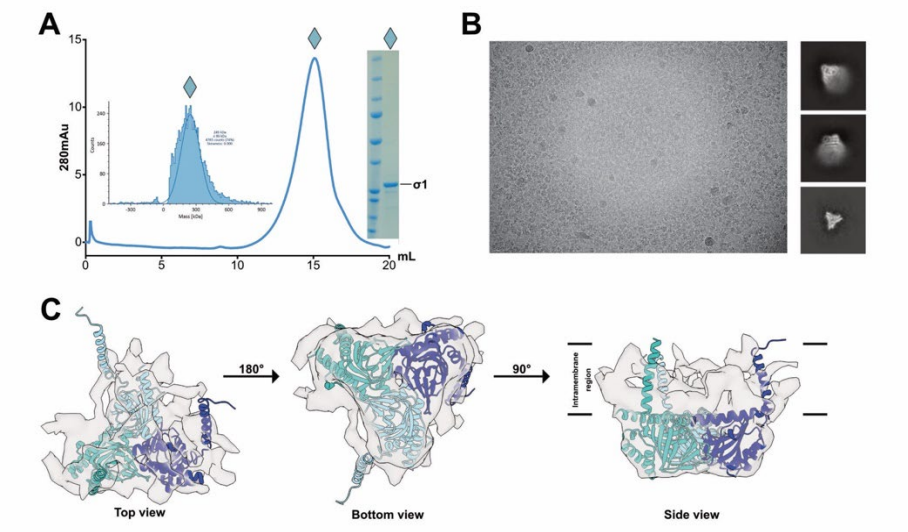

Figure 1. A) Size exclusion chromatography profile, mass photometry analysis and SDS page of σ1. B) Example of micrograph from and 2D classes from a Glacios screening. C) 3D preliminary model of APO-σ1

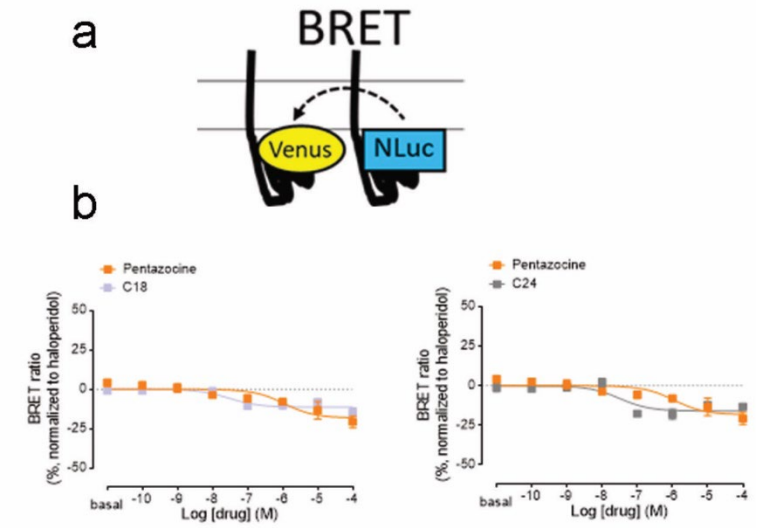

Figure 2. Compounds C18 and C24 bind to the SigmaR1 as determined by BRET assay. A) Schematic representation of the BRET assay. NIHT3T cells were transfected with the two subunits of the SigmaR1 receptor, which interact upon ligand binding. The measured fluorescence derives from the luciferase reaction achieved following the fusion of donor (luciferase) and acceptor (fluorescent) molecules to the two Sigma1R subunits. B) Titrated amounts of both compounds were incubated with the Sigma1R transfectants and fluorescence registered in biological triplicates. Both compounds are better ligands (higher affinity) than the natural SigmaR1 ligand Pentazocine. Affinity was calculated using fluorescence measurements of compound binding at titrated concentrations.

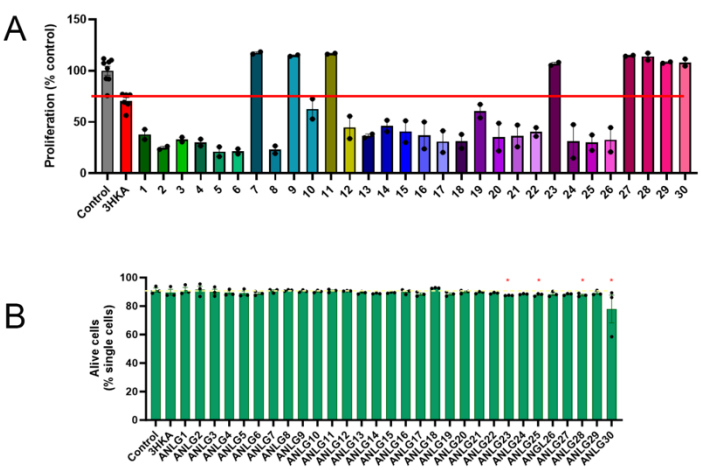

Figure 3. Figure 2 Anti-cancer proliferative activity of 3HKA-like compounds. Five different breast cancer and 5 different colorectal cancer cell lines were incubated with 3HKA or a library of analogs (1mm) for 24 hours. Proliferation was calculated using bromo bromodeoxyuridine incorporation, normalized to untreated cells. A) Results for the triple negative breast cancer cell line MDAMB468 are shown. B) cells were also tested for viability, using the MTT assay to determine whether the decrease proliferation was due to cell death. Cell death was comparable to untreated controls for all tested compounds.