**SUPPLEMENTARY DATA**

**Table S1.** Rat genomic DNA (gDNA) specific primers (within an intron of *Actb*) used in qPCR to check for gDNA contamination of extracted RNA samples. Cycling conditions: 98oC for 3 minutes, (98oC for 10 seconds, 60oC for 30 seconds) – repeated for 40 cycles, followed by melt curve: 65oC to 90oC with 0.5oC increment per 5 seconds.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| ***Gene*** | **Primers** | **Sequence (*5’* to *3’*)** | **Primer length** | **PCR product size gDNA (bp)** |
| *Actb* | *ActbFgDNA* | CACTACCTCGCTGCAGGAT | 19 | 151 |
| *ActbRgDNA* | TGTCTACACCGCGGGAAT | 18 |

**Table S2.** Primer sequences and qPCR cycling conditions for rat reference, imprinted, and epigenetic genes. Primers were optimised at the following cycling conditions: 98oC for 3 minutes, (98oC for 10 seconds, 63or 65oC for 30 seconds (\*)) – repeated for 40 cycles, followed by melt curve analysis: 65oC to 90oC with 0.5oC increment per 5 seconds.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Gene** | **Primers** | **Sequence (*5’* to *3’*)** | **Primer length** | **PCR product length (bp)** | **Annealing temperature (\*)** |
| *Hprt* | *RTHprtF* | CCTTGACTATAATGAGCACTTC | 22 | 126 | **63oC** |
| *RTHprtR* | GCCACATCAACAGGACTC | 18 |
| *Tbp* | *RTTbpF* | CTAACCACAGCACCATTG | 18 | 152 | **63oC** |
| *RTTbpR* | TTACAGCCAAGATTCACG | 18 |
| *Ywhaz* | *RTYwhazF* | ACCCACTCCGGACACAGAAT | 20 | 111 | **63oC** |
| *RTYwhazR* | GACTTCATGCAGGCTGCCA | 19 |
| *Rpl13a* | *RTRpl13aF* | GGATCCCTCCACCCTATGACA | 21 | 131 | **63oC** |
| *RTFpl13aR* | CTGGTACTTCCACCCGACCTC | 21 |
| *Sdha* | *RTSdhaF* | GATGTCACCAAGGAGCCCAT | 20 | 105 | **65oC** |
| *RTSdhaR* | CTGGCCGTTCACGTGCTT | 18 |
| *Gusb* | *RTGusbF* | CTTCCACCGGGGACCATT | 18 | 105 | **63oC** |
| *RTGusbR* | GTGCAGCCCCGCATAGTT | 18 |
| *Peg3* | *Peg3F* | AAACTCACCACTCTGTTGGAGA | 22 | 98 | **65oC** |
| *Peg3R* | CTCCCACTTCGGCTCATGTC | 20 |
| *Cdkn1c* | *Cdkn1cF* | AGGGTGTCCCTCTCCTAACG | 20 | 97 | **63oC** |
| *Cdkn1cR* | CTGGCCGTTAGGCTCTAAACT | 21 |
| *Snrpn* | *SnrpnF* | AATTGGACTTCCCCCTGCTC | 20 | 117 | **65oC** |
| *SnrpnR* | TAGGGTCTTGGTGGACGCAT | 20 |
| *Kcnq1* | *RTKcnq1F* | CTATGTCCGGAAGCCTGCTC | 20 | 118 | **63oC** |
| *RTKcnq1R* | AGGACTCAGCCCGTTATCCT | 20 |
| *Dnmt1* | *RTDnmt1F* | CTCATTGGCTTTTCTACCGCAT | 22 | 117 | **63oC** |
| *RTDnmt1R* | CTCGACCACAATCTTGCTGATG | 22 |
| *Dnmt3a* | *RTDnmt3aF* | ACGGCAGAATAGCCAAGTTC | 20 | 118 | **63oC** |
| *RTDnmt3aR* | CAGAGGATGTCTTCCTTCTC | 20 |

**Table S3.** Statistical analyses of sham and IUGR rat phenotypic data and gene expression using Tukey’s test(s) following linear mixed effects models. N/A: not applicable. \*\*\* p < 0.001, \*\* p < 0.01, \* p < 0.05, . p < 0.1.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **F1 generation** | | | | |
| Time point | Phenotype/Gene | | Linear mixed effect models | Tukey's test for post-hoc analysis |
| E20 | Pup weight | | Chisq Df Pr(>Chisq)  Control\_Restricted 44.5793 1 2.443e-11 \*\*\*  Sex 0.8343 1 0.3610  Control\_Restricted:Sex 0.2089 1 0.6476 | N/A |
| Left kidney weight (g) | | Chisq Df Pr(>Chisq)  Control\_Restricted 3.0234 1 0.08207 .  Sex 2.8296 1 0.09254 .  Control\_Restricted:Sex 0.0781 1 0.77987 | N/A |
| Left kidney weight (%) | | Chisq Df Pr(>Chisq)  Control\_Restricted 1.6041 1 0.20533  Sex 0.7769 1 0.37809  Control\_Restricted:Sex 3.1490 1 0.07597 . | N/A |
| *Dnmt1* | | Chisq Df Pr(>Chisq)  Control\_Restricted 2.7733 1 0.09585 .  Sex 3.5038 1 0.06123 .  Control\_Restricted:Sex 0.0795 1 0.77793 | N/A |
| *Dnmt3a* | | Chisq Df Pr(>Chisq)  Control\_Restricted 9.8818 1 0.001669 \*\*  Sex 0.0449 1 0.832168  Control\_Restricted:Sex 0.2779 1 0.598063 | N/A |
| *Kcnq1* | | Chisq Df Pr(>Chisq)  Control\_Restricted 2.2076 1 0.1373  Sex 0.1924 1 0.6610  Control\_Restricted:Sex 1.5595 1 0.2117 | N/A |
| *Cdkn1c* | | Chisq Df Pr(>Chisq)  Control\_Restricted 1.3072 1 0.25291  Sex 5.2082 1 0.02248 \*  Control\_Restricted:Sex 4.6166 1 0.03166 \* | Estimate Std. Error z value Pr(>|z|)  sham.female - sham.male == 0 -0.01948 0.08466 -0.230 0.99436  iugr.female - iugr.male == 0 -0.28863 0.09233 -3.126 0.00658 \*\*  sham.female - iugr.female == 0 0.21213 0.09104 2.330 0.06885 .  sham.male - iugr.male == 0 -0.05703 0.08833 -0.646 0.89512 |
| *Peg3* | | Chisq Df Pr(>Chisq)  Control\_Restricted 2.7165 1 0.09932 .  Sex 0.0045 1 0.94635  Control\_Restricted:Sex 0.1713 1 0.67898 | N/A |
| *Snrpn* | | Chisq Df Pr(>Chisq)  Control\_Restricted 0.0071 1 0.93283  Sex 3.2688 1 0.07061 .  Control\_Restricted:Sex 5.4711 1 0.01933 \* | Estimate Std. Error z value Pr(>|z|)  sham.female - sham.male == 0 0.03087 0.10522 0.293 0.9884  iugr.female - iugr.male == 0 -0.32734 0.11127 -2.942 0.0119 \*  sham.female - iugr.female == 0 0.18667 0.10862 1.718 0.2584  sham.male - iugr.male == 0 -0.17154 0.10795 -1.589 0.3238 |
| PN1 | Pup weight | | Chisq Df Pr(>Chisq)  Control\_Restricted 1.9938 1 0.15795  Sex 3.8418 1 0.04999 \*  Control\_Restricted:Sex 0.2126 1 0.64477 | N/A |
| Left kidney weight (g) | | Chisq Df Pr(>Chisq)  Control\_Restricted 6.8140 1 0.009045 \*\*  Sex 0.0067 1 0.934823  Control\_Restricted:Sex 2.4344 1 0.118700 | N/A |
| Left kidney weight (%) | | Chisq Df Pr(>Chisq)  Control\_Restricted 4.2726 1 0.03873 \*  Sex 2.7528 1 0.09708 .  Control\_Restricted:Sex 1.3077 1 0.25281 | N/A |
| *Dnmt1* | | Chisq Df Pr(>Chisq)  Control\_Restricted 0.1401 1 0.7082  Sex 0.0781 1 0.7798  Control\_Restricted:Sex 1.1364 1 0.2864 | N/A |
| *Dnmt3a* | | Chisq Df Pr(>Chisq)  Control\_Restricted 0.1920 1 0.6613  Sex 0.3201 1 0.5715  Control\_Restricted:Sex 0.5699 1 0.4503 | N/A |
| *Kcnq1* | | Chisq Df Pr(>Chisq)  Control\_Restricted 7.3854 1 0.006576 \*\*  Sex 0.8703 1 0.350875  Control\_Restricted:Sex 0.0009 1 0.976116 | N/A |
| *Cdkn1c* | | Chisq Df Pr(>Chisq)  Control\_Restricted 5.4527 1 0.01954 \*  Sex 0.1085 1 0.74183  Control\_Restricted:Sex 0.1967 1 0.65739 | N/A |
| *Peg3* | | Chisq Df Pr(>Chisq)  Control\_Restricted 0.6312 1 0.4269  Sex 0.2347 1 0.6281  Control\_Restricted:Sex 0.2670 1 0.6054 | N/A |
| *Snrpn* | | Chisq Df Pr(>Chisq)  Control\_Restricted 0.4730 1 0.4916  Sex 0.2364 1 0.6268  Control\_Restricted:Sex 0.1896 1 0.6632 | N/A |
| **F2 generation (maternal line)** | | | | |
| Time point | | Phenotype/Gene | Linear regression | Tukey's test for post-hoc analysis |
| 6 and 12 months | | Pup weight | Chisq Df Pr(>Chisq)  Control\_Restricted 0.0028 1 0.957883  Sex 516.3233 1 < 2.2e-16 \*\*\*  Timepoint 28.8380 1 7.869e-08 \*\*\*  Control\_Restricted:Sex 0.0812 1 0.775682  Control\_Restricted:Timepoint 0.0020 1 0.964664  Sex:Timepoint 8.4197 1 0.003712 \*\*  Control\_Restricted:Sex:Timepoint 0.0347 1 0.852152 | Estimate Std. Error z value Pr(>|z|)  6mo.female - 6mo.male == 0 -137.605 9.186 -14.980 <0.001 \*\*\*  12mo.female - 12mo.male == 0 -177.479 9.410 -18.861 <0.001 \*\*\*  6mo.female - 12mo.female == 0 -18.459 8.871 -2.081 0.124  6mo.male - 12mo.male == 0 -58.333 9.700 -6.014 <0.001 \*\*\* |
| Left kidney weight (g) | Chisq Df Pr(>Chisq)  Control\_Restricted 0.2811 1 0.5960  Sex 587.0468 1 < 2.2e-16 \*\*\*  Timepoint 15.5807 1 7.906e-05 \*\*\*  Control\_Restricted:Sex 2.1565 1 0.1420  Control\_Restricted:Timepoint 0.0538 1 0.8166  Sex:Timepoint 0.1282 1 0.7203  Control\_Restricted:Sex:Timepoint 2.6729 1 0.1021 | N/A |
| Left kidney weight (%) | Chisq Df Pr(>Chisq)  Control\_Restricted 0.0652 1 0.7985  Sex 0.5796 1 0.4465  Timepoint 0.2784 1 0.5977  Control\_Restricted:Sex 0.9601 1 0.3272  Control\_Restricted:Timepoint 1.4265 1 0.2323  Sex:Timepoint 2.6378 1 0.1043  Control\_Restricted:Sex:Timepoint 0.7527 1 0.3856 | N/A |
| *Dnmt1* | Chisq Df Pr(>Chisq)  Control\_Restricted 0.0886 1 0.76598  Sex 5.1559 1 0.02317 \*  Timepoint 1.7224 1 0.18938  Control\_Restricted:Sex 0.9365 1 0.33318  Control\_Restricted:Timepoint 1.8124 1 0.17821  Sex:Timepoint 0.8893 1 0.34568  Control\_Restricted:Sex:Timepoint 0.6047 1 0.43680 | N/A |
| *Dnmt3a* | Chisq Df Pr(>Chisq)  Control\_Restricted 0.0863 1 0.76890  Sex 5.5247 1 0.01875 \*  Timepoint 0.1669 1 0.68287  Control\_Restricted:Sex 1.2281 1 0.26777  Control\_Restricted:Timepoint 0.5130 1 0.47385  Sex:Timepoint 0.8796 1 0.34830  Control\_Restricted:Sex:Timepoint 0.0088 1 0.92541 | N/A |
| *Kcnq1* | Chisq Df Pr(>Chisq)  Control\_Restricted 0.0064 1 0.936318  Sex 7.7496 1 0.005372 \*\*  Timepoint 2.1290 1 0.144534  Control\_Restricted:Sex 0.6701 1 0.413031  Control\_Restricted:Timepoint 0.0318 1 0.858427  Sex:Timepoint 0.0102 1 0.919592  Control\_Restricted:Sex:Timepoint 0.4272 1 0.513359 | N/A |
| *Cdkn1c* | Chisq Df Pr(>Chisq)  Control\_Restricted 3.1730 1 0.07487 .  Sex 46.2562 1 1.038e-11 \*\*\*  Timepoint 2.3748 1 0.12331  Control\_Restricted:Sex 2.3427 1 0.12587  Control\_Restricted:Timepoint 0.3592 1 0.54897  Sex:Timepoint 0.0052 1 0.94246  Control\_Restricted:Sex:Timepoint 0.1499 1 0.69862 | N/A |
| *Peg3* | Chisq Df Pr(>Chisq)  Control\_Restricted 3.5434 1 0.059783 .  Sex 8.7722 1 0.003059 \*\*  Timepoint 0.0007 1 0.978543  Control\_Restricted:Sex 4.0768 1 0.043476 \*  Control\_Restricted:Timepoint 0.1145 1 0.735060  Sex:Timepoint 1.4295 1 0.231843  Control\_Restricted:Sex:Timepoint 0.4231 1 0.515409 | Estimate Std. Error z value Pr(>|z|)  sham.female - sham.male == 0 0.044169 0.012338 3.580 0.00169 \*\*  iugr.female - iugr.male == 0 0.008535 0.012055 0.708 0.86942  sham.female - iugr.female == 0 0.038059 0.013155 2.893 0.01449 \*  sham.male - iugr.male == 0 0.002426 0.013932 0.174 0.99758 |
| *Snrpn* | Chisq Df Pr(>Chisq)  Control\_Restricted 1.5709 1 0.21007  Sex 3.1590 1 0.07551 .  Timepoint 0.2033 1 0.65211  Control\_Restricted:Sex 1.2150 1 0.27034  Control\_Restricted:Timepoint 0.2503 1 0.61685  Sex:Timepoint 0.1516 1 0.69698  Control\_Restricted:Sex:Timepoint 0.7615 1 0.38286 | N/A |
| **F2 generation (paternal line)** | | | | |
| Time point | | Phenotype/Gene | Linear regression | Tukey's test for post-hoc analysis |
| 6 and 12 months | | Pup weight | Chisq Df Pr(>Chisq)  Control\_Restricted 2.7513 1 0.09717 .  Sex 2268.0795 1 < 2.2e-16 \*\*\*  Timepoint 109.8332 1 < 2.2e-16 \*\*\*  Control\_Restricted:Sex 1.2591 1 0.26182  Control\_Restricted:Timepoint 0.4779 1 0.48938  Sex:Timepoint 52.5351 1 4.226e-13 \*\*\*  Control\_Restricted:Sex:Timepoint 3.6796 1 0.05508 . | Estimate Std. Error z value Pr(>|z|)  6mo.female - 6mo.male == 0 -151.105 5.055 -29.894 <0.001 \*\*\*  12mo.female - 12mo.male == 0 -203.570 5.581 -36.474 <0.001 \*\*\*  6mo.female - 12mo.female == 0 -13.693 5.208 -2.629 0.0308 \*  6mo.male - 12mo.male == 0 -66.157 5.495 -12.040 <0.001 \*\*\* |
| Left kidney weight (g) | Chisq Df Pr(>Chisq)  Control\_Restricted 0.7531 1 0.38550  Sex 600.6765 1 < 2.2e-16 \*\*\*  Timepoint 9.3095 1 0.00228 \*\*  Control\_Restricted:Sex 1.3006 1 0.25410  Control\_Restricted:Timepoint 0.2790 1 0.59737  Sex:Timepoint 16.1915 1 5.725e-05 \*\*\*  Control\_Restricted:Sex:Timepoint 1.1988 1 0.27357 | Estimate Std. Error z value Pr(>|z|)  6mo.female - 6mo.male == 0 -151.105 5.055 -29.894 <0.001 \*\*\*  12mo.female - 12mo.male == 0 -203.570 5.581 -36.474 <0.001 \*\*\*  6mo.female - 12mo.female == 0 -13.693 5.208 -2.629 0.0313 \*  6mo.male - 12mo.male == 0 -66.157 5.495 -12.040 <0.001 \*\*\* |
| Left kidney weight (%) | Chisq Df Pr(>Chisq)  Control\_Restricted 0.6101 1 0.4347526  Sex 16.7718 1 4.216e-05 \*\*\*  Timepoint 13.6170 1 0.0002241 \*\*\*  Control\_Restricted:Sex 0.4429 1 0.5057165  Control\_Restricted:Timepoint 0.8549 1 0.3551804  Sex:Timepoint 0.6314 1 0.4268342  Control\_Restricted:Sex:Timepoint 0.0458 1 0.8305030 | N/A |
| *Dnmt1* | Chisq Df Pr(>Chisq)  Control\_Restricted 0.1322 1 0.71617  Sex 4.9019 1 0.02683 \*  Timepoint 0.3472 1 0.55571  Control\_Restricted:Sex 0.3214 1 0.57079  Control\_Restricted:Timepoint 0.5880 1 0.44318  Sex:Timepoint 1.6525 1 0.19862  Control\_Restricted:Sex:Timepoint 2.9119 1 0.08793 . | N/A |
| *Dnmt3a* | Chisq Df Pr(>Chisq)  Control\_Restricted 0.2415 1 0.6231  Sex 2.2698 1 0.1319  Timepoint 0.8078 1 0.3688  Control\_Restricted:Sex 0.1766 1 0.6743  Control\_Restricted:Timepoint 0.1240 1 0.7247  Sex:Timepoint 0.8828 1 0.3474  Control\_Restricted:Sex:Timepoint 0.4643 1 0.4956 | N/A |
| *Kcnq1* | Chisq Df Pr(>Chisq)  Control\_Restricted 0.1799 1 0.6714  Sex 10.6507 1 0.0011 \*\*  Timepoint 0.0150 1 0.9024  Control\_Restricted:Sex 0.5414 1 0.4619  Control\_Restricted:Timepoint 1.5421 1 0.2143  Sex:Timepoint 0.7322 1 0.3922  Control\_Restricted:Sex:Timepoint 0.1152 1 0.7343 | N/A |
| *Cdkn1c* | Chisq Df Pr(>Chisq)  Control\_Restricted 0.9137 1 0.3391  Sex 34.9325 1 3.413e-09 \*\*\*  Timepoint 2.3638 1 0.1242  Control\_Restricted:Sex 0.0251 1 0.8740  Control\_Restricted:Timepoint 0.4260 1 0.5140  Sex:Timepoint 0.1507 1 0.6978  Control\_Restricted:Sex:Timepoint 0.2698 1 0.6034 | N/A |
| *Peg3* | Chisq Df Pr(>Chisq)  Control\_Restricted 0.9355 1 0.333429  Sex 10.4275 1 0.001242 \*\*  Timepoint 0.1190 1 0.730110  Control\_Restricted:Sex 0.7633 1 0.382308  Control\_Restricted:Timepoint 0.2764 1 0.599042  Sex:Timepoint 0.0884 1 0.766169  Control\_Restricted:Sex:Timepoint 0.8262 1 0.363374 | N/A |
| *Snrpn* | Chisq Df Pr(>Chisq)  Control\_Restricted 0.0779 1 0.78019  Sex 3.3026 1 0.06917 .  Timepoint 0.2135 1 0.64408  Control\_Restricted:Sex 2.7467 1 0.09746 .  Control\_Restricted:Timepoint 2.5195 1 0.11245  Sex:Timepoint 0.4384 1 0.50788  Control\_Restricted:Sex:Timepoint 3.5347 1 0.06010 . | N/A |

A close up of a device

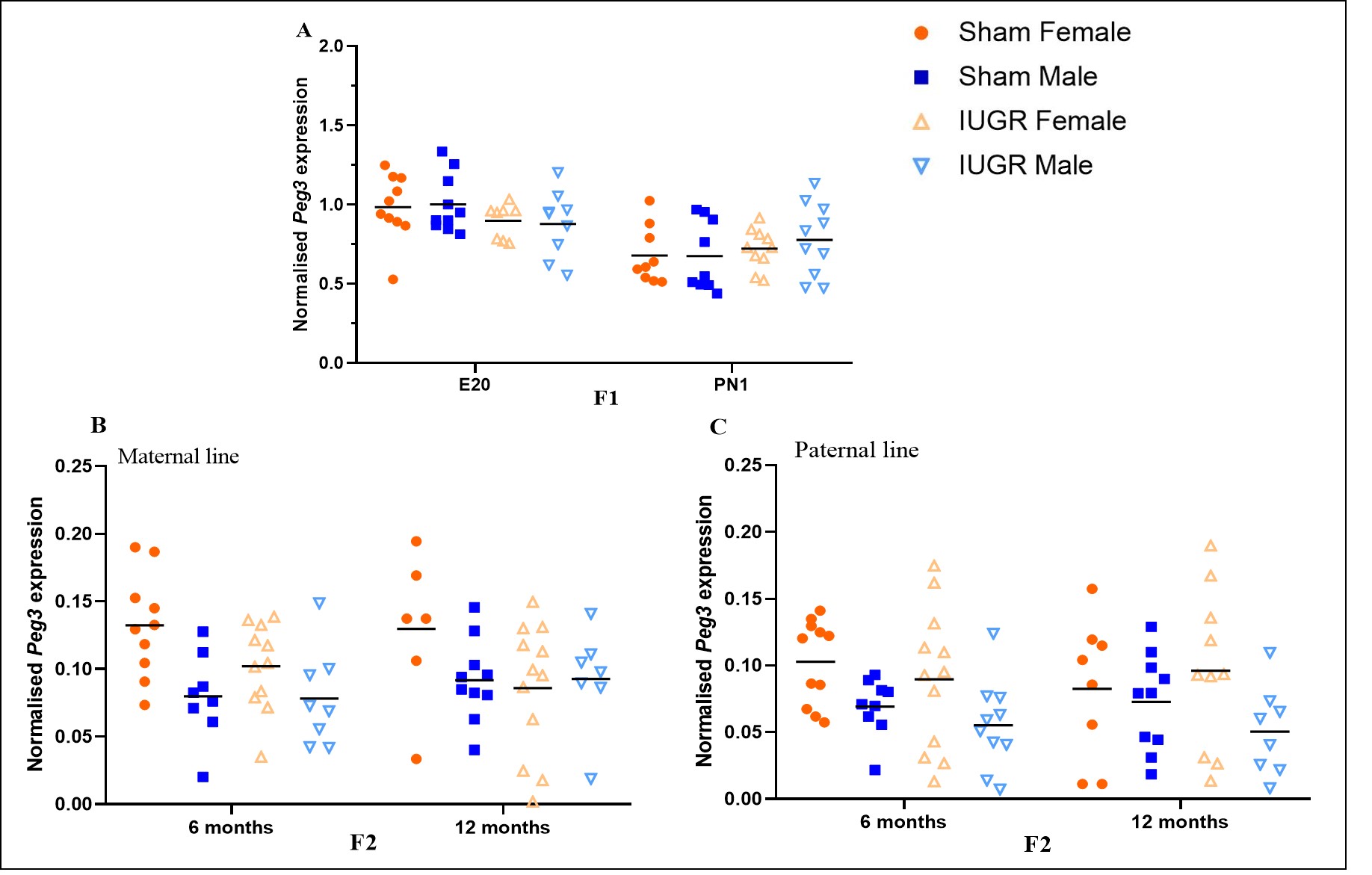
Description automatically generated

**Fig S1.1.** Example of outlier detection for the gene expression data (*Kcnq1* gene in the F1 generation) using bw plots (left) and gg plots (right) in RStudio software. Values beyond ± 3 standard deviations from the mean were indicated as an outlier and is shown in red. This outlier was removed for the statistical analysis for this gene.

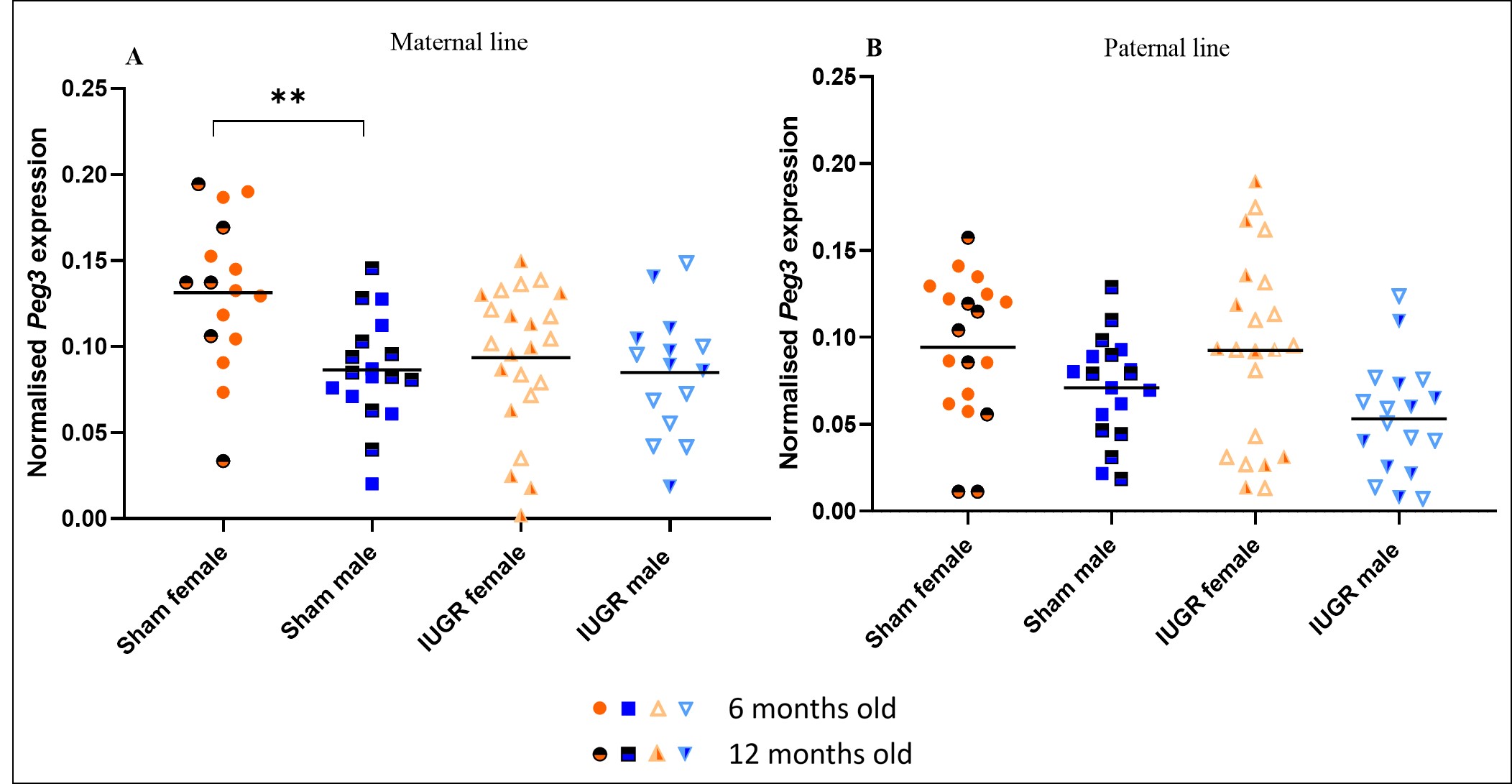
A close up of a map

Description automatically generated

**Fig S1.2.** Example of homoscedasticity assessment for the gene expression data (*Kcnq1* gene in the F1 generation) using residuals versus fits plot (top) and qq plot (bottom) in RStudio software. The data was confirmed to have a normal distribution as datapoints were normally and equally spread in the residuals versus fits plot and fall close to the reference line within the qq plot.



**Fig S2.** Normalised expression of *Peg3* in kidney tissues of sham and IUGR rat offspring in first (F1; *A*) and second (F2; *B*, *C*) generations. The mean value within each group is indicated by a black line. Significance was determined by a Tukeys post-hoc test following Linear mixed effect models in each generation and parental line (n = 6 – 13 samples per group).



**Fig S3.** Normalised expression of *Peg3* in kidney tissues of sham and IUGR rat offspring in F2 maternal (*A*) and paternal (*B*) lines. The mean value within each group is indicated by a black line. Significance was determined by a Tukeys post-hoc test following Linear mixed effect models in each generation and parental line (\*\* p < 0.01; n = 6 – 13 samples per group).