ChemMedChem

Supporting Information

The Chemerin Receptor CMKLR1 Requires Full-Length Chemerin for High Affinity in Contrast to GPR1 as Demonstrated by a New Nanoluciferase-Based Binding Assay

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Results

Tab. S1: Primer sequences used for N-terminal fusion of Nluc with Ser-Gly_4-Ser linker at CMKLR1-eYFP and GPR1-eYFP.

Code	Primer	Sequenz
1	MluI_Nluc-fw	ATATACGCGTGCCACCATGAACTCCTTCTCCACAAGCGCC
2	Nluc-SGGGGS_rev	GCTGCCTCCGCCTCCGCTCGCCAGAATGC
3	SGGGGS-CMKLR1_fw	GGAGGCGGAGGCAGCGAGGATGAAGATTACAACACTTCCATC
4	SGGGGS-GPR1_fw	GGAGGCGGAGGCAGCGAAGATTTGGAGGAAACATTATTTGAAG
5	eYFP_ XbaI_rev	ATATTCTAGACTACTTGTACAGCTCGTCCATGCCGAG

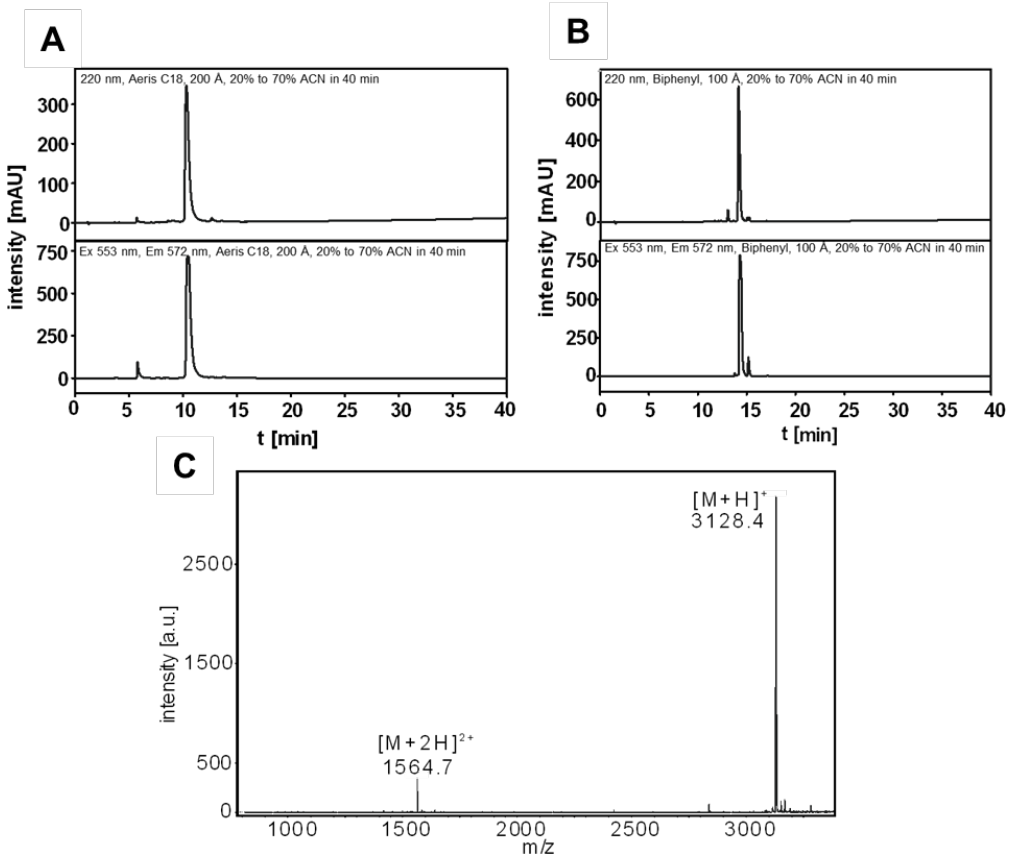


Fig. S1: Analytics of purified [K^{141}(TAMRA)]ChemS157(135-157) (part A).

A RP-HPLC chromatogram of [K^{141}(TAMRA)]ChemS157(135-157) recorded with Aeris C18, 200Å column. The peptide elutes after 10.3 min at 33 % ACN with a purity ≥95 %. The upper chromatogram shows the detection at 220 nm and the lower chromatogram shows the fluorescence channel. **B** RP-HPLC chromatogram of [K^{141}(TAMRA)]ChemS157(135-157), with biphenyl C18, 100 Å column. The peptide elutes after 14.2 min at 38 % ACN with a purity ≥95 %. The upper chromatogram shows the detection at 220 nm and the lower chromatogram shows the fluorescence channel. **C** The MALDI-ToF shows the purified peptide fragment with a calculated monoisotopic mass of 3127.4 Da.

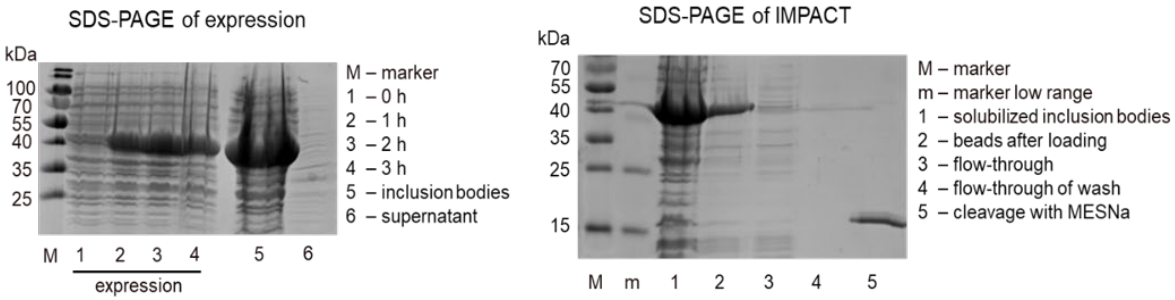


Fig. S2: SDS-PAGE analysis of ChemS157(21-134)-thioester (part B) .

Analysis was performed by SDS-PAGE using Coomassie blue staining. The calculated mass of the intein-CBD fusion protein is 43.6 kDa. The analysis of the expression shows no further increasing in band intensity at 40.0 kDa over 3 h, suggesting that the incubation time was sufficient. The fusion protein was expressed in inclusion bodies, indicated by the intense band at 40.0 kDa.

The SDS-PAGE of the IMPACT purification shows the solubilized fusion protein at 40.0 kDa and the MESNa cleaved product at around 15.0 kDa, which covers the calculated mass of 15.9 kDa.

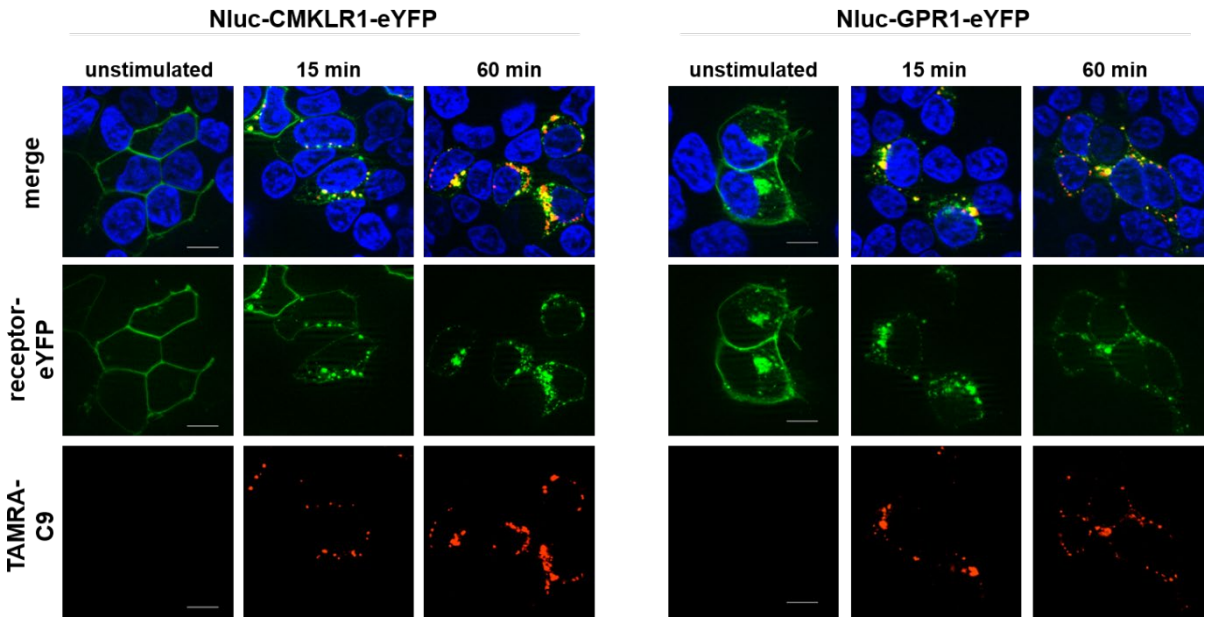
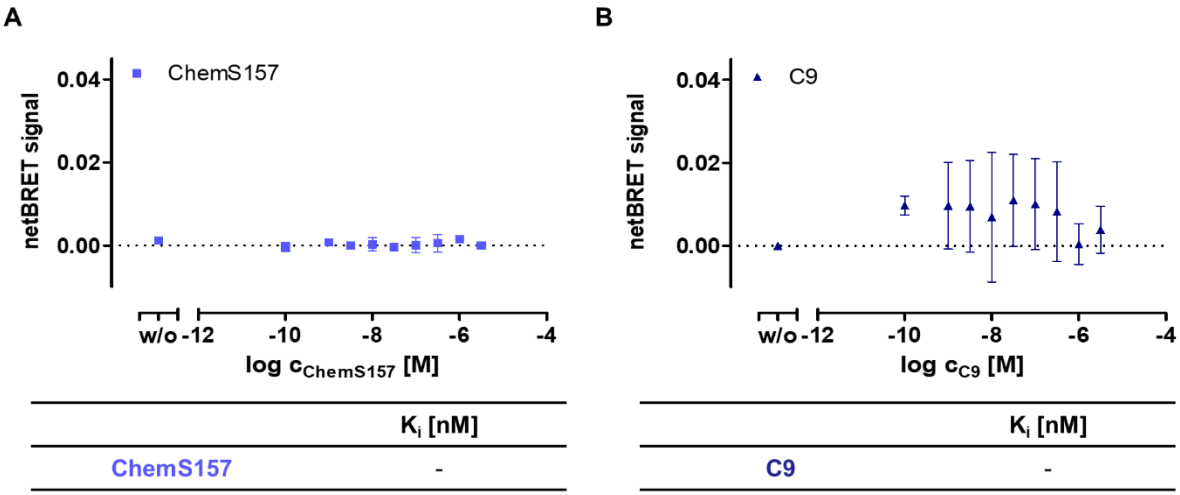


Fig. S3: Binding of TAMRA-C9 and internalization of Nluc-receptor constructs.

Due to inability of GPR1 to induce G protein signaling, binding and activation of Nluc-GPR1 (**B**) as well as Nluc-CMKLR1 (**A**) by TAMRA-C9 was additionally analyzed by fluorescence microscopy. Images were recorded before and after 15 min and 30 min stimulation with 1 µM TAMRA-C9. Both receptors (green) bind TAMRA-C9 (red) and internalize upon ligand stimulation over the incubation time. Cell nuclei were stained with Hoechst 33342 (scale bar = 10 µm).



	K_i [nM]
ChemS157	-

	K_i [nM]
C9	-

Fig. S4: Additional displacement assay at Nluc-CMKLR1. All assays were performed using transiently transfected COS-7 cells. TAMRA-ligand was added with defined concentration and unlabeled ligand was used with increasing concentrations. Both were incubated for 1 h on ice to reach an equilibrium. BRET signal was detected directly after addition of coelenterazine h. Each assay was performed at least two times in triplicates. (**A**) represents the displacement BRET assay results of TAMRA-C9 displaced with ChemS157. For (**B**) [K^{141}(TAMRA)]ChemS157 was used for labeling and competition was performed with C9.