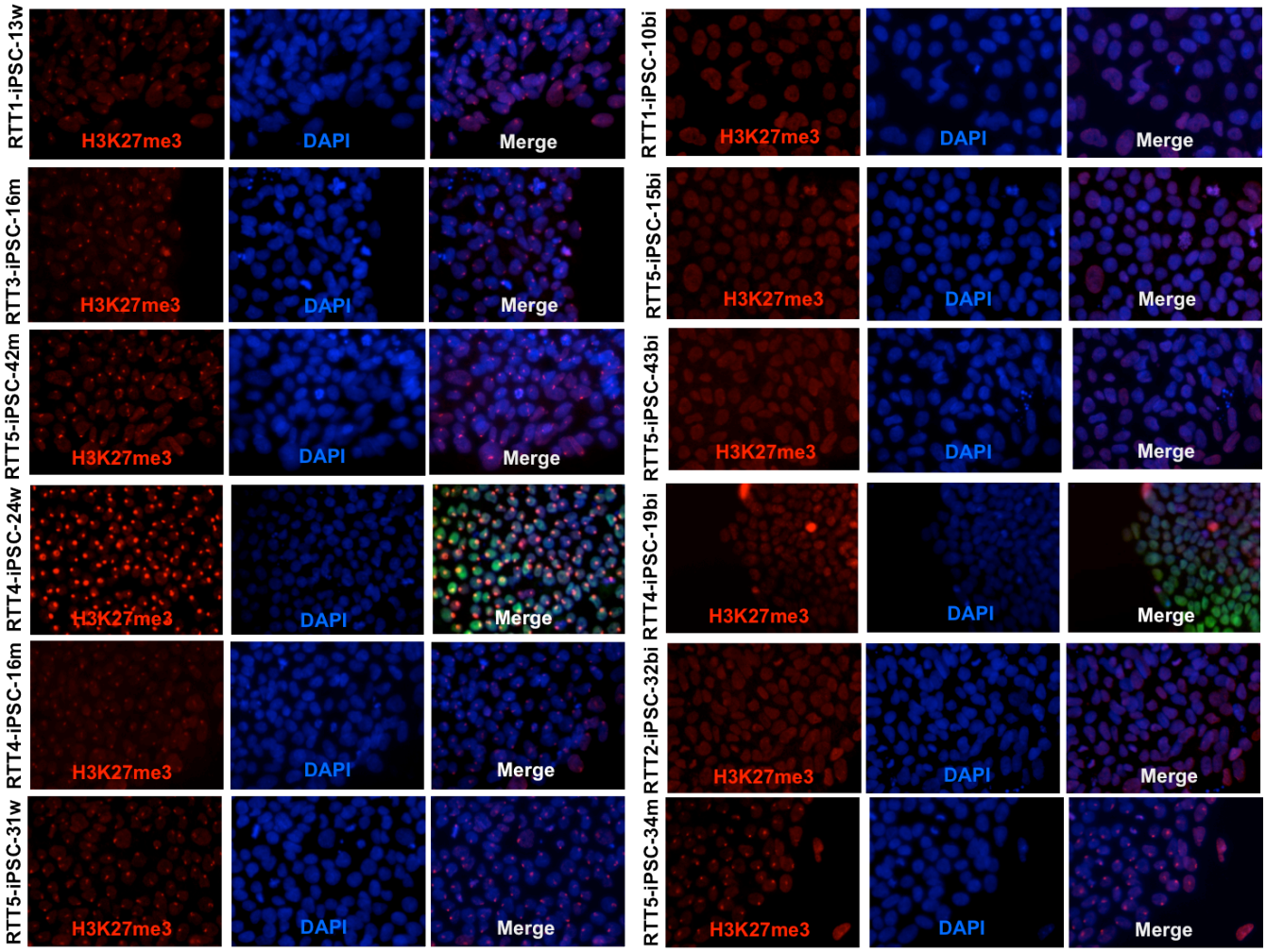
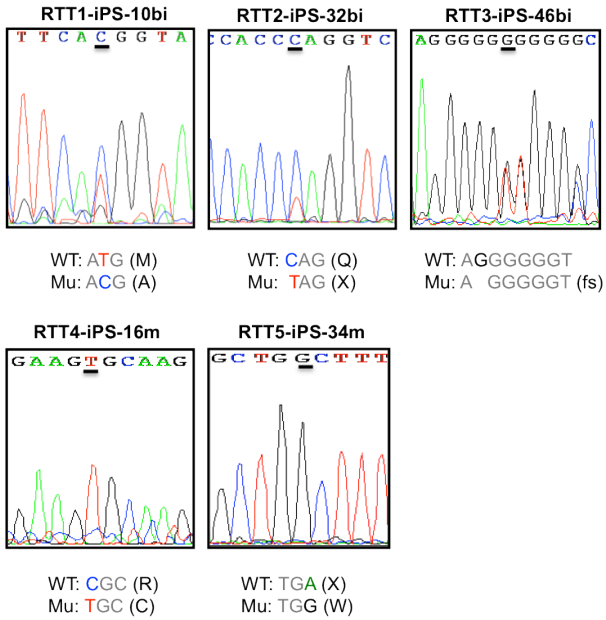
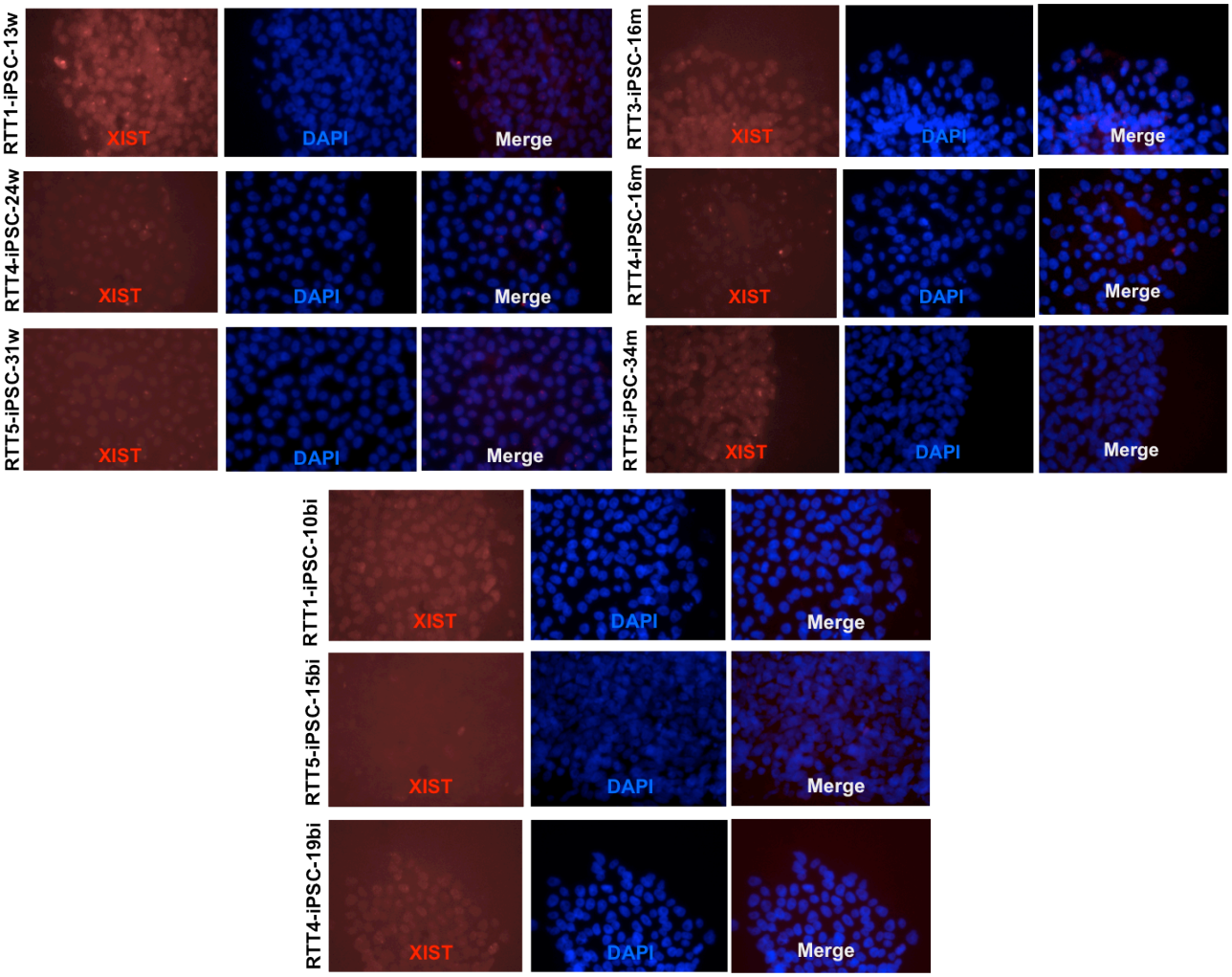
A.


RTT1-iPSC-13w
H3K27me3
DAPI
Merge
RTT3-iPSC-16m
H3K27me3
DAPI
Merge
RTT5-iPSC-42m
H3K27me3
DAPI
Merge
RTT4-iPSC-24w
H3K27me3
DAPI
Merge
RTT4-iPSC-16m
H3K27me3
DAPI
Merge
RTT5-iPSC-31w
H3K27me3
DAPI
Merge
RTT1-iPSC-10bi
H3K27me3
DAPI
Merge
RTT5-iPSC-15bi
H3K27me3
DAPI
Merge
RTT5-iPSC-43bi
H3K27me3
DAPI
Merge
RTT4-iPSC-19bi
H3K27me3
DAPI
Merge
RTT2-iPSC-32bi
H3K27me3
DAPI
Merge
RTT5-iPSC-34m
H3K27me3
DAPI
Merge

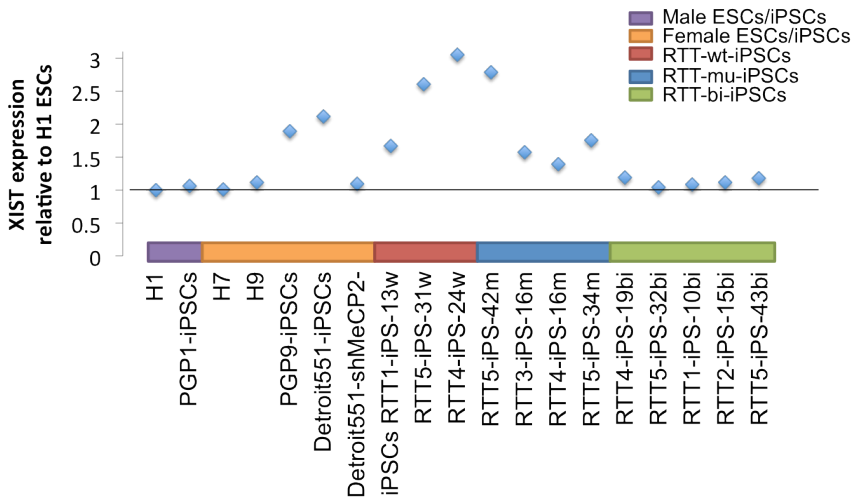
B.


RTT1-iPS-10bi
T T C A C G G T A
WT: ATG (M)
Mu: ACG (A)
RTT2-iPS-32bi
C C A C C C A G G T C
WT: CAG (Q)
Mu: TAG (X)
RTT3-iPS-46bi
A G G G G G G G G G G G C
WT: AGGGGGGT
Mu: A GGGGGT (fs)
RTT4-iPS-16m
G A A G T G C A A G
WT: CGC (R)
Mu: TGC (C)
RTT5-iPS-34m
G C T G G C T T T
WT: TGA (X)
Mu: TGG (W)

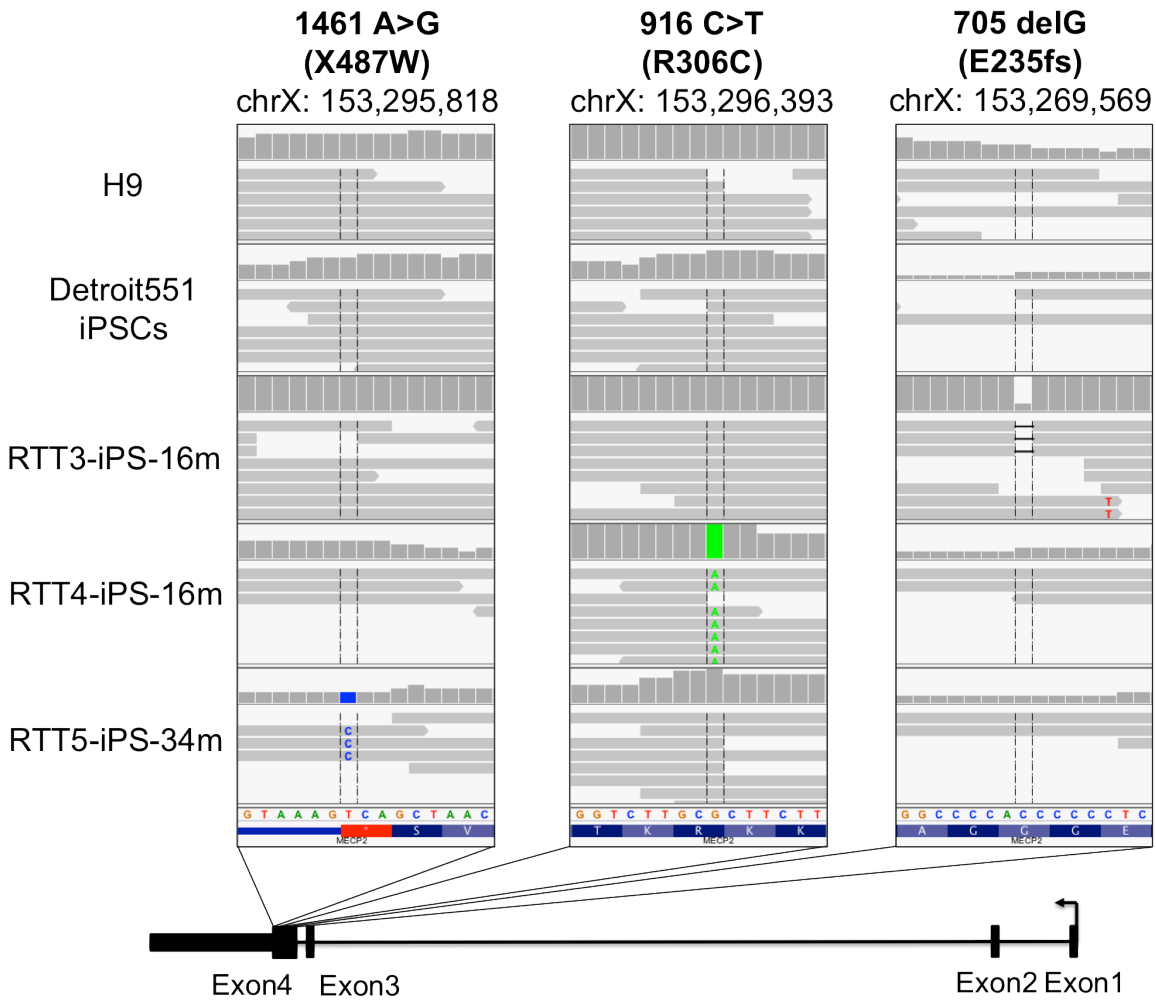
C.



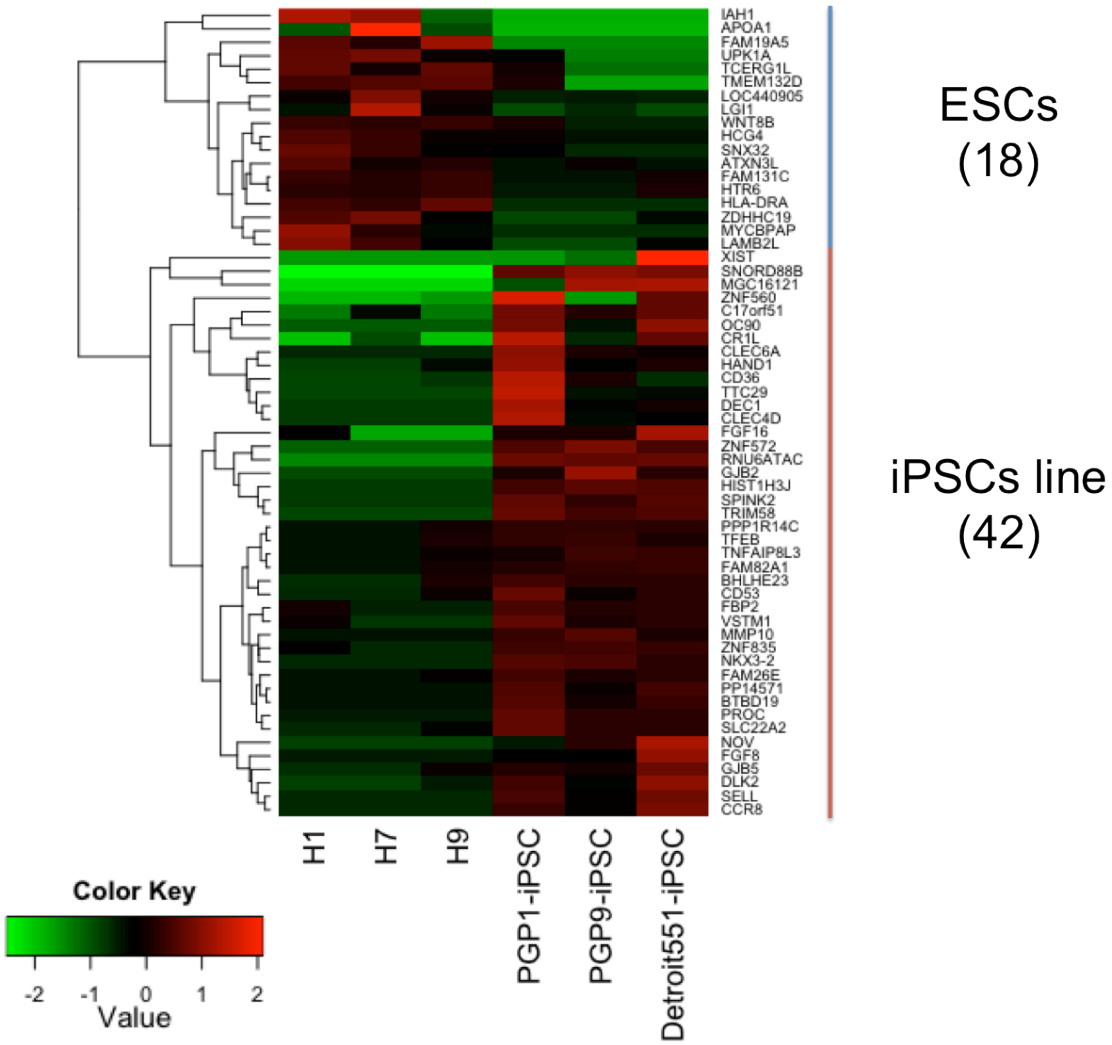
Supplementary Figure 1. X chromosome inactivation status in ESCs and iPSCs. A) H3K27me3 immunostaining in RTT-iPSCs. H3K27me3 nuclei foci were present in RTT-wt-iPSCs and RTT-mu-iPSCs, whereas RTT-bi-iPSCs displayed diffuse staining. B) MeCP2 RNA sequencing show allele specificity of monoallelic clones of RTT-iPSCs. RTT-mu-iPSCs express mutant MeCP2, whereas RTT-bi-iPSCs express both wildtype and mutant MeCP2. C) XIST expression detected by RNA FISH method. RTT-wt-iPSCs and RTT-mu-iPSCs showed XIST RNA clouds, whereas RTT-bi-iPSCs did not show them.



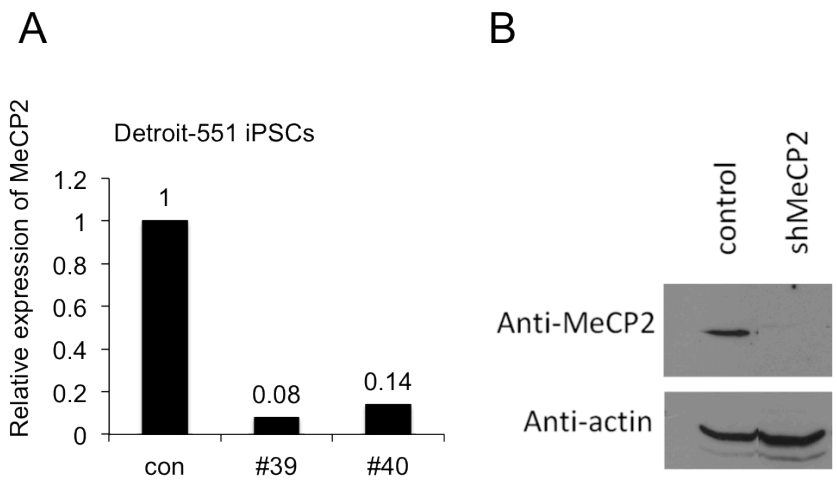
Supplementary Figure 2. XIST expression was confirmed by qPCR. XIST expression in H7, H9 and Detroit551-shMeCP2-iPSCs and RTT-bi-iPSCs are not different from that in male ESCs/iPSCs. PGP9-iPSCs, Detroit551-iPSCs, RTT-wt-iPSCs and RTT-mu-iPSCs show higher XIST expression level than male ESCs/iPSCs.



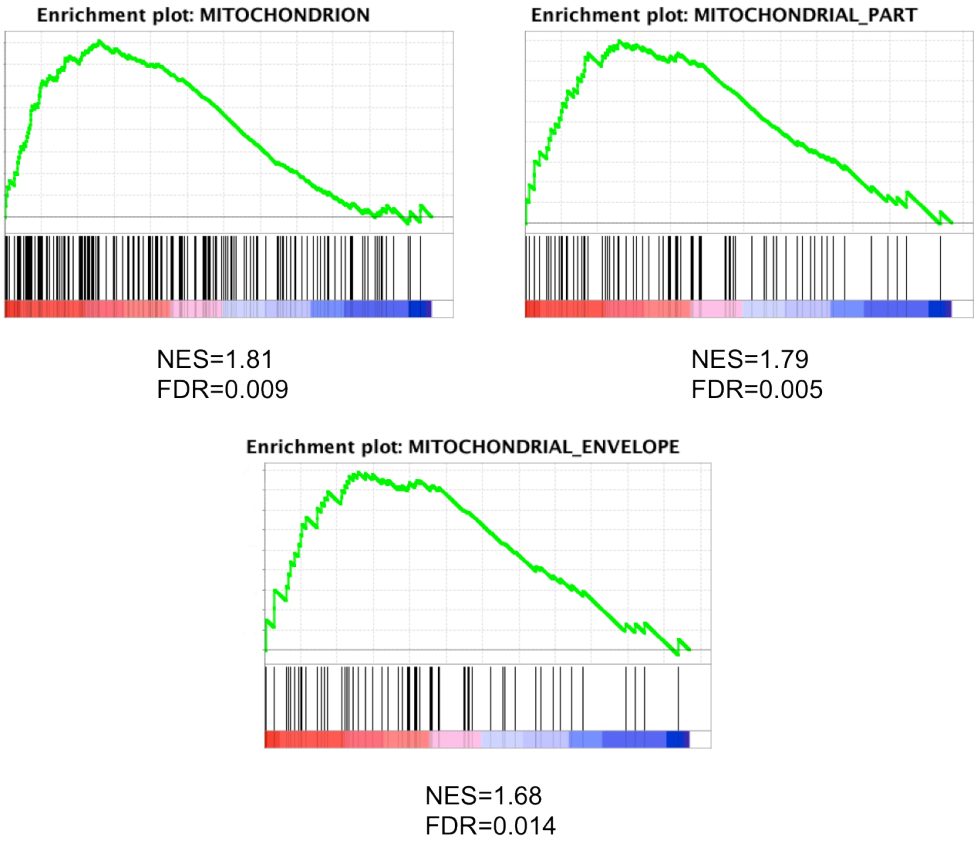
Supplementary Figure 3. Expression of mutant MeCP2 in RTT-mu-iPSCs was confirmed by RNA-seq. Snapshot of Integrative Genomics Viewer (IGV) in each mutant locus was shown.



Supplementary Figure 4. Differentially-expressed genes between ESCs and cell line-derived iPSCs. Expression values are represented to be above (red) or below (green) the average expression of all samples. 18 genes were highly expressed in ESCs and 42 genes in cell line-derived iPSCs.



Supplementary Figure 5. Knockdown efficiency of MeCP2 was confirmed by (A) qPCR and (B) Western blot. #39 and #40 represents TRCN0000021239 and TRCN0000021240 (Sigma), respectively.



Supplementary Figure 6. Enrichment of mitochondria-related genes in RTT brains. Expression data in normal and RTT brains (GSE6955) were downloaded from Gene Expression Omnibus and normalized by MAS5 method. Probesets, which correspond to RefSeq genes and show present call at least one dataset, were used for GSEA. Relative expression of RTT brains to normal brains was ranked by signal-to-noise ratios. The enrichment was evaluated with 1,000 permutation of gene set.