Table S1.

Pharmacological characteristics of SCTR and AT1aR/SCTR oligomers after various treatments. Shown are the EC_{50} values for secretin-stimulated intracellular cAMP responses in these cells, as well as E_{max} and basal values compared with the maximal cAMP concentrations achieved in the SCTR transfected cells. Data represent the SEM of data from at least three experiments performed in triplicate.

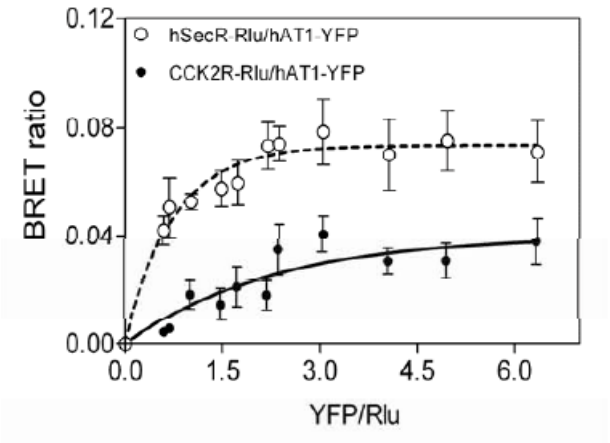
Receptors	Treatment	EC_{50} (nM)	E_{max} (% to SCTR max)	Basal (% to SCTR max)
SCTR	No	0.98	100.0±2.9	14.0±2.9
	STM-II	0.78	86.3±8.6	16.4±8.4
	STM-IV	2.48	56.7±6.0	6.7±4.1
	STM-IVm	1.95	106.0±4.4	16.7±3.7
	ATM1	0.94	97.4±4.7	11.3±5.0
	ATM4	0.56	55.1±2.1	1.2±2.5
	ATM4m	0.81	103.4±5.3	19.4±5.6
	ANGII	1.32	102.4±6.1	14.4±5.6
AT1aR/SCTR	No	0.92	66.1±3.0	6.2±2.8
	No (1μg)	1.56	81.2±4.4	12.4±3.9
	STM-II	1.61	61.7±2.7	6.3±2.3
	STM-IV	1.33	50.2±4.8	4.2±4.2
	ATM1	0.98	73.5±3.4	10.1±3.4
	ATM4	0.73	40.6±2.9	2.9±3.2
	ANGII	1.85	104.5±3.3	14.4±2.6
	ANGII+ATM1	0.91	59.0±3.4	4.8±3.7
	ANGII+ATM1m	3.31	101.2±4.2	19.4±3.2
AT2R/SCTR	No	1.49	111.2±5.4	10.3±4.7
AT1aR-CAM/SCTR	No	0.62	100.7±3.6	16.0±3.6
AT1aR-K199A/SCTR	No	0.72	73.1±2.9	11.7+2.9
	ANGII	0.94	69.0±3.7	11.9±3.8

Table S2

The sequences of the transmembrane peptides used in this study. The mutated sequences are underlined.

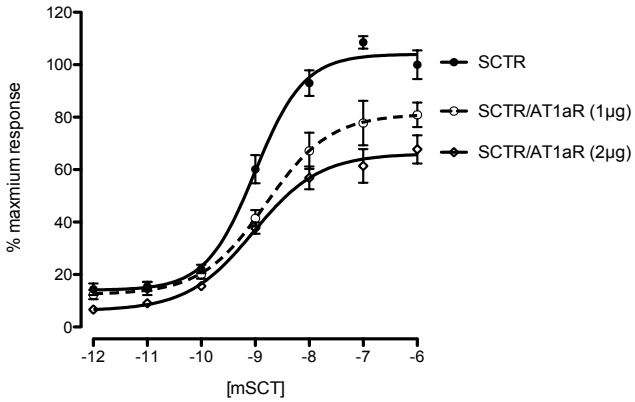
Receptor	Name	Sequence
SCTR	STM-I	KKMYTVGYSSSLAMLLVALSILCSFKK
	STM-II	KKIHMHLFVSFILRALSNFIKDAVLKK
	STM-III	KKLVMIFFQYCIMANYAWLLVEGLYKK
	STM-IV	LQAFVLFGWGSPAIFVALWAVTRHFLE
	STM-IVm	LQAFVLAGWASPAAFVALWAATRHFLE
	STM-V	KKWWVIRGPVILSIVINFIFFINILKK
	STM-VI	KKSTLLLIPLFGIHYIVFAFSPEGAKK
	STM-VII	KKLFFELALGSFQGLVVAVLYCFLNKK
AT1aR	ATM1	KKIPTLYSIIFVVGIFGNSLVVIVIKK
	ATM1m	KKIPTAYSIAFVAGIFANSLVVIVIKK
	ATM2	KKFLLNLALADLCFLLTLPLWAVYTKK
	ATM3	KKIASASVSFNLYASVFLLTCLKK
	ATM4	KKLVAKVTCIIIWLMAGLASLPAVIKK
	ATM4m	KKAVAKATCIIIWAMAGAASAPLVIKK
	ATM5	KKILGFLFPFLIILTSYTLIKK
	ATM6	KKIFRIIMAIVLFFFFSWVPHQIFTKK
	ATM7	KKAMPITICIAYFNNCLNPLFYGFLKK

SFig. 1



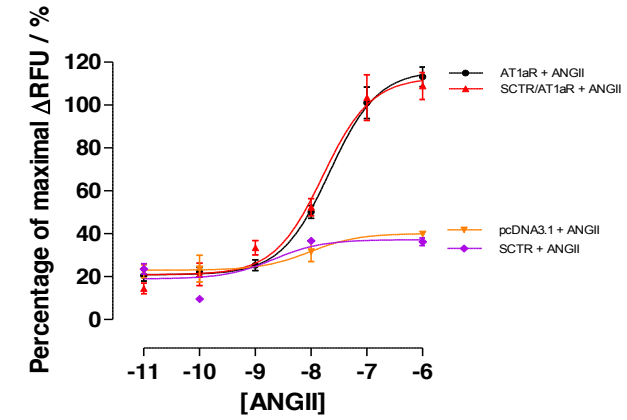
SFig. 1 ***Heteromer formation of human SCTR with AT1R.***
The BRET saturation curves are plotted as a ratio of YFP- and Rlu-tagged constructs in the transfection. A fixed amount of human SCTR-Rlu, control CCK2R-Rlu, and increasing amounts of human AT1R-YFP were co-transfected into CHO cells. Similar to data for the mouse receptors, the human SCTR-Rlu and AT1R-YFP co-transfected cells produced an exponential curve for the YFP/ Rlu ratio that reached an asymptote, consistent with a saturable BRET signal. In the control experiment, the cells with SCTR-Rlu and CCK2R-YFP did not produce a significant saturation curve. Values are expressed as mean ± SEM, with duplicate data from at least four experiments.

SFig. 2



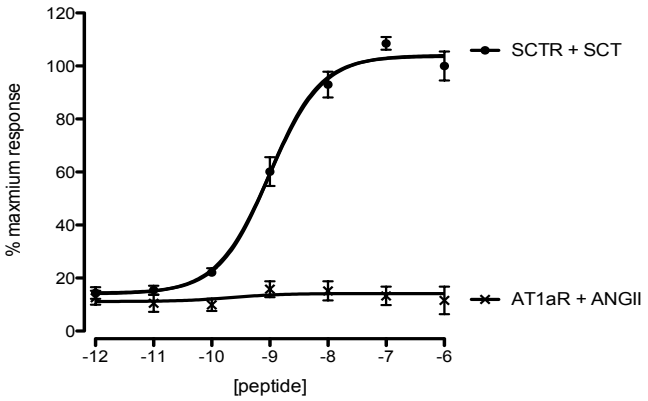
SFig. 2 ***The dose-dependent effects of AT1aR on the efficacy of cAMP production through the SCTR.***
Maximal cAMP levels induced by SCT in mouse SCTR-transfected cells were set as 100%. Co-expression of AT1aR with SCTR reduced the maximal responses in cAMP production of the SCTR. This drop in efficacy was dose-dependent, as demonstrated by using 1 mg or 2 mg of AT1aR in the co-transfection.

SFig. 3



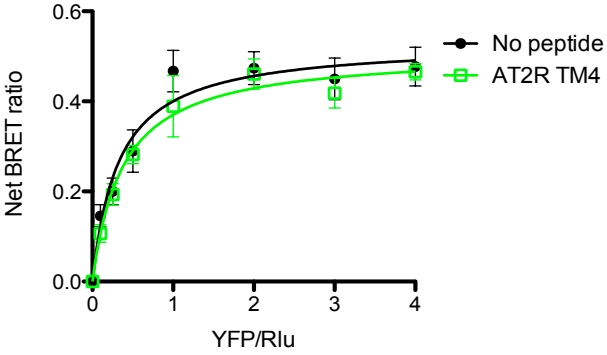
SFig. 3 ***Co-expression of SCTR and AT1aR had no effect on Ca^{2+} signaling through the AT1aR in response to ANGII.***
Effects of mouse ANGII on the intracellular $[Ca^{2+}]_i$ mobilization in the cells transfected with various combination of receptors were monitored fluorometrically by using a calcium-sensitive probe fluo 4-AM. Data were expressed in mean ± SEM of delta changes in the fluorescence signals from baseline and converted to percentages of the maximum of ANGII-induced $[Ca^{2+}]_i$ elevation in AT1aR-transfected cells. The co-expression of SCTR with AT1aR did not cause a significant change in Ca^{2+} signaling when compared to the cells transfected with AT1aR alone.

SFig. 4



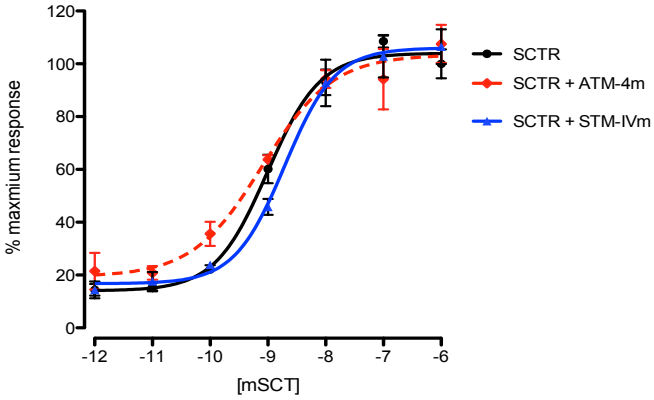
SFig. 4 ***ANGII is unable to stimulate cAMP accumulation in AT1aR-transfected cells.*** Intracellular cAMP accumulation was measured in SCTR- or AT1aR- transfected cells in response to graded concentrations of SCT or ANGII, respectively. SCT stimulated robust cAMP accumulation in SCTR-transfected cells, while ANGII was unable to activate cAMP production in AT1aR-transfected cells. Values are expressed as mean ± SEM, with duplicate data from at least three experiments.

SFig. 5



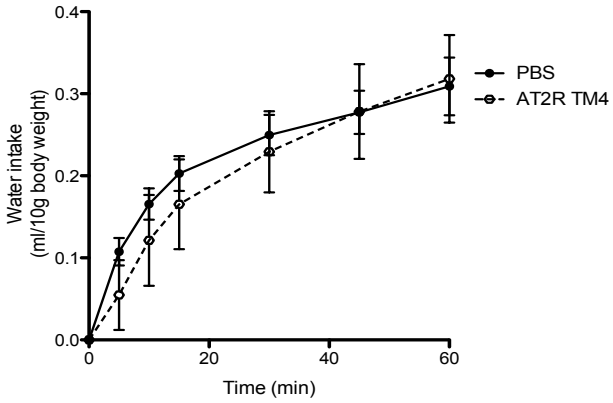
SFig. 5 ***Effects of AT2R-TM4 peptides on formation of SCTR-AT1aR hetero-complexes.*** SCTR-Rlu and AT1aR-YFP co-transfected cells were treated with AT2R-TM4 peptides. No significant change of the net BRET ratios was observed by saturation BRET studies. Values are expressed as mean ± SEM, with duplicate data from at least two experiments.

SFig. 6



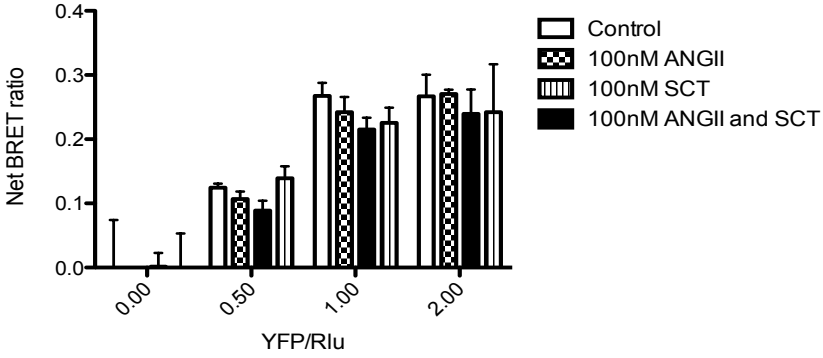
SFig. 6 ***Mutated TM4 peptides did not alter cAMP signaling of SCTR.*** The mutated TM peptides (ATM-4m and STM-IVm) were used to treat the cells expressing SCTR. Neither peptide affected the cAMP signaling of SCTR-transfected cells. Data are expressed as mean ± SEM with duplicate data points from at least three experiments.

SFig. 7



SFig. 7 ***The AT2R-TM4 peptides did not inhibit hyperosmolality-induced drinking in mice.***
The effect of AT2R-TM4 on hyperosmolality-induced drinking was studied by i.c.v. injection of the peptide (4 μg in 5 μl) vs. controls (5 μl PBS or vehicle control 20% DMSO in PBS) into the lateral ventricle 15 minutes after hyperosmotic shock. When compared with the control, no significant change in water intake can be observed after AT2R-TM4 peptide injection. Data are expressed as mean ± SEM with duplicate data points from two experiments.

SFig. 8



SFig. 8 ***SCT and ANGII treatment did not reduce the BRET signal of the heteromer.***
Fixed amount of mouse SCTR-Rlu and mouse AT1aR-YFP (0, 0.5, 1.0 and 2.0 mg) were co-transfected into CHO cells. The receptors transfected cells were treated with either SCT or ANGII or SCT and ANGII co-treatment (100 nM) for 45 min before the BRET reading. There were no significant changes of the BRET ratio after all the treatments. Values are expressed as mean ± SEM, with duplicate data from at least 2 experiments.