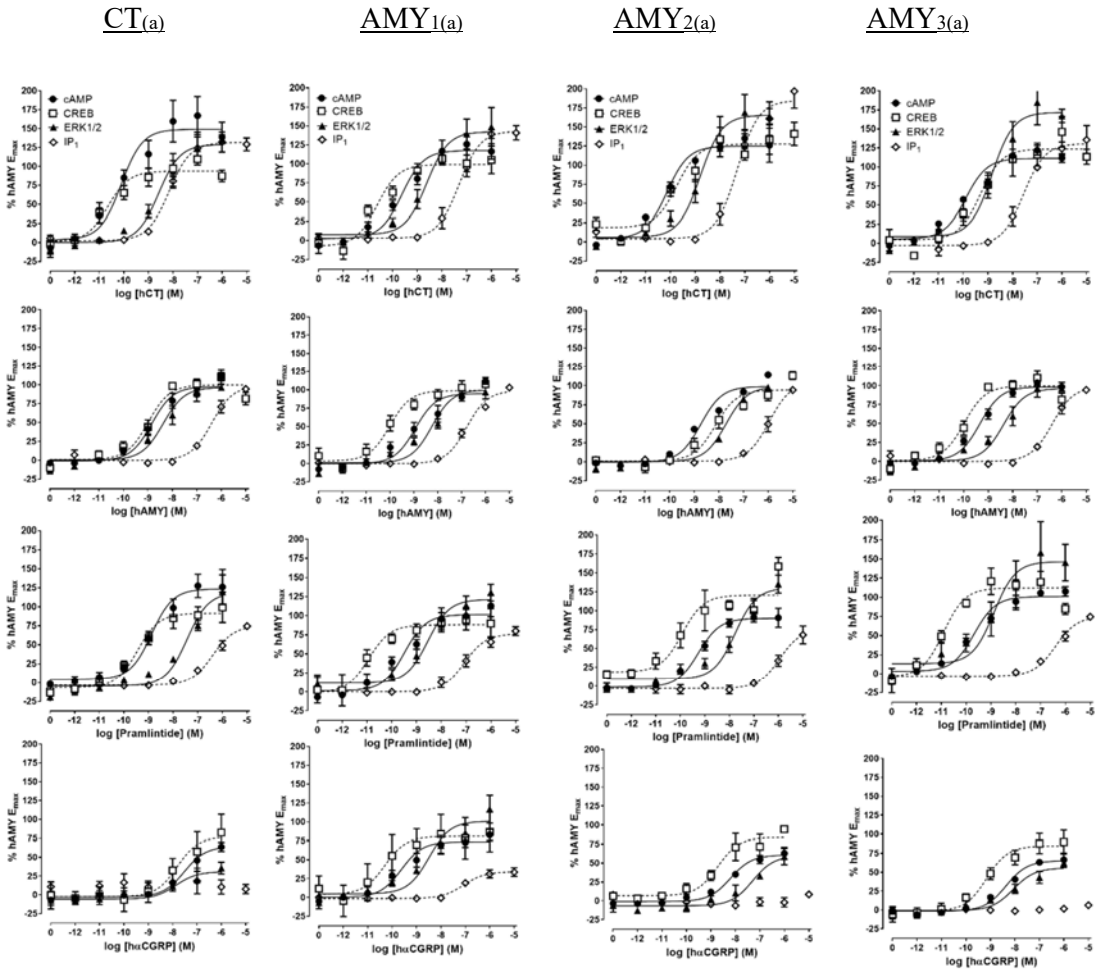
Supplementary Biology (SB)

Figure SB1. Activation of four different signaling pathways (cAMP, IP_1, ERK1/2 and CREB phosphorylation) at four different receptors (CTR, AMY_{1-3}) with four different ligands (amylin, CGRP, calcitonin and pramlintide). Data are mean ± s.e.m. of 3-5 biological replicates, as detailed in Tables SB1-4. Data were normalized to human amylin (hAMY) in each experiment. pERK1/2 data are the 15 minute time point.



Supplementary biology data tables 1-4

Data are mean ± s.e.m. of *n* biological replicates, reporting activation of four different signaling pathways (cAMP, IP_1, ERK1/2 and CREB phosphorylation) at four different receptors (CTR, AMY_{1-3}) with four different ligands (amylin, CGRP, calcitonin and pramlintide). Data were normalised to hAMY in each experiment and E_{max} values are % hAMY.

Table SB1. Signaling Pathways $CT_{(a)}$

	cAMP					IP1					ERK1/2 (7 min)					ERK1/2 (15 min)					CREB				
	pEC_{50}	**SEM**	**Emax**	**SEM**	**n**	**pEC_{50}**	**SEM**	**Emax**	**SEM**	**n**	**pEC_{50}**	**SEM**	**Emax**	**SEM**	**n**	**pEC_{50}**	**SEM**	**Emax**	**SEM**	**n**	**pEC_{50}**	**SEM**	**Emax**	**SEM**	**n**
hCT	10.2	0.07	149	23.2	5	8.16	0.02	132	12.1	4	8.29	0.12	147	13.8	4	8.68	0.08	145	10.0	4	10.9	0.18	94.4	6.90	4
hAMY	8.73	0.21	100		5	6.49	0.04	100		4	7.08	0.14	100		4	7.48	0.26	100		4	9.06	0.10	100		5
Pramlintide	8.85	0.22	127	20.2	5	6.41	0.06	84.2	12.1	4	7.04	0.18	137	15.4	4	7.50	0.29	128	32.2	4	9.47	0.22	95.5	15.9	5
hαCGRP	7.49	0.13	66.0	6.20	5	<5				4	<5				4	7.76	0.28	33.6	9.20	4	7.77	0.33	82.6	25.2	5

Table SB2. Signaling Pathways $AMY_{1(a)}$

	cAMP					IP1					ERK1/2 (7 min)					ERK1/2 (15 min)					CREB				
	pEC_{50}	**SEM**	**Emax**	**SEM**	**n**	**pEC_{50}**	**SEM**	**Emax**	**SEM**	**n**	**pEC_{50}**	**SEM**	**Emax**	**SEM**	**n**	**pEC_{50}**	**SEM**	**Emax**	**SEM**	**n**	**pEC_{50}**	**SEM**	**Emax**	**SEM**	**n**
hCT	9.62	0.11	118	12.7	5	7.35	0.09	142	5.80	4	8.36	0.10	158	16.9	4	8.55	0.17	146	22.0	4	10.6	0.24	93.7	6.60	5
hAMY	8.77	0.37	100		5	6.76	0.13	100		4	8.14	0.11	100		4	8.40	0.15	100		4	9.99	0.28	100		5
Pramlintide	9.53	0.17	102	13.0	5	7.09	0.17	76.1	5.90	4	8.22	0.16	161	14.4	4	8.43	0.17	122	11.7	4	10.9	0.21	95.8	17.6	4
hαCGRP	9.39	0.28	76.2	7.10	5	7.05	0.10	34.5	5.80	4	8.39	0.14	99.9	9.50	4	8.55	0.19	101	15.0	4	10.4	0.15	80.7	24.0	5

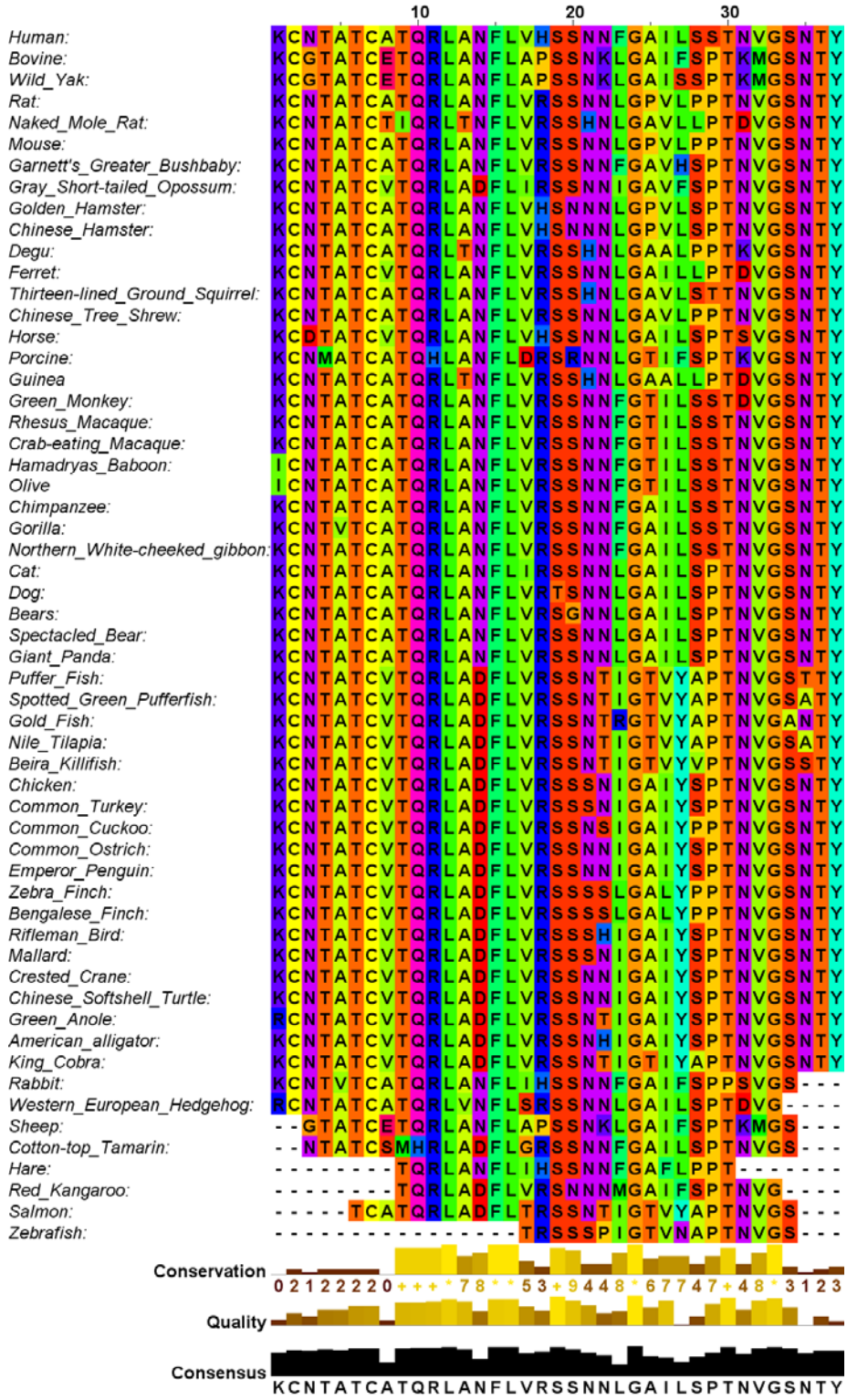
Table SB3. Signaling Pathways $AMY_{2(a)}$

	cAMP					IP1					ERK1/2 (7 min)					ERK1/2 (15 min)					CREB				
	pEC_{50}	**SEM**	**Emax**	**SEM**	**n**	**pEC_{50}**	**SEM**	**Emax**	**SEM**	**n**	**pEC_{50}**	**SEM**	**Emax**	**SEM**	**n**	**pEC_{50}**	**SEM**	**Emax**	**SEM**	**n**	**pEC_{50}**	**SEM**	**Emax**	**SEM**	**n**
hCT	10.1	0.17	126	10.0	5	7.36	0.18	195	49.1	4	8.22	0.05	172	5.90	4	8.56	0.21	171	15.7	4	10.6	0.24	133	7.70	5
hAMY	8.66	0.11	100		5	5.90	0.24	100		4	7.50	0.20	100		4	7.67	0.09	100		4	9.14	0.22	100		5
Pramlintide	9.23	0.17	91.4	6.60	5	5.95	0.05	75.0	14.4	4	7.39	0.26	126	19.7	4	7.70	0.16	132	9.30	4	9.77	0.47	133	12.4	5
hαCGRP	8.21	0.18	62.0	6.90	5	<5				4	7.49	0.38	49.3	11.7	4	7.64	0.29	57.9	12.0	4	8.45	0.29	92.0	6.80	4

Table SB4. Signaling Pathways $AMY_{3(a)}$

	cAMP					IP1					ERK1/2 (7 min)					ERK1/2 (15 min)					CREB				
	pEC_{50}	**SEM**	**Emax**	**SEM**	**n**	**pEC_{50}**	**SEM**	**Emax**	**SEM**	**n**	**pEC_{50}**	**SEM**	**Emax**	**SEM**	**n**	**pEC_{50}**	**SEM**	**Emax**	**SEM**	**n**	**pEC_{50}**	**SEM**	**Emax**	**SEM**	**n**
hCT	9.85	0.17	111	5.70	5	7.62	0.20	132	13.1	4	8.31	0.12	151	7.70	5	8.73	0.18	175	17.3	4	10.5	0.15	123	13.8	5
hAMY	9.32	0.18	100		5	6.33	0.10	100		4	7.96	0.16	100		5	8.30	0.33	100		4	10.0	0.14	100		5
Pramlintide	9.65	0.14	102	4.10	5	6.46	0.18	72.8	3.70	4	7.72	0.29	115	7.20	5	8.67	0.35	151	28.2	4	11.0	0.21	112	7.40	5
hαCGRP	8.39	0.23	66.9	5.80	5	<5				3	7.95	0.49	60.3	18.5	5	7.97	0.41	58.2	1.90	4	9.13	0.07	84.3	12.2	5

Figure SB2. Amino acid sequence alignment of amylins from different species. The majority of sequences are of pre-pro amylin with only the predicted mature fully processed sequence listed. Sequences that are not complete correspond to partial. The residues are color-coded according to their properties as follows: dark blue/purple, positive; red, negative or small polar; purple, polar; blue/cyan, aromatic; green large hydrophobic; yellow, small hydrophobic. This corresponds to the 'Taylor' scheme, as implemented in Jalview[1]. The annotations indicate the conservation, quality of the alignment and the consensus.



Figures SB3-21. cAMP production by peptide analogues or competition binding data, as indicated. Data are mean ± s.e.m. of *n* biological replicates as shown. **P* <0.05 by unpaired *t*-test for pEC_{50} or pIC_{50} or where 95% confidence intervals did not include 100 for E_{max}.

Figure SB3. *C*-terminal Alanine Analogues: $hCT_{(a)}$

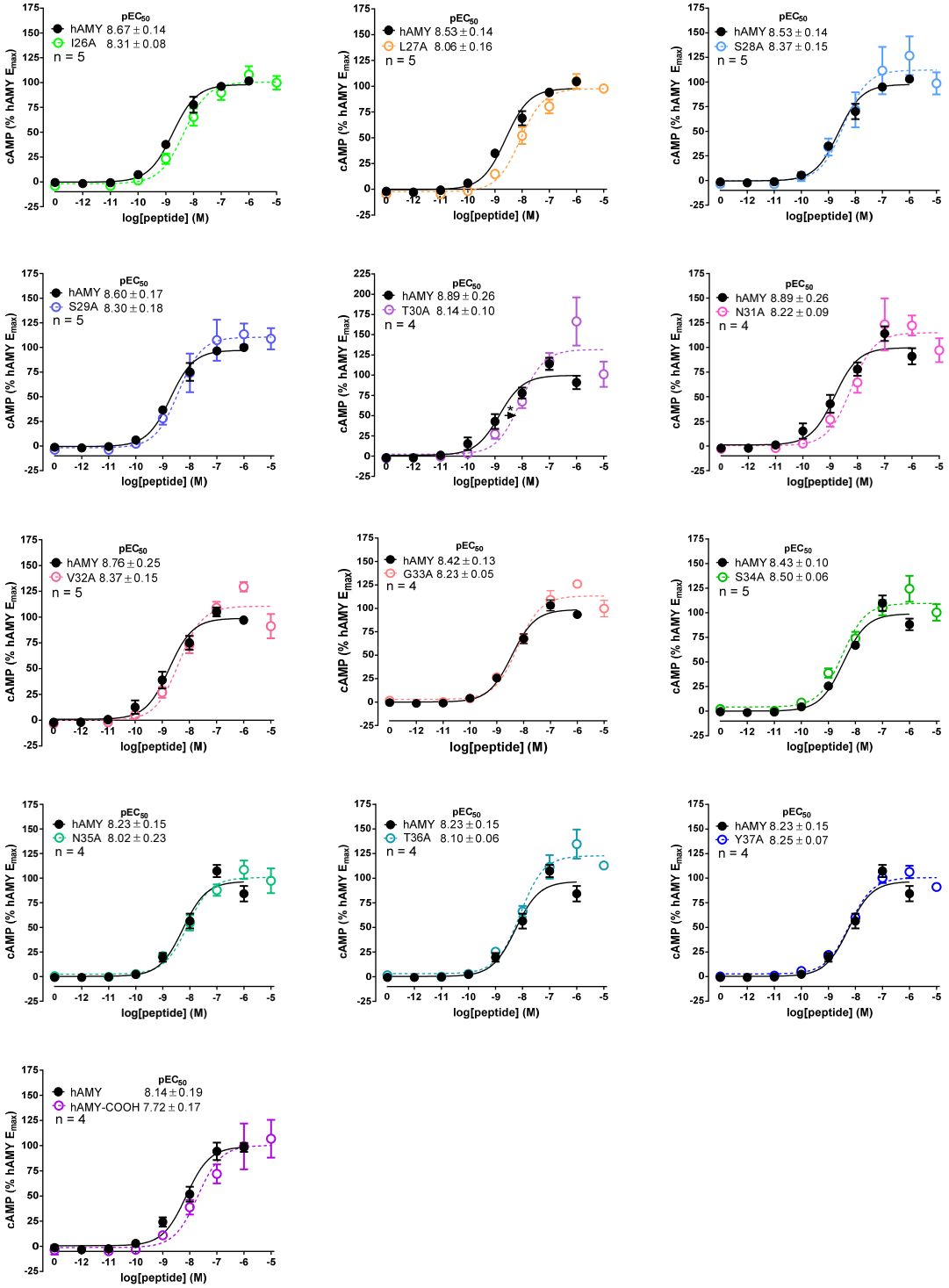


Figure SB4. *C*-terminal Alanine Analogues: hAMY$_{1(a)}$

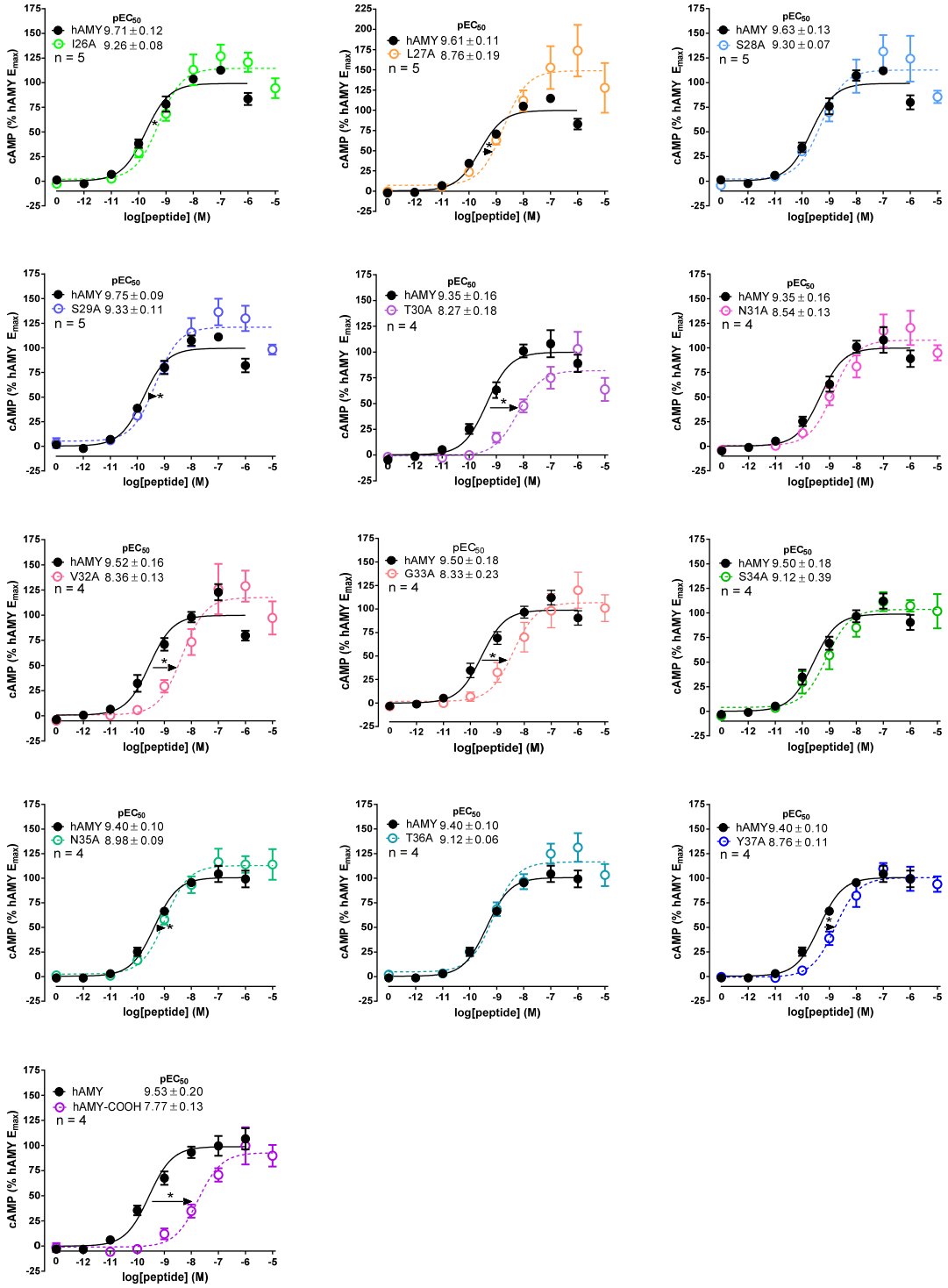


Figure SB5. *C*-terminal Analogues hAMY$_{3(a)}$

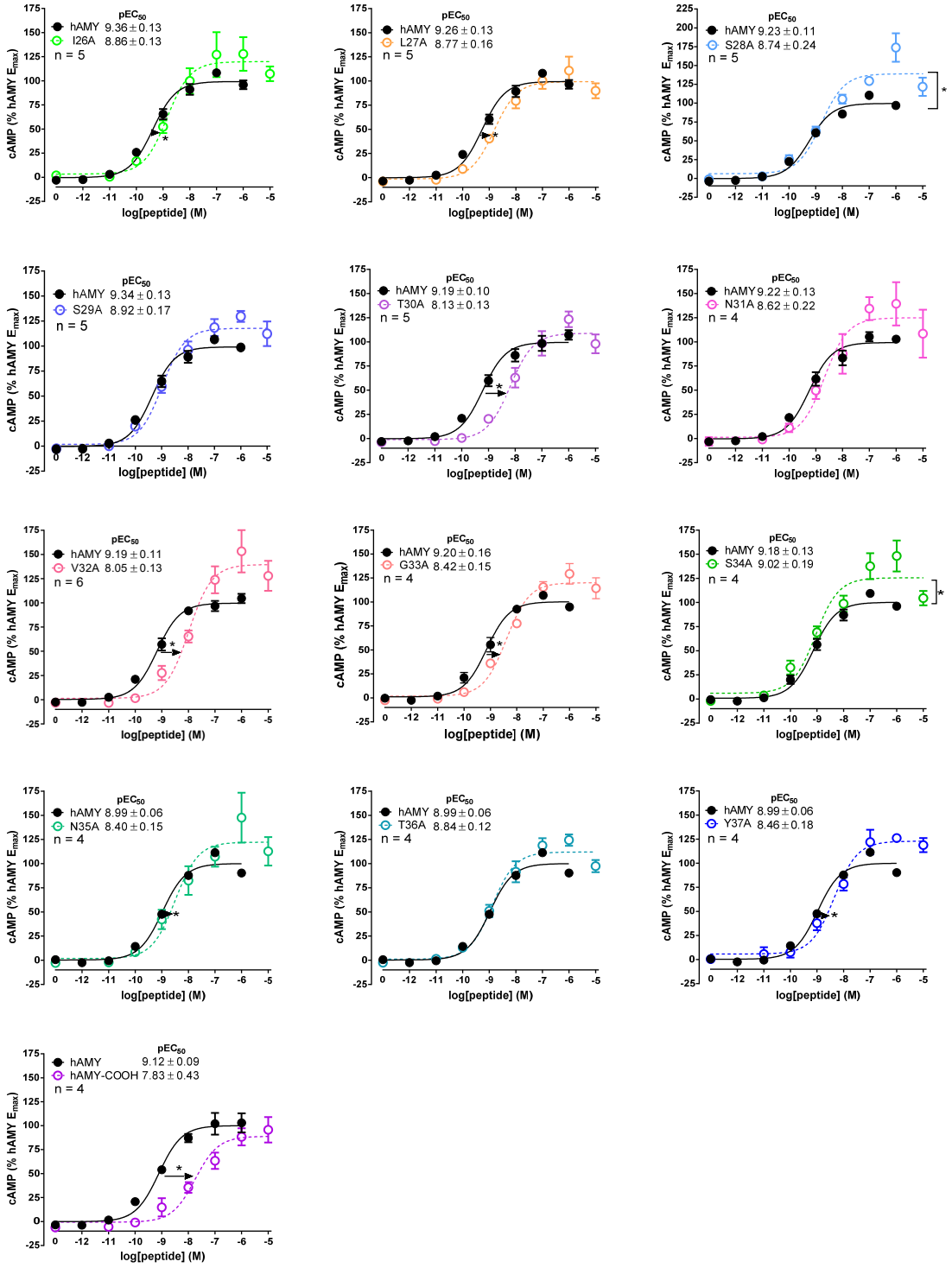


Figure SB6. Binding *C*-terminal Analogues: $AMY_{1(a)}$

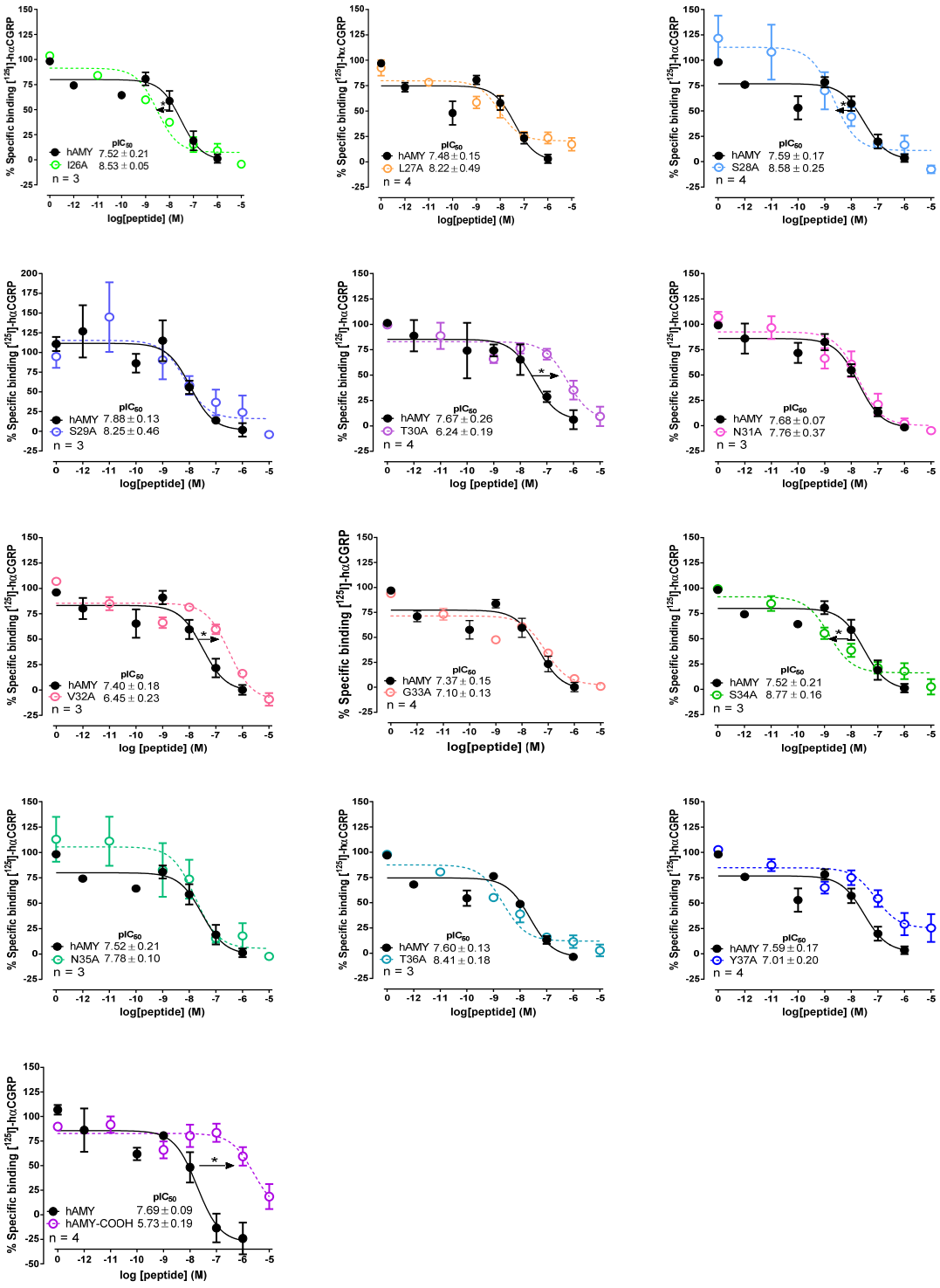


Figure SB7. *C*-terminal Exchange Between CGRP and Amylin: Y37F hAMY

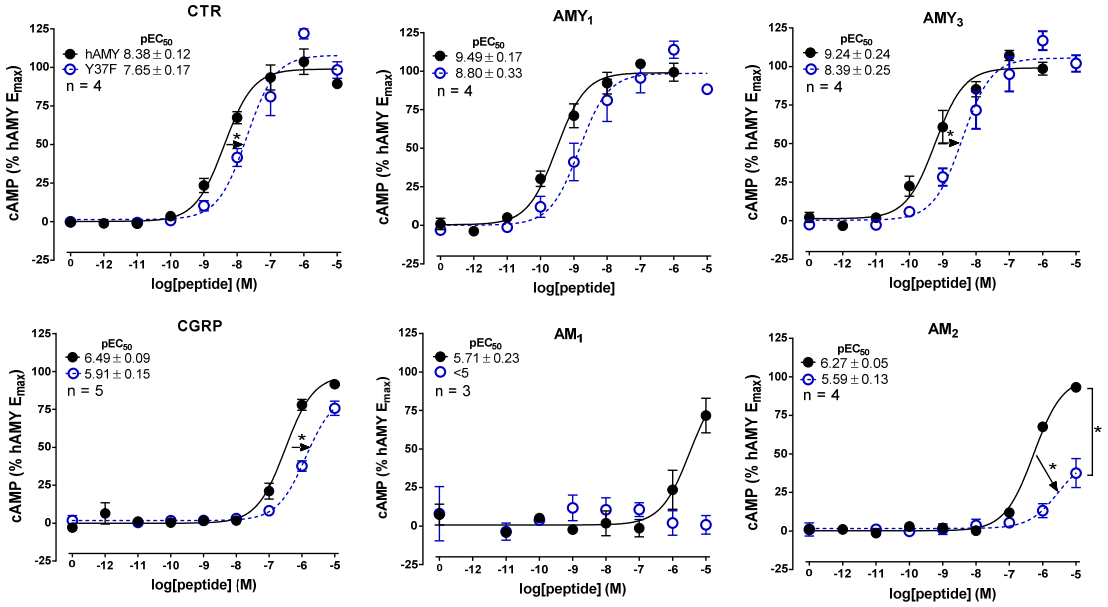


Figure SB8. *C*-terminal Exchange Between CGRP and Amylin: F37Y CGRP

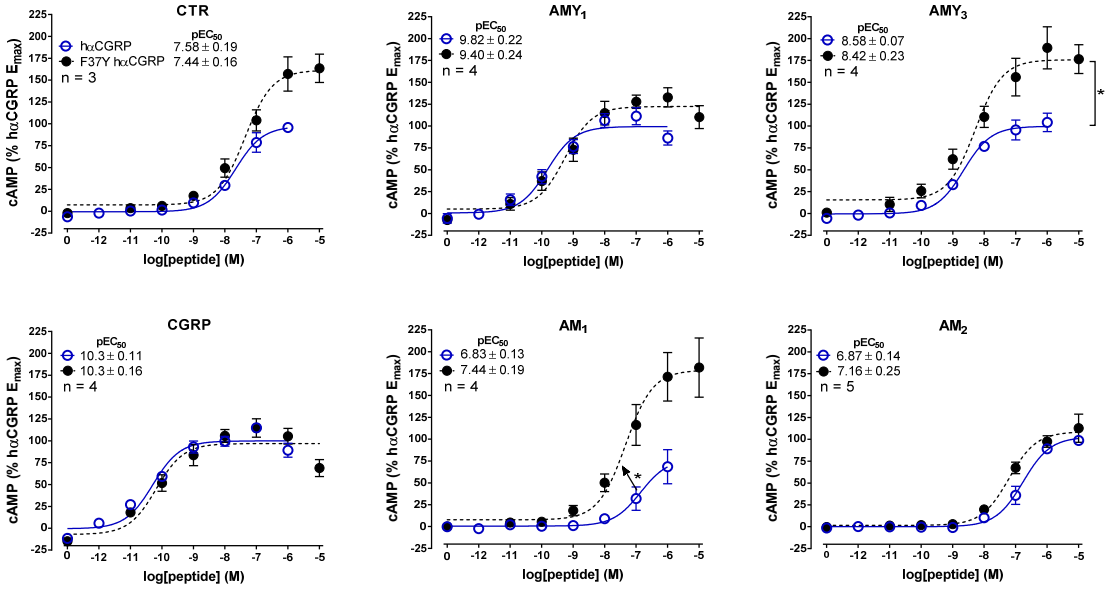
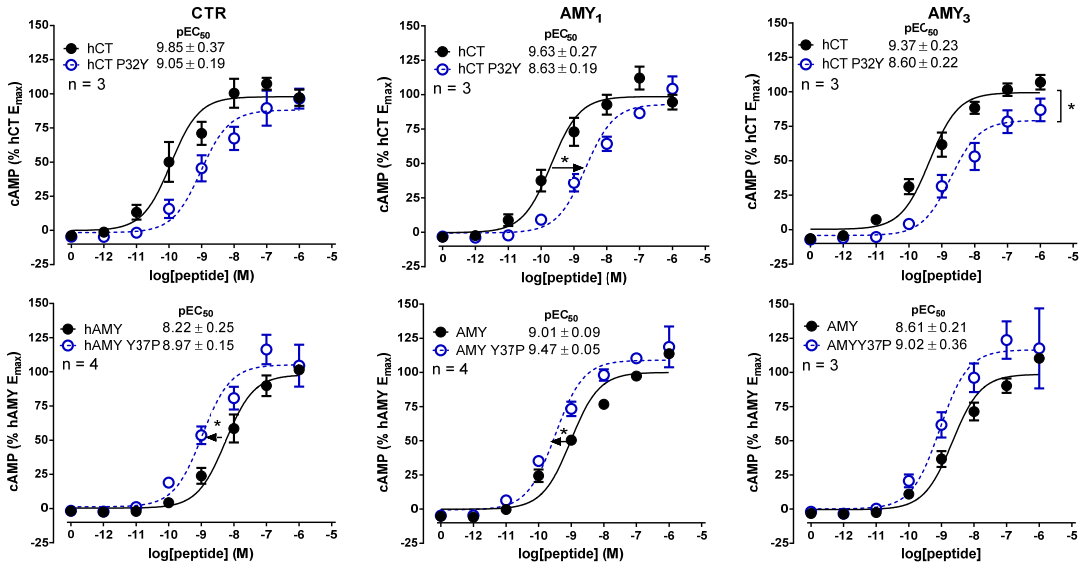


Figure SB9. *C*-terminal Exchange Between human calcitonin and amylin: P32Y hCT, Y37P hAMY. a) cAMP production and b) Binding for hAMY and Y37P hAMY or hCT and P32Y hCT at the AMY_1 receptor.

a



b

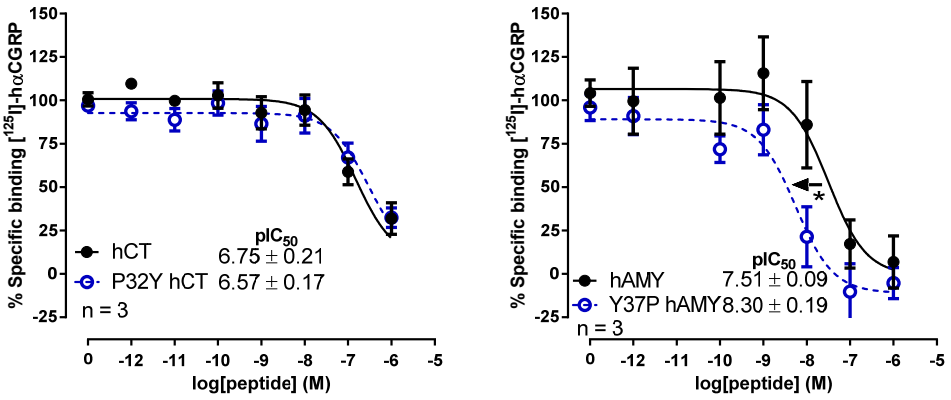


Figure SB10. *N*-terminal Loop Analogues $hCT_{(a)}$. DR, second synthesis of this peptide in DR laboratory.

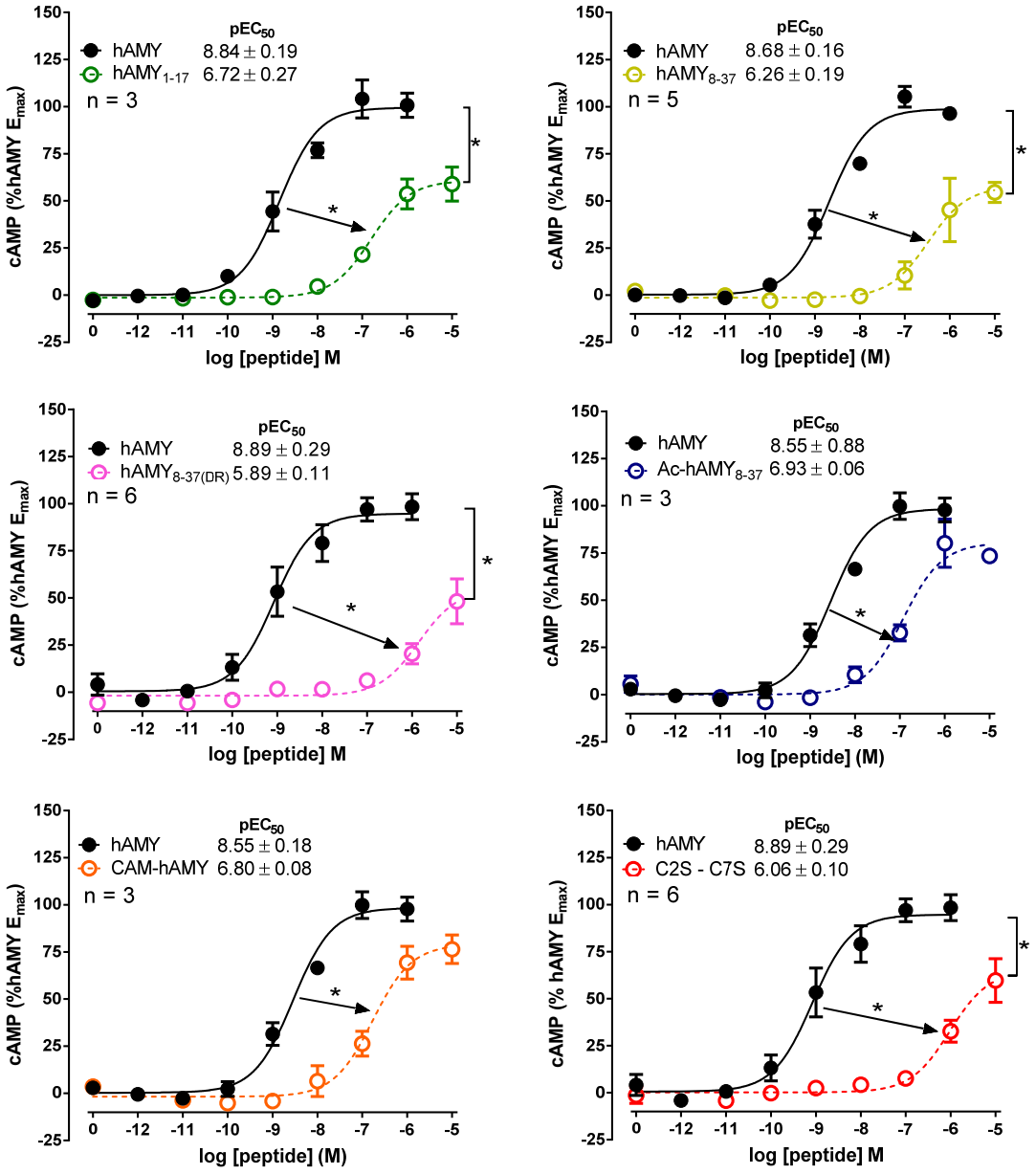


Figure SB11. *N*-terminal Loop Analogues: $hAMY_{1(a)}$

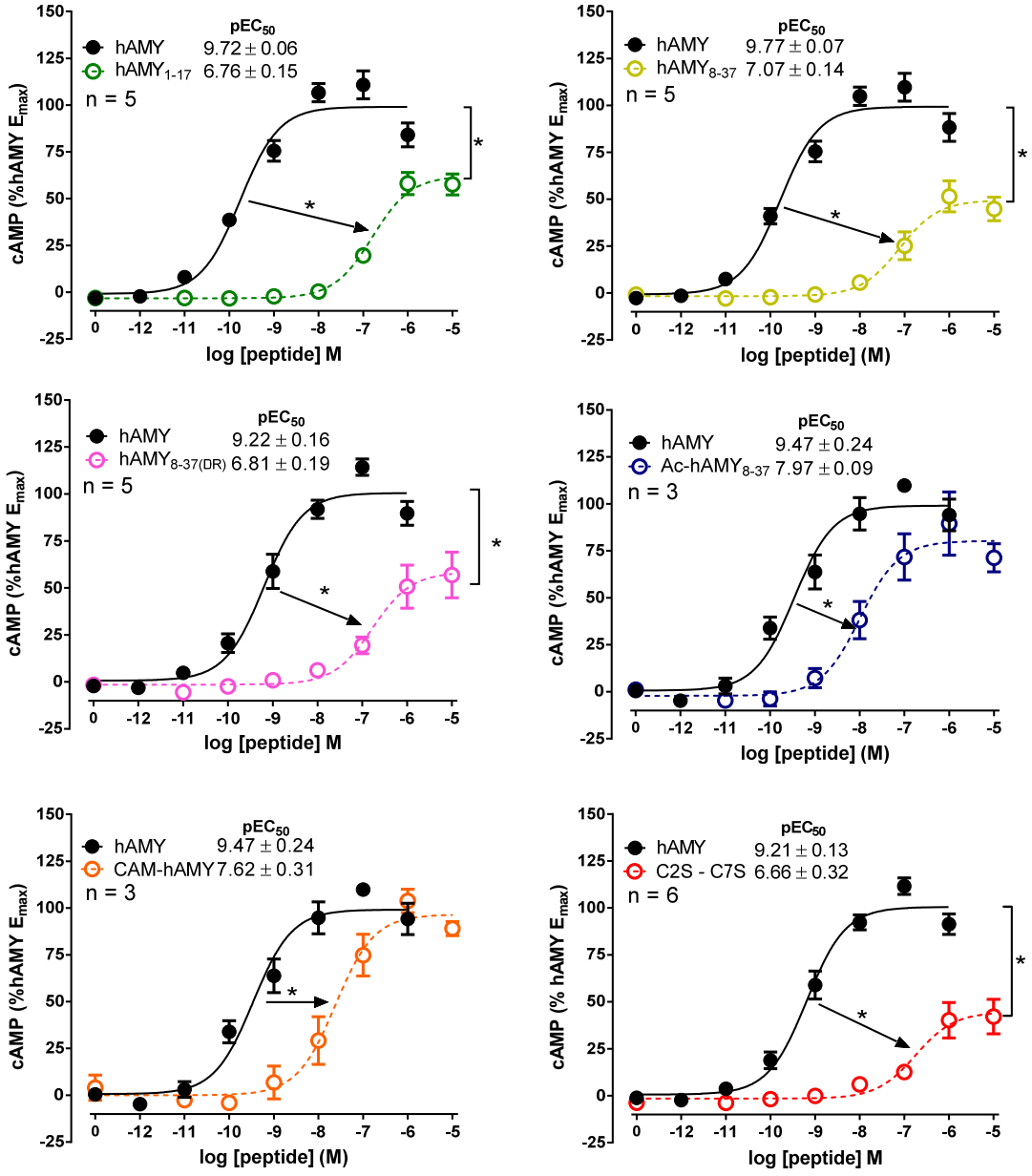


Figure SB12. *N*-terminal Loop Analogues: $hAMY_{3(a)}$

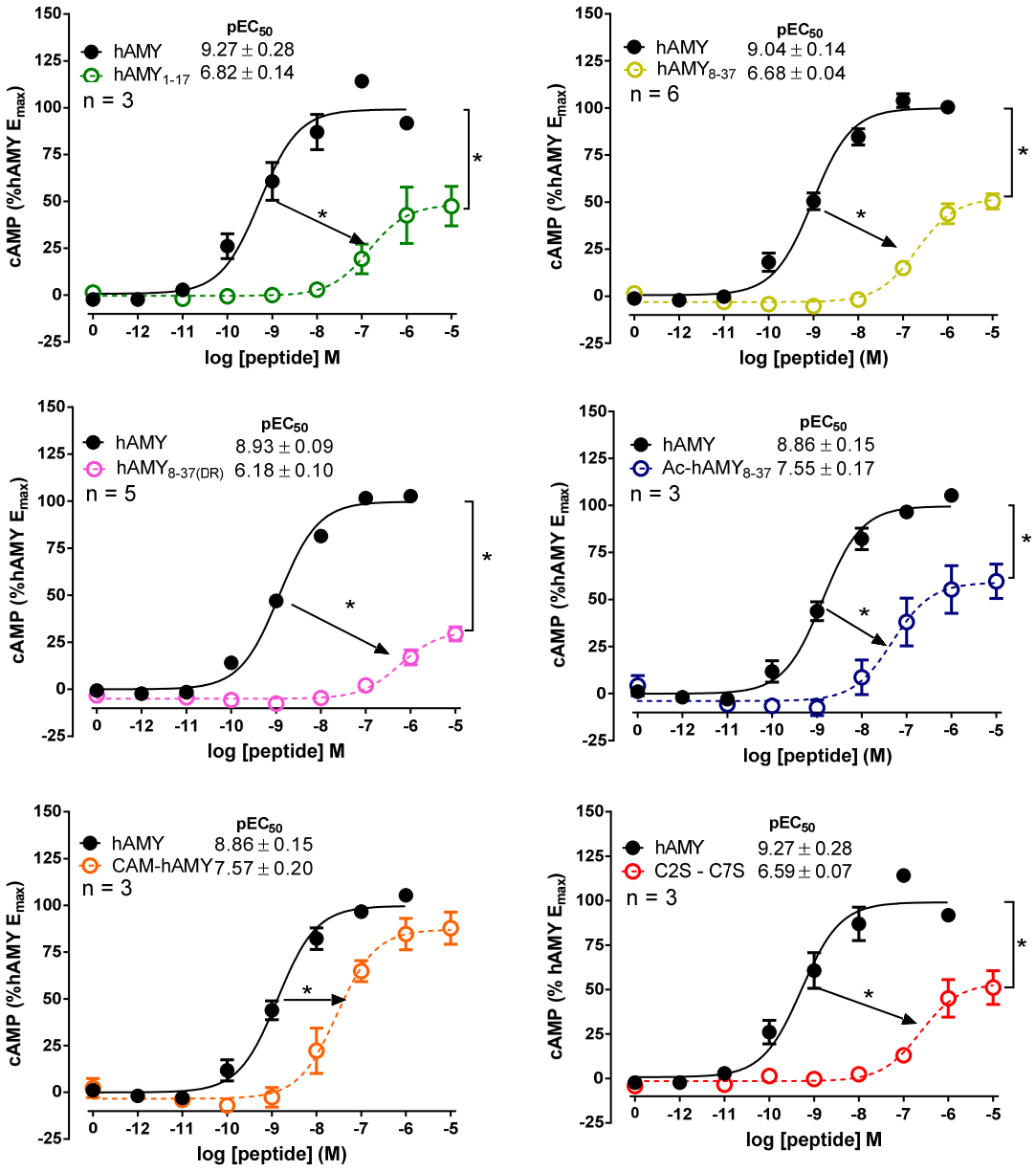


Figure SB13. *N*-terminal Alanine/Glycine Analogues: $hCT_{(a)}$

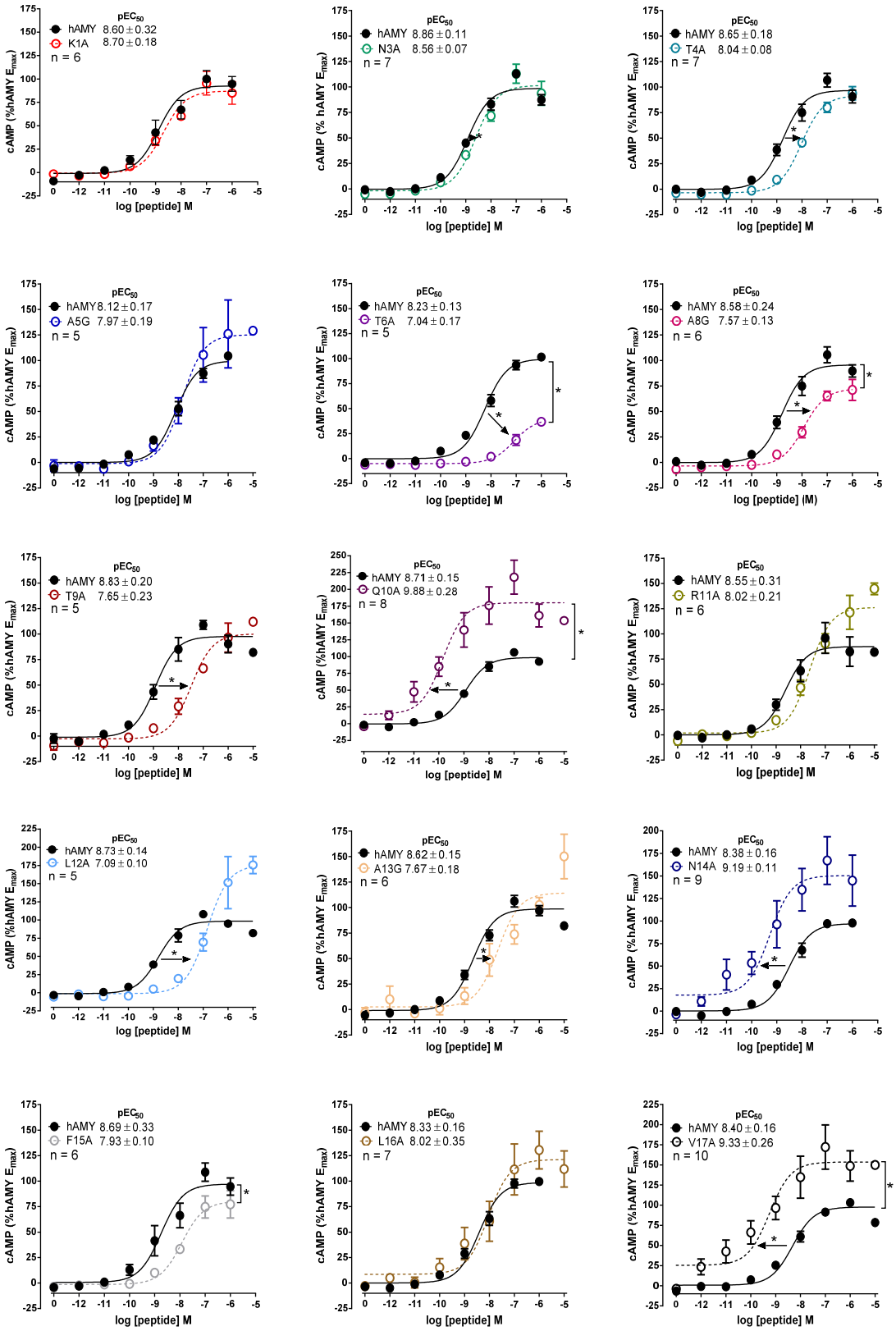


Figure SB14. *N*-terminal Alanine/Glycine Analogues: $hAMY_{1(a)}$

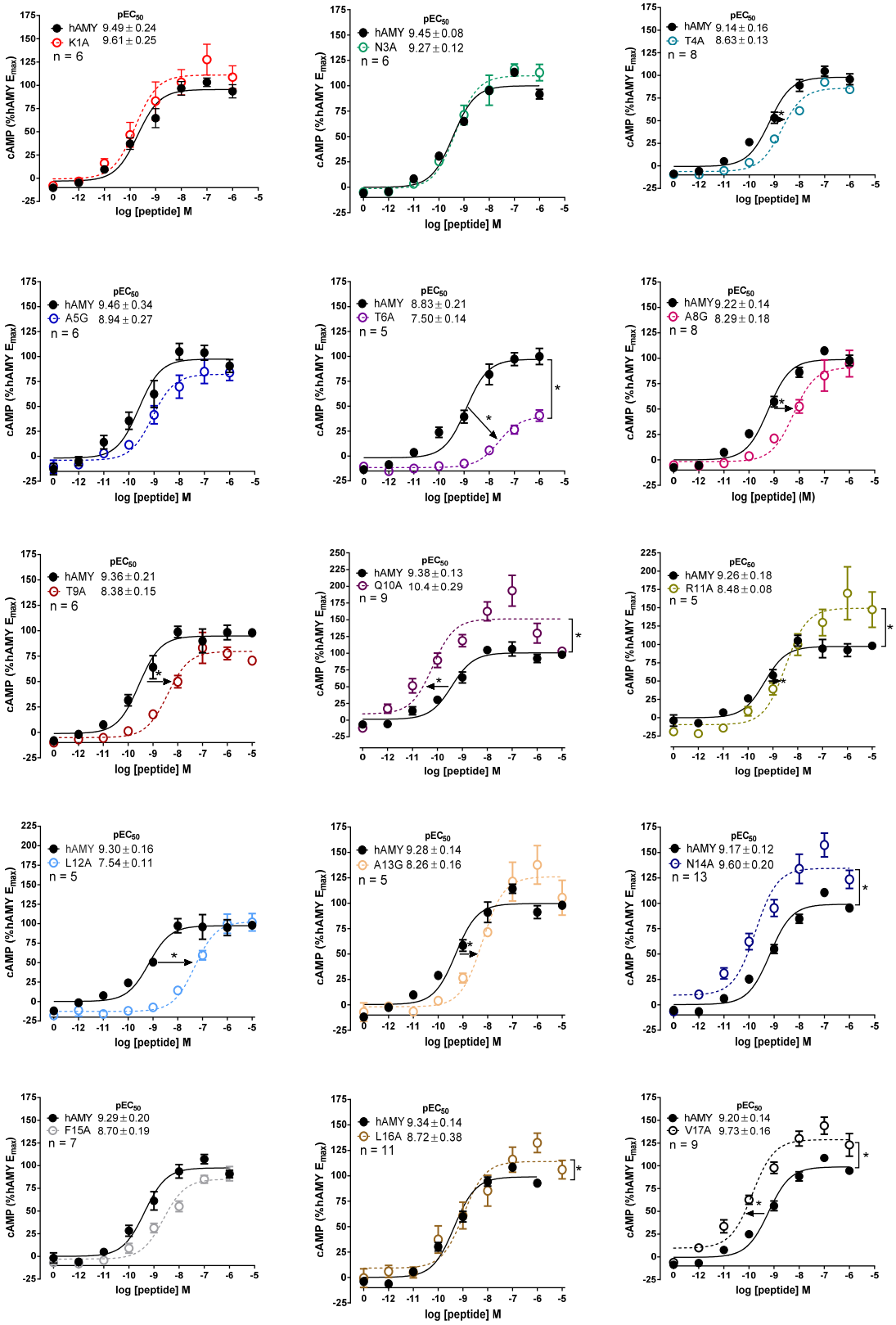


Figure SB15. *N*-terminal Alanine/Glycine Analogues: $hAMY_{3(a)}$

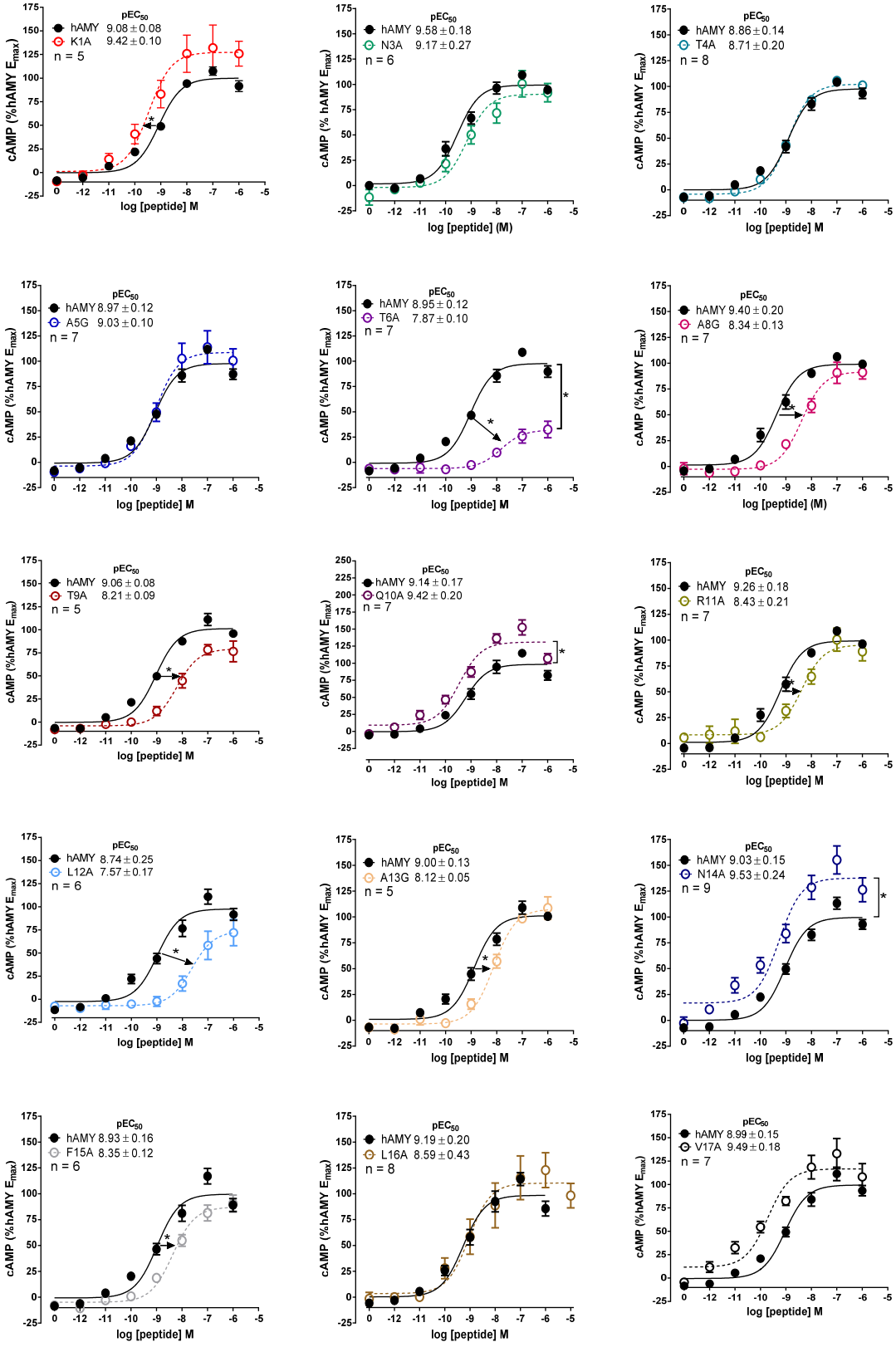


Figure SB16. Binding *N*-terminal Alanine/Glycine Analogues, $AMY_{8\text{-}37}$: $AMY_{1(a)}$

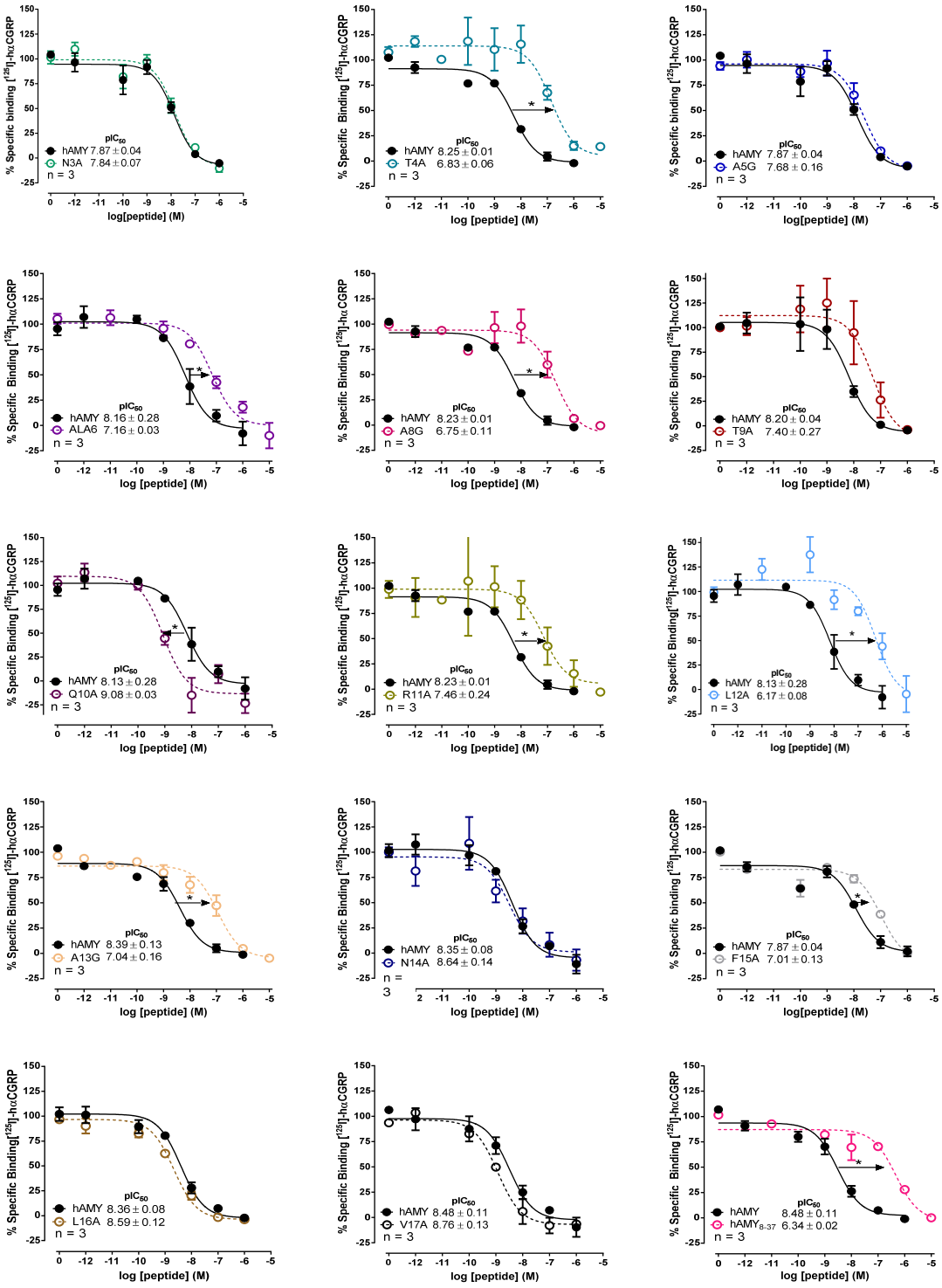


Figure SB17. Alanine to Serine Substitution: Position 5

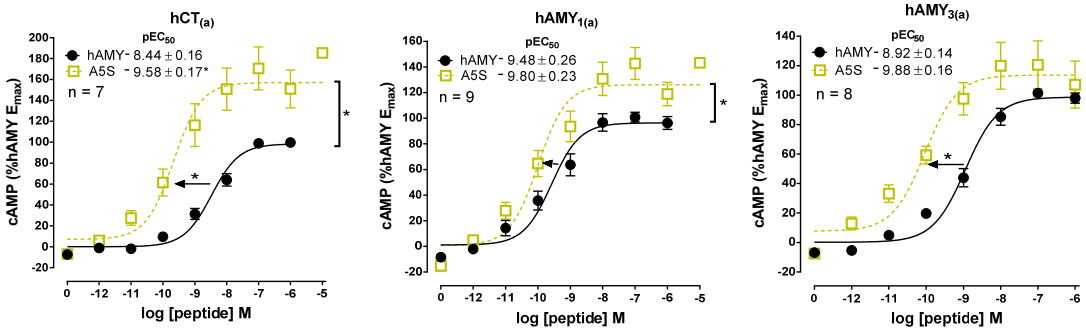
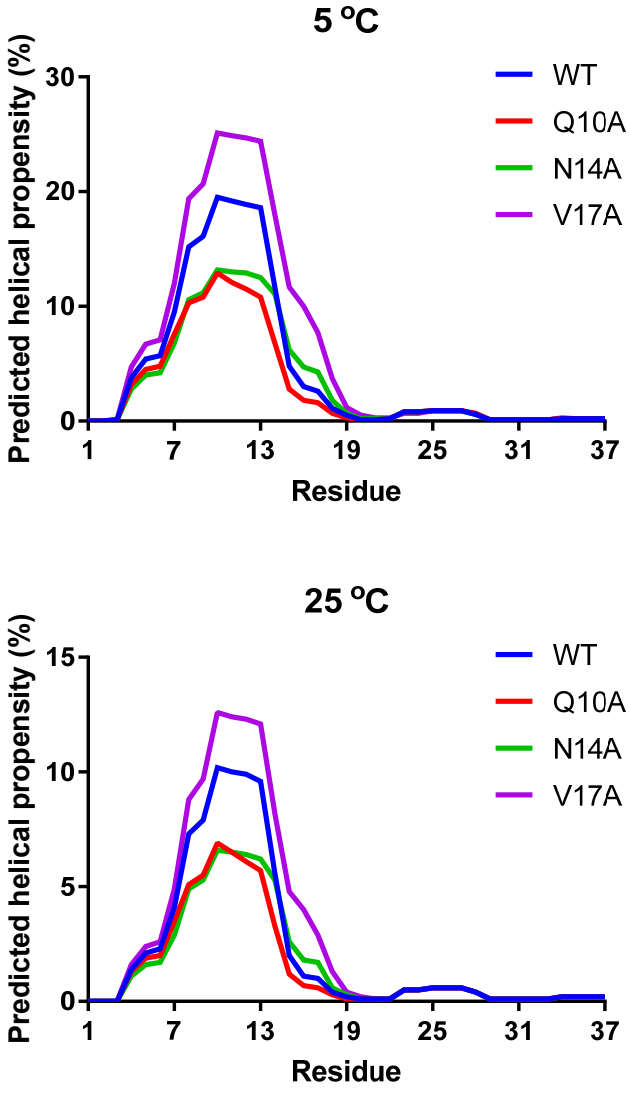


Figure SB18. Predicted helical propensity of selected peptides at 5 or 25°C, pH 7.4, and an ionic strength of .14 M



Supplementary data tables 5-7

Data are mean ± s.e.m. of *n* biological replicates, reporting cAMP production for three different receptors by peptide analogues. Data were normalized to hAMY in each experiment and E_{max} values are % hAMY, or pramlintide for the relevant analogue. For Table SB6, binding data are also shown. **P* <0.05 by unpaired *t*-test for pEC_{50} and pIC_{50} or where 95% confidence intervals did not include 100 for E_{max}. Statistically significant increases or decreases are shaded as indicated. ND, not done.

Table SB5. Analogues $CT_{(a)}$.

	decrease			increase		
	pEC_{50}	**SEM**	**Fold change**	**E_{max}**	**SEM**	**n**
hAMY	8.60	0.32	1.3	100		6
K1A	8.70	0.18		89.3	10.8	
hAMY	8.86	0.11	-2.0	100		7
N3A	8.56*	0.07		103	8.65	
hAMY	8.65	0.18	-4.1	100		7
T4A	8.04*	0.08		92.3	6.45	
hAMY	8.12	0.17	-1.4	100		5
A5G	7.97	0.19		118	28.7	
hAMY	8.23	0.13	-16	100		5
T6A	7.04*	0.17		41.8*	3.64	
hAMY	8.58	0.24	-10	100		6
A8G	7.57*	0.13		79.2*	6.27	
hAMY	8.83	0.20	-15	100		5
T9A	7.65*	0.23		98.9	13.3	
hAMY	8.71	0.15	15	100		8
Q10A	9.88*	0.28		169*	18.5	
hAMY	8.55	0.31	-3.4	100		6
R11A	8.02	0.50		113	14.0	
hAMY	8.73	0.14	-44	100		5
L12A	7.09*	0.10		151	28.4	
hAMY	8.62	0.15	-7.3	100		6
A13G	7.67*	0.18		134	26.6	
hAMY	8.38	0.16	6.5	100		9
N14A	9.19*	0.11		146	25.5	
hAMY	8.69	0.33	-5.7	100		6
F15A	7.93	0.10		74.1*	7.31	
hAMY	8.33	0.16	-2.0	100		7
L16A	8.02	0.35		119	14.7	
hAMY	8.40	0.16	8.5	100		10
V17A	9.33*	0.26		164*	26.7	
hAMY	8.67	0.14	-2.3	100		5
I26A	8.31	0.08		101	6.12	
hAMY	8.53	0.14	-3.0	100		5
L27A	8.06	0.16		98.7	5.31	
hAMY	8.53	0.14	-1.5	100		5
S28A	8.37	0.15		113	17.7	
hAMY	8.60	0.17	-2.0	100		5
S29A	8.30	0.18		102	5.92	
hAMY	8.89	0.26	-5.6	100		4
T30A	8.14*	0.10		132	19.4	
hAMY	8.89	0.26	-4.7	100		4
N31A	8.22*	0.09		114	14.7	
hAMY	8.76	0.25	-2.5	100		5
V32A	8.37	0.15		112	5.08	
hAMY	8.42	0.13	-1.6	100		4
G33A	8.23	0.05		113	5.55	
hAMY	8.43	0.10	1.2	100		5
S34A	8.50	0.06		109	7.57	
hAMY	8.23	0.15	-1.6	100		4
N35A	8.02	0.23		107	12.2	
hAMY	8.23	0.15	-1.4	100		4
T36A	8.10	0.06		123	9.20	
hAMY	8.23	0.15	1.1	100		4
Y37A	8.25	0.07		101	3.36	
hAMY	8.84	0.19	-13	100		3
hAMY1-17	6.72*	0.27		65*	6.27	

	pEC_{50}	SEM	Fold change	E_{max}	SEM	n
hAMY	8.68	0.16	-260	100		5
hAMY8-37	6.26*	0.19		64.3*	7.70	
hAMY	8.55	0.18	-42	100		3
hAMYAc8-37	6.93*	0.06		80.2	6.13	
hAMY	8.89	0.29	-680	100		6
C2S-C7S	6.06*	0.10		65.5*	13.3	
hAMY	8.55	0.18	-56	100		3
CAM	6.80*	0.08		81.2	4.7	
hAMY	8.44	0.16	14	100		7
A5S	9.58*	0.17		157*	18	
hAMY	8.14	0.19	-2.6	100		4
hAMY-COOH	7.72	0.17		102	20.9	
Pramlintide	8.82	0.23	2.9	100		6
PramQ10A	9.29	0.16		139	20.1	

Table SB6. Analogues $AMY_{1(a)}$

	decrease			increase						
	pEC_{50}	**SEM**	**Fold change**	**E_{max}**	**SEM**	**n**	**pIC_{50}**	**SEM**	**Fold change**	**n**
hAMY	9.49	0.24	1.3	100		6		ND		
K1A	9.61	0.25		113	14.8					
hAMY	9.45	0.08	-1.5	100		6	7.87	0.04	-1.1	3
N3A	9.27	0.12		111	7.25		7.84	0.07		
hAMY	9.14	0.16	-3.2	100		8	8.25	0.01	-26	3
T4A	8.63*	0.13		87.6	5.59		6.83*	0.06		
hAMY	9.46	0.34	-3.3	100		6	7.87	0.04	-1.5	3
A5G	8.94	0.27		84.8	7.64		7.68	0.16		
hAMY	8.83	0.21	-21	100		5	8.16	0.28	-10	3
T6A	7.50*	0.15		41.6*	5.89		7.16*	0.07		
hAMY	9.22	0.14	-8.5	100		8	8.23	0.01	-30	3
A8G	8.29*	0.18		96.2	13.8		6.75*	0.11		
hAMY	9.36	0.21	-9.5	100		6	8.20	0.04	-6	3
T9A	8.38*	0.15		90.7	11.8		7.40*	0.27		
hAMY	9.38	0.13	10.5	100		9	8.13	0.28	9	3
Q10A	10.4*	0.29		154*	15.2		9.08*	0.03		
hAMY	9.26	0.17	-6.0	100		5	8.23	0.01	-6	3
R11A	8.48*	0.15		150*	17.4		7.46*	0.24		
hAMY	9.30	0.16	-58	100		5	8.13	0.28	-91	3
L12A	7.54*	0.12		97.4	6.57		6.17*	0.08		
hAMY	9.28	0.14	-11	100		5	8.39	0.13	-22	3
A13G	8.26*	0.16		133	21.8		7.04*	0.16		
hAMY	9.17	0.12	2.7	100		13	8.35	0.08	2	3
N14A	9.60	0.2		142*	9.31		8.64	0.14		
hAMY	9.29	0.2	-3.9	100		7	7.87	0.04	-7.2	3
F15A	8.70	0.19		86.1	6.03		7.01*	0.13		
hAMY	9.34	0.14	-4.2	100		11	8.36	0.08	1.7	3
L16A	8.72	0.38		126*	8.28		8.59	0.12		
hAMY	9.20	0.14	3.4	100		9	8.48	0.11	2	3
V17A	9.73*	0.16		139*	9.1		8.76	0.13		
hAMY	9.71	0.12	-2.8	100		5	7.52	0.21	10.2	3
I26A	9.26*	0.08		115	9.3		8.53*	0.05		
hAMY	9.61	0.11	-7.1	100		5	7.48	0.15	5.5	4
L27A	8.76*	0.19		153	29.6		8.22	0.49		
hAMY	9.63	0.13	-2.1	100		5	7.59	0.17	9.8	4
S28A	9.30	0.07		113	15		8.58*	0.25		
hAMY	9.75	0.09	-2.6	100		5	7.88	0.13	2.3	3
S29A	9.33*	0.1		121	9.53		8.25	0.46		

	pEC_{50}	SEM	Fold change	E_{max}	SEM	n	pIC_{50}	SEM	Fold change	n
hAMY	9.35	0.16	-12	100		4	7.67	0.26	-27	4
T30A	8.27*	0.18		82.1	12.8		6.24*	0.19		
hAMY	9.35	0.16	-3.2	100		4	7.68	0.07	-1.2	3
N31A	8.84*	0.13		108	11.9		7.76	0.37		
hAMY	9.52	0.16	-15	100		4	7.40	0.18	-8.9	3
V32A	8.36*	0.13		117	17.9		6.45*	0.23		
hAMY	9.50	0.18	-15	100		4	7.37	0.15	-1.9	3
G33A	8.33*	0.23		107	12.5		7.10	0.13		
hAMY	9.50	0.18	-2.4	100		4	7.52	0.21	-18	3
S34A	9.12	0.39		108	11.5		8.77*	0.16		
hAMY	9.40	0.1	-2.6	100		4	7.52	0.21	-1.8	3
N35A	8.98*	0.09		112	8.61		7.78	0.10		
hAMY	9.40	0.1	-1.9	100		4	7.60	0.13	6.5	3
T36A	9.12	0.06		116	7.82		8.41	0.18		
hAMY	9.40	0.1	-4.4	100		4	7.59	0.17	-3.8	3
Y37A	8.76*	0.11		101	8.58		7.01	0.20		
hAMY	9.72	0.06	-910	100		5		ND		
hAMY1-17	6.76*	0.15		68.2*	5.34					
hAMY	9.77	0.07	-500	100		5	8.48	0.11	-138	3
hAMY8-37	7.07*	0.14		51.6*	6.44		6.34*	0.02		
hAMY	9.21	0.13	-250	100		6		ND		
hAMY8-37(DR)	6.81*	0.19		53.5*	12.4					
hAMY	9.47	0.24	-32	100		3		ND		
hAMYAc8-37	7.97*	0.09		82.8	10.4					
hAMY	9.21	0.13	-360	100		6	8.34	0.09	-398	3
C2S-C7S	6.66*	0.32		49.5*	9.09		5.74*	0.05		
hAMY	9.47	0.24	-71	100		3		ND		
CAM	7.62*	0.31		100	1.42					
hAMY	9.48	0.26	2.1	100		9		ND		
A5S	9.80	0.23		128*	8.8					
hAMY	9.53	0.2	-58	100		4	7.69	0.09	-91	4
hAMY-COOH	7.77*	0.13		93.5	13		5.73*	0.19		
Pramlintide	9.74	0.13	-1.9	100		7	8.54	0.12	2.6	3
PramQ10A	9.46	0.19		129*	9.84		8.96	0.09		

Table SB7. Analogues $AMY_{3(a)}$

	decrease			increase		
	pEC_{50}	**SEM**	**Fold change**	**E_{max}**	**SEM**	**n**
hAMY	9.08	0.08	-2.2	100		5
K1A	9.42*	0.10		128	16.8	
hAMY	9.58	0.18	-2.6	100		6
N3A	9.17	0.27		88.8	7.59	
hAMY	8.86	0.14	-1.4	100		8
T4A	8.71	0.20		107	16.4	
hAMY	8.97	0.12	-1.2	100		7
A5G	9.03	0.10		109	14.5	
hAMY	8.95	0.12	-12	100		7
T6A	7.87*	0.10		43.2*	11.2	
hAMY	9.40	0.20	-12	100		7
A8G	8.34*	0.13		92.3	5.44	
hAMY	9.06	0.08	-7.1	100		5
T9A	8.21*	0.09		79.5	7.90	
hAMY	9.14	0.17	1.9	100		7
Q10A	9.42	0.20		134*	5.89	
hAMY	9.26	0.18	-6.8	100		7
R11A	8.43*	0.20		96.4	8.71	
hAMY	8.74	0.25	-15	100		6
L12A	7.57*	0.17		77.3	15	
hAMY	9.00	0.13	-7.6	100		5
A13G	8.12*	0.05		108	7.04	
hAMY	9.03	0.15	3.2	100		9
N14A	9.53	0.24		136*	11.6	
hAMY	8.93	0.16	-3.8	100		6
F15A	8.35*	0.12		87.6	6.93	
hAMY	9.19	0.20	-4.0	100		8
L16A	8.59	0.43		122	12.7	
hAMY	8.99	0.15	3.2	100		7
V17A	9.49	0.18		121	13.2	
hAMY	9.36	0.13	-3.2	100		5
I26A	8.86*	0.13		121	16.0	
hAMY	9.26	0.13	-3.1	100		5
L27A	8.77*	0.16		101	8.24	
hAMY	9.23	0.11	-3.1	100		5
S28A	8.74	0.24		142*	9.09	
hAMY	9.34	0.13	-2.6	100		5
S29A	8.92	0.17		119*	7.94	
hAMY	9.19	0.10	-12	100		5
T30A	8.13*	0.13		110	6.97	
hAMY	9.22	0.13	-4.0	100		4
N31A	8.62	0.22		126	19.3	
hAMY	9.19	0.11	-13	100		6
V32A	8.05*	0.13		139	18.3	
hAMY	9.20	0.16	-6.0	100		4
G33A	8.42*	0.18		120*	8.03	
hAMY	9.18	0.13	-1.4	100		5
S34A	9.02	0.19		126*	8.73	
hAMY	8.99	0.06	-3.9	100		4
N35A	8.40*	0.15		124	16.0	
hAMY	8.99	0.06	-1.4	100		4
T36A	8.84	0.12		112*	5.09	
hAMY	8.99	0.06	-3.4	100		4
Y37A	8.46*	0.18		122	7.31	
hAMY	9.27	0.28	-280	100		3
hAMY1-17	6.82*	0.14		49.3*	11.2	
hAMY	9.04	0.14	-230	100		6
hAMY8-37	6.68*	0.04		55.5*	5.20	
hAMY	8.86	0.15	-20	100		3
hAMYAc8-37	7.55*	0.17		71.7*	4.09	
hAMY	9.27	0.28	-480	100		3
C2S-C7S	6.59*	0.07		55.5*	9.90	
hAMY	8.86	0.15	-20	100		3
CAM	7.57*	0.20		91	6.88	
hAMY	8.92	0.14	9.1	100		8
A5S	9.88*	0.16		125	20.4	

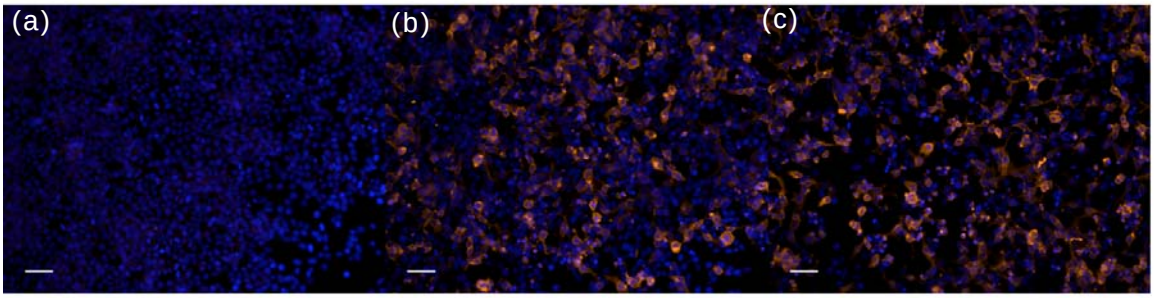
	pEC_{50}	SEM	Fold change	E_{max}	SEM	n
hAMY	9.12	0.09	-20	100		4
hAMY-COOH	7.83*	0.43		90.5	11.8	
Pramlintide	9.75	0.19	1.4	100		7
PramQ10A	9.89	0.20		129*	11.4	

Table SB8. *C*-terminal exchange peptides. Data are mean ± s.e.m. of *n* biological replicates, reporting the effect of *C*-terminal residue exchange at different receptors. Data were normalized to the relevant control in each experiment. **P* <0.05 by unpaired *t*-test for pEC_{50} or where 95% confidence intervals did not include 100 for E_{max}. Statistically significant increases or decreases are shaded as indicated.

decrease increase

	hAMY and hαCGRP *C*-terminal exchange													
Receptor		**pEC_{50}**	**SEM**	**Fold change**	**E_{max}**	**SEM**	**n**		**pEC_{50}**	**SEM**	**Fold change**	**E_{max}**	**SEM**	**n**
CT	**hAMY**	8.38	0.12	-5.4	100		3	**hαCGRP**	7.58	0.19	-1.4	100		3
	Y37F	7.65*	0.17		110	4.5		**hαCGRP F37Y**	7.44	0.16		160	15.2	
$AMY_{1(a)}$	**hAMY**	9.49	0.17	-4.9	100		4	**hαCGRP**	9.82	0.22	-2.6	100		4
	Y37F	8.80	0.33		101	3.8		**hαCGRP F37Y**	9.40	0.24		123	9.9	
$AMY_{3(a)}$	**hAMY**	9.24	0.24	-7.1	100		4	**hαCGRP**	8.58	0.07	-1.4	100		4
	Y37F	8.39*	0.25		107	4.0		**hαCGRP F37Y**	8.42	0.23		175*	16.4	
CGRP	**hAMY**	6.49	0.09	-3.8	100		5	**hαCGRP**	10.29	0.11	-1.0	100		4
	Y37F	5.91	0.15		92.5	10.1		**hαCGRP F37Y**	10.27	0.16		96.5	8.3	
AM_1	**hAMY**	<5					3	**hαCGRP**	6.83	0.13	4.0	100		4
	Y37F	<5						**hαCGRP F37Y**	7.44*	0.19		180	30.5	
AM_2	**hAMY**	6.27	0.05	-4.8	100		4	**hαCGRP**	6.87	0.14	1.9	100		4
	Y37F	5.59*	0.13		48.9*	12.8		**hαCGRP F37Y**	7.16	0.25		119	20.6	
	hAMY and hCT *C*-terminal exchange													
Receptor		**pEC_{50}**	**SEM**	**Fold change**	**E_{max}**	**SEM**	**n**		**pEC_{50}**	**SEM**	**Fold change**	**E_{max}**	**SEM**	**n**
CT	**hAMY**	8.22	0.25	5.6	100		4	**hCT**	9.85	0.37	-6.3	100		3
	Y37P	8.97*	0.15		106	11.3		**hCT P32Y**	9.05	0.19		87.4	9.2	
$AMY_{1(a)}$	**hAMY**	9.01	0.09	2.9	100		4	**hCT**	9.63	0.27	-10	100		3
	Y37P	9.47*	0.05		109	6.8		**hCT P32Y**	8.63*	0.19		94.0	4.5	
$AMY_{3(a)}$	**hAMY**	8.61	0.21	2.6	100		3	**hCT**	9.37	0.23	-5.9	100		3
	Y37P	9.02	0.36		120	18.6		**hCT P32Y**	8.60	0.22		81.3*	5.4	

Figure SB19. Specificity of anti-CTR antibody 188/10 and expression of CTR in brainstem cultures. HEK293S cells were plated into 96-well plates then transiently transfected with (a) vector only, (b) rat CTR, (c) rat CTR/rat RAMP1 or (d) CTR expression was determined in rat brainstem cultures. Cells were blocked with goat serum, then incubated with 188/10 rabbit polyclonal antibody against rat CTR (4°C overnight, 1:500 dilution) in (a)-(d) or monoclonal antibody 9B4 against CTR (4°C overnight, 1:100) in (d) only, as indicated. The secondary antibodies used were goat anti-rabbit AlexaFluor568 or goat anti-mouse Alexa Fluor 594 (Life Technologies, 1:200). Images were taken using a PerkinElmer Operetta imaging system using the 20x high numerical aperture lens. HEK293S images shown in (a)-(c) are representative of three independent experiments performed in duplicate wells. Brainstem culture images in (d) are representative of duplicate wells from one experiment, which was repeated with two (188/10) or three (9B4) other independently prepared brainstem cultures with a similar staining pattern. Scale bar shown in (a)-(c) 50 µm. DAPI is blue, antibody staining is orange.



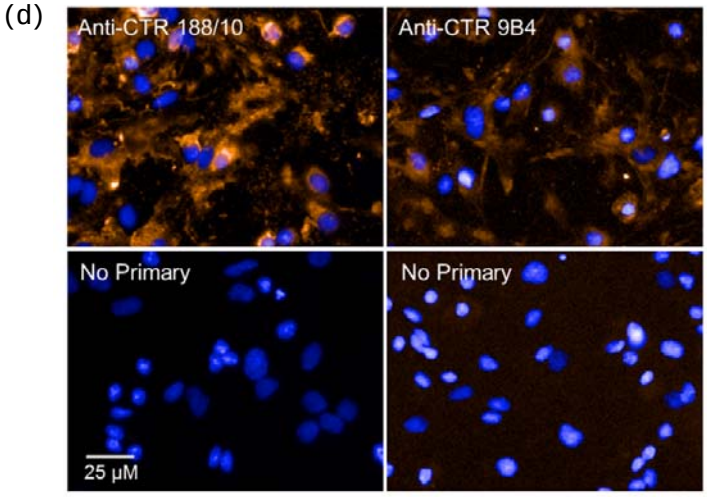


Figure SB20. Activation of four different signaling pathways at three different receptors by Q10A hAMY. Data are mean ± s.e.m. of *n* biological replicates. Data were normalized to hAMY in each experiment. **P* <0.05 by unpaired *t*-test for pEC_{50} or where 95% confidence intervals did not include 100 for E_{max}.

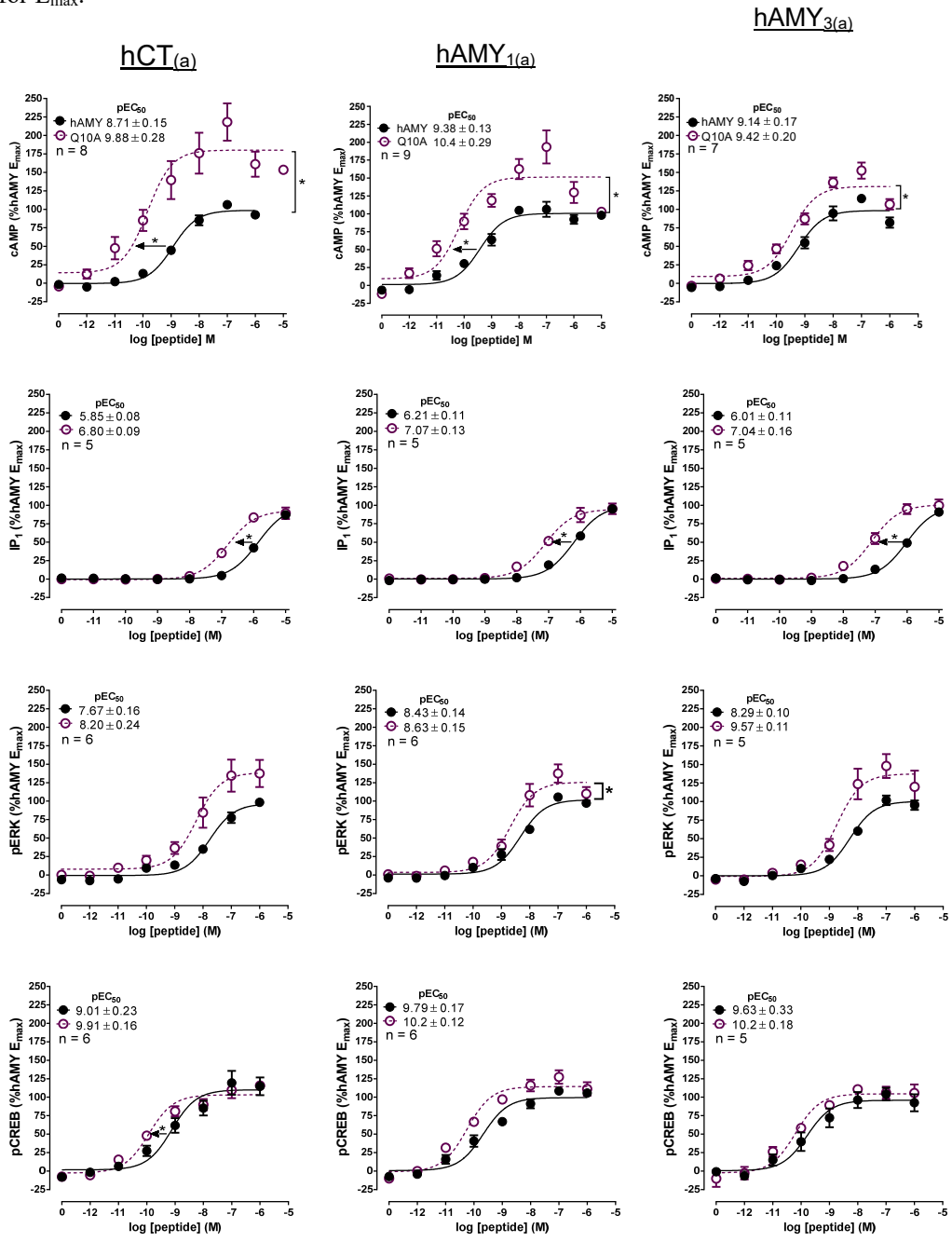
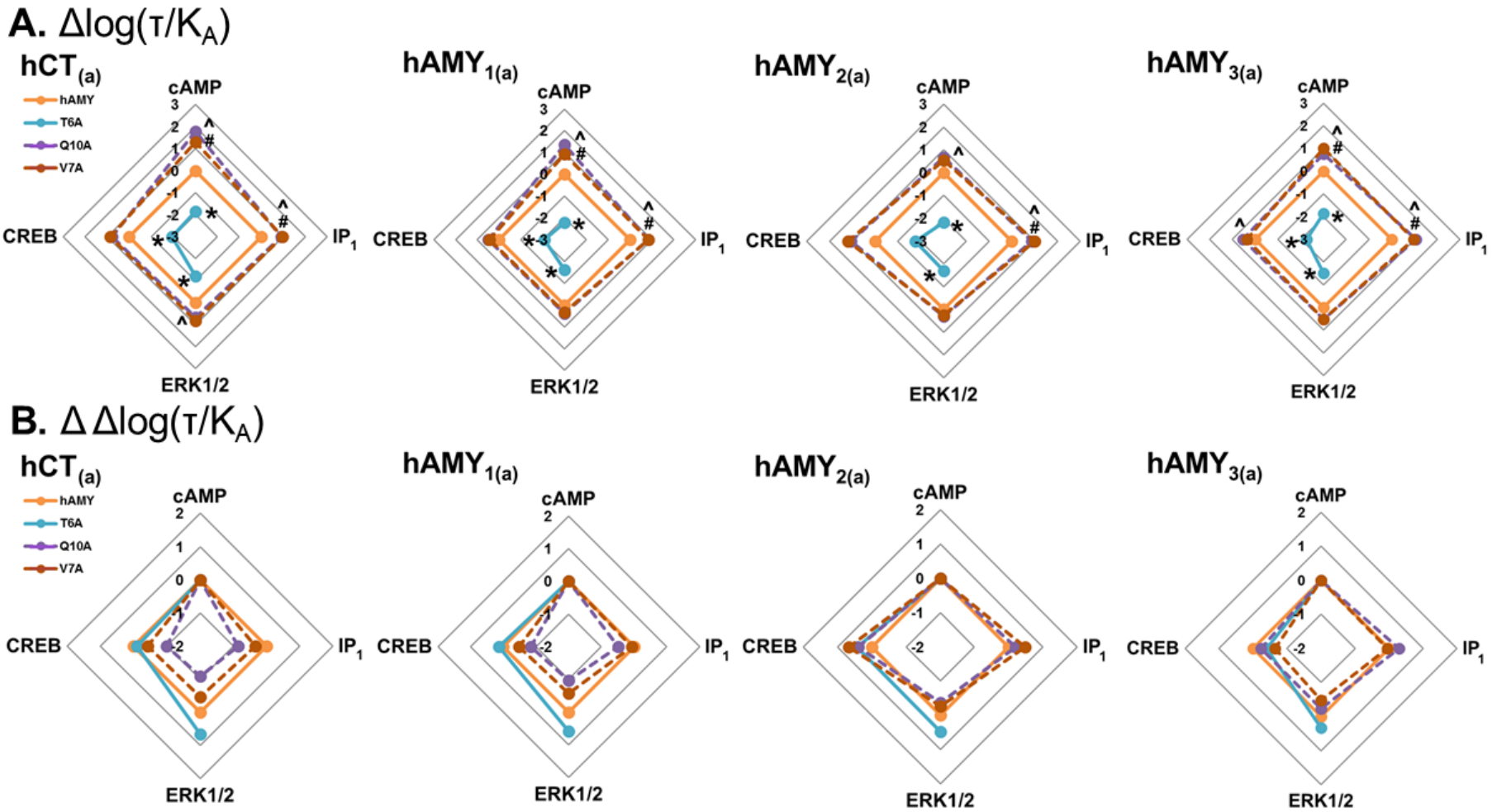


Figure SB21. Radial plots summarizing the analysis of ligand bias for hAMY, T6A, Q10A and V17A with four different signaling pathways (cAMP, IP_1, ERK1/2 and CREB phosphorylation) at four different receptors (CTR, $AMY_{1\text{-}3}$). (A) The relative effectiveness of the analogues compared to the reference ligand (hAMY) are expressed as $\Delta Log(\tau/K_A)$. (B) Signaling bias of the analogues compared to the reference pathway (cAMP) are expressed as $\Delta\Delta Log(\tau/K_A)$. T6A did not induce detectable activation of IP_1 accumulation and therefore could not be analyzed. Data points are the mean of 3-22 biological replicates. The control peptide (hAMY) was compared to the analogue by one-way ANOVA with a post-hoc Dunnet's test; $^{*}P$ <0.05 hAMY vs. T6A; $^{\#}P$ <0.05 hAMY vs. Q10A; $^{\wedge}P$ <0.05 hAMY vs. V17A.



Supplementary data tables 9-12

Data are mean ± s.e.m. of *n* biological replicates, reporting activation of four different signaling pathways (cAMP, IP_1, ERK1/2 [15 minutes] and CREB phosphorylation) at four different receptors (CTR, AMY_{1-3}) by selected peptide analogues. Data were normalized to the relevant control in each experiment and E_{max} values are % hAMY, or pramlintide for the relevant analogue. **P* <0.05 by unpaired *t*-test for pEC_{50} or where 95% confidence intervals did not include 100 for E_{max}. Statistically significant increases or decreases are shaded as indicated.

Table SB9. Analogue Signaling $CT_{(a)}$

decrease | increase

	cAMP					
	pEC_{50}	**SEM**	**Fold-Change**	**Emax**	**SEM**	**n**
hAMY	8.23	0.13	-16	100		5
T6A	7.04*	0.17		41.8*	3.60	
hAMY	8.71	0.15	15	100		8
Q10A	9.88*	0.29		169*	18.5	
hAMY	8.40	0.16	8.5	100		10
V17A	9.33*	0.26		164*	26.7	
Pramlintide	8.82	0.23	2.9	100		6
PramQ10A	9.29	0.16		139	20.1	
	IP1					
	pEC_{50}	**SEM**	**Fold-Change**	**Emax**	**SEM**	**n**
hAMY	5.85	0.08		100		5
T6A	<5					
hAMY	5.85	0.08	8.9	100		5
Q10A	6.80*	0.09		94	5.82	
hAMY	5.85	0.08	8.0	100		5
V17A	6.79*	0.07		107	5.49	
Pramlintide	5.97	0.07	3.5	100		4
PramQ10A	6.52*	0.05		112	5.98	
	ERK1/2 (15 min)					
	pEC_{50}	**SEM**	**Fold-Change**	**Emax**	**SEM**	**n**
hAMY	7.64	0.18	1.2	100		5
T6A	7.66	0.33		18.2*	4.07	
hAMY	7.67	0.16	3.4	100		6
Q10A	8.20	0.24		134	18.8	
hAMY	7.67	0.16	4.9	100		6
V17A	8.36*	0.13		133	14.3	
Pramlintide	7.74	0.13	4.2	100		6
PramQ10A	8.21*	0.09		119	11.4	
	CREB					
	pEC_{50}	**SEM**	**Fold-Change**	**Emax**	**SEM**	**n**
hAMY	9.12	0.28	-60	100		4
T6A	7.34*	0.33		62.6	17.0	
hAMY	9.01	0.23	8.1	100		6
Q10A	9.91*	0.16		105	9.33	
hAMY	8.95	0.20	6.0	100		6
V17A	9.73*	0.19		127*	10.1	
Pramlintide	9.15	0.17	8.9	100		6
PramQ10A	10.1*	0.20		99.4	10.9	

Table SB10. Analogue Signaling $AMY_{1(a)}$

decrease | increase

	cAMP					
	pEC_{50}	**SEM**	**Fold-Change**	**Emax**	**SEM**	**n**
hAMY	8.83	0.21	-21	100		5
T6A	7.50*	0.15		41.6*	5.90	
hAMY	9.38	0.13	10.5	100		9
Q10A	10.4*	0.29		154*	15.2	
hAMY	9.20	0.14	3.4	100		9
V17A	9.73*	0.16		139*	9.10	
Pramlintide	9.74	0.13	-1.9	100		7
PramQ10A	9.46	0.19		129*	9.84	
	IP1					
	pEC_{50}	**SEM**	**Fold-Change**	**Emax**	**SEM**	**n**
hAMY	6.21	0.11		100		5
T6A	<5					
hAMY	6.21	0.11	7.2	100		5
Q10A	7.07*	0.13		97	3.95	
hAMY	6.21	0.11	6.6	100		5
V17A	7.03*	0.13		110*	3.23	
Pramlintide	6.32	0.15	4.5	100		4
PramQ10A	6.97*	0.06		113	7.69	
	ERK1/2 (15 min)					
	pEC_{50}	**SEM**	**Fold-Change**	**Emax**	**SEM**	**n**
hAMY	8.21	0.12		100		5
T6A	8.06	0.30	-1.4	11*	1.99	
hAMY	8.43	0.14		100		6
Q10A	8.63	0.15	1.6	134*	9.91	
hAMY	8.43	0.14	1.4	100		6
V17A	8.59	0.10		134*	10.6	
Pramlintide	8.61	0.10	0	100		5
PramQ10A	8.61	0.10		109	5.33	
	CREB					
	pEC_{50}	**SEM**	**Fold-Change**	**Emax**	**SEM**	**n**
hAMY	9.77	0.18	-45	100		4
T6A	8.11*	0.27		60*	11.9	
hAMY	9.79	0.17	2.6	100		6
Q10A	10.2	0.12		110	5.03	
hAMY	9.79	0.17	2.6	100		6
V17A	10.2	0.12		110	11.1	
Pramlintide	10.2	0.34	3.2	100		5
PramQ10A	10.7	0.30		101	6.00	

Table SB11. Analogue Signaling $AMY_{2(a)}$

decrease | increase

	cAMP					
	pEC_{50}	**SEM**	**Fold-Change**	**Emax**	**SEM**	**n**
hAMY	8.86	0.01	-52	100		3
T6A	7.14	0.07		38.0	4.50	
hAMY	8.86	0.01	3.7	100		3
Q10A	9.38	0.07		141*	13.7	
hAMY	8.86	0.01	3.7	100		3
V17A	9.43	0.29		110	15.3	
Pramlintide	8.87	0.09	6.8	100		3
PramQ10A	9.70*	0.03		96.4	4.10	
	IP1					
	pEC_{50}	**SEM**	**Fold-Change**	**Emax**	**SEM**	**n**
hAMY	5.94	0.10		100		5
T6A	<5					
hAMY	5.94	0.10	7.9	100		5
Q10A	6.84*	0.13		99.7	10.8	
hAMY	5.94	0.10	9.1	100		5
V17A	6.90*	0.09		117	15.9	
Pramlintide	5.87	0.10	5.5	100		4
PramQ10A	6.61*	0.09		97.6	9.64	
	ERK1/2 (15 min)					
	pEC_{50}	**SEM**	**Fold-Change**	**Emax**	**SEM**	**n**
hAMY	7.72	0.10	-1.9	100		4
T6A	7.45	0.24		18*	3.41	
hAMY	8.01	0.35	1.8	100		4
Q10A	8.27	0.30		122	11.1	
hAMY	8.01	0.35	1.8	100		4
V17A	8.26	0.19		125	28.6	
Pramlintide	8.08	0.31	2.5	100		5
PramQ10A	8.48	0.30		117	16.4	
	CREB					
	pEC_{50}	**SEM**	**Fold-Change**	**Emax**	**SEM**	**n**
hAMY	8.94	0.65	-17	100		3
T6A	7.70	0.17		53*	4.62	
hAMY	9.63	0.33	3.7	100		4
Q10A	10.2	0.18		120	8.16	
hAMY	9.57	0.34	3.4	100		3
V17A	10.1	0.23		110	6.63	
Pramlintide	9.89	0.26	2.6	100		5
PramQ10A	10.3	0.20		94.9	12.7	

Table SB12. Analogue Signaling $AMY_{3(a)}$

decrease | increase

	cAMP					
	pEC_{50}	**SEM**	**Fold-Change**	**Emax**	**SEM**	**n**
hAMY	8.95	0.12	-12	100		7
T6A	7.87*	0.10		43.2*	11.2	
hAMY	9.14	0.17	1.9	100		7
Q10A	9.42	0.20		134*	5.90	
hAMY	8.99	0.15	3.2	100		7
V17A	9.49	0.18		121	13.2	
Pramlintide	9.75	0.19	1.4	100		7
PramQ10A	9.89	0.20		129*	11.4	
	IP1					
	pEC_{50}	**SEM**	**Fold-Change**	**Emax**	**SEM**	**n**
hAMY	6.01	0.11		100		5
T6A	<5					
hAMY	6.01	0.11	11	100		5
Q10A	7.04*	0.16		105	2.20	
hAMY	6.01	0.11	9.1	100		5
V17A	6.97*	0.10		100	4.58	
Pramlintide	6.31	0.15	5.4	100		4
PramQ10A	7.04*	0.08		128*	3.97	
	ERK1/2 (15 min)					
	pEC_{50}	**SEM**	**Fold-Change**	**Emax**	**SEM**	**n**
hAMY	8.14	0.08		100		4
T6A	7.94	0.35		17*	5.79	
hAMY	8.29	0.10	1.9	100		5
Q10A	8.57	0.11		148	17.9	
hAMY	8.29	0.10	1.7	100		5
V17A	8.52	0.15		173	29.6	
Pramlintide	8.21	0.24	2.6	100		5
PramQ10A	8.63	0.15		124	19.5	
	CREB					
	pEC_{50}	**SEM**	**Fold-Change**	**Emax**	**SEM**	**n**
hAMY	9.45	0.26	-32	100		4
T6A	7.94*	0.22		49.2*	8.00	
hAMY	9.63	0.33	3.7	100		5
Q10A	10.2	0.18		104	5.39	
hAMY	9.31	0.15	4.8	100		4
V17A	9.99*	0.19		109	6.52	
Pramlintide	10.2	0.28	2.5	100		6
PramQ10A	10.6	0.21		91.3	11.6	

Figure SB22. Competition binding data at AMY_1, comparing pramlintide and DAGAR1. Data are mean ± s.e.m. of *n* biological replicates. The control peptide was compared to the analogue by unpaired t test, * *P* <0.05.

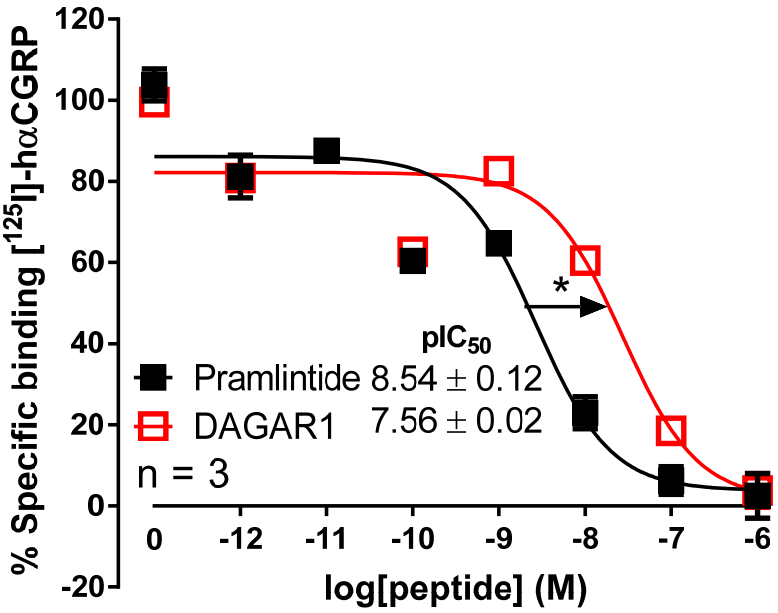


Figure SB23. Indication of variation between experiments. a) shows data for ten typical independent experiments with the AMY_1 receptor transiently transfected into Cos 7 cells and stimulated with hAMY. Each data point is an experimental replicate within an experiment b) shows the corresponding E_{max} values for each of those experiments and c) shows the corresponding pEC_{50} values.

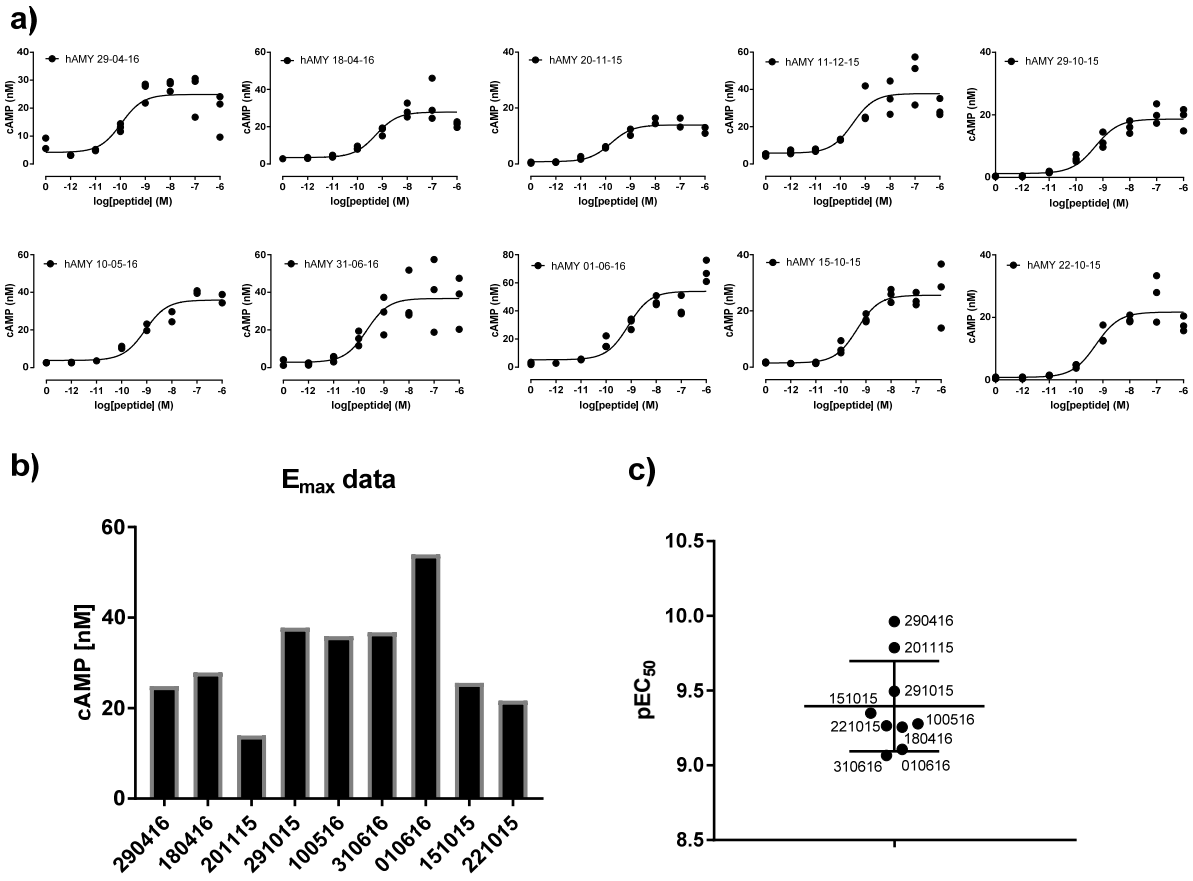
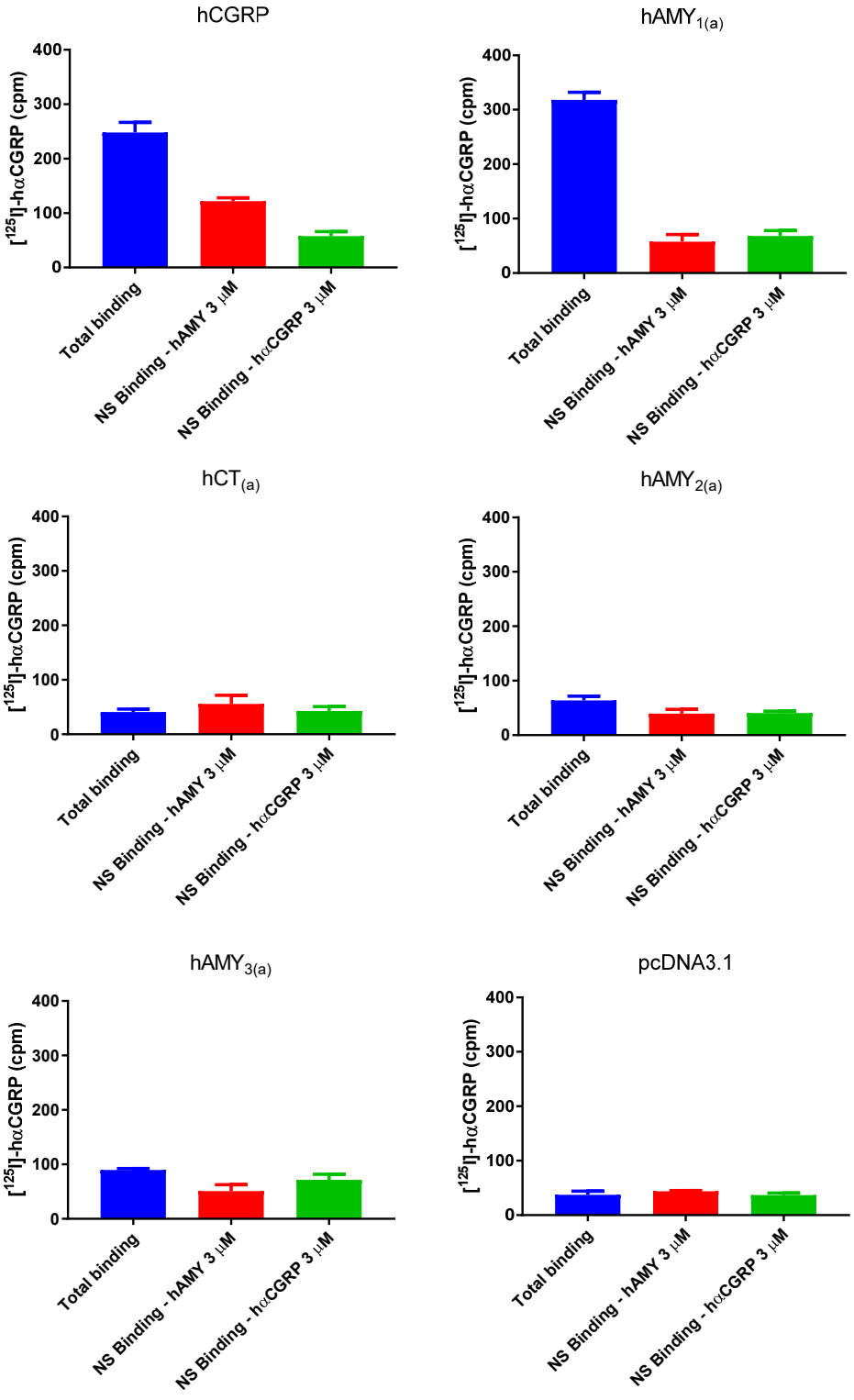


Figure SB24. ^{125}I-Calcitonin gene-related peptide (CGRP) binding to whole cells transfected with different receptors. hCGRP is the CGRP receptor comprising RAMP1 with the calcitonin-like receptor. Data are mean ± s.e.m. of duplicate or triplicate technical replicates from a single experiment. Similar results were obtained in at least four other experiments. NS, non-specific.



[1] Clamp, M., Cuff, J., Searle, S. M., and Barton, G. J. (2004) The Jalview Java alignment editor, *Bioinformatics 20*, 426-427. 10.1093/bioinformatics/btg430