

Supplementary Material: Antagonists of Growth Hormone-Releasing Hormone Inhibit the Growth of Pituitary Adenoma Cells by Hampering Oncogenic Pathways and Promoting Apoptotic Signaling

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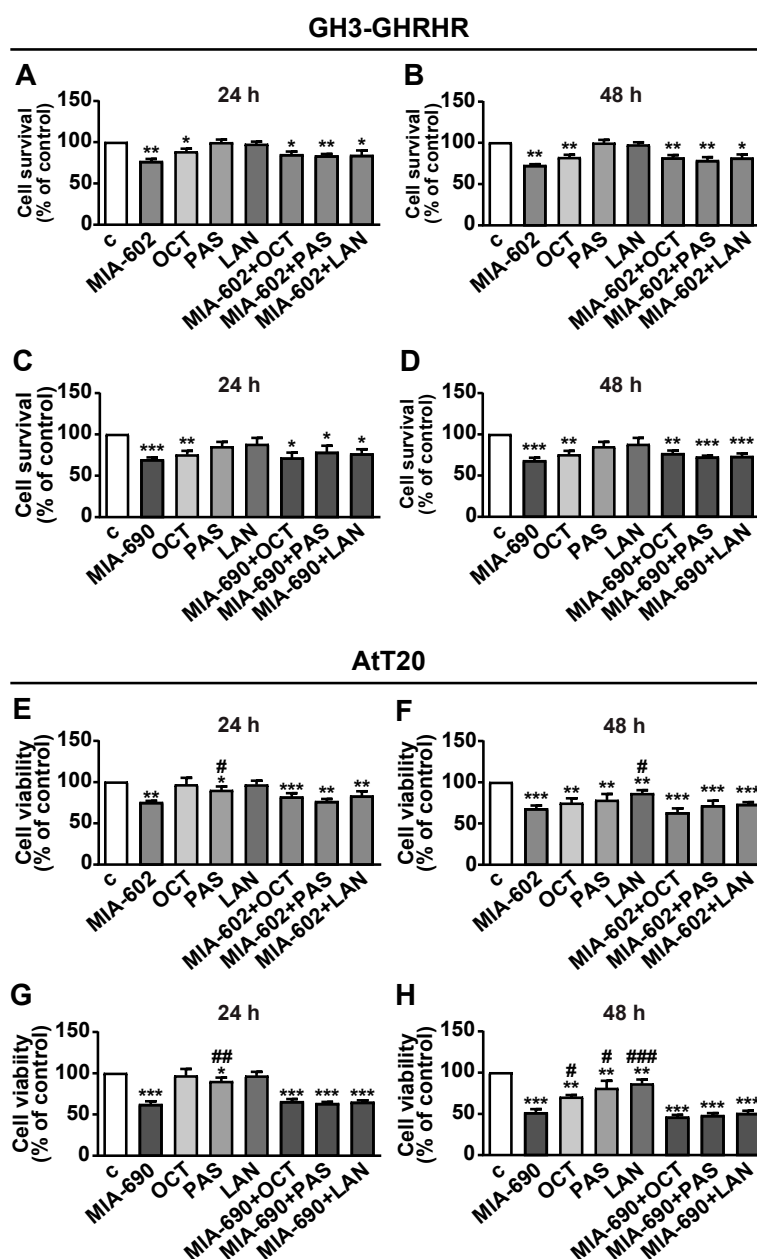


Figure S1. Inhibitory effects of GHRH antagonists in combination with somatostatin analogs in GH3-GHRHR and AtT20 cells. Cell viability assessed by MTT in GH3-GHRHR cells cultured in serum-deprived medium (c, control) for 24 h or 48 h with 1 μ M MIA-602 (A and B) or 1 μ M MIA-690

(C and D), and with either 0.1 μ M octreotide (OCT), 0.1 μ M pasireotide (PAS) and 0.1 μ M lanreotide (LAN). Results, expressed as percent of control are means \pm SEM of three replicates. $*p < 0.05$, $**p < 0.01$ and $***p < 0.001$ vs. c by unpaired Student's t-test ($n = 3$). Cell viability assessed by MTT in AtT20 cells cultured in serum-deprived medium (c, control) for 24 h or 48 h with 1 μ M MIA-602 (E and F) or 1 μ M MIA-690 (G and H), and with either 0.1 μ M octreotide (OCT), 0.1 μ M pasireotide (PAS) and 0.1 μ M lanreotide (LAN). Results, expressed as percent of control are means \pm SEM of three replicates. $*p < 0.05$, $**p < 0.01$ and $***p < 0.001$ vs. c; $*p < 0.05$, $**p < 0.01$ and $***p < 0.001$ vs. MIA-602 or MIA-690 by unpaired Student's t-test ($n = 3$).

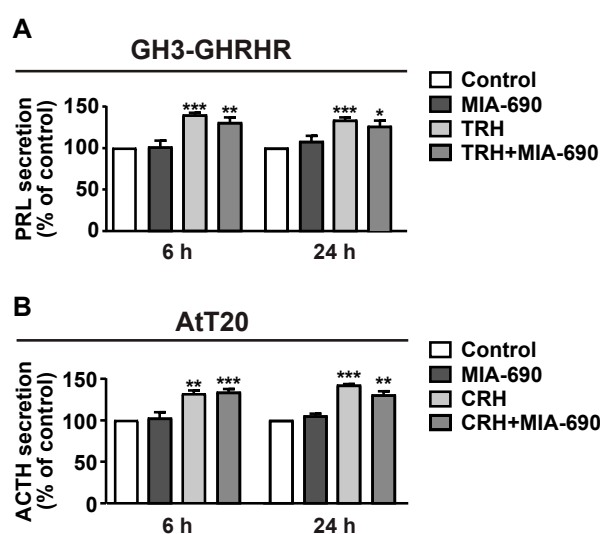


Figure S2. Effect of MIA-690 on PRL and ACTH secretion in PA cell lines. GH3-GHRHR (A) and AtT20 cells (B) were cultured in medium with 1% serum (c, control) and treated with 0.1 μ M CRH or 0.1 μ M TRH, respectively, in either absence or presence of 0.1 μ M MIA-690 for the times indicated. Secretion of PRL and ACTH was measured by ELISA. Results, expressed as percent of control, are means \pm SEM. $*p < 0.05$, $**p < 0.01$, $***p < 0.001$ vs. c by unpaired Student's t-test ($n = 3$).

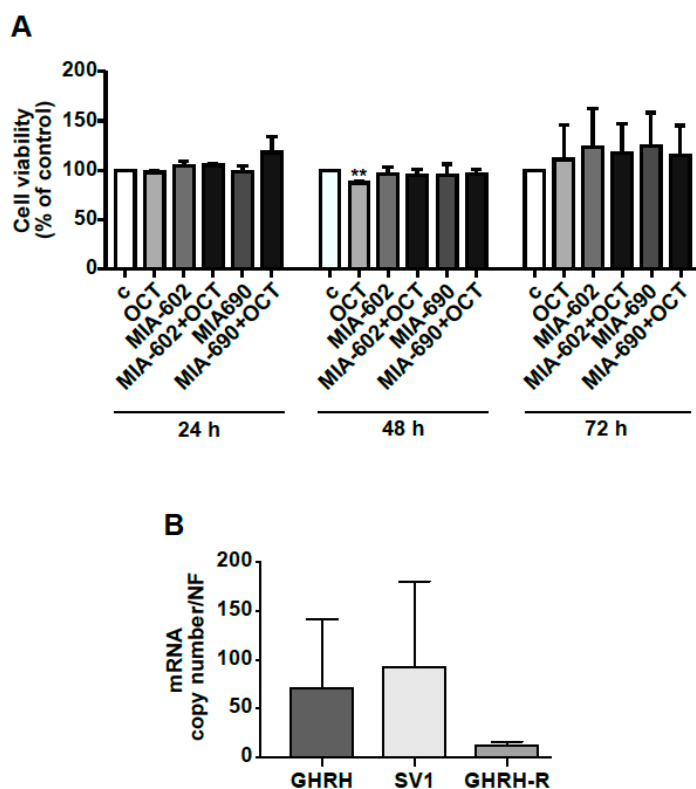


Figure S3. Effects of GHRH antagonists in NFPAs. **(A)** Cell viability, assessed by Alamar blue assay, in human primary cells derived from NFPAs. The cells were either untreated (c, control) or treated with 1 μ M MIA-602 or 1 μ M MIA-690, both alone or in combination with octreotide (OCT) for the times indicated. Results, expressed as percent of control, are means \pm SEM of three replicates. ** $p < 0.01$ vs. c by unpaired Student's t-test ($n = 3$). **(B)** Expression profile of GHRH, SV1 and GHRH-R in NFPAs cell cultures assessed by real-time PCR. Results represent absolute mRNA copy number adjusted by a normalization factor (NF), calculated from the expression levels of three control genes (GAPDH, ACTB and HPRT) and are means \pm SEM ($n = 2$).

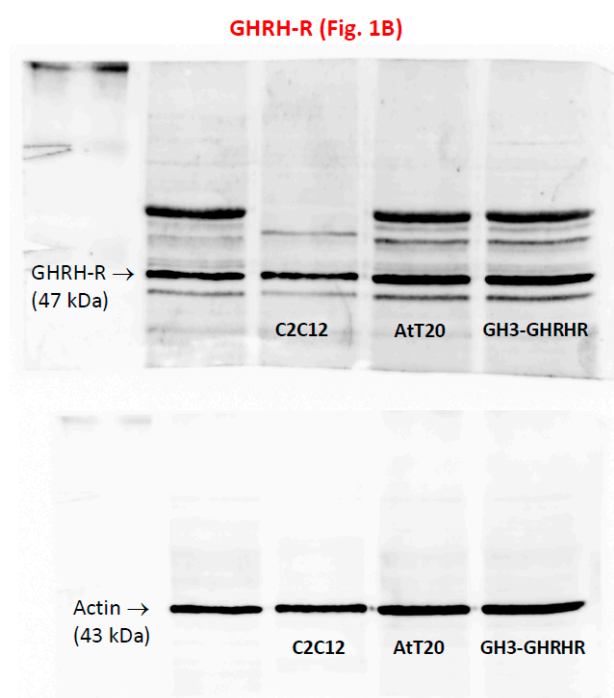


Figure S4. Uncropped Figure 1B.

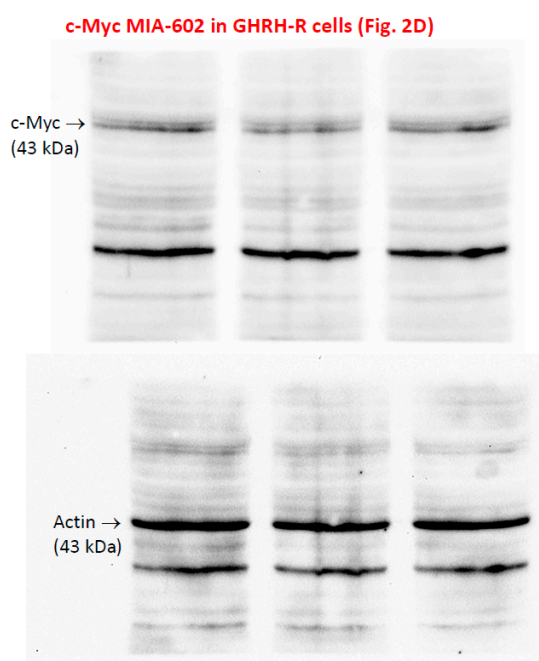


Figure S5. Uncropped Figure 2D.

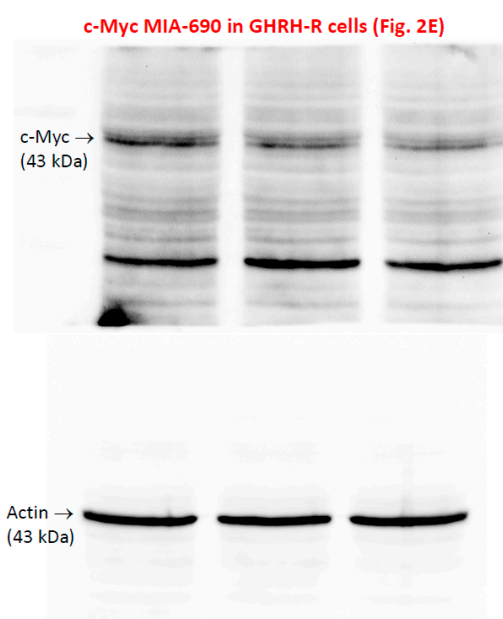


Figure S6. Uncropped Figure 2E.

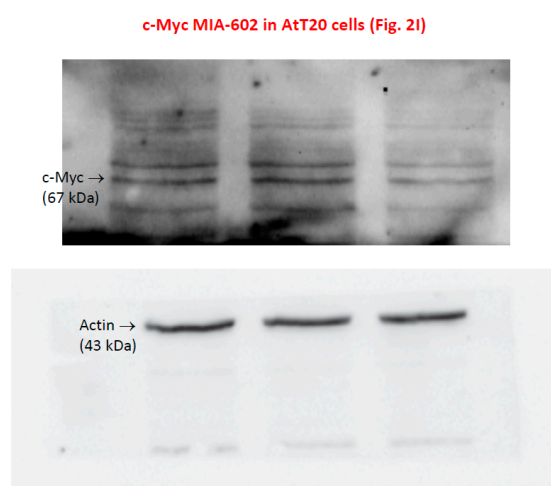


Figure S7. Uncropped Figure 2I.

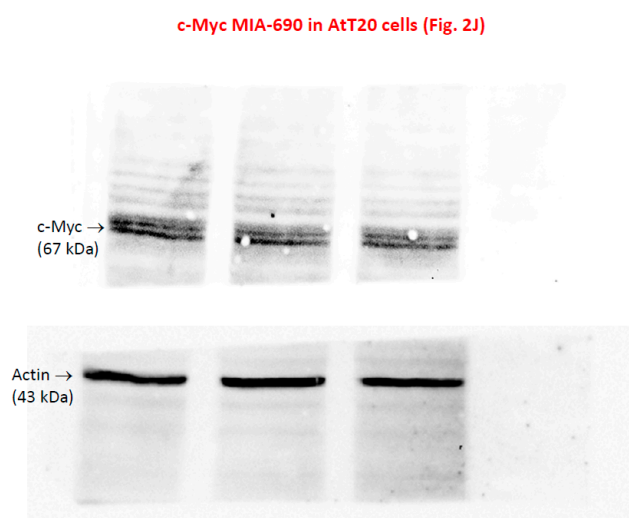


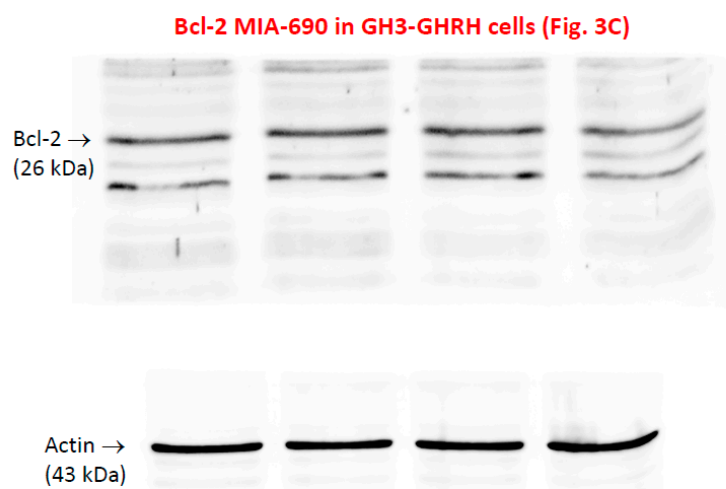
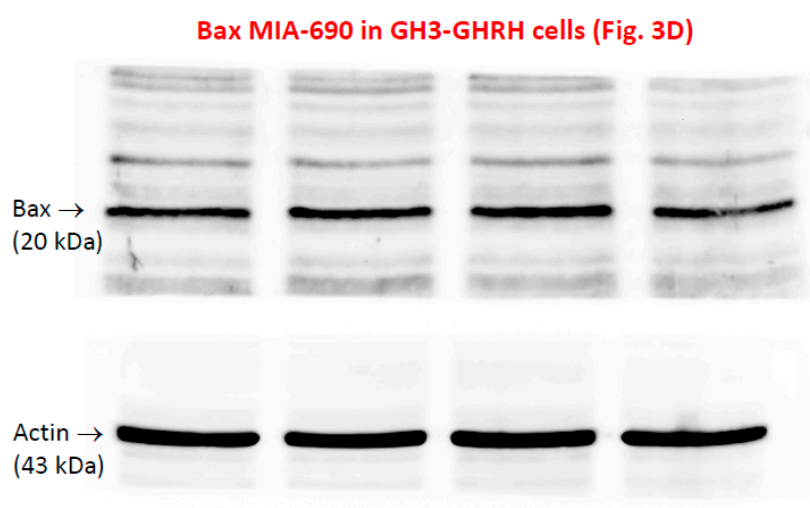
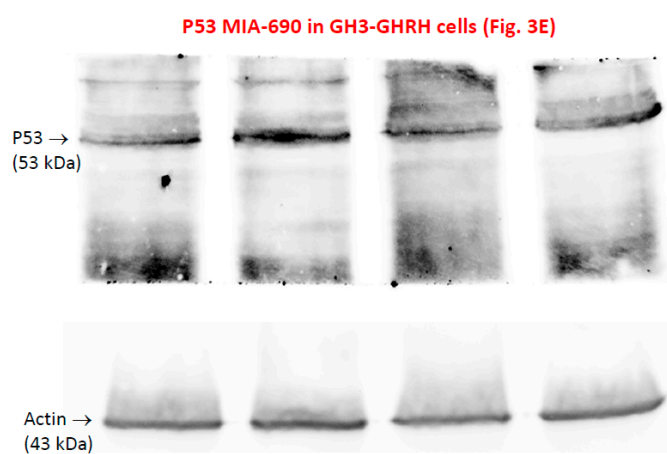
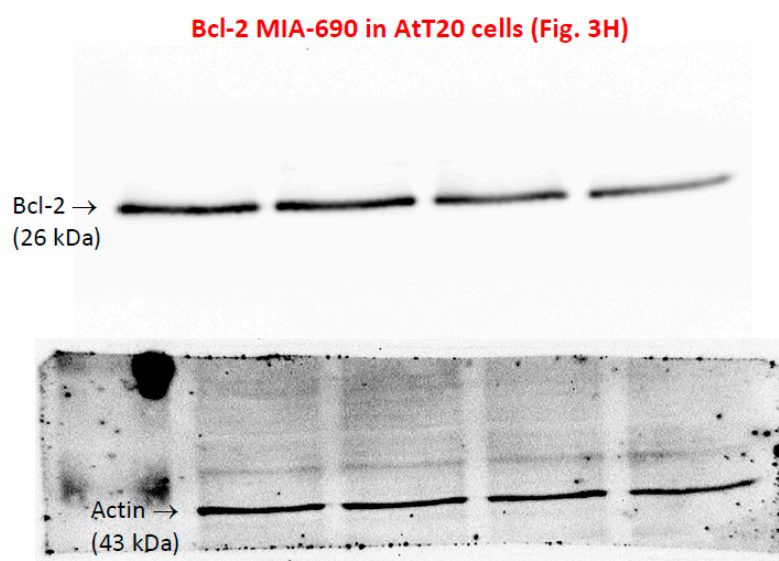
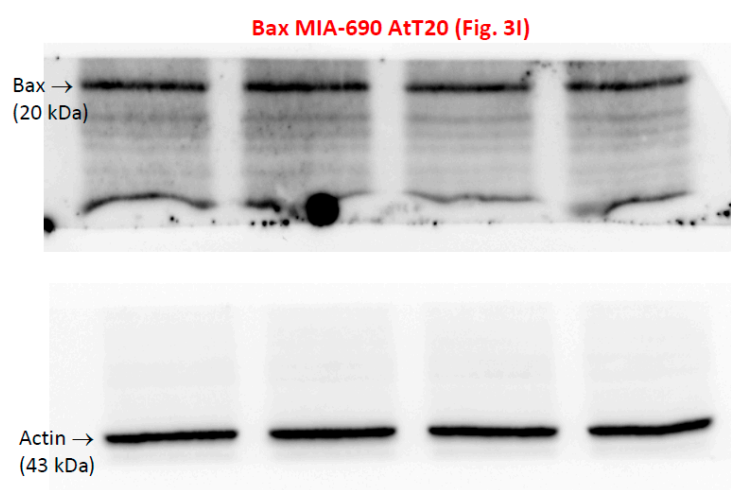
Figure S8. Uncropped Figure 2J.**Figure S9.** Uncropped Figure 3C.**Figure S10.** Uncropped Figure 3D.

Figure S11. Uncropped Figure 3E.**Figure S12.** Uncropped Figure 3H.**Figure S13.** Uncropped Figure 3I.

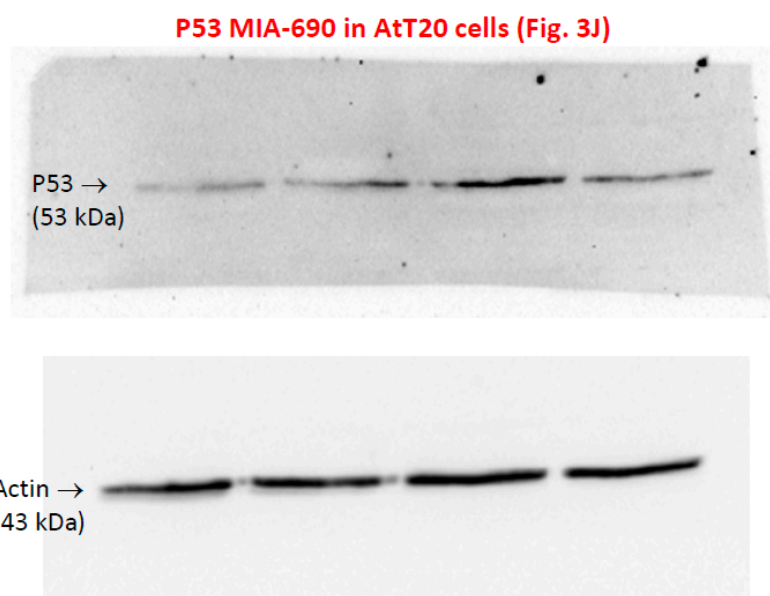


Figure S14. Uncropped Figure 3J.

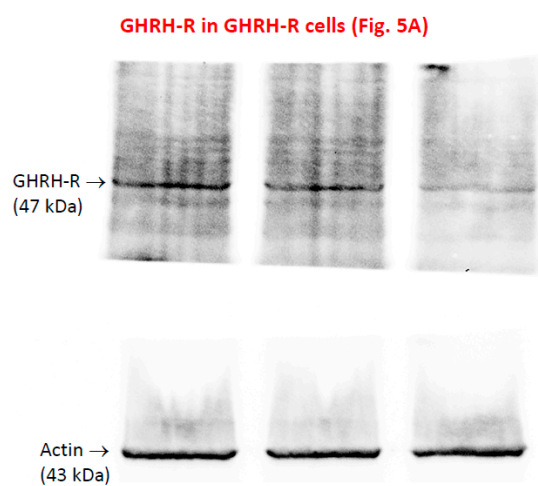


Figure S15. Uncropped Figure 5A.

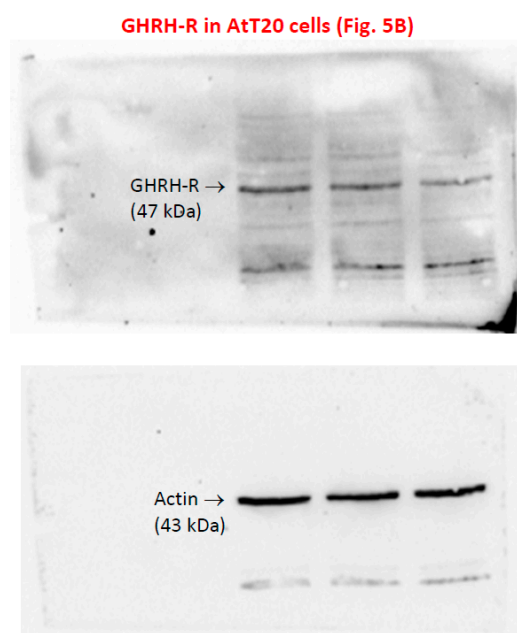


Figure S16. Uncropped Figure 5B.