

Supplementary information

Development of a selective agonist for relaxin family peptide receptor 3

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Table S1. Molecular masses of the mature R3/I5 mutants measured by electrospray mass spectrometry.

Peptide	Measured mass	Theoretical mass
R3/I5	6169.0	6168.1
[+G(B23-24)]R3/I5	6227.0	6225.1
[ΔB23]R3/I5	6113.0	6111.0
[ΔB23-24]R3/I5	6056.0	6054.0
[ΔB23-25]R3/I5	5968.0	5966.9
[G(B23)A]R3/I5	6183.0	6182.1
[G(B23)S]R3/I5	6200.0	6198.1
[G(B24)A]R3/I5	6183.0	6182.1
[G(B24)S]R3/I5	6200.0	6198.1
[G(B24)V]R3/I5	6211.0	6210.2
[G(B24)T]R3/I5	6213.0	6212.1
[G(B24)E]R3/I5	6242.0	6240.2
[G(B24)S,S(B25)A]R3/I5	6181.0	6180.2
[G(B24)S,S(B25)G]R3/I5	6169.0	6167.1
[G(B24)S,S(B25)T]R3/I5	6211.0	6211.2

	1	5	10	15	20	25
Mammal	<i>Homo sapiens</i>	RAAPYGVRLCGREF	RAVI	FTCGGSRW		
	<i>Pan troglodytes</i>	RAAPYGVRLCGREF	RAVI	FTCGGSRW		
	<i>Pan paniscus</i>	RAAPYGVRLCGREF	RAVI	FTCGGSRW		
	<i>Gorilla gorilla</i>	RAAPYGVRLCGREF	RAVI	FTCGGSRW		
	<i>Macaca mulatta</i>	RAAPYGVRLCGREF	RAVI	FTCGGSRW		
	<i>Macaca fascicularis</i>	RAAPYGVRLCGREF	RAVI	FTCGGSRW		
	<i>Cebus capucinus imitator</i>	RATSYGVKLCGREF	RAVI	FTCGGSRW		
	<i>Microcebus murinus</i>	RAAPYGVKLCGREF	RAVI	FTCGGSRW		
	<i>Cercocebus atys</i>	RAAPYGVKLCGREF	RAVI	FTCGGSRW		
	<i>Mandrillus leucophaeus</i>	RAAPYGVKLCGREF	RAVI	FTCGGSRW		
	<i>Propithecus coquereli</i>	RAAPYGVKLCGREF	RAVI	FTCGGSRW		
	<i>Aotus nancymae</i>	RAAPYGVKLCGREF	RAVI	FTCGGSRW		
	<i>Colobus angolensis palliatus</i>	RAAPYGVKLCGREF	RAVI	FTCGGSRW		
	<i>Ceratotherium simum simum</i>	GAAPYGVKLCGREF	RAVI	FTCGGSRW		
	<i>Rhinopithecus roxellana</i>	RAAPYGVKLCGREF	RAVI	FTCGGSRW		
	<i>Tarsius syrichta</i>	RVAPYGVKLCGREF	RAVI	FTCGGSRW		
	<i>Chlorocebus sabaeus</i>	RAAPYGVKLCGREF	RAVI	FTCGGSRW		
	<i>Saimiri boliviensis boliviensis</i>	RAAPYGVKLCGREF	RAVI	FTCGGSRW		
	<i>Papio anubis</i>	RAAPYGVKLCGREF	RAVI	FTCGGSRW		
	<i>Otolemur garnettii</i>	RAAPYGVKLCGREF	RAVI	FTCGGSRW		
	<i>Callithrix jacchus</i>	RAAPYGVKLCGREF	RAVI	FTCGGSRW		
	<i>Mus musculus</i>	RPAPYGVKLCGREF	RAVI	FTCGGSRW		
	<i>Rattus norvegicus</i>	RPAPYGVKLCGREF	RAVI	FTCGGSRW		
	<i>Peromyscus maniculatus bairdii</i>	RPAPYGVKLCGREF	RAVI	FTCGGSRW		
	<i>Nannospalax galili</i>	RPAGSYGVKLCGREF	RAVI	FTCGGSRW		
	<i>Dipodomys ordii</i>	RAAPYGVKLCGREF	RAVI	FTCGGSRW		
	<i>Oryzomys variegatus</i>	RAAPYGVKLCGREF	RAVI	FTCGGSRW		
	<i>Marmota marmota marmota</i>	RAAPYGVKLCGREF	RAVI	FTCGGSRW		
	<i>Oryctolagus cuniculus</i>	RTAPYAVKLCGREF	RAVI	FTCGGSRW		
	<i>Camelus ferus</i>	RTAPYRVKLCGREF	RAVI	FTCGGSRW		
	<i>Camelus bactrianus</i>	RTAPYRVKLCGREF	RAVI	FTCGGSRW		
	<i>Camelus dromedarius</i>	RTAPYRVKLCGREF	RAVI	FTCGGSRW		
	<i>Sus scrofa</i>	RASPYGVKLCGREF	RAVI	FTCGGSRW		
	<i>Erinaceus europaeus</i>	RGAPYGVKLCGREF	RAVI	FTCGGSRW		
	<i>Equus asinus</i>	RAAPYGVKLCGREF	RAVI	FTCGGSRW		
	<i>Equus caballus</i>	RAAPYGVKLCGREF	RAVI	FTCGGSRW		
	<i>Equus przewalskii</i>	RAAPYGVKLCGREF	RAVI	FTCGGSRW		
	<i>Bos mutus</i>	RATAYGVKLCGREF	RAVI	FTCGGSRW		
	<i>Bos taurus</i>	RATAYGVKLCGREF	RAVI	FTCGGSRW		
	<i>Bison bison bison</i>	RATAYGVKLCGREF	RAVI	FTCGGSRW		
	<i>Bubalus bubalis</i>	RATPYGVKLCGREF	RAVI	FTCGGSRW		
	<i>Ovis aries</i>	RATPYGVKLCGREF	RAVI	FTCGGSRW		
	<i>Pantholops hodgsonii</i>	QATPYGVKLCGREF	RAVI	FTCGGSRW		
	<i>Capra hircus</i>	QATPYGVKLCGREF	RAVI	FTCGGSRW		
	<i>Loxodonta africana</i>	RAAPYGVKLCGREF	RAVI	FTCGGSRW		
	<i>Latimeria chalumnae</i>	RNPTYGVKLCGREF	RAVI	FTCGGSRW		
	<i>Dasyatis novemcinctus</i>	RAGPYGVKLCGREF	RAVI	FTCGGSRW		
	<i>Pteropus alecto</i>	RAAPYGVKLCGREF	RAVI	FTCGGSRW		
	<i>Myotis brandtii</i>	RGAPYGVKLCGREF	RAVI	FTCGGSRW		
	<i>Myotis davidii</i>	RGAPYGVKLCGREF	RAVI	FTCGGSRW		
	<i>Fukomys damarensis</i>	RATAYGVKLCGREF	RAVI	FTCGGSRW		
	<i>Tupaia chinensis</i>	RPAPYGVKLCGREF	RAVI	FTCGGSRW		
	<i>Crictetus griseus</i>	RPAPYGVKLCGREF	RAVI	FTCGGSRW		
	<i>Felis catus</i>	RASPYGVKLCGREF	RAVI	FTCGGSRW		
	<i>Panthera tigris altaica</i>	RASPYGVKLCGREF	RAVI	FTCGGSRW		
	<i>Manis javanica</i>	RAAPYGVKLCGREF	RAVI	FTCGGSRW		
	<i>Microtus ochrogaster</i>	RPAPYGVKLCGREF	RAVI	FTCGGSRW		
	<i>Mesocricetus auratus</i>	RPAPYGVKLCGREF	RAVI	FTCGGSRW		
	<i>Mustela putorius furo</i>	RASPYGVKLCGREF	RAVI	FTCGGSRW		
	<i>Echinops telfairi</i>	RAAPYGVKLCGREF	RAVI	FTCGGSRW		
	<i>Heterocephalus glaber</i>	RAAPYGVKLCGREF	RAVI	FTCGGSRW		
	<i>Condylura cristata</i>	RATPYGVKLCGREF	RAVI	FTCGGSRW		
	<i>Jaculus jaculus</i>	QARFAYGVKLCGREF	RAVI	FTCGGSRW		
	<i>Acinonyx jubatus</i>	RASPYGVKLCGREF	RAVI	FTCGGSRW		
	<i>Lipotes vexillifer</i>	QAAPYGVKLCGREF	RAVI	FTCGGSRW		
	<i>Ictidomys tridecemlineatus</i>	RAAPYGVKLCGREF	RAVI	FTCGGSRW		
	<i>Orycteropus afer afer</i>	RAAPYGVKLCGREF	RAVI	FTCGGSRW		
	<i>Balaenoptera acutorostrata scammoni</i>	QAAPYRVKLCGREF	RAVI	FTCGGSRW		
	<i>Elephantulus edwardii</i>	RAAPYGVKLCGREF	RAVI	FTCGGSRW		
	<i>Chrysochloris asiatica</i>	RAAPYGVKLCGREF	RAVI	FTCGGSRW		
	<i>Chinchilla lanigera</i>	RAAPYGVKLCGREF	RAVI	FTCGGSRW		
	<i>Leptonyx chotes weddellii</i>	WASPYGVKLCGREF	RAVI	FTCGGSRW		
	<i>Eptesicus fuscus</i>	RGAPYGVKLCGREF	RAVI	FTCGGSRW		
	<i>Vicugna pacos</i>	RTAPYGVKLCGREF	RAVI	FTCGGSRW		
	<i>Ochotona degus</i>	RAAPYGVKLCGREF	RAVI	FTCGGSRW		
	<i>Ochotona princeps</i>	RAAPYGVKLCGREF	RAVI	FTCGGSRW		
	<i>Sorex araneus</i>	RAAPVRLKLCGREF	RAVI	FTCGGSRW		
	<i>Odobenus rosmarus divergens</i>	RASPYGVKLCGREF	RAVI	FTCGGSRW		
	<i>Ursus maritimus</i>	RASPFYGVKLCGREF	RAVI	FTCGGSRW		
	<i>Cavia porcellus</i>	RAAPYGVKLCGREF	RAVI	FTCGGSRW		
	<i>Trichechus manatus latirostris</i>	QAAPYGVKLCGREF	RAVI	FTCGGSRW		
	<i>Ailuropoda melanoleuca</i>	RASPFYGVKLCGREF	RAVI	FTCGGSRW		
	<i>Monodelphis domestica</i>	RTSPYAVKLCGREF	RAVI	FTCGGSRW		
	<i>Sarcophilus harrisii</i>	RTSPYAVKLCGREF	RAVI	FTCGGSRW		
	<i>Ornithorhynchus anatinus</i>	RSPSYGMKLCGREF	RAVI	FTCGGSRW		
Bird	<i>Gallus gallus</i>	DGDGYGVKLCGREF	RAVI	FTCGGSRW		
	<i>Colinus striatus</i>	RAEGNPVKLCGRDFVRAVFT	CGGSRW			
	<i>Cariacus cristata</i>	KGEGNTVKLCGRDFVRAVFT	CGGSRW			
	<i>Merops nubicus</i>	RGDNTVKLCGRDFVRAVFT	CGGSRW			
Reptile	<i>Protobothrops mucrosquamatus</i>	RTPPYGVKLCGREF	RAVI	FTCGGSRW		
	<i>Gekko japonicus</i>	RTPPYGVKLCGREF	RAVI	FTCGGSRW		
	<i>Alligator mississippiensis</i>	RGPPYGVKLCGREF	RAVI	FTCGGSRW		
	<i>Alligator sinensis</i>	RGPPYGVKLCGREF	RAVI	FTCGGSRW		
	<i>Python bivittatus</i>	RTPPYGVKLCGREF	RAVI	FTCGGSRW		
	<i>Chelonia mydas</i>	RNPYYGVKLCGREF	RAVI	FTCGGSRW		
	<i>Pelodiscus sinensis</i>	RNPYYGVKLCGREF	RAVI	FTCGGSRW		
Amphibian	<i>Chrysemys picta</i>	RNPYYGVKLCGREF	RAVI	FTCGGSRW		
	<i>Anolis carolinensis</i>	RTPPYGVKLCGREF	RAVI	FTCGGSRW		
	<i>Xenopus tropicalis</i>	RVPTFGVKLCGREF	RAVI	FTCGGSRW		
	<i>Nanorana parkeri</i>	RVPNIGVKLCGREF	RAVI	FTCGGSRW		
Fish	<i>Danio rerio</i>	AGPSYGVKLCGREF	RAVI	FTCGGSRW		
	<i>Clupea harengus</i>	GNPIYGVKLCGREF	RAVI	FTCGGSRW		
	<i>Astyanax mexicanus</i>	GHPYIGVKLCGREF	RAVI	FTCGGSRW		
	<i>Esox lucius</i>	ANSIYGVKLCGREF	RAVI	FTCGGSRW		
	<i>Xiphophorus maculatus</i>	HSSLYGVKLCGREF	RAVI	FTCGGSRW		
	<i>Takifugu rubripes</i>	YPSFYGVKLCGREF	RAVI	FTCGGSRW		
	<i>Poecilia mexicana</i>	HSSFYGVKLCGREF	RAVI	FTCGGSRW		
	<i>Poecilia reticulata</i>	HSSFYGVKLCGREF	RAVI	FTCGGSRW		
	<i>Fundulus heteroclitus</i>	HSSFYGVKLCGREF	RAVI	FTCGGSRW		
	<i>Notothenia coriiceps</i>	HPSFYGVKLCGREF	RAVI	FTCGGSRW		
	<i>Larimichthys crocea</i>	HPSFYGVKLCGREF	RAVI	FTCGGSRW		
	<i>Stegastes partitus</i>	HPSFYGVKLCGREF	RAVI	FTCGGSRW		
	<i>Poecilia formosa</i>	HSSFYGVKLCGREF	RAVI	FTCGGSRW		
	<i>Haplochromis burtoni</i>	HPSFYGVKLCGREF	RAVI	FTCGGSRW		
	<i>Pundamilia nyererei</i>	HPSFYGVKLCGREF	RAVI	FTCGGSRW		
	<i>Oreochromis niloticus</i>	HPSFYGVKLCGREF	RAVI	FTCGGSRW		

Fig. S1. Amino acid sequence alignment of the B-chain of relaxin-3s from different species. The two conserved Cys residues, that form interchain disulfide bonds with A-chain Cys residues, are shown in bold and indicated by asterisks. The B23–B25 region is shown in red.