

Figure S2

Amino acid sequence alignment of steroid receptor ligand-binding domains (LBD), with cephalochordate receptors aligned to LBDs of human steroid receptors. Mutations of hormone contacting residues R394C and F404L (boxed) eliminate estradiol activated transcription and generate a repressor of wild-type BfSR.

BfSR	PTLPLYNPTVPLISHLVNIEPNPILTGYNPQCTPTEGYLMALVTDLANREIEGLVDWAAR
BfER	LKALIDALDVKEGEHRGEENHPTGQQAGNWQEISNPELIESVSSLVDRELGTICWGKK
HUMANER α	LSLTADQMVSALLDAEPP-ILYSEYDPTRPFFSEASMMGLLTNLADRELVHMINWAKR
HumanER β	DALSPEQLVLTLEAEPHVLI SR--PSAPFTEASMMMSLTKLADKELVHMISWAKK
HumanGR	QLTPTLVSLLEVIEPEVLYAGYDSSVPDSTWRIMTTLNMLGGRQVIAAVKWAKA
HumanMR	RALTSPSPVMVLENIEPEIVYAGYDSSKPDTAENLLSTLNRLAGKQMIQVVVKWAKV
HumanPR	GQDIQLIPLINLLMSIEPDVIYAGHDNTKPDTSSSLTSLNQLGERQLLSVVKWSKS
HumanAR	CQPIFLNVLEAIEPGVVCAGHDNNQPDSSFAALLSSLNELGERQLVHVVKWAKA

	394	404
BfSR	LPGYGMLPMDDQVNLI RTVWLDLLMLGLVWR	RSMEHRG-EWLVPAPDLLMD-----RSLCR
BfER	IPGYSKLSLNDQVLLMESTWLDLLILDLVWC	SIRHKG-EKLLLSGGVLVNRNTISNRRNN
HUMANER α	VPGFVDLTLDHQVHLLCAWLEILMIGLVWR	RSMEHPG--KLLFAPNLLLDLDR-----NQGKC
HumanER β	IPGFVELSLFDQVRLLESCWMEVLMGLMWR	SIDHPG--KLIFAPDLVLDLDR-----DEGKC
HumanGR	IPGFRNLHLDQMTLLQYSWMFLMAFALGWR	SYRQSSANLLCFAPDLI IN-----EQRMT
HumanMR	LPGFKNLPLEDQITLIQYSWMLCSSFALS	WRSYKHTNSQFLYFAPDLVFN-----EEKMH
HumanPR	LPGFRNLHIDDQITLIQYSWMLMVFLGWR	SYKHVSGQMLYFAPDLILN-----EQRMK
HumanAR	LPGFRNLHVDDQMAVIQYSWMGLMVFAMGWR	SFTNVNSRMLYFAPDLVFN-----EYRMH

BfSR	LSG----MEYICTPMLEFARQFADLQVPQEVYVCLKALTLYTTAV-----SRLQDYRQVQR
BfER	SSGDDMEVLEMCDQILSIATKFYFDLQRREYLCLKAITLVHG-----SLKGLESdT
HUMANER α	VEG----MVEIFDMLLATSSRFMRMNLQGEFVCLKSI ILLNSGVYTFLSSTLKSLEEKD
HumanER β	VEG----ILEIFDMLLATSSRFRELKLQHKYLCVKAMILLNSSMYPLVTATQDADSSR-
HumanGR	LPC----MYDQCKHMLYVSSELHRLQVSYEEYLCMKTLLLLS-----SVPKDGLKSQE
HumanMR	QSA----MYELCQGMHQISLQFVRLQLTFFEYTIMKVLLLLS-----TIPKDGLKSQA
HumanPR	ESS----FYSLCLTMWQIPQEFVKLQVSQEEFLCMKVLLLLN-----TIPLEGLRSQT
HumanAR	KSR----MYSQCVRMRHLSQEFGLWQITPQEFELCMKALLLFS-----IIPVDGLKNQK

BfSR	LQHEINEALAEACSSSTFGFSPGNIRLMMIVSQVRQLSSLGVDHLNRLRGAETVSVEGLL
BfER	QVRQLQDDLTDALMDVCSER--HALGSRRAKMLLL-----LSHLRQVSARASSHLG
HUMANER α	HIHRVLDKITDTLIHLMAGLTLQQQHQLRAQLLLI-----LSHIRHMSNKGMEHLY
HumanER β	KLALLNNAVDALVWVIAKSGISSQQQSMRLANLLML-----LSHVRHASNKGMEHLL
HumanGR	LFDEIRMTYIKELGKAI VKREGNSSQNWRFYQLTKL-----LDSMHEVVENLLNYCF
HumanMR	AFEEMRTNYIKELRKMTKCPNNSGQSWQRFYQLTKL-----LDSMHDLVSDLLEFCF
HumanPR	QFEEMRSSYIRELIKAGLRQKGVVSSSQRFYQLTKL-----LDNLHDLVKQLHLYCL
HumanAR	FFDELRMNYIKELDRI IACKRKNTSCSRRFYQLTKL-----LDSVQPIARELHQFTF

BfSR	REIVDEPPRITEIEDSPGSAGNKMTMSRSAGKMECAASTSCTAQDEAVENSAAEAEAAE
BfER	AVRNLKVPDYDILLDILTQVSEGRDQQAHEVASSPEKER-----
HUMANER α	SMKCKNVVPDYDILLEMLDAHRLHAPTSRGGASVEETDQSHLATAGTSSSHSLQKYYITG
HumanER β	NMKCKNVVPDYDILLEMLNAHVLRGCKSSITGSECSAEDSKSKEGSQNPQSQ-----
HumanGR	QTFLD-KTMSIEFPEMLAEIITNQIPKYSNGNIKKLLFHQ-----
HumanMR	YTFRESHALKVEFPAMLVEIISDQLPKVS-GNAKPLYFHRK-----
HumanPR	NTFIQSRALSVFPEMMSEVIAAQLPKILAGMVKPLLFHKK-----
HumanAR	DLLIKSHMVSVDPEMMAEIIISVQVPKILSGKVKPIYFHTQ-----

BfSR	FAAQLKEVMEGEATTIADV KAMADQMGI EILDLD
BfER	-----
HUMANER α	EAEGFPATV-----
HumanER β	-----
HumanGR	-----
HumanMR	-----
HumanPR	-----
HumanAR	-----