

ENHANCING ANTI-TUMOR IMMUNE RESPONSE BY DNA DEMETHYLATING AGENTS

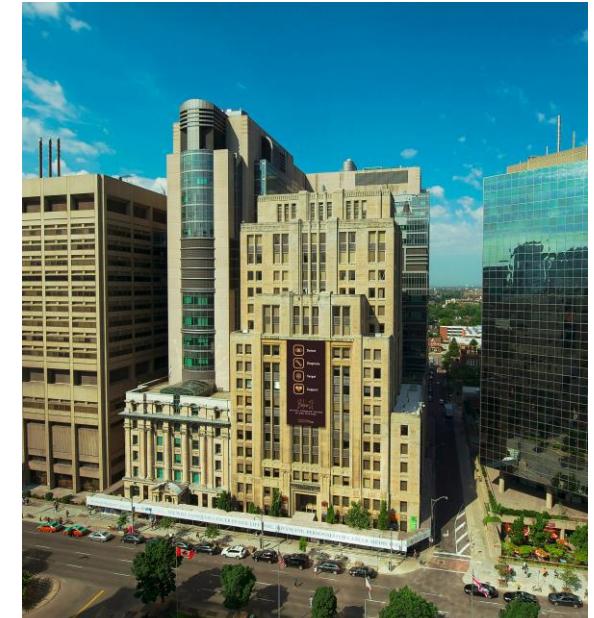
Daniel D. De Carvalho, PhD

Helen M Cooke Associate Professor, Dept of Medical Biophysics
Senior Scientist and Canada Research Chair, Princess Margaret



www.decarvalholab.org

 @decarvalho_lab



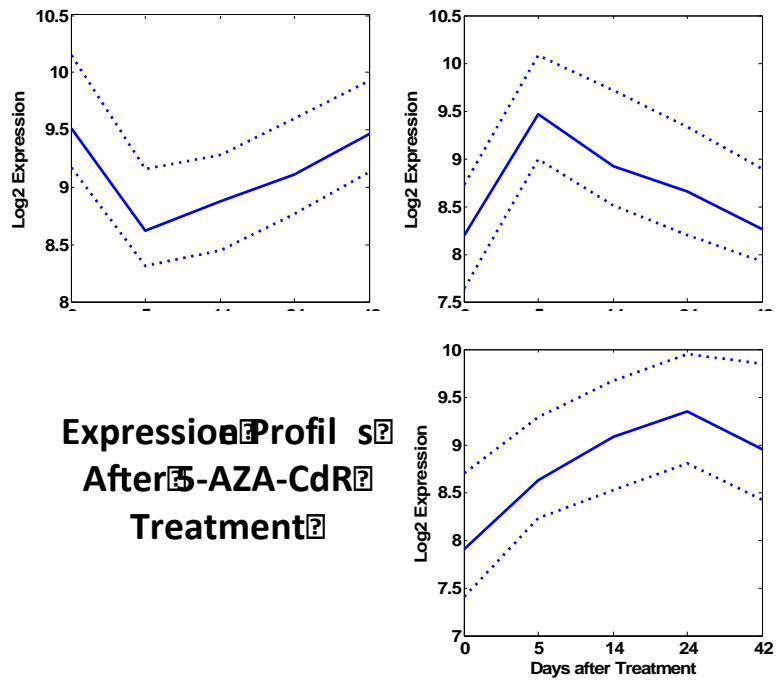
DNA Demethylating Agents: Current Paradigm

- ✓ Cancer Cells have massive DNA methylation reprogramming
(Seems to be true in ALL types of Cancers – TCGA)
- ✓ Cancer Cells depend on aberrant DNA methylation profile to survive: “Epigenetic Addiction” (De Carvalho, **Cancer Cell**, 2012)
- ✓ DNA demethylating agents may work by re-activating aberrantly methylated Tumor Suppressor Genes

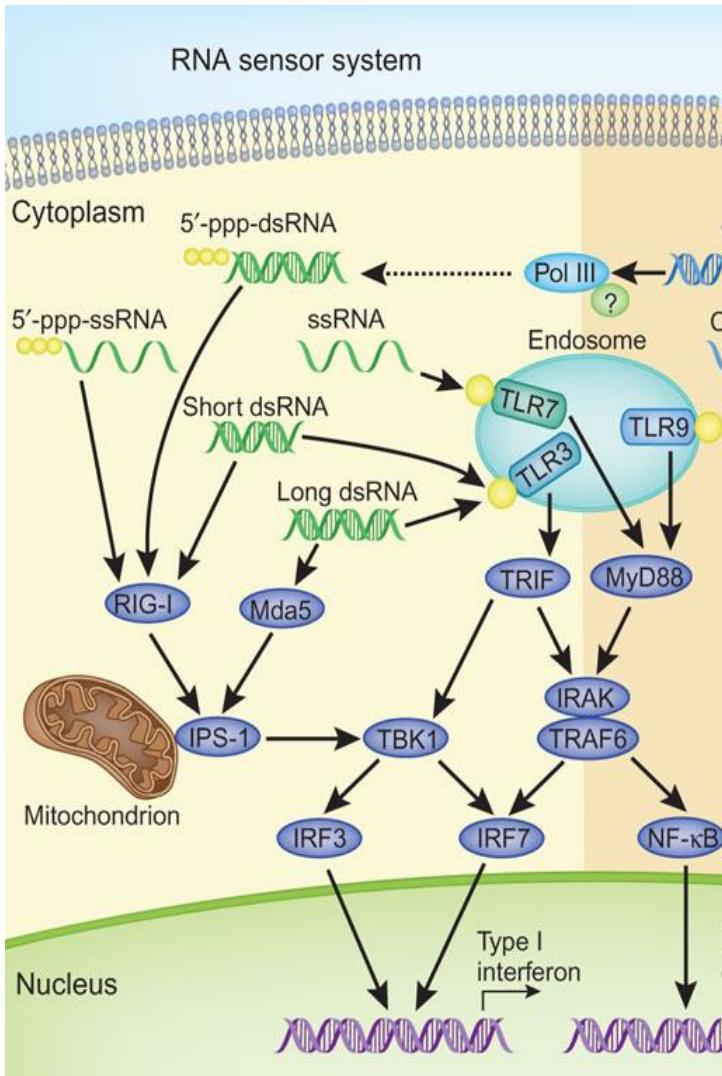
Inconsistencies in the Current Model

- If DNA demethylating agents are acting mainly by activating epigenetically silenced tumor suppressor genes, then:
 - Why clinical response is usually delayed?
 - Why DNA methylation markers can not predict clinical response?

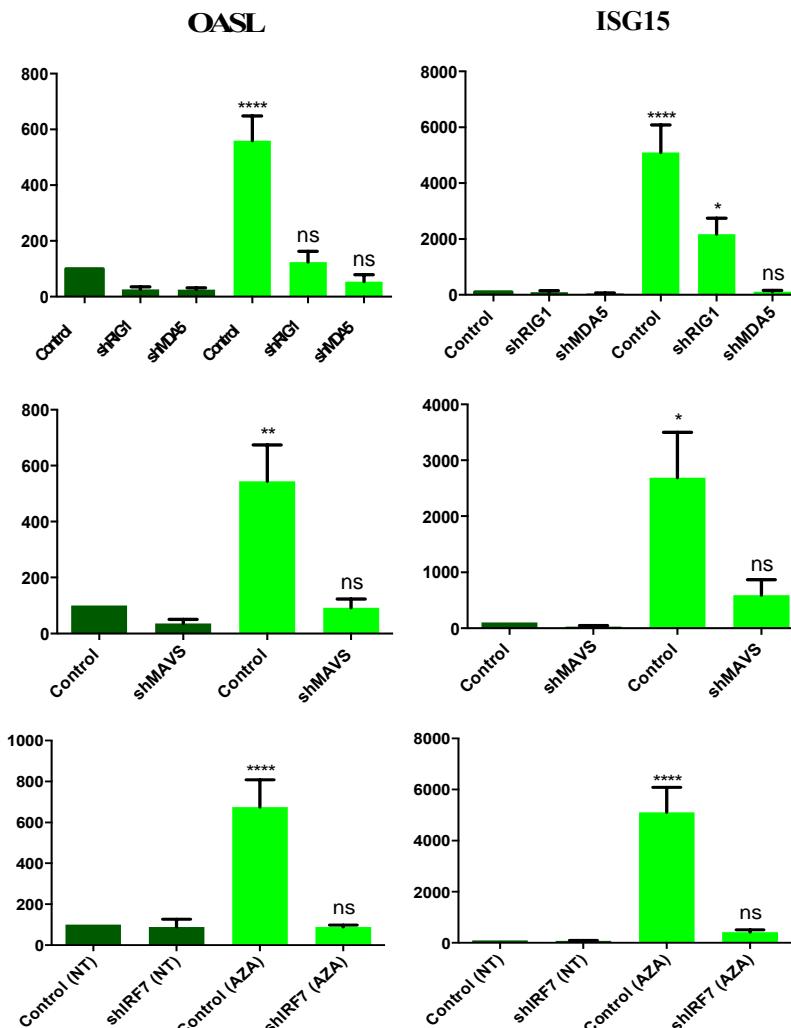
Gene Expression Profiles after 5-AZA-CdR Treatment



RNA/DNA Sensor Systems



Xuetao Cao, *Nature Immunology* 2009

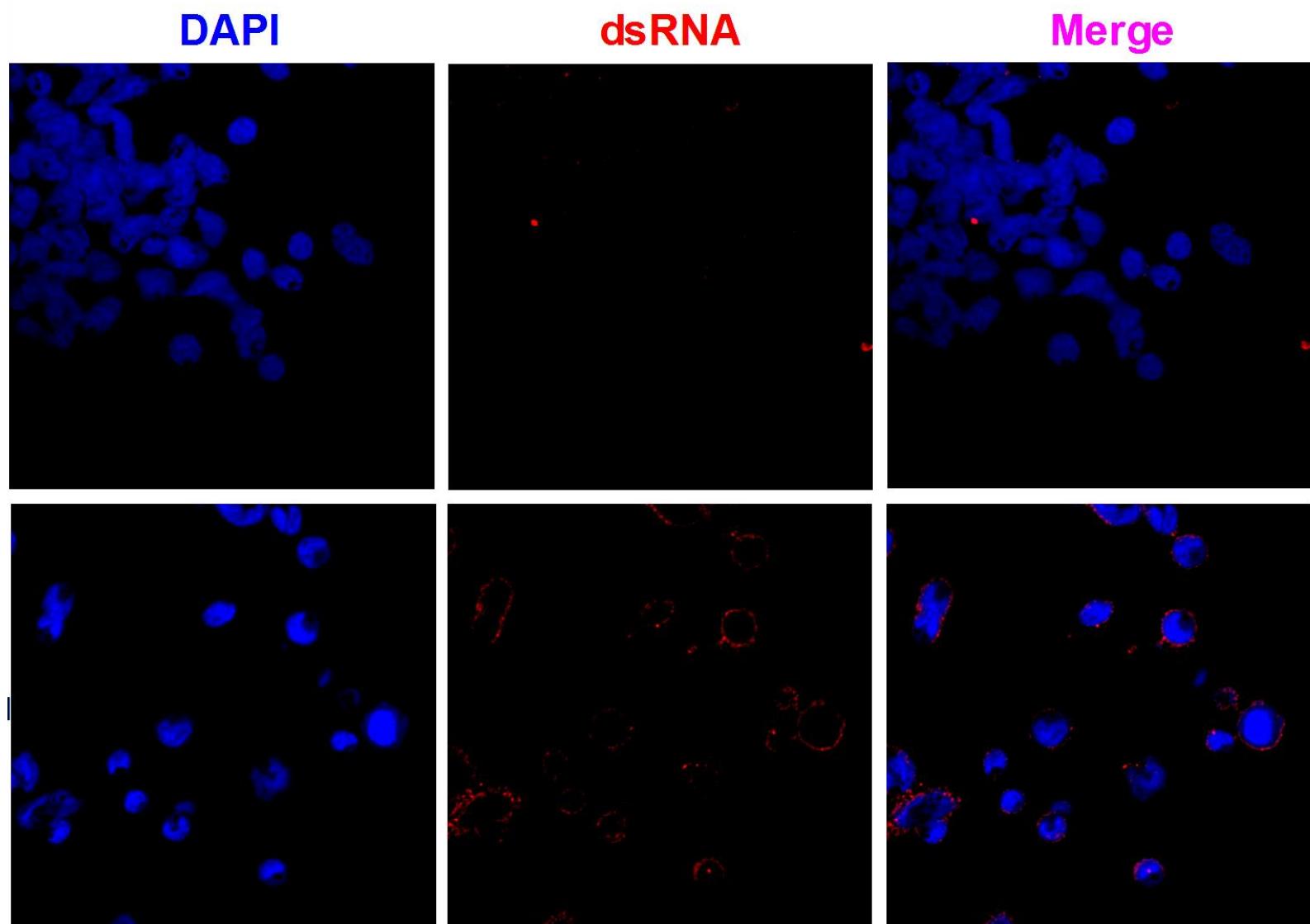


Mock treated
5-AZA-CdR treated

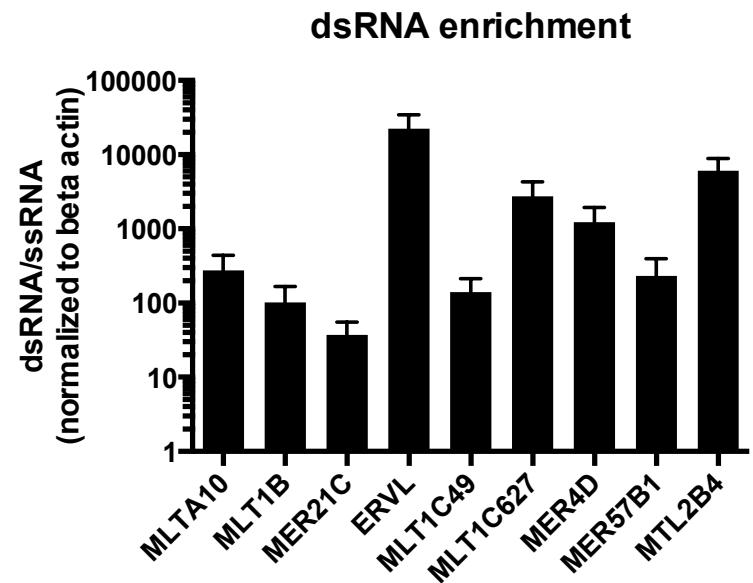
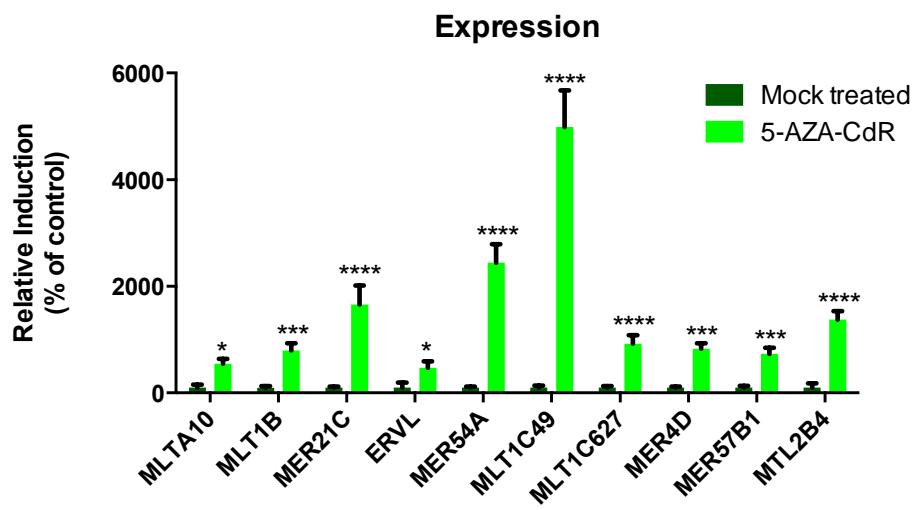
Roulois et al *Cell* 2015

5-AZA-CdR Induces Formation of dsRNA

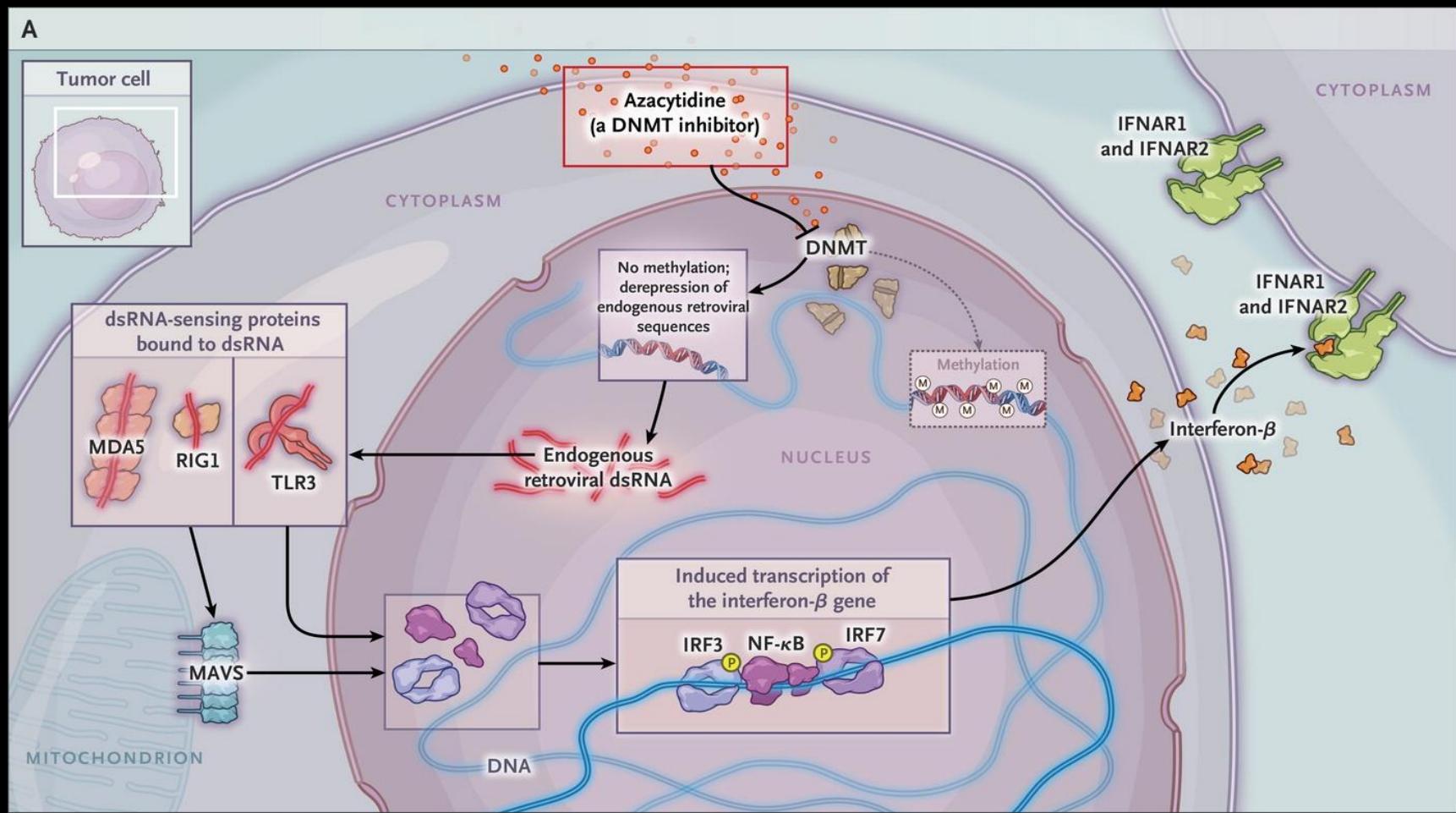
Mock
Treated



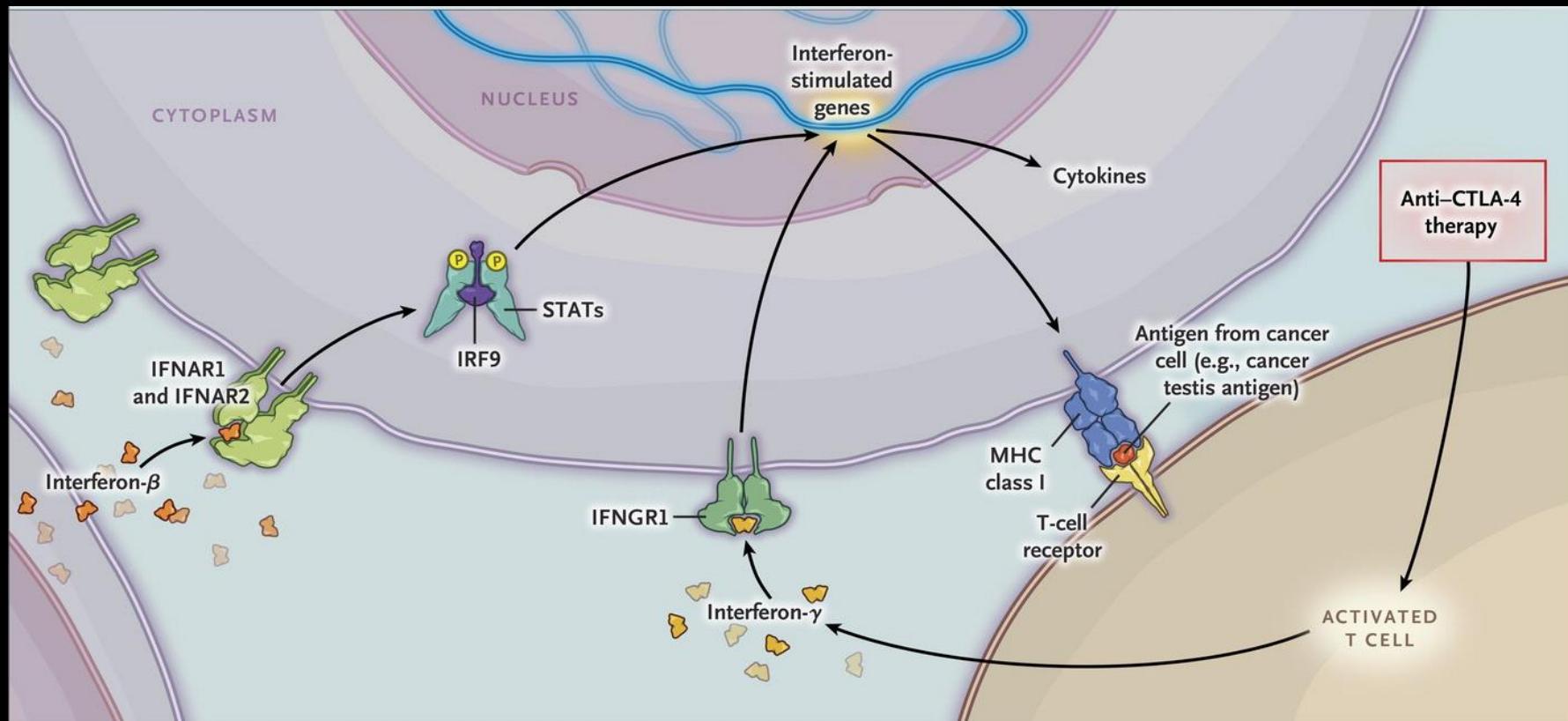
5-AZA-CdR Induces Expression of Endogenous Retrovirus



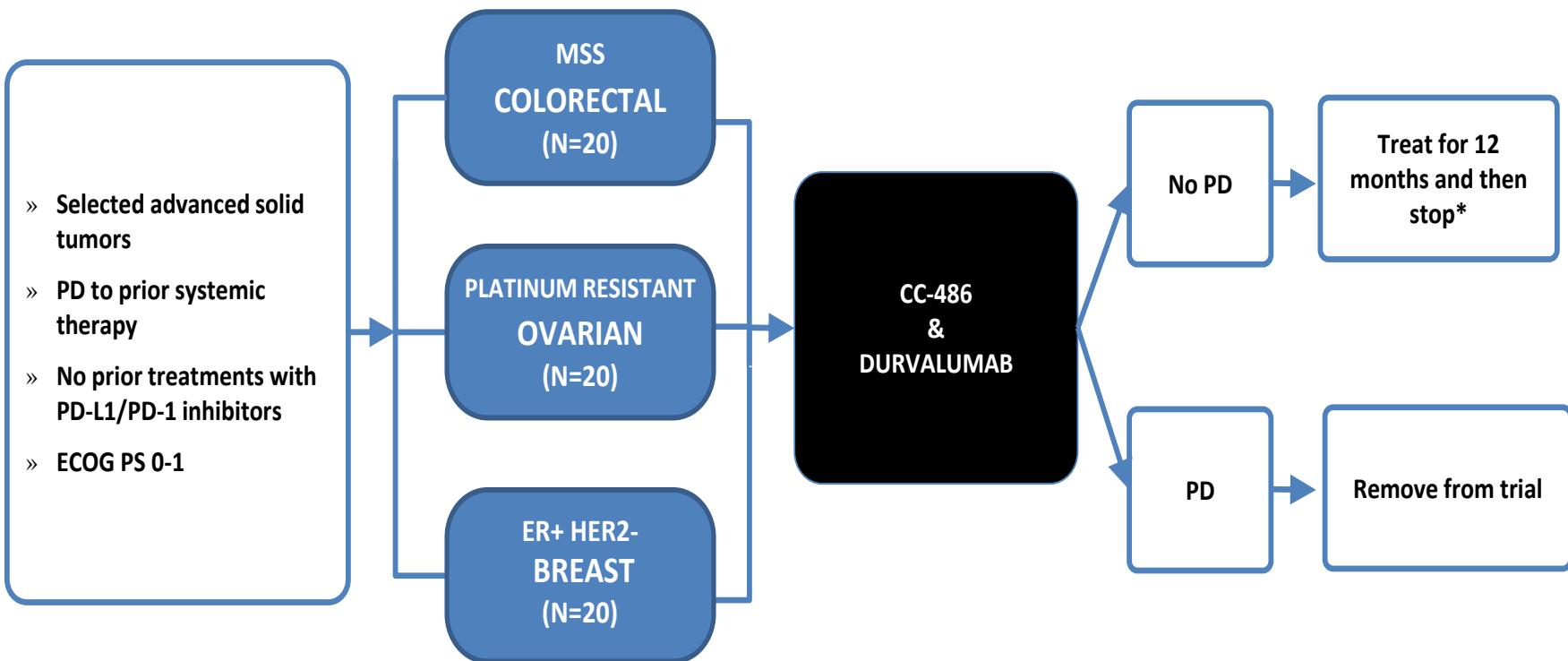
Enhancing Tumor-Cell “Visibility” to the Immune System with the Use of Viral Mimicry — A Model.



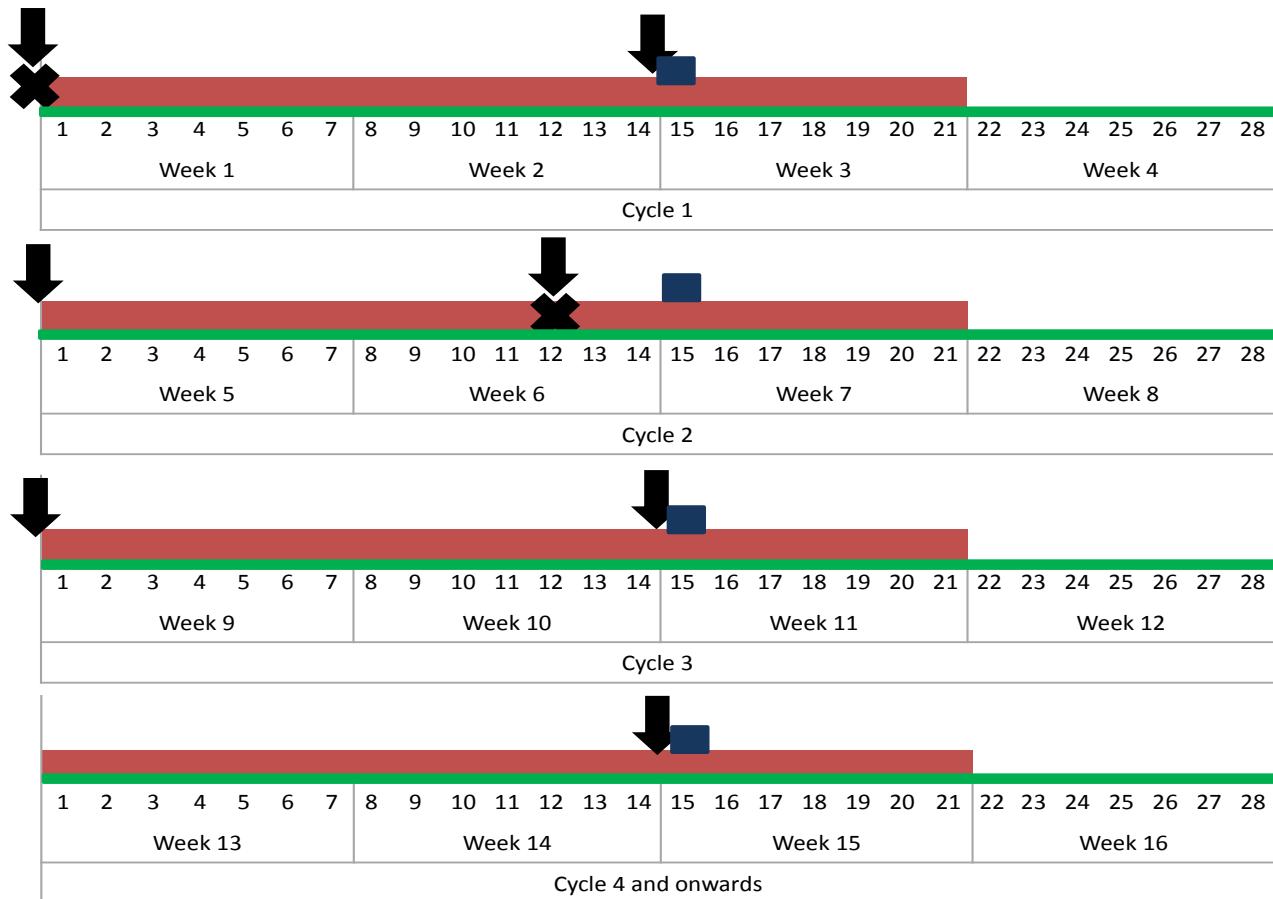
Enhancing Tumor-Cell “Visibility” to the Immune System with the Use of Viral Mimicry — A Model.



open-label, phase II basket study of a hypoMETThylating Agent oral azacitidine and DURvalumab (MEDI4736) (anti-PDL1) in advanced solid tumors (**METADUR**)



METADUR – Treatment Schema



CC-486
100mg PO
QD

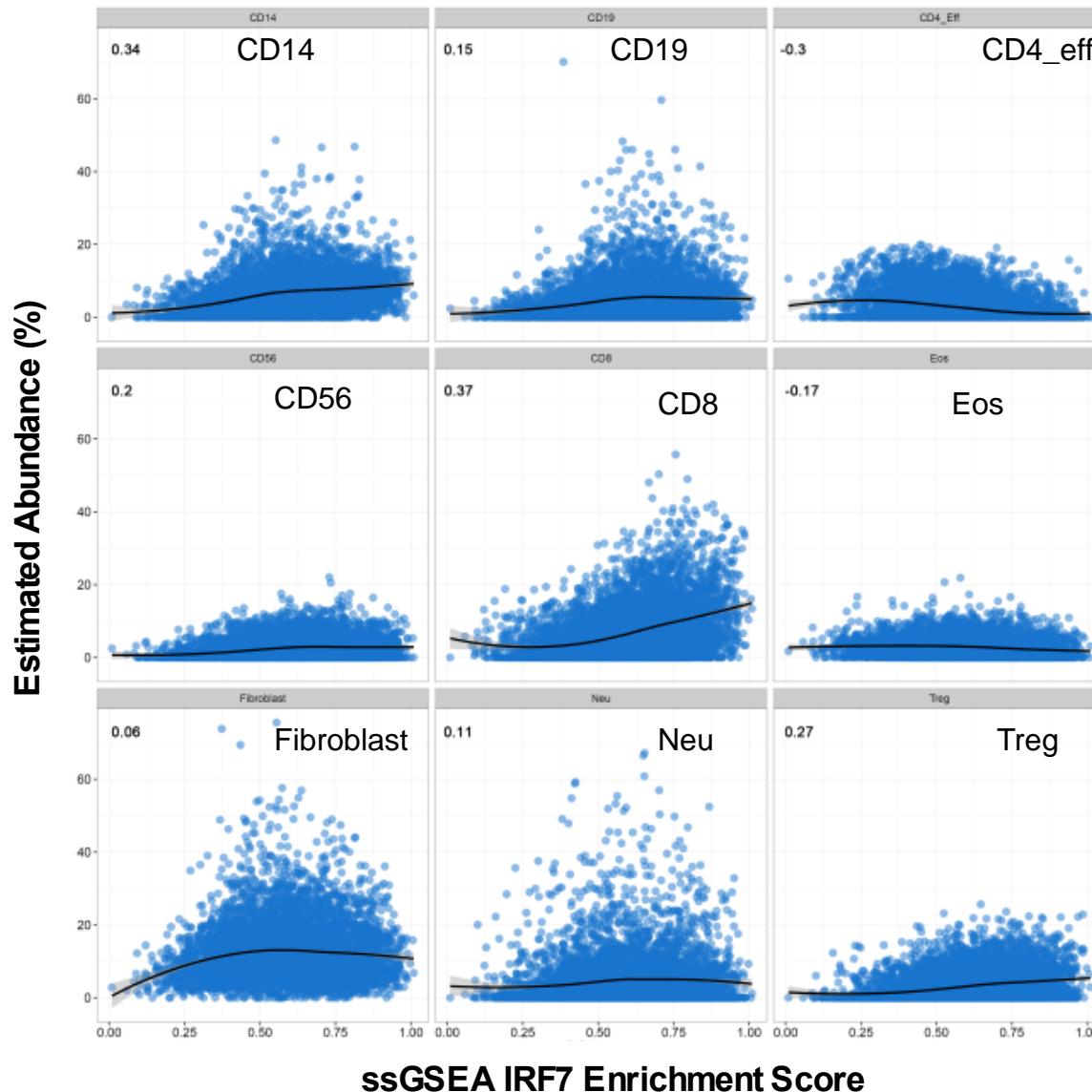
Durvalumab IV
1500 mg Q4W

Oral vitamin C
500 mg PO QD

Collection of tumor tissue (pre C1D1 and then C2D11-13 and if retreatment with CC-486, vitamin C and durvalumab)

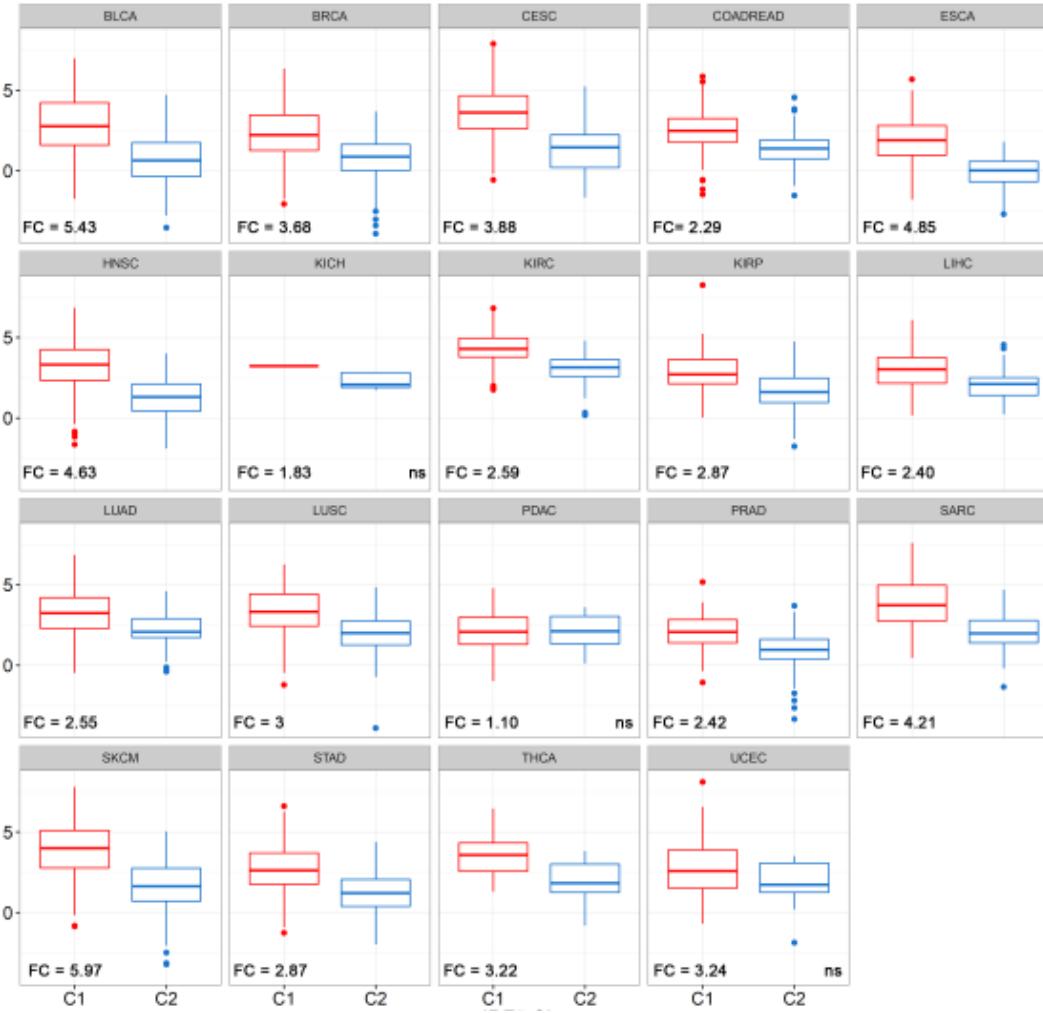
Blood collection

IRF7 high tumors have higher CD8 T cell infiltrate



IRF7 high tumors have higher cytolytic activity

Log₂ CYT



Molecular and Genetic Properties of Tumors Associated with Local Immune Cytolytic Activity

Michael S. Rooney,^{1,2} Sachet A. Shukla,^{1,3} Catherine J. Wu,^{1,3,4} Gad Getz,^{1,5} and Nir Hacohen^{1,4,6,*}

¹The Broad Institute, Cambridge, MA 02142, USA

²Harvard/MIT Division of Health Sciences and Technology, Cambridge, MA 02141, USA

³Department of Medical Oncology, Dana-Farber Cancer Institute, Boston, MA 02115, USA

⁴Department of Medicine, Harvard Medical School, Boston, MA 02115, USA

⁵Massachusetts General Hospital Cancer Center and Department of Pathology, Charlestown, MA 02129, USA

⁶Center for Immunology and Inflammatory Diseases and Department of Medicine, Massachusetts General Hospital, Charlestown, MA 02129, USA

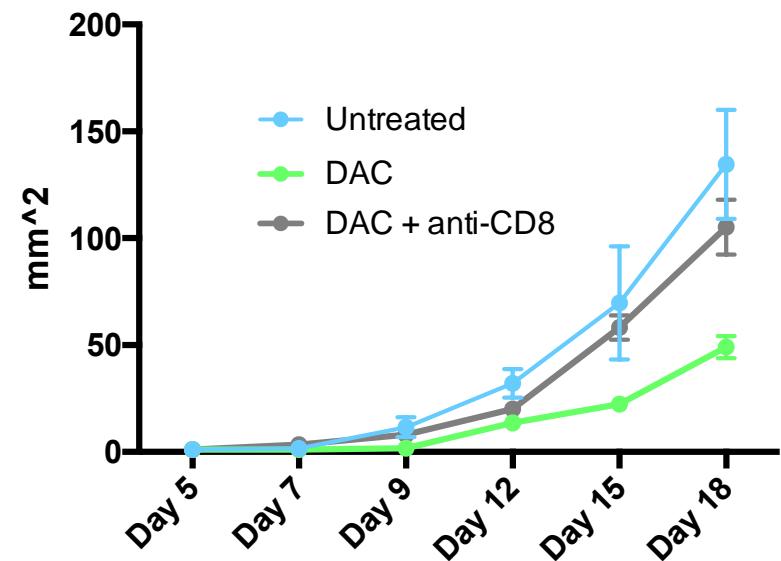
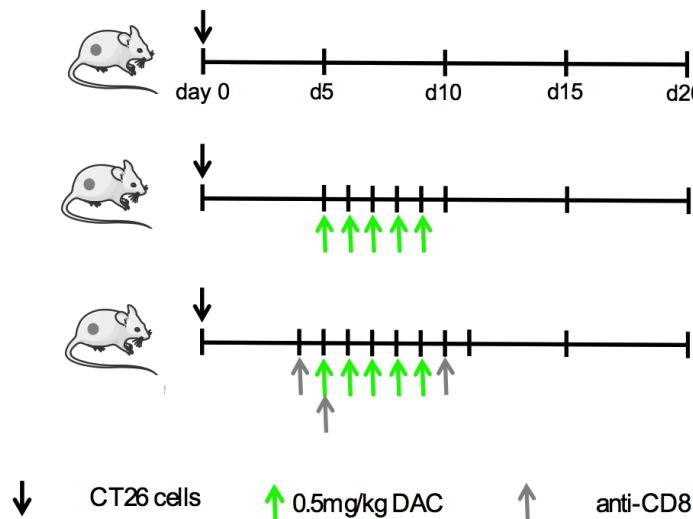
*Correspondence: nhacohen@mgh.harvard.edu

<http://dx.doi.org/10.1016/j.cell.2014.12.033>

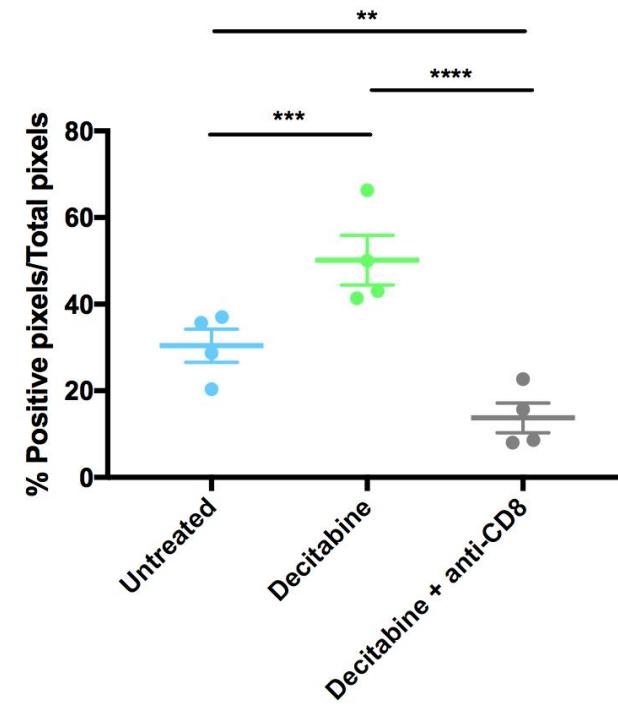
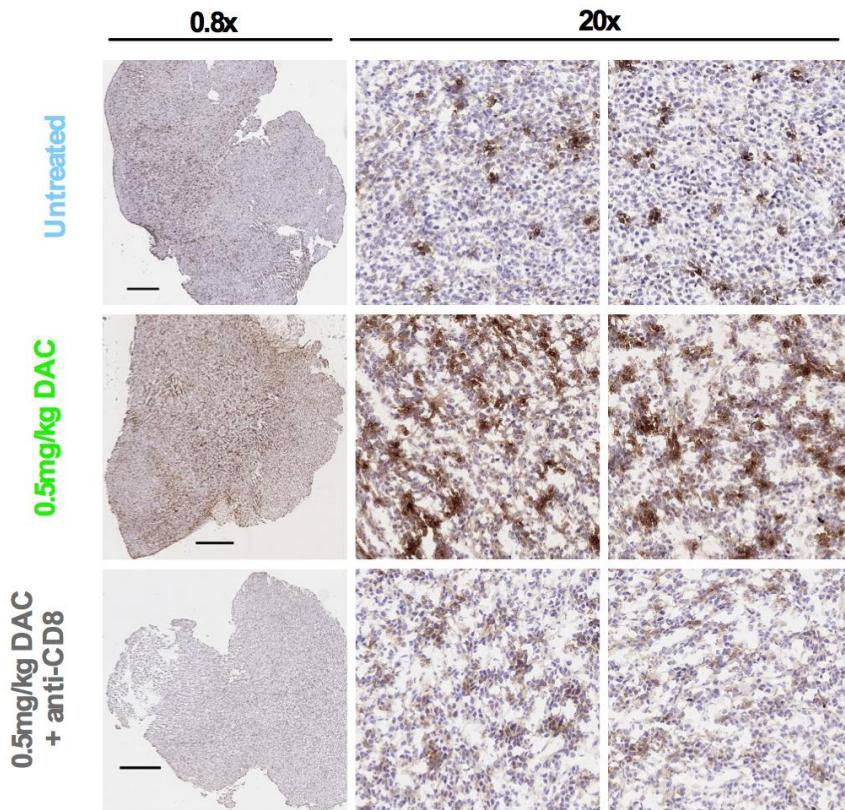
Predictions Based on pan Cancer TCGA and scRNA-seq Analyses

- IRF7 activation is associated with high tumor immune infiltration ('hot tumors')
- IRF7 activation is associated with higher cytolytic activity in CD8 T cells
- DNMTi can induce IRF7 activation (Roulois et al., Cell, 2015)
- **Prediction:** DNMTi treatment will 1) increase tumor immune infiltration and 2) increase cytolytic activity of CD8 T cells

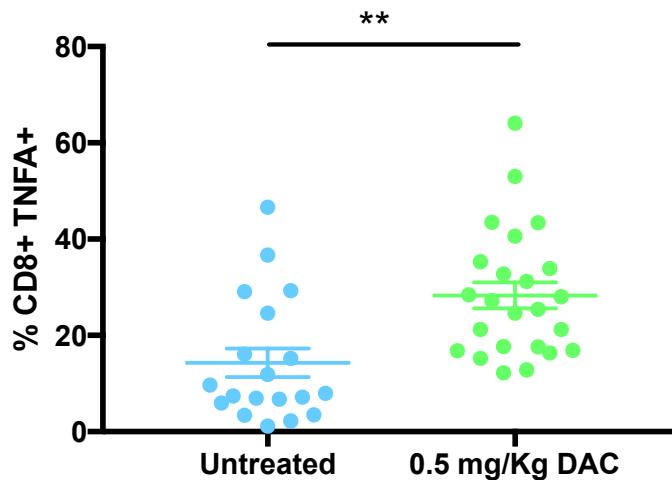
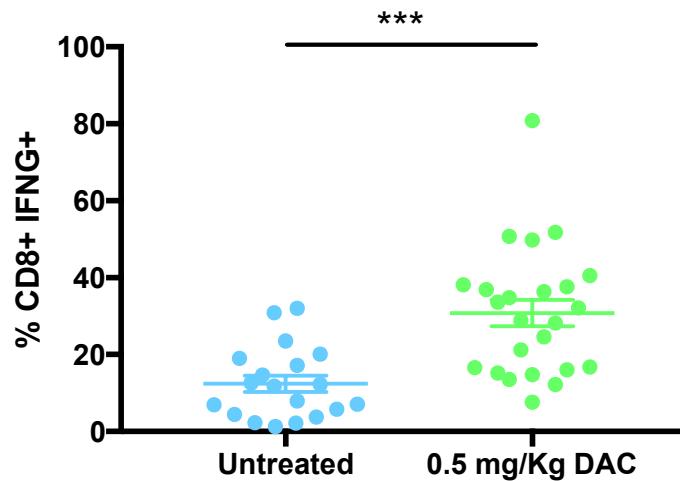
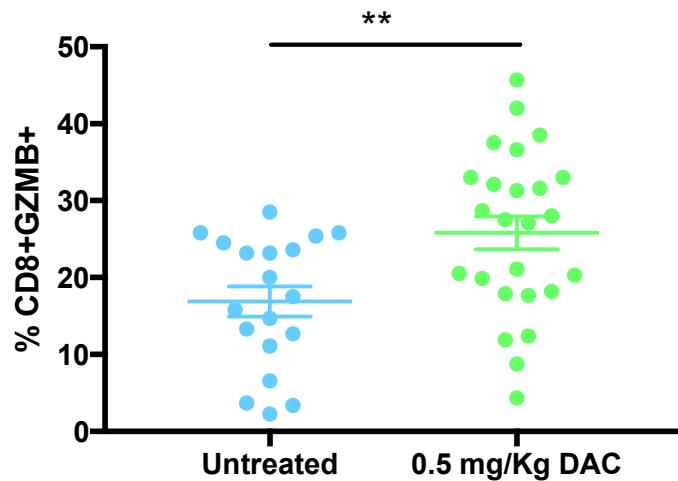
Anti-tumor response to DNMTi treatment is dependent on CD8 T Cells



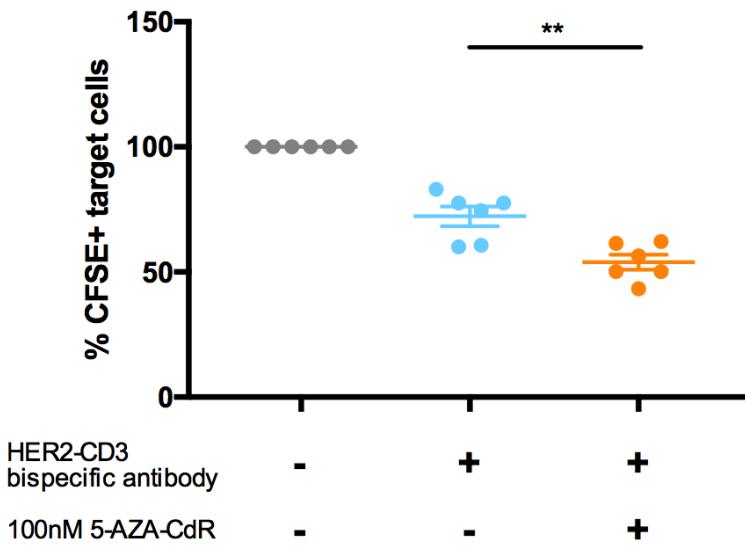
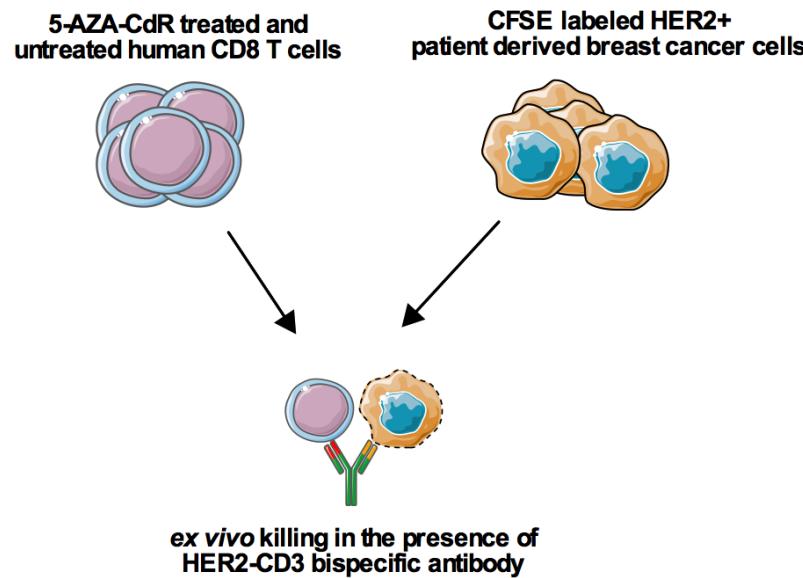
DNMTi treatment increases tumor infiltration by CD8 T cells



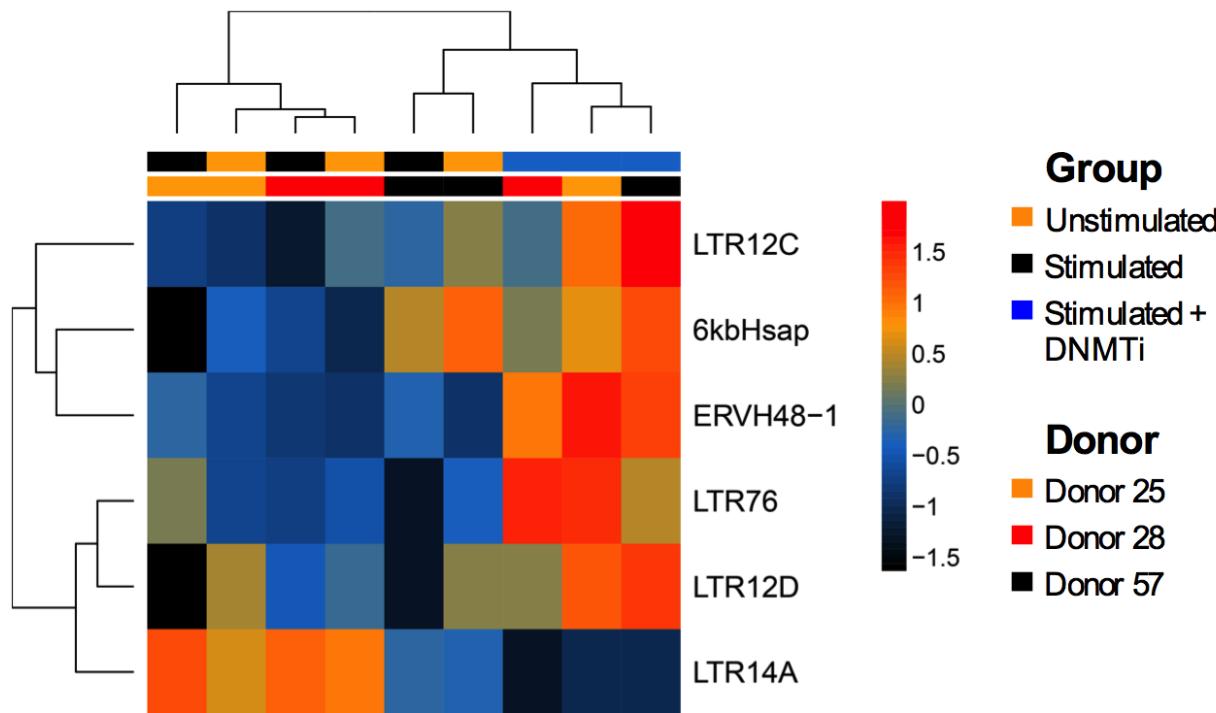
DNMTi treatment increases CD8+ T cell cytolytic activity



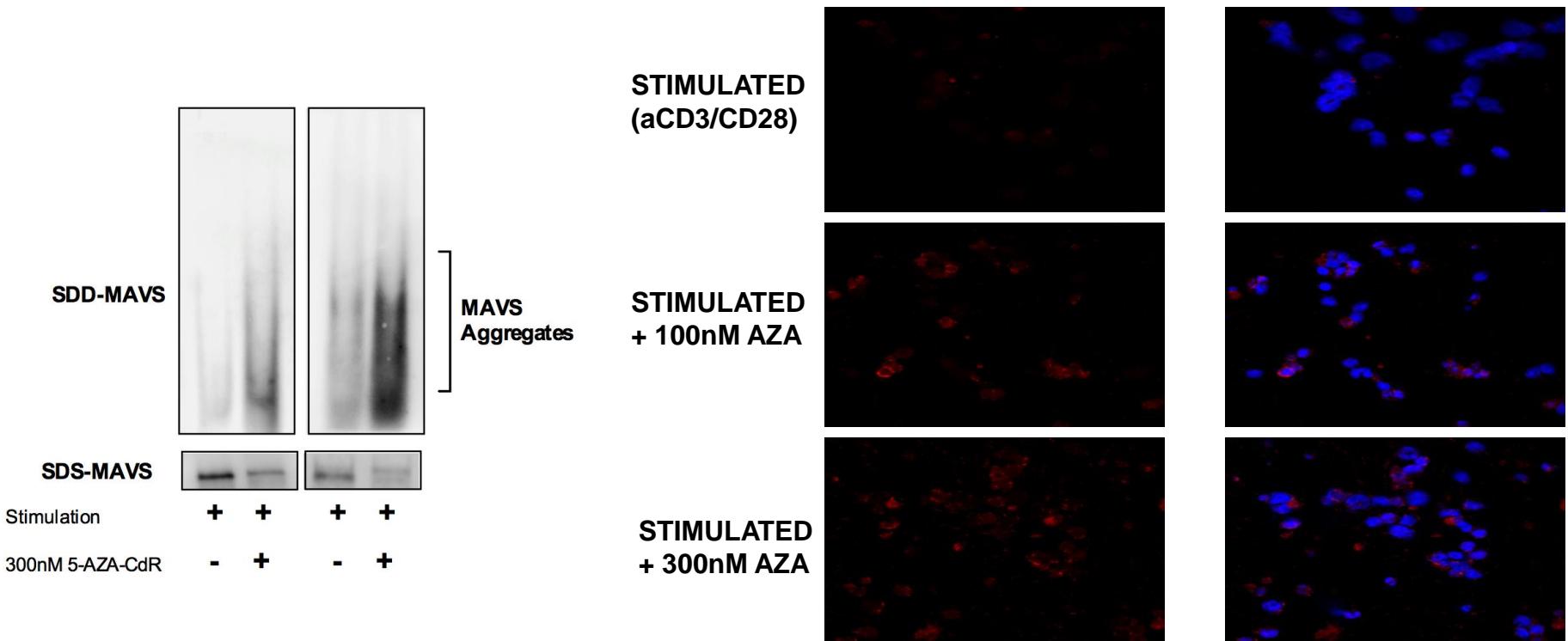
DNMTi treatment increases cytolytic activity of human CD8 T cells



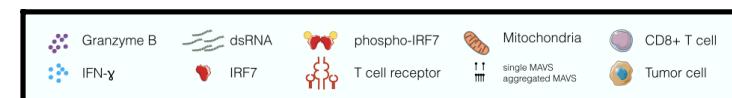
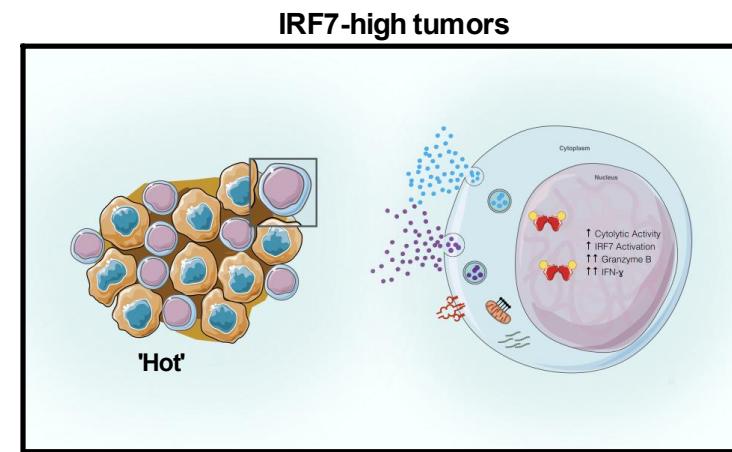
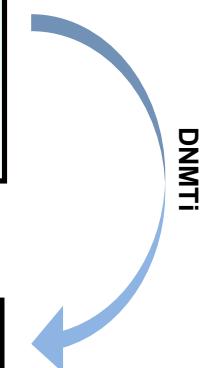
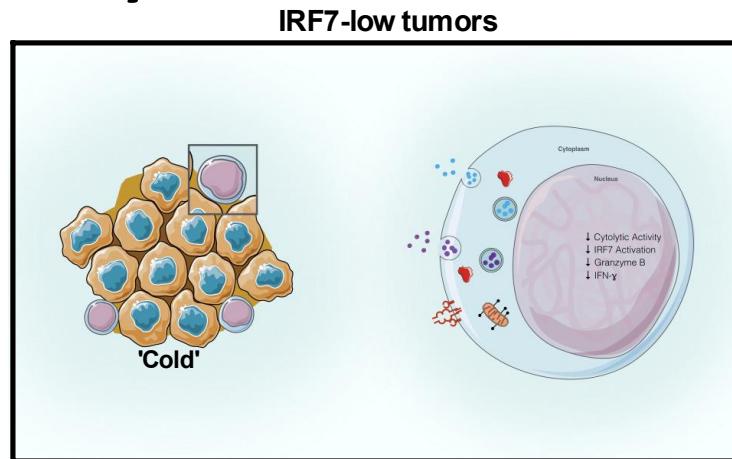
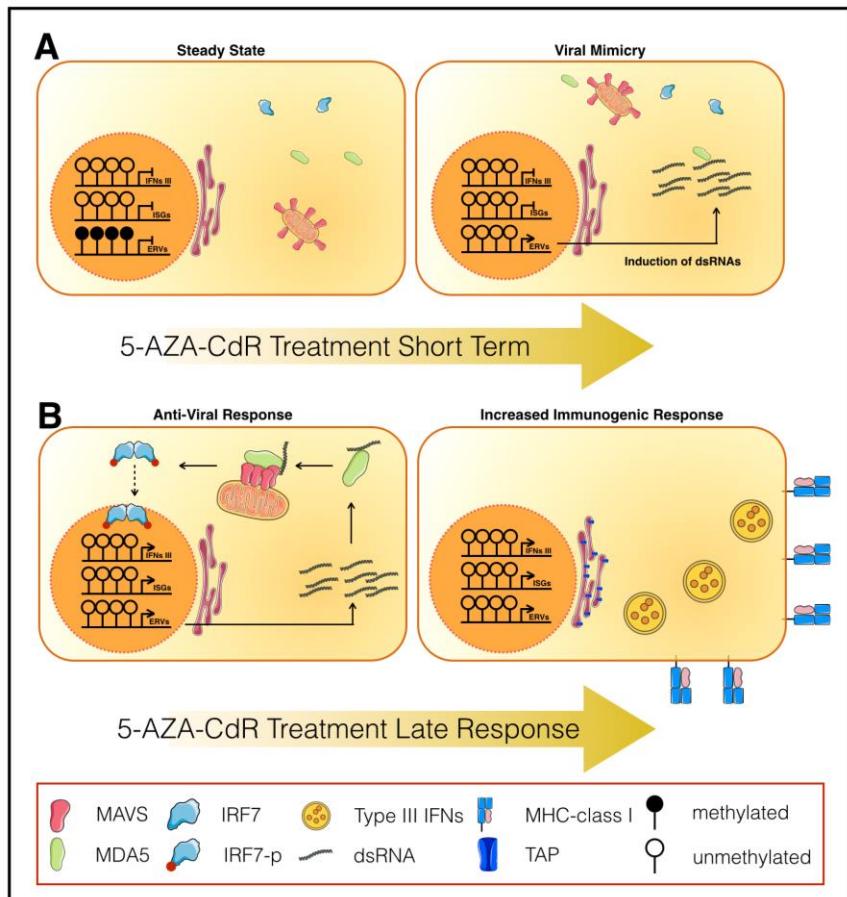
DNMTi treatment induces ERVs expression in human CD8 T cells



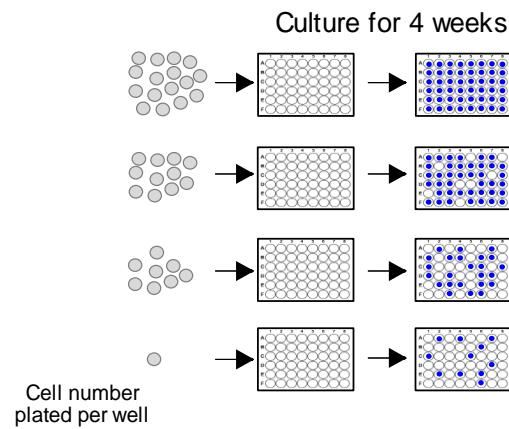
DNMTi treatment activates dsRNA sensing pathways in human CD8 T cells (viral mimicry)



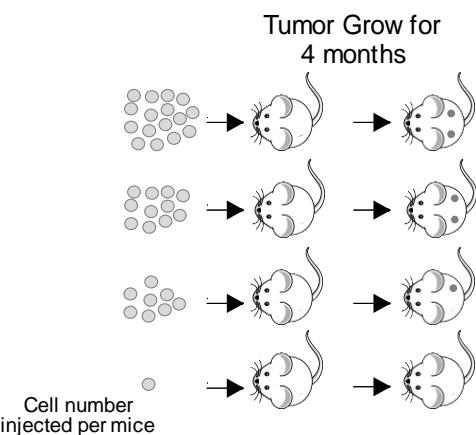
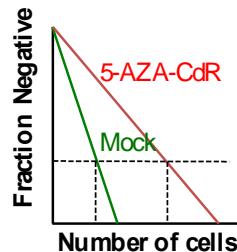
Summary



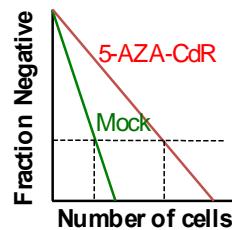
Low Dose 5-AZA-CdR Decreases Colorectal CICs



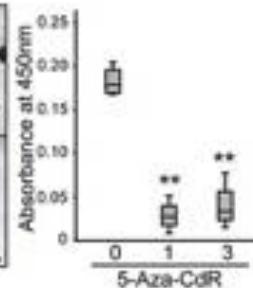
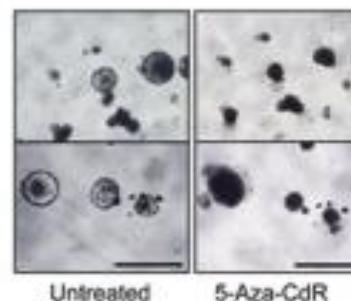
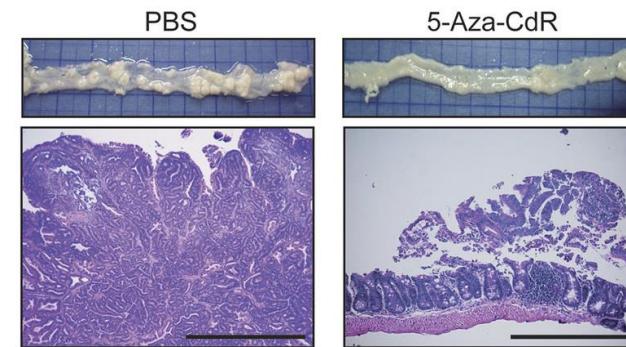
Calculate sphere-initiating frequency



Calculate cancer-initiating frequency



b

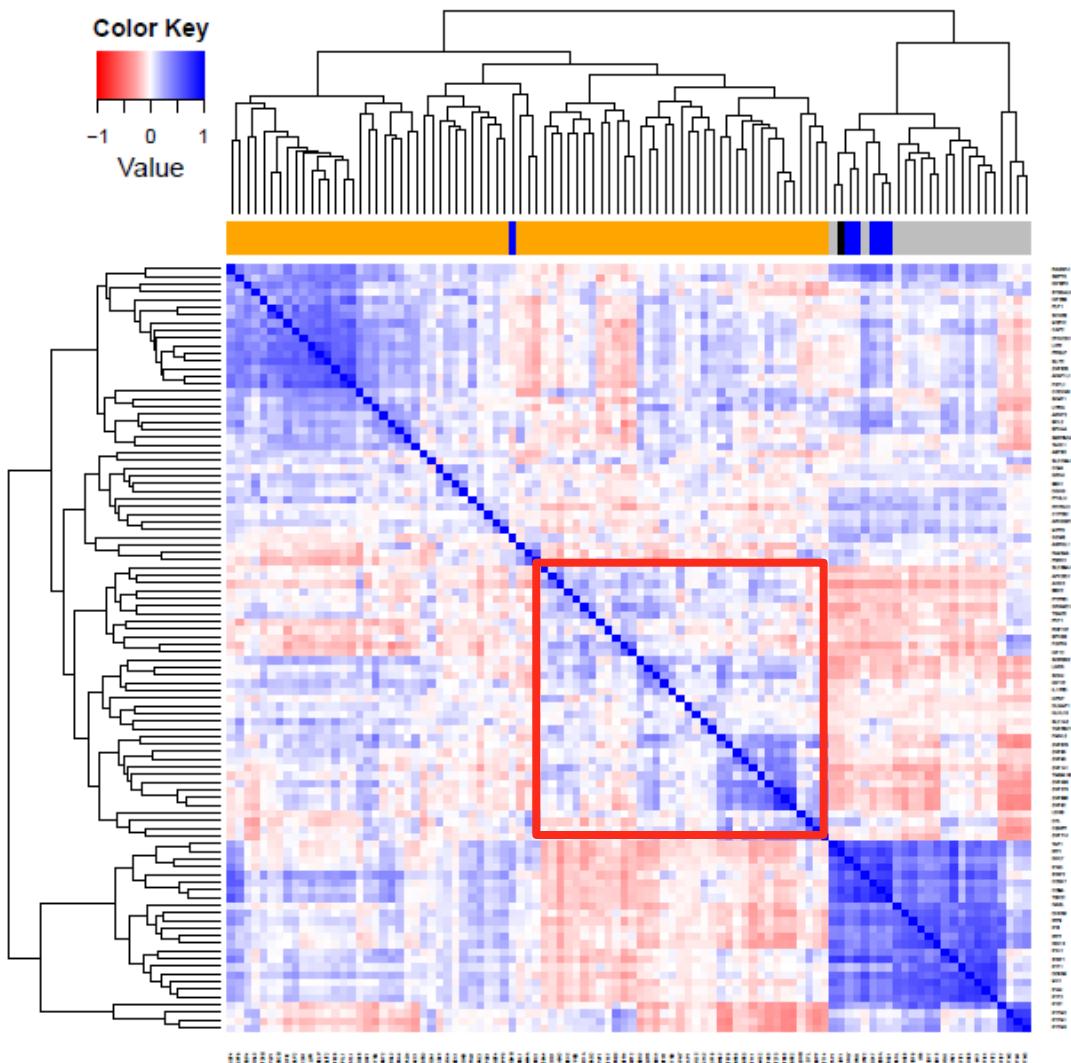


Article | OPEN

Inhibition of DNA Methylation Suppresses Intestinal Tumor Organoids by Inducing an Anti-Viral Response

Yoshimasa Saito, Toshiaki Nakao, Kasumi Sakai, Toshihide Muramatsu, Kohta Toshimitsu, Masaki Kimura, Takanori Kanai, Toshiro Sato & Hidetsugu Saito

IRF7 and CD8 T_{eff} Signatures are Positively Correlated in CRC



Ettayebi, unpublished

WNT/β-catenin
signaling

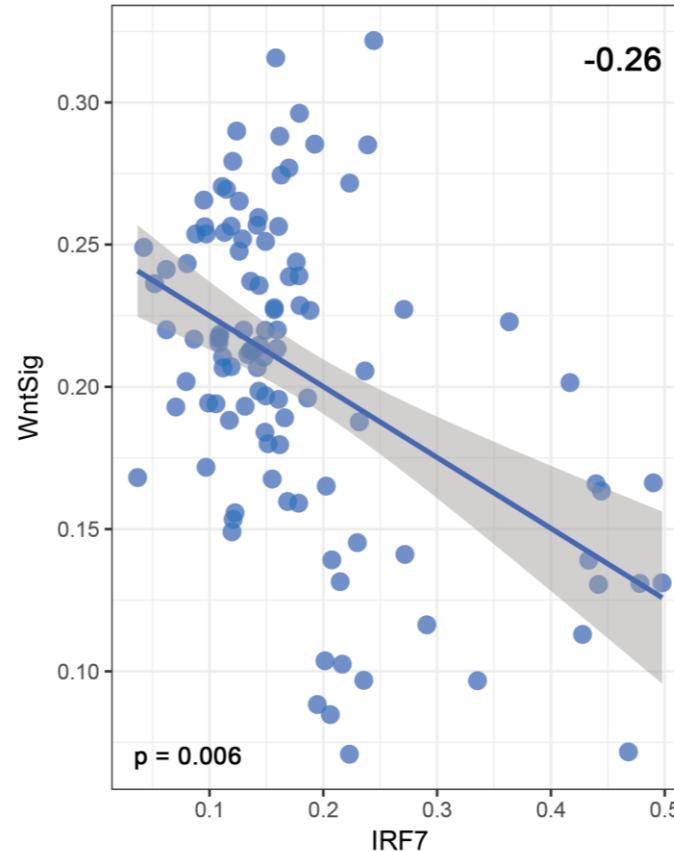
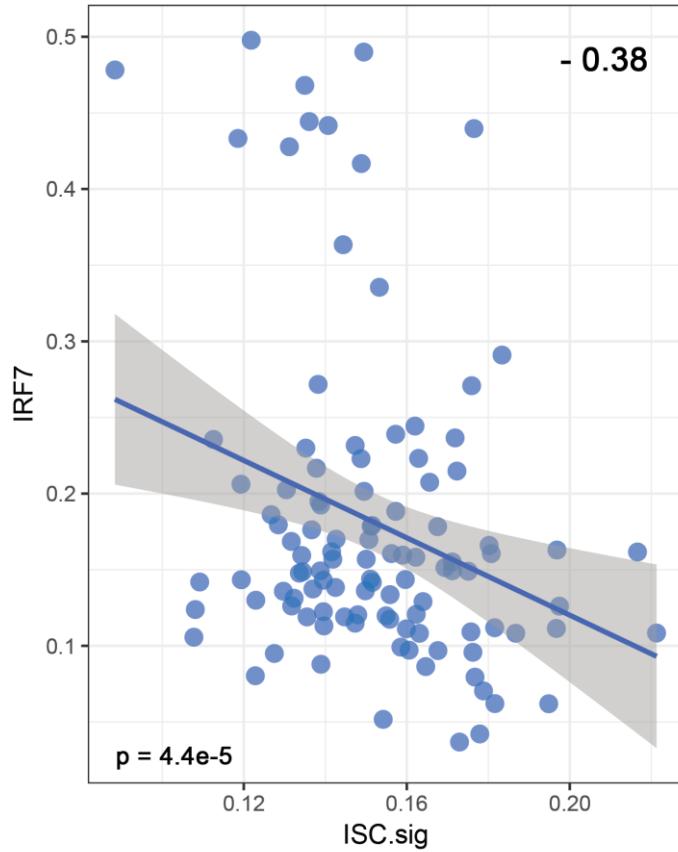
LETTER

Melanoma-intrinsic β-catenin signalling prevents anti-tumour immunity

Stefani Spranger¹, Riyue Bao² & Thomas F. Gajewski^{1,3}

doi:10.1038/nature14404

IRF7 and ISC/WNT Signatures are Negatively Correlated in CRC Organoids



5-AZA-CdR or Poly I:C (RIGI/MDA5 ligand) Decrease WNT signalling in CRC

B Activation of Wnt pathway

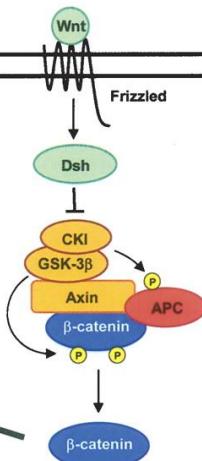
Plasma membrane

Cytoplasm

Nucleus

Tcf/Lef

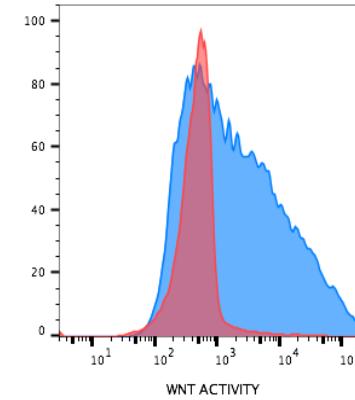
Frizzled



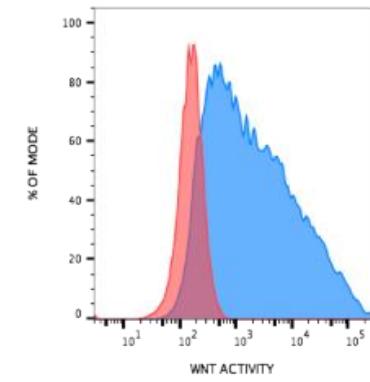
TCF/LEF Reporter (GFP)

■ Control
■ Treatment

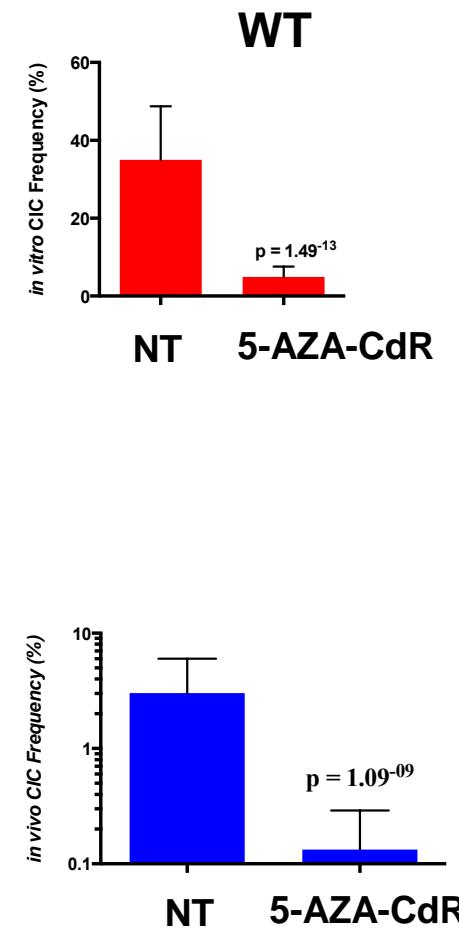
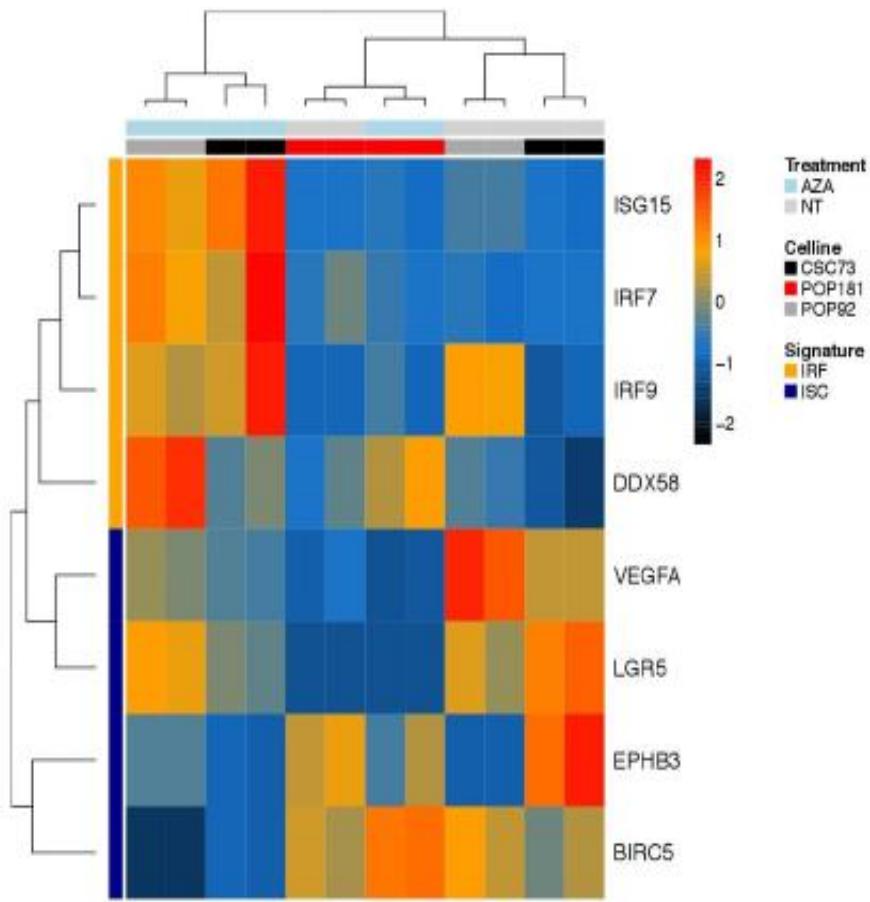
5-AZA-CdR



Poly I:C

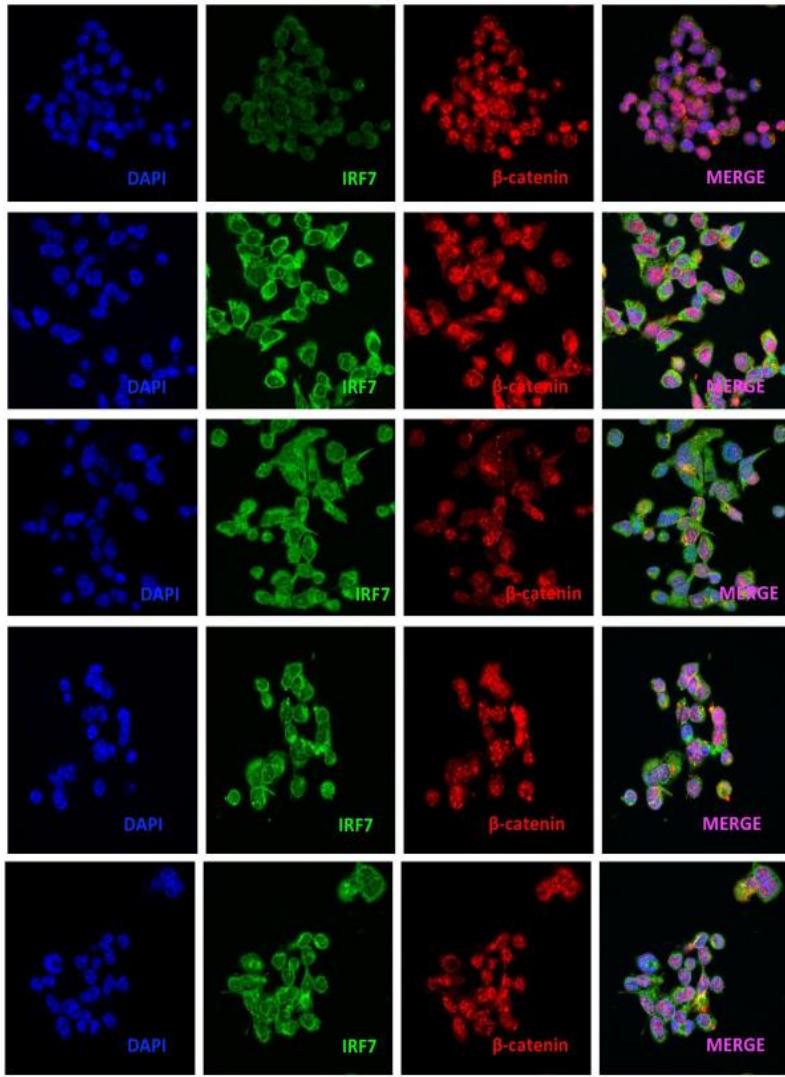


5-AZA-CdR Induce ISGs and Decrease WNT signalling in CRC

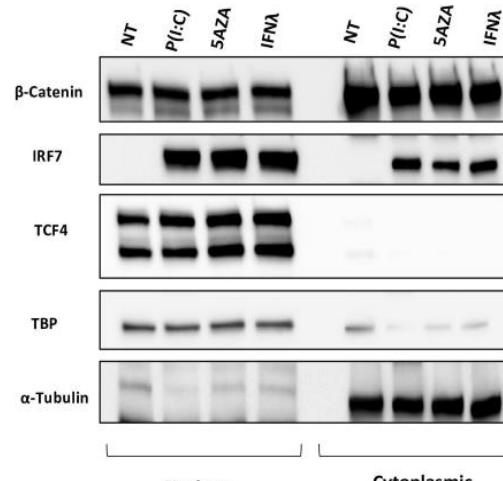
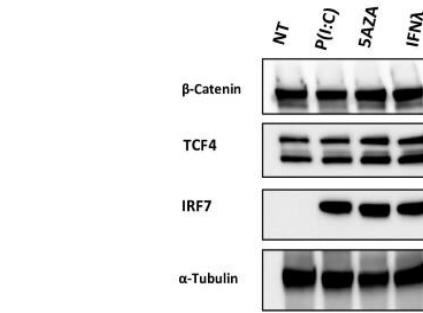


IRF7 Signalling Does Not Change β -Catenin Localization

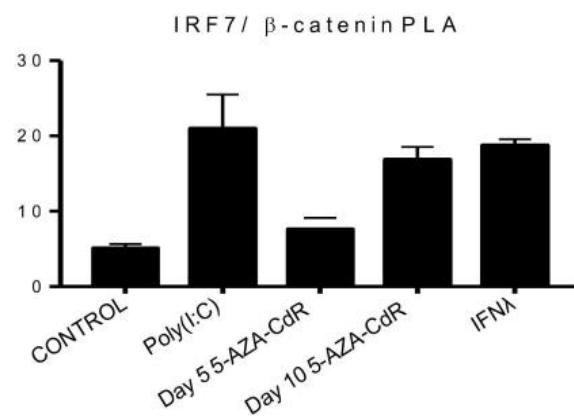
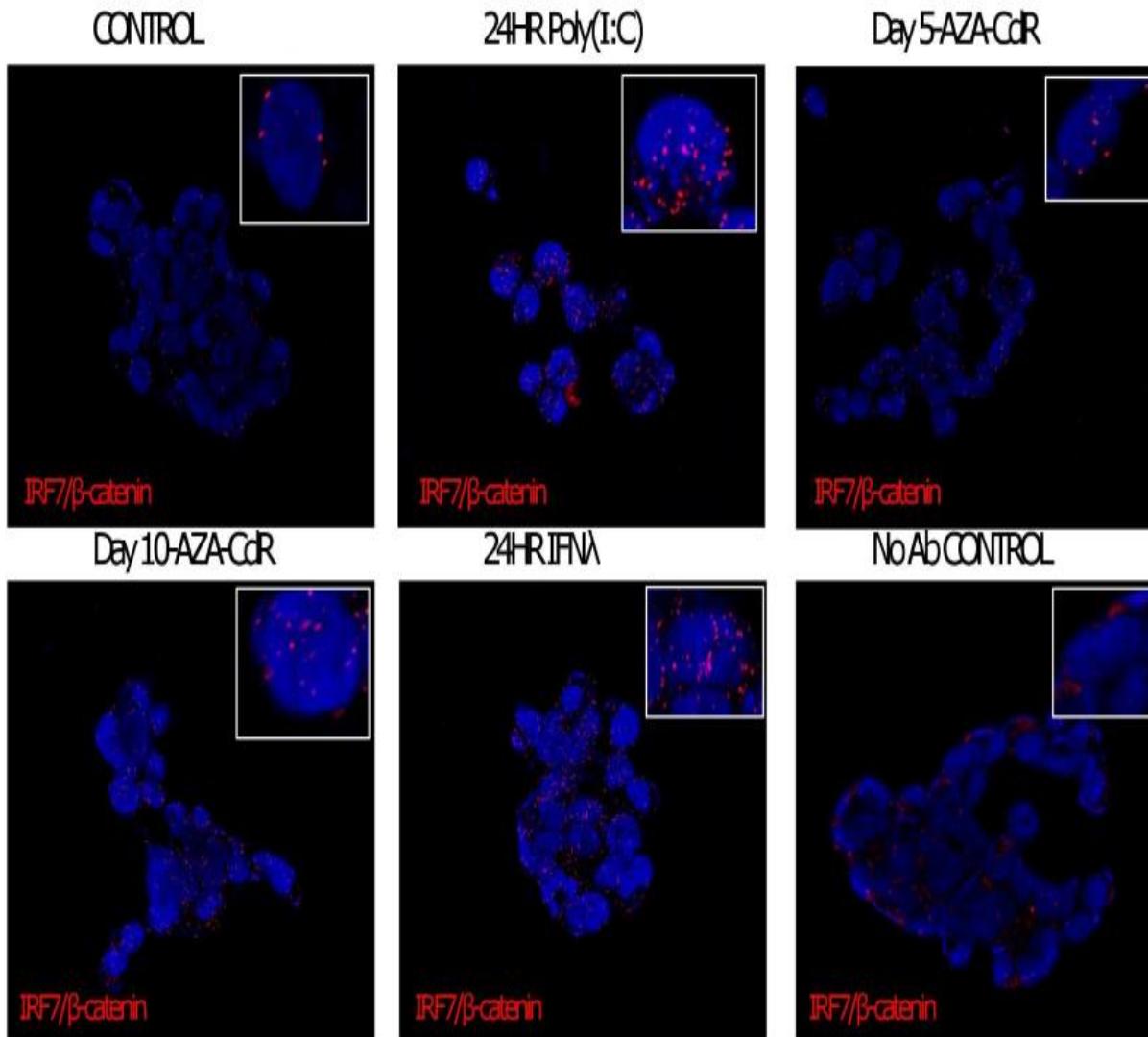
Control



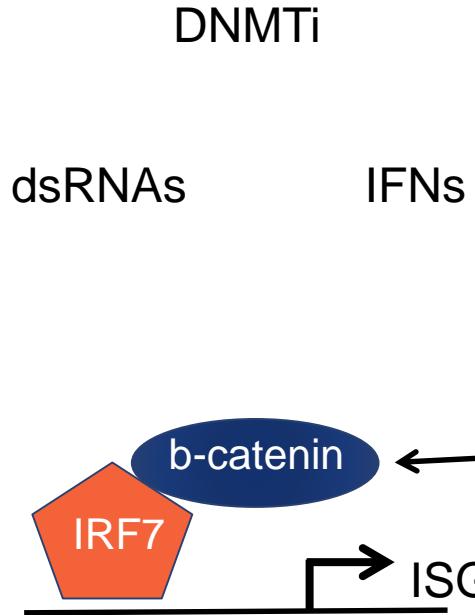
Whole cell lysate



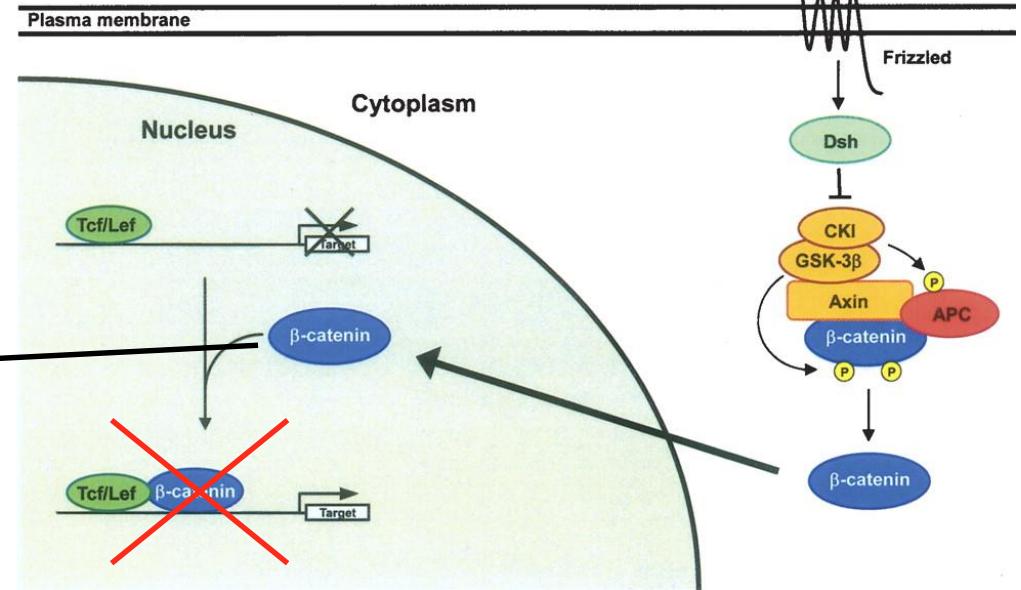
IRF7 Physically Interacts with β -Catenin



Working Model



B Activation of Wnt pathway



Immune Response
CD8 T Cell Infiltration

Stemness/Self Renewal
Low immune response

Acknowledgements

De Carvalho Lab

Lab Alumni:

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Lab Members:

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Ilias Ettayebi

Ankur Chakravarthy, PhD

Rajat Singhania, PhD

Shu Yi (Roxana) Shen, MSc

Tiago Medina, PhD

Shengrui (Frank) Feng, MSc.

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Cancer Foundation**  UHN