

# Combination therapy with reovirus and PD-1 blockade effectively establishes tumor control via innate and adaptive immune responses

Karishma Rajani<sup>1</sup>, Christopher Parrish<sup>2</sup>, Kevin Shim<sup>1</sup>, Liz Ilett<sup>2</sup>, Jill Thompson<sup>1</sup>, Tim Kottke<sup>1</sup>, Jose Pulido<sup>1</sup>, Fiona Errington-Mais<sup>2</sup>, Peter Selby<sup>2</sup>, Hardev Pandha<sup>3</sup>, Kevin Harrington<sup>4</sup>, Alan Melcher<sup>2</sup>, Rosa Maria Diaz<sup>1</sup>, Shane Zaidi<sup>1,4</sup>, Matt Coffey<sup>5</sup>, and Richard Vile<sup>1,2,6</sup>.

<sup>1</sup>Department of Molecular Medicine, Mayo Clinic, Rochester, Minnesota, USA. <sup>2</sup>Leeds Institute of Cancer and Pathology, St. James University Hospital, Leeds, UK. <sup>3</sup>University of Surrey, Guildford, UK.

<sup>4</sup>The Institute of Cancer Research, 237 Fulham Road, London, SW3. <sup>5</sup>Oncolytics Biotech Inc., Calgary, Canada. <sup>6</sup>Department of Immunology, Mayo Clinic, Rochester, Minnesota, USA

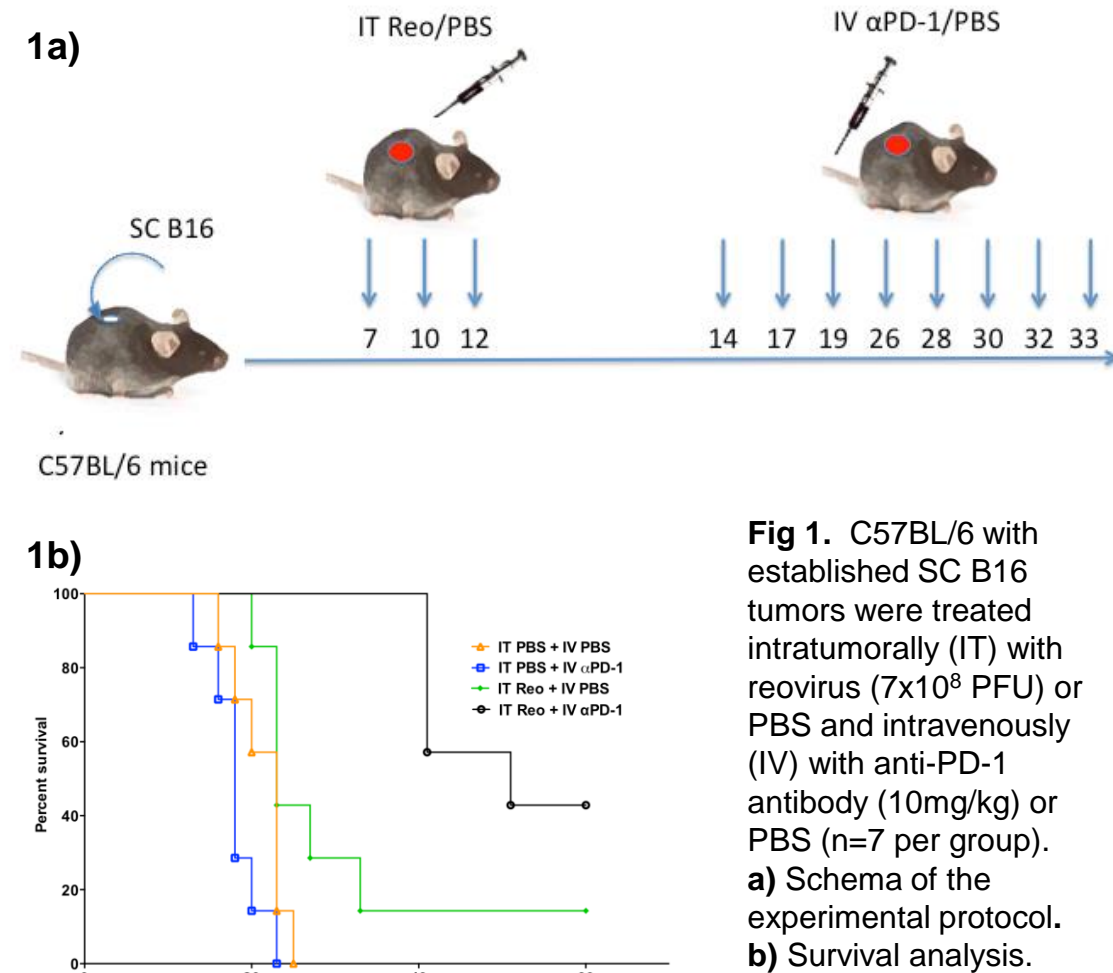
## Introduction

Previous work from our group and others has developed the use of reovirus, a naturally occurring oncolytic virus, as a systemically delivered anti-cancer agent in both pre-clinical models and early phase clinical trials. The agent has direct oncolytic activity against a wide range of human and murine tumor cells, in part due to dysfunction of the PKR-mediated anti-viral response in malignant cells. However, in addition, we have demonstrated reovirus-mediated immune activation in association with virus replication in tumor cells; these responses comprise both innate immune activation against virally infected tumor cells and generation of adaptive immune responses against tumor-associated antigens following *in vivo* priming secondary to immunogenic cell death.

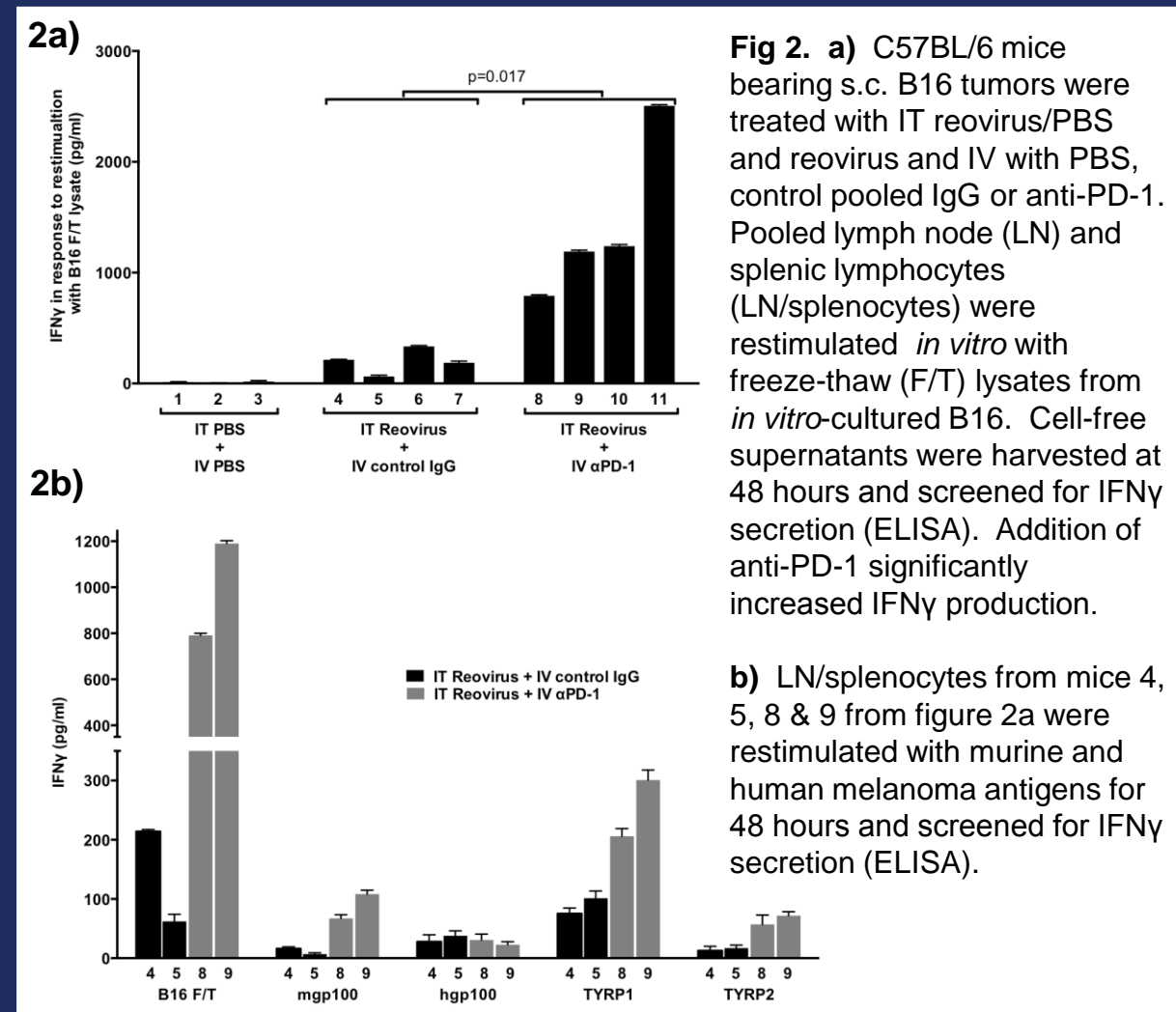
Given the multifaceted immune effects of reovirus therapy, we hypothesized that immune checkpoint inhibition would augment the efficacy of this agent. This hypothesis was tested using our immune-competent murine model in which reovirus is injected into B16 melanomas growing subcutaneously (SC) in C57BL/6 mice.

**Key result:** Combination therapy of reovirus with PD-1 blockade delivers significant survival benefit, by augmenting tumor-specific NK responses and specifically attenuating tumor-specific immunosuppression.

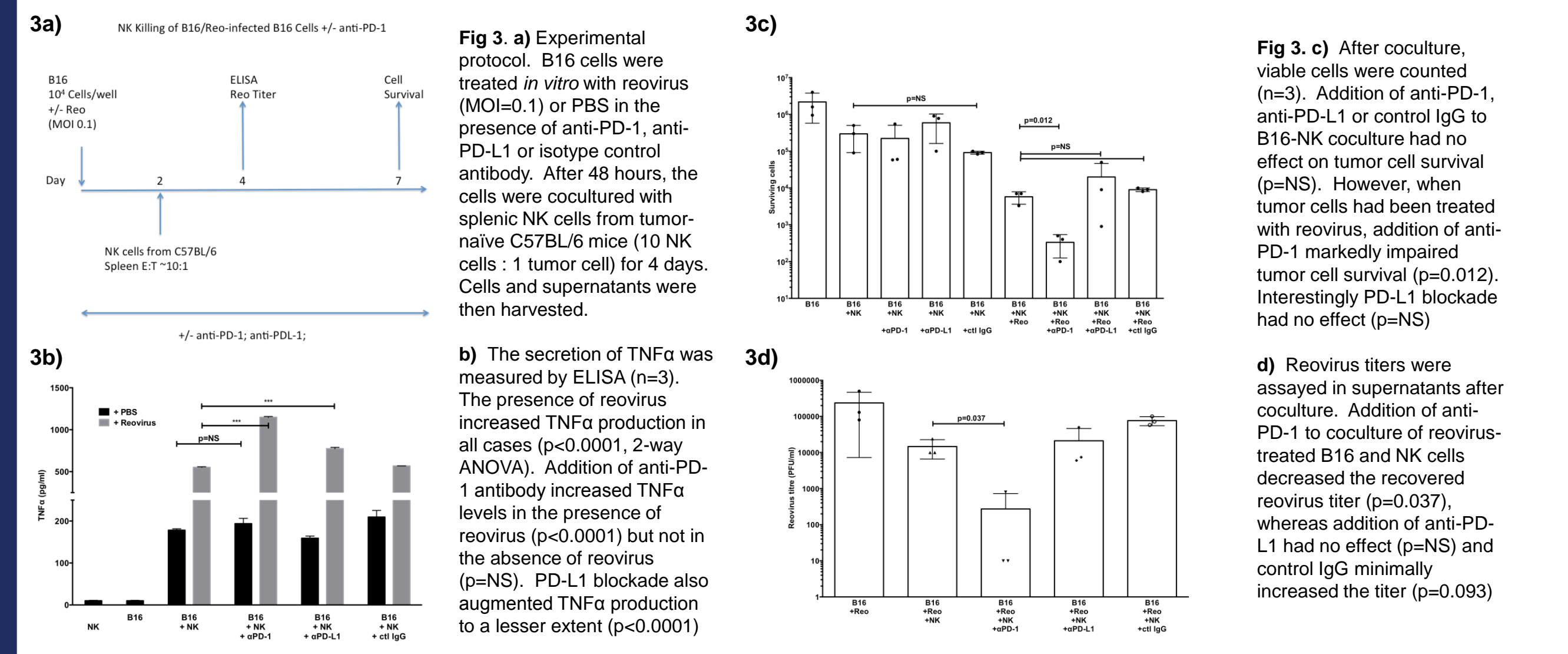
## Figure 1: Reovirus plus PD-1 blockade prolongs survival of mice with melanomas



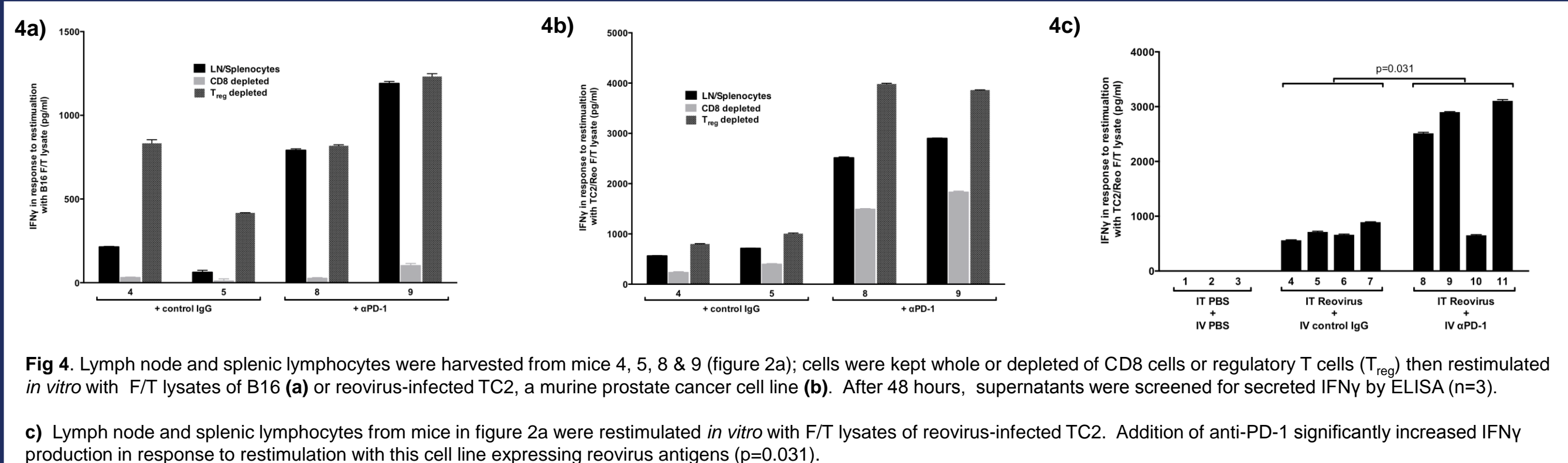
## Figure 2: Reovirus plus PD-1 blockade induces robust IFN $\gamma$ memory T cell responses



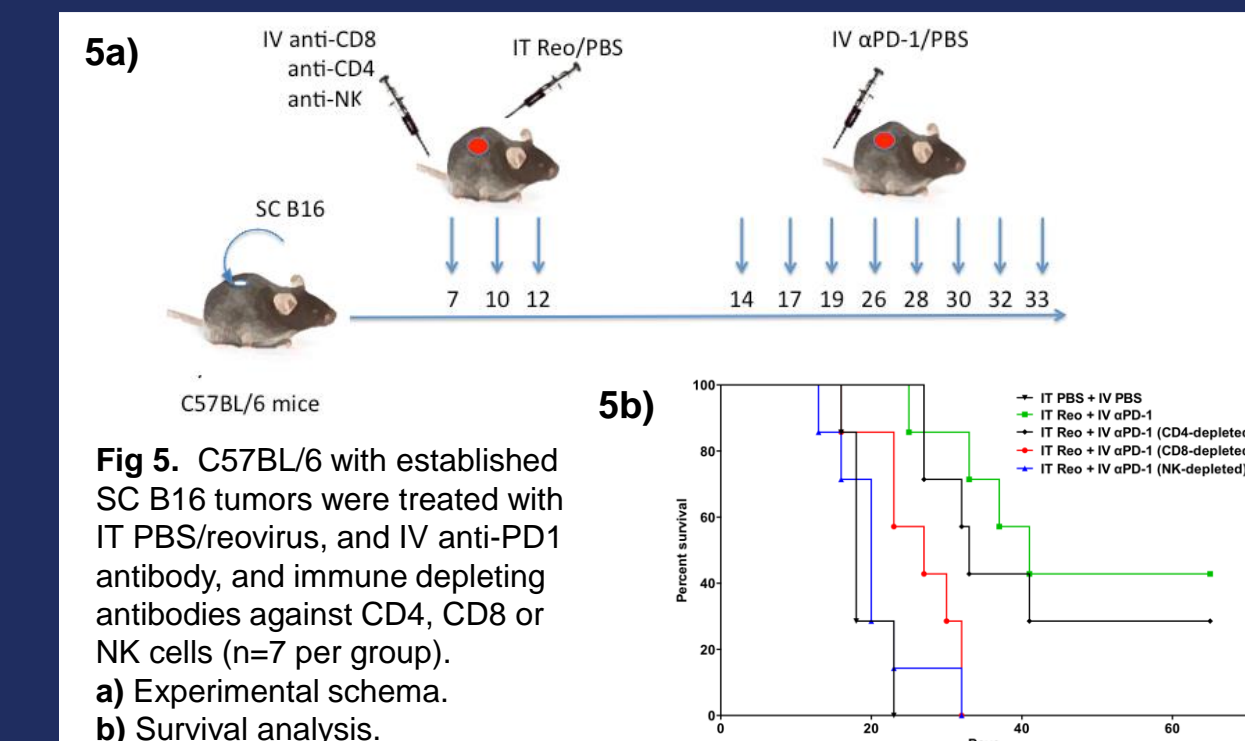
## Figure 3: PD-1 blockade augments reovirus-induced NK cytokine production and killing



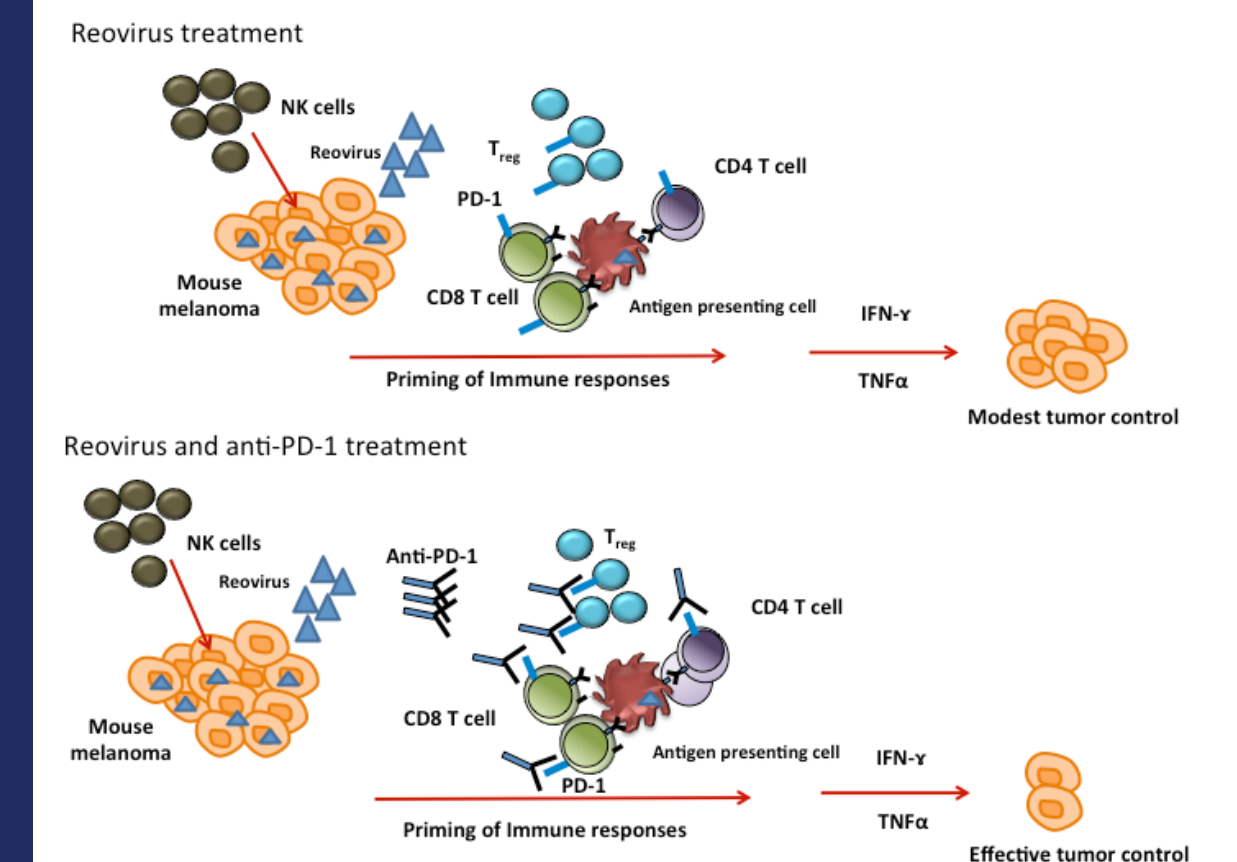
## Figure 4: PD-1 blockade ablates tumor-specific immune suppression by T<sub>reg</sub>s.



## Figure 5: Both innate and adaptive immunity mediate *in vivo* efficacy of Reovirus + alphaPD-1



## Summary



- Combining reovirus with  $\alpha$ PD-1 therapy induces durable anti-tumor responses of established tumors
- Combined therapy results in augmented T cell recall responses against both freeze-thaw tumor lysates and specific tumor-associated antigens.
- Addition of PD-1 blockade augments reovirus-induced TNF $\alpha$  secretion and tumor cell killing by NK cells; recovered viral titers were actually decreased by the combined treatment, presumably reflecting a reduced 'pool' of viable tumor cells to support replication.
- CD8-dependent IFN $\gamma$  recall responses against freeze-thaw tumor lysates were suppressed *in vitro* by T<sub>reg</sub>s – this effect was nullified by PD-1 blockade. Interestingly, T<sub>reg</sub> cells continued to suppress anti-viral adaptive immunity, even in the presence of PD-1 blockade
- In vivo* depletion of CD8 or CD4 cells reduced the efficacy of the reovirus- $\alpha$ PD-1 combination, however, NK cell depletion had an even more marked effect, confirming the central role of NK cell-mediated cell death in tumor control.

## Acknowledgements

