Immunization therapy for CADASIL

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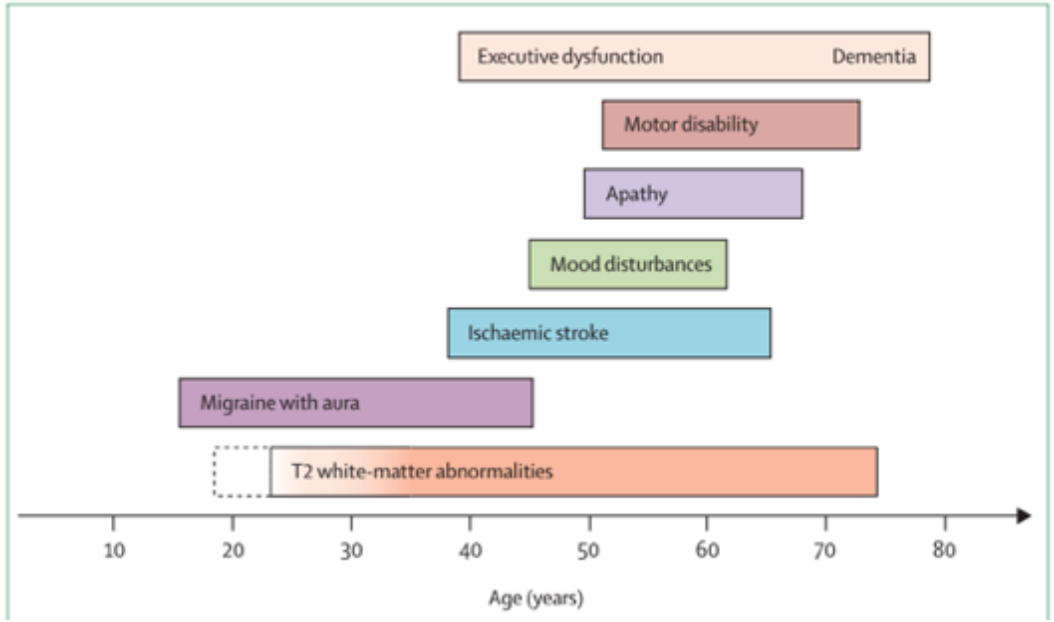
CADASIL- Cerebral Autosomal Dominant Arteriopathy with Subcortical Infarcts and Leukoencephalopathy

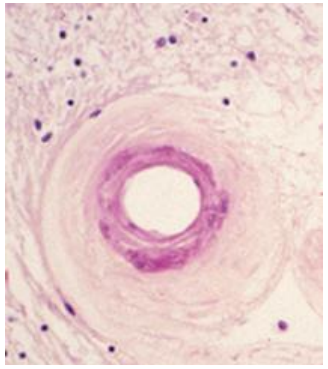
- Most common monogenic form of small vessel disease
- Caused by mutations in the NOTCH3 gene (expressed on arteries and capillaries)
- Affecting approximately 5/100 000 individuals, but most likely underdiagnosed (up to 60x more common)

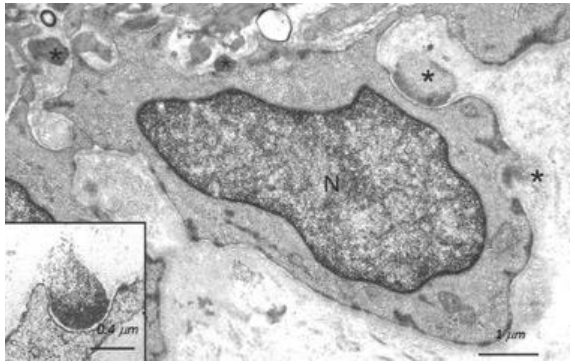


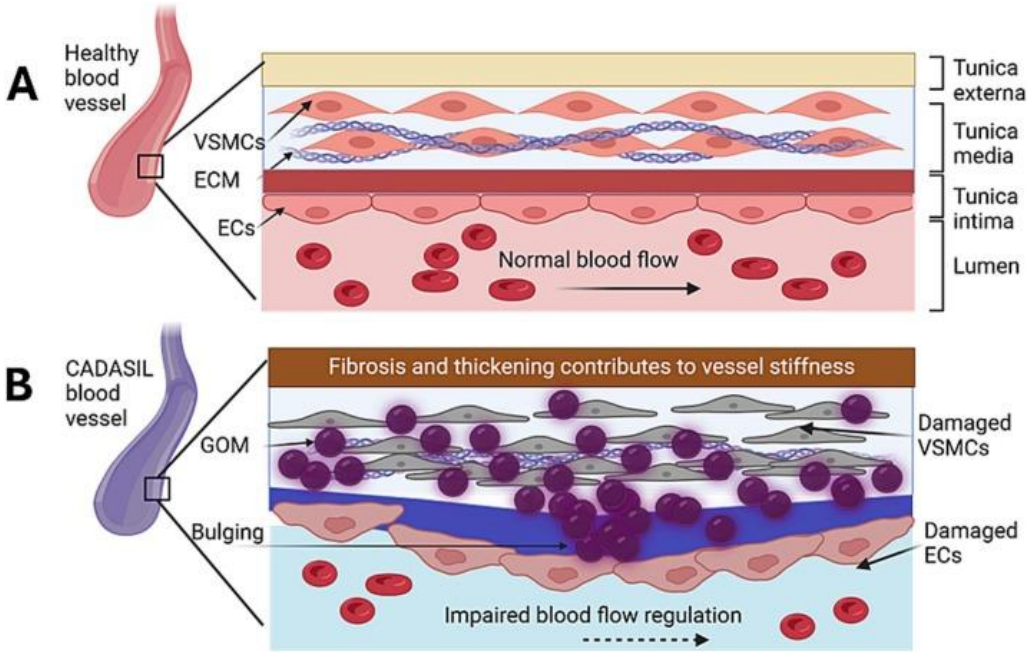
- **Currently there is NO cure!**

Pathophysiology

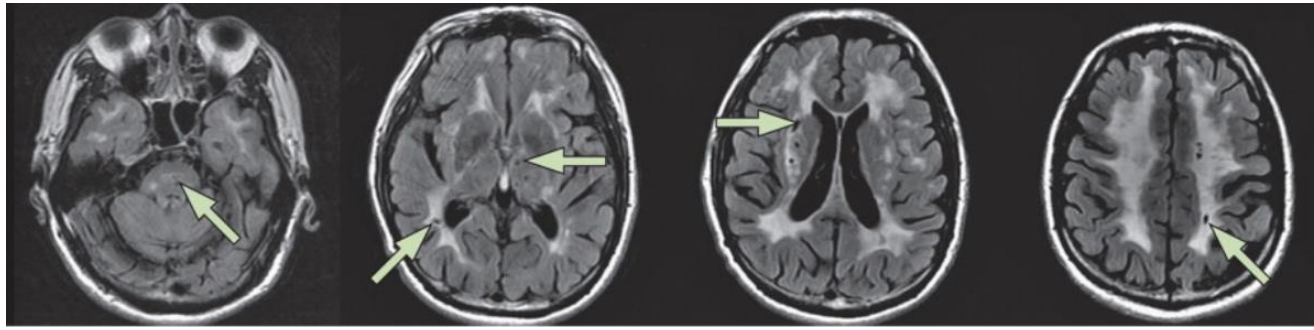
- Small vessels have GOM in basal lamina, thickened wall, decreased lumen
- Impaired cerebral blood flow





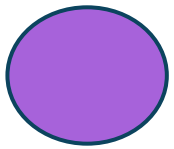


- Extensive damage to white matter

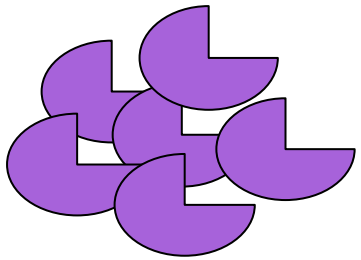


NOTCH3

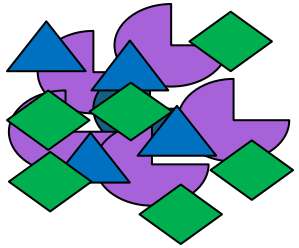
NOTCH3 protein is needed for blood vessels to work properly (contraction/dilation etc)



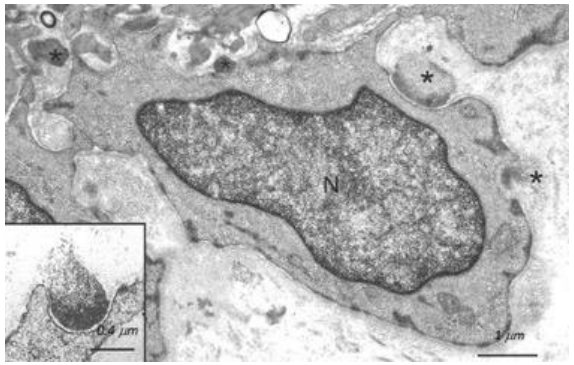
NOTCH3 protein

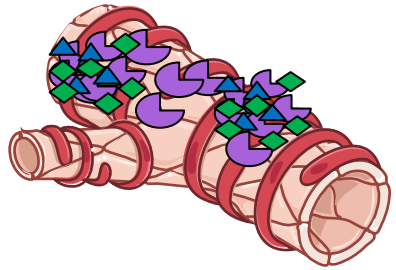


Mutant
NOTCH3 aggregation

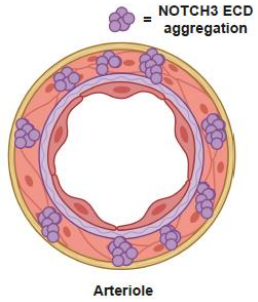


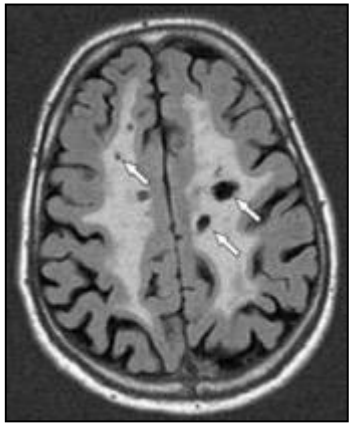
Recruitment of
other proteins





vessel wall
dysfunction

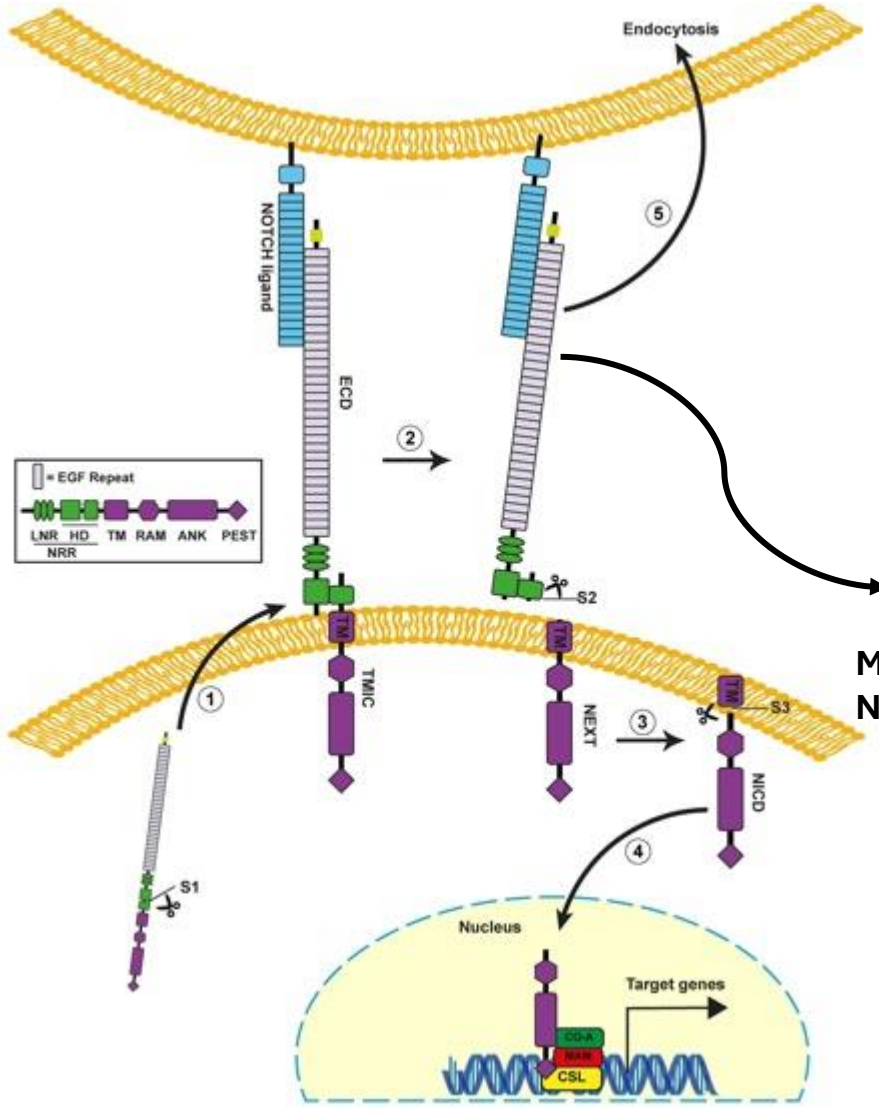




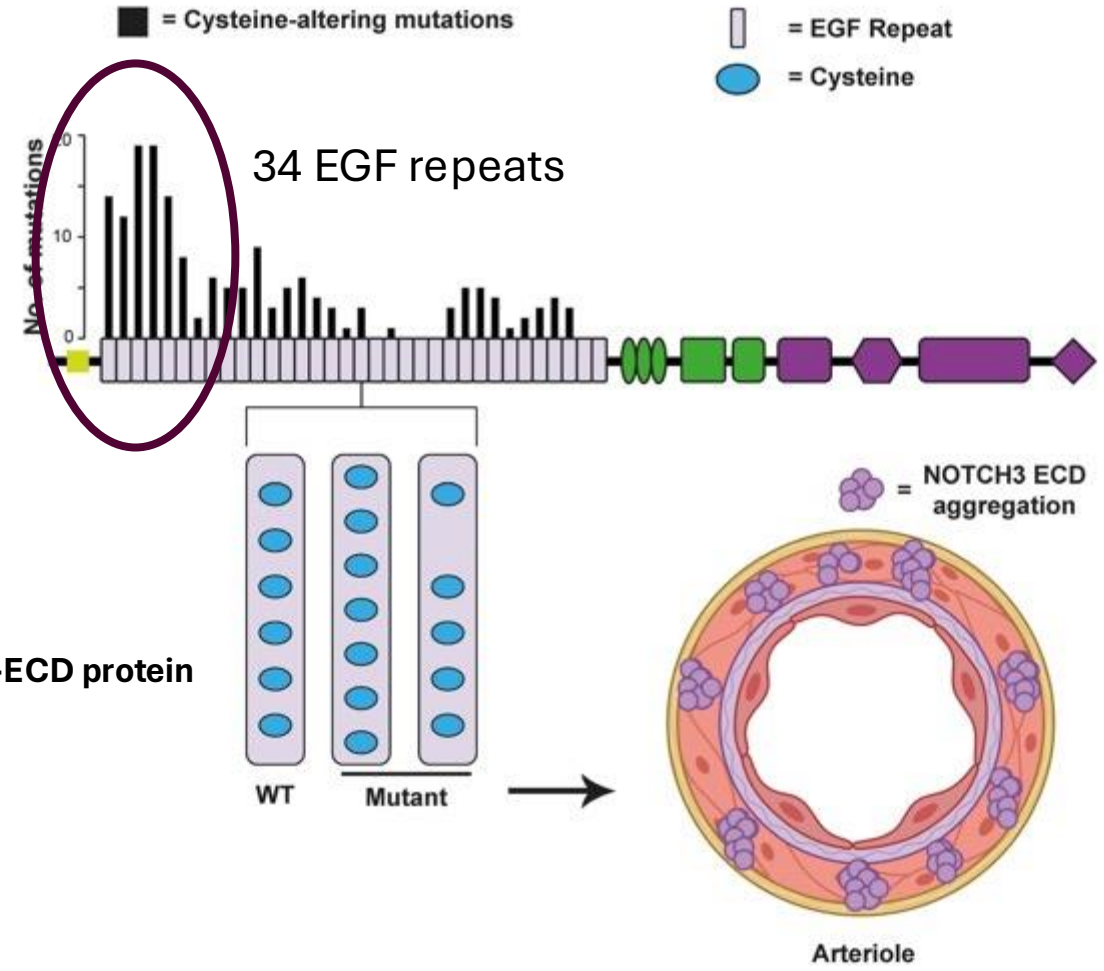
CADASIL brain MRI

Notch3 processing & signaling and CADASIL mutations

(around 300 autosomal dominant mutations exist)

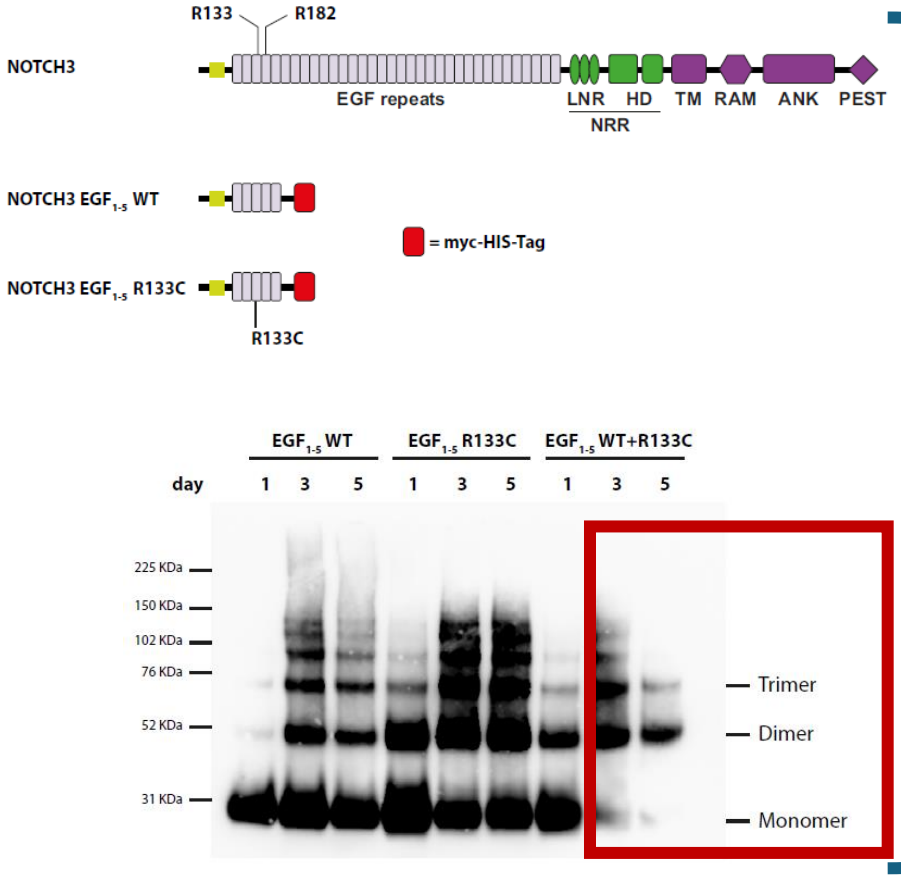


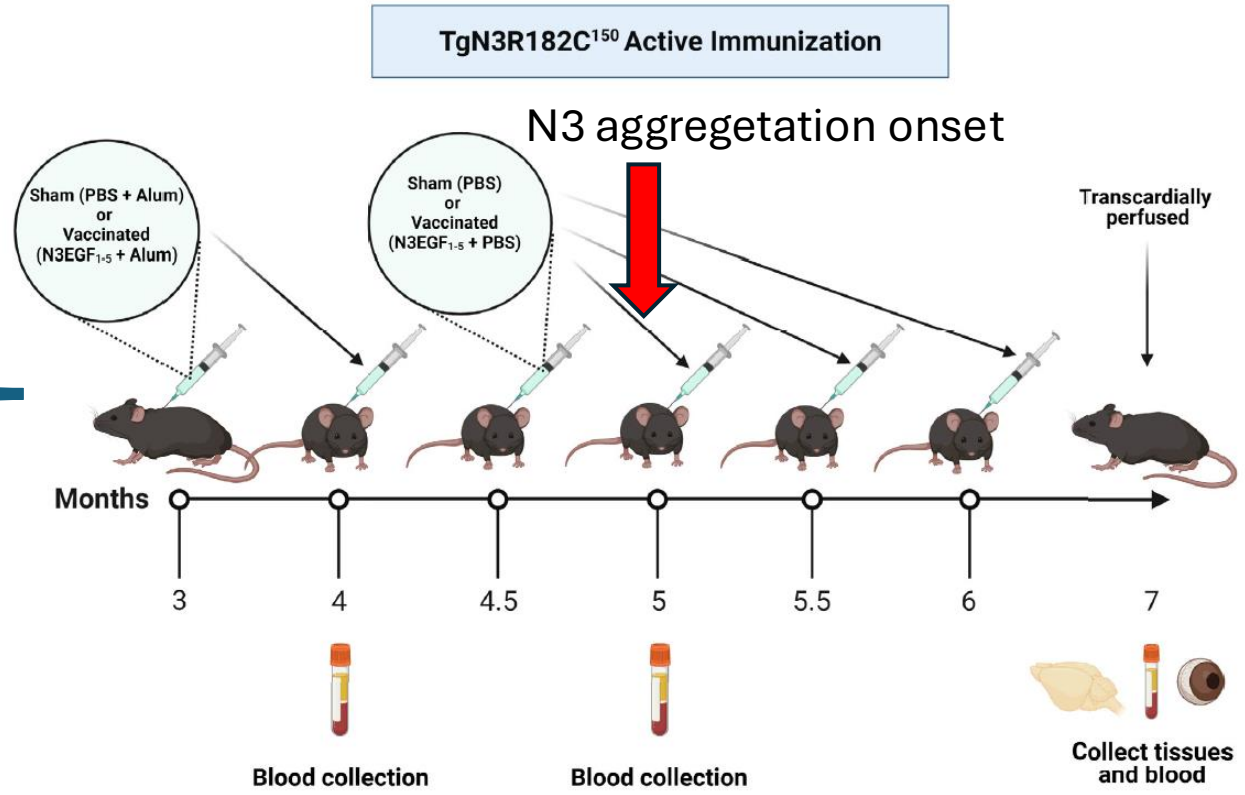
Mutated NOTCH3-ECD protein



Active immunisation 1.0 - preventive study

Oliveira et al. *Active immunotherapy reduces NOTCH3 deposition in brain capillaries in a CADASIL mouse model*. EMBO Mol Med. 2023





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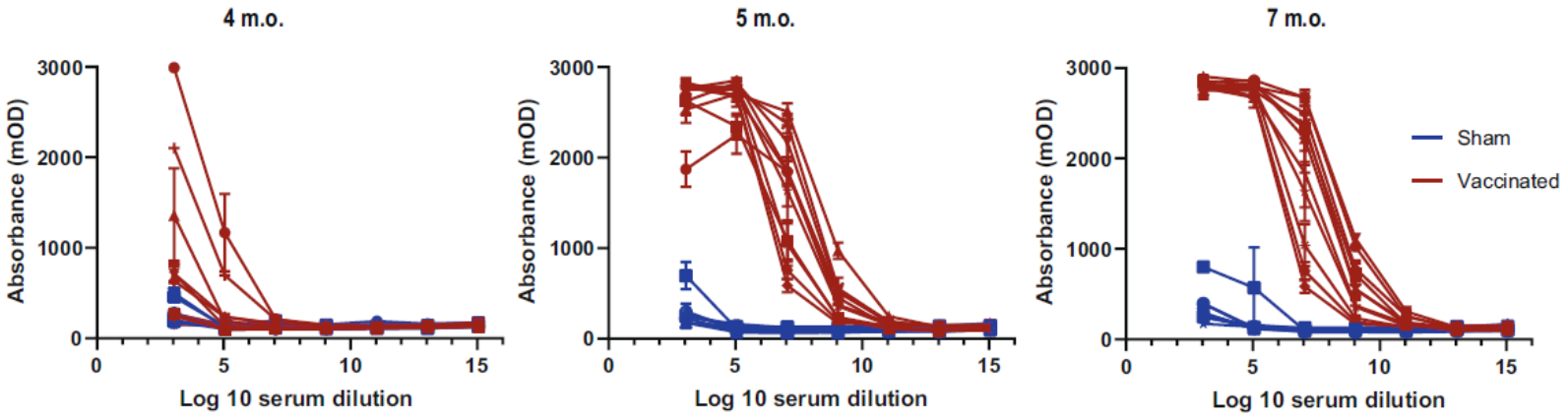
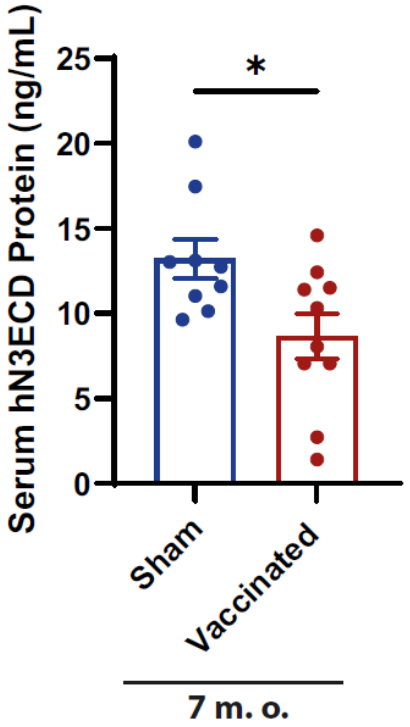


Antibody response in the serum



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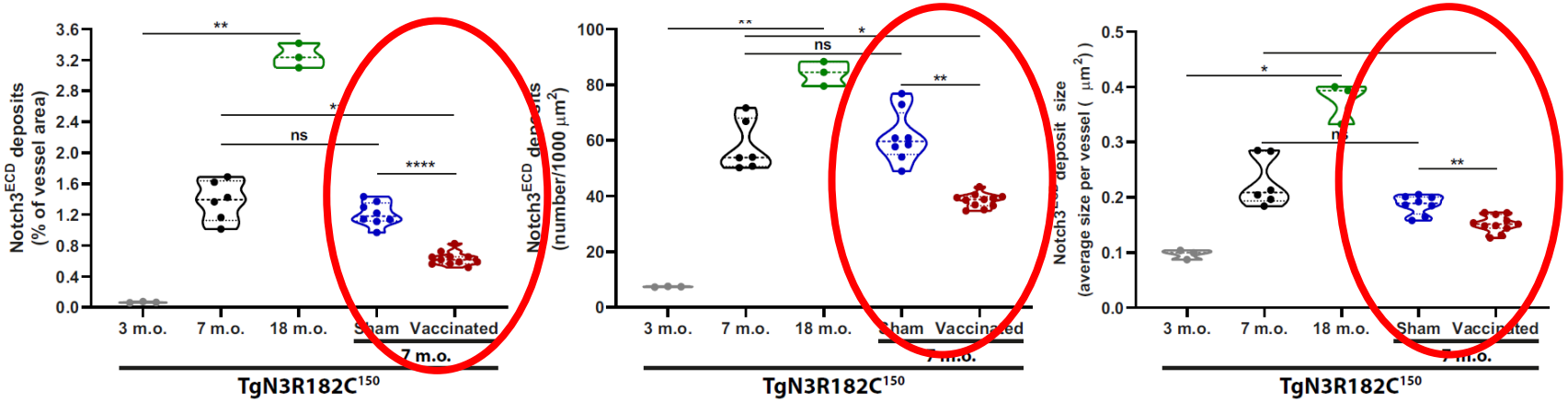
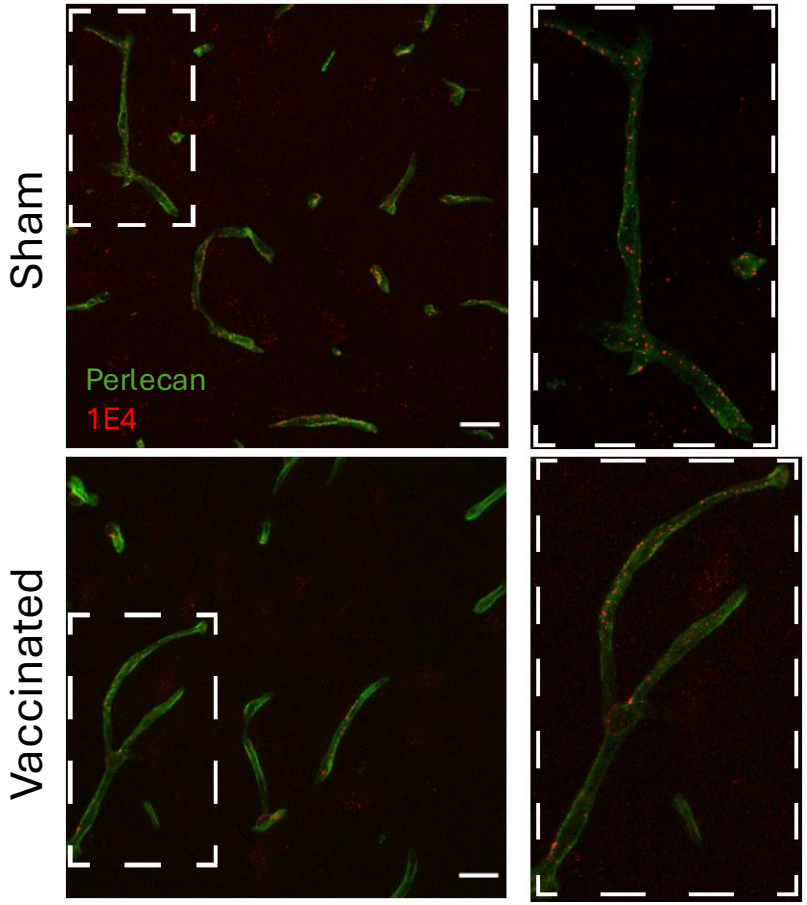


NOTCH3-ECD deposits in the brain vessels



Preventive active immunization

Oliveira et al EMBO Mol Med, 2023

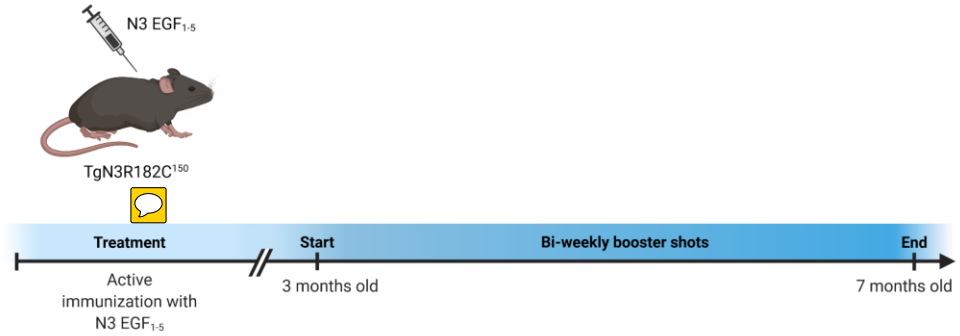
1. Vaccination give rise to a high antibody response in mice

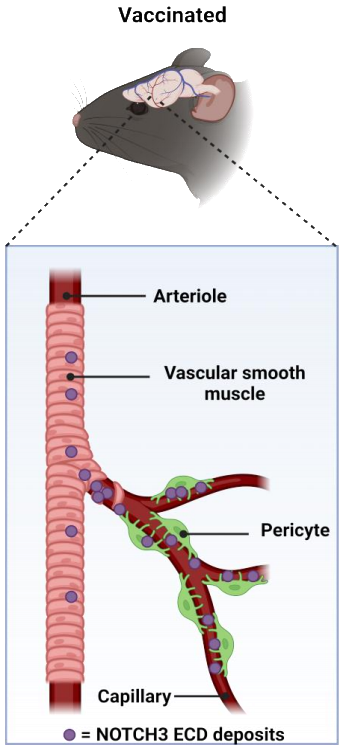
2. Vaccination reduces NOTCH3 aggregates in brain vessels and blood

3. Vaccination is activating the cleaning cells microglia to clear away the NOTCH3 aggregates

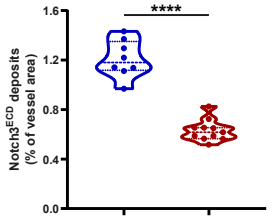
4. Vaccination seems to have cross-mutation efficacy

5. Vaccination is safe and tolerable in a pre-clinical mouse model

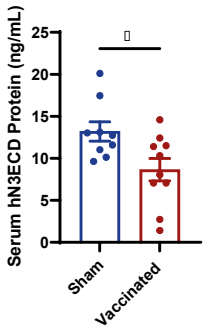




NOTCH3 ECD reduction in capillaries



NOTCH3 ECD reduction in blood serum



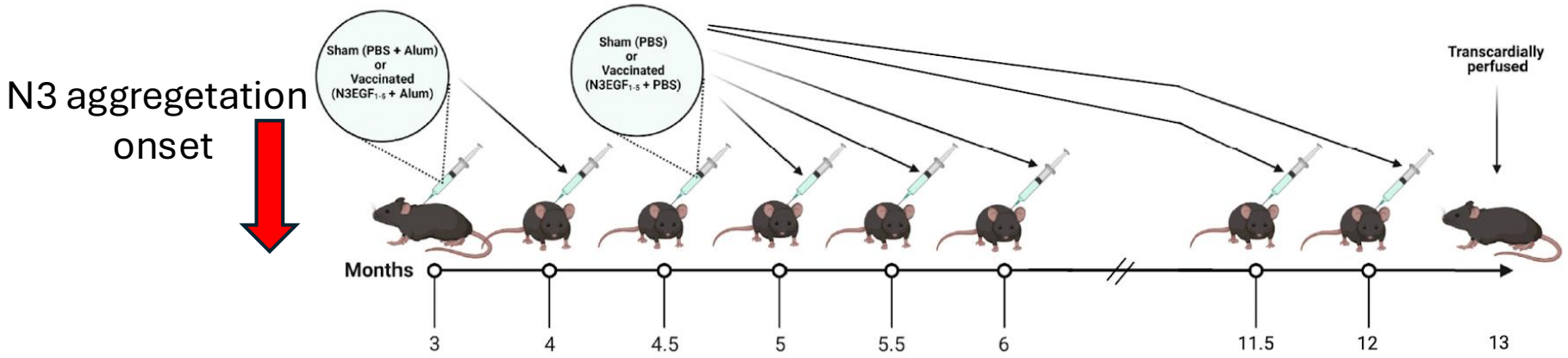
Active immunization 2.0 – therapeutic study

Differences between the studies:

- CADASIL mouse model TgN3R182C^{350} (**pathological onset, 6 w**), **10-month program** and **modified immunogen** (14 vaccinated + 14 sham mice)

Mouse organs

- Brain
- Kidney
- Serum
- Retina



Antibodies

- Spleen cells have been isolated for generation of hybridoma cells and monoclonal antibodies
- Validated with ELISA and immunohistochemistry in mice and human tissue

CADASIL patient material

- Brain
- Skin

EFFICACY

1) Does vaccination generate an immune response against the vaccine?
2) Does vaccination lead to a reduced number and size of NOTCH3-ECD aggregates in brain vessels?
3) Does vaccination lead to an increased activation of microglial cells?

APPLICABILITY

- Do the antibodies generated from vaccinated mice bind NOTCH3-ECD aggregates in human CADASIL brain and skin tissue with different CADASIL mutations?

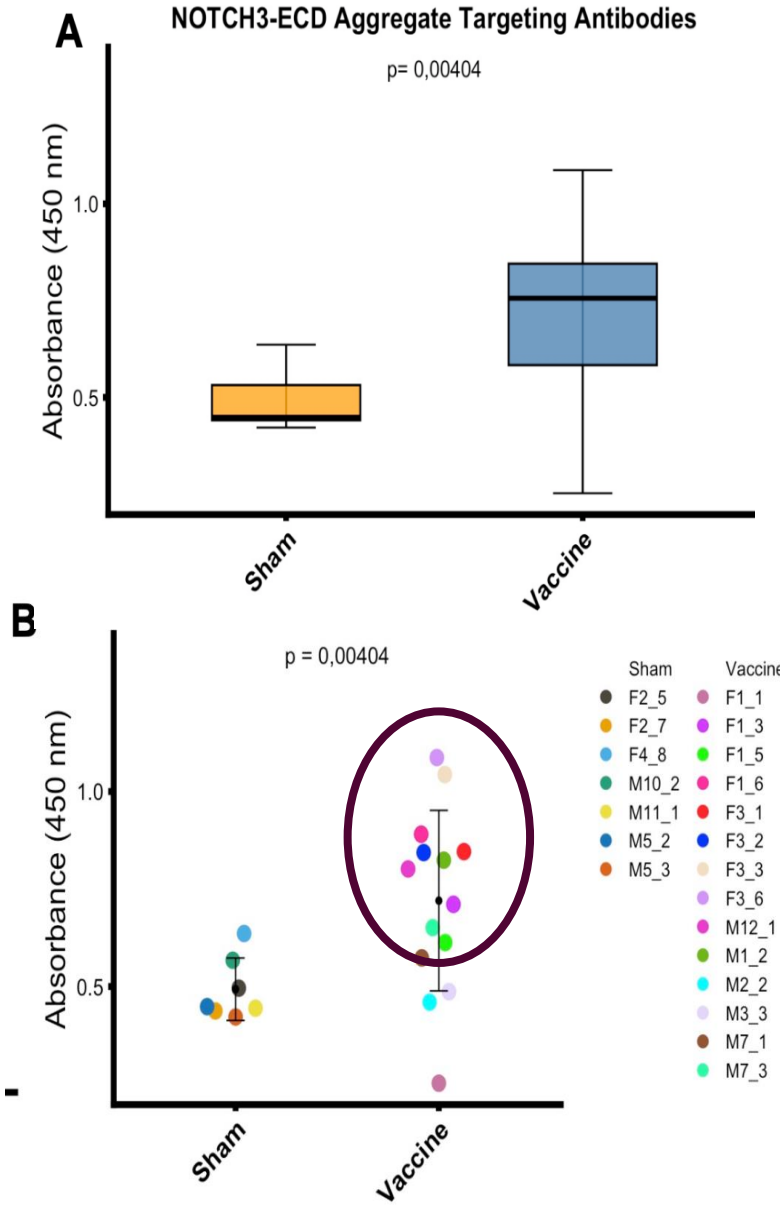
TOLERABILITY

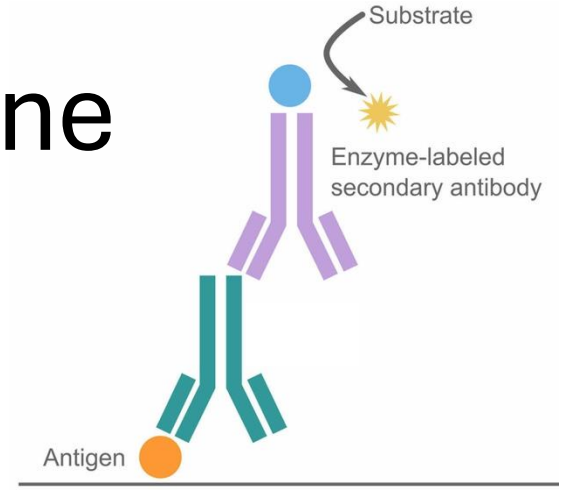
- Are there signs of toxicity occurring in vaccinated mice?

1. Does vaccination generate an immune response?

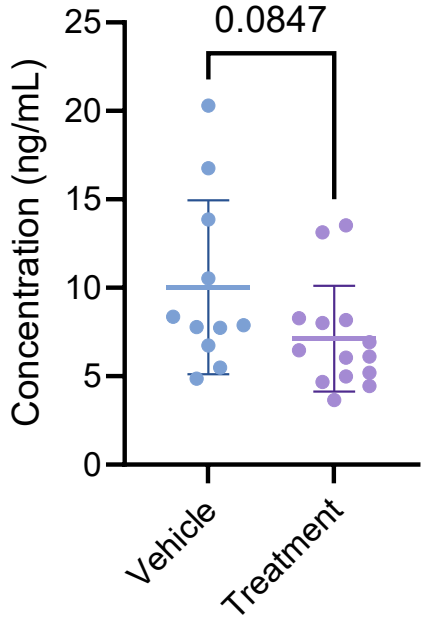
- Serum from mice
  - Vaccinated n=14, sham n=7
- **Increased** antibody response in vaccinated mice by 46 % (p= 0,00404)
- Female mice have higher antibody titer than male

➔Correlated to N3-ECD Level in serum?





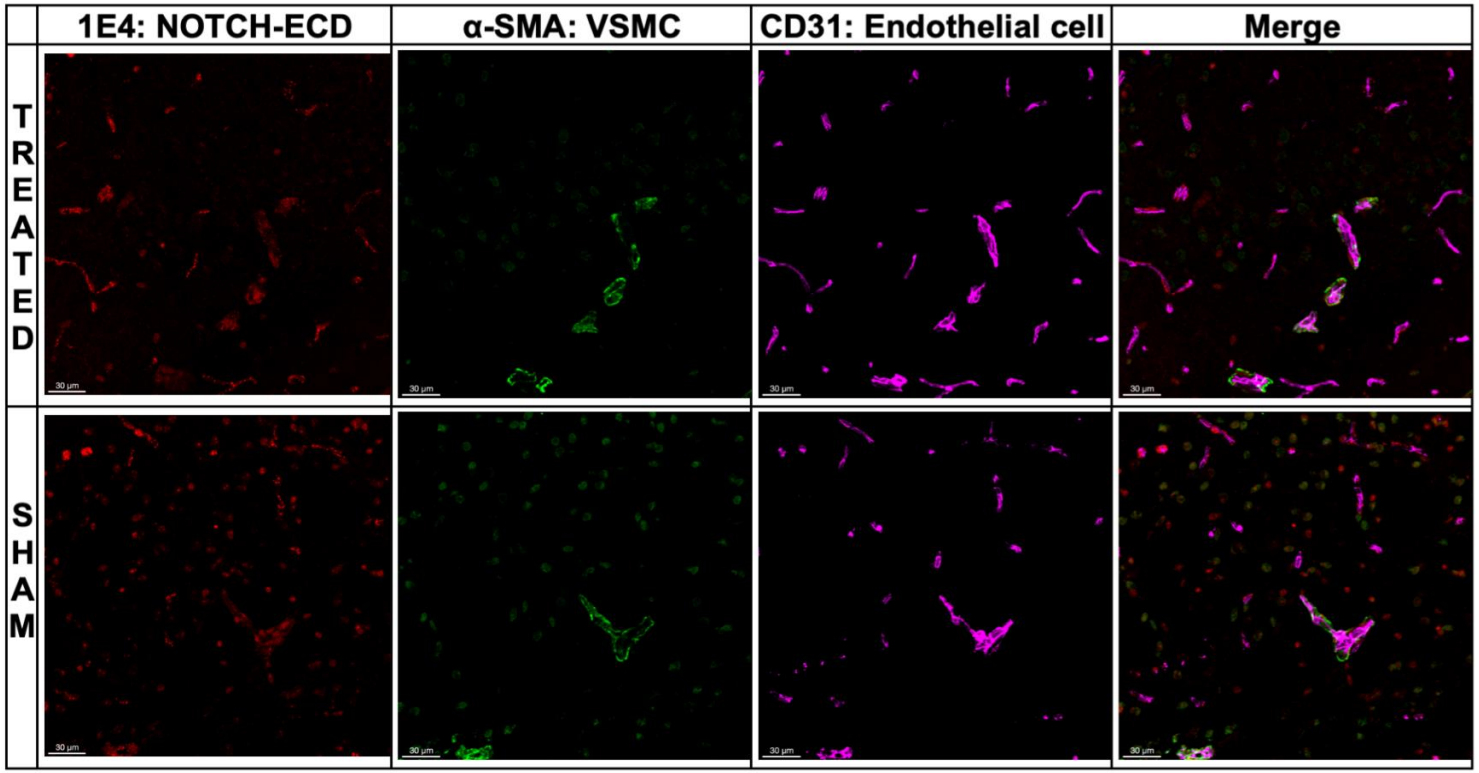
N3-ECD concentraions mouse serum



2. Does vaccination lead to a reduced number and size of NOTCH3-ECD aggregates in brain vessels?

- Immunohistochemistry on 10 um brain cryosections-staining for NOTCH3-ECD, CD31(endothelia cells)

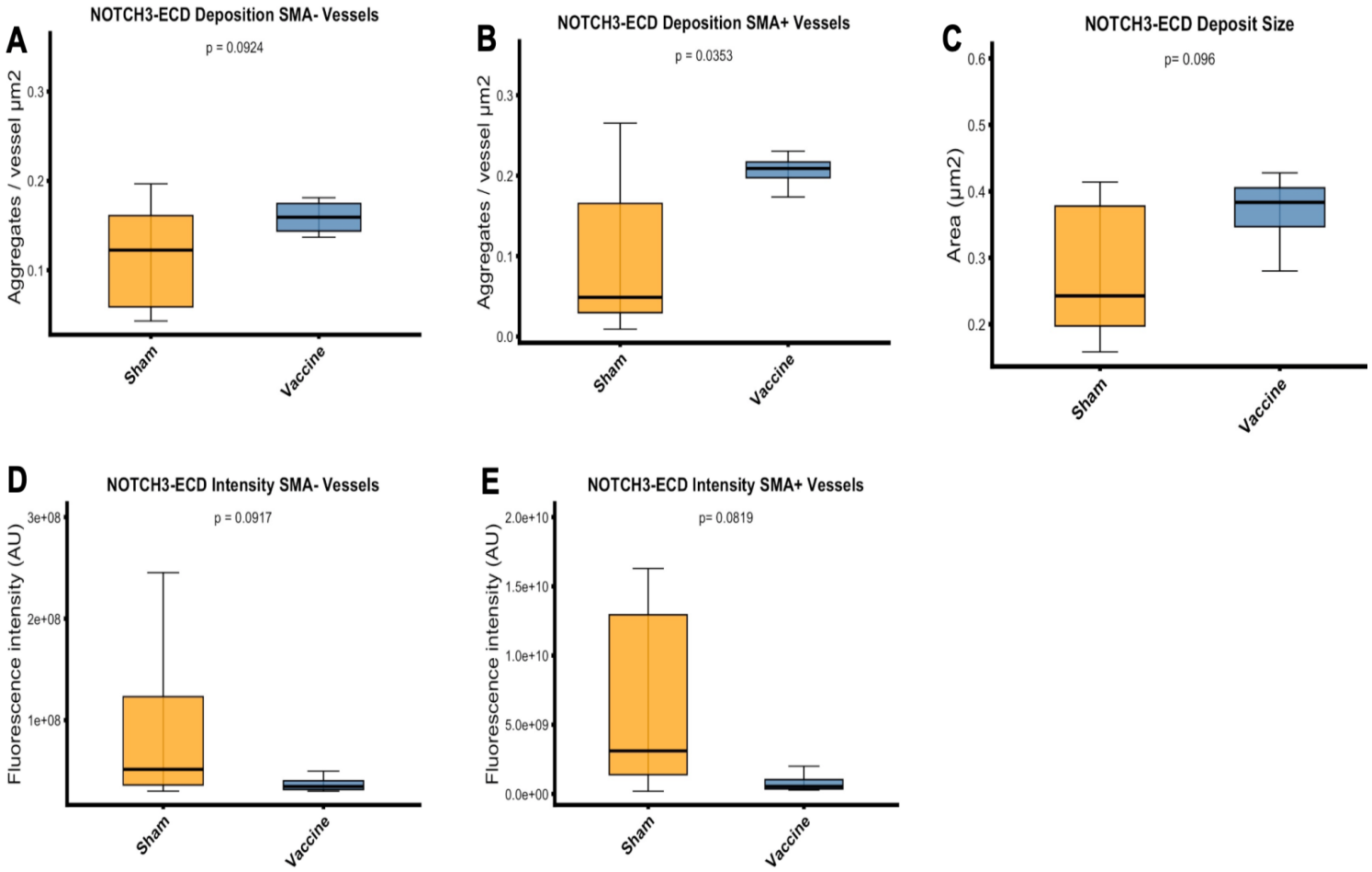
  and SMA (VSMC)
  - Treated: n=6
  - Sham: n=7



- Quantitative analysis
  - **NOTCH3-ECD deposit number in SMA+/- vessels (SMA+ are VSMC; SMA – are PC and EC)**
  - **NOTCH3-ECD deposit size**
  - **NOTCH3-ECD intensity per NOTCH3-ECD area**

Alterations in NOTCH3-ECD deposits

- **Increased** NOTCH3-ECD deposit number in SMA- and + vessels
  - SMA- 39% increase in vaccinated mice
  - SMA+ 102% increase in vaccinated mice
- **Increased** NOTCH3-ECD deposit size
  - 31% increase in vaccinated mice
- **Decreased** NOTCH3-ECD intensity
  - SMA- 60% decrease in vaccinated mice
  - SMA+ 88 % decrease in vaccinated mice



Possible explanations:

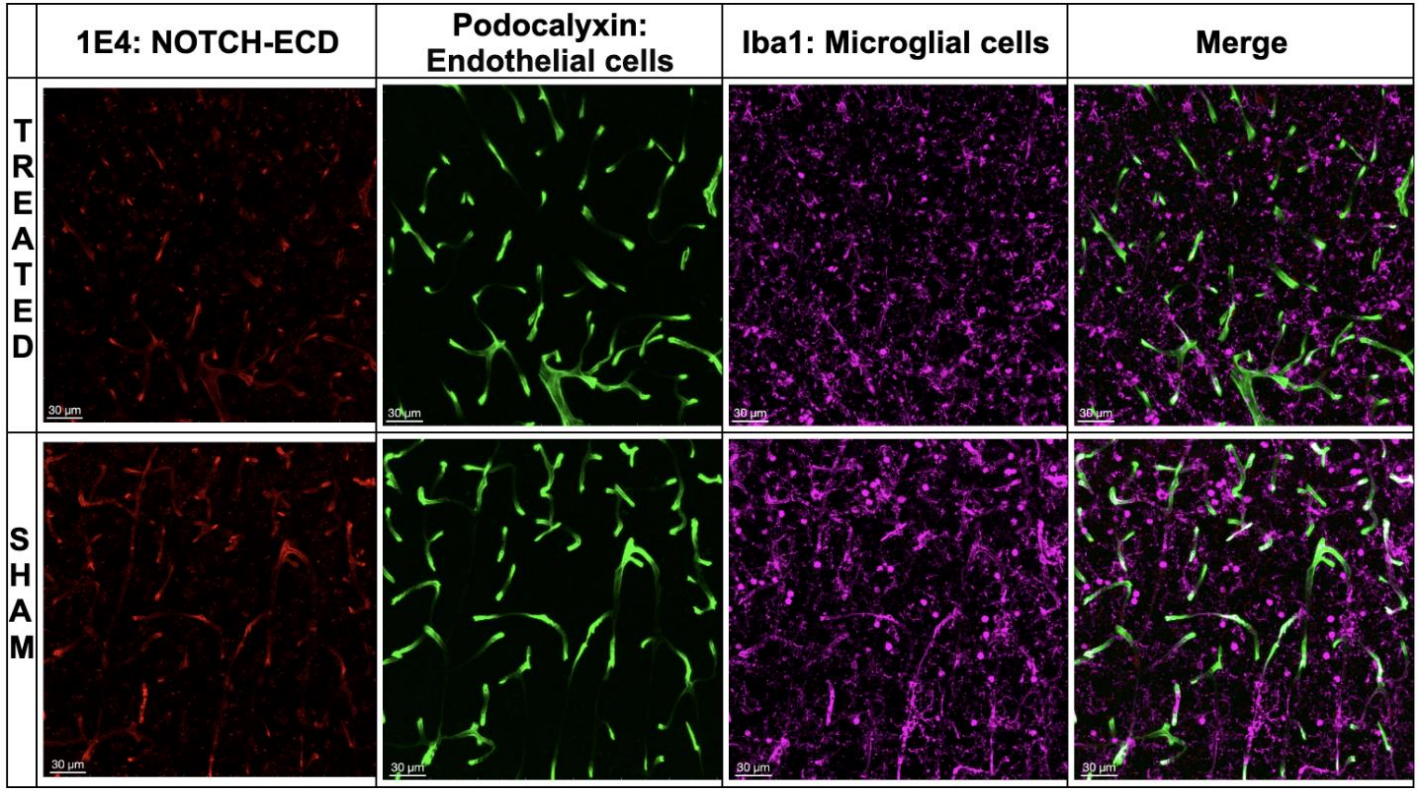
- Antibodies enhancing N3 aggregates?
- Antibodies destabilizing the N3 aggregates– make them bigger but are less dense?

Limitations:

- Spatial heterogeneity of N3 deposits-images aquired randomly
- Low sample size

3. Does vaccination lead to an increased activation of microglial cells?

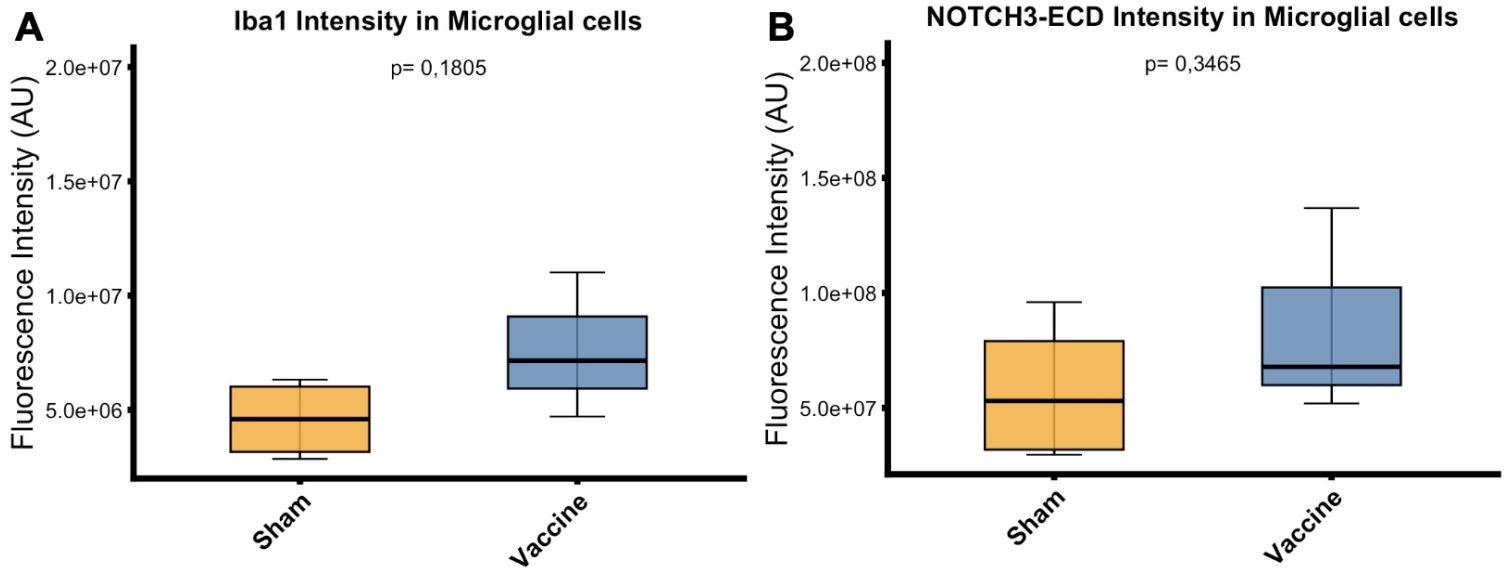
- Immunohistochemistry on 40 um brain free-floating section-staining for Iba1, NOTCH3-ECD, and podocalyxin
  - Treated: n=5
  - Sham: n=5



- Quantitative analysis
  - **Iba1 intensity per Iba1 positive cell area**
  - **NOTCH3-ECD intensity in Iba1 areas**

Increased microglial involvement

- Iba1 intensity per Iba1 positive cell area
  - **66% increase** in vaccinated mice
- NOTCH3-ECD intensity in co-localizing Iba1 positive area
  - **48% increase** in vaccinated mice



➢Activation of microglial cells

➢Phagocytosis of N3 aggregates by Microglial cells?

APPLICABILITY

Staining for NOTCH3-ECD and SMA in human patient tissue

- Immunohistochemistry on CADASIL patient brain and skin sections
  - 4 brain samples and 2 healthy controls
  - 11 skin samples
  - Total 15 mutations: EGF 1-27

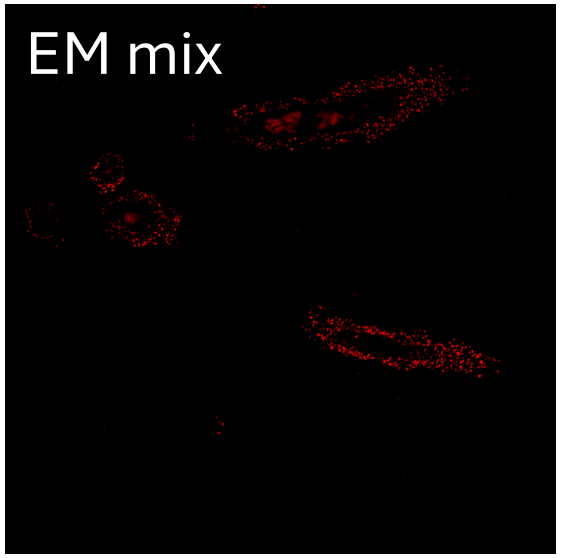
- **Mixture/pool of a number of monoclonal antibodies** generated from vaccinated mice (mimic the polyclonal ab response).

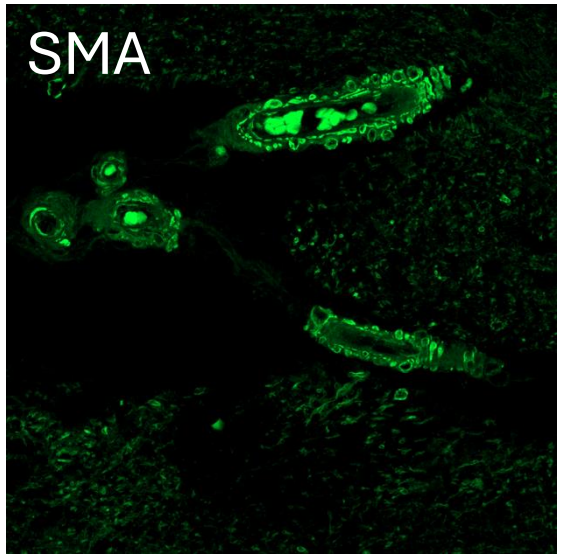
Immunohistochemistry on human brain sections

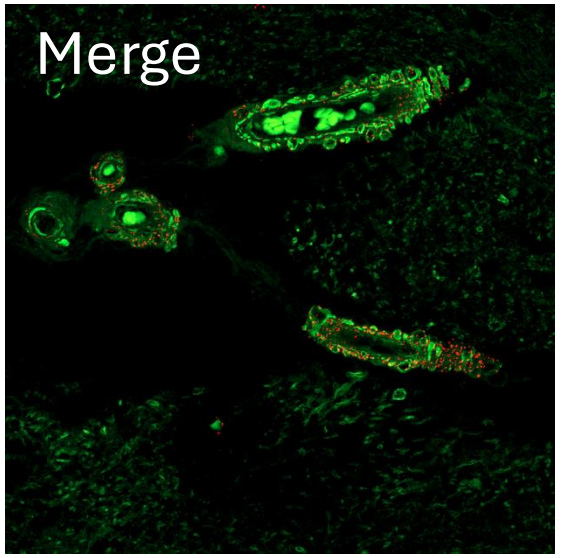
with different CADASIL mutations – (R133C, R153C, R169C)

EGFr 3

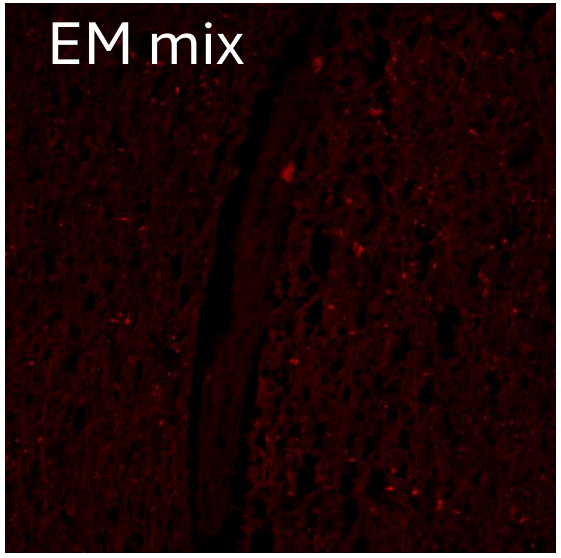
CADASIL R133C

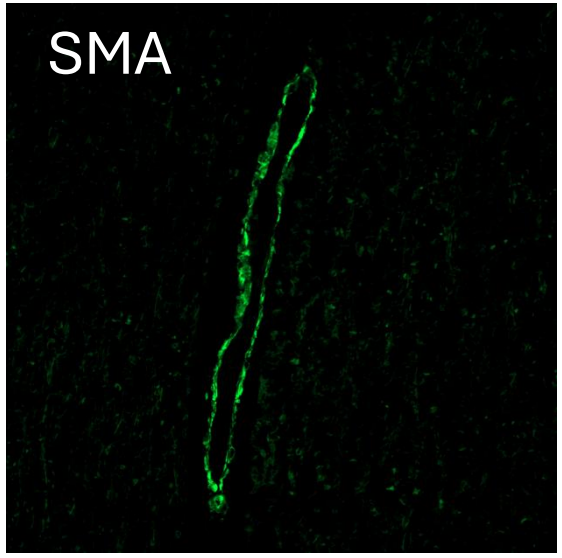


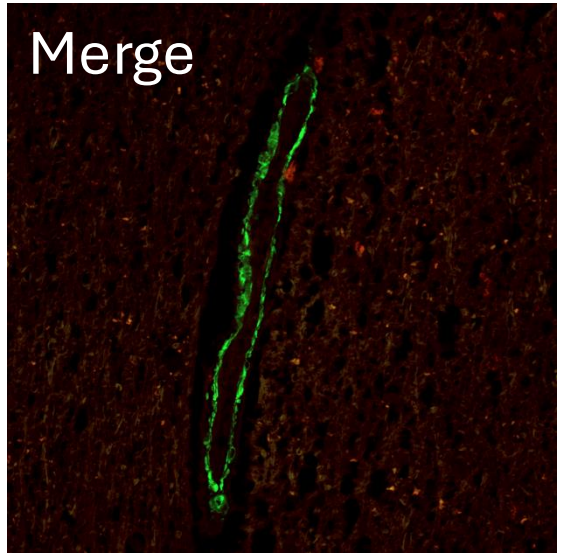




Healthy control







Human
Brain sections:

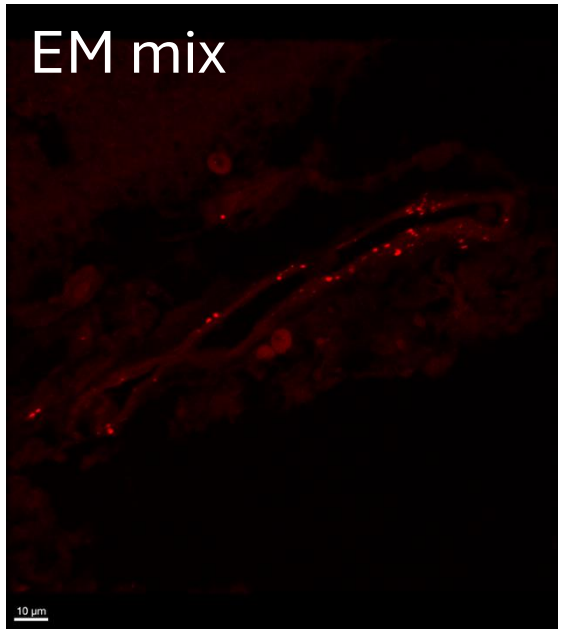
R153C

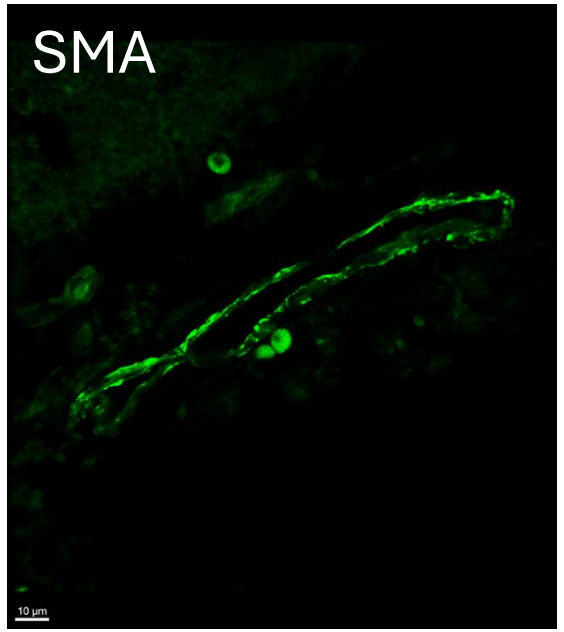
EGFr 4

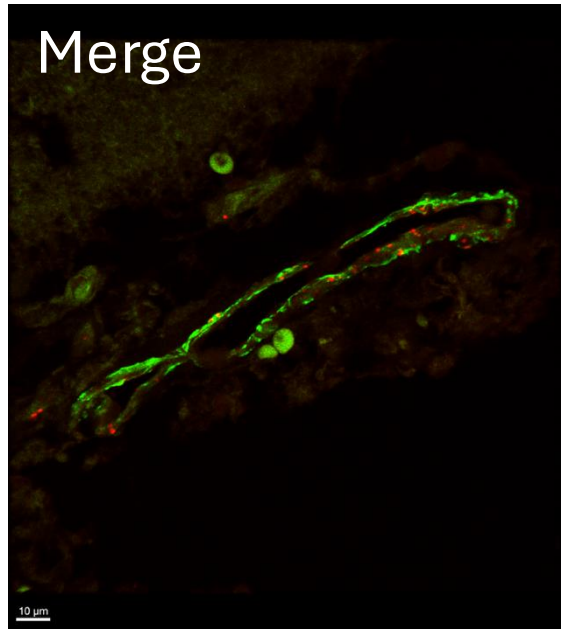
R169C

EGFr 4

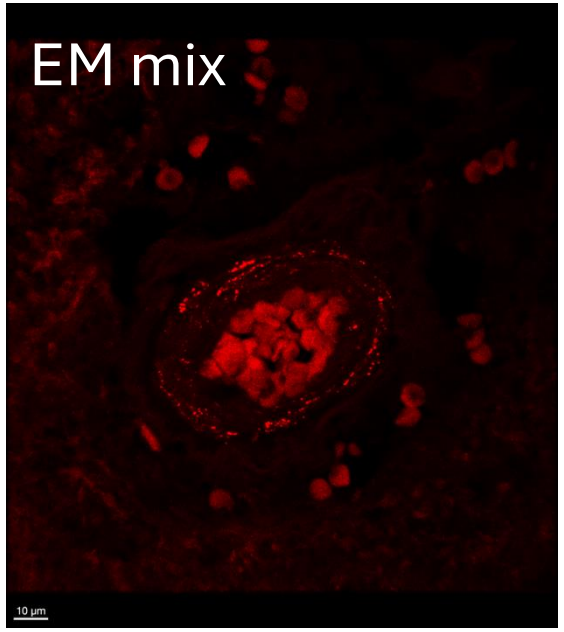
CADASIL R153C mutation

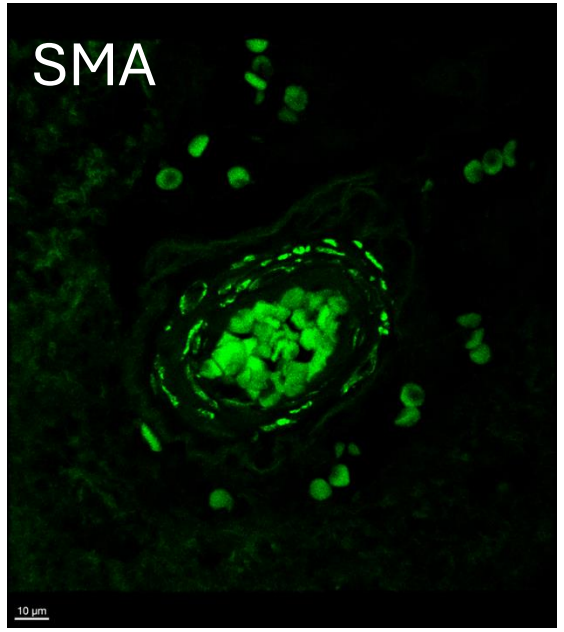


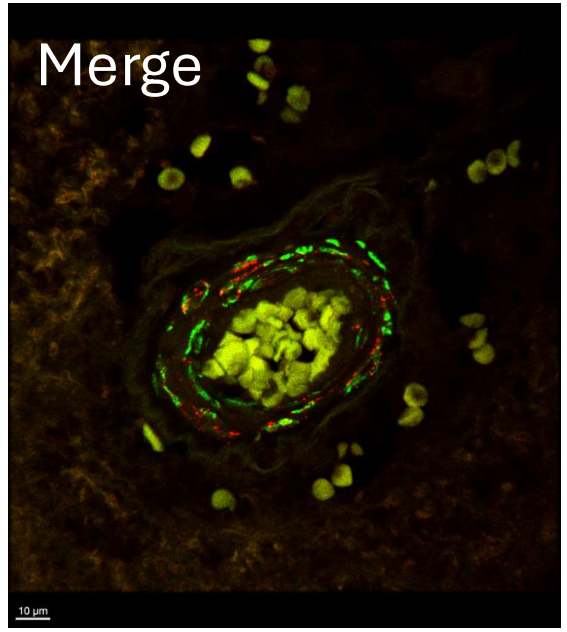




CADASIL R169C mutation



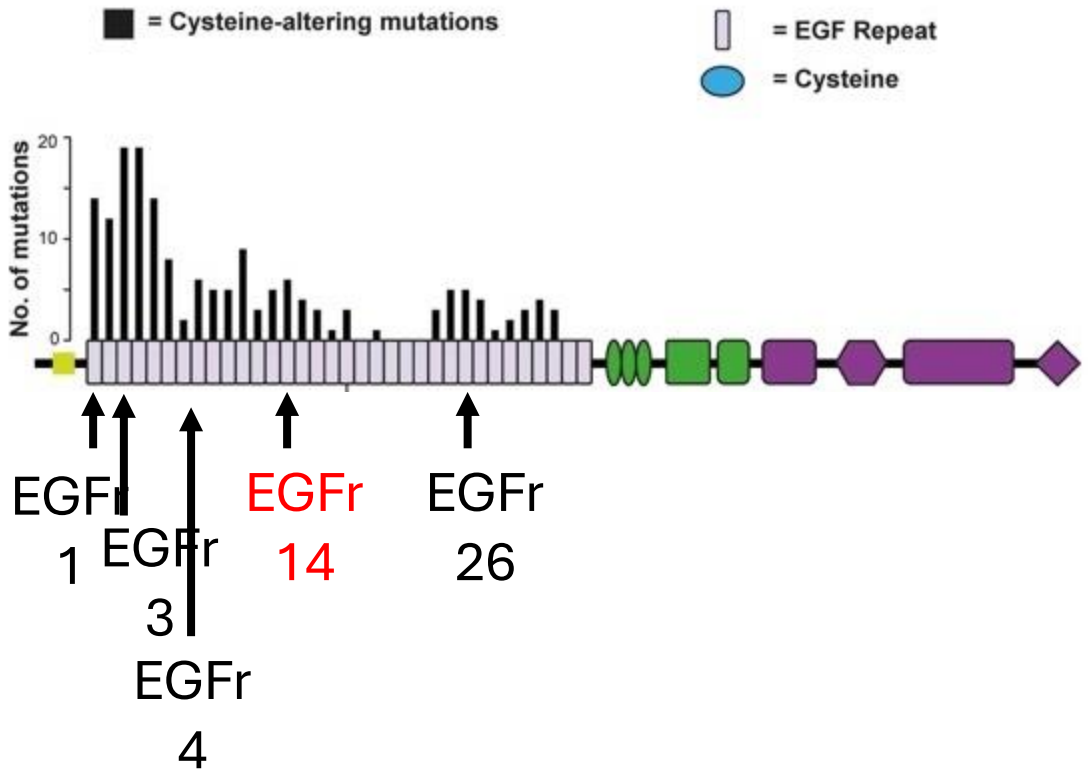




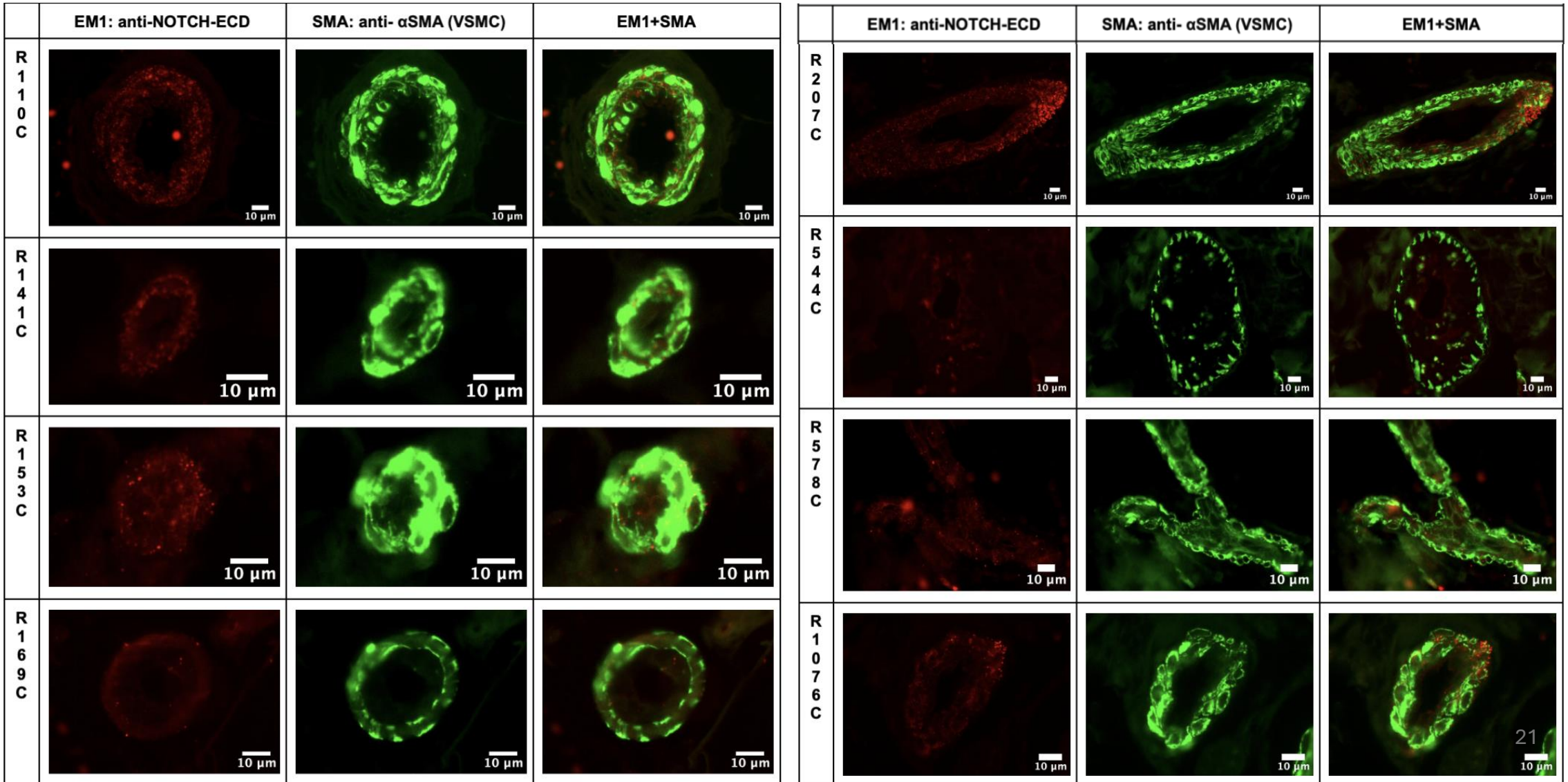
4 more human CADASIL brain sections with different mutations have been tested and most of them stain positive with EM mix ab (tot 6 are positive)

- R133C – EGFr 3
- R153C – EGFr 3
- R169C – EGFr 4

- R54C- EGFr 1
- R558C – EGFr 14?
- C144F – EGFr 3
- C1015R – EGFr 26

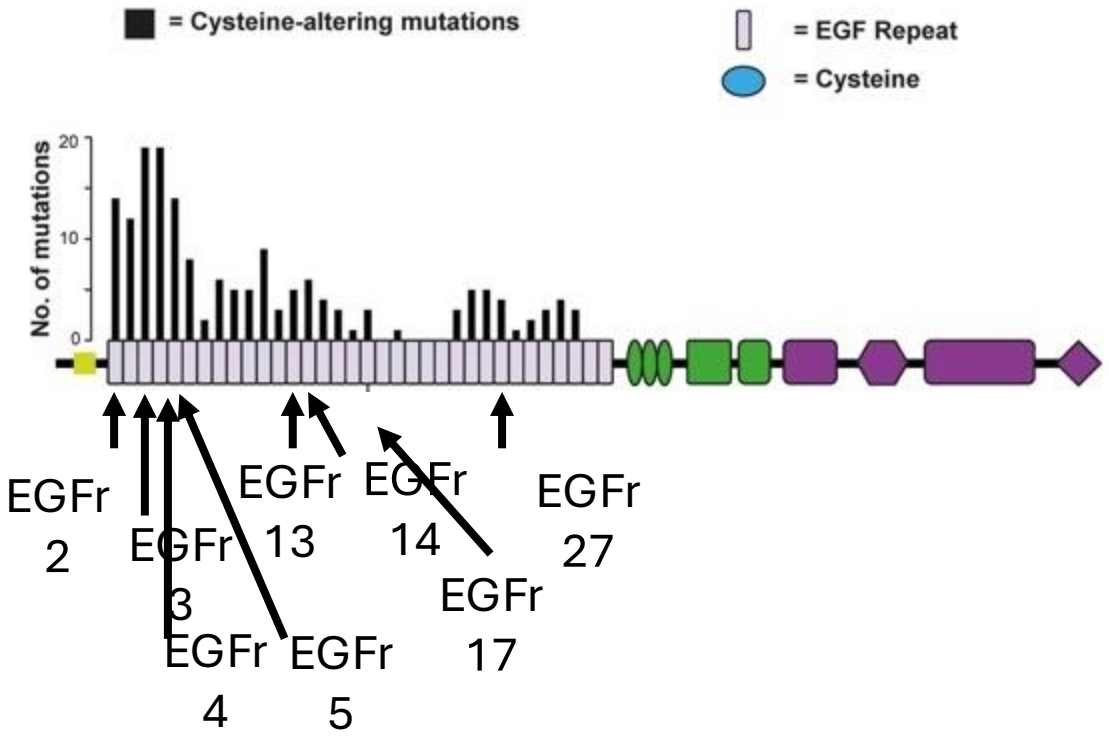


Immunohistochemistry on 8 human skin biopsies with different CADASIL NOTCH3 mutations with EM mix ab



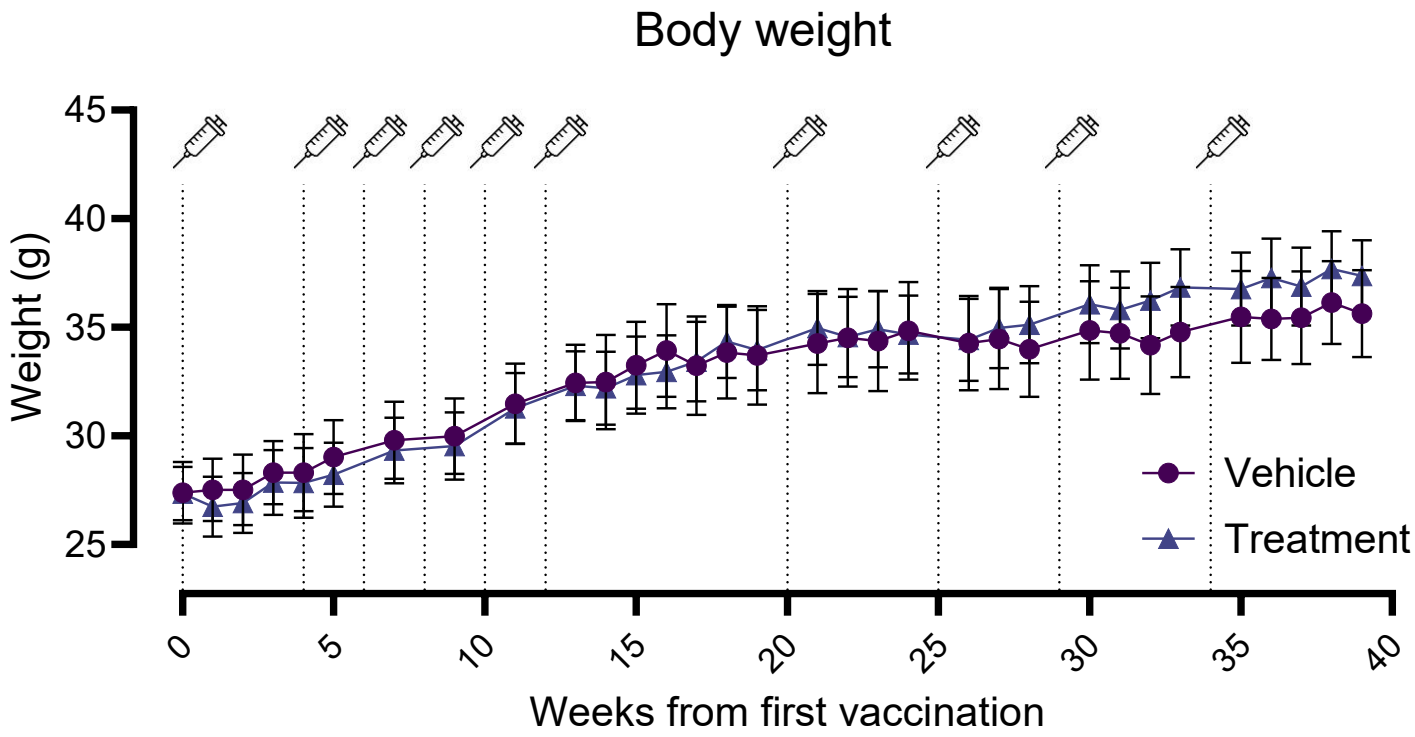
Another 3 human CADASIL skin sections with different mutations have been tested and all of them stain positive with EM mix ab (tot 11 are positive)

- R110C- EGFr 2
- R141C – EGFr 3
- R153C – EGFr 3
- R169C – EGFr 4
- R207C – EGFr 5
- C222Y – EGFr 5
- R544C – EGFr 13
- C568Y – EGFr 14
- R578C – EGFr 14
- G667C – EGFr 17
- R1076C – EGFr 27



TOLERABILITY

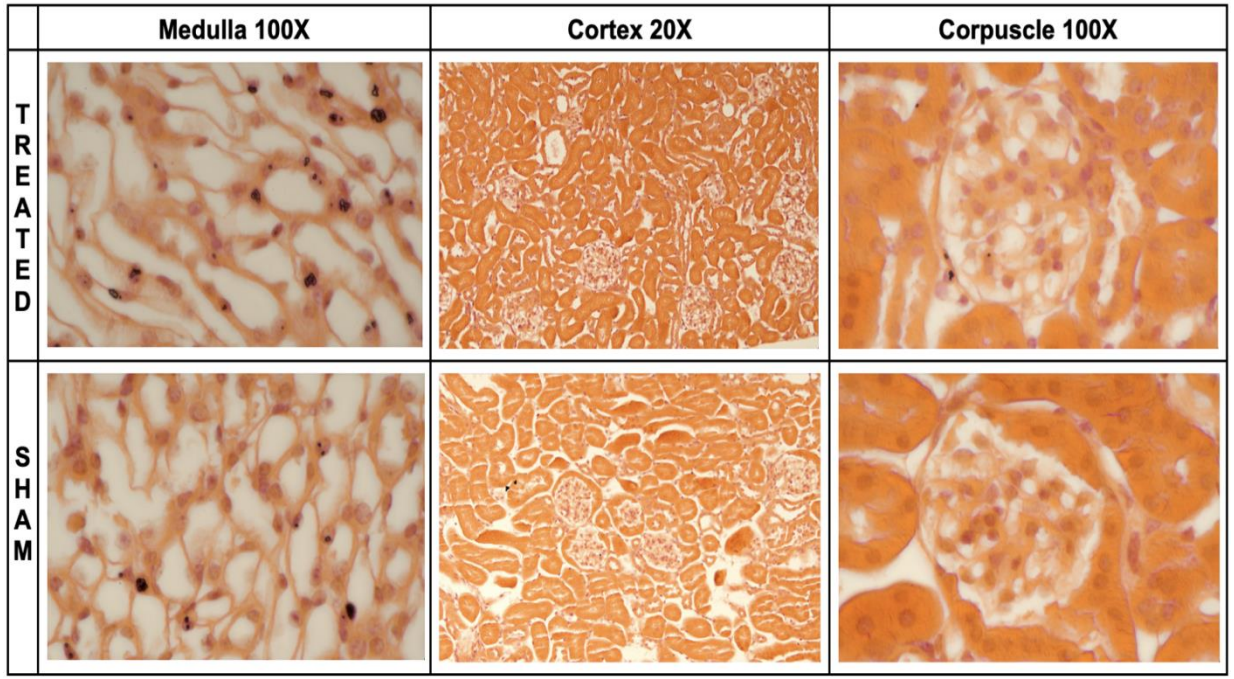
Body weight and vaccination time points (all mice)



TOLERABILITY

H&E staining on kidney- 5µm section

- Treated: n= 3
- Sham: n= 3



No observable toxicity

- No clear morphological differences
- Weak hematoxylin staining
- Distinct medulla and cortex structures

CONCLUSIONS so far…..

EFFICACY

- Partially Increased serum antibody response & decreased N3-ECD in vaccinated mice in serum
- The vaccination seems to alter the pattern of NOTCH3-ECD deposition (ab response correlation?)
- Possible activation of microglial and phagocytosis

APPLICABILITY

- Monoclonal EM mix generated from vaccinated mice recognize NOTCH3-ECD deposits across 14 different human CADASIL mutations – good applicability

TOLERABILITY

- The vaccination does not affect body weight or morphologic toxicity in kidney

NEXT STEPS

- **Continue to analyse all mice in more brain areas and increase number of sections/brain**
- **Retina-vessel analysis – does it mirror the brain vessels?**
- **Biomarker analysis in the blood (using Olink, MSD etc platforms)-disease onset, progression and therapeutic markers**
- **Evaluate the monoclonal antibodies in more detail – future Passive immunization study in mice**

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