The Role of Oncolytic Viruses in Inducing Immunogenic Tumor Cell Death

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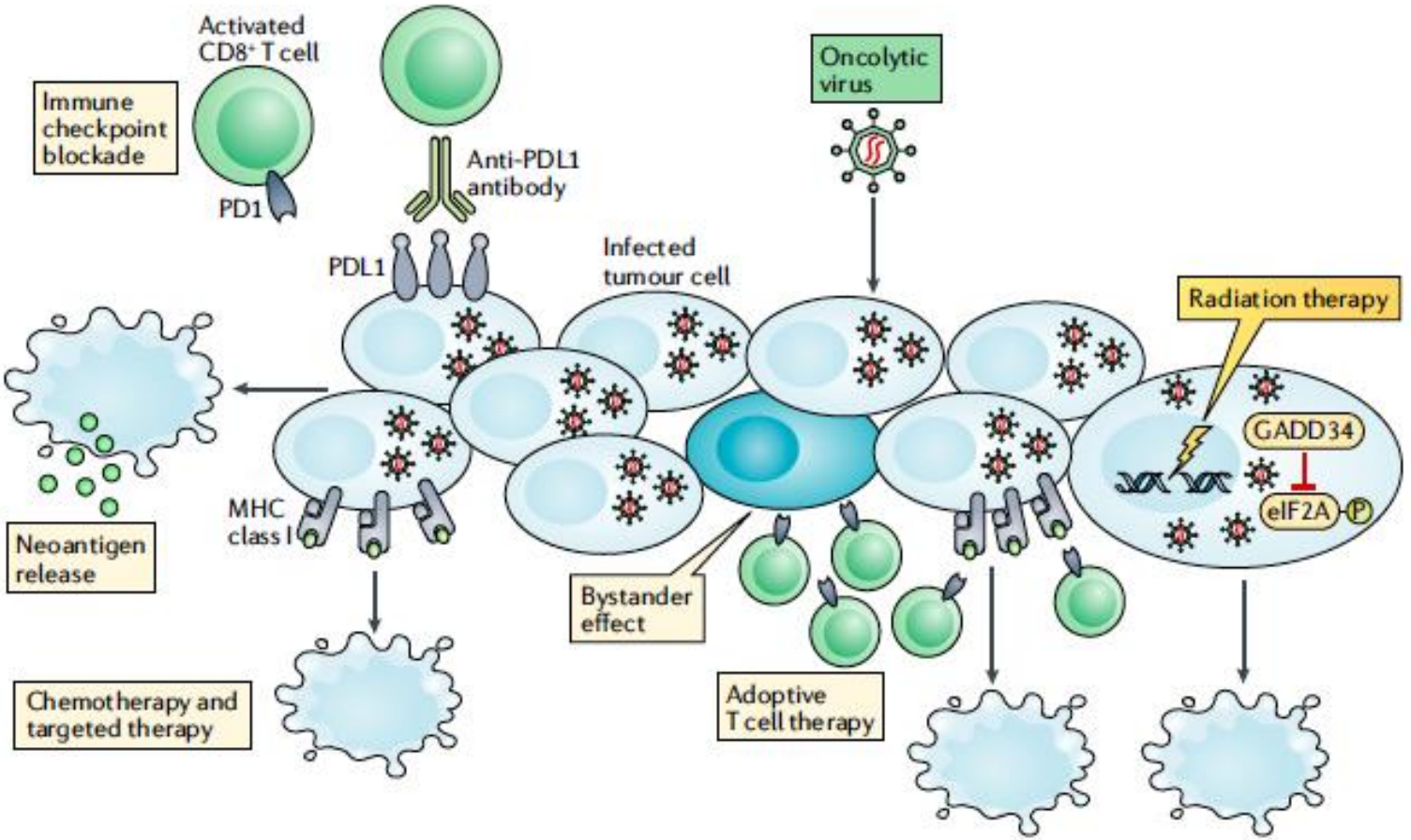
Work presented relates to prior lab at Rutgers University

Disclosures

- Employee of Replimune, Inc.
- The work presented was conducted at my prior lab at Rutgers University at which time I was an advisor to Amgen
- Work with mT-VEC was conducted under an MTA between Amgen & Rutgers University

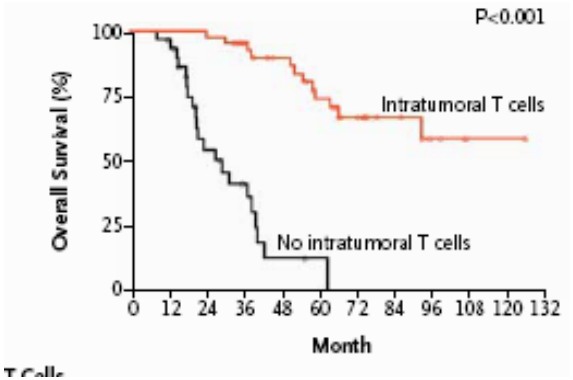
Strategies to induce immunogenic cell death



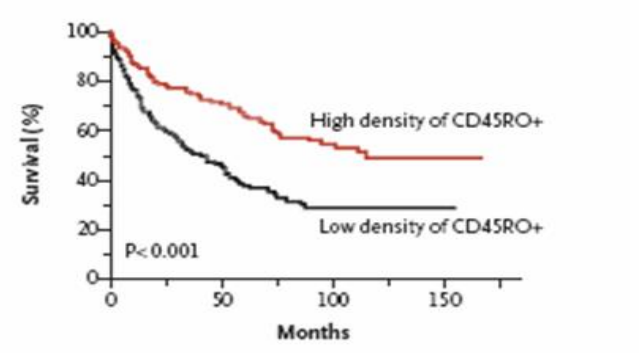
Bommareddy et al. Nature Rev Immunol. 2018

Tumor infiltrating T cells are correlated with cancer survival

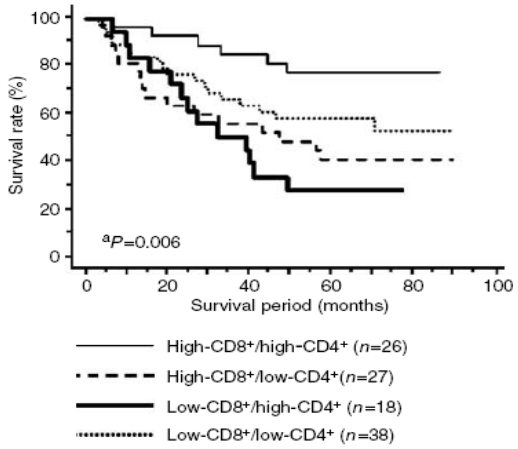
Ovarian Cancer



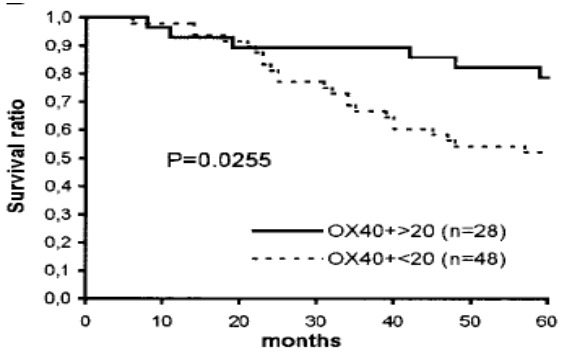
Colorectal Cancer



Lung Cancer



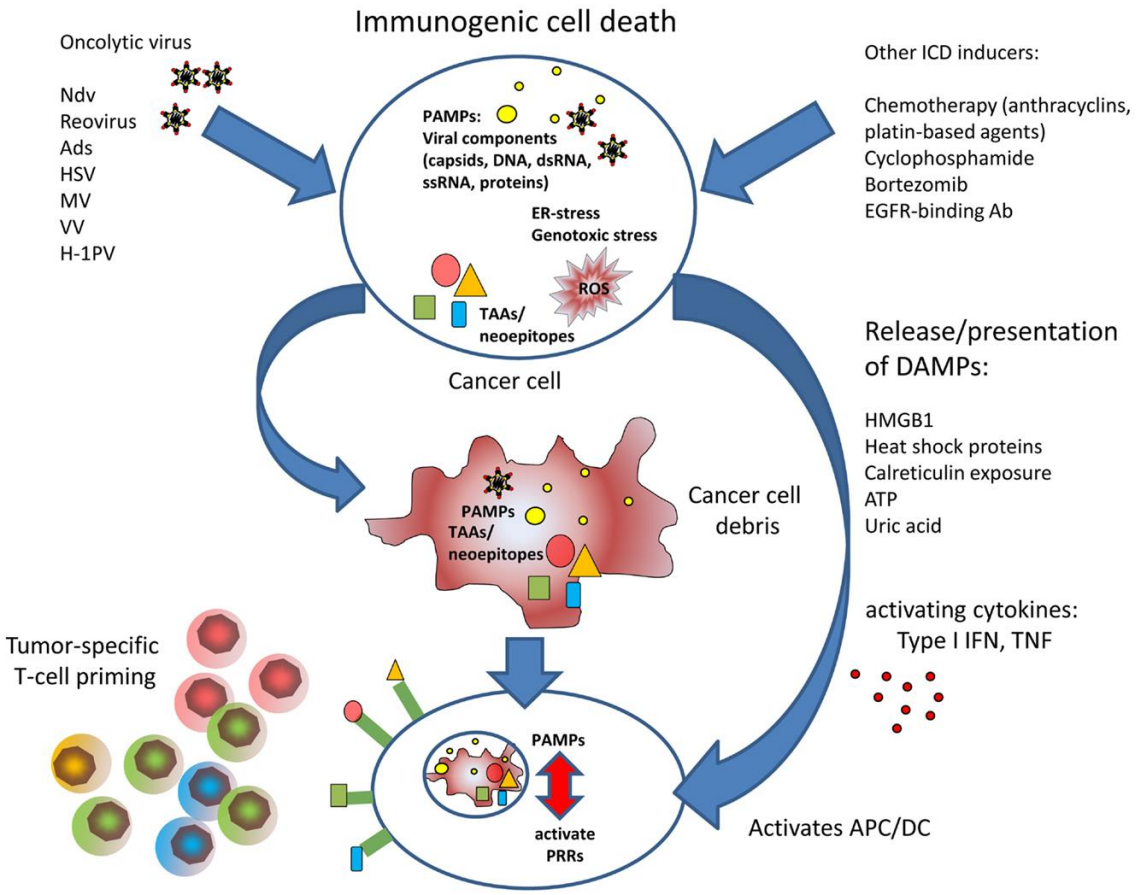
Melanoma



Zhang et al. NEJM 2003
Pages et al. NEJM 2005
Hiraoka et al. Br J Cancer 2006
Ladanyi et al. Clin Cancer Res 2004

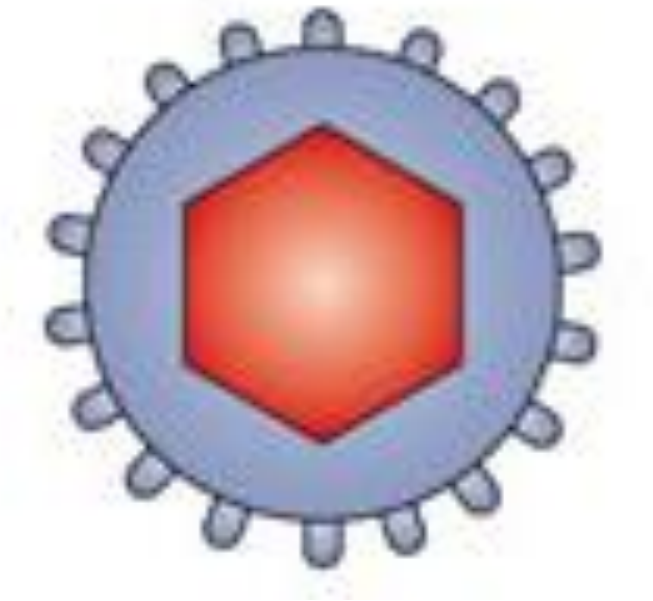
Why Oncolytic Viruses?

- Cytotoxicity
- Immunity
- Safety



Talimogene laherparepvec (T-VEC; Imlygic™): Engineered HSV-1 Oncolytic Virus

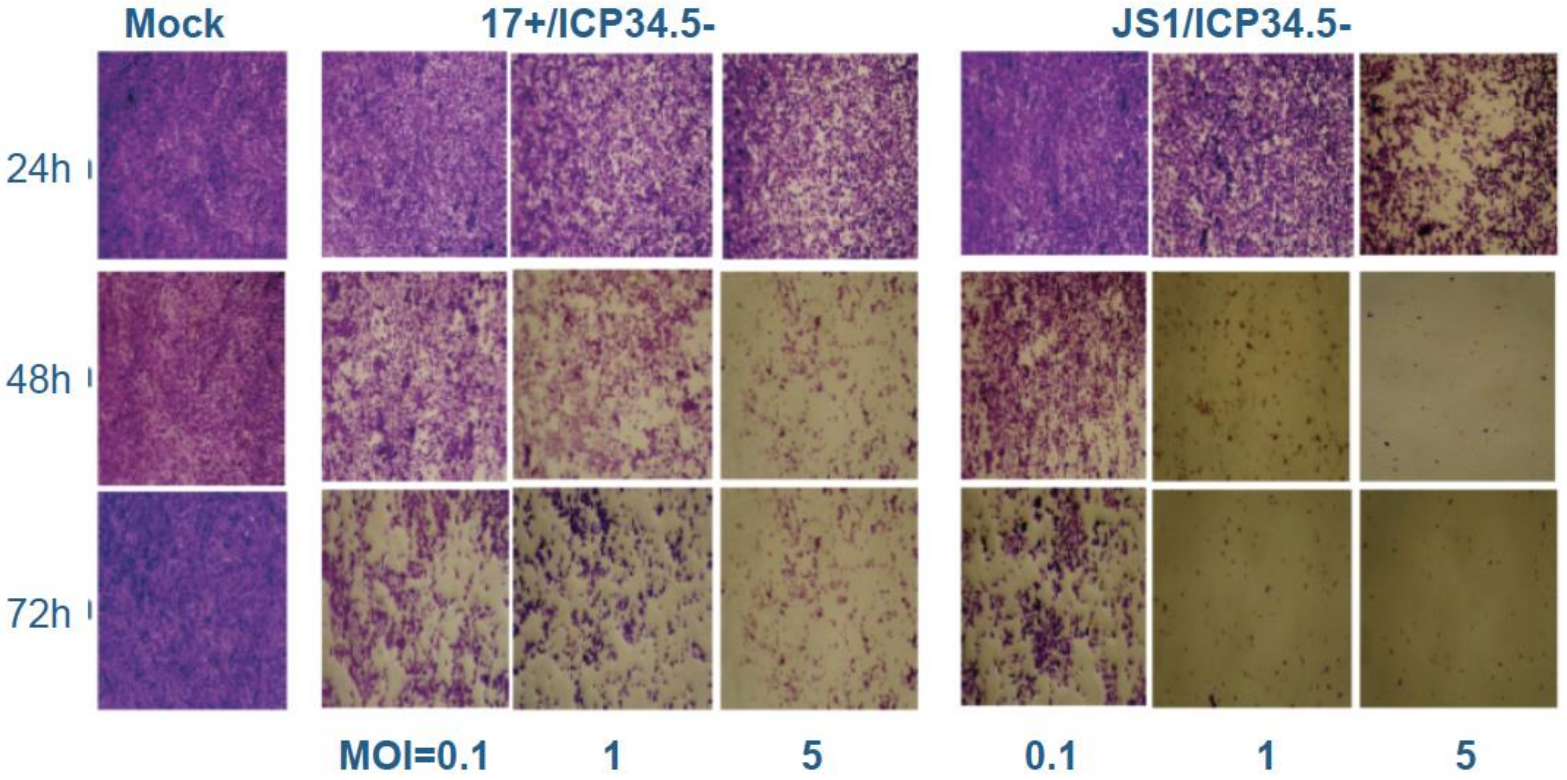
T-VEC (Herpes Virus)



Modifications	Rationale
JS-1 strain	Improved Cancer Cell Lysis
Deletion of ICP34.5	Cancer Cell Specific Replication
Early Expression of US11	Cancer Cell Specific Replication
Deletion of ICP47	Permits Antigen Presentation
Insertion of GM-CSF	Augments anti-tumor Immune response

Kaufman, Kohlhapp, and Zloza Nat Rev Drug Discov. 2015 Sep;14(9):642-62

T-VEC has profound lytic effect against SK-MEL-28 melanoma cells



Liu et al Gene Therapy 2003

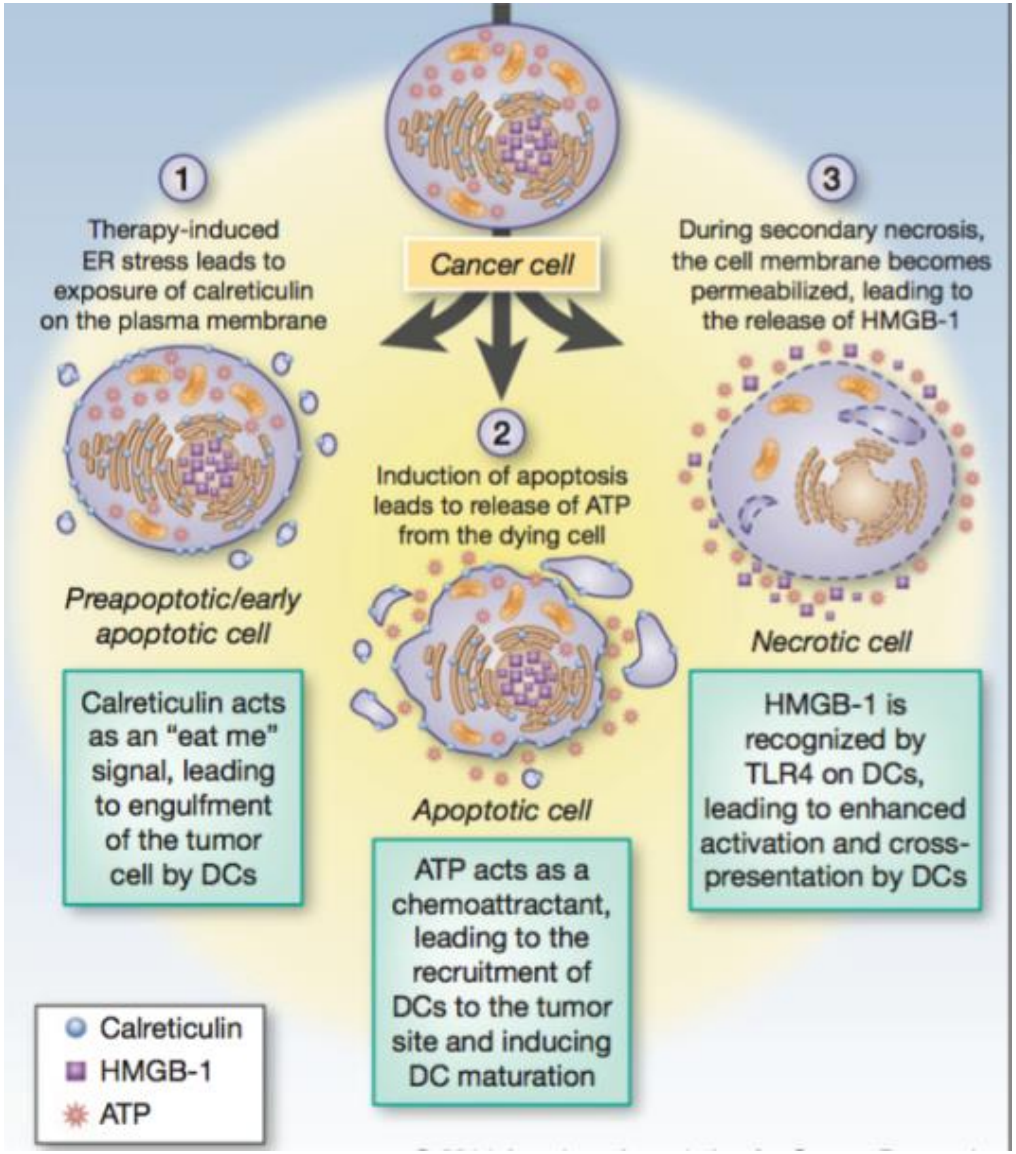
Anti-tumor activity of T-VEC in other tumor cell lines *in vitro*

Cell lines	**Tissue**	**Cell survival (%) (MOI=1)**			
		24 hrs	**48 hrs**	**3 days**	**6 days**
A549	Lung cancer	82.5	76.0	55.8	43.1
H460	Lung cancer	65.2	64.0	44.0	27.6
CALU-1	Lung cancer	71.1	60.0	41.9	40.4
PANC-1	Pancreatic cancer	74.6	57.6	24.1	9.4
MIA PACA-2	Pancreatic cancer	66.5	38.5	18.6	1.4
CAPAN-1	Pancreatic cancer	81.0	42.2	56.6	20.3
BxPC-1	Pancreatic cancer	57.6	15.1	16.1	8
HCT116	Colorectal cancer	65.7	27.4	14	1.1
HT29	Colorectal cancer	51.6	22.0	24.3	3.9
SW620	Colorectal cancer	80.4	66.8	45.0	3.9
COLO205	Colorectal cancer	49.8	20.0	9.7	3.1

MTT assays

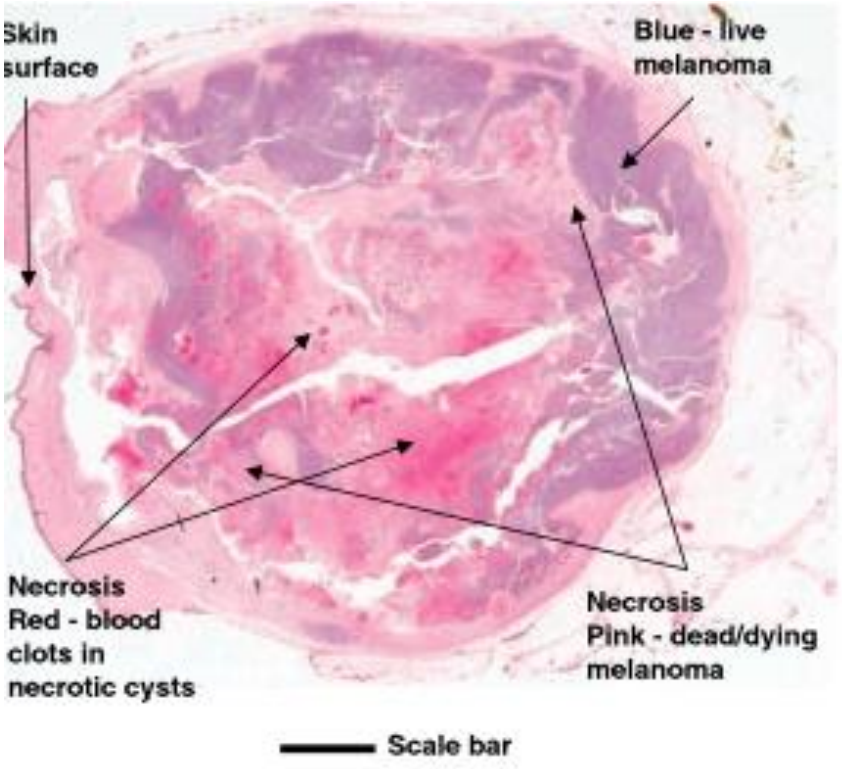
(Higher MOI's result in complete lysis of all cells)

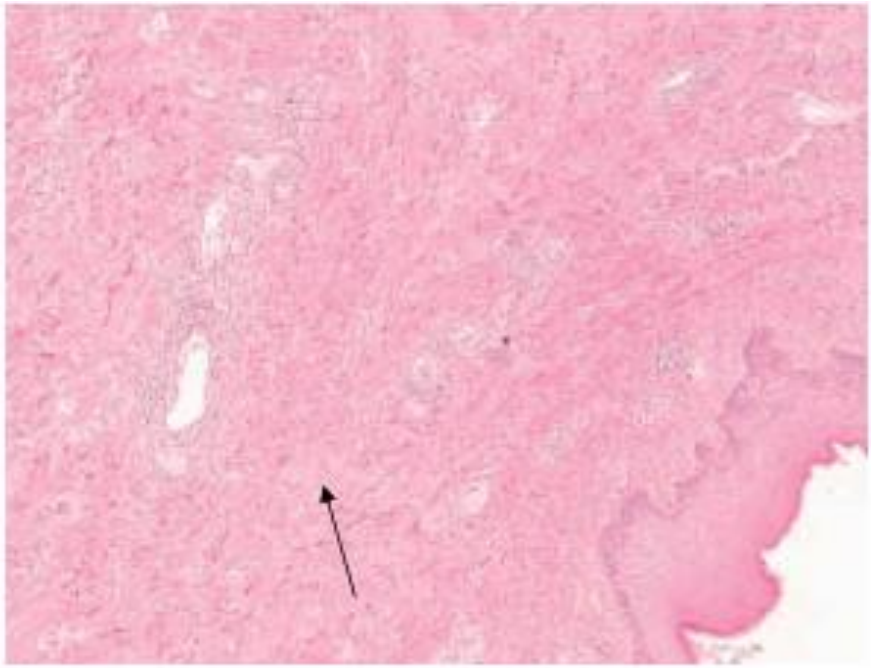
Immunogenic cell death



Stanleyson et al Clin Cancer Res 2014

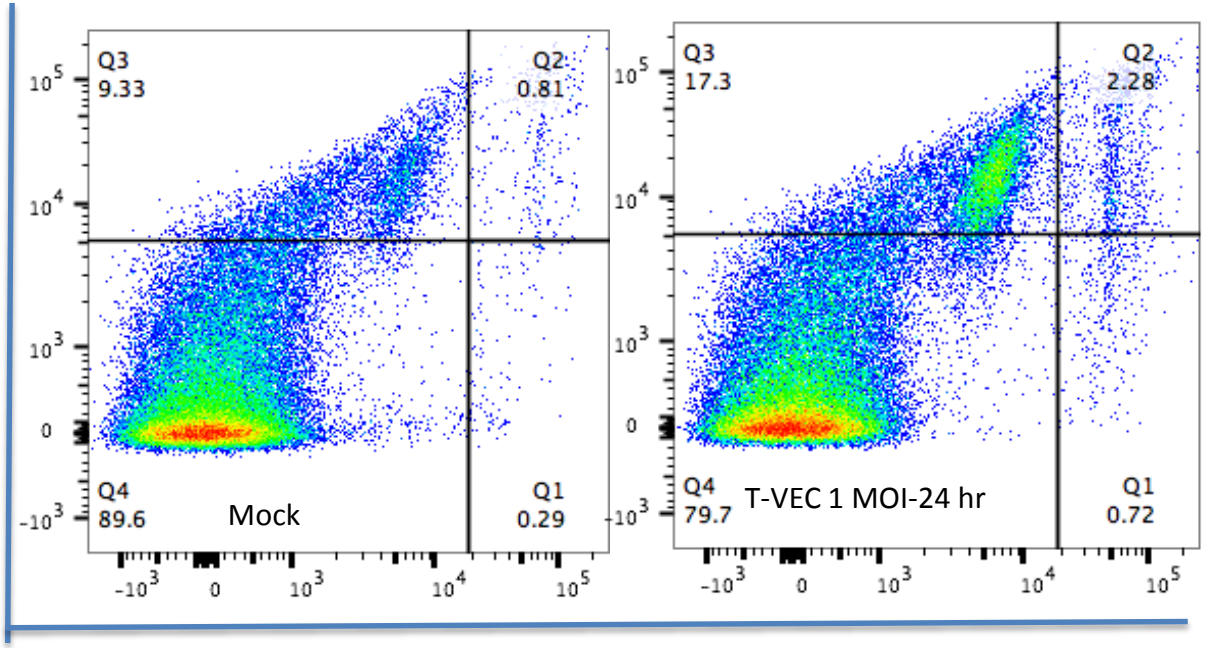
T-VEC induces central necrosis following injection

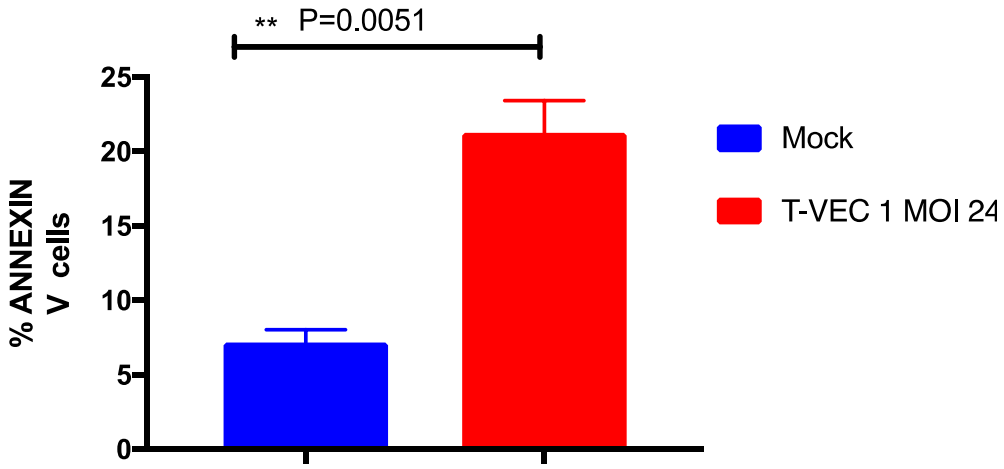


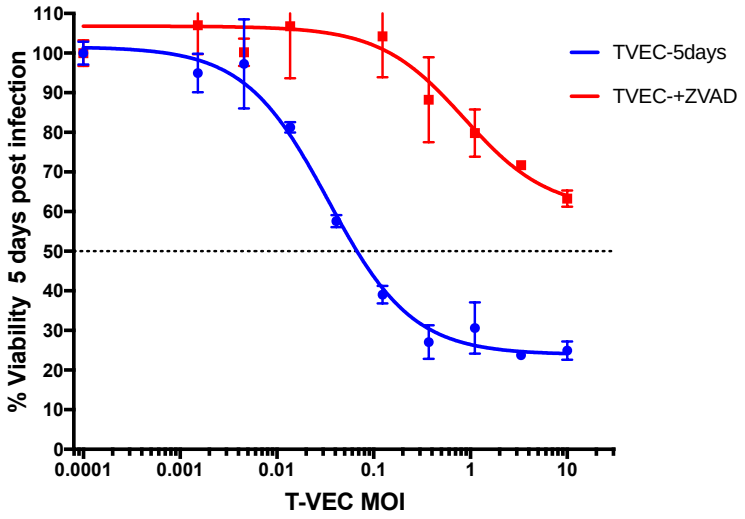


Liu et al. Clin Cancer Res 2006

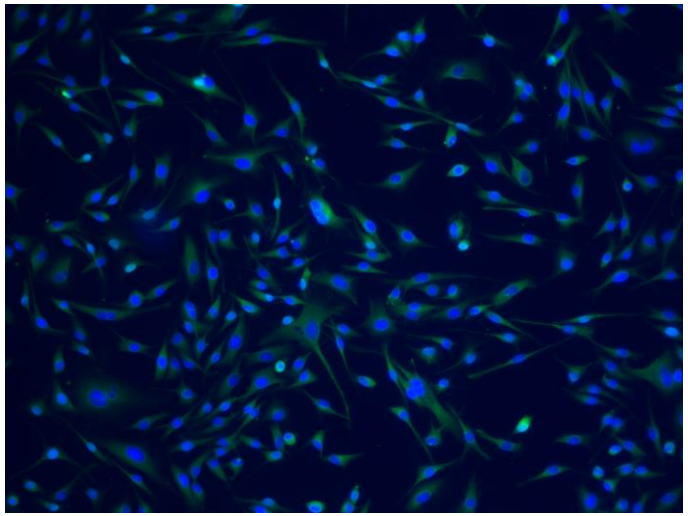
T-VEC induces apoptosis in human melanoma cell lines and can be blocked by ZVAD



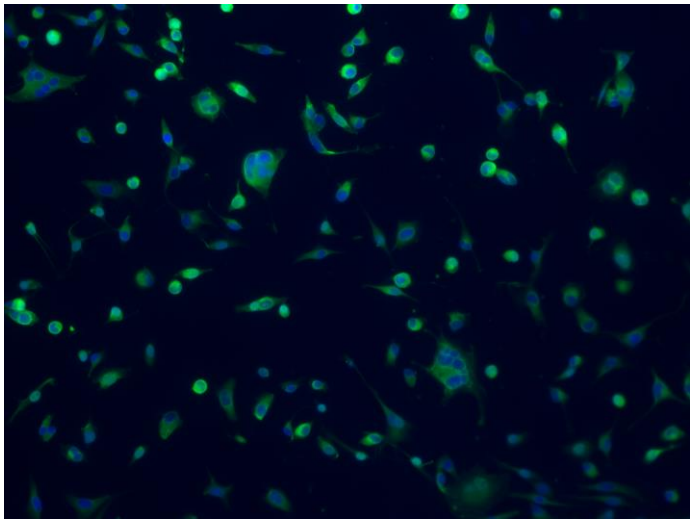




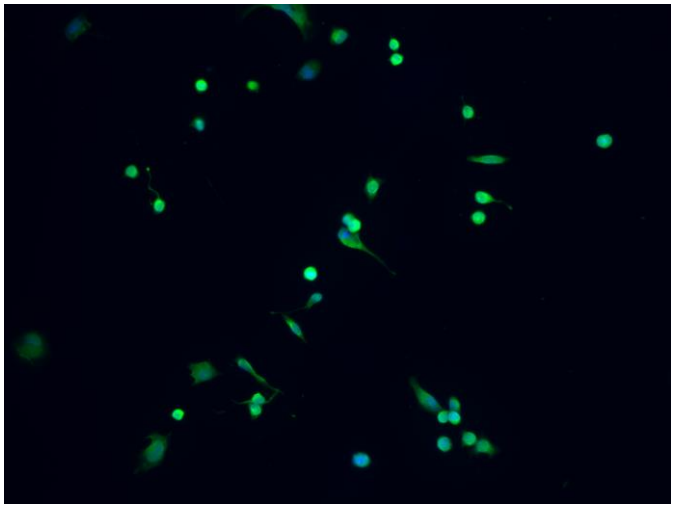
T-VEC induces calreticulin expression on the surface of SK-MEL28 cells in a dose-dependent manner*



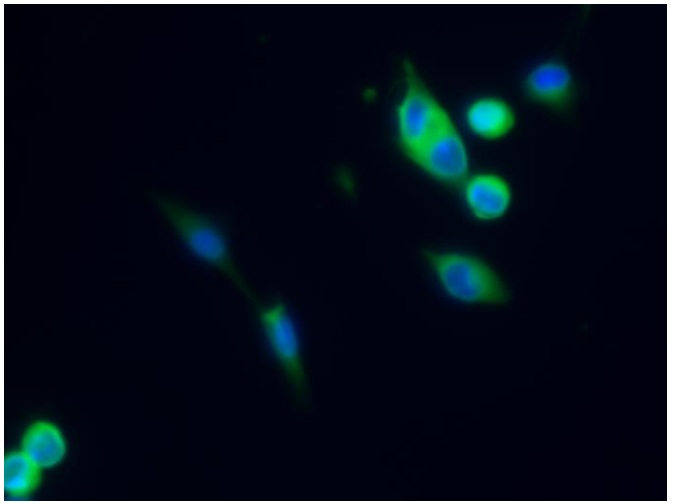
0.1 MOI (10X)



1.0 MOI (10X)



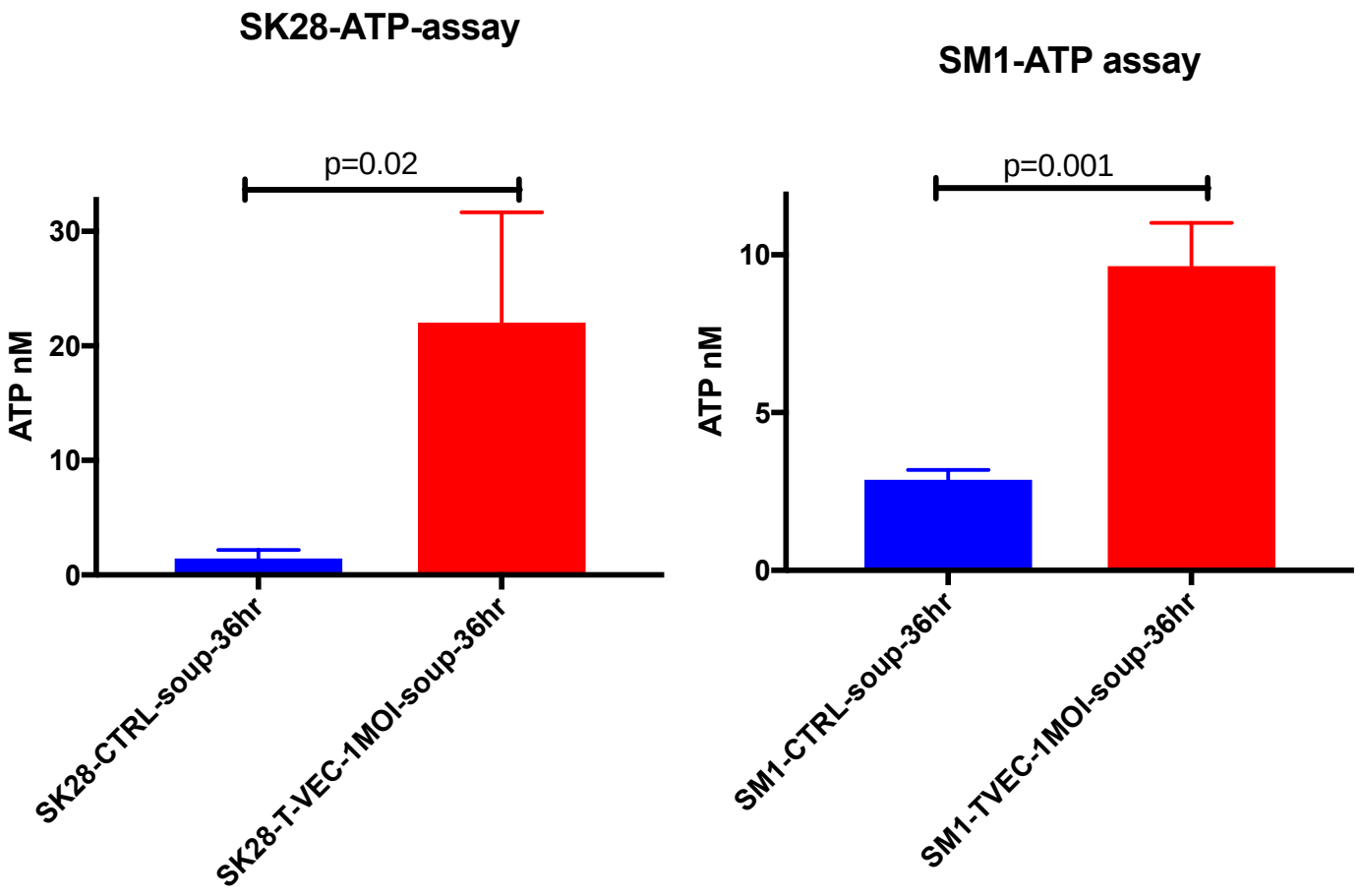
10.0 MOI (10X)



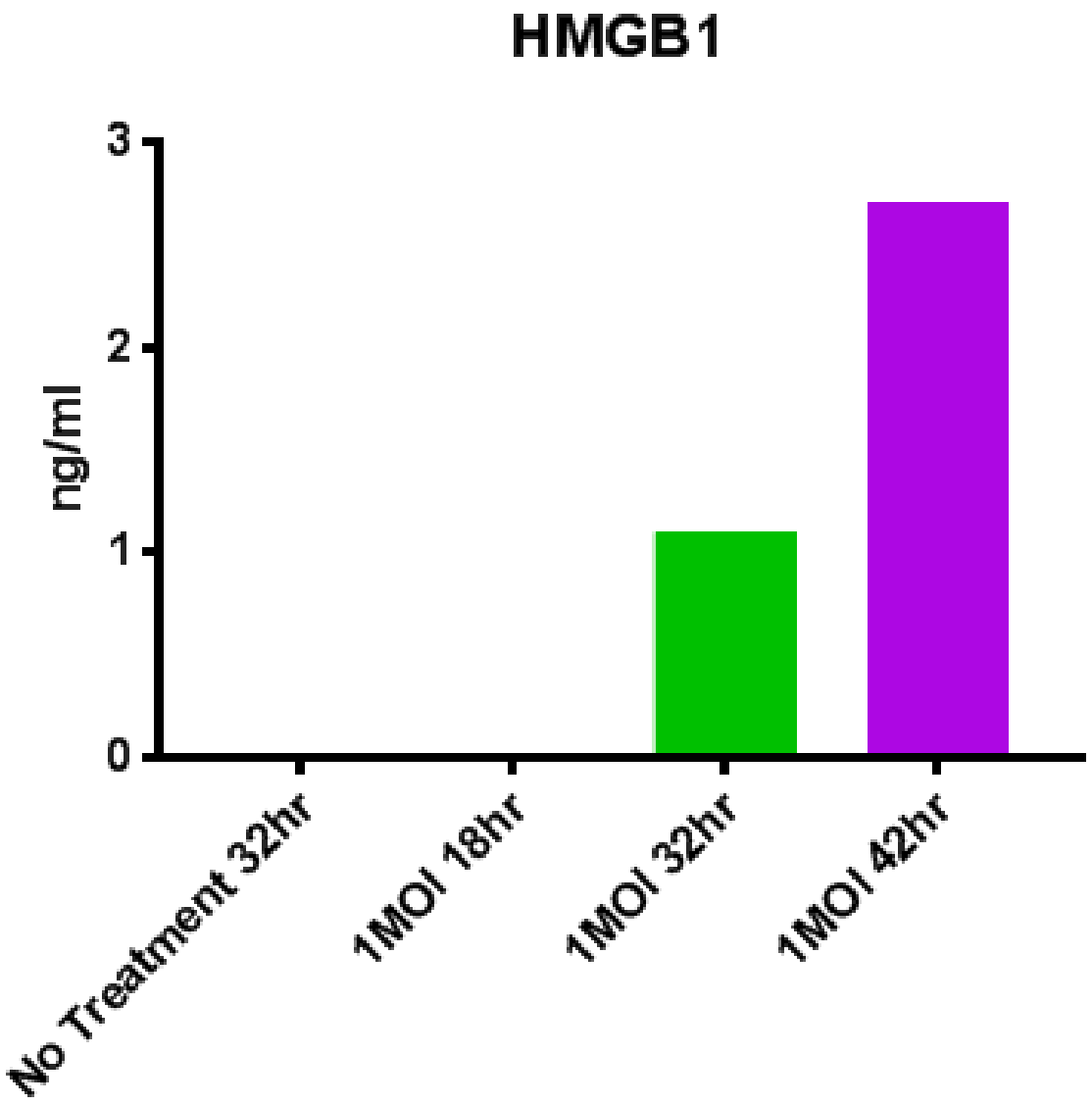
1.0 MOI (40X)

*Shown at 18 hours post-infection

T-VEC induces extracellular ATP release following infection of melanoma cells



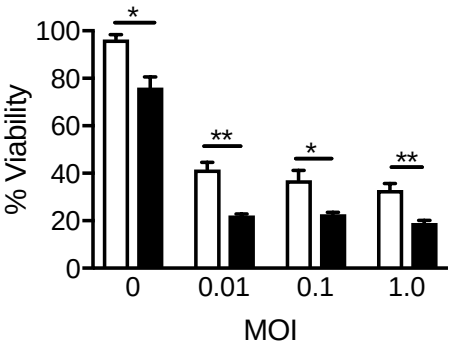
T-VEC induces release of HMGB1 from SKMEL 28 Melanoma cells



Can other cell death inducers enhance T-VEC-mediated oncolytic activity?

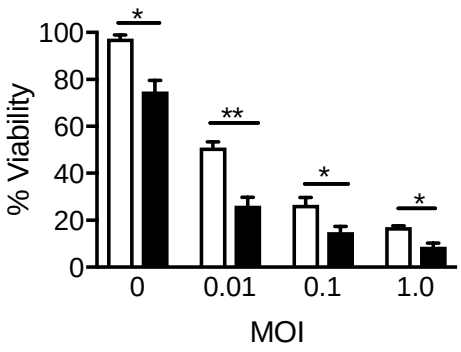
BRAF inhibition enhances T-VEC oncolytic activity in BRAF-mutant cell lines

SK-MEL-28



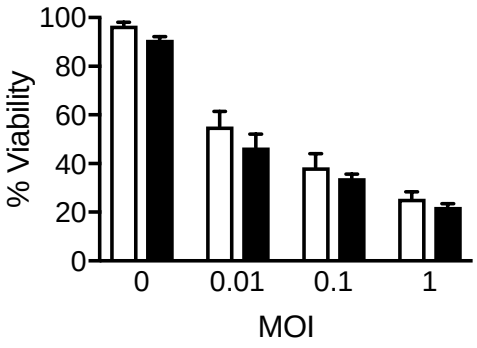
BRAF V600E
NRAS WT

SK-MEL-5



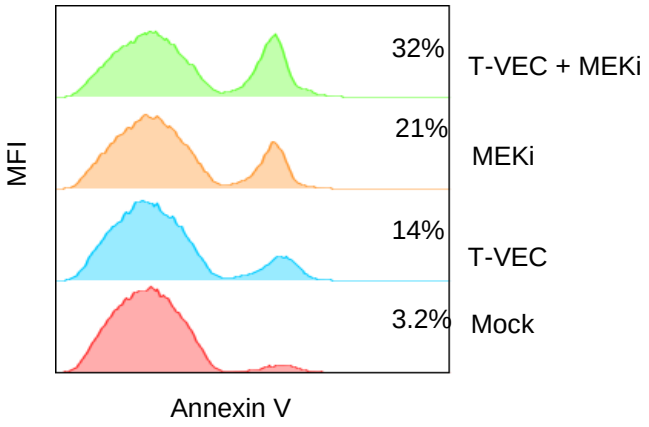
BRAFV600E
NRAS WT

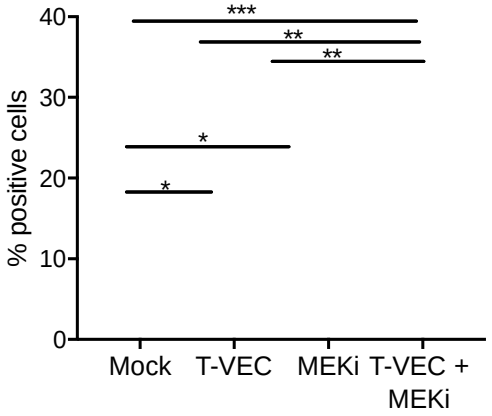
SK-MEL-2

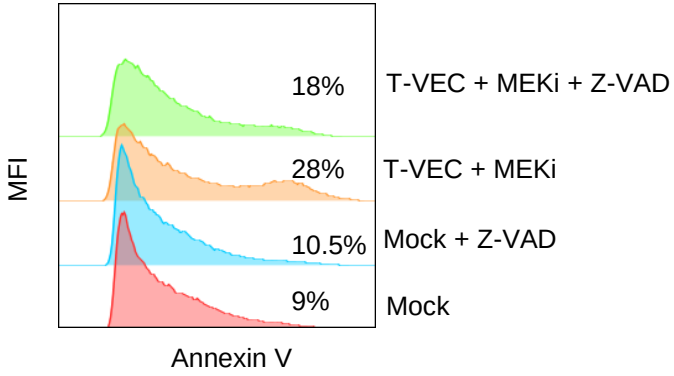


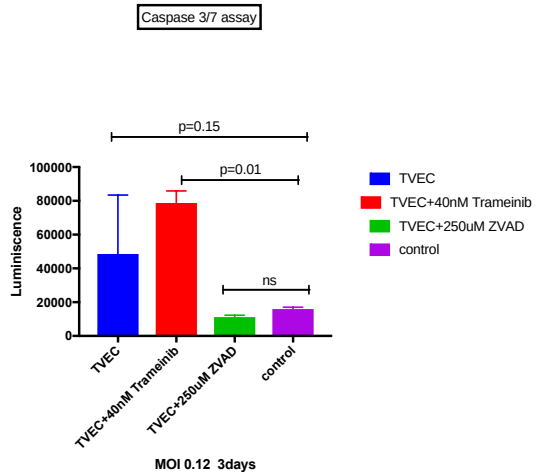
BRAF WT
NRAS Q16R

T-VEC and MEK inhibition induces apoptosis in SK-MEL28 cell line

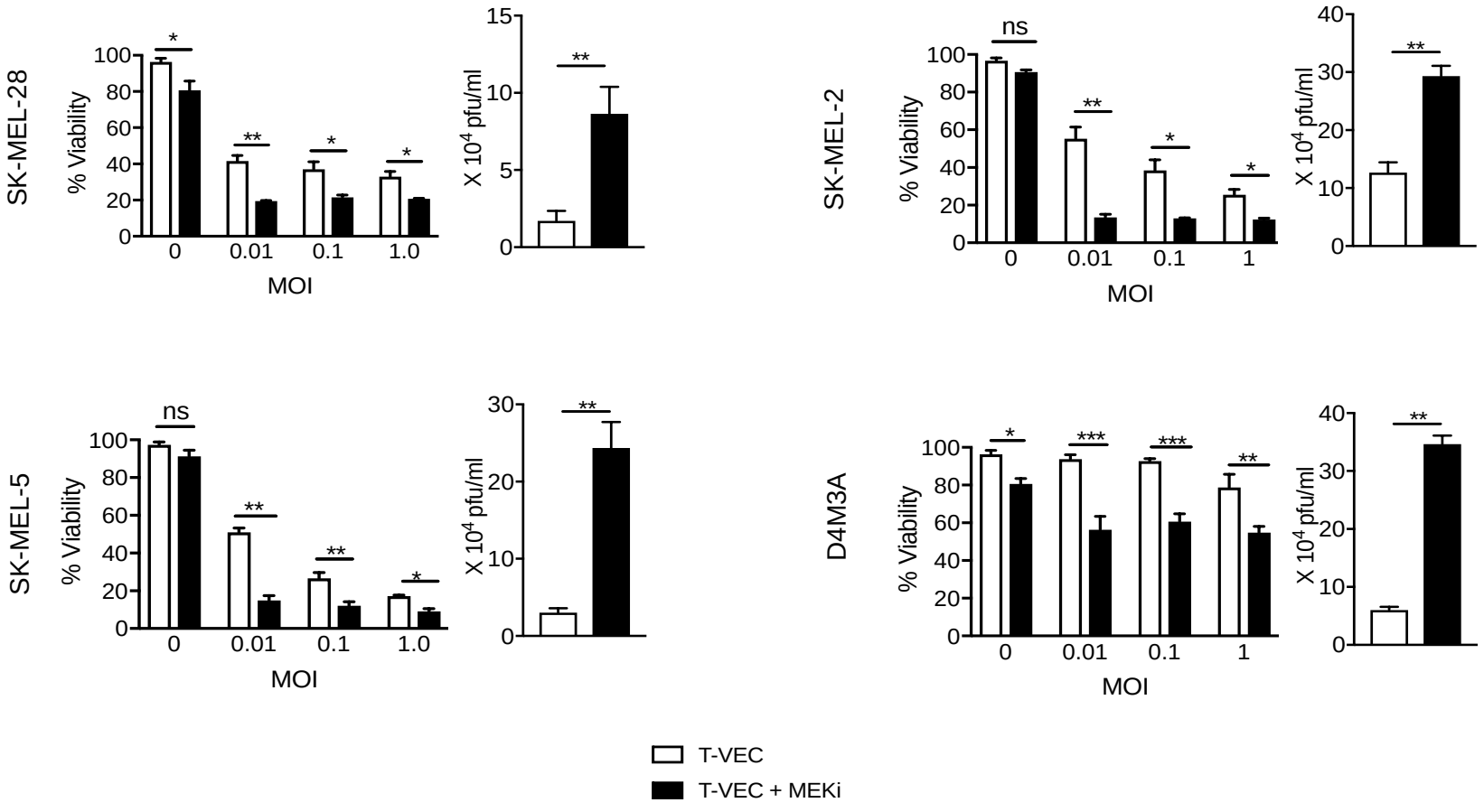




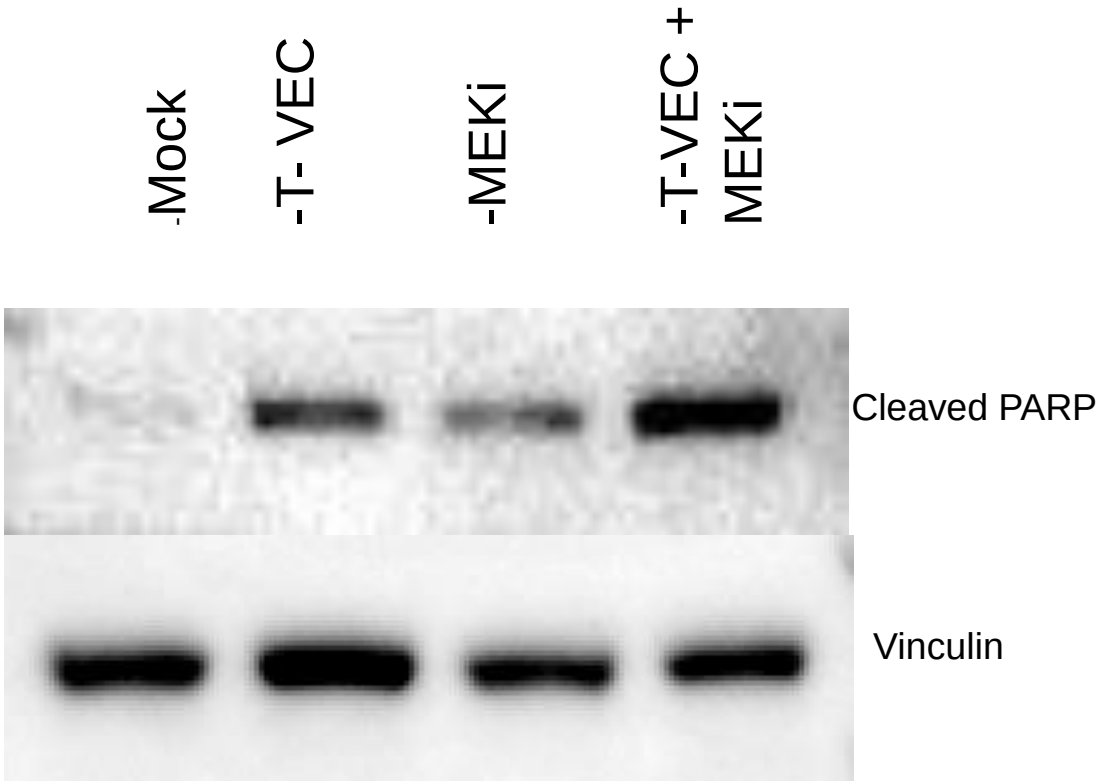




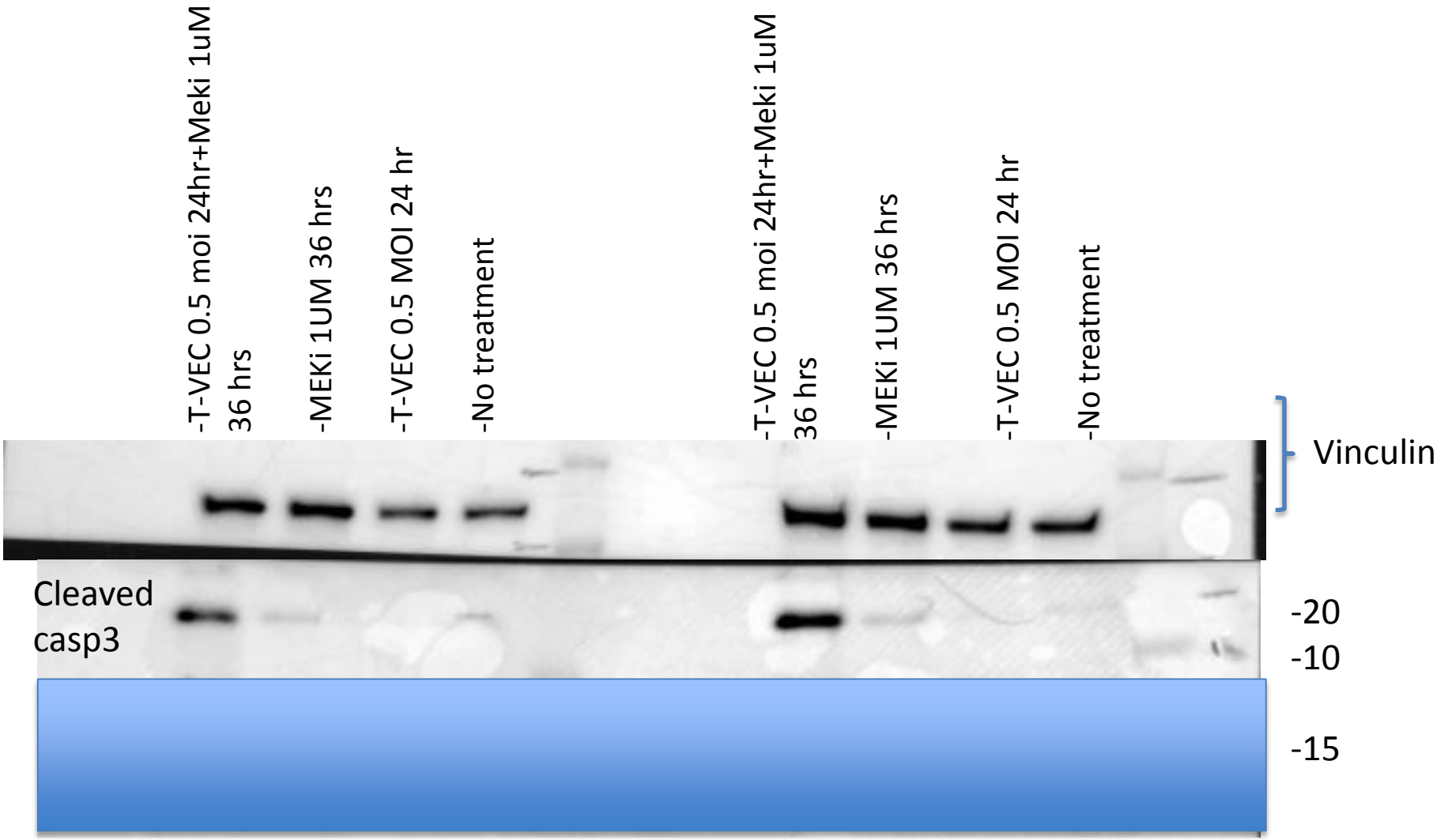
MEK inhibition enhances T-VEC-mediated cell killing and increases viral replication



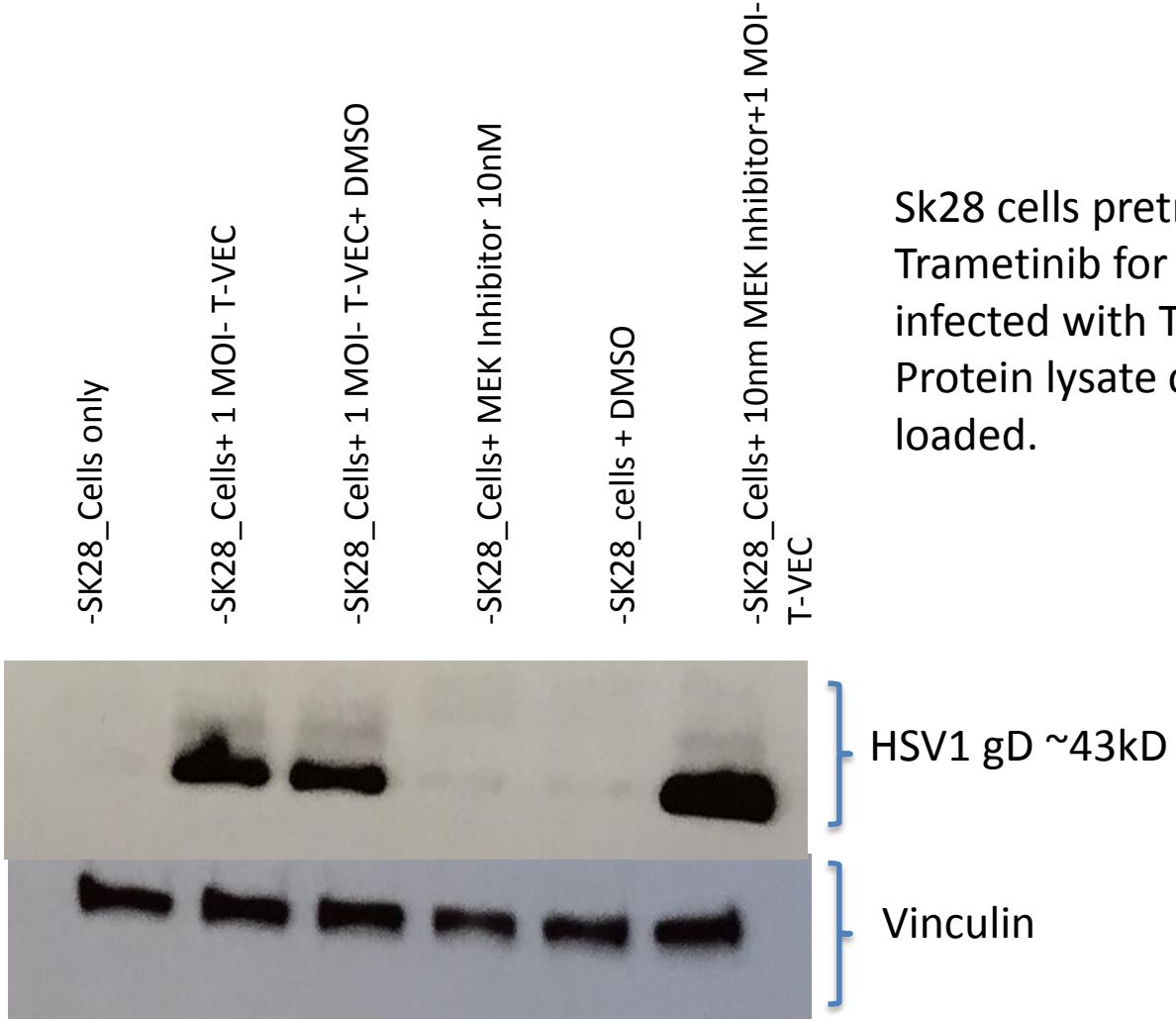
T-VEC and MEK inhibition induces PARP cleavage in SK-MEL-28 cell lines



T-VEC induces caspase 3 cleavage in the presence of MEK inhibition

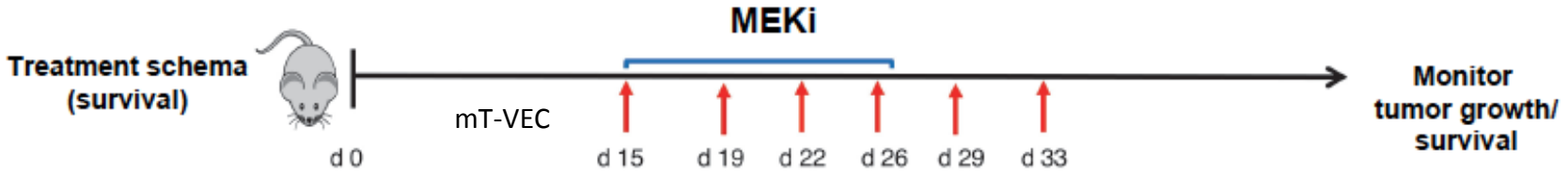


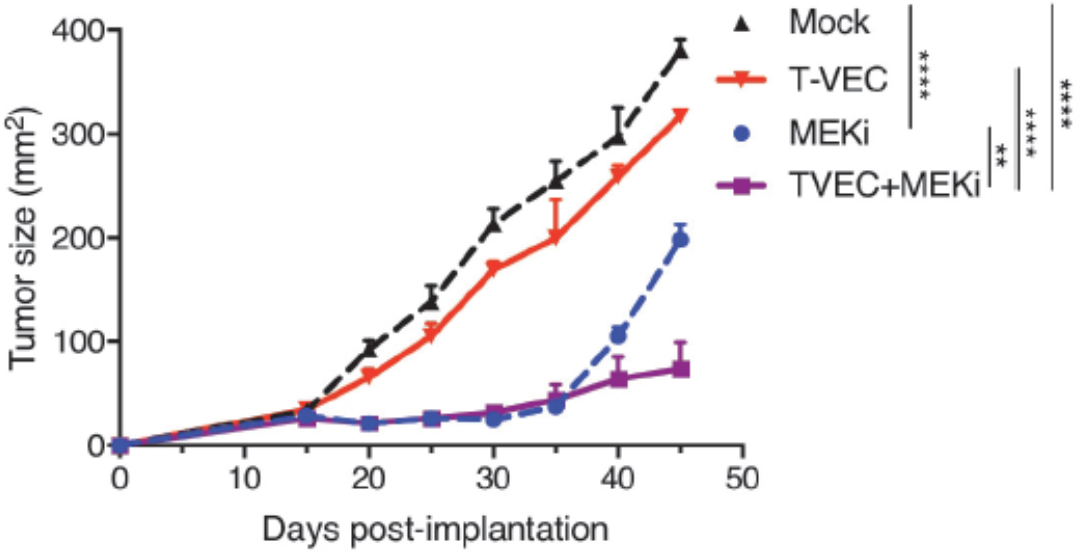
Increased cell lysis by combination T-VEC/MEKi may be due to increased viral replication



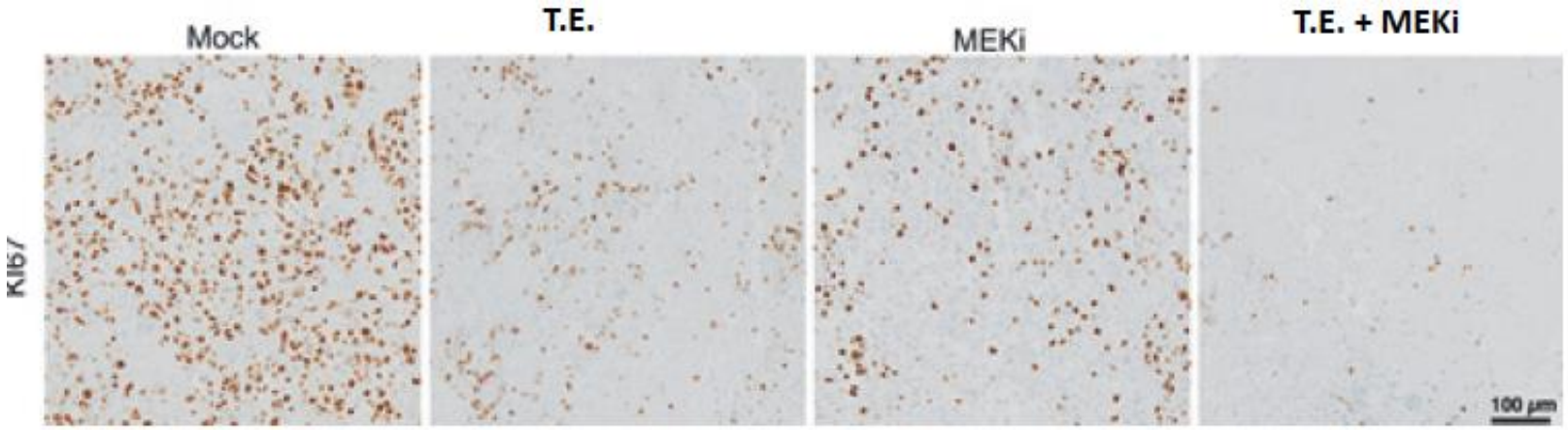
Sk28 cells pretreated with 10nM Trametinib for 12 hours and then infected with T-VEC 1 MOI for 16 hours. Protein lysate collected and 40ug is loaded.

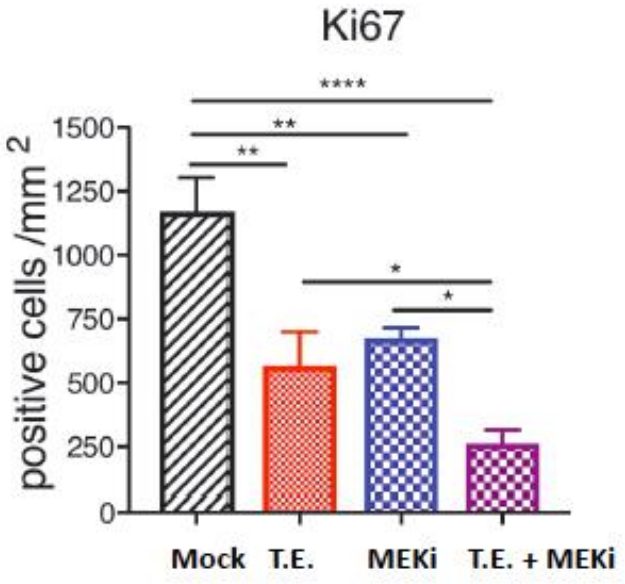
OV and MEK inhibition have therapeutic activity in melanoma xenograft model



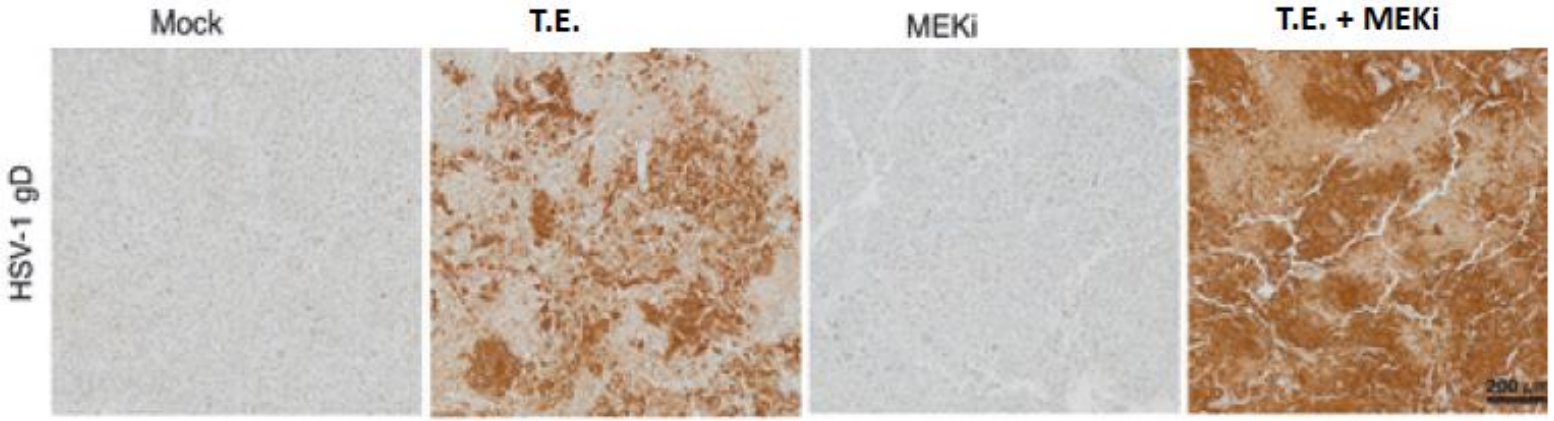


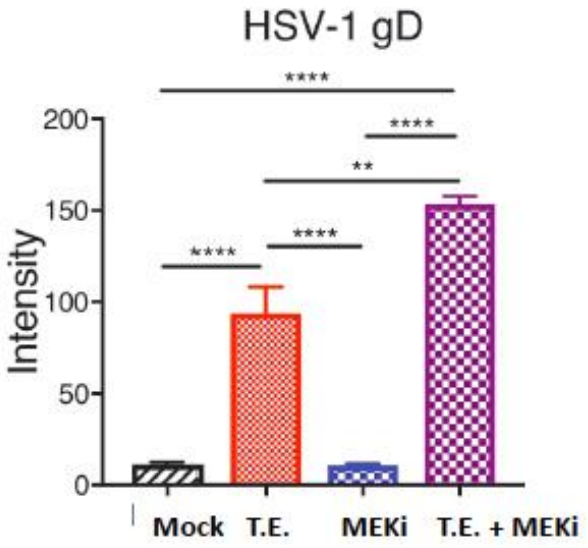
T-VEC and MEK inhibition enhances tumor cell killing in the TME



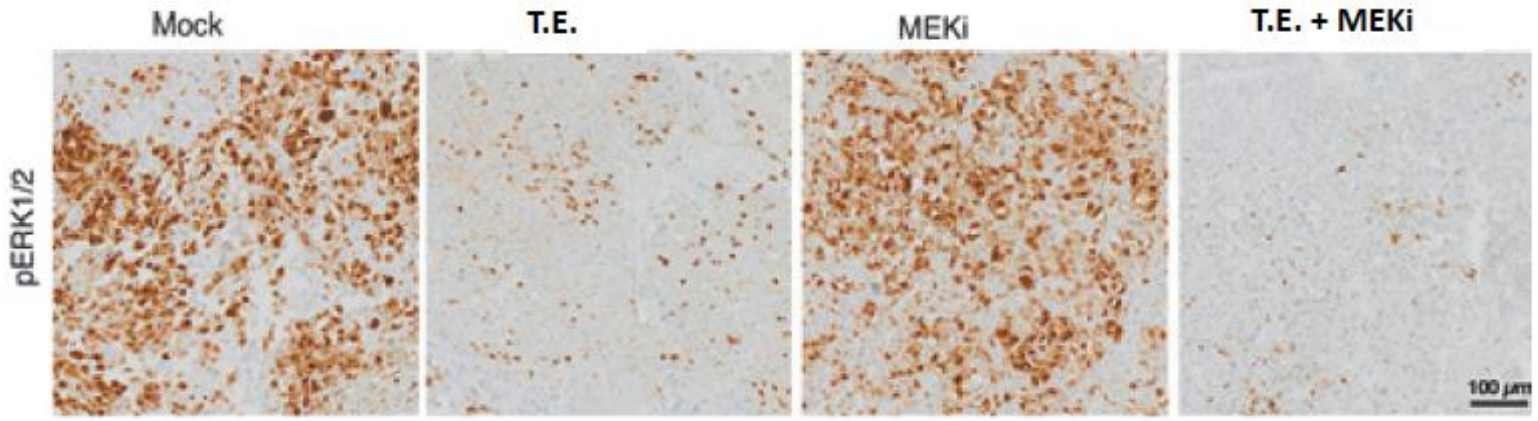


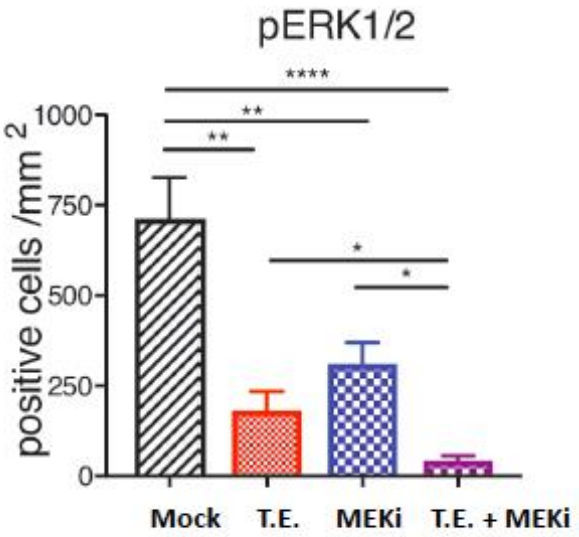
T-VEC and MEK inhibition enhances HSV-1 replication in the TME



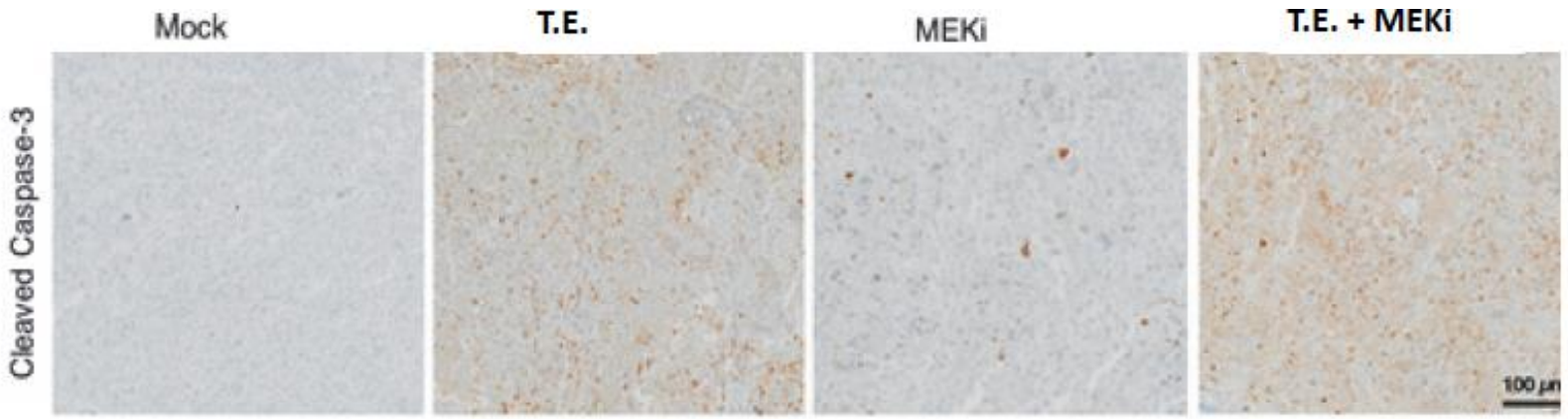


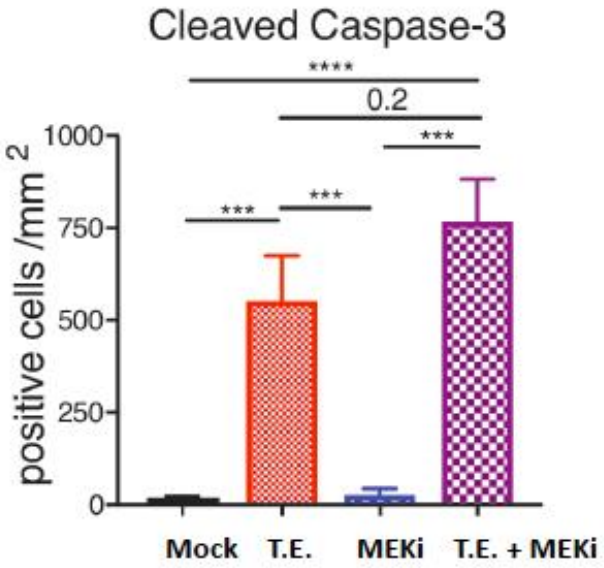
T-VEC and MEK inhibition reduces pERK1 and pERK2 in the TME



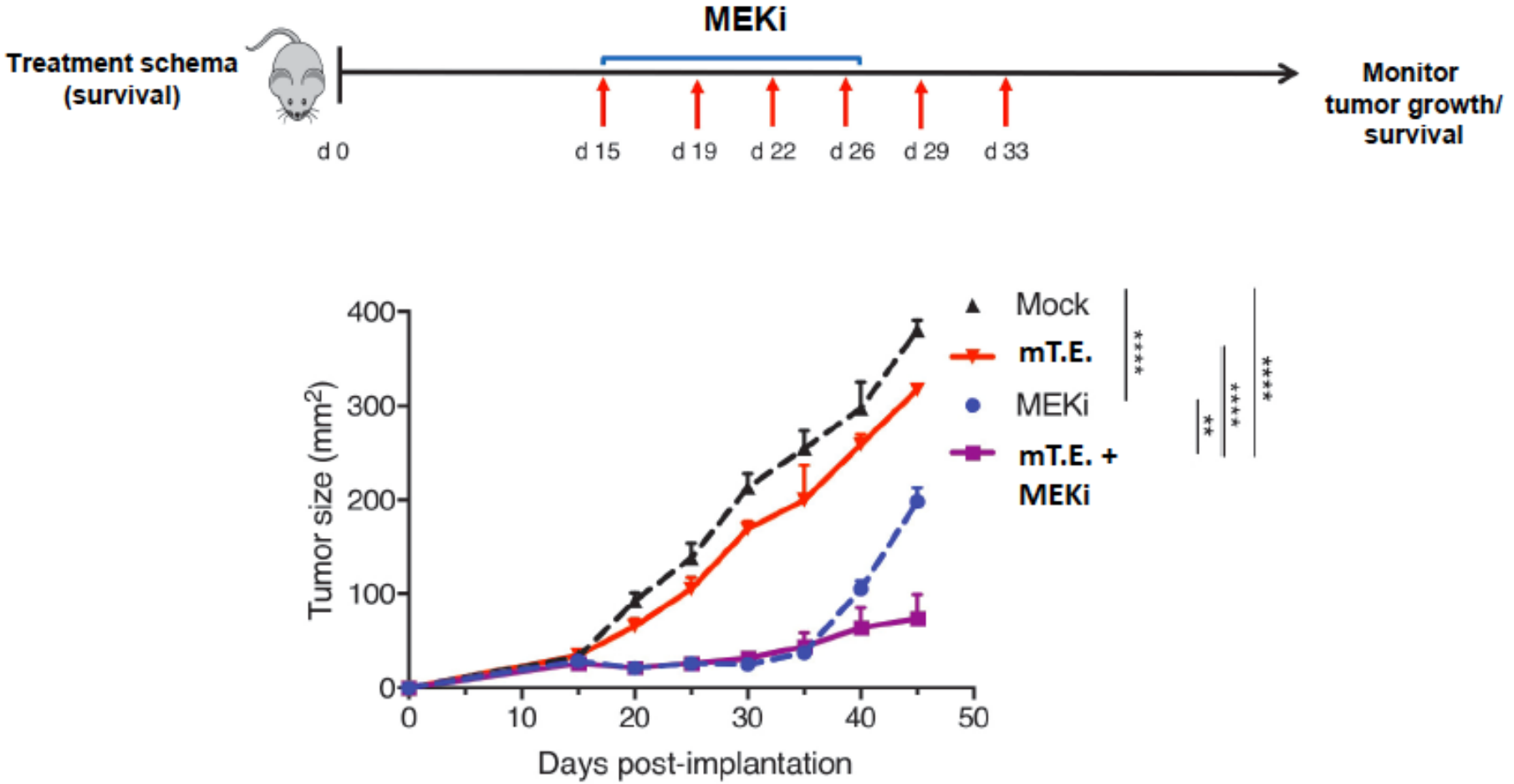


T-VEC and MEK inhibition induces caspase-3 cleavage in the TME





OV and MEK inhibition have therapeutic activity in immune competent melanoma model



T-VEC and MEK inhibition enhances CD8+ T cell recruitment into the TME

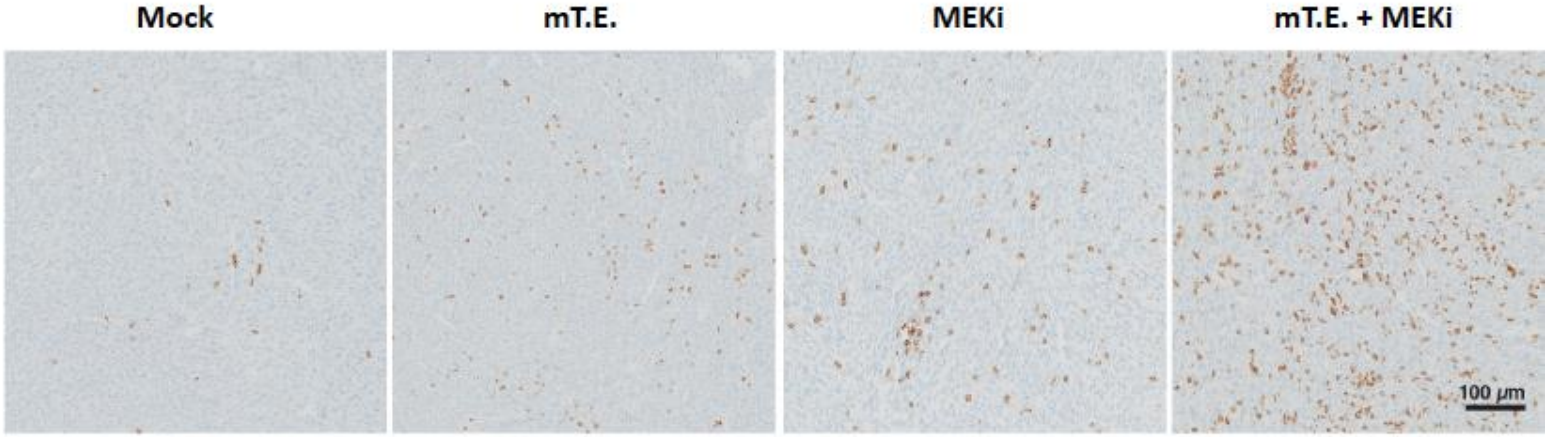
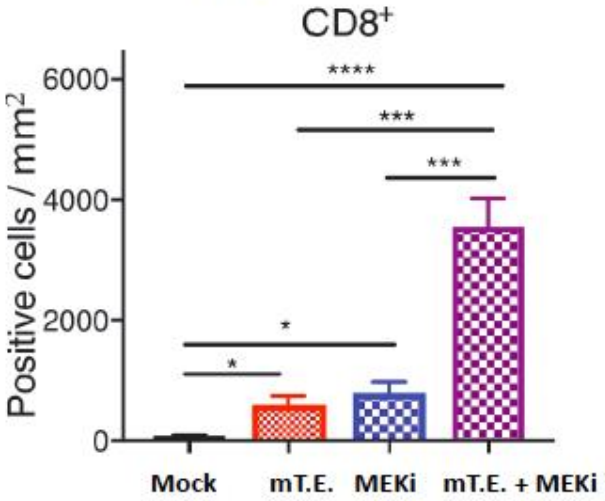
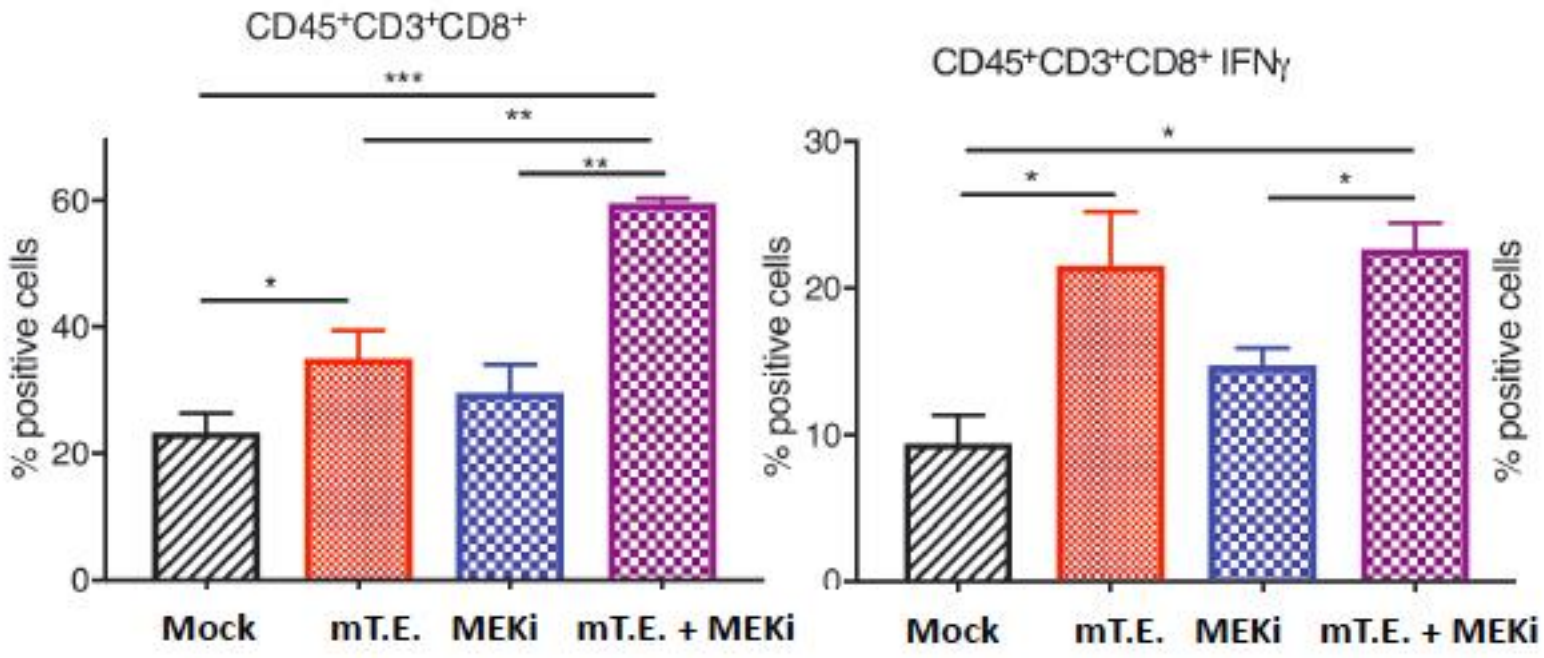


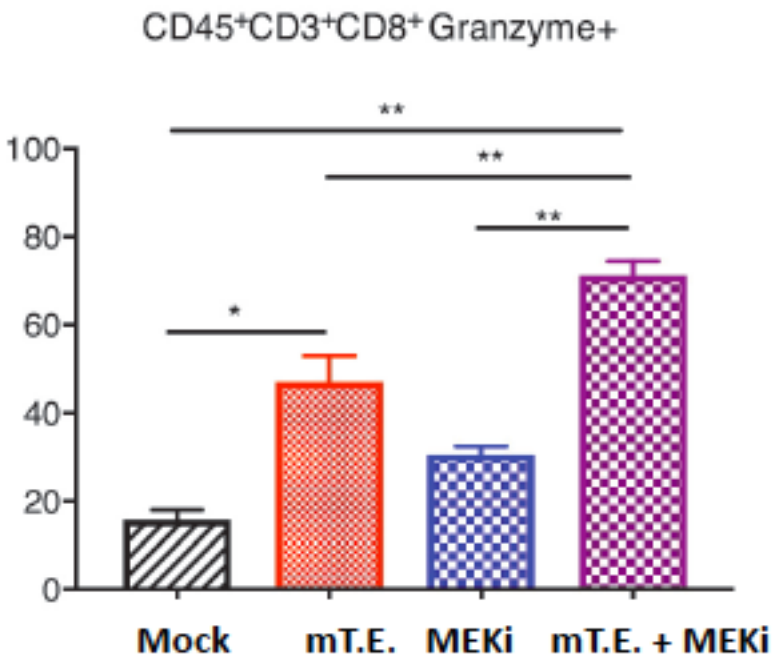
FIG. 3F

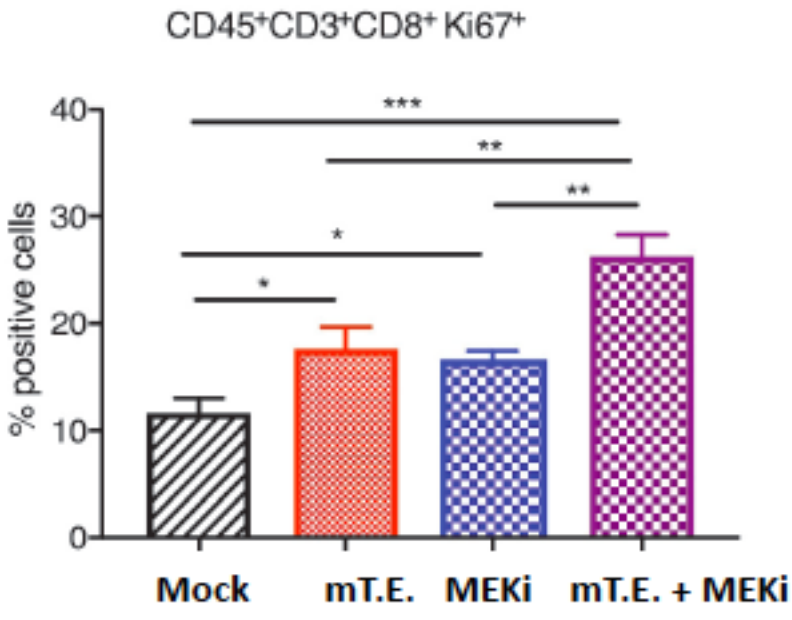


T-VEC and MEK inhibition enhance recruitment of CD8+ IFN-γ+ T cells

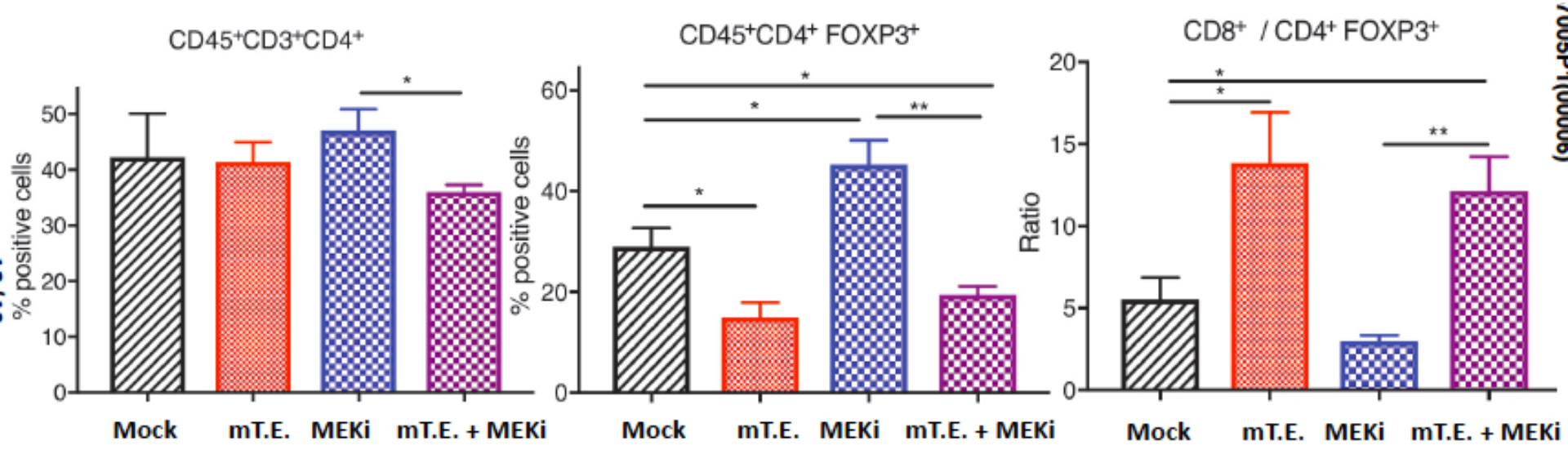


T-VEC and MEK inhibition enhances recruitment of CD8+ Granzyme B+ T cells

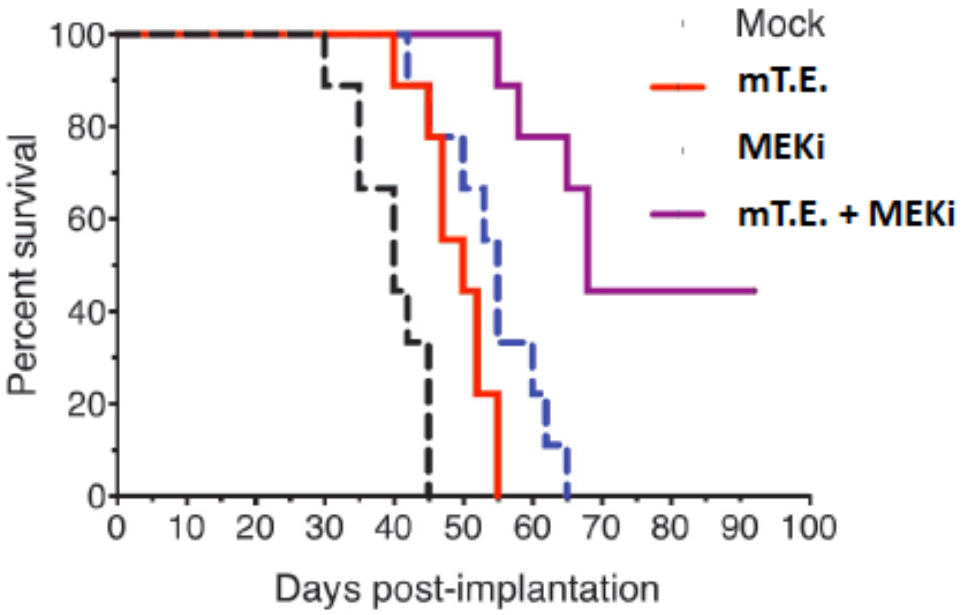


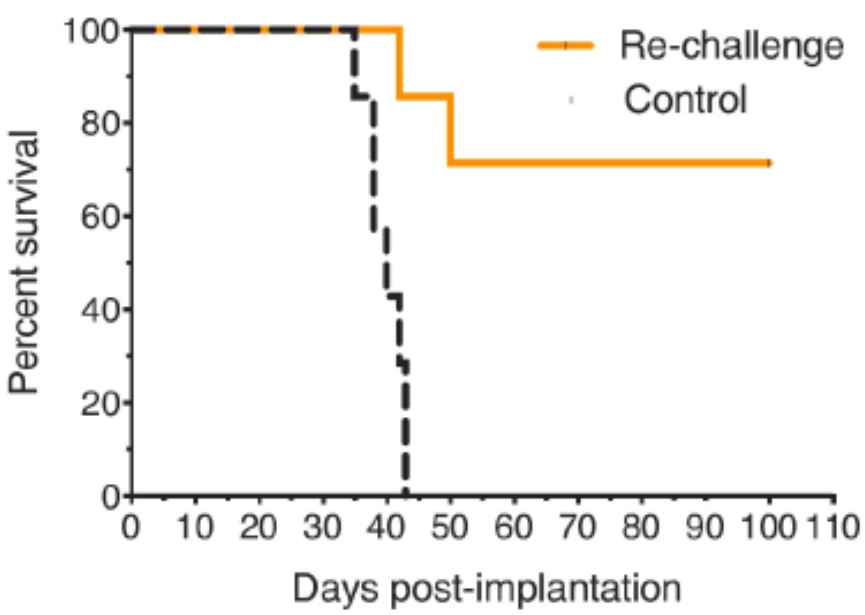


T-VEC and MEK inhibition reduces Tregs and increases the CD8+/Treg ratio in the TME

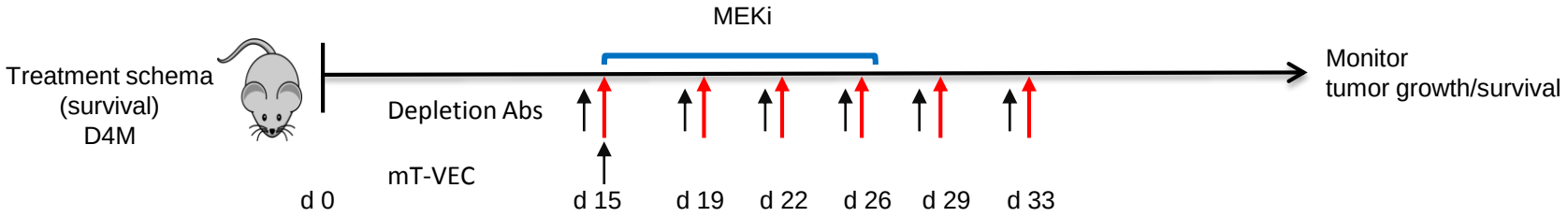


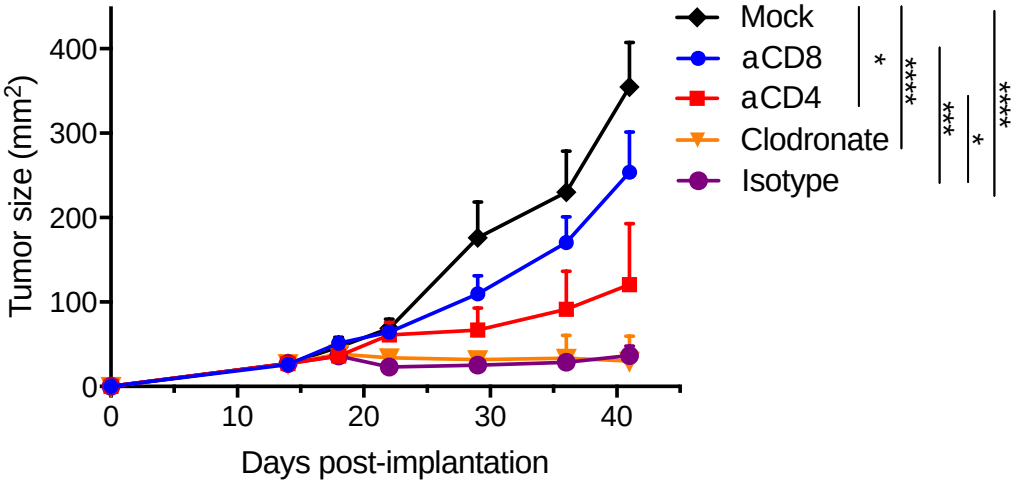
T-VEC and MEK inhibition enhances survival and results in memory

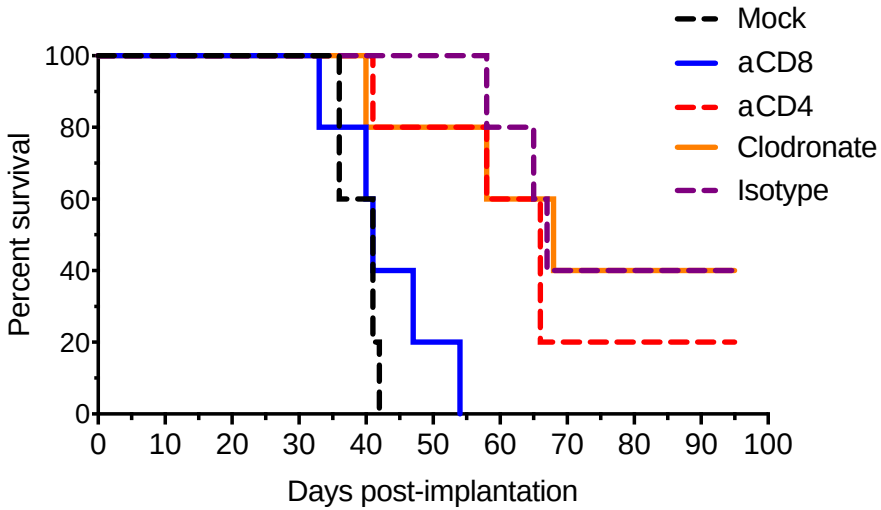




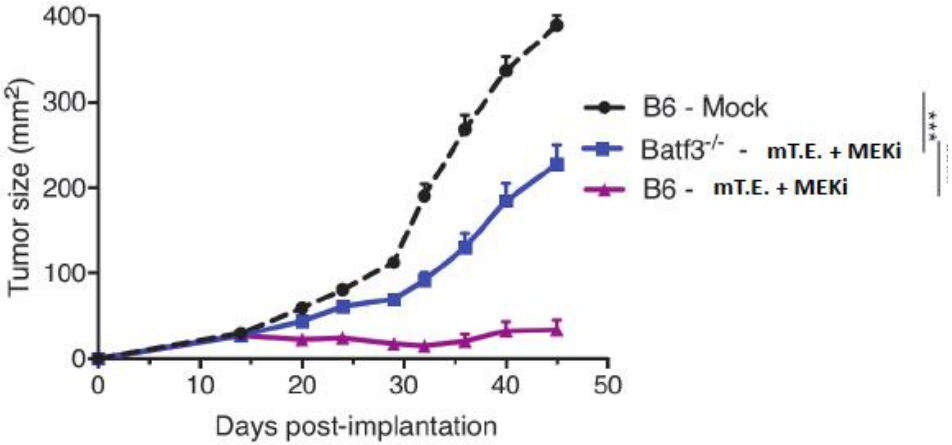
Depletion of CD8+ T cells abrogates the therapeutic effect of T-VEC and MEK inhibition

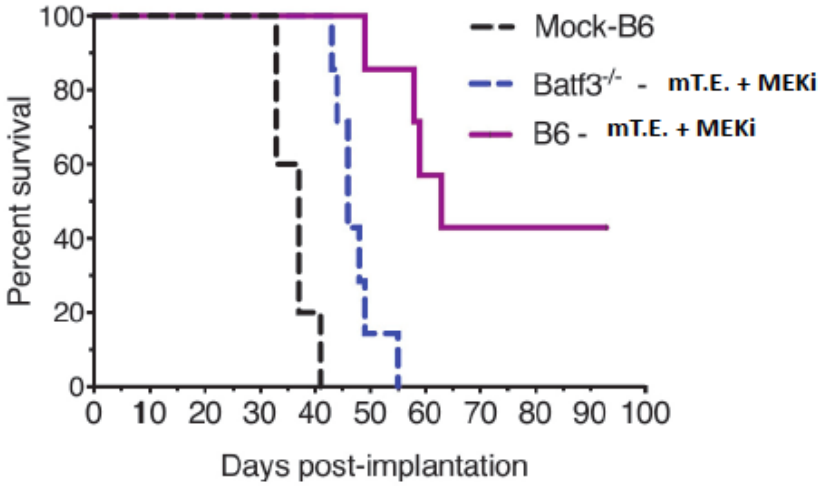




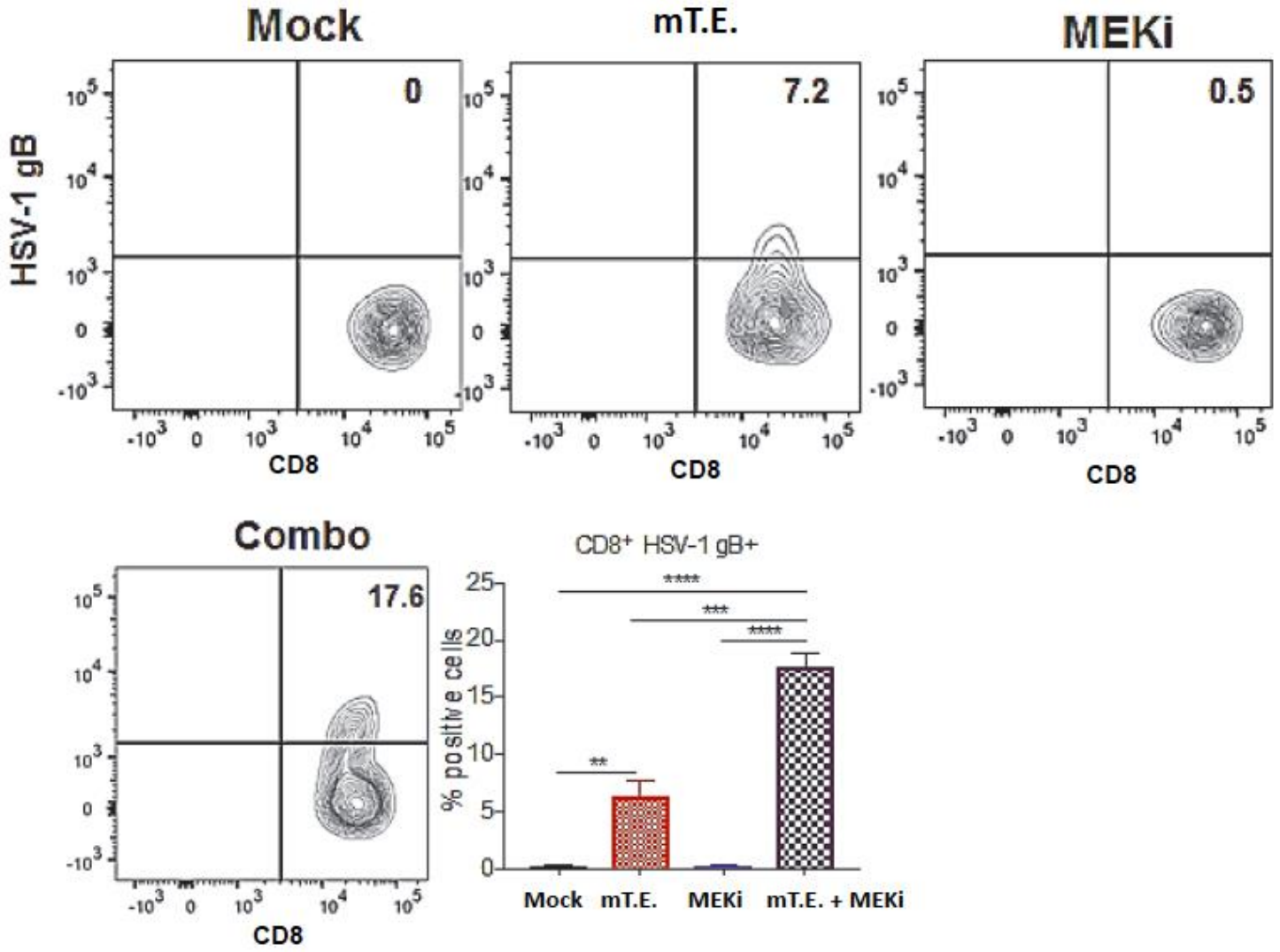


Therapeutic benefit is lost in the absence of Batf3+ dendritic cells

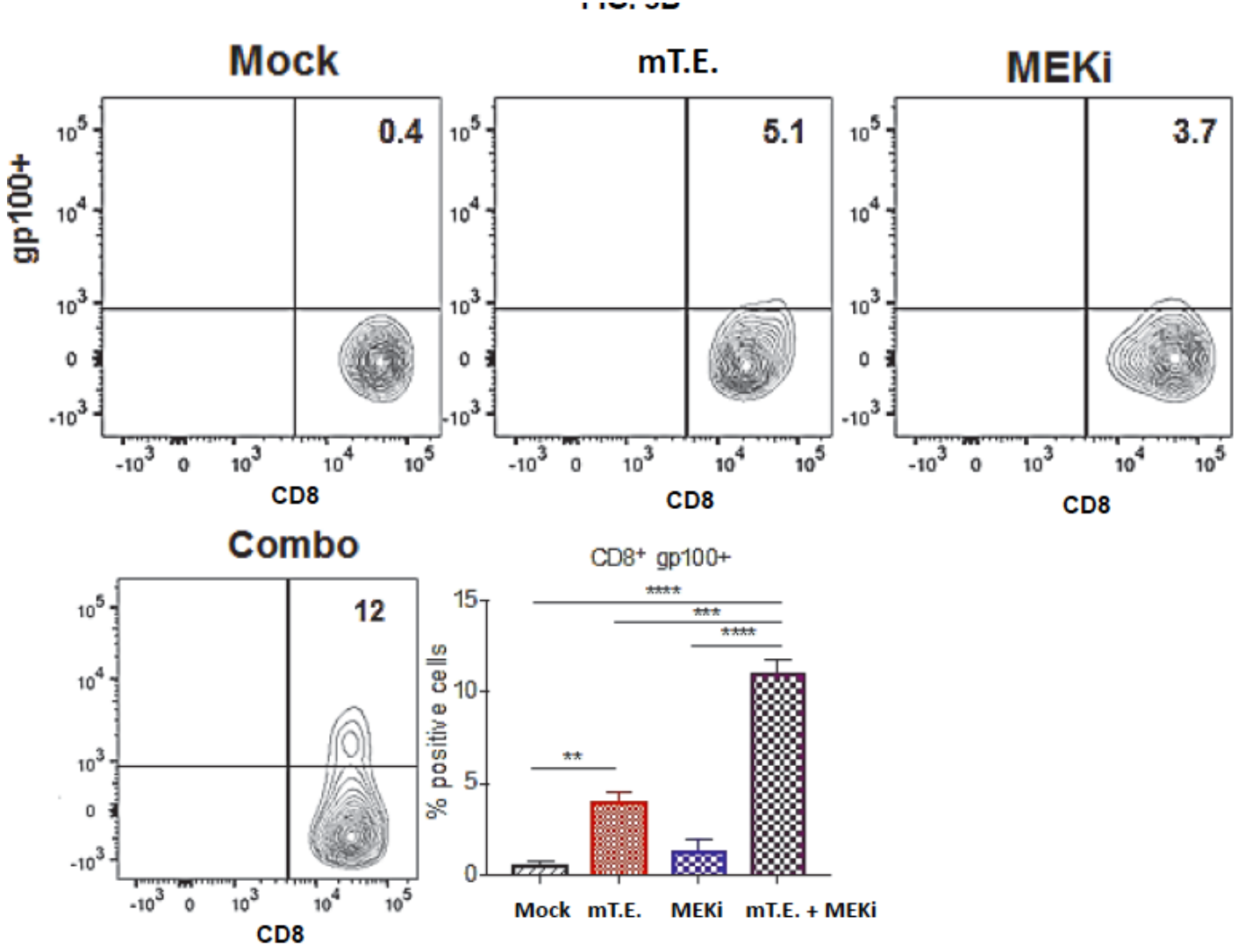




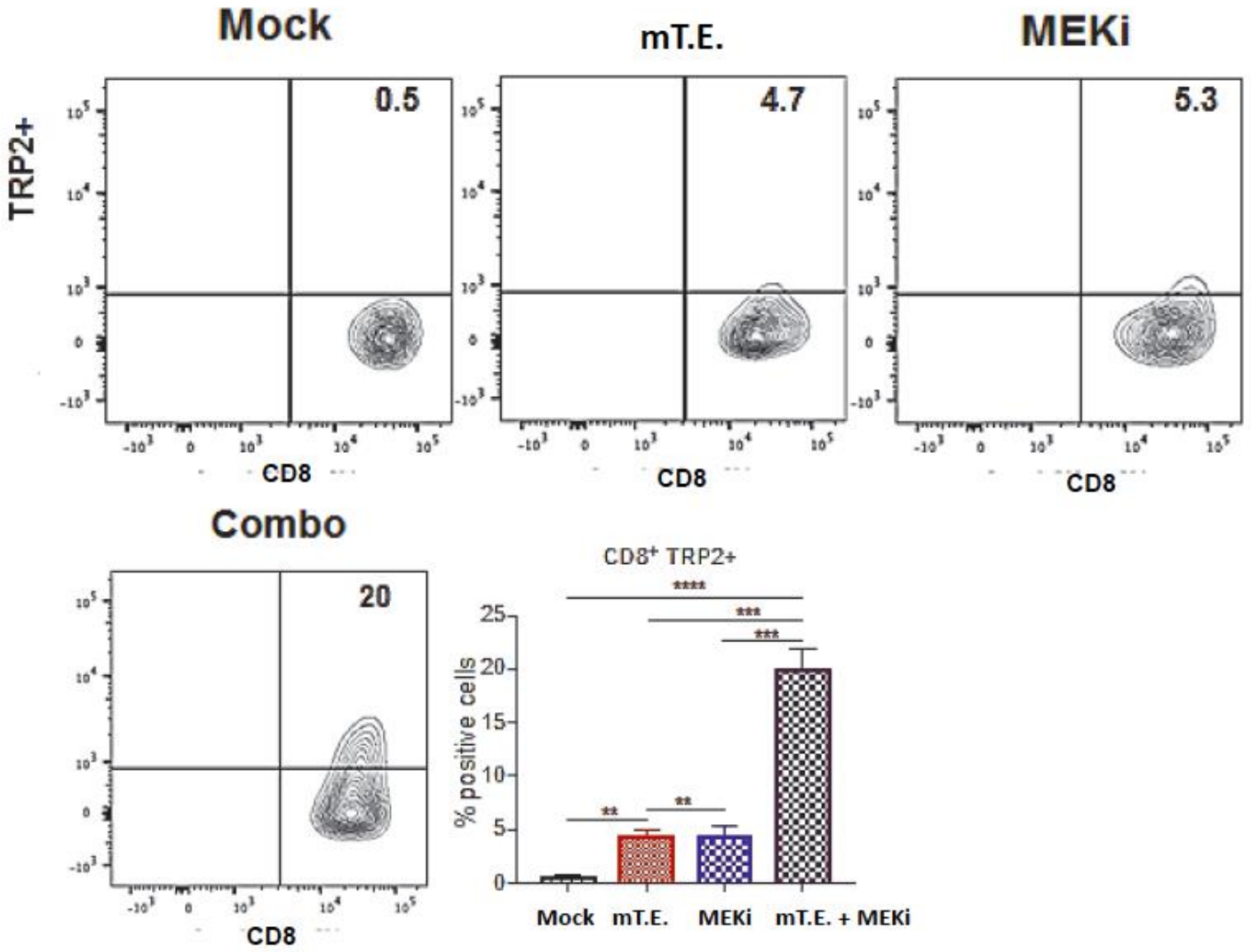
T-VEC and MEK inhibition enhances HSV-1-specific CD8+ T cells in the TME



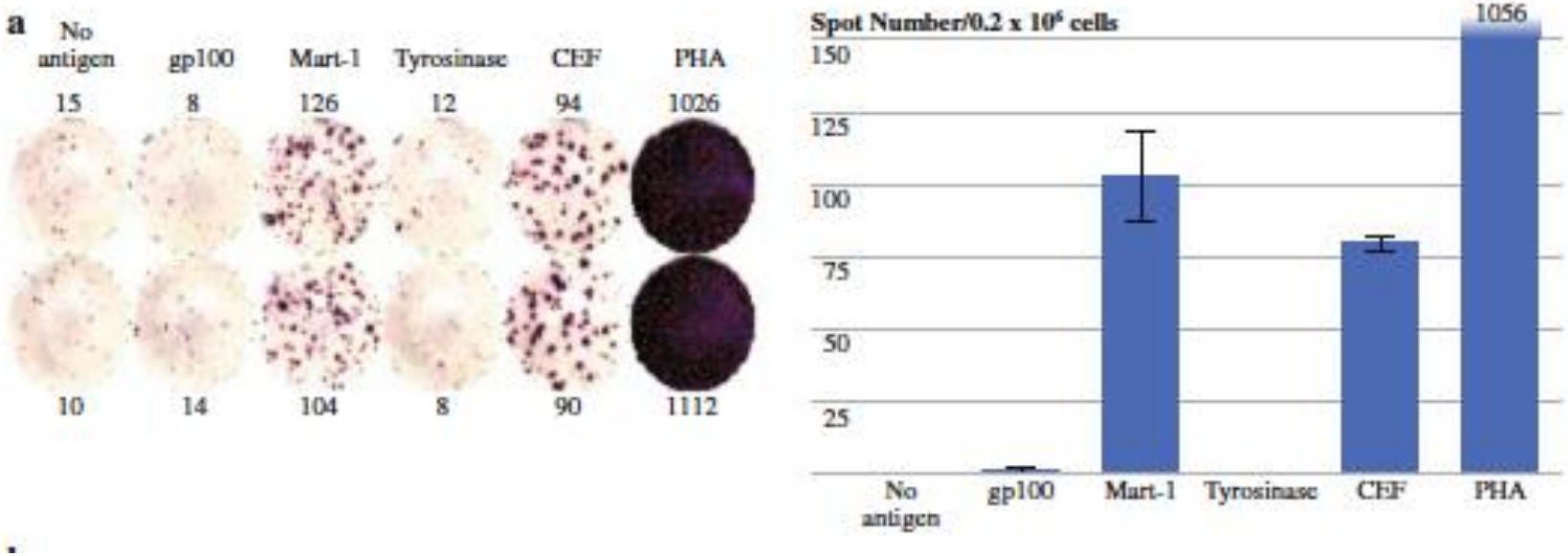
T-VEC and MEK inhibition enhances gp100-specific CD8+ T cells in the TME



T-VEC and MEK inhibition enhances TRP2-specific CD8+ T cells in the TME

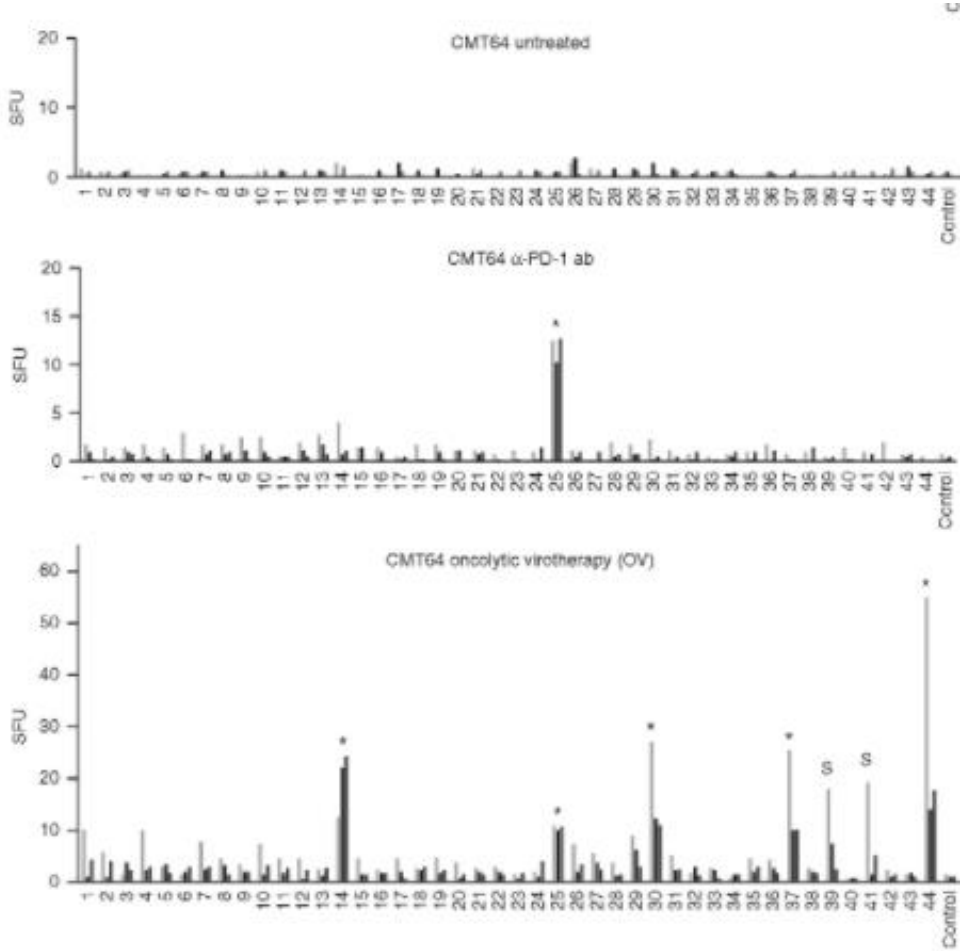


T-VEC is associated with MART-1 CD8+ T cells in tumor-infiltrating lymphocytes



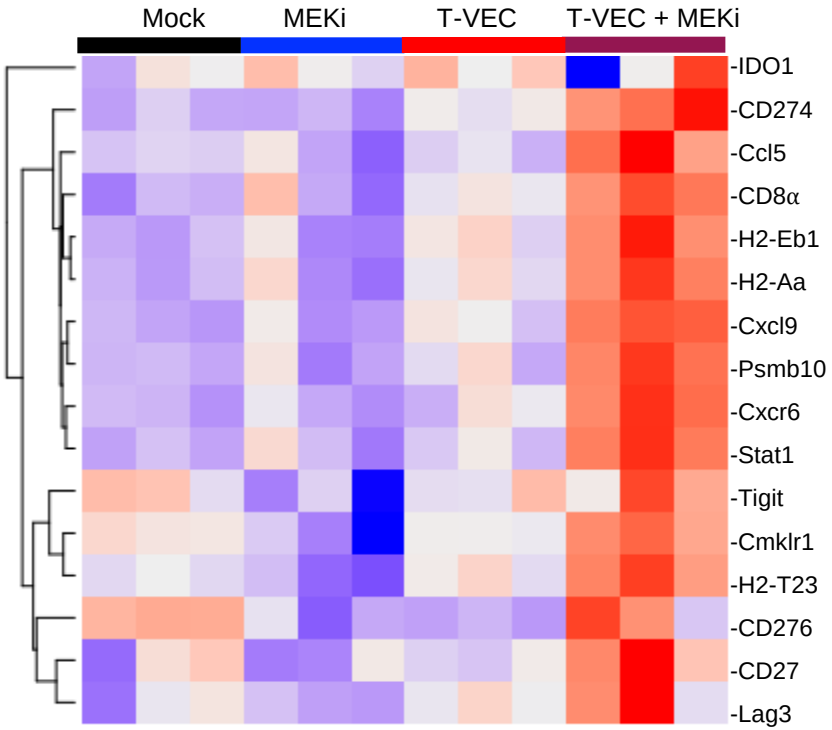
Kaufman et al. Ann Surg Oncol 2010

Oncolytic adenovirus results in expanded repertoire of neoantigen-specific CD8+ T cells

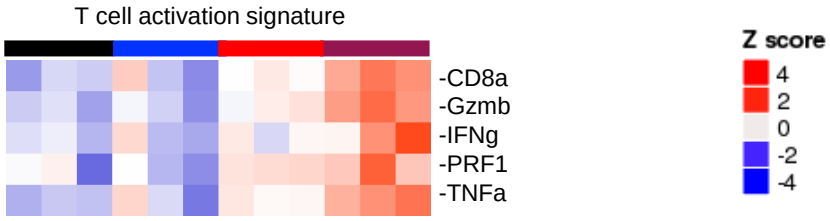


Woller et al. Mol Ther 2015

T-VEC and MEKi induces immune inflammatory and T cell activation gene signature

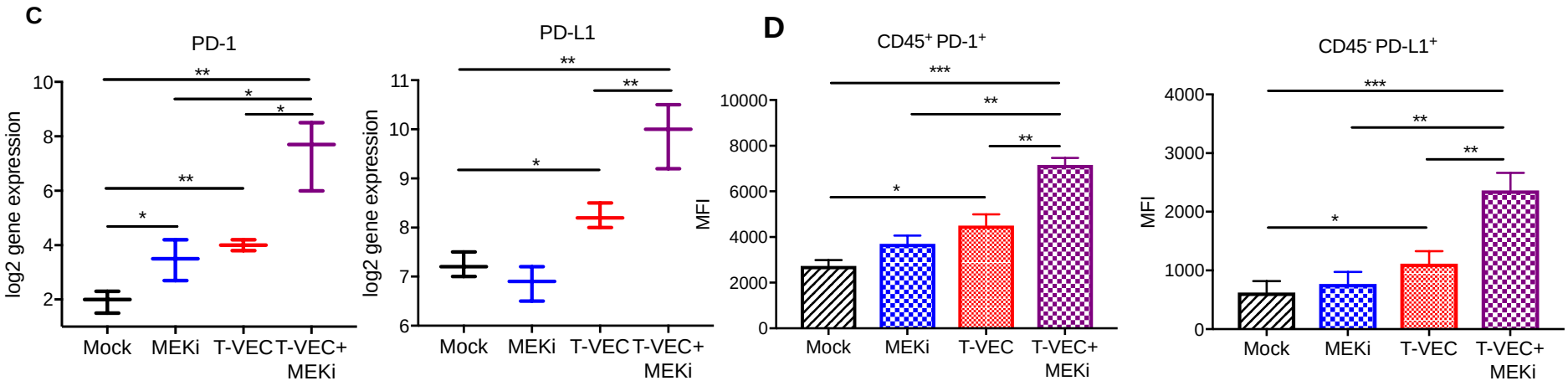


Tumor immune inflammatory signature

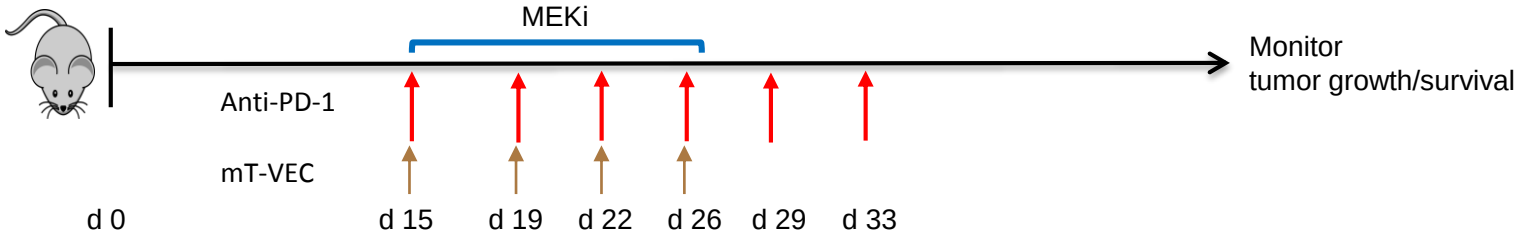


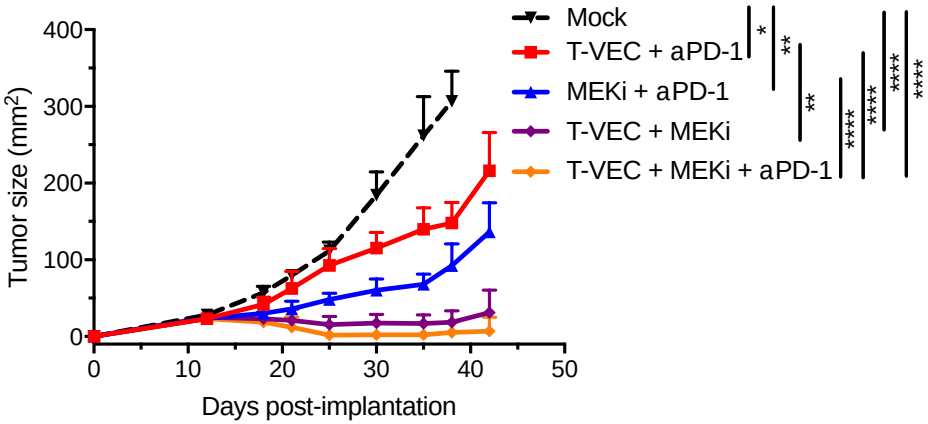
T cell activation signature

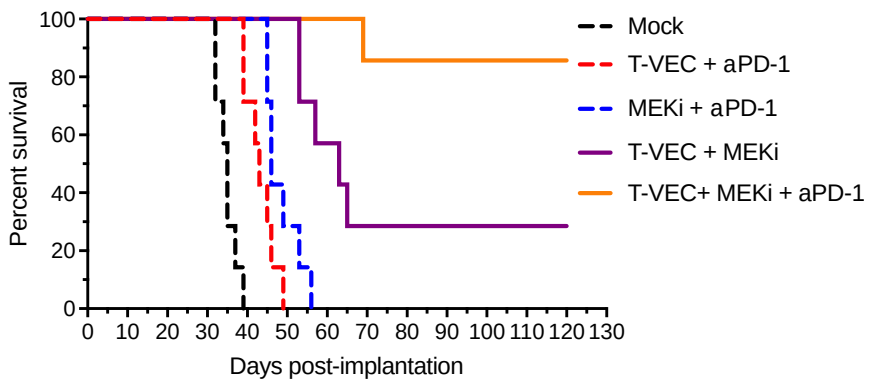
T-VEC and MEK inhibition induces strong PD-1 and PD-L1 expression



T-VEC/MEKi/anti-PD-1 triple therapy improves therapeutic responses

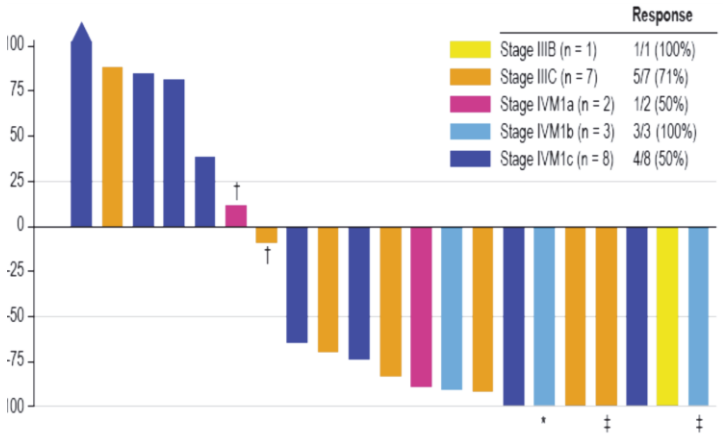


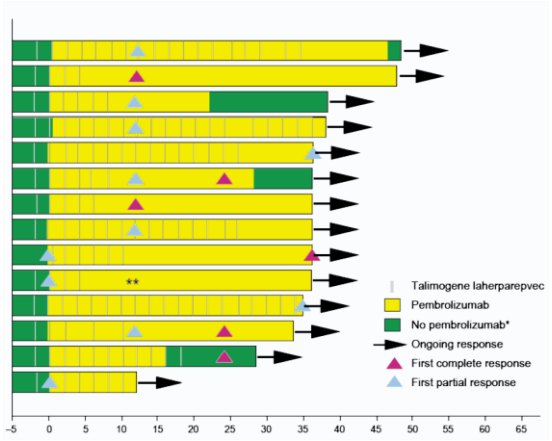




Oncolytic immunotherapy is synergistic with checkpoint blockade

T-VEC+pembrolizumab

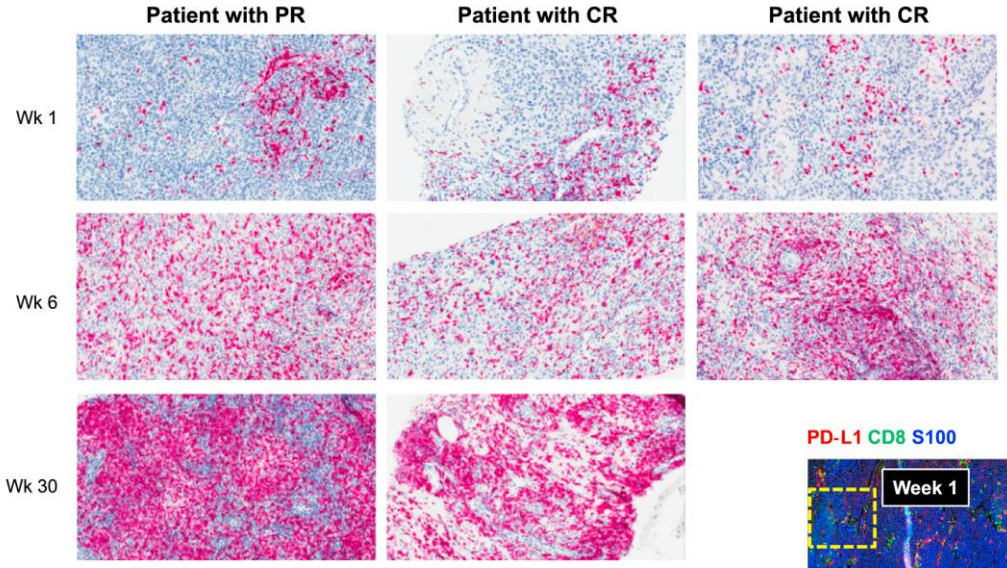


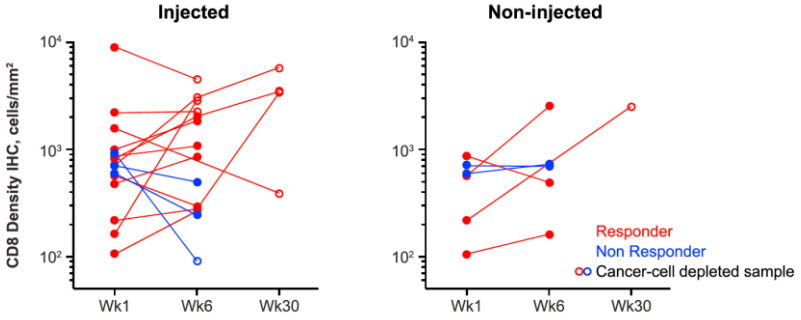


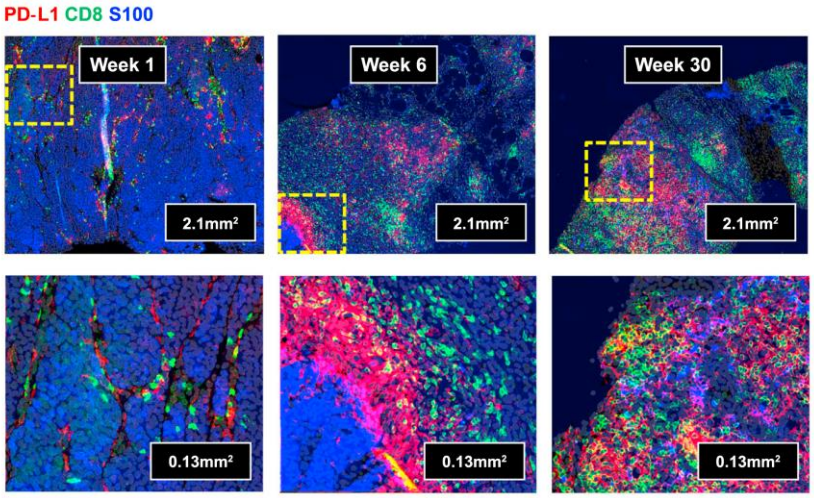
Without added toxicity

Ribas et al. Cell 2017

T-VEC and pembrolizumab increases CD8+ T cell density and PD-L1 in the TME of responding lesions

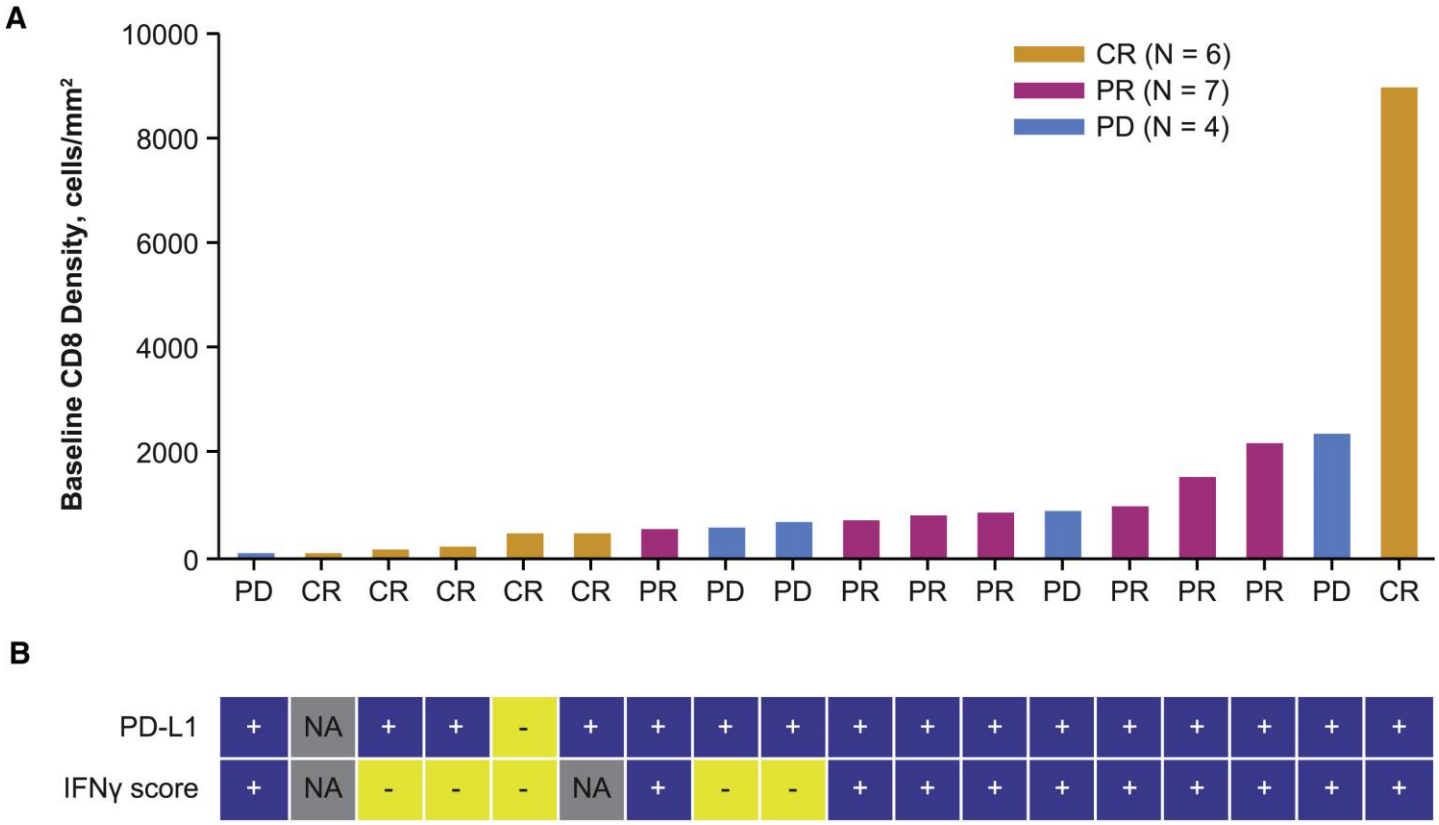






Ribas et al. Cell 2017

T-VEC + pembrolizumab increases IFN-gamma score and PD-L1 expression



Ribas et al. Cell 2017

Conclusions

- Oncolytic T-VEC induces ICD in melanoma cell lines
- Oncolytic T-VEC exhibits enhanced cell lysis *in vitro* and immune responses when combined with MEK inhibition *in vivo*
  - CD8+ T cell dependent
  - May rely on Batf3+ DC
  - Induces antigen spreading in murine models
- Gene expression profiling suggests that counter-regulatory expression of immune checkpoints may provide an opportunity for rational combinations of OV and checkpoint blockade
- Triple therapy with T-VEC, trametinib and anti-PD1 demonstrates strong therapeutic activity in mice
- Clinical studies of T-VEC and pembrolizumab appear promising
- OVs can be considered a pivotal agent to induce initial ICD as part of more rational combination strategies for tumor immunotherapy
- Further understanding of how OVs mediate anti-tumor activity are warranted; other viruses may exhibit different mechanisms

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