

Supplementary Figure Legends

Figure S1. Sequence and structure alignment of Lamtor1-3, associated with Figure 2. Structure based sequence alignments of Lamtor 1-3 were carried out by Clustal Omega.

Figure S2. Sequence and structure alignment of Lamtor4-5, associated with Figure 2. Structure based sequence alignments of Lamtor 4-5 were carried out by Clustal Omega.

Figure S3. EM Validation, associated with Figure 5. RagA/C:Ragulator complex experimental averages (A) were compared to RagA/C pre-reconstitution (B) confirming the identity of the heterodimeric GTPases in the whole complex to inform model construction. (B) The gold-standard Fourier shell correlation for the 16.2 Å structure of the RagA/C:Ragulator complex obtained by negative stain EM.

Figure S4. Preparation of active and inactive RagA/C:Ragulator complexes, associated with Figure 6. A. Purification of the RagA/C:Ragulator complex. B. Nucleotide loading of active and inactive RagA/C complexes.

Figure S5. HDX-MS of RagA/C, associated with Figure 6. The first five residues in RagC are derived from the tag.

Lamtor 1

Homo sapiens

	1	10	20	30	40	50	60	70
Homo sapiens	M	G C C Y S	S E N E D S D Q D R E E R K L L L	D P S S P P T K A L N G A E P N Y H .	.	S L P S A R T D E Q A L L S S I L A K T A S N I I D V S A A D		
Mus musculus	.	M G C C Y S	S E N E D S D Q D R E E R K L L L	D P S S P P T K A L N G A E P N Y H .	.	S L P S A R T D E Q A L L S S I L A K T A S N I I D V S A A D		
Bos taurus	.	M G C C Y S	S E N E D S D Q D R E E R K L L L	D P S S P P T K A L N G A E P N Y H .	.	S L P S A R T D E Q A L L S S I L A K T A S N I I D V S A A D		
Danio rerio	.	M G C C F S	S D S E T T D P D G D E V K P L I P D P N Q . E R K P T N G S E R N A D .	.	N L P S N R T D E Q A L L T V I L Q R T A L N I I D V S A V D			
Xenopus tropicalis	.	M G C C Y S	G E T D T G K G D Q G E R E H L L P Q S O S L P N K A P N E S E Q N S T .	.	N N P S A R T D E Q A M L S R I L A K T A S N I I D V S A V E			
Drosophila melanogaster	.	M G A V L S C C R N H S G E E N .	E A L L R E Q Q A .	G Y G S Q Q N A N D E Y D A E Q M R L K E H E H E Q K L L A R E Q E L R D I V A N T N D K L I D I S M I N	.	N A L S R L V Q N T A I N M I N V G A M D		
Yeast (EGO1)								

Homo sapiens

	80	90	100	110	120	130	140
Homo sapiens	S	Q G M E Q H E Y M D R A R Q Y S T R L A V .	L S S S L T H W K K L P P L P S L T .	S Q P H Q V L .	A S E P I P F S D L Q Q V S R I .	A A Y A Y .	
Mus musculus	S	Q G M E Q H E Y M D R A R Q Y S T R L A V .	L S S S L T H W K K L P P L P S L T .	S Q P H Q V L .	A S E P I P F S D L Q Q V S R I .	A A Y A Y .	
Bos taurus	S	Q G M E Q H E Y M D R A R Q Y S T R L A V .	L S S S L T H W K K L P P L P S L T .	S Q P H Q V L .	A S E P V P F S D L Q Q V S R I .	A A Y A Y .	
Danio rerio	S	Q G M E Q H E Y M D R A R Q Y S T R L A V .	L S R T L S Q . K K P V P L P S L T .	S Q P H Q V L .	A A D L V P H A D V Q Q V S K I .	A A Y A Y .	
Xenopus tropicalis	S	Q G M E Q H E C M D R A R Q Y S T R L A K .	L S S N L M D W K K V P P L P S L T .	S Q P H Q V I L .	A S D P V P F A D I Q Q V S K I .	A A Y A F .	
Drosophila melanogaster	C	H S L E H Q E Y A D R I R L Y S Q R L H Q .	Q W N N G Q H A . S I A P K G L L K D V P S H Q F Y .	L S K P T Y P D T A Q M K L F . T E K A H .			
Yeast (EGO1)	N S G I V I Q G T D L Q E A L D K R Q O E E G G D S R E D E R S A G D D N L S G . H S V P S S G S A Q . A T T H O T A P R T N T F T L L T S P D S A K I S K E Q L K L H S N I L N						

Homo sapiens

	150	160	
Homo sapiens	S A L S Q I R V D A K E E L V V Q F G I P .		
Mus musculus	S A L S Q I R V D A K E E L V V Q F G I P .		
Bos taurus	S A L S Q I R V D A K E E L V V Q F G I P .		
Danio rerio	S A I S Q I K V D A K E E L V V Q F G A I P .		
Xenopus tropicalis	S A L S Q I R V D A K E E D L V V Q F G I P .		
Drosophila melanogaster	I S V S H I Q I D H K E A V V V P F R I P .		
Yeast (EGO1)	E I F S Q S Q V N K P G P L T V P F		

Lamtor 2

Homo sapiens

	1	10	20	30	40	50	60
Homo sapiens	M L R P K A L T Q V L S Q A N T G G V Q S T .	L L L N N E G S L L A Y S G Y G D T D .	A R V T A A I A S N I W A A Y D R N G N .				
Mus musculus	M L R P K A L T Q V L S Q A N T G G V Q S T .	L L L N N E G S L L A Y S G Y G D T D .	A R V T A A I A S N I W A A Y D R N G N .				
Bos taurus	M L R P K A L T Q V L S Q A N T G G V Q S T .	L L L N N E G S L L A Y S G Y G D T D .	A R V T A A I A S N I W A A Y D R N G N .				
Danio rerio	M L R P K A L T Q V L S Q A N T G G V Q S T .	L L L N N E G S L L T Y S G Y G D T D .	A R V T A A I A S N I W A A Y D K N G H .				
Xenopus tropicalis	M L R P K A L T Q V L S Q A N T G G V Q S T .	L L L N N E G S L L T Y S G Y G D T D .	A R V T A A I A S N I W A A Y D K N G H .				
Drosophila melanogaster	M L K P K A L T Q V L S Q A N T G G V E N T .	L L L S Q E G A L L A Y S G Y G D K D .	A R I T A A I A S N I W A A Y E K H G R .				
Yeast (EGO3)	M V M L H S K N V K G F L E N T L K P Y D L H S V D F K T S S L Q S S M I I T A T N G G I L S Y A T S N N D V P K N S I N E I N S V N N L K M M S L I K D K W S E D E N D T E .						

Homo sapiens

	70	80	90	100	110	120
Homo sapiens	F N E D N L K F I L M D C M E G R V A I T R V A N L L C M Y A K E T V G F G M L K A K A Q A L V Q Y L E E P L T Q V A A S .					
Mus musculus	F N E D S L K F I L M D C M E G R V A I T R V A N L L C M Y A K E T V G F G M L K A K A Q A L V Q Y L E E P L T Q V A A S .					
Bos taurus	F N E D N L K F I L M D C M E G R V A I T R V A N L L C M Y A K E T V G F G M L K A K A Q A L V Q Y L E E P L T Q V A A S .					
Danio rerio	F N E D N L K F I L M D C M E G R V A I T R V A N L L C M Y A K E T V G F G M L K A K A Q A L V Q Y L E E P L T Q V A A S .					
Xenopus tropicalis	F N E D N L K F I L M D C M E G R V A I T R V A N L L C M Y A K E T V G F G M L K A K A Q A L V Q Y L E E P L T Q V A A S .					
Drosophila melanogaster	F N E D N L K F I L M D C M E G R V A I T R V S N L L C M Y A K E T V G F G M L K A K A Q A L V Y Y L E E P L N Q V S S S .					
Yeast (EGO3)	E Q H S N S C Y P V E I D S F K T K I Y T Y E M E D L . H T C V A Q I P N S D L L L F I A E G S F P Y G L L V I K E R A M R E L T D I F G Y K L G .					

Lamtor 3

Homo sapiens

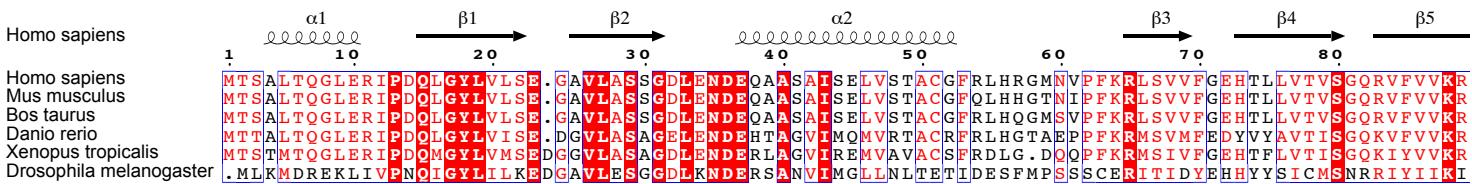
	1	10	20	30	40	50	60	70	80	90
Homo sapiens	M A D D L K R F L Y K K L P S V E G L H A I V V S D R D G V P V I K V A N D N A P E H A L R P G F L S T F A I L A T D Q G S K L G L S K N K S I I C Y Y N T Y Q V V Q F N R L P L V V .									
Mus musculus	M A D D L K R F L Y K K L P S V E G L H A I V V S D R D G V P V I K V A N D N A P E H A L R P G F L S T F A I L A T D Q G S K L G L S K N K S I I C Y Y N T Y Q V V Q F N R L P L V V .									
Bos taurus	M A D D L K R F L Y K K L P S V E G L H A I V V S D R D G V P V I K V A N D N A P E H A L R P G F L S T F A I L A T D Q G S K L G L S K N K S I I C Y Y N T Y Q V V Q F N R L P L V V .									
Danio rerio	M A D D L R L S Y L Y K K L P S V E G L H A I V V T D R D G A P V I K V A N D N A P E H A L R P A F L S T F A I L A T D Q G S K L G L S K N K S I I C Y Y N T Y Q V V Q F N R L P L V I .									
Xenopus tropicalis	M A E E L R R F L Y K K L T S V D E L H A I V V S D R D G V P V I K V A N E N A P E H A L R P A F L S T F A I L A T D Q G S K L G L S K N K S I I C Y Y S T Y Q V V Q F N Q L P L V V .									
Drosophila melanogaster	M S D D I K K Y L D G L L Q K V S G L Y V I Q I T D R D G V P L L R V S Q E K N V D F A L M P S P I P T F T T A C D Q A S K L G L I G R N K T I I S M Y S N Y Q V V Q M N K L P L I L .									

Homo sapiens

	100	110	120	
Homo sapiens	S F I A S S S A N T G L I V S L E K E L A P L F E E L R Q V V E V S .			
Mus musculus	S F I A S S S A N T G L I V S L E K E L A P L F E E L I K V V E V S .			
Bos taurus	S F I A S S S A N T G L I V S L E K E L A P L F E E L R Q V V E V S .			
Danio rerio	S F I A S S S A N T G L I F S L E K E L V P L I E E L R Q V V E V A .			
Xenopus tropicalis	S F I A S C N A N T G L I L S L E E E L G S L F K E L R Q V V E I S .			
Drosophila melanogaster	T F V G A E N C N T G H I L A L E H Q V D G Y L E D I K Q A V T E A .			

Figure S1

Lamtor 4



Homo sapiens

Sequence alignment of the REP1 domain across various species:

Species	Sequence
Homo sapiens	Q N R G R E P I D V
Mus musculus	Q N R G R E P I D V
Bos taurus	Q N R G R E P I D V
Danio rerio	Q N N O R E P V I V
Xenopus tropicalis	Q N V V R E P I S V
Drosophila melanogaster	S K S Q N G V T T T T S S S S N S V Y N D A S D S G A V L A

Lamtor 5

Homo sapiens

Homo sapiens
Mus musculus
Bos taurus
Danio rerio
Xenopus tropicalis
Drosophila melanogaster
Yeast (EGO2)

1 10 20 30 40 50 60 70 80
 MEPGAGHLDGHRAGSPSLRQALCDGSAMVFSKERRQRCTVINFVPLEAPLRSRQVTAEACGGEGRAVPLGSEPEWSVGG
MPFRKERGRGAALNFVPLETPSRRPPRSSHVIEVCGEGGSVLLRGPGGLRTACG
 r
 α1
 90

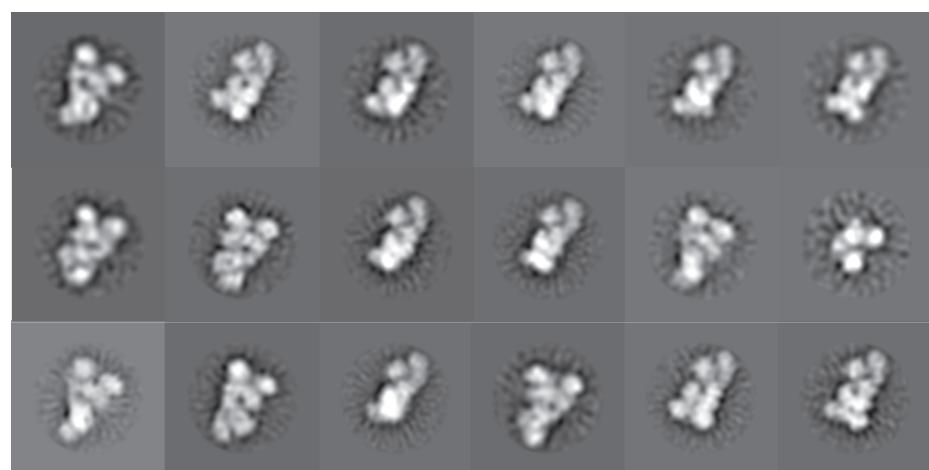
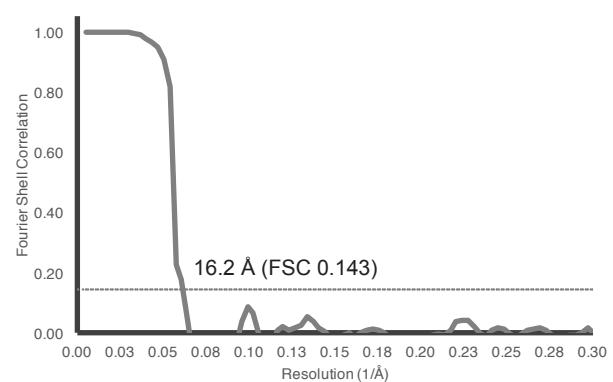
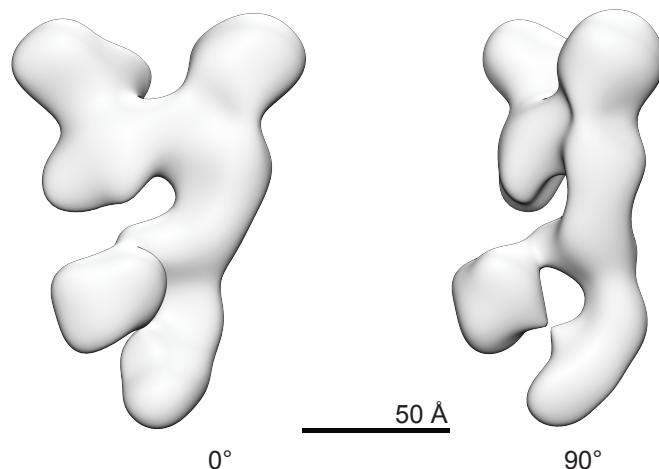
Home options

Homo sapiens	100	110	120	130	140	150	160	170
Homo sapiens	L E D T M K N P S I V G V L C T D S Q G L N L G C R G T L S D E H A G V I S V I L A Q Q A A K L T S D P T D I P V C V C L E S D N G N I M I Q K H D G I T V A V H K M A S							
Mus musculus	L E D T M K N P S I V G V L C T D S Q G L N L G C R G T L S D E H A G V I S V I L A Q Q A R L T S D P T D I P V C V C L E S D N G N I M I Q K H D G I T V A V H K M A S							
Bos taurus	L E D T M K N P S I V G V L C T D S Q G L N L G C R G T L S D E H A G V I S V I L A Q Q A A K L T S D P T D I P V C V C L E S D N G N I M I Q K H D G I T V A V H K M A S							
Danio rerio	L D D T M K N P A I V G V L C T D A Q G H N L G C R G S L S D E H G G V V S V I L A K Q A A S L T K D A T D C P T V C V C L E S D C G N I L V R R H G T I T L A V H K M A S							
Xenopus tropicalis	L E D T M K N P S I V G V L C T D S Q G L N L G C R G T L S D E H A G V I S V I L A Q Q A A K L T S D P T D I P V C V C L E S D N G N I M I Q K H D G I T V A V H K M A S							
Drosophila melanogaster	I A E I A A R Q D T V I G A L L A N R Q G L C L G T K D G I D S P D N V S S G I G M A S E P Q Y A A K L T S D P T D V P V C V C L E S D N G I V M I Q K H D H L T V A V H K V T S							
Yeast (EGO2)	. M E A E K Q S D I K G T I A F D T H G N V I E S T T G V G S O R I E D I G D . . . L S K V T I D A E G F A Q . . . V Q G D S L L V H L Y K R N D I T L A V Y T S A Q							

Homo sapiens

Homo sapiens	.
Mus musculus	.
Bos taurus	.
Danio rerio	.
Xenopus tropicalis	.
Drosophila melanogaster	S N
Yeast (EGO2)	.

Figure S2

A**B****C****Figure S3**

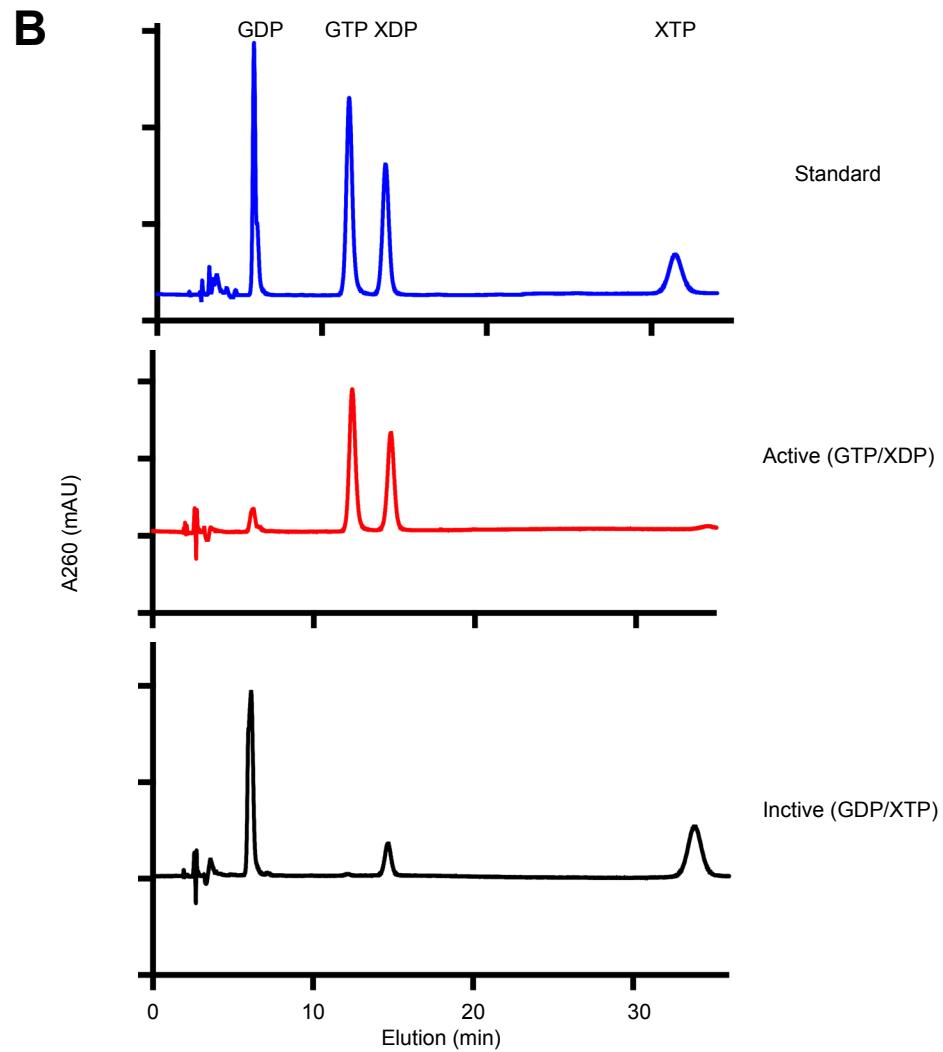
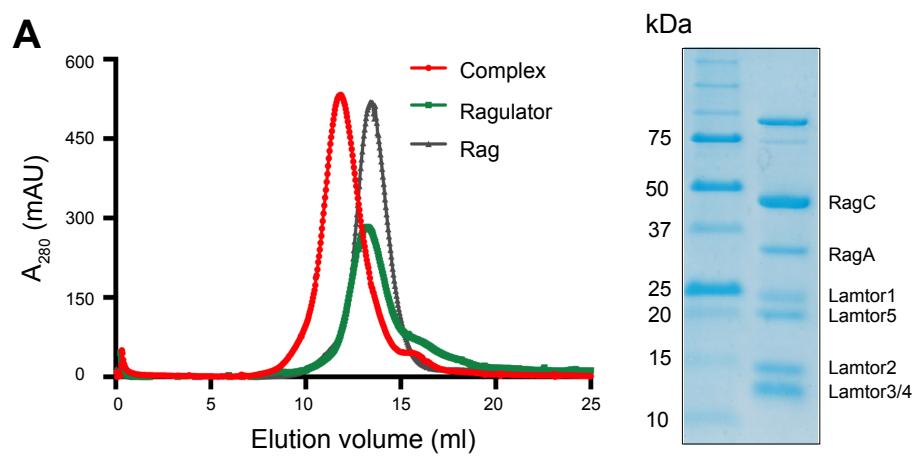
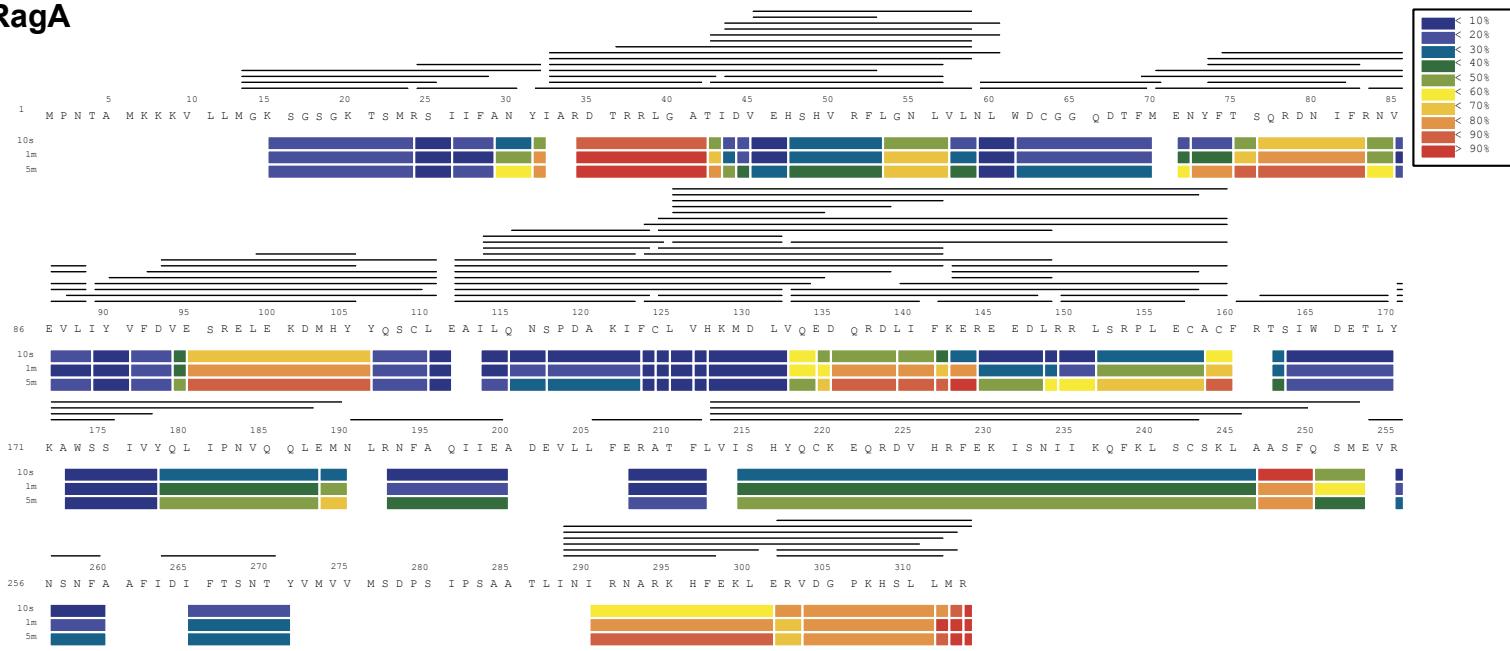


Figure S4

RagA



RagC

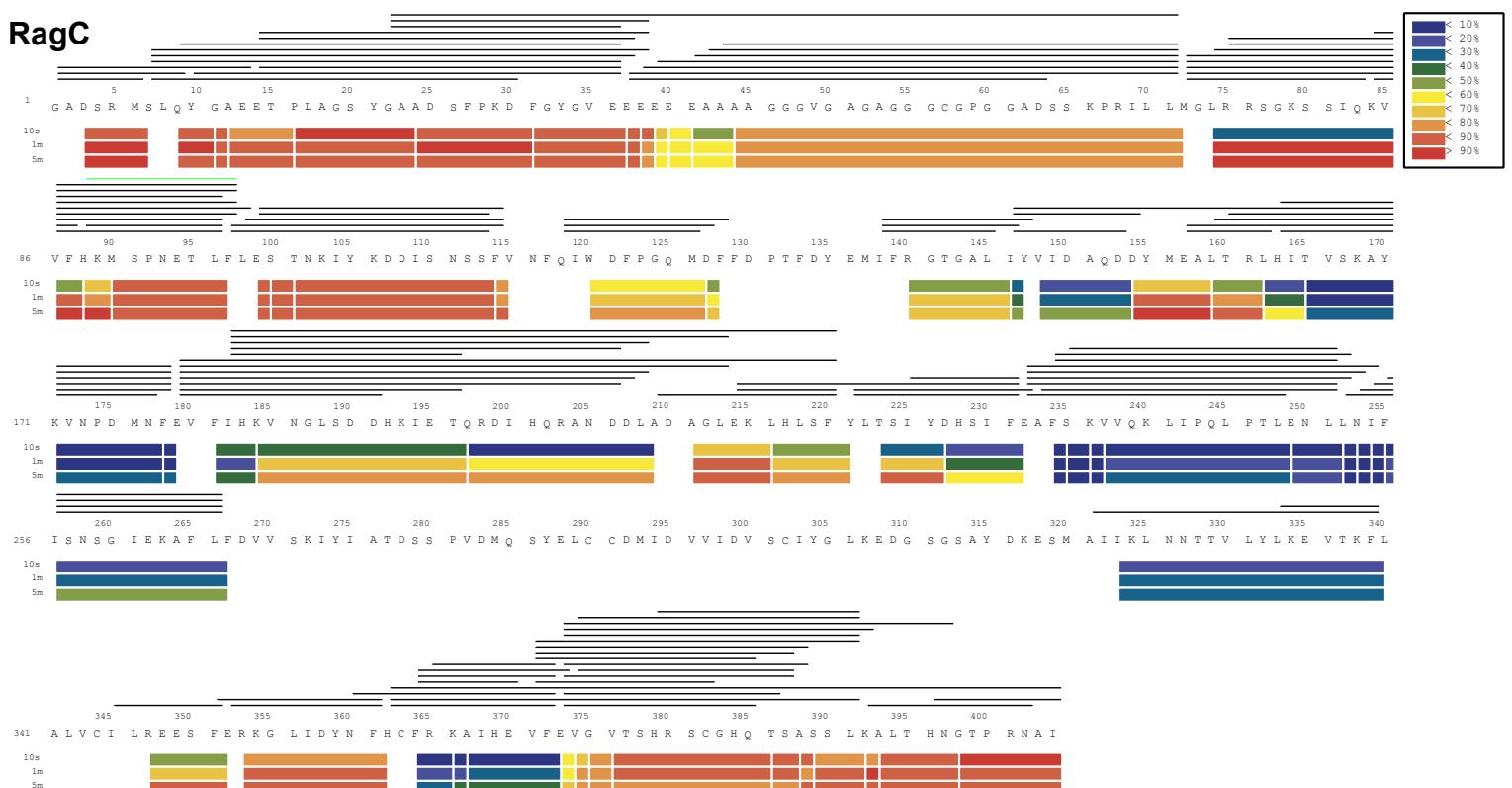


Figure S5