Supplemental Tables

SUPPLEMENTAL TABLE 1. Oligonucleotides used for site-directed mutagenesis of cDNAs encoding the human or spotted hyena SHBG precursor polypeptides.

Amino acid substitutions	Mutagenic oligonucleotides[a]
Human SHBG	
F56L	5´-TATCCCCATA**C**AAAATCACTCCCTC
W84V	5´-GTAAGCTGGGCC**AC**GTGATTGTGCAG
M139V	5´-AAGCGCAATCCTCA**C**GATGGGATGGCG
(T183 + G)	5´-CTTCTGAGGCT**GCC**AGTGGGGGC
Spotted hyena SHBG[b]	
W15 + L	5´-CCACGGCGGCGGTGGTTG**CTG**CTGCTTGTGCTGCTG
W15 + 2L	5´-GGCGGTGGTTGCTGCTGCTT**CTT**CTGCTGCTGCTGCTACCTCACAG
W15L + 2L	5´-ACATCGCCACGGCGGCGGT**T**GTTGCTGCTGCTTCTTCTGC

[a] The nucleotides added or modified are indicated in bold face type and underlined.

[b] The consensus spotted hyena SHBG cDNA sequence was produced by adding an extra Leu codon (TTG) at position 16 in the secretion signal polypeptide sequence within the original cDNA clone (Accession # DQ285473).

SUPPLEMENTAL FIG. 1. Primary structure comparison of spotted hyena (spSHBG) and human (hSHBG) SHBG precursor polypeptides. Amino acid residues within the mature human SHBG sequence that were targeted for substitution or addition of residues in the corresponding positions of spotted hyena SHBG are shown in bold. The cysteine residues that form intra-molecular disulfide bridges within the two LG domains are in bold underlined. Consensus sites for N-glycosylation are also underlined. Sequence alignment was obtained using the Clustal method. Gaps were introduced to maximize sequence similarities. Identical residues are indicted by asterisk, while conserved residues are indicated by double dots, and semi-conserved residues are indicated by single dots.

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spSHBG MEGRGPLATSPRRRWLLLLLLLPHS-----------HQRIQDPPAVHLSSASGQGP 45
hSHBG  MESRGPLATS-RLLLLLLLLLLRHTRQGWALRPVLPTQSAHDPPAVHLSNGPGQEP 55
       **.******* *   ******* *:            *  :********...** *

spSHBG VTIMTFDFTKMRKTSSSFELRTWDPEGVILYGDTDPHEDWFMLGLRGGRPEIQIHN 101
hSHBG  IAVMTFDLTKITKTSSSFEVRTWDPEGVIFYGDTNPKDDWFMLGLRDGRPEIQLHN 111
       :::****:**: *******:*********:****:*::********.******:**

spSHBG HVARLTVGAGPRLDDGKWHQVEVKVLGDLLLLTVDGEEVLCLKQVFGPLASRPQPV 157
hSHBG  HWAQLTVGAGPRLDDGRWHQVEVKMEGDSVLLEVDGEEVLRLRQVSGPLTSKRHPI 167
       * *:************:*******: ** :** ******* *:** ***:*: :*:

spSHBG VRIAVGGLPFPPSSLRLPLVPALDGCVRRGSWLDHQAQTSVSALSGSPRSCGVESQ 213
hSHBG  MRIALGGLLFPASNLRLPLVPALDGCLRRDSWLDKQAEISASAPT-SLRSCDVESN 222
       :***:*** **.*.************:**.****:**: *.** : * ***.***:

spSHBG PGSFFPPGAHAEFSLQDLPQPHAEPWAFSLDLGLQLAAGSGHLLALGTPENPPRLS 269
hSHBG  PGIFLPPGTQAEFNLRDIPQPHAEPWAFSLDLGLKQAAGSGHLLALGTPENPSWLS 278
       ** *:***::***.*:*:****************: ****************. **

spSHBG LQLQDQKVVLSSAWGPQLHLPLVLGAPLQLKLAASGVTLSQGPETEILALPLSDPG 325
hSHBG  LHLQDQKVVLSSGSGPGLDLPLVLGLPLQLKLSMSRVVLSQGSKMKALALPPLGLL 334
       *:**********. ** *.****** ******: * *.****.: : ****  .

spSHBG SLLNLWVQPHARLFLGALPGEAASASFCLDGLWAQGQKLDMDRALNRSQNIWTHSC 381
hSHBG  PSLNLWAKPQGRLFLGALPGEDSSTSFCLNGLWAQGQRLDVDQALNRSHEIWTHSC 390
       . ****.:*:.********** :*:****:*******:**:*:*****::******

spSHBG PQSLGNDTDTTH  393
hSHBG  PQSPGNGTDASH  402
       *** **.**::*
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Amino-terminal LG-domain

Carboxy-terminal LG-domain

Supplemental Figure 1