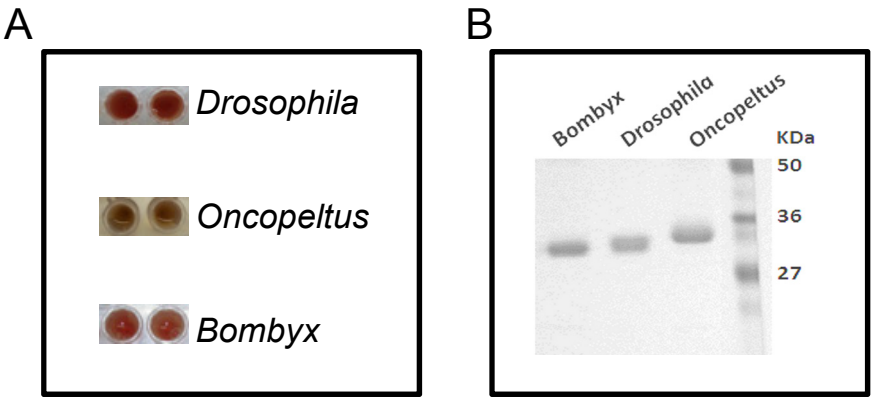
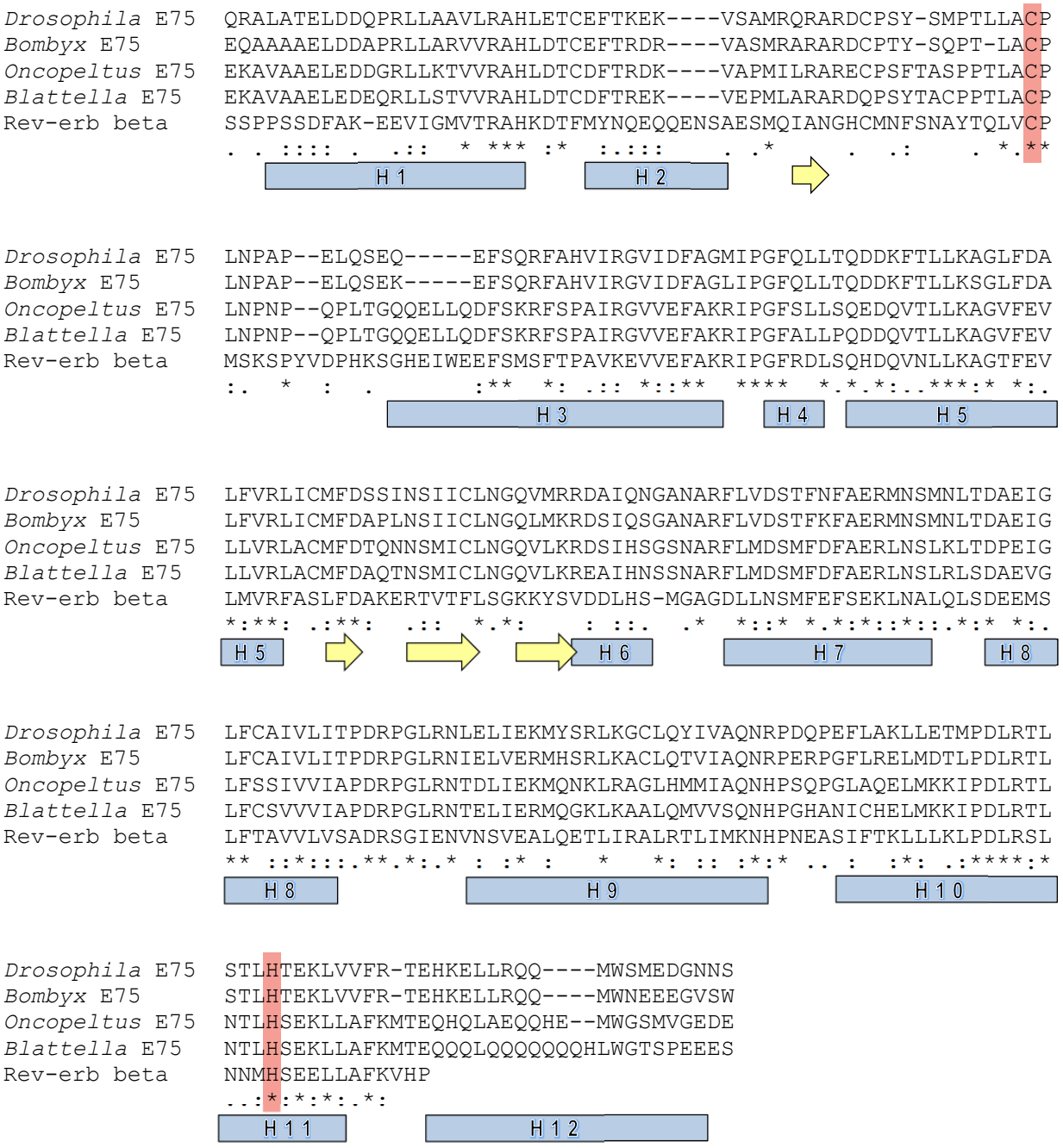
Supporting Information for Aicart-Ramos et al.,



Supplemental Figure 1: Panel A shows the colored fractions obtained of purified E75 Ligand Binding Domains of *Drosophila melanogaster*, *Oncopeltus fasciatus* and *Bombyx mori*. A Coomassie blue-stained SDS-PAGE of the purified E75 Ligand Binding Domains is shown in panel B.



Supplemental Figure 2: Sequence alignment of the E75 Ligand Binding Domains of *Drosophila melanogaster*, *Bombyx mori*, *Oncopeltus fasciatus* and *Blattella germanica* together with human Rev-erbβ LBD. Predicted α-helices and β-strands are shown at the bottom (blue rectangles and yellow arrows, respectively). The position of the conserved Cys and His residues that serve as heme ligands is marked in red. This prediction is based on the crystal structure obtained for the trypsin fragment of the heme-bound human Rev-erbβ LBD in which only the fragment comprising helices 3 through 11 are present (*1*). The crystal structure of the heme-free structure of human Rev-erbβ LBD (with only helices 3 through 11 as well) adopts a similar 3-dimensional fold (*2*). Please note that the Ligand Binding Domain of Rev-erbβ (as well as Rev-erbα) lacks the carboxy-terminal tail (helix 12) which is required for coactivator recognition (*3*), although this helix is present in insect E75 LBDs. The position of the predicted helix 1, helix 2 and helix 12 is inferred upon comparison with the atomic structure of the LBD of other crystalized nuclear receptors (*4*). The Clustal software (*5*) was used for the sequence comparison.

LBD of *Drosophila melanogaster* E75 nuclear receptor

```
        10         20         30         40         50         60         70         80         90        100
MHHHHHHNEN LYFQGHMGQQ RALATELDDQ PRLLAAVLRA HLETCEFTKE KVSAMRQRAR DCPSYSMPTL LACPLNPAPE LQSEQEFSQR FAHVIRGVID

       110        120        130        140        150        160        170        180        190        200
FAGMIPGFQL LTQDDKFTLL KAGLFDALFV RLICMFDSSI NSIICLNGQV MRRDAIQNGA NARFLVDSTF NFAERMNSMN LTDAEIGLFC AIVLITPDRP

       210        220        230        240        250        260        270        280
GLRNLELIEK MYSRLKGCLQ YIVAQNRPDQ PEFLAKLLET MPDLRTLSTL HTEKLVVFRT EHKELLRQQM WSMEDGNNSD G

Theoretical pI/Mw: 6.12 / 32149.00
```

LBD of *Oncopeltus fasciatus* E75 nuclear receptor

```
        10         20         30         40         50         60         70         80         90        100
MHHHHHHMCQ EKAVAAELED DGRLLKTVVR AHLDTCDFTR DKVAPMILRA RECPSFTASP PTLACPLNPN PQPLTGQQEL LQDFSKRFSP AIRGVVEFAK

       110        120        130        140        150        160        170        180        190        200
RIPGFSLLSQ EDQVTLLKAG VFEVLLVRLA CMFDTQNNSM ICLNGQVLKR DSIHSGSNAR FLMDSMFDFA ERLNSLKLTD PEIGLFSSIV VIAPDRPGLR

       210        220        230        240        250        260        270
NTDLIEKMQN KLRAGLHMMI AQNHPSQPGL AQELMKKIPD LRTLNTLHSE KLLAFKMTEQ HQLAEQQHEI

Theoretical pI/Mw: 6.89 / 30457.27
```

LBD of *Bombyx mori* E75 nuclear receptor

```
        10         20         30         40         50         60         70         80         90        100
MHHHHHHMQA AAAELDDAPR LLARVVRAHL DTCEFTRDRV ASMRARARDC PTYSQPTLAC PLNPAPELQS EKEFSQRFAH VIRGVIDFAG LIPGFQLLTQ

       110        120        130        140        150        160        170        180        190        200
DDKFTLLKSG LFDALFVRLI CMFDAPLNSI ICLNGQLMKR DSIQSGANAR FLVDSTFKFA ERMNSMNLTD AEIGLFCAIV LITPDRPGLR NIELVERMHS

       210        220        230        240        250        260
RLKACLQTVI AQNRPERPGF LRELMDTLPD LRTLSTLHTE KLVVFRTEHK ELLRQQMWNE EEGVF

Theoretical pI/Mw: 6.83 / 30276.04
```

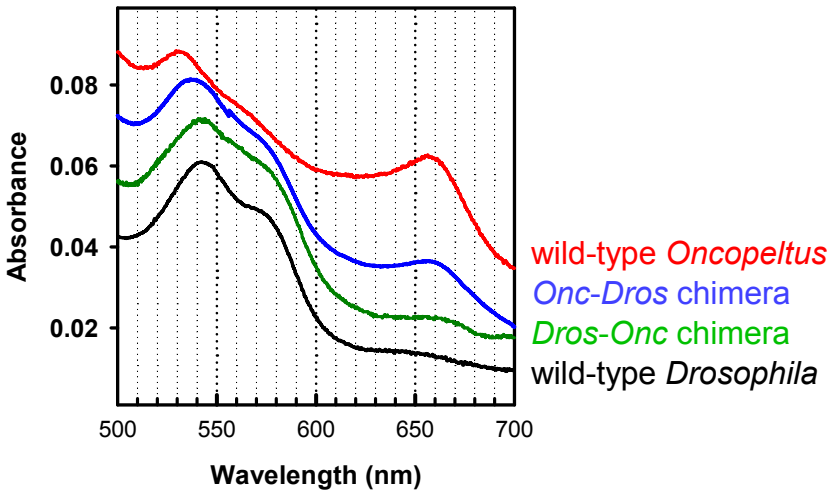
LBD of *Dros/Onc* E75 nuclear receptor chimera

```
        10         20         30         40         50         60         70         80         90        100
MHHHHHHMGQ QRALATELDD QPRLLAAVLR AHLETCEFTK EKVSAMRQRA RDCPSYSMPT LLACPLNPAP ELQSEQEFSQ RFAHVIRGVI DFAGMIPGFQ

        110        120        130        140        150        160        170        180        190        200
LLTQDDKFTL LKAGVFEVLL VRLACMFDTQ NNSMICLNGQ VLKRDSIHSG SNARFLMDSM FDFAERLNSL KLTDPEIGLF SSIVVIAPDR PGLRNTDLIE

        210        220        230        240        250        260
KMQNKLRAGL HMMIAQNHPS QPGLAQELMK KIPDLRTLNT LHSEKLLAFK MTEQHQLAEQ QHEI
```

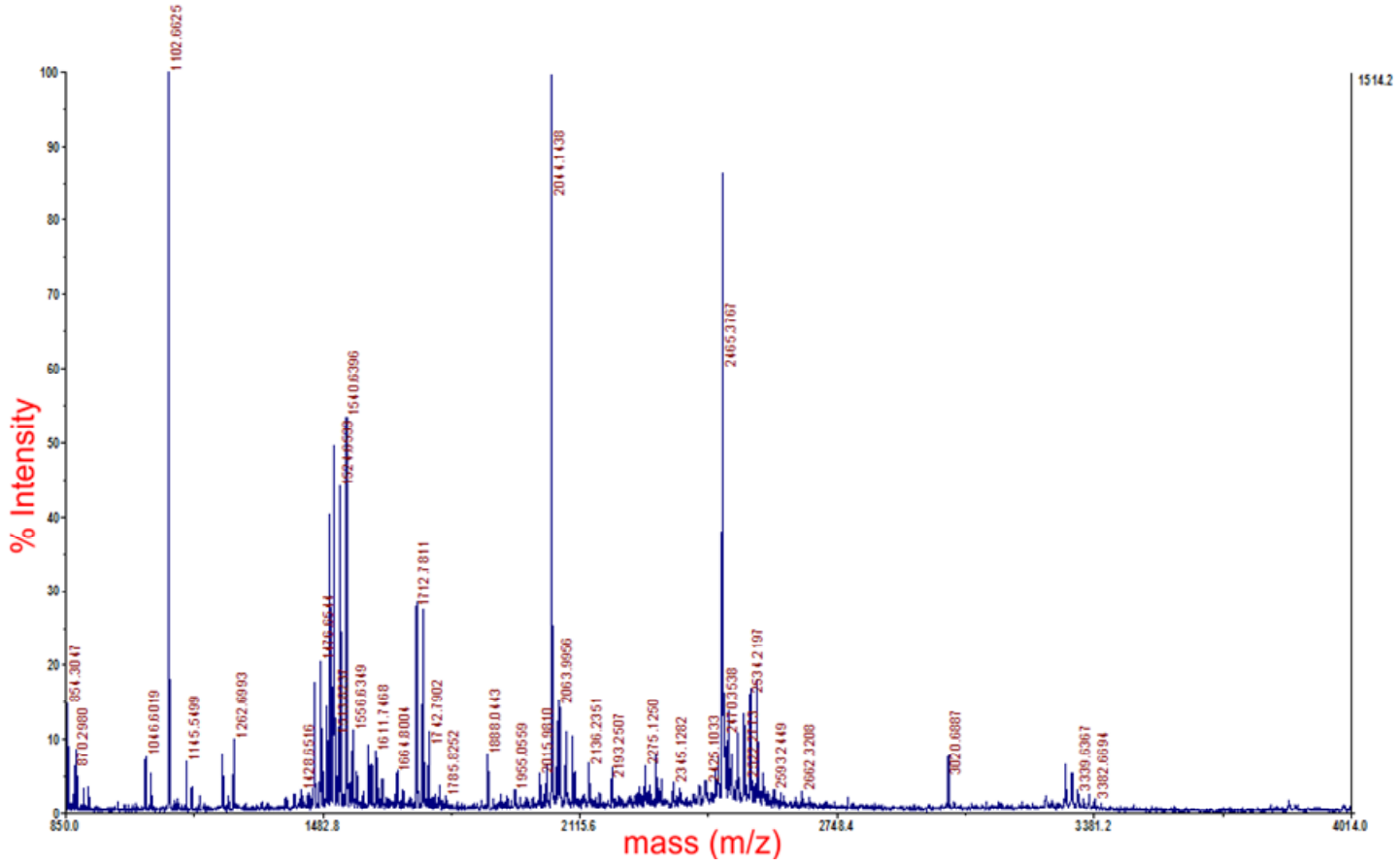
LBD of Onc*/Dros* E75 nuclear receptor chimera

```
        10         20         30         40         50         60         70         80         90        100
MHHHHHHMCQ EKAVAAELED DGRLLKTVVR AHLDTCDFTR DKVAPMILRA RECPSFTASP PTLACPLNPN PQPLTGQQEL LQDFSKRFSP AIRGVVEFAK

       110        120        130        140        150        160        170        180        190        200
RIPGFSLLSQ EDQVTLLKAG LFDALFVRLI CMFDSSINSI ICLNGQVMRR DAIQNGANAR FLVDSTFNFA ERMNSMNLTD AEIGLFCAIV LITPDRPGLR

       210        220        230        240        250        260        270
NLELIEKMYS RLKGCLQYIV AQNRPDQPEF LAKLLETMPD LRTLSTLHTE KLVVFRTEHK ELLRQQMWSM EDGNNSDG
```

Supplemental Figure 3: Amino acid sequence of the wild-type E75 Ligand Binding Domains of *Drosophila melanogaster*, *Oncopeltus fasciatus* and *Bombyx mori*. Expressed proteins have an N-terminal hexa-His tag. Note that the expressed *D. melanogaster* E75 LBD has a cleavable sequence for TEV protease (ENLYFQG) immediately after the hexa-His tag for crystallography purposes. The residue Glu158 that was mutated within the loop QQELL in the *Oncopeltus fasciatus* sequence is underlined. The sequences of the *Dros*/*Onc* and the *Onc*/*Dros* chimeras are also shown. We have underlined the sequence TLLKAG, shared by both *Drosophila* and *Oncopeltus* E75 in which a silent AflII restriction site was used to create these chimeric constructs.



Supplemental Figure 4: Absorption spectra of the E75 LBDs Fe(III) forms of *Drosophila melanogaster* (black line), *Oncopeltus fasciatus* (red line) as well as the *Drosophila-Oncopeltus* chimera (green line) and *Oncopeltus-Drosophila* chimera (blue line). The wild type *Drosophila melanogaster* E75 LBD displays prominent α/β bands at 574 and 543 nm respectively whereas wild-type *Oncopeltus fasciatus* E75 LBD displays a clear β band at 532 nm together with a CT1 band at 655 nm (high spin component). The *Drosophila-Oncopeltus* chimera displays a clear β band at 543 nm together with a small α band at 574 nm, followed by a faint but discernible CT1 band at 655 nm. The *Oncopeltus-Drosophila* chimera displays a clear β band at 537 nm together with a small α band at 574 nm, followed by a clear CT1 band at 655 nm. Spectra were vertically displaced for clarity.



Observed Mr(expt)	Peptide
1042.57	R.TLNTLHSEK.L
1043.49	R.DSIHSGSNAR.F
1058.58	R.DKVAPMILR.A + Oxidation (M)
1102.66	K.AGVFEVLLVR.L
1145.55	K.AVAAELEDDGR.L
1235.52	R.AHLDTCDFTR.D
1476.65	MHHHHHHMCQE.K
1499.78	K.AVAAELEDDGRLLK.T
1508.86	R.FLMDSMFDFAER.L
1524.62	R.FLMDSMFDFAER.L + Oxidation (M)
1540.61	R.FLMDSMFDFAER.L + 2 Oxidation (M)
1888.00	R.IPGFSLLSQEDQVTLLK.A
2044.14	K.RIPGFSLLSQEDQVTLLK.A
2063.99	R.FLMDSMFDFAERLNSLK.L
2079.94	R.FLMDSMFDFAERLNSLK.L + Oxidation (M)
2095.95	R.FLMDSMFDFAERLNSLK.L + 2 Oxidation (M)
2465.38	K.LTDPEIGLFSSIVVIAPDRPGLR.N
2502.19	R.AGLHMMIAQNHPSQPGLAQELMK.K
2518.20	R.AGLHMMIAQNHPSQPGLAQELMK.K + Oxidation (M)
2534.22	R.AGLHMMIAQNHPSQPGLAQELMK.K + 2 Oxidation (M)
3020.68	R.LNSLKLTDPEIGLFSSIVVIAPDRPGLR.N

1 40
MHHHHHHMCQEKAVAAELEDDGRLLKTVVRAHLDTCDFTR

DKVAPMILRARECPSFTASPPTLACPLNPNPQPLTGQQEL

LQDFSKRFSPAIRGVVEFAKRIPGFSLLSQEDQVTLLKAG

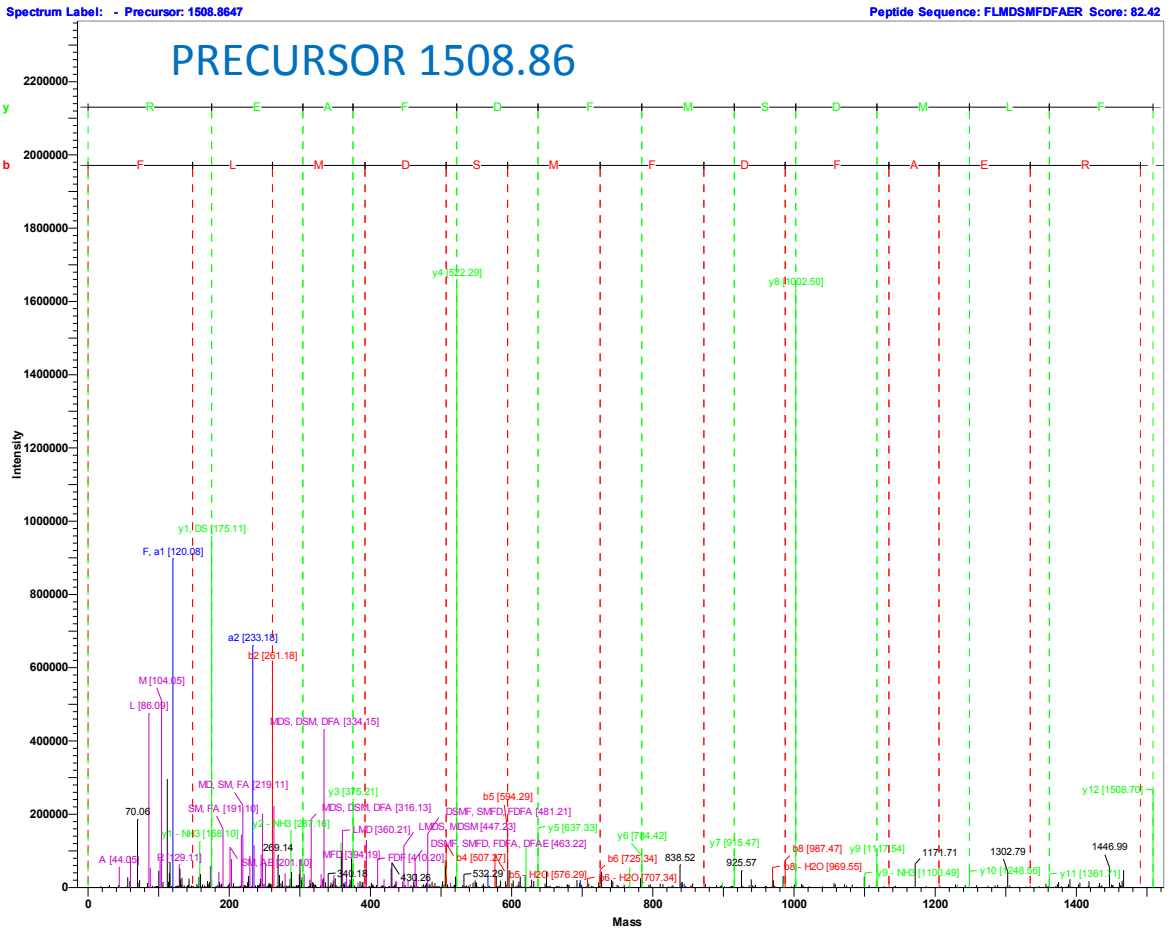
VFEVLLVRLACMFDTQNNSMICLNGQVLKRDSIHSGSNAR

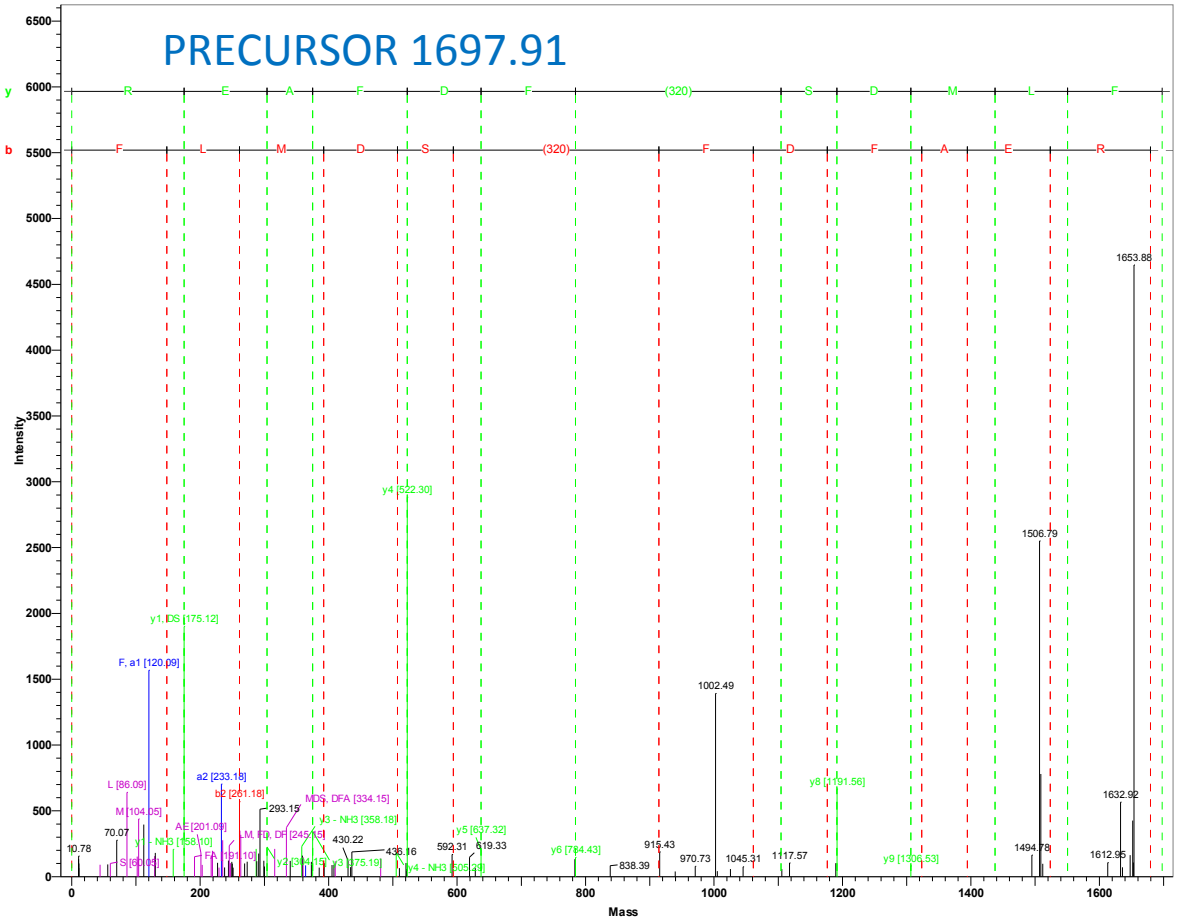
FLMDSMFDFAERLNSLKLTDPEIGLFSSIVVIAPDRPGLR

NTDLIEKMQNKLRAGLHMMIAQNHPSQPGLAQELMKKIPD

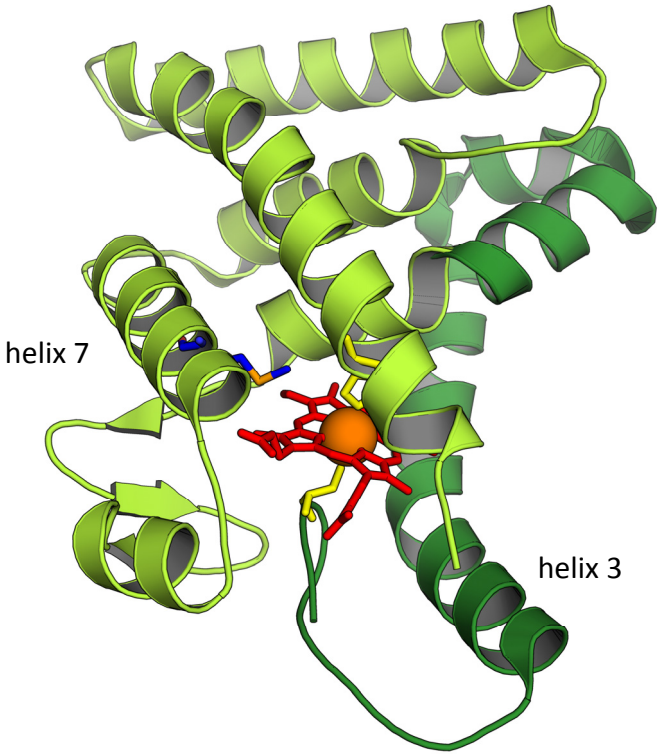
LRTLNTLHSEKLLAFKMTEQHQLAEQQHEI

Supplemental Figure 5: MALDI-TOF mass spectrum of trypsin-digested *Oncopeltus fasciatus* E75 LBD. Peptides that were identified are also shown. The coverage is depicted in the right panel with identified peptides shaded in yellow. Please note that peptide ECPSFTASPPTLACPLNPNPQPLTGQQELLQDFSK (Mass 3768.82 Da) was too large to be unambiguously identified.

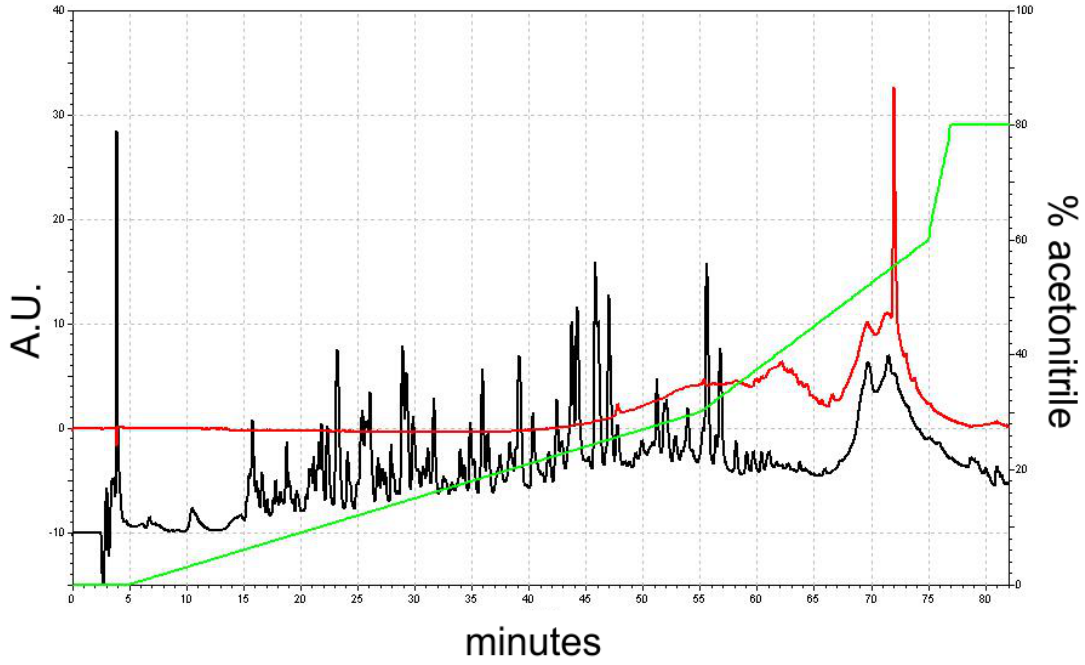

Spectrum Label: - Precursor: 1508.8647
Peptide Sequence: FLMDSMFDFAER Score: 82.42
PRECURSOR 1508.86
y R E A F D F M S D M L F
b F L M D S M F D F A E R
y4 [522.29]
y8 [1002.50]
y1-DS [175.11]
F, a1 [120.08]
a2 [233.18]
b2 [261.18]
M [104.05]
L [86.09]
MDS, DSM, DFA [334.15]
MD, SM, FA [219.11]
y3 [375.21]
MDS, DSM, DFA [316.13]
SM, FA [191.10]
y2 - NH3 [287.16]
LMD [360.21]
LMDS, MDSM [447.23]
b5 [594.29]
DSMF, SMFD, FDFA [481.21]
y5 [637.33]
y1 - NH3 [158.10]
DSMF, SMFD, FDFA, DFAE [463.22]
y6 [784.42]
MFD [304.19]
FDF [410.20]
b4 [507.27]
b6 [725.34]
838.52
y7 [915.47]
925.57
b8 [987.47]
b8 - H2O [969.55]
y9 [1117.54]
y9 - NH3 [1100.49]
1171.71
1302.79
y10 [1248.56]
1446.99
y11 [1361.71]
y12 [1508.70]
70.06
269.14
340.19
430.26
532.29
b5 - H2O [576.29]
b6 - H2O [707.34]
A [44.05]
R [129.11]
Intensity
Mass
PRECURSOR 1474.88
y R E A F D F (97.05) S D M L F
b F L M D S (97.05) F D F A E R
y4 [522.30]
y8 [988.48]
F, a1 [120.08]
y1-DS [175.12]
L [86.10]
a2 [233.20]
M [104.05]
b2 [261.19]
y2 [304.16]
y3 [375.19]
LMDS [447.24]
70.06
AE [201.08]
MD [247.08]
y3 - NH3 [358.21]
LMDS [419.29]
619.39
693.28
y7 [861.47]
y9 - NH3 [1068.48]
1343.90
1432.85
1555.51
y12 [1474.74]
1532.15
A [44.05]
R [129.12]
y1 - NH3 [158.09]
y2 - NH3 [287.16]
562.22
637.76
665.33
780.43
806.43
936.70
1040.52
a9 [1072.58]
1498.69
1412.83
1459.12
10.06
FA [191.10]
401.21
389.91
492.53
596.31
577.38
657.24
724.64
793.45
1328.86
Intensity
Mass



Supplemental Figure 6: MS/MS spectra of *Oncopeltus fasciatus* peptide FLMDSMFDFAER (Mass, 1508.8647 Da) together with peptides in which Met245 loses 33.82 Da (precursor 1474.88 Da) or gains 189.05 Da (precursor 1697.92 Da). The y-ion fragment series and the b-ion fragment series are shown on top. Fragmentation of the 1474.88 Da precursor reveals an unknown amino acid at position 6 with a mass of 97.05 Da (Met minus 34 Da) whereas fragmentation of the 1697.91 Da precursor reveals an unknown amino acid at position 6 with a mass of 320 Da (Met plus 189 Da). In all three cases the expected FLMDSMFDFAER primary sequence could be identified unambiguously.



Supplemental Figure 7: Pymol (www.pymol.org) ribbon representation of Rev-erbβ crystal structure with heme bound ((*1*), PDB 3CQV). The coordination ligands for the heme iron are Cys384 (upstream from helix 3) and His568 (in helix 11), both colored in yellow. Helices 1 and 2 (N-terminus) are missing from this crystal structure (please see Fig. S2). The N-terminal part of the polypeptide is colored in dark green whereas the C-terminal part is colored in light green. The boundary between both parts of the molecule is residues KLAG (present in both the *Drosophila melanogaster* and *Oncopeltus fasciatus* amino acids sequence) located in helix 5. These amino acids establish the boundary of our *Drosophila-Oncopeltus* and *Oncopeltus-Drosophila* chimeras (please, see main text for details). The position of Met486 in helix 7 in the proximity of one of the heme vinyl groups is shown. Considering their primary sequence homology (Fig. S2) *Oncopeltus fasciatus* Met245 (genebank ABP02025.1) would be located in the equivalent position of Rev-erbβ Met486.



Supplemental Figure 8: The purified *Oncopeltus fasciatus* E75 LBD was digested with proteinase K for 20 minutes and the digestion mixture was loaded in a 250 x 4.6-mm Beckman Coulter Ultrasphere C18 reversed phase column. Absorbance at 214 nm is shown in black and absorbance at 400 nm in red. The acetonitrile gradient is depicted in green. Please note the appearance of several hemopeptides between 68 and 72 minutes. Free heme elutes at 72 minutes.

References

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