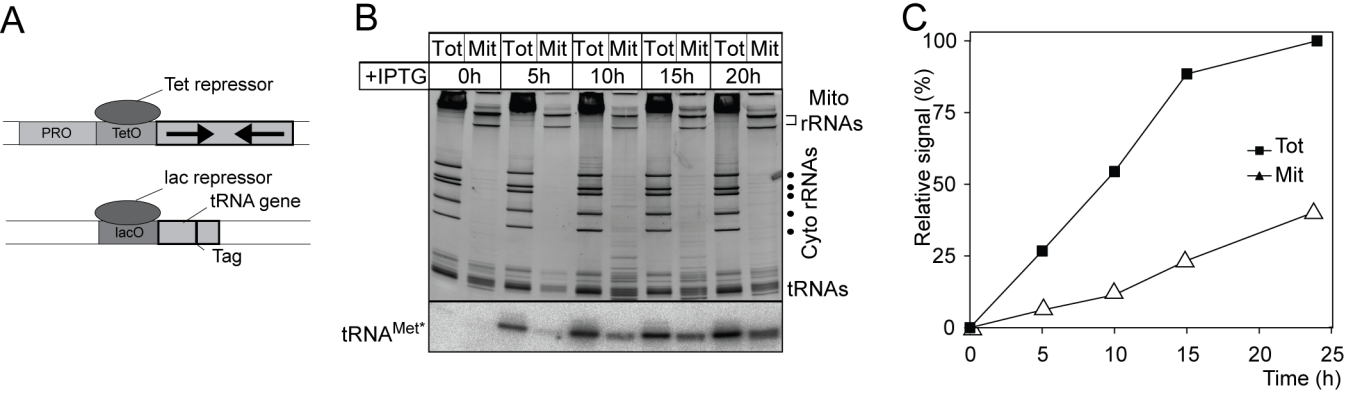
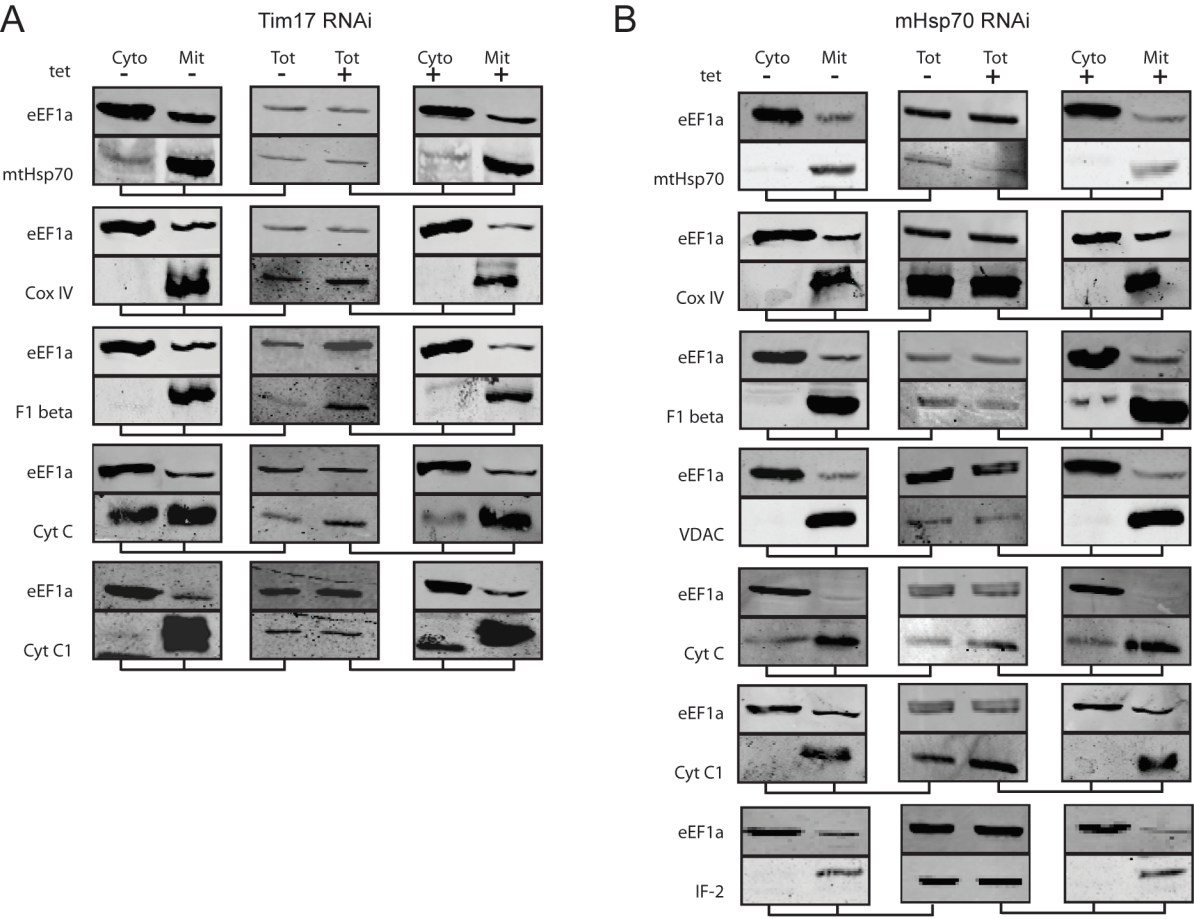
Supplementary Material

Fig. S1. Control of RNAi by Tet and tRNA expression by IPTG within the same cell line. *(A)* Scheme of the two gene expression cassettes present in the double inducible cell line. The Tet and the lac repressor genes are constitutively expressed. The Tet repressor binds to the Tet operator (TetO) and controls the procyclin promoter (pro) which transcribes the RNAi-inducing stem loop RNA. The lac-repressor controls the expression of the tagged $tRNA^{Met}$ ($tRNA^{Met*}$) gene whose 5'-end is fused to the Lac operator (LacO). *(B)* Time course of IPTG-inducible expression of the tagged tRNA. The presence of the tagged $tRNA^{Met}$ in the cytosol (Tot) and in digitonin-extracted mitochondria (Mit) was monitored by Northern analysis. The upper panel shows the the ethidium bromide-stained gel (EtBr). Positions of the mitochondrial rRNAs (Mit rRNA) and the cytosolic rRNAs (Cyt rRNA), as well as the tRNA region are indicated. The corresponding Northern blot is shown in the lower panel. *(C)* Quantitative analysis the data shown in *(B)*. The signal corresponding to the tagged $tRNA^{Met}$ at 24 h of induction in the total RNA fraction was set to 100%.

Fig. S2. Ablation of Tim17 (A) and mHsp70 (B) for 64 and 24 hours, respectively, does not influence the steady-levels of a panel of imported mitochondrial proteins. Left panels: digitonin-based fractionation of uninduced (-tet) RNAi cell lines into cytosol (Cyto) and mitochondrially enriched pellets (Mit). Middle panels: immunoblot total cellular protein for uninduced (-tet) and induced (+tet) RNAi cell lines. Right panels: digitonin-based fractionation of induced (+tet) RNAi cell lines into cytosol (Cyto) and mitochondrially enriched pellets (Mit). The following proteins were tested: mHsp70, cytochrom oxidase subunit IV (Cox IV), β-subunit of the ATP synthase (F1 beta), VDAC, cytochrome c (Cyt C), cytochrome c1 (Cyt C1), mitochondrial initiation factor 2 (IF2). For each of the tested proteins the same immunoblot was also probed for eEF1a as a loading control. Some immunoblots were reprobed with different antibodies, thus the eEF1a control panels are identical between some of mitochondrial proteins tested.


A
Tet repressor
PRO
TetO
lac repressor
tRNA gene
lacO
Tag
B
Tot Mit Tot Mit Tot Mit Tot Mit Tot Mit
+IPTG
0h
5h
10h
15h
20h
Mito rRNAs
Cyto rRNAs
tRNAs
tRNA^Met*
C
Relative signal (%)
100
75
50
25
0
0
5
10
15
20
25
Time (h)
Tot
Mit

Supp. Fig. 1


A
Tim17 RNAi
Cyto
Mit
Tot
Tot
Cyto
Mit
tet
- - - + + +
eEF1a
mtHsp70
eEF1a
Cox IV
eEF1a
F1 beta
eEF1a
Cyt C
eEF1a
Cyt C1
B
mHsp70 RNAi
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Cyt C
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Cyt C1
eEF1a
IF-2

Supp. Fig. 2