Fig. S1. Phenotypic characteristics of the RTT rat. (A–C) Effects on CNS neurite outgrowth (A) in cell culture, cortical neurons from WT P0 rat showed characteristic neurite outgrowth. (B) Neurite growth from cortical neurons of P0 Mecp2−/y KO rats were stunted. (Magnification: 100×) (C) Quantification revealed that mean neurite outgrowth in KO neurons is significantly reduced compared with the WT (*P < 0.001). (D–F) Effects on motor activity. (D) Behavioral testing was performed at 30 and 51 d of age to assess whether expected motor deficiencies occurred in rats with the Mecp2−/y genotype (30 d, n = 5 animals per group; 51 d, n = 4 animals per group). Motor coordination was tested using a Rota-Rod treadmill (MedAssociates) rotating at a speed of 4 rpm and ramping to a maximum speed of 40 rpm over an acceleration period of 300 s. Each animal was tested three times. KO rats showed a substantial deficit in motor coordination (*P = 0.005). (E) Relative activity was assessed by allowing rats 10 min of free run time in the novel environment of a force plate actimeter (BASI). Total distance traveled in meters was lower in the RTT rats (*P = 0.026). (F) Bouts of low mobility represent periods of 10 s or greater when the rat’s movements are confined to a 15-mm radius during the 10-min test period. KO rats showed more bouts than WT (*P = 0.009 by Mann–Whitney U test).
Fig. S2. MeCP2 is expressed in all dorsal root ganglion sensory neurons. (A) Staining for MeCP2 at low magnification shows overall expression in DRG cross section. (Inset) Higher magnification of same section. (B) Overlay of A with DAPI costaining confirming nuclear localization of MeCP2 in neuronal and satellite cells. (C) MeCP2 expression in cortical neurons and (D) cerebellar neurons. (Scale bars, 50 μm in A–D.) (E–P) MeCP2 expression in DRG neuronal subpopulations. Immunostaining for CGRP (E) and MeCP2 (F) and DAPI nuclear staining (G) shows nuclear MeCP2 localization in CGRP+ peptidergic neurons (H). IB4 isolectin binding (I), MeCP2 immunostaining (J), and DAPI staining (K) shows nuclear MeCP2 expression in nonpeptidergic neurons (L). Large-diameter myelinated neurons positive for NFH (M), MeCP2 (N) and DAPI (O) show nuclear localization in large neurons as well (P). (Scale bar in H, 50 μm; also for E–P.)
Fig. S3. Peptidergic and nonpeptidergic neurons are transduced with similar efficiency by lentiviral injections. (A) A section of the L4 DRG stained for IB4 and its corresponding GFP expression (B) identifies lentiviral transduction of nonpeptidergic neurons (arrows). (C) Staining for CGRP in an L4 DRG section and corresponding GFP staining (D) identifies peptidergic neurons transduced by retrogradely transported lentivirus (arrows). (E) Comparison of percentage of neurons transduced in L4-L5 DRG that are nonpeptidergic vs. peptidergic reveals no difference (i.e., subpopulation-specific bias) (n = 5 animals). (Scale bar, 50 μm.)

Fig. S4. Effect of DRG gene modulation on central projections to the spinal cord. (A and B) MeCP2 knockdown in DRG of WT rats. Analysis of lumbar spinal cord from rats in which DRG neurons were transduced for MeCP2 expression revealed no difference in gray matter area occupied by nonpeptidergic IB4+ projections (A) or CGRP-ir projections (B). Multigene knockdown in DRG of MeCP2 KO rats. (C and D) Analysis of lumbar spinal cord from KO rats in which footpad-projecting DRG neurons underwent knockdown of eight key cytoskeleton-related genes showed no changes in IB4+ (C) or CGRP-ir (D) central projections.
Fig. S5. Validation and analyses of RNA Sequencing data. (A) Principal component analysis with counts obtained from STAR mapped reads and counted with HTSeq analysis done using the EdgeR program. DRG RNA was sequenced for four animals of each genotype. (B) Smear plot of expression in counts per million (cpm) vs. fold-change shows an unskewed and uniform distribution along the x axis, reflecting good quality of analysis. (C) Volcano plot of logarithm of fold-change vs. FDR P-value of differentially expressed genes. Genes that are distributed above the blue line for FDR cut-off and beyond the red lines represent either up- or down-regulated (absolute fold-change cut-off = ±1.2) that were used in downstream analyses. Differential gene expression analysis with a P ≤ 0.05 for FDR revealed 720 up-regulated and 627 down-regulated genes at a cut-off of ≥20% (1.2-fold). (D) qPCR validations of multiple significant MeCP2-regulated candidate genes revealed by the RNA-Seq. Values are fold-change in Mecp2−/y DRG mRNA relative to Mecp2+/y mRNA (n = 4 animals per group). (E) Disease and functions pathways significantly altered by MeCP2 KO in sensory neurons, as revealed in IPA of all regulated candidate genes with ±1.2 fold-change and FDR = 0.05 cut-off.
Fig. S6. MeCP2 regulation of long genes in sensory neurons. (A) IPA of up-regulated genes with >100-kb length was conducted to examine affected biological pathways in sequencing data from KO rats. Negative logarithm to the base 10 of \( P \) values of significantly associated categories in functions. Long genes up-regulated in the category of “Cellular assembly and organization.” (B) Canonical pathways implicated in MeCP2 functions identified by up-regulation of long genes alone in KO rats. (C) qPCR of selected long genes in WT rats shows gene function was affected only at high concentrations of topotecan.
Fig. S7. MeCP2 knockdown increases neurite outgrowth in the 50B11 DRG neuronal cell line. (A) A 50B11 cell transduced by scrambled shRNA (and selected with 10 μg/mL puromycin) show basal neurite outgrowth characteristic of nontransduced cells. (B) Cells display increased neurite outgrowth upon MeCP2 knockdown. (C) Quantification reveals increased total neurite outgrowth per neuron upon MeCP2 knockdown. (*P < 0.001 in Mann–Whitney U test). (D) Outgrowth in 50B11 cells following Tiam1 knockdown. (E) Comparison of total neurite outgrowth per neuron with knockdown of multiple MeCP2-regulated candidate genes relative to scrambled shRNA (*P < 0.05, n = 50–100 neurons per group). (Scale bar in A, 50 μm; applies to all micrographs.)
**Fig. S8.** Effect of shRNA knockdown mixture in primary DRG neurons. (A) Efficiency of individual shRNAs in the KD-Mix was established in 50B11 cells using q-PCR. (B) Mecp2−/− primary DRG neurons show profuse outgrowth when cultured in the presence of scrambled (Scr) lentiviral shRNA (Scale bar, 50 μm). (C) Neurite outgrowth appears reduced in cultures containing the lentiviral shRNA knockdown mixture (KD-Mix). (D) Analysis shows that outgrowth per neuron is reduced upon knockdown of MeCP2-regulated downstream candidate genes (*P = 0.012, n = 4 per group; neurons from four animals transduced separately with either scrambled or knockdown mixture).

**Fig. S9.** Down-regulating key cytoskeletal genes has no appreciable effect on neuroanatomical and behavioral phenotypes in WT rats. (A) Following lentiviral shRNA mixture injection into the hind paw, withdrawal threshold to mechanical stimulation was not significantly different from that seen after scrambled shRNA control paw injection. (B) No change was also observed percent withdrawal to a fixed mechanical force. (C) Footpad injected with knockdown shRNA did not elicit a significant change in heat sensitivity. (D) Quantitation of PGP9.5 innervation in the footpad revealed no change in IENF per millimeter following knockdown mixture injection. (E) Quantitation showed no appreciable change of peptidergic innervation after knockdown injection. n = 6 WT rats. Data in bar graphs are presented as mean ± SEM.
Other Supporting Information Files

Dataset S1 (XLSX)