<u>Supplement to</u>: Sex-specific early growth hormone response genes in rat liver

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<u>Legends for Supplementary Figures and Supplementary Tables</u>

<u>Figure S1. Confirmatory qPCR analysis of *Igfbp1*, *Cyp2a2* and *Adh4* hnRNA</u>. qPCR assays using independent sets of hnRNA-specific primer pairs (see Table S1) were carried out for the three genes for which there was a clear indication in Fig. 2 of a discrepancy between RNA and hnRNA levels, in particular at the 30 min GH time point. qPCR analysis for Igfbp1, Adh4 and Cyp2a2 hnRNAs was carried out using the liver RNA samples described in Fig. 1, with data presentation and statistical analysis as described in Fig. 1.

<u>Figures S2, S3 and S4. qPCR analysis of six class I male genes, three class II male genes and two class I female genes.</u> Liver RNA samples described in Fig. 1 were assayed for the indicated mRNAs and hnRNAs by qPCR using primers shown in Table S1. Data presentation and statistical analysis were as described in Fig. 1.

<u>Table S1. Rat qPCR Primer Sets and GenBank Accession Numbers</u>. qPCR primer pairs were designed for the indicated mRNAs and hnRNAs, as described in "Materials and Methods." Nucleotide numbering indicating the position of the resultant PCR amplicon based on the indicated GenBank accession numbers. Primers are listed in order of their use in Figures 1, 2, S1, S2, S3 and S4.

<u>Table S2</u>. Listing of all 4150 genes of interest and subsets thereof. Table S2A, listing of 4150 genes. Included are expression ratios and raw intensity values for all 6 microarray experiments and full annotations, including Agilent probe numbers and Agilent sequence IDs, accession numbers, gene symbol and descriptions, Unigene IDs and TFS numbers. Sex-specific genes that respond to hypophysectomy are further designated according to the major gene classes delineated in Table 3; Table S2B, 471 redundant probes (defined

below), which were excluded from all subsequent analyses; Table S2C 100 top male-specific genes (M/F > ~10.1); Table S2D, 100 top female-specific genes (F/M > ~6.5); Table S2E, 100 top GH-inducible early response genes (induced at both GH time points); Table S2F, 100 top GH-suppressible early response genes (suppressed at both GH time points). The Probe Redundancy column specifies the following: Unique, indicates there are no redundant probes for the gene/transcript; Best and Diff(erent) indicate that the gene/transcript has two or more probes on the array. For those probes whose expression data indicates they are redundant, i.e., probes that fall into the same TFS group, the probe that gives the lowest *p*-value for a majority of the six microarray experiments is marked 'Best probe' and is included in the list of 4150 genes of interest. The other probes are marked Redundant and are shown on a separate Excel sheet. When two (or more) probes map to the same transcript and give different TFS numbers, both probes are retained in the 4150 genes of interest and are marked 'Diff TFS'. For some probes, no rat accession number was available from the USCS Rat Genome Browser (Nov. 2004 rat genome release), in which case the annotations shown are based on the non-rat accession numbers displayed. In some of the cases where no rat chromosomal position was available, annotations are based on a homologous mouse sequence, as identified by BLAT analysis (annotation information shown in yellow). Experimental details are provided on the first sheet.

Table S3 and Table S4. Distribution of the major groups of regulated genes within flag-based coexpression groups - Genes are grouped based on their responses to all 6 microarray experiments using the total flagging sum (TFS) system (see *Materials and Methods*). Table S3A shows major male-specific groups, Table S3B major female-specific groups (groups with ≥ 7 genes/group) and Table S3C major sex-independent groups (groups with ≥ 15 genes/group). A complete listing of TFS groupings for all 4376 genes of interest (see Table S2) is shown in Table S4.

Table S5. Effect of short term-GH treatment of Hypox male rats on pituitary-dependent genes - 2494 genes that were either up-regulated or down-regulated after hypophysectomy in males only, in females only, or in both males and females are sorted

by sex specificity and by their responses to short-term GH treatment in Hypox males. *, Genes responding to GH include genes up- or down-regulated either after one or two GH injections in Hypox males. **, Genes not responding to GH includes genes that did not respond to either GH injection, as well as genes that responded to the first GH injection but not the second GH injection, and genes that responded to both GH injections but in opposite directions (up regulation by first GH and down regulation by second, or *vice versa*).

Table S6. Effect of short term GH treatment in Hypox males - (A) Effect of short term GH treatment in Hypox males on positive pituitary-regulated genes (class I). The 1379 genes down-regulated in Hypox males only, in Hypox females only or in both Hypox males and Hypox females were sorted initially by sex specificity and secondarily by response to short term GH treatment. (B) Effect of short term GH treatment in Hypox males on negative pituitary-regulated genes (class II). The 1115 genes up-regulated in Hypox males only, in Hypox females only or in both Hypox males and Hypox females were sorted initially by sex specificity and secondarily by response to short term GH treatment. *, genes up- or down-regulated after either one or two GH injections given to Hypox males and/or females, as indicated. **, genes that did not respond to either one or two GH injections, and genes that responded to GH in an inconsistent manner, i.e., responded to the first GH injection but not the second GH injection and genes responding in two different directions to the first and second GH injection when given to Hypox male rats.

Table S7 and S8. Listing of class I sex-specific genes induced by short-term GH treatment (Table S7) and class II sex-specific genes repressed by short-term GH treatment (Table S8) – Genes in each class are subclassified as indicated in Table 3. Expression ratios shown in bold meet the fold-change and p-value thresholds specified in Materials and Methods. RefSeq/Genbank IDs and Unigene IDs shown in yellow are for homologous sequences from species other than rat, where no corresponding annotations were available for the rat, while IDs shown in blue are Agilent reporter (probe) names, in cases where no accession number is available. Several of the groups of GH-responsive genes

shown are comprised of genes from more than one TFS group. N/A, annotation information is not available.

Table S9. Listing of class I and class II sex-specific genes responsive to short-term GH treatment – Expression ratios and detailed annotation is provided for the genes listed in Tables S7 and S8.

Table S10. Listing of regulated DNA-binding proteins/transcription factors – Listed are 185 genes from the list of 4150 genes of interest (Table S2) that are identified as DNA-binding proteins by their GO Molecular Function annotation. Included are: 24 DNA-binding proteins that correspond to early GH-response genes, of which 15 were up regulated and 9 were down regulated at both GH time points; and 70 other DNA-binding proteins that were either induced (39 genes) or suppressed (31 genes) at the second GH time point only. GO annotations corresponding to 'DNA-binding', 'Transcription factor activity' and 'Regulation of transcription' were as indicated for each gene. Other annotations and other information are as described in Table S2.

Table S11. Enrichment of GO biological processes, GO molecular functions and KEGG pathways amongst class I male- and female-specific genes (Table S11A), the class II male- and female-specific genes (Table S11B), and the early GH response genes (Table S11C). Shown is the number of genes in each group that is associated with each GO term or KEGG pathway (gene count), the total number of gene in the species associated with that category (pop hits), along with the associated *p*-value. The fold-change value is derived from a comparison of the representation of each category in the list of genes in each group to its representation in the overall population of genes in the DAVID database used to carry out these analyses (see Materials and Methods). Also shown are the DAVID gene numbers and the RefSeq IDs for the genes in each enriched category. GO Biological Processes (BP), GO Molecular Functions (MF), and KEGG pathways are listed separately in each section of the table. Table S11D lists the accession numbers submitted for DAVID analysis.