

## Supplemental data

total length: 432875 bp (432875 bp excl N/X-runs)  
GC level: 41.34 %  
bases masked: 235587 bp ( 54.42 %)

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	number of elements*	length occupied	percentage of sequence
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SINEs:	144	18304 bp	4.23 %
Alu/B1	34	3449 bp	0.80 %
B2-B4	70	11450 bp	2.65 %
IDs	35	2972 bp	0.69 %
MIRs	4	376 bp	0.09 %
LINEs:	130	135855 bp	31.38 %
LINE1	123	134605 bp	31.10 %
LINE2	6	1208 bp	0.28 %
L3/CR1	1	42 bp	0.01 %
LTR elements:	144	70554 bp	16.30 %
ERVL	10	2753 bp	0.64 %
ERVL-MaLRs	28	9535 bp	2.20 %
ERV_classI	27	21232 bp	4.90 %
ERV_classII	79	37034 bp	8.56 %
DNA elements:	11	1812bp	0.42 %
hAT-Charlie	6	907 bp	0.21 %
TcMar-Tigger	2	437 bp	0.10 %
Unclassified:	13	6642 bp	1.53 %
Total interspersed repeats:		233167 bp	53.86 %
Small RNA:	14	1250 bp	0.29 %
Satellites:	5	1226 bp	0.28 %
Simple repeats:	0	0 bp	0.00 %
Low complexity:	0	0 bp	0.00 %

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**Figure S1 Repeat analysis of Contig 1.** Contig 1 of SHR Y-chromosome *Sry* region with repeat analysis done with <http://www.repeatmasker.org/>

total length: 377198 bp (377198 bp excl N/X-runs)  
 GC level: 41.85 %  
 bases masked: 197235 bp ( 52.29 %)

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                number of      length  percentage
                elements*    occupied  of sequence
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SINEs:          84           9619 bp   2.55 %
  Alu/B1        14           1289 bp   0.34 %
  B2-B4         31           4730 bp   1.25 %
  IDs           38           3567 bp   0.95 %
  MIRs          1             33 bp    0.01 %

LINEs:          100          123589 bp  32.77 %
  LINE1         99          123495 bp  32.74 %
  LINE2         1             94 bp    0.02 %
  L3/CR1        0              0 bp    0.00 %

LTR elements:  120          61699 bp  16.36 %
  ERVL          12           2597 bp   0.69 %
  ERVL-MaLRs    9           3787 bp   1.00 %
  ERV_classI    31          21450 bp   5.69 %
  ERV_classII   68          33865 bp   8.98 %

DNA elements:  1             145bp    0.04 %
  hAT-Charlie   1             145 bp   0.04 %
  TcMar-Tigger  0              0 bp    0.00 %

Unclassified:  1             1029 bp   0.27 %

Total interspersed repeats:  196081 bp  51.98 %

Small RNA:     11             1054 bp   0.28 %

Satellites:    1             100 bp   0.03 %
Simple repeats: 0              0 bp    0.00 %
Low complexity: 0              0 bp    0.00 %
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**Figure S2 Repeat analysis of Contig 2.** Contig 2 of SHR Y-chromosome *Sry* region with repeat analysis done with <http://www.repeatmasker.org/>

total length: 149399 bp (149399 bp excl N/X-runs)  
 GC level: 42.91 %  
 bases masked: 83452 bp ( 55.86 %)

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	number of elements*	length occupied	percentage of sequence
SINEs:	110	29647 bp	19.84 %
ALUs	106	29203 bp	19.55 %
MIRs	4	444 bp	0.30 %
LINEs:	35	28212 bp	18.88 %
LINE1	32	28047 bp	18.77 %
LINE2	3	165 bp	0.11 %
L3/CR1	0	0 bp	0.00 %
LTR elements:	47	22627 bp	15.15 %
ERV1	4	1439 bp	0.96 %
ERV1-MaLRs	11	3580 bp	2.40 %
ERV_classI	32	17608 bp	11.79 %
ERV_classII	0	0 bp	0.00 %
DNA elements:	16	2864 bp	1.92 %
hAT-Charlie	7	997 bp	0.67 %
TcMar-Tigger	4	1397 bp	0.94 %
Unclassified:	0	0 bp	0.00 %
Total interspersed repeats:		83350 bp	55.79 %
Small RNA:	1	102 bp	0.07 %
Satellites:	0	0 bp	0.00 %
Simple repeats:	0	0 bp	0.00 %
Low complexity:	0	0 bp	0.00 %

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**Figure S3 Repeat analysis of human SRY region.** *hSRY* flanking sequence for 74570bp 3' and 74829bp 5' of the A of ATG for *hSRY* transcript.

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sequences:          1
total length:     197889 bp (197889 bp excl N/X-runs)
GC level:        39.62 %
bases masked:    98586 bp ( 49.82 %)

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	number of elements*	length occupied	percentage of sequence
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SINEs:	41	5242 bp	2.65 %
Alu/B1	27	3083 bp	1.56 %
B2-B4	12	2011 bp	1.02 %
IDs	0	0 bp	0.00 %
MIRs	2	148 bp	0.07 %
<b>LINEs:</b>	<b>32</b>	<b>35022 bp</b>	<b>17.70 %</b>
LINE1	30	34866 bp	17.62 %
LINE2	0	0 bp	0.00 %
L3/CR1	2	156 bp	0.08 %
LTR elements:	93	54808 bp	<b>27.70 %</b>
ERVL	5	2007 bp	1.01 %
ERVL-MaLRs	17	4678 bp	2.36 %
ERV_classI	11	17213 bp	8.70 %
ERV_classII	60	30910 bp	15.62 %
DNA elements:	4	702bp	0.35 %
hAT-Charlie	2	418 bp	0.21 %
TcMar-Tigger	2	284 bp	0.14 %
Unclassified:	2	2006 bp	1.01 %
Total interspersed repeats:		97780 bp	49.41 %
Small RNA:	0	0 bp	0.00 %
Satellites:	5	806 bp	0.41 %
Simple repeats:	0	0 bp	0.00 %
Low complexity:	0	0 bp	0.00 %
=====			

**Figure S4 Repeat analysis of mouse Sry region.** Analysis of mouse *Sry* sequence for 99311bp 3' and 98578bp 5' the A of ATG for the *mSry* transcript.

**A**

	<i>Sry1</i>	<i>Sry2</i>	<i>Sry3A</i>	<i>Sry3B</i>	<i>Sry3BII</i>	<i>Sry3C</i>	<i>Sry4</i>	<i>Sry4A</i>
<i>Sry1</i>	-	421	420	11,600	11,600	11,600	11,600	10,538
<i>Sry2</i>	421	-	420	421	420	424	421	421
<i>Sry3A</i>	8,048	420	-	8,048	8,048	8,048	8,048	8,048
<i>Sry3B</i>	11,600	421	420	-	14,949	14,224	14,675	10,538
<i>Sry3BII</i>	11,600	421	420	15,235	-	14,236	14,675	10,538
<i>Sry3C</i>	11,600	421	420	14,138	13,981	-	14,675	10,538
<i>Sry4</i>	11,600	421	420	14,792	14,506	14,224	-	10,538
<i>Sry4A</i>	10,555	421	420	10,570	10,532	10,719	10,694	-

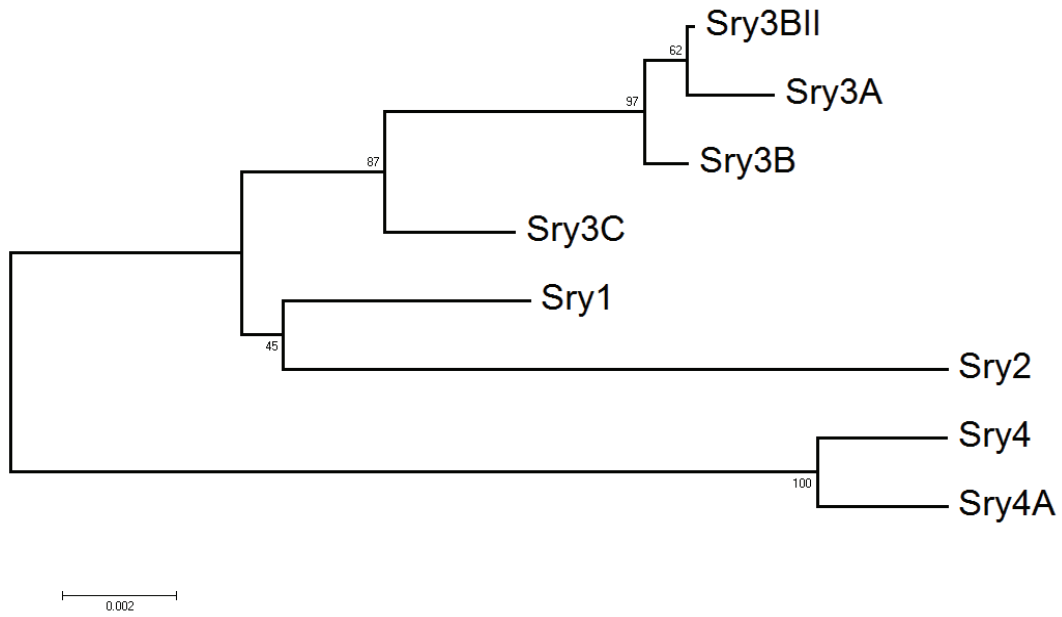
**B**

	<i>Sry1</i>	<i>Sry2</i>	<i>Sry3A</i>	<i>Sry3B</i>	<i>Sry3BII</i>	<i>Sry3C</i>	<i>Sry4</i>	<i>Sry4A</i>
<i>Sry1</i>	-	12,822	1,579	1,974	1,969	12,766	957	989
<i>Sry2</i>	12,763	-	1,579	1,974	1969	30,907	957	989
<i>Sry3A</i>	1,579	1,579	-	1,579	1,579	1,579	957	989
<i>Sry3B</i>	1,975	1936	1,579	-	17,731	1,971	15,252	15,407
<i>Sry3BII</i>	1,975	1936	1,579	15,482	-	1,971	20,389	22,094
<i>Sry3C</i>	12,763	24,564	1,579	1,974	1,969	-	957	957
<i>Sry4</i>	968	924	959	15,482	23,197	961	-	43,175
<i>Sry4A</i>	968	924	959	15,456	23,634	961	42,847	-

**C**

	<i>Sry1</i>	<i>Sry2</i>	<i>Sry3A</i>	<i>Sry3B</i>	<i>Sry3BII</i>	<i>Sry3C</i>	<i>Sry4</i>	<i>Sry4A</i>
<i>Sry1</i>	-	13,243	1,999	13,574	13,569	24,366	12,557	11,527
<i>Sry2</i>	13,184	-	1,999	2,395	2,389	31,331	1,378	1,410
<i>Sry3A</i>	9,627	1,999	-	9,627	9,627	9,627	9,005	9,037
<i>Sry3B</i>	13,575	2,357	1,999	-	32,680	16,195	29,927	25,945
<i>Sry3BII</i>	13,575	2,357	1,999	30,717	-	16,207	35,064	32,632
<i>Sry3C</i>	24,363	24,985	1,999	16,112	15,950	-	15,632	11,495
<i>Sry4</i>	12,568	1,345	1,379	30,274	37,703	15,185	-	53,713
<i>Sry4A</i>	11,523	1,345	1,379	26,026	34,166	11,680	53,541	-

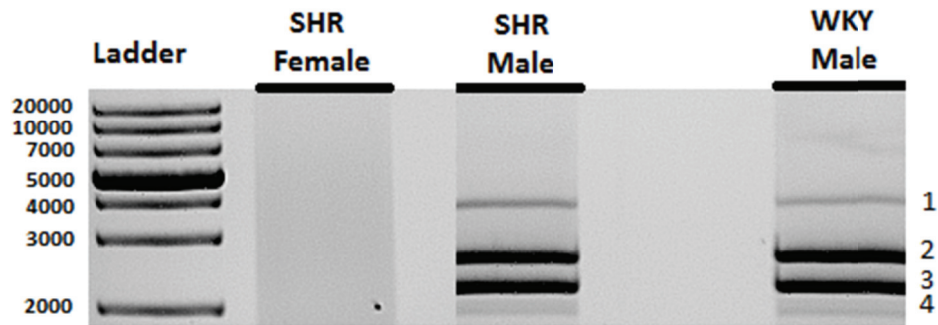
**Figure S5 Conservation analysis of the *Sry* copies.** A) Base pairs conserved 5' of the A of ATG. Highlighted in red are constructs involving *Sry1* where conservation goes to the end of the contig. Highlighted in cyan are those sequences that have a 6,809 bp transposable element inserted causing a disruption of homology that picks back up for 7,647 bp. Numbers in red are those that define the size fragment for phylogenetics. B) Base pairs conserved 3' of the A of ATG, with the green highlighted box showing a 6,386 bp insertion disrupting homology. C). Total conserved fragment length 5' and 3' of the A of ATG. At the top of each chart is the gene that the sequence was aligned to the respective *Sry* on the side.



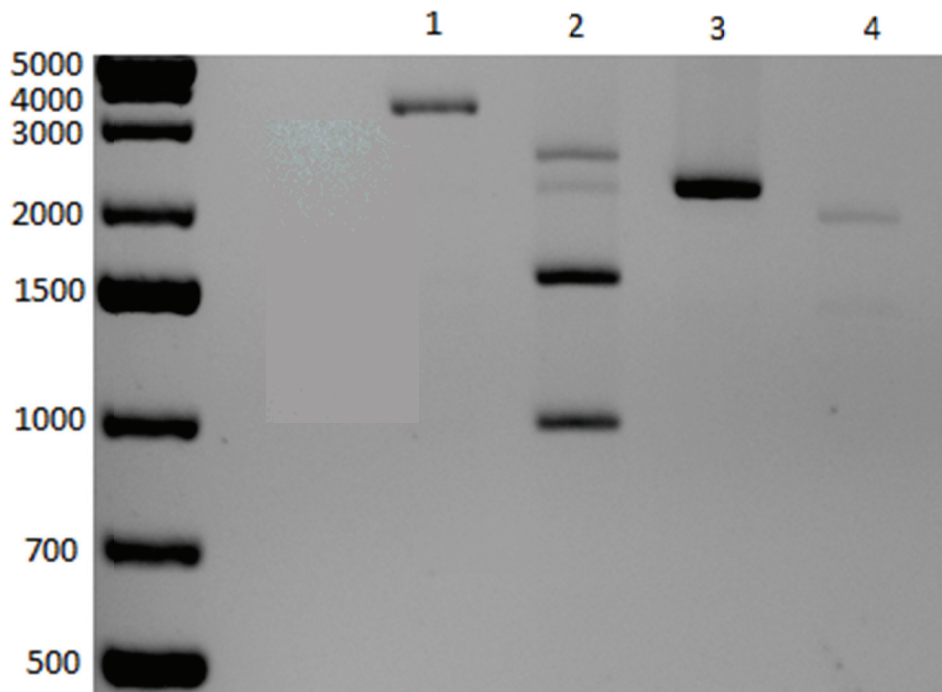
**Figure S6 Phylogenetic tree of the maximally conserved fragment of Sry copies.**

Phylogenetic tree using *Sry* genes of the maximum conserved fragment based on contig analysis of Figure S5. Numbers next to each node represent the percent of the 500 bootstrap phylogenies that were identified similar to the one shown in the figure.

A



B



**Figure S7 PCR products after amplification with JP1R and JF8L specific primers. A)** Gel of PCR amplicons from SHR and WKY genomic DNA, showing the presence of Sry3BII (4220bp, band 1), Sry4A (2031 bp, band 3), and Sry4 (2003, band 4). Band 2 is believed to be nonspecific amplification of both Sry4A and 4 based on primer mispriming around 400 base pairs upstream where JF8L should bind. **B).** Digestion of each band with AflIII. Bands 1 and 3 when digested with *AflIII* did not digest since there is no *AflIII* site found in the DNA fragments produced when amplifying either Sry3BII or Sry4A. Band 4 partially digested, suggesting further proof that it is Sry4 which contains an *AflIII* site. Band two showed similar digestion pattern as seen in band 4.

```

Med14Y  AGCCAGGCTGTGCGCCGCAATGGCTCCAGTGCAGCTGGACAACCACCAGCTCATCCCTCC
Med14X  -----ATGGCCCCAGTGCAGCTGGAGAACCACCAGCTGATCCCTCC
          *****

Med14Y  TGGCCGCGGAGGTGGCAGAGGTGGCGGCGAAGGCAGCAGCAGGGACTTGGTGTCTGCCCC
Med14X  TGGCGGCGGAGGTGGCAGCGGTGGCGGTGGAGGCAGCAGCAGCGGCTCTGCGTCTGTCCC
          **** *****

Med14Y  GGCTCCCCATCGCCCGGAGCCAT---GGCGGTGGCAGCAGCCTCCGCGACTACCCCTGG
Med14X  GGCTCCCCCGCTCTTGAGCCGCTGTGGCGGCGGCAGCTGCGGCTGCTGCTAGCCCTGG
          *****

Med14Y  ATACCGGCTTAGCACACTCATCGAATTTTGTGTCACAGGGCCTACTCAGAGCTTATGGT
Med14X  ATACCGGCTTAGCACGCTCATCGAATTTCTGCTGCACCGGGCCTACTCCGAGCTTATGGT
          *****

Med14Y  GTTGACGGACTTCTTGCCAAGGAAATCTGATGTGGAAGGAAAATAGAAATTGTACAGTT
Med14X  GTTGACGGACTTATTGCCAAGAAAATCGGATGTGGAAGGAAAATAGAAATTGTACAGTT
          *****

Med14Y  TGCTAGCCGGACACGTCAACTCTTCGTTTCGTTATTAGCTTTAGTAAAATGGGCCAGTGA
Med14X  TGCTAGCCGGACACGTCAACTCTTCGTTTCGATTATTAGCTTTAGTAAAATGGGCCAATGA
          *****

Med14Y  TGCTGGCAAGGTAGAAAAGTGTGCGATGATCACAAGCTTTTATAGATCATCAAGCCATCTT
Med14X  TGCTGGCAAGGTAGAAAAGTGTGCGATGATCTCGAGCTTTTATAGACCAGCAAGCCATCTT
          *****

Med14Y  ATTTGTGGATACCGCTGACCGCTTGGCCTCCTTAGCTAGAGATGCCCTGGTCCATGCCTG
Med14X  ATTTGTGGATACTGCTGACCGCTTGGCCTCCTTAGCTAGAGATGCCCTGGTCCATGCACG
          *****

Med14Y  CCTGCCTAGTTTTGCTATCCCATATGCCATTGATGTACTGACTACTGGCTCTTATCCAAG
Med14X  CCTGCCTAGTTTTGCTATCCCATATGCCATTGATGTACTGACTACTGGTCTTATCCACG
          *****

Med14Y  GCTTTCACCTGCATCAGGACAAAAATTATTCCTCCAGACCCTATTACCAAGATTGAGAA
Med14X  ACTTCCACCTGCATCAGAGACAAAAATTATTCCTCCAGACCCAATTACCAAAATTGAGAA
          *****

Med14Y  ACAAGCCGCACTTCATCAGCTTAATCAGATTCTTAGACATAGGCTTGTAACAGCAGATCT
Med14X  ACAAGCCACACTTCATCAGCTTAATCAGATTCTTAGACATAGGCTTGTAACGACAGATCT
          *****

Med14Y  TCCTCCACAGCTAGCAAATCTTACAGTGGCGAATGGCCGTGTGAAGTTTCGAGTTGAAAG
Med14X  TCCTCCACAGCTAGCAAATCTTACAGTGGCAAATGGCCGTGTGAAGTTTCGAGTTGAAAG
          *****

Med14Y  AGAATTGAAGCAACCTTGACAGTGATGGGTGATGACCCAAAAGTCCCATGGTGTCTTCT
Med14X  AGAATTGAAGCAACCTTGACGGTGATGGGTGATGACCCAGAAGTCCCATGGCGTCTTCT
          *****

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**Figure S8 Med14Y.** Alignment of the DNA coded on the SHR Y-chromosome (Med14Y) to the mRNA of Med14 on the X-chromosome (NCBI Gene ID: 317343). Sequence of the multiple exons are highlighted in rotating yellow and red to allow for their identification.



```

Med14Y CAAGCTAGAAATTCTAGTTGAGGATAAGCAAACGGGAGATGGGCGAGCTTTGGTTCATAG
Med14X CAAGCTAGAAATTCTAGTTGAGGATAAGGAAACAGGAGATGGGCGAGCTTTGGTTCATAG
*****

Med14Y CATGCAAATTGACTTTATCCATCAGCTAGTCCAGTCCAGGCTCTTTGCTGATGAGAAACC
Med14X CATGCAAATTGACTTTATCCATCAGCTGGTCCAGTCTAGGCTCTTTGCTGATGAGAAACC
*****

Med14Y TCTTCAGGACATGTACAACCTGCCTGCATTTTTCTGCTTATCGCTTCAATTAGAAGTATT
Med14X TCTTCAGGACATGTACAACCTGCCTACATTTGTTTCTGCTTATCACTTCAATTAGAAGTCTT
*****

Med14Y ACATTTCCAGACGCTAATGTTAATCAGAGAGAGGTGGGAGACCTTGACAGGTGGAAGG
Med14X ACATTTCCAGACACTAATGTTAATCCGAGAGAGGTGGGAGACCTTGACAGGTGGAAGG
*****

Med14Y ATACCGTGCTGGAAAGTGCCTC-----TCGGTTTGGAGCCAACAGGTTCTAGGGAAAAA
Med14X ATACCGTGCTGGAAAGTGCCTCTCCCTCTCAGTTTGGAAATCAACAGGTTCTAGGGAGAAA
*****

Med14Y AACAGGCACAGCATCTGTTTACAAAGTTACAATTAATAATCGATGAGAATGATGTCCTCAA
Med14X AACAGGCACAGCATCTGTTTACAAAGTTACAATTAATAATCGATGAGAATGATGTCCTCAA
*****

Med14Y GCCTTTACAGATTTTCCACGATCCTCCTTTACCAGCTTCTGATTCTAAATTAGTAGAAAAG
Med14X GCCTTTACAGATTTTTCATGATCCTCCTTTGCCAGCTTCTGATTCTAAATTAGTAGAAAAG
*****

Med14Y AGCCATGAAGATTGACCACTTATCAATAGAAAAGCTCCTGATTGACAGTGACACTCAGC
Med14X AGCCATGAAGATTGACCACTTATCAATAGAAAAGCTCCTGATTGACAGTGACACGCTAG
*****

Med14Y AGCCCACCAGAAGCTGCAGGAAGTGAAGGCCATTCTTAGAAGCTTCAATGCTAATGAAAA
Med14X AGCCCACCAGAAGCTGCAGGAAGTGAAGGCCATTCTTAGAAGCTTCAATGCCAACGAAAA
*****

Med14Y CTCTTCCATAGAGACTGCACTTCCAGCTCTTATTGTACCCATCTTGGAGCCCTGTGGTAA
Med14X CTCTTCCATAGAGACTGCACTTCCAGCTCTTATTGTGCCATCTTGGAGCCCTGTGGTAA
*****

Med14Y TTCGGAGTGCCTGCACATTTTTGTAGATTTGCATTCTGGAATGTTCCAATTGATGCTTTA
Med14X TTCGGAGTGCCTACACATTTTTGTAGATTTGCATTCTGGAATGTTCCAATTGATGCTTTA
*****

Med14Y TGGACTTGACACAGGCCACTCTGGAGGACATGGAAAAATCGCTGAATGATGACATGAAGCG
Med14X TGGACTTGATCAGGCCACTCTGGAGGACATGGAAAAGTCTTTGAATGATGACATGAAGCG
*****

Med14Y GATCATCCCTTGGATTCAACAACCTTAAATTTTTGGCTGGGACAACAGCGTTGCAAACAATC
Med14X GATCATCCCTTGGATTCAACAACCTTAAATTTTTGGCTGGGACAACAGCGTTGCAAACAATC
*****

Med14Y TATAAAACATCTGCCTGCAATAACCACTGAAACGTTGCAGCTCGCCAATATTCAACACA
Med14X TATAAAACATCTGCCTACCATAACCACTGAAACGTTGCAGCTTGCCCAATATTCAACACA
*****

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**Figure S8 Med14Y.** Alignment of the DNA coded on the SHR Y-chromosome (Med14Y) to the mRNA of Med14 on the X-chromosome (NCBI Gene ID: 317343). Sequence of the multiple exons are highlighted in rotating yellow and red to allow for their identification.

Med14Y TCCCATTGGTAGGCTTTCTAAGAACAAGTTGTTTATTAAGTACTCGCCTTCCGCAGTA  
Med14X TCCCATTGGAAGCCTGTCTAAGAATAAGCTGTTTATTAAGTACTCGCCTTCCACAGTA  
\*\*\*\*\*.\* \*\* \* \*\*\*\*\* \*\* \*\*\*\*\*

Med14Y CTACATTGTTGTGGAAATGCTTGAGGTTCTTAATAAGCCACCCAGCTATCATAACAAGTA  
Med14X CTACATTGTTGTGGAAATGCTTGAGGTTCTTAATAAGCCACACAGCTATCGTACAAGTA  
\*\*\*\*\*.\*\*\*\*\*.\*\*\*\*\*

Med14Y TTACTTTATGTCTGTGAGTACTGCAGATGGCGAGAACACACCTGTCATGGCACTGCTGCT  
Med14X TTACTTTATGTCTGTGAGTACTGCAGATCGTGACGACAGCCCTGCCATGGCACTGCTGCT  
\*\*\*\*\* \*\* \* \*\* .\*\*\* .\*\*\*\* \*\*\*\*\*

Med14Y GCAGCAATTCAGGACAACATCCAGGACTTGATGTCTATACAAAGATTGGAAAACAGAC  
Med14X GCAGCAATTCAGGACAACATCCAGGACTTGATGTCTGTACAAACACTGGGAAACAGAC  
\*\*\*\*\*.\*\*\*\*\* \* \*\*\*.\*\*\*\*\*

Med14Y CATAATTGGTACCAAGCACAACTTATCTGATGACCCATGTCCTGTAGAATGCAAGAAAGC  
Med14X AAGAACTGGTACCAAGCACAAAGTTGTCTGATGACTCGTGCCCGGTAGAATGTAAGAAAGC  
.\* \*\* \*\*\*\*\* \*\* .\*\*\*\*\* \*.\* \*\* \*\*\*\*\* \*\*\*\*\*

Med14Y CAAACAATCAGGAGAAATGTGTGCCTTCAATAAAGTTCTAGCTCACTTGTGCGTATGTG  
Med14X CAAACGATCAGGAGAAACGTGTGCCTTCAATAAAGTTCTAGCTCACTTGTGCGTATGTG  
\*\*\*\*\*.\*\*\*\*\* \*\*\*\*\*

Med14Y TGACACCAACATGCCATTTGTAGGACTTCGATTGGAGTTGTCCAACCTGGAGATACCACA  
Med14X TGATACCAACATGCCATTTGTAGGACTTCGATTGGAGTTGTCCAATTGGAGATACCACA  
\*\*\* \*\*\*\*\* \*\*\*\*\*

Med14Y TCAGGGAGTACAAGTGAAGGTGACGGCTTCAGCTATGCAATTCGCTTACTAAAAATTC  
Med14X TCAGGGAGTGAAGTGAAGGTGATGGCTTCAGCCATGCAATTCGCTTATAAAAATTC  
\*\*\*\*\*.\*\*\*\*\* \*\*\*\*\* \*\*\*\*\* \*\*\*\*\*

Med14Y TCCCTGTAAGGGAATAAGTGAGGAAACGCAGAAGGCTCTGGACTGCTCTTCTTGTGATTG  
Med14X TCCCTGTAAGGGAATCAGTGAGGAAACACAAAAGGCCCTGGACCGTCTCTTCTTGTGATTG  
\*\*\*\*\*.\*\*\*\*\*.\*\*\*\*\*.\* \*\* .\*\*\*\*\* \*\*\*\*\* \* \*\*\*\*\*

Med14Y CACTTTCCGATTACAAGGTAGAAATAACCGCACATGGGTGGCAGAGTTAGTGTGTTGCAA  
Med14X CACTTTCCGATTACAAGGTAGAAATAACCGCACATGGGTGGCAGAGTTAGTGTGTTGCAA  
\*\*\*\*\*

Med14Y TTGTCTCTTAATGGCACTTCTATCAGGGAGCAAGGACCATCCAGACATGTTTACCTGAC  
Med14X TTGTCCGCTTAATGGCACTTCTACCAGGGAGCAAGGACCATCCCGACATGTTTACCTGAC  
\*\*\*\*\* \*\*\*\*\* \*\*\*\*\* \*\*\*\*\* \*\*\*\*\*

Med14Y ATATGAAAATCTGTTGTCTGAACCTGTTGGTGGTAGAAGAGTAGTTGAAATGTTTCTTAA  
Med14X ATATGAAAATCTGTTGTCTGAACCTGTTGGCGGTAGAAAAGTAGTTGAAATGTTTCTTAA  
\*\*\*\*\*.\*\*\*\*\* \*\*\*\*\*

Med14Y TGACTGGAGTAGCATTCCCGATTATATGAGTGTGTGTTGGAATTTGCACATTCTCTACC  
Med14X TGACTGGAGTAGCATTGCCCGATTATATGAGTGTGTGTTGGAATTTGCACGTTCTCTACC  
\*\*\*\*\* \*\*\*\*\* \*\*\*\*\*

Med14Y AGAAATACCTGCTCATCTGAATTTTTTCTCAGAAGTTTGTGTTTACAATTATCGAAAAC  
Med14X AGAAATACCTGCTCATCTGAATTTTTTCTCAGAAGTTCTGTGTTTATAATTATCGAAAAC  
\*\*\*\*\*.\* \*\*\*\*\* \*\*\*\*\* \*\*\*\*\*

**Figure S8 Med14Y.** Alignment of the DNA coded on the SHR Y-chromosome (Med14Y) to the mRNA of Med14 on the X-chromosome (NCBI Gene ID: 317343). Sequence of the multiple exons are highlighted in rotating yellow and red to allow for their identification.

```

Med14Y TATCTTGTGTTATGGAACCACCAGAGGAAGCTCAATCAGTATCCAGTGAATTCATCA
Med14X TATCTTGTGTTATGGAACCACCAAGGAAGTTCAATTAGTATCCAGTGAATTCATCA
*****.***** **

Med14Y TCAGAAATTTTACATTTCTTTGGGAACAGTTGGCCCAAACCTCAGGTTGCAGTAATTGTCA
Med14X TCAGAAATTTTACATTTCTTTGGGAACAGTTGGCCCAAACCTCAGGTTGCAGTAACGTCA
*****.*****

Med14Y TATTACCATTCTCCATCACCTTCAAGAAATGTTCAACAAAACACCAAATGTGGTCCAGTT
Med14X CAATACCATTCTCCATCAGCTTCAAGAAATGTTCAACAAAACACCAAATGTGGTTCAGTT
*.*****

Med14Y ATTACAGGTTTTGTTTGATACTCAGGCACCATTAAATGCCATCAACAACTCCCTACTGT
Med14X ATTACAGGTTTTGTTTCGATACTCAAGCACCATTAAATGCCATCAACAACTCCCTACTGT
*****.*****

Med14Y TCCAGTGTCTGGGCTTGACTCAGAGAAGTAACACCGCCTACCAGTGTTCCTCCATATTACC
Med14X CCCAATGTTGGGCTTGACTCAGAGAAGTAACACCGCCTACCAGTGTTCCTCCATATTACC
***.***

Med14Y ACATTCATCCACCCACATCAGATTGGCCTTCAGGAACATGTACTGCATTGATATATACTG
Med14X ACAATCATCCACCCACATCAGACTGGCCTTCAGGAACATGTACTGCATTGACATATACTG
***.*****

Med14Y CCTTAGTCACGGTGTCTGTGGCAGTACGGGACGGTGCCTATAGTCTTTGTGATAACAGCAA
Med14X CCGTAGTCGAGGTGTCTGTGGCAGTACGGGACGGTGCCTATAGTCTTTGTGATAACAGCAA
** *****.*****

Med14Y GTTAGTTGAAGGCTTCTGTCTGCACCAGGATTAAGACTTTCCTGAACATGTTTGTG-
Med14X GTTAGTTGAAGGCTTCTATCCTGCACCAGGATTAAGACTTTCCTGAACATGTTTGTGGA
*****.*****

Med14Y -----ATGCTCAGAAAAGAGCTTTAAAGGAGGATGATTACCCCTCCTTCTCCTAT
Med14X TAGCAATCAAGATGCTCGGAGAAGATCTGTCAATGAGGATGATAACCCCTCCTTCTCCTAT
*****.***

Med14Y AGGAGGAGATAGGACGGACTCTTTAATATCACGACTCCAGCCACCTCAGCAACAGCCATT
Med14X AGGAGGTGACATGATGGACTCTTTAATATCACAGCTCCAGCCACCTCAGCAACAGCCATT
*****.***

Med14Y TCCAAAGCAGCCAGGGATATCGGGCGCTTACCCTCTTACTTCATCTCCTACATCCTATCG
Med14X TCCAAAGCAGCCAGGGACATCAGGCGCTTACCCTCTTACTTCACCCCTGCATCCTATCA
*****.***

Med14Y CAGCCCAGTTAATCAGCCCCCTCTATGATGCACACACAGCCTCCAG-----
Med14X CAGCACAGTTAATCAGTCCCCCTCTATGATGCACACACAGTCTCCAGGAAATCTGCATGC
****.*****

Med14Y -----
Med14X TGCCAGCTCCCCAGTGGGGCTTTGAGAGCCCCATCACCAGCGTCATTTGTTCCAACCTCC

Med14Y -----G
Med14X TCCCCGTCCTCGCATGGAATCTCAATAGGACCAGGGCCAGTTTTGCTAGTCCACATGG
*
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**Figure S8 Med14Y.** Alignment of the DNA coded on the SHR Y-chromosome (Med14Y) to the mRNA of Med14 on the X-chromosome (NCBI Gene ID: 317343). Sequence of the multiple exons are highlighted in rotating yellow and red to allow for their identification.

```

Med14Y AGCCCTTGACTCTAGTTCTCCATACACTCTTATGTCATCAAGTGA-----
Med14X AGCCCTTGACCCTAGTTCTCCATACACTATGGTGTCCACCAAGTGGACGAGCAGGAAACTG
*****
*****

Med14Y ---GGGTCTCCTCCAGTGTGGTGTCCAGACTCTCACCAGCAACCCCTTGCCCTGGAAT
Med14X GCCTGGGTCTCCTCAA-----GTGTCGGGACCCCTCACCAGCAACCCGTTGCCCGGAAT
*****
*****

Med14Y GTCACCAGCTAACCCATCTCTGCATTCAACTGTCCCAGATGTTTTTCATTCCCCTCGAGC
Med14X GTCACCAGCTAACCCATCTCTGCATTCTCTGTCCAGATGTTTCTCATTCCCCTCGAGC
*****
*****

Med14Y TGGCACAA GTTCCCAGACAATGCCAACCAACATGTCTCCACCTCGAAAACCTACCTCAGCG
Med14X AGGCACAA GTTCCCAGACAATGCCAACCAACATGCCTCCACCTCGAAAACCTACCTCAGCG
*****
*****

Med14Y CTCCTGTGCAGCATCTGTACCCACCATTCTCACTCACAGTGCCTTGAACATCTTACTGCT
Med14X CTCCTGGGCAGCATCCATACCCACCATTCTCACTCATAGTGCCTTGAACATCTTACTGCT
*****
*****

Med14Y ACCTTCTCCAATGCCAGGCCTTGTGCCTGGCCTGGCAGGTAGTTACCTTTGTTCCCCACT
Med14X ACCCTCTCCAACGCCAGGCCTTGTGCCTGGCCTGGCAGGTAGTTACCTTTGTTCCCCACT
***
*****

Med14Y TGAGAGATTCTTGGGTCTGTGCATCATGAGACGACACCTTCAAAGAATTATCCAACAGGA
Med14X CGAGAGATTCTTGGCTCTGTGCATCATGAGACGACACCTTCAAAGAATTATCCAACAGGA
*****
*****

Med14Y GACATTGCAGCTGATCAGTTCCAATGAACCTGGTGTAAATCATGTTCAAGACAGATGCCCT
Med14X AACGTTGCAGCTGATCAATTCCAATGAACCTGGTGTAAATCATGTTCAAGACAGATGCCCT
**
*****

Med14Y GAAATGCAGAGTAACCCCTAGTCCCCAAACCAACCAGACCCTTCAGCTAAAAGTGACACC
Med14X GAAGTGCAGAGTAGCCCTTAGTCCCCAAACCAACCAGACCCTTCAGCTAAAAGTGACACC
***
*****

Med14Y TGAAAATGTAGGACAGTGGAAACCTGATGAGCTTCAAGTTTGGAGAAATCTTTGAAAC
Med14X TGAAAATGCAGGACAATGGAACCTGATGAGCTTCAAGTTTGGAGAAATCTTTGAAAC
*****
*****

Med14Y AAGAGTTGCAGGACCACCATTTAAAGCCAACACACTTATAGCCTTCACCAAGCTGTTGGG
Med14X AAGAGTTGCAGGACCACCGTTTAAAGCCAACACACTTATAGCCTTCACCAAGCTGTTGGG
****
*****

Med14Y AGCGCCAACACACATCCTCCGGGACTGTGTGCATATAATGCAGTTGGAGGTGTTCCAGA
Med14X AGCACCACACACATCCTCCGGGACTGTGTGCATATCATGAAGTTGGAGCTGTTCCAGA
***
*****

Med14Y CCAAGCAACCCAGCTGAAATGGAACGTTCAATTTTGCCCTCACCATCCCTCCTAGTGCACC
Med14X CCAAGCAACGCAGCTAAAGTGGAAATGTTCAATTTTGCCCTCACCATCCCTCCCAGCGGCC
*****
*****

Med14Y ACCAATTGCAGCTCCTGGGACACCAGCCGTGGTCCTGAAATCCAAAATGCTGTTTTTTCT
Med14X ACCCATGCACCTCCTGGGACACCAGCTGTGGTCCTGAAATCCAAAATGCTCTTTTTTTCT
***
*****

```

**Figure S8 Med14Y.** Alignment of the DNA coded on the SHR Y-chromosome (Med14Y) to the mRNA of Med14 on the X-chromosome (NCBI Gene ID: 317343). Sequence of the multiple exons are highlighted in rotating yellow and red to allow for their identification.

```

Med14Y TCAGCTAACCCAGAAAACATCAGTCCCTCCCCAAGAACCTGTTAGTATAATAGTTCCAAT
Med14X TCAACTAACTCAGAAAACATCAGTCCCTCCCCAAGAACCTGTTAGTATAATAGTTCCAAT
***.*****

Med14Y TATTTATGATATGGCTTCAGGTATCACCCAGCAGGCAGACATTCCCAGACAGCAGAACTC
Med14X TATTTATGATATGGCTTCAGGTACCACTCAGCAGGCAGACATTCCCAGACAGCAGAACTC
*****

Med14Y TTCTGTTGCTGCCCCAATGATGGTCAGCACCATTCTGAAGAGGTTTGCAGAGATGAACCC
Med14X TTCTGTTGCTGCGCCAATGATGGTCAGCAACATTCTGAAGAGGTTTGCAGAGATGAACCC
*****

Med14Y TCCACGGCAAGGTGAATGTACAATATTTGCAGCTGTTTGTGATTTAATGGCACATCTTAC
Med14X TCCACGACAAGGTGAATGCACAATATTTGCAGCTGTTCGTGATTTAATGGCTAATCTTAC
*****

Med14Y ACTGCCCCCTGGTGGGTGTCCTAGACCCAATTGATTTTAAACCAGGAAGGCTGACAAAT
Med14X ACTGCCCCCTGGTGGGCGTCCATAGACACTATTGTTTTTAAACCAGGAAGGCTGACAAAT
*****

```

**Figure S8 Med14Y.** Alignment of the DNA coded on the SHR Y-chromosome (Med14Y) to the mRNA of Med14 on the X-chromosome (NCBI Gene ID: 317343). Sequence of the multiple exons are highlighted in rotating yellow and red to allow for their identification.



```

Med14Y -----ALDSSSPYTLMSSSGGS---PPVFVSRSLSPATPLPGMSPANPSLH 1085
Med14X ISIGPGASFASPHGALDPSSPYTMVSPFSGRAGNWPQVSGPSPATRLPGMSPANPSLH 1140
      ***.*****:*.** : . ** ***** *****

Med14Y STVPDVFHSPRAGTSSQTMPTNMSPPRKLQQRSCAASVPTILTHSALNILLPSPMPGLV 1145
Med14X SPVPDVSHSPRAGTSSQTMPTNMPPRKLQQRSCAASVPTILTHSALNILLPSPMPGLV 1200
*.**** *****.***** **:****** *****

Med14Y PGLAGSYLCSPLERFLGSMVIMRRHLQRRIQQETLQLISSNEPGVIMFKTDALKCRVTLSP 1205
Med14X PGLAGSYLCSPLERFLGSMVIMRRHLQRRIQQETLQLINSNEPGVIMFKTDALKCRVALSP 1260
*****.*****.*****

Med14Y QTNQTLQLKVTPEENVGQWKPDELQVLEKFFETRIVAGPPFKANTLIAFTKLLGAPTHILRD 1265
Med14X KTNQTLQLKVTPEENVGQWKPDELQVLEKFFETRIVAGPPFKANTLIAFTKLLGAPTHILRD 1320
:*****.*****

Med14Y CVHIMQLEVPDQATQLKWNVQFCLTIPPSAPPIAAGTPAVVLKSKMLFFLQLTQKTSV 1325
Med14X CVHIMKLELPDQATQLKWNVQFCLTIPPSAPPIAAGTPAVVLKSKMLFFLQLTQKTSV 1380
*****.**:*****.*****

Med14Y PPQEPVSIIVPIIYDMASGITQQADIPRQQNSSVAAPMMVSTILKRFAEMNPPRQGECTI 1385
Med14X PPQEPVSIIVPIIYDMASGITQQADIPRQQNSSVAAPMMVSNILKRFAEMNPPRQGECTI 1440
***** *****.*****

Med14Y FAAVCDLMAHLTLPPGGCP 1404
Med14X FAAVRDLMANLTLPPGGRP 1459
**** ***:***** *

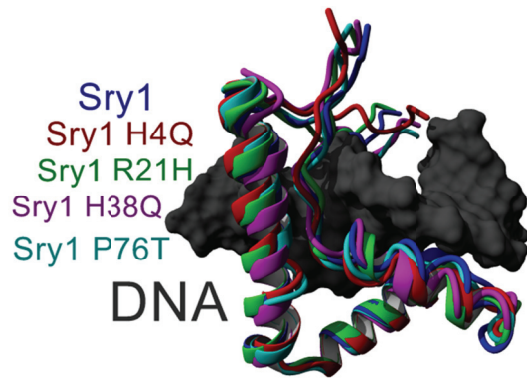
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**Figure S9 Med14Y protein.** Protein sequences coded by the mRNA of figure S8 as determined using ExPASy translate tool aligned using ClustalW. \* indicated conserved amino acids, : functionally conserved, and . slightly conserved.

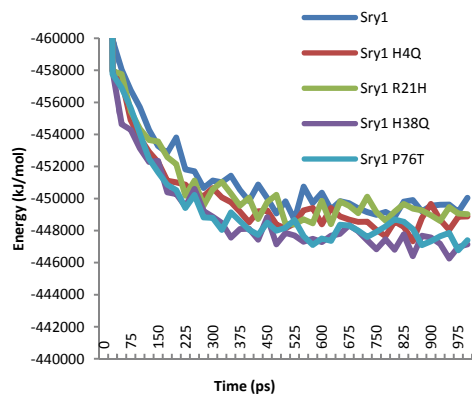




A

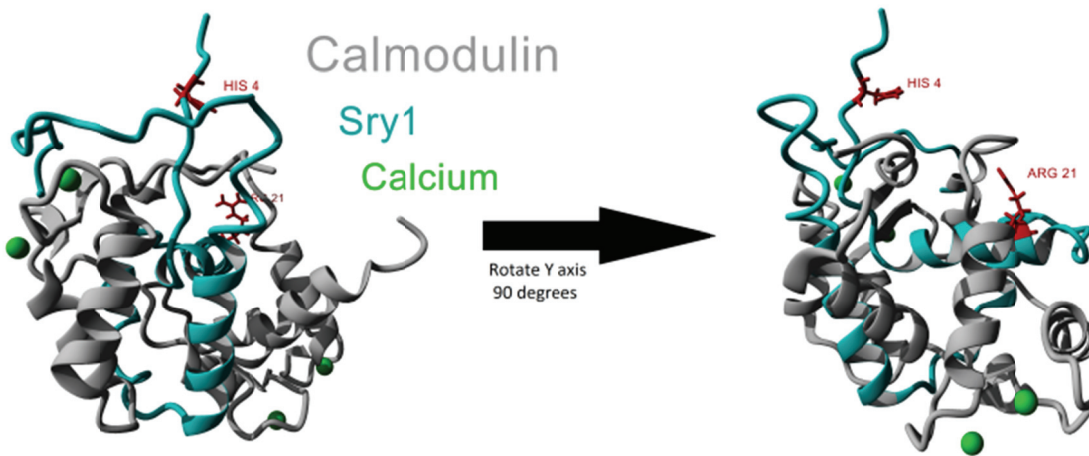


B

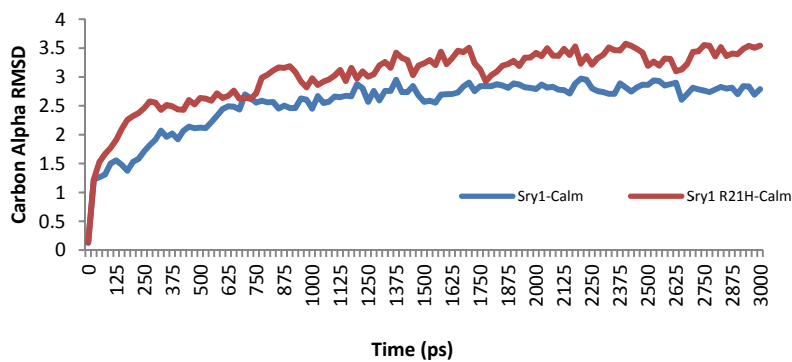


**Figure S11 Mutations to the HMG box of Sry1 based on the multiple Sry copies.** A). Energy minimized structures of the multiple models aligned together on DNA (gray). B). Energy of each model in A over the molecular dynamic simulation.

A



B



**Figure S12 Role amino acid 21 change to interaction with Calmodulin.** A). Modeled interaction between Sry and Calmodulin showing the location of amino acid 4 and 21 (red) both of which differ between all other rat Sry proteins and Sry $\beta$ . B). Molecular dynamic simulations of the modeled Sry $\alpha$ -Calmodulin interaction (A) and with amino acid 21 changed from an Arg to His leading to a decreased stability of the protein.