

Supplementary Material

Development of a bispecific antibody-based platform for retargeting of capsid modified AAV vectors

Juliane Kuklik ¹, Stefan Michelfelder ², Felix Schiele ³, Sebastian Kreuz ^{2,4}, Thorsten Lamla ⁵, Philipp Müller ^{4,†} and John E. Park ^{1,*}

¹ Division of Cancer Immunology and Immune Modulation, Boehringer Ingelheim Pharma GmbH & Co. KG, 88387 Biberach an der Riss, Germany; juliane.kuklik@boehringer-ingelheim.com

² Division of Research Beyond Borders, Boehringer Ingelheim Pharma GmbH & Co. KG, 88387 Biberach an der Riss, Germany; stefan.michelfelder@boehringer-ingelheim.com (S.M.); sebastian.kreuz@boehringer-ingelheim.com (S.K.)

³ Division of Biotherapeutics Discovery, Boehringer Ingelheim Pharma GmbH & Co. KG, 88387 Biberach an der Riss, Germany; felix.schiele@boehringer-ingelheim.com

⁴ Boehringer Ingelheim Venture Fund GmbH, 55216 Ingelheim am Rhein, Germany; philipp_3.mueller@boehringer-ingelheim.com

⁵ Division of Drug Discovery Sciences Biberach, Boehringer Ingelheim Pharma GmbH & Co. KG, 88387 Biberach an der Riss, Germany; thorsten.lamla@boehringer-ingelheim.com

* Correspondence: john.park@boehringer-ingelheim.com

† These authors contributed equally to this work.

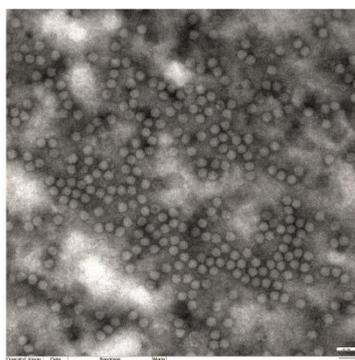


Figure 1. Transmission electron microscopy of negative stained rAAV-2E3.v6 viral particles, uncropped image. Scalebar 50 μ m.

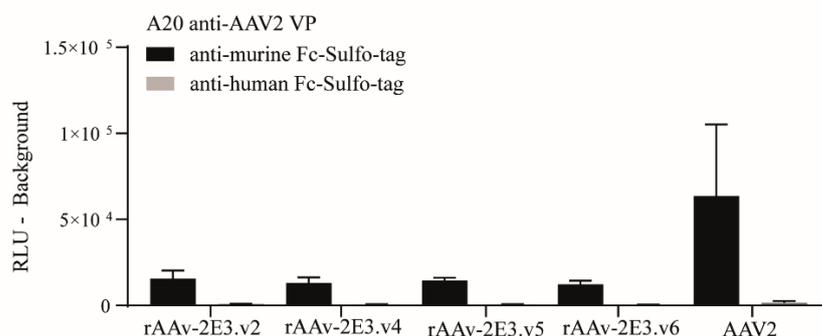


Figure 2. MSD ELISA control staining of immobilized rAAV-2E3.v2, -v4, -v5, -v6 and AAV2 following incubation with A20 anti-VP antibody and anti-murine Fc-Sulfo-tag secondary antibody. Loading control of Figure 2E. The secondary anti-human Fc-Sulfo-tag was used as isotype control. Data represent mean + SD of three independent experiments.

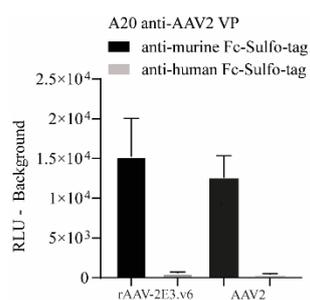


Figure 3. MSD ELISA control staining of immobilized rAAV-2E3.v6 and AAV2 following incubation with A20 anti-VP antibody and anti-murine Fc-Sulfo-tag secondary antibody. Loading control of Figure 4D The secondary anti-human Fc-Sulfo-tag was used as isotype control. Data show mean \pm SD of three independent experiments.

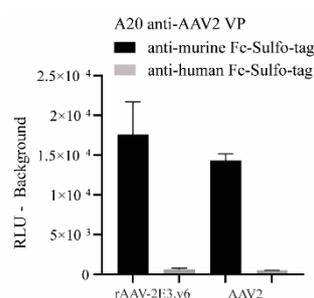


Figure 4. MSD ELISA control staining immobilized rAAV-2E3.v6 and AAV2 following incubation with A20 anti-VP antibody and anti-murine Fc-Sulfo-tag secondary antibody, loading control of Figure 7C. The secondary anti-human Fc-Sulfo-tag was used as isotype control. Data show mean \pm SD of two independent experiments.

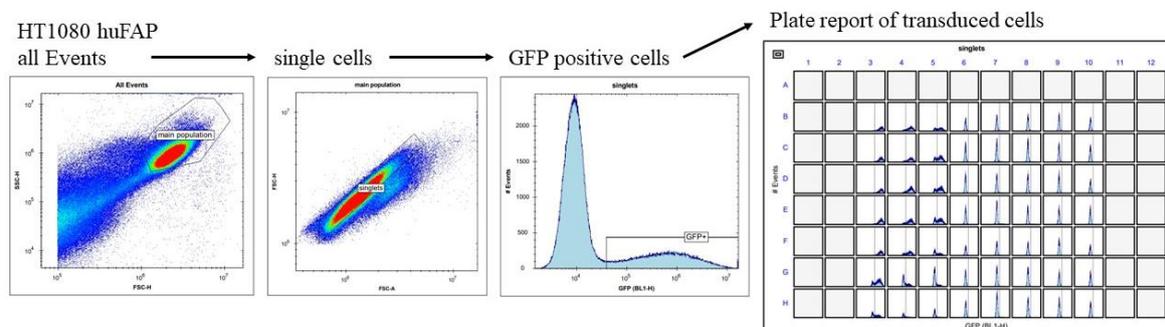


Figure 5. Exemplary overview of the flow cytometry gating strategy of HT1080 huFAP cells after transduction with a titration of rAAV-2E3.v6 and KiH-2E3-MO36. Cells were gated for GFP positive single cells. Flow cytometry and data analysis was performed using the iQue® Screener PLUS (IntelliCyt Cooperation) and ForeCyt® Software (IntelliCyt Cooperation).