## SupplementaryTables

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
|  |  | **WT** | | | **WT-TG** | | |
| *Cell* | *Subgroup* | *Mean* | *SD* | *n* | *Mean* | *SD* | *n* |
| GH+ | **Total proportion** | **0.290** | **0.023** | 7 | **0.294** | **0.020** | 7 |
| DLK1-/GH+ | 0.019 | 0.003 | 0.014 | 0.002 |
| DLK1+/GH+ | 0.271 | 0.008 | 0.280 | 0.007 |
| *% co-stained DLK1* | *93* |  | *95* |  |
| PRL+ | **Total proportion** | **0.213** | **0.012** | 4 | **0.201** | **0.018** | 4 |
| DLK1-/PRL+ | 0.135 | 0.003 | 0.130 | 0.011 |
| DLK1+/PRL+ | 0.078 | 0.004 | 0.072 | 0.002 |
| *% co-stained DLK1* | *37* |  | *36* |  |
| TSH+ | **Total proportion** | **0.096** | **0.011** | 4 | **0.091** | **0.010** | 4 |
| TSH+ | 0.057 | 0.004 | 0.056 | 0.004 |
| DLK1+/TSH+ | 0.038 | 0.002 | 0.035 | 0.002 |
| *% co-stained DLK1* | *40* |  | *38* |  |
| FSH+ | **Total proportion** | **0.096** | **0.013** | 2 | **0.092** | **0.000** | 2 |
| FSH +ve | 0.077 | 0.011 | 0.074 | 0.001 |
| DLK1+/FSH+ | 0.018 | 0.002 | 0.018 | 0.002 |
| *% co-stained DLK1* | *19* |  | *20* |  |
| ACTH+ | **Total proportion** | **0.130** | **0.010** | 4 | **0.109** | **0.016** | 4 |
| ACTH+ | 0.129 | 0.005 | 0.109 | 0.008 |
| DLK1+/ACTH+ | 0.000 | 0.000 | 0.000 | 0.000 |
| *% co-stained DLK1* | *0* |  | *0* |  |
|  | **Unclassified** | **0.177** |  |  | **0.213** |  |  |

**Supplementary File 1a**. Proportions of AP cells labelled with hormonal markers in WT and WT-TG female animals at 12 weeks of age.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
|  | **Genotype** | **Total body mass (g)** | | |  |  |
|  | *Mean* | *SD* | *n* | *p vs WT* | *p vs PAT* |
| **E11.5** | WT | 0.0464 | 0.0043 | 11 |  |  |
| WT-TG | 0.0458 | 0.0043 | 11 | ns |  |
| PAT | 0.0444 | 0.0043 | 8 | ns |  |
| PAT-TG | 0.0433 | 0.0081 | 7 | ns | ns |
| **E13.5** | WT | 0.1447 | 0.0136 | 7 |  |  |
| WT-TG | 0.1381 | 0.0157 | 17 | ns |  |
| PAT | 0.1468 | 0.0111 | 16 | ns |  |
| PAT-TG | 0.1464 | 0.0181 | 10 | ns | ns |
| **E18.5** | WT | 1.2062 | 0.1311 | 24 |  |  |
| WT-TG | 1.2110 | 0.1060 | 32 | ns |  |
| PAT | 1.0205 | 0.0711 | 15 | <0.0001 |  |
| PAT-TG | 1.1107 | 0.0808 | 20 | 0.0125 | 0.0513 |
| **P7** | WT | 4.0329 | 0.2682 | 7 |  |  |
| WT-TG | 3.8280 | 0.2197 | 5 | ns |  |
| PAT | 3.0933 | 0.2542 | 9 | 0.0003 |  |
| PAT-TG | 3.7730 | 0.5926 | 10 | ns | 0.0038 |
| **P14** | WT | 7.8124 | 0.6887 | 17 |  |  |
| WT-TG | 8.6843 | 0.6171 | 7 | 0.0161 |  |
| PAT | 5.6000 | 0.6420 | 8 | <0.0001 |  |
| PAT-TG | 6.7640 | 0.2463 | 5 | 0.0097 | 0.0105 |
| **P21** | WT | 10.7120 | 1.6084 | 10 |  |  |
| WT-TG | 11.0489 | 1.1016 | 9 | ns |  |
| PAT | 8.8117 | 1.0644 | 6 | 0.0129 | ns |
| PAT-TG | 10.1354 | 0.7752 | 13 | ns |  |

**Supplementary File 1b**. Total body mass of animals from matched litters sacrificed from E11.5 to P21. Individuals in each age group were compared by One-Way ANOVA with post-hoc pairwise testing WT vs WT-TG, PAT, PAT-TG and PAT vs PAT-TG, corrected for multiple comparisons using Bonferroni's adjustment.

|  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | **Genotype** | **AP Volume (mm^3)** | | | | **PP Volume (mm^3)** | | | |  |
|  | *Mean* | *SD* | *p vs WT* | *p vs PAT* | *Mean* | *SD* | *p vs WT* | *p vs PAT* | *n* |
| **E13.5** | WT | 0.0269 | 0.0056 |  |  | 0.0022 | 0.0005 |  |  | 9 |
| WT-TG | 0.0255 | 0.0043 | ns |  | 0.0023 | 0.0004 | ns |  | 11 |
| PAT | 0.0164 | 0.0011 | <0.0001 |  | 0.0019 | 0.0002 | ns |  | 6 |
| PAT-TG | 0.0172 | 0.0022 | <0.0001 | ns | 0.0021 | 0.0022 | ns | ns | 8 |
| **E18.5** | WT | 0.0600 | 0.0084 |  |  | 0.0092 | 0.0006 |  |  | 7 |
| WT-TG | 0.0664 | 0.0059 | ns |  | 0.0096 | 0.0006 | ns |  | 5 |
| PAT | 0.0444 | 0.0094 | 0.0025 |  | 0.0102 | 0.0016 | ns |  | 7 |
| PAT-TG | 0.0479 | 0.0044 | 0.1670 | ns | 0.0097 | 0.0020 | ns | ns | 8 |

**Supplementary File 1c.** Pituitary volumes acquired by stereological estimation in the embryo. Data from males and females is combined.

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | **Genotype** | **AL Volume (mm^3)** | | | | **IL Volume (mm^3)** | | | | **PL Volume (mm^3)** | | | |  |
|  | *Mean* | *SD* | *p vs WT* | *p vs PAT* | *Mean* | *SD* | *p vs WT* | *p vs PAT* | *Mean* | *SD* | *p vs WT* | *p vs PAT* | *n* |
| **P7** | WT | 0.0783 | 0.0069 |  |  | 0.0169 | 0.0020 |  |  | 0.0159 | 0.0022 |  |  | 5 |
| WT-TG | 0.0817 | 0.0065 | ns |  | 0.0157 | 0.0019 | ns |  | 0.0165 | 0.0024 | ns |  | 4 |
| PAT | 0.0406 | 0.0070 | <0.0001 |  | 0.0110 | 0.0020 | 0.0014 |  | 0.0138 | 0.0036 | ns |  | 8 |
| PAT-TG | 0.0565 | 0.0097 | 0.0010 | 0.0074 | 0.0143 | 0.0034 | ns | ns | 0.0157 | 0.0030 | ns | ns | 5 |
| **P14** | WT | 0.1601 | 0.0286 |  |  | 0.0221 | 0.0029 |  |  | 0.0244 | 0.0031 |  |  | 6 |
| WT-TG | 0.1795 | 0.0306 | ns |  | 0.0252 | 0.0034 | ns |  | 0.0249 | 0.0044 | ns |  | 7 |
| PAT | 0.0857 | 0.0159 | 0.0007 |  | 0.0229 | 0.0018 | ns |  | 0.0168 | 0.0010 | ns |  | 5 |
| PAT-TG | 0.0894 | 0.0165 | 0.0046 | ns | 0.0225 | 0.0025 | ns | ns | 0.0196 | 0.0041 | ns | ns | 3 |
| **P21** | WT | 0.1972 | 0.0252 |  |  | 0.0361 | 0.0036 |  |  | 0.0317 | 0.0047 |  |  | 6 |
| WT-TG | 0.2617 | 0.0494 | 0.0105 |  | 0.0359 | 0.0076 | 0.0268 |  | 0.0348 | 0.0044 | ns |  | 8 |
| PAT | 0.1542 | 0.0043 | ns |  | 0.0301 | 0.0089 | ns |  | 0.0376 | 0.0088 | ns |  | 3 |
| PAT-TG | 0.1549 | 0.0257 | ns | ns | 0.0234 | 0.0030 | ns | ns | 0.0251 | 0.0030 | ns | ns | 7 |

**Supplementary File 1d**. Pituitary volumes acquired by stereological estimation in of the intact postnatal gland. Individuals in each age group were compared by One-Way ANOVA with post-hoc pairwise testing WT vs WT-TG, PAT, PAT-TG and PAT vs PAT-TG, corrected for multiple comparisons using Bonferroni's adjustment. Data from males and females is combined.

|  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | **Genotype** | **AL Volume (mm^3)** | | | **IL Volume (mm^3)** | | | **PL Volume (mm^3)** | | |  |
|  | *Mean* | *SD* | *p vs WT* | *Mean* | *SD* | *p vs WT* | *Mean* | *SD* | *p vs WT* | *n* |
| **Males** | WT | 0.570 | 0.081 |  | 0.066 | 0.011 |  | 0.052 | 0.008 |  | 4 |
| WT-TG | 0.742 | 0.040 | 0.0126 | 0.080 | 0.014 | 0.4595 | 0.061 | 0.006 | 0.2376 | 3 |
| PAT | 0.407 | 0.031 | 0.0195 | 0.046 | 0.007 | 0.1942 | 0.050 | 0.008 | 0.9266 | 3 |
| PAT-TG | 0.555 | 0.073 | 0.9789 | 0.067 | 0.021 | 0.9972 | 0.059 | 0.007 | 0.2636 | 6 |
| **Females** | WT | 0.582 | 0.060 |  | 0.059 | 0.019 |  | 0.050 | 0.003 |  | 3 |
| WT-TG | 0.837 | 0.151 | 0.0080 | 0.065 | 0.012 | 0.8977 | 0.060 | 0.010 | 0.2003 | 3 |
| PAT | 0.509 | 0.021 | 0.4895 | 0.054 | 0.007 | 0.9503 | 0.055 | 0.004 | 0.5488 | 5 |
| PAT-TG | 0.404 | 0.034 | 0.0329 | 0.043 | 0.006 | 0.5085 | 0.048 | 0.007 | 0.9841 | 2 |

**Supplementary File 1e**. Pituitary volumes acquired by stereological estimation in of the intact adult gland. All animals were compared by Two-Way ANOVA with post-hoc pairwise testing WT vs WT-TG, PAT, PAT-TG and PAT vs PAT-TG, using Dunnett’s multiple comparison test.

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | **Genotype** | **CL % p-HH3** | | **NC % p-HH3** | | **Total % p-HH3** | |  |
|  | *Mean* | *SD* | *Mean* | *SD* | *Mean* | *SD* | *n* |
| **E11.5** | WT | - | - | - | - | 5.59 | 1.04 | 5 |
| WT-TG | - | - | - | - | 6.56 | 1.26 | 4 |
| PAT | - | - | - | - | 6.80 | 2.89 | 4 |
| PAT-TG | - | - | - | - | 7.02 | 2.27 | 4 |
| **E13.5** | WT | 24.34 | 3.42 | 4.01 | 1.91 | 11.77 | 3.09 | 7 |
| WT-TG | 24.29 | 2.68 | 4.71 | 1.29 | 12.49 | 2.88 | 8 |
| PAT | 19.89 | 2.28 | 3.53 | 1.04 | 9.69 | 0.85 | 7 |
| PAT-TG | 18.85 | 2.48 | 2.95 | 0.78 | 9.06 | 1.36 | 8 |
| **E18.5** | WT | 10.34 | 1.43 | 8.46 | 0.43 | 8.94 | 0.30 | 5 |
| WT-TG | 10.56 | 2.26 | 8.42 | 2.10 | 8.97 | 2.00 | 7 |
| PAT | 10.64 | 2.91 | 8.76 | 3.66 | 9.25 | 3.42 | 8 |
| PAT-TG | 11.19 | 2.86 | 11.02 | 3.50 | 11.01 | 3.20 | 8 |
| **P7** | WT | 12.71 | 1.22 | 4.58 | 1.63 | 5.15 | 1.59 | 6 |
| WT-TG | 5.73 | 1.15 | 2.33 | 0.25 | 2.55 | 0.29 | 3 |
| PAT | 12.91 | 3.91 | 7.17 | 0.73 | 7.59 | 0.86 | 6 |
| PAT-TG | 11.87 | 4.22 | 5.50 | 0.90 | 5.94 | 0.63 | 4 |
| **P14** | WT | 3.92 | 1.22 | 9.18 | 1.25 | 8.91 | 1.24 | 4 |
| WT-TG | 5.56 | 2.11 | 11.32 | 0.95 | 11.52 | 0.87 | 5 |
| PAT | 4.54 | 1.51 | 7.70 | 0.79 | 7.66 | 0.87 | 3 |
| PAT-TG | 4.76 | nd | 6.97 | nd | 6.87 | nd | 1 |
| **P21** | WT | 5.66 | 1.09 | 1.57 | 0.22 | 1.82 | 0.25 | 3 |
| WT-TG | 7.35 | 0.36 | 1.65 | 0.49 | 2.07 | 0.47 | 3 |
| PAT | 6.01 | 1.44 | 1.81 | 0.15 | 2.04 | 0.20 | 2 |
| PAT-TG | 6.52 | 1.58 | 2.42 | 0.37 | 2.70 | 0.46 | 3 |

**Supplementary File 1f**. Proportion of proliferating cells (IHC positive for p-HH3) in the embryonic and postnatal pituitary gland. Data from males and females is combined.

|  |  |  |
| --- | --- | --- |
| **Probe target** | **Cat#** | **Source** |
| Mm-Lef1 | 441861 | ACDBio |
| Mm-Axin2 | 400331 | ACDBio |
| Mm-Shh | 314361 | ACDBio |
| Mm-Fgf8 | 313411 | ACDBio |
| Mm-Fgf10 | 446371 | ACDBio |

**Supplementary File 1g.** RNAScope probes used in this study

|  |  |  |
| --- | --- | --- |
| **Antibody target (species)** | **Source** | **Antibody titre** |
| DLK1 (mouse, WB) | Abcam ab21682 | 1:500 |
| DLK1 (mouse, IHC) | R&D AF8277 | 1:200 |
| DLK1 (mouse, IHC) | Abcam ab210471 | 1:1000 |
| Alpha tubulin (human, WB) | Merck-SIGMA T5168 | 1:10,000 |
| GH (Rat) | National Hormone and Peptide Program (NHPP) | 1:1000 |
| PRL (Mouse) | 1:500 |
| TSHβ (Rat) | 1:500 |
| FSHβ (Rat) | 1:500 |
| ACTH (Rat) | 1:1000 |
| LH (Rat) | 1:500 |
| POU1F1 (Mouse) | A gift from S. Rhodes, Indiana University School of Medicine, Indianapolis USA | 1:300 |
| SOX2 (Mouse) | Abcam ab92494 | 1:400 |
| α-phospho-Histone H3-Ser10 (human) | Merck-SIGMA 06-570 | 1:300 |
| HES1 (human) | Cell Signaling Technologies D6P2U | 1:300 |
| Goat SOX2 | R&D AF2018 | 1:300 |

**Supplementary File 1h.** Primary antibodies used in the study.

|  |  |  |
| --- | --- | --- |
| **Target** | **Fw** | **Rv** |
| Dlk1 qPCR | GAAAGGACTGCCAGCACAAG | CACAGAAGTTGCCTGAGAAGC |
| Dlk1 splice | CTGCACACCTGGGTTCTCTG | TCCTCATCACCAGCCTCCTT |
| Ghrh | GCTGTATGCCCGGAAAAGTGAT | AATCCCTGCAAGATGCTCTCC |
| Sst | CCCAGACTCCGTCAGTTTCT | GGGCATCATTCTCTGTCTGG |
| Actb | TTCTTTGCAGCTCCTTCGTT | ATGGAGGGGAATACAGCCC |
| Tuba | AGACCATTGGGGGAGGAGAT | GTGGGTTCCAGGTCTACGAA |

**Supplementary File 1i.** RT-PCR and RT-qPCR primers used in this study.