

Ambivalent effects of 5-Fluorouracil on Anticancer Immune Responses



Lionel Apetoh

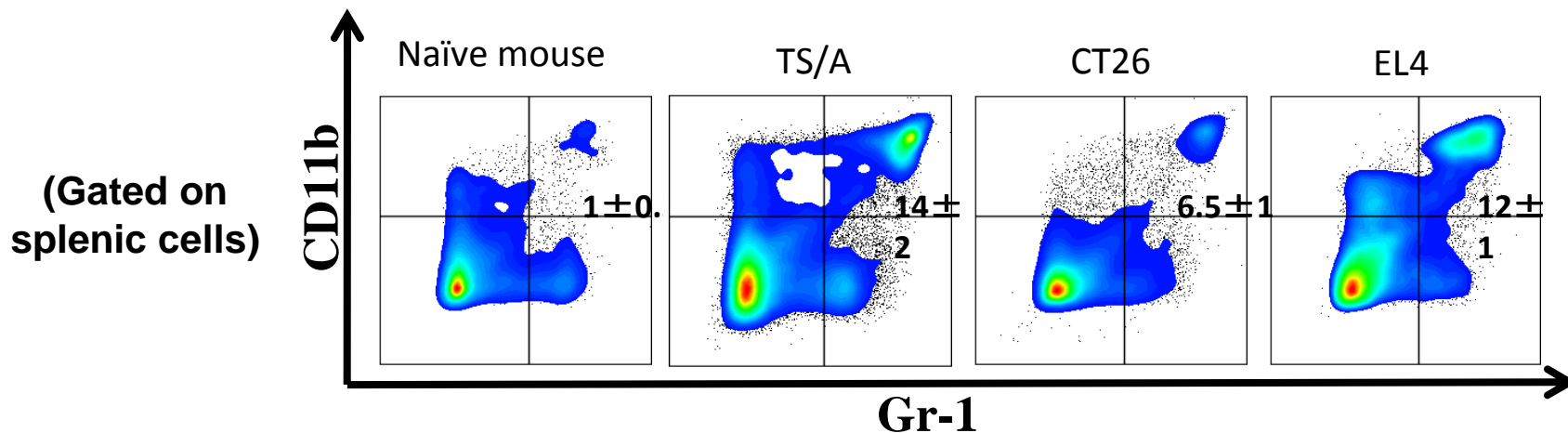
INSERM UMR866, Dijon, France

SITC annual meeting, National Harbor, November 8th 2013

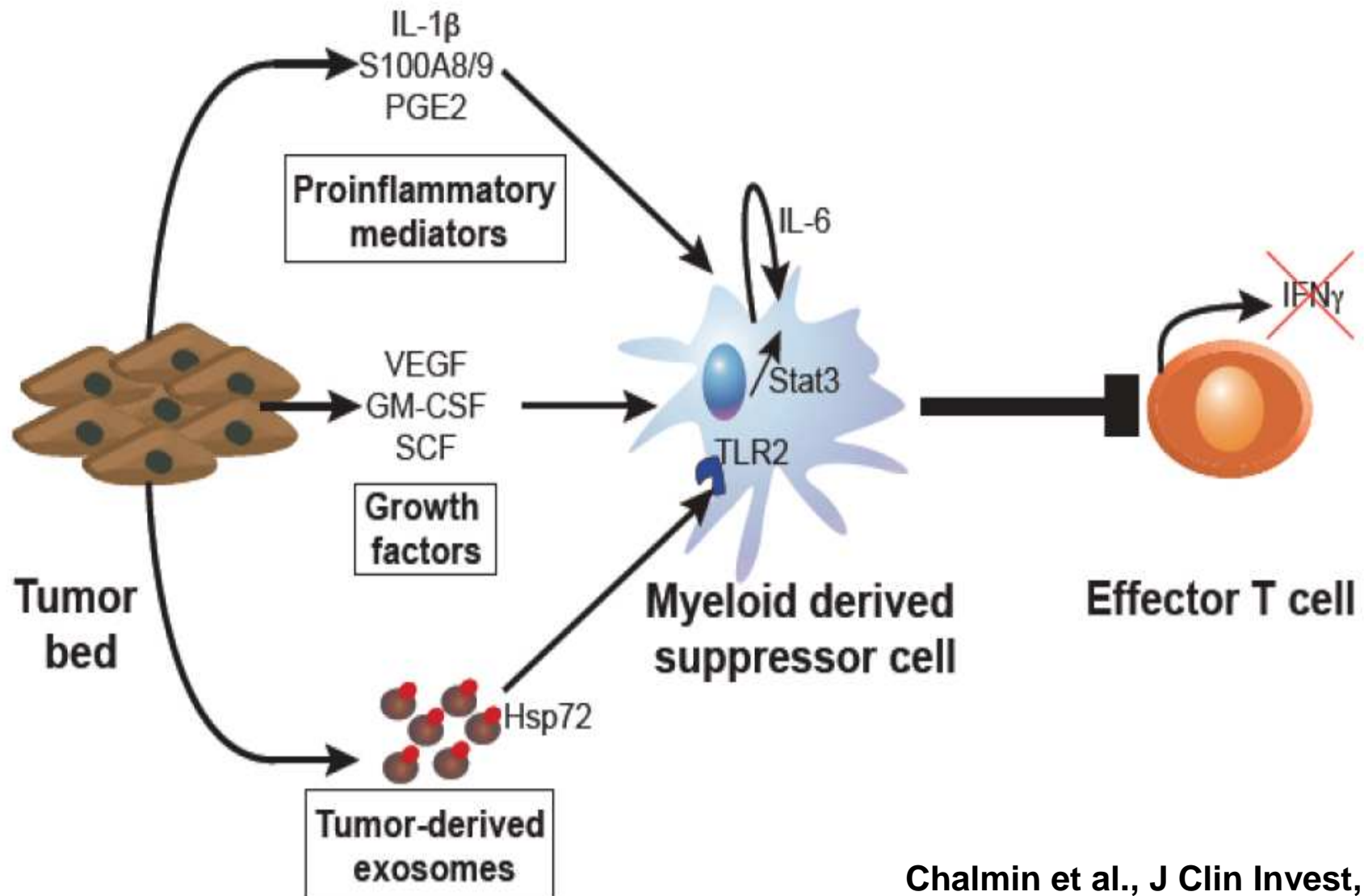
(No relevant disclosures to the presentation)

Myeloid-derived Suppressor cells

- Myeloid-derived Suppressor cells (MDSC):
 - are characterized by the expression of the CD11b and Gr-1 (Ly-6G/C) markers (Gabrilovich et al., Nat. Rev. Immunol., 2009)
 - have been shown to accumulate during tumor progression and to compromise antitumor immunity

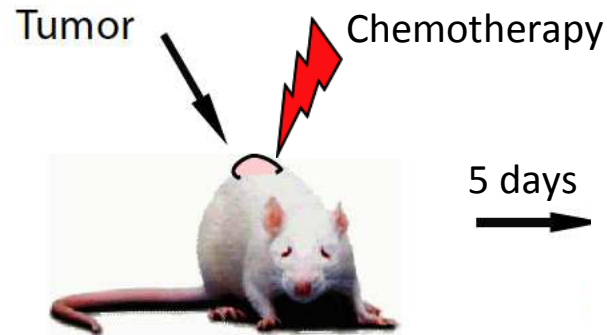


Regulatory pathways of MDSC expansion and activation

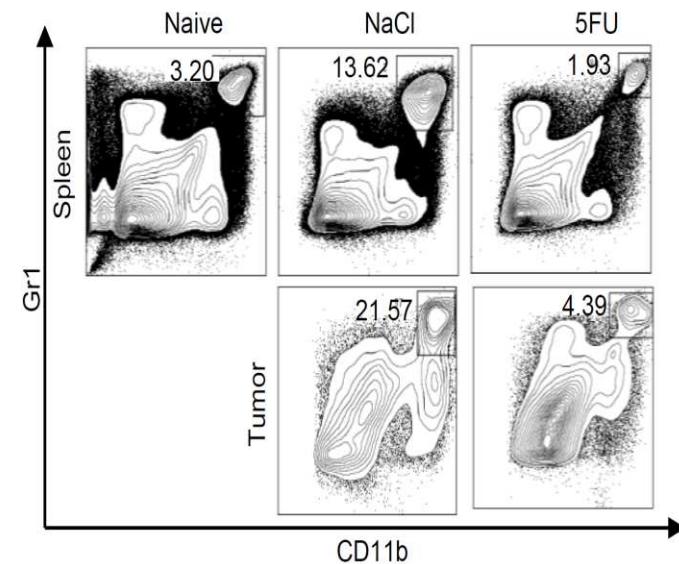
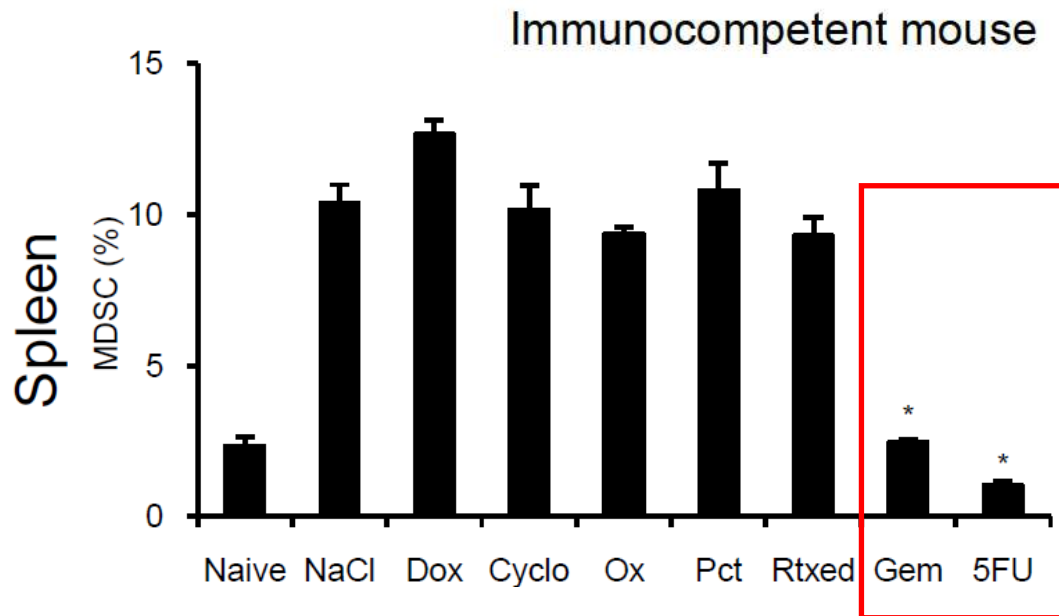


Chalmin et al., J Clin Invest, 2010
Apetoh et al., Cur Mol Med., 2011

5-Fluorouracil (5-FU) has a superior efficacy to deplete MDSC *in vivo*

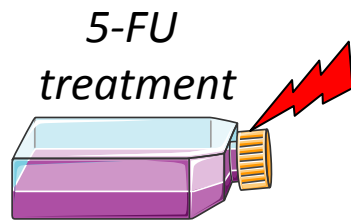


Determination of splenic and tumor-infiltrating MDSC frequency



No significant effects of 5-FU on T, B NK or dendritic cells

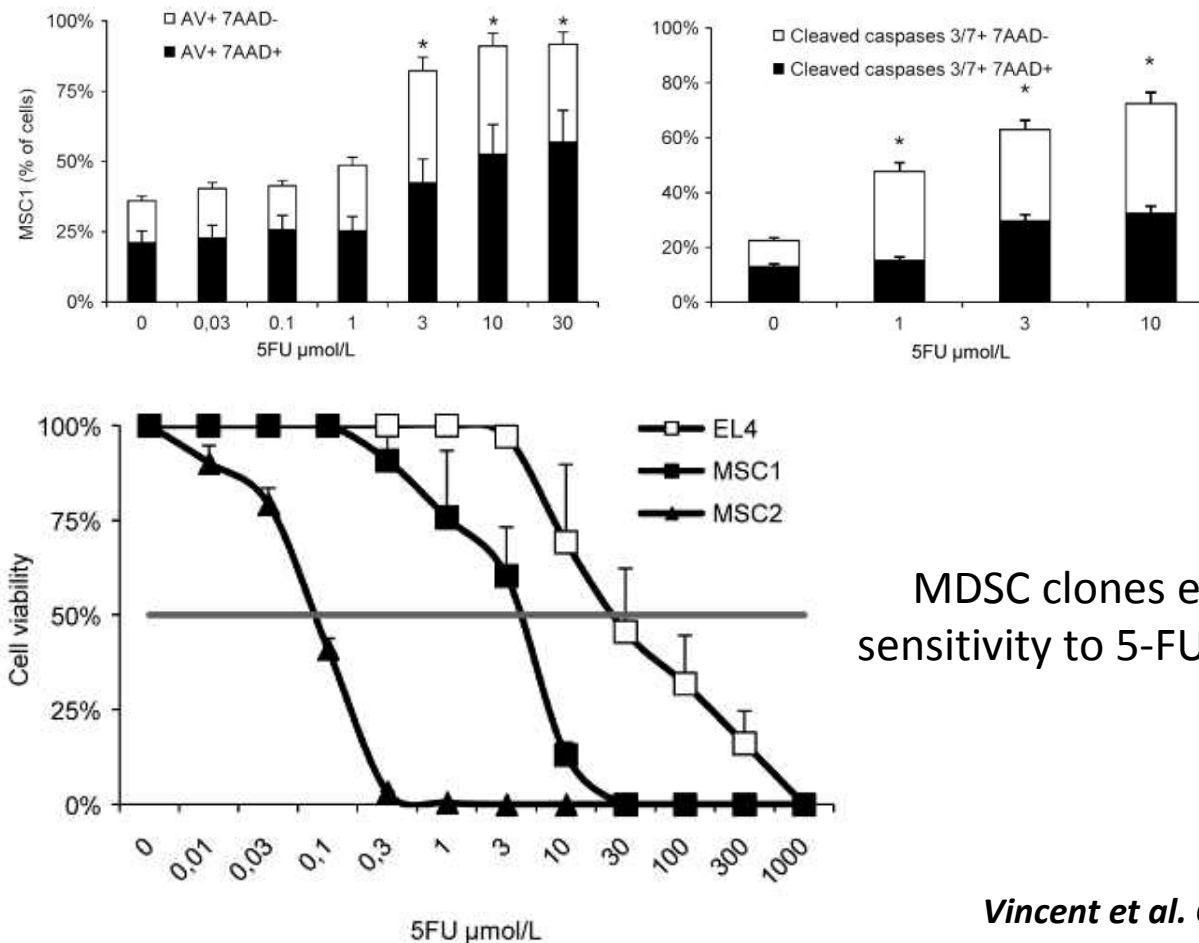
5-FU kills MDSC *in vitro*



MDSC clones vs
EL4 tumor cells

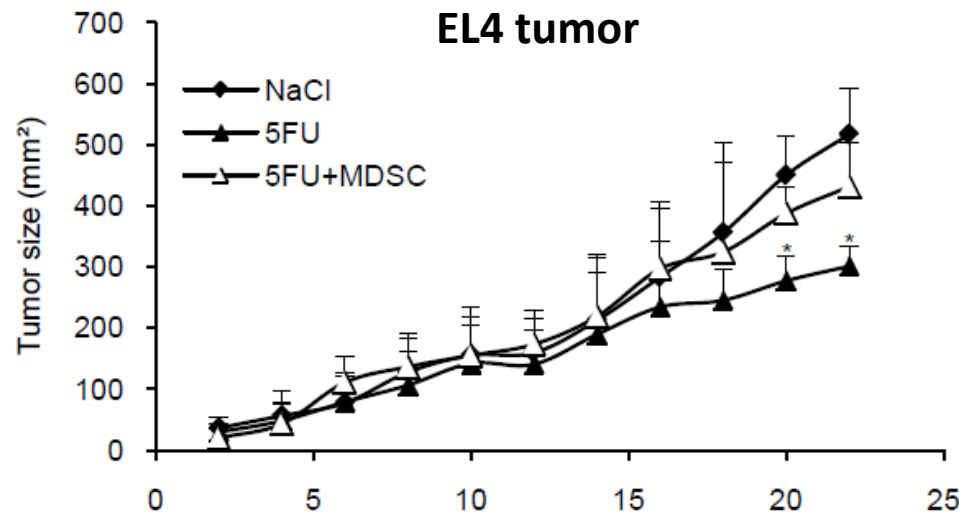
In vitro culture
(24 hours) →

Monitor cell viability

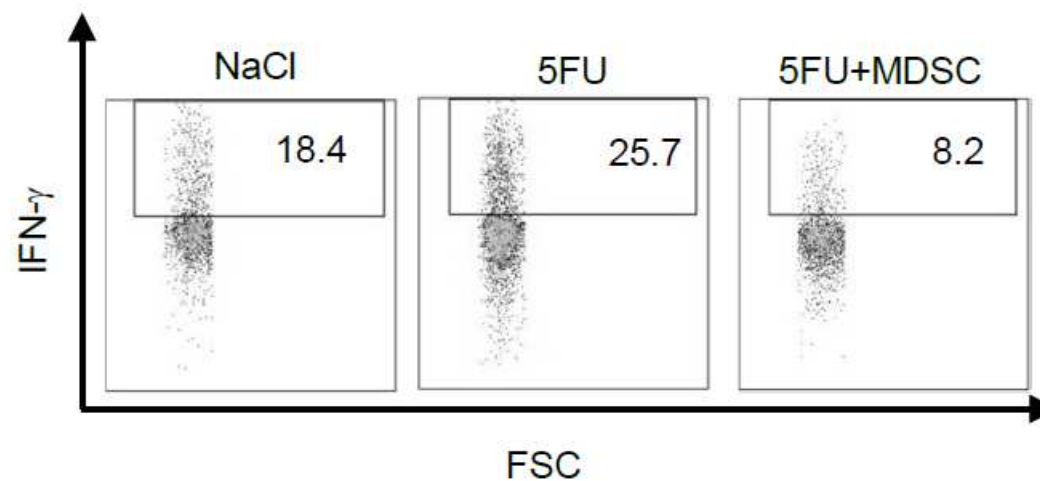


MDSC clones exhibit a higher sensitivity to 5-FU than tumor cells

5-Fluorouracil antitumor effect relies on MDSC



Gated on CD8⁺ TILs
after *in vitro*
reactivation



Can we identify additional mechanisms responsible for tumor resistance to 5-FU treatment?

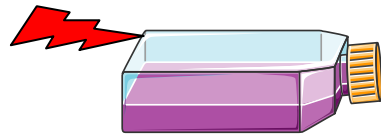
- 5-FU has cytotoxic effects on cancer cells
- 5-FU selectively kills MDSC

However, the anticancer effects of 5-FU are not long-lasting

Objective: search for additional mediators contributing to tumor resistance to 5-FU to enhance its anticancer efficacy *in vivo*

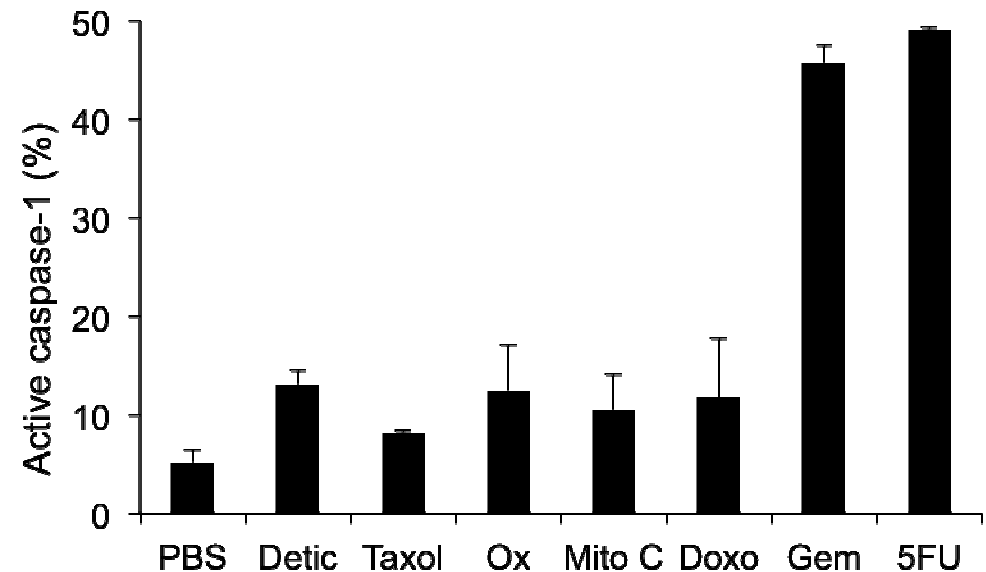
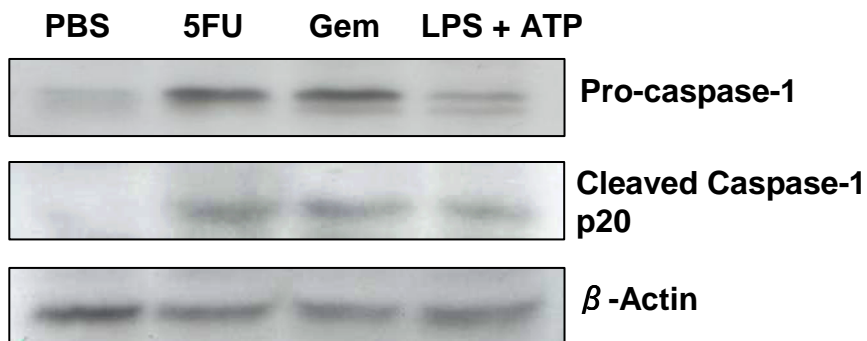
Caspase 1 is activated in MDSC by 5-FU and Gemcitabine (Gem) *in vitro*

Chemotherapeutic treatments

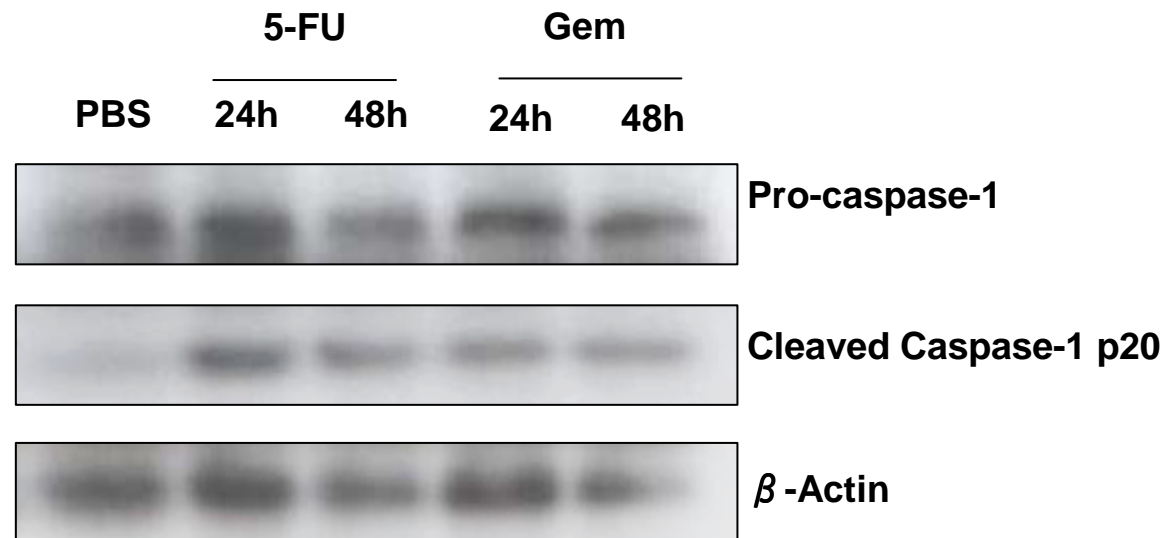
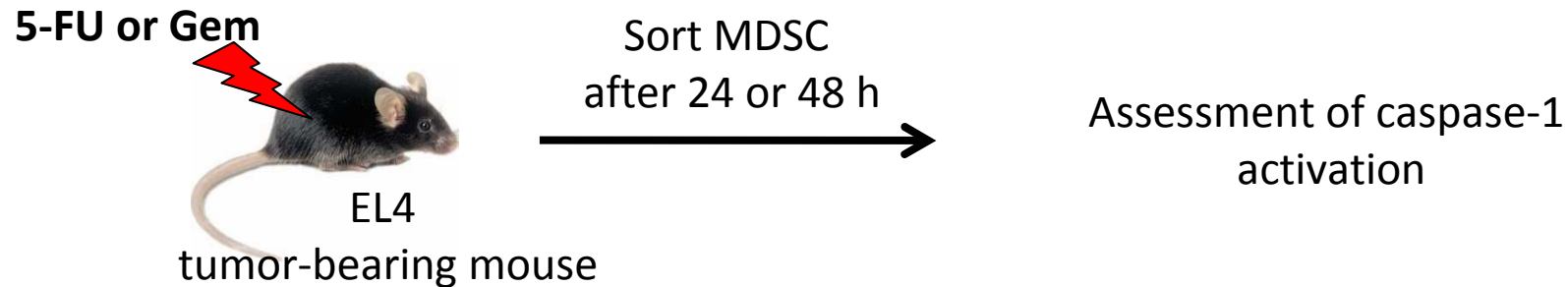


MDSC clones

Assessment of caspase-1 activation by Western Blot and flow cytometry

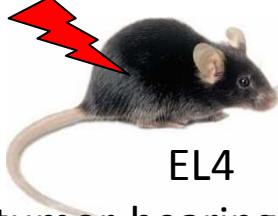


Caspase 1 is activated in MDSC by 5-FU and Gem *in vivo*



5-FU induces IL-1 β release from MDSC in a caspase-1 dependent manner

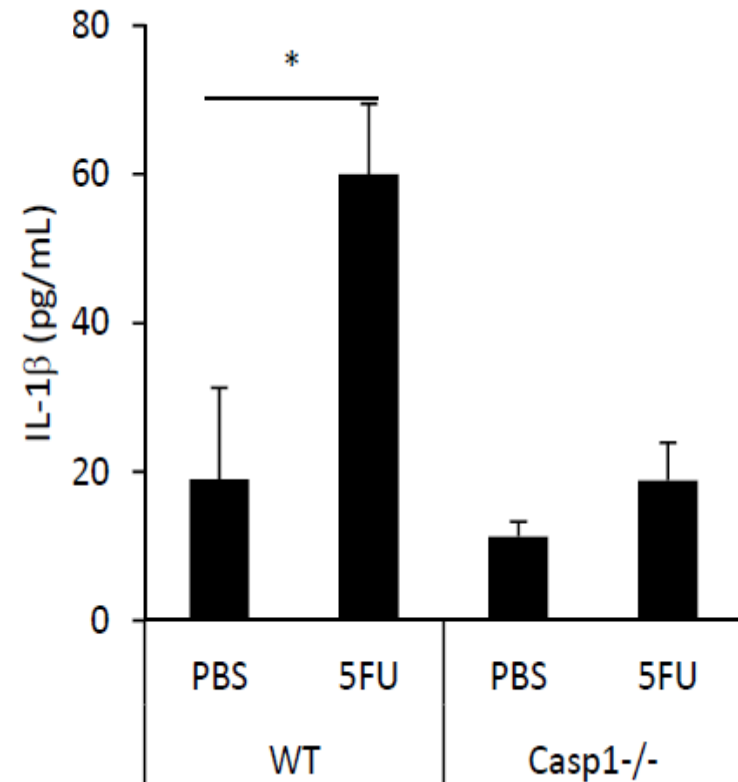
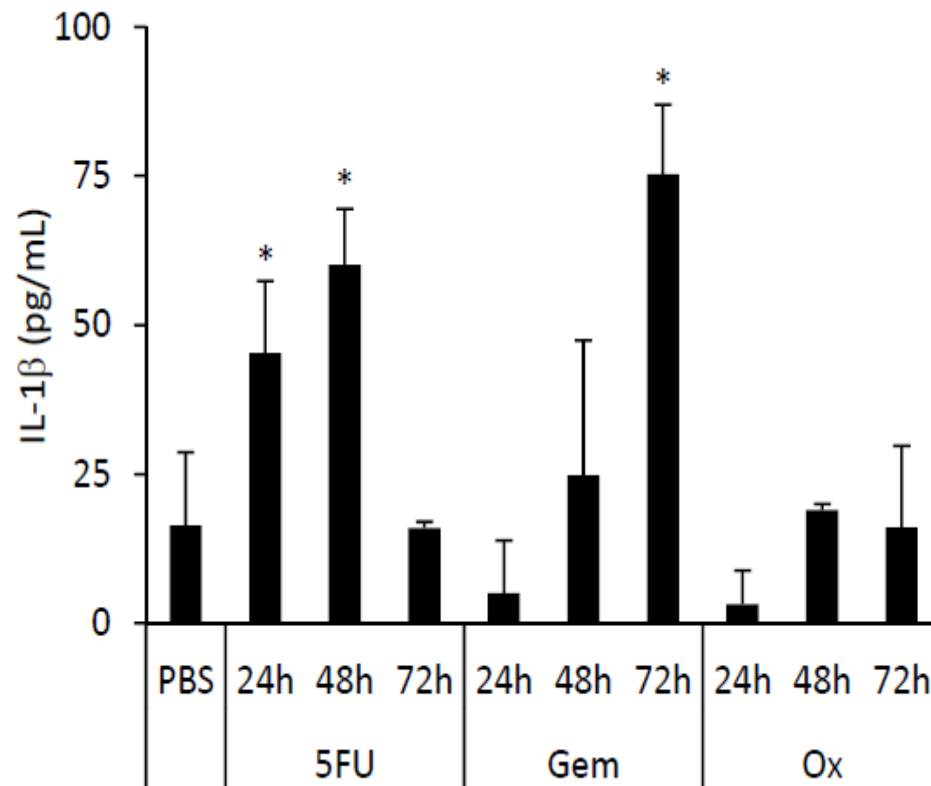
5-FU or Gem



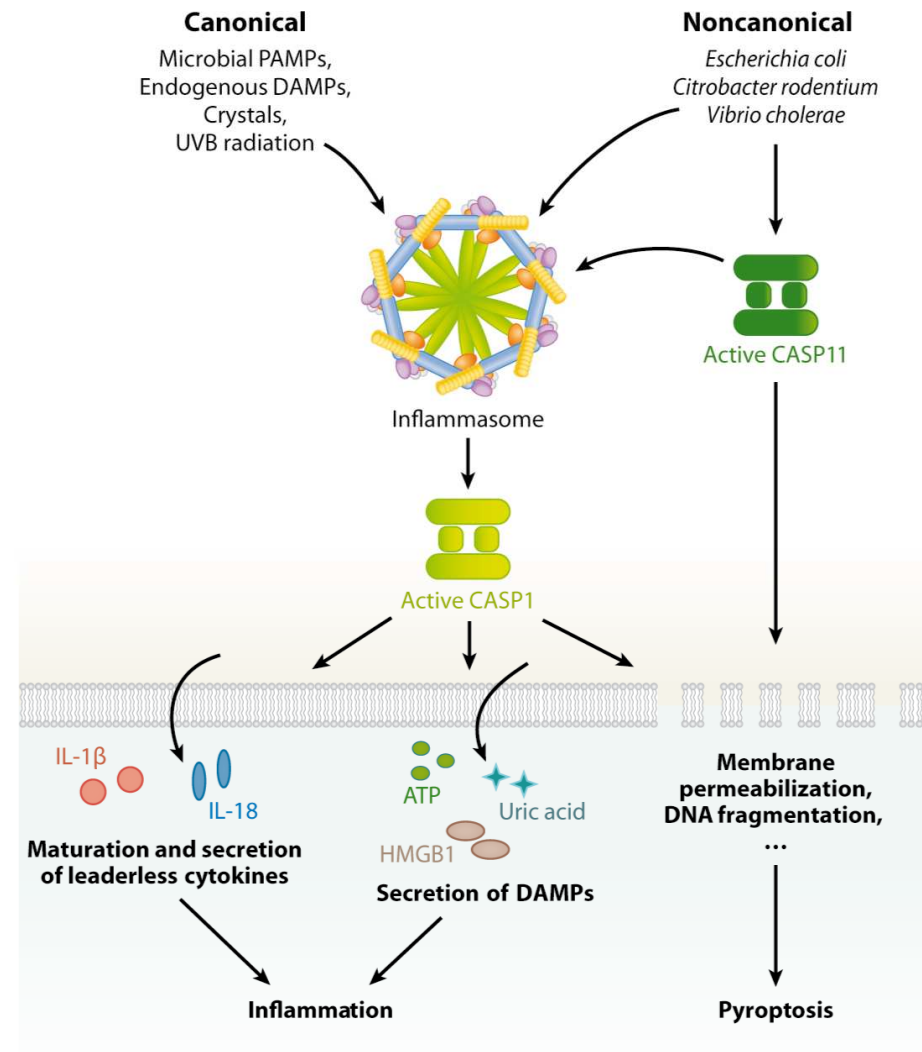
Sort MDSC
after 24 or 48 h



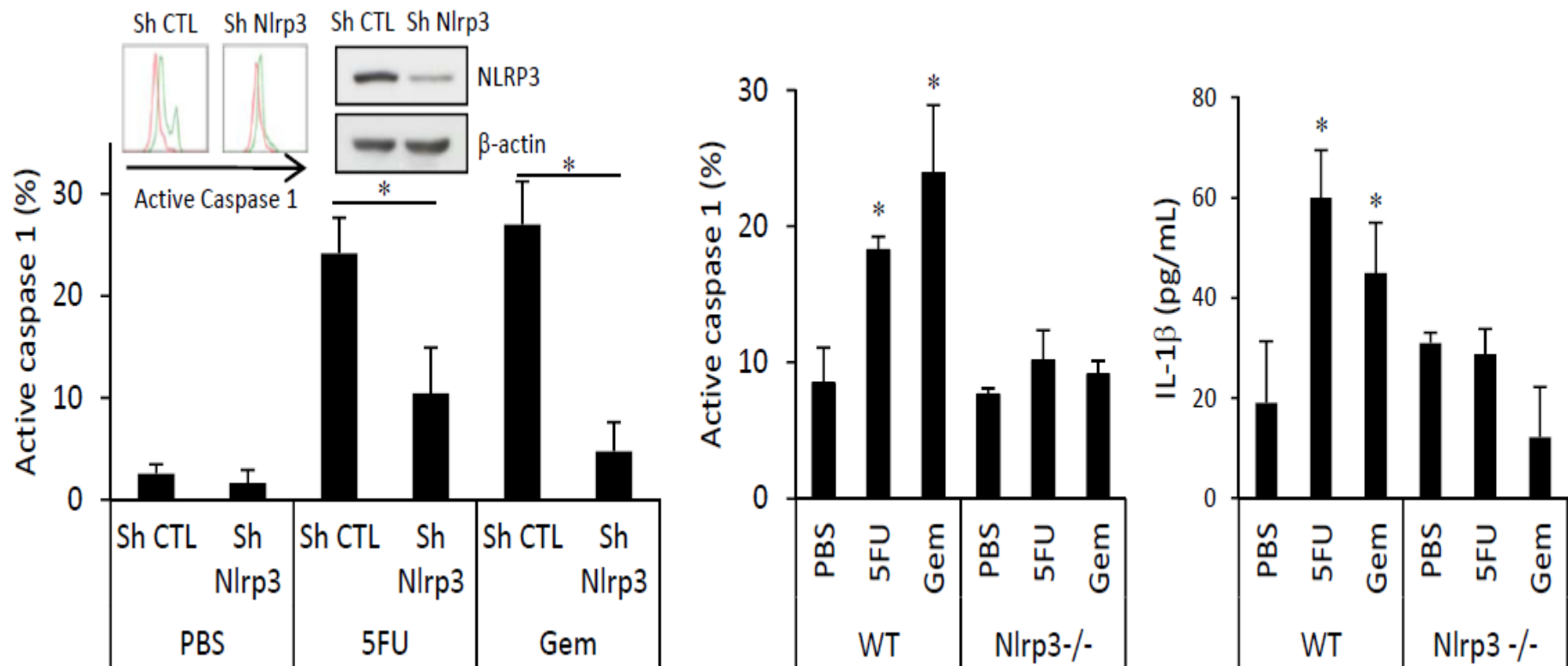
Assessment of IL-1 β secretion



Inflammasomes are macromolecular complexes leading to caspase-1 activation

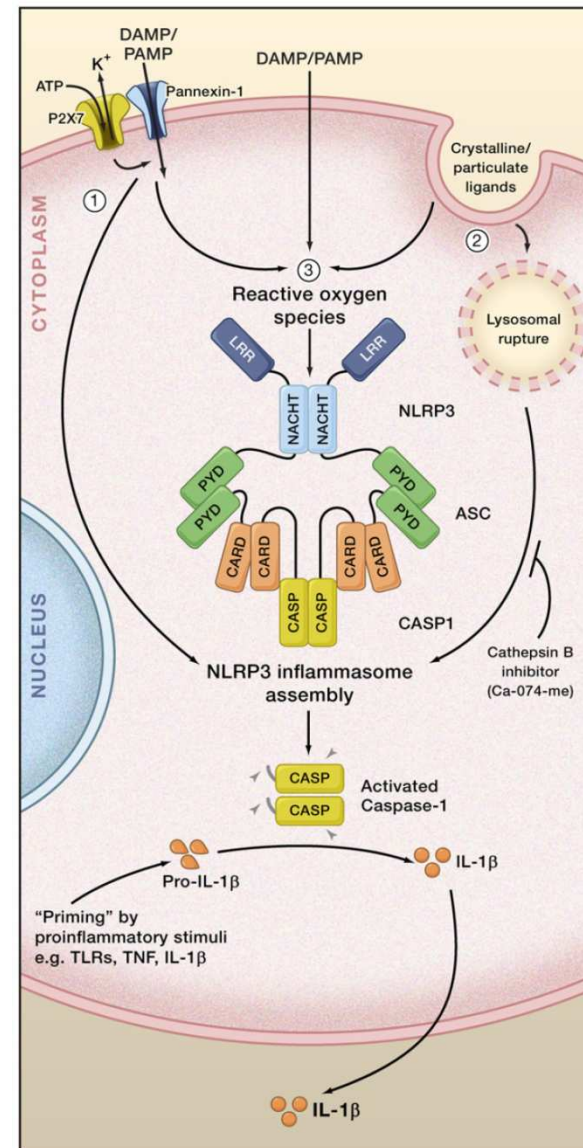


5-Fluorouracil caspase-1 activation is dependent on NLRP3



Mechanisms of NLRP3 inflammasome activation

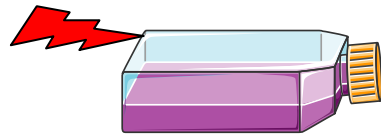
- ATP-P2rX7
- Reactive Oxygen Species
- Release of lysosomal content



*Schroder and Tschopp,
Cell, 2010*

5FU activated inflammasome via lysosome permeabilization

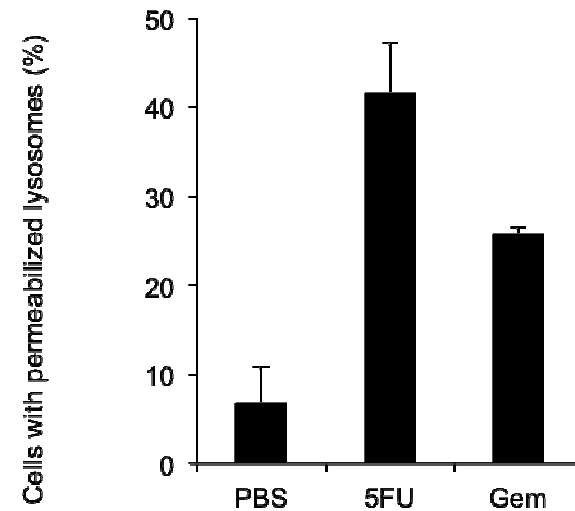
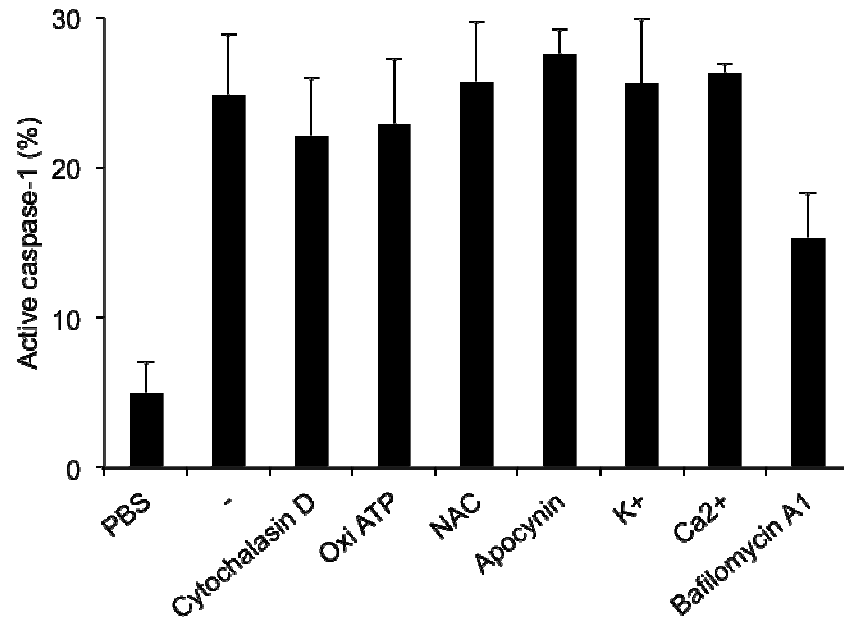
Chemotherapeutic treatments



MDSC clones

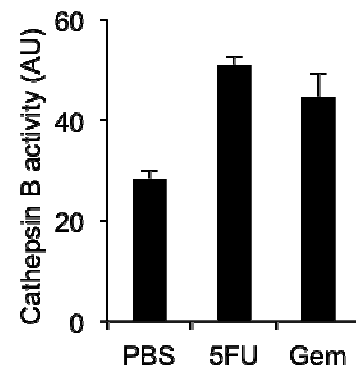


Assessment of caspase-1 activation and lysosome permeabilization

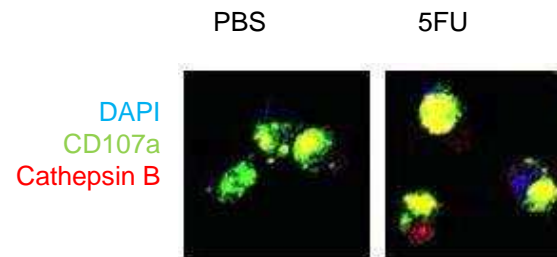


Subsequent events triggered by 5-FU-driven lysosome permeabilization

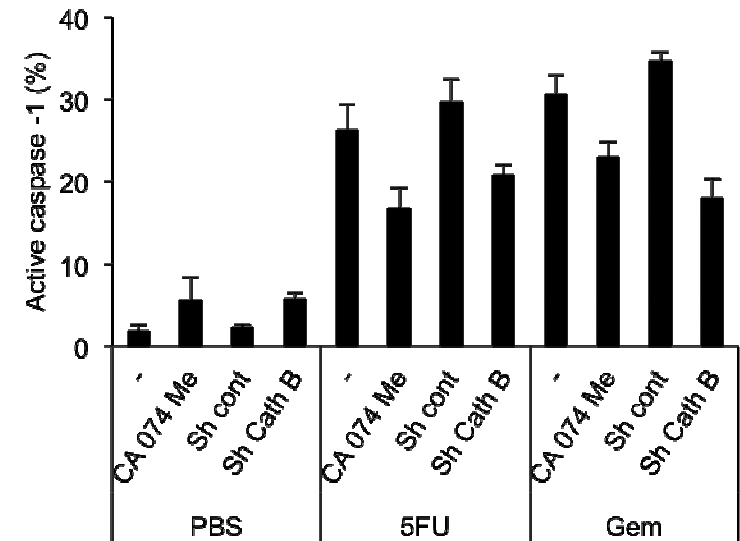
Cathepsin B activation



Cathepsin B release in the cytoplasm

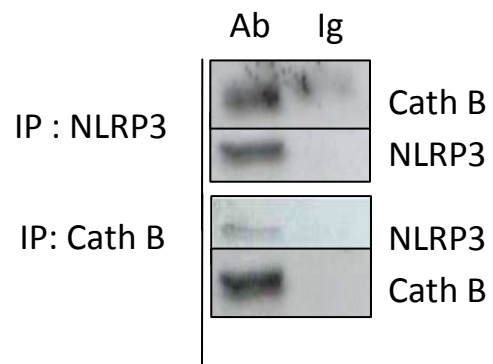


Cathepsin B dependent caspase-1 activation

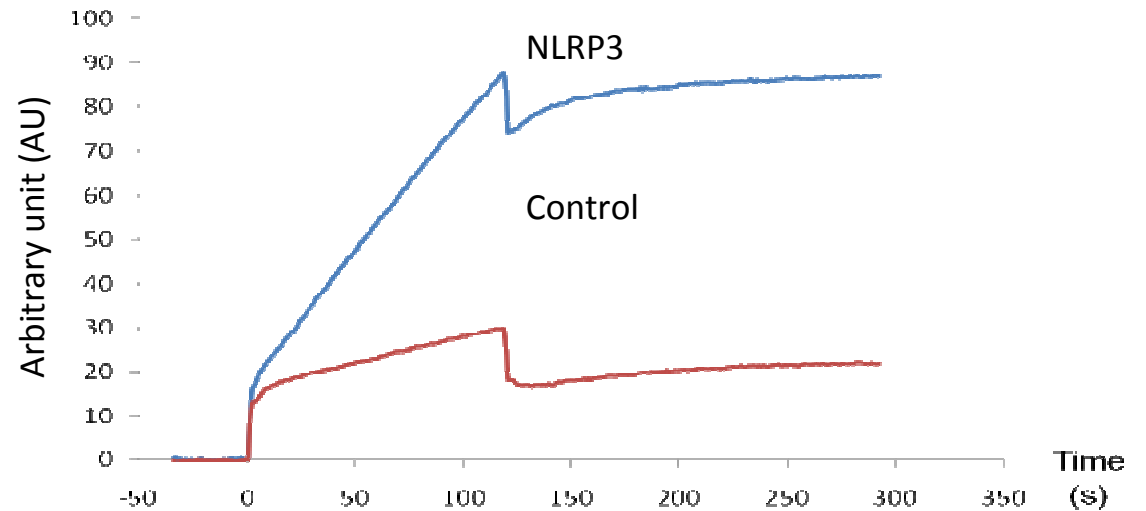


NLRP3 interacts with cathepsin B

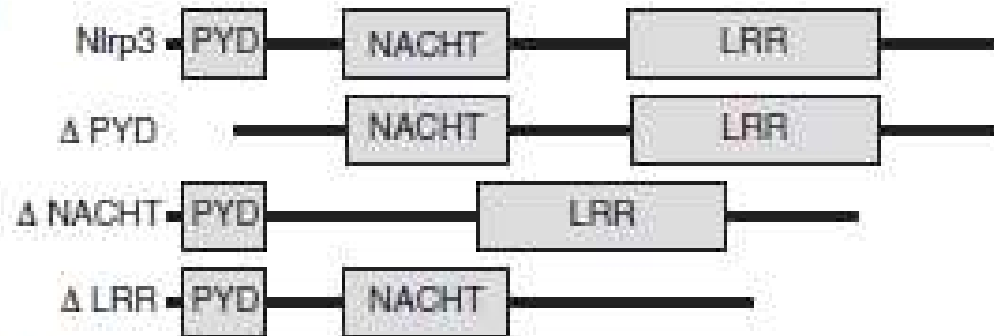
A: Immunoprecipitation



B: Surface plasmon resonance assay



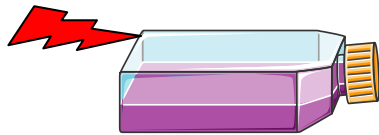
NLRP3 LRR domain is involved in NLRP3 interaction with Cathepsin B



		Δ PYD		Δ NACHT		Δ LRR		
		Ab	Ig	Ab	Ig	Ab	Ig	
IP : VSV								Cath B
								VSV
IP : Cath B								VSV
								Cath B

5-FU induces NLRP3-cathepsin B interaction

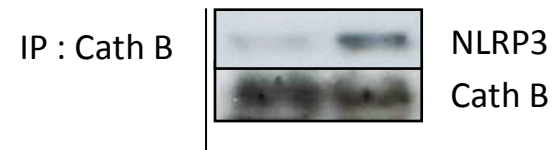
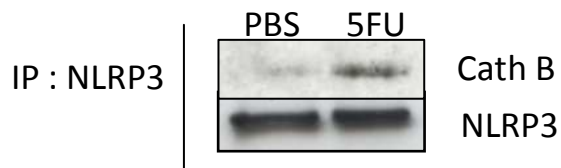
5-FU treatment



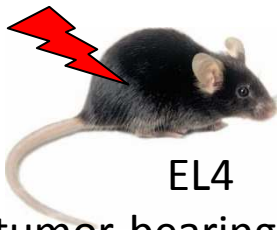
MDSC clone



Assessment of NLRP3-Cathepsin B interaction using immunoprecipitation



5-FU

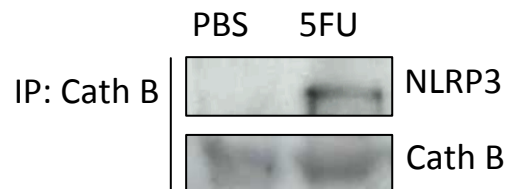


EL4

tumor-bearing mouse

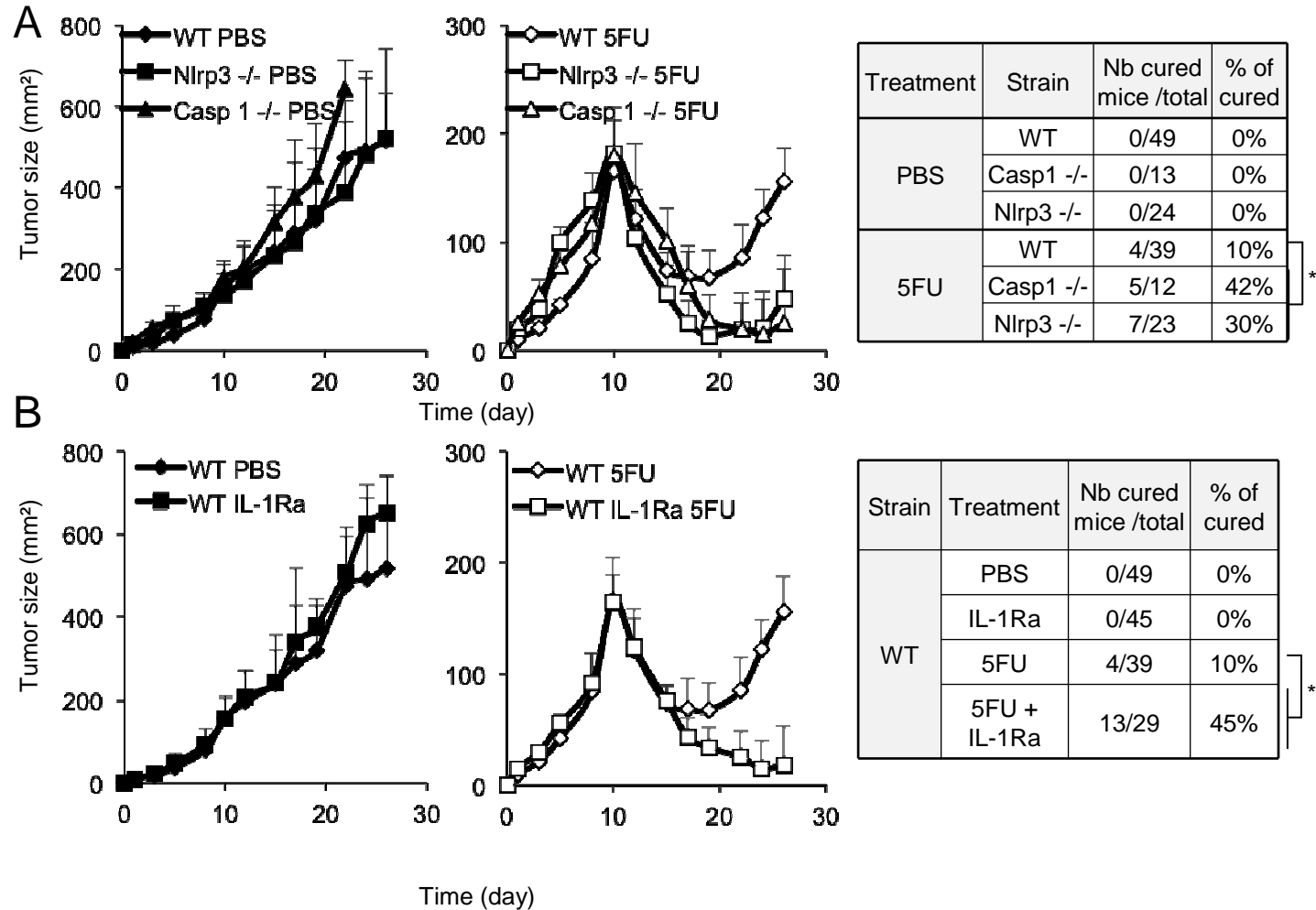
Sort MDSC
after 24 h

Assessment of NLRP3-Cathepsin B interaction using immunoprecipitation

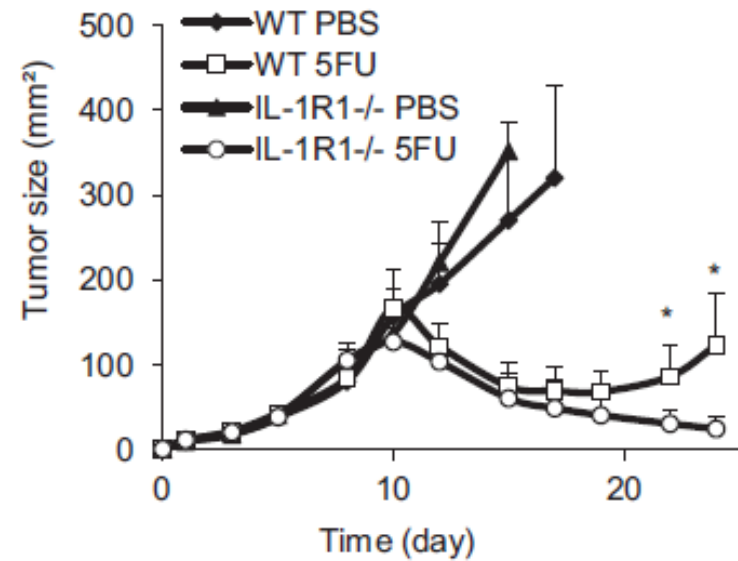
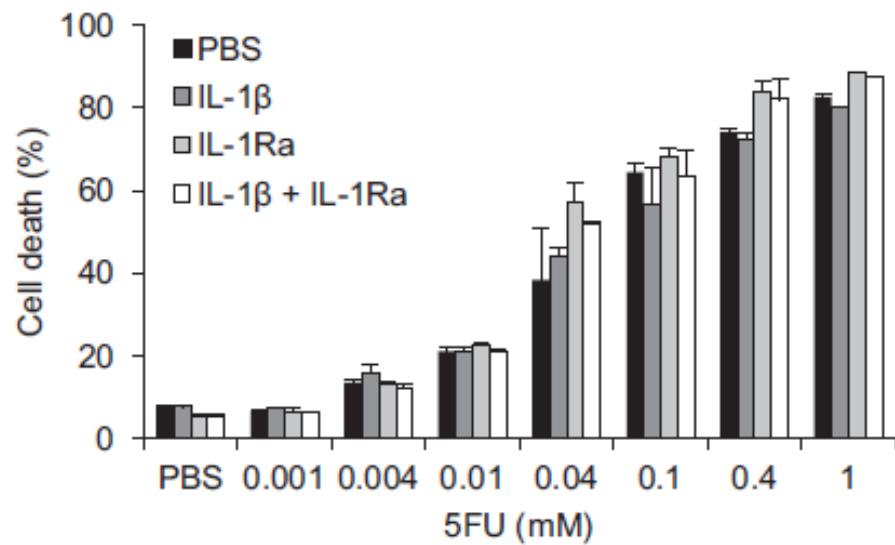


In vivo relevance of 5-FU mediated activation of inflammasome on tumor growth

EL4
tumor
model

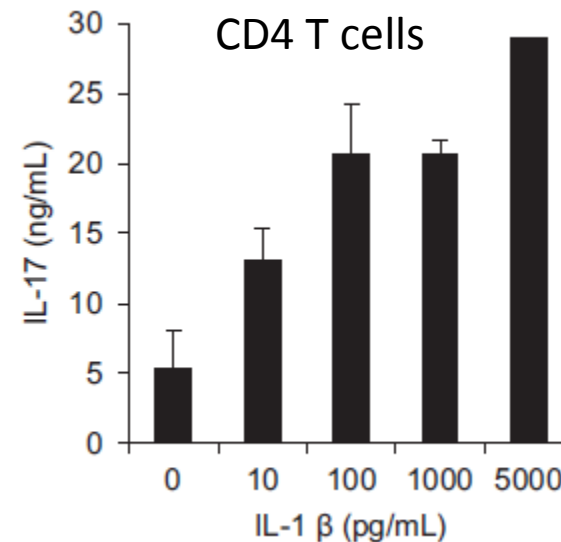
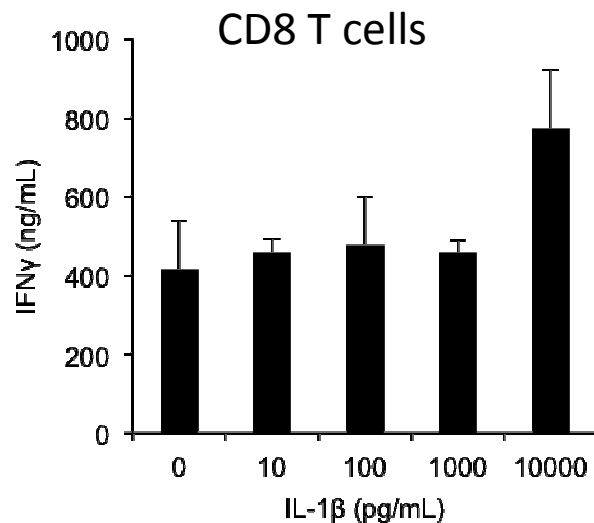


The protumor effect of IL-1 is dependent on the host



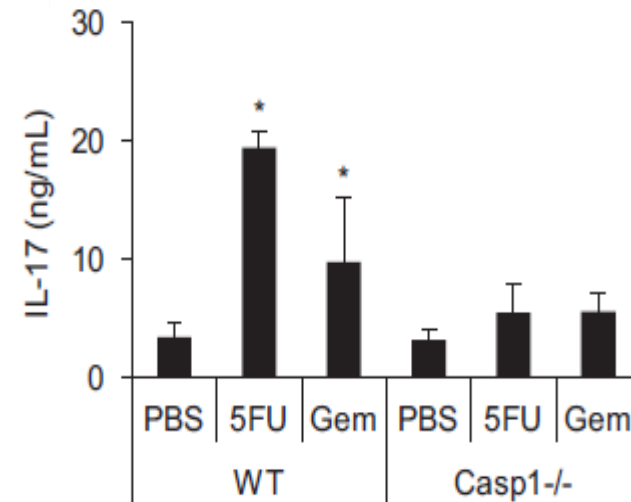
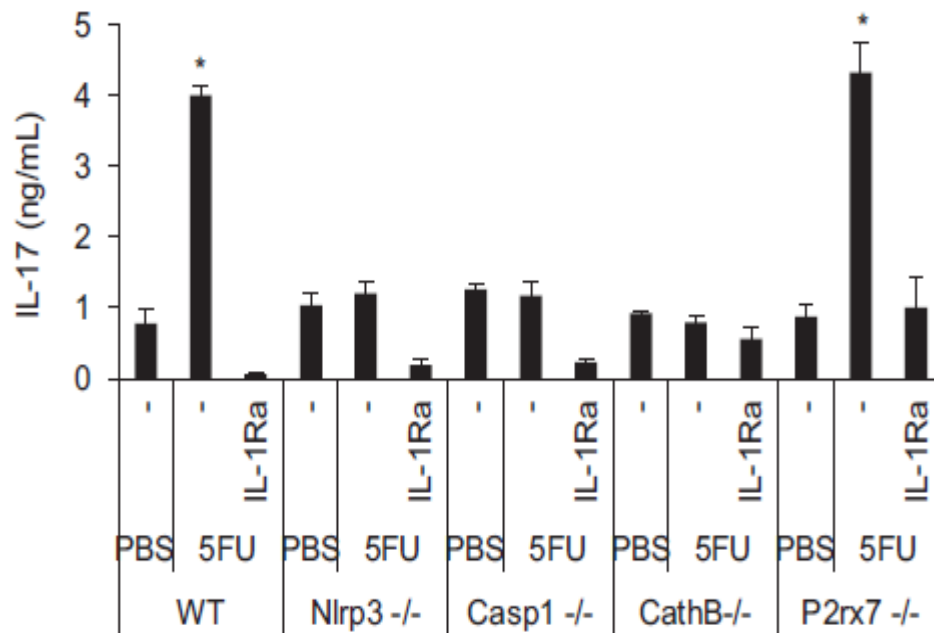
Differential effects of IL-1 β on CD4 and CD8 T cells

- Both CD4 and CD8 T cells express IL-1R1
- IL-1 β can induce secretion of IFN γ from CD8 T cells and of IL-17 from CD4 T cells



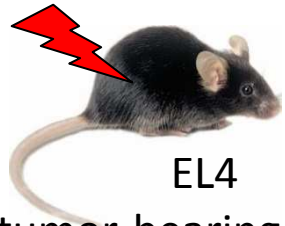
Low doses of IL-1 β favor Th17 differentiation without inducing IFN γ secretion from CD8 T cells

5-FU-driven IL-1 β release from MDSC affects CD4 T cell differentiation



In the tumor bed, the inflammasome controls Th17 generation and IL-17 dependent angiogenesis

5-FU



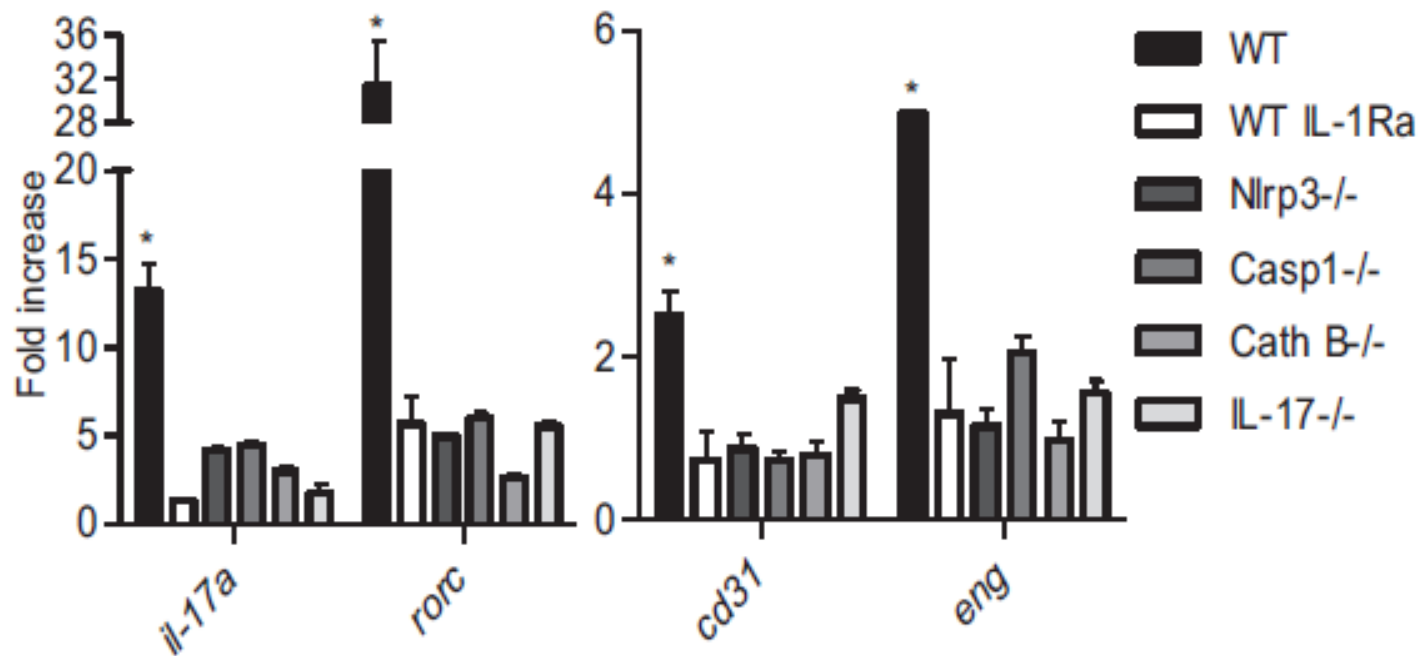
EL4

tumor-bearing mouse

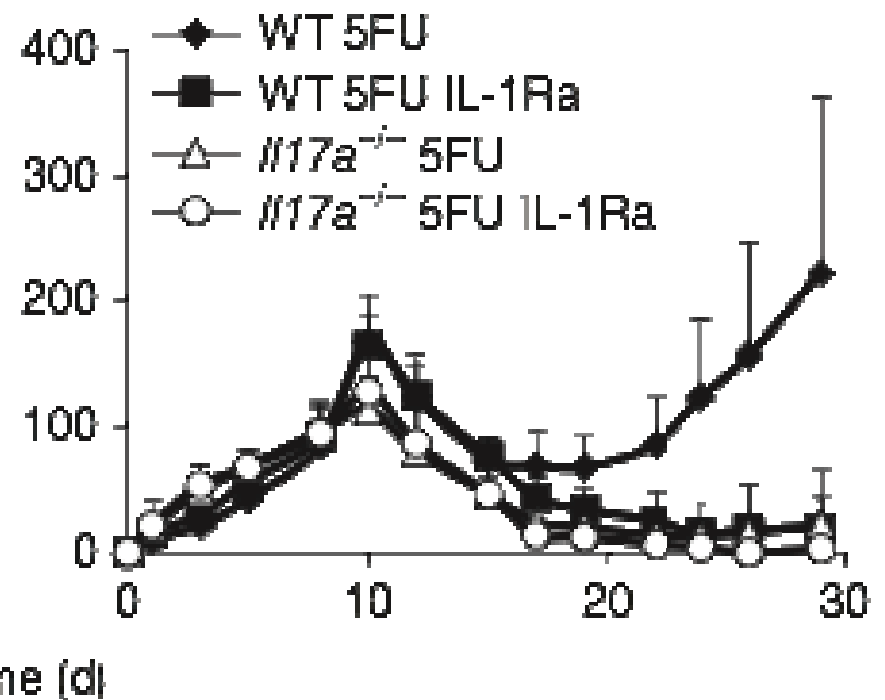
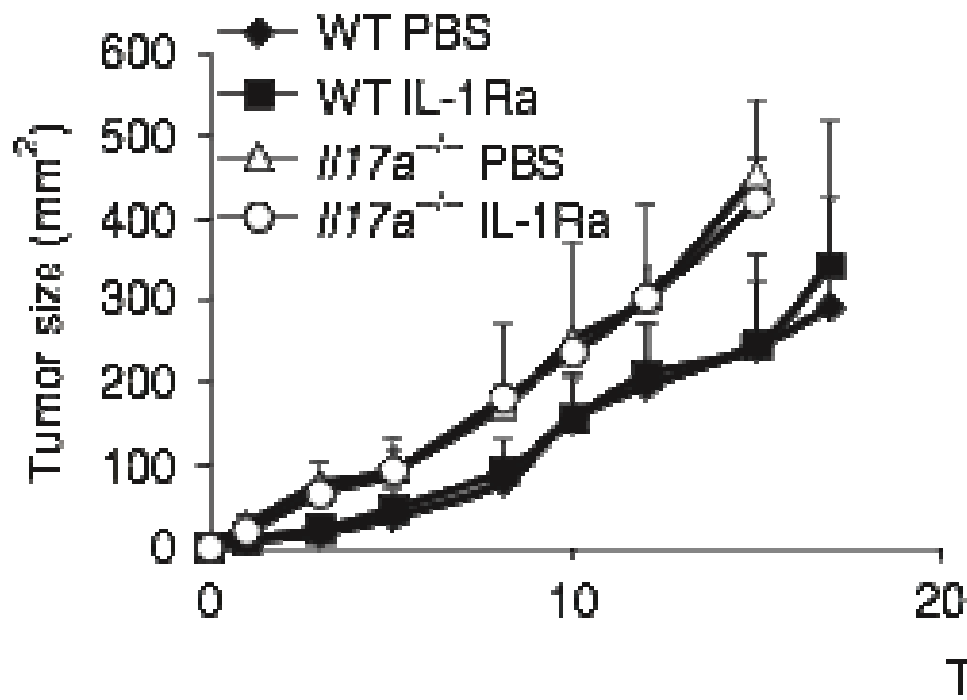
Harvest tumors after 48 h



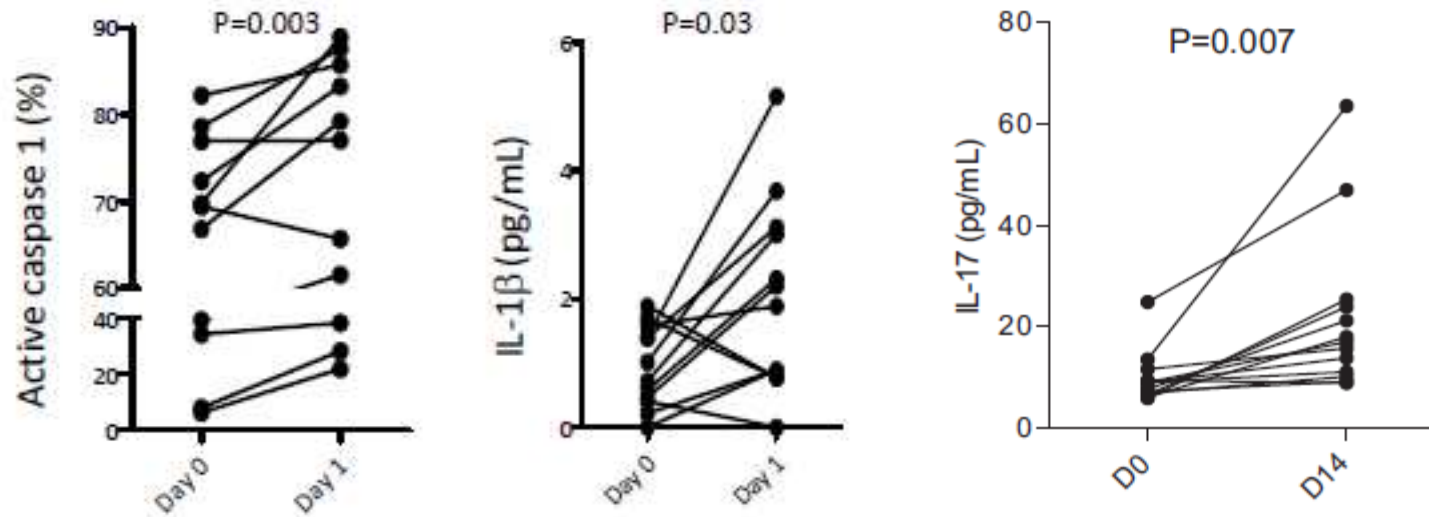
Assessment of
gene expression



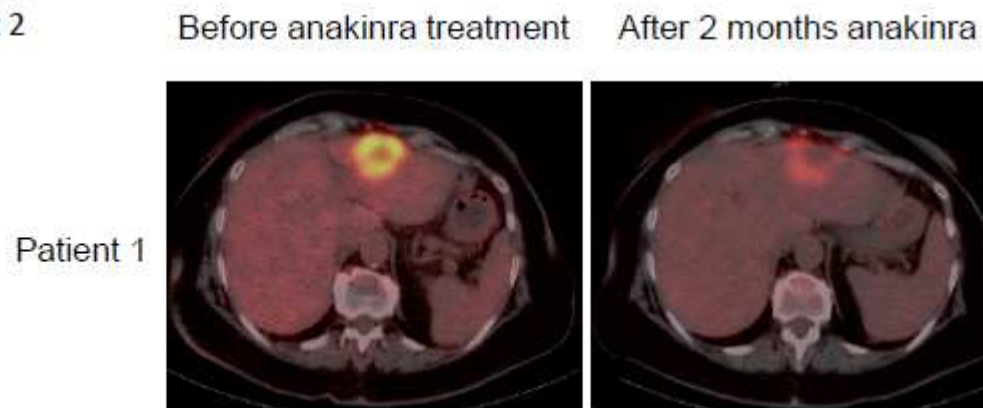
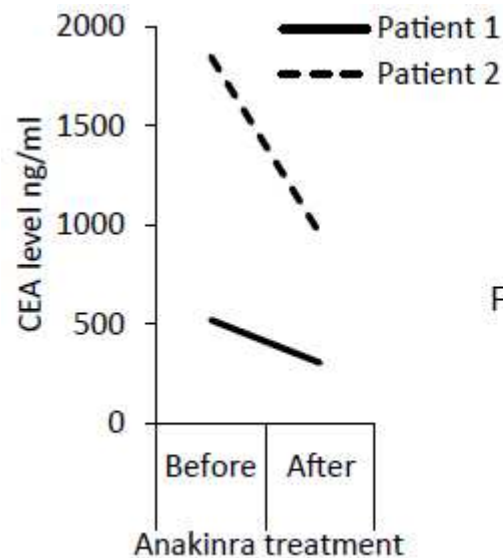
The antitumor effect of 5FU is improved in the absence of IL-17



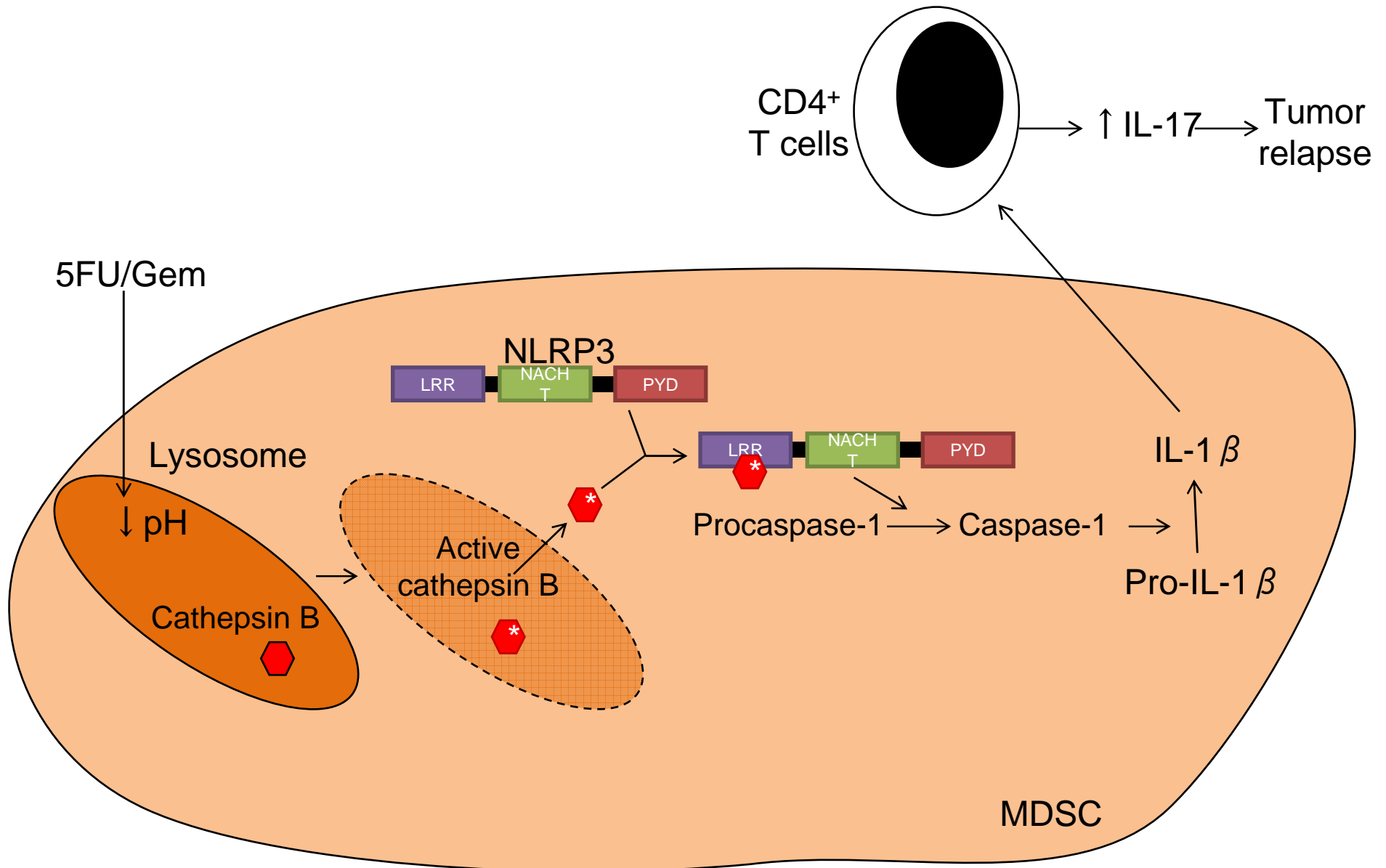
Human relevance of 5-Fu-induced caspase 1 activation and IL-17 production in colorectal cancer



Anakinra reverses resistance to 5FU



Proposed model accounting for the immunological effects of 5-FU



Acknowledgments

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- Dr. Wilfried Boireau (Besançon, France)
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