Supplementary Information

Manuscript: Retinoic acid receptor beta and angiopoietin-like protein 1 are involved in the regulation of human androgen biosynthesis

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Supplementary Table S1. List of primer sequences used for qRT-PCR
GeneBank accession numbers for source sequences are given in parentheses.

Primers for qRT-PCR	
HSD3B2 (NM_000198)	S: 5’- AAGCTGACTGTACTTGAAGG – 3’
	AS: 5’- GTGTACAAGGTATCACCATT – 3’
RARB (NM_000965)	S: 5’- GGTTTCACTGGCTTGACCAT – 3’
	AS: 5’- GGCAAAGGTGAACACAAGGT – 3’
ANGPTL1 (NM_004673)	S: 5’- CACCACCTGATCTGGCAACT – 3’
	AS: 5’- AATCCCCTGCATTTCCCTGG – 3’
PLK2 (NM_006622)	S: 5’- CCACCATCACCACCATTCGC – 3’
	AS: 5’- CAGACACAATCTGCCTGAGGT – 3’
DUSP6 (NM_001946)	S: 5’- GTTTTTCCCTGAGGCCATTTC – 3’
	AS: 5’- TCACAGTGACTGAGCGGCTAAT – 3’
DUSP10 (NM_007207)	S: 5’- CTGAACATCGGCTACG – 3’
	AS: 5’- GGTGTAAGGATTCTCGGT – 3’
NPTX2 (NM_002523)	S: 5’- CATCGAGCTGCTCATCAAC – 3
	AS: 5’- CTGCTCTTGTCCAAGGATC – 3’
ASS1 (NM_000050)	S: 5’- GCCCGCAAACAAGTGGAAAT – 3’
	AS: 5’- TCCAGGATTCCAGCCTCGTA – 3’
ENC1 (NM_001256576)	S: 5’- GTCCTTCTCCATGCCGGAAA – 3’
	AS: 5’- GGGCAGCTGGAGGAAATCTT – 3’
CFI (NM_000204)	S: 5’- GCCATGGCAGAGATGCATTG – 3’
	AS: 5’- GAAGCCTTTGCCTTGGCATG – 3’
CCDC3 (NM_031455)	S: 5’- GGGCTACTTCTCGTGCCACTC – 3’
	AS: 5’- TGCCGGTTGCGCTTCTCC – 3’
FREM2 (NM_207361)	S: 5’- AGTCGCCACTGTTGAGATCG – 3
	AS: 5’- ACATCTCCGCTCCTCCTGAT – 3’
ASCL1 (NM_004316)	S: 5’- GCGGCCAACAAGAAGATG – 3’
	AS: 5’- CAAGTCGTTGGAGTAGTTGG – 3’
CYP21A2 (NM_000500)	S: 5’- AGAGGGATCACATCGTGGAG – 3’
	AS: 5’- CTTCCAGGAGCTGTCCAGAG – 3’
Cyclophilin A (NM_021130)	S: 5’- GGCAAATGCTGGACCCAACACA – 3’
	AS: 5’- TGCTGGTCTTGCCATTCCTGGA – 3’

Supplementary Table S2. List of differentially expressed genes in H295R cells under normal growth vs starvation conditions (>1.5 fold)

By GeneChip Human Gene 1.0 ST array studies. We identified 63 genes when setting the fold change cut-off at 1.5 with an adjusted p-value < 0.05.

Order	Accession Number	Gene Symbol	Gene Title	Fold Change
1	NM_000500	CYP21A2	Cytochrome P450, family 21, subfamily A, polypeptide 2	1.83523
2	NM_170744	UNC5B	Unc-5 homolog B	1.86973
3	NM_003009	SEPW1	Selenoprotein W, 1	1.96531
4	NM_001017425	KCNK2	Potassium channel, subfamily K, member 2	-1.7602*
5	NM_020338	ZMIZ1	Zinc finger, MIZ-type containing 1	1.64539
6	NM_014848	SV2B	Synaptic vesicle glycoprotein 2B	-1.6558*
7	NM_016522	NTM	Neurotrimin	-1.6963*
8	NM_001197294	DPYSL3	Dihydropyrimidinase-like 3	1.7987
9	NM_000624	SERPINA5	Serpin peptidase inhibitor, clade A (alpha-1 antiproteinase, antitrypsin), member 5	1.5313
10	NM_001553	IGFBP7	Insulin-like growth factor binding protein 7	1.77783
11	NM_005314	GRPR	Gastrin-releasing peptide receptor	-1.8474*
12	NM_002514	NOV	Nephroblastoma overexpressed gene	1.8505
13	NM_001490	GCNT1	Glucosaminyl (N-acetyl) transferase 1, core 2	1.92664
14	NM_001128176	THRB	Thyroid hormone receptor, beta	1.67774
15	NM_018407	LAPTM4B	Lysosomal protein transmembrane 4 beta	1.86459
16	NM_006084	IRF9	Interferon regulatory factor 9	1.76116
17	NM_016546	C1RL	Complement component 1, r subcomponent-like	1.53157
18	NM_001874	CPM	Carboxypeptidase M	-1.7492*
19	NM_002934	RNASE2	Ribonuclease, RNase A family, 2 (liver, eosinophil-derived neurotoxin)	1.7072
20	NM_021202	TP53INP2	Tumor protein p53 inducible nuclear protein 2	1.6294
21	NM_030762	BHLHE41	Basic helix-loop-helix family, member e41	1.6084
22	NM_004454	ETV5	Ets variant 5	1.82798
23	NM_201555	FHL2	Four and a half LIM domains 2	1.53679
24	NM_003810	TNFSF10	Tumor necrosis factor (ligand) superfamily, member 10	1.61757
25	NM_173582	PGM2L1	Phosphoglucomutase 2-like 1	1.69809
26	NM_004460	FAP	Fibroblast activation protein, alpha	1.88712
27	NR_024101	FLJ35776	Hypothetical LOC649446	1.64384
28	NM_007053	CD160	CD160 molecule	1.67532
29	NM_007361	NID2	Nidogen 2 (osteonidogen)	-1.5459*
30	NM_201524	GPR56	G protein-coupled receptor 56	1.71853
31	NM_021643	TRIB2	Tribbles homolog 2 (Drosophila)	1.5145
32	NM_003048	SLC9A2	Solute carrier family 9 (sodium/hydrogen exchanger), member 2	-1.5989*
33	NM_005923	MAP3K5	Mitogen-activated protein kinase kinase kinase 5	1.71674
34	NM_020809	ARHGAP20	Rho GTPase activating protein 20	1.5001

35	NM 001771	CD22	CD22 molecule	1.70581
36	NM_004172	SLC1A3	Solute carrier family 1 (glial high affinity glutamate transporter), member 3	-1.5469*
37	NM_133445	GRIN3A	Glutamate receptor, ionotropic, N-methyl-D-aspartate 3A	-1.5509*
38	NM_001195032	C6orf99	Chromosome 6 open reading frame 99	-1.6939*
39	NM_002543	OLR1	Oxidized low density lipoprotein (lectin-like) receptor 1	1.52082
40	NM_153366	SVEP1	Sushi, von Willebrand factor type A, EGF and pentraxin domain containing 1	1.86166
41	NM_152637	METTL7B	Methyltransferase like 7B	1.67562
42	NM_005810	KLRG1	Killer cell lectin-like receptor subfamily G, member 1	1.66086
43	NM_024704	KIF16B	Kinesin family member 16B	1.64102
44	NM_015236	LPHN3	Latrophilin 3	1.58606
45	NM_002298	LCP1	Lymphocyte cytosolic protein 1 (L-plastin)	-1.5299*
46	NM_022843	PCDH20	Protocadherin 20	1.50787
47	NM_001013625	C1orf192	Chromosome 1 open reading frame 192	-1.5502*
48	NM_199511	CCDC80	Coiled-coil domain containing 80	1.57305
49	NM_012281	KCND2	Potassium voltage-gated channel, Shal-related subfamily, member 2	-1.5775*
50	NM_015441	OLFML2B	Olfactomedin-like 2B	1.639
51	NM_014705	DOCK4	Dedicator of cytokinesis 4	1.56717
52	NM_018672	ABCA5	ATP-binding cassette, sub-family A (ABC1), member 5	1.64576
53	NM_170672	RASGRP3	RAS guanyl releasing protein 3 (calcium and DAG-regulated)	1.5215
54	NM_001128596	TC2N	Tandem C2 domains, nuclear	1.50841
55	NM_212482	FN1	Fibronectin 1	1.59342
56	NM_001161429	RANBP3L	RAN binding protein 3-like	1.60935
57	NM_002164	IDO1	Indoleamine 2,3-dioxygenase 1	1.61038
58	NR_003530	MEG3	Maternally expressed 3 (non-protein coding)	1.64795
59	NM_001099694	ZNF578	Zinc finger protein 578	-1.7452*
60	NM_001012980	SATL1	Spermidine/spermine N1-acetyl transferase-like 1	-1.5217*
61	NM_001102562	MARCH11	Membrane-associated ring finger (C3HC4) 11	-1.5395*
62	NR_003231	SNORD113-3	Small nucleolar RNA, C/D box 113-3	1.56858
63	NR_003194	SNORD114-2	Small nucleolar RNA, C/D box 114-2	1.55025

*- Sign indicates up-regulation

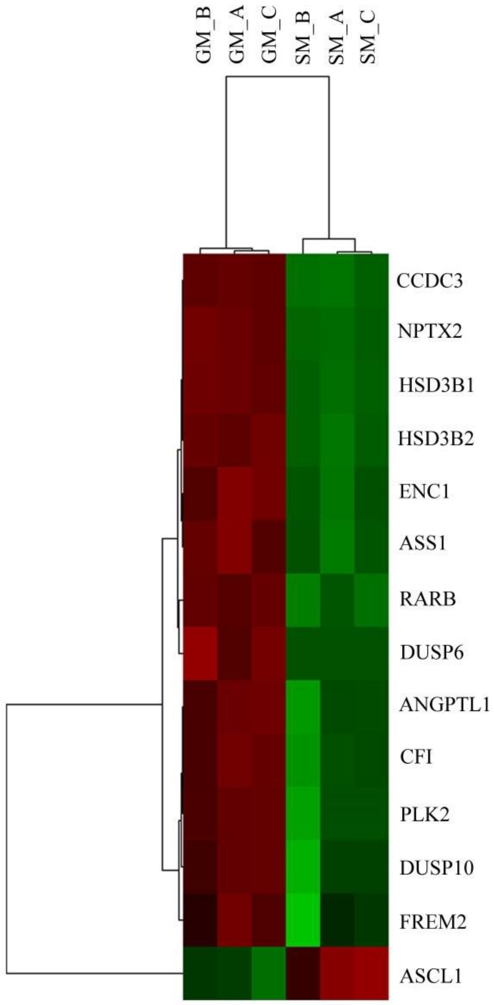
Supplementary Table S3. Suggested biological function of the 63 genes found differentially expressed at fold change > 1.5

Differentially expressed genes in normal growth vs starvation conditions were loaded into DAVID v6.5 software to perform biological functional analysis. Analysis was performed on the 63 gene transcripts identified by microarray analysis setting the fold change cut-off at 1.5 with an adjusted p-value < 0.05.

Biological Function	Overexpressed	Under expressed
Steroid biosynthetic process		CYP21A2
MAPKKK cascade		RASGRP3, MAP3K5
Signal transduction	GRPR, GRIN3A, SLC1A3	CD22, GPR56, RASGRP3, ARHGAP20, DOCK4, FAP, FN1, LPHN3, NOV, OLFML2B, PCDH20, THRB, TRIB2, TNFSF10, UNC5B
Cell adhesion	NTM, NID2	CD22, GPR56, FN1, IGFBP7, NID2, OLR1, PCDH20, SVEP1
Inflammatory response		C1RL, CFI, FN1, IDO1, KLRG1, OLR1
Glycosylation process	CPM, GRPR, GRIN3A, NID2, NTM, KCNK2, SLC9A2, SV2B	ABCA5, CD160, CD22, GPR56, PCDH20, C1RL, FN1, GCNT1, IGFBP7, KLRG1, LPHN3, NOV, OLFML2B, OLR1, RNASE2, SERPINA5, SVEP1, UNCB

Supplementary Figure S1. Heat Map of Affymetrix microarray data showing differentially expressed genes in H295R cells grown under normal and starvation conditions.

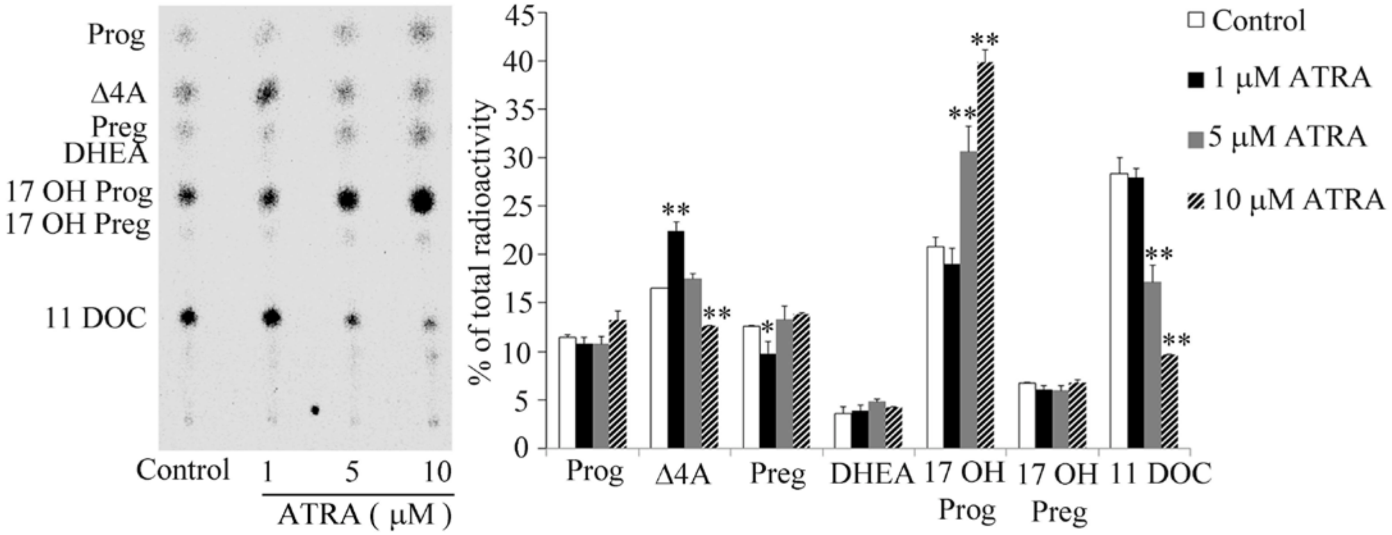
H295R cells were grown under serum-free, starvation (SM) or normal (GM) conditions. RNA was extracted and analyzed for differential gene expression by Affymetrix GeneChip Human Gene 1.0 ST arrays. Data were then analyzed by Cluster 3.0 and Jtreeview software for creating a representative heat map of the 14 genes which were identified at a significance level of fold change > 2. In the heat map graphic rows represent the individual genes. The gene expression levels are displayed for each independent sample. Both genes and samples were clustered by a complete linkage method**.** Over-expression is indicated in red, under-expression in green.



Supplementary Figure S1

Supplementary Figure S2. Effect of ATRA dose response on H295R steroid profile.

H295R cells were cultivated in serum free medium for 24 h. Thereafter, cells were grown in presence or absence of ATRA in serum-free medium for another 24 h. Steroid production was labeled with [^{3}H] pregnenolone for 90 min. Steroids were extracted and resolved by TLC. A representative TLC is shown on the left and quantitative analysis of steroids on the right side. We found ATRA treatment significantly altered the steroid production when compared to the control. Data are the mean ± SEM of two independent experiments. * p<0.05, ** p<0.01.



Spplementary Figure S2