Coevolution of Positively Selected IZUMO1 and CD9 in Rodents: Evidence of Interaction Between Gamete Fusion Proteins? Alberto Vicens and Eduardo R. S. Roldan

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Supplemental Data

Files in this data supplement:

Supplemental Figure S1. Amino acid alignment of IZUMO1. Positively selected sites with a Bayesian posterior probability > 0.95 (*) are shown. *Rattus norvegicus* was used as outgroup lineage.

Supplemental Figure S2. Amino acid alignment of CD9. Positively selected sites with a Bayesian posterior probability > 0.95 (*) are shown. *Rattus norvegicus* was used as outgroup lineage.

Supplemental Figure S3. Phylogenetic reconstructions of *Izumo1* gene using Maximum Likelihood (**A**) and Bayesian Inference (**B**) methods. Node labels indicate *P*-values from bootstrap analyses of 1000 simulations and Bayesian posterior probabilities, respectively.

Supplemental Figure S4. Phylogenetic reconstructions of *Cd9* gene using Maximum Likelihood (**A**) and Bayesian Inference (**B**) methods. Node labels indicate *P*-values from bootstrap analyses of 1000 simulations and Bayesian posterior probabilities, respectively.

Supplemental Figure S5. Analyses of robustness for alternative phylogenetic trees. Statistical results for each alternative topology are shown.

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Mus m. musculus	MGPH FTLLLAAL	ANCLCPGRPCI	KCDQFVTDAL	K T F <mark>EN</mark> T ÝLN DHL	PHDIHKN	VMRMVNHEVS	SFGVVTSA	DSYLGAVDEN	TLEOATWS FI	KDLKRÍTD
Mus m. castaneus	MGPH FTLLLAAI	ANCLCPGRPC	KC DQ FV TDAL	K T F EN T Y LN DH L	PHDIHKN	VMRMVNHEVS	SFGVVTSA	DSYLGAVDEN	TLEOATWS FI	KDLKRITD
Mus m. domesticus	MGPHFTLLLAAL	ANCLCPGRPCI	KCDOFVTDAL	K T F EN T Y LN DH L	PHDIHKN	VMRMVNHEVS	SFGVVTSA	DSYLGAVDEN	TLEOATWS FI	KDLKRITD
Mus spretus	MGPH FTLLLAAL	ANCLCPGRPCI	KCDOFVTDAL	KTFENTYLNNHL	PHDIHKN	VMRMVNLEVS	SFGVVTSA	DSYLGAVDEN	TLEOASWS FL	KDLKRITD
Mus macedonicus	MGPH FTLLLAAL	ANCLCPGRPCI	KCDOFVTDAL	KTFENTYLNDHL	PHDIHKN	VMRMVNHEVI	SFGVVTSA	DSYLGAVDEN	TLEOAAWS FI	KDLKRITD
Mus spicileaus	MGPH FTLLLAAL	ANCLCPGRPCI	KCDOFVTDAL	KTFENTYLNDHL	PHDIHKN	VMRMVNHEVI	SFGVVTSA	DSYLGAVDEN	TLEOAAWS FI	KDLKRITD
Mus pahari	MGPHFTLLLAAT	ANCLCPGRSCI	KCDPFV TAAL	KTLEDN YLPNRV	PON THEN	VMRMVNYETN	S FGEV T PD	NSFIGAVDED	TLEOAAWS FI	KDLKRTTD
Mus caroli	MGPH FTLLLAAL	ANYLCPGRPC	KCDOLVTAEL	KTLENTYLR DHL	PSEPSDALKN	VMRMVNYEVN	SFGEVNSA	DSYLGAVDEN	TLEOAVWS FI	KDLKRITD
Mus minutoides	MGPHFTLLLAAT	ANCICPGRPCI	CDPFVTAAL	KTLENTYLONRL	PSSVHKN	VMKMVNYEVN	SFGEVTSP	IDTS LGAVDED	TLEOAAWS FI	KDLKRLTD
Rattus norvegicus	MGLHFTLLLAAL	ANCLCPARLCI	ICDPFVV AAT	KTLEON YLPTHL	APEHHED	VMKRV EOEVE	NFADLPLN	NTELGVVDED	TLEOASWS FI	KDLKRITD
rtattae nerregieue	110	180	180	140	150 +	160	170	180	190	200
Muc m. mucculuc	SDIKCET	TWMEDUOKDER		TIC DNK CCUMSO	TWCTKCEK	OTUTOPKST		IDSEDIVIDCI	IS WUD ASKCT	TOVE EVEN
Mus m. musculus	S DEKGEEFEKEE									
Mus m. castaneus	S DEKGEEFEKEE								LS WHRASNGL	
Mus m. domesticus	S DEKGEEFEKEE									
Mus spretus	S DEKGEEFEKEE								LS WHH ASKGI	T DISFIRV
Mus macedonicus	SDEKGELFEKE						CGERHIEVI		LS WHHASKGI	T DISFIKV
Mus spicilegus	SULKGELFIKEL	LWMLRHQKDIF		ECPNK CGVMSQ	THEWCHACEA		CGERHIEVI		LS WHH ASKGI	TDISFIRV
Mus panari	SDVKGELFIKEL	LWMIRRQKDIF	ATLAAHFUKE	FC PNK CGV MSQ	PLIWCFKCEK		CGERHIEVI	IRLEDMV LDCL	LSWHRASKGI	TDISFIRV
Mus caroli	SULKGELFIKEL	LWMLQHQKDII		ECPNKCGVMSQ	TELWCHKCEK	QLHLCRKS LL			LS WHHASKGI	TDISFIRV
Mus minutoides	SDVKGELFIKEL	LWMIRRQKDIF	TLAEHFRKE	FCPNKCGVMSQ	PLIWCRQCEK	QUILCRKSL	CGERHIEVI	IRLEDMV LDCL	LSWHRASKGI	TDISFIRV
Rattus norvegicus	SDVKGELFVKEL	FWMLRLQKDLF	AT LVARFQKE	VIC PNQ CGIMSQ	TLIWCNKCEK	QMHFCRKSML	CGERQIEVI	IKTEDWA TDCŐ	LSWHHASEGI	TDISFIRV
	210	220	230	240 *	250	260	270	280	290	300
Mus m. musculus	210 WENSSETLIAKC	220 KEPYLTKSMVG	230 PEDAGNYR CVI	240 *	250 RYDVTVLPPK	260 HSEENQPPNI	270 TQEEHET	280 PVHVTPQTPPG	290 QEPESELY	300
Mus m. musculus Mus m. castaneus	210 WENSSETLIAKG	220 KEPYLTKSMVG KEPYLTKSMVG	230 PEDAGN YR CVI PEDAGN YR CVI	240 * LDT INOGHATVI LDT IN EGHATVI	RYDVTVEPPK RYDVTVEPPK	260 HSEENQPPNI HSEENQPPNI	270 I TQEEHE T I TQEEHET	280 P VHV TPQ TPPG P VHV TPQ TPPG	290 QEPESELY QEPESELY	300
Mus m. musculus Mus m. castaneus Mus m. domesticus	210 WENSSETLIAKO WENSSETLIAKO WENSSETLIAKO	220 KEPYLTKSMVG KEPYLTKSMVG KEPYLTKSMVG	230 PEDAGNYRCV PEDAGNYRCV PEDAGNYRCV	240 * DTINGHATVI DTINEGHATVI DTINGHATVI	SO RY DV TV LPP K RY DV TV LPP K RY DV TV LPP K	260 HSEENQPPNI HSEENQPPNI HSEENQPPNI	270 TQEEHET TQEEHET TQEEHET	280 P VHV TPQ TPPG PVHV TPQ TPPG PVHV TPQ TPPG	290 QEPESELY QEPESELY QEPESELY	300
Mus m. musculus Mus m. castaneus Mus m. domesticus Mus spretus	210 WENSSETLIAKO WENSSETLIAKO WENSSETLIAKO WENSSETLIAKO	220 KEPYUTKSMVG KEPYUTKSMVG KEPYUTKSMVG	230 PEDAGN YR CV PEDAGN YR CV PEDAGN YR CV PEDAGN YR CV	240 DT INQGHATVI DT IN EGHATVI DT INQGHATVI DT INQGHATVI	SO RYDVTVLPPK RYDVTVLPPK RYDVTVLPPK RYDVTVLPPK	260 HSEENQPPNI HSEENQPPNI HSEENQPPNI HSEENQPPNI	270 I TQEEHET I TQEEHET I TQEEHET I TQEEHET	280 P VHV TPO TPPG P VHV TPO TPPG P VHV TPO TPPG P VHV TPO TPPG	290 QEPESELY QEPESELY QEPESELY QEPESELY	300
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Supplemental Figure S1. Amino acid alignment of IZUMO1. Positively selected sites with a Bayesian posterior probability > 0.95 (*) are shown. *Rattus norvegicus* was used as outgroup lineage.

	1	10	20	30	40	50	60	70	80	90	100	11	0
Mus m. musculus	MPVKGGSKC	IKYLLFGFN	FIFWIAGIAV	LAIGLWLRFD	SOTKSIFFO	NNHSSFYTGN	YILIGAGAL	MMLVGFLG	CGAVQESQC	MIGLFFGF	LLVIFAIEIA	AAA <mark>VWGY</mark> I	HKD
Mus m. castaneus	MP <mark>V</mark> KGGSKC	IKYLLFGFN	FIFWLAGIAV	LAIGLWLRFD	SQTKSIFEQ	NNH SS FY TGV	Y I LI GA GAL	MMLVG F LG	CCGAVQESQC	MIGIFFGF	LLVI FAI EIA	AAA <mark>V</mark> WGY I	HKD
Mus . domesticus	MP <mark>V</mark> KGGSKC	IKYLLFGFN	FIFWLAGIAV	LAIGIWIRFD	SQ TKS IF EQ	NN <mark>H</mark> SSFYTG <mark>V</mark>	YILIGAGAL	MML <mark>V</mark> GFLG	CCGA <mark>V</mark> Q <mark>F</mark> SQC	MIGLFFGF	LLVI FAIEIA	AAA <mark>VWG</mark> YI	THKD
Mus spretus	MPVKGGSKC	IKYLLFGFN	FIFWLAGIAV	LAIGIWIRFD	SQ TKS IF EQ	NNH SS FY TGV	YILIGAGAL	MMLVGF LG	CCGAVQESQC	MIGIFFGF	LLVI FAI EIA	AAA <mark>VWG</mark> YI	THKD
Mus macedonicus	MPVKGGSKC	IKYLLFGFN	FIFWLAGIAV	LAIGLWLRFD	SQTKSIFEQ	NNHSS FY TGV	YILIGAGAL	MMLVGFLG	CCGAVQESQC	MIGLFFGF	LLVIFAI <mark>B</mark> IA	AAA <mark>VWG</mark> YI	THKD
Mus spicilegus	MPVKGGSKC	IKYLLFGFN	FIFWLAGIAV	LAIGLWLRFD	SQTKSIFQ	NNHSSFYTG	YILIGAGAL	MMLVGFLG	CCGAVQESQC	MIGLEEGE	LLVIFAIEIA	AAAVWGY 1	HKD
Mus caroli	MPWKGGSKC	LKYLLFGFN	FIFWLAGIAV	LAIGLWLRFD	SQTKSIFQ	TNHSSFYTG	Y I LI GAGAL	MMLVGFLG	CGAVQISQU	MIGLEEGE		AAAVWGY 1	HKD
Nus panari	MPWKGGSKC	LKILLFGFN	FIFWLAGIAV		SQIKSIF	TNHSSFITGV	I LIGAGAL	MMLVGF LG		MIGLEEGE		AAAVWGII	HKD
Rettue populations	MPWKGGSKC		F LFW AGIAV				I LIGAGAL	MMLVGF LG		MIGLEEGE			HND
Rattus noi vegicus	MLA VAADVC			LAIGLWLKI D	SY INSTICUT	11000110	I I DI GAGAD					AAAVWGII	INKU
	400	400	4.40	450	4.00	470	4.07	2	100	000	040	000	000
	120	130	140	150	* 160) 170	180) *	190	200	210	220	226
Mus m. musculus	120 EVIKELQEF	130 YKDTYQKLR	140 SKDEPQRET	150 KAIHMALDCC) 170 SDTCPKKQLL	180 I I I I I I I I I I I I I I I I I I I) EAISEVENN	190 I NKF <mark>HIIG</mark> AVO	200 GIGIAVVMI	210 F <mark>GMI</mark> FSMIL	220 CCAIRRSR	226 EMV
Mus m. musculus Mus m. castaneus	120 EMIKELQEF EMIKELQEF	130 YKDTYQKER YKDTYQKER	140 SKDEPORETI SKDEPORETI	150 KAIHMALDCC KAIHMALDCC	160 GIAGPLEOFI GIAGPLEOFI) 170 SDTCPKKQLL SDTCPKKQLL	180 SFQVK PCP SFQVK PCP) EAISEVENN EAISEVENN	190 NKFHIIGAVO NKFHIIGAVO	200 GIGIAVVMI GIGIAVVMI	210 FGMIFSMIL FGMIFSMIL	220 CAIRRSR CCAIRRSR	226 EMV EMV
Mus m. musculus Mus m. castaneus Mus . domesticus	120 IKELQEF IKELQEF IKELQEF	130 YKDTYQKER YKDTYQKER YKDTYQKER	140 SKDE POR ET SKDE POR ET SKDE POR ET	150 KAIHMALDCC KAIHMALDCC KAIHMALDCC	160 GIAGPLEOF GIAGPLEOF GIAGPLEOF) 170 SDTCPKKQLL SDTCPKKQLL SDTCPKKQLL	180 SFQVK PCP SFQVK PCP SFQVK PCP) EAISEVENN EAISEVENN EAISEVENN	190 NKFHIIGAVO NKFHIIGAVO	200 GIGIAVVMI GIGIAVVMI GIGIAVVMI	210 FGMIFSMILO FGMIFSMILO	220 CAIRRSR CAIRRSR CCAIRRSR	226 EMV EMV EMV
Mus m. musculus Mus m. castaneus Mus . domesticus Mus spretus	120 INIKELQEF INIKELQEF	130 YKDTYQKDR YKDTYQKDR YKDTYQKDR YKDTYQKDR	140 SKDPPQRTI SKDPPQRTI SKDPPQRTI SKDPPQRTI	150 KAIHMALDCC KAIHMALDCC KAIHMALDCC KAIHMALDCC	160 GIAGPLFQF GIAGPLFQF GIAGPLFQF GIAGPLFQF) 170 SDTCPKKQLL SDTCPKKQLL SDTCPKKQLL SDTCPKKQLL	180 SFQVK PCP SFQVK PCP SFQVK PCP) FAISEVENI FAISEVENI FAISEVENI FAISEVENI	190 KFHIIGA KFHIIGA KFHIIGA KFHIIGA	200 GIGIAVVMI GIGIAVVMI GIGIAVVMI GIGIAVVMI	210 FGMIFSMILO FGMIFSMILO FGMIFSMILO	220 CAIRRSR CAIRRSR CAIRRSR CAIRRSR	226 EMV EMV EMV
Mus m. musculus Mus m. castaneus Mus . domesticus Mus spretus Mus macedonicus		130 YKDTYQK YKDTYQK YKDTYQK YKDTYQK YKDTYQK YKDTYQK R	140 SKDE POR ETI SKDE POR ETI SKDE POR ETI SKDE POR ETI SKDE POR ETI	150 KAIHMALDCC KAIHMALDCC KAIHMALDCC KAIHMALDCC KAIHMALDCC	160 GLAGPLOCF GLAGPLOCF GLAGPLOCF GLAGPLOCF GLAGPLOCF GLAGPLOCF) 170 SDTCPKKQL SDTCPKKQL SDTCPKKQL SDTCPKKQL SDTCPKKQL	180 SFQVK PCP SFQVK PCP SFQVK PCP SFQVK PCP) AISPYFN AISPYFN AISPYFN AISPYFN AISPYFN	190 KFHIIGA KFHIIGA KFHIIGA KFHIIGA KFHIIGA	200 GIGIAVVMI GIGIAVVMI GIGIAVVMI GIGIAVVMI GIGIAVVMI	210 FGMIFSMIL FGMIFSMIL FGMIFSMIL FGMIFSMIL	220 CAIRRSR CAIRRSR CAIRRSR CAIRRSR CAIRRSR	226 EMV EMV EMV EMV EMV
Mus m. musculus Mus m. castaneus Mus . domesticus Mus spretus Mus macedonicus Mus spicilegus Mus caroli		130 YKDTYQKR YKDTYQKR YKDTYQKR YKDTYQKR YKDTYQKR YKDTYQKR	140 SKDE POR ETI SKDE POR ETI SKDE POR ETI SKDE POR ETI SKDE POR ETI SKDE POR ETI	150 KAIHMAIDCC KAIHMAIDCC KAIHMAIDCC KAIHMAIDCC KAIHMAIDCC KAIHMAIDCC	TAGPLOF GTAGPLOF GTAGPLOF GTAGPLOF GTAGPLOF GTAGPLOF) 170 SDTCPKKQ SDTCPKKQ SDTCPKKQ SDTCPKKQ SDTCPKKQ SDTCPKKQ SDTCPKKQ	180 SFQ K PCP SFQ K PCP SFQ K PCP SFQ K PCP SFQ K PCP SFQ K PCP) AIS M FNI AIS M FNI AIS M FNI AIS M FNI AIN M FNI AIS M FNI	190 NKFHIIGA NKFHIIGA NKFHIIGA NKFHIIGA NKFHIIGA NKFHIIGA		210 FGMIFSMIC FGMIFSMIC FGMIFSMIC FGMIFSMIC FGMIFSMIC	220 CAIRRSR CAIRRSR CAIRRSR CAIRRSR CAIRRSR CAIRRSR	226 EMV EMV EMV EMV EMV
Mus m. musculus Mus m. castaneus Mus . domesticus Mus spretus Mus macedonicus Mus spicilegus Mus caroli Mus caroli		130 YKDTYQK YKDTYQK YKDTYQK YKDTYQK YKDTYQK YKDTYQK YKDTYQK YKDTYQK	140 SKDPPQRETI SKDPPQRETI SKDPPQRETI SKDPPQRETI SKDPPQRETI SKDPPQRETI SKDPPQRETI	150 KAIHMAIDCC KAIHMAIDCC KAIHMAIDCC KAIHMAIDCC KAIHMAIDCC KAIHMAIDCC KAIHMAIDCC	H GIAGPLOF GIAGPLOF GIAGPLOF GIAGPLOF GIAGPLOF GIAGPLOF GIAGPLOF		18 SFQ K PC P SFQ K PC P	AIS VEN AIS VEN AIS VEN AIS VEN AIS VEN AIS VEN AIS VEN AIS VEN	190 KEHIIGANO KEHIIGANO KEHIIGANO KEHIIGANO KEHIIGANO KEHIIGANO	200 SI GIAVMI SI GIAVMI SI GIAVMI SI GIAVMI SI GIAVMI SI GIAVMI SI GIAVMI	210 FGMIFSMIC FGMIFSMIC FGMIFSMIC FGMIFSMIC FGMIFSMIC FGMIFSMIC	220 CALRRSR CALRRSR CALRRSR CALRRSR CALRRSR CALRRSR	
Mus m. musculus Mus m. castaneus Mus . domesticus Mus spretus Mus macedonicus Mus spicilegus Mus caroli Mus pahari Mus minutoides		130 YKDTYQKD YKDTYQKD YKDTYQKD YKDTYQKD YKDTYQKD YKDTYQKD YKDTYQKD YKDTYQKD	140 SKDEPQRETI SKDEPQRETI SKDEPQRETI SKDEPQRETI SKDEPQRETI SKDEPQRETI SKDEPQRETI	150 KAIHMAIDCC KAIHMAIDCC KAIHMAIDCC KAIHMAIDCC KAIHMAIDCC KAIHMAIDCC KAIHMAIDCC KAIHMAINCC RAIHMAINCC	Image: State of the state o		SFQ K PCP SFQ K PCP	AIS V FN AIS V FN	190 KEHIIGANO KEHIIGANO KEHIIGANO KEHIIGANO KEHIIGANO KEHIIGANO SKEHIIGANO	200 GI GI A V MI SI GI A V MI	210 FGMIFSMIC FGMIFSMIC FGMIFSMIC FGMIFSMIC FGMIFSMIC FGMIFSMIC	220 CAIRRSR CAIRRSR CAIRRSR CAIRRSR CAIRRSR CAIRRSR CAIRRSR	

Supplemental Figure S2. Amino acid alignment of CD9. Positively selected sites with a Bayesian posterior probability > 0.95 (*) are shown. *Rattus norvegicus* was used as outgroup lineage.



Supplemental Figure S3. Phylogenetic reconstructions of *Izumo1* gene using Maximum Likelihood (**A**) and Bayesian Inference (**B**) methods. Node labels indicate *P*-values from bootstrap analyses of 1000 simulations and Bayesian posterior probabilities, respectively.



Supplemental Figure S4. Phylogenetic reconstructions of *Cd9* gene using Maximum Likelihood (**A**) and Bayesian Inference (**B**) methods. Node labels indicate *P*-values from bootstrap analyses of 1000 simulations and Bayesian posterior probabilities, respectively.



Supplemental Figure S5. Analyses of robustness for alternative phylogenetic trees. Statistical results for each alternative topology are shown.