**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, cytochrome 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.