Membrane Palmitoylated Protein 1 (MPP1) acts as a tuning factor regulating phase separation abilities of Giant Plasma Membrane-derived Vesicles.

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Supporting material

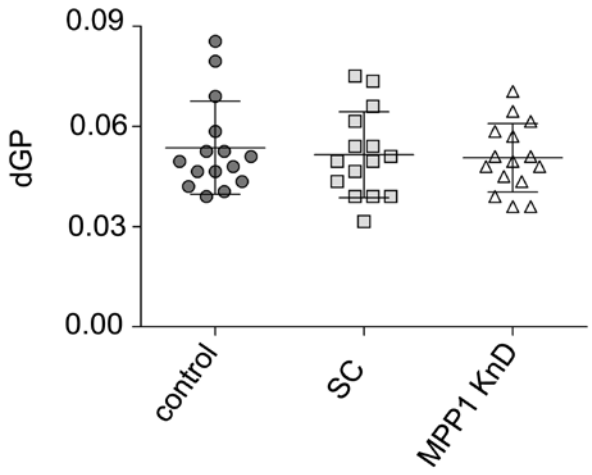

Fig. S1. ΔGP is constant for all isolated GPMVs. Quantitative analysis of ΔGP distribution in GPMVs obtained from MPP1-KnD and control cells shows no significant difference in the absence of MPP1. Each point is representative of >40 vesicles per cell-type.

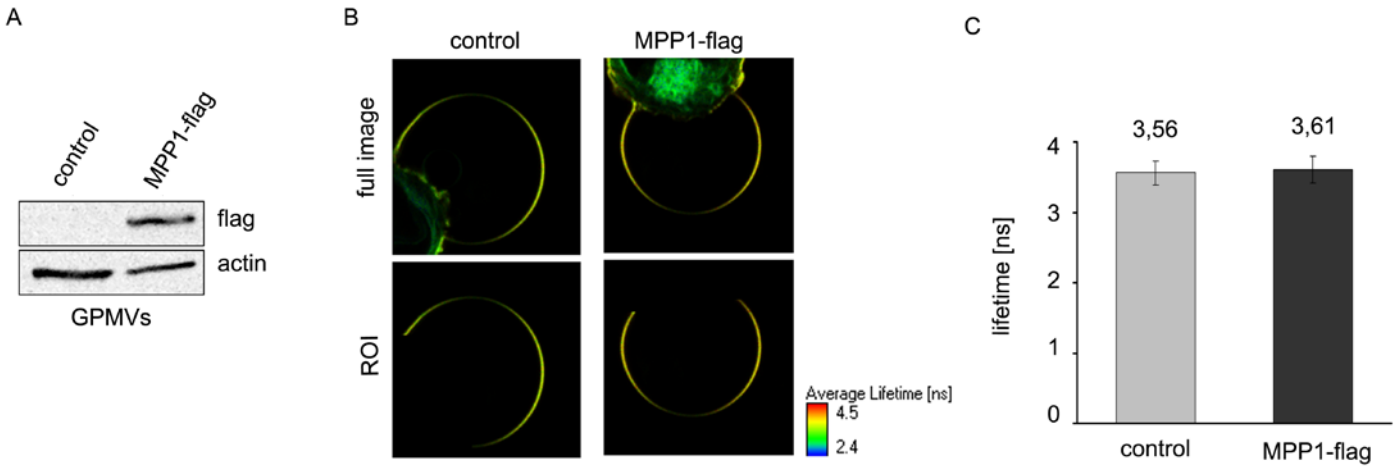

Fig. S2. MPP1 overexpression does not increase membrane order of GPMVs isolated from HEL cells. (A) Western Blot analysis of MPP1-flag expression level in GPMVs derived from HEL cells. **(B)** Representative FLIM images of di-4 stained GPMVs isolated from control and MPP1-flag transfected cells (the longer the fluorescence lifetime values, the more ordered the membrane; all images taken at 23°C). **(C)** Mean fluorescence-lifetime values averaged of >15 vesicles per cell type. ROI – region of interest.

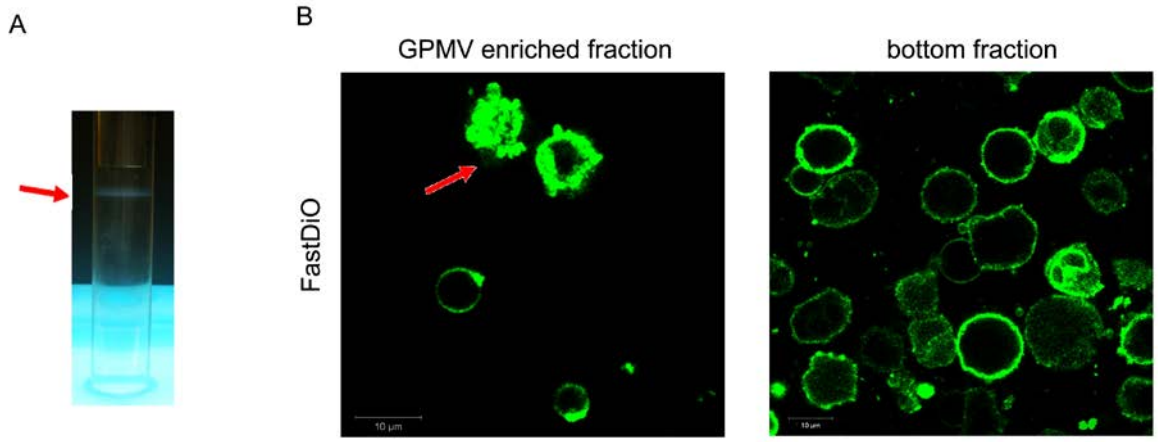

Fig. S3. GPMV enriched fraction contains also other membranes. (A) GPMV enriched fraction after gradient separation. **(B)** Representative images of Fast-DiO stained membranes. Within GPMV-enriched fraction cell debris are detected. Bottom fraction contains predominantly cells but some of retaining GPMVs are also observed.