

SUPPLEMENTARY MATERIAL

Characterization of a new potent and long-lasting single chain peptide agonist of RXFP1 in human cells and in vivo translational models

Stephane Illiano^{†*}, Bruno Poirier[†], Claire Minoletti[§], Olivier Pasquier[#], Laurence Riva[†], Xavier Chenede[†], Isabelle Menguy[§], Michel Guillotel[†], Philippe Prigent[†], Stéphane Le Claire[†], Florence Gillot[†], Gilbert Thill[§], François Lo Presti[†], Alain Corbier[†], Jean-Christophe Le Bail[†], Patrick Grailhe[†], Edith Monteagudo[‡], Raffaele Ingenito[‡], Elisabetta Bianchi[‡], Christophe Philippo[†], Olivier Duclos[§], Sergio Mallart[§], Ross Bathgate[¥], Philip Janiak[†].

[†] Cardio-Vascular and metabolism, Sanofi R&D, 1 avenue Pierre Brossolette 91385 Chilly Mazarin, France.

[§] Integrated Drug Discovery, Sanofi R&D, 1 avenue Pierre Brossolette 91385 Chilly Mazarin, France

[#] DMPK France, Sanofi R&D, 1 avenue Pierre Brossolette 91385 Chilly Mazarin, France

^{\$} Translational Science, Sanofi R&D, 1 avenue Pierre Brossolette 91385 Chilly Mazarin, France

[‡] Peptides and Small Molecules R&D Department, ≠DMPK, and Structural Biology IRBM Spa, Via Pontina Km 30 600, 00 071 Pomezia (ROME), Italy

[¥] Florey Institute of Neuroscience and Mental Health and Department of Biochemistry and Pharmacology, The University of Melbourne, Parkville, VIC 3052, Australia

Correspondence and reprints request to:

*Stephane Illiano, PhD, IRSN, B.P. 17 – 92262 Fontenay-aux-Roses Cedex, France;

Email: stephane.illiano@irsn.fr, Tel: +33-1 58 35 80 71

Supplementary Table S1

Individual and Mean plasma PK parameters of SA10SC_RLX following IV administration at 1 mg/kg to male Sprague-Dawley Rats

Animal ID	Co (μM)	AUC ($\mu\text{M}\cdot\text{h}$)	AUXext (%)	CL (mL/min/kg)	Vss (L/kg)	T1/2 (h)	number of PK points used for t1/2 calculation
1	3.02	8.48	1.01	0.48	0.14	4.16	5
2	3.36	9.32	1.01	0.44	0.11	3.49	5
3	3.28	10.9	1.02	0.38	0.12	4.11	5
Mean	3.2	9.57	1.01	0.43	0.12	3.9	
s.d.	0.2	1		0.05	0.01	0.4	

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Supplementary Table S2

Individual and Mean plasma PK parameters of SA10SC_RLX following SC administration at 3 mg/kg to male Sprague-Dawley Rats

Animal ID	Cmax (μM)	Tmax (h)	AUC ($\mu\text{M}\cdot\text{h}$)	AUC _{ext} (%)	F (%)
4	1.59	8	20.7	1.07	72
5	1.88	8	25.4	1.10	89
6	1.11	8	17.6	1.11	61
Mean	1.5	8	21.2	1.09	74
s.d.	0.4	0	4		14

Supplementary Table S3

Individual plasma PK parameters of SA10SC_RLX following IV administration at 1 mg/kg to female Göttingen minipig

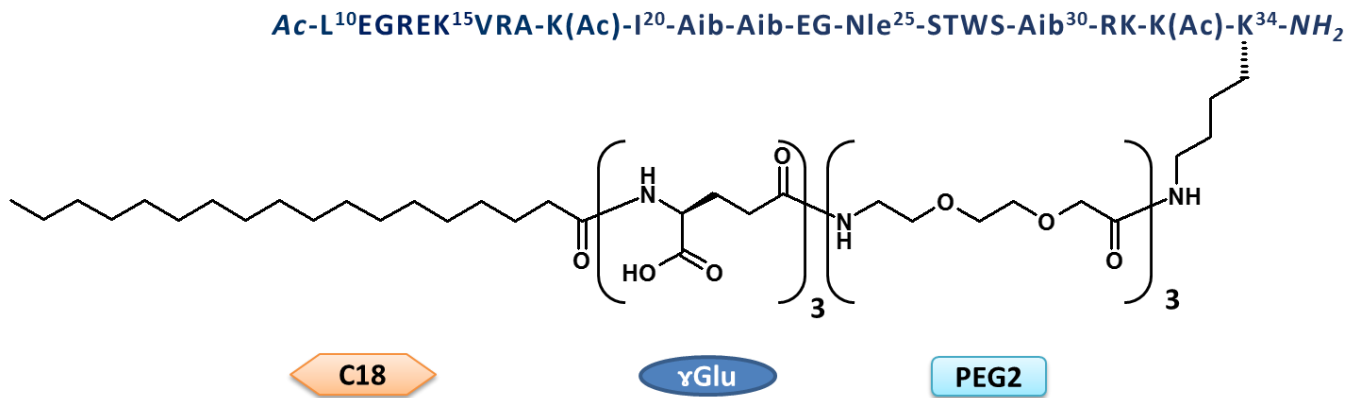
Animal ID.	C₀ (μ M)	AUC (μ M*h)	AUC_{ext} (%)	CL (mL/min/kg)	V_{ss} (L/kg)	t_{1/2} (h)	number of PK points used for t_{1/2} calculation
1	9.36	31.9	0.462	0.130	0.0439	7.06	5
2	8.57	27.1	0.479	0.151	0.0497	6.92	5

Supplementary Table S4

Individual plasma PK parameters of SA10SC_RLX following SC administration at 1 mg/kg to female Göttingen minipig

Animal ID.	C_{max} (μ M)	t_{max} (h)	AUC (μ M*h)	AUC_{ext} (%)	F (%)
3	1.42	6	23.8	3.16	81
4	1.45	4	16.6	1.83	56

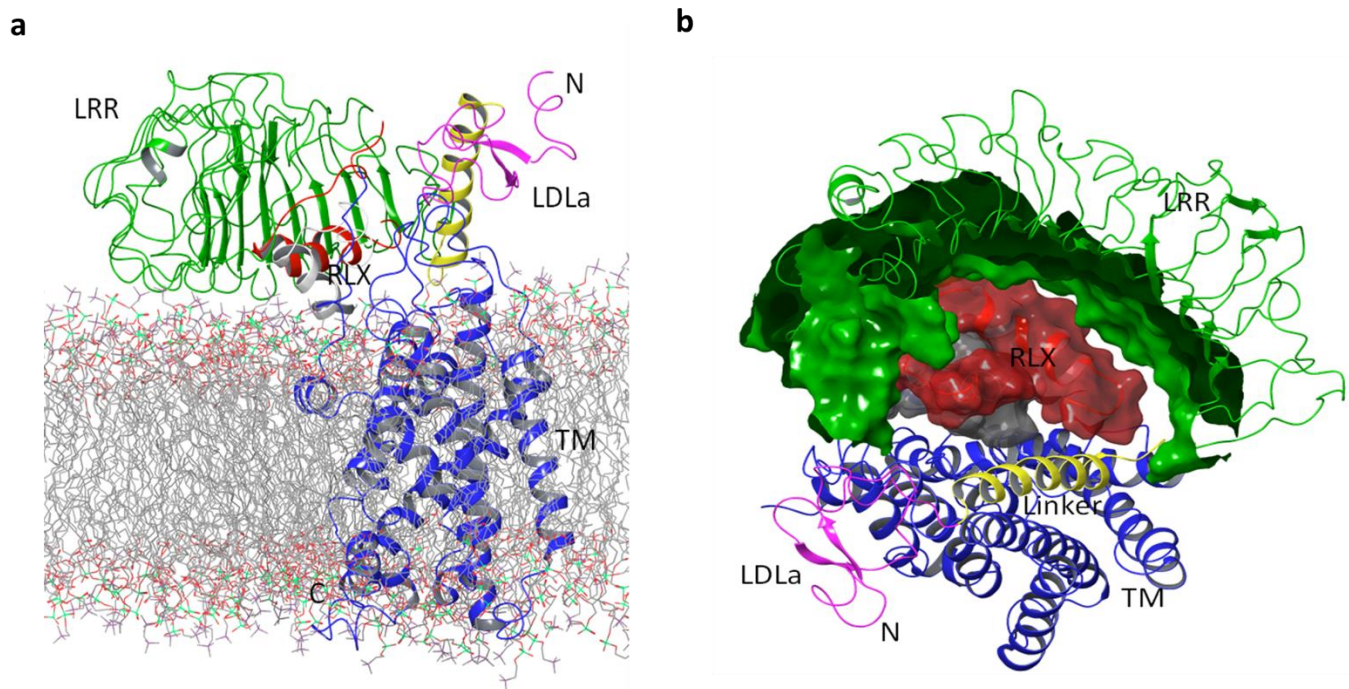
Supplementary Figure S1



Supplementary Figure S1: Structure of SA10SC-RLX.

H2 relaxin B chain numbering has been chosen based on the first description of this series of peptides by Mallart et al. (2021). PEG2 was used as a spacer and γ Glu as linker to the lipid moiety (C18, $CH_3-(CH_2)_{16}-CO-$) used to promote albumin binding and increase peptide half-life.

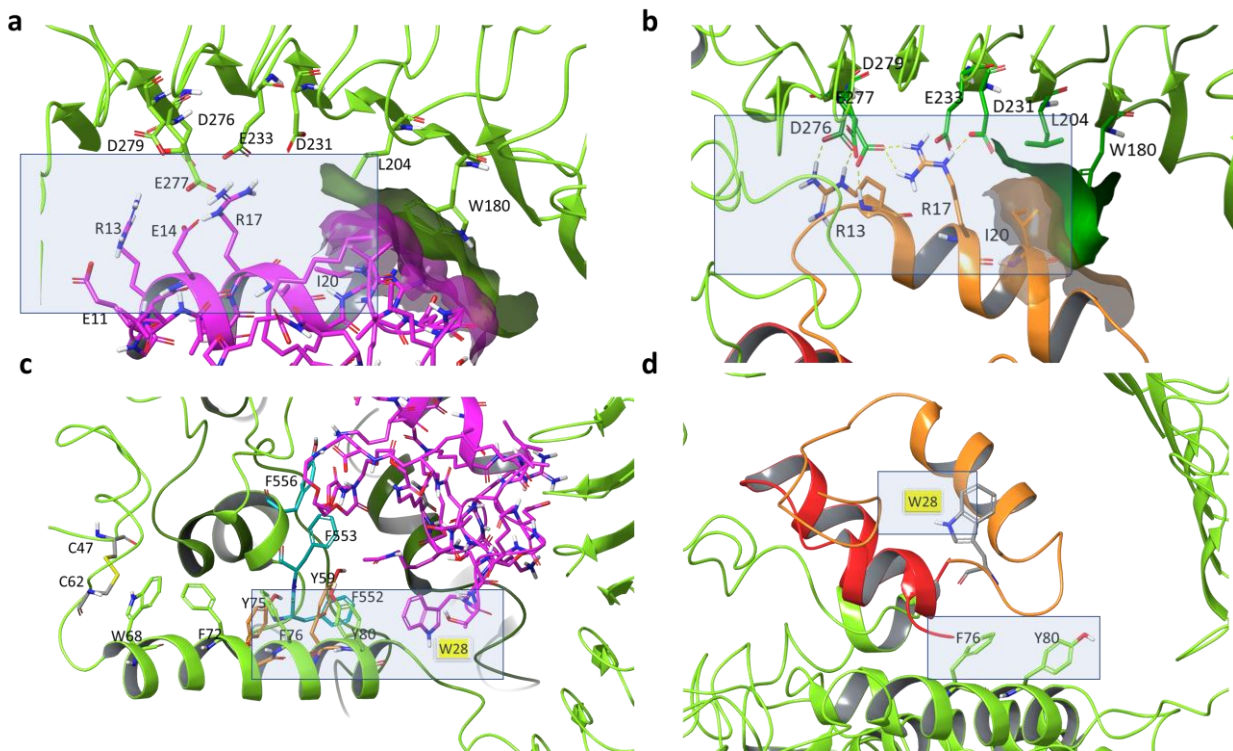
Supplementary Figure S2



Supplementary Figure S2: Model of RXFP1 in complex with relaxin

Each domain is identified by a unique color: relaxin, A chain in grey and B chain in red. For RXFP1, LDLa is in magenta, the linker with LDLa in yellow, LRR in green and Transmembrane (TM) domains in blue. (a) is a side view of the model and (b) shows the contact surface view from extracellular side of relaxin and the LRR are shown, respectively in red and green to visualize the binding cassette.

Supplementary Figure S3



Supplementary Figure S3: Interaction network of SA10SC-RLX or relaxin with RXFP1. RXFP1 is in green, SA10SC-RLX in magenta and Relaxin in Orange (B Chain) and red (A chain). The grey boxes represent the specific domains of interaction

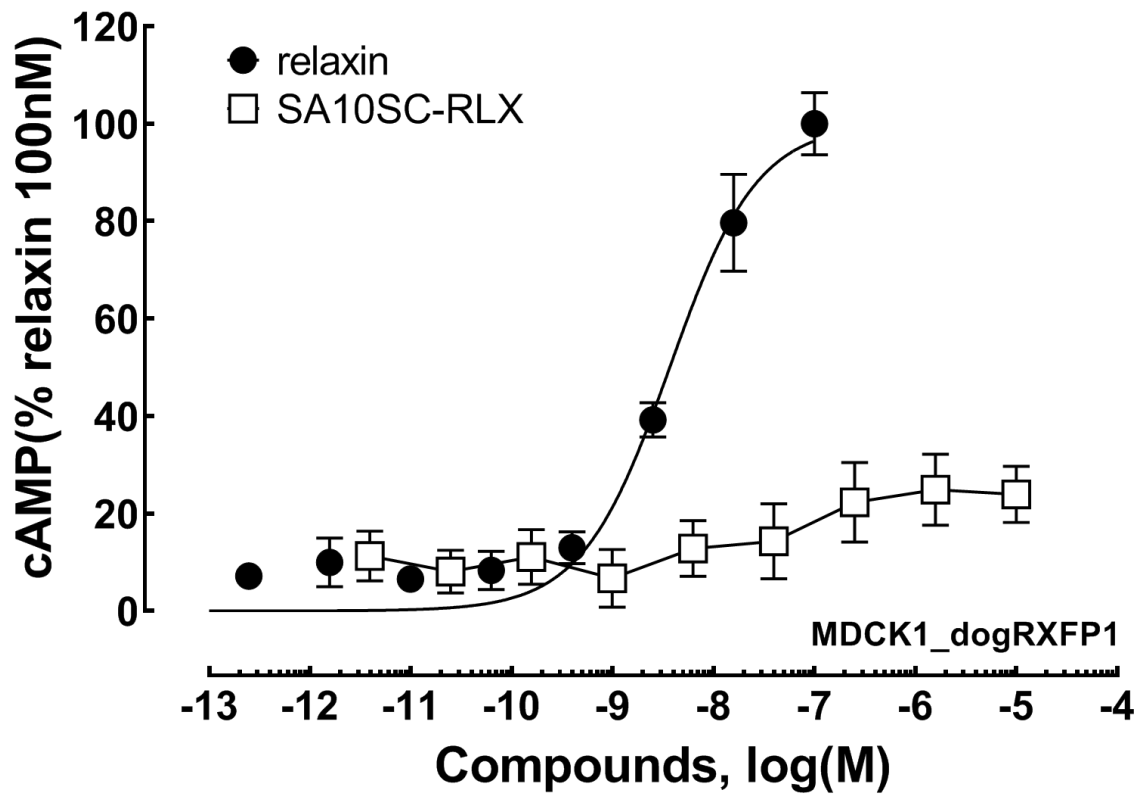
(a and b): Interaction with RXFP1 LRR domain

The three amino acids (R13-R17-I20) of the arginine binding cassette in SA10SC-RLX or relaxin are highlighted in grey boxes. The LLR is in green and SA10SC-RLX in magenta (a) and relaxin B chain in orange in (b). The contact surface between the peptides and LRR (green) is depicted respectively in orange and magenta for relaxin and SA10SC-RLX.

(c and d): Specific Interaction of W28 in SA10SC-RLX with the RXFP1 linker

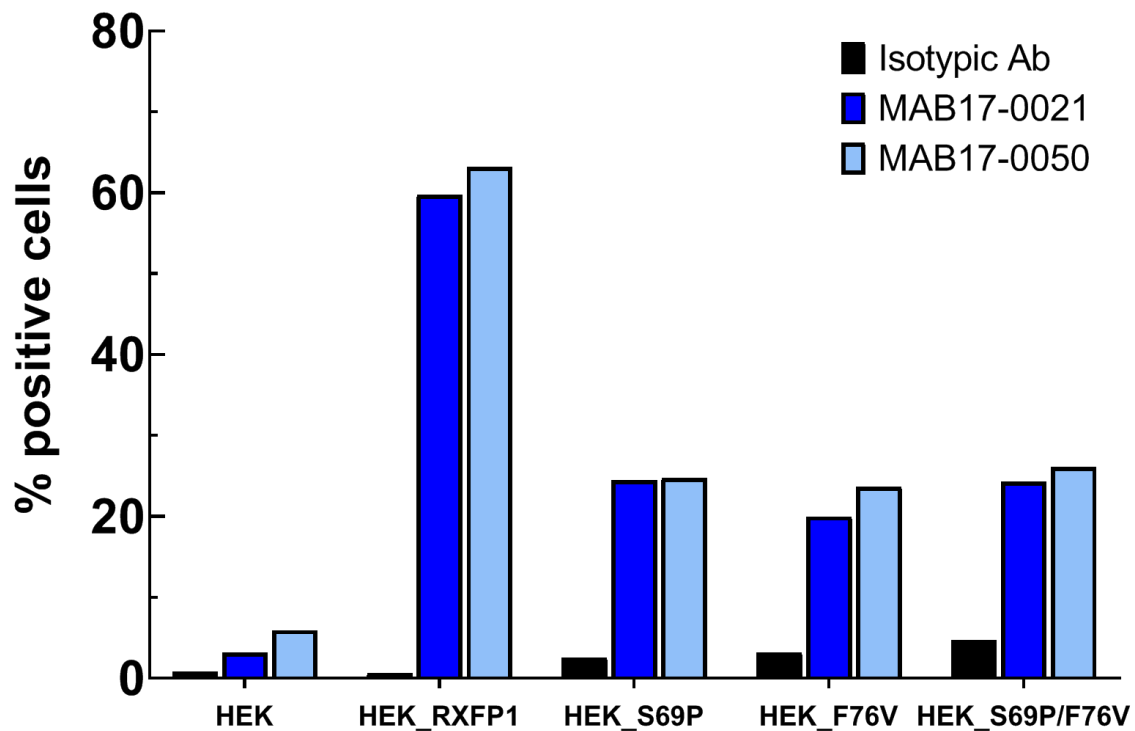
Alignment of aromatic residues of the linker from W68 to Y80 (in green) interacting with W28 (yellow box) of SA10SC-RLX (in magenta). Aromatic residues of the linker (Y59, Y75, Y76) shown in orange are also in contact with aromatic residues (cyan, F552, F553, F558) of the RXFP1 TM domain. (c). In contrast, the relaxin positioning in the RXFP1 modeling shows that W28 in the relaxin B chain is not aligned with the aromatic residues of the RXFP1 linker (d).

Supplementary Figure S4



Supplementary Figure S4: Decreased response of SA10SC-RLX compared to relaxin on cAMP production in dog MDCK.1 cells with stable expression of recombinant dog RXFP1 (sequence id. MW713050) (mean +/- sem n=6 different experiments performed in duplicate).

Supplementary Figure S5



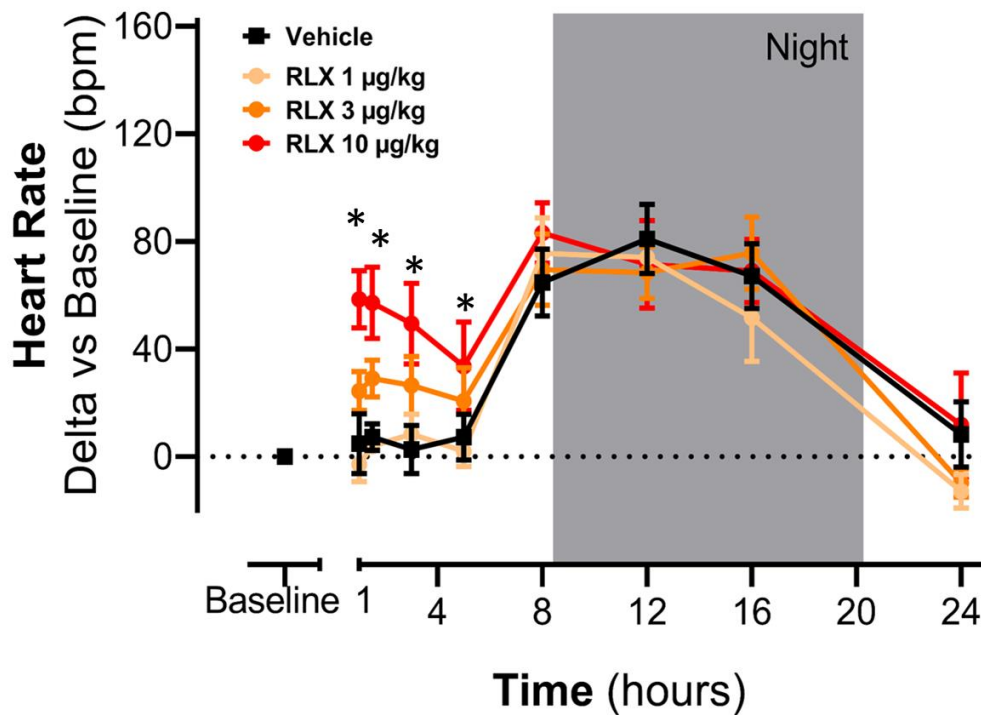
Supplementary Figure S5: Two different monoclonal anti-RXFP1 antibodies were obtained internally after immunization of rabbits with HEK_RXFP1 cells. The antibodies were utilized in FACS to detect RXFP1 and mutated RXFP1 at the surface of stable cell lines (HEK_RXFP1 / HEK_S69P /HEK_F76V or HEK_S69P/F76V). An isotypic antibody and wild type HEK cells (HEK) were used as negative controls.

Supplementary Table S5

Detection of RXFP1 and mutated RXFP1 at the surface of stable cell lines (HEK_RXFP1 / HEK_S69P /HEK_F76V or HEK_S69P/F76V) by FACS (Mean Fluorescence intensity for 3000 events/group). Same conditions as in Supplementary Figure S6.

	Ctrl Isotypic MAB + Goat anti-human-PE	MAB17-0021 + Goat anti-human-PE	MAB-0050 + Goat anti-human-PE
	Mean Fluorescence Intensity	Mean Fluorescence Intensity	Mean Fluorescence Intensity
HEK	18.1	20.9	24.0
HEK_hRXFP1 wt	14.9	225.2	276.1
HEK_hRXFP1 mut-S69P	22.6	48.6	57.3
HEK_hRXFP1 mut-F76V	20.9	43.0	51.4
HEK_HRXFP1 mut-S69P- F76V	23.8	49.1	55.5

Supplementary Figure S6



Supplementary Figure S6: Effects of a single iv administration of relaxin at 3 doses (1, 3 and 10 µg/kg) on HR in conscious healthy telemetered SD rats. Results are expressed as mean ± sem of changes from baseline for each animal (n=5/group). A significant difference (* P<0.05) between relaxin and vehicle was observed for the early time points (1-4h) at the doses of 3 and 10 µg/kg of relaxin.