

1 **Rescuing AAV gene transfer from antibody neutralization with an IgG-degrading enzyme**

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20 **Abstract**

21 Pre-existing humoral immunity to recombinant adeno-associated viral (AAV) vectors
22 restricts the treatable patient population and efficacy of human gene therapies. Approaches to
23 clear neutralizing antibodies (NAbs), such as plasmapheresis and immunosuppression are either
24 ineffective or cause undesirable side effects. Here, we describe a clinically relevant strategy to
25 rapidly and transiently degrade NAbs prior to AAV administration using an IgG degrading
26 enzyme (IdeZ). We demonstrate that recombinant IdeZ efficiently cleaves IgG in dog, monkey
27 and human antisera. Prophylactically administered IdeZ cleaves circulating, human IgG in mice
28 and prevents AAV neutralization *in vivo*. In macaques, a single intravenous dose of IdeZ rescues
29 AAV transduction by transiently reversing seropositivity. Importantly, IdeZ efficiently cleaves
30 NAbs and rescues AAV transduction in mice passively immunized with individual human donor
31 sera representing a diverse population. Our antibody clearance approach presents a new
32 paradigm for expanding the prospective patient cohort and improving efficacy of AAV gene
33 therapy.

34

35 **Introduction**

36

37 Human gene therapy using recombinant AAV vectors continues to advance steadily as a
38 treatment paradigm for rare, monogenic disorders. This is highlighted by the recent FDA approval
39 and clinical success of Zolgensma®, an intravenously dosed AAV vector delivering a functional
40 copy of the *SMN1* gene in children with Spinal Muscular Atrophy (SMA)(1). Further, the list of
41 systemically dosed AAV-based gene therapies for rare disorders such as Hemophilia A & B,
42 Duchenne Muscular Dystrophy (DMD), X-linked myotubularin myopathy (XLMTM) and Pompe

43 disease amongst others continues to grow(2, 3). These promising clinical examples have
44 concurrently highlighted important challenges that include manufacturing needs, patient
45 recruitment, and the potential for toxicity at high AAV doses. One such challenge that limits the
46 recruitment of patients for gene therapy clinical trials and adversely affects the efficacy of AAV
47 gene therapy is the prevalence of pre-existing neutralizing antibodies (NAbs) to AAV capsids in
48 the human population. Such NAbs arise due to natural infection or cross-reactivity between
49 different AAV serotypes(4–7). NAbs can mitigate AAV infection through multiple mechanisms
50 by (a) binding to AAV capsids and blocking critical steps in transduction such as cell surface
51 attachment and uptake, endosomal escape, productive trafficking to the nucleus or uncoating and
52 (b) promoting AAV opsonization by phagocytic cells, thereby mediating their rapid clearance from
53 the circulation. Multiple preclinical studies in different animal models have demonstrated that pre-
54 existing NAbs impede systemic gene transfer by AAV vectors(8–11).

55 In humans, serological studies reveal a high prevalence of NAbs in the worldwide
56 population, with about 67% of people having antibodies against AAV1, 72% against AAV2, and
57 ~ 40% against AAV serotypes 5 through 9(4, 12–14). Because of this high NAb sero-prevalence,
58 screening for AAV antisera through *in vitro* NAb assays or ELISA is common place in AAV gene
59 therapy trials and exclusion criteria can render upwards of 50% of patients ineligible for treatment
60 or admission into clinical trials(15, 16). Furthermore, vector immunogenicity represents a major
61 challenge in re-administration of AAV vectors. High titer NAbs are produced following AAV
62 vector administration, thereby preventing prospective AAV redosing(6, 17). This severely limits
63 long term gene therapy success in (a) patients in the low dose AAV cohort; (b) pediatric patients
64 who will experience tissue growth and proliferation leading to vector genome dilution and
65 potential reversal of symptoms with age, and (c) patients with degenerative disorders that might

66 require multiple AAV treatments to prevent tissue loss and sub-therapeutic transgene expression
67 levels. Taken together, NAb present a significant barrier to the broad application of AAV in the
68 clinic.

69 Strategies that are currently being evaluated to circumvent pre-existing humoral immunity
70 to AAV vectors are either early in development, ineffective or prone to causing undesirable side
71 effects. These include the engineering of new AAV variants with reduced NAb recognition(18,
72 19), plasmapheresis or immunoadsorption to reduce the overall levels of circulating antibodies in
73 patient serum prior to AAV administration(20–23), use of capsid decoys(24) or
74 immunosuppression to decrease the B cell population and consequently antibody levels in
75 general(25, 26). While these approaches have demonstrated varying success and efficiency in
76 addressing the problem of circulating antibodies and remain under evaluation, a one-solution-fits-
77 all approach that resolves this challenge is unlikely. Pertinent to this, a promising and clinically
78 validated paradigm for mitigating the effects of deleterious (auto)antibodies is the use of IgG-
79 specific proteases(27–30). In particular, the extracellular enzyme, IdeS derived from
80 *Streptococcus pyogenes*, is a 35 kDa cysteine protease that specifically cleaves IgG at the lower
81 hinge region generating one F(ab')₂ fragment and one homodimeric Fc fragment(31–34) (**Figure**
82 **1A**). IdeZ, a homolog of IdeS, was identified and characterized in *S. equi* ssp. *zooepidemicus* and
83 shown to efficiently cleave IgG in a similar manner to IdeS(35, 36). Here, we evaluate the ability
84 of IdeZ to mitigate the effect of pre-existing anti-AAV NAb in mice passively immunized with
85 human antisera and in non-human primates. First, we demonstrate the ability of IdeZ to cleave
86 antibodies in sera derived from multiple species. Next, we show that IdeZ can rescue AAV gene
87 transfer in the presence of circulating human IgG in mice and natural humoral immunity in non-

88 human primates. In addition, we demonstrate that gene transfer to the liver and heart is also rescued
89 in mice passively immunized with individual human antisera.

90

91 **Results**

92

93 **IdeZ shows robust ability to cleave antibodies in sera from multiple species.** We first
94 demonstrated that IdeZ efficiently cleaves antibodies in canine, non-human primate and human
95 sera, but not mouse serum samples *in vitro* (**Figure 1B**). The latter observation is corroborated by
96 known mutations in the hinge region of mouse IgG compared to other species that render the latter
97 resistant to IdeZ mediated degradation(35, 36). IdeZ also effectively cleaves human IgG into heavy
98 chain, light chain and Fc fragments *in vitro* (**Figure 1C**). Next, we confirmed the potency of
99 research grade, recombinant GST-tagged IdeZ produced in *E.coli* for dosing *in vivo* (**Figure 1D**).
100 Mice were first passively immunized with pooled IgG injected intraperitoneally (IP), followed by
101 a single intravenous (IV) injection of IdeZ confirming efficient cleavage into Fab and Fc fragments
102 as determined by western blotting (**Figure 1E**). Further, as shown in **Figures 1F-I**, we observed a
103 dose dependent effect in IgG degradation, with optimal clearance between 0.25-1mg/kg of IdeZ at
104 day 2-3 post-administration. Effective clearance of circulating antibodies was observed within a
105 day or two post-IdeZ administration (**Supplemental Figure S1**). Furthermore, IdeZ effectively
106 mitigated human IgG mediated neutralization of AAV8 and AAV9-Luc transduction *in vitro*
107 (**Supplemental Figure S2**) leading us to investigate the efficacy of IdeZ treatment on AAV gene
108 transfer efficiency in the presence of neutralizing antisera *in vivo*.

109

110 **IdeZ rescues AAV liver gene transfer in mice and macaques.** Based on these results, we
111 evaluated the ability of prophylactically dosing IdeZ in mice passively immunized with pooled
112 human IgG to rescue AAV transduction *in vivo*. Briefly, animals of either gender were first injected
113 IP with pooled human IgG (8 mg) on day (-1), with a single dose of IdeZ (2.5mg/kg) through the
114 tail vein on day 0 and an IV dose of AAV8 or AAV9 vectors (1×10^{13} vg/kg) packaging a CBA
115 promoter driven luciferase transgene on day 3 (**Figure 2A**). Naïve mice showed different levels of
116 AAV8 and AAV9-mediated luciferase expression in the liver (**Figures 2B-E**). In mice passively
117 immunized with pooled human IgG, luciferase expression in the liver was decreased by 10-100
118 fold due to the presence of anti-AAV NAb. In contrast, we observed rescue from AAV
119 neutralization in IdeZ treated animals, with partial to complete rescue of liver luciferase expression
120 levels. These observations were corroborated by vector genome copy numbers, which
121 corresponded with transgene expression in general; although, we observed gender-specific
122 differences (Figures 2F-I). Notably, despite restoration of AAV copy numbers in the male liver,
123 expression was not fully restored implying that other non-NAb related factors might be involved
124 in controlling liver expression (**Figure 2C,E**). While these aspects warrant further investigation
125 and dose optimization, these observations support that prophylactically administered IdeZ can
126 prevent AAV neutralization and restore liver transduction in an AAV serotype-independent
127 manner.

128 Next, we sought to evaluate whether IdeZ was effective in non-human primates. We first
129 screened male cynomolgus macaques for anti-AAV antibodies using a NAb assay to identify
130 seropositive and seronegative animals (**Supplemental Figure S3**). Animal M16561 served as the
131 naïve seronegative control, while the seropositive animals M16556 and M16558 were dosed on
132 day 0 with IV PBS or a single IV bolus dose of IdeZ (0.5mg/kg), respectively. On day 3, post-IdeZ

133 treatment, all three animals were injected with a dose of AAV9 vectors packaging the luciferase
134 transgene (5×10^{12} vg/kg) (**Figure 2J**). Evaluation of serum IgG levels at days 0, 3 and 31 post-
135 IdeZ treatment revealed selective cleavage and clearance at day 3. In addition, serum IgG levels
136 were fully restored to normal levels by day 31 corroborating the transient effect of IdeZ activity
137 (**Figure 2K**). Upon sacrifice at day 30, we observed an approximately one log order decrease in
138 luciferase gene expression and a disproportionate (~ two logs) decrease in vg copy number in the
139 liver (**Figure 2L & 2M**). Importantly, IdeZ treatment restored AAV luciferase gene expression
140 levels and partially restored vg copy numbers in the liver. Further, these results also mirrored the
141 observations in the liver of male mice injected with human IgG. While it should be noted that the
142 number of non-human primates in the current study are low, the above results underscore the
143 ability to translate the applicability of IdeZ in clearing IgG across multiple species.

144
145 **IdeZ rescues AAV liver gene transfer in mice passively immunized with individual human**
146 **sera.** To further evaluate whether IdeZ can function effectively in a clinically relevant setting, we
147 tested our approach in mice passively immunized with individual human donor sera representing
148 a diverse population. Briefly, we obtained 18 different human donor serum samples across a broad
149 demographic and displaying varying levels of AAV neutralization as determined by NAb assay
150 (**Supplemental Figure S4**). We then administered a single IP dose of donor serum in 2 animals
151 each (total 18 cohorts), following which the first animal received an IV injection of PBS and the
152 second, a single IV bolus dose of IdeZ (0.5mg/kg). The control cohort comprised of naïve mice.
153 All animals received an IV dose of AAV9-Luc vectors (1×10^{13} vg/kg) and luciferase gene
154 expression assessed in the liver and heart of the saline vs IdeZ treated cohorts (**Figure 3A**). As
155 seen in **Figures 3B-E**, the diversity of pre-existing humoral immunity to AAV transduction is well

156 represented by this small, yet diverse panel of human serum samples (**Supplemental Table 1**).
157 Notably, we observed restoration in liver luciferase expression levels in a number of animals
158 (**Figures 3B,D**). Complete restoration (100%) of liver expression to that of naïve, non-immunized
159 control animals was observed in these animals regardless of NAb titer. Some outliers were also
160 observed, where IdeZ treatment was only partially effective or adversely affected transduction.
161 One possible explanation is that these mice might have high levels of pre-existing immunity to
162 IdeZ, although the impact of such on IdeZ activity is unclear. While these aspects warrant further
163 investigation, we observed overall trends that support that IdeZ treatment can result in a
164 statistically significant improvement in liver gene expression and copy number by clearing
165 circulating antibodies (**Figures 3C,E**). These results further underscore the potential for clinical
166 translation with our approach.

167
168 **IdeZ mediated rescue of AAV cardiac gene transfer efficiency provides additional insight**
169 **into plausible neutralization mechanisms.** Concurrent to studies focused on restoring AAV gene
170 transfer in the liver, we also analyzed the heart and observed striking differences. We assessed
171 cardiac gene transfer in both mice passively immunized with pooled human IgG as well as
172 individual human sera. Notably, although pooled human IgG decreased expression and IdeZ
173 treatment restored cardiac luciferase expression levels to that of naïve mice, changes in vg copy
174 number upon IdeZ treatment were only partially rescued in females or statistically insignificant in
175 males (**Figures 4A-E**). These observations were further corroborated in mice passively immunized
176 with individual human sera. In this regard, we first observed that neutralization of cardiac
177 transduction by individual antisera does not mirror the patterns observed in the liver (**Figures 3B,D**
178 and **4G,I**, black columns). Second, only partial rescue of AAV mediated cardiac gene expression

179 is observed in most animals. In addition, although we observed some increase in vector genome
180 copy numbers within cardiac tissue, no specific correlation with luciferase expression patterns was
181 noted (**Figures 4G,I**). Assessment of overall rescue across the human sera infused cohorts
182 corroborated these trends (**Figures 4H,J**). In particular, we observed a statistically significant
183 rescue of cardiac gene transfer in cardiac luciferase expression, but not vector genome copy
184 numbers.

185

186 **Discussion**

187

188 The IgG-degrading enzyme, IdeS, also known as Imlifidase® has shown promise in a clinical trial
189 (ClinicalTrials.gov Identifier: NCT02224820) permitting successful kidney transplantation in
190 patients harboring donor-specific antibodies(37–39). Briefly, the latter study assessed the safety,
191 immunogenicity, pharmacokinetics, and efficacy of Imlifidase in an open-label, dose escalation
192 study in highly sensitized patients with anti-HLA antibodies and chronic kidney disease. This
193 approach represents a potential paradigm shifting method to desensitize patients, who would
194 otherwise not qualify to receive a lifesaving transplant. Thus, a clinical precedent for applying
195 enzymatic IgG degradation to promote rapid and transient antibody clearance already exists.
196 Further, it is noteworthy to mention that other orthogonal methods to facilitate IgG clearance using
197 soluble antibody binding bacterial proteins (e.g., Protein M(40)), FcRn (neonatal Fc receptor)
198 domains(41), anti-FcRn antibodies such as Rozanolixizumab(42), SYNT001(43, 44) etc have
199 shown promise in the clinic as well.

200 These approaches, however, have not been explored in the context of gene therapy to date.

201 The antibody degradation/clearance approach described in the current study could broadly impact

202 preclinical gene therapy studies in different large animal models, currently encumbered by pre-
203 existing NABs. For instance, pre-existing humoral immunity against different AAV serotypes in
204 macaques, dogs and pigs have been described(8, 10). Based on our earlier *in vitro* results, we
205 postulate that IdeZ could potentially be applicable for evaluating AAV gene therapies in canine
206 models of disease. These data combined with our observations in non-human primates greatly
207 expands the potential for preclinical AAV gene transfer studies, but also provides a path towards
208 safety and dose finding studies of this approach in preclinical animal models. Additional studies
209 to evaluate IdeZ dosing and kinetics of antibody clearance in such animal subjects with varying
210 anti-AAV antibody titers is likely to help optimize this approach.

211 Another important advantage of the IdeZ approach is the potential for AAV serotype-
212 independent rescue from antibody neutralization. While such will require dose optimization
213 studies with different natural and engineered AAV capsids, we postulate that the universality of
214 our antibody clearance approach is likely to broadly complement AAV gene transfer studies. One
215 possible caveat of this approach is that people may harbor antibodies against IdeZ. However, it is
216 interesting to note that IdeZ would likely degrade such antibodies as well. Another significant
217 topic that warrants further evaluation is whether IdeZ treatment can enable vector redosing. In
218 particular, IdeZ could provide an alternative solution in patients, where immunosuppression is not
219 feasible or undesirable(25, 26, 45). While we were unable to evaluate such in mice due to the
220 inability of IdeZ to cleave mouse IgG, such studies should be feasible in non-human primates or
221 other animal models. Taken together, from a clinical perspective, the current strategy has the
222 potential to significantly impact the treatable patient population and improve the efficacy of AAV
223 gene therapies.

224

225

226 **Methods**

227

228 **Plasmid Constructs and Recombinant Protein Expression**

229 IdeZ DNA sequence from *S. equi* ssp. *zooepidemicus* lacking the N-terminal signal sequence was
230 synthesized and cloned into pGEX-6P-3 expression vector using BamHI and SalI restriction sites
231 (Genscript). *E. coli* strain BL21 star (DE3) was transformed with recombinant IdeZ pGEX-6P-3
232 plasmid. A single colony was inoculated into TB medium containing ampicillin; culture was
233 incubated in 37°C at 200 rpm and then induced with IPTG. Recombinant BL21 cells stored in
234 glycerol were inoculated into TB medium containing ampicillin and cultured at 37 °C. When the
235 OD600 reached about 4, the cell culture was induced with IPTG at 37°C for 4h. Cells were
236 harvested by centrifugation. Cell pellets were resuspended with GST lysis buffer followed by
237 sonication. The supernatant after centrifugation was kept for future purification. Target protein
238 was obtained by two-step purification using a GST column and Superdex 200 column. Target
239 protein was sterilized by 0.22µm filter before stored in aliquots. The concentration was
240 determined by Bradford protein assay with BSA as standard. The protein purity and molecular
241 weight were determined by standard SDS-PAGE along with Western blot confirmation using a
242 Rabbit anti-GST pAb (GenScript, Cat.No. A00097). Recombinant GST-IdeZ was stored in 50
243 mM Tris-HCl, 150 mM NaCl, 10% Glycerol, pH 8.0. Endotoxin was removed from recombinant
244 protein using High Capacity Endotoxin Removal Spin Columns (ThermoFisher Scientific
245 Catalog #88274) following the manufacturer's instructions.

246

247 **SDS-PAGE and analysis of IdeZ enzyme activity**

248 Pooled human IgG was purchased from Sigma (I4506), Mouse and Dog serum samples were
249 obtained from lab stocks or kind gifts from David Mack (University of Washington). Individual
250 human serum samples from donors were purchased from ValleyBiomedical. Rhesus macaque
251 sera were kind gifts from Alice Tarantal (UC Davis), Yoland Smith and Adriana Galvan (Yerkes
252 National Primate Center, Emory University). Proteins analyzed by SDS-PAGE were separated
253 under reducing conditions on Nu-PAGE 4–12% Bis-Tris (Invitrogen) or on Mini-Protean TGX
254 4-15% gels (Biorad) and stained with Coomassie Blue. All *in vitro* activity assays with
255 recombinant GST-IdeZ or IdeZ (NEB Catalog #P0770S) (1ug per reaction) were performed for 3
256 h at 37°C and serum samples were diluted 50x in PBS prior to analysis by SDS-PAGE. All *in*
257 *vivo* activity assays were performed with recombinant GST-IdeZ with mouse and or NHP serum
258 samples being diluted 10x in PBS prior to analysis by SDS-PAGE and immunoblotting. Digested
259 sera was probed with Rabbit anti-Human IgG-HRP H+L Secondary Antibody (ThermoFisher
260 Scientific Catalog #A18903, 1:10,000), Rabbit anti-Human IgG Fc HRP Secondary Antibody
261 (ThermoFisher Scientific Catalog #31423, 1:10,000), and Rabbit anti-Human IgG F(ab')₂ HRP
262 Secondary Antibody (ThermoFisher Scientific Catalog #31482, 1:10,000).

263

264

265 **Cell lines and Recombinant Virus Production**

266 HEK293 were maintained in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with
267 10% fetal bovine serum (FBS), 100U/ml penicillin, 100ug/ml streptomycin. Cells were
268 maintained in 5% CO₂ at 37°C. Recombinant AAV vectors were generated using triple plasmid
269 transfection with the AAV Rep-Cap plasmid (pXR8 or pXR9 encoding AAV8 or AAV9 capsid

270 proteins, respectively), Adenoviral helper plasmid (pXX680), and a luciferase transgene cassette
271 driven by the chicken beta actin promoter (pTR-CBA-Luc), flanked by AAV2 inverted terminal
272 repeat (ITR) sequences. Viral vectors were harvested from media and purified via iodixanol
273 density gradient ultracentrifugation followed by phosphate-buffered saline (PBS) buffer
274 exchange. Titers of purified virus preparations were determined by quantitative PCR using a
275 Roche Lightcycler 480 (Roche Applied Sciences, Pleasanton, CA) with primers amplifying the
276 AAV2 ITR regions (forward, 5'-AACATGCTACGCAGAGAGGGAGTGG-3'; reverse, 5'-
277 CATGAGACAAGGAACCCCTAGTGATGGAG-3') (IDT Technologies, Ames IA).

278

279 ***In Vitro* Antibody and Serum Neutralization Assays.**

280 Pooled human IgG, (25 ug undiluted) or antiserum (25 μ l) (as specified for individual
281 experiments) was mixed with an equal volume containing recombinant AAV9-Luc vector
282 (100,000 vg/cell) in tissue culture-treated, black, glass- bottom 96-well plates (Corning) and then
283 incubated at room temperature for 30 min. For neutralization assays, 1 ug of GST-IdeZ was
284 incubated with pooled human IgG in 5% CO₂ at 37 °C for 2 h prior to addition of AAV9-CBA-
285 Luc vector. A total of 1 \times 10⁴ HEK293 cells in 50 μ L DMEM + 10% FBS + penicillin-
286 streptomycin was then added to each well, and the plates were incubated in 5% CO₂ at 37 °C
287 for 24h. Cells were then lysed with 25 μ L of 1 \times passive lysis buffer (Promega) for 30 min at room
288 temperature. Luciferase activity was measured on a Victor 3 multi-label plate reader
289 (PerkinElmer) immediately after the addition of 25 μ L of luciferin (Promega). All readouts were
290 normalized to controls with no antibody/antiserum treatment. Recombinant AAV vectors
291 packaging CBA-Luc transgenes, antibodies, sera, and GST-IdeZ were prediluted in DMEM and
292 used in this assay.

293

294 **Mouse Studies**

295 All animal experiments were performed using 6- to 8-week-old male and female C57BL/6 mice
296 purchased from Jackson Laboratories (Bar Harbor, ME). These mice were maintained and
297 treated in compliance with NIH guidelines and as approved by the UNC Institutional Animal
298 Care and Use Committee (IACUC). Mice were injected intraperitoneally with pooled human IgG
299 (8 mg). The same mice were injected intravenously 24 hours later with PBS or recombinant
300 GST-IdeZ (2.5 mg/kg). Recombinant AAV9-CBA-Luc or 1× PBS (as mock treatment) was
301 injected 72 hours post IdeZ at a dose of 1×10^{13} vg/kg. Luciferase transgene expression levels
302 were analyzed 4 weeks postinjection in the liver, heart, and kidney. Animals were sacrificed 4
303 weeks post-AAV9 injection with an intraperitoneal injection of tribromoethanol (Avertin)
304 (0.2 ml of 1.25% solution) followed by transcardial perfusion with 30 ml of 1× PBS. For human
305 serum samples/IdeZ studies, two mice were injected intraperitoneally with 200µl human sera
306 (purchased from Valley Biomedical, gift from StrideBio, Inc.). The same mice were then
307 injected intravenously 72 hours later with PBS or recombinant GST-IdeZ (2.5 mg/kg). Mice
308 were subsequently injected intravenously 72 hrs post-IdeZ treatment with AAV9-Luc (1×10^{13}
309 vg/kg).

310 **Non-human primate studies**

311 A total of 3 cynomolgus macaques (3 males) designated for use in this study were obtained from
312 Southern Research (Birmingham, AL) who obtained them from Worldwide Primates, Inc.
313 (Miami, FL). Animals were acclimated prior to study start and deemed healthy prior to study
314 initiation. On the first day of dosing, the animals were approximately 3 years of age, male gender

315 and weighed between 2.4 – 3.8 kg. Housing and animal care conformed to the guidelines of the
316 U.S. Department of Agriculture (Animal Welfare Act; Public Law 99-198) and those of the
317 *Guide for the Care and Use of Laboratory Animals* and to the applicable Standard Operating
318 Procedures (SOPs) at Southern Research. Animals were tested for pre-existing AAV9 NABs
319 using an *in vitro* NAb assay and were designated as M16561 being seronegative, M16556 being
320 seropositive and M16558 being seropositive. The seropositive NHP M16558 was administered
321 IdeZ (0.5 mg/kg) via intravenous bolus injection on Day 0. AAV9-CBA-Luc was administered
322 to all 3 NHPs via intravenous bolus injection, 72 hrs post-IdeZ injection at a dose of 5×10^{12}
323 vg/kg. All animals had blood collected for analysis on days 0, 3, and 28. On day 28, NHPs were
324 euthanized and organs collected via whole body perfusion with sterile saline while under
325 anesthesia following collection of specified blood samples.

326

327 **Tissue analysis for luciferase expression**

328 To quantify luciferase expression, animals injected with AAV9-CBA-Luc transgene were
329 sacrificed and tissues were harvested and frozen at 80C. Tissues were later thawed, weighed, and
330 lysed by adding 200µl of 1x passive lysis buffer (Promega, Madison WI) prior to mechanical
331 lysis using a Tissue Lyser II 352 instrument (QIAGEN, Valencia, CA), followed by
332 centrifugation to remove any remaining tissue debris. To measure luciferase transgene
333 expression, 15µl of supernatant from each lysate was then loaded onto an assay plate along with
334 45µl of luciferin, and luminometric analysis was performed using a Victor3 luminometer
335 (PerkinElmer, Waltham, MA). The relative luminescence units obtained for each sample were
336 then normalized to the input tissue weight for each sample, measured in grams, followed by log
337 transformation.

338

339 **Tissue analysis for vector genome biodistribution**

340 DNA was extracted and purified from tissues using a QIAamp DNA FFPE Tissue kit (catalog
341 no. 56404; Qiagen). Viral genome copy numbers were then determined for each tissue using
342 quantitative PCR with primers specific to the chicken beta actin (CBA) promoter (forward, 5'-
343 TGTTCCCATAGTAACGCCAA-3'; reverse, 5'-TGCCAAGTAGGAAAGTCCCAT-3'). These
344 viral genome copy numbers were then normalized to the level of the mouse lamin B2
345 housekeeping gene using specific primers (forward, 5'-GGACCCAAGGACTACCTCAAGGG-
346 3'; reverse, 5'-AGGGCACCTCCATCTCGGAAAC-3'). The biodistribution of viral genomes is
347 represented as the ratio of vector genomes per cell or as vector genomes per nanograms of DNA
348 extracted, followed by log transformation.

349

350 **Statistical Analysis**

351 Where appropriate, data are represented as mean or mean \pm standard deviation. Where
352 appropriate data were log transformed prior to statistical analysis. For data sets with at least three
353 groups, significance was determined by one-way ANOVA, with Tukey's post-test. For analysis
354 of the human sera data, significance was determined by the nonparametric Mann-Whitney rank
355 test. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$.

356

357 **Author Contributions**

358 ZE and AA designed all experiments, interpreted the data, and wrote the manuscript. ZE and DO
359 carried out all molecular biology, virus production and neutralization studies. KS and MF carried
360 out animal studies and assisted with tissue analysis.

361

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367

368 **Conflict of Interest**

369 AA is a co-founder at StrideBio, Inc. AA and ZE have filed patent applications on the subject
370 matter of this manuscript.

371

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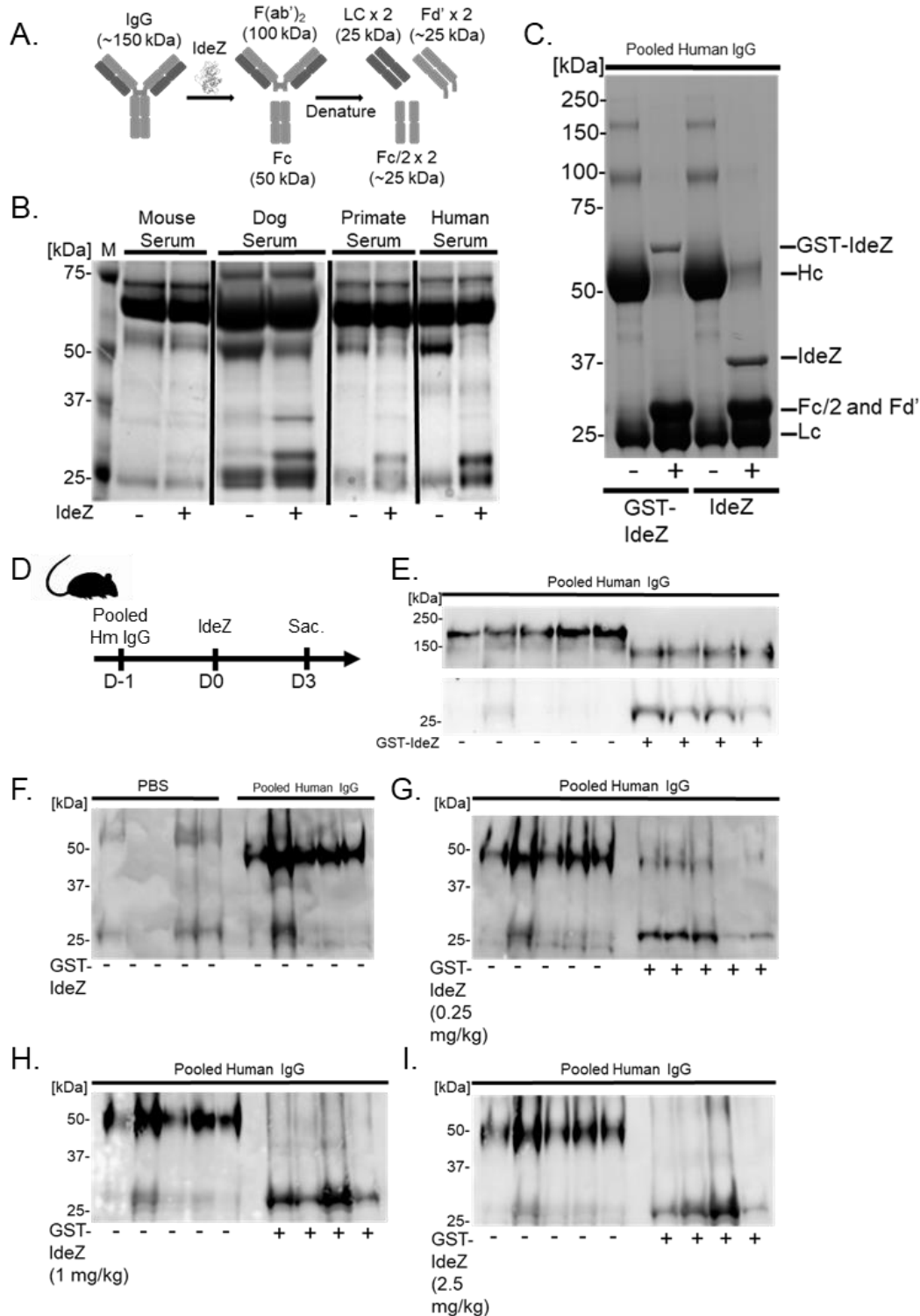
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519 **Figure 1. IdeZ cleaves serum antibodies from multiple species.**

520 **A,** Schematic outlining IdeZ cleavage of IgG below the hinge region yielding multiple F(ab')₂

521 and Fc fragments after reduction. **B,** Serum samples from mouse, dog, primate and human

522 untreated (-) or treated (+) with recombinant IdeZ and analyzed by SDS-PAGE under reducing
523 conditions. Gels were then stained with Coomassie blue. **C**, Pooled human IgG untreated (-) or
524 treated (+) with recombinant GST-IdeZ or commercial standard IdeZ (NEB) and analyzed by
525 SDS-PAGE under reducing conditions. Gels were then stained with Coomassie blue. IgG was
526 cleaved by GST-IdeZ and IdeZ into multiple fragments as indicated. **D**, Mice were injected
527 intraperitoneally first with pooled human IgG, following which they were injected intravenously
528 24 hours later with PBS (-) or recombinant GST-IdeZ (1 mg/kg) (+). Blood samples were taken
529 72 hours post injection and analyzed by SDS-PAGE under reducing conditions with
530 immunoblotting. IgG was probed with Fab (top panel) and Fc (bottom panel) specific antibodies.
531 **E**, Experimental timeline of *in vivo* GST-IdeZ dose optimization experiment. Mice were injected
532 with pooled human IgG followed 24 hrs later with no injection or injection with 3 different doses
533 of GST-IdeZ. Blood serum samples were collected 72 hours post GST-IdeZ. Sac., sacrifice
534 followed by tissue harvest. Serum samples of PBS control (**F**), 0.25 mg/kg (**G**), 1 mg/kg (**H**), and
535 2.5 mg/kg (**I**) GST-IdeZ injected mice were analyzed by SDS-PAGE under reducing conditions
536 and probed with human IgG specific antibodies to analyze IgG cleavage.

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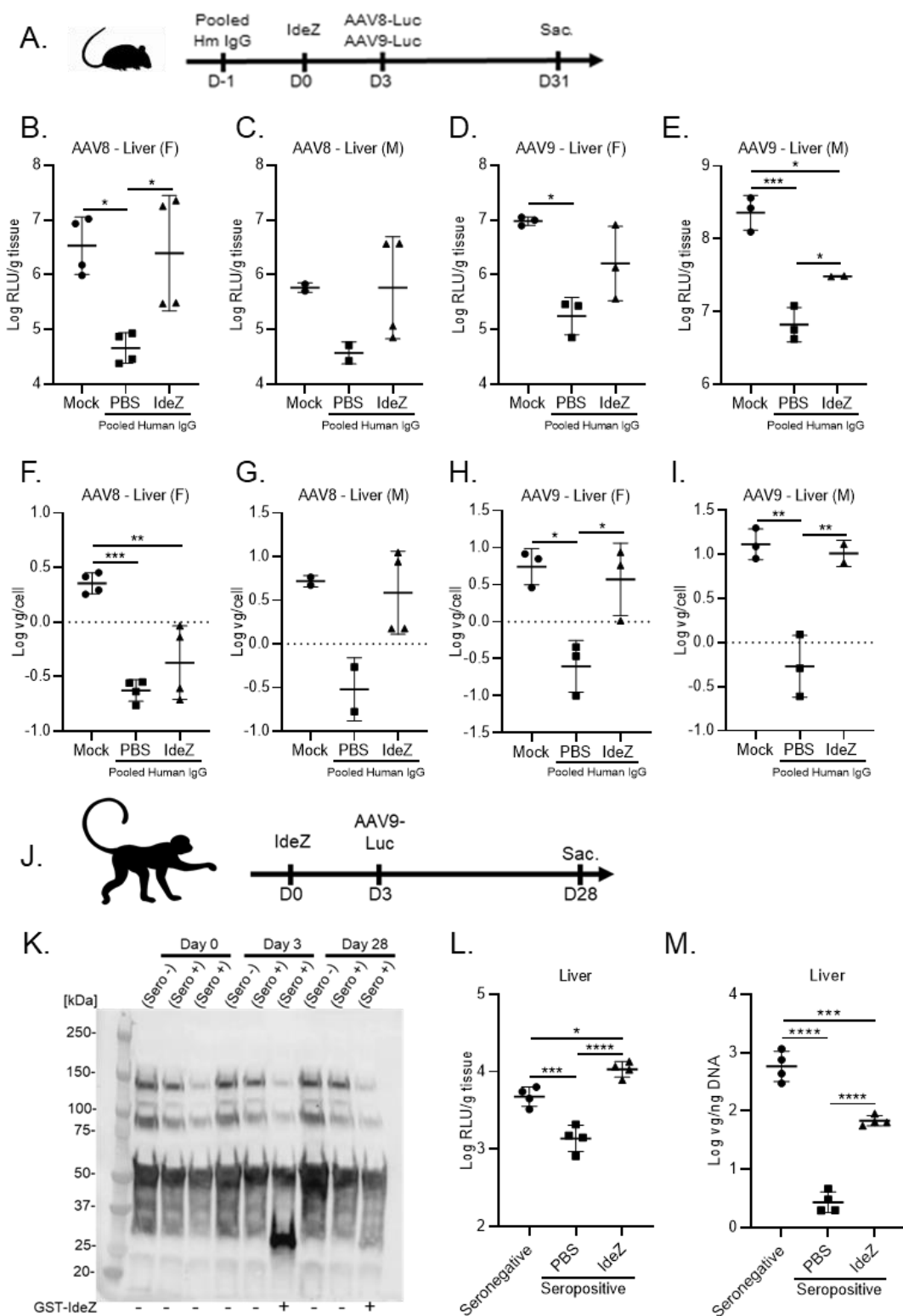
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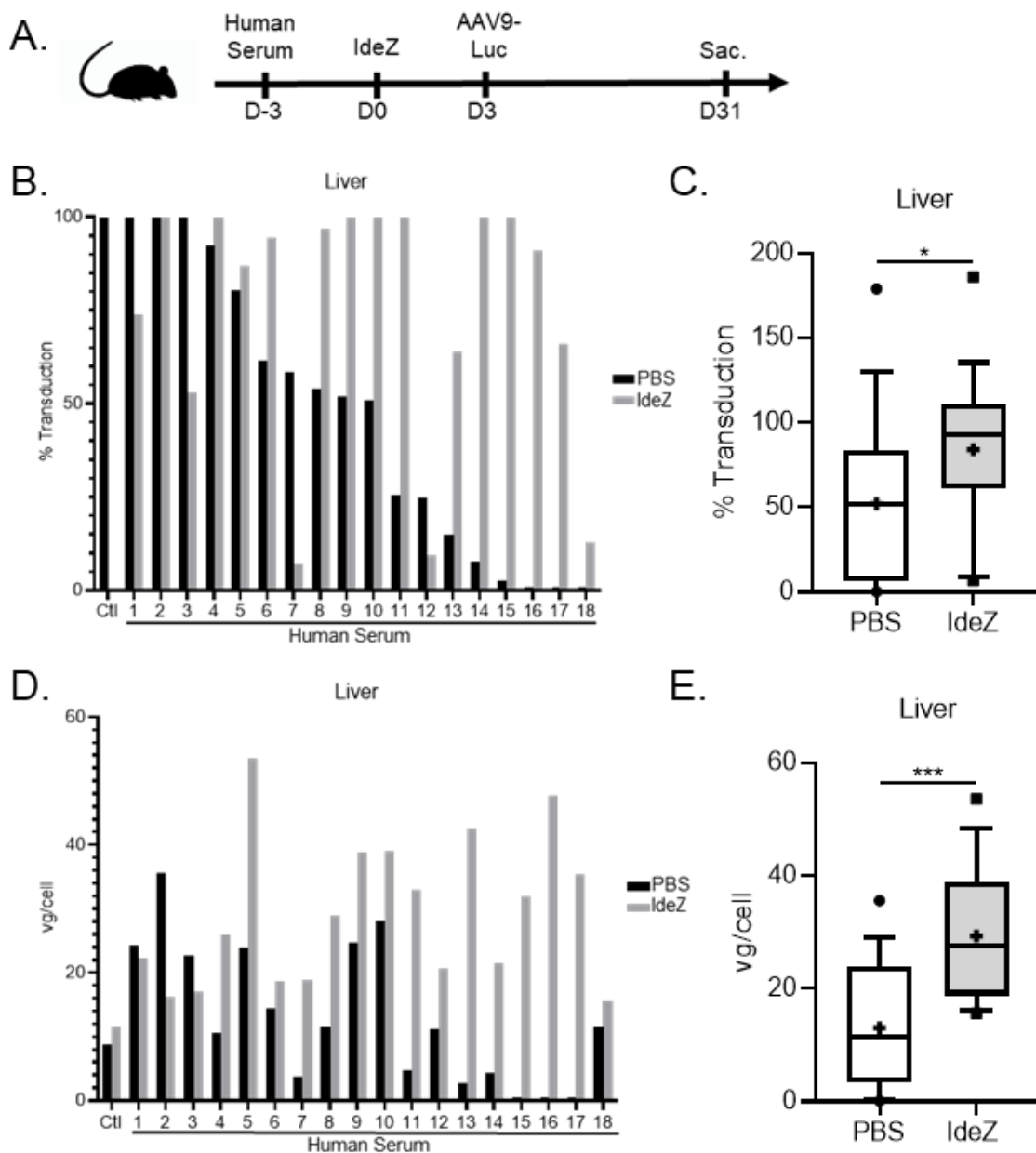
544 **Figure 2. IdeZ rescues AAV8 and AAV9 liver transduction in passively immunized mice**

545 **and cynomolgus macaques. A, Experimental timeline of IgG, IdeZ and AAV8 or AAV9-Luc**

546 injections. Sac., sacrifice followed by tissue harvest. Mice were injected intraperitoneally with
547 pooled human IgG. The same mice were injected intravenously 24 hours later with PBS or
548 recombinant GST-IdeZ (2.5 mg/kg). AAV8-Luc or AAV9-Luc was injected 72 hours post IdeZ
549 at a dose of 1×10^{13} vg/kg. Luciferase transgene expression levels were analyzed 4 weeks post-
550 injection in the liver; AAV8 (**B,C**); AAV9 (**D,E**). Luciferase expression levels were normalized
551 for total tissue protein concentration and represented as log relative luminescence units per gram
552 of tissue (log RLU/g tissue). Each dot represents the average of a technical duplicate from a
553 single animal. Biodistribution of AAV8 and AAV9 Luc vector genomes in the liver; AAV8
554 (**F,G**); AAV9 (**H,I**). Vector genome copy numbers per cell were calculated by normalizing Luc
555 copy numbers to copies of the Lamin B2 housekeeping gene and represented as log vg/cell. Each
556 dot represents a technical duplicate from a single animal, and the dash represents the mean value.
557 (F=female, M=male). **J**, Schematic demonstrating experimental timeline of IdeZ and AAV9-Luc
558 injections in NHPs. AAV9 seropositive NHP M16558 (n=1) was administered IdeZ (0.5 mg/kg)
559 via intravenous bolus injection on Day 0. AAV9-Luc was administered via intravenous bolus
560 injection 72 hrs post-IdeZ injection at a dose of 5×10^{12} vg/kg. **K**, NHP serum samples were
561 analyzed by SDS-PAGE under reducing conditions and probed with Fc specific antibodies. **L**,
562 Luciferase transgene expression levels were analyzed 4 weeks post-injection in the liver of
563 NHPs. Luciferase expression levels were normalized for total tissue protein concentration and
564 represented as log relative luminescence units per gram of tissue (Log RLU/g tissue). Each dot
565 represents a single experiment of an individual liver lobe from a single animal. **M**,
566 Biodistribution of AAV9 Luc vector genomes in the liver of NHPs. Vector genome copy
567 numbers per ng of total extracted DNA were calculated and represented as log vg/ng DNA. Each
568 dot represents a technical duplicate experiment of individual liver slices from a single animal and

569 the dash represents the mean value. Significance was determined by one-way ANOVA with
570 Tukey's post-test. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$

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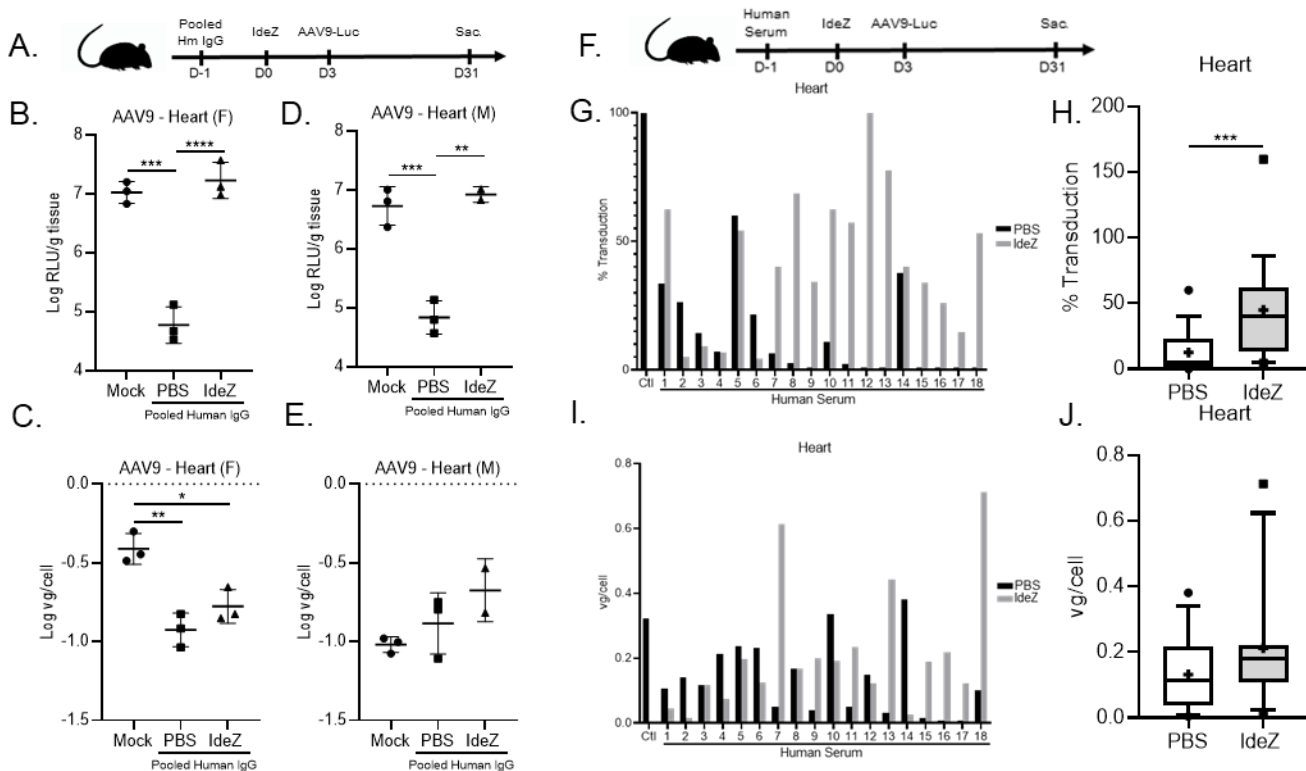
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573 **Figure 3. IdeZ rescues AAV9 liver transduction in mice passively immunized with**
574 **individual human sera.** A, Schematic demonstrating experimental timeline of human serum,
575 IdeZ and AAV9-Luc injections. 18 human serum samples were tested for their ability to

576 neutralize AAV9 transduction in the liver. Two mice per human serum sample were utilized for
577 the study and both mice were injected intraperitoneally with human serum. Mice were then
578 injected intravenously 72 hours later with PBS (black bars) or recombinant GST-IdeZ (2.5
579 mg/kg, grey bars) and subsequently injected intravenously 72 hrs post-IdeZ treatment with
580 AAV9-Luc (1×10^{13} vg/kg). Liver transduction levels were analyzed 4 weeks post-injection.
581 Sac., sacrifice followed by tissue harvest. **B**, Luciferase transgene expression levels were
582 analyzed 4 weeks post-injection in the liver of passively immunized mice treated with PBS
583 (black) or prophylactically with IdeZ (grey). Transduction levels were normalized to non-
584 immunized mice that were injected with AAV9-Luc at the same dose and represented as
585 percentage of control. Each bar represents the average of a technical duplicate from a single
586 animal. **C**, Relative liver transduction efficiency of AAV9-Luc in the entire cohort of mice
587 immunized with human sera treated with PBS control (white) or IdeZ (grey). Biodistribution of
588 AAV9 vector genomes in the liver for mice passively immunized with individual human serum
589 samples (**D**) and the entire cohort (**E**). Vector genome copy numbers per cell were calculated
590 based on normalization to copies of the Lamin B2 housekeeping gene. Each bar represents the
591 average of a technical duplicate from a single animal. Significance was determined by the
592 nonparametric Mann-Whitney rank test. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$.

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597 **Figure 4. Impact of IdeZ treatment on AAV9 cardiac transduction in passively immunized**

598 **mice. A,** Experimental timeline of pooled human IgG, IdeZ and AAV9-Luc injections. Cardiac

599 tissues were derived as outlined earlier in the liver experiment. **B,D,** Luciferase transgene

600 expression levels were analyzed 4 weeks post-injection in the heart. Luciferase expression levels

601 were normalized for total tissue protein concentration and represented as log relative

602 luminescence units per gram of tissue (log RLU/g tissue). Each dot represents the average of a

603 technical duplicate from a single animal. **C,E,** Biodistribution of AAV9 Luc vector genomes in

604 the heart. Vector genome copy numbers per cell were calculated based on normalization to

605 copies of the Lamin B2 housekeeping gene. Each dot represents the average of a technical

606 duplicate from a single animal. Female (F), Male (M). Significance was determined one-way

607 ANOVA with Tukey's post-test. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$. **F,** Schematic

608 demonstrating experimental timeline of human serum, IdeZ and AAV9-Luc injections. Cardiac

609 tissues were derived as outlined earlier in the liver experiment. **G**, Luciferase transgene
610 expression levels were analyzed 4 weeks post-injection in the heart of passively immunized mice
611 treated with PBS (black) or prophylactically with IdeZ (grey). Transduction levels were
612 normalized to non-immunized mice that were injected with AAV9-Luc at the same dose and
613 represented as percentage of control. Each bar represents the average of a technical duplicate
614 from a single animal. **H**, Relative cardiac transduction efficiency of AAV9-Luc in the entire
615 cohort of mice immunized with human sera treated with PBS control (white) or IdeZ (grey).
616 Biodistribution of AAV9 vector genomes in the heart for mice passively immunized with
617 individual human serum samples (**I**) and the entire cohort (**J**). Vector genome copy numbers per
618 cell were calculated based on normalization to copies of the Lamin B2 housekeeping gene. Each
619 bar represents the average of a technical duplicate from a single animal. Significance was
620 determined by the nonparametric Mann-Whitney rank test. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$,
621 **** $p < 0.0001$.

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