Systematic comparison of strategies for the enrichment of lysosomes by data independent acquisition

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Supplementary references:
(1) Wyant, G. A.; Abu-Remaileh, M.; Frenkel, E. M.; Laqtom, N. N.; Dharamdasani, V.; Lewis, C. A.; Chan, S. H.; Heinze, I.; Ori, A.; Sabatini, D. M. NUFIP1 is a ribosome receptor for starvation-induced ribophagy. *Science* **2018**, *360*, 751–758.
(2) Hulsen, T.; Vlieg, J. de; Alkema, W. BioVenn - a web application for the comparison and visualization of biological lists using area-proportional Venn diagrams. *BMC genomics* **2008**, 9, 488.
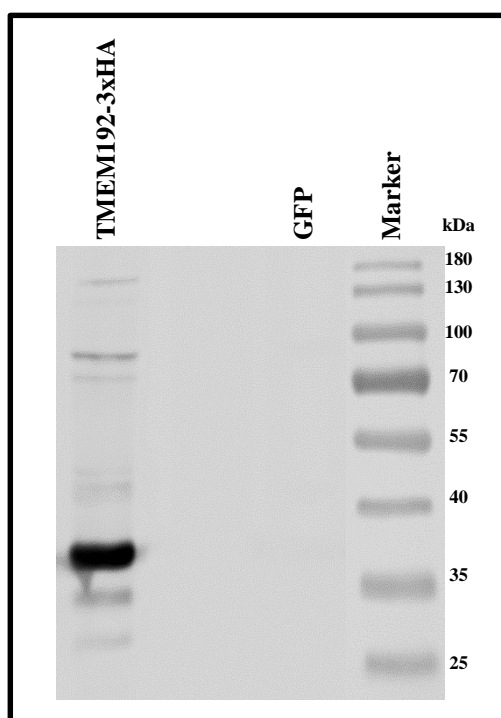
Figure S1: Western-blot verification for the stable expression of TMEM192-3xHA in HEK 293 cells. 20 µg of whole cell lysate for HEK 293 cells stably transfected with TMEM192-3xHA and HEK 293 cells transiently transfected with GFP were separated by a 10 % SDS-PAGE for 1.5 h at 120 V. Proteins separated on the gels were transferred to a nitrocellulose membrane using semi-dry electro blotter. The membrane was incubated in 5 % nonfat dry milk in tris buffer saline containing 0.05 % tween (TBS-T) for 1 h at RT. The membrane was incubated o/n at 4 °C with a primary anti HA antibody (1:2000) (self-made), washed 3x for 10 min at RT with TBS-T and incubated with a secondary goat anti mouse antibody (1:5000) (Millipore, AP180P) for 60 min at RT. Membrane was again washed 3x for 10 min at RT with TBS-T before developing the blot. Protein expression signal was detected using the enhanced chemiluminescence (ECL) kit (Bio-Rad Laboratories, Inc. (Hercules, CA, USA)), visualized with the FUSION SOLO 4M system, and illustrated/analyzed by the FusionCapt advance software.
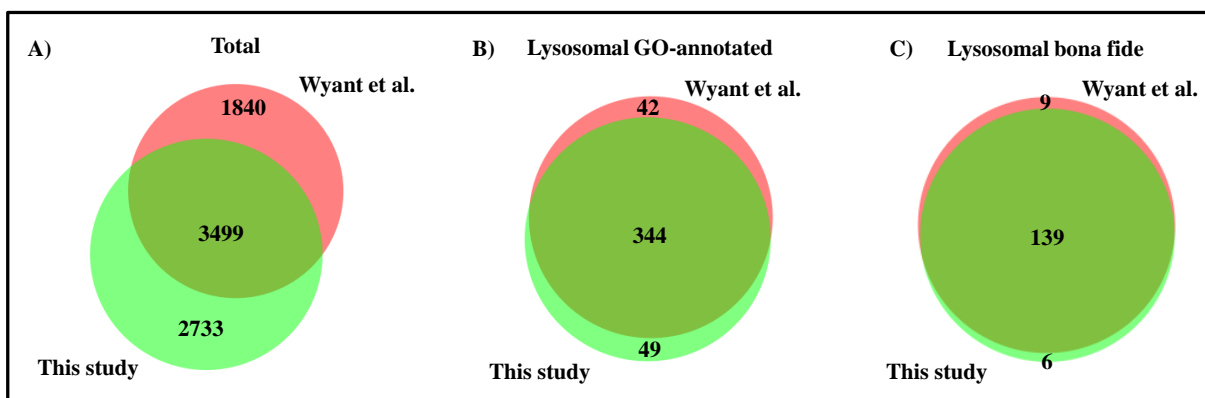
Figure S2: Comparison of TMEM-IP data with a published dataset. Comparison of data generated in this study from lysosomes isolated by TMEM-IP with a published dataset generated by Wyant et al.[1] for A) total B) lysosomal GO-annotated and C) lysosomal bona fide IDs. Venn diagram were generated with the BioVenn tool by Hulsen et al. 2008.[2]
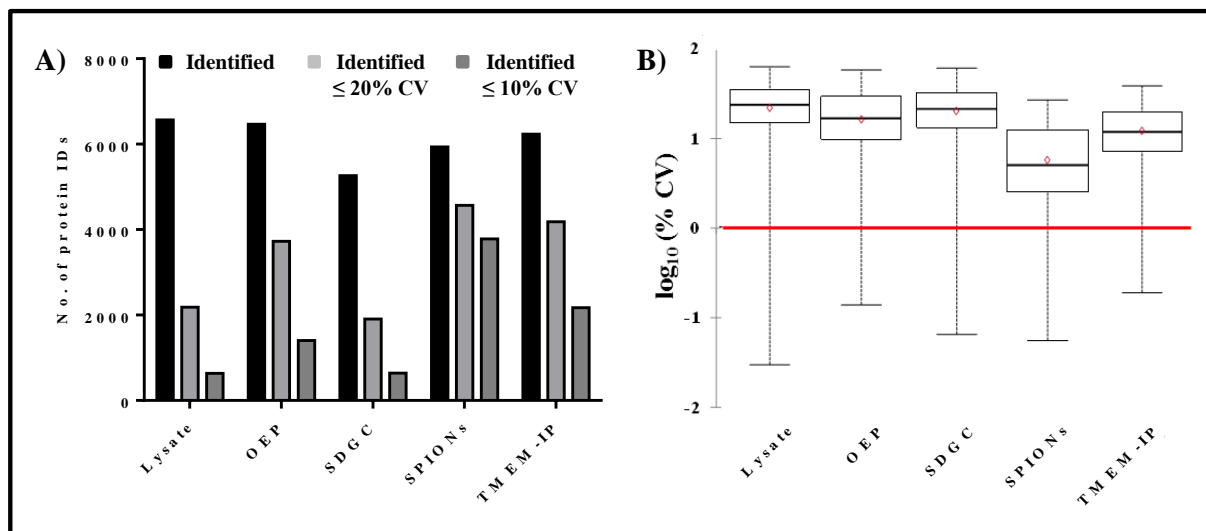
Figure S3: Variability of signal intensities for the individual datasets. Total numbers of identified proteins (black), proteins identified with a CV ≤ 20% (light grey) and a CV ≤ 10% (dark grey) for the individual datasets. B) Average CVs for the datasets of the individual methods. Lysate: whole cell lysate; OEP: organelle enriched pellet; SDGC: sucrose density gradient centrifugation; SPIONs: superparamagnetic iron oxide nanoparticles; TMEM-IP: TMEM192-3xHA immunoprecipitation.

Figure S4: Summed abundances of 70 bona fide lysosomal proteins detected in all samples. Shown are the average values (n=3) plus standard deviation for the summed abundance of signal intensities of bona fide lysosomal proteins for the individual samples. Only proteins which were quantified in each of the conditions were used for this analysis. Lysate: whole cell lysate; OEP: organelle enriched pellet; SDGC: sucrose density gradient centrifugation; SPIONs: superparamagnetic iron oxide nanoparticles; TMEM-IP: TMEM192-3xHA immunoprecipitation.
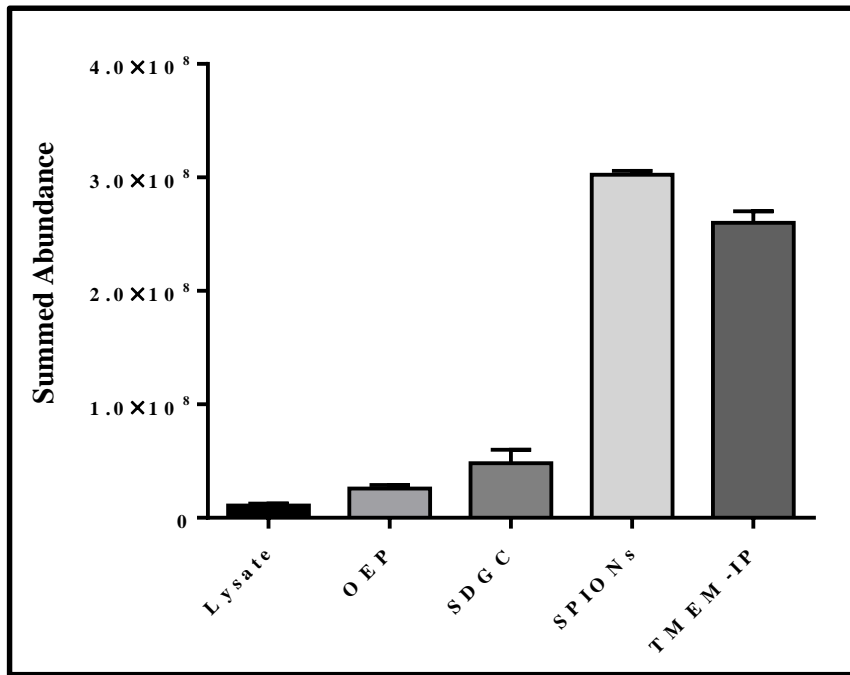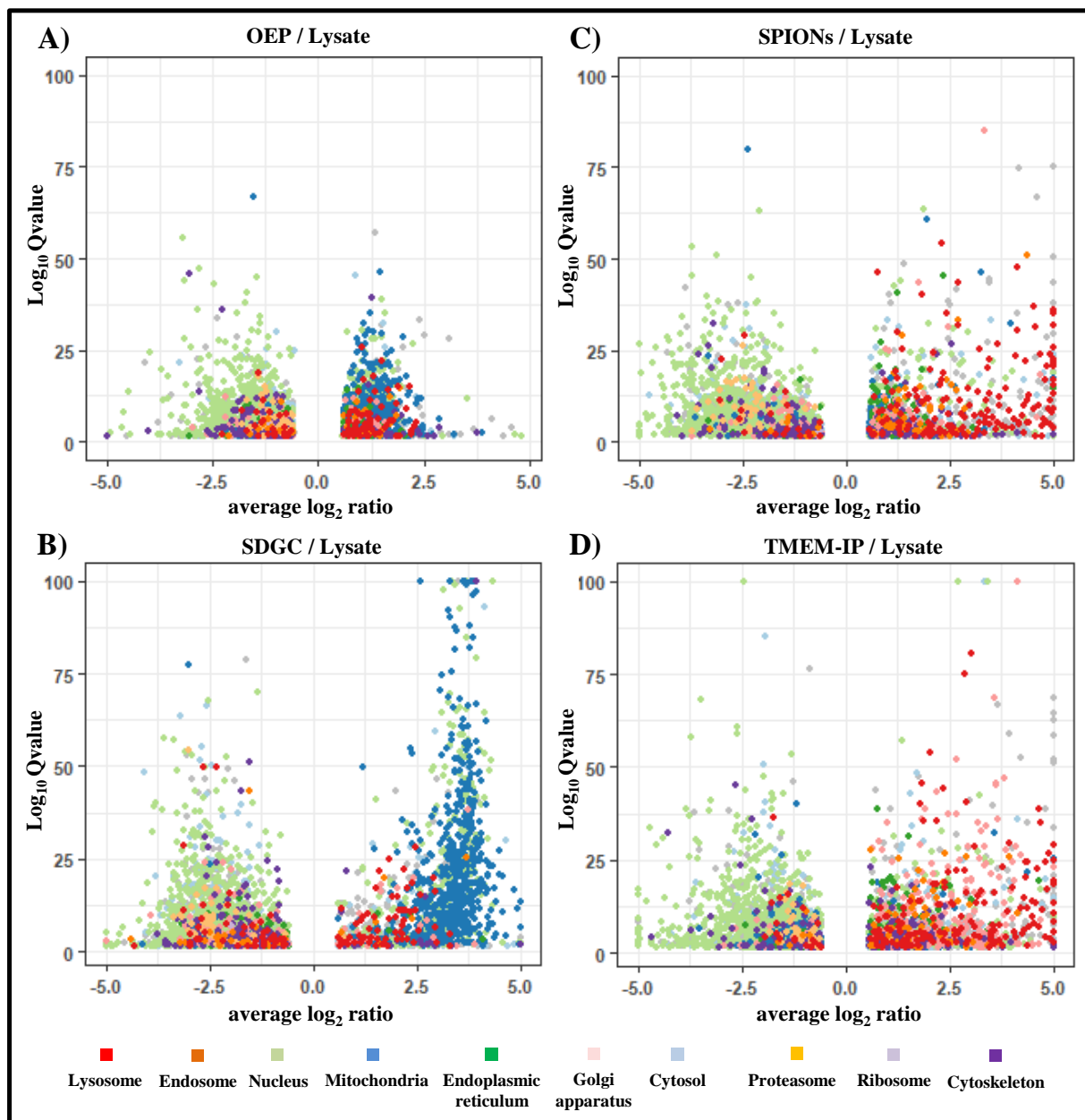
Figure S5: Enrichment factors for individual proteins detected with the four methods of lysosome enrichment relative to the whole cell lysate. Shown are the fold-change ratios of proteins passing the threshold for significant regulation with a q-value (multiple tested corrected p-value) > 0.05 and an absolute $\log_2$ ratio > 0.58 for A) OEP, B) SDGC, C) SPIONs and D) TMEM-IP. Candidates were classified based on their GO subcellular localization annotation among to the following categories: lysosome, endosome, nucleus, mitochondria, endoplasmic reticulum, Golgi apparatus, cytosol, proteasome, ribosome and cytoskeleton. OEP: organelle enriched pellet; SDGC: sucrose density gradient centrifugation; SPIONs: superparamagnetic iron oxide nanoparticles; TMEM-IP: TMEM192-3xHA immunoprecipitation.
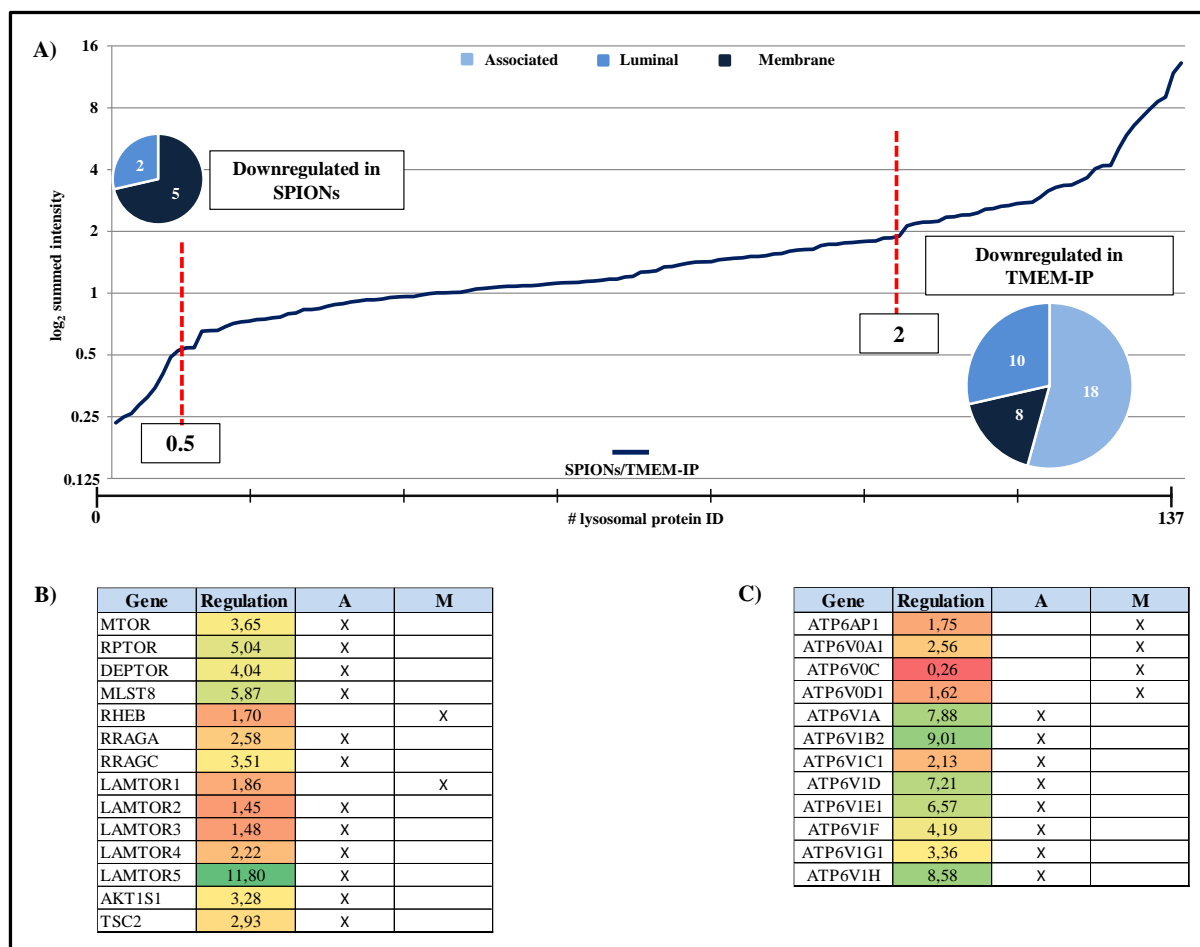
Figure S6: Variability in protein abundance between SPIONs and TMEM-IP. A) Abundance ratio values (log2) calculated for the summed intensities of bona fide lysosomal proteins (Table S3) quantified in the SPIONs and the TMEM-IP samples. Based on a 2-fold change cut off, 7 proteins were up- and 35 proteins downregulated in the TMEM-IP relative to SPIONs. The proteins were grouped based on their properties classifying them in: lysosomal associated, luminal or membrane bound. The majority of downregulated proteins consisted of members of the mTORC1 complex (B) and the vATPase complex (C). For the mTORC1 and vATPase complex, proteins were further classified in such being lysosomal membrane associated (A) or membrane bound (M) (Table S5).

B)

Gene	Regulation	A	M
MTOR	3,65	X	
RPTOR	5,04	X	
DEPTOR	4,04	X	
MLST8	5,87	X	
RHEB	1,70		X
RRAGA	2,58	X	
RRAGC	3,51	X	
LAMTOR1	1,86		X
LAMTOR2	1,45	X	
LAMTOR3	1,48	X	
LAMTOR4	2,22	X	
LAMTOR5	11,80	X	
AKT1S1	3,28	X	
TSC2	2,93	X	

C)

Gene	Regulation	A	M
ATP6AP1	1,75		X
ATP6V0A1	2,56		X
ATP6V0C	0,26		X
ATP6V0D1	1,62		X
ATP6V1A	7,88	X	
ATP6V1B2	9,01	X	
ATP6V1C1	2,13	X	
ATP6V1D	7,21	X	
ATP6V1E1	6,57	X	
ATP6V1F	4,19	X	
ATP6V1G1	3,36	X	
ATP6V1H	8,58	X	