

ONLINE SUPPLEMENT

Title: Relaxin attenuates organ fibrosis via an angiotensin-type 2 receptor mechanism in aged hypertensive female rats.

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equal contribution

Running title: RLX-AT₂R relevance in aged females

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Table S1. Effect of age and treatment on body and tissue weights recorded on day 28. Post-treatment body weight (BW) and heart and kidney weights in 6-month-old (6MO) and 15-month-old (15MO) female stroke prone-spontaneously hypertensive rats following 4-week treatment of vehicle (Veh; 20 mM sodium acetate s.c.), relaxin (RLX; serelaxin; 0.5 mg/kg/day s.c.) and relaxin+PD123319 (RLX+PD; AT₂R antagonist; 3 mg/kg/day s.c.).

	BW (g)	Total heart weight (g)	Heart:BW (g/100 g BW)	Total kidney weight (g)	Kidney:BW (g/ 100 g BW)
6MO					
Veh	219±5	1.14±0.04	0.52±0.01	0.94±0.03	0.43±0.01
RLX	229±4	1.24±0.03	0.54±0.01	1.00±0.02	0.44±0.01
RLX+PD	224±3	1.24±0.03	0.55±0.01	1.00±0.02	0.44±0.01
15MO					
Veh	259±13****	1.75±0.13****	0.69±0.06***	1.32±0.05****	0.52±0.04***
RLX	265±8	1.65±0.05	0.63±0.02	1.30±0.02	0.49±0.02
RLX+PD	278±7	1.73±0.05	0.62±0.01	1.35±0.05	0.49±0.01

Data are presented as mean ± SEM (n=6-10/group) and analyzed using a two-way ANOVA, followed by Sidak's post-hoc test to correct for multiple comparisons. ***P<0.001 and ****P<0.0001 as compared to 6MO Veh group.

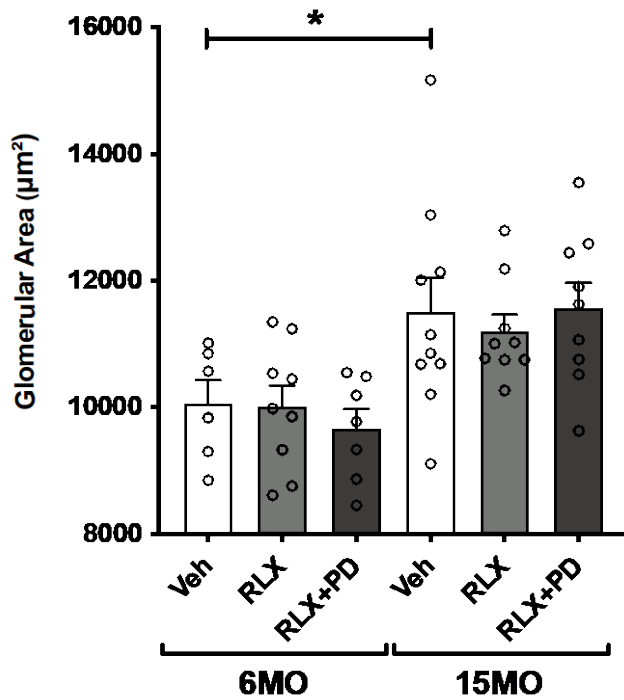


Figure S1. Glomerular area. Glomerular area (μm^2) in Masson's trichrome-stained kidney sections was measured in 6-month-old (6MO) and 15-month-old (15MO) female stroke prone-spontaneously hypertensive rats ($n=6-10/\text{group}$), after 4 weeks of vehicle (Veh; 20 mM sodium acetate s.c.), RLX (0.5 mg/kg/day s.c.) and RLX+PD123319 (AT_2R antagonist; 3 mg/kg/day s.c.) treatment. Glomerular area was quantified by tracing 30 glomerular borders per animal, when the vascular pole was evident, and measurements averaged. Data are presented as mean \pm SEM ($n=6-10/\text{group}$) and were analyzed using a Mann-Whitney test. * $P<0.05$.

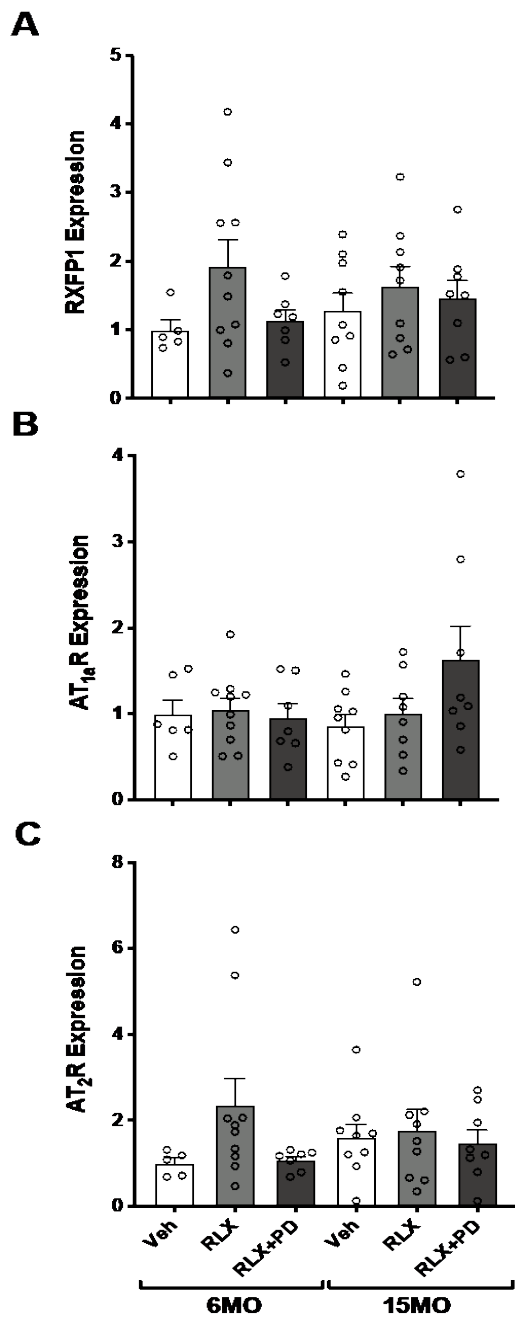


Figure S2. Relative renal gene expression of RXFP1 and angiotensin receptors. The relative renal mRNA expression of **(A)** RXFP1 **(B)** AT_{1a}R and **(C)** AT₂R in 6-month-old (6MO) and 15-month-old (15MO) female stroke-prone spontaneously hypertensive rats following 4-week treatment of vehicle (Veh; 20 mM sodium acetate s.c.), relaxin (RLX; serelaxin; 0.5 mg/kg/day s.c.) and relaxin+PD123319 (RLX+PD; AT₂R antagonist; 3 mg/kg/day s.c.). Data are presented as mean ± SEM and are expressed relative to the 6MO vehicle-treated group (n=5-10/group). 18S rRNA was used as the internal housekeeping gene. Data were analyzed using a two-way ANOVA with factors age, treatment and their interaction, followed by Sidak's multiple comparisons test.