Supplemental Figures



Supplemental Figure S1: Introduction of the RiboTag allele does not cause detectable reproductive defects.

Reproductive organ (in mg) and body weights (in g) of different control mice (blue, red and green bars, genotypes and number of animals in graph) and SCARIBO mice (purple bars) at 8 weeks of age. No significant differences were observed between reproductive organ and body weights of mice with different combinations of genetic mutations involved in this study, except for SCARIBO mice, which display the expected SCARKO phenotype with significantly reduced testis (-66%) and epididymal weight (-35%) [6]). *, p<0.05

Supplemental Tables

Sample	Read length	Reads obtained	Unmapped reads	Mapping efficiency
Input SCRIBO P10	51	32827261	1916966	94.2%
Input SCARIBO P10	51	36651489	3036189	91.7%
IP SCRIBO P10	51	32863709	2145083	93.5%
IP SCARIBO P10	51	39458359	2255450	94.3%
Input SCRIBO adult	101	53859868	6349149	88.2%
IP SCRIBO adult	101	55717788	7447655	86.6%

Supplemental Table 1 Quantitative data and mapping efficiencies of the RNA-seq experiments performed in this paper

Mapping efficiency is expressed as the percentage of total reads obtained, mapped to the reference genome.

Gene	Forward primer	Reverse primer
Ar	ATGGAGGTGCAGTTAGGGCTGGG	TCACTGTGTGTGGAAATAGATGGG
Btg3	AAGAACGAAATTGCGGCTGTT	CATCGGGATCAACTCTCTGAAAC
Corin	GCTGGTGACTGCTAACTTGCT	CCCATCAGTGACCAAAGGTTC
Drp2	GGGATGCCCTTACACCCTC	GCTTTGGACTTAGGCAGGGAT
Emilin3	GCCTCCCGTTACAGCCTCTA	GCAGGTCACATTTCTGTGTACCA
Esyt3	CACGAGCGCGAGTTCATCA	TGGATGCTCTTCTCCCGGAT
Gja6	ATGAGTGATTGGAGTGCCTTACA	CAAGCCGACTCGATAGCAGTG
Hicl	CATTCGAGGCAGCTAC	AGGTTTAGCAGGTTGTCATGC
Ky	TCATCACCTCCTACGACAACC	CGGGGTCTCATGTTTCCATTT
Mcf2	ATCCCCCGAGAGGCAAGAT	CCAGGACTAAGTGTAAGCTCCC
Nutf2	GATAACGACAGAACCCAACTAGG	GGAAGGCTAGATAGCTTCTCCA
Nxf3	GAAACTAACCCCTTGCGAAGA	GCAGGCGAGTTAAGAAATCTCT
Rhox5	GGAGGGCAACACCAGTCCCTG	CTCGGTGTCGCAAAAGGGCA
Rpl19	CTGAAGGTCAAAGGGAATGT	GGACAGAGTCTTGATGATCTC
Sept2	ATGGTGGTTGGTGAATCTGGT	AGCCTCTATCTGGACAGTTCTTT
Sept3	CGGTCAGCATCAACTCCAAC	CTTTCGGCTCACTTGGGACT
Sept6	GCGAAGATTGTCGAACTGTCC	GTCAGGCAAGCTGTCGAAC
Sept7	GTAGCTCAACCGAAGAACCTTG	AAAACTTTGGATTGCTCCACCT
Sept8	GGTCAGCAAGTCAGTCACTCA	GTCTCAAAGGTCGTGTTGAAGA
Sept9	GCTGACAACCCTAGAGATGCC	GGGCCTTTTCGTTCCGTGA
Sept11	GTGGGGAGACCGAGTAATGAA	GTTGTGAGTAGCTGGGTCACT
Wfdc15a	AGCAGCCTCCTACTGTTCACA	GGGCAGTCAAGAAGAAACTCC
Rhox2	See [1]	
Rhox3	See [1]	
Rhox4	See [1]	
Rhox5	GGAGGGCAACACCAGTCCCTG	CTCGGTGTCGCAAAAGGGCA
Rhox8	See [1]	
Rhox10	See [1]	
Rhox11	See [1]	
Rhox13	See [1]	
Sycp3	GGTTCAGAAGAAGATGTTGCTG	TTGTTGATGTCAGCTCCAAAT
Cyp17a1	GGGCACTGCATCACGATAAA	GATCTAAGAAGCGCTCAGGCA

Supplemental Table 2 List of primers used in this study

	Function	Corrected
		P-value
Adult SC genes	KEGG Pathways	
(n=501)	Glutathione metabolism	6.6e-4
	Metabolism of xenobiotics by cytochrome P450	3.4e-4
	Drug metabolism	7.3e-4
	Molecular Function	
	Glutathione transferase activity	1.8e-9
	Transferase activity, transferring alkyl or aryl (other	5.2e-6
	than methyl) groups	
	Peptidase inhibitor activity	1.6e-5
	Enzyme inhibitor activity	1.9e-5
	Endopeptidase inhibitor activity	3.6e-4
	Serine-type endopeptidase inhibitor activity	2.2e-2
	Cellular Compound	
	Extracellular region	8.4e-4
P10 SC genes	KEGG Pathways	
(n=508)	Glutathione metabolism	2.2e-4
	Metabolism of xenobiotics by cytochrome P450	4.3e-4
	Drug metabolism	4.7e-4
	Molecular Function	
	Glutathione transferase activity	1.1e-9
	Transferase activity, transferring alkyl or aryl (other	2.4e-7
	than methyl) groups	
	Peptidase inhibitor activity	4.3e-5
	Enzyme inhibitor activity	1.7e-4
	Endopeptidase inhibitor activity	9.8e-4
	Cellular Compound	
	Extracellular region	3.6e-2

Supplemental Table 3: Gene Ontology analysis of the SC fingerprint

DAVID analysis of genes predominantly expressed in SCs at P10 and adult age (EF > Stra6). P-values are corrected for multiple testing using the Benjamini technique. Corrected P<0.05.

Supplemental Table 4: Gene Ontology analysis of AR-regulated transcripts in the P10 Input but not in the P10 IP fraction.

	Function	Corrected P-value
All regulated genes	Molecular Function	
together (IP, n=445)		
	adenyl ribonucleotide binding	3.6e-5
	ATP binding	3.6e-5
	Adenyl nucleotide binding	5.0e-4
	Nucleoside binding	5.0e-2

DAVID analysis of genes significantly regulated by AR in Input but not in IP fractions at P10.

P-values are corrected for multiple testing using the Benjamini technique. Corrected P<0.05.

••	Function	Corrected P-value
Common genes	Cellular Compound	
All (n=493)	Cell junction	8.3e-3
	Cytoskeleton	1.5e-2
	Cell projection	1.6e-2
	Plasma membrane	1.7e-2
	Cell-cell junction	2.0e-2
	Stereocilium	2.1e-2
	Neuron projection	2.3e-2
	Microvillus	2.6e-2
	Stereocilium bundle	2.9e-2
	Apical junction complex	3.1e-2
	Apicolateral plasma membrane	3.2e-2
	Plasma membrane part	3.3e-2
	Molecular Function	
	Nucleoside-triphosphate regulator activity	6.3e-4
	GTPase regulator activity	9.4e-4
	Calcium ion binding	2.8e-2
	Metal ion transmembrane transporter activity	3.5e-2
	Passive transmembrane transporter activity	3.5e-2
	Channel activity	3.5e-2
	Gated channel activity	3.5e-2
	Cytoskeletal protein binding	3.9e-2
	Ion channel activity	4.0e-2
	Substrate specific channel activity	4.1e-2
	Small GTPase regulator activity	4.2e-2
	GTPase activator activity	4.3e-2
	Cation channel activity	4.5e-2
Common genes	<u>Biological Process</u>	
Ar-stimulated in IP	transmembrane transport	2.2e-2
(n=279)		
	Molecular Function	
	GTPase regulator activity	8.6e-3
	nucleoside-triphosphatase regulator activity	5.3e-3
	Metal ion transmembrane transporter activity	1.5e-2
Common genes	<u>Cellular Compound</u>	
Ar-repressed in IP	Cell-cell junction	1.2e-2
(n=214)	Anchoring junction	1.5e-2
	Cytoskeleton	1.9e-2
	Cell junction	3.9e-2
	Desmosome	4.1e-2
	Apicolateral plamsa membrane	4.5e-2
	Extracellular matrix	4.8e-2
	Proteinaceous extracellular matrix	4.9e-2
	Molecular Function	
	Calcium ion binding	2.1e-2
	KEGG Pathways	
	Arrhytmogenic right ventricular cardiomyopathy (ARVC)	1.8e-2
	Regulation of actin cytoskeleton	3.3e-2

Supplemental Table 5 GO analysis of AR-regulated genes common between P10 Input and IP fractions.

David analysis of transcripts significantly regulated by AR in both Input and IP fractions.

P-values are corrected for multiple testing using the Benjamini technique. Corrected P<0.05.

Supplemental Table 6 Functional categories overrepresented in AR-regulated genes.

Function	Corrected	Function	Corrected
	P-value		P-value
Molecular Function		Cellular Compound	
Calcium ion binding	1.3e-5	Plasma membrane	3.5e-5
Cytoskeletal protein binding	3.2e-5	Plasma mambrane part	3.6e-5
Actin binding	4.2e-4	Cell junction	4.3e-5
Cation binding	3.2e-2	Cell-cell junction	9.4e-5
Metal ion binding	3.4e-2	Anchoring junction	1.0e-3
Ion Binding	3.4e-2	Stereocilium	5.7e-3
Nucleoside-triphosphatase regulator activity	3.5e-2	Adherens junction	1.2e-2
Metal ion transmembrane transporter activity	3.6e-2	Stereocilium bundle	1.4e-2
GTPase regulator activity	3.8e-2	Cytoskeleton	1.4e-2
		Apicolateral plasma membrane	1.5e-2
Biological Process		Proteinaceous extracellular matrix	1.6e-2
Biological Adhesion	8.1e-3	Apical junction complex	1.6e-2
Cell adhesion	1.5e-2	Basement membrane	1.7e-2
Protein amino acid phosphorylation	3.4e-2	Extracellular matrix	1.7e-2
Ion transport	3.9e-2	Microvillus	2.0e-2
Actin filament-based process	4.3e-2	Actin cytoskeleton	2.4e-2
		Cell-cell adherens junction	2.6e-2
		Golgi aparatus	2.7e-2
		Extracellular matrix part	3.1e-2
		Cell projection	3.3e-2
		Basolateral plasma membrane	3.6e-2
		Neural projection	4.0e-2

DAVID analysis of all 1090 transcripts significantly differentially expressed between IP samples from SCRIBO vs. SCARIBO P10 testes. P-values are corrected for multiple testing using the Benjamini technique. Corrected P<0.05.

Supplemental References

1. MacLean J a, Hayashi K, Turner TT, Wilkinson MF: **The Rhox5 homeobox gene regulates the regionspecific expression of its paralogs in the rodent epididymis.** *Biology of reproduction* 2012, **86**:189.