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Supplemental information

Subcutaneous delivery of FGF21 mRNA therapy reverses obesity, insulin resistance, and hepatic steatosis in diet-induced obese mice

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SUPPLEMENATRY METHODS

Modeling approach

A population approach was used to fit a pharmacokinetic (PK) model to the observed plasma concentrations of either FGF21 protein or Fc-FGF21 protein and to fit a pharmacodynamic (PD) model to the observed bodyweight data. All modeling was done in Phoenix 8.1, NMLE 1.3 (Certara, L.P., 210 North Tucker Boulevard Suite 350, St. Louis, MO 63101 USA) using the FOCE-ELS estimation method. Inter-individual parameter variability was modeled by independent log-normal distributions. Proportional or additive normally distributed residual error models were used in PK and PD models, respectively. Selection of the final PK and PD models, including the addition of inter-individual parameter variability, was based on goodness-of-fit, log likelihood value, η-shrinkage, and precision of parameter estimates.

Pharmacokinetic model

A two-compartment population PK model were fit to the observed protein concentrations of either FGF21 or Fc-FGF21 in plasma following intravenous and subcutaneous administration of recombinant protein and subcutaneous administration of mRNA expressing the corresponding protein. The PK model is defined as

$$\frac{dA_p}{dt} = -k_{a,Protein} \cdot A_p, \tag{S1}$$

$$V\frac{dC}{dt} = Bolus + mRNA + F \cdot k_{a,Protein} \cdot A_p + k_e \cdot A - k_{12} \cdot A_p + k_{21} \cdot A_2, \tag{S2}$$

$$V_2\frac{dC_2}{dt} = k_{12} \cdot A_p - k_{21} \cdot A_2, \tag{S3}$$

where A_p, A and A_2 are the amount of protein in the subcutaneous dosing compartment, central compartment and peripheral compartment, and where F and $k_{a,protein}$ are the bioavailability and absorption rate of protein from the subcutaneous dosing compartment, and where *Bolus* is the intravenous dosing of protein into the central compartment, and where C and C_2 are the drug concentrations in the central and peripheral compartments, and where V and V_2 denote the central and peripheral volumes, and where k_e is the elimination rate, and where k_{12} and k_{21} are the distribution rate from the central to the peripheral compartment and the distribution rate from the peripheral to the central compartment, respectively. *mRNA* is the uptake of protein which have been secreted from mRNA transfected cells. The mRNA transfection and protein secretion into the central compartment is defined as:

$$\frac{dR_{mRNA}}{dt} = -k_{a,mRNA} \cdot R_{mRNA}, \tag{S4}$$

$$\frac{dR_{mRNA,2}}{dt} = fr \cdot k_{a,mRNA} \cdot R_{mRNA} - k_{a,mRNA} \cdot R_{mRNA,2}, \quad \text{(S5)}$$

$$\frac{dA_{mRNA}}{dt} = k_{a,mRNA} \cdot R_{mRNA,2} - k_{a,mRNA} \cdot A_{mRNA}, \quad \text{(S6)}$$

$$mRNA = k_{a,mRNA} \cdot A_{mRNA}, \quad \text{(S7)}$$

where R_{mRNA} is the subcutaneous dosing compartment of mRNA, and where R_{mRNA2} and A_{mRNA} are two transit compartments considering time delays related to cell transfection, endosomal escape, mRNA translation and protein secretion etc., and where $k_{a.\mathrm{mRNA}}$ is the transduction rate constant between the transit compartments and the arbitrary secretion rate of protein into the systemic circulations, and where *fr* is a correlation factor which can be regarded as the product of several factors such as the fraction of productive uptake of mRNA (cell transfection and endosomal escape) into the cytosol, mRNA stability in the cytosol, the translation efficiency of mRNA into proteins by the ribosomes, and the fraction protein correctly folded and successfully secreted into the systemic circulation. To account for an observed drop off in Fc-FGF21 protein exposure following repeated dosing of mRNA, the *fr* was set to be reduced with time according to:

$$\frac{dfr}{dt} = -k_{ind} \cdot fr \quad \text{(S8)}$$

where k_{ind} denotes the fractional turnover rate constant of the observed change. The number of transit compartments in the model were empirically explored.

In the analysis of the Fc-FGF21 protein data, the evaluation of the PK parameters was performed in sequence. First the intravenously and subcutaneously doses of protein was fit, and the derived population PK parameter estimates were fixed in the subsequent analysis when the mRNA produced Fc-FGF21 protein also were included in the data set. In addition, a baseline concentration (*BL*) was introduced when evaluating the Fc-FGF21 concentration, which was a consequence of a minor cross reactivity between Fc-FGF21 protein and endogenous FGF21 by the antibody used in the ELISA assay for analysis the Fc-FGF21 protein.

The empirical Bayes estimates of individual PK parameters were fixed and used as constants in the subsequent PD analysis of Bodyweight data.

Pharmacodynamic model

A turnover model was used to describe the bodyweight change (*Bw*) with time according to,

$$\frac{dBw}{dt} = k_{in} \cdot (1 - Vehicle) + k_{gro} - k_{out} \cdot S(C_p) \cdot R, \quad \text{(S9)}$$

where k_{in}, k_{out} and k_{gro} are the turnover rate, the fractional turnover rate, and the growth rate of bodyweight, respectively. At the start of the pre-dose period, the bodyweight of mice was not at steady state, but the mice were growing. Inclusions of the parameter k_{gro} allows the bodyweight to increase as the mice become older until they reached a plateau. If leaving the animals untreated, the maximal bodyweight obtained at steady state $Bw_{ss,max}$ is defined by:

$$Bw_{ss,max} = Bw_0 + \frac{k_{gro}}{k_{out}}, \quad \text{(S10)}$$

where Bw_0 is bodyweight at start of the pre-dose period. In the model k_{in} was reparametrized as:

$$k_{in} = k_{out} \cdot Bw_0 \quad \text{(S11)}$$

Vehicle relates to a transient vehicle effect, which is defined by,

$$\frac{dvehicle}{dt} = -k_{veh} \cdot Vehicle, \quad \text{(S12)}$$

where k_{veh} is the first order elimination rate of vehicle effect. The relation is activated by adding an arbitrary bolus dose (set to 1) at start of drug treatment. The amplitude of the vehicle effect is individually adjusted for by adding an inter-individual variability on a bioavailability parameter F_{Veh} on the vehicle dose.

The drug effect is added to the model as a stimulus function, *S(C)* on k_{out}, reflecting a drug induced increase in energy expenditure, which will cause the bodyweight to drop when a drug is present. The stimulus function is defined by an ordinary E_{max} function,

$$S(C) = 1 + \frac{E_{max} \cdot C}{EC_{50} + C}, \quad \text{(S13)}$$

where $1+E_{max}$ and EC_{50} are the maximal fold increase in k_{out} (energy consumption) and the drug concentration in plasma at 50% of the maximal effect (potency). The maximal reduction in bodyweight related to drug treatment is defined by,

$$Bw_{ss,min} = \left(Bw_0 + \frac{k_{gro}}{k_{out}}\right)/(1 + E_{max}), \quad \text{(S14)}$$

Besides F_{Veh}, inter-individual variability as added on R_0, k_{gro} and EC_{50}. To explore differences in potency between the two proteins (FGF21 and Fc-FGF21) and if the potency is different if dosing the drugs as recombinant protein or by expressing them with mRNA, the drug and the modality was added as covariates on the EC_{50}.

SUPPLEMENTARY TABLES.

Table S1. **Studies and treatment groups included in PD analysis**. Change in body weight and terminal plasma insulin and triglyceride (TG) levels following 2-weeks repeated dosing with the selected treatments in male diet induced obese mice (DIO). All groups are compared to their respective control group (PBS (Ctrl) for recombinant protein and Control LNP with matched lipid load in relationship to the amount of mRNA). t-test was used for comparing 2 groups and One-way ANOVA with Dunnett's multiple comparison test was used for comparing more than 3 groups. n=7-8 mice/group

Study No	Treatment	Dose (mg/kg)	Change in BW (g)	Insulin (ng/ml)	TG (mM)
1 (BE000227-19)	PBS (Ctrl)		0.8 ± 0.3	2.0 ± 0.3	0.5 ± 0.0
	recFc-FGF21 in PBS	0.15	-2.8 ± 0.5****	0.6 ± 0.1***	0.4 ± 0.0*
	recFc-FGF21 in PBS	0.5	-6.2 ± 0.6****	0.6 ± 0.2***	0.2 ± 0.0****
	recFc-FGF21 in PBS	1.5	-8.9 ± 0.5****	0.3 ± 0.1****	0.2 ± 0.0****
2 (BE000246-29)	PBS (Ctrl)		0.7 ± 0.3	3.8 ± 0.6	0.4 ± 0.0
	recFc-FGF21 in PBS	0.5	-7.3 ± 1.0****	0.5 ± 0.1****	0.2 ± 0.0***
	Control LNP 1.5		-1.9 ± 0.3	5.1 ± 0.6	0.4 ± 0.0
	mFc-FGF21 in LNP	0.15	-0.8 ± 0.5	4.0 ± 0.5	0.5 ± 0.0*
	mFc-FGF21 in LNP	0.5	-4.4 ± 0.8*	1.2 ± 0.3****	0.4 ± 0.0
	mFc-FGF21 in LNP	1.5	-8.6 ± 0.5****	0.9 ± 0.1****	0.4 ± 0.0
3 (BE000246-32)*	PBS (Ctrl)		-0.4 ± 0.3	4.8 ± 0.5	0.5 ± 0.0
	recFc-FGF21 in PBS	0.5	-8.2 ± 0.5****	0.6 ± 0.0****	0.2 ± 0.03***
	Control LNP 0.1		-1.8 ± 0.2	4.8 ± 0.5	0.6 ± 0.1
	mFGF21 in LNP	0.1	-7.3 ± 0.8****	1.0 ± 0.1****	0.3 ± 0.0**
	control LNP 0.3		-1.4 ± 0.2	6.8 ± 0.6	0.6 ± 0.1
	mFc-FGF21 in LNP	0.3	-9.3 ± 0.7****	1.4 ± 0.2****	0.4 ± 0.0**

* mRNA used in this study is mouse sequence

Table S2 Studies and treatment groups included in PK and PKPD analysis.

Study No	Strain	Treatment	Dose (mg/kg)	Dosing frequency	Route	Formulation (lipid:mRNA ratio)
BE000227-19 (Study 1)	DIO	Ctrl	-	o.d.	s.c.	PBS
		recFc-FGF21	0.15	o.d.	s.c.	PBS
		recFc-FGF21	0.5	o.d.	s.c.	PBS
		recFc-FGF21	1.5	o.d.	s.c.	PBS
BE000246-29 (Study 2)	DIO	Ctrl	-	o.d.	s.c.	PBS
		recFc-FGF21	0.5	o.d.	s.c.	PBS
		LNP 1.5[a]	-	o.d.	s.c.	LNP w 0.01 mg/kg R-C14 (20:1)
		mFc-FGF21	0.15	o.d.	s.c.	LNP w 0.01 mg/kg R-C14 (20:1)
		mFc-FGF21	0.5	o.d.	s.c.	LNP w 0.01 mg/kg R-C14 (20:1)
		mFc-FGF21	1.5	o.d.	s.c.	LNP w 0.01 mg/kg R-C14 (20:1)
BE000227-09	DIO	Ctrl	-	b.i.d	s.c.	PBS
		recFGF21	0.3	b.i.d	s.c.	PBS
		recFGF21	1	b.i.d	s.c.	PBS
BE000246-26	DIO	Ctrl	- (day 1-8)	o.d.	s.c.	TRIS
		mFGF21	0.5 (day 9)	single.		LNP w 0.01 mg/kg R-C14 (20:1)
		mFGF21	1 (day 1-5) 0.5 mg/kg (day 6-9)	o.d.	s.c.	LNP w 0.01 mg/kg R-C14 (20:1)
BE000246-32 (Study 3)*	DIO	Ctrl	-	o.d.	s.c.	PBS
		recFc-FGF21	0.5	o.d.	s.c.	PBS
		LNP 0.1[a]	-	o.d.	s.c.	LNP (10:1)
		mFGF21	0.1	o.d.	s.c.	LNP (10:1)
		LNP 0.3[a]	-	o.d.	s.c.	LNP (10:1)
		mFGF21	0.3	o.d.	s.c.	LNP (10:1)
BE000246-31	DIO	Ctrl	-	o.d.	s.c.	PBS
		recFc-FGF21	0.5	o.d.	s.c.	PBS
		LNP 0.5[a]	-	o.d.	s.c.	LNP w 0.05 mg/kg B-C14 (10:1)
		mFGF21	0.3	o.d.	s.c.	LNP w 0.05 mg/kg B-C14 (10:1)
		mFGF21	0.3	q2d	s.c.	LNP w 0.05 mg/kg B-C14 (10:1)
		LNP 0.5[a]	-	q2d	s.c.	LNP w 0.05 mg/kg B-C14 (10:1)
		mFc-FGF21	0.5	o.d.	s.c.	LNP w 0.05 mg/kg B-C14 (10:1)
		mFc-FGF21	0.5	q2d	s.c.	LNP w 0.05 mg/kg B-C14 (10:1)
BE000370-86	Lean	recFGF21	0.3	Single	i.v.	PBS
		recFGF21	0.3	Single	s.c.	PBS
BE001143-59	Lean	recFc-FGF21	0.74	Single	i.v.	PBS
		recFc-FGF21	0.74	Single	s.c.	PBS
BE001143-77	DIO	mFc-FGF21	0.5	Single	s.c.	LNP w 0.05 mg/kg B-C14 (10:1)
		mFc-FGF21	0.5	o.d.	s.c.	LNP w 0.05 mg/kg B-C14 (10:1)

[a] Control LNP containing a lipid load similar to a mRNA dose stated as number
s.c.: subcutaneous i.v.: intravenously
o.d.: once daily b.i.d: twice daily q2d: once every second day
R-C14: Rofleponide myristate
B-C14: Budesonide myristate
* mRNA used in this study is mouse sequence

Table S3 Pharmacokinetic parameters estimates, inter-individual variability (IIV), and their corresponding relative standard errors (RSE%)

				FGF21		Fc-FGF21	
Modality	Data	Parameter	Definitions	Estimate (RSE%))	IIV (RSE%)	Estimate (RSE%)	IIV (RSE%)
Protein	*Primary Parameter*	F (%)	Bioavailability	109 (13)		40.2 (14)	0.164 (68)
		t_{lag} (h)	Time until start of absorption	0.13 (20)		-	
		$k_{a,protein}$ (h^{-1})	Absorption rate of protein	1.44 (6)		0.186 (15)	
		V_1 ($L \cdot kg^{-1}$)	Volume, central compartment	1.83 (4)		0.031 (21)	
		k_e (h^{-1})	Elimination rate	1.81 (8)	0.0645 (35)	0.152 (21)	0.0024 (70)
		k_{12} (h^{-1})	Distribution rate from central into peripheral compartment	0.087 (40)		0.240 (57)	
		k_{21} (h^{-1})	Distribution rate from peripheral into central compartment	0.51 (12)		0.325 (18)	
	Secondary parameters	CL ($L \cdot h^{-1} \cdot kg^{-1}$)	Clearance	3.30 (10)		0.0047 (5)	
		CL_2 ($L \cdot h^{-1} \cdot kg^{-1}$)	Intercompartmental clearance	0.16 (43)		0.0074 (38)	
		V_2 ($L \cdot kg^{-1}$)	Volume, peripheral compartment	0.31 (34)		0.0229 (26)	
		V_{ss} ($L \cdot kg^{-1}$)	Volume, steady state	2.14 (8)		0.0539 (6)	
		MRT (h)	Mean residence time	0.65 (4)		11.4 (4)	
		$t_{½,\lambda1}$ (h)	Half-life of the first phase in the bi-exponential decline	0.36 (9)		1.08 (33)	
		$t_{½,\lambda2}$ (h)	Half-life of the second phase in the bi-exponential decline	1.5 (10)		8.9 (3)	
mRNA	*Primary Parameter*	fr (fold)	Fraction of mRNA produced protein	26.0 (12)		0.858 (11)	
		$k_{a,mRNA}$ (h^{-1})	Upptake rate of mRNA produced protein into systemic circulation	0.23 (4)		0.098 (6)	
		Ind	Rate of reduced mRNA produced protein	-		0.0041 (16)	
	Secondary parameters	$t_{½,ka,mRNA}$ (h)	Half-life of mRNA produced protein into systemic circulation	3 (4)		7.1 (6)	
All		σ (fraction)	Residual proportional error	0.33 (7)		0.39 (7)	

Table S4 Pharmacodynamic parameters estimates, inter-individual variability (IIV), and their corresponding relative standard errors (RSE%)

				Model fit		Bootstrap (n=200)	
	Data	Parameter	Definitions	Estimate	IIV	Estimate (RSE%)	IIV (RSE%)
Drug	*Primary Parameter*	$EC_{50,FGF21\ protein}$ (nmol·L^{-1})	Potency of FGF21 protein	0.135	0.629[a]	0.136 (15)	0.613 [a] (20)
		$EC_{50,\ FGF21\ mRNA}$ (nmol·L^{-1})	Potency of mRNA produced FGF21 protein	0.136	0.629[a]	0.136 (15)	0.613 [a] (20)
		$EC_{50,Fc\text{-}FGF21\ protein}$ (nmol·L^{-1})	Potency of Fc-FGF21 protein	25.8	0.629[a]	26.6 (18)	0.613 [a] (20)
		$EC_{50,\ Fc\text{-}FGF21\ mRNA}$ (nmol·L^{-1})	Potency of mRNA produced Fc-FGF21 protein	5.33	0.629[a]	5.46 (18)	0.613 [a] (20)
				Shared parameters between FGF21 and Fc-FGF21			
		E_{max} (fold)	Maximal fold increase in Energy consumption	1.70		1.76 (15)	
Vehicle	*Primary Parameter*	F_{Veh} (fraction)	Amplitude of Vehicle effect	0.318	0.512	0.326 (17)	0.525 (28)
		k_{Veh} (h^{-1})	Onset and elimination rate of vehicle effect	0.0052		0.0052 (18)	
System	*Primary Parameter*	Bw_0 (g)	Initial Bodyweight	46.3	0.0058	46.3 (1)	0.0058 (12)
		k_{growth} (g·h^{-1})	Growth rate	0.0055	0.256	0.0055 (7)	0.252 (18)
		k_{out} (h^{-1})	Fractional turnover rate of Bodyweight	0.0006		0.0006 (15)	
		σ	Residual additive error	0.54		0.54 (2)	
	Secondary parameters	$Bw_{ss,max}$ (g)	Bodyweight plateau without drug	55.5		55.7 (2)	
		$Bw_{ss,min}$ (g)	Bodyweight plateau with maximal drug response	20.6		20.4 (8)	

[a] Categorical covariates were used to calculate the difference in potency between dosed FGF21 protein and mRNA produced FGF21 protein, dosed Fc-FGF21 protein or mRNA produced Fc-FGF21 protein. The inter-individual variability is therefore shared and identical for all potency estimates.

Table S5 The amino acid sequences of recombinant proteins or mRNA constructs used in the in vivo studies.

Name	Description	Amino acid sequence of mature protein (signal sequence not shown)	Comments
mFGF21	mRNA wild-type human FGF21	HPIPDSSPLLQFGGQVRQRYLYTDDAQQTEAHLEIREDGTVGGAADQSPES LLQLKALKPGVIQILGVKTSRFLCQRPDGALYGSLHFDPEACSFRELLLEDG YNVYQSEAHGLPLHLPGNKSPHRDPAPRGPARFLPLPGLPPAPPEPPGILAP QPPDVGSSDPLSMVGPSQGRSPSYAS	Common allele rs739320 L174P
recFGF21	recombinant wild-type human FGF21 produced in E coli	MHHHHHHENLYFQHPIPDSSPLLQFGGQVRQRYLYTDDAQQTEAHLEIRED GTVGGAADQSPESLLQLKALKPGVIQILGVKTSRFLCQRPDGALYGSLHFDP EACSFRELLLEDGYNVYQSEAHGLPLHLPGNKSPHRDPAPRGPARFLPLPG LPPAPPEPPGILAPQPPDVGSSDPLSMVGPSQGRSPSYAS	His-TEV sequence at amino terminus
mFc-FGF21	mRNA fusion of Fc with wild-type human FGF21	METDTLLLMVLLLWVPGSTGDKTHTCPPCPAPELLGGPSVFLFPPKPKDTL MISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRV VSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPS RDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFL YSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGKGGGGGSGG GSGGGGSHPIPDSSPLLQFGGQVRQRYLYTDDAQQTEAHLEIREDGTVGG AADQSPESLLQLKALKPGVIQILGVKTSRFLCQRPDGALYGSLHFDPEACSF RERLLEDGYNVYQSEAHGLPLHLPGNKSPHRDPAPRGPARFLPLPGLPPAP PEPPGILAPQPPDVGSSDPLSMVGGSQGRSPSYAS	2 point mutations were introduced into FGF21 to block the FAPa proteolytic site (P171G, FGF21 numbering) and to reduced aggregation (L98R)
recFc-FGF21	recombinant fusion of Fc with wild-type human FGF21 purified from supernatant of transient plasmid transfected HEK293	ATHTCPPCPAPEFEGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEV KFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKV SNKALPASIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPS DIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCS VMHEALHNHYTQKSLSLSPGGGGGGSGGGSGGGGSHPIPDSSPLLQFGG QVRQRYLYTDDAQQTEAHLEIREDGTVGGAADQSPESLLQLKALKPGVIQIL GVKTSRFLCQRPDGALYGSLHFDPEACSFRERLLEDGYNVYQSEAHGLPLH LPGNKSPHRDPAPRGPARFLPLPGLPPAPPEPPGILAPQPPDVGSSDPLSM VGGSQGRSPSYAS	2 point mutations were introduced into FGF21 to block the FAPa proteolytic site (P171G, FGF21 numbering) and to reduced aggregation (L98R)

Table S6 List of TaqMan Gene Expression Assays.

Mouse Assays
DUSP4: Mm00723761_m1 DUSP6: Mm00518185_m1 SPRY4: Mm00442345_m1 Spred1: Mm01277511_m1 KLB: Mm00473122_m1 TBP: Mm00446973_m1 GAPDH: Mm99999915_g1 FGFr1c: Mm00442345_m1
Human Assays
DUSP4: Hs01027785_m1 SPRY4: Hs01935412_s1 TBP: Hs00427620_m1

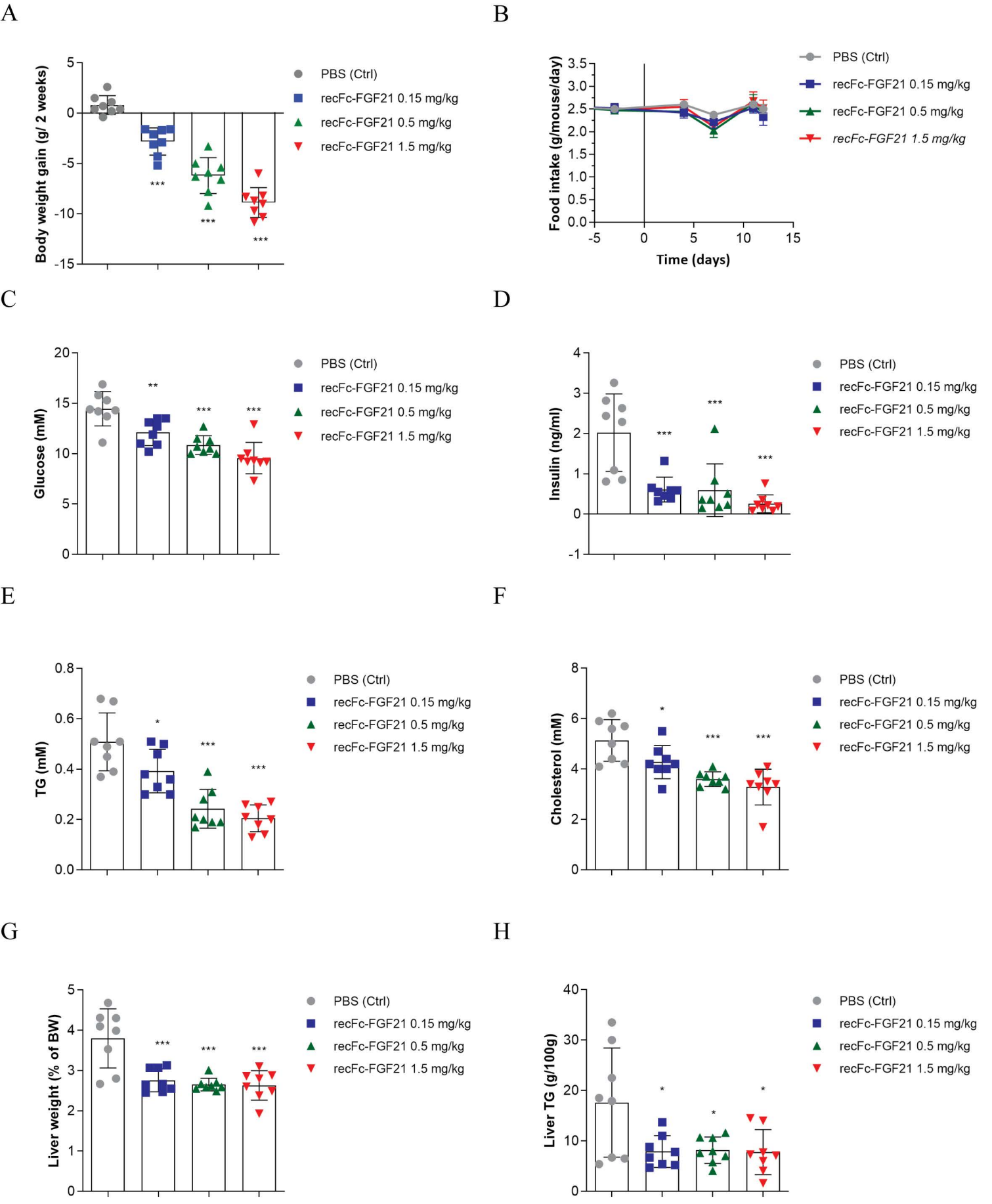


Figure S1. Dose dependent improvement in metabolic endpoints following repeated administration of recFC-FGF21 to DIO mice. Two weeks repeated dosing with recFc-FGF21 (0.15, 1.5 and 1.5 mg/kg, qd) to DIO mice reduces body weight (A) without affecting food intake (B) and reduces plasma glucose (C), insulin (D), TG (E), cholesterol (F), liver weight (G) and liver TG (H) compared to PBS treated mice. Figures show mean ±SEM. N=8, * P<0.05, ** P<0.01, and ***P<0.001 vs PBS. Ordinary one-way ANOVA was performed followed by Dunnett's test.

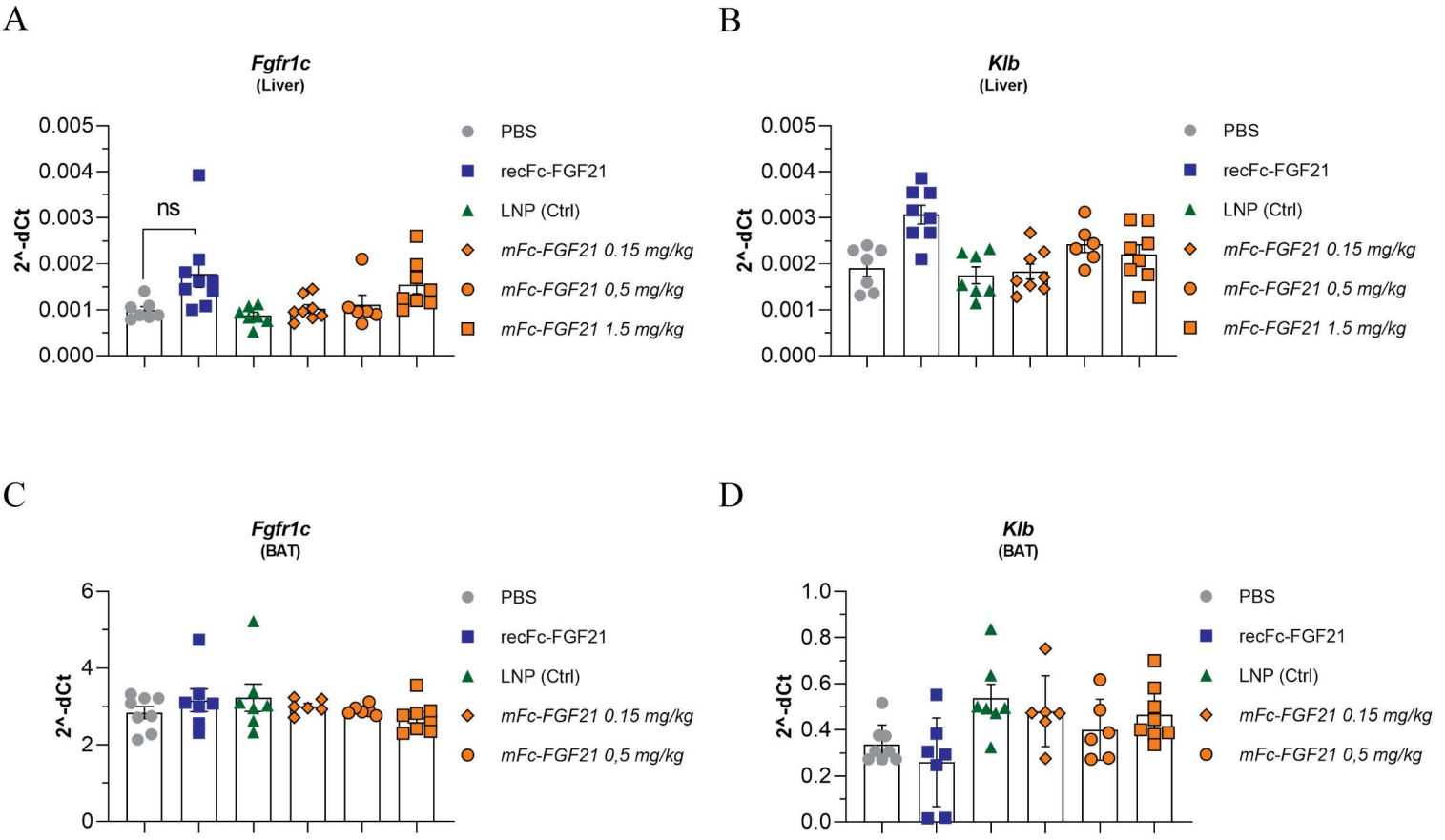


Figure S2. mRNA encoding FGF21 does not alter expression of *Fgfr1* and *β-Klotho* in target tissues. Two weeks repeated dosing with protein (recFcFGF21) or mRNA encoding FcFGF21(mFc-FGF21) to DIO mice translates to FGF21 signal / target engagement in liver (A-B) and brown adipose tissue (BAT; C-D). Figures show mean ±SEM. N=4-8. Ordinary one-way ANOVA was performed followed by Sidak's multiple comparison test. Fgfr1c and Klb (β-Klotho) gene expression, as expected, remained unchanged.

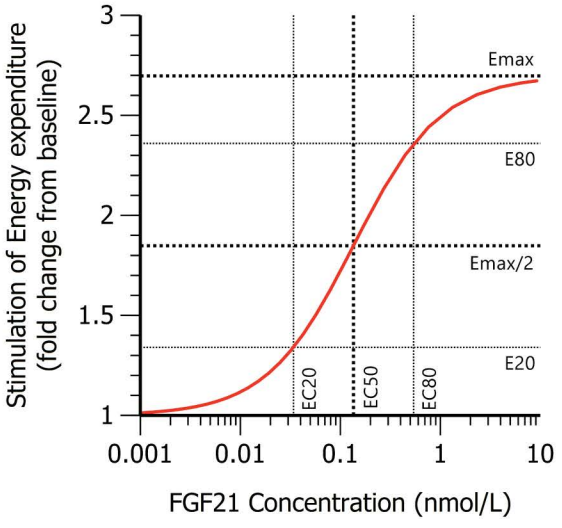
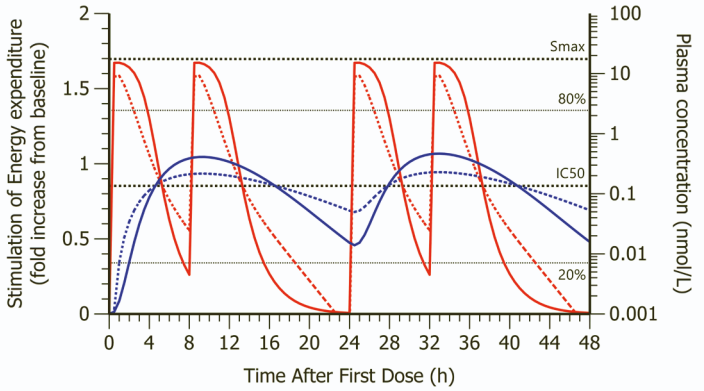


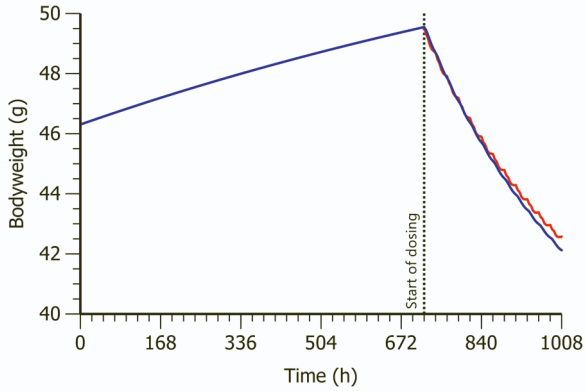
Figure S3. Relationship between drug concentration and drug effect.

A - Scenario 1

1 mg/kg recFGF21 b.i.d. and 0.13 mg/kg *mFGF21* o.d.



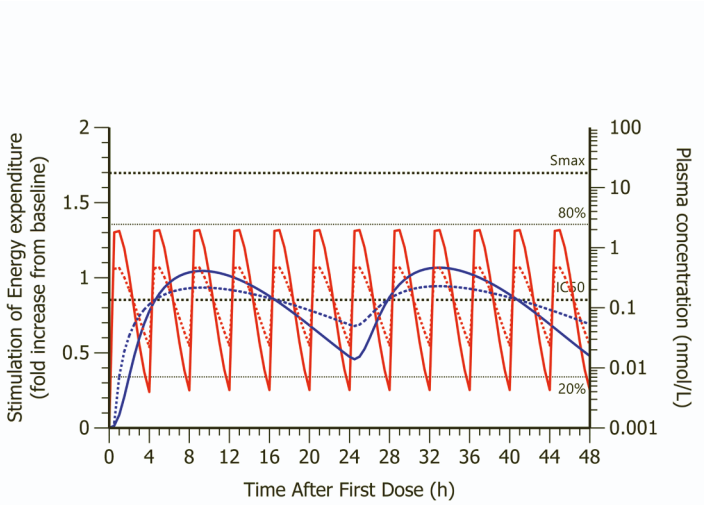
Predicted body weight reduction



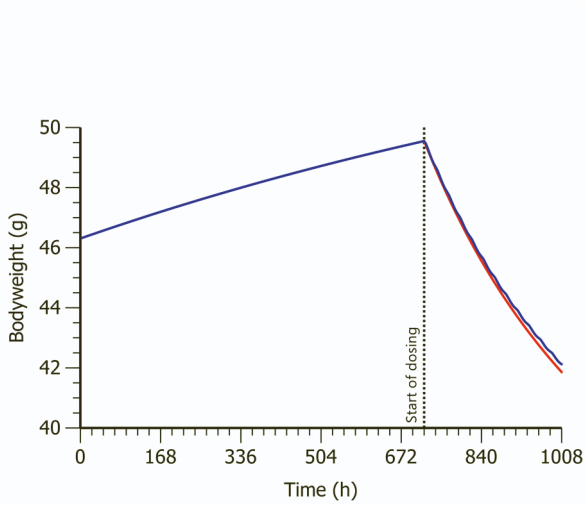
$C_{ss,av\ protein}$ = 1.41nM $E_{ss,av\ protein}$ = 0.75 (44% of Emax)
$C_{ss,av\ mRNA}$ = 0.145nM $E_{ss,av\ mRNA}$ = 0.84 (49% of Emax)

B - Scenario 2

0.05 mg/kg recFGF21 q6d and 0.13 mg/kg *mFGF21* o.d.



Predicted body weight reduction



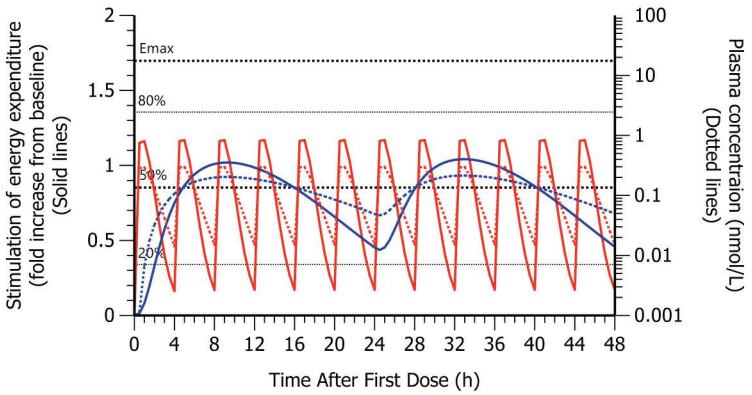
$C_{ss,av\ protein}$ = 0.212nM $E_{ss,av\ protein}$ = 0.75 (44% of Emax)
$C_{ss,av\ mRNA}$ = 0.145nM $E_{ss,av\ mRNA}$ = 0.84 (49% of Emax)

Solid lines - stimulation of energy expenditure
Dotted lines - FGF21 protein concentration

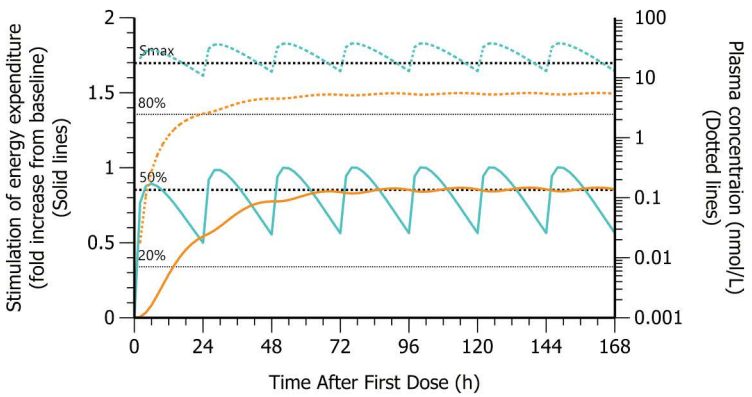
Red - recombinant protein administration
Blue - mRNA administration

Figure S4. Simulation of protein exposure, stimulation of energy expenditure and body weight change using different dosing regimens of recFGF21 compared to mFGF21. Scenario 1: A high daily dose of recFGF21 (2 mg/kg) is required to generate similar bodyweight change as mFGF21 (0.13 mg/kg, qd) if using a dosing regimen (1 mg/kg b.i.d) which results in drug concentrations outside the EC_{20}-EC_{80} range for a substantial period of time of the daily dosing interval. Scenario 2: The daily dose of the recFGF21 can be reduced (0.3 mg/kg) by using a dosing regimen (0.05 mg/kg, q6d) which results in protein concentrations within the EC_{20}-EC_{80} range during the main part of the daily dosing interval.

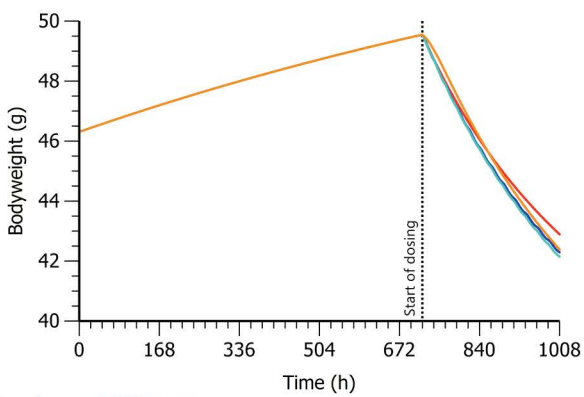
A



B



C



0.032 mg/kg recFGF21 q6d
0.125 mg/kg *mFGF21* qd
0.357 mg/kg recFc-FGF21 qd
0.368 mg/kg *mFc-FGF21* qd*

* the observed drop-off in exposure following repeated dosing of *mFc-FGF21* was ignored in simulation.

Figure S5. Simulation of protein exposure, stimulation of energy expenditure and body weight change using doses of recFGF21, mFGF21, recFc-FGF21 or mFc-FGF21 targeting in vivo EC50 as average concentration during the dosing interval at steady state. (A) Simulation of FGF21 protein exposure (dotted lines) and stimulation of energy expenditure (filled lines) using 0.032 mg/kg recFGF21 q6d (red) or 0.125 mg/kg mFGF21 qd (blue), (B) Simulation of Fc-FGF21 protein exposure (dotted lines) and stimulation of energy expenditure (filled lines) using 0.357 mg/kg recFc-FGF21 qd (turquoise) or 0.368 mg/kg mFc-FGF21 qd (amber), (C) Predicted bodyweight change using the dosing regimes in A and B.

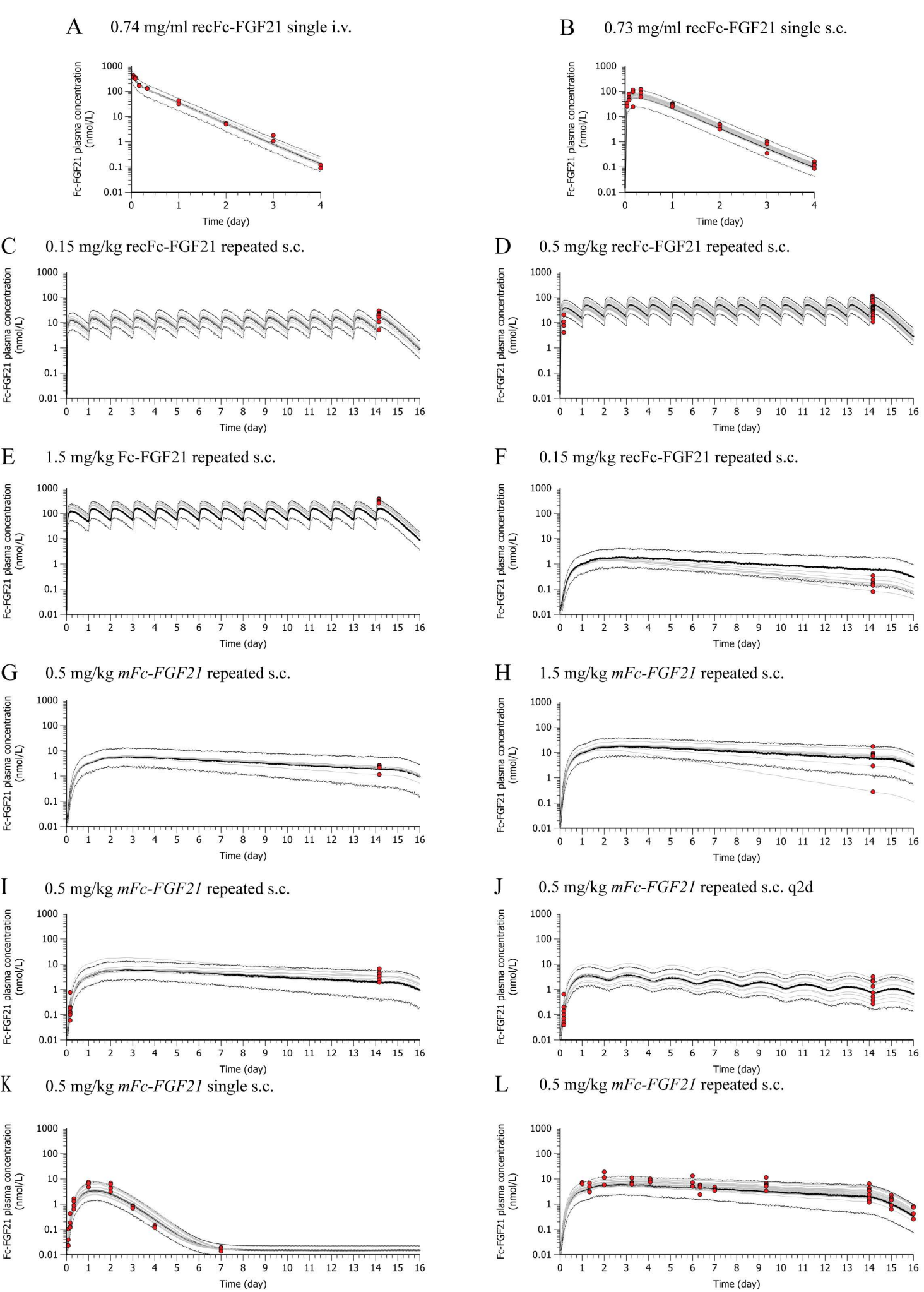


Figure S6 Pharmacokinetics.Visual predictive check of model fit to Fc-FGF21 protein concentrations vs. time following recombinant Fc-FGF21 (recFc-FGF21) administration or mRNA encoding Fc-FGF21 (*mFGF21*)

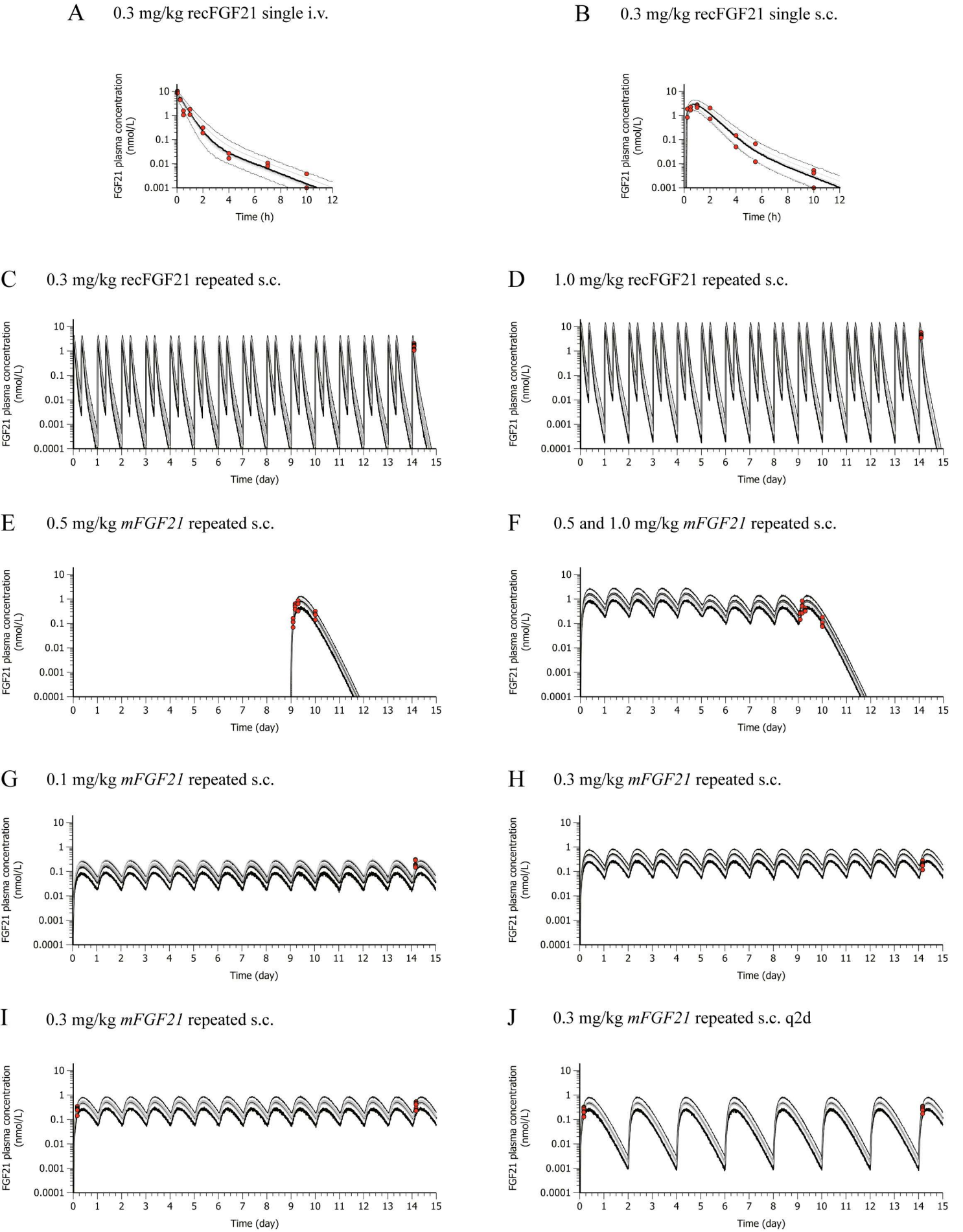


Figure S7 Pharmacokinetics. Visual predictive check of model fit to FGF21 protein concentrations vs. time following recombinant FGF21 (recFGF21) administration or mRNA encoding FGF21 (*mFGF21*)

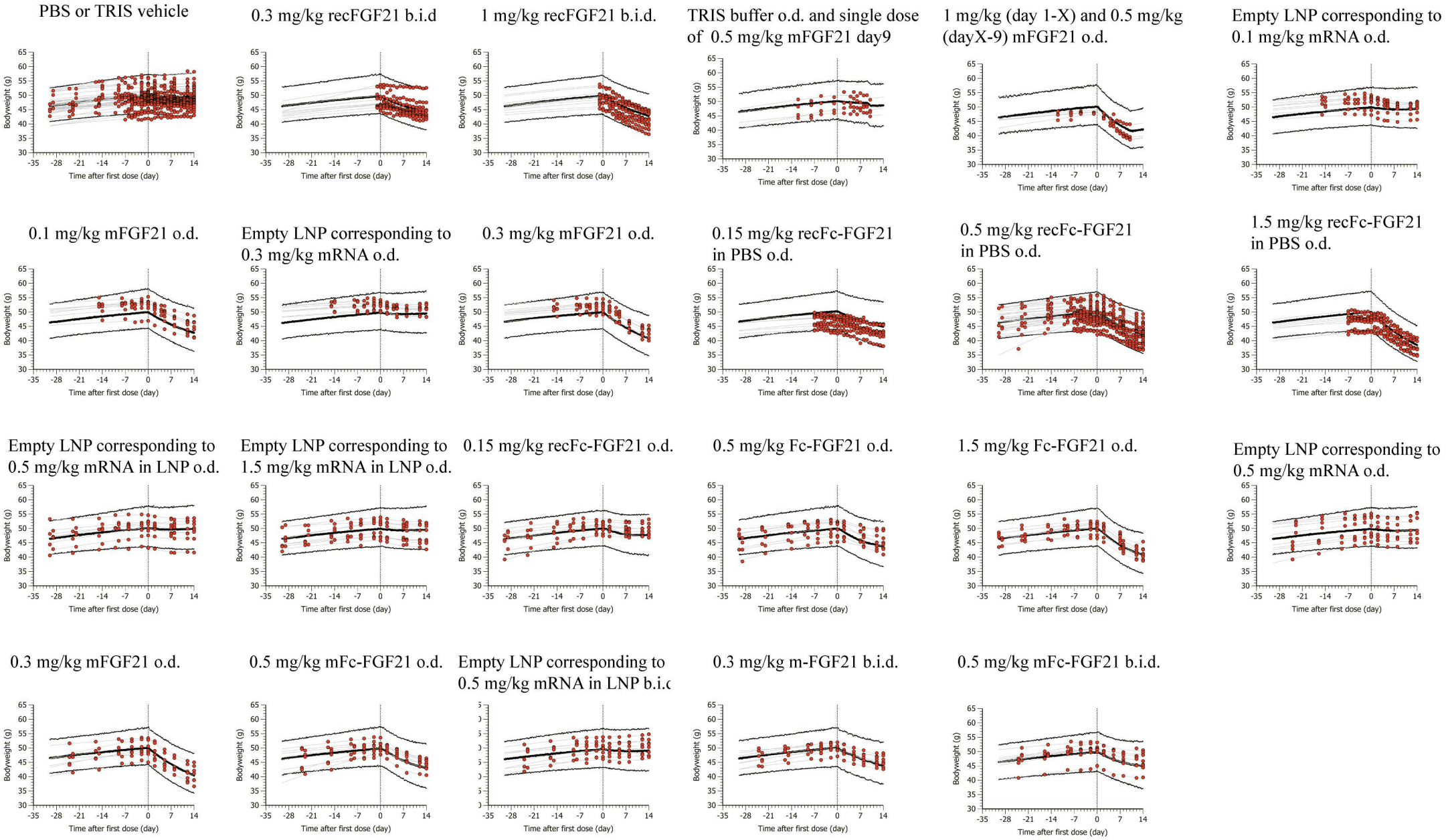


Figure S8 Visual predictive check of bodyweight changes following vehicle, FGF21 and Fc-FGF21 protein or mRNA (mFGF21, and mFc-FGF21) treatments.