

# Cytometric methods for developing and validating a Tasmanian devil facial tumour vaccine

Australasian Cytometry  
Society 2024

A/Prof Andrew Flies

\*Dr Ruth Pye

\*Dr Chrissie Ong

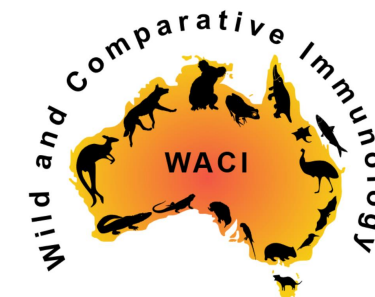
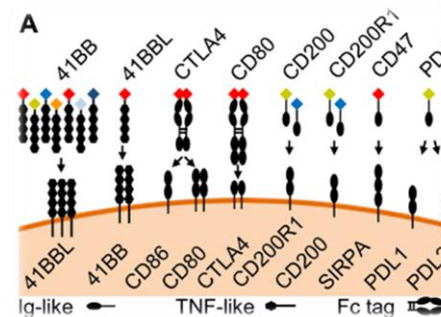
Menzies Institute for Medical Research

University of Tasmania

 @WildImmunity

 WildImmunity.com

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# Two independent transmissible cancers

**nature**

Published: 01 February 2006

Allograft theory

## Transmission of devil facial-tumour disease

[A.-M. Pearse](#) & [K. Swift](#)

- Cancer
- Allograft
- Infectious disease
  - biting
  - mating



Ruth Pye

DFT1



Image credit: C Baars



Image credit: AS Flies

## A second transmissible cancer in Tasmanian devils

Ruth J. Pye<sup>a</sup>, David Pemberton<sup>b</sup>, Cesar Tovar<sup>a</sup>, Jose M. C. Tubio<sup>c</sup>, Karen A. Dun<sup>d</sup>, Samantha Fox<sup>b</sup>, Jocelyn Darby<sup>a</sup>, Dane Hayes<sup>e</sup>, Graeme W. Knowles<sup>e</sup>, Alexandre Kreiss<sup>a</sup>, Hannah V. T. Siddle<sup>f</sup>, Kate Swift<sup>g</sup>, A. Bruce Lyons<sup>g</sup>, Elizabeth P. Murchison<sup>c,1,2</sup>, and Gregory M. Woods<sup>a,1,2</sup>

<sup>a</sup>Menzies Institute for Medical Research, University of Tasmania, Hobart, TAS 7000, Australia; <sup>b</sup>Save the Tasmanian Devil Program, Tasmanian Department of Primary Industries, Parks, Water and the Environment, Hobart, TAS 7000, Australia; <sup>c</sup>Department of Veterinary Medicine, University of Cambridge, Cambridge CB3 0ES, United Kingdom; <sup>d</sup>Department of Cyto genetics, Royal Hobart Hospital, Hobart, TAS 7000, Australia; <sup>e</sup>Mount Pleasant Laboratories, Tasmanian Department of Primary Industries, Parks, Water and the Environment, Prospect, TAS 7250, Australia; <sup>f</sup>Centre for Biological Science, University of Southampton, Highfield Campus, Southampton SO17 1BJ, United Kingdom; and <sup>g</sup>School of Medicine, University of Tasmania, Hobart, TAS 7000, Australia

Edited by Stephen P. Goff, Columbia University College of Physicians and Surgeons, New York, NY, and approved November 30, 2015 (received for review

DFT2

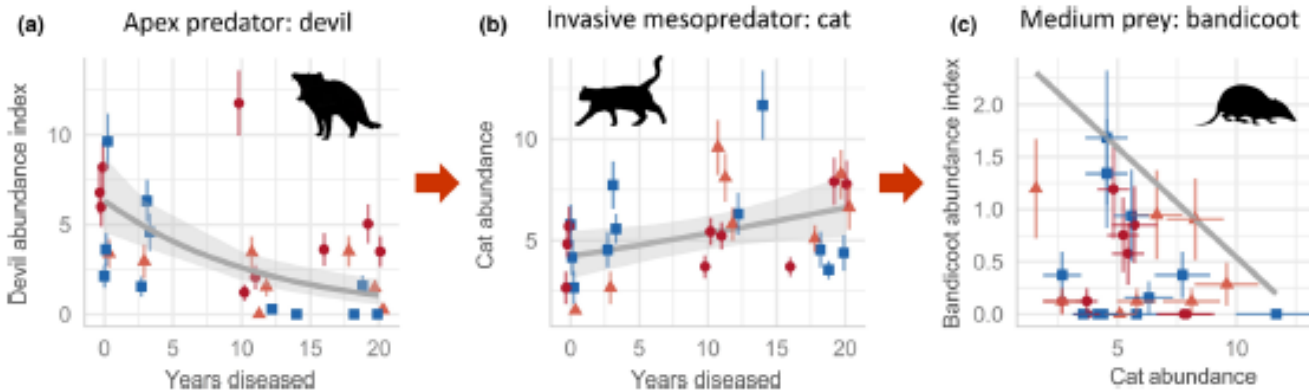
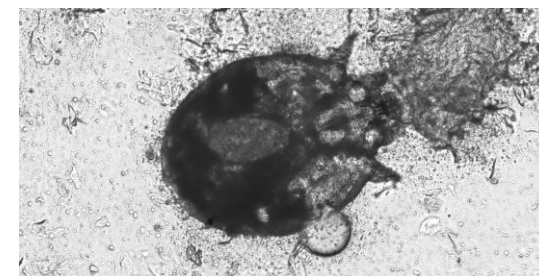
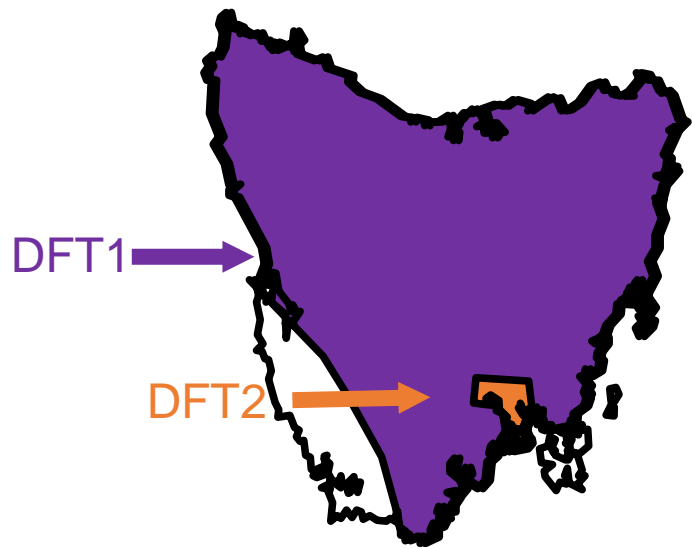
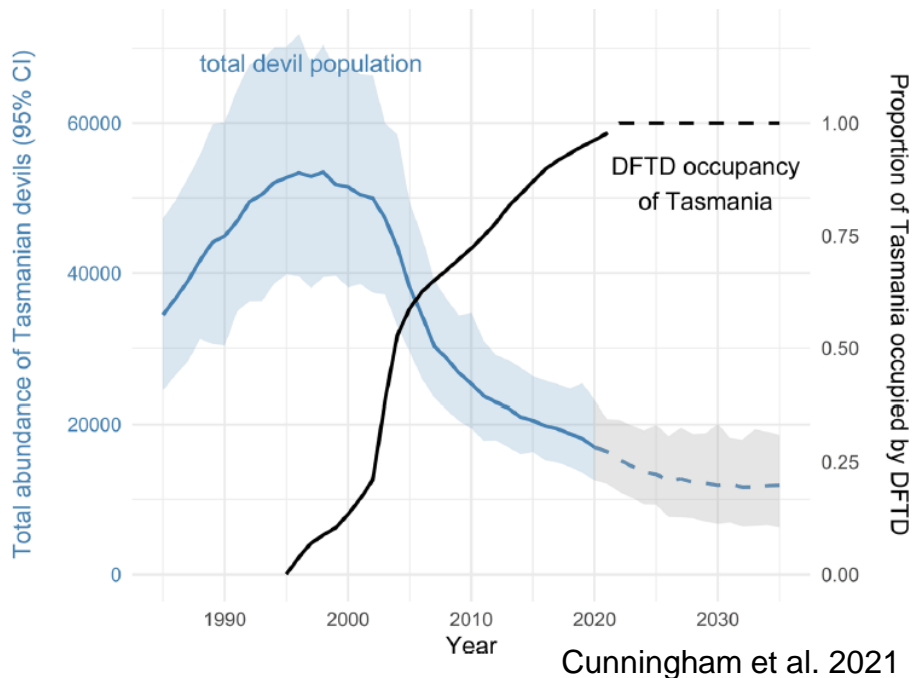


Image credit: AS Flies



Image credit: GG Russell

# Devil facial tumour (DFT) disease



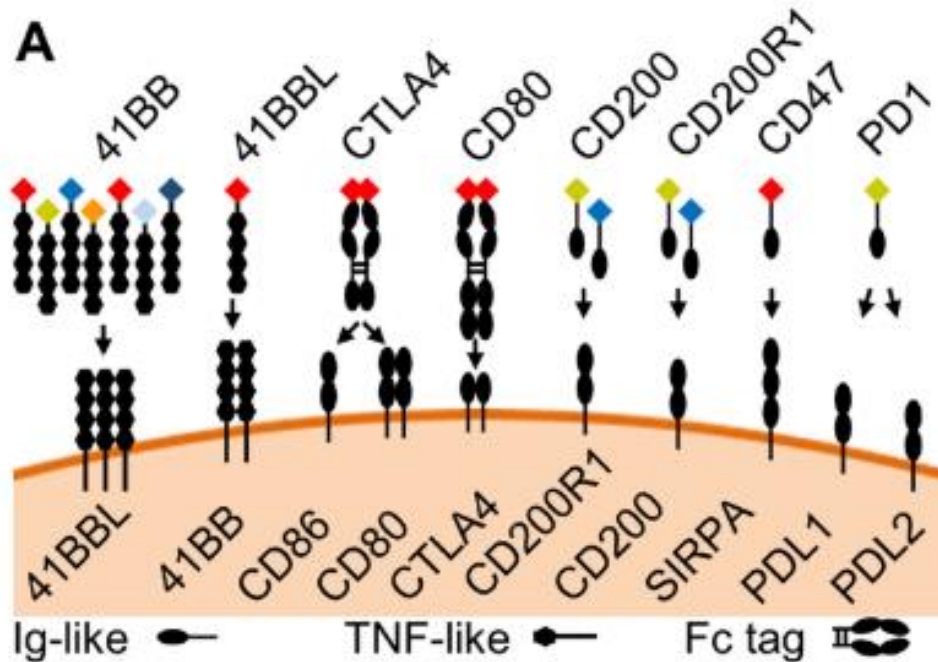
Cunningham et al. 2020

# Mapping immune checkpoint interactions

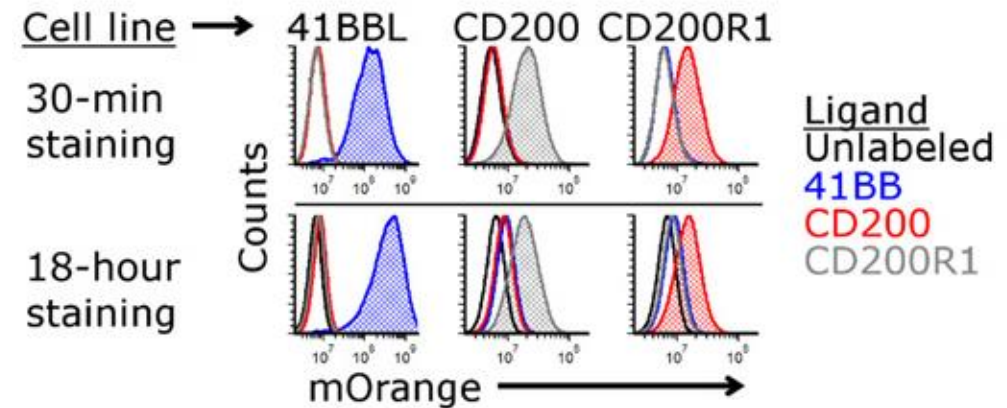
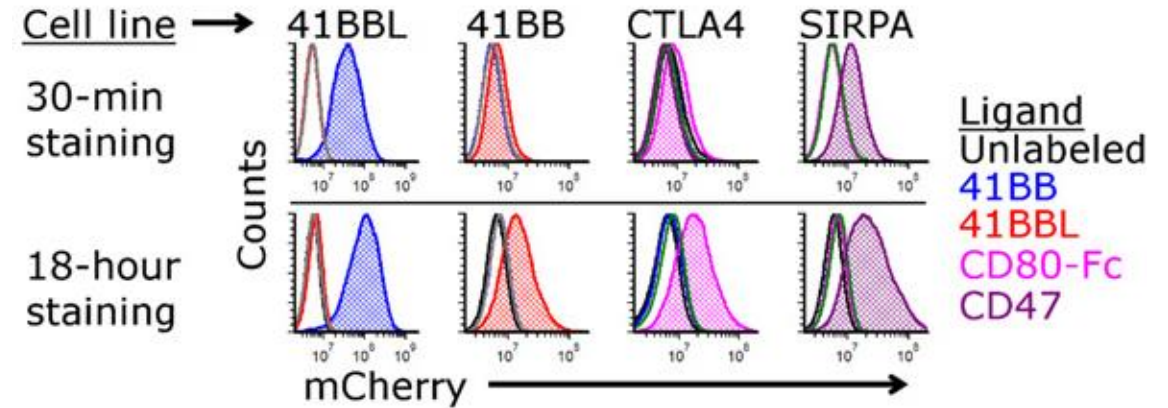
A novel system to map protein interactions reveals evolutionarily conserved immune evasion pathways on transmissible cancers

Andrew S. Files<sup>1,\*</sup>, Jocelyn M. Darby<sup>1</sup>, Patrick R. Lennard<sup>1,2,†</sup>, Peter R. Murphy<sup>1,2,†</sup>, Chrissie E. B. Ong<sup>1</sup>, Terry L. Pinfeld<sup>2</sup>, Alana D...

Science Advances 01 Jul 2020  
Vol. 6, no. 27, eaba5031  
DOI: 10.1126/sciadv.aba5031



## B

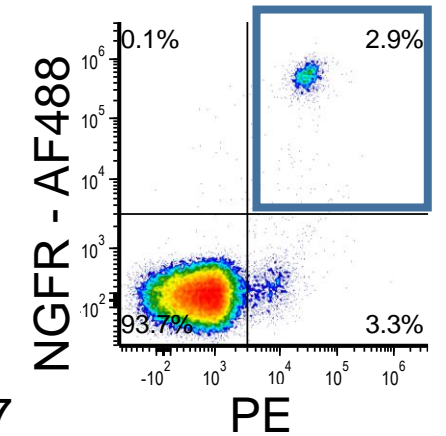
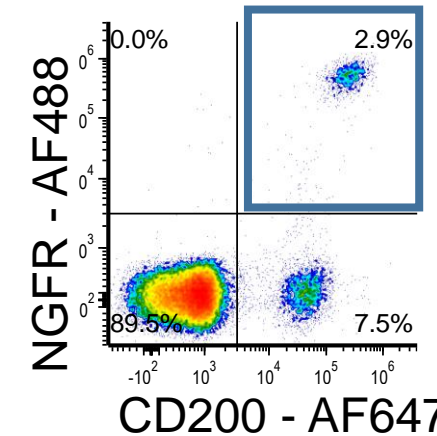
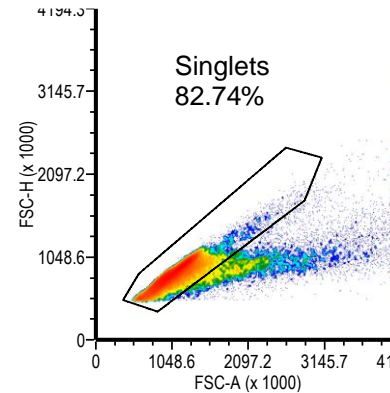
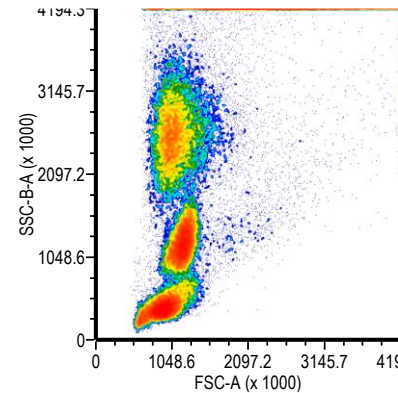


# Sorting circulating tumour cells from blood and biopsies?

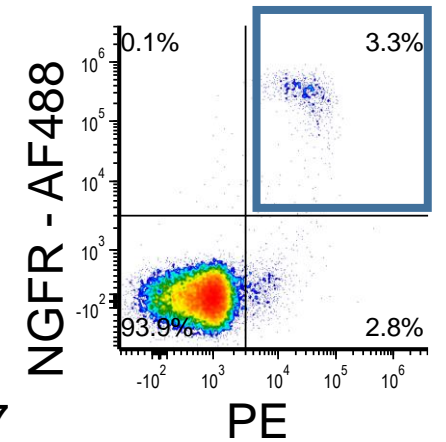
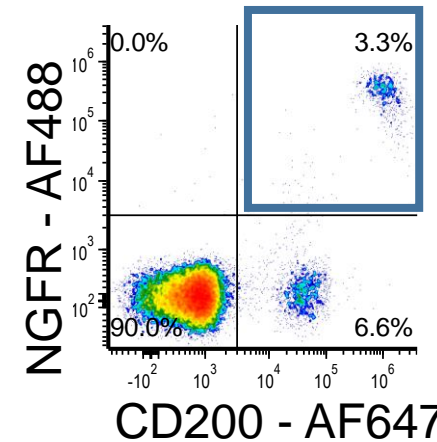
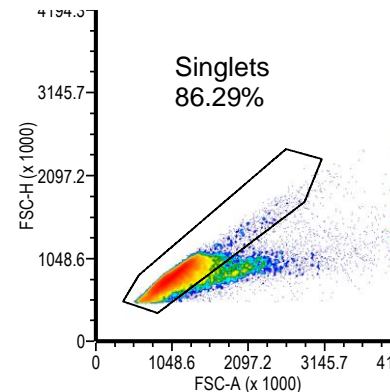
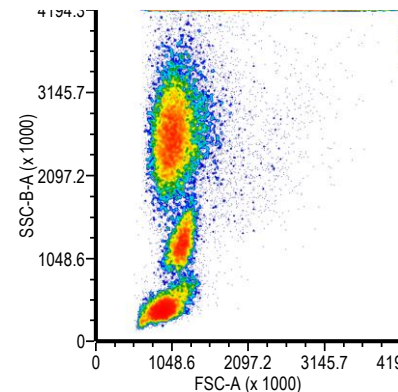


- DFT1 or DFT2 cells spiked into whole blood
- Stained with 3 antibodies
- Red blood cells lysed
- DFT1 and DFT2 sorted from blood

DFT1



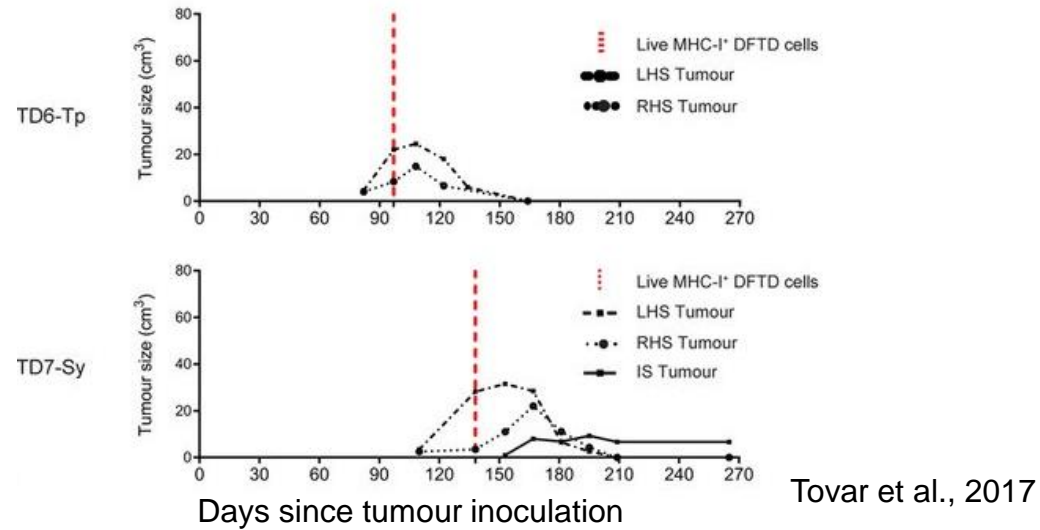
DFT2



Anuk Kruawan

# Devil immune system can kill DFT1 cells

## Captive devils with DFT1 regressions



## Wild devil with DFT1 regression

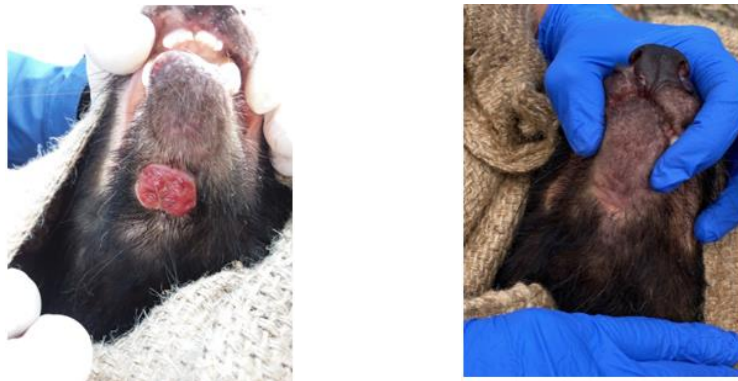
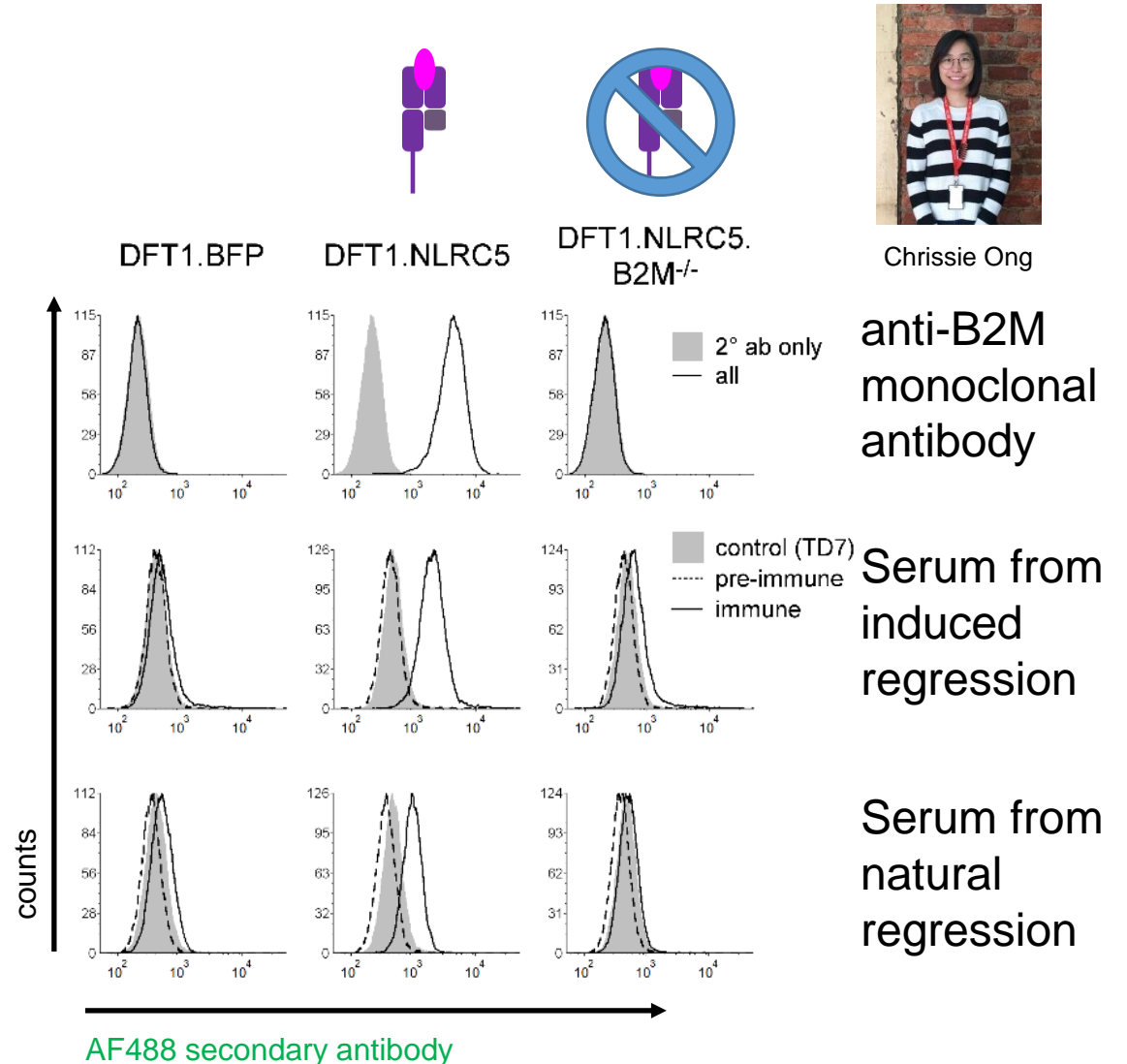


Image credit: NRE Tasmania STDP



Adapted from Ong et al., 2021

# How do you vaccinate a wild devil?



# We need a prophylactic vaccine that can be delivered in edible baits

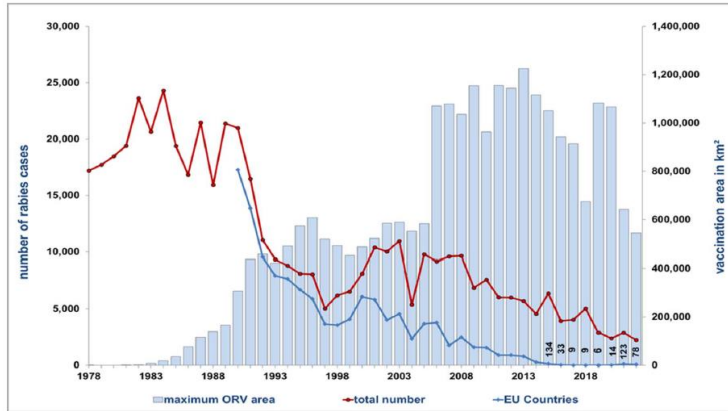


FIGURE 1. Development of rabies cases and vaccination areas in Europe between 1978 and 2022 (bat rabies cases not included). The total number of rabies cases for 2015–2022 is an approximation that may be underestimated because of irregular or missing data reporting from Belarus, Russia, and Ukraine, where rabies virus (RABV) remains endemic and is not efficiently controlled. For better visualization RABV cases in EU countries for the years 2015–2022 are indicated by numbers.

Rupprecht et al., 2024





# Human adenovirus can transduce devil facial tumour cells and express transgenes

JOURNAL OF  
GENERAL VIROLOGY

Volume 103, Issue 11

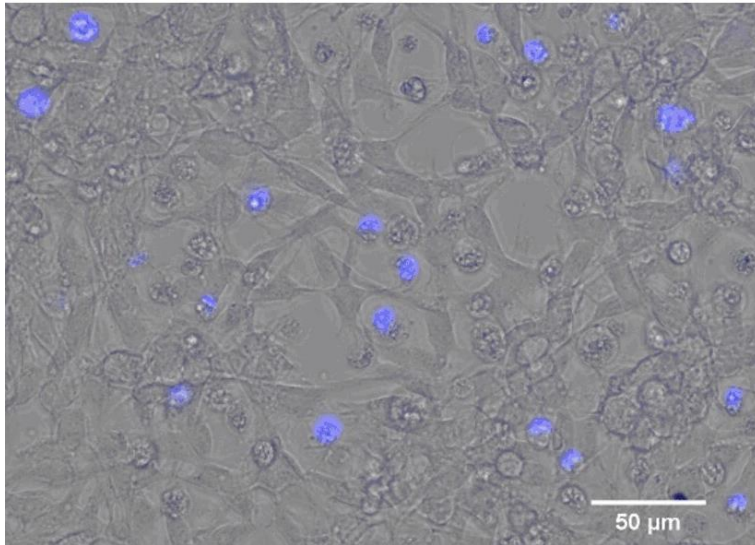
Short Communication | Open Access

**A human adenovirus encoding IFN- $\gamma$  can transduce Tasmanian devil facial tumour cells and upregulate MHC-I**

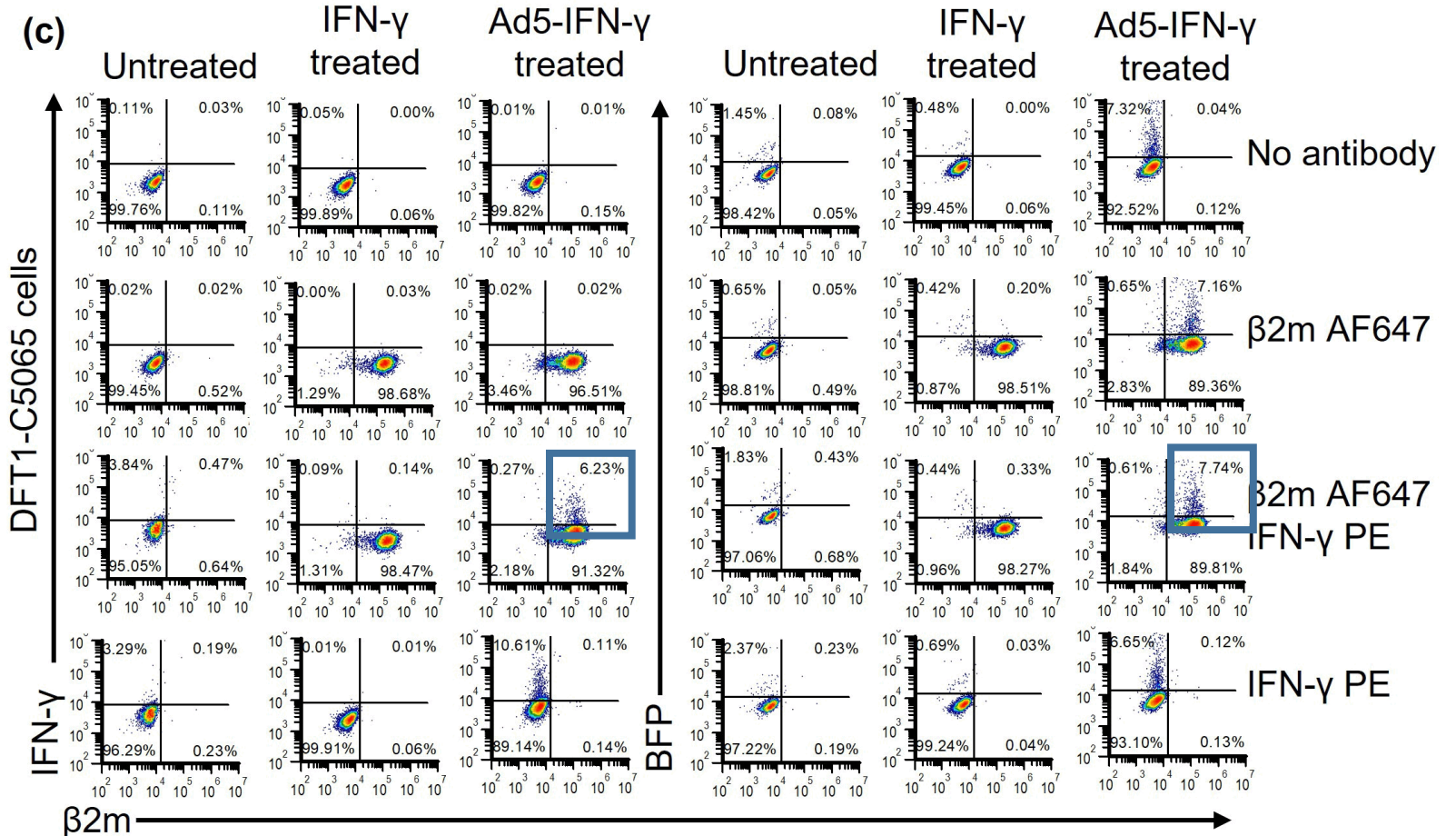
Ahab N. Kayigwe<sup>1,2</sup>, Jocelyn M. Darby<sup>1</sup>, A. Bruce Lyons<sup>3</sup>, Amanda L. Patchett<sup>1</sup>, Leszek Lisowski<sup>4,5</sup>, Guei-Sheung Liu<sup>1,6</sup>  
 and Andrew S. Flies<sup>1</sup>

View Affiliations

Published: 16 November 2022 | <https://doi.org/10.1099/jgv.0.001812>



Ahab Kayigwe

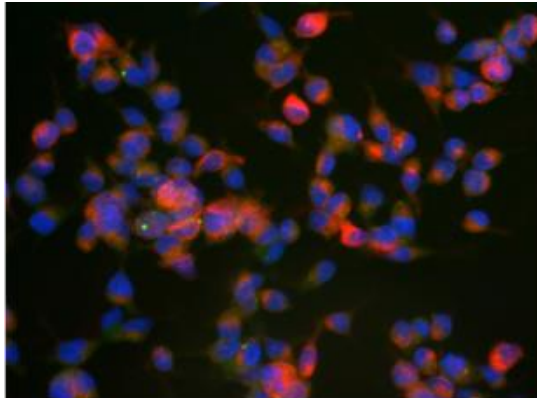


Need a different approach for a prophylactic vaccine

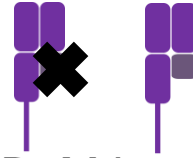
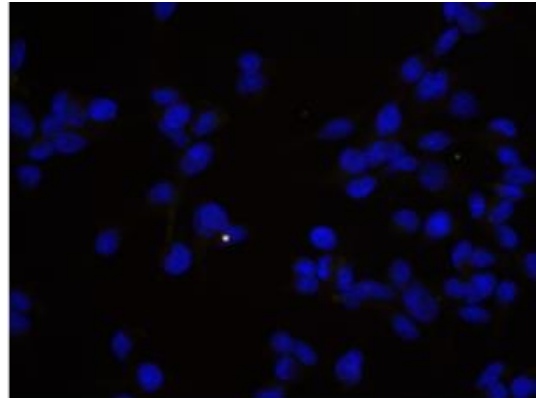
# Vector-encoded MHC-I and B2M



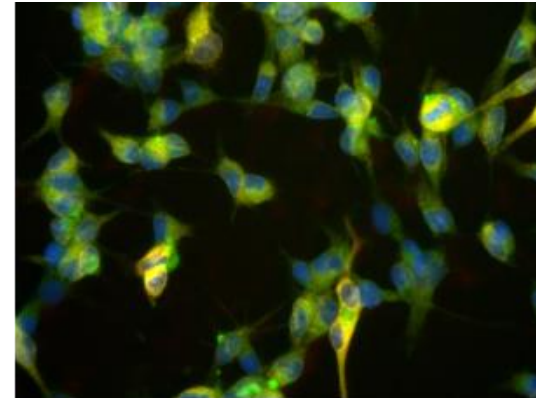
DFT1



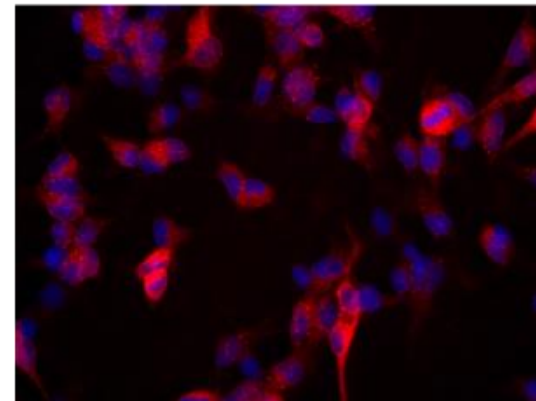
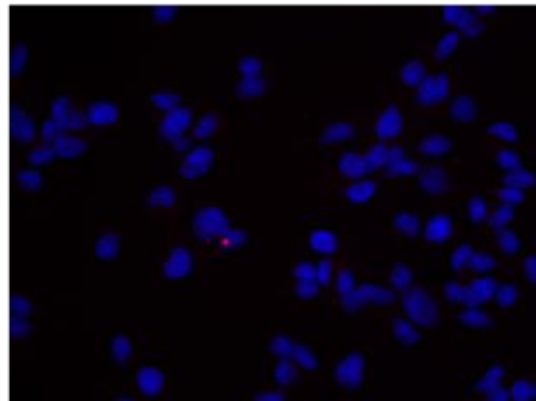
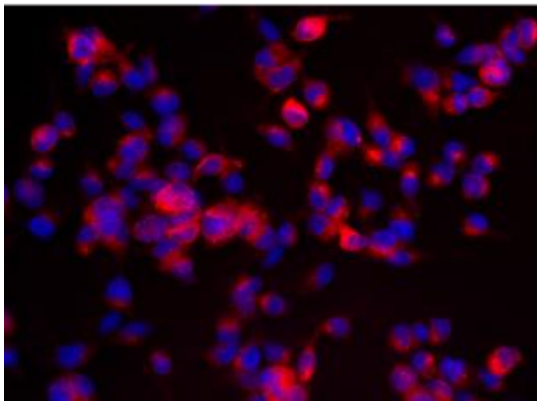
DFT1 B2M knockout



DFT1 B2M knockout  
+ B2M & MHC-I knock-in



Blue – DAPI  
Red – B2M Alexa 594  
Green – pAF350 B2M GFP



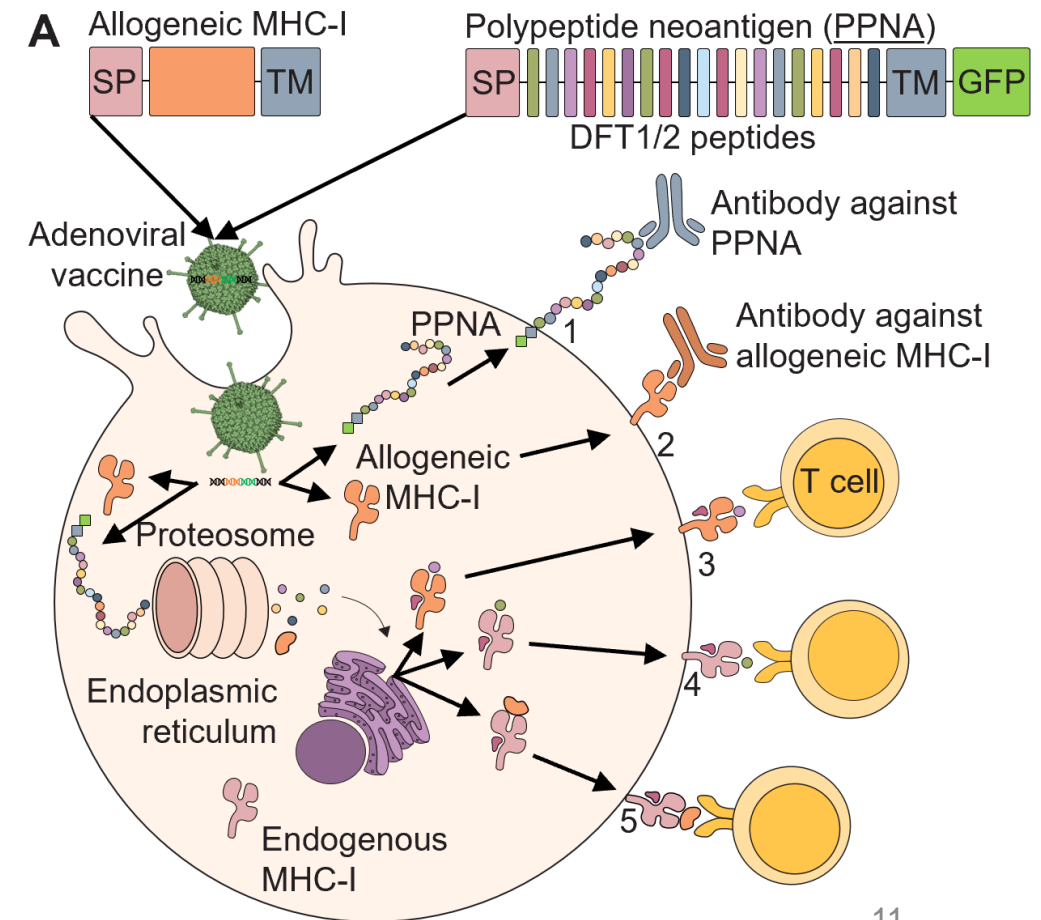
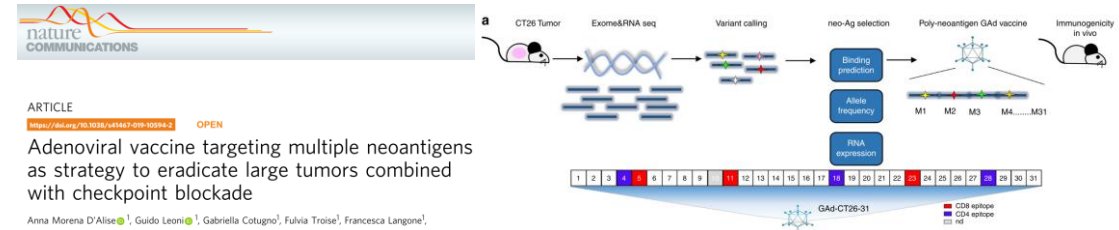
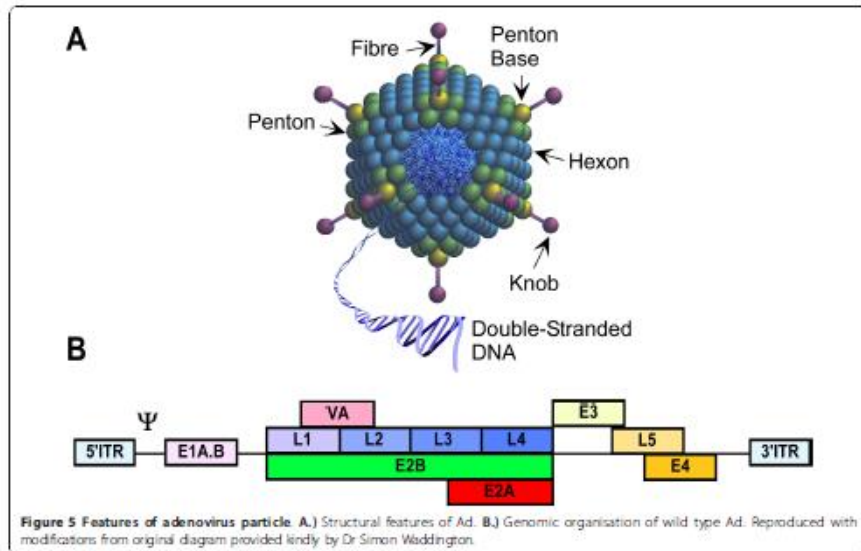
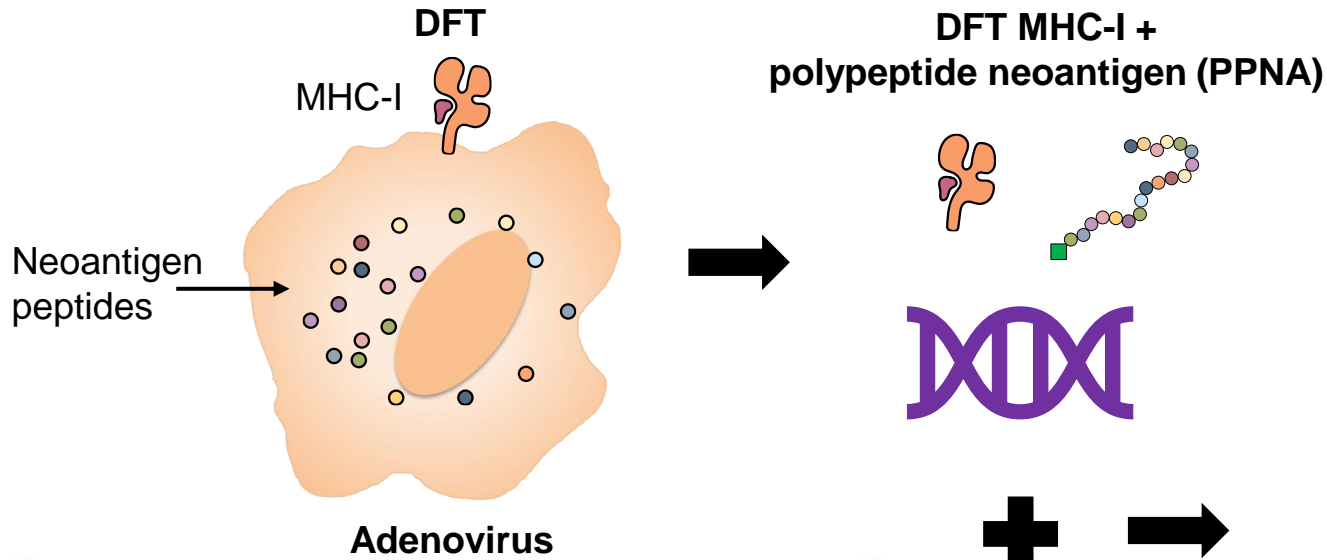
Blue – DAPI  
Red – B2M Alexa 594



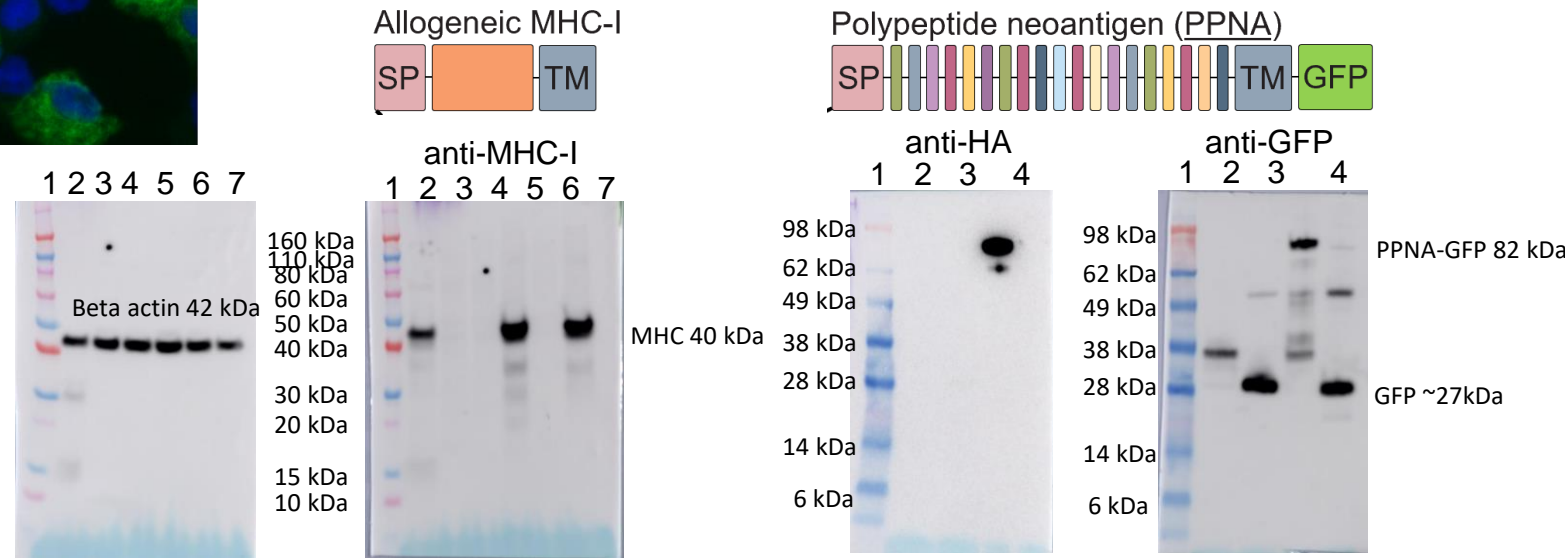
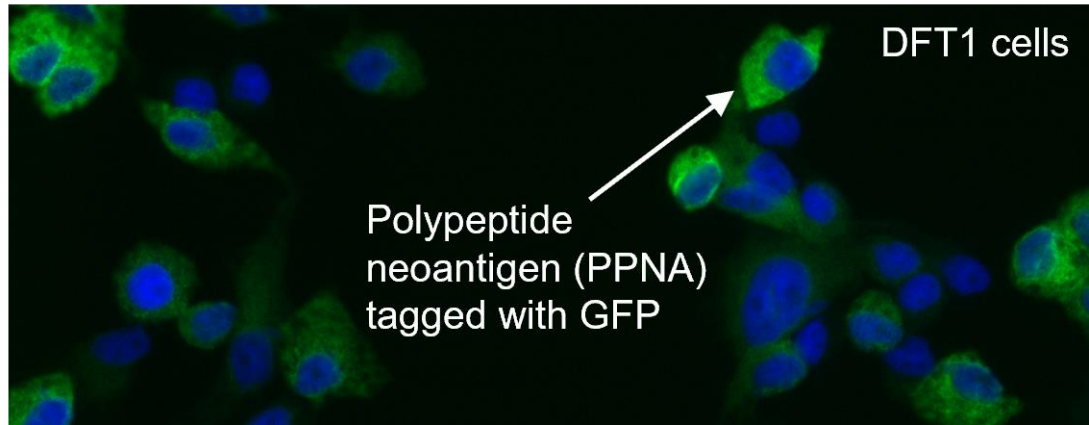
Jocelyn Darby



# Vaccine design to hit multiple immune targets and effector pathways



# Western blots for virus-encoded MHC-I allele and polypeptide neoantigen-GFP



- 1 Novex sharp prestained protein standard
- 2 Spleen
- 3 HEK293
- 4 HEK293 WIVA5.VS1
- 5 HEK293 WIVA12.VS1
- 6 HEK293 WIVA21.VS1
- 7 HEK293 WIVA20.VS3

- 1 Seebule plus 2 prestained markers
- 2 WIVA20.VS3 eluate
- 3 WIVA21.VS1 eluate
- 4 WIVA20.VS3 beads post elution
- 5 WIVA21.VS1 beads post elution

Permits for *in vivo* >2 years and counting;  
test virus in primary tissues in lab

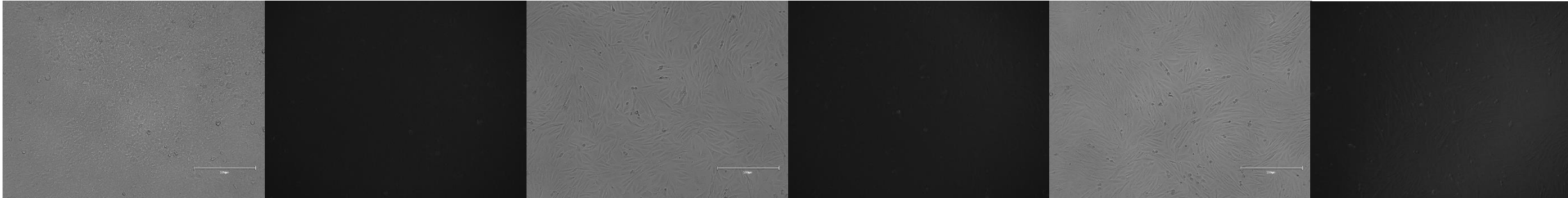


HEK293A

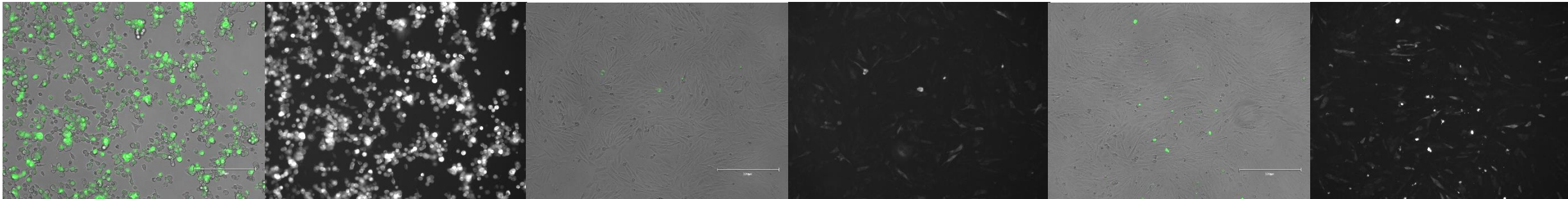
TD656 fibroblast – gum tissue

TD656 fibroblast - lip tissue

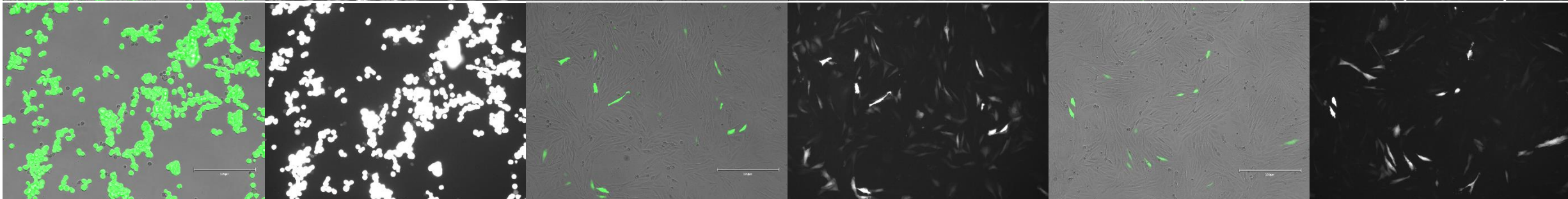
untreated



WIVA20

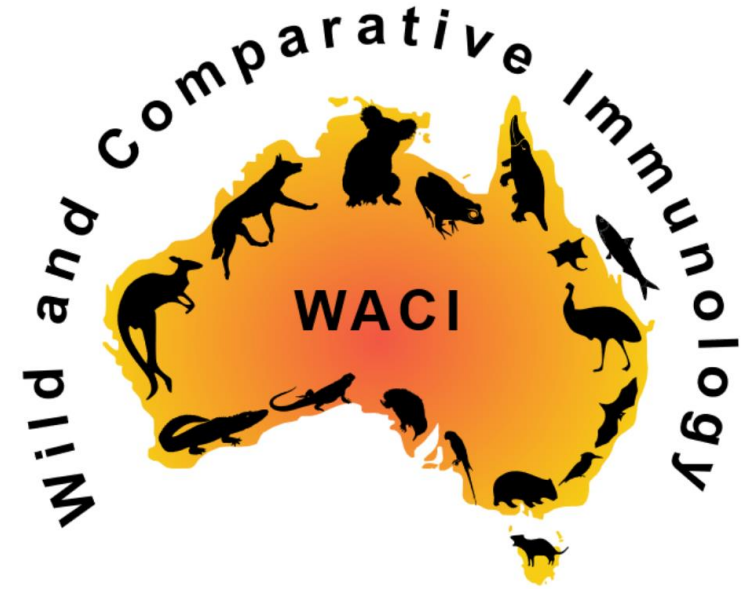


WIVA21



# Summary

- Devil immune system can kill DFT1 cells
- Oral vaccines effective for humans and wildlife
- Adenoviral vectors can transduce devil cells
- Experimental vaccines in development



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## Rewilding immunology

Integrating comparative immunology can improve human, animal, and ecosystem health

[ANDREW S. FLIES](#) AND [WILD COMPARATIVE IMMUNOLOGY CONSORTIUM](#) [Authors Info & Affiliations](#)

SCIENCE • 3 Jul 2020 • Vol 369, Issue 6499 • pp. 37-38 • [DOI:10.1126/science.abb8664](https://doi.org/10.1126/science.abb8664)

# Thank you!

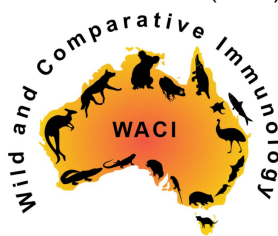
## Tasmanian community and devil supporters around the world!

### Devil group

Ai-Mei Chang  
 Alex Kreiss  
 Amanda Patchett  
 Anuk Kruawan  
 Bruce Lyons  
 Chrissie Ong  
 Grace Russell  
 Greg Woods  
 Jocelyn Darby  
 Khal Glinada  
 Prithul Chaturvedi  
 Ruth Pye  
 Sally Nofs  
 Tom Walsh

### Menzies/UTAS

Alex Hewitt  
 Alex Sella  
 Anne Lloyd-Jones  
 Bill Connelly  
 Bryce Lockhart-Gillett  
 Dave Gell  
 David Steele  
 Guna Karupiah  
 Jac Charlesworth  
 Kathryn Burdon  
 Kirsten Fairfax  
 Louise Cate  
 Mark Bennett  
 Nick Blackburn  
 Rebecca Cuthill  
 Research office  
 Stewart Wells  
 Tim Lennard



### Collaborators

Alexandra Sharland (USyd)  
 Amy Gilbert (USDA NWRC)  
 Andreas Bergthaler (Vienna)  
 Anna Schönichler (Vienna)  
 Billie Lazenby (NRE STDP)  
 Chiara Palmieri (U Queensland)  
 Drew Lee (NRE STDP)  
 David Schaap (NRE STDP)  
 Debbie Burnett (Garvan Institute)  
 Caren Han (Uni Melb)  
 Carolyn Hogg (USyd)  
 Dallas Flies (NextCure)  
 David Chung (Fortifyedge)  
 David Tschärke (ANU)

### Collaborators

Eddie Holmes (USyd)  
 Emily Flies (UTAS)  
 Erin Harvey (USyd)  
 Fern Koay (PDI)  
 Fred Bunz (Johns Hopkins)  
 Hannah Siddle (UQ)  
 Julien Melade (USyd)  
 Katherine Kedzierska (PDI)  
 Linton Staples (ACTA)  
 Liz Murchison (Cambridge)  
 Louise Rowntree (PDI)  
 Maria Croyle (U Texas)  
 Megan Powell (UNC-Ashville)  
 Michael Barry (Mayo Clinic)  
 Michael Souter (PDI)  
 Michaela Lucas (UWA)  
 Nick Gherardin (PDI)  
 Peter Padd (Fortifyedge)  
 Richard Moriggl (Vienna)  
 Rick Liu (UniMelb)  
 Sam Fox (NRE STDP)

### Support

Bonnie Sveen  
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## We need PhD students!

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 Integrating comparative immunology can improve human, animal, and ecosystem health  
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**SAVE THE TASMANIAN DEVIL APPEAL**

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**SAVE THE TASMANIAN DEVIL PROGRAM**  
**SAVE THE TASMANIAN DEVIL.**  
 Devil Facial Tumour Disease threatens the existence of this internationally-recognised icon. In some areas more than 90% of the Tasmanian devil population has been wiped out. [Read more...](#)

Wildcare Tasmania

Saffire  
 FREYCINET

TREADRIGHT FOUNDATION  
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TROWUNNA WILDLIFE SANCTUARY

BONORONG Wildlife Sanctuary

Smitten merino  
 MADE IN TASMANIA

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NextCure

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Australian Government

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 Australian Research Council

Australian Government

Business  
 business.gov.au

Entrepreneurs' Programme

Morris Animal FOUNDATION

Morris Animal FOUNDATION

Animal Control Technologies Australia



# Vaccine facility construction and permits for vaccine testing

## Permits

- Office of the Gene Technology Regulator (approved June 2023)
- Animal Ethics Committee (approved 2022; update in progress)
- Captive Research Advisory Group
- Australian Pesticides and Veterinary Medicines Authority
- Biosecurity / NRE Tasmania / Parks & Wildlife Service



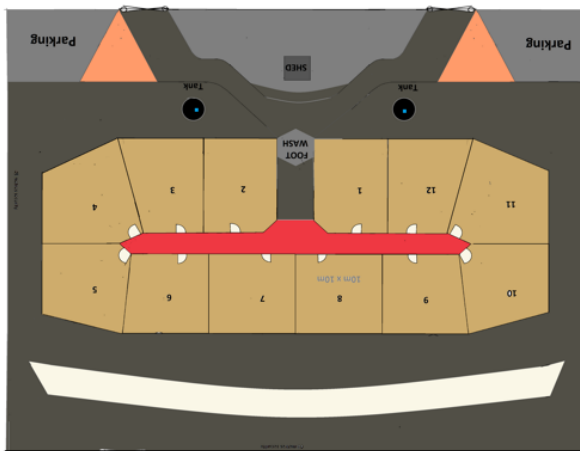
**Licence for dealings involving an intentional release of a GMO into the environment**

Licence No.: DIR 195

Licence Holder: University of Tasmania

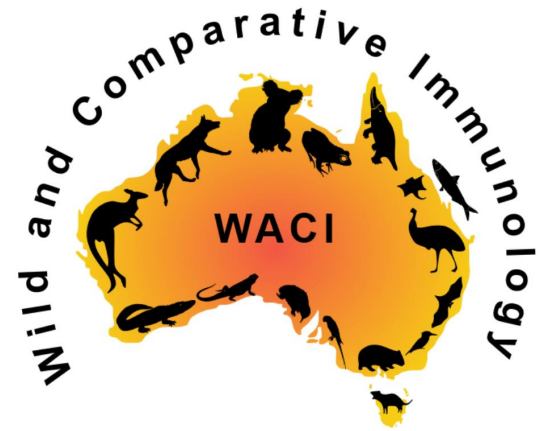
Trial of a genetically modified vaccine against devil facial tumour disease in Tasmanian devils

Issued: 14 June 2023





# Diagnostic tests and advanced immunology tools for non-model species



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## Rewilding immunology

Integrating comparative immunology can improve human, animal, and ecosystem health

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Health



Disease



Antibody isotypes

IgA



IgE



IgM



IgG



B cells



Nanobody toolbox



Immune profile

Immuno-phenotype



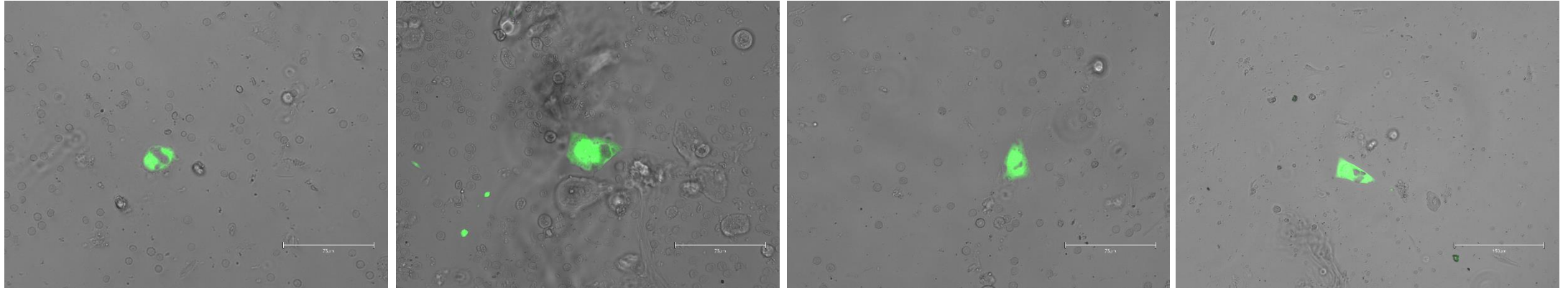
Andy.Flies@utas.edu.au



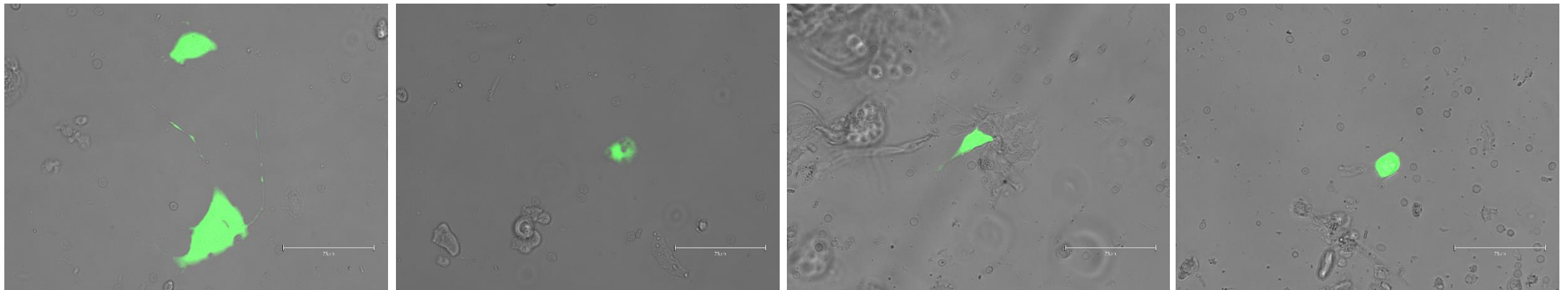
Grace Russell

# Transduction of lip tissue

WIVA20

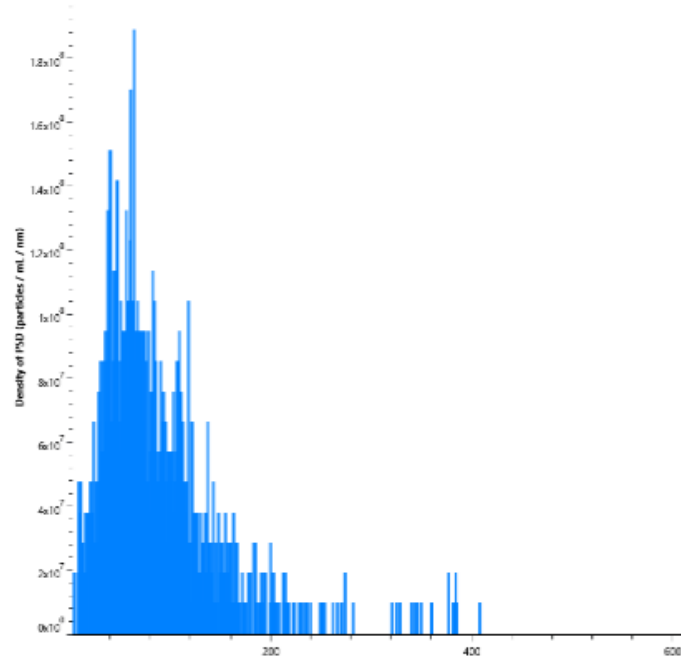


WIVA21



# Quantification of virus particles and infectious units

## Multispectral particle analysis

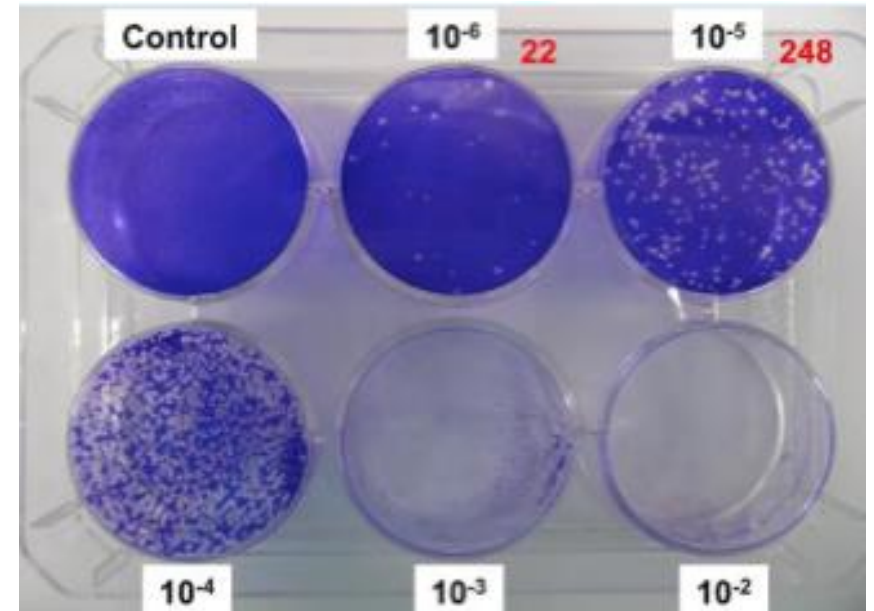


Parameter	Result
Mean	89.16 nm
Mode	64.50 nm
D10	32.12 nm
D50	72.05 nm
D90	155.33 nm
Span	1.710 nm
Concentration (sample)	$7.83 \times 10^9$ particles/mL
Concentration (minus blank)	$7.83 \times 10^9$ particles/mL
Integration range	3 - 981 nm
Chart type	ConstantBinsTable
Volume calibration	None



Jocelyn Darby

## Plaque assay

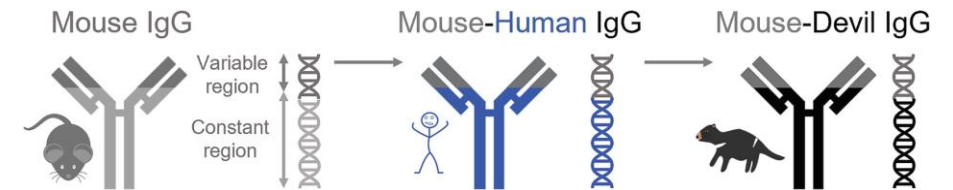
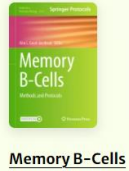


Ai-Mei Change

# PD1/PDL1 immunotherapy

## Conversion of Mouse-Derived Hybridomas to Tasmanian Devil Recombinant IgG Antibodies

Protocol | First Online: 18 July 2024  
pp 231–249 | Cite this protocol



PD-L1 Is Not Constitutively Expressed on Tasmanian Devil Facial Tumor Cells but Is Strongly Upregulated in Response to IFN- $\gamma$  and Can Be Expressed in the Tumor Microenvironment

Andrew S. Flies<sup>1,2,3\*</sup> A. Bruce Lyons<sup>4</sup> Lynn M. Corcoran<sup>5,6</sup> Anthony T. Papenfuss<sup>5,6,7</sup>  
James M. Murphy<sup>5,6</sup> Graeme W. Knowles<sup>8</sup> Gregory M. Woods<sup>1</sup> John D. Hayball<sup>2,3,9</sup>

