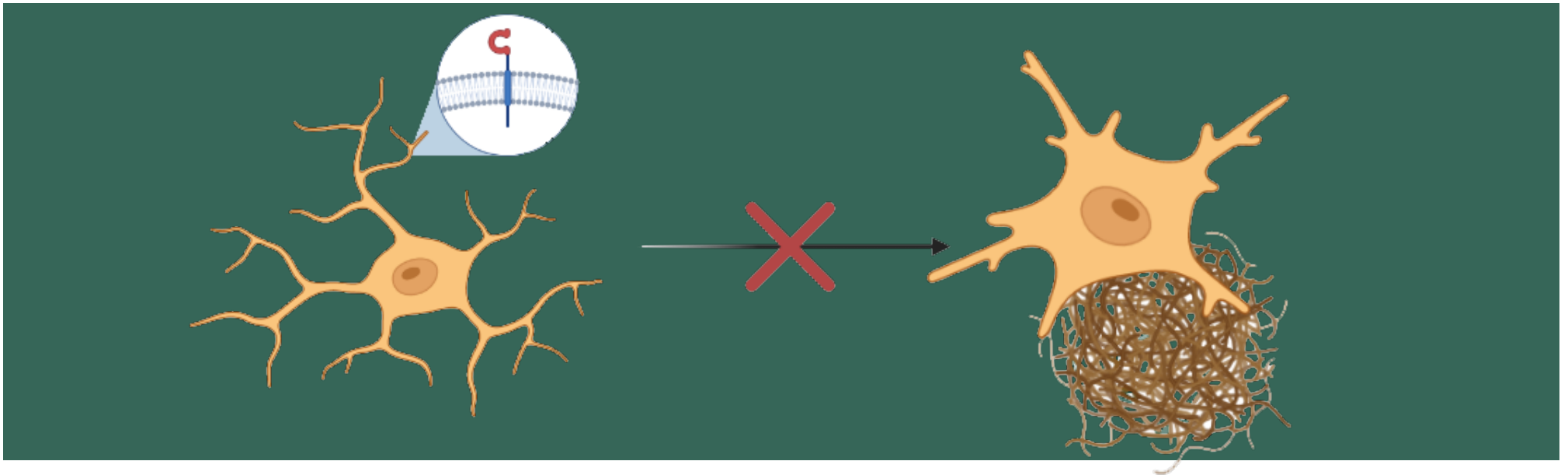


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# Alzheimer's Disease

Rescuing Microglial response by restoring TREM2 correct expression via gene therapy



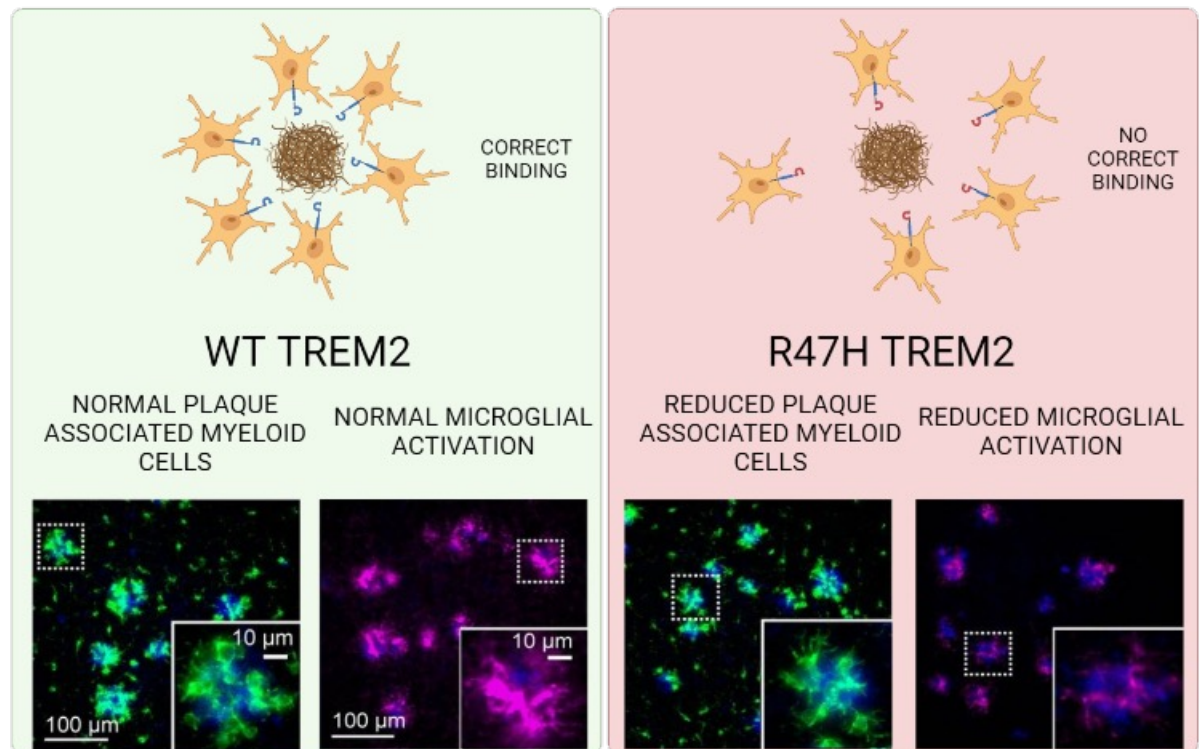
Eminente Sofia, Fenili Gianmarco, De Vincenzi Eleonora, Prosperi Giorgio

# BACKGROUND

R47H mutation in TREM2 has been strongly associated with an increased risk of Alzheimer's disease.

R47H mutation in TREM2 has an AD-related effect size similar to the well-characterized ApoE  $\epsilon$ 4 allele.

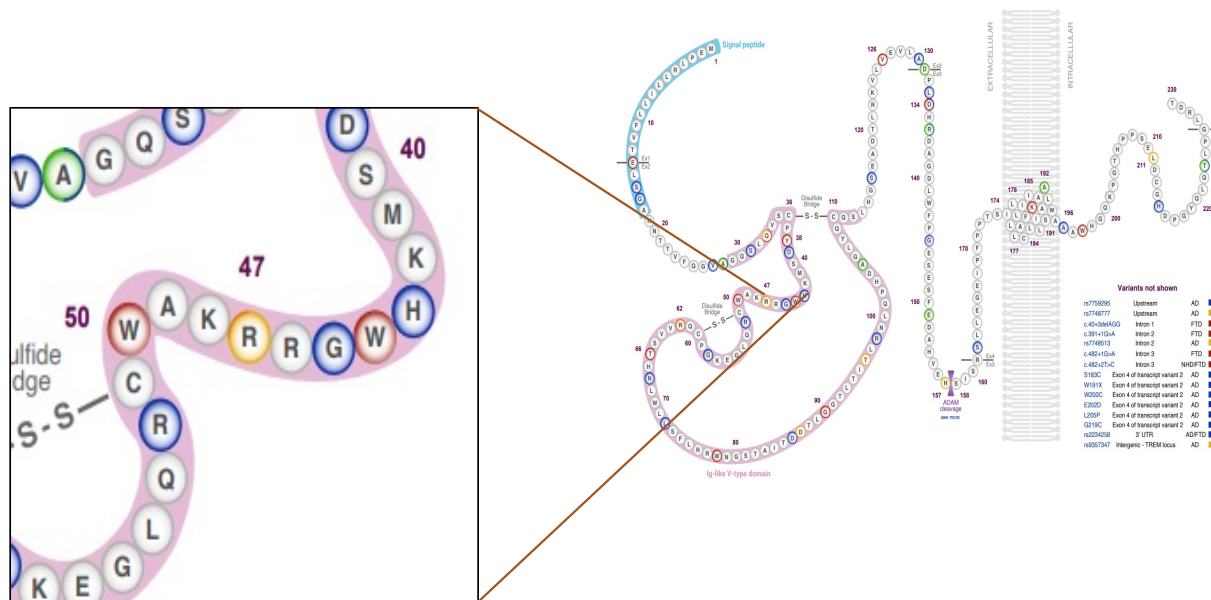
Trem2<sup>+/R47H</sup> variant compromises myeloid cell response to AD-like amyloid pathology.



MICROGLIA (IBA1) AROUND PLAQUES (6 E10) AND THEIR ACTIVATION (CD45)

# AIM OF THE PROJECT

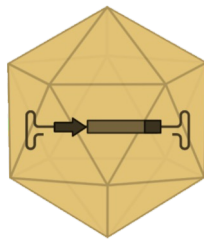
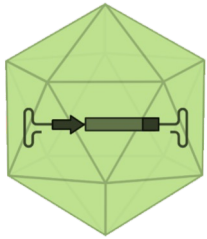
Can gene therapy rescue the DAM-phenotype in  $TREM2^{R47H}$  positive microglia?



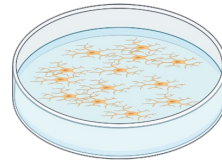
Our aim is to repair the SN mutation through Crispr/Cas9 in AD patients microglia therefore promoting A $\beta$  phagocytosis and slowing down the progression of the disease.

# MATERIALS AND METHODS

Modified AAVcMGs  
AAV-cMG.WPP    AAV-cMG.QRP



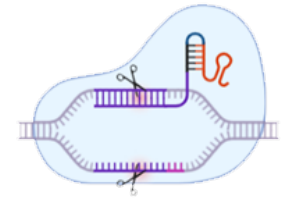
Cell lines: primary  
Microglial culture



Animal model: Trem2<sup>R47H</sup>-  
5xFAD mouse



Sa-Cas9



Stereotactic delivery

Immunohistochemistry

Western blot

GUIDE-Seq

Behavioral tests

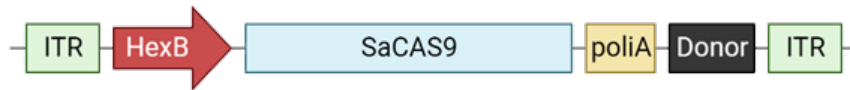
rtPCR

gRNA selection

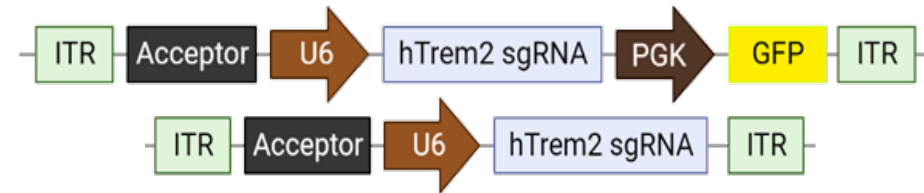
Citofluorometry

# AAV-CMG DOUBLE INJECTION

## AAV-cMG.VWPP



## AAV-cMG.QRP



## Transfection

HEK 293



AAV Vector



AAV Helper



## TREM2 sgRNA

TEMPLATO

PAM

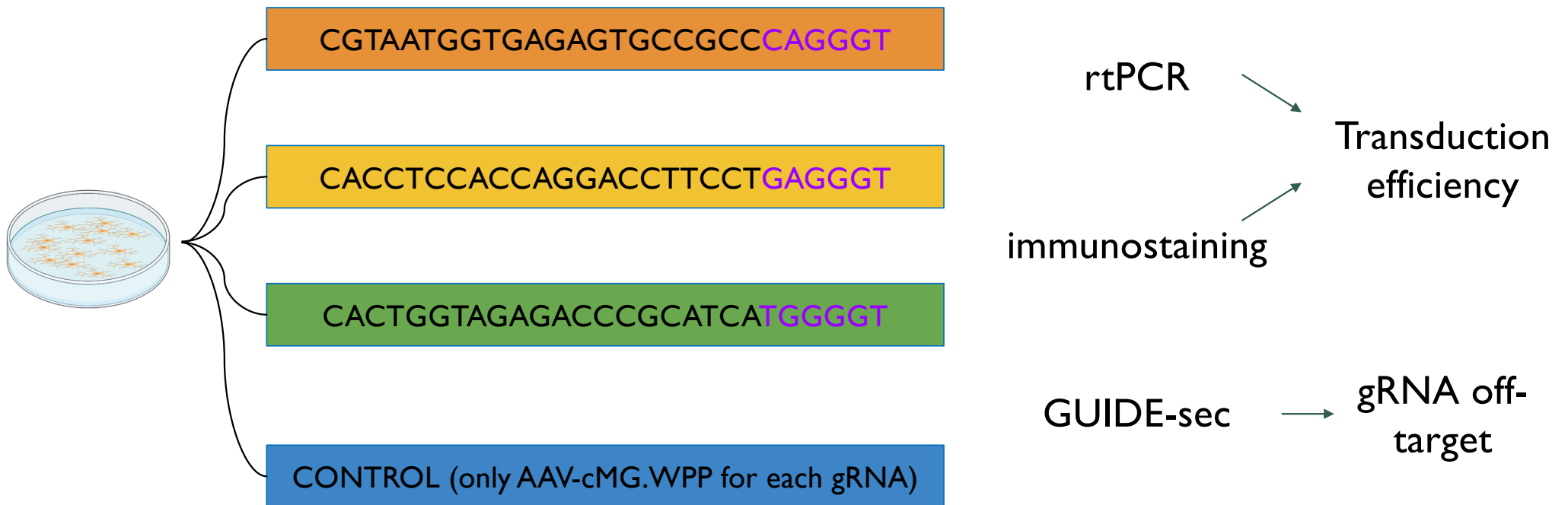
CGTAATGGTGAGAGTGCCGCC**CAGGGT**

CACCTCCACCAGGACCTTCCT**GAGGGT**

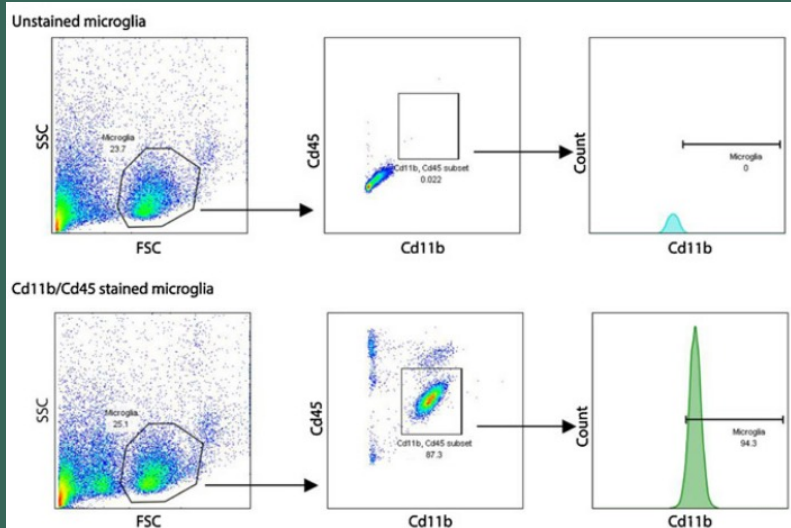
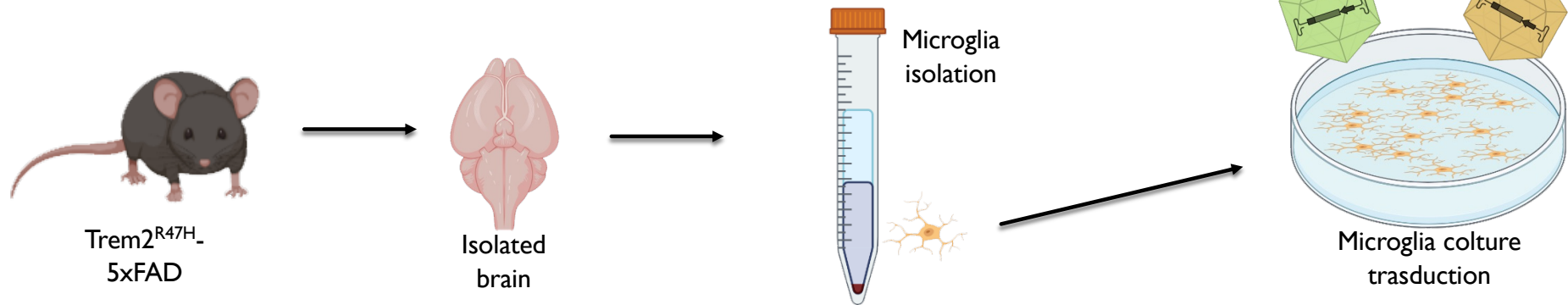
CACTGGTAGAGACCCGCATCAT**GGGGT**

# PRELIMINARY CONTROLS

AAV-cMG.WPP and AAV-cMG.QRP cotransduction

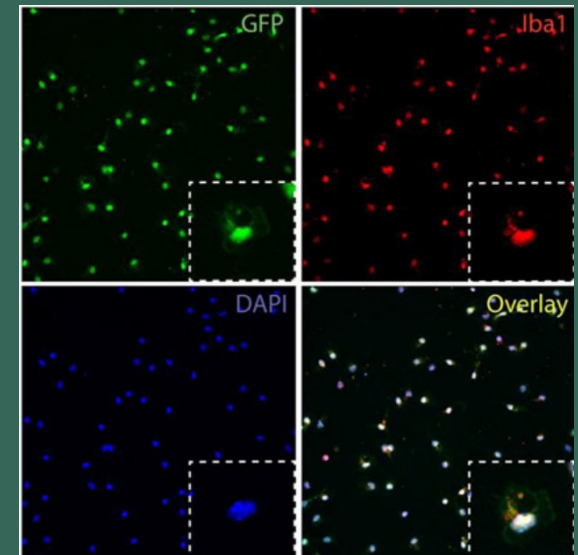


# EXPERIMENTAL PLAN- IN VITRO VECTOR CONTROL



Flow  
cytometry

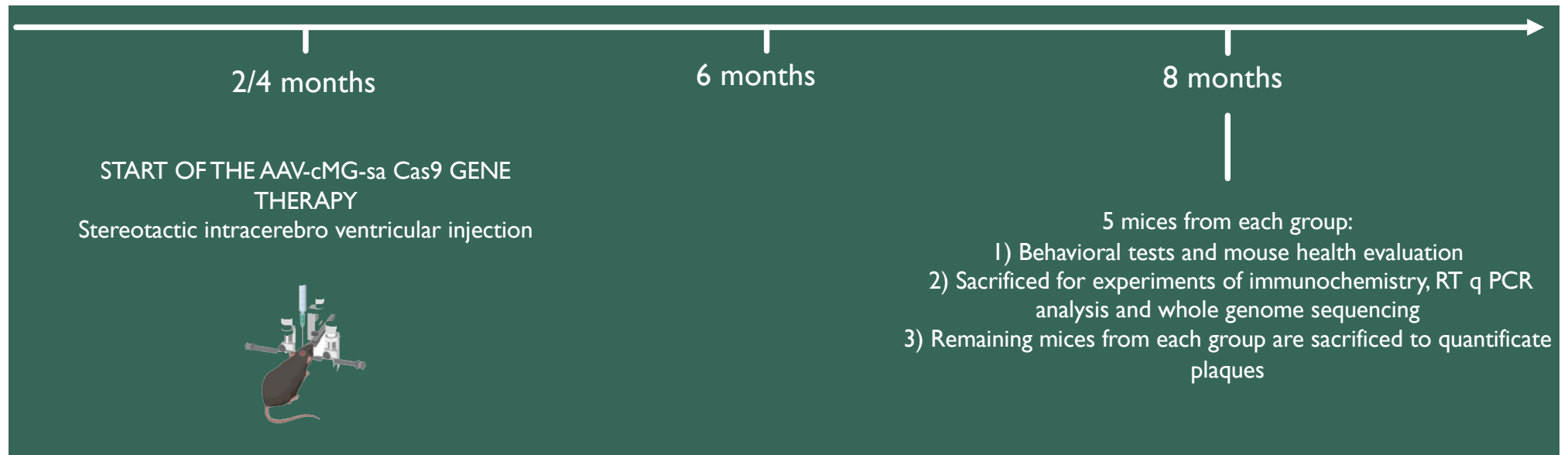
Immunostaining



# EXPERIMENTAL PLAN – IN VIVO

## OUR SUBJECTS:

- Trem2<sup>+/R47H</sup>5XFAD treated with AAV cMG - saCas9 (n=14)
- Trem2<sup>+/R47H</sup>5XFAD treated with AAV cMG.WPP (no AAV cMG.QRP) (n=12)
  - Trem2<sup>+/R47H</sup>5XFAD untreated (n=12)
  - Trem2<sup>+/+</sup>-5XFAD (n=10)





# RESULTS – IN VIVO

Phenotype profiles were compared using immunohistochemistry, WGS and RT q PCR showing a similar phenotype between groups, in line with the mutation correction.

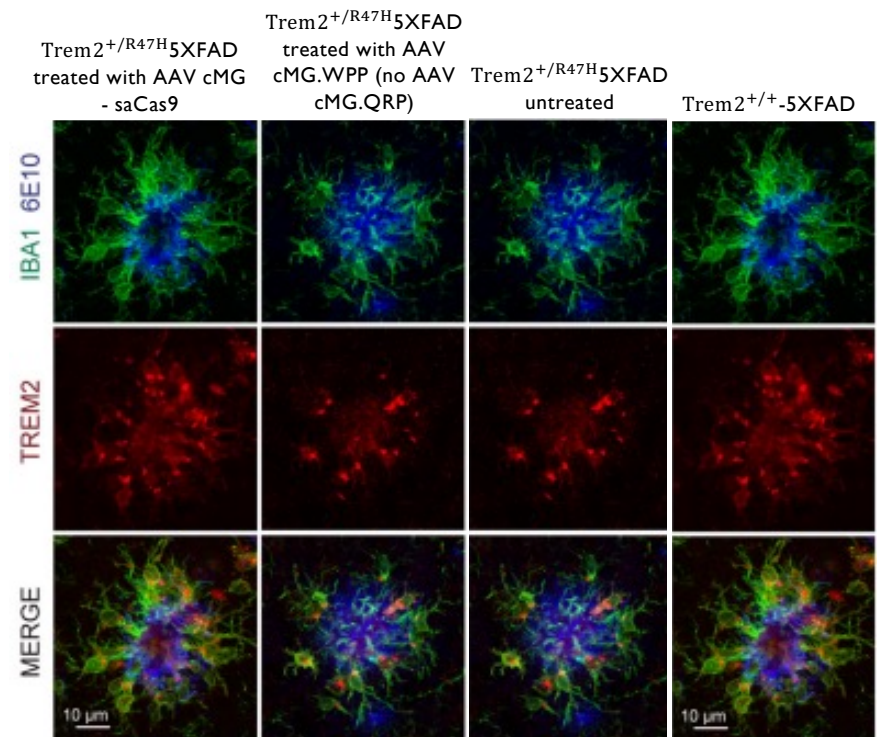
## WHOLE GENOME SEQUENCING

No off-targets  
Successful correction of Trem2 in  
Trem2<sup>+/R47H</sup>5XFAD treated with AAV cMG -  
saCas9.

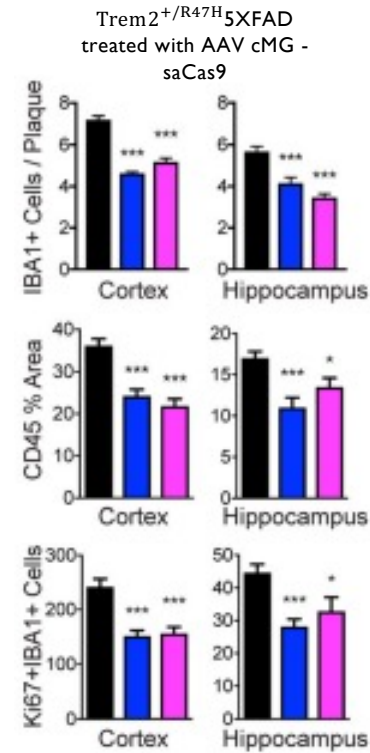
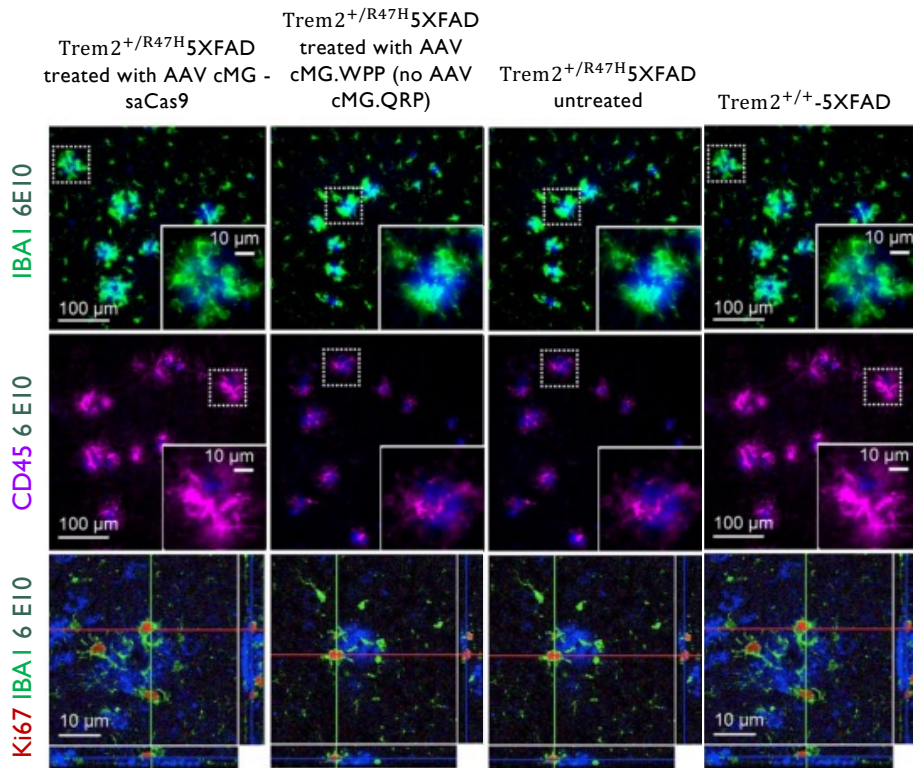
## RT – q PCR ANALYSIS

Upregulation of microglia activation-  
related transcripts in  
Trem2<sup>+/R47H</sup>5XFAD treated with AAV  
cMG -saCas9 compared to control.

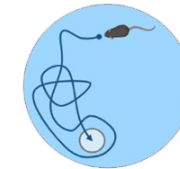
## IMMUNOISTOCHEMISTRY IN CORTEX



# RESULTS - IN VIVO



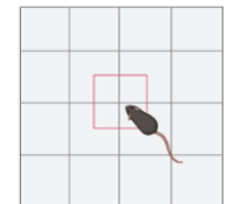
## BEHAVIORAL TESTS AND MOUSE HEALTH EVALUATION



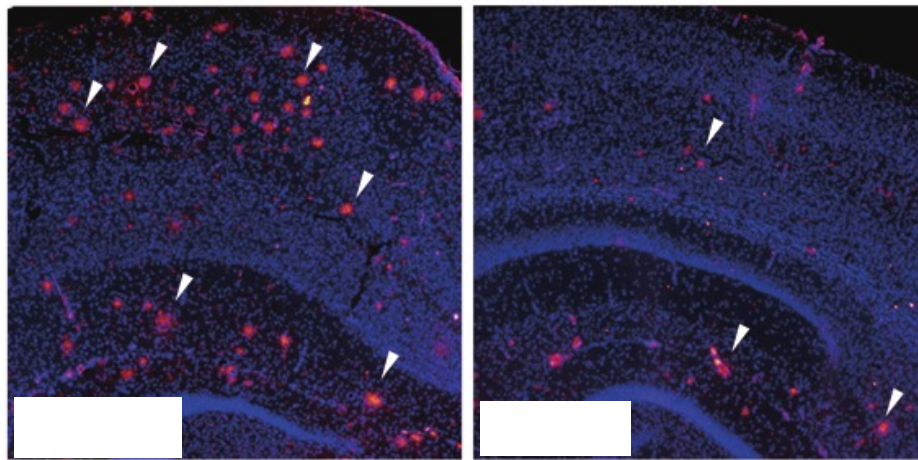
Morris Water Maze

Novel Object Recognition

Open Field

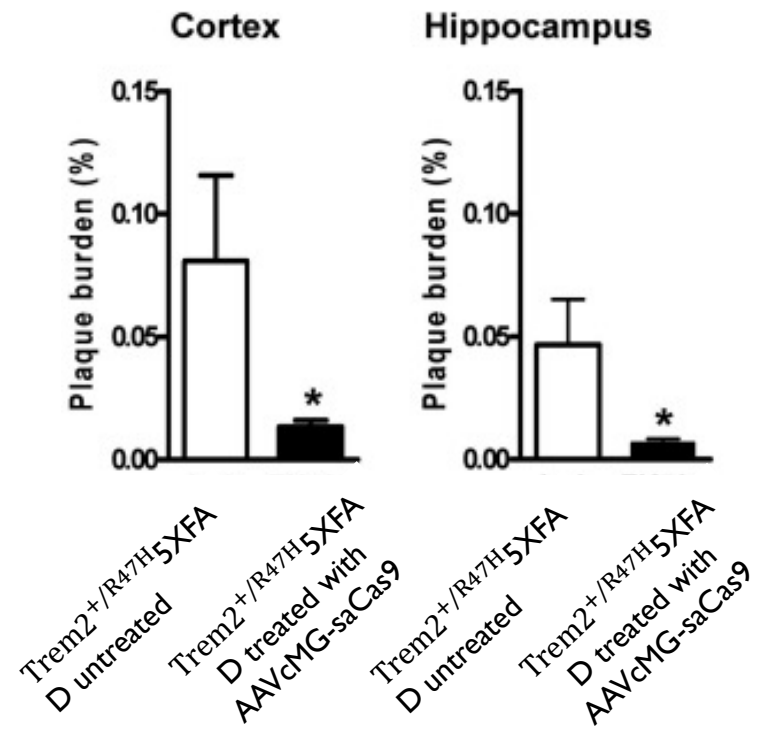


# RESULTS - IN VIVO



Trem2<sup>+</sup>/R47H5XFAD untreated

Trem2<sup>+</sup>/R47H5XFAD treated with AAVcMG-saCas9



## CONCLUSIONS

Treatment with AAVcMG-saCas9 successfully restores Trem2 expression in both primary microglial cultures and Trem2<sup>+ / R47H</sup>5XFAD mice.

Restoring Trem2 expression in Trem2<sup>+ / R47H</sup>5XFAD mice significantly increases DAM profile in cortex and hippocampus microglial population and reduces  $\beta$  amyloid plaques in cortex and hippocampus.

## PITFALLS

To this day it is inefficient to deliver our gene therapy without neurosurgery.

This treatment is possible only in subjects with TREM2 R47H mutation.

Double transduction widely decrease transduction efficiency



## FUTURE PERSPECTIVES

This will be the first gene therapy approach to target specifically and directly microglia.

Development of modified aav capsids that can both overcome the BBB and target microglia will be key to bring this kind of therapy in humans.

## COSTS FOR A 2 YEARS WORK

	SOURCE	COST
Mice	Colonna lab, Washington university.	Collaboration with Colonna lab
Whole Genome Seq		4500 euro/sample
Antibodies	<a href="https://www.antibodies-online.com/">https://www.antibodies-online.com/</a>	5700 euro
rtPCR kit	<a href="https://www.sigmaaldrich.com/">https://www.sigmaaldrich.com/</a>	890 euro
Manpower (1 PI, 2 doctorates, 1 lab tec)		6500 euro/month
AAV-MG	National Institute of Biological Sciences (NIBS), Beijing, China.	3650 euro
Plasmids		10000 euro
Citofluorometria		1600 euro