SUPPLEMENTARY INFORMATION:

LIGAND-SPECIFIC CONFORMATION OF THE EXTRACELLULAR LOOP-2 IN ANGIOTENSIN II TYPE 1 RECEPTOR

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SUPPLEMENTARY TABLES

Supplementary Table I. Characterization of ECL2 single cysteine mutants.

	Bmax (mean±SEM)	~Molecules/cell*	ΔKd**	MAPK activation***
HA-CYS¯AT1R	5.80 ± 0.12	350,000	1.0	Yes
I172C	0.36 ± 0.07	21,500	1.3	Yes, p = 0.20
E173C	0.34 ± 0.03	20,600	1.4	Yes, p = 0.44
N174C	0.66 ± 0.05	40,000	1.4	Yes, p = 0.69
T175C	0.72 ± 0.01	43,000	1.4	Yes, p = 0.69
N176C	0.71 ± 0.12	43,000	1.3	Yes, p = 0.35
I177C	0.26 ± 0.05	15,800	1.3	Yes, p = 0.88
T178C	2.32 ± 0.32	139,600	1.6	Yes, p = 0.79
V179C	0.12 ± 0.02	7,500	2.3	Yes, p = 0.39
A181C	0.38 ± 0.03	22,700	2.1	Yes, p = 0.60
F182C	0.71 ± 0.11	42,800	1.4	Yes, p = 0.34
H183C	1.94 ± 0.47	116,700	1.4	Yes, p = 0.66
Y184C	0.34 ± 0.04	20,600	1.8	Yes, p = 0.54
E185C	0.11 ± 0.03	6,700	2.3	Yes, p = 0.51
S186C	0.91 ± 0.32	55,100	1.9	Yes, p = 0.55
R187C	6.50 ± 0.20	391,700	1.6	Yes, p = 0.51
N188C	3.36 ± 0.21	202,600	1.4	Yes, p = 0.35
S189C	0.78 ± 0.05	46,700	2.1	Yes, p = 0.69
T190C	0.61 ± 0.05	36,800	1.9	Yes, p = 0.98
L191C	1.59 ± 0.23	95,600	2.2	Yes, p = 0.90
P192C	0.03 ± 0.01	1,700	3.0	Yes, p = 0.68
HA-AT1R	3.5 ± 0.15	175,000	0.8	Yes, p = 0.65

* Number of receptors on the cell surface calculated by the formula: [(B_{max}*Avogadro's number) / number of cells].

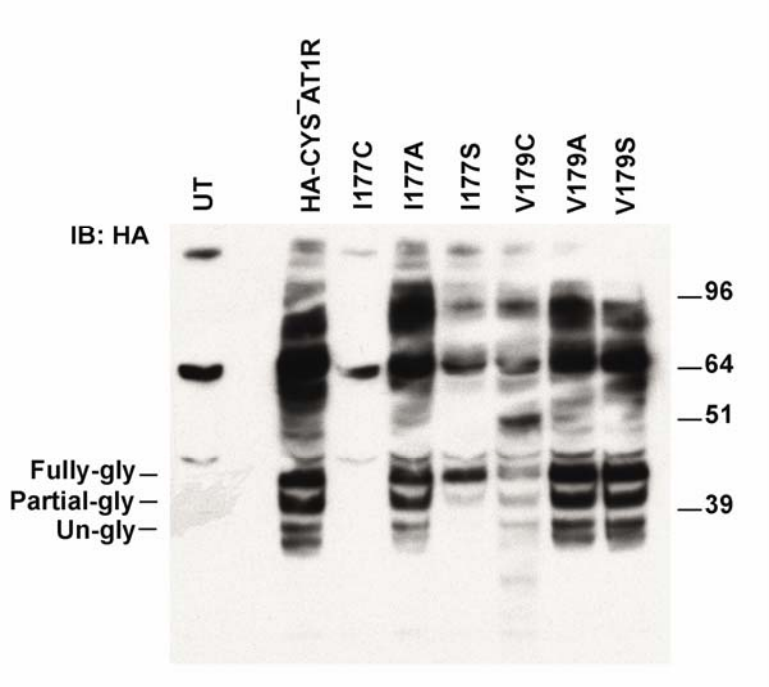
** K_d value for HA-CYS ¯AT1R is 4.4 nm. K_d value for HA-AT1R is 3.5 nm.

*** MAPK activation indicates that ERK is phosphorylated upon treatment with 1μM Ang II, demonstrating the ability of mutants to assume active conformation upon binding Ang II. P-values are derived from two-tailed, unpaired t-test.

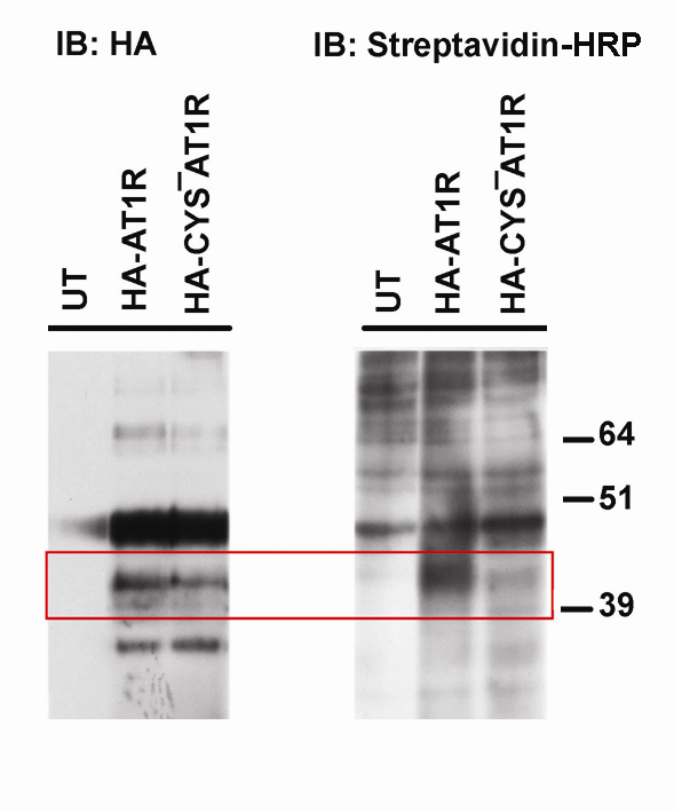
Supplementary Table II. Comparison of MTSEA-biotin reactivity of fully-glycosylated monomeric receptor and multimeric forms of the receptor.

	41.9 kDa Monomeric form Relative Accessibility		**50-80 kDa Multimeric Relative Accessibility**	
Mutants	**Mean**	**S.E.M.**	**Mean**	**S.E.M.**
HA-CYS^{-}AT1R	1.00	0.24	1.00	0.28
I172	1.39	0.40	1.24	0.24
E173	1.86	0.48	1.53	0.79
N174	3.08	0.60	1.52	1.21
T175	3.18	0.49	1.24	0.97
N176	1.61	0.06	1.07	0.35
I177	0.58	0.17	1.31	0.38
T178	1.63	0.09	1.16	0.26
V179	0.73	0.16	0.94	0.10
A181	0.1	0.01	0.54	0.21
F182	0.81	0.20	0.83	0.42
H183	0.42	0.06	0.88	0.32
Y184	1.04	0.17	1.56	1.04
E185	2.03	0.56	1.67	0.62
S186	2.38	0.47	1.91	1.46
R187	3.52	0.23	1.29	0.92
N188	0.57	0.15	0.55	0.13
S189	2.08	0.28	1.56	1.43
T190	1.57	0.34	0.90	0.17
L191	0.92	0.20	0.97	0.75
P192	1.21	0.23	0.99	0.96

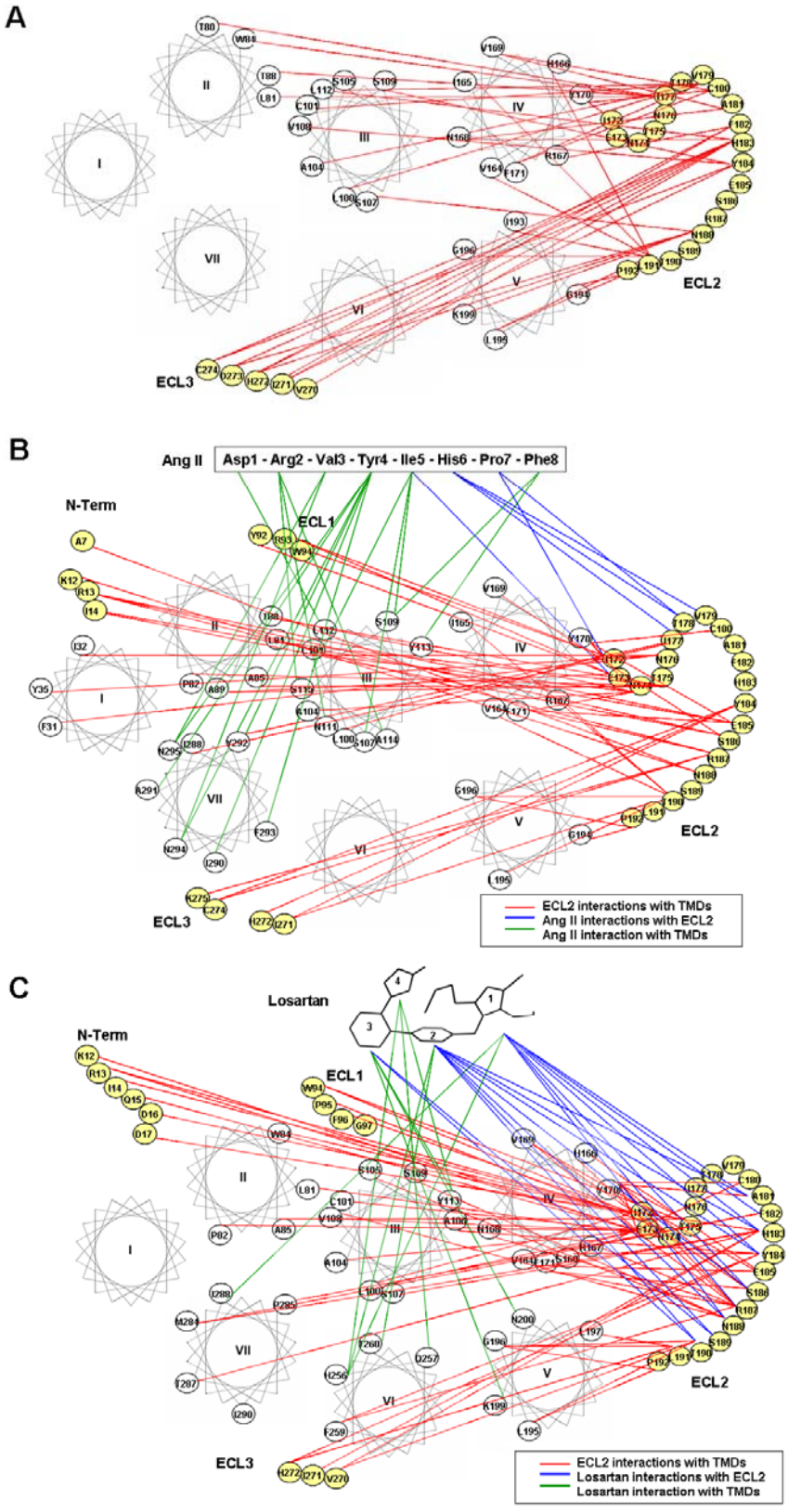
SUPPLEMENTARY FIGURES



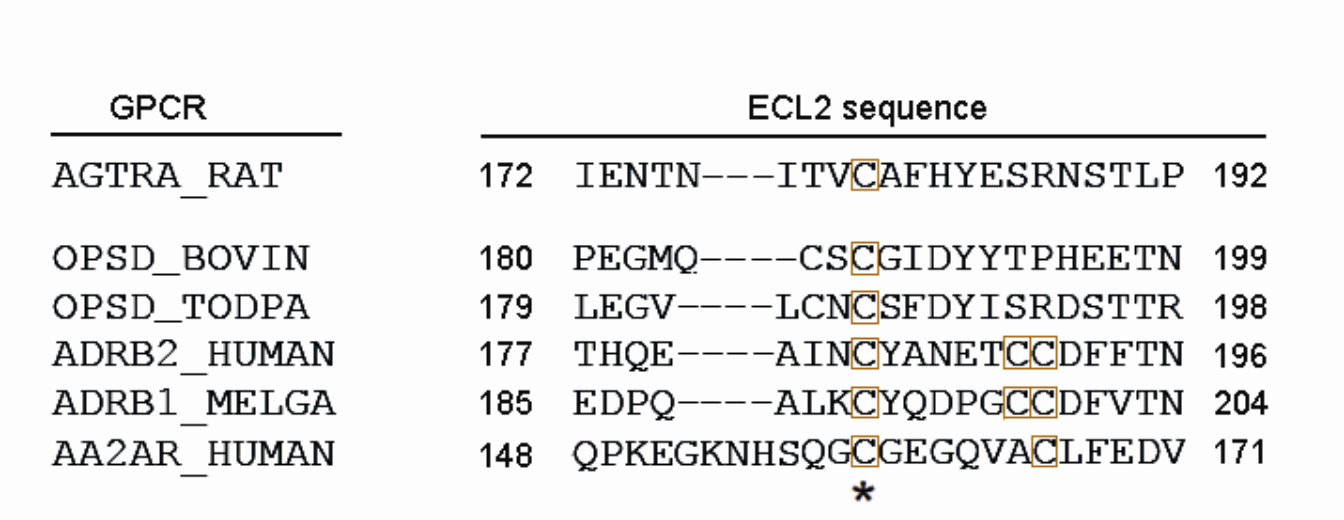
Supplementary Figure 1. The expression of I177 and V179 mutants with cysteine, alanine and serine substitutions.



Supplementary Figure 2. MTSEA-biotin accessibity of HA-AT1R and HA-CYS¯AT1R. HA and Streptavidin-HRP blots are shown on the left and right respectively. The band representing the fully-glycosylated, mature form of the receptor that has been used for quantification of MTSEA-biotin relative accessibility is shown in red box.


A
I
II
III
IV
V
VI
VII
ECL2
ECL3
B
Ang II
Asp1 - Arg2 - Val3 - Tyr4 - Ile5 - His6 - Pro7 - Phe8
N-Term
ECL1
ECL2
ECL3
ECL2 interactions with TMDs
Ang II interactions with ECL2
Ang II interaction with TMDs
C
Losartan
N-Term
ECL1
ECL2
ECL3
ECL2 interactions with TMDs
Losartan interactions with ECL2
Losartan interaction with TMDs

Supplementary Figure 3. Predicted interactions of AT1R and Ang II. *A*. Predicted interactions of ECL2 with the TM helices (red) in the absence of ligand. TM helices are depicted as helical wheel diagram generated using Helical Wheel Projections (http://rzlab.ucr.edu/scripts/wheel/). *B*. Predicted interactions of ECL2 with the TM helices (red) in the presence of Ang II. Predicted interactions of Ang II with ECL2 (blue) and TM helices (green) are also shown. *C*. Predicted interactions of ECL2 with the TM helices (red) in the presence of losartan. Predicted interactions of losartan with ECL2 (blue) and TM helices (green) are also shown. Loop and N-terminal residues are highlighted in yellow. 1; imidazole ring, 2; phenyl ring 1, 3; phenyl ring 2, 4; tetrazole ring.



Supplementary Figure 4. Alignment of ECL2 of AT1R with the ECL2 sequences of GPCRs for which crystal structures are available. The conserved cysteine is indicated by asterisk. The cysteines which are involved in disulfide bond formation in each GPCR is boxed in gold (1-7). The alignment was generated by using ClustalW2 (http://www.ebi.ac.uk/clustalw/).

ECL2*	Ile Ile Val Phe His Tyr Asn Pro	
Ang II	Asp-Arg-Val-Tyr-Ile-His-Pro-Phe	Agonist
[Ile^4] Ang II	Asp-Arg-Val-***Ile***-Ile-His-Pro-Phe	Partial Agonist
[Ile^8] Ang II	Asp-Arg-Val-Tyr-Ile-His-Pro-***Ile***	Antagonist
[Ile^4, Ile^8] Ang II	Asp-Arg-Val-***Ile***-Ile-His-Pro-***Ile***	Antagonist

Supplementary Figure 5. Ang II analogous ECL2 Pharmacophore. *The inaccessible residues of ECL2 in the empty-state which are similar to Ang II residues are shown. Three Ang II analogs with different properties are also shown where the scrambled residues are indicated in bold italic.

SUPPLEMENTARY REFERENCES

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