1 **SUPPLEMENTARY FIGURES**

2 **Figure S1. Genomic organization of the MMTR gene. (A)** Schematic representation
3 of the MMTR coding sequence structure. Bipartite short coiled-coil motifs are located
4 between amino acid 170 and 420. Putative nuclear localization signals (dotted box) are
5 observed at the start of the amino acid 33 (PDKKKSK), 224 (RRRK), 289 (PKKKLPQ)
6 and 447 (PNSRKRR). **(B)** Chromosomal locations of human and mouse MMTR. The
7 human MMTR gene is consisted of 11 exons and located in chromosome 1P34, whereas
8 the mouse MMTR gene is consisted of 10 exons and located in chromosome 4D1.
9 Marked boxes represent the location and size of genes around MMTR.

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11 **Figure S2. The high evolutionary conservation of MMTR. (A)** Phylogenetic tree of
12 MMTR orthologues at the amino acid level. **(B)** Amino acid sequence identity scores of
13 MMTR homologues. **(C)** Alignment of MMTR amino acid sequences in vertebrates.
14 ClustalW (EBI) was used to align amino acid sequences, draw a phylogenetic tree, and
15 calculate amino acid sequence identity scores of mouse MMTR (GenBank accession
16 number AF438610) to its homologues from Yeast (S. cerevisiae and S. pombe), fungi,
17 plants, to human (identified by BLASTP). (!, anyone of IV; $, anyone of LM; %,
18 anyone of FY; #, anyone of NDQEBZ; +, anyone of KRQ; capital letter, high consensus
19 sequence; lower case letter, low consensus sequence)

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21 **Figure S3. Expression profiles of MMTR in differentiating mESCs and nuclear**
22 **expression of MMTR in various tissues. (A)** Analyses of MMTR expression during
23 differentiation of mouse ES cells in vitro. Embryoid bodies were generated by the
24 conventional hanging-drop method. Total RNAs were isolated from embryoid bodies in

each differentiation day and subjected to semiquantitative RT-PCR assays. RT-PCR showed that MMTR mRNA increases during *in vitro* differentiation of ES cells. The expression levels of MMTR during differentiation were determined after normalizing by that of day 0. **(B)** Relative expression index of MMTR transcript in various mouse tissues as measured by semiquantitative RT-PCR. The expression level of MMTR was normalized with endogenous HPRT transcripts. **(C)** In situ hybridization showing ubiquitous expression of MMTR in the mouse embryo (left panel, longitudinal section; right panel, horizontal section). **(D)** Subcellular localization of MMTR protein. HIB-1B cells were reacted with the polyclonal anti-mouse MMTR antibody and FITC-conjugated secondary antibody. Nuclei were stained with ethidium bromide.

Figure S4. Reduced expression of MMTR in differentiating mESCs causes the elevated expression of randomly chosen genes. Using reverse-Northern slot blots, we measured transcript levels of randomly isolated mouse ES cDNA clones (3) in 6th day embryoid bodies of a mESC line into which the antisense MMTR expression vector was stably transfected (see Supplemental Materials and Methods). At differentiation day 6, total transcripts of both wild type and antisense MMTR RNA expressing mouse ESC embryoid bodies were hybridized onto nylon membrane bound each mouse ES cDNA clones. Each number below the graph is the clone ID and the value on left side is the expression rate of each clone in antisense-MMTR transfected mESCs compared to the wild type cells.

SUPPLEMENTAL MATERIALS & METHODS

Mouse embryonic stem cell culture. The 129/sv-derived mouse embryonic stem cell line CCE that was adapted to grow in the presence of leukemia inhibitory factor (LIF) without feeder cells (5) was maintained and differentiated as described elsewhere (2). All other culture solutions were from Gibco BRL. Antisense MMTR expressing mouse ES cell lines were generated by stable transfection of the antisense MMTR expression vector (pBS-antisense MMTR) into mouse ESC (CCE line) by electroporation (250 uF, 400 V) and selected by G418 (500 ug/ml) for 2 weeks. Integrity of insert DNA in antisense MMTR cell lines was determined by genomic PCR. Suppression of MMTR expression in antisense MMTR cell lines was confirmed by semiquantitative RT-PCR to total RNA samples prepared in 3rd and 6th day of embryoid bodies.

Reverse-Northern Slot blot analyses. Plasmid DNA from each clone was prepared from the *E. coli* strain XL-10 Gold using the alkaline lysis method and 100 ng of each DNA was slot blotted to the nylon membrane (Zeta-probe GT membrane, Bio-Rad) (3). Each of multiple sets of blots was then hybridized with [^{32}P]-labeled cDNA probes. To make [^{32}P]-labeled probes, mRNA (1 μg) isolated from the cells was subjected to oligo-dT-primed first strand cDNA synthesis reaction in the presence of [α-^{32}P] dCTP. Hybridization and washing of the membrane were performed according to the procedures described elsewhere (1). The relative expression level of each clone to the internal control, HPRT (hypoxanthine-guanine phosphoribosyltransferase), was calculated after measuring the intensity of hybridization signal by densitometric scanning of each slot.

Immunohistochemistry. Immunohistochemistry for the Paraffin-embedded mouse tissues was carried out according to the procedure of Peter *et al* (4). Conventional immunohistochemistry was employed. Samples were reacted with the polyclonal anti-mouse MMTR antibody and FITC-conjugated secondary antibody. Polyclonal rabbit anti mouse MMTR antibodies were generated by immunization of the purified GST-MMTR fusion proteins with Freund's Complete Adjuvant (Sigma, USA).

SUPPLEMENTAL REFERENCES

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