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**Strong antigen-specific T-cell immunity induced by a recombinant human TERT measles virus vaccine and amplified by a DNA/viral vector prime boost in IFNAR/CD46 mice**

Elodie Pliquet, Claude Ruffie, Marie Escande, Jessie Thalmensi, Valérie Najburg, Chantal Combredet, Thomas Bestetti, Marion Julithe, Christelle Liard, Thierry Huet, et al.

► **To cite this version:**

Elodie Pliquet, Claude Ruffie, Marie Escande, Jessie Thalmensi, Valérie Najburg, et al.. Strong antigen-specific T-cell immunity induced by a recombinant human TERT measles virus vaccine and amplified by a DNA/viral vector prime boost in IFNAR/CD46 mice. *Cancer Immunology, Immunotherapy*, 2019, 68 (4), pp.533-544. 10.1007/s00262-018-2272-3 . pasteur-03232227

**HAL Id: pasteur-03232227**

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Submitted on 9 Jun 2021

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# Cancer Immunology, Immunotherapy

## Strong antigen specific T-cell immunity induced by a recombinant human TERT measles virus vaccine and amplified by a DNA/viral vector prime-boost in IFNAR/CD46 mice

--Manuscript Draft--

<b>Manuscript Number:</b>	CIIM-D-18-00105R2	
<b>Full Title:</b>	Strong antigen specific T-cell immunity induced by a recombinant human TERT measles virus vaccine and amplified by a DNA/viral vector prime-boost in IFNAR/CD46 mice	
<b>Article Type:</b>	Original Article	
<b>Funding Information:</b>	Association Nationale de la Recherche et de la Technologie (2012/0152)	Dr. Elodie PLIQUET
<b>Abstract:</b>	<p>Cancer immunotherapy is seeing an increasing focus on vaccination with tumor-associated antigens (TAAs). Human telomerase (hTERT) is a TAA expressed by most tumors to overcome telomere shortening. Tolerance to hTERT can be easily broken both naturally and experimentally and hTERT DNA vaccine candidates have been introduced in clinical trials. DNA prime/boost strategies have been widely developed to immunize efficiently against infectious diseases. We explored the use of a recombinant measles virus (MV) hTERT vector to boost DNA priming as recombinant live attenuated measles virus has an impressive safety and efficacy record. Here, we show that a MV TERT vector can rapidly and strongly boost DNA hTERT priming in MV susceptible IFNAR/CD46 mouse models. The cellular immune responses were Th1 polarized. No humoral responses were elicited. The 4 kb hTERT transgene did not impact MV replication or induction of cell mediated responses. These findings validate the MV TERT vector to boost cell-mediated responses following DNA priming in humans.</p>	
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<b>Additional Information:</b>	
<b>Question</b>	<b>Response</b>
Please provide one or two short sentences highlighting what makes your manuscript especially interesting. This précis is meant for our table of contents and must not exceed 250 characters, including spaces. Please include an identical précis in your manuscript text, following the abstract	Heterologous prime/boost strategies provide exciting opportunities for use in tumor patients because higher immune response to the target antigen can be induced. MV vaccine has impressive safety record.



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#### FOR THE REVIEWERS

**Reviewer #1: The authors have responded to comments in a satisfactory way.**

**Reviewer #2: The revised manuscript is substantially improved and therefore I am happy with it being accepted for publication without the need for any further revision.**

*Response: We are happy that our additional responses and modifications improved the manuscript and were satisfied for you.*

#### FOR THE EDITORIAL OFFICE

4) “in IFNAR/CD46 mouse model” is not quite correct.

→ Indeed, “IFNAR/CD46 mice” is more appropriate because there are two different mouse models in this manuscript with IFNAR/CD46 background.

7) Look at your “Author contributions” section.

→ Done

9) please make sure that both the contents and the English of your entire paper (including all parts) are correct [...] Maybe your co-author Simon Wain-Hobson is a native English speaker who could correct the entire final (!) version.

→ Done

Best regards,

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8 IFNAR/CD46 mice  
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## Abstract

Cancer immunotherapy is seeing an increasing focus on vaccination with tumor-associated antigens (TAAs). Human telomerase (hTERT) is a TAA expressed by most tumors to overcome telomere shortening. Tolerance to hTERT can be easily broken both naturally and experimentally and hTERT DNA vaccine candidates have been introduced in clinical trials. DNA prime/boost strategies have been widely developed to immunize efficiently against infectious diseases. We explored the use of a recombinant measles virus (MV) hTERT vector to boost DNA priming as recombinant live attenuated measles virus has an impressive safety and efficacy record. Here, we show that a MV-TERT vector can rapidly and strongly boost DNA hTERT priming in MV susceptible IFNAR/CD46 mouse models. The cellular immune responses were Th1 polarized. No humoral responses were elicited. The 4 kb hTERT transgene did not impact MV replication or induction of cell mediated responses. These findings validate the MV-TERT vector to boost cell-mediated responses following DNA priming in humans.

**Key words:** Cancer, immunotherapy, hTERT, measles virus vaccine, T-cell responses

## Précis

Heterologous prime/boost strategies provide exciting opportunities for use in tumor patients because higher immune response to the target antigen can be induced. MV vaccine has impressive safety record.

## Abbreviations

AP	Alkaline phosphatase
ATU	Additional transcription unit
BCIP	5-Bromo-4-chloro-3-indolyl phosphate
CBA	Cytometric beads array
MOI	Multiplicity of infection
MV	Measle virus
NBT	Nitro blue tetrazolium chloride
PVDF	Polyvinylidene fluoride
ROI	Region of interest
Tg	Transgenic
TMB	3,3',5,5'-Tetramethylbenzidine
Ubi	Ubiquitin
UCP	Universal cancer peptide

## Introduction

1 Tumors manipulate their microenvironment and the adaptive immune response. With this  
2 understanding, immunotherapy has not surprisingly come to the fore as a means to treat cancer  
3 [1,2]. To stimulate anti-tumoral adaptive immunity, novel vaccine strategies are required.  
4 Attenuation and reverse genetics have allowed the development of viral vectors to deliver  
5 tumor-associated antigens (TAAs) [3]. Some live attenuated viral vectors can deliver  
6 transgenes directly to professional antigen-presenting cells (APCs) and dendritic cells (DCs)  
7 [4] allowing enhanced specific cytotoxic T-cells (CTLs) frequencies [5].

16 Attenuated measles virus (MV), a member of *Morbillivirus* genus in the *Paramyxoviridae*  
17 family, is an enveloped virus with a non-segmented negative single-stranded RNA genome  
18 which replicates exclusively in the cytoplasm [6]. Today the attenuated Schwarz/Moraten  
19 vaccine strain has a longstanding efficacy and an excellent safety record [7,8]. This vaccine  
20 induces strong cellular and humoral immune responses after one or two low-dose injections.  
21 Indeed, MV specific CD8 T-cells and antibodies persist for life [9]. The cloned MV Schwarz  
22 strain has proved to be an excellent vector allowing the incorporation of up to an additional 6  
23 kb, or ~40% of its genome [10,11]. Proof of concept in humans has recently been demonstrated  
24 for a measles vector-based Chikungunya vaccine (MV-CHIK). The phase I clinical trial of this  
25 candidate showed that the vaccine was well tolerated and induced robust and functional  
26 antibody responses in 100% of volunteers after 2 immunizations [12]. This trial also  
27 demonstrated that pre-existing measles antibodies (anti-vector immunity) did not impair the  
28 immunogenicity of the heterologous antigen, paving the way for using recombinant MV as a  
29 vaccine vector [13,12]. Some TAAs like carcinoembryonic antigen (CEA) have also been  
30 efficiently expressed in this vector [14,15].

45 Telomerase reverse transcriptase (TERT) has emerged as a near universal tumor antigen and is  
46 actively investigated as a target for cancer immunotherapy [16]. Human telomerase (hTERT)  
47 is the rate-limiting catalytic subunit of the telomerase enzyme that synthesizes telomere DNA  
48 at chromosome ends [17]. Telomerase transcriptional activation has become the most important  
49 tumor escape mechanism to circumvent telomere-dependent pathways of cell death [18].  
50 Indeed, hTERT is overexpressed in >85% human tumors regardless of their origin [19] and is  
51 associated with poor prognosis [20,21]. Natural anti-hTERT immune responses in some cancer  
52 patients and a pre-existing anti-hTERT repertoire in healthy volunteers show that tolerance to  
53 telomerase may be readily overcome [22]. Towards this end, we have developed a hTERT

1 modified DNA plasmid (INVAC-1) vaccine candidate that elicited strong specific immune  
2 responses and reduced tumor growth in mice [23]. INVAC-1 is currently in an ongoing phase I  
3 clinical trial (NCT02301754).  
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7 DNA vaccines offer numerous advantages such as their capacity to readily incorporate multiple  
8 genes, their easy engineering and their stability [24]. Heterologous prime-boost strategies using  
9 a DNA prime followed by boosting with viral vectors have been shown to increase the  
10 magnitude of immune responses to HIV [25], Chikungunya virus [26], melanoma [27] or breast  
11 cancer [28]. Here, we have developed a recombinant MV vector expressing the insert of  
12 INVAC-1, which is a modified ubiquitin-hTERT fusion protein, as immunotherapeutic agent.  
13 In mouse models susceptible for MV infection, we demonstrate that immunization with a single  
14 low-dose of this construct referred to as MV-TERT, elicited strong hTERT specific cytotoxic  
15 cellular immune responses. The vector could expand primary hTERT memory responses  
16 induced by DNA priming.  
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## 27 **Materials and Methods**

### 28 **Construction of measles virus telomerase construct**

29 The pTM-MV Schw-ATU2 plasmid encodes the Ubi-hTERT insert of INVAC-1 [23] cloned  
30 into the additional transcription unit (ATU) inserted between the P and M genes of an infectious  
31 molecular clone of the Schwarz MV vaccine strain [11]. The INVAC-1 insert carries a 9 bp  
32 deletion that removes three amino acids (867VDD869) crucial to hTERT catalytic activity [29].  
33 The first 47 residues encoding the nucleolar localization sequence (NoLS) [30] were replaced  
34 by human ubiquitin (76 residues) according to the ubiquitin-fusion approach [31] along with an  
35 HLA-A\*0201 restricted influenza A virus epitope and V5 tag was added at the carboxy  
36 terminus to facilitate characterization. The entire transgene was codon optimized. The length  
37 of the insert (3576 bp) was such that the “rule of six” was respected - the number of nucleotides  
38 in a MV genome must be a multiple of 6 to allow efficient replication [32]. The transgene was  
39 synthesized by GeneCust (Luxembourg) and subcloned into the BsiWI/BssHII restriction sites  
40 of the pTM-MV Schw-ATU2 vector generating a plasmid designated pTM-MV-TERT.  
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### 54 **Rescue of recombinant MV-TERT and MV Schw from cloned cDNAs**

55 Recombinant MV-TERT and MV Schwarz (MV Schw) viruses were recovered using a helper  
56 cell-based rescue system [33,34]. HEK-293-T7-MV cells stably expressing both the T7-RNA  
57 polymerase and MV Schw N and P proteins, were co-transfected with 5 µg of pTM-MV-TERT  
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or pTM-MV Schw DNA and 20 ng of the pEMC-L plasmid that expresses the MV polymerase L gene. After incubation overnight at 37°C, the cells were heat shocked at 43°C for 3 hours and transferred onto Vero cell monolayers and incubated at 37°C for two days. Individual syncytia were isolated and transferred to 35 mm wells with a new monolayer of Vero cells and then expanded in 25 cm<sup>2</sup> and finally 150 cm<sup>2</sup> flasks. Viruses were harvested when syncytia reached 90% of the culture and titers were determined by an endpoint limit dilution assay on Vero cells. Viral titers were calculated using the Kärber method and were expressed as TCID<sub>50</sub>/mL.

### Cell lines

HEK293-T7-MV helper cells were cultured in DMEM (Gibco) without sodium pyruvate supplemented with 10% heat-inactivated fetal calf serum and 1% penicillin/streptomycin (Life Technologies, Saint-Aubin, France). Vero cells were maintained in DMEM without sodium pyruvate supplemented with 10% heat-inactivated fetal calf serum and 1% penicillin/streptomycin (Life Technologies, Saint-Aubin, France). For co-culture, Vero cells were seeded at 5 x 10<sup>6</sup> cells and grown as monolayers in 10 cm dishes at 37°C and 5% CO<sub>2</sub> until single syncytia formation. For amplification, 3 x 10<sup>5</sup> Vero cells were seeded in 12 well plates and grown to 80-90% confluence, at which time each syncytium was then transferred.

### Western blotting

Vero cells were seeded at 2 x 10<sup>6</sup> cells and grown as monolayers in T-25 flasks and infected at a multiplicity of infection (MOI) of 0.1. Forty-eight hours later, when syncytia reached 80-90% confluence, cells were lysed in RIPA buffer (Sigma-Aldrich, St. Louis, USA) supplemented with a protease inhibitor cocktail (Roche Diagnostic, Indianapolis, USA). Proteins were separated on Nu-PAGE<sup>®</sup> Novex 4-12% Bis-Tris gels (Invitrogen, Carlsbad, USA) and electroblotted onto Polyvinylidene fluoride (PVDF) membranes (iBlot<sup>®</sup> device, Invitrogen, Carlsbad, USA). hTERT proteins were detected with primary mouse anti-V5 monoclonal antibody (R960-25; Invitrogen, Carlsbad, USA) and nucleoproteins with a primary mouse anti-MV N monoclonal antibody (Abcys, Courtaboeuf, France). A sheep anti-mouse IgG-HRP conjugate (NA931; GE Healthcare, Buckinghamshire, UK) was used as secondary antibody. Novex<sup>®</sup> Sharp Prestained Protein Ladder (Invitrogen, Carlsbad, USA) were used to determine molecular weight. β-Actin was used as loading control. Peroxidase activity was detected on films with chemiluminescence ECL HRP substrate reagent kit (GE Healthcare, Buckinghamshire, UK).

## Immunization

1 MV susceptible IFNAR/CD46 and HHD/IFNAR/CD46 mice were obtained as described [34-  
2 36] and were housed under specific pathogen-free conditions at the Institut Pasteur animal  
3 facility. The IFNAR/CD46 strain has been accepted by the FDA as a model of measles toxicity  
4 prior to initiation of clinical trials of engineered MV strains. Transgenic mice between 6 and 16  
5 weeks of age were inoculated intraperitoneally (i.p.) once or twice with  $10^5$  TCID<sub>50</sub> of  
6 recombinant MV-TERT or MVSchw. To evaluate induction of humoral specific response,  
7 transgenic (Tg) mice were inoculated with  $10^5$  TCID<sub>50</sub> (D0) and  $8 \times 10^4$  TCID<sub>50</sub> (D28) of  
8 MVSchw and MV-TERT at 1 month intervals. For the heterologous prime-boost, priming was  
9 performed via the intradermal (i.d.) route at the base of the tail (bilateral injections) using 25  
10  $\mu$ g of INVAC-1 plasmid coding for Ubi- $\Delta$ hTERT [23] or PBS, as control. Directly after DNA  
11 vaccination, electroporation was performed using CLINIPORATOR<sup>®</sup> 2 (IGEA, Carpi, Italy).  
12 The following train of pulses was applied using non-invasive plate electrodes (P-30-8G, IGEA,  
13 0.5 cm apart); one high voltage pulse (100  $\mu$ s duration; 1,000 V/cm) followed 1 s later by one  
14 low voltage pulse (400 ms duration; 140 V/cm). Twenty-one days later, mice received an i.p.  
15 boost injection with  $10^5$  TCID<sub>50</sub> of MV-TERT or MVSchw.  
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## ELISpot assays

31 HLA-A\*0201 restricted hTERT peptides have been described [37,38]. H2-K/Db and H2-IAb  
32 restricted hTERT peptides were determined *in silico* using the following online epitope  
33 prediction algorithms: Syfpeithi (<http://www.syfpeithi.de/>), Bimas ([http://www-  
34 bimas.cit.nih.gov/](http://www-bimas.cit.nih.gov/)), NetMHCpan and SMM (<http://tools.immuneepitope.org/main/>). All  
35 synthetic peptides were purchased lyophilized (>90% purity) from Proimmune (Oxford, United  
36 Kingdom) and are described in Table 1. Peptides were dissolved in sterile water at 2 mg/mL  
37 and stored at -80°C or -20°C prior use.  
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47 Murine IFN- $\gamma$  kits were purchased from Diaclone (Eurobio, Courtaboeuf, France) and used  
48 following the manufacturer's instructions. Ficoll-purified lymphocyte cell suspensions from  
49 peripheral blood or spleen were stimulated in triplicate at  $2 \times 10^5$  cells/well with pools of HLA-  
50 A\*0201, H2-K/Db or H2-IAb restricted hTERT peptides at 5  $\mu$ g/mL, with serum free RPMI  
51 culture medium (as negative control), with MVSchw at MOI = 1 (as immunization control) or  
52 with PMA-Ionomycin as positive control (0.1  $\mu$ M and 1  $\mu$ M respectively). After 19 hours, spots  
53 were revealed with the biotin-conjugated detection antibody followed by streptavidin-alkaline  
54 phosphatase (AP) and 5-Bromo-4-chloro-3-indolyl phosphate/Nitro blue tetrazolium chloride  
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(BCIP/NBT) substrate solution. Spots were counted using the Immunospot ELISpot counter and software (Cellular Technology Limited, Bonn, Germany).

### **Bioluminescence imaging**

Transgenic mice were immunized with  $10^5$  TCID<sub>50</sub> of recombinant MV-Luc expressing the luciferase gene [39] or MVSchw as control. Immunized mice received i.p. 150 mg/kg body weight of a D-luciferin potassium salt solution at 30 mg/mL (Perkin Elmer Life Sciences, Villebon-sur-Yvette, France). Five minutes later, they were anesthetized with 2–3% isoflurane (Attane Isoflurane, JD Medical Dist. Co., Inc., Phoenix, AZ, USA) delivered in 100% oxygen at a flow rate of 0.8 liter/min and imaged using an IVIS Lumina while the results were analyzed using Living Image software (both Caliper Life Sciences, Hopkinton, MA, USA). Luciferin signals were followed during 8 days until the mice were euthanized using region of interest (ROI) drawing. Bioluminescence signal was expressed as average radiance (ph/sec/cm<sup>2</sup>/sr).

### ***In vivo* cytotoxicity assay**

The capacity of CD8 CTLs to kill peptide loaded target cells *in vivo* was assessed as described [40]. Briefly, splenocytes from naive IFNAR/CD46 mice were split in three and labeled with high (5  $\mu$ M), medium (1  $\mu$ M) or low (0.2  $\mu$ M) concentrations of CFSE (Vybrant CFDA-SE cell-tracer kit; Life Technologies, Saint-Aubin, France). Subsequently, CFSE<sup>high</sup>-labeled cells were pulsed with the immunodominant hTERT p660 peptide and CFSE<sup>medium</sup>-labeled cells were pulsed with the subdominant p1021 hTERT peptide for 1.5 hours whereas CFSE<sup>low</sup>-labeled cells were left unpulsed. Cells were mixed in a 1:1:1 ratio and  $6.8 \times 10^6$  cells in 50  $\mu$ L of PBS were intravenously injected at day 7 into mice previously vaccinated with MV-TERT, MVSchw or PBS. Fifteen hours later, single-cell suspensions from immunized mice spleen were analyzed by MACSQuant® flow cytometer (Miltenyi, Germany). The percentage of specific killing was determined as follows:

$$[1 - [\text{mean}(\% \text{CFSE}^{\text{low}} / \text{CFSE}^{\text{high or medium}})_{\text{CONTROL}} / (\% \text{CFSE}^{\text{low}} / \text{CFSE}^{\text{high or medium}})_{\text{IMMUNIZED}}]] \times 100.$$

### **ELISA for humoral responses**

Sera collected before immunization (D0) and 1 month after each vaccination (D28 and D49) were heat inactivated. MV-specific Ig antibodies were measured by using commercial ELISA kit (Trinity Biotech, USA). Briefly, plates were coated overnight with 50 ng of MV antigens and D28 and D49 sera were serially diluted to determine the end point positive limit dilution.

1 An anti-mouse antibody-HRP conjugate (Amersham) was used as secondary antibody with  
2 3,3',5,5'-Tetramethylbenzidine (TMB) substrate to obtain sample absorbance. Anti-MV titers  
3 were calculated as the highest serum dilution between maximum and minimum absorbance  
4 value of a 1/100 dilution of mixture control sera (mean OD+5SD).  
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9 The presence of hTERT-specific binding antibodies in sera was performed by Bertin Pharma  
10 (France) according to a validated qualitative ELISA immunoassay. Sera were incubated with a  
11 recombinant hTERT peptide (P165-S348, GenWay, San Diego, USA). A secondary goat anti-  
12 mouse IgG conjugated to AP was added. Antigen-Ab complexes were visualized by addition  
13 of a chromogenic substrate. Results are expressed as mean ratios (R) where R = OD values/cut  
14 off point; cut off point = normalized cut off x mean of eight determinations of the negative pool  
15 of matrices. Positive or negative results were obtained according the control (QC) ratio with an  
16 anti-hTERT monoclonal antibody (MAB6595, R&D Systems) diluted 1:4000 (Low QC) and  
17 1:50 (High QC) in pool of negative serum samples from non-immunized mice.  
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### 27 **T-cell cytokine secretion**

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29 For the heterologous prime-boost, at day 28, ficoll-purified splenocytes ( $6 \times 10^5$  cells) from  
30 vaccinated IFNAR/CD46 mice were cultured for 24 h at 37°C with H2-K/Db-restricted hTERT  
31 peptides (429, 660, 1021, 1034) at 5 µg/mL or with MVSchw at an MOI = 1. IL-2, IFN-γ, TNF-  
32 α, IL-4, IL-6, IL-17a and IL-10 were quantified simultaneously on supernatants using the  
33 Cytometric beads array mouse kit (CBA, BD biosciences) according to the manufacturer's  
34 instructions. Flow cytometry acquisition was performed using the FACScan LSR Fortessa flow  
35 cytometer (BD Biosciences); quantitative analyses were performed using the FCAP Array TM  
36 Software version 3.0 (BD Biosciences).  
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### 45 **Statistical analyses**

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47 GraphPad Prism 6.0 software was used for data handling, analysis and graphic representations.  
48 Data are represented as the mean ± standard deviation. Statistical analyses were performed  
49 using a two-tailed Mann Whitney test. Significance was set at p-value ≤ 0.05.  
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## 54 **Results**

### 55 **Efficient expression of hTERT from recombinant MV vector**

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57 The Ubi-ΔhTERT-Flu-V5 transgene was cloned into pTM-MV Schw-ATU2 (Fig. 1a).  
58 Expression of hTERT fusion protein was assessed by Western blot using MV-TERT infected-  
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1 cell lysates with V5-specific antibodies (Fig. 1b). The full length Ubi-ΔhTERT-Flu-V5 fusion  
2 protein was identified at the predicted size of 130.9 kDa. The strongest band at 122.5 kDa  
3 corresponds to processing and removal of the ubiquitin moiety from the fusion protein as  
4 previously reported for INVAC-1 [23]. There is an additional, weaker N-terminal degradation  
5 product around 100 kDa, which is not surprising given that ubiquitin was added to facilitate  
6 degradation. Both MV-TERT and MVSchw constructs expressed the 57.7 kDa MV  
7 nucleoprotein to comparable levels (Fig. 1b) indicating the stability of MV-TERT construct.  
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### 13 **Immunization elicited high frequencies of hTERT specific CD8 T-cells**

14 The immunogenicity of MV-TERT recombinant vector was assessed using MV susceptible  
15 IFNAR/CD46 and HHD/IFNAR/CD46 mice. Mice were immunized i.p. with 10<sup>5</sup> TCID<sub>50</sub> of  
16 each virus. Both hTERT and MV specific T-cell responses were monitored in splenocytes  
17 collected at 7 or 14 days post-immunization using an IFN-γ ELISpot assay with specific hTERT  
18 peptides for *ex vivo* re-stimulation and MVSchw virus for control stimulation (Fig. 2). To assess  
19 MV-TERT immunogenicity in HHD/IFNAR/CD46 mice, splenocytes were stimulated *in vitro*  
20 with a mix of HLA-A\*0201 restricted hTERT peptides for a week. As expected, strong hTERT  
21 specific CD8 T-cell responses were observed in IFNAR/CD46 or HHD/IFNAR/CD46  
22 MV-TERT immunized mice at 7 and 14 days after the last immunization compared to the  
23 MVSchw controls (p<0.01) (Figs. 2a and b). Similarly, hTERT specific CD4 T-cells were  
24 detected in MV-TERT immunized mice for all H2-restricted hTERT peptides tested, as  
25 compared to the MVSchw controls (Fig. 2c). Interestingly, the strength of MV specific T-cell  
26 responses was comparable for the two viruses indicating that the hTERT transgene did not  
27 impact the existing immunogenicity of MV (Figs. 2a and c).  
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43 Specific T-cells frequencies were highest on day 7 for control MVSchw and MV-TERT, while  
44 their frequencies decreased slightly by day 14 (i.e. Fig. 2a) indicating that both MVSchw and  
45 MV-TERT replicate efficiently and probably cleared after 6-7 days post-immunization due to  
46 the ramping up of immune responses. To corroborate this interpretation, vector replication and  
47 clearance were measured *in vivo* using a recombinant MV-luciferase vector (pTM2-MV/Luc  
48 [39]) and monitored by bioluminescence imaging (Fig. 2d). Luciferase activity was detected  
49 between 1-6 days post-infection (maximum average radiance at D3 = 2.01 x 10<sup>5</sup> p/s/cm<sup>2</sup>/sr; Fig.  
50 2d left) and had virtually disappeared by D8 when sacrificed. Even dissection of the animals  
51 failed to identify more luciferin activity at day 8 (D8) (Fig. 2d far right).  
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### **MV-TERT specific CD8 T-cells are cytolytic *in vivo***

To demonstrate that recombinant MV-TERT induces cytolytic hTERT specific CD8 T-cells, an *in vivo* killing assay was performed in IFNAR/CD46 mice using, CFSE-labeled and hTERT peptide pulsed (p660 and p1021) splenocytes or unpulsed splenocytes as target cells. Flow cytometry showed a strong decrease of p660 and p1021 pulsed CFSE cells in MV-TERT immunized mice compared to MVSchw controls illustrating that specific target cells were lysed when they were adoptively transferred into congenic recipient MV-TERT immunized mice (Fig. 3a). Human TERT specific CTLs from MV-TERT immunized mice killed ~50% of p660 pulsed cells and ~14% of p1021 pulsed cells (Fig. 3 b).

### **MV-TERT did not elicit hTERT antibodies**

As MV infection is cytolytic, we wondered whether MV-TERT vaccination might induce hTERT antibodies. To explore this, IFNAR/CD46 and HHD/IFNAR/CD46 mice were immunized twice at a one month interval. MV and TERT specific ELISAs were performed using sera collected at D0, D28 and D49 (Fig. 4). The TERT ELISA plates were coated with a recombinant protein, residues 165-348, as target. As positive or negative results were determined with  $R = OD \text{ values} / \text{cut off point}$  and according the control (QC) ratio (IFNAR/CD46:  $R \text{ QC high} = 7.27-7.67$ ;  $R \text{ QC low} = 1.46-1.50$ ; cut-off = 0.252. HHD/IFNAR/CD46:  $R \text{ QC high} = 8.30-8.99$ ;  $R \text{ QC low} = 1.43-1.44$ ; cut-off = 0.279), no anti-hTERT antibodies were detected in both mouse strains after one or two immunizations (Figs. 4a and b). By contrast, for both mouse strains, anti-MV antibodies were detected after one MV-TERT immunization (D28: IFNAR/CD46 = 81,000; HHD/IFNAR/CD46 = 6,800) followed by a 3-7-fold increase after the second immunization (D49: IFNAR/CD46 = 280,000; HHD/IFNAR/CD46 = 52,000) (Figs. 4c and d). These titers did not differ significantly to those generated by the control MVSchw virus (Figs. 4a and b). These findings confirm that the TERT transgene did not impact MV humoral immunogenicity.

### **Heterologous prime-boost elicited enhanced and multifunctional hTERT responses**

Heterologous prime-boost vaccination can enhance vaccine specific immune responses in infectious diseases and cancer [41]. To test this strategy, we vaccinated a group of ten IFNAR/CD46 mice with INVAC-1 DNA and boosted six with MV-TERT and four with MVSchw 21 days later (Fig. 5). TERT specific CD8 T-cells were monitored by an IFN- $\gamma$  ELISpot assay in PBMCs over time (Fig. 5a) and in splenocytes at each end-point (Fig. 5b). Priming with a single shot of INVAC-1 resulted in weak to no hTERT specific CD8 T-cell

1 responses at 14 days post-immunization; only 2/10 mice developed weak responses in blood  
2 (Fig. 5a). This is not surprising given that DNA priming is known to be poor in IFNAR KO  
3 mice [42,43]. Animals were boosted with MV constructs 21 days post DNA prime  
4 immunization. One week later strong hTERT specific CD8 T-cell responses were detected in  
5 the PBMCs of MV-TERT boosted group in comparison of MVSchw boosted mice (Fig. 5a,  
6 mean #spots: 118.5; n=6). In the spleen, significant numbers of hTERT specific CD8 T-cells  
7 were detected after boosting with MV-TERT compared to controls (Fig. 5b, mean #spots: 453.7  
8 (DNA+MV-TERT; n=6) versus 0.7 (DNA; n=6) or 22.5 (DNA+MVSchw; n=4); p=0.0022 and  
9 0.0095) and compared to only one MV-TERT injection (Fig. 5b, mean #spots: 453.7  
10 (DNA+MV-TERT; n=6) versus 160.4 (MV-TERT; n=13); p=0.0047). A significant difference  
11 in MV specific T-cell responses was observed between a MV-TERT prime and  
12 DNA+MV-TERT boost as expected from the immunization strategy. In keeping with this, no  
13 difference in MV specific T-cell responses between DNA+MVSchw or DNA+MV-TERT boost  
14 were found (Fig. 5b).  
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27 In order to demonstrate the functionality of the hTERT and MV specific T-cells after  
28 heterologous prime-boost vaccination, secreted cytokines were assessed using CBA with  
29 overnight stimulation supernatant. Significant concentrations of IL-6, IFN- $\gamma$  and TNF were  
30 secreted by hTERT specific CD8 T-cells induced by MV-TERT compared to MVSchw, (Fig.  
31 5c). MV specific T-cells secreted the same cytokine, IL-2, IL-6, IL-10, IFN- $\gamma$  and TNF, whether  
32 boosted by MV-TERT or MVSchw (Fig. 5d). Taken together, these results demonstrated that  
33 priming with INVAC-1 DNA and boosting by MV-TERT elicit a powerful TERT specific Th1  
34 response while a more mixed polarized response was induced to MV.  
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## 44 Discussion

45 With a heterologous prime-boost strategy in mind, we developed a recombinant MV vaccine  
46 vector expressing the modified ubiquitin-hTERT fusion protein, to increase the magnitude of  
47 hTERT specific immune responses. This choice reflects promising vector properties of the MV  
48 vaccine that is characterized by long standing safety and efficacy with immunity persisting for  
49 up to 25-30 years [8,14]. As MV targets the human CD46 receptor, it allows hTERT antigen  
50 production directly within macrophages, DCs and lymphocytes [8]. The recombinant MV-  
51 vectored Ubi- $\Delta$ hTERT-Flu-V5 fusion protein was efficiently expressed and presented a similar  
52 degradation profile compared to native INVAC-1 reflecting ubiquitin-fusion [44]. As the  
53 construct carried a deletion in the catalytic site resulting in complete activation, there was no  
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1 risk of cellular transformation. This is further compounded by the fact that MV infection is lytic  
2 while infected cells are marked out for destruction by viral antigen presentation.  
3 Bioluminescence imaging data demonstrated efficient vector replication two days after  
4 immunization and its partial clearance six days later by the ramping up of MV specific immune  
5 response in Tg IFNAR/CD46 mice, confirming the transitory nature of MV transgene delivery  
6 *in vivo*.  
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12 The MV-TERT construct induced high levels of MV and hTERT specific CD8 and CD4 T-cells  
13 seven and fourteen days after a low dose ( $10^4$ - $10^5$  TCID<sub>50</sub>) immunization. The hTERT specific  
14 CD8 T-cells were able to recognize and lyse specific target cells *in vivo*, which is crucial to the  
15 induction of antitumor immunity. An important consideration in this study for the recombinant  
16 MV kinetics is the choice of IFNAR KO mice model that allows powerful and rapid viral  
17 replication in the absence of IFN type I context and subsequently the induction of specific  
18 immune response within seven days [45]. Similar experiments using a recombinant wild-type  
19 MV-EGFP in macaques have demonstrated that the levels of MV-infected lymphocytes  
20 increase exponentially during the first 7–9 days due to viral replication but decrease rapidly  
21 during the subsequent week with the appearance of MV specific T-cells [46].  
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33 The primary goal of the present study was to test a heterologous INVAC-1 DNA prime – MV-  
34 TERT boost strategy to expand hTERT specific immune responses. As expected for IFNAR  
35 KO mice, the present findings reiterate the observation that DNA vaccination is weak in this  
36 background [42,43] compared to our previous work with INVAC-1 DNA on syngeneic or HLA  
37 Tg mice only [23]. It also should be noted that hTERT specific CD8 T-cell response induced  
38 by heterologous prime-boost was 2-3 fold higher than the response after one MV-TERT  
39 immunization (Fig. 5b vs. Fig. 2a). This suggests that INVAC-1 DNA vaccination in  
40 IFNAR/CD46 mice induced hTERT specific CD8 T-cell repertoires but did not enable their  
41 expansion, in contrast to MV-TERT immunization. Indeed, low dose of DNA and its adjuvant  
42 properties are generally efficient to initiate and expand primary immune responses but they are  
43 less relevant to raise the level of secondary memory responses in contrast of live attenuated  
44 viral vectors [47].  
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56 This, along with our results, shows that INVAC-1 DNA prime and MV-TERT boost elicit  
57 hTERT specific and effective CD8 T-cells with Th1 polarization in a mixed response to MV  
58 [48]. In addition, differences in MV specific T-cell responses observed between a MV-TERT  
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1 prime and DNA+MV-TERT prime-boost but not observed between MVSchw or MV-TERT  
2 boost confirm a DNA-activated-immune context. These results open the way to undertaking a  
3 phase I combination DNA prime, MV boost clinical trial.  
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### 6 7 **Author contributions**

8  
9 Simon Wain-Hobson, Frédéric Tangy and Pierre Langlade-Demoyen conceived the project.

10 They also shared their knowledge and experience to help Elodie Pliquet with the experimental  
11 program design.  
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13  
14 Elodie Pliquet performed and analyzed the experiments under the supervision of Christelle  
15 Liard and Thierry Huet. Elodie Pliquet and Claude Ruffie carried out mice experiments. Valérie  
16 Najburg and Chantal Combredet constructed the recombinant measles-telomerase virus. Marie  
17 Escande, Jessie Thalmensi, Thomas Bestetti and Marion Julithe provided technical help.  
18

19  
20 Elodie Pliquet analysed all the data and wrote the manuscript together with Simon Wain-  
21 Hobson and Frédéric Tangy. All authors viewed and approved the final version of the  
22 manuscript.  
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### 27 28 **Acknowledgments**

29 The authors would like to thank Ludovic Bourré, Anne-Sophie Pailhes-Jimenez, Emanuèle  
30 Bourges, Rahima Youssi and Pascal Clayette for experimental and editorial help, and the staff  
31 of the Institut Pasteur's animal facilities and imaging platform.  
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### 37 38 **Funding**

39 We would like to thank Shannon A. Fairbanks and Bernardo Fort Brescia for their unwavering  
40 support. Elodie Pliquet was supported by an industrial PhD fellowship from the French National  
41 Association of Research and Technology (ANRT).  
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### 47 48 **Compliance with ethical standards**

#### 49 **Conflict of interest**

50 The authors declare that they have no conflict of interest.  
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### 54 55 **Ethical approval and ethical standards**

56 All protocols have been submitted and approved by the Pasteur Institute office of laboratory  
57 animal care CETEA N°89 under the references 2013-0026 on February 01<sup>st</sup>, 2013 and 2014-  
58 0061 on October 07<sup>th</sup>, 2015 and by French Committee for Hygiene, Safety and Working  
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Conditions (CHSCT) under the reference 07.157 on April 10<sup>th</sup>, 2007 and 16.0013 on January 8<sup>th</sup>, 2016.

All *in vivo* experiments were conducted in strict accordance with good animal practice and complied with local animal experimentation and ethics committee guidelines of the Pasteur Institute (agreement N°75-15-01 delivered on the 06<sup>th</sup> September 2013 and N°A75-15-01-1 on 18<sup>th</sup> August 2014) and Directive 2010/63/EU on the harmonization of laws, regulations and administrative provisions relating to the protection of animals.

### Animal source

IFNAR/CD46 (IFN- $\alpha$ / $\beta$ R<sup>-/-</sup> CD46<sup>+/-</sup>) mouse strain used for immunization experiments was generated by Frédéric Tangy's team by cross-breeding and backcross between FVB/CD46<sup>+/-</sup> mice (gift from F. Grosveld, Erasmus University, Rotterdam, The Netherlands) and 129sv IFN- $\alpha$ / $\beta$ R<sup>-/-</sup> mice which lack the type I IFN receptor (gift from M. Aguet, Swiss Institute for Experimental Cancer Research, Epalinges, Switzerland).

HHD/IFNAR/CD46 (HHD<sup>+/+</sup> CD46<sup>+/-</sup> IFN- $\alpha$ / $\beta$ R<sup>-/-</sup>) mouse strain was generated by Frédéric Tangy's team by cross-breeding and backcross between HHD mice (human  $\beta$ 2m-HLA-A2.1  $\alpha$ 1 $\alpha$ 2-H-2D<sup>b</sup>  $\alpha$ 3-transmembrane and H-2Db<sup>-/-</sup>  $\beta$ 2mb<sup>-/-</sup> double knockout; mice generated at Pasteur Institute by François Lemonnier) and IFNAR/CD46 mice.

### Cell line authentication

HEK-293-T7-MV cell line was produced, provided and patented by Frédéric Tangy and the Institut Pasteur. Vero cells were provided by the American Type Culture Collection (ATCC®) under reference number CCL-81™.

### Figure legends

#### Fig. 1 Recombinant MV-TERT construction and expression

(a) Schematic maps of the pTM-MV-TERT plasmid. The MV orfs are: N, nucleoprotein; P, phosphoprotein including C and V proteins; M, matrix; F, fusion; H, hemagglutinin; L, polymerase. T7, T7 RNA polymerase promoter; hh, hammerhead ribozyme; h $\delta$ v, hepatitis delta virus ribozyme; T7t, T7 RNA polymerase terminator. (b) Western blot of hTERT transgene and MV nucleoproteins.  $\beta$ -Actin served as loading control.

#### Fig. 2 Induction of hTERT specific CD8 and CD4 T-cells

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(a) MV and hTERT CD8 specific T-cell responses were evaluated seven or fourteen days post-immunization (MVSchw n=4/day; MV-TERT n=6/day) by IFN- $\gamma$  ELISpot assay with MV or a pool of H2-K/Db restricted hTERT specific peptides. (b) IFN- $\gamma$  ELISpot assay was performed using a mix of hTERT specific peptides restricted to HLA-A\*0201 on HHD/IFNAR/CD46 mice seven days post-immunization (MVSchw n=4; MV-TERT n=6) after one week of *in vitro* stimulation with the same peptides. (c) Seven days post-immunization (n=3 mice/group), MV, hTERT CD8 and CD4 specific T-cell responses were evaluated using MV, pool of H2-K/Db restricted hTERT peptides or individual H2-IAb restricted hTERT peptides. (a, b, c) MV stimulation was used as immunization control. Data are represented as mean $\pm$ SD. Mann Whitney non-parametric test against mice control (MVSchw), \*\*p<0.01. (d) Kinetics of recombinant MV-Luc replication cycle on IFNAR/CD46 mice (MVSchw n=1; MV-TERT n=5, data shown 2/5 mice).

### Fig. 3 MV-TERT induces hTERT specific cytotoxic T-cells *in vivo*

(a) The disappearance of naive peptide-pulsed-splenocyte in spleens of IFNAR/CD46 mice immunized once with PBS, MVSchw or MV-TERT was analyzed by flow cytometry (mean data shown per group). Gating was based on FSC/SSC parameters with doublet discrimination. Viable single stained cells were analyzed with FITC channel corresponding to CFSE fluorescence. (b) Percent killing for p660 (black bars) and p1021 (hatched bars) was calculated using cytometry data and was presented as mean $\pm$ SD (MVSchw n=3; MV—TERT n=5). Mann Whitney non-parametric test against mice control (MVSchw), \*p<0.05.

### Fig. 4 Antibody responses following MV-TERT immunization

(a, b) Detection of anti-hTERT antibody after MV-TERT prime-boost immunization (before immunization (D0) and one month after last immunization (D28 and D49)). Results are expressed as mean ratio (R) $\pm$ SD. Mann Whitney non-parametric test against MVSchw control was performed for each day. (c, d) Anti-MV antibody titers detected according the same schedule. Results are expressed as mean values $\pm$ SD determined in serial dilutions of sera. Mann Whitney non-parametric test against MVSchw control was performed. (a, c) Data on IFNAR/CD46 mice (MVSchw n=3; MV-TERT n=5) and (b, d) on HHD/IFNAR/CD46 (MVSchw n=4; MV-TERT n=6).

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**Fig. 5 Heterologous DNA prime / MV-TERT boost induced multiple patterns of T-cell responses**

(a) Individual hTERT CD8 specific T-cell response at days 0, 7, 14 and 21 post-priming and at day 7 post-boost (D28) on PBMCs stimulated with pool of hTERT specific peptides restricted to H2-K/Db. Black arrows indicate vaccination. (b) At day 28, MV specific T-cells and hTERT specific CD8 T-cells were detected on ficoll-purified splenocytes with MV or pool of hTERT specific peptides restricted to H2-K/Db. Data for prime with PBS (n=4), INVAC-1 (n=6) or MV-TERT (3 experiments, n=13) (hatched bars) and prime-boost with INVAC-1 + MVSchw (n=4) or MV-TERT (n=6) (grey bars) are represented as mean±SD. MV specific T-cell stimulation was used as immunization control. Mann Whitney non-parametric test between INVAC-1+MV-TERT and INVAC-1 DNA or MV-TERT alone, \*\*p<0.01. (c) At day 28, concentration of different cytokines secreted by hTERT specific CD8 T-cells and (d) by MV specific T-cells was evaluated using CBA assay. Cytokine concentrations in pg/mL are represented as mean±SD. Mann Whitney non-parametric test against mice control (MVSchw), \*p<0.05; \*\*p<0.01.

## References

1. Eggermont AMM, Kroemer G, Zitvogel L (2013) Immunotherapy and the concept of a clinical cure. *Eur J Cancer* 49 (14):2965-2967. <https://doi.org/10.1016/j.ejca.2013.06.019>
2. Bluestone JA, Small EJ (2012) The Future of Cancer Treatment: Will It Include Immunotherapy? *Cancer Cell* 22 (1):7-8. <https://doi.org/10.1016/j.ccr.2012.06.009>
3. Draper SJ, Heeney JL (2010) Viruses as vaccine vectors for infectious diseases and cancer. *Nat Rev Microbiol* 8 (1):62-73. <https://doi.org/10.1038/nrmicro2240>
4. Bonnet MC, Tartaglia J, Verdier F, Kourilsky P, Lindberg A, Klein M, Moingeon P (2000) Recombinant viruses as a tool for therapeutic vaccination against human cancers. *Immunol Lett* 74 (1):11-25. [https://doi.org/10.1016/S0165-2478\(00\)00244-3](https://doi.org/10.1016/S0165-2478(00)00244-3)
5. Larocca C, Schlom J (2011) Viral Vector –based Therapeutic Cancer Vaccines. *Cancer J* 17 (5):359-371. <https://doi.org/10.1097/PPO.0b013e3182325e63>
6. Moss WJ, Griffin DE (2012) Measles. *Lancet* 379 (9811):153-164. [https://doi.org/10.1016/S0140-6736\(10\)62352-5](https://doi.org/10.1016/S0140-6736(10)62352-5)
7. Hilleman MR (2001) Current overview of the pathogenesis and prophylaxis of measles with focus on practical implications. *Vaccine* 20 (5–6):651-665. [https://doi.org/10.1016/S0264-410X\(01\)00384-X](https://doi.org/10.1016/S0264-410X(01)00384-X)
8. Guillerme J-B, Gregoire M, Tangy F, Fonteneau J-F (2013) Antitumor Virotherapy by Attenuated Measles Virus (MV). *Biology* 2 (2):587. <https://doi.org/10.3390/biology2020587>
9. Amanna IJ, Carlson NE, Slifka MK (2007) Duration of humoral immunity to common viral and vaccine antigens. *N Engl J Med* 357 (19):1903-1915. <https://doi.org/10.1056/NEJMoa066092>
10. Zuniga A, Wang Z, Liniger M, Hangartner L, Caballero M, Pavlovic J, Wild P, Viret JF, Glueck R, Billeter MA, Naim HY (2007) Attenuated Measles Virus as a Vaccine Vector. *Vaccine* 25 (16):2974-2983. <https://doi.org/10.1016/j.vaccine.2007.01.064>
11. Brandler S, Tangy F (2008) Recombinant vector derived from live attenuated measles virus: potential for flavivirus vaccines. *Comp Immunol Microbiol Infect Dis* 31 (2-3):271-291. <https://doi.org/10.1016/j.cimid.2007.07.012>
12. Ramsauer K, Schwameis M, Firbas C, Mullner M, Putnak RJ, Thomas SJ, Despres P, Tauber E, Jilma B, Tangy F (2015) Immunogenicity, safety, and tolerability of a recombinant measles-virus-based chikungunya vaccine: a randomised, double-blind, placebo-controlled, active-comparator, first-in-man trial. *Lancet Infect Dis* 15 (5):519-527. [https://doi.org/10.1016/S1473-3099\(15\)70043-5](https://doi.org/10.1016/S1473-3099(15)70043-5)
13. Knuchel MC, Marty RR, Morin TN, Ilter O, Zuniga A, Naim HY (2013) Relevance of a pre-existing measles immunity prior immunization with a recombinant measles virus vector. *Hum Vaccin Immunother* 9 (3):599-606. <https://doi.org/10.4161/hv.23241>

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14. Russell SJ, Peng KW (2009) Measles virus for cancer therapy. *Curr Top Microbiol Immunol* 330:213-241
  15. Msaouel P, Iankov ID, Allen C, Morris JC, von Messling V, Cattaneo R, Koutsilieris M, Russell SJ, Galanis E (2009) Engineered measles virus as a novel oncolytic therapy against prostate cancer. *Prostate* 69 (1):82-91. <https://doi.org/10.1002/pros.20857>
  16. Zanetti M (2017) A second chance for telomerase reverse transcriptase in anticancer immunotherapy. *Nat Rev Clin Oncol* 14 (2):115-128. <https://doi.org/10.1038/nrclinonc.2016.67>
  17. Collins K, Mitchell JR (2002) Telomerase in the human organism. *Oncogene* 21 (4):564-579. <https://doi.org/10.1038/sj.onc.1205083>
  18. Low KC, Tergaonkar V (2013) Telomerase: central regulator of all of the hallmarks of cancer. *Trends Biochem Sci* 38 (9):426-434. <https://doi.org/10.1016/j.tibs.2013.07.001>
  19. Shay JW, Bacchetti S (1997) A survey of telomerase activity in human cancer. *Eur J Cancer* 33 (5):787-791. [https://doi.org/10.1016/S0959-8049\(97\)00062-2](https://doi.org/10.1016/S0959-8049(97)00062-2)
  20. Zhu CQ, Cutz JC, Liu N, Lau D, Shepherd FA, Squire JA, Tsao MS (2006) Amplification of telomerase (hTERT) gene is a poor prognostic marker in non-small-cell lung cancer. *Brit J Cancer* 94 (10):1452-1459. <https://doi.org/10.1038/sj.bjc.6603110>
  21. Lü M-H, Deng J-Q, Cao Y-L, Fang D-C, Zhang YAO, Yang S-M (2012) Prognostic role of telomerase activity in gastric adenocarcinoma: A meta-analysis. *Exp Ther Med* 3 (4):728-734. <https://doi.org/10.3892/etm.2012.471>
  22. Karanikas V, Zamanakou M, Soukou F, Kerenidi T, Gourgoulisianis KI, Germenis AE (2010) Naturally occurring tumor-specific CD8+ T-cell precursors in individuals with and without cancer. *Immunol Cell Biol* 88 (5):575-585. <https://doi.org/10.1038/icb.2010.8>
  23. Thalmensi J, Pliquet E, Liard C, Escande M, Bestetti T, Julithe M, Kostrzak A, Pailhes-Jimenez A-S, Bourges E, Loustau M, Caumartin J, Lachgar A, Huet T, Wain-Hobson S, Langlade-Demoyen P (2016) Anticancer DNA vaccine based on human telomerase reverse transcriptase generates a strong and specific T cell immune response. *Oncoimmunology* 5 (3):e1083670. <https://doi.org/10.1080/2162402X.2015.1083670>
  24. Rice J, Ottensmeier CH, Stevenson FK (2008) DNA vaccines: precision tools for activating effective immunity against cancer. *Nat Rev Cancer* 8 (2):108-120. <https://doi.org/10.1038/nrc2326>
  25. Brown SA, Surman SL, Sealy R, Jones BG, Slobod KS, Branum K, Lockey TD, Howlett N, Freiden P, Flynn P, Hurwitz JL (2010) Heterologous Prime-Boost HIV-1 Vaccination Regimens in Pre-Clinical and Clinical Trials. *Viruses* 2 (2):435-467. <https://doi.org/10.3390/v2020435>
  26. Hallengård D, Lum F-M, Kümmerer BM, Lulla A, Lulla V, García-Arriaza J, Fazakerley JK, Roques P, Le Grand R, Merits A, Ng LFP, Esteban M, Liljeström P (2014) Prime-Boost

1 Immunization Strategies against Chikungunya Virus. *J Virol* 88 (22):13333-  
2 13343. <https://doi.org/10.1128/JVI.01926-14>

3  
4 27. Smith CL, Dunbar PR, Mirza F, Palmowski MJ, Shepherd D, Gilbert SC, Coulie P,  
5 Schneider J, Hoffman E, Hawkins R, Harris AL, Cerundolo V (2005) Recombinant modified  
6 vaccinia Ankara primes functionally activated CTL specific for a melanoma tumor antigen  
7 epitope in melanoma patients with a high risk of disease recurrence. *Int J Cancer* 113 (2):259-  
8 266. <https://doi.org/10.1002/ijc.20569>

9  
10  
11 28. Kim SB, Ahn JH, Kim J, Jung KH (2015) A phase 1 study of a heterologous prime-boost  
12 vaccination involving a truncated HER2 sequence in patients with HER2-expressing breast  
13 cancer. *Mol Ther Methods Clin Dev* 2:15031. <https://doi.org/10.1038/mtm.2015.31>

14  
15  
16 29. Drosopoulos WC, Prasad VR (2007) The active site residue Valine 867 in human  
17 telomerase reverse transcriptase influences nucleotide incorporation and fidelity. *Nucleic*  
18 *Acids Res* 35 (4):1155-1168. <https://doi.org/10.1093/nar/gkm002>

19  
20  
21 30. Yang Y, Chen Y, Zhang C, Huang H, Weissman SM (2002) Nucleolar localization of  
22 hTERT protein is associated with telomerase function. *Exp Cell Res* 277 (2):201-  
23 209. <https://doi.org/10.1006/excr.2002.5541>

24  
25  
26 31. Bachmair A, Finley D, Varshavsky A (1986) In vivo half-life of a protein is a function of  
27 its amino-terminal residue. *Science* 234 (4773):179-  
28 186. <https://doi.org/10.1126/science.3018930>

29  
30  
31 32. Bankamp B, Takeda M, Zhang Y, Xu W, Rota PA (2011) Genetic Characterization of  
32 Measles Vaccine Strains. *J Infect Dis* 204 (suppl 1):S533-  
33 S548. <https://doi.org/10.1093/infdis/jir097>

34  
35  
36 33. Radecke F, Spielhofer P, Schneider H, Kaelin K, Huber M, Dotsch C, Christiansen G,  
37 Billeter MA (1995) Rescue of measles viruses from cloned DNA. *The EMBO journal* 14  
38 (23):5773-5784

39  
40  
41 34. Combredet C, Labrousse V, Mollet L, Lorin C, Delebecque F, Hurtrel B, McClure H,  
42 Feinberg MB, Brahic M, Tangy F (2003) A molecularly cloned Schwarz strain of measles  
43 virus vaccine induces strong immune responses in macaques and transgenic mice. *J Virol* 77  
44 (21):11546-11554. <https://doi.org/10.1128/JVI.77.21.11546-11554.2003>

45  
46  
47 35. Lorin C, Delebecque F, Labrousse V, Da Silva L, Lemonnier F, Brahic M, Tangy F  
48 (2005) A recombinant live attenuated measles vaccine vector primes effective HLA-A0201-  
49 restricted cytotoxic T lymphocytes and broadly neutralizing antibodies against HIV-1  
50 conserved epitopes. *Vaccine* 23 (36):4463-4472. <https://doi.org/10.1016/j.vaccine.2005.04.024>

51  
52  
53 36. Pascolo S, Bervas N, Ure JM, Smith AG, Lemonnier FA, Perarnau B (1997) HLA-A2.1-  
54 restricted education and cytolytic activity of CD8(+) T lymphocytes from beta2 microglobulin  
55 (beta2m) HLA-A2.1 monochain transgenic H-2Db beta2m double knockout mice. *J Exp Med*  
56 185 (12):2043-2051. <https://doi.org/10.1084/jem.185.12.2043>

57  
58  
59 37. Hernandez J, Garcia-Pons F, Lone YC, Firat H, Schmidt JD, Langlade-Demoyen P,  
60 Zanetti M (2002) Identification of a human telomerase reverse transcriptase peptide of low  
61

1 affinity for HLA A2.1 that induces cytotoxic T lymphocytes and mediates lysis of tumor cells.  
2 Proc Natl Acad Sci USA 99 (19):12275-12280.<https://doi.org/10.1073/pnas.182418399>

3  
4 38. Dosset M, Godet Y, Vauchy C, Beziaud L, Lone YC, Sedlik C, Liard C, Levionnois E,  
5 Clerc B, Sandoval F, Daguindau E, Wain-Hobson S, Tartour E, Langlade-Demoyen P, Borg  
6 C, Adotevi O (2012) Universal cancer peptide-based therapeutic vaccine breaks tolerance  
7 against telomerase and eradicates established tumor. *Clinical cancer research : an official*  
8 *journal of the American Association for Cancer Research* 18 (22):6284-  
9 6295.<https://doi.org/10.1158/1078-0432.CCR-12-0896>

10  
11 39. Komarova AV, Combredet C, Meyniel-Schicklin L, Chapelle M, Caignard G, Camadro  
12 JM, Lotteau V, Vidalain PO, Tangy F (2011) Proteomic analysis of virus-host interactions in  
13 an infectious context using recombinant viruses. *Mol Cell Proteomics* 10 (12):M110  
14 007443.<https://doi.org/10.1074/mcp.M110.007443>

15  
16 40. Durward M, Harms J, Splitter G (2010) Antigen specific killing assay using CFSE labeled  
17 target cells. *Journal of visualized experiments : JoVE* (45).<https://doi.org/10.3791/2250>

18  
19 41. Lu S (2009) Heterologous prime-boost vaccination. *Current opinion in immunology* 21  
20 (3):346-351.<https://doi.org/10.1016/j.coi.2009.05.016>

21  
22 42. Ishii KJ, Kawagoe T, Koyama S, Matsui K, Kumar H, Kawai T, Uematsu S, Takeuchi O,  
23 Takeshita F, Coban C, Akira S (2008) TANK-binding kinase-1 delineates innate and adaptive  
24 immune responses to DNA vaccines. *Nature* 451 (7179):725-  
25 729.<https://doi.org/10.1038/nature06537>

26  
27 43. Coban C, Kobiyama K, Aoshi T, Takeshita F, Horii T, Akira S, Ishii KJ (2011) Novel  
28 strategies to improve DNA vaccine immunogenicity. *Curr Gene Ther* 11 (6):479-  
29 484.<https://doi.org/10.2174/156652311798192815>

30  
31 44. Tasaki T, Sriram SM, Park KS, Kwon YT (2012) The N-end rule pathway. *Annu Rev*  
32 *Biochem* 81:261-289.<https://doi.org/10.1146/annurev-biochem-051710-093308>

33  
34 45. Mrkic B, Pavlovic J, Rulicke T, Volpe P, Buchholz CJ, Hourcade D, Atkinson JP, Aguzzi  
35 A, Cattaneo R (1998) Measles virus spread and pathogenesis in genetically modified mice. *J*  
36 *Virol* 72 (9):7420-7427

37  
38 46. Ludlow M, de Vries RD, Lemon K, McQuaid S, Millar E, van Amerongen G, Yuksel S,  
39 Verburgh RJ, Osterhaus AD, de Swart RL, Duprex WP (2013) Infection of lymphoid tissues  
40 in the macaque upper respiratory tract contributes to the emergence of transmissible measles  
41 virus. *J Gen Virol* 94 (Pt 9):1933-1944.<https://doi.org/10.1099/vir.0.054650-0>

42  
43 47. Woodland DL (2004) Jump-starting the immune system: prime-boosting comes of age.  
44 *Trends Immunol* 25 (2):98-104.<https://doi.org/10.1016/j.it.2003.11.009>

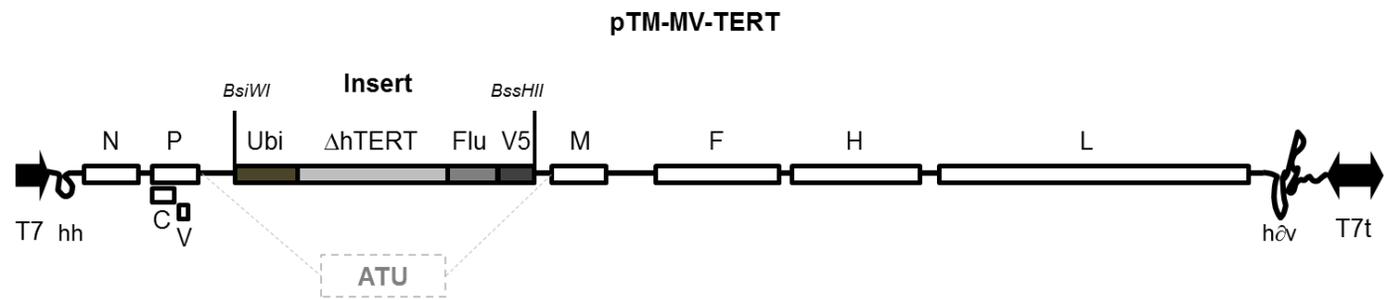
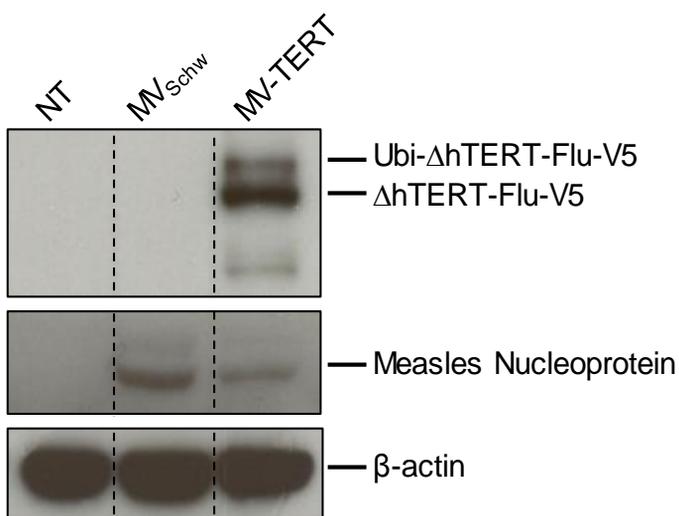
45  
46 48. Palucka K, Ueno H, Banchereau J (2011) Recent developments in cancer vaccines. *J*  
47 *Immunol* 186 (3):1325-1331.<https://doi.org/10.4049/jimmunol.0902539>

Tables

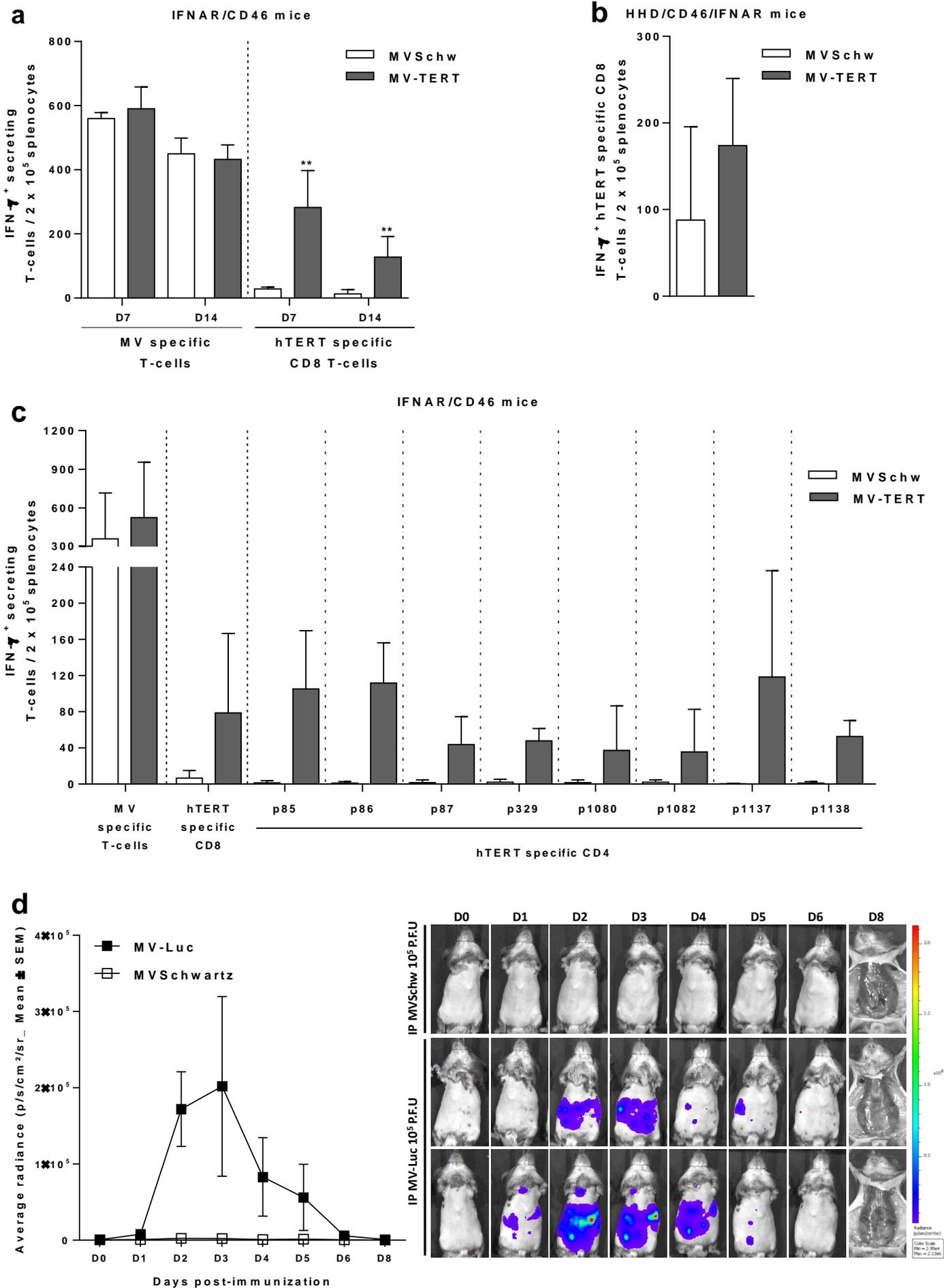
Table 1. hTERT peptides

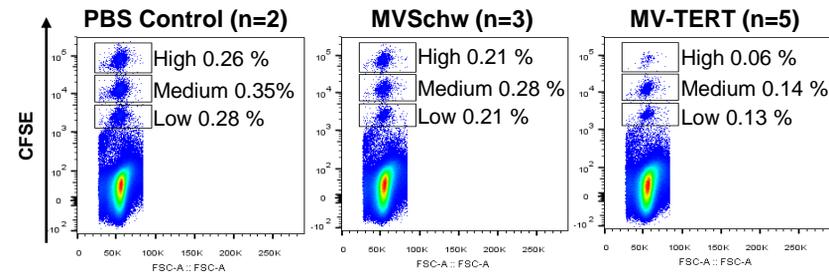
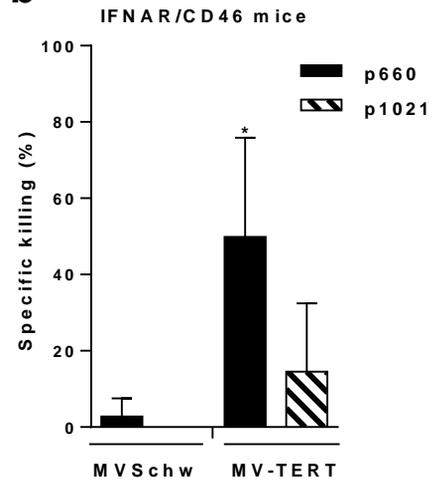
Peptide	Numbered to	Sequence	MHC	Mouse Strain	
429	Ubi-ΔhTERT	HAQCPYGVL	H2-Kb	IFNAR/CD46	
1034		QAYRFHACVL			
660		RPIVNMDYV			
1021		QTVCTNIYKI	H2-Db		
85		VCPWDARPPPAAPS	H2-IAb		IFNAR/CD46
86		CVPWDARPPPAAPSF			
87		VPWDARPPPAAPSF			
329		GRQHHAGPPSTSRPP			
1080		MSLGAKGAAGPLPSE			
1082		LGAKGAAGPLPSEAV			
1137		TLTALEAAANPALPS			
1138		LTALEAAANPALPSD			
540		hTERT	ILAKFLHWL	HLA-A*0201	HHD/IFNAR/CD46
[37]					
Y572	YLFFYRKS				
[37]					
Y988	YLQVNSLQTV				
UCP2.1					
[38]		SVWSKLQSI			
UCP4.1					
[38]		SLCYSILKA			

Following H2 restricted Ubi-ΔhTERT peptides have been predicted *in silico*; HLA-A\*0201 restricted hTERT peptides were previously described [37,38]. Peptides were used either in ELISpot assay, CBA or *in vivo* cytotoxicity assay according to the strains of mouse.

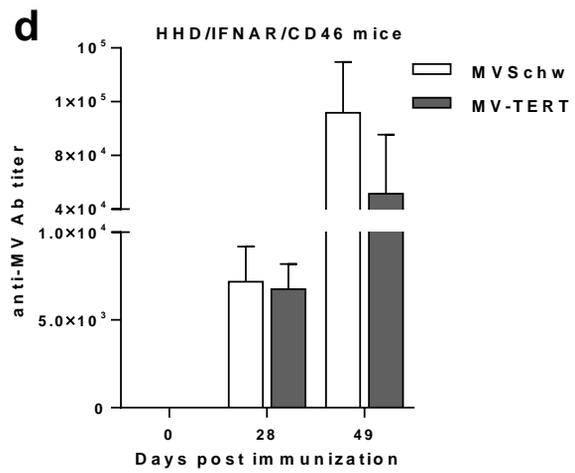
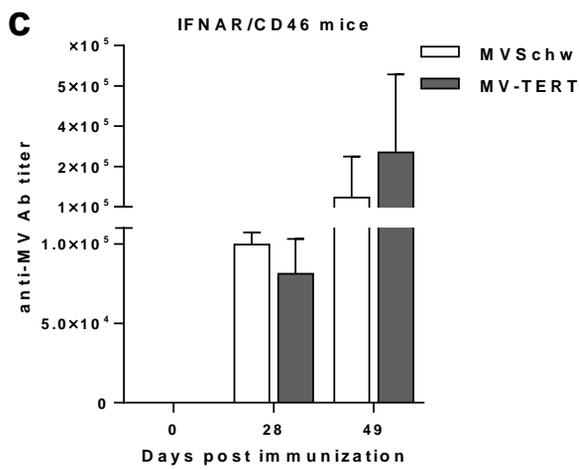
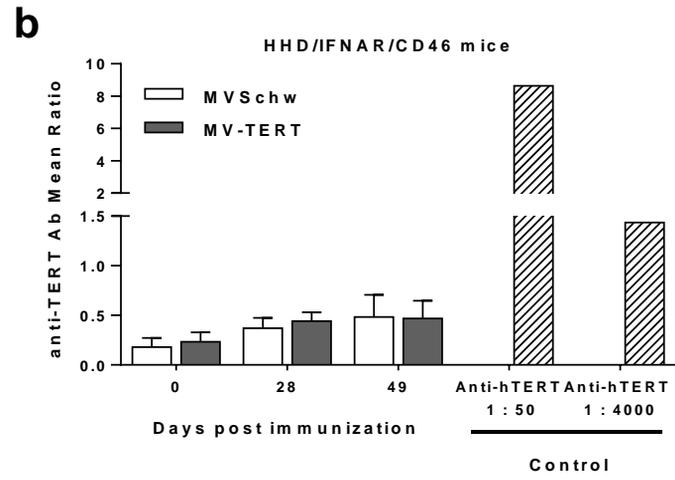
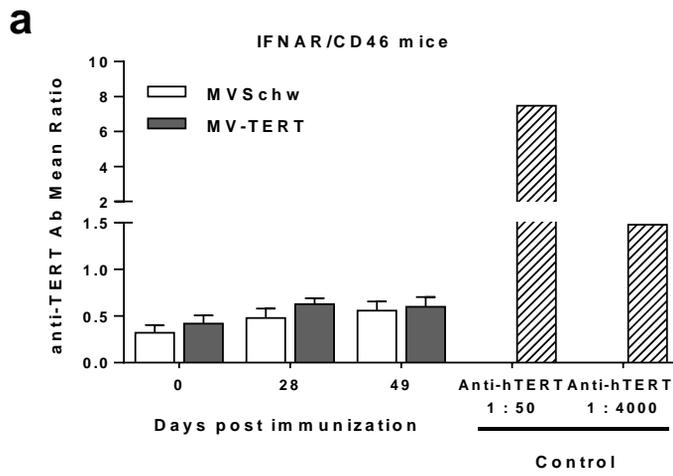
**FIGURE 1****a****b**

## FIGURE 2



**FIGURE 3****a****b**

# FIGURE 4



## FIGURE 5

