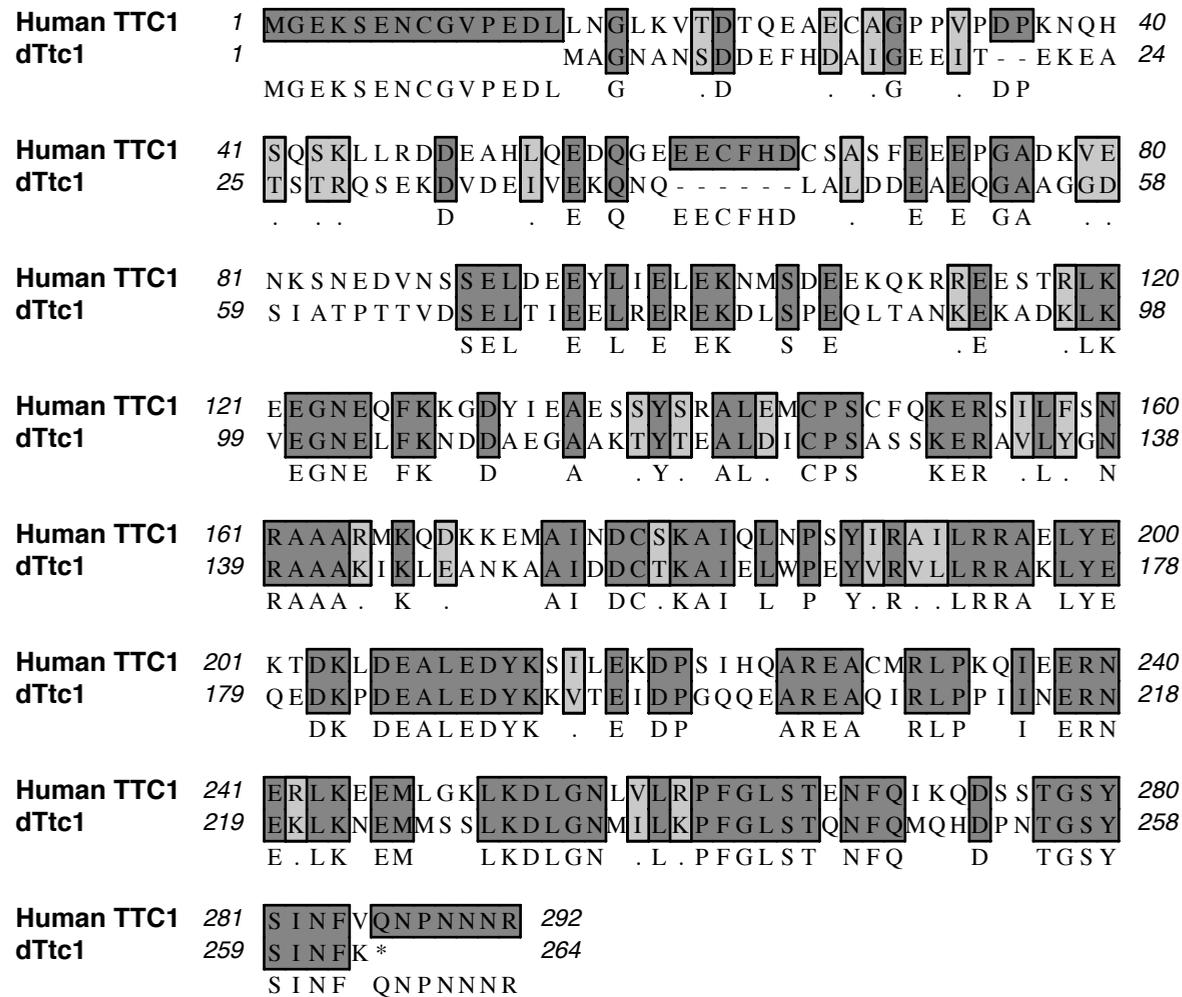


Fig. S1. (A) A cellular compartment GO enrichment analysis of the Egl interactome. **(B)** Egg chambers expressing a control shRNA against *eb1* (B) or an shRNA against *egl* (B') were fixed and processed for immunofluorescence using an antibody against Me31b. Me31b signal is shown using a red to white LUT. Although P bodies are present in Egl depleted egg chambers, diffuse cytoplasmic Me31b localization is increased. **(C)** Lysates were also prepared from ovaries of these same genotypes. The lysates were analyzed by western blotting using an antibody against Me31b and Khc as a loading control. The expression of Me31b is reduced in Egl depleted ovaries.

A.

Formatted Alignments



B.

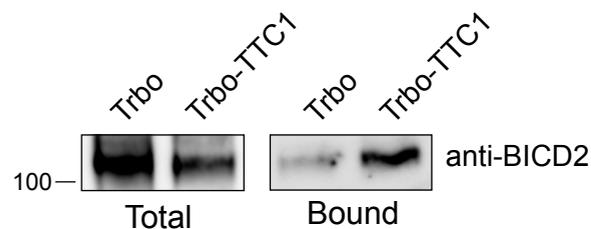


Fig. S2. (A) A schematic showing an alignment between human TTC1 and *Drosophila* dTtc1. Identical residues are shaded in dark grey and conserved residues are shaded in light grey. **(B)** HeLa cells were transiently transfected with constructs expressing either Trbo alone or Trbo-TTC1. Lysates were prepared from these cells and biotinylated proteins were purified using streptavidin agarose. Bound proteins were eluted and analyzed by western blotting using an antibody against BicD2.

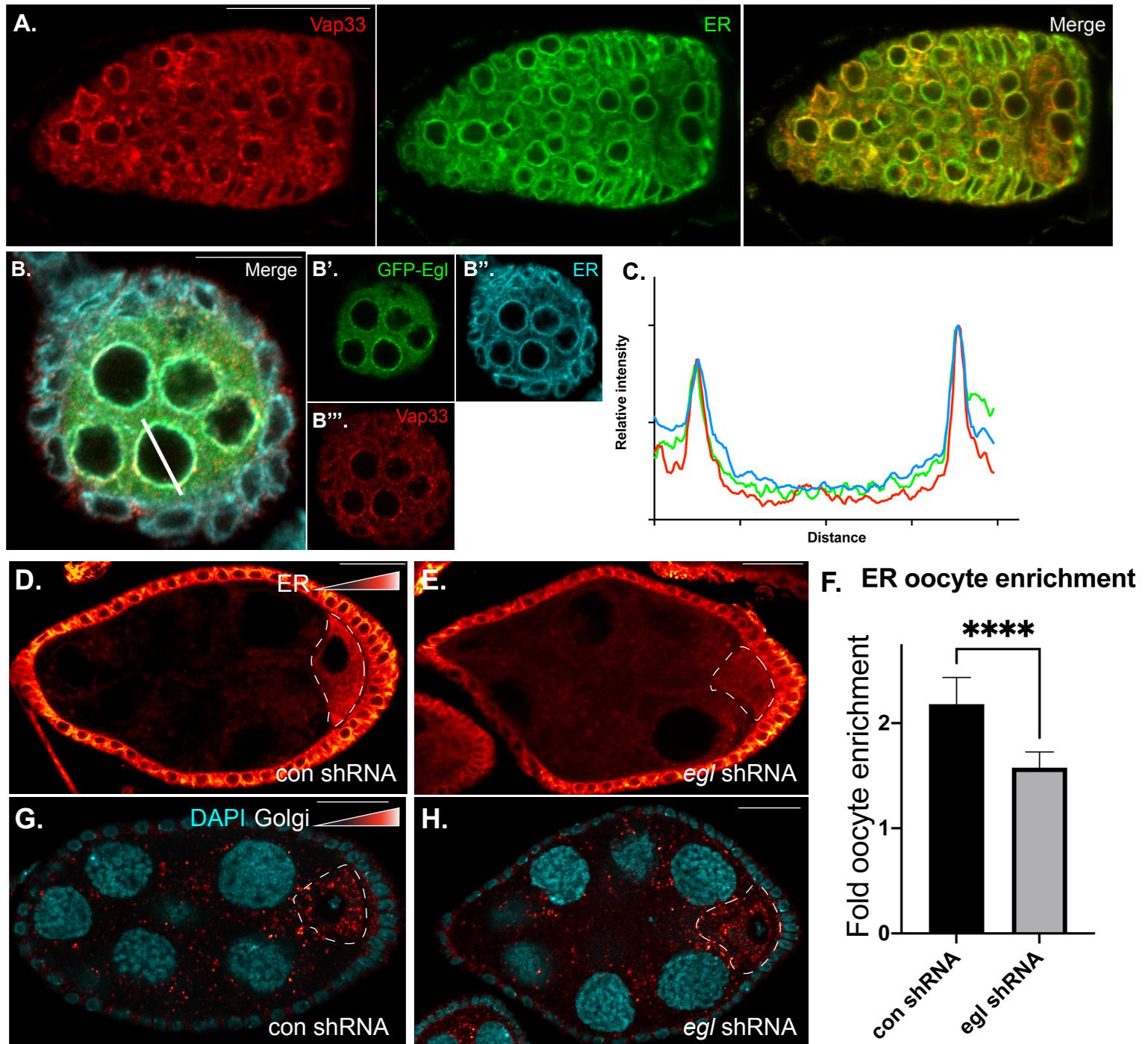


Fig. S3. (A) Egg chambers from wild-type flies were fixed and processed for immunofluorescence using antibodies against Vap33 (red) and Cnx99A (green), an ER vesicle maker. A merged image is also shown. (B-C) Egg chambers from flies expressing Egl-GFP (green) were fixed and processed for immunofluorescence using antibodies against Vap33 (Red) and Cnx99A (cyan). All three proteins were abundantly localized in a peri-nuclear region. Panel C shows the intensity profile of the line depicted in panel B. (D-H) Egg chambers expressing a control shRNA (D, G) or an shRNA against *egl* (E, H) were fixed and processed using antibodies against ER vesicles (D, E) and Golgi vesicles (G, H). Signal for these proteins is represented using the red to white LUT. Oocyte enrichment of ER was dependent upon Egl, whereas the modest oocyte-enrichment of Golgi vesicles was not Egl-dependent. The oocyte is indicated by dashed lines. Quantification of the ER localization data is shown in panel F (n=15 egg chambers per genotype). Unpaired *t* tests were used for these analyses; *****p*<0.0001. The scale bar is 20 microns.

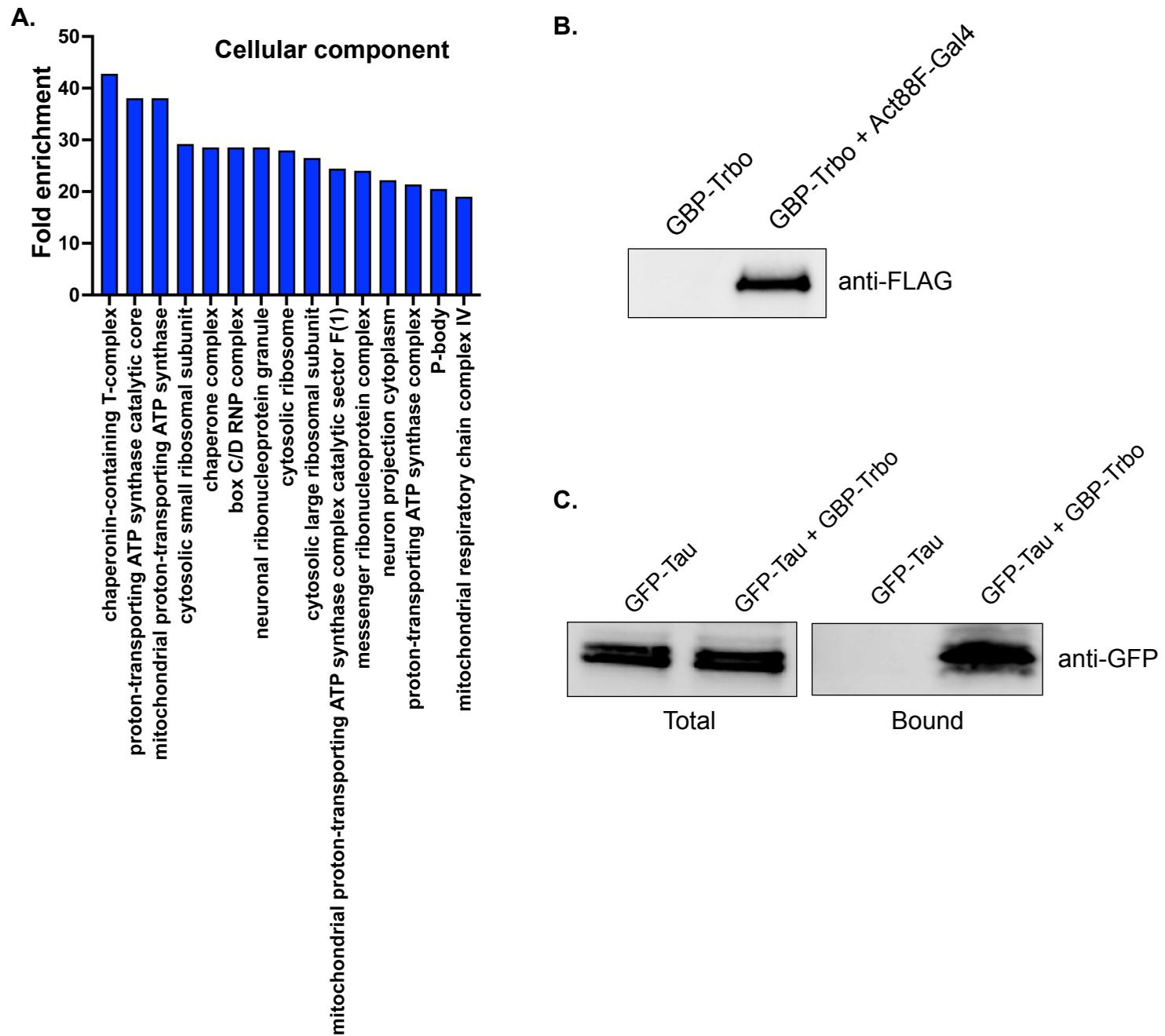


Fig. S4. (A) A cellular compartment GO enrichment analysis of the Me31b interactome. **(B)** Whole fly lysates were prepared from control flies (no driver) or flies expressing GBP-Trbo using the Act88-Gal4 driver. The lysates were analyzed by western blotting using a FLAG antibody. The Trbo fusion also contains a FLAG tag. **(C)** Whole fly lysates were prepared from strains expressing GFP-Tau or GFP-Tau and GBP-Trbo. GBP-Trbo was driven using the Act88-Gal4 driver. The lysates were incubated with Streptavidin conjugated beads. After binding and wash steps, bound proteins were eluted and analyzed by western blotting using an antibody against GFP. GFP-Tau is specifically precipitated in lysates expressing GBP-Trbo.

Trbo construct used for expression in flies.

```
GGATCCGGAGGGGGAGGAAGTGGTGGTGGAGGAAGCGGAGGGGGTGGATCGGGCGGCGGGCGGCA
GCGGCGGAGGCGGCTCCATGAAGGACAACACCGTTCCGCTGAAGCTGATTGCCTTGCTGGCAAA
TGGGGAGTTCCACAGCGGCGAGCAGCTTGGAGAGACGCTGGGCATGTCCC GCGCCGCCATCAAC
AAGCACATACAGACGCTGCGCGATTGGGGCGTTGACGTGTTTACAGTGCCGGAAAGGGCTACA
GCTTGCCAGAGCCAATACCTCTTCTGAACGCTAAGCAGATCCTGGGCCAGTTGGATGGTGGATC
GGTCGCAGTGCTCCCAGTCGTGGATAGCACTAATCAGTACTTGCTGGATAGGATCGGTGAGCTG
AAGAGTGGCGATGCTTGCATCGCGGAATATCAGCAAGCCGGCCGCGGCTCCCCTGGCAGGAAGT
GGTTCAGCCCCTTCGGCGCCAACCTATATCTGTCGATGTTCTGGCGCTTGAAGCGCGGCCCCGC
TGCCATCGGGCTGGGACCGGTCATTGGCATCGTAATGGCAGAGGCGCTGCGGAAACTCGGTGCC
GATAAGGTGCGAGTGAAATGGCCCAACGACCTGTACTTACAAGATCGCAAACCTGGCAGGTATAC
TAGTGGAGCTGGCTGGAATCACCGGCGACGCGGCCAGATTGTGATCGGAGCCGGTATCAATGT
GGCGATGCGCCGAGTGGAGGAGTCAGTGGTTAACCAGGGCTGGATTACCCTACAGGAGGCCGGT
ATCAACTTGGACAGAAATACCTTGGCGGCCACCCTCATCCGCGAACTTCGTGCCGCCCTGGAAC
TCTTCGAACAAGAGGGACTGGCCCCCTACCTCCCCCGCTGGGAGAAGTTAGATAATTTTCATCAA
CCGTCCCCTAAAGCTGATTATTGGTGATAAAGAGATCTTTGGCATCAGCCGCGGCATAGACAAA
CAAGGCGCCCTCCTGCTTGAAGCAGGACGGAGTCATTAAGCCGTGGATGGGAGGCGAAATTTCCC
TGCGGTCTGCTGAAAAGATGGACTACAAAGACCATGACGGTGATTATAAAGATCATGACATCGA
TTACAAGGATGACGATGACAAGTAATCTAGA
```

BamH1

25 amino acid linker

Trbo

3xFLAG tag

XbaI

Fig. S5. Sequence of Trbo used in these studies.

GBP construct used for expression of GBP-Trbo in flies.

```
GGTACCATGGCCGATGTGCAGTTGGTGGAGTCAGGCGGCGCCCTGGTTCAACCCGGAGGTAGCC
TGCGACTGAGCTGCGTCGCCTCGGGCTTCCCCGTAATCGCTACTCGATGCGCTGGTATCGCCA
GGCCCCGGCAAGGAACGTGAGTGGGTGGCCGGAATGTCCAGTGCGGGTGACCGGAGCAGCTAC
GTTGATTCCGTGAAGGGCCGCTTCACCATCCGCCGCGATGATGCAAGGAACACCGTCTATCTGC
AGATGAATAGCCTGAAGCCGGAGGACACGGCTGTGTACTACTATAACGTAAACGTGGGATTCGA
GTACTGGGGCCACGGCACCCAGGTGACCGTCAGCTTTGGGGGAAGCGGCGGATCTGGAGGATCG
GGCGGCAGCGGCGGCTCCATGGCCGATGTGCAGTTGGTTCGAATCCGGCGGAGCTCTGGTACAGC
CCGGTGGCAGCCTGCGTCTGAGTTGCGTGGCCTCCGGCTTTCCCGTGAATCGCTATAGCATGCG
CTGGTATCGCCAAGCCCCGGCAAGGAGCGGGAGTGGGTGGCCGGTATGTCCAGCGCGGGTGAT
AGGTTCGTCATACGTGGATAGCGTCAAGGGCCGCTTCACCATCCGACGCGACGATGCCCGCAACA
CCGTCTACCTGCAGATGAACTCGCTGAAGCCCCGAGGACACCGCAGTGTATTACTACAATGTTAA
CGTTGGATTTCGAGTACTGGGGTCACGGCACGCAGGTGACCGTGAGTTTCGGAGGGGGAGGAAGT
GGTGGTGGAGGAAGCGGAGGGGGTGGATCGGGCGGCGGCGGCAGCGGCGGAGGCGGCTCCGGAT
CC
```

Kpn1

GBP

Amino acid linker

BamH1

Fig. S6. Sequence of GBP-Trbo used for expression in *Drosophila* tissue.

GBP construct used for expression of Trbo-GBP in mammalian cells.

```
ATGGCCGACGTGCAGCTCGTGGAATCCGGAGGAGCACTGGTCCAGCCTGGGGGATCACTGAGAC
TGTCTTGTGTCGCTTCTGGCTTTCCTGTGAATCGGTACTCTATGAGATGGTACAGGCAGGCACC
TGGAAAAGAGCGAGAATGGGTGCGCCGGAATGTCATCTGCTGGCGATAGGAGTTCCTACGTGGAT
AGCGTGAAAGGACGGTTCACCATTAGAAGGGACGATGCTAGAAACACCGTGTACCTCCAGATGA
ACTCCCTGAAACCTGAGGACACTGCTGTGTACTACTACAACGTCAATGTGGGCTTTGAATACTG
GGGACATGGCACACAAGTGAAGTGTAGTTTTGCATGCGGGGAAGCGGCGGATCTGGAGGATCG
GGCGGCAGCGGCGGCTCCATGGCCGACGTGCAGCTGGTGGAGAGCGGCGGCGCCCTGGTGCAGC
CCGGCGGCAGCCTGCGGCTGAGCTGCGTGGCCAGCGGCTTCCCCGTGAACCGGTACAGCATGCG
GTGGTACCGGCAGGCCCGGCAAGGAGCGGGAGTGGGTGGCCGGCATGAGCAGCGCCGGCGAC
CGGAGCAGCTACGTGGACAGCGTGAAGGGCCGGTTCACCATCCGGCGGGACGACGCCCGGAACA
CCGTGTACCTGCAGATGAACAGCCTGAAGCCCAGGACACCGCCGTGTACTACTACAACGTGAA
CGTGGGCTTCGAGTACTGGGGCCACGGCACCCAGGTGACCGTGAGCTTCGCCTGC
```

GBP
25 amino acid linker

Fig. S7. Sequence of Trbo-GBP used for expression in mammalian cells.

Table S1. The Egl interactome.

[Click here to download Table S1](#)

Table S2. The Me31B interactome identified using GFP-Trbo.

[Click here to download Table S2](#)

Table S3. Previously identified Me31b interacting proteins.

[Click here to download Table S3](#)

Table S4. The dTtc1 interactome.

[Click here to download Table S4](#)