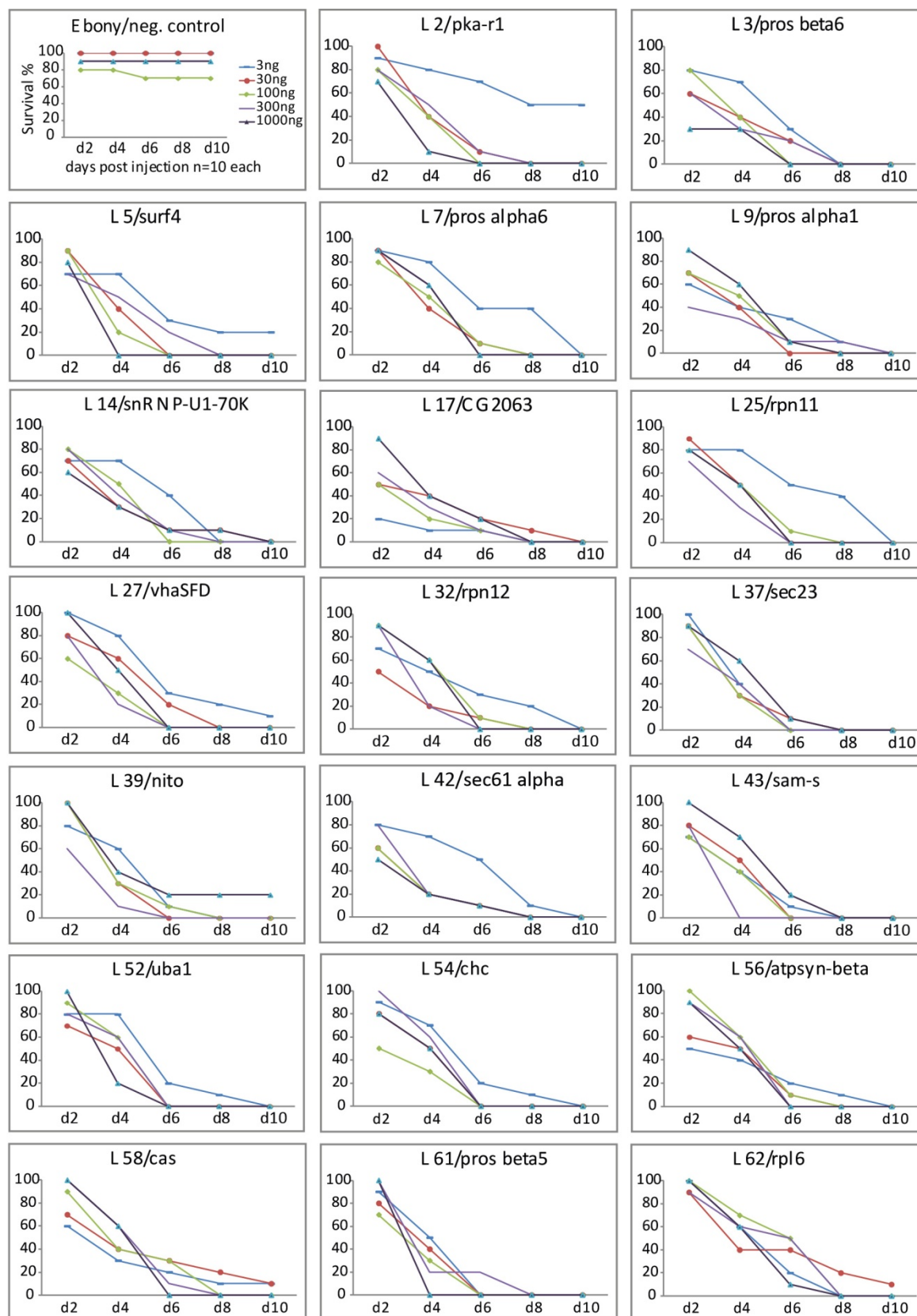
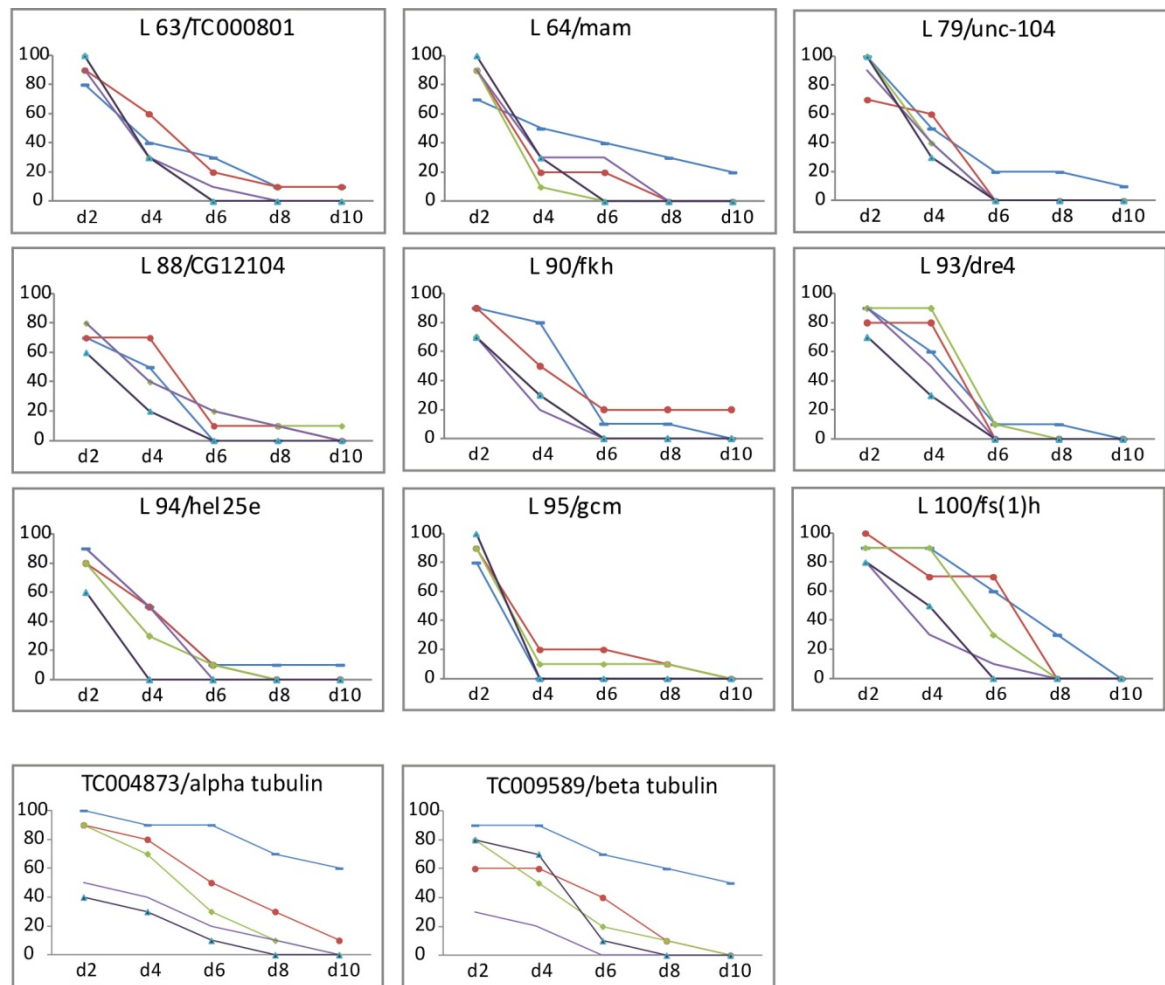


## Additional File 2

**Figure S1: Identification of forty novel RNAi target genes in *Tribolium castaneum***

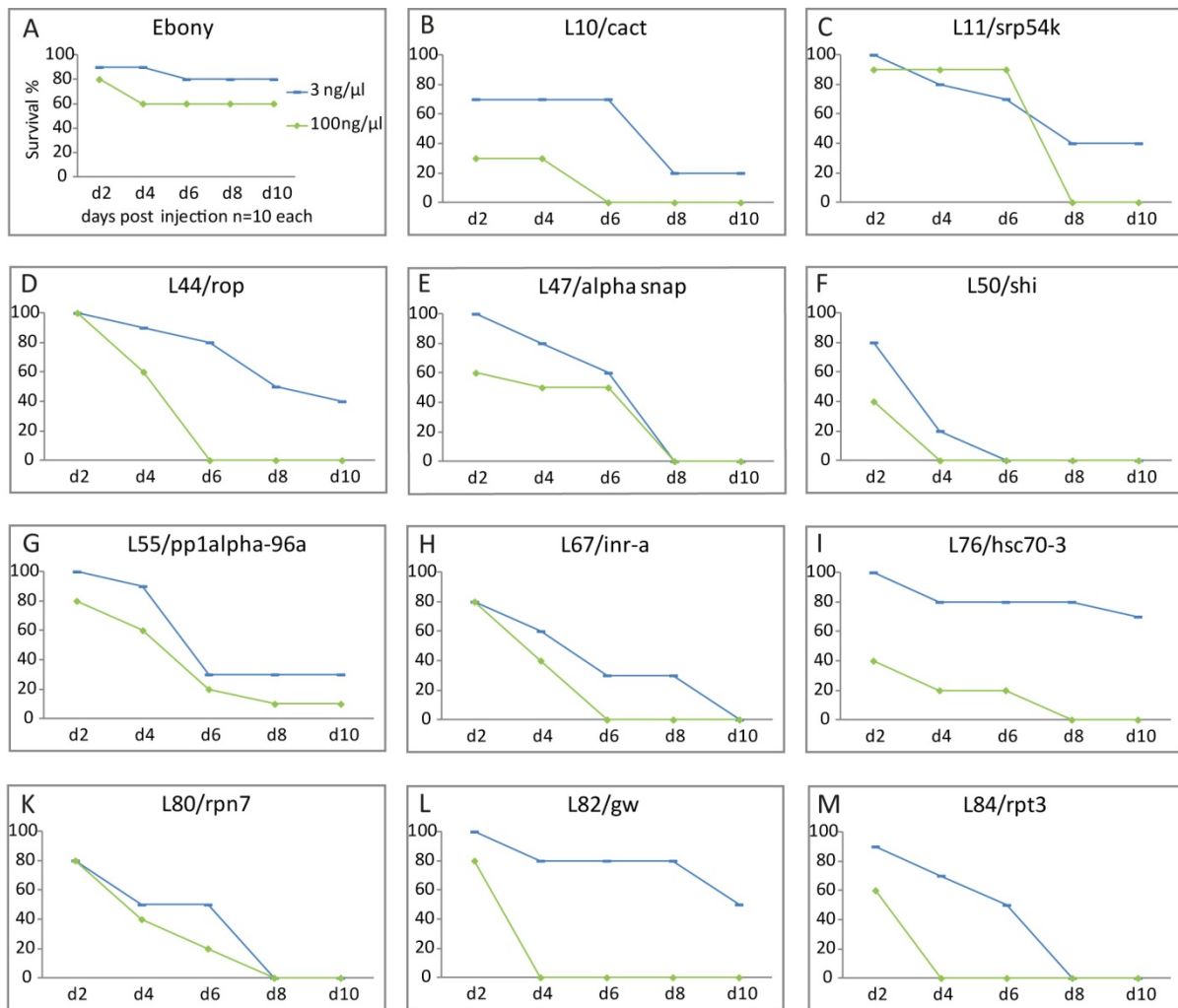
**A**





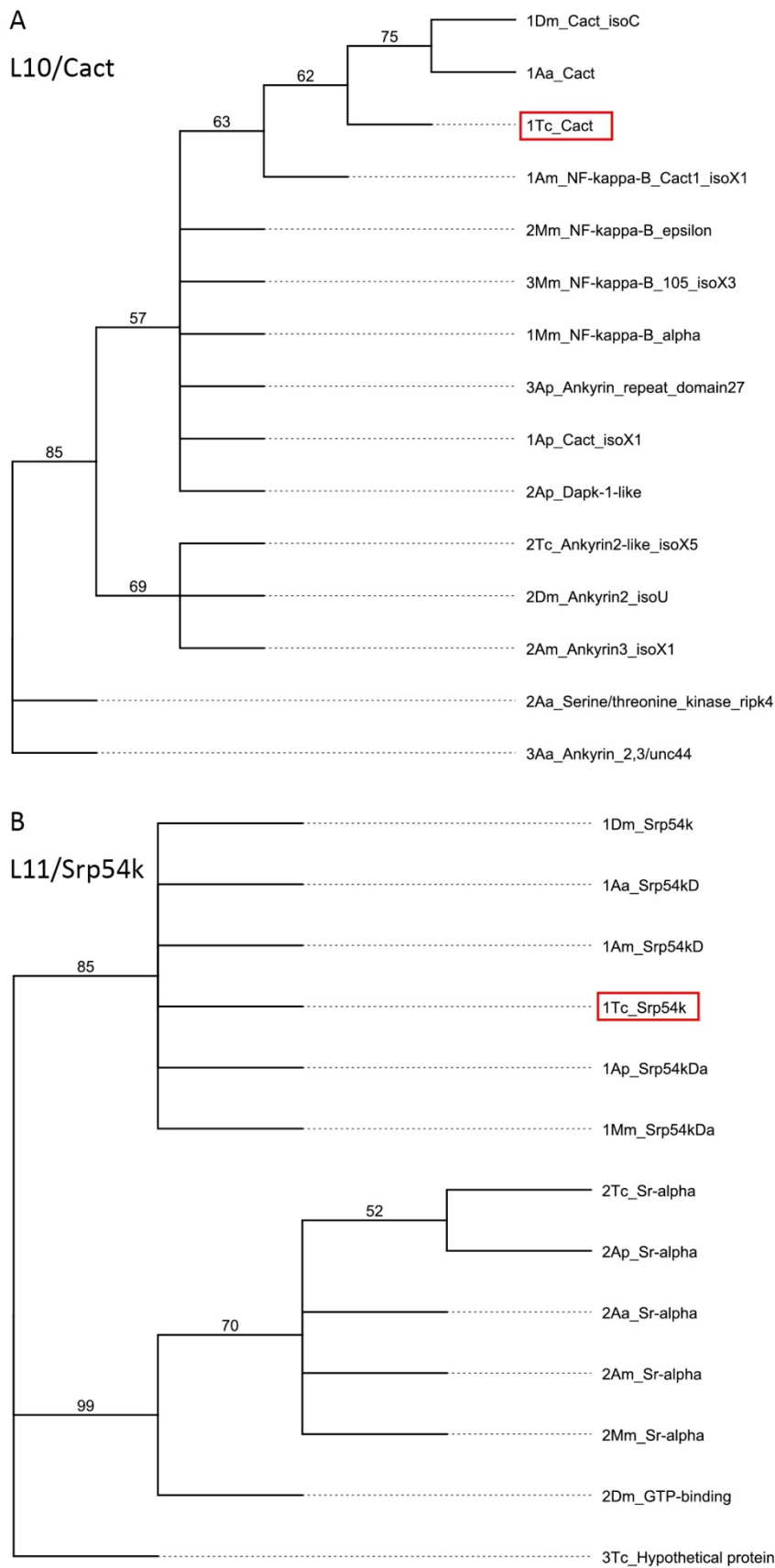
Based on data of the iBeetle screen, the 100 most efficient RNAi target genes were selected and retested using different dsRNA concentrations. (A) The results for the most efficient 40 lethal genes are shown. The corresponding results of the top eleven candidates are shown in Figure 1. See further details in figure legend of Figure 1. (B) The *Tribolium* orthologs of the two less effective RNAi target genes published by Baum et al., 2007 [1] are displayed.

**Figure S2: Lethality is induced in adult stages as well**



The most efficient eleven RNAi target genes were also tested at two different concentrations (3ng/μl, 100ng/μl) by injections into adult beetles. (A) *Tc-ebony* was used as negative control. (B-M) All RNAi target genes are lethal in adult stages as well but at lower concentrations, the efficiency was slightly lower compared to larval injections shown in Figure 1.

Figure S3: Phylogenetic trees of the novel RNAi target genes



L44/Rop



D

L47/Snap

Phylogenetic tree D showing relationships between various Snap protein isoforms. The tree is rooted on the left and branches to the right. Bootstrap values are indicated at the nodes. The taxa are listed on the right, with some names truncated. The taxa 1Tc\_Snap and 1Am\_Snap\_isoX2 are highlighted with a red box.

1Tc\_Snap

1Am\_Snap\_isoX2

1Dm\_alpha\_Snap

1Aa\_Snap

1Ap\_Snap

4Mm\_beta\_Snap\_isoX1

2Mm\_beta\_Snap

3Mm\_alpha\_Snap\_isoX1

1Mm\_alpha\_Snap

2Tc\_Snap

2Dm\_Super\_sex\_combs\_isoB

5Mm\_gamma\_Snap

2Ap\_gamma\_Snap

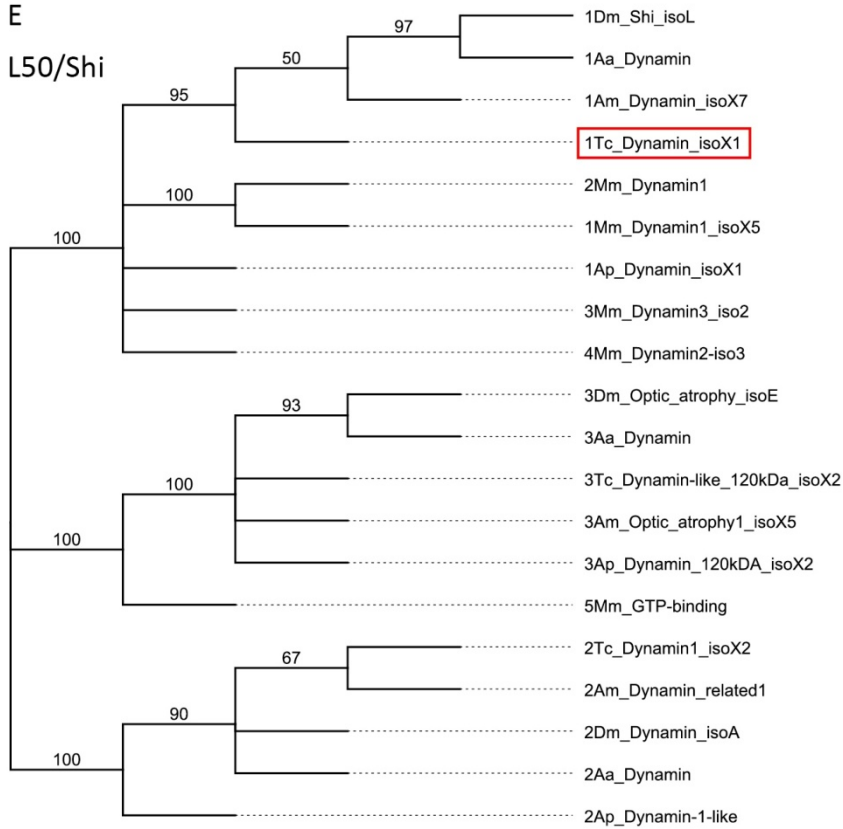
3Tc\_gamma\_Snap

2Aa\_gamma\_Snap

2Am\_LOC409363

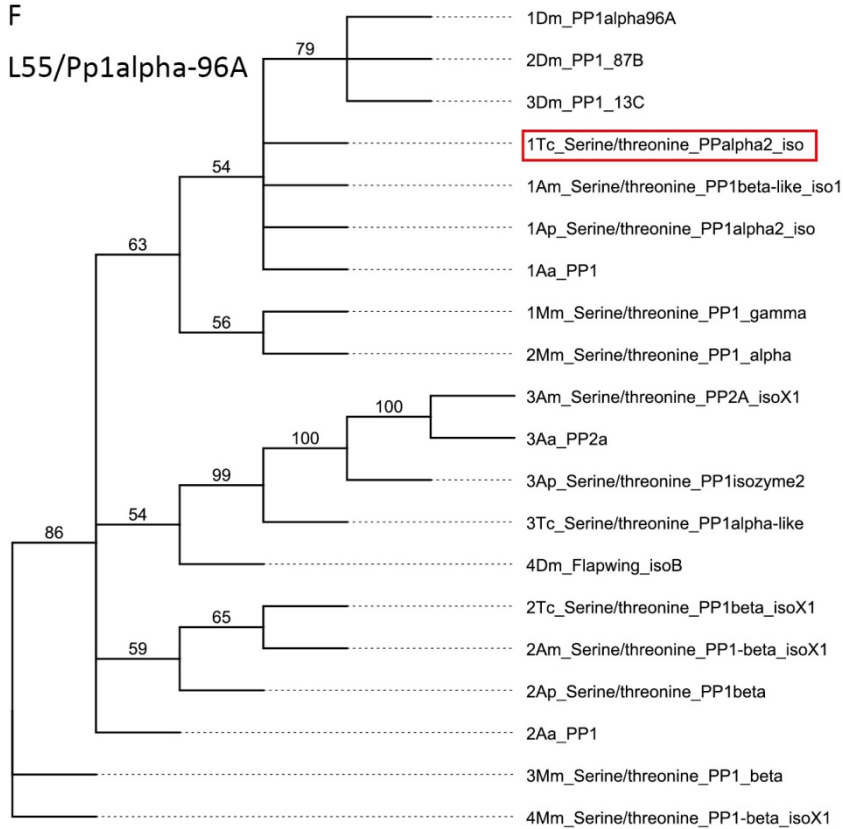
E

L50/Shi



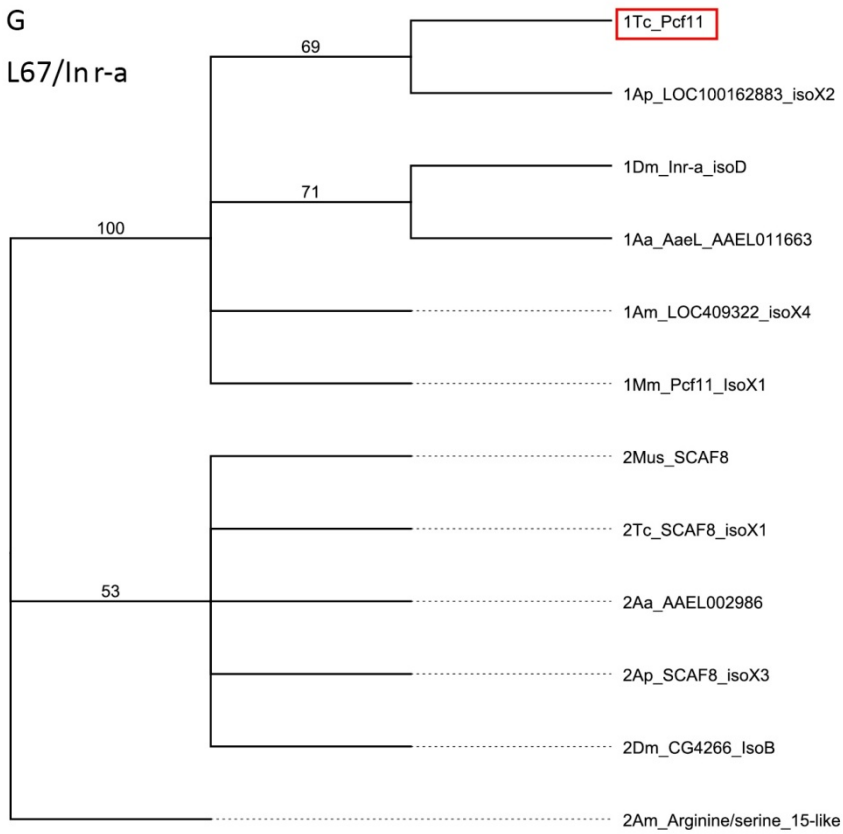
F

L55/Pp1alpha-96A



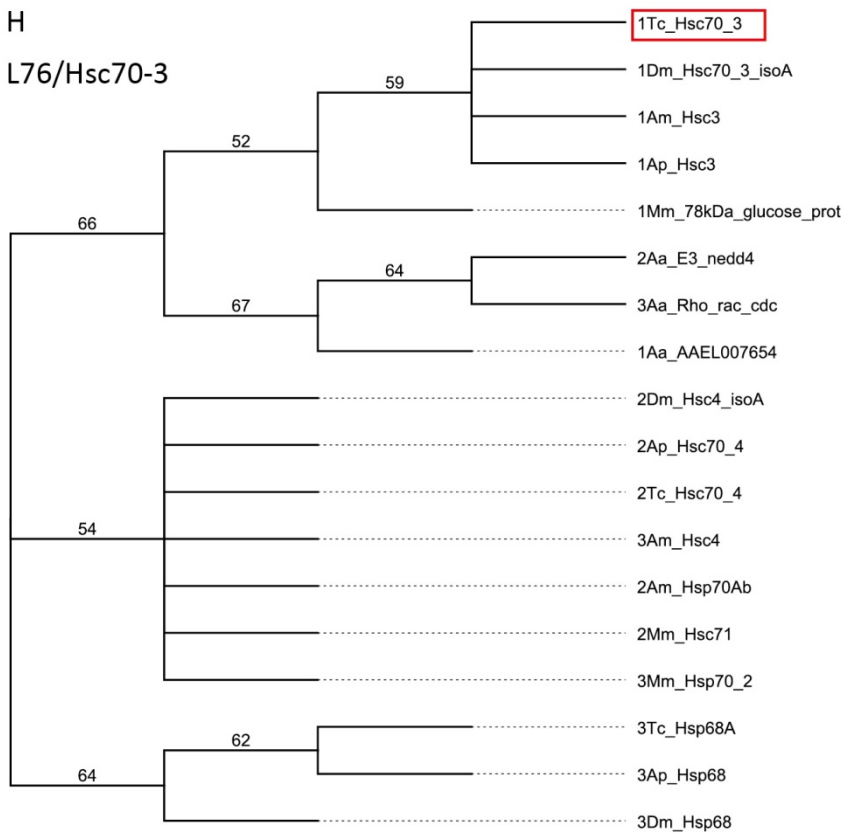
G

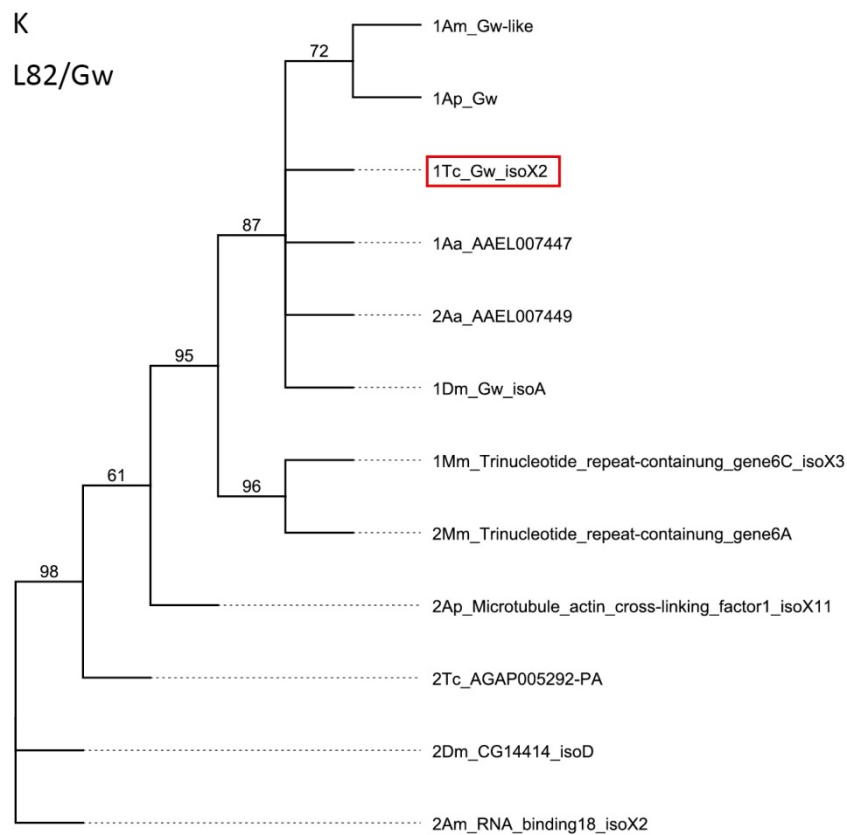
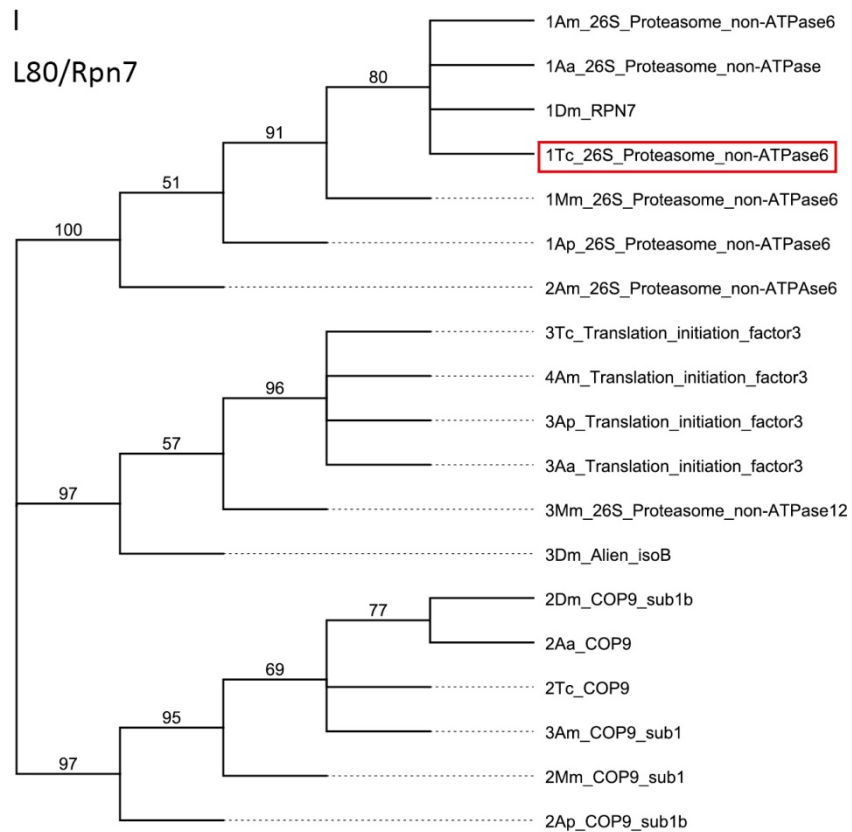
L67/Inr-a

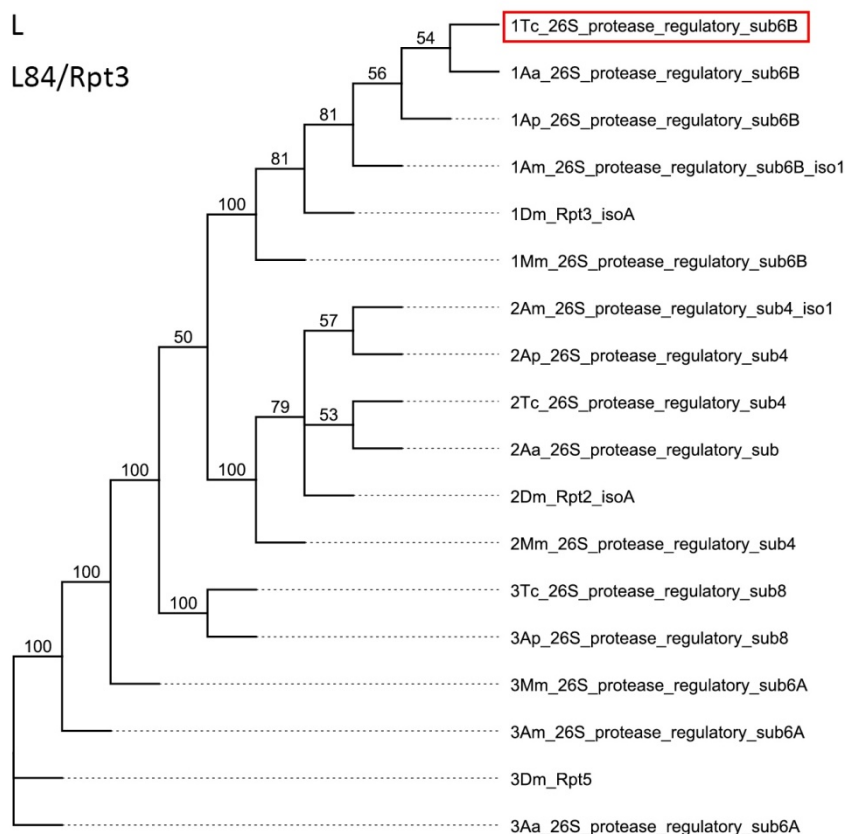


H

L76/Hsc70-3







M

| Tribolium Expression Level        | L10 | L11 | L44 | L47 | L50 | L55 | L67 | L76 | L80 | L82 | L84 |
|-----------------------------------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| adult male body                   |     |     |     |     |     |     |     |     |     |     |     |
| Drosophila Expression Level       |     |     |     |     |     |     |     |     |     |     |     |
| adult male body                   |     |     |     |     |     |     |     |     |     |     |     |
| Drosophila Expression Level       |     |     |     |     |     |     |     |     |     |     |     |
| central nervous system, larvae L3 |     |     |     |     |     |     |     |     |     |     |     |
| salivary gland, larvae L3         |     |     |     |     |     |     |     |     |     |     |     |
| digestive system, larvae L3       |     |     |     |     |     |     |     |     |     |     |     |
| fat body, larvae L3               |     |     |     |     |     |     |     |     |     |     |     |
| carcass, larvae L3                |     |     |     |     |     |     |     |     |     |     |     |
| low expression                    |     |     |     |     |     |     |     |     |     |     |     |
| moderate expression               |     |     |     |     |     |     |     |     |     |     |     |
| high expression                   |     |     |     |     |     |     |     |     |     |     |     |
| very high expression              |     |     |     |     |     |     |     |     |     |     |     |

(A-L) The *Tribolium* protein sequences were blasted against *Drosophila melanogaster*, *Apis mellifera*, *Aedes aegypti*, *Acyrtosiphon pisum* and *Mus musculus* RefSeq protein collection [2] at NCBI (<http://blast.ncbi.nlm.nih.gov/Blast.cgi>). The alignments were done using ClustalW as implemented in the Geneious program (v.5.6.4) (Biomatters, Auckland, New Zealand) and trimmed to remove unclear parts of the alignments. The phylogenetic trees were calculated using the Geneious Tree Builder with the Jukes Cantor genetic distance model, neighbor-joining [3] as tree building method, and a number of 10.000 replicates for

creation of the bootstrap consensus tree [4]. Dm *Drosophila melanogaster*, Am *Apis mellifera*, Aa *Aedes aegypti*, Ap *Acyrtosiphon pisum*, Mm *Mus musculus*.

(M) Expression levels of the eleven RNAi target genes of *Tribolium* adult male body were obtained from RNA-Seq data of the *Tribolium* au2 gene set (<http://bioinf.uni-greifswald.de/tcas/genes/au2/>) and compared to the expression levels of the orthologous *Drosophila* genes in the adult male body obtained from modENCODE high-throughput RNA-Seq data in Flybase [5]. *Tribolium* expression levels were calculated and categorized into four expression strengths: low expression with 0-1.5, moderate expression with 1.6-3, high expression with 3.1-4.5 and very high expression with  $\geq 4.6$  number of reads per position. The comparison revealed similar expression levels only for L10, L11 and L76 in the adult male body of *Tribolium* and *Drosophila*.

**Figure S4: GO term analysis of top 40 RNAi target genes reveals additional targets**

| Annotation Cluster 1  | Enrichment Score: 4,26                                                             |       |    |          |
|-----------------------|------------------------------------------------------------------------------------|-------|----|----------|
| Category              | Term                                                                               | Count | %  | PValue   |
| SP_PIR_KEYWORDS       | proteasome                                                                         | 7     | 19 | 1,61E-09 |
| GOTERM_CC_FAT         | GO:0000502~proteasome complex                                                      | 7     | 19 | 1,03E-06 |
| KEGG_PATHWAY          | dme03050:Proteasome                                                                | 7     | 19 | 1,37E-06 |
| GOTERM_MF_FAT         | GO:0004175~endopeptidase activity                                                  | 7     | 19 | 1,21E-02 |
| GOTERM_BP_FAT         | GO:0006508~proteolysis                                                             | 8     | 22 | 2,39E-02 |
| GOTERM_MF_FAT         | GO:0070011~peptidase activity, acting on L-amino acid peptides                     | 7     | 19 | 4,36E-02 |
| Genes                 | CG16916, CG5378, CG18174, CG4157, CG4904, CG1782,CG4097, CG12323                   |       |    |          |
| Annotation Cluster 2  | Enrichment Score: 2,37                                                             |       |    |          |
| SP_PIR_KEYWORDS       | threonine protease                                                                 | 3     | 8  | 1,72E-03 |
| INTERPRO              | IPR001353:Proteasome, subunit alpha/beta                                           | 3     | 8  | 3,46E-03 |
| GOTERM_MF_FAT         | GO:0004298~threonine-type endopeptidase activity                                   | 3     | 8  | 5,05E-03 |
| GOTERM_MF_FAT         | GO:0070003~threonine-type peptidase activity                                       | 3     | 8  | 5,05E-03 |
| GOTERM_CC_FAT         | GO:0005839~proteasome core complex                                                 | 3     | 8  | 9,76E-03 |
| Genes                 | CG4904, CG4097, CG12323                                                            |       |    |          |
| Annotation Cluster 3  | Enrichment Score: 2,22                                                             |       |    |          |
| GOTERM_BP_FAT         | GO:0008104~protein localization                                                    | 7     | 19 | 4,24E-03 |
| GOTERM_BP_FAT         | GO:0015031~protein transport                                                       | 6     | 16 | 4,71E-03 |
| GOTERM_BP_FAT         | GO:0006886~intracellular protein transport                                         | 5     | 14 | 4,97E-03 |
| GOTERM_BP_FAT         | GO:0045184~establishment of protein localization                                   | 6     | 16 | 5,23E-03 |
| GOTERM_BP_FAT         | GO:0034613~cellular protein localization                                           | 5     | 14 | 5,49E-03 |
| GOTERM_BP_FAT         | GO:0070727~cellular macromolecule localization                                     | 5     | 14 | 1,59E-02 |
| Genes                 | CG4659, CG15811, CG9539, CG6625, CG9012, CG5848, CG13281                           |       |    |          |
| Annotation Cluster 4  | Enrichment Score: 2,10                                                             |       |    |          |
| GOTERM_BP_FAT         | GO:0007268~synaptic transmission                                                   | 5     | 14 | 6,65E-03 |
| GOTERM_BP_FAT         | GO:0019226~transmission of nerve impulse                                           | 5     | 14 | 7,55E-03 |
| GOTERM_BP_FAT         | GO:0007267~cell-cell signaling                                                     | 5     | 14 | 9,74E-03 |
| Genes                 | CG15811, CG18102, CG42341, CG6625, CG9012                                          |       |    |          |
| Annotation Cluster 5  | Enrichment Score: 2,09                                                             |       |    |          |
| GOTERM_BP_FAT         | GO:0030163~protein catabolic process                                               | 6     | 16 | 1,12E-03 |
| GOTERM_BP_FAT         | GO:0044265~cellular macromolecule catabolic process                                | 6     | 16 | 1,89E-03 |
| GOTERM_BP_FAT         | GO:0051603~proteolysis involved in cellular protein catabolic process              | 5     | 14 | 5,94E-03 |
| GOTERM_BP_FAT         | GO:0044257~cellular protein catabolic process                                      | 5     | 14 | 5,94E-03 |
| GOTERM_BP_FAT         | GO:0019941~modification-dependent protein catabolic process                        | 4     | 11 | 2,99E-02 |
| GOTERM_BP_FAT         | GO:0043632~modification-dependent macromolecule catabolic process                  | 4     | 11 | 3,04E-02 |
| SP_PIR_KEYWORDS       | Protease                                                                           | 4     | 11 | 3,37E-02 |
| Genes                 | CG16916, CG18174, CG4904, CG1782, CG4097, CG12323, CG31992                         |       |    |          |
| Annotation Cluster 6  | Enrichment Score: 2,01                                                             |       |    |          |
| SP_PIR_KEYWORDS       | nucleotide-binding                                                                 | 9     | 24 | 6,56E-04 |
| GOTERM_MF_FAT         | GO:0032555~purine ribonucleotide binding                                           | 10    | 27 | 1,35E-02 |
| GOTERM_MF_FAT         | GO:0032553~ribonucleotide binding                                                  | 10    | 27 | 1,35E-02 |
| GOTERM_MF_FAT         | GO:0017076~purine nucleotide binding                                               | 10    | 27 | 2,06E-02 |
| GOTERM_MF_FAT         | GO:0032559~adenyl ribonucleotide binding                                           | 8     | 22 | 3,71E-02 |
| Genes                 | CG4659, CG16916, CG18102, CG4147, CG42341, CG11154, CG1782, CG7269, CG2674, CG8566 |       |    |          |
| Annotation Cluster 7  | Enrichment Score: 1,92                                                             |       |    |          |
| GOTERM_BP_FAT         | GO:0000226~microtubule cytoskeleton organization                                   | 6     | 16 | 6,31E-03 |
| GOTERM_BP_FAT         | GO:0007052~mitotic spindle organization                                            | 5     | 14 | 7,28E-03 |
| GOTERM_BP_FAT         | GO:0007051~spindle organization                                                    | 5     | 14 | 1,21E-02 |
| GOTERM_BP_FAT         | GO:0007010~cytoskeleton organization                                               | 6     | 16 | 3,67E-02 |
| Genes                 | CG8749, CG42341, CG4157, CG12323, CG7269, CG11522                                  |       |    |          |
| Annotation Cluster 8  | Enrichment Score: 1,66                                                             |       |    |          |
| GOTERM_BP_FAT         | GO:0043623~cellular protein complex assembly                                       | 4     | 11 | 4,40E-03 |
| GOTERM_BP_FAT         | GO:0034622~cellular macromolecular complex assembly                                | 4     | 11 | 2,82E-02 |
| GOTERM_BP_FAT         | GO:0006461~protein complex assembly                                                | 4     | 11 | 2,90E-02 |
| GOTERM_BP_FAT         | GO:0070271~protein complex biogenesis                                              | 4     | 11 | 2,90E-02 |
| GOTERM_BP_FAT         | GO:0034621~cellular macromolecular complex subunit organization                    | 4     | 11 | 4,75E-02 |
| Genes                 | CG4659, CG15811, CG6625, CG13281                                                   |       |    |          |
| Annotation Cluster 9  | Enrichment Score: 1,62                                                             |       |    |          |
| GOTERM_BP_FAT         | GO:0010324~membrane invagination                                                   | 5     | 14 | 1,92E-02 |
| GOTERM_BP_FAT         | GO:0006897~endocytosis                                                             | 5     | 14 | 1,92E-02 |
| GOTERM_BP_FAT         | GO:0016044~membrane organization                                                   | 5     | 14 | 3,80E-02 |
| Genes                 | CG18102, CG6625, CG9012, CG5848, CG13281                                           |       |    |          |
| Annotation Cluster 10 | Enrichment Score: 1,56                                                             |       |    |          |
| GOTERM_BP_FAT         | GO:0050657~nucleic acid transport                                                  | 3     | 8  | 2,64E-02 |
| GOTERM_BP_FAT         | GO:0050658~RNA transport                                                           | 3     | 8  | 2,64E-02 |
| GOTERM_BP_FAT         | GO:0051236~establishment of RNA localization                                       | 3     | 8  | 2,72E-02 |
| GOTERM_BP_FAT         | GO:0015931~nucleobase, nucleoside, nucleotide and nucleic acid transport           | 3     | 8  | 3,04E-02 |
| Genes                 | CG17332, CG9012, CG7269                                                            |       |    |          |

The functional clusters as revealed by GO term analysis (DAVID database) using our top forty RNAi target genes are displayed. GOTERM\_BP\_FAT where BP means biological process; GOTERM\_CC\_FAT where CC means cellular component; GOTERM\_MF\_FAT where MF means molecular function; SP\_PIR\_KEYWORDS where PIR means protein information resource.

## Supplementary References

1. Baum JA, Bogaert T, Clinton W, Heck GR, Feldmann P, Ilagan O, Johnson S, Plaetinck G, Munyikwa T, Pleau M, Vaughn T, Roberts J: **Control of coleopteran insect pests through RNA interference.** *Nat Biotechnol* 2007, **25**:1322–1326.
2. Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ: **Basic local alignment search tool.** *J Mol Biol* 1990, **215**:403–410.
3. Saitou N, Nei M: **The neighbor-joining method: a new method for reconstructing phylogenetic trees.** *Mol Biol Evol* 1987, **4**:406–25.
4. Felsenstein J: **Confidence limits on phylogenies: An approach using the bootstrap.** *Evolution* 1985, **39**:783–791.
5. Graveley BR, Brooks AN, Carlson JW, Duff MO, Landolin JM, Yang L, Artieri CG, van Baren MJ, Boley N, Booth BW, Brown JB, Cherbas L, Davis CA, Dobin A, Li R, Lin W, Malone JH, Mattiuzzo NR, Miller D, Sturgill D, Tuch BB, Zaleski C, Zhang D, Blanchette M, Dudoit S, Eads B, Green RE, Hammonds A, Jiang L, Kapranov P, et al.: **The developmental transcriptome of *Drosophila melanogaster*.** *Nature* 2011, **471**:473–479.