Supplemental Figure 1. Comparison of GH immunoreactivities (wt-GH and GH Zn mutant) in DSL-GH ELISA.

(A) Concentrated stock of wt-GH or GH Zn mutant produced in CHO cells was further diluted in complete medium, Zn^{2+}-depleted medium or Zn^{2+}-enriched medium (with increasing amounts of Zn^{2+}) up to approx. the same concentration and dilutions were again re-measured by DSL-GH ELISA. The results are given as the means ± SD of three independent experiments.

(B) A series of dilutions of wt-GH or GH Zn mutant was made up to the concentrations of 50, 100, 250, 500, 1000, 3000, 6000 ng/ml in complete medium, Zn^{2+}-depleted medium or Zn^{2+}-enriched medium and measured by DSL-GH ELISA. The results are given as the means ± SD of three independent experiments.
Supplemental Figure 2. The capacity of wt-GH and GH Zn mutant to induce Jak2/Stat5 signaling in 293GHR cells.

wt-GH and GH Zn mutant were produced in CHO cells after transient transfection and 3 days of culturing in serum free Ham’s F12 medium. Then both GH variants were concentrated from culture medium and their concentration was measured by the DSL-hGH ELISA. 293HEK cells stably expressing the hGHR (293GHR cells) were used to assay signal transduction activities (Jak2/Stat5 activation) of the wt-GH and GH Zn mutant as previously described (Von Laue et al. J Endocrinol 2000; 165; 301-311, Ross et al. Mol Endocrinol 1997; 11; 265-273). Briefly, cells were transfected with a Stat5-responsive luciferase reporter gene construct and treated with increasing amounts of GH (wt-GH or GH Zn mutant) for 6 hours. Luciferase expression was then measured by the dual luciferase reporter assay (Promega) on a luminometer (Mediators PhL, Aureon Biosystems, Vienna, Austria). Results are expressed as a fold induction relative to the basic activity of unstimulated cells (SM).