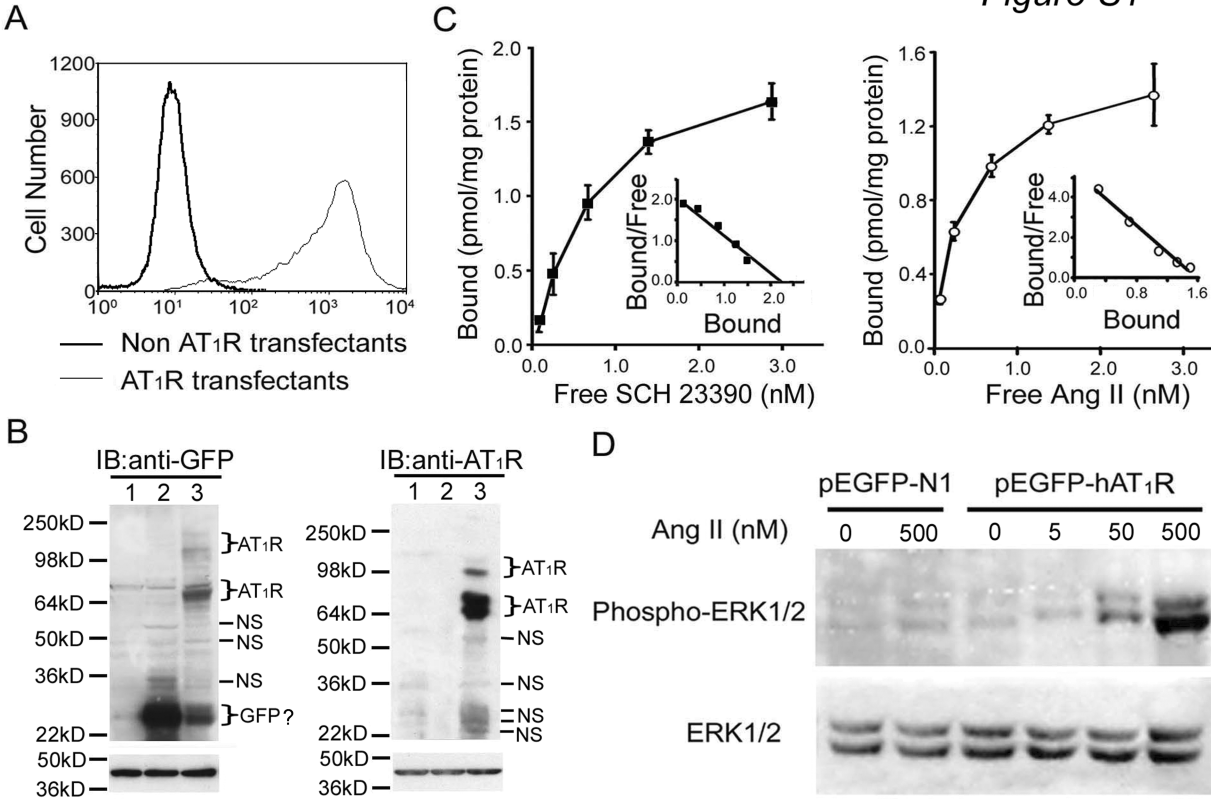
Figure S1


A
Cell Number
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900
600
300
0
10^0
10^1
10^2
10^3
10^4
Non AT1R transfectants
AT1R transfectants
C
Bound (pmol/mg protein)
Free SCH 23390 (nM)
Bound/Free
Bound
Free Ang II (nM)
B
IB:anti-GFP
IB:anti-AT1R
1 2 3
250kD
98kD
64kD
50kD
36kD
22kD
AT1R
NS
GFP?
D
pEGFP-N1
pEGFP-hAT1R
Ang II (nM)
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Phospho-ERK1/2
ERK1/2

Figure S2

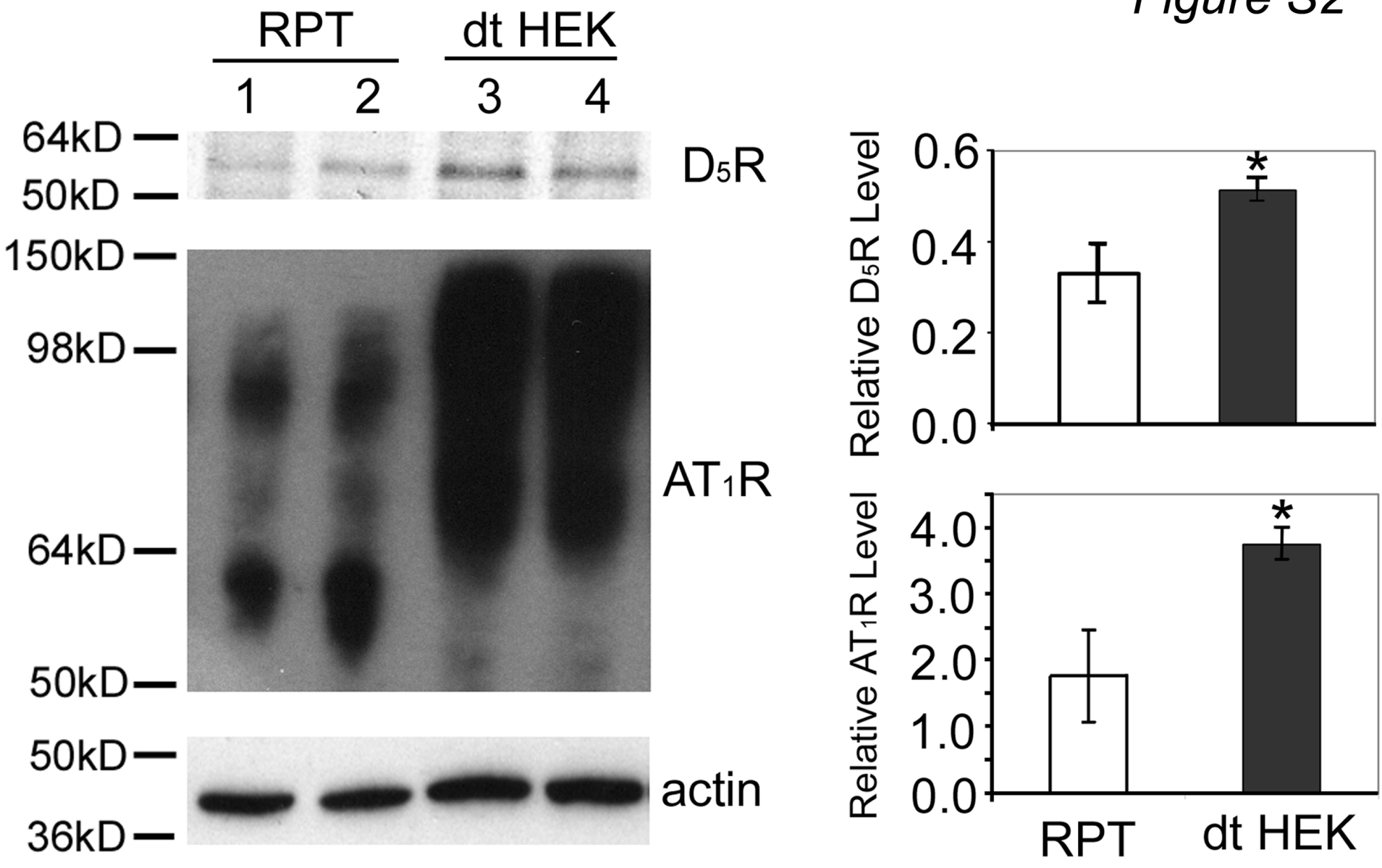
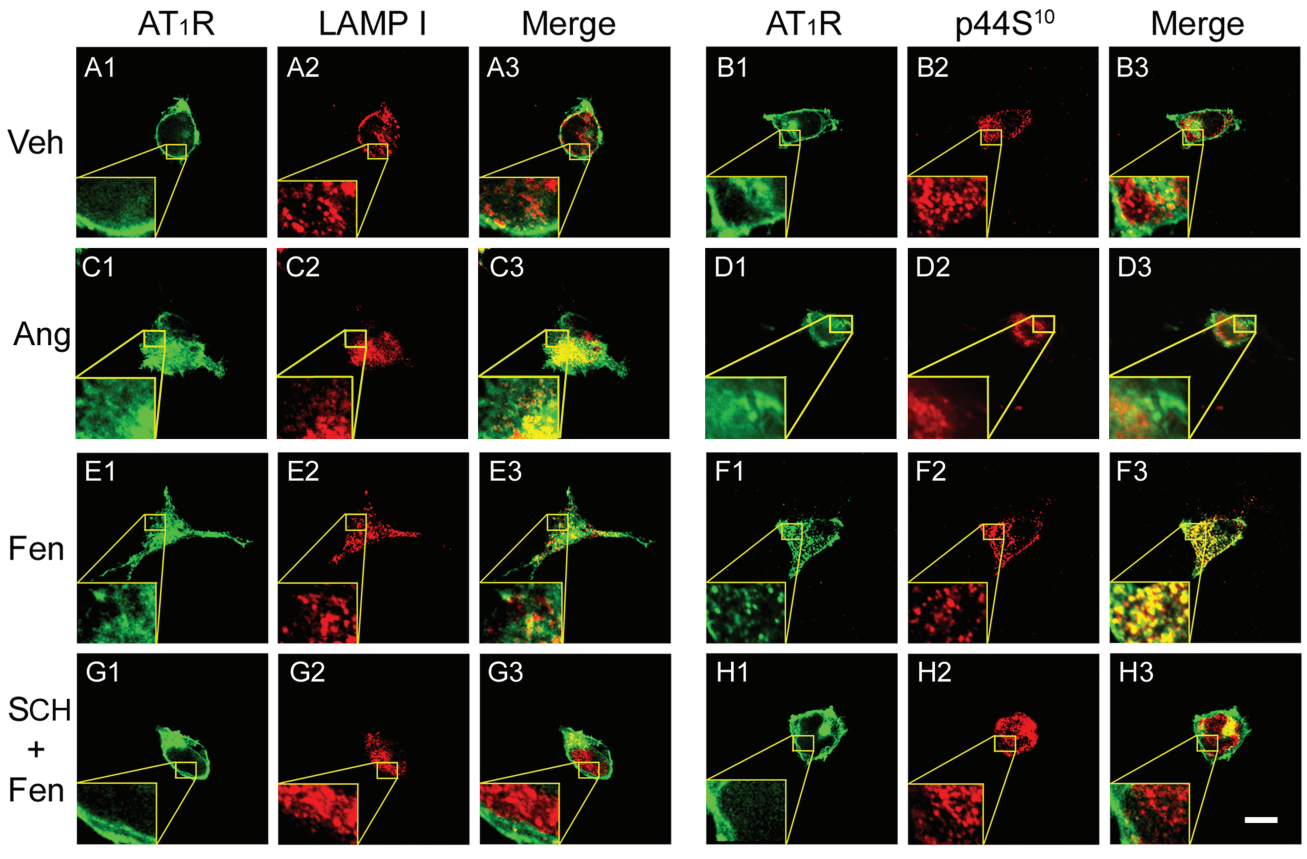

RPT
dt HEK
1
2
3
4
64kD
50kD
150kD
98kD
64kD
50kD
50kD
36kD
D5R
AT1R
actin
Relative D5R Level
0.6
0.4
0.2
0.0
Relative AT1R Level
4.0
3.0
2.0
1.0
0.0
RPT
dt HEK

Figure S3


AT1R
LAMP I
Merge
AT1R
p44S10
Merge
Veh
Ang
Fen
SCH
+
Fen
A1
A2
A3
B1
B2
B3
C1
C2
C3
D1
D2
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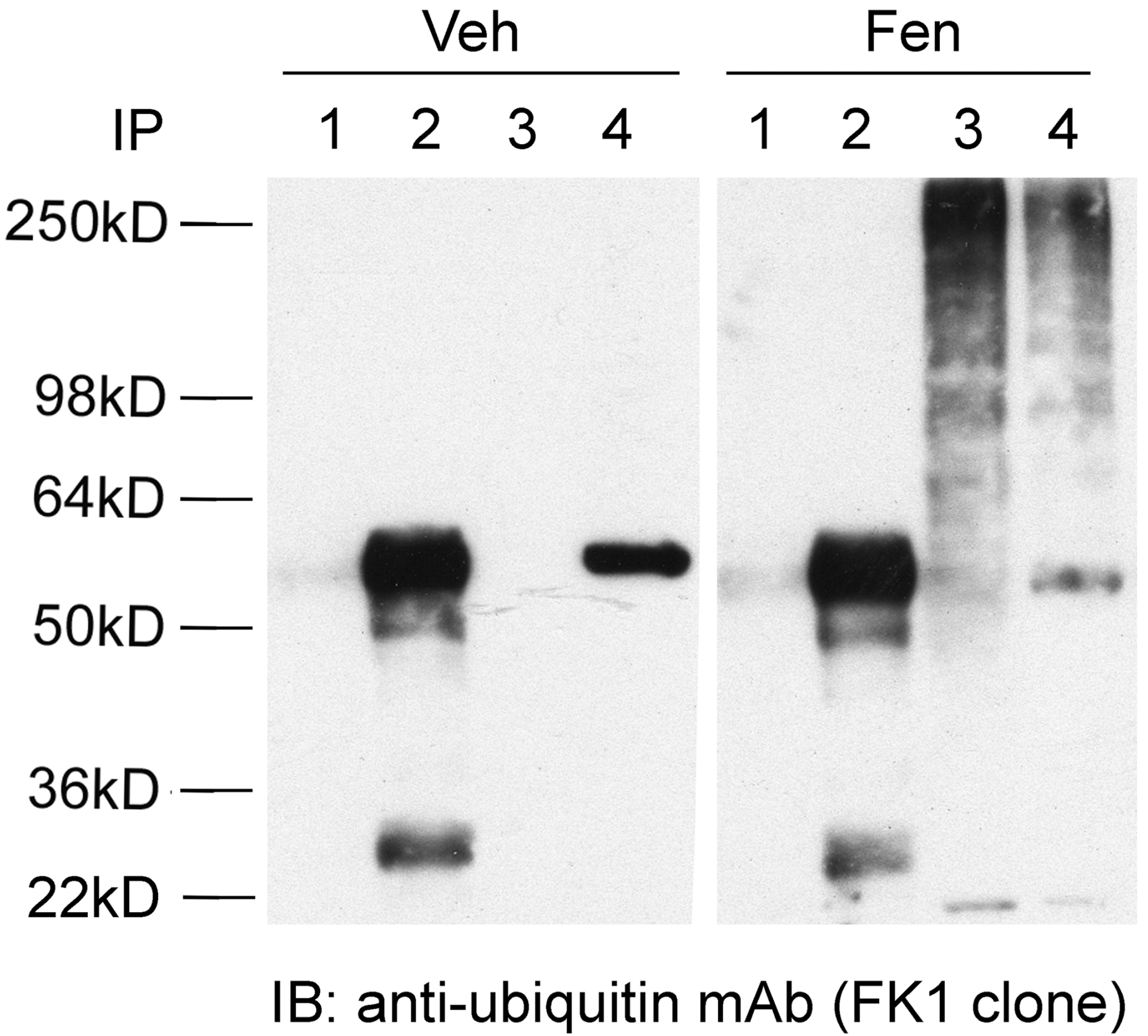
G1
G2
G3
H1
H2
H3

Figure S4


Veh
Fen
IP
1
2
3
4
1
2
3
4
250kD
98kD
64kD
50kD
36kD
22kD
IB: anti-ubiquitin mAb (FK1 clone)

Supplemental data

Supplemental Methods

Antibodies and reagents

The polyclonal rabbit GFP, monoclonal mouse Ub antibody (clone P4D1), polyclonal goat p44S[10] antibody, normal mouse, rabbit, goat IgGs, normal mouse IgM, and HRP-conjugated anti-sheep IgG were obtained from Santa Cruz Biotechnology; the Ub monoclonal antibody (clone FK1) was purchased from Biomol International LP; the AT_1R monoclonal antibody was from GeneTex Inc.; the polyclonal rabbit LAMP 1 antibody was from Affinity Bioreagents, Inc.; the polyclonal rabbit p42/44 MAPK antibody and its corresponding phospho-p42/44 MAPK antibody were from Cell Signaling Technology; the polyclonal rabbit D_5R antibody was generated as reported (1); the polyclonal sheep renin antibody was purchased from Innovative Research, Inc.; the horseradish peroxidase-conjugated anti-rabbit IgG, anti-mouse IgG, and anti-goat IgG were from Promega; Alexa-633, Alexa-555, and Alexa-546 protein labeling kits were from Molecular Probes. The TRAN^{35}S-LABEL^{TM} metabolic labeling reagent was from MP Biomedicals. ^{125}I-Sar^1-Ang II was from Peptide Radioiodination Service Center at University of Mississippi. ^{3}H-SCH 23390 was from Perkin Elmer.

Cell lines and transfection

Human RPT cells were immortalized by infecting the cells in culture with two HIV-based lentiviral constructs that contain the neomycin resistance gene, one containing SV40 large T antigen, and the other containing the catalytic subunit of human telomerase

(2). The cells were then selected with 600 μg/ml G418 for 10 days or until the non-infected control cells were dead. Cells were verified to be of RPT origin by staining with antibodies against γ-glutamyl transpeptidase and LTA lectin; 100% of the cells stained for both markers. The immortalized human RPT cells were cultured in a 1:1 mixture of Dulbecco's modified Eagle's medium (DMEM) and Ham's F-12 medium (Invitrogen) supplemented with 5% FBS, selenium (5 ng/ml), insulin (5 μg/ml), transferrin (5 μg/ml), hydrocortisone (36 ng/ml), triiodothyronine (4 pg/ml), and epidermal growth factor (10 ng/ml).

Human embryonic kidney (HEK) 293 cells and the HEK 293 cells stably over-expressing full length D_5R tagged with V5 and His (1) were cultured in DMEM medium containing 4.5 g/L glucose, 10% FBS, 2 mM L-glutamine and 1 mM sodium pyruvate. Human AT_1R-tagged EGFP or its empty pEGFP-N1 vectors (3) were transfected into D_5R HEK 293 cells using Lipofectamine™ 2000 transfection reagents (Invitrogen), according to the manufacturer's protocol. Transfectants were selected with G418. The stable transfectants were designated as AT_1R/D_5R HEK 293 cells.

Radioligand binding

Membrane pellets prepared from AT_1R/D_5R HEK 293 cells were suspended in 50 mM Tris buffer (pH 7.4). The AT_1R radioligand binding assay was carried out as follows: 20 μg of membranes were incubated for 2 h at room temperature in 10 mM phosphate-buffered saline (PBS), pH 7.4, containing 120 mM NaCl, 5 mM ethylenediaminetetraacetic acid (EDTA), 0.005 % bacitracin, 0.2% protease free bovine serum albumin (BSA) and 0.25 to 4 nM ^{125}I-Sar^1-Ang II (Specific activity: 2176

Ci/mmol). Non-specific binding was determined by the addition of 10 μM losartan. The radioligand binding was terminated by filtration through glass-fiber filters (Whatman GF/C, Hillsboro, OR). The filters were washed 3 times with 50 mM Tris (pH 7.4) and counted in a gamma spectrophotometer (Packard). The D_1-like receptor radioligand binding assay was carried out as described above except that the binding buffer was 50 mM Tris containing 120 mM NaCl, 5 mM KCl, 1 mM $MgCl_2$, 2 mM $CaCl_2$, 0.005% bacitracin and 0.1% protease free BSA. ^{3}H-SCH 23390 (0.06 to 2.9 nM) was used as the radioligand. Non-specific binding was determined in the presence of 5 μM unlabeled SCH 23390.

Radioligand binding autoradiography

Radioligand binding autoradiography was performed in kidney sections from $D_5R^{-/-}$ and $D_5R^{+/+}$ mice as described previously (4). Sixteen-micrometer-thick kidney sections were cut in a cryostat, thaw-mounted on poly-1-lysine-coated slides (Labscientific Inc), dried overnight in a desiccator at 4 °C, and stored at _ 80 °C until use. Sections were labeled in vitro with 0.5 nM of ^{125}I-Sar1–Ang II, iodinated to a specific activity of 2176 Ci/mmol. Sections were preincubated for 15 min at 22 °C in 10 mM Na phosphate buffer, pH 7.4, containing 120 mM NaCl, 5 mM Na2EDTA, 0.005% bacitracin, and 0.2% protease-free BSA, followed by incubation for 120 min in fresh buffer containing 0.5 nM of ^{125}I-Sar1–Ang II. Nonspecific binding was determined in consecutive sections incubated as above in the presence of 1 _M unlabeled Ang II. To determine selective binding to the Ang II AT_1R, we incubated consecutive sections with 0.5 nM of ^{125}I-Sar1–Ang II in the presence of antagonist losartan (10 _M) to give maximum specific displacement. The

number of AT_1Rs was defined as the binding displaced by the AT_1R antagonist (4). After incubation, slides were rinsed, and the sections were dried under air. Sections were exposed to X-ray film (Eastman Kodak Company) together with ^{14}C-labeled microscales (American Radiolabeled Chemicals). Optical densities of autoradiograms generated by incubation with the ^{125}I-labeled ligand were quantified by computerized densitometry after calibration with ^{14}C-labeled standards as described (4). Each mouse was quantified independently.

Biotinylation and immunoprecipitation

Following treatment with Veh, Fen, and/or tunicamycin after 2 hr starvation, human RPT or AT_1R/D_5R HEK 293 cells were washed with PBS twice and treated with EZ-Link Sulfo-NHS-SS-Biotin (Pierce) by gentle rocking at 4°C for 30 min; un-reacted biotin was quenched with cold 50 mM glycine in PBS. The biotinylated cells were harvested by washing with TBS (0.025M Tris, 0.15 M NaCl; pH 7.2) three times and the cell pellets were sonicated (20 sec x 5, on ice) in lysis buffer containing 20 mM Tris·HCl, pH 8.0, 1 mM EDTA, 1 mM NaN_3, 2 mM DTT, 0.25 M sucrose, 1 mM benzamidine, 1μg/ml soybean and lima bean trypsin inhibitors. The cell lysates were mixed with NeutrAvidin™ gel slurry (Pierce) for 60 min at room temperature. The biotinylated proteins were eluted with 50 mM DTT buffer. The isolated plasma membrane proteins were concentrated and adjusted to 1 mg protein/ml in a buffer containing 20 mM Tris·HCl (pH 8.0), 0.25 M sucrose, 0.2 mM $MgCl_2$, 0.1 mM ATP, 1mM creatine phosphate, 10 units of creatine phosphokinase and 50% volume of rabbit reticulocytes (Promega), 0.5 mM benzamidine hydrochloride, and 1 μg/ml soybean and lima bean

trypsin inhibitors, and were incubated (rocking, 4°C, 6 hr) with 6 μg of anti-AT_1R monoclonal antibody or 6 μg of affinity-purified anti-D_5R polyclonal antibody. Controls were normal rabbit or mouse IgG or IgM. After adding 40 μl of a 50% slurry of protein G-Sepharose CL-4B (Amersham Pharmacia) in PBS and incubation at 4°C overnight, beads were washed three times with 1 ml of ice-cold PBS containing protease inhibitors. Proteins bound to beads were eluted in 50 μl of loading buffer at 65°C for 30 min, separated by SDS-PAGE gels, and transferred onto nitrocellulose membrane for incubation with anti-Ub mAb (clone FK1) or anti-AT_1R mAb, followed by appropriate horseradish peroxidase-conjugated secondary antibodies and detection using Super Signal Chemiluminescent substrate (Pierce).

Deglycosylation

Ninety percent confluent AT_1R/D_5R HEK 293 cells were washed with PBS twice. Cell pellets were sonicated (20 sec x 5, on ice) in a 50 mM phosphate buffer containing 1 mM EDTA, 2 mM dithiothreitol (DTT), 1 mM benzamidine, 1μg/ml soybean and lima bean trypsin inhibitors. Fifty μg cell lysates were deglycosylated with 10 mU of PNGase F (Sigma) in phosphate buffer (pH 5.6) containing 2 μM $MgCl_2$, 0.1 M β-mercaptoethanol, 25 mM EDTA, and 0.5% NP-40 at 37°C for the indicated time. Equivalent samples were subjected to 10% SDS-PAGE and transferred onto nitrocellulose membranes. The blots were incubated with antibody against GFP, and subsequently with goat anti-rabbit horseradish peroxidase-conjugated secondary antibody, before developing using ECL substrate (Pierce).

Proteasome isolation

Proteasomes were isolated from human RPT cells by affinity purification using a human proteasome isolation kit (Calbiochem, EMD Biosciences) following the manufacturer's instruction. Briefly, human RPT cells were lysed in a lysis buffer containing 150 mM NaCl, 5 mM EDTA, 1% Triton X-100, 2 mM ATP and 50 mM HEPES, pH 7.5). One mg (in 1 ml volume) protein samples were incubated at 4 °C (4 h) with 100 µl of control or proteasome-affinity agarose beads. The affinity matrix was washed three times and eluted with SDS sample buffer at 85°C for 15 minutes. The protein complexes were resolved by SDS-PAGE and transferred to nitrocellulose membranes, followed by immunoblotting with anti-AT_1R mAb or antibody against proteasome marker protein, p44S[10].

Flow cytometry

AT_1R/D_5R HEK 293 cells, in six-well plates, were washed three times with PBS, and the cell pellets resuspended in ice-cold PBS at a density of 4 _ 10^6 cells per ml. Relative total AT_1R protein expression was determined by measuring the total EGFP fluorescence on a FACSort flow cytometer (BD Biosciences). 20,000-30,000 live cells were acquired for processing and analysis (FCS Express, DeNovo Software).

Determination of catecholamine levels

Catecholamine levels were measured as previously reported from our laboratory (5). Kidneys were homogenized with a buffer containing 0.05 $MHClO_4$ /1 M $NaHSO_3$/0.1 M EDTA (2.8:0.1:0.1 ml), centrifuged at 6,000 *g* for 20 min at 4°C. The

catecholamine levels in the renal supernatant and plasma samples were determined by HPLC and electrochemical detection (5).

Drd5$^{-/-}$ mice and blood pressure measurement

The generation and genotyping of *Drd5*$^{-/-}$ mice have been previously described (5). 6-mo-old six generation (F6) *Drd5*$^{-/-}$ mice (>98% congenic) and sex-matched $D_5R^{+/+}$ littermates (5) were used in this study. Vehicle or the AT_1R antagonist, losartan (20 mg/kg/day) was injected intraperitoneally in 6-month old mice daily for 5-7 days. The mice were anesthetized with pentobarbital (50 mg/kg), tracheotomized, and placed on a heated board to maintain rectal temperature at 37°C. Catheters were inserted into the femoral vessels towards the aortae for immediate pressure measurement, as described previously (5).

Supplemental Reference

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3. Lanctôt, P.M., Leclerc, P.C., Escher, E., Leduc, R., and Guillemette, G. 1999. Role of N-glycosylation in the expression and functional properties of human AT_1 receptor. *Biochemistry.* **38**: 8621-8627.
4. Armando, I., Carranza, A., Nishimura, Y., Hoe, K.L., Barontini, M., Terrón, J.A., Falcón-Neri, A., Ito, T., Juorio, A.V., Saavedra, J.M. 2001. Peripheral administration of an angiotensin II AT_1 receptor antagonist decreases the hypothalamic-pituitary-adrenal response to isolation stress. *Endocrinology.* **142**:3880-3889.
5. Hollon, T.R., Bek, M.J. Lachowicz, J.E., Ariano, M.A., Mezey, E., Ramachandran, R., Wersinger, S.R., Soares-da-Silva, P., Liu, Z.F., Grinberg, A., Drago, J., Young, W.S., Westphal, H., Jose, P.A., and Sibley, D.R. 2002. Mice lacking D5 dopamine receptors have increased sympathetic tone and are hypertensive. *J. Neurosci.* **22**: 10801-10810.

Supplemental Figure Legends

Figure S1. Heterologous overexpression of the AT_1R and the D_5R in HEK 293 cells. (A) Flow cytometric analysis of the AT_1R-EGFP transfectants in D_5R HEK 293 cells. The green fluorescence signal intensity provided an estimate of the total AT_1R protein expression. The shift in green fluorescence of transfectants (thinner tracing) from the non-transfected D_5R HEK 293 cells (thicker tracing) confirmed the stable expression of AT_1R in D_5R HEK 293 cells.

(B) Immunoblotting of the AT_1R using anti-GFP (left panel) and anti-AT_1R (right panel) antibodies in the AT_1R/D_5R HEK 293 cells. Lane 1, HEK 293 cells; lane 2, empty pEGFP-N1 vector (Clontech)-transfected D_5R HEK 293 cells; and lane 3, AT_1R/D_5R HEK 293 cells (heterologously overexpressed AT_1R and D_5R in HEK 293 cells). Immunoblotting for actin in the lower panel of each blot showed the sample protein loading. Both anti-GFP and anti-AT_1R antibodies recognize two specific AT_1R-EGFP bands: one band is ~65-85 kDa, the other band is ~100-120 kDa, indicating two potentially different forms (non-glycosylated and glycosylated) of the AT_1R in the AT_1R/D_5R HEK 293 cells. GFP?"indicates our uncertainty of the identity of the band; NS, non specific bands; kD, kilodalton.

(C) Radioligand binding assays in AT_1R/D_5R HEK 293 cells. All assays were performed in triplicate in a final volume of 200 μl. Specific binding was defined by subtracting nonspecific binding from total binding. The B_{max} and K_d were determined from Scatchard plots.

Left panel: Saturation curve and Scatchard plot (insert) of the binding of ^{3}H-SCH 23390 to cell membranes. Non-specific binding was determined in the presence of 5 μM unlabeled SCH 23390. B_{max} = 2.25 pmol/mg membrane protein, K_d= 0.8 nM (n=3). Right panel: Saturation curve and Scatchard plot (insert) of the binding of ^{125}I-Sar^{1}-Ang II to cell membranes. Non-specific binding was determined in the presence of 10 μM losartan (AT_1R antagonist). B_{max}=1.57 pmol /mg membrane protein; K_d= 0.28 nM (n=3). (D) Ang II-induced ERK1/2 phosphorylation in the D_5R HEK 293 cells transfected with AT_1R-EGFP or the corresponding empty vector pEGFP-N1. The transfected cells were split into 60-mm Petri dishes and incubated with increasing concentrations of Ang II, as indicated for 5 min at 37 °C. ERK1/2 activation was determined by immunoblotting with phospho-specific ERK1/2 antibodies. Blots of phospho-ERK 1/2 (upper panel) and total ERK1/2 expression (lower panel) are shown. The experiment was repeated twice with similar results.

Figure S2. Comparison of D_5R and AT_1R protein expression in human RPT cells and AT_1R/D_5R HEK 293 cells. Cell pellets were suspended in lysis buffer (20 mM Tris·HCl pH 8.0, 0.25 M sucrose, 2 mM $MgCl_2$, 100 mM NaCl, 1 mM EDTA, 1 mM NaN_3, 1 mM DTT, 0.5 mM AEBSF, and 0.5% 3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate). After centrifugation of the cell lysates (1,000 _ *g* for 3 min), the supernatants were centrifuged (158,000 _ *g* for 90 min), and the pellets homogenized in the same buffer. Thirty μg protein samples were subjected to SDS/PAGE and transferred onto nitrocellulose membranes, which were incubated with anti-D_5R (top bands), anti-AT_1R (middle bands) and anti-actin (bottom bands) antibodies, followed by the

appropriate secondary antibodies conjugated to horseradish peroxidase (Promega). The bands were detected using Super Signal Chemiluminescent substrate (Pierce) and quantified by densitometry. Data are mean±SEM, Student's t test, *P<0.05. RPT, renal proximal tubule cells; dtHEK, AT_1R/D_5R HEK 293 cells.

Figure S3. Subcellular distribution of AT_1R in AT_1R/D_5R HEK 293 cells. Cells were treated with Veh (images A1-A3 and B1-B3), Ang II (50 nM, 15min) (C1-C3 and D1-D3), Fen (1 μM, 20 min) (E1-E3 and F1-F3) or Fen pretreated with the D_5R antagonist, SCH 23390 (1 μM, 30 min) (SCH+ Fen, G1-G3 and H1-H3). Slides were prepared as described in the "Methods" section. Green, AT_1R-EGFP; red, lysosomes (A2, C2, E2, and G2) or proteasomes (B2, D2, F2, and H2), were detected by immunostaining with anti-LAMP 1 or anti-p44S[10] antibodies, respectively, conjugated with Alexa Fluor® 633*;* yellow, co-localization of the AT_1R with lysosomes (A3, C3, E3, G3) or proteasomes (B3, D3, F3, H3). Scale bar = 10 μm. The magnification of inserts is 4X.

Figure S4. Ubiquitination of AT_1R upon D_5R stimulation with fenoldopam in AT_1R/D_5R HEK 293 cells. Cells were treated with vehicle or fenoldopam (1 μM/5 min), and cell membrane fractions, isolated by biotinylation, were immunoprecipitated with normal rabbit Ig G (lane 1), normal mouse IgG (lane 2), anti-GFP IgG (lane 3, for AT_1R) or anti-V_5 mAb (lane 4, for D_5R) and immunoblotted with anti-Ub mAb (clone FK1). kD, kilodalton.

Supplemental tables

Table S1. Comparison of AT_1R protein expression levels in the glomeruli and the cortex tubules in *Drd5*$^{+/+}$and *Drd5*$^{-/-}$mice.

Mouse Strain	*Drd5*$^{+/+}$	*Drd5*$^{-/-}$	P value
Glomeruli	248.1±15.4	237.3±16.7	0.677
Cortex Tubules	73.0±7.8	109.3±5.5	0.019*

Binding assays were performed in 16 μm kidney sections, as described in Methods (Supplemental data). All values are mean±SEM in fmol/mg protein (n=4/group). P values were determined by Student's *t* test. * $p<0.05$.

Table S2. Plasma and renal catecholamine levels in *Drd5*$^{+/+}$and *Drd5*$^{-/-}$mice

	Plasma (ng/ml)			Kidney (ng/g)		
	Drd5$^{+/+}$	*Drd5*$^{-/-}$	P	*Drd5*$^{+/+}$	*Drd5*$^{-/-}$	P
L-DOPA	3.1±0.5	3.6±0.9	0.66	137.5±19.8	99.1±7.1	0.10
Norepinephrine	20.9±5.4	14.4±1.7	0.28	583.3±90.0	650.5±41.3	0.52
Epinephrine	4.9±4.1	6.5±4.9	0.81	32.4±13.6	73.3±26.8	0.21
Dopamine	3.0±1.0	4.5±1.6	0.57	44.5±7.3	90.7±14.2	0.02

All values are mean±SEM (n=5/group). P values were determined by Student's *t* test. L-DOPA, 3,4-dihydroxy-L-phenylalanine.

Table S3. Percentage of AT_1R-EGFP fluorescence localized at the plasma membrane in AT_1R/D_5R HEK 293 cells

Treatment	Number	Mem localization (%)	P value (*vs.* Veh)	P value (*vs.* Fen)	P value (*vs.* Chlor+Fen)
Veh	6	43.47±7.0			
Fen	12	12.10±4.9	<0.001		
SCH + Fen	10	52.25±18.2	0.265	<0.001	
Chlor + Fen	10	16.92±8.6	<0.001	0.308	
CLBL + Fen	10	42.40±11.8	0.983	<0.001	<0.001

AT_1R/D_5R HEK 293 cells were randomly chosen. Images were thresholded to exclude background pixels. Regions delineating the plasma membrane or the entire cell were drawn manually after zooming in the images. The percentage of AT_1R fluorescence localized to the plasma membrane was defined as the integrated gray value within the plasma membrane region over the integrated gray value of the entire cell times 100. Images were analyzed using MetaMorph 6.1 (Molecular Devices). Data are mean±SD. Significant differences were analyzed using ANOVA, Student-Newman-Keuls test (SigmaStat 3.0). Veh, vehicle; Fen, fenoldopam; SCH, SCH 23390; Chlor, chloroquine; CLBL, clasto-lactacystin beta-lactone; Mem, plasma membrane.

Table S4. Co-localization analysis of AT_1R with the lysosome marker protein LAMP 1, and the proteasome marker protein $p44S^{10}$ in AT_1R/D_5R HEK 293 cells

Treatment	% co-localization of AT_1R over LAMP 1	P *vs.* Veh	% co-localization of AT_1R over $p44S^{10}$	P *vs.* Veh
Veh	23.19±4.0, n=3		11.26±2.3, n=3	
Fen	21.47±3.3, n=6	0.599	56.12±5.8, n=6	<0.001
SCH+Fen	16.41±3.6, n=6	0.311	9.34±3.0, n=4	0.587
Chlor+Fen	20.11±3.7, n=6	0.612	51.31±7.7, n=4	<0.001
CLBL+Fen	19.87±6.1, n=5	0.754	16.66±3.1, n=4	0.250

AT_1R/D_5R HEK 293 cells were randomly chosen. Images were thresholded to non background pixels. The percentage of co-localization was defined as the AT_1R pixels over the total number of pixels positive for lysosomes or proteasomes markers times 100. Images were analyzed using MetaMorph 6.1 (Molecular Devices). Data are mean±SD. Significant differences were analyzed using ANOVA, Student-Newman-Keuls test (SigmaStat 3.0), n = number of studies, Abbreviations as in Table S3.

Table S5. Co-localization of AT_1R and Ubiquitin (Ub) with the proteasome marker $p44S^{10}$ in AT_1R/D_5R HEK 293 cells

Treat-ment	AT_1R over $p44S^{10}$(%)		Ub over AT_1R (%)		Ub over $p44S^{10}$ (%)	
	Mem	Cyto	Mem	Cyto	Mem	Cyto
Veh	NA	8.92±1.7 (n=6)	NA	14.46±2.6 (n=11)	NA	16.45±2.4 (n=7)
Fen	9.51±1.0 (n=6)	13.98±2.0 (n=6) P<0.05	35.35±3.2 (n=5)	17.31±3.3 (n=5) P>0.05	29.21±2.5 (n=6)	18.56±1.6 (n=6) P>0.05

Veh- or Fen-treated AT_1R/D_5R HEK 293 cells were randomly chosen. Images were thresholded to exclude background pixels. Co-localization was defined as the number of pixels of the AT_1R or Ub localized over the positive pixels of proteasome marker $p44S^{10}$ expressed as a percentage of the total pixels of proteasome marker. The co-localization of AT_1R and Ub was defined as the pixels of Ub over pixels of AT_1R expressed as a percent. Pixels containing Ub and $p44S^{10}$ at the membrane were far fewer than in the cytoplasm, and for this reason the co-localization in vehicle-treated cells was not analyzable (NA). Images were analyzed using MetaMorph 6.1 (Molecular Devices). Data are mean±SD. Significant differences of co-localization were analyzed using ANOVA, Student-Newman-Keuls test (SigmaStat 3.0). Mem, membrane; Cyto, cytoplasm; Fen, fenoldopam. Other abbreviations as in Tables S3 and S4.