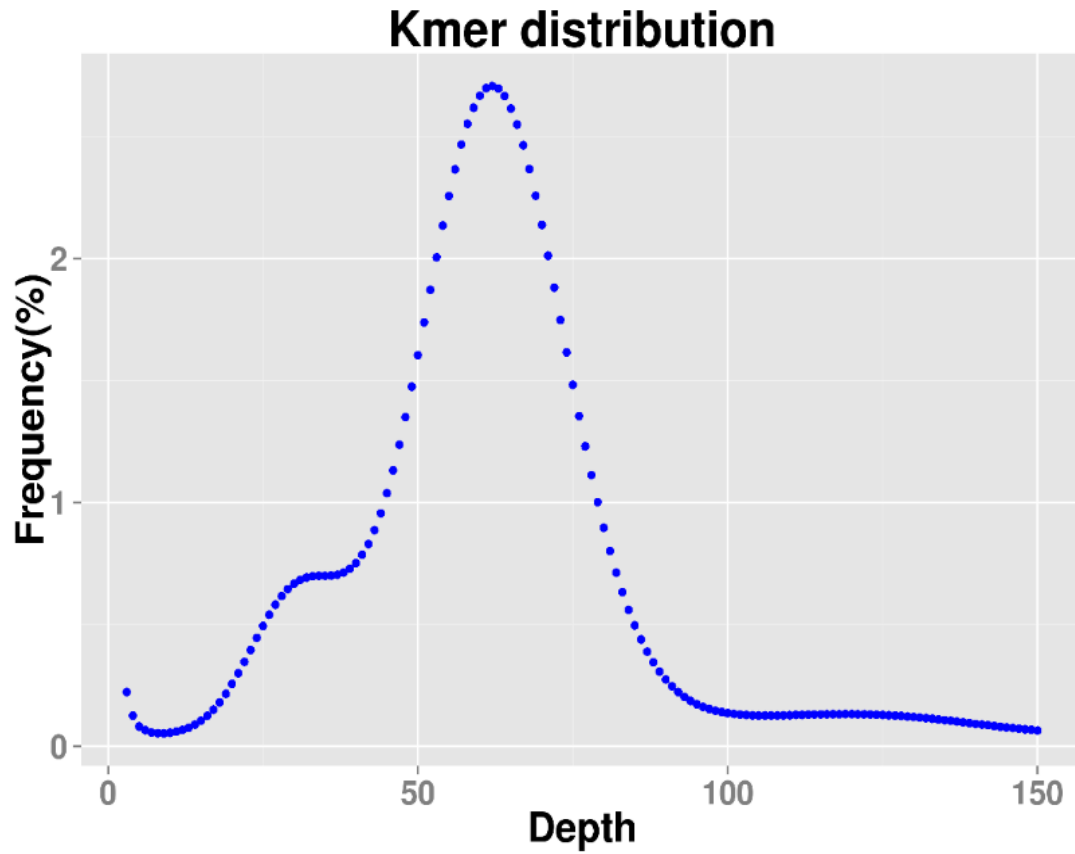

Supplementary information

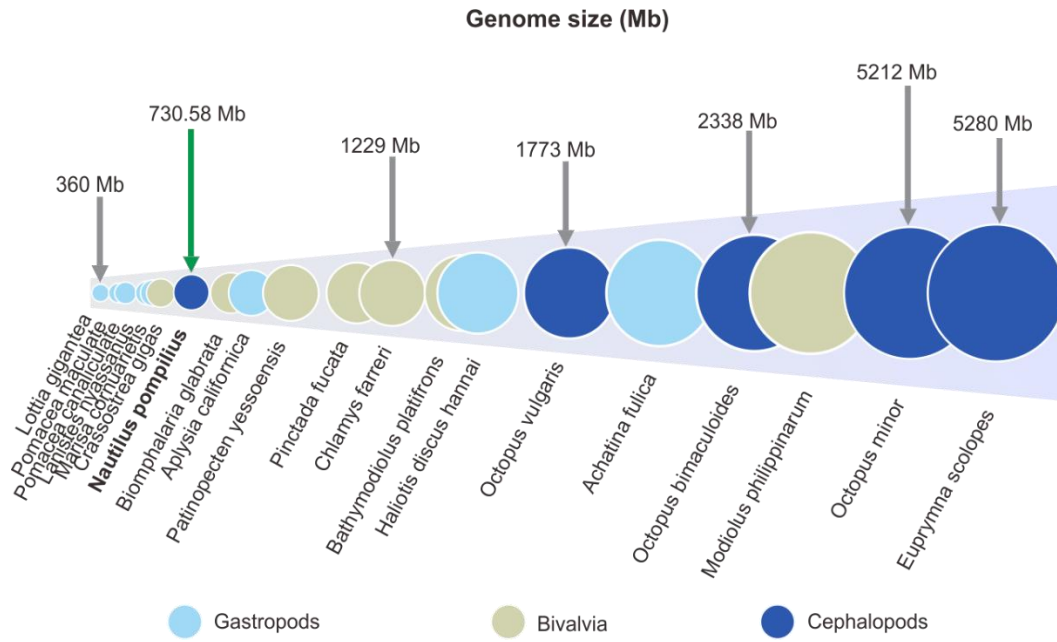
**The genome of *Nautilus pompilius*
illuminates eye evolution and
biomineralization**

In the format provided by the
authors and unedited

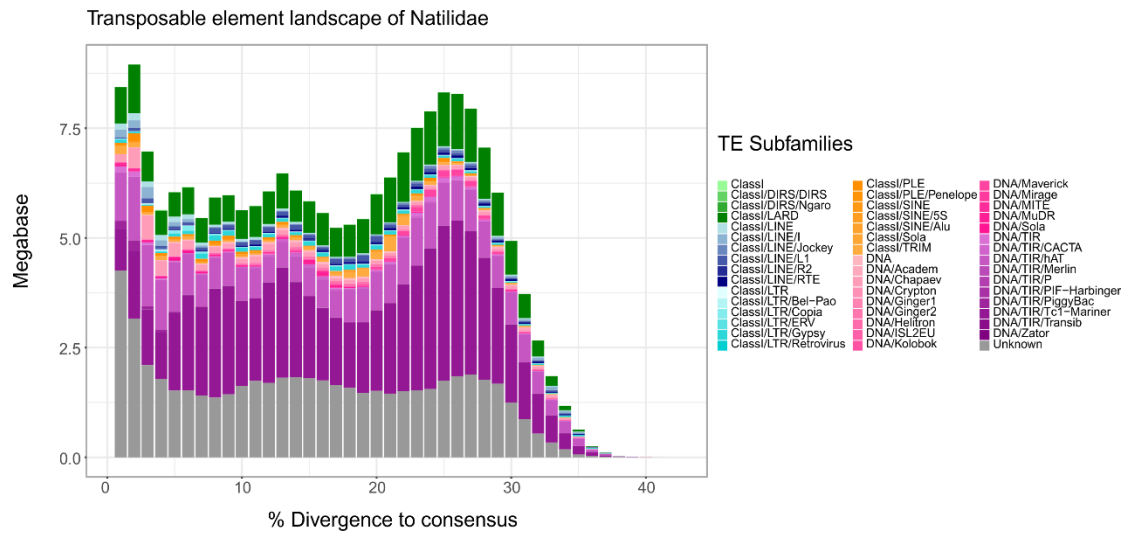
Supplementary Figures



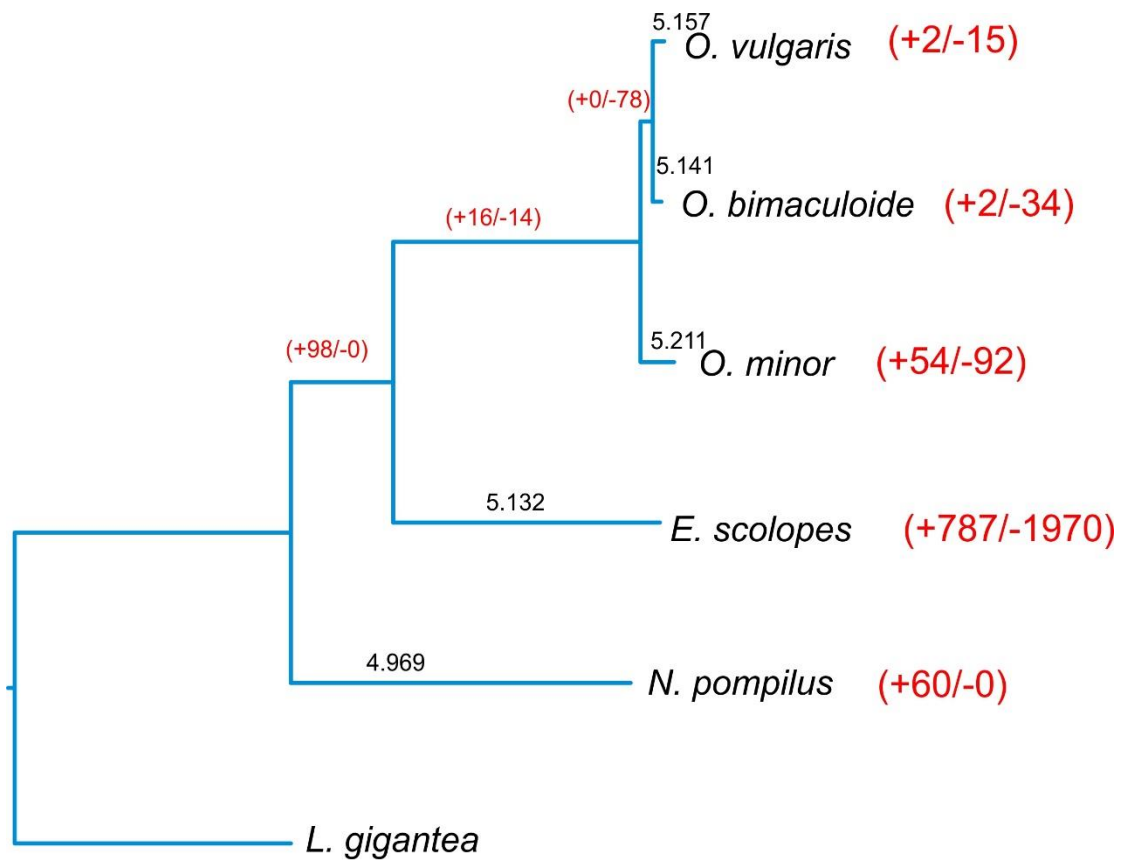
Supplementary Fig. 1 | k-mer distribution of the *N. pompilius* genome. Genome size estimation was performed by the k-mer analysis, and about 59.78 Gb corrected Illumina reads were selected to estimate the genome size. The genome size of *N. pompilius* thus estimated is 753.09 Mb.



Supplementary Fig. 2 | Distribution of genome size in different molluscan lineages. Molluscan species are lined up according to their genome sizes, ranging from 360 Mb (*L. gigantea*) to 5.28 Gb (*E. scolopes*)¹⁻¹¹. Gastropods, bivalvia and cephalopods are indicated by different colors. Notably, the genome size of *N. pompilius* is the smallest among known cephalopods.

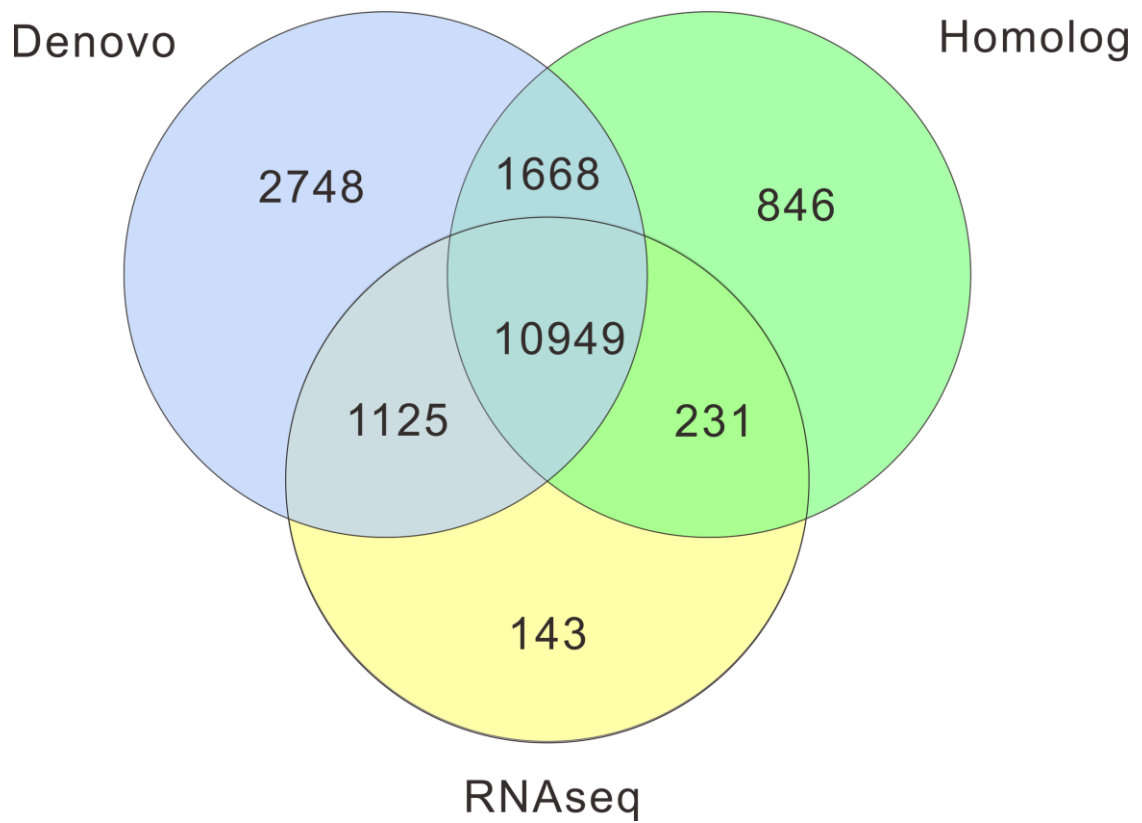


Supplementary Fig. 3 | History of transposable element (TE) accumulation in the *N. pompilius* genome. Temporal changes in transposable element (TE) accumulation in the *N. pompilius* genome based on a Kimura distance-based copy divergence analysis of TEs, with Kimura substitution level (CpG adjusted) illustrated on the *x*-axis, and percentage of the genome represented by each repeat type on the *y*-axis. Repeat type is indicated by different colored bars.

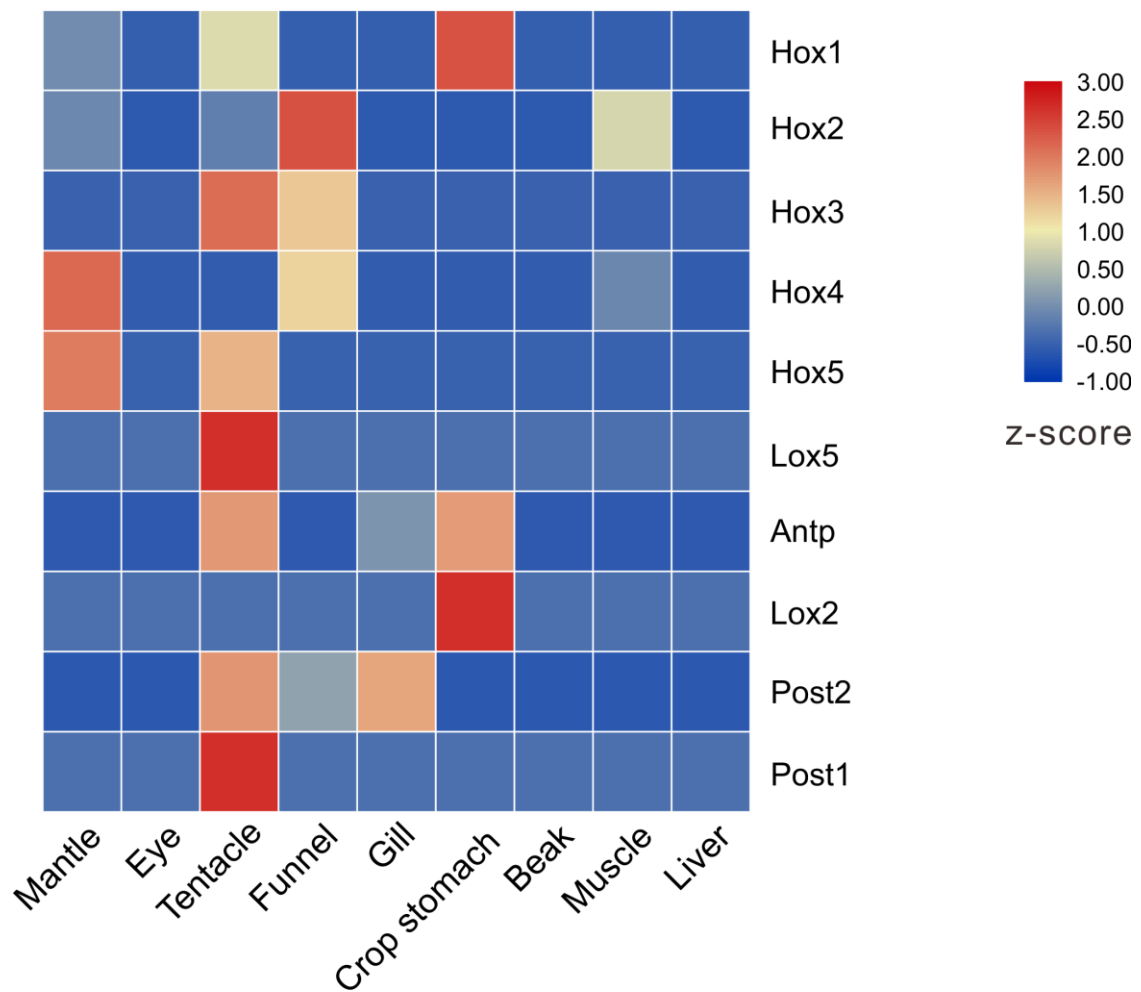


0.4

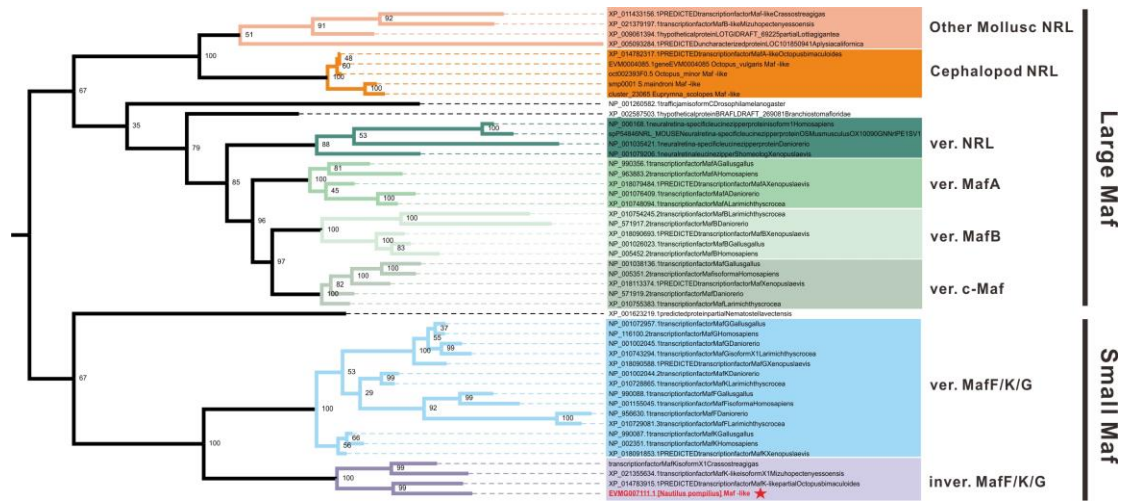
Supplementary Fig. 4 | Neutral tree and intron gain/loss event. Neutral tree of five cephalopods and *L. gigantea* is based on fourfold degenerate sites and pairwise distances to *L. gigantea* are shown for each species above their respective branches. Intron gain/loss events are shown in red besides taxon labels and at the ancestral nodes.



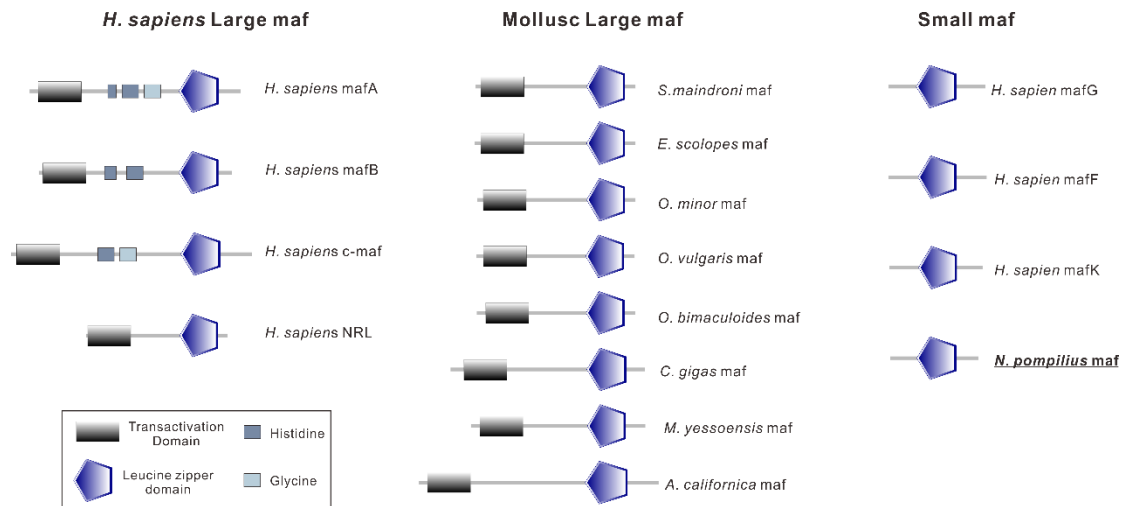
Supplementary Fig. 5 | Genome annotation in *N. pompilius*. Whole-genome annotation was performed by integrating multiple methods, which eventually generated 17,710 protein coding genes.



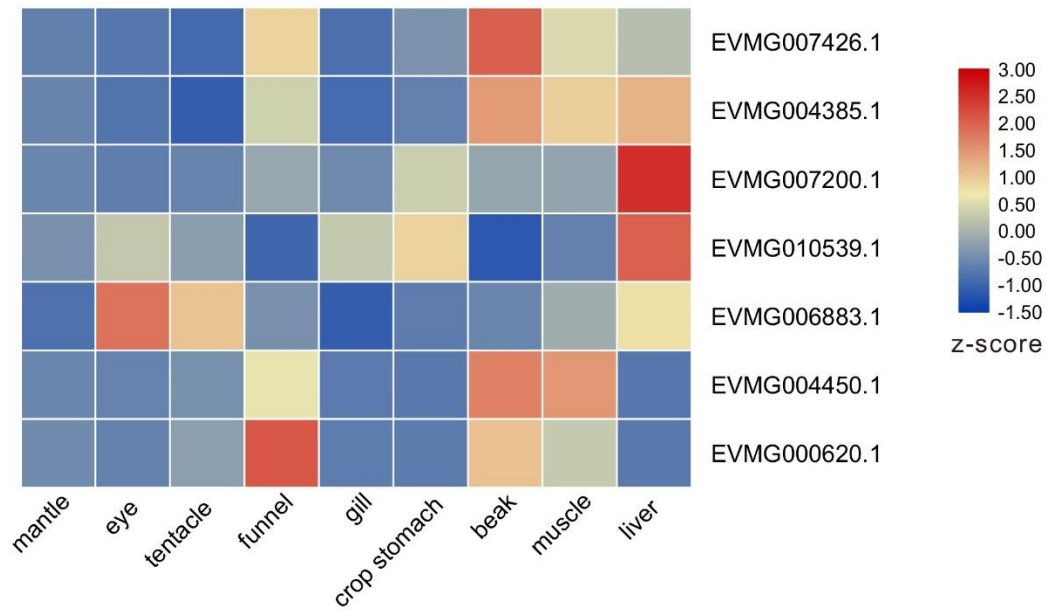
Supplementary Fig. 6 | Tissue distribution of Hox cluster in *N. pompilius*. Heatmap shows the expression profile of Hox cluster genes in different tissues. *x*-axis displays different tissues and *y*-axis shows the degree of expression of different Hox genes. Colored bars represent Z-score calculated from RPKM-values of a target gene in different tissues.



Supplementary Fig. 7 | Phylogenetic tree of the Maf/NRL superfamily. Multiple alignment was performed by using three methods including MAFFT 7.221¹², MUSCLE¹³ and T-coffee¹⁴. The best alignment was applied to phylogenetic analysis beads on MUMSA scores¹⁵. Then, the phylogenetic tree was constructed by MrBayes 3.2.1¹⁶ under a mixed model of amino acid substitution. Two independent runs with one cold and three heated chains were set for 15,000,000 generations. Starting trees were random and the trees were sampled every 1,000th generation. The ancestor of Maf/NRL was divided into the large and the small Maf clades. Each clade evolves independently and expands specifically in vertebrates. In the large Maf clades, the ancestor of Maf was continuously duplicated three times and generated four members (NRL, Maf A, Maf B and c-Maf) in vertebrates, but preserved one copy of NRL in mulloscan lineages. Similarly, the small Maf clade was divided into Maf F, Maf G and Maf K after vertebrate-specific duplications, while one copy of Maf K was preserved in mulloscan lineages. In contrast, the *N. pompilius* genome only encodes one Maf K gene but lost NRL. ver., vertebrate; inver., invertebrate.

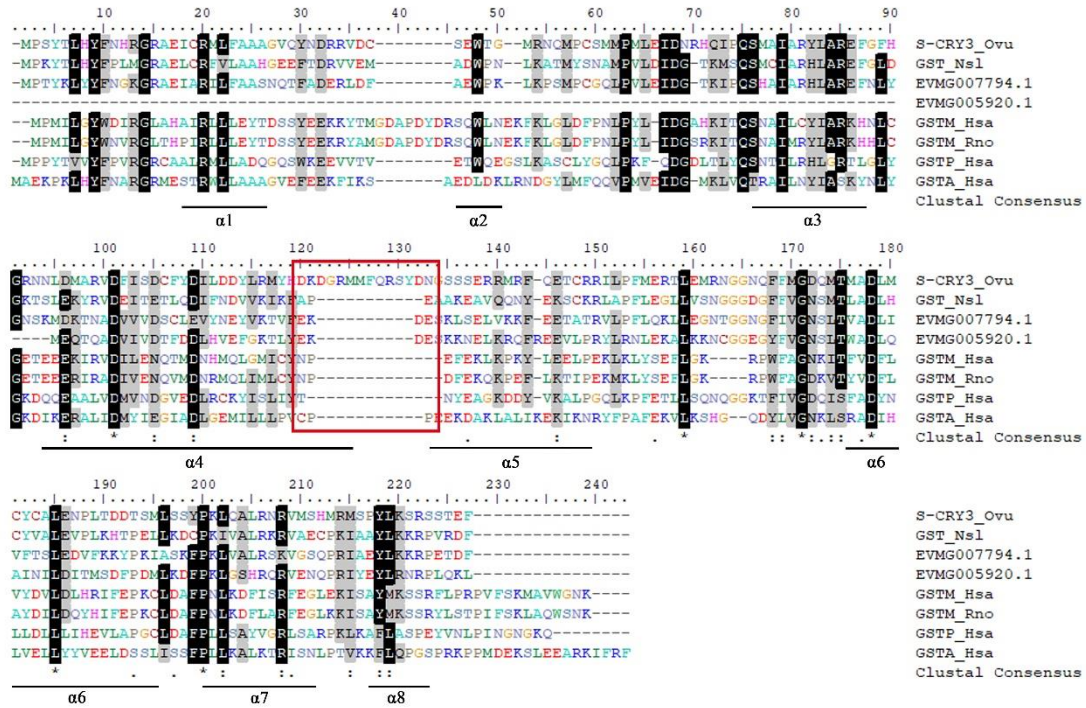


Supplementary Fig. 8 | Domain composition of Maf/NRL family members across different metazoans. MafA, MafB, c-Maf and NRL belong to large Maf family, and Maf K Maf G and Maf F belong to the small Maf family. The only extant homologue of Maf in the *N. pompilus* genome is the member of small Maf. Domain architecture was predicted and constructed by the software SMART¹⁷.

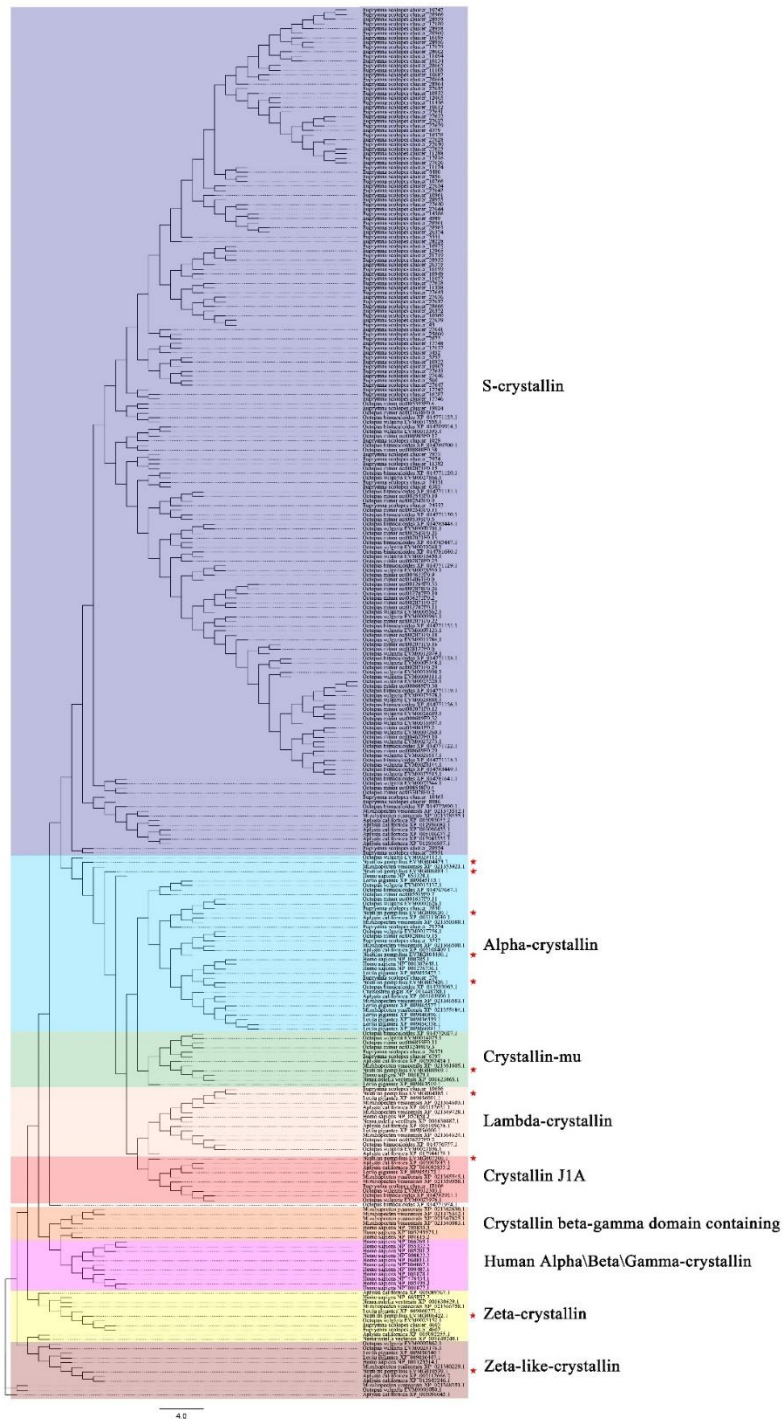


Supplementary Fig. 9 | Tissue distribution of crystallin-like genes in *N. pompilius*.

Heatmap shows the expression profile of crystallin genes in different tissues, in which 3 of crystallin genes without expression are excluded. *x*-axis displays different tissues and *y*-axis shows the degree of expression of different crystallin genes. Colored bars represent Z-score calculated from RPKM-values of a target gene in different tissues.

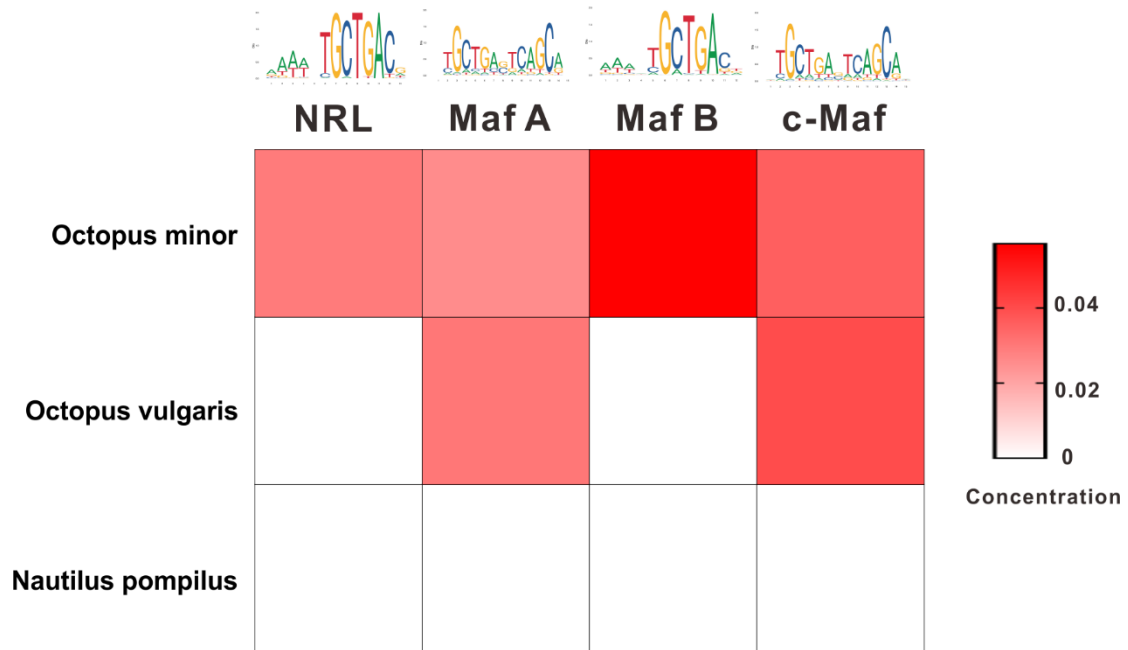


Supplementary Fig. 10 | Pairwise alignment of the potential S-crystallin of *N. pompilius* with cephalopods S-crystallin, Glutathione S-transferase (GST) and other classes of GST. The a-helices in S-crystallin are underlined and labeled. Compared with other classes of GST, the cephalopods S-crystallin has an 11-amino acid residues insertion between the conserved a4 and a5 helices (red box). Ovu, *Octopus vulgaris*; Nsl, *Nototodarus sloanii*; Has, *Homo sapiens*; Rno, *Rattus norvegicus*.

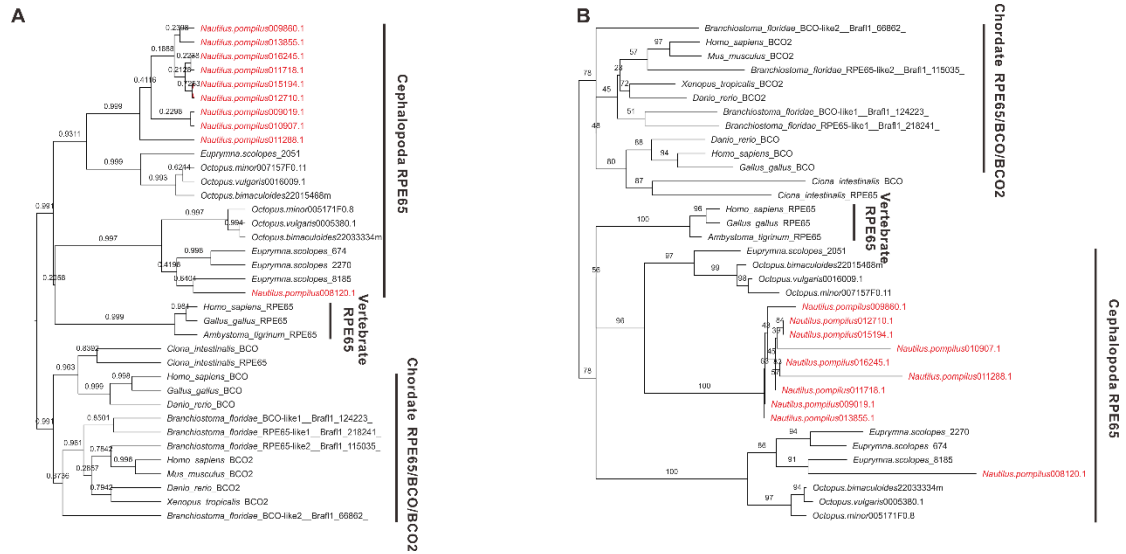


Supplementary Fig. 11 | Phylogenetic tree of the crystallin gene family.

Phylogenetic tree of crystallin family were constructed by MrBayes methods as described above. Crystallin genes from *Homo sapiens*, *Euprymna scolopes*, *Octopus minor*, *Octopus bimaculoides*, *Octopus vulgaris*, *Nautilus pompilius*, *Aplysia californica*, *Lottia gigantea*, *Mizuhopecten yessoensis*, *Crassostrea gigas* and *Nematostella vectensis* are used. Different types of crystallin are labeled with separate colors. *N. pompilius* genome only contains a total of 10 crystallin genes (by red pentacle) and lacking S-crystallin which constitutes the major lens protein in cephalopods, featuring the least number of crystallins in metazoans.



Supplementary Fig. 12 | Enrichment analysis on NRL/MAF binding motifs on the promoter of the cephalopod crystallin gene family. 2,000 bp of 5'-flanking regions of crystallin genes were extracted from the genomes of *O. minor*, *O. vulgaris* and *N. pompilus*. NRL, Maf A, Maf B and c-Maf binding motif matrices were downloaded from a JASPAR database. Enrichment analysis for NRL/MAF binding motifs in the crystallin promoter regions were analyzed by CentriMo¹⁸. Search parameters are set as follows: (1) 0-order background model generated from supplied sequences; (2) motif sites on either strand is considered; (3) motif sites only are considered, if they have a match score ≥ 5 ; and (4) regions are only reported, if they have a E -value ≤ 1 .



Supplementary Fig. 13 | RPE65 family expansion in *N. pompilus*. RPE65 domain containing proteins in cephalopods were applied to construct a phylogenetic tree by MrBayes method (A) as described above and ML method (B, model: LG+G4; bootstrap: 1000), respectively. The cephalopod RPE65 are homologs of vertebrates RPE65. Moreover, the *N. pompilus* genome contains a total of 10 RPE65 proteins, among which 9 of them were expanded and specifically clustered into one independent clade, and 1 of them was clustered with coleoids and formed one other clade.

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      10      20      30      40      50      60      70
H. sapiens      -----MSIQVEHPA GGYKLFETV EELSSPLTAH VTGRIPLWLT GSLLRGPGGL FEVGESEFFYH 59
EVMG013855.1  1 -----MALVRSFSLP LETKQPVKTT ITGSIPIHWLS GSLFRNGPGV QKVDGFKLNH 50
EVMG012710.1  1 -----
EVMG008120.1  1 MKPLLLITVF IIVAKAEDPD VGFNLLYTSN EKEFRDVPVR FEYPLPKWLE GTLVRNNGGGG FEMGKRKLLH 70
EVMG016245.1  1 -----
EVMG009860.1  1 -----MALVRSFSLQ LETKRPVKTT ITGSIPIHWLS GSLFRNGPGV QKVDGFKLNH 50
EVMG010907.1  1 -----MSSHSDGCFP DQEQHPAQP-----FT LDVPTADIGS QKVDGFKLNH 41

      80      90     100     110     120     130     140
H. sapiens     60 LFDGQALLHK FDFK-EGHVT YHRRFIRIDA YVRAMTEKRI VITEFGTCAF PDPCKNIFSR FFSYFRGVEV 128
EVMG013855.1  51 LFDGFAVVHR FDIK-DREVL YQNKILKTED WLFPAIKTHRL LATHFGTVPV PDPCKCLFSR HFSFYF--KM 117
EVMG012710.1  1 -----MYQ NKIL-KTEDW LS-----AA HRLL ATQLV-----LYL HFSFYFNSKR 38
EVMG008120.1  71 AFDAYSKLTG WKFHNGSVS FSTVFLKTES YNRSAAQDV APYLLLLGVN PP-----FSA LQRTAALLRG 135
EVMG016245.1  1 -----
EVMG009860.1  51 LFDGFAVVHR FDIK-DGEVM YQNKILKTED WLSAIKTHRL LATQFGTVSV PDPCKCLFSR HFSFYFNSKR 119
EVMG010907.1  42 LFDGFAVVHR FDIK-DGEVI LV-----VCCSPL VSDSVGTVSV PDPCKCLFSR HFSFYFNSKR 98

      150     160     170     180     190     200     210
H. sapiens     129 TDNALNVVYP V----GEDY YACTETNFIIT KINPETLETI KOVDLCNYVS VNGATAHPHI ENDGTVYVIG 193
EVMG013855.1  118 TDNVSNVIFR H----GDGL FAVSEIDNIW RIDPQSLGTV EKTAVSDHMA VHMATAHPLI DRNGMIVYNG 182
EVMG012710.1  39 TDVSGFAVHR H----GDGL FAVSEIDNIW RIDPQSLDTV EKTAVSDHMA VHMATAHPLI DRNGMIVYNG 103
EVMG008120.1  136 IDNMNVNFR YPTDDGGTGY FALNDYMKVY EFSIGRLDVL GGPVNPPIFS RPPASPAEAI GVLSSQISSAH 205
EVMG016245.1  1 -----MA VHMATAHPLI DRNGMIVYNG 22
EVMG009860.1  120 TDNVSNVIFR H----GDGL FAVSEIDNIW RIDPQSLDTV EKTAVSDHMA VHMATAHPLI DRNGMIVYNG 184
EVMG010907.1  99 TDNVSNVIFR H----GDGL FAVSEIDNIW RIDPQSLDTV EKMAVSDHMA VHMATAHPLI DRNGMIVY-VG 162

      220     230     240     250     260     270     280
H. sapiens     194 NCFGKMFSTIA YNIVKIPPLQ ADKEDPISK EIVVQFPCSD RFKPS----YVHSFGLTPN YIVFVETPVK 258
EVMG013855.1  183 SNY-RDYKRA FNILAFPQPA NGELDSMKTG RIVASIPSRW KFHYG----YIHSFGMAEK YFVLLBQPCT 246
EVMG012710.1  104 SNY-RDYKRP FNILAFPQPA NGELDSMKTG RIVASIPSRW KFHCG----YIHSFGMAER YFVLLBQPCT 167
EVMG008120.1  206 PLPEPRRPSF LTFLEIRLL PGEKDAISLV RIHTVTRREV VARWEVDVVP YVHSFVSTEN HAVVFPASPYH 275
EVMG016245.1  23 SNY-RDYKRP FNILAFPQPA NGELDSMKTG RIVASIPSRW KFHYG----YIHSFGMAER YFVLLBQPCT 86
EVMG009860.1  185 SNY-RDYKRA FNILAFPQPA NGELDSMKTG RIVASIPSRW KFHYG----YIHSFGMAEK YFVLLBQPCT 248
EVMG010907.1  163 SNY-RDYKRP FNILAFPQPA NGRISTST--FIISTLREN KHKWR----LINSF---K CLHCKEQ-ED 218

      290     300     310     320     330     340     350
H. sapiens     259 INLFKFLSSW SLWGANYMDC FESNETMGVW LHIADKKRKK YLNNKYRTSP FN-LFHHINT YEDNGFLIVD 327
EVMG013855.1  247 YSLFKLFRQ -VHKYSPLEA MENYENQML FHIIRKSDGK RLSTTYKSSD VKFCFHHINT YEEEGHLVVD 315
EVMG012710.1  168 FSIFKLIHQ -VCKYSPLEA MENYENQML FHIIRKSDGK RLSTTYKSSD VKFCFHHINT YEEEGHLVVD 236
EVMG008120.1  276 VSVLKMVDTA ----RALDS LEWRGDSPCM IYVVDLRSQG --VHTLKTEA MFFMHHANAF ELDDSRLLVD 338
EVMG016245.1  87 YSIFKLIHQ -VCKYSPLEA MENYENQML FHIIRKSDGK RLSTTYKSSD VKFCFHHINT YEEEGHLVVD 155
EVMG009860.1  249 YSIFKLIHQ -VCKYSPLEA MENYENQML FHIIRKSDGK RLSTTYKSSD VKFCFHHINT YEEEGHLVVD 275
EVMG010907.1  219 DTVLHCIF--ACPLN--IE MRPLVRDSML FHIIRKSDGK RLSTTYKSSD VKFCFHHINT YEEEGHLVVD 283

      360     370     380     390     400     410     420
H. sapiens     328 LCCWKGFEFV YNYLYLANLR ENWEEVKKNA RKAPQPEVRR YVLPINIDKA DTGKNLVTLN NTTATAILCS 397
EVMG013855.1  316 ICGYNDFSIV NNFRLN----LRLFS DTSTATFKRF VLPIDVTEKM PLGKNLVNLK YTTATAVKQP 377
EVMG012710.1  237 ICGYNDFNIV NNFRLN----LRLSS DTSTATFKRF VLPIDVTEKM PLGKNLVNLK DTTATAVKQP 298
EVMG008120.1  339 IAVYEDPSFI NQMTLEN----L--D PNKRNGIDLA PQLKRYTLNI 378
EVMG016245.1  156 ICGYNDFNIV NNFRLN----LRLSS DTSTATFKRF VLPIDVTEKM PLGKNLVNLK DTTATAVKQP 217
EVMG009860.1  275 -----V TN----- SV----- YLAVLDAHTL E----- 278
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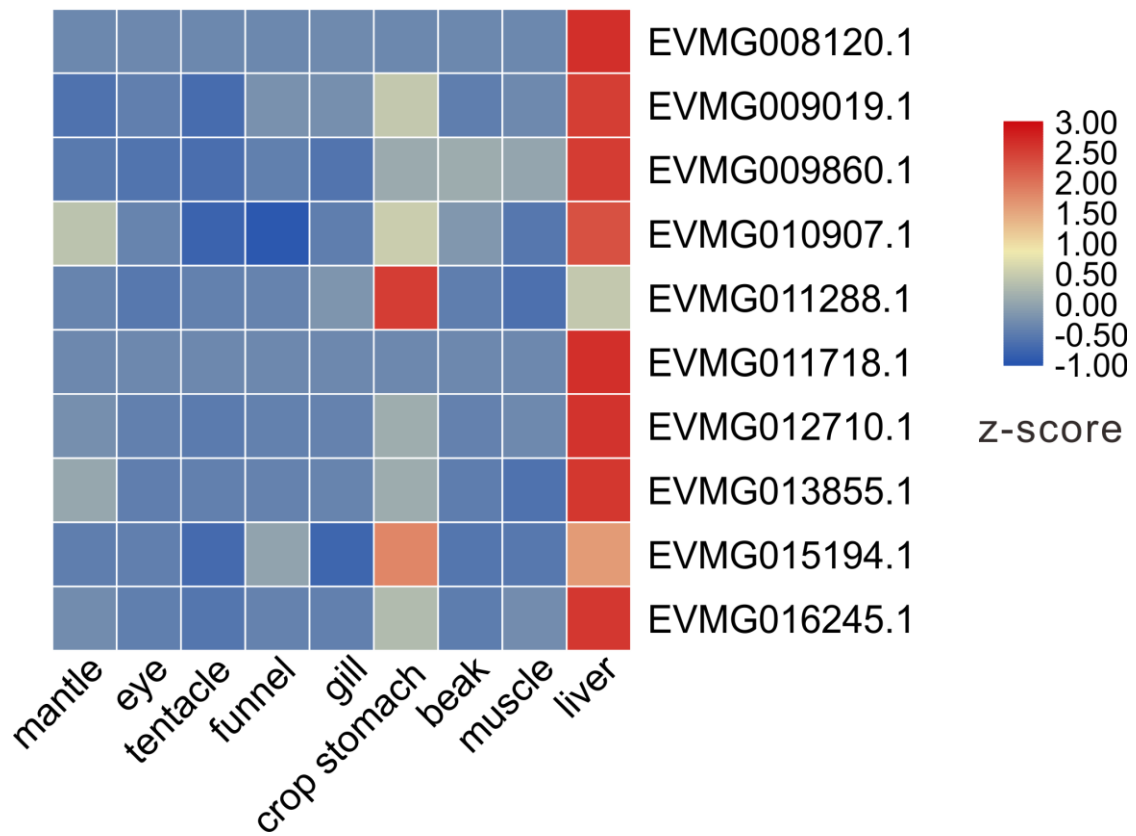
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H. sapiens     398 DETIWLPEEV LFGSP--RQA EEFQPIYQK YCGKPYTYAY GLG---LNH FVPDRLCKLN VKTKETWVWQ 461
EVMG013855.1  378 DGSVLLCCDN LTEDPACPIY LELPQINYEA CAGRNYRYVY GT----- LLKTEIHKID ILMRTVNWKV 439
EVMG012710.1  299 DGSVLLCCDN LTEDPARPVY LELRQINYEA RAGRNYRYVY GT----- LLKTEIHKID ILMRTVNWKV 360
EVMG008120.1  379 TTKAVSIQTF HTKRDRFISR LEFPTIN-ED YRARNYCYVY GFVQKMDGKR LATNALVKKD LCGNGMDRYW 447
EVMG016245.1  218 DGSVLLCCDN LTEDPARPVY LELPQINYEA RAGRNYRYVY GT----- LLKTEIHKID ILMRTVNWKV 279
EVMG009860.1  278 -----PRQ LQVPKICLEG SV----- YLAVLDAHTL E----- 293
EVMG010907.1  346 DGSVLLCCDN LTEDPARPVY LELPQINYEA RAGRNYRYVY GT----- LLKTEVGV---LG 396

      500     510     520     530     540     550     560
H. sapiens     462 EPD-SYSPSE IFVSHPDAL EDDGVVLSV VSPGAGQKPA YLLILNAKDL SEVARAEVEI N--IPVTFHG 528
EVMG013855.1  440 EPNGFLAAEP VFVPRPSED EDDGVILIPV TSSDP-ERPS YLAILDAHTL EEVAKADVPT DTFIPITFHG 508
EVMG012710.1  361 EPNGFLAAEP VFVPRPSED EDDGVILIPV TSSDP-ERPS YLAILDAHTL EEVAKADVPT DTFIPITFHG 429
EVMG008120.1  448 SESYHYGSEL WFVVPNAGTR EDDGVLLSPV LNGTK--GQS YLAVFDARSM KLMNVG--YL PTYIPITFHG 513
EVMG016245.1  280 EPNGFLAAEP VFVPRPSED EDDGVILIPV TSSDP-ERPS YLAVLDAHTL E----- 329
EVMG009860.1  293 -----
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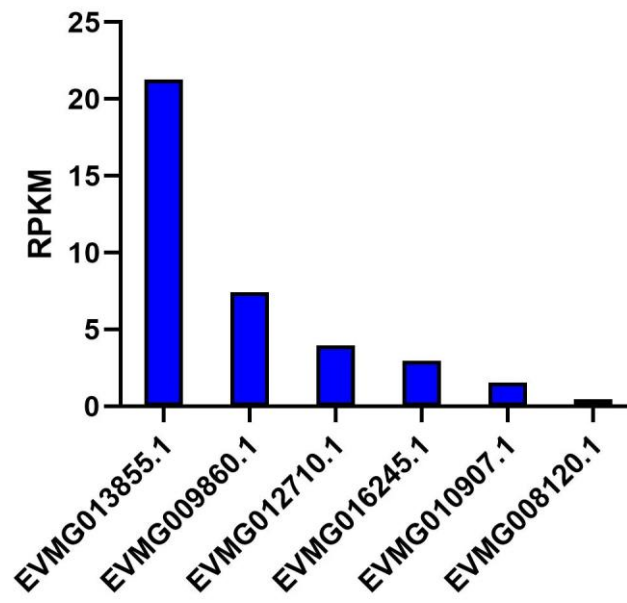
      570
H. sapiens     529 LFKKS----- 533
EVMG013855.1  509 FFDPPQQQL- ---- 517
EVMG012710.1  430 FFDPPQQQL- ---- 438
EVMG008120.1  514 RFFENI----- 519
EVMG016245.1  329 ----- 329
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EVMG010907.1  466 FFDPSSSCKL WVVGE 480

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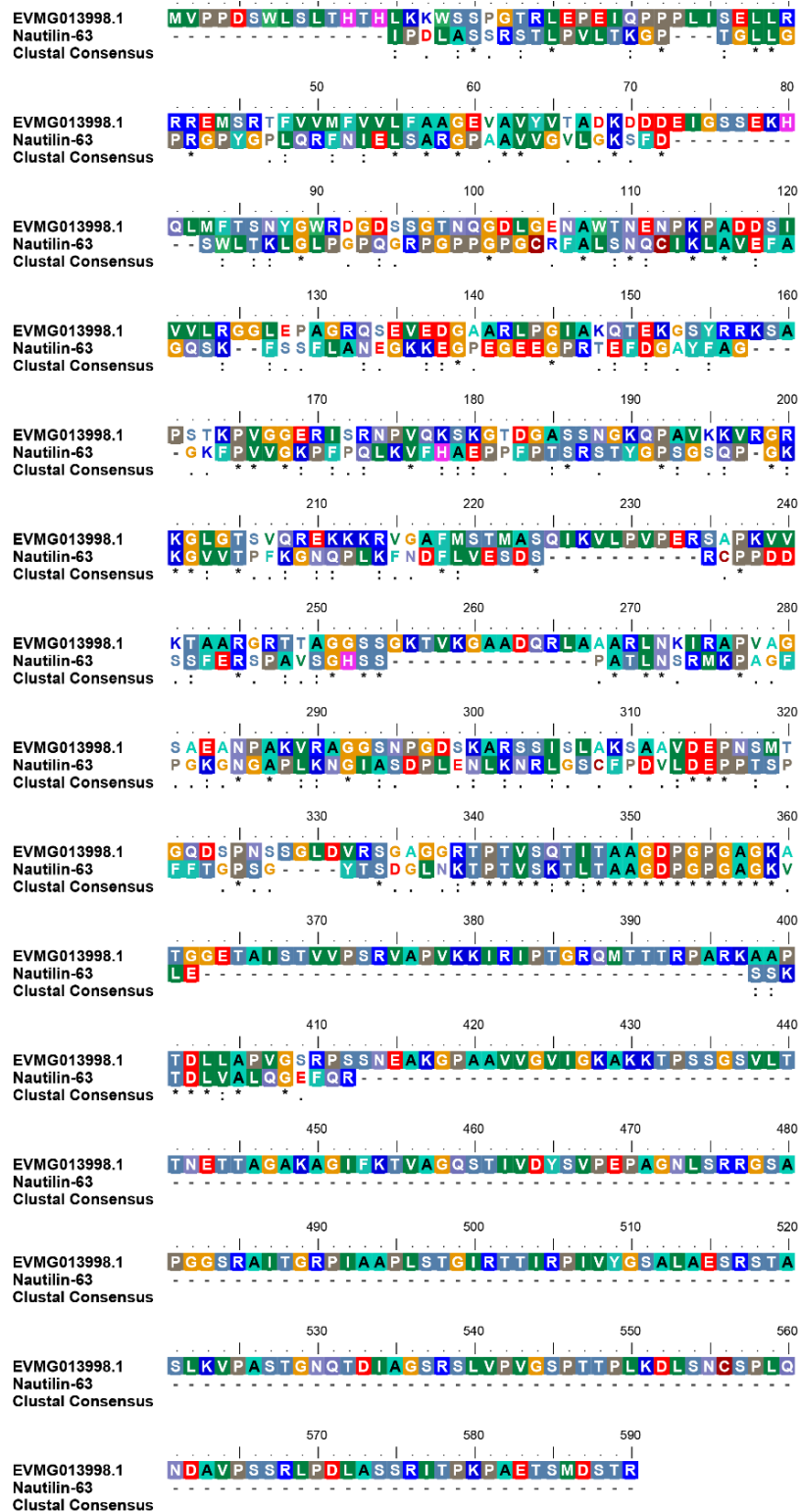
Supplementary Fig. 14 | Sequence alignment of RPE65 of *N. pompilius* and RPE65 of *H. sapiens*. Sequence alignment was conducted and displayed using Bioedit software, between six RPE65 sequences of *N. pompilius* and one of *H. sapiens*. The conserved residues in RPE65 were marked with color background, and EVMG013855.1 retained the conserved domains as in *H. sapiens* RPE65.



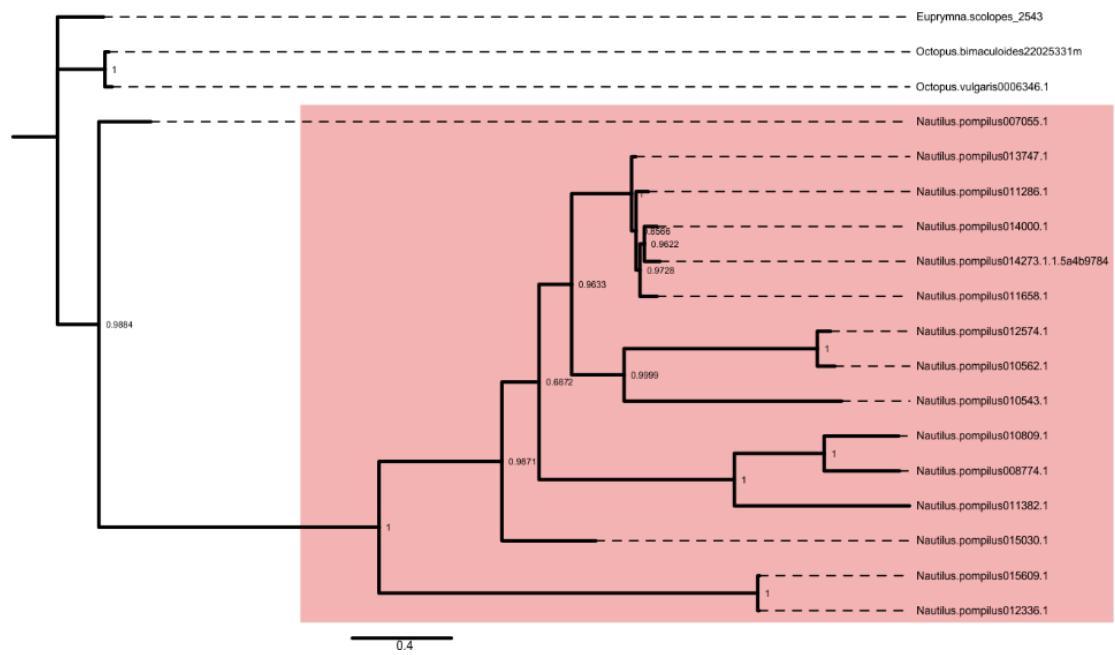
Supplementary Fig. 15 | Expression pattern of RPE65 family in *N. pompilus*. Expression level of RPE65 gene family were analyzed by using transcriptomic data in different tissues, which showed high expression of RPE65 genes in the liver.



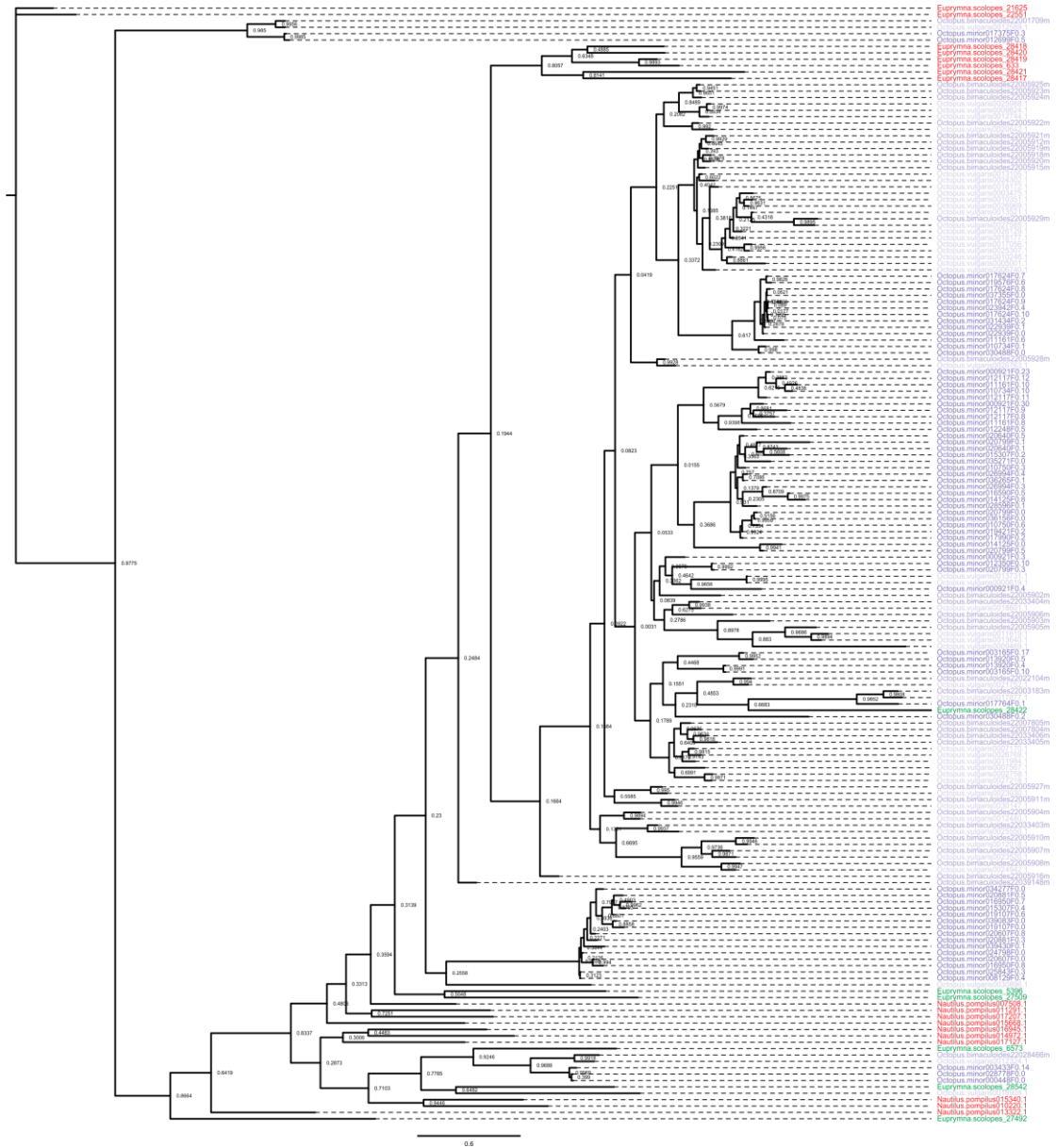
Supplementary Fig. 16 | The expression pattern of RPE65 families in the Nautilus eye. Six members of the RPE65 family genes were detected to be expressed in the eye.



Supplementary Fig. 17 | Sequence alignment between Nautilin-63 in *Nautilus macromphalus* and EVMG013998.1 in *N. pompilius*. The identical, highly conserved, and less conserved amino acid residues are indicated by ‘*’, ‘:’, and ‘.’, respectively.



Supplementary Fig. 18 | Expansion of IFN-inducible GTPases (IIG) gene family in the *N. pompilus* genome. Phylogenetic tree of IIG proteins in cephalopods was constructed by using MrBayes methods as described above, and contains 15 of IIG proteins in *N. pompilus*, and only single IIG proteins in other cephalopods.



Supplementary Fig. 19 | Phylogenetic tree of interleukin-17 (IL-17) gene family in cephalopods. Phylogenetic tree of IL-17 was constructed by MrBayes method as described above, and includes 10 of IL-17 in *N. pompilus*, 14 of IL-17 in *E. scolopes*, 34 of IL-17 in *O. bimaculoides*, 72 of IL-17 in *O. minor* and 45 of IL-17 in *O. vulgaris*. Independent expansion of IL-17 gene family was found in three octopus species, strongly suggestive of a crucial role of IL-17 in octopus immune defense.

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