

Additional File 1 (Supplementary Figures)

This .pdf file contains the following supplementary figures: S1-S8. Legends for these figures are below.

S1 The *S. scovelli* genetic maps. a) The consensus map, which combines markers from both maps for each linkage group (LG), was used to link the scaffolds into chromosomes. b) Regional patterns of reduced recombination in males (inferred to be centromeric) and reduced recombination in females (inferred to be telomeric) were used to orient of centromeres to the top in the LGs in panel (a). Blue marks regions of relative expansion in the female map; red marks relative expansion in the male map for the markers they have in common on this example linkage group. c) On LG 1 a large block of markers (shown in red) aligning to about 17 Mb of scaffold length is non-recombining in both the male and female in this genetic cross. A possible explanation for this is that both parents carry a large inversion across this region. This is one of the two chromosomes formed by fusion of two ancestral chromosomes.

S2 Length distributions for unlinked scaffolds and for those captured by linkage groups show that the majority of large, gene-containing scaffolds are incorporated into the chromosome models. a) Boxplots and individual points reflect scaffold length distributions. Individual points (scaffolds) are coded by gene density (grading from yellow for no genes to red for high gene density). b) Scaffold length distributions in histogram form.

S3 VISTA plots suggest pipefish *dlx* clusters are missing CNEs (red arrows) that are conserved between other percomorphs and cod, a non-percomorph. The reference for the *dlx3/4a* and *3/4b* plots is stickleback (Gac) and for *dlx5/6a* is medaka. Hsa, human; Dre, zebrafish; Gmo, cod; Ssc, pipefish; Ola, medaka; Tru, pufferfish. Exons are highlighted in blue, CNEs in pink. Zebrafish has evolutionarily lost *dlx3a*. See Fig. 3 for the *dlx1/2a* VISTA comparison.

S4 Gene co-expression patterns reveal brood pouch transcriptome differences between pregnant and non-pregnant males. a) nMDS ordination of samples highlighting differences in reduced (2)-dimensional space. Orange circles represent pregnant male fish and blue boxes non-pregnant fish. b) Heatmap including all 15,253 pouch-expressed genes with sample (column) and gene (row) clustering. c) Heatmap including 70 randomly selected pouch-expressed genes with KEGG orthology (KO) assignments. d) Heatmap including the 70 pouch-expressed genes with “natural killer cell mediated cytotoxicity” KO assignments. Pink boxes highlight KO descriptions for clusters of genes showing pregnancy-depressed (above) and pregnancy-enriched (below) expression. Color keys for all heatmaps show expression values, scaled for each gene to have a mean of 0 and a standard deviation of 1, and dashed portions of clustering dendrograms represent non-pregnant males.

S5 Pathview [132] KEGG pathway renderings with scaled pregnancy-specific \log_2 fold changes mapped to KOs. Blue hues represent pregnancy-depressed KOs, while orange hues represent pregnancy-enriched KOs. a) The “coagulation and complement cascades” KEGG pathway. The red asterisk marks pregnancy-enriched components of the Membrane Attack Complex (see text). b) The “cytokine-cytokine receptor interaction” KEGG map, with red asterisk marking the chemokines, of which pro-inflammatory chemokines are pregnancy-enriched.

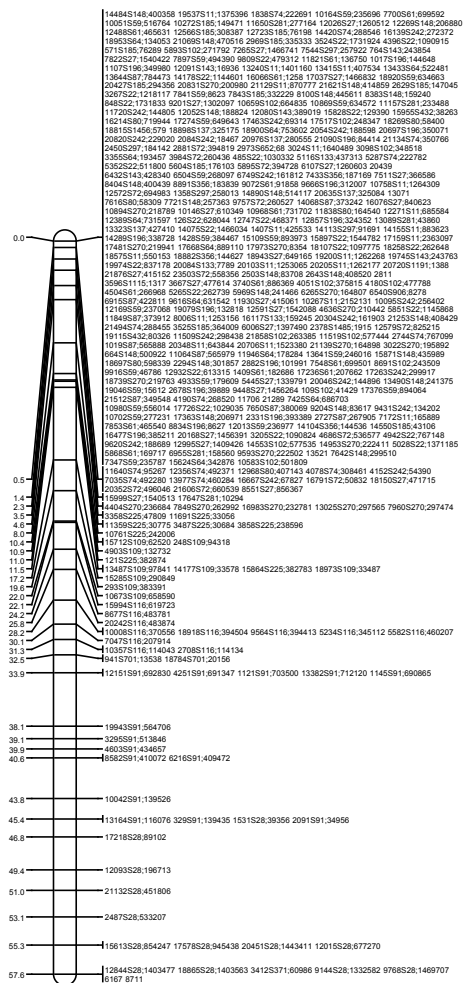
S6 Heatmaps comparing pregnancy-specific expression patterns for an innate immunity KEGG pathway [a], “coagulation and complement cascades”] and an adaptive immunity KEGG pathway [b], “antigen processing and presentation”]. The pink box highlights pipefish genes in the coagulation and complement cascades that are distinctly pregnancy enriched. No such strong patterns are observed for antigen processing and presentation. Color keys for heatmaps show expression values, scaled for each gene to have a mean of 0 and a standard deviation of 1, and dashed portions of clustering dendrograms represent non-pregnant males.

S7 *S. scovelli* has atypical craniofacial development from early developmental stages in which cartilages can first be detected by alcian blue staining. a) Shown is a developmental series of pipefish embryos from within the brood pouch and a juvenile (one day after emergence from the pouch, at 16 days after fertilization), stained with alcian blue for cartilage and alizarin red for mineralized bone. Approximately similar developmental stages of stickleback, another percomorph, are shown. Note the upturned jaws (formed by Meckel’s and palatoquadrate cartilages), the unusually curved ethmoid cartilage, and the elongating hyosymplectic cartilage in pipefish compared to stickleback, which has less derived development and morphology. b) A ventral view of pharyngeal cartilages of an 11 day old pipefish (stained with alcian and alizarin). In all panels, anterior is to the left. m, Meckel’s cartilage; pq, palatoquadrate; et, ethmoid; ch, ceratohyal; hs, hyosymplectic.

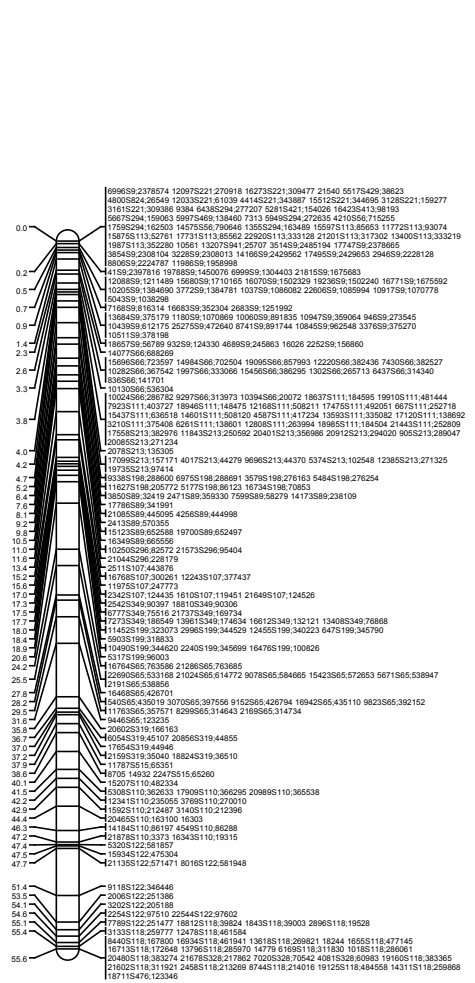
S8 Maximum Likelihood (PhyML) tree from Fig. 7, including complete accession numbers for all amino acid sequences. Pastristacins are in purple. Clade support values are SH-aLRT, and number of bootstrap replicates (of 500 total) in which the clade was recovered. See Fig. 7 for context.

a

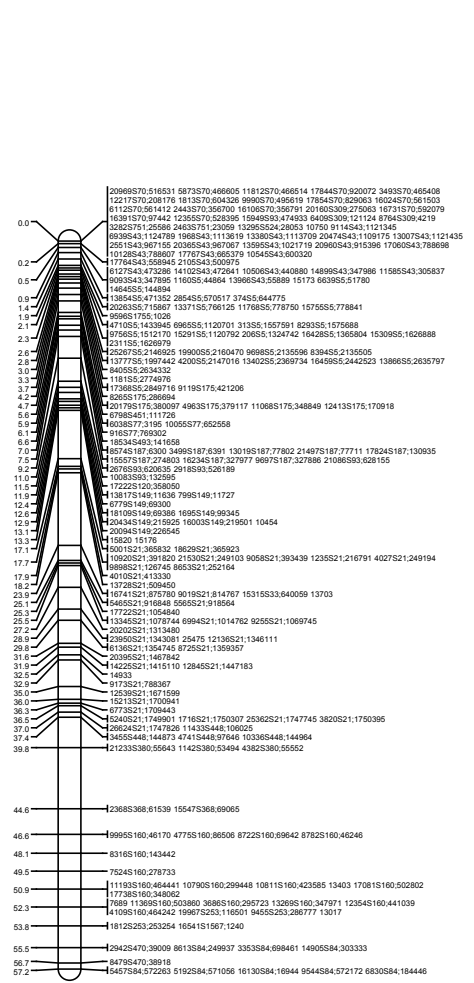
SscLG1



SscLG2

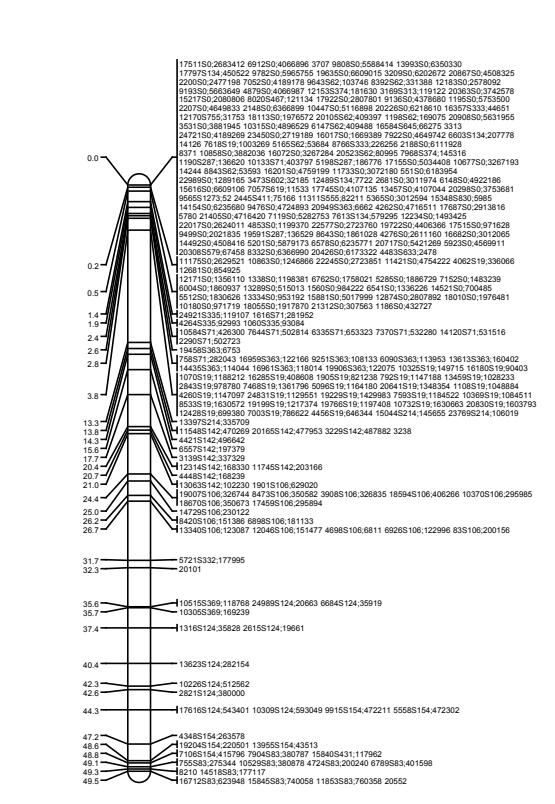


SscLG3

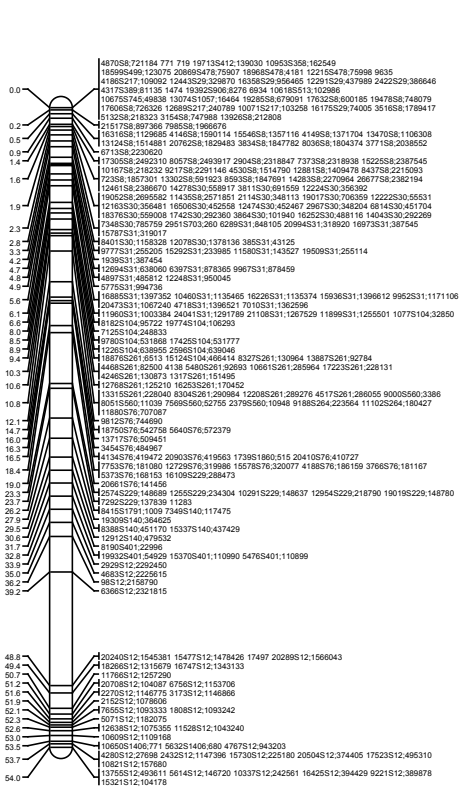


Additional File 1

SscLG4



SscLG5



SscLG6

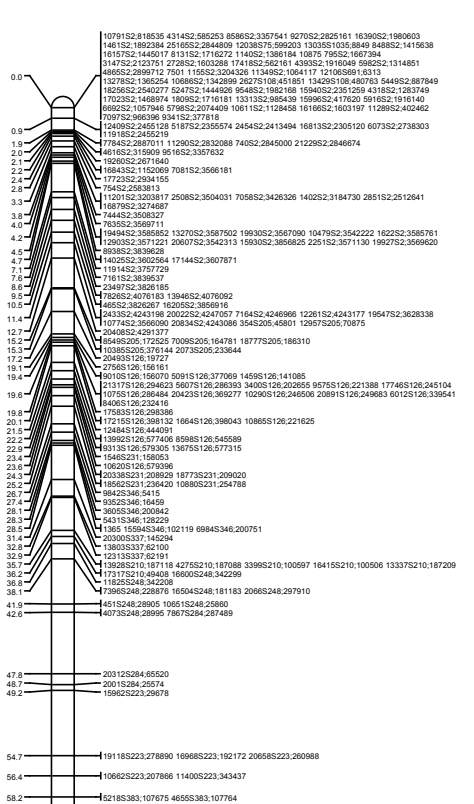
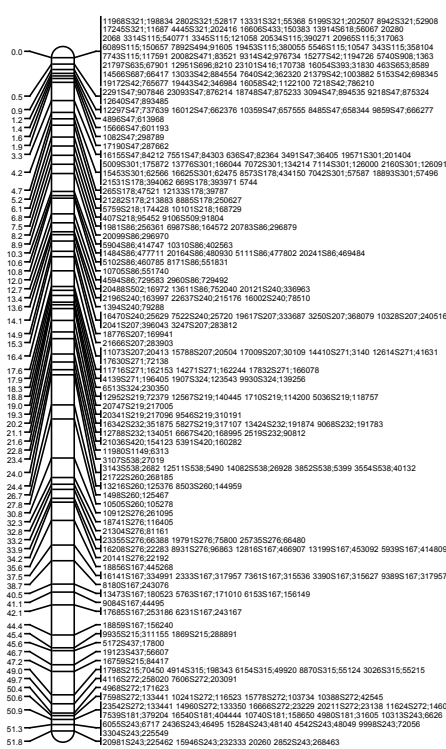


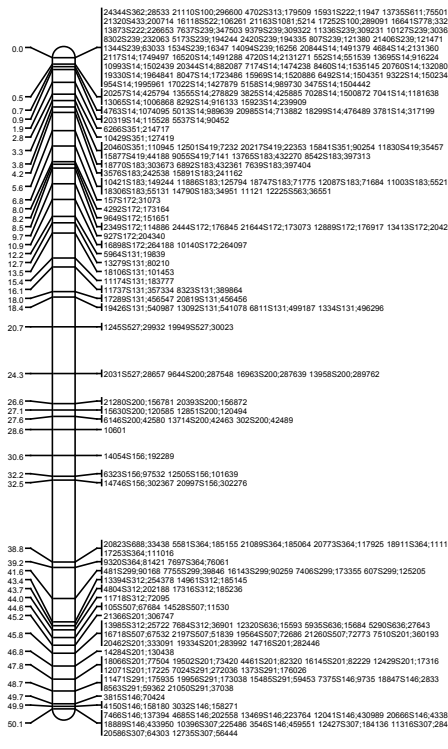
Fig. S1

a

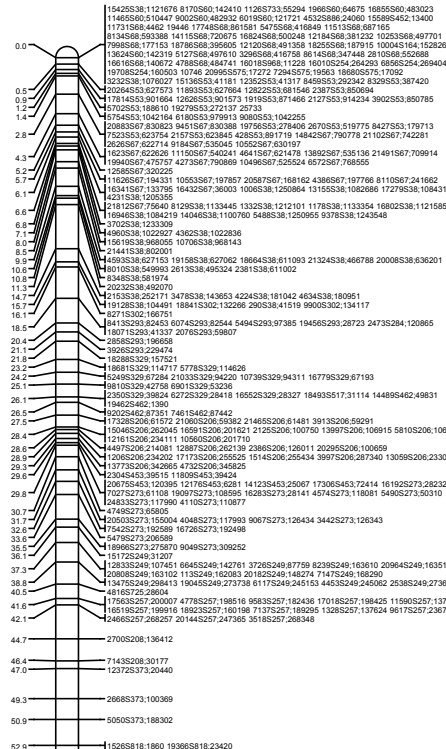
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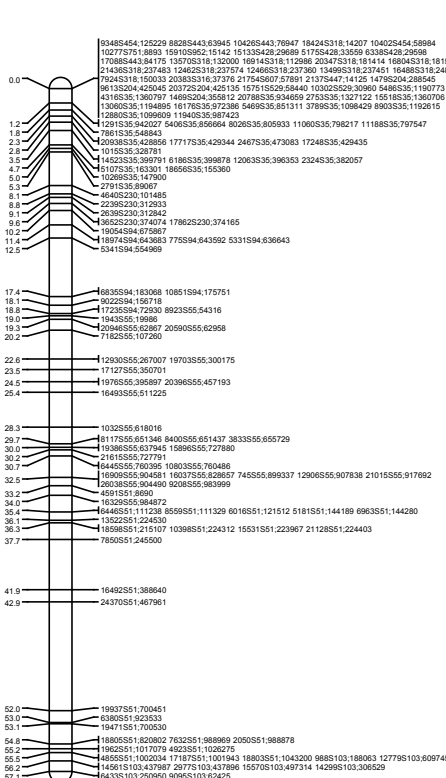
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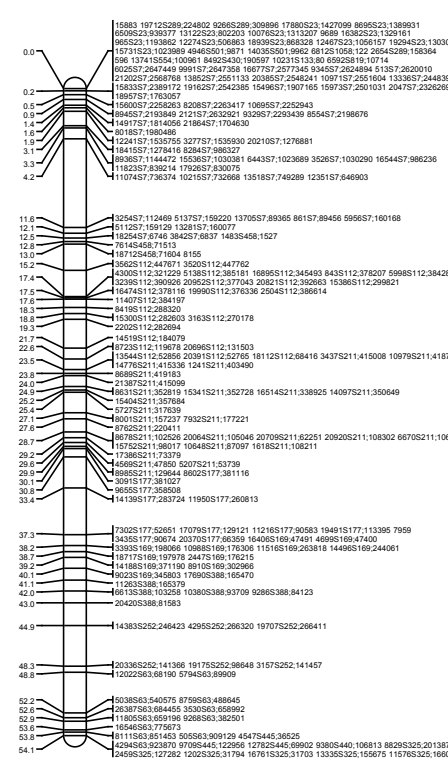
SscLG9



SscLG10



SscLG11



SscLG12

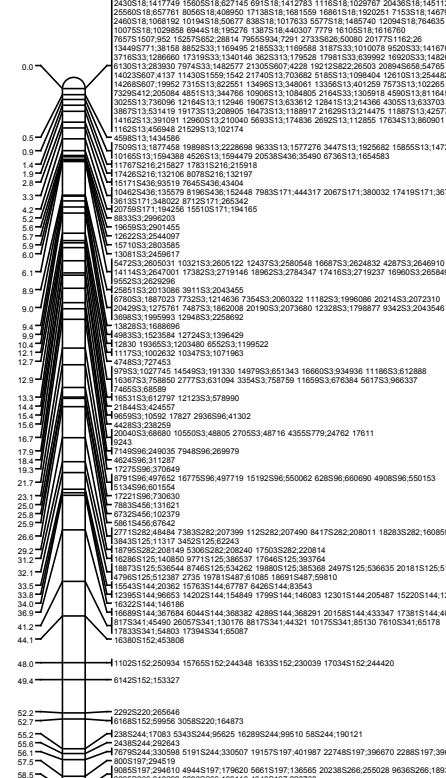


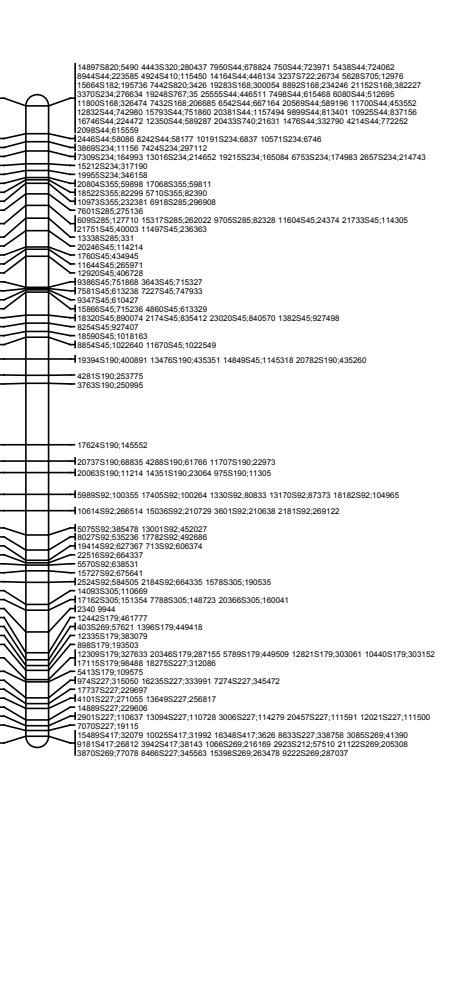
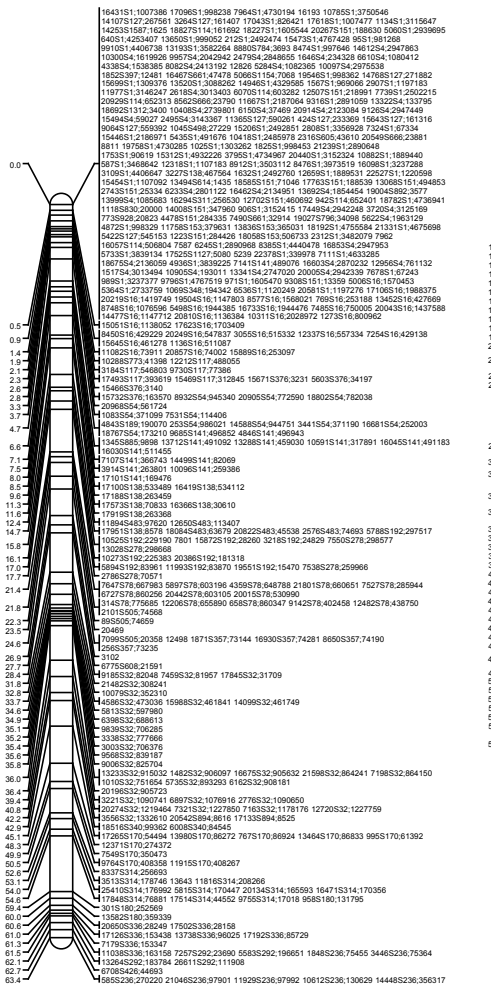
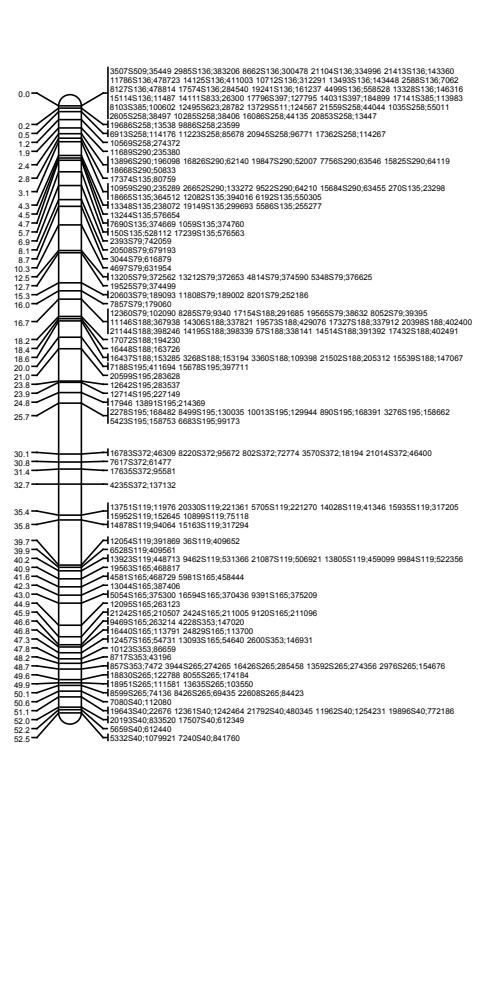
Fig. S1

a

SscLG13

SscLG14

SscLG16



SscLG 6

SscLG17

SscLG18

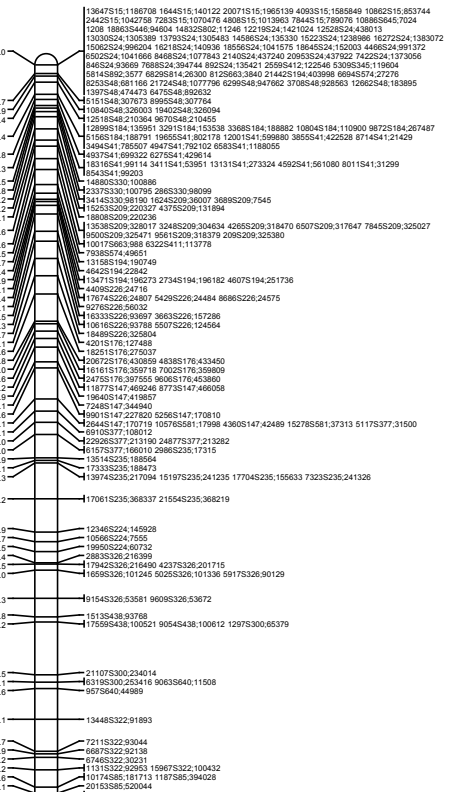
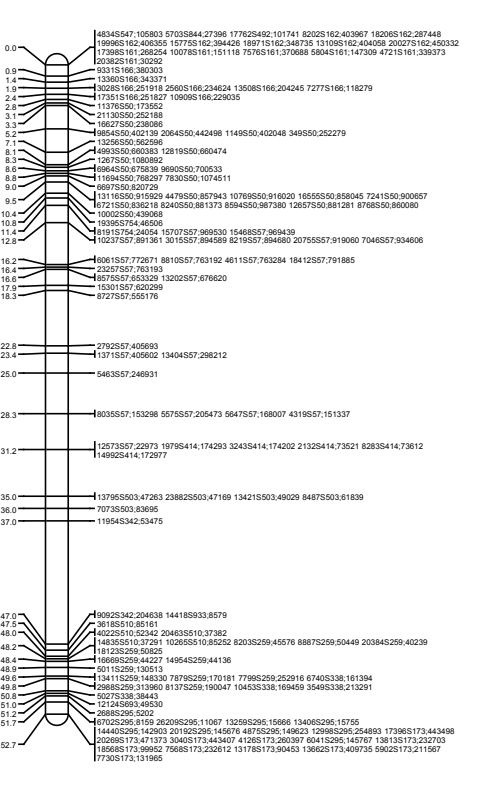
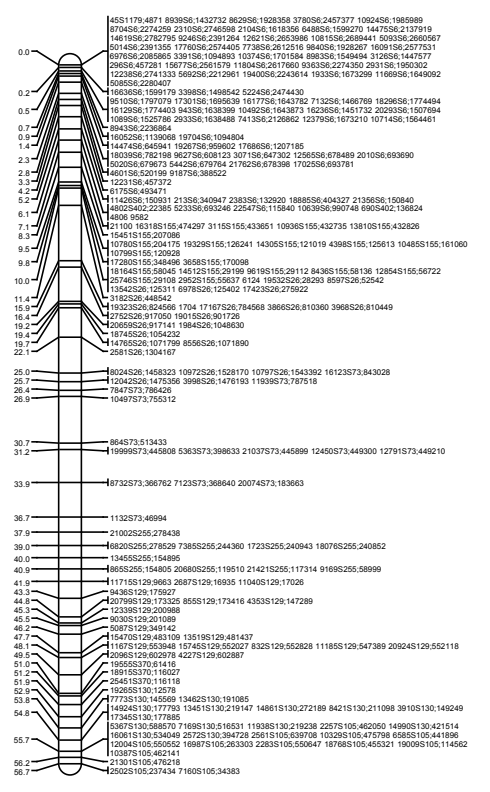


Fig. S1

a

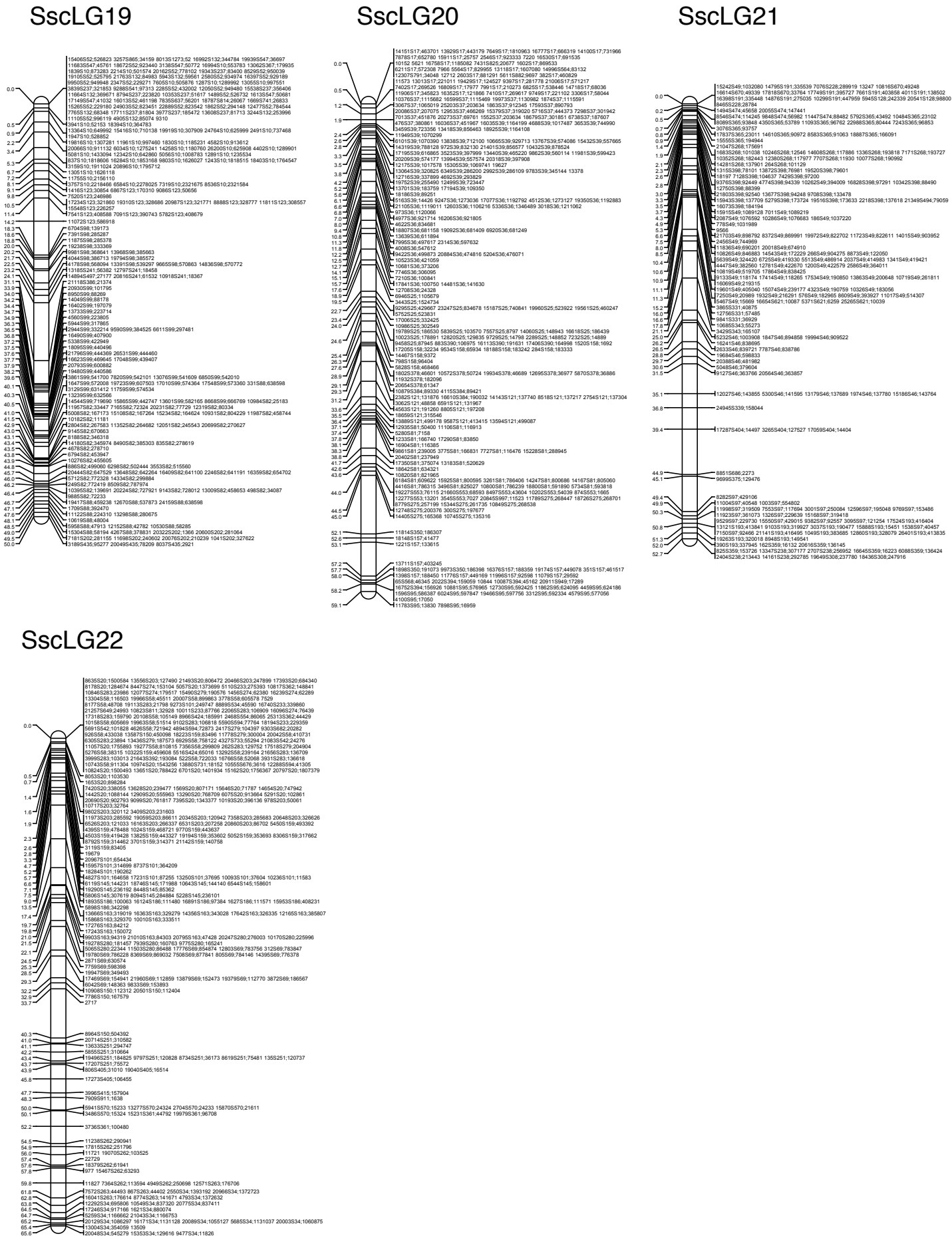
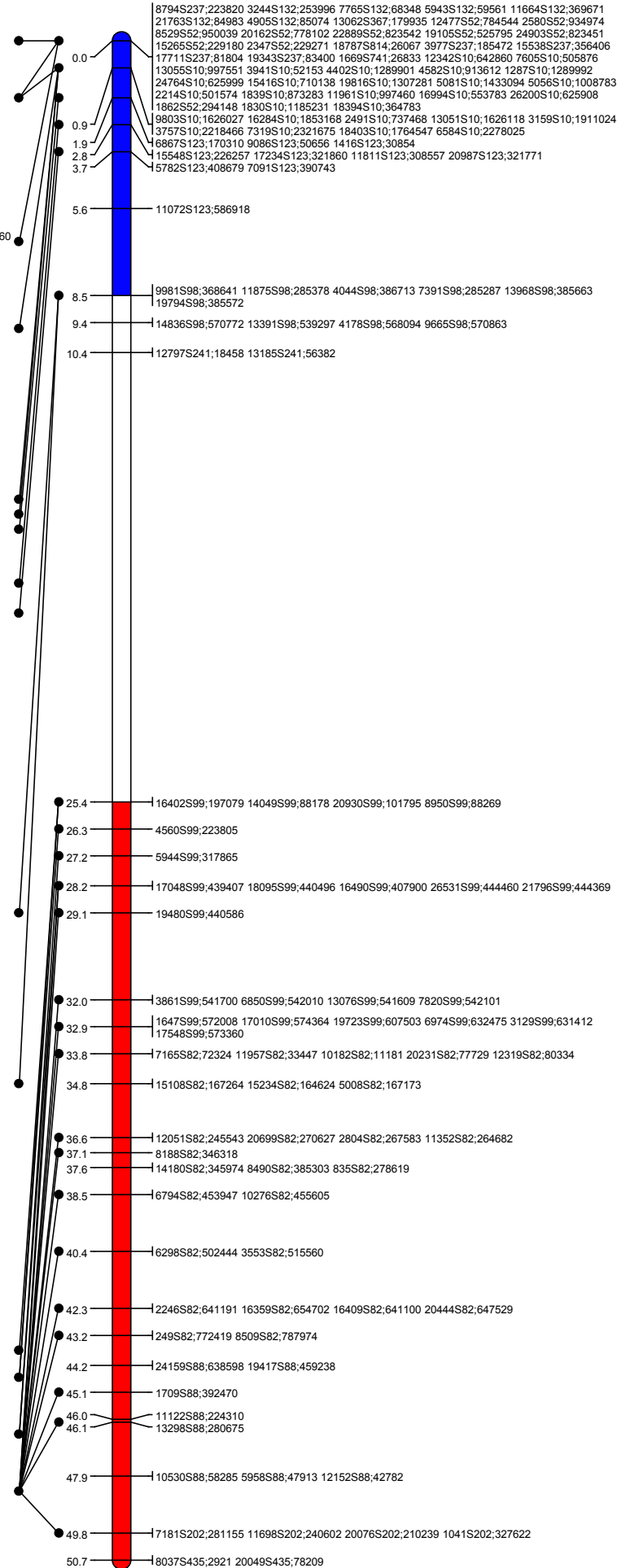
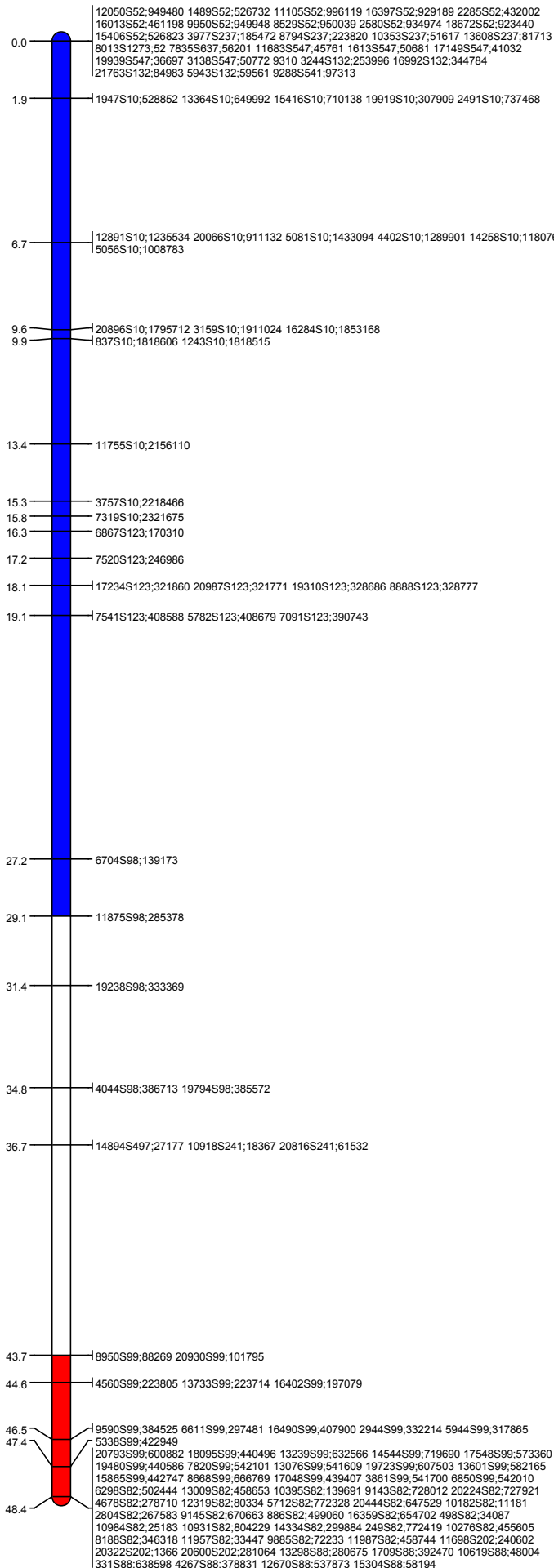


Fig. S1

b**SscLG19Female****SscLG19Male****Fig. S1**

c LG1Female

LG1Male

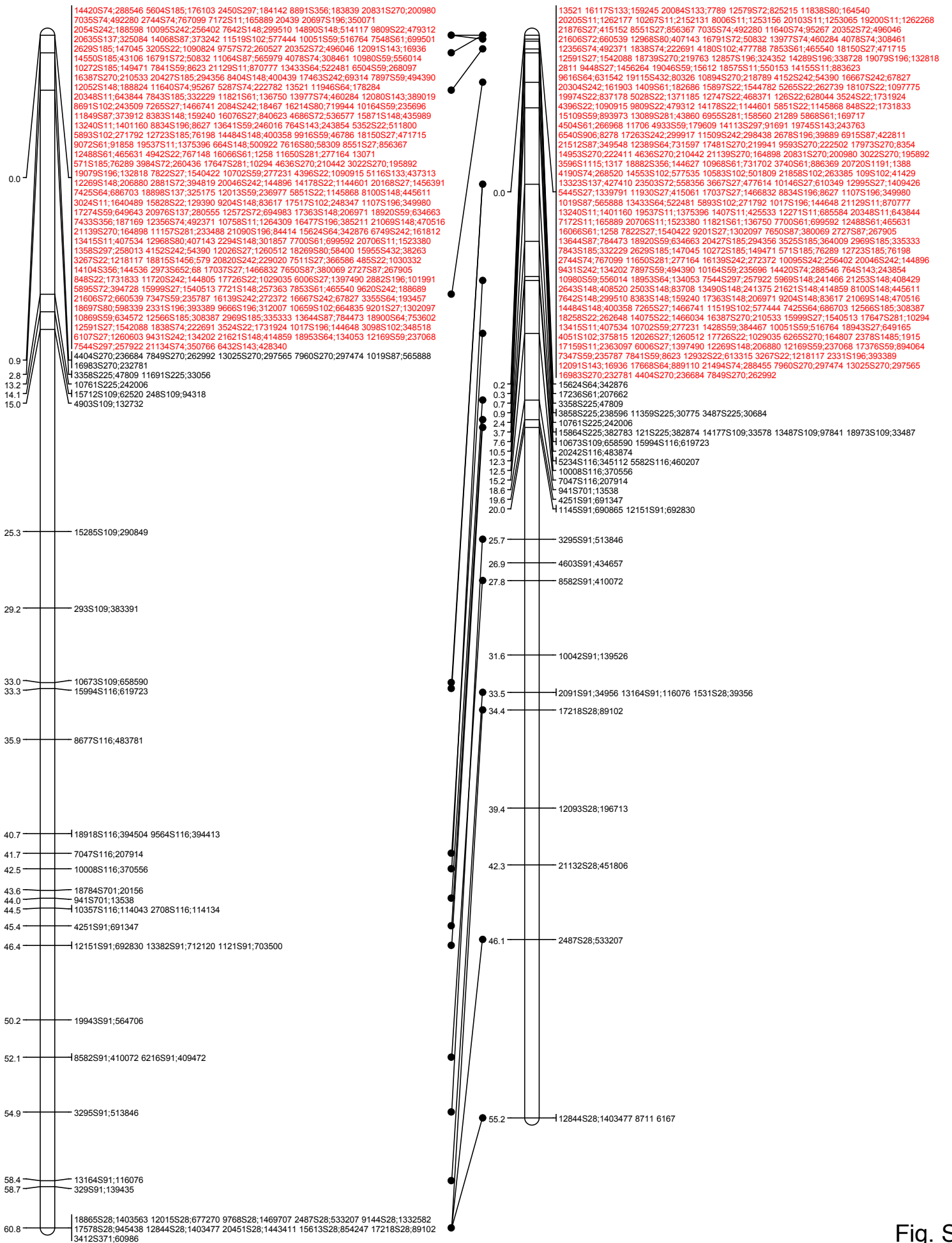


Fig. S1

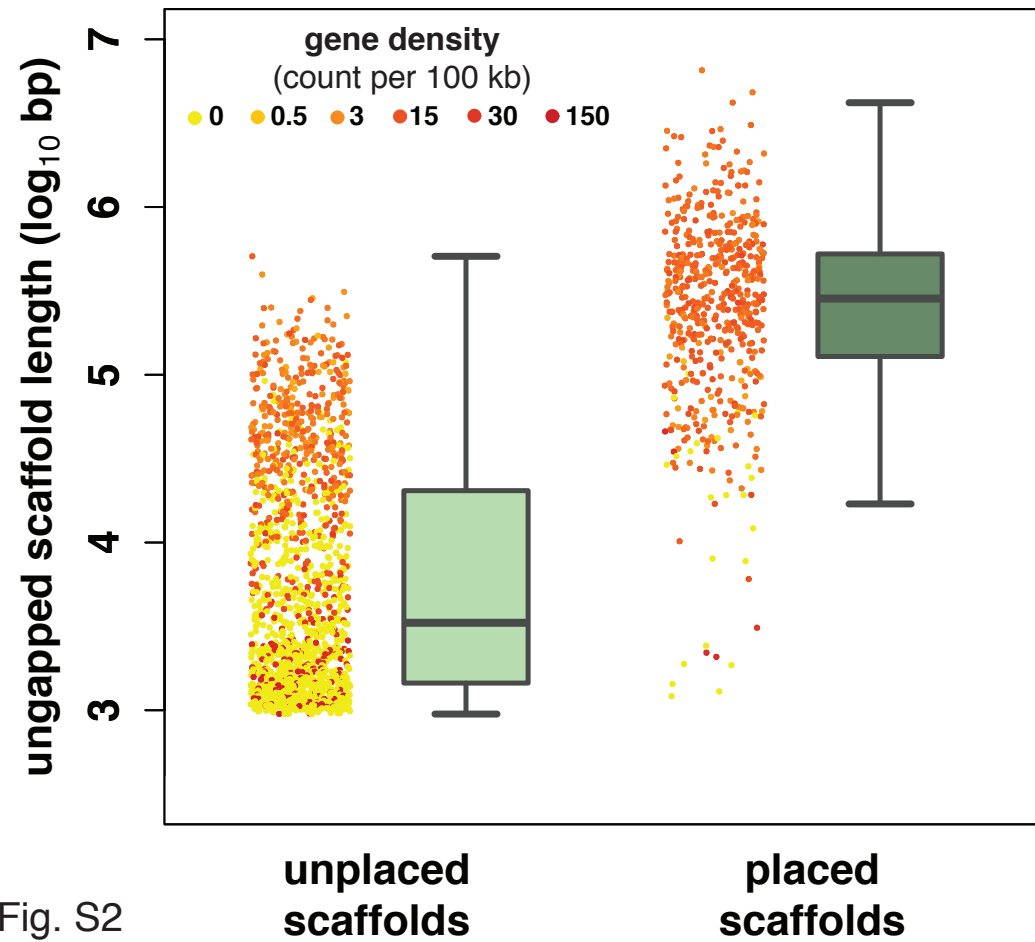
a

Fig. S2

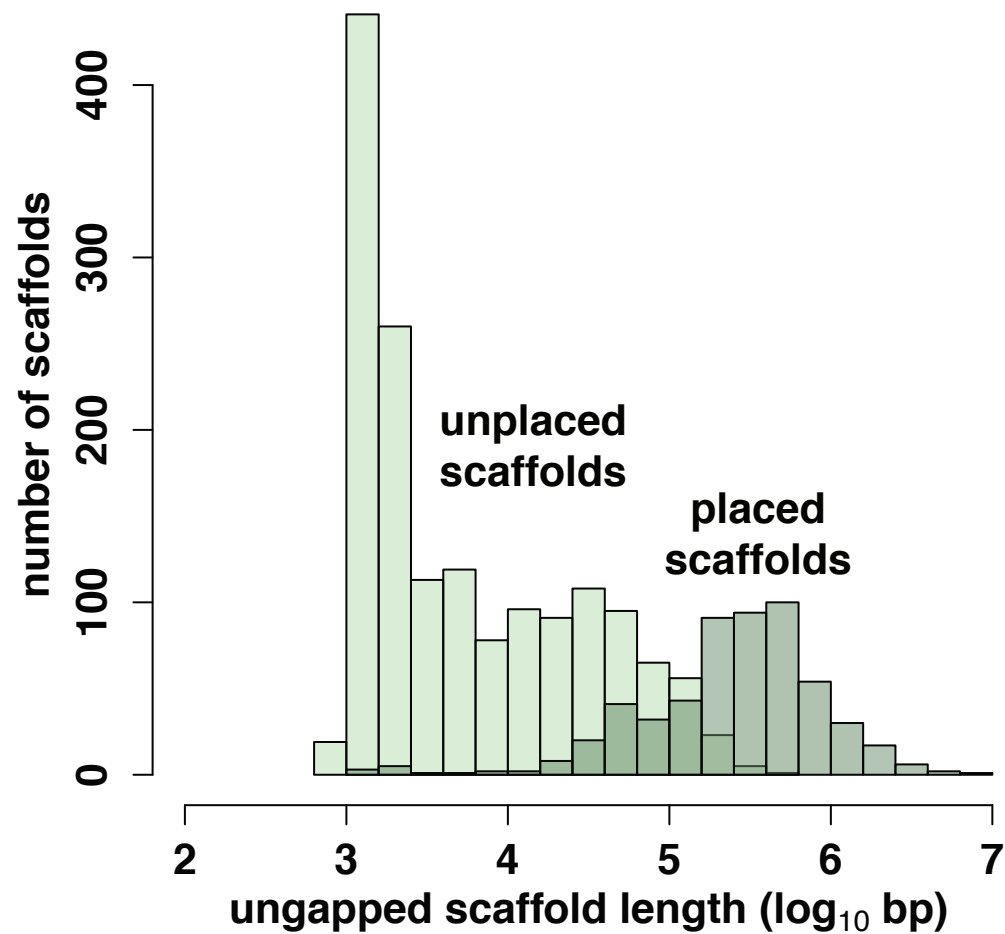
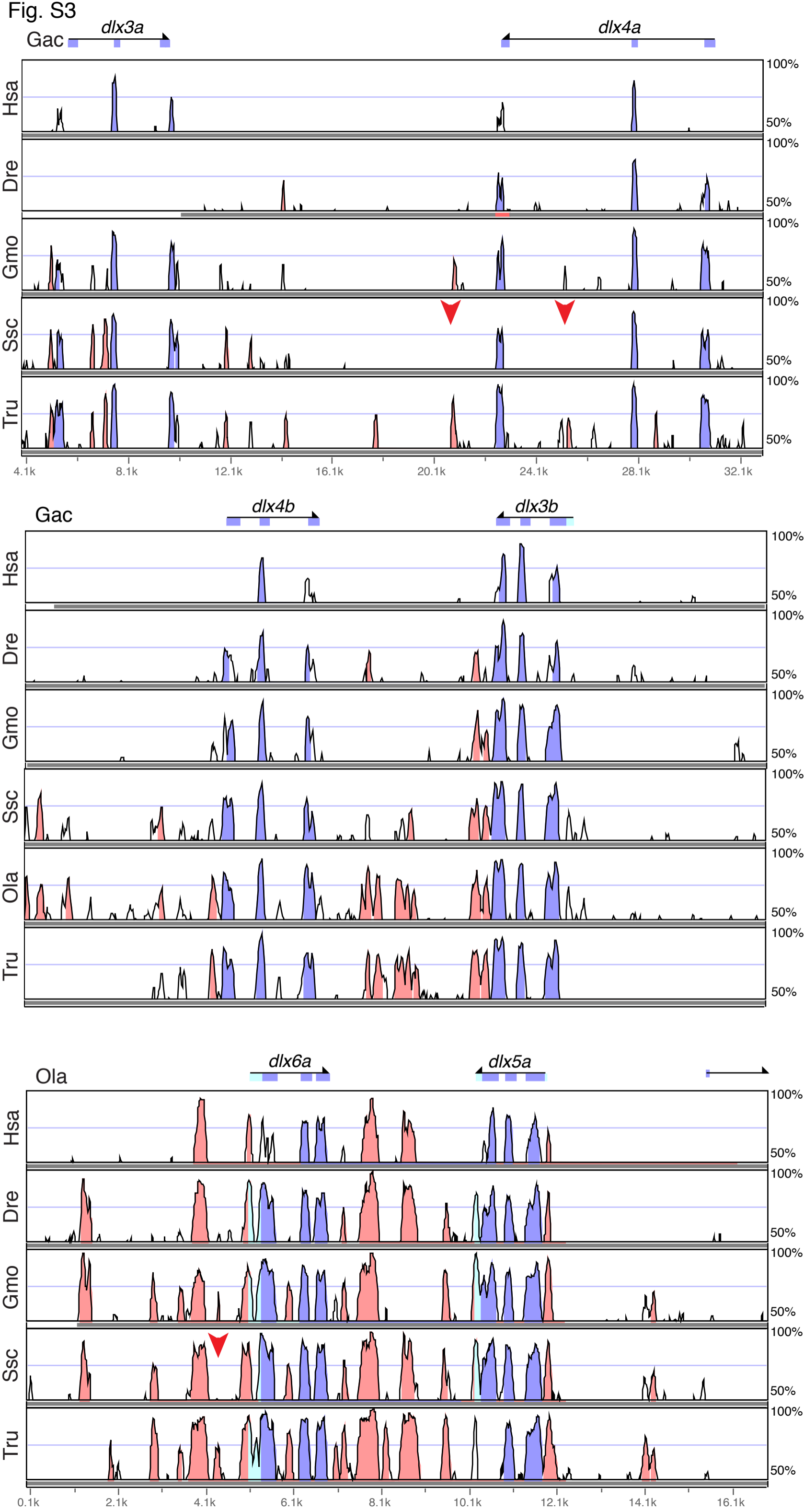
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Fig. S3



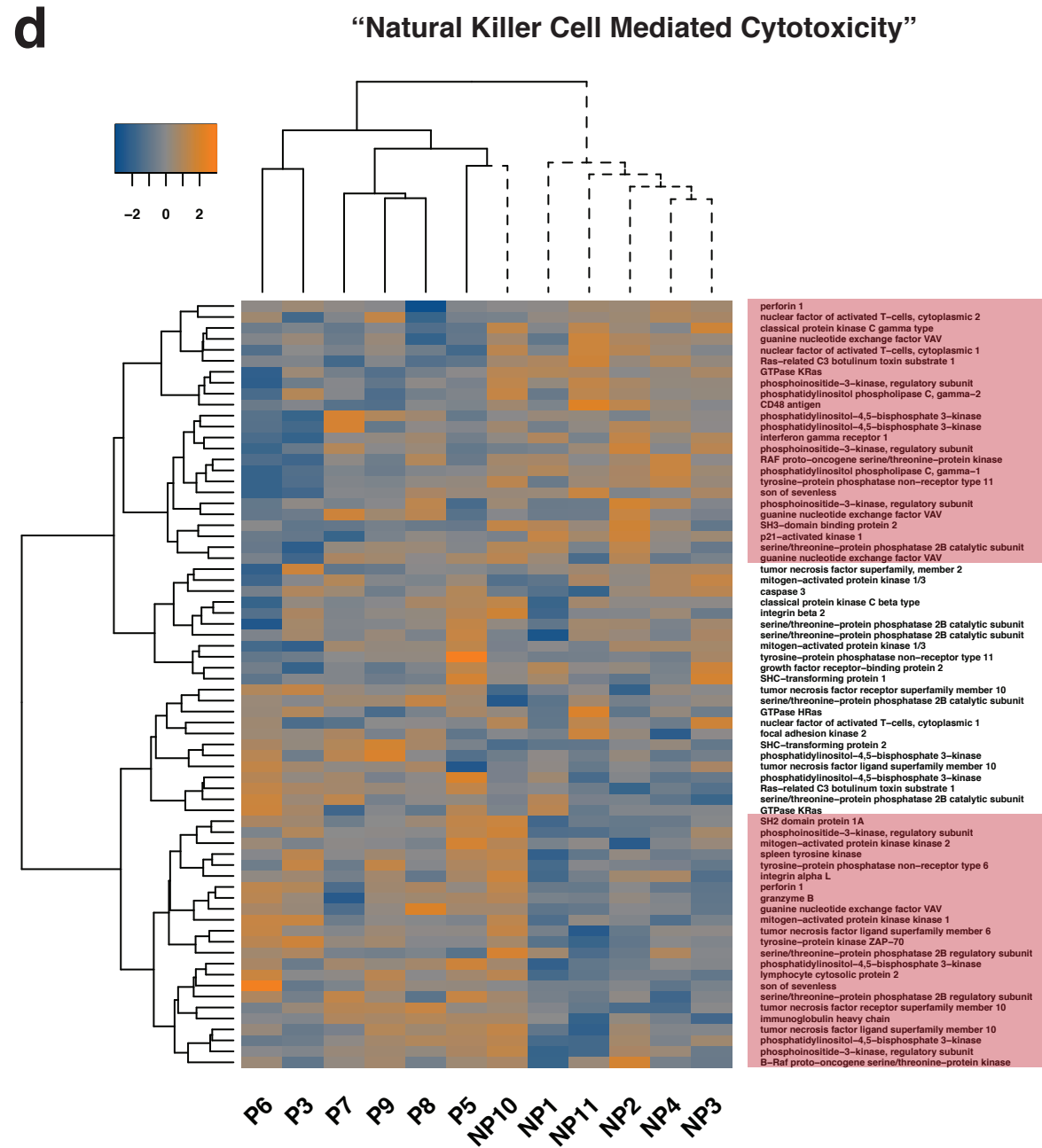
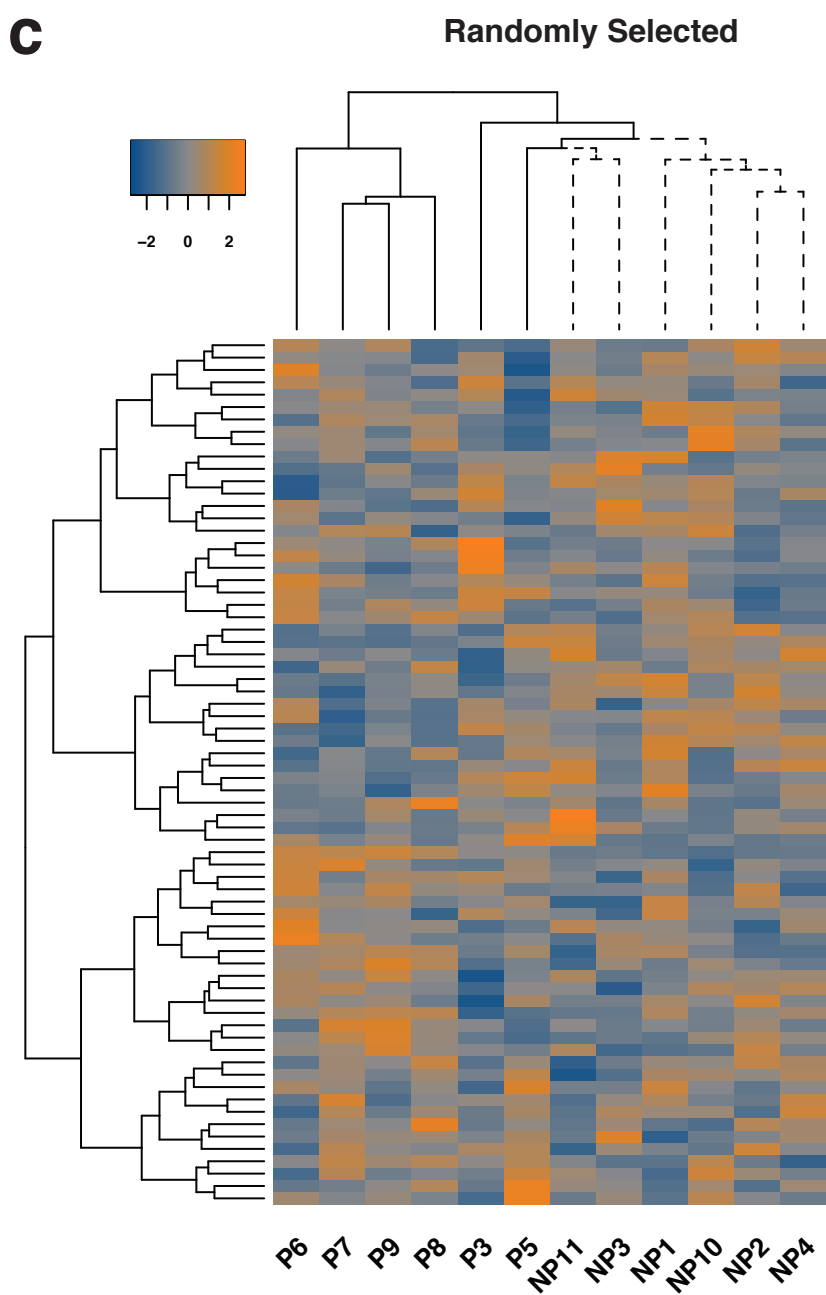
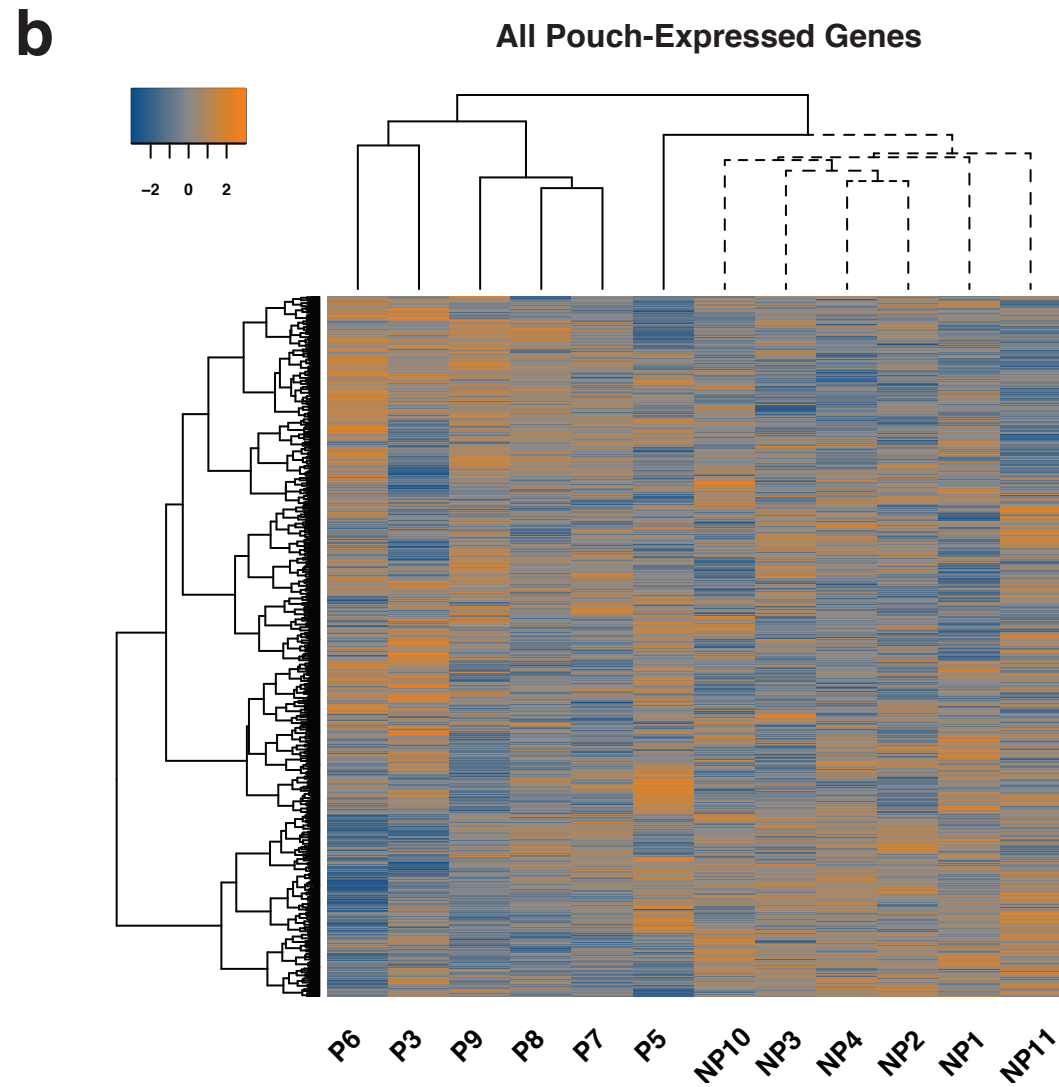
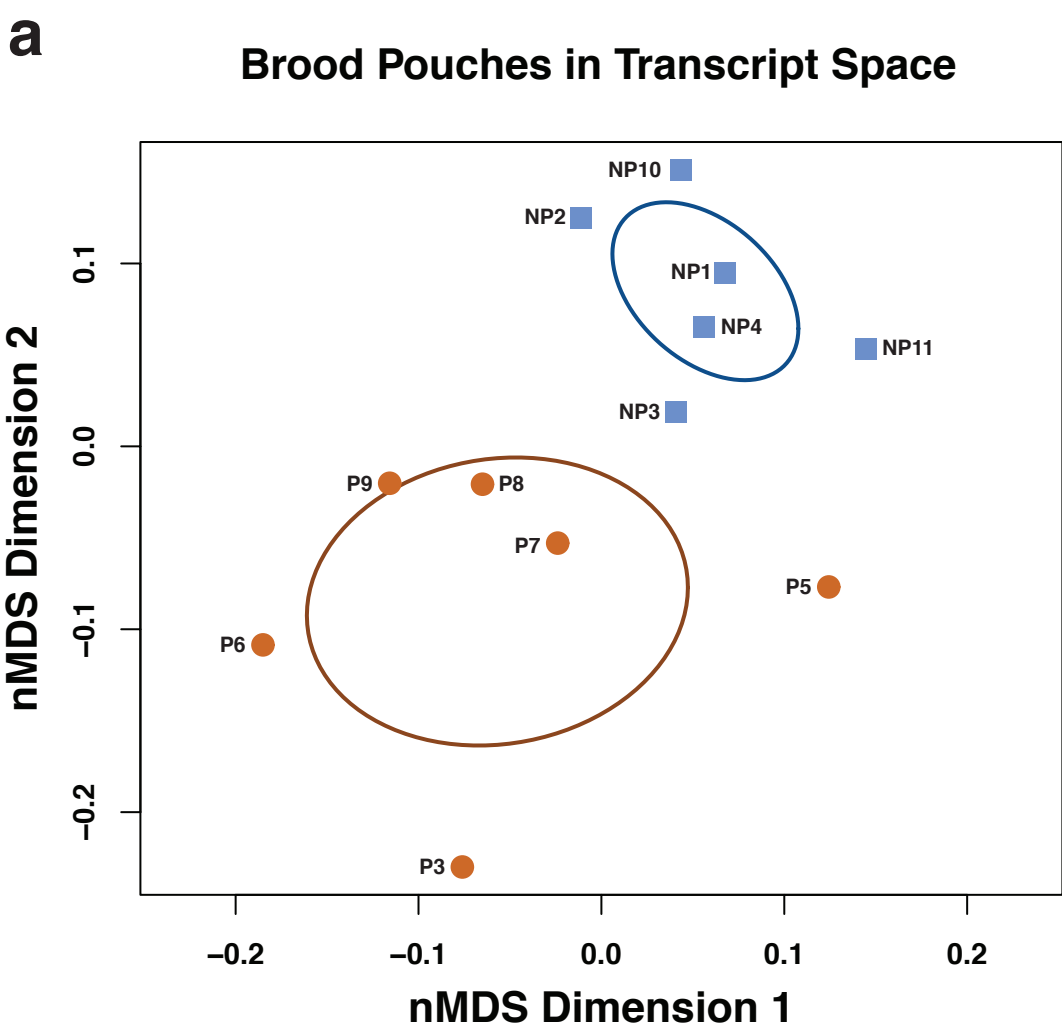
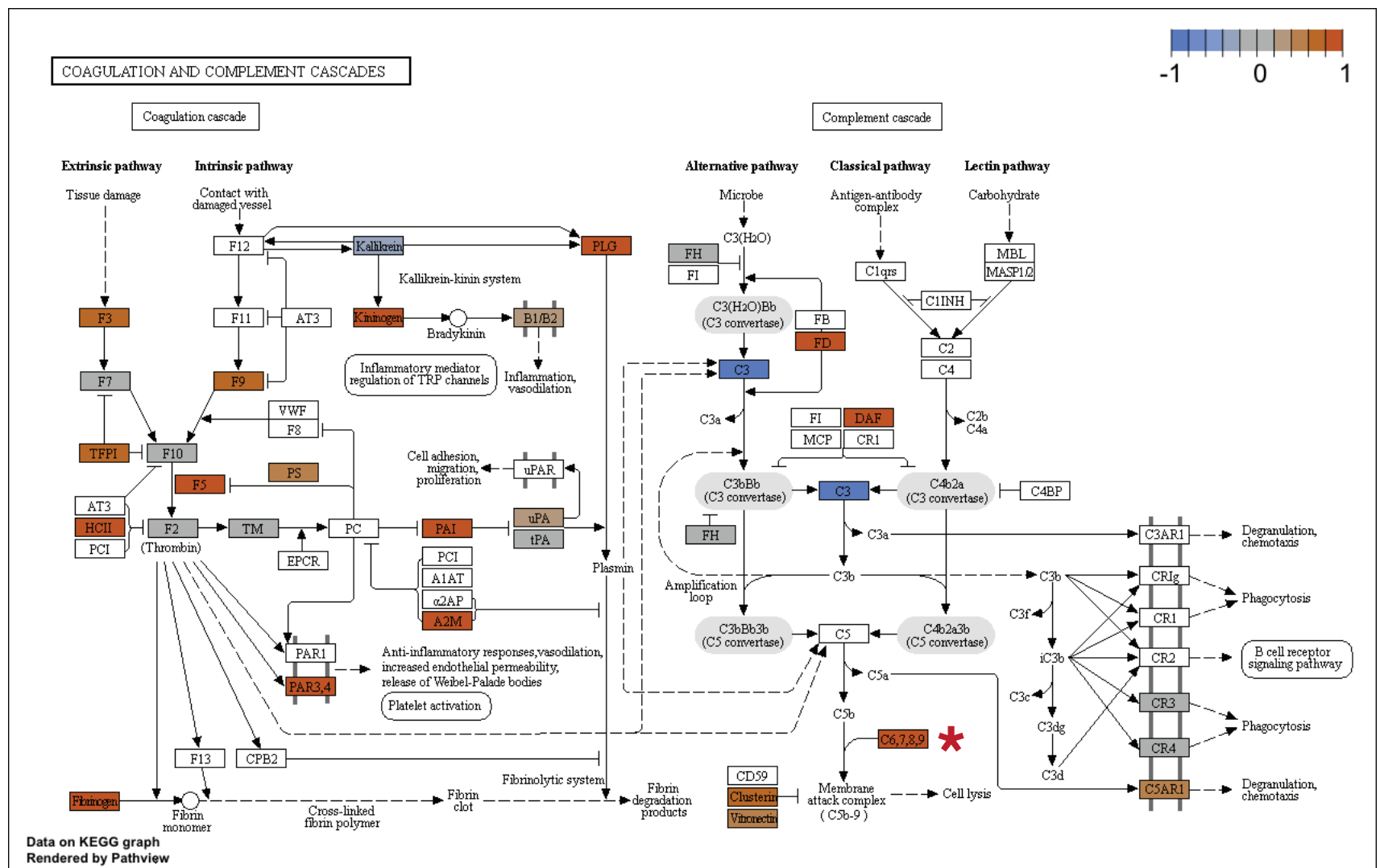


Fig . S4

a



b

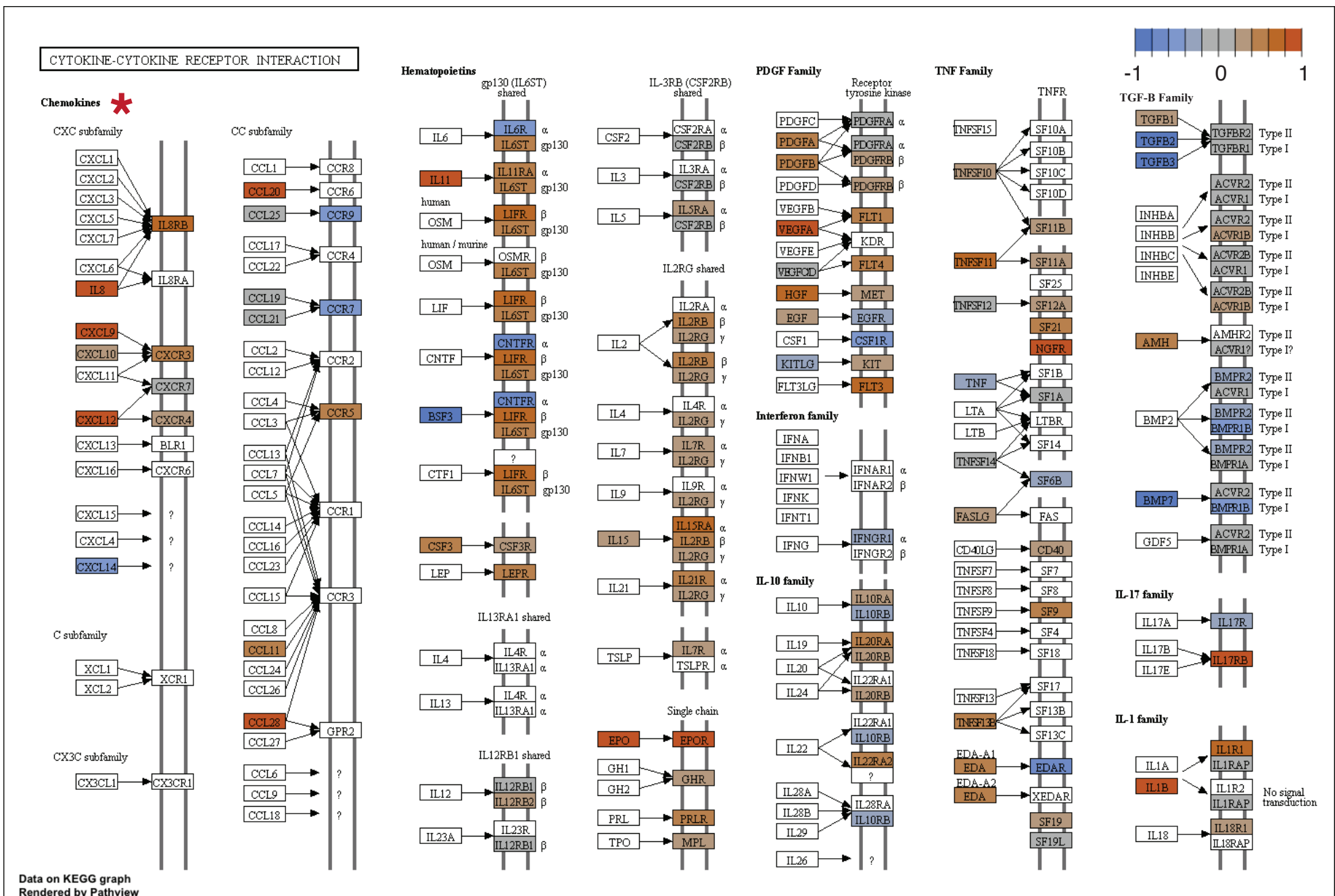
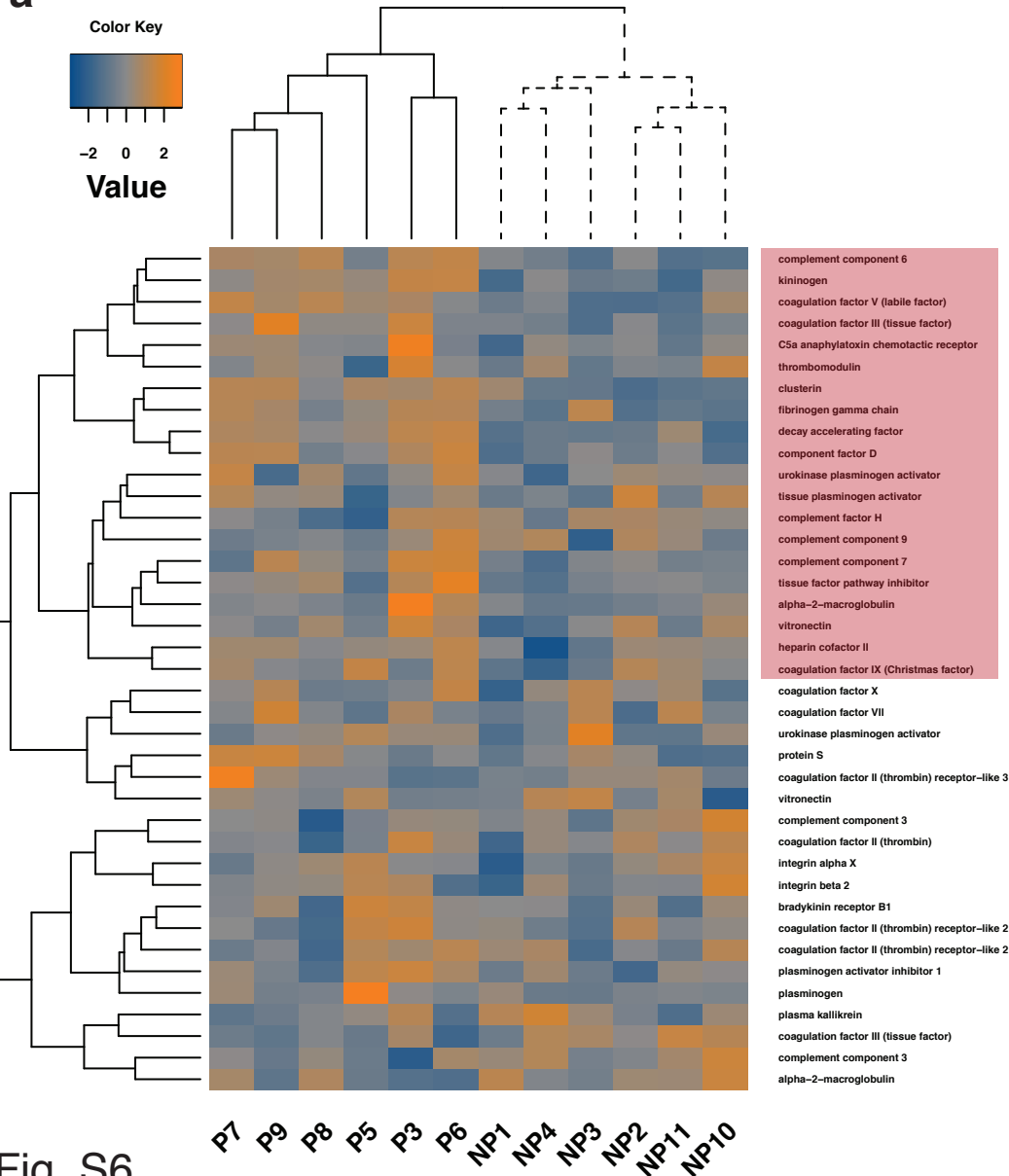


Fig. S5

a coagulation and complement cascades



b antigen processing and presentation

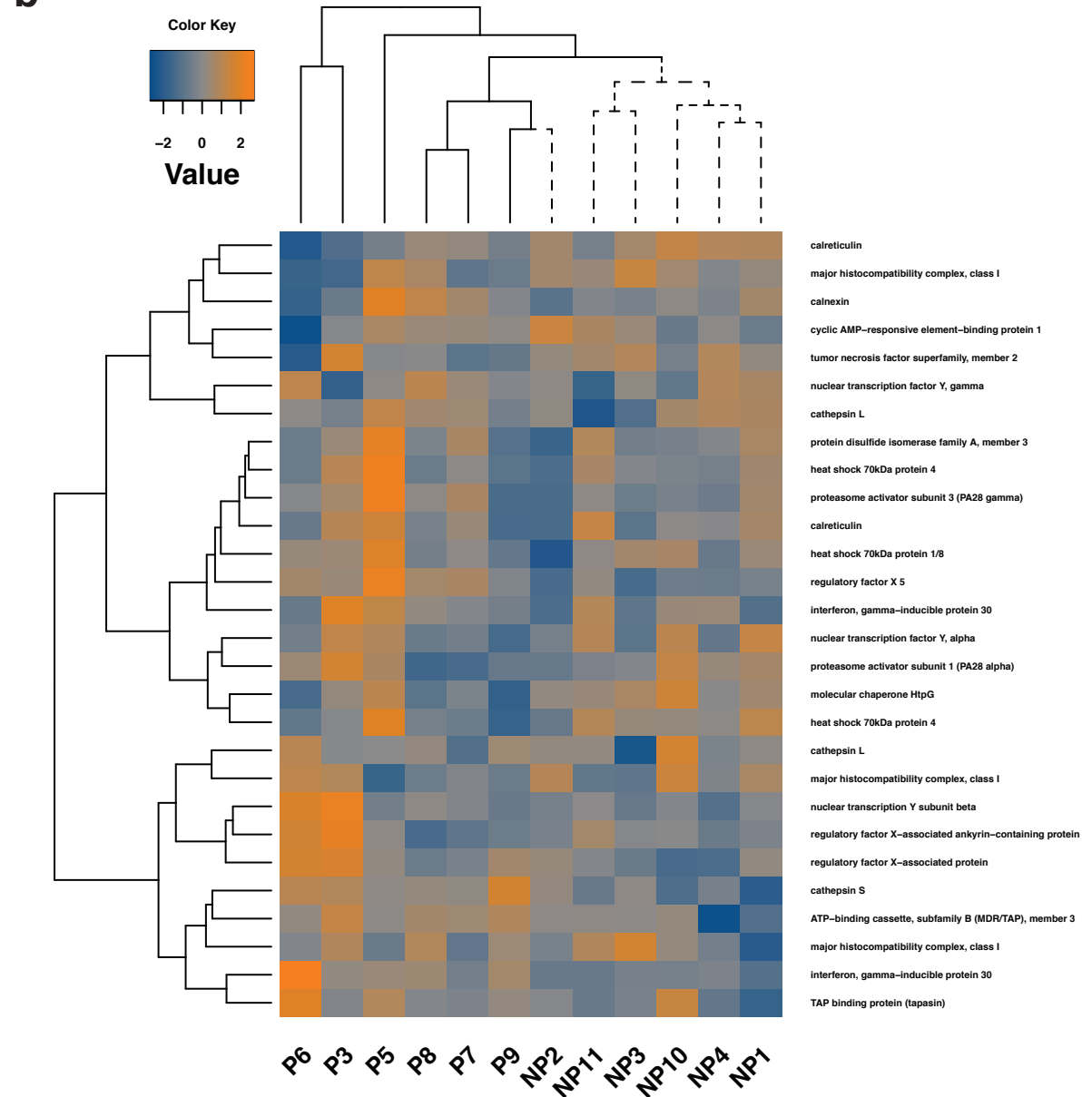


Fig. S6

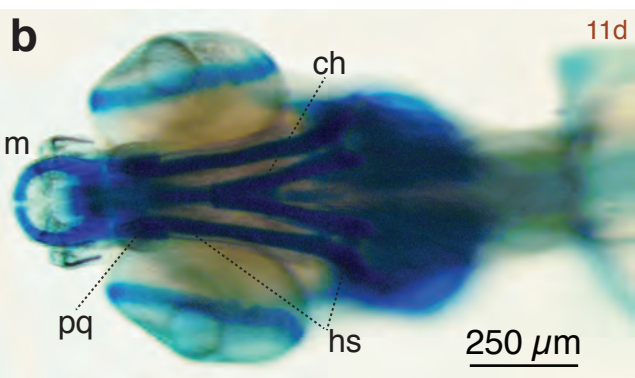
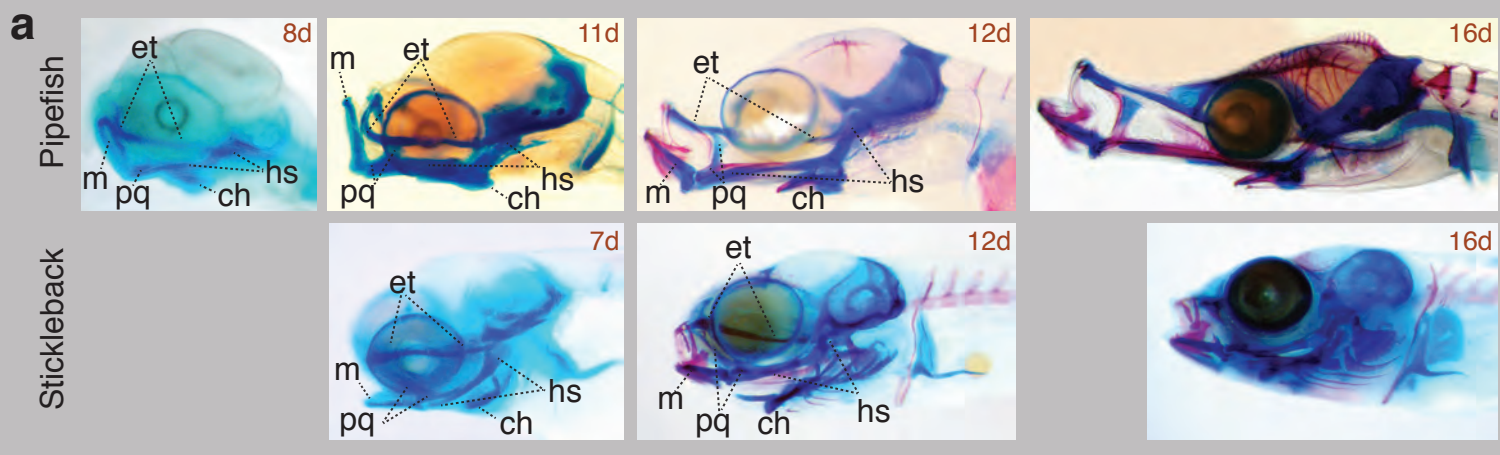


Fig. S7

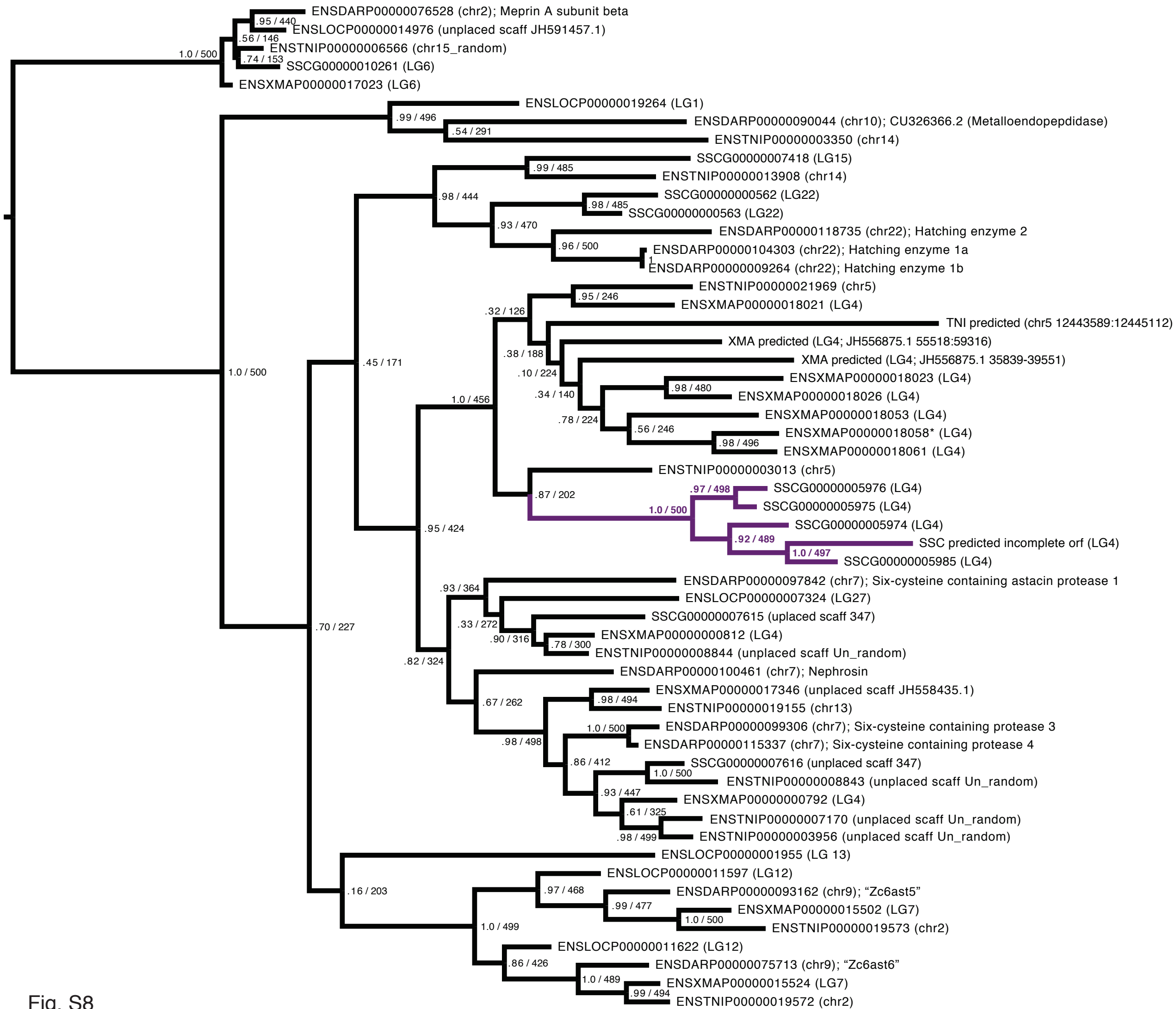


Fig. S8