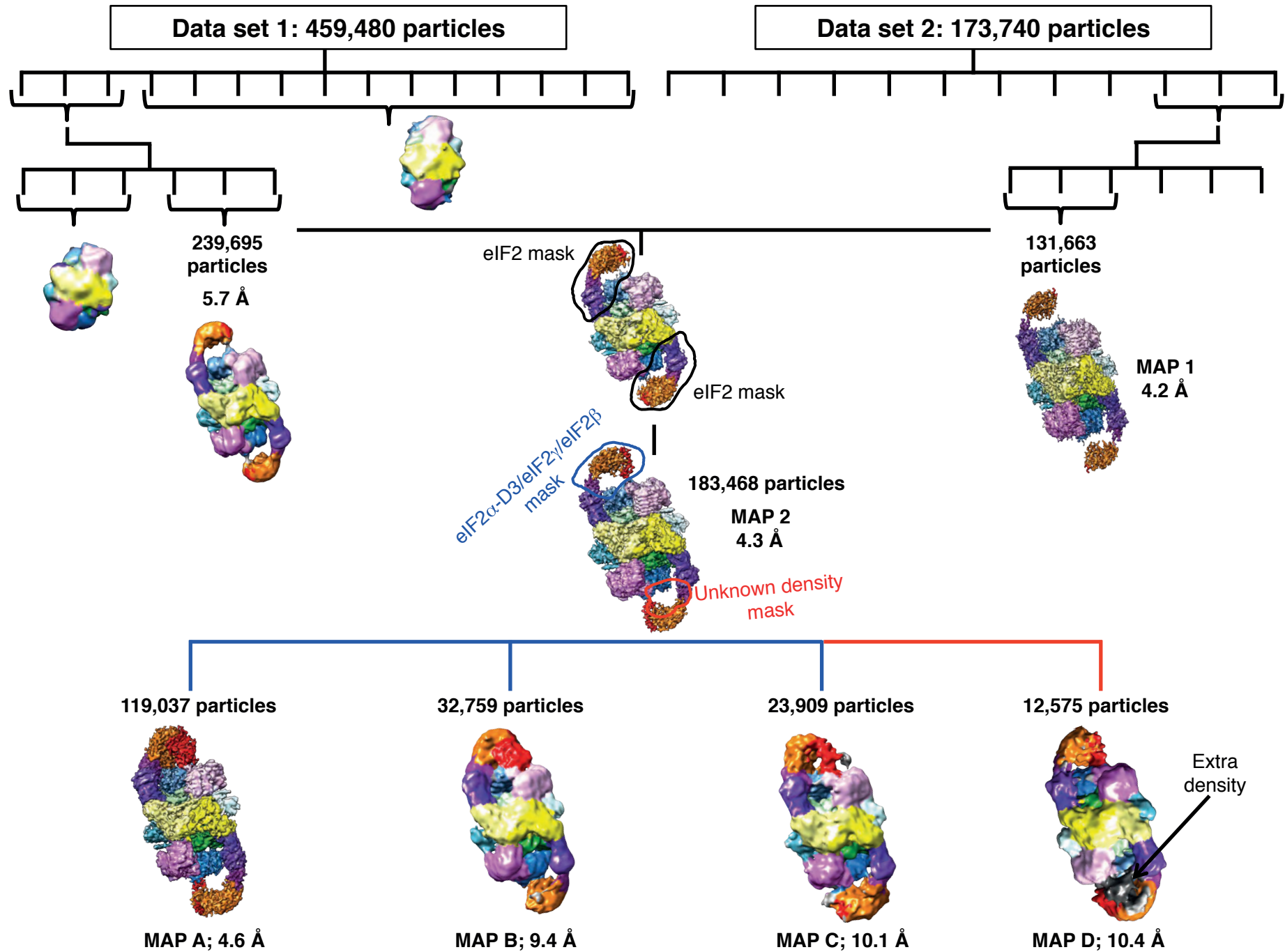
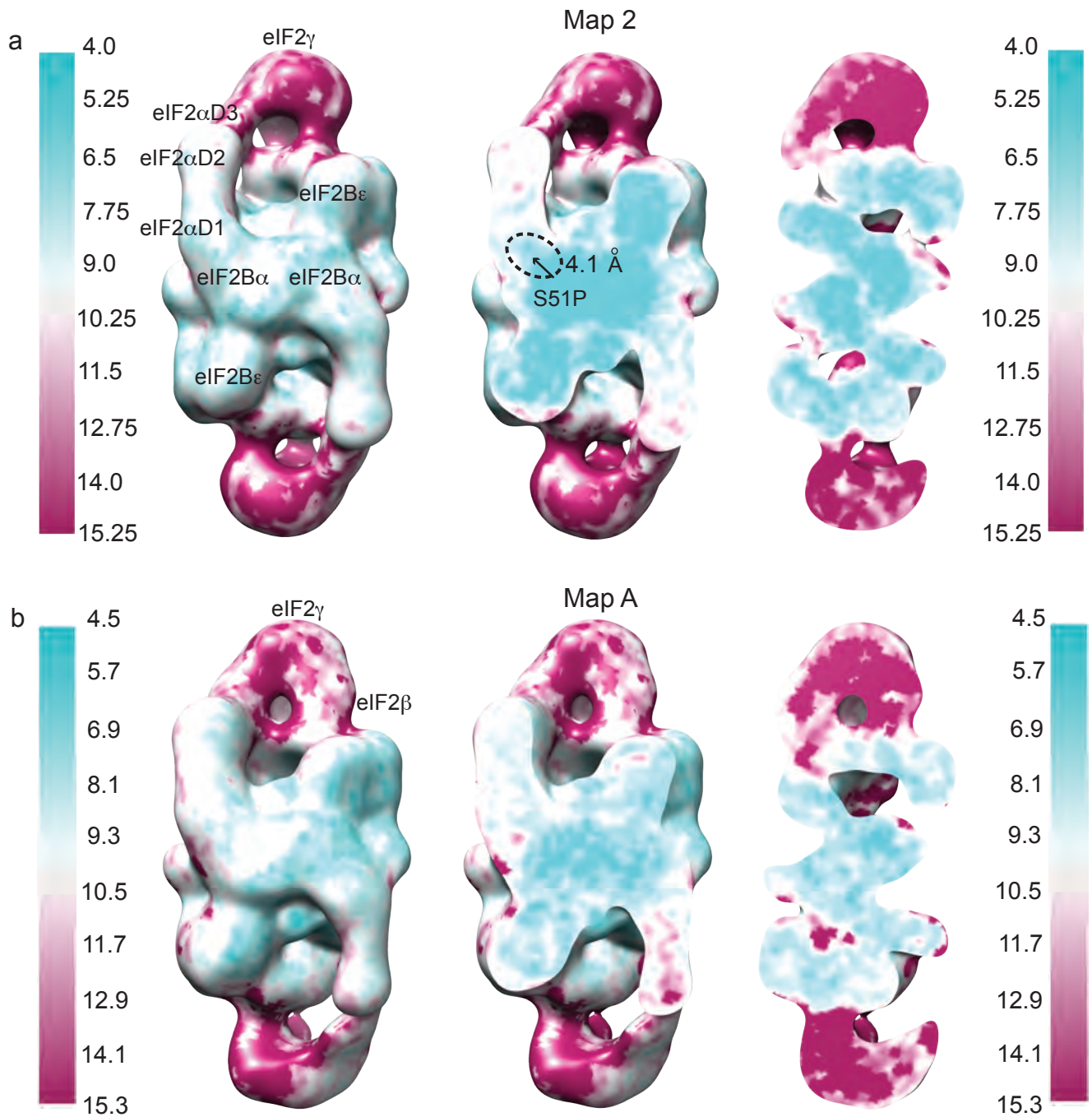


Supplementary Information
Supplementary Figure 1. 3D- classification scheme.



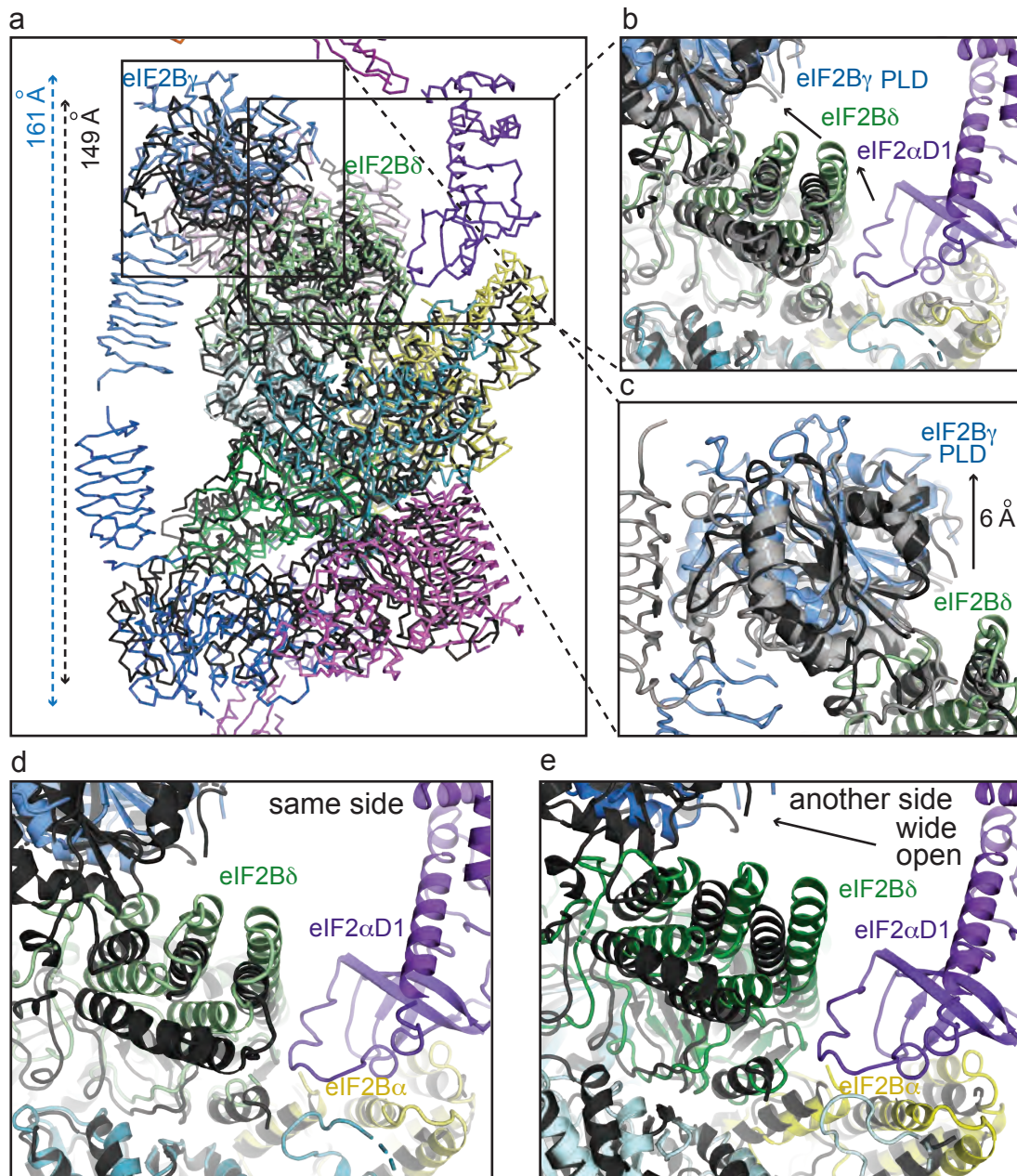
Supplementary Figure 2. Local resolutions of eIF2B - eIF2(α P) complex.



(a) Local resolutions of eIF2B - eIF2(α P) complex in map 2 (related to Fig. 1a) showing surface (left) and two cross-sections around eIF2 α -D1 binding site and location of Ser-51(52 sequence numbering).

(b) Same as in (a), but in in map A (related to Fig. 1b).

Supplementary Figure 3. Superposition of the ISRIB bound human eIF2B to eIF2B - eIF2(α P) complex.



(a) Superposition of eIF2B-eIF2(α P) complex with human ISRIB bound eIF2B (black) (PDB 6CAJ) showing elongation of eIF2B hetero-decamer towards catalytic poles upon binding of eIF2 by ~ 12 Å.

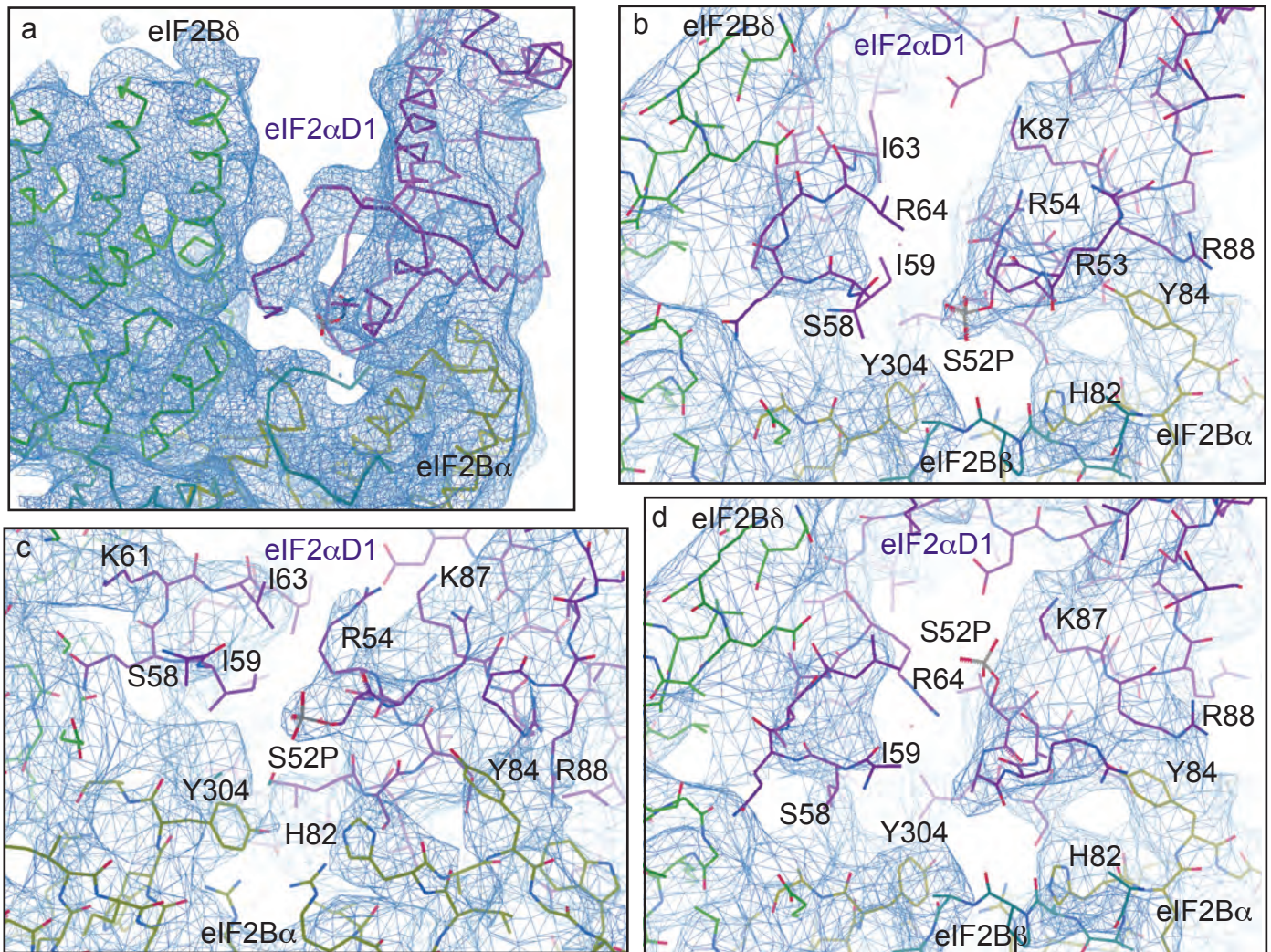
(b) Same superposition as in (a), but also including *S. pombe* eIF2B structure (grey) (PDB 5B04), shows that elongation of eIF2B is mainly induced by closure eIF2B α and δ around eIF2 α -D1 displacing eIF2B γ outwards.

(c) Same superposition as in (a), showing displacement of eIF2B γ in eIF2B-eIF2(α P) complex in one of the eIF2B poles.

(d) The binding site of eIF2 α -D1 contacting the superposed eIF2B α subunit showing reduced contacts with eIF2B δ and local rearrangement of eIF2B α interacting helices (indicated with an arrow).

(e) The other binding site of eIF2 α -D1 showing that on this side the binding pocket for eIF2 α -D1 formed by eIF2B α and δ is wide open.

Supplementary Figure 4. Structural model fitting of eIF2 α -D1 in a density map.



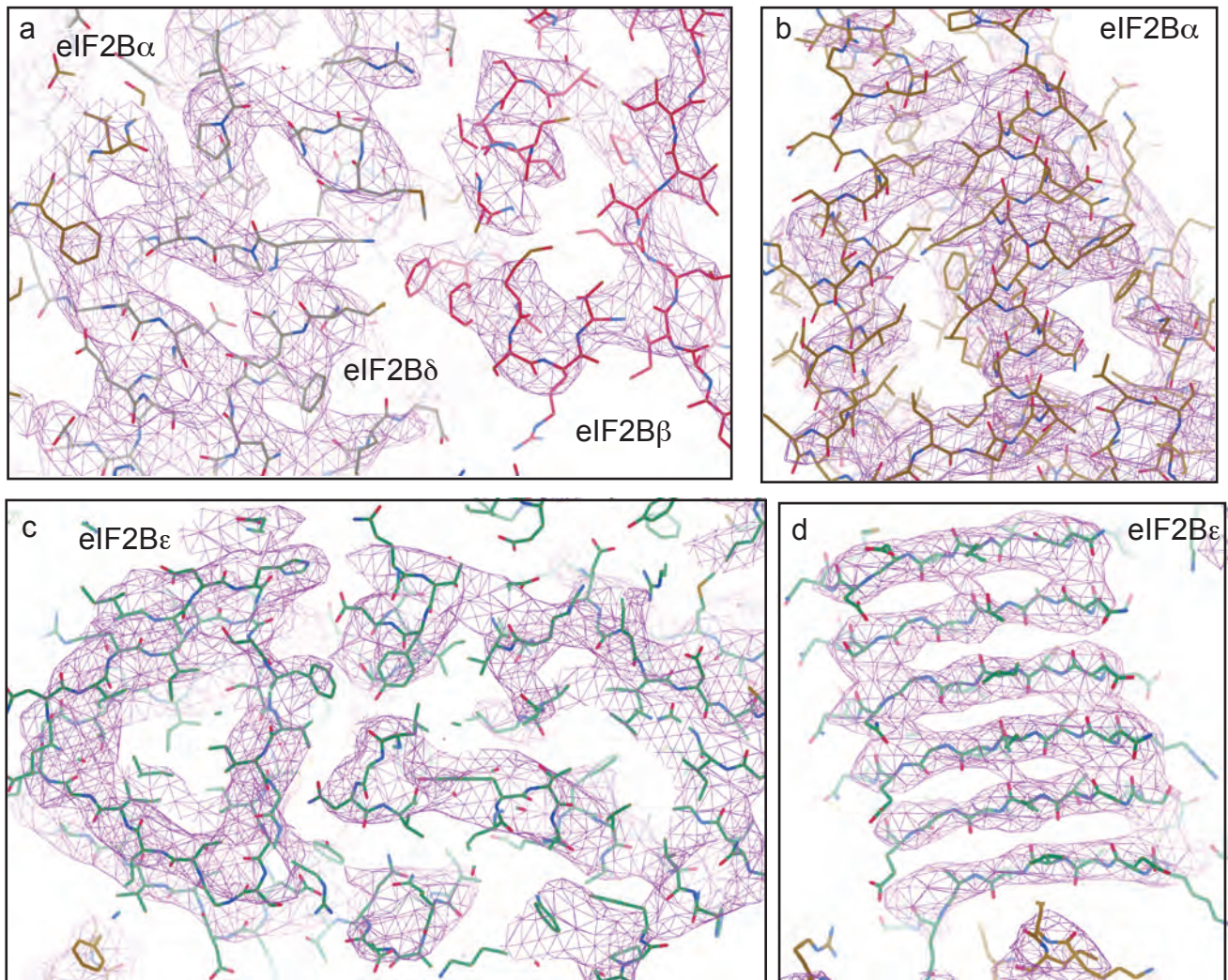
(a) Overall density for the contact of eIF2 α -D1 with eIF2B α and δ subunits.

(b) Close view of the density around Ser51(P) (S52 sequence numbering) and our structural model fitting into the density.

(c) Same as in (b) but at different orientation.

(d) Fitting of c structural model cut through of the same region as in (b) in our density map.

Supplementary Figure 5. Examples of structural models fitting in density maps.



(a) Density for eIF2B δ in contact with eIF2B β and eIF2B α subunits.

(b) Density for eIF2B α subunit.

(c) Density for eIF2B ϵ PLD domain.

(d) Density for eIF2B ϵ L β H domain.

Supplementary Figure 6. Multiple sequence alignment of eIF2B regulatory subunits using Clustal Omega.

a

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eIF2B $\alpha$ _HUMAN 40 E T I Q G L R A N L T S A I E T - L C G V D S S V A V S S G G E L F L R F I S L A S L E Y S D 85
eIF2B $\alpha$ _YEAST 40 E T A A E M I N T I K S S T E E L I K S I P N S V S L R A G C D I F M R F V L R N L H L Y G D 86
eIF2B $\alpha$ _SCHPO 53 K T I S E F M D I L Q N G S N T L K E G V Q N N I S L S A G C D I F Q R F V T R S L H D V G D 99
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b

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residue interacting
with eIF2 $\alpha$  in eIF2B
 $\beta/\delta$  binding site

eIF2B $\delta$ _HUMAN 248 L S R D L V N K L K P Y M S F L T Q C R P L S A S M H N A I K F L N K E 283
eIF2B $\delta$ _YEAST 315 L S R N L T S Y L S H Q I D L L K K A R P L S V T M G N A I R W L K Q E 350
eIF2B $\delta$ _SCHPO 186 L S R H L T T H I N S Q I A Y L V S T R P L S I S M G N A I R F L K L E 221

eIF2B $\delta$ _HUMAN 284 I T S V G S S K R E E E A K S E L R A A I D R Y V Q E K I V L A A Q A I 319
eIF2B $\delta$ _YEAST 351 I S L I D P S T P D K A A K K D L C E K I G Q F A K E K I E L A D Q L I 386
eIF2B $\delta$ _SCHPO 222 I S V L D I D L T D D E G K E L L E K I D S Y I R D R I I I A G Q V I 257

residues interacting
with eIF2 $\alpha$  in eIF2B
 $\alpha/\delta$  binding site
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c

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eIF2B $\beta$ _HUMAN 71 A Q P S E T T V G N M V R R V L K I I R E E Y G R L H G R S D - - - - - E - S D - 104
eIF2B $\beta$ _MOUSE 71 A Q P S E T T V G N M V R R V L K I I R E E Y G R L H G R S D - - - - - E - S D - 104
F1NAP1_CHICK 71 A Q P S E T T V G N M V R R V L K V I R E E Y G R L H G R S E - - - - - E - S D - 104
M7B0Y2_CHEMY 80 P E P S D G - - - - - - - - - - - V R D H E V P H E P L R G R S E - - - - - E - S D - 104
I3KDJ8_ORENI 74 A Q P S E T T V G N M I R R V L K I I R E E Y A R S R G S S E - - - - - E - A D - 107
O76863_DROME 66 A L P Q E T V T S N I A R R I L K L T R E E F D L L H A K V Q H F A D - - - - - D - S Q - 101
Q9XWD5_CAEEL 6 A E R S E L I I N I G L M I T K L A R D E V L Q K K G G G - - - - - - - - - - - 97
eIF2B $\beta$ _YEAST 77 A H P T A F S C G N V I R R I L A V L R D E V E E D T M S T T V - - - - - - - - - - 108
eIF2B $\beta$ _SCHPO 68 A Q P T E F S C G N I I R R I L R L I R E E Y Q E L L K T A D E N E K L I V S S S N S 110

eIF2B $\beta$ _HUMAN - - - - - Q Q E S L H K L L T S G G L N - - - - - - - - - - - 119
eIF2B $\beta$ _MOUSE - - - - - Q Q E S L H K L L T S G G L S - - - - - - - - - - - 119
F1NAP1_CHICK - - - - - Q Q D S L H K L L T S G G L S - - - - - - - - - - - 119
M7B0Y2_CHEMY - - - - - Q Q E S L H K L L T S G G L S - - - - - - - - - - - 119
I3KDJ8_ORENI - - - - - Q E S L H K L L T S G G L S - - - - - - - - - - - 121
O76863_DROME - - - - - A S L S L H K L V T Q T S E S - - - - - - - - - - - 117
Q9XWD5_CAEEL - - - - - - - - - - - - - - - - E P E P I Y T L W R D - - - - - D D - - - E T 112
eIF2B $\beta$ _YEAST - - - - - - - - - - - T S T - S V A E P L I S S M F N L L Q K P E Q P H Q N R K N S 138
eIF2B $\beta$ _SCHPO S S P S Q K R D I P S N E K L V Q S H E P V S V Q M Y S S M L N L L G R P T L E S P T H S K T 157

human eIF2 $\alpha$  R52 R53
N132 E135

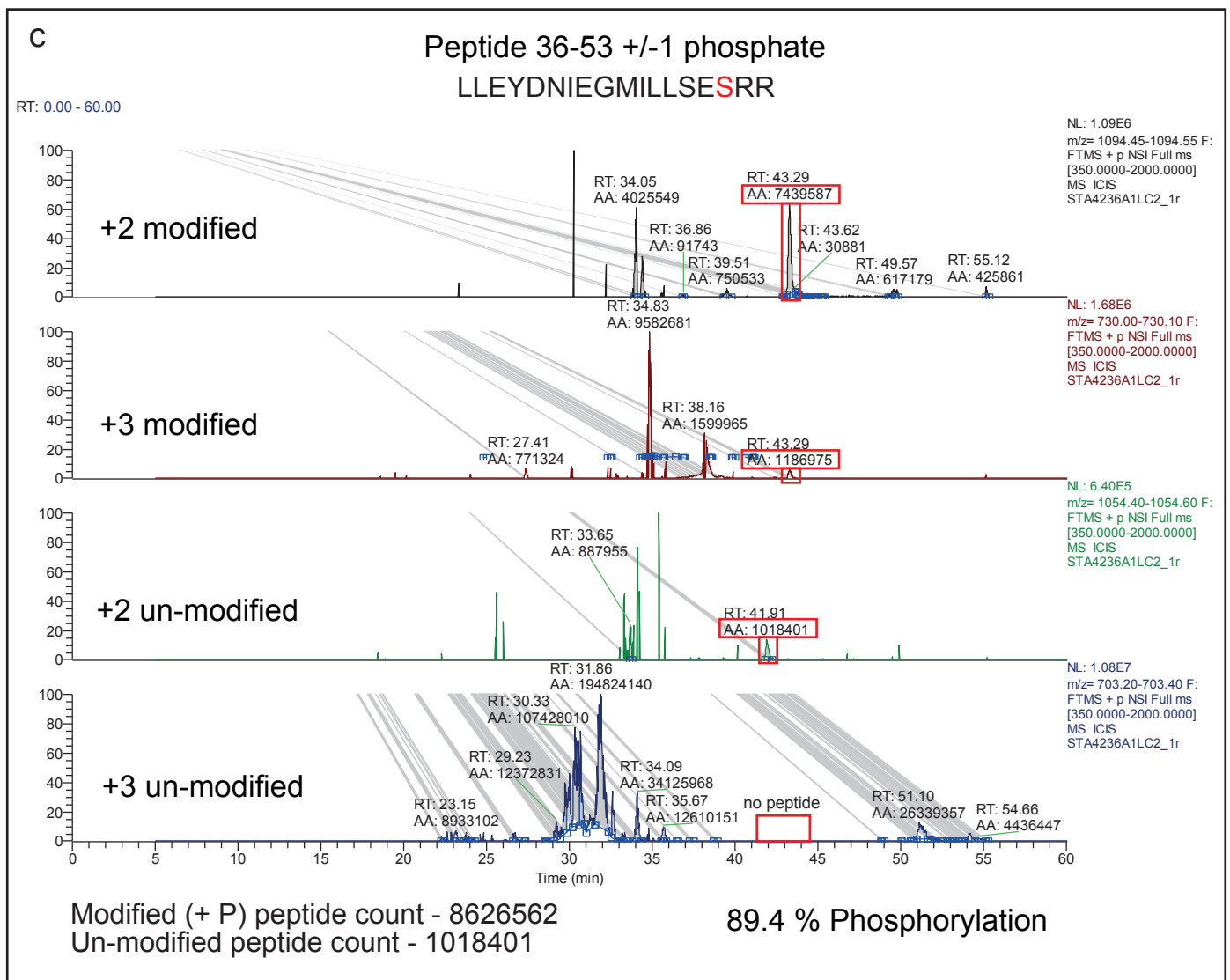
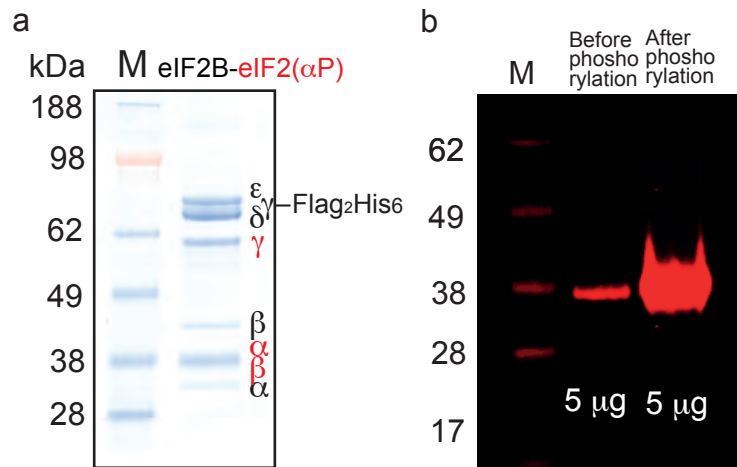
eIF2B $\beta$ _HUMAN - E - D F S F H Y A Q L Q S N I I E A I N E L L V E L E G T M E N I A A Q A L E H I H S N E V 164
eIF2B $\beta$ _MOUSE - E - D F S F H F A P L K A N I I E A I N E L L V E L E G T M E N I A A Q A L E H I H S N E V 164
F1NAP1_CHICK - E - D F S T P Y P S L R A N V I E A I N E L L I E L E G T T D N I A M Q A L E H I H S N E V 164
M7B0Y2_CHEMY - E - D F S T P Y P P L R A N V I E A I S E L L I E L E G T T D N I A M Q A L E H I H S N E V 164
I3KDJ8_ORENI - E E N F R Q H F A P L K A N V I E A I N E L L T E L E G T T D N I A M Q A L E H I H S S E I 167
O76863_DROME V S V D Y S V P Q H G L R E A L L D H L Q E V E T E L E T S S E N I C V Q A E H I H S S E I 164
Q9XWD5_CAEEL I K K L K T A D M K K I K K D L Q A S I K E L V T E I E S S R E C I A S Q S T E L L F N N D V 158
eIF2B $\beta$ _YEAST S G S S S M K T K T D Y R Q V A I Q G I K D L I D E I K N I D E G I Q Q I A I D L I H D H E I 185
eIF2B $\beta$ _SCHPO V G D S R V T G G M D M R A V I I S G I Q D V I D E L D K I N T D I E V Q S M D H L H S N E I 204
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(a) Alignment of human, *S. cerevisiae* and *S. pombe* eIF2B α sequences in the region of eIF2 α binding site between α and δ subunits of eIF2B. Residues important for the interaction with eIF2 α [1] and this paper are boxed.

(b) Alignment of human, *S. cerevisiae* and *S. pombe* eIF2B δ sequences in the region of both eIF2 α binding sites. Residues that important for the interaction with eIF2 α [1] and this paper are boxed.

(c) Alignment of human (*Homo sapiens*), mouse (*Mus musculus*), chicken (*Gallus gallus*), green sea-turtle (*Chelonia mydas*), fish (*Oreochromis niloticus*), fruit fly (*Drosophila melanogaster*), *Caenorhabditis elegans*, *S. cerevisiae* and *S. pombe* eIF2B β sequences in the region of alternative eIF2 α binding site between β and δ subunits of eIF2B. Residues important for the interaction in in this site based on the structures of human eIF2B-eIF2 complex [1, 5] are boxed. Alignment shows conservation of these residues (N132 and E135 according to human numbering) in vertebrates, however they are not conserved in yeast. Also part of the loop - tether (boxed), which in yeast interacts with eIF2B α , is missing in the species where alternative binding site evolved. *Drosophila melanogaster* lost the tether, however alternative binding site is not entirely conserved.

Supplementary Figure 7. Biochemical and MS analysis of eIF2B-eIF2 α (P) complex.

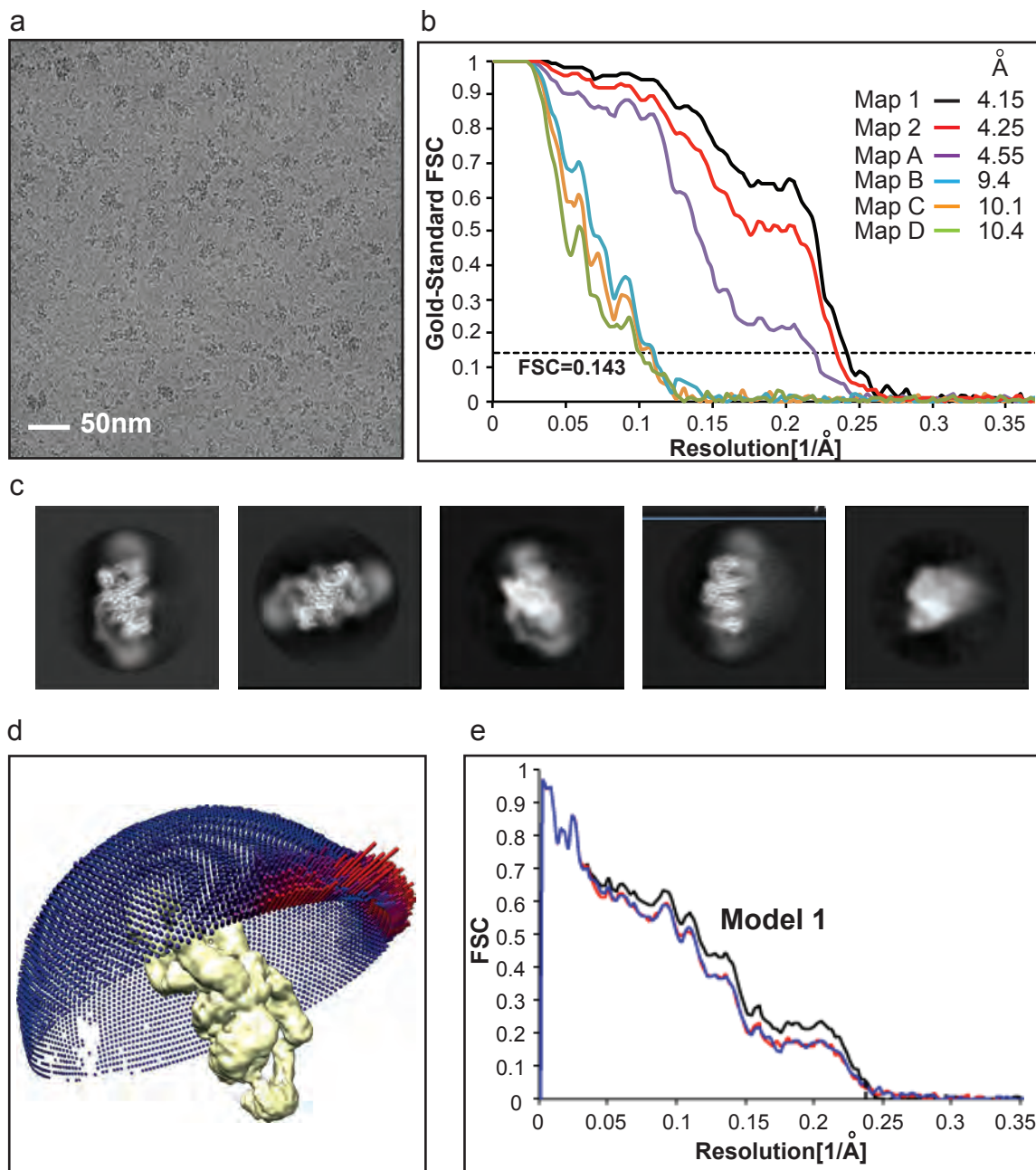


(a) SDS PAGE of eIF2B - eIF2(α P) complex with eIF2B subunits labelled in black and eIF2 subunits in red.

(b) Western blotting of eIF2 using antibodies specific against human eIF2 α (P) (Invitrogen 44-728G) before and after phosphorylation with PKR (5 μ g of eIF2 protein was loaded in each lane).

(c) Mass spectra of the 2+ and 3+ charge states of eIF2 α peptide 36-53 (LLEYDNIIEGMILLSESRR) after eIF2 was phosphorylated with PKR. Phosphorylation (89.4 %) was calculated based on the area of the peaks of modified (phosphorylation) and non-modified peptides in both charge states.

Supplementary Figure 8. Cryo-EM methods.



(a) Typical micrograph of eIF2B - eIF2(α P) complex (see methods for details).

(b) FSC curves.

(c) Representative 2-D classes after 2-D classification of eIF2B - eIF2(α P) complex.

(d) Orientation distribution plot for the eIF2B-eIF2 complex map from dataset I. Efficiency of the reconstruction measured with cryoEF⁴ is 0.62 (good > 0.8; bad < 0.5) which shows that the complex suffers from some preferred orientation.

(e) FSC curve of the model versus map.

1. Adomavicius, T., Guaita M., Zhou Y, Jennings, M.D., Latif Z, Roseman, A.M., Pavitt, G.D. The structural basis of translational control by eIF2 phosphorylation. *BioRxiv*, doi: <https://doi.org/10.1101/501411> (2018).
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