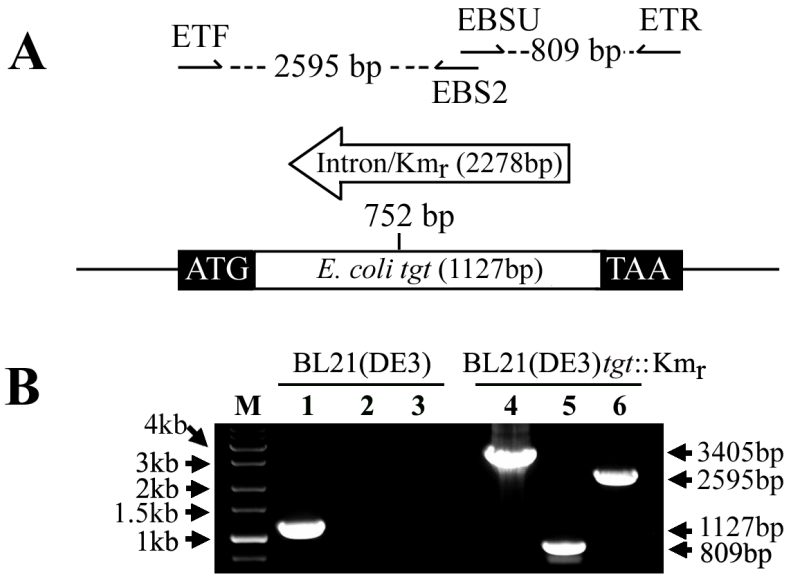
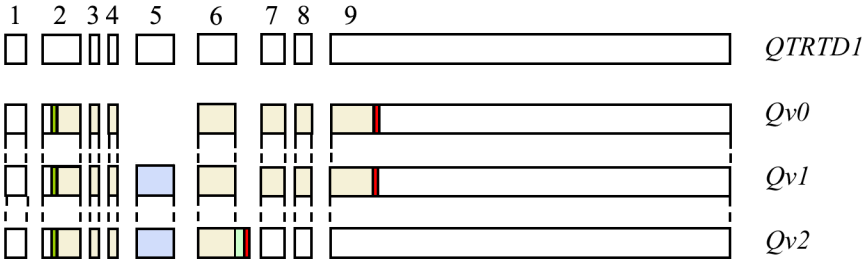
Supplementary Figure 1



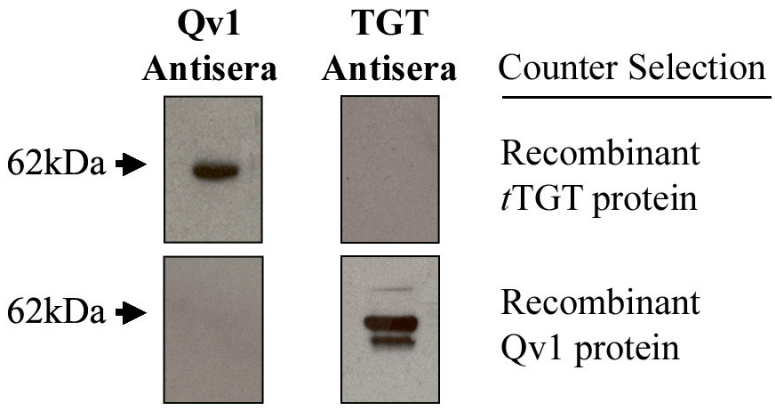
Sup. Fig.1. Intron-based mutagenesis of the *tgt* gene in *E. coli* BL21(DE3). (A) Graphical depiction of the wild-type *tgt* gene and the insertion of the kanamycin resistant group II intron (Intron/ Km_r) at position 752 bp, according to cDNA numbering, in the reverse direction (upper panel). (B) An analysis of wild-type BL21(DE3) and BL21(DE3) *tgt*::Km_r cells by PCR using the ETF and ETR primer pair (lanes 1 and 4) showing the absence (1127 bp) and presence (3405 bp) of the intron, respectively; the origin and size of the amplicon from the ESBU and ETR primer pair (lanes 2 and 5) and the ETF and EBS2 primer pair (lanes 3 and 6) are shown in the upper panel. M denotes DNA molecular weight markers.

Supplementary Figure 2



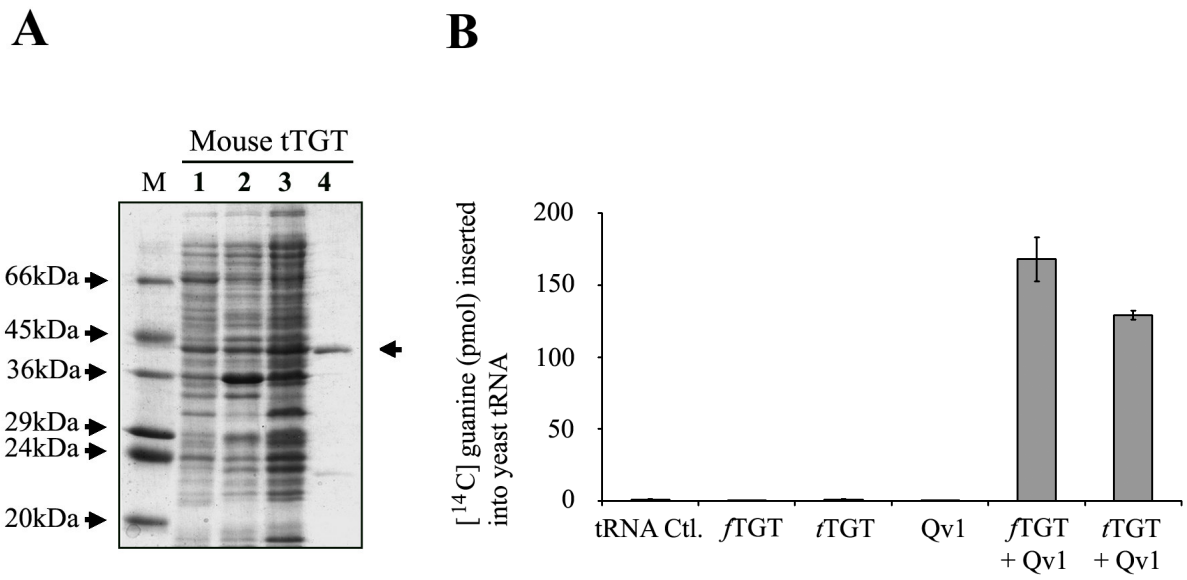
Sup. Fig. 2. Alternative spliced forms of the mouse *QTRTD1* gene. The genomic structure of *QTRTD1* is presented on the top line, with the various alternative spliced products illustrated underneath. Coding exons are shown in yellow and the 5' and 3' UTRs as white boxes. Note that the sizes of the exon boxes are only broadly proportional to the actual length of the sequences. Start codons are shown in green whereas stop codons are marked in red. Exon 5, shown in blue, is skipped in the Qv0 transcript whereas intron 6 has been partially retained in the Qv2 transcript. Qv0, NM029128; Qv1, FM985872; Qv2, BC017628.

Supplementary Figure 3



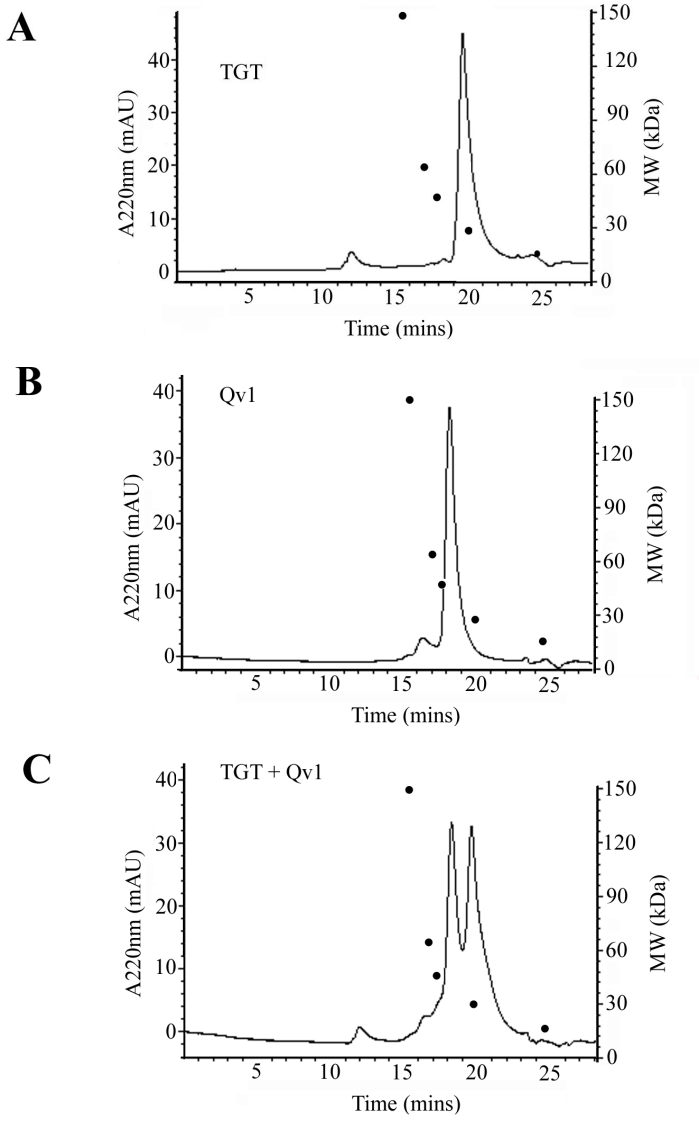
Sup. Fig. 3. Western blot analysis of purified TGT and Qv1 antisera used in confocal studies. Antisera were raised to recombinant *t*TGT and Qv1 and purified on Protein A. The antisera were then selected on resin bound to the opposing member protein (used for subsequent confocal analysis and immunoblotting) or to the protein to which the antisera was raised (to examine specificity). For immunoblott analysis, total cell extracts from Cos7 cells were resolved on SDS-PAGE and transferred to Immobilon P membrane (Millipore). Qv1 antisera selected on resin bound recombinant *t*TGT (that would lead to the removal of antibody idiotypes capable of binding TGT) gave an expected band of 62 kDa in Cos7 total cell extracts (left-hand side, upper panel), due to exclusive recognition of endogenous Qv1 protein. When selected on Qv1 bound resin the Qv1 antisera did not recognise proteins in Cos7 extract, demonstrating the specificity of the antisera (left-hand side lower panel). Similarly, TGT antisera selected on resin bound recombinant Qv1 (leading to the removal of antibody idiotypes capable of binding Qv1) gave an expected band of 62 kDa in Cos7 total cell extracts (right-hand side lower panel) but when TGT antisera was selected against resin bound *t*TGT no band was observed on immunoblots of Cos7 total cell extract (right-hand side, upper panel).

Supplementary Figure 4



Sup. Fig. 4. Recombinant expression and enzymatic assay of truncated mouse TGT. (A) Purification of recombinant mouse truncated TGT (*t*TGT) from BL21(DE3) *tgt*::Km_r cells. Note that the hexa-histidine tag was not removed. Lane 1, uninduced *E.coli*; lane 2 IPTG induced *E.coli* grown overnight; lane 3, insoluble pellet fraction; lane 4, protein purified by nickel-agarose chromatography. M denotes protein molecular weight markers. (B) Comparison of the ability of *t*TGT and *f*TGT alone and in combination with Qv1 to insert radiolabelled guanine into yeast tRNA (tRNA*).

Supplementary Figure 5



Sup. Fig. 5. Size-exclusion gel-chromatography of *t*TGT and Qv1. (A) *t*TGT and (B) Qv1 were chromatographed on a TSK-Gel G3000SW$_{XL}$ column by HPLC (Shimadzu) in 20 mM Tris-HCl pH 7.5, 200 mM NaCl and 10% glycerol, individually giving sizes of 26 kDa and 37.5 kDa, respectively, or (C) pre-incubated and run together yielding two peaks identical to individual runs. Calibration of the column was made with alcohol dehydrogenase (150 kDa), albumin fraction V (67 kDa), ovalbumin (43 kDa), carbonic anhydrase (29 kDa), cytochrome C (12.4 kDa), as indicated by the black dots. The elution of protein was recorded at A_{220}.