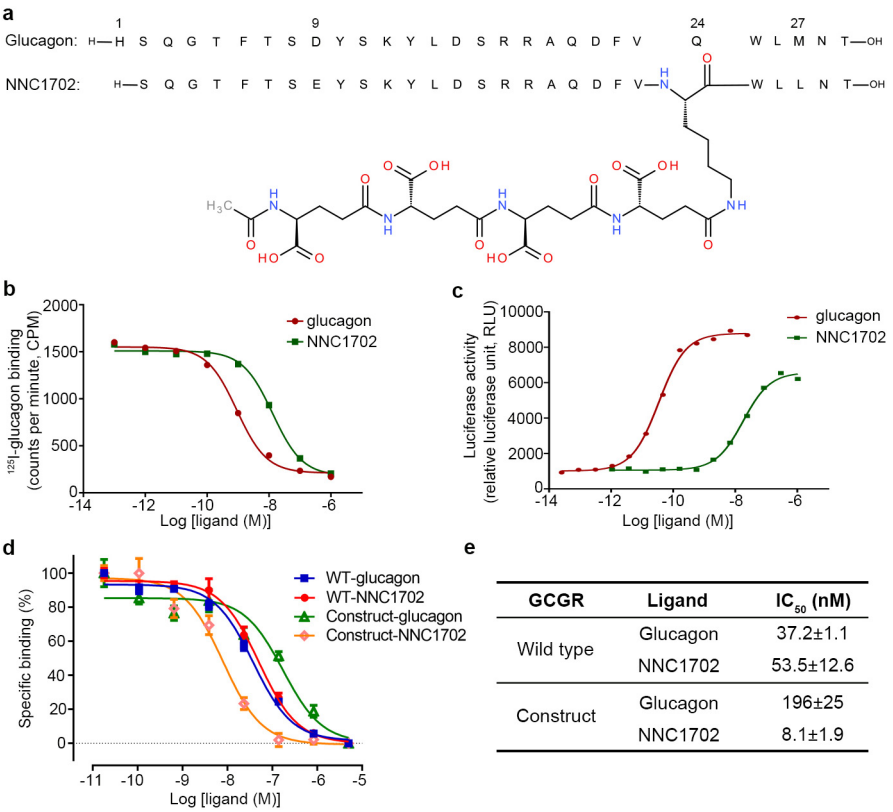

e

GCGR	Ligand	IC_{50} (nM)
Wild type	Glucagon	37.2±1.1
	NNC1702	53.5±12.6
Construct	Glucagon	196±25
	NNC1702	8.1±1.9

Extended Data Figure 1 | Binding affinity and potency of NNC1702. **a**, Sequences of glucagon and NNC1702. **b**, Binding assay of NNC1702. Competitive binding of human glucagon (red dots) and NNC1702 (green squares) to membranes from BHK cells that stably express human GCGR, on WGA-coated SPA beads. Glucagon labelled with ^{125}I (60 pM), and increasing concentrations of human glucagon and NNC1702, were used to generate the binding curves (representative example shown) and calculate IC_{50} values (glucagon: 1.2 ± 0.5 nM, NNC1702: 12.8 ± 6.6 nM). At least three independent experiments were performed with technical duplicates. **c**, Potency of NNC1702. The potencies of human glucagon (red dots) and NNC1702 (green squares) were determined by luciferase assays using BHK cells stably transfected with the human GCGR and CRE luciferase. Serial dilutions were prepared in medium (with 1 μM as the highest final concentration). Plate luminescence was read and EC_{50} values (glucagon: 22.8 ± 18.2 pM, NNC1702: 16.2 ± 8.4 nM) were calculated from the activation curves. At least three independent experiments were performed with technical duplicates (representative example shown). **d**, **e**, Inhibition of ^{125}I-labelled glucagon binding to CHO-K1 cells expressing wild-type (WT) and the engineered GCGR used for crystallization by glucagon and NNC1702. Data are shown as mean ± s.e.m. from three independent experiments performed in duplicate. 'Construct' indicates the GCGR construct used for crystallization. The IC_{50} values are listed in **e**.