SI Experimental Procedures

LHR cDNA Mutants. Oligonucleotide-mediated site-directed mutagenesis was performed by using the QuickChangeII Site-Directed Mutagenesis Kit (Stratagene) according to the manufacturer's instructions. Clones selected by ampicillin were directly sequenced to verify that they contained the correct mutation. The entire sequence of each mutant cDNA was determined.

The LHR mutants (LHR^{LH−} and LHR^{AMP−}) as cDNAs were then subcloned into an expression vector pDNA3.1(-) (Invitrogen) between the restriction site of EcoRI and BamHI and tagged with HA or FLAG into the N-terminal coding sequence just after the signal peptide (referred to as FLAG-LHR^{AMP−} and HA-LHR^{LH−}). The entire coding region of each cDNA construct was sequenced to verify the different modifications.

BACs and Recombination. A BAC clone carrying the entire mouse LHR gene (RPC123-18D7) was obtained from the BAC PAC resources of Oakland Children's Hospital in E.coli strain H8996. The C22A mutation (LHR^{LH−}) was adjusted by a mouse codon usage program (www.entelechon.com). The point mutation in LHR^{LH−} and ΔTM67 deletion in LHR^{AMP−} were achieved by inserting and replacing a selection/counter selection cassette (RpsL-Neo) with single strand oligonucleotides (1) (see below for primer sequences). Bicistronic reporter cassettes were inserted by Red/ET recombination and screened with specific primers. Pro-lactin signal sequence was inserted by the ALFIRE procedure (2), first by inserting an RpsL-Neo cassette flanked by ISce-I restriction sites and homology arms to the bovine PRL signal sequence from a previously described vector (3). All areas of modification were sequenced to ensure correctness. PCR amplification for recombineering was performed using TripleMaster polymerase mix and buffers (Eppendorf). BAC DNA was propagated in bacteria by standard procedures and purified using a Large construct Maxiprep kit (Qiagen), linearized, PFGE gel-purified and injected into the pronucleus of fertilized mouse oocytes using standard procedures. Additional details about the experimental settings and oligos are below.

Tissues for Immunofluorescence, RT-PCR, and Receptor Binding Assays. Blood, testes, seminal vesicles, and other organs were collected, and their weights were recorded. Serum was separated by centrifugation, frozen, and stored at -20 °C until used for hormone assays. After removal, one of each pair of testes was immediately frozen in liquid nitrogen and stored at -70 °C until used for LHR binding and mRNA measurements. The other testis was fixed for 30 min with 4% paraformaldehyde at 4 °C, followed by incubation for an additional 15 min in 0.1% Triton after dehydration with ethanol, and embedded in paraffin for immunofluorescence or immunohistochemistry.

Immunofluorescence of Histological Sections. Whole-mount fixed testes sections were boiled in citric buffer (10 mM sodium citrate, 0.05% Tween-20, pH 6.0) for 10 min, washed and blocked with 10% normal goat serum (NGS) in PBS. Then, double immunofluorescent staining was performed using standard protocols and commercial mouse anti-RFP and rabbit anti-GFP (which also detects cCFP) antibodies (both MBL International Antibodies) diluted in PBS (0.05% goat serum) 1:100. Sections were incubated with primary antibodies overnight at 4 °C, and thereafter washed twice in PBS before exposure to the secondary antibodies (1:500) (Molecular Probes), goat anti-rabbit conjugated to Alexafluor-488 (green) for cCFP and goat anti-mouse Alexafluor-594 (red) for RFP, for 1h at room temperature. Nuclei were permeabilized in the last wash with PBS-Triton (0.1%) and stained with DAPI (Vector Laboratories) (dilution 1:10,000) for 5 min before mounting with Vectashield antifading medium (Vector Laboratories). Images were captured with a Leica DMRB fluorescent microscope (Leica Microsystems), imaged with a CCD camera using IM500 (Leica) and cropped in Adobe Photoshop.

Determination of BAC Transgene Copy Number by qPCR. The number of copies of each transgene in the genome of founder animals was measured by qPCR using DyNAmo SYBR Green (Finzymes) and standard procedures. Genomic DNA was isolated as described (4), precipitated with ethanol and resuspended in 10 mM Tris-HCl, pH 8.5. Genomic DNA from different mouse samples was measured by UV spectrophotometer and adjusted to 5 ng/μL. PCR reactions were performed in duplicate in 20 μL using 96-well plates (Bio-Rad) in a qPCR thermocycler (Chromo4 with OpticonMonitor software, Bio-Rad), containing primers that recognize both WT and TG sequences. Specific primers that detect only endogenous WT receptor gene (as for genotyping LuRKO mice) were also used for normalization of the samples and standardization of TG copy numbers. A linear standard curve was drawn using WT genomic DNA at different dilutions. Considering that WT genome contains 2 copies of the LHR gene, the extra number of copies in the founder TG animals was considered as TG copies according to the trend line equation and dilution used. The following conditions were used: 95 °C for 15 min, followed by 40 cycles of 94 °C for 20 s, 56 °C for 45 s, 72 °C for 1 min, 80 °C for 1 s.

Cell Cultures and Transfections. To analyze the ability of the LHR^{LH−} and LHR^{AMP−} mutants to generate cAMP signal, either basally or in response to hCG, HEK-293 cells previously seeded onto 96 well plates were transfected with high dose of 100 ng of the receptor cDNAs, using Lipofectamine 2000 (Invitrogen) transfection reagent. cAMP generation was monitored by cotransfection with 40 ng of a cre-luciferase plasmid DNA, and using 1 ng of pRL-CMV as a transfection control. The total concentration of transfected DNA was standardized using pcDNA 3.1. Forty-eight hours after transfection, cells were stimulated with 0, 0.05, 0.5 and 5 nM hCG for 4 h and assayed for cre-luciferase and pRL-CMV activity using Luciferase substrate solution (Perkin-Elmer), and coelenterazine substrate (Calbiochem) respectively. Luminescence was determined using a Victor2 plate-reading Luminometer (Perkin-Elmer). In another experiment, plasmids encoding β2-adrenergic receptor (β2-AR) and the LHR^{AMP−} mutant were cotransfected (100 ng of each) with cre-luciferase plasmid as above, and assayed for luciferase activity in the absence and presence of 0.05 and 0.5 nM hCG.

Immunofluorescence Staining of Tagged Receptors by Confocal Imaging. Visualization of the tagged LHR molecules was carried out using indirect immunofluorescence microscopy of HEK-293 cells stably transfected with N-terminally tagged LHR either HA-WT, HA-LHR^{LH−} or FLAG-LHR^{AMP−}. Surface receptors were labeled by ‘feeding’ the live intact cells either with a rabbit anti-FLAG antibody (1:500, Sigma) or mouse anti-HA antibody (1:250, Covance) for 20 min at 37 °C, before fixation (4% paraformaldehyde in PBS, 20 min), followed by incubation for an additional 15 min in the absence (nonpermeabilized) or presence of the 0.02% Nonidet P-40 in PBS with 2% FBS for 15 min (permeabilized). Cells were then washed extensively with PBS and further incubated for 30 min with goat anti-mouse or goat anti-rabbit Alexa Fluor 488-conjugated antibodies (1:1000, Invitrogen) in blocking solution. Specimens were washed extensively in PBS, mounted on
glass slides, and examined using a Leica SP5 laser scanning confocal microscope with a 63x/1.4NA oil immersion objective, using instrument settings verified to produce negligible bleed through between channels and an estimated section thickness of 1 μm. Micrographs shown are representative optical sections imaged through the center of the cell.

**Immunoprecipitation.** HEK-293 cells expressing either or both LHR mutants, or both LHR+cAMP and β2-AR (a gift from Mark von Zastrow, University of California San Francisco) were washed 48 h after tranfection, and collected using lysis buffer with protease inhibitors (50 mM Tris-HCl, pH 7.4, with 150 mM NaCl, 1 mM EDTA, and 1% TRITON X-100). The extracts were incubated on ice for 20 min followed by microfuge centrifugation for 15 min at maximal speed. Protein concentrations in the supernatants were measured using the Bradford assay. For compunonprecipitation of differentially tagged LHRs, and differentially tagged LHR+cAMP and β2-AR, one-mL aliquots of cell lysates were incubated overnight with 40 μL of anti-FLAG agarose affinity gel (A2220; Sigma) and then eluted with FLAG peptide (F4799; Sigma).

Immunoprecipitates were separated by SDS polyacrylamide-gel electrophoresis (SDS/PAGE) under reducing conditions, electroblotted onto a nitrocellulose membrane, probed with either an anti-FLAG antibody (F7425; Sigma) or an anti-HA antibody (sc-805; Santa Cruz Biotechnology), and horseradish peroxidase-conjugated goat anti-mouse IgG (1:1000; DAKO). The immunoreactive bands were visualized using an ECL detection system (Amersham Biosciences).

**mRNA Expression by qPCR.** Quantitative PCR was performed by standard protocols using DyNAmo SYBR Green (Finnzymes) kit. PCR reactions were performed in triplicate in 20 μL using 96-well plates (Bio-Rad) in a qPCR thermocycler (Chromo4 with OpticonMonitor software, Bio-Rad), using primers that recognize LHR (WT and mutants, see below). For normalization of the samples a housekeeping gene (Ppia) was used. A linear standard curve was drawn using different dilutions of a plasmid containing the cDNA of the LHR. Results are expressed as the number of LHR/Ppia versus WT control.

The same procedure was used to determine the level of expression of LH-dependent Leydig cell-specific StAR and Cyp-17α1 genes in testes of the different mutant mice, and results are presented below as percentage of WT controls.

**LHR Binding Measurements.** [125]I-hCG binding to testicular homogenates was measured as previously described (5, 6). Briefly, a piece of the frozen testis (see above) was homogenized with an Ultra-Turrax 18/10 homogenizer in Dulbecco’s PBS (100 mg testis/mL) containing 0.1% (wt/vol) BSA (Sigma Chemical), and testis/mL) containing 0.1% (wt/vol) BSA (Sigma Chemical), and was then centrifuged for 20 min followed by microfuge centrifugation for 15 min at maximal speed. Protein concentrations in the supernatants were measured using the Bradford assay. For competition binding-inhibition curves, 100 μL aliquots of LHR cDNA-transfected HEK-293 cells (6x10^5 cells) were incubated in triplicates) in the presence of 0.3 nM [125]I-hCG and increasing concentrations of cold hCG (0-10 nM). Incubations were terminated by addition of 4 mL ice-cold Dulbecco’s PBS-BSA. Bound and free hormone were separated by centrifugation at 3,000 g for 30 min. The supernatants were discarded, and the radioactivity in the pellets was measured in a γ-counter (1470 Wizard, Wallac).

**Measurement of Tubule Diameter and Leydig Cell Volume Density.** Tubule diameters and Leydig cell volume densities of testis samples were measured from paraffin sections stained with cosin-hematoxylin using a Leica microscope and Leica MI1000 software (Leica). Sections from three tubules per group were analyzed, all tubules in three sections were measured. In the same way, Leydig cell volume densities were measured and calculated as percentage (%) of the total area of the section.

**Oligonucleotides.** All of the short primers (20–30 nts) described in this paper were purchased from TAG. All longer primers (60–140 nts) were purchased from Thermo.

**LHR cDNA mutants.** The primers used to create the C22A, (LHR^LH−) mutant:

- 5′-GCCGGATGTTGCGCCTGCGACCCCTGCTGCGCCCTGCGTGTCGGGCACGC-3′ and 5′-GCCAGCTCGAGGCGCCATGCGGCCGCAAGGAGATGCGGCGCAGGAGACCATC-3′.

The primers used to create DTM67 (LHR^AMP−) mutant:

- 5′-AGCTTAGATATTTGACCAAACCTACGCTCAGGAGAGAAACCATGCGC-3′ and 5′-CCTTGGAGGGGTTGATGAGTTGGGTAAGATGGTGTGGTATGAAC-3′.

Primers used to insert FLAG epitope:

- 5′-CACAGTCGACCTCTTTACATCCATGCTGAGTACCGGAGACAGGATCAGTGTCAGCGCTTGGACG-3′.

Primers used to insert HA epitope:

- 5′-CACAGTCGACCTCTTTACATCCATGCTGAGTACCGGAGACAGGATCAGTGTCAGCGCTTGGACG-3′.

### For modification of the LHR gene in BAC clones using Red/ET recombination

Selection/counter selection cassette, Rpssl-Neo, (CS) was amplified with primers containing homology arms for the region to be modified, then Rpssl-Neo cassette was exchanged by a single strand (ss) oligonucleotide.

**For point mutation, LHRH−.**

- CS-LHRH−-F 5′-AGCGTGGCGCGCCGCCTGCGACCCCTGCTGCGCCCTGCGTGTCGGGCACGC-3′ and 5′-GCCAGCTCGAGGCGCCATGCGGCCGCAAGGAGATGCGGCGCAGGAGACCATC-3′.

**For insertion of the reporter genes.** Both reporter genes (eCFP and RFP) were amplified by PCR from bicistronic vectors containing a common IRES and polya, which were used for the primer binding sites. Reporter gene insertion after the LHR gene were performed before other modifications using the following primers:

- IRES-XFP-LHR-R 5′-CCATAGTGCACTGTCAACAACCTACACTCAAACAGCGTGGTCTACTTGTTAGATGATATTTGACCAAACCTACGCTCAGGAGAGAAACCATGCGC-3′.

Insertion of the PRL signal sequence by ALFIRE

ALFIRE-LHR-F 5′-GAGCTGGCGCGCCGCCTGCGACCCCTGCTGCGCCCTGCGTGTCGGGCACGC-3′ and ALFIRE-LHR-R 5′-GGGGGGAACATATTTAGATACAATTCA-3′.

Notation for the reporter genes. Both reporter genes (eCFP and RFP) were amplified by PCR from bicistronic vectors containing a common IRES and polya, which were used for the primer binding sites. Reporter gene insertion after the LHR gene were performed before other modifications using the following primers:

- IRES-XFP-LHR-R 5′-CCATAGTGCACTGTCAACAACCTACACTCAAACAGCGTGGTCTACTTGTTAGATGATATTTGACCAAACCTACGCTCAGGAGAGAAACCATGCGC-3′.

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- IRES-XFP-LHR-F 5′-CCATAGTGCACTGTCAACAACCTACACTCAAACAGCGTGGTCTACTTGTTAGATGATATTTGACCAAACCTACGCTCAGGAGAGAAACCATGCGC-3′.
Seminal vesicles had similar weights of the LuRKO, LHR\textsuperscript{LH\textsubscript{−}/cAMP\textsubscript{−}} mice, whereas the WT and LHR\textsuperscript{LH\textsubscript{+}/cAMP\textsubscript{+}} seminal vesicles had similar weights (Table S3).

Seminiferous tubular diameters and Leydig cell volume densities of the LuRKO, LHR\textsuperscript{LH\textsubscript{−}/cAMP\textsubscript{−}}, and LHR\textsuperscript{LH\textsubscript{−}/cAMP\textsubscript{−}}-plasmids were transgressed into HEK-293 cells, no cAMP signal (measured by cec-luciferase activity) was detected either in the absence of hCG (0.05, 0.5, and 5 nM) (Fig. S2), indicating that overexpression of one of the mutants alone is not able to activate cAMP generation, which was readily detected in cells expressing WT receptor. Likewise, cotransfection (100 ng) each of the LHR\textsuperscript{cAMP\textsubscript{−}}, mutant and β2-AR expression plasmids did not produce cAMP signal basally or in response to hCG stimulation (Fig. S2). Robust activation of signaling was observed in each case when WT LHR was transfected.

Table S1 Results

Weights of the LuRKO, LHR\textsuperscript{LH\textsubscript{−}} and LHR\textsuperscript{cAMP\textsubscript{−}} tests were approximately one third of those of WT mice, whereas the LH\textsuperscript{LH\textsubscript{−}/cAMP\textsubscript{−}} tests did not differ from the latter (Table S3). Seminal vesicles of the LuRKO, LHR\textsuperscript{LH\textsubscript{−}} and LHR\textsuperscript{cAMP\textsubscript{−}} mice were rudimentary, whereas the WT and LHR\textsuperscript{LH\textsubscript{+}/cAMP\textsubscript{+}} seminal vesicles had similar weights (Table S3).

With maximal amounts (100 ng) of LHR\textsuperscript{LH\textsubscript{−}} or LHR\textsuperscript{cAMP\textsubscript{−}}-plasmids were transgressed into HEK-293 cells, no cAMP signal (measured by cec-luciferase activity) was detected either in the absence of hCG (0.05, 0.5, and 5 nM) (Fig. S2), indicating that overexpression of one of the mutants alone is not able to activate cAMP generation, which was readily detected in cells expressing WT receptor. Likewise, cotransfection (100 ng) each of the LHR\textsuperscript{cAMP\textsubscript{−}}, mutant and β2-AR expression plasmids did not produce cAMP signal basally or in response to hCG stimulation (Fig. S2). Robust activation of signaling was observed in each case when WT LHR was transfected.

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Fig. S1. Details of the BAC constructs used to express binding- and signaling-deficient LHR mutants in transgenic mice. BAC clones containing the entire LHR genomic gene (exons numbered) were modified by point mutations, insertions, and deletions of specific areas. (A) One receptor mutant harbored an inactivating mutation in the LH/hCG ligand binding region (LHR LH−), where Cys22 was replaced by Ala22 (C22A) through a TGC/GCA point mutation. As reporter gene, a bicistronic cassette containing an internal ribosomal entry site (IRES) and enhanced cyan fluorescent protein (eCFP) were inserted after the LHR gene. (B) The other mutant contained a deletion of transmembrane domains 6 and 7 (ΔTM67) in exon 11 (amino acid deletion Val553 to Ala689), which are involved in G protein coupling and second messenger production (7) (LHR cAMP−) but retaining the intracellular domain. To ensure translocation of the latter LHR mutant to the cell membrane, a bovine prolactin (PRL) leader signal was inserted into the N-terminus of the mature receptor. As reporter gene, a bicistronic cassette containing an IRES and Dicosoma sp. red fluorescent protein (RFP), was inserted after the LHR gene. Primer positions are marked, for genotyping: IRESF for either transgene, with RFP-R for LHR LH−-IRES-eCFP, and with CFP-R for LHR cAMP−-IRES-RFP. (C) WT and LHR-knockout (LuRKO) alleles, and resulting WT receptor, no protein is generated by the LuRKO allele. Primers for genotyping the LHR knockout (LuRKO) and WT alleles were as previously described and shown in the figure to demark a different position. Primers F1-R1 were used for RT-PCR amplification of either transgene because LHR cAMP− encoded a shorter product. Schematic representation of each of the receptor mutants is portrayed on the right of the BAC diagrams marking, with blue and red colors the areas of modification.

Fig. S2. Inactive LHR mutants are unable to respond to hCG or to trans-activate other GPCRs. LHR LH− (A) or LHR cAMP− (B) plasmids (100 ng per well) were transfected into HEK-293 cells together with the cAMP-cre-luciferase activity plasmid, and then cells were stimulated with different concentrations of hCG (0.05, 0.5, and 5 nM) but showed no cAMP response. (C) Cotransfection of LHR cAMP− with β2 adrenergic receptor (β2R) expression plasmids in HEK-293 cells did not produce cAMP signal basally or in response to hCG stimulation (0, 0.05, 0.5, and 5 nM). A clear cAMP response was observed in each experiment in cells transfected with WT LHR.

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Table S1. Transgene copy number

<table>
<thead>
<tr>
<th>TG founder</th>
<th>TG copy number</th>
</tr>
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<tbody>
<tr>
<td>LHR\textsuperscript{LH−}</td>
<td>3–11</td>
</tr>
<tr>
<td>LHR\textsuperscript{cAMP−}</td>
<td>2–8</td>
</tr>
</tbody>
</table>

Genomic DNA of two founder mice per group in duplicate was analyzed by real-time PCR. Copy numbers were calculated after subtraction of the WT copy number (considered = 2) and calculated on a linear standard curve created by dilutions of WT genomic DNA.

Table S2. LHR expression

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Total LHR expression, times WT</th>
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<tbody>
<tr>
<td>LuRKO</td>
<td>N/A</td>
</tr>
<tr>
<td>LHR\textsuperscript{LH−}</td>
<td>0.58 ± 0.24\textsuperscript{a}</td>
</tr>
<tr>
<td>LHR\textsuperscript{cAMP−}</td>
<td>0.68 ± 0.29\textsuperscript{b}</td>
</tr>
<tr>
<td>LHR\textsuperscript{LH−/cAMP−}</td>
<td>2.33 ± 0.64\textsuperscript{c}</td>
</tr>
<tr>
<td>WT</td>
<td>1.00 ± 0.19\textsuperscript{b}</td>
</tr>
</tbody>
</table>

Testicular mRNA was isolated and analyzed by real-time PCR for LHR expression, normalized with a housekeeping gene (Ppia), and presented as total LHR expression as compared to control (WT). Results are the mean ± SD of at least three different samples per group, each sample measured in triplicate. Values with different superscript letters differ statistically significantly (P < 0.05).

Table S3. Testis and seminal vesicles weights

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Testis, mg</th>
<th>Seminal vesicles, mg</th>
</tr>
</thead>
<tbody>
<tr>
<td>LuRKO</td>
<td>53.6 ± 5.7\textsuperscript{a}</td>
<td>N/A</td>
</tr>
<tr>
<td>LHR\textsuperscript{LH−}</td>
<td>52.1 ± 8.3\textsuperscript{a}</td>
<td>N/A</td>
</tr>
<tr>
<td>LHR\textsuperscript{cAMP−}</td>
<td>54.9 ± 6.7\textsuperscript{a}</td>
<td>N/A</td>
</tr>
<tr>
<td>LHR\textsuperscript{LH−/cAMP−}</td>
<td>145.3 ± 44.0\textsuperscript{h}</td>
<td>539.8 ± 37.7\textsuperscript{a}</td>
</tr>
<tr>
<td>WT</td>
<td>147.9 ± 40.2\textsuperscript{b}</td>
<td>535.2 ± 26.6\textsuperscript{a}</td>
</tr>
</tbody>
</table>

Each value is the mean ± SD of measurements from at least four mice. Groups with different superscripts differ significantly (P < 0.001). N/A, not applicable because the size of the seminal vesicles in LuRKO, LHR\textsuperscript{LH−}, and LHR\textsuperscript{cAMP−} mice was too small for accurate measurement.

Table S4. Seminiferous tubule diameter and Leydig cell volume densities

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Seminiferous tubule diameter, nm</th>
<th>Leydig cell volume density, as % of the total area of a testicular section</th>
</tr>
</thead>
<tbody>
<tr>
<td>LuRKO</td>
<td>98.9 ± 11.0\textsuperscript{a}</td>
<td>1.58 ± 0.18\textsuperscript{a}</td>
</tr>
<tr>
<td>LHR\textsuperscript{LH−}</td>
<td>100.4 ± 13.9\textsuperscript{a}</td>
<td>2.69 ± 0.16\textsuperscript{a}</td>
</tr>
<tr>
<td>LHR\textsuperscript{cAMP−}</td>
<td>95.4 ± 12.2\textsuperscript{a}</td>
<td>2.70 ± 0.20\textsuperscript{a}</td>
</tr>
<tr>
<td>LHR\textsuperscript{LH−/cAMP−}</td>
<td>138.2 ± 11.0\textsuperscript{b}</td>
<td>5.49 ± 0.52\textsuperscript{b}</td>
</tr>
<tr>
<td>WT</td>
<td>127.8 ± 8.1\textsuperscript{b}</td>
<td>5.47 ± 0.36\textsuperscript{b}</td>
</tr>
</tbody>
</table>

Each value is the mean ± SD of at least three samples. Groups with different superscripts differ significantly (P < 0.001 for seminiferous tubular data; P < 0.05 for Leydig cell data). Leydig cell volume density (% of total section) was calculated as the area covered by Leydig cells in a testicular histological section divided by the total area of the section.

Table S5. LH-dependent gene expression

<table>
<thead>
<tr>
<th>Genotype</th>
<th>STAR/Ppia</th>
<th>Cyp17a1/Ppia</th>
</tr>
</thead>
<tbody>
<tr>
<td>LuRKO</td>
<td>0.059 ± 0.017\textsuperscript{a}</td>
<td>0.077 ± 0.034\textsuperscript{a}</td>
</tr>
<tr>
<td>LHR\textsuperscript{LH−}</td>
<td>0.062 ± 0.015\textsuperscript{a}</td>
<td>0.096 ± 0.027\textsuperscript{a}</td>
</tr>
<tr>
<td>LHR\textsuperscript{cAMP−}</td>
<td>0.076 ± 0.036\textsuperscript{a}</td>
<td>0.053 ± 0.006\textsuperscript{a}</td>
</tr>
<tr>
<td>LHR\textsuperscript{LH−/cAMP−}</td>
<td>0.85 ± 0.15\textsuperscript{b}</td>
<td>0.60 ± 0.13\textsuperscript{b}</td>
</tr>
<tr>
<td>WT</td>
<td>1.0 ± 0.084\textsuperscript{b}</td>
<td>1.0 ± 0.17\textsuperscript{b}</td>
</tr>
</tbody>
</table>

Isolated mRNA from testis of different animals was analyzed by real-time PCR, normalized with a housekeeping gene (Ppia), and presented as percentage of WT control. Results are the mean ± SD of at least three different animals per group, each analyzed in triplicate. Values with different superscript letters differ statistically significantly (P < 0.05).