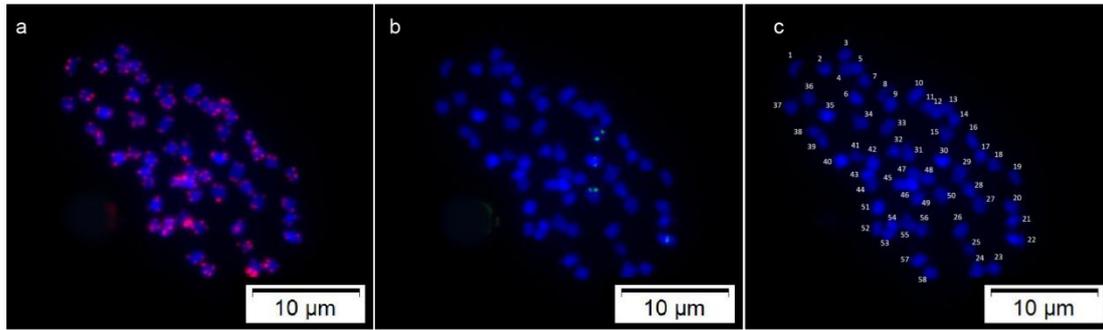
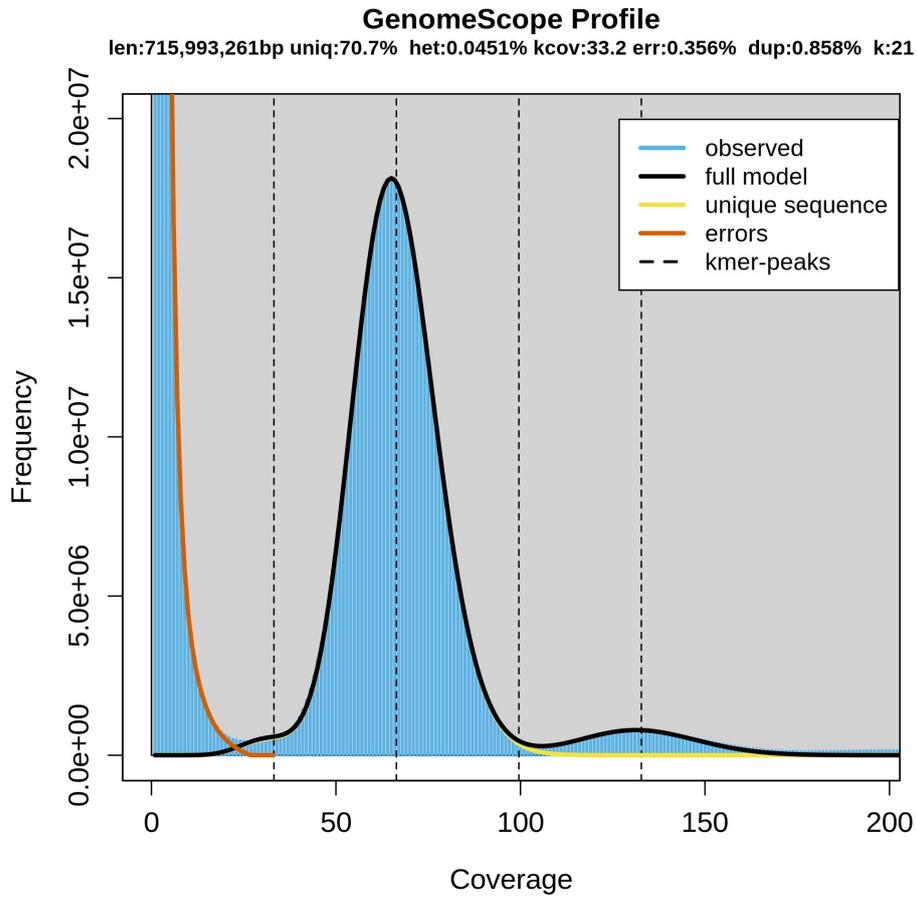


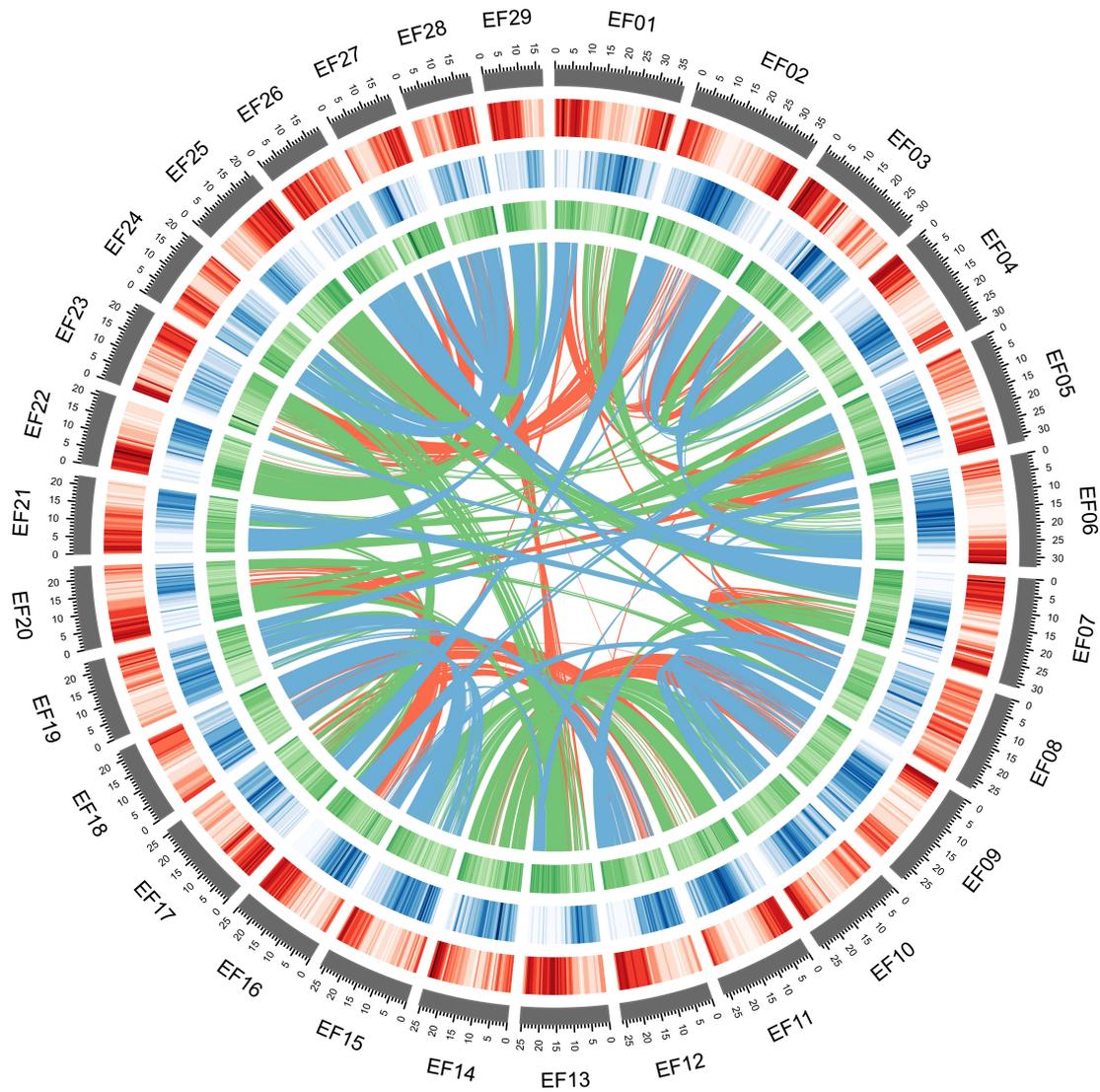
Supplementary Figure 1. Hi-C based clustering of the *E. ferox* chromosomes. Heat map showing the density of Hi-C interactions (calculated by pair-end reads) between contigs, the level of red color indicates the level of density of interactions.



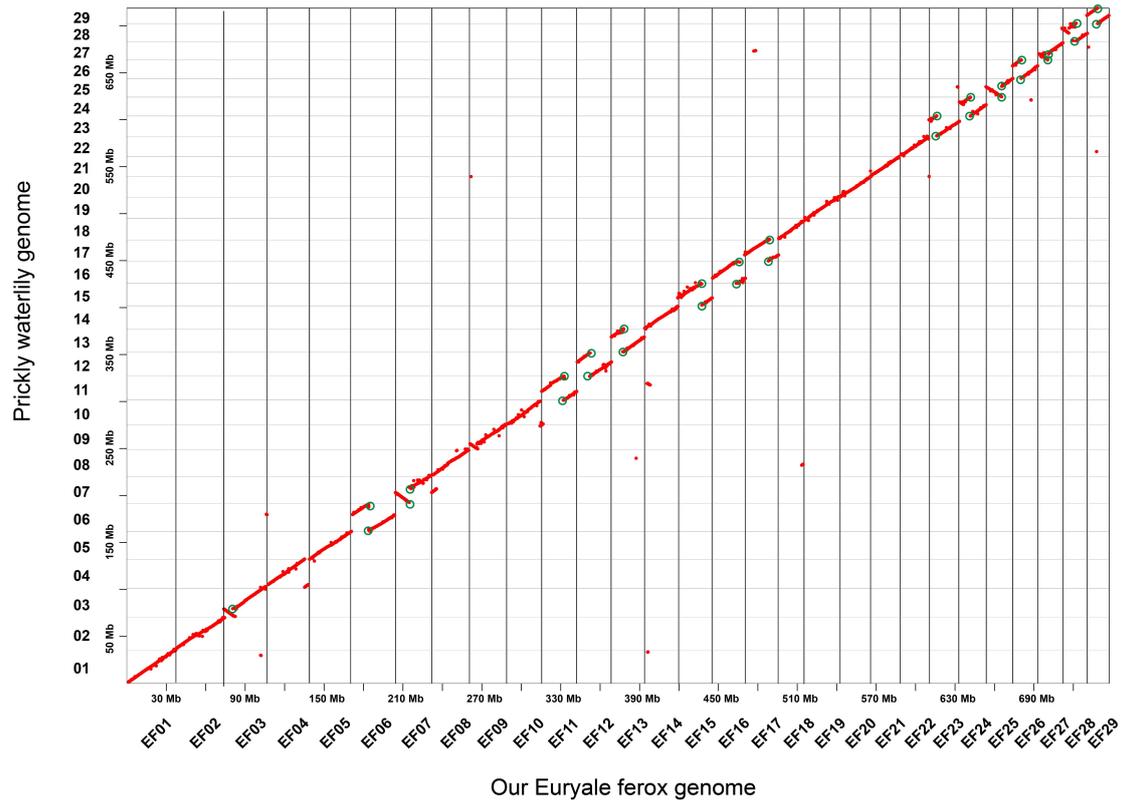
Supplementary Figure 2. Visualization of metaphase chromosomes of *E. ferox* after fluorescence in situ hybridization (FISH). (a) Texas Red-dUTP-labelled telomere of chromosomes (red). (b) Alexa Fluor 488-dUTP labelled a 410-bp BamHI fragment of 5S rDNA (green) of chromosomes. (c) Number the chromosomes in the (c).



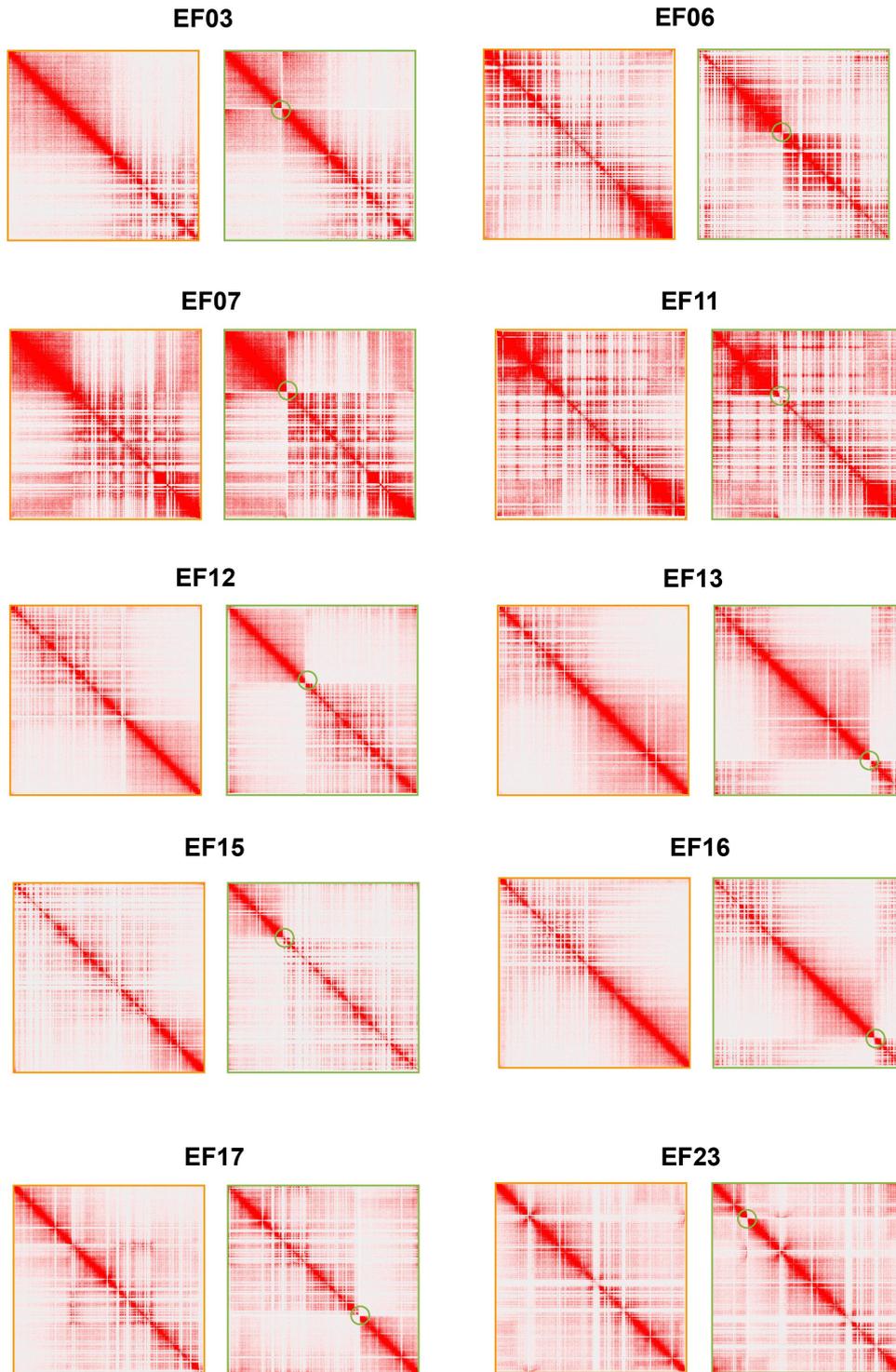
Supplementary Figure 3. *E. ferox* genome size and heterozygosity estimation results.



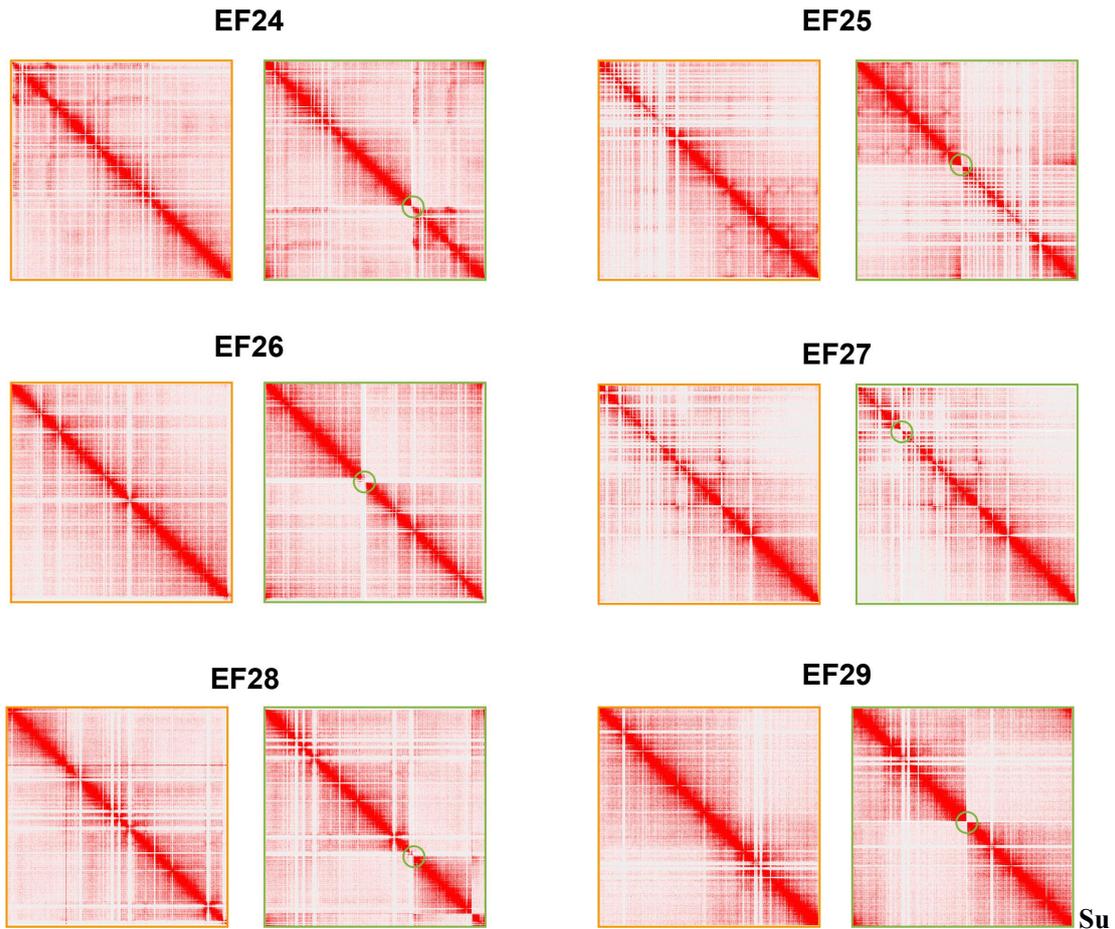
Supplementary Figure 4. Characterization of the *E. ferox* genome with circos plotting. The concentric circles from outermost to innermost show (1) the 29 pseudo-chromosomes, (2) heatmap of the gene density (the colors red-white denote high-low levels of gene density), (3) heatmap of the TE density (the colors blue-white denote high-low levels of the density of repeat sequences), and (4) heatmap of the GC content (the colors green-white denote high-low levels of GC content). The linking lines indicating the paralogous fragments between either two of the three sub-genomes that were generated from the whole genome triplication event.



Supplementary Figure 5. Chromosomal synteny comparison between two *E. ferox* genomes, the green circles mark the breakpoints of chromosome structural variants.

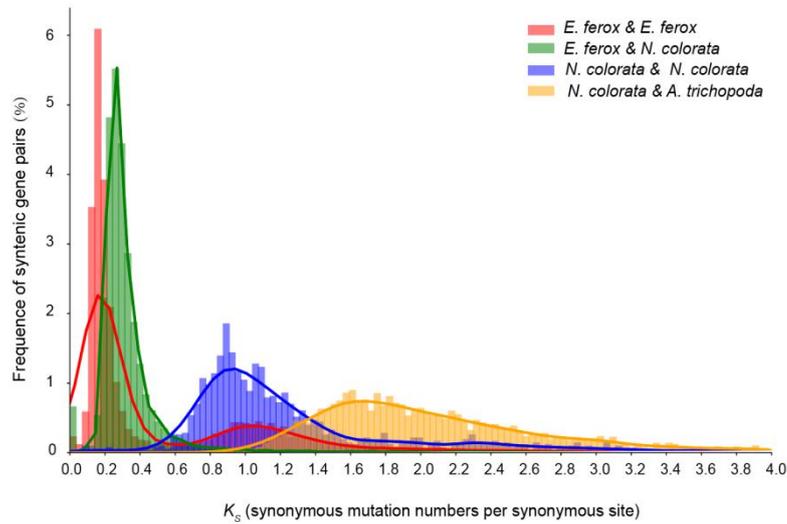


Supplementary Figure 6. HIC-heatmap of our *E. ferox* genome before and after adjustment according to the prick waterlily genome. The yellow box denotes before adjustment, the green box denotes after adjustment, the green circle represents the adjusted connection position.



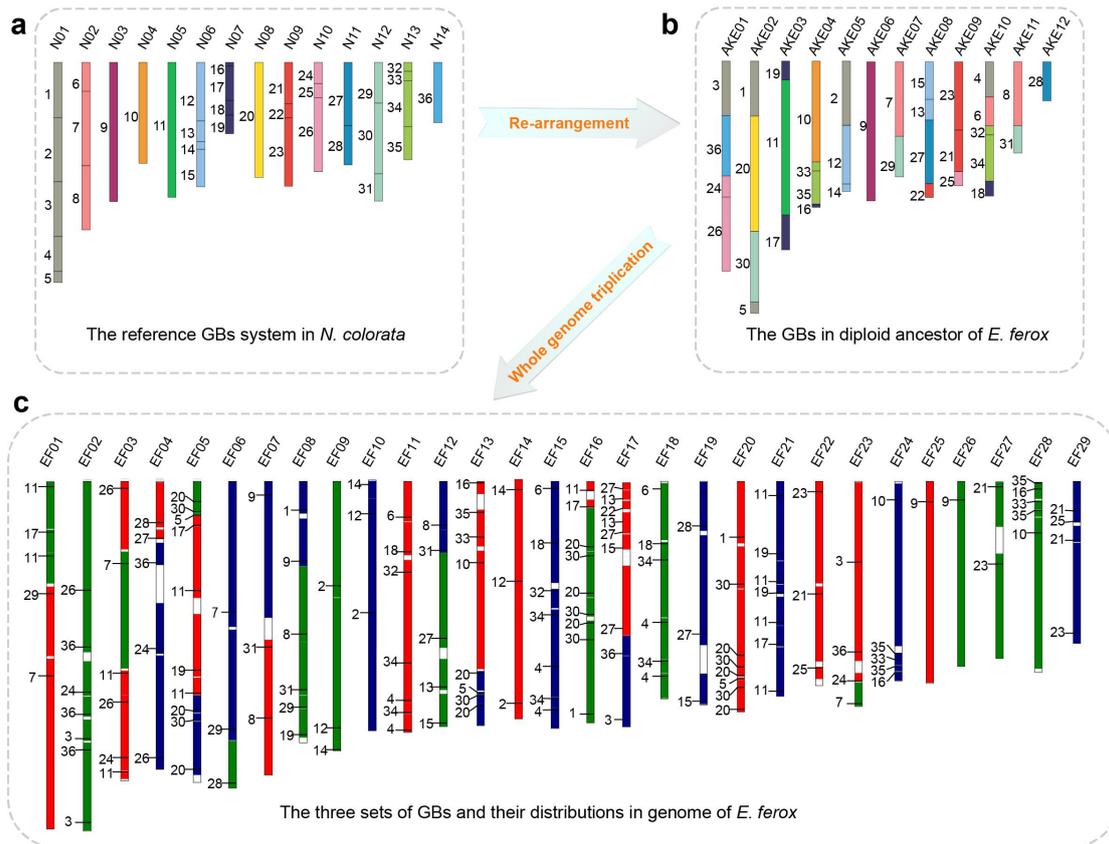
pplementary Figure 7. Hi-C-heatmap of our *E. ferox* genome before and after adjustment according to the prick waterlily genome. The yellow box denotes before adjustment, the green box denotes after adjustment, the green circle represents the adjusted connection position.

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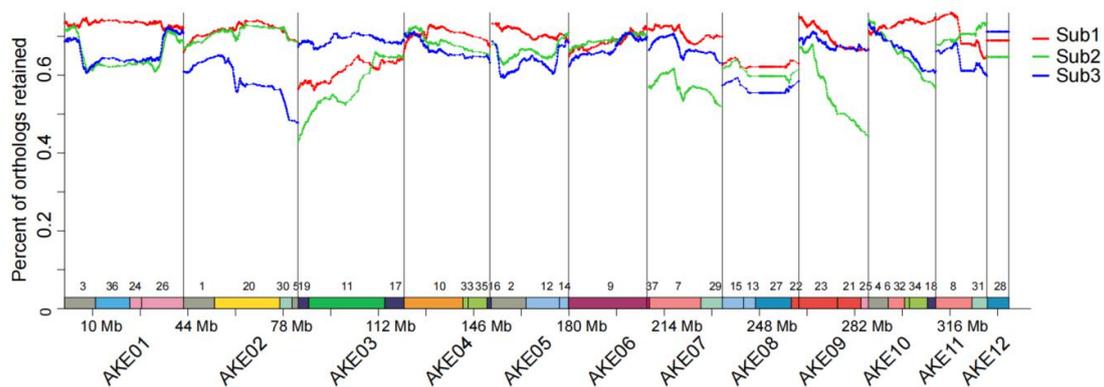


Supplementary Figure 8. Frequency distribution of K_s values between homologs genes.

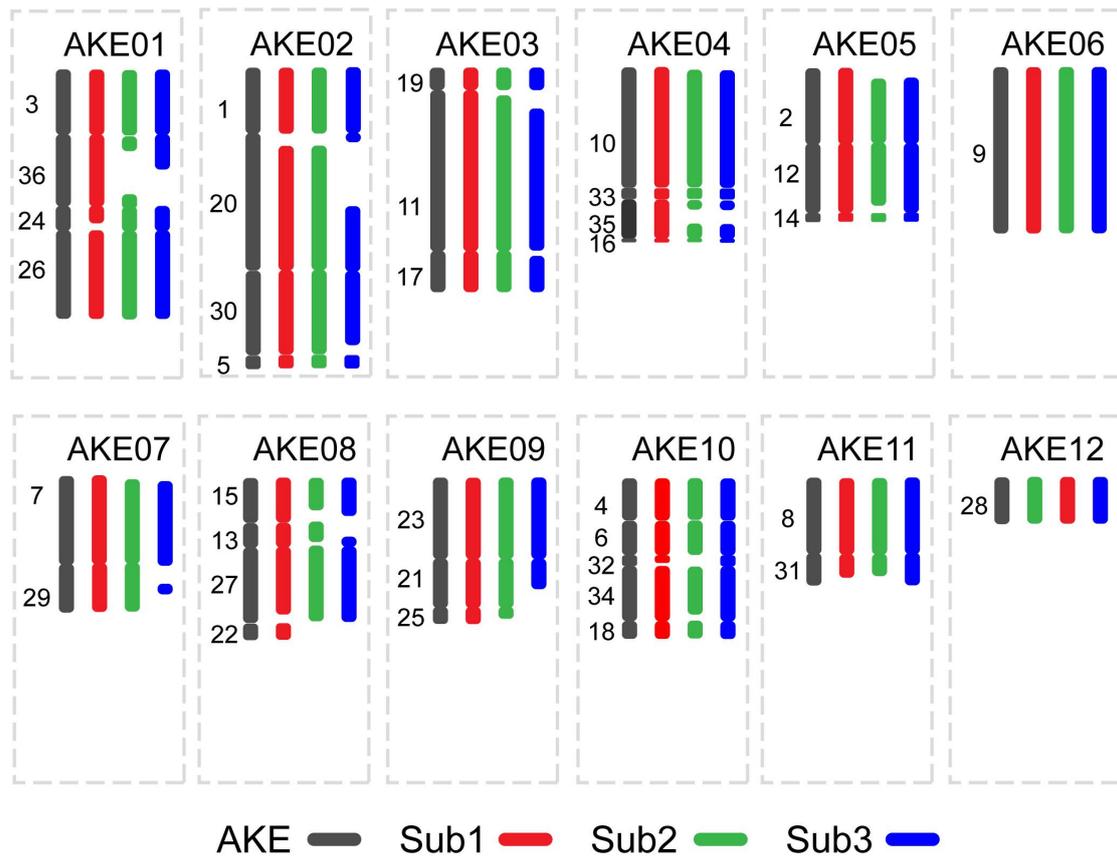
Red line show K_s distribution of paralogous gene pairs in *E. ferox* genome; green line show K_s distribution of orthologous gene pairs between *E. ferox* and *N. colorata* genome; blue line show K_s distribution of paralogous gene pairs in *N. colorata* genome; yellow line show K_s distribution of orthologous gene pairs between *N. colorata* and *A. trichopoda* genome.



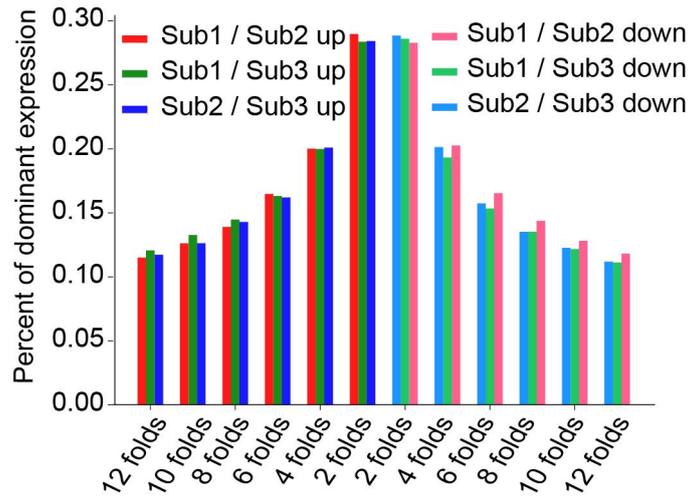
Supplementary Figure 9. Genome evolution trajectory of *E. ferox*. (a) Distribution of 36 genomic blocks (GBs) in the 14 chromosomes of *N. colorata*, the number next to the chromosome is the id of GBs. (b) Distribution of 36 GBs in the deduced 12 ancestral chromosomes of the diploid ancestor of *E. ferox*. (c) Distribution of the triplicated 36 genomic blocks in the 29 chromosomes of *E. ferox*, the colors red, green, and blue indicate GBs from sub-genomes Sub1, Sub2, and Sub3.



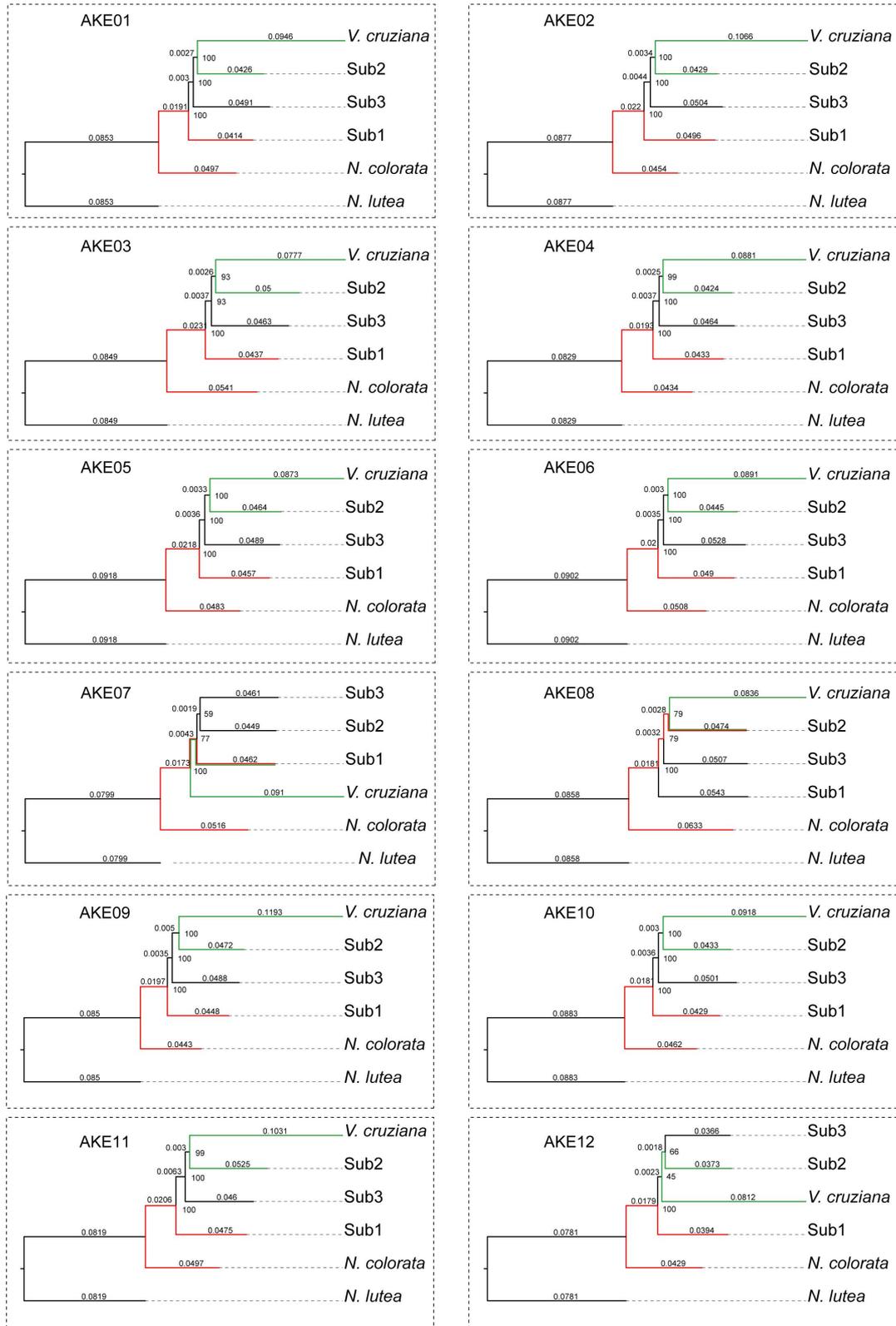
Supplementary Figure 10. Sub-genome features observed in the paleo-allopolyploidy genome of *E. ferox*. (a) The density of syntenic genes in three sub-genomes of *E. ferox*, compared to the deduced diploid ancestor of *E. ferox*. (genes of diploid genome *N. colorata* were used as the reference). The *x*-axis denotes the physical position of each gene locus in the genome of the diploid ancestor. The *y*-axis denotes the percentage of retained homologous genes in *E. ferox* sub-genomes around each diploid ancestor gene, where 500 genes flanking each side of a certain gene locus were analyzed, giving a total window size of 1,001 genes. (b) The number of dominantly expressed paralogous gene pairs between sub-genomes in the leaves, with no differences between the three sub-genomes. (c) The difference in average TE density in the vicinity regions of paralogous genes from the three sub-genomes of *E. ferox*.



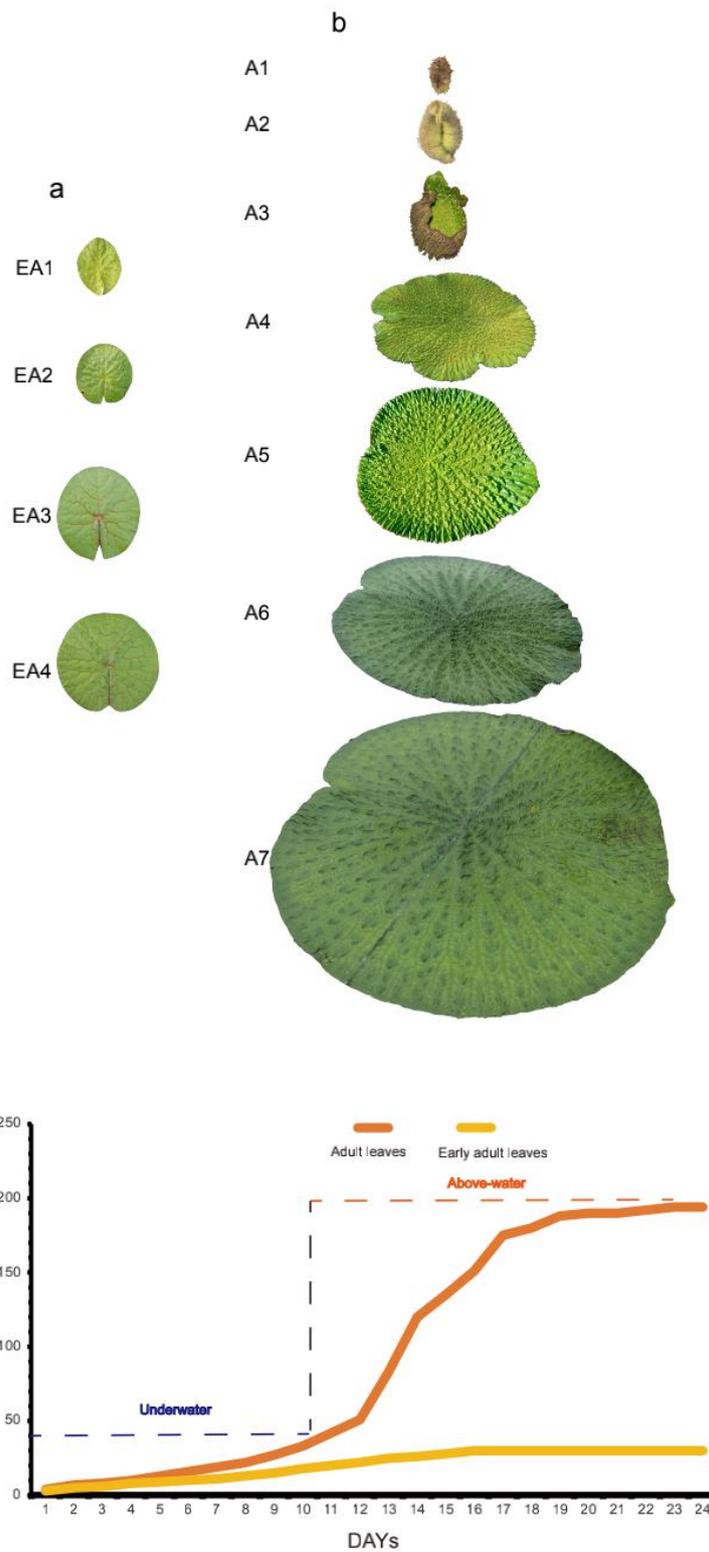
Supplementary Figure 11. *E. ferox* diploid ancestor karyotype and three sub-genomes. The black bars represent ancestral chromosomes, the numbers are GB ids, and the red, green and blue bars represent ancestor chromosomes of the three sub-genomes, blank areas represent large fragments that have been lost.



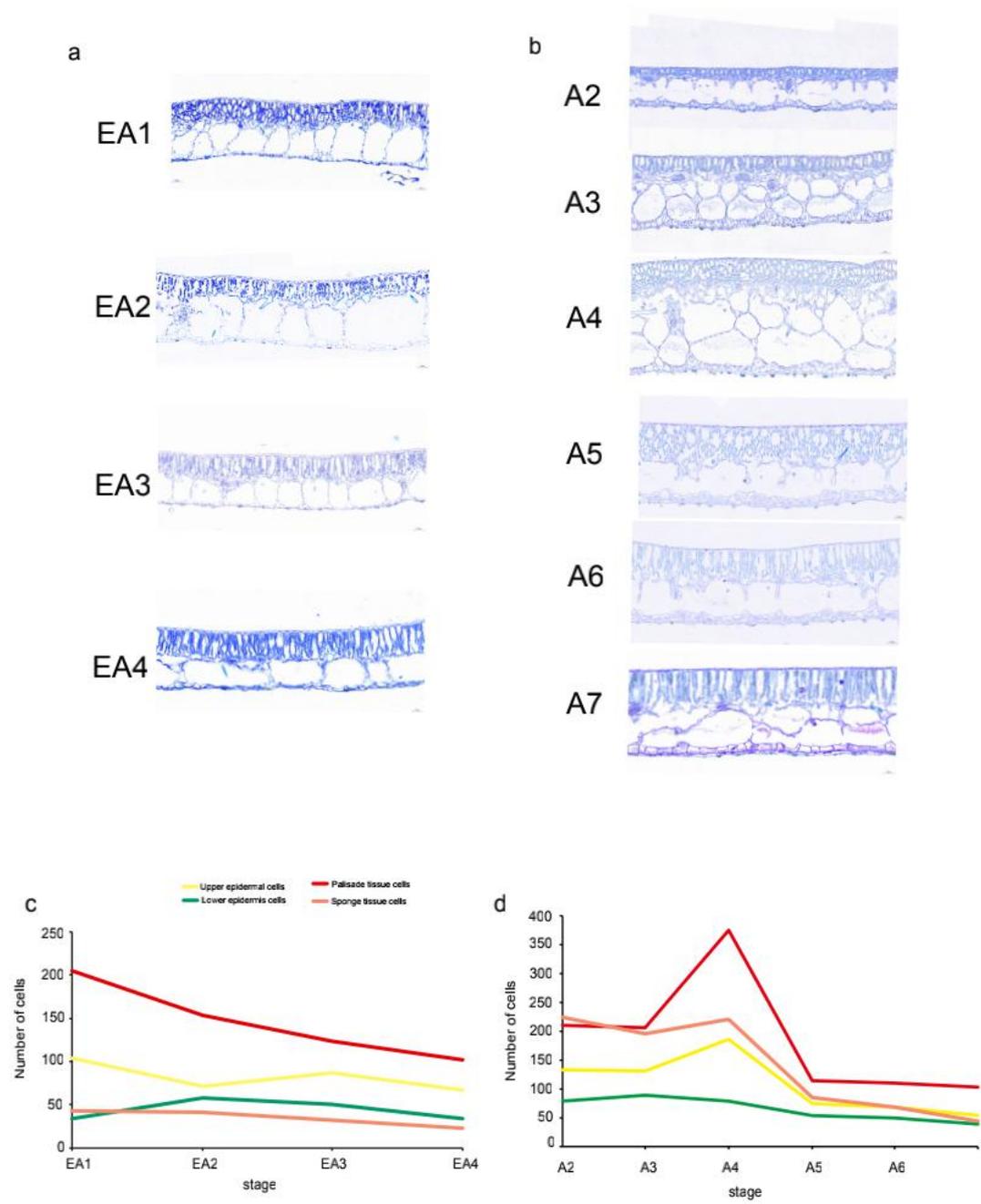
Supplementary Figure 12. Differential expression of paralogous gene pairs. The number of dominantly expressed paralogous gene pairs between sub-genomes in the leaves, with no differences between the three sub-genomes.



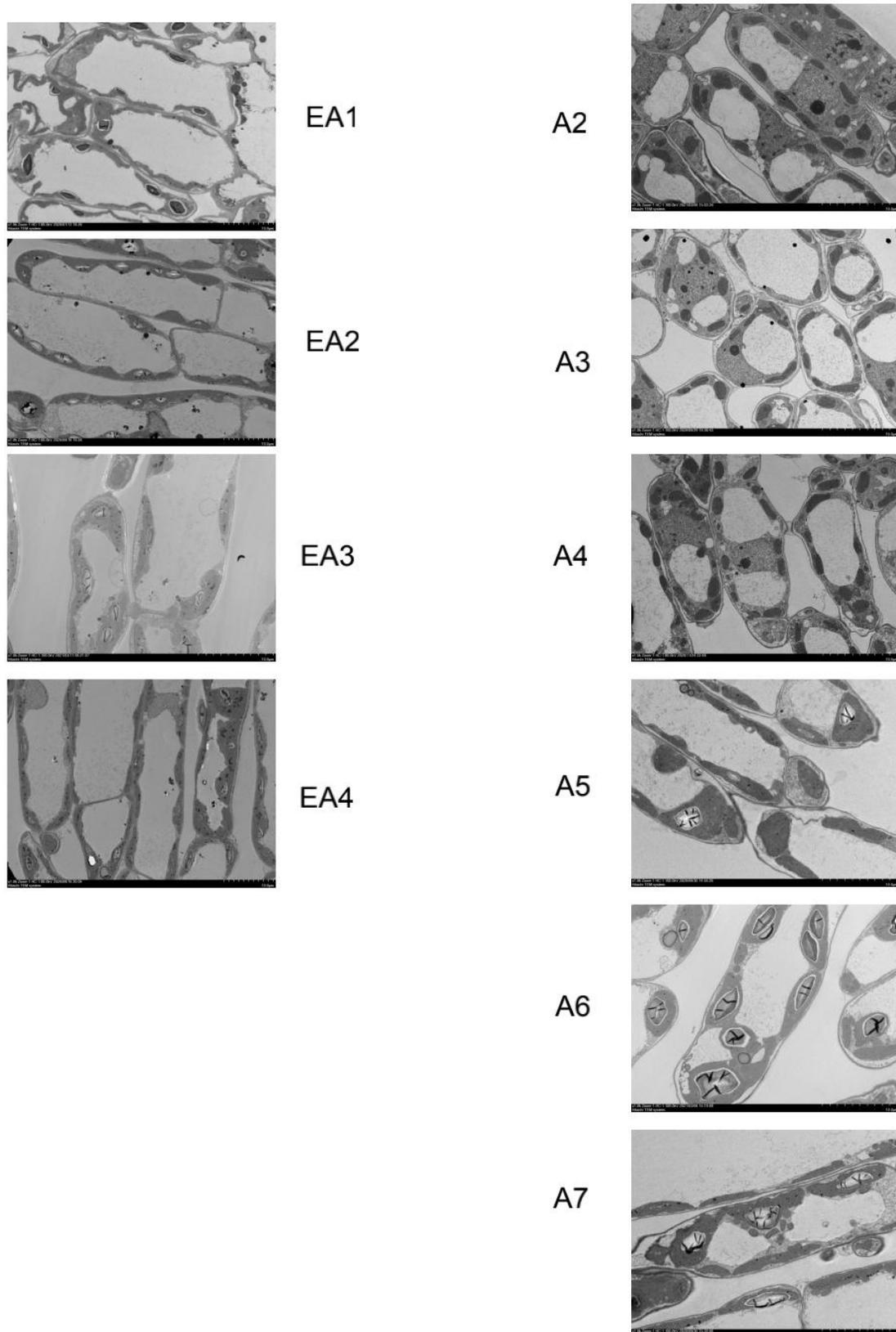
Supplementary Figure 13. Phylogenetic trees of 12 AKECs. Phylogenetic trees built by orthologous groups among *N. lutea*, *N. colorata*, *V. cruziana*, and sub-genomes of *E. ferox*. Red lines indicate the sub-genome that has shorter branch length to *N. colorata*, green lines indicate the sub-genome that has shorter branch length to *V. cruziana*.



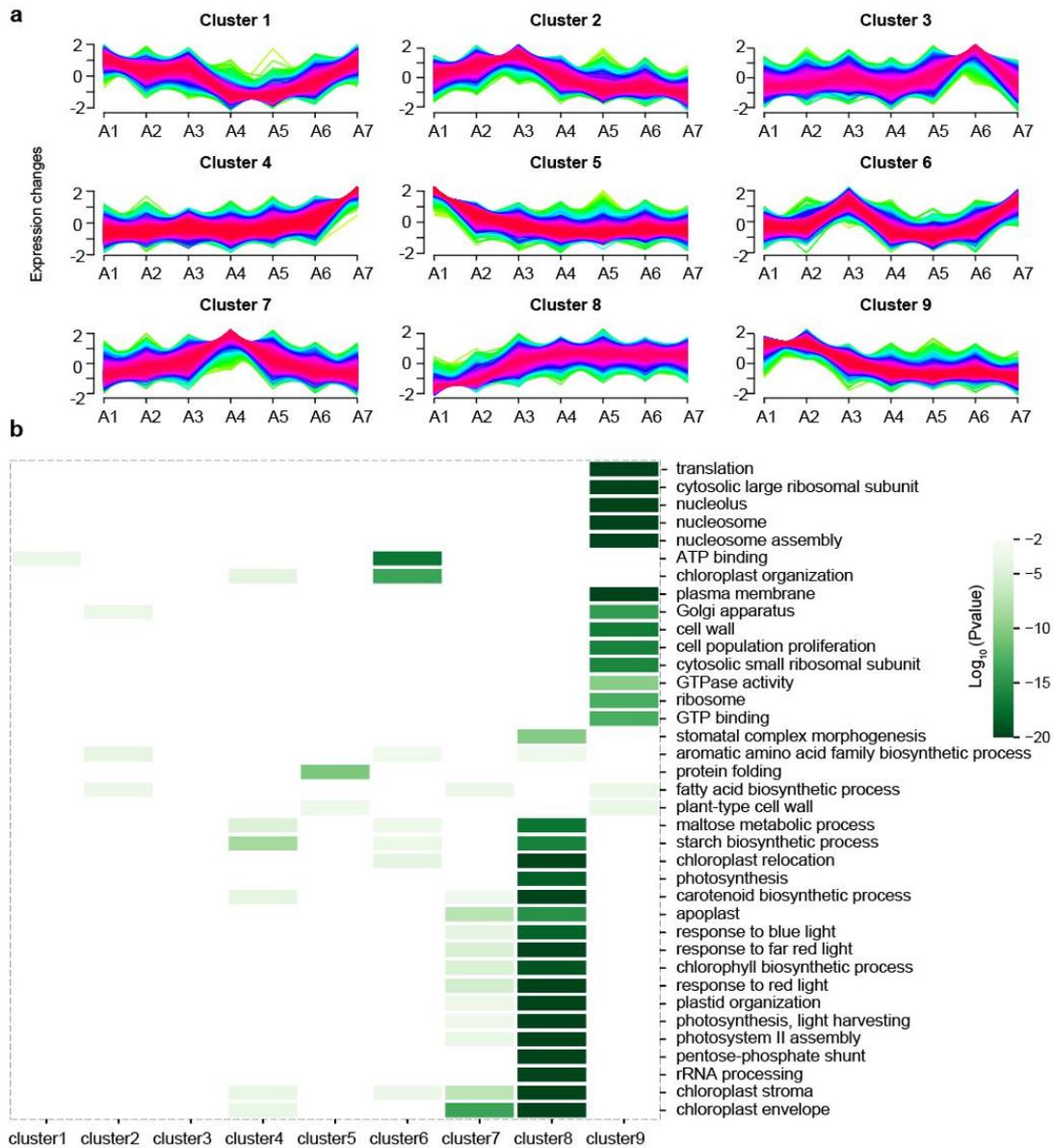
Supplementary Figure 14. (a) *E. ferox* early adult leaves of different stage (EA1- EA4). (b) *E. ferox* adult leaves of different stage (A1- A7). (c) Daily diameter length of the two types of leaves of *E. ferox*.



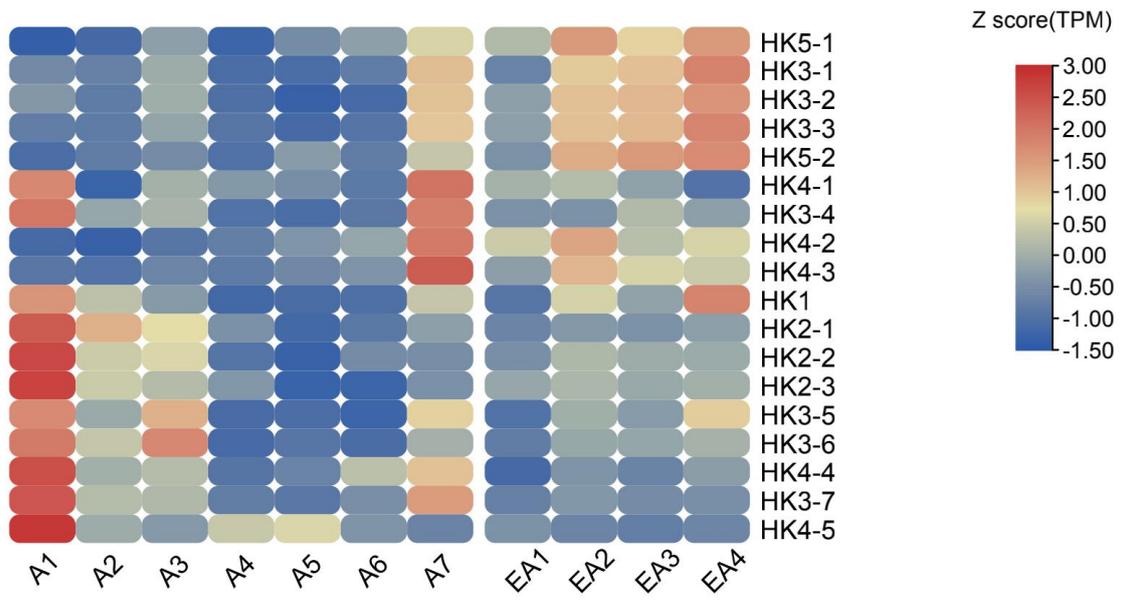
Supplementary Figure 15. (a,b) The Paraffin section of *E. ferox* early adult leave (EA1-EA4) and adult leaves cross section (A2- A7). (c,d) The cell number of *E. ferox* different leaf tissues at different development stage.



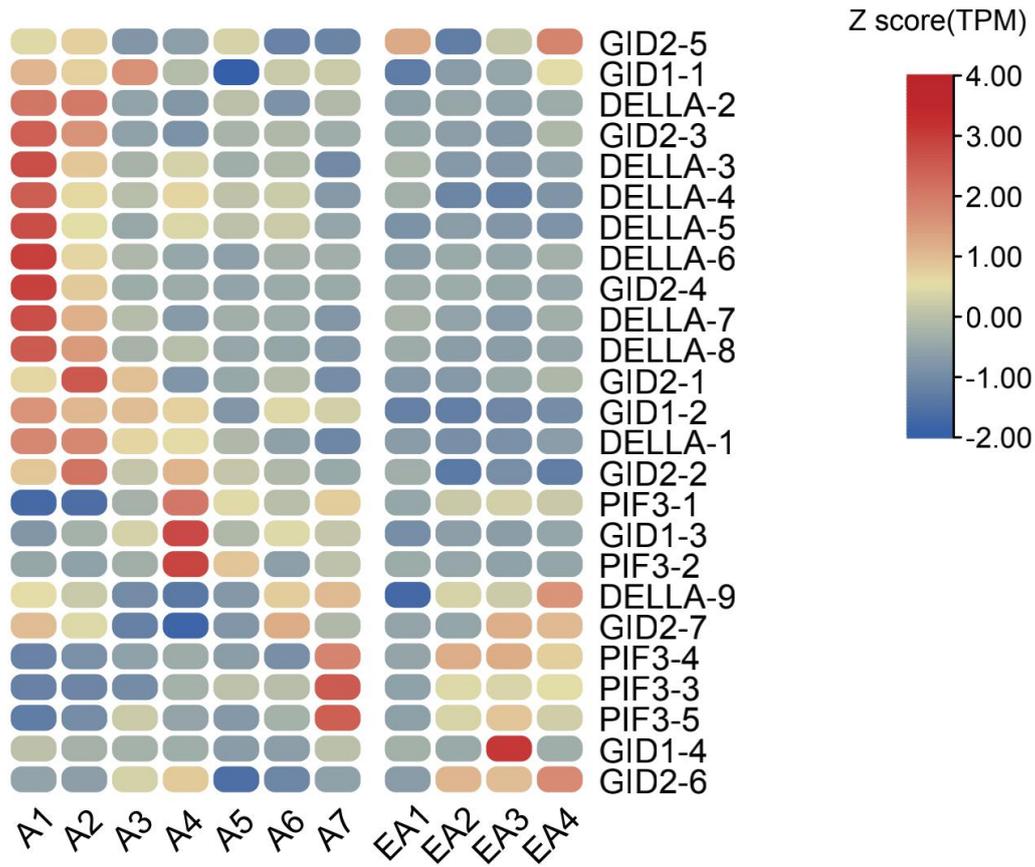
Supplementary Figure 16. The ultrastructure of *E. ferox* palisade tissue cell early adult leaves (EA1- EA4) and adult leaves (A2- A7).



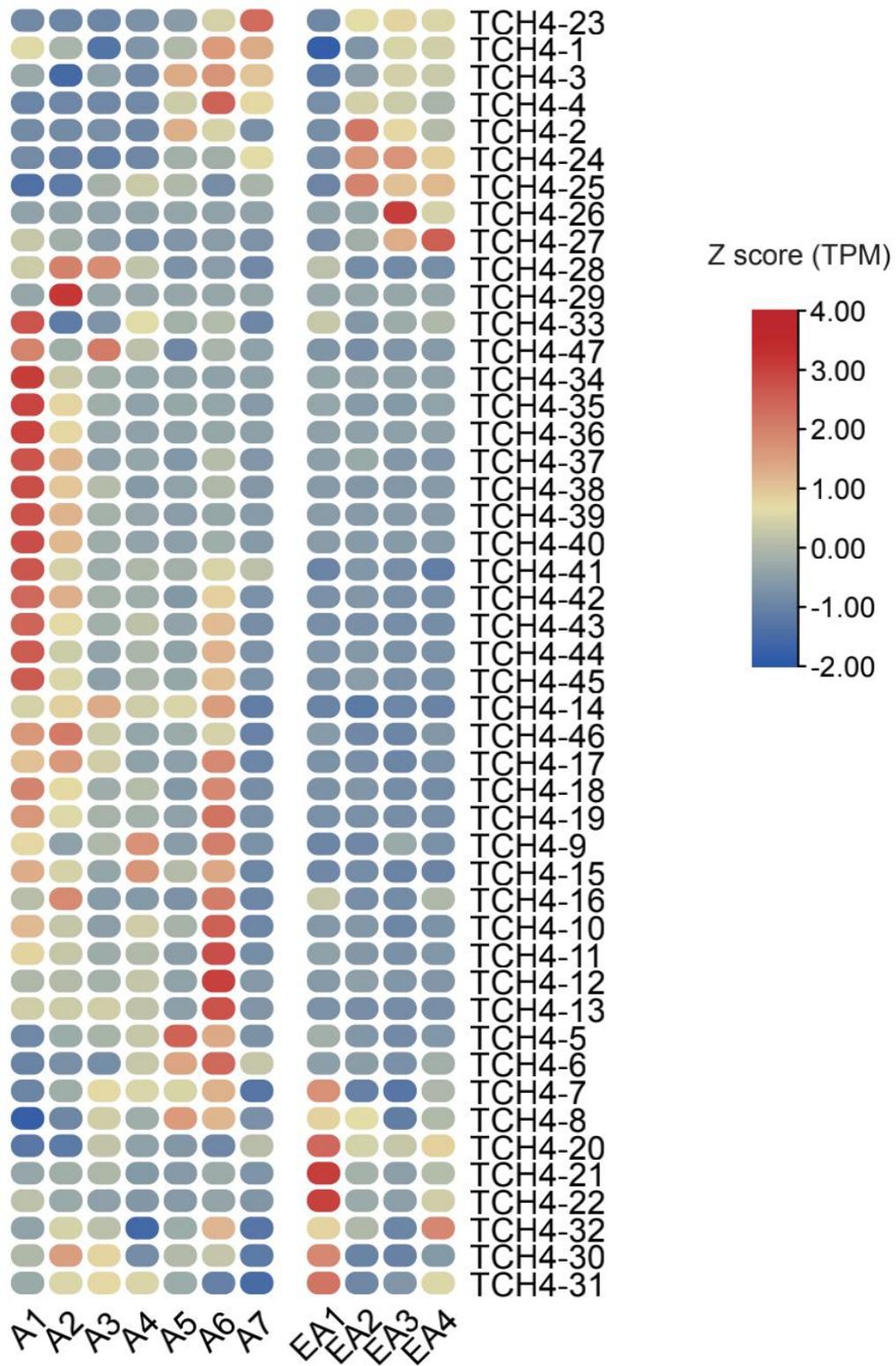
Supplementary Figure 17. Clustering and GO enrichment analyses of differentially expressed genes at adult leaves. (a) Nine clusters of differentially expressed genes at adult leaves by tool Mfuzz. (b) Heat map show *P* value of different GO items of nine clusters.



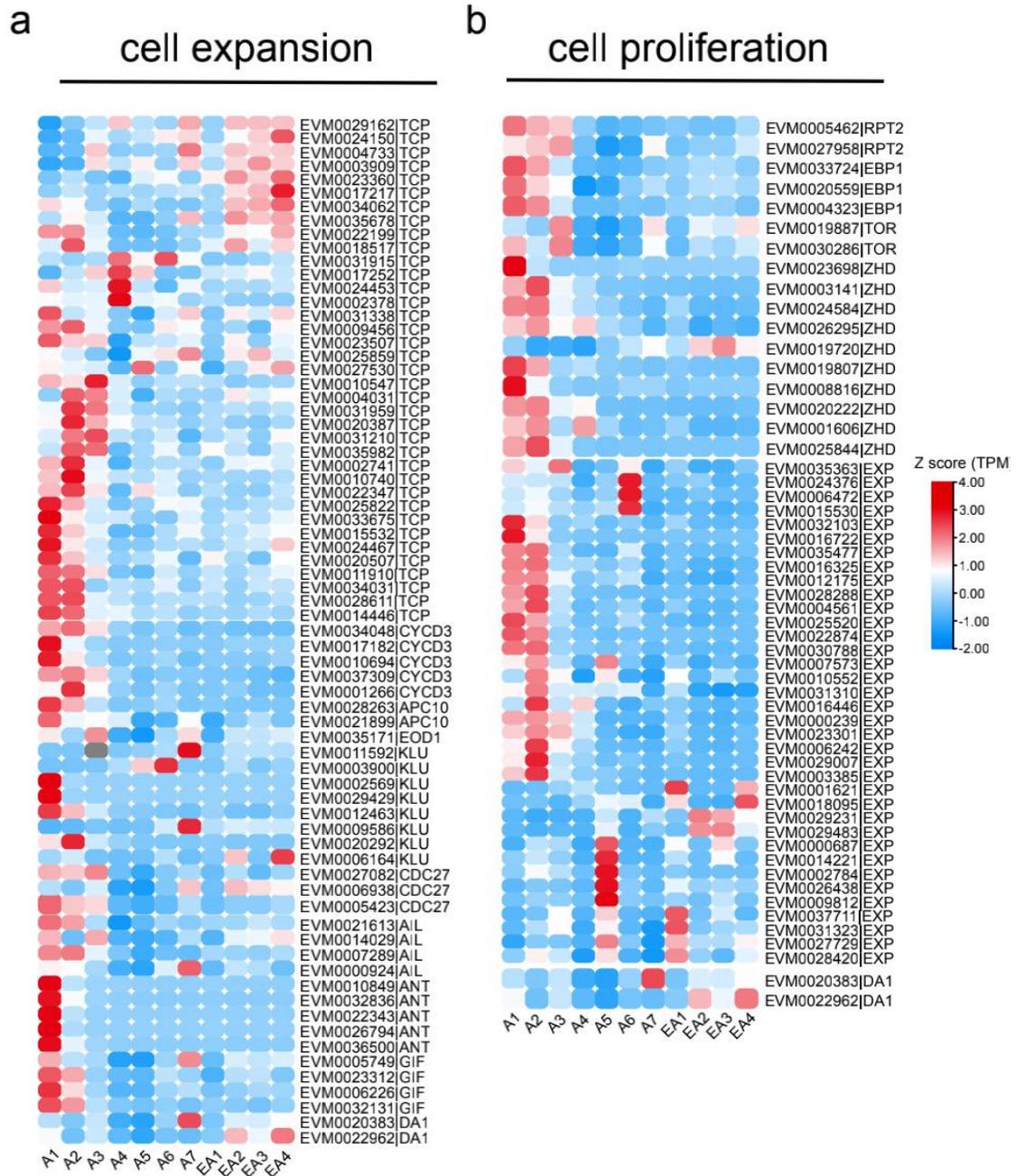
Supplementary Figure 18. Expression of HK gene family of *E. ferox*.



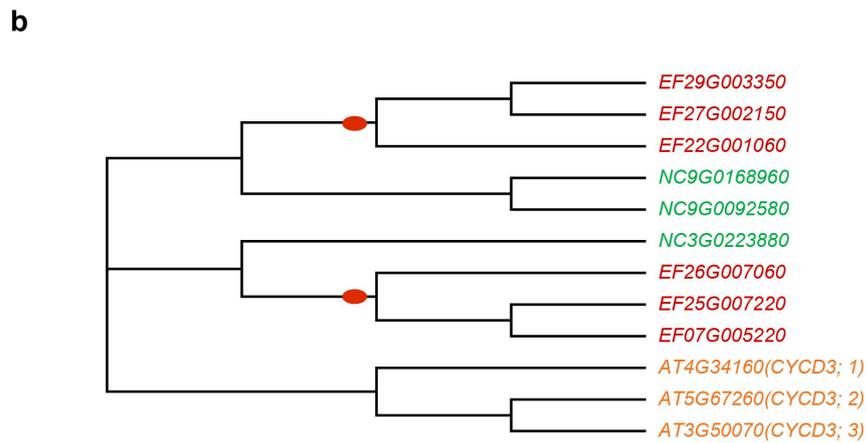
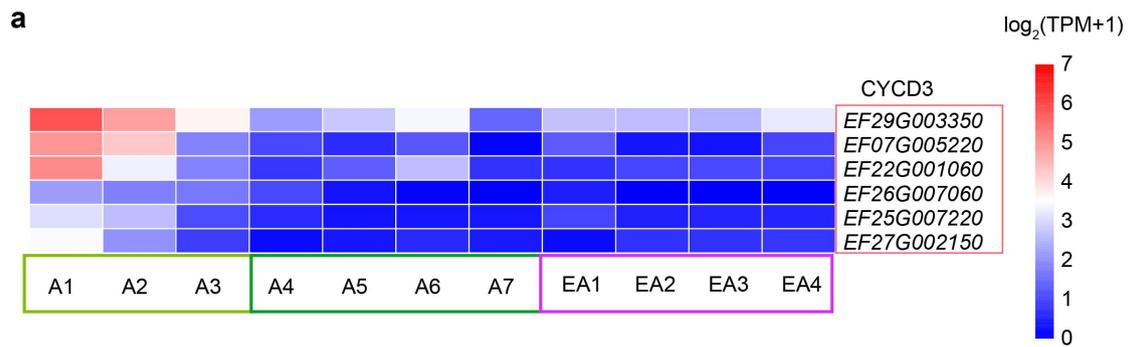
Supplementary Figure 19. Expression of GID, DELLA and PIF3 gene families of *E. ferox*.



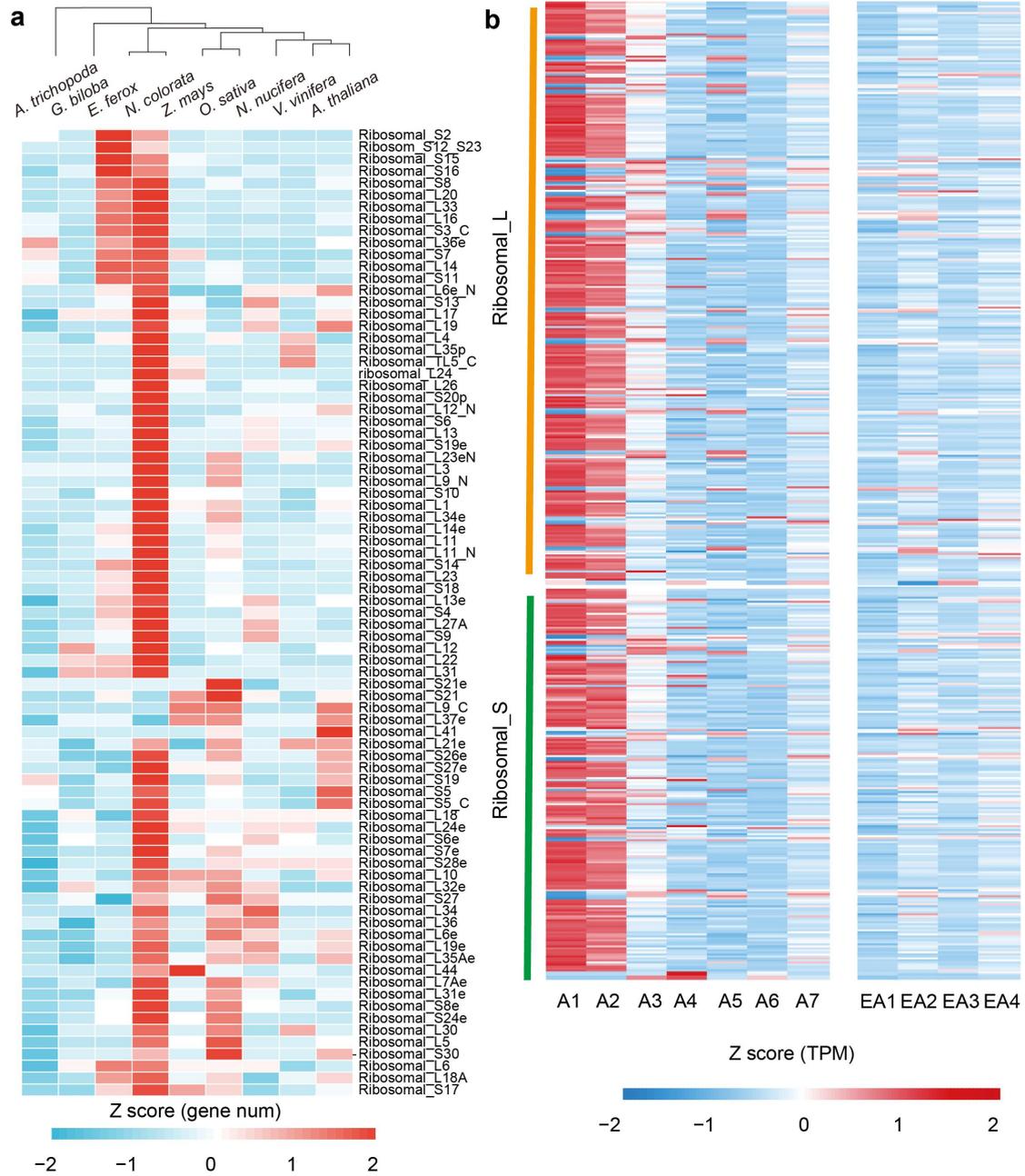
Supplementary Figure 20. Expression of TCH4 gene family of *E. ferox*.



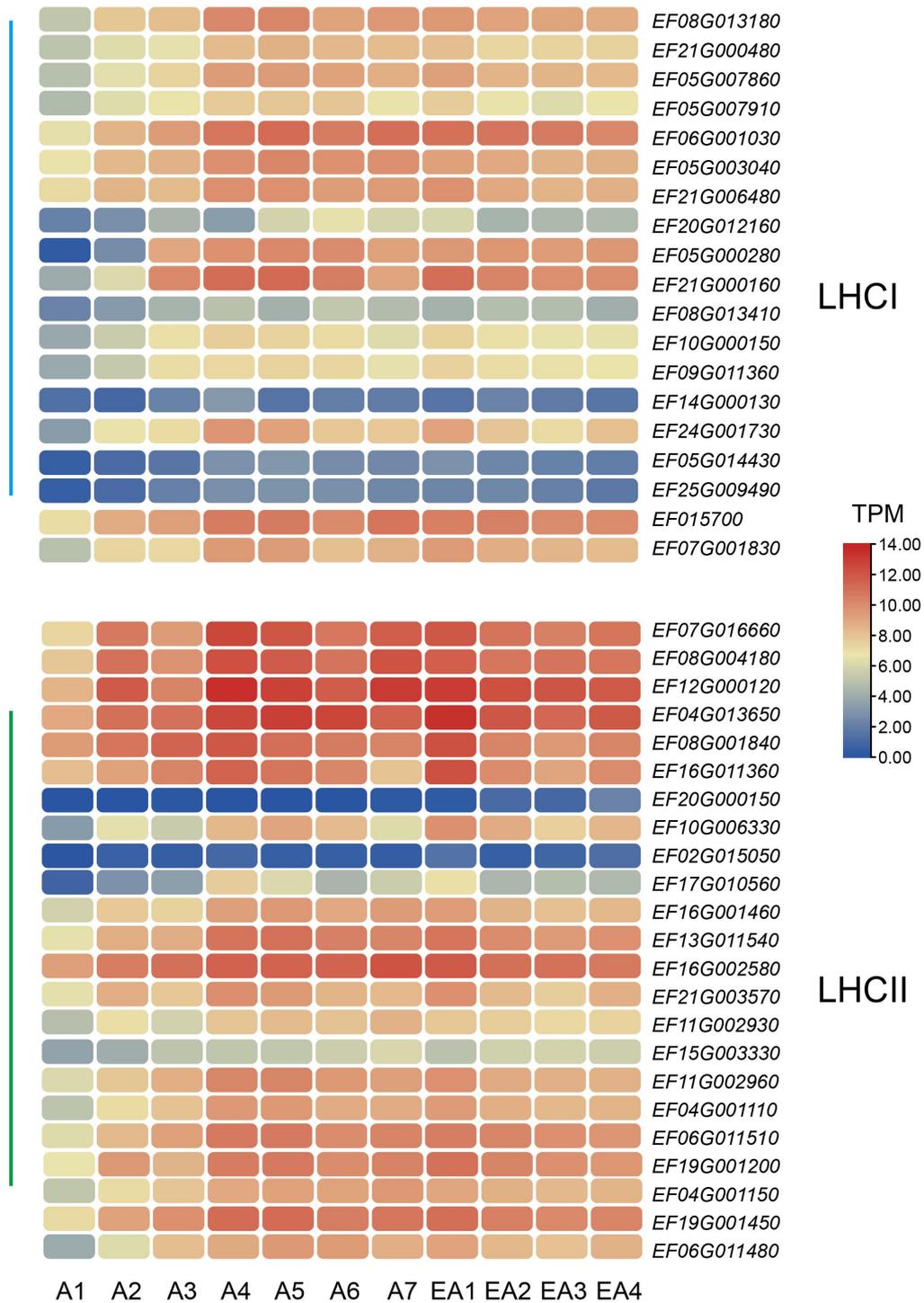
Supplementary Figure 21. Expression of cell expansion and cell proliferation relation of gene family of *E. ferox*.



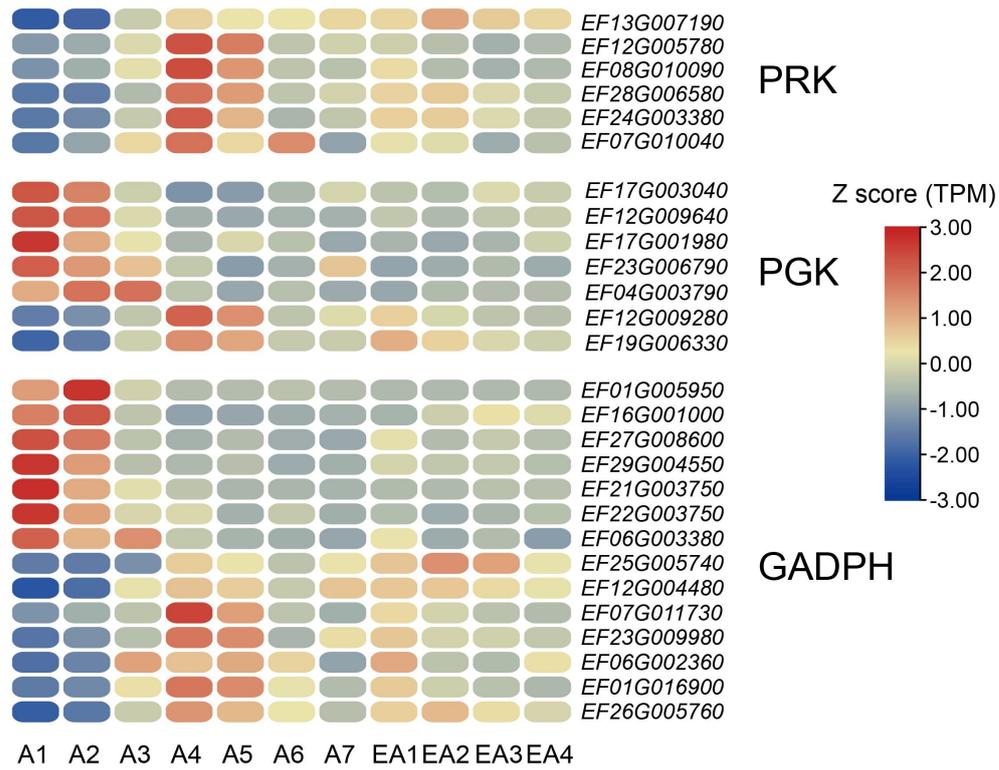
Supplementary Figure 22. The CYCD3 gene family. (a) Phylogenetic tree of CYCD3 gene homologous in *E. ferox*, *N. colorata* and *A. thaliana*. (b) Expression of CYCD3 genes in *E. ferox*.



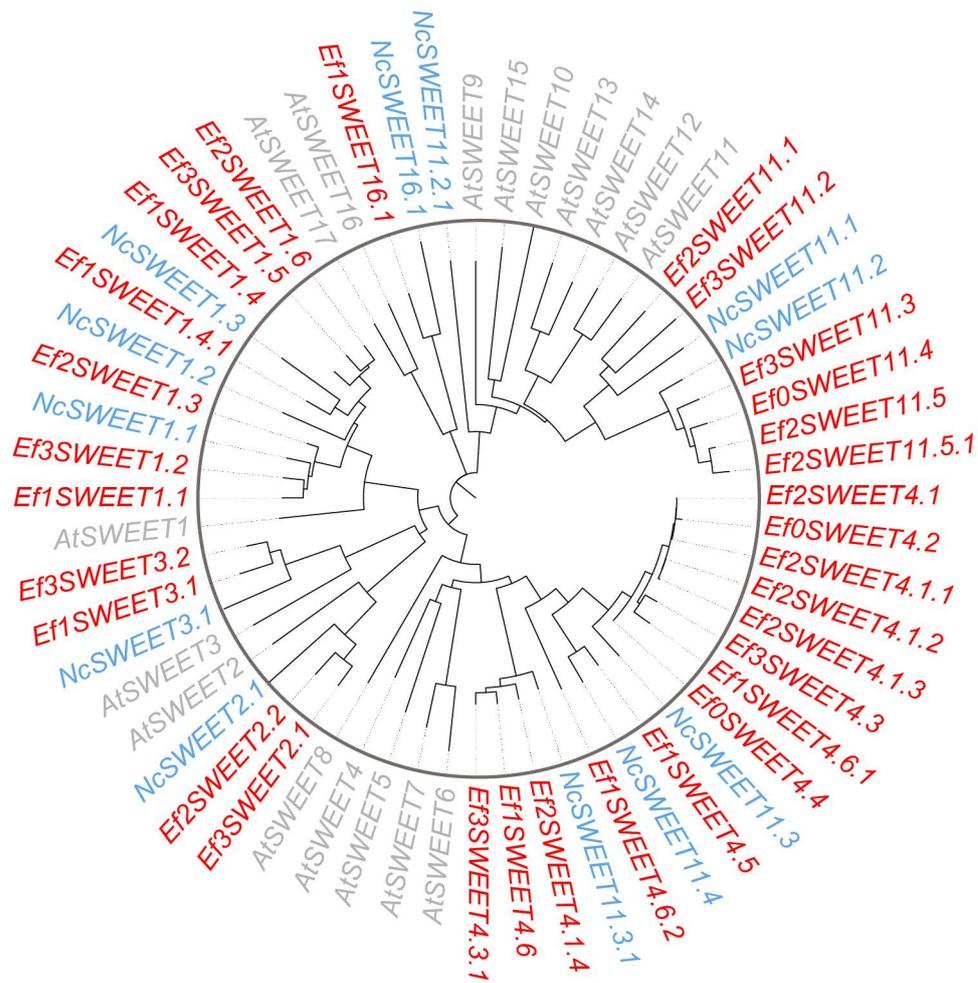
Supplementary Figure 23. Gene families for ribosomal protein. (a) Copy number of ribosomal protein gene families in *Ginkgo biloba*, *Amborella trichopoda*, *N. colorata*, *E. ferox*, *Oryza sativa*, *Zea mays*, *Nelumbo nucifera*, *Vitis vinifera*, and *Arabidopsis*. (b) Expression of ribosomal protein genes in *E. ferox*.



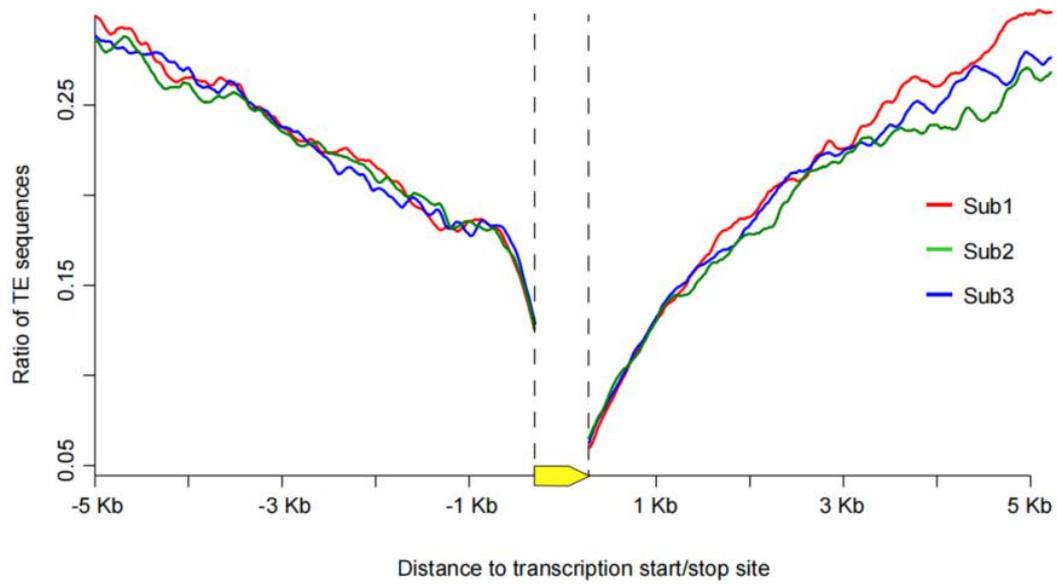
Supplementary Figure 24. Expression of LHC gene family of *E. ferox*.



Supplementary Figure 25. Expression of PRK, PGK and GAPDH gene families of *E. ferox*.



Supplementary Figure 26. Phylogenetic tree of SWEET gene homologous in *E. ferox*, *N. colorata* and *A. thaliana*.



Supplementary Figure 27. The difference in average TE density in the vicinity regions of paralogous genes from the three sub-genomes of *E. ferox*.