**Supplementary Information for:**

**Genome-scale single nucleotide resolution analysis of DNA methylation in human autosomal dominant polycystic kidney disease.**

**Sarah A Bowden1#, Euan J Rodger1,2#, Michael Bates1#,Aniruddha Chatterjee1,2, Michael R Eccles1,2, Cherie Stayner1\***

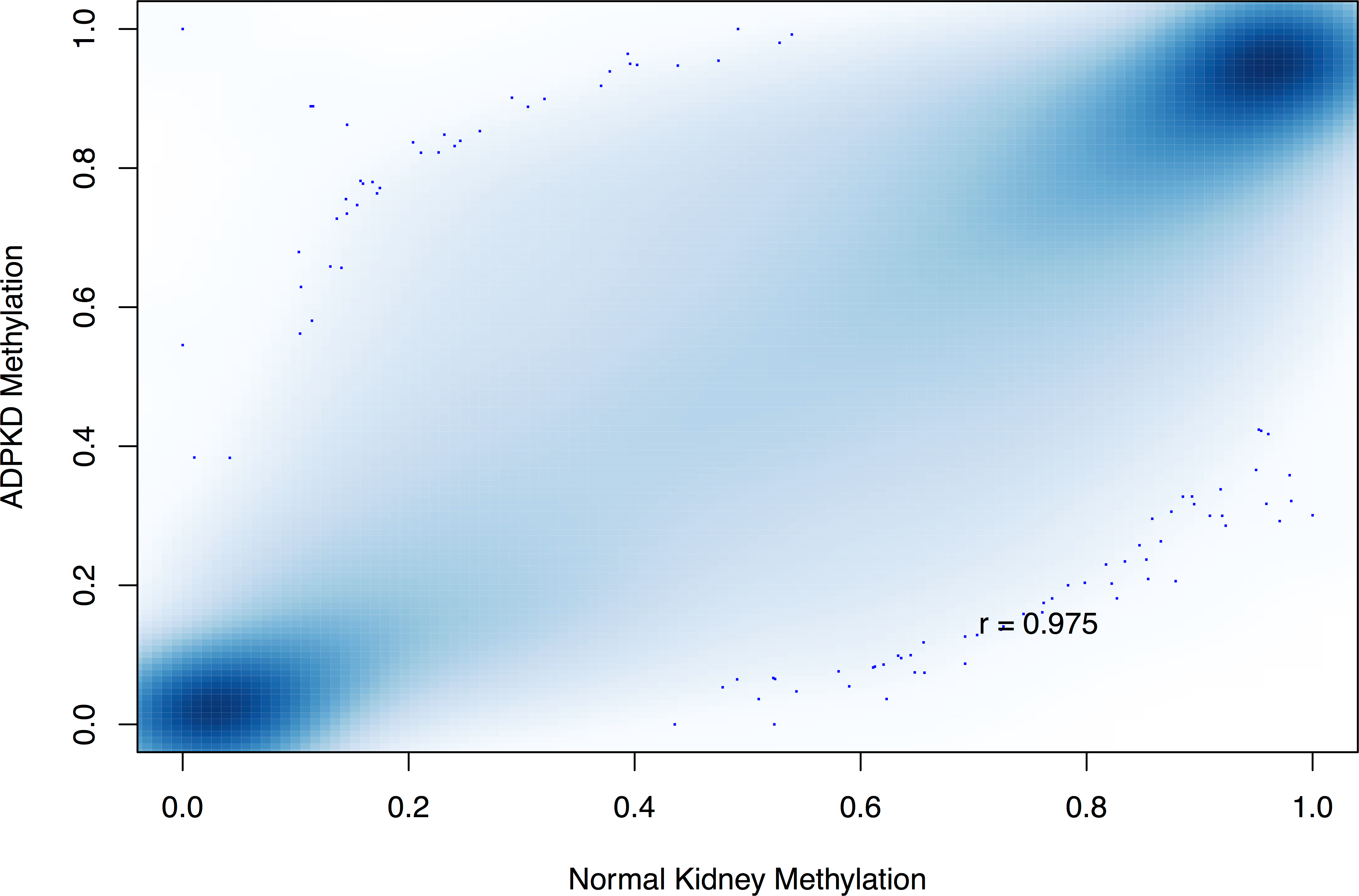
1Department of Pathology, Dunedin School of Medicine, University of Otago, 270 Great King Street, Dunedin 9054, New Zealand. 2Maurice Wilkins Centre for Molecular Biodiscovery, Level 2, 3A Symonds Street, Auckland 1010, New Zealand.

# These authors contributed equally to this work

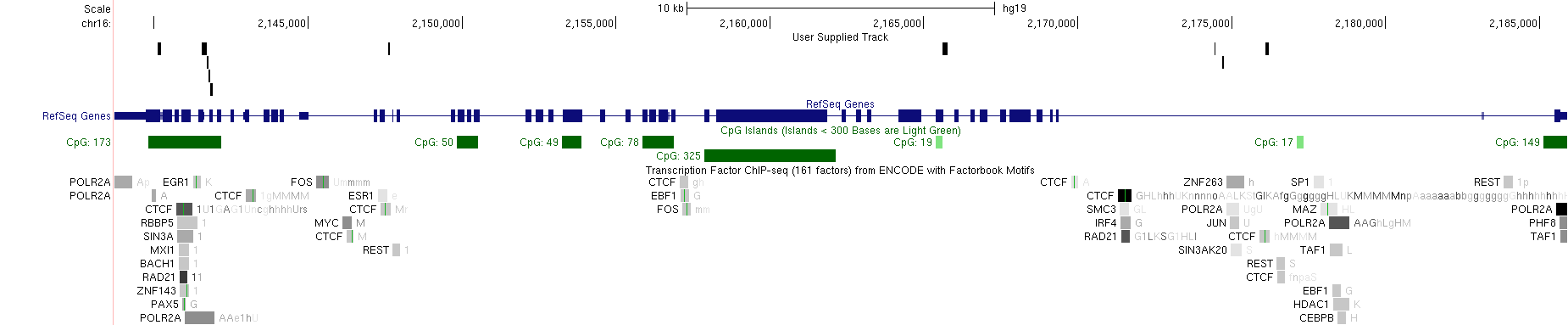
**Running title:** DNA methylation in human ADPKD

\*Correspondence: Dr. Cherie Stayner, Department of Pathology, Dunedin School of Medicine, University of Otago, P.O. Box 56, Dunedin 9054, New Zealand, Phone: +64 3 470 3453, Fax: +64 3 479 7136 email: [cherie.stayner@otago.ac.nz](mailto:cherie.stayner@otago.ac.nz).

**Supplementary Figures S1-S3**



**Figure S1. Correlation of DNA methylation in ADPKD compared to non-ADPKD kidney.** A smoothed colour density scatterplot of the 345711 commonly analysed RRBS *Msp*I fragments shows the correlation of methylation in ADPKD (y-axis) compared to non-ADPKD (x-axis).



**Figure S2. Gene map of *PKD1* in ADPKD and non-ADPKD kidney.** ANOVA analysis of RRBS *Msp*I fragment-based methylomes from renal cortex of non-ADPKD (n=3) compared to ADPKD kidney (n=4) identified 10 fragments (shown in black on top track) with a nominal raw P-value threshold of <0.05 and methylation differences greater than 0.10. The bottom track shows predicted transcription factor binding sites from ENCODE.

**Supplementary Tables S1-S7**

**Table S1. Summary of Bismark genome alignment for RRBS libraries**

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | **ADPKD 07** | **ADPKD 08** | **ADPKD 05** | **ADPKD 16** | **Non-ADPKD E1** | **Non-ADPKD G2** | **Non-ADPKD H3** | **TOTAL** | **MEDIAN** |
| **Sequences analysed in total** | 4.69.E+07 | 5.72.E+07 | 1.86E+07 | 1.42E+07 | 4.25.E+07 | 3.50.E+07 | 4.15.E+07 | 2.56E+08 | 4.15.E+07 |
| **No. of alignments with a unique best hit** | 3.13.E+07 | 3.78.E+07 | 1.01E+07 | 7.70E+06 | 2.59.E+07 | 2.34.E+07 | 2.83.E+07 | 1.65E+08 | 2.83.E+07 |
| **Mapping efficiency** | 66.70% | 66.20% | 54.20% | 54.20% | 61.00% | 67.00% | 68.30% |  | 66.20% |
| **Sequences with no alignments under any condition** | 6.90E+06 | 9.41E+06 | 5.09E+06 | 3.86E+06 | 8.81E+06 | 5.94E+06 | 5.38E+06 | 4.54E+07 | 5.94E+06 |
| **Sequences did not map uniquely** | 8.71E+06 | 9.93E+06 | 3.41E+06 | 2.63E+06 | 7.75E+06 | 5.59E+06 | 7.76E+06 | 4.58E+07 | 7.75E+06 |
| **Total number of Cs analysed** | 6.38E+08 | 8.06E+08 | 2.01E+08 | 1.37E+08 | 5.50E+08 | 4.92E+08 | 5.93E+08 | 3.42E+09 | 5.93E+08 |
| **C meth in CpG context** | 35.80% | 31.90% | 39.00% | 35.90% | 40.90% | 40.00% | 39.00% |  | 39.00% |
| **C meth in CHG context** | 0.80% | 0.90% | 0.40% | 0.80% | 1.00% | 1.20% | 1.00% |  | 1.00% |
| **C meth in CHH context** | 0.50% | 0.40% | 0.40% | 0.70% | 0.50% | 0.70% | 0.60% |  | 0.50% |

**Table S2. Global methylation analysis of ADPKD and non-ADPKD kidney tissue**

|  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | **Genome-wide** | | **Promoter** | | **Intron** | | **Exon** | | **Intron/Exon Boundary** | | **Intergenic** | |
|  | Non-ADPKD | ADPKD | Non-ADPKD | ADPKD | Non-ADPKD | ADPKD | Non-ADPKD | ADPKD | Non-ADPKD | ADPKD | Non-ADPKD | ADPKD |
| Median methylation | 0.7833 | 0.7619 | 0.0380 | 0.0278 | 0.8202 | 0.7985 | 0.2521 | 0.2204 | 0.5425 | 0.5396 | 0.8706 | 0.8507 |
| Mean methylation | 0.5778 | 0.5633 | 0.1790 | 0.1655 | 0.6048 | 0.5892 | 0.4399 | 0.4250 | 0.4869 | 0.4737 | 0.6785 | 0.6648 |
| Analysed fragments | 345711 | | 72182 | | 129949 | | 33199 | | 15764 | | 131420 | |
| Wilcoxon rank sum test | W = 63754556349  P-value < 2.2e-16 | | W = 2914702038  P-value < 2.2e-16 | | W = 9036317200  P-value < 2.2e-16 | | W = 590623184  P-value < 2.2e-16 | | W = 132954508  P-value < 2.2e-16 | | W = 9293002131  P-value < 2.2e-16 | |

**Table S3. Global CpG island methylation analysis of ADPKD and non-ADPKD kidney tissue**

|  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | **Genome-wide** | | **CGI core** | | **CGI shore** | | **CGI shelf** | | **Open sea** | |
|  | Non-ADPKD | ADPKD | Non-ADPKD | ADPKD | Non-ADPKD | ADPKD | Non-ADPKD | ADPKD | Non-ADPKD | ADPKD |
| Median methylation | 0.7833 | 0.7619 | 0.0293 | 0.0215 | 0.4423 | 0.4207 | 0.7985 | 0.7751 | 0.8818 | 0.8605 |
| Mean methylation | 0.5778 | 0.5633 | 0.0819 | 0.0705 | 0.4802 | 0.4664 | 0.6153 | 0.5969 | 0.6933 | 0.6782 |
| Analysed fragments | 345711 | | 56922 | | 12602 | | 8291 | | 263957 | |
| Wilcoxon rank sum test | W = 63754556349  P-value < 2.2e-16 | | W = 1881819234  P-value < 2.2e-16 | | W = 82547192  P-value < 2.2e-16 | | W = 36694268  P-value < 2.2e-16 | | W = 37679062783  P-value < 2.2e-16 | |

**Table S4. Genehancer analysis on differentially methylated fragments (DMFs) in ADPKD**

|  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **ADPKD DMFs** | | | **Genehancer analysis** | | | | | | | |
| **Chr** | **Start** | **End** | **Confidence**  **score** | **ID** | **Connected**  **gene 1**  **(assoc. score)** | **Connected**  **gene 2**  **(assoc. score)** | **Connected**  **gene 3**  **(assoc. score)** | **Connected**  **gene 4**  **(assoc. score)** | **Connected**  **gene 5**  **(assoc. score)** | **Connected**  **gene 6**  **(assoc. score)** |
| 4 | 793929 | 794143 |  |  |  |  |  |  |  |  |
| 7 | 922176 | 922249 | 0.38 | GH07F000881 | *PRKAR1B* (15.26) | *DNAAF5* (11.4) |  |  |  |  |
| 7 | 6511106 | 6511153 |  |  |  |  |  |  |  |  |
| 7 | 6495310 | 6495378 |  |  |  |  |  |  |  |  |
| 10 | 14979136 | 14979987 |  |  |  |  |  |  |  |  |
| 12 | 3862528 | 3862582 | 0.25 | GH12F003751 | *CRACR2A* (6.18) | *TULP3* (5.31) |  |  |  |  |
| 17 | 40683608 | 40683711 | 1.09 | GH17F042528 | *TUBG2* (20.8) | *NAGLU* (15.87) | *RAB5C* (10.61) | *HSD17B1* (8.99) | *MLX* (8.7) | *BECN1* (5.8) |
| 22 | 37499386 | 37499523 |  |  |  |  |  |  |  |  |
| 2 | 771624 | 771728 |  |  |  |  |  |  |  |  |
| 9 | 130768316 | 130768366 | 1.39 | GH09F128004 | *LOC101929314* (11.5) | *FAM102A* (11.47) | *PIP5KL1* (10.81) |  |  |  |
| 18 | 10271529 | 10271568 |  |  |  |  |  |  |  |  |
| 19 | 789109 | 789181 | 0.87 | GH19F000788 | *CFD* (4.83) |  |  |  |  |  |
| 20 | 2513933 | 2513980 |  |  |  |  |  |  |  |  |

**Table S5. OregAnno analysis on differentially methylated fragments (DMFs) in ADPKD**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **ADPKD DMFs** | | | **OregAnno analysis** | | |
| **Chr** | **Start** | **End** | **ID** | **Target Gene** | **Transcription Factors** |
| 4 | 793929 | 794143 |  |  |  |
| 7 | 922176 | 922249 | OREG1180168, OREG1530622 | *GET4* | TFAP2C, ESR1 |
| 7 | 6511106 | 6511153 |  |  |  |
| 7 | 6495310 | 6495378 |  |  |  |
| 10 | 14979136 | 14979987 | OREG1772349 | *DCLRE1C* | MITF |
| 12 | 3862528 | 3862582 | OREG1511145 | *CRACR2A* | EGR1 |
| 17 | 40683608 | 40683711 | OREG1267929 | *ATP6V0A1* | SMARCA4 |
| 22 | 37499386 | 37499523 |  |  |  |
| 2 | 771624 | 771728 |  |  |  |
| 9 | 130768316 | 130768366 | OREG1885090, OREG1174681, OREG1639121, OREG1675784, OREG0298345 | *FAM102A* | STAT1, TFAP2C, FOXA1, GATA2, STAT1 |
| 18 | 10271529 | 10271568 |  |  |  |
| 19 | 789109 | 789181 | OREG1487706, OREG1365666 | *PTBP1* | EGR1, CTCF |
| 20 | 2513933 | 2513980 |  |  |  |

**Table S6. All RRBS MspI fragments spanning the *PKD1* gene**

|  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Chr** | **Start** | **End** | **Fragment**  **Length** | **No. of CpGs** | **Raw P-value**  **(ANOVA)** | **Mean non-ADPKD**  **methylation**  **(n=3)** | **Mean ADPKD**  **methylation**  **(n=4)** | **Methdiff 1** | **Overlap** | **Genehancer ID** |
| 16 | 2189554 | 2189620 | 67 | 9 | 0.597 | 0.96 | 0.95 | -0.01 | Promoter |  |
| 16 | 2185561 | 2185611 | 51 | 7 | 0.271 | 0.07 | 0.05 | -0.02 | E1 |  |
| 16 | 2185297 | 2185356 | 60 | 9 | 0.292 | 0.00 | 0.00 | 0.00 | I1 |  |
| 16 | 2185140 | 2185253 | 114 | 11 | 0.183 | 0.00 | 0.01 | 0.01 | I1 |  |
| 16 | 2183963 | 2184050 | 88 | 1 | 0.064 | 0.82 | 0.55 | -0.27 | I1 |  |
| 16 | 2180046 | 2180194 | 149 | 14 | 0.343 | 0.57 | 0.42 | -0.15 | I1 | GH16H002129 |
| 16 | 2176100 | 2176196 | 97 | 5 | 0.010 | 0.75 | 0.50 | -0.25 | I1 | GH16H002121 |
| 16 | 2175156 | 2175228 | 73 | 3 | 0.145 | 0.30 | 0.11 | -0.19 | I1 | GH16H002121 |
| 16 | 2174754 | 2174804 | 51 | 5 | 0.068 | 0.61 | 0.17 | -0.44 | I1 | GH16H002121 |
| 16 | 2174683 | 2174753 | 71 | 5 | 0.004 | 0.55 | 0.11 | -0.44 | I1 | GH16H002121 |
| 16 | 2174553 | 2174682 | 130 | 2 | 0.051 | 0.62 | 0.25 | -0.36 | I1 | GH16H002121 |
| 16 | 2174493 | 2174552 | 60 | 2 | 0.165 | 0.33 | 0.20 | -0.13 | I1 | GH16H002121 |
| 16 | 2174437 | 2174483 | 47 | 2 | 0.042 | 0.60 | 0.39 | -0.21 | I1 | GH16H002121 |
| 16 | 2174236 | 2174288 | 53 | 5 | 0.507 | 0.23 | 0.14 | -0.09 | I1 | GH16H002121 |
| 16 | 2173064 | 2173148 | 85 | 3 | 0.322 | 0.94 | 0.84 | -0.10 | I1 | GH16H002121 |
| 16 | 2171927 | 2171991 | 65 | 3 | 0.102 | 0.91 | 0.69 | -0.22 | I1 | GH16H002121 |
| 16 | 2170942 | 2171087 | 146 | 4 | 0.683 | 0.94 | 0.91 | -0.03 | I1 |  |
| 16 | 2170874 | 2170941 | 68 | 4 | 0.268 | 0.92 | 0.83 | -0.09 | I1 |  |
| 16 | 2170696 | 2170740 | 45 | 2 | 0.503 | 0.85 | 0.80 | -0.05 | I1 |  |
| 16 | 2168570 | 2168666 | 97 | 1 | 0.470 | 0.30 | 0.27 | -0.04 | I4 |  |
| 16 | 2168488 | 2168569 | 82 | 5 | 0.277 | 0.57 | 0.67 | 0.10 | I4 |  |
| 16 | 2167293 | 2167354 | 62 | 2 | 0.215 | 0.97 | 0.93 | -0.05 | I6 |  |
| 16 | 2167179 | 2167240 | 62 | 2 | 0.046 | 0.96 | 0.88 | -0.08 | I6 |  |
| 16 | 2166837 | 2166896 | 60 | 6 | 0.054 | 0.63 | 0.76 | 0.13 | E7 |  |
| 16 | 2165619 | 2165775 | 157 | 4 | 0.045 | 0.94 | 0.81 | -0.14 | E10/I9 |  |
| 16 | 2165447 | 2165585 | 139 | 8 | 0.004 | 0.96 | 0.87 | -0.09 | E10 |  |
| 16 | 2164268 | 2164373 | 106 | 6 | 0.491 | 0.93 | 0.91 | -0.02 | E11 |  |
| 16 | 2162624 | 2162769 | 146 | 5 | 0.698 | 0.96 | 0.98 | 0.02 | I13 |  |
| 16 | 2162506 | 2162579 | 74 | 5 | 0.762 | 0.92 | 0.90 | -0.02 | I13 |  |
| 16 | 2161327 | 2161399 | 73 | 5 | 0.959 | 0.95 | 0.94 | -0.01 | E15 |  |
| 16 | 2161213 | 2161313 | 101 | 13 | 0.822 | 0.97 | 0.97 | 0.00 | E15 |  |
| 16 | 2159464 | 2159573 | 110 | 7 | 0.062 | 0.96 | 0.89 | -0.07 | E15 |  |
| 16 | 2159371 | 2159427 | 57 | 5 | 0.025 | 0.98 | 0.95 | -0.03 | E15 |  |
| 16 | 2158574 | 2158720 | 147 | 13 | 0.016 | 0.98 | 0.96 | -0.02 | E15 |  |
| 16 | 2156939 | 2157066 | 128 | 2 | 0.759 | 0.92 | 0.90 | -0.01 | E17/I16 | GH16H002106 |
| 16 | 2155697 | 2155768 | 72 | 3 | 0.273 | 0.94 | 0.90 | -0.04 | I20 |  |
| 16 | 2155553 | 2155685 | 133 | 6 | 0.062 | 0.97 | 0.91 | -0.06 | I20 |  |
| 16 | 2153303 | 2153403 | 101 | 8 | 0.201 | 0.96 | 0.93 | -0.03 | E23 |  |
| 16 | 2152911 | 2153002 | 92 | 5 | 0.025 | 0.97 | 0.93 | -0.04 | E24/I23 |  |
| 16 | 2152842 | 2152910 | 69 | 3 | 0.961 | 0.94 | 0.92 | -0.02 | E24 |  |
| 16 | 2152709 | 2152816 | 108 | 4 | 0.579 | 0.97 | 0.94 | -0.03 | I24/E24 |  |
| 16 | 2152070 | 2152144 | 75 | 7 | 0.406 | 0.97 | 0.95 | -0.02 | E26 |  |
| 16 | 2151079 | 2151145 | 67 | 4 | 0.126 | 0.95 | 0.97 | 0.02 | I26 |  |
| 16 | 2150522 | 2150683 | 162 | 6 | 0.920 | 0.93 | 0.94 | 0.01 | E27/I26 |  |
| 16 | 2150459 | 2150509 | 51 | 4 | 0.567 | 0.96 | 0.97 | 0.01 | E27 |  |
| 16 | 2147955 | 2148077 | 123 | 5 | 0.625 | 0.91 | 0.88 | -0.03 | E31/I30 |  |
| 16 | 2147611 | 2147671 | 61 | 3 | 0.001 | 0.16 | 0.78 | 0.62 | I32 |  |
| 16 | 2146813 | 2146886 | 74 | 3 | 0.968 | 0.78 | 0.81 | 0.03 | I34 |  |
| 16 | 2146379 | 2146422 | 44 | 8 | 0.670 | 0.93 | 0.96 | 0.04 | I34 |  |
| 16 | 2146263 | 2146378 | 116 | 4 | 0.174 | 0.58 | 0.81 | 0.23 | I34 |  |
| 16 | 2145756 | 2145856 | 101 | 7 | 0.643 | 0.85 | 0.85 | 0.00 | I34 |  |
| 16 | 2144777 | 2144863 | 87 | 7 | 0.043 | 0.97 | 0.93 | -0.04 | I34 |  |
| 16 | 2144074 | 2144242 | 169 | 5 | 0.598 | 0.72 | 0.77 | 0.05 | I35/E35/I34 | GH16H002086 |
| 16 | 2143980 | 2144073 | 94 | 3 | 0.223 | 0.93 | 0.86 | -0.06 | E36/I35 | GH16H002086 |
| 16 | 2143265 | 2143404 | 140 | 2 | 0.746 | 0.96 | 0.97 | 0.00 | I37 | GH16H002086 |
| 16 | 2142169 | 2142212 | 44 | 1 | 0.289 | 0.92 | 0.82 | -0.10 | E40/I39 | GH16H002086 |
| 16 | 2142059 | 2142168 | 110 | 10 | 0.938 | 0.88 | 0.86 | -0.02 | E40 | GH16H002086 |
| 16 | 2141958 | 2142026 | 69 | 5 | 0.476 | 0.91 | 0.92 | 0.01 | I40 | GH16H002086 |
| 16 | 2141821 | 2141911 | 91 | 6 | 0.006 | 0.73 | 0.86 | 0.14 | E41/I40 | GH16H002086 |
| 16 | 2141754 | 2141820 | 67 | 2 | 0.034 | 0.61 | 0.76 | 0.15 | I41/E41 | GH16H002086 |
| 16 | 2141710 | 2141753 | 44 | 2 | 0.012 | 0.38 | 0.66 | 0.28 | I41 | GH16H002086 |
| 16 | 2141559 | 2141709 | 151 | 15 | 0.049 | 0.14 | 0.51 | 0.37 | E42/I41 | GH16H002086 |
| 16 | 2141514 | 2141558 | 45 | 8 | 0.093 | 0.21 | 0.45 | 0.24 | E42 | GH16H002086 |
| 16 | 2141409 | 2141506 | 98 | 15 | 0.121 | 0.20 | 0.53 | 0.34 | I42/E42 | GH16H002086 |
| 16 | 2141283 | 2141351 | 69 | 10 | 0.067 | 0.06 | 0.20 | 0.14 | I42 | GH16H002086 |
| 16 | 2140121 | 2140224 | 104 | 6 | 0.008 | 0.69 | 0.87 | 0.18 | E46/I45 | GH16H002086 |
| 16 | 2140032 | 2140120 | 89 | 5 | 0.365 | 0.59 | 0.76 | 0.17 | E46 | GH16H002086 |
| 16 | 2139882 | 2140031 | 150 | 7 | 0.196 | 0.45 | 0.56 | 0.11 | E46 | GH16H002086 |

1 Methdiff = average methylation difference between ADPKD and non-ADPKD tissue (a negative value indicates hypomethylation in ADPKD compared to non-ADPKD). Fragments with Methdiff >0.10 and a nominal raw P-value <0.05 are highlighted in yellow.

**Table S7. Correlation of RRBS fragment methylation with *PKD1* expression**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **RRBS**  **Fragment** | **Spearman r** | **P value** | **P value summary**  **(alpha = 0.05)** | **Number of XY Pairs** |
| 1 | 0.9286 | 0.0067 | \*\* | 7 |
| 2 | 1 | 0.0028 | \*\* | 6 |
| 3 | 0.8214 | 0.0341 | \* | 7 |
| 4 | 0.5714 | 0.2000 | ns | 7 |
| 5 | 0.9 | 0.0833 | ns | 5 |
| 6 | 0.8857 | 0.0333 | \* | 6 |
| 7 | -0.6 | 0.4167 | ns | 4 |
| 8 | -0.9643 | 0.0028 | \*\* | 7 |
| 9 | -0.7714 | 0.1028 | ns | 6 |
| 10 | -0.8286 | 0.0583 | ns | 6 |

|  |  |  |
| --- | --- | --- |
| Target |  | Primer Sequence (5’ to 3’) |
| *GET4* | F | GAGCCCTGAAGTGGTCCAG |
| R | GTGCAGAAAATGATACCTCGACT |
| *NAGLU* | F | TGGCACATCAAGCAGCTTTA |
| R | GTGACAGCCTCGGGAACAT |
| *PKD1* | F | CTGCAGGAAGCACTCTACCC |
| R | CTCCCAGCCAACGTCGTAAT |
| *B2M* | F | GAGTGCTGTCTCCATGTTTGATGT |
| R | AAGTTGCCAGCCCTCCTAGAG |
| *EEFA1A* | F | CTGCCACCCCACTCTTAATCA |
| R | GGCCAATTGAAACAAACAGTTCT |

**Table S8: RT-PCR primer sequences**