**Figure legends**

**Figure 1: Miro1 and Miro2 are localised to peroxisomes.** A) Representative zooms of WT MEFs transfected with GFP-tagged Miro1 and Miro2. GFPTom70(1-70) is a GFP tagged version of the first 70 amino acids of Tom70. Tom20 (red) and catalase (magenta) stain mitochondria and peroxisomes, respectively. Merge is of Miro (GFP) and catalase (magenta) with colocalisation shown as white. B) Quantification of the extent of GFP signal on peroxisomes, comparing all constructs. C) Pulldown of overexpressedPex19 in Cos7 cells with a Pex19 antibody shows interaction with full-length Miro1, but not Miro1 lacking the transmembrane domain. One-way ANOVA with Tukey post hoc test was used for all comparisons (*n* = 18 cells per condition over 3 independent experiments. \*\*\* denote *p* < 0.001 in comparison to GFPTom70(1-70). Scale bar is 5 μm.

**Figure 2: The first GTPase domain of Miro1 negatively regulates the extent of peroxisomal localisation.** A) Schematic of Miro1 truncation mutants used in B-D. B) Representative images of Miro1 truncation constructs in DKO MEFs. Mitochondria and peroxisomes are stained with Tom20 and catalase, respectively. C) Quantification of the extent of peroxisomal localisation of the Miro1 truncation constructs. D) Co-immunoprecipation of Miro1 truncation constructs and mycPex19 following transfection into Cos7 cells. GFP-tagged Miro1 truncation constructs were pulled down with GFP-Trap agarose beads and Pex19 was probed with myc antibody. One-way ANOVA was used to quantify difference in C (*n* = 18 cells over three independent experiments \*\*\* represents *p* < 0.001 in comparison to GFPTom70(1-70). ### is *p* < 0.001 in comparison to GFPMiro1. Scale bar is 5 μm.

**Figure 3: Loss of Miro1/2 does not affect saltatory peroxisomal trafficking or peroxisomal distribution**. A) Six consecutive frames of live trafficking of peroxisomes (pxDsRed signal at 1.5 seconds per frame). Yellow arrows show the trajectory of a fast moving peroxisome in both WT and DKO MEFs. Scale bar is 5 μm. WT and DKO MEFs were blind scored using pxDsRed signal (*n* = 23 for WT and 21 for DKO over three independent experiments). B) Representative images of WT and DKO MEFs seeded onto Y-shaped fibronectin micropatterns stained for mitochondria (Tom20 in red) and peroxisomes (Catalase in white). C) Normalised cumulative distribution curves comparing peroxisome subcellular distribution from the centre to the periphery of WT and DKO MEFs. D) Bar graph comparing the average distance at which 95% of the peroxisomal signal is distributed (Perox95). B), C) and D) is *n* = 60 cells over three independent experiments. Two-tailed Student’s t-test was used to test for statistical significance; *ns* = not significant.

**Figure 4: Miro regulates peroxisome oscillatory motility and peroxisome size**. A) Snapshots and tracks of pxDsRed signal in WT and DKO MEFs following live imaging at 1.5 seconds per frame for two minutes. B) Quantification of median net displacement of individual peroxisomes (pxDsRed signal) for WT and DKO MEFs (*n* = 23 cells for WT and 21 cells for DKO over three independent experiments). C) Representative images of catalase stained (peroxisomes) WT and DKO MEFs. D) Bar graph comparing average peroxisome area of WT and DKO MEFs (*n* = 60 cells over three independent experiments). E) Representative images of GFPTom70(1-70) and GFPMiro1 in WT MEFs stained with catalase (peroxisomes). F) Bar graph comparing average peroxisome area of GFPTom70(1-70) and GFPMiro1 overexpressing WT MEFs (*n* = 60 cells over three independent experiments). Statistical test used on B), D) and F) was a two-tailed Student’s t test \*, \*\* and \*\*\* denotes *p* < 0.05, *p* < 0.01 and *p* < 0.001, respectively. Scale bar is 10 μm. Scale bar in zooms is 5 μm.

**Supplementary figure legends**

**Supplementary figure 1: Miro is localised to peroxisomes in Cos7 cells**. A) Representative image of Cos7 cells transfected with GFPTom70(1-70), GFPMiro1 and GFPEF1-EF2-GTP2 truncation construct of Miro1. Mitochondria and peroxisomes are stained with Tom20 (red) and Catalase (magenta), respectively. B) mycMiro1 localises to Pex14 positive structures (peroxisomes) in WT MEFs. White box denotes the area for the zoomed image. Scale bar is 10 μm and 5 μm in zooms.

**Supplementary figure 2: Peroxisomal trafficking in WT and DKO MEFs following disruption of microtubules.** A) Snapshots and tracks of representative movies of peroxisome motility (pxDsRed) of untreated and vinblastine treated (1 μM for one hour) WT and DKO MEFs. Movies were acquired at 1.5 seconds per frame for two minutes. B) Blind scoring of long-ranged, fast-moving peroxisomes events per cell over a two minute movie. C) Median net displacement of peroxisomes over a two minute period. B) and C) UT=Untreated and Vin=Vinblastine treated. Two-way ANOVA was used to test for significance; \*\*\* denotes *p* < 0.001.

**Supplementary figure 3: Sholl-like organelle distribution analysis.** A) Shape of fibronectin micropattern and representation of Sholl-like analysis. B) Analysis of the distance at which 50% of peroxisomes are distributed (Perox50) in WT and DKO MEFs. C) Normalised cumulative distribution of mitochondria in WT and DKO MEFs (n=40 cells over three independent experiments). D) Distance at which 95% of mitochondria are distributed (Mito95) for WT and DKO MEFs. Statistical test used on B), and D) was a two-tailed Student’s t test \*\*\* denotes *p* < 0.001.

**Supplementary figure 4: Overexpression of Miro does not affect peroxisome distribution.** A) Representative images of overexpression of Miro constructs in WT MEFs stained for catalase (peroxisomes). B) and C) Quantification of the distance at which 50% (Perox50)and 95%(Perox95)of peroxisomes are distributed, respectively. D) Normalised cumulative distribution of peroxisomes for the conditions in A). Two-way ANOVA was used to test for significance. N = 60 cells over three independent experiements for each condition.

**Supplementary movie 1: Representative movie of peroxisomal trafficking in WT MEFs**. Peroxisomal trafficking by imaging pxDsRed at 1.5 seconds per frame for two minutes.

**Supplementary movie 2: Representative movie of peroxisomal trafficking in DKO MEFs**. Peroxisomal trafficking by imaging pxDsRed at 1.5 seconds per frame for two minutes.

**Supplementary movie 3: Example movie of long-ranged peroxisomal trafficking in WT MEFs**. Zoom of peroxisomal trafficking by imaging pxDsRed at 1.5 seconds per frame for two minutes.

**Supplementary movie 4: Example movie of long-ranged peroxisomal trafficking in DKO MEFs**. Zoom of peroxisomal trafficking by imaging pxDsRed at 1.5 seconds per frame for two minutes.

**Supplementary movie 5: Representative movie of peroxisomal trafficking in vinblastine treated WT MEFs**. Peroxisomal trafficking by imaging pxDsRed at 1.5 seconds per frame for two minutes. Cells were treated with 1 μM vinblastine for 1 hour before imaging.

**Supplementary movie 6: Representative movie of peroxisomal trafficking in vinblastine treated DKO MEFs**. Peroxisomal trafficking by imaging pxDsRed at 1.5 seconds per frame for two minutes. Cells were treated with 1 μM vinblastine for 1 hour before imaging.