Additional File 2

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

A

| Gene     | Survival % | Days post injection | Doses | Legend |
|----------|------------|---------------------|-------|--------|
| Ebony/neg. control | ![Graph](image1.png) | d2, d4, d6, d8, d10 | 3ng, 30ng, 100ng, 300ng, 1000ng |        |
| L 2/pka-r1 | ![Graph](image2.png) | d2, d4, d6, d8, d10 |        |        |
| L 3/pros beta6 | ![Graph](image3.png) | d2, d4, d6, d8, d10 |        |        |
| L 5/surf4 | ![Graph](image4.png) | d2, d4, d6, d8, d10 |        |        |
| L 7/pros alpha6 | ![Graph](image5.png) | d2, d4, d6, d8, d10 |        |        |
| L 9/pros alpha1 | ![Graph](image6.png) | d2, d4, d6, d8, d10 |        |        |
| L 14/snRN P-U1-70K | ![Graph](image7.png) | d2, d4, d6, d8, d10 |        |        |
| L 17/C G 2063 | ![Graph](image8.png) | d2, d4, d6, d8, d10 |        |        |
| L 25/rpn11 | ![Graph](image9.png) | d2, d4, d6, d8, d10 |        |        |
| L 27/vhaSFD | ![Graph](image10.png) | d2, d4, d6, d8, d10 |        |        |
| L 32/rpn12 | ![Graph](image11.png) | d2, d4, d6, d8, d10 |        |        |
| L 37/sec23 | ![Graph](image12.png) | d2, d4, d6, d8, d10 |        |        |
| L 39/nito | ![Graph](image13.png) | d2, d4, d6, d8, d10 |        |        |
| L 42/sec61 alpha | ![Graph](image14.png) | d2, d4, d6, d8, d10 |        |        |
| L 43/sam-s | ![Graph](image15.png) | d2, d4, d6, d8, d10 |        |        |
| L 52/uba1 | ![Graph](image16.png) | d2, d4, d6, d8, d10 |        |        |
| L 54/chc | ![Graph](image17.png) | d2, d4, d6, d8, d10 |        |        |
| L 56/atpsyn-beta | ![Graph](image18.png) | d2, d4, d6, d8, d10 |        |        |
| L 58/cas | ![Graph](image19.png) | d2, d4, d6, d8, d10 |        |        |
| L 61/pros beta5 | ![Graph](image20.png) | d2, d4, d6, d8, d10 |        |        |
| L 62/pl6 | ![Graph](image21.png) | d2, d4, d6, d8, d10 |        |        |
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.
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

A
L10/Cact

B
L11/Srp54k
(A-L) The *Tribolium* protein sequences were blasted against *Drosophila melanogaster*, *Apis mellifera*, *Aedes aegypti*, *Acyrthosiphon pisum* and *Mus musculus* RefSeq protein collection [2] at NCBI ([http://blast.ncbi.nlm.nih.gov/Blast.cgi](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

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| Tribolium Expression Level | L10 | L11 | L44 | L47 | L50 | L55 | L67 | L76 | L80 | L82 | L84 |
|---------------------------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| adult male body           |     |     |     |     |     |     |     |     |     |     |     |

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| Drosophila Expression Level | L10 | L11 | L44 | L47 | L50 | L55 | L67 | L76 | L80 | L82 | L84 |
|-----------------------------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| adult male body             |     |     |     |     |     |     |     |     |     |     |     |

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| Drosophila Expression Level | L10 | L11 | L44 | L47 | L50 | L55 | L67 | L76 | L80 | L82 | L84 |
|-----------------------------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| central nervous system, larvae L3 |     |     |     |     |     |     |     |     |     |     |     |

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| Drosophila Expression Level | L10 | L11 | L44 | L47 | L50 | L55 | L67 | L76 | L80 | L82 | L84 |
|-----------------------------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| salivary gland, larvae L3   |     |     |     |     |     |     |     |     |     |     |     |

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| Drosophila Expression Level | L10 | L11 | L44 | L47 | L50 | L55 | L67 | L76 | L80 | L82 | L84 |
|-----------------------------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| digestive system, larvae L3 |     |     |     |     |     |     |     |     |     |     |     |

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| Drosophila Expression Level | L10 | L11 | L44 | L47 | L50 | L55 | L67 | L76 | L80 | L82 | L84 |
|-----------------------------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| fat body, larvae L3         |     |     |     |     |     |     |     |     |     |     |     |

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| Drosophila Expression Level | L10 | L11 | L44 | L47 | L50 | L55 | L67 | L76 | L80 | L82 | L84 |
|-----------------------------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| carcass, larvae L3          |     |     |     |     |     |     |     |     |     |     |     |

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| Drosophila Expression Level | L10 | L11 | L44 | L47 | L50 | L55 | L67 | L76 | L80 | L82 | L84 |
|-----------------------------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| low expression              |     |     |     |     |     |     |     |     |     |     |     |

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| Drosophila Expression Level | L10 | L11 | L44 | L47 | L50 | L55 | L67 | L76 | L80 | L82 | L84 |
|-----------------------------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| moderate expression         |     |     |     |     |     |     |     |     |     |     |     |

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| Drosophila Expression Level | L10 | L11 | L44 | L47 | L50 | L55 | L67 | L76 | L80 | L82 | L84 |
|-----------------------------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| high expression             |     |     |     |     |     |     |     |     |     |     |     |

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| Drosophila Expression Level | L10 | L11 | L44 | L47 | L50 | L55 | L67 | L76 | L80 | L82 | L84 |
|-----------------------------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| very high expression        |     |     |     |     |     |     |     |     |     |     |     |
creation of the bootstrap consensus tree [4]. Dm *Drosophila melanogaster*, Am *Apis mellifera*, Aa *Aedes aegypti*, Ap *Acyrthosiphon 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.unigreifswald.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 ≥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        | 1.6E-09     |
| GOTERM_CC_FAT       | GO:0000502*proteasome complex | 7     | 19        | 1.0E-06     |
| KEGG_PATHWAY        | dme03505:Proteasome      | 7     | 19        | 1.3E-06     |
| GOTERM_MF_FAT       | GO:0004175*endopeptidase activity | 7     | 19        | 1.2E-02     |
| GOTERM_BP_FAT       | GO:0006508*proteolysis    | 8     | 22        | 2.3E-02     |
| GOTERM_MF_FAT       | GO:0070011*peptidase activity, acting on L-amino acid peptides | 7     | 19        | 4.3E-02     |
| Genes               | CG16916, CG5378, CG18174, CG4157, CG4904, CG1782, CG4097, CG12323 |

| Annotation Cluster 2 | Enrichment Score: 3.37 |
|---------------------|--------------------------|
| Category            | Term                      | Count | %         | PValue      |
| SP_PIR_KEYWORDS     | threonine protease        | 3     | 8         | 1.7E-03     |
| INTERPRO            | IP001353:Proteasome, subunit alpha/beta | 3     | 8         | 3.4E-03     |
| GOTERM_MF_FAT       | GO:0004298*threonine-type endopeptidase activity | 3     | 8         | 5.0E-03     |
| GOTERM_MF_FAT       | GO:0070003*threonine-type peptidase activity | 3     | 8         | 5.0E-03     |
| GOTERM_CC_FAT       | GO:0005839*proteasome core complex | 3     | 8         | 9.7E-03     |
| Genes               | CG4904, CG4097, CG12323 |

| Annotation Cluster 3 | Enrichment Score: 7.22 |
|---------------------|--------------------------|
| Category            | Term                      | Count | %         | PValue      |
| GOTERM_BP_FAT       | GO:0008104*protein localization | 7     | 19        | 4.2E-03     |
| GOTERM_BP_FAT       | GO:0015031*protein transport | 6     | 16        | 4.7E-03     |
| GOTERM_BP_FAT       | GO:0006886*intracellular protein transport | 5     | 14        | 4.9E-03     |
| GOTERM_BP_FAT       | GO:0045184*establishment of protein localization | 6     | 16        | 5.2E-03     |
| GOTERM_BP_FAT       | GO:0034613*cellular protein localization | 5     | 14        | 5.4E-03     |
| Genes               | CG4659, CG15811, CG9539, CG6625, CG9012, CG5848, CG13281 |

| Annotation Cluster 4 | Enrichment Score: 2.10 |
|---------------------|--------------------------|
| Category            | Term                      | Count | %         | PValue      |
| GOTERM_BP_FAT       | GO:0007268*synaptic transmission | 5     | 14        | 6.6E-03     |
| GOTERM_BP_FAT       | GO:0019226*transmission of nerve impulse | 5     | 14        | 7.5E-03     |
| GOTERM_BP_FAT       | GO:0007267*cell-cell signaling | 5     | 14        | 9.7E-03     |
| Genes               | CG15811, CG18102, CG42341, CG6625, CG9012 |

| Annotation Cluster 5 | Enrichment Score: 2.09 |
|---------------------|--------------------------|
| Category            | Term                      | Count | %         | PValue      |
| GOTERM_BP_FAT       | GO:0030163*protein catabolic process | 6     | 16        | 1.2E-03     |
| GOTERM_BP_FAT       | GO:0044265*cellular macromolecule catabolic process | 6     | 16        | 1.9E-03     |
| GOTERM_BP_FAT       | GO:0051602*protein synthesis involved in cellular protein catabolic process | 5     | 14        | 5.9E-03     |
| GOTERM_BP_FAT       | GO:0044257*cellular protein catabolic process | 5     | 14        | 5.9E-03     |
| Genes               | CG4659, CG16916, CG18102, CG4147, CG42341, CG11154, CG1782, CG7269, CG2674, CG8566 |

| Annotation Cluster 6 | Enrichment Score: 2.01 |
|---------------------|--------------------------|
| Category            | Term                      | Count | %         | PValue      |
| SP_PIR_KEYWORDS     | nucleotide-binding       | 9     | 24        | 6.5E-04     |
| GOTERM_MF_FAT       | GO:0032555*purine ribonucleotide binding | 10    | 27        | 1.3E-02     |
| GOTERM_MF_FAT       | GO:0032553*ribonucleotide binding | 10    | 27        | 1.3E-02     |
| GOTERM_MF_FAT       | GO:0017076*purine nucleotide binding | 10    | 27        | 2.0E-02     |
| GOTERM_MF_FAT       | GO:0032559*adenylnucleotide binding | 8     | 22        | 3.7E-02     |
| Genes               | CG4659, CG16916, CG18102, CG4147, CG42341, CG11154, CG1782, CG7269, CG2674, CG8566 |

| Annotation Cluster 7 | Enrichment Score: 1.92 |
|---------------------|--------------------------|
| Category            | Term                      | Count | %         | PValue      |
| GOTERM_BP_FAT       | GO:000226*microtubule cytoskeleton organization | 6     | 16        | 6.3E-03     |
| GOTERM_BP_FAT       | GO:0007052*mitotic spindle organization | 5     | 14        | 7.2E-03     |
| GOTERM_BP_FAT       | GO:0007051*spindle organization | 5     | 14        | 1.2E-02     |
| Genes               | CG8749, CG42341, CG4157, CG12323, CG7269, CG11522 |

| Annotation Cluster 8 | Enrichment Score: 1.66 |
|---------------------|--------------------------|
| Category            | Term                      | Count | %         | PValue      |
| GOTERM_BP_FAT       | GO:0043623*cellular protein complex assembly | 4     | 11        | 4.4E-03     |
| GOTERM_BP_FAT       | GO:0034622*cellular macromolecular complex assembly | 4     | 11        | 2.8E-02     |
| GOTERM_BP_FAT       | GO:0006461*protein complex assembly | 4     | 11        | 2.9E-02     |
| GOTERM_BP_FAT       | GO:0070271*protein complex biogenesis | 4     | 11        | 2.9E-02     |
| Genes               | CG4659, CG15811, CG6625, CG13281 |

| Annotation Cluster 9 | Enrichment Score: 1.62 |
|---------------------|--------------------------|
| Category            | Term                      | Count | %         | PValue      |
| GOTERM_BP_FAT       | GO:0010324*membrane invagination | 5     | 14        | 1.9E-02     |
| GOTERM_BP_FAT       | GO:0006897*endocytosis     | 5     | 14        | 1.9E-02     |
| GOTERM_BP_FAT       | GO:0016044*membrane organization | 5     | 14        | 3.8E-02     |
| Genes               | CG18102, CG6625, CG9012, CG5848, CG13281 |

| Annotation Cluster 10 | Enrichment Score: 1.56 |
|-----------------------|--------------------------|
| Category              | Term                      | Count | %         | PValue      |
| GOTERM_BP_FAT         | GO:0050657*nucleic acid transport | 3     | 8         | 2.6E-02     |
| GOTERM_BP_FAT         | GO:0050658*RNA transport   | 3     | 8         | 2.6E-02     |
| GOTERM_BP_FAT         | GO:0051236*establishment of RNA localization | 3     | 8         | 2.7E-02     |
| GOTERM_BP_FAT         | GO:0015931*nucleoside, nucleotide and nucleic acid transport | 3     | 8         | 3.0E-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

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