



Oncogene-induced maladaptive activation of Trained Immunity in the pathogenesis and treatment of Erdheim-Chester disease

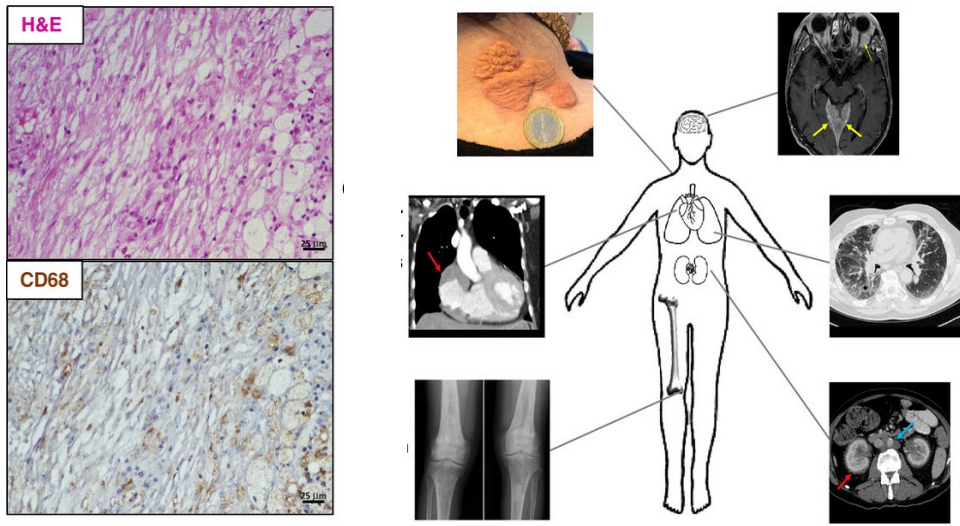


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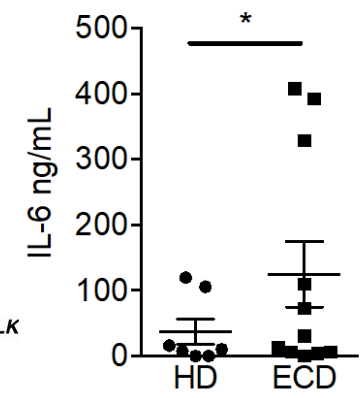
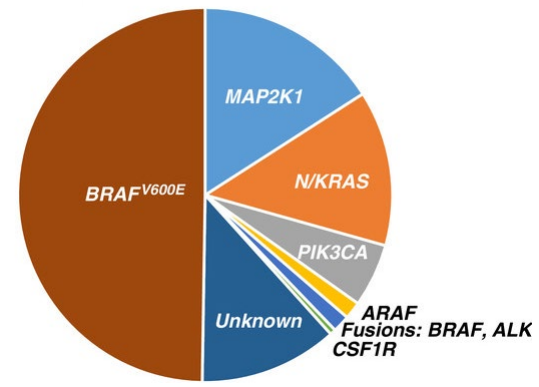
ECD: Inflammatory myeloid neoplasm

Missing link between oncogenic mutation and inflammatory activation

Infiltration of multiple tissues with foamy macrophages

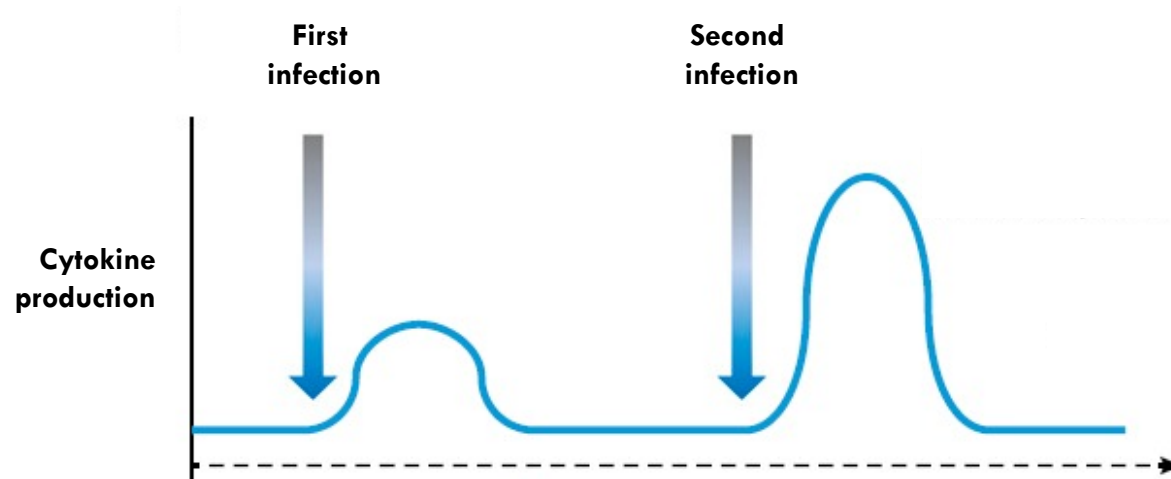


MAPK pathway activation, enhanced cytokine production



Trained immunity (TI)

Functional and mechanistic features

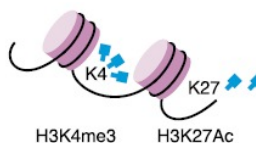


Immunometabolic rewiring



- Increased glycolysis
- Increased glutamine metabolism
- Increased cholesterol synthesis

Modulation of epigenetics



- H3K4Me3 and H3K27Ac on the promoters of genes encoding cytokines

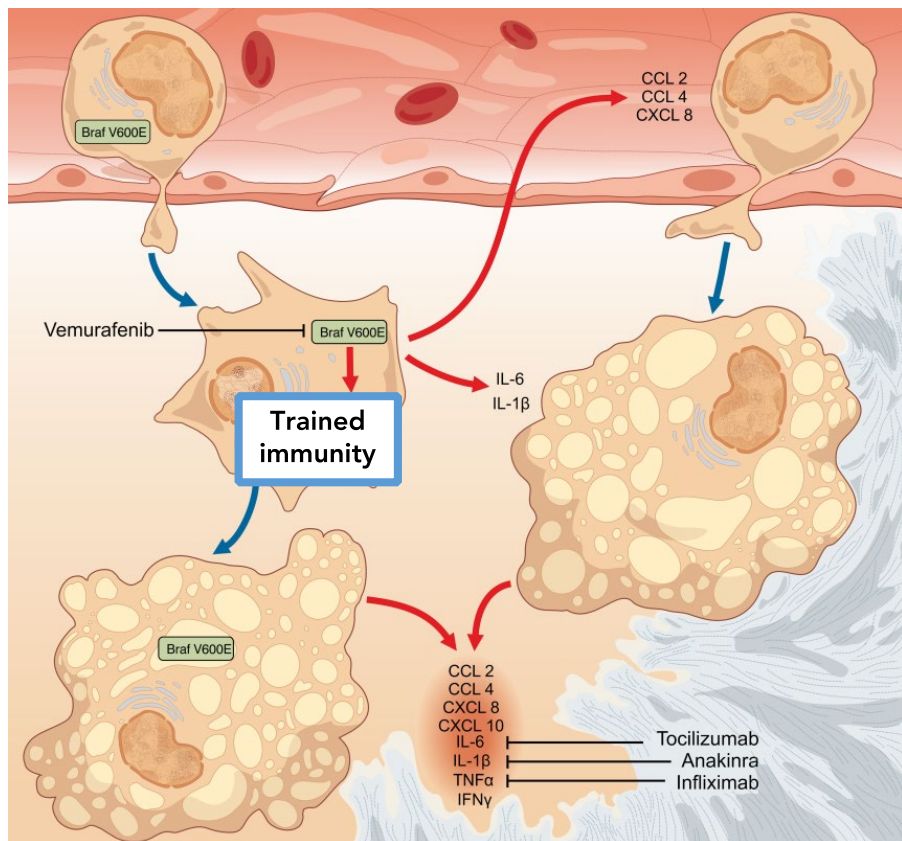
Macrophage similarities in ECD and trained immunity

Similarities

1. Morphologic changes: large size, foamy macrophages
2. Cytokine secretion pattern
3. Activation of the MAPK pathway

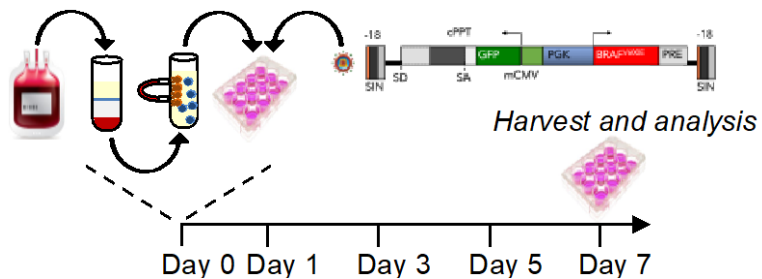
Hypothesis

Maladaptive activation of TI as the missing link between oncogenic mutation and inflammation in ECD



Transduction of primary human monocytes with BRAFV600E recapitulates key features ECD

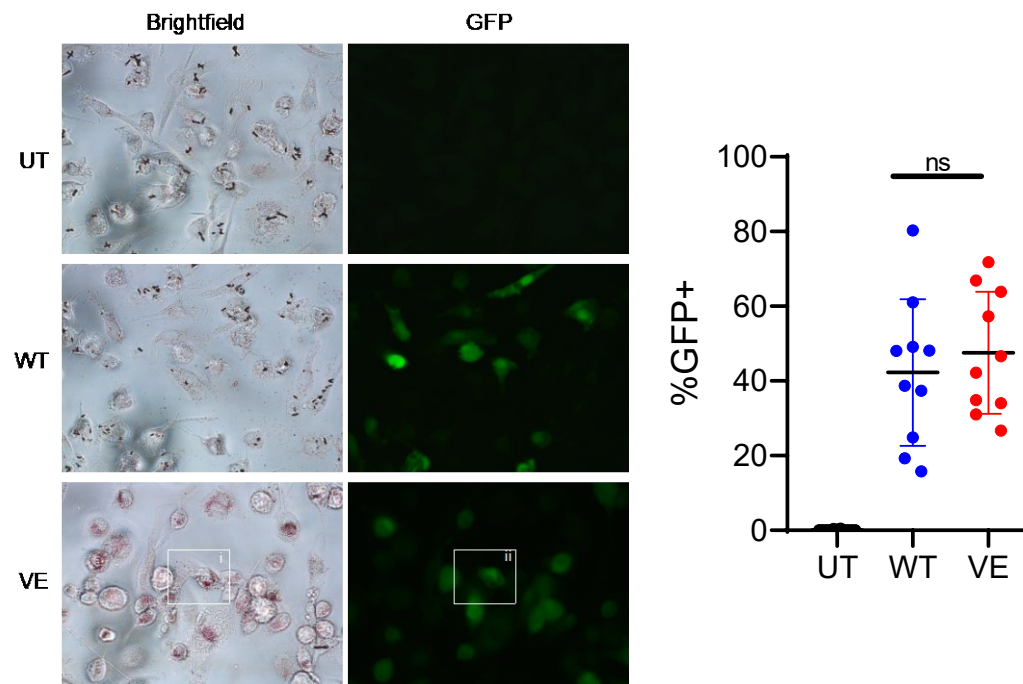
Experimental setup



Lentiviral transduction of *BRAFV600E* into healthy monocytes

Conditions:

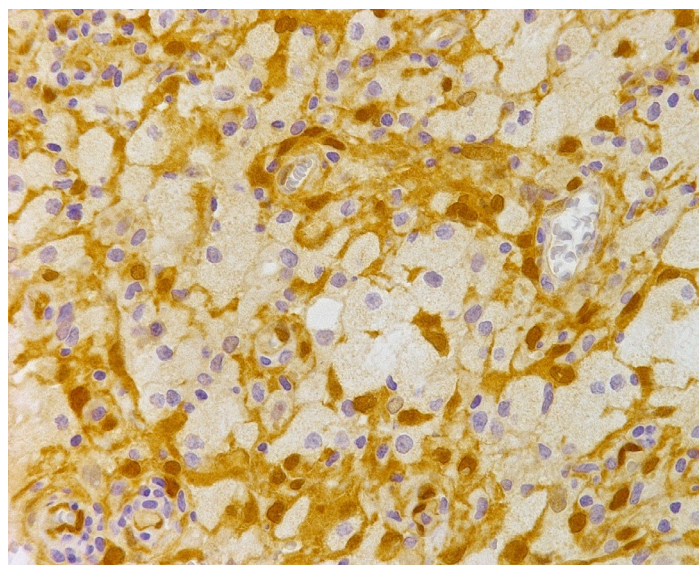
1. untransduced (UT)
2. Wild type BRAF (WT, control)
3. BRAFV600E (VE)



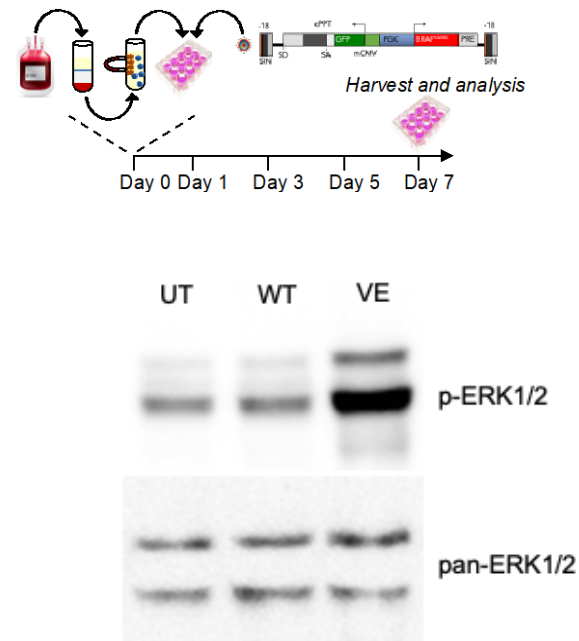
GFP enables tracking of mutated cells (FACS, brightfield microscopy)

Transduction of primary human monocytes with BRAFV600E recapitulates key features of ECD

1) Activation of the MAPK pathway



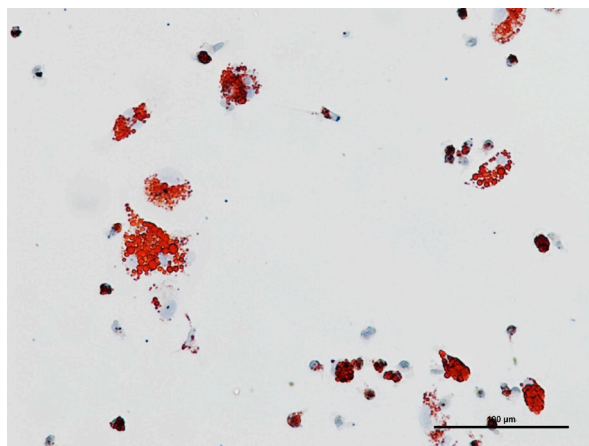
Macrophages infiltrating ECD lesions exhibit pERK (IHC)



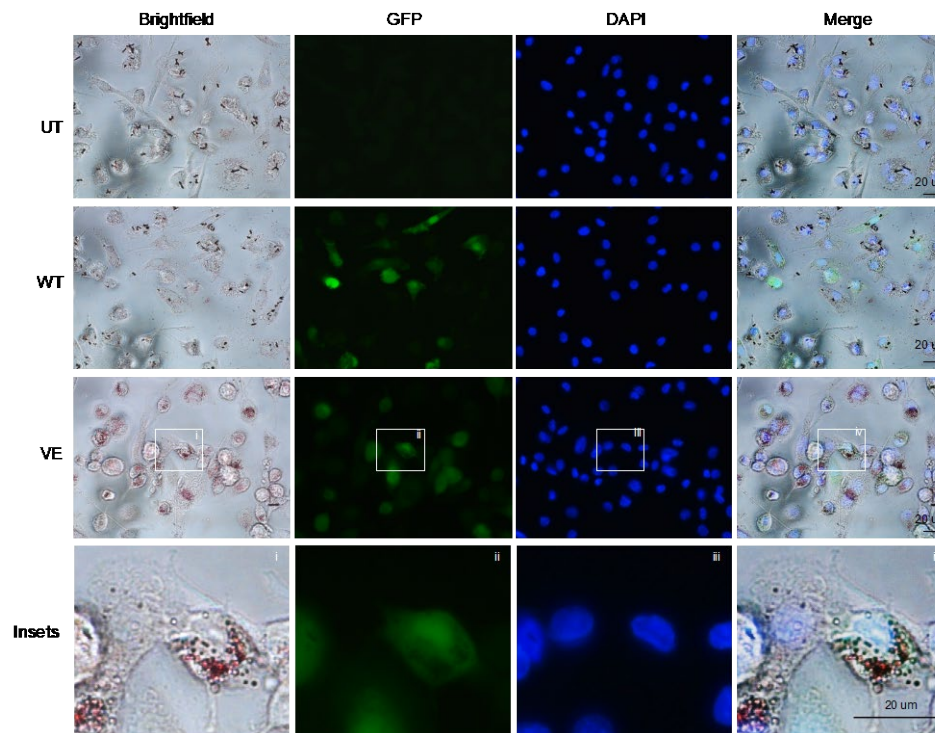
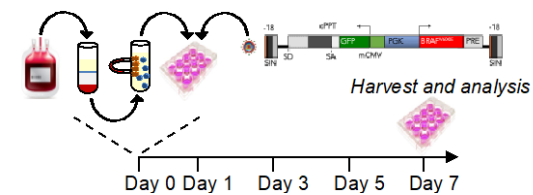
Macrophages expressing BRAFV600E exhibit constitutive activation of the downstream MAPK pathway intermediates ERK1/2 (WB)

Transduction of primary human monocytes with BRAFV600E recapitulates key features of ECD

2) Morphologic changes (large size and foamy appearance)



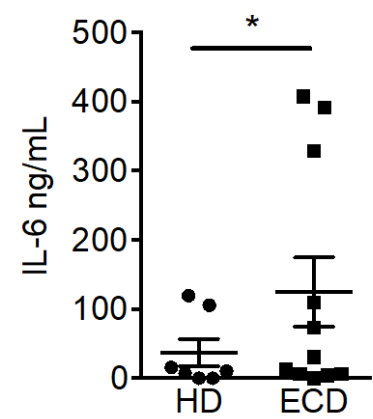
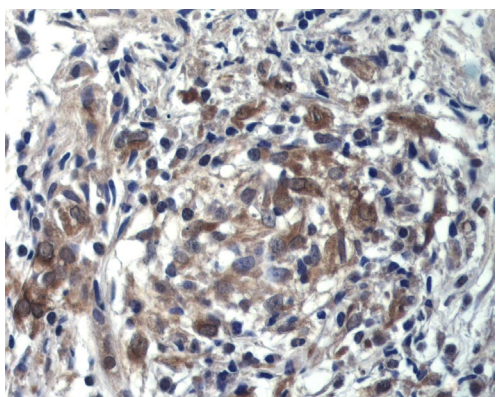
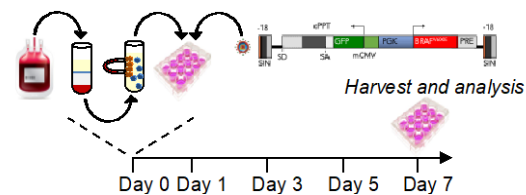
Macrophages isolated from ECD lesion exhibit a large size and a foamy, lipid laden appearance (Oil Red O)



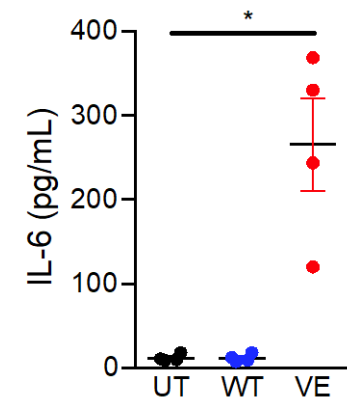
Macrophages expressing BRAFV600E undergo morphologic changes leading to a large size, foamy appearance, and increase in cytoplasmic lipid content (Brightfield microscopy)

Transduction of primary human monocytes with BRAFV600E recapitulates key features of ECD

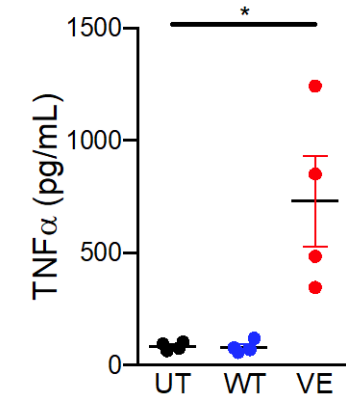
3) Spontaneous cytokine production



Spontaneous cytokine production by ECD macrophages in lesion microenvironment or following isolation (IHC, ELISA)



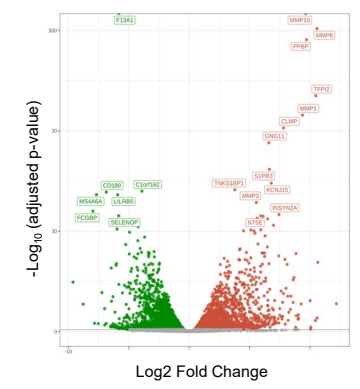
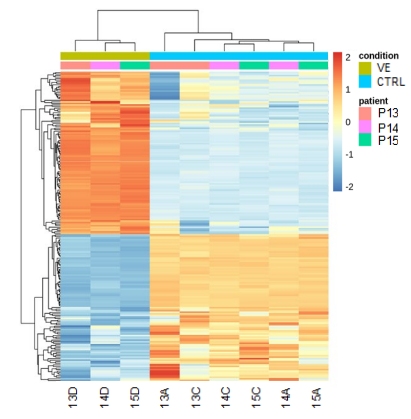
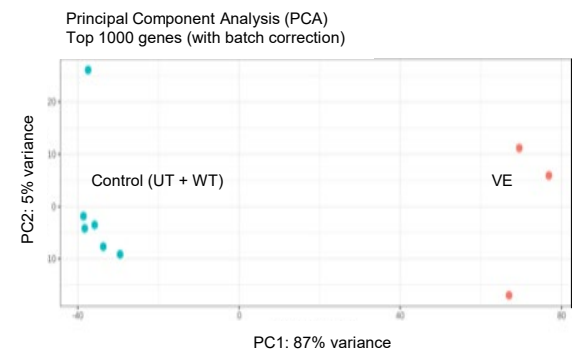
Macrophages expressing BRAFV600E exhibit spontaneous production of IL-6 (and TNFα (ELISA))



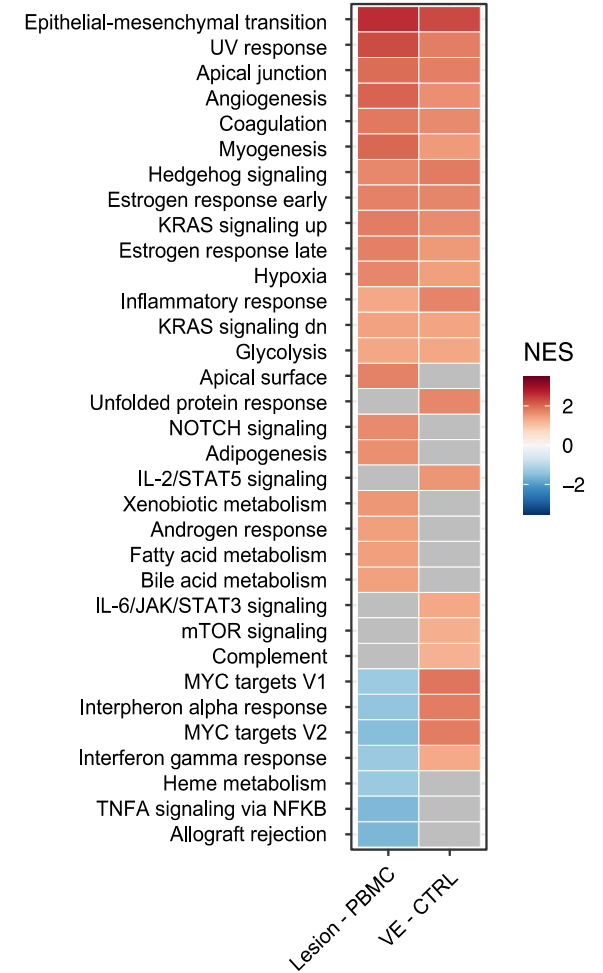
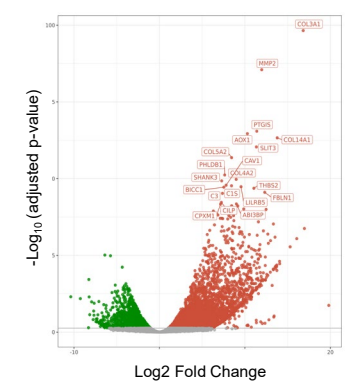
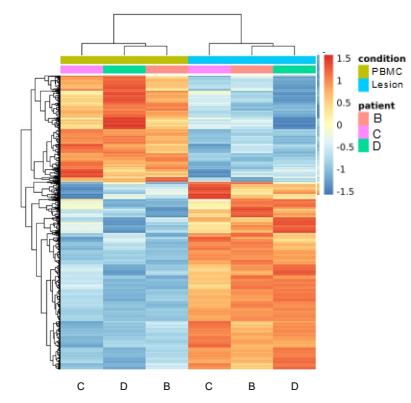
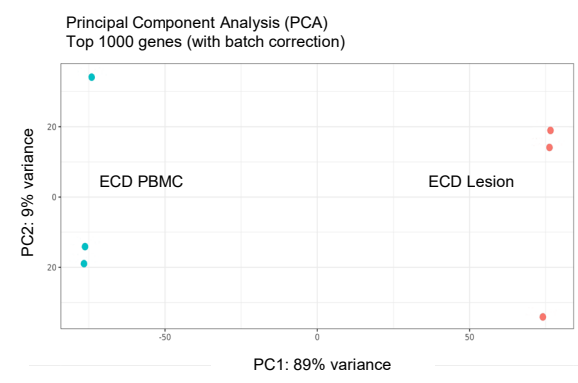
Transduction of primary human monocytes with BRAFV600E recapitulates key features of ECD

Global gene expression analysis

ECD in vitro (model)



ECD lesion (biopsy)





Transduction of primary human monocytes with BRAFV600E recapitulates key features of ECD

Results Recap

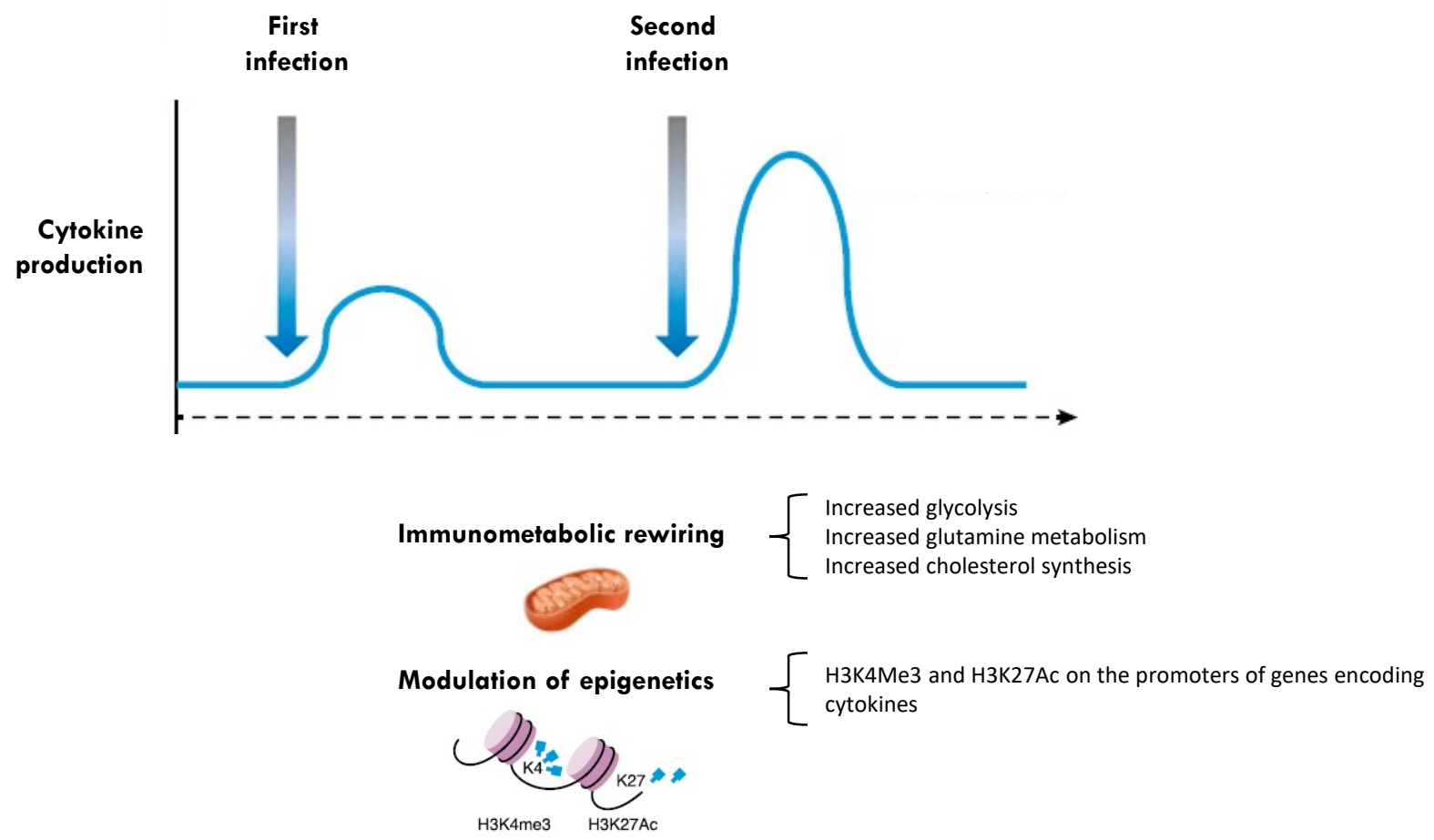
Macrophages expressing ectopic *BRAFV600E* exhibit:

1. constitutive activation of the MAPK pathway
2. transformation into foamy macrophages with a large lipid-laden cytoplasm
3. production of pro-inflammatory cytokines
4. Activation of pro-inflammatory transcriptional programs

The *in vitro* model recapitulates genetic, phenotypic and functional features of ECD.

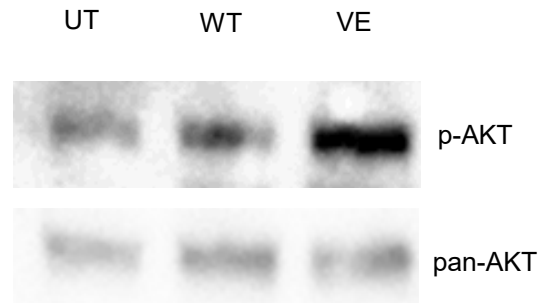
Hypothesis

Maladaptive activation of TI: missing link between BRAFV600E and inflammation in ECD

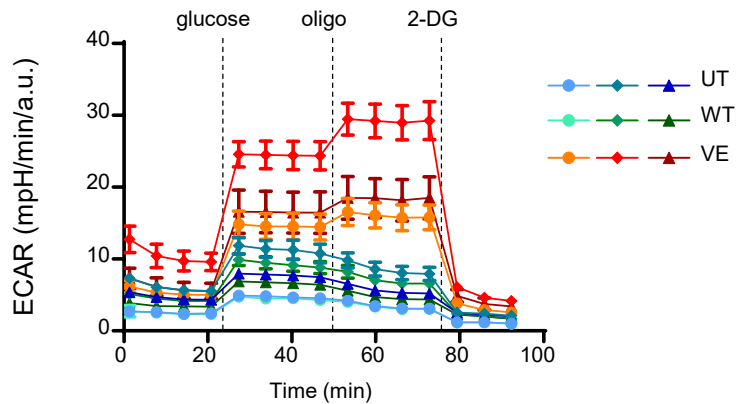


BRAFV600E induces immunometabolic changes indicative of TI in macrophages

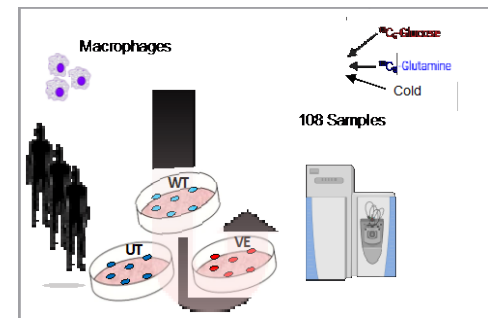
Activation of Akt-mTOR pathway, increased glycolysis, immunometabolic rewiring



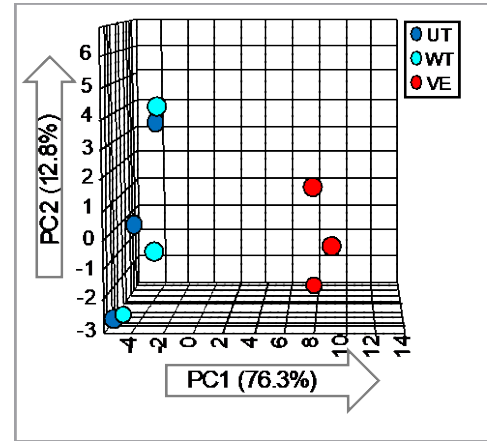
Macrophages expressing *BRAFV600E* exhibit constitutive p-AKT (WB)



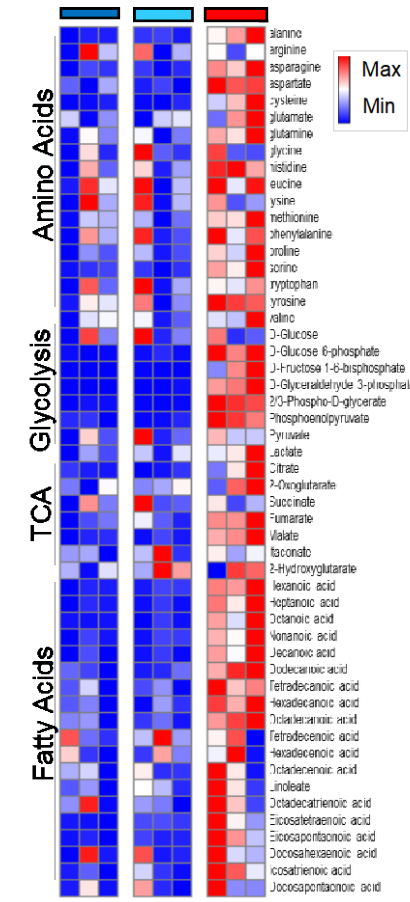
Seahorse Flux analyzer confirmed downstream induction of glycolysis



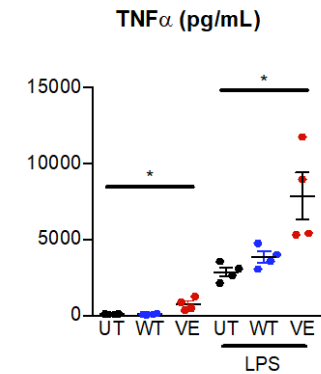
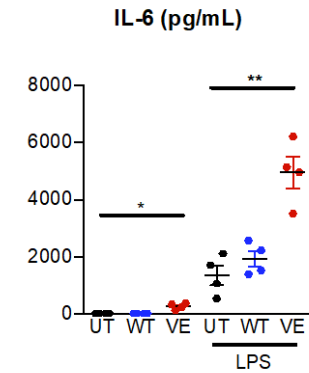
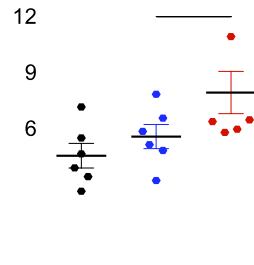
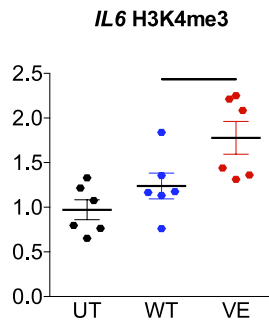
Cells were cultured in culture media, cold or enriched with ¹³C₆-glucose or ¹³C₅-glutamine



The metabolome of macrophages expressing *BRAFV600E* clustered independently from controls, indicative of profound metabolic rewiring, particularly in pathways relevant to TI.



BRAFV600E induces epigenetic and functional secretory features of T1 in macrophages



Epigenetic markers of T1: H3K4me3 and H3K27ac on the promoters of genes encoding cytokines (ChIP PCR)

Enhanced cytokine production following stimulation with LPS



BRAFV600E induces features indicative of TI in macrophages

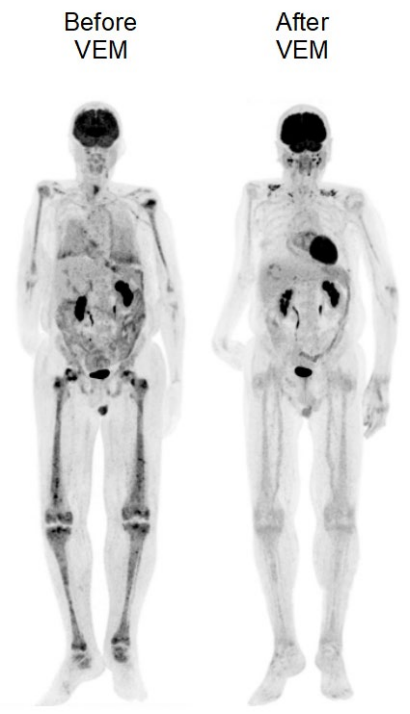
Results Recap

BRAFV600E in macrophages induces:

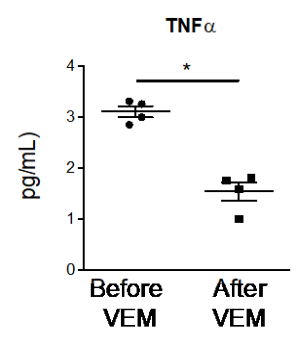
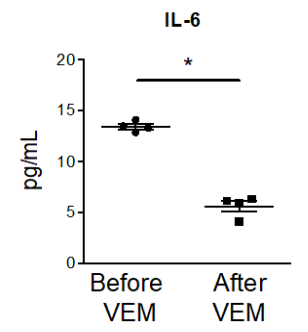
1. immunometabolic changes indicative of TI (glycolysis, glutaminolysis through the TCA cycle, cholesterol synthesis)
2. epigenetic changes indicative of TI (H3K4me3 and H3K27ac on cytokine gene promoters)
3. functional hallmark of TI (hyper-responsiveness to inflammatory triggers)

Therapeutic targeting of trained immunity in ECD

Effective therapeutic strategies contrast maladaptive TI phenotype

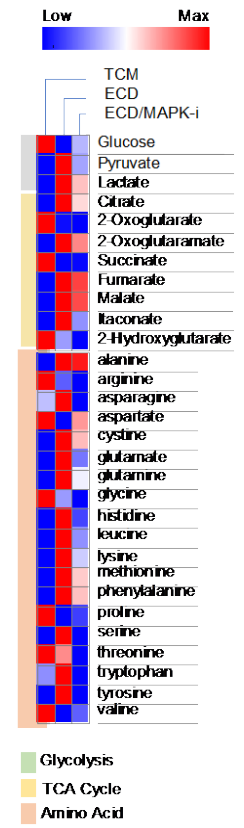
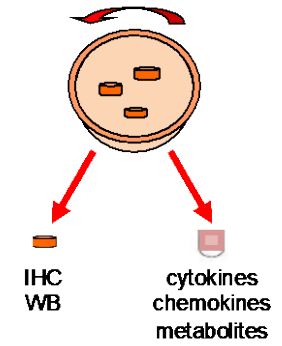
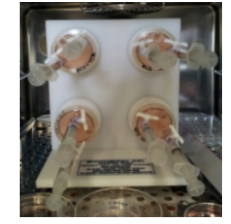


Glucose uptake by ECD lesions (FDG-PET)



Serum cytokines (ELISA)

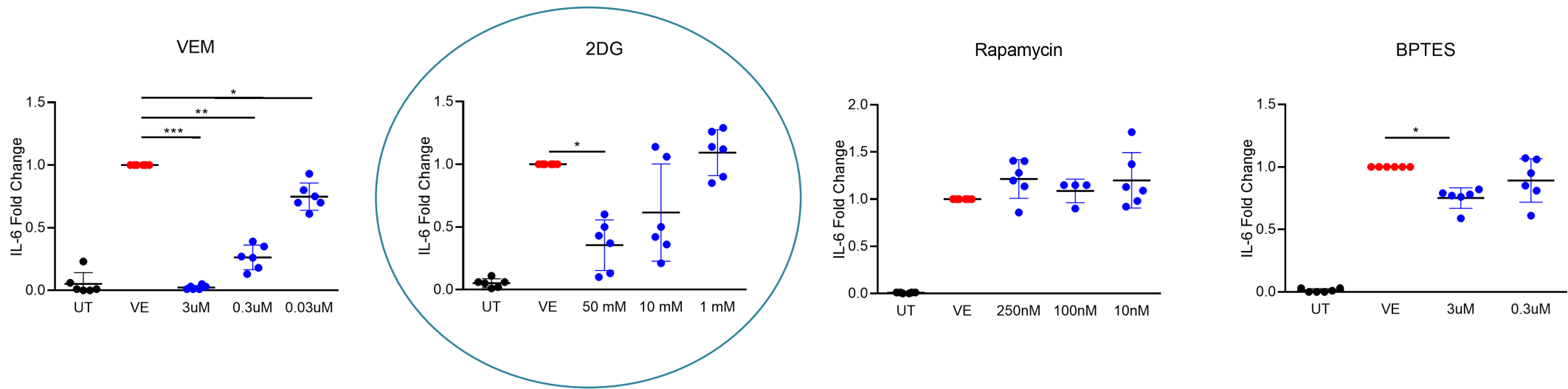
3D culture +/- treatments



Immunometabolic changes of TI occur in ECD lesions in vivo and are reverted by MAPK inhibition

Therapeutic targeting of trained immunity suppresses inflammation in ECD

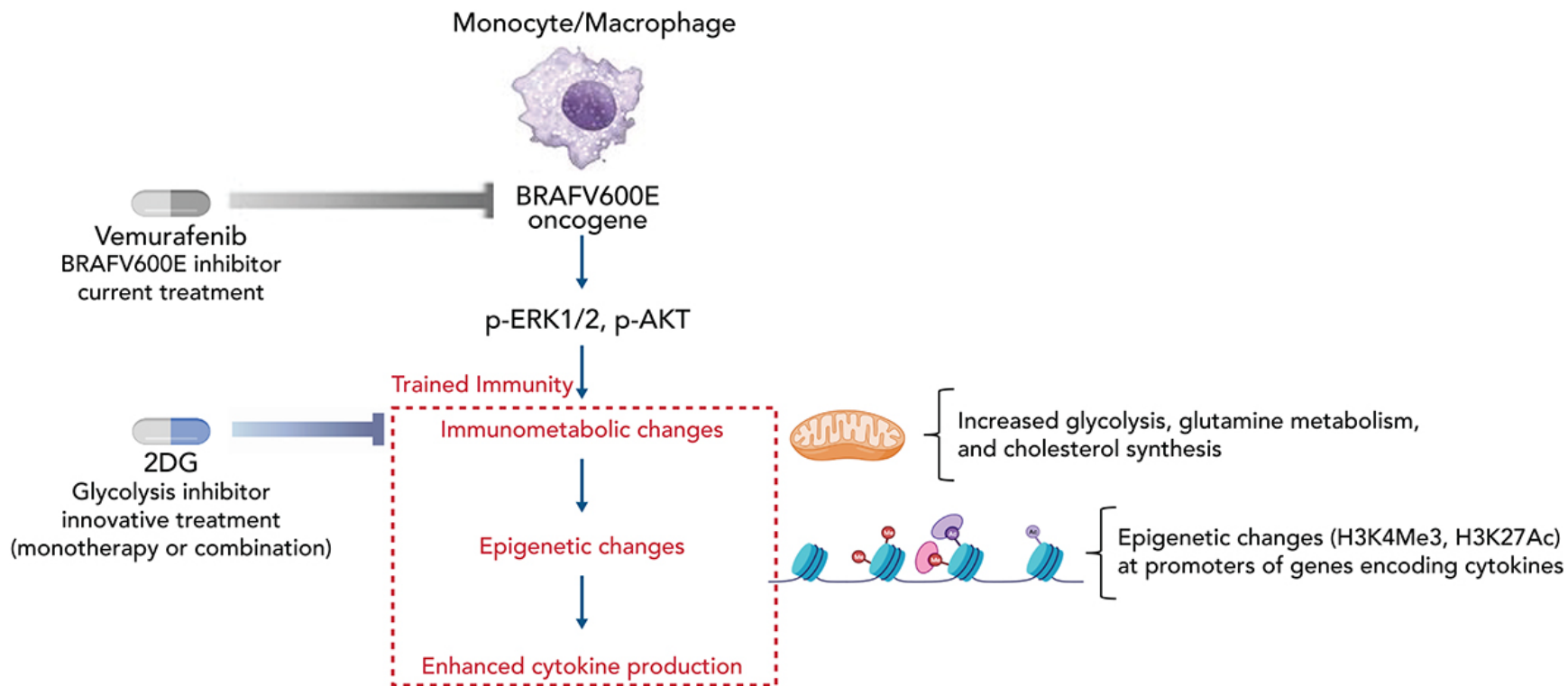
The glycolysis inhibitor 2DG has therapeutic potential in ECD



Inhibition of glycolysis with 2DG suppresses cytokine production

Conclusions

Oncogene-induced maladaptive activation of TI is a feature and therapeutic target of ECD



Oncogene-induced maladaptive activation of Trained Immunity in the pathogenesis and treatment of Erdheim-Chester Disease

Molteni R*, Biavasco R*, Stefanoni D, Panigada M, Cantoni E, Nemkov T, Domínguez-Andrés J, Arts RJW, Merelli I, Mazza D, Zambrano S, Tengesdal IW, Maksud P, Piras F, De Luca G, Cassina L, Distefano G, Loffreda A, Gnani D, Doglioni C, Joosten LAB, Kajaste-Rudnitski A, Haroche J, Dagna L, Dinarello CA, Ferrarini M, Ferrero E, Cardaci S, Boletta A, Cenci S, D'Alessandro A, Montini E, Netea MG, **Cavalli G**

Blood, 2021

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Cavalli Lab

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Thank you

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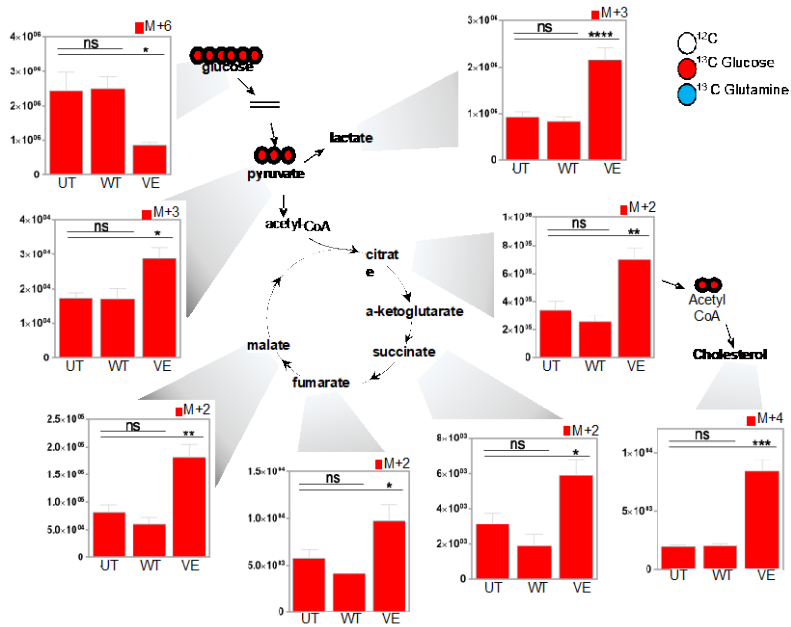
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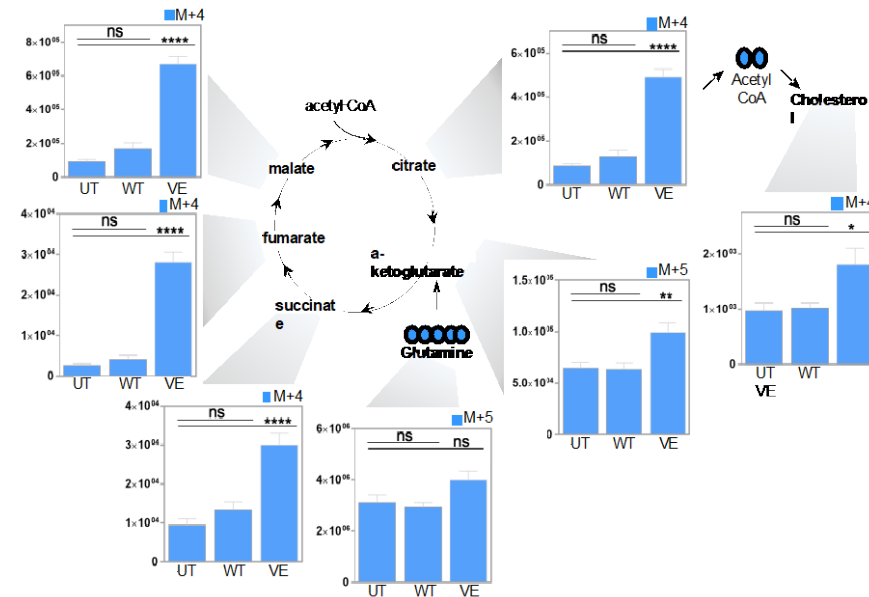
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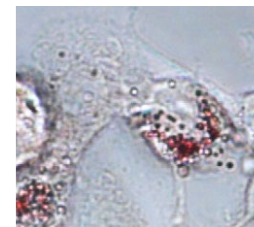
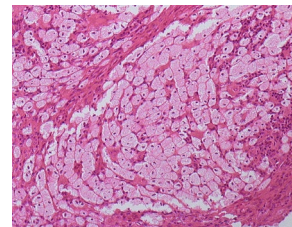
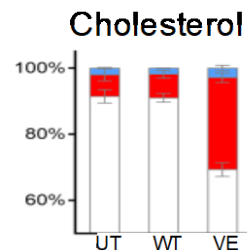
Glycolysis, glutaminolysis through the TCA cycle, cholesterol synthesis



¹³C₆-glucose tracing revealed increased generation of glycolysis intermediates as well as end products pyruvate and lactate



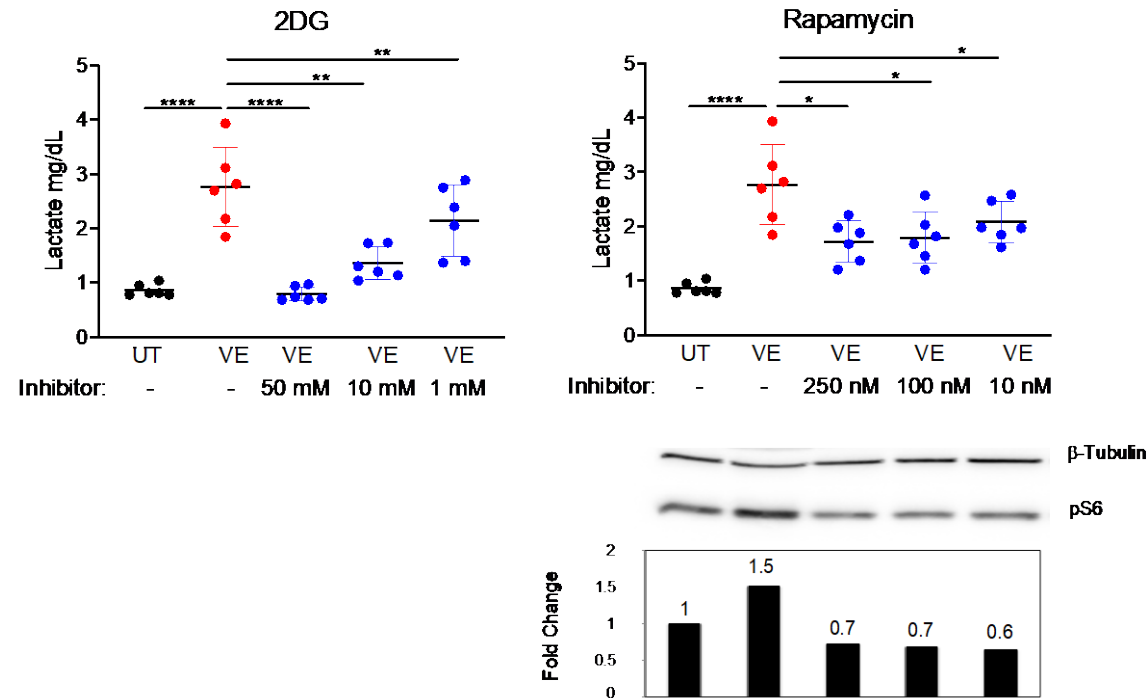
¹³C₅-glutamine tracing reveals accumulation of TCA intermediates α-ketoglutarate, citrate, malate, fumarate, and succinate



Tracking incorporation of carbons derived from ¹³C₆-glucose or ¹³C₅-glutamine revealed increased de novo cholesterol synthesis

Therapeutic targeting of trained immunity suppresses inflammation in ECD

Rapamycin is ineffective despite partial inhibition of mTOR and glycolysis



Sirrolimus plus prednisone for Erdheim-Chester disease: an open-label trial

Davide Gianfreda,¹ Maria Nicastro,¹ Maricla Galetti,^{1,2} Federico Alberici,¹ Domenico Corradi,³ Gabriella Becchi,³ Giorgio Baldari,⁴ Massimo De Filippo,⁵ Stefania Ferretti,⁶ Gabriella Moroni,⁷ Rosario Foti,⁸ Marcella Di Gangi,⁸ Guido Jeannin,⁹ Raphael Saffroy,¹⁰ Jean-François Emile,¹¹ Carlo Buzio,¹ and Augusto Vaglio¹