Supplementary Material, Methods and Figures

MS Western, a Method of Multiplexed Absolute Protein Quantification is a Practical Alternative to Western Blotting

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Supplementary Material and Methods

Protein analysis by GeLC-MS/MS

Proteins were *in-gel* digested with trypsin (Shevchenko et al, 2006) and peptides extracted from a gel matrix were dried down in a vacuum centrifuge, re-dissolved in 5% aqueous FA and 5 µL were injected using an autosampler into a Dionex Ultimate 3000 nano-HPLC system equipped with 300 µm i.d. × 5 mm trap column and 75 µm × 15 cm Acclaim PepMap100 C18 analytical column. 0.1% aqueous FA and acetonitrile were used as solvents A and B, respectively. Samples were loaded onto the trap column for 5 min with the solvent A at the flow rate of 20 µL/min. Then the trap column was switched on-line to the separation column and flow rate was set to 200nL/min. Peptides were separated using 180 min gradient elution program: a linear gradient from 0% to 30% B was delivered in 145 min and increased to 100% B in 10 min and maintained for another 5 min, dropped to 0%B in 10 min and maintained for another 10 min for column equilibration.

Expression of chimeric proteins

Synthetic genes produced by GeneArt (Thermo Fisher Scientific) or GenScript (Piscataway NJ) were sub-cloned into pET expression vector and transformed into an *E.coli* strain that was dual auxotroph for arginine and lysine ($^{\Delta}$Arg$^{\Delta}$LysBL21 (DE3) T1 pRARE). Fresh transformants were inoculated into a synthetic media MDAG-135 (Studier F.W, 2005) supplemented with antibiotics (100 µg/ml ampicillin and 15 µg/ml chloramphenicol) and incubated overnight at 37°C on a shaking platform. This overnight starting culture was further diluted 1000-fold by PA-5052 and MDAG-135 media supplemented with the same antibiotics and incubated at 37°C until OD_{600} =0.5. Cells in MDAG-135 media were induced by 0.2mM isopropyl β-d-1-thiogalactopyranoside (IPTG), while this was not required in PA-5052 auto-inducing media. After 4 to 6 hr post induction cells were pelleted, re-suspended in

2x phosphate-buffered saline (PBS), aliquoted, snap frozen in liquid nitrogen and stored at -80°C until analysed. Prior analyses frozen aliquots were thawed and cell lysed in an equal volume of 2x Laemmli buffer by incubating at 80°C for 15 minutes. Samples were clarified by centrifugation and the supernatant subjected to 1D SDS PAGE on a 4-20% pre-cast gradient gel. Proteins were visualized by Coomassie staining and full length expression of corresponding synthetic genes was validated by *in-gel* digestion and LC-MS/MS of recovered peptides.

Quantification of target proteins in HeLa cells extracts

HeLa cells were cultured in Dulbecco's modified Eagle's medium supplemented with 10% fetal calf serum and 1% penicillin-streptomycin (Gibco™ Life Technologies). $1x10^7$ HeLa cells were re-suspended in 100 µL RIPA buffer containing Complete Protease Inhibitor Cocktail, EDTA-free (Roche). Equal volume of 2x Laemmli buffer was added and incubated at 80°C for 15 minutes with intermittent vortexing. Next, the sample was incubated with benzonase at 37°C for 30 minutes. The sample was centrifuged and supernatant was transferred to a new vial and further diluted with RIPA / 2x Laemmli buffer (1:1, v/v). The sample obtained at each dilution step was subjected to 1D SDS PAGE on two different gels. One gel was analyzed by WB utilizing the LI-COR Odyssey system and another by MS Western using bands of isotopically labelled CP03 and BSA as the internal standard and reference protein, respectively. To equalize the protein amount analyzed by both methods 50 µL of the extract were loaded on a gel processed by MS Western protocol and an aliquot corresponding to 20% of recovered tryptic peptides was analysed by LC-MS/MS. For the analysis on an Odyssey system 10 µL of extracts were loaded onto 4-12% Bis-Tris gel (NuPAGE, Life Technologies) and proteins blotted onto a nitrocellulose membrane (Amersham Protran 0.45µm, GE Healthcare). Primary antibodies (Supplementary Table S6) were applied and incubated overnight at 4°C. After washing the secondary antibodies

(Supplementary Table S6) were applied in the dark at room temperature for 30 min and the membrane was washed three times. Membranes were scanned with Odyssey Infrared Imager (LI-COR) at the channel 700 for ladder and GAPDH and channel 800 for GOI at highest possible intensity without saturation. Signals were measured using the Image Studio Software (version 2.1) by applying rectangles with background correction 'median left/right 3'. The GOI was normalized to GAPDH.

Knockdown experiments in HeLa cells

For knock-down (KD) experiments $2x10^5$ HeLa cells were seeded in 6 well plates containing 2ml of supplemented DMEM and after 24h the medium was exchanged to 2ml antibiotic-free DMEM. For each transfection, 300 ng of esiRNA (Eupheria Biotech, Dresden, Germany) against the genes of interest (AKT1, CAT, PLK1, and TUBA4A) were dissolved in 200 µl OptiMEM (Gibco™, Life Technologies), combined with 7 µl Oligofectamine reagent (Invitrogen) in 70 µl OptiMEM, incubated for 20 min and then pipetted into the individual wells. All KDs were performed in triplicate; renilla luciferase transfections as RNAi specificity controls were performed on a separate plate. Cells transfected with AKT1-, CAT-, and RLuc-esiRNA were harvested 72h post-transfection (except PLK1 and TUBA4A that were harvested after 24h and 48h, respectively, to avoid cell death) by washing with PBS and detaching cells with 0.05% Trypsin-EDTA (Gibco™ Life Technologies) for 5 min at 37°C. Cells were re-suspended in 1 ml culture media followed by manual counting using disposable chambers FastRead 102 (Biosigma S.r.l., Cona, Italy. Cell suspension contained in each well was transferred into a 1.5 ml Eppendorf tube and pelleted by spinning at 0.1 rcf at room temperature. The supernatant was aspirated and discarded and cells were washed twice with 1ml PBS. Cell pellets were re-suspended in 100 µl RIPA-buffer containing protease inhibitors, aliquoted and stored at -20°C until analysis. Aliquots of the thawed pellet were analysed by MS Western and Odyssey WB as described above. Further details on the

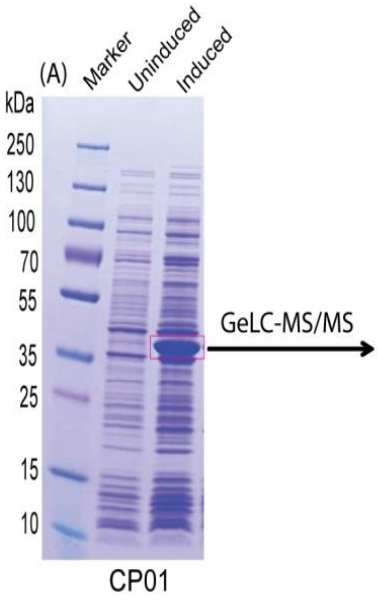
employed antibodies and esiRNA probes are provided in Supplementary Tables S6 and S7, respectively.

Supplementary Figures

Twin strep tag-3C cleavage site- GP (1-8)-Ubiquitin (9-12)-ADH (13-18)-Enolase (19-25)-BSA (26-31)-His-tag

MGSAWSHPQFEKGGGSGGGSGGSAWSHPQFEKLEVLFQGPAAAKVFADYEEYVK$_{1}$DFYELEPH
K$_{2}$VAAAFPGDVDR$_{3}$GLAGVENVTELK$_{4}$IGEEYISDLDQLR$_{5}$VIFLENYR$_{6}$LLSYVDDEAFIR$_{7}$VLVDLE
R$_{8}$TLSDYNIQK$_{9}$ESTLHLVLR$_{10}$TITLEVEPSDTIENVK$_{11}$EGIPPDQQR$_{12}$IGDYAGIK$_{13}$VLGIDGGEGK
$_{14}$EALDFFAR$_{15}$VVGLSTLPEIYEK$_{16}$ANELLINVK$_{17}$GVIFYESHGK$_{18}$GNPTVEVELTTEK$_{19}$TFAEALR
$_{20}$AADALLLK$_{21}$TAGIQIVADDLTVTNPK$_{22}$NVNDVIAPAFVK$_{23}$VNQIGTLSESIK$_{24}$AVDDFLISLDGT
ANK$_{25}$HLVDEPQNLIK$_{26}$YLYEIAR$_{27}$QTALVELLK$_{28}$LGEYGFQNALIVR$_{29}$DAFLGSFLYEYSR$_{30}$LVN
ELTEFAK$_{31}$GSGHHHHHH

Fig. S1: Sequence design of the chimeric protein CP01. The proteotypic peptides were selected from five commercially available proteins: Glycogen phosphorylase (GP), Ubiquitin, Alcohol dehydrogenase (ADH), Enolase and BSA. The peptides originating from the respective proteins are colour coded as exemplified in the header. Numbers in parenthesis represent the peptides numbers in the assembled chimera sequence which belongs to the corresponding proteins, e.g. peptide 1 to 8 belong to GP; peptides 9 to 12 belong to Ubiquitin *etc*. The sequence string assembled from sequences of proteotypic peptides (here peptide 1 to peptide 31) is flanked with twin-strep-tag and his-tag at the N- and C-terminus, respectively. The cleavage site for the 3C protease is placed after the twin-strep-tag.



(B)

Nominal mass (M_r): **42298**; Calculated pI value: **4.78**

Fixed modifications: Carbamidomethyl(C), Label: 13C (6) (K), Label: 13C (6)15N (4) (R)

Variable modifications: Acetyl (Protein N-term), Oxidation (M)

Cleavage by Trypsin: cuts C-term side of KR unless next residue is P

Sequence Coverage: **99%**

Matched peptides shown in **Bold Red**

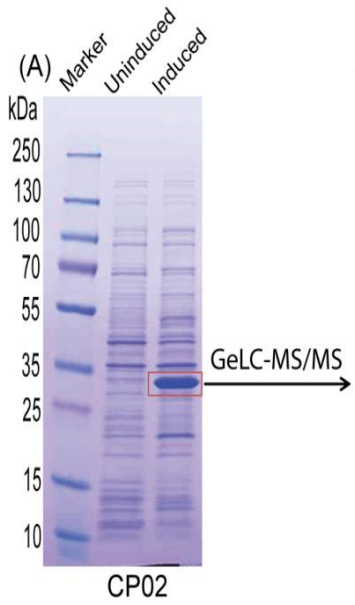
1 MGSAWSHPQFEKGGGSGGGSGGSAWSHPQFEKLEVLFQGPAAAKVFADYE
51 EYVKDFYELEPHKVAAAFPGDVDRGLAGVENVTELKIGEEYISDLDQLRV
101 IFLENYRLLSYVDDEAFIRVLVDLERTLSDYNIQKESTLHLVLRTITLEV
151 EPSDTIENVKEGIPPDQQRIGDYAGIKVLGIDGGEGKEALDFFARVVGLS
201 TLPEIYEKANELLINVKGVIFYESHGKGNPTVEVELTTEKTFAEALRAAD
251 ALLLKTAGIQIVADDLTVTNPKNVNDVIAPAFVKVNQIGTLSESIKAVDD
301 FLISLDGTANKHLVDEPQNLIKYLYEIARQTALVELLKLGEYGFQNALIV
351 RDAFLGSFLYEYSRLVNELTEFAKGSGHHHHHH

Fig. S2: Expression and validation of the sequence of chimeric protein CP01. (**A**) 1D SDS-PAGE analysis (4-20% gradient gel) showing the overexpressed CP01 (**B**) in-gel digestion of CP01 with trypsin followed by LC-MS/MS analysis of recovered tryptic peptides confirms its accurate full length expression. MS/MS spectra were mapped to the CP01 sequence by the Mascot search (mass accuracy 5 ppm for precursor and 0.3Da for fragment ions; two missed cleavage site allowed) and indicated its 100% coverage.

Twin strep tag-3C cleavage site-GP (1-4)-H4 (5-9)-H2B (10-14)-H2A (15-16, 26)-H3 (17-19)-BSA (20-25)-His tag

MGSAWSHPQFEKGGGSGGGSGGSAWSHPQFEKLEVLFQGPAAAKVFADYEEYVK$_{1}$DFYELEPH
K$_{2}$VAAAFPGDVDR$_{3}$GLAGVENVTELK$_{4}$VFLENVIR$_{5}$DAVTYTEHAK$_{6}$ISGLIYEETR$_{7}$TVTAMDVVY
ALK$_{8}$DNIQGITKPAIR$_{9}$ESYAIYVYK$_{10}$AMGIMNSFVNDIFER$_{11}$LLLPGELAK$_{12}$QVHPDTGISSK$_{13}$EIQ
TAVR$_{14}$AGLQFPVGR$_{15}$HIQLAVR$_{16}$STELLIR$_{17}$EIAQDFK$_{18}$YRPGTVALR$_{19}$HLVDEPQNLIK$_{20}$YLYEI
AR$_{21}$QTALVELLK$_{22}$LGEYGFQNALIVR$_{23}$DAFLGSFLYEYSR$_{24}$LVNELTEFAK$_{25}$GSGHHHHHHREP
NAGTEAQSQDF$_{26}$

Fig. S3: Sequence design of the chimeric protein CP02. The proteotypic peptides were selected from four histones (H2A, H2B, H3 and H4) from zebrafish (*D.rerio*). The reference peptides were selected from glycogen phosphorylase (GP) and BSA and were placed at the N- and C-terminus respectively. The peptides originating from the respective proteins are colour coded as exemplified in the header. Numbers in parenthesis represent the peptides numbers in the assembled chimera sequence which belongs to the corresponding proteins, e.g. peptide 1 to 4 belong to GP; peptides 5 to 9 belong to Histone H4 *etc*. The sequence string assembled from sequences of proteotypic peptides (here peptide 1 to 25) is flanked with twin-strep-tag and his-tag at the N- and C-terminus, respectively. The cleavage site for the 3C protease was placed after the twin-strep-tag.



(B)

Nominal mass (M_r): **35036**; Calculated pI value: **5.72**

Fixed modifications: Carbamidomethyl(C), Label: 13C (6) (K), Label: 13C (6)15 N (4) (R)

Variable modifications: Acetyl (Protein N-term), Oxidation (M)

Cleavage by Trypsin: cuts C-term side of KR unless next residue is P

Sequence Coverage: **100%**

Matched peptides shown in **Bold Red**

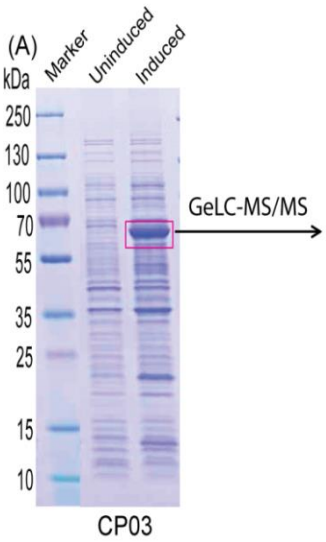
1 **MGSAWSHPQFEKGGGSGGGSGGSAWSHPQFEKLEVLFQGPAAAKVFADYE**
51 **EYVKDFYELEPHKVAAAFPGDVDRGLAGVENVTELKVFLENVIRDAVTYT**
101 **EHAKISGLIYEETRTVTAMDVVYALKDNIQGITKPAIRESYAIYVYKAMG**
151 **IMNSFVNDIFERLLLPGELAKQVHPDTGISSKEIQTAVRAGLQFPVGRHI**
201 **QLAVRSTELLIREIAQDFKYRPGTVALRHLVDEPQNLIKYLYEIARQTAL**
251 **VELLKLGEYGFQNALIVRDAFLGSFLYEYSRLVNELTEFAKGSGHHHHHH**
301 **REPNAGTEAQSQDF**

Fig. S4: Expression and validation of the sequence of chimeric protein CP02. (**A**) 1D SDS-PAGE analysis (4-20% gel) showing the overexpressed CP02 (**B**) in-gel digestion of CP02 with trypsin followed by LC-MS/MS analysis of recovered tryptic peptides confirms its accurate full length expression. MS/MS spectra were mapped to the CP02 sequence by the Mascot search (mass accuracy 5 ppm for precursor and 0.3Da for fragment ions; two missed cleavage site allowed) and indicated its 100% coverage.

Twin strep tag-3C cleavage site-GP (1-4)-PLK1 (5-10)-AKT1 (11-16)-PTEN (17-22)-Catalase (23-29)-GAPDH (30-35)-Tubulin (36-41)-BSA (42-47)-His tag

MGSAWSHPQFEKGGGSGGGSGGSAWSHPQFEKLEVLFQGPAAAK<u>VFADYEEYVK</u>$_1$<u>DFYELEPH</u>
<u>K</u>$_2$<u>VAAAFPGDVDR</u>$_3$<u>GLAGVENVTELK</u>$_4$<u>LILYNDGDSLQYIER</u>$_5$<u>AGVPGVAAPGAPAAAPPAK</u>$_6$<u>LGN</u>
<u>LFLNEDLEVK</u>$_7$<u>QEEAEDPACIPIFWVSK</u>$_8$<u>HINPVAASLIQK</u>$_9$<u>FSIAPSSLDPSNR</u>$_{10}$<u>LFELILMEEIR</u>$_{11}$<u>DE</u>
<u>VAHTLTENR</u>$_{12}$<u>NDGTFIGYK</u>$_{13}$<u>CLQWTTVIER</u>$_{14}$<u>TFCGTPEYLAPEVLEDNDYGR</u>$_{15}$<u>HPFLTALK</u>$_{16}$<u>IYS</u>
<u>SNSGPTR</u>$_{17}$<u>YVYYYSYLLK</u>$_{18}$<u>FMYFEFPQPLPVCGDIK</u>$_{19}$<u>NNIDDVVR</u>$_{20}$<u>AQEALDFYGEVR</u>$_{21}$<u>IYNLCA</u>
<u>ER</u>$_{22}$<u>LSQEDPDYGIR</u>$_{23}$<u>NAIHTFVQSGSHLAAR</u>$_{24}$<u>DLFNAIATGK</u>$_{25}$<u>FNTANDDNVTQVR</u>$_{26}$<u>NLSVEDAA</u>
<u>R</u>$_{27}$<u>AFYVNVLNEEQR</u>$_{28}$<u>DPILFPSFIHSQK</u>$_{29}$<u>VPTANVSVVDLTCR</u>$_{30}$<u>GALQNIIPASTGAAK</u>$_{31}$<u>VIHDNF</u>
<u>GIVEGLMTTVHAITATQK</u>$_{32}$<u>LVINGNPITIFQER</u>$_{33}$<u>VGVNGFGR</u>$_{34}$<u>LISWYDNEFGYSNR</u>$_{35}$<u>VGINYQPP</u>
<u>TVVPGGDLAK</u>$_{36}$<u>TIGGGDDSFNTFFSETGAGK</u>$_{37}$<u>AVFVDLEPTVIDEVR</u>$_{38}$<u>NLDIERPTYTNLNR</u>$_{39}$<u>IHF</u>
<u>PLATYAPVISAEK</u>$_{40}$<u>QLFHPEQLITGK</u>$_{41}$<u>HLVDEPQNLIK</u>$_{42}$<u>YLYEIAR</u>$_{43}$<u>QTALVELLK</u>$_{44}$<u>LGEYGFQN</u>
<u>ALIVR</u>$_{45}$<u>DAFLGSFLYEYSR</u>$_{46}$<u>LVNELTEFAK</u>$_{47}$GSGHHHHHH

Fig. S5: Sequence design of the chimeric protein CP03. The proteotypic peptides were selected from six proteins (PLK1, AKT1, PTEN, Catalase, GAPDH and α-Tubulin) from HeLa cells. The reference peptides were selected from glycogen phosphorylase (GP) and BSA and were placed at the N- and C-terminus respectively. The peptides originating from the respective proteins are colour coded as exemplified in the header. Numbers in parenthesis represent the peptides numbers in the assembled chimera sequence which belongs to the corresponding proteins, e.g. peptide 1 to 4 belong to GP; peptides 5 to 10 belongs to PLK1 *etc*. The sequence string assembled from sequences of proteotypic peptides (here peptide 1 to 47) is flanked with twin-strep-tag and his-tag at the N- and C-terminus, respectively. The cleavage site for the 3C protease was placed after the twin-strep-tag.



(B)

Nominal mass (M_r): **72398**; Calculated pI value: **5.01**

Fixed modifications: Carbamidomethyl(C), Label: 13 C (6) (K), Label: 13 C (6)15 N (4) (R)

Variable modifications: Acetyl (Protein N-term), Oxidation (M)

Cleavage by Trypsin: cuts C-term side of KR unless next residue is P

Sequence Coverage: **98%**

Matched peptides shown in **Bold Red**

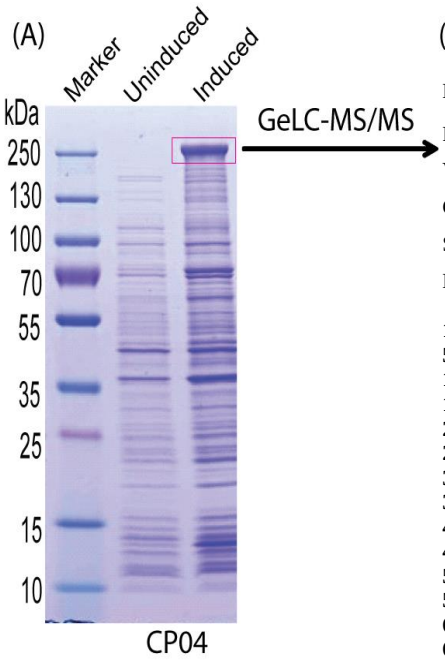
1 M**GSAWSHPQFEKGGGSGGGSGGSAWSHPQFEKLEVLFQGPAAAKVFADYE**
51 **EYVKDFYELEPHKVAAAFPGDVDRGLAGVENVTELKLILYNDGDSLQYIE**
101 **RAGVPGVAAPGAPAAAPPAKLGNLFLNEDLEVKQEEAEDPACIPIFWVSK**
151 **HINPVAASLIQKFSIAPSSLDPSNRLFELILMEEIRDEVAHTLTENRNDG**
201 **TFIGYKCLQWTTVIERTFCGTPEYLAPEVLEDNDYGRHPFLTALKIYSSN**
251 **SGPTRYVYYYSYLLKFMYFEFPQPLPVCGDIKNNIDDVVRAQEALDFYGE**
301 **VRIYNLCAERLSQEDPDYGIRNAIHTFVQSGSHLAARDLFNAIATGKFNT**
351 **ANDDNVTQVRNLSVEDAARAFYVNVLNEEQRDPILFPSFIHSQKVPTANV**
401 **SVVDLTCRGALQNIIPASTGAAKVIHDNFGIVEGLMTTVHAITATQKLVI**
451 **NGNPITIFQERVGVNGFGRLISWYDNEFGYSNRVGINYQPPTVVPGGDLA**
501 **KTIGGGDDSFNTFFSETGAGKAVFVDLEPTVIDEVRNLDIERPTYTNLNR**
551 **IHFPLATYAPVISAEKQLFHPEQLITGKHLVDEPQNLIKYLYEIARQTAL**
601 **VELLKLGEYGFQNALIVRDAFLGSFLYEYSRLVNELTEFAK**GSGHHHHHH
651

Fig. S6: Expression and validation of the sequence of chimeric protein CP03. (**A**) 1D SDS-PAGE analysis (4-20% gel) showing the overexpressed CP03 (**B**) in-gel digestion of CP03 with trypsin followed by LC-MS/MS analysis of recovered tryptic peptides confirms its accurate full length expression. MS/MS spectra were mapped to the CP03 sequence by the Mascot search (mass accuracy 5 ppm for precursor and 0.3Da for fragment ions; two missed cleavage site allowed) and indicated its 100% coverage.

Twin strep tag-3C cleavage site-GP (1-4)-187 target peptides (5-191) from 43 different eye proteins from *Drosophila melanogaster*-BSA (192-197)-His tag

MGSAWSHPQFEKGGGSGGGSGGSAWSHPQFEKLEVLFQGPAAAKVFADYEEYVK$_{1}$DFYELEPHK$_{2}$VAAAFPGDVDR$_{3}$GLAG
VENVTELK$_{4}$GSTLSAQQGSQFK$_{5}$NEPVSYILELINGR$_{6}$NIGDSYVAAK$_{7}$LDNGYNHLIEVVR$_{8}$LVLGIPTYGR$_{9}$FSPLVASNER$_{10}$LT
EAEGSSLYIGGR$_{11}$STDAEEDPQVIK$_{12}$FADQDNDLVNLR$_{13}$LSNFVDTTVAWLNYR$_{14}$FFVSVTR$_{15}$VQLTDLNR$_{16}$DVFIPDVFNNY
K$_{17}$ILAIFPFPGPSQYINVVPYLK$_{18}$VPIWPYTLYFR$_{19}$GTFFVSHK$_{20}$GHEISVFSLTHK$_{21}$APLGLHFHASWLK$_{22}$IINNPEATQR$_{23}$AV
YWVEHVSR$_{24}$SHYHVGSALAK$_{25}$AEQNGYGVTVHYEELSSAK$_{26}$GSAYAHAENTLR$_{27}$SEGDLTQITPPSALGVQVR$_{28}$AEVEAVQI
IAESLK$_{29}$AANQLTDRPTIINVAISPSSDR$_{30}$GHGAGGADVLTYK$_{31}$DLNQGNSYVQDK$_{32}$SLVFVDNHDNQR$_{33}$SEYTGLGAITEFR$_{34}$
VVEFLDHLIDLGVAGFR$_{35}$IGALDTSR$_{36}$LIELLVR$_{37}$LADLLLQEER$_{38}$VNGEPVDVGAFFIR$_{39}$ITPDVVGAIVQEATTYFR$_{40}$ATQLS
EQLGSELTPK$_{41}$LLAADYADGVSQPR$_{42}$ILSWNAVNLYGLK$_{43}$ASEQPGLTAIHTAFLR$_{44}$LLPAQYEDGISAPR$_{45}$GIATNDVGIAK$_{46}$
DTFSFLTGSGR$_{47}$AINEGGFQSLPQK$_{48}$FGLNEGSEPQAYGIGLK$_{49}$ITTHYTLNPR$_{50}$AILVYLVEK$_{51}$ITPGWEENWAGALDVK$_{52}$IN
PQHSIPTLVDNGFTIWESR$_{53}$ALGLEFNK$_{54}$INLDPYALYVIVEK$_{55}$TNFDDSALFYASGESLK$_{56}$VLTTGGDAGSNR$_{57}$LGSDVQPPG
R$_{58}$VFLQLLENQR$_{59}$NLSQTFGNIWR$_{60}$VFDLPDFLTWR$_{61}$GSTVQFAAR$_{62}$DGAISHVVFK$_{63}$YFNAYLSDR$_{64}$FSEPYDSTLSDVIK$_{65}$N
VANLEATK$_{66}$DADAIVQQTLAK$_{67}$GTQAEIVVADVTK$_{68}$VGDVTEVAEAVAFLASSK$_{69}$SVLLTLR$_{70}$IDALEGVYK$_{71}$ALNLDFDYK$_{7}$
$_{2}$AITFPLFWENK$_{73}$LTLYGIDGSPPVR$_{74}$ELPYYEEANGSR$_{75}$AVGVELNK$_{76}$APADPEAFK$_{77}$AIQVYLVEK$_{78}$LLNLQAGEHLKPEFL
K$_{79}$INPQHTIPTLVDNGFALWESR$_{80}$GLVVSGTR$_{81}$SLSDVSLTGR$_{82}$GNSYLILPPPR$_{83}$ISGPSNHVTVVR$_{83}$NAAFSGDSYVSHR$_{84}$SV
VNVGLGQALR$_{85}$STQEEVDHIR$_{86}$SAVHDVEVFLK$_{87}$VSTAIDAISVSGR$_{88}$VALQIQDVATSSR$_{89}$LEEISLR$_{90}$LLELHDNR$_{91}$LFNNFD
VLR$_{92}$LAVLDLSHNR$_{93}$ASLSGIQSHAFK$_{94}$TFFDGNPIHTLR$_{95}$DFGVELEDLQITR$_{96}$STISSTTTVR$_{97}$TSTTSSLTGNPR$_{98}$TSVATVA
GGAVVGATK$_{99}$LLVPGSSSTTTTSSLR$_{100}$LLPDTTDEQLLTSALEEK$_{101}$STGNIFAAK$_{102}$VFAVNSAGR$_{103}$IFADNVYGR$_{104}$GVAED
FAPSFVK$_{105}$HDGGSPITGYIIEK$_{106}$HYPNPAVR$_{107}$VVGSEADTGR$_{108}$DAPTTESYLR$_{109}$STVPFATSESNR$_{110}$LVGGNDLDTPLIITR$_{11}$
$_{1}$IAELEGLGSR$_{112}$IQYLEDSTR$_{113}$IGEAISQSSIR$_{114}$FITQIVDVTK$_{115}$AEDAGVYLVVAR$_{116}$TYYDFGFVALDIIK$_{117}$VWQVSVSAHA
LLWDLNDGK$_{118}$DVLSVAFSADNR$_{119}$LWDLAAGK$_{120}$LNNDLIAR$_{121}$FQEALAGLSK$_{122}$LAAHDALGGAAK$_{123}$SAGSGVSTTAIEK$_{12}$
$_{4}$IVQVQIDDVGK$_{125}$SELDVFSDWLQVAR$_{126}$FSQSDFGLDQGETLLR$_{127}$ESLLEITIYHQK$_{128}$GAAYQEAPVDAEVAVTPK$_{129}$ISAFG
LFTYSVFSIIAGSLK$_{130}$VFANPVQLEFYGFVPWSIIQR$_{131}$QVGASSGSSGAVSK$_{132}$IVQIQQVGPAHLNVK$_{133}$VQDNSVLVEGNHE
ER$_{134}$VLPAIEAFQK$_{135}$FSEATLDEIIR$_{136}$FHGVALAFNALDSK$_{137}$ENAILTDIWNITPFK$_{138}$VIVDILLK$_{139}$TILVDLQVGK$_{140}$LAELH
AASVVAK$_{141}$EAGLEIELAPK$_{142}$AGFAGDDAPR$_{143}$SYELPDGQVITIGNER$_{144}$VAPEEHPVLLTEAPLNPK$_{145}$GYSFTTTAER$_{146}$EITS
LAPSTIK$_{147}$EITALAPSTIK$_{148}$QEYDESGPGIVHR$_{149}$QEYDESGPSIVHR$_{150}$SIELLEK$_{151}$LQASGDFPTTK$_{152}$EQNSPIYISR$_{153}$ATVAA
FAASEGHAHPR$_{154}$YQVIVK$_{155}$SSDAQSQATASEAESK$_{156}$ALSASSLIALSSR$_{157}$DPVLTAFQLSWELK$_{158}$DDYGITEDDIIEVR$_{159}$AT
AWVIVHR$_{160}$VELELWLK$_{162}$VVLPDLAVLR$_{163}$ALGIEEQSGGAAR$_{164}$VDEYGFFLYWK$_{165}$YYLSDLAR$_{166}$IEQADYLPTEQDILR$_{167}$
VPTTGILEYPFDLDGIVFR$_{168}$TIITYPWFQNSSVILFLNK$_{169}$AGIAVEGDIK$_{170}$DAFGIIVSYAVK$_{171}$DTALASTTLIASQDAR$_{172}$DFL
LSPGELELEVTLDK$_{173}$VFGQLATTYR$_{174}$LQYAPLNR$_{175}$ISLEVTLDR$_{176}$LLQPAPGTIEK$_{177}$FYPEDVAEELIQDITLR$_{178}$IGFPWSE
IR$_{179}$TTHTAGFLANDR$_{180}$LFYLQVK$_{181}$AAIELNR$_{182}$GDNILLTK$_{183}$VPPEVPPSK$_{184}$LVDFIINR$_{185}$SLVGVQK$_{186}$QIDVETVADR$_{187}$YI
QATDLSEASR$_{188}$NAEYDQFLEDIQK$_{189}$ATAWVIVHR$_{190}$ALSASSLIALSSR$_{191}$HLVDEPQNLIK$_{192}$YLYEIAR$_{193}$QTALVELLK$_{194}$LG
EYGFQNALIVR$_{195}$DAFLGSFLYEYSR$_{196}$LVNELTEFAK$_{197}$GSGHHHHHH

Fig. S7: Sequence design of the chimeric protein CP04. The proteotypic peptides were selected from 43 proteins (For list of proteins see supplementary Table S4) from the eye of *Drosophila melanogaster*. The reference peptides were selected from glycogen phosphorylase (GP) and BSA and were placed at the N- and C-terminus respectively. The sequence string assembled from sequences of proteotypic peptides (here peptide 1 to 197) is flanked with twin-strep-tag and his-tag at the N- and C-terminus, respectively. The cleavage site for the 3C protease was placed after the twin-strep-tag.



(B)

Nominal mass (M_r): **264624**; Calculated pI value: **5.00**

Fixed modifications: Carbamidomethyl(C), Label: 13C (6) (K), Label: 13C (6)15 N (4) (R)

Variable modifications: Acetyl (Protein N-term), Oxidation (M)

Cleavage by Trypsin: cuts C-term side of KR unless next residue is P

Sequence Coverage: **98%**

Matched peptides shown in **Bold Red**

1M**GSAWSHPQFEKGGGSGGGSGGSAWSHPQFEKLEVLFQGPAAAKVFADYE**
51EYVKDFYELEPHKVAAAFPGDVDRGLAGVENVTELKGSTLSAQQGSQFKN
101EPVSYILELINGRNIGDSYVAAKLDNGYNHLIEVVRLVLGIPTYGRFSPL
151VASNERLTEAEGSSLYIGGRSTDAEEDPQVIKFADQDNDLVNLRLSNFVD
201TTVAWLNYRFFVSVTRVQLTDLNRDVFIPDVFNNYKILAIFPFPGPSQYI
251NVVPYLKVPIWPYTLYFRGTFFVSHKGHEISVFSLTHKAPLGLHFHASWL
301KIINNPEATQRAVYWVEHVSRSHYHVGSALAKAEQNGYGVTVHYEELSSA
351KGSAYAHAENTLRSEGDLTQITPPSALGVQVRAEVEAVQIIAESLKAANQ
401LTDRPTIINVAISPSSDRGHGAGGADVLTYKDLNQGNSYVQDKSLVFVDN
451HDNQRSEYTGLGAITEFRVVEFLDHLIDLGVAGFRIGALDTSRLIELLVR
501LADLLLQEERVNGEPVDVGAFFIRITPDVVGAIVQEATTYFRATQLSEQL
551GSELTPKLLAADYADGVSQPRILSWNAVNLYGLKASEQPGLTAIHTAFLR
601LLPAQYEDGISAPRGIATNDVGIAKDTFSFLTGSGRAINEGGFQSLPQKF
651GLNEGSEPQAYGIGLKITTHYTLNPRAILVYLVEKITPGWEENWAGALDV
701KINPQHSIPTLVDNGFTIWESRALGLEFNKINLDPYALYVIVEKTNFDDS
751ALFYASGESLKVLTTGGDAGSNRLGSDVQPPGRVFLQLLENQRNLSQTFG
801NIWRVFDLPDFLTWRGSTVQFAARDGAISHVVFKYFNAYLSDRFSEPYDS
851TLSDVIKNVANLEATKDADAIVQQTLAKGTQAEIVVADVTKVGDVTEVAE
901AVAFLASSKSVLLTLRIDALEGVYKALNLDFDYKAITFPLFWENKLTLYG
951IDGSPPVRELPYYEEANGSRAVGVELNKAPADPEAFKAIQVYLVEKLLNL
1001QAGEHLKPEFLKINPQHTIPTLVDNGFALWESRGLVVSGTRSLSDVSLTG
1051RGNSYLILPPPRISGPSNHVTVVRNAAFSGDSYVSHRSVVNVGLGQALRS
1101TQEEVDHIRSAVHDVEVFLKVSTAIDAISVSGRVALQIQDVATSSRLEEI
1151SLRLLELHDNRLFNNFDVLRLAVLDLSHNRASLSGIQSHAFKTFFDGNPI
1201HTLRDFGVELEDLQITRSTISSTTTVRTSTTSSLTGNPRTSVATVAGGAV
1251VGATKLLVPGSSSTTTTSSLRLLPDTTDEQLLTSALEEKSTGNIFAAKVF
1301AVNSAGRIFADNVYGRGVAEDFAPSFVKHDGGSPITGYIIEKHYPNPAVR
1351VVGSEADTGRDAPTTESYLRSTVPFATSESNRLVGGNDLDTPLIITRIAE
1401LEGLGSRIQYLEDSTRIGEAISQSSIRFITQIVDVTKAEDAGVYLVVART
1451YYDFGFVALDIIKVWQVSVSAHALLWDLNDGKDVLSVAFSADNRLWDLAA
1501GKLNNDLIARFQEALAGLSKLAAHDALGGAAKSAGSGVSTTAIEKIVQVQ
1551IDDVGKSELDVFSDWLQVARFSQSDFGLDQGETLLRESLLEITIYHQKGA
1601AYQEAPVDAEVAVTPKISAFGLFTYSVFSIIAGSLKVFANPVQLEFYGFV
1651PWSIIQRQVGASSGSSGAVSKIVQIQQVGPAHLNVKVQDNSVLVEGNHEE
1701RVLPAIEAFQKFSEATLDEIIRFHGVALAFNALDSKENAILTDIWNITPF
1751KVIVDILLKTILVDLQVGKLAELHAASVVAKEAGLEIELAPKAGFAGDDA
1801PRSYELPDGQVITIGNERVAPEEHPVLLTEAPLNPKGYSFTTTAEREITS
1851LAPSTIKEITALAPSTIKQEYDESGPGIVHRQEYDESGPSIVHRSIELLE
1901KLQASGDFPTTKEQNSPIYISRATVAAFAASEGHAHPRYQVIVKSSDAQS
1951QATASEAESKALSASSLIALSSRDPVLTAFQLSWELKDDYGITEDDIIEV
2001RATAWVIVHRVELELWLKVVLPDLAVLRALGIEEQSGGAARVDEYGFFLY
2051WKYYLSDLARIEQADYLPTEQDILRVPTTGILEYPFDLDGIVFRTIITYP
2101WFQNSSVILFLNKAGIAVEGDIKDAFGIIVSYAVKDTALASTTLIASQDA
2151RDFLLSPGELELEVTLDKVFGQLATTYRLQYAPLNRISLEVTLDRLLQPA
2201PGTIEKFYPEDVAEELIQDITLRIGFPWSEIRTTHTAGFLANDRLFYLQV
2251KAAIELNRGDNILLTKVPPEVPPSKLVDFIINRSLVGVQKQIDVETVADR
2301YIQATDLSEASRNAEYDQFLEDIQKATAWVIVHRALSASSLIALSSRHLV
2351DEPQNLIKYLYEIARQTALVELLKLGEYGFQNALIVRDAFLGSFLYEYSR
2401LVNELTEFAKGSGHHHHHH

Fig. S8: Expression and validation of the sequence of chimeric protein CP04. (**A**) 1D SDS-PAGE analysis (4-20% gel) showing the overexpressed CP04 (**B**) in-gel digestion of CP03 with trypsin followed by LC-MS/MS analysis of recovered tryptic peptides confirms its accurate full length expression. MS/MS spectra were mapped to the CP04 sequence by the Mascot search (mass accuracy 5 ppm for precursor and 0.3Da for fragment ions; two missed cleavage site allowed) and indicated its 100% coverage.

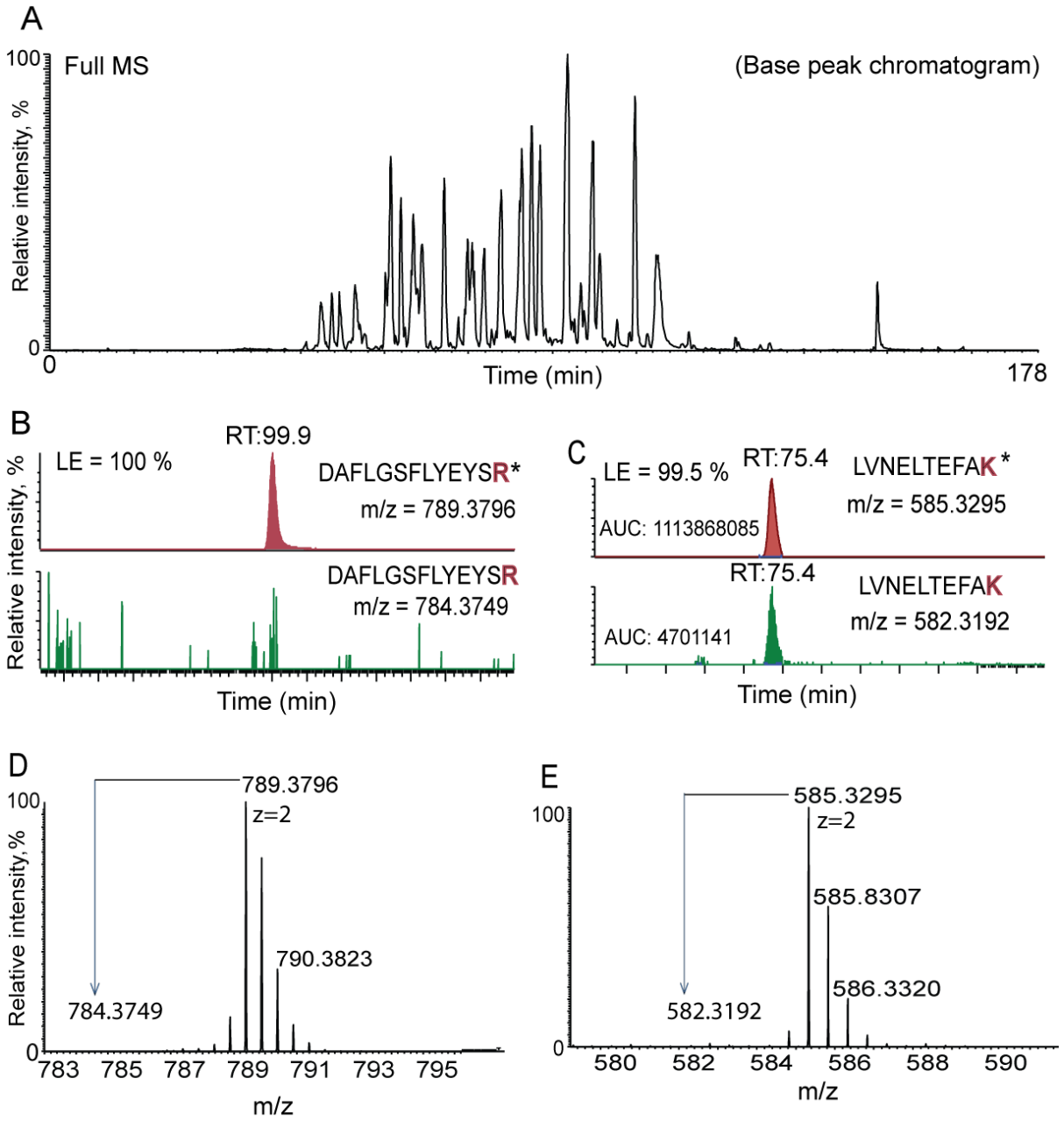


Fig. S9: Efficient incorporation of heavy isotope labelled Arginine ($^{13}C_6$ $^{15}N_4$-Arg) and Lysine ($^{13}C_6$-Lys) amino acids into a chimeric protein. **A**, shows the base peak chromatogram of full LC-MS/MS run of in-gel digested heavy isotope labelled chimeric protein CP01. **B,** shows the extracted ion chromatogram (XIC) of one of the arginine containing peptide pair: heavy*(labelled) and light (unlabelled). As evident by the XICs, even the trace of unlabelled peptide was not detected (B, lower panel) confirming the labeling efficiency (LE) of ca 100%. Similarly, the LE of lysine was calculated and exemplified here with one of the lysine containing peptide in panel **C**, the LE of lysine was found to be 99.5%. The mass spectrum of heavy isotope labelled arginine and lysine containing peptide is shown in panel **D** and **E** respectively.

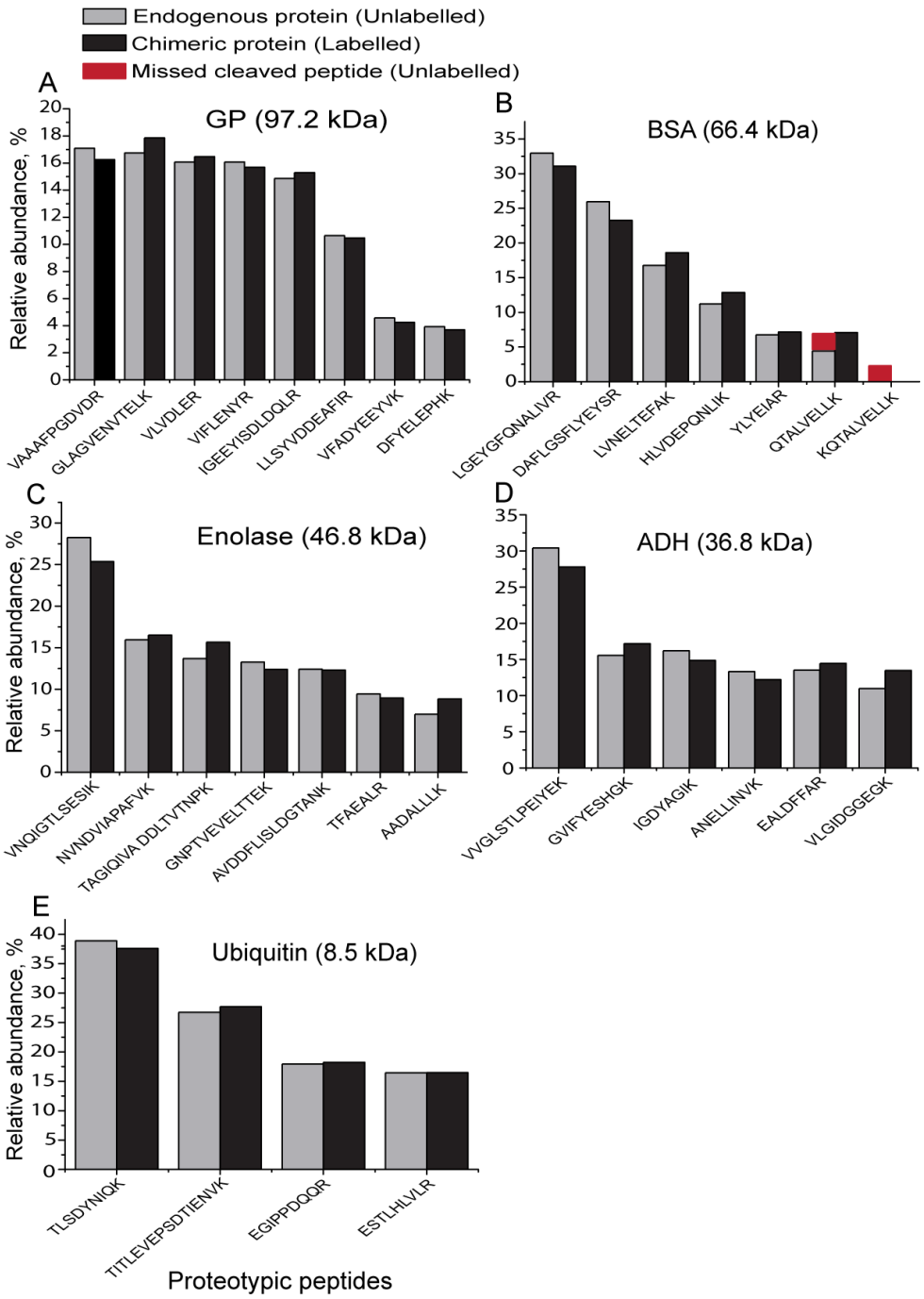


Fig. S10: Relative abundance of proteotypic peptides from endogenous and chimeric protein CP01. Isotopically labelled and unlabelled peptides were produced by in-gel co-digestion of CP01 and corresponding native proteins, respectively. The relative abundance of peptide pairs resulting from native full length and single CP01 differed by less than 10%. The abundance of a complementary peptide resulting from an incomplete cleavage of native BSA is highlighted in red.

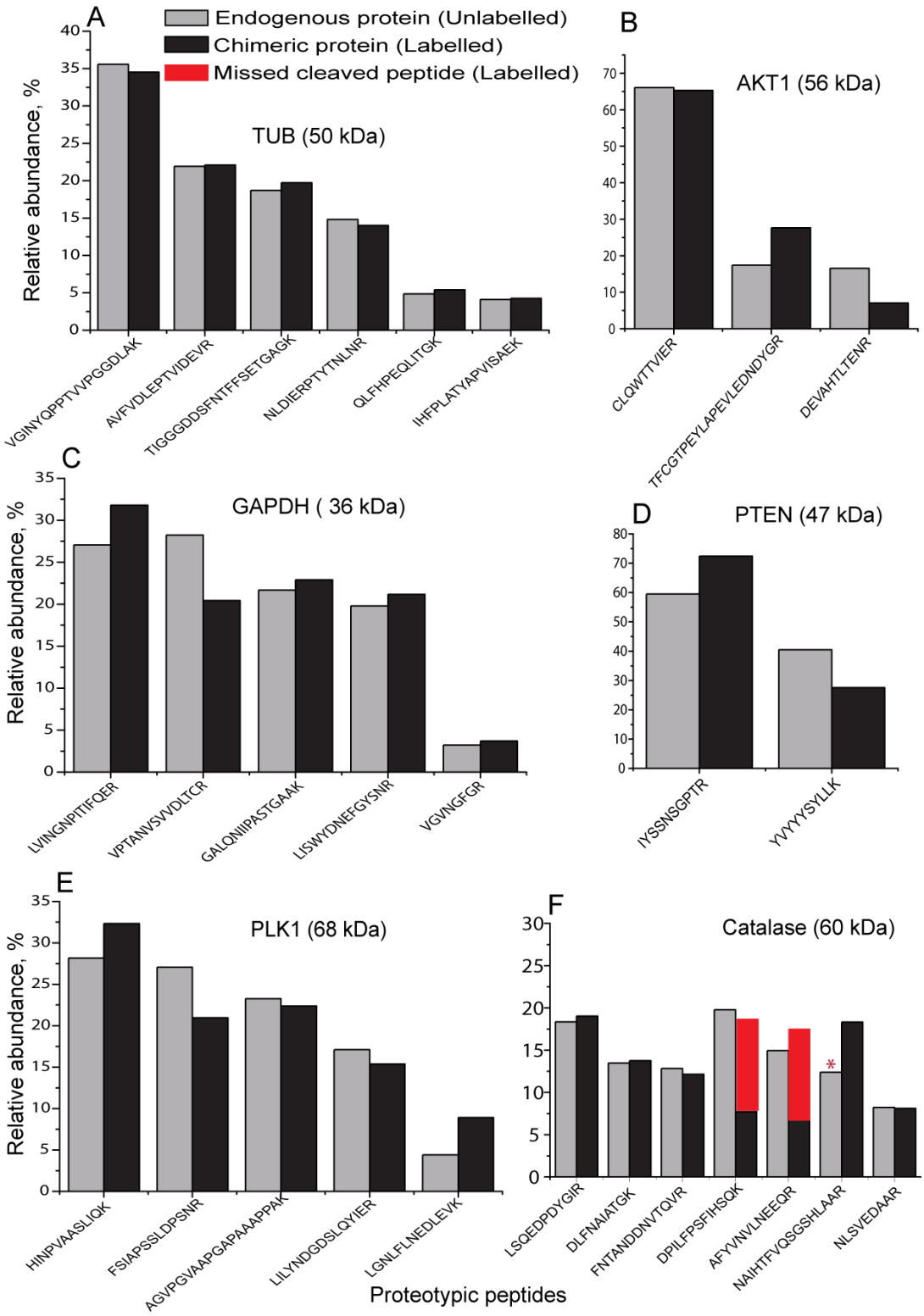


Fig. S11: Relative abundance of proteotypic peptides from endogenous and chimeric protein (CP03). The relative abundance of peptide pairs resulting from native full length and single CP03 differed by less than 10%. The abundance of “complementary” peptide resulting from incomplete cleavage of two of the catalase peptide is coloured. The peptide marked with (*) is known to have phosphorylation (Mertins et al., 2013)

Histone H4 Zebrafish (Uniprot accession # A4VAK6)

MSGRGKGSKGLGKGGAKRHRKVLRDNIQGITKPAIRRLARRGGVKRISGLIYEETRGVLKVF
LENVIRDAVTYTEHAKRKTVTAMDVVYALKRQGRTLYGFGG

Histone H4 Human, Recombinant (NEB Catalog # M2504S)

SGRGKGGKGLGKGGAKRHRKVLRDNIQGITKPAIRRLARRGGVKRISGLIYEETRGVLKVFL
ENVIRDAVTYTEHAKRKTVTAMDVVYALKRQGRTLYGFGG

Fig. S12: Amino acid sequence of Histone H4 from Zebrafish and Human recombinant as supplied by NEB. Both these sequences share 99% identity.

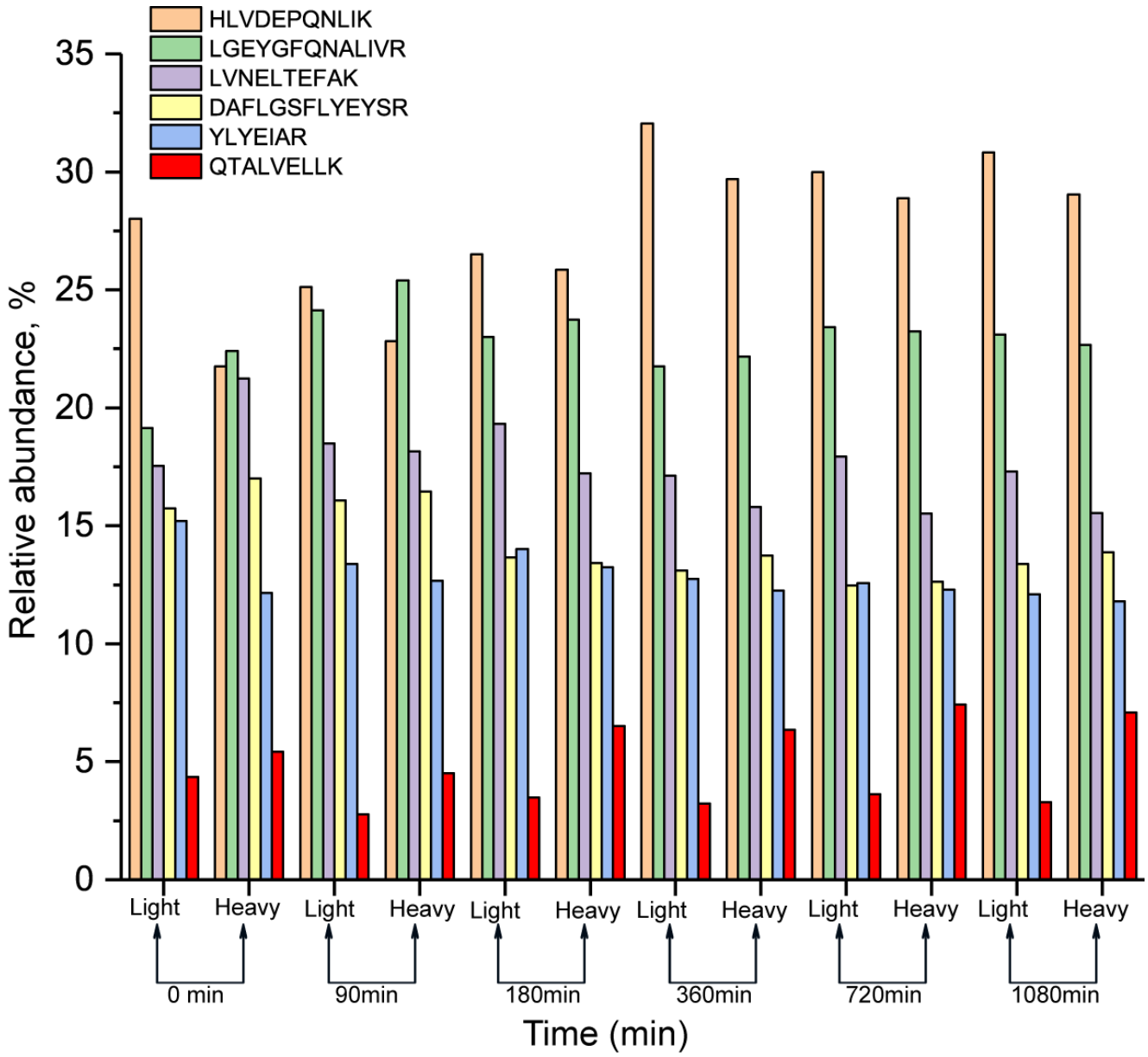


Fig. S13: Relative abundance of proteotypic peptides from full length BSA and chimeric protein CP03. The yield of unlabelled (light) and isotopically labelled (heavy) peptide pairs from full length BSA and CP03, respectively, at different digestion time point are shown. After 90 minutes of digestion, the relative abundance of all the light and heavy peptide pairs, except QTALVELLK, differed by less than 5%. Low abundance of light QTALVELLK compared to its heavy counterpart is due to missed cleavage (KQTALVELLK) owing to the presence of dibasic residue at the peptide N-terminus (KKQTALVELLK).

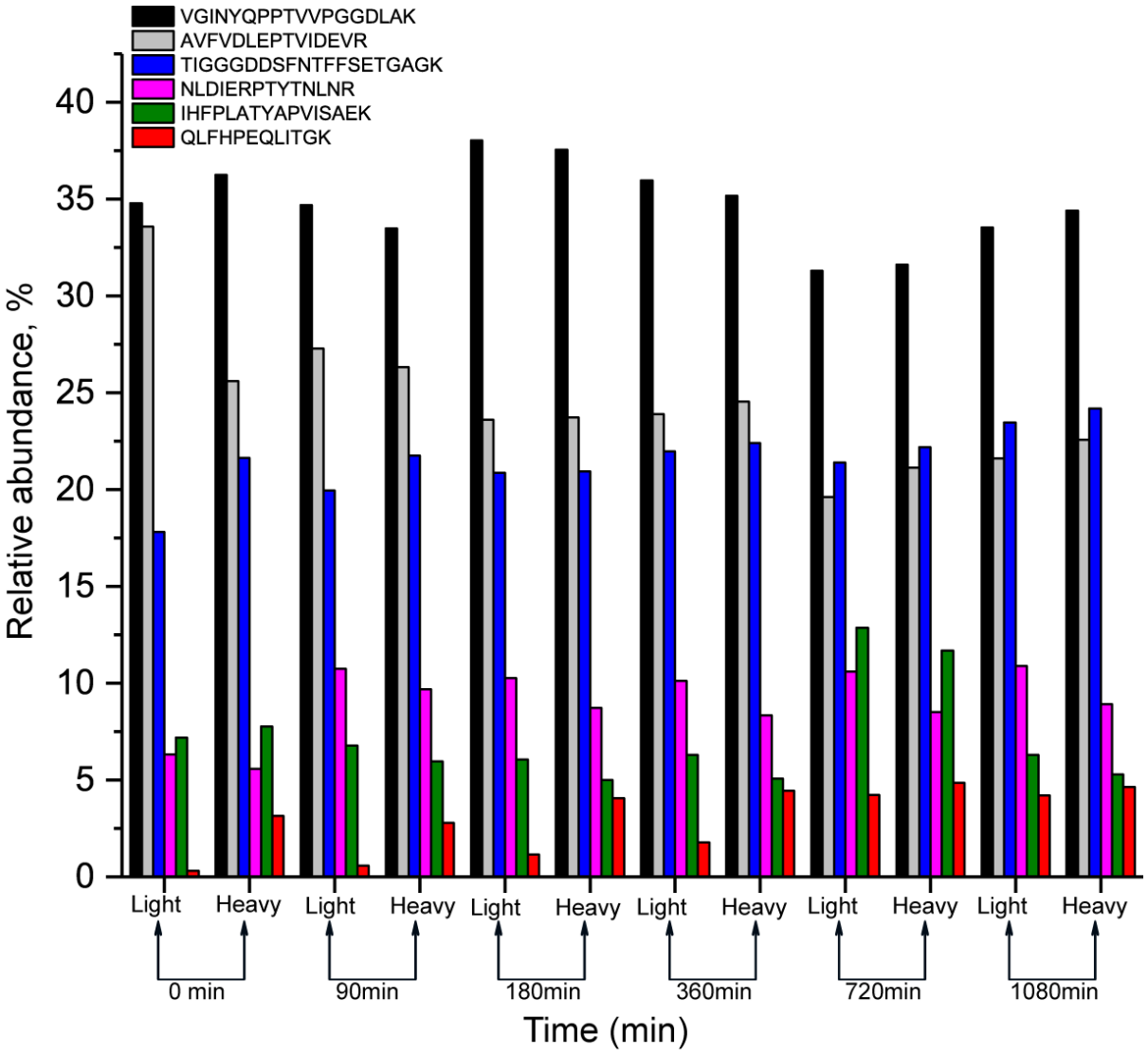
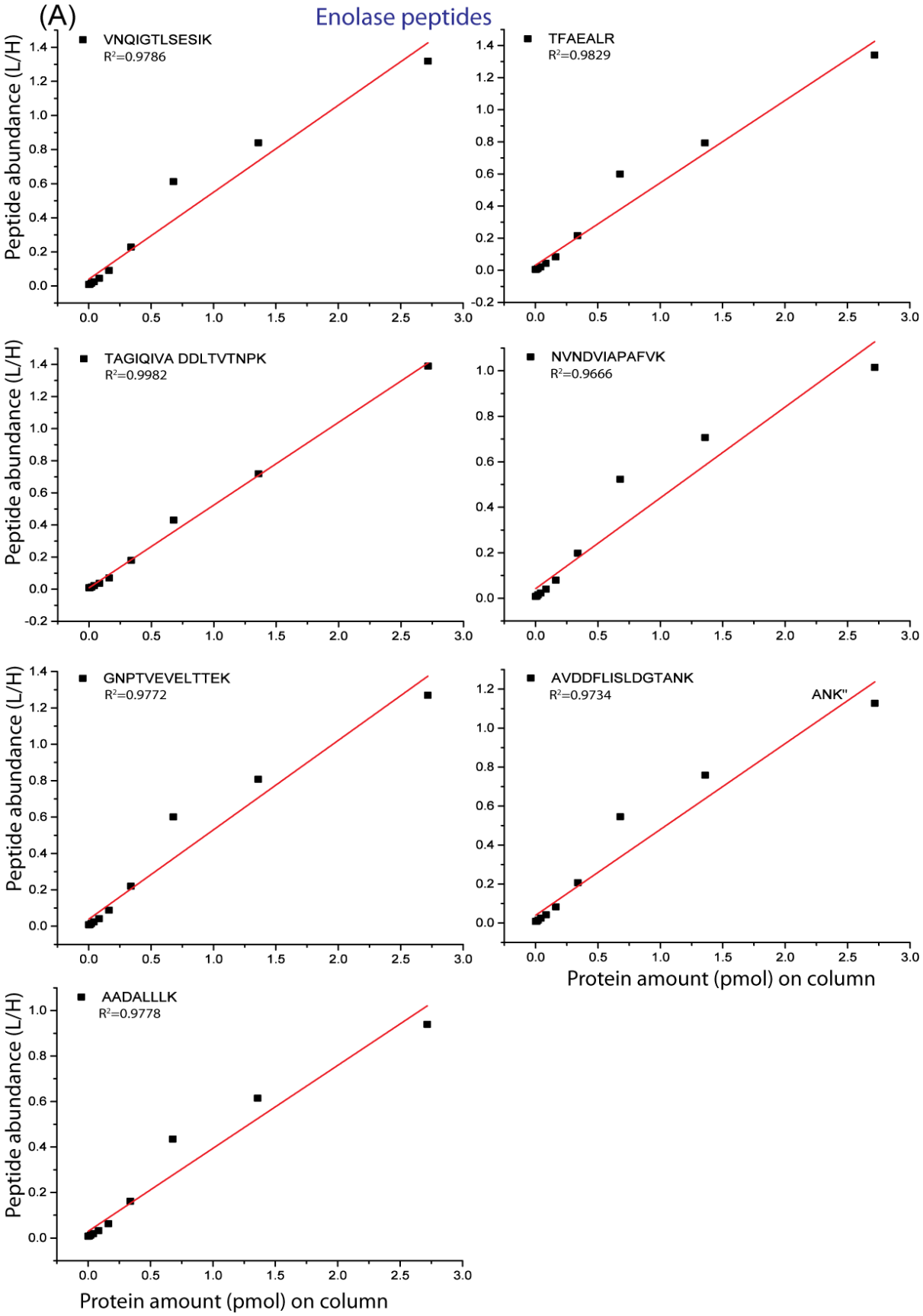
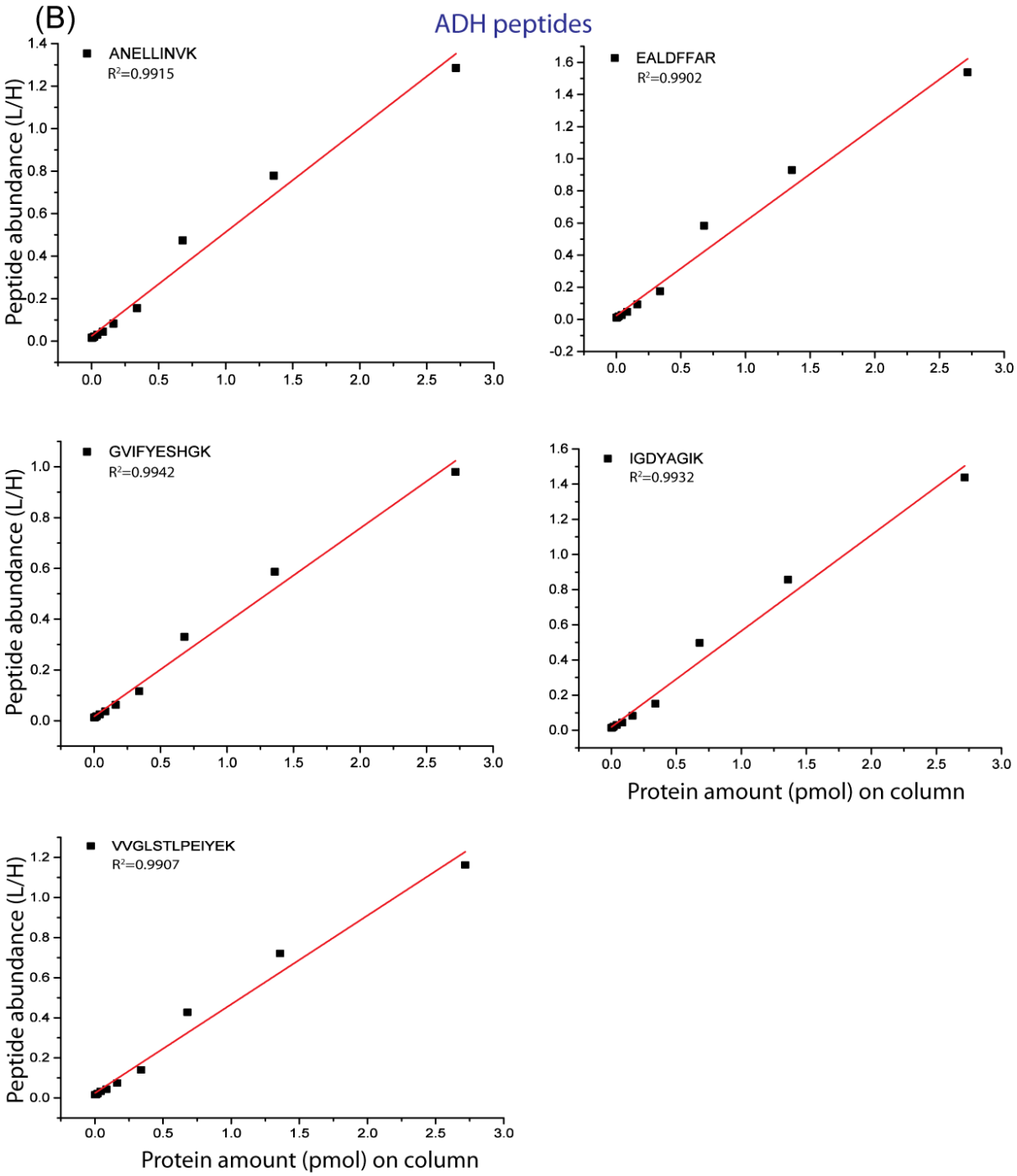
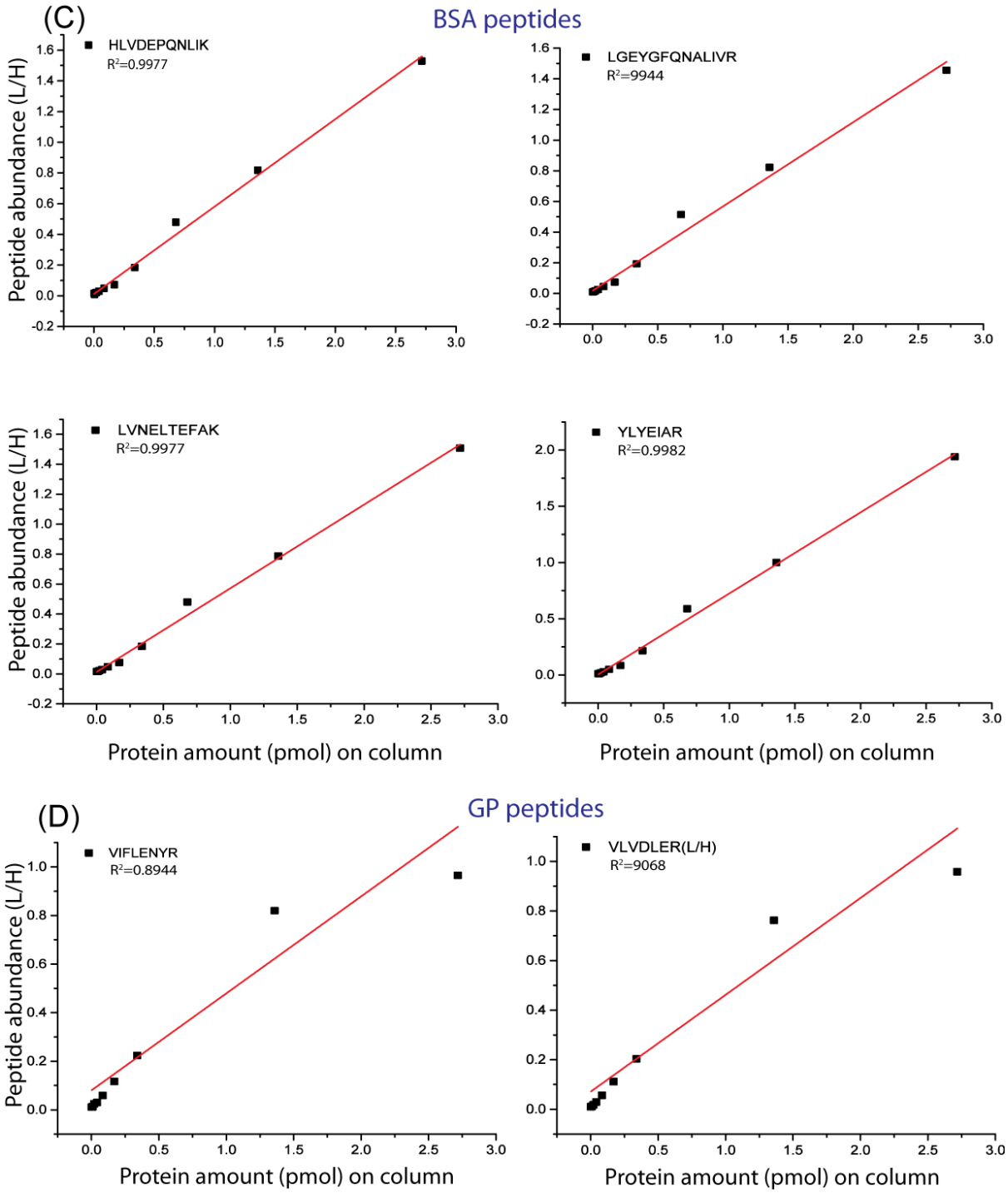


Fig. S14: Relative abundance of proteotypic peptides from endogenous α-Tubulin (TUBA) and chimeric protein CP03. The yield of unlabelled (light) and isotopically labelled (heavy) peptide pairs from endogenous TUBA and CP03, respectively, at different digestion time point are shown. After 90 minutes of digestion, the relative abundance of all the light and heavy peptide pairs differed by less than 5%. Low abundance of light QLFHPEQLITGK compared to its heavy counterpart is due to slow release and after 12hrs of digestion the abundance of light QLFHPEQLITGK matched to its heavy counerparts.


(A)
Enolase peptides
VNQIGTLSESIK
R²=0.9786
TFAEALR
R²=0.9829
TAGIQIVA DDLTVTNPK
R²=0.9982
NVNDVIAPAFVK
R²=0.9666
GNPTVEVELTTEK
R²=0.9772
AVDDFLISLDGTANK
R²=0.9734
ANK"
AADALLLK
R²=0.9778
Peptide abundance (L/H)
Protein amount (pmol) on column


(B)
ADH peptides
ANELLINVK
R²=0.9915
EALDFFAR
R²=0.9902
GVIFYESHGK
R²=0.9942
IGDYAGIK
R²=0.9932
VVGLSTLPEIYEK
R²=0.9907
Peptide abundance (L/H)
Protein amount (pmol) on column


(C)
BSA peptides
HLVDEPQNLIK
R²=0.9977
LGEYGFQNALIVR
R²=9944
LVNELTEFAK
R²=0.9977
YLYEIAR
R²=0.9982
Peptide abundance (L/H)
Protein amount (pmol) on column
(D)
GP peptides
VIFLENYR
R²=0.8944
VLVDLER(L/H)
R²=9068
Peptide abundance (L/H)
Protein amount (pmol) on column

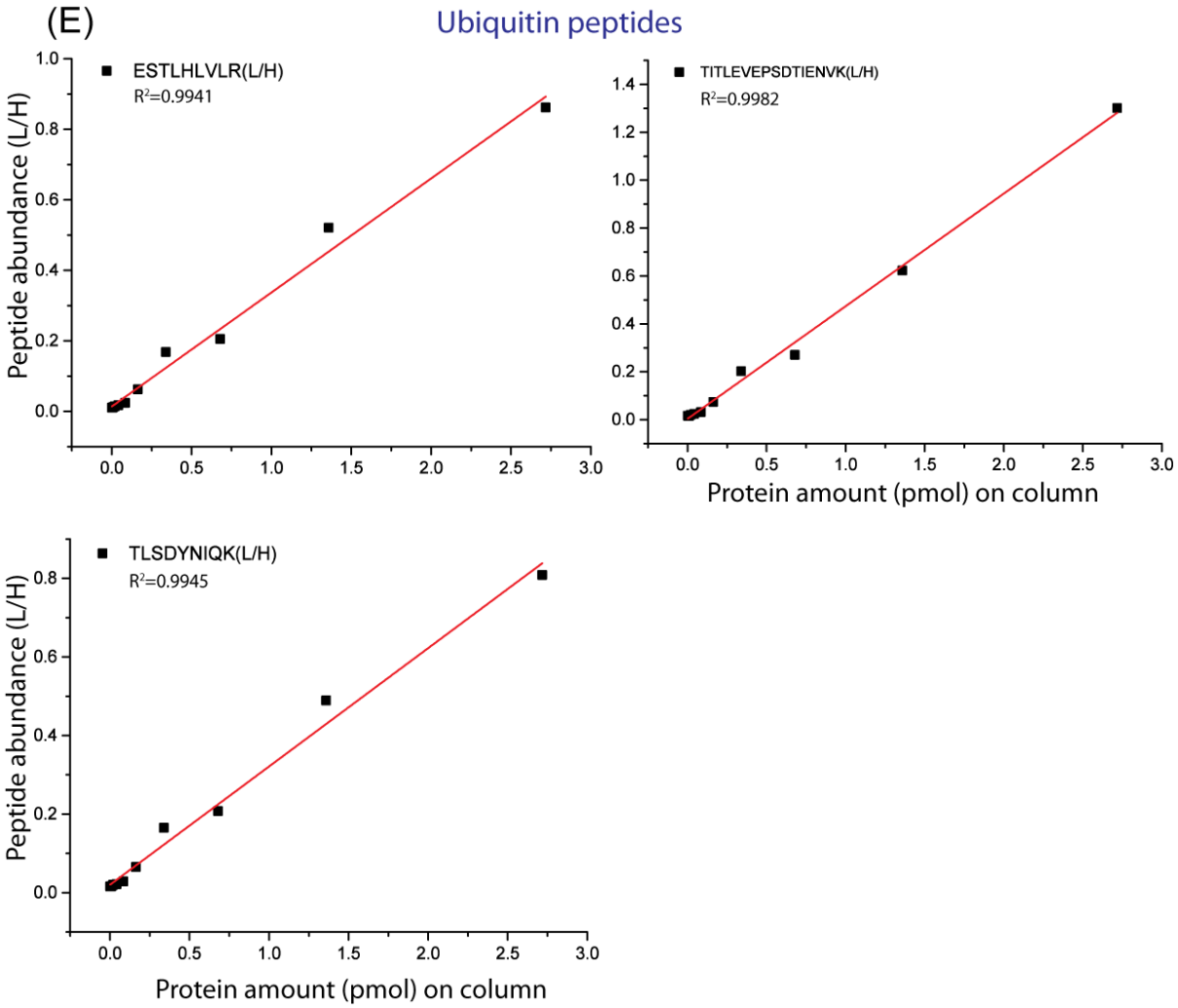


Fig. S15: MS Western quantification of proteins spiked into the constant background of E.coli lysate . **A-E,** respectively shows the quantification of Enolase, ADH, BSA, GP and UBI based on different peptides.

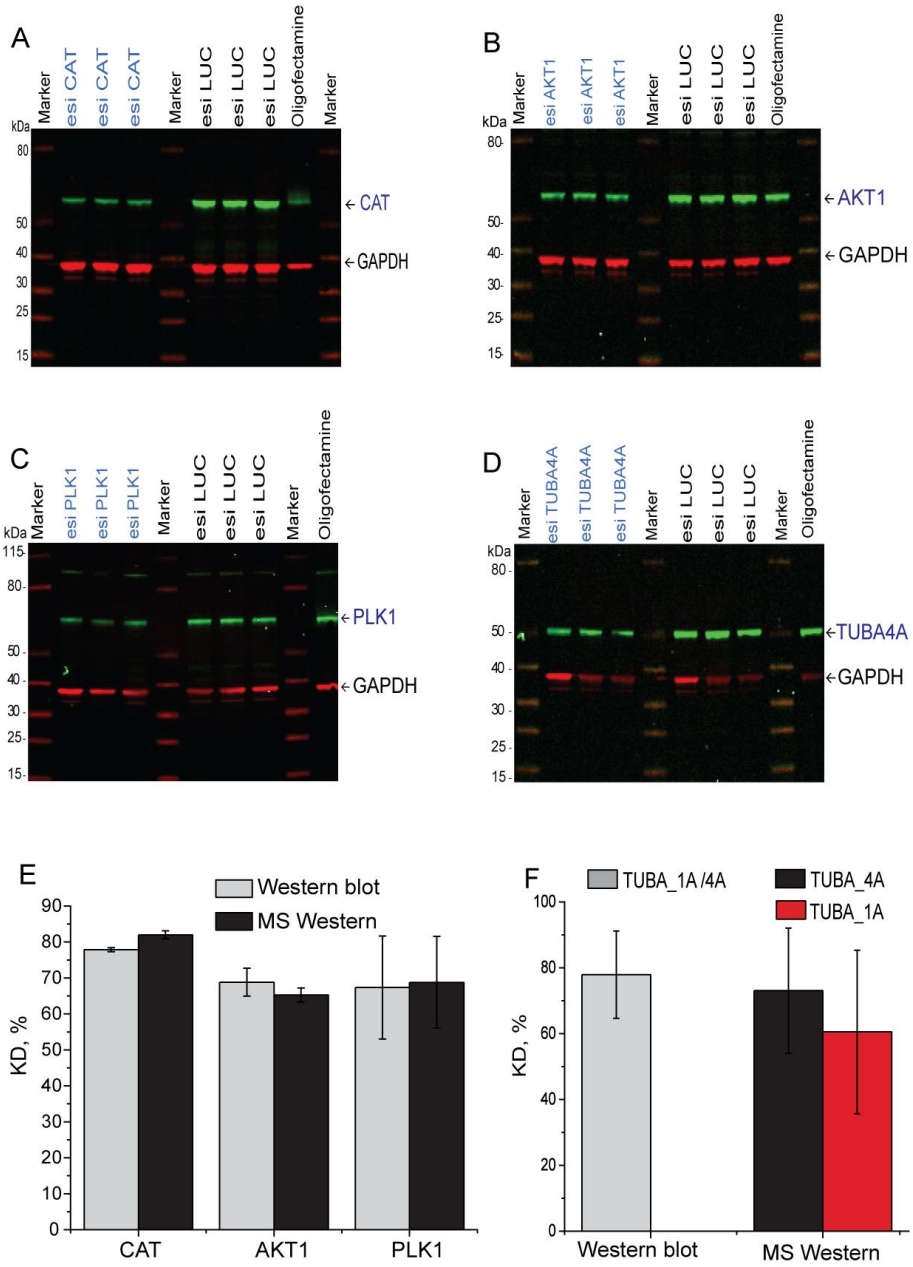


Fig. S16: Quantification by MS Western and Odyssey is quantitatively concordant.

A-D, the Odyssey images of total protein extract of HeLa cell subjected to RNAi. Knock-down (KD) experiments were performed in triplicate and compared against non-targeting luciferase esiRNA (esiLUC) control. **E**, KD efficiency by esiRNA against CAT, AKT1 and PLK1 proteins determined by MS Western and Odyssey. **F**, KD efficiency by esiRNA against TUBA4A determined by MS Western and Odyssey. MS Western recognized that TUBA1A, along with TUB4A, was also KD with the similar efficiency. In panels E and F values are mean ±SD (n=3), where “n” represents number of biological replicates.

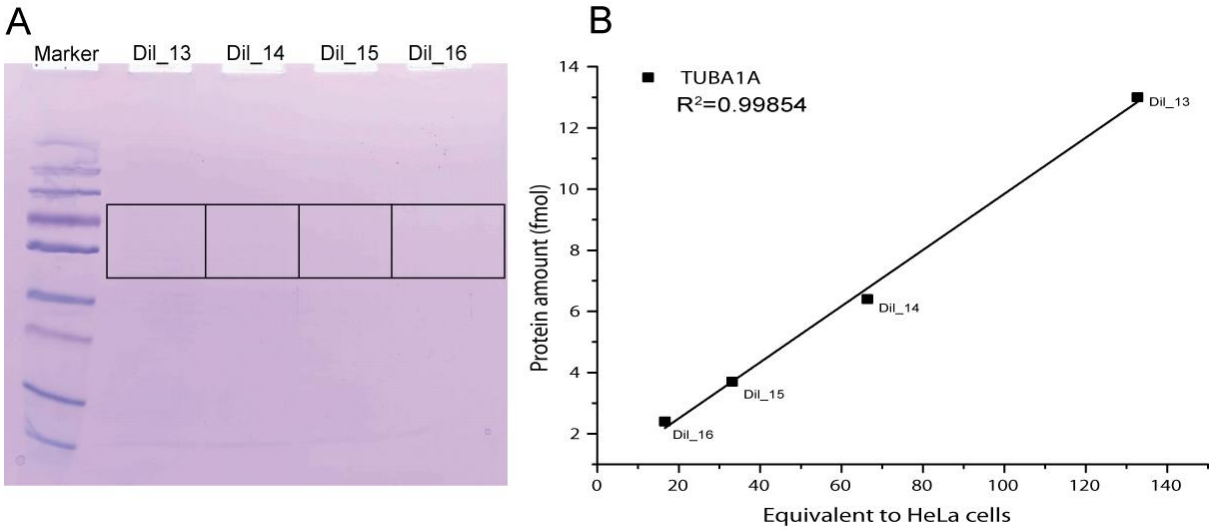


Fig. S17: Quantification of protein from the gel where nothing is visible. **A**, shows the electrophoresed 1D SDS PAGE gel loaded with serially diluted total protein extract from HeLa cells. The gel slice corresponding to the apparent molecular weight of α-Tubulin (TUBA) were cut from different dilutions (marked with box) and codigested with BSA and CP01 gel band. **B,** shows the absolute quantification of TUBA1A from four different dilutions. The y-axis shows the amount (fmol) of TUBA1A quantified and the x-axis shows the corresponding equivalent amount of HeLa cells.

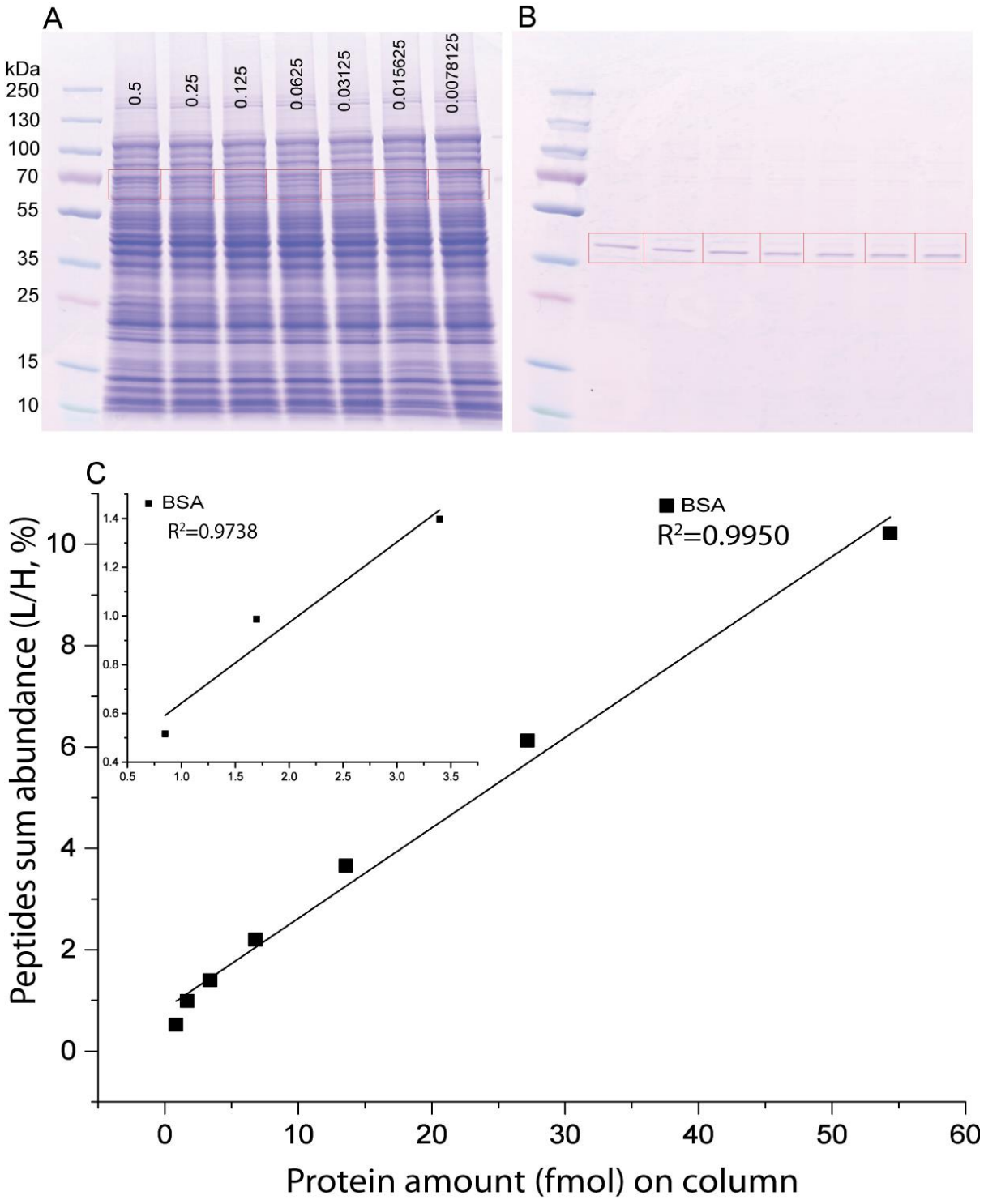
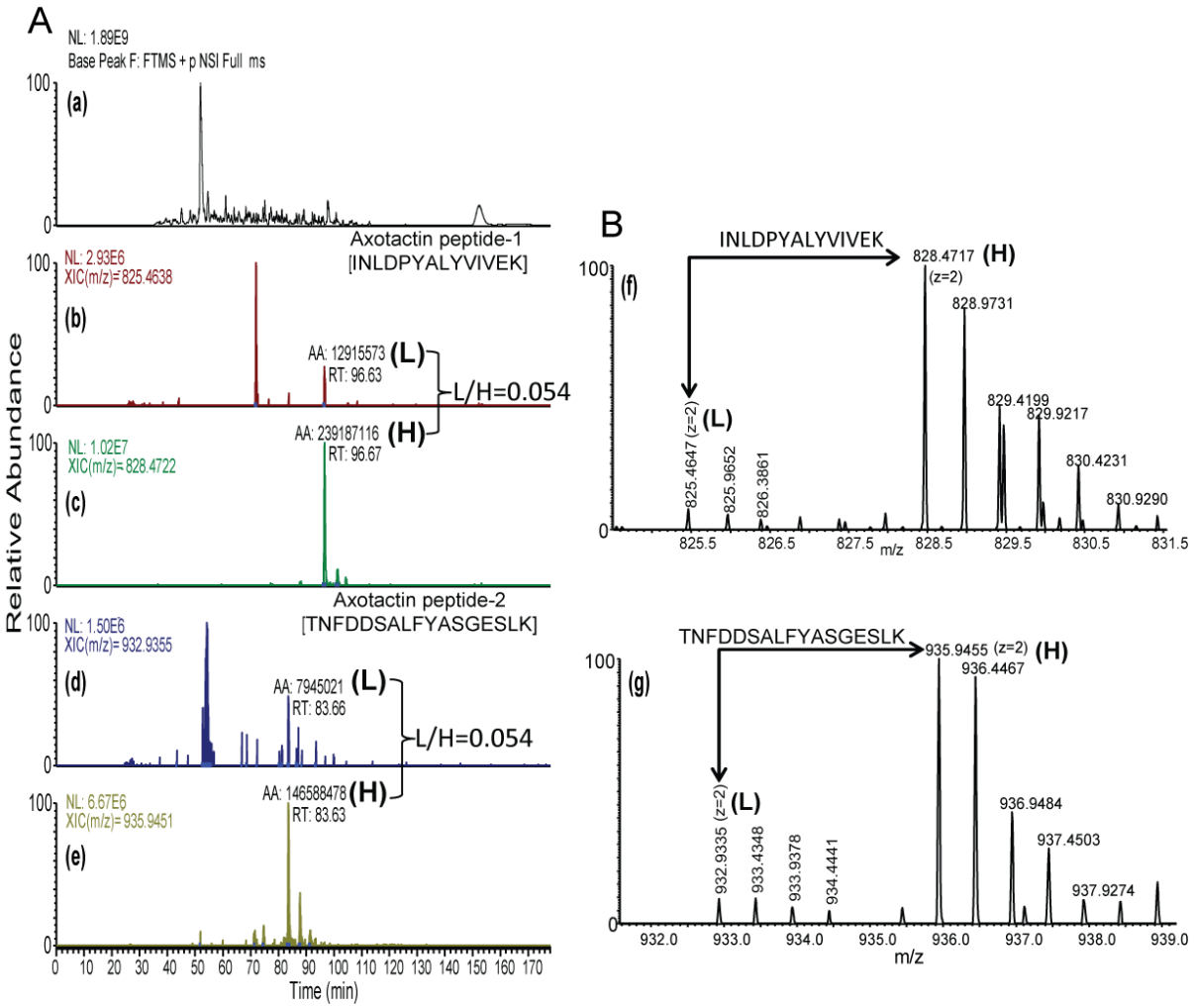


Fig. S18: Sub-femtomole quantification of protein spiked in large excess of complex background. **A**, shows the electrophoresed 1D SDS PAGE gel loaded with serially diluted BSA protein spiked in constant 50µg *E.coli* total protein extract. The pmol amount of BSA protein spiked in 50µg *E.coli* total protein extract loaded in each gel lane is indicated. **B,** shows the electrophoresed 1D SDS PAGE gel loaded with the heavy isotope labelled chimeric protein CP01 (same amount in all lanes). The gel slice corresponding to the apparent molecular weight of BSA were cut (gel A) from different dilutions (marked with colour box) and codigested with CP01 gel band (gel B) (marked with colour box). After in-gel digestion and extraction the peptides were recovered in 46µl of 5% aqueous formic acid and

5µl was injected on to the HPLC column and analysed by LC-MS/MS (only 10.8% of total protein amount loaded on the gel was injected into HPLC column). **C**, shows the calibration plot of serially diluted BSA. The y-axis shows the abundance of light BSA peptide (coming from serially diluted BSA) normalised to the corresponding heavy BSA peptide (coming from CP01). The x-axis shows the amount (fmol) of protein loaded on to the HPLC column. Inset to the pane C, shows the calibration plot of three lower dilutions at 0.8, 1.6 and 3.3fmol.


A
NL: 1.89E9
Base Peak F: FTMS + p NSI Full ms
(a)
Axotactin peptide-1
[INLDPYALYVIVEK]
NL: 2.93E6
XIC(m/z)= 825.4638
(b)
AA: 12915573 (L)
RT: 96.63
L/H=0.054
AA: 239187116 (H)
RT: 96.67
NL: 1.02E7
XIC(m/z)= 828.4722
(c)
Axotactin peptide-2
[TNFDDSALFYASGESLK]
NL: 1.50E6
XIC(m/z)= 932.9355
(d)
AA: 7945021 (L)
RT: 83.66
L/H=0.054
AA: 146588478 (H)
RT: 83.63
NL: 6.67E6
XIC(m/z)= 935.9451
(e)
Relative Abundance
Time (min)
B
INLDPYALYVIVEK
828.4717 (H)
(z=2)
828.9731
829.4199
829.9217
830.4231
830.9290
(f)
825.4647 (z=2) (L)
825.9652
826.3861
m/z
TNFDDSALFYASGESLK
935.9455 (z=2) (H)
936.4467
(g)
932.9335 (z=2) (L)
933.4348
933.9378
934.4441
936.9484
937.4503
937.9274
m/z
C
Chimeric protein (Labelled)
Endogenous target protein (Unlabelled)
Relative abundance, %
TNFDDSALFYASGESLK
INLDPYALYVIVEK
Axotactin proteotypic peptides
D
Axotactin (MW=229 kDa)
Protein amount (amol)
Condition_1
Condition_2
Drosophila eye

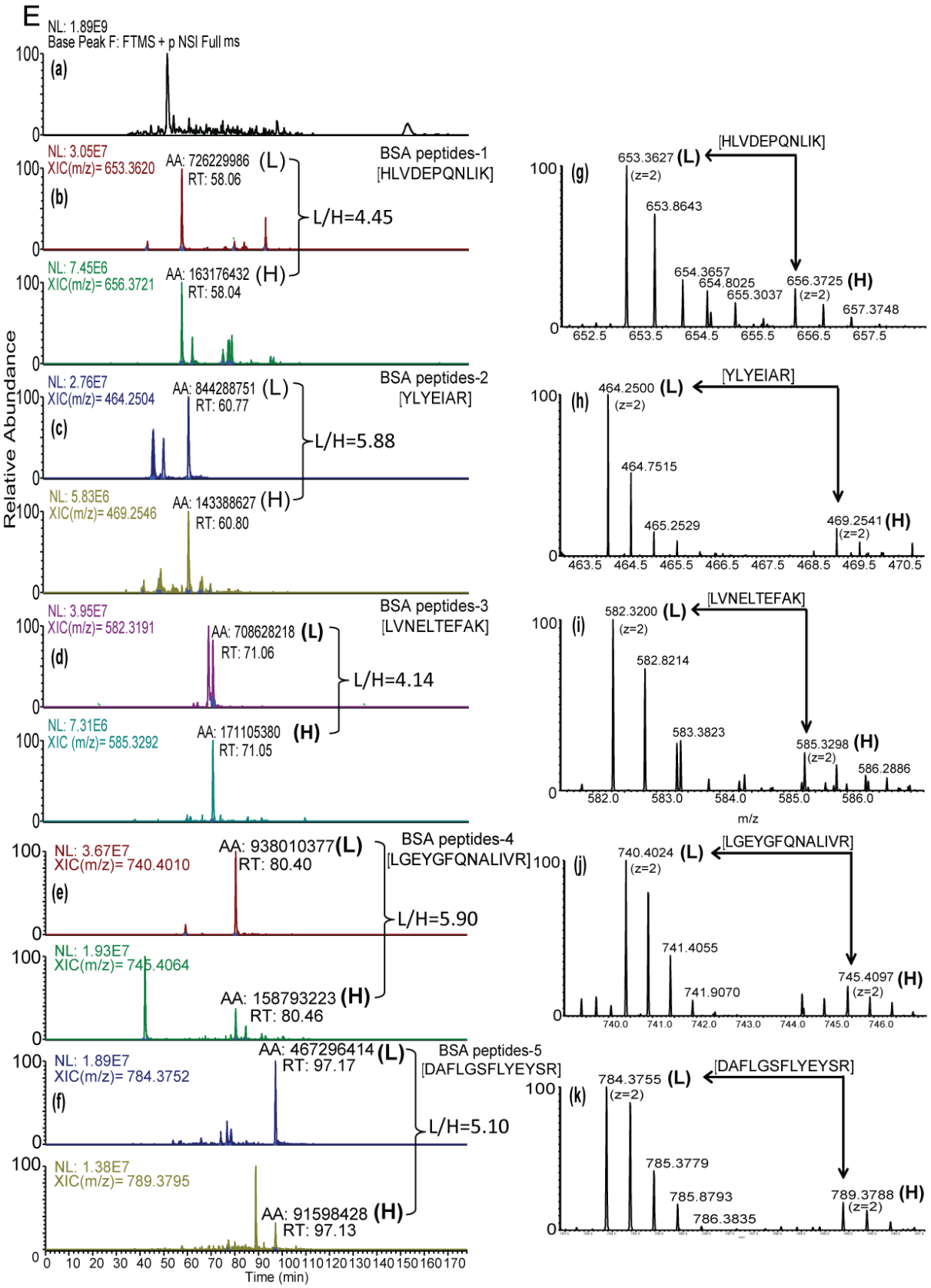
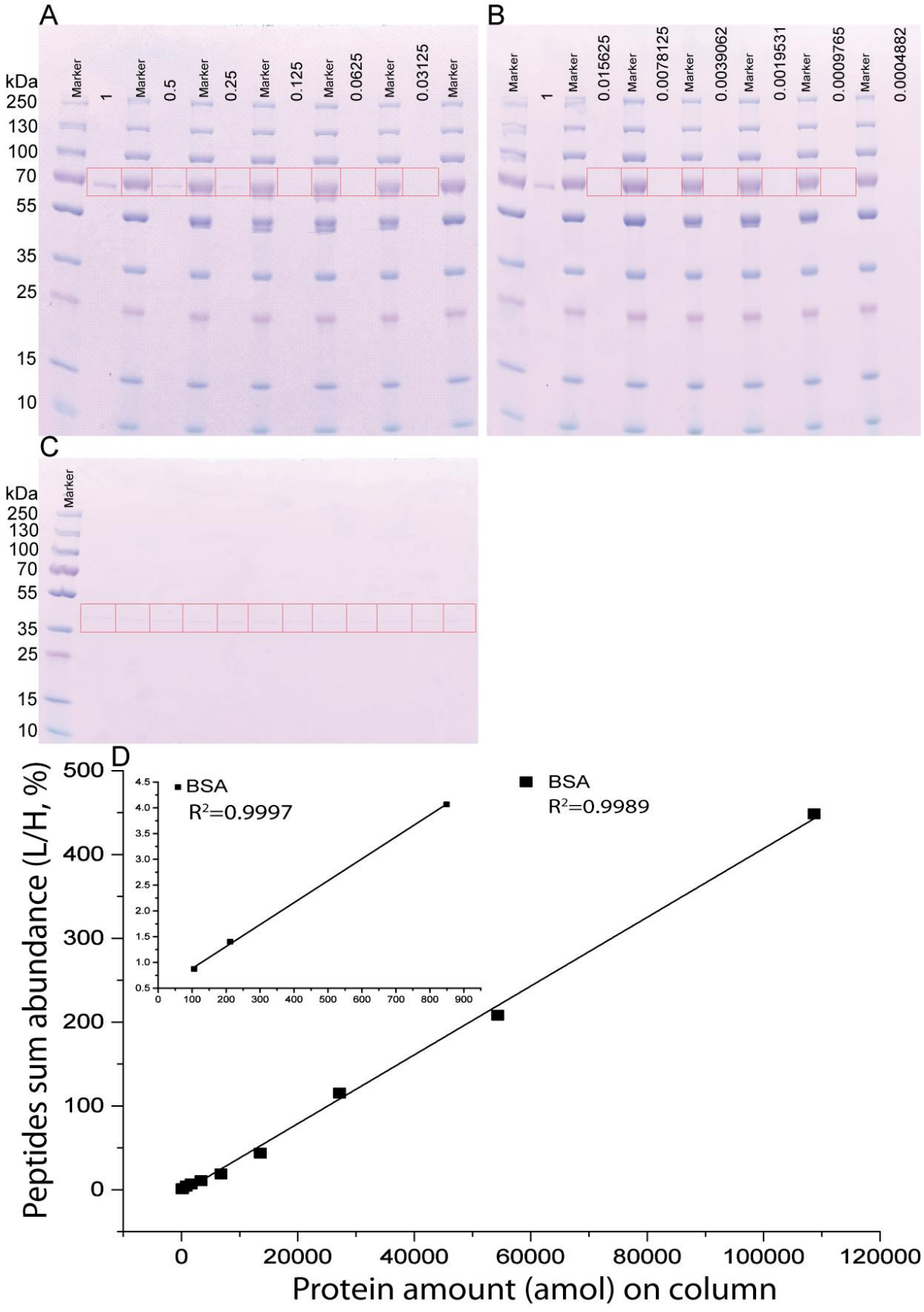


Fig. S19: Attomole quantification of axotactin protein from *Drosophila* eye. **A**, (**a**) shows the base peak chromatogram of full LC-MS/MS run of in-gel codigest of total protein extract of *Drosophila* eye, reference protein BSA and CP04. (**b-e**) shows the XICs of light (L) and heavy (H) peptide pairs of

two of the axotactin peptides of sequence INLDPYALYVIVEK and TNFDDSALFYASGESLK at m/z 825.4627 (L), 828.4717(H) and 932.9331(L), 935.9446(H) respectively. **B**, (**f-g**) shows the corresponding mass spectrum of INLDPYALYVIVEK and TNFDDSALFYASGESLK at m/z 825.4627 (L), 828.4717(H) and 932.9331(L), 935.9446(H) respectively. **C**, shows the relative abundance of heavy and light peptides yielding from CP04 and endogenous axotactin protein respectively. **D**, shows the attomole quantification of axotactin protein from the eye of *Drosophila* raised under two different conditions. Error bar indicates ±SD (n=2) **E**, (**a**) shows the base peak chromatogram of full LC-MS/MS run of in-gel codigest of total protein extract of *Drosophila* eye, known amount of BSA and heavy isotope labelled chimeric protein CP04. (**b-f**) shows the XICs of light and heavy pairs of five BSA peptides and the corresponding mass spectra are shown in panel (**g-k**)


A
kDa
250
130
100
70
55
35
25
15
10
Marker
1
0.5
0.25
0.125
0.0625
0.03125
B
0.015625
0.0078125
0.0039062
0.0019531
0.0009765
0.0004882
C
D
Peptides sum abundance (L/H, %)
Protein amount (amol) on column
BSA
R²=0.9997
BSA
R²=0.9989

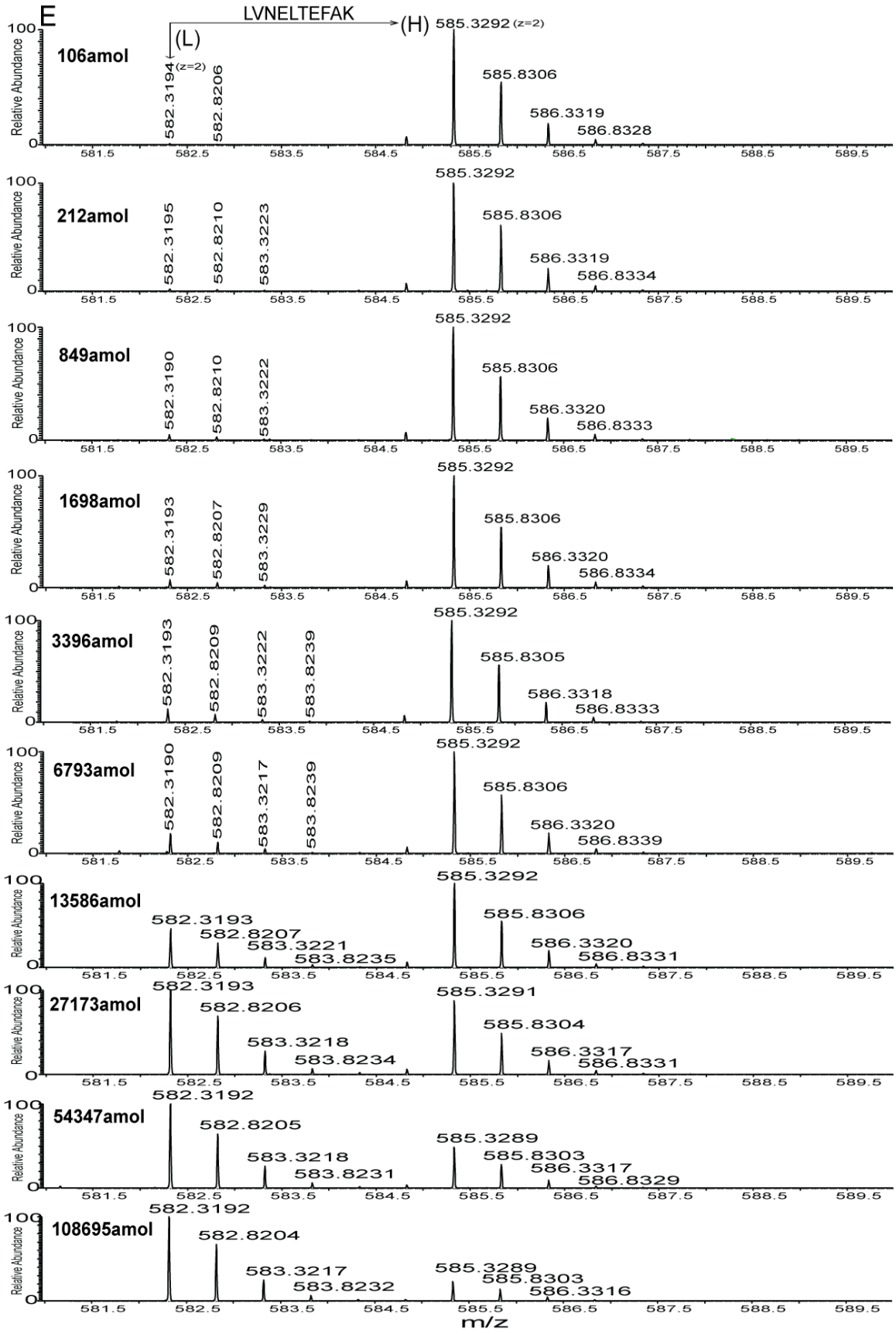


Fig. S20: Attomole quantification of protein. **A**, **B,** shows the electrophoresed 1D SDS PAGE gel loaded with serially diluted BSA protein. The pmol amount of protein loaded in each gel lane is

indicated. **C,** shows the electrophoresed 1D SDS PAGE gel loaded with the heavy isotope labelled chimeric protein CP01 (same amount in all lanes). The BSA gel band corresponding to different dilutions (gel A&B) were cut (marked with colour box) and codigested with CP01 gel band (gel C) (marked with colour box). After in-gel digestion and extraction the peptides were recovered in 46µl of 5% aqueous formic acid and 5µl was injected on to the HPLC column and analysed by LC-MS/MS (only 10.8% of total protein amount loaded on the gel was injected into HPLC column). **D**, shows the calibration plot of serially diluted BSA. The y-axis shows the abundance of light BSA peptide (coming from serially diluted BSA) normalised to the corresponding heavy BSA peptide (coming from CP01). The x-axis shows the amount (amol) of protein loaded on to the HPLC column. Inset to the pane D, shows the calibration plot of three lower dilutions at 106, 212 and 849amol. **E**, exemplify the mass spectra of light and heavy BSA peptide of sequence LVNELTEFAK at m/z 582.3190 and 585.3290 respectively. The masses were extracted using Xcalibur software with 5ppm mass tolerance.

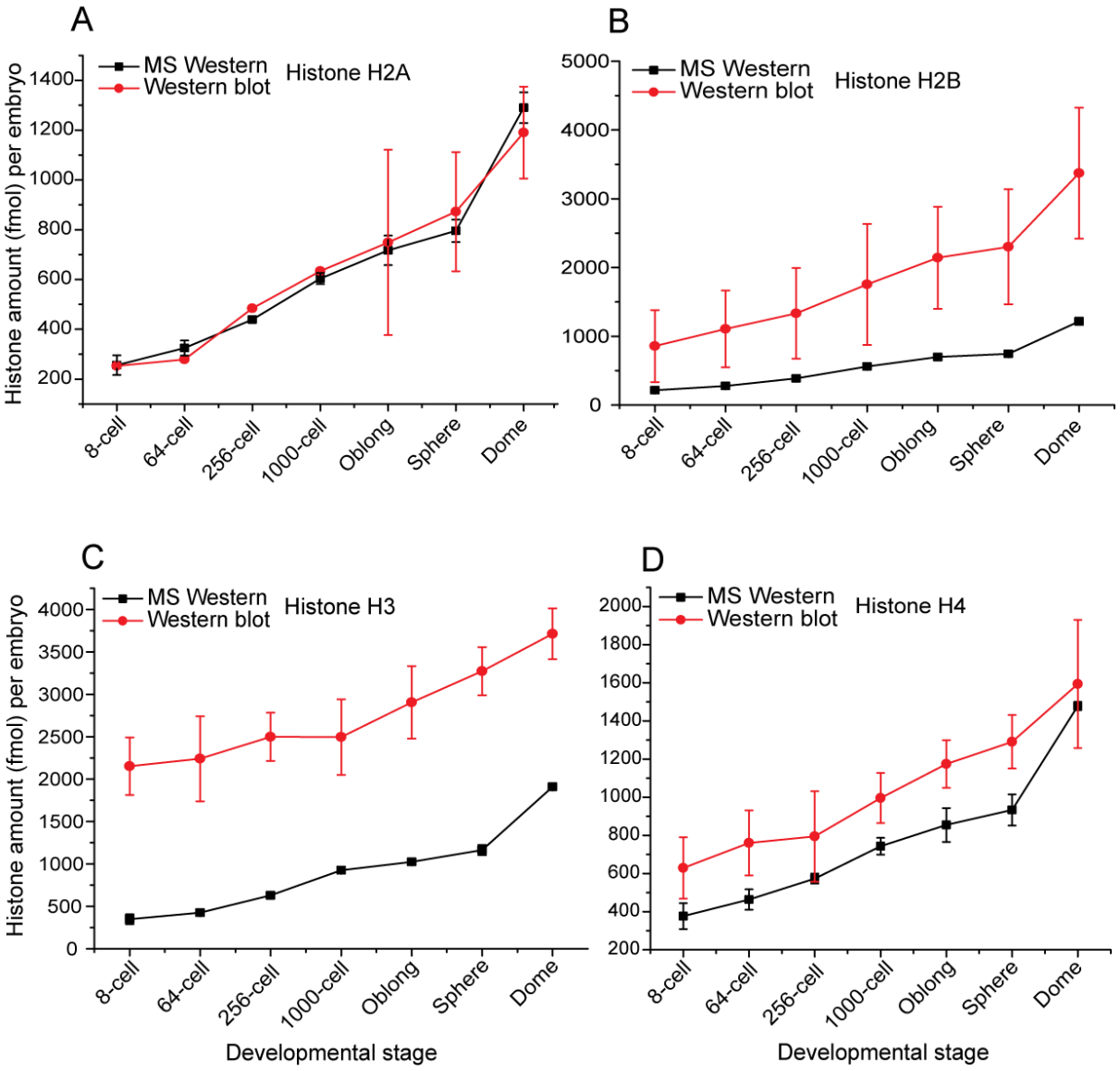


Fig. S21: Comparison of histone quantification by MS Western and by Western blot. The quantification of histone molecules from different developmental stages of zebrafish embryo was carried out by MS Western and Western blot (Li-COR Odyssey system). **A**, The number of histone H2A per embryo determined by MS Western and Western blot fits very well. **B and D,** the number of histone H2B and H4 per embryo determined by MS Western and Western blot is generally matching. **C**, the number of histone H3 per embryo determined by MS Western and Western blot did not match. The western blot reports higher number of molecules compared to MS Western. The values are mean ±SD (n=3), where “n” represents number of biological replicates.

Supplementary Tables

Table S1: Target proteins and corresponding proteotypic peptides selected for chimeric protein CP01.

S.No	Protein UniProt accession number	Protein Name (Gene Name)	Peptide sequence	$[M+2H]^2$ m/z^+
1.	P00489 (PYGM_RABIT)	Glycogen phosphorylase, muscle form (PYGM)	GLAGVENVTELK	615.3408
			VLVDLER	422.2505
			VAAAFPGDVDR	559.2859
			IGEEYISDLDQLR	775.8889
			LLSYVDDEAFIR	720.8722
			VIFLENYR	527.2903
			VFADYEEYVK	631.8024
			DFYELEPHK	589.2796
2.	P02769 (ALBU_BOVIN)	Bovine Serum Albumin (ALB)	LVNELTEFAK	582.319
			YLYEIAR	464.2505
			DAFLGSFLYEYSR	784.3771
			HLVDEPQNLIK	653.3619
			LGEYGFQNALIVR	740.4016
			QTALVELLK	507.8128
3.	P00924 (ENO1_YEAST)	Enolase 1 (ENO1)	GNPTVEVELTTEK	708.8649
			NVNDVIAPAFVK	643.8589
			AVDDFLISLDGTANK	789.9055
			TFAEALR	404.2218
			TAGIQIVADDLTVTNPK	878.478
			AADALLLK	407.7553
			VNQIGTLSESIK	644.8593
4.	P00330 (ADH1_YEAST)	Alcohol dehydrogenase 1 (ADH1)	GVIFYESHGK	568.7909
			ANELLINVK	507.3033
			IGDYAGIK	418.7294
			VLGIDGGEGK	472.7569
			EALDFFAR	484.7457
			VVGLSTLPEIYEK	724.406
5.	P63048 (RL40_BOVIN)	Ubiquitin (UBA52)	TITLEVEPSDTIENVK	894.4675
			TLSDYNIQK	541.2798
			ESTLHLVLR	534.3139
			EGIPPDQQR	520.2618

Table S2: Target proteins and corresponding proteotypic peptides selected for chimeric protein CP02. Histone peptides were selected from *Danio rerio (Zebrafish)*

S.No	Protein UniProt accession number	Protein Name (Gene Name)	Peptide sequence	$[M+2H]^{2+}$ *m/z*
1.	P00489 (PYGM_RABIT)	Glycogen phosphorylase, muscle form (PYGM)	GLAGVENVTELK	615.3405
			VAAAFPGDVDR	559.2855
			DFYELEPHK	589.2796
			VFADYEEYVK	631.8006
2	P02769 (ALBU_BOVIN)	Bovine Serum Albumin (ALB)	LVNELTEFAK	582.3193
.			YLYEIAR	464.2504
			DAFLGSFLYEYSR	784.3754
			HLVDEPQNLIK	653.3617
			LGEYGFQNALIVR	740.4018
			QTALVELLK	507.8128
3	A4VAK6	Histone H4 (hist1h4l)	DNIQGITKPAIR	663.3809
.			ISGLIYEETR	590.8141
			VFLENVIR	495.2926
			DAVTYTEHAK	567.7749
			TVTAMDVVYALK	655.8549
4.	B3DJF8	Histone H2B (si:dkey-108k21.22)	ESYAIYVYK	568.2872
			EIQTAVR	408.7324
			LLLPGELAK	477.3051
			AMGIMNSFVNDIFER	872.4133
			QVHPDTGISSK	584.8015
5.	Q7ZTT0	Histone H2A (phc2b)	AGLQFPVGR	472.7693
			HIQLAVR	418.759
			EPNAGTEAQSQDF	697.2966
6.	Q6PI20	Histone H3.3 (h3f3a)	YRPGTVALR	516.8011
			STELLIR	416.2504
			EIAQDFK	425.7191

Table S3: Target proteins and corresponding proteotypic peptides selected for chimeric protein CP03.

S.No	Protein UniProt accession number	Protein Name (Gene Name)	Peptide sequence	$[M+2H]^{2+}$ *m/z*
1.	P00489 (PYGM_RABIT)	Glycogen phosphorylase, muscle form (PYGM)	GLAGVENVTELK	615.3405
			VAAAFPGDVDR	559.2855
			DFYELEPHK	589.2796
			VFADYEEYVK	631.8006
2.	P02769 (ALBU_BOVIN)	Bovine Serum Albumin (ALB)	LVNELTEFAK	582.3193
			YLYEIAR	464.2504
			DAFLGSFLYEYSR	784.3754
			HLVDEPQNLIK	653.3617
			LGEYGFQNALIVR	740.4018
			QTALVELLK	507.8128
3.	P04040	Catalase (CAT)	DPILFPSFIHSQK	764.912
			NLSVEDAAR	487.7483
			LSQEDPDYGIR	646.8105
			DLFNAIATGK	525.2853
			FNTANDDNVTQVR	747.3531
			AFYVNVLNEEQR	741.3729
			NAIHTFVQSGSHLAAR	859.9518
4.	P53350	Serine/threonine-protein kinase PLK1 (PLK1)	AGVPGVAAPGAPAAAPPAK	785.4403
			LGNLFLNEDLEVK	752.4067
			HINPVAASLIQK	645.8803
			FSIAPSSLDPSNR	695.8518
			QEEAEDPACIPIFWVSK	1009.9782
			LILYNDGDSLQYIER	906.4623
5.	Q71U36	Tubulin alpha-1A chain (TUBA1A)	TIGGGDDSFNTFFSETGAGK	1004.451
			AVFVDLEPTVIDEVR	851.4547
			QLFHPEQLITGK	705.8925
			NLDIERPTYTNLNR	859.9448
			IHFPLATYAPVISAEK	878.9846
			VGINYQPPTVVPGGDLAK	912.9968
6.	P60484	Phosphatase and tensin homolog (PTEN)	NNIDDVVR	472.7435
			IYNLCAER	519.7557
			AQEALDFYGEVR	699.3384
			YVYYYSYLLK	687.8529
			IYSSNSGPTR	541.2681
			FMYFEFPQPLPVCGDIK	1052.5011
7.	P31749	RAC-alpha serine/threonine-protein kinase (AKT1)	NDGTFIGYK	507.7482
			CLQWTTVIER	653.3343
			DEVAHTLTENR	642.812
			HPFLTALK	463.7757
			TFCGTPEYLAPEVLEDNDYGR	1223.5483
			LFELILMEEIR	703.3916
8.	P04406	Glyceraldehyde-3-phosphate dehydrogenase (GAPDH)	VGVNGFGR	403.2192
			LVINGNPITIFQER	807.4531
			VIHDNFGIVEGLMTTVHAITATQK	1298.1836
			GALQNIIPASTGAAK	706.3984

			VPTANVSVVDLTCR	765.9009
			LISWYDNEFGYSNR	882.4048

Table S4: Target proteins and corresponding proteotypic peptides selected for chimeric protein CP04.

S.No	Protein UniProt accession number	Protein Name (Gene Name)	Peptide sequence	$[M+2H]^{2+}$ *m/z*
1.	P00489 (PYGM_RABIT)	Glycogen phosphorylase, muscle form (PYGM)	GLAGVENVTELK	615.3405
			VAAAFPGDVDR	559.2855
			DFYELEPHK	589.2796
			VFADYEEYVK	631.8006
2.	P02769 (ALBU_BOVIN)	Bovine Serum Albumin (ALB)	LVNELTEFAK	582.3193
			YLYEIAR	464.2504
			DAFLGSFLYEYSR	784.3754
			HLVDEPQNLIK	653.3617
			LGEYGFQNALIVR	740.4018
			QTALVELLK	507.8128
3	A0A0B4K7R8	Crumbs (crb)	GSTLSAQQGSQFK	669.836
			NEPVSYILELINGR	808.939
			NIGDSYVAAK	519.267
			LDNGYNHLIEVVR	514.607
4	A8JUP3	Cht6 (Cht6)	LVLGIPTYGR	544.827
			FSPLVASNER	560.295
			LTEAEGSSLYIGGR	726.87
			STDAEEDPQVIK	666.32
			FADQDNDLVNLR	710.347
5	Q9VGT0	UDP-glycosyltransferase 35b (Ugt35b)	LSNFVDTTVAWLNYR	899.962
			FFVSVTR	428.24
			VQLTDLNR	479.769
			DVFIPDVFNNYK	735.867
			ILAIFPFPGPSQYINVVPYLK	1188.67
6	E1JI40	Vermiform (verm)	VPIWPYTLYFR	727.895
			GTFFVSHK	461.743
			GHEISVFSLTHK	452.242
			APLGLHFHASWLK	492.942
7	Q9VGT3	GM04645p (Ugt86Da)	IINNPEATQR	578.309
			AVYWVEHVSR	623.323
			SHYHVGSALAK	390.541
			AEQNGYGVTVHYEELSSAK	694.664
8	Q7K3B7	LD40177p (CG11208)	GSAYAHAENTLR	645.316
			SEGDLTQITPPSALGVQVR	984.523
			AEVEAVQIIAESLK	771.426
			AANQLTDRPTIINVAISPSSDR	780.418
9	A0A0B4LGS0	Amylase proximal (Amy-p)	GHGAGGADVLTYK	623.316
			DLNQGNSYVQDK	690.825

			SLVFVDNHDNQR	722.354
			SEYTGLGAITEFR	722.36
			VVEFLDHLIDLGVAGFR	950.52
10	Q9VJ86	Bicoid stability factor (bsf)	IGALDTSR	416.731
			LIELLVR	428.287
			LADLLLQEER	600.335
			VNGEPVDVGAFFIR	760.401
			ITPDVVGAIVQEATTYFR	990.527
			ATQLSEQLGSELTPK	801.423
11	Q9VJ80	LD42267p (CG10211)	LLAADYADGVSQPR	738.381
			ILSWNAVNLYGLK	745.922
			ASEQPGLTAIHTAFLR	571.309
			LLPAQYEDGISAPR	765.404
12	Q7JWF1	Electron transfer flavoprotein-ubiquinone oxidoreductase, mitochondrial (Etf-QO)	GIATNDVGIAK	529.796
			DTFSFLTGSGR	594.289
			AINEGGFQSLPQK	694.862
			FGLNEGSEPQAYGIGLK	890.45
			ITTHYTLNPR	608.331
13	Q9VG97	Inactive glutathione S-transferase D3 (GstD3)	AILVYLVEK	524.326
			ITPGWEENWAGALDVK	893.443
			INPQHSIPTLVDNGFTIWESR	808.750
			ALGLEFNK	446.251
14	M9NF15	Axotactin (axo)	INLDPYALYVIVEK	825.462
			TNFDDSALFYASGESLK	932.933
			VLTTGGDAGSNR	574.289
15	Q9VZJ8	RH40737p (CG11594)	LGSDVQPPGR	513.272
			VFLQLLENQR	630.359
			NLSQTFGNIWR	668.344
			VFDLPDFLTWR	704.866
16	Q9VF24	Crossveinless d (cv-d)	GSTVQFAAR	468.748
			DGAISHVVFK	536.792
			YFNAYLSDR	574.773
			FSEPYDSTLSDVIK	800.889
17	Q9U1L2	CG3699 (EG:BACR7A4.14)	NVANLEATK	480.262
			DADAIVQQTLAK	636.844
			GTQAEIVVADVTK	665.864
			VGDVTEVAEAVAFLASSK	896.972
18	A1ZB68	FI01423p (GstE3)	SVLLTLR	401.264
			IDALEGVYK	504.274
			ALNLDFDYK	549.777
			AITFPLFWENK	683.364
			LTLYGIDGSPPVR	694.383
			ELPYYEEANGSR	714.325
19	P20432	Glutathione S-transferase D1 (GstD1)	AVGVELNK	415.243
			APADPEAFK	473.237
			AIQVYLVEK	531.813
			LLNLQAGEHLKPEFLK	925.531
			INPQHTIPTLVDNGFALWESR	803.417
20	A0A1F4	Protein eyes shut (eys)	GLVVSGTR	394.735

			SLSDVSLTGR	517.777
			GNSYLILPPPR	613.849
			ISGPSNHVTVVR	633.351
			NAAFSGDSYVSHR	705.824
21	A0A0B4LGM0	Prominin (prom)	SVVNVGLGQALR	606.857
			STQEEVDHIR	607.294
			SAVHDVEVFLK	415.229
			VSTAIDAISVSGR	638.349
			VALQIQDVATSSR	694.381
22	A0A0B4KI35	Chaoptin (chp)	LEEISLR	430.248
			LLELHDNR	505.275
			LFNNFDVLR	569.306
			LAVLDLSHNR	569.323
			ASLSGIQSHAFK	623.333
			TFFDGNPIHTLR	709.366
			DFGVELEDLQITR	767.892
23	A0A0B4KI71	Microtubule-associated protein 205 (Map205)	STISSTTTVR	526.783
			TSTTSSLTGNPR	611.307
			TSVATVAGGAVVGATK	694.891
			LLVPGSSSTTTTSSLR	803.935
			LLPDTTDEQLLTSALEEK	1008.53
24	L0MN91	Bent (bt)	STGNIFAAK	454.746
			VFAVNSAGR	460.751
			IFADNVYGR	527.769
			GVAEDFAPSFVK	633.823
			HDGGSPITGYIIEK	743.881
25	E1JI76	Z band alternatively spliced PDZ-motif protein 66 (Zasp66)	HYPNPAVR	477.251
			VVGSEADTGR	495.746
			DAPTTESYLR	576.78
			STVPFATSESNR	648.315
			LVGGNDLDTPLIITR	798.953
26	M9PBI9	Titin (sls)	IAELEGLGSR	522.788
			IQYLEDSTR	562.783
			IGEAISQSSIR	580.816
			FITQIVDVTK	582.338
			AEDAGVYLVVAR	631.841
			TYYDFGFVALDIIK	832.936
27	M9PCC1	Receptor of activated protein kinase C 1 (Rack1)	VWQVSVSAH	506.763
			ALLWDLNDGK	572.804
			DVLSVAFSADNR	647.327
			LWDLAAGK	437.246
28	A1Z9J3	Short stop (shot)	LNNDLIAR	464.764
			FQEALAGLSK	532.295
			LAAHDALGGAAK	365.537
			SAGSGVSTTAIEK	604.312
			IVQVQIDDVGK	607.344
			SELDVFSDWLQVAR	832.921
			FSQSDFGLDQGETLLR	906.943

29	E1JJE6	Uncharacterized protein (CG42492)	ESLLEITIYHQK	737.402
			GAAYQEAPVDAEVAVTPK	908.461
			ISAFGLFTYSVFSIIAGSLK	1061.09
			VFANPVQLEFYGFVPWSIIQR	1255.67
30	M9NE68	Heat shock protein 23 (Hsp23)	QVGASSGSSGAVSK	611.308
			IVQIQQVGPAHLNVK	822.484
			VQDNSVLVEGNHEER	575.612
31	Q9W3C3	CG2004 (CG2004)	VLPAIEAFQK	558.327
			FSEATLDEIIR	647.339
			FHGVALAFNALDSK	745.395
			ENAILTDIWNITPFK	887.975
32	Q9VBS7	Uncharacterized protein (CG10550)	VIVDILLK	456.811
			TILVDLQVGK	543.332
			LAELHAASVVAK	604.854
			EAGLEIELAPK	585.325
33	P53501	Actin-57B (Act57B)	AGFAGDDAPR	488.728
			SYELPDGQVITIGNER	895.95
			VAPEEHPVLLTEAPLNPK	977.537
			GYSFTTTAER	566.767
			EITSLAPSTIK	580.332
34	A0A0B4LH50	Actin 87E (Act87E)	EITALAPSTIK	572.335
			QEYDESGPGIVHR	743.85
35	X2JCP8	Actin 5C (Act5C)	QEYDESGPSIVHR	758.856
36	Q8IMT8	Veli (veli)	SIELLEK	416.245
			LQASGDFPTTK	582.799
			EQNSPIYISR	603.81
			ATVAAFAASEGHAHPR	531.603
37	P06002	Opsin Rh1 (ninaE)	YQVIVK	375.232
			SSDAQSQATASEAESK	798.854
38	P19334	Transient receptor potential protein (trp)	ALSASSLIALSSR	638.368
			DPVLTAFQLSWELK	823.945
			DDYGITEDDIIEVR	826.888
			ATAWVIVHR	526.803
39	X2JCI4	Phosphoinositide phospholipase C (norpA)	VELELWLK	515.303
			VVLPDLAVLR	547.851
			ALGIEEQSGGAAR	629.824
			VDEYGFFLYWK	733.854
40	P23625	G protein alpha q subunit (Galphaq)	YYLSDLAR	500.758
			IEQADYLPTEQDILR	902.459
			VPTTGILEYPFDLDGIVFR	1076.58
			TIITYPWFQNSSVILFLNK	1142.62
41	M9NES0	Arrestin 1 (Arr1)	AGIAVEGDIK	486.771
			DAFGIIVSYAVK	641.856
			DTALASTTLIASQDAR	817.424
			DFLLSPGELELEVTLDK	959.507
42	P19107	Phosrestin-1 (Arr2)	VFGQLATTYR	578.311

			LQYAPLNR	487.774
			ISLEVTLDR	523.298
			LLQPAPGTIEK	583.842
43	C7LAH9	Moesin/ezrin/radixin homolog 1 (Moe)	FYPEDVAEELIQDITLR	1026.02
			IGFPWSEIR	552.796
			TTHTAGFLANDR	652.324
			LFYLQVK	455.774
44	P10676	Neither inactivation nor afterpotential protein C (ninaC)	AAIELNR	393.728
			GDNILLTK	437.256
			VPPEVPPSK	475.272
			LVDFIINR	495.293
45	P45594	Cofilin/actin-depolymerizing factor homolog (tsr)	SLVGVQK	365.727
			QIDVETVADR	573.295
			YIQATDLSEASR	677.336
			NAEYDQFLEDIQK	806.879

Table S5: Data-dependent acquisition (DDA) settings used in this study

Data dependent acquisition setting for LTQ Orbitrap Velos mass spectrometer	
Parameter	Value
Scan range	350-1700 m/z
Microscans	1
Nominal resolution (at 400 m/z)	60,000
Lock mass	445.120025 m/z (Siloxane)
Data mode	Centroid
Target ion count	1x10E6 (FT) and 5x10E4 (IT)
Max. fill time	500 ms (FT) and 100 ms (IT)
Automatic gain control (AGC)	ON
Activation type (MS/MS)	CID
Intensity threshold for DDA	5000
Isolation width	2.0 m/z
Normalized collision energy (NCE)	35%
Activation Q	0.25
Activation time	30 ms
Dynamic exclusion list size	500
Dynamic exclusion time	30s
Exclusion mass width	5ppm
Data dependent acquisition setting for Q Exactive HF mass spectrometer	
Parameter	Value
Full scan	
Scan range	350-1700 m/z
Microscans	1
Resolution	60,000
Lock mass	445.120025 m/z (Siloxane)
Data mode	Profile
AGC target	3e6
Maximum IT	50 ms
dd-MS2	
Microscans	1
Resolution	15,000
Data mode	Centroid
AGC target	1e5
Maximum IT	30 ms
TopN (no msx)	12
Isolation window	1.6 m/z
Isolation offset	0.0 m/z
Fixed first mass	140 m/z
NCE (no stepped NCE)	25
Apex trigger	Off
Charge exclusion	Unassigned, 1 and >8
Peptide match	Off
Exclude isotopes	On
Dynamic exclusion	30s

Table S6: Antibodies used for quantifying proteins in HeLa cells

Primary antibodies			
Target	**Company**	**Code**	**dilution in WB**
Serine/Threonine Kinase 1 (AKT1), mouse monoclonal	Sigma-Aldrich	P2482	1:5000
Catalase (CAT), mouse monoclonal	Sigma-Aldrich	C0979	1:2000
Polo like Kinase 1(PLK1), mouse monoclonal	Sigma-Aldrich	P5998	1:5000
α-Tubulin (TUBA4A), mouse monoclonal	Sigma-Aldrich	T6199	1:5000
Glyceraldehyde-3-Phosphate Dehydrogenase (GAPDH), goat polyclonal	Acris Antibodies	AP16240PU-N*	1:7000

*****at present discontinued by the manufacturer

Secondary antibodies			
Name	**Company**	**Code**	**dilution in WB**
IRDye® 680LT Donkey anti-Goat (H+L)	LI-COR	925-68024	1:15000
IRDye® 800CW Donkey anti-Mouse (H+L)	LI-COR	926-32212	1:15000

Table S7: **Human esiRNAs used to knock-down target proteins in HeLa cells**

esiRNAs		
Target	**Company**	**Code**
RLUC	Eupheria Biotech	EHURLUC
AKT1	Eupheria Biotech	EHU083501
CATALASE	Eupheria Biotech	EHU048671
PLK1	Eupheria Biotech	EHU051011
TUBA4A	Eupheria Biotech	EHU159831

Table S8: Antibodies used for quantifying zebrafish histones

Primary antibodies			
Target	**Company**	**Code**	**WB**
H3	Abcam	ab1791	1:10,000
H4	Abcam	ab10158	1:1,000
H2A	Abcam	ab18255	1:1,000
H2B	Abcam	ab1790	1:3,000
α-tubulin	Sigma	T6074	1:20,000

Secondary antibodies			
Name	**Company**	**Code**	**WB**
IRDye® 800CW donkey anti-rabbit	LI-COR	P/N 925-32213	1:20,000
IRDye® 800CW donkey anti-mouse	LI-COR	P/N 925-32212	1:20,000

Table S9: Absolute quantification of four histone proteins from zebrafish embryo at ten different developmental stage (BR=Biological replicate and each biological replicate value represents average of two technical replicates). The value in BR1, BR2 and BR3 columns represents amount of histone protein (in fmol) per embryo. The average CV and rCV is <10%

Histone H4	Stage	BR1	BR2	BR3	Average	STDEV	%CV	Median	MAD	rSD	%rCV
	1Cell	457.7922	404.7025	391.4224	417.9723	28.6741	6.860287	404.7025	13.28008	19.68905	4.865068
	8Cell	329.6173	326.3716	471.9903	375.9931	67.89325	18.05705	329.6173	3.245678	4.812042	1.459888
	64Cell	416.8338	435.7922	538.8788	463.8349	53.62552	11.56134	435.7922	18.9584	28.10773	6.449801
	256Cell	580.0844	538.1631	602.0019	573.4164	26.48514	4.618833	580.0844	21.91748	32.49486	5.601747
	1K Cell	800.1541	692.1338	737.5328	743.2736	44.28553	5.958174	737.5328	45.39893	67.30845	9.126165
	HIGH	791.3026	814.9278	699.5248	768.5851	49.77634	6.476361	791.3026	23.62529	35.02685	4.42648
	OBLONG	973.007	829.0689	760.894	854.3233	88.41688	10.34935	829.0689	68.17483	101.076	12.19151
	SPHERE	1007.126	973.5415	819.5227	933.3967	81.68011	8.750846	973.5415	33.58461	49.79255	5.114579
	DOME	1505.467	1451.782	1478.131	1478.46	21.91767	1.482466	1478.131	26.34882	39.06477	2.642848
	SHIELD	2227.938	2311.965	2303.745	2281.216	37.82236	1.657991	2303.745	8.21964	12.18644	0.528984
Histone H2B	Stage	BR1	BR2	BR3	Average	STDEV	%CV	Median	MAD	rSD	%rCV
	1Cell	194.2716	145.65	143.8195	161.247	23.36384	14.48947	145.65	1.830496	2.713893	1.863298
	8Cell	183.294	201.006	263.9764	216.0921	34.62278	16.02223	201.006	17.71202	26.25984	13.0642
	64Cell	259.3253	260.5473	314.4543	278.109	25.70486	9.24273	260.5473	1.221982	1.81171	0.695348
	256Cell	394.2926	369.6656	402.8801	388.9461	14.07693	3.61925	394.2926	8.58745	12.73175	3.229011
	1K Cell	590.3178	547.9452	546.5898	561.6176	20.30162	3.614848	547.9452	1.355396	2.00951	0.366735
	HIGH	602.5109	635.9684	526.4676	588.3156	45.81657	7.787753	602.5109	33.45759	49.60423	8.232919
	OBLONG	738.5126	732.591	625.5263	698.8766	51.92286	7.429474	732.591	5.921552	8.779293	1.198389
	SPHERE	762.2935	781.0886	690.0224	744.4682	39.25619	5.273052	762.2935	18.79515	27.86569	3.655507
	DOME	1193.542	1171.266	1289.459	1218.089	51.279	4.209791	1193.542	22.27623	33.02674	2.76712
	SHIELD	1720.818	2105.001	2407.573	2077.797	281.0254	13.52516	2105.001	302.5716	448.5927	21.3108
Histone H2A	Stage	BR1	BR2	BR3	Average	STDEV	%CV	Median	MAD	rSD	%rCV
	1Cell	249.3922	197.9448	188.0373	211.7914	26.89365	12.69818	197.9448	9.907552	14.68894	7.420724
	8Cell	215.3167	242.5229	309.7799	255.8732	39.70305	15.51669	242.5229	27.2062	40.33591	16.6318
	64Cell	301.5995	305.8671	367.6506	325.0391	30.18125	9.285421	305.8671	4.267666	6.327242	2.068624
	256Cell	432.3453	430.5412	453.6402	438.8423	10.48962	2.390293	432.3453	1.804121	2.67479	0.61867
	1K Cell	633.8255	597.9493	579.814	603.8629	22.44311	3.71659	597.9493	18.13531	26.88742	4.496605
	HIGH	632.6728	665.1393	563.4623	620.4248	42.40335	6.834568	632.6728	32.46651	48.13485	7.608174
	OBLONG	779.6588	734.8612	637.3996	717.3065	59.38882	8.27942	734.8612	44.79764	66.41698	9.038031
	SPHERE	825.9572	828.6819	731.978	795.539	44.95818	5.651286	825.9572	2.724675	4.039603	0.489081
	DOME	1374.179	1228.439	1266.959	1289.859	61.66213	4.780532	1266.959	38.51926	57.10866	4.507539
	SHIELD	1718.778	2144.869	1903.66	1922.435	174.4568	9.074783	1903.66	184.8823	274.1065	14.39892
Histone H3	Stage	BR1	BR2	BR3	Average	STDEV	%CV	Median	MAD	rSD	%rCV
	1Cell	244.9543	220	260.0746	241.6763	16.52378	6.837154	244.9543	15.12029	22.41734	9.151641
	8Cell	298.6748	317.298	430.7783	348.917	58.38181	16.73229	317.298	18.62317	27.61071	8.701823
	64Cell	413.4805	387.1543	479.8892	426.8413	39.01986	9.141538	413.4805	26.32627	39.03133	9.439702
	256Cell	682.2903	582.4125	624.1576	629.6201	40.95749	6.505112	624.1576	41.74512	61.89132	9.915977
	1K Cell	927.4467	1290.012	926.6482	1048.036	171.1033	16.3261	927.4467	0.798579	1.183973	0.127659
	HIGH	968.1659	893.3207	922.0678	927.8515	30.8279	3.322504	922.0678	28.74711	42.62046	4.62227
	OBLONG	1049.45	976.9009	1047.411	1024.587	33.72979	3.292036	1047.411	2.039448	3.023686	0.288682
	SPHERE	1229.202	1178.453	1083.159	1163.605	60.53903	5.202714	1178.453	50.74838	75.23955	6.384602
	DOME	1916.443	1943.526	1867.927	1909.299	31.27372	1.637969	1916.443	27.08336	40.15378	2.095225
	SHIELD	3209.92	ND	3467.35	3338.635	128.715	3.855317	3338.635	#VALUE!	#VALUE!	#VALUE!