SUPPLEMENTARY TEXT

a) MIAME description for gene expression profiling of H295R TR SF-1 clones.

Experiment design

Gene expression profiles were analyzed in two different H295R TR/SF-1 WT clones overexpressing SF-1 in a tetracycline-regulated fashion cultured in basal conditions or after three days of doxycycline treatment. For each condition, two biological replicates were examined.

Array #22354	Clone #1	replicate 1	basal Cy3/Dox Cy5
Array #22416	Clone #1	replicate 2	basal Cy5/Dox Cy3
Array #22446	Clone #2	replicate 1	basal Cy3/Dox Cy5
Array #22447	Clone #2	replicate 2	basal Cy5/Dox Cy3

Samples and labeling

Two different H295R TR/SF-1 clones were cultured in 6-well plates in complete medium [DMEM/F-12 supplemented with 2% NuSerum (Becton Dickinson), 1% ITS Plus (Becton Dickinson) and antibiotics]. For each clone two wells were cultured in basal conditions while doxycycline (1 μg/mL) was added to two other wells. Cells were left in culture for 72 hours. Total RNA was extracted by the Trizol (Gibco BRL) method and purified on RNeasy columns (Qiagen). RNA concentration was measured by spectrophotometry and its integrity checked using an Agilent Bioanalyzer instrument. 1 μg total RNA was amplified and labelled with Cy3 and Cy5 fluorochromes using the Amino Allyl MessageAmp aRNA kit according to the manufacturer's (Ambion) protocol. Cy3-and Cy5-labeled cRNAs were fragmented using Fragmentation Buffer (Agilent), dissolved in Hybridization Buffer (Agilent) and hybridized to pan-genomic human microarrays of the RNG/MRC resource (http://www.microarray.fr). These microarrays harbor 25342 50mer oligonucleotides[1]. Probe sequences are available on the MEDIANTE web site (http://www.microarray.fr:8080/merge/index), and are archived at GEO (http://www.ncbi.nlm.nih.gov/projects/geo/query/acc.cgi?acc=GPL1456) as platform GPL1456.

Hybridization procedures

Cy3-and Cy5-labeled cRNAs dissolved in hybridization buffer were hybridized on previously processed slides (treated with 50 mM ethanolamine in 50 mM borate buffer, pH 9.0 at 20°C for 1 hour) in a total volume of 500 μL using the Microarray Hybridization Chamber (Agilent) at 62°C for 17 hours using a 4 rpm agitation. Slides were washed with washing buffer #1 (6X SSC, 0.005% Triton X-102) at 20°C for 10 min and with washing buffer #2 (0.1X SSC, 0.005% Triton X-102) at 4°C for 5 min, dried and stocked under vacuum. Fluorescence data were acquired from slides using a GenePix 4000B instrument (Molecular Devices).

Measurement data

Data are deposited in the GEO database under the accession number GSE5911.

Data analysis

TIF images containing the data from each fluorescence channel were quantified with the GenePix Pro 6.0 program (Axon Instruments). First, data were log-transformed, mean-centered and reduced for an equal standard deviation between each slide (Z-score), for subsequent analysis using the GeneANOVA software[2]. Second, normalization was also independently performed using the limma package available on the bioconductor web site (http://www.bioconductor.org)[3,4]. A given gene was considered to be differentially expressed between H295R TR/SF-1 WT cells in basal conditions and treated with doxycycline when its absolute value of log-fold change was >0.7, its mean log-expression level was >9 and its statistical score (log-odds of differential expression) was >0.

b) MIAME description for gene expression profiling of Sf-1 transgenic mice adrenals

Experiment design
Gene expression profiles were analyzed by two-color microarrays in transgenic mice from line #56 heterozygous or homozygous for the transgene, male or female and of different ages. Profiles from each transgenic mouse were directly compared to a sex- and age-matched wild-type littermate

For each experimental point, two technical replicates (dye-swaps) were examined.

Array #	**Replicate**	**Age**	**Sex**	**Transgene copy number**	**Cy3**	**Cy5**
16682	A1	10 days	M	1	WT	transgenic
16479	A2	10 days	M	1	transgenic	WT
16683	B1	10 days	M	1	WT	transgenic
16625	B2	10 days	M	1	transgenic	WT
16685	C1	10 days	F	1	WT	transgenic
16680	C2	10 days	F	1	transgenic	WT
16516	D1	4 months	M	2	WT	transgenic
16452	D2	4 months	M	2	transgenic	WT
16517	E1	4 months	M	2	WT	transgenic
16453	E2	4 months	M	2	transgenic	WT
16518	F1	4 months	F	2	WT	transgenic
16475	F2	4 months	F	2	transgenic	WT

Samples and labeling
Adrenal glands were rapidly dissected from wild-type and transgenic mice. Total RNA was extracted by the Trizol (Gibco BRL) method and purified on RNeasy columns (Qiagen). RNA concentration was measured by spectrophotometry and its integrity checked using an Agilent Bioanalyzer instrument. 1 mg total RNA was amplified and labelled with Cy3 and Cy5 fluorochromes using the Amino Allyl MessageAmp aRNA kit according to the manufacturer's (Ambion) protocol. Cy3-and Cy5-labeled cRNAs were fragmented using Fragmentation Buffer (Agilent), dissolved in Hybridization Buffer (Agilent) and hybridized to pan-genomic mouse microarrays of the RNG/MRC resource (http://www.microarray.fr). These microarrays harbor 24109 50mer oligonucleotides[1]. Probe sequences are available on the MEDIANTE web site (http://www.microarray.fr:8080/merge/index). They are archived at GEO (http://www.ncbi.nlm.nih.gov/projects/geo/query/acc.cgi?acc=GPL1476) as platform GPL1476.

Hybridization procedures
Cy3-and Cy5-labeled cRNAs dissolved in hybridization buffer were hybridized on previously processed slides (treated with 50 mM ethanolamine in 50 mM borate buffer, pH 9.0 at 20°C for 1 hour) in a total volume of 500 µL using the Microarray Hybridization Chamber (Agilent) at 62°C for 17 hours using a 4 rpm agitation. Slides were washed with washing buffer #1 (6X SSC, 0.005% Triton X-102) at 20°C for 10 min and with washing buffer #2 (0.1X SSC, 0.005% Triton X-102) at 4°C for 5 min, dried and stocked under vacuum. Fluorescence data were acquired from slides using a GenePix 4000B instrument (Molecular Devices).

Measurement data

Data are deposited in the GEO database under the accession number GSE5912.

Data analysis

TIF images containing the data from each fluorescence channel were quantified with the GenePix Pro 6.0 program (Axon Instruments).

Data were analyzed using programs of the TM4 software suite (http://www.tm4.org)[5]. Data were imported from the MEDIANTE database as .gpr files and converted into .mev files using the ExpressConverter software. They were susequently normalized using the lowess method and the flip dye routine on the MIDAS software. Analysis was performed using the Significance Analysis of Microarrays (SAM)[6] method on MeV 4.0 after percentage cutoff filtering (=100%). SAM parameters were set as follows:

10 days old group of mice	FDR=18.4%	# falsely significant genes=2.2
4 month old group of mice	FDR=24.4%	# falsely significant genes=5.1

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2. Didier, G., Brezellec, P., Remy, E. & Henaut, A. GeneANOVA – gene expression analysis of variance. *Bioinformatics* **18**, 490-491 (2002).
3. Smyth, G.K. Linear models and empirical Bayes methods for assessing differential expression in microarray experiments. *Stat. Appl. Genet. Mol. Biol.* **3**, 3 (2004).
4. Wettenhall, J.M. & Smyth, G.K. limmaGUI: a graphical user interface for linear modeling of microarray data. *Bioinformatics* **20**, 3705-3706 (2004).
5. Saeed, A.I. *et al.* TM4: a free, open-source system for microarray data management and analysis. *Biotechniques* **34**, 374-378 (2003).
6. Tusher, V.G., Tibshirani, R. & Chu, G. Significance analysis of microarrays applied to the ionizing radiation response. *Proc. Natl. Acad. Sci. USA* **98**, 5116-5121 (2001).

Supplementary Table I.
Transcripts significantly regulated in H295R cells upon SF-1 overexpression.

Abbreviation	Name	log2(intensity)	log2(ratio)	statistical score
NOV	nephroblastoma overexpressed gene/CCN3	14,04	-1,94	1,60
DUSP6	dual specificity phosphatase 6	10,32	-1,81	0,69
COL15A1	collagen, type XV, alpha 1	8,79	-1,50	0,04
CHAD	chondroadherin	11,23	-1,48	0,14
TM4SF12	transmembrane 4 superfamily member 12	10,69	-1,37	1,68
BHLHB3	basic helix-loop-helix domain containing, class B, 3	11,66	-1,29	1,62
CYP21A2	cytochrome p450, family 21, subfamily A, polypeptide 2	9,66	-1,20	0,75
FLJ38335	ankyrin-repeat and fibronectin type III domain containing 1 (ANKFN1)	10,29	-1,09	0,16
MGC4504	ChaC, cation transport regulator homolog 1 (E. coli) (CHAC1)	12,32	-1,08	0,34
ENPP2	ectonucleotide pyrophosphatase/phosphodiesterase 2 (autotaxin)	9,33	-1,03	0,61
TM4SF13	transmembrane 4 superfamily member 13	10,95	-0,95	0,32
FGF13	fibroblast growth factor 13	10,38	-0,93	1,50
PDGFD	platelet derived growth factor D	11,89	-0,92	1,00
DDIT4L	DNA-damage-inducible transcript 4-like	13,25	-0,92	0,22
NPTX2	neuronal pentraxin 2	13,37	-0,89	1,31
ETV1	Ets variant gene 1	8,98	-0,88	1,97
TNFRSF19	tumor necrosis factor receptor superfamily, member 19	12,03	-0,87	1,27
ABCA3	ATP-binding cassette, sub-family A (ABC1), member 3	10,04	-0,86	0,25
C9orf58	chromosome 9 open reading frame 58	12,56	-0,85	0,52
AKAP7	A kinase (PRKA) anchor protein 7	8,99	-0,85	0,70
ACPL2	acid phosphatase-like 2	12,49	-0,84	1,64
DKFZP566N034	transmembrane protein 163 (TMEM163)	7,64	-0,83	0,51
SYTL2	synaptotagmin-like 2	12,18	-0,83	1,11
TAS2R49	taste receptor, type 2, member 49	5,19	-0,82	0,58
IFIT3	interferon-induced protein with tetratricopeptide repeats 3	11,13	-0,80	0,91
ITGA1	integrin alpha 1	10,89	-0,79	1,87
TM7SF3	transmembrane 7 superfamily member 3	11,72	-0,77	0,98
SERPINA5	serpin peptidase inhibitor, clade A (alpha-1 antiproteinase, antitrypsin), member 5	13,49	-0,76	1,05
LCP1	lymphocyte cytosolic protein 1 (L-plastin)	10,36	-0,75	0,43
CDW92	solute carrier family 44, member 1 (SLC44A1)	8,74	-0,74	1,90
PDK4	pyruvate dehydrogenase kinase, isozyme 4	10,14	-0,73	0,68
NR0B1	nuclear receptor subfamily 0, group B, member 1 (DAX-1)	10,71	0,75	0,47
PLXNB2	plexin B2	9,74	0,75	0,51
AKR1B1	aldo-keto reductase family 1, member B1 (aldose reductase)	14,17	0,76	2,17
TBC1D13	TBC1 domain family, member 13	12,01	0,76	1,71
SHOX	short stature homeobox	5,56	0,77	1,58
HSD11B2	hydroxysteroid (11-beta) dehydrogenase 2	9,35	0,77	0,10
DCBLD2	discoidin, CUB and LCCL domain containing 2	11,97	0,77	0,84
PPP1R13B	protein phosphatase 1, regulatory (inhibitor) subunit 13B	11,97	0,78	0,84
LOC283514	similar to seven in absentia 2	7,99	0,78	0,78
AMIGO2	adhesion molecule with Ig-like domain 2	8,62	0,79	0,81
CIDEA	cell death-inducing DFFA-like effector A	5,09	0,80	0,61
CCND3	cyclin D3	11,04	0,81	0,81
LOC151162	encoding hypothetical protein LOC151162	10,18	0,81	0,72
VAV2	Vav 2 oncogene	11,77	0,83	1,41
MGC16121	encoding hypothetical protein MGC16121	10,97	0,83	1,41
SNF1LK	SNF1-like kinase	9,48	0,87	1,44
RIMS3	regulating synaptic membrane exocytosis 3	11,43	0,88	1,25
SLC16A10	solute carrier family 16 (monocarboxylic acid transporters), member 10	8,18	0,89	0,70
CDKN2D	cyclin-dependent kinase inhibitor 2D (p19)	10,17	0,89	2,23
COL11A1	collagen, type XI, alpha 1	12,69	0,91	0,41
DDX48	DEAD (asp-glu-ala-asp) box polypeptide 48	12,93	0,93	1,20
EMID1	EMI domain containing 1	11,51	0,94	2,79
KIAA1695	formin homology 2 domain containing 1 (FHOD1)	10,59	0,94	1,50
KIAA1644	encoding KIAA1644 protein	9,51	0,96	0,19
SULT2A1	sulfotransferase family, cytosolic, 2A, dehydroepiandrosterone (DHEA)-preferring, member 1	11,63	0,98	1,89
KIAA1145	transmembrane and coiled-coil domain family 3 (TMCC3)	8,95	1,01	0,84
CYP3A7	cytochrome p450, family 3, subfamily A, polypeptide 7	9,53	1,05	0,75
FLJ10094	ecto-NOX disulfide-thiol exchanger 1 (ENOX1)	6,11	1,05	0,89
ACSL6	acyl-CoA synthetase long-chain family member 6	8,06	1,06	0,36
GATA3	GATA binding protein 3	10,55	1,09	0,77
BMP6	bone morphogenetic protein 6	9,41	1,10	1,84
PJA1	praja 1	13,42	1,11	1,53
CDH22	cadherin-like 22	8,48	1,14	0,89
GATA3	GATA binding protein 3	10,26	1,14	1,01
NS5ATP13TP2	OAF homolog (Drosophila)	11,38	1,14	1,70
SLC31A2	solute carrier family 31 (copper transporters), member 2	9,84	1,15	0,48
AXL	AXL receptor tyrosine kinase	9,35	1,15	0,59
HSPB7	heat shock 27kDa protein family, member 7	11,75	1,16	2,71
FXYD6	FXYD domain containing ion transport regulator 6	13,28	1,19	1,74
DKFZp434C1915	encoding hypothetical protein DKFZp434c1915	14,04	1,20	1,63
TUBB5	tubulin, beta	12,60	1,28	0,29
LOC375682	encoding hypothetical protein LOC375682	10,16	1,29	0,53
TMOD1	tropomodulin 1	7,53	1,29	2,47
MGC45871	family with sequence similarity 101, member B (FAM101B)	10,98	1,29	1,58
SCEL	sciellin	7,89	1,34	1,89
RAB31	Rab31, member of ras oncogene family	9,55	1,39	2,56
EML1	echinoderm microtubule associated protein like 1	8,60	1,39	0,74
PTPRZ1	protein tyrosine phosphatase, receptor-type, Z polypeptide 1	10,15	1,47	0,47
NXN	nucleoredoxin	11,46	1,49	2,39
KIAA0802	encoding KIAA0802 protein	9,14	1,49	2,48
LOC388394	encoding reprimo-like	9,98	1,49	1,67
APOA1	apolipoprotein A1	9,81	1,55	2,62
LOC283157	encoding hypothetical protein LOC283157	9,41	1,58	1,16
C20orf160	chromosome 20 open reading frame 160	8,91	1,58	0,73
PPP2R5A	protein phosphatase 2, regulatory subunit B (B56), alpha isoform	10,55	1,59	2,47
EPAS1	endothelial PAS domain protein 1	10,91	1,63	0,93
KIAA1913	encoding KIAA1913 protein	13,02	1,67	1,47
DAAM2	dishevelled associated activator of morphogenesis 2	12,44	1,70	1,92
C21orf25	chromosome 21 open reading frame 25	11,88	1,73	1,43
FATE1	fetal and adult testis expressed 1	12,27	1,81	3,28
HES6	hairy and enhancer of split 6 (Drosophila)	10,96	2,07	1,65
SORL1	sortilin-related receptor, l (DLR class) A repeats-containing	8,42	2,14	1,48
MLLT7	myeloid/lymphoid or mixed-lineage leukemia (trithorax homolog, Drosophila); translocated to, 7	10,91	2,25	2,74
ATP1B1	ATPase, Na+/K+ transporting, beta 1 polypeptide	8,79	2,29	1,81
C21orf15	chromosome 21 open reading frame 15	10,66	2,33	1,09
TUBB	tubulin, beta	11,66	2,60	1,29
COL18A1	collagen, type XVIII, alpha 1	10,66	2,93	1,65

Supplementary Table II.

Amh and Gata-4 expression in the adrenal cortex of *Sf-1* transgenic mice.

Animal tag number	Tg line	Age	Immunostaining results
561	56	21 days	Amh+/Gata-4 -
562	56	25 days	Amh+/Gata-4 -
10233	56	6 mo	Amh-/Gata-4-
10932	37	3,5 mo	Amh-/Gata-4 -
9442	37	5 mo	Amh-/Gata-4 -
11076	27	25 days	Amh-/Gata-4 -
7111	14	12 mo	Amh-/Gata-4 +
7425	14	12 mo	Amh-/Gata-4 +
6068	14	9 mo	Amh+/Gata-4 -
WT		1 mo	Amh-/Gata-4 -
WT		3 mo	Amh-/Gata-4 -

Animal tag number	Tg line	Age	Immunoblot results
A571*	56	25 days	Gata-4 -
A439*	56	4 mo	Gata-4 -
11924*	56	8 mo	Gata-4 +
11823*	37	12 mo	Gata-4 -
11007*	37	12 mo	Gata-4 +
A600*	27	25 days	Gata-4 -
A602*	27	25 days	Gata-4 -
A604, A605, A606**	27	25 days	Gata-4 -
A209*	27	7 mo	Gata-4 +
A212*	27	7 mo	Gata-4 -
A213*	27	7 mo	Gata-4 +
A579*	14	25 days	Gata-4 -
A581*	14	25 days	Gata-4 -
A283*	14	7 mo	Gata-4 +
A280*	14	7 mo	Gata-4 -
A239*	7	7 mo	Gata-4 +
A241*	7	7 mo	Gata-4 +
WT**		2 mo	Gata-4 -

*Final volume of adrenal extract was 100 μl. Full size of sample was loaded onto the SDS PAGE gel for Western blot analysis with anti Gata-4 antibody

**Adrenals from 3 animals were combined in the volume 100 μl

Supplementary Table III.
Sequences of primers used for qRT-PCR.

TBP
5’ GAACATCATGGATCAGAACAACAG 3’
5’ ATTGGTGTTCTGAATAGGCTGTG 3’

CYP21A2
5’ GTCATCATTCCGAACCTCCAA 3’
5’ GAACTCATGTGGCCTCTCCC 3’

HSD3B2
5’ CGGGCCCAACTCCTACAAG 3’
5’ GCCATGTGTTTTCCAGAGGC 3’

NOV
5’ GATGGGCAGATTGGCTGTGT 3’
5’ GGCAGTTAGGCTCAGGCAGT 3’

DUSP6
5’ AGATACGCTCAGACCCGTGC 3’
5’ AGCCACGCCACCGTCTT 3’

TRB3
5’ ACTGTCACCAGCACGGTCTG 3’
5’ CTCACGGTCAGCGAAGACAA 3’

DDIT3
5’ AGAACCAGGAAACGGAAACAGA 3’
5’ TTCATGCGCTGCTTTCCA 3’

FXYD5
5’ TCGCAGCTGTGCTGTTCATC 3’
3’ GGGACAGCTGCCTGCACT 3’

CDKN1A
5’ CGCTAATGGCGGGCTG 3’
5’ CGGTGACAAAGTCGAAGTTCC 3’

BBC3
5’ GGACGACCTCAACGCACAGT 3’
5’ AGATTGTACAGGACCCTCCAGG 3’

C21orf25
5’ GCCAGGCTATTCAGTTCTTGGT 3’
5’ CGTACAGCCGGCATCCAG 3’

COL18A1
5’ TCCAGAAGTGAAGAAGTCGAGGA 3’
5’ GACAGAATCTGAGCCAGGAAGTG 3’

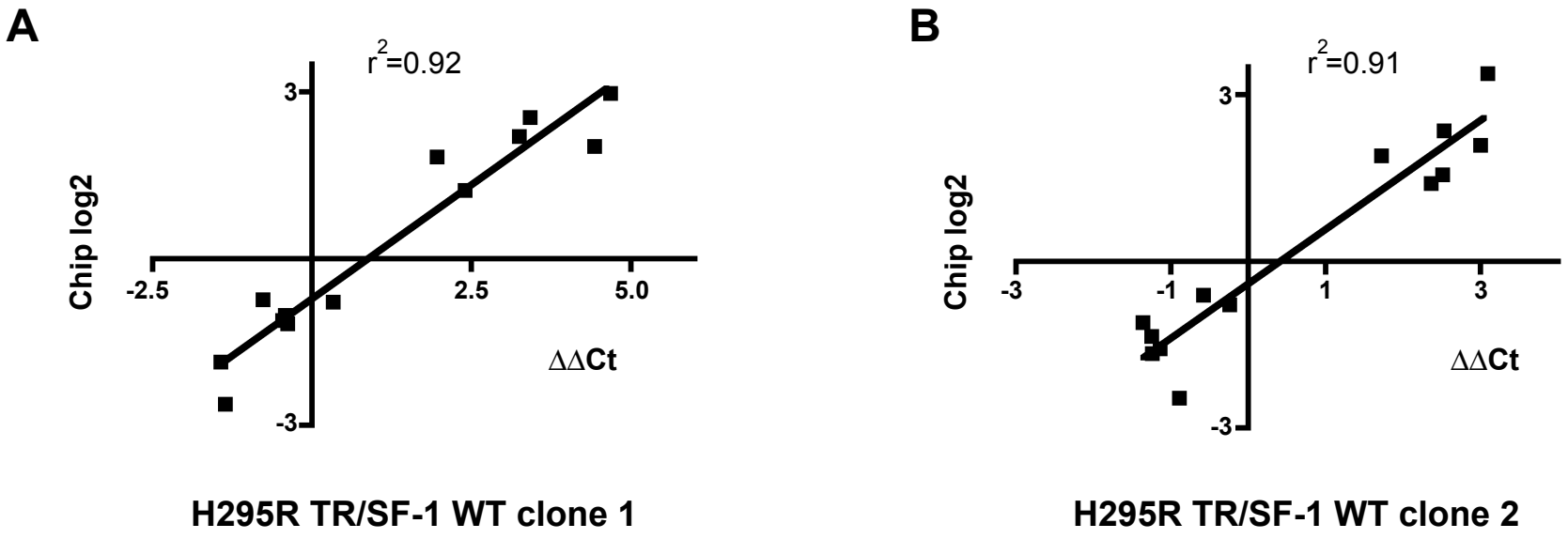
HES6
5’ CGAGCTCCTGAACCATCTGC 3’
5’ CCTGGAAGCTGCTGCCC 3’

FATE1
5’ GGCAATTTCCAAGGCATACG 3’
5’ CTAGTCTGCGCCACTGCATC 3’

GATA3
5’ AAATGAACGGACAGAACCGG 3’
5’ TGCTCTCCTGGCTGCAGAC 3’

MLLT7
5’ GAGCCCTGTCGGCCACT 3’
5’ TCTTCACGGTTTCGAGAGCA 3’

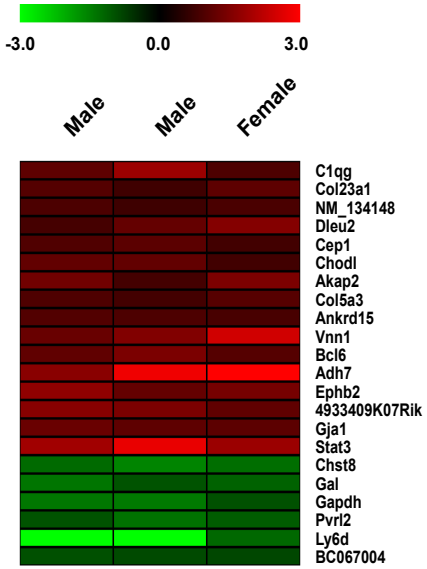
Supplementary Figure 1



H295R TR/SF-1 WT clone 1

H295R TR/SF-1 WT clone 2

Supplementary Figure 2



LEGENDS TO SUPPLEMENTARY FIGURES

Supplementary Figure 1. Correlation between microarray and qRT-PCR results. A, H295R TR/SF-1 WT clone 1 and B, H295R TR/SF-1 WT clone 2 cells. Transcripts analyzed were: *NOV, DUSP6, TRB3, DDIT3, FXYD5, CDKN1A*, *BBC3, C21orf25, COL18A1, HES6, FATE1, GATA-3, MLLT7.*

Supplementary Figure 2. Transcripts modulated in *Sf-1* transgenic mice adrenals (line #56) vs. wild-type littermates. For each group of animals (see MIAME description for experiments details) gene expression profiles were compared in two male and one female animals of each genotype.