Supplementary Materials and Methods

Cell Culture and Other Reagents

HEK293.RXFP1 and 293T.RXFP1 stably-transfected cell lines were obtained from parental HEK293 and 293T cell lines, respectively, by transfection of the pCDNA3.1/Zeo-LGR7 (RXFP1) plasmid, followed by selection under 1,000 ug/ml of Zeocin (Life Technologies, Burlington, ON), single cell cloning, and maintenance under 250 ug/ml of Zeocin. Successful transfection was verified by flow cytometric analysis of the cells with the mouse monoclonal anti-FLAG-M2 antibody (3.2 ug/ml, Clone M2, Sigma-Aldrich, St. Louis, MO) using the secondary goat anti-mouse IgG Alexa Fluor 488-conjugated antibody (2 ug/ml, Life Technologies, Burlington, ON) for detection. HEK293.RXFP1.mGL.2 (Clone #1) cell line was derived by stable transfection of the HEK293.RXFP1 cells with the pCRE-mGL.2 plasmid (Xactagen, Shoreline, WA), carrying membrane-anchored *Gaussia* luciferase. Cells were selected under 1,000 ug/ml of G418 (Sigma-Aldrich, St. Louis, MO) while being maintained under 250 ug/ml of Zeocin, single cell cloned, and maintained under double selection with 250 ug/ml of Zeocin and 250 ug/ml of G418. Clone #1 was selected based on high luciferase activity following stimulation with 10uM forskolin (Cayman Chemical, Ann Arbour, MI) in the presence of 50uM isobutylmethylxanthine (IBMX, Cayman Chemical, Ann Arbour, MI).

Synthesis of AT-001

The two A and B peptide chains of AT-001 were synthesized by standard Fmoc Solid-Phase Peptide Synthesis (SPPS), incorporating t-Butyl (tBu), Acm, or Trt (trityl) protecting groups on the Cys residues for subsequent sequential assembly of intrachain and interchain disulfide bonds. The intrachain disulfide was formed first, followed by the sequential formation of the two interchain disulfide bonds to yield the final molecule. The final product was purified by C18 RP-HPLC, and lyophilized with acetate as counter ion. Final product was analyzed by HPLC (Column: Discovery C18, 4.6mm x 250 mm, 5 micron (Sigma-

Aldrich, St. Louis, MO); Linear Gradient: 25%-45% Buffer B in 20 min.; Buffer A: 0.1% TFA in Water; Buffer B: 0.1% TFA in Acetonitrile; Room Temperature), Amino Acid Analysis (EZChrom Elite, Agilent, Pleasanton, CA), and LC-ESI-MS (Finnigan LCQ Advantage LC/MS/MS, Thermo Fisher Scientific, Rockford, IL). Peptide purity was 95.9% by HPLC with a peptide content of 89%.

Radioiodination of H2 Relaxin

H2 relaxin (2 ug in 90 ul of phosphate-buffered saline (PBS), pH 7.4) was added to a Pierce Iodination Tube, pre-coated with the iodination reagent, followed by the addition of 10ul of $Na^{125}I$ (1mCi, Perkin Elmer, Waltham, MA) and incubation for 10 min. at room temperature with periodic agitation. A solution of tyrosine was added to quench residual iodinating species. Sample was diluted with bovine serum albumin (BSA, Sigma-Aldrich, St. Louis, MO) in PBS to a final concentration of 0.1% BSA. ^{125}I-labeled H2 relaxin was cleaned up by buffer exchange on a polyacrylamide desalting column (1.8K MWCO, 5ml, Thermo Fisher Scientific, Rockford, IL) following the manufacturer's instructions. Pooled fractions containing protein were measured for protein concentration by BCA Protein Assay (Pierce, Thermo Fisher Scientific, Rockford, IL) and radioactivity on a GammaMaster 1277 gamma counter (LKB Wallac, Finland).