DNA-based delivery of incretin receptor agonists using MYO Technology leads to durable weight loss in a diet-induced obesity model

Linda Sasset[1], Andrew Cameron[1], Carleigh Sussman1, Luisa Rubinelli[1], Debnath Maji[1,2], Robert Miller[1], Andy Thompson[1], Delcora Campbell[1], Melanie R. Walker[1], Marek M. Drozdz[1,*], Rachel A Liberatore[1,*]

[1]RenBio Inc., Long Island City, New York, NY 11101, USA.

[2] Department of Biological and Agricultural Engineering, Louisiana State University, Baton Rouge, LA 70803, USA

* Correspondence should be addressed to R.A.L. (rachel.liberatore@renbio.com) or M.M.D. (marek.drozdz@renbio.com)

ABSTRACT

Therapeutic proteins have seen a substantial increase in clinical development and use across many disease areas. Despite their broad applicability, significant drawbacks limit access to many of these drugs, including: i) high manufacturing costs; ii) administration via time-consuming infusions; iii) frequent dosing, sometimes even daily; and iv) requirements for low temperature storage.

MYO Technology was developed to overcome these barriers. The MYO Technology platform consists of therapeutic-encoding plasmid DNA (pDNA), and a proprietary medical device for intramuscular injection and delivery of electrical pulses. These pulses enable the *in vivo* electroporation of muscle cells and uptake of injected pDNA, leading to the production, secretion, and delivery of the therapeutic protein into peripheral circulation. MYO

Technology offers several advantages over standard delivery of therapeutic proteins; pDNA manufacturing is a simpler and less specialized process compared to protein manufacturing, and pDNA is very stable and lacks most cold chain requirements. Furthermore, administration using MYO Technology takes only a few minutes, and the serum level of a therapeutic protein can potentially be maintained for many months without the need for redosing.

Incretin receptor agonists (IRAs) are a class of therapeutic proteins that have recently come to prominence as powerful weight and glucose control drugs, and are used for the treatment of type 2 diabetes (T2D) and obesity. Semaglutide and tirzepatide, currently the most widely used within this class, are both potent molecules, but have a short half-life, requiring weekly administration by subcutaneous injections. Moreover, since their clinical benefits rapidly disappear upon treatment cessation, T2D and obese patients may have a life-long dependency on IRAs, and the requirement for weekly injections can negatively affect the quality of life and the adherence to therapy, as well as create a significant financial burden. Therefore, increasing the interval between injections has become one of the major goals in the field.

Here, we present our preclinical studies on the delivery of IRAs with MYO Technology. Animal proof-of-concept studies demonstrate that MYO Technology-delivered IRAs are functional, and efficacious in promoting long-lasting weight and glucose control in mouse models of diet-induced obesity. Moreover, engineering the IRAs to facilitate blood–brain barrier penetration further enhances treatment efficacy, with benefits persisting beyond one year following a single administration. Together, these findings highlight MYO Technology's potential to transform care for patients with T2D and obesity by enabling long-lasting therapeutic effects with minimal dosing, ultimately improving quality of life and treatment adherence.

53 **INTRODUCTION**

54 Glucagon like peptide-1 (GLP-1) is an emerging glucose control and weight loss drug, which
55 is used in the treatment of type 2 diabetes (T2D) and obesity. This peptide is administered
56 subcutaneously to patients, and upon binding to its receptor in different organs, GLP-1
57 exerts several effects: in the pancreas it enhances insulin secretion and inhibits glucagon
58 release, in the stomach it delays gastric emptying, and in the hypothalamus it reduces
59 hunger, increases the feeling of satiety, and alters feeding behavior, overall decreasing body
60 weight and ameliorating metabolic dysregulation[1].

61 A significant downside of GLP-1 is its short half-life, which is about 2-5 minutes in vivo[2] .
62 Despite significant progress made in the field to incorporate modifications capable of
63 improving its durability, the current GLP-1 class of drugs used in the clinic still has a half-life
64 of only about one week, making weekly dosing necessary to maintain its therapeutic effects[3].

65 Several molecules are currently approved for both obesity and T2D: liraglutide (Saxenda) and
66 semaglutide (Ozempic/Wegovy) are GLP-1 receptor agonists, and tirzepatide
67 (Mounjaro/Zepbound) is a double GLP-1 and GIP receptor agonist. All these molecules have
68 an added fatty acid moiety, which binds albumin as a mechanism for half-life extension[4].
69 Some earlier generation molecules are only approved for T2D, such as dulaglutide (Trulicity),
70 which incorporates an Fc fusion as a strategy for half-life extension[5].

71 RenBio has evaluated intramuscular electroporation (IM-EP)-mediated delivery of plasmid
72 DNA (pDNA), encoding GLP-1, using its MYO (Make Your Own) Technology, as a new
73 therapeutic strategy. With this approach, GLP-1 is constantly produced for an extended
74 period, overcoming the necessity for weekly dosing. This technology has been successfully
75 employed in animal models of neutropenia for the delivery of granulocyte colony stimulating
76 factor (G-CSF), another small therapeutic protein with a short (~3.5 hours) half-life[6].

77 MYO Technology takes advantage of the efficiency of in vivo transfection of skeletal muscle
78 cells enabled by EP, and the durable protein production by those cells following transfection[7].
79 Indeed, expression of a monoclonal antibody or other therapeutic protein can be sustained

80 for several months to a year or more, both in animal models, and most importantly, in a
81 clinical setting[6]. Because of this durability, the potential exists to make a significant impact
82 on the treatment of chronic diseases, including obesity[7].

83 The GLP-1 class of drugs has recently transformed the treatment of obesity, though
84 questions remain regarding the full suite of mechanisms of action of these molecules. In
85 addition to direct action on the gastrointestinal tract, data suggest that targets in the brain
86 are important for their activity[8]. Angiopep-2 is a peptide that has been shown to increase
87 penetration of the blood-brain barrier (BBB) when fused to heterologous proteins[9]. Using a
88 mouse model of diet-induced obesity (DIO), we demonstrate sustained weight loss and
89 improved glucose metabolism for more than one year following a single dose of a GLP-1-Fc
90 fusion protein with an appended Angiopep-2 moiety. This approach raises the prospect of
91 dramatically reduced dosing frequency of this important class of therapeutics.

92

93 **RESULTS**

94 **DNA-based delivery of GLP-1 peptide is efficacious in a diet induced obesity model**

95 To evaluate the feasibility of DNA-based delivery of GLP-1 with MYO Technology we used a
96 DIO mouse model[10–12]. Recombinant GLP-1 currently used in clinic has an added fatty acid
97 moiety, which binds albumin and aids in half-life extension[4] but this is incompatible with a
98 DNA-based approach. We were able to alter the cleavage site recognized by DPP-4 in GLP-1,
99 another strategy incorporated into the current class of GLP-1 drugs. DPP-4 is a protease that
100 breaks down incretin hormones like GLP-1 and GIP, and plays a key role in the regulation of
101 GLP-1 activity[13].

102 To evaluate the effect of MYO-delivered GLP-1, mice were fed a high fat diet (HFD) for 8 weeks
103 and then divided into four groups: two groups were kept on HFD and two groups switched
104 to a standard diet (SD). We reasoned that the cohorts fed SD would better resemble a clinical
105 scenario where obesity patients make dietary changes in addition to the drug treatment.
106 Within each diet group, one subgroup was electroporated with the pDNA encoding GLP-1

107 and one kept as a control (Figure 1A). Mice that remained on HFD and received GLP-1 showed
108 reduced weight gain in the first two weeks compared to the placebo controls (Figure 1B). This
109 effect was transient, and after 2 weeks, all animals on HFD gained weight at the same rate.
110 The therapeutic effect of MYO-delivered GLP-1 was much more pronounced in mice switched
111 to SD (Figure 1C). The experimental cohort started losing weight immediately after IM-EP,
112 showing over 15% weight loss compared to placebo control by week 2. Between week 3 and
113 5, however, animals started regaining weight, and by the end of the experiment, the placebo
114 adjusted weight loss was at ~5%.

115 Another important effect of GLP-1 molecules is the improvement of glycemic control. We
116 therefore performed a glucose tolerance test (GTT) assay at different time points post IM-EP.
117 GTT is a test that measures how the body regulates blood sugar levels after consuming a
118 known amount of glucose[14]. Two weeks after IM-EP delivery, mice that received GLP-1
119 showed improved glucose clearance compared to controls in HFD groups (Figure 1D),
120 demonstrating that the MYO-delivered GLP-1 is biologically active. We also examined
121 glycemia at a later timepoint, when any weight loss benefit was already diminished. Not
122 surprisingly, at eight weeks after IM-EP, we did not detect any difference in the glucose
123 clearance rate (Figure 1E). The same was true for the animals switched to SD; two weeks after
124 EP, animals that received GLP-1 showed improved GTT, but the effect had disappeared by
125 week 8 (Figure 1F and G, respectively).

126

127 **GLP-1 fused to an Fc domain exhibits therapeutic effects comparable to those of**
128 **semaglutide**

129 Since the therapeutic effect of native GLP-1 peptide was limited in time, we decided to fuse
130 GLP-1 with the sequence encoding an IgG Fc region in order to extend the molecule's half-
131 life and boost its bioavailability[5]. We focused on a murine Fc sequence to allow proof-of-
132 concept studies in a DIO mouse model without evoking an anti-drug antibody response. The
133 encoded protein is referred to as GLP-1-mIgG1(Fc) (Figure 2A).

134 First, we compared the potency of GLP-1-mIgG1(Fc) with that of semaglutide. To do so, we
135 developed an in vitro reporter assay[15]. We co-expressed GLP-1 receptor (GLP-1R) and a
136 luciferase reporter under the control of a cAMP-responsive element (CRE) in HEK293T cells.
137 GLP-1R activation leads to the activation of the adenylyl cyclase and consequent increase in
138 cAMP generation which, in turn, activates CRE-dependent transcription. Using this assay, we
139 evaluated GLP-1R activation by recombinant GLP-1-mIgG1(Fc) or semaglutide (Figure 2B). We
140 determined semaglutide EC50 at 0.29 nM, which is comparable to 0.15 nM reported by Novo
141 Nordisk in their FDA application (application #210913Orig1s000). GLP-1-mIgG1(Fc), albeit
142 less potent than semaglutide, activated GLP-1R at low nanomolar ranges (EC50 = 7.35 nM).

143 Next, we evaluated GLP-1-mIgG1(Fc) compared to semaglutide in DIO mice when delivered
144 as purified protein, instead of IM-EP of pDNA. We followed the same diet protocol described
145 in Figure 1A. After randomization into HFD and SD groups, animals received either molecule
146 subcutaneously at 30nmol/kg over a period of 10 days, then observed for another 11 days
147 to monitor the effects of drug withdrawal.

148 In both HFD and SD groups, GLP-1-mIgG1(Fc) and semaglutide demonstrated comparable: i)
149 reduction of body weight (Figure 2C and D, top panels); ii) weight rebound post-withdrawal
150 (Figure 2C and D, bottom panels); and iii) improved glucose clearance (Figure 2E and F). These
151 data confirm that the GLP-1 Fc fusion and semaglutide have comparable effects on weight
152 and glucose metabolism in mice.

153

154 **DNA-based delivery of GLP-1 Fc fusion improves weight loss compared to native GLP-1**

155 After confirming comparable potency of recombinant GLP-1-mIgG(Fc) and semaglutide, we
156 delivered DNA-encoded GLP-1-mIgG1(Fc) with MYO Technology, following the same diet
157 protocol as before (Figure 1A). Similar to the first study (Figure 1B), the cohort kept on a HFD
158 lost minimal weight compared to controls (Figure 3A). The cohort switched to SD, however,
159 started losing weight immediately after EP and showed much bigger weight loss than GLP-1
160 alone (Figure 3B). Importantly, even after animals started regaining weight past week 3, the

161 placebo adjusted weight loss remained at ~10% by the end of the experiment at week 6,
162 which was a two-fold improvement from the GLP-1 alone. Assessment of the GTT showed a
163 significant improvement in the glycemic control in groups that received GLP-1 Fc fusion. The
164 difference was significant at 2 weeks after EP regardless of the diet and was maintained at
165 week 6 in the SD cohort (Figure 3C-F).

166

167 **Enhanced BBB crossing dramatically improves GLP-1-mIgG1(Fc) efficacy**

168 One of the mechanisms by which GLP-1 promotes weight loss is by binding to GLP-1R in the
169 brain, particularly in the hypothalamus, and enhancing the feeling of satiety[1,16]. GLP-1-
170 mIgG1(Fc) fusion is a relatively large protein and presumably unable to easily penetrate the
171 BBB. The inability to pass through the BBB is an issue for several large molecules (e.g.
172 antibodies) as well as for adeno-associated viral vectors, and several strategies have been
173 recently developed to overcome this. One such strategy is the addition of Angiopep-2, a 19
174 amino acid peptide that binds to the low-density lipoprotein receptor related protein-1 (LRP-
175 1) expressed by the endothelial cells of the BBB; Angiopep-2 binding to LRP-1 facilitates the
176 transcytosis of the molecules conjugated to the peptide[17,18]. Therefore, we decided to assess
177 whether the addition of Angiopep-2 to GLP-1-mIgG1(Fc) could improve the molecule's
178 efficacy in treatment of obesity. This new molecule is referred to as GLP-1-mIgG1(Fc)-Angp2
179 (Figure 4A).

180 First, we evaluated the potency of GLP-1-mIgG1(Fc)-Angp2 on GLP-1R and compared it to that
181 of semaglutide and GLP-1-mIgG1(Fc), using the in vitro reporter assay described earlier in
182 this manuscript. While addition of Angiopep-2 to GLP-1-mIgG1(Fc) led to reduction of the
183 potency, it remained at a low EC50 of 31.1 nM (Figure 4B). Thus, we decided to evaluate DNA-
184 based delivery of this molecule in the DIO model, following the same diet protocol described
185 in Figure 1A. Mice that remained on HFD lost ~7% placebo adjusted weight in the first 2
186 weeks, and for the following 16 weeks they gained weight at a slower rate compared to
187 controls (Figure 4C), but the weight started rebounding after week 18. Mice switched to SD,

188 however, exhibited a much more robust therapeutic effect of GLP-1-mIgG1(Fc)-Angp2 after
189 MYO delivery. They started losing weight immediately after IM-EP with ~15% placebo
190 adjusted weight reduction, sustained for over one year after a single administration (Figure
191 4D). We also assessed the response to glucose via GTT assay at different time points.
192 Importantly, mice that received DNA-encoded GLP-1-mIgG1(Fc)-Angp2 showed an improved
193 glucose clearance compared to the controls over an extended period (Figure 4E and F). The
194 effect in animals on SD was more durable, and at week 45 still significantly differentiated
195 from placebo. Interestingly, in the HFD cohort, by week 24 no difference in GTT was detected
196 between treated and control animals, which correlated with weight rebound in the treated
197 animals. These data suggest that targeting GLP-1-mIgG1(Fc) to the BBB, thereby enabling
198 access to hypothalamic GLP-1R, is critical for sustained weight loss and long-term glucose
199 control.

200

201 **Human Fc fusion demonstrates similar effects in an obesity model**

202 Given the ultimate goal of developing a molecule suitable for clinical use, we replaced the
203 murine IgG1 Fc domain with that of human IgG4, and retained the Angiopep-2 fusion. This
204 molecule is referred to as GLP-1-hIgG4(Fc)-Angp2 (Figure 5A).

205 First, we evaluated the potency of GLP-1-hIgG4(Fc)-Angp2 on GLP-1R and compared it to the
206 potency of semaglutide, as described before. We determined that GLP-1-hIgG4(Fc)-Angp2
207 binds GLP-1R and exhibits low EC_{50} of 4.3 nM (Figure 5B).

208 We then evaluated the delivery of GLP-1-hIgG4(Fc)-Angp2 with MYO Technology in mice,
209 following the same experimental design as before (Figure 1A). Mice that were administered
210 DNA-encoded GLP-1-hIgG4(Fc)-Angp2 and kept on a HFD gained weight at a slower rate
211 compared to controls, showing ~7% placebo adjusted weight loss (Figure 5C). Mice that were
212 switched to SD showed over 10% placebo adjusted weight loss (Figure 5D). Furthermore, GTT
213 tests performed at week 4 showed a significantly improved glucose clearance compared to
214 control, regardless of the diet (Figure 5E and 5G). The effect in animals on SD was maintained

also at week 12, while in the HFD cohort, at week 12 no difference in GTT was detected between treated and control animals, which correlated with weight rebound in the treated animals (Figure 5F and 5H).

These data are consistent with our hypothesis that targeting a GLP-1Fc fusion to the brain via the addition of Angiopep-2 promotes a sustained weight loss and a better glucose clearance over time.

MATERIAL AND METHODS

Animals

All animal procedures were performed at Mispro facilities and overseen by the Mispro Institutional Animal Care and Use Committee (IACUC), with the animals cared for in accordance with all federal and state regulations. The mice were housed in disposable, individually ventilated, filter-top cages (Innocage®, Innovive Inc., San Diego, CA, USA).

6/8-week-old C57BL/6J (The Jackson Laboratory, strain #000664) male mice were fed high fat diet (HFD. ResearchDiet, cat# D12451) for 8 weeks. The day before study initiation all mice were weighed and randomized into treatment groups based on body weight (BW) so that all groups had similar starting BW before treatment (n = 5/group). Immediately after IM/EP, mice were either kept on HFD or switched to a standard diet (SD. ResearchDiet, cat# D12450H), and monitored as indicated in the result section. During the course of the study mice weight was recorded weekly. For the % of weight gain/loss (top panels), individual mouse weight recorded the day before IM/EP was considered 100%, and the weight at the following time-points calculated relative to this 100% baseline. For weight loss placebo adjusted, for each week the mouse average weight in the control cohort was considered 100%. Then, the individual weights in the treated cohort calculated as a percentage of this 100% baseline. The difference between this percentage and 100% represents the percentage of weight loss compared to the placebo group.

241 **Electroporation**

242 Electroporation was performed bilaterally in the tibialis anterior (TA) muscle. Target muscle
243 was injected with a 40 µL mixture consisting in 50 µg of pDNA and 3U of Hylenex®
244 (hyaluronidase human injection, Halozyme Therapeutics), formulated in 0.45% saline, with
245 pDNA at a final concentration of 1.25 µg/µL. This formulation was injected using a 29-gauge
246 syringe (Becton Dickinson, cat# 324702), at a depth of 1.9 ± 0.2 mm. The pDNA injection was
247 immediately followed by electroporation using an in-house-developed needle-based
248 electroporator. The electroporator consists of four 0.3 mm diameter gold acupuncture
249 needle electrodes (Asiamed, cat# AMG-3030), each at the corner of a 2 × 2 mm square. A
250 series of pulses at 150 V/cm was applied using a RenBio custom-built generator (RenBio Inc).

251

252 **Glucose Tolerance Test (GTT)**

253 After overnight fasting, basal glucose levels were recorded by using an Auvon glucose meter.
254 Mice were then injected with a glucose (Sigma-Aldrich, cat# G7021) solution intraperitoneally
255 (1.5 g/kg BW in water) and blood glucose measured at the indicated times.

256

257 **GLP-1R in vitro reporter assays**

258 HEK293T (cat#) were seeded at 1.8e6 cells in a 10 cm plate in DMEM (Corning, cat# 10-017-
259 CV) completed with 10% FBS (Corning, cat# 35-011-CV) and 1x pen/strep (Corning 30-002-CI).
260 After 24h 2.5 ug of pDNA expressing GLP-1R and 2.5 ug of pGL4.29[*luc2P*/CRE/Hygro]
261 (Promega, cat# E8471) were transiently transfected with PEI. DNA encoding GLP-1R
262 (GenBank, Gene ID: 2740) was cloned into the pCBAINA, a gWiz-derived expression construct.
263 The backbone of pCBAINA includes a cytomegalovirus (CMV) enhancer, chicken beta actin
264 promoter, CMV intron A, bovine growth hormone polyadenylation (BGH-PolyA) sequence,
265 and kanamycin resistance gene. GLP-1R sequence was synthesized by Twist Bioscience (San
266 Francisco, CA, USA). Medium was changed 6h post-transfection to selection medium

containing 0.5 ug/mL of puromycin (Boston Bioproducts, cat# ABT-440). 24h post-transfection cells were seeded at 70000/well in a 96 multiwell plate. 48h post-transfection cells were incubated with different concentrations of IRAs. After 3h of incubation cells were lysed with Bright-Glo™ Luciferase Assay System (Promega, cat# E2610) according to the manufacturer's protocol, and the luminescence was measured using GloMax Navigator Microplate Luminometer (Promega, cat# GM2000). Luminescence data were normalized as % activation compared to the one of semaglutide at the maximum concentration.

DISCUSSION

In recent years, the IRA class of drugs such as GLP-1 have transformed the treatment of obesity and T2D[3]. It is clear that these molecules not only have the potential to help patients lose weight, but also to make inroads in tackling many of the comorbidities associated with these diseases, including cardiovascular and kidney diseases[19–21]. A major drawback to these drugs, however, is their relatively short half-life, despite both genetic and biochemical modifications aimed at improving their pharmacokinetic (PK) profile. Because of this, the majority of the current generation of approved GLP-1 drugs must be dosed weekly by subcutaneous injection. And as a result of the expense associated with manufacturing protein biologics, as well as the frequent dosing regimen required, these drugs can exact a high cost from patients, often in the range of $1000 per month.

In order to address these barriers of frequent dosing and high costs, we have evaluated a DNA-based delivery approach. Previous work with our MYO Technology platform for the delivery of another small therapeutic protein with a short half-life, G-CSF, yielded very encouraging results regarding the ability of this approach to overcome the poor PK profile of some small proteins[6] Given these results, we sought to determine if this approach could similarly improve the outcomes of treatment with IRAs.

We found that delivery of a GLP-1 RA alone could indeed lead to weight loss in a mouse DIO model. However, these effects were transient, and only significantly different from untreated

294 controls when the animals were also switched to a standard diet following treatment. In
295 order to probe the effect of modifications that would improve the half-life of these
296 molecules, we generated and evaluated Fc-fusion variants, initially appending a murine IgG1
297 Fc. This Fc-fusion protein demonstrated a modest improvement in both weight loss and
298 glucose metabolism as compared to the unmodified GLP-1 RA molecule.

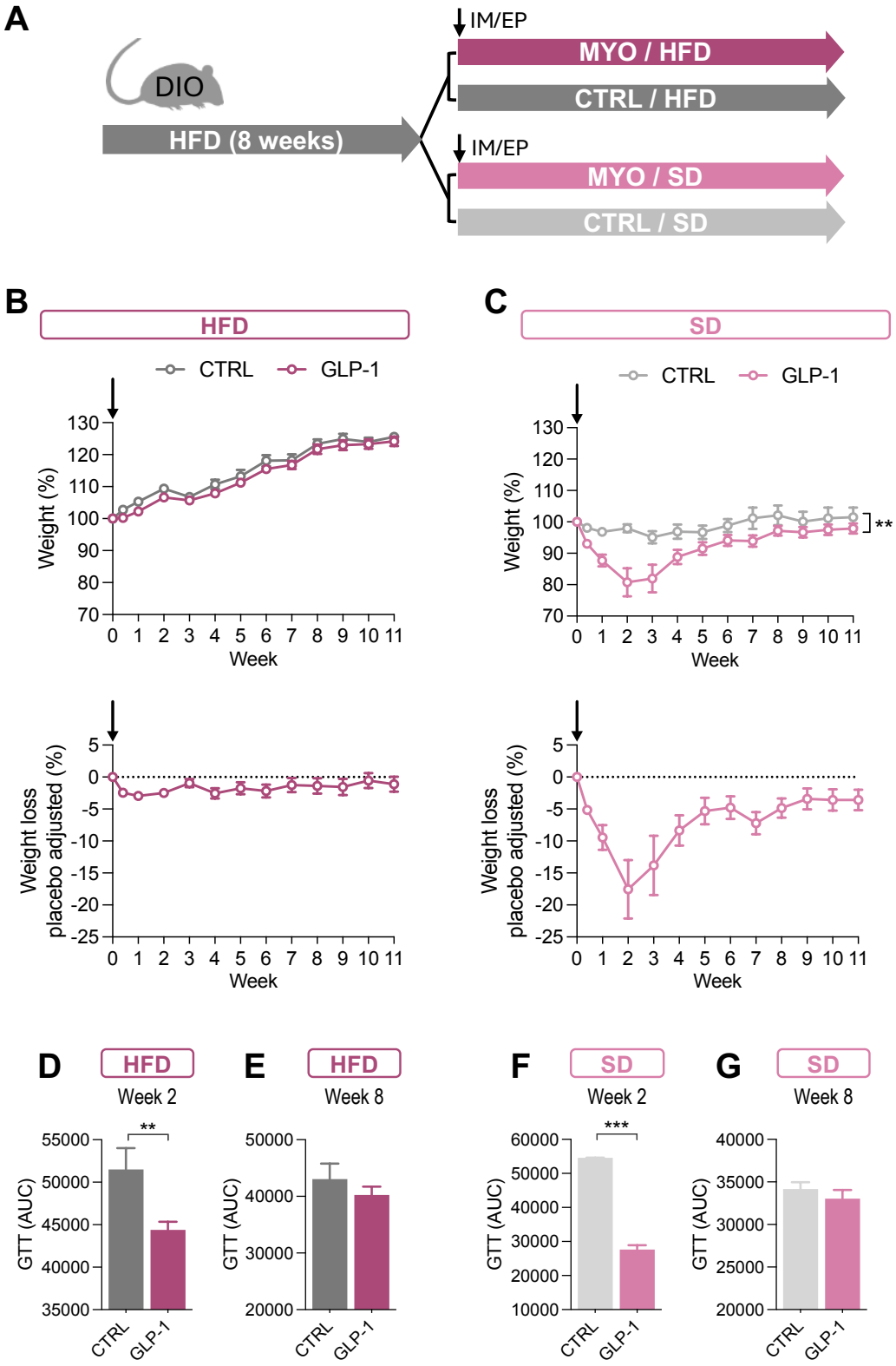
299 Though questions remain about the various mechanisms by which the GLP-1 class of
300 molecules exert their effects on weight loss, strong data indicate that targets in the brain
301 play a role[1]. Thus, we sought to understand whether increased access to the brain, via
302 improved penetration of the BBB, may lead to improved efficacy. Indeed, by appending
303 Angiopep-2, a peptide known to increase BBB targeting[9], we significantly improved the
304 magnitude and duration of weight loss following a single administration, an effect that
305 persisted for over one year. This was also accompanied by a durable improvement in glucose
306 metabolism. Importantly, this effect was also apparent in the context of a human IgG1 fusion,
307 though this study has not yet been followed as long.

308 These results clearly demonstrate that the addition of Angiopep-2 to a GLP-1 RA improves
309 both the magnitude and durability of weight loss in a mouse DIO model, when delivered as
310 a DNA-based drug with MYO Technology. In order to further probe the mechanism of this
311 effect, we plan to evaluate the biodistribution of this new molecule, with a particular focus
312 on the central nervous system.

313 Although the GLP-1 class of drugs has clearly demonstrated potency in terms of overall
314 weight loss, improving the quality of weight loss remains one of the next important hurdles.
315 Many groups are evaluating the ability of additional therapeutics to reduce lean muscle loss,
316 primarily in the form of monoclonal antibodies targeting pathways involved in muscle
317 maintenance and growth, such as myostatin or activin[22,23]. MYO Technology-based delivery
318 is particularly well-suited to such multiplexing, in that delivery of multiple genes can be easily
319 accomplished either on a single plasmid or via incorporation of multiple plasmids expressing
320 different genes.

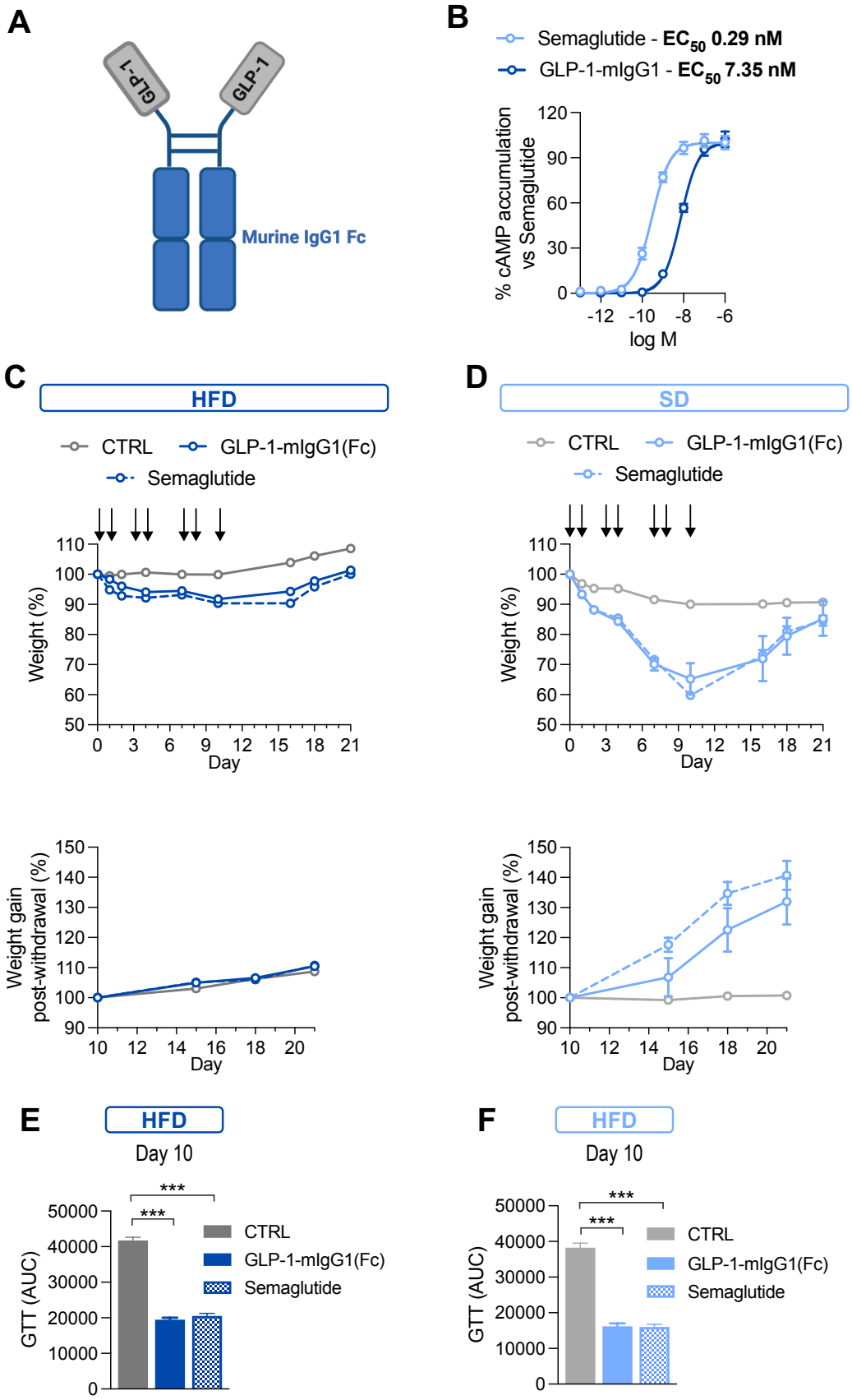
GLP-1 drugs have ignited a dramatic shift in obesity research and treatment, revealing both impressive efficacy and areas clearly needing improvement. We have demonstrated that a DNA-based delivery approach, using MYO Technology, has the potential to overcome the poor PK profile associated with these drugs. Additionally, by taking advantage of the ease of co-delivery of multiple biologics using this approach, we present an opportunity to evaluate many of the molecules aimed at improving the quality of weight loss achieved by these remarkable drugs.

FIGURES


A
DIO
HFD (8 weeks)
IM/EP
MYO / HFD
CTRL / HFD
IM/EP
MYO / SD
CTRL / SD
B
HFD
CTRL
GLP-1
Weight (%)
Week
Weight loss placebo adjusted (%)
C
SD
CTRL
GLP-1
Weight (%)
**
Week
Weight loss placebo adjusted (%)
D
HFD
Week 2
GTT (AUC)
**
CTRL
GLP-1
E
HFD
Week 8
GTT (AUC)
CTRL
GLP-1
F
SD
Week 2
GTT (AUC)
***
CTRL
GLP-1
G
SD
Week 8
GTT (AUC)
CTRL
GLP-1

331 **Figure 1. DNA-based delivery of GLP-1 peptide is efficacious in a diet induced obesity**
332 **model. (A)** Experimental design. Percentage of absolute (top panel) and placebo adjusted
333 weight gain/loss (bottom panel) relative to the time of the EP (Week 0) in mice fed **(B)** high
334 fat diet (HFD) (n=5), and **(C)** standard diet (SD) (n=5). Area under the curve (AUC) derived from
335 glucose tolerance test (GTT) performed at **(D)** week 2, and **(E)** week 8 in mice fed HFD (n=5).
336 AUC derived from GTT performed at **(F)** week 2, and **(G)** week 8 in mice fed SD (n=5). Data
337 are expressed as mean ± SEM. ** $p<0.01$, *** $p<0.01$. Statistical significance was determined
338 by Two-way mixed-effects ANOVA (**B-C**) and unpaired t-test (**D-G**).

339


A
GLP-1
GLP-1
Murine IgG1 Fc
B
Semaglutide - EC50 0.29 nM
GLP-1-mIgG1 - EC50 7.35 nM
% cAMP accumulation vs Semaglutide
log M
C
HFD
CTRL
GLP-1-mIgG1(Fc)
Semaglutide
Weight (%)
Day
Weight gain post-withdrawal (%)
Day
D
SD
CTRL
GLP-1-mIgG1(Fc)
Semaglutide
Weight (%)
Day
Weight gain post-withdrawal (%)
Day
E
HFD
Day 10
GTT (AUC)
CTRL
GLP-1-mIgG1(Fc)
Semaglutide
F
HFD
Day 10
GTT (AUC)
CTRL
GLP-1-mIgG1(Fc)
Semaglutide

340

341 **Figure 2. GLP-1 fused to an Fc domain exhibits therapeutic effects comparable to those**
342 **of semaglutide. (A)** Schematic of the protein. **(B)** Intracellular cAMP accumulation measured
343 in HEK293T cells expressing GLP-1R. Data are expressed as % of cAMP relative to the highest
344 semaglutide concentration (n=10 from 3 independent experiments). Percentage of absolute
345 weight gain/loss relative to the time of the first injection (Day 0, top panel), and percentage
346 of absolute weight gain relative to the time of the last injection (Day 10, bottom panel) in
347 mice fed **(C)** HFD (n=5), and **(D)** SD (n=5). Area under the curve (AUC) derived from glucose
348 tolerance test (GTT) performed at day 10 in mice fed **(E)** HFD (n=5), or **(F)** SD (n=5). Data are
349 expressed as mean ± SEM. *** $p<0.01$. Statistical significance was determined by One-way
350 ANOVA (**E-F**).

351

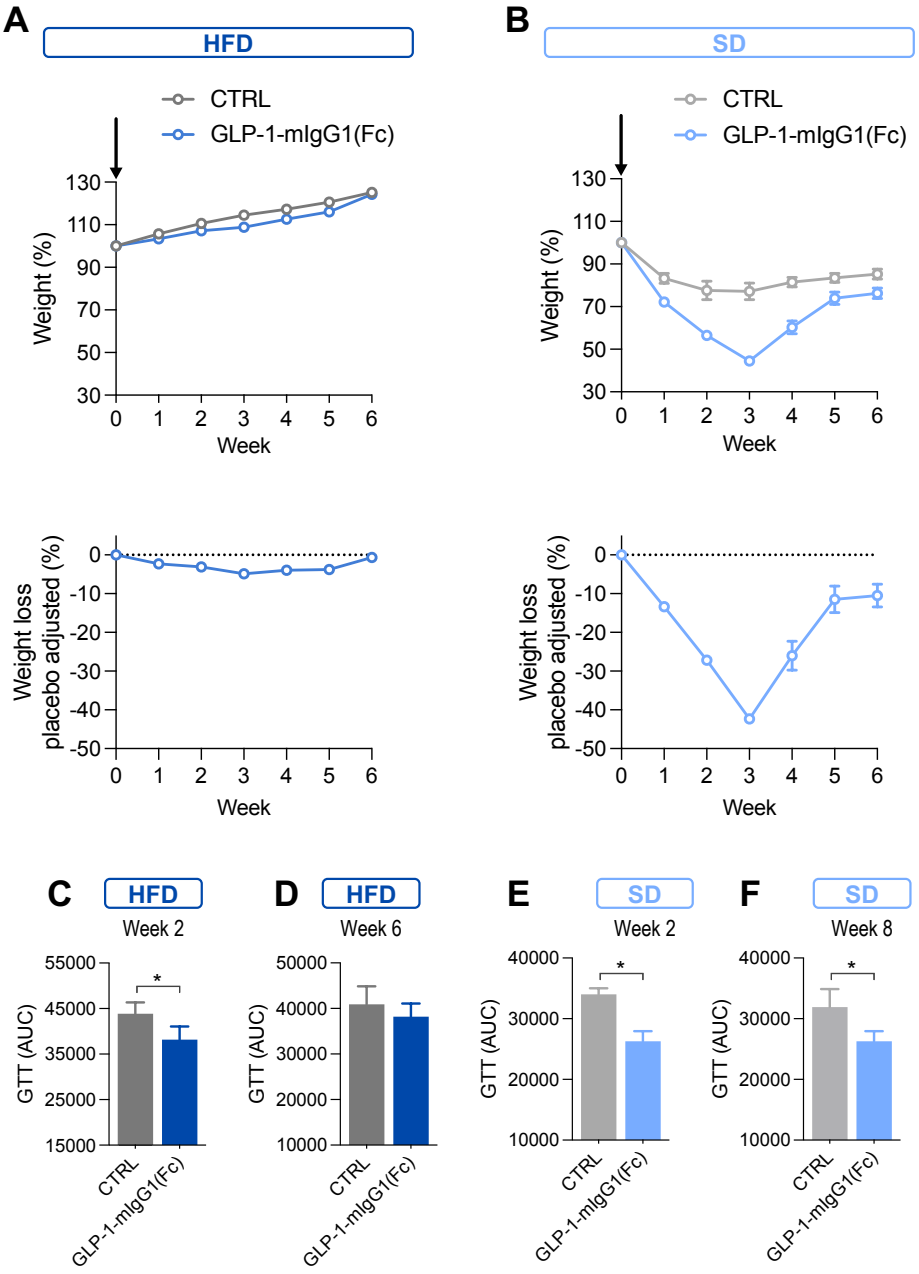


Figure 3. DNA-based delivery of GLP-1 Fc fusion improves weight loss compared to native GLP-1. Percentage of absolute (top panel) and placebo adjusted weight gain/loss (bottom panel) relative to the time of the EP (Week 0) in mice fed in mice fed **(B)** HFD (n=5), and **(C)** SD (n=5). Area under the curve (AUC) derived from glucose tolerance test (GTT) performed at **(C)** week 2, and **(D)** week 6 in mice fed HFD (n=5), and at **(E)** week 2, and **(F)** week 6 in mice fed SD (n=5). Data are expressed as mean ± SEM. * $p<0.05$. Statistical significance was determined by unpaired t-test (**C-F**).

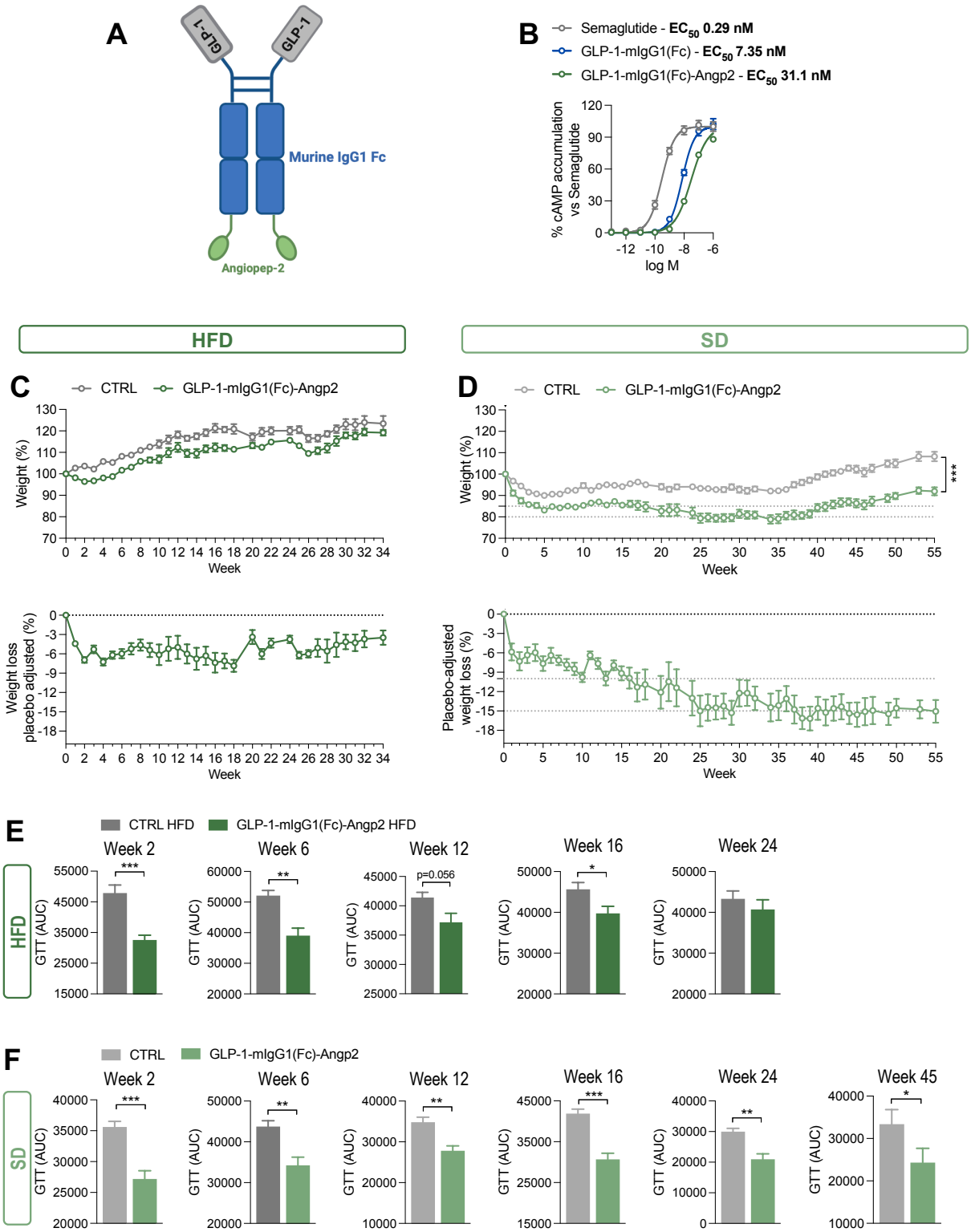
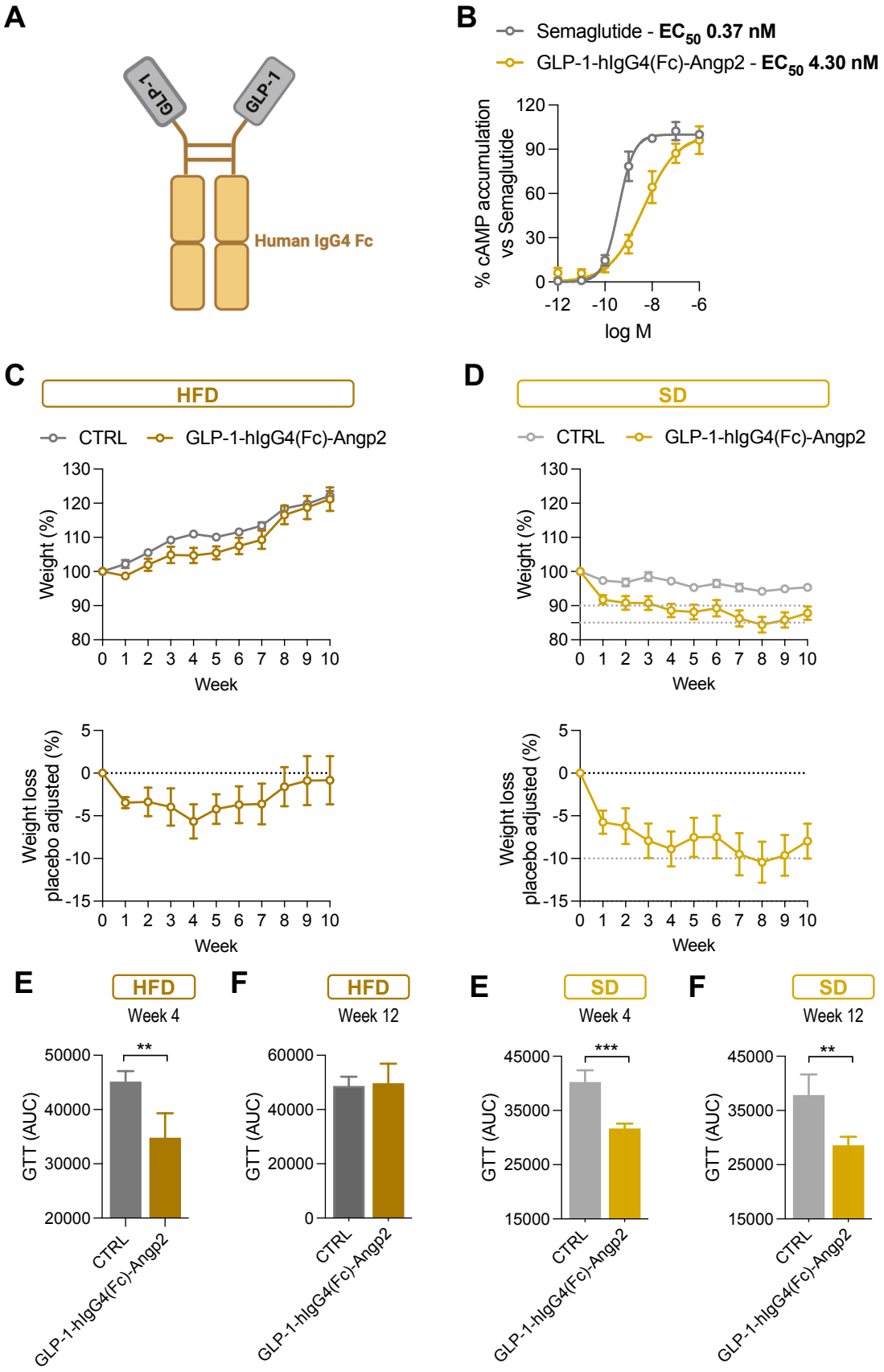


Figure 4. Enhanced BBB crossing dramatically improves GLP-1-mIgG1(Fc) efficacy. (A) Schematic of the protein. **(B)** Intracellular cAMP accumulation measured in HEK293T cells expressing GLP-1R. Data are expressed as % of cAMP relative to the highest semaglutide

363 concentration (n≥4 from 3 independent experiments). Percentage of absolute (top panel)
364 and placebo adjusted weight gain/loss (bottom panel) relative to the time of the EP (Week 0)
365 in mice fed **(C)** high fat diet (HFD) (n=5), and **(D)** standard diet (SD) (n=5). Area under the
366 curve (AUC) derived from glucose tolerance test (GTT) performed at the time points indicated
367 in the figure in mice fed **(E)** HFD (n=5), or **(F)** SD (n=5). Data are expressed as mean ± SEM. *
368 $p<0.05$, ** $p<0.01$, *** $p<0.01$. Statistical significance was determined by Two-way mixed-
369 effects ANOVA (**C-D**) and unpaired t-test (**E-F**).

370


A
GLP-1
GLP-1
Human IgG4 Fc
B
Semaglutide - EC50 0.37 nM
GLP-1-hIgG4(Fc)-Angp2 - EC50 4.30 nM
% cAMP accumulation vs Semaglutide
log M
C
HFD
CTRL
GLP-1-hIgG4(Fc)-Angp2
Weight (%)
Week
Weight loss placebo adjusted (%)
D
SD
E
F
Week 4
Week 12
GTT (AUC)
CTRL
GLP-1-hIgG4(Fc)-Angp2

372 **Figure 5. Human Fc fusion demonstrates similar effects in an obesity model. (A)**
373 Schematic of the protein. **(B)** Intracellular cAMP accumulation measured in HEK293T cells
374 expressing GLP-1R. Data are expressed as % of cAMP relative to the highest semaglutide
375 concentration (n=3). Percentage of absolute (top panel) and placebo adjusted weight
376 gain/loss (bottom panel) relative to the time of the EP (Week 0) in mice fed **(C)** high fat diet
377 (HFD) (n=5), and **(D)** standard diet (SD) (n=5). Area under the curve (AUC) derived from
378 glucose tolerance test (GTT) performed at **(E)** week 4, and **(F)** week 12 in mice fed HFD (n=5),
379 and at **(G)** week 4, and **(H)** week 12 in mice fed SD (n=5). Data are expressed as mean ± SEM.
380 ** $p<0.01$, *** $p<0.01$. Statistical significance was determined by unpaired t-test (**E-G**).

381

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