Zoological Research

Supplementary Materials

Long-read genome assemblies reveal a *cis*-regulatory landscape associated with phenotypic divergence in two sister *Siniperca* fish species

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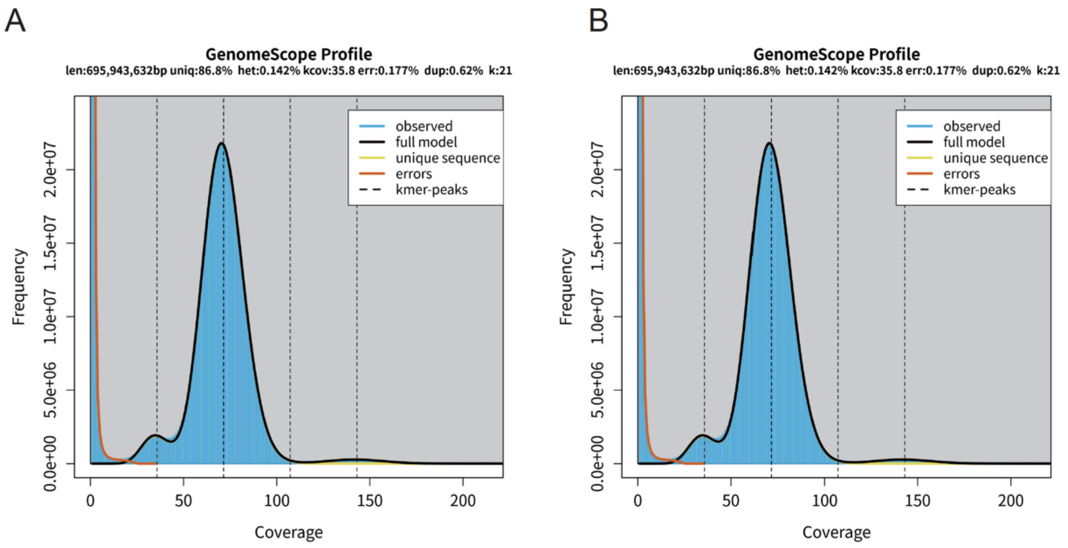
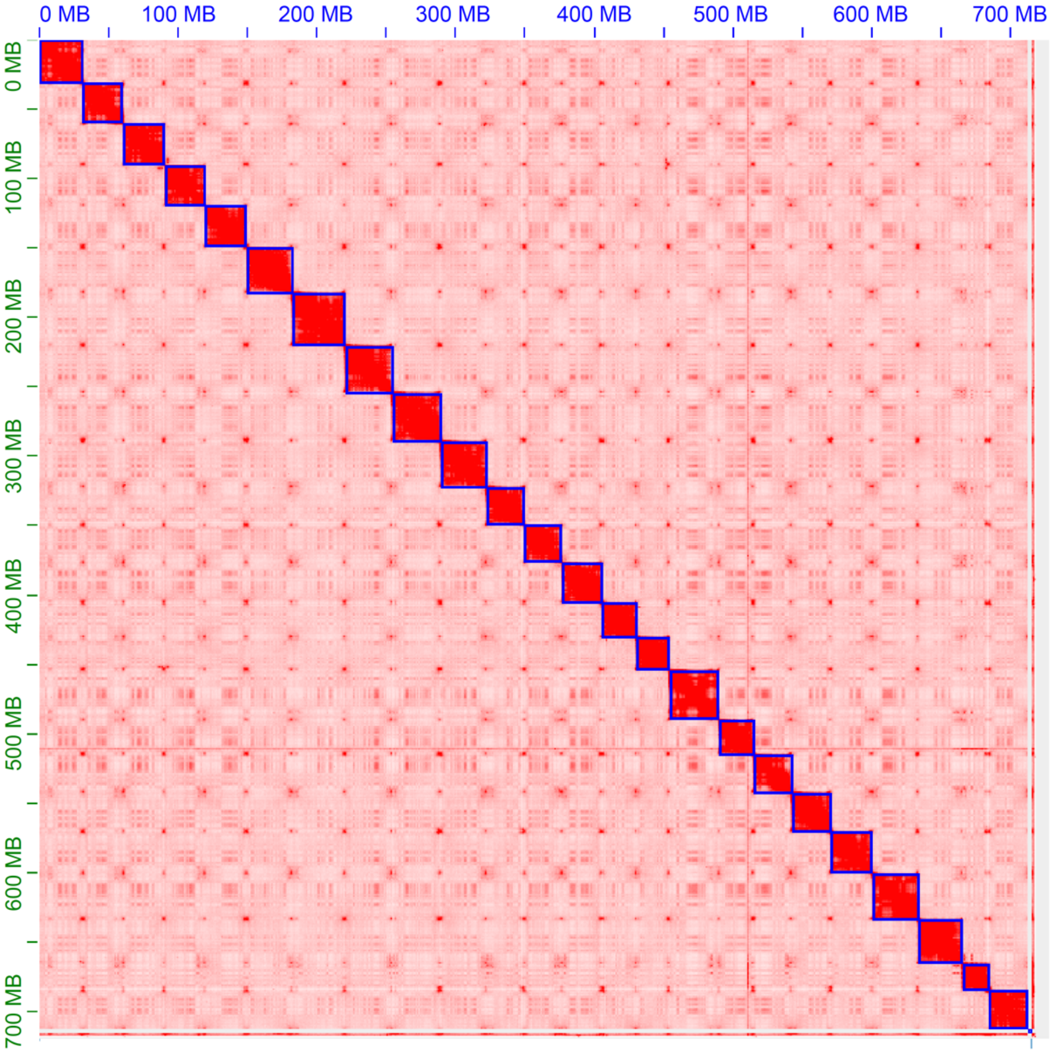


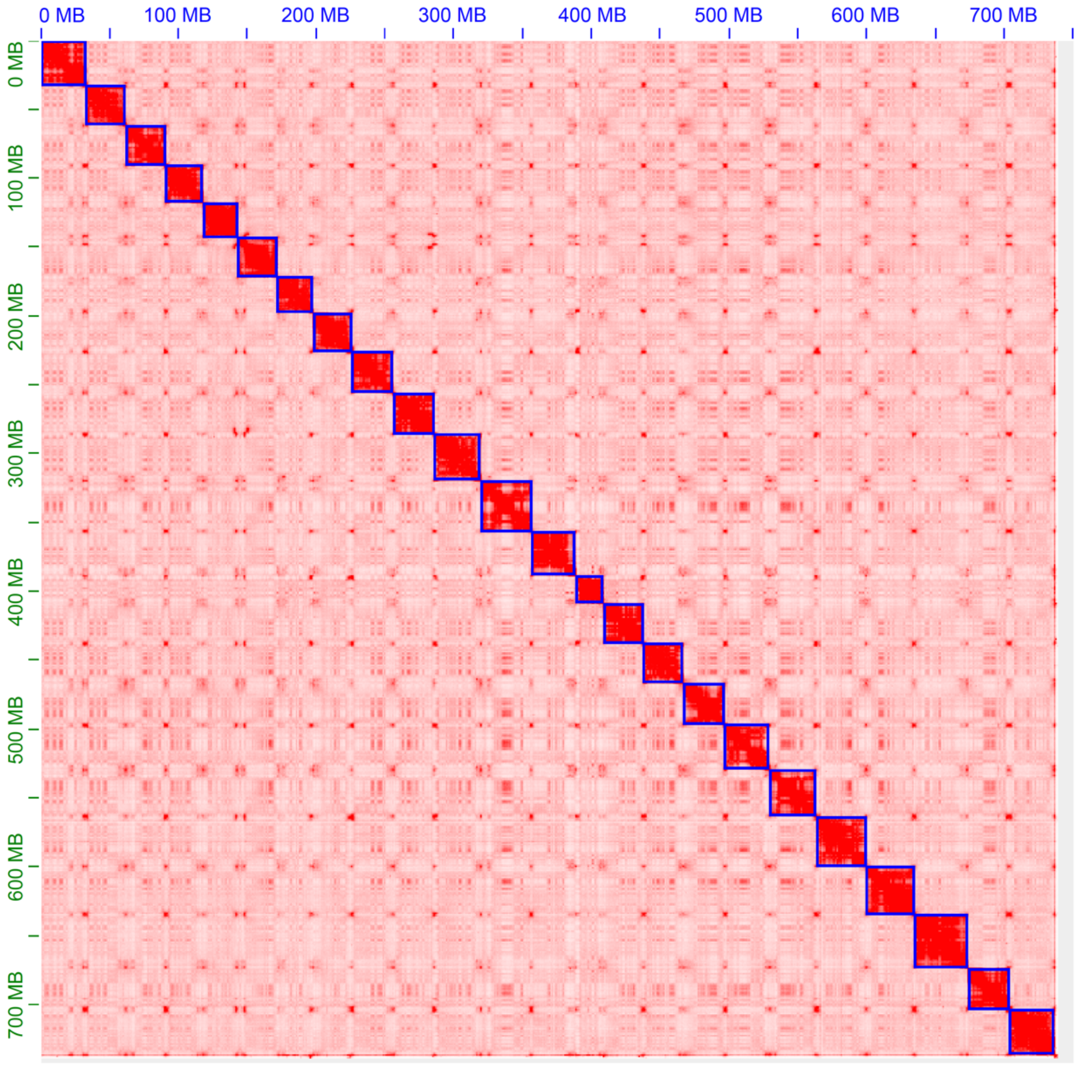
Supplementary Figure S1 Distribution of 21-mer frequency in *S. chuatsi* (A) and *S. scherzeri* (B) genomes

Sizes of *S. chuatsi* and *S. scherzeri* genomes were estimated at 695.9 Mb and 708.4 Mb, respectively. Heterozygous rates of *S. chuatsi* and *S. scherzeri* genomes were estimated at 0.142% and 0.231%, respectively. (len: haploid length; uniq: percentage non-repetitive sequence; het: percentage estimated heterozygosity; kcov: average *k*-mer coverage for heterozygous bases; err: percentage sequencing error rate; dup: mean read duplication rate; k: *k*-mer size).



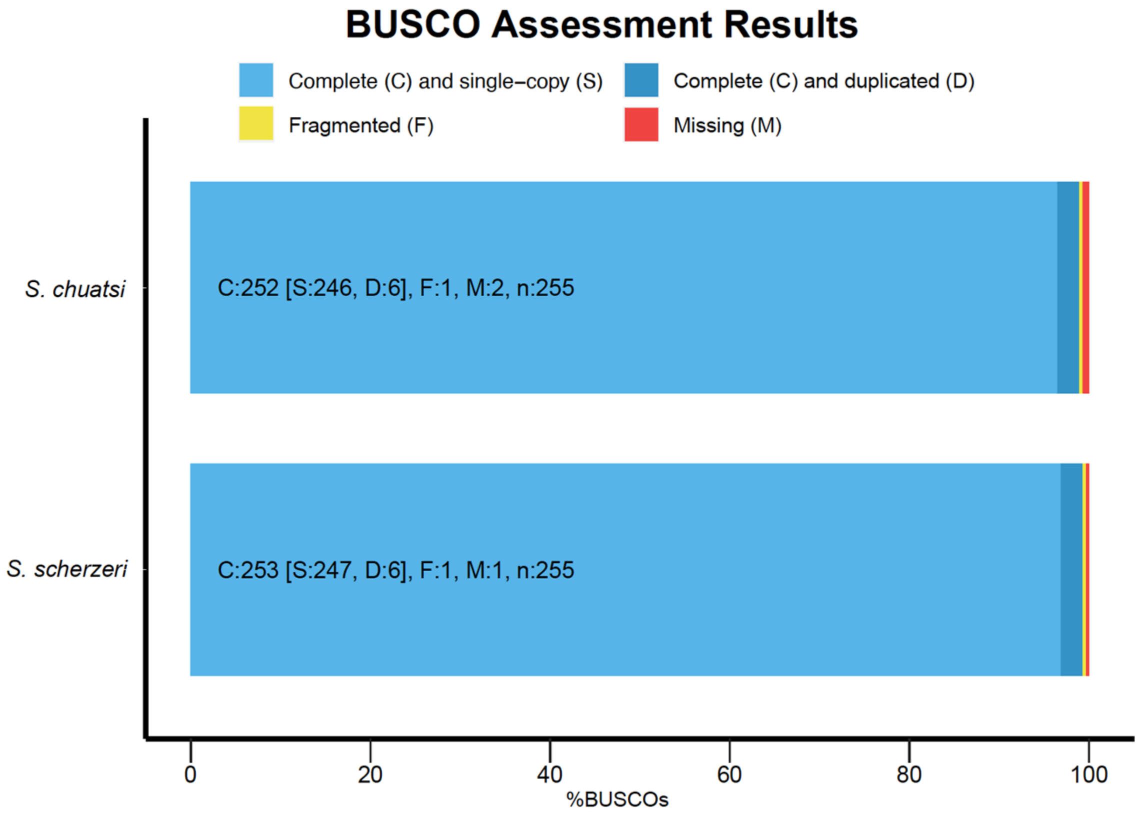
Supplementary Figure S2 Hi-C interaction matrix for assembled *S. chuatsi* scaffolds generated using Juicebox Hi-C visualization

Darker colors indicate higher frequency of chromatin interactions. Plot shows clear separation of chromosomal boundaries (blue rectangles) and limited off-diagonal interactions, supporting global structure of chromosome-scale scaffolds.

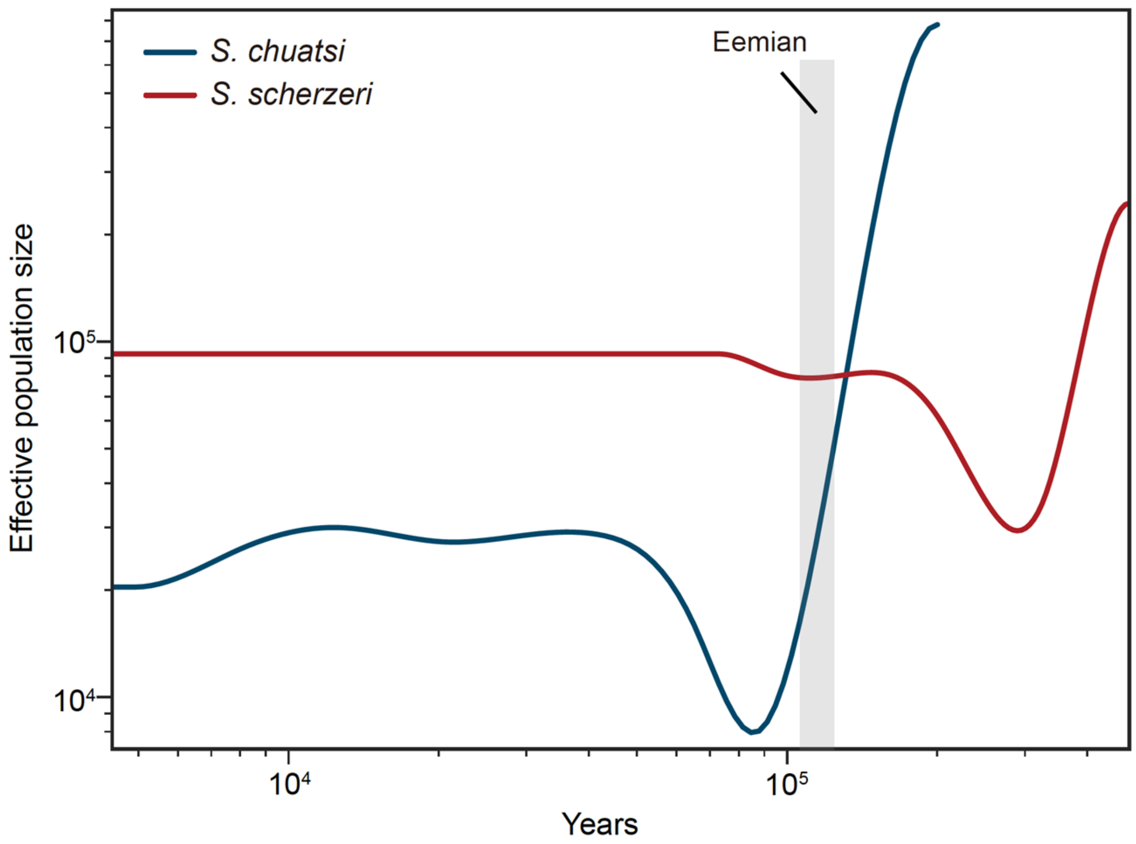


Supplementary Figure S3 Hi-C interaction matrix for assembled *S. scherzeri* scaffolds generated using Juicebox Hi-C visualization

Darker colors indicate higher frequency of chromatin interactions. Plot shows clear separation of chromosomal boundaries (blue rectangles) and limited off-diagonal interactions, supporting global structure of chromosome-scale scaffolds.

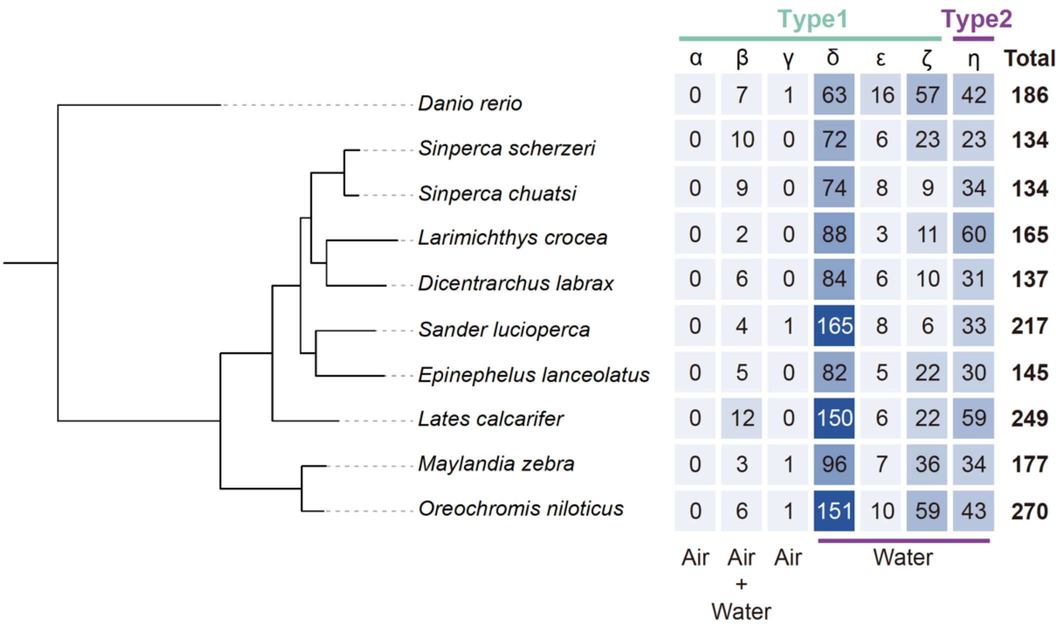


Supplementary Figure S4 BUSCO assessment of predicted gene models in *S. chuatsi* and *S. scherzeri*



Supplementary Figure S5 Ancestral population sizes of *S. chuatsi* and *S. scherzeri* in North China (Jilin Province)

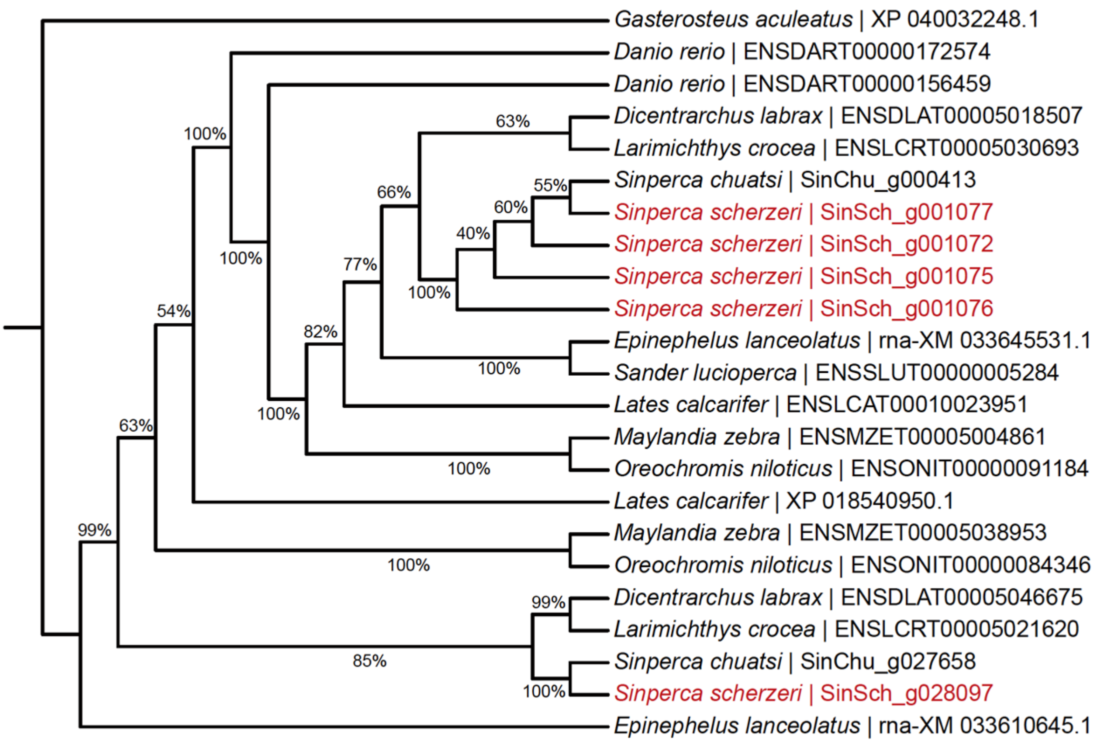
Ancestral population size of *S. scherzeri* from North China declined at ~300 ka.



	α	β	γ	δ	ε	ζ	η	Total
Danio rerio	0	7	1	63	16	57	42	**186**
Sinperca scherzeri	0	10	0	72	6	23	23	**134**
Sinperca chuatsi	0	9	0	74	8	9	34	**134**
Larimichthys crocea	0	2	0	88	3	11	60	**165**
Dicentrarchus labrax	0	6	0	84	6	10	31	**137**
Sander lucioperca	0	4	1	165	8	6	33	**217**
Epinephelus lanceolatus	0	5	0	82	5	22	30	**145**
Lates calcarifer	0	12	0	150	6	22	59	**249**
Maylandia zebra	0	3	1	96	7	36	34	**177**
Oreochromis niloticus	0	6	1	151	10	59	43	**270**

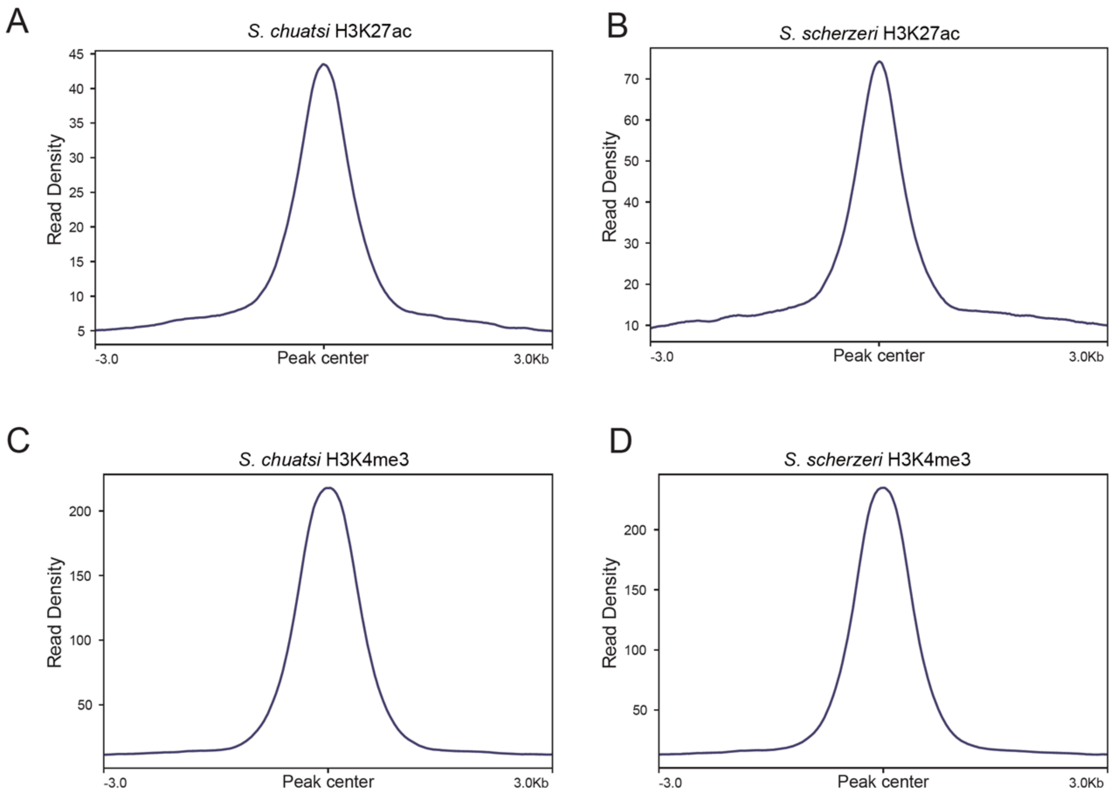
Supplementary Figure S6 Functional olfactory receptor (OR) genes identified in *S. chuatsi* and *S. scherzeri* genomes as well as seven other perciform fish species

Siniperca chuatsi and *S. scherzeri* have fewer OR genes than other perciform species.



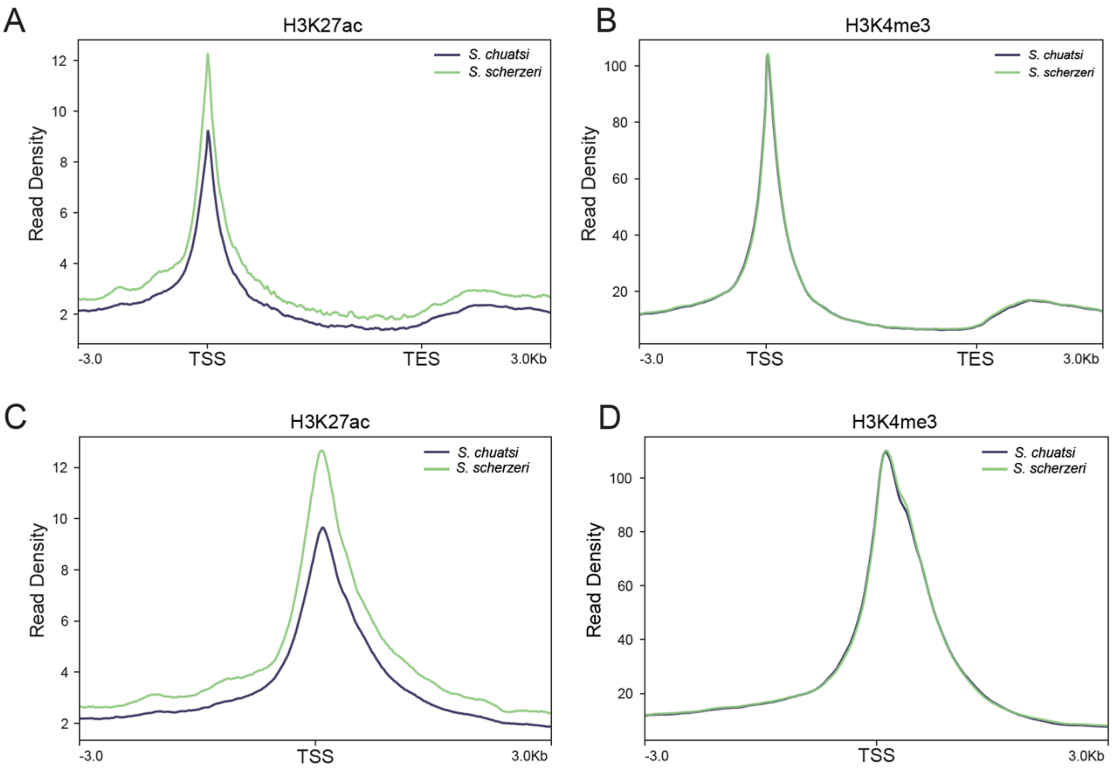
Supplementary Figure S7 Phylogenetic tree of p300 genes in 10 perciform fish species

p300 genes were significantly expanded in *S. scherzeri* genome (five copies) compared with all other perciform fish (two copies).



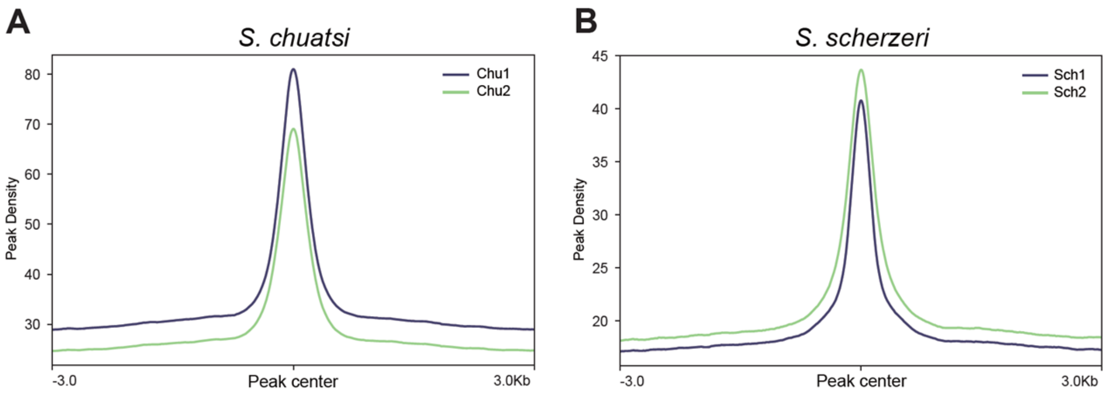
Supplementary Figure S8 CUT&Tag signals of H3K27ac and H3K4me3

H3K27ac CUT&Tag signals over replicated H3K27ac peaks in genomes of *S. chuatsi* (**A**) and *S. scherzeri* (**B**). H3K4me3 CUT&Tag signals over replicated H3K4me3 peaks in genomes of *S. chuatsi* (**C**) and *S. scherzeri* (**D**).

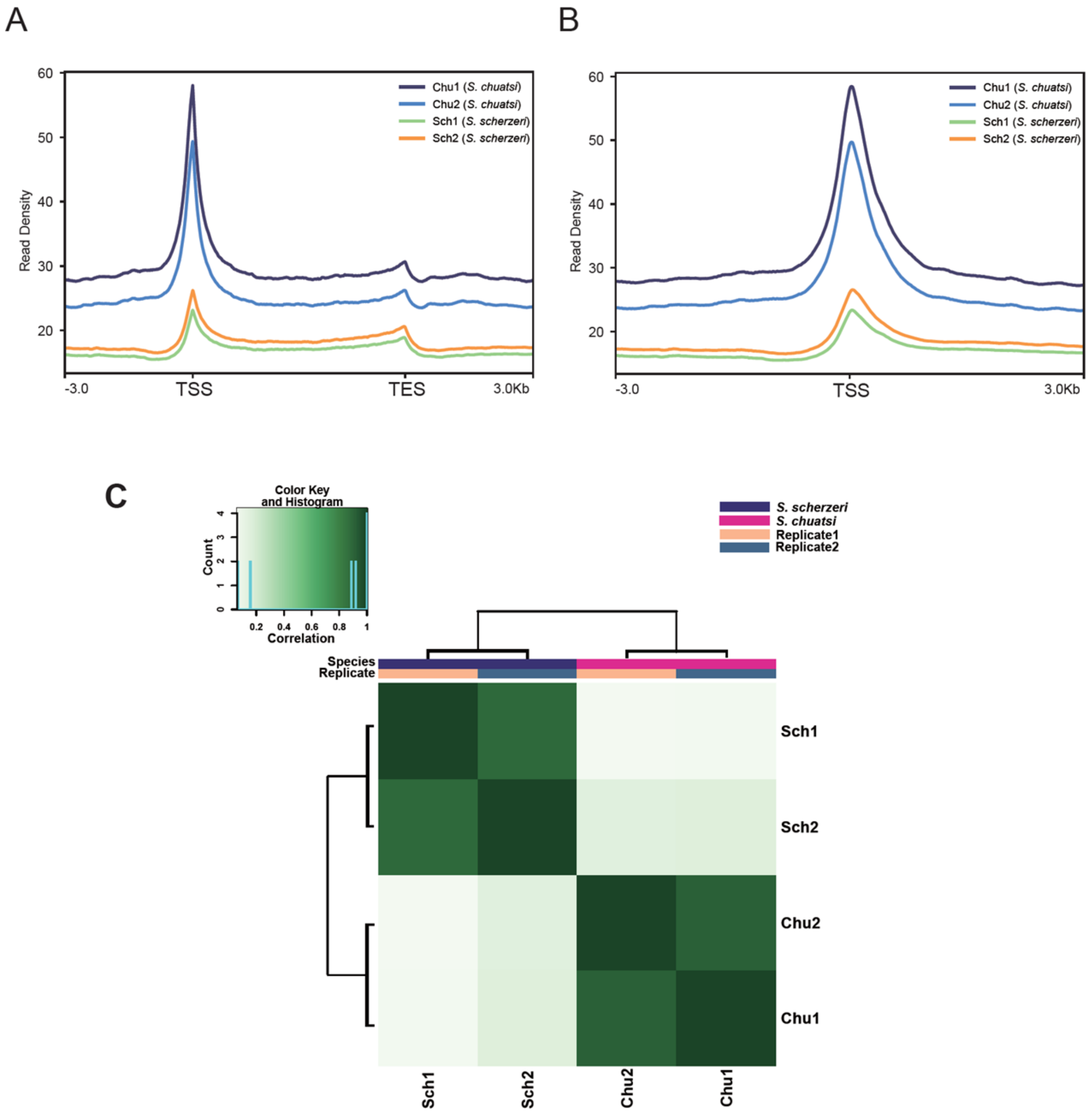


Supplementary Figure S9 Quality control for CUT&Tag data

A: Most *S. chuatsi* and *S. scherzeri* genes show increased H3K27ac signals. **B:** Most *S. chuatsi* and *S. scherzeri* genes show increased H3K4me3 signals. **C:** Transcription start sites (TSSs) show particularly high H3K27ac signals in *S. chuatsi* and *S. scherzeri* genomes. **D:** TSSs show particularly high H3K4me3 signals in *S. chuatsi* and *S. scherzeri* genomes.

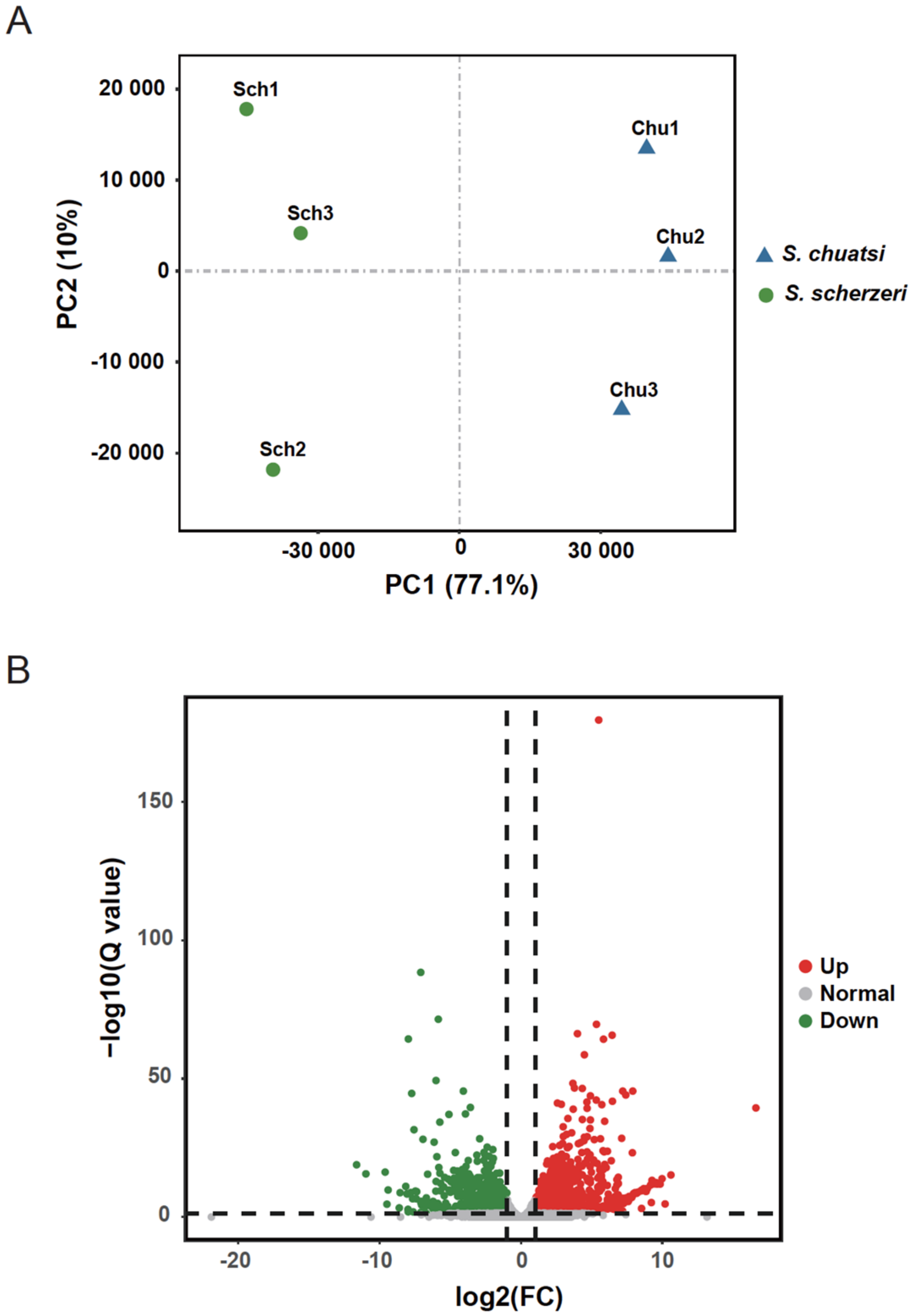


Supplementary Figure S10 ATAC-seq signals in *S. chuatsi* and *S. scherzeri* genomes



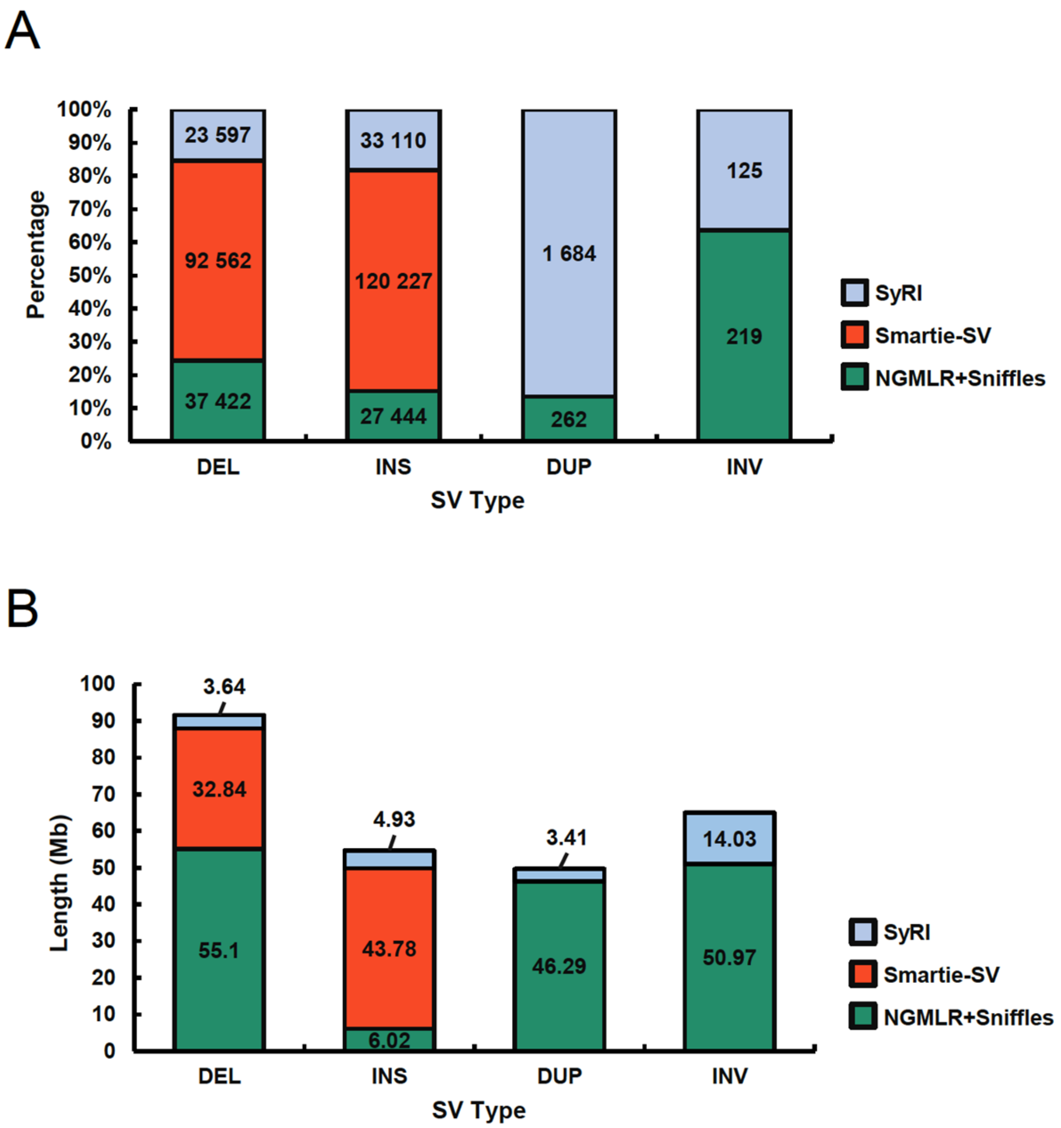
Supplementary Figure S11 Quality control for ATAC-seq data

A: Most *S. chuatsi* and *S. scherzeri* genes show increased ATAC-seq signals. **B:** TSSs show particularly high ATAC-seq signals in *S. chuatsi* and *S. scherzeri* genomes. **C:** Correlation between two biological replicates of ATAC-seq of *S. chuatsi* (Chu1, Chu2) and *S. scherzeri* (Sch1, Sch2).



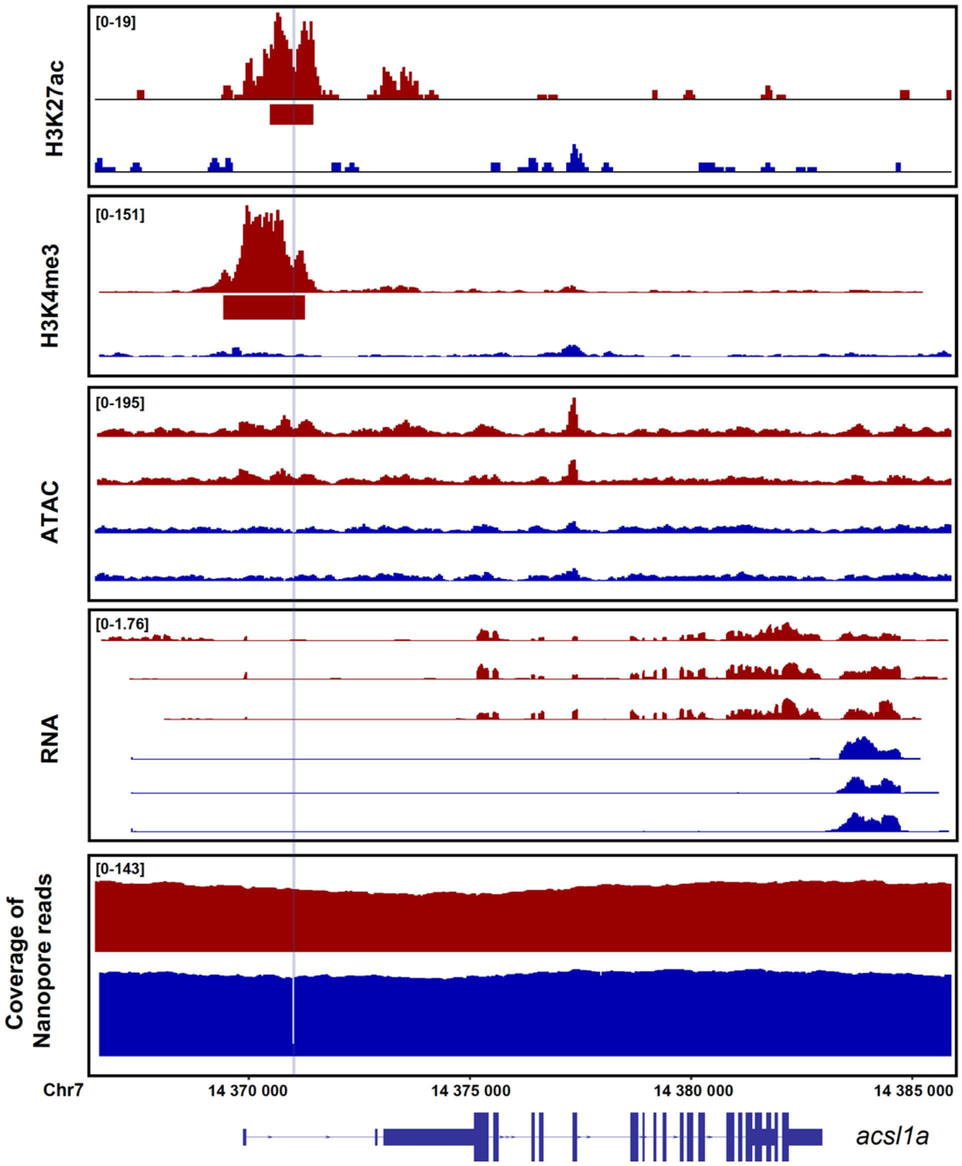
Supplementary Figure S12 Identification of differentially expressed genes (DEGs) in liver of *S. chuatsi* and *S. scherzeri*

A: Principal component analysis (PCA) of RNA-seq data revealed expression divergence between *S. chuatsi* and *S. scherzeri*. **B:** In total, 2 099 genes are up-regulated and 1 771 genes are down-regulated in *S. chuatsi* compared with *S. scherzeri*.



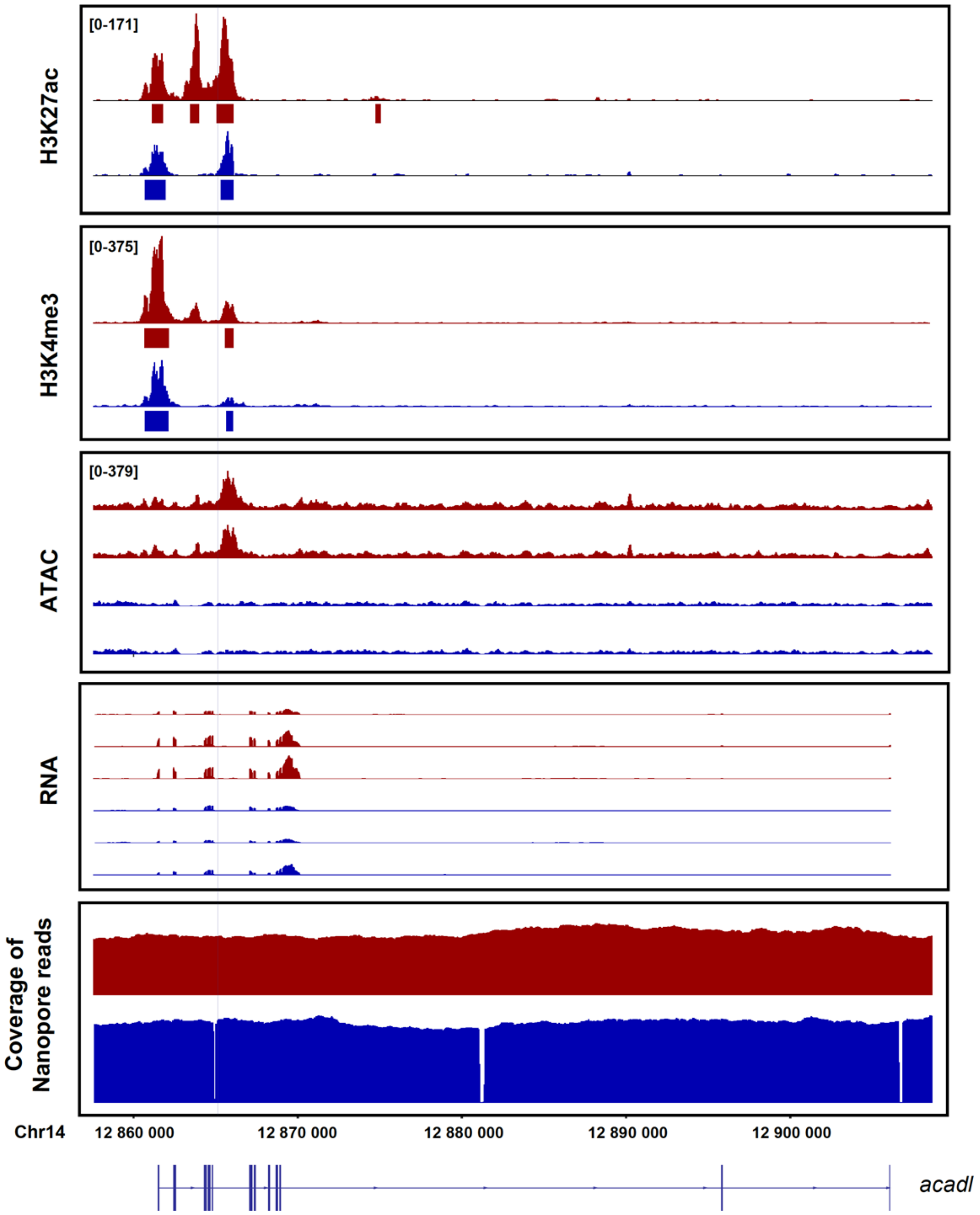
Supplementary Figure S13 Summary of genomic structural variants (SVs) detected using three different programs

A: Number of identified SVs. **B:** Length of identified SVs.



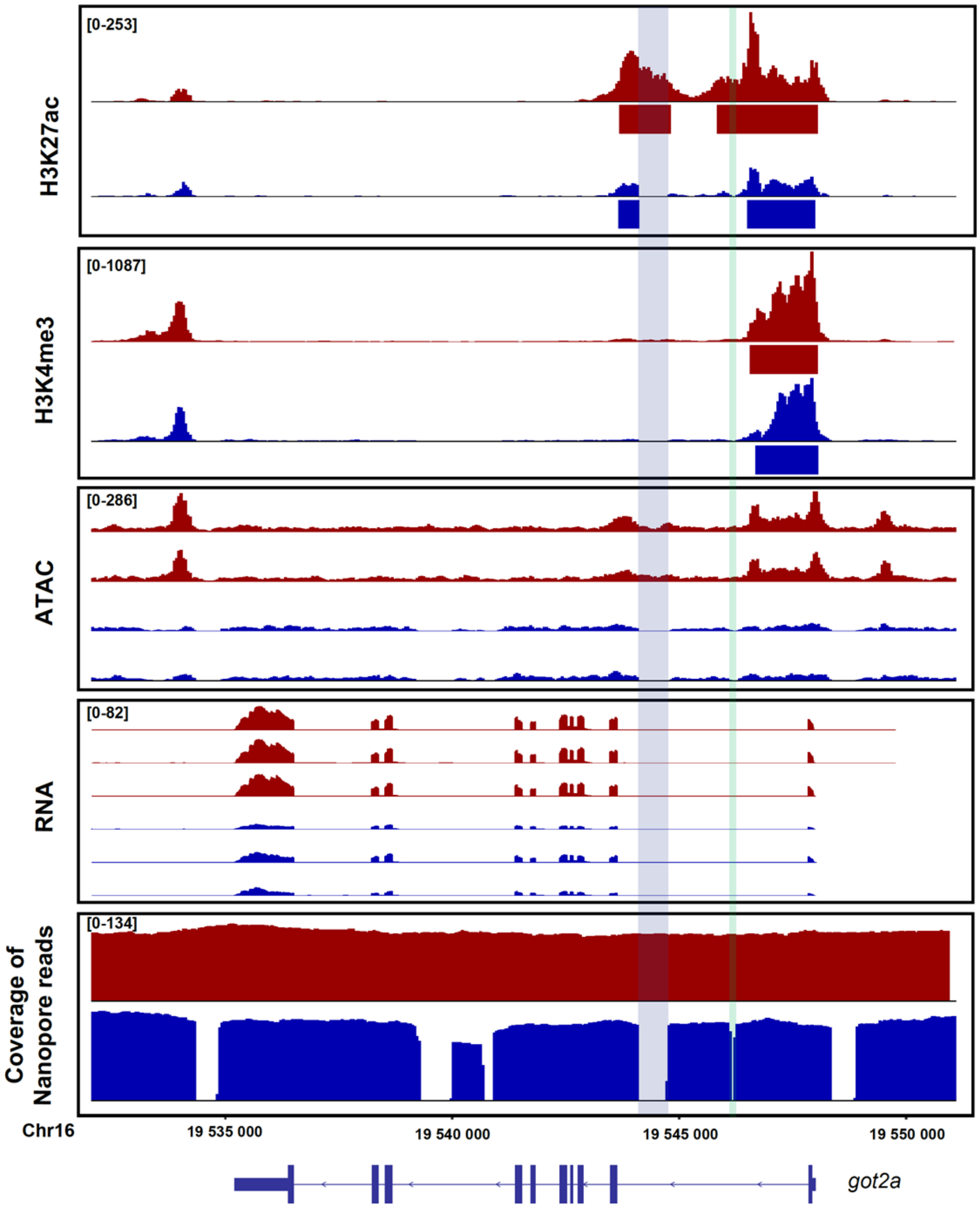
Supplementary Figure S14 ***Cis*****-regulatory divergence between** ***S. chuatsi*** **(red) and** ***S. scherzeri*** **(blue) at** ***acsl1a*** **gene**

Intensities of H3K27ac, H3K4me3, ATAC-seq, and gene expression are shown. Mapping coverage of Nanopore reads is plotted to indicate genomic SVs. Deletion in *S. scherzeri* is denoted in light blue. MACS2-identified H3K27ac and H3K4me3 peaks are denoted with rectangles below tracks. Transcripts (with exons as boxes) are depicted.



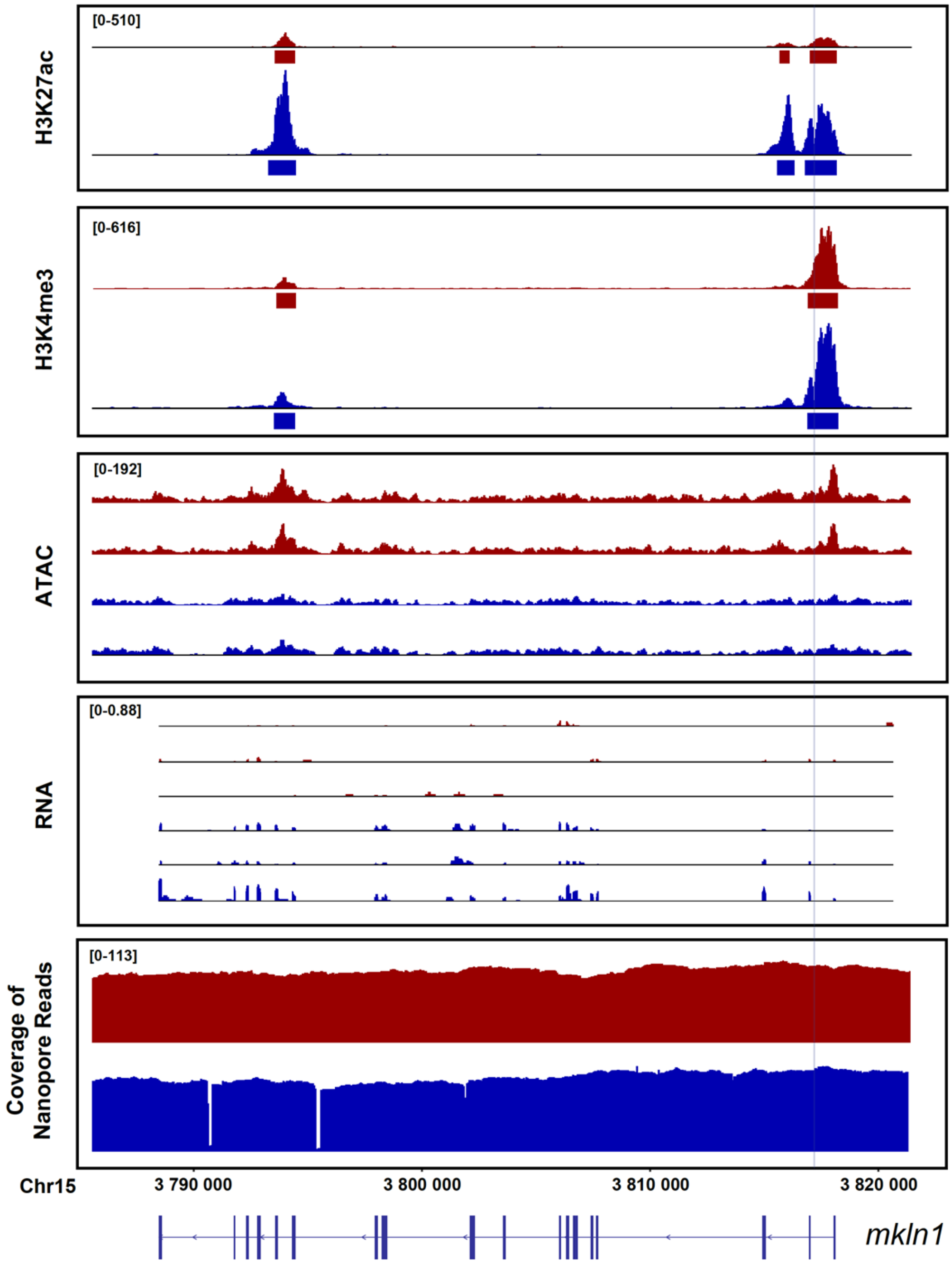
Supplementary Figure S15 ***Cis*****-regulatory divergence between** ***S. chuatsi*** **(red) and** ***S. scherzeri*** **(blue) at** ***acadl*** **gene**

Intensities of H3K27ac, H3K4me3, ATAC-seq, and gene expression are shown. Mapping coverage of Nanopore reads is plotted to indicate genomic SVs. Deletion in *S. scherzeri* is denoted in light blue. MACS2-identified H3K27ac and H3K4me3 peaks are denoted with rectangles below tracks. Transcripts (with exons as boxes) are depicted.



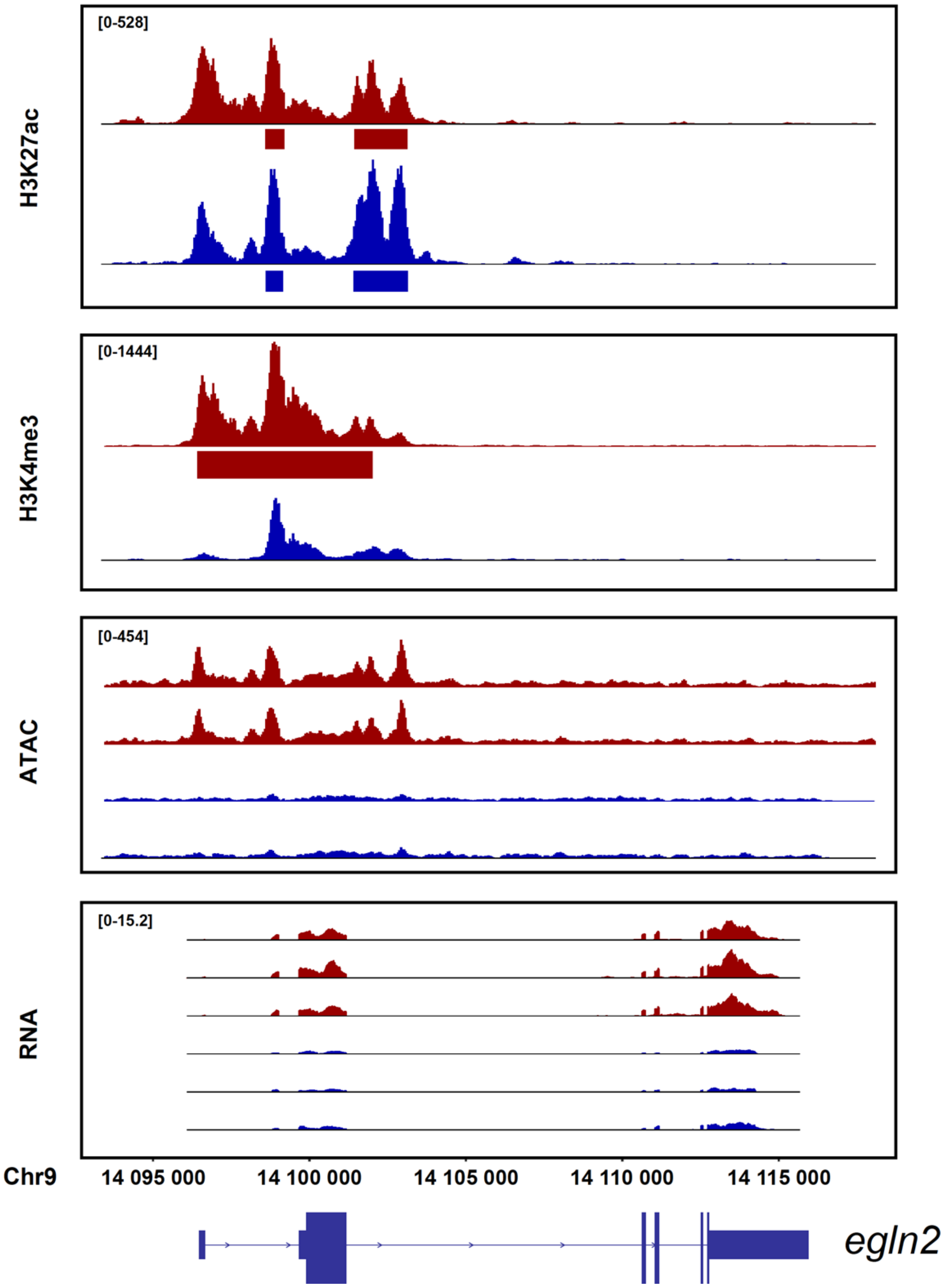
Supplementary Figure S16 ***Cis*****-regulatory divergence between** ***S. chuatsi*** **(red) and** ***S. scherzeri*** **(blue) at** ***got2a*** **gene**

Intensities of H3K27ac, H3K4me3, ATAC-seq, and gene expression are shown. Mapping coverage of Nanopore reads is plotted to indicate genomic SVs. Two deletions in *S. scherzeri* are denoted in light blue and green, respectively. MACS2-identified H3K27ac and H3K4me3 peaks are denoted with rectangles below tracks. Transcripts (with exons as boxes) are depicted.



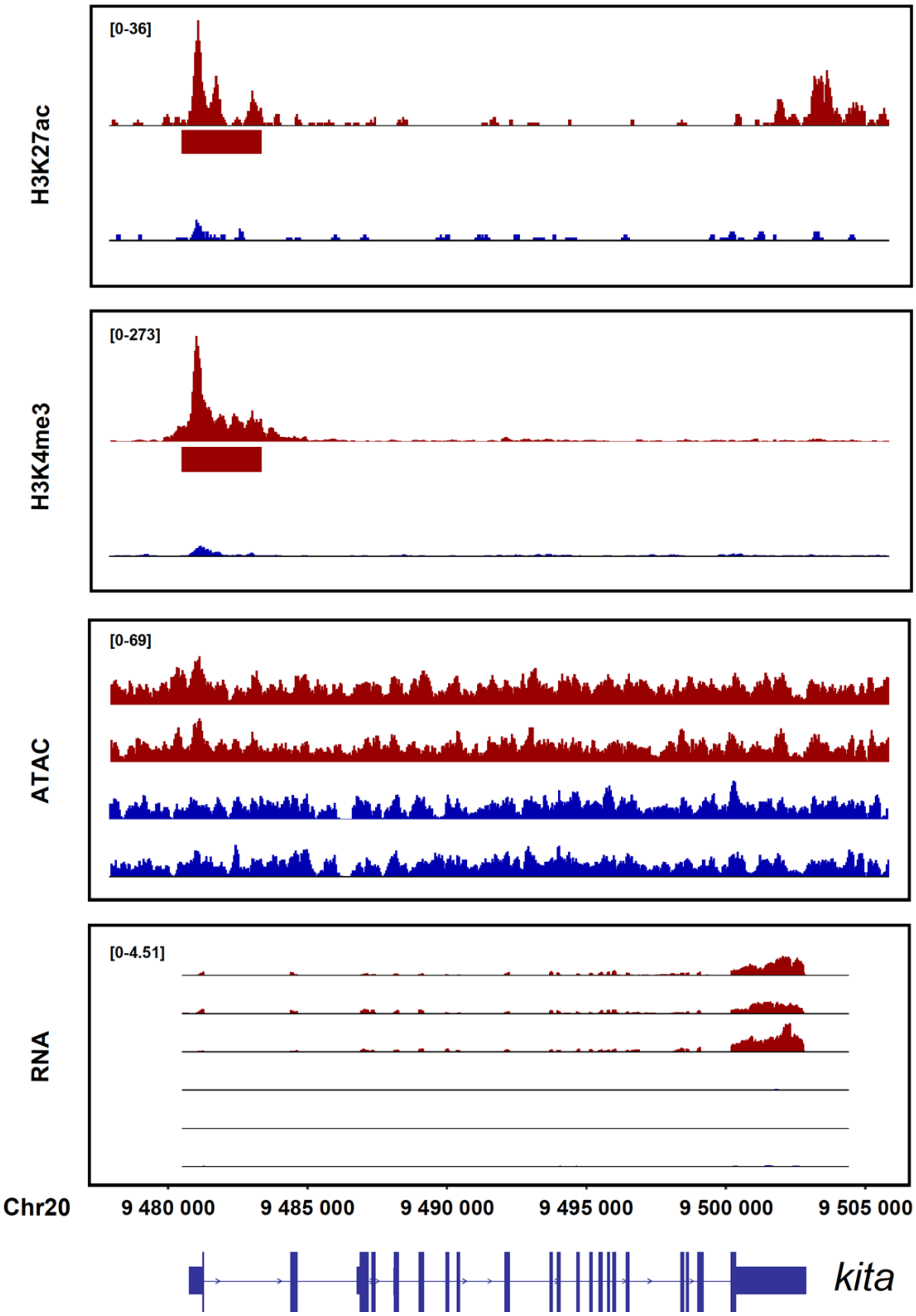
Supplementary Figure S17 ***Cis*****-regulatory divergence between** ***S. chuatsi*** **(red) and** ***S. scherzeri*** **(blue) at** ***mkln1*** **gene**

Intensities of H3K27ac, H3K4me3, ATAC-seq, and gene expression are shown. Mapping coverage of Nanopore reads is plotted to indicate genomic SVs. Deletion in *S. chuatsi* is in light blue. MACS2-identified H3K27ac and H3K4me3 peaks are denoted with rectangles below tracks. Transcripts (with exons as boxes) are depicted.



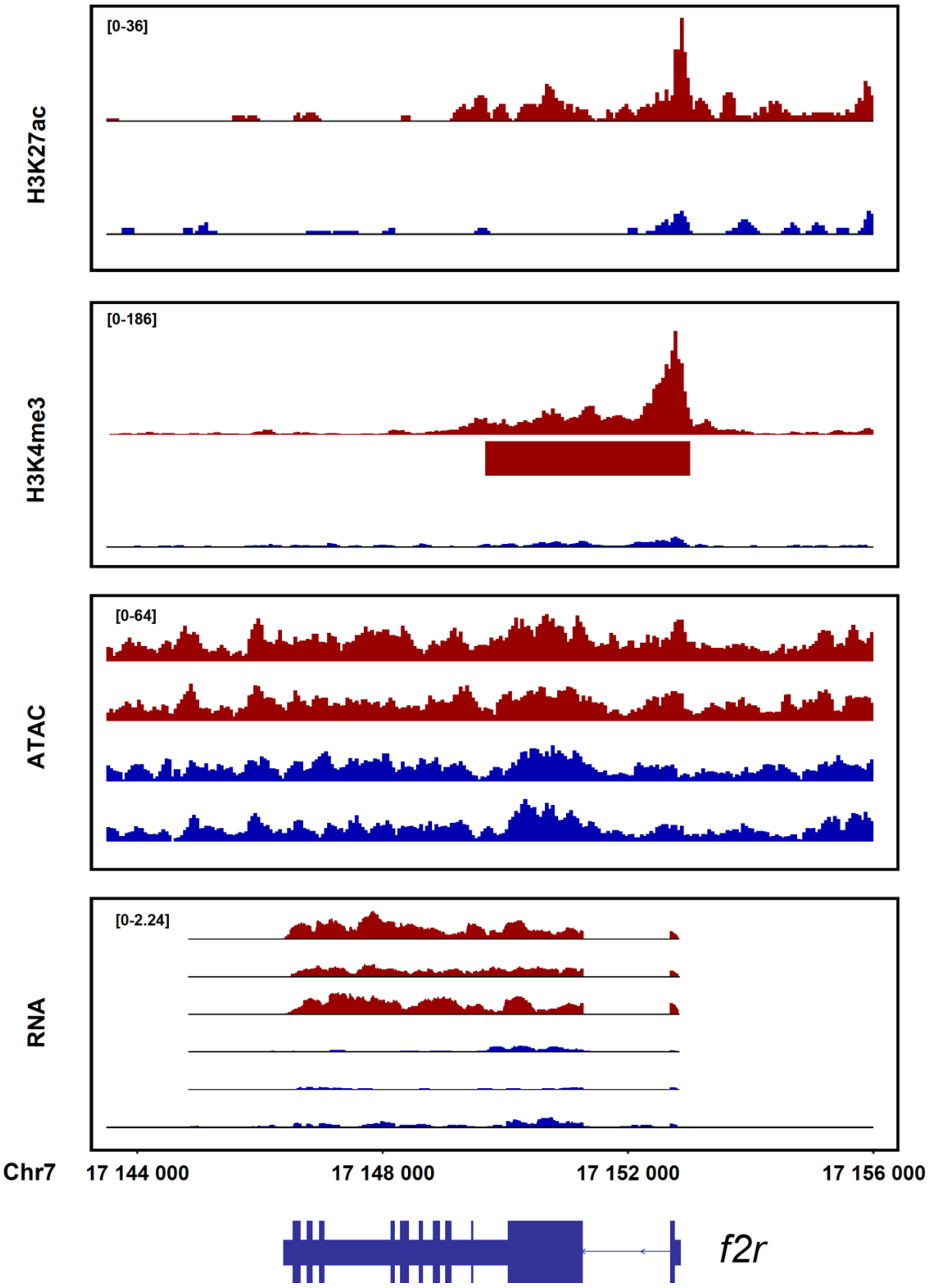
Supplementary Figure S18 ***Cis*****-regulatory divergence between** ***S. chuatsi*** **(red) and** ***S. scherzeri*** **(blue) at** ***egln2*** **gene**

Intensities of H3K27ac, H3K4me3, ATAC-seq, and gene expression are shown. MACS2-identified H3K27ac and broad H3K4me3 peaks are denoted with rectangles below track. Transcripts (with exons as boxes) are depicted.



Supplementary Figure S19 ***Cis*****-regulatory divergence between** ***S. chuatsi*** **(red) and** ***S. scherzeri*** **(blue) at** ***kita*** **gene**

Intensities of H3K27ac, H3K4me3, ATAC-seq, and gene expression are shown. MACS2-identified H3K27ac and broad H3K4me3 peaks are denoted with rectangles below tracks. Transcripts (with exons as boxes) are depicted.



Supplementary Figure S20 ***Cis*****-regulatory divergence between *S. chuatsi* (Red) and *S. scherzeri* (Blue) at *f2r* gene**

Intensities of H3K27ac, H3K4me3, ATAC-seq, and gene expression are shown. MACS2-identified H3K27ac and broad H3K4me3 peaks are denoted with rectangles below track. Transcripts (with exons as boxes) are depicted.

Supplementary Table S1 Basic statistics of Illumina reads

	Number of reads	**Total bases (bp)**	**Depth of coverage （×）**
S. chuatsi	412 923 280	61 938 492 000	86.5
S. scherzeri	308 477 640	46 271 646 000	62.5

Supplementary Table S2 Basic statistics of Nanopore reads

	Total number of reads	**Total number of bases (Gb)**	**Maximum length of reads (bp)**	**Minimum length of reads (bp)**	**Average length of reads (bp)**	**Depth of coverage (×)**
S. chuatsi	4 978 504	97.7	240 827	42	19 630	136.4
S. scherzeri	3 926 889	94.3	243 874	105	24 010	127.3

Supplementary Table S3 Basic statistics of Hi-C reads

	Total bases (bp)	**Depth of coverage (×)**	**cis-chromosomal interaction (> 20 kb)**	**cis-chromosomal interaction (< 20 kb)**
S. chuatsi	94 818 391 200	132.4	24 %	7 %
S. scherzeri	102 637 460 100	138.6	19 %	7%

Supplementary Table S4 Statistics of *S. chuatsi* genome assembly

	Contig		Scaffold	
	Size (bp)	Number	Size (bp)	Number
N50	21 551 097	14	29 956 575	11
N60	19 623 084	18	29 175 850	14
N70	13 306 200	22	28 362 969	16
N80	8 640 736	29	27 500 176	19
N90	3 004 397	42	24 860 938	21
Total	716 210 655	328	716 348 155	191

Supplementary Table S5 Statistics of *S. scherzeri* genome assembly

	Contig		Scaffold	
	Size (bp)	Number	Size (bp)	Number
N50	16 036 871	15	30 486 584	11
N60	14 480 942	19	30 021 833	14
N70	9 520 673	26	29 387 193	16
N80	5 357 922	36	28 699 791	19
N90	3 383 819	53	27 297 733	21
Total	740 384 376	406	740 538 376	252

Supplementary Table S6 BUSCO evaluation of *S. chuatsi* and *S. scherzeri* genome assemblies

	S. chuatsi	*S. scherzeri*
Complete BUSCOs	3562 (97.9%)	3593 (98.7%)
Complete and single-copy BUSCOs	3540 (97.3%)	3567 (98.0%)
Complete and duplicated BUSCOs	22 (0.6%)	26 (0.7%)
Fragmented BUSCOs	16	17
Missing BUSCOs	62	30
Total BUSCO groups searched	3640	3640

Supplementary Table S7 RNA-seq reads alignment statistics of two *S. chuatsi* assemblies

	Assembly	**Unmapped reads (%)**	**Uniquely mapped reads (%)**	**Multi-mapping reads (%)**	**Mapping rate (%)**
Eye	sinChu7	15.50%	81.50%	2.42%	86.13%
	This assembly	6.79%	86.72%	5.88%	95.00%
Gill	sinChu7	20.19%	75.80%	3.48%	81.64%
	This assembly	7.23%	85.40%	6.86%	95.10%
Heart	sinChu7	14.38%	82.07%	3.17%	87.52%
	This assembly	6.85%	87.71%	5.05%	95.17%
Intestine	sinChu7	20.03%	76.12%	3.42%	82.23%
	This assembly	9.05%	84.01%	6.56%	93.71%
Kidney	sinChu7	14.61%	82.01%	2.87%	87.22%
	This assembly	9.09%	86.13%	4.28%	93.21%
Liver	sinChu7	6.56%	86.74%	6.38%	94.57%
	This assembly	5.59%	88.67%	5.40%	95.80%
Spleen	sinChu7	7.94%	84.68%	6.96%	94.42%
	This assembly	7.39%	85.05%	7.15%	95.36%
Stomach	sinChu7	19.85%	75.06%	4.78%	81.72%
	This assembly	7.51%	83.84%	8.36%	94.51%
Testis	sinChu7	9.31%	86.75%	3.43%	92.57%
	This assembly	6.73%	88.36%	4.35%	95.39%
Brain* (SRR10867828)	sinChu7	28.86%	67.91%	1.74%	78.14%
	This assembly	21.72%	72.20%	4.52%	86.40%
Intestine* (SRR10867827)	sinChu7	36.82%	59.18%	2.68%	69.86%
	This assembly	22.28%	67.67%	8.65%	86.76%
Liver* (SRR10867826)	sinChu7	33.45%	59.68%	5.52%	72.34%
	This assembly	19.70%	67.72%	11.12%	88.45%
Muscle* (SRR10867825)	sinChu7	31.39%	58.95%	8.38%	75.42%
	This assembly	25.37%	65.10%	8.16%	84.25%

Note: RNA-seq reads from He *et al.* (2019) [1] were denoted with *

Reference

He S, Li L, Lv LY, Cai WJ, Dou YQ, Li J, Tang SL, Chen X, Zhang Z, Xu J *et al.* 2020. Mandarin fish (Sinipercidae) genomes provide insights into innate predatory feeding. *Communications Biology*, **3**(1): 361.

Supplementary Table S8 RNA-seq reads alignment statistics of two *S. scherzeri* assemblies

	Assembly	Unmapped reads (%)	Uniquely mapped reads (%)	Multi-mapping reads (%)	Mapping rate (%)
Eye	sinSch6b	6.17%	89.64%	3.91%	96.09%
	This assembly	5.39%	91.81%	2.52%	96.74%
Gill	sinSch6b	10.35%	86.09%	3.20%	92.71%
	This assembly	7.16%	88.14%	4.35%	95.44%
Heart	sinSch6b	6.77%	87.17%	5.77%	95.61%
	This assembly	5.46%	90.75%	3.48%	96.74%
Intestine	sinSch6b	10.29%	85.27%	4.03%	93.01%
	This assembly	6.92%	88.92%	3.79%	95.83%
Kidney	sinSch6b	8.65%	85.76%	5.28%	94.18%
	This assembly	6.53%	89.94%	3.20%	96.02%
Liver	sinSch6b	8.16%	85.66%	5.75%	94.26%
	This assembly	4.55%	88.77%	6.39%	97.29%
Spleen	sinSch6b	11.06%	85.30%	3.27%	92.60%
	This assembly	8.10%	87.76%	3.78%	95.10%
Stomach	sinSch6b	7.05%	88.67%	3.96%	95.32%
	This assembly	4.98%	90.95%	3.76%	97.01%
Testis	sinSch6b	7.91%	86.49%	5.35%	94.78%
	This assembly	5.94%	90.74%	3.07%	96.40%

Supplementary Table S9 Statistics of assembly quality and completeness evaluation using Mercury

		QV	Error rate	Completeness (%)
S. chuatsi	This assembly	36.9142	0.000203508	96.397
	sinChu7	32.3679	0.000579707	97.1919
S. scherzeri	This assembly	35.7313	0.000267218	95.7469
	sinSch6	30.9618	0.000801352	93.7093

Supplementary Table S10 Summary of annotated repeats in *S. chuatsi* genome

	Number	Length (bp)	Percentage (%)
Retroelements			
SINEs:	25 488	2 507 374	0.35
MIRs	8 935	1 040 905	0.15
LINEs:	151 607	37 269 631	5.20
LINE1	2 091	351 931	0.05
LINE2	73 943	17 149 829	2.39
LTR elements:	24 221	6 034 393	0.84
ERV_classI	1 217	622 070	0.09
DNA transposons：	**383 869**	**55 184 211**	**7.70**
hAT-Charlie	18 456	2 008 459	0.28
Unclassified:	**600 490**	**76 225 084**	**10.64**
Total interspersed repeats		**177 220 693**	**24.74**
Small RNA:	**4 196**	**441 630**	**0.06**
Satellites:	**643**	**169 440**	**0.02**
Simple repeats:	**318 886**	**16 965 675**	**2.37**
Low complexity	**37 739**	**2 290 874**	**0.32**
Total		**196 347 086**	**27.41**

Supplementary Table S11 Summary of annotated repeats in *S. scherzeri* genome

	Number	Length (bp)	Percentage (%)
Retroelements			
SINEs:	28 055	2 578 008	0.35
MIRs	11 449	1 294 403	0.17
LINEs:	154 990	43 019 685	5.81
LINE1	2 382	514 798	0.07
LINE2	77 893	19 455 186	2.63
LTR elements:	34 632	7 746 310	1.05
ERV_classI	4128	1 000 999	0.14
ERV_classII	570	86 334	0.01
DNA transposons：	**393 505**	**55 646 827**	**7.51**
hAT-Charlie	12 997	1 541 780	0.21
TcMar-Tigger	957	69 671	0.01
Unclassified:	**645 653**	**80 168 797**	**10.83**
Total interspersed repeats		**189 159 627**	**25.54**
Small RNA:	**3 604**	**646 701**	**0.09**
Satellites:	**2 528**	**273 497**	**0.04**
Simple repeats:	**329 210**	**17 681 281**	**2.39**
Low complexity	**38 168**	**2 295 851**	**0.31**
Total		**209 398 734**	**28.28**

Supplementary Table S12 Whole-genome gene annotation for *S. chuatsi* using different prediction approaches

Gene set		Gene Number	Exon Number
De novo based	Braker2 (Illumina)	38 330	392 512
	Braker2 (PacBio Iso-seq)	37 689	246 953
Homolog based	*Danio rerio*	31 454	290 167
	Oryzias latipes	39 946	353 988
	Takifugu rubripes	25 652	252 838
	Gasterosteus aculeatus	32 183	280 964
	Tetraodon nigroviridi	21 112	229 856
RNA-seq based	Stringtie (Illumina)	26 700	705 699
	Stringtie (Iso-seq)	5 251	50 443
	PASA	176 040	2 194 858
EVM Integration		28 905	255 740
PASA Update		29 278	585 487

Supplementary Table S13 Whole-genome gene annotation for *S. scherzeri* using different prediction approaches

Gene set		Gene Number	Exon Number
Ab initio based	Braker2 (Illumina)	39 599	396 977
	Braker2 (PacBio Iso-seq)	42 435	271 365
Homolog based	*Danio rerio*	30 977	291 720
	Oryzias latipes	41 571	357 174
	Takifugu rubripes	25 743	253 974
	Gasterosteus aculeatus	31 746	281 875
	Tetraodon nigroviridi	21 220	231 206
RNA-seq based	Stringtie (Illumina)	23 844	741 156
	Stringtie (Iso-seq)	11 771	94 293
	PASA	135 467	1 718 623
EVM Integration		29 497	256 599
PASA Update		29 543	604 121

Supplementary Table S14 BUSCO evaluation of predicted gene models of *S. chuatsi* and *S. scherzeri*

	S. chuatsi	*S. scherzeri*
Complete BUSCOs	3353 (92.1%)	3337 (91.7%)
Complete and single-copy BUSCOs	2136 (58.7%)	2158 (59.3%)
Complete and duplicated BUSCOs	1217 (33.4%)	1179 (32.4%)
Fragmented BUSCOs	44	73
Missing BUSCOs	243	230
Total BUSCO groups searched	3640	3640

Supplementary Table S15 Summary of gene functional annotations

		S. chuatsi	***S. scherzeri***
Total		29 278	29 543
Annotated	InterPro	25 507 (87.12%)	25 899 (87.67%)
	NCBI NR	23 064 (78.78%)	23 444 (79.36%)
	KEGG	16 119 (55.05%)	16 544 (56.00%)
	Swiss-Prot	20 051 (68.48%)	20 501(69.39%)
	eggNOG	20 796 (71.03%)	21 192 (71.73%)
	Total	26 623 (90.93%)	27 024 (91.47%)
Unannotated		2 655 (9.07%)	2 519 (8.53%)

Supplementary Table S16 Summary of *S. chuatsi* and *S. scherzeri* individuals for resequencing

Sample name	species	Sampling site	Tissue of samples	Total bases (Gb)	Sequencing depth (×)
SinChu1	*S. chuatsi*	Hunan	Dorsal Fin	8.70	12.15
SinChu2	*S. chuatsi*	Hunan	Dorsal Fin	9.53	13.31
SinChu3	*S. chuatsi*	Hunan	Dorsal Fin	8.84	12.34
SinChu4	*S. chuatsi*	Hunan	Dorsal Fin	8.19	11.43
SinChu5	*S. chuatsi*	Hunan	Dorsal Fin	8.87	12.38
SinChu6	*S. chuatsi*	Hunan	Dorsal Fin	8.59	12.00
SinSch1	*S. scherzeri*	Jilin	Dorsal Fin	8.14	11.37
SinSch2	*S. scherzeri*	Jilin	Dorsal Fin	8.69	12.13
SinSch3	*S. scherzeri*	Jilin	Dorsal Fin	8.36	11.67
SinSch4	*S. scherzeri*	Jilin	Muscle	9.89	13.80
SinSch5	*S. scherzeri*	Jilin	Muscle	10.04	14.02
SinSch6	*S. scherzeri*	Jilin	Muscle	9.54	13.32
SinSch7	*S. scherzeri*	Guangdong	Dorsal Fin	9.04	12.62
SinSch8	*S. scherzeri*	Guangdong	Dorsal Fin	9.57	13.36
SinSch9	*S. scherzeri*	Guangdong	Dorsal Fin	9.45	13.19
SinSch10	*S. scherzeri*	Guangdong	Dorsal Fin	9.33	13.02
SinSch11	*S. scherzeri*	Guangdong	Dorsal Fin	8.57	11.96
SinSch12	*S. scherzeri*	Guangdong	Dorsal Fin	8.57	11.96

Supplementary Table S17 Positively selected genes (PSGs) in *S. chuatsi*

No.	**Gene ID**	**Gene Name**	**Abbreviation**	**Omega**	***P*-value**
1	SinChu_g028183	*phosphofructokinase, platelet*	*pfkp*	12.777	6.83E-12
2	SinChu_g012873	*Rap guanine nucleotide exchange factor (GEF) 3*	*rapgef3*	1.543	9.17E-06
3	SinChu_g008662	*arrestin domain containing 3a*	*arrdc3a*	1	1.45E-05
4	SinChu_g005846	*sphingomyelin phosphodiesterase 3*	*smpd3*	1.963	1.92E-05
5	SinChu_g005932	*membrane-bound transcription factor peptidase, site 1*	*mbtps1*	2.892	1.27E-04
6	SinChu_g006396	*tropomyosin 1*	*tpm1*	37.621	1.53E-04
7	SinChu_g014068	*eukaryotic translation initiation factor 2-alpha kinase 3*	*eif2ak3*	1.542	3.94E-04
8	SinChu_g022725	*ubiquitin carboxyl-terminal esterase L3*	*uchl3*	1.603	2.71E-03
9	SinChu_g019912	*activation-induced cytidine deaminase*	*aicda*	5.854	2.96E-03
10	SinChu_g019441	*chymotrypsinogen B*	*ctrb*	1.632	5.49E-03
11	SinChu_g022935	*crystallin, gamma M3*	*crygm3*	5.738	7.58E-03
12	SinChu_g001073	*titin-cap*	*tcap*	5.131	7.83E-03
13	SinChu_g016175	*notch receptor 1*	*notch1*	2.341	8.93E-03
14	SinChu_g004528	*peroxiredoxin like 2B*	*prxl2b*	1.017	1.48E-02
15	SinChu_g017335	*solute carrier family 25 member 3a*	slc25a3a	2.022	4.56E-02

Supplementary Table S18 Positively selected genes (PSGs) in *S. scherzeri*

No	Gene ID	Gene Name	Abbreviation	Omega	*P*-value
1	SinSch_g018173	*Kin17 DNA and RNA binding protein*	*kin*	16.209	2.032E-14
2	SinSch_g027281	*prolyl 4-hydroxylase, alpha polypeptide I a*	*p4ha1a*	14.815	1.32E-13
3	SinSch_g004881	*calcium channel, voltage-dependent, L type, alpha 1D subunit, a*	*cacna1da*	3.126	1.63E-06
4	SinSch_g015059	*EYA transcriptional coactivator and phosphatase 4*	*eya4*	2.176	4.79E-06
5	SinSch_g004606	*mitogen-activated protein kinase 14b*	*mapk14b*	10.548	1.79E-05
6	SinSch_g021541	*ribosomal protein S8b*	*rps8b*	5.625	2.52E-05
7	SinSch_g021470	*integrin, beta 1a*	*itgb1a*	1.786	2.57E-05
8	SinSch_g004447	*STIM activating enhance*	*stimate*	6.274	4.06E-05
9	SinSch_g005151	*cadherin 22*	*cdh22*	2.009	1.11E-04
10	SinSch_g016577	*syntaxin binding protein 1a*	*stxbp1a*	3.702	8.13E-04
11	SinSch_g004114	*cell division cycle 42*	*cdc42*	1.487	9.01E-04
12	SinSch_g015537	*5'-3' exoribonuclease 2*	*xrn2*	1.457	1.40E-03
13	SinSch_g002350	*DENN domain containing 10*	*dennd10*	3.309	3.15E-03
14	SinSch_g007414	*recombination signal binding protein for immunoglobulin kappa J region b*	*rbpjb*	10.465	3.89E-03
15	SinSch_g010804	*chloride channel, nucleotide-sensitive, 1A*	*clns1a*	1.226	4.74E-03
16	SinSch_g001009	*solute carrier family 25 member 17*	*slc25a17*	3.178	5.36E-03
17	SinSch_g021912	*ring finger and CCCH-type domains 1b*	*rc3h1b*	1.265	5.39E-03
18	SinSch_g007217	*protein phosphatase 1, regulatory (inhibitor) subunit 14Bb*	*ppp1r14bb*	8.416	5.82E-03

19	SinSch_g003579	*N-acetylneuraminic acid synthase a*	*nansa*	7.354	6.93E-03
20	SinSch_g027795	*elongin B*	*elob*	26.514	1.03E-02
21	SinSch_g010515	*proteasome 20S subunit beta 4*	*psmb4*	1.014	1.17E-02
22	SinSch_g017936	*ubiquitin-conjugating enzyme E2Nb*	*ube2nb*	13.274	1.51E-02
23	SinSch_g001810	*protein phosphatase, Mg2+/Mn2+ dependent, 1Ba*	*ppm1ba*	1.737	1.66E-02
24	SinSch_g008885	*bri3 binding protein*	*bri3bp*	4.148	2.38E-02
25	SinSch_g014370	*attractin*	*atrn*	2.439	4.54E-02

Supplementary Table S19 Summary of sequencing and peak calling of CUT&Tag

	Species	**Mapped pair-reads**	**Mapping rate**	**PCR repeat rate**	**Uniquely mapped reads**	**Number of peaks**	**Average length of peaks (bp)**	**FRiPs**
H3K27ac	*S. chuatsi*	22 383 313	97.90%	86.62%	2 762 865	17,015	789.66	66.05%
	S. scherzeri	25 938 076	96.18%	80.85%	3 655 502	15,150	742.97	69.99%
H3K4me3	*S. chuatsi*	52 063 564	95.14%	73.09%	13 173 930	16,955	1061.62	77.32%
	S. scherzeri	25 585 943	93.21%	39.33%	13 785 016	16,239	1014.29	72.05%
IgG (Control)	*S. chuatsi*	165 620	26.77%	96.07%	2 627	2	329.50	2.00%
	S. scherzeri	451 411	55.46%	95.91%	7 697	4	322.00	0.22%

Supplementary Table S20 Summary of sequencing and peak calling of ATAC-Seq

Sample	Species	Mapped pair-reads	Mapping rate	PCR repeat rate	Uniquely mapped reads	Number of peaks	Average length of peaks (bp)	FRiPs
Chu1	*S. chuatsi*	91 891 999	97.29%	25.10%	61 507 958	140 942	371.16	22.13%
Chu2	*S. chuatsi*	75 938 882	97.35%	22.29%	52 783 582	133 094	362.69	21.14%
Sch1	*S. scherzeri*	67 435 897	93.63%	18.70%	40 670 224	172 513	267.22	18.72%
Sch2	*S. scherzeri*	62 998 480	94.40%	15.77%	39 595 802	161 158	306.56	20.62%

Supplementary Table S21 Summary of *cis*-regulatory regions identified in the genomes of *S. chuatsi* and *S. scherzeri*

	Distal enhancers	Promotors			Non-redundant *cis*-regulatory regions
	H3K27ac	**H3K4me3**	**H3K27ac**	**Total promotors**	
S. chuatsi	4 965	16 953	12 048	17 623	22 588
S. scherzeri	3 132	16 239	11 164	16 726	19 858

Supplementary Table S22 Differentially expressed immune genes associated with *cis*-regulatory regions affected by SVs

Gene ID	Abbreviation	Gene name	Ortholog in Human	log2FC (H3K27ac Peak Intensity)	FPKM (Spleen)	
					S. chuatsi	***S. scherzeri***
SinChu_g011534	*prss16*	*serine protease 16*	*PRSS16*	-1.93689	27.62	71.85
SinChu_g015362	*lama4*	*laminin, alpha 4*	*LAMA4*	-1.15982	2.2	30.09
SinChu_g020408	*cd22*	*cd22 molecule*	*CD22*	-1.58585	0.63	1.66
SinChu_g027583	*tecpr1b*	*tectonin beta-propeller repeat containing 1b*	*TECPR1*	-9.46312	1.27	3.86

Supplementary Table S23 Characterization of broad H3K4me3 peaks

	Number of broad peaks	**H3K27ac peaks**	
		Overlap	**Percentage**
S. chuatsi	491	459	93.50%
S. scherzeri	481	444	92.30%

Supplementary Table S24 Summary of top 20 enriched KEGG pathways of genes associated with differential broad H3K4me3 peaks

	Pathway ID	**Pathway**	**Candidate genes with pathway annotation**	**All genes with pathway annotation**	***P*-value**	**Q-value**
1	ko04072	Phospholipase D signaling pathway	10 (12.5%)	144 (2.29%)	0.000012	0.002434
2	ko05206	MicroRNAs in cancer	8 (10%)	134 (2.13%)	0.000275	0.027607
3	ko05200	Pathways in cancer	15 (18.75%)	454 (7.22%)	0.000501	0.03359
4	ko04910	Insulin signaling pathway	7 (8.75%)	139 (2.21%)	0.001833	0.0729
5	ko04151	PI3K-Akt signaling pathway	11 (13.75%)	318 (5.06%)	0.002123	0.0729
6	ko05231	Choline metabolism in cancer	6 (7.5%)	106 (1.69%)	0.002176	0.0729
7	ko05222	Small cell lung cancer	5 (6.25%)	76 (1.21%)	0.002678	0.076894
8	ko05221	Acute myeloid leukemia	4 (5%)	50 (0.79%)	0.003585	0.079814
9	ko04218	Cellular senescence	7 (8.75%)	157 (2.5%)	0.003645	0.079814
10	ko05202	Transcriptional misregulation in cancers	7 (8.75%)	165 (2.62%)	0.004791	0.079814
11	ko04211	Longevity regulating pathway - mammal	5 (6.25%)	87 (1.38%)	0.004806	0.079814
12	ko04015	Rap1 signaling pathway	8 (10%)	210 (3.34%)	0.00497	0.079814
13	ko05310	Asthma	3 (3.75%)	28 (0.45%)	0.005162	0.079814
14	ko04213	Longevity regulating pathway - multiple species	4 (5%)	60 (0.95%)	0.006899	0.099043
15	ko05230	Central carbon metabolism in cancer	4 (5%)	62 (0.99%)	0.007742	0.101914
16	ko05169	Epstein-Barr virus infection	7 (8.75%)	182 (2.89%)	0.008113	0.101914
17	ko05163	Human cytomegalovirus infection	7 (8.75%)	189 (3%)	0.009887	0.116899
18	ko05162	Measles	5 (6.25%)	114 (1.81%)	0.014648	0.163572
19	ko04630	Jak-STAT signaling pathway	5 (6.25%)	120 (1.91%)	0.017933	0.186168
20	ko04152	AMPK signaling pathway	5 (6.25%)	121 (1.92%)	0.018524	0.186168

Supplementary Table S25 Differential broad H3K4me3 peaks associated genes that are enriched in pathways in cancer (ko05200)

Gene ID	Abbreviation	Gene Name	Ortholog in Human	log2FC (Broad H3K4me3 Intensity)	log2FC (Gene Expression Level)
SinChu_g011111	*egln2*	*egl-9 family hypoxia-inducible factor 2*	*EGLN2*	1.17304	2.23113776
SinChu_g008717	*f2r*	*coagulation factor II (thrombin) receptor*	*F2R*	2.08824	3.19522938
SinChu_g023426	*kita*	*KIT proto-oncogene, receptor tyrosine kinase a*	*KIT*	3.00812	4.68452923
SinChu_g001477	*ncoa4*	*nuclear receptor coactivator 4*	*NCOA4*	1.19068	2.291397069
SinChu_g017824	*ccnd2a*	*cyclin D2, a*	*CCND2*	1.90242	1.93104124
SinChu_g022510	*cyc1*	*cytochrome c-1*	*CYC1*	-2.17504	-6.141871664
SinChu_g010322	*e2f3*	*E2F transcription factor 3*	*E2F3*	-1.47952	-2.294534034
SinChu_g016271	*pik3r1*	*phosphoinositide-3-kinase, regulatory subunit 1 (alpha)*	*PIK3R1*	-1.92386	-1.4228252