Tolerogenic APC-targeted Vaccibody™ Vaccines Treat Disease in Mouse Models of Experimental Autoimmune Encephalomyelitis and Non-Obese Diabetes

Louise Bjerkan, Anthony Ravussin, Joel B. Heim, Dipankar Manna, Aleksandra Urban, Emmanuelle Benard, Linn Guro Olsen, Ruixia Huang, Audun Bersaas, Stine Granum, Mikkel W. Pedersen, Håkan Norell, Agnete B. Fredriksen and *Henrik Søndergaard

*Corresponding author: hsondergaard@nykode.com
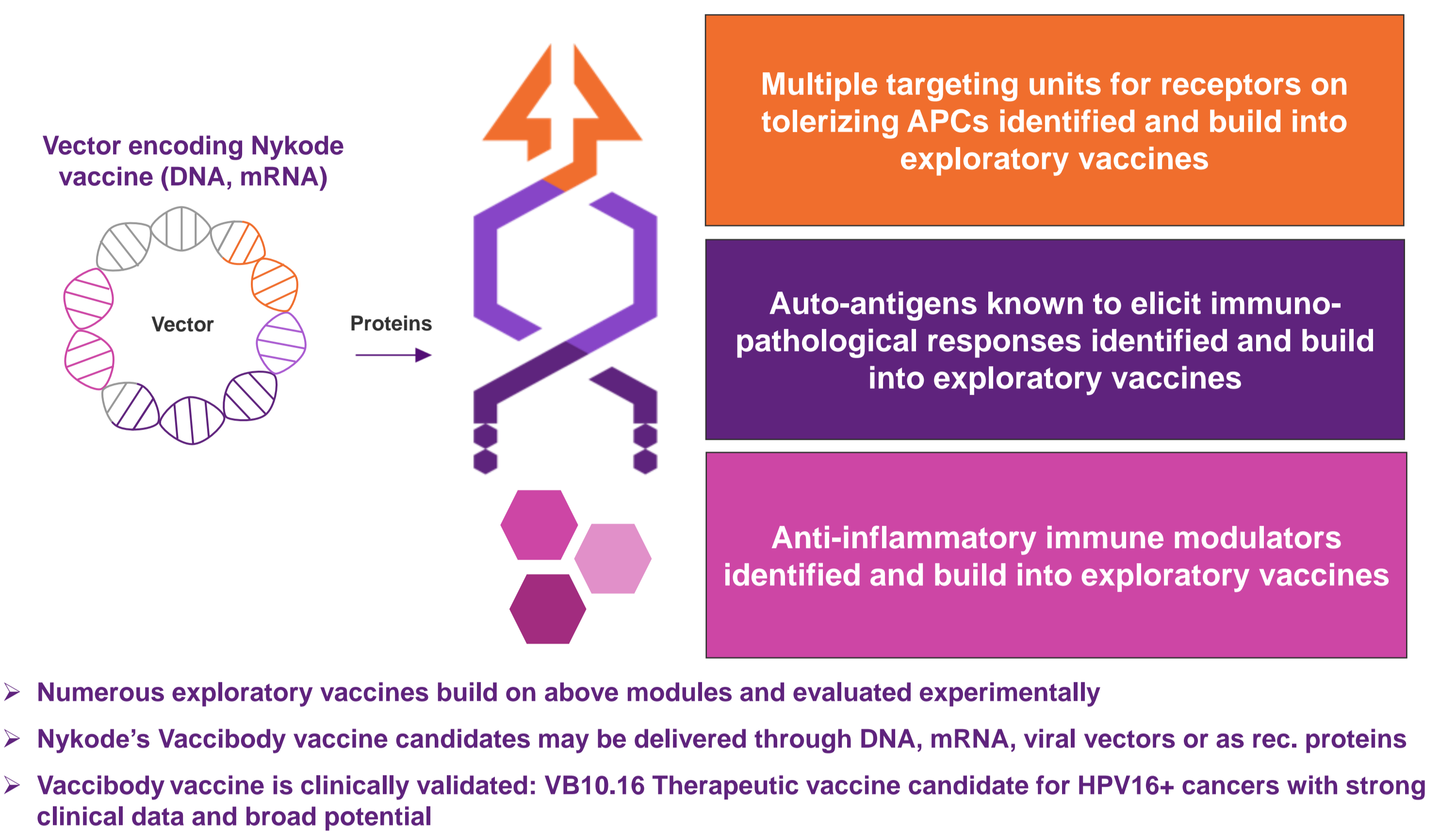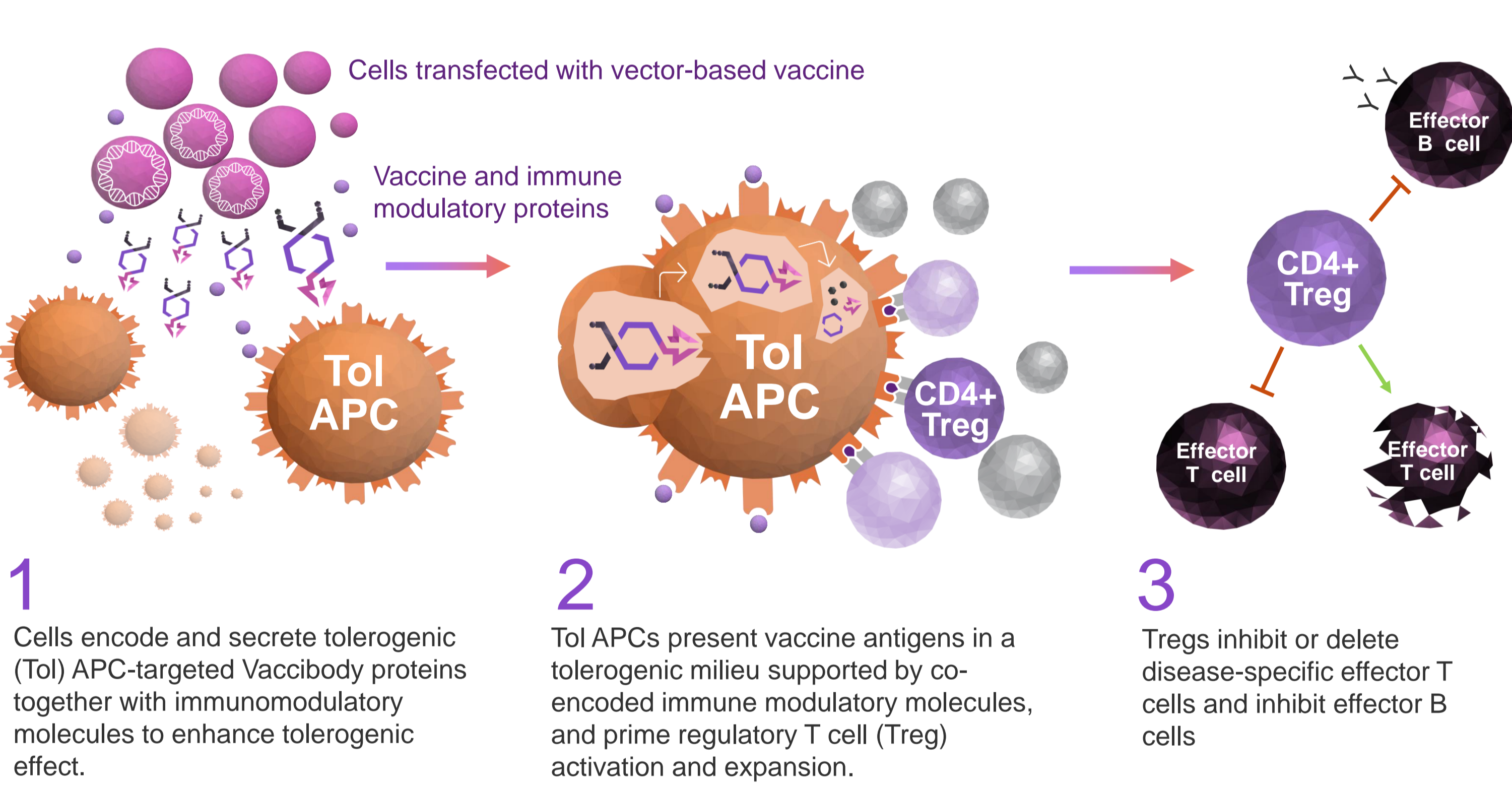Nykode Therapeutics ASA, Oslo, Norway

Poster #Th112

Introduction

Nykode Therapeutics has developed a platform that targets antigens directly to antigen presenting cells (APCs) using a modular dimeric protein format known as a Vaccibody™.

Here, Vaccibody vaccines were designed to deliver a tolerogenic response toward disease-associated antigens via specific APC-receptor-targeting. The vaccines were tested for their tolerogenic potential in the Experimental Autoimmune Encephalomyelitis (EAE) model and in Non-Obese Diabetic (NOD) mice either alone or combined with co-expression of immune-modulatory proteins in a multicistronic plasmid DNA.
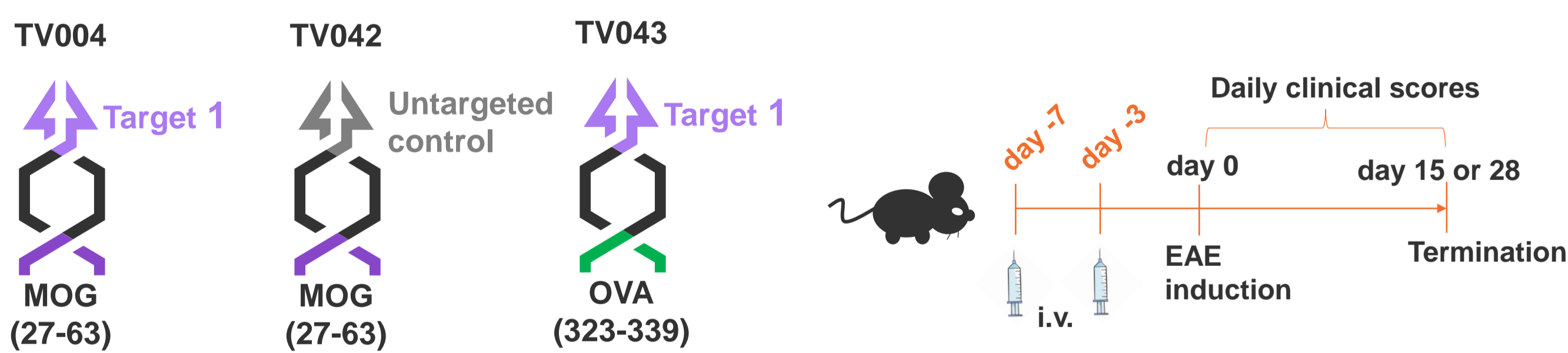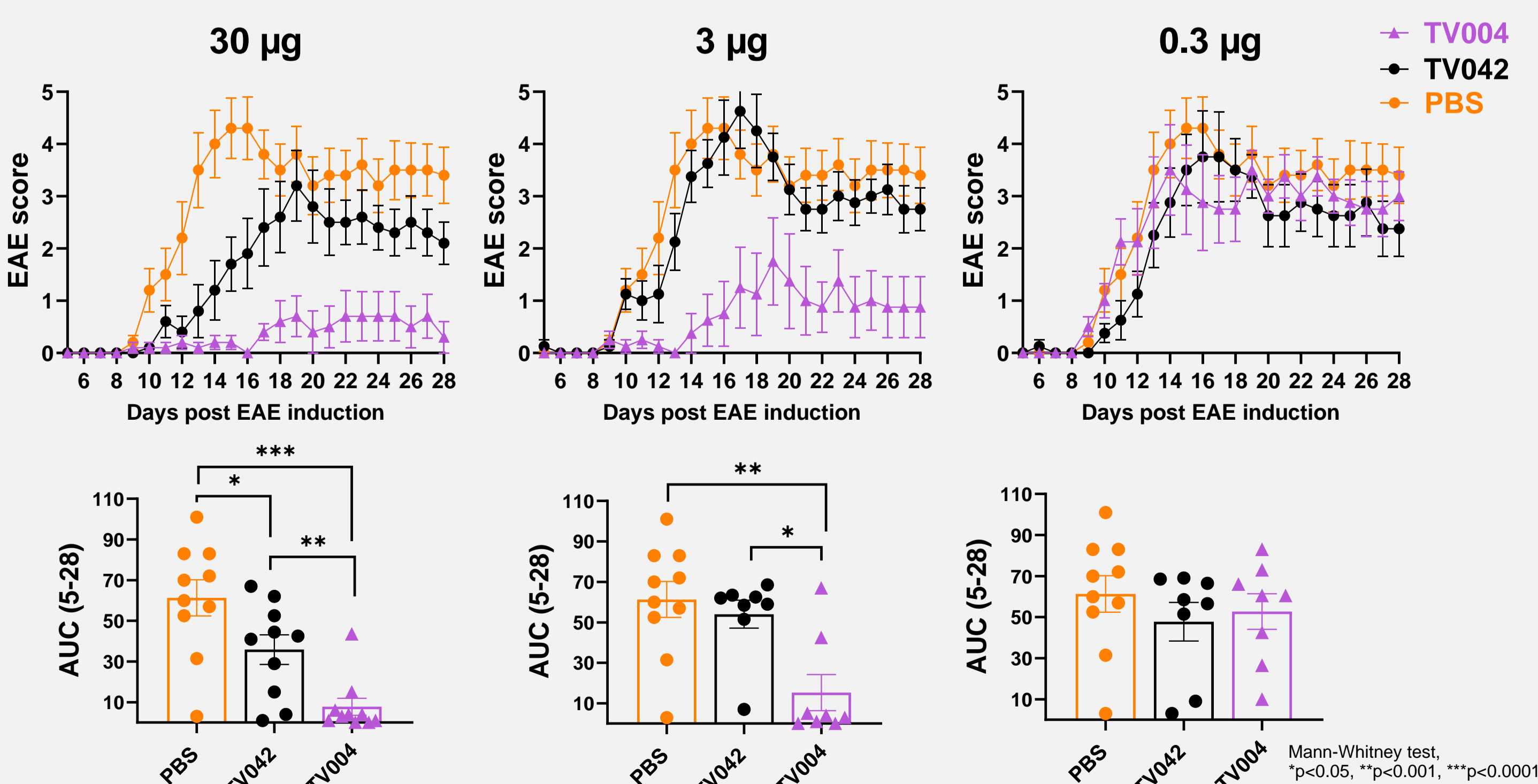
NYKODE MODULAR VACCINE DESIGN

Vector encoding Nykode vaccine (DNA, mRNA) → Proteins

- Multiple targeting units for receptors on tolerizing APCs identified and build into exploratory vaccines
- Auto-antigens known to elicit immuno-pathological responses identified and build into exploratory vaccines
- Anti-inflammatory immune modulators identified and build into exploratory vaccines

➢ Numerous exploratory vaccines build on above modules and evaluated experimentally
➢ Nykode's Vaccibody vaccine candidates may be delivered through DNA, mRNA, viral vectors or as rec. proteins
➢ Vaccibody vaccine is clinically validated: VB10.16 Therapeutic vaccine candidate for HPV16+ cancers with strong clinical data and broad potential

NYKODE: TOLERANCE INDUCTION MOA

Cells transfected with vector-based vaccine
Vaccine and immune modulatory proteins

1 Cells encode and secrete tolerogenic (Tol) APC-targeted Vaccibody proteins together with immunomodulatory molecules to enhance tolerogenic effect.

2 Tol APCs present vaccine antigens in a tolerogenic milieu supported by co-encoded immune modulatory molecules, and prime regulatory T cell (Treg) activation and expansion.

3 Tregs inhibit or delete disease-specific effector T cells and inhibit effector B cells

EFFECT OF NYKODE VACCINE IN EAE MOUSE MODEL

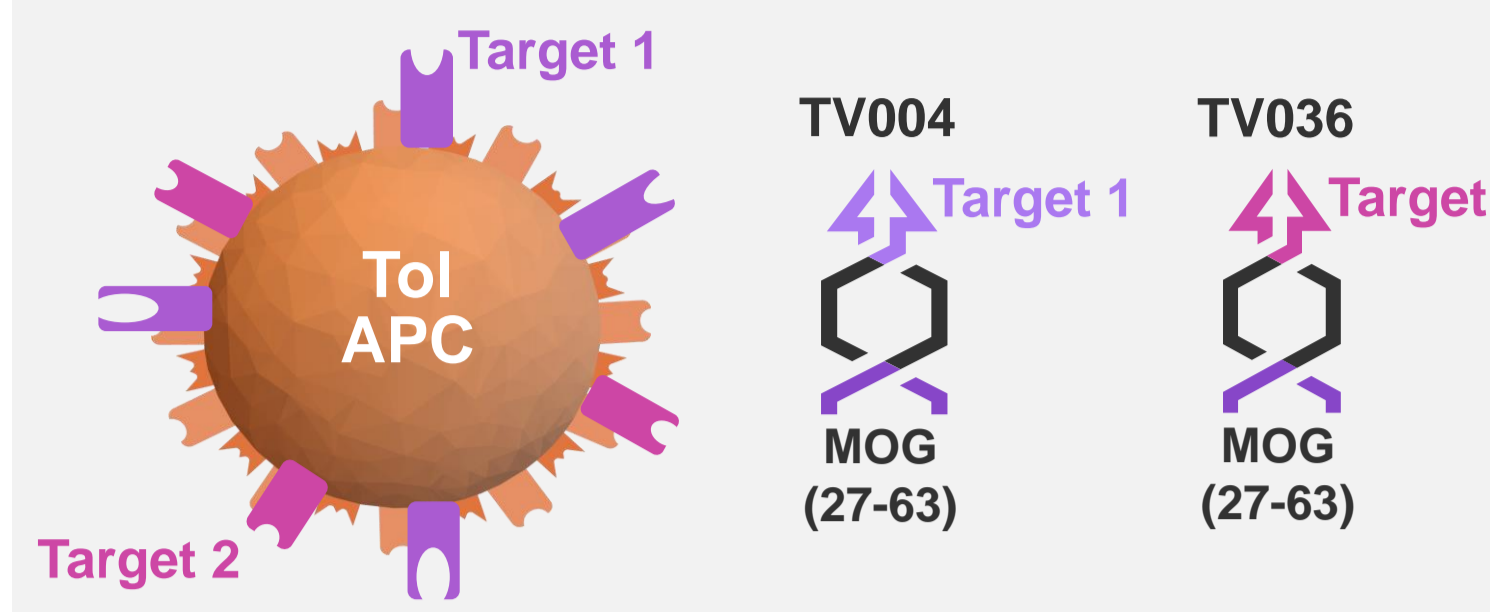➢ Recombinant Vaccibody in MOG(35-55)/CFA + PTX-induced EAE in C57BL/6 mice

TV004 — Target 1 — MOG (27-63)
TV042 — Untargeted control — MOG (27-63)
TV043 — Target 1 — OVA (323-339)

day -7, day -3 i.v. → day 0 EAE induction → Daily clinical scores → day 15 or 28 Termination

1. APC targeting by Vaccibody is required for effective disease protection in the EAE model

30 µg / 3 µg / 0.3 µg — TV004, TV042, PBS
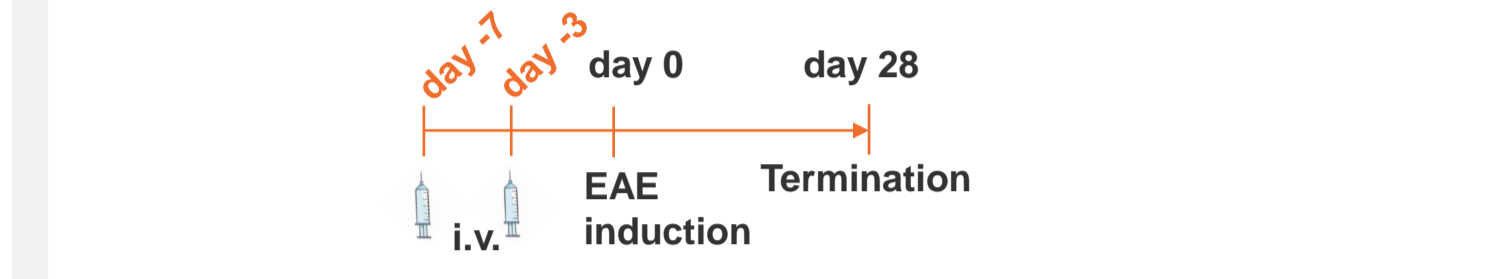
Mann-Whitney test, *p<0.05, **p<0.001, ***p<0.0001

2. Vaccibody delivers antigen-specific suppression of EAE

Paralysis — TV004-MOG, TV043-OVA, PBS

3. Vaccibody vaccine delivers dose-dependent effect on antigen-specific disease-associated cytokine-release

4 µg TV004, 12 µg TV004, 35 µg TV004, PBS

IFNγ [pg/ml] — PBS, TV004 4µg, TV004 12µg, TV004 35µg
IL-17 [pg/ml] — PBS, TV004 4µg, TV004 12µg, TV004 35µg

Mann-Whitney test, **P < 0.01, ***P < 0.001.

5. Vaccibody delivers therapeutically effective disease protection, in contrast to equimolar dose of antigen peptide alone

day 0 → day7, day10 i.v. → day 20
EAE induction → Termination

Paralysis — TV004 30 µg, TV004 3 µg, MOG(27-63) 3 µg, PBS
AUC (5-15) — PBS, MOG(27-63) 3 µg, TV004 30 µg, TV004 3 µg
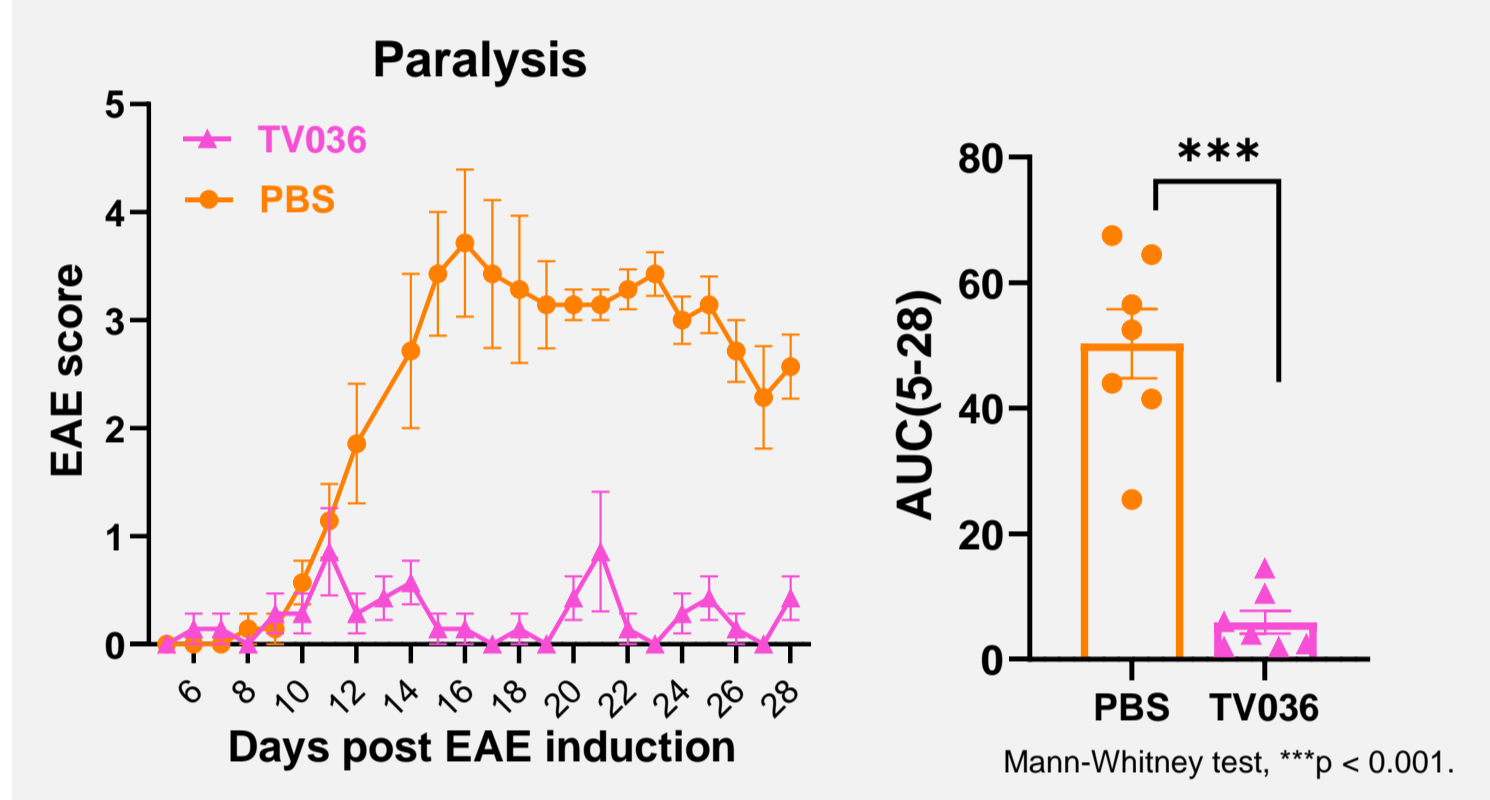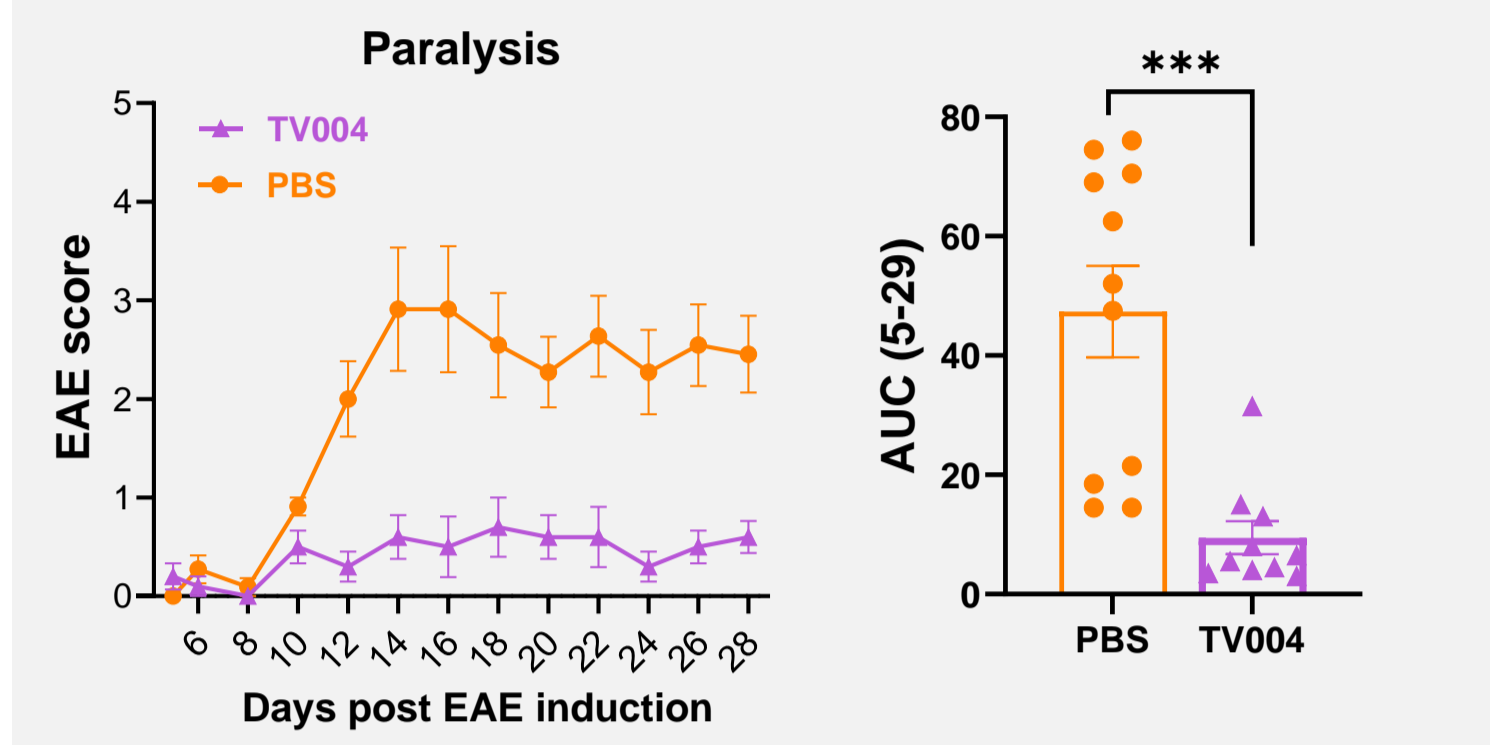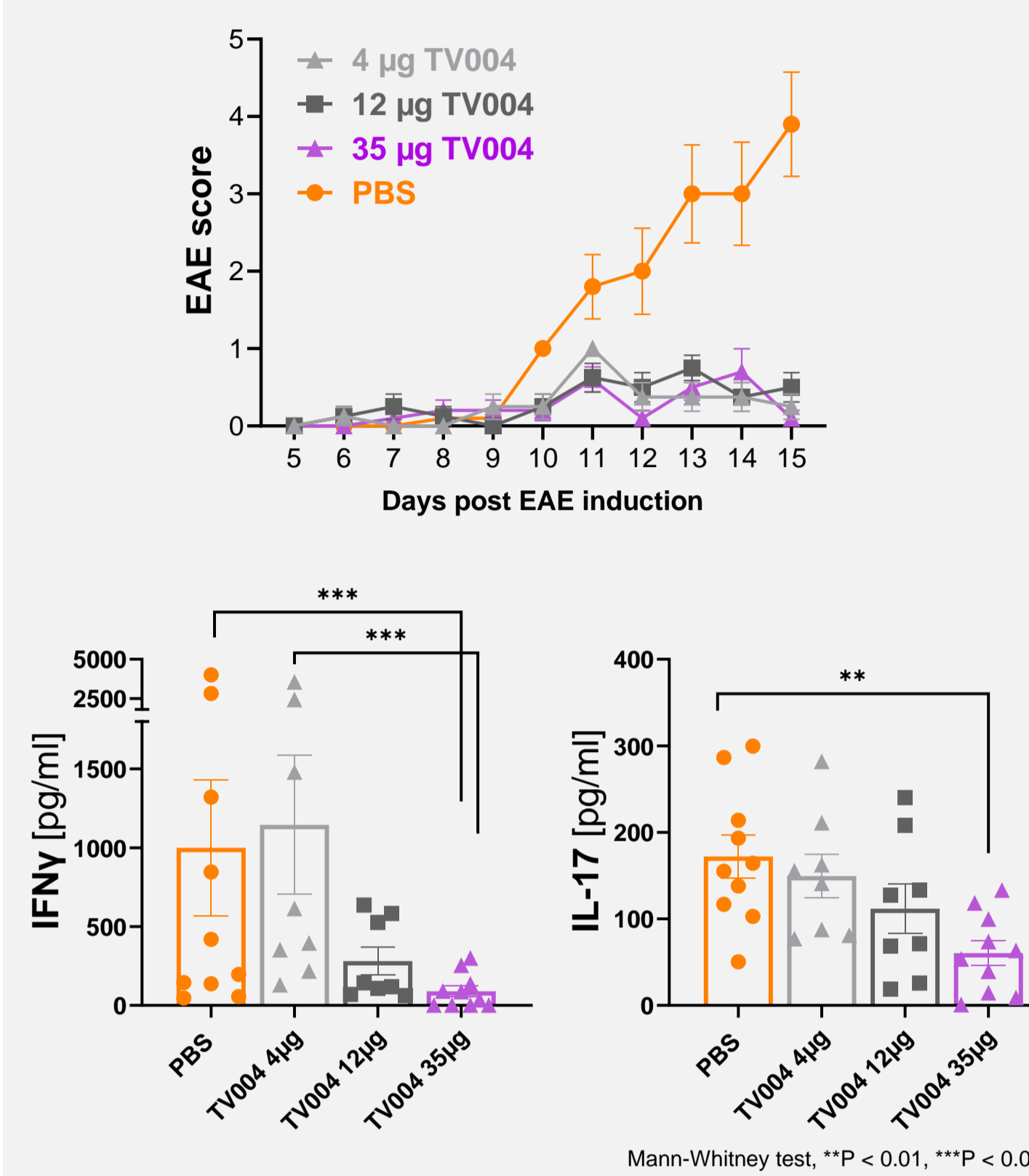
Mann-Whitney test, **p<0.01

4. Vaccibody targeting different receptors on APCs is effective in both a preventive and an early therapeutic setting in EAE

Tol APC — Target 1, Target 2
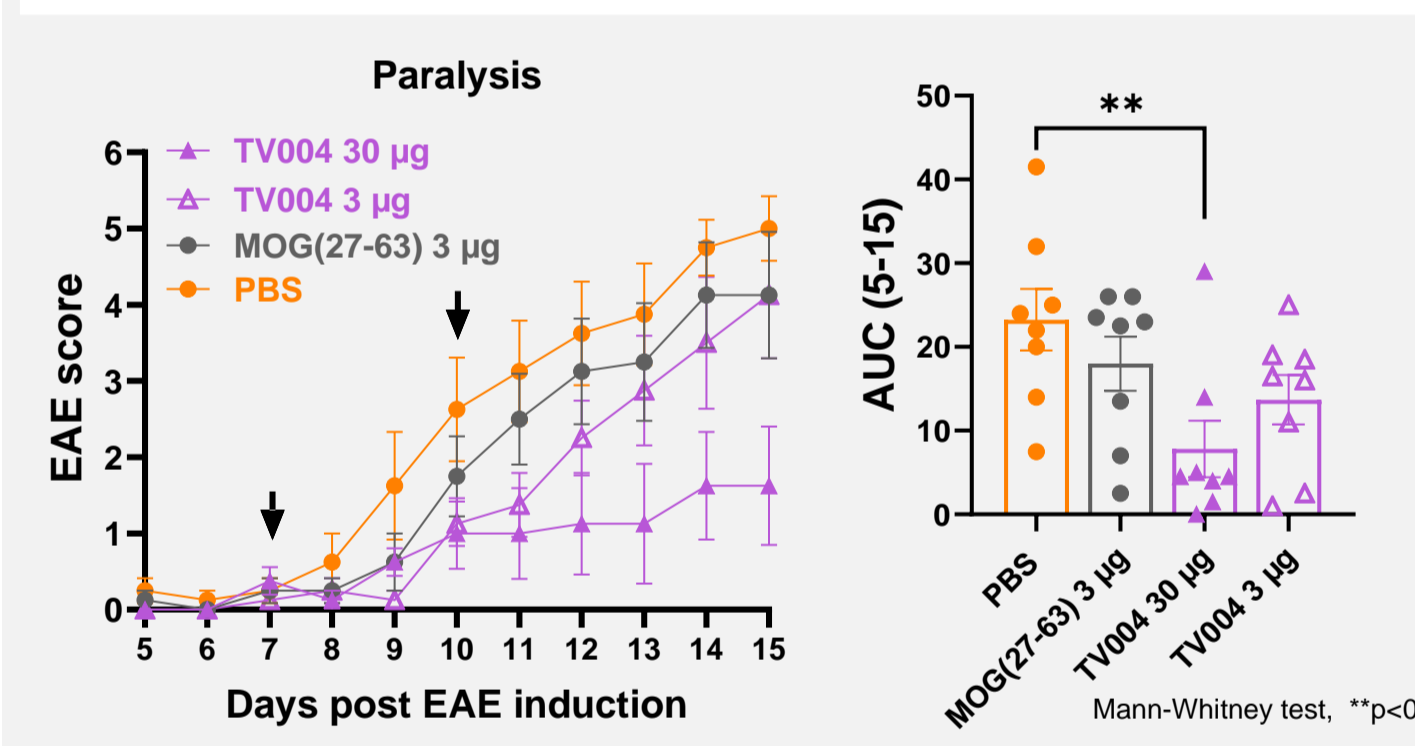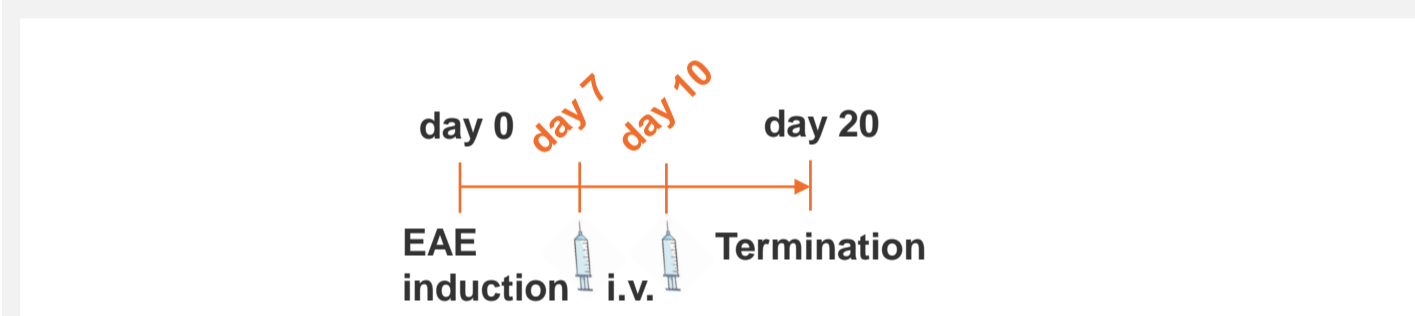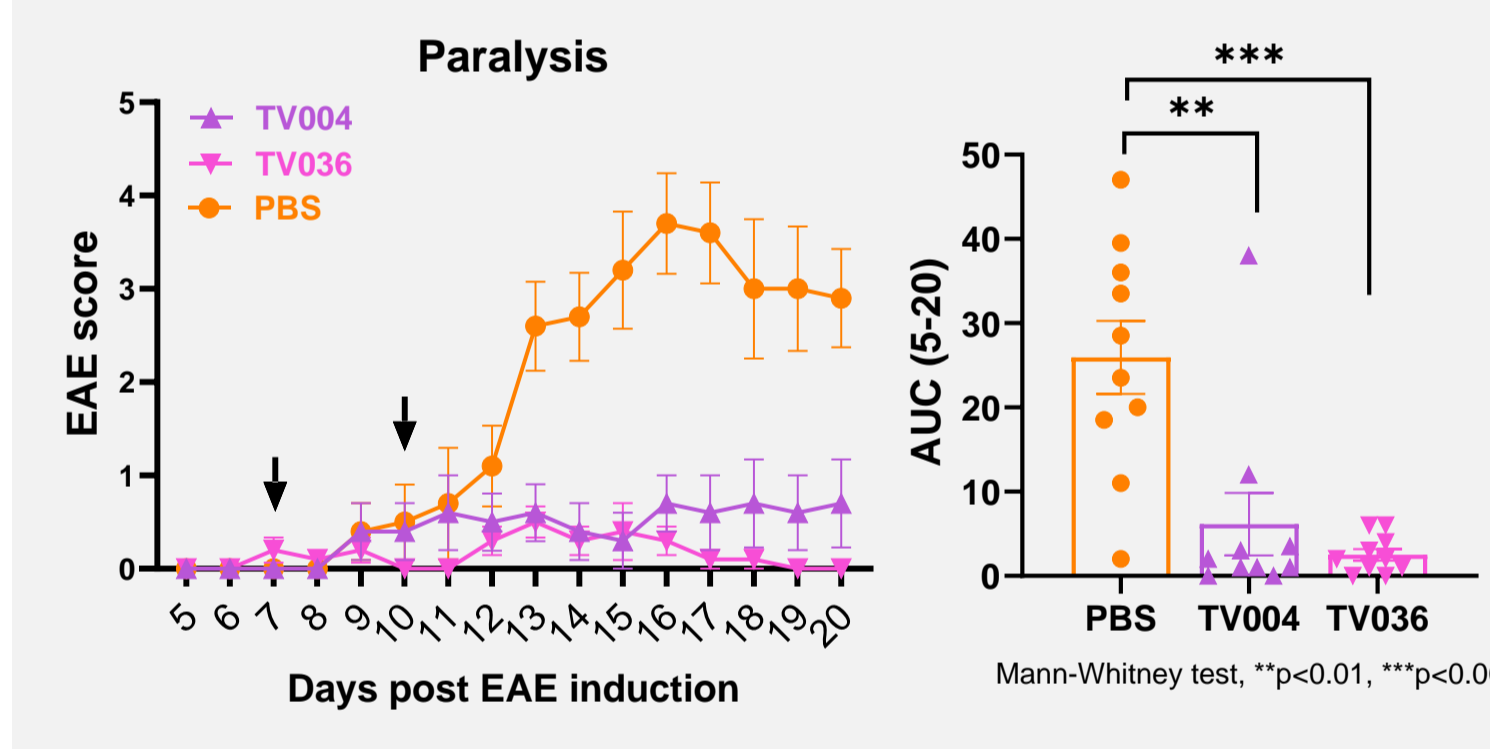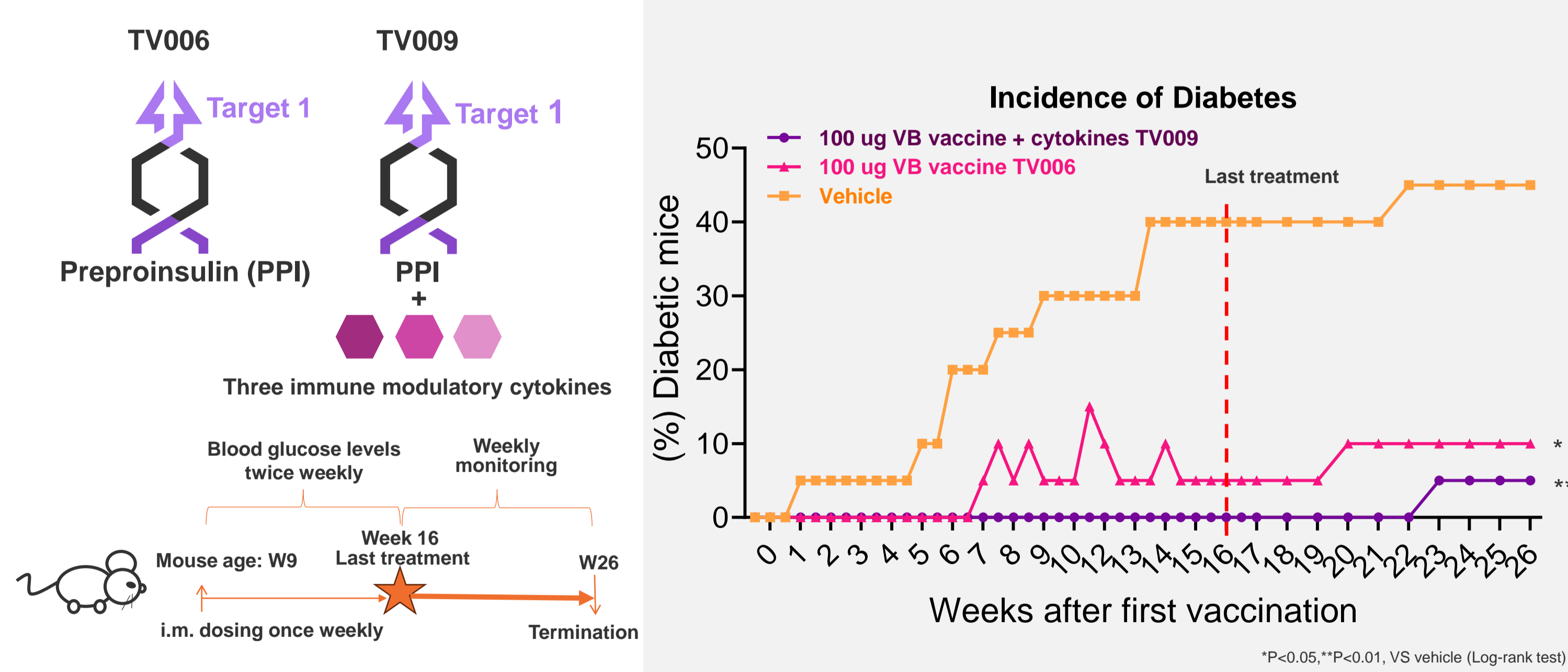
TV004 — Target 1 — MOG (27-63)
TV036 — Target 2 — MOG (27-63)

Preventive vaccination

day -7, day -3 i.v. → day 0 EAE induction → day 28 Termination

Paralysis — TV004, PBS — AUC (5-29) — PBS, TV004 ***
Paralysis — TV036, PBS — AUC (5-28) — PBS, TV036 ***

Mann-Whitney test, ***p < 0.001.

Early therapeutic vaccination

day 0 EAE induction → day7, day10 i.v. → day 20 Termination

Paralysis — TV004, TV036, PBS — AUC (5-20) — PBS, TV004, TV036

Mann-Whitney test, **p<0.01, ***p<0.001.
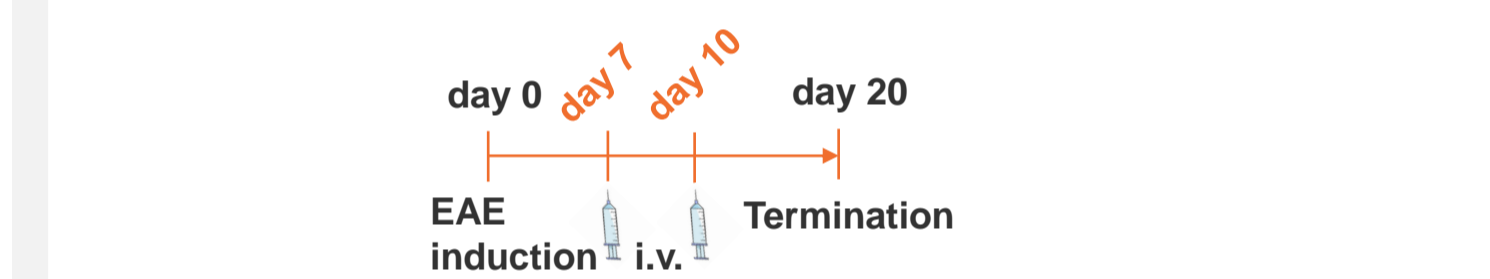
EFFECT OF NYKODE VACCINE IN NOD MOUSE MODEL

➢ DNA vaccination with Vaccibody targeting tolerizing APCs show durable prevention of diabetes in NOD mice

TV006 — Target 1 — Preproinsulin (PPI)
TV009 — Target 1 — PPI + Three immune modulatory cytokines

Blood glucose levels twice weekly, Weekly monitoring
Mouse age: W9 | Week 16 Last treatment | W26
i.m. dosing once weekly → Termination

Incidence of Diabetes
- 100 ug VB vaccine + cytokines TV009
- 100 ug VB vaccine TV006
- Vehicle

*P<0.05,**P<0.01, VS vehicle (Log-rank test)

CONCLUSION

These data demonstrate the flexibility of novel Vaccibody vaccines tailored to target specific APC receptors and deliver potent tolerogenic responses in two different mouse models of autoimmune disease.

NEXT AND ONGOING

In-depth analysis to further dissect the immunological and molecular mechanisms behind the disease control mediated by tolerogenic Vaccibody vaccine, including:

➢ Further evaluate potency and efficacy of later therapeutic delivery of Vaccibody in the EAE and NOD model
➢ Immunological mode of action with focus on Tregs
➢ Assess Vaccibody potential for inducing bystander suppression