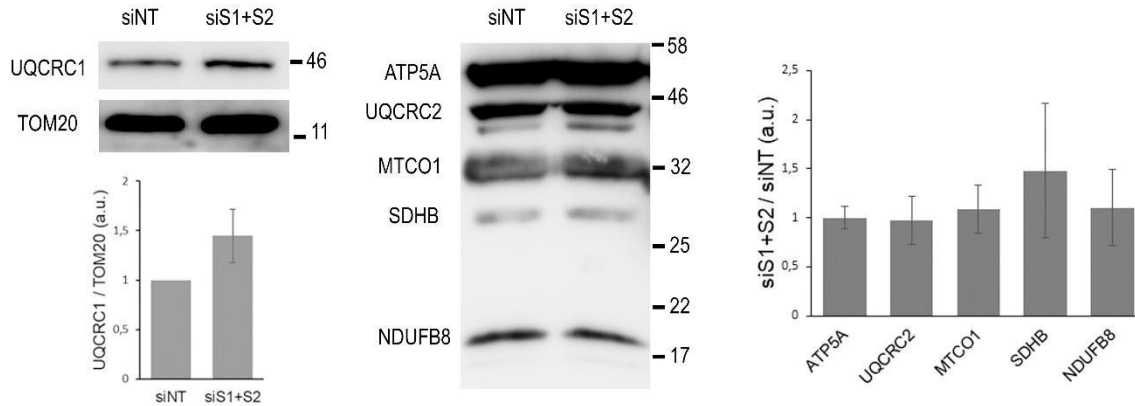


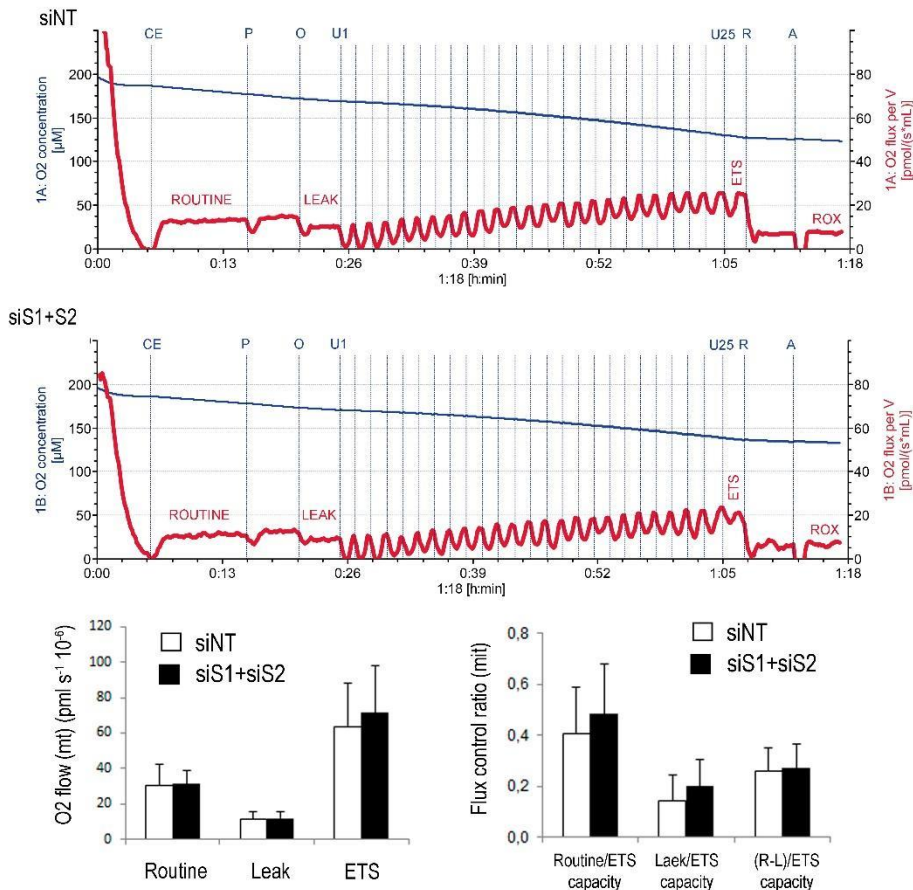
**Fig. S1. A. Smaug1-EYFP bodies exclude ubiquitin and small ribosomal subunits (linked to Figure 1C).** Top, Smaug1-V5-transfected U2OS cells were immunostained for V5 and ubiquitin. Bottom, FISH with a ribosomal 18S riboprobe was performed in Smaug1-EYFP-transfected cells as described in Materials and Methods. Representative images from two independent stainings are shown in each case. Both ubiquitin and 18S rRNA are excluded from Smaug1-EYFP bodies.

**B. Expression levels and recovery of Smaug1 constructs (linked to Figure 1D).** Expression levels of the indicated constructs tagged with MBP-V5 and their recovery after pull-down were analyzed by western blot using an anti-V5 antibody. Tubulin was used as loading control for the input samples.

**A**

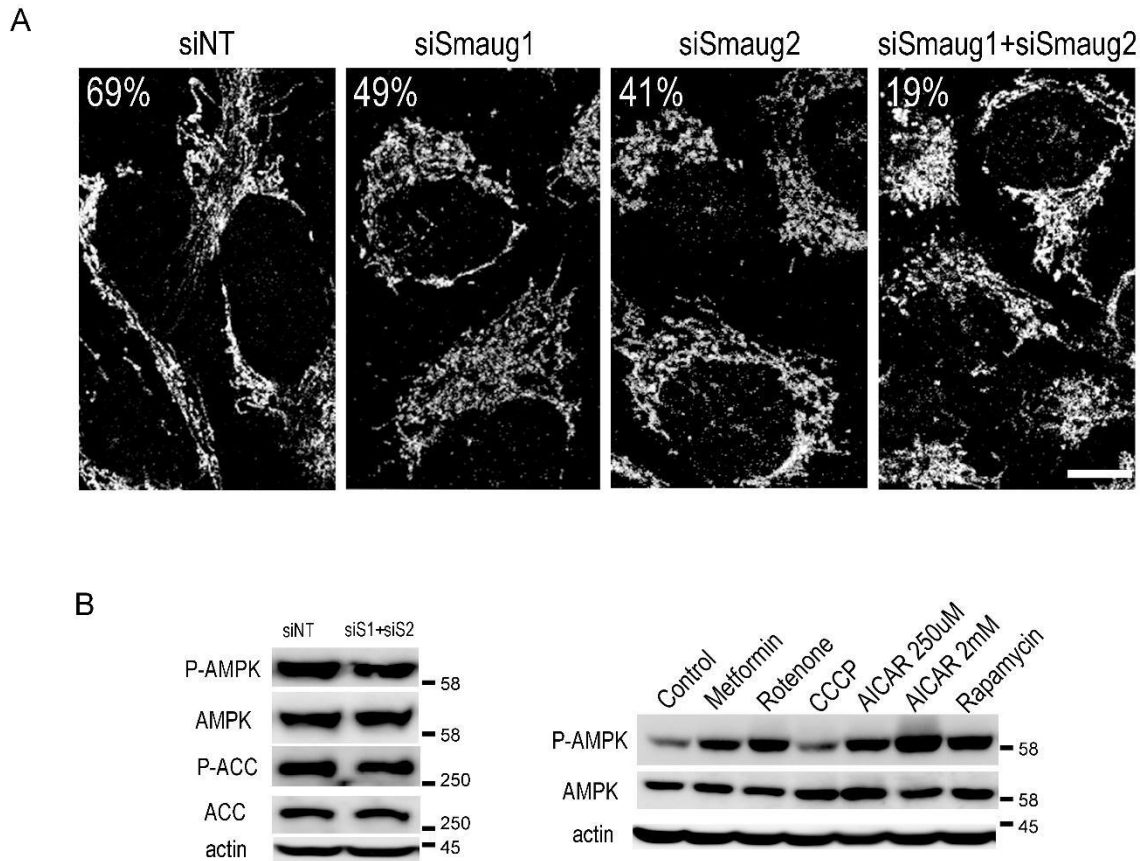


**B**



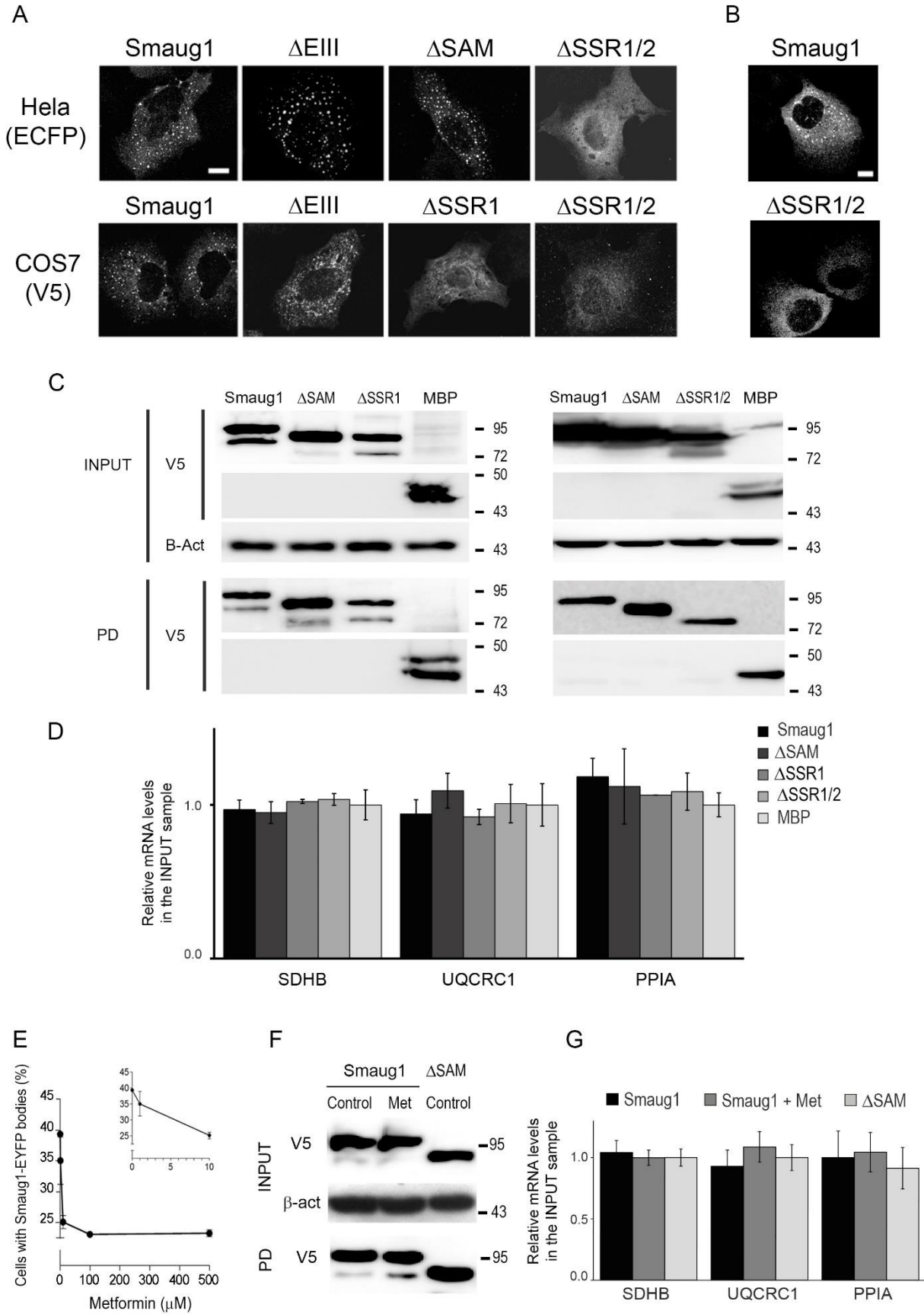
**Fig. S2. A Expression levels of mitochondrial enzymes upon Smaug1+2 knockdown.** The indicated proteins were analyzed by western blot of mitochondrial protein extracts obtained as described in Materials and Methods. Three independent experiments were performed and the media value is plotted. A representative image is shown. Error bars, standard deviation.

**B. Respiratory capacity of Smaug1+2 knockdown U2O2 cells (linked to Figure 2C)** U2OS were treated with non-targeting (NT) siRNA or with siRNAs against both Smaug1 and Smaug2 (siS1+S2) and respiration in intact cells ( $1 \times 10^6$  cells/mL) was examined in growth medium at 37 °C as described in Materials and Methods. Oxygen flow ( $\text{pmol O}_2 \times \text{s}^{-1} \times 10^{-6}$  cells) and total oxygen concentration (nmol/mL) in the Oxygraph chamber are indicated as red and blue traces, respectively. CE: cellular substrate, P: Pyruvate, O: Oligomycin, U: Uncoupler (CCCP) R: Rotenone, A: Antimycin A. After measuring routine oxygen consumption, the adenosine triphosphate (ATP)-synthase inhibitor oligomycin was added to evaluate proton LEAK. The uncoupler carbonyl cyanide m-chlorophenylhydrazone (CCCP) allowed the measurement of maximal oxygen consumption (Max) stimulating maximal respiration assuming all required substrates are present. Finally, rotenone (complex I inhibitor) and antimycin A (complex III inhibitor), which completely prevent oxygen consumption through the ETC were added. Oxygen flow per cell was corrected for ROX at the indicated mitochondrial respiration state. Calculated mitochondrial (mt) flux control ratios show basal cellular routine respiration (R), leak respiration (L) and fraction of respiration ( $\text{netR} = \text{R}-\text{L}$ ) used for ATP production normalized to ETS capacity.



**Fig. S3. A Mitochondrial network disruption upon Smaug1 and Smaug2 knockdown (linked to Figure 3A).** U2OS cells were treated with the indicated siRNAs and live-stained with MitoTracker™ Red CMXRos. At least 200 cells per treatment were analyzed and the percentage of cells with elongated mitochondria is indicated. A representative experiment out of three is shown. Scale bars, 10  $\mu$ m.

**B. Phosphorylation levels of AMPK and ACC remained unchanged upon Smaug1+2 knockdown.** U2OS cells were treated with the indicated siRNAs or exposed to metformin, AICAR or rapamycin. Whole cell protein extracts were obtained by lysis with 1XSDS loading buffer as described in Materials and Methods. Phosphorylation of AMPK $\alpha$  and of its target ACC, and their total protein levels were analyzed by western blot. Three independent experiments were performed and a representative western blot is shown.



**Fig. S4. A Smaug-MLO formation in COS7 and HeLa cells (linked to Figure 5D).** Representative images of cells transfected with the indicated constructs tagged with V5-SBP or ECFP are shown. At least two independent transfection experiments were performed in each case.

**B Smaug MLO formation in tethering assay (linked to Figure 6B).** U2Os cells were co-transfected with the firefly reporter carrying a tandem array of 6 MS2 binding sites and the indicated constructs tagged with MS2-HA. Three independent experiments were performed and representative cells are depicted .

**C,D Expression levels of Smaug1 constructs and target mRNAs (linked to Figure 6C).** (C) Expression levels of the indicated constructs tagged with MBP-V5 and their recovery after pull-down were analyzed by western blot using anti-V5 antibody.  $\beta$ -actin was used as loading control for the input samples. (D) Levels of the indicated transcripts in the input samples depicted in Figure 6C were determined by RT-qPCR and normalized to RpLp0 mRNA values.

**E Response to metformin in U2OS cells.** Cells transfected with Smaug1-EYFP were exposed during 1 h at the indicated metformin concentrations. More than 100 cells from duplicate coverslips were analyzed for each experimental point. Error bars, standard deviation.

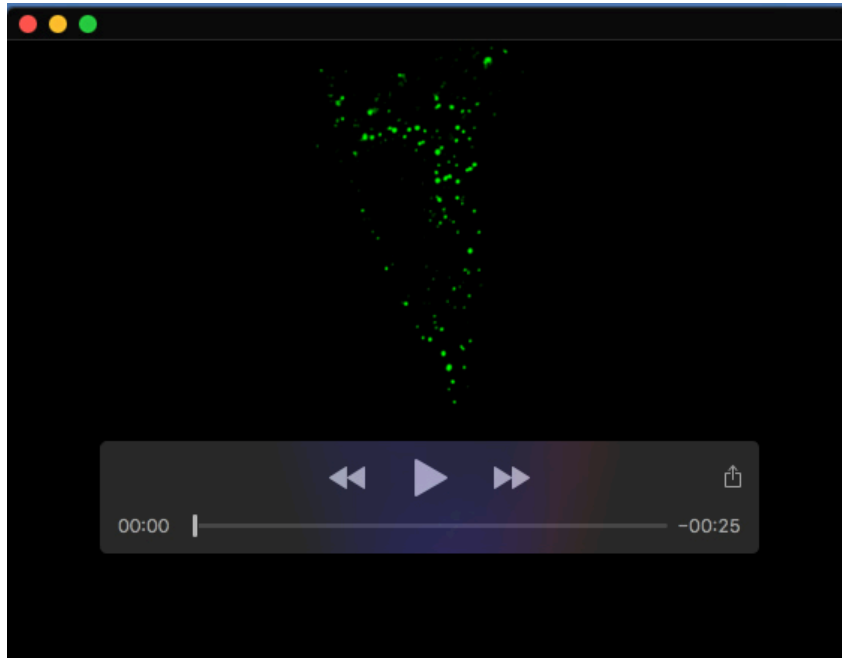
**F,G Expression levels of Smaug1 constructs and of target mRNAs upon metformin treatment (linked to Figure 8C).** (F) Western blot of input and pull-down (PD) samples depicted in Figure 8C. (G) Levels of the indicated transcripts in the input samples depicted in Figure 8C were determined by RT-qPCR and normalized to RpLp0 mRNA values.



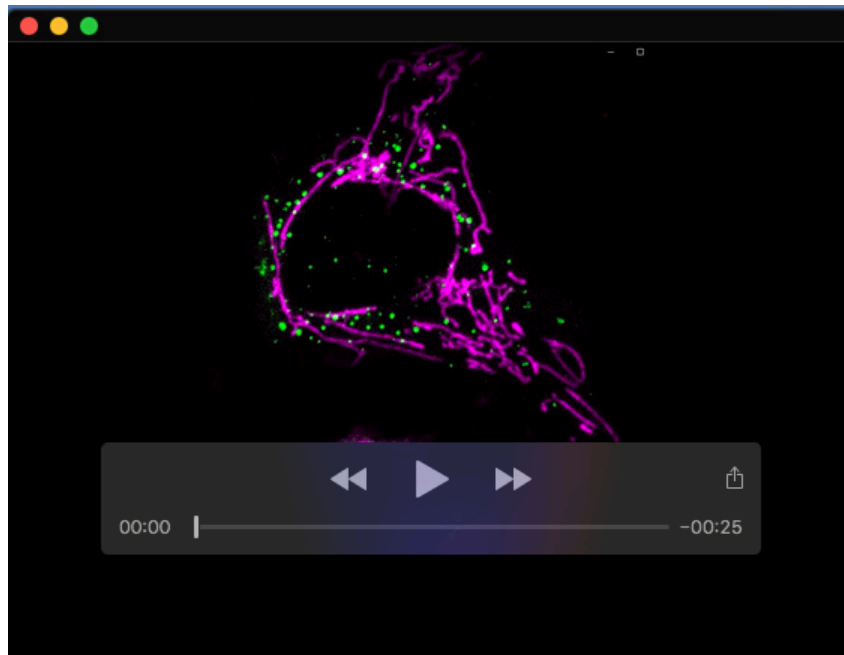
### Table S1. A putative mitochondrial regulon linked to Smaug

Chen et al. (2014) reported 340 transcripts bound to Smaug and 1918 mRNAs translationally repressed by Smaug in Drosophila embryos (additional data files 2 and 7 in (Chen et al., 2014)). Among these Smaug targets, several mRNAs are linked to mitochondrial function. In addition, Chartier et al. (2015) reported that mRNAs of TCE components are bound to Drosophila Smaug in adult thorax (Chartier et al., 2015).

Function	Name		REF
ETC	SDHB	bound	mammalian U2OS cells (this work)
ETC	UQCRC1	bound, repressed?	mammalian U2OS cells (this work)
ACYL-CoA DEHYDROGENASES	Butyryl-CoA dehydrogenase;DmelCG6638;ortholog	bound, repressed	Drosophila embryo (Chen et al., 2014)
alpha-amino acid metabolic process	Probable methylmalonate-semialdehyde dehydrogenase [acylating], mitochondrial;FlyBase:FBgn0023537;ortholog	bound, repressed	Drosophila embryo (Chen et al., 2014)
ETC	Succinate dehydrogenase [ubiquinone] flavoprotein subunit, mitochondrial;sdhA;ortholog	bound, repressed	Drosophila embryo (Chen et al., 2014)
ETC	Ferredoxin, mitochondrial;Fcd;ortholog	bound, repressed	Drosophila embryo (Chen et al., 2014)
ETC and OXPHOS	BcDNA, GH02220;OXAL1;ortholog	bound, repressed	Drosophila embryo (Chen et al., 2014)
fatty acid beta-oxidation	GH06693p;15207b;at;ortholog	bound, repressed	Drosophila embryo (Chen et al., 2014)
fatty acid catabolic process	Arc42;Arc42;ortholog	bound, repressed	Drosophila embryo (Chen et al., 2014)
Iron-sulfur cluster assembly-ETC related process	Cysteine desulfurase, mitochondrial;Nfs1;ortholog	bound, repressed	Drosophila embryo (Chen et al., 2014)
misc	RE8077p;ENIP3;ortholog	bound, repressed	Drosophila embryo (Chen et al., 2014)
misc	ATG11 domain-containing protein;Atg17;ortholog	bound, repressed	Drosophila embryo (Chen et al., 2014)
mitochondrial translation	Mitochondrial ribosomal protein L33;mrpL33;ortholog	bound, repressed	Drosophila embryo (Chen et al., 2014)
mitochondrial translation	Probable 26S ribosomal protein S25, mitochondrial;mrpS25;ortholog	bound, repressed	Drosophila embryo (Chen et al., 2014)
mitochondrial translation	CG6469 protein;P32;ortholog	bound, repressed	Drosophila embryo (Chen et al., 2014)
mitochondrial translation	Mitochondrial ribosomal protein L53;mrpL53;ortholog	bound, repressed	Drosophila embryo (Chen et al., 2014)
OxPhos	ATP synthase subunit beta, mitochondrial;ATP5b;ortholog	bound, repressed	Drosophila embryo (Chen et al., 2014)
OxPhos	ATP synthase subunit alpha, mitochondrial;blw;ortholog	bound, repressed	Drosophila embryo (Chen et al., 2014)
protein import	Mitochondrial import inner membrane translocase subunit TIM44;CG117798;ortholog	bound, repressed	Drosophila embryo (Chen et al., 2014)
protein import	CHCH domain-containing protein;DmelCG7950;ortholog	bound, repressed	Drosophila embryo (Chen et al., 2014)
pyruvate metabolic process	Pyruvate dehydrogenase E1 component subunit beta;Pdhb;ortholog	bound, repressed	Drosophila embryo (Chen et al., 2014)
Redox homeostasis	Thioredoxin domain-containing protein;DmelCG8983;ortholog	bound, repressed	Drosophila embryo (Chen et al., 2014)
TCA	Iso_dh domain-containing protein;DmelCG5026;ortholog	bound, repressed	Drosophila embryo (Chen et al., 2014)
tRNA metabolism	Tryptophanyl-tRNA synthetase e-TrpRS-m;ortholog	bound, repressed	Drosophila embryo (Chen et al., 2014)
channel	Voltage-dependent anion-selective channel;parin;ortholog	bound	Drosophila embryo (Chen et al., 2014)
ETC	GH01077p;UQCR-C3;ortholog	bound	Drosophila embryo (Chen et al., 2014)
ETC	AT02348p;UQCR-C2;ortholog	bound	Drosophila embryo (Chen et al., 2014)
ETC	GE011443p;1;UQCR-6;ortholog	bound	Drosophila embryo (Chen et al., 2014)
fatty acid beta-oxidation	Uncharacterized protein;DmelCG9146;ortholog	bound	Drosophila embryo (Chen et al., 2014)
fatty acid beta-oxidation	F10602p;GH07925p;ortholog	bound	Drosophila embryo (Chen et al., 2014)
metabolic enzyme	Aspartate aminotransferase;Gat2;ortholog	bound	Drosophila embryo (Chen et al., 2014)
misc	NFU1 iron-sulfur cluster scaffold homolog, mitochondrial;FlyBase:FBgn0052657;ortholog	bound	Drosophila embryo (Chen et al., 2014)
mitochondrial organization	Coiled-coil-helix-coiled-coil-helix domain containing 2, isoform A;Chchd2;ortholog	bound	Drosophila embryo (Chen et al., 2014)
mitochondrial translation	RH08993p;BcDNA;RH08992;ortholog	bound	Drosophila embryo (Chen et al., 2014)
mitochondrial translation	38S ribosomal protein L22, mitochondrial;mrpL22;ortholog	bound	Drosophila embryo (Chen et al., 2014)
mitochondrial translation	Mitochondrial ribosomal protein S2, mrpS2;ortholog	bound	Drosophila embryo (Chen et al., 2014)
OxPhos	Cytochrome c oxidase subunit 7A, mitochondrial;COX7A;ortholog	bound	Drosophila embryo (Chen et al., 2014)
OxPhos	RES7459p;CG6922;ortholog	bound	Drosophila embryo (Chen et al., 2014)
protein import	Heat shock protein 60A;Hsp60A;ortholog	bound	Drosophila embryo (Chen et al., 2014)
protein import	Mitochondrial import inner membrane translocase subunit TIM50-C;ttm50;ortholog	bound	Drosophila embryo (Chen et al., 2014)
protein import	DNL-type domain-containing protein;DmelCG9206;ortholog	bound	Drosophila embryo (Chen et al., 2014)
pyruvate metabolic process	Pyruvate carboxylase;PCB;ortholog	bound	Drosophila embryo (Chen et al., 2014)
TCA	Probable isocitrate dehydrogenase [NAD] subunit alpha, mitochondrial;idh3a;ortholog	bound	Drosophila embryo (Chen et al., 2014)
ETC complex I	NADH dehydrogenase (ubiquinone) 24 kDa subunit	bound	Drosophila adult thorax (Chartier et al., 2015)
ETC complex I	NADH dehydrogenase (ubiquinone) 8S subunit	bound	Drosophila adult thorax (Chartier et al., 2015)
ETC complex I	NADH dehydrogenase (ubiquinone) 7S kDa subunit	bound	Drosophila adult thorax (Chartier et al., 2015)
ETC complex II	Succinate dehydrogenase, subunit B (iron-sulfur)	bound	Drosophila adult thorax (Chartier et al., 2015)
ETC complex II	Succinate dehydrogenase, subunit A (flavoprotein)	bound	Drosophila adult thorax (Chartier et al., 2015)
ETC complex II	Succinate dehydrogenase, subunit D	bound	Drosophila adult thorax (Chartier et al., 2015)
ETC complex III	Rieske iron-sulfur protein	bound	Drosophila adult thorax (Chartier et al., 2015)
ETC complex III	Ubiquinol-cytochrome c reductase	bound	Drosophila adult thorax (Chartier et al., 2015)
ETC complex IV	Cytochrome c oxidase subunit 7C	bound	Drosophila adult thorax (Chartier et al., 2015)
ETC complex IV	Cytochrome c oxidase subunit 7A	bound	Drosophila adult thorax (Chartier et al., 2015)
ETC complex V	ATP synthase, beta subunit	bound	Drosophila adult thorax (Chartier et al., 2015)
ETC complex V	ATP synthase, subunit F	bound	Drosophila adult thorax (Chartier et al., 2015)

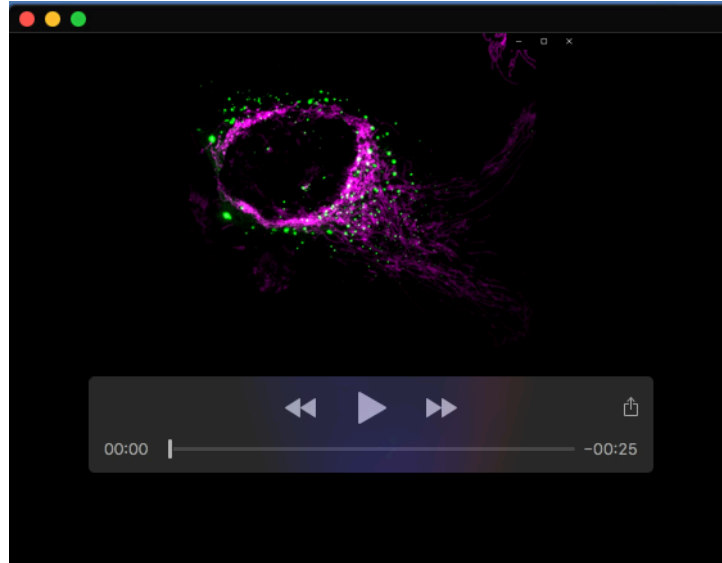


**Movie 1.** Live cell imaging of the Smaug1-EYFP expressing cell depicted in Figure 1A recorded during 30 min (0.5 frames per minute) as described in Materials and Methods.



**Movie 2.** Live cell imaging of the control cell depicted in Figure 8A. Smaug1-EYFP (green) and Mito-dsRED (magenta) were co-transfected. One frame each 2 min during 32 min.





**Movie 3.** Live cell imaging of the metformin-exposed cell depicted in Figure 8A. Smaug1-EYFP (green) and Mito-dsRED (magenta) were co-transfected. One frame each 2 min during 32 min.