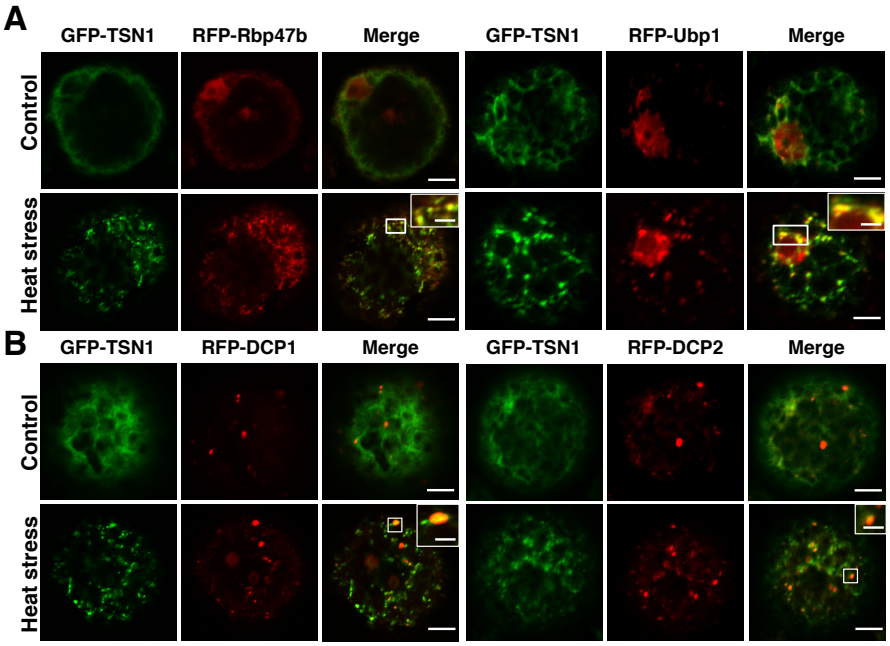



**Figure S1.** TSN1 localizes to SG and PB.
**(A)** and **(B)** Co-localization of GFP-TSN1 (green) with the SG **(A)** and PB **(B)** marker proteins (red) RFP-Rbp47b, RFP-Ubp1 and RFP-DCP1, RFP-DCP2, respectively, in *N. benthamiana* protoplasts at 23ºC (control) or after 30 min at 39ºC (heat stress). N-terminal GFP and RFP fusion proteins were expressed under the control of the 35S promoter. Boxes delimited with white line indicate the areas which are depicted magnified in the insets ('Merge'). Scale bars, 5 µm (2 µm in insets) .

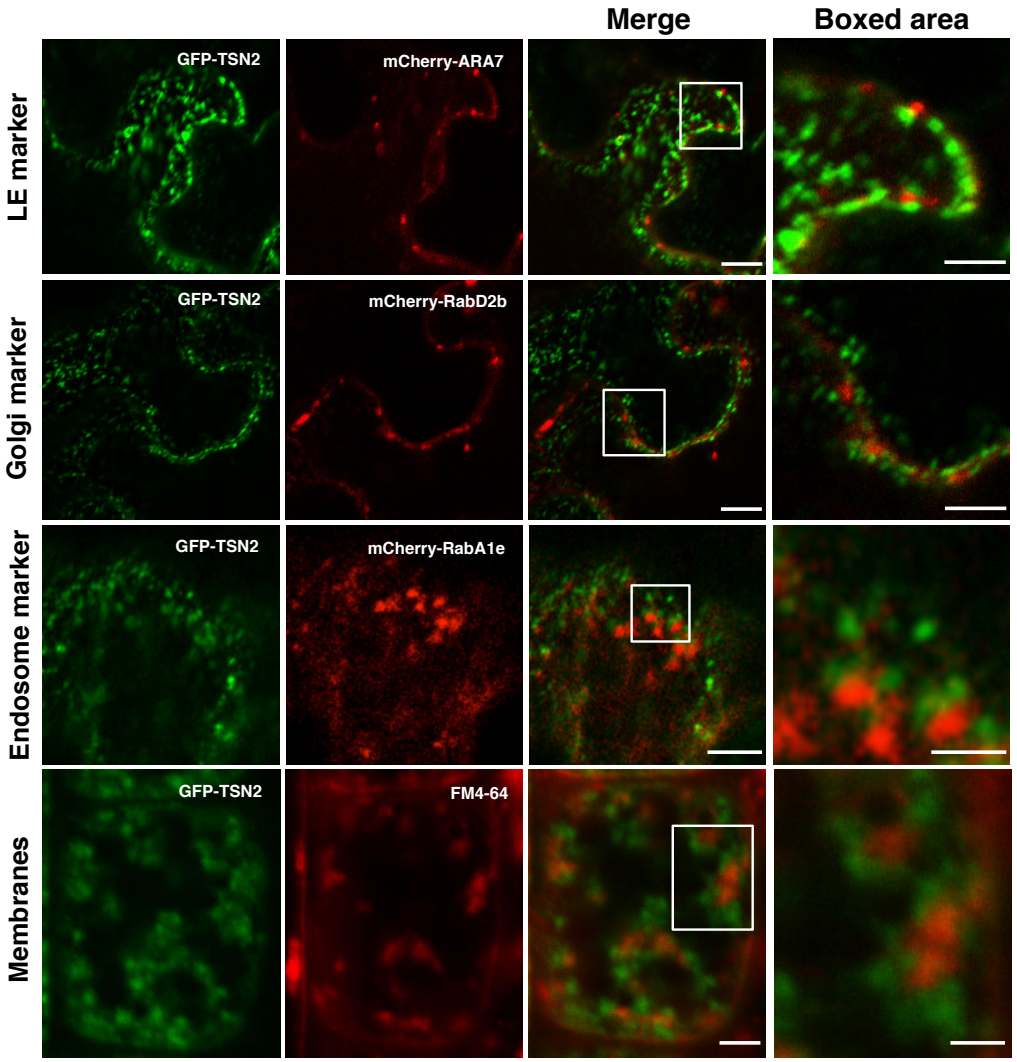



**Figure S2**: Co-localization analysis of GFP-TSN2 with endosome, Golgi, late endosome (LE) and membrane markers.

For co-localization analysis of Golgi, endosome and LE markers, *N. benthamiana* leaves were separately agro-infiltrated with mCherry-ARA7, mCherry-RabD2b or mCherry-RabA1e fusion proteins, respectively, together with GFP-TSN2. After two days, leaves were heat stressed and the fluorescence was monitored using confocal laser scanning microscopy. Scale bars, 10 µm (5 µm in insets). For membrane staining, 5-day-old seedlings expressing *ProTSN2:GFP-TSN2* were incubated with FM4-64 for 15 min and then heat-treated (39ºC) for 40 min. Boxes delimited with white line indicate the areas which are depicted magnified in boxed areas. Scale bars, 5 µm (2 µm in inset).

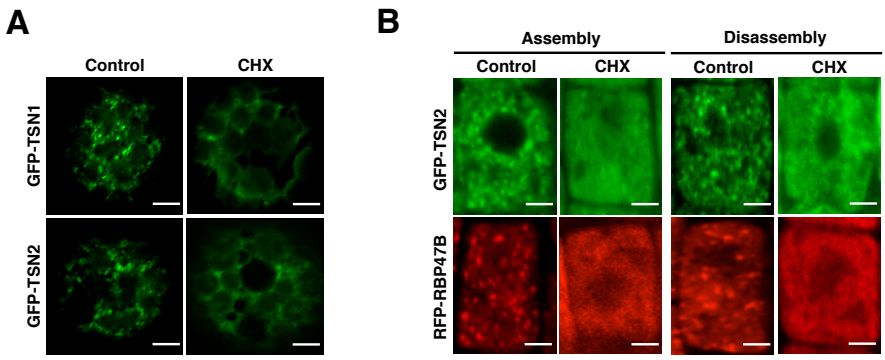



**Figure S3**: Assembly and disassembly of TSN-containing foci is cycloheximide (CHX) dependent.
**(A)** Localization of GFP-TSN1 and GFP-TSN2 in protoplasts treated with CHX and subjected to heat stress. Protoplasts were incubated in 35 µM CHX or DMSO (control) for 15 min at 23ºC and then incubated for an additional 30 min at 39ºC. N-terminal GFP and fusion proteins were expressed under the control of the 35S promoter. Scale bars, 5 µm.
**(B)** Assembly and disassembly of GFP-TSN2 (green) and RFP-Rbp47b (red) foci in the root tip cells treated with CHX and subjected to heat stress. In the assembly experiments, 5-day-old seedlings expressing *ProTSN2:GFP-TSN2* and *Pro35S:RFP-Rbp47b* fusion proteins were incubated with 35 µM CHX or DMSO (control) for 15 min and then heat stressed for 40 min at 39ºC. In the disassembly experiments, seedlings were heat-stressed for 40 min at 39ºC, treated with 35 µM CHX or DMSO (control) and incubated for an additional 290 min at 23ºC. Scale bars, 2 µm.

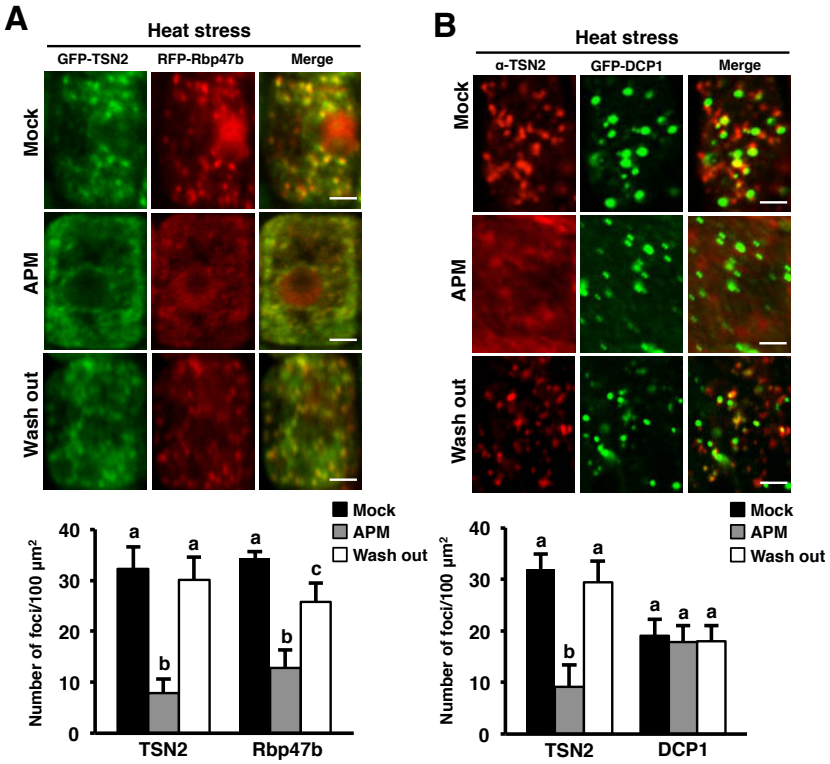



**Figure S4**: Co-localization analysis of TSN2 with RFP-Rbp47b and GFP-DCP1 in heat-stressed root tip cells after APM treatment.

**(A)** Five-day-old seedlings expressing *ProTSN2:GFP-TSN2* (green) and SG marker *Pro35S:RFP-Rbp47b* (red) were treated with 10 µM APM or DMSO (mock) for 5 h at 23ºC. APM-treated seedlings were washed and incubated for 2 h in liquid MS medium (wash out). Heat stress was induced for 40 min at 39ºC. Scale bars, 2 µm. Graph shows mean number ± SD of TSN2 and Rbp47b foci per 100 µm$^2$ of cytoplasm counted in 5 to 10 plants in each triplicate experiment. Means with different letters are significantly different at $p<0.05$; Student's *t*-test.

**(B)** Five-day-old seedlings expressing PB marker *Pro35S:GFP-DCP1* (green) were treated as described in **(A)**. TSN was immunostained with anti-TSN2 (red). Scale bars, 2 µm. Graph shows mean number ± SD of TSN2 and DCP1 foci per 100 µm$^2$ of cytoplasm counted in 5 to 10 plants in each triplicate experiment. Means with different letters are significantly different at $p<0.05$; Student's *t*-test.

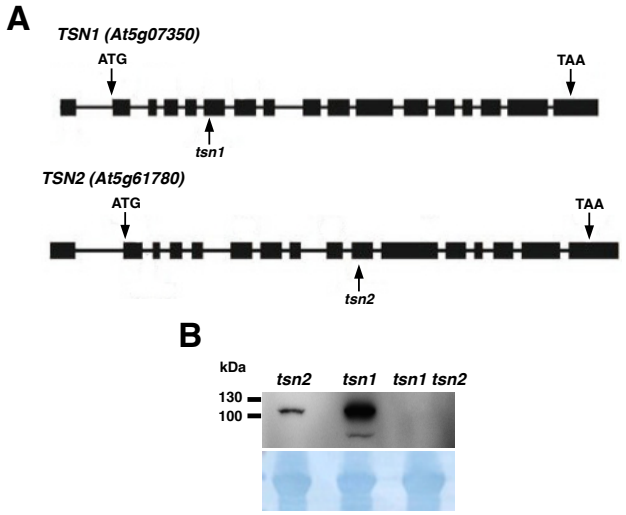



**Figure S5**: Characterisation of *tsn1 tsn2* double knockout Arabidopsis line.
**(A)** The position of T-DNA insertions in *TSN1* and *TSN2* (arrows). Boxes and lines indicate exons and introns, respectively.
**(B)** Immunoblot analysis of TSN in *tsn2*, *tsn1* and *tsn1 tsn2* using rabbit anti-TSN (top panel). Coomassie Brilliant Blue R-250 staining was used as a loading control (bottom panel).

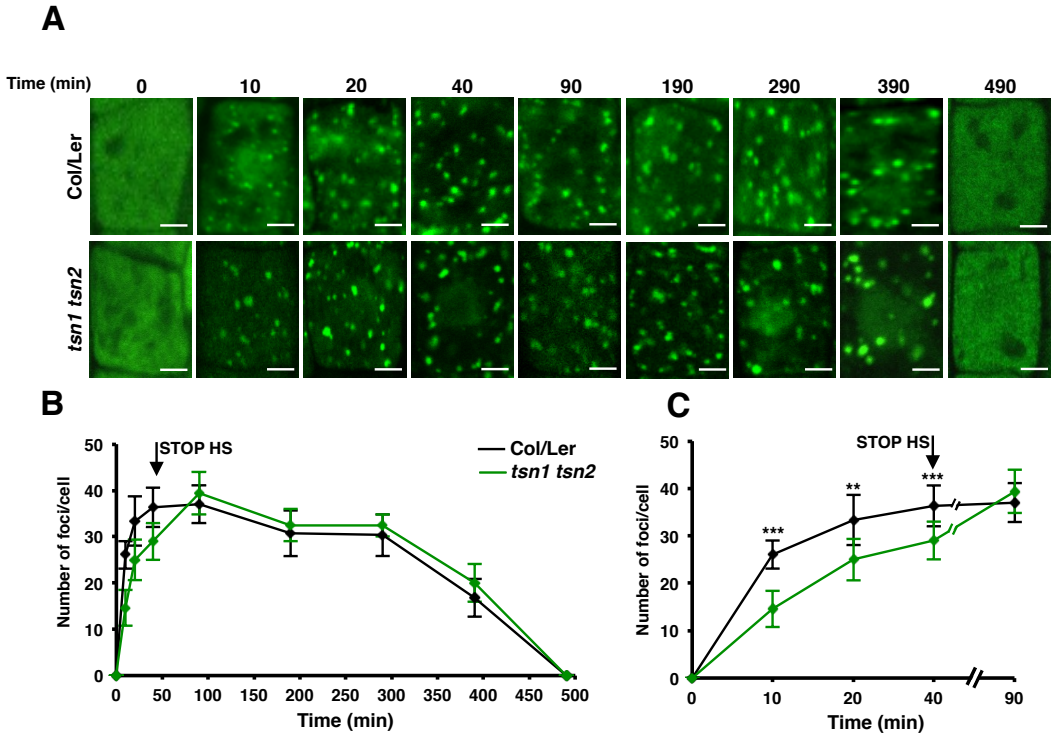



**Figure S6**: Kinetics of assembly and disassembly of GFP-Rbp47b foci in WT (Col/Ler) and *tsn1 tsn2* root tip cells.

**(A)** Five-day-old Col/Ler and *tsn1 tsn2* seedlings expressing *Pro35S:GFP-Rbp47b* were heat-stressed at 39ºC for 40 min followed by incubation at 23ºC for up to 500 min. Scale bars, 2 µm.

**(B)** Kinetics of assembly and disassembly of Rbp47b foci.

**(C)** Fragment of graph shown in **(B)** corresponding to the first 90 min.

Graphs in (**B**) and (**C**) show mean ± SD of triplicate experiments, each including 5 to 10 plants per line. STOP HS, time point when the plants were transferred from 39ºC to 23ºC. **, $p<0.05$; ***, $p<0.001$; Student's *t*-test.

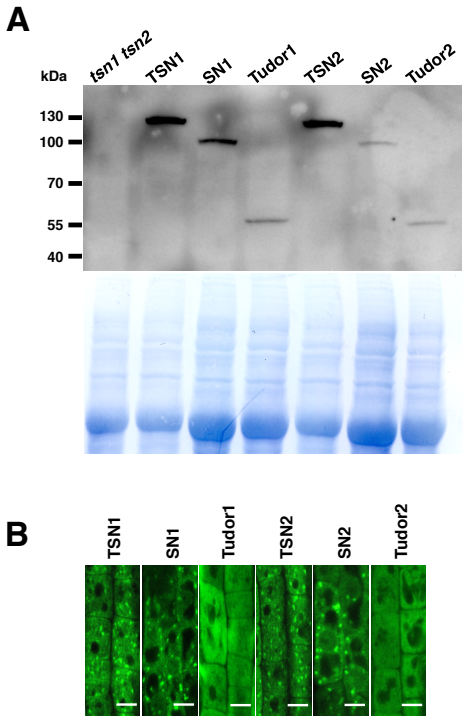



**Figure S7**: Protein expression levels in *tsn1 tsn2* double knockout complemented lines.
**(A)** Immunoblot analysis of GFP-TSN, GFP-SN and GFP-Tudor fusion proteins expressed in the *tsn1 tsn2* background. Anti-GFP was used as a primary antibody (top panel). Coomassie Brilliant Blue R-250 staining was used as a loading control (bottom panel).
**(B)** Localization of GFP fusion proteins shown in (**A**). Five-day-old *tsn1 tsn2* seedlings expressing full-length (*ProTSN:GFP-TSN*) and truncated versions (*Pro35S:GFP-SN* and *Pro35S:GFP-Tudor*) of TSN were heat stressed for 40 min at 39ºC. Scale bars, 25 µm.

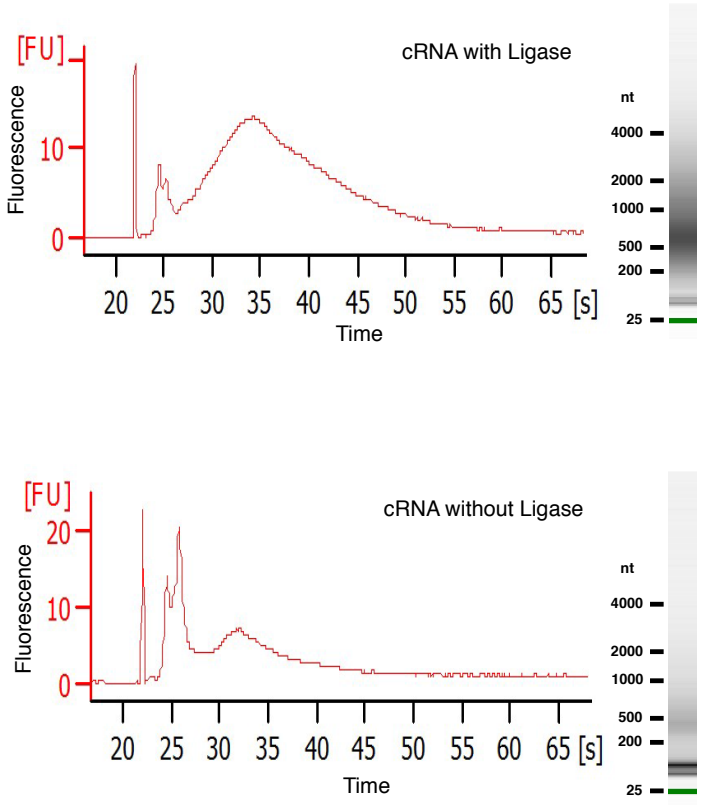



**Figure S8**: Bioanalyzer electropherograms of amplified cRNA samples.
Uncapped mRNA was isolated from total mRNA by adding an RNA adaptor and using T4 RNA ligase and subsequent cRNA synthesis. The cRNA distribution in the probes prepared with and without T4 RNA ligase was evaluated using an Agilent 2100 bioanalyzer. The resulting electropherograms from the positive experiment (upper panel) shows the expected profile, with a size distribution ranging from 250 to 5,000 nucleotides (nt). In contrast, the resulting electropherograms from the control experiment (lower panel) shows predominant presence of short cRNA.

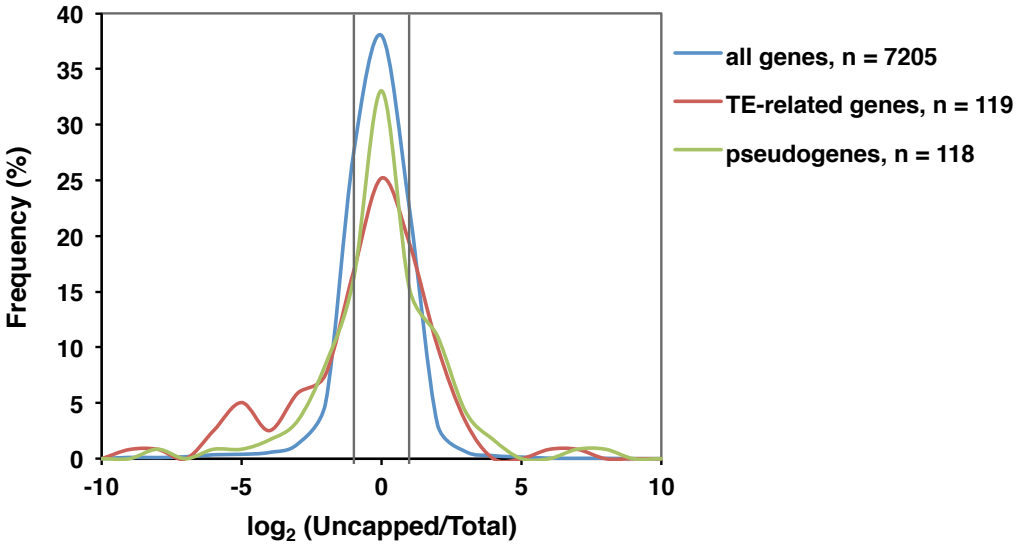



**Figure S9**: Distribution profiles of mRNA decapping levels in heat-stressed Col plants. Grey vertical lines indicate uncapped/total ratios of 0.5 and 2 that were set as cutoffs for depletion and enrichment, respectively.

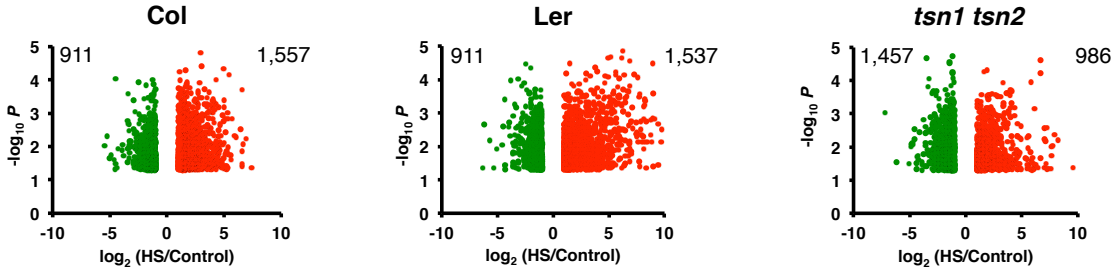



**Figure S10**: Expression profile of uncapped mRNAs in Col, Ler and *tsn1 tsn2* plants under heat stress *versus* control conditions.
Volcano plots where $\log_2$-transformed ratios of uncapped mRNA signals under heat stress (HS) to control conditions were plotted against the negative $\log_{10}$ transformed *p* values. Only transcripts differentially accumulated under heat stress and control conditions are shown ($p<0.05$). Green and red dots denote enriched and depleted uncapped mRNAs with heat stress/control ratios of >2 and <0.5, respectively.

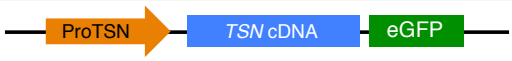
| Gene constructs | Figures |
|---|---|
|  | 1 |
|  | 1,4,3,5,10, S3B, S4A, S6, S7 |
|  | 2,3,5,4, S1, S3B, S4A |
|  | 2, S1 |
|  | 4 |
|  | 8,10, S7 |
|  | 5,6,7,9,S5 |
|  | 5,6,7, S4B |
|  | 2,S1,S2, S3A |

**Table S1**: Gene constructs used in this study.

| Primers used in this work | |
|---|---|
| **Cloning** | |
| egb1 (TSN1 -F) | 5´-GGGGACCACTTTGTACAAGAAAGCTGGGTATTACCGGCGACCACCAGCA-3´ |
| egb2 (TSN1 -R) | 5´-GCCTGCTGGTGGTCGCCGGTACCCAGCTTTCTTGTACAAAGTGGTCCCC-3´ |
| egb3 (TSN2 -F) | 5´-GGGGACAAGTTTGTACAAAAAAGCAGGCTTGATGGCGACTGGGGCAGCA-3´ |
| egb4 (TSN2 -R) | 5´-GAAACCGGGTCGCGGGTAATACCCAGCTTTCTTGTACAAAGTGGTCCCC-3´ |
| egb5 (SN1 -R) | 5´-GGGGACCACTTTGTACAAGAAAGCTGGGTATTATCCGTTTGAGACTTCTTCTCCTTCA-3´ |
| egb6 (Tudor1 -F) | 5´-GGGGACAAGTTTGTACAAAAAAGCAGGCTTGATGAATACTAACACCGTAGAAACCA-3´ |
| egb7 (SN2 -R) | 5´-GGGGACCACTTTGTACAAGAAAGCTGGGTATTAGTTTACTACTTCCTCTCCTTCA-3´ |
| egb8 (Tudor2 -F) | 5´-GGGGACAAGTTTGTACAAAAAAGCAGGCTTGATGGGTAGTTCTAAAGTAGAAACCAGGCA-3´ |
| egb9 (Rbp47b -F) | 5´-GGGGACAAGTTTGTACAAAAAAGCAGGCTTGATGCAGACAACCAACGGC-3´ |
| egb10 (Rbp47b -R) | 5´-GGGGACCACTTTGTACAAGAAAGCTGGGTATCAATTCTCCCCATGATAGTTG-3´ |
| egb11 (Ubp1 -F) | 5´-GGGGACAAGTTTGTACAAAAAAGCAGGCTTGATGCAGAGGTTGAAGCAGCA-3´ |
| egb12 (Ubp1 -R) | 5´-GGGGACCACTTTGTACAAGAAAGCTGGGTATTACTGGTAGTACATGAGCTGCT-3´ |
| egb13 (DCP1 -F) | 5´-GGGGACAAGTTTGTACAAAAAAGCAGGCTTG ATGTCTCAAAACGGGAAGA-3´ |
| egb14 (DCP1 -R) | 5´-GGGGACCACTTTGTACAAGAAAGCTGGGTATCATTGTTGAAGTGCATT-3´ |
| egb15 (DCP2 -F) | 5´-GGGGACAAGTTTGTACAAAAAAGCAGGCTTGATGTCGGGCCTCCATCGA -3´ |
| egb16 (DCP2 -R) | 5´-GGGGACCACTTTGTACAAGAAAGCTGGGTATCAAGCTGAATTACCAGA -3´ |
| egb17 (ProTSN1 -F) | 5´-GGGGACAAGTTTGTACAAAAAAGCAGGCTTGGTCTCAATGAGTGATCAAGTCAAT-3´ |
| egb18 (ProTSN1 -R) | 5´-GGGGACCACTTTGTACAAGAAAGCTGGGTACGCTGCCATGTTCACTAGCTAACCTTAGAC-3´ |
| egb19 (ProTSN2 -F) | 5´-GGGGACAAGTTTGTACAAAAAAGCAGGCTTGTGACAATATGAAAATATGAGCTAAGT-3´ |
| egb20 (ProTSN2 -R) | 5´-GGGGACCACTTTGTACAAGAAAGCTGGGTACGCCATGTTGGTGATTGGCTAAGACCTGC-3´ |
| egb21 (GFP -F) | 5´-GGGGACAAGTTTGTACAAAAAAGCAGGCTTGATGGTGAGCAAGGGCGAG-3´ |
| egb22 (GFP -R) | 5´-GGGGACCACTTTGTACAAGAAAGCTGGGTACTTGTACAGCTCGTCCAT-3´ |
| **Fusion PCR for ProTSN-TSN-GFP and ProTSN-GFP-TSN** | |
| egb23 (TSN1 -R) | 5´-TAGCTAGTGAACATGGCAACGGGGGCTGAGAACCAATGGCT-3´ |
| egb24 (TSN1 -F) | 5´-AGCCATTGGTTCTCAGCCCCCGTTGCCATGTTCACTAGCTA-3´ |
| egb25 (TSN2 -F) | 5´-CCAATCACCAACATGGCGACTGGGGCAGCAACTGAGAA-3´ |
| egb26 (TSN2 -R) | 5´-TTCTCAGTTGCTGCCCCAGTCGCCATGTTGGTGATTGG-3´ |
| egb27 (ProTSN1 -R) | 5´-TCGCCCTTGCTCACCATGTTCACTAGCTAACCTTAGAC-3´ |
| egb28 (GFP -F) | 5´-GTCTAAGGTTAGCTAGTGAACATGGTGAGCAAGGGCGA-3´ |
| egb29 (GFP -R) | 5´-GGTGGCGACCGGTGGATCCTTGTACAGCTCGTCCAT-3´ |
| egb30 (TSN1 -F) | 5´-AAGGATCCACCGGTCGCCACCATGGCAACGGGGGCTGA-3´ |
| egb31 (ProTSN2 -R) | 5´-TCGCCCTTGCTCACCATGTTGGTGATTGGCTAAGAC-3´ |
| egb32 (GFP -F) | 5´-GTCTTAGCCAATCACCAACATGGTGAGCAAGGGCGA-3´ |
| egb33 (TSN2 -F) | 5´-AAGGATCCACCGGTCGCCACCATGGCGACTGGGGCAGC-3´ |

**Table S2**: Primers used in this study.