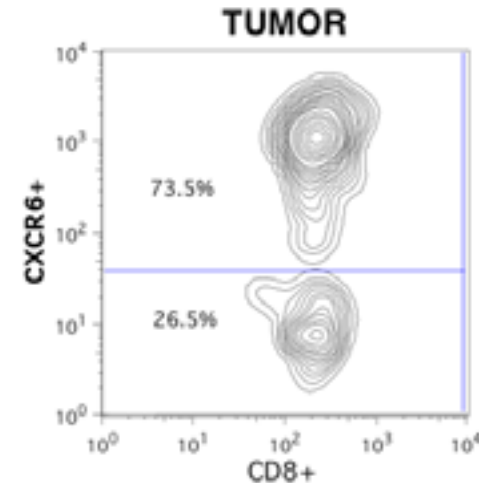
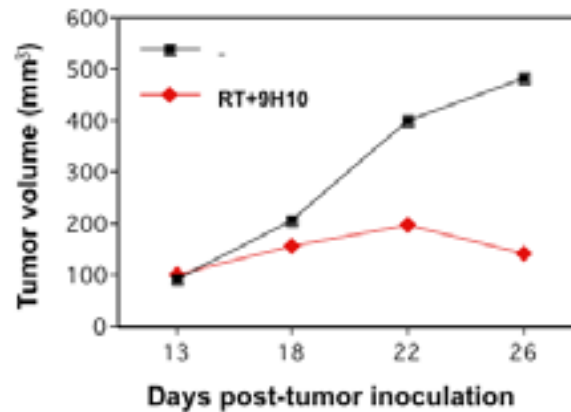
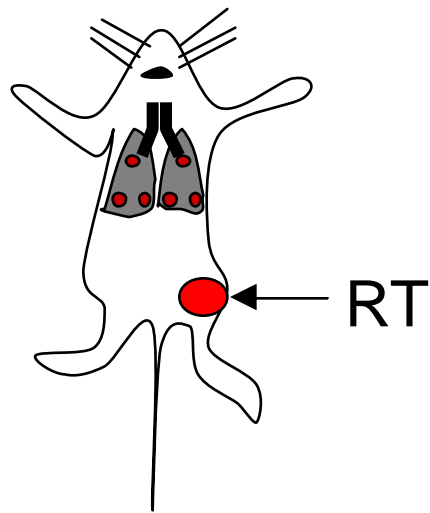


# **Stress-induced signals promote T cell-tumor cell interactions following anti-CTLA4 therapy**

**Maria Grazia Ruocco  
NYU School of Medicine**

# 4T1 mouse breast cancer model



**Anti-CTLA4 mAb 9H10 in combination with local RT but NOT alone induced anti-tumor CD8 T cell responses that inhibited the primary tumor and its metastases**

**CD8 T cells infiltrating the regressing tumors were CXCR6+**

*Demaria et al., Clin. Cancer Res. 11:728-34, 2005*

*Matsumura et al., J Immunol 181:3099-3107 2008*

# Hypothesis

Ionizing radiation alters the expression of cell surface molecules on tumor cells, resulting in changes in the interactions between tumor cells and T cells.

## Aim

A better understanding of tumor cell-T cell interactions will contribute to improve clinical approaches.

# Experimental model



injection of  
4T1 tumor cells

*short term*

RT

RT

9H10

16 imaging

*long term*

RT

RT

9H10

9H10

22 imaging

day

13

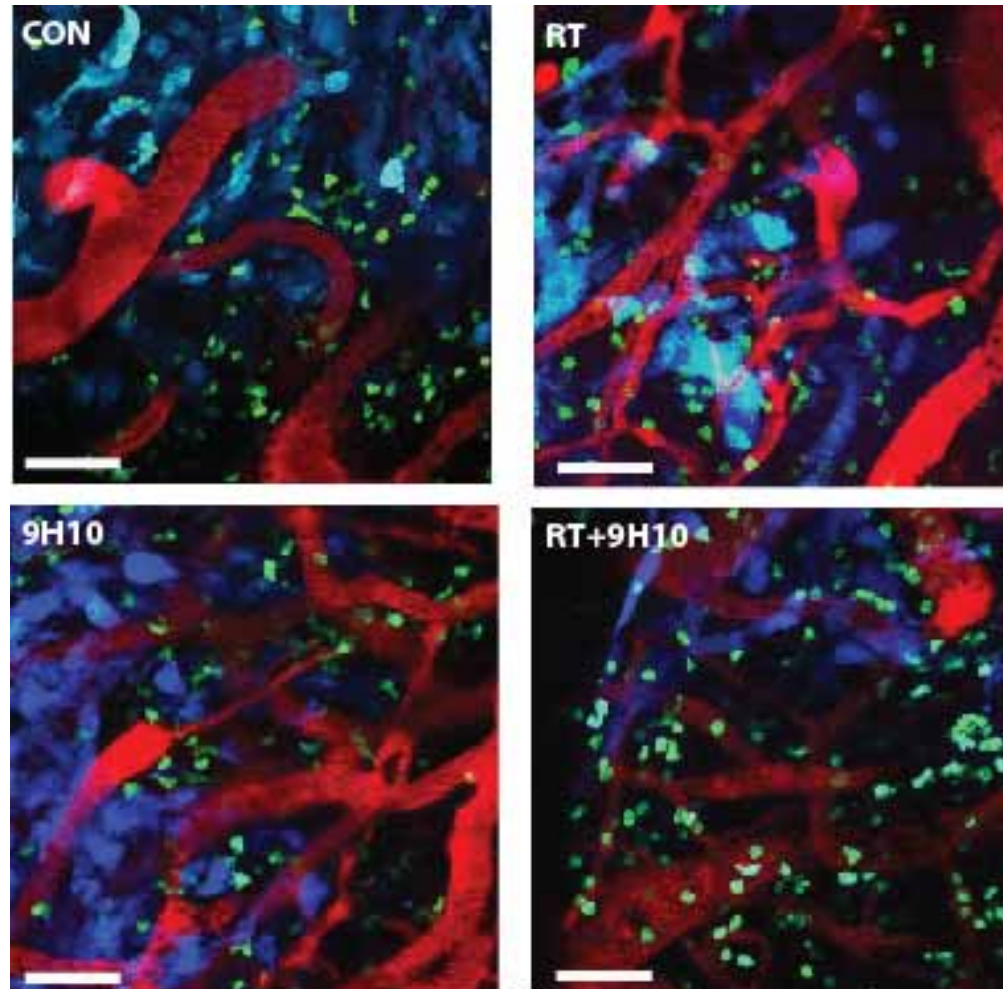
14

15

18

Blood vessels  
T cells  
2nd harmonic

# T cells infiltrate the tumor microenvironment



# Control

Blood vessels  
T cells  
4T1 tumor cells

RT

Blood vessels  
T cells  
4T1 tumor cells



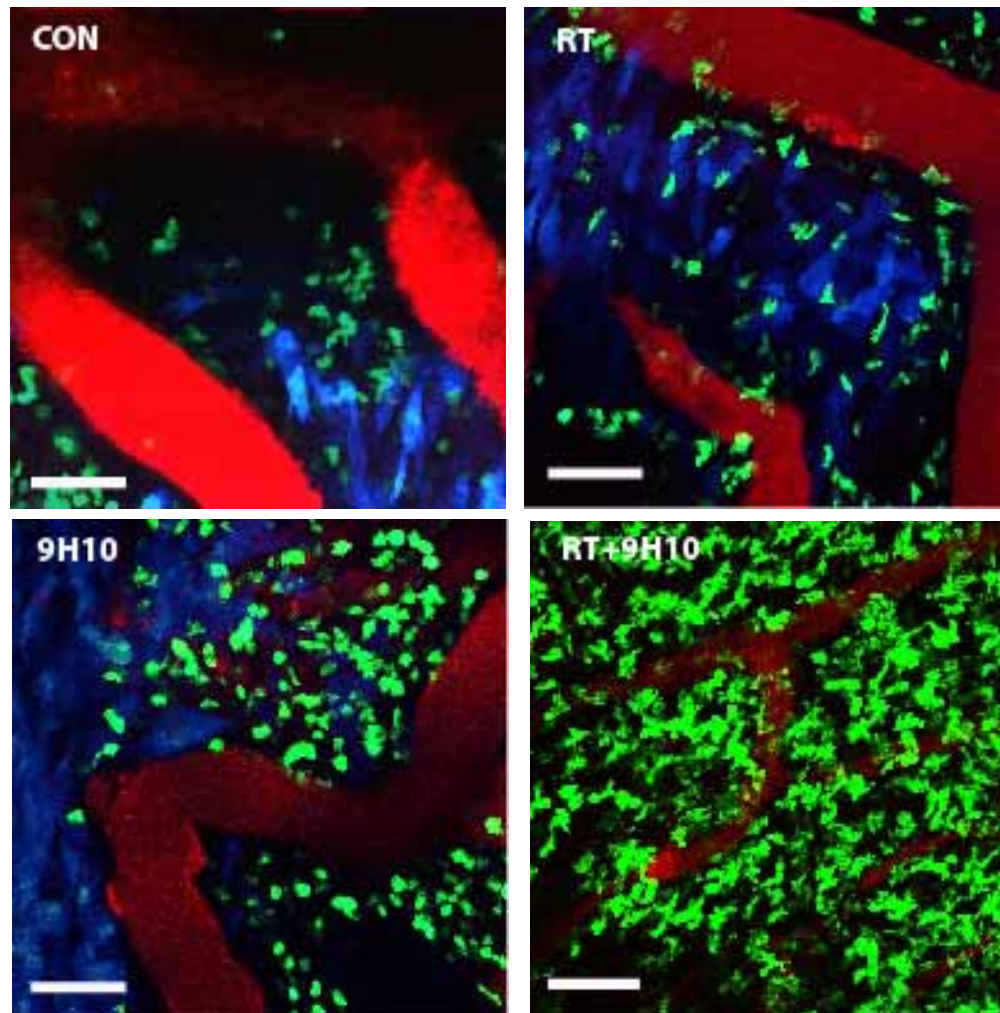
# 9H10

Blood vessels  
T cells  
4T1 tumor cells

# RT+9H10

Blood vessels  
T cells  
4T1 tumor cells

# RT+9H10 leads to tumor eradication



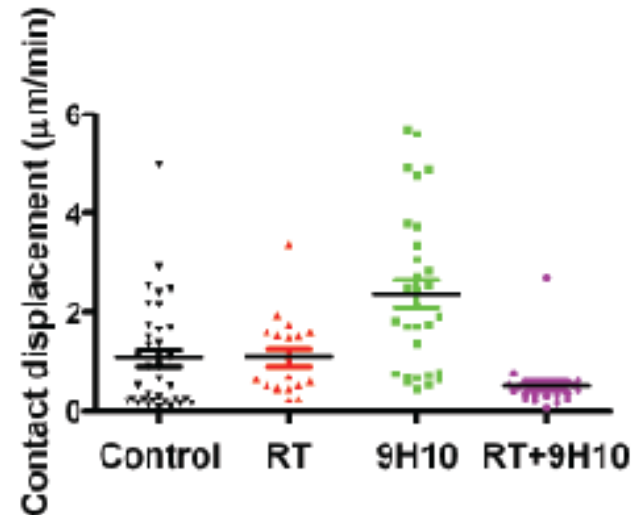
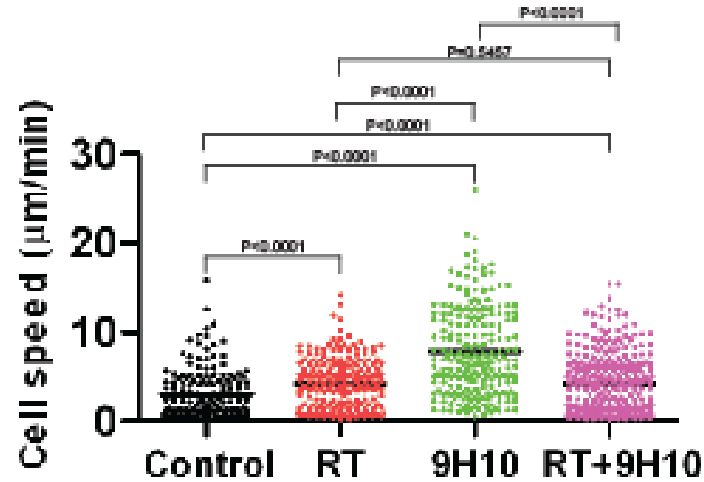
RT

Blood vessels  
T cells  
4T1 tumor cells

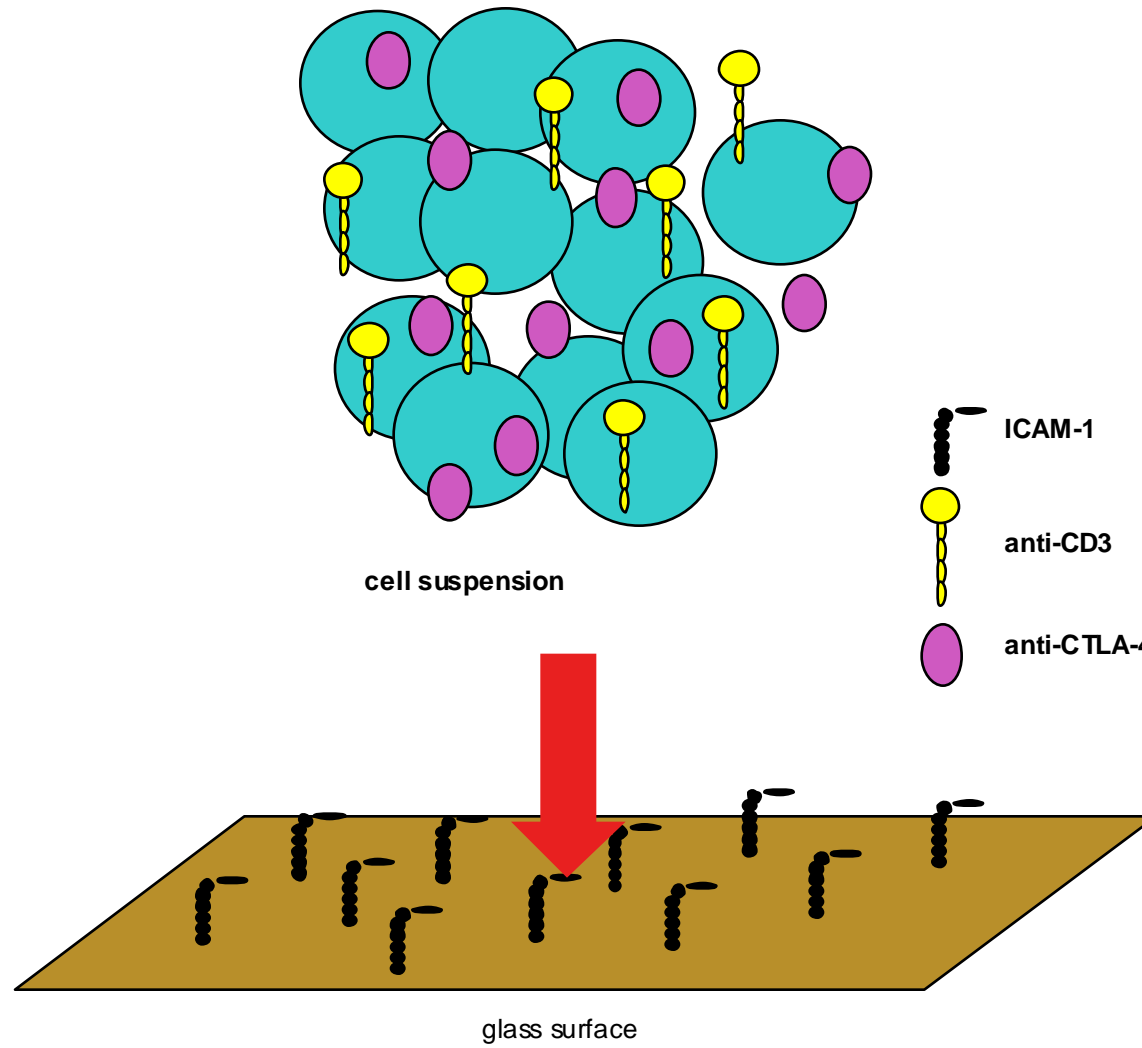
# RT+9H10

Blood vessels  
T cells  
4T1 tumor cells

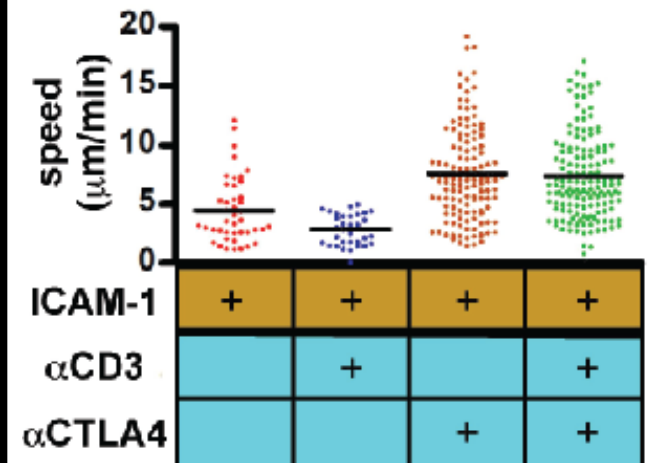
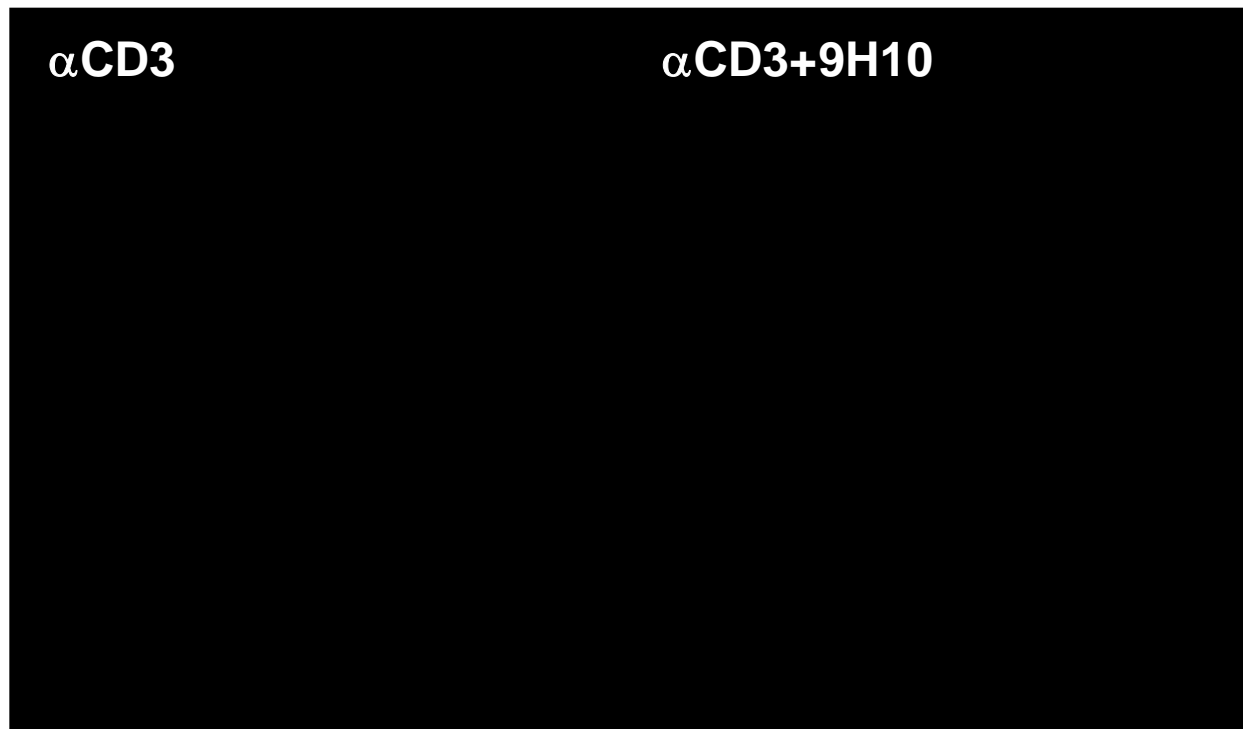
# RT and 9H10 combined treatment increases the duration of T cells-tumor cell interactions



# *In vitro* T cell migration assay

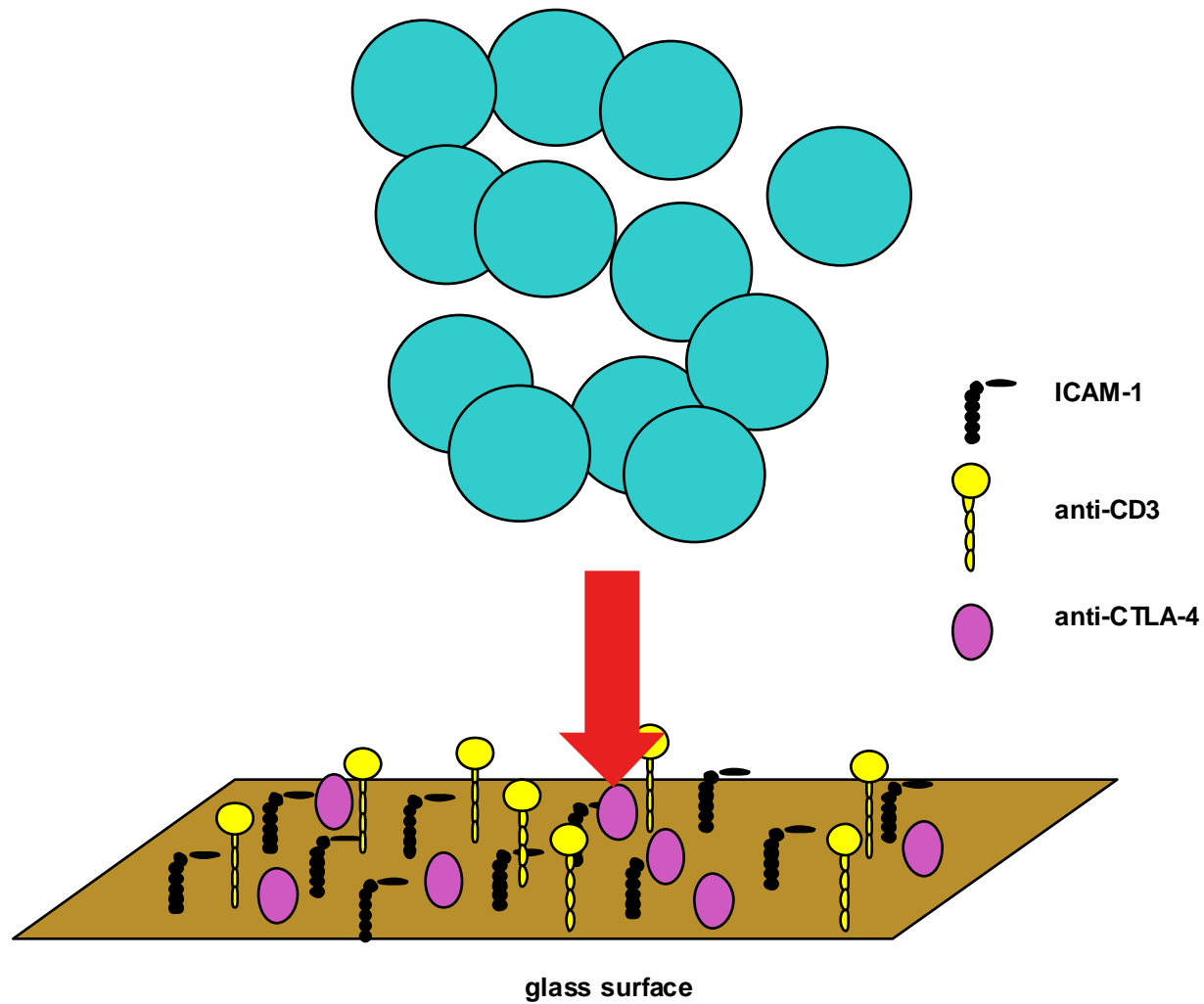


# Anti-CTLA4 induces high T cell motility and prevents stable T cell-tumor cell interactions



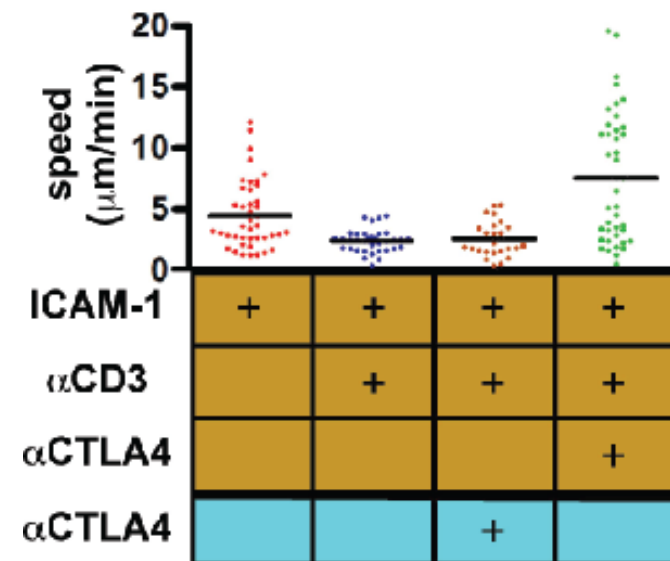


# *In vitro* T cell migration assay

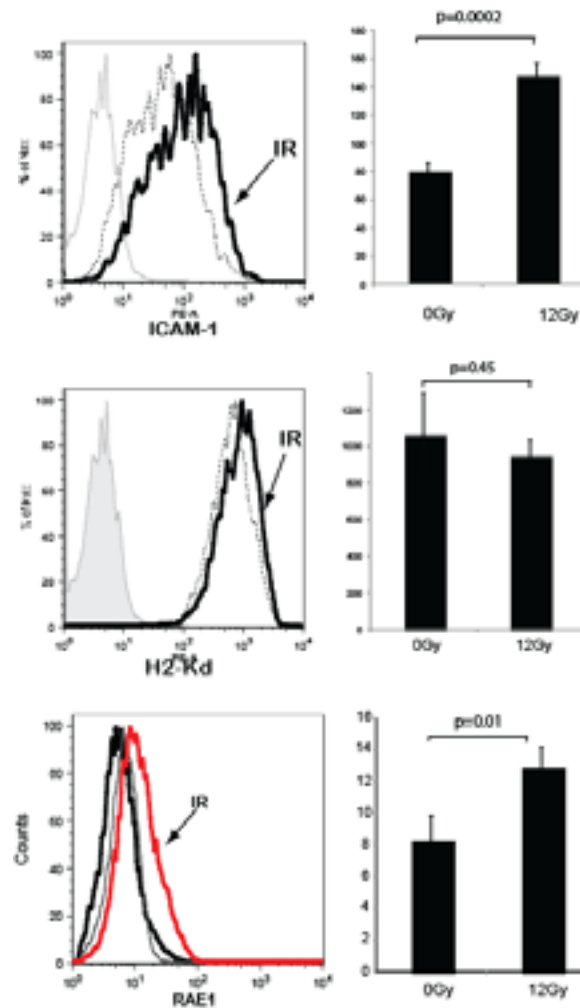


# Soluble anti-CTLA4 fails to override a bound anti-CD3 stop signal

bound $\alpha$ CD3+sol9H10



# ICAM-1 and Rae1 expression is increased in 4T1 tumor cells following *in vivo* RT



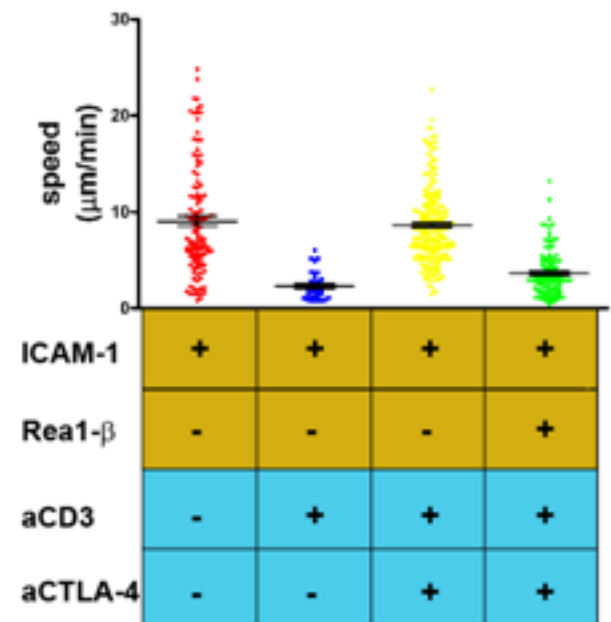
# Rae1- $\beta$

- A stress-activated molecule, expressed on transformed cells.
- Binds to NKG2D, expressed on effector cells including activated cytolytic CD8 T cells.
- Interaction of Rae1 with NKG2D expressed on anti-tumor CD8+ T cells is important for immune-mediated tumor inhibition in the 4T1 tumor model (Nam JS et al. Cancer Res., 2008).

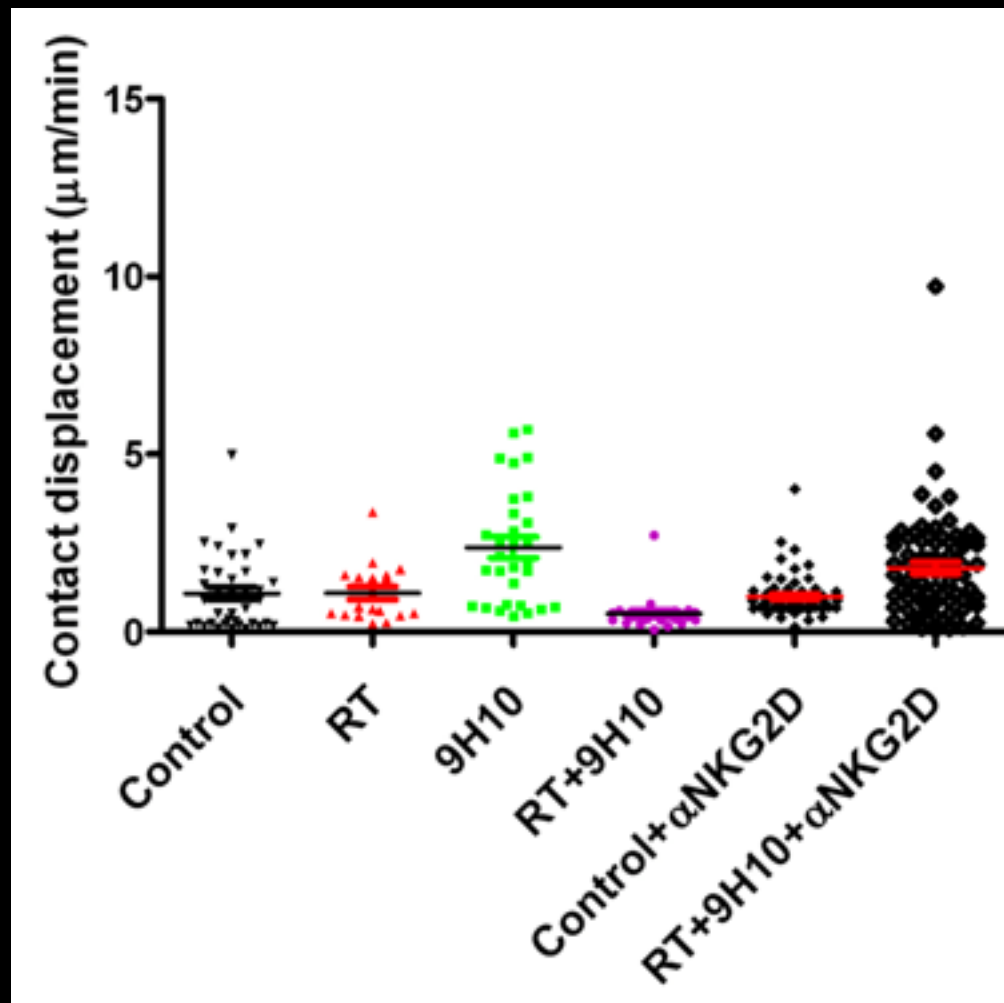
# Rae1- $\beta$ converts the anti-CTLA4 'go' signal into a 'stop' signal

$\alpha$ CD3+9H10

$\alpha$ CD3+9H10+ $\alpha$ NKG2D



*In vivo* anti-NKG2D antibody treatment:  
reverses the effects of RT+9H10



# Conclusions

- RT+9H10 increases the duration of T cell-tumor cell interactions *in vivo*.
- 9H10 treatment alone increases T cell migration decreasing the duration of T cell- tumor cell interactions.
- RT upregulates ICAM1 and Rae1 on tumor cells
- Blocking the interactions between Rae1 and NKG2D prevents stable interactions suggesting that these molecules are important for formation of stable IS and T cell activation.

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