Supplementary Information

Induction of androgen formation in the male by a peptide blocking 14-3-3ε protein adaptor and mitochondrial VDAC1 interactions

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Figure Legends

Figure S1. Sequences of 14-3-3ε specific siRNA.

Figure S2. TV fusion peptide properties (**a**) TSPO, STAR and VDAC1 each contain 2 to 3 *in silico* predicted 14-3-3 binding motif as indicated. The amino acid sequence of these motifs is shown based on the sequence homology with mode I (RSXpSXP) or II: RXXXpSXP) of the classic 14-3-3 motifs. These motifs on all three proteins are suboptimal, varying by 1 to 2 amino acids from the classic 14-3-3 motifs. (**b**) MA-10 cells were treated with 8-Br-cAMP for 0, 15, 30, 60, and 120 minutes. Cross-linking (CL) was performed with photo-activatable leu and met and UV light. Cell lysates were immunoprecipitated with 14-3-3ε anti-sera (IP). Immunoblot analysis confirms the previous results (Fig 2) and shows the dynamics of 14-3-3ε interactions with other 14-3-3 isoforms (14-3-3 pan), TSPO, STAR, and VDAC1 during steroidogenesis, C lane indicates these interactions in native MA-10 cell protein lysates.

Figure S3. Sequence of TV fusion peptides. (**a**) The table provides the name of the TAT-VDAC1 chimeric peptides. The amino acid sequence of the TV peptide and the location, on the mouse VDAC1 protein (Table a) are shown. (**b**) Immunofluorescence image of control MA-10 cells and MA-10 cells treated with the fluorescent FAM-TVS167 chimeric peptide, indicating the high efficiency of these peptides to penetrate cell membranes.

Figure S4. 14-3-3ε regulates the affinity of TSPO for its drug ligand PK11195. (**a**) Levels of TSPO bound to PK11195 in the absence or presence of the TVS167 peptide at different doses of ^{3}H PK 11195. (**b**) Kd, affinity^{-1} of TSPO for PK 11195, was measured in the absence or presence of TVS167. (**c**) Bmax, the available binding site of TSPO for PK 11195, was measured in the absence or presence of TVS167.

Figure S5. TVS167 induces T levels in a dose response manner. One testis of each adult Sprague-Dawley rat was injected with a bolus of water, TVG167, or different doses of TVS167. The other testis was used as a control. T levels were measured after 2 hrs in the intratesticular fluid of each treated testis and, control testis (**a**). Serum LH levels (**b**) were also monitored.

Figure S6. Administration of the TVS167 peptides does not induce toxic histological modifications to the testis. Rat testes sections were obtained from wild type adult Sprague-Dawley rats and rats injected with a bolus does of H2O or 150 ng TVS167 following implantation of H2O or TVS167 (75ng/24h) releasing pump. The pumps were directed to one testis. Animals were dissected 24 h post pump implantation and sectioned. Hematoxylin staining of these sections indicates no histological difference between testes of these animals.

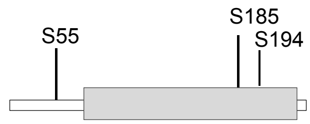
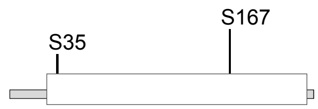
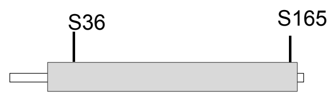
Figure S7. Alignment of amino acid sequences of 14-3-3ε and VDAC1 showing high degree of conservation for both proteins and that the 14-3-3 binding motif is conserved in the three mammalian species as indicated.

Figure S1

siRNA sequence
5’-GCAAGAUCAUCAUUAGAA -3’, 3’-UUUCCAUUUCUAAUGAUG -5’
5’-GGGAGGAGAGGACAAAUU -3’, 3’-CAUCUUUAAUUUGUCCUC -5’
5’-AGAUGAGAAUCAGUGAGA -3’, 3’-UAUUUCGUCUCACUGAUU -5’

Figure S2

a

Protein	14-3-3 motif	Motif mode	On the protein structure
STAR	RRRSSLL	I or II	
	RDFVSVR	II	
	RRGSTC	I	
VDAC1	KTKSEN	I	
	RVTQSNF	II	
TSPO	RWYASLQ	II	
	RGGSRLP	II	

b

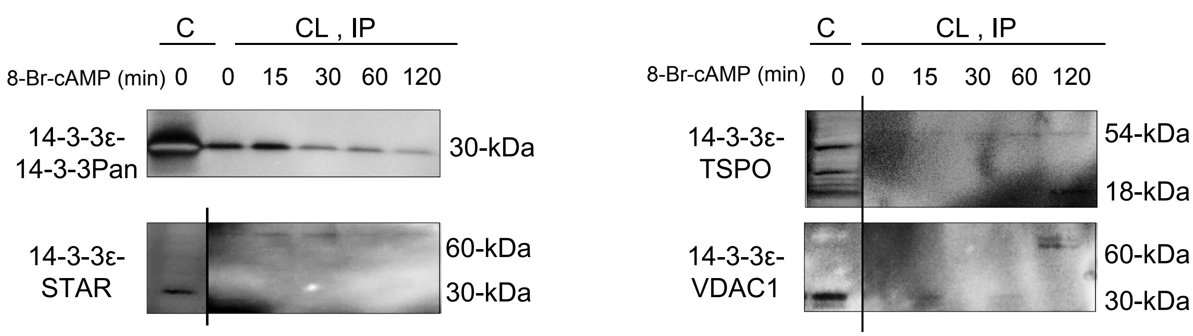


Figure S3

a

Peptide name	**Peptide sequence**	**14-3-3 motif (aa #)**
TVS35	YGRKKRRQRRR-G-KLDLKTKSENGLE	KTKSEN (32-37)
TVS35G	YGRKKRRQRRR-G-KLDLKTKGENGLE	
TVS167	YGRKKRRQRRR-G-SKSRVTQSNFAVG	RVTQSNF (163-169)
TVS167G	YGRKKRRQRRR-G-SKSRVTQGNFAVG	

b

Control FAM-TVS167

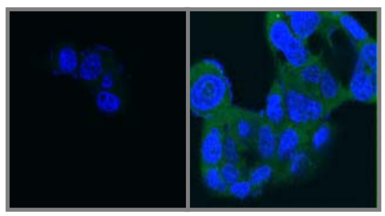
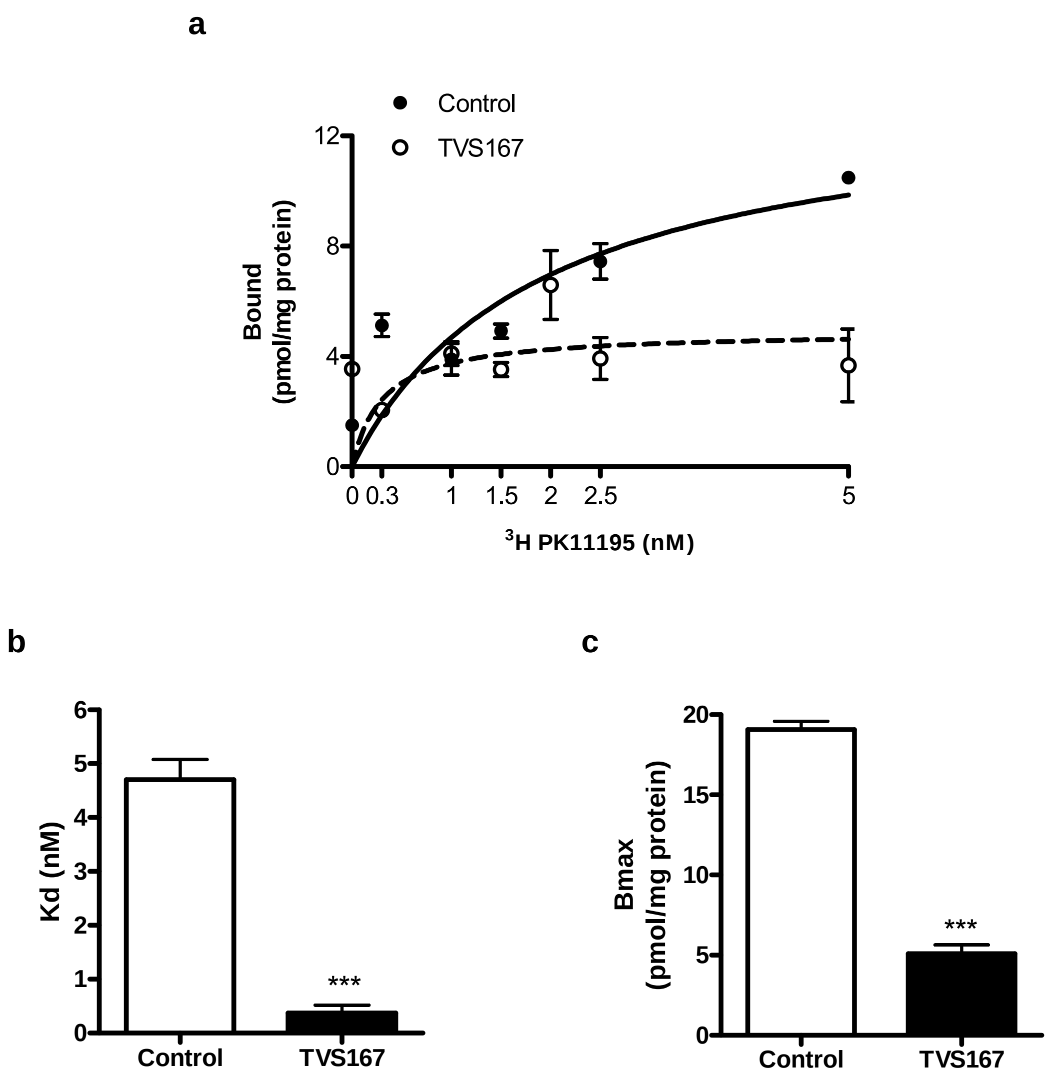


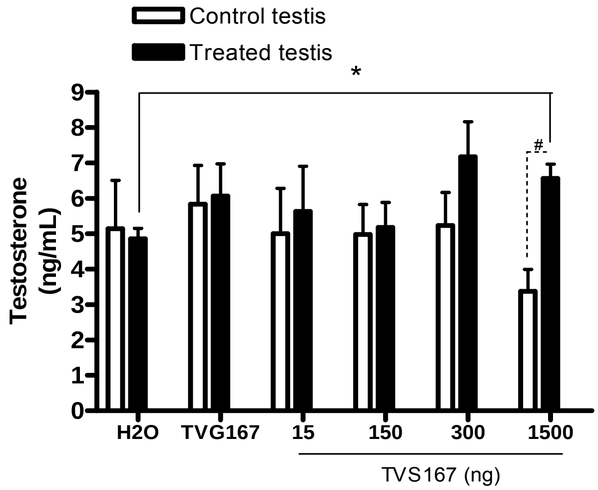
Figure S4



a
Control
TVS167
Bound
(pmol/mg protein)
12
8
4
0
0 0.3 1 1.5 2 2.5 5
^{3}H PK11195 (nM)
b
Kd (nM)
6
5
4
3
2
1
0
***
Control
TVS167
c
Bmax
(pmol/mg protein)
20
15
10
5
0
***
Control
TVS167

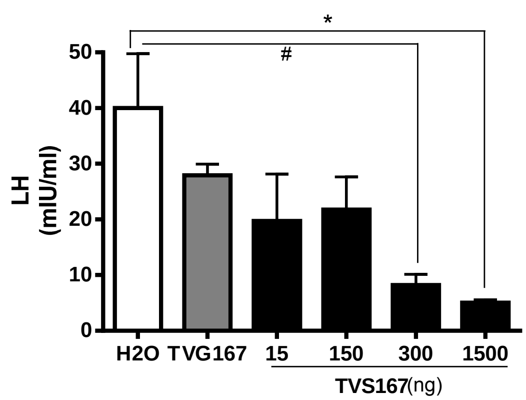
Figure S5

a



Control testis
Treated testis
*
#
Testosterone (ng/mL)
9
8
7
6
5
4
3
2
1
0
H2O
TVG167
15
150
300
1500
TVS167 (ng)

b



*
#
LH (mIU/ml)
50
40
30
20
10
0
H2O
TVG167
15
150
300
1500
TVS167(ng)

Figure S6

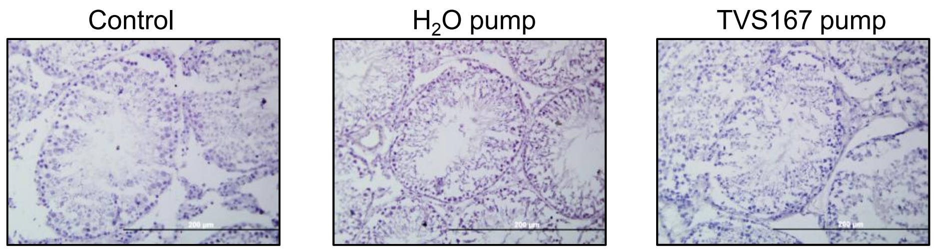


Figure S7

14-3-3ε

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Mus         MDDREDLVYQAKLAEQAERYDEMVESMKKVAGMDVELTVEERNLLSVAYKNVIGARRASW
Homo        MDDREDLVYQAKLAEQAERYDEMVESMKKVAGMDVELTVEERNLLSVAYKNVIGARRASW
Rattus      MDDREDLVYQAKLAEQAERYDEMVESMKKVAGMDVELTVEERNLLSVAYKNVIGARRASW
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Mus         RIISSIEQKEENKGGEDKLKMIREYRQMVETELKLICCDILDVLDKHLIPAANTGESKVF
Homo        RIISSIEQKEENKGGEDKLKMIREYRQMVETELKLICCDILDVLDKHLIPAANTGESKVF
Rattus      RIISSIEQKEENTGGEDKLKMIREYRQMVETELKLICCDILDVLDKHLIPAANTGESKVS
            ************.**********************************************

Mus         YYKMKGDYHRYLAEFATGNDRKEAAENSLVAYKAASDIAMTELPPTHPIRLGLALNFSVF
Homo        YYKMKGDYHRYLAEFATGNDRKEAAENSLVAYKAASDIAMTELPPTHPIRLGLALNFSVF
Rattus      YYYMKGDYHRYLAEFATGNDRKEAAENSLVAYKAASDIAMTELPPTHPIRLGLALNFSVF
            ** *********************************************************

Mus         YYEILNSPDRACRLAKAAFDDAIAELDTLSEESYKDSTLIMQLLRDNLTLWTSDMQGDGE
Homo        YYEILNSPDRACRLAKAAFDDAIAELDTLSEESYKDSTLIMQLLRDNLTLWTSDMQGDGE
Rattus      YYEILNSPDRACRLAKAAFDDAIAELDTLSEESYKDSTLIMQLLRDNLTLWTSDMQGDGE
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Mus         EQNKEALQDVEDENQ
Homo        EQNKEALQDVEDENQ
Rattus      EQNKEALQDVEDENQ
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VDAC1

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Mus         MAVPPTYADLGKSARDVFTKGYGFGLIKLDLKTKSENGLEFTSSGSANTETTKVNGSLET
Rattus      MAVPPTYADLGKSARDVFTKGYGFGLIKLDLKTKSENGLEFTSSGSANTETTKVNGSLET
Homo        MAVPPTYADLGKSARDVFTKGYGFGLIKLDLKTKSENGLEFTSSGSANTETTKVTGSLET
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Mus         KYRWTEYGLTFTEKWNTDNTLGTEITVEDQLARGLKLTFDSSFSPNTGKKNAKIKTGYKR
Rattus      KYRWTEYGLTFTEKWNTDNTLGTEITVEDQLARGLKLTFDSSFSPNTGKKNAKIKTGYKR
Homo        KYRWTEYGLTFTEKWNTDNTLGTEITVEDQLARGLKLTFDSSFSPNTGKKNAKIKTGYKR
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Mus         EHINLGCDVDFDIAGPSIRGALVLGYEGWLAGYQMNFETSKSRVTQSNFAVGYKTDEFQL
Rattus      EHINLGCDVDFDIAGPSIRGALVLGYEGWLAGYQMNFETSKSRVTQSNFAVGYKTDEFQL
Homo        EHINLGCDMDFDIAGPSIRGALVLGYEGWLAGYQMNFETAKSRVTQSNFAVGYKTDEFQL
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Mus         HTNVNDGTEFGGSIYQKVNKKLETAVNLAWTAGNSNTRFGIAAKYQVDPDACFSAKVNNS
Rattus      HTNVNDGTEFGGSIYQKVNKKLETAVNLAWTAGNSNTRFGIAAKYQVDPDACFSAKVNNS
Homo        HTNVNDGTEFGGSIYQKVNKKLETAVNLAWTAGNSNTRFGIAAKYQIDPDACFSAKVNNS
            **********************************************:*************

Mus         SLIGLGYTQTLKPGIKLTLSALLDGKNVNAGGHKLGLGLEFQA
Rattus      SLIGLGYTQTLKPGIKLTLSALLDGKNVNAGGHKLGLGLEFQA
Homo        SLIGLGYTQTLKPGIKLTLSALLDGKNVNAGGHKLGLGLEFQA
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