

Table S1: AT1R ligand panel. Related to Figures 3, 4, S1, and S5.

Ligand	Log Ki		Cellular signaling bias (Rajagopal et al., 2011)			Molecular efficacy bias (Strachan et al., 2014)			NNMF component amplitude (% population)			
	Wild-type	Minimal cysteine	σ_{IP1}	$\sigma_{\beta arr}$	Bias factor (β) $((\sigma_{IP1} - \sigma_{\beta arr})/\sqrt{2})$	Log α_{Gq}	Log $\alpha_{\beta arr2}$	Molecular bias $(\text{Log}(\alpha_{Gq}/\alpha_{\beta arr2}))$	I	II	III	IV
Candesartan	ND	-7.93 ± 0.05	ND	ND	ND	ND	ND	ND	95.7	1.6	2.6	0
Olmesartan	See below ^a	See below ^a	ND	ND	ND	ND	ND	ND	89.2	4.3	0	6.6
Apo	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	65.4	27.6	0	6.9
TRV026	-7.42 ± 0.02	-6.90 ± 0.04	-1.983 ± 0.188	-0.290 ± 0.107	-1.197 ± 0.217	0.20 ± 0.02	1.26 ± 0.02	-1.061 ± 0.080	21.8	78.3	0	0
TRV027	-6.58 ± 0.04	-6.06 ± 0.01	ND	ND	ND	ND	ND	ND	17.1	42.8	31.7	8.4
TRV023	-6.66 ± 0.04	-6.33 ± 0.02	ND	ND	ND	0.28 ± 0.04	1.14 ± 0.04	-0.864 ± 0.090	27.4	16.2	44.5	11.9
TRV034	-6.57 ± 0.04	-6.14 ± 0.02	-1.892 ± 0.166	-0.105 ± 0.112	-1.264 ± 0.201	0.35 ± 0.08	1.11 ± 0.08	-0.761 ± 0.090	22.1	18.2	51.3	8.4
AngII	-7.76 ± 0.07	-7.11 ± 0.01	0.000 ± 0.126	0.000 ± 0.121	0.000 ± 0.174	1.63 ± 0.02	1.47 ± 0.02	0.162 ± 0.040	17.5	7.5	48.5	26.6
TRV055	-5.26 ± 0.07	-4.92 ± 0.01	1.582 ± 0.111	1.010 ± 0.120	0.404 ± 0.163	2.62 ± 0.02	1.51 ± 0.02	1.106 ± 0.030	44.3	12.2	1.1	42.3
TRV056	-4.67 ± 0.15	-3.95 ± 0.05	1.119 ± 0.111	0.510 ± 0.119	0.431 ± 0.163	2.59 ± 0.02	1.35 ± 0.02	1.245 ± 0.100	48.0	15.0	0	37.0

Inhibitory constants (Ki), transducer coupling efficacies, and NNMF component amplitudes for AT1R ligands used in this study. Log Ki values (logarithm of the equilibrium dissociation constant in molar, shown graphically in Figure S1C) were determined for purified AT1R wild-type or minimal cysteine using the Kd values of [³H]-olmesartan shown below. Values of σ are those previously reported (Rajagopal et al., 2011) and represent the log ($\tau_{ligand}/\tau_{AngII}$), where τ is the transducer coupling efficiency for Gq-mediated IP1 generation and β -arrestin (βarr) recruitment derived from the operational model. Cellular signaling data for TRV027 and TRV023 are reported elsewhere (Strachan et al., 2014; Violin et al., 2010). Log α_{Gq} and log $\alpha_{\beta arr}$ values are those previously reported (Strachan et al., 2014) and are molecular efficacy values describing the allosteric coupling between ligands and transducers in AT1R-Gq and AT1R- $\beta arr2$ fusion proteins. NNMF component amplitudes are the values shown graphically in Figure 4B. Error in all values reported represents standard error determined from at least three independent experiments. ND = not determined.

^aAT1R wild-type [³H]-olmesartan Kd = 4.43 ± 0.36 nM, log Kd = -8.35; AT1R minimal cysteine [³H]-olmesartan Kd = 6.65 ± 0.65 nM, log Kd = -8.18

Table S2: Ligand-binding properties of spin-labeled AT1R mutants. Related to Figure 1 and Figure S1.

Construct	Log IC₅₀ (M)
Minimal Cysteine	-6.24 ± 0.15
TM1-ICL2 (55V1 139V1)	-6.73 ± 0.13
TM1-TM6 (55V1 236V1)	-6.55 ± 0.05
TM1-TM7 (55V1 304V1)	-6.54 ± 0.11
TM1-H8 (55V1 311V1)	-6.69 ± 0.06
TM5-H8 (220V1 311V1)	-6.19 ± 0.16
ICL2-TM5 (139V1 220V1)	-5.94 ± 0.19
ICL2-TM6 (139V1 236V1)	-6.69 ± 0.06
ICL2-TM7 (139V1 304V1)	-6.43 ± 0.26
ICL2-H8 (139V1 311V1)	-6.48 ± 0.18
TM6-H8 (236V1 311V1)	-6.45 ± 0.23

Log IC₅₀ values for AngII binding to spin-labeled AT1R constructs determined by [³H]-olmesartan competition radioligand binding in the scintillation proximity assay. Data are the mean and standard error of the log IC₅₀ values determined in at least three independent experiments.

Table S3: Oligonucleotides used in this study. Related to Key Resources Table.

Oligonucleotide	Sequence
C76A_fwd	gaatttagcactggctgacttaGCcttttactgactttgccacta
C121A_fwd	ctagtgtgttctactcacgGCctcagcattgatcgatacc
C149A_fwd	gctttagcctaaagtcaccGCcatcatcattggctgctg
C289A_fwd	cacggccatgcctatcaccattGCtatagcttatttaacaattgcc
C296A_fwd	cctatcaccattgctatagcttatttaacaatGCcctgaatcctctttttatg
C355A_fwd	accaagaagcctgcaccaGCtttgagggtgagtgagg
F55C_fwd	ttggtggtgatagtcatttactGttatatgaagctgaagactgtg
R139C_fwd	cccaatgaagtcctcctTgCcgcaaatgctttag
K220C_fwd	attcttacaagtatactcttatttggTGCgccctaaagaaggcttatgaaattcag
D236C_fwd	aaggcttatgaaattcagaagaacaaaccaagaaatTGtgatattttaagataattatggca
F304C_fwd	tgccctgaatcctctttttatggctGtctggggaaaaaat
R311C_fwd	ctctttttatggcttctggggaaaaaatTaaTgCtattttctccagctctaaaatatattc