

1 Supplemental Figure Legends

2 **Suppl. Fig. 1.** Tissue distribution of riGnRHRs. Total RNAs from various tissues were subjected to
3 RT-PCR analysis. For the negative control, cDNA was replaced by distilled water. M, 100-bp DNA
4 size markers; Ad, adrenal gland; CB, cerebellum; CTL, control; DL, distal lobe of the pituitary;
5 GAPDH, glyceraldehyde 3-phosphate dehydrogenase; HYP, hypothalamus; I/NP, neurointermediate
6 lobe of the pituitary; Kd, kidney; Liv, liver; Lu, lung; MC, mesencephalon; OL, olfactory lobe; TC,
7 telencephalon; RC, rhombencephalon; SC, spinal cord; Tes, testis.

8 **Suppl. Fig. 2.** Labeling of consecutive frontal sections through the anterior preoptic area (Poa)
9 illustrating the specificity control of the GnRH1 immunoreaction. **A-C:** Adjacent sections were
10 incubated with the GnRH1 antibody (A) or with the antiserum preincubated with 10^{-5} M synthetic
11 GnRH1 (B) or 10^{-5} M GnRH2 (C). X300. III, third ventricle.

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SUPPLEMENTAL TABLE 1.

Sequence of the specific primers used for the amplification of the cDNAs encoding GnRHR in *Pelophylax ridibundus* and for the RT-PCR.

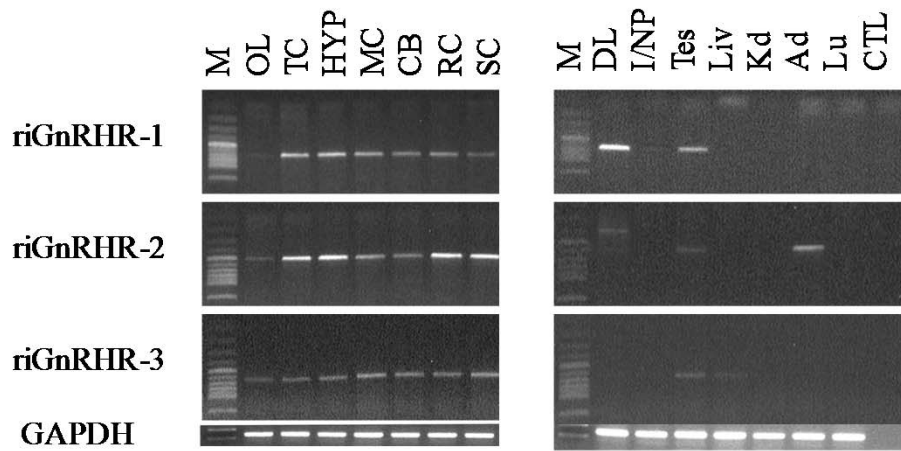
Sequence of the specific primers used for the amplification of the cDNAs encoding GnRHR in <i>Pelophylax ridibundus</i>	
FR-1F	(5'-CGCG <u>AATTCGCCACCA</u> TGAATATCTCAAAGGAAGTTAGCAT-3')
FR-1R	(5'-CGCCTCTAGATTCATATTATACACATCTGTAATTGACT-3')
FR-2F	(5'-CGCG <u>AATTCGCCACCA</u> TGCAGCCAGCGATCGTAAATCGAAG-3')
FR-2R	(5'-CGCCTCTAGATTCAAAAGACAGACTGTACTGTGGTGGCC-3')
FR-3F	(5'-CGCG <u>AATTCGCCACCA</u> TGAACGCTAGTGACCAACCTATGG-3')
FR-3R	(5'-CGCCTCTAGATTCACATAAAGCTCTCTACGATTTGAC-3')
Sequence of the specific primers used for the RT-PCR	
3'-GSP (I)	5'-CCAAGCGCATGAGCAAAGGAACACTTTC-3' and FR-1R
3'-NGSP (II)	5'-GCACCCTATTTGTCACTCCGCTAGGCGTCA-3' and FR-2R
3'-GSP (III)	5'-TGTTTGTGTTCCACACGGTGAGCCGGTC-3' and FR-3R

The FR-1F, FR-2F and FR-3F primers incorporate an *EcoRI* restriction enzyme site (*underlined*) and a Kozak translation initiation site (*italics*). The FR-1R, FR-2R and FR-3R primers incorporate an *XbaI* site (*underlined*).

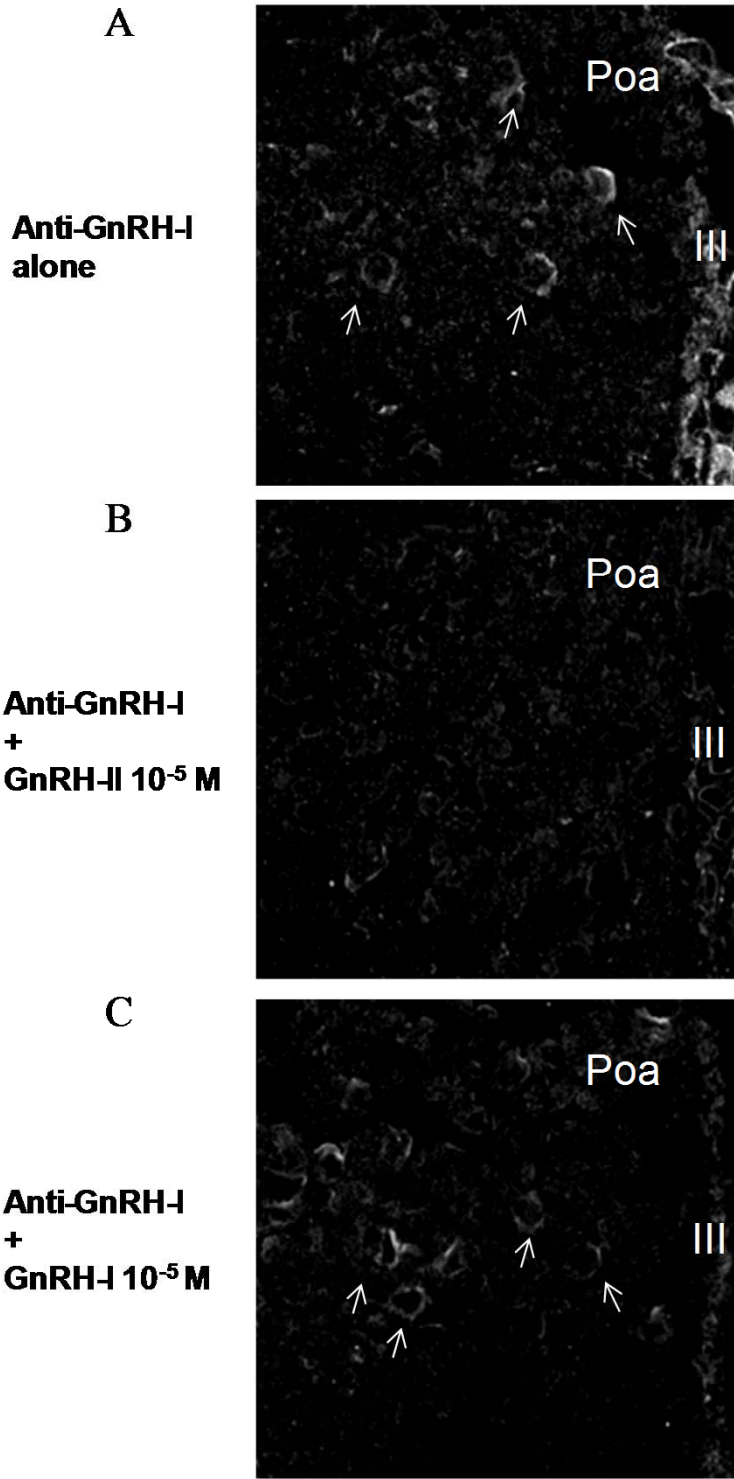
SUPPLEMENTAL TABLE 2.Log EC₅₀ and E_{max} values of riGnRHRs for GnRH1 and GnRH2

		GnRH1		GnRH2	
		Log EC ₅₀	E _{max}	Log EC ₅₀	E _{max}
c-fos	riGnRHR-1	-6.99 ± 0.10	14.4 ± 0.55	-8.13 ± 0.08	13.0 ± 0.30
	riGnRHR-2	-6.61 ± 0.05	58.0 ± 1.32	-9.26 ± 0.21	69.6 ± 3.59
	riGnRHR-3	-6.24 ± 0.03	42.5 ± 0.73	-8.11 ± 0.08	40.1 ± 0.97
CRE	riGnRHR-1	-7.39 ± 0.13	4.33 ± 0.18	-7.55 ± 0.09	7.04 ± 0.23
	riGnRHR-2	-6.74 ± 0.08	46.4 ± 1.47	-9.63 ± 0.08	53.8 ± 1.06
	riGnRHR-3	-6.68 ± 0.15	16.6 ± 1.03	-8.28 ± 0.16	16.8 ± 0.68

CV-1 cells were cotransfected with individual riGnRHR together with the CRE- or c-fos-luc reporter. Luciferase activity was determined after GnRH stimulation for 6 h. Data are the mean ± SEM of three independent experiments. Log EC₅₀ and E_{max} values were obtained from the dose-response curves of each receptor for ligand using the PRISM2 program.



Supplemental Figure 1
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Supplemental Figure 2
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