

## Supplementary Material

### Post-release immune responses of Tasmanian devils vaccinated with an experimental devil facial tumour disease vaccine

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**Table S1. Sex, year of birth, house names and microchip numbers of all the individual Tasmanian devils, and origin of the vaccinated devils referred to in the manuscript.**  
 NA = not applicable (incumbent devils In1-In9, by definition, were wild born)

<b>Devil identification in manuscript</b>	<b>Sex</b>	<b>Year of birth</b>	<b>House name</b>	<b>Microchip number</b>	<b>Captive (C) or wild (W) born</b>
<b>Va1</b>	M	2013	Akaroa	982000153632053	C
<b>Va2</b>	M	2014	Askja	982000191016491	W
<b>Va3</b>	M	2014	Geysir	982000123208479	W
<b>Va4</b>	F	2013	Guernsey	982000191016028	W
<b>Va5</b>	M	2013	Macca	982000191010136	W
<b>Va6</b>	M	2014	Moffett	982000123209291	W
<b>Va7</b>	F	2012	Nutella	982009106163346	C
<b>Va8</b>	F	2014	Ventoux	982000363283037	W
<b>In1</b>	M	2015	Brimstone	982000405800521	NA
<b>In2</b>	M	2016	Clay	982000405978328	NA
<b>In3</b>	F	2016	Lapis	982000363454835	NA
<b>In4</b>	M	2016	Mercury	982000405977541	NA
<b>In5</b>	M	2016	Obsidian	982000405977892	NA
<b>In6</b>	M	2015	Pumpkin	982000405978897	NA
<b>In7</b>	F	2016	Ruby	982000405800801	NA
<b>In8</b>	F	2015	Zuzanna	982000363410824	NA
<b>In9</b>	F	2013	Tilly	982000405949360	NA

**Table S2. Summary of the Animal Health Laboratory\* histopathology reports for the DFT1 biopsies collected from the individual devils referred to in the manuscript. Vaccinated devils, Va1 to Va8; and incumbent devils, In1 to In8.**

\* Animal Health Laboratory, Department of Primary Industries, Parks, Water and the Environment, Prospect, Tasmania, 7250

<b>Devil ID</b>	<b>Date of tumour biopsy</b>	<b>Number of mitotic figures per high power field</b>	<b>Comments</b>
<b>Va4</b>	Aug 2018	Rare	Moderate anisokaryosis & karyomegaly
<b>Va5</b>	Sep 2017	2	Minimal pleomorphism
<b>Va6</b>	June 2017	Rare	Mild anisocytosis and anisokaryosis
	Sep 2017	1-3	Mild to moderate anisokaryosis and karyomegaly, moderate pleomorphism
<b>Va7</b>	June 2017	1-2	Mild anisocytosis and anisokaryosis
	Sep 2017	2	Pleomorphism increased compared to June 2017
	Feb 2018	1-2	Mild to moderate anisokaryosis and karyomegaly
<b>In1</b>	Feb 2018	1-2	Mild to moderate anisokaryosis and karyomegaly
<b>In2</b>	May 2018	1-2	Mild to moderate anisokaryosis and karyomegaly
<b>In3</b>	May 2018	1-2	Mild to moderate anisokaryosis and karyomegaly
<b>In4</b>	May 2018	1-2	Mild to moderate anisokaryosis and karyomegaly
<b>In5</b>	May 2018	1-2	Mild to moderate anisokaryosis and karyomegaly
<b>In6</b>	Feb 2018	1-2	Mild to moderate anisokaryosis and karyomegaly
<b>In7</b>	Feb 2018	1-2	Mild to moderate anisokaryosis and karyomegaly
	May 2018	1-2	Mild to moderate anisokaryosis and karyomegaly Extensive inflammation expanding the connective tissue between nodules
<b>In8</b>	Feb 2018	1-2	Mild to moderate anisokaryosis and karyomegaly Apoptotic tumour cells in centre of multiple nodules

**Table S3. Summary of immunohistochemistry analysis of DFT1 biopsies for devils trapped once, showing the average number of tumour infiltrating cells with standard deviations. The number in brackets is the number of high powered fields counted for each tumour. Note not all biopsies had enough tumour tissue to count 10 fields\*. Vaccinated devils, Va4 and Va5; and incumbent devils, In1-In6, In8.**

NA = not available

\*Method according to (Zhang et al., 2003)

Devil ID	Tumour	IHC stain		
		CD3	MHC-II	PD-1
<b>Va4</b>	<b>T1</b>	11 ± 5 (3)	17 ± 9 (2)	9 (1)
<b>Va5</b>	<b>T1</b>	15 ± 6 (9)	20 ± 8 (8)	2 ± 2 (8)
	<b>T2</b>	14 ± 11 (6)	16 ± 6 (4)	4 ± 4 (4)
<b>In1</b>	<b>T2</b>	2 ± 1 (10)	5 ± 3 (8)	0 (5)
<b>In2</b>	<b>T1</b>	3 ± 1 (8)	4 ± 2 (7)	0 (5)
	<b>T4</b>	1 ± 1 (6)	7 ± 2 (6)	NA
<b>In3</b>	<b>T1</b>	0 (8)	2 ± 2 (7)	0 (5)
<b>In4</b>	<b>T1</b>	1 ± 1 (8)	1 ± 1 (7)	0 (5)
<b>In5</b>	<b>T1</b>	0 (8)	3 ± 3 (8)	0 (5)
<b>In6</b>	<b>T2</b>	2 ± 2 (7)	6 ± 2 (6)	3 ± 2 (6)
<b>In8</b>	<b>T1</b>	2 ± 1 (8)	10 ± 3 (7)	0 (5)

**Table S4. Summary of the immunohistochemistry analysis of tumours biopsied from vaccinated devils, Va6 and Va7, and incumbent devil, In7, on sequential monitoring trips, showing the degree of immune cell infiltration in the tumour biopsies.**

T1: tumour 1; T3: tumour 3

NA: not applicable

Intratumoural immune cells graded as 0, +, ++, or +++ (0, 1-5, 6 to 19, or  $\geq 20$  cells per high-power field, respectively) (Zhang et al., 2003)

Biopsy date		June 2017			Sep 2017			Feb 2018			May 2018		
Immunohistochemistry stain		CD3	MHC II	PD1	CD3	MHCII	PD1	CD3	MHCII	PD1	CD3	MHC II	PD1
<b>Va6</b>	T1	0	+	0	+	+	0	Not trapped			Not trapped		
<b>Va7</b>	T1	+	+	0	++	++	0	+++	+++	++	Not trapped		
	T3	NA	NA	NA	NA	NA	NA	++	++	+			
<b>In7</b>	T1	Not trapped			Not trapped			+	+	0	++	++	++

**Table S5. Summary of immunohistochemistry analysis of tumours biopsied from vaccinated devils, Va6 and Va7, and incumbent devil, In7, on sequential monitoring trips showing the average number of infiltrating tumour cells with standard deviations. The number in brackets is the number of high powered fields counted for each tumour\*. Note not all biopsies had enough tumour tissue to count 10 fields.**

NA = not available

\*Method according to (Zhang et al., 2003)

Biopsy date	06/17			09/17			02/18			05/18			
IHC stain	CD3	MHC II	PD1	CD3	MHCII	PD1	CD3	MHCII	PD1	CD3	MHC II	PD1	
<b>Va6</b>	<b>T1</b>	0 (6)	3 ± 2 (5)	NA	1 ± 1 (8)	5 ± 5 (6)	0 (5)	Not trapped again					
<b>Va7</b>	<b>T1</b>	2 ± 2 (10)	5 ± 2 (10)	NA	10 ± 6 (10)	13 ± 6 (8)	NA	31 ± 4 (5)	33 ± 9 (6)	15 ± 1 (3)	Not trapped again		
	<b>T3</b>	NA	NA	NA	NA	NA	NA	8 ± 3 (6)	13 ± 3 (5)	4 ± 2 (3)			
<b>In7</b>	<b>T1</b>	Not trapped until 02/18						1 ± 1 (8)	5 ± 4 (7)	NA	12 ± 10 (8)	19 ± 5 (9)	8 ± 2 (5)

**Table S6. Summary of the serum antibody responses against MHC-I<sup>-ve</sup> and MHC-I<sup>+ve</sup> DFT1 cells of all vaccinated devils (Va1 to Va8), and of the incumbent devils that had a positive result (In1, In8, In9). Results are shown for the serum samples collected on the last date the devils were trapped post-release. Antibody responses were classified as negative ‘-’ (MFIR < 1.5), or positive ‘+’ (MFIR ≥ 1.5) (Pye et al., 2018).**

<b>Devil ID</b>	<b>Sex; age (in years) at time of sample collection</b>	<b>DFT1 status, date of DFT1 diagnosis (or, if healthy, date last trapped)</b>	<b>Serum antibodies against MHC-I<sup>-ve</sup> DFT1 cells</b>	<b>Serum antibodies against MHC-I<sup>+ve</sup> DFT1 cells</b>
<b>Va1</b>	M 4y	Healthy 09/17	+	+
<b>Va2</b>	M 3y	DFT1 07/17	+	+
<b>Va3</b>	M 3 y	Healthy 09/17	+	+
<b>Va4</b>	F 5 y	DFT1 08/18	-	+
<b>Va5</b>	M 4y	DFT1 09/17	+	+
<b>Va6</b>	M 3y	DFT1 07/17	+	+
<b>Va7</b>	F 5y	DFT1 07/17	+	+
<b>Va8</b>	F 3y	DFT1 Trapped healthy 07/17. Found dead with DFT1 08/18	+	+
<b>In1</b>	M 3y	DFT1 02/18	+	+
<b>In8</b>	F 3y	DFT1 02/18	-	+
<b>In9</b>	F 5y	Healthy 03/19	-	+

**Table S7. Summary of the age, sex and DFT1 status of the 76 incumbent devils that were tested for serum antibody against DFT1 cells. Three devils (In1, In8, In9) out of the 76 devils were positive for serum antibody and these are referred to in Tables S2 and S8**

Age	Healthy		DFT1	
	Male	Female	Male	Female
<b>Subadult (&lt;2yo)</b>	26	26	2	2
<b>Adult (≥2yo)</b>	4	6	4	6

## References

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