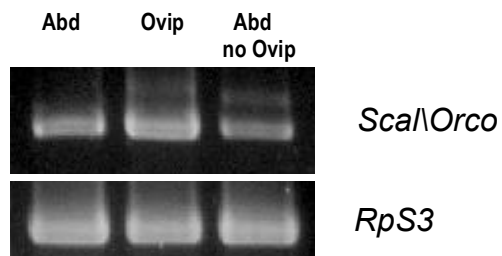


Supplemental Figure 1. Detection of ScOrco (A) and HirrOrco (A) by immunoblotting. Insoluble protein fractions were isolated separately from stable fly and horn fly heads (H) and thoraces (T). The anti-Orco antibody labeled two distinct fragments in each lane that were 75 kDa and ~130kDa in size possibly representing Orco protein monomer and Or-Orco dimers, respectively. The estimated molecular mass of ScOrco and Hirr Orco is ~54kDa, suggesting a gel migration shift that is typical of membrane protein fractions (Rath et al., 2009). Protein equivalent to a single head or thorax was resolved by PAGE on a NuPAGE[®] 4-12% Bis-Tris gel (Invitrogen) in MOPS/SDS running buffer (Invitrogen) under reducing conditions. The Precision Plus Protein[™] Standard (MW; Bio-Rad Laboratories, Hercules, CA) was included, and a recombinant tick protein was resolved alongside the fractions as an irrelevant negative control (Neg). Proteins were transferred to a PVDF membrane using a TransBlot[™] SD Semi-Dry Transfer Cell (Bio-Rad Laboratories, Hercules, CA), washed in PBS/0.3% Tween 20 (PBS-Tw20), blocked in 10% goat milk, and incubated in biting fly anti-Orco (1:500) at 4 °C overnight. Blots were rinsed in PBS-Tw20, incubated in HRP-conjugated goat anti-rabbit secondary antibody (1:20,000; Bio-Rad Laboratories), and developed for 30 mins using an amplified Opti-4CN[™] substrate colorimetric detection assay (Bio-Rad Laboratories). Three faint, lower molecular mass bands developed in the stable fly thorax lane after 20 mins of incubation in developer.

Rath, A, Glibowicka, M, Nadeau, VG, Chen, G, and Deber, CM (2009) Detergent binding explains anomalous SDS-PAGE migration of membrane proteins. *Proc Nat Acad Sci USA* **106**: 1760-1765.



Supplemental Figure 2. Expression of *Sca\Orco* in female abdomen and ovipositor. Presence of the transcript was evaluated using primer pair ScOr83b-Fwd4/R7 and template synthesized separately from abdomens (Abd), dissected ovipositors (Ovip) and abdomens with the ovipositors removed (Abd no Ovip). The transcript was detected in all templates evaluated. Expression of biting fly ribosomal protein S3 (*RpS3*) was used as a positive control for the presence of template.