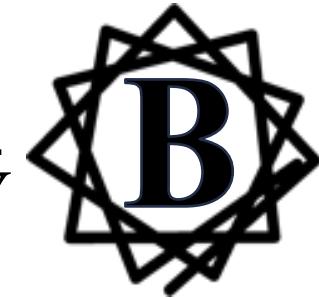


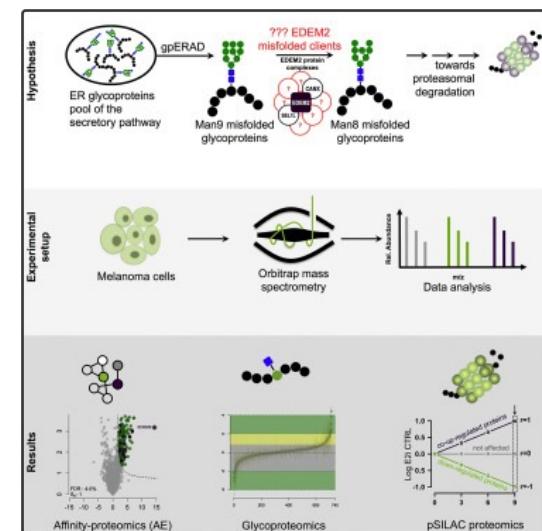


ROMANIAN ACADEMY INSTITUTE OF BIOCHEMISTRY



Affinity Proteomics and (De)glycoproteomics Uncover Novel EDEM2 Endogenous Substrates and an Integrative ERAD Network

Cristian VA Munteanu*, Gabriela N Chiritoiu*, Marioara B. Chiritoiu, Simona Ghenea, Andrei-Jose Petrescu, Stefana M Petrescu[#]

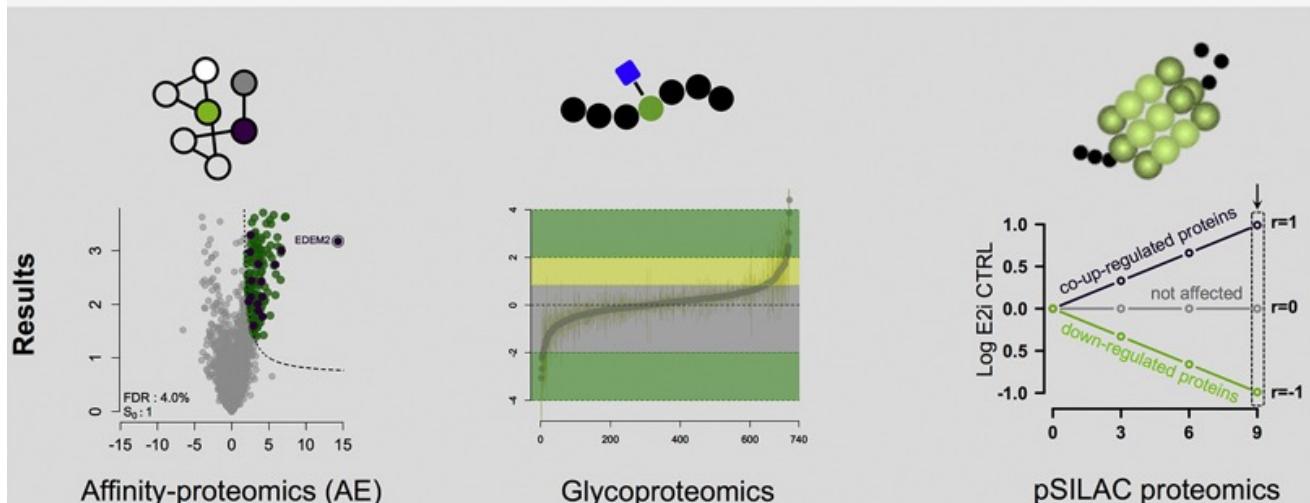
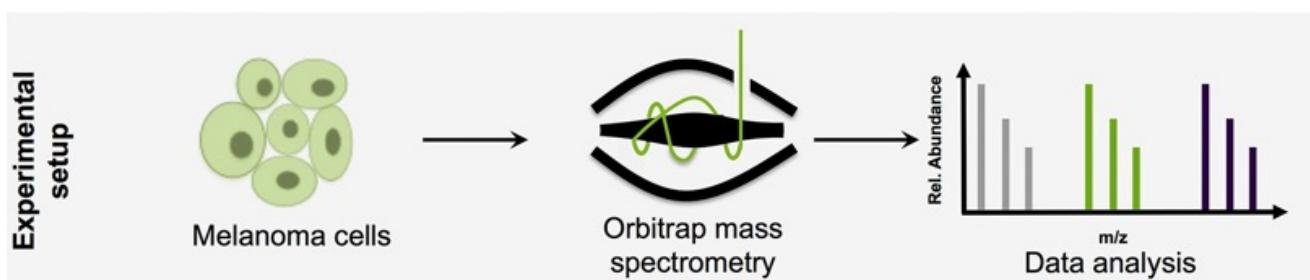
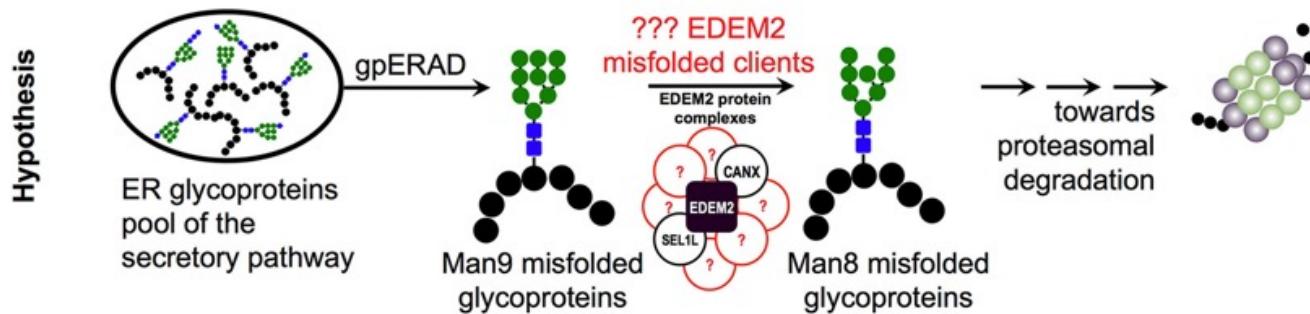


Bioinformatics and
Structural Biochemistry
Department

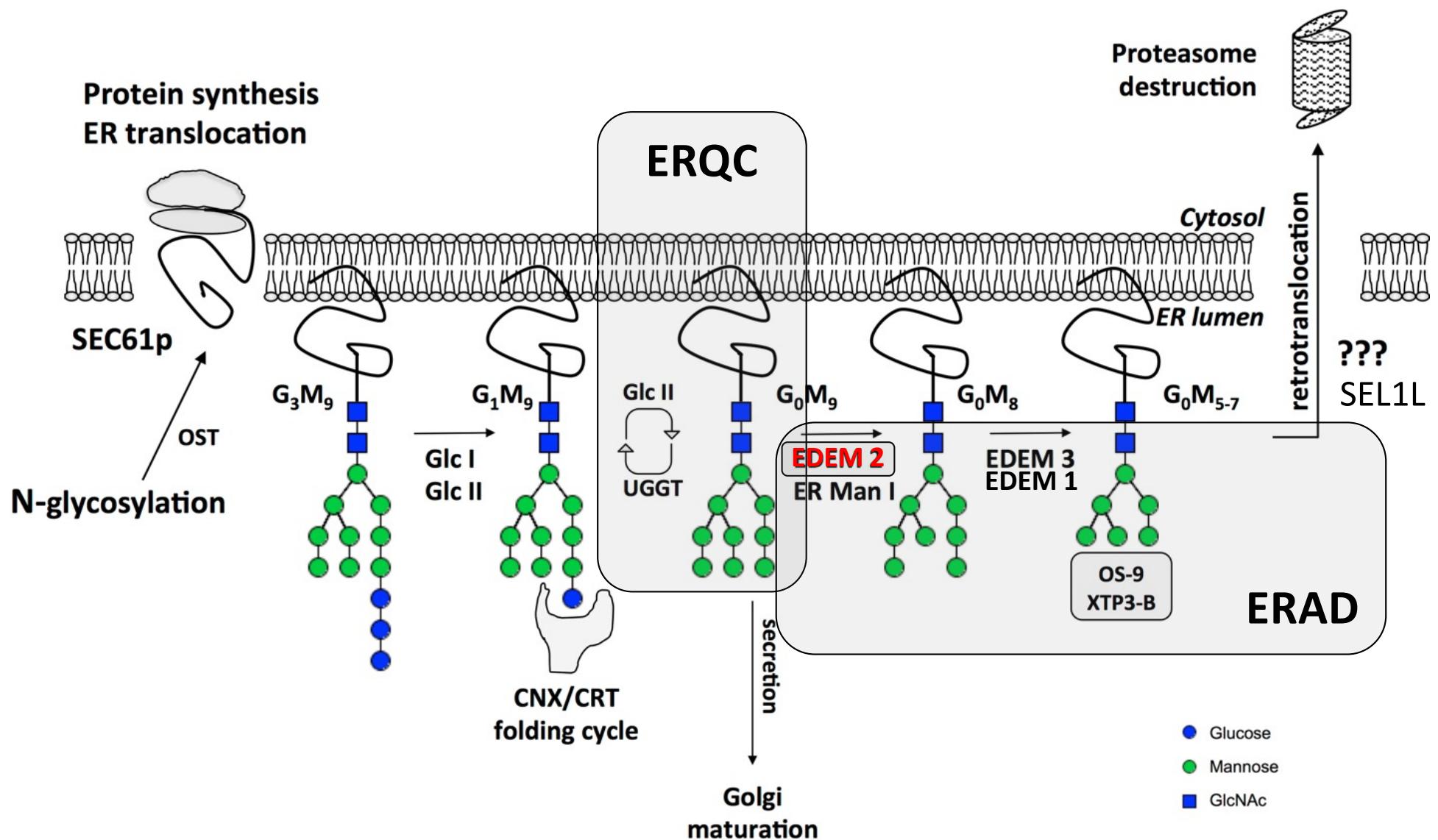
IBAR Meeting
March 10th, 2023 Bucharest

PCF
Protein Chemistry Facility
INSTITUTE OF BIOCHEMISTRY

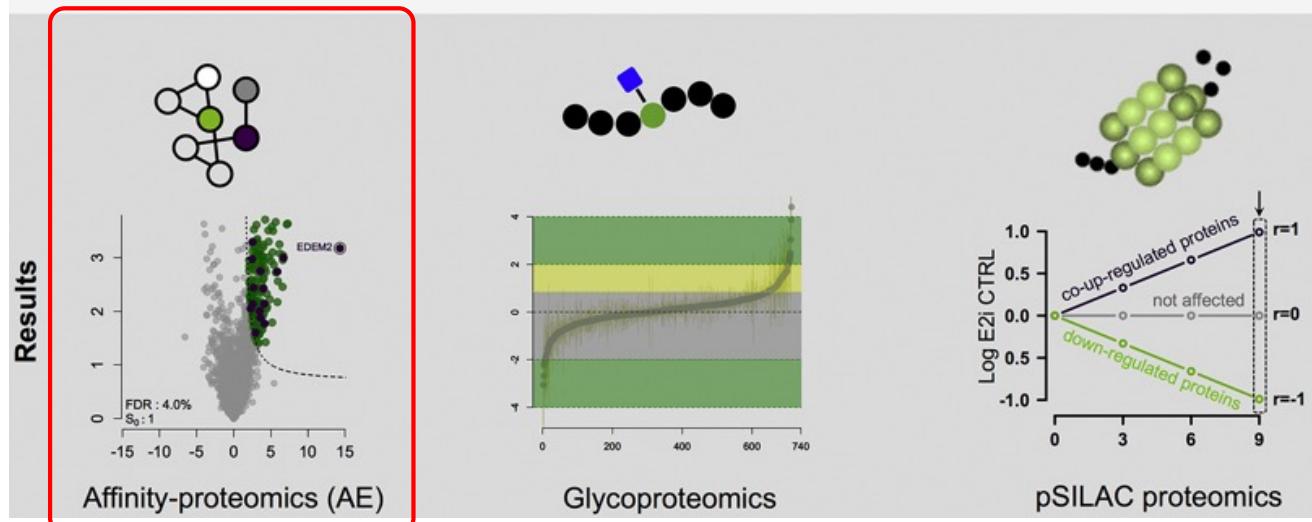
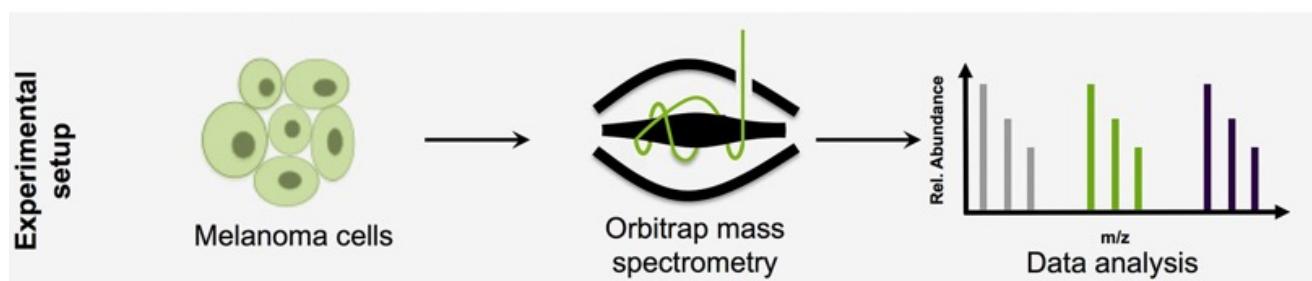
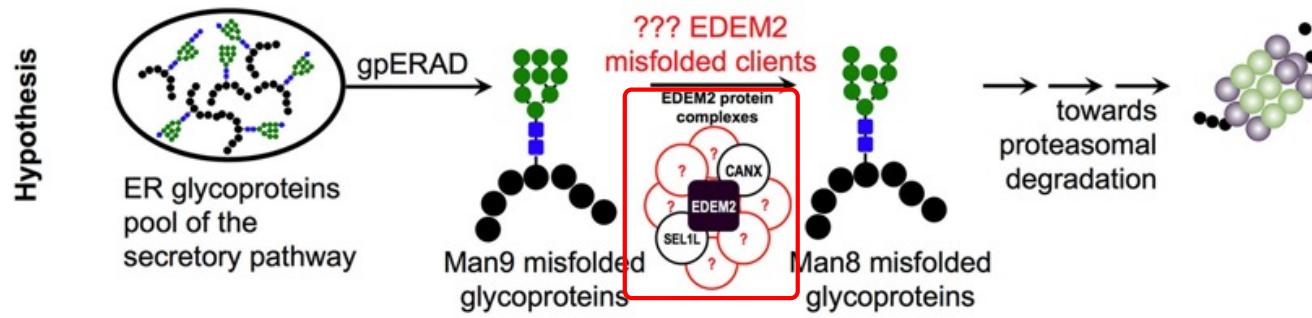
Multiomics and biochemical study of EDEM2 clients



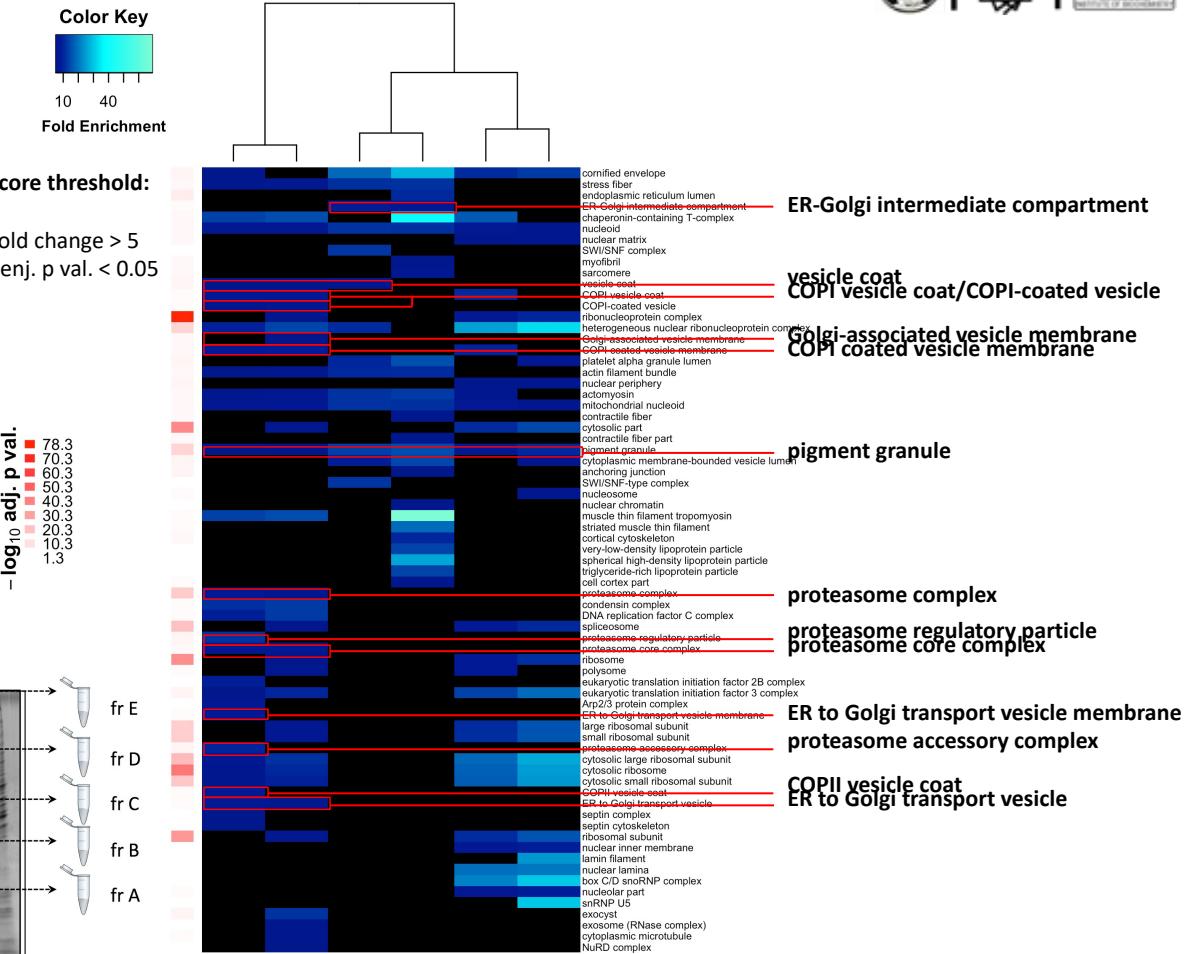
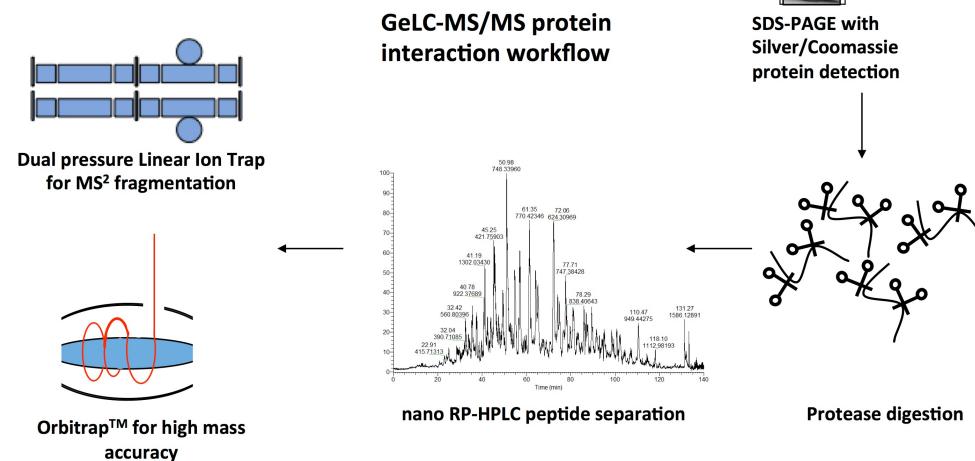
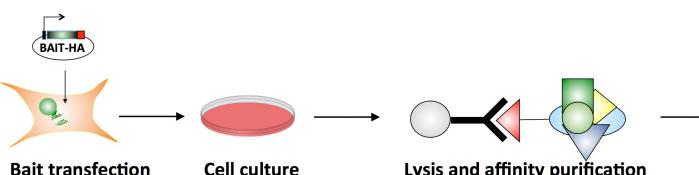
ERQC/ERAD – a complex landscape



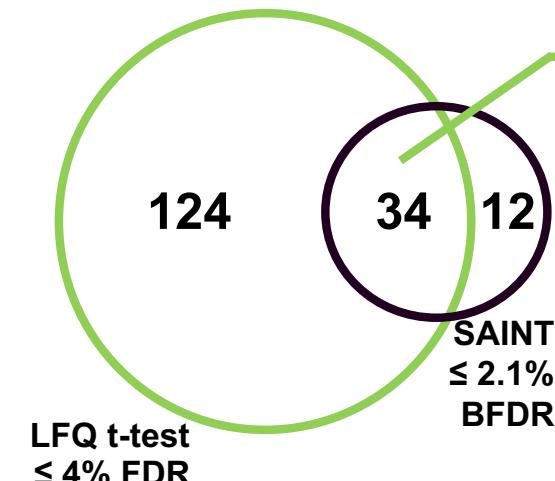
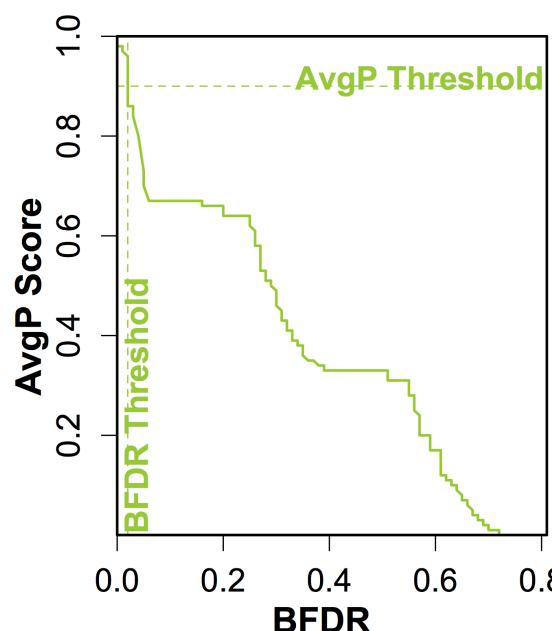
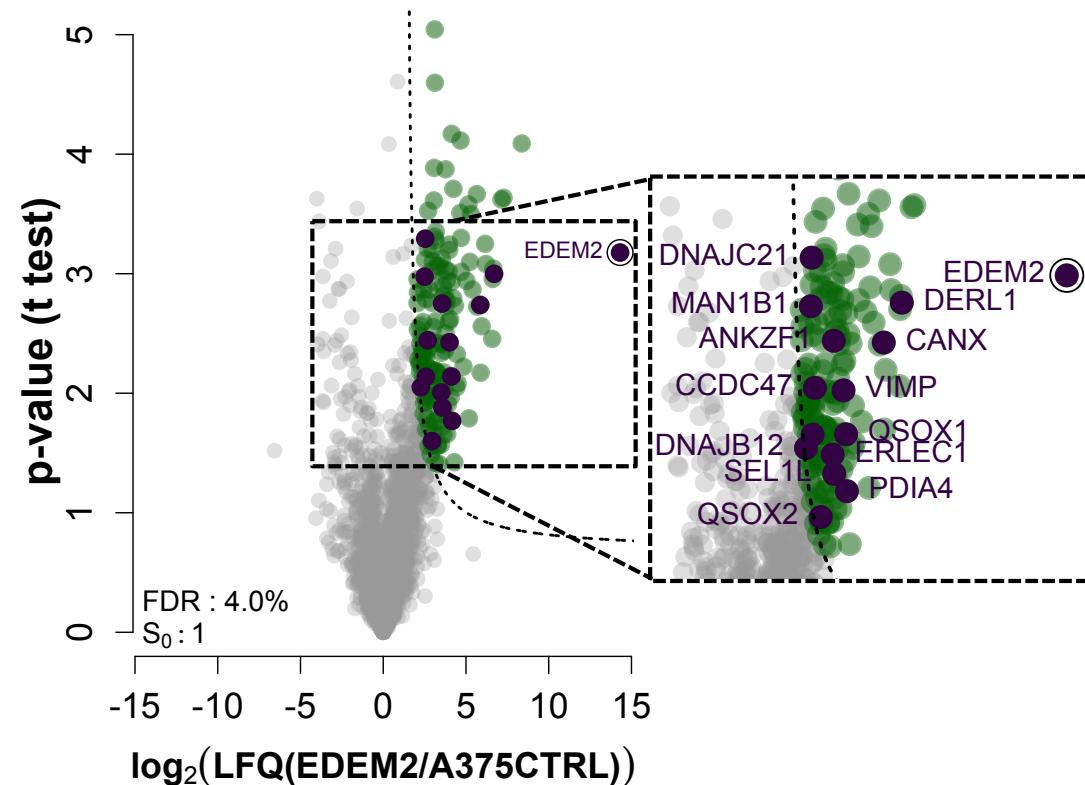
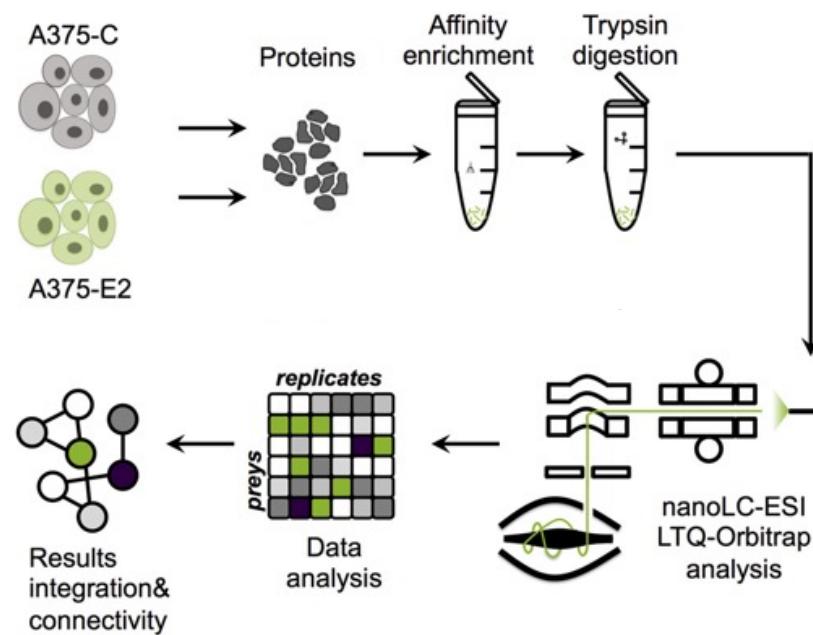
Multiomics and biochemical study of EDEM2 clients



AFFINITY-ENRICHMENT (AE) PROTEOMICS WORKFLOW

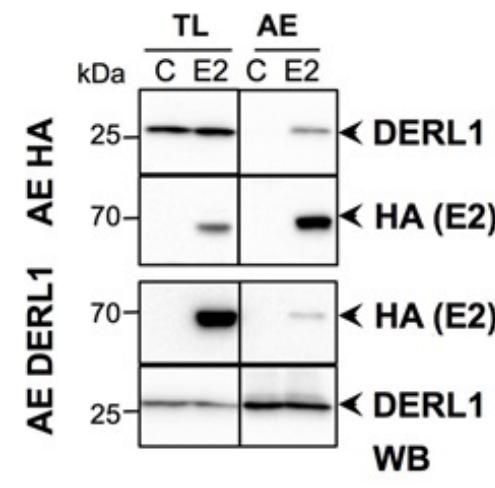
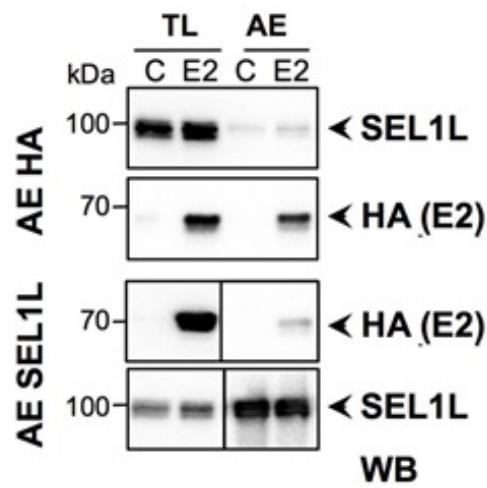
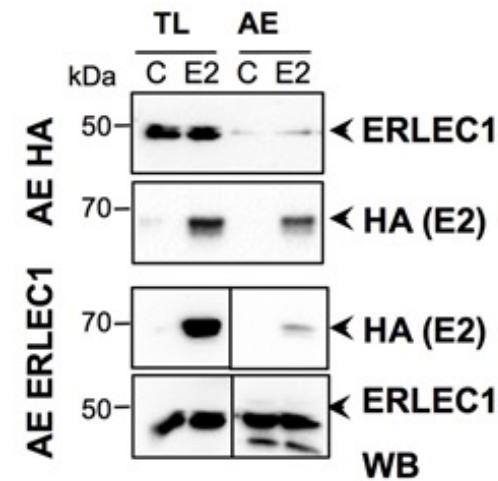
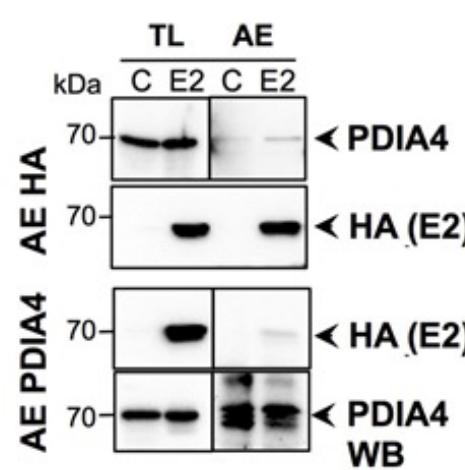
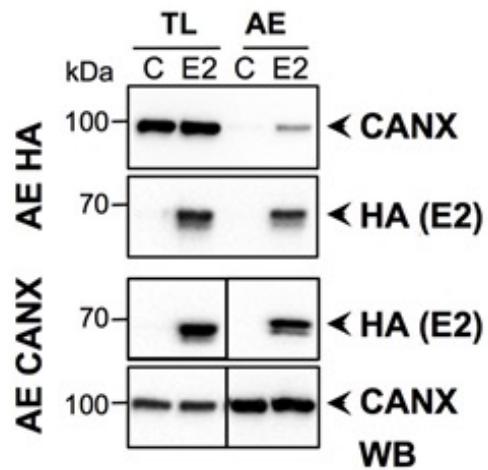


EDEM2 Affinity Enrichment retrieved complexes

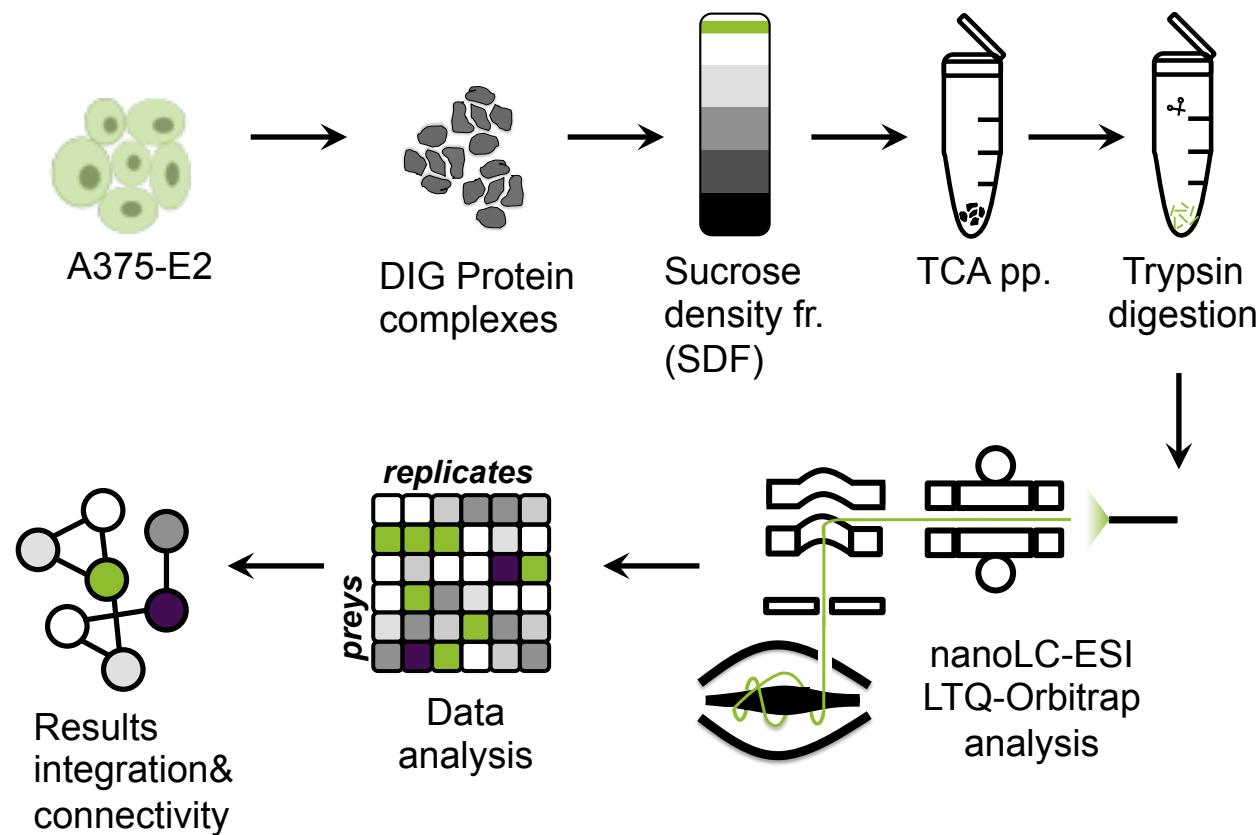


CANX	EDEM2
TXNDC11	MAN1B1
UGGT2	ERLEC
QSOX2	DERL1
GALNT2	DNAJB12
B3GAT3	CCDC47

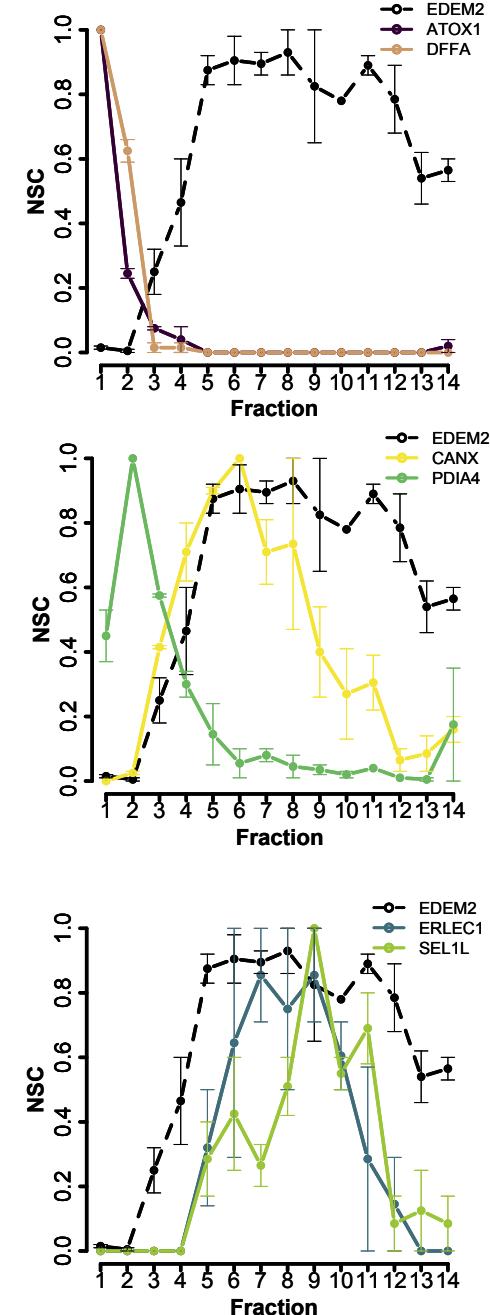
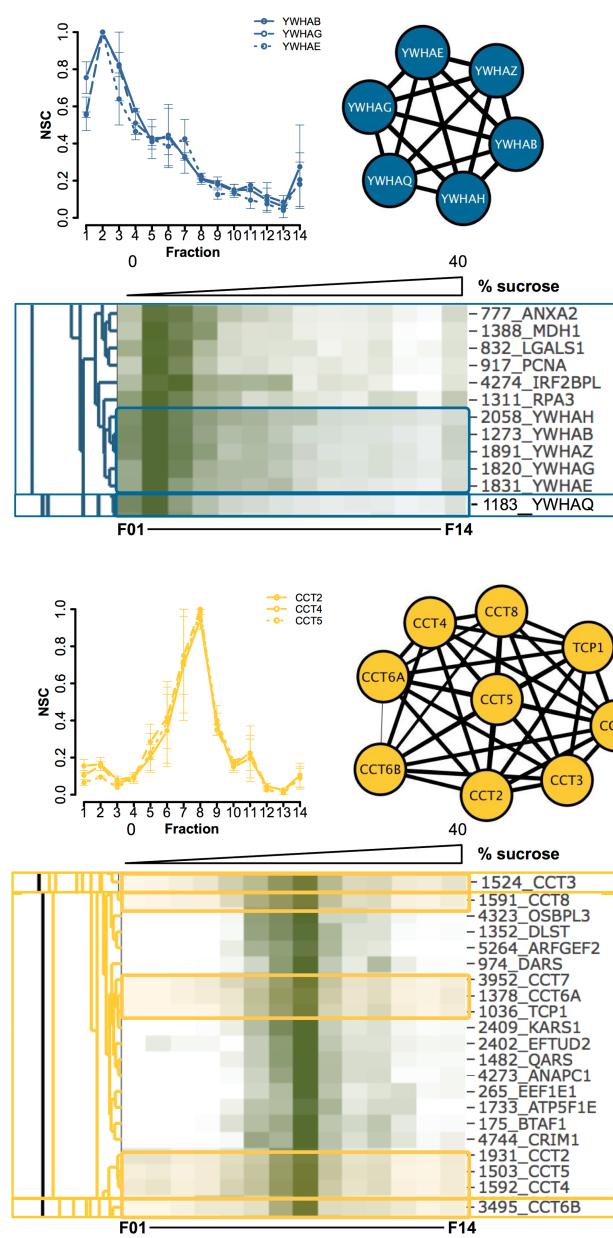
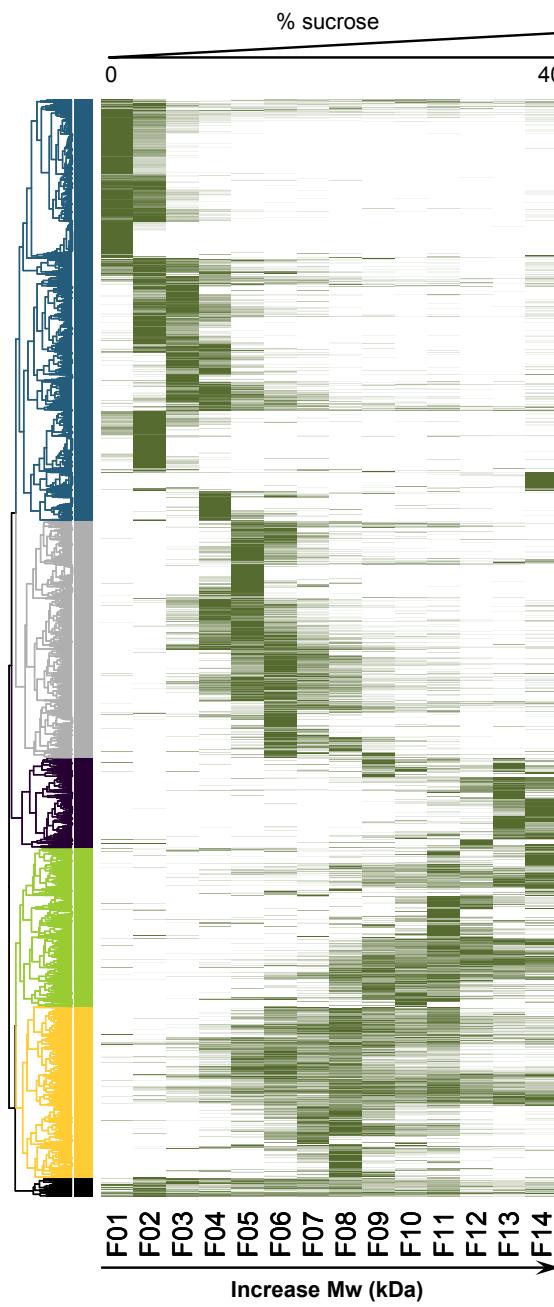
WB analysis of EDEM2 affinity co-enriched proteins



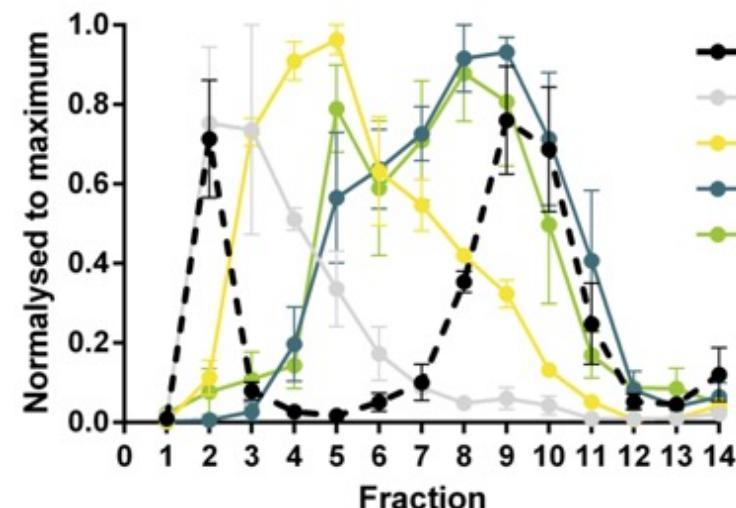
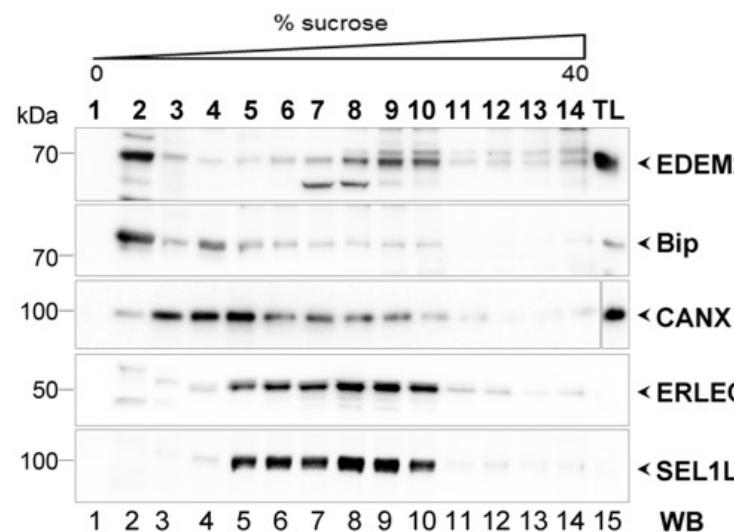
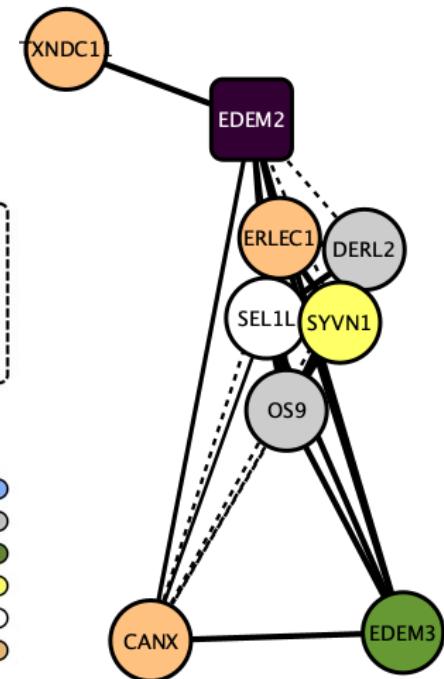
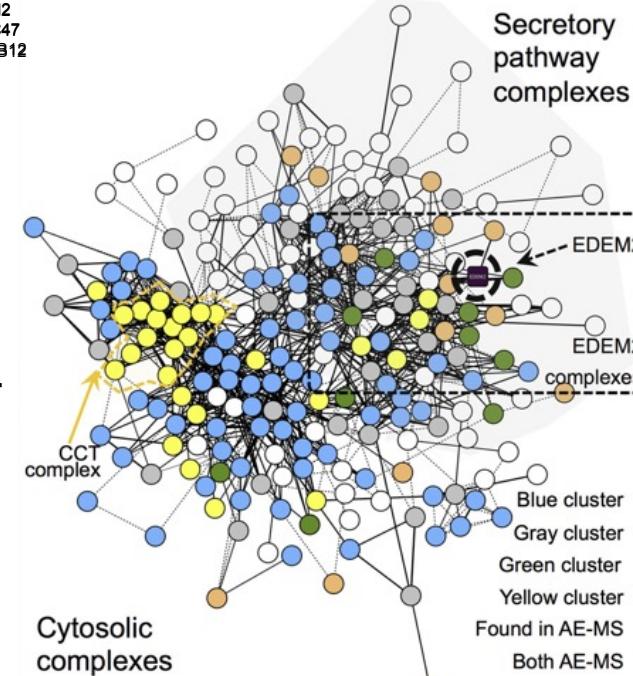
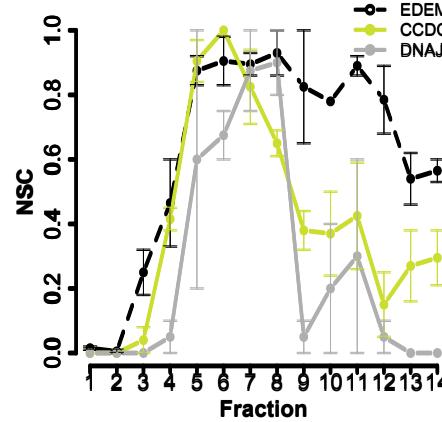
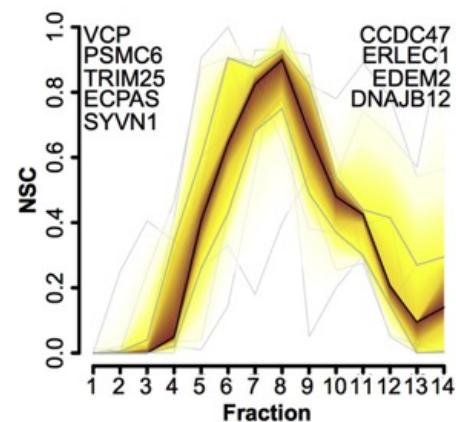
Sucrose density fractionation workflow



Sucrose density fractionation of known protein complexes



Sucrose density fractionation of EDEM2 complexes



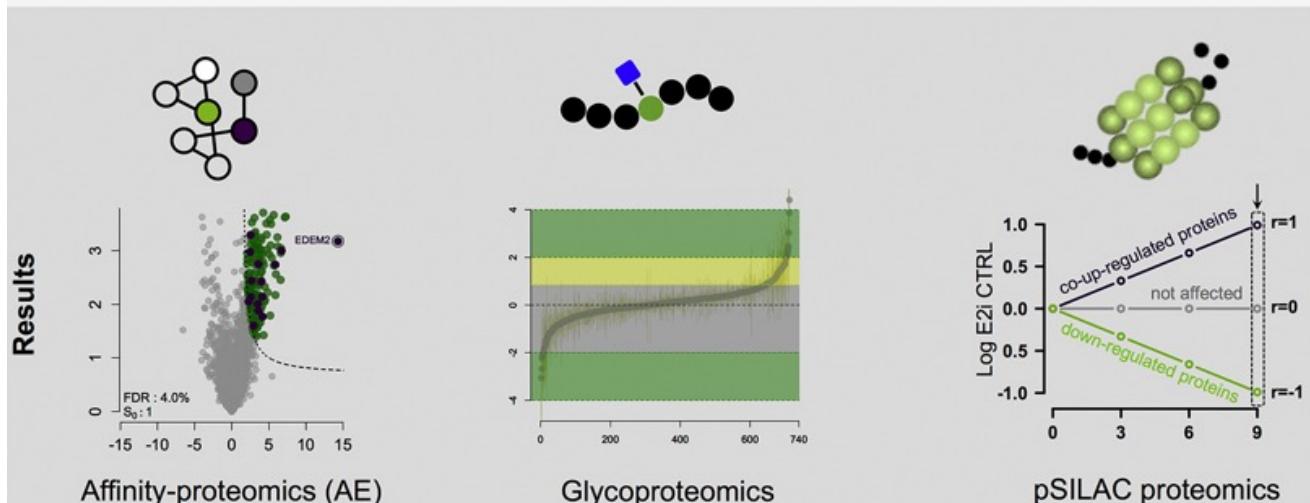
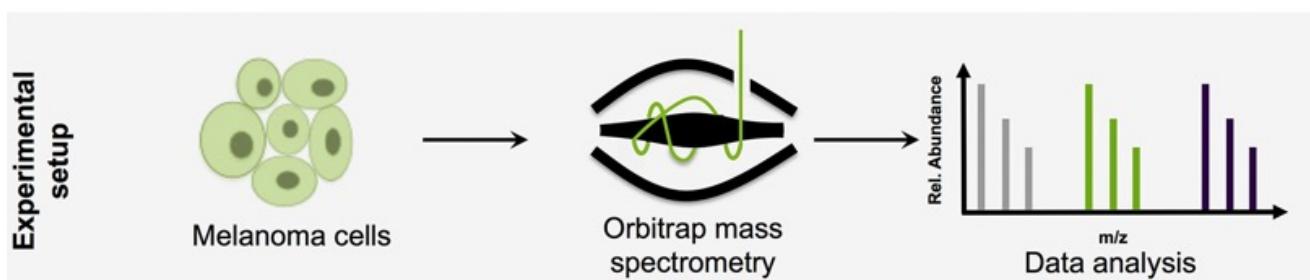
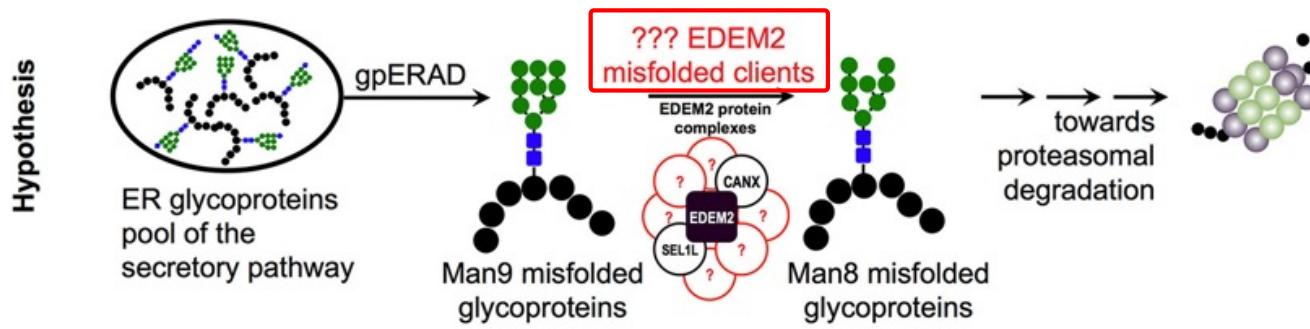
EDEM2 interaction proteomics

CONCLUSIONS



- both spectral counts and ion current are suitable for quantitative interaction proteomics for co-enriched proteins delineation in probabilistic or deterministic models
- EDEM2 is part of a multiprotein complex containing both ERQC and ERAD members
- EDEM2 is co-enriched with CANX, SEL1L, ERLEC1, DERL1, PDIA4 and UGGT2 from A375 melanoma cell lysates as confirmed in at least one of the computational analyses of MS data but also by WB experiments
- ERAD protein complexes distribute to higher molecular weights in SDF, compared with ERQC associated proteins
- results suggest a possible ERLEC1/SEL1L/DERL1 dependent degradation of EDEM2 and its substrates
- TXNDC11 was also evidenced as a co-enriched protein in spectral count based analysis of data

Multiomics and biochemical study of EDEM2 clients



Human soluble tyrosinase – EDEM2 target substrate

Canonical EDEM2 glycoprotein substrates:

Beta-secretase (BACE476), Alpha-1 antitrypsin (NHK, PI Z), Sonic Hedgehog (both glycosylated and non-glycosylated SHH), ATF6 α

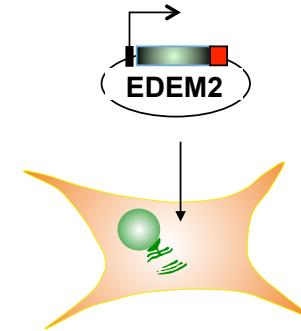
NOT involved in:

BACE476NOG, PI Z(NOG)

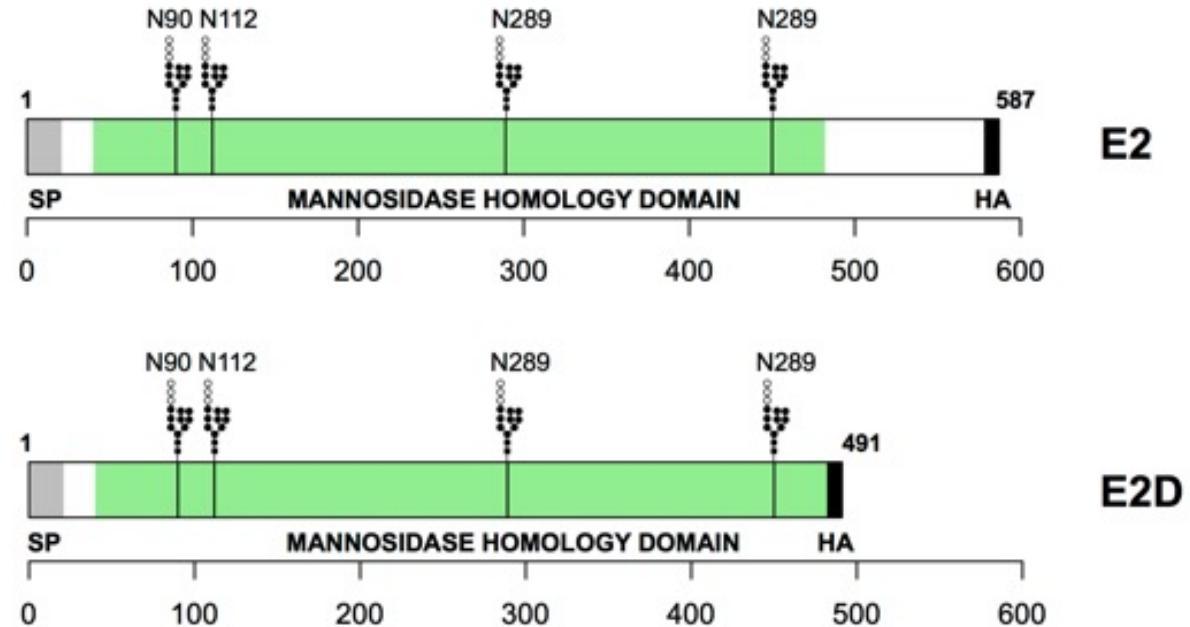
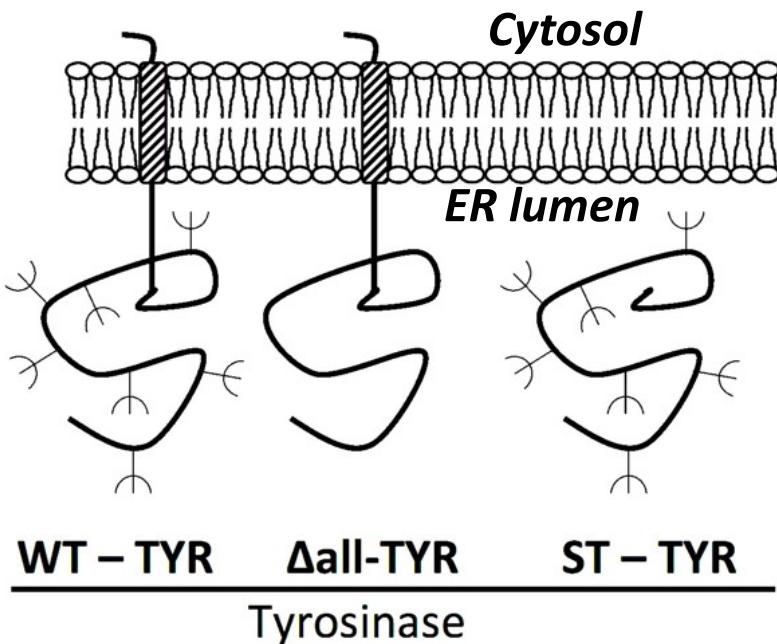
Mast SW et. al, *Glycobiology*. 2005 Apr;15(4):421-36.

Olivari S. et al., *J Biol Chem*. 2005 Jan 28;280(4):2424-8.

Ninagawa S. et al., *J Cell Biol*. 2014 Aug 4;206(3):347-56.



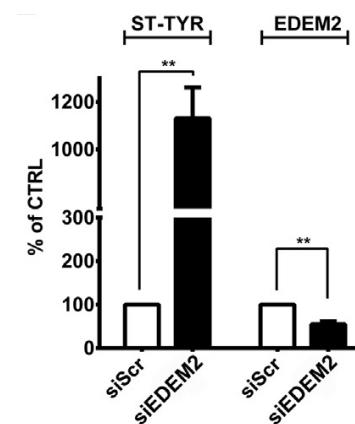
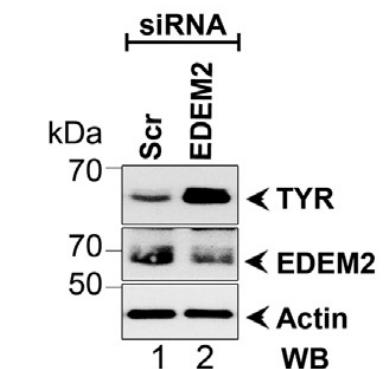
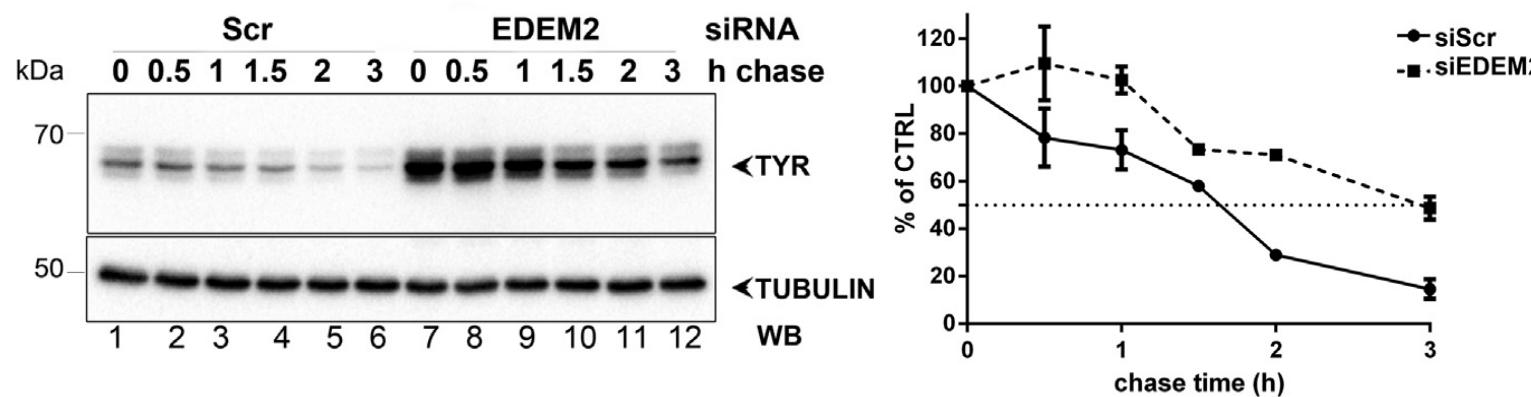
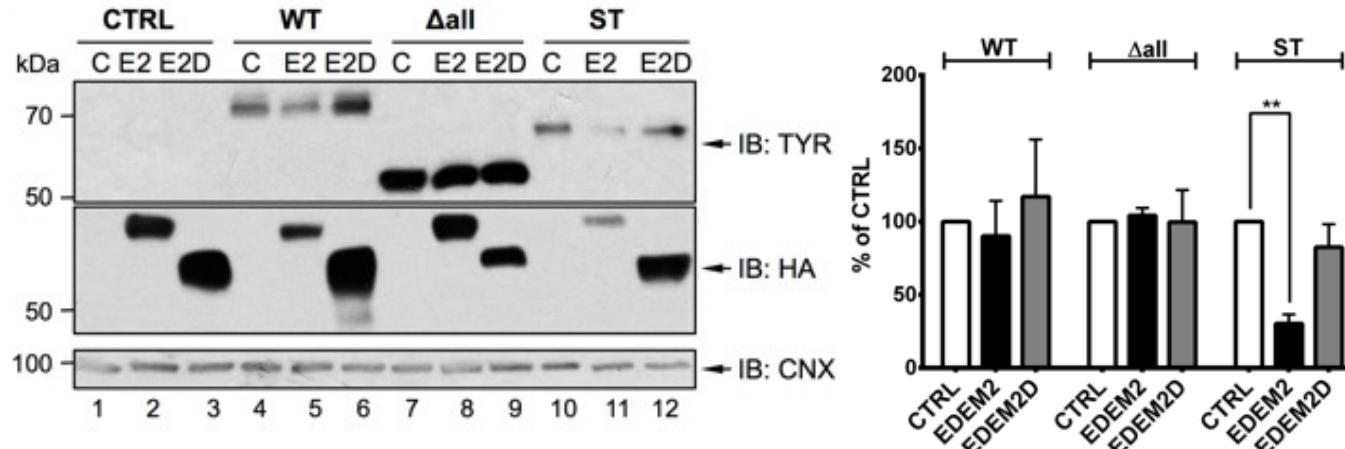
Cell transfection
A375 melanoma cells



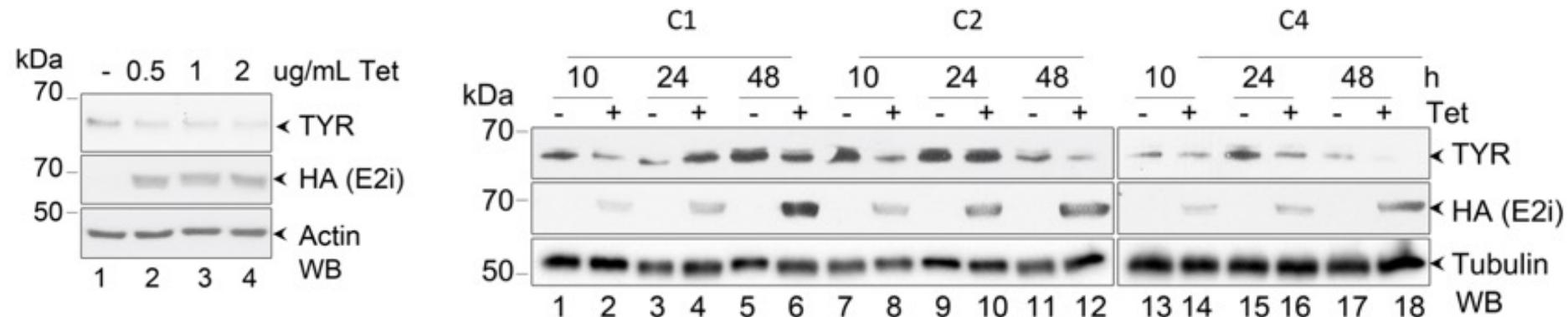
Popescu et al., *J Biol Chem*. 2005 Apr 8;280(14):13833-40.

Marin MB. et al, *PLoS One*. 2012;7(8):e42998

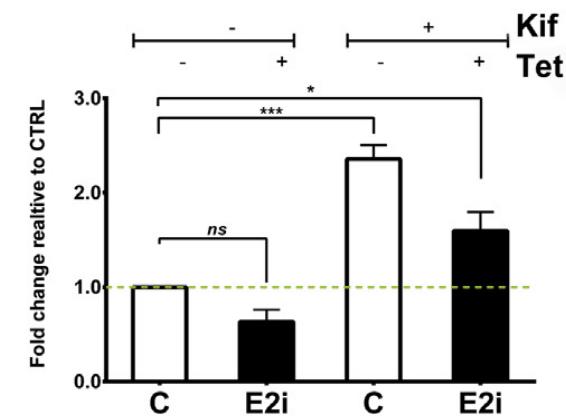
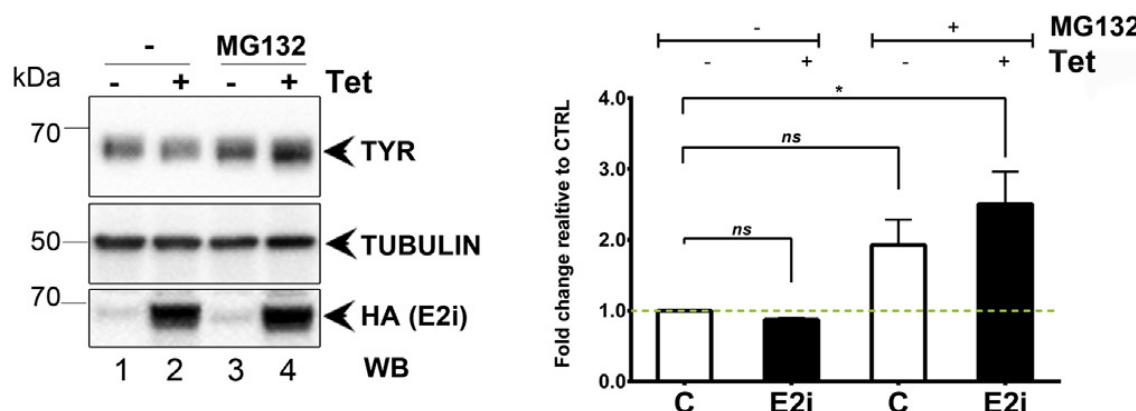
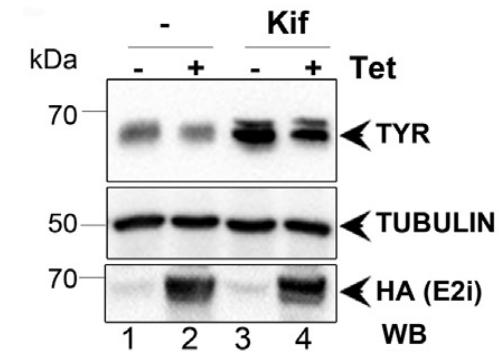
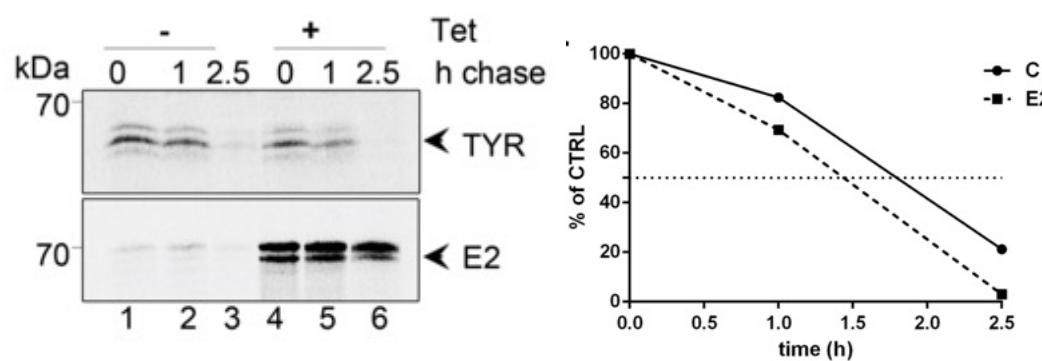
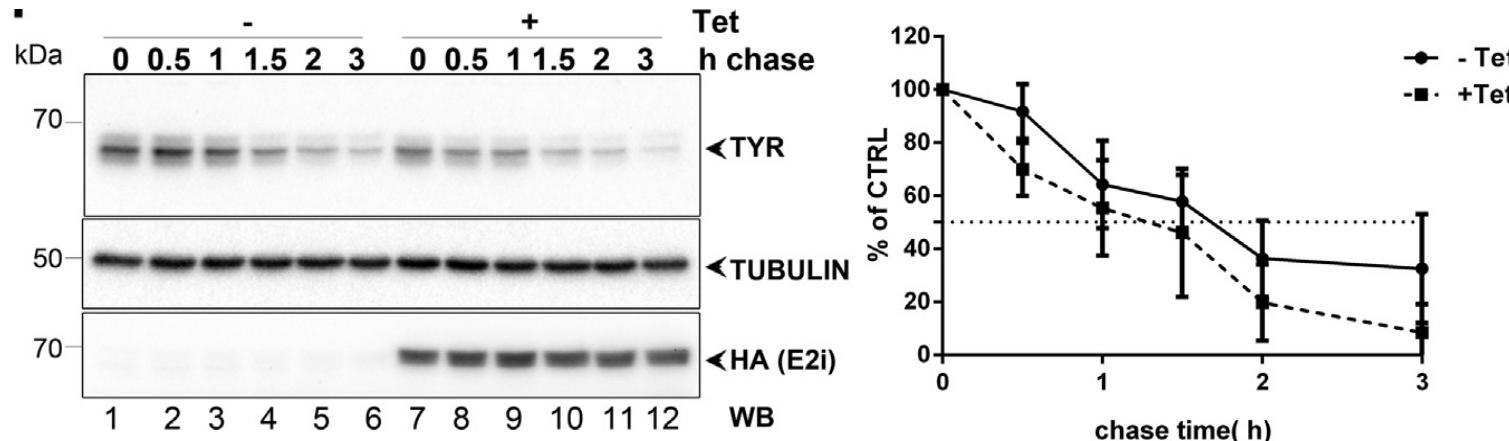
Human soluble tyrosinase – EDEM2 target substrate



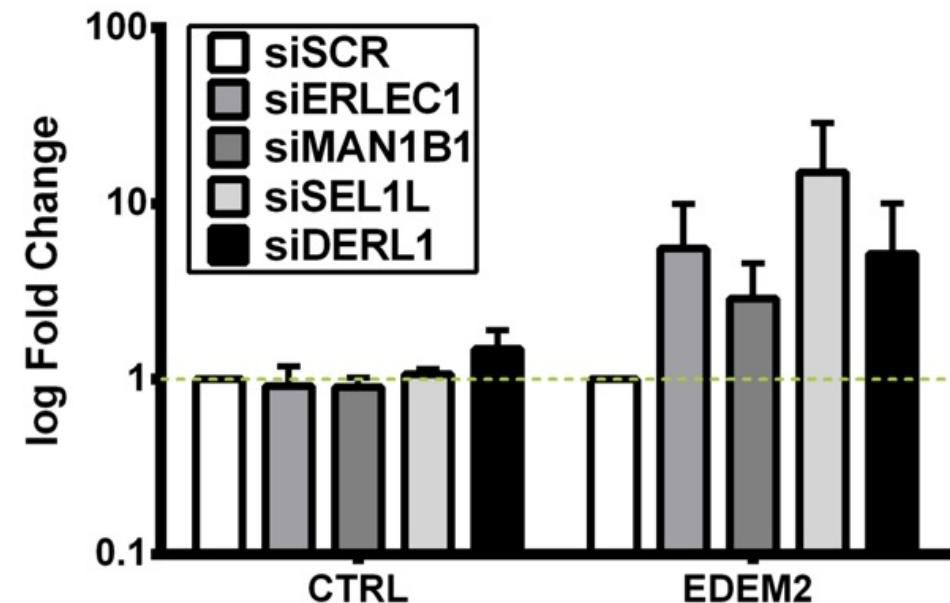
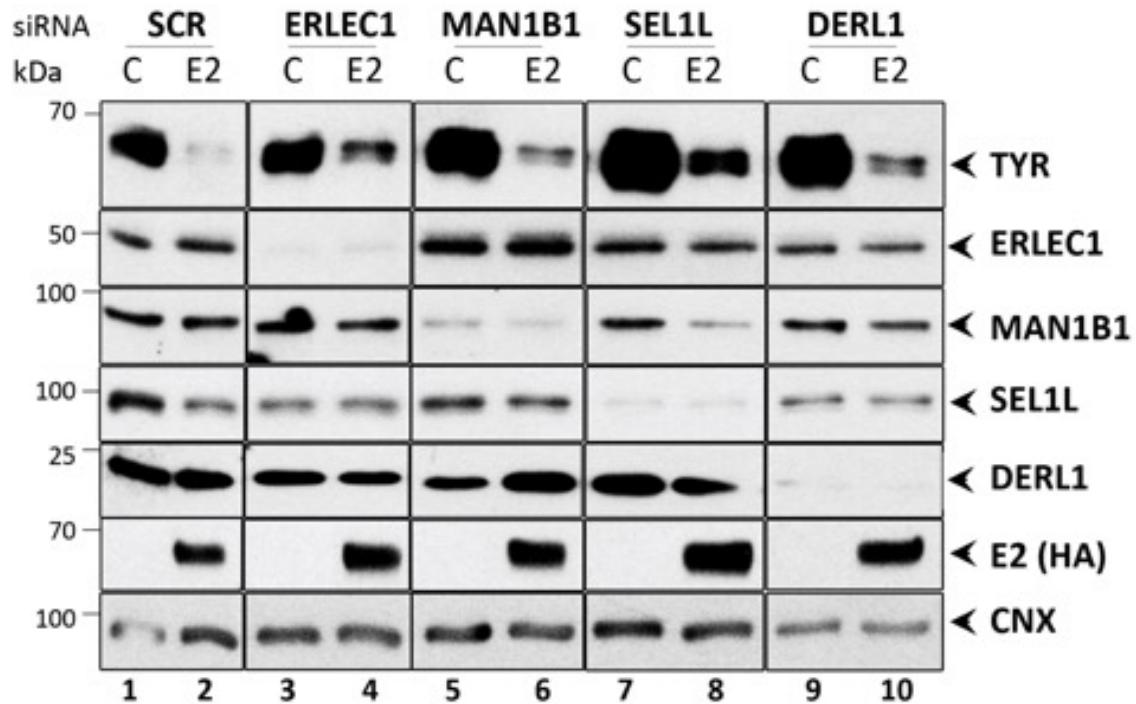
similar phenotype for Tet-inducible human EDEM2



Human soluble tyrosinase – EDEM2 target substrate



EDEM2 complexes are involved in ST-TYR levels



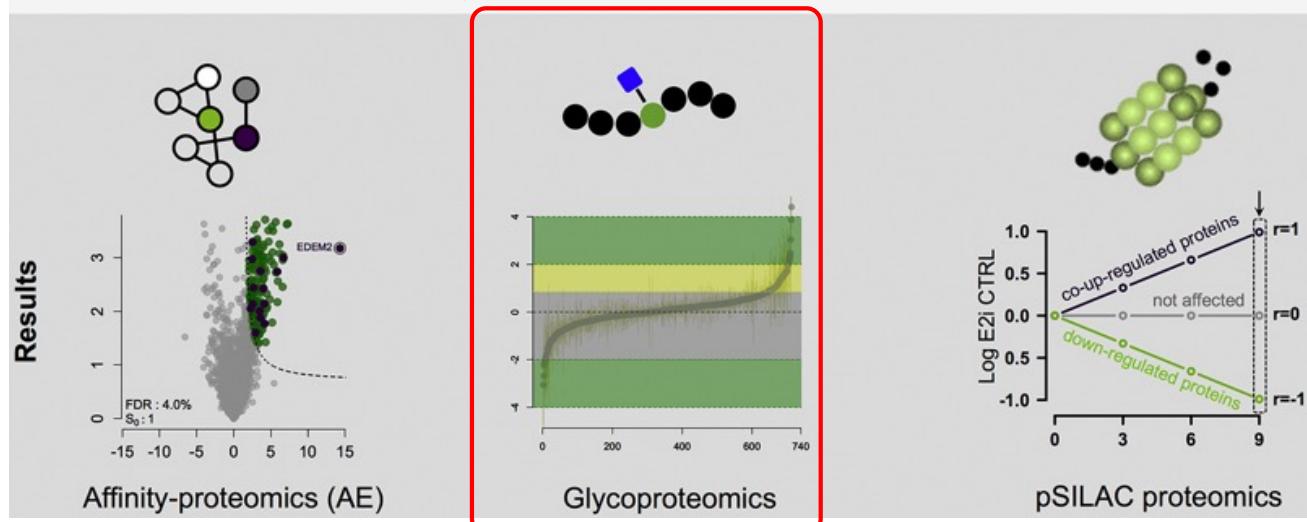
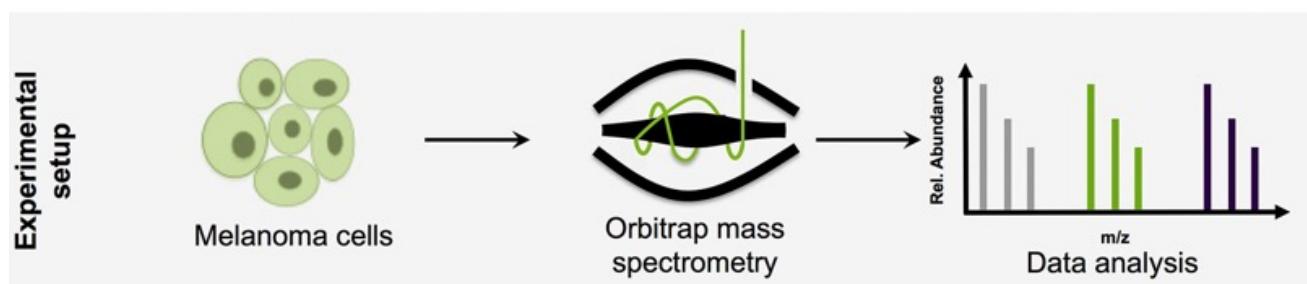
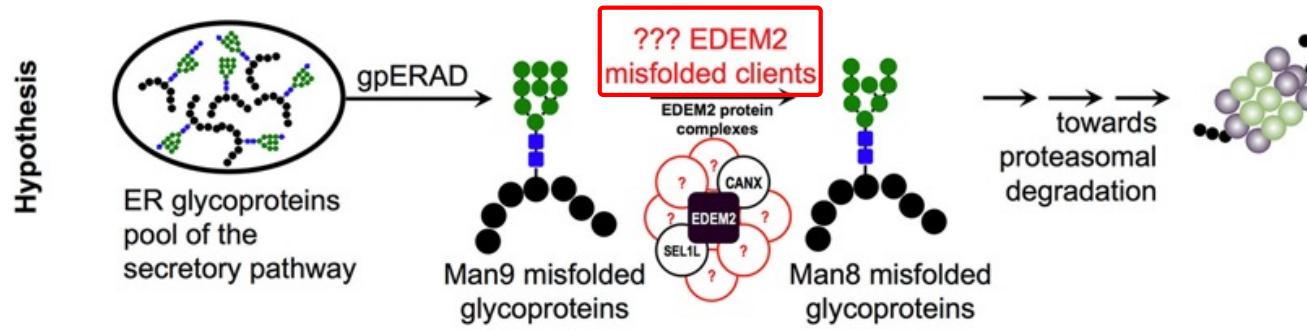
HA-tagged EDEM2 is functional in A375 melanoma cells

CONCLUSIONS

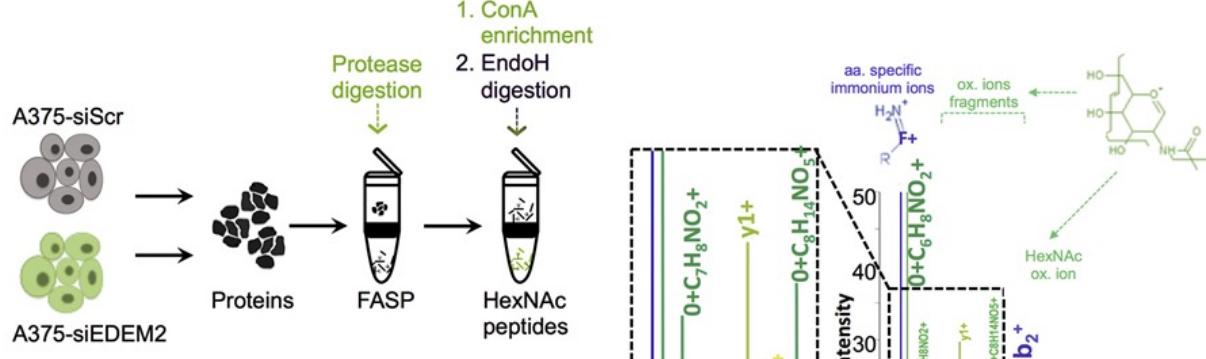


- ST-Tyr is a canonical EDEM2 substrate in A375 melanoma cells
- HA-tagged overexpressed EDEM2 displays a similar activity with the endogenous EDEM2 in A375 melanoma cells
- EDEM2 induced degradation of ST tyrosinase is mannosidase dependent (Kifunensine exps)
- EDEM2 accelerates ST tyrosinase degradation via the ubiquitin-proteasome pathway (MG132 exps)
- EDEM2-dependent misfolded substrates degradation could involve the participation of ERLEC1, SEL1L and DERL1 proteins (co-enrichment and siRNA experiments) -> at least for ST-Tyr degradation

Multiomics and biochemical study of EDEM2 clients



(De)glycoproteomics of A375-ST melanoma cells

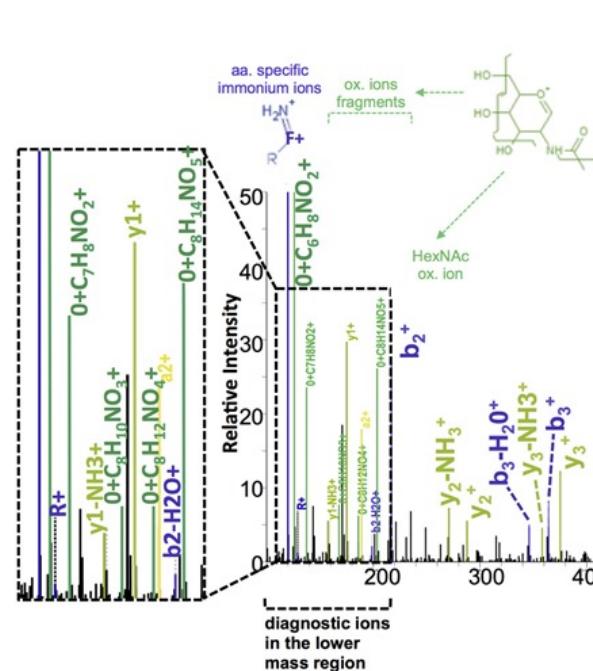
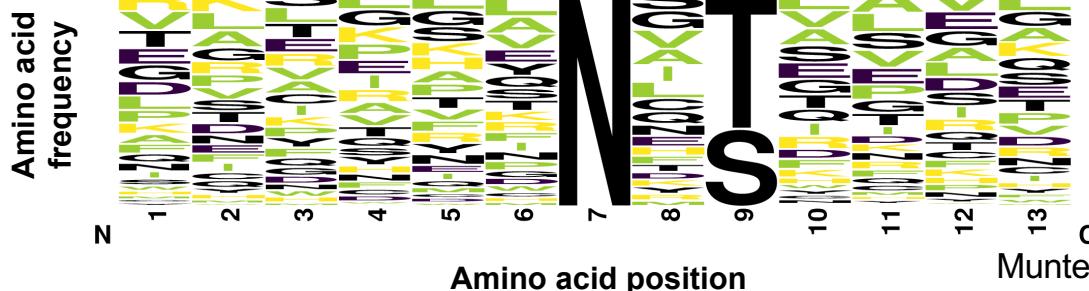


endocytic part lumen vesicle secretory

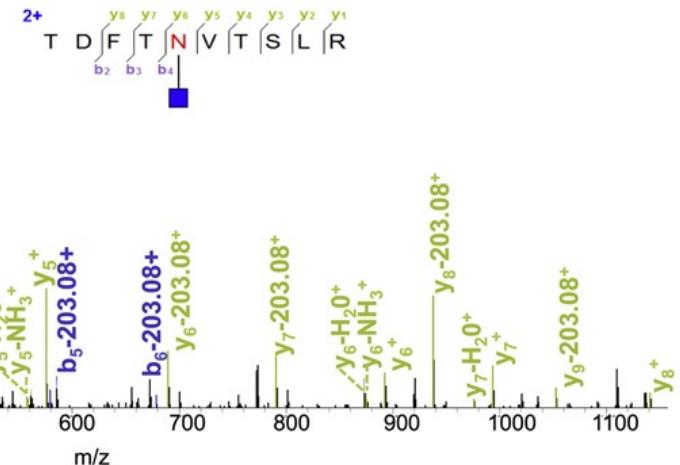
cytoplasmic endoplasmic transport platelet vacuole granule component

membrane

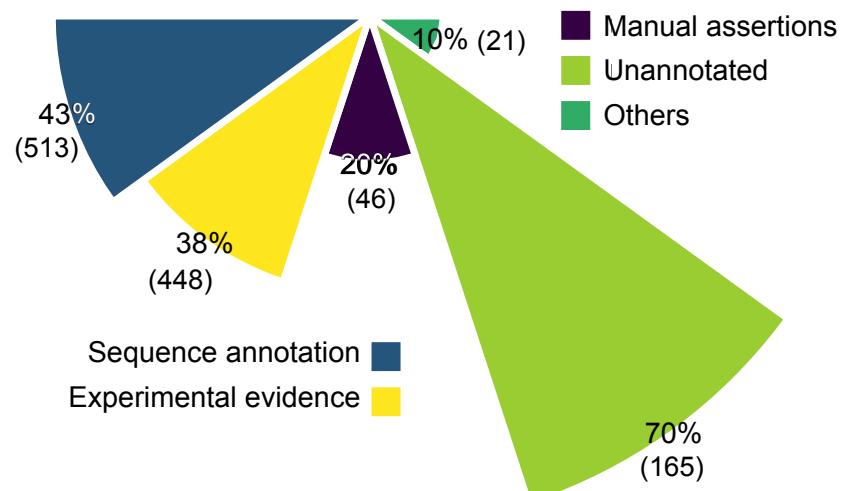
complex endosome Golgi reticulum plasma intrinsic
integral side vacuolar nuclear MHC alpha inner protein organelle compartment



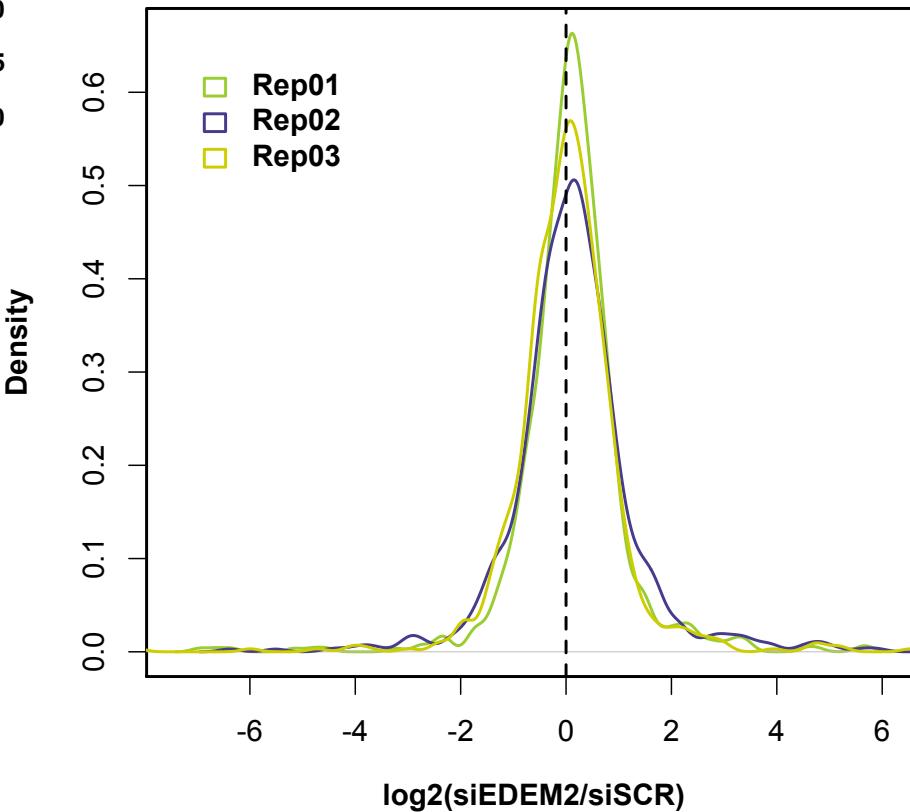
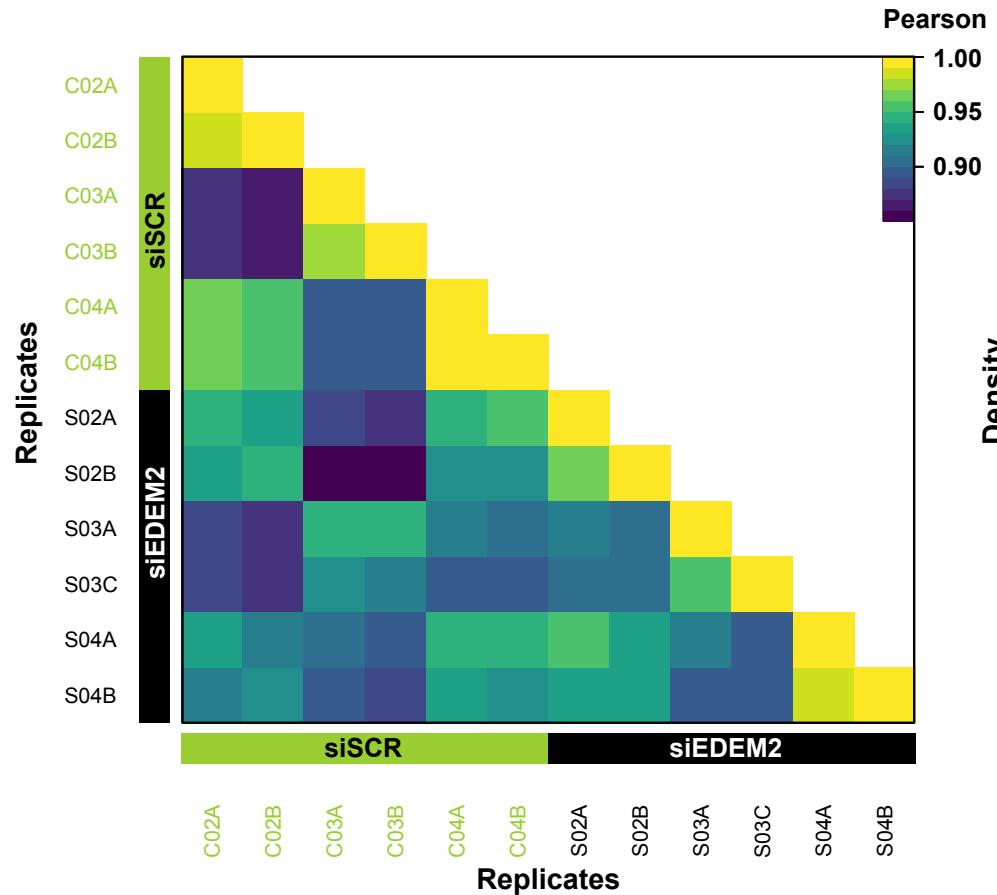
HexNAc modification of SORT1 N406
ms2 678.85@hcd40.00



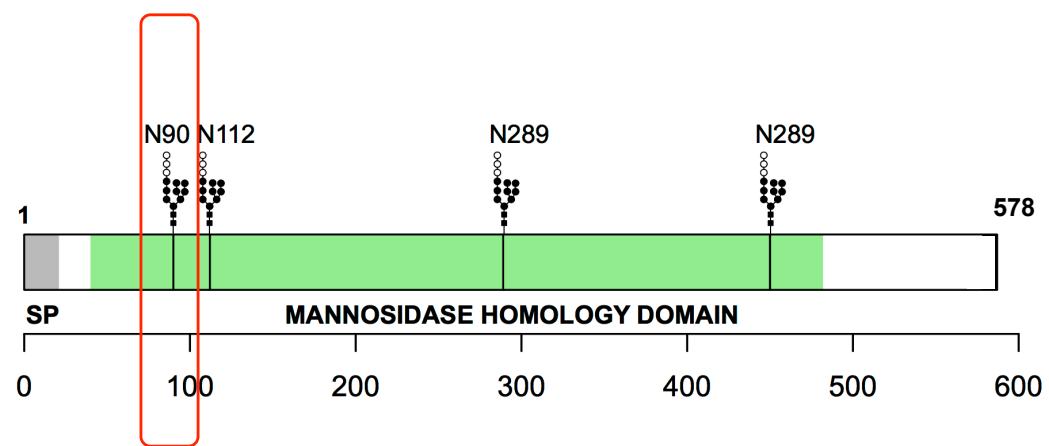
Seq. analysis + Experimental ev. 81% 19% No annotation + Manual assertions



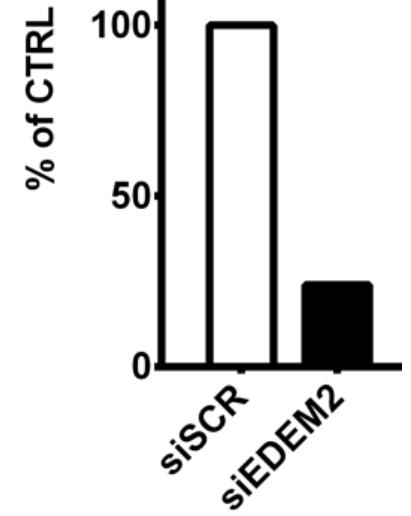
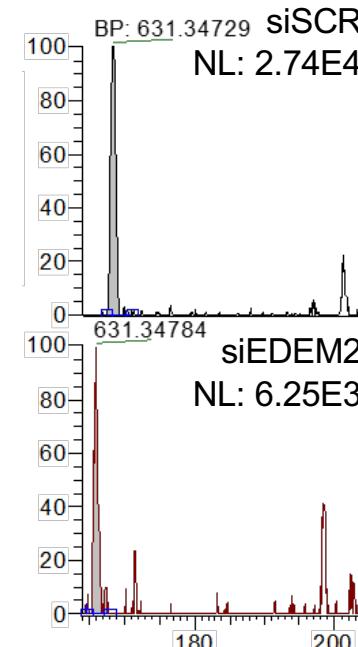
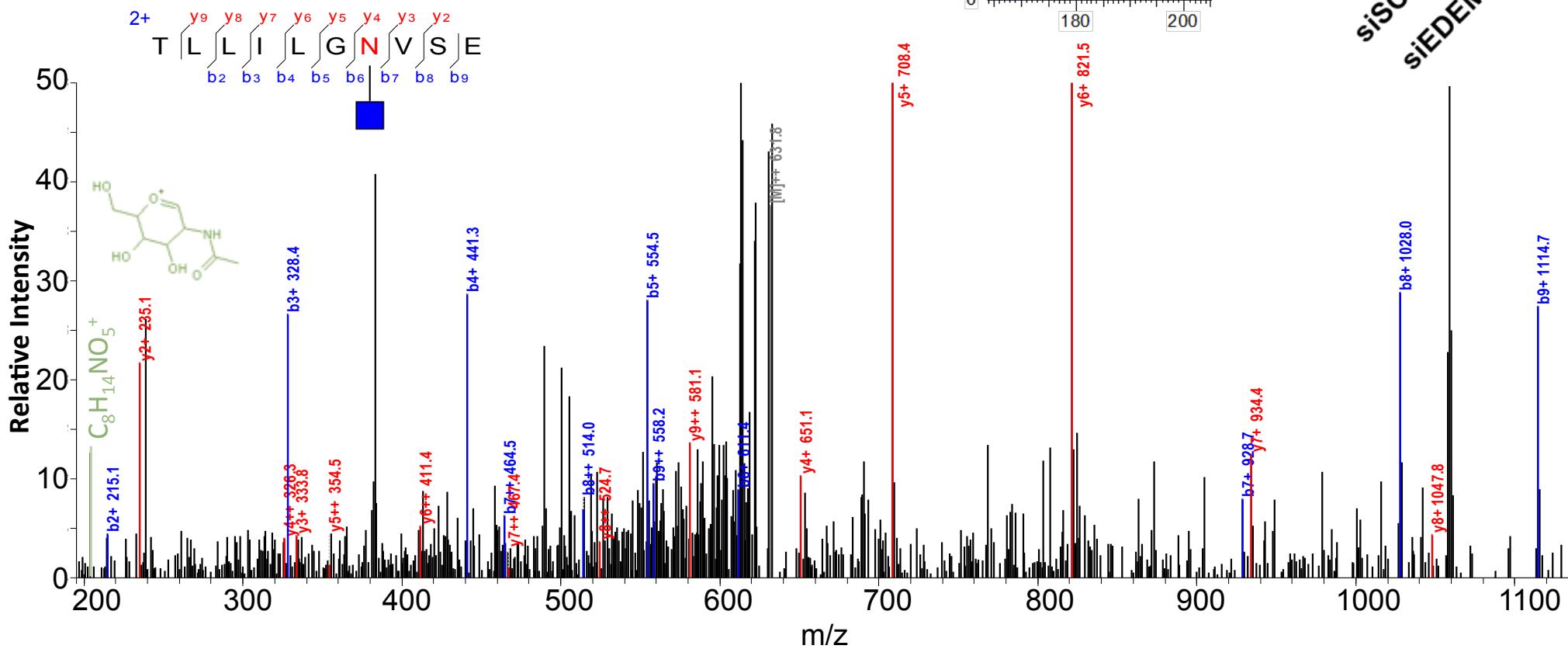
(De)glycoproteomics goes quantitative



Endogenous EDEM2 in A375-ST melanoma cells



HexNAc modification of EDEM2 N90
ms2 631.35@cid35.00



Differential (de)glycoproteomics at glycosite resolution

SAM - Significance Analysis of Microarrays

Observed

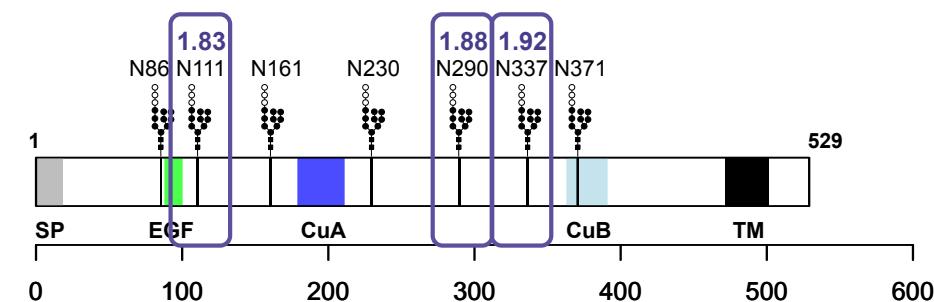
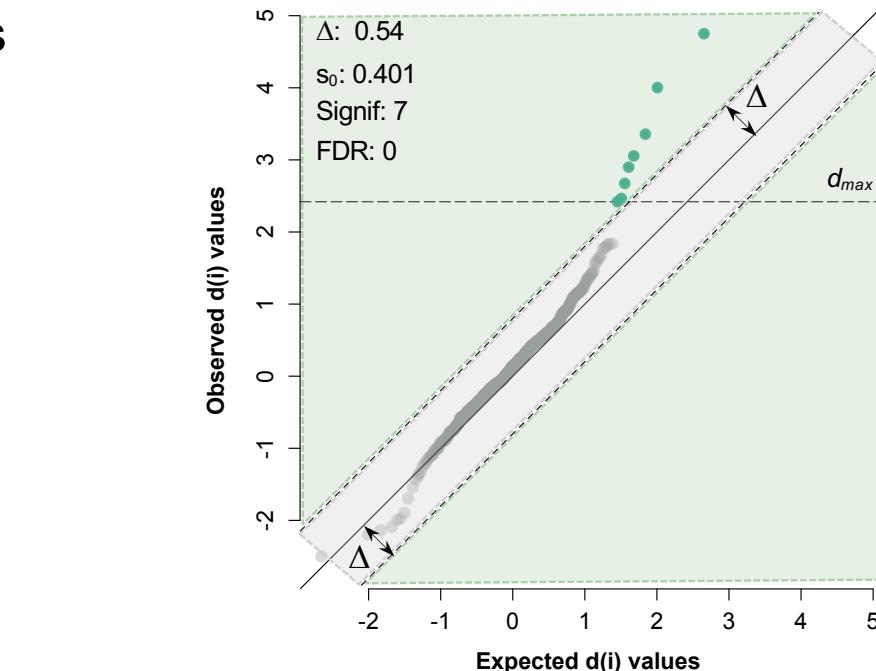
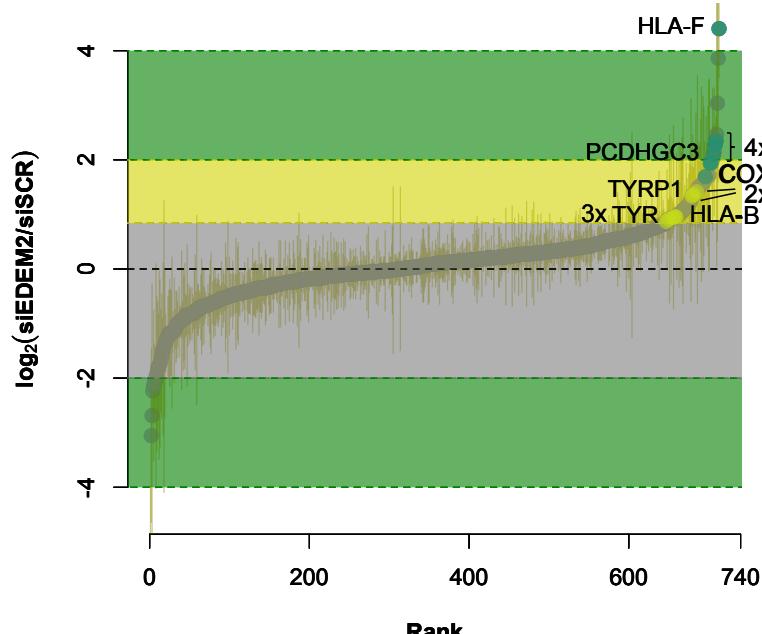
$$d(i) = \frac{\bar{x}_I(i) - \bar{x}_U(i)}{s(i) + s_0}$$

Expected

$$\bar{d}_{(i)} = (1/B) \sum_b d_{(i)}^{*b}$$

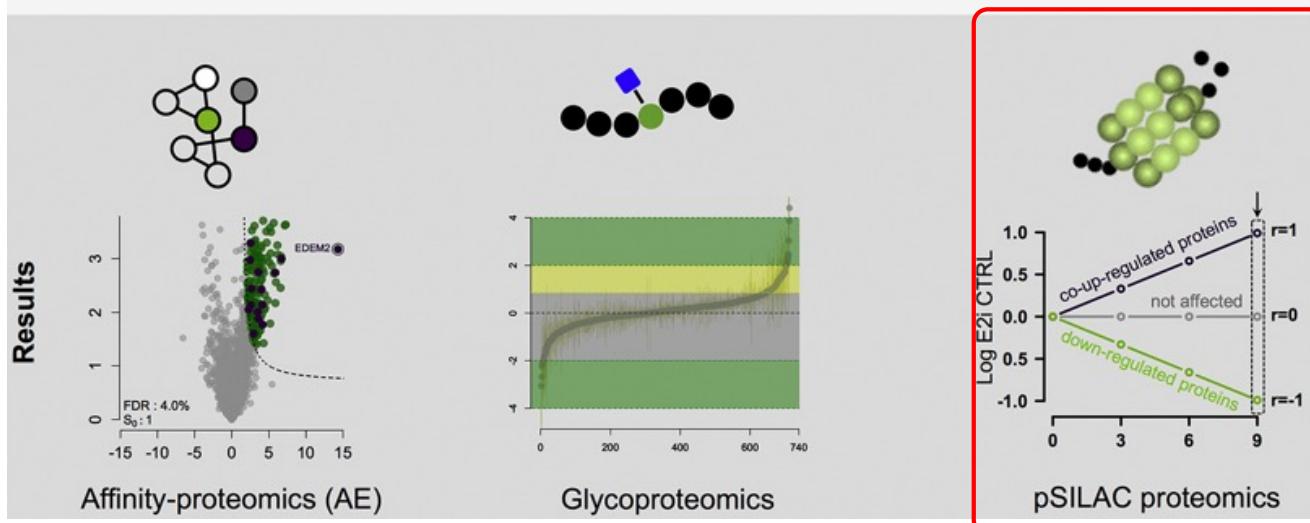
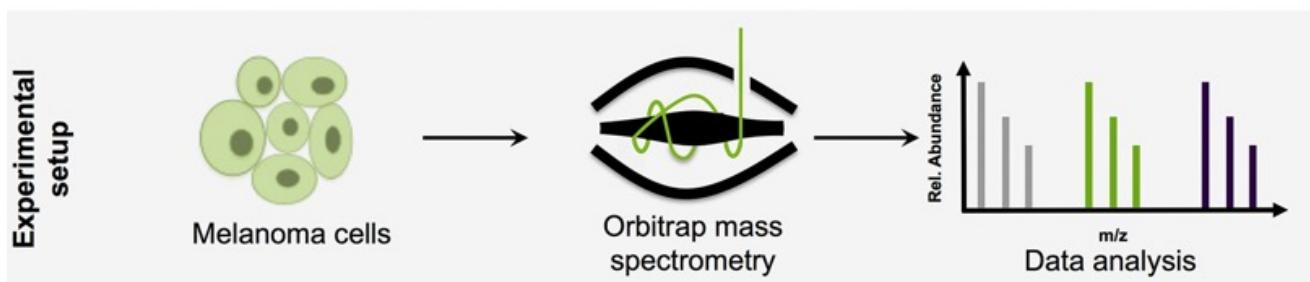
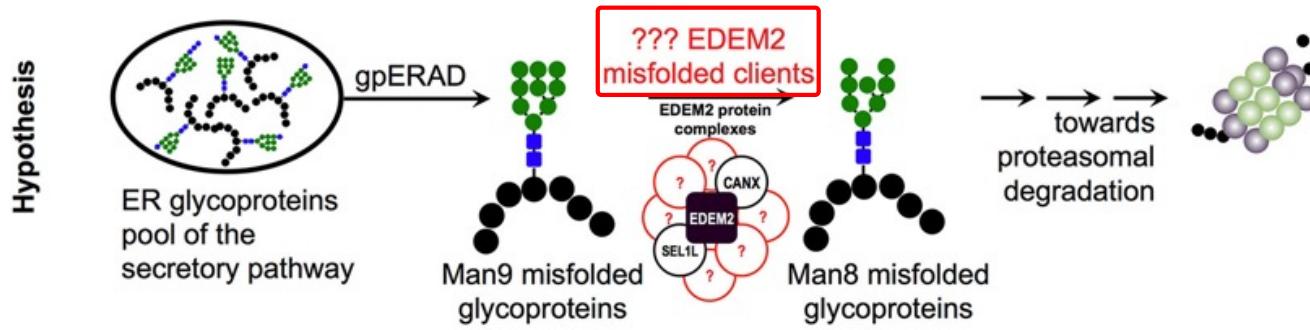
↑
no. of permutations

Tusher VG, Tibshirani R, Chu G., *Proc Natl Acad Sci U S A*. 2001 Apr 24;98(9):5116-21.

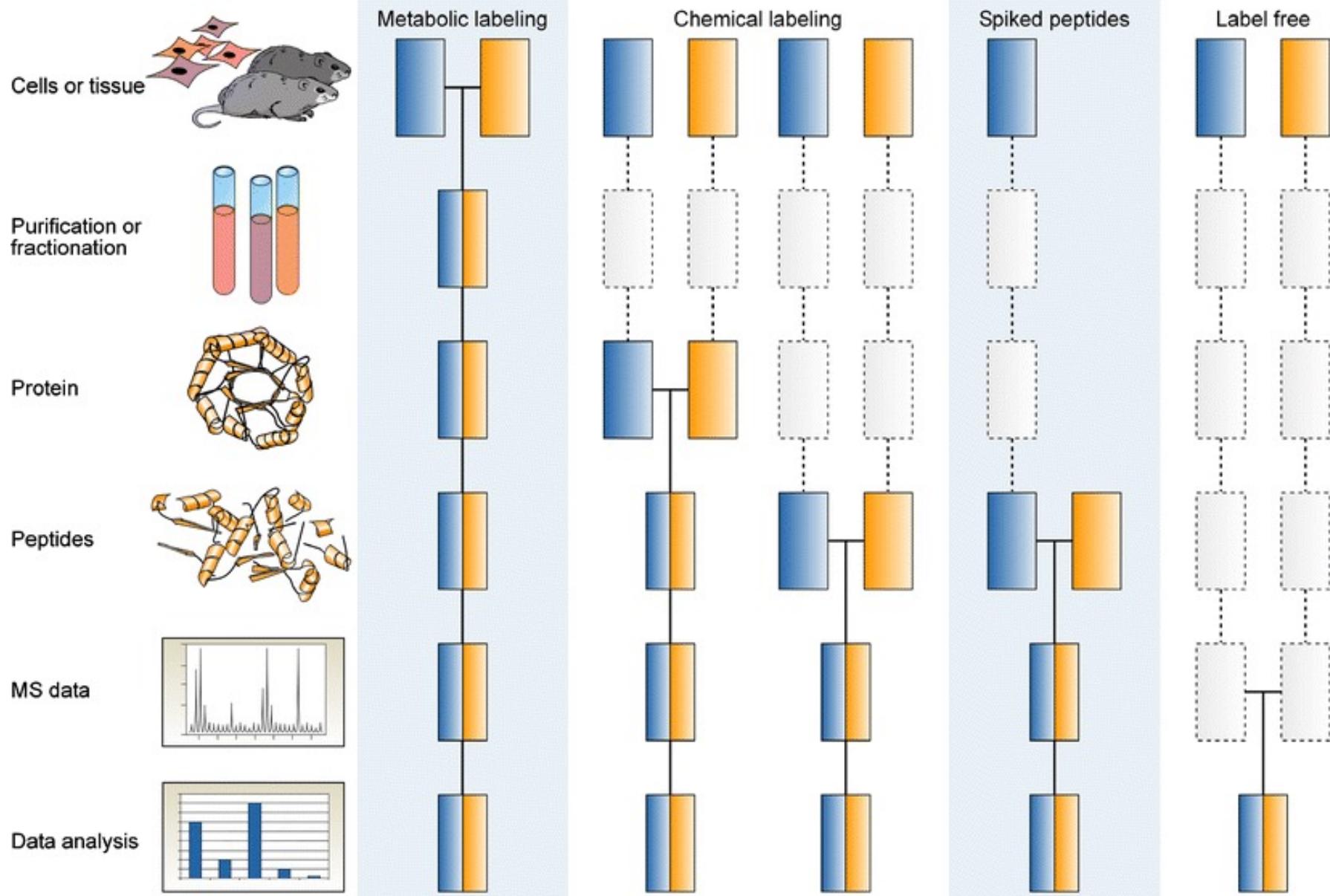


GeneName	ProteinName	Score(d)	FoldChange	#Glycosites
HLA-F	HLA class I histocompatibility antigen, alpha chain F	2.90	15.99	1
ITGA1	Integrin alpha-1	2.36	3.81	5
TYR	Tyrosinase	1.33	1.88	3
HLA-B	HLA class I histocompatibility antigen, B-44/58 alpha chain	0.88	1.94	1

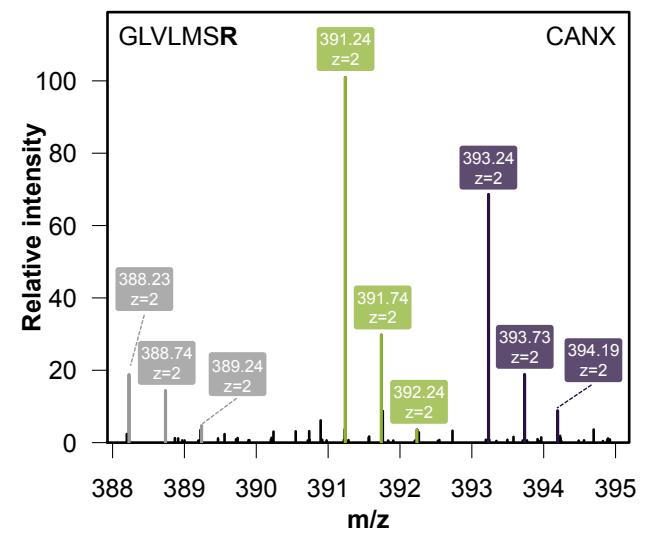
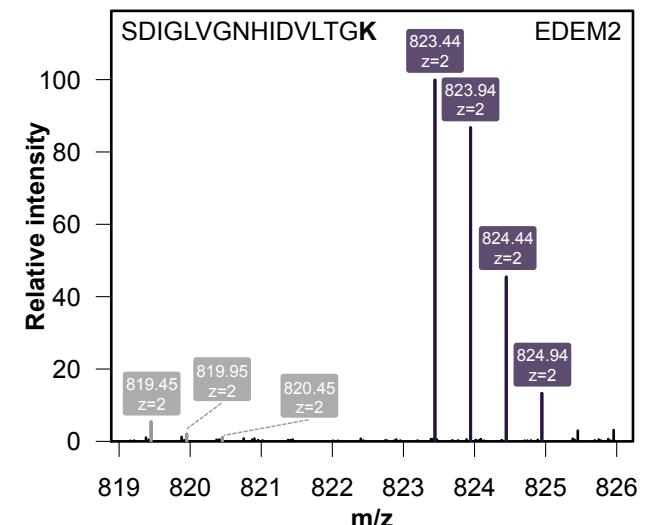
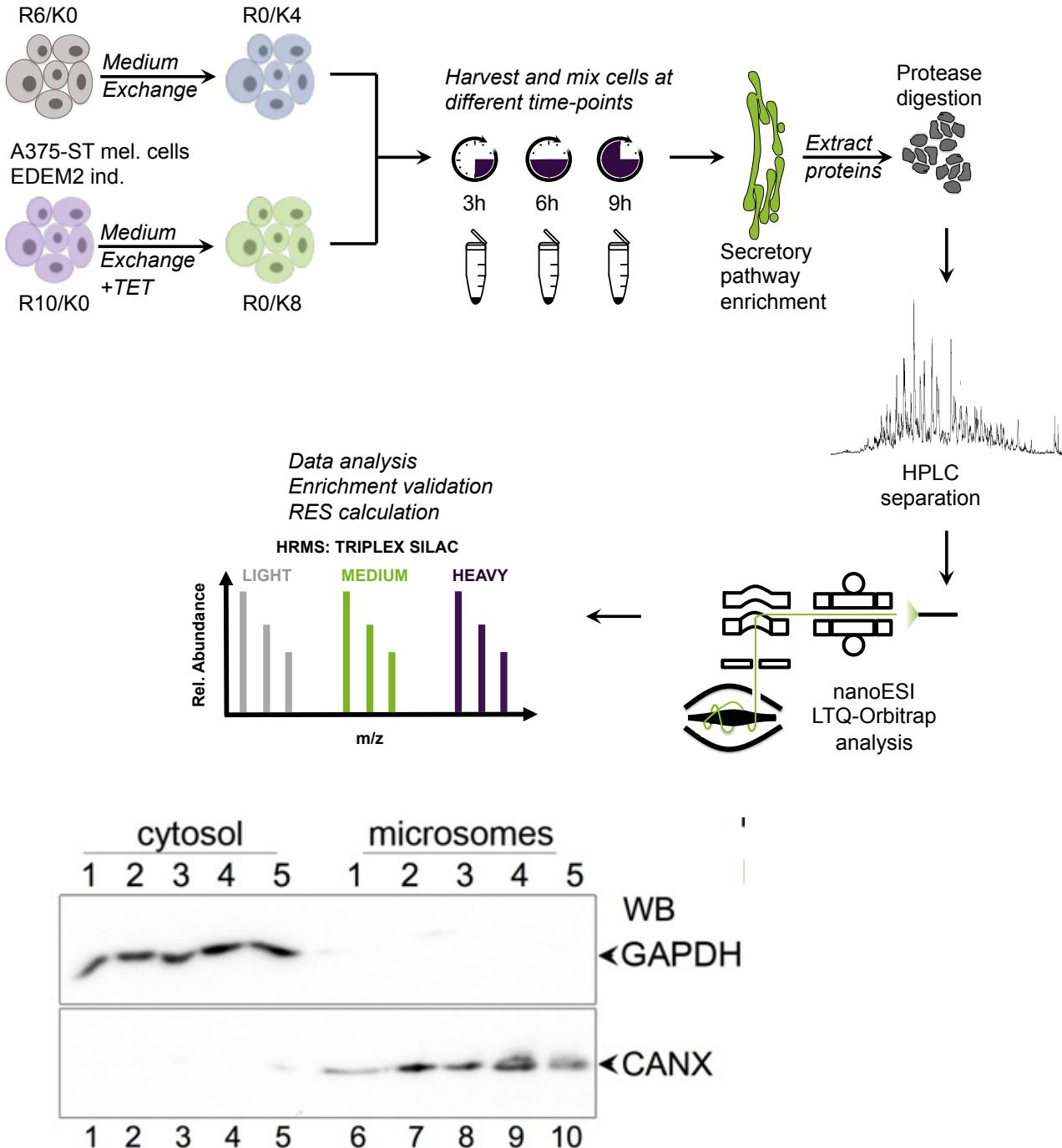
Multiomics and biochemical study of EDEM2 clients



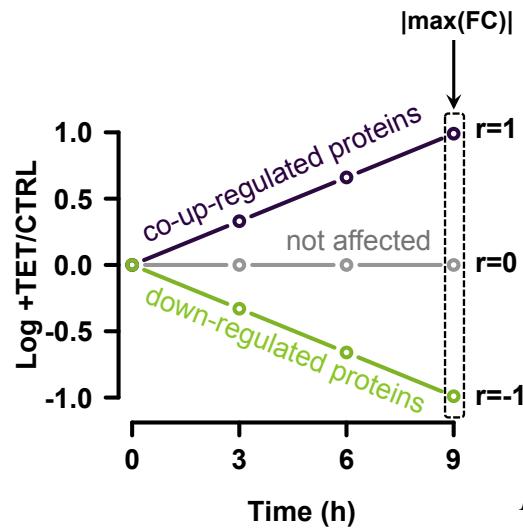
SILAC (Stable Isotope Labeling by Aas. in Cell culture)



Candidate analysis by pSILAC

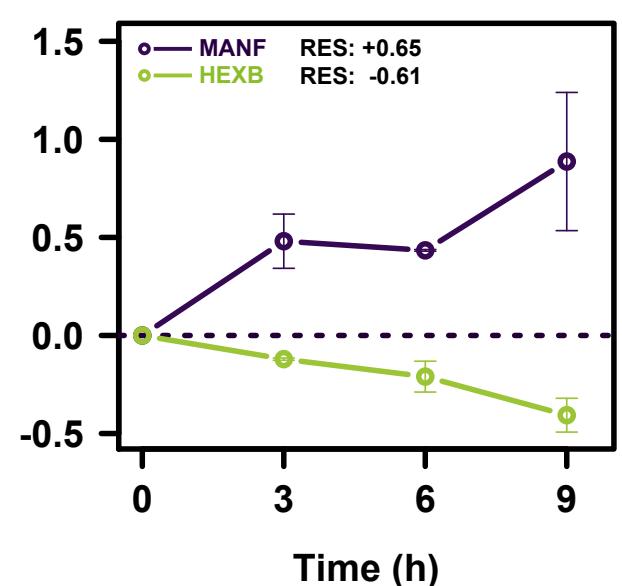
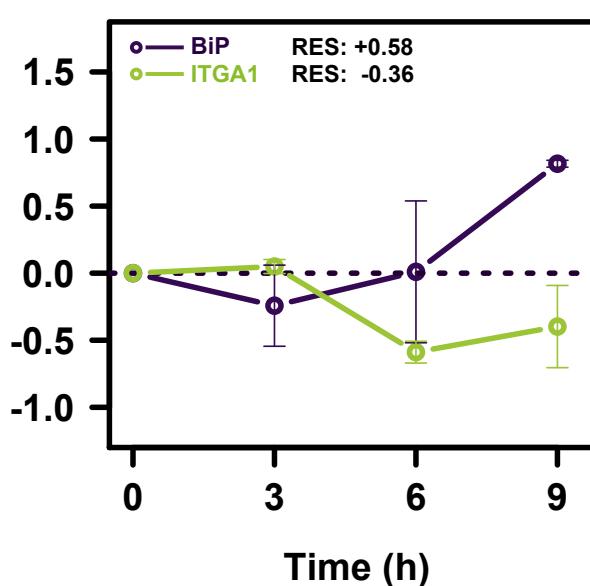
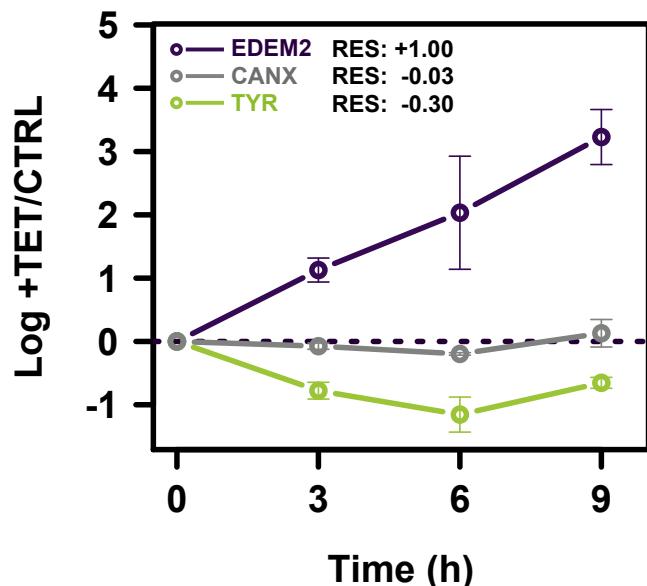
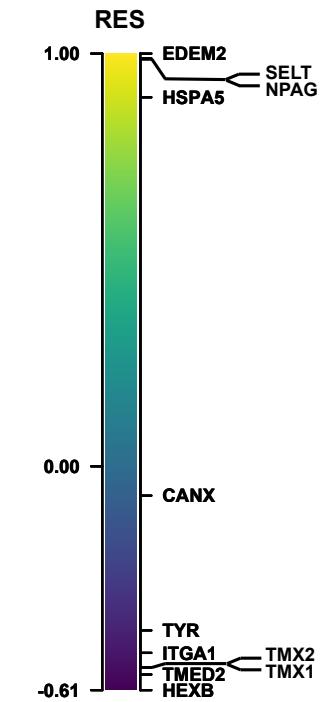
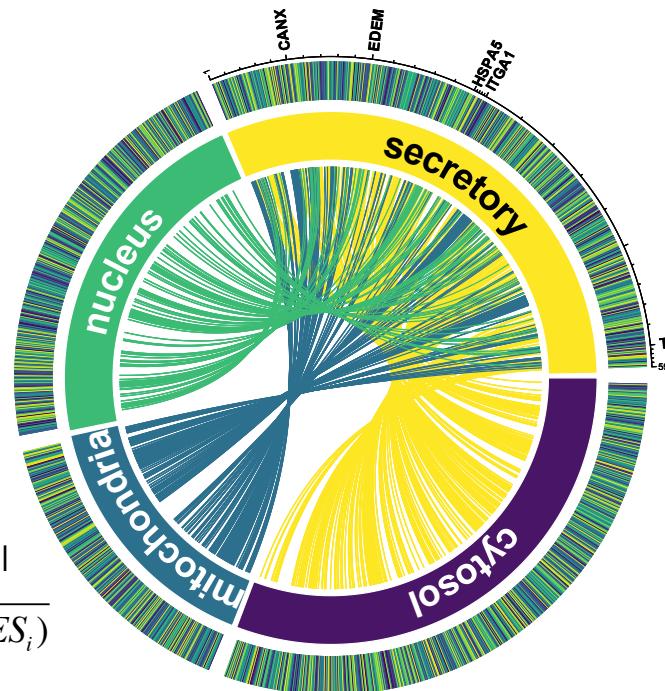


Candidate analysis by pSILAC

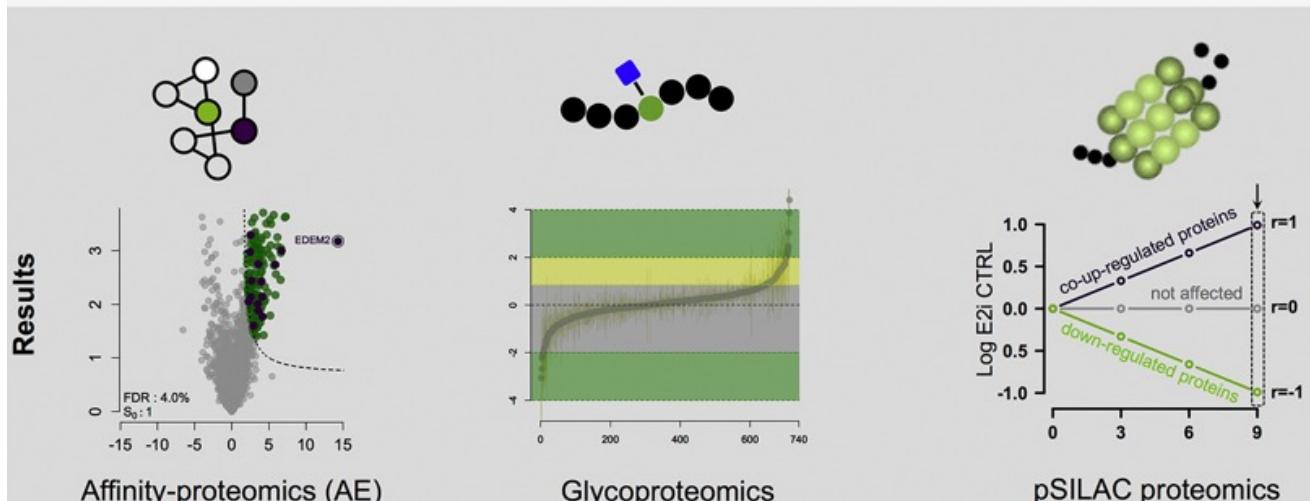
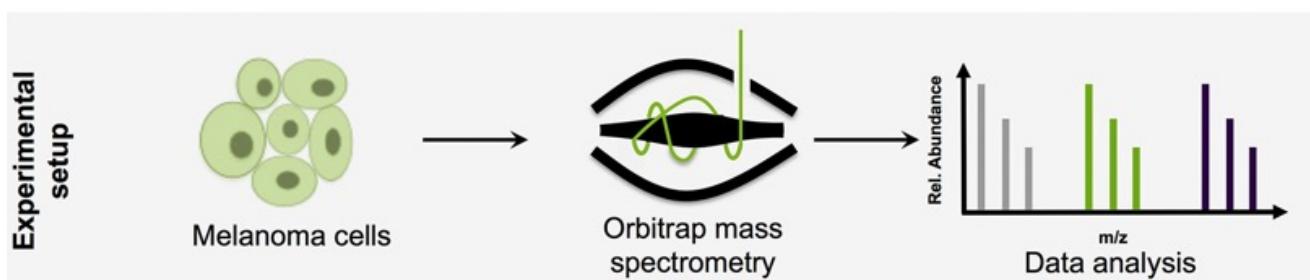
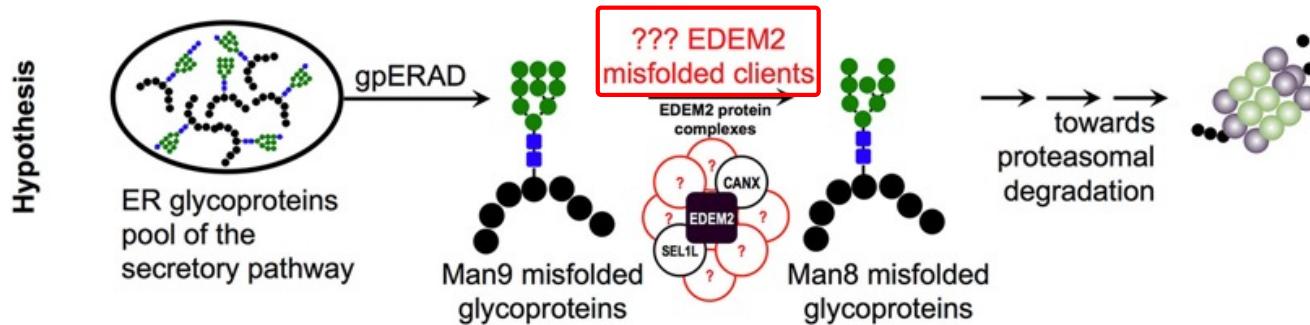


$$ES_i = \frac{1}{2} r_i + \frac{1}{2} |\max(FC_i)|$$

$$RES_i = \frac{\frac{1}{2} r_i + \frac{1}{2} |\max(FC_i)|}{\max(ES_1, ES_2, ES_3, \dots, ES_i)}$$

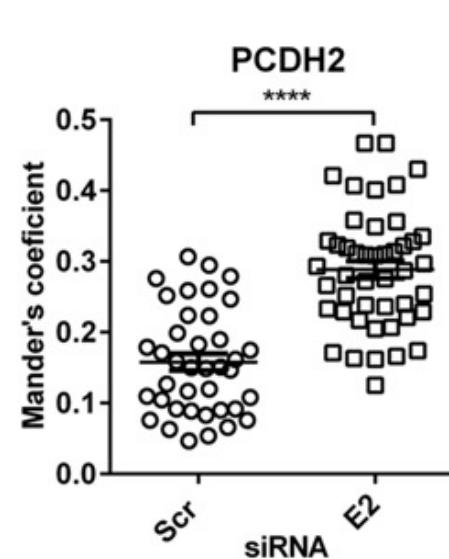
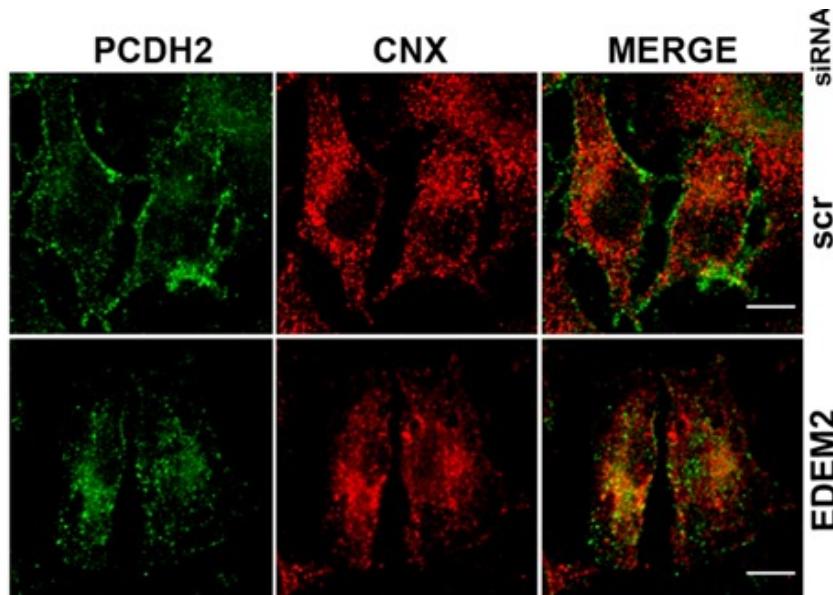
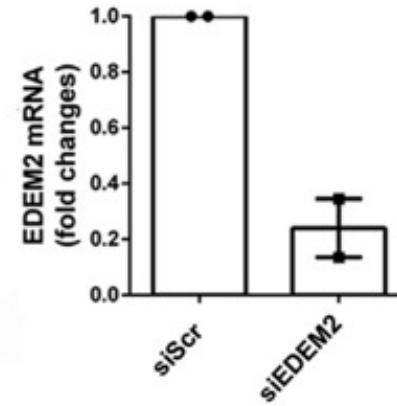
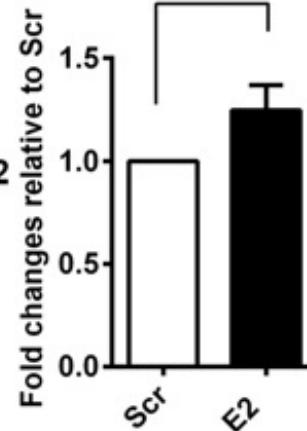
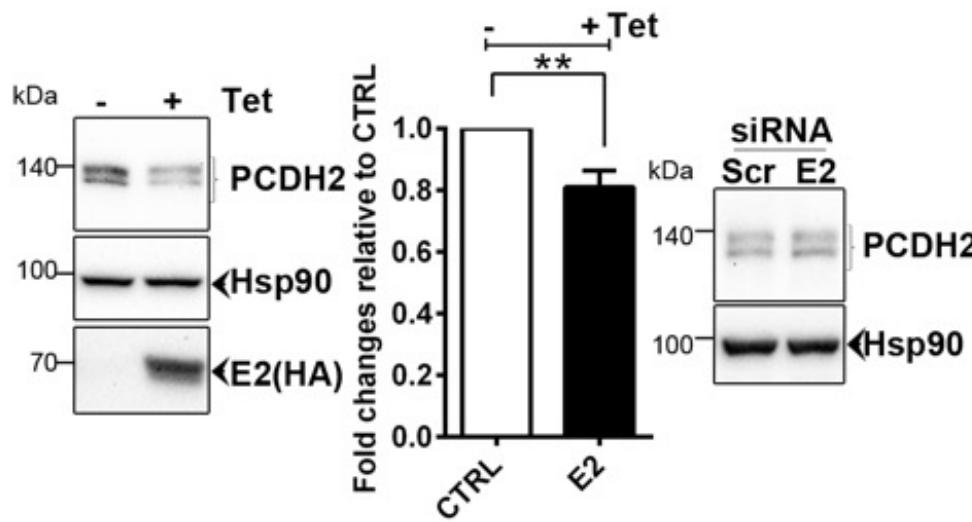


Multiomics and biochemical study of EDEM2 clients



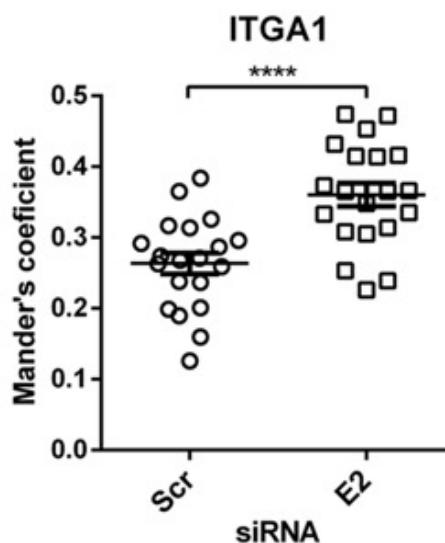
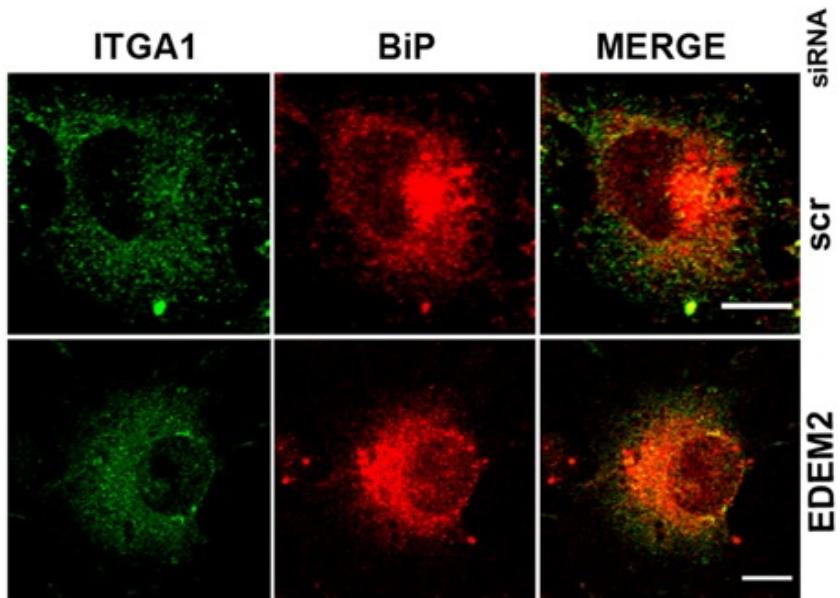
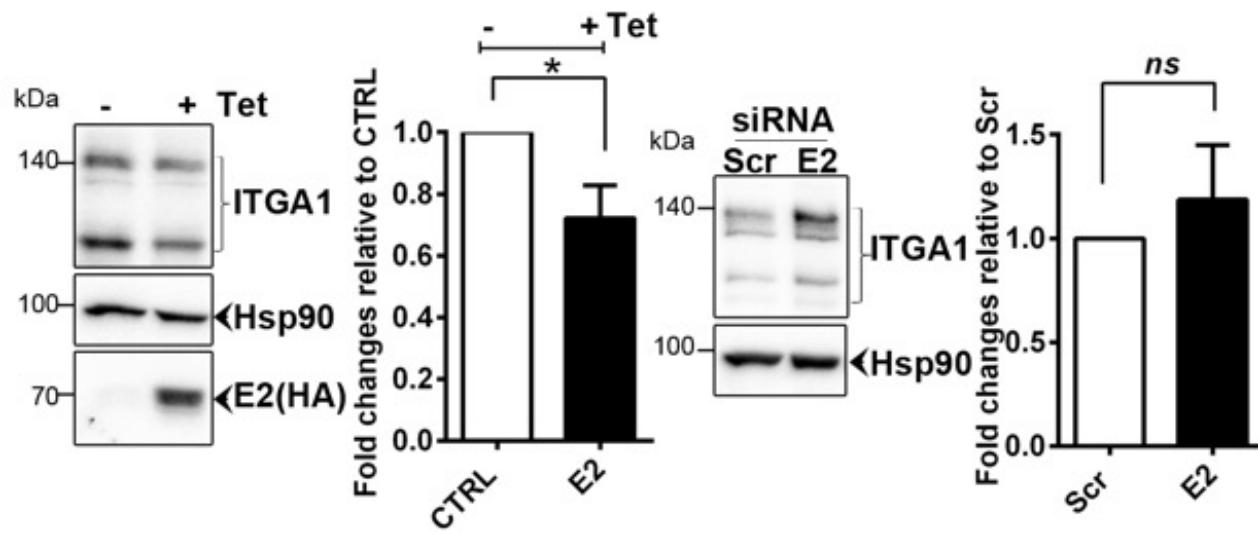
Biochemical validation of EDEM2 candidate clients

PCDH2



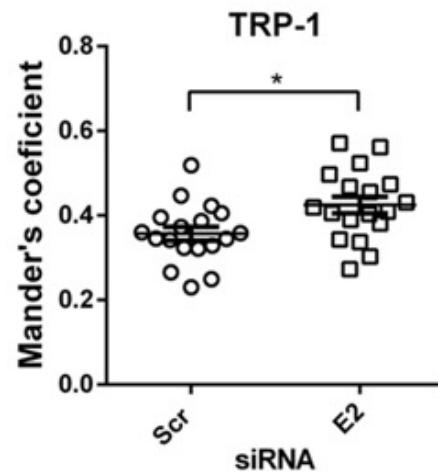
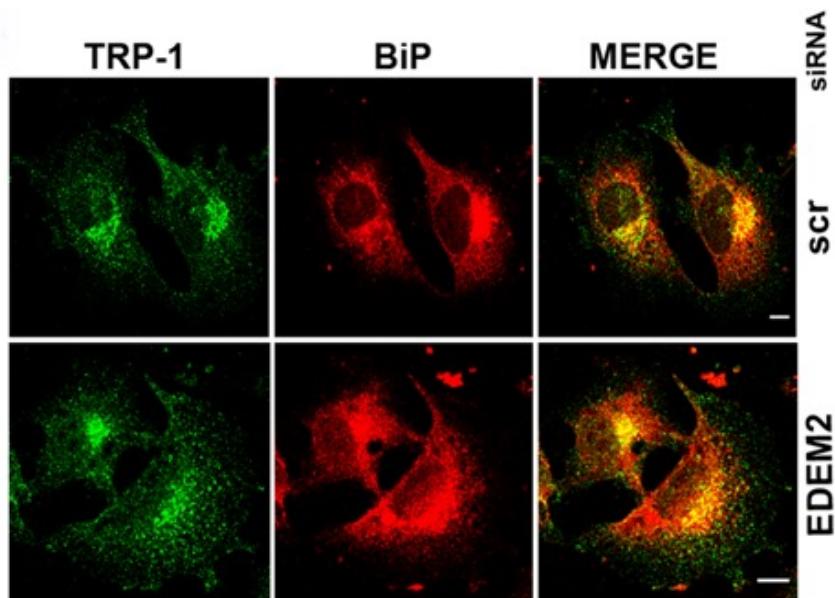
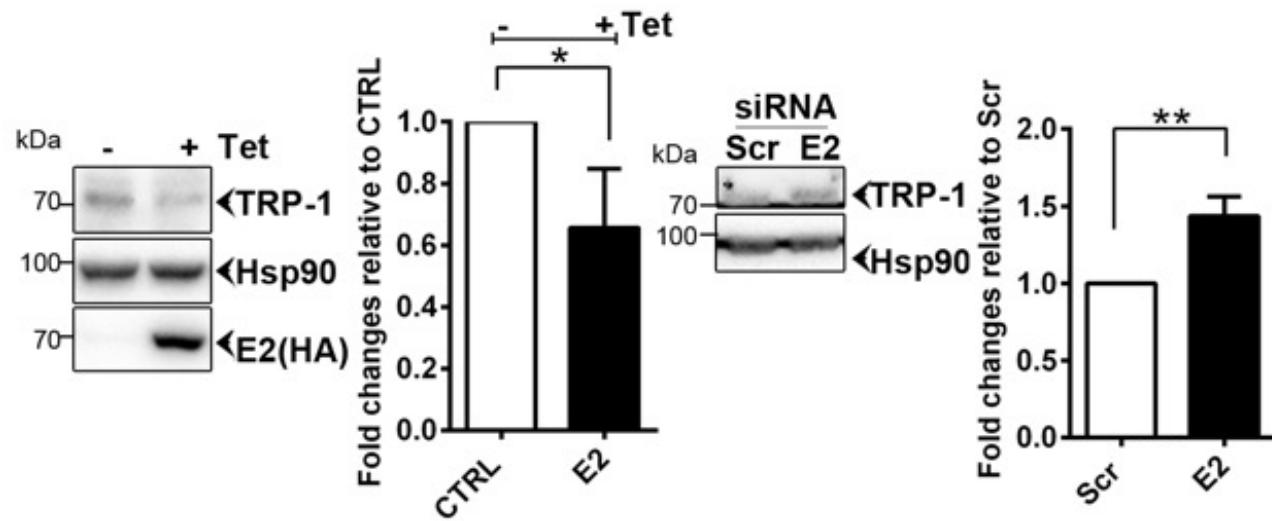
Biochemical validation of EDEM2 candidate clients

ITGA1



Biochemical validation of EDEM2 candidate clients

TRP-1



CONCLUSIONS

- >1000 N-glycosylation sites of proteins were identified in the (De)glycoproteomics workflow
- relative quantification using lectin enrichment and high-resolution mass spectrometry confirmed ST tyrosinase as a potential substrate of EDEM2 in A375 melanoma cells
- potential EDEM2 new endogenous substrates in melanoma cells were mapped with glycosite-specific resolution
- pSILAC analysis confirmed ST-Tyr and ITGA1 as novel EDEM2 substrate candidates
- alternative methods confirmed the expected trends for ST-Tyr, ITGA1, PCDH2 and TRP-1 in A375 melanoma cells with altered EDEM2 levels

Acknowledgments

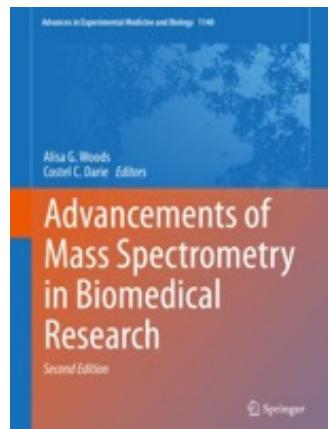
Dr. Ștefana M. Petrescu

Dr. Andrei J. Petrescu

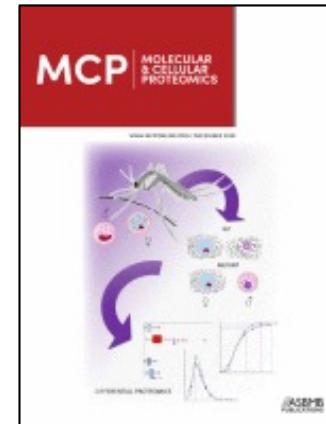
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