Expanded View Figures

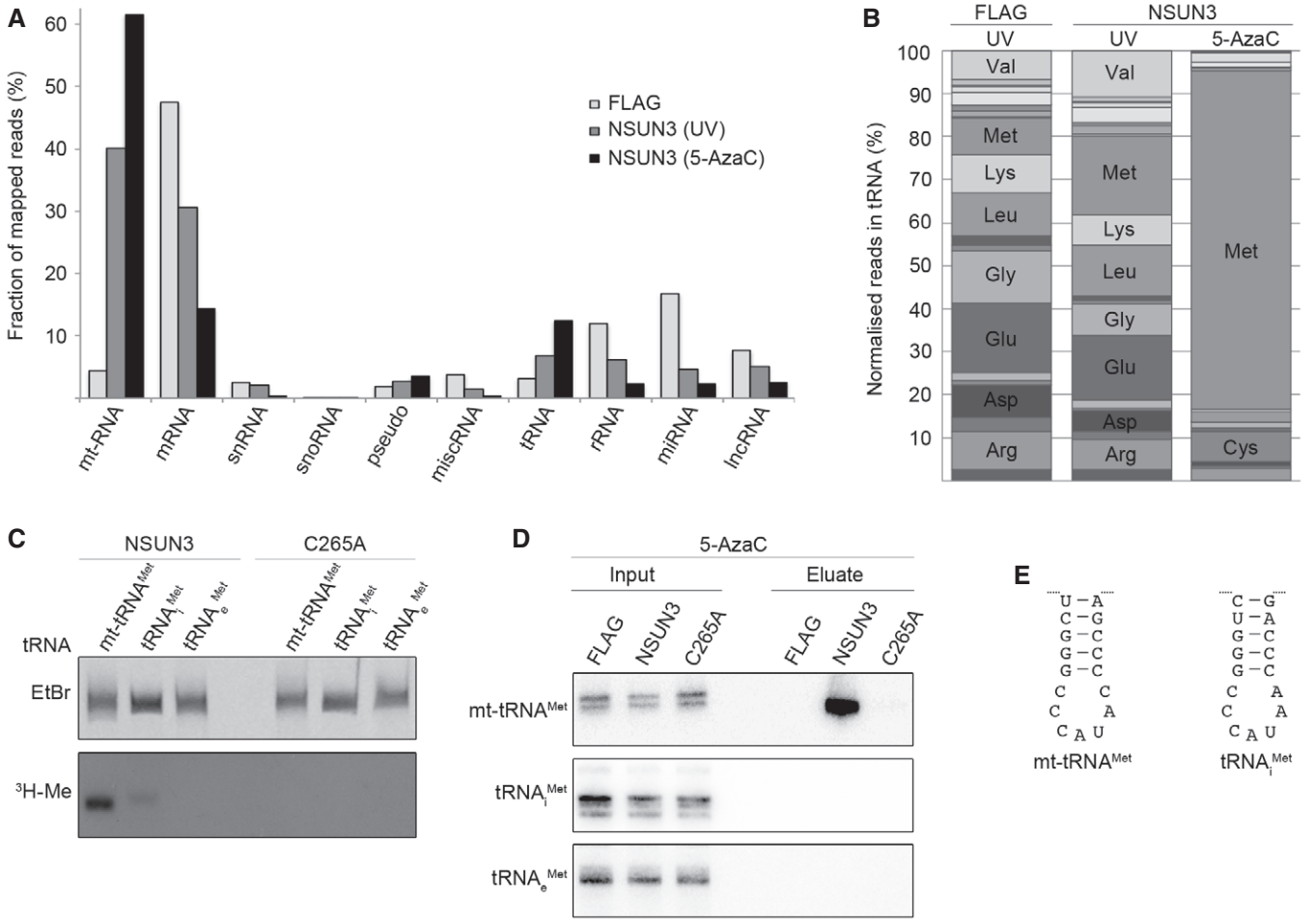

Figure EV1. The cytoplasmic tRNA Met-i and Met-e do not represent substrates of NSUN3 *in vivo*.

A, B The UV or 5-AzaC cross-linking and analysis of cDNA (CRAC) experiments with NSUN3-HisPrcFLAG or FLAG control cells were performed as described for Fig 2. (A) The percentages of the Illumina sequence reads mapped to individual classes of RNA are given graphically for each sample. Abbreviations: tRNA, transfer RNA; snRNA, small nuclear RNA; snoRNA, small nucleolar RNA; rRNA, ribosomal RNA; mtRNA, mitochondrial-encoded RNA; miscRNA, miscellaneous RNA; miRNA, microRNA; lncRNA, long non-coding RNA. (B) The relative distribution of cytoplasmic tRNA sequence reads obtained from the CRAC experiments is shown. Only tRNAs that were represented by more than 5% of all cytoplasmic tRNA reads are labelled.

C *In vitro* methylation reactions were performed using recombinant His_{14}-MBP-NSUN3 (NSUN3) or the catalytically inactive mutant His_{14}-MBP-NSUN3-C265A (C265A), [^{3}H-methyl]-labelled S-adenosylmethionine as a methyl group donor and *in vitro*-transcribed mitochondrial mt-tRNA^{Met}, cytoplasmic tRNA$_i^{Met}$ and tRNA$_e^{Met}$. The RNA was then separated on a denaturing polyacrylamide gel, stained with ethidium bromide (EtBr) to indicate inputs and exposed to an X-ray film to analyse methylation (^{3}H-Me).

D 5-AzaC cross-linking was performed and RNA-associated with wild-type NSUN3, the catalytic NSUN3 mutant (C265A) or the FLAG tag alone was isolated as described in (A). The RNA was isolated from the purified protein–RNA complexes and analysis by Northern blot using probes against the mt-tRNA^{Met}, mt-tRNA$_i^{Met}$ and mt-tRNA$_e^{Met}$. Inputs are shown on the left and eluates on the right. The mt-tRNA^{Met} panel is identical to that shown in Fig 2G.

E The nucleotide sequences of the anticodon stem loops of mt-tRNA^{Met} (left) and tRNA$_i^{Met}$ (right) are shown.

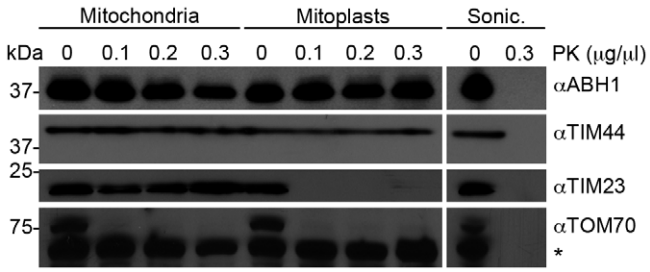

Figure EV2. The mitochondrial pool of ABH1 is localised in the matrix.
To analyse submitochondrial localisation of ABH1, human mitochondria were isolated and either left untreated, swollen in hypotonic buffer (Mitoplasts) or disrupted by sonication (Sonic.) before treatment with different amounts of proteinase K (PK) where indicated, followed by SDS–PAGE and Western blotting using antibodies against human TIM44, TIM23, TOM70 or FLAG-tagged NSUN3. Note that TIM44 extrudes into the matrix, while a major domain of TIM23 localises to the intermembrane space and TOM70 is largely exposed on the mitochondrial surface. The asterisk indicates a cross-reaction of the TOM70 antibody. The panels of TIM44, TIM23 and TOM70 are identical with those shown in Fig 1B.

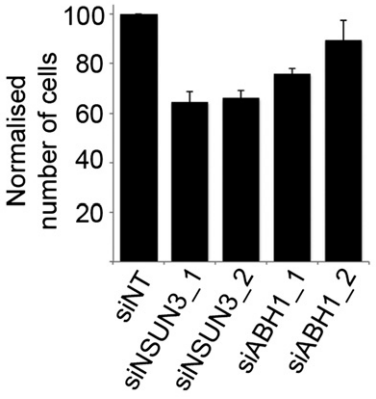

Figure EV3. RNAi-mediated depletion of NSUN3 or ABH1 leads to reduced cell growth.
HeLa cells that had been transfected with siRNAs against NSUN3 (siNSUN3_1 or siNSUN3_2), ABH1 (siABH1_1 or siABH1_2) or non-target siRNAs (siNTs) were harvested and counted. Cell numbers from three experiments were normalised to the non-target control, and results are given graphically as mean ± SD.