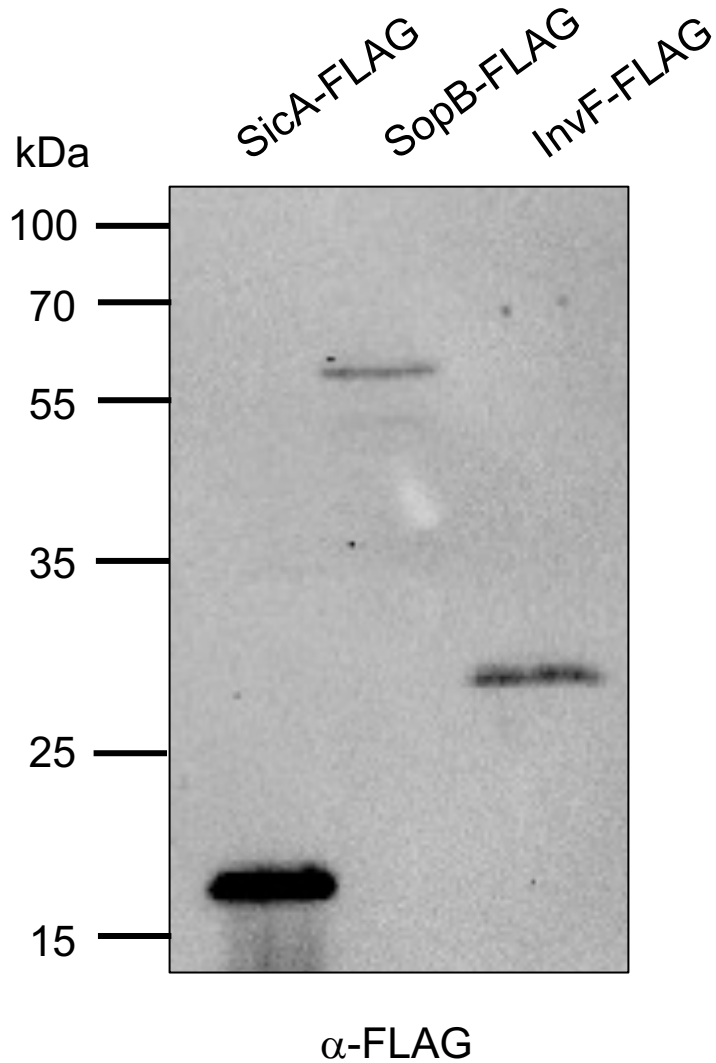


Supplementary material for the manuscript:

Interactions between the AraC/XylS-like transcriptional activator InvF of *Salmonella*
Typhimurium, the RNA polymerase alpha subunit and the chaperone SicA

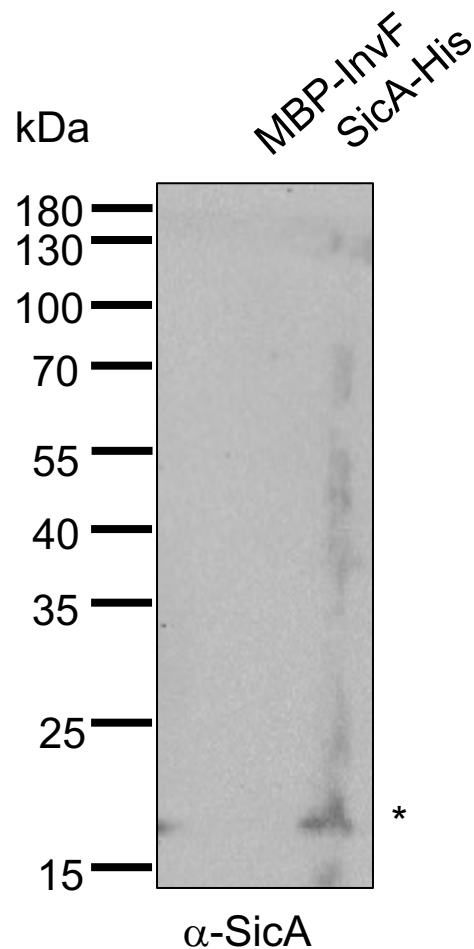
Daniel Cortés-Avalos, André Borges Farias, Luis E. Romero-González, Cristina Lara-
Ochoa, Lourdes Villa-Tanaca, Francisco García-del Portillo, Vanessa López-Guerrero,
Víctor H. Bustamante, Ernesto Pérez-Rueda and J. Antonio Ibarra

Supplementary Figure 1



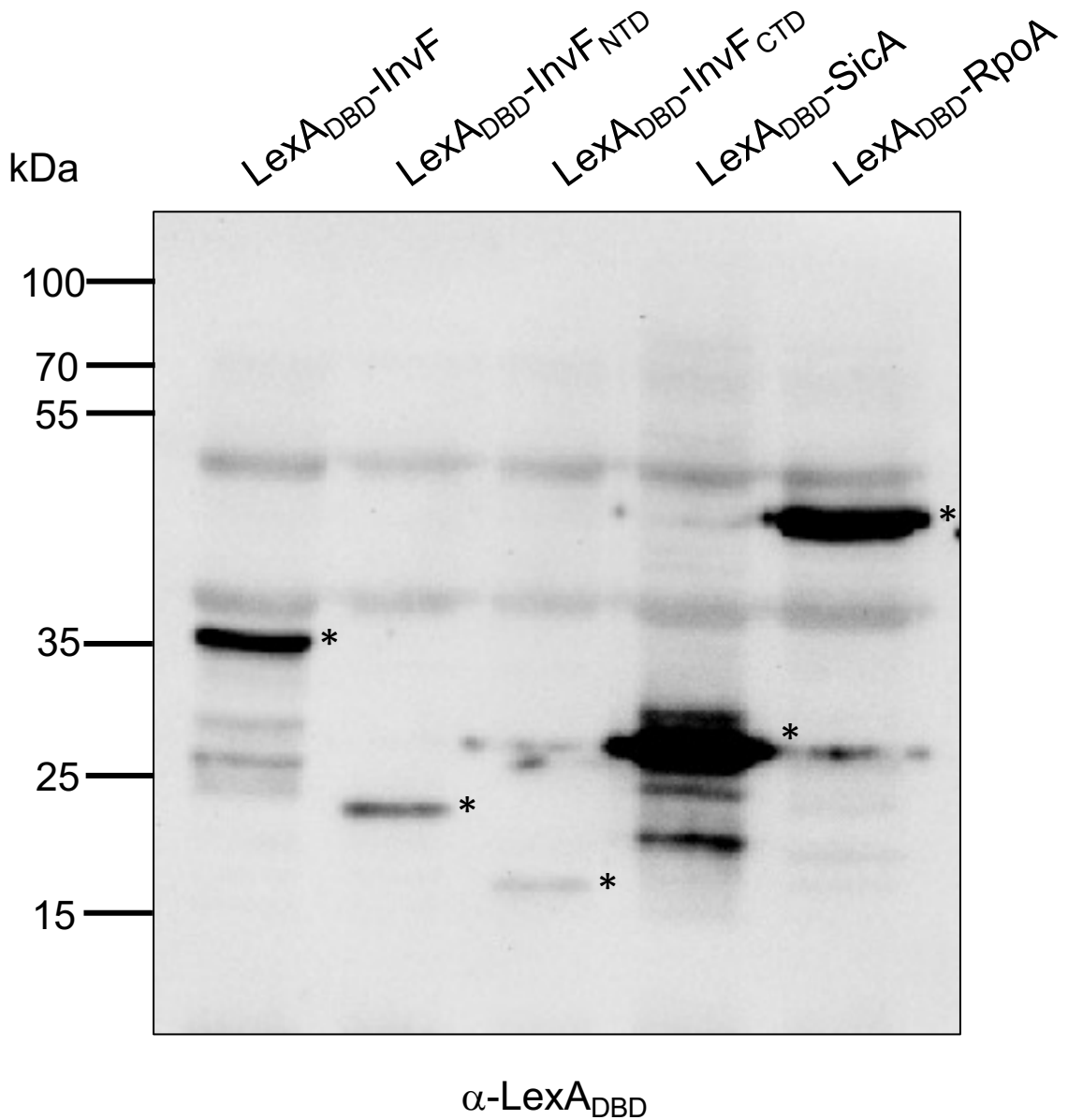
Supplementary figure S1. Expression of FLAG-fused proteins. Proteins were overexpressed with IPTG 1 mM and identified by Western blot with anti-FLAG-HRP as described in the methods section.

Supplementary Figure 2



Supplementary figure S2. Characterization of anti-SicA antibodies. Purified SicA-His6 protein was used to generate antibodies in mice. Purified proteins His6-RpoA, MBP-InvF and SicA-His6 were used to test mouse polyclonal anti-SicA antibodies by Western blot. Recombinant protein G-HRP was used as a secondary antibody.

Supplementary Figure 3



Supplementary figure S3. Expression of LexA-fused proteins. Proteins were overexpressed with IPTG 1 mM and identified by Western blot with anti-LexA antibodies and recombinant protein G-HRP.

Supplementary table S1. Strains and plasmids used in this work.

Strain or plasmid	Genotype or description	Reference ^a
<i>Salmonella</i> Typhimurium strains		
SL1344	Wild-type SL1344, Sm ^R	[1]
SL1344 ΔSPI-1	Wild-type SL1344 isogenic mutant, Km ^R	[2]
SL1344 <i>invF::Tn5</i>	SL1344 <i>invF::Tn5</i> mutant, Km ^R	[3]
SL1344 <i>invF::Tn5 sopB::FLAG</i>	Obtained by transducing <i>invF::Tn5</i> from SL1344 <i>invF::Tn5</i> into SL1344 <i>sopB::FLAG</i> Km ^R	This work
SL1344 <i>invF::3xFLAG-kan</i>	Obtained by transducing <i>invF::3xFLAG-kan</i> from <i>Salmonella</i> strain 14028 into SL1344, Km ^R	[4]
SL1344 <i>invF::3xFLAG</i>	Derivative SL1344 <i>invF::3xFLAG-kan</i> , Sm ^R	[4]
MD1163	SL1344 <i>sopB::3xFLAG-Km</i>	This work
MD1180	SL1344 <i>sopB::3xFLAG</i>	This work
<i>Escherichia coli</i> strains		
DH10B	Laboratory cloning strain, Sm ^R	Invitrogen
BL21	Strain for expression of recombinant proteins	Invitrogen
SU202	Reporter strain harboring a <i>sulA::lacZ</i> transcriptional fusion	[5]
Plasmids		
pMal-InvF	pMAL-c2X derivative expressing MBP-InvF from a <i>tac</i> promoter, Ap ^R	[6]
pMal-c2xa	Low copy number plasmid to generate fusions to the <i>malE</i> gene for expressing MBP, Ap ^R	New England Biolabs
pTOPO-SicA	pCRTOPPO derivative expressing SicA-His ₆ from the T7 promoter, Km ^R Ap ^R	[6]
pET28-RpoA	pET28a derivate expressing His ₆ -RpoA, Km ^R	[7]
pINIIIA1	pBR322 derivate cloning vector for mutants of <i>rpoA</i> , Ap ^R	[8]
pLAX185	pINIIIA1derivate expressing wild type RpoA, Ap ^R	[9]
pLAD235	pINIIIA1derivate expressing RpoA truncated from the carboxy end, which retain 235 amino acids, Ap ^R	[9]
pLAD256	pINIIIA1derivate expressing RpoA truncated from the carboxy end, which retain 256 amino acids, Ap ^R	[9]
pTOPO-SicA-FLAG	pCRTOPPO derivative expressing SicA-FLAG from the T7 promoter, Km ^R Ap ^R	This work

pSR658	ColE1 derivative cloning vector, <i>lac</i> promoter, Tc ^R	[10]
pSR659	p15A derivative low-copy-number cloning vector, <i>lac</i> promoter, Ap ^R	[10]
pSR658-InvF	pSR658 derivative expressing LexA _{DBDwt} -InvF from the <i>lac</i> promoter	[6]
pSR658-SicA	pSR658 derivative expressing LexA _{DBDwt} -SicA from the <i>lac</i> promoter	[6]
pSR658-NTDInvF	pSR658 derivative expressing LexA _{DBDwt} -InvF _{NTD} from the <i>lac</i> promoter	This work
pSR658-CTDInvF	pSR658 derivative expressing LexA _{DBDwt} -InvF _{CTD} from the <i>lac</i> promoter	This work
pSR659-SicA	pSR659 derivative expressing LexA _{DBDmut} -SicA from the <i>lac</i> promoter	[6]
pSR659-RpoA	pSR659 derivative expressing LexA _{DBDmut} -RpoA from the <i>lac</i> promoter	This work
pSR658-HilD	pSR658 derivative expressing LexA _{DBDwt} -HilD from the <i>lac</i> promoter	[11]
pSR659-HilE	pSR659 derivative expressing LexA _{DBDmut} -HilE from the <i>lac</i> promoter	[11]

^a. References for Supplementary table 1.

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Supplementary table S2. Oligonucleotides used in this work.

Primer name	Sequence (5'-3)'	Tm (°C)
sicA-RBS-Fw	CTCGAGATTAAAGGAGGTAAATAATGGATTATCAAAT AATGTCAGCG	53
SicA-FLAGrv	TTACTTGTCGTCATCGTCTTTGTAGTCTTCCTTTTCTTG TTCAGTGTGC	53
RpoALexAFw	CCGCTCGAGATGCAGGGTTCTGTGAC	52
RpoALexA Rv	GGGGTACTTAGTCGTCAGCGATGC	52
invf-NDT-Rv	GTCTCTAGATCAGAAGGCCGAGAAGGCGT	60
lexA-InvF-Fw	CCGCTCGAGATGCTAAATACGCAGGAAGTAC	52
CTDInvFw	CCGCTCGAGAATAAGGTACTGGCGCTG	52
InvFrevLexA	GGGGTACCTCATTTGTCTGCCAATTG	48
sopB-RT-Fw	AAGCAGCTTAATAACCAGCCC	55
sopB-RT-Rv	ACCGTCCTCATGCACACTCAC	59
sopBFlag-1	TTGGCAGTCAGTAAAAGGCATTTCTTCATTAATCACATC TGACTACAAAGACCATGACGG	
sopBFlag-2	TAAACGATTTAATAGACTTTCCATATAGTTACCTCAAGA CCATATGAATATCCTCCTTAG	
gyrB-Fw	TGGTTTCCCACAGCTGATCC	59
gyrB-Rv	TTCAATCAGACCACGCAGCT	58

Supplementary file 1

Jose_20180130_Refseq_Salmonella_Typhimurium_20180219_InvF-Pulldown_SCAFF4, Samples report created on 07/27/2023

Experiment: Jose_20180130_Refseq_Salmonella_Typhimurium_20180219_InvF-Pulldown_SCAFF4

Peak List Generator: unknown

Version: unknown

Charge States Calculated: unknown

Deisotoped: unknown

Textual Annotation: unknown

Database Set: 1 Database

Database Name: Refseq_Salmonella_Typhimurium

Version: unknown

Taxonomy: All Entries

Number of Proteins: 0

Explain Database w/ < 1000 entries:

Does database contain common contaminants?: unknown

Search Engine Set: 1 Search Engine

Search Engine: Mascot (Ion Score Only)

Version: Mascot in Proteome Discoverer 2.1.0.81

Samples: All Samples

Fragment Tolerance: 0.60 Da (Monoisotopic)

Parent Tolerance: 10.0 PPM (Monoisotopic)

Fixed Modifications: +57 on C (Carbamidomethyl)

Variable Modifications: +16 on M (Oxidation)

Database: Refseq_Salmonella_Typhimurium (unknown version, 0 entries)

Digestion Enzyme: Trypsin

Max Missed Cleavages: 2

Probability Model:

VL_20180216_ADH_37: Peptide Prophet with Delta Mass Correction [+2 and below,+3,+4,+5,+6,+7,+8,+9,+10,+11,+12,+13,+14,+15,+16,+17,+18,+19,+20,+21,+25 and above]

VL_20180219_IBA_01: Peptide Prophet with Delta Mass Correction [+2 and below,+3,+4,+5,+6,+7,+8,+10,+12,+19 and above]

VL_20180219_ADH_01: Peptide Prophet with Delta Mass Correction [+2 and below,+3,+4,+5,+6,+7,+8,+11,+12,+13,+14,+15,+16,+17,+18,+19,+20,+21,+23,+24 and above]

VL_20180219_IBA_02: Peptide Prophet with Delta Mass Correction [+2 and below,+3,+4,+5,+6,+7,+8,+9,+10,+11,+12,+13,+16,+17,+18,+19,+21 and above]

VL_20180219_ADH_02: Peptide Prophet with Delta Mass Correction [+2 and below,+3,+4,+5,+6,+7,+8,+9,+10,+11,+12,+13,+14,+16,+17,+19,+20,+21 and above]

VL_20180219_IBA_03: Peptide Prophet with Delta Mass Correction [+2 and below,+3,+4,+5,+6,+7,+8,+9,+10,+12,+13,+14,+15,+16,+17,+18,+19,+22 and above]

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VL_20180219_IBA_05: Peptide Prophet with Delta Mass Correction [+2 and below,+3,+4,+5,+6,+7,+8,+9,+11 and above]

VL_20180219_ADH_06_2: Peptide Prophet with Delta Mass Correction [+2 and below,+3,+4,+5,+6,+7,+8,+10,+11,+12,+13,+14,+15,+17,+19,+20,+21 and above]

VL_20180219_IBA_06: Peptide Prophet with Delta Mass Correction [+2 and below,+3,+4,+5,+6,+7,+8,+9,+10,+11,+12,+22 and above]

Scaffold: Version: Scaffold_5.2.0

Modification Metadata Set: 2334 modifications

Source: C:\Program Files\Scaffold 4\parameters\unimod.xml

Comment:

Protein Grouping Strategy: Experiment-wide grouping with protein cluster analysis

Peptide Thresholds: 95.07% minimum

Protein Thresholds: 95.07% minimum and 2 peptides minimum

Peptide FDR: 0.07% (Decoy)

Protein FDR: 0.07% (Decoy)

GO Annotation Source(s):

Pathway Annotation Source(s): Unknown

Alternate ID Source(s):

Jose_20180130_Refseq_Salmonella_Typhimurium_20180219_InvF-Pulldown_SCAFF4										Stimated size (kDa)		40	50	100	15	50	70			
#	Visible?	Starred?	Identified Protein	Accession Number	Alternate ID	Molecular Weight	Protein Group	Taxonomy	00-BLK before	01-Band 5	02-BLK before	03-Band 4	04-BLK before	05-Band 1	06-BLK before	07-Band 6	08-BLK before	09-Band 3	10-BLK before	11-Band 2
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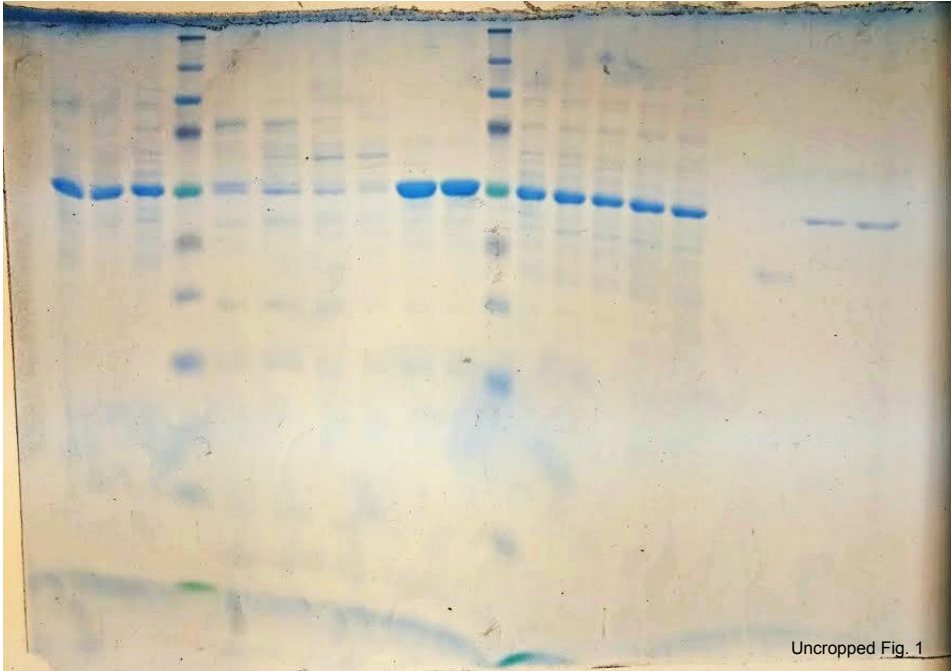
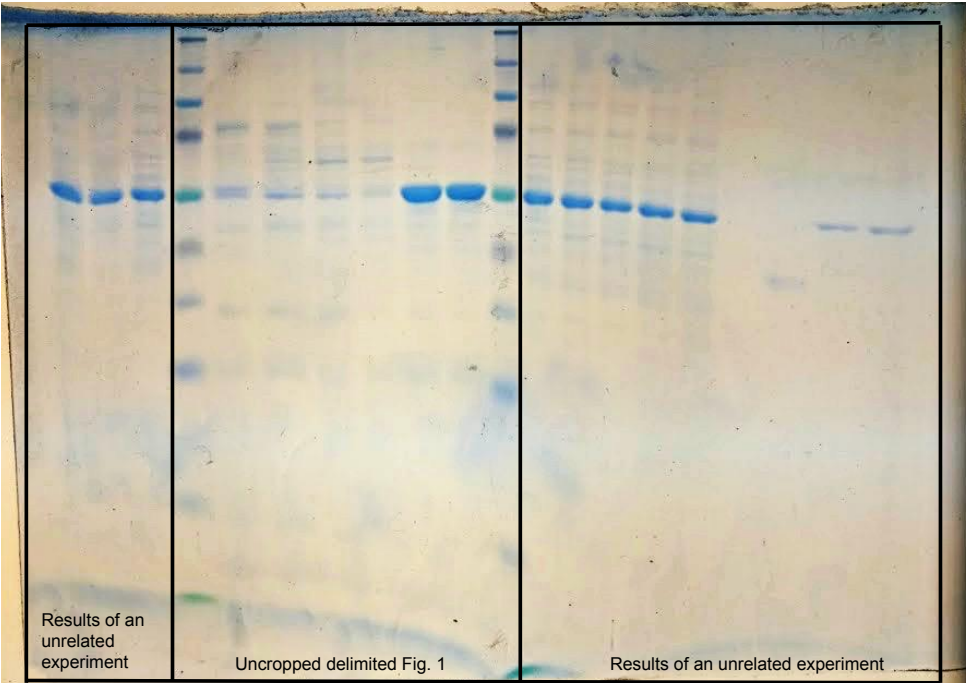
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29	true	Empty	MULTISPECIE WP_001238917.1	15 kDa		unknown	0	0	0	2	0	2	0	50	8	1	2	3
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37	true	Empty	MULTISPECIE WP_000940121.1	18 kDa		unknown	0	0	0	0	0	0	0	47	6	0	0	0
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39	true	Empty	phosphate a: CBW18409.1	77 kDa		unknown	0	0	0	0	0	42	1	0	0	0	0	6
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51	true	Empty	anaerobic gh CBW18357.1	44 kDa		unknown	0	27	0	0	0	0	0	0	0	0	0	0
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67	true	Empty	outer membe WP_001240935.1	90 kDa		unknown	0	0	0	0	0	20	0	0	0	0	0	0
68	true	Empty	energy-depe: WP_000046770.1	62 kDa		unknown	0	0	0	0	0	0	0	0	0	24	0	0

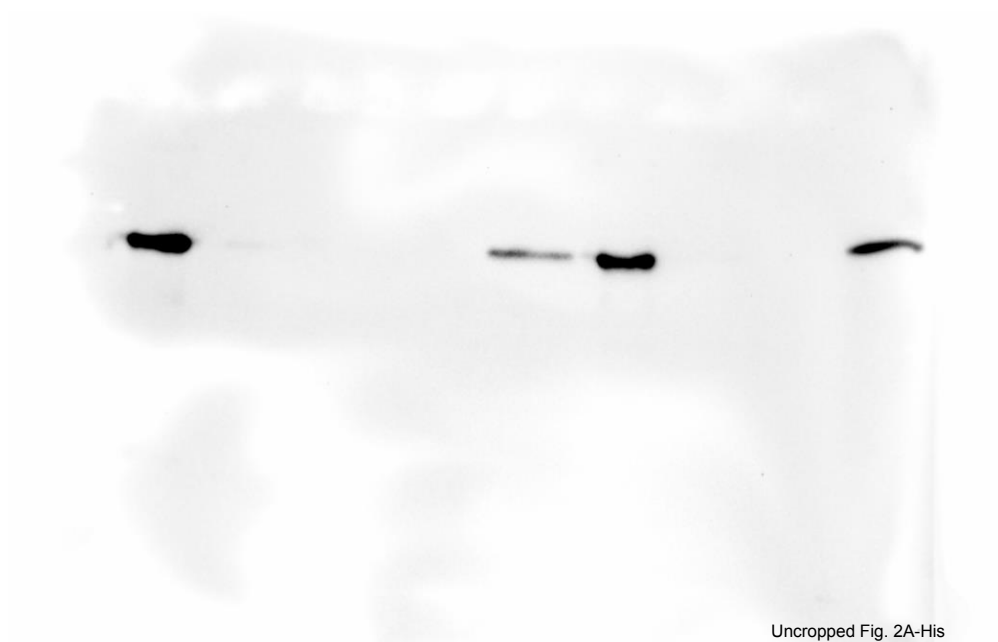
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70	true	Empty	polynucleotic WP_000174614.1	55 kDa	unknown	0	0	0	0	0	8	0	0	0	0	0	11
71	true	Empty	fructose-bisph WP_001250625.1	36 kDa	unknown	0	23	0	0	0	0	0	0	0	0	0	0
72	true	Empty	2-amino-3-k CBW19769.1	43 kDa	unknown	0	21	0	0	0	0	0	0	0	0	0	0
73	true	Empty	50s ribosomi CBW20413.1	16 kDa	unknown	0	0	0	0	0	0	0	21	2	0	0	0
74	true	Empty	membrane-t WP_000829731.1	86 kDa	unknown	0	0	0	0	0	19	0	0	0	0	0	0
75	true	Empty	anaerobic glh CBW18355.1	59 kDa	unknown	0	0	0	0	0	0	0	0	0	18	0	0
76	true	Empty	chaperone pr WP_000758966.1	18 kDa	unknown	0	0	0	0	0	0	0	17	2	0	0	0
77	true	Empty	D-alanine ca: CBW16725.1	44 kDa	unknown	0	19	0	0	0	0	0	0	0	0	0	0
78	true	Empty	MULTISPECIF WP_000447529.1	12 kDa	unknown	0	0	0	0	0	0	0	15	0	1	0	0
79	true	Empty	hypothetical CBW18440.1	36 kDa	unknown	0	8	4	1	0	0	0	0	0	0	0	0
80	true	Empty	sulphate trar CBW18506.1	41 kDa	unknown	0	16	0	0	0	0	0	0	0	0	0	0
81	true	Empty	GTP-binding CBW20045.1	67 kDa	unknown	0	0	0	0	0	0	0	0	0	0	0	17
82	true	Empty	tRNA uridine WP_000499872.1	70 kDa	unknown	0	0	0	0	0	0	0	0	0	0	0	10
83	true	Empty	50S ribosomi WP_000579838.1	22 kDa	unknown	0	0	0	0	0	2	0	14	0	0	0	0
84	true	Empty	glutamate 1- CAC03102.1	45 kDa	unknown	0	15	1	0	0	0	0	0	0	0	0	0
85	true	Empty	hypothetical CBW19566.1 (+1)	85 kDa	unknown	0	0	0	0	0	15	0	0	0	0	0	0
86	true	Empty	major outer i CBW17407.1	8 kDa	unknown	0	8	0	2	0	2	0	0	0	1	0	0
87	true	Empty	lysine-N-met CBW17982.1	45 kDa	unknown	0	11	0	0	0	0	0	0	0	0	0	0
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91	true	Empty	outer memb: CBW19259.1	54 kDa	unknown	0	0	0	12	0	0	0	0	0	0	0	0
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94	true	Empty	formate acet CBW17006.1	85 kDa	unknown	0	0	0	0	0	10	0	0	0	0	0	2
95	true	Empty	ATP-depende CBW19349.1 (+1)	70 kDa	unknown	0	0	0	0	0	11	0	0	0	0	0	0
96	true	Empty	hypothetical CBW19752.1	43 kDa	unknown	0	10	0	0	0	0	0	0	0	0	0	0
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103	true	Empty	alcohol dehy: CBW17775.1	96 kDa	unknown	0	0	96	0	0	4	0	0	0	0	0	5
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105	true	Empty	D-alanyl-D-al CBW16936.1	44 kDa	unknown	0	8	44	0	0	0	0	0	0	0	0	0
106	true	Empty	chemotaxis f CBW17950.1	73 kDa	unknown	0	0	0	0	0	0	0	0	0	0	0	8
107	true	Empty	UDP-glucose CBW18153.1	44 kDa	unknown	0	6	0	0	0	0	0	0	0	0	0	0
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111	true	Empty	non-heme fe WP_000920611.1	19 kDa	unknown	0	0	0	0	0	0	0	8	0	0	0	0
112	true	Empty	SipA [Salmo: AAA86618.1 (+1)	74 kDa	unknown	0	0	0	0	0	5	0	0	0	0	0	0
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131	true	Empty	DNA ligase (WP_000433266.1	73 kDa	unknown	0	0	0	0	0	0	0	0	0	0	0	5
132	true	Empty	DUF945 dom WP_000753326.1	54 kDa	unknown	0	0	0	4	0	0	0	0	0	0	0	0
133	true	Empty	glycogen phc WP_000993428.1	93 kDa	unknown	0	0	0	0	0	4	0	0	0	0	0	0
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135	true	Empty	Methionyl-tr CBW18228.1	76 kDa	unknown	0	0	0	0	0	0	0	0	0	0	0	4
136	true	Empty	MULTISPECIE WP_000081498.1	38 kDa	true unknown	0	5	0	0	0	0	0	0	0	0	0	0
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152	true	Empty	MULTISPECIE WP_000065257.1	13 kDa	unknown	0	0	0	0	0	0	0	3	0	0	0	0
153	true	Empty	cytoplasmic CBW19547.1	45 kDa	unknown	0	0	0	0	0	0	0	0	0	0	0	3
154	true	Empty	MULTISPECIE WP_000753958.1	49 kDa	unknown	0	3	0	0	0	0	0	0	0	0	0	0
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156	true	Empty	30S ribosom: CBW19488.1	14 kDa	unknown	0	0	0	0	0	0	0	2	0	0	0	0
157	true	Empty	type III restri WP_000910371.1	73 kDa	unknown	0	0	0	0	0	0	0	0	0	0	0	3
158	true	Empty	redox-regula WP_000505857.1	40 kDa	unknown	0	2	0	0	0	0	0	0	0	0	0	0
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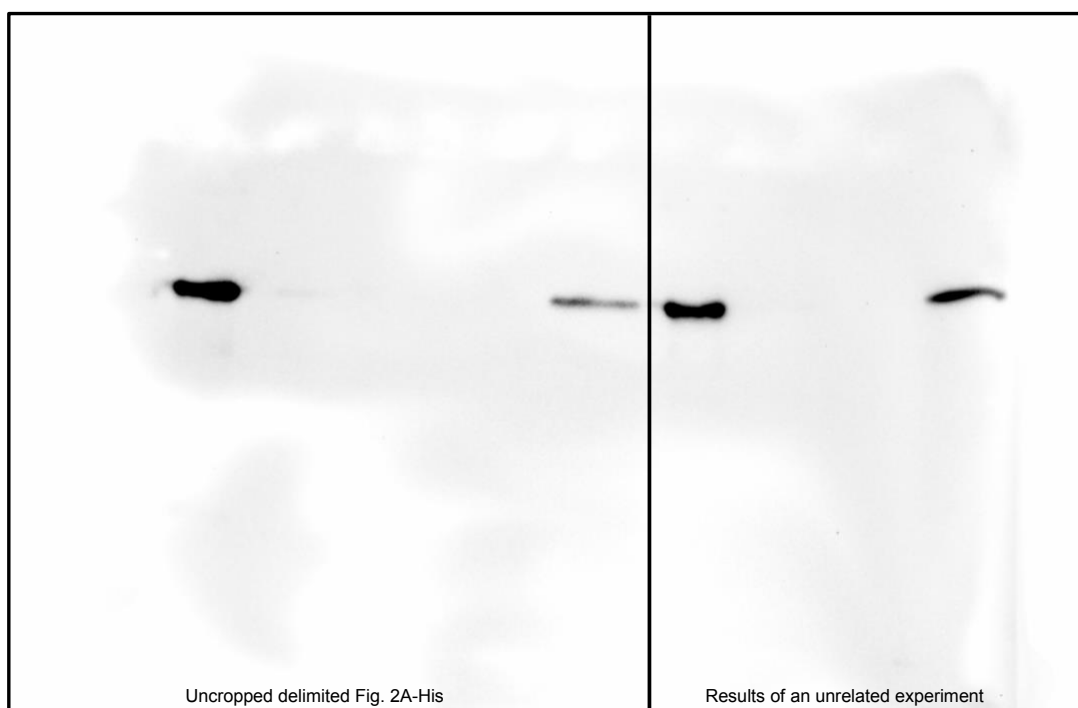
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Uncropped figures



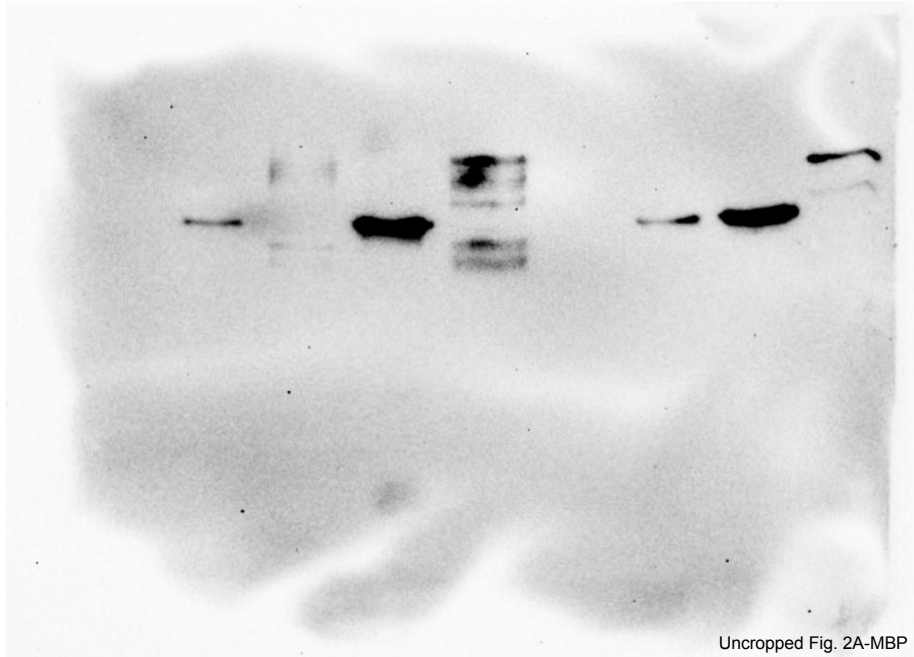


Uncropped Fig. 2A-His

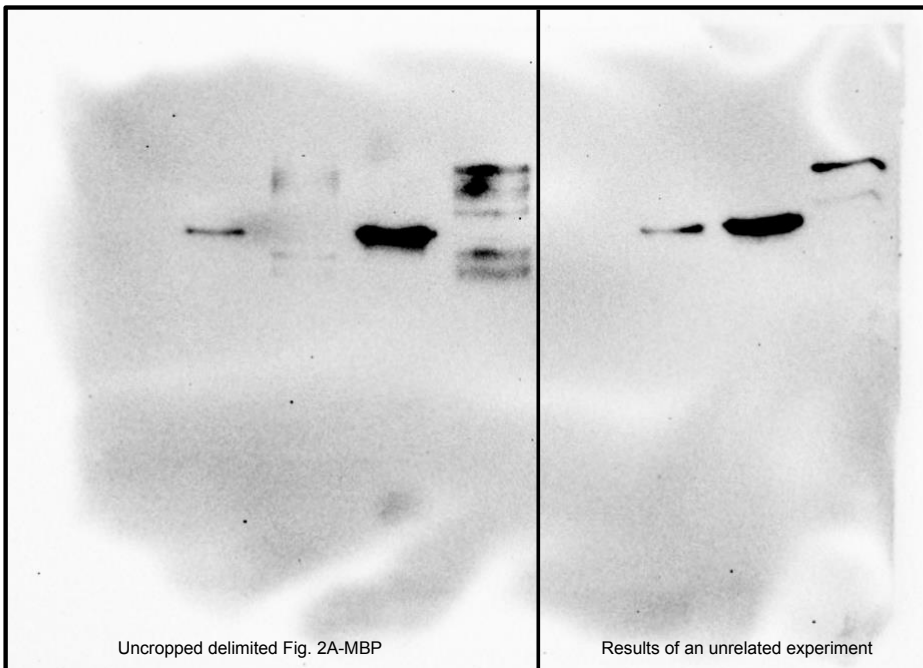


Uncropped delimited Fig. 2A-His

Results of an unrelated experiment

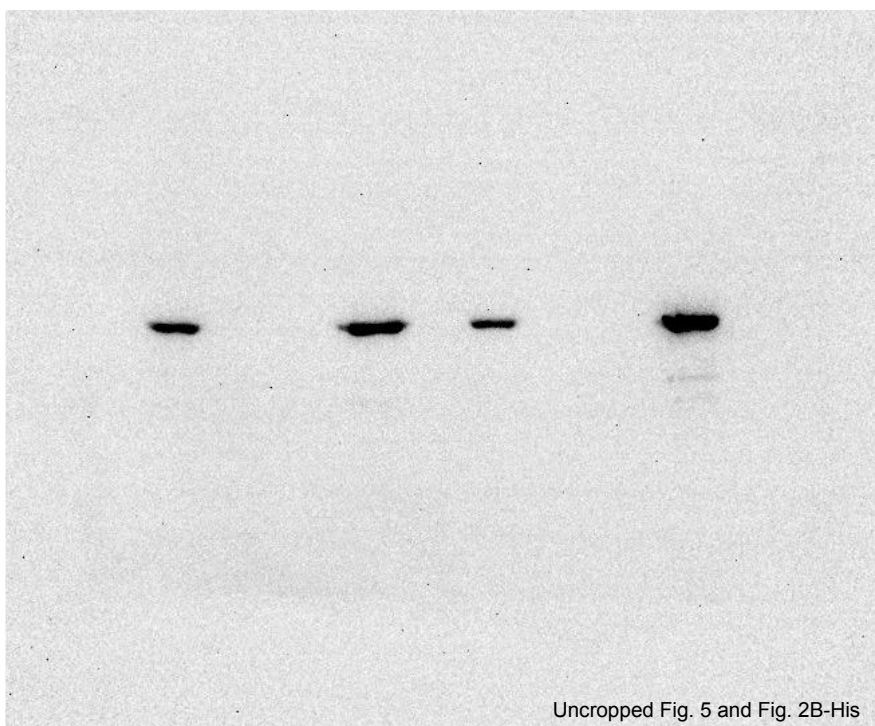


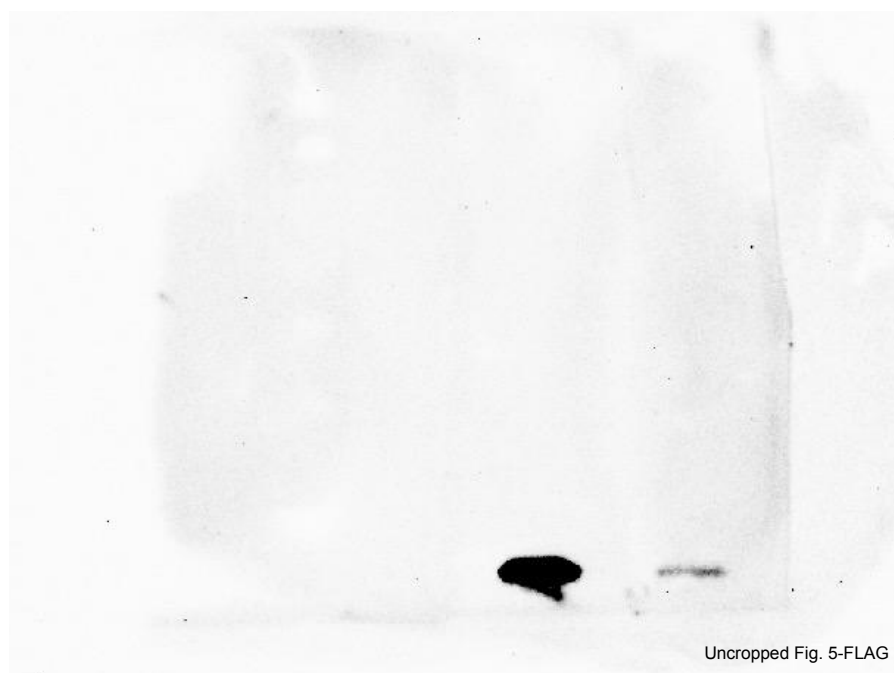
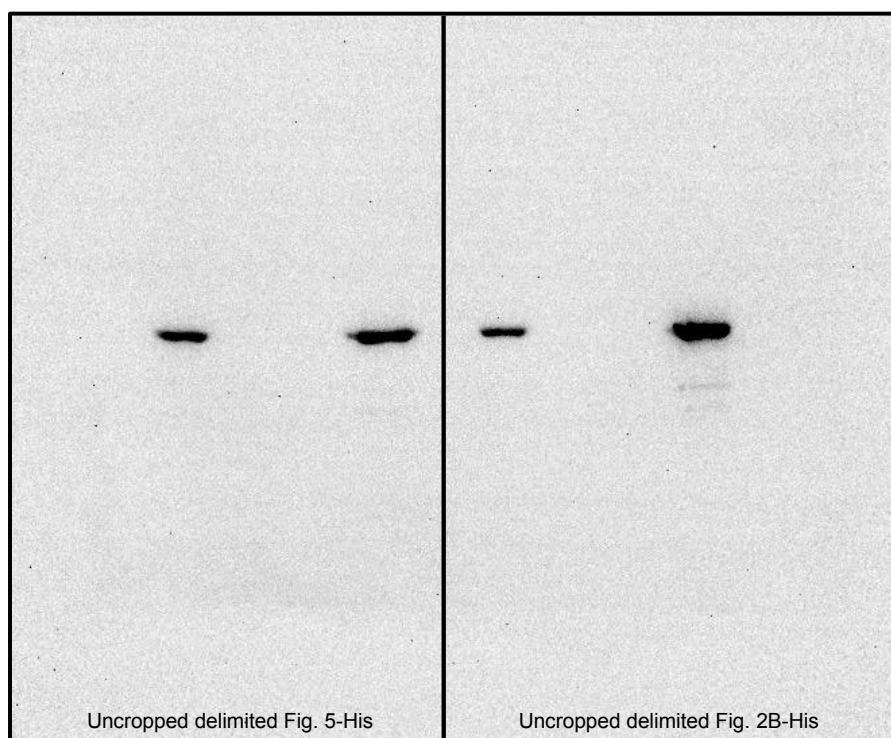
Uncropped Fig. 2A-MBP

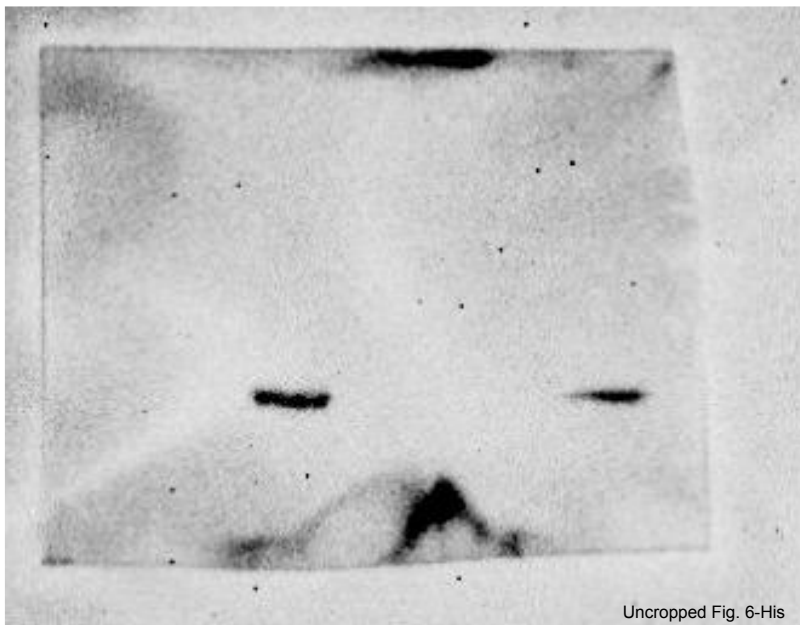


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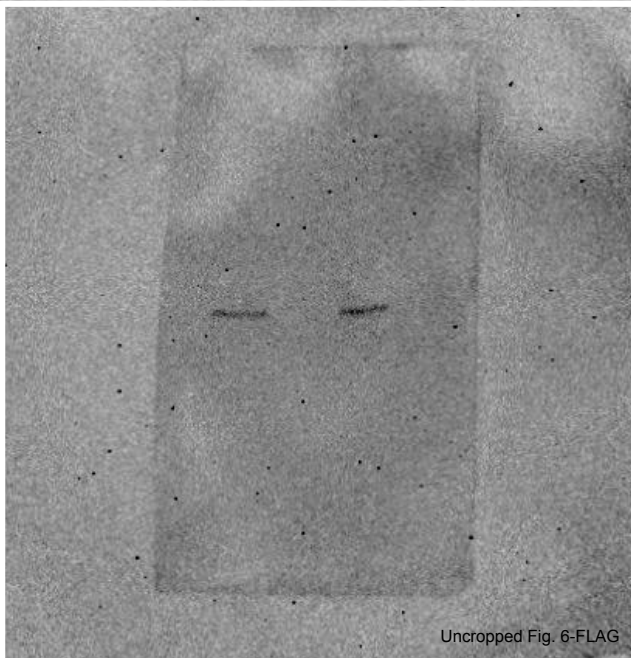
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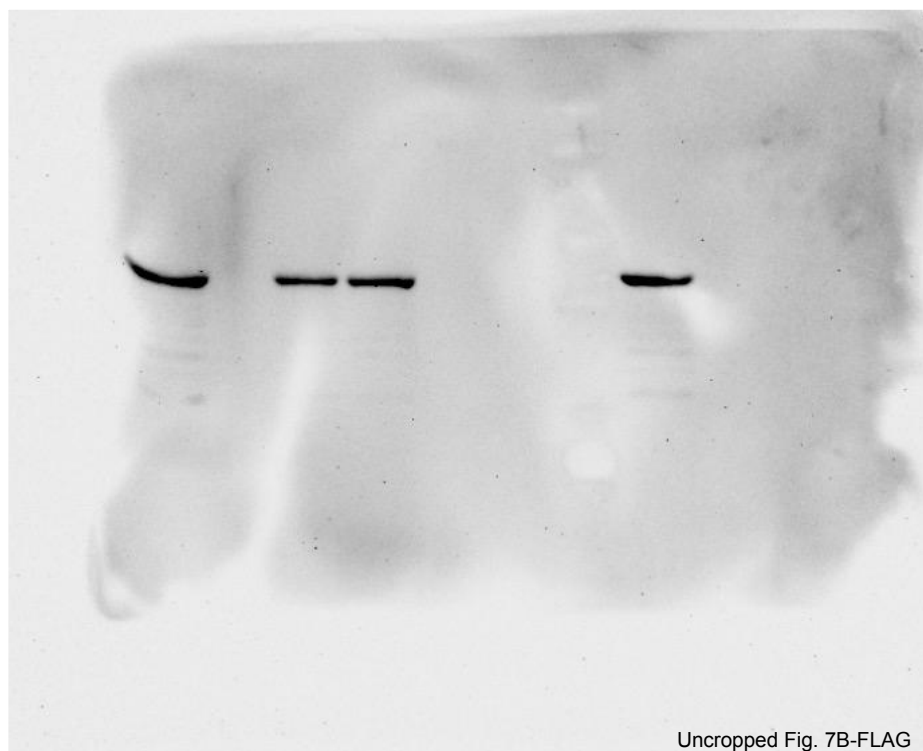
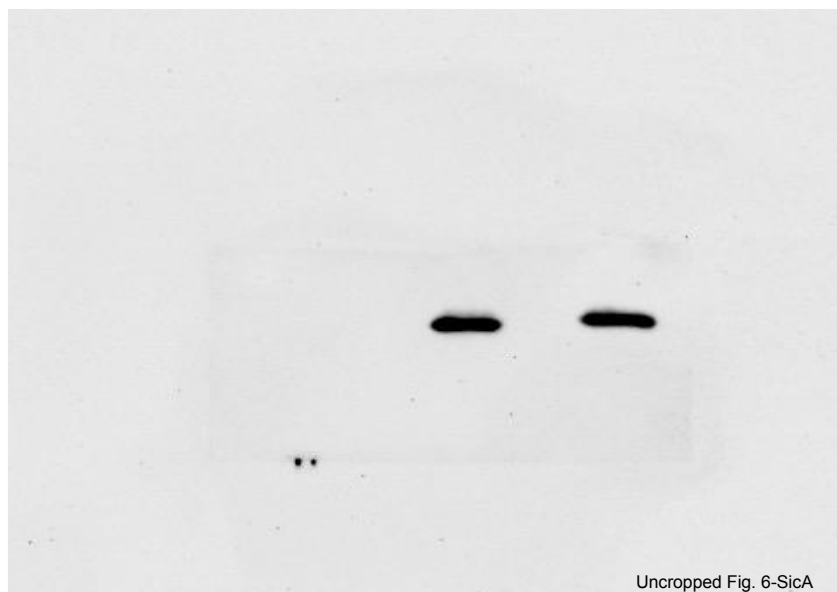


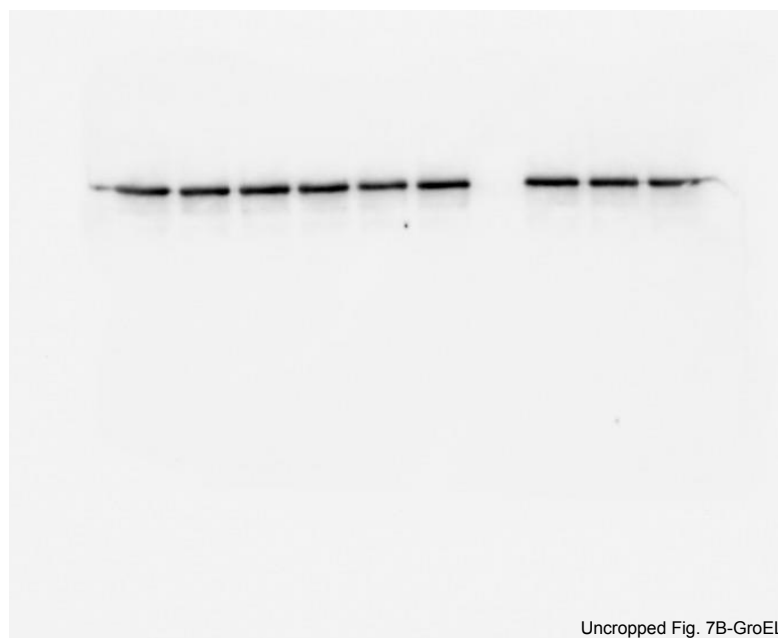
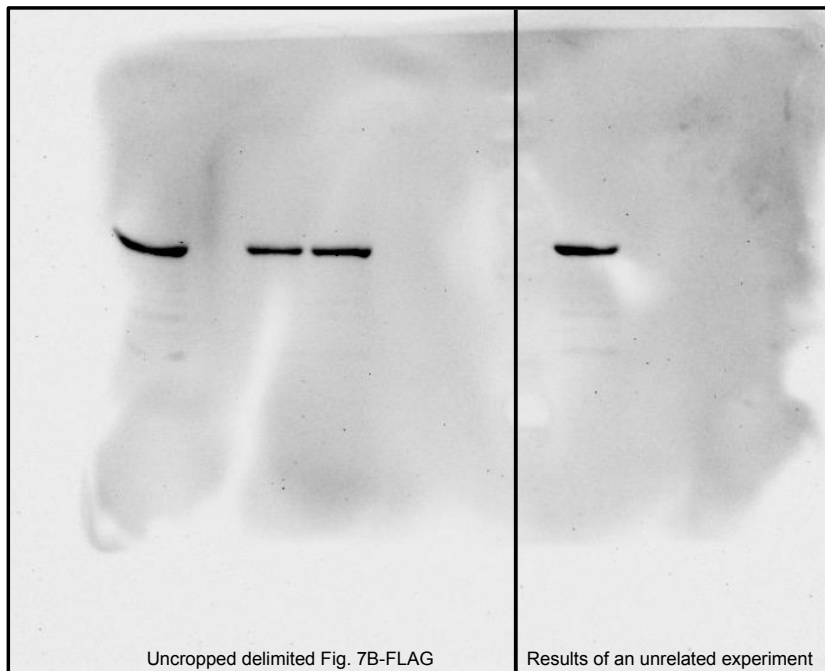


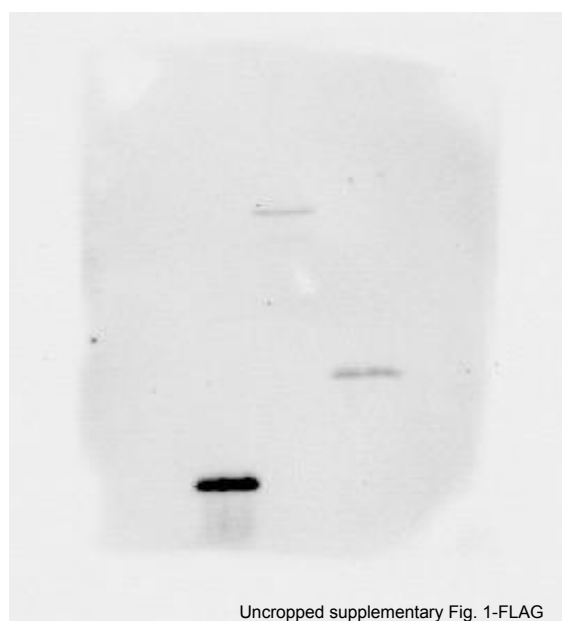
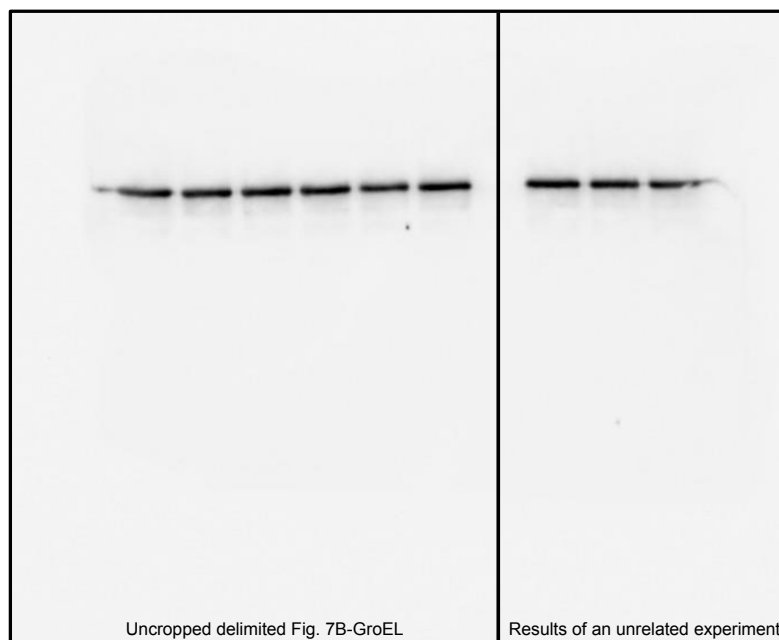
Uncropped Fig. 6-His

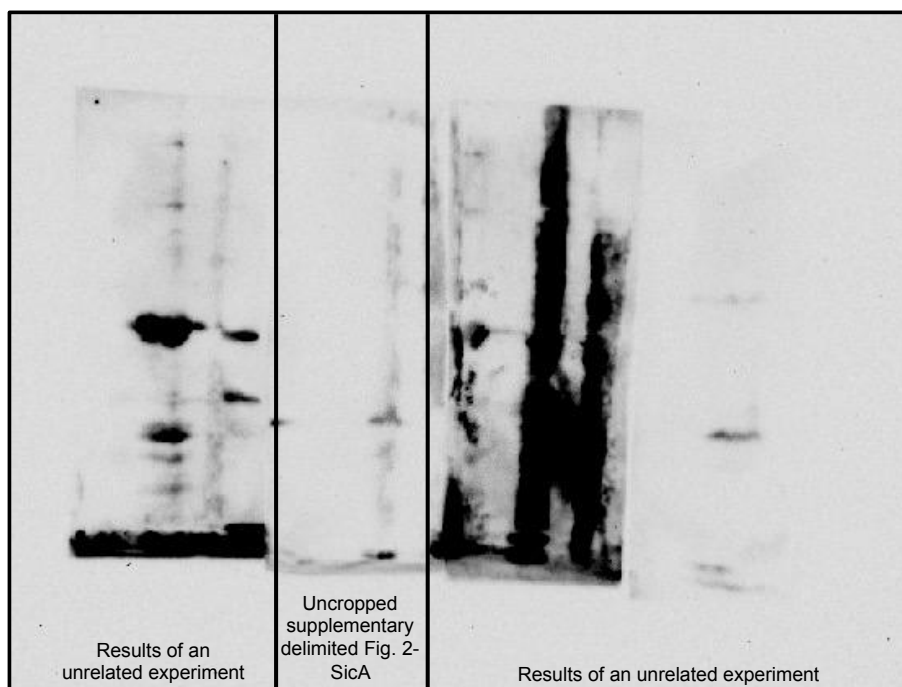
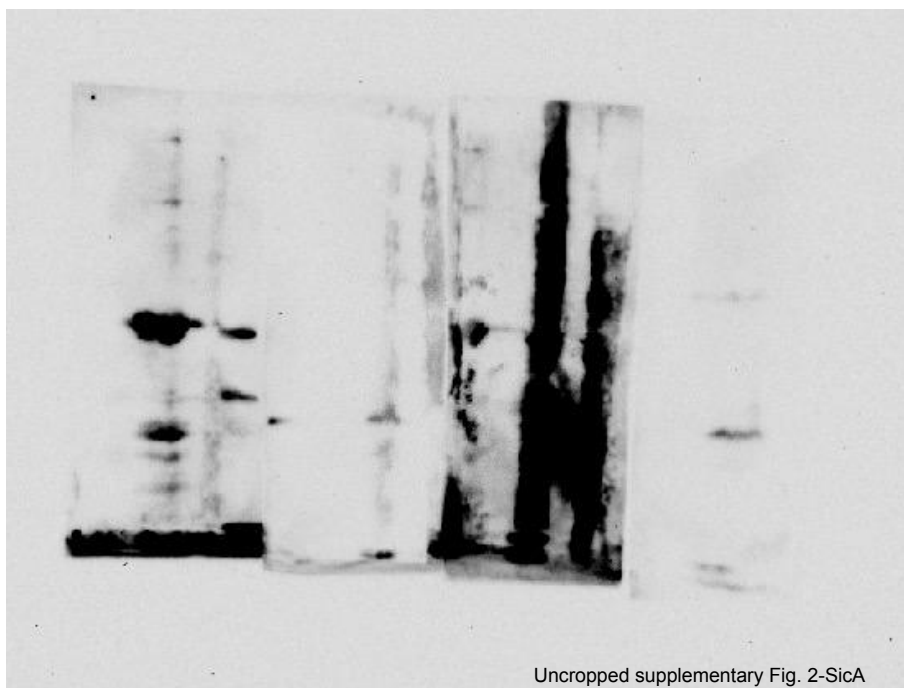


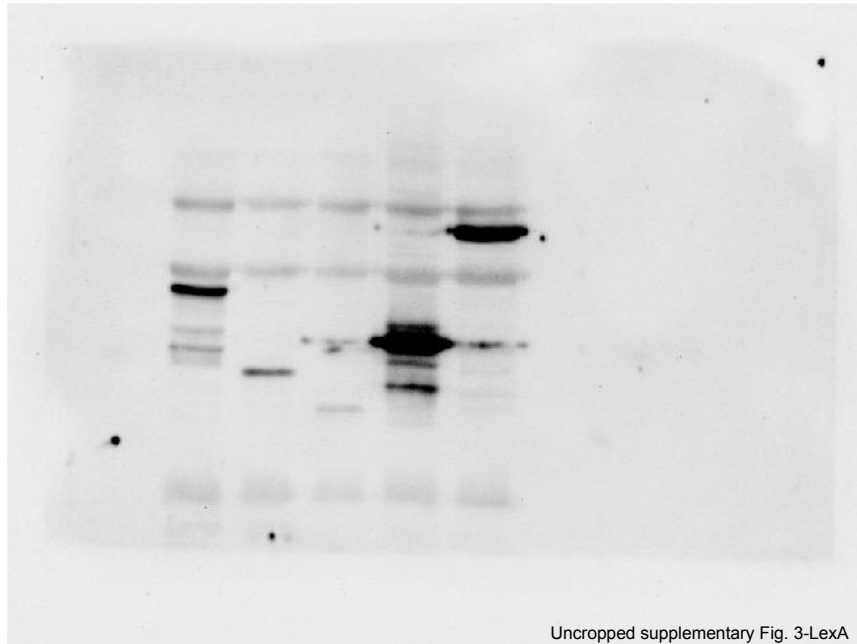
Uncropped Fig. 6-FLAG











Uncropped supplementary Fig. 3-LexA