Table S1. Maximum likelihood reconstruction of ancestral steroid receptor AncSR1.

New AncSR1 was inferred from alignment of 210 steroid and related receptor sequences
Old AncSR1 was inferred from a smaller dataet in Thornton et al, Science 2003
Ligand contacting sites are in red or green; residues that distinguish estrogens from other steroids are in green.
A dot in sequence indicates state identical to that in new AncSR1
Alt state is the state with the second highest posterior probability

Contact ligand?	Site #	New AncSR1 ML state	Probability	homoERa	Old SR1 ML state	homoERb	state in newSR1= homoERa ?	state in newSR1= homoERb ?	state in newSR1 = homoERa or b?	state in oldSR1= homoERa ?	state in oldSR1= homoERb ?	state in oldSR1 matches homoERa or b?	notes
	1	**E**	0.999	.	.	.	1	1	1	1	1	1	
	2	**P**	1	.	.	.	1	1	1	1	1	1	
	3	**P**	0.558	.	E	.	1	1	1	0	0	0	
	4	**N**	0.257	I	T	H	0	0	0	0	0	0	
	5	**L**	0.393	.	V	V	1	0	1	0	1	1	
	6	**Y**	0.69	.	.	L	1	0	1	1	0	1	
	7	**S**	0.663	.	A	I	1	0	1	0	0	0	
	8	**H**	0.209	E	S	S	0	0	0	0	1	1	
	9	**H**	0.951	Y	.	-	0	0	0	0	0	0	
	10	**D**	0.983	.	.	R	1	0	1	1	0	1	
	11	**P**	0.938	.	.	.	1	1	1	1	1	1	
	12	**S**	0.328	T	D	.	0	1	1	0	0	0	
	13	**K**	0.2	R	.	A	0	0	0	0	0	0	
	14	**P**	1	.	.	.	1	1	1	1	1	1	
	15	**P**	0.206	F	D	F	0	0	0	0	0	0	
	16	**T**	0.987	S	.	.	0	1	1	0	1	1	
	17	**E**	0.791	.	.	.	1	1	1	1	1	1	
	18	**A**	0.326	.	V	.	1	1	1	0	0	0	
	19	**H**	0.248	S	S	S	0	0	0	1	1	1	
	20	**L**	0.709	M	M	M	0	0	0	1	1	1	
1	21	**M**	0.58	.	L	.	1	1	1	0	0	0	
	22	**T**	0.668	G	.	M	0	0	0	0	0	0	
	23	**S**	0.932	L	T	.	0	1	1	0	0	0	
2	24	**L**	0.995	.	.	.	1	1	1	1	1	1	
	25	**T**	0.96	.	.	.	1	1	1	1	1	1	
	26	**N**	0.519	.	.	K	1	0	1	1	0	1	
3	27	**L**	1	.	.	.	1	1	1	1	1	1	
4	28	**A**	1	.	.	.	1	1	1	1	1	1	
	29	**D**	0.999	.	.	.	1	1	1	1	1	1	
	30	**R**	0.944	.	.	K	1	0	1	1	0	1	
5	31	**E**	0.999	.	.	.	1	1	1	1	1	1	
	32	**L**	0.995	.	.	.	1	1	1	1	1	1	
	33	**V**	0.99	.	.	.	1	1	1	1	1	1	
	34	**H**	0.381	.	.	.	1	1	1	1	1	1	
	35	**I**	0.658	M	.	M	0	0	0	0	0	0	
	36	**I**	0.92	.	.	.	1	1	1	1	1	1	
	37	**N**	0.384	.	.	S	1	0	1	1	0	1	
	38	**W**	1	.	.	.	1	1	1	1	1	1	
	39	**A**	1	.	.	.	1	1	1	1	1	1	
	40	**K**	1	.	.	.	1	1	1	1	1	1	
	41	**K**	0.419	R	.	.	0	1	1	0	1	1	
	42	**I**	0.899	V	.	.	0	1	1	0	1	1	
	43	**P**	1	.	.	.	1	1	1	1	1	1	
	44	**G**	1	.	.	.	1	1	1	1	1	1	
	45	**F**	0.682	.	.	.	1	1	1	1	1	1	
	46	**S**	0.869	V	T	V	0	0	0	0	0	0	
	47	**D**	0.452	.	.	E	1	0	1	1	0	1	
	48	**L**	1	.	.	.	1	1	1	1	1	1	
	49	**S**	0.856	T	.	.	0	1	1	0	1	1	
	50	**L**	0.996	.	.	.	1	1	1	1	1	1	
	51	**N**	0.538	H	G	F	0	0	0	0	0	0	
	52	**D**	1	.	.	.	1	1	1	1	1	1	
	53	**Q**	1	.	.	.	1	1	1	1	1	1	
	54	**V**	0.959	.	M	.	1	1	1	0	0	0	
	55	**H**	0.58	.	.	R	1	0	1	1	0	1	
	56	**L**	1	.	.	.	1	1	1	1	1	1	
	57	**L**	0.954	.	.	.	1	1	1	1	1	1	
	58	**Q**	0.848	E	.	E	0	0	0	0	0	0	
	59	**S**	0.802	C	C	.	0	1	1	1	0	1	
	60	**C**	0.794	A	.	.	0	1	1	0	1	1	
	61	**W**	1	.	.	.	1	1	1	1	1	1	
6	62	**M**	0.933	L	.	.	0	1	1	0	1	1	
	63	**E**	0.98	.	.	.	1	1	1	1	1	1	
	64	**L**	0.762	I	I	V	0	0	0	1	0	1	
7	65	**L**	1	.	.	.	1	1	1	1	1	1	
8	66	**I**	0.809	M	.	M	0	0	0	0	0	0	I in CcapER, lampreyER, haq
	67	**L**	0.82	I	.	M	0	0	0	0	0	0	
	68	**G**	0.999	.	.	.	1	1	1	1	1	1	
9	69	**L**	0.984	.	.	.	1	1	1	1	1	1	
	70	**A**	0.485	V	V	M	0	0	0	1	0	1	
	71	**W**	1	.	.	.	1	1	1	1	1	1	
10	72	**R**	1	.	.	.	1	1	1	1	1	1	
	73	**S**	1	.	.	.	1	1	1	1	1	1	
	74	**M**	0.553	.	I	I	1	0	1	0	1	1	
	75	**D**	0.393	E	.	.	0	1	1	0	1	1	
	76	**H**	0.984	.	.	.	1	1	1	1	1	1	
	77	**K**	0.272	P	G	P	0	0	0	0	0	0	
	78	**G**	0.965	.	E	.	1	1	1	0	0	0	
	79	**-**	0.118	-	.	-							
	80	**-**	0.089	-	-	-							
	81	**K**	0.715	.	.	.	1	1	1	1	1	1	
	82	**-**	0.498	-	T	-							
	83	**L**	0.999	.	.	.	1	1	1	1	1	1	
	84	**V**	0.541	L	I	I	0	0	0	0	1	1	
11	85	**F**	1	.	.	.	1	1	1	1	1	1	
	86	**A**	1	.	.	.	1	1	1	1	1	1	
	87	**P**	0.998	.	.	.	1	1	1	1	1	1	
	88	**D**	0.998	N	.	.	0	1	1	0	1	1	
	89	**L**	0.997	.	.	.	1	1	1	1	1	1	
	90	**I**	0.693	L	.	V	0	0	0	0	0	0	
	91	**L**	0.776	.	.	.	1	1	1	1	1	1	
	92	**D**	0.997	.	.	.	1	1	1	1	1	1	
	93	**-**		-	-	-							
	94	**-**		-	-	-							
	95	**-**		-	-	-							
	96	**-**		-	-	-							
	97	**-**		-	-	-							

	98	**-**		-	-	-							
	99	**R**	0.884	.	.	.	1	1	1	1	1	1	
	100	**D**	0.428	N	E	.	0	1	1	0	0	0	
	101	**Q**	0.469	.	.	E	1	0	1	1	0	1	
	102	**S**	0.649	G	G	G	0	0	0	1	1	1	
	103	**K**	0.551	.	R	.	1	1	1	0	0	0	
	104	**C**	0.719	.	.	.	1	1	1	1	1	1	
	105	**V**	0.609	.	.	.	1	1	1	1	1	1	
	106	**A**	0.931	E	.	E	0	0	0	0	0	0	
	107	**G**	1	.	.	.	1	1	1	1	1	1	
	108	**-**		-	-	-							
	109	**-**		-	-	-							
	110	**-**		-	-	-							
	111	**-**		-	-	-							
12	112	**M**	0.905	.	.	I	1	0	1	1	0	1	
	113	**L**	0.179	V	.	.	0	1	1	0	1	1	
	114	**E**	0.756	.	.	.	1	1	1	1	1	1	
13	115	**I**	0.968	.	L	.	1	1	1	0	0	0	
14	116	**F**	0.53	.	.	.	1	1	1	1	1	1	
	117	**D**	0.498	.	.	.	1	1	1	1	1	1	
	118	**Q**	0.736	M	.	M	0	0	0	0	0	0	
15	119	**I**	0.925	L	L	L	0	0	0	1	1	1	I in CcapER and PdER
	120	**L**	0.934	.	.	.	1	1	1	1	1	1	
	121	**-**		-	-	-							
	122	**-**		-	-	-							
	123	**-**		-	-	-							
	124	**-**		-	-	-							
	125	**-**		-	-	-							
	126	**-**		-	-	-							
	127	**-**		-	-	-							
	128	**-**		-	-	-							
	129	**-**		-	-	-							
	130	**Q**	0.265	A	.	A	0	0	0	0	0	0	
	131	**I**	0.574	T	.	T	0	0	0	0	0	0	
	132	**A**	0.769	S	V	T	0	0	0	0	0	0	
	133	**Q**	0.371	S	R	S	0	0	0	0	0	0	
	134	**Q**	0.532	R	R	R	0	0	0	1	1	1	
	135	**F**	0.997	.	.	.	1	1	1	1	1	1	
	136	**R**	0.472	.	.	.	1	1	1	1	1	1	
	137	**E**	0.664	M	.	.	0	1	1	0	1	1	
	138	**L**	0.993	M	.	.	0	1	1	0	1	1	
	139	**Q**	0.623	N	K	K	0	0	0	0	1	1	
	140	**V**	0.662	L	L	L	0	0	0	1	1	1	
	141	**Q**	0.534	.	E	.	1	1	1	0	0	0	
	142	**K**	0.506	G	R	H	0	0	0	0	0	0	
	143	**E**	0.96	.	.	K	1	0	1	1	0	1	
	144	**E**	1	.	.	.	1	1	1	1	1	1	
	145	**F**	0.698	.	.	Y	1	0	1	1	0	1	
	146	**V**	0.948	.	.	L	1	0	1	1	0	1	
	147	**C**	0.999	.	.	.	1	1	1	1	1	1	
	148	**L**	1	.	.	V	1	0	1	1	0	1	
	149	**K**	0.998	.	.	.	1	1	1	1	1	1	
	150	**A**	0.999	S	.	.	0	1	1	0	1	1	
	151	**I**	0.641	.	L	M	1	0	1	0	0	0	
	152	**T**	0.343	I	V	I	0	0	0	0	0	0	
	153	**L**	1	.	.	.	1	1	1	1	1	1	
	154	**L**	0.799	.	.	.	1	1	1	1	1	1	
	155	**N**	0.997	.	.	.	1	1	1	1	1	1	
	156	**A**	0.75	S	S	S	0	0	0	1	1	1	
	157	**S**	0.342	G	D	.	0	1	1	0	0	0	
	158	**V**	0.203	.	.	M	1	0	1	1	0	1	
	159	**-**		-	-	-							
	160	**-**		-	-	-							
	161	**F**	0.331	Y	L	Y	0	0	0	0	0	0	
	162	**T**	0.297	.	P	-	1	0	1	0	0	0	
	163	**S**	0.203	F	F	P	0	0	0	1	0	1	
	164	**L**	0.271	.	.	.	1	1	1	1	1	1	
	165	**S**	0.418	.	.	V	1	0	1	1	0	1	
	166	**A**	0.328	S	D	T	0	0	0	0	0	0	
	167	**D**	0.307	T	T	A	0	0	0	1	0	1	
	168	**A**	0.399	L	L	T	0	0	0	1	0	1	
	169	**E**	0.514	K	.	Q	0	0	0	0	0	0	
	170	**R**	0.509	S	E	D	0	0	0	0	0	0	
	171	**L**	0.975	.	.	A	1	0	1	1	0	1	
	172	**E**	0.967	.	.	D	1	0	1	1	0	1	
	173	**N**	0.392	E	.	S	0	0	0	0	0	0	
	174	**H**	0.507	K	L	S	0	0	0	0	0	0	
	175	**D**	0.24	.	E	R	1	0	1	0	0	0	
	176	**K**	0.437	H	L	.	0	1	1	0	0	0	
	177	**V**	0.879	I	I	L	0	0	0	1	0	1	
	178	**Q**	0.872	H	.	A	0	0	0	0	0	0	
	179	**K**	0.323	R	.	H	0	0	0	0	0	0	
	180	**L**	0.932	V	M	.	0	1	1	0	0	0	
	181	**Q**	0.998	L	R	L	0	0	0	0	0	0	
	182	**D**	0.556	.	.	N	1	0	1	1	0	1	
	183	**A**	0.202	K	K	.	0	1	1	1	0	1	
	184	**I**	0.682	.	.	V	1	0	1	1	0	1	
	185	**H**	0.476	T	T	T	0	0	0	1	1	1	
	186	**D**	0.815	.	.	.	1	1	1	1	1	1	
	187	**A**	1	T	.	.	0	1	1	0	1	1	
	188	**L**	1	.	.	.	1	1	1	1	1	1	
	189	**V**	0.389	I	.	.	0	1	1	0	1	1	
	190	**D**	0.711	H	.	W	0	0	0	0	0	0	
	191	**T**	0.318	L	Y	V	0	0	0	0	0	0	
	192	**V**	0.363	M	I	I	0	0	0	0	1	1	
	193	**A**	0.413	.	.	.	1	1	1	1	1	1	
	194	**K**	0.542	.	G	.	1	1	1	0	0	0	
	195	**S**	0.185	A	Q	.	0	1	1	0	0	0	
	196	**H**	0.786	G	R	G	0	0	0	0	0	0	
	197	**P**	0.565	L	G	I	0	0	0	0	0	0	
	198	**S**	0.297	T	E	.	0	1	1	0	0	0	
	199	**S**	0.33	L	G	.	0	1	1	0	0	0	
	200	**-**		-	S	-							
	201	**P**	0.994	Q	Q	Q	0	0	0	1	1	1	
	202	**Q**	0.476	.	.	.	1	1	1	1	1	1	
	203	**Q**	0.679	.	.	.	1	1	1	1	1	1	
	204	**S**	0.466	H	W	.	0	1	1	0	0	0	

	205	**R**	0.913	Q	.	M	0	0	0	0	0	0
	206	**R**	1	.	.	.	1	1	1	1	1	1
	207	**L**	0.649	.	F	.	1	1	1	0	0	0
	208	**A**	0.903	.	.	.	1	1	1	1	1	1
	209	**K**	0.477	Q	Q	N	0	0	0	1	0	1
	210	**L**	0.979	.	.	.	1	1	1	1	1	1
	211	**L**	0.993	.	.	.	1	1	1	1	1	1
	212	**M**	0.787	L	L	.	0	1	1	1	0	1
	213	**L**	0.831	I	.	.	0	1	1	0	1	1
	214	**L**	0.999	.	.	.	1	1	1	1	1	1
	215	**S**	0.602	.	.	.	1	1	1	1	1	1
	216	**H**	0.908	.	.	.	1	1	1	1	1	1
	217	**I**	0.769	.	.	V	1	0	1	1	0	1
	218	**R**	1	.	.	.	1	1	1	1	1	1
	219	**Q**	0.942	H	.	H	0	0	0	0	0	0
	220	**V**	0.767	M	I	A	0	0	0	0	0	0
	221	**S**	0.946	.	.	.	1	1	1	1	1	1
	222	**T**	0.256	N	N	N	0	0	0	1	1	1
	223	**K**	0.754	.	.	.	1	1	1	1	1	1
16	224	**G**	0.713	.	.	.	1	1	1	1	1	1
	225	**I**	0.662	M	.	M	0	0	0	0	0	0
	226	**E**	0.622	.	.	.	1	1	1	1	1	1
17	227	**H**	0.999	.	.	.	1	1	1	1	1	1
18	228	**L**	0.891	.	F	.	1	1	1	0	0	0
	229	**Y**	0.971	.	.	L	1	0	1	1	0	1
	230	**S**	0.318	.	.	N	1	0	1	1	0	1
	231	**M**	0.574	.	.	.	1	1	1	1	1	1
	232	**K**	0.957	.	.	.	1	1	1	1	1	1
	233	**S**	0.441	C	L	C	0	0	0	0	0	0
	234	**E**	0.851	K	Q	K	0	0	0	0	0	0
	235	**G**	0.701	N	S	N	0	0	0	0	0	0
	236	**-**		-	G	-						
	237	**-**		-	-	-						
	238	**-**		-	-	-						
	239	**V**	0.23	.	.	.	1	1	1	1	1	1
	240	**V**	1	.	.	.	1	1	1	1	1	1
	241	**P**	0.998	.	.	.	1	1	1	1	1	1
	242	**L**	0.695	.	.	V	1	0	1	1	0	1
	243	**H**	0.556	Y	.	Y	0	0	0	0	0	0
	244	**D**	0.929	.	.	.	1	1	1	1	1	1
	245	**L**	1	.	.	.	1	1	1	1	1	1
	246	**L**	0.999	.	.	.	1	1	1	1	1	1
	247	**L**	0.77	.	.	.	1	1	1	1	1	1
	248	**E**	1	.	.	.	1	1	1	1	1	1
	249	**M**	0.995	.	.	.	1	1	1	1	1	1
	250	**L**	0.997	.	.	.	1	1	1	1	1	1
	251	**D**	0.926	.	.	N	1	0	1	1	0	1
	average or total		0.729080717				0.59	0.58	160	0.61	0.55	156

Table S2. Reconstruction of ancestral function from data on extant receptors by parsimony.

The ligand regulation of various extant receptors was coded as follows: E, estrogen-activated; S, activation by other steroids; C, constitutive activity; R, transcriptionally inactive repressor. These states can be represented in the following matrix:

Vertebrate ERs	E
Branchiostoma ER	R
Mollusk ERs	C
Annelid ERs	E
Vertebrate SRs	E
Branchiostoma SR	S

The rescaled consistency index (rci) is a measure of how parsimoniously a phylogenetic hypothesis explains the distribution of character states as due to common descent. It ranges from 0 (the least parsimonious possible history) to 1 (the most parsimonious). The rci is defined as

$$rci = ci * ri$$

where ci is the consistency index and ri the retention index. The ci is defined as min/s, where min is the minimum number of character changes on the most parsimonious phylogeny possible, and s is the actual number required on a given phylogeny. The ri is defined as ri = (max – s) / (max – min), where max is the maximum number of steps required on the least parsimonious possible phylogeny.

The minimum number of possible changes for this character on any phylogeny is 3 (on a phylogeny that groups all Es together), and the maximum is 5 (on an unresolved phylogeny).

If AncSR1 is estrogen-activated (E), the character requires 3 changes of state – gain of constitutive activity in the mollusk ER, loss of activity to R in the Branciostoma ER, and modification of ligand sensitivity from estrogens to 3-ketosteroids in the vertebrate SRs. This scenario is shown in Fig. 1C in the main article.

If AncSR1 is constitutive (C) on the same phylogeny, the same character requires 5 changes of state.

Comparing the two hypotheses using these metrics:

Metric	E in AncSR1	C in AncSR1
Steps	3	5
Ci	1.0	0.6
Ri	1.0	0.0
Rci	1.0	0.0

For more information on the rci, see Farris JS (1989) The retention index and the rescaled consistency index. *Cladistics* 5:417–419.

Table S3. Accession numbers and species names for sequences used in phylogenetic analyses.

sequence name	species name	accession number
eelAR	Anguilla japonica	BAA75464
eelER	Anguilla japonica	BAA19851
eelPR	Anguilla japonica	BAA89539
anolisER	Anolis carolinensis	AAC64412
aplysiaER	Aplysia californica	AY327135
haplochromisAR	Astatotilapia burtoni	AAD25074
BfER	Branchiostoma floridae	EU371730
BfSR	Branchiostoma floridae	EU371729
capitellaER	Capitella capitata	EU497681
goldfishER	Carassius auratus	AAD26921
whiptaillizardERa	Cnemidophorus uniparens	AAB35739
whiptaillizardPR	Cnemidophorus uniparens	AAB35740
quailERb	Coturnix japonica	AAC36463
CragigER	Crassostreas gigas	BAF45381
crocodilePR	Crocodylus siamensi	AAB81722
zebrafishSF1	Danio rerio	AAC60274
fruitflyERR	Drosophila melanogaster	AAF50473
chickER	Gallus gallus	P06212
chickPR	Gallus gallus	P07812
chickSF1	Gallus gallus	BAA76713
humanRXRb	Homo sapiens	P28702
humanAR	Homo sapiens	NP_000035
humanCOUP1	Homo sapiens	NP_005645
humanCOUP2	Homo sapiens	P24468
humanERa	Homo sapiens	P03372
humanERb	Homo sapiens	BAA24953
humanERRa	Homo sapiens	P11474
humanERRb	Homo sapiens	NP_004443
humanERRg	Homo sapiens	NP_001429
humanGR	Homo sapiens	P04150
humanLRH1	Homo sapiens	AAD03155
humanMR	Homo sapiens	P08235
humanPR	Homo sapiens	NP_000917
humanRXRa	Homo sapiens	P19793
humanRXRg	Homo sapiens	P48443
catfishERa	Ictalurus punctatus	AAC69548
skateAR	Leucoraja erinacea	Q1KXY2
skateMR	Leucoraja erinacea	Q1KXY3

skatePR	Leucoraja erinacea	Q1KXY1
skateGR	Leucoraja erinacea	Q1KXY4
MarcorER	Marisa cornuarietis	ABI97119
croakerERa	Micropogonias undulatus	AAG16713
croakerERb	Micropogonias undulatus	AAG16711
croakerERg	Micropogonias undulatus	AAG16712
mouseAR	Mus musculus	P19091
mouseSF1	Mus musculus	P33242
mouseERa	Mus musculus	P19785
mouseERb	Mus musculus	AAB51132
mouseERR2	Mus musculus	NP_036064
mouseERR3	Mus musculus	AAD48369
mouseERRa	Mus musculus	AAB51250
mouseLRH1	Mus musculus	P45448
mousePR	Mus musculus	Q00175
hagfishSR2	Myxine glutinosa	ABD46743
hagfishCR	Myxine glutinosa	ABD46742
hagfishER	Myxine glutinosa	EU439936
NuclapER	Nucella lapillus	ABQ96884
octopusER	Octopus vulgaris	Q19AB0
troutARa	Oncorhynchus mykiss	BAA32784
troutARb	Oncorhynchus mykiss	BAA32785
troutGR	Oncorhynchus mykiss	P49843
TilapiaER1	Oreochromis niloticus	AAD00245
TilapiaER2	Oreochromis niloticus	AAD00246
BluetilapiaER	Oreochromus aureus	P50240
MedakaER	Oryzias latipes	P50241
sheepERb	Ovis aries	AAD10826
redseabreamAR	Pagrus major	BAA33451
redseabreamER	Pagrus major	O42132
flounderGR	Paralichthys olivaceous	O73673
halibutGR	Paralichthys olivaceus	BAA25997
lampreyER	Petromyzon marinus	AAK20929
lampreyGR	Petromyzon marinus	AAK20930
lampreyPR	Petromyzon marinus	AAK20931
PlatynereisER	Platynereis dumerilii	EU482033
ratERa	Rattus norvegicus	CAA43411
ratERb	Rattus norvegicus	AAC52602
ratMR	Rattus norvegicus	P22199
salmonER	Salmo salar	P50242
canaryAR	Serinus canaria	AAA17402

gildedseabreamERa	Sparus aurata	AAD31032
gildedseabreamERB	Sparus aurata	AAD31033
squalusER	Squalus acanthias	AAK57823
zebrafinchERa	Taeniopygia guttata	AAB81108
thaisER	Thais clavigera	BAC66480
turtleSF1	Trachemys scripta	AAD01975
xenopusAR	Xenopus laevis	AAC97386
xenopusERa	Xenopus laevis	P81559
xenopusGR	Xenopus laevis	P49844
xenopusMR	Xenopus laevis	Q91573
xenopusPR	Xenopus laevis	AAG42362