**Supplemental material**

**Figure S1**

PCA plot showing sample relationships based on the filtered, CPM-TMM and log2 transformed centered dataset.

**Figure S2**

Visualization of expression patterns with the number of genes per cluster.

**Figure S3**

BUSCO assessment of *Spodoptera exigua* compared to other Lepidoptera genomes for ortholog presence and copy number. The relationships are according to Figure 4.

**Figure S4**

Clustered heatmap showing Pearson’s correlation for pairwise sample comparisons based on differentially expressed genes.

**Figure S5**

Gene tree of the nuclear pore complex sequences consisting of: the *Spodoptera*-specific orthogroup sequences (OG0013351) and the Lepidoptera sequences from the protein group 'DDB\_G0274915' as derived from the NCBI protein database. The sequences from the *Spodoptera*-specific orthogroup are marked by the red asterisks, the red frame highlights the *Spodoptera*-specific clade, the red arrow indicates the specific *Spodoptera exigua* differentially expressed gene from expression cluster 4. All sequences derived from the *S.* exigua genome (this study) are underlined. Sequences derived from the protein group 'DDB\_G0274915' are given their full species names and gene ID.

**Figure S6**

Gene tree of the mg7 sequences (reduced dataset) consisting of: the *Spodoptera*-specific orthogroup sequences (OG0014260) and sequences from OrthoDB cluster ‘15970at7088’. The sequences from the *Spodoptera*-specific orthogroup are marked by the red asterisks, the red frame highlights the *Spodoptera-*specific clade, the red arrow indicates the specific *Spodoptera exigua* differentially expressed gene from expression cluster 4. All sequences derived from the *Spodoptera* exigua genome (this study) are underlined. Sequences derived from the OrthoDB cluster are given their full species names.

**Figure S7**

Gene tree of the mg7 sequences (extended dataset) consisting of: the *Spodoptera*-specific orthogroup sequences (OG0014260), sequences from OrthoDB cluster ‘15970at7088’ and all ‘mg’ gene sequences from He et al. (2012). The sequences from the *Spodoptera*-specific orthogroup are marked by the red asterisks, the red frame highlights the clade, the red arrow indicates the specific *Spodoptera exigua* differentially expressed gene from expression cluster 4. All sequences derived from the *Spodoptera* *exigua* genome (this study) are underlined. Sequences derived from the OrthoDB cluster are given their full species names only. Genes from He et al. (2012) are given their species names, followed by their gene ID.

**Figure S8**

Gene tree of βREPAT sequences (reduced dataset) consisting of the *Spodoptera*-specific orthogroup (OG0014254), sequences from OrthoDB cluster ‘16151at7088 - "MBF2"’, and βREPAT gene sequences from Navarro-Cerrillo et al. (2013). The sequences from the *Spodoptera*-specific orthogroup are marked by the red asterisks, the red frame highlights the *Spodoptera*-specific clade, while the red arrow indicates the specific *Spodoptera exigua* differentially expressed gene from expression cluster 4. All sequences derived from the *S. exigua* genome (this study) are underlined. Sequences derived from the OrthoDB cluster are given their full species names and cluster ID "MBF2". Genes from Navarro-Cerrillo et al. (2013) are given the initials of the species names, followed by the REPAT identifier.

**Figure S9**

Gene tree of the REPAT sequences (extended dataset) consisting of: the *Spodoptera*-specific orthogroup sequences (OG0014254), sequences from OrthoDB cluster ‘16151at7088 - "MBF2"’, and REPAT gene sequences derived from Navarro-Cerrillo et al. (2013). Both βREPAT and REPAT sequences are included in this gene tree. The sequences from the *Spodoptera*-specific orthogroup are marked by the red asterisks, the red frame highlights the *Spodoptera*­specific clade, the red arrow indicates the specific *Spodoptera exigua* differentially expressed gene from expression cluster 4. All sequences derived from the *S.* *exigua* genome (this study) are underlined. Sequences derived from the OrthoDB cluster are given their full species names and cluster ID "MBF2". Genes from Navarro-Cerrillo et al. (2013) are given the initials of the species names, followed by the REPAT identifier.

**Figure S10**

Gene tree of the trypsin sequences consisting of: the *Spodoptera*-specific orthogroup sequences (OG0014208) and sequences from OrthoDB cluster ‘118933at50557’. The sequences from the *Spodoptera*-specific orthogroup are marked by the red asterisks, the red frame highlights the *Spodoptera*-specific clade, the red arrow indicates the specific *Spodoptera exigua* differentially expressed gene from expression cluster 4. All sequences derived from the *Spodoptera* *exigua* genome (this study) are underlined. Sequences derived from the OrthoDB cluster are given their full species names and gene ID.

**Table S1**

Overview of developmental stages used for the RNA-Seq approach and sequencing results.

Table S1.1: Developmental stages and samples used for the RNA-Seq approach.

Table S1.2: Quality of developmental stages and samples used for the RNA-Seq approach.

Table S1.3: Details on RNA-Seq run results.

**Table S2**

Overview of DNA-Seq approach and sequencing results.

Table S2.1: Results of the Oxford Nanopore long read sequencing run

Table S2.2: Results of the Illumina short read sequencing run

Table S2.3: Results of the trimming and cleaning steps.

**Table S3**

InterProScan annotation report of *Spodoptera exigua* proteins from the official gene set OGS v. 1.1.

**Table S4**

BLASTX annotation report of *Spodoptera exigua* proteins from the official gene set OGS v1.1 using local Arthropoda database.

**Table S5**

BLASTP annotation report of *Spodoptera exigua* proteins from the official gene set OGS v1.1 using local Arthropoda database.

**Table S6**

Raw read counts matrix of isoforms in the different samples.

**Table S7**

CPM, TMM cross-sample normalized and filtered count matrix.

**Table S8**

Genes with included in the 14 clusters.

**Table S9**

Cluster membership with GO annotations

**Table S10**

GO slims and frequency in respective clusters.

**Table S11**

Overview of Lepidoptera genomes and protein sequence files including source location and accession date used for the phylogenomic analyses.

**Table S12**

Gene family identifiers, using InterProScan identifiers and UniRef cluster terms, used to identify putative gene members from families P450, CCE, UGT, GST and ABC.

**Table S13**

Table S13.1: List of 119 *Spodoptera*-specific orthogroups (OG) with gene IDs.

Table S13.2: Within the 119 OGs, seven *Spodoptera exigua* genes were differentially expressed and clustered in expression cluster 4.

**Table S14**

Comparison of differential gene expression between *Spodoptera exigua* developmental stages.

**Table S15**

List of DE genes identified per pairwise comparison of the developmental stages.

**Table S16**

Proteins annotated using InterProScan for the five main detoxification gene families P450 monooxygenases (P450s), carboxyl- and choline esterases (CCEs), UDP-glycosyltransferases (UGTs), glutathione S-transferases (GSTs), ATP-binding cassettes (ABCs).

**Table S17**

Read counts of all samples for the seven selected genes based on the ortholog search and specificity for cluster 4.

**Table S18**

BLASTP annotated REPAT genes in the *Spodoptera exigua* protein set using reference REPAT sequences from Navarro-Cerrillo et al. (2013).