Supplementary Methods

Subfractionation of Mitochondria

Purified mitochondria were incubated with an equal volume of 2X lysis solution (600 mM NaCl, 20 mM CaCl$_2$, 200 mM Tris-HCl, pH 8.5, 1% NP-40, 2 mM phenylmethylsulfonyl fluoride) at RT for 10 minutes$^1$, and then spun for 35 minutes at maximum speed in an Eppendorf 5417R centrifuge. The supernatant yields the soluble fraction and the pellet yields the membrane fraction.

Generation of S2 cells expressing ANT-V5

Full-length sesB (ANT) cDNA was generated by RT-PCR and subsequently cloned into the pAc5.1/V-5-His vector (Invitrogen) at KpnI and XhoI sites. The expression plasmid was transfected into Drosophila S2 cells (Invitogen) according to manufacturer's protocol. Cells were harvested 3 days following transfection, and the expression level of ANT-V5 was determined by Western blots using the V5 antibody (Invitrogen).

Generation of deletion constructs and co-immunoprecipitation

The coding sequences of full-length and truncated nebula were cloned into PET15b vector (Novagen). E. Coli BL21(DE3) strains containing the expression plasmids were grown at 37 °C in LB containing 100 g/ml ampicillin until $A_{600}$ of the culture reached 0.6. Expression of His-tagged proteins was induced by the addition of isopropyl β-D-thiogalactopyranoside (IPTG) to a final concentration of 0.5 mM. After growth at 30 °C for 4 hours, cells were harvested and stored at
-70 °C until purification. His-tagged proteins were purified using the ProBond Purification System (Invitrogen). To detect interaction, protein extract from S2 cells expressing ANT-V5 were incubated with 1 g of purified His-nebula at 4 °C for 2 hours, and then immunoprecipitated following addition of agarose beads-coupled V5 antibody (Sigma Aldrich). Interactions between ANT-V5 and the nebula constructs were confirmed by detecting the immunoprecipitated product on a Western blot with the nebula antibody (1:10,000).

Supplementary References