

Supplementary figure legends

Fig. S1: *Rhox5* Expression in Sertoli Cell Lines. qPCR analysis of total cellular RNA from 15P-1, MSC-1, and Tm4 Sertoli cells. Adult testes and spleen served as positive and negative controls, respectively (1). All values were quantified as described in Fig. 1B. The values denote the mean fold change \pm SEM.

Fig. S2: RHOX5 Protein is Undetectable in Parental 15P-1 Cells. Western blot analysis of RHOX5 protein expression in 15P-1 cells, performed as described in Fig. 1A. SL12.4 cells serves as a positive control.

Fig. S3: *Rhox5*-Regulated Genes in 15P-1 Cells. Classification of proteins encoded by genes regulated by 2-fold or more in response to *Rhox5* (from Supplementary table 1 and 2), determined using OntoExpress software.

Fig. S4: *Rhox5*-Upregulated Genes in 15P-1 Cells. Classification of proteins encoded by genes upregulated by 2-fold or more in response to *Rhox5* (from Supplementary table 1), determined using OntoExpress software.

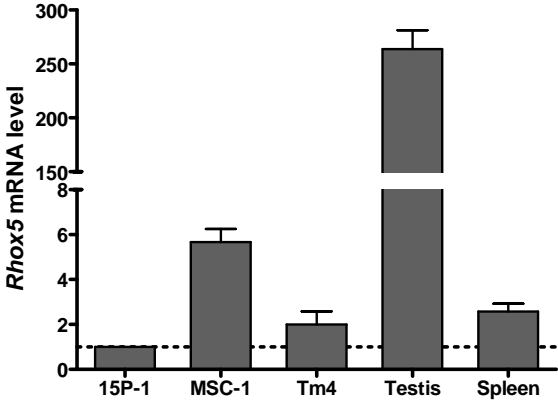
Fig. S5: *Rhox5*-Downregulated Genes in 15P-1 Cells. Classification of proteins encoded by genes downregulated by 2-fold or more in response to *Rhox5* (from Supplementary table 1), determined using OntoExpress software.

Fig. S6: Developmental Expression Pattern of *Rhox5*-Regulated Genes. qPCR analysis of testes RNA from *Rhox5*-null (KO) and wild-type (WT) littermate mice of the indicated postnatal ages (6 mice per time point), quantified and normalized as described in Fig. 5. The genes shown in this figure did not exhibit an appreciable change in developmental expression pattern in *Rhox5*-null mice relative to control mice.

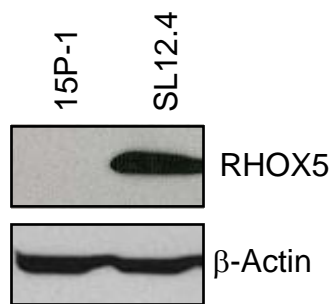
Fig. S7. The Candidate SAR Genes do Not Respond to AR and Androgen *In Vitro*. qPCR analysis of total cellular RNA from 15P-1 cells in the presence or absence of AR and androgen, quantified and normalized as described in Fig. 1B. “+AR” denotes cells cotransfected with an AR expression vector (100 ng) and “+T” denotes cells incubated with the synthetic androgen R1881.

1. MacLean JA, Chen MA, Wayne CM, Bruce SR, Rao MK, Meistrich ML, MacLeod CL, Wilkinson MF 2005 *Rhox*: A new homeobox gene cluster. *Cell* 120:369-382

Supplemental Figure 1



Supplemental Figure 2



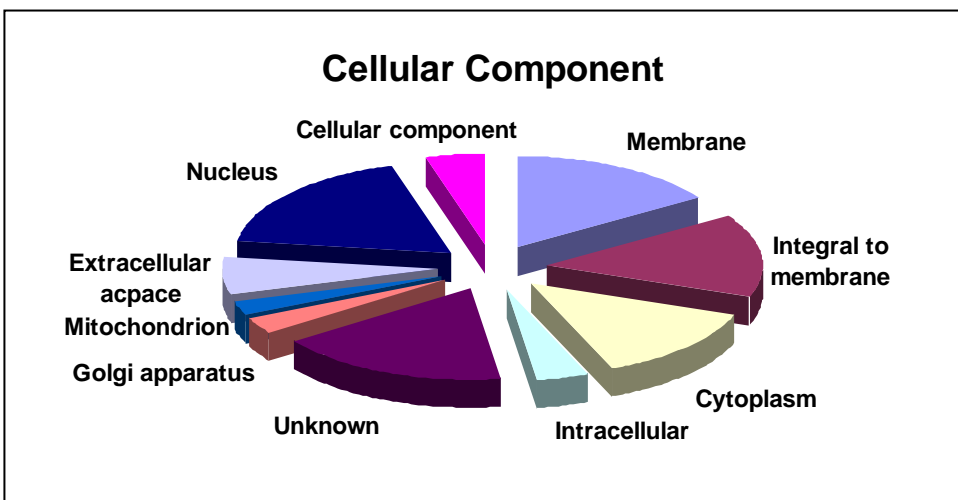
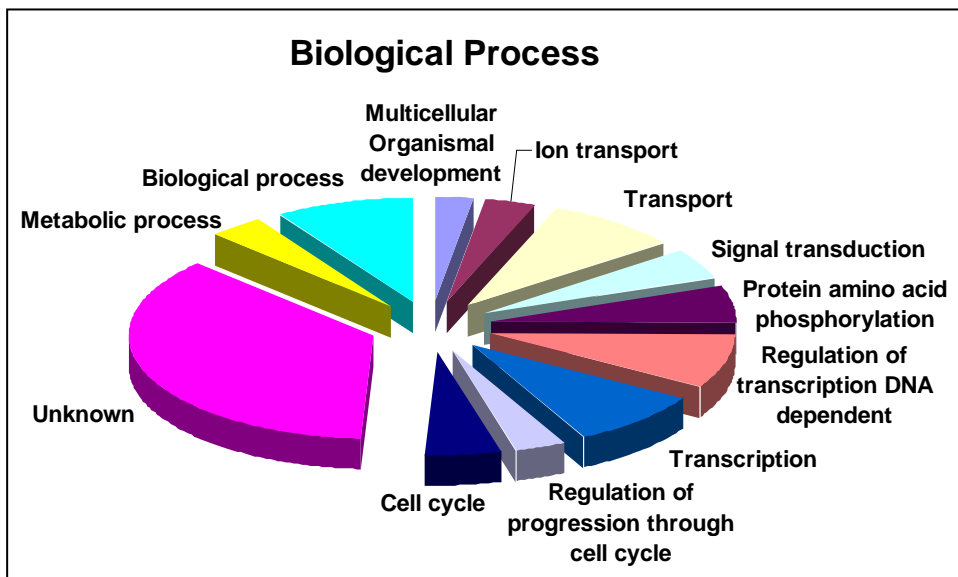
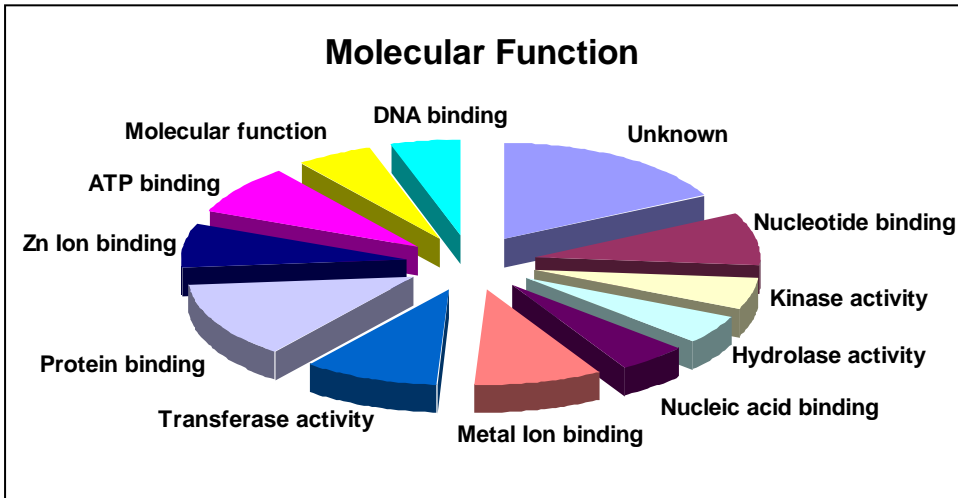
Supplemental Table 1. Transcripts downregulated by *Rhox5* (p<0.05)

Probe set ID	fold	Gene symbol	Description
1455622 at	77.37	ESTs	Weakly similar to endoglycan; podocalyxin-like 2 (Homo sapiens) (H.sapiens)
1438799 at	28.01	ESTs	ESTs
1438870 at	24.61	/	expressed sequence A1536462
1437153 at	18.74	/	expressed sequence C85069
1438248 at	15.7	Pcsk5	proprotein convertase subtilisin/kexin type 5
1441168 at	13.86	ESTs	ESTs, Weakly similar to B21124 Bkm-like sex-determining region hypothetical protein CS314 - fruit fly (D.melanogaster)
1443211 at	12.79	ESTs	ESTs
1441184 at	10.98	ESTs	ESTs
1442378 x at	10.95	ESTs	ESTs, Weakly similar to env polyprotein, retrovirus-related (M.musculus)
1429072 at	10.19	/	clone:1110001D15:KunitzBovine pancreatic trypsin inhibitor domain containing protein, full insert sequence.
1439177 at	9.466	ESTs	ESTs
1426188 s at	9.318	/	dexamethasone induced product, complete cds.
1440597 at	8.94	ESTs	ESTs
1439914 at	8.83	ESTs	ESTs
1431220 at	8.778	/	RIKEN cDNA 2810416G20 gene
1448901 at	8.061	Cpxm1	Mus musculus carboxypeptidase X 1 (M14 family) (Cpxm1), mRNA.
1435931 at	8.02	ESTs	ESTs
1458331 x at	7.441	/	Expressed sequence BB081581
1423469 at	5.897	/	Mus musculus adult male testis cDNA, RIKEN full-length enriched library, clone:1700018E14.gb:NM_026461.1
1421692 at	5.274	/	calcium channel, voltage-dependent, R type, alpha 1E subunit
1444288 at	5.025	ESTs	ESTs
1440764 at	4.986	ESTs	ESTs, Weakly similar to S26689 hypothetical protein hc1 - mouse (M.musculus)
1435396 at	4.852	ESTs	expressed sequence C85317
1446290 at	4.822	ESTs	ESTs
1420787 at	4.726	/	RIKEN cDNA 4933436101
1431905 s at	4.6	/	Mus musculus adult male testis cDNA, RIKEN full-length enriched library, clone:4933427G17
1445154 at	4.537	ESTs	ESTs
1425292 at	4.518	Dtna	Mus musculus alpha-dystrobrevin 2a mRNA, complete cds.
1427425 at	4.462	/	Unknown (protein for IMAGE:4237122)
1429210 at	4.328	/	RIKEN cDNA 2810458L13 gene
1427313 at	3.857	Ptgir	prostaglandin I receptor (IP)
1428780 at	3.801	/	RIKEN full-length enriched library, clone:1300017K07
1458053 at	3.735	ESTs	ESTs
1451462 a at	3.521	IFNAR2	Mus musculus type I interferon receptor soluble isoform precursor (IFNAR2) mRNA, complete cds
1449522 at	3.491	Unc5c	Mus musculus unc5 homolog (C. elegans) 3 (Unc5h3), mRNA.
1456341 a at	3.417	Klf9	Kruppel-like factor 9
1435723 at	3.334	ESTs	ESTs
1436763 a at	3.181	Klf9	Kruppel-like factor 9
1419689 at	3.162	Gpc6	Mus musculus glypican 6 (Gpc6), mRNA.
1417963 at	2.878	Pltp	Mus musculus phospholipid transfer protein (Pltp), mRNA.
1445667 at	2.85	ESTs	ESTs, Highly similar to S14234 hypothetical protein
1428802 at	2.817	/	RIKEN cDNA 1110038J12 gene
1426894 s at	2.716	/	Unknown (protein for IMAGE:5009979)
1419359 at	2.704	Clp1	Mus musculus cardiac lineage protein 1 (Clp1), mRNA.
1452939 a at	2.689	/	RIKEN full-length enriched library, clone:1110020B03:homolog to RETINAL DEGENERATION B BETA, full insert sequence.
1437667 a at	2.638	Bach2	BTB and CNC homology 2
1434123 at	2.631	/	expressed sequence A1317183
1453241 a at	2.616	/	RIKEN cDNA 2810047C21 gene
1456689 at	2.582	ESTs	ESTs
1421053 at	2.55	Kif1a	Mus musculus kinesin heavy chain member 1A (Kif1a), mRNA.
1436994 a at	2.5	H1f2	H1 histone family, member 2
1459895 at	2.494	ESTs	ESTs, Weakly similar to A57514 RNA helicase HEL117 - rat (R.norvegicus)
1440831 at	2.442	ESTs	ESTs
1448398 s at	2.438	RPI22	ribosomal protein L22
1437874 s at	2.416	/	Mus musculus, Similar to elongation factor G2,
1441367 a at	2.413	ESTs	ESTs
1437683 x at	2.401	Serf2	small EDRK-rich factor 2
1424694 at	2.397	/	RIKEN cDNA 2010011I20 gene.
1458318 at	2.335	ESTs	ESTs
1441059 at	2.324	ESTs	ESTs
1439060 s at	2.322	/	Mus musculus, Similar to hypothetical protein FLJ10055
1436938 at	2.3	ESTs	ESTs, Weakly similar to RNA binding motif, single stranded interacting protein 1 (Mus musculus)
1450923 at	2.296	Tgfb2	transforming growth factor, beta 2
1429303 at	2.289	/	RIKEN full-length enriched library, clone:7420700M05:Zinc finger, C2H2 type containing protein, full insert sequence.
1458941 at	2.262	ESTs	ESTs
1435998 at	2.25	ESTs	ESTs, Moderately similar to T47153 hypothetical protein DKFZp564A0772.1 (H.sapiens)
1455300 at	2.248	ESTs	ESTs
1426306 a at	2.241	Maged2	Mus musculus mage-d2 mRNA, complete cds
1417879 at	2.239	/	Mus musculus RIKEN cDNA 1110060M21 gene
1451386 at	2.232	/	Unknown (protein for MGC:27866)
1441763 at	2.215	ESTs	ESTs
1423909 at	2.209	/	RIKEN cDNA 0610011I04 gene
1452952 at	2.165	/	RIKEN full-length enriched library, clone:9030418K01
1436085 at	2.159	ESTs	ESTs
1421451 at	2.135	Crb1	Mus musculus crumbs homolog 1 (Drosophila) (Crb1), mRNA.
1431067 at	2.128	/	RIKEN cDNA 6330404A07 gene
1416332 at	2.115	Cirbp	Mus musculus cold inducible RNA binding protein (Cirbp), mRNA.
1418004 a at	2.101	LR8	Mus musculus RIKEN cDNA 1810009M01 gene (1810009M01Rik), mRNA.
1418534 at	2.092	Fzd2	frizzled homolog 2 (Drosophila)
1426624 a at	2.091	/	RIKEN cDNA 0610043B10 gene
1460499 at	2.079	/	Mus musculus adult male epididymis cDNA, RIKEN full-length enriched library, clone:9230110I02
1438127 at	2.079	/	ESTs
1447509 at	2.076	/	ESTs
1456624 at	2.067	/	Mus musculus, Similar to hypothetical protein FLJ10055
1436933 at	2.067	/	ESTs
1421048 a at	2.06	/	Mus musculus cardiac Abnormalityabnormal facies (CATCH22), microdeletion syndrome (Dgl1-pending), mRNA.
1415961 at	2.059	Itm3-pending	Mus musculus integral membrane protein 3 (Itm3-pending), mRNA.
1427139 at	2.057	Adams10	Mus musculus zinc metalloproteinase (Adams10) mRNA, partial cds
1451017 at	2.032	Sdbcag84	serologically defined breast cancer antigen 84
1444406 at	2.018	ESTs	ESTs
1451674 at	2.009	Sic12a5	Mus musculus strain ILS K-CI cotransporter (Sic12a5) mRNA, complete cds

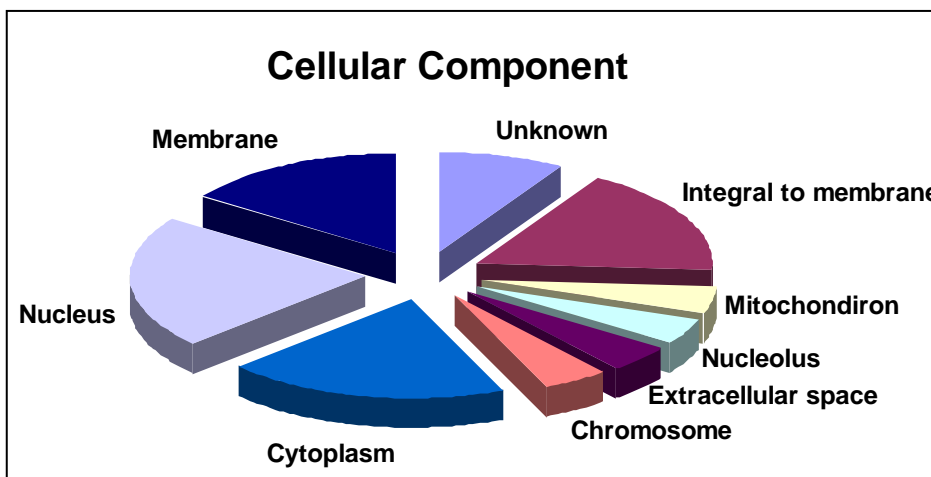
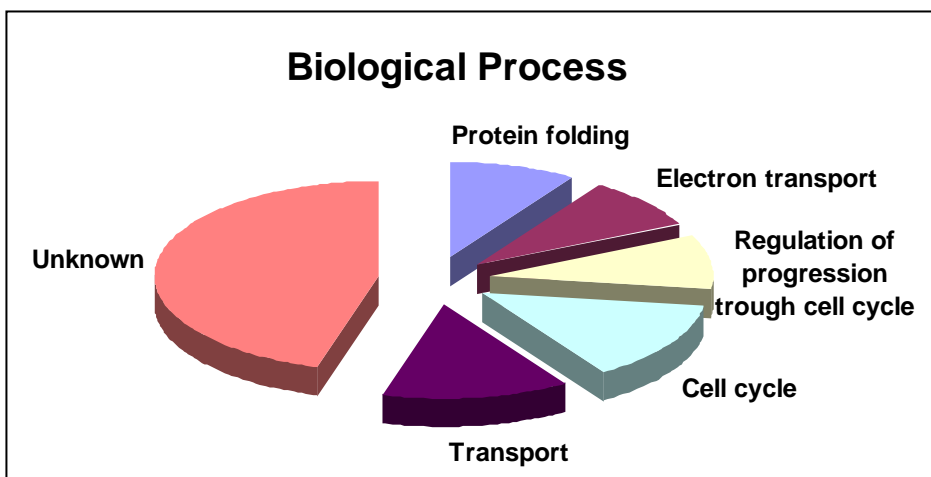
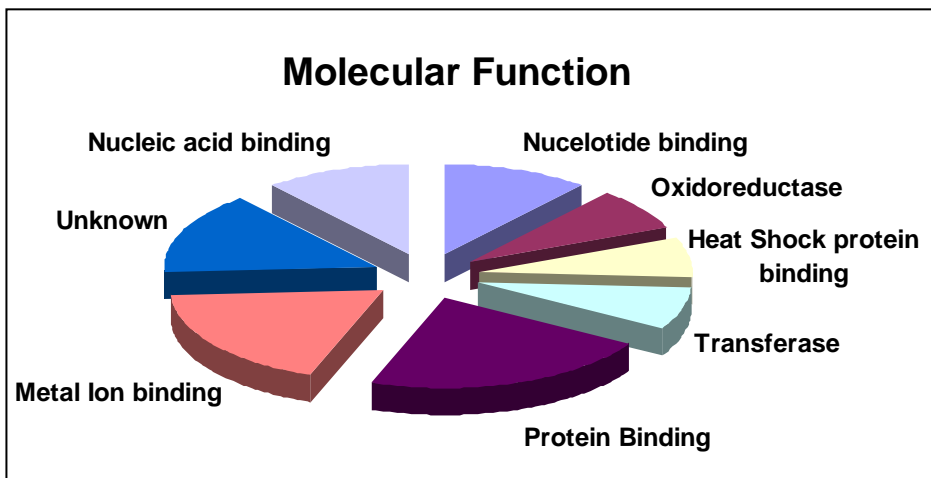
Supplemental Table 2. Transcripts upregulated by *Rhox5* (p<0.05)

Probe set ID	fold	Gene symbol	Description
1423614 at	2.000	/	expressed sequence AI326115
1419077 at	2.004	Mpp3	Mus musculus membrane protein, palmitoylated 3 (MAGUK p55 subfamily member 3) (Mpp3), mRNA.
1456083 at	2.004	/	expressed sequence AI315324
1443050 at	2.008	ESTs	ESTs, Moderately similar to RIKEN cDNA 5730493B19
1457281 at	2.008	ESTs	ESTs, Weakly similar to T24396 hypothetical protein T03F6.2 - <i>Caenorhabditis elegans</i> (<i>C.elegans</i>)
1427739 a at	2.028	Trp53	Mus musculus mRNA for transformation related protein 53 (Trp53 gene).
1450090 at	2.033	Zfp101	Mus musculus zinc finger protein 101 (Zfp101), mRNA.
1424832 at	2.041	/	Unknown (protein for MGC:30299)
1424201 a at	2.045	/	RIKEN cDNA 2610007A16 gene
1439563 at	2.053	/	expressed sequence AI854635
1431315 at	2.062	/	RIKEN cDNA 3010015K02 gene
1437700 at	2.070	ESTs	ESTs
1438196 at	2.083	/	DNA segment, Chr 9, ERATO Doi 660, expressed
1426339 at	2.101	/	Mus musculus AK5 mRNA for adenylate kinase isozyme 5, complete cds.
1426631 at	2.101	/	Mus musculus, Similar to hypothetical protein, clone MGC:7703 IMAGE:3497634, mRNA, complete cds
1420646 at	2.101	/	hypothetical protein, MGC:7199
1421015 s at	2.105	Pole3	Mus musculus DNA polymerase epsilon, subunit 3 (Pole3), mRNA. /PROD=NF-YB-like protein
1417767 at	2.110	/	RIKEN cDNA 1810044O22 gene
1451056 at	2.110	Psmid7	proteasome (prosome, macropain) 26S subunit, non-ATPase, 7
1419749 at	2.114	Dnmt2	Mus musculus DNA methyltransferase 2 (Dnmt2), mRNA.
1456192 x at	2.132	ESTs	ESTs
1438003 at	2.137	ESTs	ESTs, Highly similar to LAK-1 (<i>H.sapiens</i>)
1417183 at	2.160	DnaJA2	DnaJ (Hsp40) homolog, subfamily A, member 2
1430778 a at	2.160	/	nucleotide binding protein 1
1444676 at	2.174	ESTs	ESTs, Weakly similar to TYROSINE-PROTEIN KINASE JAK3 (<i>M.musculus</i>)
1421055 at	2.174	/	exportin 4
1421972 s at	2.183	Hcfc1	host cell factor C1
1458541 at	2.183	ESTs	ESTs
1431073 at	2.198	/	Protein prenyltransferase alpha subunit repeat containing protein,
1454006 a at	2.198	/	RIKEN full-length enriched library, clone:4933428E22:DNA segment, Human S2298E, full insert sequence.
1457528 at	2.217	ESTs	ESTs, Weakly similar to T14031 sodium bicarbonate cotransporter, pancreatic - mouse (<i>M.musculus</i>)
1434653 at	2.222	Ptk2b	protein tyrosine kinase 2 beta
1454036 a at	2.304	/	RIKEN full-length enriched library, clone:4921514G19:homolog to DEUBIQUITINATING ENZYME UBP109, full insert sequence.
1433942 at	2.304	ESTs	ESTs
1459265 at	2.304	ESTs	ESTs, Weakly similar to MYOSIN I ALPHA (<i>M.musculus</i>)
1448119 at	2.392	Bpgm	2,3-bisphosphoglycerate mutase
1454955 at	2.410	/	RIKEN cDNA 5730403B10 gene
1454090 at	2.415	/	RIKEN full-length enriched library, clone:2700031G06:trans-prenyltransferase, full insert sequence.
1428968 at	2.427	/	Mus musculus adult male testis cDNA, RIKEN full-length enriched library, clone:4921511I07
1449645 s at	2.469	/	expressed sequence AL024092
1418023 at	2.519	/	NMDA receptor-regulated gene 1
1425373 a at	2.519	/	Mus musculus, Similar to x 003 protein, clone MGC:27732 IMAGE:2646038, mRNA, complete cds.06.1
1452232 at	2.532	/	expressed sequence AI225872
1453435 a at	2.551	/	Mus musculus adult male tongue cDNA, RIKEN full-length enriched library, clone:2310042I22
1427253 s at	2.571	/	Mus musculus, clone IMAGE:3485208, mRNA, partial cds
1452012 a at	2.611	/	Mus musculus, RIKEN cDNA 2610104C07 gene
1450986 at	2.618	Nol5	nucleolar protein 5
1432016 a at	2.632	ldh3a	isocitrate dehydrogenase 3 (NAD+) alpha
1453983 a at	2.646	/	RIKEN cDNA 2810013M15 gene
1442549 at	2.674	ESTs	ESTs
1426908 at	2.681	/	expressed sequence AI225872
1416756 at	2.688	DnaJB1	DnaJ (Hsp40) homolog, subfamily B, member 1
1437179 at	2.710	ESTs	ESTs, Moderately similar to unnamed protein product (<i>H.sapiens</i>)
1453854 at	2.717	/	Mus musculus adult male small intestine cDNA, RIKEN full-length enriched library, clone:2010013L17
1452983 at	2.825	/	RIKEN cDNA 4931428M20 gene
1447717 x at	2.849	ESTs	ESTs
1458447 at	2.874	ESTs	ESTs, Moderately similar to CENF_HUMAN CENP-F KINETOCHORE PROTEIN (<i>H.sapiens</i>)
1451026 at	3.021	/	ectoplacental cone, invasive trophoblast giant cells, extraembryonic ectoderm and chorion sequence 3
1429228 at	3.030	/	RIKEN cDNA 4930534B04 gene
1415864 at	3.175	Bpgm	Bpgm /PROD=2,3-bisphosphoglycerate mutase
1452115 a at	3.215	sk-18	serinethreonine kinase 18
1422802 at	3.279	Defcr3	Mus musculus defensin related cryptdin 3 (Defcr3), mRNA.
1425388 a at	3.311	/	Unknown (protein for MGC:31041)
1458599 at	3.344	ESTs	ESTs
1445251 at	3.401	ESTs	ESTs
1429625 at	3.676	/	RIKEN cDNA 2900054C01 gene
1441205 at	3.817	ESTs	ESTs
1450087 a at	3.876	Nolc1	Mus musculus nucleolar and coiled-body phosphoprotein 1 (Nolc1), mRNA. /PROD=RIKEN cDNA 3230402K17
1428838 a at	4.329	Dck	Dck /UG TITLE=deoxycytidine kinase
1422168 a at	4.854	Bdnf	Mus musculus brain derived neurotrophic factor (Bdnf), mRNA. /PROD=brain derived neurotrophic factor
1450091 at	5.051	Ighmbp2	Mus musculus immunoglobulin mu binding protein 2 (Ighmbp2), mRNA. /PROD=immunoglobulin S mu binding protein 2
1429734 at	5.376	/	Mus musculus 0 day neonate skin cDNA, RIKEN full-length enriched library, clone:4632434I11
1429658 a at	5.682	/	RIKEN cDNA 5730502P04 gene
1433902 at	6.061	/	expressed sequence AU040152
1440864 at	6.061	/	expressed sequence AI836737
1429660 s at	6.135	/	RIKEN cDNA 5730502P04 gene
1424967 x at	6.536	/	Mus musculus cardiac troponin T isoform A2b mRNA, complete cds. /PROD=cardiac troponin T isoform A2b
1454480 at	7.353	/	Mus musculus adult male testis cDNA, RIKEN full-length enriched library, clone:4933439N06
1422966 a at	8.475	Trfr	Mus musculus transferrin receptor (Trfr), mRNA.
1446523 at	9.009	ESTs	ESTs
1454437 at	9.615	/	RIKEN full-length enriched library, clone:4933412F11:similar to Y-LINKED TESTIS-SPECIFIC PROTEIN, full insert sequence.
1450940 at	10.493	Gdap1	ganglioside-induced differentiation-associated-protein 1
1430268 at	10.718	/	RIKEN cDNA 9630005C17 gene
1445974 at	11.507	ESTs	ESTs
1431539 at	14.164	Samsn1	SAM domain, SH3 domain and nuclear localisation signals, 1
1446889 at	14.245	/	DNA segment, Chr 2, ERATO Doi 105, expressed
1416034 at	16.584	CD24a	CD24a antigen
1447565 at	25.000	ESTs	ESTs
1455179 at	29.499	/	RIKEN cDNA 1110068J02 gene
1426712 at	50.761	/	Mus musculus, clone IMAGE:5361283, mRNA, partial cds
1420514 at	253.165	Tm4sf10	transmembrane 4 superfamily member 10

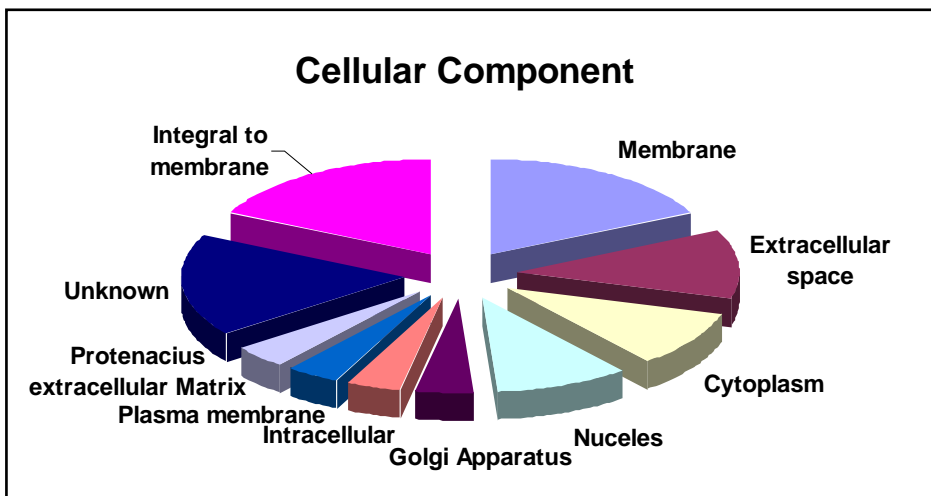
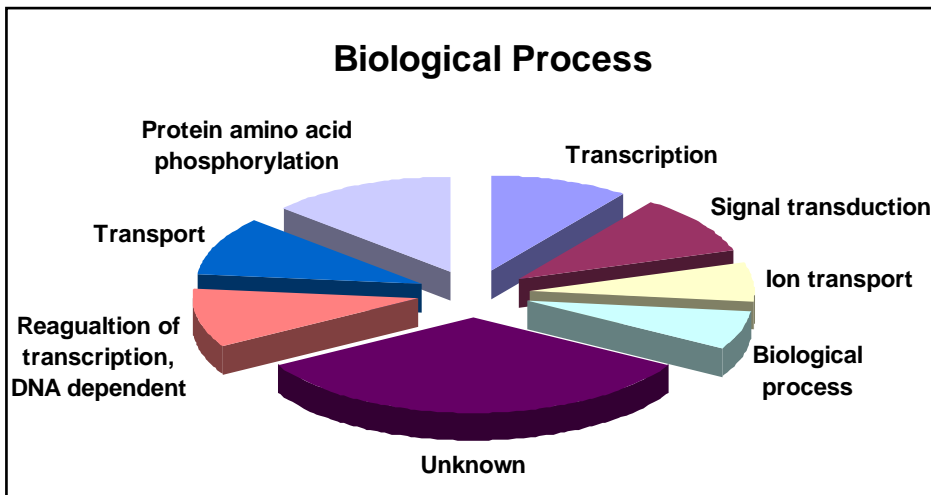
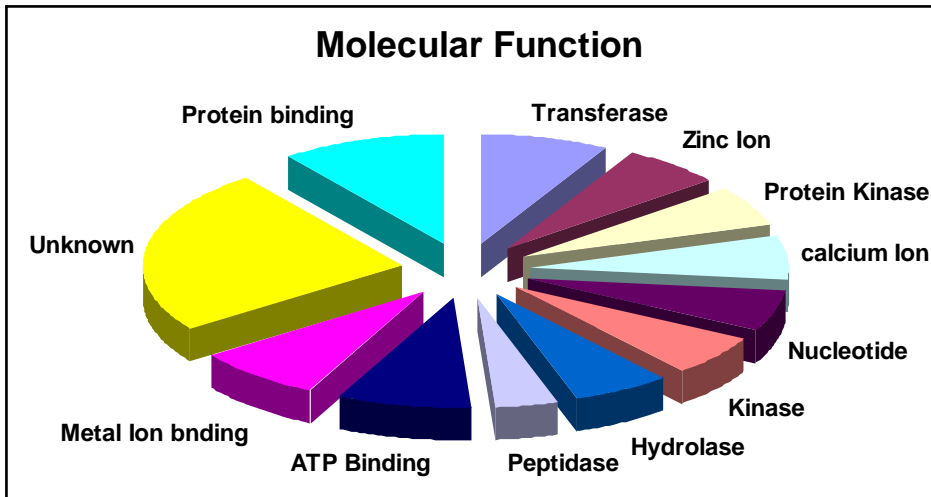
Supplemental Figure 3



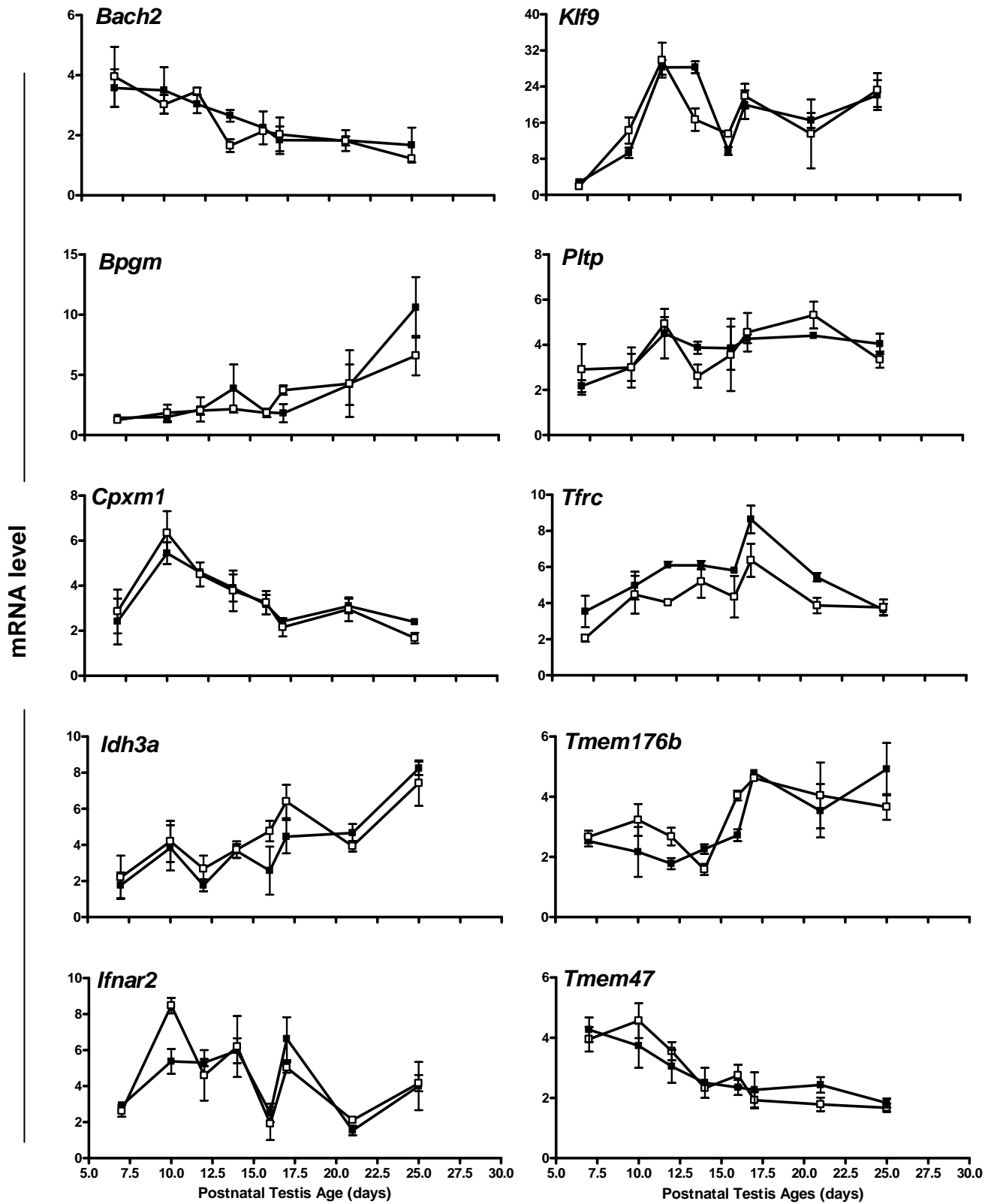
Supplemental Figure 4



Supplemental Figure 5



Supplemental figure 6.

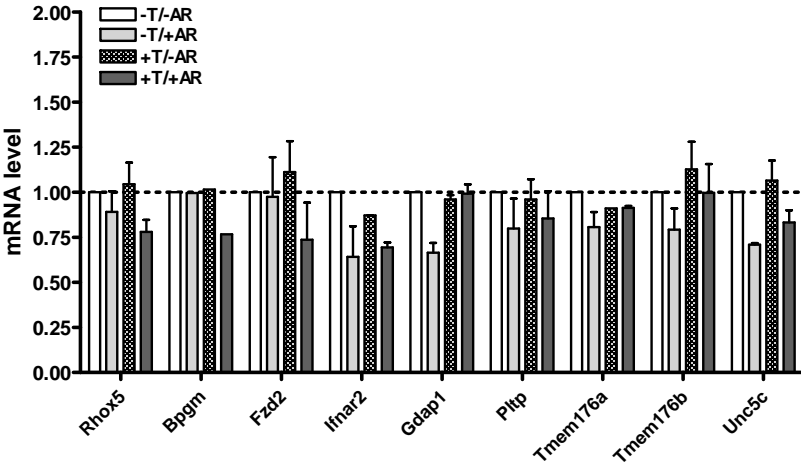


Supplemental Table 3. Consensus AREs in candidate SAR genes

Gene symbol	AREs	
	5'- flanking	3'- flanking
<i>Bpgm</i>	none	1 site
<i>Fzd2</i>	none	none
<i>Ifnar2</i>	2 sites	none
<i>Gdap1</i>	none	none
<i>Pltp</i>	none	1 site
<i>Tmem176a</i>	1 site	1 site
<i>Tmem176b</i>	none	none
<i>Unc5c</i>	none	none

Consensus AREs in the 5' and 3' flanking regions (1 kb) determined using the Genomatix program.

Supplemental Figure 7



Supplemental Table 4. Oligonucleotides

Name	Orientation	Sequence
MDA-1287	Sense	5' - GCCTGGGAGTCAAGGAA
MDA-1444	Sense	5' - TGGGCACTTTCCATACCTTC
MDA-1445	Antisense	5' - GTTGCCACGTTTCATCACTTG
MDA-1556	Sense	5' - CCAGTGTCCCCATGCTCAAC
MDA-1557	Antisense	5' - TGCATGGTCCTTCCAGGTCT
MDA-1558	Sense	5' - GACCAAAAGCTGAGGCTGAGA
MDA-1559	Antisense	5' - CAATGTGGCCATCAGGGTAGA
MDA-1625	Sense	5' - CTGAAGGTCAAAGGGAATGT
MDA-1626	Antisense	5' - GGACAGAGTCTTGATGATCTC
MDA-1784	Sense	5' - CAGCATTATTCCAGTGGATG
MDA-1785	Antisense	5' - GGGCACTTGCACAGAGATG
MDA-1904	Antisense	5' - ATTCTTCTCCTTCGCCTTC
MDA-2097	Sense	5' - GAGTTTGCTGACACCATCAA
MDA-2098	Antisense	5' - TAGTGTTTCATCTCGCCAGAC
MDA-2099	Sense	5' - CCATTACAGAGTGCATACAG
MDA-2100	Antisense	5' - AATCTCTTCTCACACAGTGG
MDA-2101	Sense	5' - AGTGAGTCGTGTCCTGTGC
MDA-2102	Antisense	5' - GGATGTCGTGGATGAACTC
MDA-2103	Sense	5' - GACAATTTGCCCAATGTCTTg
MDA-2104	Antisense	5' - CTGCCATCCTCATAATCGTA
MDA-2105	Sense	5' - GAGAGTAGATGAAACCTCTG
MDA-2106	Antisense	5' - ACGTATGCCTTCCGACTCT
MDA-2107	Sense	5' - CATTGCGAATCCACTCTCG
MDA-2108	Antisense	5' - CATGACAGCAACAGGTCTG
MDA-2109	Sense	5' - AGACCTGGAGGAGCTGGA
MDA-2110	Antisense	5' - TAACAGTGGCAGTGTCGG
MDA-2113	Sense	5' - CGTCCGAAGCTGGAAATG
MDA-2114	Antisense	5' - CTATGGAGATCAGGTTCAA
MDA-2115	Sense	5' - TTCAGCCTCAGTCATGAAGA
MDA-2116	Antisense	5' - CCCATCTTGTTCTTATCCAT
MDA-2223	Sense	5' - CTGAATCCTCTGCATCAACAA
MDA-2224	Antisense	5' - GTTGAGCAGTGGATACACCTG
MDA-3628	Sense	5' - GACGTGGATGTGCAGAAGAT
MDA-3629	Antisense	5' - GTGGTGGAGGAGGAGGATTA
MDA-3662	Sense	5' - TTGGAGCAGCTGCTGGG
MDA-3663	Antisense	5' - GCACAGCTCAGTCCAAGG
MDA-3689	Sense	5' - GTTCTGGAGAGACTTCTTC
MDA-3690	Antisense	5' - CCAGCAGAATCGGAACTC
MDA-3693	Sense	5' - GTGGTGCAGACTGTGCTG
MDA-3694	Antisense	5' - CCAGCAGGTACCACCCC
MDA-4077	Sense	5' - TTTGCAGCGCACTAATTCCTT
MDA-4080	Antisense	5' - CCGCAGCCCTCCTGATCT
MDA-4388	Sense	5' - CTTCTGGCACTGCTCCTA
MDA-4389	Antisense	5' - ACCAGCTGTGGACTGCAG
MDA-4390	Sense	5' - TGAGCAGACTTTTCTGGATG
MDA-4391	Antisense	5' - ACAGTGAGCTCAGGATGCAA
MDA-4392	Sense	5' - CGACTTCACAGTCTACATG
MDA-4393	Antisense	5' - CGCTTCACACAGTGGTCTC
MDA-4394	Sense	5' - GAGACCATCACCATCCAG
MDA-4395	Antisense	5' - CGTTGGAGATGTTACAGCAG
MDA-4396	Sense	5' - CAAAGATGCTCTTCTCACCT
MDA-4397	Antisense	5' - CTGTTGTAACATCGTGGTTAA