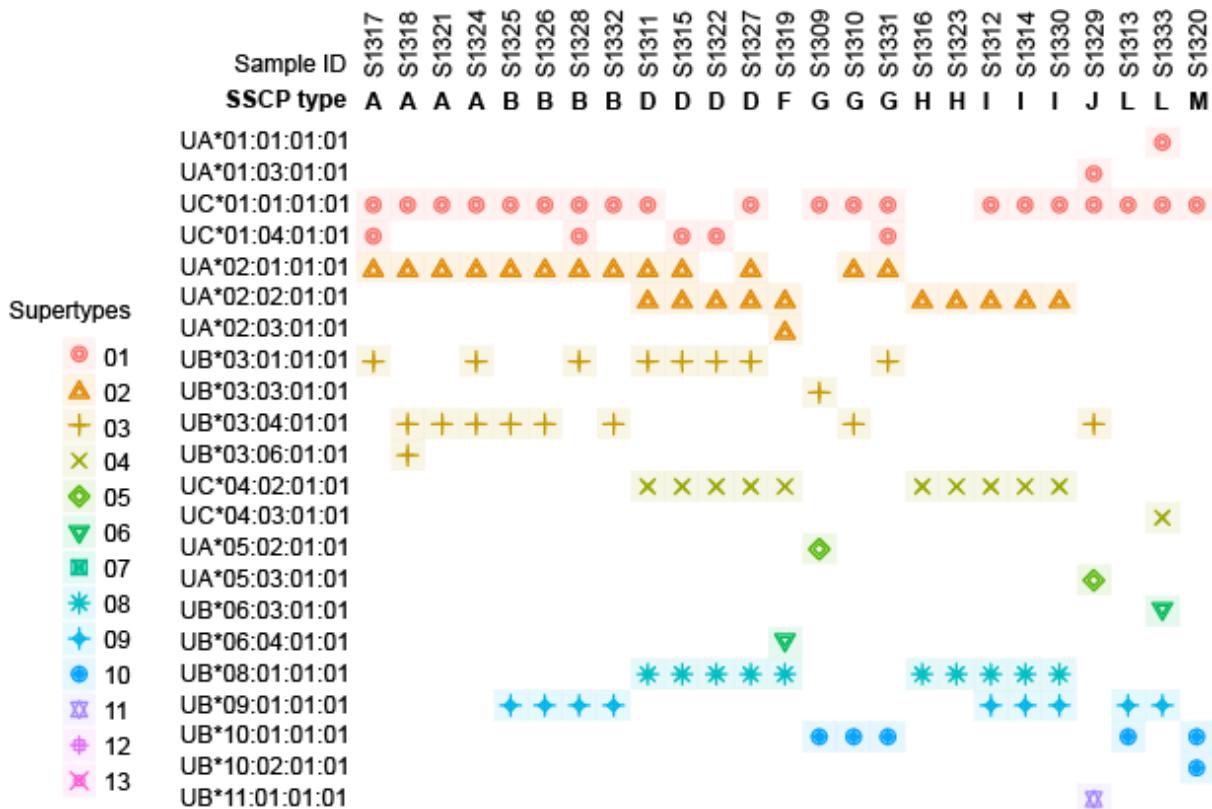


Supplementary Data 2. Limited resolution of SSCP method



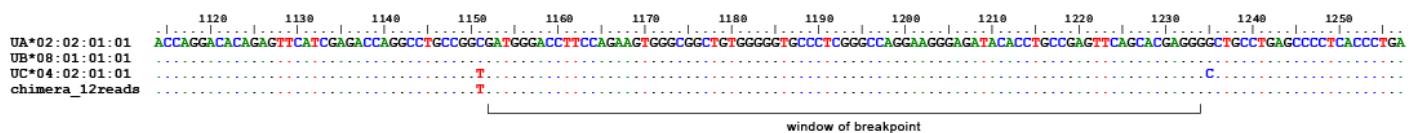
Each column in the table represents an individual Tasmanian devil with SSCP type and MHC-I alleles shown. Before the development of the new method described in this paper, previous genotyping efforts in the Tasmanian devil were mainly based on amplicon cloning and Sanger sequencing, which is a laborious, time-consuming, and costly method. As an alternative, the single-strand conformation polymorphism (SSCP) analysis, which is still a relatively common method for MHC typing in non-model species nowadays, was used as a trade-off for simplicity and suitability for population-scale surveys. In this study, we analysed 25 Tasmanian devils representing 10 different SSCP patterns¹. While the SSCP method appeared to provide high consistency with different SSCP types indicating different MHC genotypes, it was insufficient for differentiating certain MHC variants, especially those within the same supertype groups, which resulted in some animals with different genotypes sharing the same SSCP type.

Supplementary Data 3. PacBio read statistics

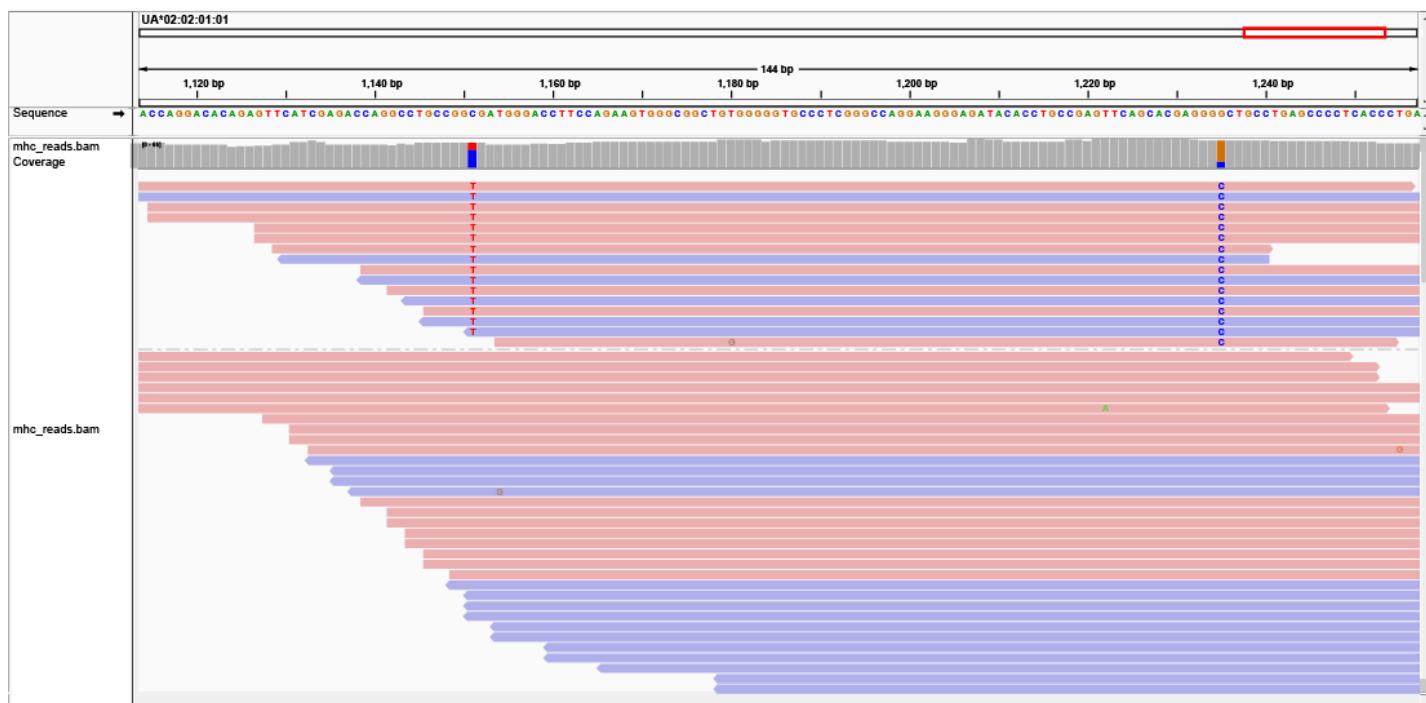
	Total	SMRTcell_1	SMRTcell_2	SMRTcell_3	SMRTcell_4
Number of polymerase reads	1,989,412	496,556	510,674	473,285	508,897
Avg. polymerase read length (bp)	35,912	37,456	36,928	35,571	33,703
Number of subreads	49,125,512	12,735,113	12,843,178	11,669,501	11,877,720
Avg. subread length (bp)	1,413	1,419	1,427	1,401	1,403
Number of stranded CCS reads	1,910,014	492,646	498,965	453,530	464,873
Number of reads per sample	3,868 ± 1,086				
Number of refined reads per sample	3,850 ± 1,085				
Number of aligned reads per sample	3,846 ± 1,082				

Supplementary Data 4. PCR chimera analysis

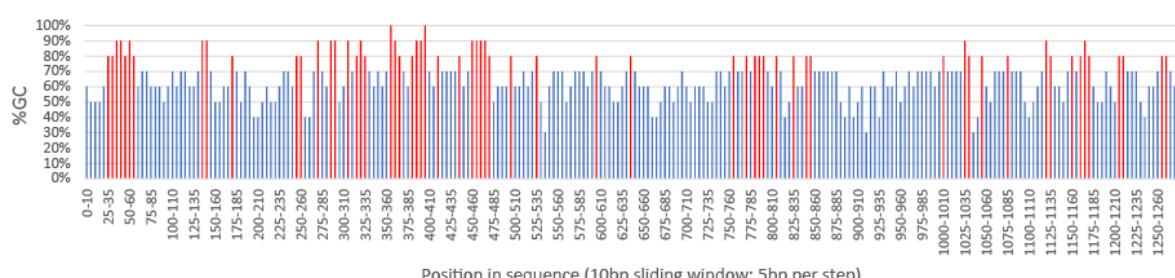
a



b



c



SD4_Fig1. Analysis of MHC PCR chimera formation in a Tasmanian devil. a) Partial sequence alignment of three MHC-I alleles and one recurring chimeric variant, with the region of chimera formation shown; b) Whole genome sequencing reads aligned to the region of chimera formation, as visualised in IGV; c) GC content distribution in devil MHC-I gene, with %GC greater than 80% highlighted in red.

It has been observed in bacterial 16S rRNA gene amplicons that PCR chimeras can reproducibly form among independent PCR amplifications². To investigate if this can occur in MHC gene amplicons, we genotyped one Tasmanian devil (Maria Island; microchip number 982000123209291) whose genome had been sequenced for another study (unpublished data). Two independent PCR amplifications were carried out, one using Phusion Hot Start II High Fidelity PCR Master Mix (Thermo Scientific) and the other using Platinum SuperFi II PCR Master Mix (Invitrogen), both with 20 PCR cycles performed. The generated amplicons were sequenced and data analysed using the methods described in the main article.

Both sets of sequencing data suggested that the individual has three MHC-I alleles, including UA*02:02:01:01, UB*08:01:01:01, and UC*04:02:01:01. One putative chimeric sequence variant, which appears to be a combination of UC*04:02:01:01 and either of the other two alleles (SD4_Fig1a), was found in both results supported by 12 reads and five reads, respectively. Based on whole genome resequencing data generated with Illumina sequencing, this chimeric sequence variant, which contains a nucleotide T at sequence position 1151 and G at 1235, does not exist in the genome of this individual (SD4_Fig1b). This result along with the low read counts suggest that this sequence variant is a technical artefact, which formed repeatedly in independent PCR amplifications carried out using different kits. One possible cause of this issue is the high abundance of GC-rich regions in the target MHC genes (SD4_Fig1c). High GC content in PCR templates can adversely affect the efficiency and success rate of amplification due to increased regional secondary structure formation. Although both PCR kits that were used are designed to improve the amplification of templates with high GC content, the GC-rich regions within the target genes may still increase the chance of generating partial amplicons, which will facilitate the formation of recurring chimeric artefacts.

These results demonstrate that the identification of a sequence variant in multiple samples or PCR amplifications is not a sufficient criterion for MHC allele calling, and that a stringent chimera removal strategy is needed during MHC amplicon data processing.

Supplementary Data 5. Nucleotide sequences of 61 Tasmanian devil MHC-I alleles

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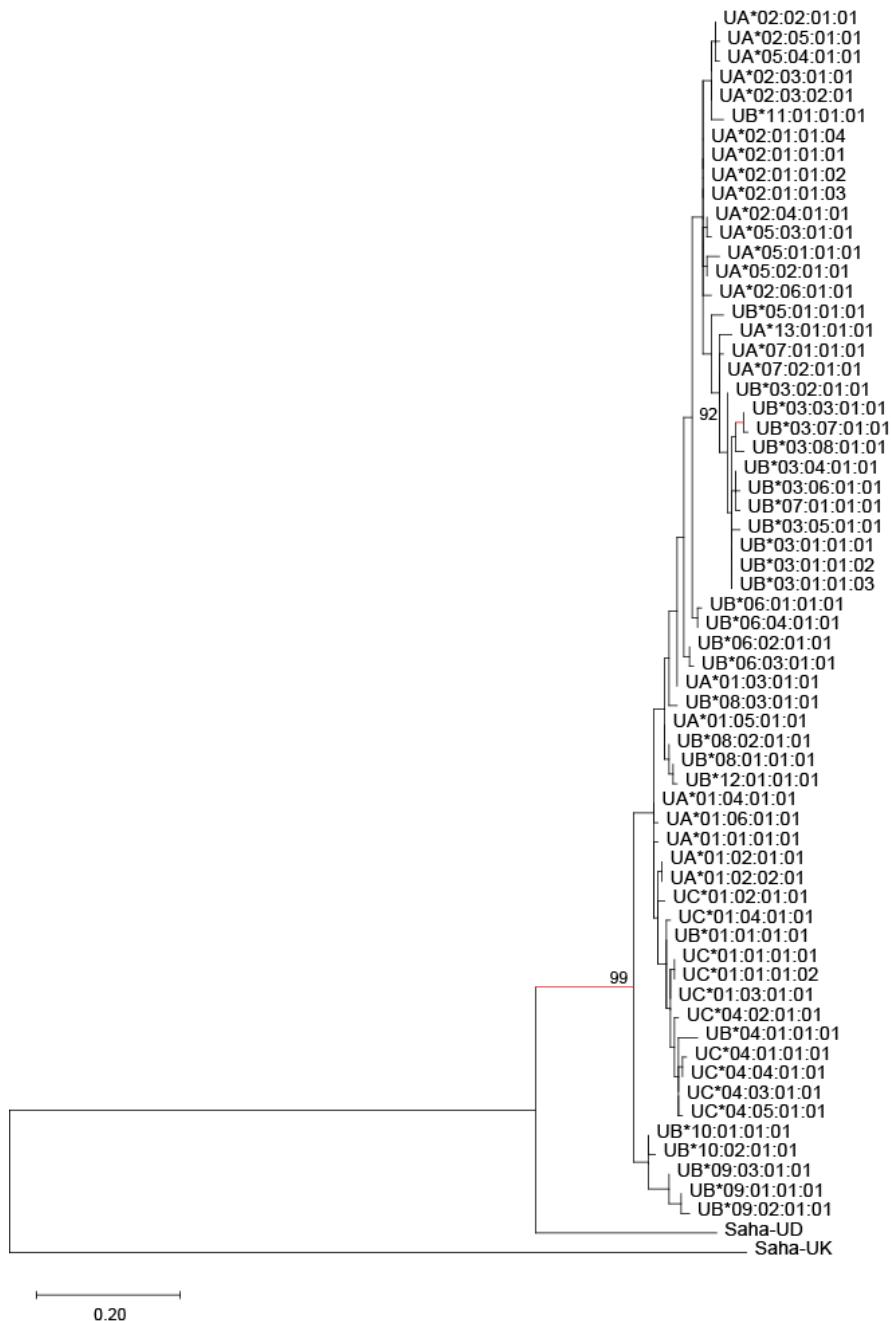
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CGGGCCAGGAAGGGAGATAACCTGCCAGTTCAGCACGAGGGCTGCCCTGAGCCCTCACCTGAAATGGGTAAGCACCGTGAGGAGGGGTTGGGGGATCCCCACCTCC

Supplementary Data 6. Phylogenetic relationships among Tasmanian devil MHC-I alleles



The evolutionary history among the Tasmanian devil MHC-I alleles was inferred based on amino acid sequences using the Maximum Likelihood method and Whelan And Goldman model³ in MEGA X⁴. The tree with the highest log likelihood (-2192.52) is shown. A discrete Gamma distribution was used to model evolutionary rate differences among sites (5 categories (+G, parameter = 0.3083)). The rate variation model allowed for some sites to be evolutionarily invariable ([+I], 30.40% sites). The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. Two non-classical MHC-I genes, *Saha-UK* and *Saha-UD*, were included for rooting the tree. The percentage of replicate trees in which the associated sequences clustered together in the bootstrap test (200 replicates) are shown for branches with > 60% support. The evolutionary relationships among alleles of the three devil MHC-I genes are very difficult to resolve due to high sequence similarities, with the majority of the branches having low bootstrap values.

Supplementary Data 7. Evidence of individual residues under selection

Codon ^a	Selection ^b	Evidence ^c			
		MEME	IFEL	REL	EVF
24	+	+	+	+	+
44				+	
56					+
65			+		
66	+	+			+
70				+	
73	-		-	-	-
76				+	
77	+	+	+	+	
78				+	
80	+	+	+	+	+
83	+	+	+	+	+
100				+	
102		+			
127				+	
156	+	+	+	+	+
172				+	
192				+	
195	-		-	-	-
240				-	
241	-		-	-	
264				+	
269				-	

"+": positively selected sites

"-": negatively selected sites

^a Codons correspond to amino acid sites starting from alpha 1 domain of the MHC-I molecules (i.e. signal peptide excluded).

^b Sites that have evidence of selection inferred by more than one method are considered under selection.

^c Statistical significance: one-tailed p-value <= 0.05 for MEME⁵ and IFEL⁶, posterior probability >= 0.95 for REL⁶ and EVF⁷.

Supplementary Data 8. Tasmanian devil MHC-I haplotypes

Supplementary Data 8a. List of inferred haplotypes

Haplotype ID	MHC-I loci ^a		
	Saha-UA	Saha-UB	Saha-UC
hap_1	x	UB*10:01:01:01	UC*04:01:01:01
hap_2	UA*02:02:01:01	UB*08:01:01:01	UC*04:02:01:01
hap_3	x	UB*09:01:01:01	UC*04:01:01:01
hap_4	UA*02:01:01:01	UB*03:01:01:01	UC*01:01:01:01
hap_5	UA*07:01:01:01	UB*03:02:01:01	UC*01:01:01:01
hap_6	UA*05:01:01:01	UB*11:01:01:01	UC*01:01:01:01
hap_7	UA*02:03:01:01	UB*06:01:01:01	UC*04:02:01:01
hap_8	UA*02:02:01:01	UB*12:01:01:01	UC*04:02:01:01
hap_9	UA*02:01:01:01	UB*03:04:01:01	UC*01:01:01:01
hap_10	UA*02:01:01:01	UB*03:03:01:01	UC*01:01:01:01
hap_11	x	UB*10:02:01:01	UC*04:01:01:01
hap_12	x	x	UC*01:01:01:01
hap_13	UA*02:03:02:01	UB*06:02:01:01	UC*04:03:01:01
hap_14	UA*02:01:01:01	UB*03:05:01:01	UC*01:02:01:01
hap_15	UA*02:01:01:01	UB*03:01:01:02	UC*01:03:01:01
hap_16	UA*02:01:01:01	UB*03:01:01:01	UC*01:04:01:01
hap_17	UA*02:01:01:01	UB*03:01:01:03	UC*01:01:01:01
hap_18	UA*05:02:01:01	UB*03:03:01:01	UC*01:01:01:01
hap_19	UA*02:04:01:01	UB*03:05:01:01	UC*01:02:01:01
hap_20	UA*05:03:01:01	UB*11:01:01:01	UC*01:01:01:01
hap_21	UA*01:01:01:01	UB*06:03:01:01	UC*04:03:01:01
hap_22	x	UB*04:01:01:01	UC*04:01:01:01
hap_23	UA*02:02:01:01	UB*08:02:01:01	UC*04:02:01:01
hap_24	UA*02:01:01:01	UB*03:06:01:01	UC*01:01:01:01
hap_25	UA*02:01:01:01	UB*03:07:01:01	UC*01:01:01:01
hap_26	UA*01:02:01:01	x	x
hap_27	UA*02:03:01:01	UB*06:04:01:01	UC*04:02:01:01

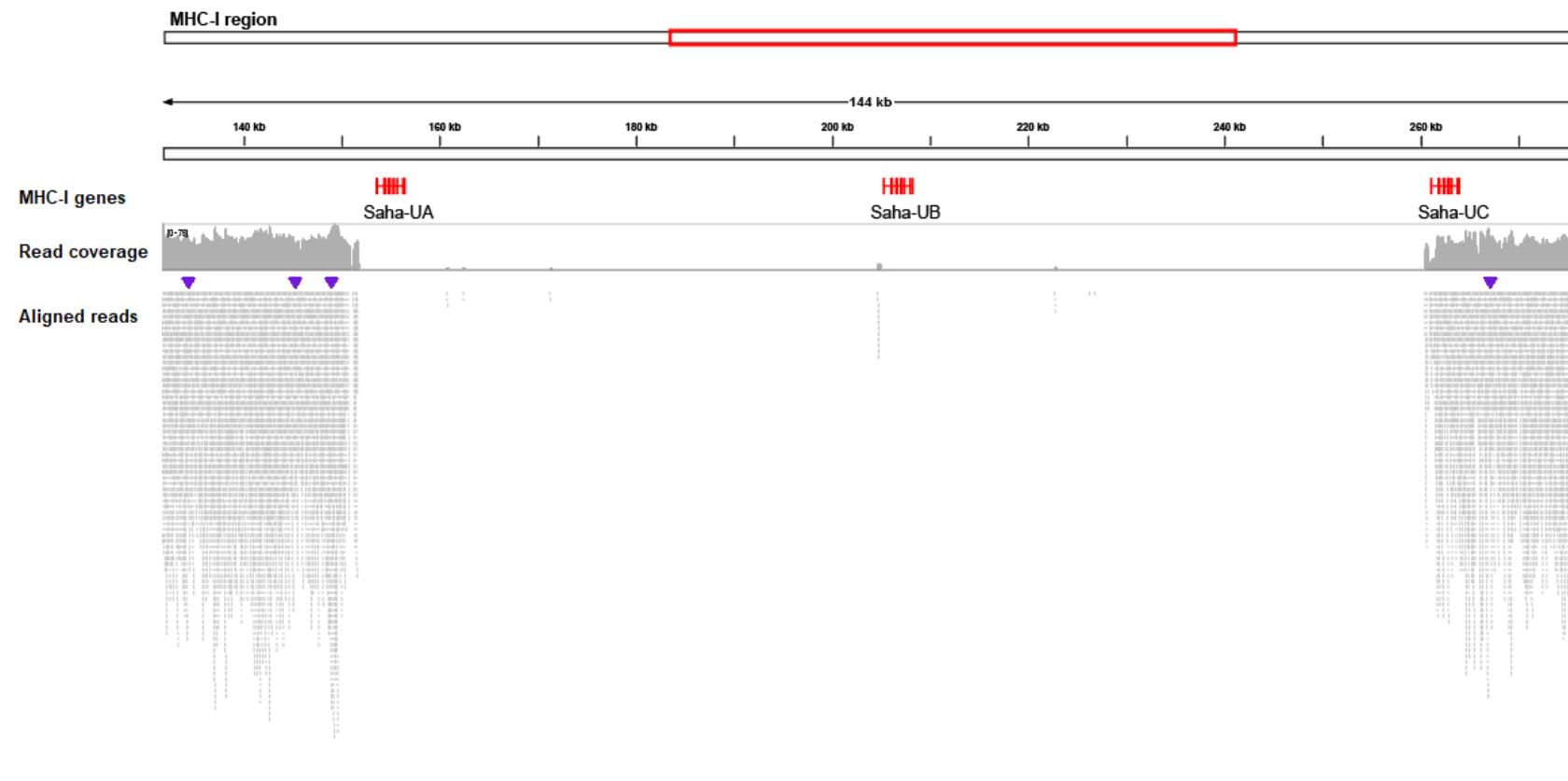
hap_28	UA*07:02:01:01	UB*03:02:01:01	UC*01:01:01:01
hap_29	UA*01:02:02:01	UB*06:02:01:01	UC*04:03:01:01
hap_30	x	UB*10:02:01:01	UC*04:04:01:01
hap_31	x	UB*09:02:01:01	UC*04:01:01:01
hap_32	UA*02:01:01:01	UB*03:03:01:01	UC*01:01:01:02
hap_33	UA*02:01:01:01	UB*08:03:01:01	UC*01:01:01:01
hap_34	UA*02:01:01:01	UB*05:01:01:01	UC*01:01:01:01
hap_35	UA*02:01:01:04	UB*03:01:01:02	UC*01:03:01:01
hap_36	UA*02:02:01:01	UB*01:01:01:01	UC*01:01:01:01
hap_37	x	UB*09:01:01:01	UC*04:02:01:01
hap_38	x	UB*09:03:01:01	UC*04:01:01:01
hap_39	UA*02:01:01:01	UB*07:01:01:01	UC*01:01:01:01
hap_40	UA*02:01:01:02	UB*03:01:01:01	UC*01:01:01:01
hap_41	UA*02:01:01:02	UB*03:04:01:01	UC*01:01:01:01
hap_42	UA*02:01:01:03	UB*03:03:01:01	UC*01:01:01:01
hap_43	UA*02:03:01:01	UB*06:03:01:01	UC*04:05:01:01
hap_44	UA*05:04:01:01	UB*12:01:01:01	UC*04:02:01:01
hap_45	UA*13:01:01:01	UB*03:05:01:01	UC*01:02:01:01
hap_46	UA*01:05:01:01	UB*03:01:01:02	UC*01:03:01:01
hap_47	UA*02:05:01:01	UB*12:01:01:01	UC*04:02:01:01
hap_48	UA*01:03:01:01	UB*03:08:01:01	UC*01:03:01:01
hap_49	UA*01:06:01:01	UB*06:01:01:01	UC*04:02:01:01
hap_50	UA*02:06:01:01	UB*03:03:01:01	UC*01:01:01:01

^a The Tasmanian devil MHC has been found to contain genomic structural variants resulting in some haplotypes lacking certain MHC-I gene(s)^{1,8,9}, which are indicated with “x”.

Supplementary Data 8b. Confirmation of hap_12

One of the two inferred haplotypes that contain only one copy of MHC-I gene, hap_12, was observed in multiple devil subpopulations and was relatively common in a certain subpopulation (e.g. frequency = 0.15 in wukalina; see also Supplementary Data 9). To further confirm this haplotype, we sequenced the genome of one individual from wukalina (microchip number 982000365591975) which was found to have only one MHC allele (UC*01:01:01:01) and was predicted to have two copies of hap_12.

The sequencing library was constructed from genomic DNA using the KAPA HyperPlus Kit (Roche) and was sequenced on one lane in a NovaSeq SP 2x150bp run. Raw reads were trimmed using program Trimmomatic¹⁰ v0.38 (LEADING:20 TRAILING:20 SLIDINGWINDOW:4:15 MINLEN:40) and data quality was checked with FastQC v0.11.8. Trimmed reads were aligned to the Tasmanian devil reference genome (accession number: GCF_902635505.1) using BWA¹¹ v0.7.17 and PCR or optical read duplicates were removed using Picard¹² v2.21.7. A total of 934,975,126 reads (average read length 147.6 bp) were aligned to the genome. Reads aligned to the MHC-I region were visualised in IGV v2.5.2, as shown in the screenshot below.



In this sequenced individual, two of the three MHC-I genes, *Saha-UA* and *Saha-UB*, are completely missing due to a deletion with the size of approximately 109kb within the genomic region. No single nucleotide variation was detected in gene *Saha-UC*, with read sequences matching the sequence of allele UC*01:01:01:01. This data confirms that this individual has only one MHC-I allele in its genome, consistent with the genotyping and haplotyping results generated using our new MHC typing method.

Supplementary Data 9. MHC-I haplotype, allele, and supertype frequencies in nine Tasmanian devil subpopulations

	Haplotype/ Allele/ Supertype	East						North west	South west	Insurance population
		wukal ina	Stony Head	Nara wnta pu	Bront e	Buckl and	Fento nbury	Wool north	South west	Maria Island
Haplotype frequencies	hap_1	0.250	0.167	0.342	0.167	0.026	0.235	0.179	0.000	0.102
	hap_2	0.050	0.000	0.105	0.033	0.000	0.000	0.238	0.143	0.102
	hap_3	0.175	0.000	0.000	0.033	0.079	0.177	0.048	0.000	0.159
	hap_4	0.025	0.104	0.026	0.067	0.237	0.000	0.131	0.000	0.034
	hap_5	0.025	0.000	0.053	0.267	0.184	0.235	0.048	0.000	0.000
	hap_6	0.000	0.021	0.132	0.000	0.079	0.000	0.000	0.607	0.091
	hap_7	0.050	0.104	0.000	0.067	0.211	0.000	0.000	0.000	0.023
	hap_8	0.000	0.125	0.105	0.067	0.000	0.118	0.012	0.000	0.068
	hap_9	0.000	0.000	0.000	0.000	0.000	0.000	0.191	0.000	0.057
	hap_10	0.025	0.104	0.000	0.000	0.000	0.000	0.012	0.000	0.000
	hap_11	0.000	0.000	0.105	0.100	0.000	0.059	0.000	0.036	0.068
	hap_12	0.150	0.083	0.053	0.067	0.026	0.000	0.012	0.000	0.000
	hap_13	0.025	0.042	0.000	0.000	0.000	0.059	0.000	0.000	0.000
	hap_14	0.050	0.063	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	hap_15	0.000	0.000	0.000	0.100	0.053	0.088	0.000	0.071	0.000
	hap_16	0.000	0.000	0.000	0.000	0.000	0.000	0.012	0.000	0.023
	hap_17	0.000	0.063	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	hap_18	0.000	0.000	0.000	0.000	0.000	0.000	0.036	0.000	0.023
	hap_19	0.000	0.042	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	hap_20	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.034
	hap_21	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.057
	hap_22	0.000	0.021	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	hap_23	0.000	0.000	0.000	0.000	0.000	0.000	0.012	0.000	0.046
	hap_24	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.034
	hap_25	0.000	0.021	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	hap_26	0.000	0.000	0.026	0.000	0.000	0.000	0.000	0.000	0.000
	hap_27	0.000	0.000	0.000	0.000	0.000	0.000	0.012	0.000	0.011
	hap_28	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.107	0.000
	hap_29	0.075	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	hap_30	0.000	0.000	0.026	0.000	0.026	0.000	0.000	0.000	0.000
	hap_31	0.000	0.000	0.000	0.000	0.000	0.000	0.024	0.000	0.000
	hap_32	0.025	0.021	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	hap_33	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.023
	hap_34	0.000	0.000	0.000	0.000	0.000	0.000	0.024	0.000	0.000
	hap_35	0.000	0.000	0.000	0.000	0.053	0.000	0.000	0.000	0.000
	hap_36	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.023
	hap_37	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.036	0.000
	hap_38	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.011
	hap_39	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	hap_40	0.000	0.000	0.000	0.000	0.000	0.000	0.012	0.000	0.000
	hap_41	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.011
	hap_42	0.000	0.021	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	hap_43	0.000	0.000	0.000	0.000	0.026	0.000	0.000	0.000	0.000

Allele frequencies	hap_44	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	hap_45	0.025	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	hap_46	0.025	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	hap_47	0.000	0.000	0.000	0.033	0.000	0.000	0.000	0.000	0.000
	hap_48	0.000	0.000	0.000	0.000	0.000	0.029	0.000	0.000	0.000
	hap_49	0.000	0.000	0.026	0.000	0.000	0.000	0.000	0.000	0.000
	hap_50	0.025	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	UA*01:01:01:01	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.057
	UA*01:02:01:01	0.000	0.000	0.026	0.000	0.000	0.000	0.000	0.000	0.000
	UA*01:02:02:01	0.075	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	UA*01:03:01:01	0.000	0.000	0.000	0.000	0.000	0.029	0.000	0.000	0.000
	UA*01:05:01:01	0.025	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	UA*01:06:01:01	0.000	0.000	0.026	0.000	0.000	0.000	0.000	0.000	0.000
	UA*02:01:01:01	0.125	0.375	0.026	0.167	0.289	0.088	0.369	0.071	0.170
	UA*02:01:01:02	0.000	0.000	0.000	0.000	0.000	0.012	0.000	0.011	
	UA*02:01:01:03	0.000	0.021	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	UA*02:01:01:04	0.000	0.000	0.000	0.000	0.053	0.000	0.000	0.000	0.000
	UA*02:02:01:01	0.050	0.125	0.211	0.100	0.000	0.118	0.262	0.143	0.239
	UA*02:03:01:01	0.050	0.104	0.000	0.067	0.237	0.000	0.012	0.000	0.034
	UA*02:03:02:01	0.025	0.042	0.000	0.000	0.000	0.059	0.000	0.000	0.000
	UA*02:04:01:01	0.000	0.042	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	UA*02:05:01:01	0.000	0.000	0.000	0.033	0.000	0.000	0.000	0.000	0.000
	UA*02:06:01:01	0.025	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	UA*05:01:01:01	0.000	0.021	0.132	0.000	0.079	0.000	0.000	0.607	0.091
	UA*05:02:01:01	0.000	0.000	0.000	0.000	0.000	0.036	0.000	0.023	
	UA*05:03:01:01	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.034
	UA*05:04:01:01	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	UA*07:01:01:01	0.025	0.000	0.053	0.267	0.184	0.235	0.048	0.000	0.000
	UA*07:02:01:01	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.107	0.000
	UA*13:01:01:01	0.025	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	UB*01:01:01:01	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.023
	UB*03:01:01:01	0.025	0.104	0.026	0.067	0.237	0.000	0.155	0.000	0.057
	UB*03:01:01:02	0.025	0.000	0.000	0.100	0.105	0.088	0.000	0.071	0.000
	UB*03:01:01:03	0.000	0.063	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	UB*03:02:01:01	0.025	0.000	0.053	0.267	0.184	0.235	0.048	0.107	0.000
	UB*03:03:01:01	0.075	0.146	0.000	0.000	0.000	0.000	0.048	0.000	0.023
	UB*03:04:01:01	0.000	0.000	0.000	0.000	0.000	0.000	0.190	0.000	0.068
	UB*03:05:01:01	0.075	0.104	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	UB*03:06:01:01	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.034
	UB*03:07:01:01	0.000	0.021	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	UB*03:08:01:01	0.000	0.000	0.000	0.000	0.000	0.029	0.000	0.000	0.000
	UB*04:01:01:01	0.000	0.021	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	UB*05:01:01:01	0.000	0.000	0.000	0.000	0.000	0.000	0.024	0.000	0.000
	UB*06:01:01:01	0.050	0.104	0.026	0.067	0.211	0.000	0.000	0.000	0.023
	UB*06:02:01:01	0.100	0.042	0.000	0.000	0.000	0.059	0.000	0.000	0.000
	UB*06:03:01:01	0.000	0.000	0.000	0.000	0.026	0.000	0.000	0.000	0.057
	UB*06:04:01:01	0.000	0.000	0.000	0.000	0.000	0.012	0.000	0.011	
	UB*07:01:01:01	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	UB*08:01:01:01	0.050	0.000	0.105	0.033	0.000	0.000	0.238	0.143	0.102
	UB*08:02:01:01	0.000	0.000	0.000	0.000	0.000	0.012	0.000	0.045	

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