

## **Legends for Supplementary Figures**

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**Supplementary Figure 2. A)** Correlation of counts of number of peptides per protein with the number of proteins in each category. **B)** Distribution of peptide coverage per protein from nine different tissues.

**Supplementary Figure 3. Coverage of sequence by mass spectrometry data.** A) AGAP009323-PA was identified with 100% sequence coverage including an N-terminal acetylated peptide. B) Extensive sequence coverage of AGAP003153 (VATA\_ANOGA) gene is shown that is based on 69 unique peptides that validated each of its five exons and four junctional peptides that confirmed all exon-exon junctions.

**Supplementary Figure 4. Change in the structure of the AGAP010657 gene based on GSSPs.**

A) Seven peptides mapped to intergenic and intragenic region of the AGAP010657 gene. The SNAP prediction model supported a C-terminal exon extension of AGAP010657 and the presence of novel exon in the intronic region of gene AGAP010657. B) The MS/MS spectra of two genome search specific peptides, DYFGFLEVVAR and DVLIFHSDEFK, are shown.

**Supplementary Figure 5. Joining of two genes.**

A) A single gene structure was identified in place of two separate predicted genes using 11 intergenic peptides mapped between the two Ensembl annotated genes, AGAP011872 and AGAP011873. B) Representative MS/MS spectra for peptides SDVLISGLGGLGVEVAK and HNIALIVADTR supporting the novel gene are shown.

**Supplementary Figure 6. Identification of novel genes using peptides identified in intergenic regions.**

A) Thirteen peptides were identified in the intergenic region on chromosome 2L where intron of VectorBase gene model AGAP007548-RB is annotated on the opposite strand. Presence of a novel gene is also predicted by SNAP prediction program. B) Representative MS/MS spectra for peptides THFYNATQSVGENVTQWY and FGSNLEAILLDK supporting novel gene are shown.

**Supplementary Figure 7. Change in the gene structure using peptide in the intron of the annotated gene.**

A) Fifteen peptides were identified in 3 different introns of the gene AGAP010021 which suggest change in the gene structure. These intronic peptides and other intergenic peptides support longer gene model supported the SNAP and Fgenesh models. B)

Representative MS/MS spectra for peptides LCRPVCANDQSCLNNER and ASCSCFSGMVPSPTAK supporting change in gene structure are shown.

**Supplementary Figure 8.** Confirmation of splice sites from peptides spanning exon-exon junctions. Peptides identified in protein database search but not detected in tblastn search were further investigated. In the case of the AGAP011026 gene, four such exon spanning peptides were mapped to exon1-exon2, exon4-exon5, exon5-exon6 and exon6-exon7 junctions. Exons are marked in alternate black and blue letters to distinguish the junctions.

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**Supplementary Data.** 1) Details of the contribution of proteomic data to VectorBase. 2) Selected MS/MS spectra of genome search specific peptides. 3) Details of sequences submitted to Genbank along with a list of the sequences, primers and *Anopheles gambiae* mosquito organs used for the analysis.

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**Supplementary Table 6.** The list of 616 N-terminally acetylated unique peptides with their corresponding protein entries.

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