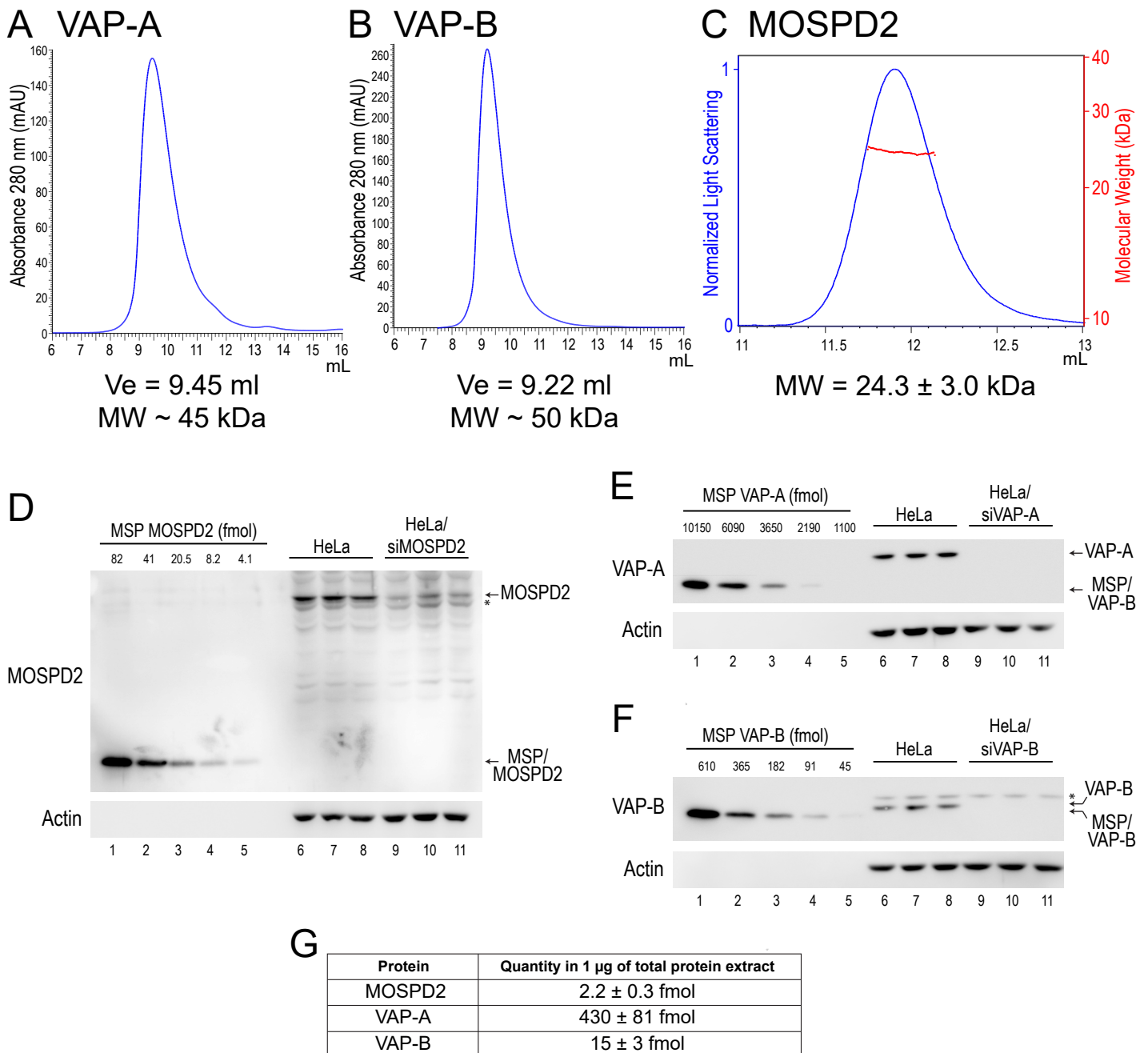


APPENDIX

Table of contents

Appendix Figure S1: The recombinant MSP domain of MOSPD2 is monomeric, while recombinant VAP-A and VAP-B are dimeric.	2
Appendix Figure S2: MOSPD2 recruitment to inter-organelle contact sites does not require VAP proteins	3
Appendix Figure S3: MOSPD2 modulates inter-organelle contacts	4
Appendix Table S1: Proteins co-precipitated with GFP-MOSPD2 and not with GFP-MOSPD2 RD/LD	5-6

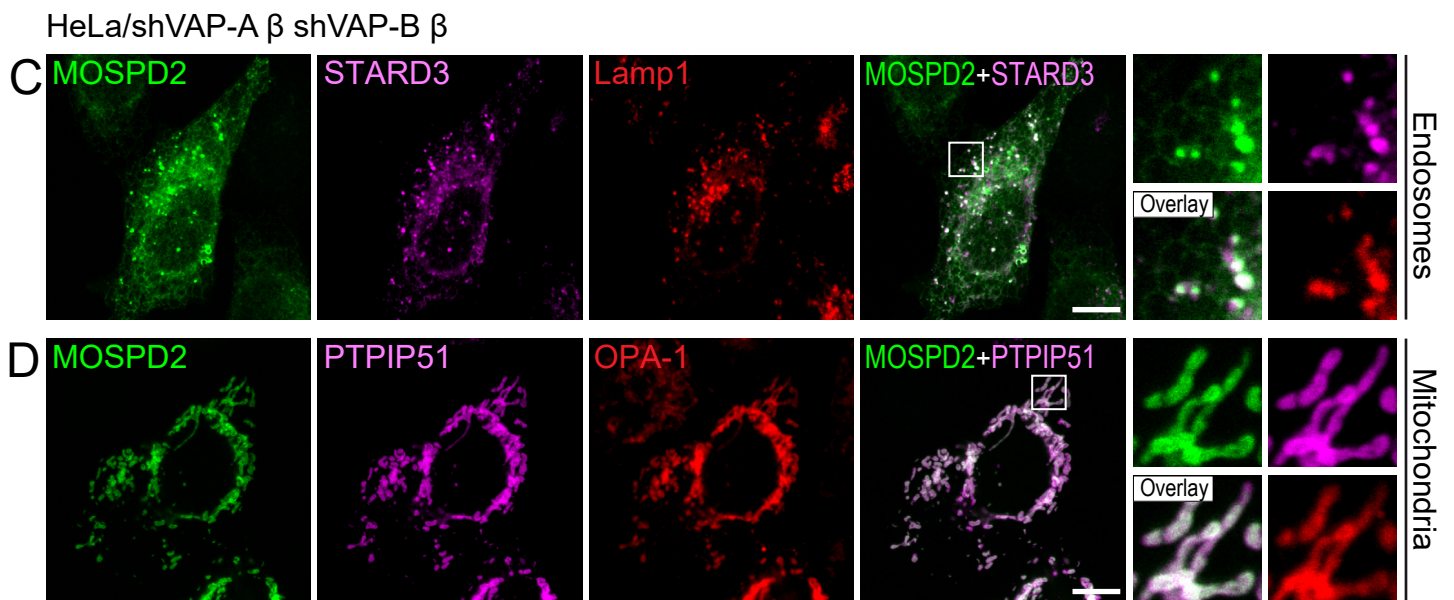
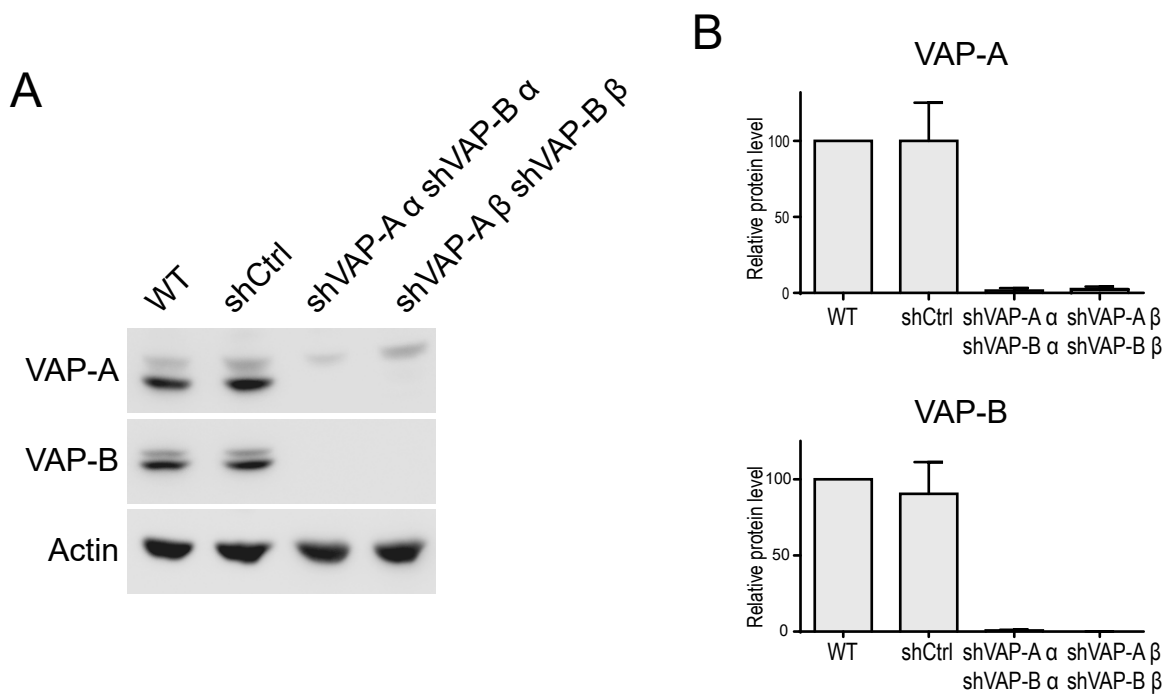


Appendix Figure S1: The recombinant MSP domain of MOSPD2 is monomeric, while recombinant VAP-A and VAP-B are dimeric.

A, B: Gel filtration profiles of recombinant VAP-A (A) and VAP-B (B) proteins. The proteins were loaded on a Superdex 75 10/300 GL column equilibrated with 20 mM Tris pH7.5, 150 mM NaCl. The column was calibrated with a mixture of bovine serum albumin, ovalbumin and ribonuclease (data not shown). The apparent molecular weights of VAP-A (theoretical MW: 24.6 kDa) and VAP-B (theoretical MW: 24.6 kDa) correspond to dimeric forms of the proteins.

C: Size Exclusion Chromatography with Multi-Angle Light Scattering (SEC-MALS) analysis of MOSPD2. The protein was loaded on a Superdex 75 10/300 GL column equilibrated with 20 mM Tris pH7.5, 150 mM NaCl, coupled to a MALS system (Wyatt technology). The MW detected corresponds to a monomer of MSP domain of MOSPD2 (theoretical MW: 24.2 kDa).

D-G: Quantification of MOSPD2, VAP-A and VAP-B protein levels in HeLa cells. Dilution series of recombinant MSP domains of MOSPD2 (D, lanes 1-5), VAP-A (E, lanes 1-5), and VAP-B (F, lanes 1-5) were analyzed by Western blot, together with protein extracts from HeLa cells (triplicate; lanes 6-8) and HeLa cells treated with siRNAs targeting MOSPD2 (D; triplicate, lanes 9-11), VAP-A (E; triplicate, lanes 9-11), and VAP-B (F; triplicate, lanes 9-11) as negative controls. Quantity of total protein extracts loaded: D: 13 μ g; E: 10 μ g and F: 10 μ g. Actin detection is shown as loading control. Chemiluminescence signals were quantified; standard curves established with recombinant proteins dilutions allowed the determination of MOSPD2, VAP-A and VAP-B quantities in protein extracts from HeLa cells (G). Experiments performed in triplicate.

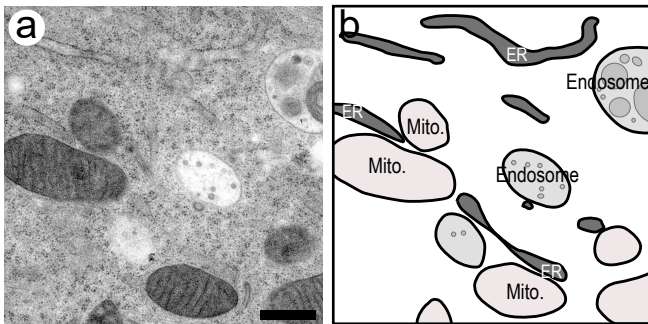
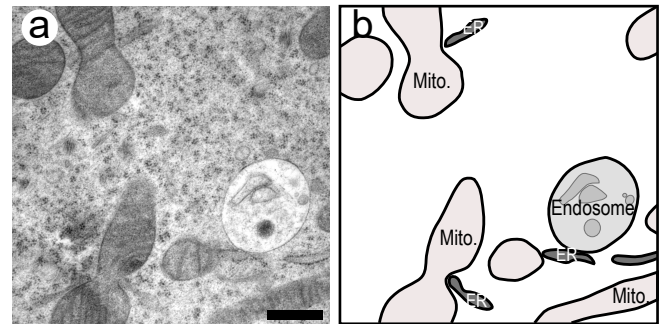
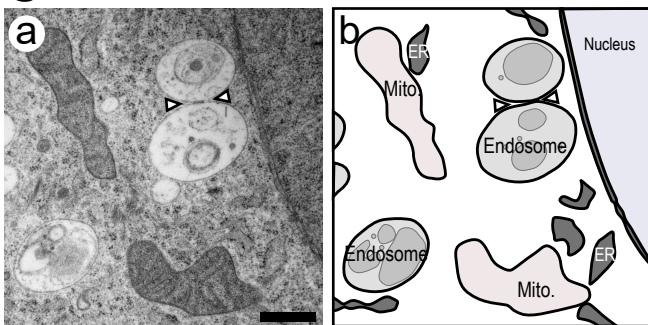
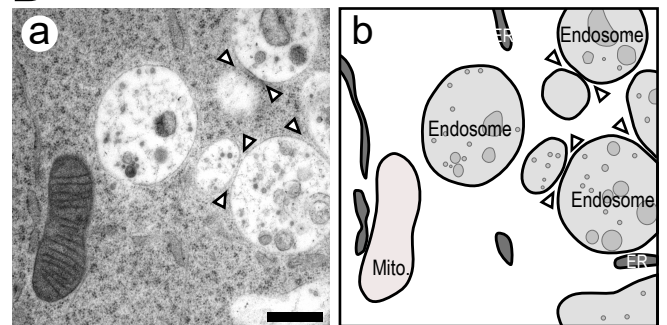


Appendix Figure S2: MOSPD2 recruitment to inter-organelle contact sites does not require VAP proteins

A: Western blot analysis of VAP protein level in control HeLa cells (WT) and in HeLa cells expressing a control shRNA (shCtrl) and two pairs of shRNAs targeting VAP-A and VAP-B (shVAP-A α /shVAP-B α or shVAP-A β /shVAP-B β).

B: Quantification of VAP-A and VAP-B protein levels by Western blot. Means and error bars (SD) are shown. n: three independent experiments.

C,D: GFP-MOSPD2 (green) was co-expressed with Flag-STARD3 and HA-PTPIP51 in HeLa/shVAP-A β shVAP-B β cells, and labelled using anti-Flag (C; magenta), anti-HA (D; magenta), and anti-Lamp1 (C; red) or OPA-1 (D; red) as markers of late endosomes/lysosomes and mitochondria, respectively. The subpanels on the right are higher magnification (3.5x) images of the area outlined in white. The Overlay panel shows merged green and magenta images. Scale bars: 10 μ m.

A HeLa**B** HeLa/shCtrl**C** HeLa/shMOSPD2- α **D** HeLa/shMOSPD2- β **Appendix Figure S3: MOSPD2 modulates inter-organelle contacts**

A-D: TEM images of control HeLa cells (A: HeLa; B: HeLa/shCtrl) and MOSPD2-silenced HeLa cells (C: HeLa/shMOSPD2- α ; D: HeLa/shMOSPD2- β) is shown on the left (a). An interpretation scheme representing contacts between organelles is shown on the right (b); the ER, endosomes and ILV are in dark, light and medium gray, respectively. Mitochondria are in pink. Scale bars: 500 μ m.

Appendix Table S1

Accession Number	Identity	Ratio
Q13509	Tubulin beta-3 chain OS=Homo sapiens GN=TUBB3 PE=1 SV=2 - [TBB3_HUMAN]	206,613333
Q14764	Major vault protein OS=Homo sapiens GN=MVP PE=1 SV=4 - [MVP_HUMAN]	96,1966667
P42166	Lamina-associated polypeptide 2, isoform alpha OS=Homo sapiens GN=TMPO PE=1 SV=2 - [LAP2A_HUMAN]	89,8
Q96A33	Coiled-coil domain-containing protein 47 OS=Homo sapiens GN=CCDC47 PE=1 SV=1 - [CCDC47_HUMAN]	70,2766667
Q9JHW5	GPN-loop GTPase 3 OS=Homo sapiens GN=GPN3 PE=1 SV=2 - [GPN3_HUMAN]	35,5766667
Q8TD16	Protein bicaudal D homolog 2 OS=Homo sapiens GN=BICD2 PE=1 SV=1 - [BICD2_HUMAN]	34,0666667
Q96R06	Sperm-associated antigen 5 OS=Homo sapiens GN=SPAG5 PE=1 SV=2 - [SPAG5_HUMAN]	31,4333333
P52597	Heterogeneous nuclear ribonucleoprotein F OS=Homo sapiens GN=HNRNPF PE=1 SV=3 - [HNRNPF_HUMAN]	26,92
P16989	Y-box-binding protein 3 OS=Homo sapiens GN=YBX3 PE=1 SV=4 - [YBOX3_HUMAN]	26,2366667
Q96TC7	Regulator of microtubule dynamics protein 3 OS=Homo sapiens GN=RMDN3 PE=1 SV=2 - [RMD3_HUMAN]	23,7266667
F8WJN3	Cleavage and polyadenylation-specificity factor subunit 6 OS=Homo sapiens GN=CPSP6 PE=1 SV=1 - [F8WJN3_HUMAN]	22,4066667
E9PDF6	Unconventional myosin-Ib OS=Homo sapiens GN=MYO1B PE=1 SV=1 - [E9PDF6_HUMAN]	21,7666667
P08670	Vimentin OS=Homo sapiens GN=VIM PE=1 SV=4 - [VIME_HUMAN]	19,84
Q9BXW6	Oxysterol-binding protein-related protein 1 OS=Homo sapiens GN=OSBPL1A PE=1 SV=2 - [OSBL1_HUMAN]	19,5566667
Q5JR95	40S ribosomal protein S8 OS=Homo sapiens GN=RPS8 PE=1 SV=1 - [Q5JR95_HUMAN]	18,26
P27694	Replication protein A 70 kDa DNA-binding subunit OS=Homo sapiens GN=RPA1 PE=1 SV=2 - [RFA1_HUMAN]	17,9233333
P42704	Leucine-rich PPR motif-containing protein, mitochondrial OS=Homo sapiens GN=LRPPRC PE=1 SV=3 - [LRPPRC_HUMAN]	17,5533333
P17706	Tyrosine-protein phosphatase non-receptor type 2 OS=Homo sapiens GN=PTN2 PE=1 SV=2 - [PTN2_HUMAN]	17,4366667
P40939	Trifunctional enzyme subunit alpha, mitochondrial OS=Homo sapiens GN=HADHA PE=1 SV=2 - [ECHA_HUMAN]	16,73
G3V576	Heterogeneous nuclear ribonucleoproteins C1/C2 OS=Homo sapiens GN=HNRNPC PE=1 SV=1 - [G3V576_HUMAN]	16,6266667
A0A087WXX9	Collagen alpha-1(V) chain OS=Homo sapiens GN=COL5A1 PE=1 SV=1 - [A0A087WXX9_HUMAN]	16,5733333
P35244	Replication protein A 14 kDa subunit OS=Homo sapiens GN=RPA3 PE=1 SV=1 - [RFA3_HUMAN]	14,7
P42167	Lamina-associated polypeptide 2, isoforms beta/gamma OS=Homo sapiens GN=TMPO PE=1 SV=2 - [LAP2B_HUMAN]	14,6366667
P20042	Eukaryotic translation initiation factor 2 subunit 2 OS=Homo sapiens GN=EIF2S2 PE=1 SV=2 - [IF2B_HUMAN]	14,5533333
V9GY01	Chromosome 15 open reading frame 23, isoform CRA_d OS=Homo sapiens GN=KNSTRN PE=1 SV=1 - [V9GY01_HUMAN]	14,3233333
Q15155	BET1 homolog OS=Homo sapiens GN=BET1 PE=1 SV=1 - [BET1_HUMAN]	13,3133333
P50991	T-complex protein 1 subunit delta OS=Homo sapiens GN=CCT4 PE=1 SV=4 - [TCPD_HUMAN]	13,1166667
FSRHW4	Erlin-2 (Fragment) OS=Homo sapiens GN=ERLIN2 PE=1 SV=1 - [FSRHW4_HUMAN]	12,93
X1WI28	60S ribosomal protein L10 (Fragment) OS=Homo sapiens GN=RPL10 PE=1 SV=7 - [X1WI28_HUMAN]	12,8133333
Q15545	Transcription initiation factor TFIID subunit 7 OS=Homo sapiens GN=TAF7 PE=1 SV=1 - [TAF7_HUMAN]	11,63
C9JPK5	MLN64 N-terminal domain homolog (Fragment) OS=Homo sapiens GN=STARD3NL PE=1 SV=1 - [C9JPK5_HUMAN]	11,3766667
Q13835	Plakophilin-1 OS=Homo sapiens GN=PKP1 PE=1 SV=2 - [PKP1_HUMAN]	11,1466667
Q8NC51	Plasminogen activator inhibitor 1 RNA-binding protein OS=Homo sapiens GN=SERBP1 PE=1 SV=2 - [PAIRB_HUMAN]	11,0933333
J3QR09	Ribosomal protein L19 OS=Homo sapiens GN=RPL19 PE=1 SV=1 - [J3QR09_HUMAN]	10,9966667
P54136	Arginine-tRNA ligase, cytoplasmic OS=Homo sapiens GN=RARS PE=1 SV=2 - [SYRC_HUMAN]	10,8
M0R0F0	40S ribosomal protein S5 (Fragment) OS=Homo sapiens GN=RPS5 PE=1 SV=1 - [M0R0F0_HUMAN]	10,57
Q96AG4	Leucine-rich repeat-containing protein 59 OS=Homo sapiens GN=LRRC59 PE=1 SV=1 - [LRC59_HUMAN]	10,5433333
J3QQQ9	Uncharacterized protein OS=Homo sapiens PE=4 SV=1 - [J3QQQ9_HUMAN]	10,5
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P62750	60S ribosomal protein L23a OS=Homo sapiens GN=RPL23A PE=1 SV=1 - [RL23A_HUMAN]	9,9633333
P62424	60S ribosomal protein L7a OS=Homo sapiens GN=RPL7A PE=1 SV=2 - [RL7A_HUMAN]	9,8366667
Q8WXX5	DnaJ homolog subfamily C member 9 OS=Homo sapiens GN=DNAJC9 PE=1 SV=1 - [DNJC9_HUMAN]	9,6166667
P09923	Intestinal-type alkaline phosphatase OS=Homo sapiens GN=ALPI PE=1 SV=2 - [PPBI_HUMAN]	9,54
A0A0D9SF54	Spectrin alpha chain, non-erythrocytic 1 OS=Homo sapiens GN=SPTAN1 PE=1 SV=1 - [A0A0D9SF54_HUMAN]	9,5066667
P22695	Cytochrome b-c1 complex subunit 2, mitochondrial OS=Homo sapiens GN=UQCRC2 PE=1 SV=3 - [QCR2_HUMAN]	9,28
E7ETK0	40S ribosomal protein S24 OS=Homo sapiens GN=RPS24 PE=1 SV=1 - [E7ETK0_HUMAN]	9,2366667
F8WAE5	Eukaryotic translation initiation factor 2A OS=Homo sapiens GN=EIF2A PE=1 SV=1 - [F8WAE5_HUMAN]	9,1866667
P33993	DNA replication licensing factor MCM7 OS=Homo sapiens GN=MCM7 PE=1 SV=4 - [MCM7_HUMAN]	9,0733333
Q15717	ELAV-like protein 1 OS=Homo sapiens GN=ELAVL1 PE=1 SV=2 - [ELAV1_HUMAN]	9,0733333
O43752	Syntaxin-6 OS=Homo sapiens GN=STX6 PE=1 SV=1 - [STX6_HUMAN]	9,0466667
P62829	60S ribosomal protein L23 OS=Homo sapiens GN=RPL23 PE=1 SV=1 - [RL23_HUMAN]	8,91
Q92841	Probable ATP-dependent RNA helicase DDX17 OS=Homo sapiens GN=DDX17 PE=1 SV=2 - [DDX17_HUMAN]	8,8466667
H0YEN5	40S ribosomal protein S2 (Fragment) OS=Homo sapiens GN=RPS2 PE=1 SV=1 - [H0YEN5_HUMAN]	8,6533333
P49848	Transcription initiation factor TFIID subunit 6 OS=Homo sapiens GN=TAF6 PE=1 SV=1 - [TAF6_HUMAN]	8,4866667
P15927	Replication protein A 32 kDa subunit OS=Homo sapiens GN=RPA2 PE=1 SV=1 - [RPA2_HUMAN]	8,14
Q15042	Rab3 GTPase-activating protein catalytic subunit OS=Homo sapiens GN=RAB3GAP1 PE=1 SV=3 - [RB3GP_HUMAN]	8,1066667
O60506	Heterogeneous nuclear ribonucleoprotein Q OS=Homo sapiens GN=SYNCRIP PE=1 SV=2 - [HNRPO_HUMAN]	8,1058558
B4DUR8	T-complex protein 1 subunit gamma OS=Homo sapiens GN=CCT3 PE=1 SV=1 - [B4DUR8_HUMAN]	8,0966667
O95573	Long-chain-fatty-acid-CoA ligase 3 OS=Homo sapiens GN=ACSL3 PE=1 SV=3 - [ACSL3_HUMAN]	8,0766667
P36578	60S ribosomal protein L4 OS=Homo sapiens GN=RPL4 PE=1 SV=5 - [RL4_HUMAN]	8,07
P60842	Eukaryotic initiation factor 4A-1 OS=Homo sapiens GN=EIF4A1 PE=1 SV=1 - [IF4A1_HUMAN]	8,0233333
A0A0D9SFB3	ATP-dependent RNA helicase DDX3X OS=Homo sapiens GN=DDX3X PE=1 SV=1 - [A0A0D9SFB3_HUMAN]	7,8555556
Q9BSD7	Cancer-related nucleoside-triphosphatase OS=Homo sapiens GN=NTPCR PE=1 SV=1 - [NTPCR_HUMAN]	7,8366667
A0A087WXM6	60S ribosomal protein L17 (Fragment) OS=Homo sapiens GN=RPL17 PE=3 SV=1 - [A0A087WXM6_HUMAN]	7,8033333
H7BY29	Mitochondrial amidoxime-reducing component 1 (Fragment) OS=Homo sapiens GN=MARC1 PE=1 SV=1 - [H7BY29_HUMAN]	7,73
G5E9W7	28S ribosomal protein S22, mitochondrial OS=Homo sapiens GN=MRPS22 PE=1 SV=1 - [G5E9W7_HUMAN]	7,5533333
P02545	Prelamin-A/C OS=Homo sapiens GN=LMNA PE=1 SV=1 - [LMNA_HUMAN]	7,50962038
Q08211	ATP-dependent RNA helicase A OS=Homo sapiens GN=DHX9 PE=1 SV=4 - [DHX9_HUMAN]	7,3966667
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P19338	Nucleolin OS=Homo sapiens GN=NCL PE=1 SV=3 - [NUCL_HUMAN]	7,0782347

Q96KK5	Histone H2A type 1-H OS=Homo sapiens GN=HIST1H2AH PE=1 SV=3 - [H2A1H_HUMAN]	7,0227577
Q96CS3	FAS-associated factor 2 OS=Homo sapiens GN=FAF2 PE=1 SV=2 - [FAF2_HUMAN]	6,92333333
B1AK87	Capping protein (Actin filament) muscle Z-line, beta, isoform CRA_a OS=Homo sapiens GN=CAPZB PE=1 SV=1 - [B1AK87_HUMAN]	6,91333333
F5GY55	DNA damage-binding protein 1 OS=Homo sapiens GN=DDB1 PE=1 SV=1 - [F5GY55_HUMAN]	6,83666667
P61026	Ras-related protein Rab-10 OS=Homo sapiens GN=RAB10 PE=1 SV=1 - [RAB10_HUMAN]	6,78
Q13200	26S proteasome non-ATPase regulatory subunit 2 OS=Homo sapiens GN=PSMD2 PE=1 SV=3 - [PSMD2_HUMAN]	6,78
P61353	60S ribosomal protein L27 OS=Homo sapiens GN=RPL27 PE=1 SV=2 - [RL27_HUMAN]	6,58333333
P09874	Poly [ADP-ribose] polymerase 1 OS=Homo sapiens GN=PARP1 PE=1 SV=4 - [PARP1_HUMAN]	6,57666667
Q7Z406	Myosin-14 OS=Homo sapiens GN=MYH14 PE=1 SV=2 - [MYH14_HUMAN]	6,45
P05141	ADP/ATP translocase 2 OS=Homo sapiens GN=SLC25A5 PE=1 SV=7 - [ADT2_HUMAN]	6,39215686
Q13049	E3 ubiquitin-protein ligase TRIM32 OS=Homo sapiens GN=TRIM32 PE=1 SV=2 - [TRI32_HUMAN]	6,35
Q8IVT2	Mitotic interactor and substrate of PLK1 OS=Homo sapiens GN=MISP PE=1 SV=1 - [MISP_HUMAN]	6,34333333
A0A0G2JRY5	Transcription initiation factor TFIID subunit 4 (Fragment) OS=Homo sapiens GN=TAF4 PE=1 SV=1 - [A0A0G2JRY5_HUMAN]	5,84
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Q9UJ50	Calcium-binding mitochondrial carrier protein Aralar2 OS=Homo sapiens GN=SLC25A13 PE=1 SV=2 - [CMC2_HUMAN]	5,69
P51570	Galactokinase OS=Homo sapiens GN=GALK1 PE=1 SV=1 - [GALK1_HUMAN]	5,64666667
Q07666	KH domain-containing, RNA-binding, signal transduction-associated protein 1 OS=Homo sapiens GN=KHDRBS1 PE=1 SV=1 - [KHDR1_HUMAN]	5,54
Q15526	Surfeit locus protein 1 OS=Homo sapiens GN=SURF1 PE=1 SV=1 - [SURF1_HUMAN]	5,47333333
P0CW18	Serine protease 56 OS=Homo sapiens GN=PRSS56 PE=1 SV=1 - [PRSS56_HUMAN]	5,42
Q15637	Splicing factor 1 OS=Homo sapiens GN=SF1 PE=1 SV=4 - [SF01_HUMAN]	5,4
Q13283	Ras GTPase-activating protein-binding protein 1 OS=Homo sapiens GN=G3BP1 PE=1 SV=1 - [G3BP1_HUMAN]	5,35547122
P55072	Transitional endoplasmic reticulum ATPase OS=Homo sapiens GN=VCP PE=1 SV=4 - [TERA_HUMAN]	5,34295416
I3L0K7	Heat shock protein 75 kDa, mitochondrial OS=Homo sapiens GN=TRAP1 PE=1 SV=1 - [I3L0K7_HUMAN]	5,3
F5GYQ2	Multifunctional methyltransferase subunit TRM112-like protein OS=Homo sapiens GN=TRMT112 PE=1 SV=1 - [F5GYQ2_HUMAN]	5,12
Q95249	Golgi SNAP receptor complex member 1 OS=Homo sapiens GN=GOSR1 PE=1 SV=1 - [GOSR1_HUMAN]	5,11378978
Q96IR7	4-hydroxyphenylpyruvate dioxygenase-like protein OS=Homo sapiens GN=HPDL PE=1 SV=1 - [HPDL_HUMAN]	5,07
P62701	40S ribosomal protein S4, X isoform OS=Homo sapiens GN=RPS4X PE=1 SV=2 - [RS4X_HUMAN]	5,06640927
H9KV75	Alpha-actinin-1 OS=Homo sapiens GN=ACTN1 PE=1 SV=1 - [H9KV75_HUMAN]	5,03
Q13885	Tubulin beta-2A chain OS=Homo sapiens GN=TUBB2A PE=1 SV=1 - [TBB2A_HUMAN]	5,0245483
Q9H2M9	Rab3 GTPase-activating protein non-catalytic subunit OS=Homo sapiens GN=RAB3GAP2 PE=1 SV=1 - [RBGPR_HUMAN]	4,97
P06899	Histone H2B type 1-J OS=Homo sapiens GN=HIST1H2BJ PE=1 SV=3 - [H2B1J_HUMAN]	4,93666667
C9JZ20	Prohibitin (Fragment) OS=Homo sapiens GN=PHB PE=1 SV=1 - [C9JZ20_HUMAN]	4,93666667
Q96I24	Far upstream element-binding protein 3 OS=Homo sapiens GN=FUBP3 PE=1 SV=2 - [FUBP3_HUMAN]	4,89333333
Q15046	Lysine--tRNA ligase OS=Homo sapiens GN=KARS PE=1 SV=3 - [SYK_HUMAN]	4,88666667
Q04837	Single-stranded DNA-binding protein, mitochondrial OS=Homo sapiens GN=SSBP1 PE=1 SV=1 - [SSBP_HUMAN]	4,87731629
HOYLY7	Calcineurin B homologous protein 1 (Fragment) OS=Homo sapiens GN=CHP1 PE=1 SV=1 - [HOYLY7_HUMAN]	4,86333333
Q14849	StAR-related lipid transfer protein 3 OS=Homo sapiens GN=STARD3 PE=1 SV=2 - [STAR3_HUMAN]	4,84

Proteins possessing an FFAT motif and shown to interact with VAP in the literature

Proteins investigated in our study

Proteins identified as MOSPD2 partner in databases (<https://thebiogrid.org/>)

Proteins identified as VAP-A or VAP-B partner in databases

Protein extracts from HeLa cells stably expressing GFP-MOSPD2 or GFP-MOSPD2 RD/LD were immunoprecipitated using a GFP-trap resin. Eluted protein were analyzed by tandem mass spectrometry. For the analysis, mass spectrometry scores were corrected by adding 1 to each score. Proteins were filtered for a ratio (Score Protein X in GFP-MOSPD2 sample)/(Score Protein X in GFP-MOSPD2 RD/LD sample)>4.84.