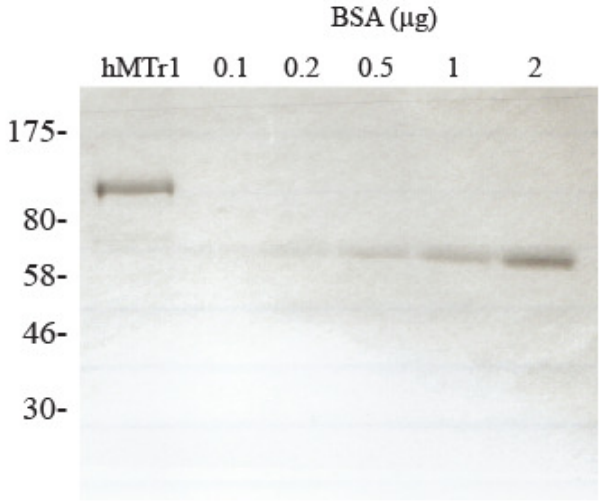
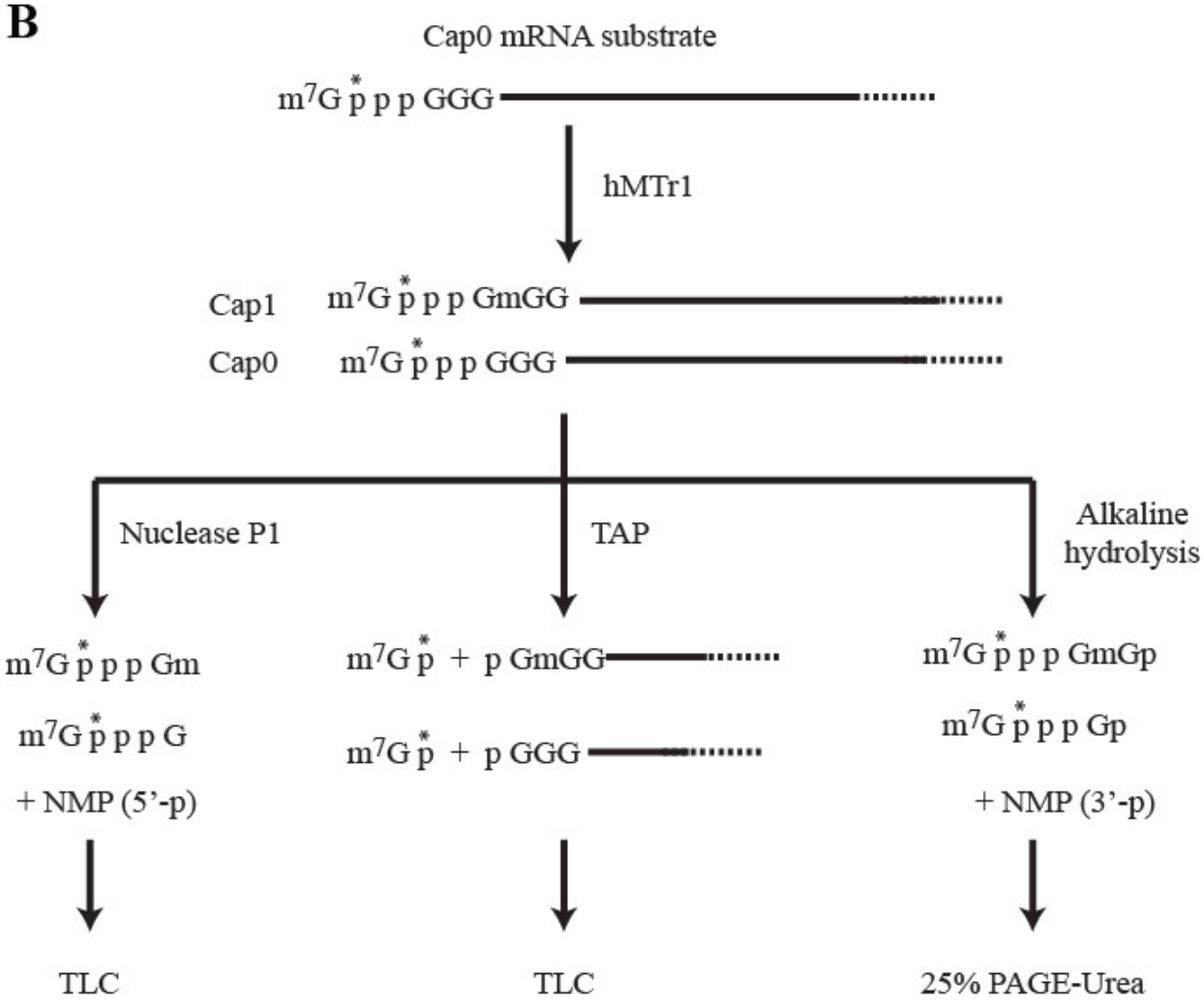
Supplementary Figure 1

(A) hMTr1 was overexpressed and purified from *E. coli*. Recombinant protein was analyzed by SDS-PAGE and stained with Coomassie. **(B)** Schematics of the assays used for characterization of hMTr1 activity *in vitro*. The capped CAT mRNA substrate is methylated *in vitro* with recombinant hMTr1 (* indicates the radiolabeled phosphate). Digestion with Nuclease P1 results in the release of the labeled cap di-nucleotide and free nucleotides. Digestion with TAP releases the cap moiety attached to the radiolabeled phosphate. Nuclease P1 and TAP digests are analysed by TLC. Digestion by alkaline hydrolysis results in labeled fragments of different length depending on the methylation of the 2'O-ribose position. These fragments are resolved with a 25% polyacrylamide-Urea gel.

A


BSA (µg)
hMTr1
0.1
0.2
0.5
1
2
175-
80-
58-
46-
30-

B


Cap0 mRNA substrate
m7G*ppp GGG
hMTr1
Cap1
m7G*ppp GmGG
Cap0
m7G*ppp GGG
Nuclease P1
TAP
Alkaline hydrolysis
m7G*ppp Gm
m7G*ppp G
+ NMP (5'-p)
m7G*p + p GmGG
m7G*p + p GGG
m7G*ppp GmGp
m7G*ppp Gp
+ NMP (3'-p)
TLC
TLC
25% PAGE-Urea