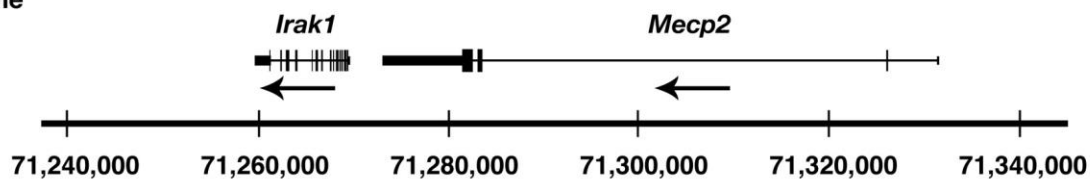


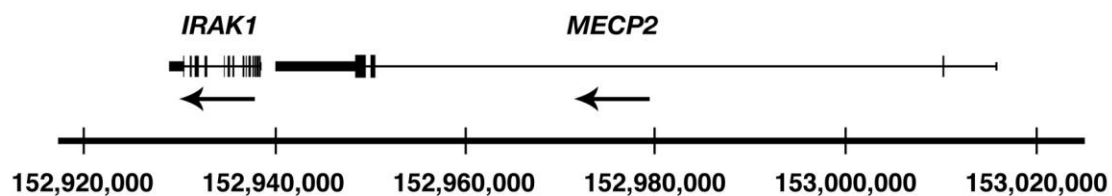
Supplementary Figure 1: Identification of dysregulated genes in *Mecp2*-null FACS-purified CPN

(A-B) At P3, green fluorescent microspheres were injected in one hemisphere of the neocortex of wild-type and *Mecp2*-null mice in CPN axonal terminal fields, and were retrogradely transported to the somata of contralateral CPN. The brackets in B indicate retrogradely labeled layer 2/3 and 5 CPN. (C-D) FACS plot of the population of purified CPN; CPN were selected based on (C) size (forward scatter) and surface characteristics (side scatter), and (D) high intensity green fluorescence. (E-F) Dissociated P14 cells before (E) and after (F) FACS. Arrows in E and F indicate CPN identified by green fluorescent microspheres. (G) Intensity plots of all genes in pair-wise comparisons of wild-type vs. *Mecp2*-null CPN at P14. We performed microarray analysis on three sets of independent biological replicates, using Affymetrix M430 2.0 GeneChips, which include probe sets for over 39,000 mouse genes and ESTs; differentially expressed genes (p -value < 0.001) indicated by red crosses (over-expressed) and green crosses (under-expressed). Red lines mark the 2-fold change interval. Red circles indicate *Mecp2* probe sets. Scale bar 200 μ m in B, 50 μ m in E and F.

Mouse X chromosome

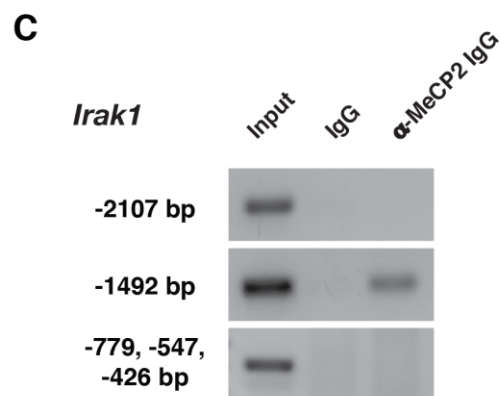
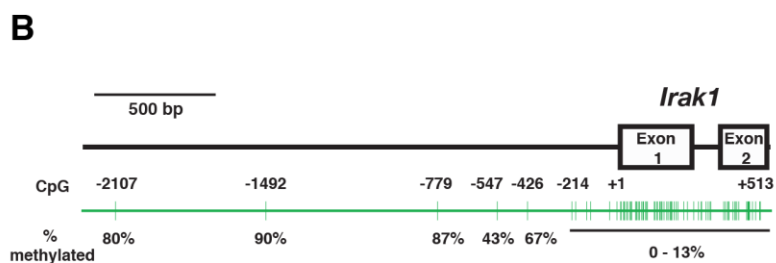
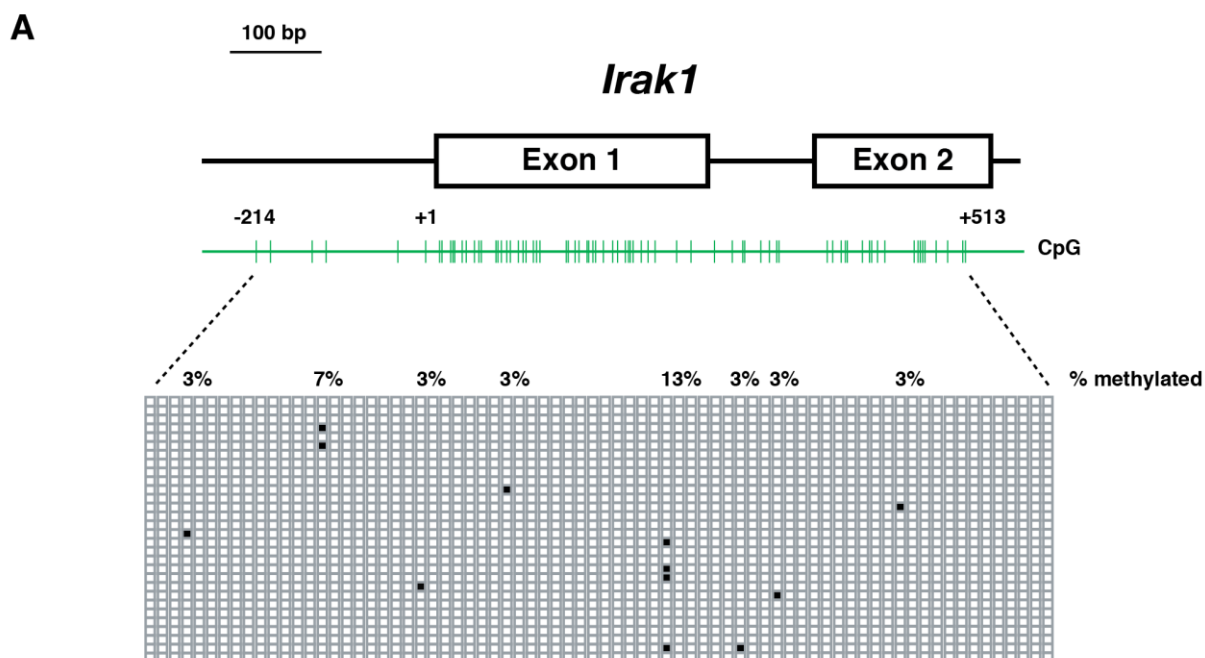


Human X chromosome



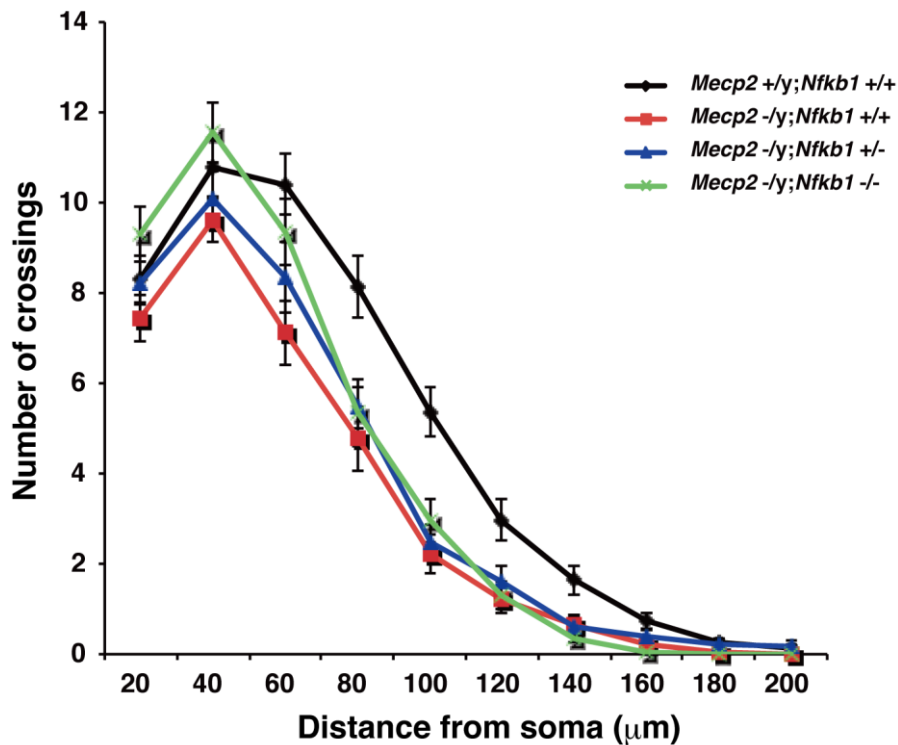
10 kb

Supplementary Figure 2: Location of *Irak1*/*IRAK1* and *Mecp2*/*MECP2* on mouse and human X chromosome
The *Irak1*/*IRAK1* locus is located downstream of the *Mecp2*/*MECP2* locus on both the mouse and human X chromosome. Positions on the X chromosome are based on the NCBI Build 37 assembly for the mouse genome, and the NCBI Build 36.1 assembly for the human genome.



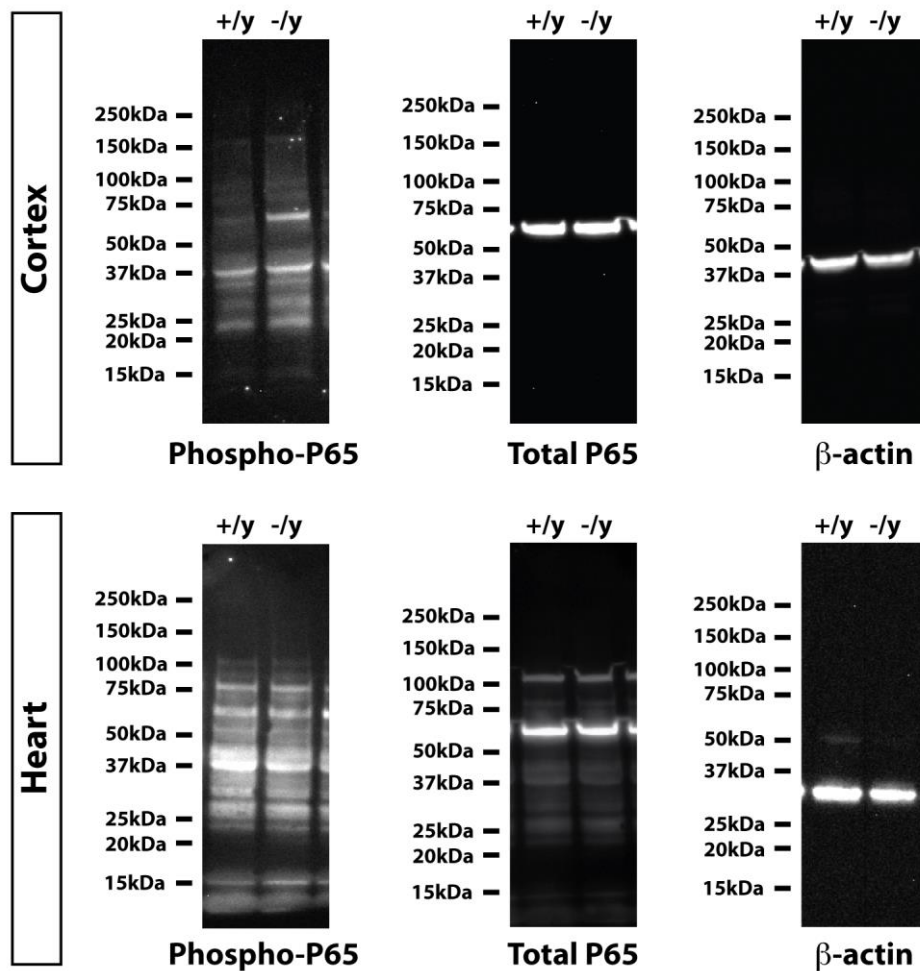
Supplementary Figure 3: Methylation status of CpGs in exons 1 and 2 of *Irak1*

(A) Methylation status of 74 CpGs near the transcription initiation site of *Irak1* in the cortex of P14 male mice was analyzed by bisulfite genomic sequencing. In contrast to the 5 CpGs (-2107, -1492, -779, -547, and -426 bp) on the *Irak1* promoter region, these CpGs were methylated at very low levels. Closed boxes, methyl-CpG; open boxes, unmethyl-CpG. Locations of CpG sites in the *Irak1* promoter region are relative to the transcription initiation site. (B) While most of the CpGs on or near exons 1 and 2 of *Irak1* are methylated at very low levels in P14 cortex, 5 CpGs upstream of the TIS of *Irak1* (-2107, -1492, -779, -547, and -426 bp) are highly methylated. Closed boxes, methylated-CpG; open boxes, unmethylated-CpG. (C) ChIP with anti-MeCP2 IgG identifies that MeCP2 specifically binds to the CpG located 1492 bp upstream of the TIS of *Irak1*, and not to the other 4 highly methylated CpGs. There is no binding with control IgG to any of these sites.



Supplementary Figure 4: Sholl analysis does not detect improvement of *Mecp2*-null dendritic complexity by attenuated NF-κB signaling

To investigate whether modification of NF-κB signaling rescue dendritic complexity of *Mecp2*-null layer 2/3 pyramidal neurons, we crossed *Mecp2* mutant mice with *Nfkb1* mutant mice. We visualized the dendritic morphology of *Mecp2*^{+/y};*Nfkb1*^{+/+} (black line), *Mecp2*^{-/y};*Nfkb1*^{+/+} (red line), *Mecp2*^{-/y};*Nfkb1*^{+/-} (blue line), and *Mecp2*^{-/y};*Nfkb1*^{-/-} (green line) layer 2/3 pyramidal neurons by Golgi staining. Although we analyzed an initial (relatively low sensitivity) screen of dendritic complexity by Sholl analysis, we failed to detect significant differences in *Mecp2*-null dendritic complexity with additional disruption of *Nfkb1*.



Supplementary Figure 5: Phosphorylated P65 is increased relative to total P65 in cortex of Mecp2-nulls

We investigated NF- κ B activation in vivo in 8 week neocortex and heart by Western blot for phosphorylated p65/RelA, relative to total P65/RelA, and normalized to B-actin loading control. We identified an approximately 2-fold up-regulation of phospho-P65 in Mecp2-*y* cortex compared to littermates controls, but there was no significant difference in heart. Blots display representative data from one pair of littermates. Quantification was performed on three independent pairs of littermates. These images have been cropped for presentation in Figure 3E.

Supplementary Table 1: Genes over-expressed by *Mecp2*-null P14 CPN (fold change > 2.0, *P* < 0.001)

| Gene Symbol | NCBI Accession | Description | Fold Change <i>Mecp2</i>-null vs. WT | <i>P</i> Value |
|----------------------|-----------------------|--|---|-----------------------|
| <i>Rab39b</i> | AK020665 | RAB39B, member RAS oncogene family | 3.90 | 4.53E-08 |
| <i>Kif1b</i> | BE199508 | Kif1b kinesin family member 1B | 6.25 | 2.91E-07 |
| <i>Irak1</i> | AV091354 | interleukin-1 receptor-associated kinase 1 | 2.80 | 2.09E-06 |
| <i>Plekha1</i> | BB475301 | Pleckstrin homology domain containing, family A (phosphoinositide binding specific) member 1 | 5.28 | 2.00E-05 |
| <i>Cpm</i> | BB549552 | carboxypeptidase M | 7.83 | 3.00E-05 |
| <i>Cst12</i> | AF440737 | cystatin 12 | 3.97 | 6.00E-05 |
| | BB102018 | BB102018, EST | 2.25 | 1.70E-04 |
| <i>Igh-VJ558</i> | AK007826 | immunoglobulin heavy chain (J558 family) | 4.50 | 2.30E-04 |
| <i>5830434F19Rik</i> | AK017969 | RIKEN cDNA 5830434F19 gene | 5.32 | 2.90E-04 |
| <i>Utp23</i> | AK005831 | rRNA-processing protein UTP23 homolog | 5.69 | 4.50E-04 |
| <i>Tas2r119</i> | NM_020503 | taste receptor, type 2, member 119 | 4.71 | 4.70E-04 |
| <i>1700082M22Rik</i> | NM_028564 | RIKEN cDNA 1700082M22 gene | 3.98 | 6.70E-04 |
| <i>Gpatch8</i> | BC019948 | G patch domain containing 8 | 2.03 | 6.70E-04 |
| <i>Klra12</i> | AF204265 | killer cell lectin-like receptor subfamily A, member 12 | 3.09 | 7.90E-04 |
| <i>Ptprk</i> | BB667296 | protein tyrosine phosphatase, receptor type, K | 6.10 | 8.00E-04 |
| <i>2300010F08Rik</i> | AK009054 | RIKEN cDNA 2300010F08 gene | 4.12 | 9.30E-04 |
| <i>Dep1</i> | BB626684 | diabetic embryopathy 1 | 3.71 | 9.80E-04 |
| <i>V1rd1</i> | NM_030742 | vomer nasal 1 receptor, D1 | 3.02 | 9.90E-04 |

Supplementary Table 2: Genes under-expressed by *Mecp2*-null P14 CPN (fold change < -2.0, *P* < 0.001)

| Gene Symbol | NCBI Accession | Description | Fold Change <i>Mecp2</i> -null vs. WT | <i>P</i> Value |
|----------------------|----------------|---|--|----------------|
| <i>Mecp2</i> | BB130002 | methyl CpG binding protein 2 | -83.78 | 0.00E+00 |
| <i>Mecp2</i> | BC027153 | methyl CpG binding protein 2 | -12.71 | 1.28E-16 |
| <i>Cd99l2</i> | BB334959 | CD99 antigen-like 2 | -3.43 | 1.36E-06 |
| <i>Cd99l2</i> | BB334959 | CD99 antigen-like 2 | -5.79 | 4.33E-06 |
| <i>Ankrd17</i> | BG065567 | Ankyrin repeat domain 17 | -3.66 | 6.39E-06 |
| <i>Pde4b</i> | BG793493 | phosphodiesterase 4B, cAMP specific | -4.77 | 2.00E-05 |
| <i>A630023P12Rik</i> | AI451630 | RIKEN cDNA A630023P12 gene | -5.26 | 7.00E-05 |
| <i>Igfbp1b</i> | AK006203 | immunoglobulin (CD79A) binding protein 1b | -5.67 | 2.10E-04 |
| <i>Gsn</i> | NM_010354 | gelsolin | -3.65 | 2.50E-04 |
| <i>Gad1</i> | AF326547 | glutamic acid decarboxylase 1 | -2.84 | 2.80E-04 |
| | AW912607 | uf54a03.x1, EST | -3.82 | 3.10E-04 |
| | BQ031094 | UI-1-CF0-alz-c-03-0-UI.s1, EST | -2.14 | 4.50E-04 |
| <i>Nfatc1</i> | BB356861 | nuclear factor of activated T-cells, cytoplasmic, calcineurin-dependent 1 | -4.60 | 4.60E-04 |
| <i>D3Erttd300e</i> | NM_019995 | DNA segment, Chr 3, ERATO Doi 300, expressed | -6.54 | 4.70E-04 |
| <i>C330006K01Rik</i> | AV263612 | RIKEN cDNA C330006K01 gene | -4.92 | 4.80E-04 |
| <i>Hsd17b13</i> | AV050442 | hydroxysteroid (17-beta) dehydrogenase 13 | -4.13 | 5.10E-04 |
| <i>Thumpd2</i> | AK012806 | THUMP domain containing 2 | -4.11 | 5.80E-04 |
| <i>Mcf2</i> | NM_133197 | mcf.2 transforming sequence | -3.99 | 6.10E-04 |
| | BB830387 | BB830387, EST | -2.46 | 6.60E-04 |
| | BB341238 | BB341238, EST | -5.42 | 9.10E-04 |
| <i>2210010C04Rik</i> | AK008695 | RIKEN cDNA 2210010C04 gene | -5.22 | 9.50E-04 |

Supplementary Table 3: Genes over-expressed by *Mecp2*-null P14 CPN (fold change > 1.5, *P* < 0.005)

| Gene Symbol | NCBI Accession | Description | Fold Change <i>Mecp2</i>-null vs. WT | <i>P</i> Value |
|----------------------|-----------------------|--|---|-----------------------|
| <i>Rab39b</i> | AK020665 | RAB39B, member RAS oncogene family | 3.90 | 4.53E-08 |
| <i>Nhs12</i> | XM_00147828 | NHS-like 2 | 1.58 | 1.78E-07 |
| <i>Kif1b</i> | BE199508 | Kif1b kinesin family member 1B | 6.25 | 2.91E-07 |
| <i>Irak1</i> | AV091354 | interleukin-1 receptor-associated kinase 1 | 2.80 | 2.09E-06 |
| <i>Plekha1</i> | BB475301 | Pleckstrin homology domain containing, family A (phosphoinositide binding specific) member 1 | 5.28 | 2.00E-05 |
| <i>Fbxw10</i> | NM_001033669 | F-box and WD-40 domain protein 10 | 1.78 | 2.00E-05 |
| <i>Cpm</i> | BB549552 | carboxypeptidase M | 7.83 | 3.00E-05 |
| <i>Cst12</i> | AF440737 | cystatin 12 | 3.97 | 6.00E-05 |
| <i>Usp6nl</i> | NM_001080548 | USP6 N-terminal like | 1.51 | 1.20E-04 |
| | BB102018 | BB102018, EST | 2.25 | 1.70E-04 |
| <i>Igh-VJ558</i> | AK007826 | immunoglobulin heavy chain (J558 family) | 4.50 | 2.30E-04 |
| <i>5830434F19Rik</i> | AK017969 | RIKEN cDNA 5830434F19 gene | 5.32 | 2.90E-04 |
| <i>Utp23</i> | AK005831 | rRNA-processing protein UTP23 homolog | 5.69 | 4.50E-04 |
| <i>Pcdh17</i> | NM_001013753 | protocadherin 17 | 1.76 | 4.60E-04 |
| <i>Tas2r119</i> | NM_020503 | taste receptor, type 2, member 119 | 4.71 | 4.70E-04 |
| <i>1700082M22Rik</i> | NM_028564 | RIKEN cDNA 1700082M22 gene | 3.98 | 6.70E-04 |
| <i>Gpatch8</i> | BC019948 | G patch domain containing 8 | 2.03 | 6.70E-04 |
| <i>Klra12</i> | AF204265 | killer cell lectin-like receptor subfamily A, member 12 | 3.09 | 7.90E-04 |
| <i>Ptpnk</i> | BB667296 | protein tyrosine phosphatase, receptor type, K | 6.10 | 8.00E-04 |
| <i>2300010F08Rik</i> | AK009054 | RIKEN cDNA 2300010F08 gene | 4.12 | 9.30E-04 |
| <i>Dep1</i> | BB626684 | diabetic embryopathy 1 | 3.71 | 9.80E-04 |
| <i>V1rd1</i> | NM_030742 | vomer nasal 1 receptor, D1 | 3.02 | 9.90E-04 |
| <i>Il18bp</i> | NM_010531 | Il18bp interleukin 18 binding protein | 4.04 | 1.16E-03 |
| <i>Irak1</i> | NM_008363 | interleukin-1 receptor-associated kinase 1 | 2.79 | 1.24E-03 |
| <i>Naaladl2</i> | XM_91083 | N-acetylated alpha-linked acidic dipeptidase-like 2 | 3.83 | 1.29E-03 |

| Gene Symbol | NCBI Accession | Description | Fold Change <i>Mecp2</i> -null vs. WT | P Value |
|----------------------|----------------|---|--|----------|
| | BM249512 | BM249512, EST | 3.98 | 1.37E-03 |
| <i>Unc5b</i> | NM_029770 | Unc5b unc-5 homolog B (<i>C. elegans</i>) | 4.80 | 1.59E-03 |
| <i>Tmem2</i> | NM_001033759 | transmembrane protein 2 | 1.79 | 1.61E-03 |
| <i>Gpr107</i> | NM_178760 | G protein-coupled receptor 107 | 1.66 | 1.63E-03 |
| <i>Efcab3</i> | NM_001081046 | EF-hand calcium binding domain 3 | 3.03 | 1.67E-03 |
| <i>Ttll10</i> | NM_029264 | tubulin tyrosine ligase-like family, member 10 | 2.44 | 1.70E-03 |
| <i>Atp2c1</i> | NM_175025 | ATPase, Ca ⁺⁺ -sequestering | 2.85 | 1.96E-03 |
| <i>Mei1</i> | XM_901649 | Mei1 meiosis defective 1 | 4.08 | 1.97E-03 |
| <i>A630038E17Rik</i> | BC038285 | RIKEN cDNA A630038E17 gene | 3.91 | 2.03E-03 |
| <i>Irak1</i> | NM_008363 | interleukin-1 receptor-associated kinase 1 | 3.17 | 2.09E-03 |
| <i>Nrip1</i> | NM_173440 | nuclear receptor interacting protein 1 | 1.97 | 2.23E-03 |
| <i>LOC552912</i> | XR_005134 | hypothetical LOC552912 | 1.54 | 2.36E-03 |
| <i>Camk2d</i> | NM_001025438 | calcium/calmodulin-dependent protein kinase II, delta | 2.00 | 2.40E-03 |
| <i>Wdfy3</i> | XM_152907 | WD repeat and FYVE domain containing 3 | 2.05 | 2.60E-03 |
| | AK045858 | AK045858, EST | 2.53 | 2.64E-03 |
| <i>Klk5</i> | NM_008456 | Klk1b5 kallikrein 1-related peptidase b5 | 4.44 | 2.86E-03 |
| | BE995622 | UI-M-CG0p-bia-e-07-0-UI.s1, EST | 3.47 | 2.89E-03 |
| <i>4933439C20Rik</i> | NR_003518 | RIKEN cDNA 4933439C20 gene | 1.64 | 3.39E-03 |
| <i>Lpin2</i> | NM_022882 | Lipin 2 | 4.01 | 3.49E-03 |
| <i>4933402E13Rik</i> | XM_889482 | RIKEN cDNA 4933402E13 gene | 2.89 | 3.74E-03 |
| <i>5033405D04Rik</i> | AK019887 | RIKEN cDNA 5033405D04 gene | 2.38 | 3.85E-03 |
| <i>Neurl3</i> | NM_153408 | Neurl3 neuralized homolog 3 homolog (<i>Drosophila</i>) | 4.92 | 3.94E-03 |
| <i>Obox5</i> | NM_145709 | oocyte specific homeobox 5 | 2.71 | 4.04E-03 |
| <i>Grip1</i> | AK086934 | Glutamate receptor interacting protein 1 | 4.15 | 4.13E-03 |
| <i>D18Erttd653e</i> | NM_172631 | DNA segment, Chr 18, ERATO Doi 653, expressed | 3.56 | 4.31E-03 |
| <i>Ovgp1</i> | NM_007696 | oviductal glycoprotein 1 | 2.36 | 4.47E-03 |
| <i>Casr</i> | NM_013803 | calcium-sensing receptor | 3.95 | 4.49E-03 |
| <i>Rab34</i> | NM_033475 | RAB34, member of RAS oncogene family | 1.84 | 4.68E-03 |

| Gene Symbol | NCBI Accession | Description | Fold Change <i>Mecp2</i>-null vs. WT | P Value |
|--------------------|---------------------------|--------------------------|---|----------------|
| | AK084017 | AK084017, EST | 1.60 | 4.69E-03 |
| <i>Adora2a</i> | NM_009630 | adenosine A2a receptor | 2.18 | 4.82E-03 |
| <i>Tcfe2a</i> | NM_011548 | transcription factor E2a | 5.06 | 4.92E-03 |

Supplementary Table 4: Genes under-expressed by *Mecp2*-null P14 CPN (fold change < -1.5, *P* < 0.005)

| Gene Symbol | NCBI Accession | Description | Fold Change <i>Mecp2</i> -null vs. WT | <i>P</i> Value |
|----------------------|----------------|---|---------------------------------------|----------------|
| <i>Mecp2</i> | BB130002 | methyl CpG binding protein 2 | -83.78 | 0.00E+00 |
| <i>Mecp2</i> | BC027153 | methyl CpG binding protein 2 | -12.71 | 1.28E-16 |
| <i>Cd99l2</i> | BB334959 | CD99 antigen-like 2 | -3.43 | 1.36E-06 |
| <i>Cd99l2</i> | BB334959 | CD99 antigen-like 2 | -5.79 | 4.33E-06 |
| <i>Ankrd17</i> | BG065567 | Ankyrin repeat domain 17 | -3.66 | 6.39E-06 |
| <i>Pde4b</i> | BG793493 | phosphodiesterase 4B, cAMP specific | -4.77 | 2.00E-05 |
| <i>A630023P12Rik</i> | AI451630 | RIKEN cDNA A630023P12 gene | -5.26 | 7.00E-05 |
| <i>Mycn</i> | NM_008709 | v-myc myelocytomatosis viral related oncogene | -1.93 | 1.40E-04 |
| <i>Igfbp1b</i> | AK006203 | immunoglobulin (CD79A) binding protein 1b | -5.67 | 2.10E-04 |
| <i>Ccdc126</i> | NM_175098 | coiled-coil domain containing 126 | -1.90 | 2.40E-04 |
| <i>Gsn</i> | NM_010354 | gelsolin | -3.65 | 2.50E-04 |
| <i>Gad1</i> | AF326547 | glutamic acid decarboxylase 1 | -2.84 | 2.80E-04 |
| | AW912607 | uf54a03.x1, EST | -3.82 | 3.10E-04 |
| | BQ031094 | UI-1-CF0-alz-c-03-0-UI.s1, EST | -2.14 | 4.50E-04 |
| <i>Nfatc1</i> | BB356861 | nuclear factor of activated T-cells, cytoplasmic, calcineurin-dependent 1 | -4.60 | 4.60E-04 |
| <i>D3Erttd300e</i> | NM_019995 | DNA segment, Chr 3, ERATO Doi 300, expressed | -6.54 | 4.70E-04 |
| <i>C330006K01Rik</i> | AV263612 | RIKEN cDNA C330006K01 gene | -4.92 | 4.80E-04 |
| <i>Hsd17b13</i> | AV050442 | hydroxysteroid (17-beta) dehydrogenase 13 | -4.13 | 5.10E-04 |
| <i>Thumpd2</i> | AK012806 | THUMP domain containing 2 | -4.11 | 5.80E-04 |
| <i>Mcf2</i> | NM_133197 | mcf.2 transforming sequence | -3.99 | 6.10E-04 |
| | BB830387 | BB830387, EST | -2.46 | 6.60E-04 |
| | BB341238 | BB341238, EST | -5.42 | 9.10E-04 |
| <i>2210010C04Rik</i> | AK008695 | RIKEN cDNA 2210010C04 gene | -5.22 | 9.50E-04 |
| <i>Eps15l1</i> | NM_001122832 | epidermal growth factor receptor pathway substrate 15-like 1 | -4.34 | 1.12E-03 |
| | | | | 1.17E-03 |
| <i>Zfp287</i> | NM_133208 | Zinc finger protein 287 | -5.57 | |

| Gene Symbol | NCBI Accession | Description | Fold Change <i>Mecp2</i> -null vs. WT | P Value |
|----------------------|----------------|---|--|----------|
| <i>6430537H07Rik</i> | NM_178689 | RIKEN cDNA 6430537H07 gene | -3.00 | 1.21E-03 |
| <i>Nfatc2</i> | NM_001037177 | nuclear factor of activated T-cells, cytoplasmic, calcineurin-dependent 2 | -4.70 | 1.36E-03 |
| <i>Ints7</i> | NM_178632 | integrator complex subunit 7 | -3.21 | 1.49E-03 |
| <i>Sidt1</i> | NM_198034 | SID1 transmembrane family, member 1 | -5.49 | 1.52E-03 |
| | C76313 | C76313, EST | -3.58 | 1.52E-03 |
| | AK044288 | AK044288, EST | -3.45 | 1.89E-03 |
| <i>Tgm2</i> | NM_009373 | transglutaminase 2, C polypeptide | -7.72 | 1.93E-03 |
| | AA241963 | AA241963, EST | -2.57 | 2.17E-03 |
| <i>Lmcd1</i> | NM_144799 | LIM and cysteine-rich domains 1 | -1.90 | 2.26E-03 |
| <i>Thap7</i> | NM_026909 | THAP domain containing 7 | -1.72 | 2.30E-03 |
| | AW124522 | AW124522, EST | -4.60 | 2.41E-03 |
| <i>Gpr150</i> | NM_175495 | G protein-coupled receptor 150 | -5.80 | 2.46E-03 |
| <i>Wdr92</i> | NM_178909 | WD repeat domain 92 | -2.08 | 2.49E-03 |
| <i>Btb11</i> | NM_001017525 | BTB (POZ) domain containing 11 | -2.35 | 2.57E-03 |
| <i>Gja1</i> | NM_010288 | gap junction protein, alpha 1 | -2.64 | 2.65E-03 |
| <i>Ccnd2</i> | NM_009829 | cyclin D2 | -3.55 | 2.72E-03 |
| <i>5330425B07Rik</i> | AK019908 | RIKEN cDNA 5330425B07 gene | -4.24 | 2.73E-03 |
| <i>Sumf2</i> | NM_026445 | sulfatase modifying factor 2 | -6.44 | 2.92E-03 |
| <i>Il13ra1</i> | NM_133990 | interleukin 13 receptor, alpha 1 | -4.77 | 2.99E-03 |
| | BB810913 | BB810913, EST | -5.25 | 3.21E-03 |
| <i>E130317F20Rik</i> | AK148204 | RIKEN cDNA E130317F20 gene | -6.10 | 3.28E-03 |
| <i>Snmp70</i> | NM_009224 | small nuclear ribonucleoprotein 70 (U1) | -4.92 | 3.31E-03 |
| <i>Traf4</i> | NM_009423 | Tnf receptor associated factor 4 | -4.22 | 3.32E-03 |
| <i>Gsn</i> | NM_146120 | gelsolin | -1.96 | 3.33E-03 |
| <i>Prl7a1</i> | NM_008930 | prolactin family 7, subfamily a, member 1 | -4.06 | 3.38E-03 |
| <i>Plp1</i> | NM_011123 | proteolipid protein (myelin) 1 | -2.33 | 3.39E-03 |
| | T25656 | T25656, EST | -4.30 | 3.42E-03 |
| <i>Plp1</i> | NM_011123 | proteolipid protein (myelin) 1 | -5.19 | 3.46E-03 |

| Gene Symbol | NCBI Accession | Description | Fold Change <i>Mecp2</i>-null vs. WT | P Value |
|----------------------|-----------------------|---|---|----------------|
| <i>Ccnf</i> | NM_007634 | cyclin F | -2.32 | 3.59E-03 |
| <i>A330068G13Rik</i> | XR_002354 | RIKEN cDNA A330068G13 gene | -5.53 | 3.89E-03 |
| <i>Lcp2</i> | NM_010696 | lymphocyte cytosolic protein 2 | -4.28 | 3.92E-03 |
| <i>Figl1</i> | NM_021891 | fidgetin-like 1 | -6.56 | 4.03E-03 |
| <i>Mycn</i> | NM_008709 | v-myc myelocytomatosis viral related oncogene | -3.46 | 4.24E-03 |
| <i>Zxdc</i> | NM_030260 | ZXD family zinc finger C | -2.00 | 4.42E-03 |
| <i>2610002M06Rik</i> | NM_025921 | RIKEN cDNA 2610002M06 gene | -1.61 | 4.64E-03 |
| <i>Bcl7c</i> | NM_009746 | B-cell CLL/lymphoma 7C | -2.72 | 4.75E-03 |
| | AK079106 | AK079106, EST | -9.10 | 4.77E-03 |
| <i>Cdad1</i> | NM_027986 | cytidine and dCMP deaminase domain containing 1 | -2.45 | 4.77E-03 |
| <i>Higd1c</i> | NM_001002900 | HIG1 domain family, member 1C | -2.16 | 4.90E-03 |
| <i>Mecp2</i> | NM_001081979 | methyl CpG binding protein 2 | -6.08 | 4.97E-03 |

Supplementary Table 5: Functional categorization of dysregulated genes in *Mecp2*-null P14 CPN (fold change > 1.5 or < -1.5, P < 0.005)

Dendritic, cytoskeletal, and synaptic formation, maturation, and maintenance

| Gene Symbol | NCBI Accession | Description | Fold Change <i>Mecp2</i> -null vs. WT | P Value |
|-----------------|----------------|---|---------------------------------------|----------|
| <i>Mecp2</i> | BB130002 | methyl CpG binding protein 2 | -83.78 | 0.00E+00 |
| <i>Mecp2</i> | BC027153 | methyl CpG binding protein 2 | -12.71 | 1.28E-16 |
| <i>Kif1b</i> | BE199508 | Kif1b kinesin family member 1B | 6.25 | 2.91E-07 |
| <i>Pde4b</i> | BG793493 | phosphodiesterase 4B, cAMP specific | -4.77 | 2.00E-05 |
| <i>Gsn</i> | NM_010354 | gelsolin | -3.65 | 2.50E-04 |
| <i>Pcdh17</i> | NM_001013753 | protocadherin 17 | 1.76 | 4.60E-04 |
| <i>Mcf2</i> | NM_133197 | mcf.2 transforming sequence | -3.99 | 6.10E-04 |
| <i>Naaladl2</i> | XM_91083 | N-acetylated alpha-linked acidic dipeptidase-like 2 | 3.83 | 1.29E-03 |
| <i>Unc5b</i> | NM_029770 | Unc5b unc-5 homolog B (C. elegans) | 4.80 | 1.59E-03 |
| <i>Tmem2</i> | NM_001033759 | transmembrane protein 2 | 1.79 | 1.61E-03 |
| <i>Camk2d</i> | NM_001025438 | calcium/calmodulin-dependent protein kinase II, delta | 2.00 | 2.40E-03 |
| <i>Gsn</i> | NM_146120 | gelsolin | -1.96 | 3.33E-03 |
| <i>Plp1</i> | NM_011123 | proteolipid protein (myelin) 1 | -2.33 | 3.39E-03 |
| <i>Plp1</i> | NM_011123 | proteolipid protein (myelin) 1 | -5.19 | 3.46E-03 |
| <i>Grip1</i> | AK086934 | Glutamate receptor interacting protein 1 | 4.15 | 4.13E-03 |
| <i>Adora2a</i> | NM_009630 | adenosine A2a receptor | 2.18 | 4.82E-03 |
| <i>Mecp2</i> | NM_001081979 | methyl CpG binding protein 2 | -6.08 | 4.97E-03 |

Standardly viewed as immune system-related

| Gene Symbol | NCBI Accession | Description | Fold Change <i>Mecp2</i> -null vs. WT | P Value |
|------------------|----------------|--|---------------------------------------|----------|
| <i>Cd99l2</i> | BB334959 | CD99 antigen-like 2 | -3.43 | 1.36E-06 |
| <i>Irak1</i> | AV091354 | interleukin-1 receptor-associated kinase 1 | 2.80 | 2.09E-06 |
| <i>Cd99l2</i> | BB334959 | CD99 antigen-like 2 | -5.79 | 4.33E-06 |
| <i>Igfbp1b</i> | AK006203 | immunoglobulin (CD79A) binding protein 1b | -5.67 | 2.10E-04 |
| <i>Igh-VJ558</i> | AK007826 | immunoglobulin heavy chain (J558 family) | 4.50 | 2.30E-04 |

| | | | | |
|----------------|-----------|---|-------|----------|
| <i>Klra12</i> | AF204265 | killer cell lectin-like receptor subfamily A, member 12 | 3.09 | 7.90E-04 |
| <i>Il18bp</i> | NM_010531 | Il18bp interleukin 18 binding protein | 4.04 | 1.16E-03 |
| <i>Zfp287</i> | NM_133208 | Zinc finger protein 287 | -5.57 | 1.17E-03 |
| <i>Irak1</i> | NM_008363 | interleukin-1 receptor-associated kinase 1 | 2.79 | 1.24E-03 |
| <i>Irak1</i> | NM_008363 | interleukin-1 receptor-associated kinase 1 | 3.17 | 2.09E-03 |
| <i>Il13ra1</i> | NM_133990 | interleukin 13 receptor, alpha 1 | -4.77 | 2.99E-03 |
| <i>Lcp2</i> | NM_010696 | lymphocyte cytosolic protein 2 | -4.28 | 3.92E-03 |
| <i>Zxdc</i> | NM_030260 | ZXD family zinc finger C | -2.00 | 4.42E-03 |

Metabolism, endocrine

| Gene Symbol | NCBI Accession | Description | Fold Change Mecp2-null vs. WT | P Value |
|----------------------|----------------|--|-------------------------------|----------|
| <i>Cpm</i> | BB549552 | carboxypeptidase M | 7.83 | 3.00E-05 |
| <i>Plekha1</i> | BB475301 | Pleckstrin homology domain containing, family A (phosphoinositide binding specific) member 1 | 5.28 | 2.00E-05 |
| <i>Lpin2</i> | NM_022882 | Lipin 2 | 4.01 | 3.49E-03 |
| <i>Casr</i> | NM_013803 | calcium-sensing receptor | 3.95 | 4.49E-03 |
| <i>Dep1</i> | BB626684 | diabetic embryopathy 1 | 3.71 | 9.80E-04 |
| <i>V1rd1</i> | NM_030742 | vomeronasal 1 receptor, D1 | 3.02 | 9.90E-04 |
| <i>6430537H07Rik</i> | NM_178689 | RIKEN cDNA 6430537H07 gene | -3.00 | 1.21E-03 |

Reproduction

| Gene Symbol | NCBI Accession | Description | Fold Change Mecp2-null over WT | P Value |
|--------------|----------------|---|--------------------------------|----------|
| <i>Cst12</i> | AF440737 | cystatin 12 | 3.97 | 6.00E-05 |
| <i>Mei1</i> | XM_901649 | Mei1 meiosis defective 1 | 4.08 | 1.97E-03 |
| <i>Obox5</i> | NM_145709 | oocyte specific homeobox 5 | 2.71 | 4.04E-03 |
| <i>Ovgp1</i> | NM_007696 | oviductal glycoprotein 1 | 2.36 | 4.47E-03 |
| <i>Cdad1</i> | NM_027986 | cytidine and dCMP deaminase domain containing 1 | -2.45 | 4.77E-03 |

Transcription, RNA processing, DNA modification

| Gene Symbol | NCBI Accession | Description | Fold Change Mecp2-null vs. WT | P Value |
|----------------|----------------|---|----------------------------------|----------|
| <i>Utp23</i> | AK005831 | rRNA-processing protein UTP23 homolog | 5.69 | 4.50E-04 |
| <i>Nfatc1</i> | BB356861 | nuclear factor of activated T-cells, cytoplasmic, calcineurin-dependent 1 | -4.60 | 4.60E-04 |
| <i>Nfatc2</i> | NM_001037177 | nuclear factor of activated T-cells, cytoplasmic, calcineurin-dependent 2 | -4.70 | 1.36E-03 |
| <i>Ints7</i> | NM_178632 | integrator complex subunit 7 | -3.21 | 1.49E-03 |
| <i>Tll10</i> | NM_029264 | tubulin tyrosine ligase-like family, member 10 | 2.44 | 1.70E-03 |
| <i>Lmcd1</i> | NM_144799 | LIM and cysteine-rich domains 1 | -1.90 | 2.26E-03 |
| <i>Thap7</i> | NM_026909 | THAP domain containing 7 | -1.72 | 2.30E-03 |
| <i>Wdr92</i> | NM_178909 | WD repeat domain 92 | -2.08 | 2.49E-03 |
| <i>Snrnp70</i> | NM_009224 | small nuclear ribonucleoprotein 70 (U1) | -4.92 | 3.31E-03 |
| <i>Tcfe2a</i> | NM_011548 | transcription factor E2a | 5.06 | 4.92E-03 |

Miscellaneous

| Gene Symbol | NCBI Accession | Description | Fold Change Mecp2-null vs. WT | P Value |
|----------------------|----------------|---|----------------------------------|----------|
| <i>Rab39b</i> | AK020665 | RAB39B, member RAS oncogene family | 3.90 | 4.53E-08 |
| <i>Nhsl2</i> | XM_00147828 | NHS-like 2 | 1.58 | 1.78E-07 |
| <i>Ankrd17</i> | BG065567 | Ankyrin repeat domain 17 | -3.66 | 6.39E-06 |
| <i>Fbxw10</i> | NM_001033669 | F-box and WD-40 domain protein 10 | 1.78 | 2.00E-05 |
| <i>A630023P12Rik</i> | AI451630 | RIKEN cDNA A630023P12 gene | -5.26 | 7.00E-05 |
| <i>Usp6nl</i> | NM_001080548 | USP6 N-terminal like | 1.51 | 1.20E-04 |
| <i>Mycn</i> | NM_008709 | v-myc myelocytomatosis viral related oncogene | -1.93 | 1.40E-04 |
| | BB102018 | BB102018, EST | 2.25 | 1.70E-04 |
| <i>Ccdc126</i> | NM_175098 | coiled-coil domain containing 126 | -1.90 | 2.40E-04 |
| <i>5830434F19Rik</i> | AK017969 | RIKEN cDNA 5830434F19 gene | 5.32 | 2.90E-04 |
| | AW912607 | uf54a03.x1, EST | -3.82 | 3.10E-04 |
| | BQ031094 | UI-1-CF0-alz-c-03-0-UI.s1, EST | -2.14 | 4.50E-04 |
| <i>Tas2r119</i> | NM_020503 | taste receptor, type 2, member 119 | 4.71 | 4.70E-04 |
| <i>D3Erttd300e</i> | NM_019995 | DNA segment, Chr 3, ERATO Doi 300, expressed | -6.54 | 4.70E-04 |
| <i>C330006K01Rik</i> | AV263612 | RIKEN cDNA C330006K01 gene | -4.92 | 4.80E-04 |

| | | | | |
|----------------------|--------------|--|-------|----------|
| <i>Hsd17b13</i> | AV050442 | hydroxysteroid (17-beta) dehydrogenase 13 | -4.13 | 5.10E-04 |
| <i>Thumpd2</i> | AK012806 | THUMP domain containing 2 | -4.11 | 5.80E-04 |
| | BB830387 | BB830387, EST | -2.46 | 6.60E-04 |
| <i>1700082M22Rik</i> | NM_028564 | RIKEN cDNA 1700082M22 gene | 3.98 | 6.70E-04 |
| <i>Gpatch8</i> | BC019948 | G patch domain containing 8 | 2.03 | 6.70E-04 |
| <i>Ptpk</i> | BB667296 | protein tyrosine phosphatase, receptor type, K | 6.10 | 8.00E-04 |
| | BB341238 | BB341238, EST | -5.42 | 9.10E-04 |
| <i>2300010F08Rik</i> | AK009054 | RIKEN cDNA 2300010F08 gene | 4.12 | 9.30E-04 |
| <i>2210010C04Rik</i> | AK008695 | RIKEN cDNA 2210010C04 gene | -5.22 | 9.50E-04 |
| <i>Eps15l1</i> | NM_001122832 | epidermal growth factor receptor pathway substrate 15-like 1 | -4.34 | 1.12E-03 |
| | BM249512 | BM249512, EST | 3.98 | 1.37E-03 |
| | C76313 | C76313, EST | -3.58 | 1.52E-03 |
| <i>Sidt1</i> | NM_198034 | SID1 transmembrane family, member 1 | -5.49 | 1.52E-03 |
| <i>Gpr107</i> | NM_178760 | G protein-coupled receptor 107 | 1.66 | 1.63E-03 |
| | AK044288 | AK044288, EST | -3.45 | 1.89E-03 |
| <i>Tgm2</i> | NM_009373 | transglutaminase 2, C polypeptide | -7.72 | 1.93E-03 |
| <i>Atp2c1</i> | NM_175025 | ATPase, Ca ⁺⁺ -sequestering | 2.85 | 1.96E-03 |
| <i>A630038E17Rik</i> | BC038285 | RIKEN cDNA A630038E17 gene | 3.91 | 2.03E-03 |
| | AA241963 | AA241963, EST | -2.57 | 2.17E-03 |
| <i>LOC552912</i> | XR_005134 | hypothetical LOC552912 | 1.54 | 2.36E-03 |
| | AW124522 | AW124522, EST | -4.60 | 2.41E-03 |
| <i>Gpr150</i> | NM_175495 | G protein-coupled receptor 150 | -5.80 | 2.46E-03 |
| <i>Btbd11</i> | NM_001017525 | BTB (POZ) domain containing 11 | -2.35 | 2.57E-03 |
| <i>Wdfy3</i> | XM_152907 | WD repeat and FYVE domain containing 3 | 2.05 | 2.60E-03 |
| | AK045858 | AK045858, EST | 2.53 | 2.64E-03 |
| <i>Gja1</i> | NM_010288 | gap junction protein, alpha 1 | -2.64 | 2.65E-03 |
| <i>Ccnd2</i> | NM_009829 | cyclin D2 | -3.55 | 2.72E-03 |
| <i>5330425B07Rik</i> | AK019908 | RIKEN cDNA 5330425B07 gene | -4.24 | 2.73E-03 |
| <i>Klk5</i> | NM_008456 | Klk1b5 kallikrein 1-related peptidase b5 | 4.44 | 2.86E-03 |

| | | | | |
|----------------------|--------------|--|-------|----------|
| | BE995622 | UI-M-CG0p-bia-e-07-0-UI.s1, EST | 3.47 | 2.89E-03 |
| <i>Sumf2</i> | NM_026445 | sulfatase modifying factor 2 | -6.44 | 2.92E-03 |
| | BB810913 | BB810913, EST | -5.25 | 3.21E-03 |
| <i>E130317F20Rik</i> | AK148204 | RIKEN cDNA E130317F20 gene | -6.10 | 3.28E-03 |
| <i>Traf4</i> | NM_009423 | Tnf receptor associated factor 4 | -4.22 | 3.32E-03 |
| <i>Prl7a1</i> | NM_008930 | prolactin family 7, subfamily a, member 1 | -4.06 | 3.38E-03 |
| <i>4933439C20Rik</i> | NR_003518 | RIKEN cDNA 4933439C20 gene | 1.64 | 3.39E-03 |
| | T25656 | T25656, EST | -4.30 | 3.42E-03 |
| <i>Ccnf</i> | NM_007634 | cyclin F | -2.32 | 3.59E-03 |
| <i>4933402E13Rik</i> | XM_889482 | RIKEN cDNA 4933402E13 gene | 2.89 | 3.74E-03 |
| <i>5033405D04Rik</i> | AK019887 | RIKEN cDNA 5033405D04 gene | 2.38 | 3.85E-03 |
| <i>A330068G13Rik</i> | XR_002354 | RIKEN cDNA A330068G13 gene | -5.53 | 3.89E-03 |
| <i>Neur13</i> | NM_153408 | Neur13 neuralized homolog 3 homolog (Drosophila) | 4.92 | 3.94E-03 |
| <i>Figl1</i> | NM_021891 | fidgetin-like 1 | -6.56 | 4.03E-03 |
| <i>Mycn</i> | NM_008709 | v-myc myelocytomatosis viral related oncogene | -3.46 | 4.24E-03 |
| <i>D18Ert653e</i> | NM_172631 | DNA segment, Chr 18, ERATO Doi 653, expressed | 3.56 | 4.31E-03 |
| <i>2610002M06Rik</i> | NM_025921 | RIKEN cDNA 2610002M06 gene | -1.61 | 4.64E-03 |
| <i>Rab34</i> | NM_033475 | RAB34, member of RAS oncogene family | 1.84 | 4.68E-03 |
| | AK084017 | AK084017, EST | 1.60 | 4.69E-03 |
| <i>Bcl7c</i> | NM_009746 | B-cell CLL/lymphoma 7C | -2.72 | 4.75E-03 |
| | AK079106 | AK079106, EST | -9.10 | 4.77E-03 |
| <i>Higd1c</i> | NM_001002900 | HIG1 domain family, member 1C | -2.16 | 4.90E-03 |

Supplementary Methods:

Bisulfite genomic sequencing

Genomic DNA was extracted from three independent cortices of wild-type P14 male C57BL/6 mice using the DNeasy Blood & Tissue kit according to the manufacturer's instructions (Qiagen, Valencia, CA). 500 ng of genomic DNA was used for bisulfite treatment using the EZ DNA Methylation-Gold kit (Zymo Research, Orange, CA). Bisulfite-modified DNA was amplified by PCR using the following six primer pairs covering 79 CpGs on the promoter region of *Irak1*.

| | | |
|---------|---------|---|
| Pair 1: | Forward | 5'- GAG GAT AAT GGT ATG TTA TAA -3' |
| | Reverse | 5'- TTA ACC ATA TAC TAA ACT TCC -3' |
| Pair 2: | Forward | 5'- GTT AGT TAT GGA TTT TGT GTA -3' |
| | Reverse | 5'- CTA AAC CCA ACT CCT CTC TTA -3' |
| Pair 3: | Forward | 5'- GTG TAT TTA TAT GAA GAG GAT -3' |
| | Reverse | 5'- AAT ATA TAC CAT ACT ACT ACT-3' |
| Pair 4: | Forward | 5'- GAT TGA TTA ATA ATT TGG TTG -3' |
| | Reverse | 5'- AAT CTATAA TCC TAC ACAAAC -3' |
| Pair 5: | Forward | 5'- TTA GAT GTT TGT GTA GGA TTA TAG TTT -3' |
| | Reverse | 5'- ACA CAT AAC CCA AAA TAA CAC CT -3' |
| Pair 6: | Forward | 5'- AGG TGT TAT TTT GGG TTA TGT -3' |
| | Reverse | 5'- TCA CAA ACT ATA ATA ATA TCC -3' |

The PCR products were subcloned into the pCRII-TOPO vector (Invitrogen), and 10 clones were sequenced for each PCR product (for a total of 30 clones from 3 independent P14 cortical samples).

Chromatin immunoprecipitation (ChIP)

Cortices were dissected from three P14 mice for each ChIP experiment, and dissociated as described above. ChIP was performed three times using the EZ ChIP kit (Millipore, Billerica, MA). Approximately six million dissociated cortical cells were fixed in 1% paraformaldehyde at room temperature for 10 min, and unreacted paraformaldehyde was then quenched by adding glycine solution (final 0.125 M). After washing the cross-linked dissociated cells with ice-cold PBS twice, the cells were lysed in 600 μ l SDS lysis buffer [1% SDS, 10 mM EDTA, 50 mM Tris-HCl (pH 8.1), protease inhibitor cocktail]. The lysate was sonicated on ice with a Branson Sonifier 450 (Branson, Danbury, CT) using a double stepped microtip (tip diameter 3 mm) for 10 sec, 12 times, at power level 1, to produce fragments with an average length of ~100 to 1,000 bp. 100 μ l of sonicated chromatin was added to 900 μ l of ChIP dilution buffer (Millipore). The sample was precleared with Protein G Agarose (Millipore) for one hour at 4 °C, and 1% of the sample was set aside as an input sample. The precleared lysate was incubated with either 5 μ g of rabbit polyclonal anti-MeCP2 antibody (Millipore) or 5 μ g of rabbit IgG (Sigma-Aldrich, St. Louis, MO) overnight at 4°C. Protein-DNA-antibody complexes were precipitated with Protein G Agarose, and were washed sequentially with low salt buffer, high salt buffer, and TE buffer (Millipore). The precipitated protein-DNA-antibody complexes were eluted from Protein G Agarose with 1% SDS and 0.1 M NaHCO₃, and then incubated at 65 °C for 5 hours in 200 mM NaCl to reverse formaldehyde cross-links. Following RNase A and proteinase K treatment, the sample was purified by phenol/chloroform extraction and ethanol precipitation. The IP samples were subjected to PCR amplification using the following primer pairs that cover the 5 highly methylated CpGs on the *Irak1* promoter:

Pair 1 (for -2107 bp CpG)

Forward 5'- ATT GTG AGC ATA CCT AGA AGC -3'

Reverse 5'- CCA AGA GGT TAA CCATAT GCT -3'

Pair 2 (for -1492 bp CpG)

Forward 5'- TCT GAA GAC ATA CTA TAA GTT -3'

Reverse 5'- TGA ACA CAC CTT GAG CTT GCT -3'

Pair 3 (for -779, -547, and -426 bp CpGs)

Forward 5'- CAG AGA GCA TAA TGA GAA AGT -3'

Reverse 5'- TTG TGT GCT AGA TCT CCA TGC -3'