# E07-12-1287 van der Bliek

## Supplemental Table

**Suppl. Table 1.** Genes identified in the *Drosophila* siRNA screen for mitochondrial morphology defects. *Drosophila* gene names, name of the closest Human homologues, GO annotations and a brief description of the siRNA phenotypes are given.

| *Drosophila* Gene | Human Homolog | GO annotation (function) | siRNA Phenotype |
|---|---|---|---|
| Drp1 | DRP1 | GTPase activity | Clumped Mitochondria |
| Lis-1 | PAFAH1B1 | Dynein binding | Clumped Mitochondria |
| par-1 | MARK3 | ATP binding, Serine/Threonine kinase | Clumped Mitochondria |
| nud E | NDE1 | Microtubule binding | Clumped Mitochondria |
| alpha Tub84B | TUBA1A | GTP binding | Clumped Mitochondria, Cell death |
| beta Tub97EF | TUBB8 | GTP binding | Clumped Mitochondria, Cell death |
| betaTub60D | TUBB3 | GTP binding. Structural component of cytoskeleton | Clumped Mitochondria, Cell death |
| CG3625 | AIG1 | Integral to membrane | Perinuclear Mitochondria |
| CG9339 | TBC1D24 | Rab GTPase activator activity | Perinuclear Mitochondria |
| sktl | PIP5K1A | 1Phodphatidyl inositol-4-phosphate 5- Kinase activity | Perinuclear Mitochondria |
| CG33214 | GLG1 | Fibroblast growth factor binding | Clumped Mitochondria |
| mys | ITGB1 | Protein hetero-dimerization activity | Clumped Mitochondria |
| CG12203 | NDUFS4 | NADH dehydrogenase (Ubiquinone) activity | Clumped Mitochondria, Cell death |
| sbr | NXF1 | Nucleocytoplasmic transporter activity | Clumped Mitochondria, Cell death |
| Tango11 | C2orf33 | Integral to membrane | Clumped Mitochondria |

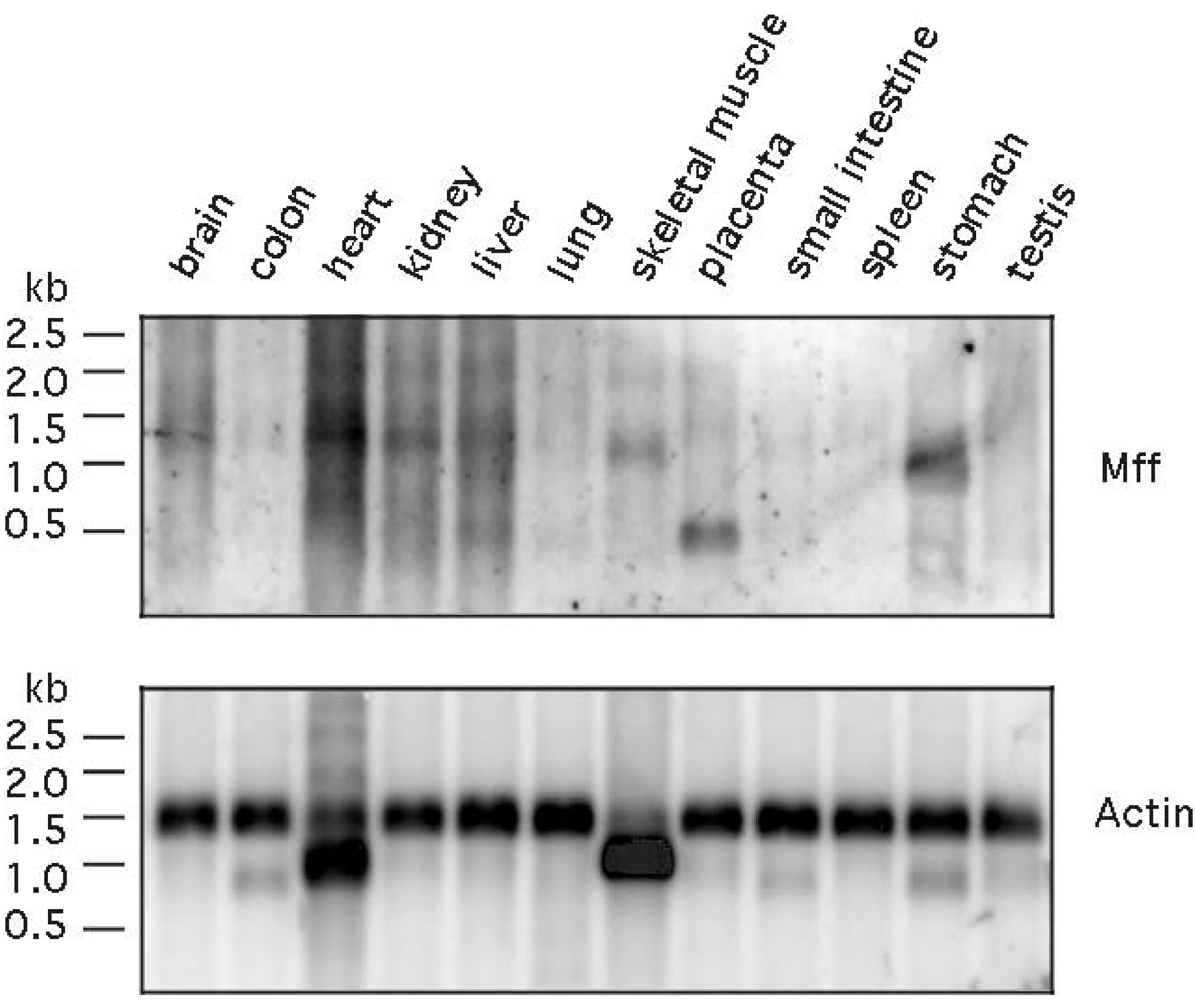
## Supplemental Figures

**Supplemental Figure 1.** Northern blot analysis of Mff expression. A blot containing polyA+ RNA from a range of human tissues was probed with radiolabeled Mff cDNA and actin cDNA as a loading control. The autoradiogram shows a prominent RNA species of 1.2 kb in brain, heart, kidney, liver and skeletal muscle, a slightly smaller RNA of around 1.0 kb in stomach tissue and a much smaller RNA of around 0.5 kb in placenta. Other tissues have low levels of the 1.2 kb RNA, suggesting that Mff is ubiquitously expressed.

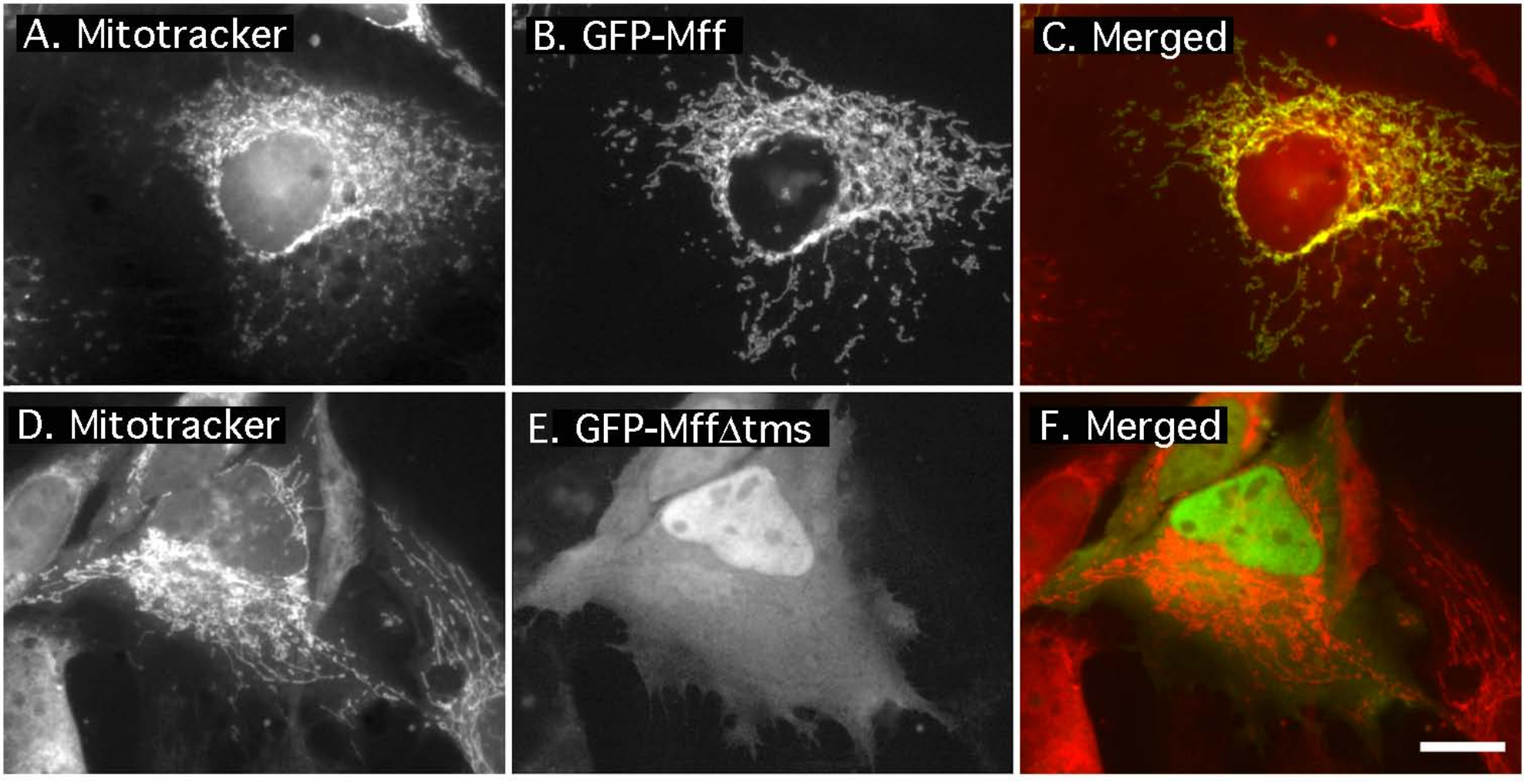
**Supplemental Figure 2.** Localization of Mff requires the transmembrane segment. A construct expressing GFP-tagged Mff was transfected into HeLa cells. Panel A shows Mitotracker staining, panel B shows GFP fluorescence and panel C shows the merged image with Mitotracker in red and GFP in green. A second construct expressing GFP-tagged Mff with a carboxy terminal deletion of 20 amino acids, which removes the putative transmembrane segment, shows mitochondria stained with Mitotracker (D), but diffuse cytosolic and nuclear fluorescence with GFP fused to truncated Mff (E). Mislocalization of truncated Mff is also evident in the merged image with Mitotracker in red and GFP in green (F). The constructs used for these experiments encode isoform 8, which lacks exons 5, 6 and 7 (Fig. 1). The scale bar is 10 μm.

**Supplemental Figure 3.** Peroxisomal localization of Mff. HeLa cells were cotransfected with a myc-tagged Mff expression construct and Drp1 siRNA oligonucleotides. Panel A shows a cell stained with myc antibody, panel B shows the same cell stained with Catalase antibody and Panel C shows the merged image with myc in green and catalase in red. The insets are enlargements showing that myc-Mff colocalizes with Catalase. Panel D shows a cell stained with myc antibody, panel E shows the same cell stained with Mitotracker and Panel F shows the merged image with myc in green and Mitotracker in red with enlargements of selected areas in the insets. This experiment shows that areas devoid of mitochondria still have myc-Mff. Panel G – I are controls showing that Catalase staining does not bleed through in the FITC channel, which was used in the other images for detection of myc-Mff. The scale bar is 10 μm.

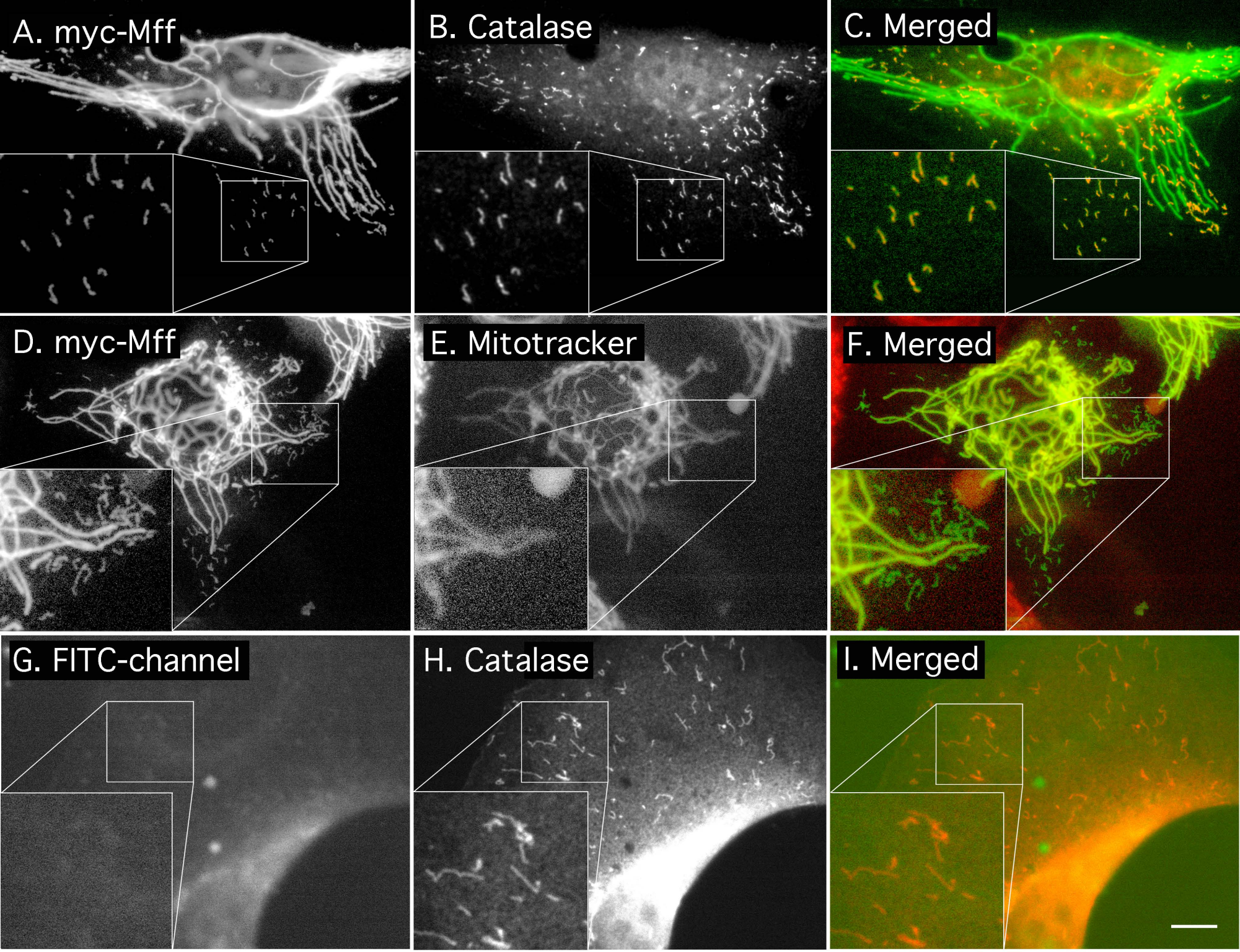
**Supplemental Figure 4.** Delay of Actinomycin-D-induced cytochrome c release from mitochondria. Hela cells were transfected with scrambled, Mff, Fis1 and Drp1 siRNA oligonucleotides. Apoptosis was induced by treating the cells with actinomycin D. The effects were monitored by staining the cells with cytochrome c antibody. Cytochrome c was released from mitochondria several hours after inducing apoptosis, but this release was substantially delayed by Mff, Fis1 and Drp1 siRNA. The percentages were determined with 300 – 400 cells.



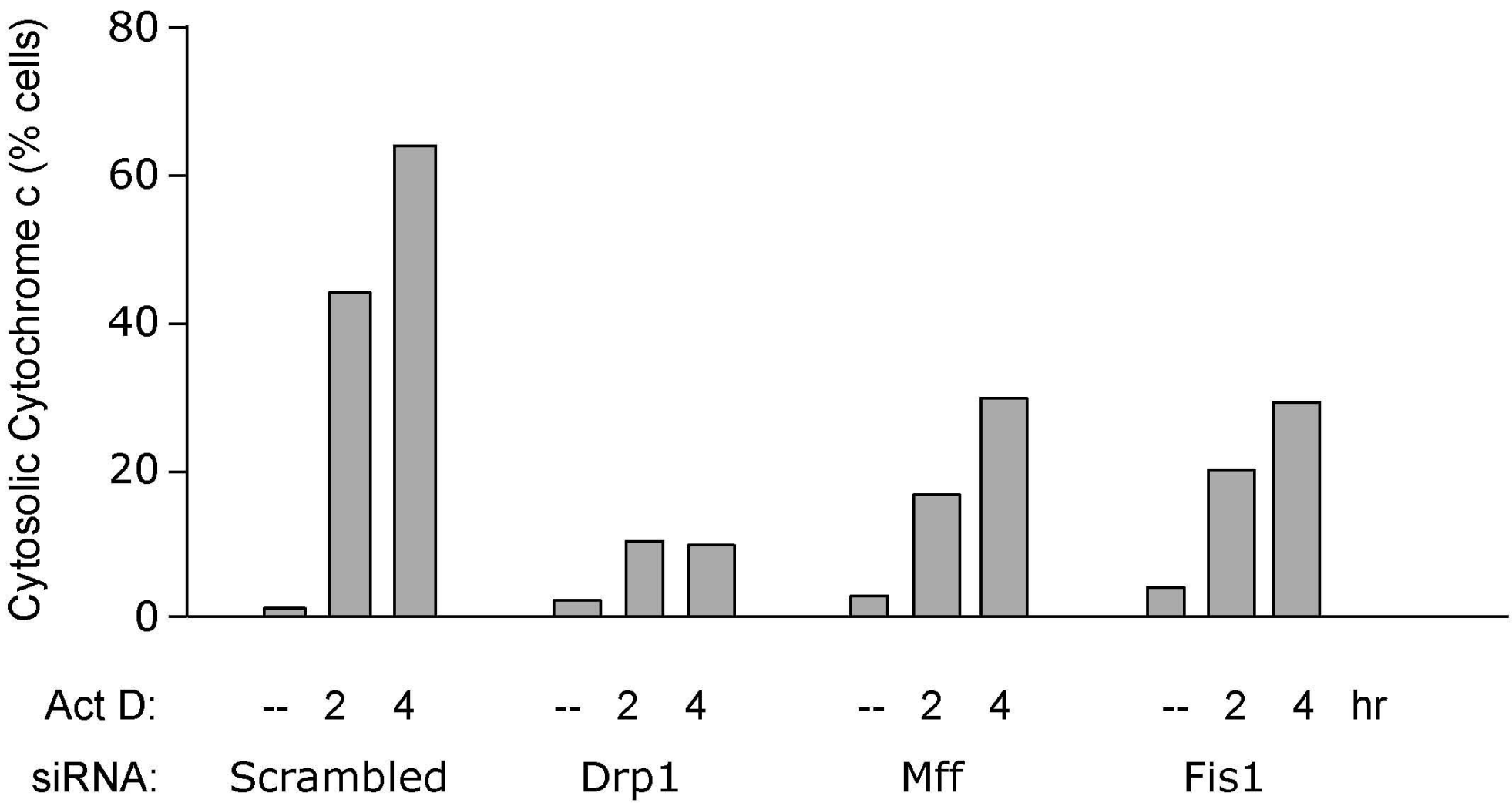

Suppl. Fig. 1. Gandre-Babbe and van der Bliek




Suppl. Fig. 2. Gandre-Babbe and van der Bliek




Suppl. Fig. 3. Gandre-Babbe and van der Bliek




Suppl. Fig. 4. Gandre-Babbe and van der Bliek