Supplemental Material

High-Throughput Fluorescence Polarization Assay to Identify Ligands using purified G Protein-coupled Receptor

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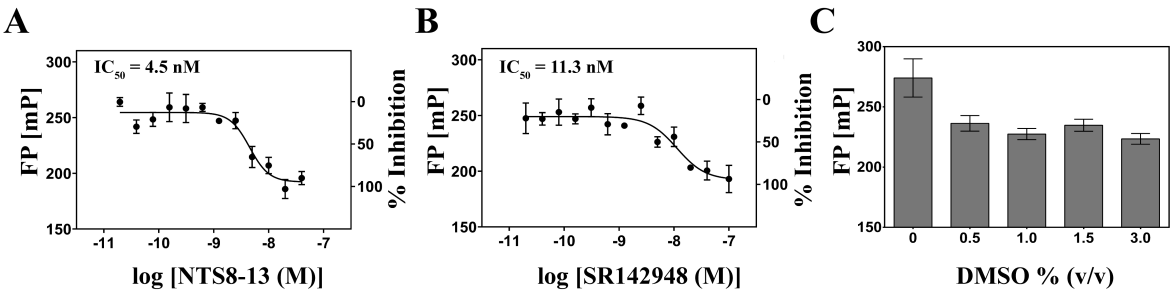

Figure S1. FP assay development**.** (**A**) Competitive binding experiment of a NTS1 agonist (NTS8-13) in presence of 1% (v/v) DMSO. (**B**) Competitive binding experiment of a NTS1 antagonist (SR142948) in presence of 1% (v/v) DMSO. Data were fitted using a three-parameter curve fit in GraphPad Prism. (**C**) The effect of 0-3% (v/v) DMSO (solvent of compounds) on the maximal signal response (in the absence of any competitor) was investigated. All measurements were performed with 5 nM NT-HiLyte647 and 12.5 nM NTS1. Data points represent the mean ± SEM from duplicate measurements.

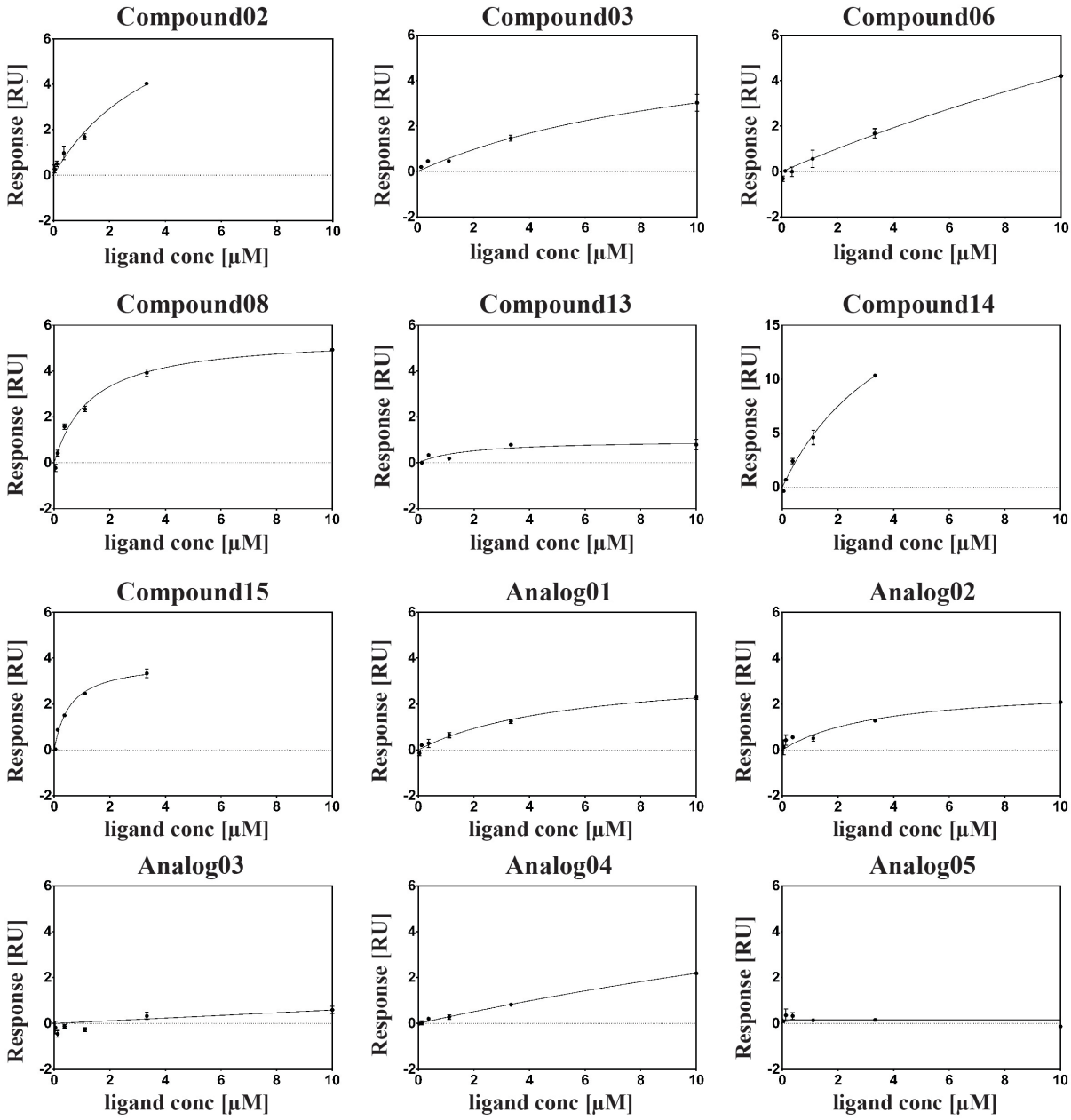

Figure S2. Equilibrium fits of all tested compounds in SPR. Equilibrium fits of the SPR titrations of Compound 02, 03, 06, 08, 13, 14, 15, and the five analogs, Analog 01-05. Data points represent the mean ± SEM from duplicate measurements. Curves were fitted using a non-linear-specific binding fit in GraphPad Prism.

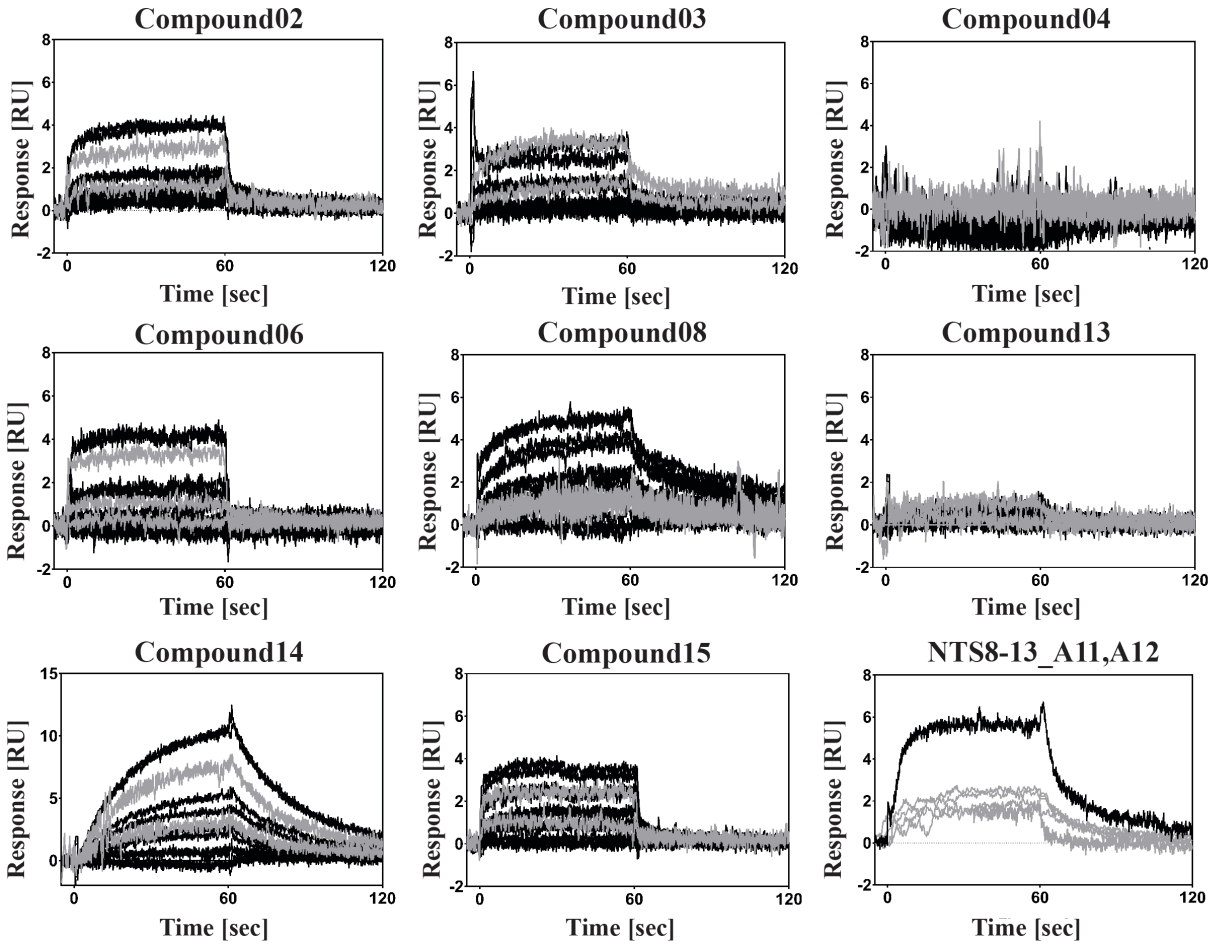

Figure S3. SPR kinetic binding data for the compounds 02, 03, 04, 06, 08, 13, 14, and 15 with and without competition by a tight-binding orthosteric antagonist. Black curves represent titration series of each compound in duplicates on a free receptor surface, grey curves represent the three highest concentrations as single injections on an antagonist-blocked (SR142948) receptor surface. The control NTS8-13_A11,A12 was injected at a concentration of 200 nM before (black) and after (grey) the blocking procedure.

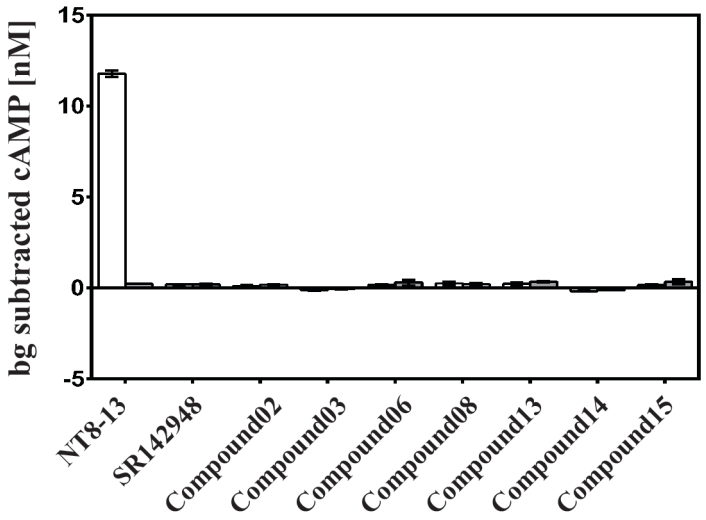

Figure S4. Functional assay on cells. G_s signaling by measuring cAMP level of NTS8-13 (1 μM); SR142948 (1 μM); and compounds 02, 03, 06, 08, 13, 14, and 15 (all at 100 μM) in HEK293 cells, either expressing (white) or non-expressing NTS1 (grey) All data points are background-corrected and represent mean ± SEM from duplicate measurements.

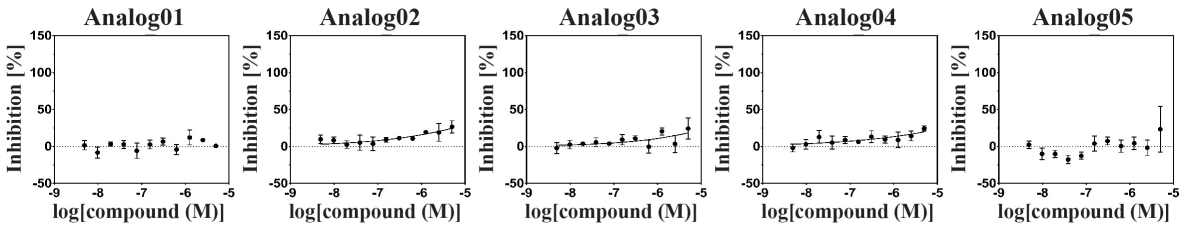

Figure S5. Results of the Fluorescence Polarization analog profiling against NTS1. The analogs were measured in titration curves up to 10 µM. Data points represent the mean ± SEM from duplicate measurements. Curves were fitted using a sigmoidal four-parameter fit in Graphpad Prism.

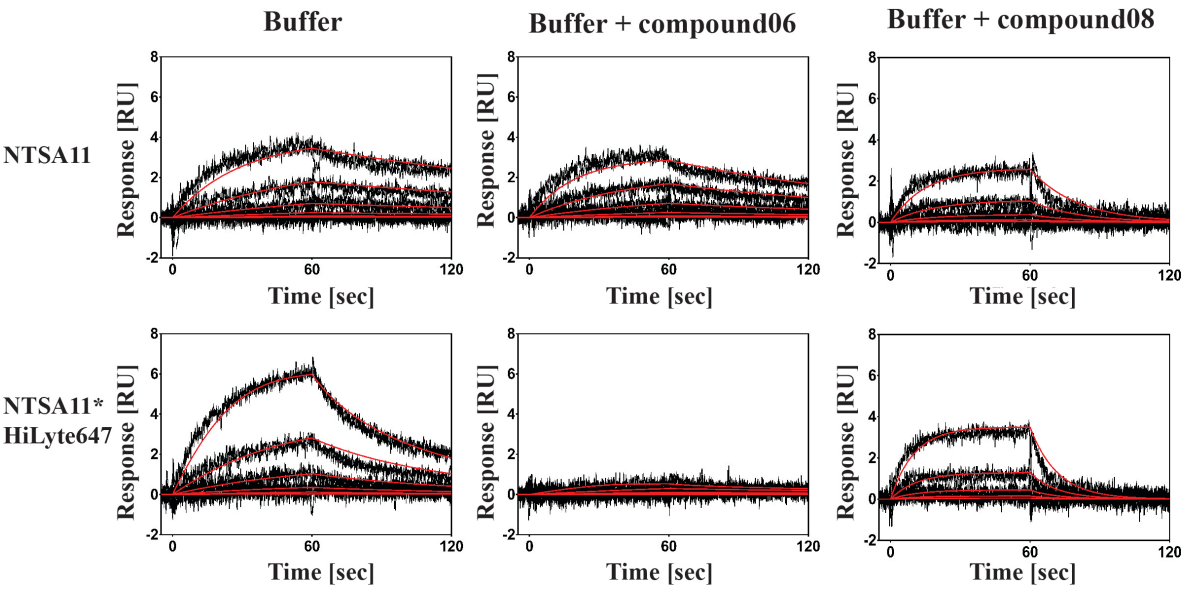

Figure S6. Binding data of NT-peptide in SPR. Curves represent titration series (from 0.12 to 30 nM) of NTSA11 (=NTS8-13 peptide with point mutation at position 11 (Y to A)), and the same peptide labelled with a HiLyte647 dye, either on a free receptor surface (left), in the presence of 10 µM compound 06 (middle), or in the presence of 10 µM compound 08 (right).

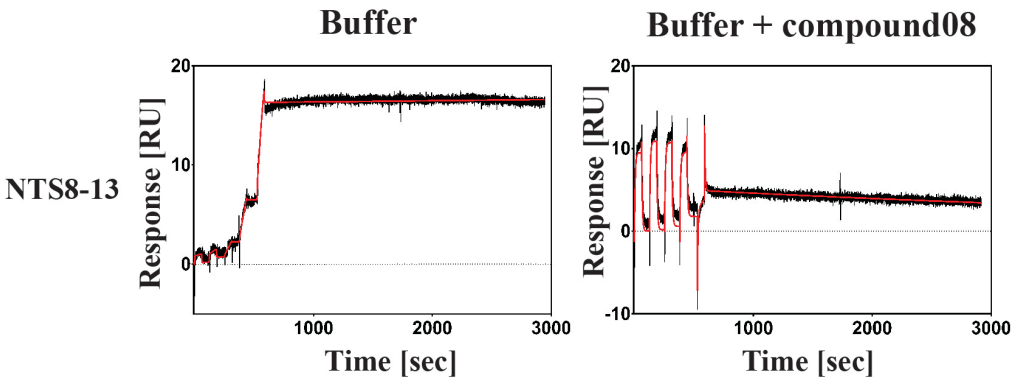

Figure S7. Binding data of NTS8-13 in SPR. Curves represent single cycle kinetics (from 0.111 to 9 nM) of NTS8-13 either on a free receptor surface (left), or in presence of 10 µM compound 08 (right).

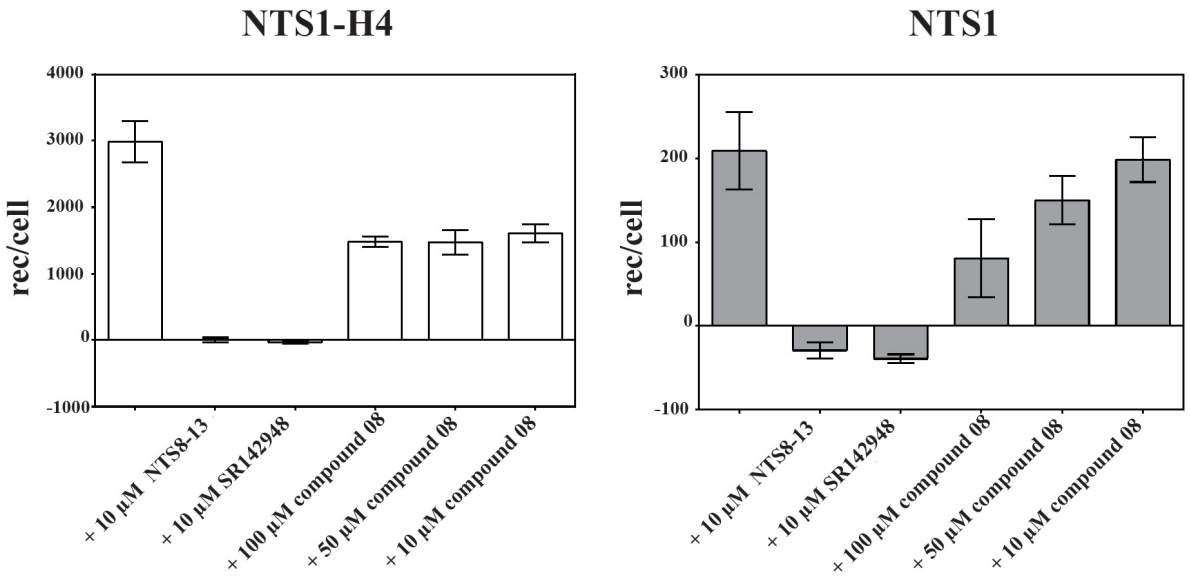

Figure S8. Inhibition of NTS binding on cells. Tritium-labeled NTS competing with NTS8-13, SR142948 (both 10 µM) and compound 08 (10, 50, and 100 µM) on NTS1-H4 expressing *E. coli* cells (left) and on NTS1 wild type expressing *E. coli* cells (right).