Supporting Information

Inoue et al. 10.1073/pnas.1323416111

SI Materials and Methods

Fertility. Each male mouse was allowed to breed with two wild-type (WT) female mice for 2 wk. Females were checked every morning for copulatory plugs. Females were separated from males after 2 wk and monitored to assess pregnancy and litter size.

Sperm Numbers and Motility. Sperm were isolated from epididymides and incubated for 30 min in warm Embryomax Human Tubal Fluid medium [Millipore; catalog no. MR-070-D(CH)]. Sperm numbers and motility were evaluated under a light microscope according to standard protocols.

Histology and Immunohistochemistry. Hematoxylin/eosin (H&E) staining and immunohistochemistry (IHC) were examined as previously described (1). Briefly, samples isolated testes and epididymides for immunohistochemistry were fixed in either Bouin's solution (Sigma; catalog no. HT10132) or formalin. Immunostaining was performed with antibodies recognizing the following proteins: TAp73 (Santa Cruz Biotechnology; catalog no. sc-7957), protamin-1 (Santa Cruz Biotechnology; catalog no. sc-23105), Ki67 (Dako; catalog no. M7249), γH2AX (Millipore; catalog no. 07-164), and 8-hydroxy-2′-deoxyguanosine (Abcam; catalog no. AB48508). All samples for IHC were formalin-fixed, except samples for IHC of γH2AX or 8-hydroxy-2′-deoxyguanosine, which were fixed in Bouin's solution. Images were digitized using an Olympus-Hamamatsu Nanozoomer (2.0HT) slide scanner.

***N*-Acetyl-L-Cysteine Administration.** Sterile drinking water containing 10 mg/mL *N*-acetyl-L-cysteine (NAC) (Sigma; catalog no. A7250) was adjusted to pH 7.4. Mice were allowed free access to either standard drinking water or NAC-supplemented drinking water from the age of 3 wk. The NAC-supplemented water was replaced every 2 d. These experiments were approved by a University Health Network Animal Care Committee (ID: AUP1996).

Electron Microscopy. Testes were excised, bisected, and fixed overnight at 4 °C in 2% (wt/vol) glutaraldehyde in 0.1 M sodium cacodylate buffer (pH 7.4). Fixed samples were cut into small cubes and postfixed for 1 h at room temperature in 1% osmium tetroxide/1% potassium ferrocyanide. Cells were then stained en bloc overnight in 5% (wt/vol) aqueous uranyl acetate, dehydrated, and embedded in Taab epoxy resin (Taab Laboratories Equipment). Ultrathin sections were stained with lead citrate, and examined using a Jeol 100-CXII electron microscope (Jeol). Images were recorded and analyzed with a Megaview 3 digital camera and iTEM software (Olympus Soft Imaging Solutions).

Measurement of Metabolites in Serum. Serum from male mice were evaluated for level of several metabolites. Analyses were done by the Center for Modeling Human Disease (CMHD) and CMHD Physiology Core Department (Toronto, Canada).

ChIP. Saos-2 tet-on inducible cells for HA-TAp73β expression were exposed to 2 μg/mL doxycycline for 16 h. Cells were collected at 80% confluence and fixed in 1% formaldehyde. Chromatin was sheared by sonication and immunoprecipitated for 2 h with anti-HA antibody (Covance) or mouse IgG according to MAGnify ChIP system procedure (Invitrogen). The coimmunoprecipitated genomic DNA fragments and input DNA were amplified by PCR and run on agarose gel. Primers used were as follows: MDM2, GGTTGACTCAGCTTTTCCTCTTG (forward) and GGAAAATGCATGGTTTAAATAGCC (reverse); p21, GGGTCTGCTACTGTGTCCTC (forward) and GCAGAGGATGGATGGTTGTTCATCT (reverse); and MMP13, TCAGGTAGACACAAGACATCTC (forward) and GTGGGAAGAAGCAGAGAGTAG (reverse).

1. Inoue S, et al. (2013) Mule/Huwe/Arf-BP1 suppresses Ras-driven tumorigenesis by preventing c-Myc/Miz1-mediated down-regulation of p21 and p15. *Gnes Dev* 27(10):1101–1114.

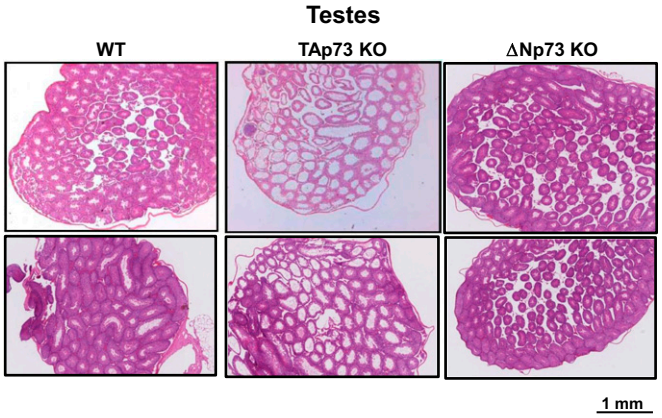

Fig. S1. Unique histological alterations are observed in testes of TAp73 KO mice. Histological sections of testes from WT, TAp73 KO, and ΔNp73 KO mice at 11 wk of age stained with H&E. Only in TAp73 KO mice are elongated spermatids or mature spermatozoa missing from the seminiferous tubules.

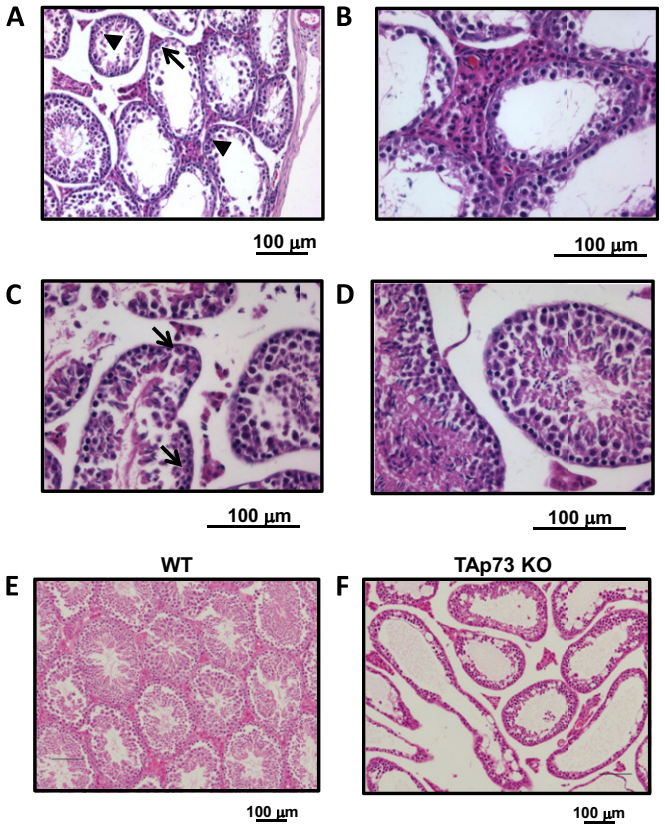

Fig. S2. Severity of the histological alterations in TAp73 KO testes increases with age. (*A–D*) Representative H&E-stained histological sections of seminiferous tubules from 11-wk-old TAp73 KO mice. (*A*) Seminiferous tubules contain a hypoplastic epithelium comprised of Sertoli cells and one or two layers of germ cells/spermatocytes but no or very few spermatids or mature spermatozoa. Although early synchronous maturation is present in the tubules, the majority of cells present are either early germ cells (arrows) or early round spermatocytes (arrow heads). (*B*) Higher magnification of *A* shows seminiferous tubules with marked deficit in elongating spermatids, degenerating tubule epithelium, and decreased number of germ cells. (*C*) Seminiferous tubules with scattered apoptotic spermatogonia (arrows). (*D*) Sections of testes from 11-wk-old ΔNp73 KO control mouse are not different from wild type. (*E* and *F*) H&E-stained histological sections of testes from 36-wk-old WT (*E*) and TAp73 KO (*F*) mice.

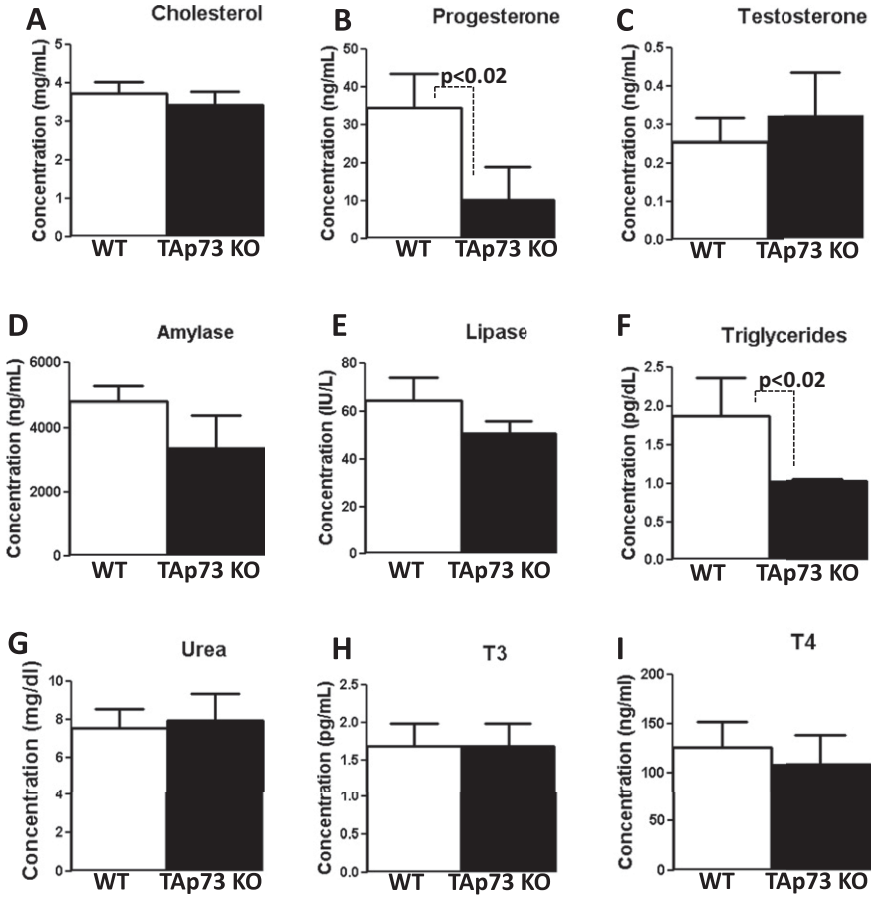

Fig. S3. Decreased serum progesterone in TAp73 KO male mice. (*A–I*) Serum levels of the indicated metabolites were determined in 36-wk-old WT and TAp73 KO mice ($n = 3$ mice/group). Data are the mean ± SD. *P* values were determined according to unpaired Student *t* test.

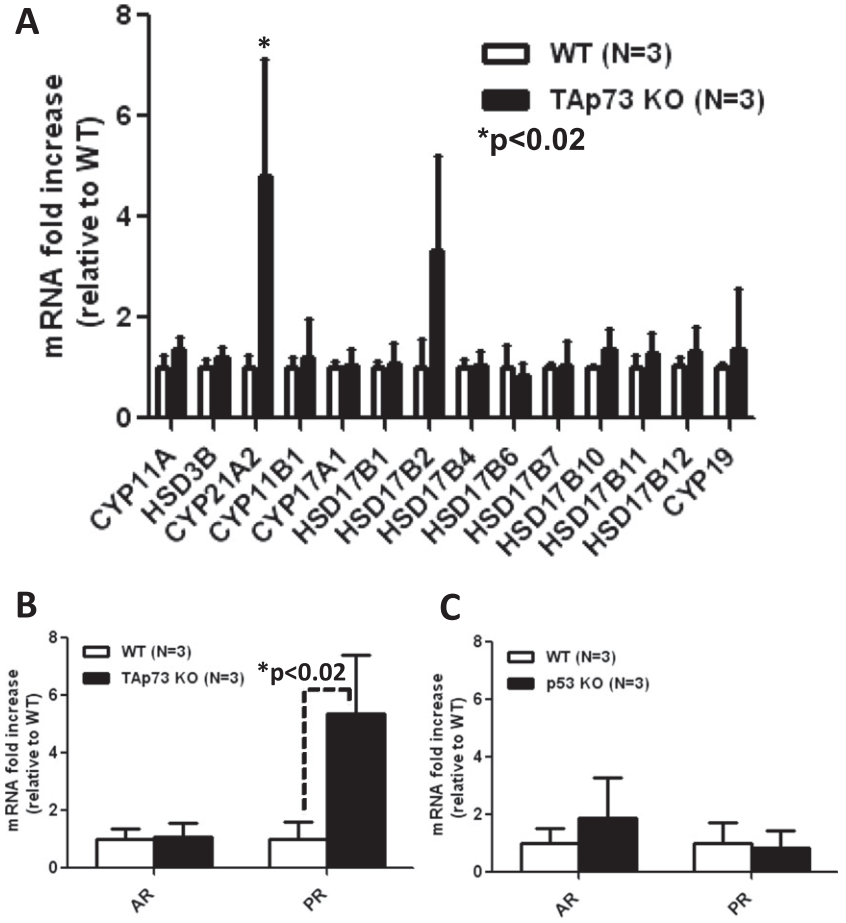

Fig. S4. CYP21A2 mRNA is up-regulated in testes of TAp73 KO mice. (*A–C*) Quantitative RT-PCR determination of levels of the indicated mRNAs related to steroidogenesis from 36-wk-old WT ($n = 3$) and TAp73 KO ($n = 3$) mice (*A*) and androgen (AR) and progesterone receptor (PR) in whole testes from 36-wk-old WT ($n = 3$) and TAp73 KO ($n = 3$) mice (*B*) and 18-wk-old WT ($n = 3$) and p53 KO ($n = 3$) mice (*C*). Data are the means ± SD and are expressed relative to WT levels after normalization to expression of the housekeeping gene encoding 18S RNA. *P* values were determined according to unpaired Student *t* test.

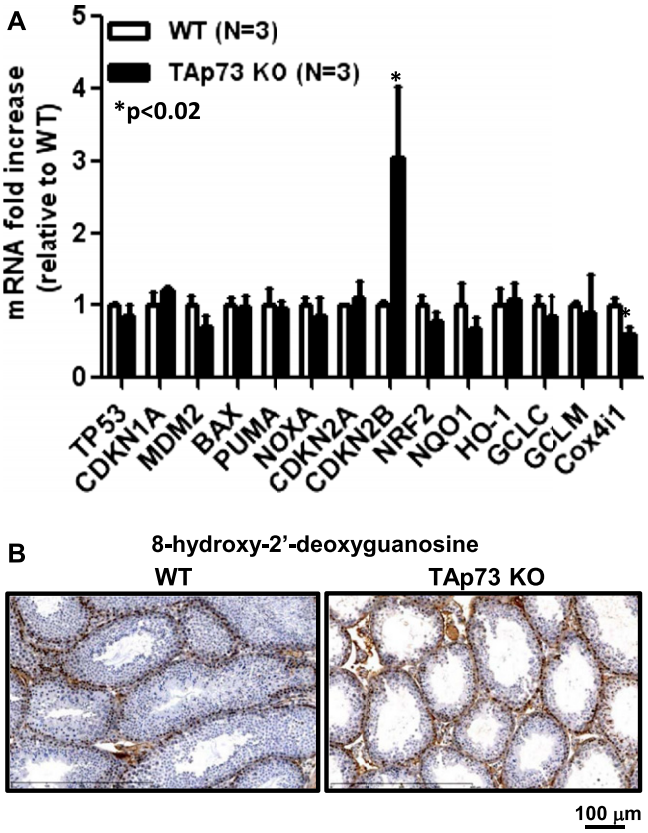

Fig. S5. No evidence for oxidative stress in testes of TAp73 KO mice. (*A*) Quantitative RT-PCR determination of the levels of the indicated mRNAs in testes from 36-wk-old WT ($n = 3$) and TAp73 KO ($n = 3$) mice. Data are the means ± SD and are expressed relative to WT levels after normalization to expression of the housekeeping gene encoding 18S RNA. *P* values were determined according to unpaired Student *t* test. (*B*) Representative immunohistochemical analysis to detect 8-hydroxy-2′-deoxyguanosine, a marker of oxidative DNA damage, in testes of 16-wk-old WT and TAp73 KO mice. Results are representative of seven mice examined per group.

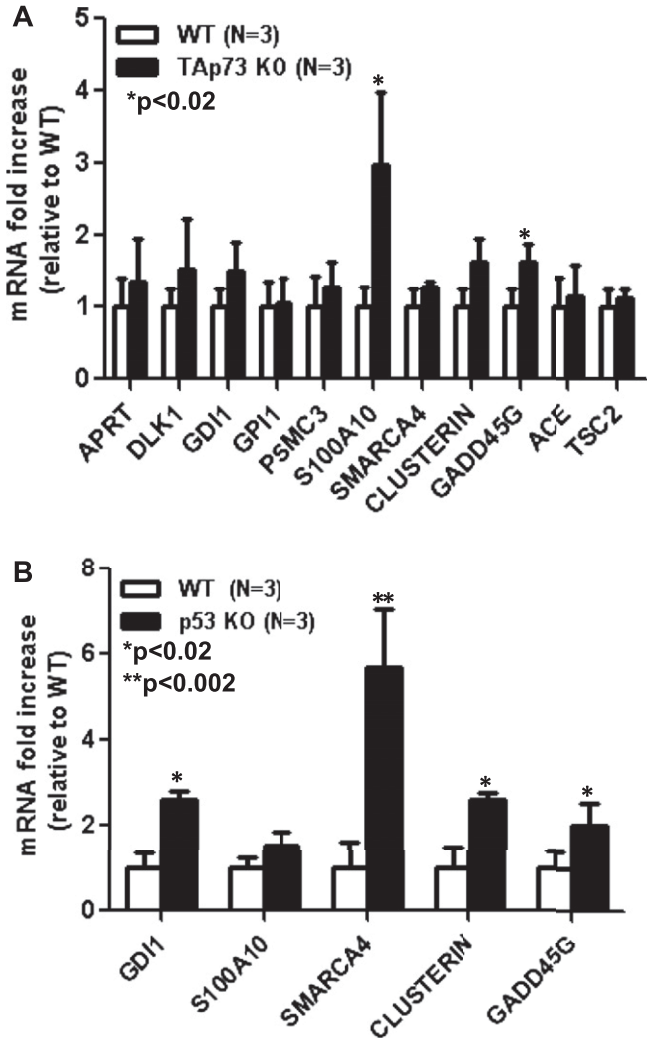

Fig. S6. S100A10 mRNA is up-regulated in testes of TAp73 KO but not p53 KO mice. (*A* and *B*) Quantitative RT-PCR determinations of levels of the indicated genes, which were listed in microarray analysis using human clinical and infertile gene knockout mice samples in testes of 36-wk-old WT ($n = 3$) and TAp73 KO ($n = 3$) mice (*A*) and 18-wk-old WT ($n = 3$) and p53 KO ($n = 3$) mice (*B*). Data are the means ± SD and are expressed relative to WT levels after normalization to expression of the housekeeping gene encoding 18S RNA. *P* values were determined according to unpaired Student *t* test.

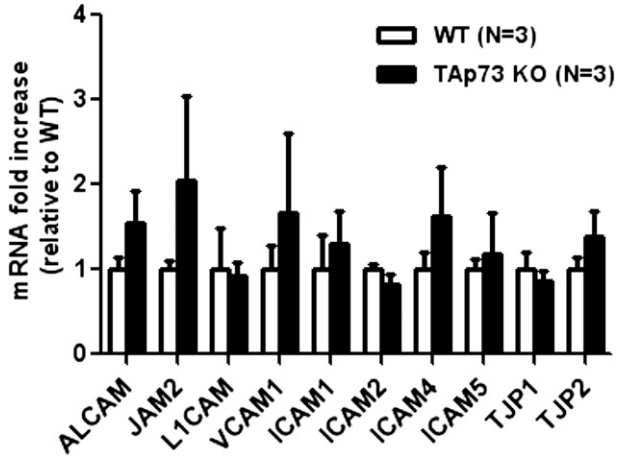

Fig. S7. Normal expression of genes related to cell adhesion in testes of TAp73 KO mice. Quantitative RT-PCR determination of levels of the indicated mRNAs in testes from 36-wk-old WT ($n = 3$) and TAp73 KO ($n = 3$) mice. Data are the means ± SD and are expressed as in Fig. S4. *P* values were determined according to unpaired Student *t* test.

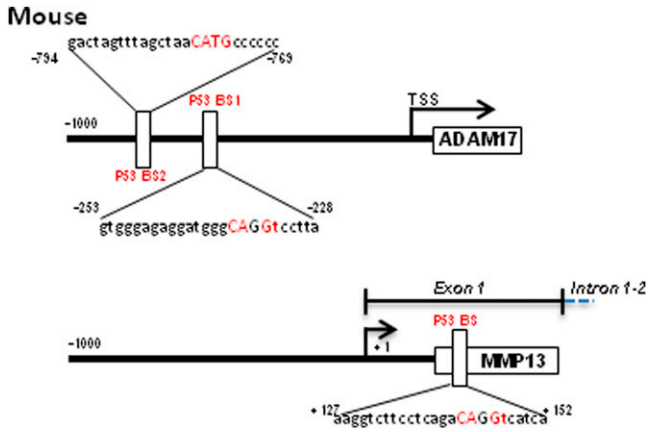

Fig. S8. Positive p53-binding sites in mouse ADAM17 and MMP13. Bioinformatics analysis of mouse ADAM17 and MMP13 promoters using the MatInspector program by Genomatrix has been analyzed for putative p53-binding sites (p53BS). Two p53 BSs in mouse ADAM17 were found; the first is located at −794/−769 bp from the ATG and the second at −253/−228 bp from the ATG. One p53BS in mouse MMP13 was found that is located at +127/+152 bp from the ATG.

Table S1. Male fertility data from TAp73 KO mice at different age and genetic background

*Strain	Genotype	Age (wk)	*n*	Plugs	Fertility	Littersize	†Sperm
TAp73 (F1)	WT	8	7	100% (7/7)	100% (7/7)	7.4	Yes/moving well
	TAp73 KO		6	100% (6/6)	33.3% (2/6)	2	Yes/partially moving
	WT	16	5	‡n.t.	‡n.t.	7.4	Yes/moving well
	TAp73 KO		3	‡n.t.	‡n.t.	0	Yes/Not moving
	WT	36	10	‡n.t.	‡n.t.	‡n.t.	Yes/moving well
	TAp73 KO		10	‡n.t.	‡n.t.	‡n.t.	Yes/Not moving
TAp73 (F6)	WT	7	10	90% (9/10)	90% (9/10)	6.3	Yes/moving well
	TAp73 KO		10	80% (8/10)	40% (4/10)	3.8	Yes/moving well
	WT	11	8	87.5% (7/8)	87.5% (7/8)	6	Yes/moving well
	TAp73 KO		11	81.8% (9/11)	0 % (0/11)	0	No sperms
	WT	16	4	100% (4/4)	100% (4/4)	5.3	Yes/moving well
	TAp73 KO		4	50% (2/4)	0% (0/4)	0	No sperms
TAp73 (F9)	WT	11	9	100% (9/9)	100% (9/9)	6.1	Yes/moving well
	TAp73 KO		10	90% (9/10)	50% (5/10)	4	Yes/moving well
	WT	16	10	90% (9/10)	80% (8/10)	5.4	Yes/moving well
	TAp73 KO		10	80% (8/10)	30% (3/10)	3.7	less/moving
ΔNp73 (F9)	Δp73 KO	11	6	100% (6/6)	100% (6/6)	5.8	Yes/moving well
p53 (F9)	P53 KO	11	6	83.3% (5/6)	83.3% (5/6)	5.2	Yes/moving well

*C57BL6:129Ola
†Motility of isolated sperms from epididymides was assessed in under microscope.
‡Not tested.

Table S2. NAC administration does not rescue male infertility of TAp73 KO

Treatment	TAp73 genotype	Age, wk	*n*	Plugs	Fertility
Without NAC	WT	16	4	100% (4/4)	100% (4/4)
	TAp73 KO		5	100% (5/5)	0% (5/5)
With NAC	WT	16	4	100% (4/4)	100% (4/4)
	TAp73 KO		4	100% (4/4)	0% (4/4)

Table S3. List of primers for qPCR for mouse genes

Primer for qPCR	Forward	Reverse
18S rRNA	CGGCGACGACCCATTCGAAC	GAATCGAACCCTGATTCCCCGTC
CDH1	CAGGTCTCCTCATGGCTTTGC	CTTCCGAAAAGAAGGCTGTCC
PLZF	CCCAGTTCTCAAAGGAGGATG	TTCCCACACAGCAGACAGAAG
DMC1	ATGAAGGAGGATCAAGTTGTGC	CATGCTTCTGCAACAGGTCAA
ID2	ATGAAAGCCTTCAGTCCGGTG	AGCAGACTCATCGGGTCGT
PGK2	TTCTGCTAAGTTGACTCTGGACA	AGCCTTGATTCTCTGGTTGTTTG
PRM1	CCGTCGCAGACGAAGATGTC	CACCTTATGGTGTATGAGCGG
ALCAM	ATGGCATCTAAGGTGTCCCCT	CTGAGTTGACAGTGTACCATCC
JAM2	GTGCCCACTTCTGTTATGACTG	TTCCCTAGCAAACTTGTGCCA
VCAM1	AGTTGGGGATTCGGTTGTTCT	CCCCTCATTCCTTACCACCC
ICAM1	GTGATGCTCAGGTATCCATCCA	CACAGTTCTCAAAGCACAGCG
ICAM2	TGGTCCGAGAAGCAGATAGTAG	GAGGCTGGTACACCCTGATG
ICAM4	GGCCACAAGTACACTCTGC	GGCTTAAAGCGAGGACTGTCA
ICAM5	TCCGAACTTTCCAGCGACC	CTACGAAACTGCGGCGAATC
TJP1	GCCGCTAAGAGCACAGCAA	TCCCCACTCTGAAAATGAGGA
TJP2	ATGGGAGCAGTACACCGTGA	TGACCACCCTGTCATTTTCTTG
SERPINE1	TTCAGCCCTTGCTTGCCTC	ACACTTTTACTCCGAAGTCGGT
SERPINE2	CACATGGGATCGCGTCCATC	CAGCACTTTACCAACTCCGTTTA
SERPINA5	AGAAGAAGGCTAAAGAGTCCTCG	CTCATAGACACGCTCAAGGGG
SPINK2	CATGAGACTCTCGACTCTTCCG	CGCACACAGGGTTGAGGTT
SPINK3	TTTGGCCCTGCTGAGTTTAGC	TGGCATAAGTAATTCCGTCAGTC
SPINT1	GTCGGCGTATGGCTCCTTT	GCTTCGGTGTCCAGCACAA
ADAM10	ATGGTGTTGCCGACAGTGTTA	GTTTGGCACGCTGGTGTTTTT
ADAM12	CACACGGATCATTGTTACTACCA	ATTGGCTCTAAGCTGTACGTTTT
ADAM17	GTACGTCGATGCAGAGCAAA	AAACCAGAACAGACCCAACG
ADAM19	TCAGTGGCGGACTTCAGAAAG	GCAAAAAGGTGCTCGTTCTTC
MMP1B	GCTCATGCTTTTCTGCCAGG	TAGAATGGGAGAGTCCAAGGG
MMP2	CAAGTTCCCCGGCGATGTC	TTCTGGTCAAGGTCACCTGTC
MMP13	CTTCTTCTTGTTGAGCTGGACTC	CTGTGGAGGTCACTGTAGACT
MMP14	CAGTATGGCTACCTACCTCCAG	GCCTTGCCTGTCACTTGTAAA
TIMP1	GCAACTCGGACCTGGTCATAA	CGGCCCGTGATGAGAAACT
TIMP2	TCAGAGCCAAAGCAGTGAGC	GCCGTGTAGATAAACTCGATGTC
TIMP3	CTTCTGCAACTCCGACATCGT	GGGGCATCTTACTGAAGCCTC
TIMP4	TGTGGCTGCCAAATCACCA	TCATGCAGACATAGTGCTGGG
TP53	CTCTCCCCCGCAAAAGAAAAA	CGGAACATCTCGAAGCGTTA
CDKN1A	CCACTTTGCCAGCAGAATAA	ACGGGACCGAAGAGACAAC
MDM2	CCAGCTTCGGAACAAGAGAC	GTCGTTTTGCGCTCCAAC
BAX	TGGAGATGAACTGGACAGCA	GAAGTTGCCATCAGCAAACA
PUMA	TCACCCTGGAGGGTCATGTA	GCGGGTGTAGGCACCTAGT
NOXA	ATCAAGGGCTGAAGGGATTT	AGAGAGGCACCTGGGATATG
CDKN2A	CATGTTGTTGAGGCTAGAGAGG	TCGAATCTGCACCGTAGTTG
CDKN2B	ATCTGGAGCAGCATGGAGTC	TCGAATCTGCACCGTAGTTG
NRF2	GCCCACATTCCCAAACAAGAT	CCAGAGAGCTATTGAGGGACTG
NQO1	AGGATGGGAGGTACTCGAATC	AGGCGTCCTTCCTTATATGCTA
HO-1	AAGCCGAGAATGCTGAGTTCA	GCCGTGTAGATATGGTACAAGGA
GCLC	GGCTCTCTGCACCATCACTT	GTTAGAGTACCGAAGCGGGG
GCLM	AGGAGCTTCGGGACTGTATCC	GGGACATGGTGCATTCCAAAA
Cox4i1	TCACTGCGCTCGTTCTGAT	CGATCGAAAGTATGAGGGAT
CYP11A1	AGGTCCTTCAATGAGATCCCTT	TCCCTGTAAATGGGGCCATAC
HSD3B1	TGGACAAAGTATTCCGACCAGA	GGCACACTTGCTTGAACACAG
CYP21A2	AGACCCTTCACGACTGTGTC	CCGACTCTCTTGGATCTGCTT
CYP11B1	CAGATTGTGTTTGTGACGTTGC	CGGTTGAAGTACCATTCTGGC
CYP17A1	GCCCAAGTCAAAGACACCTAAT	GTACCCAGGCGAAGAGAATAGA
HSB17B1	ACTTGGCTGTTCGCCTAGC	GAGGGCATCCTTGAGTCCTG
HSB17B2	ATGAGCCCGTTTGCCTCTG	CCACAGGTAACAAGTCTTGGTC
HSD17B4	AGGGGACTTCAAGGGAATTGG	GCCTGCTTCAACTGAATCGTAA
HSD17B6	GGAGCGTGTTGGAGACAGAG	GAGGTTCACTTGAAAGATAGGCA
HSD17B7	TTTTTCTGCGGCATCTTTTCAAG	AGTGACCGAGTCATTCTGGGT
HSD17B10	GCTTGGTCGCGGTAGTAACTG	TGGGGCAAATATGCAGCTTTC
HSD17B11	AAGAACGGCATCGAGGAAACA	TCCTGCGTAGCAAATAAGTCTG
HSD17B12	GGTCTTGAGATTGGCGTTTTAGT	GTCCAAGTCGGGAATTTCCAG
CYP19	ATGTTCTTGGAAATGCTGAACCC	AGGACCTGGTATTGAAGACGAG
AR	CTGGGAAGGGTCTACCCAC	GGTGCTATGTTAGCGGCCTC

Table S3. Cont.

Primer for qPCR	Forward	Reverse
PR	CTCCGGGACCGAACAGAGT	ACAACAACCCTTTGGTAGCAG
APRT	CCCTCTTGAAAGACCCGGAC	TCCAGAGAATAGGAGGCTGAC
DLK1	AGTGCGAAACCTGGGTGTC	GCCTCCTTGTTGAAAGTGGTCA
GDI1	GGGACAGGTCTTACCGAATGC	AGCTCTCACCCCCATAGTAGG
PSMC3	GACCGTGTGGGATGAAGCTG	CGCTGGACAATCTCTTCCGTG
S100A100	TGGAAACCATGATGCTTACGTT	GAAGCCCACTTTGCCATCTC
SMARCA4	CAAAGACAAGCATATCCTAGCCA	CACGTAGTGTGTGTTAAGGACC
CLUSTERIN	AGCAGGAGGTCTCTGACAATG	GGCTTCCTCTAAACTGTTGAGC
GADD45G	GGGAAAGCACTGCACGAACT	AGCACGCAAAAGGTCACATTG
ACE	AGGTTGGGCTACTCCAGGAC	GGTGAGTTGTTGTCTGGCTTC
TSC2	GTTCCCGTGCTAACAGCATTA	TGGCGCAGCGGTAGATAAGGC

qPCR, quantitative RT-PCR.

Table S4. List of primers for qPCR for human genes

Primer for qPCR	Forward	Reverse
MMP13	CCAGTCTCCGAGGAGAAACA	AAAAACAGCTCCGCATCAAC
ADAM17	CCTTTCTGCGAGAGGGAAC	CACCTTGCAGGAGTTGTCAGT
TIMP4	ATCTGTGCAACTACATCGAGC	CGAGATGGTACAGGGTACTGTG
SPINK2	TCTCTGATCCCTCAATTTGGTCT	CCACACACAGGGTTAAAGTGTC
SPINT1	AACTACCTCACGAGGGAAGTG	GGTTGTACCTTCAAGTCTATGCC
CYP21A2	CTCACCTTCGGAGACAAGATCA	TCCACAATTTGGATGGACCAG