# Inhibition of CD39 to Promote Anti-Tumor Immune Responses

Christine Bowman, PhD

Associate Director, Biology

October 13, 2023

ARCUS BIOSCIENCES

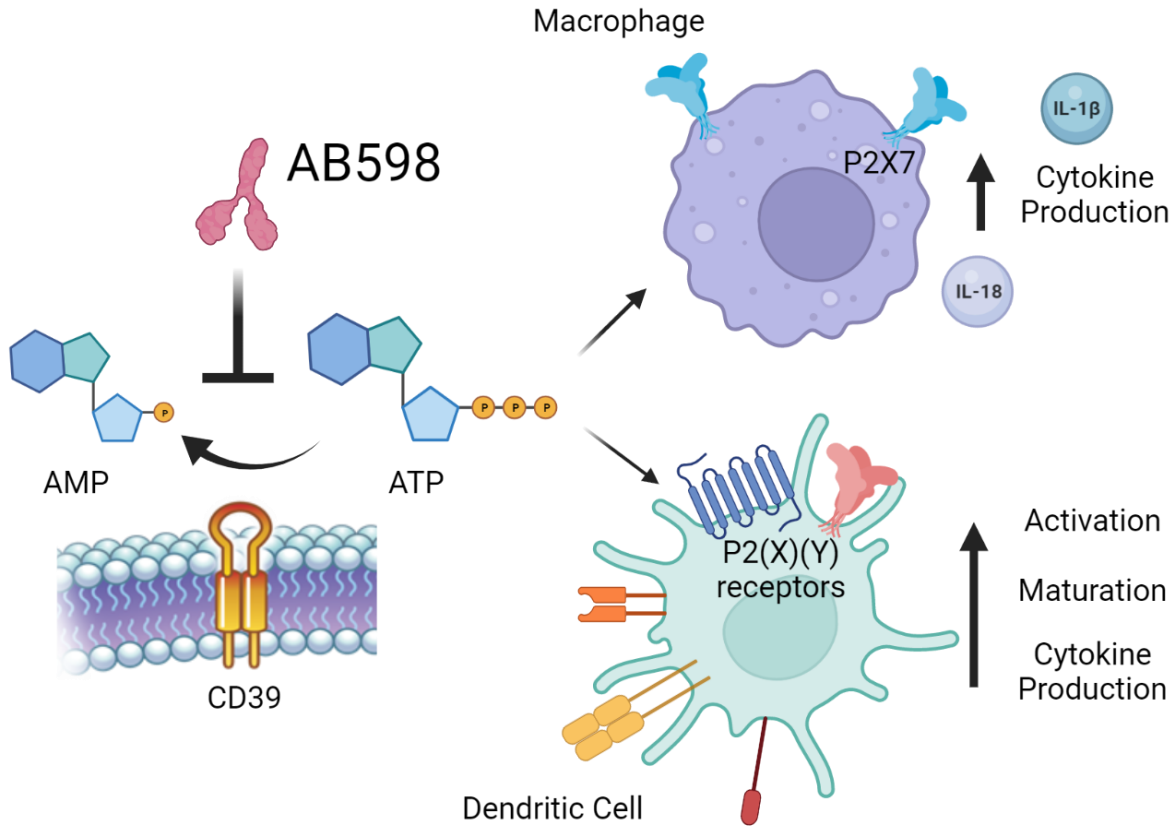
# CD39 Inhibition Through AB598 Will Result in a Heightened Anti-Tumor Immune Response

## Therapeutic Hypothesis

Inhibition of CD39 enzymatic activity will increase local levels of ATP leading to an immunostimulatory TME

## Translational Hypothesis

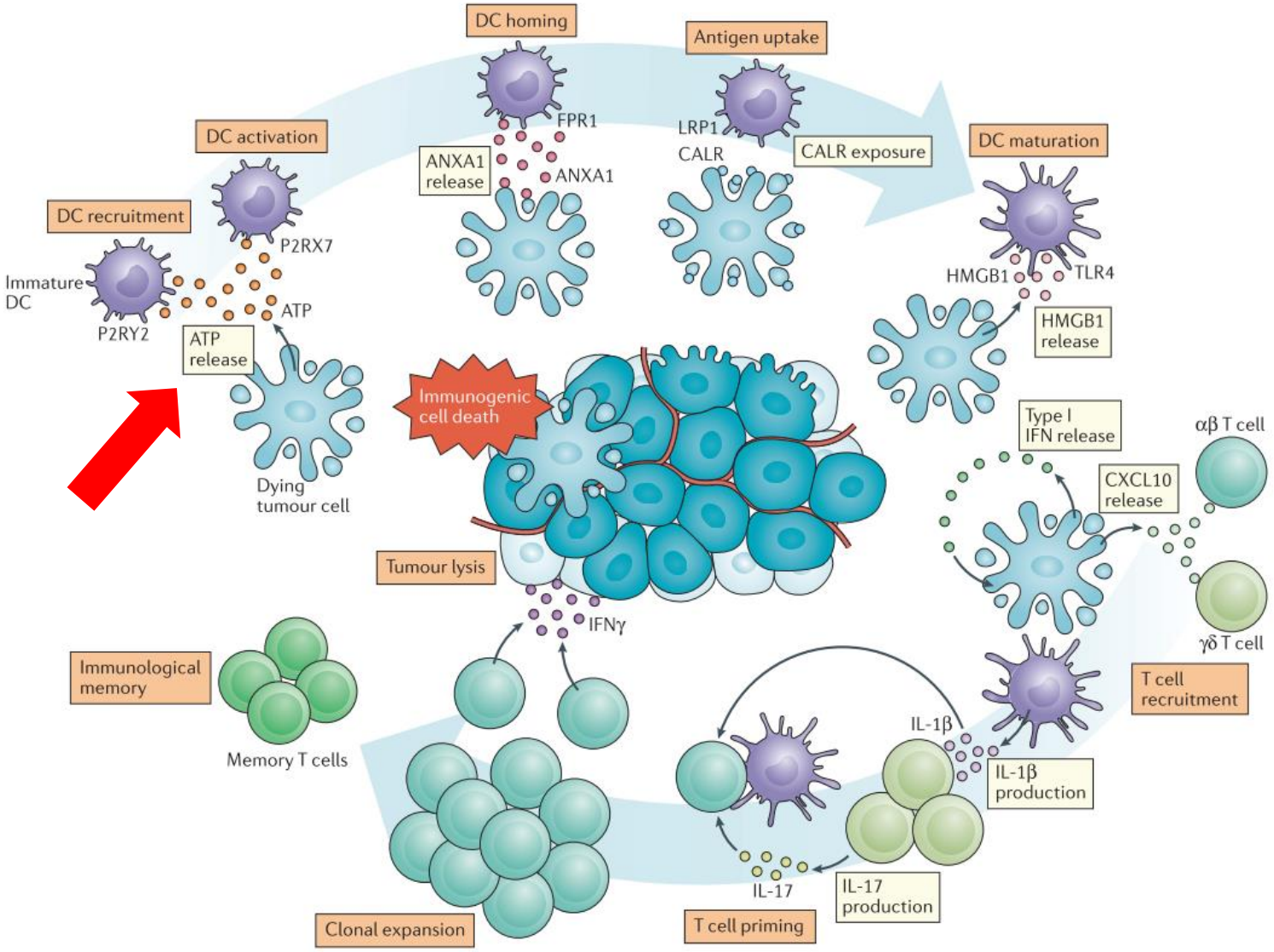
Inhibition of CD39 in combination with chemotherapy and/or radiation, agents that induce immunogenic cell death (ICD), will increase local levels of ATP, and enhance clinical response to immunotherapies




*AB598 is a highly potent and specific IgG1 Fc-silent antibody targeting CD39*

Schematic created with BioRender.com.

# CD39 Inhibition Elevates Extracellular ATP to Activate the Immune System




*Elevated levels of ATP can result in:*

**Dendritic Cell Activation**

↓

**Antigen Presentation**

↓

**T cell Recruitment and Expansion**

↓

**Tumor Cell Killing**

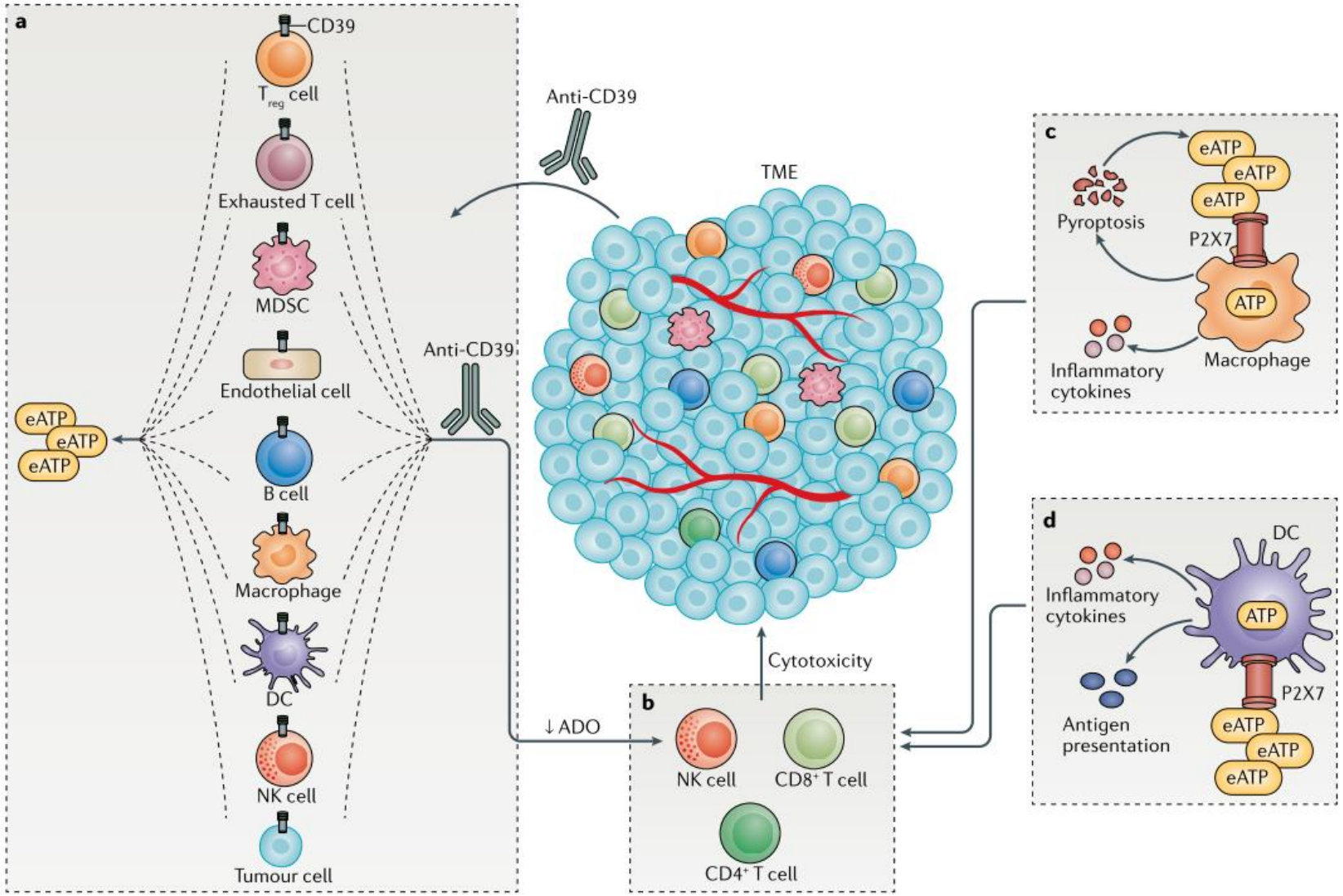
↓

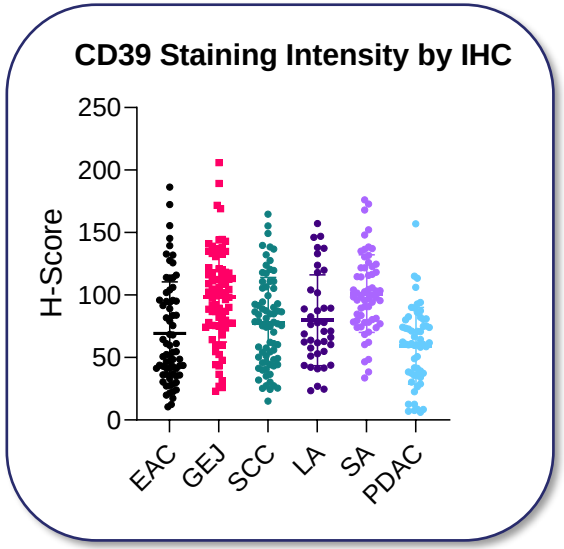
**Immunologic Memory**



Galluzzi, L. (2017) Nat. Rev. Immunol.

# CD39 Is Expressed Within the Immune and Stromal Compartments of the TME




- CD39 inhibition can act in cis or trans, allowing for increased ATP in the TME



Moesta, A.K. (2020) Nat. Rev. Immunol.



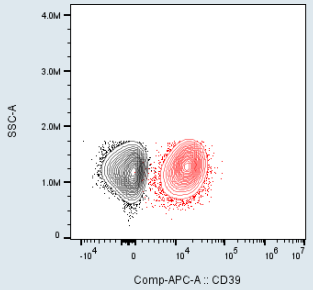
EAC = Esophageal Adenocarcinoma; GEJ = Gastroesophageal Junction Adenocarcinoma; SCC = Lung Squamous Cell Carcinoma; LA = Lung Adenocarcinoma; SA = Stomach Adenocarcinoma; PDAC = Pancreatic Ductal Adenocarcinoma

# CD39 Is Highly Expressed Predominantly on the Surface of Myeloid Cells in Healthy Human Whole Blood

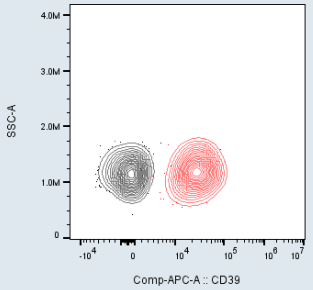
## *Myeloid Cells*

CD11b+

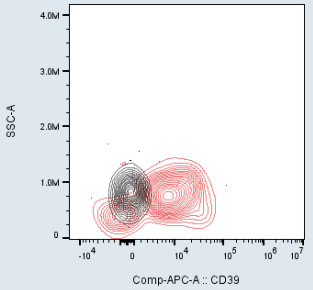
**Classical Monocytes**
HLA-DR+ CD14+ CD16-



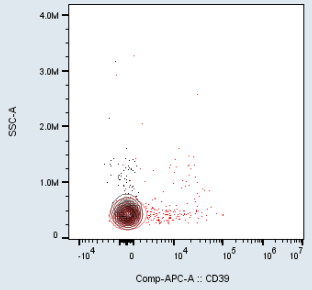

**Intermediate Monocytes**
HLA-DR+ CD14+ CD16+



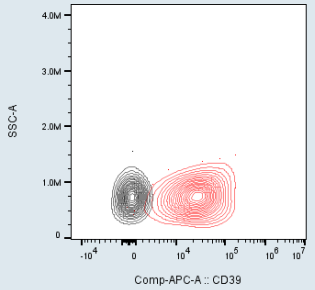

**Non-Classical Monocytes**
HLA-DR+ CD14- CD16+



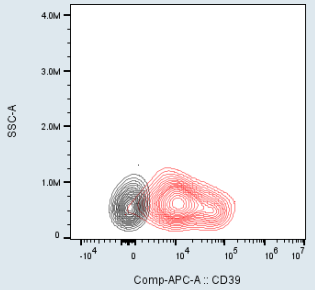

**NK Cells**
CD3- CD56+



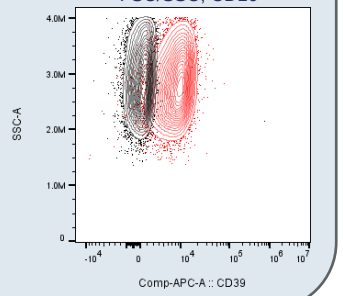

**Conventional DC1**
CD14- CD16- CD11c+ CD141+




**Conventional DC2**
CD14- CD16- CD11c+ CD141-



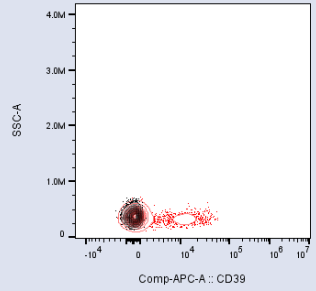

**Neutrophils**
FSC/SSC, CD16+



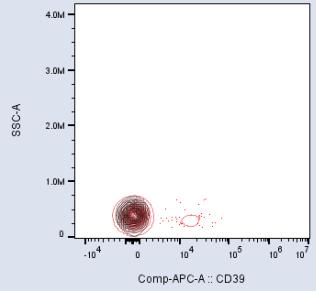

## *Lymphoid Cells*

CD11b-

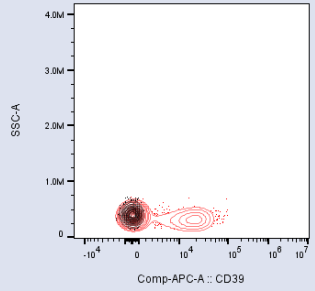
**CD4 CEM**
CD3+ CD4+ CD45RA- CCR7+



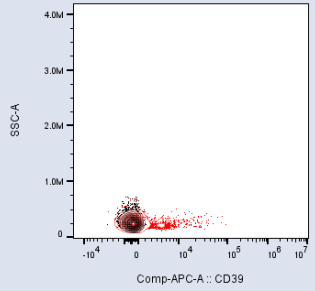

**CD4 Effector**
CD3+ CD4+ CD45RA+ CCR7-



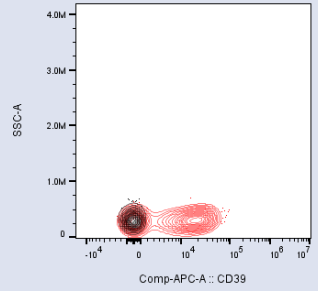

**CD4 EM**
CD3+ CD4+ CD45RA- CCR7-



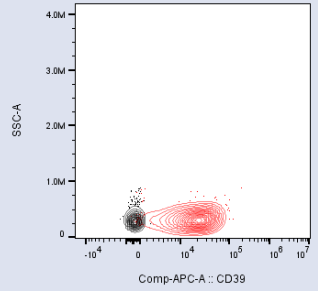

**CD4 Naive**
CD3+ CD4+ CD45RA+ CCR7+



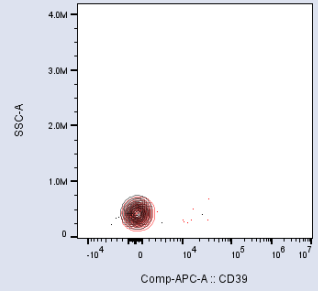

**Tregs**
CD3+ CD4+ CD127lo CD25hi



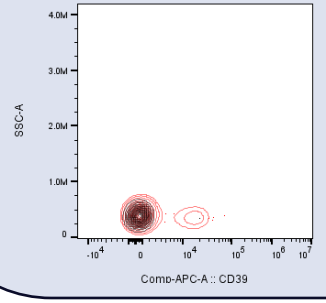

**B Cells**
CD3- CD19+



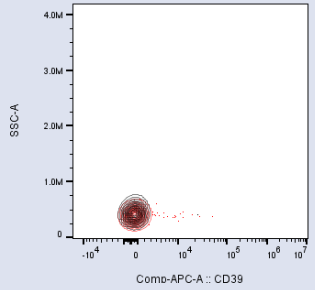

**NKT**
CD3+ CD56+



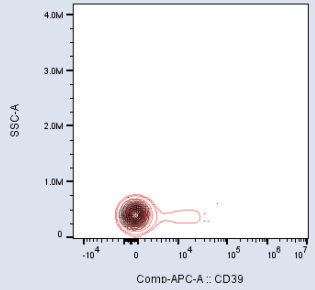

**CD8 CEM**
CD3+ CD8+ CD45RA- CCR7+



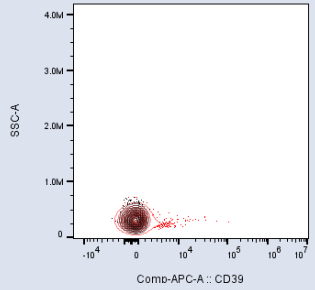

**CD8 Effector**
CD3+ CD8+ CD45RA+ CCR7-




**CD8 EM**
CD3+ CD8+ CD45RA- CCR7-




**CD8 Naive**
CD3+ CD8+ CD45RA+ CCR7+




Isotype control
Anti-CD39

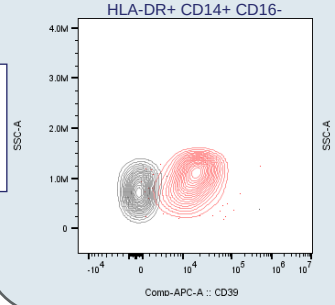
# CD39 Is Highly Expressed on Human Gastric Tumor-Infiltrating Immune Cells

Isotype control
Anti-CD39

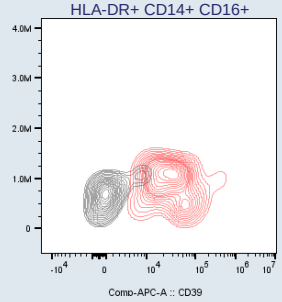
## *Myeloid Cells*

CD11b+

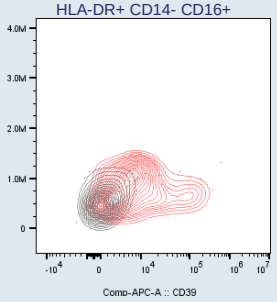
**Classical Monocytes**
HLA-DR+ CD14+ CD16-



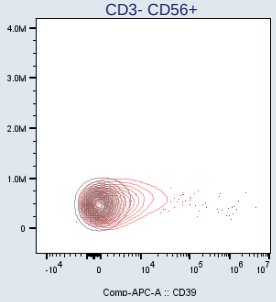
**Intermediate Monocytes**
HLA-DR+ CD14+ CD16+



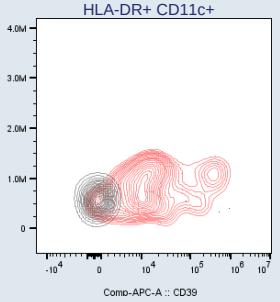
**Non-Classical Monocytes**
HLA-DR+ CD14- CD16+



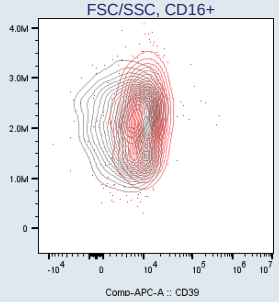
**NK Cells**
CD3- CD56+



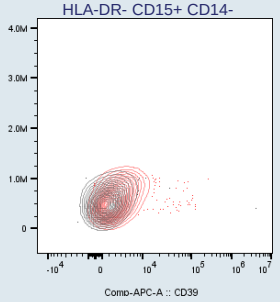
**DC**
HLA-DR+ CD11c+



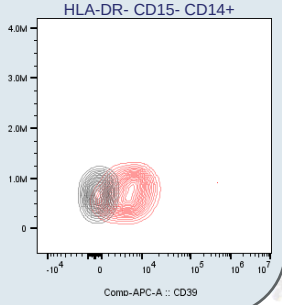
**Neutrophils**
FSC/SSC, CD16+



**pMDSC**
HLA-DR- CD15+ CD14-



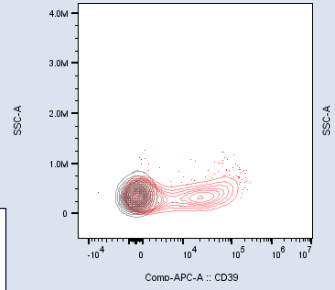
**mMDSC**
HLA-DR- CD15- CD14+



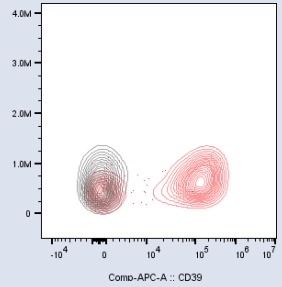
## *Lymphoid Cells*

CD11b-

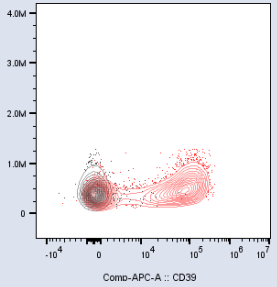
**CD4 CEM**
CD3+ CD8- CD56- CD45RA- CCR7+



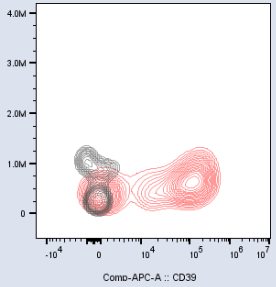
**CD4 Effector**
CD3+ CD8- CD56- CD45RA+ CCR7-



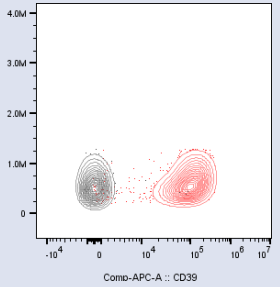
**CD4 EM**
CD3+ CD8- CD56- CD45RA- CCR7-



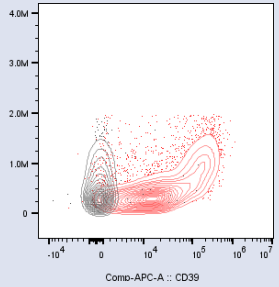
**CD4 Naive**
CD3+ CD8- CD56- CD45RA+ CCR7+



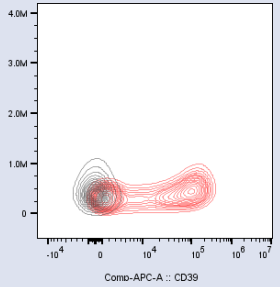
**Tregs**
CD3+ CD8- CD56- FOXP3+



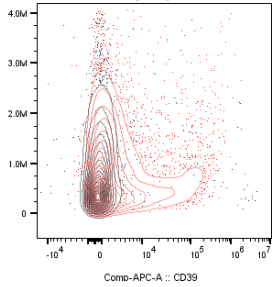
**B Cells**
CD3- CD19+



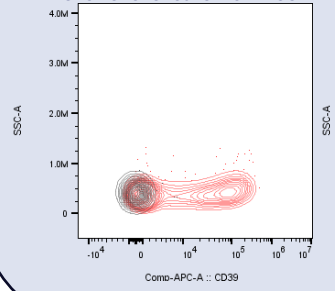
**NKT**
CD3+ CD56+



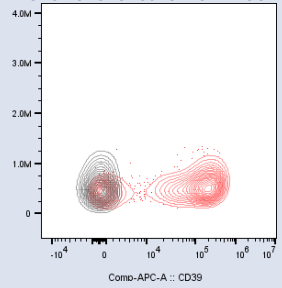
**Non-Immune Cells**
CD45-



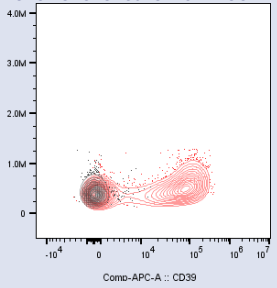
**CD8 CEM**
CD3+ CD8- CD56- CD45RA- CCR7+



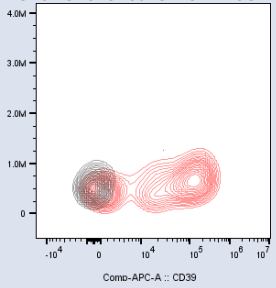
**CD8 Effector**
CD3+ CD8- CD56- CD45RA+ CCR7-



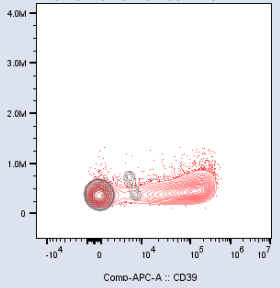
**CD8 EM**
CD3+ CD8- CD56- CD45RA- CCR7-



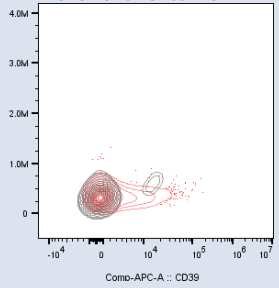
**CD8 Naive**
CD3+ CD8- CD56- CD45RA+ CCR7+



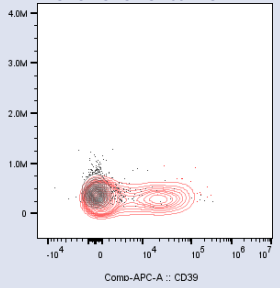
**CD8 Prog1**
CD3+ CD8+ CD69+ TCF1+



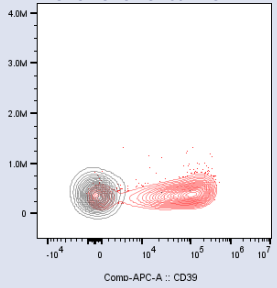
**CD8 Prog2**
CD3+ CD8+ CD69- TCF1+



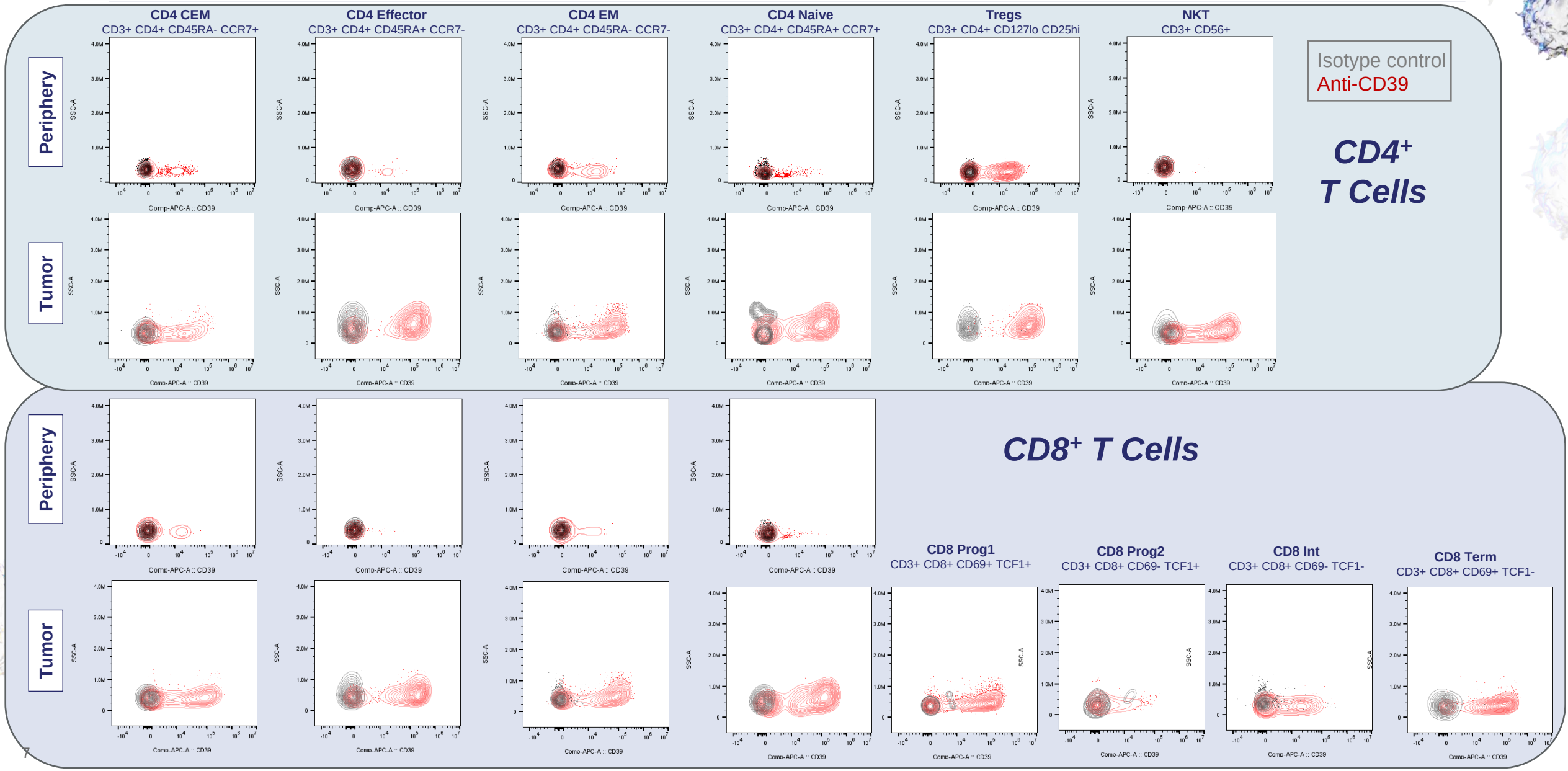
**CD8 Int**
CD3+ CD8+ CD69- TCF1-



**CD8 Term**
CD3+ CD8+ CD69+ TCF1-



# Intratumoral T Cell Activation Leads to Increased Expression of CD39



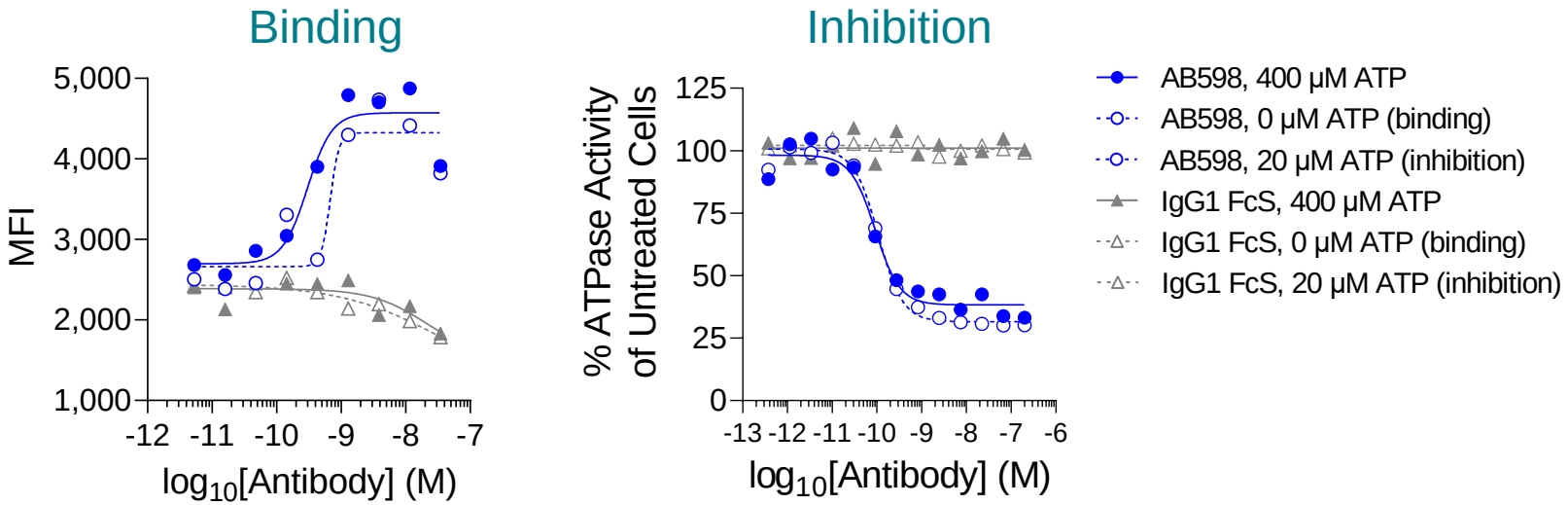
# AB598 is a Potent CD39 Inhibitory Antibody

## BINDING TO RECOMBINANT CD39 BY SPR:

| | $k_{on}$ ($M^{-1}s^{-1}$) | $k_{off}$ ($s^{-1}$) | $K_D$ (M) |
|---|---|---|---|
| AB598 | $3.0 \times 10^{5}$ | $7.2 \times 10^{-5}$ | $2.4 \times 10^{-10}$ |
| IPH5201[1] | $2.0 \times 10^{5}$ | $1.6 \times 10^{-3}$ | $3.2 \times 10^{-9}$ |
| TTX-030[2] | $1.78 \times 10^{5}$ | $2.02 \times 10^{-3}$ | $1.13 \times 10^{-8}$ |

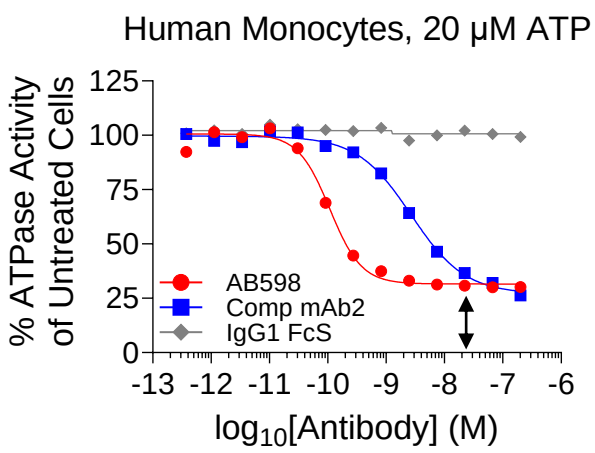
[1]Perrot, I. (2019) Cell Rep. [2]Spatola, B. (2020) mAbs.

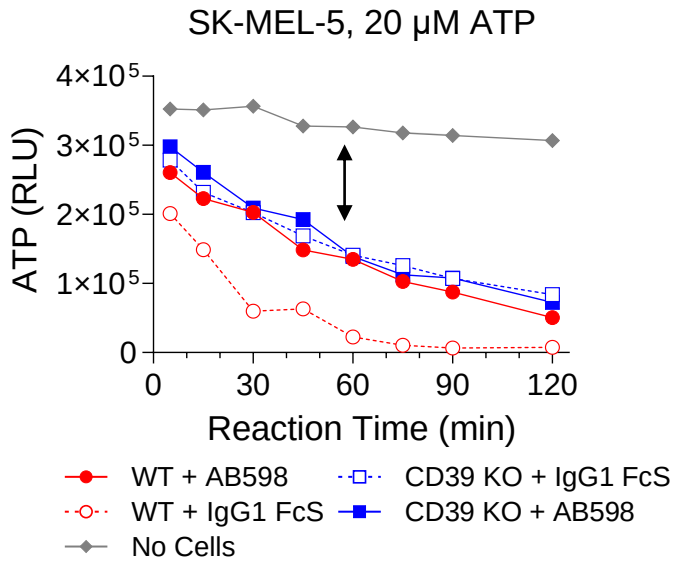
## BINDING AND INHIBITION OF CELL SURFACE CD39 ON PRIMARY HUMAN MONOCYTES:



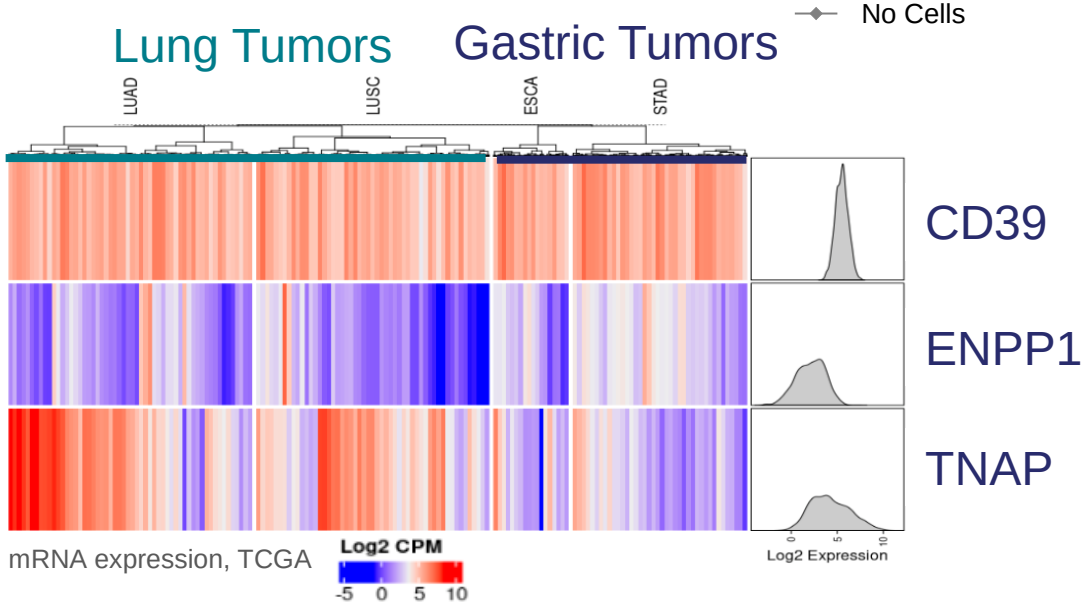

- AB598 binds with a fast on-rate to CD39 and dissociates with a slow off-rate, resulting in a long residence time and high, sub-nanomolar affinity to CD39
- AB598 has sub-nanomolar binding and inhibition of membrane-bound CD39
- Potent binding and inhibition of CD39 are maintained in the presence of high ATP

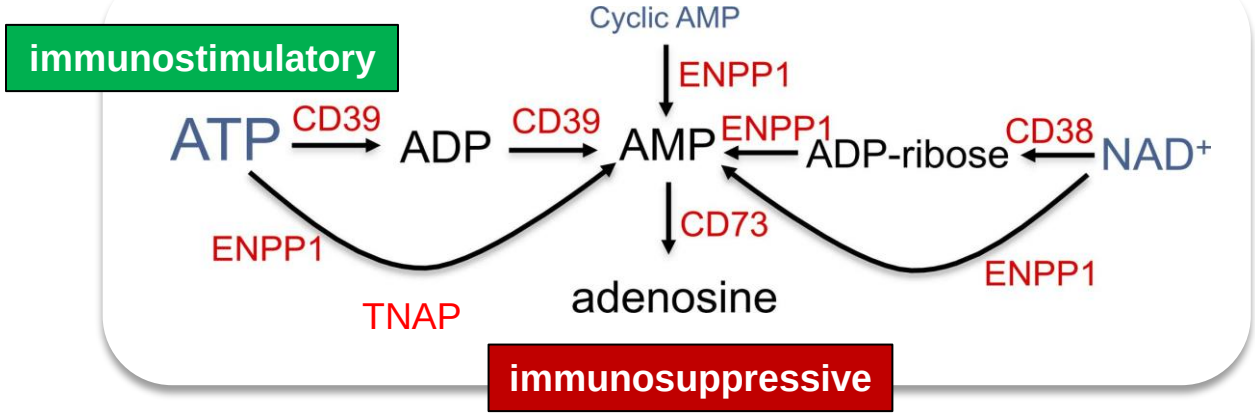
# AB598 Fully Inhibits CD39 Enzymatic Activity




- AB598 fully inhibits CD39 enzymatic activity but does not completely block ATP degradation
- CD39, ENPP1, and TNAP contribute to extracellular ATP degradation

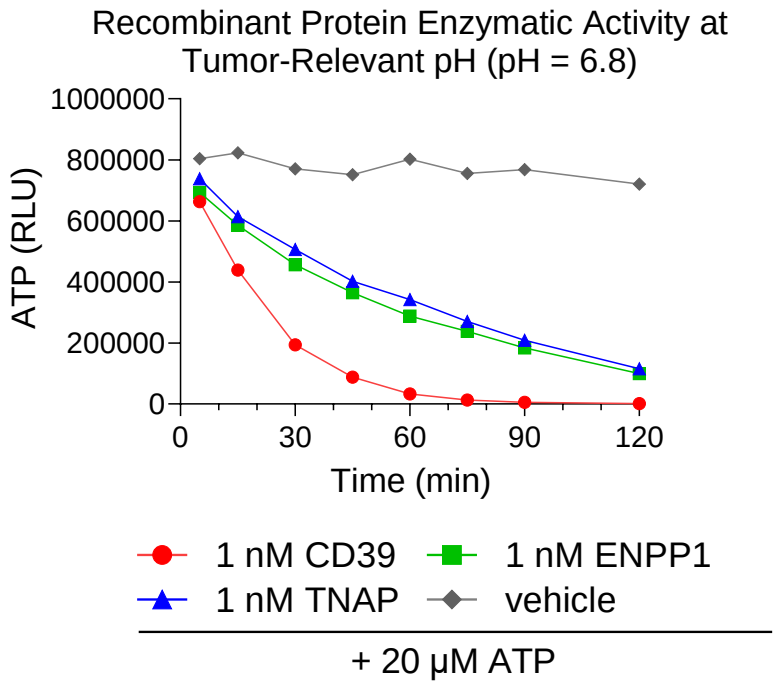


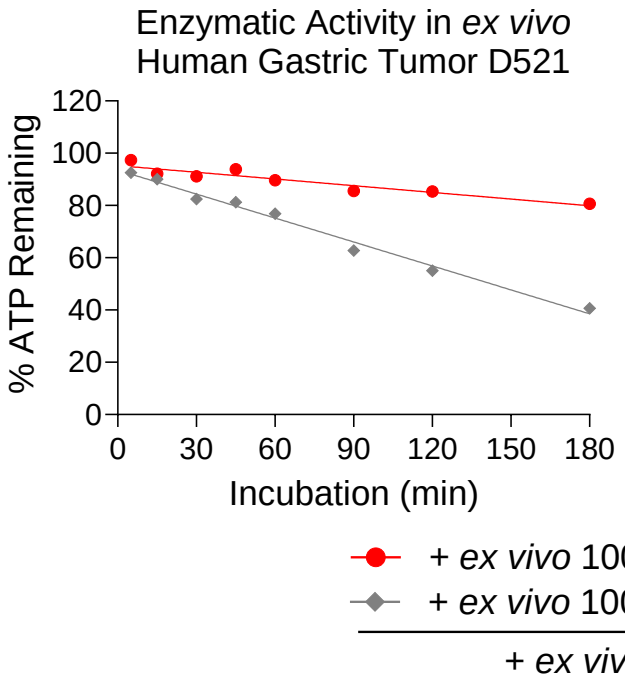



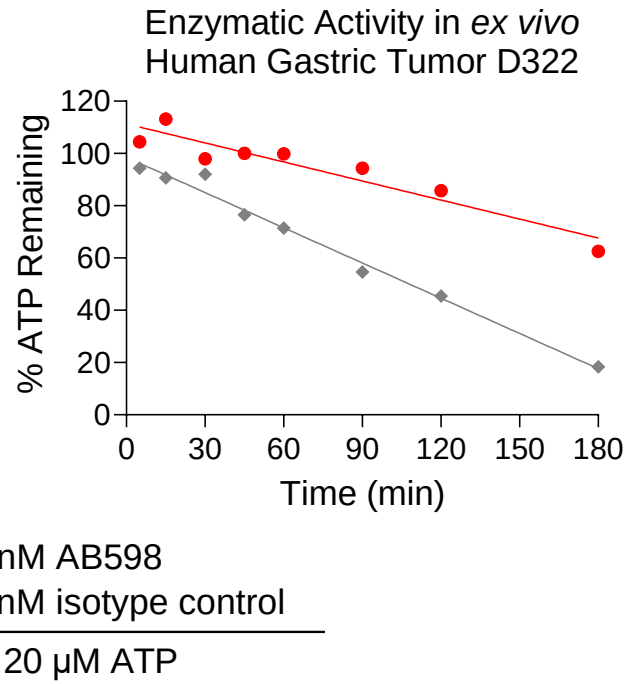



Schematic modified from Yu, J.C. (2018) JCI Insight.

# CD39 is the Dominant ATP Degrading Enzyme in the Tumor Microenvironment



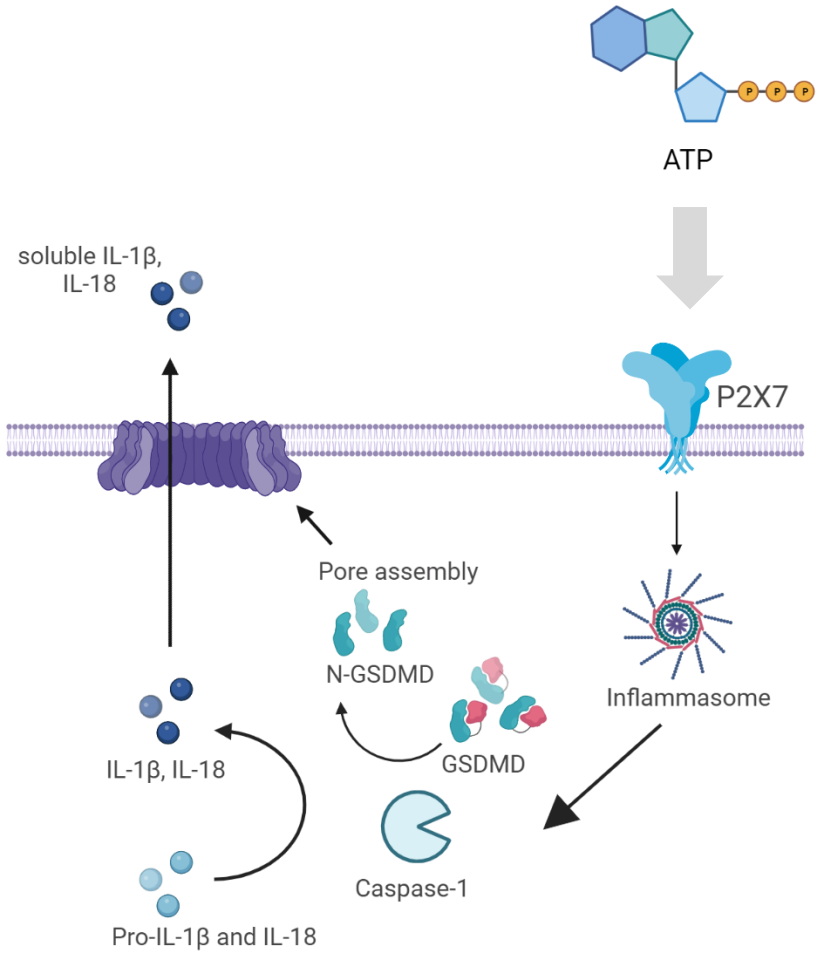



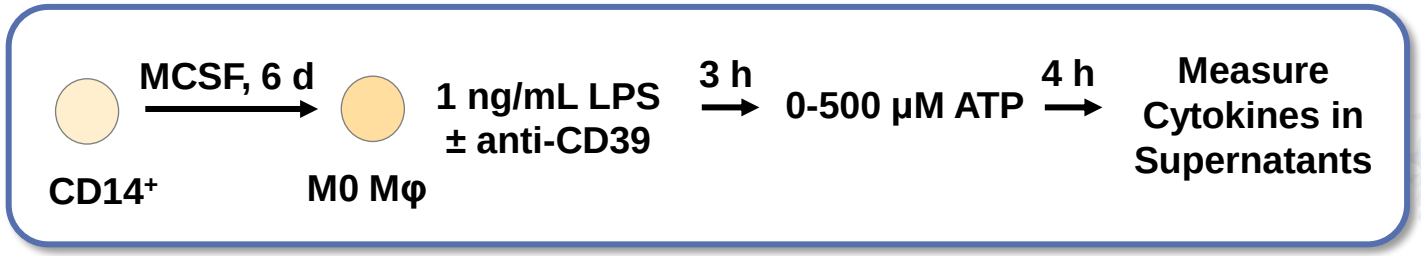



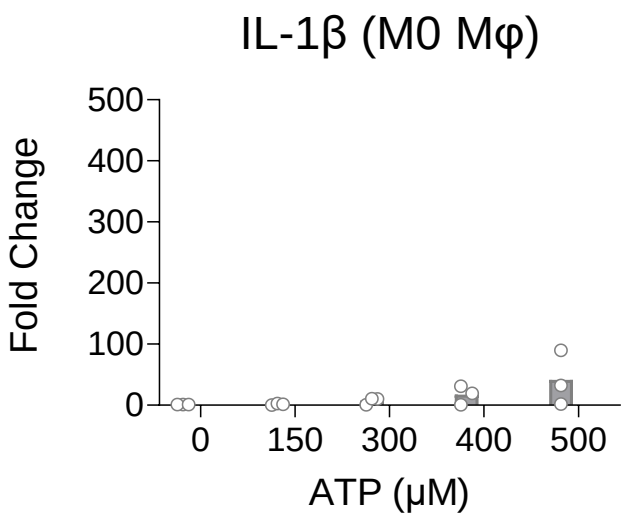

- At the slightly acidic pH of the TME, CD39 is the most active of the extracellular ATP-degrading enzymes
- In primary *ex vivo* gastric tumors, AB598 inhibited ATPase activity, allowing for elevated extracellular ATP

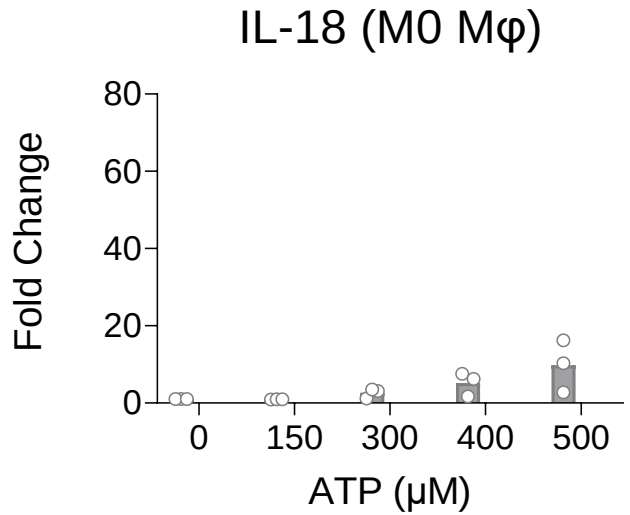
# Increasing ATP Promotes Inflammasome Activation

- Elevated ATP activates the inflammasome to promote cytokine release



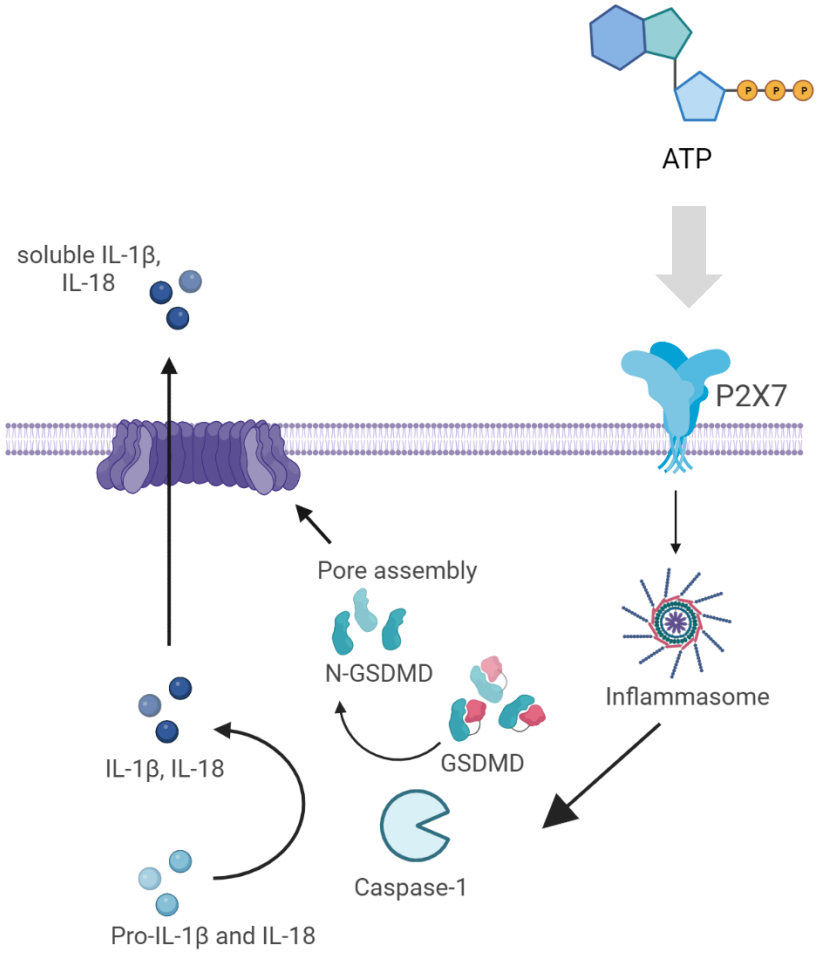



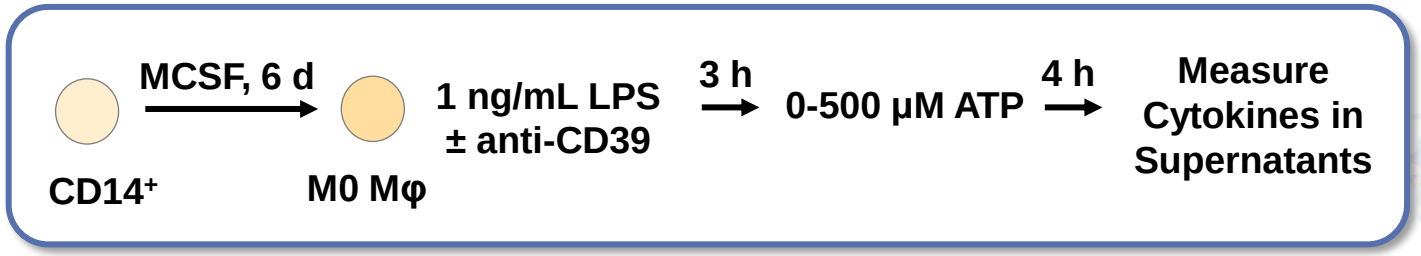


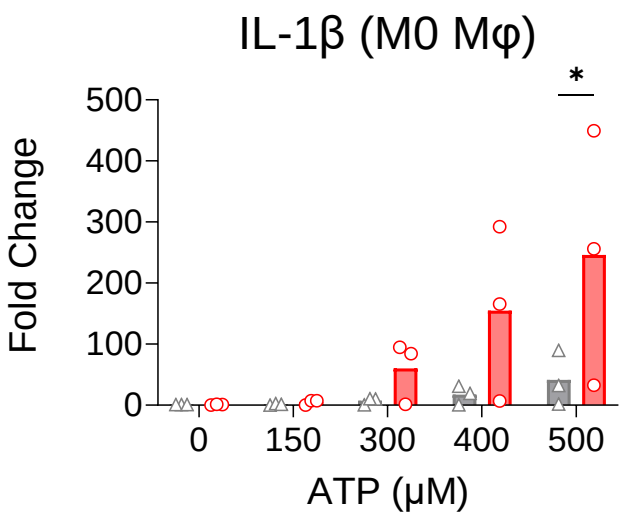
Fold change was calculated per donor to the 0 µM ATP, IgG1 FcS-treated condition. Schematic created with BioRender.com.

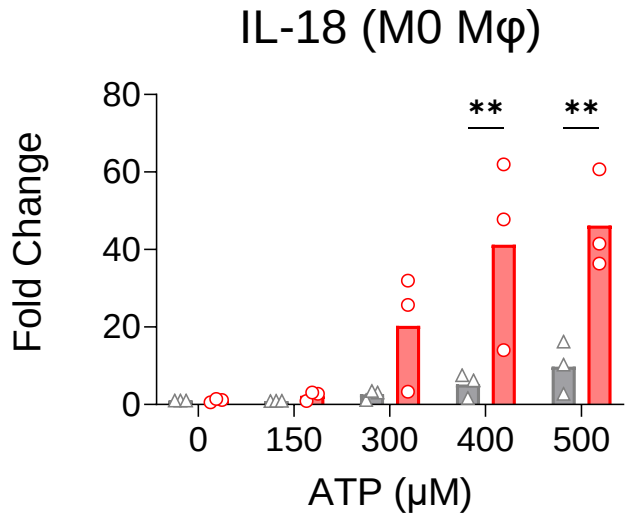
# AB598 Promotes ATP-Dependent Inflammasome Activation

- Elevated ATP activates the inflammasome to promote cytokine release



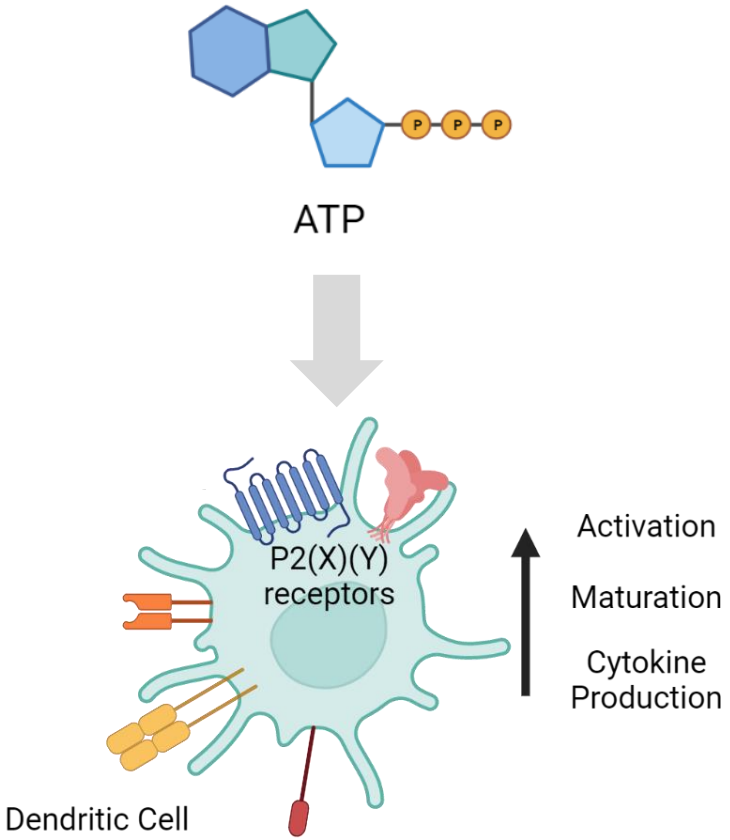



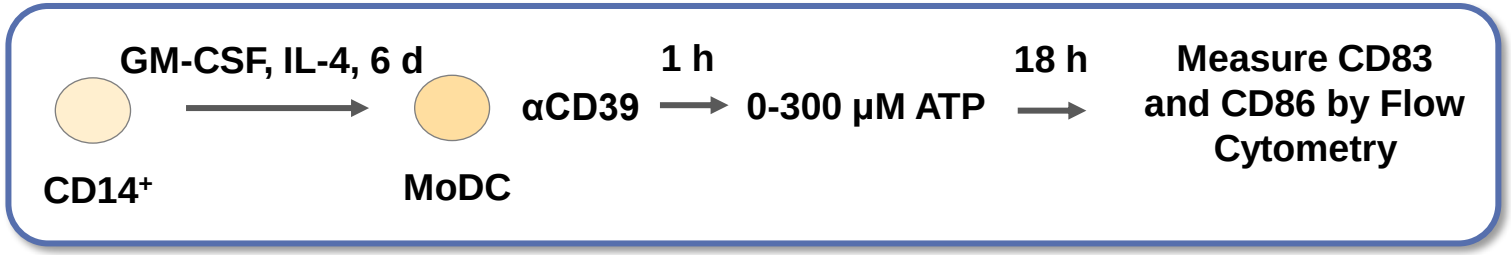





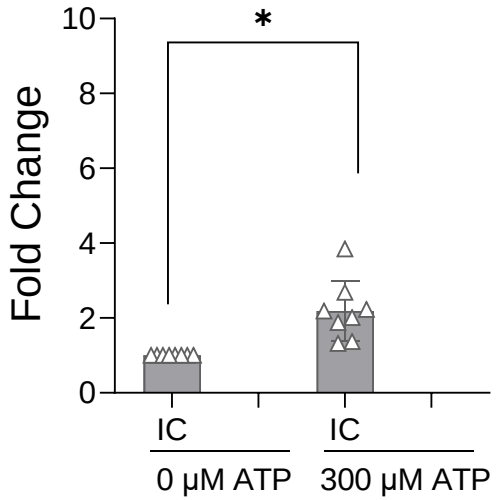
Statistical significance calculated with a two-way ANOVA using Šídák's multiple comparisons test, *P ≤ 0.05, ** P ≤ 0.01. Fold change was calculated per donor to the 0 µM ATP, IgG1 FcS-treated condition. Schematic created with BioRender.com.

# AB598 Promotes ATP-Dependent Monocyte-Derived Dendritic Cell (moDC) Activation

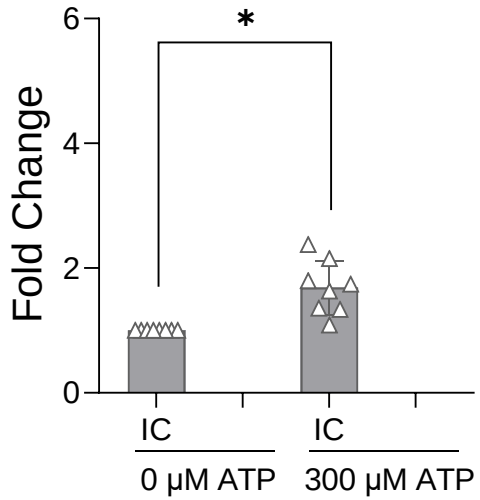



CD83, % Positive




CD86, MFI

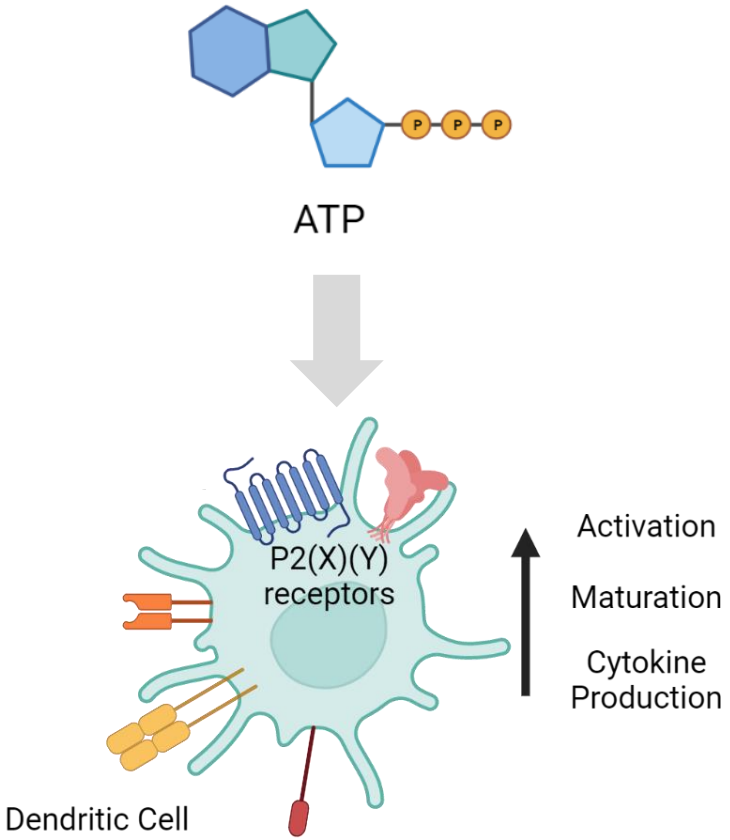


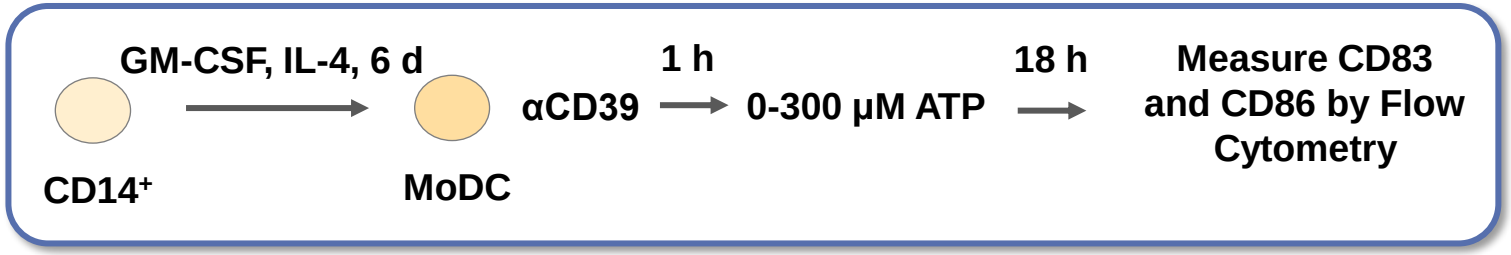

△ IgG1 FcS

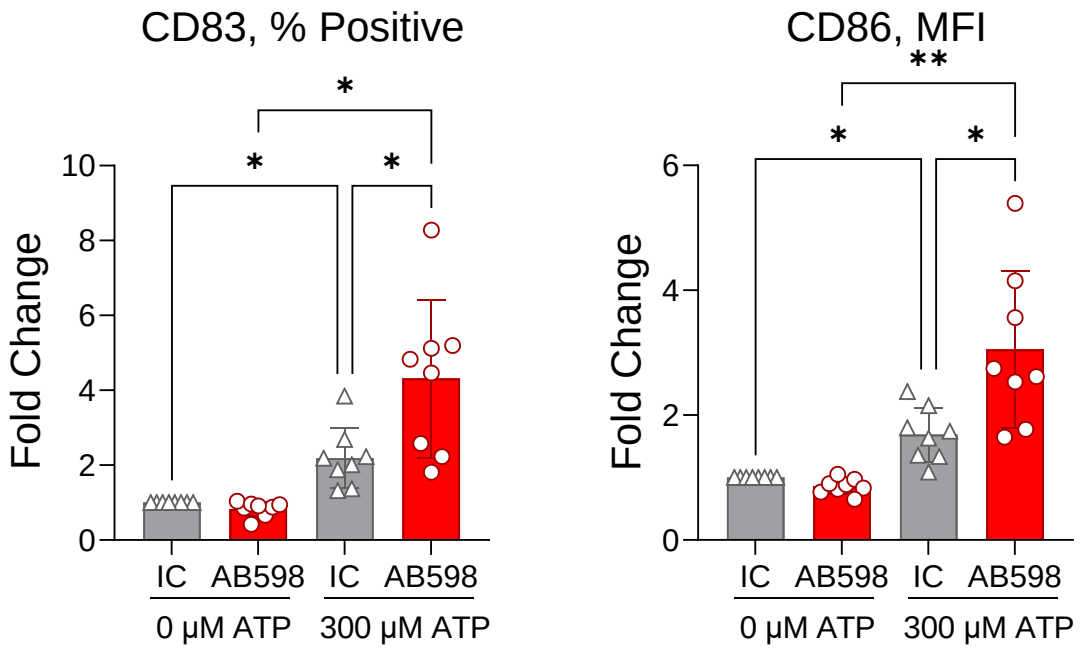
Statistical significance was calculated with a two-way ANOVA using Šídák's multiple comparisons test, *P ≤ 0.05, ** P ≤ 0.01, *** P ≤ 0.001. Fold change calculated per donor to the 0 μM ATP, IgG1 FcS-treated condition. Schematic created with BioRender.com.

# AB598 Promotes ATP-Dependent Monocyte-Derived Dendritic Cell (moDC) Activation

- ATP promotes moDC maturation, an effect amplified by anti-CD39



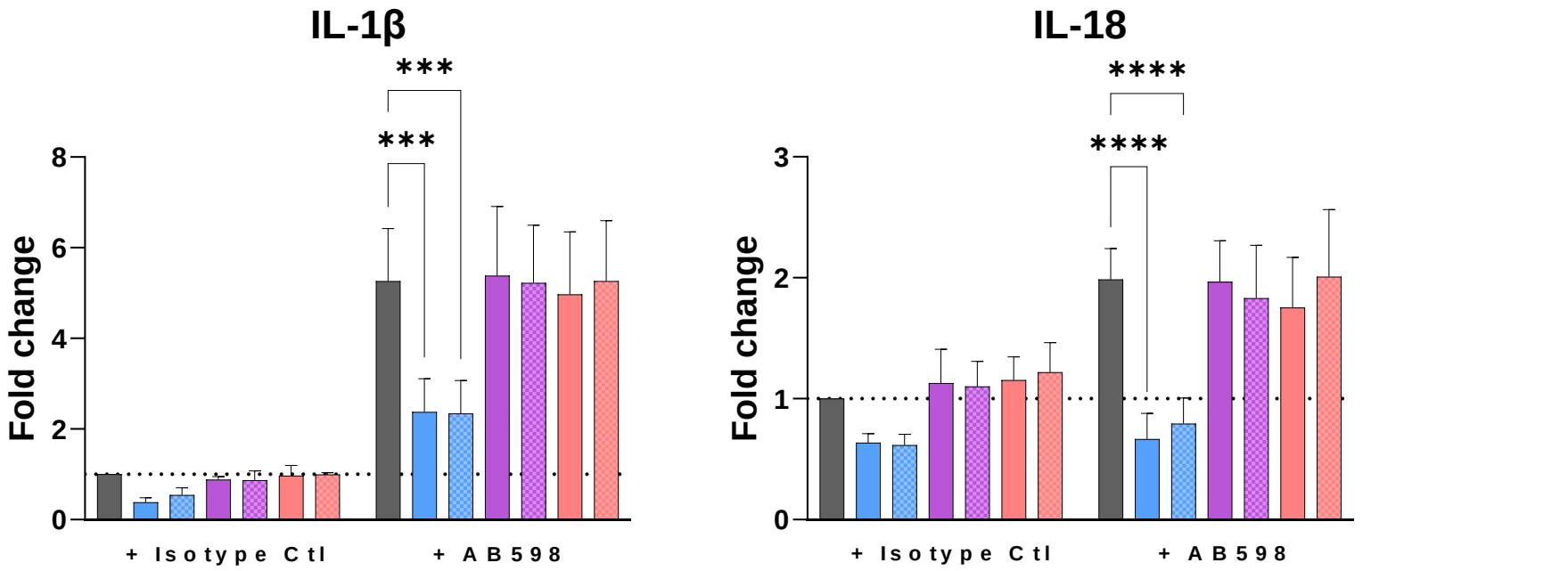



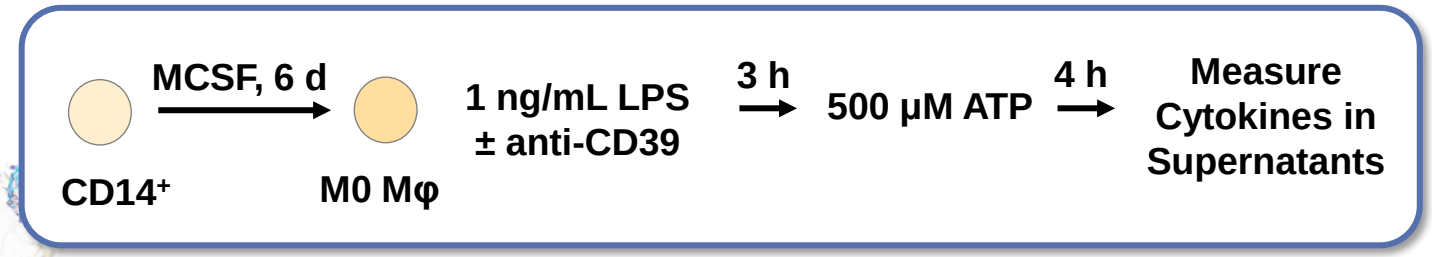





Statistical significance was calculated with a two-way ANOVA using Šídák's multiple comparisons test, *P ≤ 0.05, ** P ≤ 0.01, *** P ≤ 0.001. Fold change calculated per donor to the 0 μM ATP, IgG4-treated condition. Schematic created with BioRender.com.

# AB598 Promotes ATP-Dependent Inflammasome Activation Through P2X7



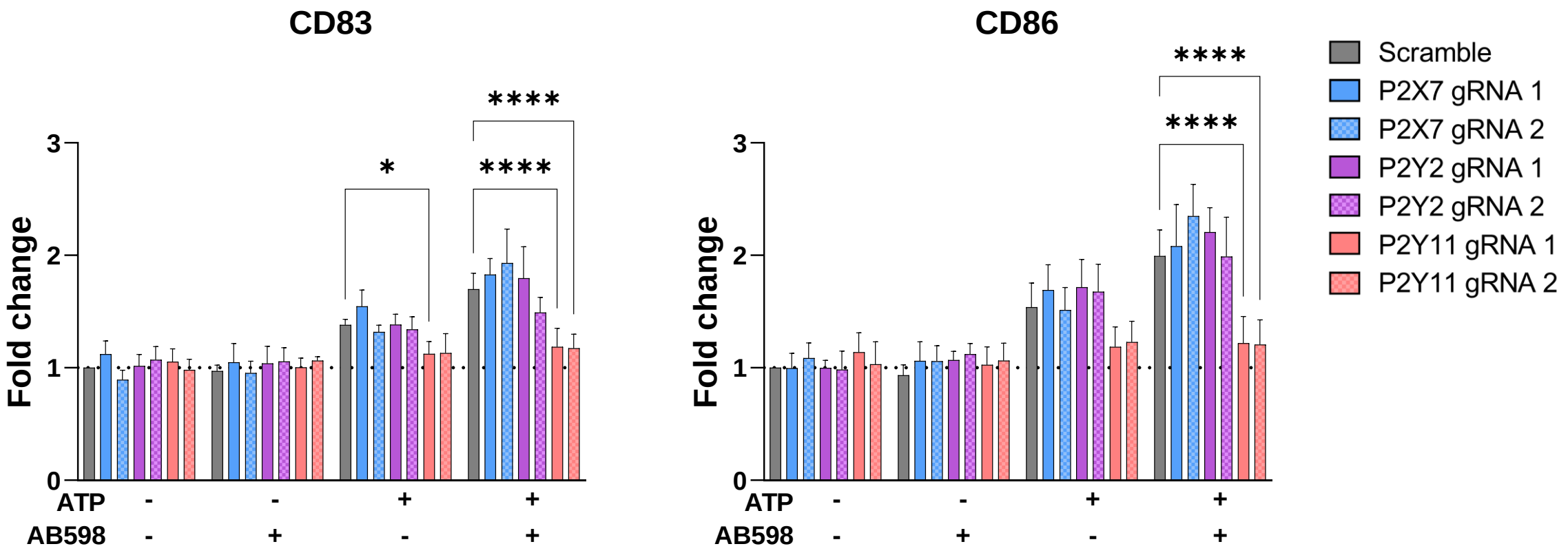





- High ATP signals through P2X and P2Y receptors on the cell surface
- The P2X7 receptor is responsible for the ATP-dependent release of IL-18 and IL-1β

Statistical significance was calculated with a two-way ANOVA using Dunnett's multiple comparisons test, *** P ≤ 0.001, **** P ≤ 0.0001. Error bars represent the SD. Fold change was calculated per donor to the IgG1 FcS-treated condition.

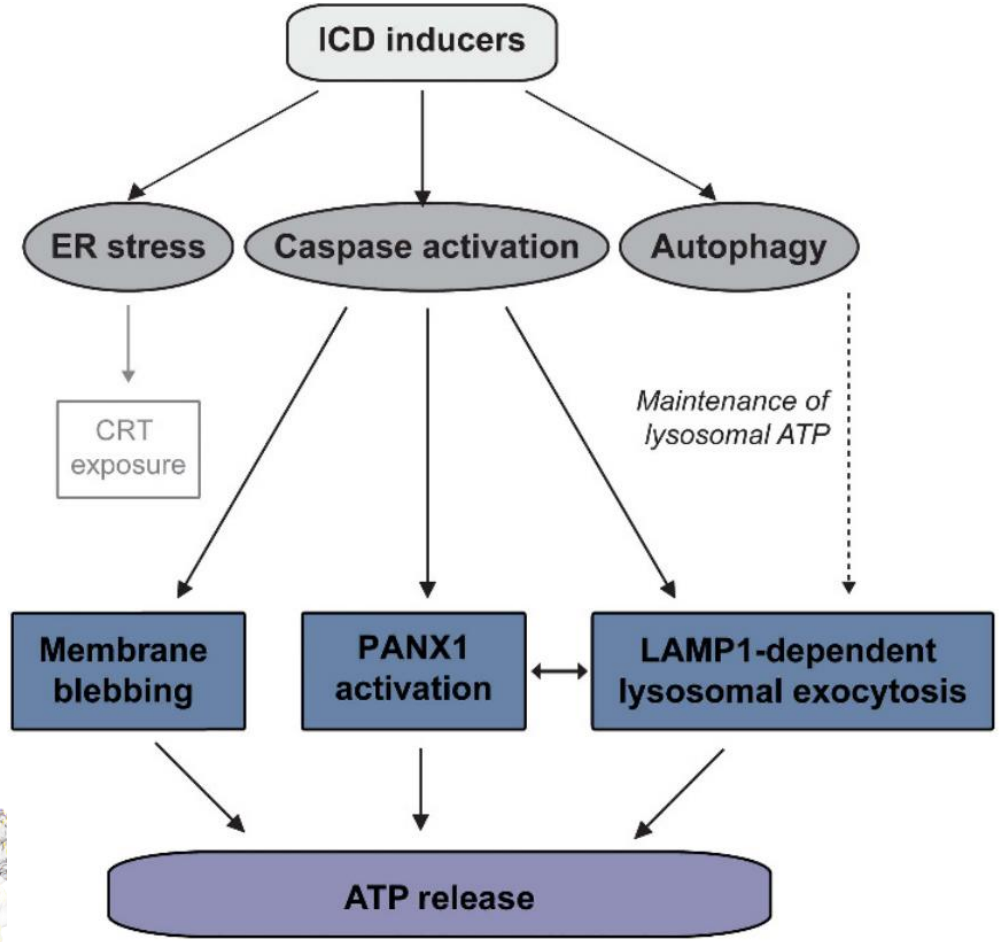
# AB598 Promotes ATP-Dependent Monocyte-Derived Dendritic Cell Maturation Through P2Y11



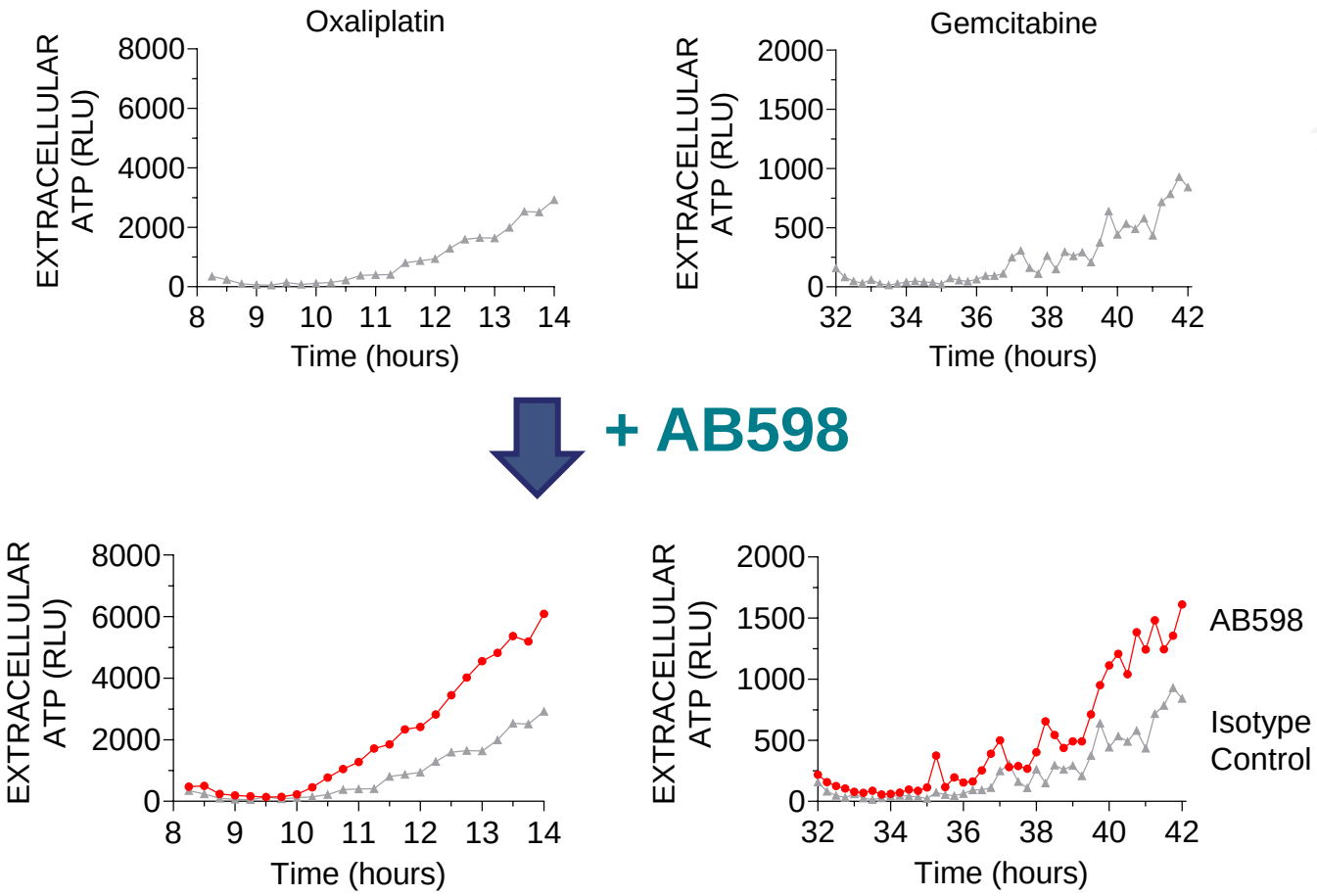

- High ATP signals through P2X and P2Y purinergic receptors on the cell surface
- The P2Y11 receptor is responsible for the ATP-dependent elevation of CD83 and CD86 seen on monocyte-derived dendritic cells

Statistical significance was calculated with a two-way ANOVA using Dunnett's multiple comparisons test, *P ≤ 0.05, **** P ≤ 0.0001. Error bars represent the SD. Fold change was calculated per donor to the 0 µM ATP, IgG1 FcS-treated condition.

# Chemotherapy Can Induce ATP Release, an Effect Amplified by AB598




## SK-MEL-5, CD39+ Tumor Cells

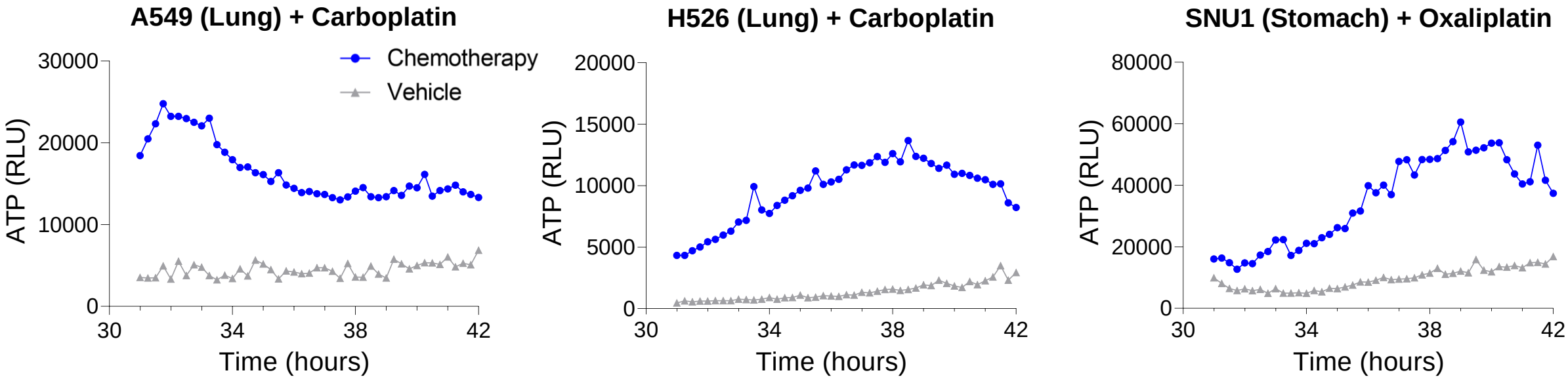





Schematic: Martins, I. (2014) Cell Death and Differ.

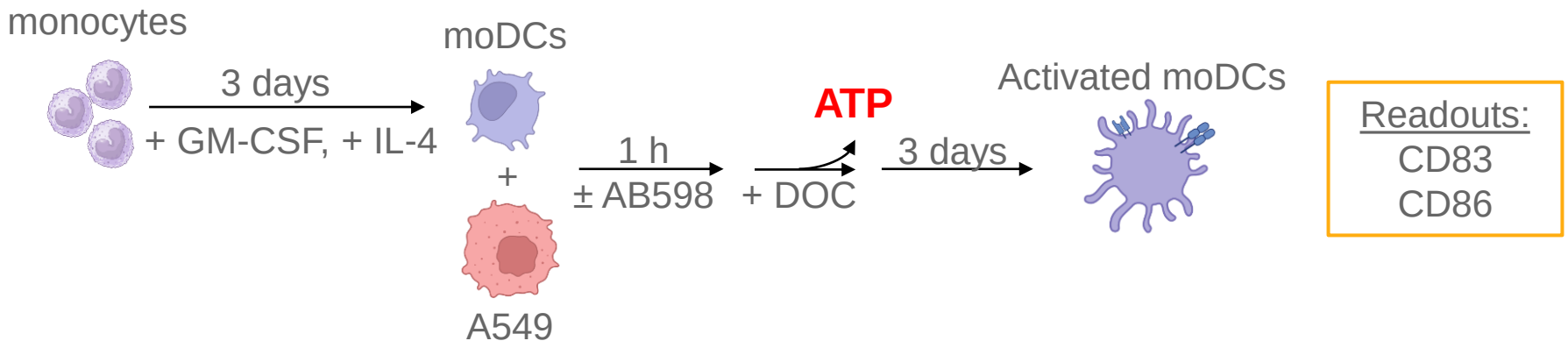
# Clinically-Relevant Chemotherapies Induce ATP Release in Lung and Stomach Cancer Cell Lines

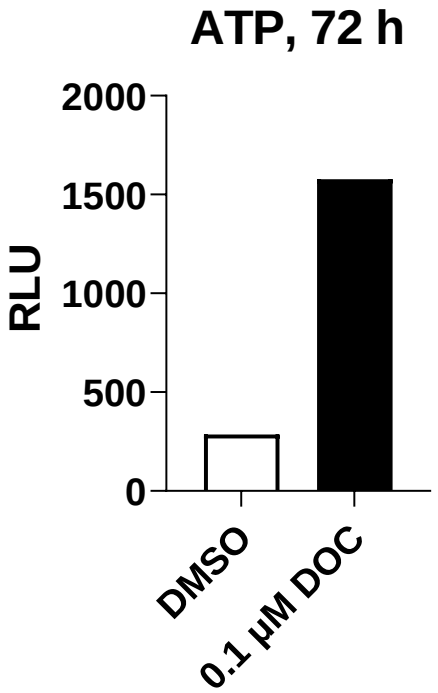
*Human Cancer Cell Lines*

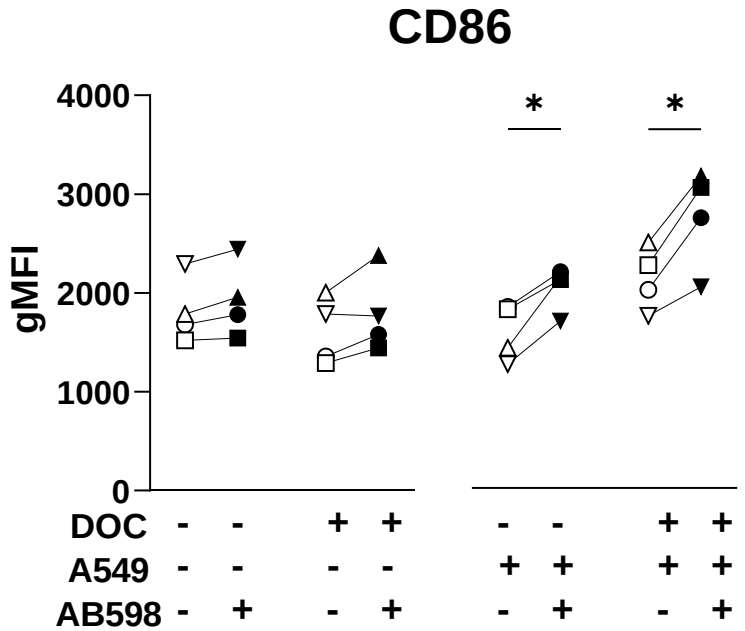


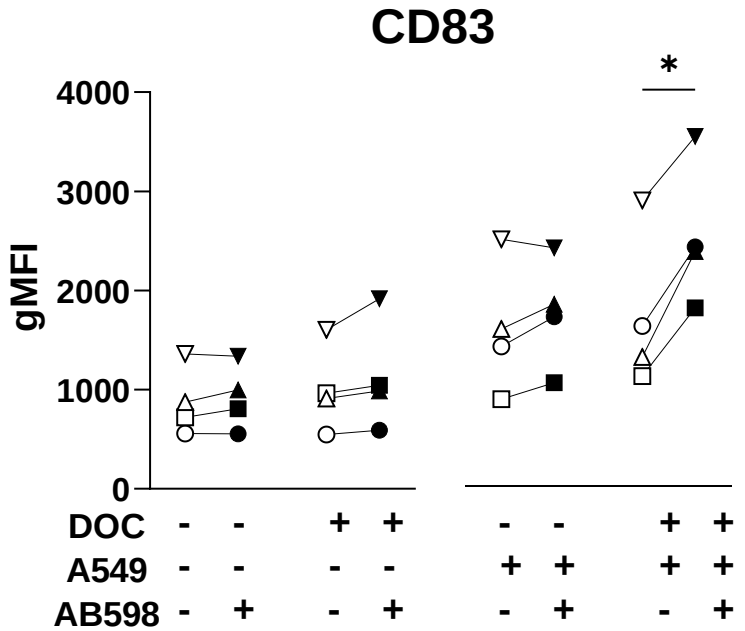

- Carboplatin is used in first line treatment of metastatic NSCLC
- FOLFOX (contains oxaliplatin) is used in first line treatment of gastric, GEJ, and colorectal cancers
- Chemotherapies tested at $IC_{90}$ concentrations for each cell line

# AB598 Boosts the Effect of Chemotherapy to Promote MoDC Activation



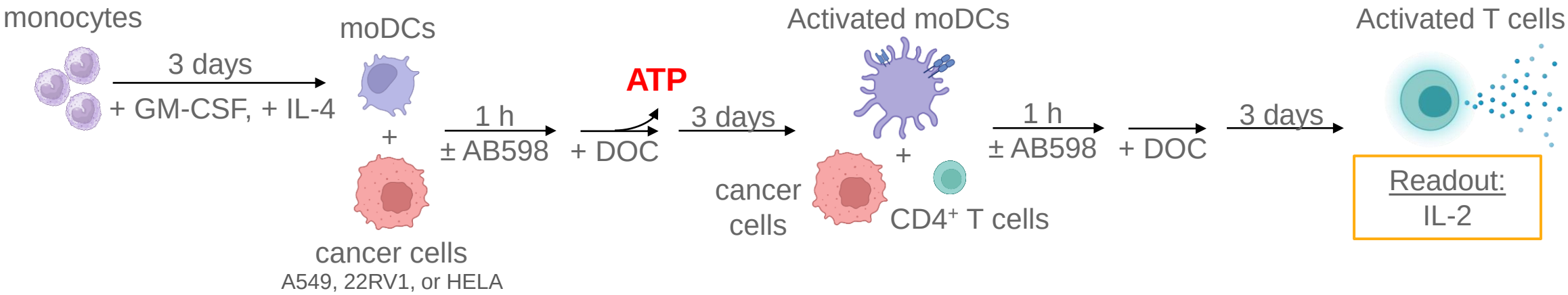



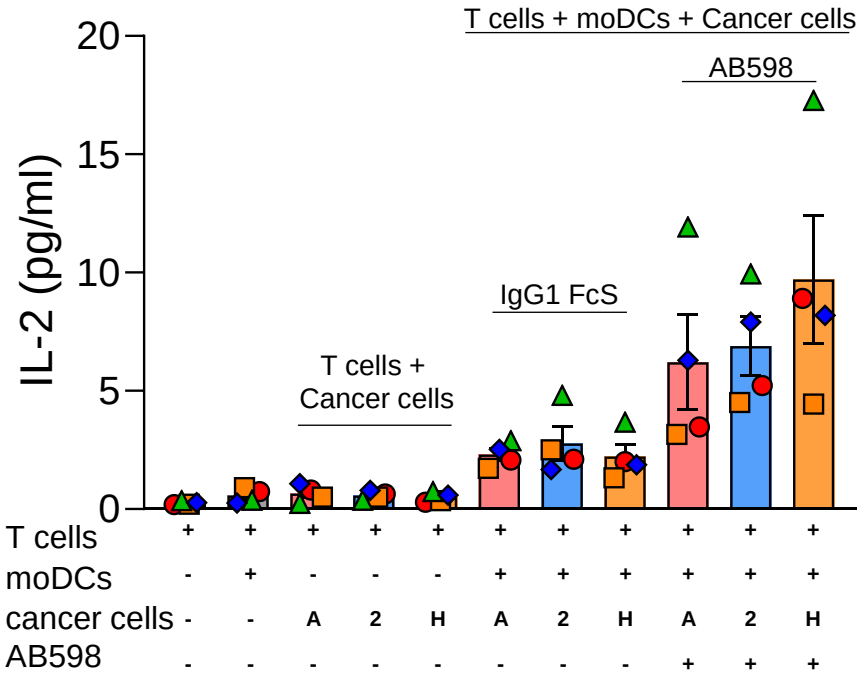



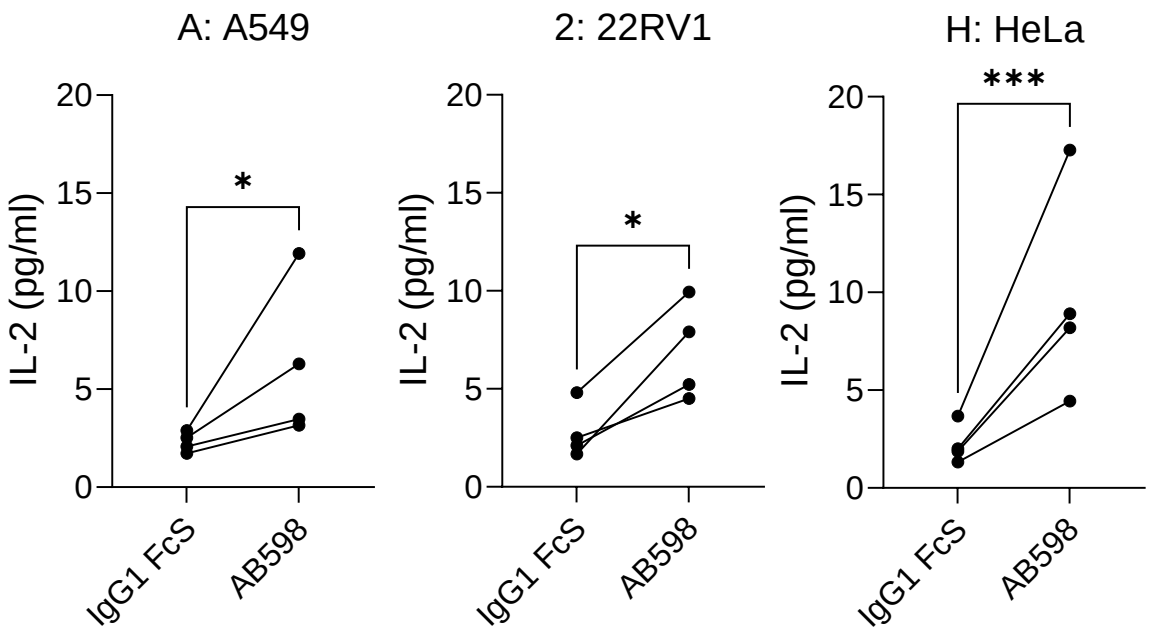




Statistical significance was calculated with a two-way ANOVA using Tukey's multiple comparisons test and the Geisser-Greenhouse correction, *P ≤ 0.05. Cell type images created with Biorender.com. DOC = docetaxel.

# AB598 Boosts the Effect of Chemotherapy to Promote Myeloid-Driven T Cell Activation

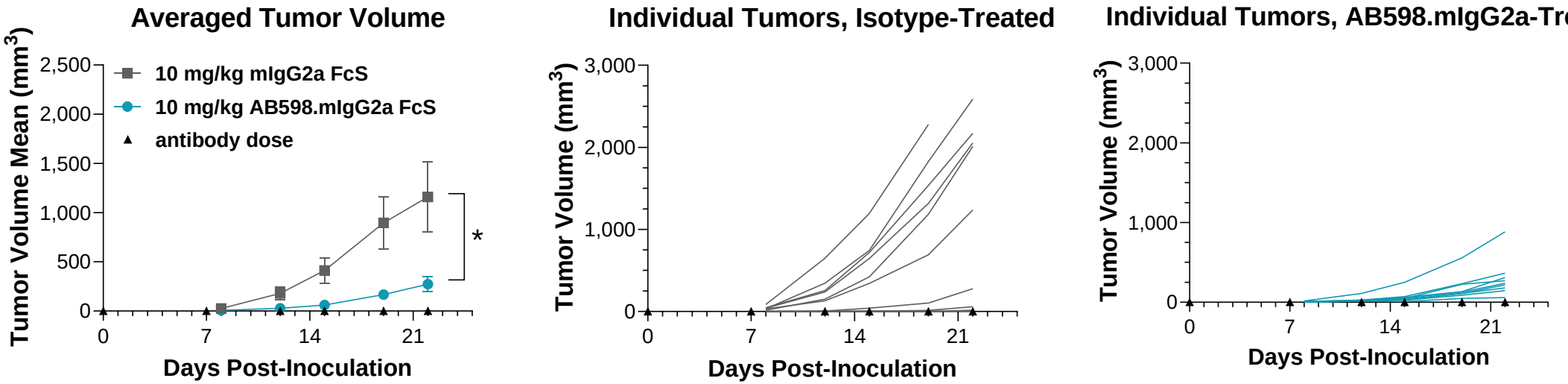


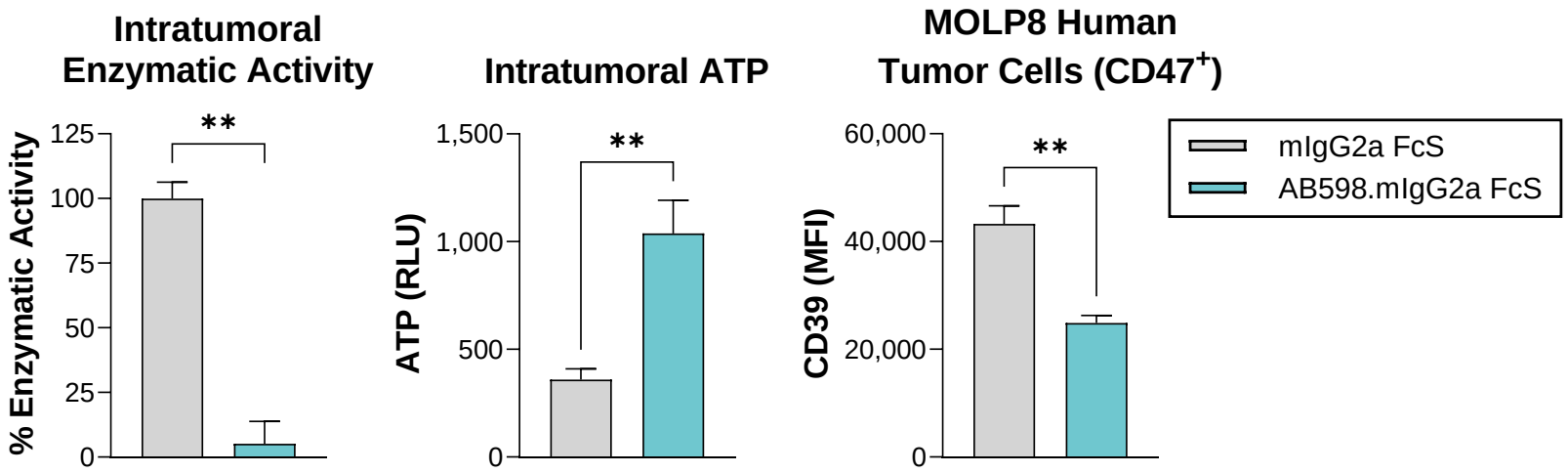




Four healthy donors. T cells and monocytes matched from the same donor.
Statistical significance was calculated using a ratio-paired T test. *P ≤ 0.05, *** P ≤ 0.001.

# AB598 Inhibits CD39 to Increase Intratumoral ATP in a MOLP8 Xenograft Model




- MOLP8, a human multiple myeloma cell line that naturally expresses CD39, inoculated into immunocompromised SCID mice

Statistical significance was calculated with (top panel) a two-way ANOVA with Šidák's multiple comparison test, N = 10 / group or (bottom panel) an unpaired T-test, N = 2 - 4 / group. Error bars represent the SEM. ns = non-significant, *P ≤ 0.05, ** P ≤ 0.01, *** P ≤ 0.001. Samples collected after 48 h for enzymatic activity and intratumoral ATP and 72 h for flow cytometry.

# In a C57BL/6 Human CD39 Knock-In Mouse Model, Tumor Infiltrating Immune Cells are CD39+

## *Myeloid Cells*

CD11b+

**Monocytes**
F4-80- Ly6c+

**M1-like Macrophages**
F4-80+ CD206-

**M2-like Macrophages**
F4-80+ CD206+

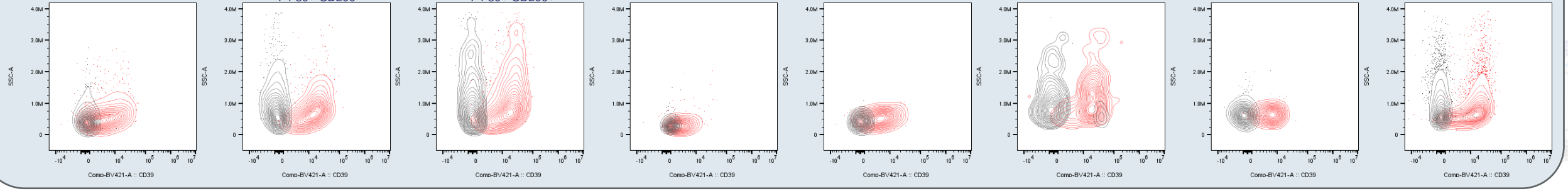
**NK Cells**
F4-80- Nk1.1+

**DC**
F4-80- CD11c+

**Neutrophils**
F4-80+ Ly6G+

**pMDSC**
F4-80- Ly6G+

**mMDSC**
F4-80+ LY6C+




## *Lymphoid Cells*

CD11b-

**CD4 T Cells**
CD3+ CD4+

**CD4 CM**
CD3+ CD4+ CD62L+ CD44+

**CD4 Effector**
CD3+ CD4+ CD62L- CD44+

**CD4 Naive**
CD3+ CD4+ CD62L+ CD44-

**Tregs**
CD3+ CD4+ FOXP3+

**B Cells**
CD3- CD19+

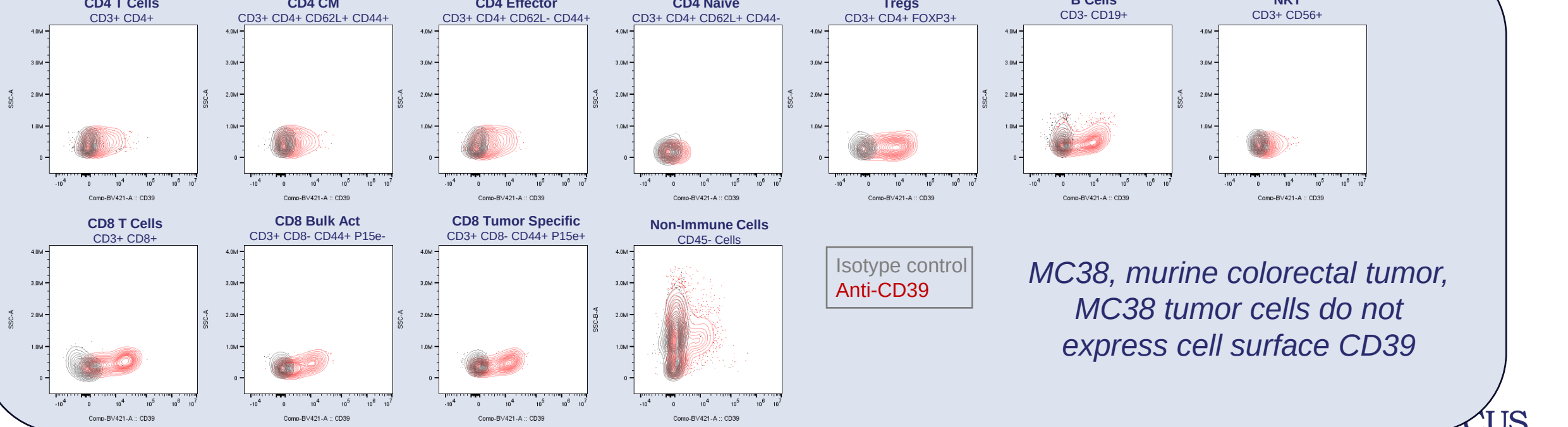
**NKT**
CD3+ CD56+

**CD8 T Cells**
CD3+ CD8+

**CD8 Bulk Act**
CD3+ CD8- CD44+ P15e-

**CD8 Tumor Specific**
CD3+ CD8- CD44+ P15e+

**Non-Immune Cells**
CD45- Cells

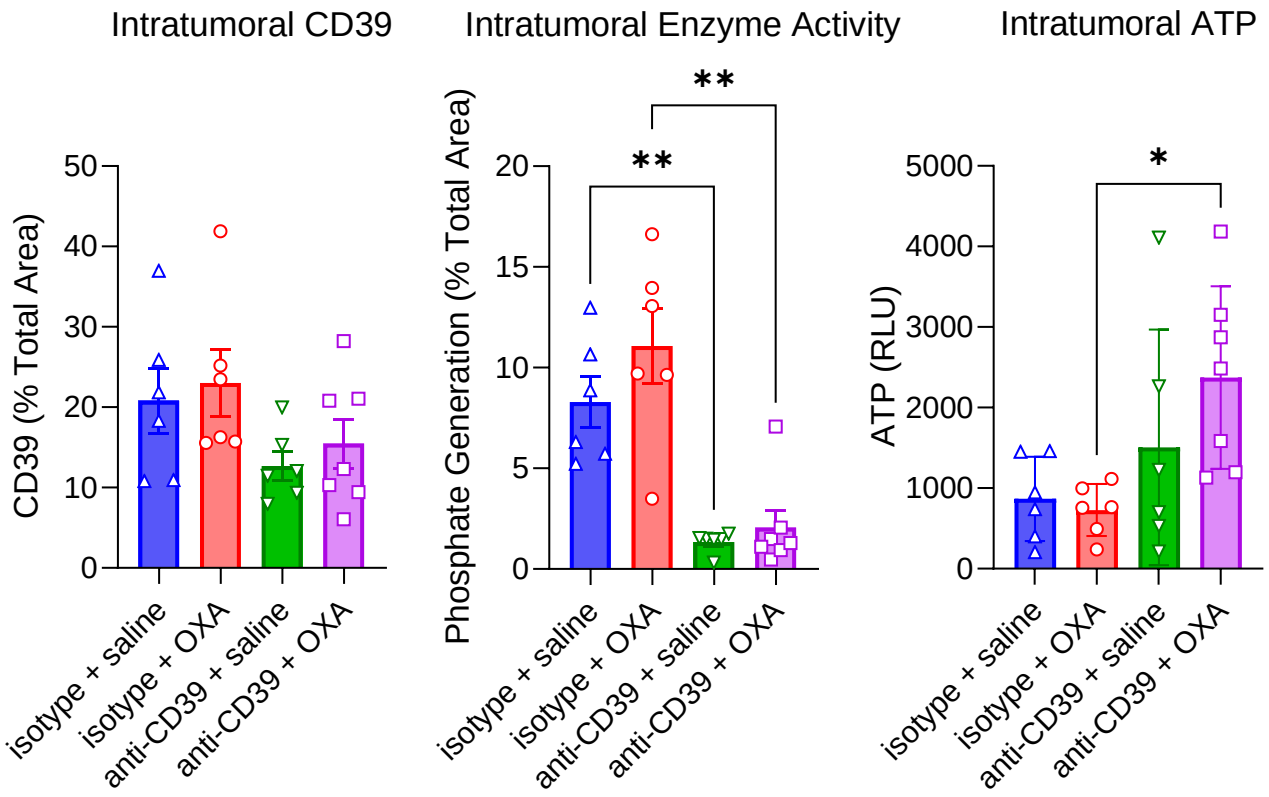



Isotype control
Anti-CD39

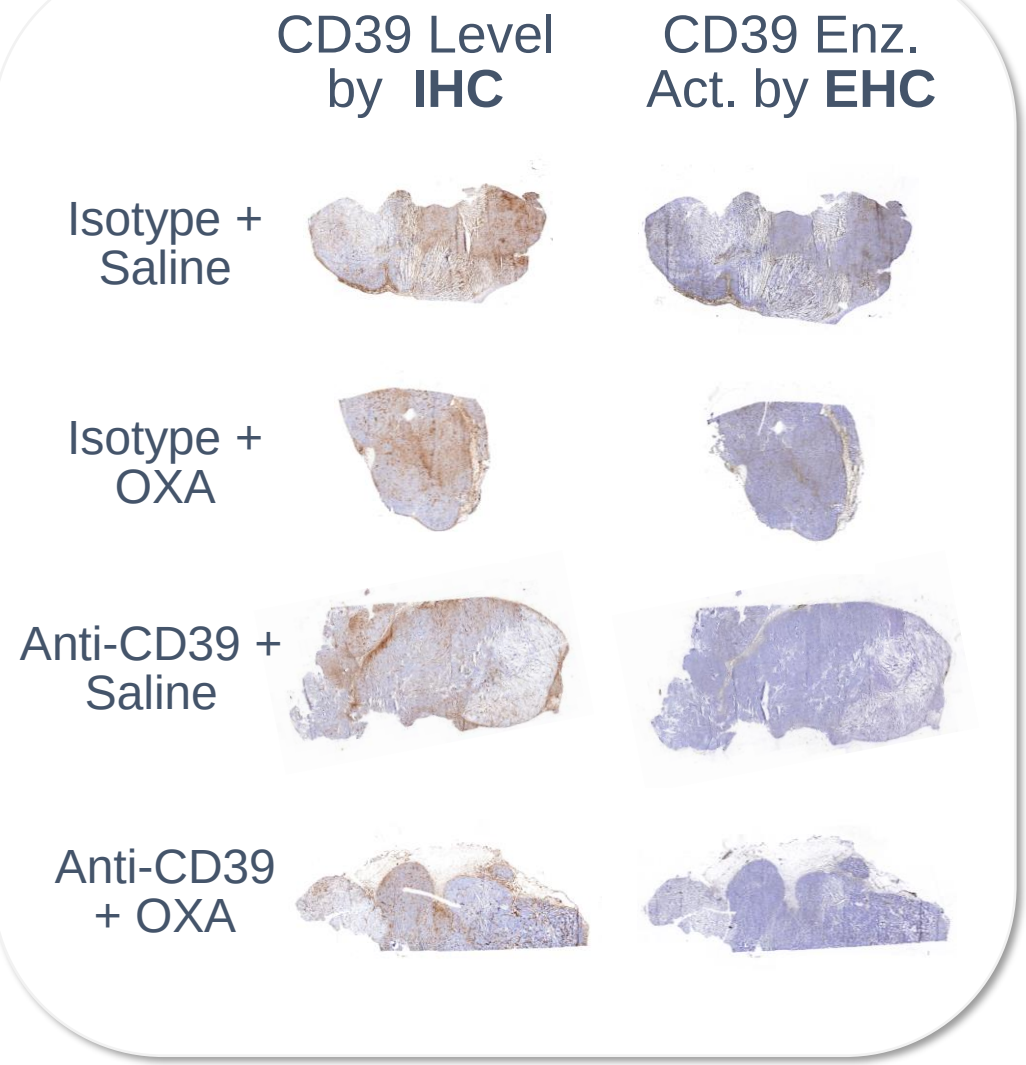
*MC38, murine colorectal tumor, MC38 tumor cells do not express cell surface CD39*

C57BL/6 human CD39 knock-in (hCD39KI) mice provided by Biocytogen.

# AB598.mIgG2a FcS in Combination with OXA Inhibits CD39 Enzymatic Activity Leading to Increases in Intratumoral ATP




- Intratumoral enzymatic inhibition measured using an enzyme histochemistry (EHC) lead phosphate deposition assay

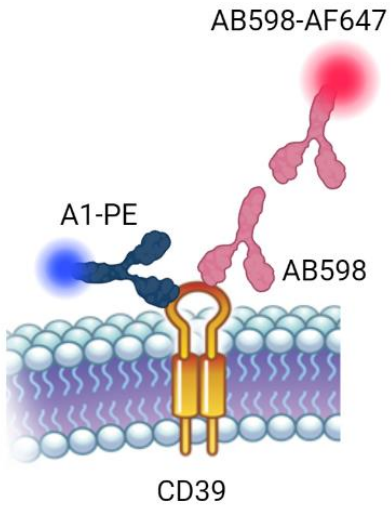


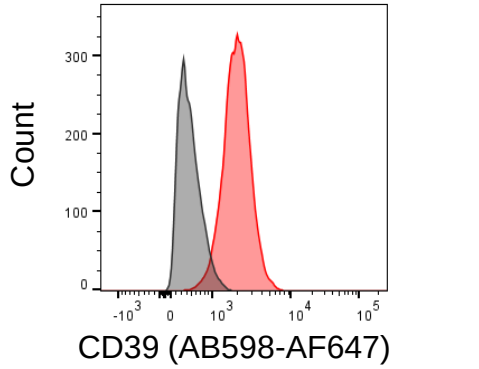

Statistical significance was calculated with a one-way ANOVA with Dunnett's T3 multiple comparisons test, and Brown-Forsythe and Welch ANOVA tests. Error bars represent the SEM. *P ≤ 0.05, ** P ≤ 0.01, *** P ≤ 0.001.

C57BL/6 human CD39 knock-in (hCD39KI) mice provided by Genoway. Mice have MC38 tumors. All images taken at 1.5x magnification.

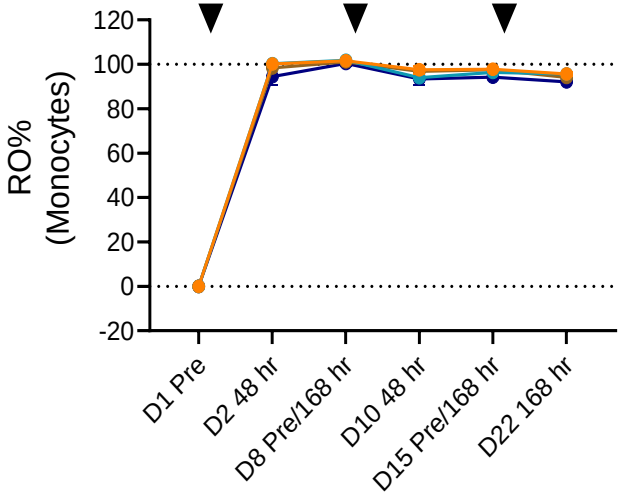
# Full Receptor Occupancy in Peripheral Monocytes of Cynomolgus Monkeys Treated with AB598

100% Receptor Occupancy in Peripheral Cyno Monocytes

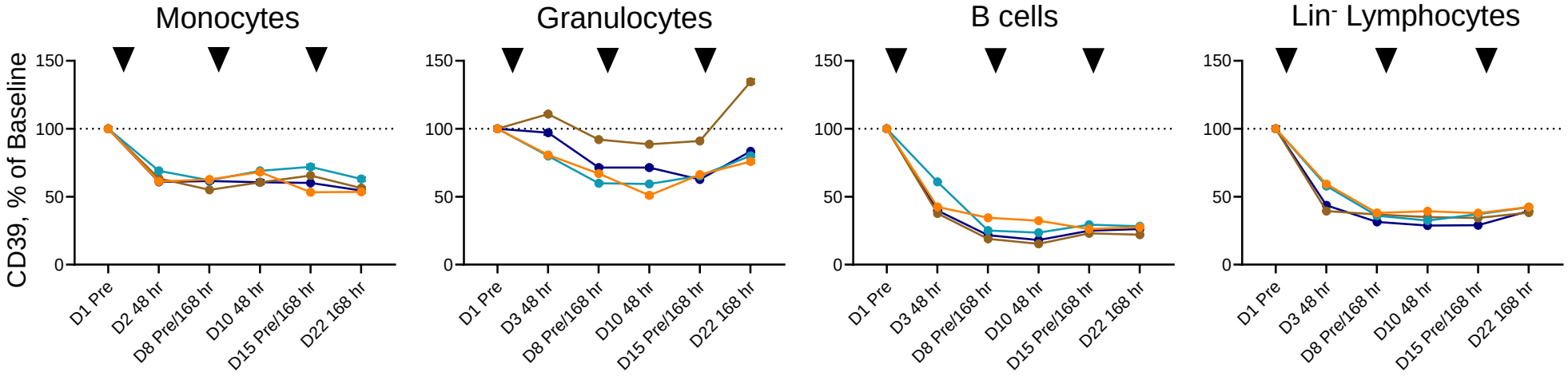



Day 1 (Predose)
Day 8 (168 hr Post-dose)

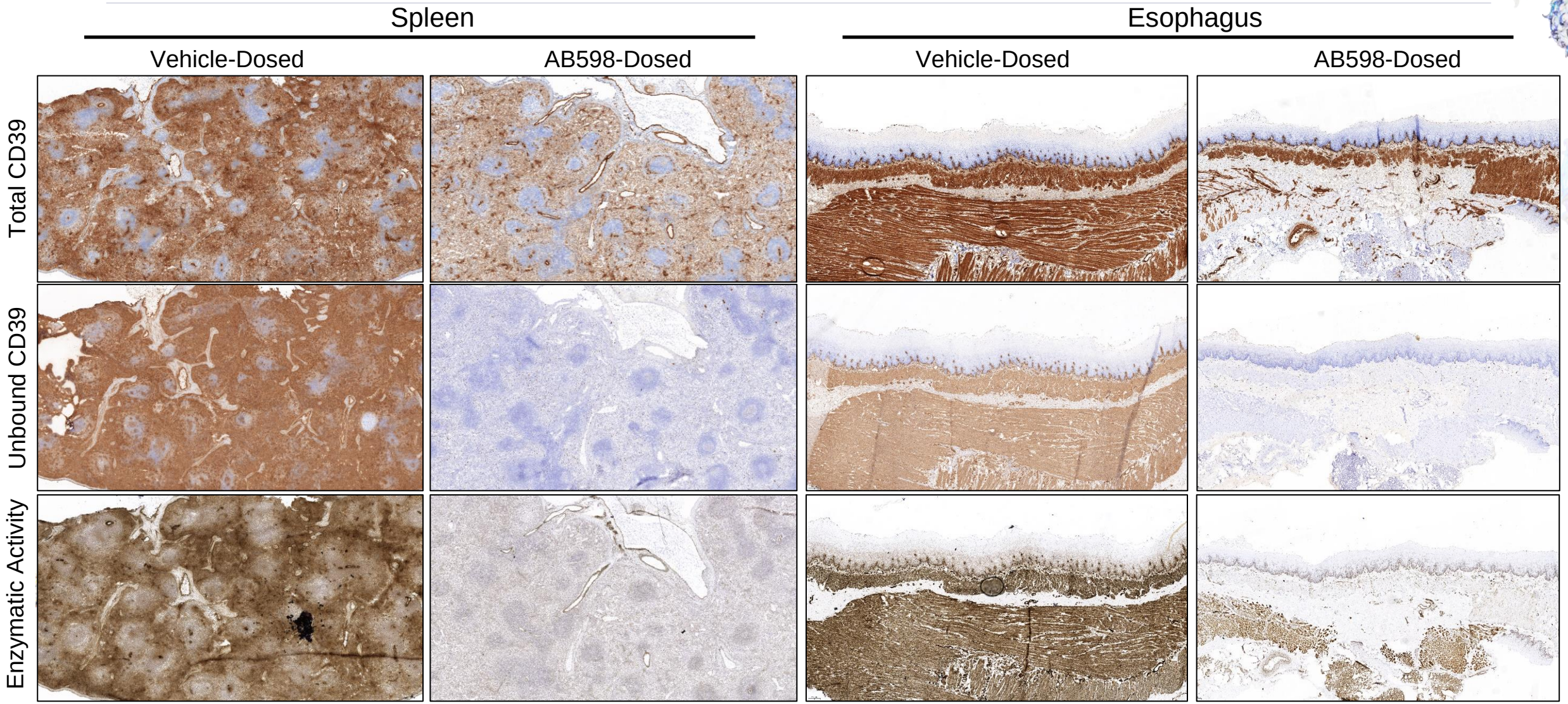



Decrease in Cell Surface CD39 with AB598 Treatment




▼ AB598 Dosed (Weekly)

# Robust Receptor Occupancy and Enzymatic Inhibition of CD39 in Tissues of AB598-Dosed Cynomolgus Monkeys



# Robust Receptor Occupancy and Enzymatic Inhibition of CD39 in Tissues of AB598-Dosed Cynomolgus Monkeys

Spleen

Esophagus

Vehicle-Dosed

AB598-Dosed

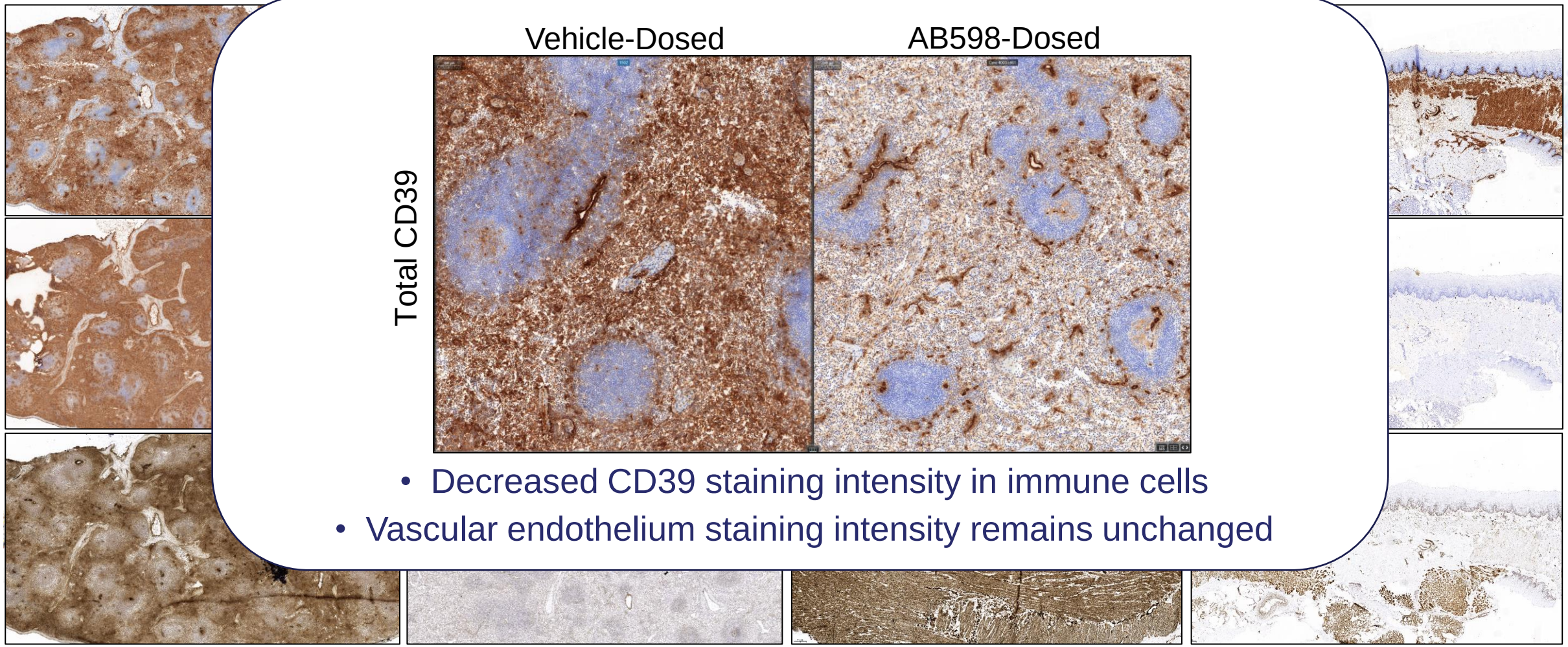
Vehicle-Dosed

AB598-Dosed

Total CD39

Unbound CD39

Enzymatic Activity



Vehicle-Dosed

AB598-Dosed

Total CD39



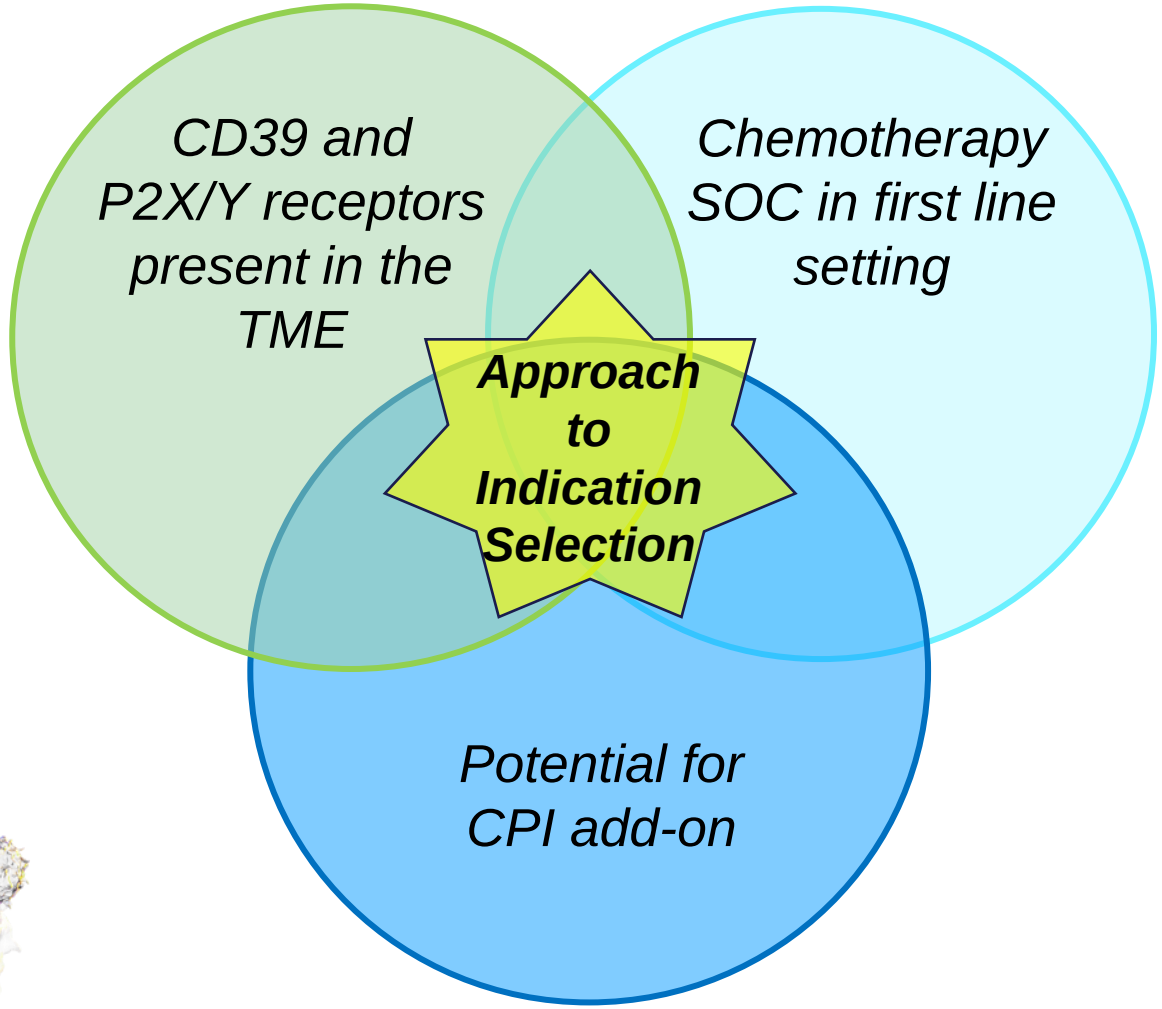
- Decreased CD39 staining intensity in immune cells
- Vascular endothelium staining intensity remains unchanged



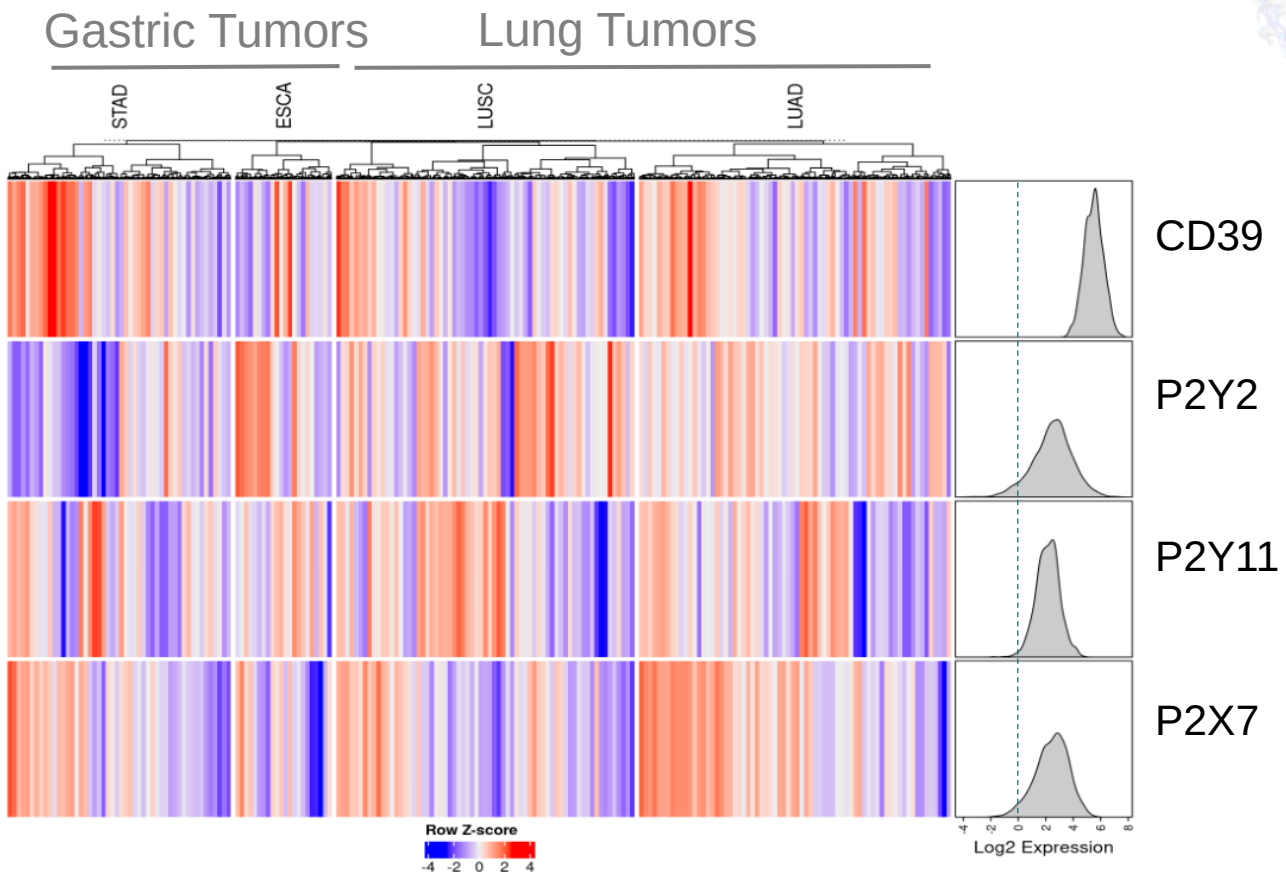


ARCUS BIOSCIENCES

# Scientific Rationale Supports Clinical Utility of AB598 in Several Solid Tumor Settings

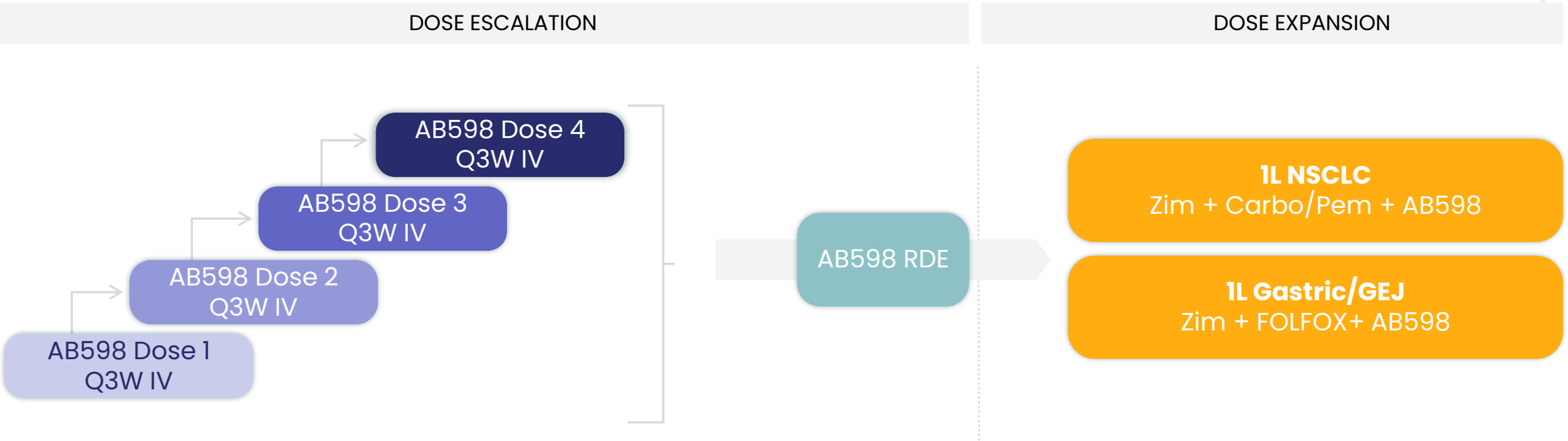



- Dose Escalation: Late-line all comers
- Dose Expansion: Lung and Gastric




mRNA expression, TCGA, normalized per gene

# ARC-25 Phase 1a/1b Study Design (NCT05891171)




- Primary Outcomes
  - Number of participants with Adverse Events (AEs) and Serious Adverse Events (SAEs)
  - Dose Escalation Cohorts: Number of participants with Dose-Limiting Toxicities (DLTs)
- Secondary Outcomes
  - Evaluation of AB598 PK in humans
  - Antidrug Antibodies (ADAs) to AB598
  - Objective Response Rate (ORR)
  - Dose Expansion Cohorts: Duration of Response (DOR)



RDE: recommended dose for expansion

# Summary

- AB598 is a highly potent and specific anti-CD39 antibody which acts by fully inhibiting CD39 enzymatic activity and increasing immunostimulatory ATP levels
- High ATP, which can be achieved intratumorally with chemotherapy in combination with AB598, can activate myeloid cells for a pro-inflammatory anti-tumor response
- Ph1 study in progress in advanced cancer patients