ARISTOTLE UNIVERSITY OF THESSALONIKI

RNA editing enzyme APOBEC3A promotes pro-inflammatory M1 macrophage polarization

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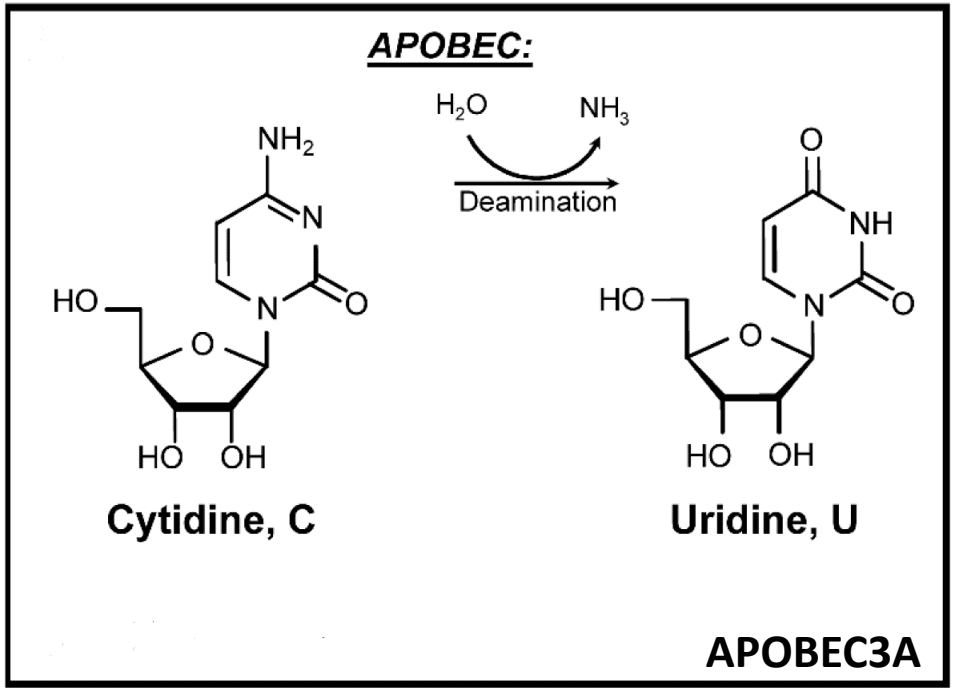
MAIN MODULES

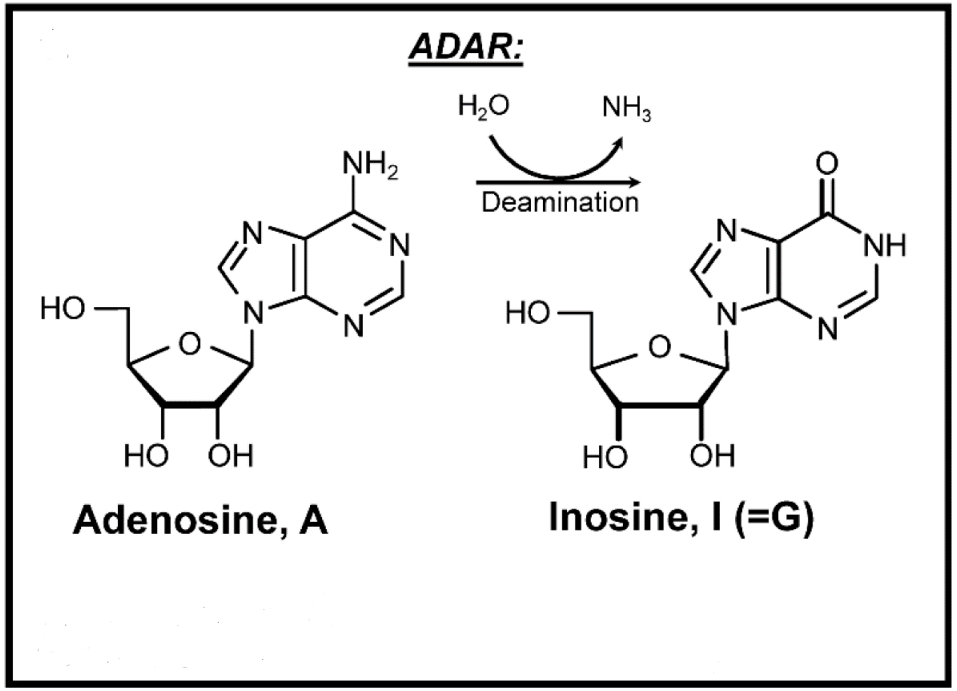
1. INTRODUCTION
2. RESULTS
3. LAB RESEARCH

Introduction

RNA Editing

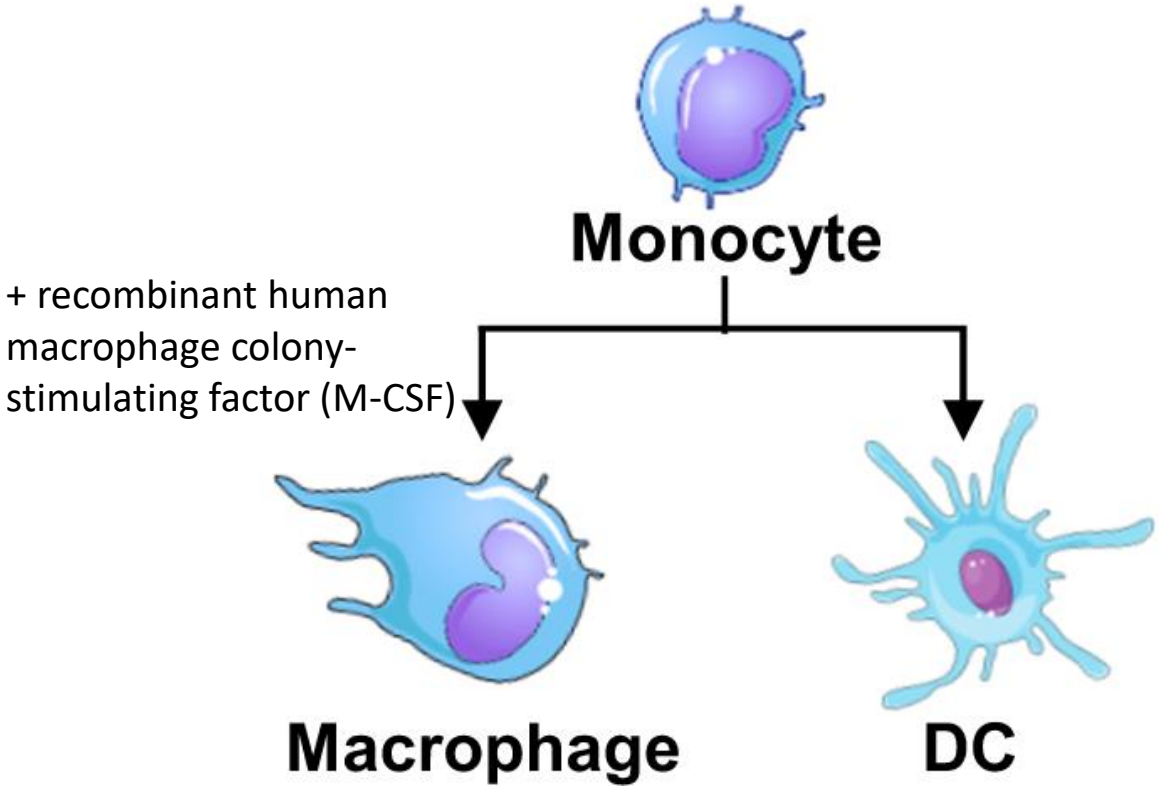
- Post-transcriptional mechanism
- Protein diversity
- Two main protein families





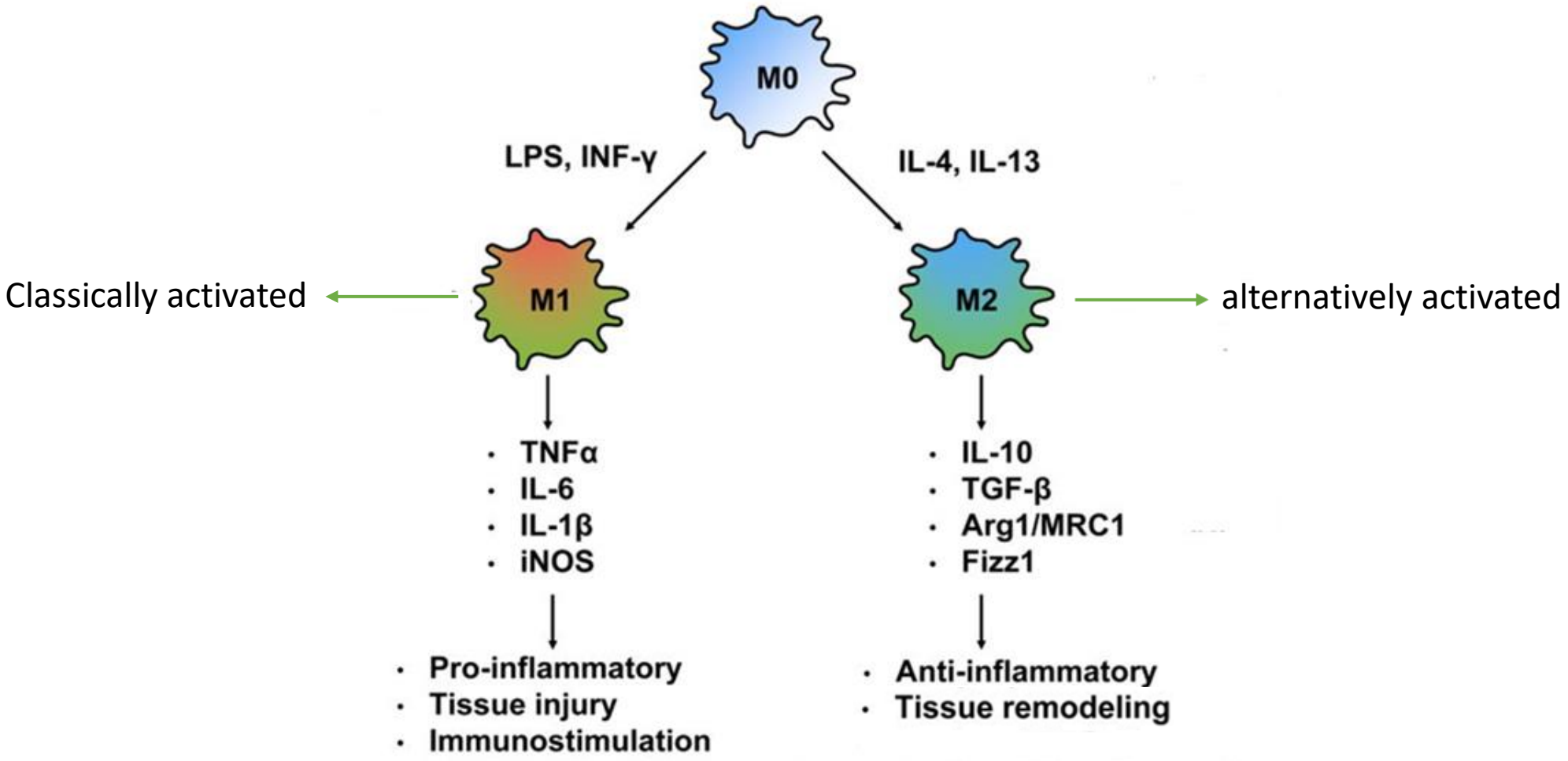
Introduction

Origin of the macrophage



Introduction

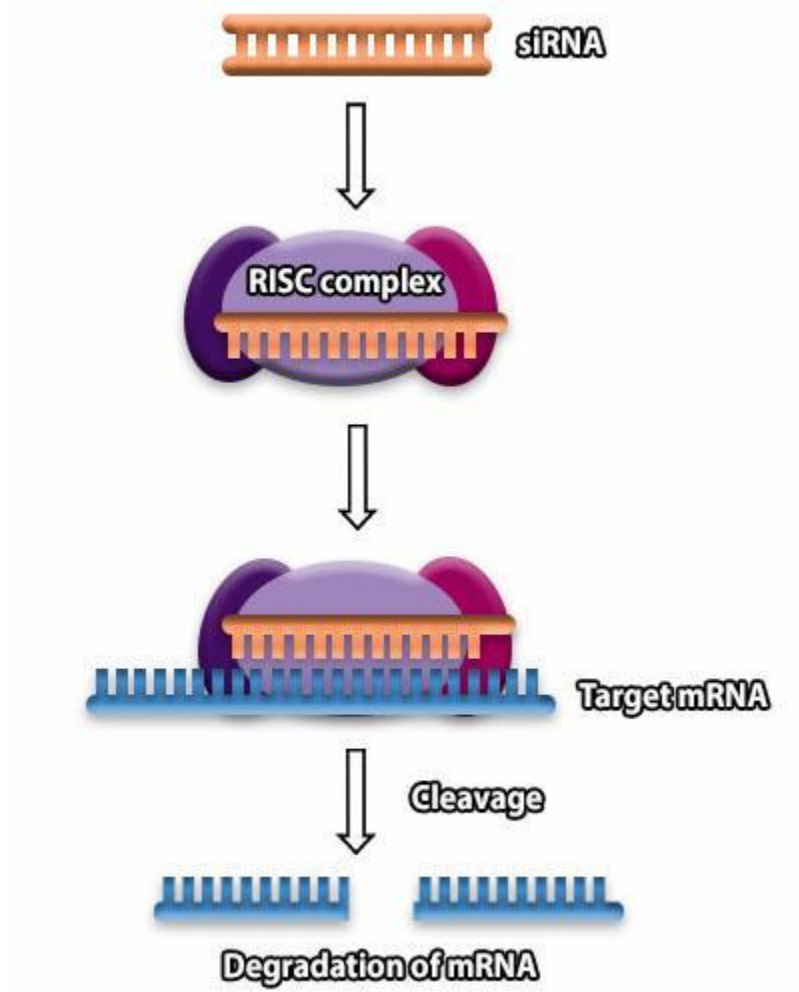
Polarization of the Macrophage



Introduction

siRNA Knockdown (KD)

- short interfering RNA (siRNA)
- RNA-silencing complex (RISC)



Introduction

Scrambled cells Knockdown cells

- Both transfected

- siRNA that don't recognize any sequence
- siRNA that recognizes specific sequence

invitrogen

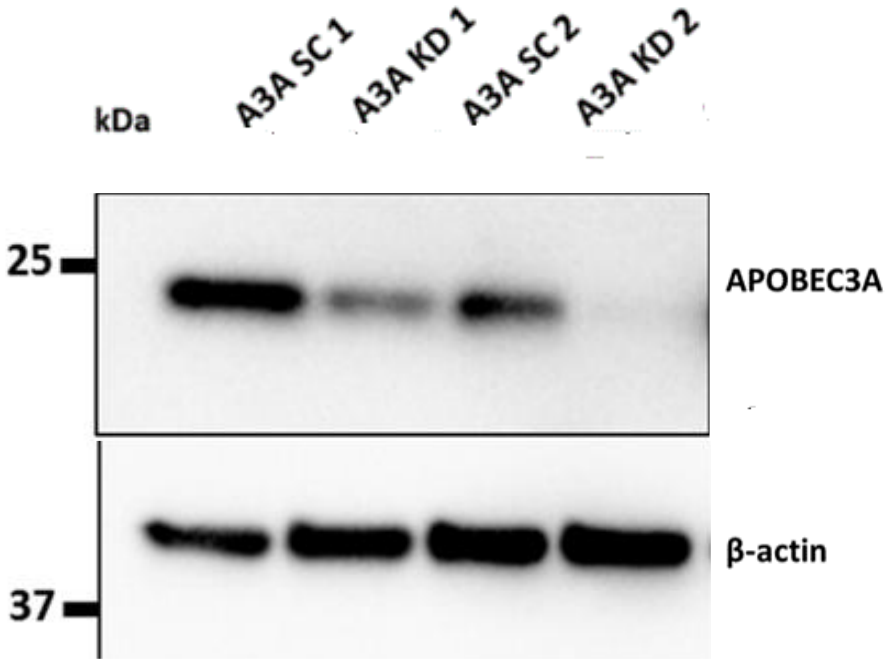
Silencer™ Negative Control No. 1 siRNA Invitrogen™

Ambion® Silencer® Negative Control #1 siRNA has no significant sequence similarity to mouse, rat, or human gene sequences. The control has also been tested in cell-based screens and proven to have no significant effect on cell proliferation, viability, or morphology.

Results

- Reduced expression of APOBEC3A protein by siRNA KD

Western blot analysis of M1 SC control and M1 APOBEC3A KD samples



SC=scrambled cells

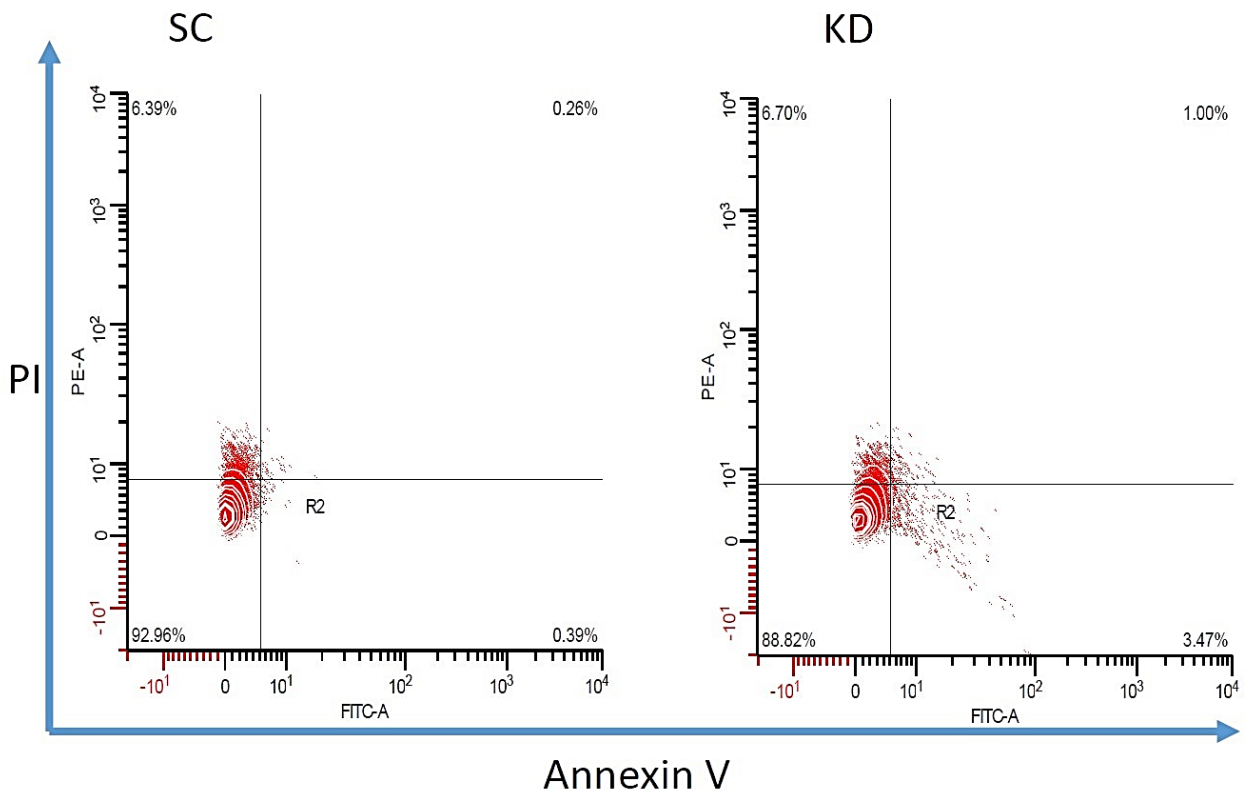
KD= Knockdown cells

A3A= APOBEC3A

Results

- M0 → M1 using LPS and IFN-γ
- Percent viable cells are similar in SC (93%) and KD (89%).
- APOBEC3A does not affect cell viability in M1 macrophages.

Assessment of the macrophages' viability using flow cytometry



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Propidium Iodide (PI) = DNA binding-dye

Annexin V = binding protein

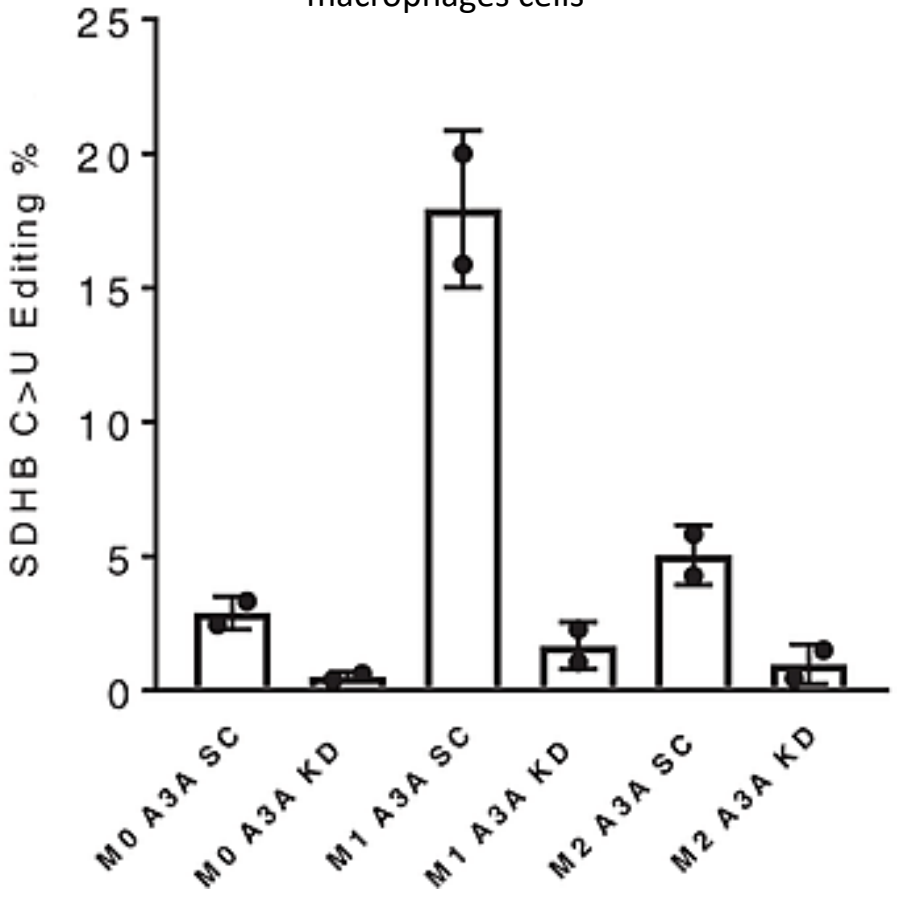
Results

SDHB c.C136U (R46X)

At the 136 nucleotide of the coding sequence, C → U

- SDHB c.C136U RNA editing occurs in M1 macrophages but not in M0 or M2 macrophages

The percentage of the R46X event in macrophages cells



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Annexin V = binding protein

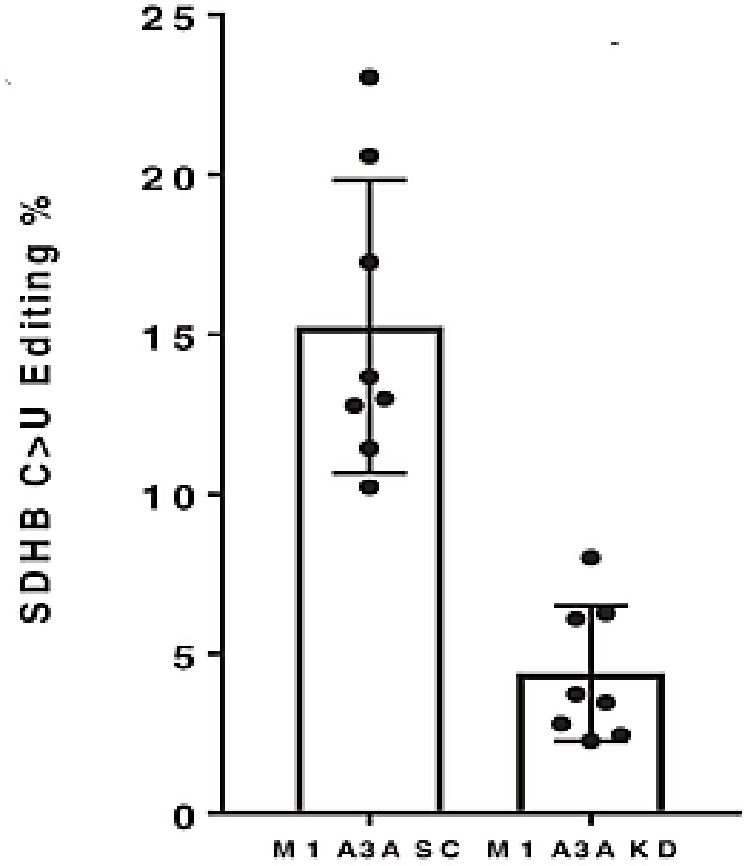
SDHB=succinate dehydrogenase subunit B gene

R46X= the editing event that occurs in SDHB c.C136U

Results

- In eight additional donor samples
- APOBEC3A responsible for the observed SDHB c.C136U RNA editing in M1 macrophages.

The percentage of the R46X event in M1 macrophages cells



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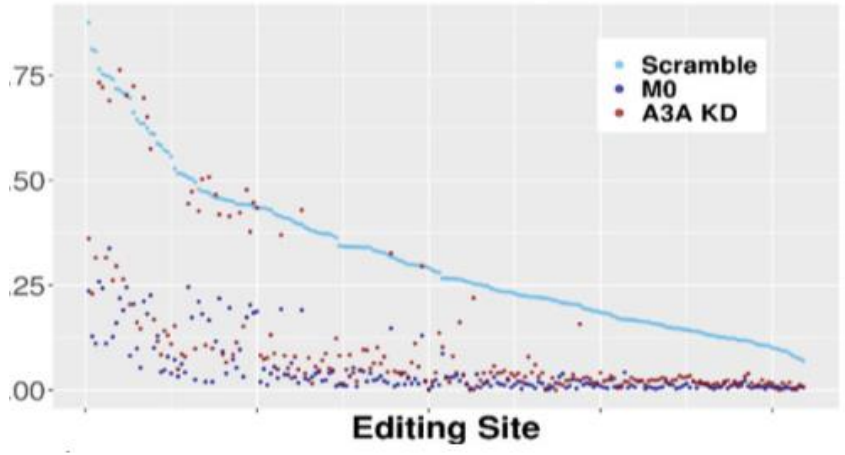
Results

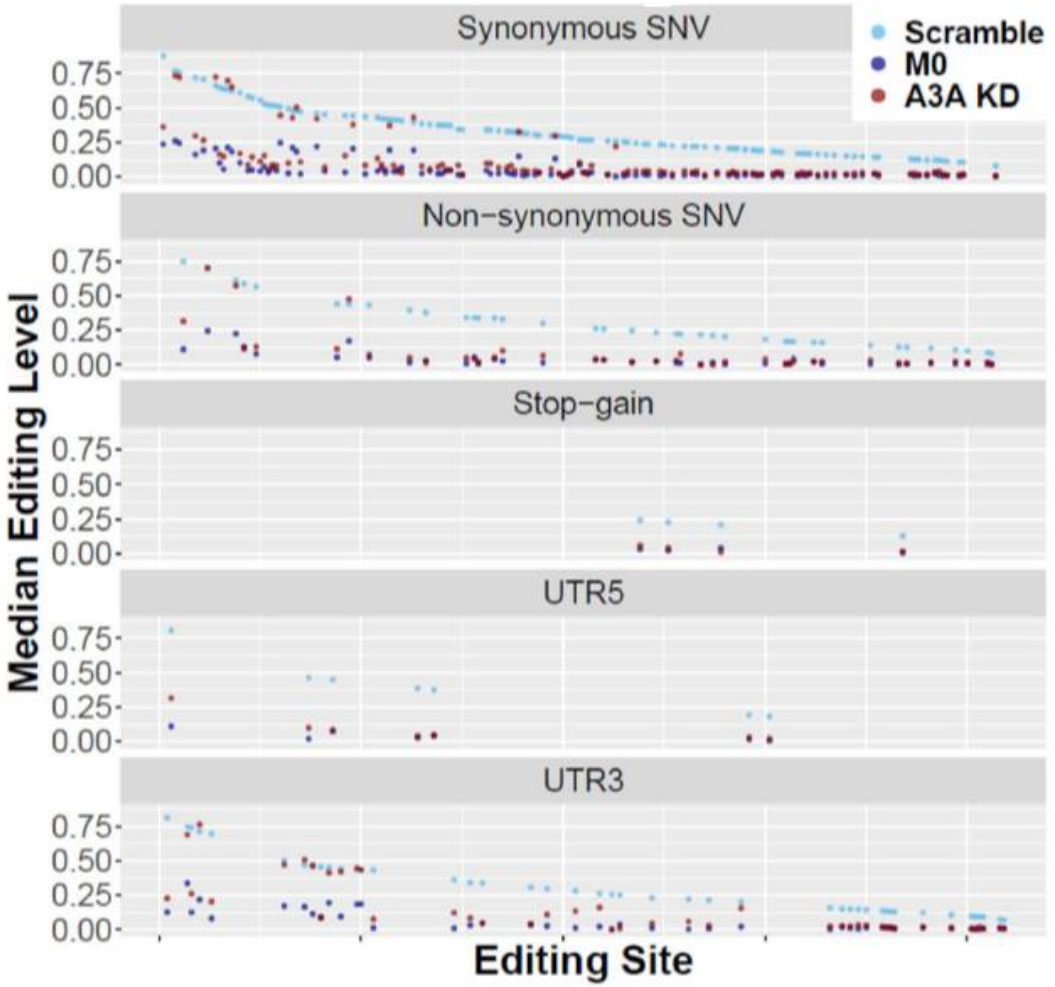
Editing level in 209 editing sites



- KD of APOBEC3A during M1 polarization reduced RNA editing levels in 180 of these 209 (~86%) sites

- APOBEC3A catalyzes the majority of C>U RNA editing sites during M1 polarization

- Synonymous (98/180 = 54.4%)
- Non-synonymous (36/180 = 20%)
- 3'-UTR (33/180 = 18.3%)



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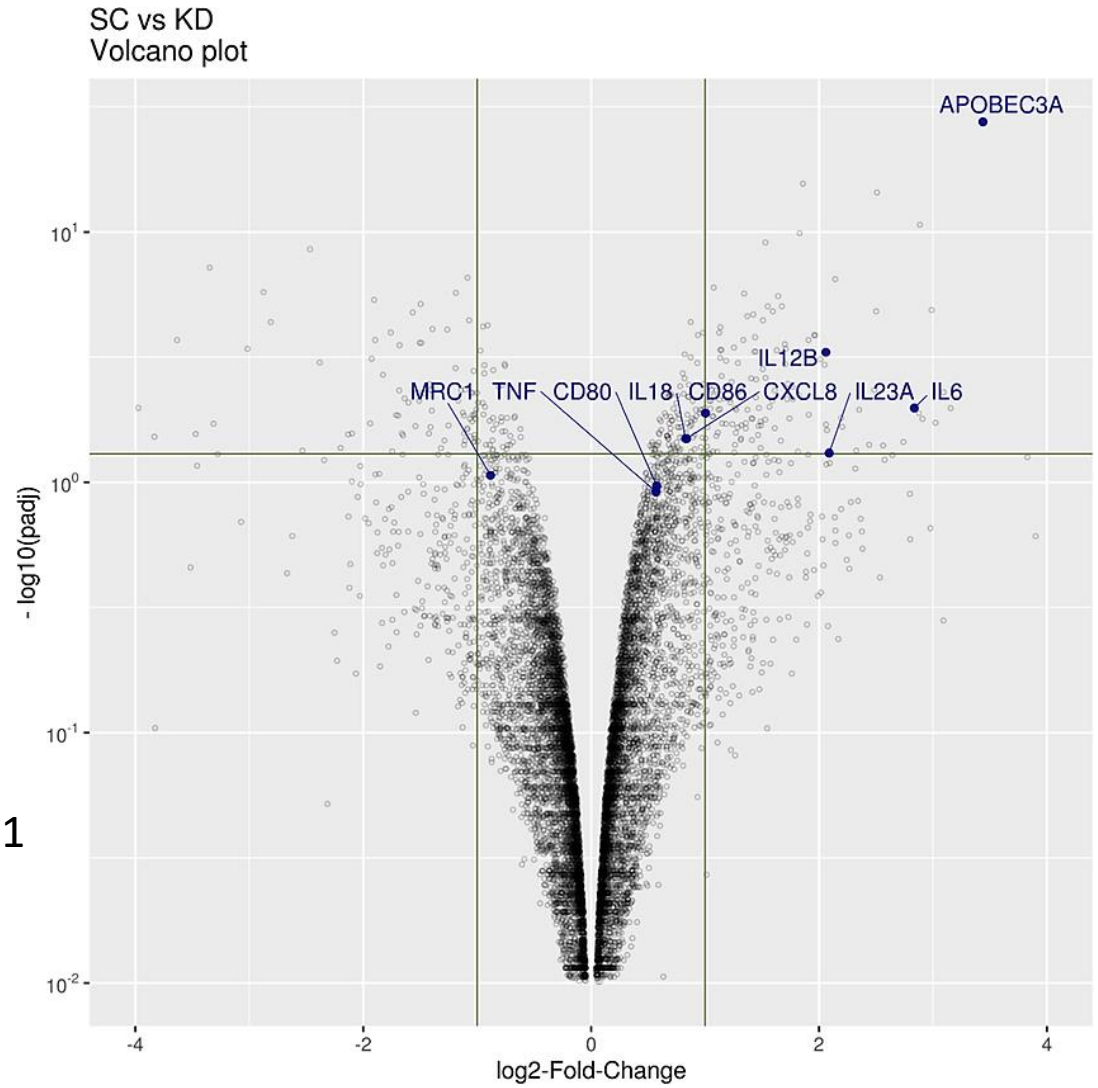
Results

- APOBEC3A expression is significantly enriched in SC relative to KD

1. Effective knockdown and

2. APOBEC3A is required for high expression levels of pro-inflammatory genes IL6, IL23A, IL12B and CD86 in M1 macrophages



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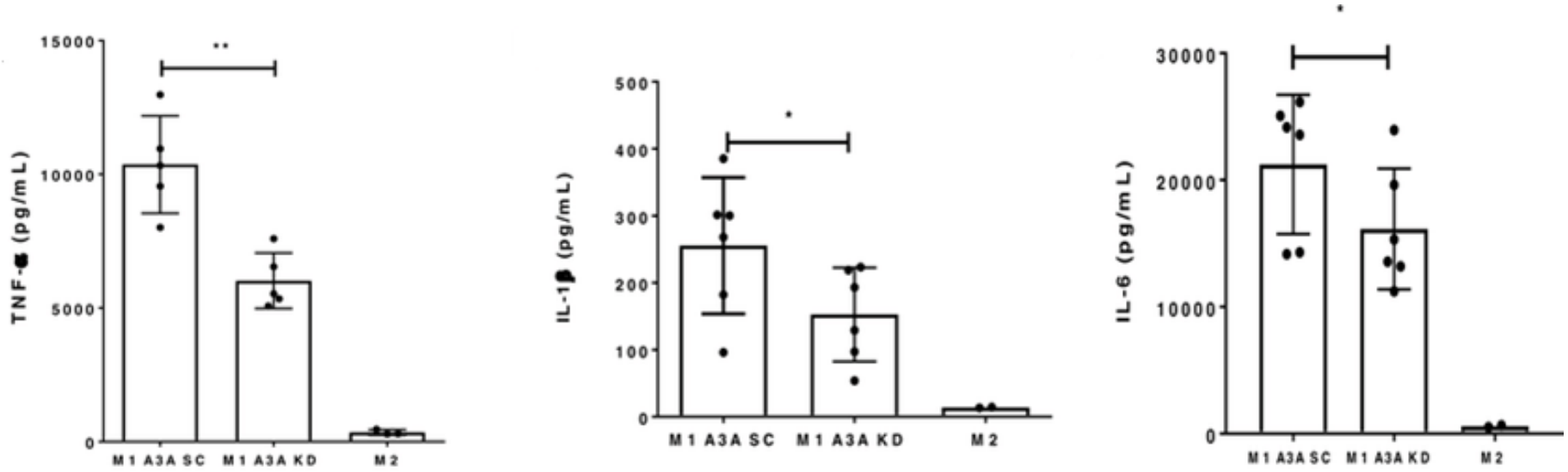
Results

Cytokine production by M1 APOBEC3A SC and M1 APOBEC3A KD macrophages was assessed by ELISA



- APOBEC3A KD decreased TNF-α, IL-1, and IL-6 secretion by M1 macrophages

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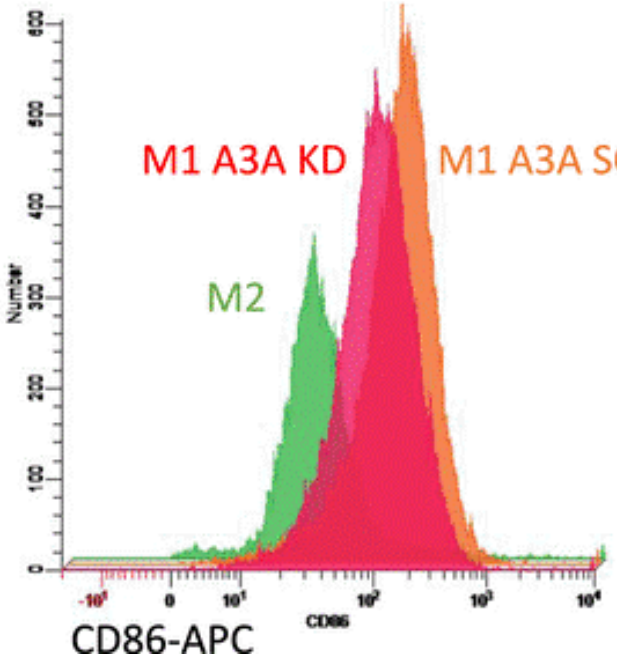
Results

- CD86 → marker for M1 macrophages

- Compared to M1 APOBEC3A SC macrophages, M1 APOBEC3A KD macrophages showed a reduction in CD86 expression

Assessment of the role of A3A in modulating the surface expression of CD86 as markers for M1 macrophages by flowcytometry



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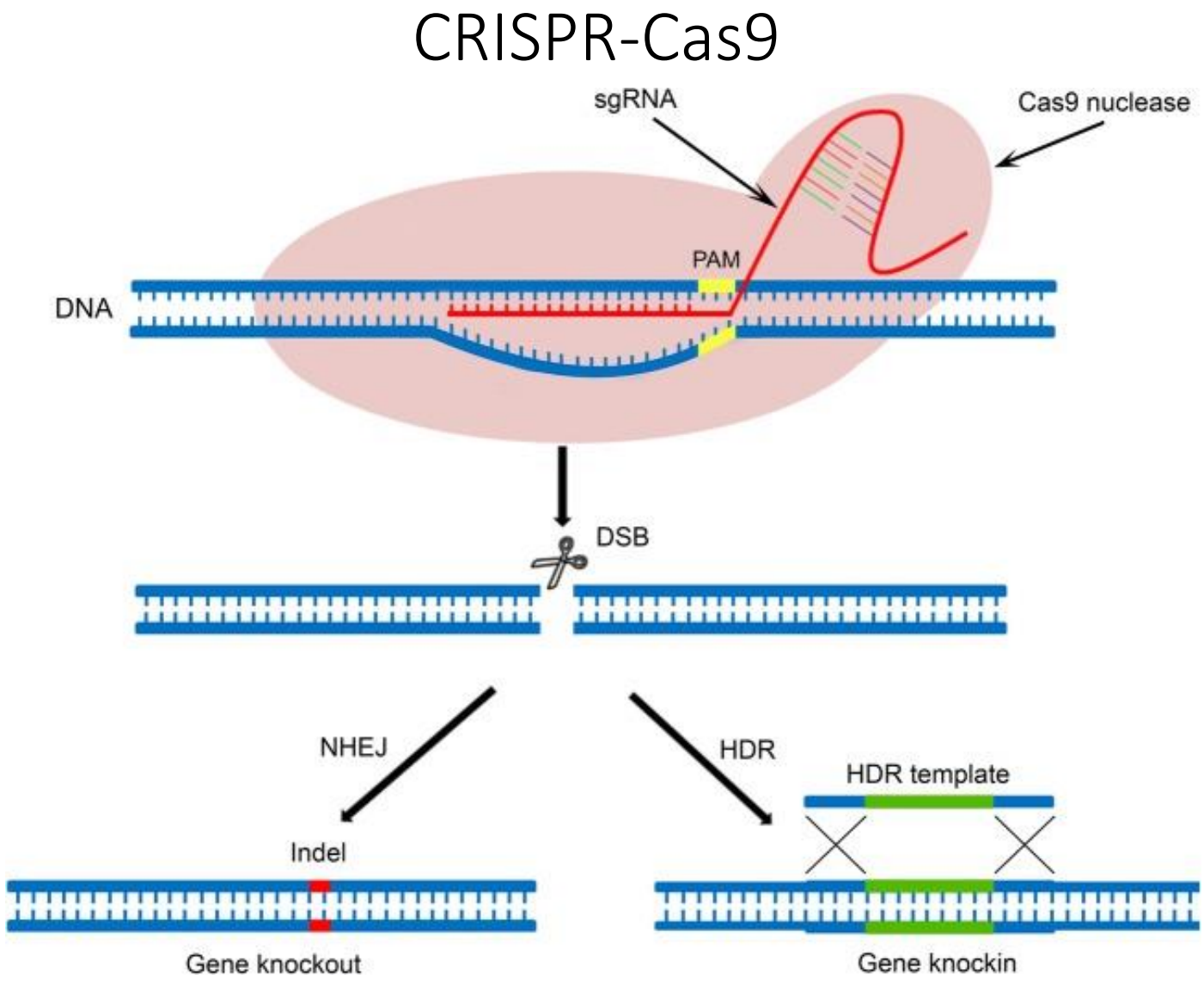
Lab research

Macrophages —siRNA→ Macrophages APOBEC3A KD

BV2 cell line —CRISPR-Cas9→ BV2 ADAR-1 KO
ADAR-2 KO
APOBEC-1 KO

Lab research

- Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)
- Protospacer Adjacent Motif (PAM)
- Double-Strand Break (DSB)
- Non-Homologous End Joining (NHEJ)
- Homology-directed repair (HDR)



Lab research

After the creation of the cell lines…

- Western blot
- Flow cytometry
- Activate M1 and M2 state
- RT-PCR
- ELISA
- Bioinformatics analyses

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