

APPENDIX

DPP8/9 processing of human AK2 unmask an IAP binding motif

Kim J. Lapacz^{1,#}, Konstantin Weiss^{1,#}, Franziska Mueller^{2,3,#}, Yuxing Xue³, Simon Poepfel^{4,5}, Matthias Weith¹, Tanja Bange^{3,*}, Jan Riemer^{1,5,*}

1, Institute for Biochemistry, University of Cologne, 50674 Cologne, Germany

2, Department of Mechanistic Cell Biology, Max Planck Institute of Molecular Physiology, 44227 Dortmund

3, Institute of Medical Psychology and Biomedical Center (BMC), Faculty of Medicine, LMU Munich, 80336 Munich, Germany

4, Center for Molecular Medicine Cologne (CMMC), Faculty of Medicine and University Hospital, University of Cologne, D-50931 Cologne, Germany.

5, Cologne Excellence Cluster on Cellular Stress Responses in Aging-Associated Diseases (CECAD), University of Cologne, 50931 Cologne, Germany.

#, these authors contributed equally

* address correspondence to

J.R.: jan.riemer@uni-koeln.de, +49-221-470-7306, ORCID 0000-0002-7574-8457

T.B.: tanja.bange@med.uni-muenchen.de, +49-89-218075654, ORCID 0000-0002-9680-8586

Condensed title: DPP8 and DPP9 unmask IAP binding motifs

TABLE OF CONTENT

APPENDIX FIGURES

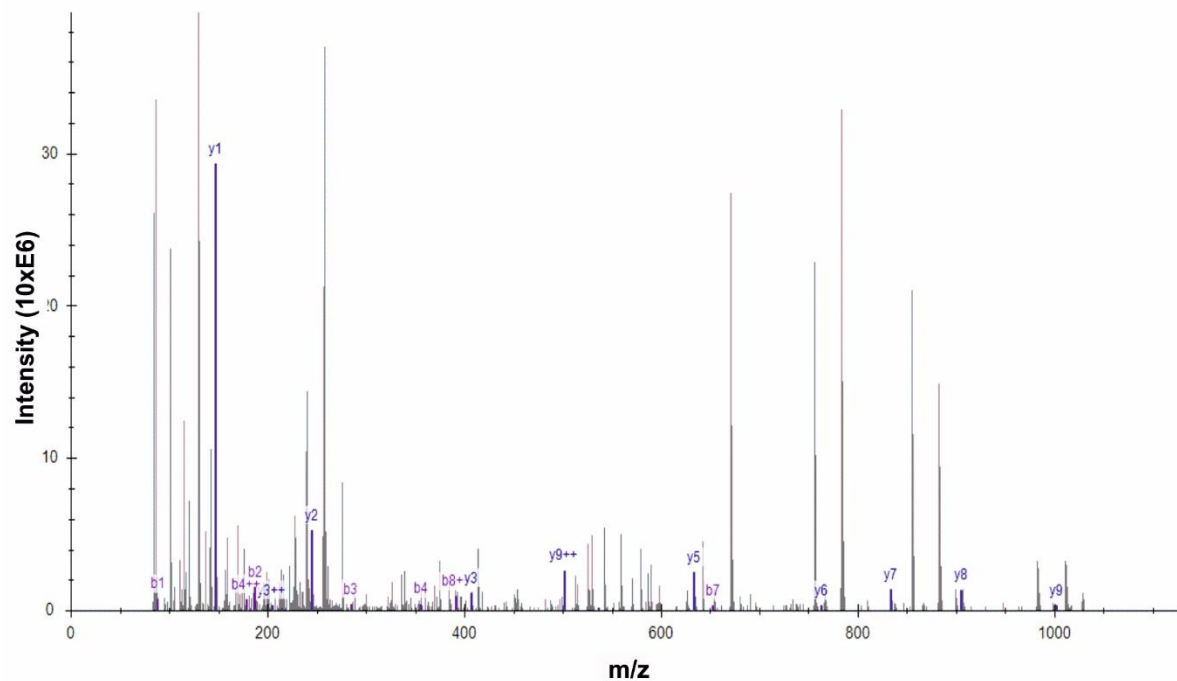
Appendix Figure S1	page 3
Appendix Figure S2	page 7
Appendix Figure S3	page 8
Appendix Figure S4	page 9
Appendix Figure S5	page 10
Appendix Figure S6	page 11
Appendix Figure S7	page 12
Appendix Figure S8	page 13

APPENDIX REFERENCES	page 18
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APPENDIX FIGURES

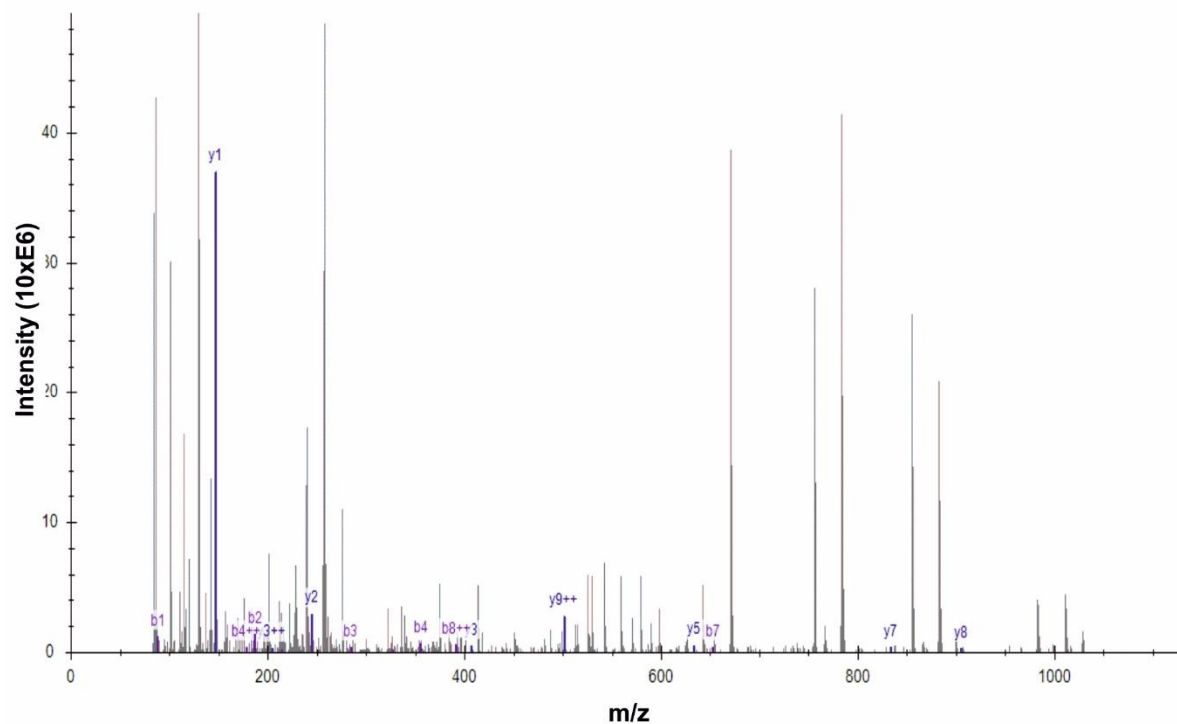
A

SVPAAEPEYPK₂⁺ (PEP 4.51E⁻⁰⁵) - DDP8/9 cleaved N-terminus in HEK WT
20240425_TB_SA_WTprot_2.raw (33.69 min)



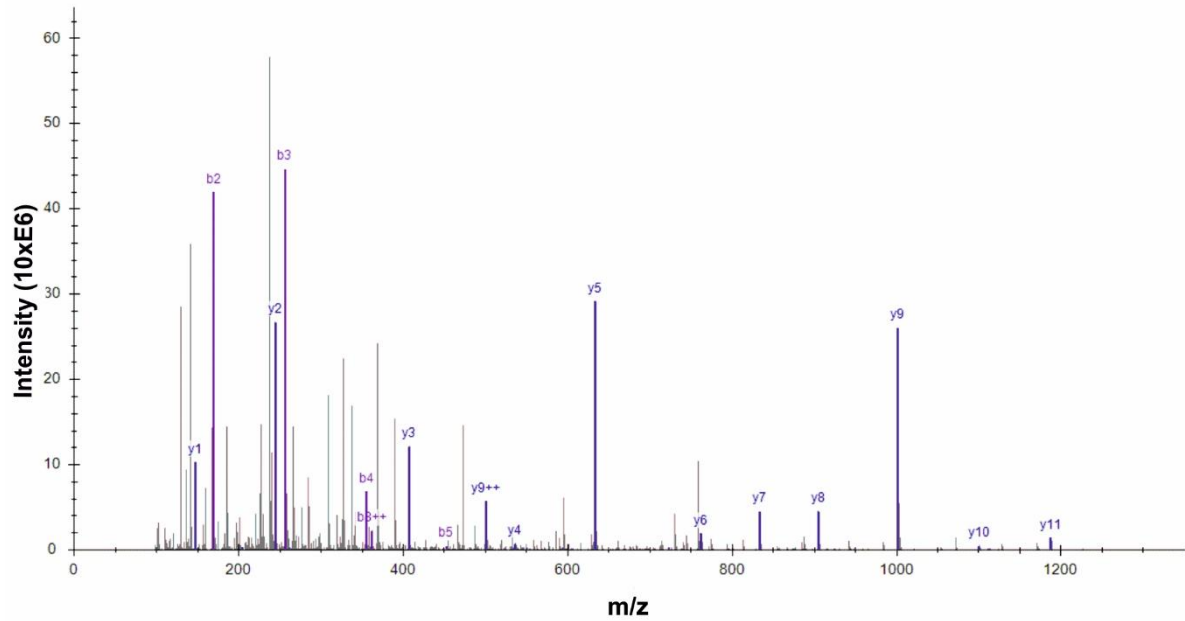
B

SVPAAEPEYPK₂⁺ (PEP 2.75E⁻⁰⁷) - DPP8/9 cleaved N-terminus in HEK DPP9 KO
2024_TB_SA_KOprot_2.raw (33,76min)

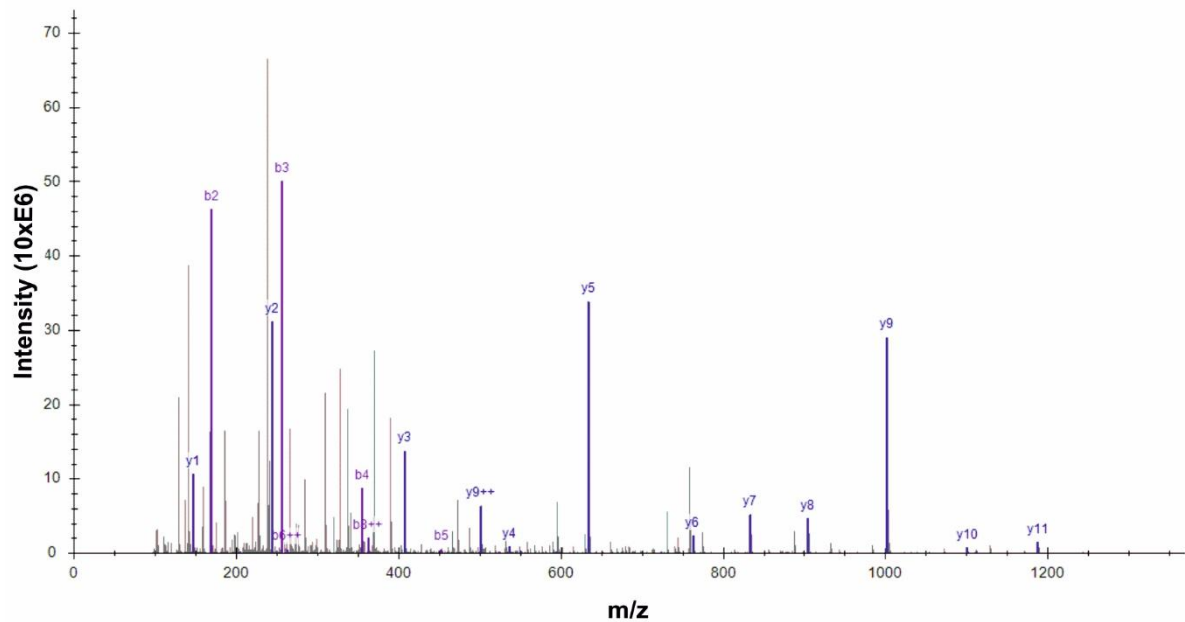


C

APSVPAAEPEYPK²⁺ (PEP 8.24E⁻⁰⁵) - MetAP cleaved N-terminus in HEK WT
20240425_TB_SA_WTprot_2.raw (41.75 min)

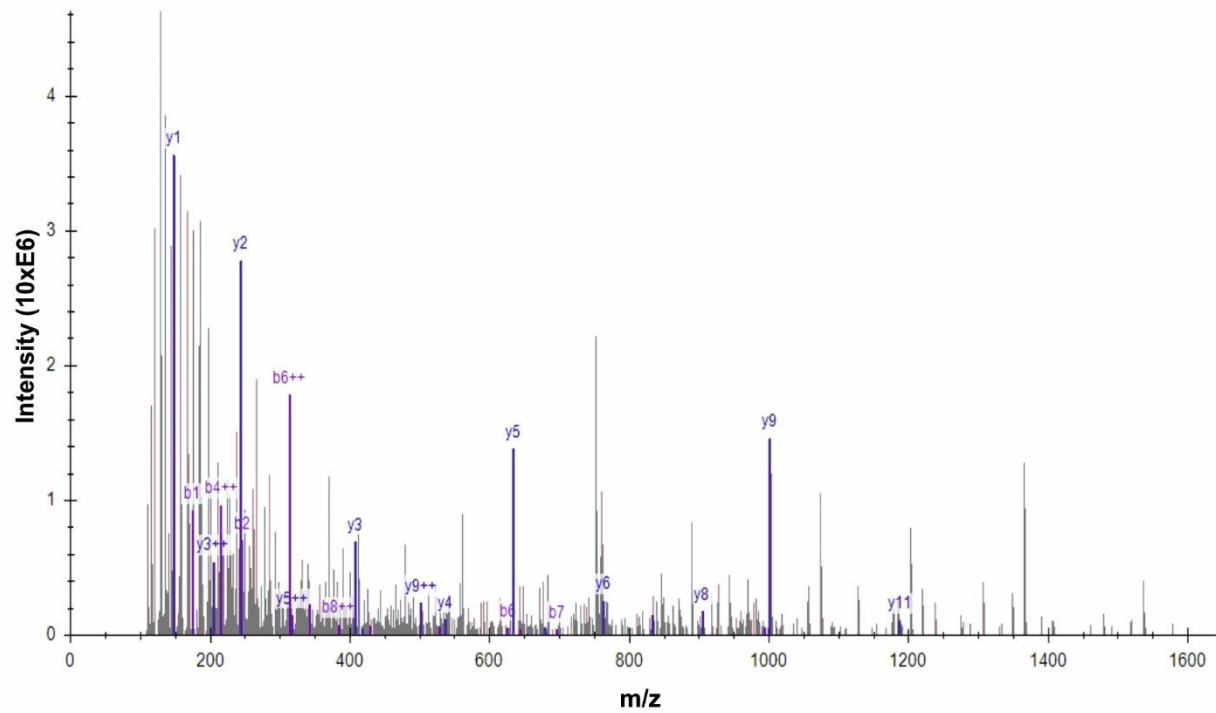
**D**

APSVPAAEPEYPK²⁺ (PEP 5.15E⁻⁰⁵) - MetAP cleaved N-terminus in HEK DPP9 KO
2024_TB_SA_KOprot_2.raw (41.58min)

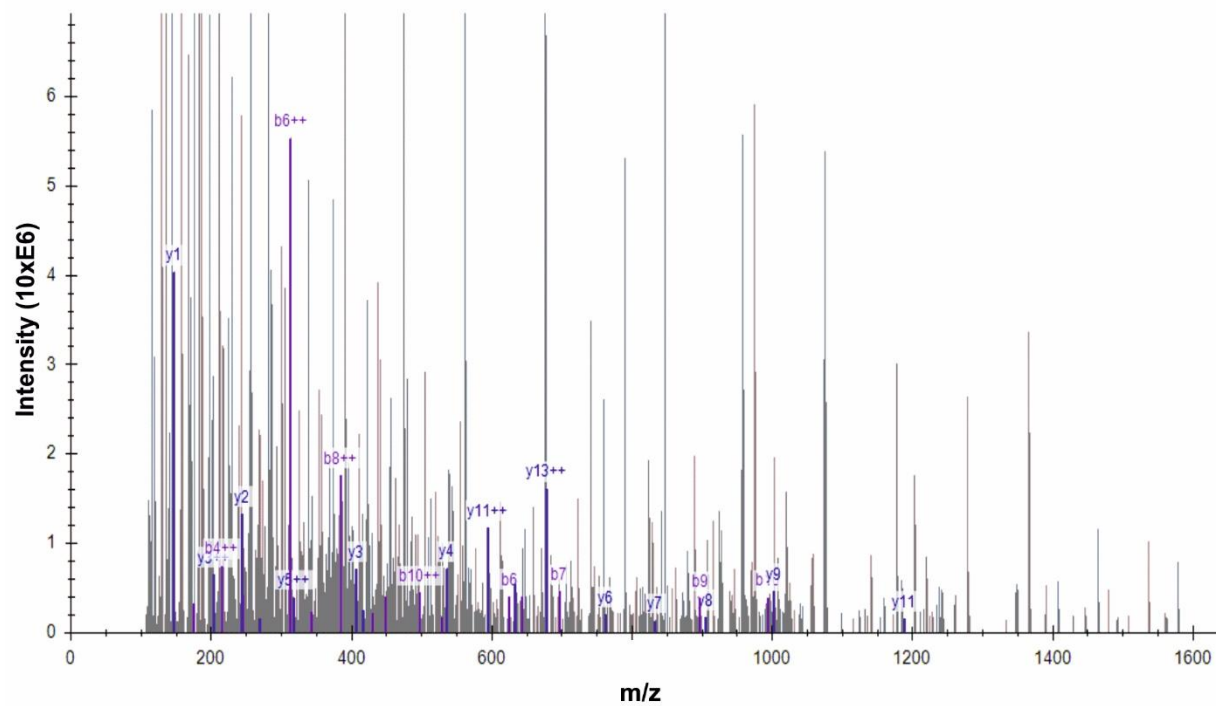


E

M(ac)APSVPAAEPEYPK₂⁺ (PEP 8.9E⁻⁰⁵) - unprocessed N-terminus in HEK WT
20240425_TB_SA_WTprot_3.raw (64.18 min)

**F**

M(ac)APSVPAAEPEYPK₂⁺ (PEP 0.0007) - unprocessed N-terminus in HEK DPP9 KO
2024_TB_SA_KOprot_3.raw (64.21min)

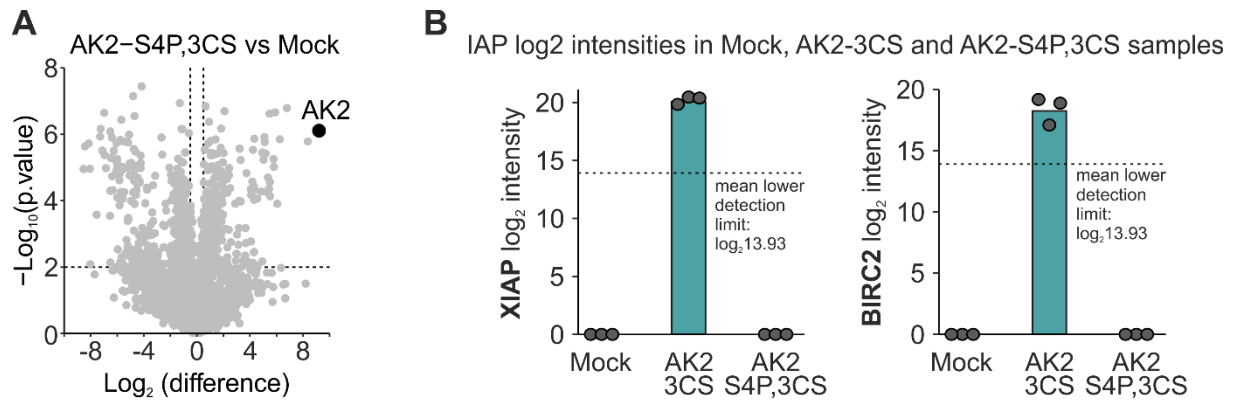


Appendix Figure S1 (related to Figure 1F). MS2 spectra of AK2 N-terminal peptides identified from whole cellular lysates of HEK293 WT and DPP9 KO cells.

A,B. AK2 N-terminal peptide NH₂-SVPAAEPEYPK in WT (A) and DPP9 KO (B) lysate.

C,D. AK2 N-terminal peptide NH₂-APSVPAEPEYPK in WT (C) and DPP9 KO (D) lysate.

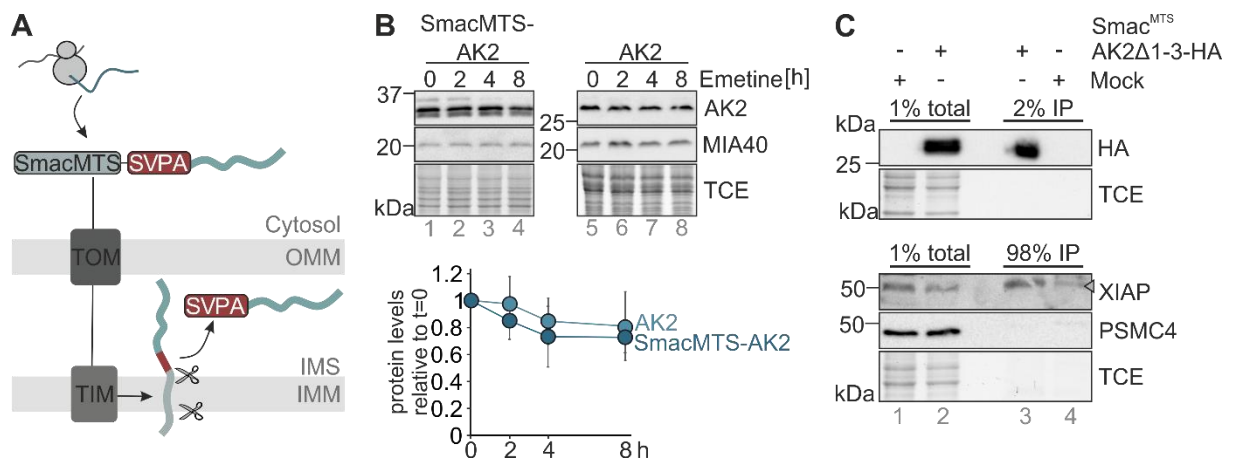
E,F. AK2 N-terminal peptide ac-NH₂-MAPSVPAEPEYPK in WT (E) and DPP9 KO (F) lysate.



Appendix Figure S3 (related to Figure 2M,N). The IAPs XIAP and BIRC2 can bind to AK2 but not to the S4P mutant of AK2.

A. Neither the terminal mutation of DPP8/9 processed AK2 (S4P) nor the mock control bind to XIAP and BIRC2. AK2 S4P, C40,42,92S-HA (S4P,3CS) and mock treated cells were immunoprecipitated and binding partners were quantified by MS. 2,623 proteins were quantified, number of biological replicates $n=3$. Data were imputed at the lower end of the distribution (downshift: 2; width:0.3). AK2 and IAPs are highlighted.

B. XIAP and BIRC2 were identified as interaction partners only in cytosolic WT AK2. Measured $\log_2$ intensities of coprecipitates of mock, AK2 C40,42,92S-HA (3CS) and AK2 S4P, C40,42,92S-HA (S4P,3CS) for XIAP and BIRC2 respectively ($n=3$ biological replicates). Data from **Fig. 2M,N** and **S3A** before data imputation. The lowest measured value for quantified proteins in this data set is inserted as a dashed line.

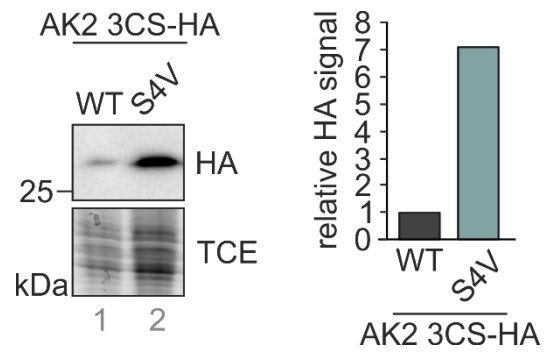


Appendix Figure S4 (related to Figure 2). The IAP XIAP can bind to AK2.

A. Smac^{MTS}-AK2 $\Delta 1-3$ is translated in the cytosol. It is then imported into mitochondria where the MTS is removed leaving soluble AK2 exposing its IBM.

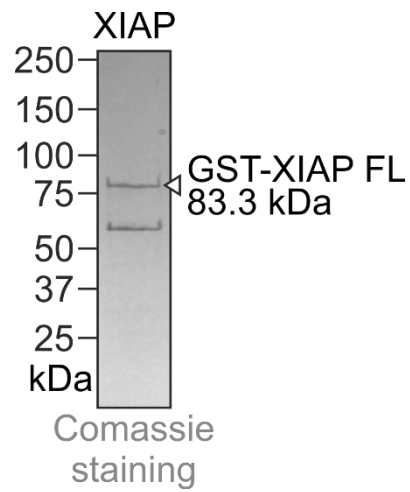
B. AK2 with and without Smac^{MTS} is stable in the IMS over time. Stable inducible cell lines expressing AK2 with or without the Smac^{MTS} were tested in an Emetine chase for the indicated amount of time. Samples were lysed and analyzed by SDS-PAGE and immunoblot. n=3 biological replicates.

C. The IAP XIAP can bind to AK2. Stable inducible HeLa cell lines in an AK2 KO background were lysed. Smac^{MTS}-AK2 $\Delta 1-3$ -HA was precipitated using HA-beads and the resulting precipitate was analysed for XIAP. XIAP was coprecipitated with AK2 indicating interaction of both proteins after cell lysis. Analyses was done by SDS-PAGE and immunoblot. n=3 biological replicates.



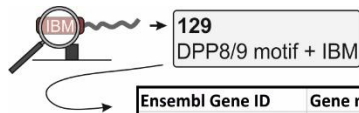
Appendix Figure S5 (related to Figure 3). Mutation of the AK2 IBM stabilizes the protein.

Stable inducible cell lines expressing either the WT or the S4V variant of AK2 were lysed and analyzed via SDS-PAGE and immunoblot. n=4 biological replicates; one replicate is shown, the three other replicates were used in (Finger *et al.*, 2020) for quantification.



Appendix Figure S6 (related to Figure 3H,I and 4J). Purification of human full-length GST-XIAP.

Analysis of GST-tagged XIAP purification by Coomassie staining of SDS-PAGE.



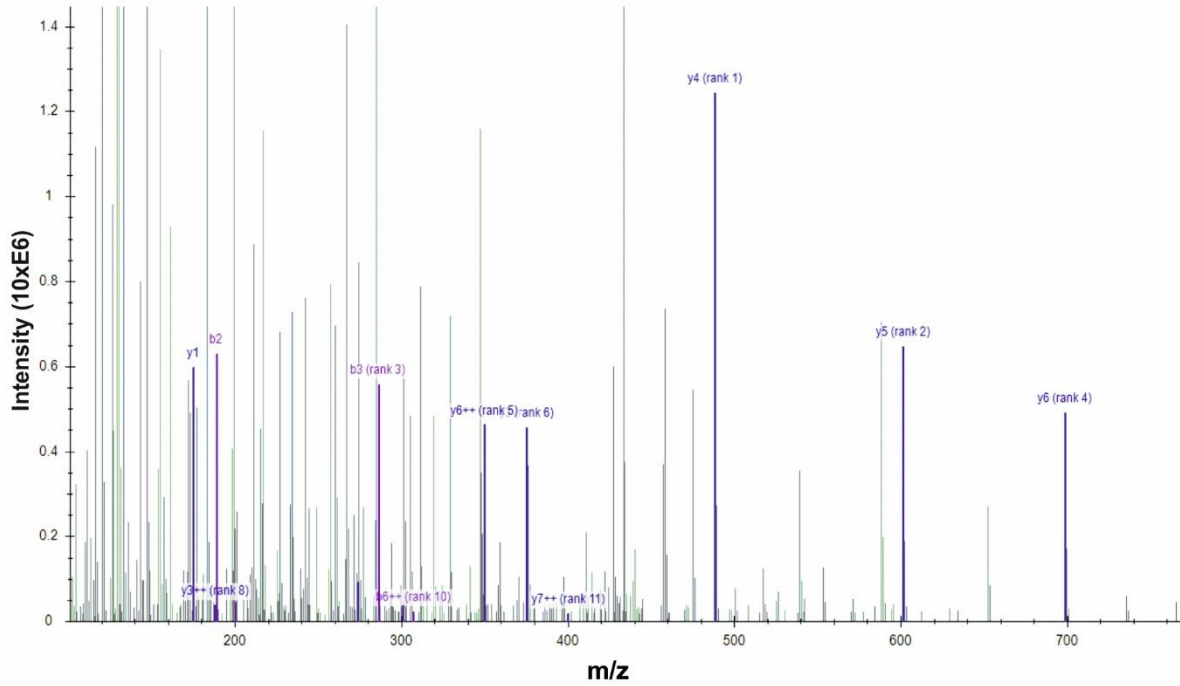
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ENSG00000004455	AK2	CCDS374
ENSG00000004455	AK2	CCDS81294
ENSG00000004455	AK2	CCDS81295
ENSG00000004455	AK2	CCDS81296
ENSG00000002726	AOC1	CCDS43679
ENSG00000002726	AOC1	CCDS64797
ENSG00000105290	APLP1	CCDS32997
ENSG00000105290	APLP1	CCDS92600
ENSG00000132254	ARFIP2	CCDS55739
ENSG00000100325	ASCC2	CCDS13869
ENSG00000100325	ASCC2	CCDS56226
ENSG00000173918	C1QTNF1	CCDS11762
ENSG00000205129	C4orf47	CCDS47169
ENSG00000129993	CBFA2T3	CCDS10972
ENSG00000065883	CDK13	CCDS5461
ENSG00000065883	CDK13	CCDS5462
ENSG00000273777	CEACAM20	CCDS74390
ENSG00000273777	CEACAM20	CCDS74391
ENSG00000273777	CEACAM20	CCDS74392
ENSG00000273777	CEACAM20	CCDS74393
ENSG00000090539	CHRD	CCDS3266
ENSG00000090539	CHRD	CCDS77868
ENSG00000154080	CHST9	CCDS42422
ENSG00000154080	CHST9	CCDS58618
ENSG00000176435	CLEC14A	CCDS9667
ENSG00000114646	CSPG5	CCDS56252
ENSG00000155368	DBI	CCDS42741
ENSG00000188542	DUSP28	CCDS33418
ENSG00000144895	EIF2A	CCDS46935
ENSG00000144895	EIF2A	CCDS82859
ENSG00000144895	EIF2A	CCDS82860
ENSG00000144895	EIF2A	CCDS82861
ENSG00000178568	ERBB4	CCDS2394
ENSG00000178568	ERBB4	CCDS42811
ENSG00000178607	ERN1	CCDS45762
ENSG00000158869	FCER1G	CCDS1225
ENSG00000089327	FXR1	CCDS12447
ENSG00000089327	FXR1	CCDS82328
ENSG00000111087	GLI1	CCDS53807
ENSG00000125772	GPCPD1	CCDS13090
ENSG00000133937	GSC	CCDS9930
ENSG00000128731	HERC2	CCDS10021
ENSG00000197921	HES5	CCDS41233
ENSG00000160223	ICOSLG	CCDS93102
ENSG00000168811	IL12A	CCDS93420
ENSG00000068745	IP6K2	CCDS2777
ENSG00000068745	IP6K2	CCDS33752
ENSG00000068745	IP6K2	CCDS54579
ENSG00000132321	IQCA1	CCDS74677
ENSG00000161999	JMJD8	CCDS45369
ENSG00000161999	JMJD8	CCDS92074
ENSG00000161999	JMJD8	CCDS92075
ENSG00000144445	KANSL1L	CCDS33370
ENSG00000144445	KANSL1L	CCDS77519
ENSG00000135519	KCNH3	CCDS8786
ENSG00000087299	L2HGDH	CCDS9698
ENSG00000143815	LBR	CCDS1545
ENSG00000140506	LMAN1L	CCDS10270
ENSG00000254726	MEX3A	CCDS53377
ENSG00000178053	MLF1	CCDS93416
ENSG00000103152	MPG	CCDS32345
ENSG00000101181	MTG2	CCDS13492

Ensembl Gene ID	Gene name	CCDS
ENSG00000114026	OGG1	CCDS2576
ENSG00000114026	OGG1	CCDS2577
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ENSG00000114026	OGG1	CCDS2580
ENSG00000114026	OGG1	CCDS2581
ENSG00000114026	OGG1	CCDS43046
ENSG00000114026	OGG1	CCDS46742
ENSG00000116774	OLFM13	CCDS870
ENSG00000076641	PAG1	CCDS6227
ENSG00000188677	PARVB	CCDS74874
ENSG00000056487	PHF21B	CCDS63504
ENSG00000130827	PLXNA3	CCDS14752
ENSG00000009830	POMT2	CCDS9857
ENSG00000189099	PRSS48	CCDS47145
ENSG00000103479	RBL2	CCDS10748
ENSG00000089902	RCOR1	CCDS9974
ENSG00000167771	RCOR2	CCDS8052
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ENSG00000197747	S100A10	CCDS1008
ENSG00000135622	SEMA4F	CCDS1955
ENSG00000135622	SEMA4F	CCDS62942
ENSG00000135622	SEMA4F	CCDS74529
ENSG00000099995	SF3A1	CCDS13875
ENSG00000113558	SKP1	CCDS4171
ENSG00000113558	SKP1	CCDS4172
ENSG00000155380	SLC16A1	CCDS858
ENSG00000173638	SLC19A1	CCDS13725
ENSG00000173638	SLC19A1	CCDS56218
ENSG00000205060	SLC35B4	CCDS34756
ENSG00000121871	SLITRK3	CCDS3197
ENSG00000132207	SLX1A	CCDS32431
ENSG00000132207	SLX1A	CCDS32432
ENSG00000181625	SLX1B	CCDS10648
ENSG00000181625	SLX1B	CCDS10649
ENSG00000198732	SMOC1	CCDS32110
ENSG00000198732	SMOC1	CCDS9798
ENSG00000152377	SPOCK1	CCDS4191
ENSG00000160075	SSU72	CCDS32
ENSG00000158246	TENT5B	CCDS294
ENSG00000129028	THAP10	CCDS10237
ENSG00000151952	TMEM132D	CCDS9266
ENSG00000125895	TMEM74B	CCDS13011
ENSG00000139921	TMX1	CCDS41953
ENSG00000284844	TOMT	CCDS91529
ENSG00000111669	TPI1	CCDS8566
ENSG00000239264	TXNDC5	CCDS4505
ENSG00000115446	UNC50	CCDS2035
ENSG00000104219	ZDHHC2	CCDS47810
ENSG00000156599	ZDHHC5	CCDS7965
ENSG00000166140	ZFYVE19	CCDS58353
ENSG00000164134	NAA15	CCDS43270
ENSG00000164134	NAA15	CCDS93635
ENSG00000117650	NEK2	CCDS1500
ENSG00000117650	NEK2	CCDS55682
ENSG00000117650	NEK2	CCDS73024
ENSG00000131196	NFATC1	CCDS12015
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ENSG00000131196	NFATC1	CCDS62467
ENSG00000131196	NFATC1	CCDS62468
ENSG00000171246	NPTX1	CCDS32762
ENSG00000174145	NWD2	CCDS47040

Appendix Figure S7 (related to Figure 4A,B). DPP8 and DPP9 potentially unmask IBMs at the N-terminus of 129 cellular proteins.

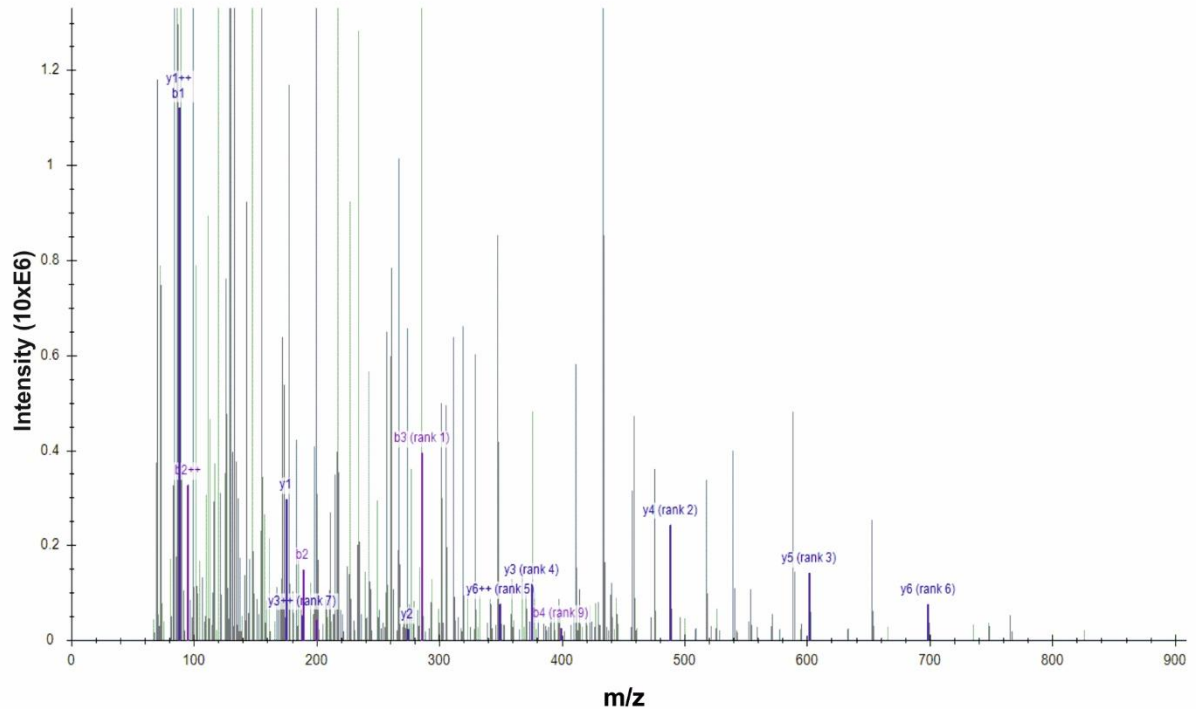
A

STPLLTVR2⁺ (PEP 1.32E⁻⁷) - DPP8/9 cleaved N-terminus in HEK WT
 20240425_TB_SA_EIF2A_WT_20240428150220.raw (44.72 min)



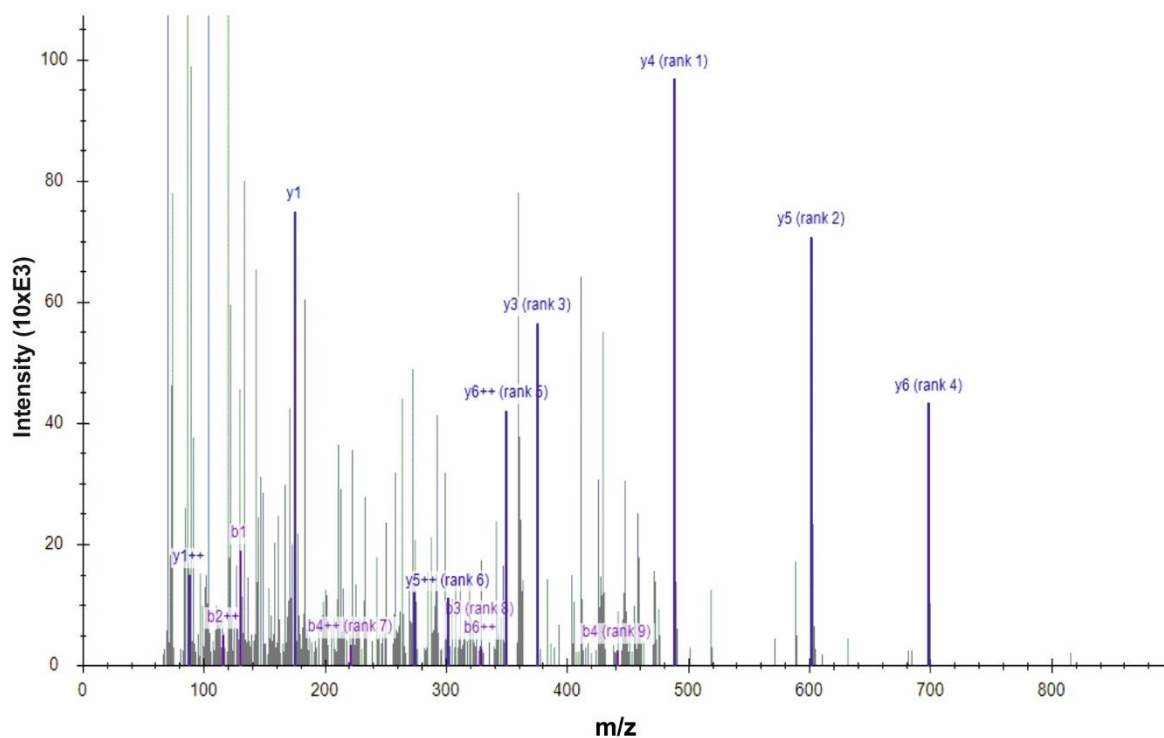
B

STPLLTVR2⁺ (PEP 2.59E⁻⁶) - DPP8/9 cleaved N-terminus in HEK DPP9 KO
 2024_TB_SA_EIF2A_KO_20240428165944.raw (44.41 min)



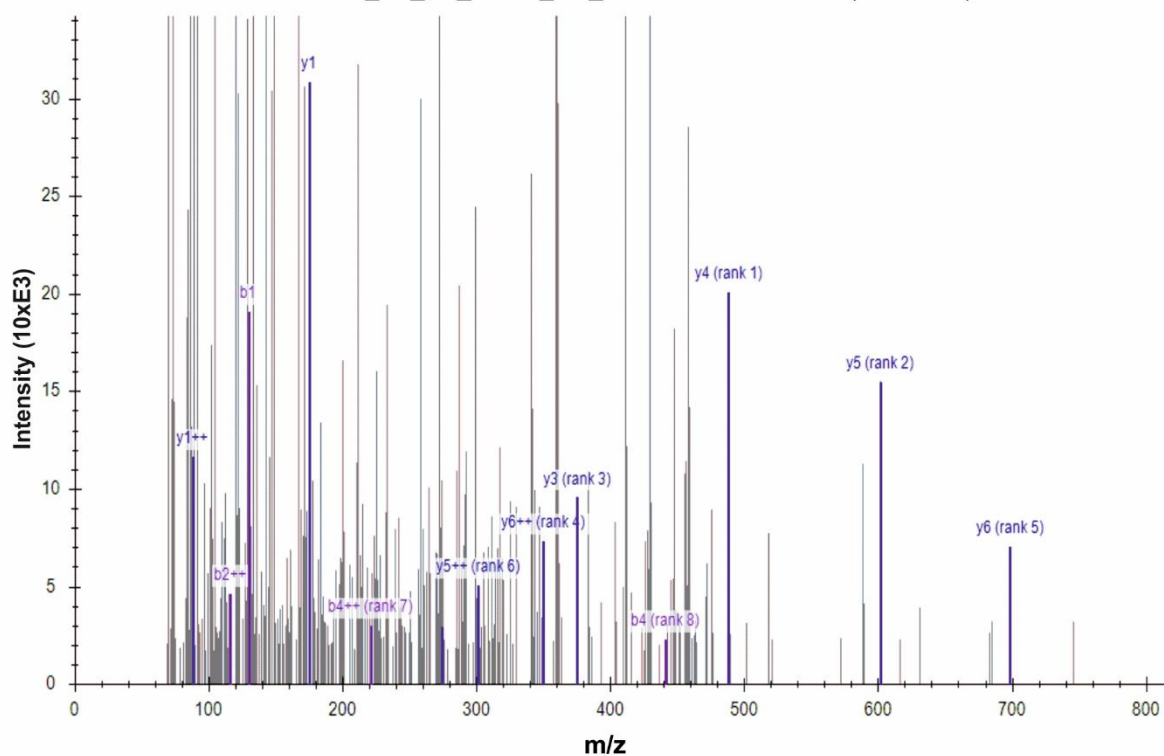
C

acSTPLLTVR2⁺ (PEP 2.56E⁻⁶) - acetylated DPP8/9 cleaved N-terminus in HEK WT
 20240425_TB_SA_EIF2A_WT_20240428150220.raw (65.17 min)



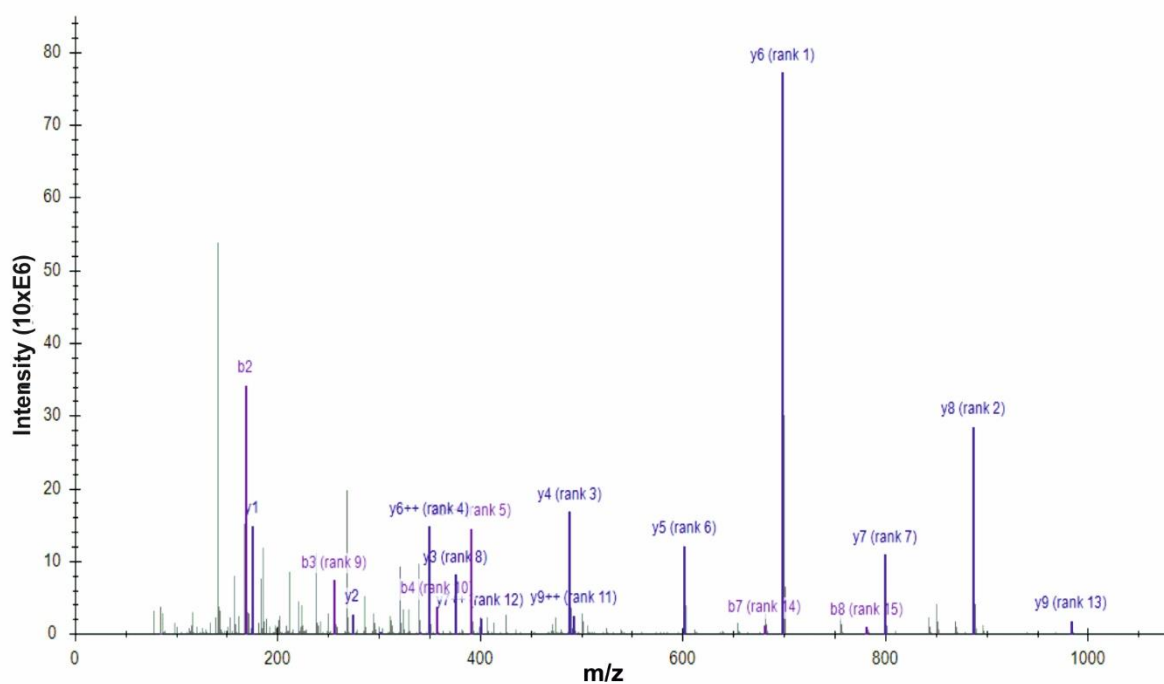
D

acSTPLLTVR2⁺ (PEP 2.60E⁻⁶) - acetylated DPP8/9 cleaved N-terminus in HEK DPP9 KO
 20240425_TB_SA_EIF2A_KO_20240428165994.raw (64.82 min)



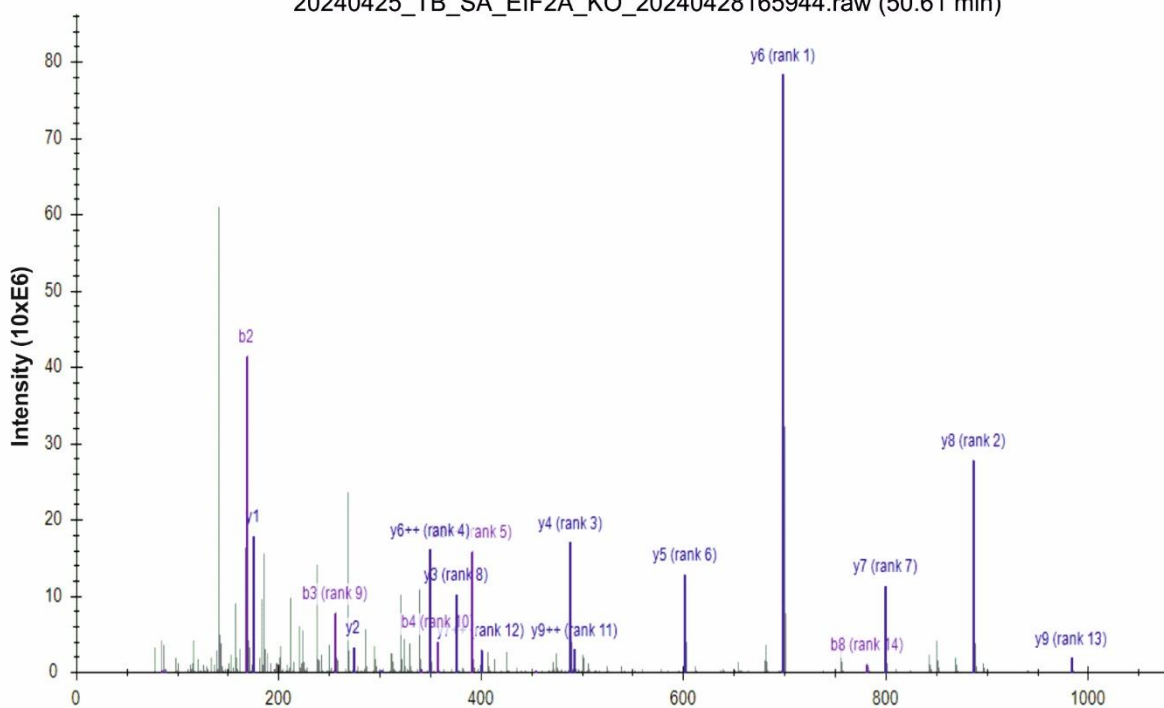
E

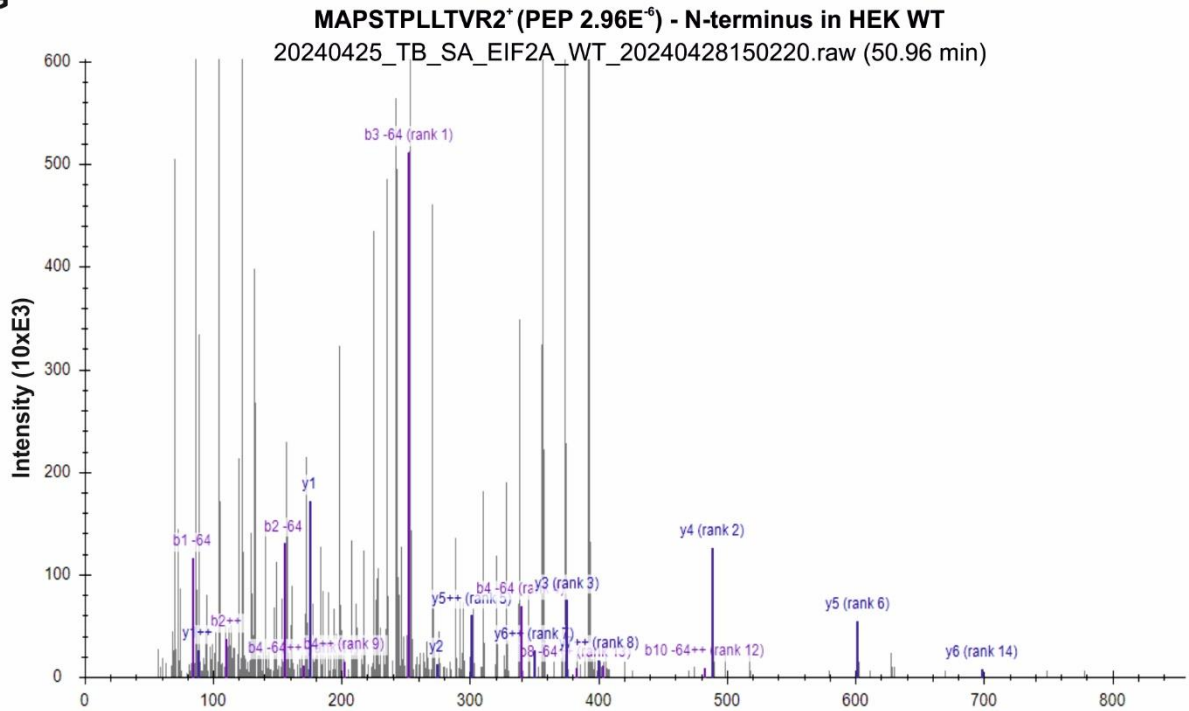
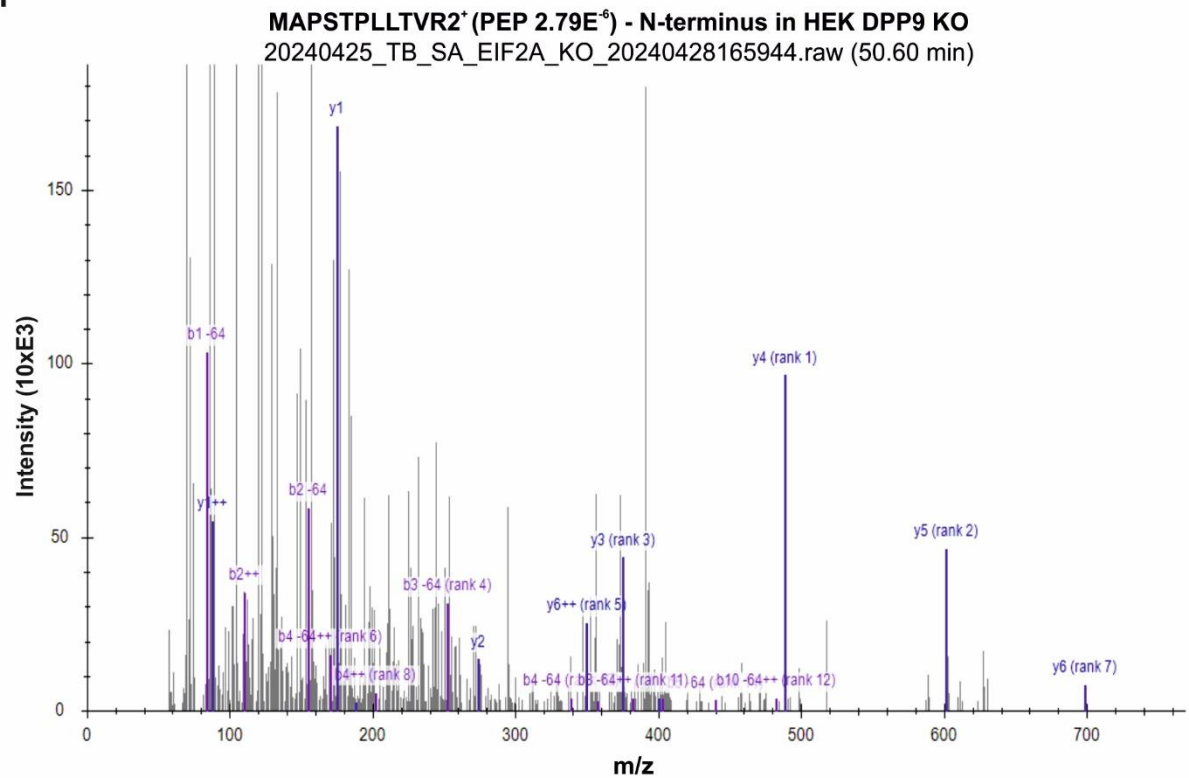
APSTPLLTVR2⁺ (PEP 0.09)- MetAP cleaved N-terminus in HEK WT
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F

APSTPLLTVR2⁺ (PEP 0.09) - MetAP cleaved N-terminus in HEK DPP9 KO
 20240425_TB_SA_EIF2A_KO_20240428165944.raw (50.61 min)



G**H**

Appendix Figure S8 (related to Figure 4C). MS2 spectra for EIF2A peptides identified in whole cellular lysates of HEK293 WT and DPP9 KO cells.

A,B. EIF2A N-terminal peptide NH₂-STPLLTVR in WT (A) and DPP9 KO (B) lysate.

C,D. EIF2A N-terminal peptide ac-NH₂-STPLLTVR in WT (C) and DPP9 KO (D) lysate.

E,F. EIF2A N-terminal peptide NH₂-APSTPLLTVR in WT (E) and DPP9 KO (F) lysates.

G,H. EIF2A N-terminal peptide NH₂-MAPSTPLLTVR in WT (G) and DPP9 KO (H) lysates.

APPENDIX REFERENCES

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- Finger Y, Habich M, Gerlich S, Urbanczyk S, van de Logt E, Koch J, Schu L, Lapacz KJ, Ali M, Petrunaro C *et al* (2020) Proteasomal degradation induced by DPP9-mediated processing competes with mitochondrial protein import. *The EMBO Journal* 39
- Jumper J, Evans R, Pritzel A, Green T, Figurnov M, Ronneberger O, Tunyasuvunakool K, Bates R, Zidek A, Potapenko A *et al* (2021) Highly accurate protein structure prediction with AlphaFold. *Nature* 596: 583-589
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