# **Supporting Information Appendix**

## Reversible epigenetic down-regulation of MHC molecules by Devil Facial Tumour Disease illustrates immune escape by a contagious cancer

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### **Supplementary Material**

### **Supplementary Materials and Methods**

### Cell Culture

DFTD (1426, 4906 and C5065) cell lines were cultured at  $35^{\circ}$ C and 5% CO<sub>2</sub> in "complete medium" composed of RPMI 1640 (Invitrogen) with 10% heat inactivated foetal bovine serum (FBS, Invitrogen), L-glutamine (2 mM, PAA) and kanamycin (100 µg/ml, PAA). A devil fibroblast cell line was cultured in DMEM (high glucose, PAA) with 10% FBS (Invitrogen), penicillin/streptomycin (0.1 units/ml penicillin and 0.1 mg/ml streptomycin, PAA) and L-glutamine (2 mM, PAA). Cells were split 1:3 every 72 hours. CHO cells were cultured in Ham's F12 media with 5% penicillin/streptomycin (0.1 units/ml penicillin and 0.1 mg/ml streptomycin, PAA), 10% FBS (Invitrogen) and L-glutamine (2 mM, PAA) at 37 °C with 5% CO<sub>2</sub>.

### Reverse-transcriptase quantitative PCR (RT-qPCR)

RT-qPCR was carried out using the Absolute Blue Sybr Green Fluorescein qPCR mix (Thermo Scientific) with primers at 250 nM each and the following cycling conditions; 95°C for 15 min, followed by 40 cycles of 95°C for 15 s, 59°C for 30 s and 72°C for 30 s. A number of housekeeping genes were trialled, including 28S ribosomal RNA (28S), glyceraldehyde 3-phosphate dehydrogenase (GAPDH), TATA-binding protein (TBP), ribosomal protein L13a (RPL13A) and hypoxanthine phosphoribosyltransferase 1 (HRPT1), but RPL13A gave the most consistency with equal amounts of cDNA across the samples. The tumour cDNA samples were tested in triplicate with no-DNA controls for all master mixes. Fibroblast cDNA at four dilutions (1, 0.25, 0.04 and 0.008) was used to create a standard curve for the amplification of RPL13A, MHC class I and  $\beta_2$ -microglobulin ( $\beta_2$ m) in each RT-qPCR experiment.

## PCR conditions for rapid amplification of cDNA ends (RACE) PCRs

A 5' RACE PCR was carried out to amplify the 5' end of MHC class I with a 5' RACE primer (GeneRacer, Invitrogen) and MHC class I reverse primer 5'GTCGTAGGCGAACTGAAG 3'. 3' RACE PCR was carried out using a 3' RACE primer and MHC class I forward primer 5' CAGATTTCCCGAGTGGAC 3'. Similarly, 5' and 3' RACE PCRs were carried out to amplify  $\beta_2$ m transcripts using forward (5'TGTGCATCCTTCCCTACCTGGAGG 3') and reverse (5' CATTGTTGAAAGACAGATCGGACCGC 3')  $\beta_2$ m specific primers in exon 2 of  $\beta_2$ m and either a 5' or 3' RACE primer. The optimal RACE PCR conditions were as follows: 1 x buffer, 1.6 mM MgCl<sub>2</sub>, 200 µM dNTP, 0.5 µM RACE primer, 2 µM reverse/forward primer and 0.3 µl of Taq polymerase (Expand High Fidelity Taq, Roche). PCR cycles were performed with initial denaturation at 94°C for 3 min, followed by 30 cycles of 94°C for 30 s, 65°C for 30 s, and 72°C for 2 min, and a final extension at 72°C for 10 min.

#### β<sub>2</sub>m expression and purification

Bacterial colonies containing the pET22b<sup>+</sup>-  $\beta_2$ m construct were cultured to OD<sub>600</sub> = 0.6 and recombinant  $\beta_2$ m expression was induced with 1 mM IPTG. Inclusion bodies were dissolved in 8 M urea, and  $\beta_2$ m was purified using a Hi-Trap nickel affinity column (BD Biosciences) according to the manufactures instructions. The expression of HIS-tagged  $\beta_2$ m was confirmed using western blot as described below, using an anti-HIS antibody (Roche cat no. 04905318001) according to the manufacturer's instructions. The denatured  $\beta_2$ m was refolded overnight at 4°C in a refold buffer (100 mM Tris-HCI pH 8.2, 400 mM arginine, 2 mM EDTA, 0.5 mM oxidized glutathione and 5 mM reduced glutathione) and purified by size exclusion chromatography using the AKTA FPLC system with an S75 10/30 Superdex column (Amersham).

### Specificity of MHC class I and β<sub>2</sub>m antibodies

The specificity of the TD50 antibody was tested in three independent experiments. First, a full length devil MHC class I transcript (Saha\*01, a previously published full length class I transcript (NCBI accession: EF591089)) was amplified using the following primers (F - 5'

GCGGAGACCCGGGCGGGCTCTTACCCATACGATGTTCCA 3' and R - 5' TACTTCTAGATTATTTGGCTGTCAGAGAGACATCTGACCCC 3') and cloned into the pcDNA 3.1 expression vector (Invitrogen) using standard molecular biology procedures. The resulting construct was sequenced in both directions with primers T7 and bovine growth hormone (BGH) to ensure no errors were introduced during amplification or cloning. DFTD cells were then transiently transfected using Fugene transfection reagent according to the manufacturers instructions (Promega) with either the MHC class I- pcDNA 3.1 construct or no DNA (a mock transfection as a control). DFTD cells were incubated for 30 h at 35°C before cells were harvested and a cell lysate was prepared as described in the main text. A western blot was performed as described in the main text using the antibody TD50. Second, a western blot was performed using fibroblast cell line lysate (prepared as described in the main text) and probed with either the TD50 antibody or with the TD50 antibody preblocked by adding 2 mg of the immunisation peptide (GGKGGDYVPAAGN) and incubating on ice for 30 min before use as a probe. Third, fibroblast cell line lysate (prepared as described in the main text) was deglycosylated using peptide-N glycosidase F (PNGase F; NEB) according to the manufacturer's instructions. Untreated and deglycosylated fibroblast lysates were then probed on a western blot with TD50 as described in the main text.

## **Supplementary Figures**



Figure S1. Additional DFTD biopsies stained for periaxin and  $\beta_2 m$ . IHC on serial sections of primary DFTD biopsies from wild devils (DFTD\_365, DFTD\_344 and DFTD\_287) stained for; **a - c**) periaxin, a marker specific for DFTD cells, at 20 x magnification, **d - f**)  $\beta_2 m$  at 20 x magnification, **g - i**)  $\beta_2 m$  at 40 x magnification, **j - l**) negative controls stained with pre-immune rat serum at 40 x magnification. Boxes indicate areas shown at 40 x magnification and arrow heads indicate similar positions in the serial sections, pointing towards DFTD cells. Positive cells for each marker are stained brown, nuclei are stained blue. Scale bars are 50 µm for 20 x magnification and 20 µm for 40 x magnification.



**Figure S2. RT-PCR amplification of MHC class II genes**. RT-PCR amplification of MHC class IIB, MHC class IIA and DMB from RNA from spleen, fibroblast cell line and DFTD cell lines. A no-cDNA negative control was included in each experiment. Markers show that amplicons are between 100 and 300 bp.

SahaUB 211H13 . . . SahaUC 161A10 ..... .G.....A..A....A...G..A.T......G. 110 \*Sahal ID isolate2 \*SahaUC isolate3 - - - -\* SahaUB isolate4 - -SahaUA 211H13 GGATACTGGGAGCAGGAGAGACACAGATCATTAAGGAGACTGCACAGATTTCCCGAGTGGACCTGCAGAGCCGGGGCTACTACAACCAGAGCGAGGGCGGGGCCCACAC 220 \*SahaUA isolate1 . . . . . . . . . . . . . . . . 196 SahaUA 211H13 CTTCCAGCGCATGTACGGCTGCGAGGTTTCCCCCGGAGCTCTCCTTCCAGCGCGGGTTTCTTCAGTACGCCTACGAC 296 \*SahaUB isolate4 в SahaDAB1'01 AGCATTGGAACAGCCAGAAGGATTTCATGGAGCAGAGAGGGCCGCGGGGGCCGCGGTGGACACTTACTGCAGGCACAACTACGGAATATCTGAGTCCTTCTTAGTGCGCAGGCCGCGTC 110 SahaDAB1'01 CAGCCCGAGGTGACTGTGTATCCATCCAAGATGGCTCCCCTGGGACACCACAACCTGCTTGTCTGCTCCGTCAGCGGTTTCT 192 SahaDAB3\*05 \*SahaDAB isolate1 \*SahaDAB isolate2 С SahaDAA CTAAGAGCCCAGCGGAGATGGACCAGCCCAATGTCCTTATCTGCTTCATTGACAAGTTCTCTCCCCCCGTACTTAATGTGACATGGCTTCGTAATGGGCAGCCCATCACT 110 SahaDAA TCACTGGGGACTGGAACAACCTCTTATGAAACATTGGGAACCAGAAATACGAACCCCCCTGCCAGAGACAACAGAGACTGTGGGTCT 306 \*SahaDAA isolate1

Figure S3. Alignments of MHC class I, class II B and class II A sequences amplified during RT-PCR. A) Nucleotide alignment comparing sequences from four previously described devil MHC class I genes (including the polymorphic loci, SahaUA, UB and UC, and the nonpolymorphic UD) with class I sequences isolated from DFTD cells during RT-PCR. The top four sequences are derived from BACs described by Cheng et al. (2012) (Accession numbers - 211H13 FQ482146.2, 161A10 FQ482138.3, 491I11 FQ482142.7). The bottom four sequences were isolated from DFTD cells as described for Figure 2 of this paper, and are marked with a star. Primer sites are shown in red. B) Nucleotide alignment comparing sequences from three previously described devil MHC class II B genes from BACs described in Cheng et al. (2012) with class II B sequences isolated from DFTD cells during RT-PCR, as described for Figure S2 of this paper. The top four sequences are previously described MHC class II B genes with the following accession numbers, SahaDAB1\*01 – EF591102.1, SahaDAB2\*03 – EF591104.1, SahaDAB3\*05 – EF591105.1. The bottom four sequences were isolated from DFTD cells and are marked with a star. Primer sites are shown boxed in red. C) Nucleotide alignment comparing the single devil class II A gene (Accession number FQ790241:10943-10728) with a single class II A sequence isolated from devil spleen, as described for Figure S2 of this paper. Primer sites are shown in red.

А



Figure S4. RT-PCR experiments on additional DFTD biopsies. RT-PCR amplification of RPL13A, MHCI,  $\beta_2$ m, TAP1, TAP2 and tapasin from RNA of primary DFTD biopsies from wild devils (DFTD\_CS, DFTD\_UBA, DFTD\_Vesna, DFTD\_307). A no cDNA negative control was included in each experiment. Markers show that amplicons are between 100 and 300 bp.



Figure S5. Antigen processing genes are not deleted from the genome of DFTD cells. PCR amplification of RPL13A, MHC class I,  $\beta_2$ m, TAP1 and TAP2, MHC class IIB, MHC class IIA and DMB from DNA of DFTD and fibroblast cells. A no DNA negative control was included in each PCR. Markers show four bands at 400, 300, 200 and 100 bp.

<sup>20</sup> 1 SahaUA\*01 MGSPARALFLLTALAALAETRAGSHSLRYFDTAVSRPGLGEPRFLSVGYVDDQQFVRFDSDSASQ 65 
 STBS2
 A
 G

 STPTD8
 A
 G

 STPTD3
 A
 G

 STPTD4
 A
 G

 3'Fibs1
 G
 G
 65 65 ------3'DFTD7 3'DFTDB6 3'DFTDB3 -----SahaUA\*01 SEEPRAPWMEKVKDVDPGYWEQETQIIKETAQISRVDLQTLRGYYNQSEGGAHTFQRMYGCEVSP 130 130 130 130 130 3'0FTD7 3'DFTDB6 26 3'DFTDB3 -----140 160 180 SahaUA\*01 ELSFQRGFLQFAYDGQDYIALDTETLTWTAAQNEAVNTKRKWEAERSYAERDKAYLEETČVLWVQ 195 ahaUA\*01 ELSFQRGFLQFAYDGQDY1ALDTETLTWTAAQNEAVNTKRKWEAEHSYAERDKAYLEETCVLWVQ 5%bs2 57PFD8 57DFTD3 57DFTD5 57DFTD5 57DFTD4 3%PETD7 144 144 144 5'Fibs4 5'Fibs2 5°DFTD8 5°DFTD3 5°DFTD5 5°DFTD5 5°DFTD4 144 156 

 3'DFTDB3
 156

 280
 \*

 1
 \*

 SahaUA\*01
 PAGDGTFQKWAAVGVPSGQEGRYTCRVQHEGLPEPLTLKWEPESSLPWIIVGVLAAVLLLTAVIA

5'Fibs4 5'Fibs2 

 SFibs2

 5'DFTD8

 5'DFTD5

 5'DFTD5

 5'DFTD4

 3'Fibs1

 3'DFTD7

 S'DETD8

144 221 221 3'DETDB6 ..... 5 <sup>340</sup> **p** <sup>360</sup> SahaUA\*01 GAVVWRKKTSGGKGGDYVPAAGNDSAQGSDVSLTAK 361 
 SFibs2
 144

 S'Fibs2
 144

 S'DFTD8
 144

 S'DFTD8
 144

 S'DFTD5
 144
 5'DFTD4 ------3'Fibs1 ..... 257 3'DFTD7 ..... 3'DETDB6 3'DFTDB3

**Figure S6.** Amino acid alignment of MHC class I transcripts isolated by 3' and 5' **RACE PCRs from DFTD cells (4906) and fibroblast cells**. Transcripts are aligned to Saha\*01, a previously published full length MHC class I transcript (NCBI accession: EF591089). Sequences isolated from fibroblast cells are designated by 5'Fibs or 3'Fibs. Sequences isolated from DFTD cells are designated 5'DFTD or 3'DFTD. The black arrows indicate the sites of the reverse and forward primers used for 5' and 3' RACE respectively. The 5' and 3' transcripts could not be aligned using overlapping sequence due to the amplification of multiple loci and alleles, so 5' and 3' transcripts are given separately in the alignment. The first amino acid of each domain is given by a numeral in grey. A glycosylation site is indicated by brackets, asterisks indicate cysteines expected to be in disulphide bridges, and p indicates a phosphorylation site.

Α				
	5'UTR 2	0 4		
DFTD_Str4	TAGAAAAGGTCTACCCAAG	AGATCCGACAGGGAGCGGAG	acgtctctgtcctcggtcatg	60
Fibs2				60
FIDST		0 10		00
DETD Str4	GCGAACTTCTTGCTAATTG			120
Fibs2				120
Fibs1		GC		120
	14	40 16 I	60 180 I I	
DFTD_Str4	GTCACAAGTCCTCCCAGAG	TCAGGTTTATTCCCGTCAC	CCAGTGGACAGTGACAAGGAT	180
Fibs2 Fibs1				180
	20	00 22	20 240	
DFTD Str4	AACTACATAAATTGTTATG	GTCAGGATTTCACCCACCA	LAGATCACCATTGAGCTTCTA	240
 Fibs2				240
Fibs1		20		240
	20	20 20		
DFTD_Str4 Fibs2	AAGGATGGTGAAAAAATTG	AAAAGTAGAGCGGTCCGATC	CTGTCTTTCAACAATGATTGG	300 300
Fibs1				300
	33	20 34	40 360	
DFTD_Str4	ACCTTCTATCAGCTTGTCT	TGCACCTTTAGAGCGAAACT	тстаааадтдаттттдтттдс	360
Fibs2	•••••			360 360
FIDST	38	30 40		500
DETD Str4	COCOTOGTOCATTCAACCO			420
Fibs2				420
Fibs1				420
	4 3'UTR 4'	40 46 I	60 480 I I	
DFTD_Str4	TAATCTGCATTATGAAGAG	TTGGTGAGTTTGAATATTTT	AAGGCTTCAAGAAATCTAGCT	480
Fibs2				460
	50	0 52	20 540	
DFTD_Str4	TAACCCGAGAGGATTTAAAA	ATCTACCAGGATTTTCAGAT	ттасатттааттдтдаттса	540
Fibs2				540
FIDS1	54	30 55		520
				600
Fibs2				600
Fibs1				580
	62	20 64 I	40 660 I I	
DFTD_Str4	TTTCTTAGTTTTACTTTCT	TGCATATTTGCAAATATTTC	GCAGATATCTCCTTAAGTACA	660
Fibs2				640
	61	30 70	00 720	
DFTD_Str4	TGCATTTGTCATTCAATAT	CTAGACTTTAGATTTTGTAAC	ĠGAATATACATTTTGAGGGGĠ	720
Fibs2				720
FIDS1	74	10 76	60 780	700
DETD Str4	CANATGAATTTCAAAATT			790
Fibs2				780
Fibs1				760
	80	00 8; I	20 840 I I	
DFTD_Str4	TCTTCACAAAGAATGGCTAA	ATTGACTACATTATATTCAAT	TAAACCTCTGAAATTGGCTGA	840
Fibs2				820
	84	50		
DFTD_Str4	ТАААААААААААААААА	AAAAAAAA 869		
Fibs2		865		
Fibs1	CTT.TC.GTGCTC.T			
<b>_</b>				
В		20	40	60
DFTD Str4	MANFLLIALLGOLCIIP	YLEAVTS PPRVOVYS	RHPVDSDKDNYINCYVSGF	HPPQITIEL 60
Fibs2	· · · · · · · · · · · · · · · · · · ·			60
Fibs1	RS			60
ModoB2N			. Y . P S . FL	
	80 I		100 	120 
DFTD_Str4	LKDGEKIEKVERSDLSF	NNDWTFYQLVSAPLERNS	KSDFVCRVVHSTLKEPKVI	KWDPEQN 121
Fibs2				
ModoB2N	IK.M.NQ	SKNLFDP	N.E.A.K.TNV	KDN* 124

Figure S7. Alignment of full length  $\beta$ 2m transcripts from DFTD (4906) and fibroblast cells. A) Nucleotide alignment of  $\beta_2$ m from DFTD (DFTD\_str4) and fibroblast cells (Fibs1 and Fibs2), with the black arrows indicating start and stop codons, B) amino acid alignment of  $\beta_2$ m from DFTD (DFTD\_str4), fibroblast cells (Fibs1 and Fibs2) and the grey short-tailed opossum (*Monodelphis domesticas*, ModoB2m).

А	
Fib B2	من من من المعلم المع المعلم المعلم
 DFTD 1426 B2	۲m
MaeuB2	rmTCT.AT.AA.AC
	-220 -200 -160 -160 -140 X Box I I Y Box I TATA box I I -140 - 140
FID_62	
DFTD_1420_B2	
Maeubz	
Fib_B2	m AAGGGGTCCCTCGCAAGATGAGCGGAGCGT 226
DFTD1426_B2	.m
MaeuB2	m GCT.TT.G.G.TC.TCGCGCCTCTTCCTATTTGCCCTCTTGGGGCATTTGTGCTTCCTTC
Fib_B2	Start site m CTCTGTCCTCGGTCATGCCGAACTTCT 253
DFTD1426_B2	lm
MaeuB2	mT.TGGTCTCGAAA.GGT 342
В	
FTAP1 prm	400
DFTD TAP1 prm	
MaeuTAP1	.TC.AGC
ModoTAP1	CGACCCAGGAC.TGAGCA.CGGTTTAT.AC.A.TTCGG.A.G. 109
FTAP1 prm	320      300      280      260      200
DFTD TAP1 prm	192
MaeuTAP1	
ModoTAP1	AA.G.CAT.G.AGGAGGAGGAGGAGGAGGAGGAGGGC.GAG.ATGCC
ETAP1 pm	Potential X-box      -180      -160      -140      -120        Potential X-box      I
DFTD TAP1 prm	C
MaeuTAP1	.GC.CCCC.CCCCGCACAGTTTGTTGAGGTTATCTGAGACGGCCGCCT.T.TCCA.TCC.T.TCCTCTCC.AGGC.CT
ModoTAP1	CGC.TATT.GCTTC.TGAGCTTCCCAGAGAGGCCGCGTC.TCTA.TCCTG.GCCCTGTCCT.G.G.A.C.CA.AA.TC 315
FTAP1 prm	1 1 1 26-box 1 26-box 1 1 1 1 1 1 1 1 1 1 1 1 1
DFTD TAP1 prm	
MaeuTAP1	CGCC.GG.CTTTA.TTTTG.CGAC.CAGA
ModoTAP1	TGCTCC.A.C.CA.CCGTACTGA.TA.TCATAG.T.GATAT
C	
FTAP2 prm	-540 -480 -440 -440 -440 I I TA ACTGGGAAGATGGGGGGGGGGGGCCATATTTGGGGGGTTTTAA - AGGTGCCCTCCTCCCCCTTCATCGTCCACCATATGTCGGGGGATTTCTCTGCCCCTTCCA 106
DFTD_TAP2_prm	
MaeuTAP2	GGAAAGAAGTAGGATCTTACAAATCCG 110
ETAP2 pro	420 400 380 360 340 1 I Interferon Response Element I A GA A GE CA
DETD TAP2_prim	
MaeuTAP2	
	-320 -300 -280 -260 -220 I I Potential X-box I I I I
FTAP2_prm	AGACGCTTCATTTCTCTGAGCCTCGGTTACCGCAGAAACGGTAGAAATGGTGGGGTTGGACTAAAT-GGTACTTATCCAGGACAGCCCTAATTCCTATGAGCCCAT 321
MaeuTAP2	
mada i Ar Z	
FTAP2_prm	······GACTGGGAACCTACTGATGAGTTTGGGGTTGAGTT-GGGGCTCCTCTGGAAAGGAGGGAAGAGATCTTGG······GTGGTCCTGATTGGAAGT 408
UFID_TAP2_prm	
maeu I AP2	- 100 - 40 - 40 - 40 - 20 1 I I I Startsite
FTAP2_prm	GTA - • TCCTTATATTA - • • • TATCCTTAATATTAT - • CTTAATAATAATG • • • • GTGGTGGTAATGATAATCTTTCTGATTCTTTTACAGACACCAACATGCAGCT 504
DFTD_TAP2_prm	
MaeuTAP2	ACIUC

**Figure S8.** Nucleotide alignment of the  $\beta$ 2m, TAP1 and TAP2 promoter regions from DFTD (1426). A) Nucleotide alignment of the  $\beta_2$ m promoter region from devil fibroblast (Fib\_B2m), DFTD (DFTD\_1426\_B2m) and tammar wallaby (*Macropus eugenii*, MaeuB2m). A putative IRE site, S, X and Y boxes, TATA motif and start codon for the devil  $\beta_2$ m are indicated. **B)** Nucleotide alignment of 362 base pairs upstream from the transcription start site for TAP1 from devil fibroblast (FTAP1\_prm), DFTD (DFTD\_TAP1\_prm), tammar wallaby (MaeuTAP1) and opossum (ModoTAP1). A putative IRE site, potential X box, GC box and transcription start site are highlighted. **C)** Nucleotide alignment of 496 base pairs upstream from the transcription start site for TAP2 from devil fibroblast (FTAP2\_prm), DFTD (DFTD\_TAP2\_prm) and tammar wallaby (MaeuTAP2). A putative IRE site and transcription start site, X box and transcription start site are highlighted.





А					20 I		40 I			60 I			80 I		
	FTAP1prom7	TTTGA	GGGGGAGAG	GTAATT	TTTGGGG	3TTGAGGTTG	TGGTTGG	GAGGAGTG	AGGTAAG	GGGAGGGGT	AAGTTGG	******	TTTTTTTA	AGTTTTAG	95 05
	FIAP1prom11	TTTGA	GGGGGAGAG		TTTCCCC	TIGAGGIIG	TGGTTGG	AGGAGIG		GGGAGGGGT	AAGIIGG	******	TTTTTTT	AGITTAG	95
	ETAP1prom1	TTTCA	GGGGAGAG	31 AA 1 1 3 T A A T T	TTTCCCC		TGGTTGG	AGGAGTG		GGGAGGGGT	AGTTGG	 	TTTTTTTT		95 05
	FTAP1prom2	TTTGA	GGGGGAGAG	STAATI		TTGAGGTTG	TGGTTGG	AGGAGTG		GGAGGGGT	AGTTGO	,,,,,,,,,, ,,,,,,,,,,,	TTTTTTTT		95
	FTAP1prom5	TTTGA	GGGGAGAGA	GTAATT			TGGTTGG	AGGAGTG		GGGAGGGGT	AGTTGG	 	TTTTTTTT		95
	FTAP1prom9	TTTGA	GGGGGAGAG	GTAATT	TTTGGGG	TTGAGGTTG	тааттаа	AGGAGTG	AGGTAAG	GGGAGGGGT	AAGTTGG	ттттттт	TTTTTTTA	AGTTTTAG	95
	FTAP1prom8	TTTGA	GGGGAGAG	GTAATT	TTTGGGG	TTGAGGTTG	TGGTTGG	AGGAGTG	AGGTAAG	GGGAGGGGT	AAGTTGG	тттттт	TTTTTTTA	AGTTTTAG	95
	REF	TTTGA	GGGGGAGAG	GTAATT	TTCGGGG	TTGAGGTTG	тааттаа	AGGAGTG	AGGTAAG	GGGAGGGGT	AAGTCG	тттттт	TTTTTTTA	AGTTTTAG	95
		10	0		12	20		140		1	60		180		
	FTAP1prom7	ATTTA	GGTTTGG	ΑΑΤΤΤΤ	TTGAGTO	GGG <mark>TG</mark> GTTT	TGGTGGG	ATTGAGT	TAAGGAA	ATGCGGTAG	TTTTGA	тттттт	TTTTGGAAA	TTTTTTA 1	190
	FTAP1prom11	ATTTA	GGTTTGG	ΑΑΤΤΤΤ	TTGAGTO	GGGG <mark>TG</mark> GTTT	TGGTGGG	GATTGAGT	TAAGGAA	A TGTGGTAG	TTTTGA	тттттт	TTTTGGAAA	TTTTTTTA 1	190
	FTAP1prom3	ATTTA	GGTTTGG	ΑΑΤΤΤΤ	TTGAGTO	GGGTGGTTT	TGGTGGG	GATTGAGT	FAAGGAA	ATGTGGTAG	TTTT <b>TG</b> A	тттттт	TTTTGGAAA	TTTTTTA 1	190
	FTAP1prom1	ATTTA	GGTTTGG	ΑΑΤΤΤΙ	TTGAGTO	GGGTGGTTT	TGGTGGG	GACTGAGT	TAAGGAA	ATGCGGTAG	TTTTGA	тттттт	TTTTGGAAA	TTTTTTA 1	190
	FTAP1prom2	ATTTA	GGTTTGG	ΑΑΤΤΤΤ	TTGAGTO	GGGTGGTTT	TGGTGGG	GATTGAGT	TAAGGAA	ATGTGGTAG	TTTTGA	тттттт	TTTTGGAAA	TTTTTTA 1	190
	FTAP1prom5	ATTTA	GGTTTGG	AATTTT	TTGAGTO	GGGTGGTTT	TGGTGGG	GATTGAGT	FAAGGAA/	ATGTGGTAG	TTTTTGA	*****	TTTTGGAAA	TTTTTTTA 1	190
	FTAP1prom9	ATTTA	GGTTTGG	AAIIII AATTTT	TTGAGIO	GGGTGGTTT	TGGTGGG	GACIGAGI		ATGTGGTAG	OTTTTCA		TTTTTCCAAA		190
	PTAP Ipromo	ATTTA	GGTTTGG	4 A I I I I A A T T T T	TCGAGTO	GCCCCCTTT	TGGCGGG	ATTGAGT		ACCCCCCTAC		 	TTTTGGAAA		190
	KEF	ATT 14	200	ATTT	GAGIO	220	laacaaa	ATTGAGT	240	ACGCGGTAG	260		TTTTGGAAA		190
	FTAP1prom7	ттттт	TGTTATT	GGGTAA	TEGGT	TOTTOOTO	тттттт		GTTEGTT	OT <b>DI</b> TOTT	GTTATTT			277	
	FTAP1prom11	TTTTT	CGTTATT	GGGTAA	TTGGGTT	GTGGTTTGT	TTTTTT	ATTTTA	GTTTGTT	TCTGTTGTG	GTTATTT	AAGGAAT	TTTTTGGC	à 277	
	FTAP1prom3	ттттт	TGTTATT	GGGTAA	TEGGT	GTGGTTTGT	тттттт	ATTTTA	GTT <b>TG</b> TT	тттөт <b>тө</b> тө	GTTATT	AAGGAAT	TTTTTGGG	277	
	FTAP1prom1	ттттт	TGCTATTO	GGGTAA	TEGGT	GTGGTTTGT	тттттт	ΑΤΤΤΤΤΑ	GTT <b>TG</b> TT	тттөт <b>тө</b> тө	GTTATTT	AAGGAAT	TTTTTGGG	277	
	FTAP1prom2	ттттт	TGCTATTO	GGGTAA	TGGGT	GTGGTTTGT	тттттт	ΑΤΤΤΤΤΑ	GTT <b>TG</b> TT	тттат <b>та</b> та	GTTATTT	AAGGAAT	TTTTTGGG	277	
	FTAP1prom5	сттт	TGTTATTO	GGGTAA	TGGGTT	GTGGTTTGT	тттттт	ΑΤΤΤΤΤΑ	GTT <b>TG</b> TT	TTTGT <b>TG</b> TG	GTTATTT	AAGGAAT	TTTTTGGG	277	
	FTAP1prom9	ттттт	TGTTATT	GGGTAA	TGGGT	GTGGTTTGT	тттттт	ΑΤΤΤΤΤΑ	GTT <b>TG</b> TT	тттат <b>та</b> та	GTTATTT	AAGGAAT	TTTTTGGG	277	
	FTAP1prom8	ттттт	TGTTATTO	GGGTAA	TGGGT	GTGGTTTGT	тттттт	ΑΤΤΤΤΤΑ	GTT <b>TG</b> TT	TTTGT <b>TG</b> TG	GTTATTT	AAGGAAT	TTTTTGGG	i 277	
	REF	ттттт	CGTTATTO	GGGTAA	TCGGGT	GCGGTTCGT	тттттт	ΑΤΤΤΤΤΑ	GTTCGTT	TTTGTCGTG	GTTATTT	AAGGAAT	TTTTTGGG	277	
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	DFTD1426TA	P1prom9	TTTGAGG	GGAGA	GTAATTT	TTGGGGTTG	AGGTTGT	GGTTGGAG	GAGTGAG	GGTAAGGGG	AGGGGTA	AGTTGGT		TTTTTTAAGT	F 90
	DETD1426TAD	P1prom3	TTTCACC	GGAGAG	GIAAIII	TTGGGGTTG	AGGIIGI	GGTTGGAC	GAGIGAG	GGTAAGGGG	AGGGGTP	AGIIGGI			1 90
	DF1D14201AP	PEE	TTTGAGG	GGAGAG	GTAATTI	1 I G G G G T I G/		COTTOCAL		COTAACCCC	ACCCCT	ACTICCI	*******		r 00
			TTTGAGG	GGAGA	GTAATTT	TCCCCCTTC	AGGIIGI	GGTTGGAG	GAGIGAG	GGTAAGGGG		AGTTGGT	****		T 90
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	DFTD1426TA	P1prom1	TTTGAGG	GGAGAGA	GTAATTT TTTGGAA	TCGGGGTTG	AGGTTGT AGGTTGT 120 TGGGGGTG	GGTTGGAC		GGTAAGGGG GGTAAGGGG 40 J GAGTTAAGG	AGGGGTA AGGGGTA AAATGTC	AGTTGG1 AGTCGG1 160 GTAGTT1	TTTTTTTTT TTTTTTTTTT TTGATTTT	TTTTTTAAG TTTTTTAAG1 180 TTTTTTTGGA	T 90 T 90
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**Figure S10. Bisulphite sequencing of the TAP1 promoters in DFTD cells and fibroblast cells. A)** Nucleotide alignment of bisulphite-treated fibroblast DNA from the TAP1 transcription start site to 277 bp upstream with CpG sites highlighted in grey, **B)** Schematic of the methylation pattern of the fibroblast TAP1 promoter. Each CpG site is shown as a box, methylated CpG sites are represented by dark grey boxes, **C)** Nucleotide alignment of bisulphite-treated DFTD DNA from the TAP1 transcription start site to DNA 277 bp upstream with CpG sites highlighted in grey, **D)** Schematic of the methylation pattern of the DFTD TAP1 promoter. Each CpG site is shown as a box, methylated CpG sites are represented by dark grey boxes.

	Leader peptide	20 I	Helix A	40 I	Helix B	60 I	Helix C	80 I	Helix D	3
Saha IFNy	MNYSSYLLASFI	LCVILSSSGCLS	QVNLREDMQT	LHNYFNATKSD\	SDGSSLFMDM	MKTWKEGNC	DKKILMSHV	VAVYFKIFEIF	KNNSIVKRSMEHI	REDMIM-KLF 106
Hosa IFNy	K . T I FQ .	I V . G . L YC	. DPYVKEAEN	. КК GН	A.NGHS.LRH	F ES -	. R MQ . Q I	. SF L . KN.	. DDQSIQK.V.T.	K NVF. 100
Patr IFNy	K . T I FQ .	I V . G . L YC	. DPYVKEAEN	. KK GH	. A . NGT LG I	L.NES-	. R MQ . Q I	. SF L . KN.	. DDQSIQK.V.T.	KNVF. 105
Mumu IFNy	ATHCI LQ .	FLMAV YCI	HGTVI.SLES	. N SSG I	. EEK L . I'	WRN.QKDG-	. M Q. QI	ISF.LRLVL	. D. QAISNNISV.	ESHL.T-TF. 103
Bota IFNy	K.TFLL.	GL.GFSYG	. GQFFREIEN	. KE SSP	. AK.GP SEI	L.NDES-	IQ.QI	. SF L NL	. D . QV I Q D I .	KQFQFL 105
Ptal IFNy	M T I FQ.	G S . YC	. ATFLKEIEN	. KE SN . N .	. A GN L . I	L . N . R . ES -	Q.QI	. SF L NL	. D . P . IQS . VQI .	KLRVF. 106
Gaga IFNy	. TCQT . NLFV . SVIN	AIYYGHTASS.NI	L.Q.QD.IDK	.KADSSH	. A GPIIVEK	L.N.T.R	E.R.IL.QI	. SM. LEML.NT	DKS PHIK	S.ELYTL.NN 106
	Helix E <sup>120</sup>	Helix F	140 I		160 I					
Saha IFNy	PNNTASSVDDFEAL	INTQVNDLKVQRI	KAMFELVYVF	RNLSPKPHLTGF	RRRQNKSQGK	ITQ 167				
Hosa IFNy	SNKKKRK.1	Г.ҮЅ.ТN	IHIQ.M.	AEAAKTGK.	.K.S.MLFR.R	RASQ* 162				
Patr IFNy	SNKKKRK.1	Г.ҮЅ.ТN	IHIQ.M.	AEAVKTGK	. K . S . MLFR . R	RASQ* 167				
Mumu IFNy	- S. SKAKK. A. MSIA	AKFENPQ(	Q.FNIR.V	HQ.L.ESS.RK.	. K . SRC *	156				
Bota IFNy	GSSEKLEKK	.QIP.DQI	INIK.M	NDSN.RK	. K . S L F R . R	RAST* 167				
Ptal IFNy	SNN.KLEKKV	. Q I P NQ T	ISFK.M	TDSNQRK	. K . S . SLFR . W	<b>KA*</b> 166				
Gaga IFNy	LPDGVKK.K.IMD.	AKLPMRI	AN FSIL	QK.VDP.SF-K	. K . S . SQ R	RCNC* 165				

Figure S11. Amino acid alignment of IFN $\gamma$  sequences from Tasmanian devil and other species. Amino acid alignment comparing the devil IFN $\gamma$  sequence used for expression of protein with IFN $\gamma$  sequences from other vertebrate species. Saha – Sarcophilus harissi (Tasmanian devil), Hosa – Homo sapien (human), Patr - Pan troglodytes (chimpanzee), Mumu – Mus musculus (mouse), Bota – Bos tauros (cow), Ptal - Pteropus alecto (black flying fox, a megabat), Gaga – Gallus gallus (chicken). Identical residues are represented as a dot, gaps are represented as a dash and stop codons (when present in the alignment) are represented by a star. Helices are indicated by text.



**Figure S12. Western blots illustrating specificity of the MHC class I monoclonal antibody. A)** Western blot on whole cell detergent lysates from DFTD cells either transfected with an MHC class I construct (*Saha-UB*) or mock transfected with no DNA, and probed with MHCI-mAb clone TD50. A specific band is present around 40 kDa only in the transfected DFTD cells. **B)** Western blot on whole cell detergent lysates from fibroblast cells either untreated or deglycosylated with Peptide N-glycosidase F and then probed with MHCI-mAb antibody clone TD50. A band is present around 40 kDa in the untreated fibroblast cell lysates, which drops to around 37 kDa in the deglycosylated lysate (indicating that the protein recognised has a single N-linked glycan as expected based on devil class I sequences). **C)** Western blot on whole cell detergent lysates from fibroblast cells probed with MHCI-mAb clone TD50 directly or pre-blocked with the peptide used for mouse immunisations. A band is present around 40 kDa in the untreated fibroblast cell lysates, but is not present when the MHCI-mAb is blocked with the immunogen (indicating that specific Ab in the tissue culture supernatants is reacting with the protein in the lysate).

# Supplementary Tables

GENE	SEQUENCE (5'-3')
RPL13A F	CCCCACAAGACCAAGCGAGGC
RPL13A R	ACAGCCTGGTATTTCCAGCCAACC
MHClex1F	CCGTGGGCTACGTGGACGATCAGC
MHClex2R	GTCGTAGGCGAACTGAAG
TAP2ex6F	TGTGGGCTAAGGCAGATTCTGG
TAP2ex7R	ATTCCCAGGAGGAGCTAAGCG
TAP1ex2F	ACAGACTGGATCCTGCAGGATGAAG
TAP1ex4R	GAGACGTGATAGCACCTGTTTGG
B2Mex1F	TGTGCATCCTTCCCTACCTGGAGG
B2Mex2R	CATTGTTGAAAGACAGATCGGACCGC
Tapasin ex3F	AGCCTCTTGCAGCTGTCTCAGTCC
Tapasin ex4R	TGGCCACCCAGTCCTGGAGTCAC
CIITAF	ACCCTTGTCCAACTTGGTTGTGTTACC
CIITAR	ACCAGGCTACAAAGGTCCTCTACATCC
Class IIb2F	AGCATTGGAACAGCCAGAAGG
Class IIb3R	AGAAACCGCTGACGGAGCAGACAAGC
Class IIa3F	CTAAGAGCCCAGCGGAGATGG
Class IIa4R	AGACCACAGTCTCTGTTGTCTCTGG
DMBF	CGAATATTGCATCTCCTTCAACAAGG
DMBR	ATAGAAGCCCCATACATAACAGGCC

Supplementary Table 1. Primers used for RT-PCR on cDNA

# Supplementary Table 2. Primers used for PCR on DNA

GENE	SEQUENCE (5'-3')
MHClex1.1F	GGAGCCGCGGGCGGCGTGG
MHClex1.1R	CCTGGCTCTGGTTGAAGTAGCC
TAP2 ex5F	TGTGGGCTAAGGCAGATTCTGGCAGGG
TAP2 ex6R	TCCCAGGAGGAGCTAAGCGTGG
TAP1 ex7F	TGCCTCAGTATCAGCACCGGTATCTGC
TAP1 ex8.1R	CTTCCATGACCTCCTCTAGCTCTGG
B2M ex2.1F	CTCCCAGAGTTCAGGTTTATTCCC
B2M ex2.1R	AGGTTCTTTGAGGGTTGAATGGACC
Tapasin ex3F	AGCCTCTTGCAGCTGTCTCAGTCC
Tapasin ex4R	TGGCCACCCAGTCCTGGAGTCAC
CIITAF	ACCCTTGTCCAACTTGGTTGTGTTACC
CIITAR	ACCAGGCTACAAAGGTCCTCTACATCC
Class IIb2.1F	GACATAGCCCAGAGCACTTCACG
Class IIb2.1R	GCCTGCGCACTAAGAAGGACTCA
Class IIa2.1F	CCTCCCGTACTTAATGTGACATGGC
Class IIa2.1R	TCATAAGAGGTTGTTCCAGTCCCC
DMBF	CGAATATTGCATCTCCTTCAACAAGG
DMBR	TGACTGGCACAATCCTGGAGTCC
B2m promoter F	ATCGTGCCTAAGCGTTTGAGAAGGCGA
B2m promoter R	AGGAGAAGCCGGCCAGTGGAGC
TAP1 promoter F	TCTGAGGGGAGAGCAACTTTCGGG

TAP1 promoter R	GTTGGAGAGATGCGAGAGACTTCG
TAP2 promoter F	GAGCACTCAGTACTAGGAAGCGA
TAP2 promoter R	AAGCAGGATCAGGTCAGTCAGG

Supplementary Table 3. Primers used for bisulphite sequencing

GENE	SEQUENCE (5'-3')
B2m F	ATTTATATGAGGTTTGGTAGTTTTTAAGTA
B2m R	CAAAATAAAACAATTAACAAAAAATTC
TAP1 F	AAGTTTTTTTGAGGGGAGAGTAATT
TAP1 R	ACCCAAAAAAATTCCTTAAATAACC

GENE	BUFFER	MgCl <sub>2</sub> (mM)	dNTPS (µM)	PRIMERS (mM)	ENZYME	TEMPLATE	ANNEALING TEMP
RT-PCR	1		I	I			
RPL13A	1x	3	200	0.6	Phusion (NEB)	100	60°C
class I	1x	3	200	0.6	Phusion (NEB)	100	60°C
B2m	1x	3	200	0.6	Phusion (NEB)	100	60°C
TAP1	1x	3	200	0.4	Phusion (NEB)	200	61°C
TAP2	1x	3	200	0.2	Phusion (NEB)	200	59°C
Tapasin	1x	3	200	0.6	Phusion (NEB)	100	61°C
CIITA	1x	3	200	0.4	Phusion (NEB)	200	60°C
Class IIB	1x	3	200	0.5	Phusion (NEB)	200	61°C
DMB	1x	3	200	0.5	Phusion (NEB)	200	62ºC
Class IIA	1x	3	200	0.4	Phusion (NEB)	200	61°C
DNA PCF	R	I	1		l	1	
28S	1x	1.4	200	0.6	Taq (Invitrogen)	100	60°C
Class I	1x	1.4	200	0.6	Taq (Invitrogen)	100	60°C
B2m	1x	1.4	200	0.6	Taq (Invitrogen)	100	60°C
TAP1	1x	1.4	200	0.6	Taq (Invitrogen)	100	60°C
TAP2	1x	1.4	200	0.6	Taq (Invitrogen)	100	60°C
Amplifica	tion of p	romote	ers	1	1		
B2m	1x	1.4	200	0.6	Taq (Invitrogen)	100	60°C
TAP1	1x	1.4	200	0.6	Taq (Invitrogen)	100	60°C

# Supplementary Table 4. Amplification reactions and conditions for PCR

TAP2	1x	1.4	200	0.6	Taq (Invitrogen)	100	60°C
Amplifica	tion of bi	isulpha	ate treate	d DNA			
B2m	1x	2	200	0.6	Expand HF (Roche)	100	58.5°C
TAP1	1x	2	200	0.6	Expand HF (Roche)	100	58.5 °C

# Supplementary Table 5. Antibodies

ANTIBODY	CLONE OR CAT NUMBER	SUPPLIER (WHERE
		APPLICABLE)
Periaxin	HPA 001868	Sigma
β-actin	Clone AC-15 (A1978)	Sigma
CD3ɛ	A0452	Dako
MHC class I	TD50	NA (this paper)
B2m	polyclonal	NA (this paper)