

Supplemental Figure 1.

B-Chain

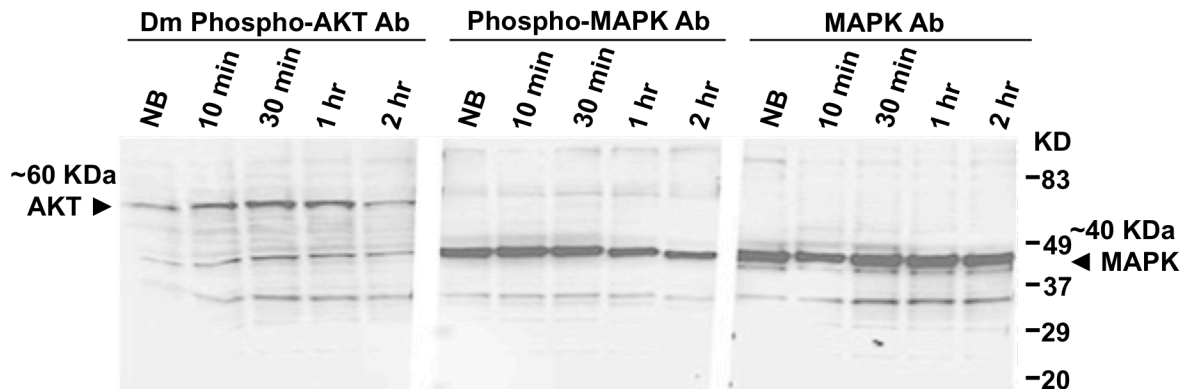
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ILP1      TENRGS GAEQRNSTETQFHRVTRDRRH YCGKAL TDTLALVCNSY P SPW
ILP3      ADQRF CGKQLVLTLSMLCDEF PDLHYGA
ILP4      SQKQKF CGPKLARALAE L C DAYPTLS P PPM
ILP7      QRF CGKVL TDTLTAYCEIFPT PRPSQRFCGKVL TDTLTAYCEIFPT PRPS
ILP8      ERVCGPKLVKTMYNVCPNGFYGPQT
Bv-Ins    FVNQHLCGSHLVEALYLVCGERGFFYIPKA
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A-Chain

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ILP1      QIVDECCRKS . CT LKTLKQ . YCAD
ILP3      SPRGIVDECCLRP . CSINQLLK . YCKTIA
ILP4      PGKGIVEECCRKG . CTYEY LMLNYCA
ILP7      GVVDDCCYKP . CT LQYLLKNYCG
ILP8      NIPTGLAHECCQKS . CTYEEMES . YCIT
Bv-Ins    GIVEQCCA . SVCSLYQLEN . YCN
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Sequence alignment of the A and B chains for five predicted ILPs (accession nos. DQ845750, DQ845752, DQ845753, DQ845757, DQ845758) expressed in the the *Ae. aegypti* brain with bovine insuline (Bv-Ins: PO1317). Conservation of cysteine positions for inter- and intra-chain disulfide bond formation are near identical for all *Aedes* ILPs and insulin, with the exception of an extra residue (Asn) between the third and fourth Cys residues in the A chain of ILP4. The motif (GFFY) near the carboxyl (C-) terminus of the insulin B chain is considered important for binding of mammalian insulins to the IR (see Brown et al., 2008), but this motif is absent from all insect ILPs. ILP3 and ILP4 exhibit several differences in primary structure of their A and B chains. The isoelectric point of ILP3 is 6.75 and 8.24 for ILP4 versus 5.39 of insulin. Solubility reflects these differences with ILP3 and ILP4 being soluble in water and bovine insulin being soluble in weak acid. The distribution of positively charged residues in the A chains is also different: ILP3 with four; ILP4, three; and bovine insulin with none.

Supplemental Figure 2.



The insulin signaling pathway is rapidly activated in the ovaries of blood fed female *Ae. aegypti* in response to ILPs released from the brain, as indicated by phosphorylation of the Akt homolog (~60 kDa). Phosphorylation of the MAPK homolog (~40 kDa) in ovaries was not affected by blood ingestion. Ovaries were dissected from non-blood fed females (NB) and 10 min to 2 h PBM, pooled, and extracted for immunoblotting (3 ovary pair equivalents/lane) as described for Fig. 5A with antibodies to *Drosophila* phospho-Akt (ser505), human P44/42 MAPK, and phospho-P44/42 MAPK (Thr202/Tyr204).