

Expanded View Figures

Figure EV1. LETM1 repression perturbs mtDNA organization.

- A Further examples of the mtDNA abnormalities are shown in Fig 3A. *LETM1* expression was suppressed in HeLa cells by transfection with targeted siRNAs (siR1, siR2, or siR3). A non-target dsRNA (NT) served as control. Cells were fixed and immunolabeled with anti-DNA antibody (green). Scale bar: 15 μ m.
- B HeLa cells stained with anti-LETM1 antibody (red) and anti-BrdU (green) after labeling with 5 mM BrU for 60 min. In other images, LETM1 was stained green and other proteins stained red using antibodies to the RNA granule protein GRSF1, the 55S ribosome component MRPL45, or the outer mitochondrial membrane protein TOM20. Scale bars are 12 μ m in the main images and 3 μ m in offset magnification.

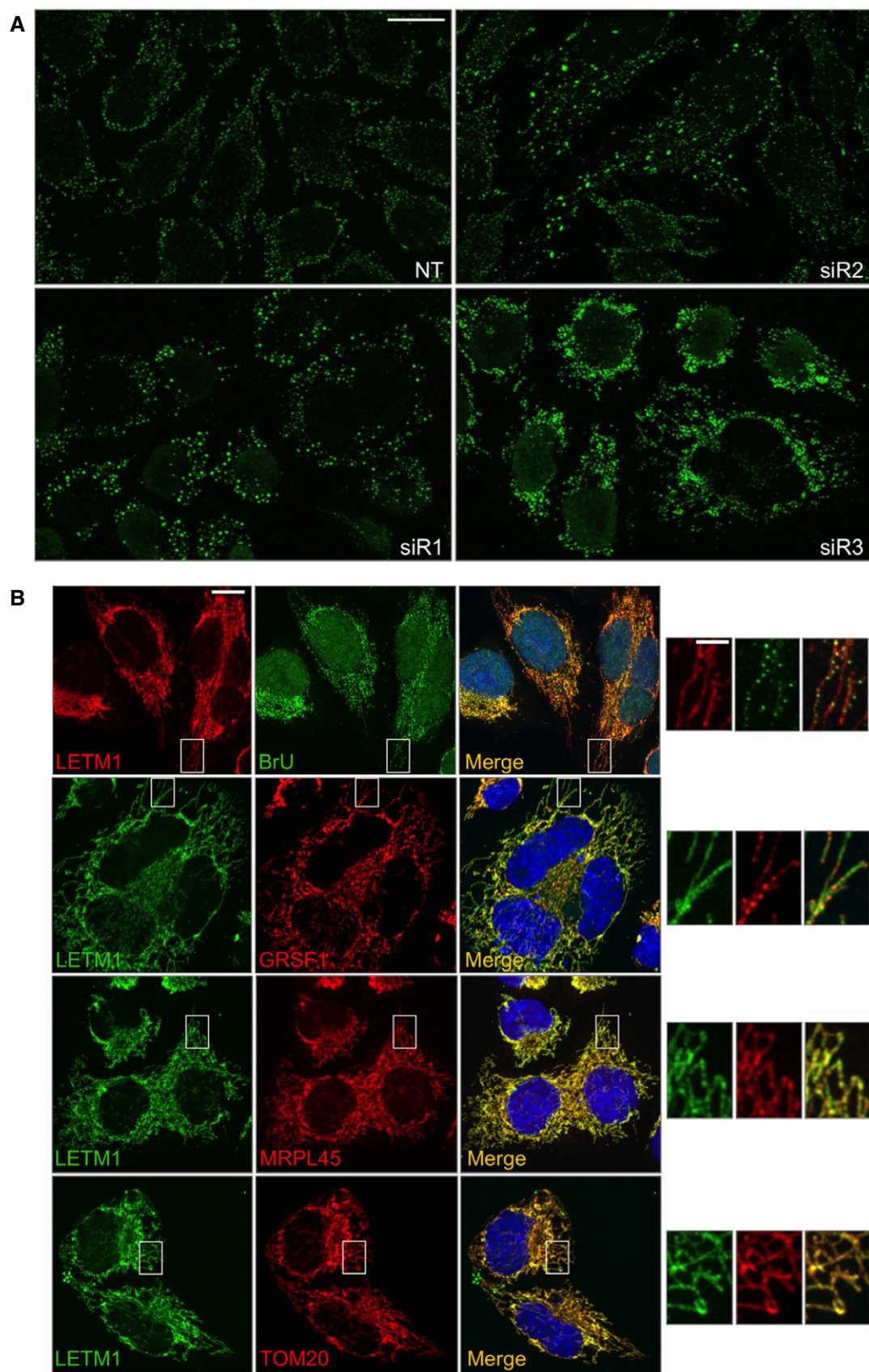


Figure EV1.

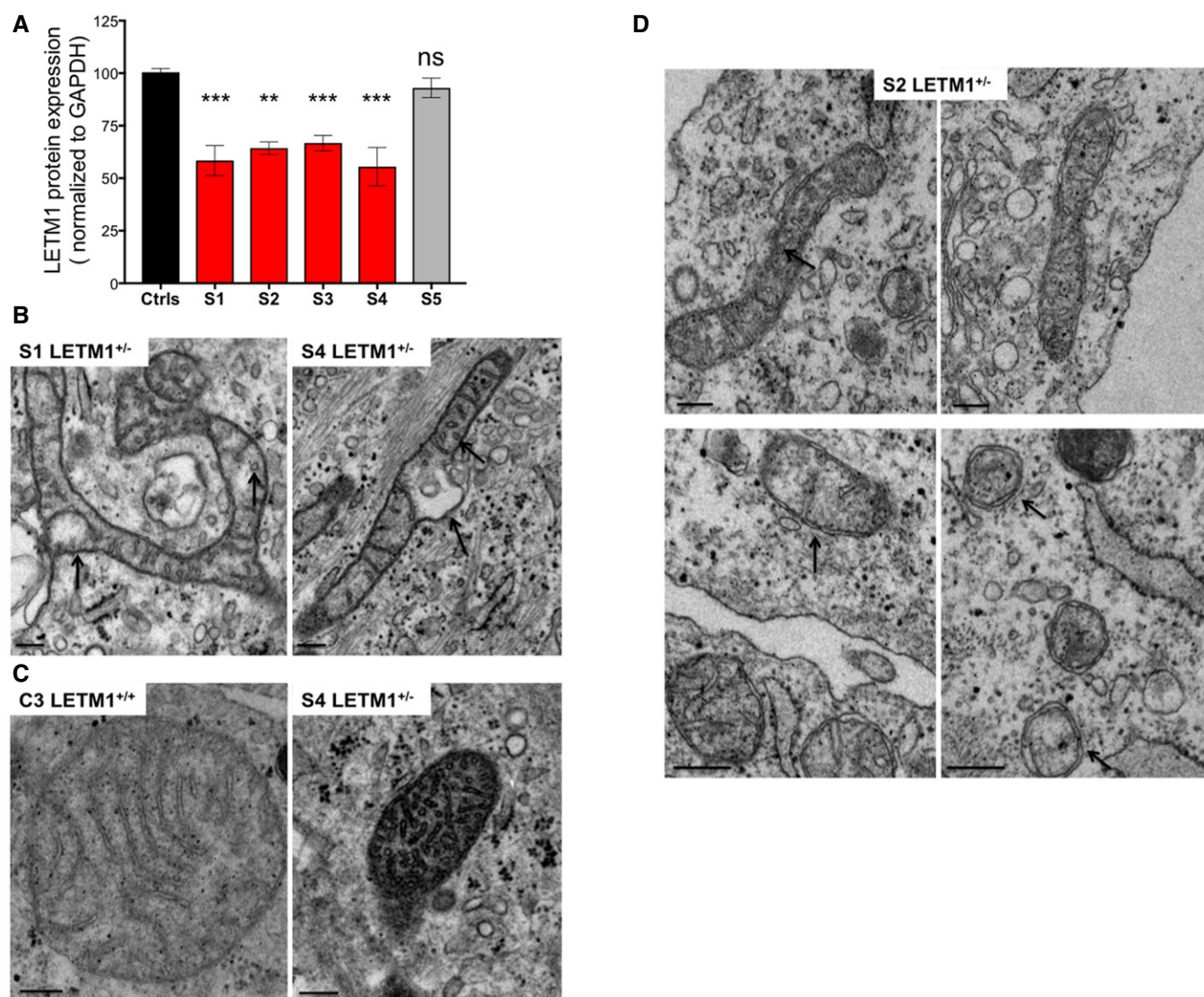


Figure EV2. LETM1 haploinsufficiency in WHS is associated with mitochondrial ultrastructure abnormalities.

A Quantification of LETM1 expression in fibroblasts. Data from control cell lines (ctrls) C1–C3 were pooled. All data are expressed as mean $\pm$ SEM ($n = 4$ or 5). ** $P < 0.01$, *** $P < 0.001$. ns, not statistically significant (one-way ANOVA).
B Magnification of WHS LETM1^{+/-} S1 and S4 mitochondria. Black arrows indicate circular cristae and matrix distensions. Scale bar: 0.2 μ m.
C Magnification of control C3 and WHS LETM1^{+/-} S4 mitochondria showing dense matrix and circular cristae exclusively in the latter. Scale bar: 0.2 μ m.
D Electron micrographs of WHS LETM1^{+/-} (S2) fibroblasts. Black arrows indicate circular cristae in the upper 2 images and mitochondria with sparse cristae in the lower 2 images. Scale bar: 0.2 μ m.

Figure EV3. PDH is repressed in WHS LETM1^{+/-} fibroblasts, and unlike controls cells, they are able to grow on medium containing KB in place of glucose and pyruvate.

A Quantification of total PDH, PDH^{S293}, and PDK3 immunoblots. Data are expressed as mean $\pm$ SEM of $n = 5$ independent experiments. * $P < 0.05$, ** $P < 0.01$. ns, not statistically significant; t -test with Welch's correction for PDH and PDH^{S293}, one-way ANOVA for PDK3.
B Light microscope images of control (C2), WHS LETM1^{+/+} (S5), and WHS LETM1^{+/-} (S1, S3, and S4) cells growing in 0.3 mM BHB (KB) for 6 days, after a switch from 25 mM glucose and 1 mM pyruvate at 40% confluence. Scale bar: 300 μ m.
C S4 cells grown to confluency in KB medium (as per panel A) were passaged and cultured for a further 6 days.

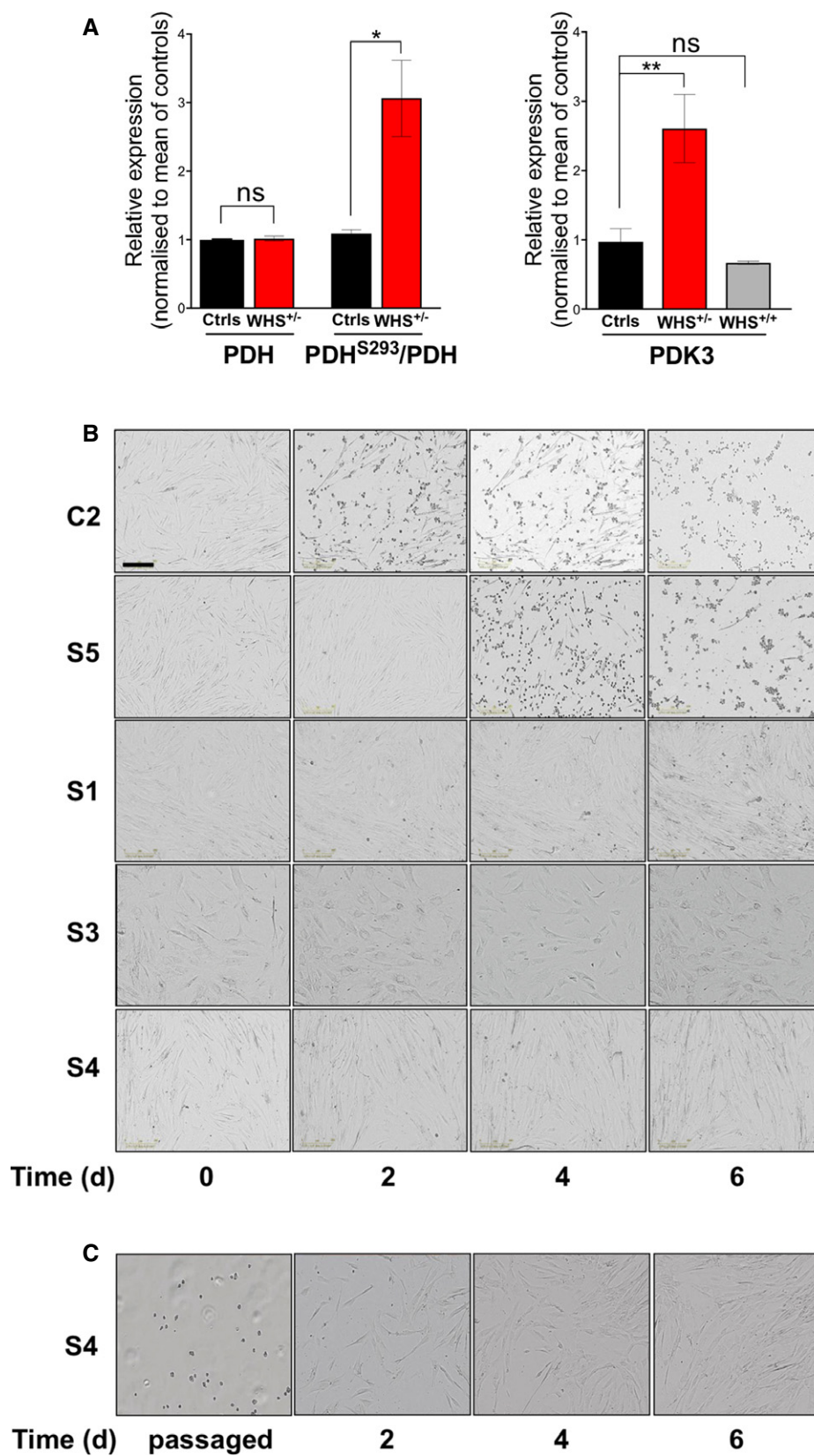


Figure EV3.