Supplementary Figure 1

The pedigrees of families with 16p11.2 microdeletion (family 1, 2) or microduplication.

Two 16p11.2 deletion families and one 16p11.2 duplication family used as controls are shown. Individuals with 16p11.2 rMDS are presented as shaded squares. Subject information of 16p11.2 microdeletion Family 1 was reported in Shen et al.23. Probands are indicated by arrows.
Supplementary Figure 2

CRISPR strategy and validation

(a) Strategy for PCR characterization of 16p11.2 rMDS in iPSC lines (575 kb deletion). The ~1 kb PCR product is specific to the iPSC harboring 16p11.2 rMDS. (b) Left: PCR characterization reveals a clear ~1 kb band for the CRISPR generated 16p11.2 rMDS iPSC "+" compared to the control iPSC "C" and the CRISPR treated line that failed to generate an rMDS "-". Right: Sanger sequencing of the 575 kb rMDS deletion (red dashes = deleted bases; red bases = insertion sequence; blue bases = PAM sequence).
Supplementary Figure 3

Overview of the RNAseq experiment.

RNAseq was performed on CRISPR-treated lines harboring putative CNV (one for 575kb deletion, three for 740 kb deletion, five for 740 kb duplication), CRISPR-treated lines without CNV (Cas9 1-3), untreated 8330-controls (Control 1-3), as well as family lines. Those samples labeled * were selected for genome-wide DNA microarray analysis.
Supplementary Figure 4

Representative array comparative genomic hybridization (aCGH).

aCGH results generated at Jackson Laboratories are shown for four representative lines harboring putative CNV, and for the iPSCs from the family lines harboring CNV. Normalized log2 ratios are provided for comparison of the CRISPR treated line to controls for each probe. Probes with log2 ratio less than −0.25 are indicated as red dots for deletion, and probes with ratios greater than 0.25 are indicated as green dots for duplication. (a) CRISPR/Cas9 treated 740 Kb microdeletion line and (b) family line harboring deletion. (c) CRISPR/Cas9 740 kb microduplication line and (d) family line harboring duplication.
Supplementary Figure 5

The junction sequences of deletions/duplications in 16p11.2 rMDS iPSC lines created by the SCORE approach.

Schematic illustrating the junction sequences found in the individual iPSC lines harboring 16p11.2 microdeletion and microduplication. (a) In the 16p11.2 microdeletion iPSC lines, three different junction sequences observed in the individual iPSC lines (Del 1-3), two different junction sequences were found in the Del 4 line, a single-nucleotide T insertion was detected in four deletion lines (Del 5-8), and the remaining deletion lines were perfectly homologous with the reference (Del 9-12). (b) In the 16p11.2 microduplication iPSC lines, only one aberrant junction sequence found in four of the five iPSC lines. The ratio of certain junction sequence in all amplicons. (Red dashes = deleted bases; red bases = insertion bases; gray triangle = insertion point; orange arrow = CRISPR/Cas9 cut site; those samples labeled * were selected for RNAseq analysis)
Supplementary Figure 6

MAPK3 protein expression level in 16p11.2 rMDS iPSC lines.

Representative gel pattern and statistics for MAPK3 protein level (indicated as arrow) in the iPSC lines harboring 16p11.2 microdeletion (575 kb deletion: n = 1, Del 1; 740 kb deletion: n = 3, Del 2-4), microduplication (n = 5, Dup1-5), and 8330 control iPSC lines. All iPSC lines used in western blot analyses were confirmed by RNAseq. β-actin was used as an internal control. Results from western blot showed that the protein level of MAPK3 (MAPK3/β-actin) was significantly increased in 740kb duplication lines ($q = 4.391, p = 0.004$) but decreased in 740kb deletion lines ($q = 2.93, p = 0.031$) compared with 8330 control lines ($F_{2,8} = 43.548, P<0.001$). Experiments are in triplicate. Data are mean ± SEM. *$p <0.05$ and **$p<0.01$ compared with the control group from One-Way ANOVA. The full, uncropped blots are shown in Supplementary Figure 8.
Supplementary Figure 7

Copy number analysis of SCG5 and TJP1 genes.

Compared with 8330 control, there is no significant dosage difference in SCG5 and TJP1 genes (directly outside the CRISPR target region), suggesting the size of deletion/duplication made by CRISPR in our 15q13.3 rMDS iPSC lines are specific to the predicted region. The relative copy number was determined using relative quantitation method (See Online Method). For each gene, experiments are in triplicate. Data are mean ± SEM.
Supplementary Figure 8

MAPK3 protein expression level in 16p11.2 rMDS iPSC lines.

The full, uncropped blots are shown here.