Structural insights into cytokine cleavage by inflammatory caspase-4

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Supplementary Figure 1: Uncropped gels and blots.

Supplementary Video 1: 3D variability analysis of caspase-4/pro-IL-18 complex.

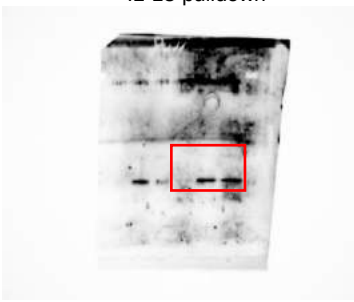
Supplementary Video legends

Supplementary Video 1: 3D variability analysis of caspase-4/pro-IL-18 complex. Domain flexibility within caspase-4/pro-IL-18 complex indicated by 3D variability analysis.

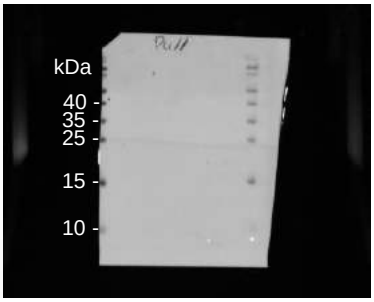
Supplementary Fig. 1 | Uncropped SDS-PAGE gels and immunoblots.

Figure 1g

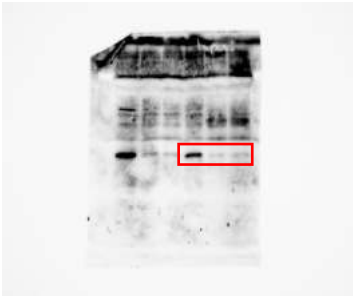
IL-18 pulldown



Colorimetric



IL-18 lysate



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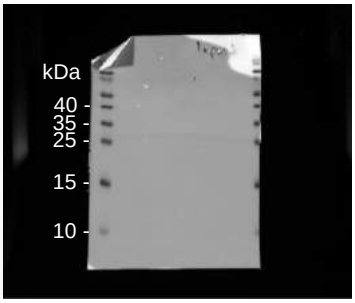
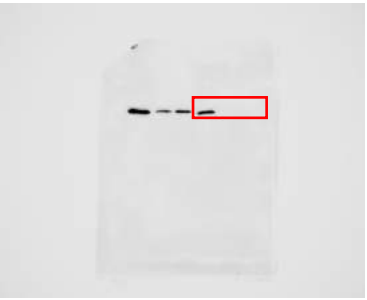


Actin



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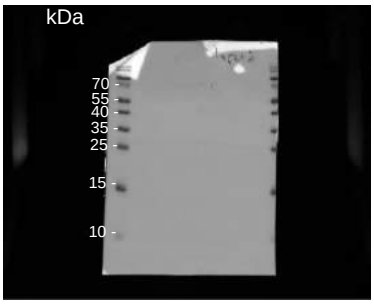
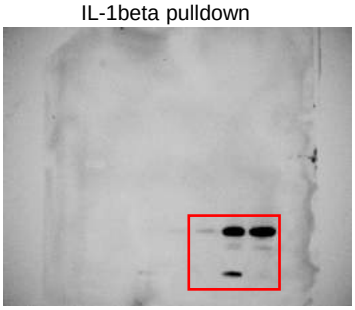


Figure 1h

IL-1beta pulldown



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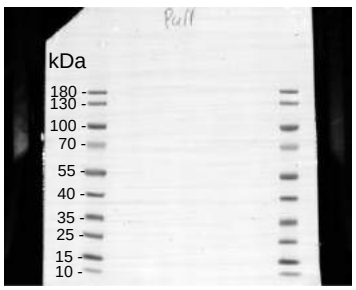

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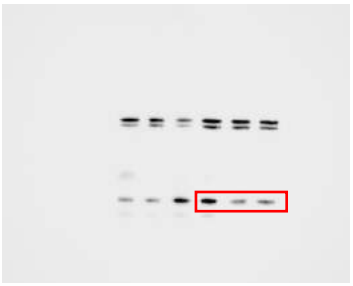

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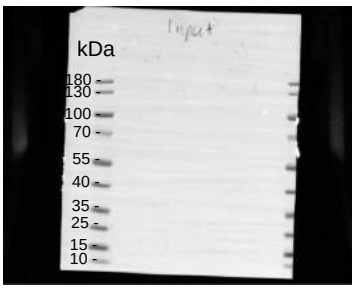

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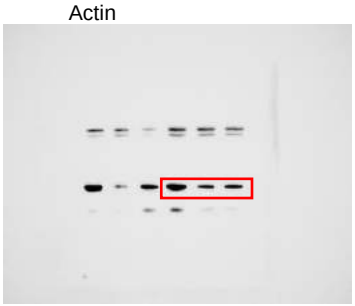

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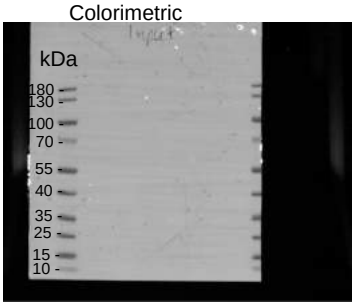
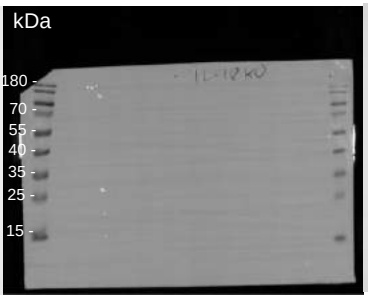


Figure 4d

Myc

Colorimetric



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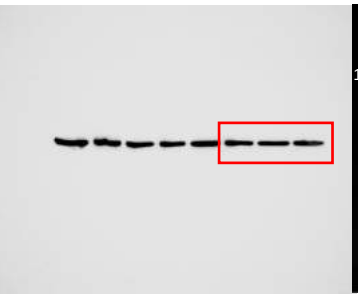

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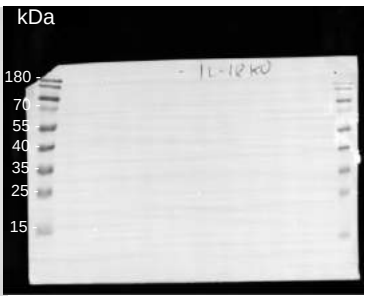
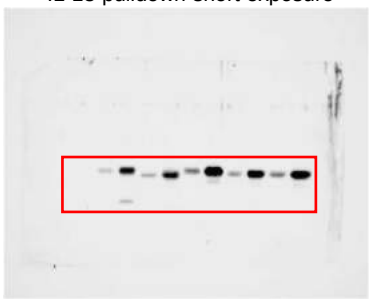
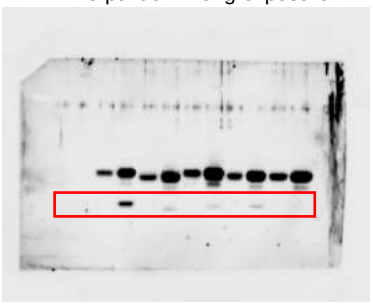


Figure 5i

IL-18 pulldown short exposure



IL-18 pulldown long exposure



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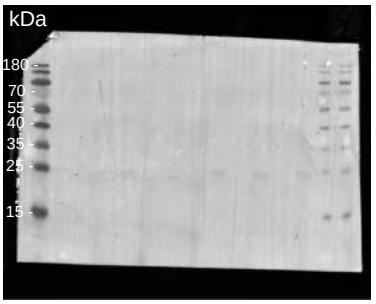

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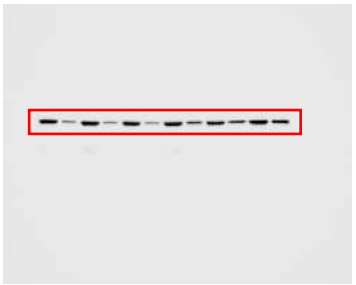

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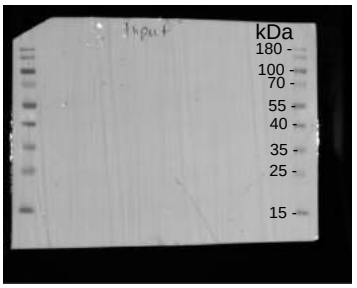
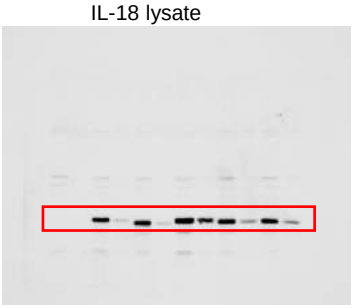


IL-18 lysate



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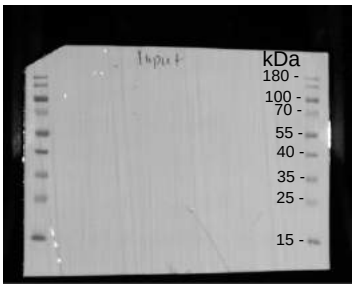
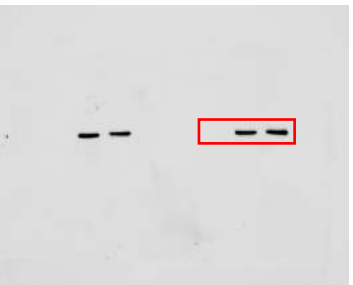


Figure 6d

Myc



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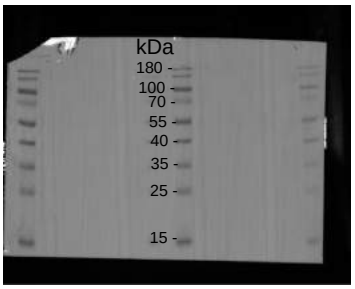
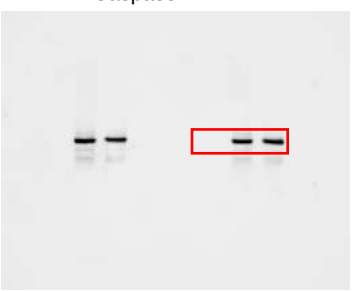


Caspase-11



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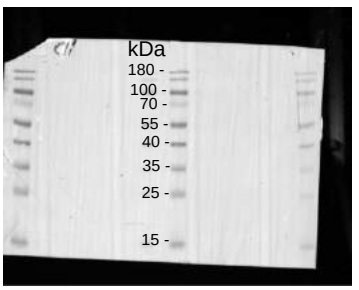


actin

Colorimetric

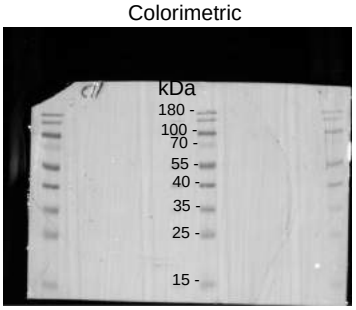
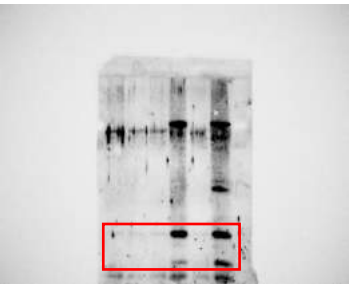
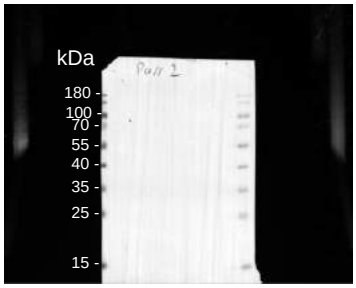


Figure 6e

IL-18 pulldown

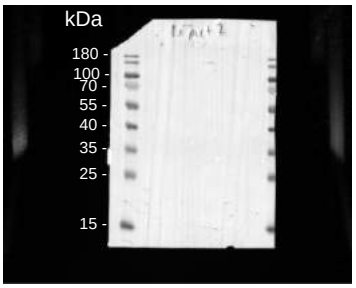


Colorimetric



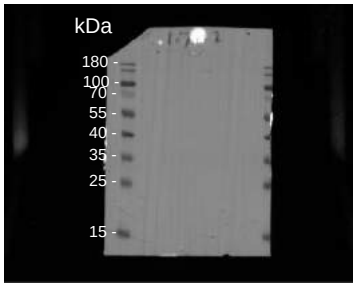
IL-18 lysate

Colorimetric

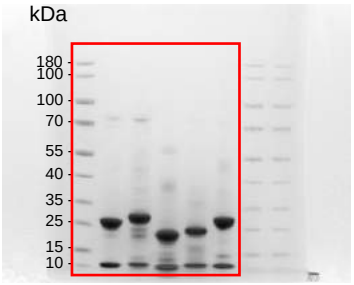


actin

Colorimetric

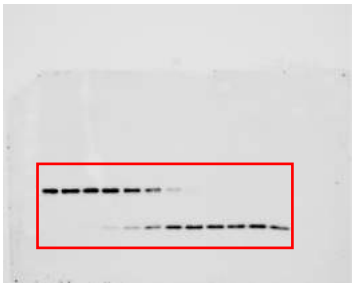


Extended Data Figure 1a



Extended Data Figure 1b

IL-18



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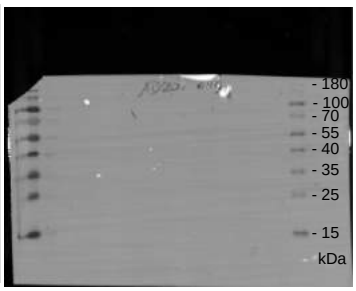
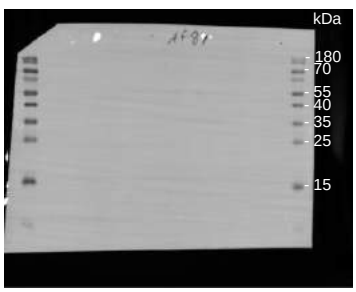


Extended Data Figure 1c

Myc

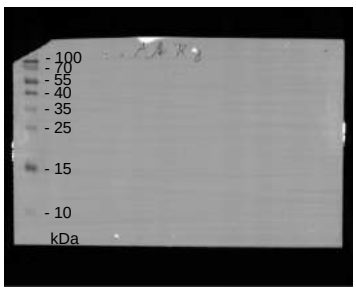
Colorimetric



Extended Data Figure 1d

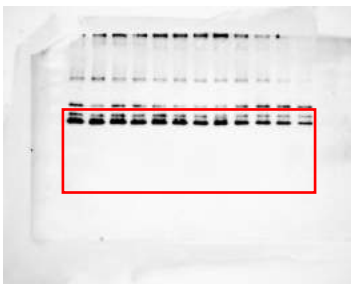
IL-1beta human

Colorimetric

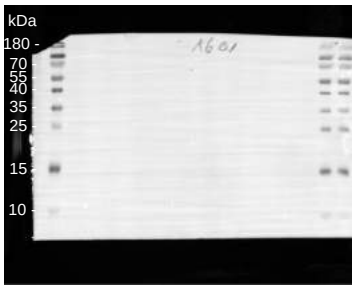


Extended Data Figure 1e

Myc

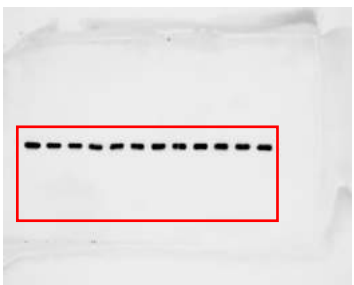


Colorimetric



Extended Data Figure 1f

Myc

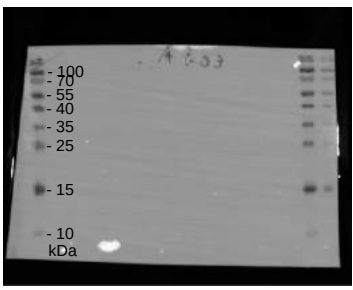


Colorimetric

Extended Data Figure 1g

Myc

Colorimetric



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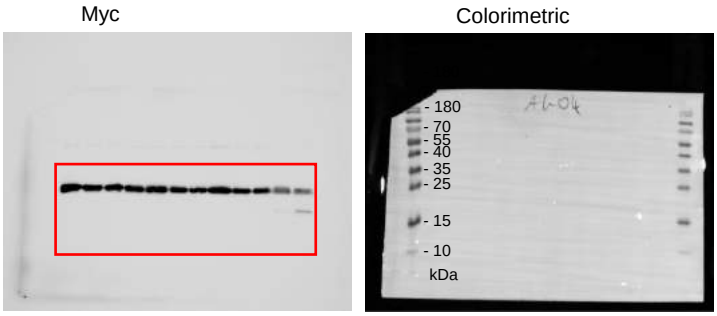

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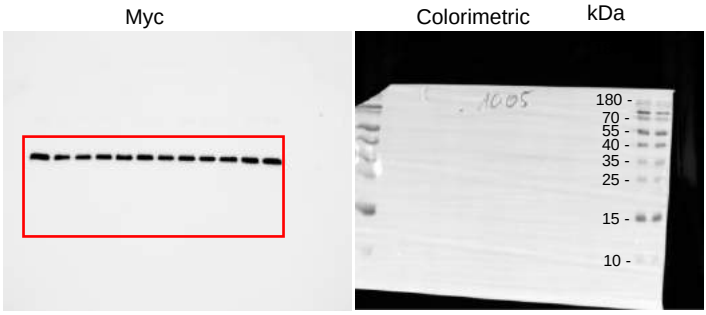

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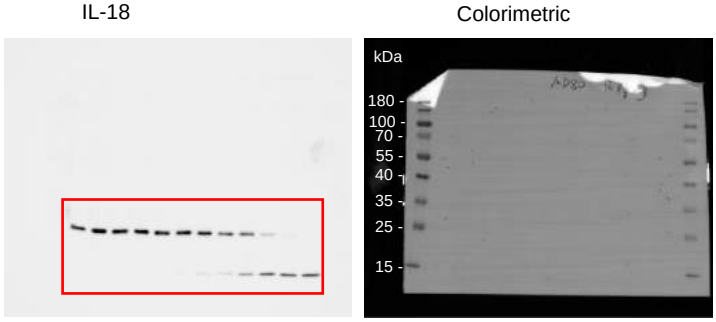

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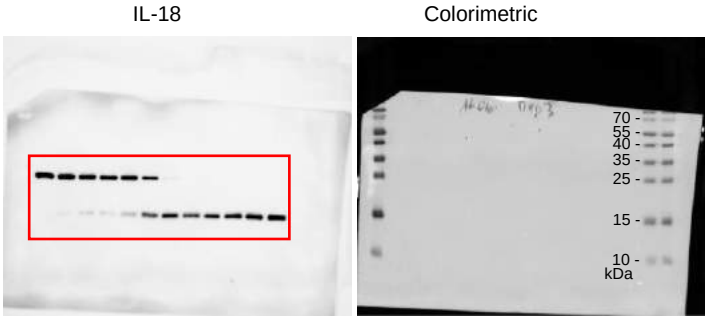
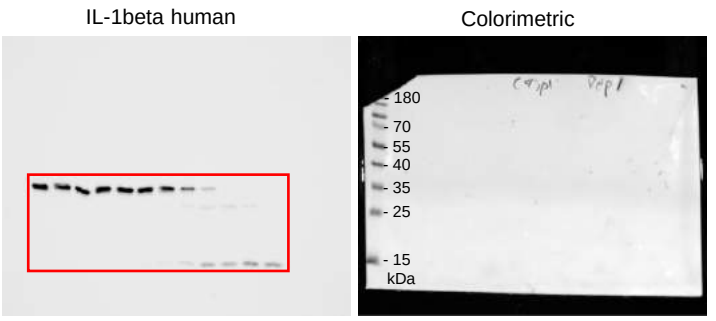
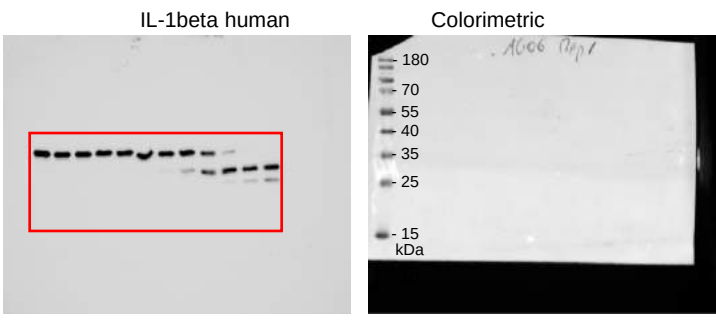


Extended Data Figure 1l

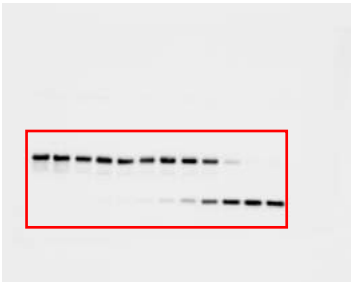


Extended Data Figure 1m

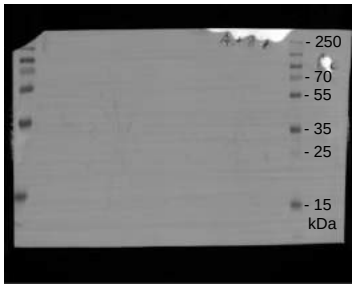


Extended Data Figure 1n

IL-18 murine



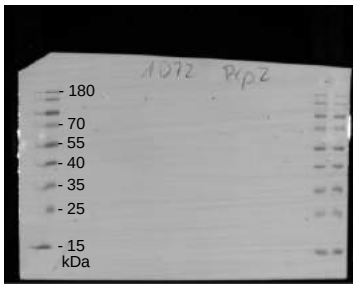
Colorimetric



Extended Data Figure 1o

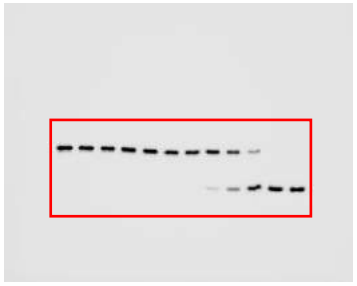
IL-18 murine

Colorimetric

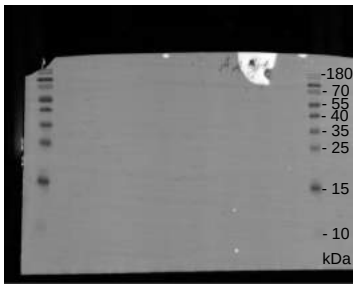


Extended Data Figure 1p

IL-18 murine

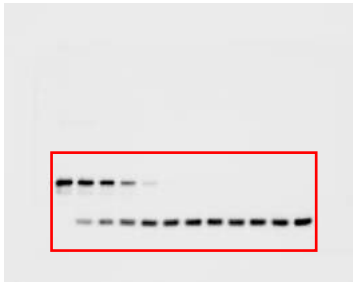


Colorimetric



Extended Data Figure 1q

IL-18 murine



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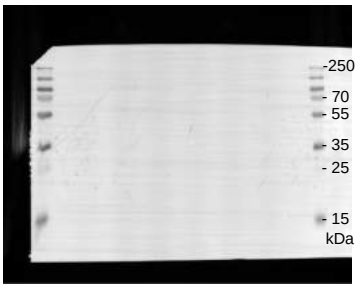
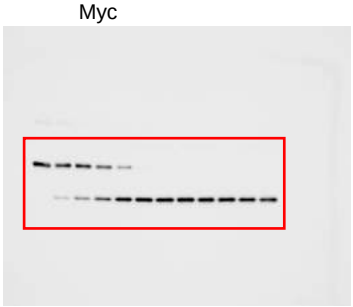


Extended Data Figure 1r

Myc



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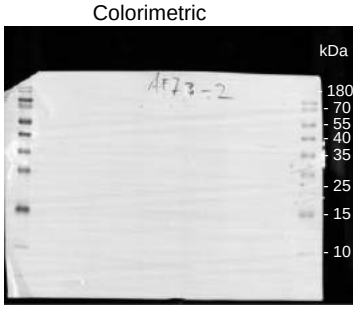
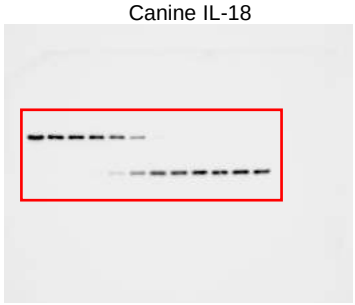
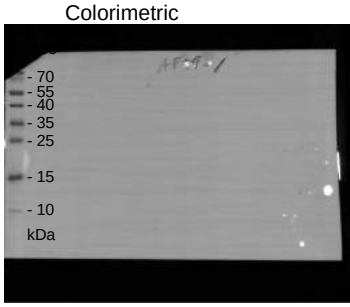


Extended Data Figure 1s

Canine IL-18

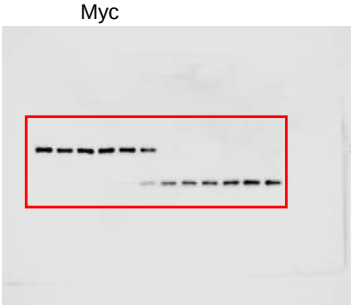


Colorimetric

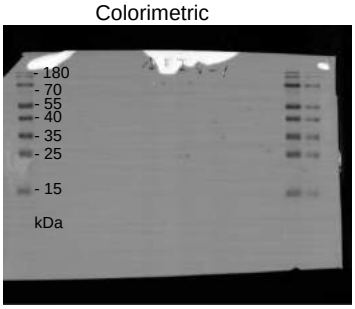


Extended Data Figure 1t

Myc

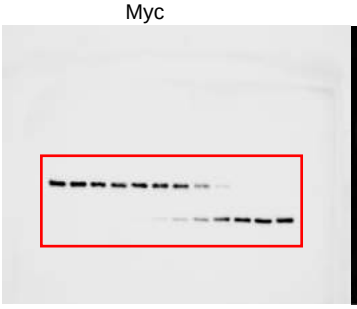


Colorimetric

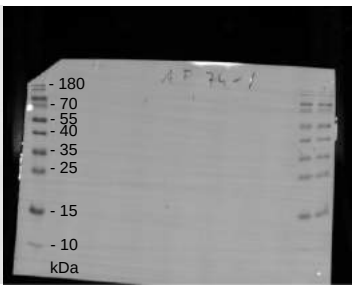


Extended Data Figure 1u

Myc



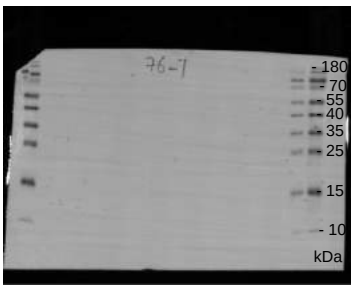
Colorimetric



Extended Data Figure 1v

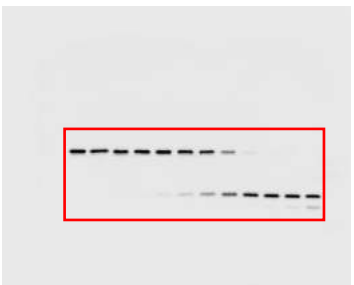
Myc

Colorimetric

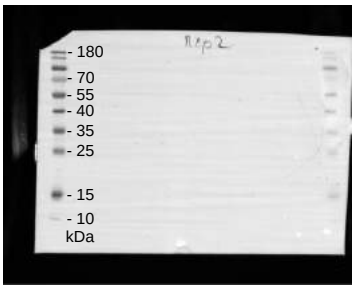


Extended Data Figure 1w

IL-18

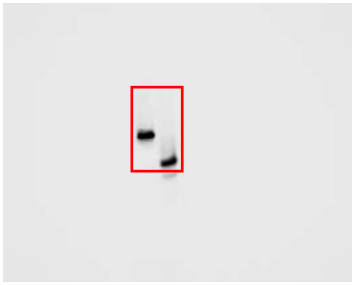


Colorimetric

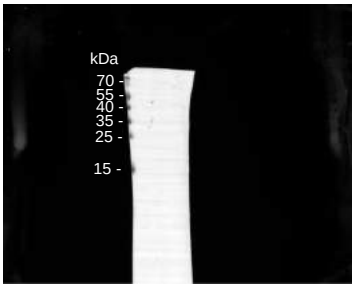


Extended Data Figure 2c

IL-18

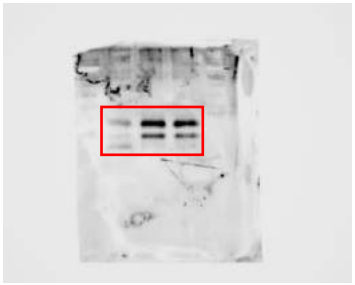


Colorimetric

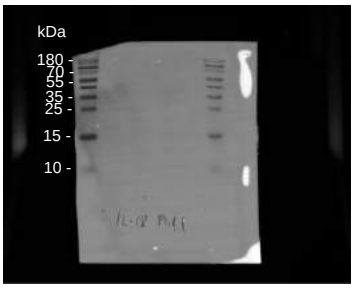


Extended Data Figure 2d

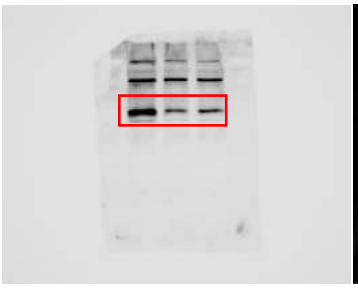
IL-18 pulldown



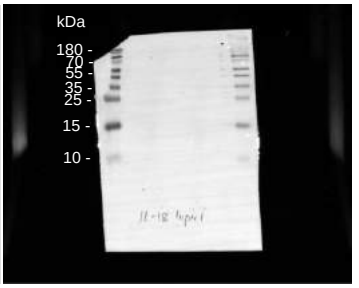
Colorimetric



IL-18 lysate

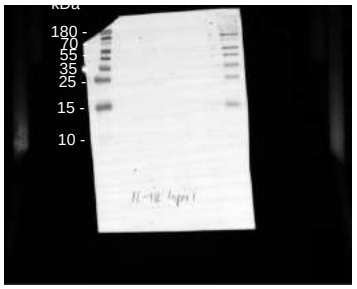


Colorimetric



actin

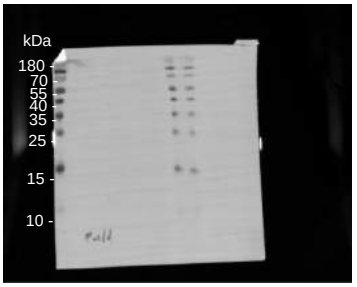
Colorimetric



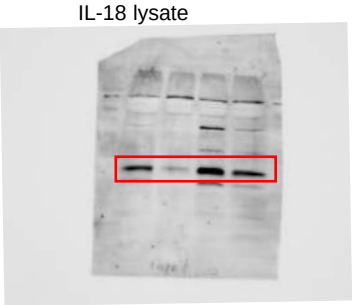
Extended Data Figure 2e

IL-18 pulldown

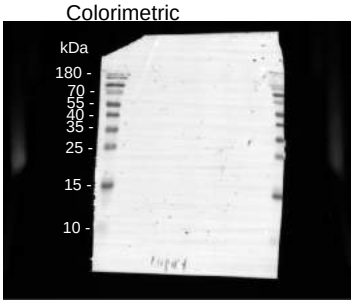
Colorimetric



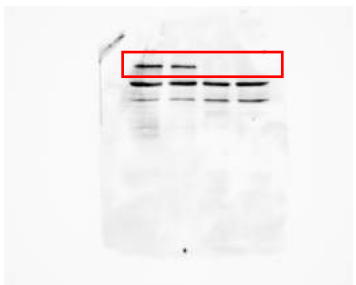
IL-18 lysate



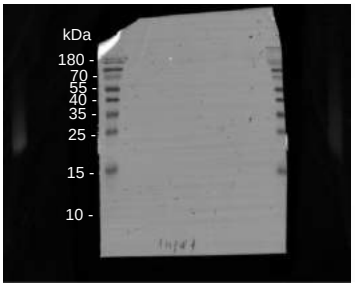
Colorimetric



NLRP3

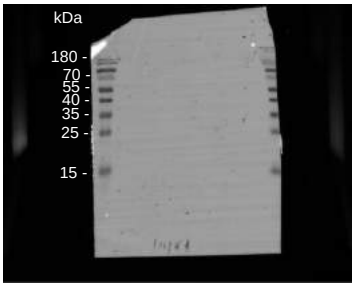


Colorimetric



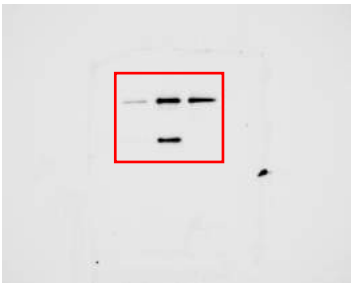
Actin

Colorimetric

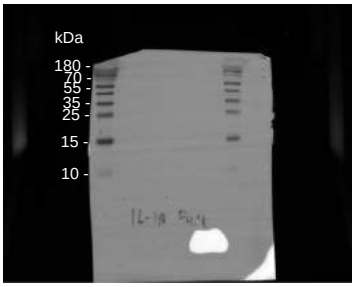


Extended Data Figure 2f

IL-1beta human pulldown

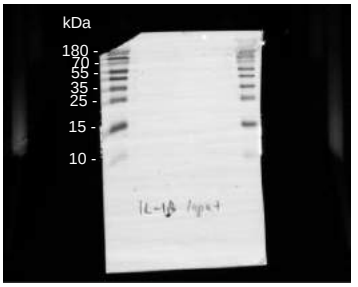


Colorimetric



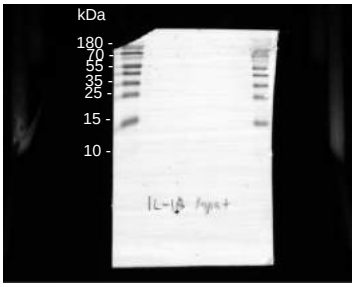
IL-1beta human lysate

Colorimetric



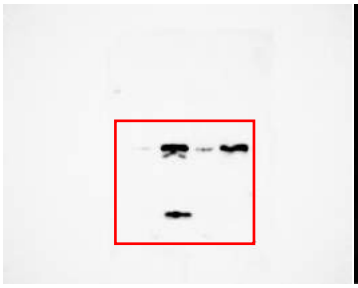
actin

Colorimetric

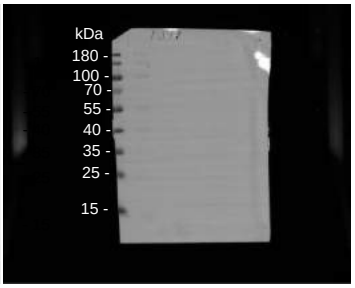


Extended Data Figure 2g

IL-1beta human pulldown

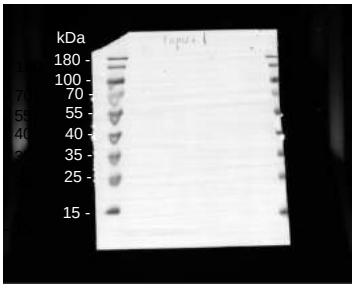


Colorimetric



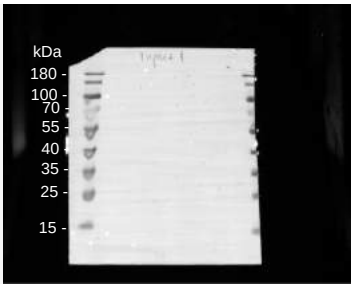
IL-1beta human lysate

Colorimetric



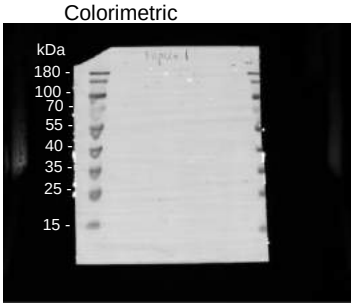
NLRP3

Colorimetric



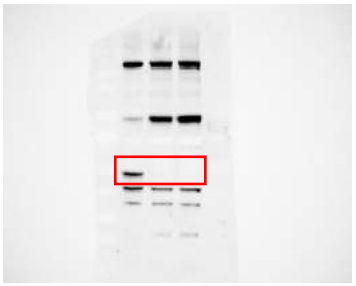
Actin

Colorimetric

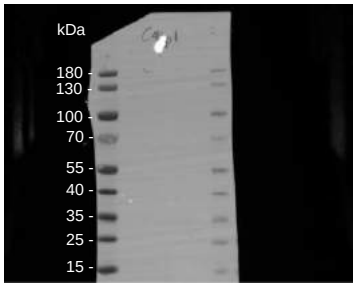


Extended Data Figure 2h

Caspase-1

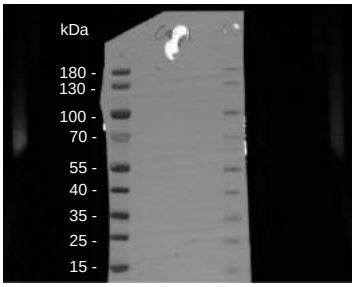


Colorimetric

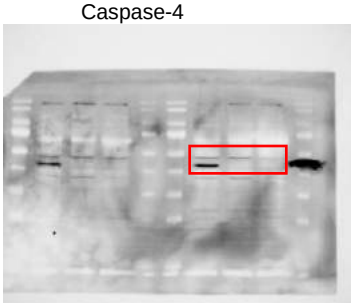


Actin

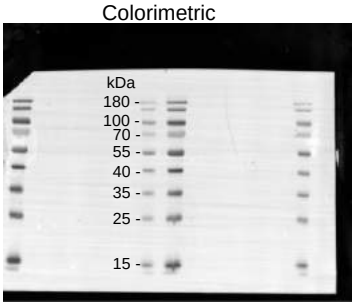
Colorimetric



Caspase-4

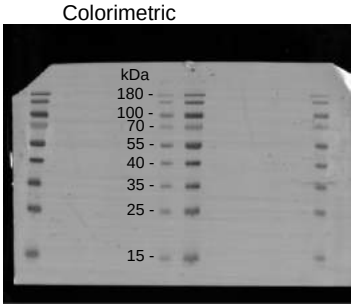


Colorimetric



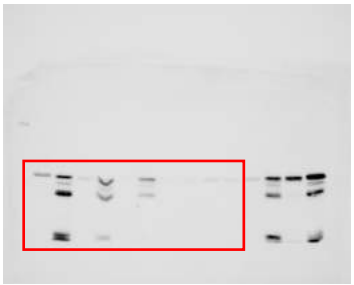
Actin

Colorimetric

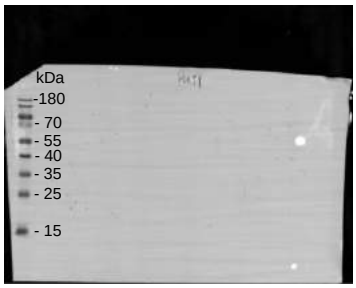


Extended Data Figure 2j

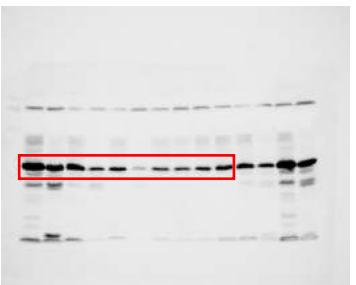
IL-1beta human pulldown



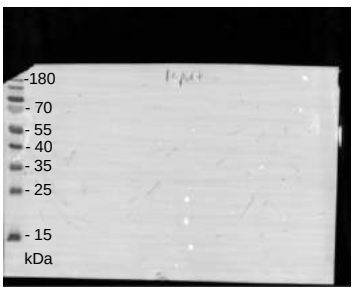
Colorimetric



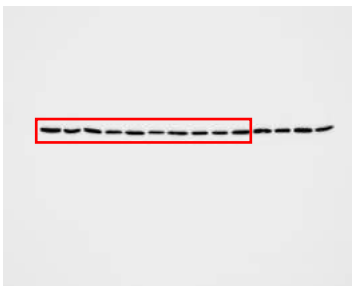
IL-1beta human lysate



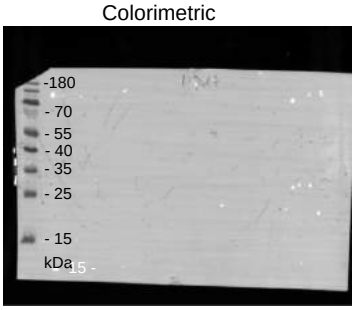
Colorimetric



actin

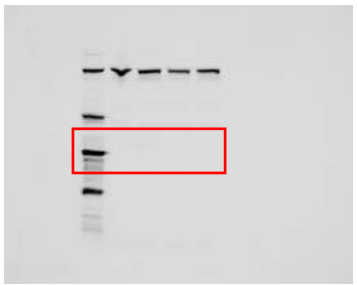


Colorimetric

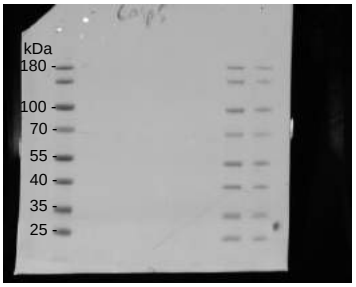


Extended Data Figure 2k

Caspase-5

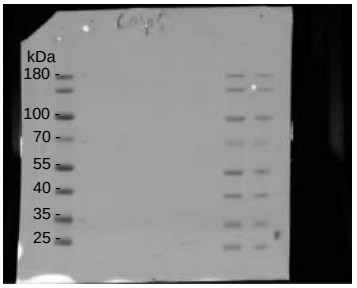


Colorimetric



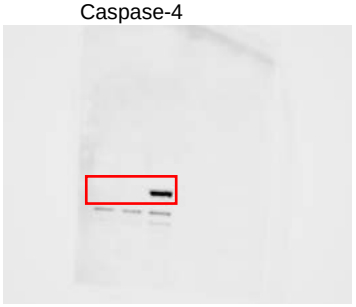
Actin

Colorimetric

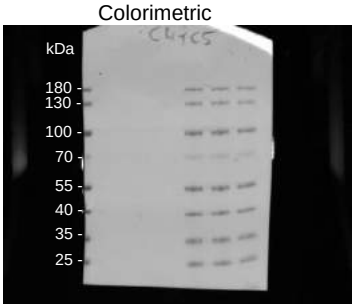


Extended Data Figure 2l

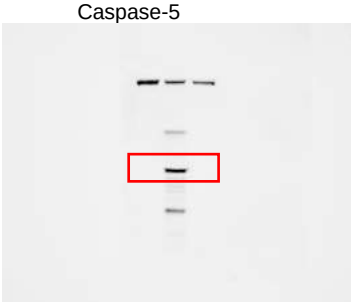
Caspase-4



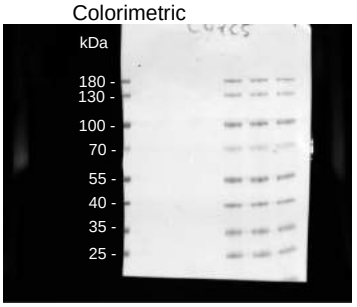
Colorimetric



Caspase-5

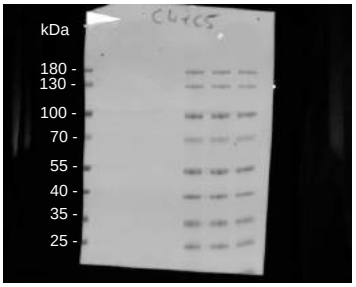


Colorimetric



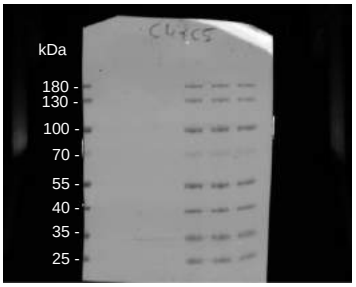
Myc

Colorimetric



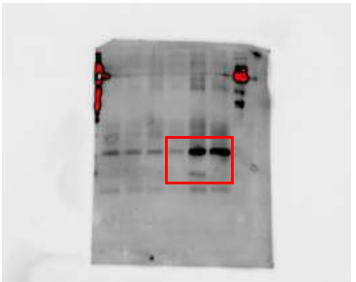
Actin

Colorimetric

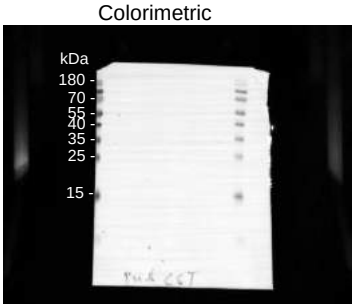


Extended Data Figure 3d

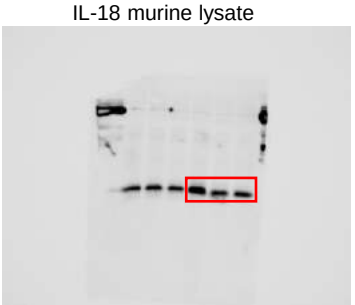
IL-18 murine pulldown



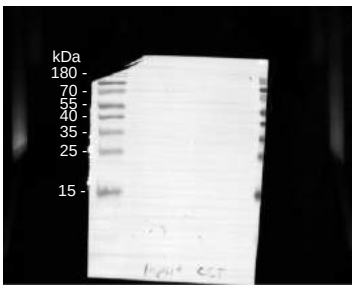
Colorimetric



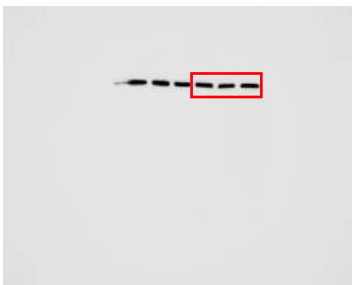
IL-18 murine lysate



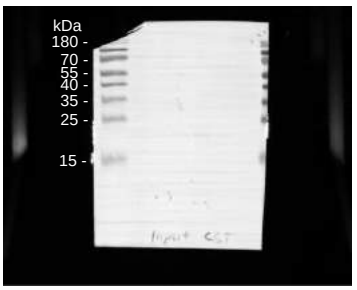
Colorimetric



actin



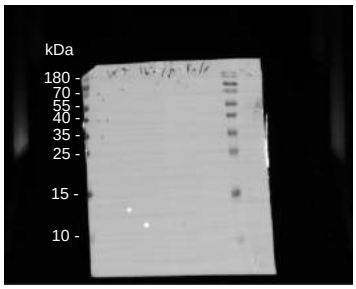
Colorimetric



Extended Data Figure 3e

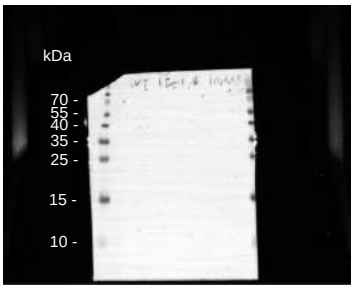
IL-1beta murine pulldown

Colorimetric

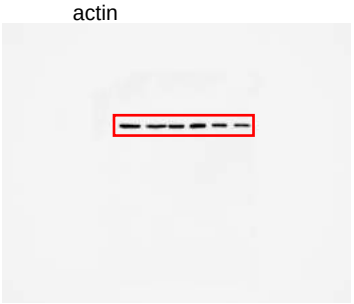


IL-1beta murine lysate

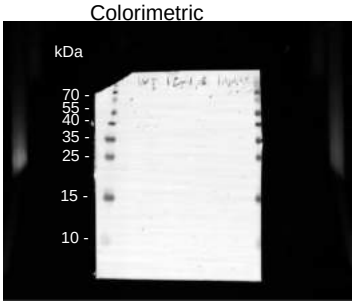
Colorimetric



actin

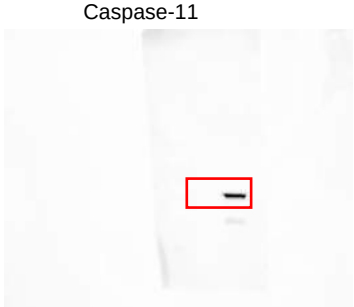


Colorimetric

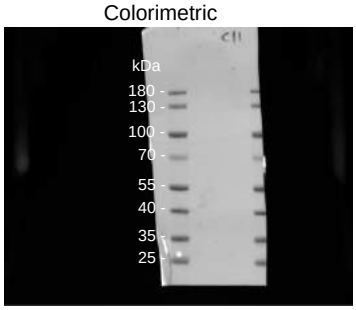


Extended Data Figure 3f

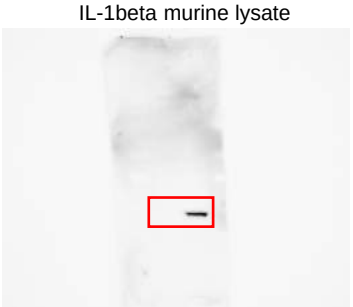
Caspase-11



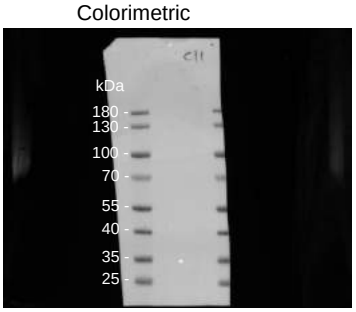
Colorimetric



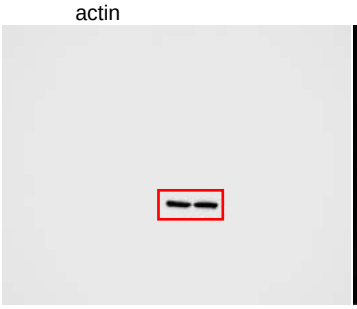
IL-1beta murine lysate



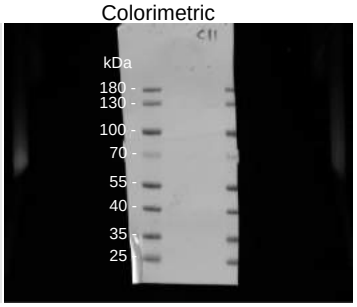
Colorimetric



actin

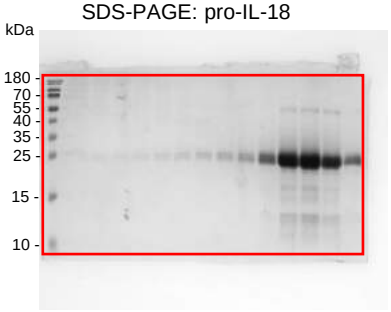


Colorimetric



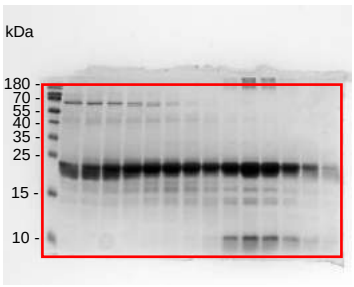
Extended Data Figure 4a

SDS-PAGE: pro-IL-18



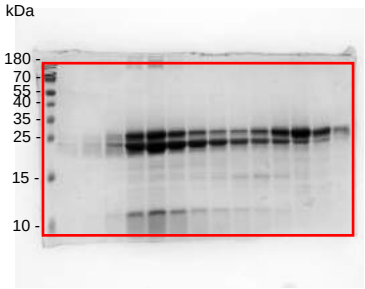
Extended Data Figure 4b

SDS-PAGE: Caspase-4 p20/p10



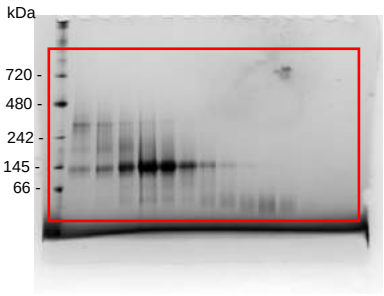
Extended Data Figure 4c

SDS-PAGE: pro-IL-18 + caspase-4



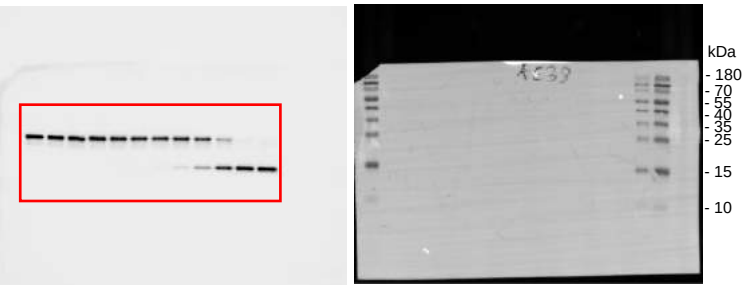
Extended Data Figure 4d

Native PAGE: pro-IL-18 + caspase-4 + BS3

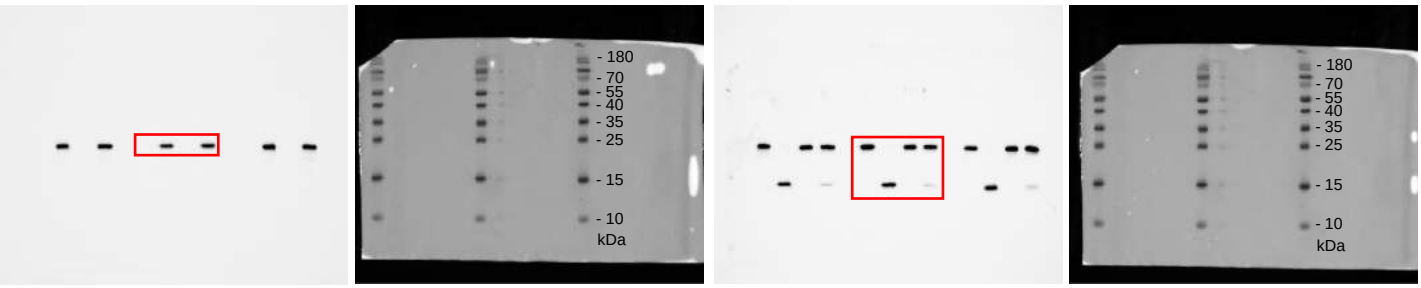


Extended Data Figure 7a

IL-18 Colorimetric

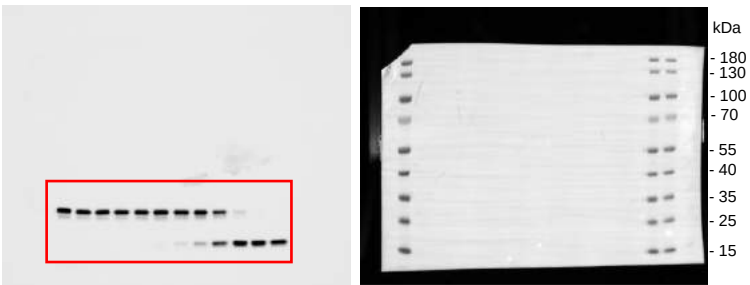


Caspase-4 Colorimetric IL-18 Colorimetric



Extended Data Figure 7c

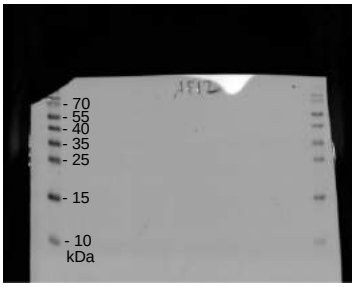
IL-18 Colorimetric



Extended Data Figure 7d

IL-18 murine

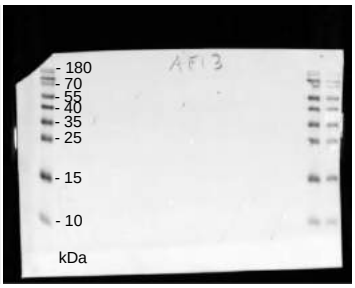
Colorimetric



Extended Data Figure 7f

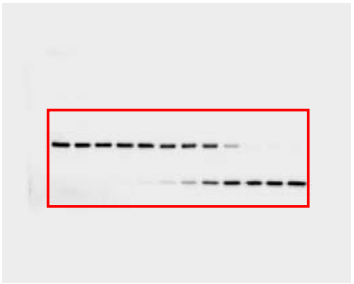
IL-18 murine

Colorimetric

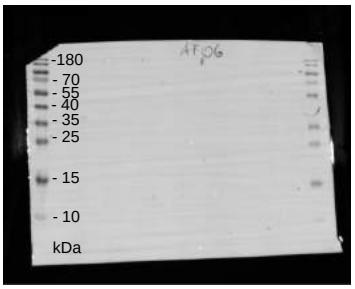


Extended Data Figure 7e

IL-18 murine

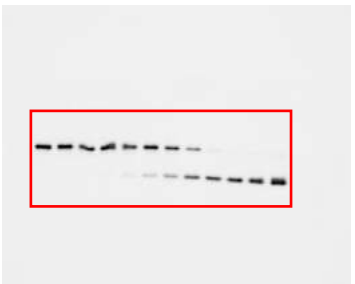


Colorimetric

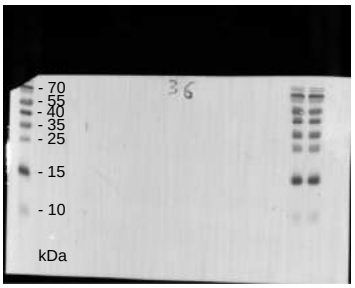


Extended Data Figure 7h

IL-18 murine

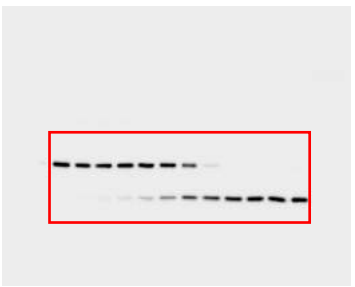


Colorimetric

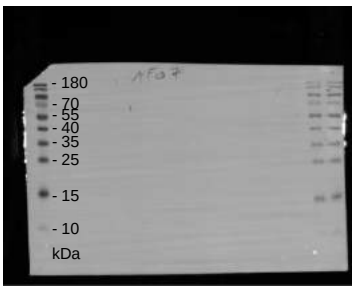


Extended Data Figure 7g

IL-18 murine

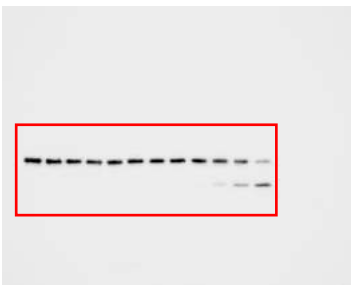


Colorimetric

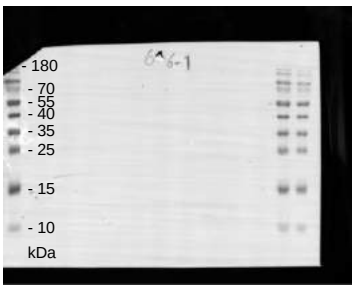


Extended Data Figure 7j

IL-18 murine

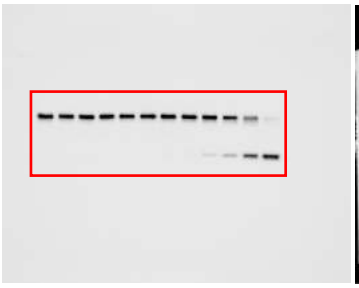


Colorimetric

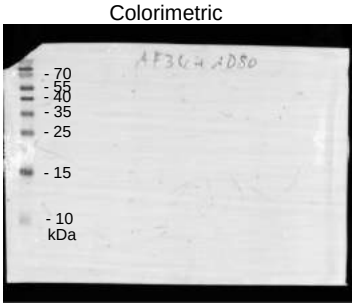


Extended Data Figure 8c

IL-18 human



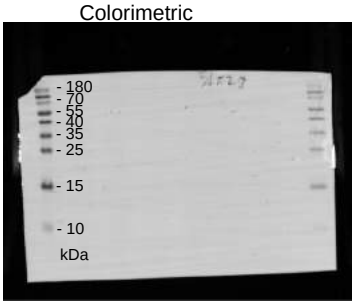
Colorimetric



Extended Data Figure 8d

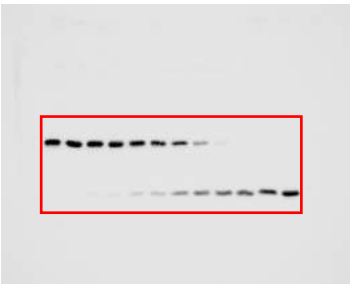
IL-18 human

Colorimetric

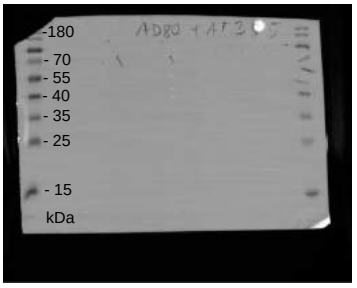


Extended Data Figure 8e

IL-18 human

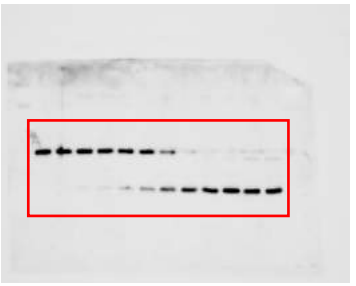


Colorimetric

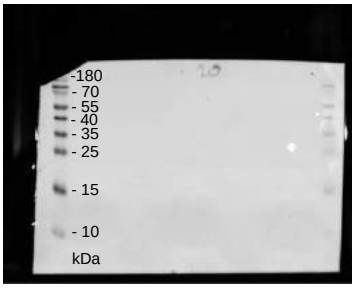


Extended Data Figure 8h

IL-18 human

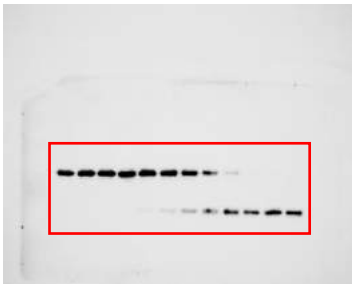


Colorimetric

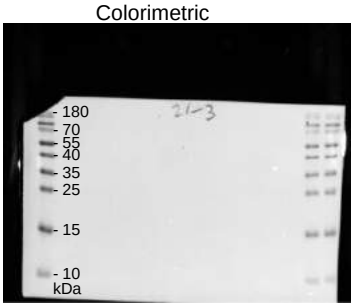


Extended Data Figure 8i

IL-18 human

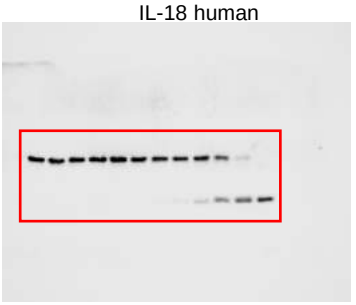


Colorimetric

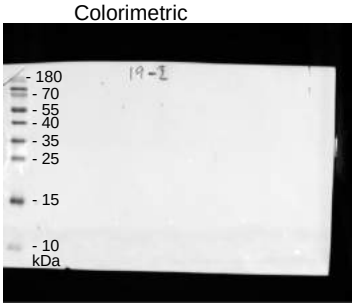


Extended Data Figure 8k

IL-18 human

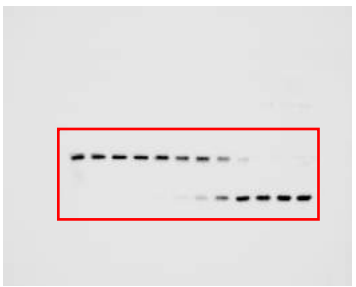


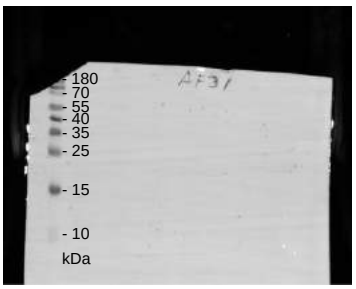
Colorimetric



Extended Data Figure 8j

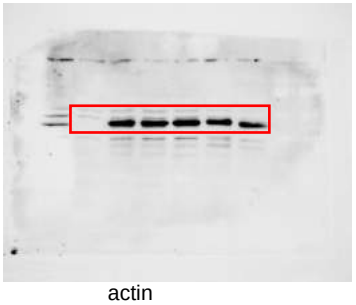
IL-18 human



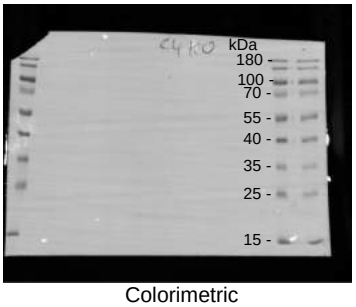


Extended Data Fig 9a

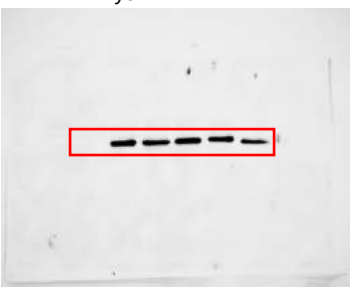
Caspase-4



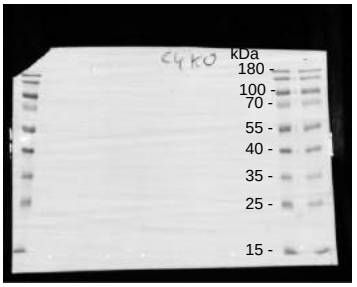
Colorimetric



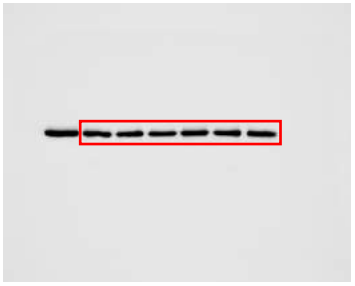
Myc



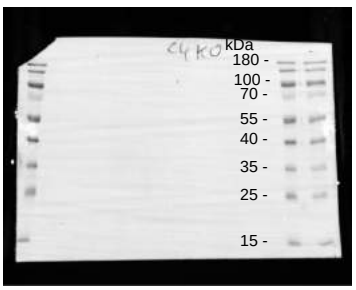
Colorimetric



actin

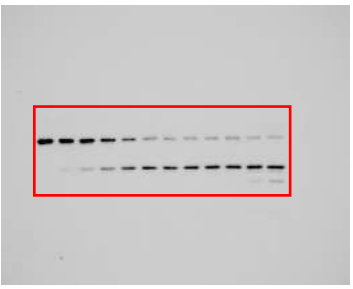


Colorimetric

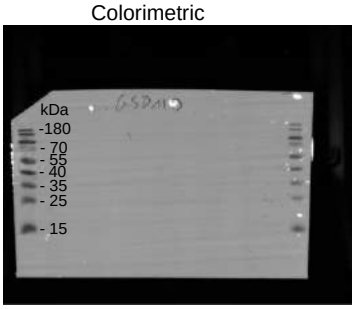


Extended Data Figure 9c

GSDMD

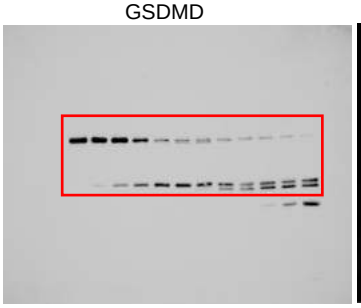


Colorimetric

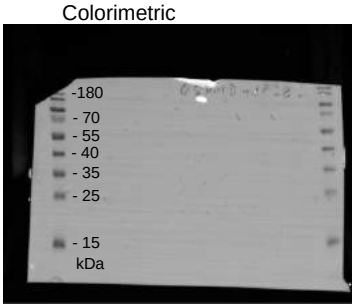


Extended Data Figure 9d

GSDMD



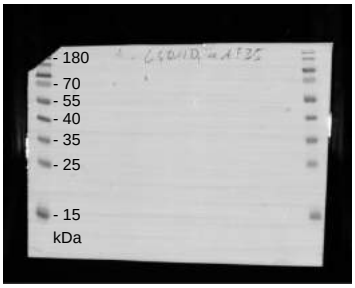
Colorimetric



Extended Data Figure 9e

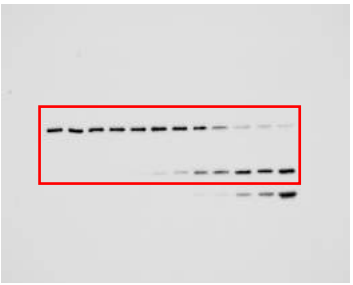
GSDMD

Colorimetric

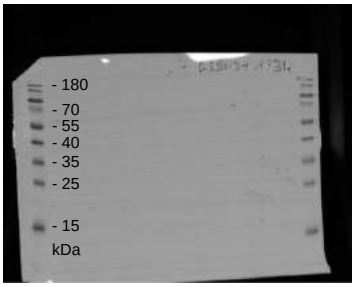


Extended Data Figure 9f

GSDMD

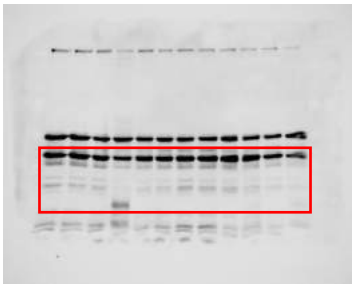


Colorimetric

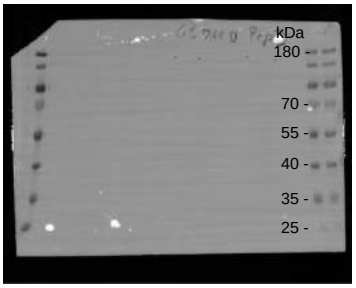


Extended Data Fig 9g

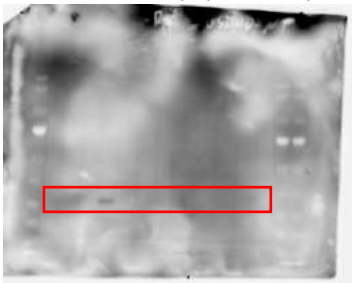
GSDMD



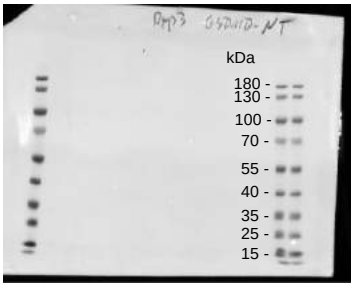
Colorimetric



NT-GSDMD (separate blot)

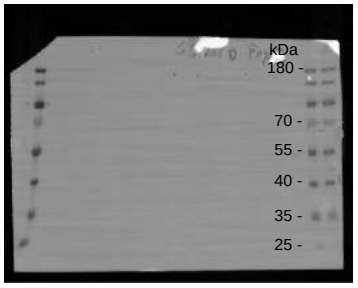


Colorimetric



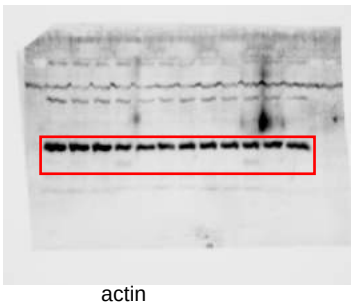
actin

Colorimetric

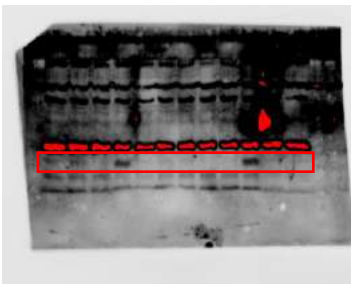


Extended Data Fig 9h

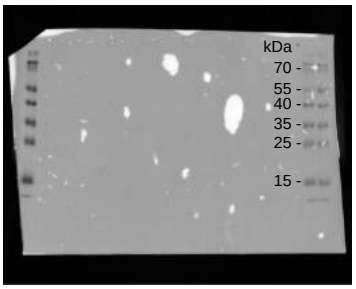
IL-18 short exposure



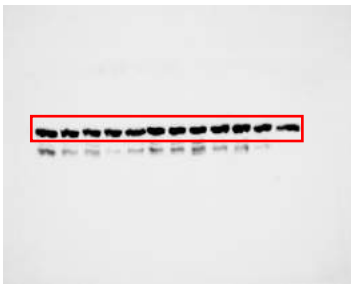
IL-18 long exposure



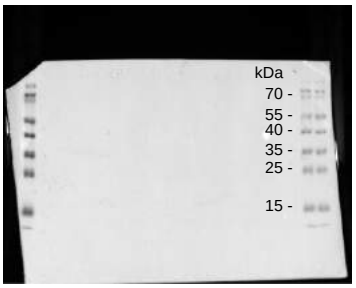
Colorimetric



actin



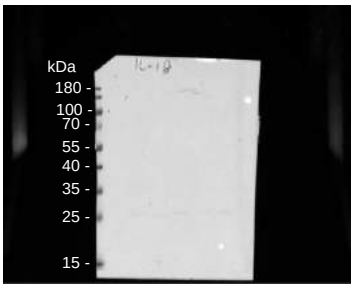
Colorimetric



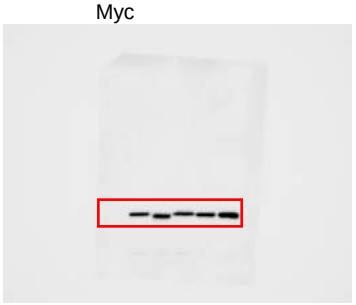
Extended Data Fig 9i

IL-18

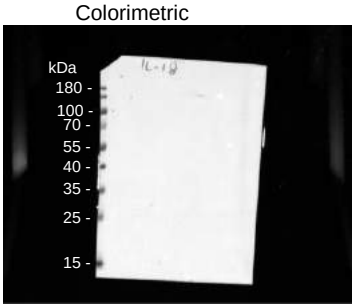
Colorimetric



Myc

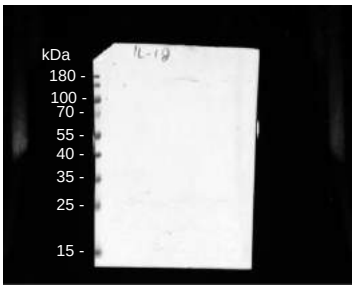


Colorimetric

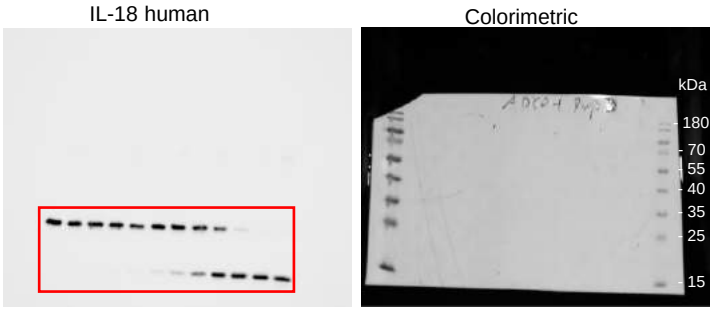


actin

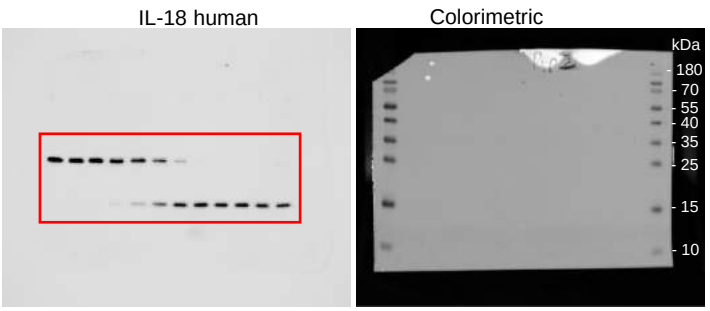
Colorimetric



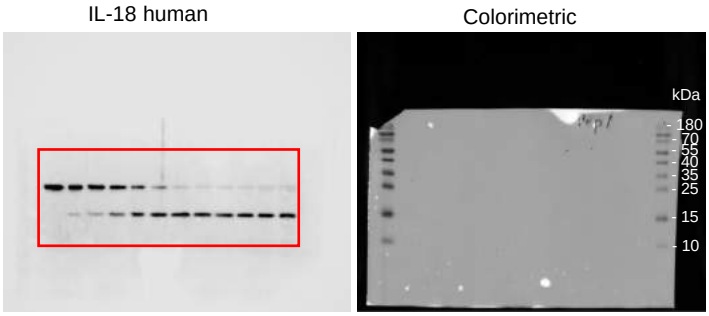
Extended Data Figure 10a



Extended Data Figure 10b



Extended Data Figure 10c



Full wwPDB EM Validation Report ⓘ

May 4, 2023 – 12:17 PM EDT

PDB ID	:	8SPB
EMDB ID	:	EMD-40678
Title	:	Caspase-4/Pro-IL-18 complex
Deposited on	:	2023-05-02
Resolution	:	3.20 Å(reported)

This wwPDB validation report is for manuscript review

This is a Full wwPDB EM Validation Report.

This report is produced by the wwPDB biocuration pipeline after annotation of the structure.

We welcome your comments at *validation@mail.wwpdb.org*
A user guide is available at
https://www.wwpdb.org/validation/2017/EMValidationReportHelp
with specific help available everywhere you see the ⓘ symbol.

The types of validation reports are described at
http://www.wwpdb.org/validation/2017/FAQs#types.

The following versions of software and data (see references ⓘ) were used in the production of this report:

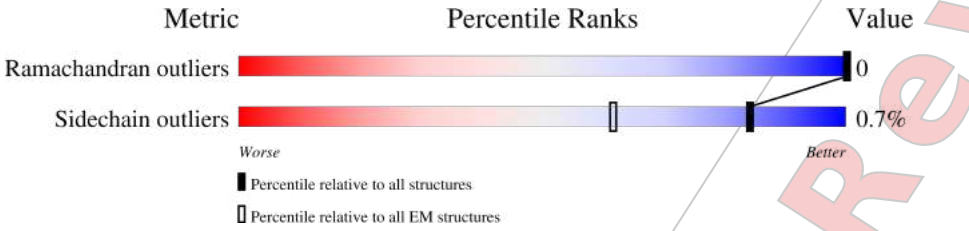
EMDB validation analysis	:	0.0.1.dev50
MolProbity	:	4.02b-467
Percentile statistics	:	20191225.v01 (using entries in the PDB archive December 25th 2019)
MapQ	:	1.9.9
Ideal geometry (proteins)	:	Engh & Huber (2001)
Ideal geometry (DNA, RNA)	:	Parkinson et al. (1996)
Validation Pipeline (wwPDB-VP)	:	2.32.2

1 Overall quality at a glance (i)

The following experimental techniques were used to determine the structure:
ELECTRON MICROSCOPY

The reported resolution of this entry is 3.20 Å.

Percentile scores (ranging between 0-100) for global validation metrics of the entry are shown in the following graphic. The table shows the number of entries on which the scores are based.



Metric	Whole archive (#Entries)	EM structures (#Entries)
Ramachandran outliers	154571	4023
Sidechain outliers	154315	3826

The table below summarises the geometric issues observed across the polymeric chains and their fit to the map. The red, orange, yellow and green segments of the bar indicate the fraction of residues that contain outliers for >=3, 2, 1 and 0 types of geometric quality criteria respectively. A grey segment represents the fraction of residues that are not modelled. The numeric value for each fraction is indicated below the corresponding segment, with a dot representing fractions <=5% The upper red bar (where present) indicates the fraction of residues that have poor fit to the EM map (all-atom inclusion < 40%). The numeric value is given above the bar.

Mol	Chain	Length	Quality of chain
1	A	207	80% 20%
1	a	207	80% 20%
2	C	220	8% 69% 30%
2	c	220	8% 69% 30%
3	B	88	100%
3	b	88	99%

2 Entry composition (i)

There are 3 unique types of molecules in this entry. The entry contains 6636 atoms, of which 0 are hydrogens and 0 are deuteriums.

In the tables below, the AltConf column contains the number of residues with at least one atom in alternate conformation and the Trace column contains the number of residues modelled with at most 2 atoms.

- Molecule 1 is a protein called Caspase-4 subunit p20.

Mol	Chain	Residues	Atoms					AltConf	Trace
1	A	166	Total	C	N	O	S	0	0
			1332	836	237	251	8		
1	a	166	Total	C	N	O	S	0	0
			1332	836	237	251	8		

There are 62 discrepancies between the modelled and reference sequences:

Chain	Residue	Modelled	Actual	Comment	Reference
A	64	MET	-	expression tag	UNP P49662
A	65	GLY	-	expression tag	UNP P49662
A	66	SER	-	expression tag	UNP P49662
A	67	SER	-	expression tag	UNP P49662
A	68	HIS	-	expression tag	UNP P49662
A	69	HIS	-	expression tag	UNP P49662
A	70	HIS	-	expression tag	UNP P49662
A	71	HIS	-	expression tag	UNP P49662
A	72	HIS	-	expression tag	UNP P49662
A	73	HIS	-	expression tag	UNP P49662
A	74	SER	-	expression tag	UNP P49662
A	75	SER	-	expression tag	UNP P49662
A	76	GLY	-	expression tag	UNP P49662
A	77	LEU	-	expression tag	UNP P49662
A	78	VAL	-	expression tag	UNP P49662
A	79	PRO	-	expression tag	UNP P49662
A	80	ARG	-	expression tag	UNP P49662
A	81	GLY	-	expression tag	UNP P49662
A	82	SER	-	expression tag	UNP P49662
A	83	HIS	-	expression tag	UNP P49662
A	84	MET	-	expression tag	UNP P49662
A	85	GLU	-	expression tag	UNP P49662
A	86	ASN	-	expression tag	UNP P49662
A	87	LEU	-	expression tag	UNP P49662
A	88	TYR	-	expression tag	UNP P49662
A	89	PHE	-	expression tag	UNP P49662

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Chain	Residue	Modelled	Actual	Comment	Reference
A	90	GLN	-	expression tag	UNP P49662
A	91	GLY	-	expression tag	UNP P49662
A	92	GLY	-	expression tag	UNP P49662
A	93	SER	-	expression tag	UNP P49662
A	258	ALA	CYS	conflict	UNP P49662
a	64	MET	-	expression tag	UNP P49662
a	65	GLY	-	expression tag	UNP P49662
a	66	SER	-	expression tag	UNP P49662
a	67	SER	-	expression tag	UNP P49662
a	68	HIS	-	expression tag	UNP P49662
a	69	HIS	-	expression tag	UNP P49662
a	70	HIS	-	expression tag	UNP P49662
a	71	HIS	-	expression tag	UNP P49662
a	72	HIS	-	expression tag	UNP P49662
a	73	HIS	-	expression tag	UNP P49662
a	74	SER	-	expression tag	UNP P49662
a	75	SER	-	expression tag	UNP P49662
a	76	GLY	-	expression tag	UNP P49662
a	77	LEU	-	expression tag	UNP P49662
a	78	VAL	-	expression tag	UNP P49662
a	79	PRO	-	expression tag	UNP P49662
a	80	ARG	-	expression tag	UNP P49662
a	81	GLY	-	expression tag	UNP P49662
a	82	SER	-	expression tag	UNP P49662
a	83	HIS	-	expression tag	UNP P49662
a	84	MET	-	expression tag	UNP P49662
a	85	GLU	-	expression tag	UNP P49662
a	86	ASN	-	expression tag	UNP P49662
a	87	LEU	-	expression tag	UNP P49662
a	88	TYR	-	expression tag	UNP P49662
a	89	PHE	-	expression tag	UNP P49662
a	90	GLN	-	expression tag	UNP P49662
a	91	GLY	-	expression tag	UNP P49662
a	92	GLY	-	expression tag	UNP P49662
a	93	SER	-	expression tag	UNP P49662
a	258	ALA	CYS	conflict	UNP P49662

- Molecule 2 is a protein called Interleukin-18.

Mol	Chain	Residues	Atoms					AltConf	Trace
			Total	C	N	O	S		
2	C	154	1255	797	198	250	10	0	0

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Mol	Chain	Residues	Atoms					AltConf	Trace
			Total	C	N	O	S		
2	c	154	1255	797	198	250	10	0	0

There are 56 discrepancies between the modelled and reference sequences:

Chain	Residue	Modelled	Actual	Comment	Reference
C	-26	MET	-	expression tag	UNP Q14116
C	-25	SER	-	expression tag	UNP Q14116
C	-24	TYR	-	expression tag	UNP Q14116
C	-23	TYR	-	expression tag	UNP Q14116
C	-22	HIS	-	expression tag	UNP Q14116
C	-21	HIS	-	expression tag	UNP Q14116
C	-20	HIS	-	expression tag	UNP Q14116
C	-19	HIS	-	expression tag	UNP Q14116
C	-18	HIS	-	expression tag	UNP Q14116
C	-17	HIS	-	expression tag	UNP Q14116
C	-16	ASP	-	expression tag	UNP Q14116
C	-15	TYR	-	expression tag	UNP Q14116
C	-14	ASP	-	expression tag	UNP Q14116
C	-13	ILE	-	expression tag	UNP Q14116
C	-12	PRO	-	expression tag	UNP Q14116
C	-11	THR	-	expression tag	UNP Q14116
C	-10	THR	-	expression tag	UNP Q14116
C	-9	GLU	-	expression tag	UNP Q14116
C	-8	ASN	-	expression tag	UNP Q14116
C	-7	LEU	-	expression tag	UNP Q14116
C	-6	TYR	-	expression tag	UNP Q14116
C	-5	PHE	-	expression tag	UNP Q14116
C	-4	GLN	-	expression tag	UNP Q14116
C	-3	GLY	-	expression tag	UNP Q14116
C	-2	ALA	-	expression tag	UNP Q14116
C	-1	MET	-	expression tag	UNP Q14116
C	0	GLY	-	expression tag	UNP Q14116
C	1	SER	-	expression tag	UNP Q14116
c	-26	MET	-	expression tag	UNP Q14116
c	-25	SER	-	expression tag	UNP Q14116
c	-24	TYR	-	expression tag	UNP Q14116
c	-23	TYR	-	expression tag	UNP Q14116
c	-22	HIS	-	expression tag	UNP Q14116
c	-21	HIS	-	expression tag	UNP Q14116
c	-20	HIS	-	expression tag	UNP Q14116
c	-19	HIS	-	expression tag	UNP Q14116

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Chain	Residue	Modelled	Actual	Comment	Reference
c	-18	HIS	-	expression tag	UNP Q14116
c	-17	HIS	-	expression tag	UNP Q14116
c	-16	ASP	-	expression tag	UNP Q14116
c	-15	TYR	-	expression tag	UNP Q14116
c	-14	ASP	-	expression tag	UNP Q14116
c	-13	ILE	-	expression tag	UNP Q14116
c	-12	PRO	-	expression tag	UNP Q14116
c	-11	THR	-	expression tag	UNP Q14116
c	-10	THR	-	expression tag	UNP Q14116
c	-9	GLU	-	expression tag	UNP Q14116
c	-8	ASN	-	expression tag	UNP Q14116
c	-7	LEU	-	expression tag	UNP Q14116
c	-6	TYR	-	expression tag	UNP Q14116
c	-5	PHE	-	expression tag	UNP Q14116
c	-4	GLN	-	expression tag	UNP Q14116
c	-3	GLY	-	expression tag	UNP Q14116
c	-2	ALA	-	expression tag	UNP Q14116
c	-1	MET	-	expression tag	UNP Q14116
c	0	GLY	-	expression tag	UNP Q14116
c	1	SER	-	expression tag	UNP Q14116

- Molecule 3 is a protein called Caspase-4 subunit p10.

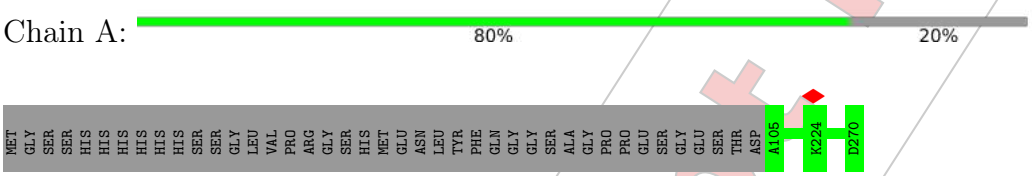
Mol	Chain	Residues	Atoms					AltConf	Trace
3	B	88	Total	C	N	O	S	0	0
			731	472	123	129	7		
3	b	88	Total	C	N	O	S	0	0
			731	472	123	129	7		

3 Residue-property plots (i)

These plots are drawn for all protein, RNA, DNA and oligosaccharide chains in the entry. The first graphic for a chain summarises the proportions of the various outlier classes displayed in the second graphic. The second graphic shows the sequence view annotated by issues in geometry and atom inclusion in map density. Residues are color-coded according to the number of geometric quality criteria for which they contain at least one outlier: green = 0, yellow = 1, orange = 2 and red = 3 or more. A red diamond above a residue indicates a poor fit to the EM map for this residue (all-atom inclusion < 40%). Stretches of 2 or more consecutive residues without any outlier are shown as a green connector. Residues present in the sample, but not in the model, are shown in grey.

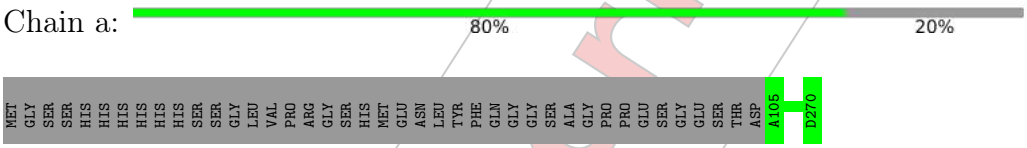
- Molecule 1: Caspase-4 subunit p20

Chain A:



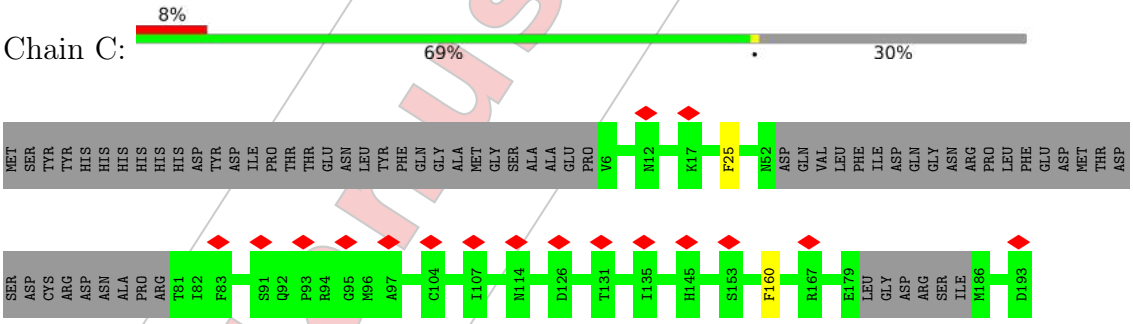
- Molecule 1: Caspase-4 subunit p20

Chain a:



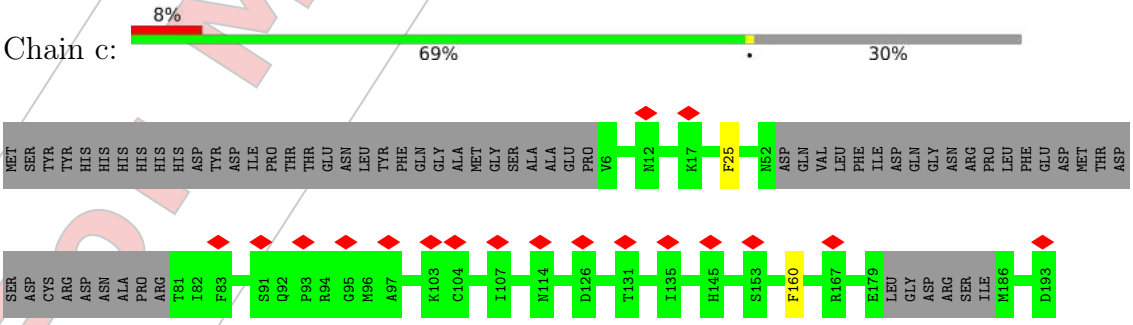
- Molecule 2: Interleukin-18

Chain C:



- Molecule 2: Interleukin-18

Chain c:



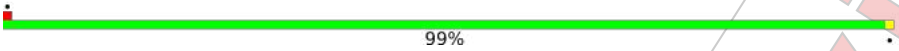
- Molecule 3: Caspase-4 subunit p10

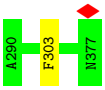
Chain B:

There are no outlier residues recorded for this chain.

- Molecule 3: Caspase-4 subunit p10

Chain b:





4 Experimental information (i)

Property	Value	Source
EM reconstruction method	SINGLE PARTICLE	Depositor
Imposed symmetry	POINT, Not provided	
Number of particles used	200000	Depositor
Resolution determination method	FSC 0.143 CUT-OFF	Depositor
CTF correction method	NONE	Depositor
Microscope	FEI TITAN KRIOS	Depositor
Voltage (kV)	300	Depositor
Electron dose ($e^-/Å^2$)	55	Depositor
Minimum defocus (nm)	1000	Depositor
Maximum defocus (nm)	2500	Depositor
Magnification	Not provided	
Image detector	GATAN K3 (6k x 4k)	Depositor
Maximum map value	3.493	Depositor
Minimum map value	-2.387	Depositor
Average map value	-0.000	Depositor
Map value standard deviation	0.065	Depositor
Recommended contour level	0.15	Depositor
Map size (Å)	222.44, 222.44, 222.44	wwPDB
Map dimensions	268, 268, 268	wwPDB
Map angles (°)	90.0, 90.0, 90.0	wwPDB
Pixel spacing (Å)	0.83, 0.83, 0.83	Depositor

5 Model quality

5.1 Standard geometry

The Z score for a bond length (or angle) is the number of standard deviations the observed value is removed from the expected value. A bond length (or angle) with $|Z| > 5$ is considered an outlier worth inspection. RMSZ is the root-mean-square of all Z scores of the bond lengths (or angles).

Mol	Chain	Bond lengths		Bond angles	
		RMSZ	#$\|Z\|>5$	RMSZ	#$\|Z\|>5$
1	A	0.27	0/1355	0.56	0/1828
1	a	0.26	0/1355	0.52	0/1828
2	C	0.25	0/1274	0.55	0/1706
2	c	0.25	0/1274	0.55	0/1706
3	B	0.27	0/753	0.51	0/1018
3	b	0.29	0/753	0.53	0/1018
All	All	0.26	0/6764	0.54	0/9104

There are no bond length outliers.

There are no bond angle outliers.

There are no chirality outliers.

There are no planarity outliers.

5.2 Too-close contacts

Due to software issues we are unable to calculate clashes - this section is therefore empty.

5.3 Torsion angles

5.3.1 Protein backbone

In the following table, the Percentiles column shows the percent Ramachandran outliers of the chain as a percentile score with respect to all PDB entries followed by that with respect to all EM entries.

The Analysed column shows the number of residues for which the backbone conformation was analysed, and the total number of residues.

Mol	Chain	Analysed	Favoured	Allowed	Outliers	Percentiles	
1	A	164/207 (79%)	160 (98%)	4 (2%)	0	100	100

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Mol	Chain	Analysed	Favoured	Allowed	Outliers	Percentiles	
1	a	164/207 (79%)	157 (96%)	7 (4%)	0	100	100
2	C	148/220 (67%)	129 (87%)	19 (13%)	0	100	100
2	c	148/220 (67%)	129 (87%)	19 (13%)	0	100	100
3	B	86/88 (98%)	83 (96%)	3 (4%)	0	100	100
3	b	86/88 (98%)	81 (94%)	5 (6%)	0	100	100
All	All	796/1030 (77%)	739 (93%)	57 (7%)	0	100	100

There are no Ramachandran outliers to report.

5.3.2 Protein sidechains (i)

In the following table, the Percentiles column shows the percent sidechain outliers of the chain as a percentile score with respect to all PDB entries followed by that with respect to all EM entries.

The Analysed column shows the number of residues for which the sidechain conformation was analysed, and the total number of residues.

Mol	Chain	Analysed	Rotameric	Outliers	Percentiles	
1	A	147/180 (82%)	147 (100%)	0	100	100
1	a	147/180 (82%)	147 (100%)	0	100	100
2	C	146/204 (72%)	144 (99%)	2 (1%)	67	86
2	c	146/204 (72%)	144 (99%)	2 (1%)	67	86
3	B	82/82 (100%)	82 (100%)	0	100	100
3	b	82/82 (100%)	81 (99%)	1 (1%)	71	88
All	All	750/932 (80%)	745 (99%)	5 (1%)	84	94

All (5) residues with a non-rotameric sidechain are listed below:

Mol	Chain	Res	Type
2	C	25	PHE
2	C	160	PHE
3	b	303	PHE
2	c	25	PHE
2	c	160	PHE

Sometimes sidechains can be flipped to improve hydrogen bonding and reduce clashes. There are no such sidechains identified.

5.3.3 RNA

There are no RNA molecules in this entry.

5.4 Non-standard residues in protein, DNA, RNA chains

There are no non-standard protein/DNA/RNA residues in this entry.

5.5 Carbohydrates

There are no monosaccharides in this entry.

5.6 Ligand geometry

There are no ligands in this entry.

5.7 Other polymers

There are no such residues in this entry.

5.8 Polymer linkage issues

There are no chain breaks in this entry.

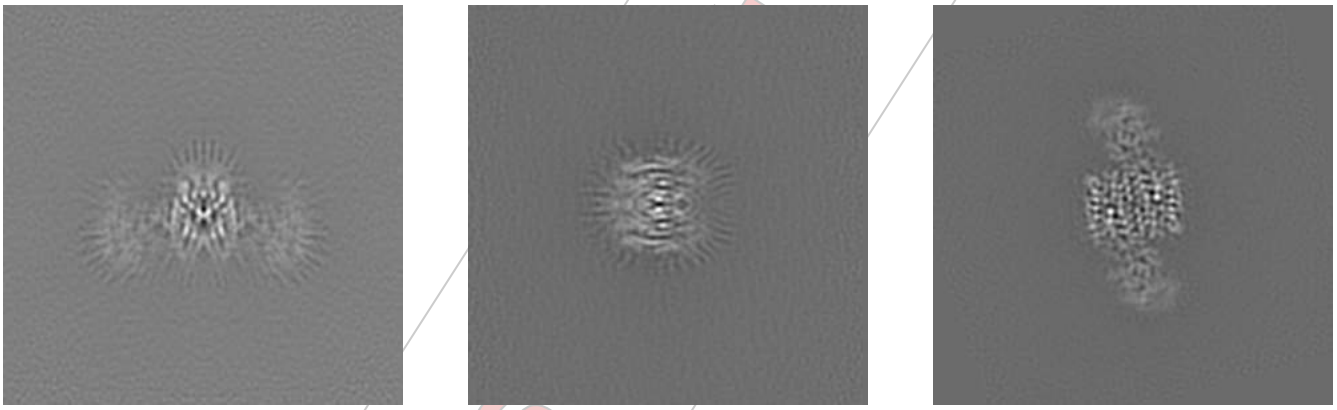
6 Map visualisation

This section contains visualisations of the EMDB entry EMD-40678. These allow visual inspection of the internal detail of the map and identification of artifacts.

Images derived from a raw map, generated by summing the deposited half-maps, are presented below the corresponding image components of the primary map to allow further visual inspection and comparison with those of the primary map.

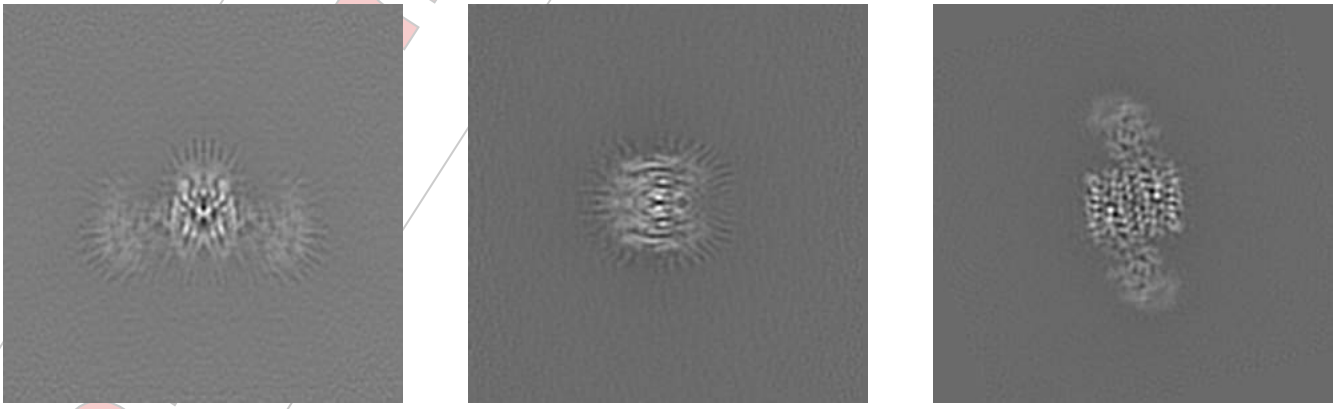
6.1 Orthogonal projections

6.1.1 Primary map



X Y Z

6.1.2 Raw map

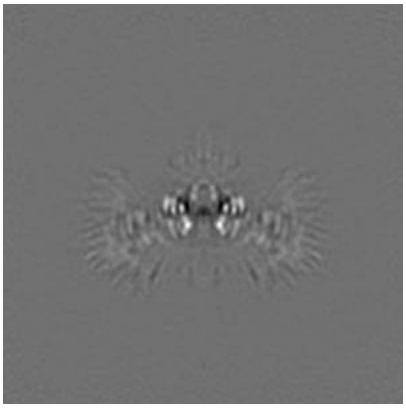


X Y Z

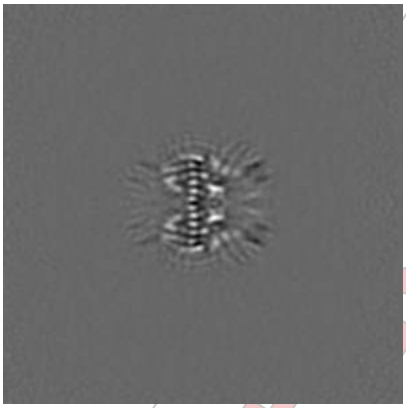
The images above show the map projected in three orthogonal directions.

6.2 Central slices (i)

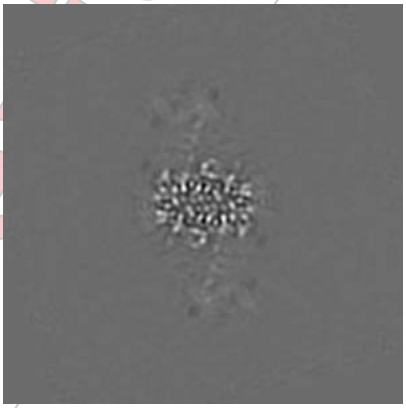
6.2.1 Primary map



X Index: 134

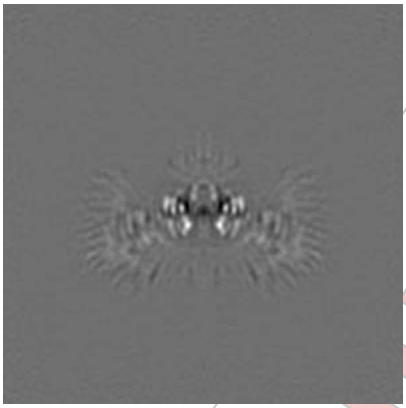


Y Index: 134

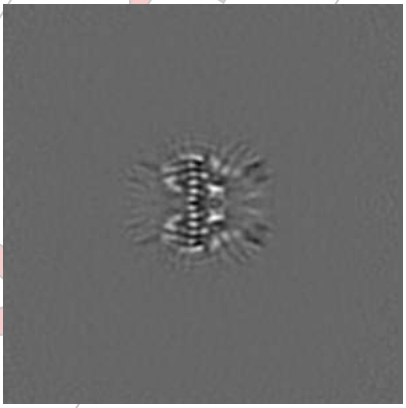


Z Index: 134

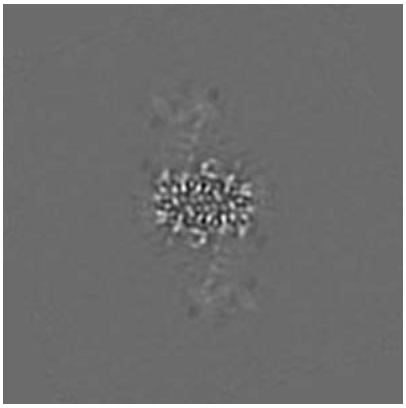
6.2.2 Raw map



X Index: 134



Y Index: 134

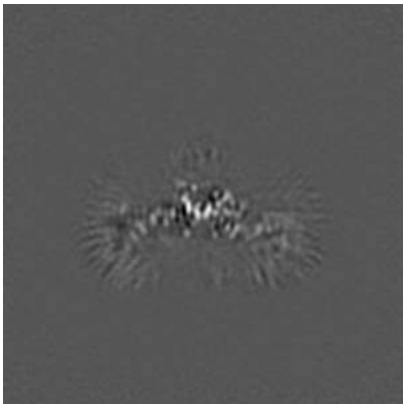


Z Index: 134

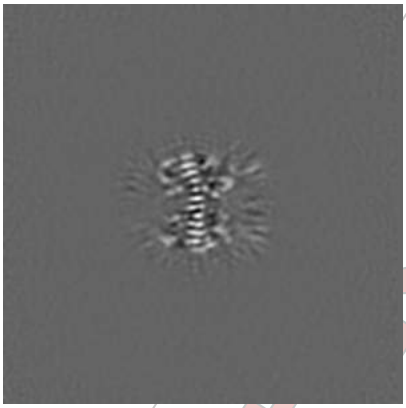
The images above show central slices of the map in three orthogonal directions.

6.3 Largest variance slices (i)

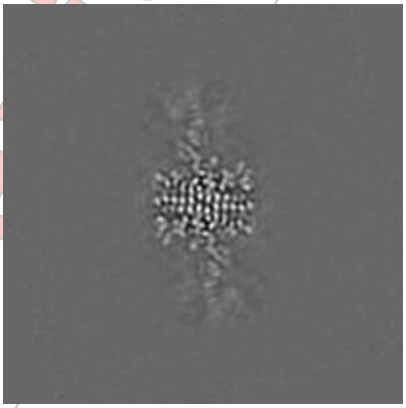
6.3.1 Primary map



X Index: 137

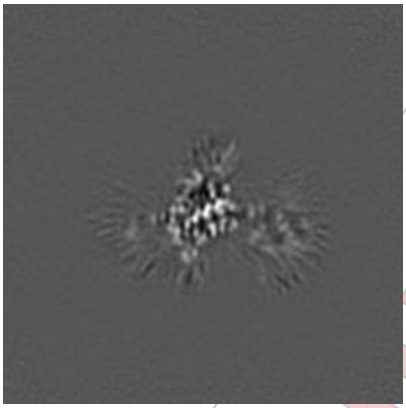


Y Index: 137

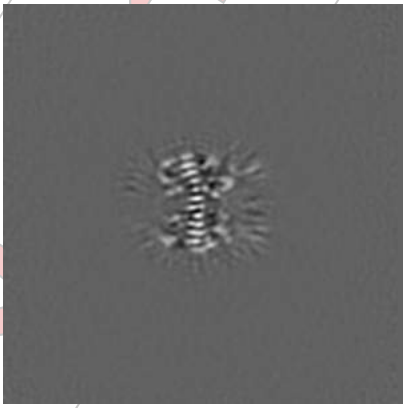


Z Index: 129

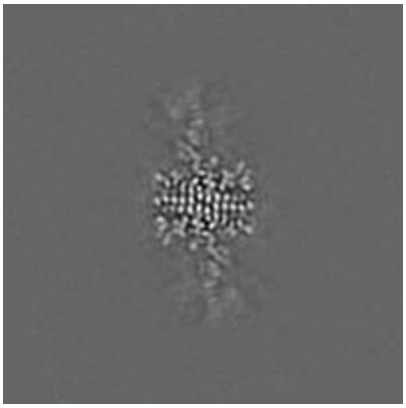
6.3.2 Raw map



X Index: 120



Y Index: 137

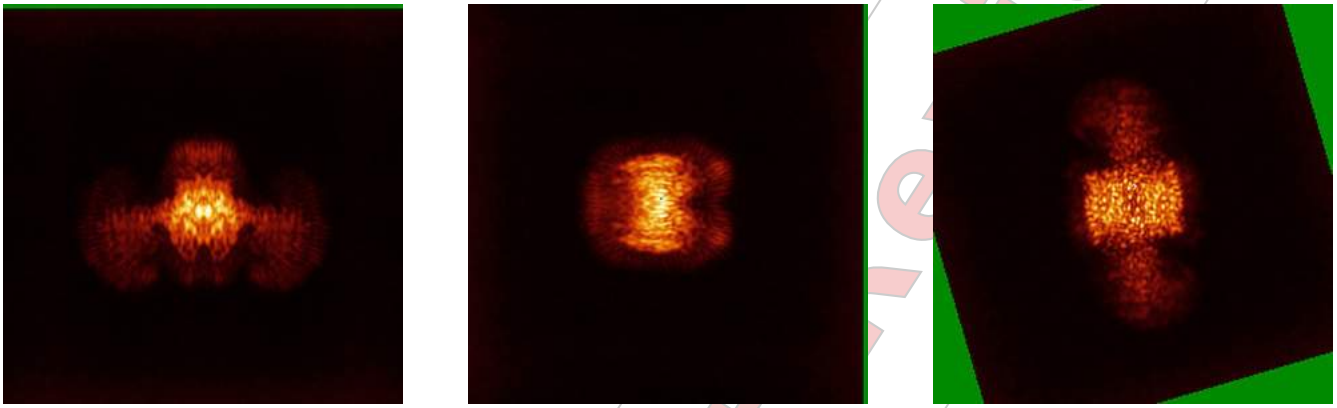


Z Index: 129

The images above show the largest variance slices of the map in three orthogonal directions.

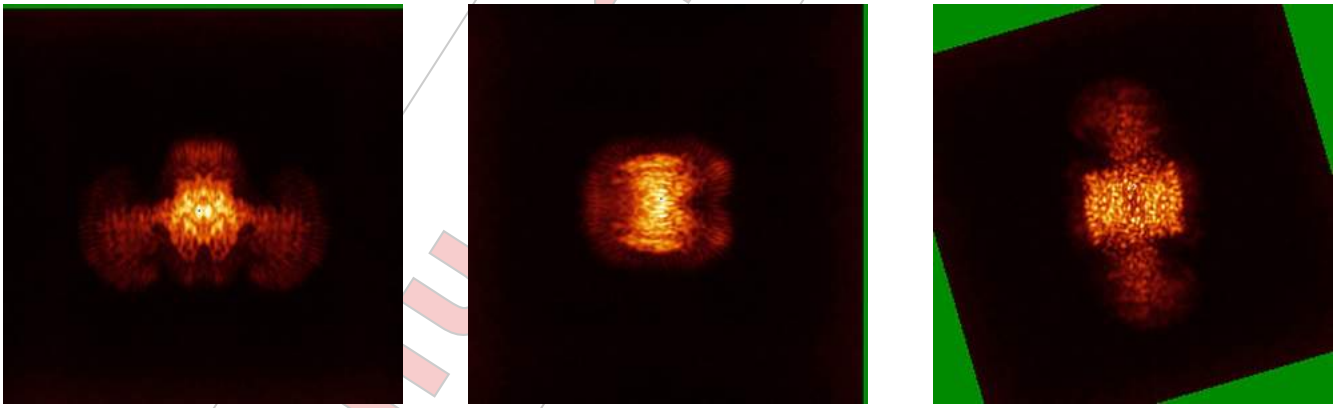
6.4 Orthogonal standard-deviation projections (False-color)

6.4.1 Primary map



X Y Z

6.4.2 Raw map

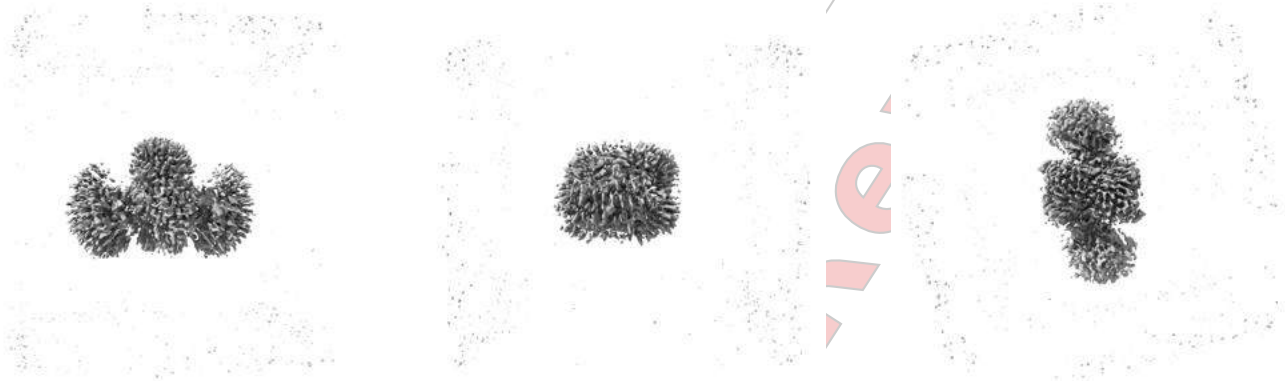


X Y Z

The images above show the map standard deviation projections with false color in three orthogonal directions. Minimum values are shown in green, max in blue, and dark to light orange shades represent small to large values respectively.

6.5 Orthogonal surface views (i)

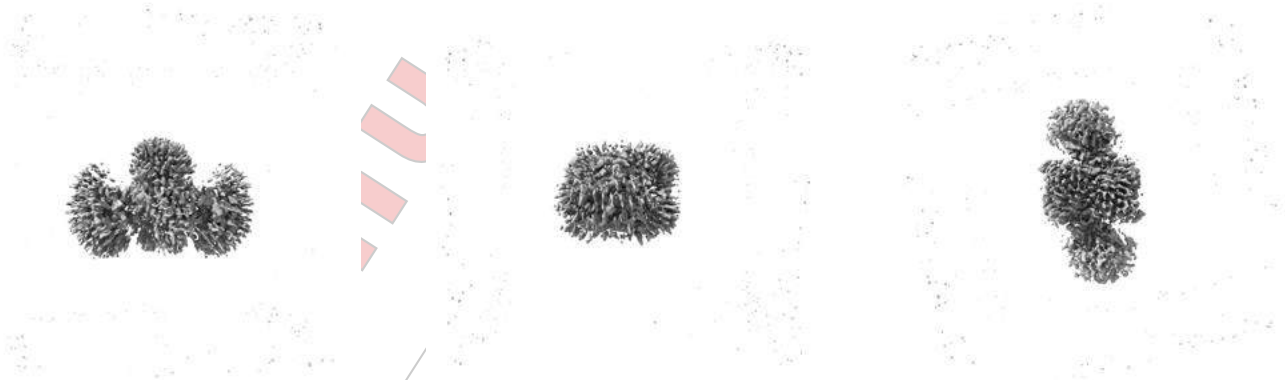
6.5.1 Primary map



X Y Z

The images above show the 3D surface view of the map at the recommended contour level 0.15. These images, in conjunction with the slice images, may facilitate assessment of whether an appropriate contour level has been provided.

6.5.2 Raw map



X Y Z

These images show the 3D surface of the raw map. The raw map's contour level was selected so that its surface encloses the same volume as the primary map does at its recommended contour level.

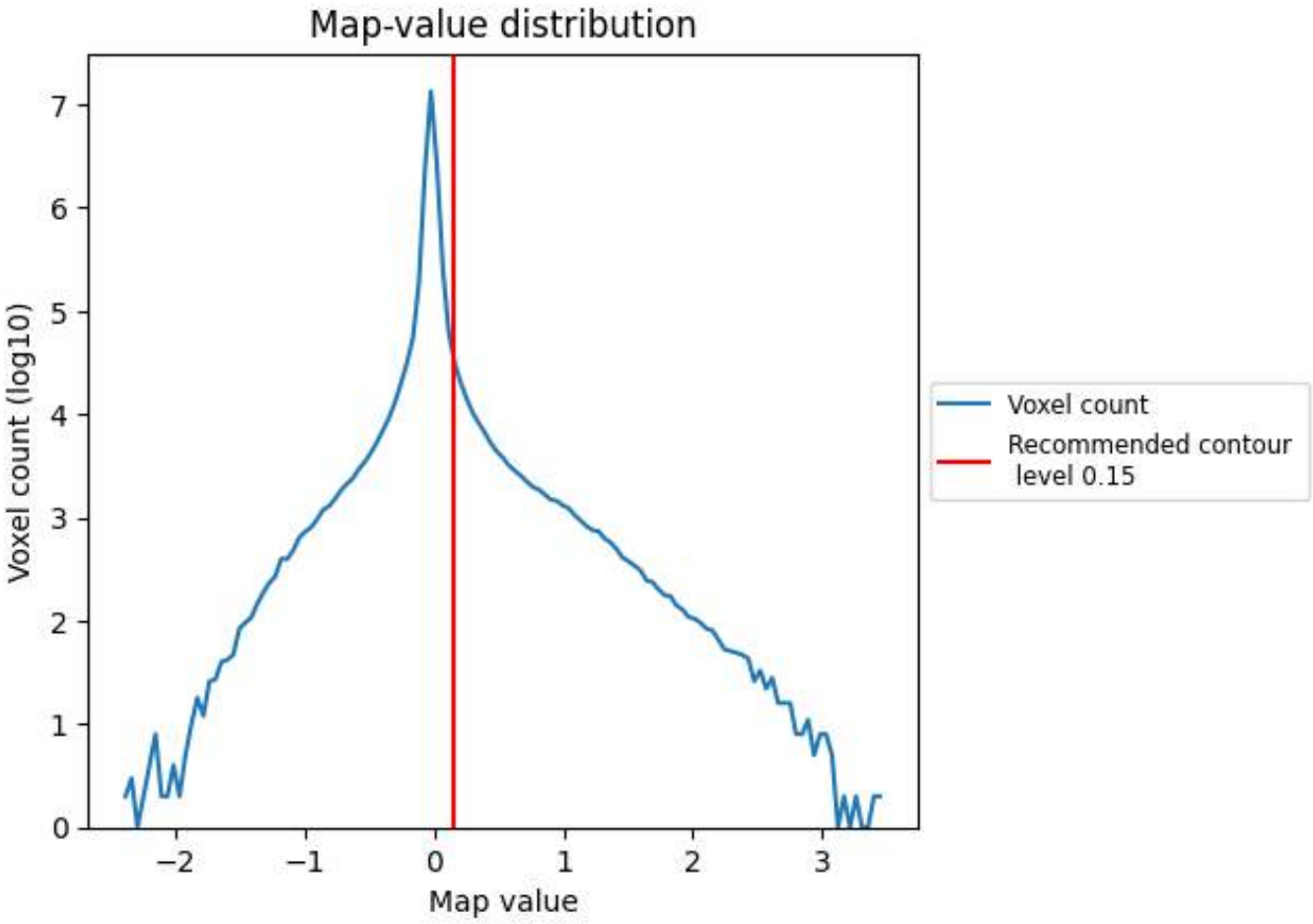
6.6 Mask visualisation (i)

This section was not generated. No masks/segmentation were deposited.

7 Map analysis

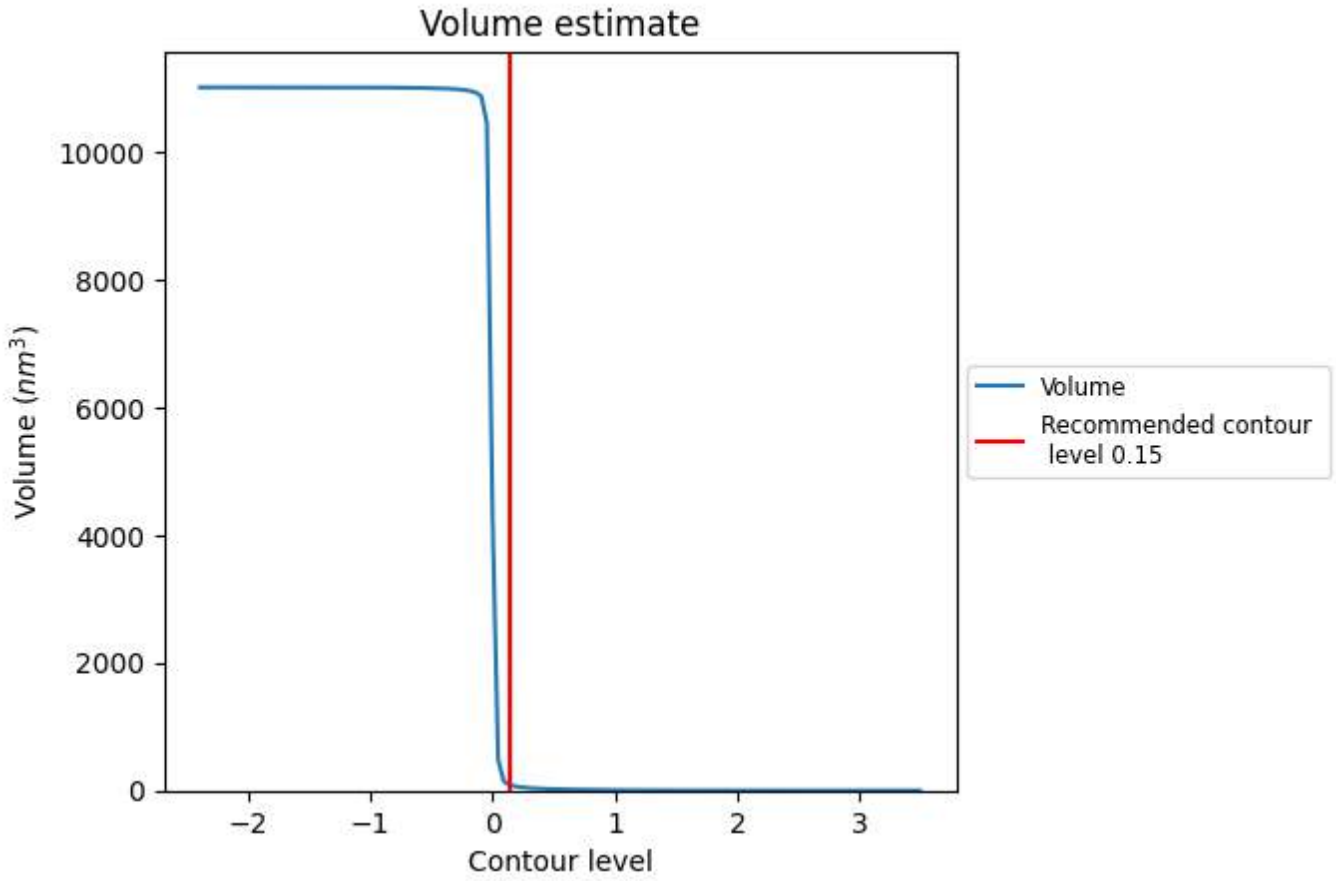
This section contains the results of statistical analysis of the map.

7.1 Map-value distribution



The map-value distribution is plotted in 128 intervals along the x-axis. The y-axis is logarithmic. A spike in this graph at zero usually indicates that the volume has been masked.

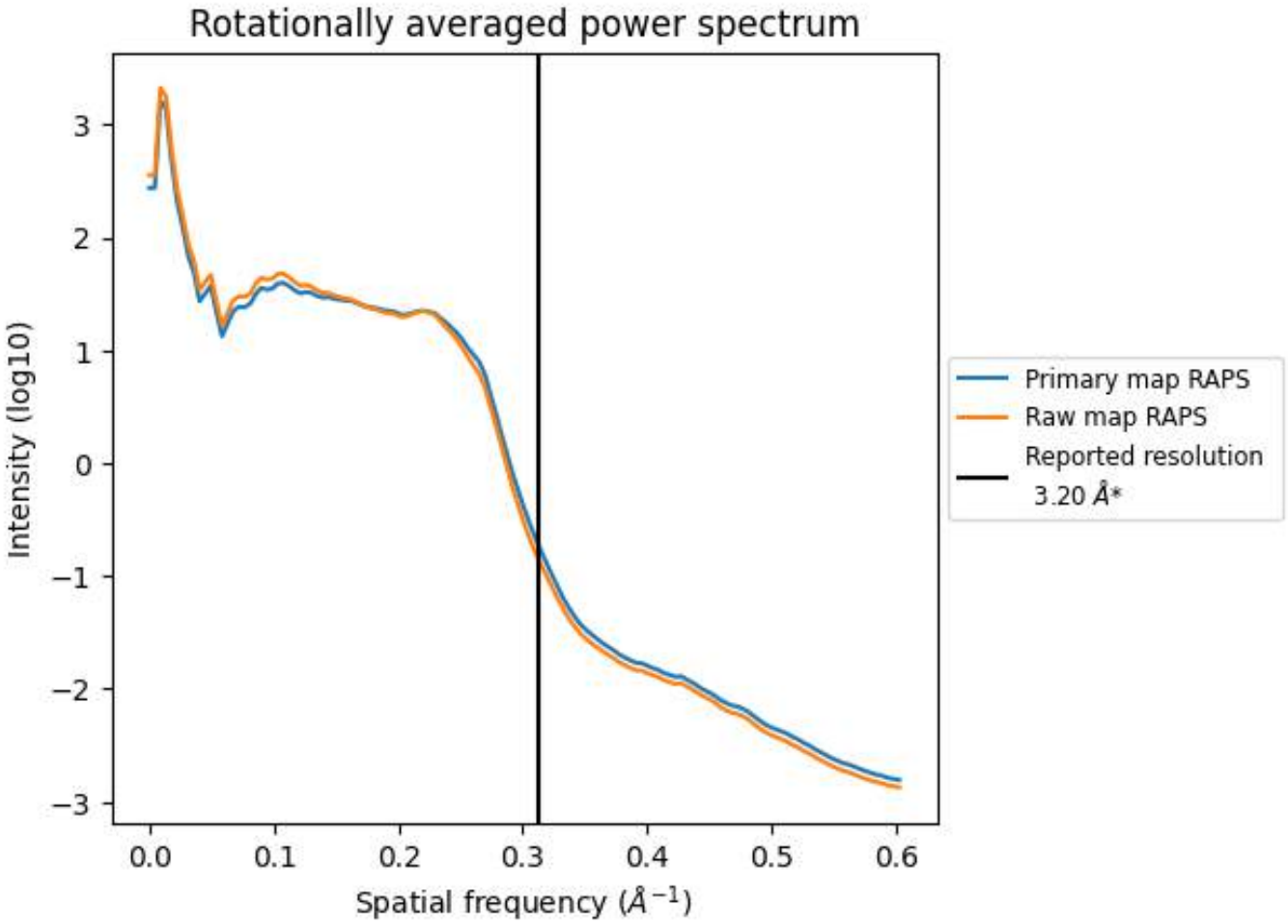
7.2 Volume estimate (i)



The volume at the recommended contour level is 84 nm^3; this corresponds to an approximate mass of 75 kDa.

The volume estimate graph shows how the enclosed volume varies with the contour level. The recommended contour level is shown as a vertical line and the intersection between the line and the curve gives the volume of the enclosed surface at the given level.

7.3 Rotationally averaged power spectrum (i)

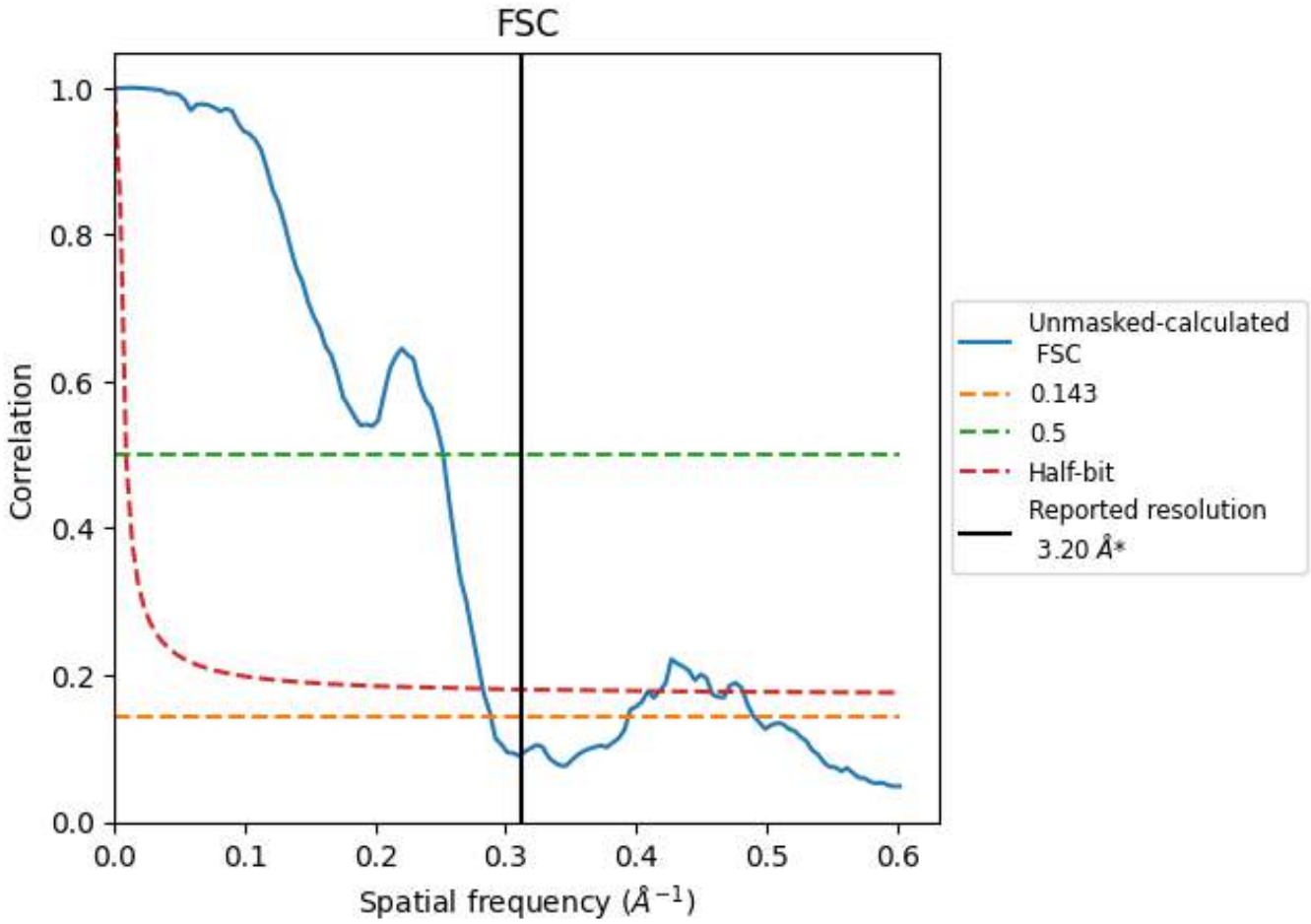


*Reported resolution corresponds to spatial frequency of 0.312 Å^{-1}

8 Fourier-Shell correlation

Fourier-Shell Correlation (FSC) is the most commonly used method to estimate the resolution of single-particle and subtomogram-averaged maps. The shape of the curve depends on the imposed symmetry, mask and whether or not the two 3D reconstructions used were processed from a common reference. The reported resolution is shown as a black line. A curve is displayed for the half-bit criterion in addition to lines showing the 0.143 gold standard cut-off and 0.5 cut-off.

8.1 FSC



*Reported resolution corresponds to spatial frequency of 0.312 Å^{-1}

8.2 Resolution estimates (i)

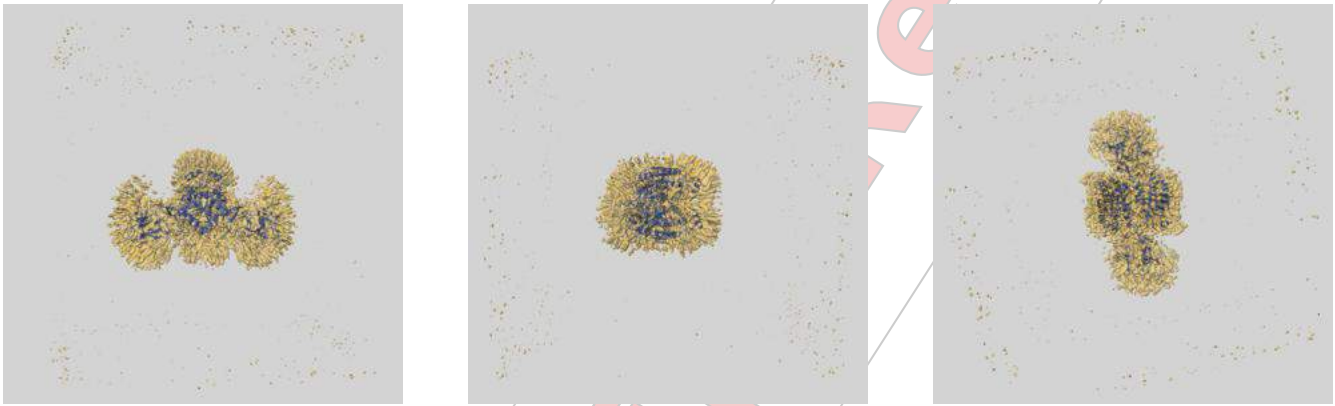
Resolution estimate (Å)	Estimation criterion (FSC cut-off)		
	0.143	0.5	Half-bit
Reported by author	3.20	-	-
Author-provided FSC curve	-	-	-
Unmasked-calculated*	3.47	3.97	3.54

*Resolution estimate based on FSC curve calculated by comparison of deposited half-maps.

9 Map-model fit

This section contains information regarding the fit between EMDB map EMD-40678 and PDB model 8SPB. Per-residue inclusion information can be found in section 3 on page 7.

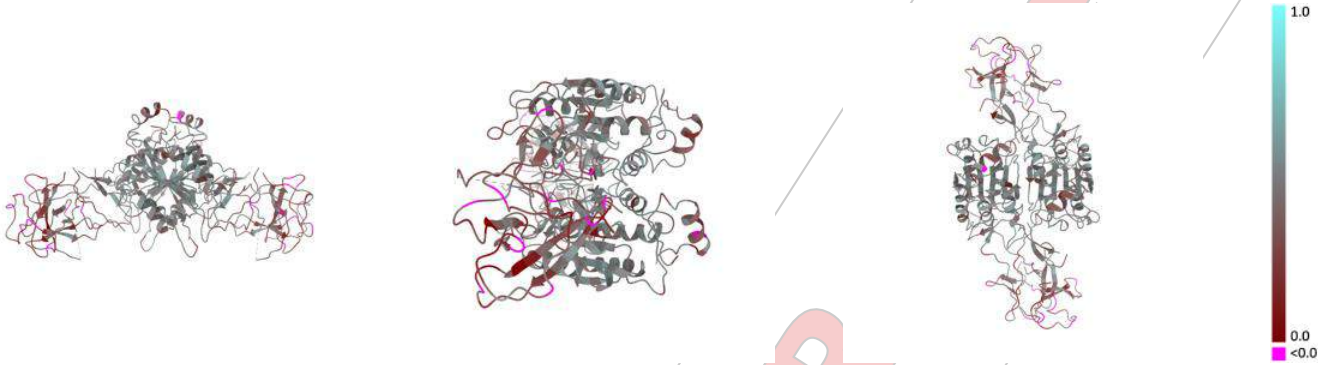
9.1 Map-model overlay



X Y Z

The images above show the 3D surface view of the map at the recommended contour level 0.15 at 50% transparency in yellow overlaid with a ribbon representation of the model coloured in blue. These images allow for the visual assessment of the quality of fit between the atomic model and the map.

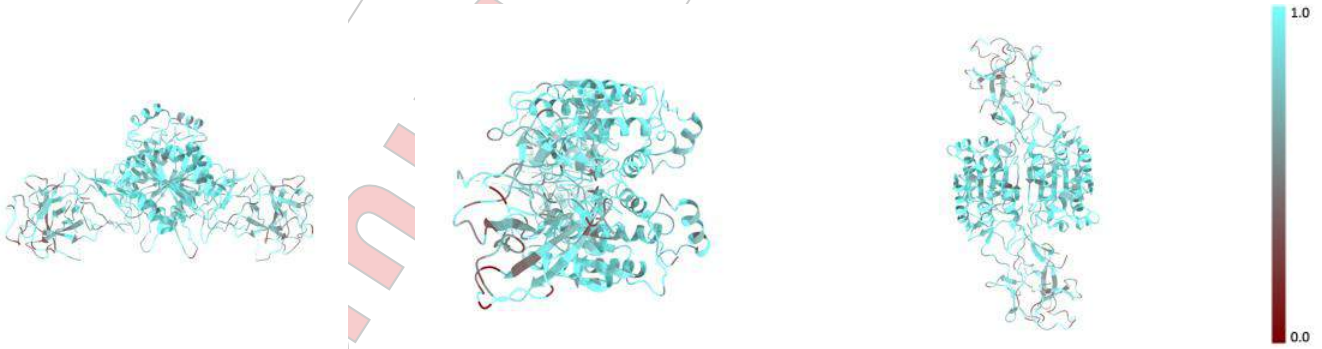
9.2 Q-score mapped to coordinate model (i)



X Y Z

The images above show the model with each residue coloured according its Q-score. This shows their resolvability in the map with higher Q-score values reflecting better resolvability. Please note: Q-score is calculating the resolvability of atoms, and thus high values are only expected at resolutions at which atoms can be resolved. Low Q-score values may therefore be expected for many entries.

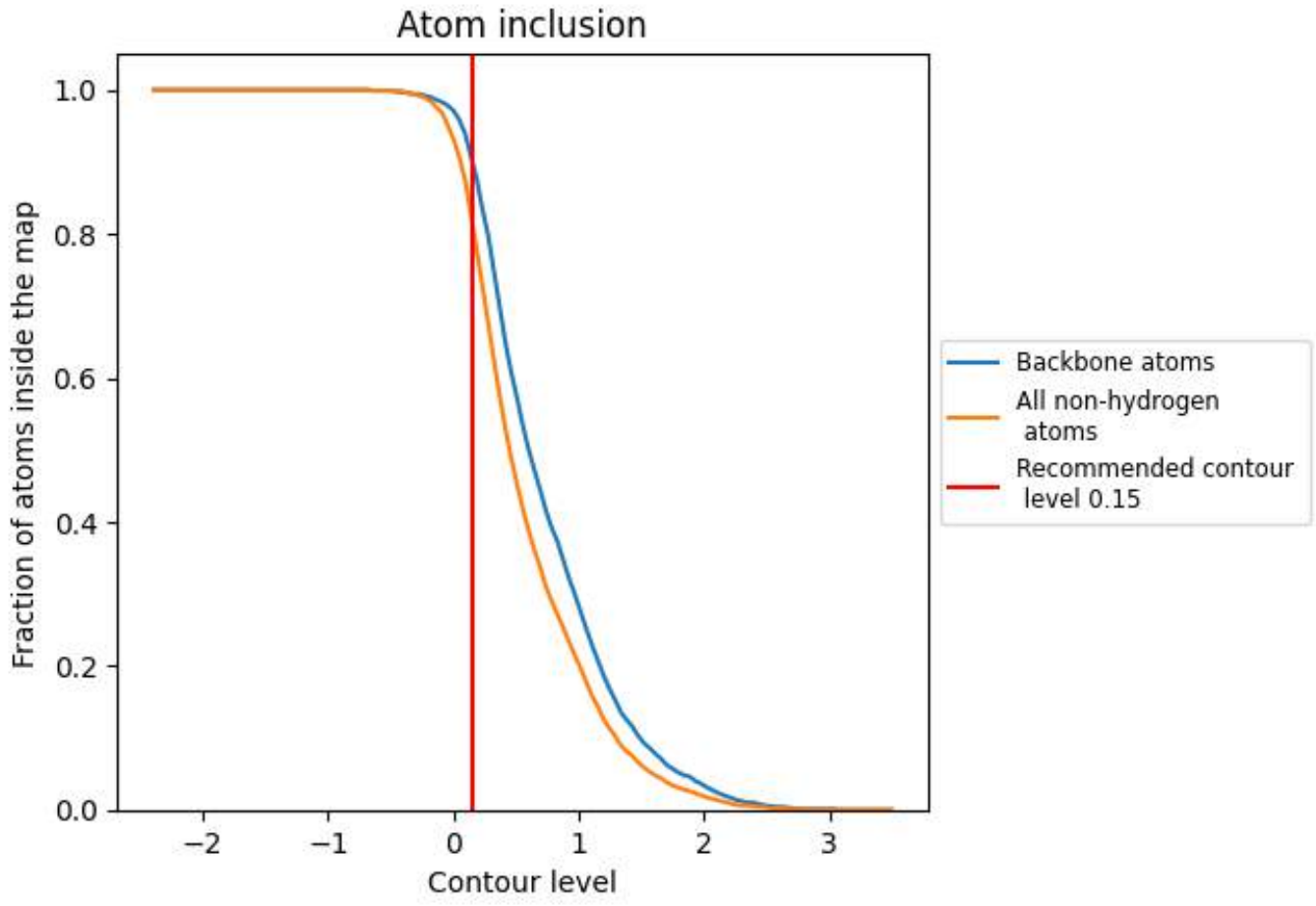
9.3 Atom inclusion mapped to coordinate model (i)



X Y Z

The images above show the model with each residue coloured according to its atom inclusion. This shows to what extent they are inside the map at the recommended contour level (0.15).

9.4 Atom inclusion (i)



At the recommended contour level, 90% of all backbone atoms, 82% of all non-hydrogen atoms, are inside the map.

9.5 Map-model fit summary (i)

The table lists the average atom inclusion at the recommended contour level (0.15) and Q-score for the entire model and for each chain.

Chain	Atom inclusion	Q-score
All	0.8150	0.4050
A	0.8830	0.4570
B	0.9120	0.4860
C	0.6900	0.3000
a	0.8750	0.4560
b	0.9090	0.4940
c	0.6960	0.3020