Supplementary Information

Reduced Activity of SRY and its Target Enhancer *Sox9*-TESCO in a Mouse Species with X*Y Sex Reversal

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	-40	-30	-20	-10	1	10	20	30	40	50	60	70	80	90	100
Consensus	aagagaa	caagttttgg	gactggtgac	ааттбтстас	GAGAGC <u>ÅTG</u> GA	GGGCCATGTC	AAGCGCCCT	ATGAATGCATT	TATGGTGTG	атсстатаат	GAGAGGCAAAA	AGTTGGCCCA	GCAGAATCCCA	CCATGCAAA	ACACAGAGATCAGCA
Clone#1 Clone#2 Clone#3 Clone#4 Clone#5 Clone#6 Clone#7										C					
Clone#8							• • • • • • • • • • •						•••••		
	110	120	130	140	150	160	170	180	190	200	210	220	230	240	250
Consensus	AACTGC	TGGGATGCAG	GTGGAAAAGC	CTTACAGAAG	ACGAAAAAA	GCCCTTTTTC	CAGGAGGCA	CAGAGACTGAA	GACCCTACA	CAGAGAGAAA	ГАСССАААТТИ	ΑΤΑΑΑΤΑΤCA	бсстсатсее	GGGCTAAA	GTGCCACAGAGGAGTG
Clone#1 Clone#2 Clone#3 Clone#4 Clone#5 Clone#6 Clone#7 Clone#8															
	260	270	280	290	300	310	320	330	340	350	360	370	380	390	400
Consensus	ĠĠĠĊŢŢŢ	TACAGCCTGC	AGTTACCTCA	ACAAAACTGT	ACAACCTGCT	GCAGTGGGAC	AGGAÁCCCA	CACACCATCAC	ATACAGACA	AGACTGGACT	AGAGCTGCAC	асствтсстс	CAAAAACCAGC	AAAGĊTTTT	ATTGTCAGCCTGTGG
Clone#1 Clone#2 Clone#3 Clone#4 Clone#5 Clone#6 Clone#7 Clone#8			· · · · · · · · · · · · · · · · · · ·							G					
	410	420	430	440	450	460	470	480	490	500	510	520	530	540	550
Consensus	ATATCO	CCACTGGGAA	CCCACTACAG	CAGAGGCTGC	TCCAAGACCA	GCGCCAACTG	AAGCAGCAA	CTTCAGCACGA	GTTCCAACA	CCAACGCCAG	CAAGAGCTGG	TTTCCCCGCA	GCAAGAGCTGT	TTCCCCACA	AGCAGCTCTCAGACAA
Clone#1 Clone#2 Clone#3 Clone#4 Clone#5 Clone#6 Clone#7 Clone#8	G														
	560	570	580	590	600	610	620								
Consensus	GAGGTT	GGTGGGTTTT	CTCCTGAGGA	CATCTGGCTC	Ctctcctaco	tatgccaaca	ctcccct								
Clone#1 Clone#2 Clone#3 Clone#4 Clone#5 Clone#6 Clone#7 Clone#8		C.													

Supplementary Figure S1. Nucleotide sequence alignment of eight independent *M. minutoides Sry* **clones.** Five haplotypes were identified (see also Table 1). The consensus sequence is displayed above the alignment. Sequences identical to the consensus are indicated by dots, while missing nucleotides are depicted by hyphens. Binding sites of PCR primers are in lower case. Start and stop codons are double-underlined. Sequences encoding the HMG, Bridge, and polyQ domains are boxed, underlined or in grey, respectively.

	-40	-30	-20	-10	1	10	20	30	40	50	60	70	80	90	100
Consensus	aagagaa	caagttttgg	gactggtga	caATTGTCTA	GAGAGC <u>ATG</u> O	GAGGGCCATGT	CAAGCGGCCT	ATGAATGCAT	TTATGGTGTG	бтсстотобо	GAGAGGCAGAA	GTTGGCCCA	GCAGAATCCC	AGCATGCAA	AACACAGAGATCAGCA
Clone#1				•••••									••••		
Clone#2 Clone#3					· · · · · · · · · · · · ·				 						
Clone#4	•••••		• • • • • • • • • •	•••••	• • • • • • • • • • •					• • • • • • • • • • • •		• • • • • • • • • •	•••••		
Clone#6														· · · · · · · · · · · ·	
Clone#7				•••••						• • • • • • • • • • •			••••		• • • • • • • • • • • • • • • • • • • •
Clotte#0															
	110	120	130	140	150	160	170	180	190	200	210	220	230	240	250 I
Consensus	AGCTGCI	TGGGATGCAG	GTGGAAAAG	CCTTACAGAAG	GGCGAAAAAA	AGGCCCTTTTT	CCAGGAGGCA	CAGAGACTGA	AGACCCTACA	CAGAGAGAAA	ГАСССАААСТА	ΤΑΑΑΤΑΤCΑ	GCCTCATCGG	AGGGCTAAA	<u>GTGCCACAGAGGAGTG</u>
Clone#1 Clone#2					· · · · · · · · · · · · ·										
Clone#3	•••••		• • • • • • • • • •	••••						• • • • • • • • • • •			•••••		
Clone#5		 			· · · · · · · · · · · · ·						 				
Clone#6 Clone#7			•••••	•••••	• • • • • • • • • • •	• • • • • • • • • • • • •	• • • • • • • • • • •	• • • • • • • • • • •	• • • • • • • • • • • •	••••	• • • • • • • • • • • •	•••••	• • • • • • • • • • • •	• • • • • • • • • • •	• • • • • • • • • • • • • • • • • • • •
Clone#8															
	260	270	280	290	300	310	320	330	340	350	360	370	380	390	400
Consensus	GTGCTT	TACAGACTGC	AGTTACCTC	AACAAAACTG	TACAACCTG	TGCAGTGGGA	CAGGAACCCA		CATACAGACA	AGACTGGACT		CCTGTCCTC			TATTGTCAGCCTGTGG
Clone#1															
Clone#2 Clone#3	•••••		•••••	•••••	• • • • • • • • • • •					• • • • • • • • • • •		• • • • • • • • • •	•••••		
Clone#4															
Clone#5 Clone#6				•••••						•••••			•••••		
Clone#7															
Clone#8	•••••		•••••	•••••	• • • • • • • • • • • •	• • • • • • • • • • • • •				•••••	• • • • • • • • • • • • •	•••••	•••••		
	410	420	430	440 I	450 I	460 I	470 I	480 I	490 I	500 I	510	520 I	530	540 I	550
Consensus	ATATCCO	CCACTGGGAA	CCCACTACA	GCAGCAGCAG	CAGCAGCAGC	CAGCAGCACCA	GTTCCACCAC	CACCAGCAGC	AGCAGCAGCA	CCAGTTCCAC	CAGCAGCAGCA	GCTGAAGCA	TCAGCATCAG	CACCAGTTCT	TACCAGCAGCAGCAGC
Clone#1 Clone#2															
Clone#3	•••••			•••••											
Clone#5		 													• • • • • • • • • • • • • • • • • • • •
Clone#6 Clone#7										G.					
Clone#8					<u>T</u> .										
	560	570	580	590	600	610	620	630	640	650	660	670	680	690	700
Consensus	560 I AGCAGCA	570 I ACCAGTTCCA	580 I ICCAGCAGCA	590 I GCAGCAGCAG	600 I CAGCAGCAGC	610 I CAGCAGCAGCA	620 ACAATTCCAC	630 I CAGCAGCAGC	640 I AGCAGCAACA	650 I ATTCCACCAG	660 I CAGCAGCAACA	670 I IGAAGCAGCA	680 I GCAGCAACAGT	690 I ITCCACCACC	700 I CAGCACCAGCAGCAAC
Consensus Clone#1	560 I AGCAGCA	570 I ACCAGTTCCA	580 I ICCAGCAGCA	590 I GCAGCAGCAGG	600 I CAGCAGCAGC	610 I CAGCAGCAGCA	620 I ACAATTCCAC	630 I CAGCAGCAGC	640 I AGCAGCAACA	650 I ATTCCACCAG	660 I CAGCAGCAACA	670 I IGAAGCAGCA	680 I GCAGCAACAGT	690 I ITCCACCACC	700 L CAGCACCAGCAGCAAC
Consensus Clone#1 Clone#2 Clone#3	560 AGCAGCA	570 I ACCAGTTCCA	580 I ICCAGCAGCA	590 GCAGCAGCAG	600 I CAGCAGCAGC	610 L CAGCAGCAGCA	620 I ACAATTCCAC	630 I CAGCAGCAGC	640 I AGCAGCAACA	650 I ATTCCACCAG	660 I CAGCAGCAACA	670 I IGAAGCAGCA	680 I GCAGCAACAGT	690 I ITCCACCACC	700 I CAGCACCAGCAGCAAC
Consensus Clone#1 Clone#2 Clone#3 Clone#4 Clone#5	560 AGCAGCA	570 I ACCAGTTCCA	580 I SCCAGCAGCA	590 J GCAGCAGCAGC	600 I CAGCAGCAGC	610 I CAGCAGCAGCAGCA	620 ACAATTCCAC	630 I CAGCAGCAGC	640 I AGCAGCAACA	650 I ATTCCACCAG	660 I CAGCAGCAACA	670 I IGAAGCAGCA	680 I GCAGCAACAGT	690 I ITCCACCACC	700 I CAGCACCAGCAGCAAC
Consensus Clone#1 Clone#2 Clone#3 Clone#4 Clone#5 Clone#6	560 AGCAGCA 	570 I ACCAGTTCCA	580 I ICCAGCAGCA	590 I GCAGCAGCAGC	600 I CAGCAGCAGC	610 I CAGCAGCAGCAG	620 ACAATTCCAC	630 I CAGCAGCAGC	640 I AGCAGCAACA	650 I ATTCCACCAG	660 I CAGCAGCAACA	670 I IGAAGCAGCA	680 I GCAGCAACAGT	690 I ITTCCACCACC	700 CAGCACCAGCAGCAAC
Consensus Clone#1 Clone#2 Clone#3 Clone#4 Clone#5 Clone#6 Clone#7 Clone#8	560 AGCAGC/	570 I ACCAGTTCCA	580 I ICCAGCAGCA	590 GCAGCAGCAGG	600 I CAGCAGCAGC	610 I CAGCAGCAGCA	620 I ACAATTCCAC	630 L CAGCAGCAGC	640 I AGCAGCAACA	650 ATTCCACCAG	660 I CAGCAGCAACA	670 I GAAGCAGCA	680 GCAGCAACAGT	690 I ITTCCACCACC	700 CAGCACCAGCAAC
Consensus Clone#1 Clone#2 Clone#3 Clone#4 Clone#5 Clone#6 Clone#7 Clone#8	560 I AGCAGC/ 	570 I ACCAGTTCCA	580 I.CCAGCAGCAGCA	590 GCAGCAGCAGC	600 I CAGCAGCAGC	610 I CAGCAGCAGCAGCAG	620 I ACAATTCCAC	630 CAGCAGCAGC	640 I AGCAGCAACA	650 ATTCCACCAG	660 LAGCAGCAACA	670 GAAGCAGCA	680 GCAGCAACAGT	690 1 TTCCACCACC	700 CAGCACCAGCAGCAAC
Consensus Clone#1 Clone#2 Clone#3 Clone#4 Clone#5 Clone#6 Clone#7 Clone#8	560 AGCAGCA 	570 1 ACCAGTTCCA 	580 	590 GCAGCAGCAGC 740	600 CAGCAGCAGC 750	610 I CAGCAGCAGCAGCA 760 I AGCGCCAGCAGCA	620 ACAATTCCAC	630 CAGCAGCAGC 780	640 1 AGCAGCAACA 790 1 AGCAGCAAGCA	650 	660 CAGCAGCAACA 810 TACTACTTCC	670 IGAAGCAGCA 	680 GCAGCAACAGT	690 ITTCCACCACC 840	700 CAGCACCAGCAGCAAC 850 CCAGCAGCAGCAGGTTC
Consensus Clone#1 Clone#2 Clone#3 Clone#4 Clone#5 Clone#6 Clone#7 Clone#8 Consensus Clone#1	560 AGCAGCA 	570 1 ACCAGTTCCA 	580 	590 GCAGCAGCAGCAGC 740 CCACCAGCAGC	600 I CAGCAGCAGC 750 I CAGCAGAAGC	610 I CAGCAGCAGCAG A A T CAGCAGCAGCAGCAG A CAGCGCCAGCAG	620 ACAATTCCAC 770 GCACCAGTTC	630 CAGCAGCAGC 780 1 CACCAGCAG <u>T</u>	640 AGCAGCAACA 790 A <u>GC</u> AGCAAGC	650 ATTCCACCAGG	660 CAGCAGCAACA 810 GTACTAGTTCC	670 	680 GCAGCAACAGT 830 AGCAGCAGCAJ	690 ITCCACCACC 840 I ACAGTTCCAC	700 CAGCACCAGCAGCAAC 850 CCAGCAGCACAAGTTC
Consensus Clone#1 Clone#2 Clone#3 Clone#5 Clone#6 Clone#7 Clone#8 Consensus Consensus Clone#1 Clone#2	560 AGCAGCA 	570 4ACCAGTTCCA 720 720 4GCAGCAGCA	580 , CCCAGCAGCA ,	590 GCAGCAGCAGCAGC 740 CCACCAGCAGC	600 CAGCAGCAGC 750 CAGCAGAAAGC	610 I CAGCAGCAGCAGCA 760 I CAGCGCCAGCA	620 ACAATTCCAC 770 GCACCAGTTC	630 CAGCAGCAGC 780 CACCAGCAG <u>T</u>	640 AGCAGCAACA 	650 ATTCCACCAG	660 CAGC AGC A CAGC AGC A CAGC AGC A STACTAGTTCC	670 .GAAGCAGCA 	680 GCAGCAACAGT 830 AGCAGCAGCAJ	690 ITTCCACCACC 840 ACACAGTTCCAC	700 CAGCACCAGCAGCAAC 850 CCAGCAGCACAAGTTC
Consensus Clone#1 Clone#2 Clone#3 Clone#5 Clone#6 Clone#7 Clone#8 Consensus Clone#1 Clone#3 Clone#4	560 AGCAGCA 	570 	580 .ccagcagca 	590 GCAGCAGCAGG 740 CCACCAGCAGG	600 CAGCAGCAGC 750 CAGCAGAAGC	610 CAGCAGCAGCAG CAGCAGCAGCA 760 - AGCGCCCAGCA	620 ACAATTCCAC 770 GCACCAGTTC	630 30 30 30 30 30 780 780 780 1 30 50 50 50 50 50 50 50 50 50 50 50 50 50	640 AGCAGCAACA 790 A <u>GC</u> AGCAAGC	650 ATTCCACCAG 	660 CAGCAGCAACA 810 GTACTAGTTCC	670 .GAAGCAGCA 	680 GCAGCAACAGT 830 AGCAGCAGCAA	690 ITTCCACCACC	700 CAGCACCAGCAGCAAC 850 CCAGCAGCACAAGTTC
Consensus Clone#1 Clone#2 Clone#3 Clone#5 Clone#6 Clone#7 Clone#8 Consensus Clone#1 Clone#3 Clone#4 Clone#5 Clone#6	560 AGCAGCA 	570 I ACCAGTTCCA 720 720 AGCAGCAGCA	580 .ccagcagca 	590 GCAGCAGCAGG 740 CCCACCAGCAGG	600 CAGCAGCAGC 750 CAGCAGAAGG	610 ICAGCAGCAGCA CAGCAGCAGCA 760 CAGCGCCAGCA	620 ACAATTCCAC 770 GCACCAGTTC	630 CAGCAGCAGC 780 1 CACCAGCAGT	640 AGCAGCAACA 790 AGCAGCAAGC	650 ATTCCACCAG4 800 AGCAGCAGCAGCA	660 CAGCAGCAAC7 810 STACTAGTTCC	670 .GAAGCAGCA 	680 GCAGCAACAGT 830 AGCAGCAGCAGCAJ	690 ITTCCACCACC 840 ACACAGTTCCAC	700 CAGCACCAGCAGCAAC 850 CCAGCAGCACAAGTTC
Consensus Clone#1 Clone#2 Clone#3 Clone#6 Clone#6 Clone#7 Clone#8 Consensus Clone#1 Clone#3 Clone#4 Clone#6 Clone#6 Clone#7	560 AGCAGC/ 	570 ACCAGTTCCA	580 .ccagcagca 	590 GCAGCAGCAGG 740 CCCACCAGCAGG	600 CAGCAGCAGC 750 CAGCAGAAGC	610 ICAGCAGCAGCA CAGCAGCAGCA 760 CAGCGCCAGCA	620 ACAATTCCAC 770 GCACCAGTTC	630 CAGCAGCAGC 780 CACCAGCAGT	640 AGCAGCAACA 790 4 <u>GC</u> CAGCAAGC	650 ATTCCACCAG4 	660 CAGCAGCAAC7 810 STACTAGTTCC	670 .GAAGCAGCA 	680 GCAGCAACAGT 830 AGCAGCAGCAGCAJ	690 ITTCCACCACC 840 ACCAGTTCCAC	700 CAGCACCAGCAGCAAC 850 CCCAGCAGCACCAAGTTC
Consensus Clone#1 Clone#2 Clone#3 Clone#6 Clone#6 Clone#7 Clone#8 Consensus Clone#1 Clone#2 Clone#4 Clone#5 Clone#4 Clone#5 Clone#7 Clone#8	560 AGCAGCA 710 ATAAGCA	570 ACCAGTTCCA	580 .ccagcagca 	590 GCAGCAGCAGG 740 CCCACCAGCAGG	600 CAGCAGCAGC 750 CAGCAGAAGC	610 ICAGCAGCAGCA CAGCAGCAGCA 760 CAGCGCCAGCA	620 ACAATTCCAC 770 GCCACCAGTTC	630 CAGCAGCAGC 780 CACCAGCAGT	640 AGCAGCAACA 790 4 6 6 6 7 790 1 1 7 7 90 1 1 7 7 0 1 1 7 90 1 1 7 90 1 1 1 7 90	650 ATTCCACCAG4 800 AGCAGCAGCAGCA	660 CAGCAGCAAC7 810 STACTAGTTCC	670 .GAAGCAGCA 	680 GCAGCAACAGT 830 AGCAGCAGCAGCAJ	690 ITTCCACCACC 840 4 ACAGTTCCAC	700 CAGCACCAGCAGCAAC 850 CCAGCAGCACAAGTTC
Consensus Clone#1 Clone#2 Clone#3 Clone#6 Clone#7 Clone#8 Consensus Clone#1 Clone#2 Clone#4 Clone#5 Clone#4 Clone#5 Clone#6 Clone#7 Clone#8	560 AGCAGCA 710 ATAAGCA 860	570 ACCAGTTCCA 720 AGCAGCAGCA AGCAGCAGCA 870	580 .ccagcagcagca 	590 GCAGCAGCAGCA 740 CCCACCAGCAGCA 890	600 CAGCAGCAGC 750 CAGCAGAAGC CAGCAGAAGC 900	610 [:AGCAGCAGCA. 760 [:AGCGCCAGCA 910 1	620 ACAATTCCAC 770 GCACCAGTTC G 920	630 (CAGCAGCAGC 780 (CACCAGCAGT CACCAGCAGT 930	640 AGCAGCAACA 790 1 4 4 4 5 7 90 1 1 7 1 7 1 7 1 7 90 1 1 7 90 1 7 90 1 940	650 ATTCCACCAG4 800 AGCAGCAGCAGCAGCAGCAGCAGCAGCAGCAGCAGCAGCA	660 CAGCAGCAAC7 810 STACTAGTTCC 960	670 .GAAGCAGCA 	680 GCAGCAACAGT 830 AGCAGCAGCAGCAG 980	690 ITTCCACCACC 840 1 ACAGTTCCAC 990	700 CAGCACCAGCAGCAAC 850 CCAGCAGCAGCAAGTTC T 1000
Consensus Clone#1 Clone#2 Clone#3 Clone#6 Clone#7 Clone#8 Consensus Clone#1 Clone#2 Clone#4 Clone#5 Clone#5 Clone#6 Clone#7 Clone#8	560 AGCAGCA 710 ATAAGCA 860 CACCAGG	570 ACCAGTTCCA 720 AGCAGCAGCAGCA 870 CAGCAGCAGCAGCA	580 .ccagcagcagca 730 .ccagtrcca 	590 GCAGCAGCAGCA 740 CCCACCAGCAGCA 890 ACCAGCAGCAGCA	600 CAGCAGCAGC 750 CAGCAGAAGC CAGCAGAAGC	610 [TAGCAGCAGCA 760 [CAGCGCCAGCA 910 [CCAGCAATAGC/	620 ACAATTCCAC 770 GCACCAGTTC G. G. G. G.	630 CAGCAGCAGC 780 CACCAGCAGT CACCAGCAGT 930 5CAGCAGCAGCAA	640 AGCAGCAACA 790 4 4 4 4 5 7 90 1 1 7 7 90 1 4 6 7 7 90 1 7 7 90 4 6 6 7 7 90 4 6 7 7 90 4 6 7 7 90 4 7 7 90 4 6 7 7 90 4 7 7 90 4 7 7 8 7 8 7 8 7 8 7 8 7 8 7 8 7 8 7 8	650 ATTCCACCAGG 800 AGCAGCAGCAGCAG 950 AGCAGCAGCAGCAGCAG	660 CAGCAGCAAC7 810 STACTAGTTCC 960 5CAGCAGCAGCAGC	670 .GAAGCAGCA 	680 GCAGCAACAGT 830 AGCAGCAGCAGCAJ 980 TCCACCACCAC	690 ITTCCACCACC 840 ACCAGTTCCAC 990 5CAGCTAACC	700 CAGCACCAGCAGCAAC 850 CCAGCAGCAGCAAGTTC T
Consensus Clone#1 Clone#2 Clone#3 Clone#6 Clone#7 Clone#8 Consensus Clone#1 Clone#8 Clone#6 Clone#7 Clone#8 Clone#6 Clone#7 Clone#8 Clone#1 Clone#1 Clone#1 Clone#1 Clone#1 Clone#1 Clone#1 Clone#2	560 AGCAGCA 710 ATAAGCA 860 CACCAGC	570 ACCAGTTCCA 720 AGCAGCAGCAGCA 870 CAGCAGCAGCAGCAGCA	580 .ccagcagcagca 730 .ccagticca .ccagticca 	590 GCAGCAGCAGC 740 CCCACCAGCAGCA 890 ACCAGCAGCAGCA	600 CAGCAGCAGC 750 CAGCAGAAGC 900 CCAGTTCCAC	610 [TAGCAGCAGCA 760 [AGCGCCAGCA 200 [CAGCGCATAGC/	620 ACAATTCCAC 770 GCACCAGTTC G. G. G. G.	630 CAGCAGCAGC 780 CACCAGCAGT CACCAGCAGT 930 GCAGCAGCAGCAA	640 AGCAGCAACA 790 4 4 4 4 5 7 7 90 1 1 7 7 90 1 4 6 7 7 90 1 7 7 90 1 4 6 7 7 90 1 7 7 90 1 7 7 90 1 7 7 90 1 7 7 90 1 7 7 90 1 7 7 90 1 7 7 90 1 7 7 7 90 1 7 7 7 90 1 7 7 7 90 1 7 7 7 90 1 7 7 7 90 1 7 7 7 90 1 7 7 90 1 7 7 90 1 7 7 90 1 7 7 90 1 7 7 90 1 7 7 90 1 7 7 90 1 7 7 90 1 7 7 90 1 7 7 7 90 1 7 7 90 1 7 7 90 1 7 7 7 90 1 7 7 90 1 7 7 90 1 7 7 90 1 7 7 90 1 7 7 7 90 1 7 7 90 1 7 7 90 1 7 7 7 7 90 1 7 7 7 7 90 1 7 7 7 7 90 1 7 7 7 90 1 7 7 7 7 7 90 1 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7	650 ATTCCACCAG4 800 AGCAGCAGCAGCAG 950 AGCAGCAGCAGCAG	660 CAGCAGCAAC7 810 STACTAGTTCC 960 5CAGCAGCAGC	670 .GAAGCAGCA 	680 GCAGCAACAGT 830 AGCAGCAGCAGCAJ 980 TCCACCACCACCAC	690 ITTCCACCACC 840 ACCAGTTCCAC 990 5CAGCTAACC	700 CAGCACCAGCAGCAAC 850 CCAGCAGCACAAGTTC T
Consensus Clone#1 Clone#2 Clone#3 Clone#4 Clone#5 Clone#6 Clone#7 Clone#8 Consensus Clone#1 Clone#8 Consensus Clone#1 Clone#1 Clone#2 Clone#2 Clone#3 Clone#4	560 AGCAGCA 710 ATAAGCA 860 CACCAGC	570 ACCAGTTCCA 720 AGCAGCAGCAGCA 870 CAGCAGCAGCAGCAGC	580 .ccagcagcagca 730 .ccagticca .ccagticca 	590 GCAGCAGCAGC 740 CCCACCAGCAGCA 890 ACCAGCAGCAGCA	600 CAGCAGCAGC 750 CAGCAGAAGC CAGCAGAAGC	610 [TAGCAGCAGCA 760 [CAGCGCCAGCA 200 [CAGCGCATAGC/ 200 [CAGCGATAGC/	620 ACAATTCCAC 770 GCACCAGTTC G. G. G. G.	630 CAGCAGCAGC 780 CACCAGCAGT CACCAGCAGT 930 5CAGCAGCAGCAA	640 AGCAGCAACA 790 46 46 46 46 47 40 40 40 40 40 40 40 40 40 40 40 40 40	650 ATTCCACCAGG 800 AGCAGCAGCAGCAG 950 AGCAGCAGCAGCAG	660 CAGCAGCAAC7 810 STACTAGTTCC 960 5CAGCAGCAGC	670 .GAAGCAGCA 	680 GCAGCAACAGT 830 AGCAGCAGCAGCAJ 980 TCCACCACCACCAC	690 ITTCCACCACC 840 ACCAGTTCCAC 990 5CAGCTAACC	700 CAGCACCAGCAGCAAC 850 CCAGCAGCACAAGTTC T
Consensus Clone#1 Clone#2 Clone#3 Clone#6 Clone#5 Clone#7 Clone#8 Consensus Clone#1 Clone#8 Clone#6 Clone#7 Clone#8 Clone#6 Clone#7 Clone#8 Clone#4 Clone#2 Clone#3 Clone#4 Clone#4 Clone#4 Clone#4 Clone#4 Clone#4 Clone#4	560 AGCAGCA 710 ATAAGCA 860 CACCAGC	570 ACCAGTTCCA 720 AGCAGCAGCAGCA 870 CAGCAGCAGCAGC	580 .ccagcagcagca 730 .ccagttcca 	590 GCAGCAGCAGC 740 CCCACCAGCAGCAGCA 890 ACCAGCAGCAGCA	600 CAGCAGCAGC 750 CAGCAGAAGC CAGCAGAAGC	610 17.66CAGCAGCA 7.60 10.0000 10.0000 10.000 10.000 10.000 10.000 10.000 10.000 10.000 10	620 ACAATTCCAC 770 GCACCAGTTC G. G. G. G.	630 CAGCAGCAGC 780 CACCAGCAG CACCAGCAG 500 500 500 500 500 500 500 500 500 50	640 AGCAGCAACA 790 AGCACCAAGC AGCACCAAGC	650 ATTCCACCAG6 800 AGCAGCAGCAGCAG 950 AGCAGCAGCAGCA	660 CAGCAGCAAC7 810 STACTAGTTCC 960 5CAGCAGCAGC	670 .GAAGCAGCA 	680 GCAGCAACAGT 830 AGCAGCAGCAGCA 980 TCCACCACCAC	690 ITTCCACCACC 840 ACCAGTTCCAC 990 5CAGCTAACC	700 CAGCACCAGCAGCAAC 850 CCAGCAGCACAAGTTC
Consensus Clone#1 Clone#2 Clone#3 Clone#4 Clone#5 Clone#6 Clone#7 Clone#2 Clone#4 Clone#3 Clone#4 Clone#7 Clone#8 Clone#6 Clone#1 Clone#3 Clone#1 Clone#3 Clone#3 Clone#4 Clone#2 Clone#4 Clone#5 Clone#6 Clone#6 Clone#7	560 AGCAGCA 710 ATAAGCA 860 CACCAGC	570 ACCAGTTCCA 720 AGCAGCAGCAGCA 870 CAGCAGCAGCAGC	580 .ccagcagcagca 730 .ccagtrcca 	590 GCAGCAGCAGC 740 CCCACCAGCAGCAGC ACCAGCAGCAGCA	600 CAGCAGCAGC 750 CAGCAGAAGC CAGCAGAAGC	610 EAGCAGCAGCA 760 CAGCGCCAGCA CAGCGCCAGCA	620 ACAATTCCAC 770 GCACCAGTTC G 920 AGCAGCAGCA	630 CAGCAGCAGC 780 CACCAGCAG CACCAGCAG 500 500 500 500 500 500 500 500 500 50	640 AGCAGCAACA 790 AGCACCAAGC 	650 ATTCCACCAG6 800 AGCAGCAGCAGCAG 950 AGCAGCAGCAGCAG	660 CAGCAGCAAC7 810 STACTAGTTCC 960 5CAGCAGCAGC	670 .GAAGCAGCA 	680 GCAGCAACAGT 830 AGCAGCAGCAGCAJ 980 TCCACCACCAC	690 ITTCCACCACC 840 ACCAGTTCCAC 990 5CAGCTAACC	700 CAGCACCAGCAGCAAC 850 CCAGCAGCACAAGTTC
Consensus Clone#1 Clone#2 Clone#4 Clone#5 Clone#6 Clone#7 Clone#8 Consensus Clone#1 Clone#6 Clone#7 Clone#3 Clone#8 Consensus Clone#1 Clone#3 Clone#6 Clone#7 Clone#4 Clone#7 Clone#7 Clone#7 Clone#7 Clone#8	560 AGCAGCA 710 ATAAGCA 860 CACCAGG	570 ACCAGTTCCA 720 AGCAGCAGCAGCA 870 CAGCAGCAGCAGC	580 .ccagcagcagca 730 .cccagttcca 	590 GCAGCAGCAGCA 740 CCCACCAGCAGCA ACCAGCAGCAGCA	600 CAGCAGCAGC 750 CAGCAGAAGC CAGCAGAAGC	610 IIIAGCAGCAGCAA 760 CAGCGCCAGCAA CAGCGCCAGCAA CCAGCATAGC/	620 ACAATTCCAC 770 GCACCAGTTC G. G. G. G.	630 CAGCAGCAGC 780 CACCAGCAG CACCAGCAG 500 500 500 500 500 500 500 500 500 50	640 AGCAGCAACA 790 AGCAGCAAGC 	650 ATTCCACCAG6 800 AGCAGCAGCAGCAG 950 AGCAGCAGCAGCA	660 CAGCAGCAAC7 810 STACTAGTTCC 960 5CAGCAGCAGC	670 .GAAGCAGCA 	680 GCAGCAACAGT 830 AGCAGCAGCAGCA 980 TCCACCACCAC	690 ITTCCACCACC 840 ACCAGTTCCAC	700 CAGCACCAGCAGCAAC 850 CCAGCAGCAACAAGTTC
Consensus Clone#1 Clone#2 Clone#4 Clone#5 Clone#6 Clone#7 Clone#8 Consensus Clone#1 Clone#6 Clone#7 Clone#8 Consensus Clone#1 Clone#3 Clone#3 Clone#3 Clone#3 Clone#4 Clone#2 Clone#4 Clone#5 Clone#6 Clone#7 Clone#7 Clone#8	560 AGCAGCA 710 ATAAGCA 860 CACCAGC 1010	570 ACCAGTTCCA 720 AGCAGCAGCAGCA CAGCAGCAGCAGCA CAGCAGCAGCAGCA 1020	580 .ccagcagcagca 730 .ccagtrcca 	590 GCAGCAGCAGC 740 CCCACCAGCAGC ACCAGCAGCAGCA ACCAGCAGCAGCA 1040	600 CAGCAGCAGC 750 CAGCAGAAGC CAGCAGAAGC CCAGTTCCAC	610 17.AGCAGCAGCAA 7.60 10.CAGCCAGCAA 10.CAGCAATAGC/ 10.60	620 ACAATTCCAC 770 GCACCAGTTC G. 	630 CAGCAGCAGC 780 CACCAGCAG 500 500 500 500 500 500 500 500 500 50	640 AGCAGCAACA 790 AGCAGCAAGC AGCAGCAAGC CAGCAACAGC	650 ATTCCACCAGA 800 AGCAGCAGCAGCAG 950 AGCAGCAGCAGCAGCAG 1100	660 CAGCAGCAAC7 810 STACTAGTTCC 960 5CAGCAGCAGCAGC	670 .GAAGCAGCA 	680 GCAGCAACAGT 830 AGCAGCAGCAGCAJ 980 TCCACCACCAC	690 ITTCCACCACC 840 ACCAGTTCCAC	700 CAGCACCAGCAGCAAC 850 CCAGCAGCACAAGTTC
Consensus Clone#1 Clone#2 Clone#3 Clone#4 Clone#5 Clone#6 Clone#7 Clone#2 Clone#4 Clone#3 Clone#4 Clone#5 Clone#6 Clone#7 Clone#3 Clone#8 Consensus Clone#1 Clone#3 Clone#8 Clone#8 Clone#7 Clone#8 Clone#4 Clone#5 Clone#4 Clone#5 Clone#8 Clone#4 Clone#5 Clone#4 Clone#3 Clone#4 Clone#5 Clone#3 Clone#3 Clone#4 Clone#2 Clone#3 Clone#4 Clone#3 Clone#4 Clone#5 Clone#6 Clone#7 Clone#8 Clone	560 AGCAGCA 710 ATAAGCA 860 CACCAGG 1010 GACATCA	570 ACCAGTTCCA 720 AGCAGCAGCAGCA CAGCAGCAGCAGCA CAGCAGCAGCAGCA 1020 ACTGGTGAGC	580 .ccagcagcagca 730 .ccagtrcca 	590 GCAGCAGCAGC 740 CCCACCAGCAGCAGC ACCAGCAGCAGCAG 1040 ATCAGGAGCAT	600 CAGCAGCAGC 750 CAGCAGAAGC CAGCAGAAGC CCAGTTCCAC	610 17.66CAGCAGCAA 7.60 10.00 10.	620 ACAATTCCAC 770 GCACCAGTTC G. 	630 CAGCAGCAGC 780 CACCAGCAG 500 500 500 500 500 500 500 500 500 50	640 AGCAGCAACA 790 AGCAGCAAGC AGCAGCAAGC CAGCAACAGC 1090 CCTTT+ctccc	650 ATTCCACCAGA 800 AGCAGCAGCAGCAG AGCAGCAGCAGCAGCAGCA 1100 ttacctatgcca	660 CAGCAGCAAC7 810 STACTAGTTCC 960 5CAGCAGCAGCAGC 1110 1110	670 GAAGCAGCA 	680 GCAGCAACAGT 830 AGCAGCAGCAGCAJ 980 TCCACCACCAC	690 ITTCCACCACC 840 ACCAGTTCCAC	700 CAGCACCAGCAGCAAC 850 CCAGCAGCAACAAGTTC
Consensus Clone#1 Clone#2 Clone#3 Clone#4 Clone#5 Clone#6 Clone#7 Clone#2 Clone#4 Clone#3 Clone#4 Clone#5 Clone#6 Clone#7 Clone#3 Clone#8 Consensus Clone#1 Clone#3 Clone#4 Clone#3 Clone#4 Clone#5 Clone#4 Clone#5 Clone#6 Clone#7 Clone#8 Clone#6 Clone#7 Clone#8 Clone#6 Clone#7 Clone#8 Clone#6 Clone#7 Clone#8 Clone#1 Clone#2 Clone#3 Clone#1 Clone#2 Clone#3 Clone#4 Clone#2 Clone#3 Clone#4 Clone#5 Clone#6 Clone#6 Clone#7 Clone#8 Clone#6 Clone#7 Clone#8 Clone#7 Clone#8 Clone#1 Clone#8 Clone#6 Clone#1 Clone#8 Clone#1 Clone#8 Clone#1 Clone#8 Clone#1 Clone#8 Clone#1 Clone#8 Clone#8 Clone#8 Clone#8 Clone#1 Clone#8 Clone#8 Clone#8 Clone#1 Clone#8 Clone#8 Clone#8 Clone#1 Clone#3 Clone#8 Clone#1 Clone#8 Clone#1 Clone#8 Clone#8 Clone#8 Clone#8 Clone#1 Clone#8 Clone Clone Clone Clone Clone Clone Clone Clone Clone8 Clone8 Clone88 Clone88 Clone88 Clone88 Clon	560 AGCAGCA 710 ATAAGCA 860 CACCAGG 	570 ACCAGTTCCA 720 AGCAGCAGCAGCA CAGCAGCAGCAGCA CAGCAGCAGCAGCA 1020 ACTGGTGAGC	580 .ccagcagcagca 730 .ccagtrcca 	590 GCAGCAGCAGCAGC 740 CCCACCAGCAGCAGCA ACCAGCAGCAGCAGCA 1040 ATCAGGAGCAT	600 CAGCAGCAGC 750 CAGCAGAAGC CAGCAGAAGC CCAGTTCCAC	610 17.66CAGCAGCAA 7.60 10.00 10.	620 ACAATTCCAC 770 GCACCAGTTC G. 	630 CAGCAGCAGC 780 CACCAGCAG 500 500 500 500 500 500 500 500 500 50	640 AGCAGCAACA 790 AGCAGCAAGC AGCAGCAAGC CAGCAACAGC 1090 CCTTT+c+ccc	650 ATTCCACCAGA 800 AGCAGCAGCAGCAG 950 AGCAGCAGCAGCAGCAG 1100 ttactatgccc	660 CAGCAGCAAC7 810 STACTAGTTCC 960 5CAGCAGCAGCAGC 1110 1110	670 GAAGCAGCA 	680 GCAGCAACAGT 830 AGCAGCAGCAGCAJ 980 TCCACCACCAC	690 ITTCCACCACC 840 ACAGTTCCAC	700 CAGCACCAGCAGCAAC 850 CCAGCAGCAACAAGTTC
Consensus Clone#1 Clone#2 Clone#3 Clone#4 Clone#5 Clone#6 Clone#7 Clone#2 Clone#4 Clone#3 Clone#4 Clone#7 Clone#8 Consensus Clone#1 Clone#3 Clone#3 Clone#4 Clone#5 Clone#4 Clone#5 Clone#6 Clone#7 Clone#8 Clone#4 Clone#2 Clone#8	560 AGCAGCA 710 ATAAGCA 710 ATAAGCA 860 CACCAGG 	570 ACCAGTTCCA 720 AGCAGCAGCAGCA CAGCAGCAGCAGCA CAGCAGCAGCAGCA 1020 ACTGGTGAGC	580 .ccagcagcagca 730 .ccagtrcca 	590 GCAGCAGCAGCA 740 CCCACCAGCAGCAGCA ACCAGCAGCAGCA 1040 ATCAGGAGCAI	600 CAGCAGCAGC 750 CAGCAGAAGC CAGCAGAAGC CAGCAGAAGC 1050 TCTCAGGAAA	610 EAGCAGCAGCA 760 CAGCCCCAGCA CAGCCCCAGCA 1060 1060 1060	620 ACAATTCCAC 770 GCACCAGTTC G. 	630 CAGCAGCAGC 780 CACCAGCAG 500 500 500 500 500 500 500 500 500 50	640 AGCAGCAACA 790 AGCAGCAAGC AGCAGCAAGC CAGCAACAGC 1090 CCTTT+c+ccc	650 ATTCCACCAGA 800 AGCAGCAGCAGCAG AGCAGCAGCAGCAGCAGCA 1100 ttactatgcca	660 CAGCAGCAAC7 810 STACTAGTTCC 960 5CAGCAGCAGCAGC 1110 1110	670 GAAGCAGCA 	680 GCAGCAACAGT 830 AGCAGCAGCAGCAJ 980 TCCACCACCAC	690 ITTCCACCACC 840 ACAGTTCCAC	700 CAGCACCAGCAGCAAC 850 CCAGCAGCAACAAGTTC
Consensus Clone#1 Clone#2 Clone#3 Clone#4 Clone#5 Clone#6 Clone#7 Clone#8 Consensus Clone#1 Clone#4 Clone#6 Clone#7 Clone#8 Consensus Clone#4 Clone#2 Clone#8 Clone#6 Clone#7 Clone#8 Clone#6 Clone#7 Clone#8 Clone#6 Clone#7 Clone#8 Clone#6 Clone#7 Clone#8 Clone#6 Clone#7 Clone#8 Clone#6 Clone#7 Clone#8 Clone#6 Clone#7 Clone#8 Clone#6 Clone#7 Clone#8 Clone#7 Clone#8 Clone#6 Clone#7 Clone#8 Clone#7 Clone#8 Clone#6 Clone#7 Clone#8 Clone#7 Clone#8 Clone#7 Clone#8 Clone#8 Clone#6 Clone#7 Clone#8 Clone#8 Clone#8 Clone#7 Clone#8 Clone88 Clone88 Clone88 Clone88 Clone88 Clone88 Clone88 Clone88 Clone88 Clone88 Clone88 Clone88 Clone88 Clone88	560 AGCAGCA 710 ATAAGCA 710 ATAAGCA 860 CACCAGG 	570 ACCAGTTCCA 720 AGCAGCAGCAGCA CAGCAGCAGCAGCA CAGCAGCAGCAGCA 1020 ACTGGTGAGC	580 .ccagcagcagca 730 .ccagtrcca 	590 GCAGCAGCAGCA 740 CCCACCAGCAGCAGCA ACCAGCAGCAGCAGCA 1040 ATCCAGGAGCAT	600 CAGCAGCAGC 750 CAGCAGAAGC CAGCAGAAGC CAGCAGAAGC 1050 TCTCAGGAAA	610 [TAGCAGCAGCA 760 CAGCCCCAGCA CAGCCCCAGCA 1060 1060 1060	620 ACAATTCCAC 770 GCACCAGTTC G. 	630 CAGCAGCAGC 780 CACCAGCAG 500 500 500 500 500 500 500 500 500 50	640 AGCAGCAACA 790 AGCACCAAGC AGCACCAAGC 	650 ATTCCACCAGA 800 AGCAGCAGCAGCAG 950 AGCAGCAGCAGCAGCA 1100 tacctotgccc	660 CAGCAGCAAC7 810 STACTAGTTCC 960 960 5CAGCAGCAGCAGC 1110 1110	670 .GAAGCAGCA 	680 GCAGCAACAGT 830 AGCAGCAGCAGCAJ 980 TCCACCACCAC	690 ITTCCACCACC 840 ACAGTTCCAC	700 CAGCACCAGCAGCAAC 850 CCAGCAGCACAAGTTC
Consensus Clone#1 Clone#2 Clone#4 Clone#5 Clone#6 Clone#7 Clone#8 Consensus Clone#1 Clone#6 Clone#7 Clone#8 Consensus Clone#4 Clone#3 Clone#4 Clone#5 Clone#8 Clone#4 Clone#5 Clone#3 Clone#4 Clone#3 Clone#4 Clone#5 Clone#4 Clone#4 Clone#4 Clone#3 Clone#4 Clone#4 Clone#5 Clone#4 Clone#4 Clone#3 Clone#4 Clone#3 Clone#4 Clone#4 Clone#3 Clone#4 Clone#4 Clone#3 Clone#4 Clone#4 Clone#3 Clone#4 Clone#5 Clone#4 Clone#4 Clone#3 Clone#4 Clone#5 Clone#4 Clone#4 Clone#5 Clone#4 Clone#3 Clone#4 Clone#5 Clone#4 Clone#5 Clone#4 Clone#3 Clone#4 Clone#5 Clone#6	560 AGCAGCA 710 ATAAGCA 860 CACCAGC 1010 GACATCA	570 ACCAGTTCCA 720 AGCAGCAGCAGCA CAGCAGCAGCAGCA CAGCAGCAGCAGCA 1020 ACTGGTGAGC	580 .ccagcagcagca 730 .ccagtrcca 	590 GCAGCAGCAGCA 740 CCCACCAGCAGCA ACCAGCAGCAGCA 1040 ATCAGGAGCAT	600 CAGCAGCAGC 750 CAGCAGAAGC CAGCAGAAGC 2000 CCAGTTCCAC	610 [TAGCAGCAGCA 760 CAGCCCCAGCA (CAGCGCCAGCA 1060 1060 1060	620 ACAATTCCAC 770 GCACCAGTTC G. 	630 CAGCAGCAGC 780 CACCAGCAG 500 500 500 500 500 500 500 500 500 50	640 AGCAGCAACA 790 AGCAGCAAGC AGCAGCAAGC 	650 ATTCCACCAGA 800 AGCAGCAGCAGCAG 950 AGCAGCAGCAGCAGCAG 1100 ttacctotgccc	660 CAGCAGCAAC7 810 STACTAGTTCC 960 960 5CAGCAGCAGCAGC 1110 1110	670 .GAAGCAGCA 	680 GCAGCAACAGT 830 AGCAGCAGCAGCAJ 980 TCCACCACCAC	690 ITTCCACCACC 840 ACAGTTCCAC	700 CAGCACCAGCAGCAAC 850 CCAGCAGCAGCAAGTTC

Supplementary Figure S2. Nucleotide sequence alignment of eight independent *M. mattheyi* Sry clones. Seven haplotypes were identified (see also Table 1). The consensus sequence is displayed above the alignment. Sequences identical to the consensus are indicated by dots, while missing nucleotides are depicted by hyphens. Binding sites of PCR primers are in lower case. Start and stop codons are double-underlined. Sequences encoding the HMG, Bridge, and polyQ domains are boxed, underlined or in grey, respectively.



Supplementary Figure S3. Alignment of the deduced SRY peptide sequences in *M. minutoides*.

The consensus sequence is displayed above the alignment. Residues identical to the consensus are indicated by dots, while missing residues are depicted by hyphens. The HMG, Bridge, and polyQ domains are boxed, underlined or in grey, respectively. A deletion in clones #1 and #2 (E170_Q176del; *M. minutoides Sry* haplotype a, Table 1) resulted in the loss of one polyglutamine block.

	10	20	30	40	50	60	70	80	90	100
Consensus	MEGHVKRPMNAFMVW	SCGERQKLA	QNPSMQNTE:	ESKLLGCRWK	SLTEGEKRPF	FQEAQRLKTL	HREKYPNYKY	QPHRRAKVPQI	RSGALQTAVTS	TKLYN
Clone#1										
Clone#2										
Clone#3										
Clone#4										
Clone#5										
Clone#6										
Clone#7										
Clone#8		•••••								• • • • •
	110	120	130	140	150	160	170	180	190	200
Consensus	LLQWDRNPHTITYRQ	DWTRAAHLS	SKNQQSFYCQI	PVDIPTGNPL	<u> 2000 τόσο τη 2000 το </u>	QFHHHQQQQQ	HQFHQQQQLKI	HQHQHQFYQQ	QQQHQFHQQQ	QQQQQ
Clone#1										
Clone#2									–	
Clone#3										
Clone#4										
Clone#5									–	
Clone#6							R			
Clone#7										
Clone#8		•••••			• • • • • •					
	210	220	230	240	250	260				
Consensus	QQQQQQFHQQQQQQ	FHQQQQQKQ	<u></u> 2000 εΗΗΩΗQ	<u> </u> <u> </u> <u> </u> <u></u>	HQQQQKQRQ	QHQFHQQ				
Clone#1										
Clone#2										
Clone#3						.R				
Clone#4										
Clone#5										
Clone#6										
Clone#7		•••••								
Clone#8										

Supplementary Figure S4. Alignment of the deduced SRY peptide sequences in *M. mattheyi*.

The consensus sequence is displayed above the alignment. Residues identical to the consensus are indicated by dots, while missing residues are depicted by hyphens. The HMG, Bridge, and polyQ domains are boxed, underlined or in grey, respectively. A deletion in clone #3 (Q172_Q195del; M. *mattheyi Sry* haplotype c, Table 1) resulted in the loss of two polyglutamine block, while the polyQ tract is almost completely lost in clone #8 (M. *mattheyi Sry* haplotype g, Table 1).

	-40	-30	-20	-10	1	10	20	30	40	50	60	70	80	90	100
Consensus	aagaga	caagttttgg	gactggtga	caATTGTCTA	GAGAGC <u>ATG</u> O	GAGGGCCATGTC	AAGCGCCCT	ATGAATGCAT	TTATGGTGTGG	тсстотосто	GAGAGGCAXAA	бттобссси	AGCAGAATCCCA	GCATGCAAA	ACACAGAGATCAGCA
M. musculus					· · · · · · · · · · · · · · ·		c			c	c				.T
M. minutoides	····· · · · · · · · · ·										A			с	
	110	120	130	140	150	160	170	180	190	200	210	220	230	240	250
Consensus	AGCTGC	TGGGATGCAG	GTGGAAAAG		GXCGAAAAAA					AGAGAGAAAT		ΤΑΑΑΤΑΤΟ		GGGCTAAAG	TGCCACAGAGGAGTG
M. musculus	A				.c			T	T						
M. mattheyi					.G						· · · · · · · · · · · · · · · · · · ·				
w. minutoides	S .A	• • • • • • • • • • • •	•••••	• • • • • • • • • • • •	.A	• • • • • • • • • • • • • •						•••••			
	260	270	280	290	300	310	320	330	340	350	360	370	380	390	400 I
Consensus	GXGCTT	TACAGCCTGC	AGTTACCTC	AACAAAACTG	TACAACCTG	TGCAGTGGGAC	AGGAACCCA	CACACCATCA	CATACAGACAA	GACTGGACTA	GAGCTGCACA	CCTGTCCT	CCAAAAACCAGC	AAAGCTTTT	ATTGTCAGCCTGTGG
M. musculus M. mattheyi M. minutoides	.CAT .T G	A	G		т.		· · · · · · · · · · · · · · · · · · ·	TG	G	G		A 		· · · · · · · · · · · · · · · · · · ·	
	410	420	430	440	450	460	470	480	490	500	510	520	530	540	550
Consensus	I ATATCC	ı CCACTGGGAA	I CCCACTACA	GCAGCAGCAG	I CAGCAGCAG	I CAGCAGCA	CCAXXTC	I CACCACCAXC	I AGCAGCAGCAG	TTCCAXCACO	I ACCACCAGCA	I GCAGCAGC	I TGXAGCAGCAGC	I AXCAGCAGC	
M. musculus		c.	TG			GCAG	TTTAA.	G.	A	T.TG			A.C	.G	TG
M. mattheyi M. minutoides		• • • • • • • • • • •					GT	C.		CAGCG1	Τ	 мс т.с		.TC.	TC.GG.
w. minutoluez							AC.0	A.UUA.	11			A0		.AG	11CCAd.Ad.1
	560 I	570 I	580 I	590	600 I	610	620 I	630 I	640 I	650 I	660 I	670 I	680 I	690 I	700 I
Consensus	AXCAGC	AGAAGCAXCA	GTTGX	XXXCCACCAG	CAGCAACAG	CAG	CAGCAGCAX	CAXCAXCAGX	AGCAXCAXTTO	CACCAX	AXCAGCAGCA	XCAXCAXT	TCCACC	AGCAGCAGC	AXCAGXAGCAGCAGC
M. musculus M. matthevi	.C		TCAT.A		G	TICCATGAC	ccc		GG	TGAC.	.C	AGG. GAA.	ATGACC		.GC
M. minutoides	S CTA	CA.GAGGTTG	GG	TTTT.T <u>T./</u>	<u>4</u> G⊤.	.т	T								
	710	720	730	740	750	760	770	780	790	800	810	820	830	840	850
Consensus	AGCAXC	AGTTCCAXXA	CCAXCACCA	GCAGXAXCAX	۲ «AG		CAXCAG	CAGCAXCAGT	TCCACCAC	CAGCAGCAG	AGXAGCAGXX	CCA	GC	AGCAXCAGT	TCCACC
M. musculus	G.	TG.	C	A.GG	CTTCCATO	GACCACCACCAC	CACA	G	TGA		CTT	TGACC	ACCAGCAGCA	T	TGACCACCC
M. mattheyi M. minutoides	A. 3	CC.	G	C.AT/	4		G	C						C	
	860	870	880	890	900	910	920	930	940	950	960	970	980	990	1000
Consensus	AGCAGX		۱ ۱			آ ۱۵۵۵۵۵			ΧΧΓΑΧΓΑGXXX		ΑΓΓΑΓΓΑΓ	GCAGCAG-	XAXCAXC	Ι ΑΓΓΑGΓΑ	
M. musculus	A	GTT	CCATGACC.	c.cc	AC.GC	TGA		GA	AGGTTC	TG.CCA	A.	T	TCCATG.CC.	GA	AGTG
M. mattheyi	<u>⊺</u>	<u></u>		G.AG	GT.CT		GG	AT	тсссас	CA.GTT	c.		A.GG.		CCC
w. minutolaes	5														
	1010	1020	1030	1040	1050	1060	1070	1080	1090	1100	1110	1120	1130	1140 I	1150
Consensus	AXCAXX	AXCAGCAGCA	XCAGCAG	CAX	CAXCAGCAX	CAGCAGCAGCAG	CAGCAGCAG	CAGCAGCAGC	AGTTCCACXAC	CAGCAGCTX	ССТАСТТАСТ	AACAGCTG	ACATCACTGGTG	AGCATAXAC	XXTAXCAGGAGCAXC
M. musculus M. mattheyi M. minutoides	.CCC .GAT	.C	ATT G	CCATGACC	CG.			· · · · · · · · · · · · · · · · · · ·	G C	A		· · · · · · · · · · · · · · · · · · ·	· · · · · · · · · · · · · · · · · · ·	C T	CACC. TGTT.
	1160	1170	1180	1190	1200	1210	1220	1230							
Consensus	TCAGXA		GTXGGCAGT	CTCATGACAC	TGGCCTTTta	tcctacctata	ccaacactc	cccct							
M. musculus	C.	C													
M. mattheyi M. minutoides	G.	A	A					· · · · · -							

Supplementary Figure S5. Nucleotide sequence comparison of *Sry* sequences in *M. minutoides*, *M. mattheyi*, and *M. musculus*. Reference *Sry* sequences in *M. minutoides* (clone #7, Supplementary Fig S1; see also Table 1) and *M. mattheyi* (clone #4, Supplementary Fig S2) are used here. *M. musculus Sry* sequence is retrieved from mm10 reference genome. The consensus is displayed above the alignment. Sequences identical to the consensus are indicated by dots, while missing nucleotides are depicted by hyphens. Binding sites of PCR primers are in lower case. Start and stop codons are double-underlined. Sequences encoding the HMG, Bridge, and polyQ domains are boxed, underlined or in grey, respectively.

	10	2 0	30	40	50	60	70	80	90	100
Consensus	MEGHVKRPMNA	MVWSCGERQKL	QQNPSMQNTE	ISKLLGCRWK	SLTEXEKRPF	FQEAQRLKTL	HREKYPNYKY	QPHRRAKVPQF	RSGALQPAVT	STKLYN
M. musculus M. mattheyi M. minutoides		RH		Q	A G	I.	· · · · · · · · · · · · · · · · · · ·		A	
Consensus	110 I LLQWDRNPHTI	120 I TYRQDWTRAAHLS	130 SSKNQQSFYCQ	140 I PVDIPTGNPL		160 I (QFHXHXQQQQ	170 I XXXHQQQQ	180 I LKXQXQXQFXX	190 I KXQQQQHQFH	200 I IXQXQQQ
M. musculus M. mattheyi M. minutoides	A	S	(W.	Н.		QN.H HH.Q	QFYDH HQF D.R.	QQQ.Q.QHL H.H.HY(ОННКQ 2Q LЕ.Q	DHH Q.Q H.RE
Consensus	210 I QXXXQ(220 I QQQFHXXXQQQQ	230 I XHQQQQQX	240 I XQQQQQFHXXH	250 I IQQX	260 I HXQQQXQFHX	270 I XQQQX	280 I -QRQQHQFHXX	290 I (300
M. musculus M. mattheyi M. minutoides	.QFHDHHHHH.E QQQ. LVSP.	EDHH(QQQ .EL.PHSS	QFHDQ FK S	0DH. (HQ.	KQQFHDHH	H.HQD KHH	HQQQFHD QK	H.QDH QC -T.GWWV.S	IPQQKQQFHD }	HPQQQQ
_	310 I	320 I	330 I	340 I	350 I	360 I	370 I	380 I	390 I	
Consensus										
M. musculus M. mattheyi M. minutoides	QFHDHHHQQQQI	KQQFHDHHQQKQG)FHDHHQQKQQ	<u>FHDHHQQQQ</u> Q)FHDHHQQQQQ	QQQQQQQQQFH	DQQLTYLLTA	DITGEHTPYQE	HLSTALWLA	.VS

Supplementary Figure S6. Comparison of deduced SRY peptide sequences in *M. musculus*, *M.*

mattheyi, and *M. minutoides*. Reference SRY sequences in *M. minutoides* (clone #7, Supplementary Fig S1; see also Table 1) and *M. mattheyi* (clone #4, Supplementary Fig S2) are used here. *M. musculus* SRY sequence is retrieved from mm10 reference genome. The consensus sequence is displayed above the alignment. Residues identical to the consensus are indicated by dots, while missing residues are depicted by hyphens. The HMG, Bridge, and polyQ domains are boxed, underlined or in grey, respectively. * indicates an amino acid substitution (R17C) in *M. mattheyi* and *M. minutoides*, compared with *M. musculus*.

Consensus	10 GCGGACGCCG			30 I CGTACGACGA	40 GGACCTGGAG	50 GAGCTGTGTC	60 CGGTGTGTGG	70 I TGACAAGGTO	80 I STCGGGATACO	90 ACTACGGGC	100 TGCTCACGTG	110 CGAGAGCTGC		130 I ICAAGCGCACA	140 I AGTCCAGAACA	150 I ACAAG
M. musculus M. mattheyi M. minutoides a M. minutoides b							.A		C							
Consensus M. musculus M. mattheyi	160 I CATTACACGTO	GCACCGA	170 GAGTCAGA	180 GCTGCAAAAT	190 CGACAAGACO	200 GCAGCGTAAGC	210 GCTGTCCCTT	220 	230 I CCAGAAGTGCO	240 I CTGACGGTGG	250 GCATGCGCCT	260 GGAAGCTGTG	270 CGTGCTGATCO	280 GAATGCGGGGT	290 I IGGCCGGAACA	300 AGTTT
M. minutoides a M. minutoides b	310	, 	320	330	340	350	360	370	380	390	400	410	420	430	440	 450
Consensus M. musculus M. mattheyi M. minutoides a M. minutoides b	GGGCCCATGT	ACAAGAG	AGACCGGG	CCTTGAAGCA	GCAGAAGAAA	AGCACAGATTC	GGGCCAATGO	GCTTCAAGCTC	GGAGACCGGA	CCACCAATGG	GGGTGĊCCCC	GCCACCTCCT		ACTACATGTT4	CCCCCTAGCC	.TGCAT
Consensus M. musculus M. mattheyi M. minutoides a M. minutoides h	466 I GCACCGGAGCO) CCAAGGX	470 CCTGGTCT	480 I CTGGCCCACC	490 CAGTGGGCCC	500 GCTGGGTGACT	510 TTGGAGCCCC	520 I ATCTCTGCCC	530 I CATGGCTGTGG	540 I CCTGGTCCCC	550 ACGGGCCTCT	560 GGCTGGCTAC	570 I CTCTATCCTG	580 I CCTTCTCTAAC	590 I CCGCACCATCA	600 AGTCT
M. minutoides b Consensus M. musculus M. mattheyi M. minutoides e	610 GAGTATCCAGA	AGCCCTA	620 TGCCAGCC	630 CCCCTCAACA	640 GCCAGGGCCA	650 ACCCTACAGCT	660 I ATCCAGAGCC	670 CCTTCTCXXXC	680 GGGAGGGCCCA	690 AATXTACCAG	700 AGCTCATATT	710 GCAGCTGCTT(720 I CAACTAGAGCO	730 CAGAGGAGGAG	740 CCAGGTGCGTG	750 GCTCGC
M. minutoides a M. minutoides b Consensus M. musculus M. mattheyi M. mattheyi	766 I ATCGTGGGCT	GTCTXCA	770 IGGAGCCAG	780 CCAAAAGCCG	790 TTCTGACCAC	800 GCCAGCGCCCT	810 TCAGCCTCCT	820 CTGCAGAATO	830 5GCCGACCAG/	840 ACCTTTATCT	850 CCATTGTCGA	860 CTGGGCACGAA	870 AGGTGCATGG	880 FCTTTAAGGAG	890 I SCTGGAGGTGG	900 GCTGAC
M. minutoides b Consensus M. musculus M. mattheyi M. minutoides a M. minutoides a	91(CAGATGACACT	GCTXCA	920 GAACTGTT	930 GGAGCGAGCT	940 GCTGGTGTTC	950 I GGACCACATCT	960 ACCGCCAGGT	970 I CCCAGTACGGG	980 I CAAGGAAGACA	990 I AGCATCCTGC	1000 TGGTTACTGG	1010 ACAGGAGGTG	1020 I GAGCTGAGCAG	1030 CAGTGGCTGTC	1040 I GCAGGCTGGCT	1050 СССТС
Consensus M. musculus M. mattheyi M. minutoides a M. minutoides b	106 CTGCACAGCCT	Ø	1070 GCGGGCCC	1080 AAGAGTTAGT	1090 GCTCCAGTTC	1100 GCATGCGCTGC	1110 AGCTGGACCC	1120 GCCAGGAGTTO	1130 CGTCTGTCTC/	1140 AAGTTCCTCA	1150 TCCTCTTCAG	1160 CCTCGATGTG	1170 AAATTCCTGA/	1180 ACAACCACAGO	1190 CCTGGTAAAGG	1200 ATGCC .C
Consensus	121 CAGGAGAAGGG	Ø CCAACGC	1220 CGCCCTGT	1230 I TGGATTACAC	1240 CTTATGTCAG	1250 I CTACCCACACT	1260 XCGGGGACAA	1270 ATTCCAGCAG	1280 I STTGCTATTG	1290 I IGCCTGGTGG	1300 AGGTGCGGGC	1310 L CCTGAGCATG	1320 I CAGGCCAAGG/	1330 AGTACCTGTAC	1340 I CCACAAGCATT	1350 TGGGC
M. musculus M. mattheyi M. minutoides a M. minutoides b	A		T		G	T	G G T T									
Consensus M. musculus M. mattheyi M. minutoides a M. minutoides b	136 AACGAGATGCO	Ø	1370 CAACCTTC	1380 TCATTGAGAT	1390 GCTGCAGGCC	1400 T CAAGCAGACTT	1410 GAGCCTGGGT	1420 GCCAGGCAGT	1430 I GGGCAGTAGG	1440 I GCAGGGATGC	1450 CACTGCCTCC	1460 AAAAAGACTCC	1470 I TTGCATTAGG	1480 I I I GATCCAGG		

Supplementary Figure S7. Nucleotide sequence alignment of *Sf1* coding sequences in *M. musculus*, *M. mattheyi*, and *M. minutoides*. *M. mattheyi* and *M. minutoides Sf1* coding sequences are assembled from sequencing results of individual *Sf1* exons 2–7. The *M. mattheyi* individual analysed appeared to carry two identical *Sf1* alleles, whereas two different *Sf1* alleles (*a*, *b*) were identified in the *M. minutoides* individual. *M. musculus Sf1* sequence is retrieved from mm10 reference genome. The consensus sequence is displayed above the alignment. Sequences identical to the consensus are indicated by dots, while missing nucleotides are depicted by hyphens. Start and stop codons are double-underlined.

	10	20	30	40	50	60	70	80	90	100
Consensus	MDYSYDEDLDELCP	CGDKVSGYH	GLLTCESCK	GFFKRTVQNNI	KHYTCTESQS	CKIDKTQRKR	CPFCRFQKCL	TVGMRLEAVR	ADRMRGGRNKF	GPMYK
M. musculus										••••
M. mattheyi			•••••	• • • • • • • • • • •				• • • • • • • • • • •		••••
M. minutoides	•••••	• • • • • • • • • • •	• • • • • • • • • • •	• • • • • • • • • • • •	• • • • • • • • • • • •	• • • • • • • • • • •	• • • • • • • • • • • •	• • • • • • • • • • • •		••••
	110	120	130	140	150	160	170	180	190	200
Consensus										
M. musculus									· · · · · · · · · · · · · · ·	
M. mattheyi			A							••••
M. minutoides			••••••	•••••	V		• • • • • • • • • • •			••••
	210	220	220	240	250	260	270	200	200	200
	1	220	230	1	250	200	270 I	200	290 I	500
Consensus	YASPPQQPGPPYSYF	PEPFS-GGPN	/PELILQLLQI	LEPEEDQVRAI	RIVGCLQEPA	KSRSDQPAPF:	SLLCRMADQTI	-ISIVDWARR(CMVFKELEVAD	QMTLL
M. matthevi				• • • • • • • • • • • • •				· · · · · · · · · · · ·	 	••••
M. minutoides		S]	[
	310	320	330	340	350	360	370	380	390	400
Consensus	QNCWSELLVLDHIYF	QVQYGKEDSI	LLVTĠQEVEI	LSTVAVQAGSI	LHSLVLRAQI	ELVLQLHALQI		FLILFSLDVK	FLNNHSLVKDA	QEKAN
M. musculus				•••••						•••••
M. mattheyi M. minutoides	• • • • • • • • • • • • • • • • • •	• • • • • • • • • • •	•••••	•••••	• • • • • • • • • • •	• • • • • • • • • • •	• • • • • • • • • • • •	• • • • • • • • • • • •		••••
w. minutolues			•••••	• • • • • • • • • • • •	• • • • • • • • • • • •		• • • • • • • • • • •	• • • • • • • • • • • •		••••
	410	420	430	440	450	460				
Consensus				7 1 1 1						
M. musculus										
M. mattheyi										
M. minutoides	F.		• • • • • • • • • • •	•••••						

Supplementary Figure S8. Deduced peptide sequence alignment of SF1 in M. musculus, M.

mattheyi, and *M. minutoides*. Note the two *M. minutoides Sf1* alleles (S7 Fig) encode the same protein. *M. musculus* SF1 sequence is retrieved from mm10 reference genome. The consensus sequence is displayed above the alignment. Residues identical to the consensus are indicated by dots, while missing residues are depicted by hyphens.



Supplementary Figure S9. SF1 proteins from *M. musculus*, *M. mattheyi*, and *M. minutoides* show similar activities in musTESCO-Luc reporter assays. (a) Schematic of SF1 proteins from three *Mus* species: *M. musculus* (musSF1), *M. mattheyi* (matSF1), and *M. minutoides* (minSF1). DBD, DNA binding domain; LBD, ligand-binding domain. Non-synonymous sequence changes compared with musSF1 are indicated in matSF1 and minSF1. (b) Synergistic activation of musTESCO-Luc by various combinations of SRY (including two chimeric mutants) and SF1 from *M. musculus*, *M. mattheyi*, and *M. minutoides* were tested using *in vitro* reporter assays. The luciferase activity of musTESCO-Luc co-transfected with the empty vector in the absence of SF1 was set to 1. For simplicity, only the +SF1 data are presented here as mean \pm s.e.m (n = 3), as the -SF1 data essentially showed unchanged base level activities of musTESCO-Luc reporter. Dashed line indicates the level of empty vector + musSF1. (*) *P* < 0.05, Tukey's multiple comparisons test. ns, not significant. (c) Summary of two-way repeated measures ANOVA results of (b).

	10	20	30	40	50	60	70	80	90	100	110	120	130	140
Consensus	ccagcaagatttct	gcatatgtcAC	атасстаа	GGTGAAAATA	татдадссст	GTCTAAATCGGA	астссаахс	хтдтассат	тттхсттаадд	ccccacago	iaagaaaaaag	JAAAAAAACAAA	аааасаа́аааас	
M. musculus					c		c.	Δ	c			. G G . G .	G	
M. mattheyi M. minutoides a				• • • • • • • • • • • •		A.	C	A	С т	A		۸A . ۲	A	
M. minutoides b						· · · · · · · · · · · · · · · · ·		G						
	150	160	170	190	100	200	210	220	220	240	250	V1	270	280
Conconsus	TCACACCCCCAC													200
M musculus	IGAGAGCCCGAC	ICHAITGITG		TAGCAGAACT	CAGCIGIAAI	ACAGAGECATIT	GAAAGGAAT	GACAATIGA	GITCIGCCCAG	CCTGAAGAA			ICCIAACCIGG	
M. matthevi	AAC					A	· · · · · · · · · · ·	.c						
M. minutoides a			A.					. T						
W. Minutoides D		<u> </u> R4	A.									FCRi	·]· · · · · · · · · · · · · ·	
	290	300	310	320	330	340	350	360	370	380	390	400	410	420
Consensus	ттсасаааатааса	атассттсттт	ГСАБАААСТ	TTAGGGCTAA	GGAAGGXACA	астссастстсо	CAGATAAGG	бстббсаба	AGAGGTGGCAG	стасса́аст	ACAGAGAGAG	rggctgcaggx	GTTCCCAGGGTC	AAACA
M. musculus		G			. A AG . AG	G				A	G	A		
M. mattheyi		• • • • • • • • • • • • •	A	• • • • • • • • • • • •	G	A		.			A	A		
M. minutoides a						· · · · · · · · · · · · · · · · · · ·						G		
	R5/EC	Rii EC	Riii				ECRiv	ECRv						
0	430 I	440 I	450	460	470	480 I	490 I	500	510	520	530	540	550	560
Consensus	CAAGTGCCTXGCTT	CTTGGTGAGAG	GCATTAGG	CA-GGAAGAG	cerredicce	AGGAACTTAAAA	GCTCXTACC	CCAGCCCCT	GTGCTGACGTG	CGCXXAAGG	AGACCTCTT	GCCCAAGCAGA	AGAAGCCTAA-C	GGTCT
M. MUSCUIUS M. matthevi	G		AA	AG. -	• • • • • • • • • • • • • • • • • • • •	G	c.c.cc	CA.T				TA	T.C	A .
M. minutoides a	A						T			GC				
M. minutoides b	A		· · · · · · · · · · · · · · · · · · ·	<u></u>	<u></u>		T			GC			· · · · · · <u> · · · · -</u>	<u></u> .
	570	580	590	600	610	620	630	640	650	660	670	680	690	J 700
Consensus	CCAAAAAXATCTCC-	I TTCGCCATCTC	GGTACXTT	TGCAGTTGGG	GGCTATGTCT	ACAXCTGACTTC	TTCCAAGAC	I TCTGAGGTT	TAGAGTTTGAG	TGAGCTTGG	тоостоост	ттстстстст	Ι ΓΑCCTTTTTATT	I ICAAAG
M. musculus	.TGC	A.AT	TG		c	G		c						
M. mattheyi	GCC.		G	G		G								
M. minutoides a M. minutoides b		· · · · · · · · · · · · · ·				A	· · · · · · · · · · ·							
	710	720	730	740	750	760	770	780	790	800	810	820	830	840
Consensus	710 I TTTCCAACACACAAA	720 I AGCGCTTTAGA	730 I AGTATCCAT	740 I GGAAACTTCC	750 I ATAGCCACGG	760 I GCTCAGAATGAG	770 I GCTGTGAGC	780 I AAAGTGTCA	790 I GCAGCCTGGAA	800 I GTCACCCCA	810 I AGAGCATCAA	820 I AGTCCC-GGTG	830 I GCATGAATXTGT	840 I ICACTT
Consensus M. musculus	710 I TTTCCAACACACAAA	720 I AGCGCTTTAGA	730 I AGTATCCAT	740 I GGAAACTTCC/	750 I ATAGCCACGG	760 I GCTCAGAATGAG	770 I GCTGTGAGC	780 I AAAGTGTCA	790 I IGCAGCCTGGAA	800 I GTCACCCCA	810 I AGAGCATCAA	820 I AGTCCC-GGTG	830 GCATGAATXTGT	840
Consensus M. musculus M. mattheyi M. minutoides a	710 I TTTCCAACACACAAA	720 I AGCGCTTTAGA	730 I AGTATCCAT	740 GGAAACTTCC/	750 I ATAGCCACGG	760 I GCTCAGAATGAG A	770 I GCTGTGAGC	780 I AAAGTGTCA	790 I IGCAGCCTGGAA	800 I GTCACCCCA	810 I AGAGCATCAA	820 I AGTCCC-GGTG CA	830 I GCATGAATXTGT G G	840 I ICACTT
Consensus M. musculus M. mattheyi M. minutoides a M. minutoides b	710 I TTTCCAACACACAAA	720 I AGCGCTTTAGA	730 I AGTATCCAT	740 GGAAACTTCC/	750 I ATAGCCACGG	760 I GCTCAGAATGAG	770 I GCTGTGAGC	780 I AAAGTGTCA	790 I GCAGCCTGGAA	800 I GTCACCCCA	810 I AGAGCATCAA	820 I AGTCCC-GGTG CA 	830 I GCATGAATXTGT G C	840 I TCACTT
Consensus M. musculus M. mattheyi M. minutoides a M. minutoides b	710 TTTCCAACACACACAAA	720 I AGCGCTTTAGA	730 I AGTATCCAT	740 I GGAAACTTCC/	750 I ATAGCCACGG	760 I GCTCAGAATGAG A	770 I GCTGTGAGC.	780 AAAGTGTCA 	790 I GCAGCCTGGAA	800 I GTCACCCCA	810 I AGAGCATCAA	820 I AGTCCC-GGTGG CA 960	830 I GCATGAATXTGT G C 970	840 I TCACTT
Consensus M. musculus M. maitheyi M. minutoides a M. minutoides b	710 TTTCCAACACAAAA 	720 H AGCGCTTTAGA G 860 XGAGCCACAAA	730 I AGTATCCAT 	740 	750 I ATAGCCACGG 	760 I GCTCAGAATGAG A 900 GACATGAGCCCA	770 I GCTGTGGAGC. 	780 AAAGTGTCA 	790 IGCAGCCTGGAA	800 I GTCACCCCA 	810 	820 I AGTCCC-GGTG CA 960 I GGAATTIGGGC	830 I GCATGAATXTGT G G G C 970 I XGGTAATGTGG	840 TCACTT
Consensus M. musculus M. mattheyi M. minutoides a M. minutoides b	710 TTTCCAACACACACAA 	720 AGCGCTTTAGA G 860 1 XGAGCCACAAA C.G.G.G.G.G.	730 I AGTATCCAT 	740 GGAAACTTCC/ 	750 ATAGCCACGG 	760 GCTCAGAATGAG A	770 I GCTGTGAGC. 910 GCTAAAAAA	780 AAAGTGTCA 	790 I GCAGCCTGGAA 930 I CCTACTGATAGG	800 GTCACCCCA	810 I AGAGCATCAA 	820 	830 	840 I TCACTT 980 GCCAGT
Consensus M. musculus M. mattheyi M. minutoides a M. minutoides b Consensus M. musculus M. mattheyi	710 TTTCCAACACACAAA 	720 I AGCGCTTTAGA G 860 XGAGCCACAAA G.GGGC G	730 I AGTATCCAT 870 I AGTGCCATT	740 GGAAACTTCC/ 	750 ATAGCCACGG 	760 I GCTCAGAATGAG A	770 I GCTGTGAGC. 	780 I AAAGTGTCA 920 -GGGAGTAG G	790 .GCAGCCTGGAA 	800 I GTCACCCCA 	810 IAGAGCATCAA 	820 I AGTCCC-GGTG 	830 GCATGAATXTGT GG. G C 970 XGGTAAATGTGC T	840 FCACTT 980 5CCAGT T
Consensus M. musculus M. mattheyi M. minutoides a M. minutoides b Consensus M. musculus M. minutoides a M. minutoides a M. minutoides a	710 TTTCCAACACACAAA 	720 AGCGCTTTAGA G 860 1 XGAGCCACAAA G.GGGC G	730 AGTATCCAT 	740 GGAAACTTCC/ 	750 ATAGCCACGG 	760 GCTCAGAATGAG A	770 I GCTGTGAGC. 	780 1 AAAGTGTCA 920 -GGGAGTAG G A	790 I GCAGCCTGGAA 930 I CCTACTGATAGG	800 GTCACCCCA 940 ATGAATTTG	810 IAGAGCATCAA 	820 	830 G	840
Consensus M. musculus M. mattheyi M. minutoides a M. minutoides b Consensus M. musculus M. mattheyi M. minutoides a M. minutoides b	710 TTTCCAACACACAAA 	720 AGCGCTTTAGA G 860 1 XGAGCCAAA G.GGGC GA.	730 1 AGTATCCAT 870 1 AGTGCCATT 5	740 GGAAACTTCC/ 	750 ATAGCCACGG 	760 GCTCAGAATGAG A	770 I GCTGTGAGC. 910 I GCTAAAAAA G	780 1 920 	790 GCAGCCTGGAA 930 CTACTGATAGG	800 GTCACCCCA 940 ATGAATTTG	810 	820 	830 J GCATGAATXTG1 G G 	840
Consensus M. musculus M. mattheyi M. minutoides a M. minutoides b Consensus M. musculus M. mattheyi M. minutoides a M. minutoides b	710 TTTCCAACACACAAA 	720 1 AGCGCTTTAGA G 860 XGAGCCACAAA G.GGGC G A 1000 1	730 AGTATCCAT 870 AGTGCCATT 5 1010	740 GGAAACTTCC/ 	750 ATAGCCACGG 890 GGGCCACACC A	760 GCTCAGAATGAG A	770 I GCTGTGAGC. 910 GCTAAAAA GCTAAAAA GCTAAAAA GCTAAAAA	780 AAAAGTGTCA 	790 GCAGCCTGGAA 930 CTACTGATAGG 	800 GTCACCCCA 940 ATGAATTTG C.C. 1080	810 AGAGCATCA/ 950 1 1 1090 1090 1	820 JAGTCCC-GGTG CA CA 960 IGGAATTTGGGC: 1100	830 GCATGAATXTG1 G G G 	840 TCACTT 980 5CCAGT .T 11120
Consensus M. musculus M. mattheyi M. minutoides a M. minutoides b Consensus M. musculus M. minutoides a M. minutoides a M. minutoides b	710 TTTCCAACACACAAA 	720 4GCGTTTAGA 8660 1 XGAGCACAAA G. G	730 1 AGTATCCAT 870 1 AGTGCCATT 5 1010 1 ACTGCAGXAA	740 GGAAACTTCC/ 	750 ATAGCCACGG 890 GGGCCACACC A	760 GCTCAGAATGAG A	770 I GCTGTGAGC. 910 GCTAAAAAA GCTAGAAAAA G 1050 I CCTGATAAA	780 AAAAGTGTCA 	790 GCAGCCTGGAA 930 CTACTGATAGG 10/0 CTXCTAGAGGG	800 GTCACCCCA 940 ATGATTTG C.C. 10080 4 AGGTGTATC	810 AGAGCATCA 950 IGACTGCCGTT AA. (1090 ATCGTCCCA(820 	830 GCATGAATXTG1 G G G 	840 TCACTT 980 50CCAGT T 1120 50CTTCC
Consensus M. musculus M. mattheyi M. minutoides a M. minutoides b Consensus M. musculus M. minutoides a M. minutoides a M. minutoides b Consensus M. musculus	710 TTTCCAACACACAAA 	720 GCTTTAGA AGCGCTTAGA 860 XGAGCACAAA G.GGGC G 1000 GGACTCAGGCA	730 1 AGTATCCAT 870 1 AGTGCCATT 5 1010 1 ACTGCAGXA	740 GGAAACTTCC/ 	750 ATAGCCACGG 890 GGGCCACACC A	760 GCTCAGAATGAG A	770 GCTGTGAGC. 910 GCTAAAAAA GCTAAAAAAA G 1050 CCTGATAAAA	780 1 AAAAGTGTCA 920 1 -GGGAGTAG 	790 I GCAGCCTGGAA 930 CTACTGATAGG 10/0 CCTXCTAGAGGG 	800 GTCACCCCA 940 ATGAATTTG C.C. 1080 AGGTGTATC	810 AGAGCATCA 950 GACTGCCGTT AA. C 1090 ATCGTCCCAC C. T	820 	830 GCATGAATXTG1 G G G 	840 TCACTT 980 50CCAGT T 1120 50CTTCC
Consensus M. musculus M. mattheyi M. minutoides a M. minutoides b Consensus M. musculus M. minutoides a M. minutoides a M. minutoides b Consensus M. musculus M. musculus M. mattheyi M. minutoides a	710 TTTCCAACACACAAA 	720 GCTTTAGA AGCGCTTAGA 860 XGAGCCACAAA G.GGGC G.GGGC A 1000 GGACTCAGGCA A.	730 1 1 3GTATCCAT 870 1 3GTGCCATT 5 1010 1 ACTGCAGXA A. A.	740 GGAAACTTCC/ 	750 ATAGCCACGG 890 GGGCCACACC A	760 GCTCAGAATGAG A	770 GCTGTGAGC. 910 GCTAAAAAA GCTAAAAAAA G 1050 CCTGATAAAA	780 AAAAGTGTCA 920 -GGGAGTAG 	790 I GCAGCCTGGAA 930 I CTACTGATAGG 10/0 CTXCTAGAGGG 	800 GTCACCCCA 940 ATGAATTTG C.C. 1080 AGGTGTATC	810 AGAGCATCA 950 1090 ATCGTCCCAC C.TA	820 	830 GCATGAATXTG1 G G G 	840 TCACTT 980 55CCAGT T 1120 55CTTCC
Consensus M. musculus M. mattheyi M. minutoides a M. minutoides b Consensus M. musculus M. minutoides a M. minutoides a M. musculus M. musculus M. musculus M. mattheyi M. minutoides a M. minutoides a M. minutoides b	710 TTTCCAACACACAAA 	720 GCTTTAGA 8660 XGAGCCACAAA G. G	730 1 1 3GTATCCAT 870 1 3GTGCCATT 5	740 GGAAACTTCC/ 880 TCTTTCCCAA 10/20 ATGCACCATCC TGC. 	750 ATAGCCACGG 890 GGGCCACACC A	760 GCTCAGAATGAG A	770 GCTGTGAGC. 910 GCTAAAAAA GCTAAAAAAA G 1050 CCTGATAAAA	780 AAAAGTGTCA 920 -GGGAGTAG 	790 I GCAGCCTGGAA 930 I CTACTGATAGG 10/0 CCTXCTAGAGGG 	800 GTCACCCCA 940 ATGAATTTG C.C. 1080 AGGTGTATC	810 AGAGCATCA 950 GGACTGCCGTT AAC 1090 AAC C.TA	820 JAGTCCC-GATG CA 960 IGAATTTGGGC: 1100 1100 CTCCCCACCTCC	830 GCATGAATXTG1 G G G 	840 TCACTT 980 56CCAGT T 1120 56CTTCC
Consensus M. musculus M. mattheyi M. minutoides a M. minutoides b Consensus M. musculus M. minutoides a M. minutoides b Consensus M. musculus M. musculus M. minutoides a M. minutoides a M. minutoides a	710 TTTCCAACACACAAA 	720 AGCGCTTTAGA G 860 XGAGCCACAAA G.GGGC GGGC G.A 10000 J GGACTCAGGCA A 10400 J A 1140	730 1 AGTATCCAT 870 1 AGTGCCATT 1010 1 ACTGCAGXA A. A. G. 1150	740 GGAAACTTCC 880 TCTTTGCCAA 1020 ATGCACCATCC TGC. F 1160	750 ATAGCCACGG 890 GGGCCACACC A	760 GCTCAGAATGAG A	770 GCTGTGAGC. 910 1050 CCTGATAAAAA 1050 CCTGATAAAA 	780 1 1 920 -GGGAGTAG 	790 I GCAGCCTGGAA 930 1 CCTACTGATAGG 10/0 CCTXCTAGAGGG 	800 GTCACCCCA 940 ATGAATTTG C.C. 1080 AGGTGTATC 	810 AGAGCATCA/ 950 GGACTGCCGTT AA.C 1090 ATCGTCCCAC C.TA	820 JGTCCC-GGTG CA 960 IGAATTTGGGC 1100 1100 1100 1240	830 GCATGAATXTG1 G G G 	840 TCACTT 980 50CAGT T 11260 1260
Consensus M. musculus M. maitheyi M. minutoides a M. minutoides b Consensus M. musculus M. minutoides a M. minutoides a M. musculus M. maitheyi M. minutoides a M. minutoides a M. minutoides a M. minutoides a M. minutoides a M. minutoides a	710 TTTCCAACACACAAA 	720 AGCGCTTTAGA 860 XGAGCCACAAA G.GGGC GGGC G.A 10000 1 GGGACTCAGGCA A 11400 GGGACTCAGGCA	730 1 1 1 1 1 1 1 1 1 1 1 1 1	740 1 GGAAACTTCC/ 880 TCTTTGCCAA 1020 ATGCACCATTC/ ATGCACCATTC/ F 1150 AGTGTGTCA	750 ATAGCCACGG 600 600 600 600 600 100 100 100 100 100	760 GCTCAGAATGAG A	770 GCTGTGAGC. 910 GCTAAAAAA GCTAAAAAA CCTGATAAA CCTGATAAA 1050 I CCTGATAAA CCTGATAAA CCTGATAAA	780 1 1 920 -GGGAGTAG 	790 GCAGCCTGGAA 930 CTACTGATAGG 1070 1070 CCTXCTAGAGG 	800 GTCACCCCA 940 ATGAATTTC 1080 AGGTGTATC 1220 CATTGTTC	810 AGAGCATCA/ 950 GGACTGCGGTT AA.C 1090 ATCGTCCCAC C.T A 1230 GGAGTCTCC	820 1 AGTCCC-GGTG 	830 GCATGAATXTGT 	840 rcactt 980 980 555 11266 555 11266 1126
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Consensus M. musculus M. maitheyi M. minutoides a M. minutoides b Consensus M. musculus M. minutoides a M. minutoides a	710 TTTCCAACACACAAA 	720 AGCGCTTTAGA S60 XGACCCACAAA G.GGCG GA 10000 GGGACTCAGGCA AA 10000 GGGACTCAGGCC I280 -GGGATGCCCA CAA	730 1 AGTATCAT 870 1 AGTGCCATT 5	740 GGAAACTCCA 880 TCTTTGCCAAA A 1020 ATGCACCATCC TGC. F 1160 AGTGTGCTACC 1300 ATAAAACAGCC	750 ATAGCCACGG 890 GGGCCACCC 	760 GCTCAGAATGAG A	770 GCTGTGAGC. 910 GCTAAAAAA GCTAAAAAAA GCTAAAAAAA CCTGATAAAA 1050 100	780 AAAAGTGTCA 920 	790 GGAGCCTGGAA 930 CTACTGATAGG 1070 CCTXCTAGAGG 	800 GTCACCCA 940 ATGAATTTG 1080 AGGTGTATC 1080 AGGTGTATC CATTGTTCT CATTGTTCT 1220 CATTGTTCT 1210 R6 1360 TTAACATAT	810 AGAGCATCA/ 950 1 GACTGCGGTT 	820 1 AGTCCC-GGTG CA 960 1 GAATTTGGGC: 1100 CCTCCCACCTCC 1240 GCCAGCATTGG A 1380 gaggtgctggg	830 GCATGAATXTG1 GG 970 KGGTAAATGTGC T 1110 	840 1 TCACTT 980 5CCAGT 11226 5CTTCC 12266 127

Supplementary Figure S10. Nucleotide sequence alignment of TESCO sequences in *M. musculus*, *M. mattheyi*, and *M. minutoides*. The *M. mattheyi* individual analysed appeared to carry two identical copies of TESCO, whereas two TESCO haplotypes were identified in *M. minutoides*. The binding sites of SRY and SF1 identified in *M. musculus* (R4-5 and F1-6, respectively), the evolutionarily conserved sites (ECRi-v), and the sequence variations between the two haplotypes of minTESCO (V1-3) are highlighted in boxes. SRY binding sites and most of the ECR sites remain unchanged in mat/minTESCO, whereas several SF1 binding sites showed sequence variations in *M*. *mattheyi* and *M*. *minutoides*. The consensus sequence is displayed above the alignment. Identical residues are indicated by dots, while missing residues are depicted by hyphens. Binding sites of PCR primers are in lower case.



Supplementary Figure S11. TESCO enhancer in *M. minutoides* and *M. mattheyi* showed reduced response to musSF1. Mutations of SF1 binding sites F1-4 in musTESCO to the corresponding sequence in matTESCO (musTESCO.mut1-Luc) or minTESCO (musTESCO.mut2-Luc) caused mildly reduced response to musSF1. Data are presented as fold induction in the presence versus the absence of musSF1. Error bars: s.e.m. (n = 3). Dashed line indicates the level of musTESCO. (**) P < 0.01 vs. musTESCO, one-way repeated measures ANOVA with Dunnett's multiple comparisons test.



Supplementary Figure S12. Sequence variations at the V3 site between two haplotypes of *M. minutoides* TESCO results in reduced response to musSF1. minTESCO.b.mut with V3 site mutated to the corresponding sequence in minTESCO.a showed fully restored response to musSF1. Data are presented as TESCO luciferase activity normalized to co-transfected CMV-renilla luciferase activity. Error bars: s.e.m. (n = 3). (**) P < 0.01, one-way repeated measures ANOVA with Dunnett's multiple comparisons test. ns, not significant.



Supplementary Figure S13. SF1 proteins from *M. musculus*, *M. mattheyi*, and *M. minutoides* show similar activities in min/matTESCO reporter assays. (a,b) Activation of TESCO by SF1 from three Mus species were tested using *in vitro* reporter assays. Different TESCO reporter constructs contribute to > 98% of total variation (c). Compared with matSF1 and musSF1, minSF1 showed mildly higher activation of both musTESCO and minTESCO.a, but not minTESCO.b, suggesting that *Sf1* is unlikely to have co-evolved with TESCO in *M. minutoides* (see also Supplementary Fig. S14). The luciferase activity of each TESCO-Luc co-transfected with the empty vector in the absence of SF1 was set to 1. Error bars: s.e.m (n=3). (**) P < 0.01, Tukey's multiple comparisons test. ns, not significant. (c) Summary of two-way repeated measures ANOVA results of (a,b).



Supplementary Figure S14. SF1 proteins from *M. musculus*, *M. mattheyi*, and *M. minutoides* show similar activities in synergizing with different SRY in activating various TESCO reporters. Synergistic activation of different TESCO luciferase reporters by various combinations of SRY and SF1 from *M. musculus*, *M. mattheyi*, and *M. minutoides* were tested using *in vitro* reporter assays (a-c). With all three TESCO reporters, different SRY constructs contribute to > 95% of total variation (d). Compared with matSF1 and musSF1, minSF1 showed mildly higher activation of minTESCO.a-Luc (a) and matTESCO-Luc (c) in the presence of musSRY. However, minSF1 did not outperform musSF1 or matSF1 in activating its cognate minTESCO.a/b-Luc reporters in the presence of cognate minSRY, suggesting that *Sf1* is unlikely to have co-evolved with *Sry* or TESCO in *M. minutoides*. The luciferase activity of each TESCO-Luc co-transfected with the empty vector in the absence of SF1 was set to 1. For simplicity, only the +SF1 data are

presented here as mean \pm s.e.m (n=3). Dashed lines indicate the levels of empty vector + musSF1. (**) P < 0.01, Tukey's multiple comparisons test. ns, not significant. (d) Summary of two-way repeated measures ANOVA results of (a-c).



Supplementary Figure S15. Full-length Western blots in Fig. 2b. Predicted molecular weight: g-musSRY, 77.1 kDa; g-musSRY ΔQ , 47.9 kDa; g-minSRY, 50.7 kDa; g-matSRY, 59.9 kDa; α -Tubulin, 50 kDa.

Supplementary Table S1. PCR primers.

Primer	Sequence	Note
Sry.Fw	AGATCTAAGAGACAAGTTTTGGGACTGGTGACA	BglII site underlined
Sry.Rv	CTCGAGAGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGG	XhoI site underlined
TESCO.Fw	CCAGCAAGATTTCTGCATATGTC	
TESCO.Rv	CACTTACCCAGCACCTCCATC	
Sf1exon2_3.Fw	agGCGGACGCCGCGGGCATGGACTATT	Intronic sequence in
Sf1exon2_3.Rv	acCTTCCAGGCGCATGCCCACCGTCA	small letters
Sf1exon4.Fw	agCTGTGCGTGCTGATCGAATG	Intronic sequence in
Sf1exon4.Rv	acCTCCAGCTCCTTAAAGACCATGCA	small letters
Sf1exon5.Fw	agGTGGCTGACCAGATGACACTGCT	Intronic sequence in
Sf1exon5.Rv	acCTCCTGTCCAGTAACCAGCAGGAT	small letters
Sf1exon6.Fw	agGTGGAGCTGAGCACAGTGGCTGT	Intronic sequence in
Sf1exon6.Rv	acCGAGGCTGAAGAGGATGAGGAACTT	small letters
Sflexon7.Fw	gcagATGTGAAATTCCTGAACAACCACA	Intronic sequence in
Sf1exon7.Rv	CCTGGATCACCTAATGCAAGGAGTCTT	small letters