

Additional files 3 for Joo et al.

Article title: Common ancestry of heterodimerizing TALE homeobox transcription factors across Metazoa and Archaeplastida.

Authors: Sunjoo Joo, Ming Hsiu Wang, Gary Lui, Jenny Lee, Andrew Barnas, Eunsoo Kim, Sebastian Sudek, Alexandra Z. Worden, and Jae-Hyeok Lee.

The following Supporting Information is available for this article:

Fig. S1. Alignment of TALE homeodomain sequences of the Archae-algal collection.

Fig. S2. Homology domain alignment of KNOX sequences.

Fig. S3. GLX class is defined by PBL-Chloro domain.

Fig. S4. Extensive homology of Picsa_04995 and Klefl_00021_0250 to Chocr_41034 indicates their classification as PBX-Red.

Fig. S5. Intron-retention pattern suggests parallel evolution of KNOX and non-KNOX group classes from common duplicated TALE ancestors.

Fig. S6. Alignment of Class-B TALE proteins in volvocales.

Fig. S7. Alignment of Mam-A TALE proteins in mamiellophyceae.

Fig. S8. Alignment of Mam-B TALE proteins in mamiellophyceae.

Fig. S9. Full-length proteins are necessary for mamiellophyceae non-KNOX TALE proteins to form heterodimers.

Fig. S10. TALE interaction network defined by this study using yeast-two-hybrid assays.

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Fig. S15. ELK-domain alignment.

Fig. S2. Homology domain alignment of KNOX sequences. MEIS class outgroup sequences are included at the bottom. Class label is on the left. KN-A, KN-B, ELK, HOMEODOM, KN-C1, and KN-C2 domains are labeled on the top. Class groups are labeled by colored bars on the left next to the gene names. Yellow, light green, and green shades in sequences show more than 60%, 80%, or 100% similarity in each column. Gaps between KN-A and KN-B and between KN-B and ELK have been eliminated.

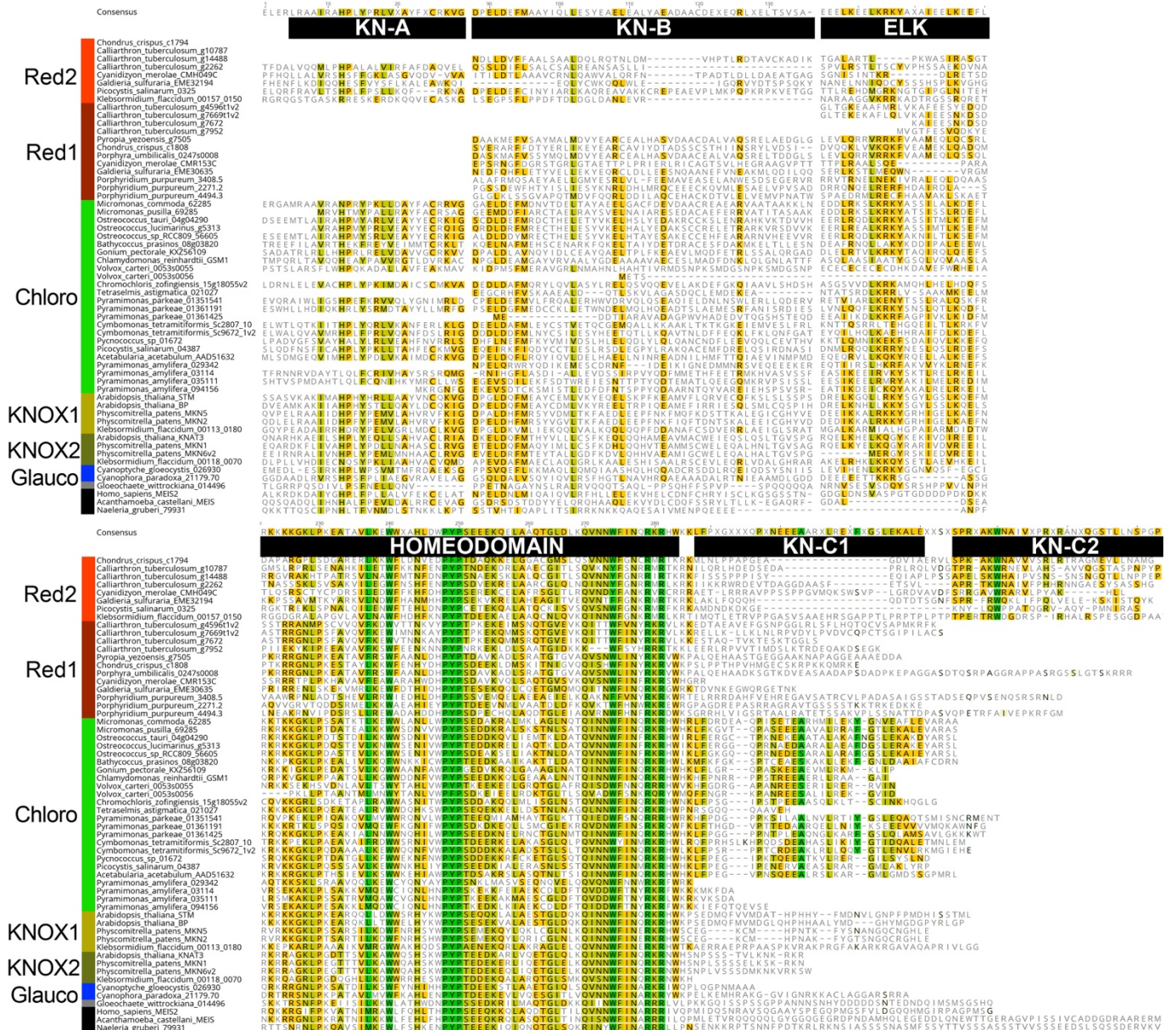


Fig. S3. GLX class is defined by PBL-Chloro domain. (A-C) Three alignments are adjusted with inserting gaps for direct comparison among different PBL-Chloro domains. (A) GLX-Chloro class members. (B) GLX-Basal class members. (C) Three Viridiplantae sequences with strong MEINOX homology domain. PBC-homology is shared among the Chlorophyta non-KNOX sequences.

(a)

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Picocystis_salinarum_02499      VQKQEDVHPL-----VQKIYDLQNKYVKLIVRLDECGQLCRGLVY-----
Chlamydomonas_reinhardtii_GSP1 KKKGAAAAA-----AAQP P P P P L P R T M D R R A A L S G A A G-----
Gonium_pectorale_KXZ41783      AGSGAGVGG-----GLAAPAPVLVWTTERRAAQSAAVS-----
Volvox_carteri_0026s0144       PPVAGRVAHA-----DLPL P P P P P S P S Q E L Q M A K Y T P A I S-----
Chromochloris_zofingiensis_15g18055v2 L R K W G L V G T V-----T D G T F S S P-----E E L A-----
Klebsormidium_flaccidum_00010_0090 E L V S E V V D T C-----L G G G A P G P G A V A-----E E L A-----

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(b)

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Crustomastix_stigmata_064173    I Q Q P E E T R S S-----C S K A R G K P E P Q S A N I - V E N N R Y P - N F S N S F C-----
Dolichomastix_tenuilepis_064008 M P S G V N G D L-----L R R K L E S R V E R T R N A I I C A A E E A E G - R G E E G E Q-----
Cymbomonas_tetramitiformis_Sc10992_1 I A V T S A P G A-----S Q Y Y P P P P M A - V R L E D V-----N P-----
Pyramimonas_amyliifera_049838  I M E P E A D G S-----G G T K F R S P G K A T F-----Q R A P-----S Q-----
Pyramimonas_parkeae_01336589   M M N H A M D G G-----L S L S S E E Q K P I I - V E T K S L-----Q K D D A-----

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(c)

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Picocystis_salinarum_02499      VQKQEDVHPL-----VQKIYDLQNKYVKLIVRLDECGQLCRGLVY-----
Prasinococcus_capsulatus_017584 V I K Q S D M E P L K A I Q A N A S I - V E E V K H L M S I Y K K E L E K V D R T C Y E Q G H R L L D E S N R I M D A G A D V E M-----
Klebsormidium_flaccidum_00159_0100 A V E R P T R G G R E G P K A S A V I D I G G R R K S K R G H T T A E E E A I Q T G V P Q R E R P R R K S A R L S K S R T D V E L-----
Guillardia_theta_137502        M K K T S E S E S S L T L Q L N T P V - M Q Q I K L I I E N Q F N S E K C K V D Q V C E D V C Q R M-----

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MEINOX homology Cys-rich

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Picocystis_salinarum_02499      T Y V G R S -- A V E G I I P F Q H E F Q T M R C R G - H G Q L-----G L S ----A E I Q L K Q A T I R Q M L D K - K - Q K C L E H F E R H V E
Chlamydomonas_reinhardtii_GSP1 A F M A A C D E A V R E I L L A M N F S D H D A E A - I I T G-----A A P ----P Q I E H A A S G V V S L V R L - K - S S V L S H Y I K H I N
Gonium_pectorale_KXZ41783      R E M V Q S D R L I T E I L L H L G E S E A Q A E A - A I Q S-----L L S A ----F P G A A A N V V N L V R L - R - S S L I S A M I R H I D
Volvox_carteri_0026s0144       H E M E K S D D L I R D M L Q A A C F G K A R I E E - A L S A-----I I S G ----G S I E A F S P F T L Q M I Y D V A R - S S L I M L E L E Y I E
Chromochloris_zofingiensis_15g18055v2 E Y M R Q D Q A I A A L L G A L S L P D A D L H A - L L A G-----Q L T ----D M D L K K A A V L Q A V I D L - K - L R F I E L Y K K L I G
Klebsormidium_flaccidum_00010_0090 T C I K K H T Q L I K E V E K E Y G R R E A D L K A T H E K Q-----D A L Y ----G V K A E A R A S K Q S Q I D G L R - V C F V I Q H T V A K

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Crustomastix_stigmata_064173    S F E D S L G C I V A E L C T V E H L V F Q R I N T - V L R T-----R A Q ----A I G G Q K N L S D D S I V N S L K - T R F V R I K S K I N
Dolichomastix_tenuilepis_064008 E E E E A E E A R Q Q M P P R M M P P P P K P V A R P - Q P S N N P R R S D R I I ----S A I E H L A A A A E A A F E D - T - G H F - S L Y N G D D E
Cymbomonas_tetramitiformis_Sc10992_1 A N L K Q Y A A L Q T V T L A R Q E F Y T R M A T L A S T Q-----K K I F N ----S T Y T N G P G Q P T E I I V T - V - R R Y - N A M V N T L R
Pyramimonas_amyliifera_049838  E V Y R Q L K E I C A Q K I Q D V R K L R E H I A E M T E E L-----N T I M N ----P T I H A G S V R V L D Q M V S M - D - E K L - A S H I K Y F Q
Pyramimonas_parkeae_01336589   D V L K R L E D I Q A H A E Q R N E Y C K R I E K A S E E Q-----R V I F D ----P D I H S S S T S V L S S I T E K - A - E R E K N I H L K V T H

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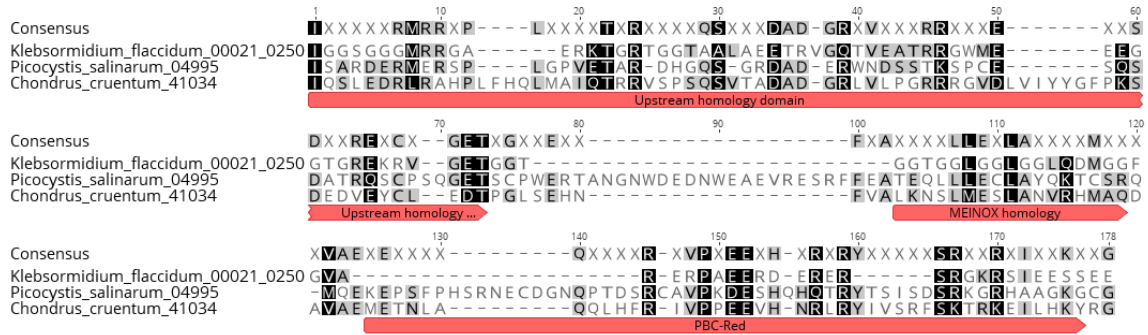
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Picocystis_salinarum_02499      T Y V G R S -- A V E G I I P F Q H E F Q T M R C R G - H G Q L-----G L S ----A E I Q L K Q A T I R Q M L D K - K - Q K C L E H F E R H V E
Prasinococcus_capsulatus_017584 T A V E R A -- I A L A A N S T T Q G E N T R A N I - H R Q F L E R V G Q I R V Q T K V A D P A A A T I T G O R T S N S O I I P G S - N I T S Y L K A Y D T I V A
Klebsormidium_flaccidum_00159_0100 H I L E E I -- N Q L A L V S P E R A F A E R A G L - N R E L V I R R N E L E S E L E V S G A D R Q T I A S E Q T K K M E Y I H E N - D V L S Y M K G P R E I V A
Guillardia_theta_137502        ---R T---I I N S R K R T R H V S E Q E E N V - H C Q A -- I Q A K F Q Y Q E Y V N D C L - N Q I N L N P D G S V S Q W P S A S P - H K L I K L S A G S A G

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PBC-B homology

Fig. S4. Extensive homology of Picsa_04995 and Klefl_00021_0250 to Chocr_41034 indicates their classification as PBX-Red. MEINOX-homology and PBL-Red domains are indicated by red bars below the alignment.



(b)

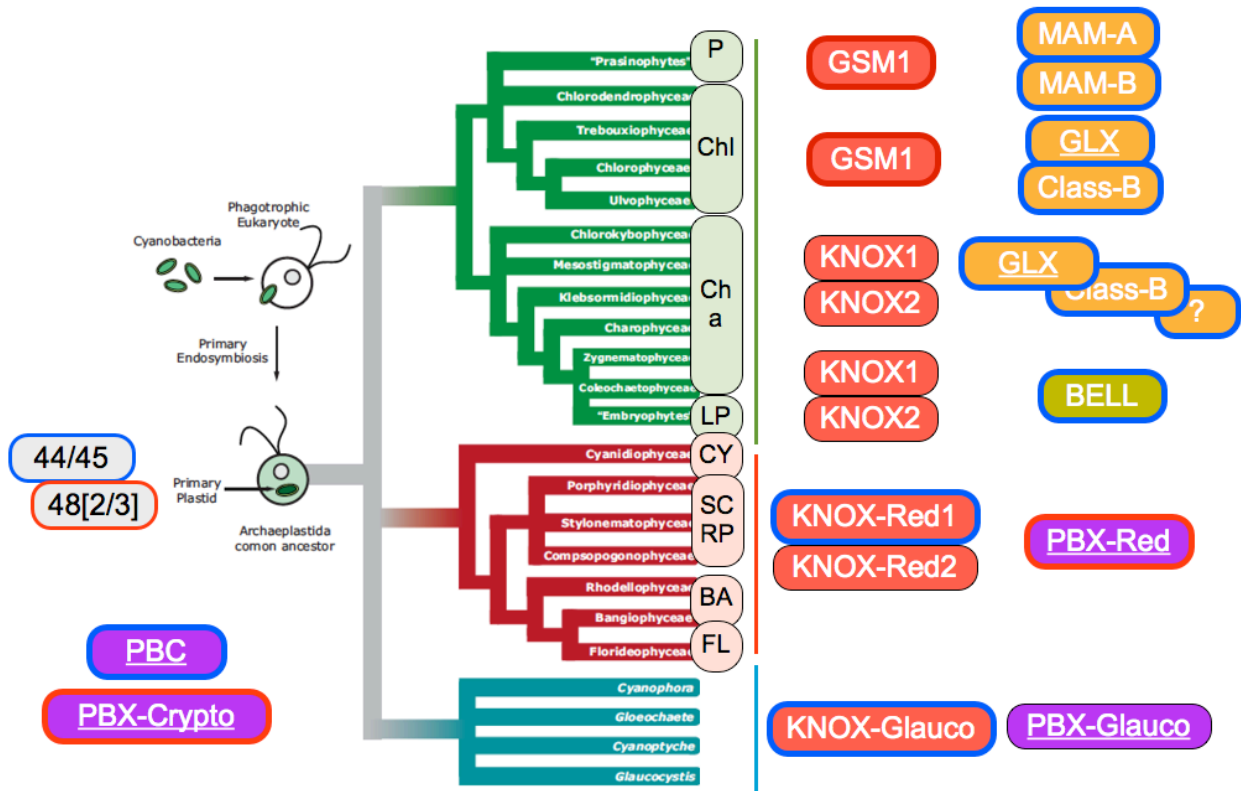


Fig. S7. Alignment of Mam-A TALE proteins in mamiellophyceae. Short motifs (Box1-4) are conserved among all members in this class over the entire length of the sequence. Red reverse triangle at 548-549 shows the truncation position of Micco_Mam-A-tr used in Yeast-two-hybrid analysis.

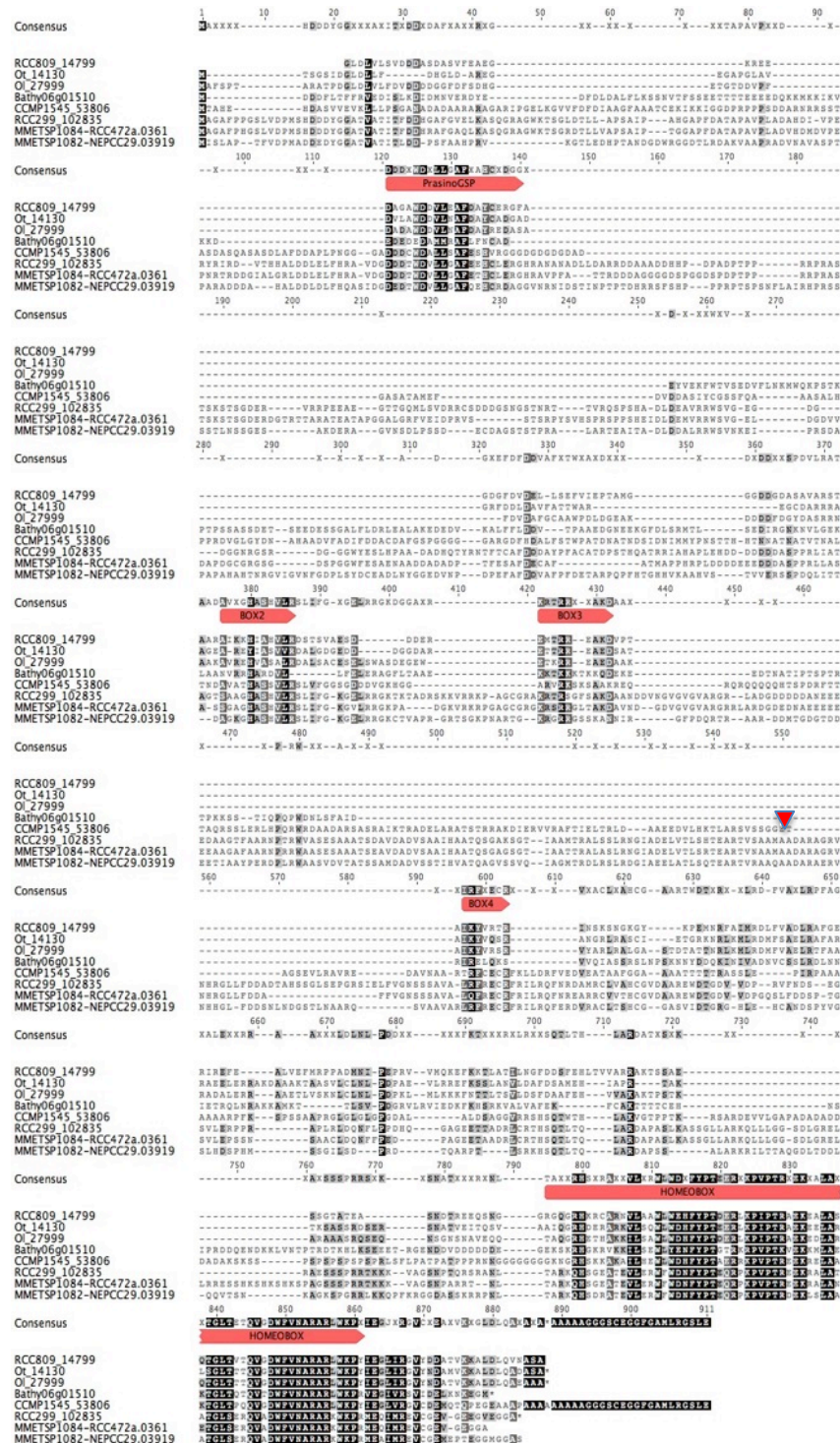


Fig. S8. Alignment of Mam-B TALE proteins in mamiellophyceae. A conserved motif is found between 180-197 amino acids in the alignment. Red reverse triangle at 100-101 shows the truncation position of Ostta_Mam-B-tr used in Yeast-two-hybrid analysis. Homology is restricted to a single homology-A domain outside the homeodomain.

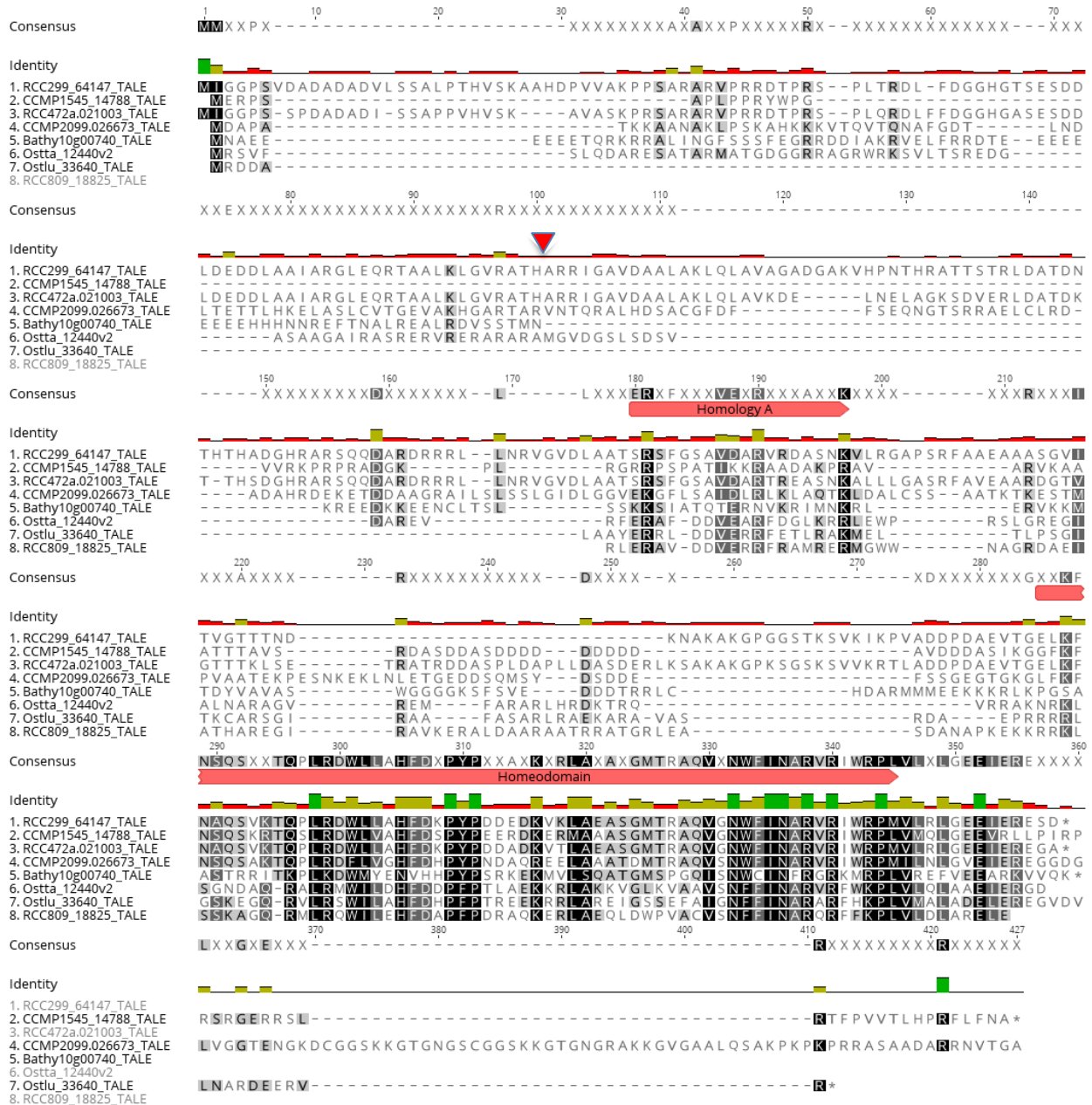
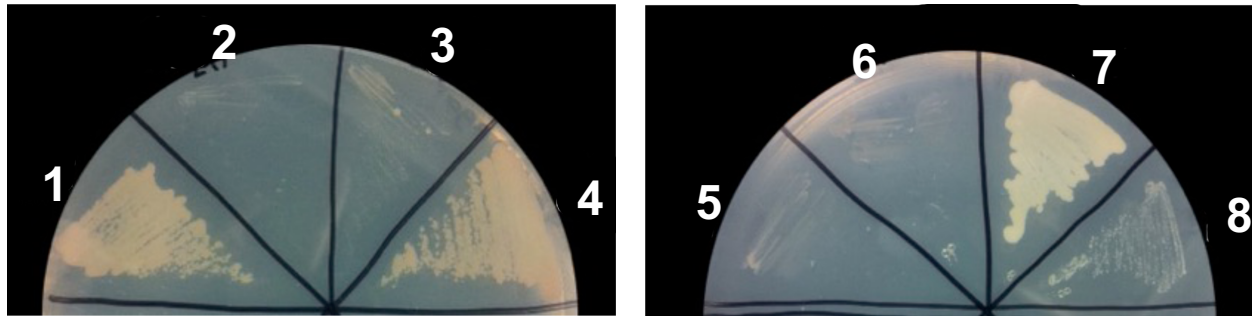


Fig. S9. Full-length proteins are necessary for mamiellophyceae non-KNOX TALE proteins to form heterodimers. Left and Right: Yeast-two-hybrid assays on Ade-/His-/Leu-/Trp- medium. The construct information for the prey conjugated with the GAL4 DNA-binding domain and for the bait conjugated with the GAL4 transcriptional activation domain is given in the table below.



Sector	DBD	AD	Sector	DBD	AD
1	Ostta_KNOX	Ostta_Mam-A	5	Micco_Mam-B	T-Ag
2	Ostta_KNOX	Ostta_Mam-B-tr	6	Micco_Mam-A-tr	Micco_KNOX
3	Ostta_Mam-A	Ostta_Mam-B-tr	7	Micco_Mam-B	Micco_KNOX
4	Ostta_Mam-A	Ostta_KNOX	8	Micco_Mam-B	Micco_Mam-A-tr

Fig. S10. TALE interaction network defined by this study using yeast-two-hybrid assays.

(A) Summary diagram for the TALE interaction network. (B) Yeast-two-hybrid assays for the cross-species interaction of TALE proteins. Only one of the possible reciprocal combinations of GAL4 domain conjugations is provided for simplicity. Large X indicates no yeast in the sector. -LTHA: Leu-/Trp-/His-/Ade- medium; -LT: Leu-/Trp- medium.

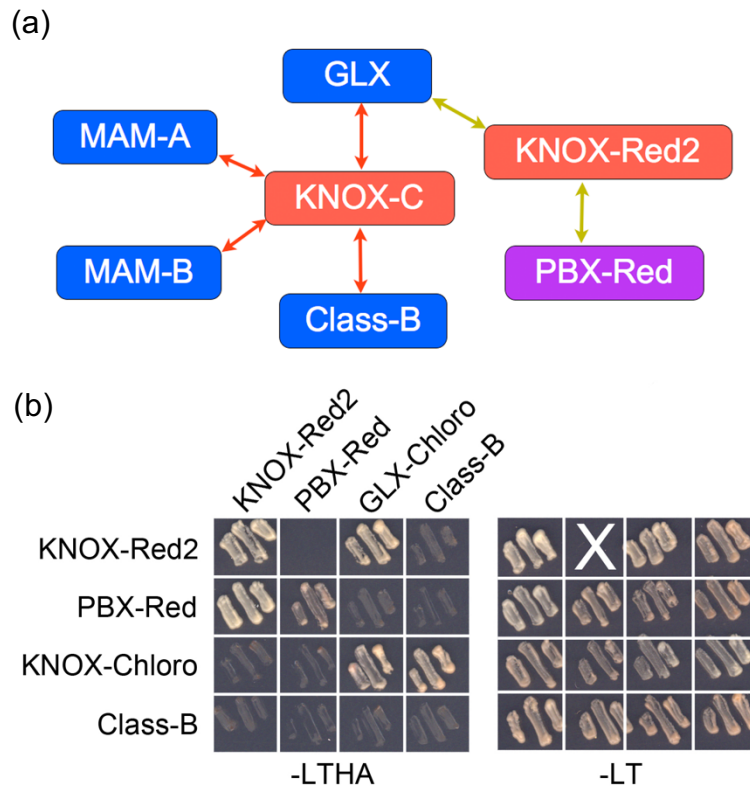


Fig. S11. Identification of MEIS homologs in choanoflagellates.

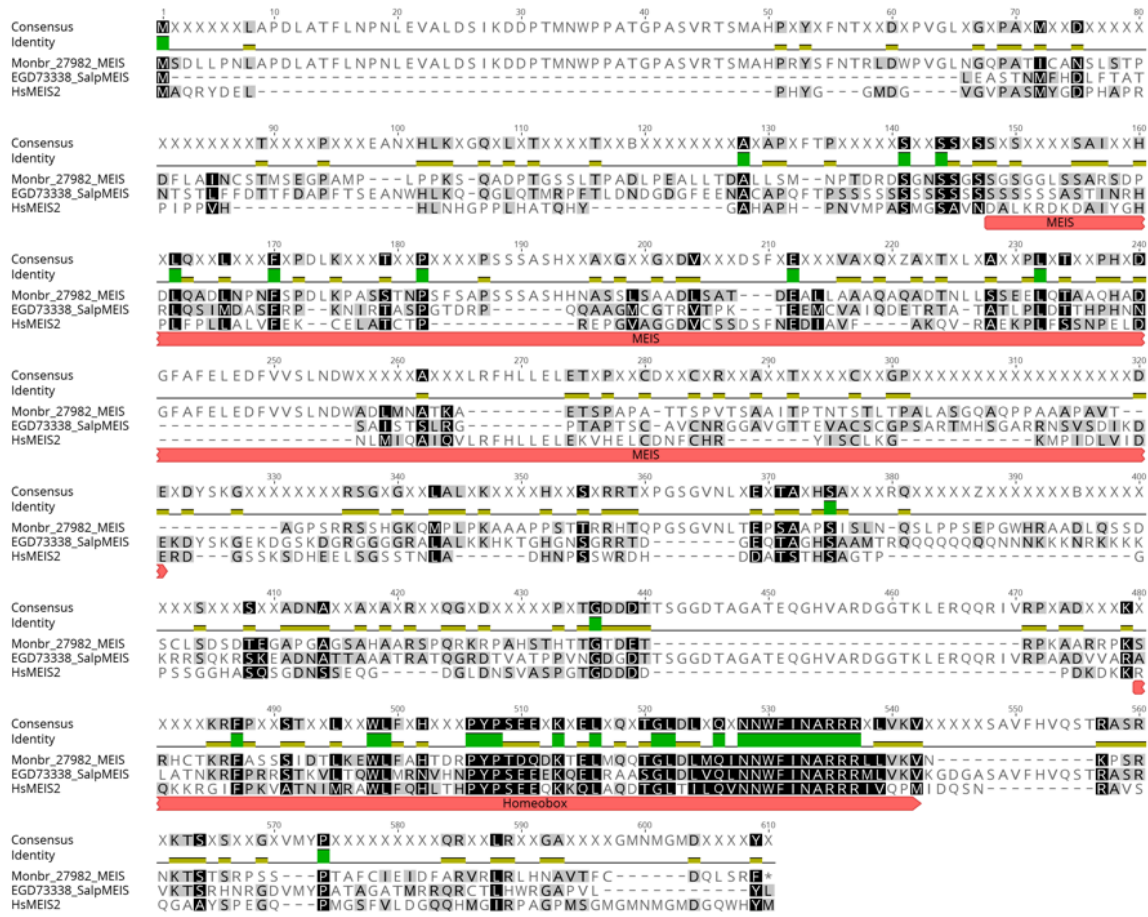


Fig. S12. Identification of PBX homologs in choanoflagellates.

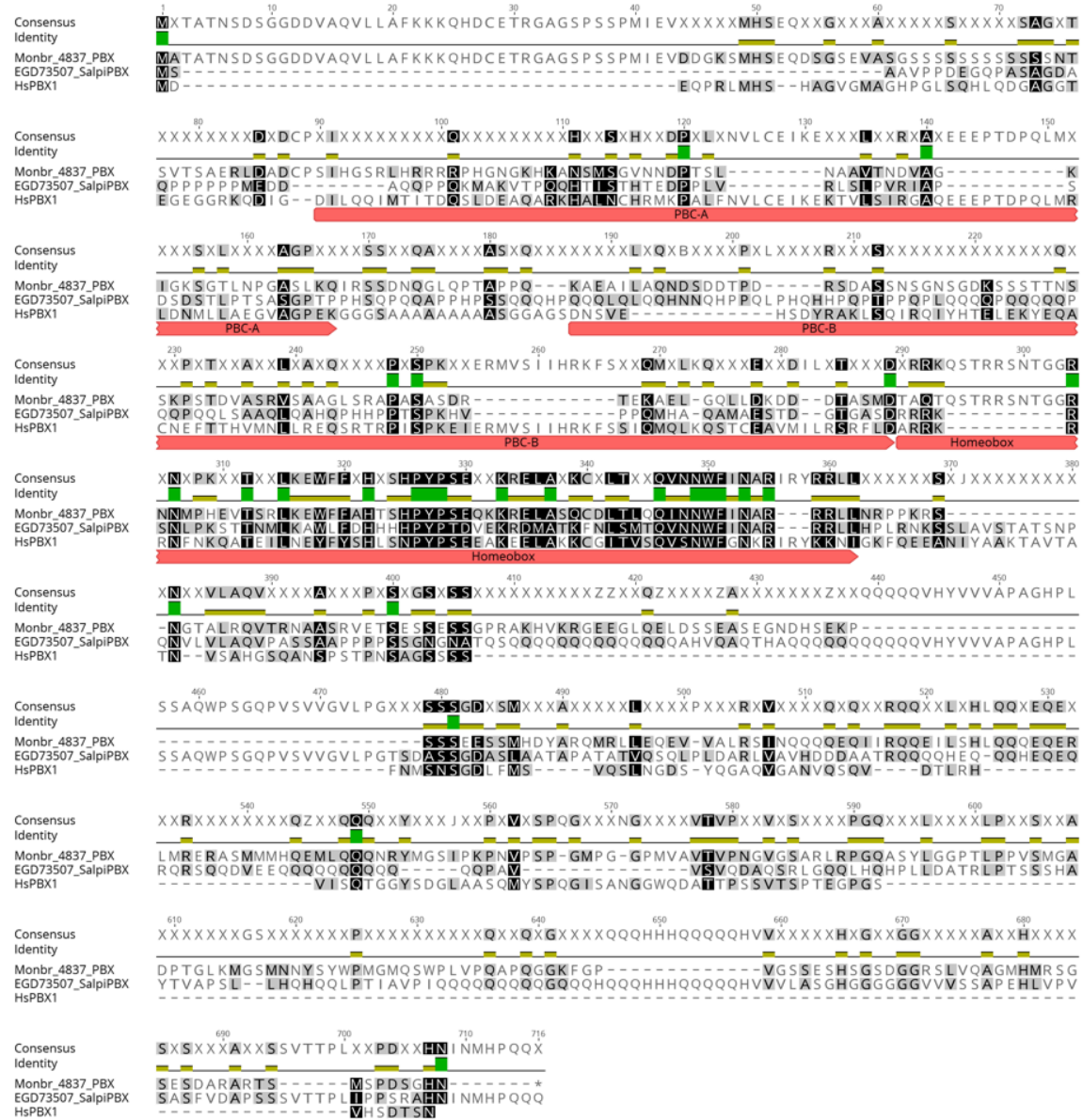


Fig. S14. Alignment of the PBX homologs in Amorphea.

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1. Capowc_EFW47516_TALE 1 TPTNRVGFASHDVTAAQLDVCIAQERAACAASETPSLAEIRGLFAFLDMQLKVAHR--L
2. Spharc_KNC86410_TALE 10 SEQARQYDFLIHRQIHH--QLLELLADGPERNARD-SEAK-----QMLDRITHNLIN
3. Salros_EGD73507_PBX 20 PQPLQQQPPQQQPPQPQ--QLSAAQLQAHPHHP--TSPK-----HVP PQMHAQ-A
4. Monbr_4837_PBX 30 SSSTTNS SKPSTDVASRV SAAGLSRAPASASDRTEK--AELG-----QLDKDDD--T
5. HsPBX1 40 HTELEKYEQACNEFTTHVM--NLLREQSRT-RPISP--KEIE-----RMVSI IHRK-F

1. Capowc_EFW47516_TALE 70 SSLEYQLKRKVVYTKVIFLKSSTFLDSRKRRINLSREAQOVLNDWFLAHIIEHPYPSESEKEQL
2. Spharc_KNC86410_TALE 80 DSIDEALQKH-----GTGASTGAKRRRQLPPAAHSLKWL YENQDCPYPSDEQKQOL
3. Salros_EGD73507_PBX 90 MAESTD-----GTGASDRRRKRSNLPKSTTNMLKAWLFDHHHPYPTDVEKRD
4. Monbr_4837_PBX 100 ASMDTAQT-----QSTRRSNTGGRNMPHEVTSRLKEWFEAHTSHPYPSEQKREL
5. HsPBX1 110 SSIQMLKQSTCEAVMILRSRFLDARRKRRNENKQATEILNEYFYSHLSNPYPSEEAKEEL

1. Capowc_EFW47516_TALE 130 ADQTNLTMRQISTWFANKRNRQAQD
2. Spharc_KNC86410_TALE 140 AEFCOLEITQQIRNWFANTRNRKRHLF
3. Salros_EGD73507_PBX 150 ATKFNLSMTQVNNWFI NARRRL LHP LRNK S
4. Monbr_4837_PBX 152 ASQCDLTLQQI NNWFI NARRRL LNR PPKRS
5. HsPBX1 AKKCGITVSVQVSNWFGNKRIRYKKN

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Fig. S15. ELK-domain alignment.

