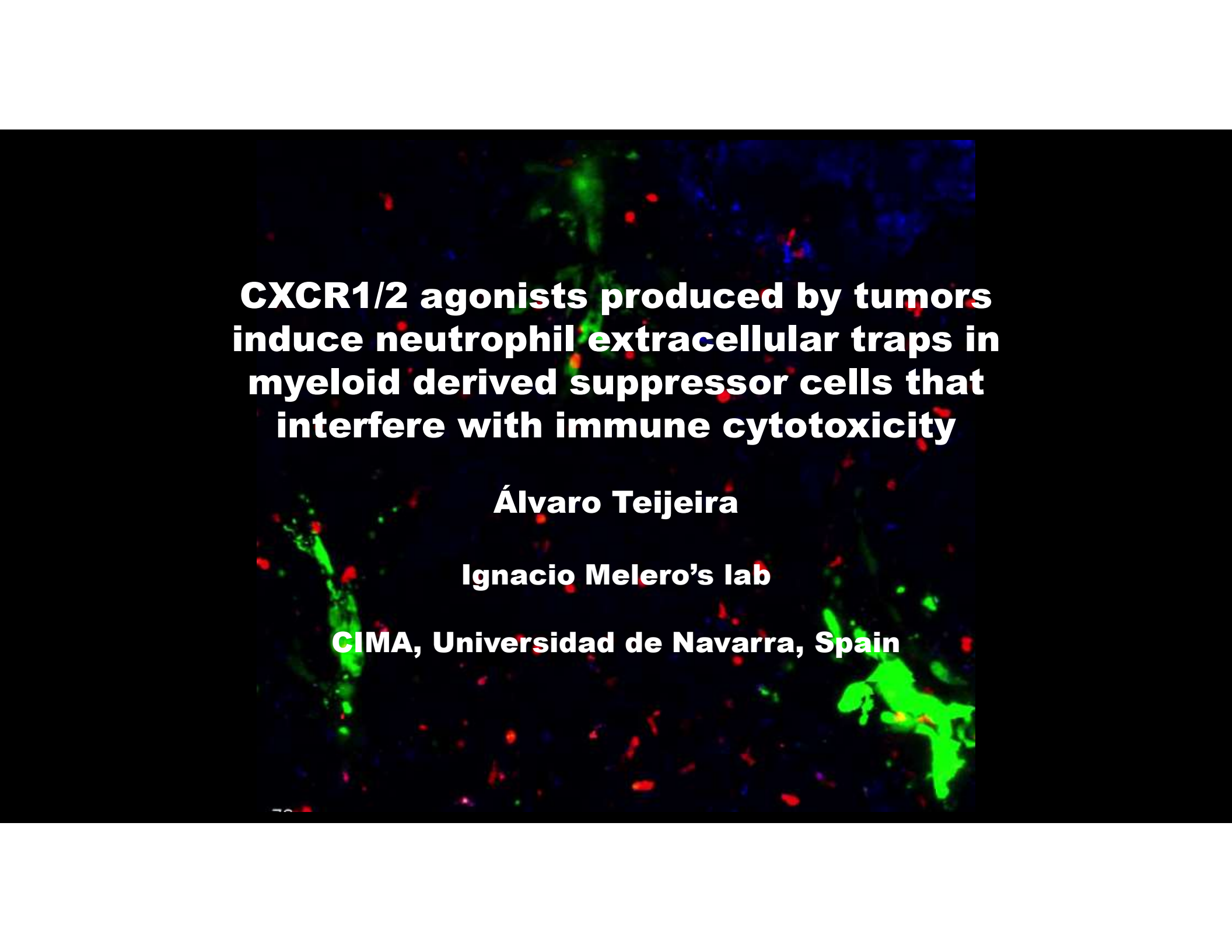




**CXCR1/2 agonists produced by tumors
induce neutrophil extracellular traps in
myeloid derived suppressor cells that
interfere with immune cytotoxicity**

Álvaro Teixeira

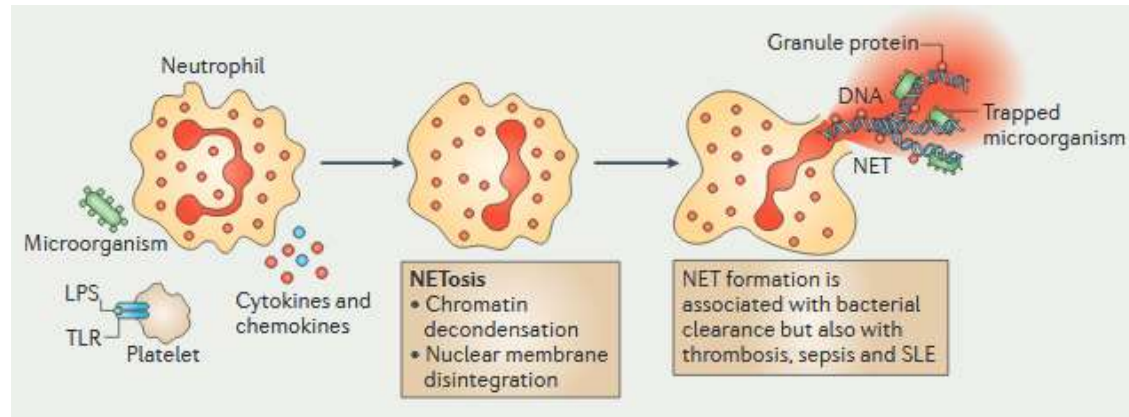
Ignacio Melero's lab

CIMA, Universidad de Navarra, Spain

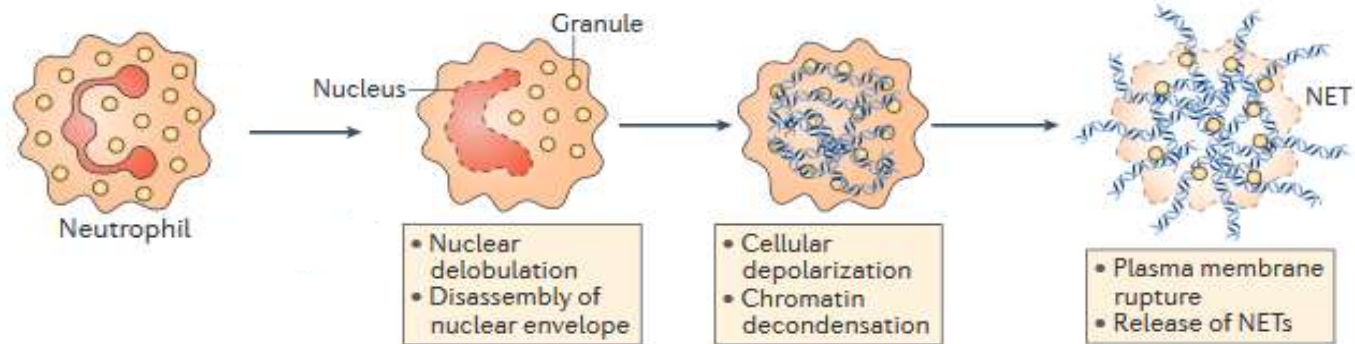
DISCLOSURES

- The presenter declare no conflicts of interest
- This study has been partially funded by Bristol Myers Squibb through II-ON Network

Neutrophil Extracellular Traps

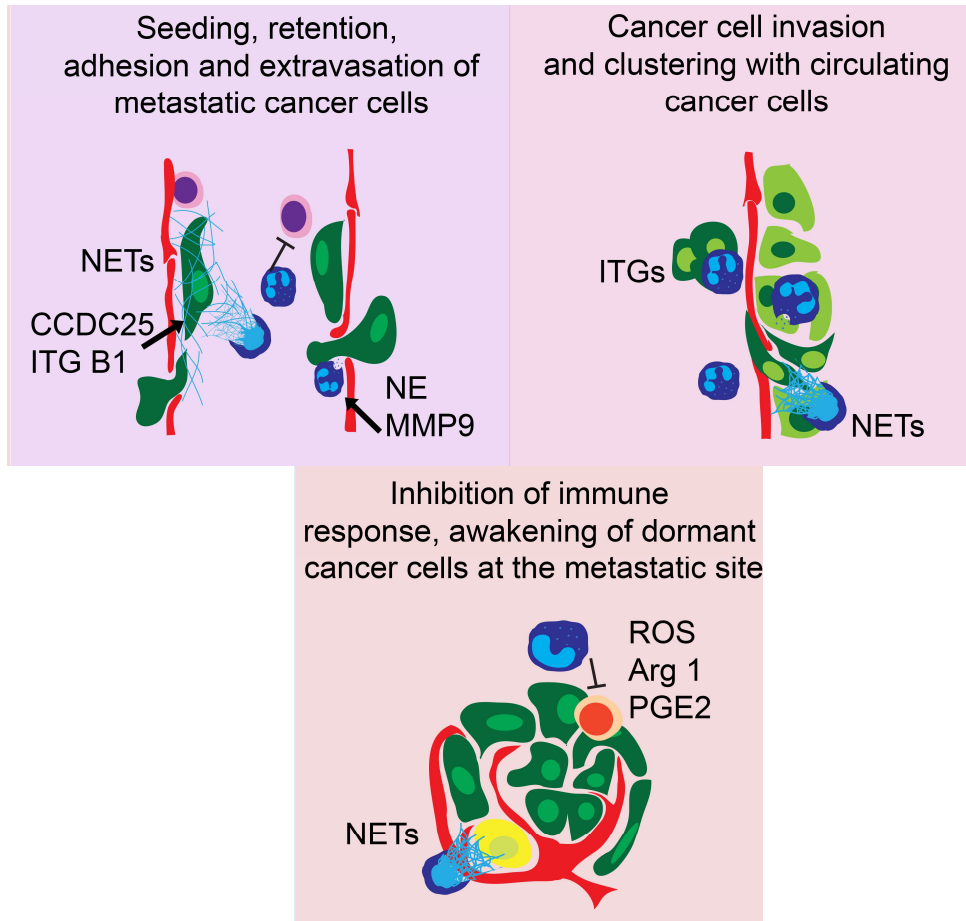


Mantovani et al. 2011



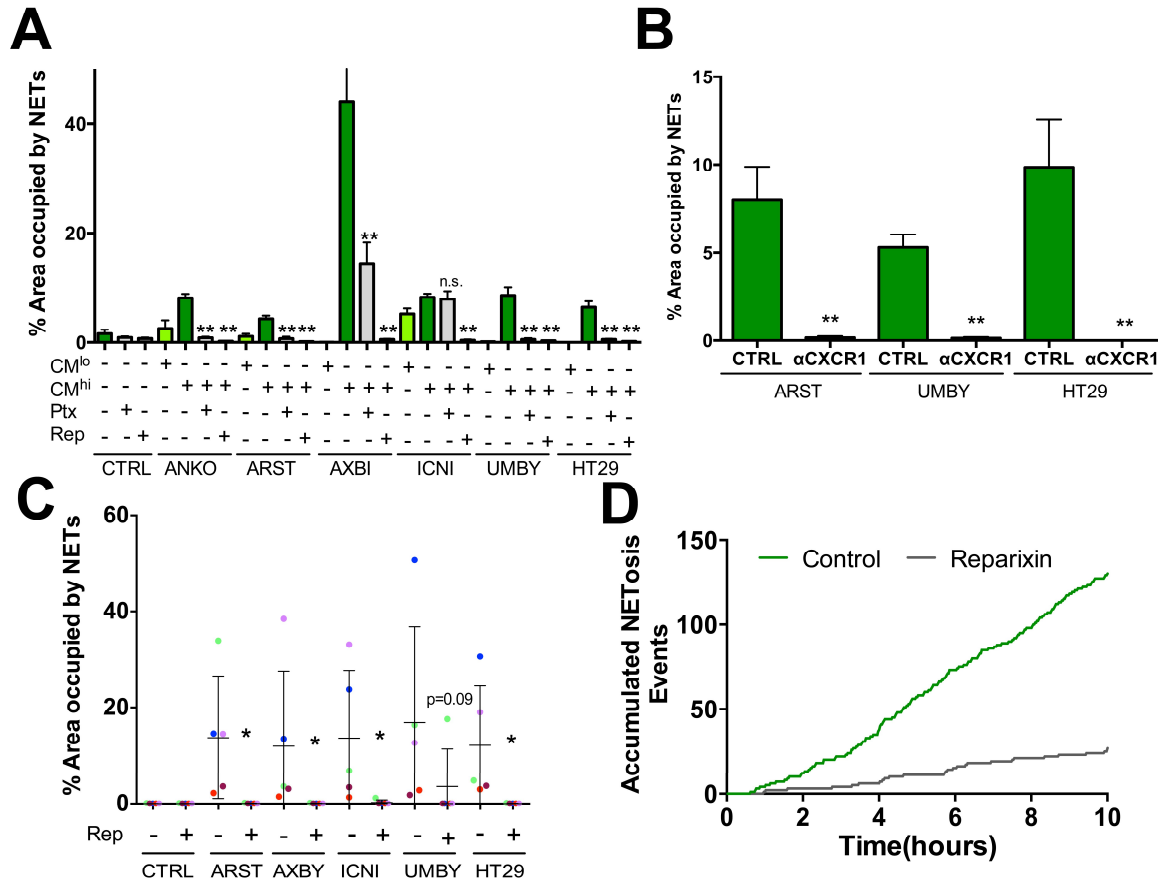
Papayannopoulos 2018

NETosis in cancer



Malignancy	Technique	Observations
Ovarian cancer	H3-Cit IF	N/A
Large B cell lymphoma	H3-Cit IF, MPO-DNA ELISA	Increased NETs in advanced stages, worse PFS, OS
Lung adenocarcinoma	MPO-DNA ELISA	Increased NETs in advanced stages, correlation with liver metastases
Esophagogastric adenocarcinoma	MPO-DNA ELISA	Increased NETs in advanced stages
Breast cancer	MPO-DNA ELISA	Increased liver metastases, increased NETs in triple-negative BC
Colorectal cancer	MPO-DNA ELISA	Correlation with pro-coagulation parameters
Gastric Adenocarcinoma	NE-DNA ELISA	Correlation with worse PFS in HER2 negative patients only
Oral squamous cell carcinoma	MPO-DNA ELISA	Increased NETs in advanced stages
Pancreatic Cancer	H3-Cit IF	Association with bad clinical prognosis after surgery
Liver metastases of colon and breast cancer	H3-Cit IF, MPO-DNA ELISA	Correlation with metastasis-free survival
Pan-cancer	H3-Cit and MPO-DNA ELISAs	Increased in cancer patients, correlates with bad poor prognosis

Tumor-Derived Factors Induce NETosis in Neutrophils and GR-MDSCs by Activating CXCR1 and CXCR2 Receptors

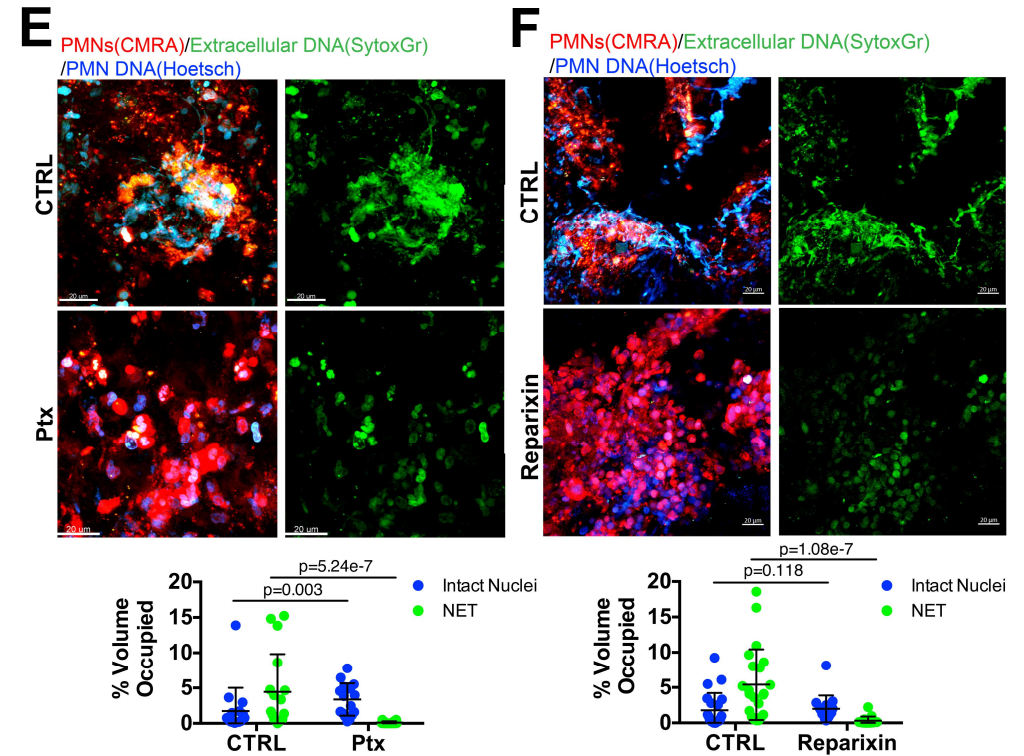
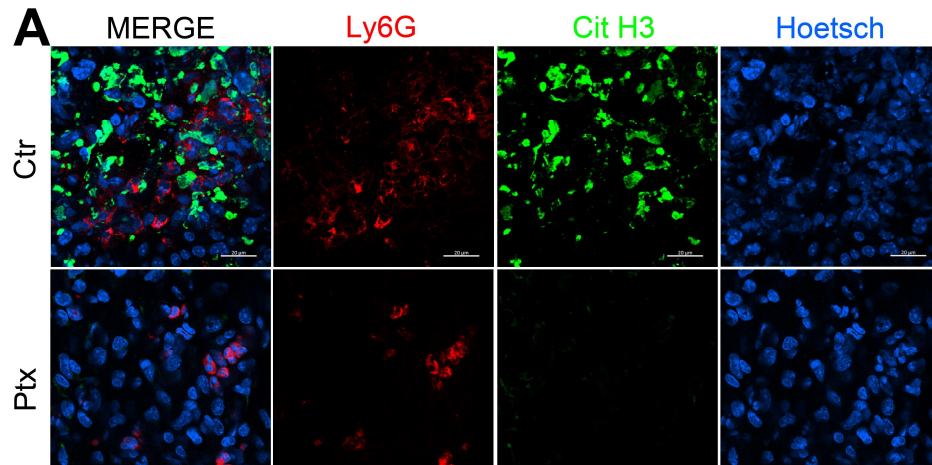


HT29 Spheroids in
Co-culture with Human Neutrophils

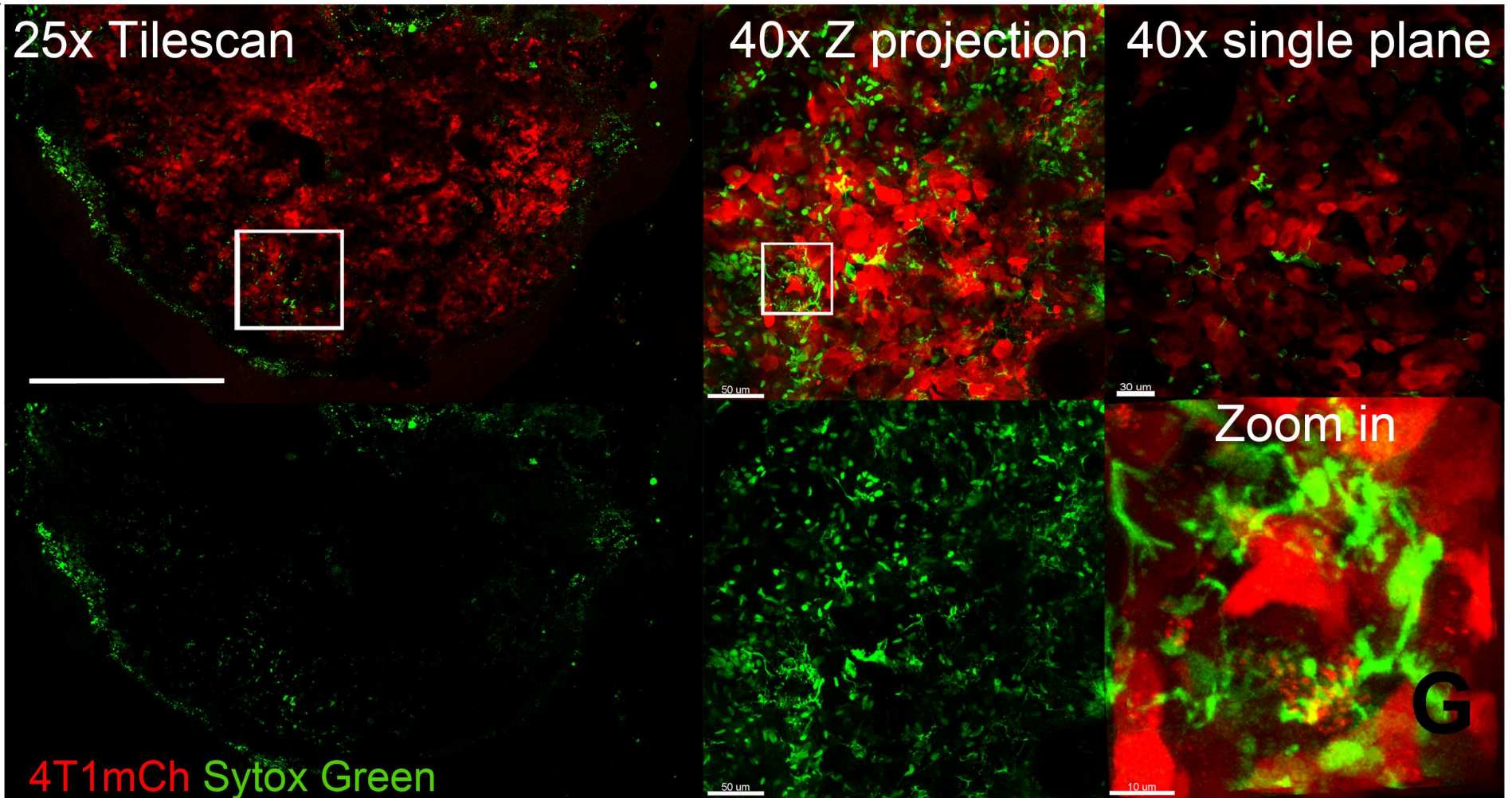
HT29/ CMRA
Neutrophils/ Cell Tracker Deep Red
NETs/ Sytox Green

10 hours video. 450x accelerated

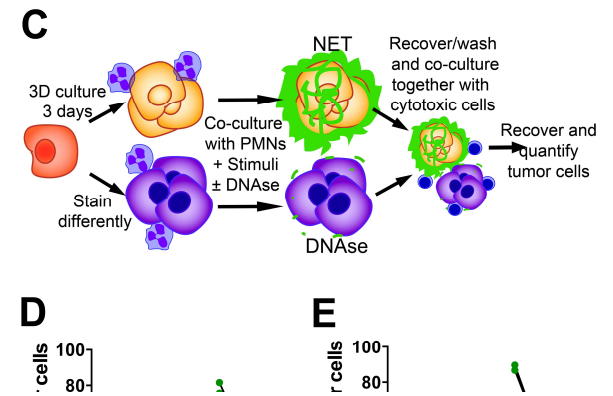
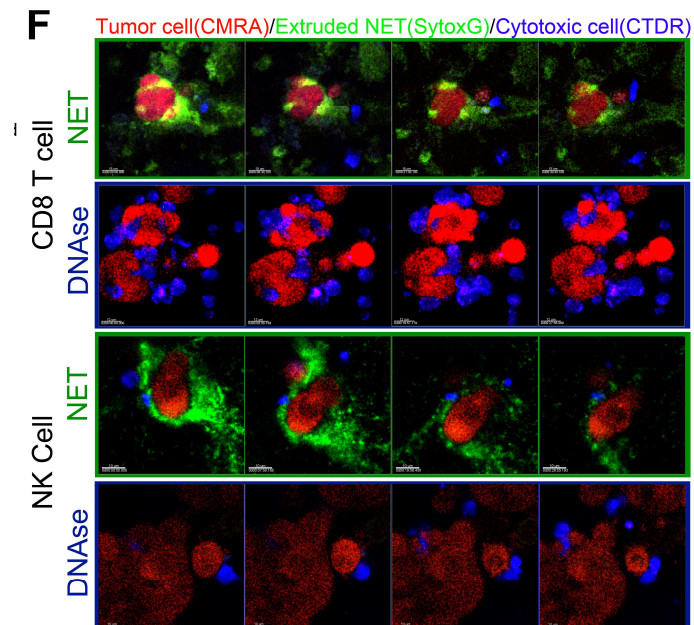
Tumors Induce NETosis by Activation of CXCR1 and CXCR2 Chemokine Receptors



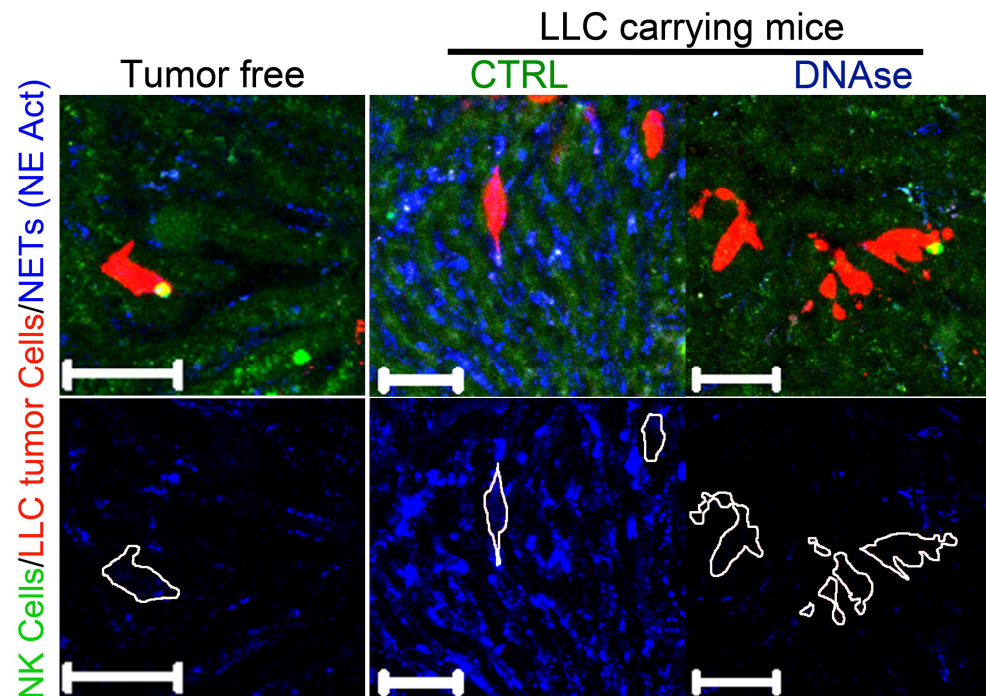
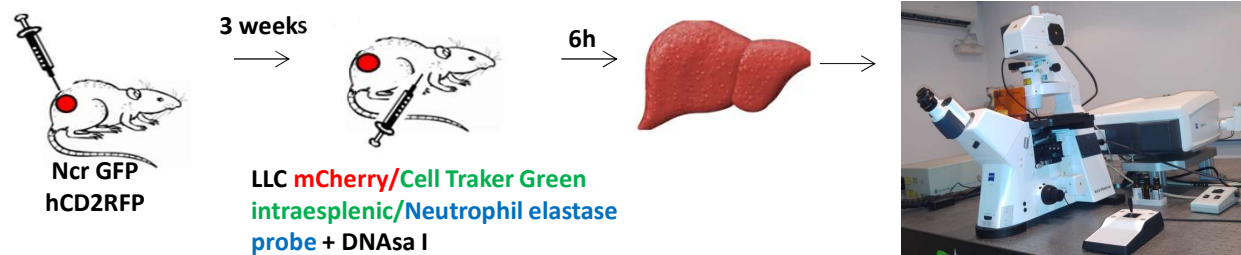
NETs Inhibit Immune Cell Cytotoxicity by
Impeding Contact with Tumor Cells



NETs Inhibit Immune Cell Cytotoxicity by Impeding Contact with Tumor Cells



NETs Impair Cytotoxic Cell Contact with Tumor Cells in the Metastatic Intravascular Niche



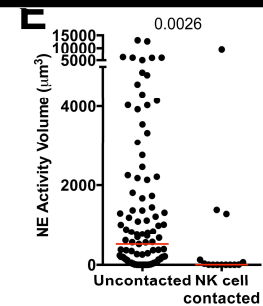
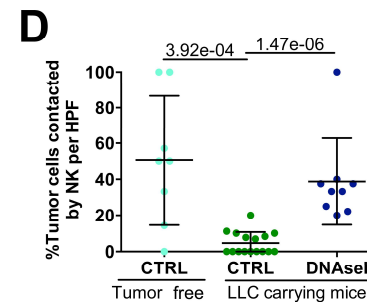
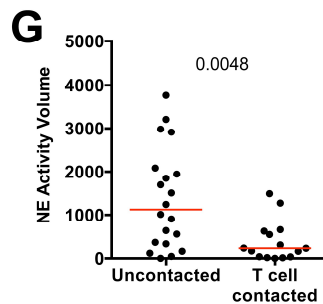
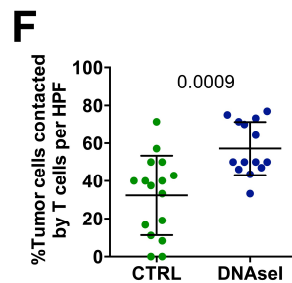
NETs Impair Cytotoxic Cell Contact with Tumor Cells in the Metastatic Intravascular Niche

Intravital imaging of LLC tumor cells in the liver sinusoids of hCD2RFP mice carrying LLC subcutaneous tumors.

T-Cells
LLC tumor cells
NETs (NE activity)

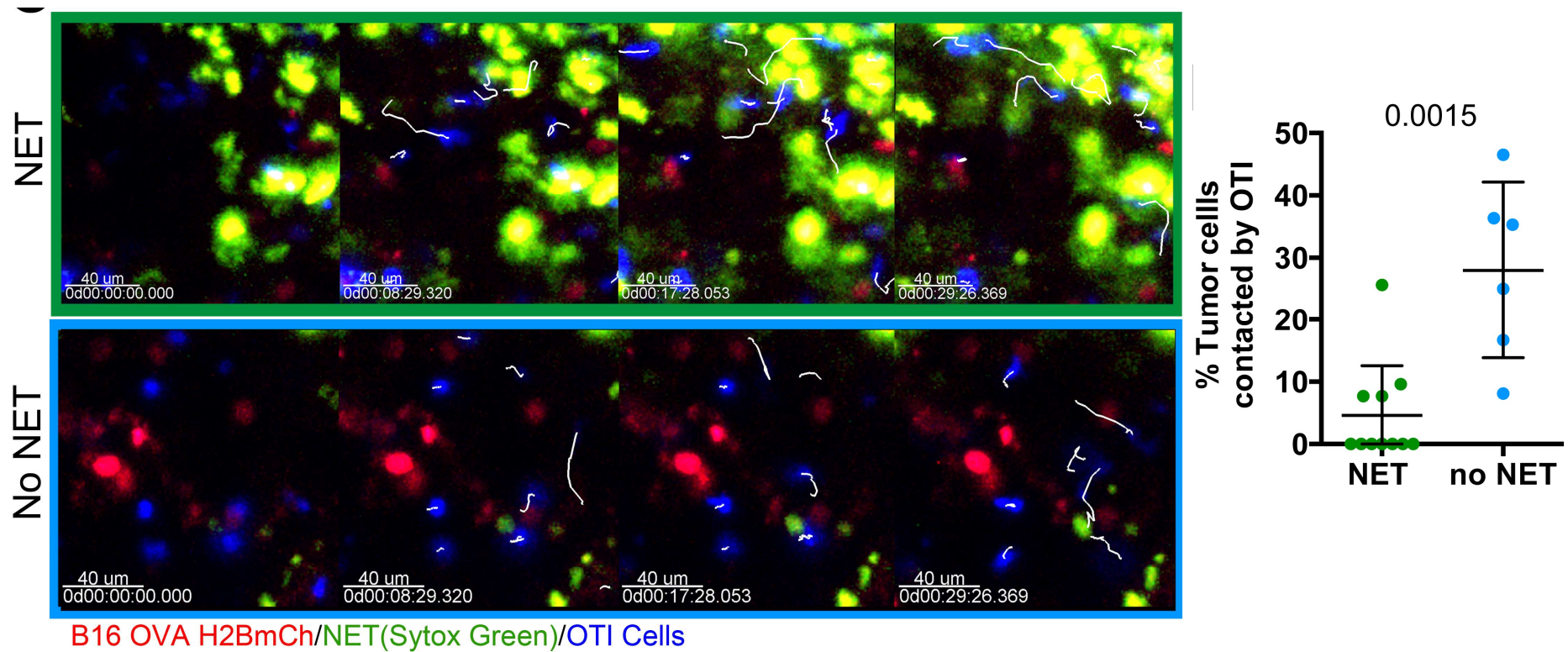
Intravital imaging of LLC tumor cells in the liver sinusoids of Ncr1 GFP mice

NK Cells (Intense green)
LLC tumor cells
NETs (NE activity)
Liver (autofluorescence, dim green)



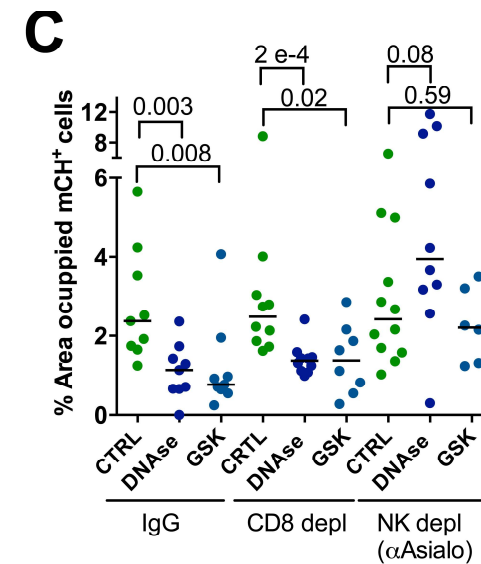
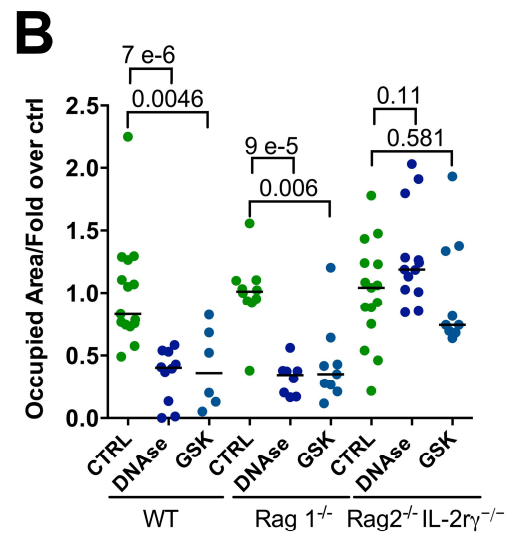
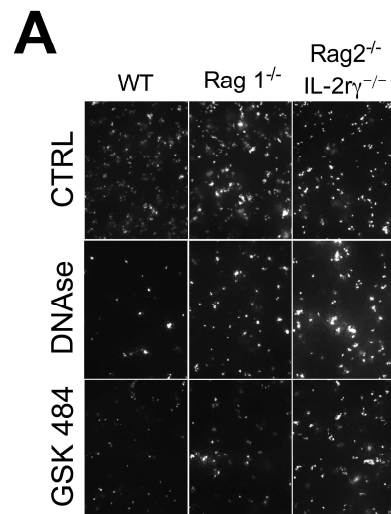
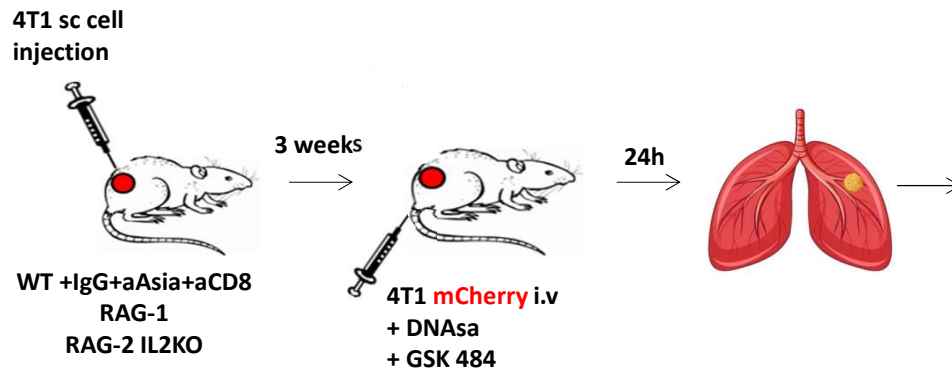
Intravital microscopy of B16 OVA tumor (**H2B mCherry**)
infiltrated by OTI T-Cells (**Cell tracker Deep Red**)
24h after NET (**Sytox Green**) injection

NETs Impair Cytotoxic Cell Contact with Tumor Cells in Subcutaneous Tumors

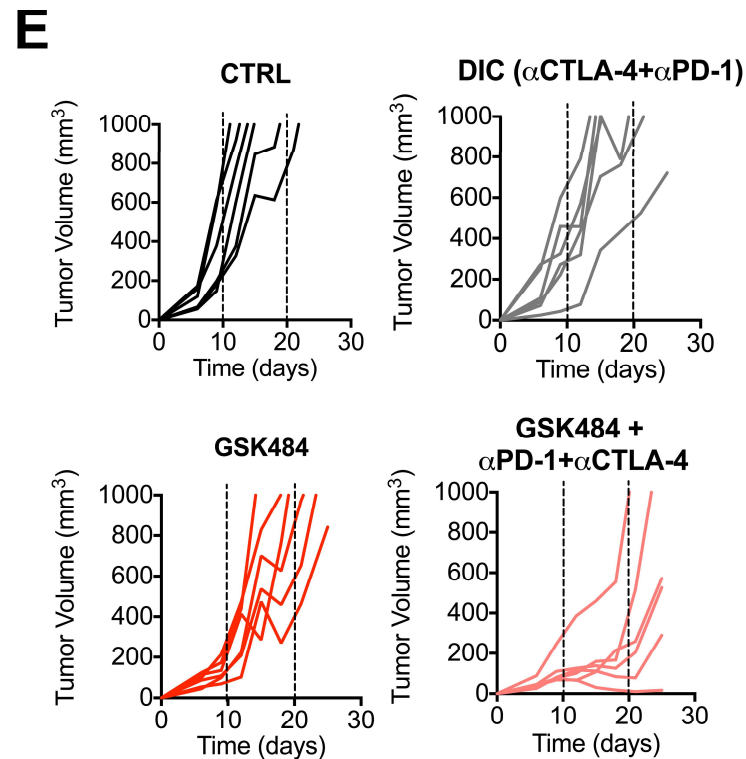
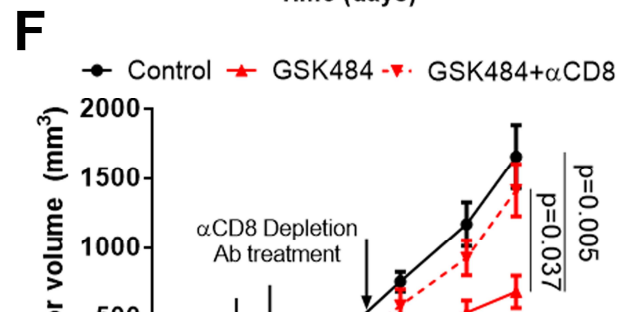
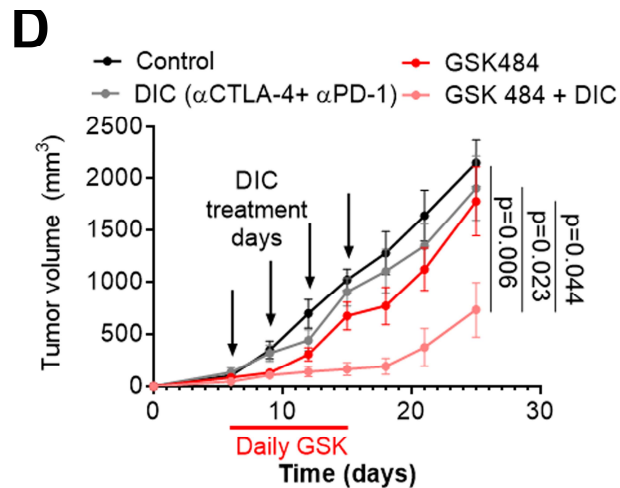


LLC GFP tumor cells (green) implanted in the ear of
hCD2RFP mice (T-cells, Red) in the presence of
NE fluorescent substrate to visualize NETs(blue)

NETs Limit Immune Responses and Checkpoint-Based Immunotherapy against 4T1 Tumors

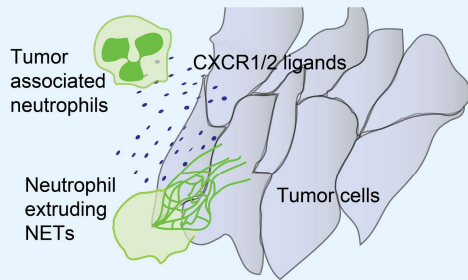


NETs Limit Immune Responses and Checkpoint-Based Immunotherapy against 4T1 Tumors

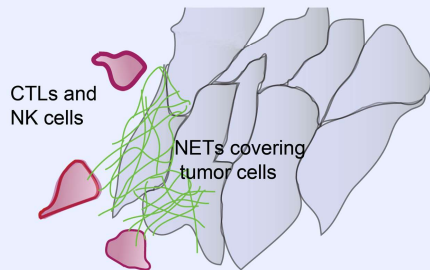


Conclusions

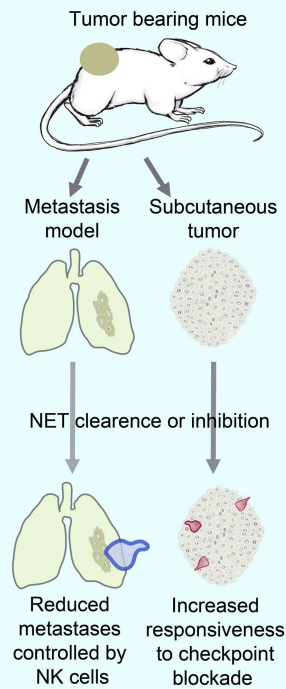
Tumor derived CXCR1/2 ligands induce tumor associated neutrophil release of extracellular traps



Neutrophil extracellular traps block cytotoxic immune cell contact with target tumor cells



NETs limit immune response to cancer



- Tumor-secreted CXCR1 and CXCR2 ligands induce extrusion of NETs
- NETs protect tumor cells from CTL and NK cytotoxicity in 3D cultures
- NETs impair contact of immune cytotoxic cells with tumor cells in living mice
- Inhibition of NETosis sensitizes tumors to PD-1+CTLA-4 dual checkpoint blockade



"la Caixa" Foundation



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