Table S1. Oligonucleotides used for qRT-PCR experiments

Target	qRT-PCR oligonucleotides
SelT	Fw : TCCAGATTTGTGTTTCCTGAGG Rev : CTGGGTACCGCTGGCTAATA
Sel15	Fw : CGGACAGTTCAACCTGCTTC Rev : AATACAGGGTCTGAACCACG
SelM	Fw : TCCCGATGAGCCTCCTGTTG Rev : ATGGAATGTCCTGCGTGACG
SelW	Fw : ACGTGGACACAGAAAGCAAG Rev : ACAGCAGCCACGAGAACATC
SelN	Fw : AGCTTCATCAGCACCTGGTC Rev : CGGAGGTGATGTCCAAGAAG
SelR	Fw : CAGGTTTTCCAGAATCACTTTG Rev : GGCCATGGAGACGAGTGT
SelK	Fw : ATCTGATTCCAGATATGATGAT Rev : TGATTGATTCTACCCATTCTTC
SelO	Fw : GAGGAGTTTGACGCCGAGTTC Rev : GCTCAGCAAGTAGAAGGTGTTTGTG
GPx3	Fw : GGAGTACATCCCCTTCAAGC Rev : CGAATGGTGCAAGCTCTTCC
GPx4	Fw : CGGGCTACAACGTCAAATTCG Rev : GGGGCAGGTCCTTCTCTATCA
GPx1	Fw : TGCAACCAGTTTGGGCATCA Rev : ACCGTTCACCTCGCACTTC
TrxR1	Fw : CCTTATCATCATTGGAGGTGG Rev : AAGAGGGGTGGGAGTGACAAA
SMN	Fw : CTGATGCTTTGGGAAGTAT Rev : GCCAGCATTTCTCCTTAA
Nop58	Fw : CCATGAACCGCTGGCAGTAG Rev : CCAATCCAAGACACATAGCTG
TGS1	Fw : GGCTATTACATCAGAGACAGTG Rev : GAATCAAGTTCACTTTCATCCA
snARN U2	Fw : TTCTCGGCCTTTTGGCTAAG Rev : CTCCCTGCTCCAAAAATCCA
snoARN U3	Fw : TTCTCTGAACGTGTAGAGCACC Rev : GATCATCAATGGCTGACGGCAG
SBP2	Fw : GGTTGAGGACAAGTCTGAAGAGCC Rev : CTTTGAGTAGGTCGGTAACACAAGC
eIF4E	Fw : GGCCCACTCTGTAATAGTTC Rev : CCATCAGCAAGAGTACAGC
β-actin	Fw : CCTTCTACAATGAGCTGCGT Rev : CTCCTTAATGTCACGCACGAT
LDHA	Fw : TGGCAGCCTTTTCCTTAGAACA Rev : ACGGCTTTCTCCCTCTTGCTGA
HPRT	Fw : TGACACTGGCAAAACAATGCA Rev : GGTCCTTTTCACCAGCAAGCT

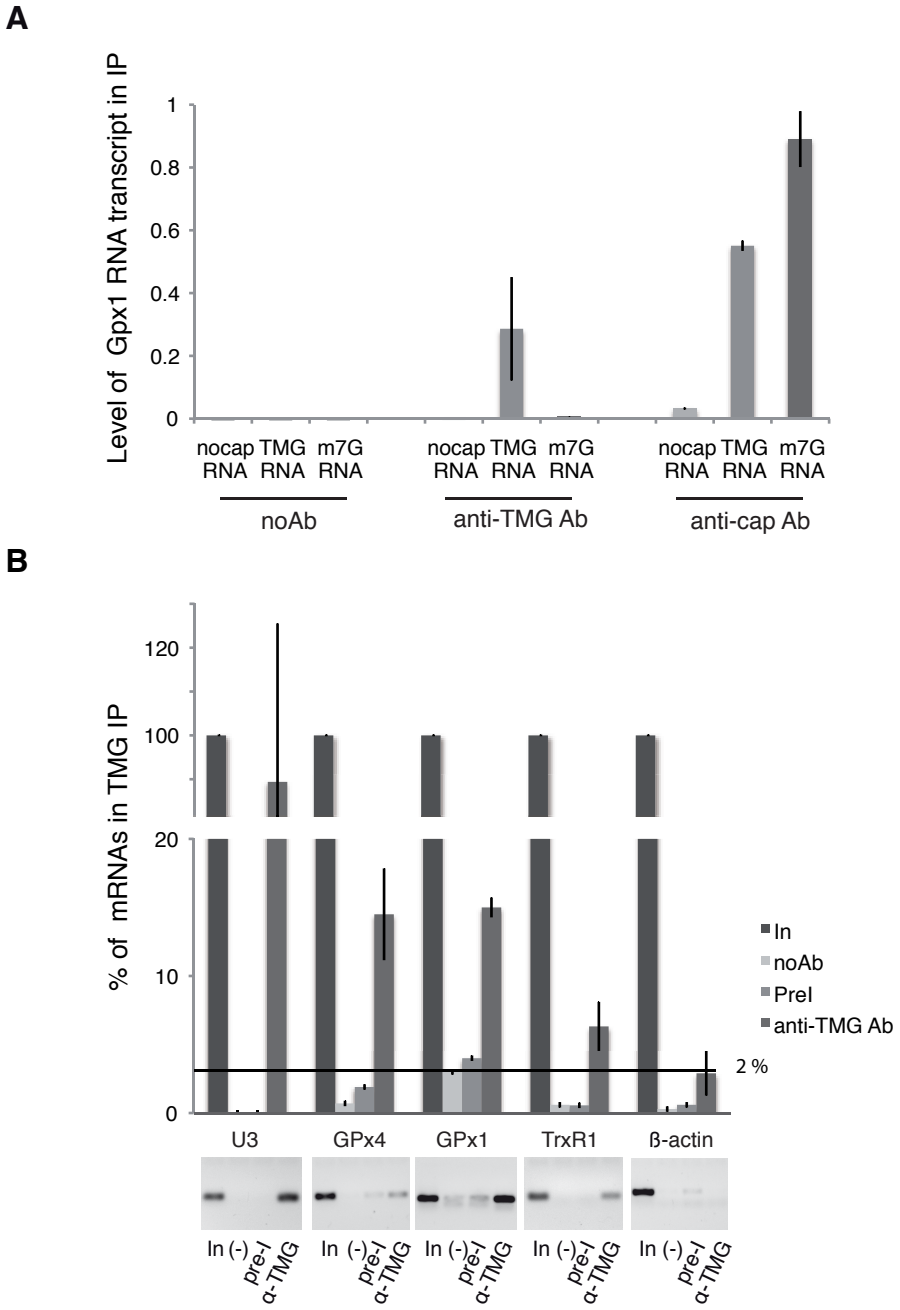

Figure S1: Validation of the anti-TMG serum specificity for TMG-capped RNAs. (A) In vitro transcribed GPx1 RNAs, capped co-transcriptionaly with either m3G (TMG RNA) or m7G (m7G RNA) cap analogs were used to test the specificity of TMG-IPs. Non-capped RNAs (nocap RNA) were used as negative controls. Immunoprecipitations were performed with no antibodies, specific anti-TMG serum (anti-m3G R1131 serum, Synaptic Systems) and anti-cap antibodies (anti-m7(m3)G antibodies (Synaptic Systems). Bound GPx1 RNA transcripts were analyzed by qRT-PCR. Maximum RNA binding levels were set to 1. RNA IPs reveal that the anti-TMG serum specifically recognizes TMG-capped RNA but not m7G-capped RNA transcripts while anti-cap antibodies recognize both RNAs. (B) Example total RNA TMG-IP quality control. Total RNA extracted from HEK293FT cells was immunoprecipitated with anti-TMG serum (anti-TMG Ab), no antibodies (noAb or (-)) or unrelated rabbit pre-immune serum (PreI). Bound RNA was analyzed by qRT-PCR . The graphs represents the % of mRNAs in IP compared to the input RNA. The horizontal line represents the level of housekeeping mRNA binding and background (1-2% in average). Background values due to beads or pre-immune serum are retrieved from specific binding values in all subsequent representations. (A, B) Error bars represent standard deviation of an average of 3 independent experiments. Panels below the graph show the RT-PCR products obtained after 35 PCR cycles. In: input 10 %.

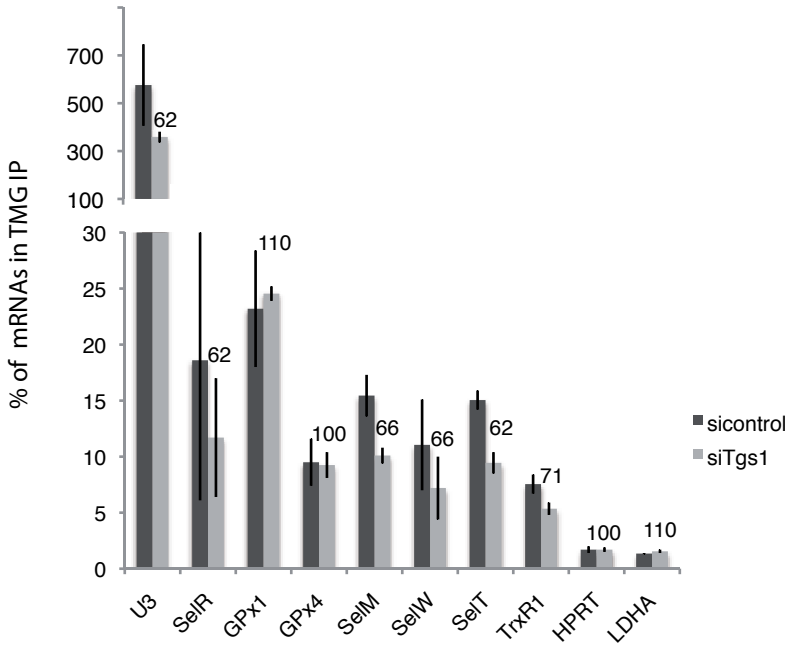

Figure S2: Tgs1 is required for selenoprotein mRNA cap hypermethylation. RNA-IP using anti-TMG serum was performed as described in Fig. 1. The housekeeping HPRT and LDHA mRNAs are negative controls for the TMG-IP.

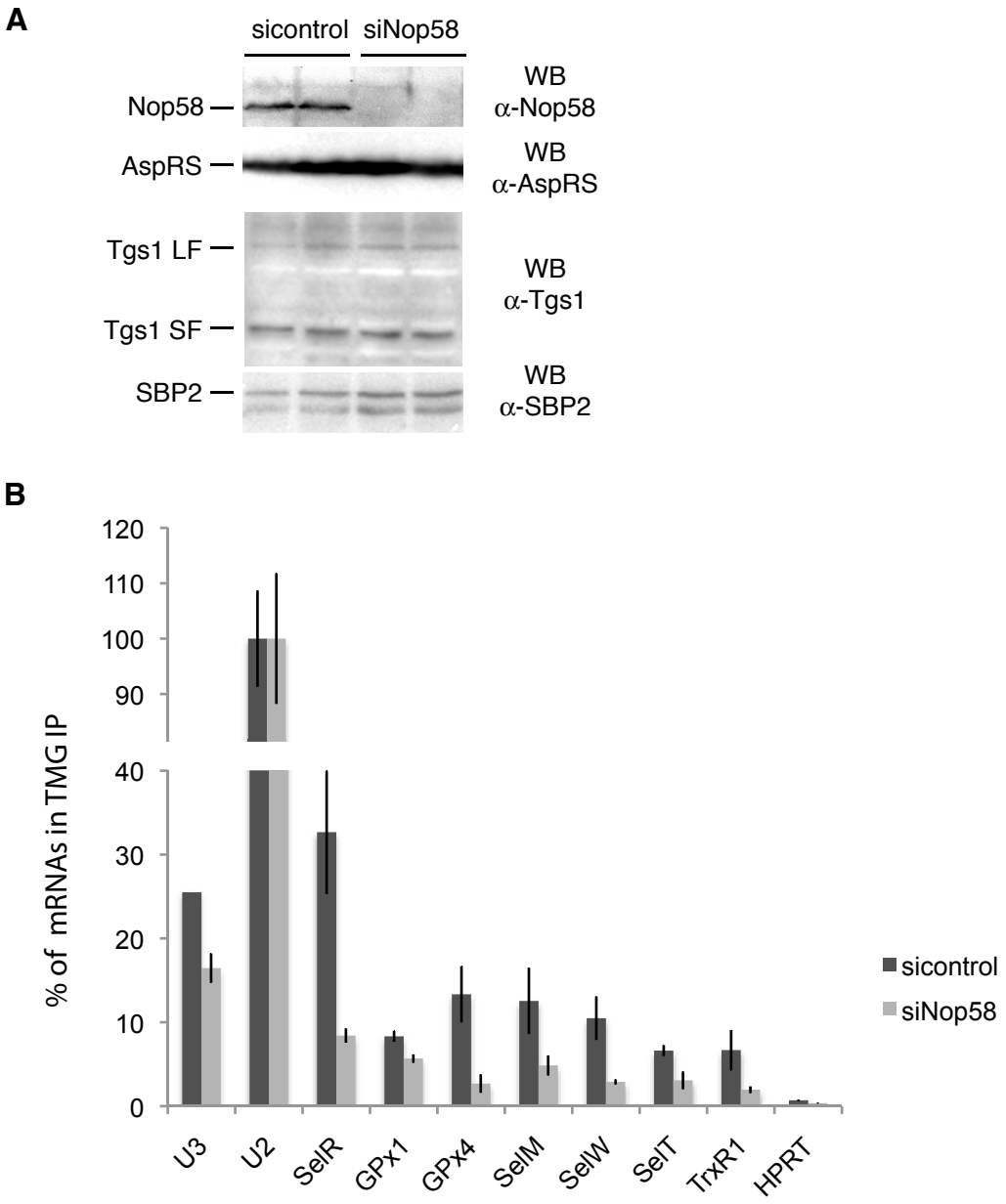

Figure S3: Nop58 is required for cap hypermethylation. (A) Western blot analysis of siNop58 efficiency. Levels of Nop58 protein were reduced to 2% compared to the control, Tgs1 and SBP2 were unaffected. (B) Nop58 contributes to selenoprotein mRNA hypermethylation. RNA-IP using anti-TMG serum was performed as described in Fig. 1.

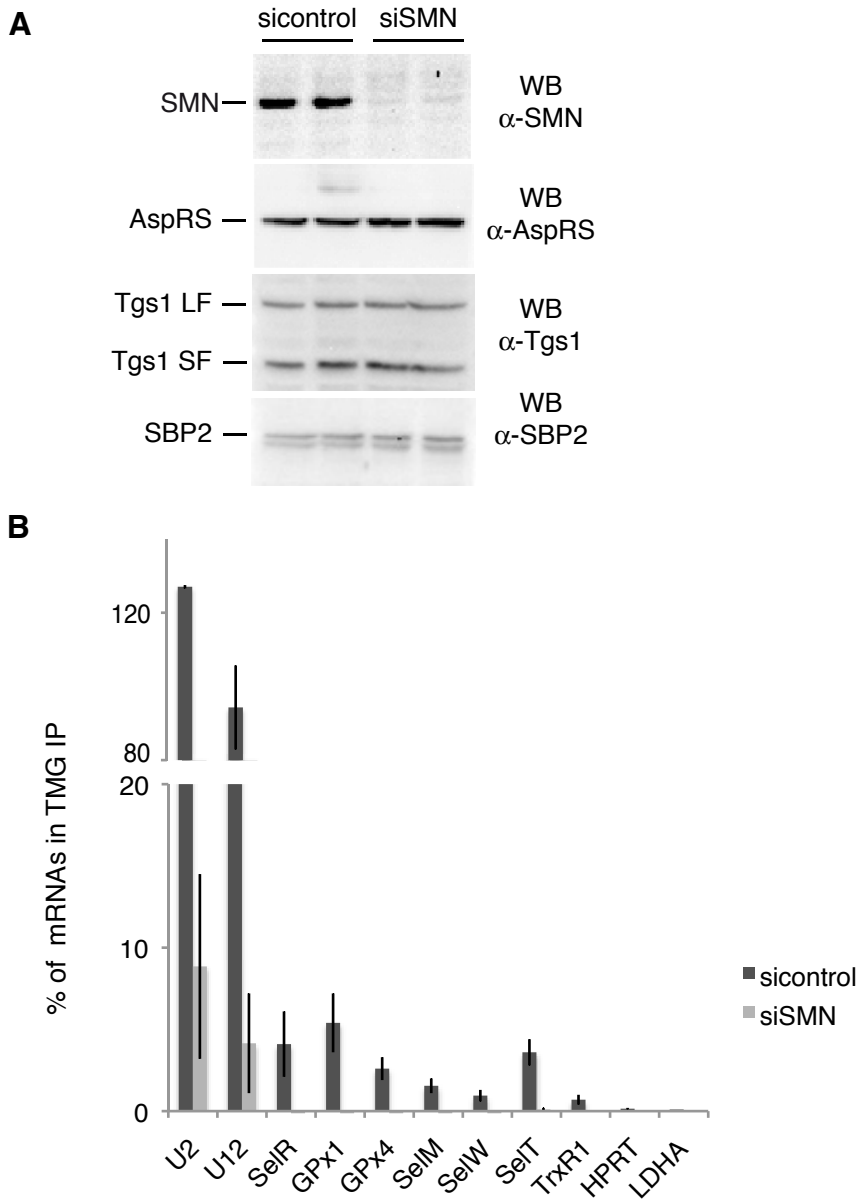

Figure S4: SMN is required for cap hypermethylation. (A) Western blot analysis of siSMN efficiency. Levels of SMN protein were reduced to 8% compared to the control, Tgs1 and SBP2 were unaffected. (B) SMN is required for selenoprotein mRNA hypermethylation. RNA-IP using anti-TMG serum in siSMN conditions were performed as described in Fig. 1.

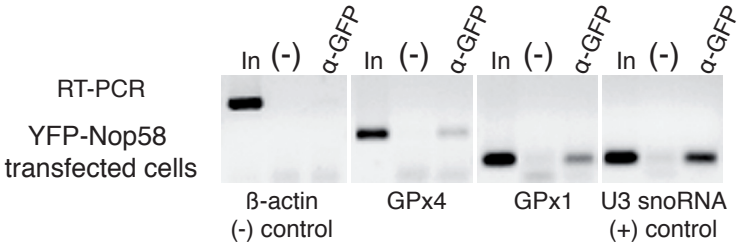

Figure S5: Nop58 interacts with selenoprotein mRNAs in vivo. (A) HEK293FT cells transfected with SBP2 and Nop58-YFP were immunoprecipitated using anti-GFP antibodies. Detection of bound RNAs was done by RT-PCR. In: input 15%; (-) no antibody; U3: positive control; ß-actin: negative control.

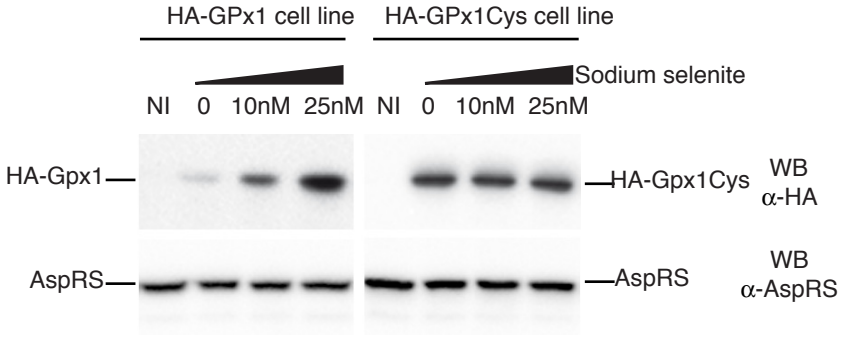

Figure S6: Experimental validation of the stable cell lines HA-GPx1 and HA-GPx1Cys.
Western blot analysis of the expression of HA-GPx1 and HA-GPx1Cys proteins using anti-HA antibodies, after doxycycline induction. NI: non induced. The levels of HA-GPx1 protein expression, but not of HA-GPx1Cys, are selenium dependant. Sodium selenite concentrations are indicated above the panels. Western blot using anti-AspRS antibodies were used as control.