## Supplementary Figure 1

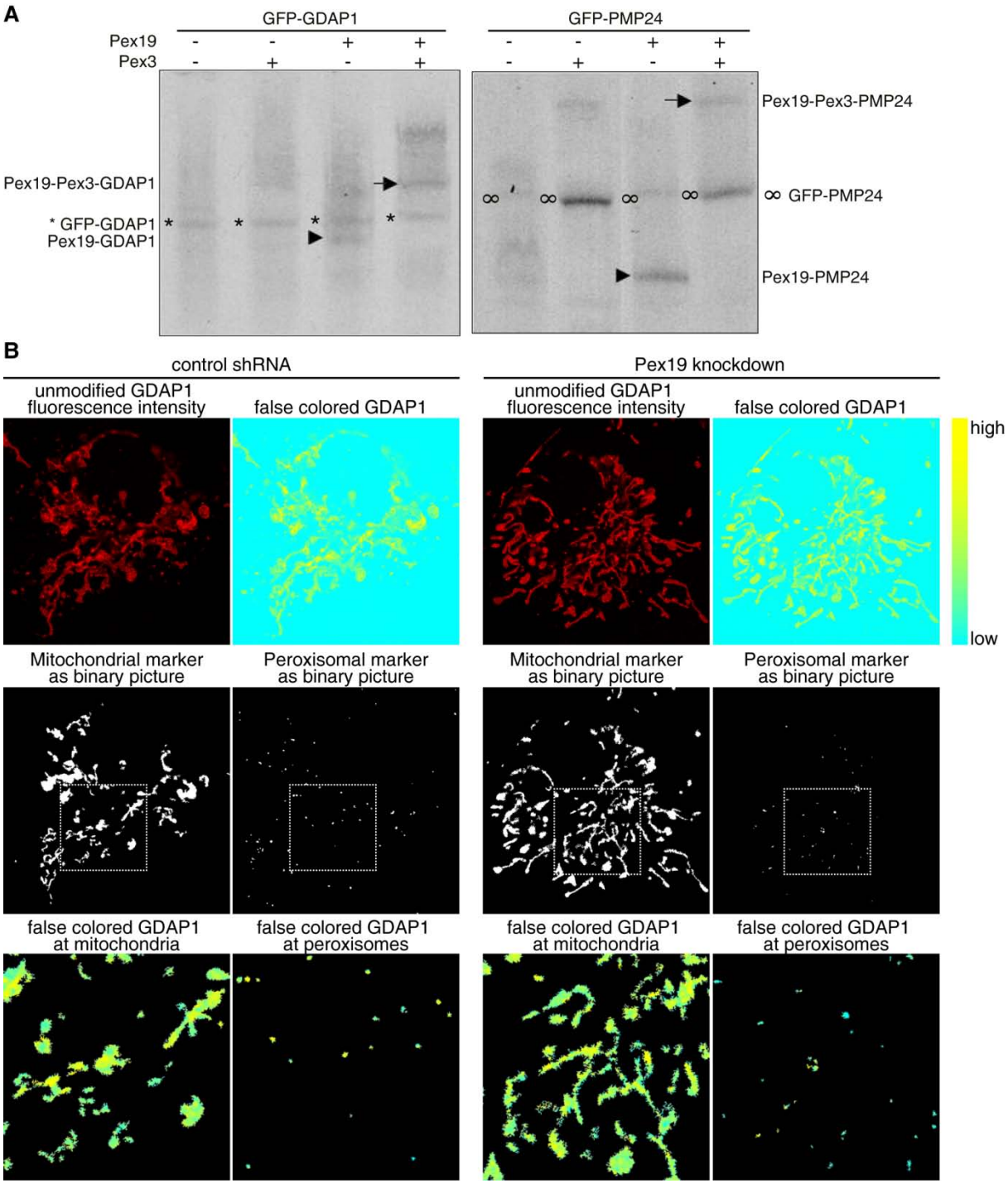


**Fig. S1: Peroxisomal targeting of GDAP1 depends on Pex19.** (A) To test whether Pex19, the peroxisomal shuttle receptor for membrane proteins can form a complex with GDAP1, GFP-GDAP1 was *in vitro* translated using labelled ($^{35}$S) amino acids. Recombinant, non-labelled Pex19, Pex3, or both were added, and subsequently the proteins were separated on a native gel and detected by autoradiography. As positive control, the same experiment was performed using radioactively labelled GFP-PMP24 [1]. In the presence of recombinant Pex19, a faint faster migrating band appeared (arrow heads) in addition to the main GFP-GDAP1 (*) signal or the GFP-PMP24 (∞ ) signal, indicating a complex of GFP-GDAP1 and Pex19. The addition of

only Pex3 - the docking protein for cargo-loaded Pex19 - did not alter the migration of GFP-GDAP1. However, in combination with Pex19, a band shift of GFP-GDAP1 occurs (arrow), similar to the shift in the GFP-PMP24 positive control. This suggests that GDAP1, Pex19 and Pex3 form a trimeric complex. (B) To quantify the intensity of GDAP1 at peroxisomes we infected COS7 cells stably expressing GFP-SKL with lentiviral particles encoding shRNA targeting Pex19 or a non-silencing control shRNA. Three days after infection, the cells were transiently co-transfected with GDAP1 and mtDsRed expression constructs. After 16 h, the cells were fixed, immunostained for GDAP1, and single-plane confocal images were acquired. We converted the signals of the mitochondrial marker (mtDsRed) and the peroxisomal marker (GFP-SKL) into binary images and quantified the intensities of GDAP1 for all mtDsRed and GFP-SKL positive objects (here shown in false color for illustration purposes: yellow – highest GDAP1 fluorescence intensity; turquoise – lowest GDAP1 fluorescence intensity; quantification shown in Fig.1C).

**Supplementary Figure 2**

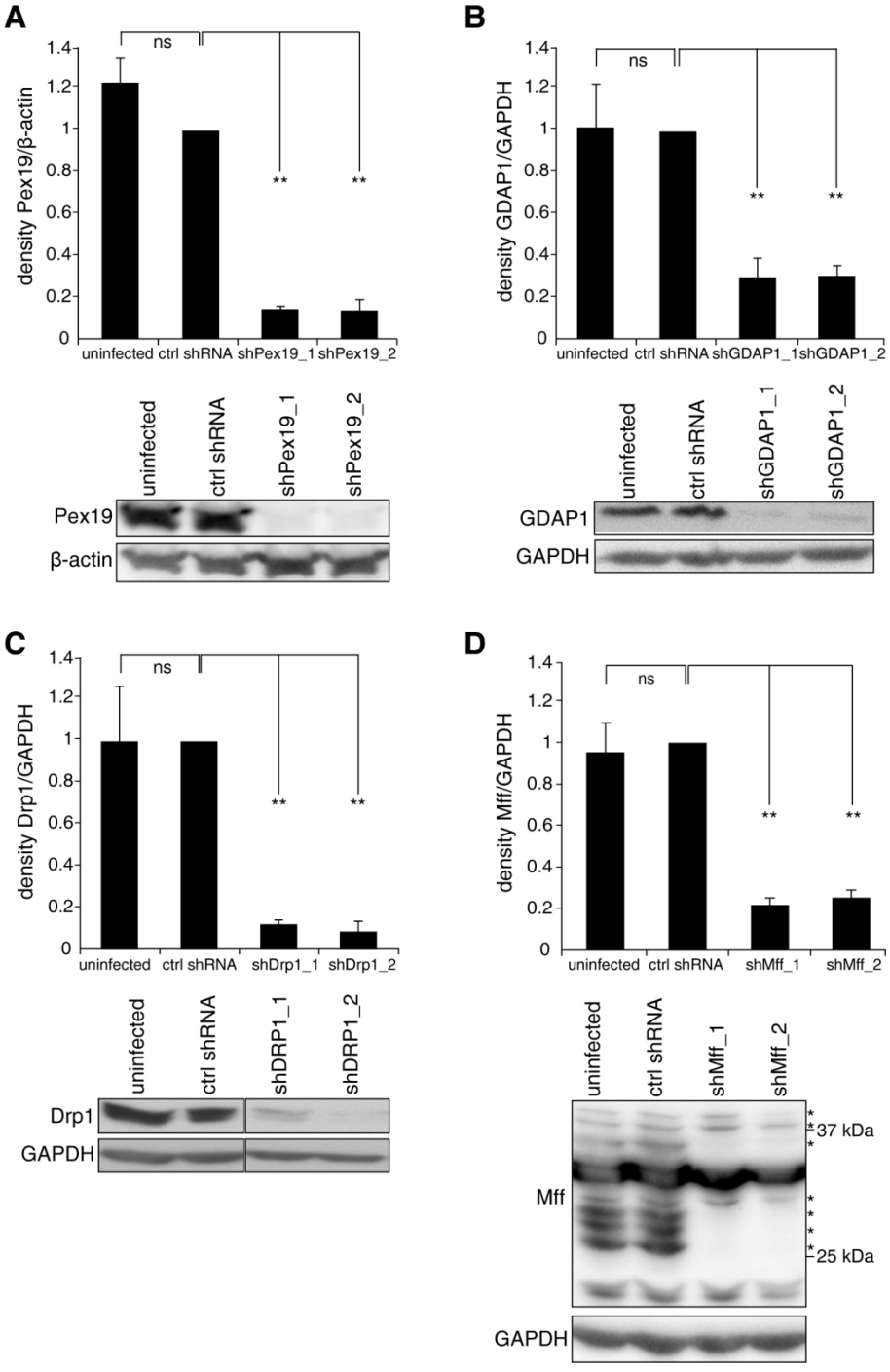


**Fig. S2: shRNA-mediated knockdown of Pex19, GDAP1, Drp1, and Mff.** COS7 cells (A) or N1E-115 cells (B-D) were infected with lentiviral particles encoding for non-silencing (ctrl) shRNA, Pex19-specific shRNAs, GDAP1-specific shRNAs, Drp1-specific shRNAs, Mff-specific shRNAs, or were left uninfected. Five days after start of infection, the loss of protein was quantified on Western Blots, for (A) Pex19, (B) GDAP1, (C) Drp1, or (D) Mff signal in relation to the β-actin or GAPDH expression. Values represent means and s.e.m. of at least three independent experiments: ** $p<0.01$, two-tailed unpaired t-test.

**Supplementary Figure 3**

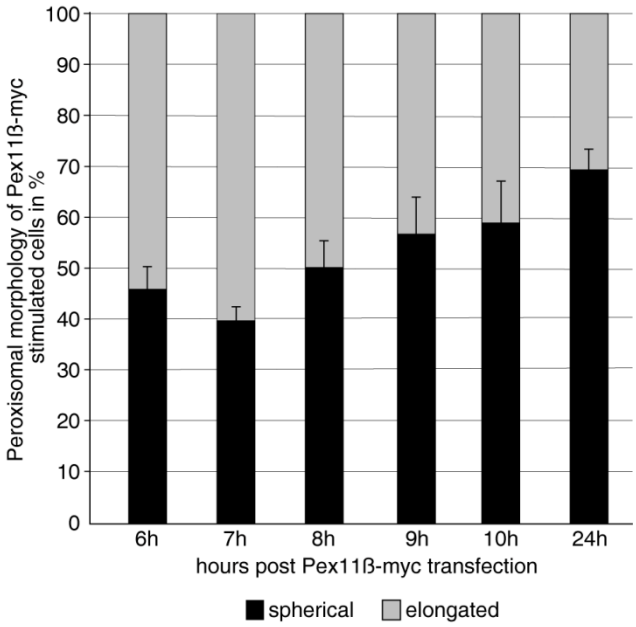


**Fig. S3: Time course of changes in the peroxisomal morphology following transient expression of Pex11β-myc.** N1E-115 cells were transiently transfected with Pex11β-myc expression constructs, fixed at the indicated time points after start of transfection, stained using antibodies for myc and analysed according to the three peroxisomal categories (Fig.2A). Note that the category “tubular” was only observed in Drp1 or Mff knockdown cells and thus is not represented in this analysis. The quantifications of the peroxisomal morphologies at different time points after Pex11β-myc transfection reveals cells with elongated peroxisomes at early time points of Pex11β-myc expression with a maximum at 7 h after start of transfection. Later time points (8 h – 24 h) show an increasing percentage of Pex11β-myc positive cells with spherical peroxisomal morphology. Values represent means and s.e.m. of three independent experiments, 100 cells were counted per condition per experiment.

**Supplementary Figure 4**

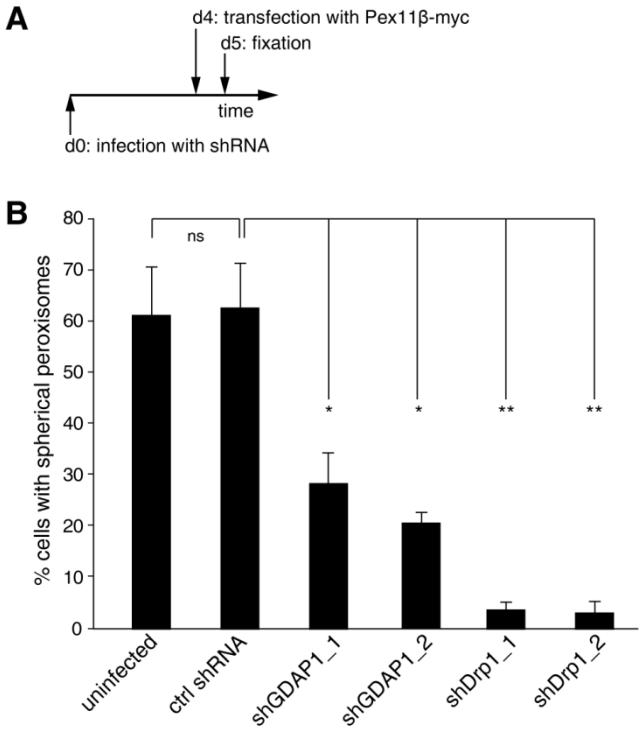


**Fig. S4: Loss of GDAP1 in Pex11β-myc stimulated cells leads to peroxisomal elongation.** (A) The knockdown experiment was performed as described before (Fig. 2A). In addition, Pex11β-myc was overexpressed at day 4 after infection (d4). Cells were fixed, immunostained for myc and the myc-positive cells were analysed according to Fig. 2A. (B) Loss of GDAP1 leads to a significant reduction of cells with spherical peroxisomes. This is even more pronounced upon loss of Drp1. Values represent means and s.e.m. of three independent experiments, 100 cells were counted per condition per experiment. * $p<0.05$, ** $p<0.01$, two-tailed unpaired t-test.

## Supplementary Figure 5

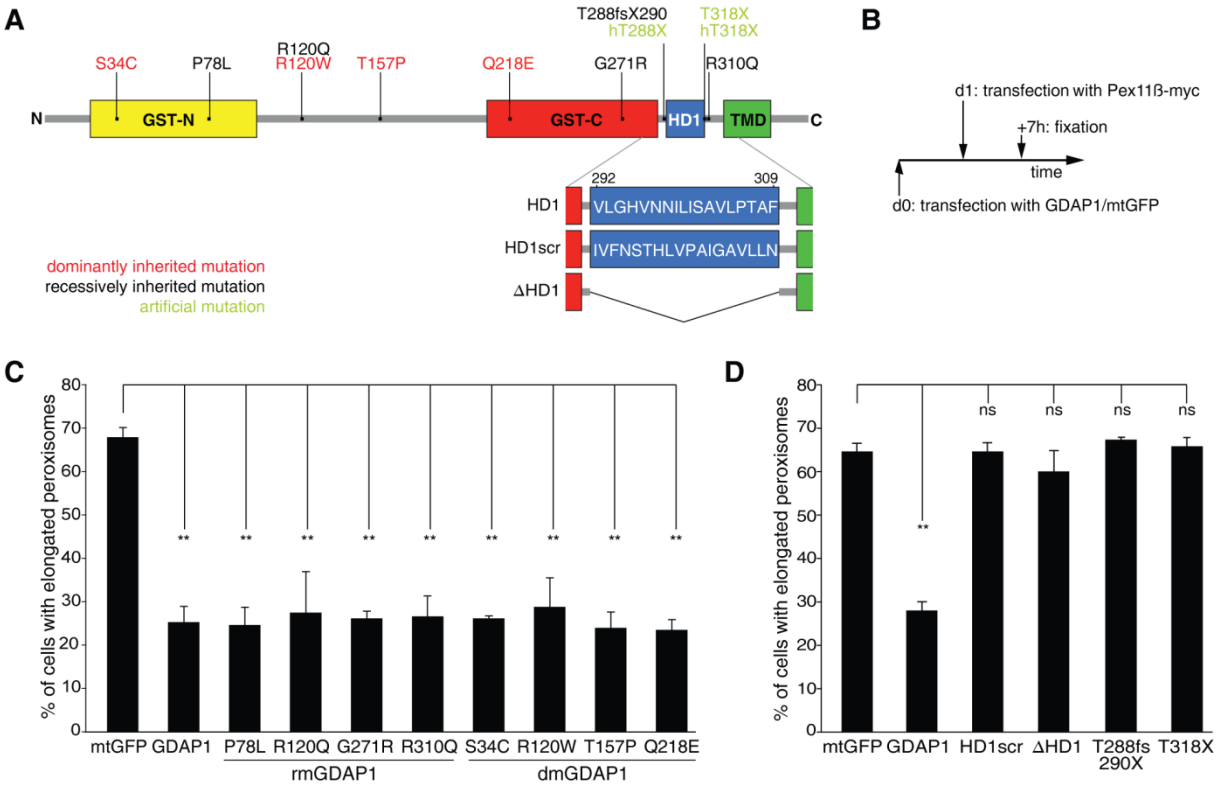


**Fig. S5: N-terminal CMT-associated GDAP1 missense mutants are still able to induce peroxisomal fragmentation, while HD1 mutants and C-terminal truncation mutants fail to do so.** (A) Domain structure of GDAP1 with the localisation of the tested mutations used in this study. (B) N1E-115 cells were transfected with GDAP1 wildtype or a mutant expression construct (d0). mtGFP served as negative control. 24 h later (d1), cells were transfected again to express Pex11β-myc. After 7 h, cells were fixed, stained for myc and analysed. (C) Fewer cells co-expressing Pex11β-myc and GDAP1 wildtype are displaying elongated peroxisomes compared to control cells expressing mtGFP and Pex11β-myc. Also all tested disease-related missense mutants (dominantly inherited mutation (dm), recessively inherited mutation (rm)) show a peroxisomal fission activity similar to that of GDAP1 wildtype. (D) Pex11ß-myc-positive cells overexpressing GDAP1 with scrambled HD1 (HD1scr), deleted HD1 (ΔHD1), or C -terminal truncated GDAP1 (T288fs290X and T318X) display mainly elongated peroxisomal morphologies like the mtGFP expressing control cells. Only GDAP1 wildtype overexpressing cells have less elongated peroxisomes as shown before in C (positive control). Values represent means and s.e.m. of at least three independent experiments, 80 to 100 cells were counted per condition per experiment: ** $p<0.01$, two-tailed unpaired t-test.

**Supplementary Figure 6**

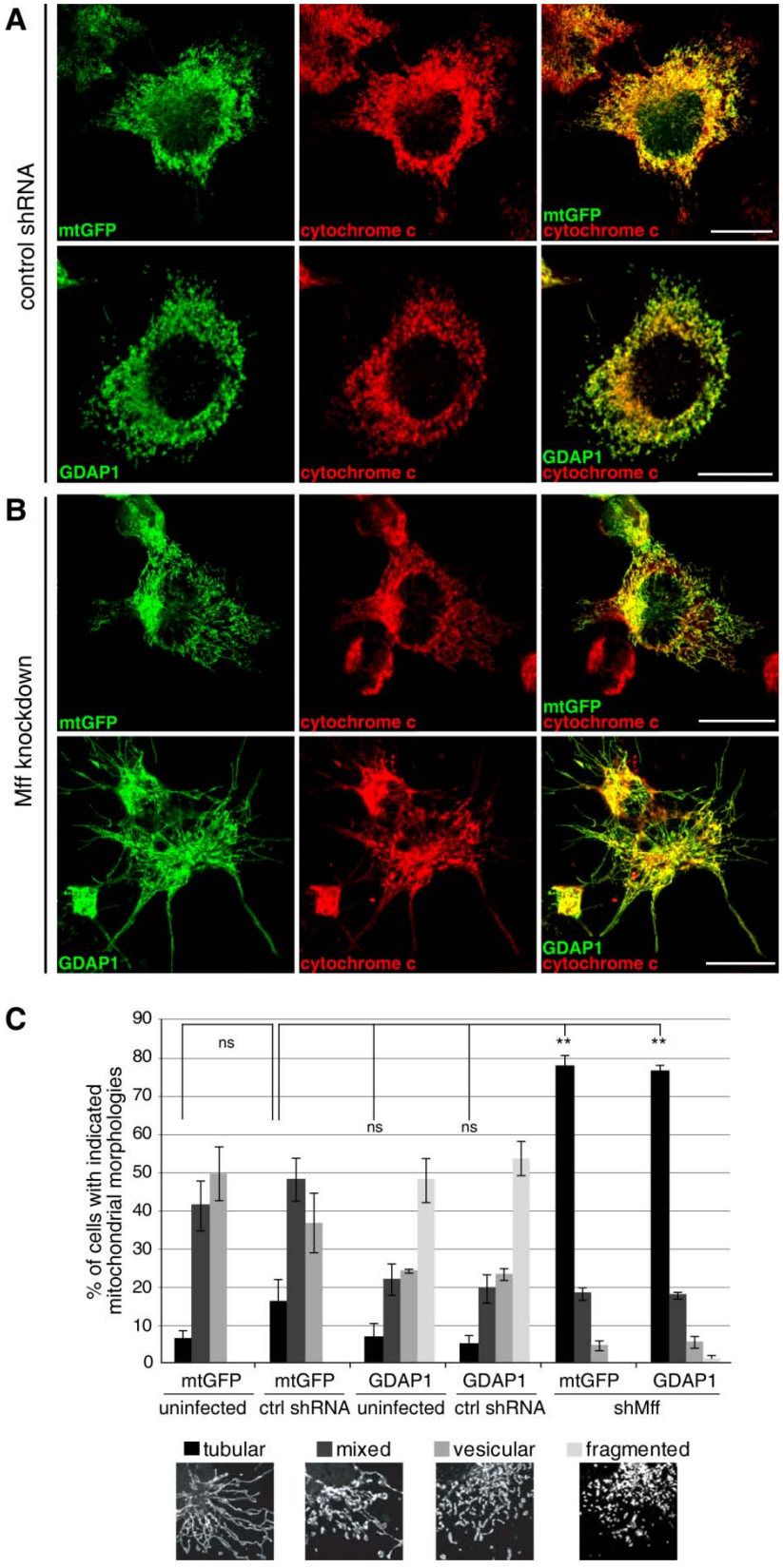


**Fig. S6: GDAP1-induced mitochondrial fragmentation depends on Mff**. N1E-115 cells were infected with lentiviral particles encoding non-silencing control shRNA (A,C), shRNA against Mff (B,C), or were left uninfected (C). Four days post infection, cells were transiently transfected with expression constructs encoding mtGFP or

GDAP1. 24 h later, cells were fixed and stained for GDAP1 and cytochrome *c*. (A,B) Confocal images represent the predominant mitochondrial morphology of the different conditions. (C) The mitochondrial morphology of transfected cells was quantified in blinded countings. The knockdown of Mff leads to elongated mitochondria and the expression of GDAP1 in Mff knockdown cells does not cause mitochondrial fragmentation. Values represent means and s.e.m. of three independent experiments, 100 cells were counted per condition per experiment: ** $p<0.01$, two-tailed unpaired t-test. Bars, 10 µm.

**Supplementary Table I**

| | Overexpression of the modified protein… induces peroxisomal fission | induces mitochondrial fission | allows fusion of mitochondrial matrix | **Expression constructs used in this study** |
|---|---|---|---|---|
| **GDAP1 wildtype** | yes | yes | yes | GDAP1 |
| **Disease-causing mutants** | | | | |
| Dominant missense mutants | yes | yes | no | S34C, R120W, T157P, Q218E |
| Recessive N-terminal missense mutants | yes | no | yes | P78L, R120Q, R310Q, G271R |
| Recessive mutation leading to C-terminal truncation | no | no | yes | T288fs290X |
| **Artificial mutants** | | | | |
| Deletion of tail-anchor domain (TA) | no | no | yes | T318X |
| Deletion of HD1 and TA-domain | no | no | yes | hT288X |
| Deletion of HD1 | no | no | yes | ΔHD1 |
| Scrambling of HD1 | no | no | yes | HD1scr |

**Tab. SI: Summary of the effects of mutated forms of GDAP1 on peroxisomal and mitochondrial dynamics.** The table summarizes results presented in this manuscript and compares them to data using the same experimental approaches and constructs to investigate mitochondrial dynamics in previous publications [2-4]. The T318X mutant refers to the human and mouse form of GDAP1. Yellow indicates that mutants have an altered effect on peroxisomal dynamics; blue denotes that mutants have an altered effect on mitochondrial dynamics.

**Reference list for supplemental information**

1. Pinto MP, Grou CP, Alencastre IS, Oliveira ME, Sa-Miranda C, Fransen M, Azevedo JE (2006) The import competence of a peroxisomal membrane protein is determined by Pex19p before the docking step. *J Biol Chem* **281:** 34492-34502

2. Niemann A, Ruegg M, La Padula V, Schenone A, Suter U (2005) Ganglioside-induced differentiation associated protein 1 is a regulator of the mitochondrial network: new implications for Charcot-Marie-Tooth disease. *J Cell Biol* **170:** 1067-1078

3. Niemann A, Wagner KM, Ruegg M, Suter U (2009) GDAP1 mutations differ in their effects on mitochondrial dynamics and apoptosis depending on the mode of inheritance. *Neurobiol Dis* **36:** 509-520

4. Wagner KM, Ruegg M, Niemann A, Suter U (2009) Targeting and function of the mitochondrial fission factor GDAP1 are dependent on its tail-anchor. *PLoS One* **4:** e5160