Y	437.2507	219.1290	420.2241	210.6157	3
18	1961.9136	981.4604	1944.8870	972.9472	V	274.1874	137.5973	257.1608	129.0840	2
19					R	175.1190	88.0631	158.0924	79.5498	1

(c)



(b)



Supplementary information - Model validation.

After regularization in COOT, sidechain packing was optimised and total energy compared with the unphosphorylated IRAK4 structure (pdb id: 3mop, chain G) using the FoldX atomic forcefield. (FoldX version 4.0 running under Win10)¹. Sidechain clashes were checked with 'findclash' function in UCSF-Chimera².

Neglecting the new stabilizing interactions of the phospho-serine residue, the helical refolding of the N-terminal 16 residues was calculated by FoldX to be comparable in energy to the crystal structure. Compared with the equivalent optimised crystal structure (minimised with the FoldX 'Repair' protocol), the refolded IRAK4 had an overall $\Delta\Delta G$ stabilisation of -0.2 kcal/mol (neglecting any phospho-serine contributions). This favourable change in estimated stability on helical refolding is for the IRAK4 in isolation, but no unfavourable contacts are generated when packed with other IRAK4 and IRAK2 chains to form the myddosome assembly.

The geometry and sidechain packing of the refolded IRAK4 were validated with WHATCHECK10.1 (<u>http://swift.cmbi.ru.nl/gv/whatcheck/</u>). Ramachandran analysis (with COOT) showed no new outliers were generated by the helix refolding: the IRAK4 crystal structure had 98 residues in preferred or allowed regions, and 7 outliers. None of the outliers is in the N-terminal region and refolding of the N-terminal region does not introduce any new Ramachandran outliers.

References

- 1. Kiel, C., Serrano, L. & Herrmann, C. A detailed thermodynamic analysis of ras/effector complex interfaces. *J. Mol. Biol.* **340**, 1039-1058 (2004).
- Yang, Z. *et al.* UCSF Chimera, MODELLER, and IMP: an integrated modeling system. *J. Struct. Biol.* 179, 269-278 (2012).