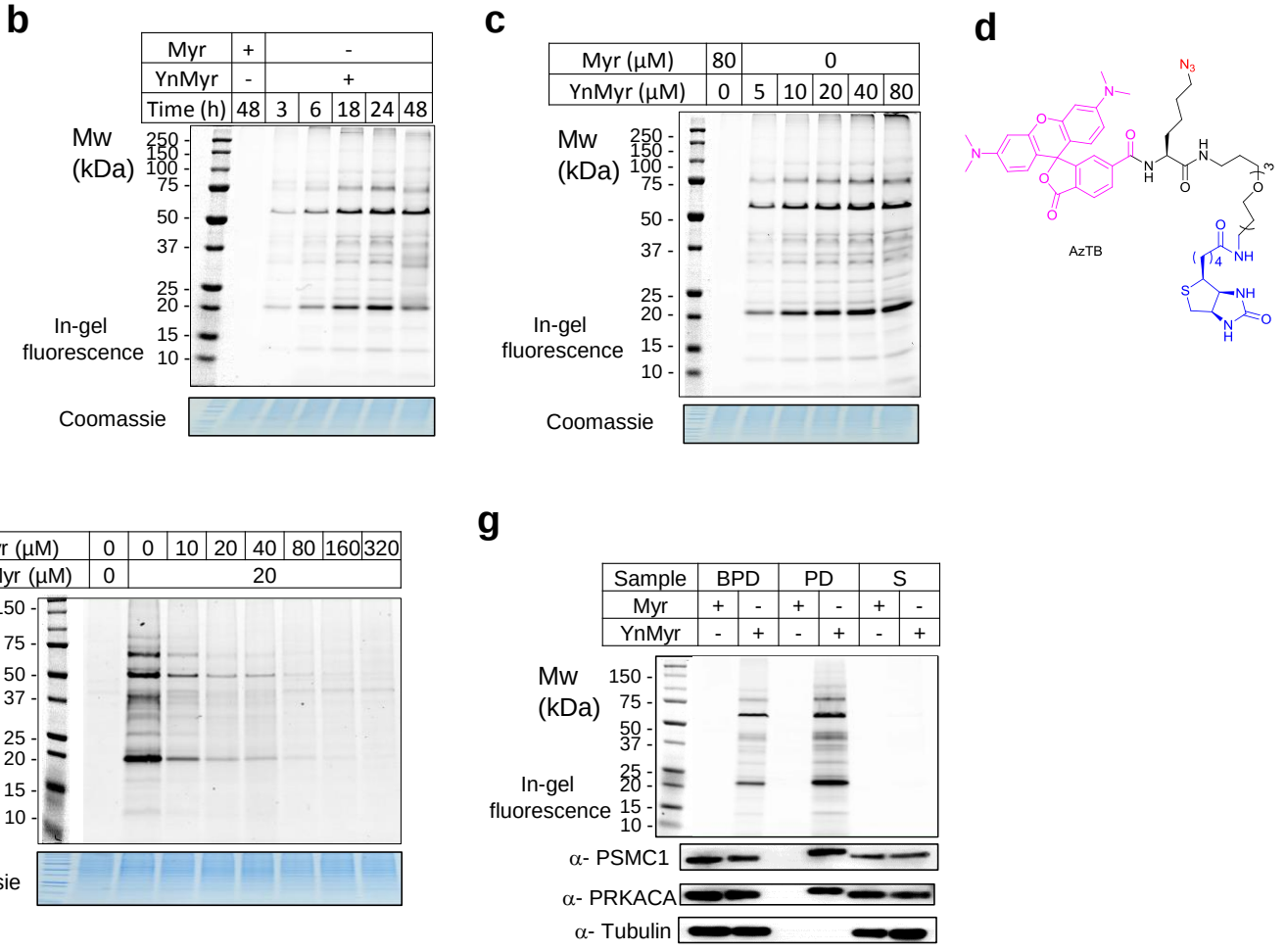
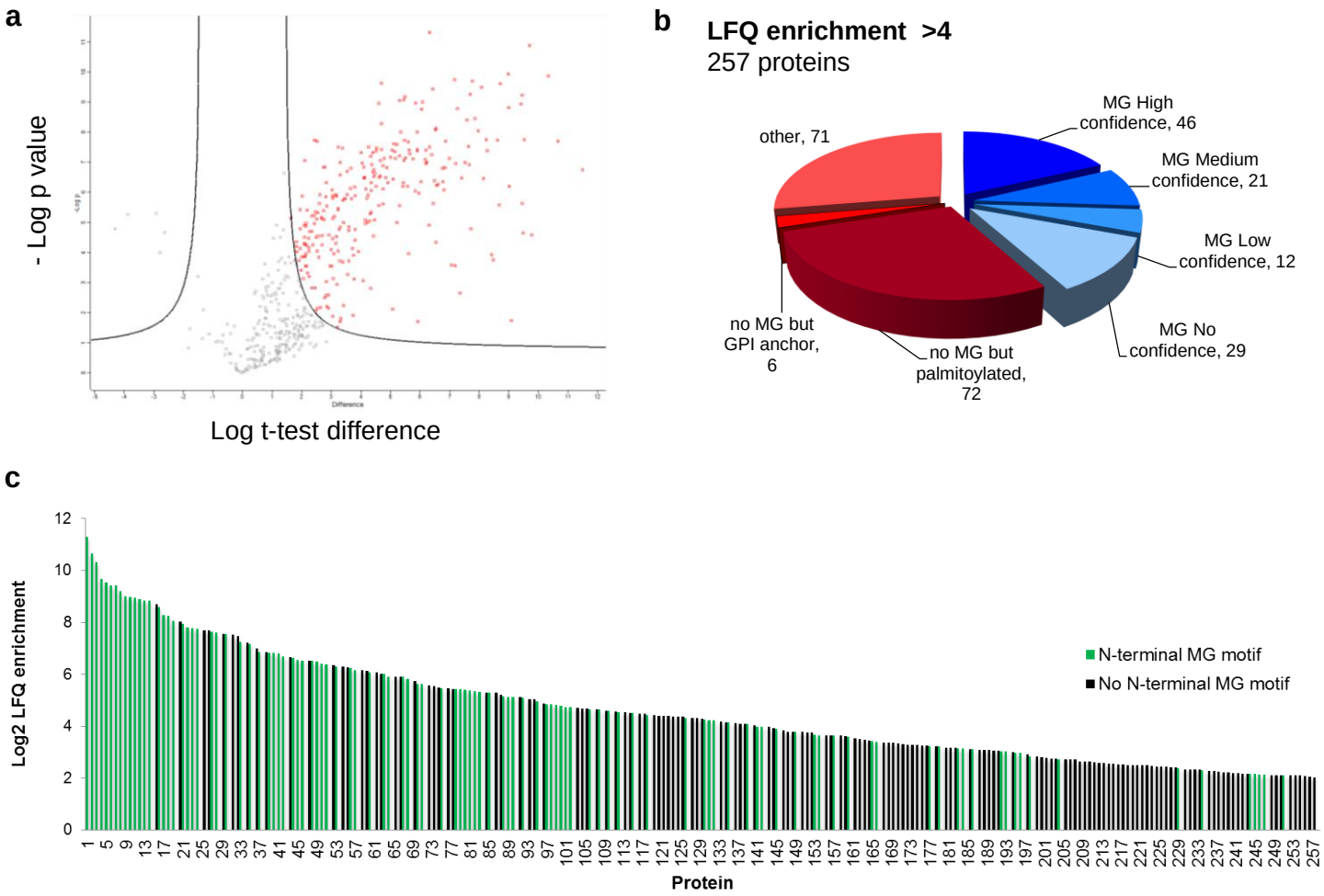
a

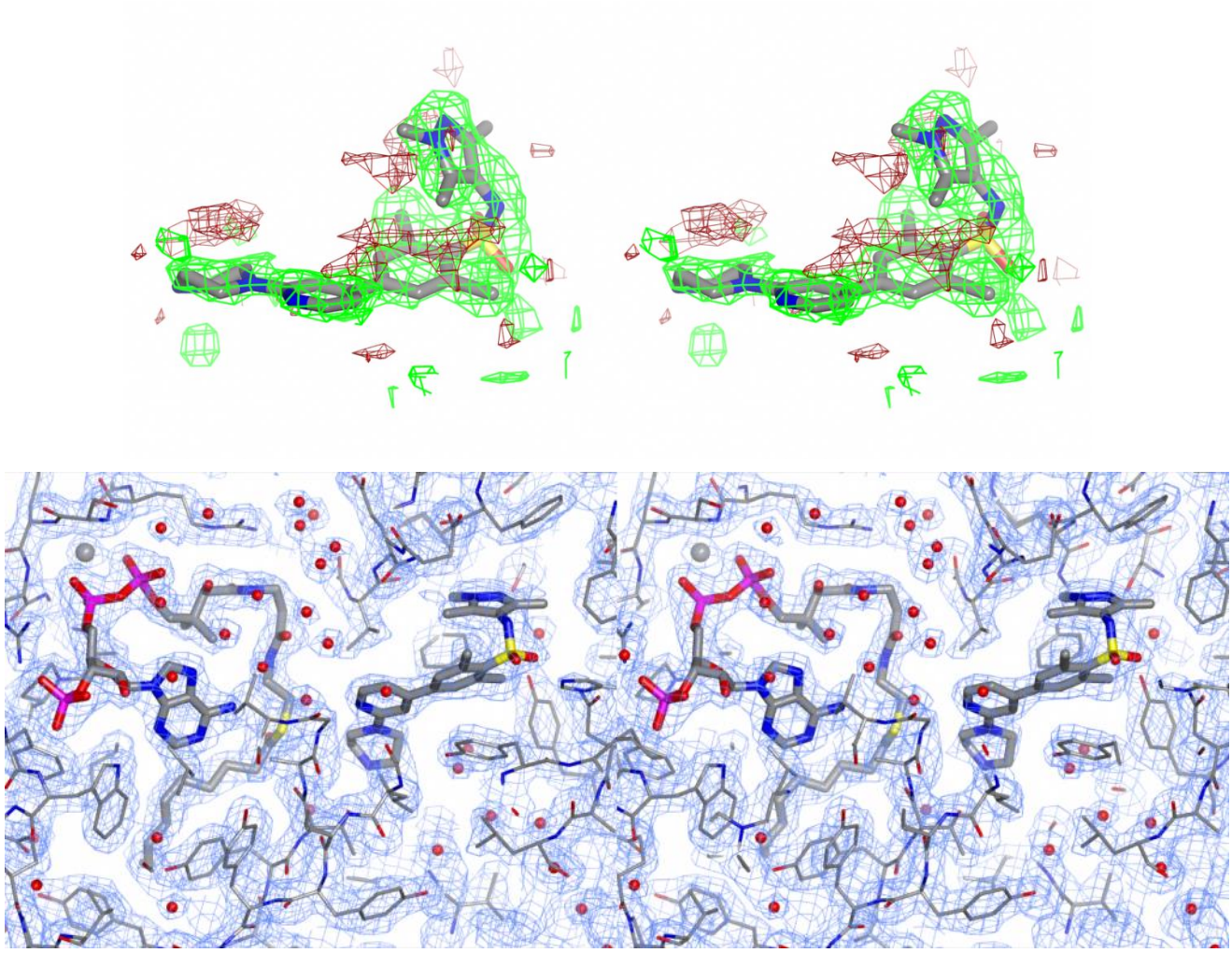
	K_m c-Src 8mer peptide		K_m Myr-CoA	K_m YnMyr-CoA
	With Myr-CoA	With YnMyr-CoA		
NMT1	3.8 ± 0.8 (V_{max} = 26389 ± 1725)	9.8 ± 1.6 (V_{max} = 22953 ± 1480)	4.1 ± 1.1 (V_{max} = 30795 ± 2537)	0.7 ± 0.3 (V_{max} = 17383 ± 1210)
NMT2	1.1 ± 0.2 (V_{max} =15591 ± 650)	2.0 ± 0.6 (V_{max} = 17586 ± 1319)	2.9 ± 0.9 (V_{max} = 19487± 1735)	1.2 ± 0.1 (V_{max} = 15888 ± 373)



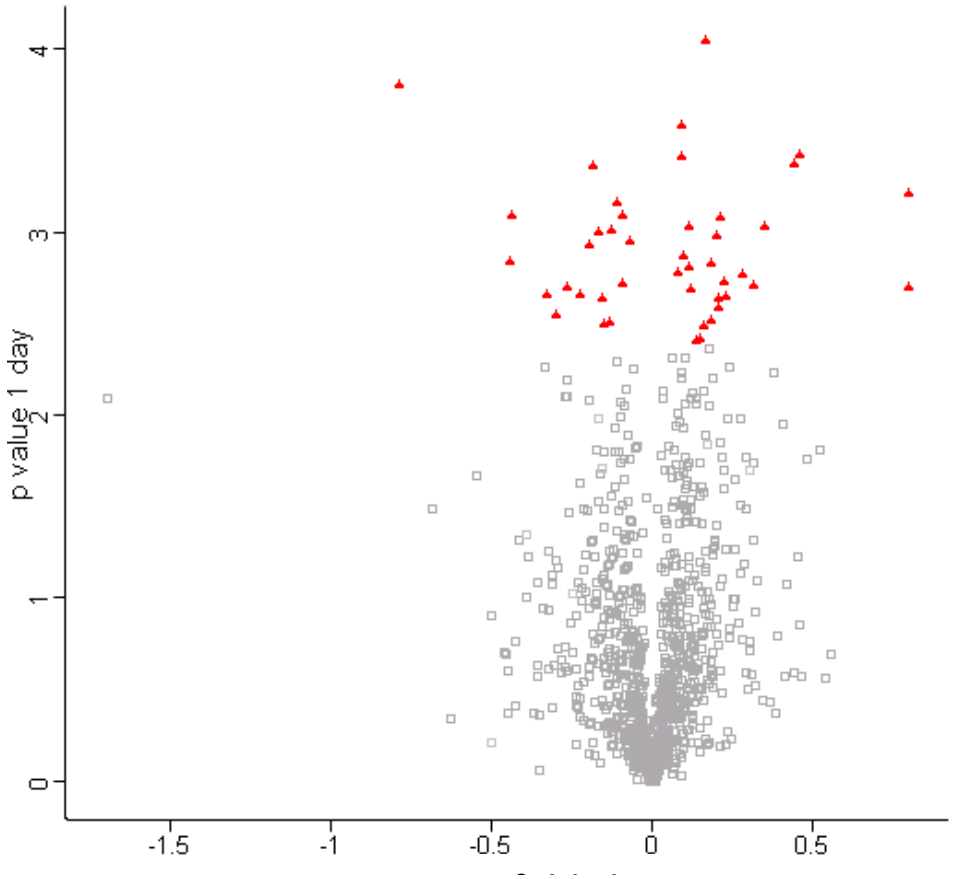
Supplementary Figure 1: YnMyr labeling in mammalian cells. a) K_m of 8AA N-terminal c-SRC was determined in the presence of NMT1 or NMT2 and in the presence of Myr-CoA or YnMyr-CoA using a fluorogenic assay [1]. The data was fitted to the Michaelis–Menten equation using GraFit (7.0). The K_m of Myr-CoA and YnMyr-CoA were also determined in presence of 8AA N-terminal c-Src and NMT1/2; errors, s.e.m. (*n*= 3) b) Time-course labeling with YnMyr (20 µM) in HeLa. c) Concentration series of YnMyr in HeLa. Cells were labeled for 24 h. d) Structure of the capture reagent AzTB (azido-tamra-biotin) [2]. e) Cell viability assay (MTS assay (Promega)) for 72 h in HeLa cells treated with increasing amount of YnMyr; errors s.d. (n = 3). f) Competition experiment: YnMyr was shown to compete with the natural lipid substrate (Myr). g) Samples were analyzed by in-gel fluorescence (top) or enriched by pull-down on streptavidin beads and analyzed by Western blot (WB; bottom). The sample before pull-down (BPD), pull-down sample (PD) and the supernatant from the pull-down (S) were analyzed. PSMC1 and PRKACA were enriched in the pull-down samples. Tubulin: loading control.



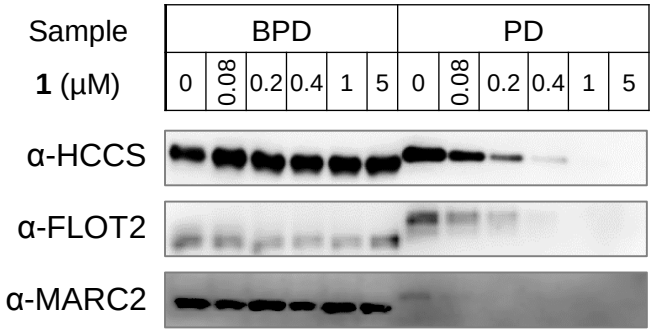
Supplementary Figure 2: Label-free quantification (LFQ) of enrichment of YnMyr-labeled proteins in mammalian cells. a) Two-sample test. To select the significantly enriched proteins in the YnMyr biological replicates (n=4), a two-sample test was carried out in Perseus (modified t-test (permutation-based FDR)) using the following criteria: 250 permutations; FDR 0.001; S0 = 1. The –log p value was plotted against the difference of mean of the Myr biological replicates (n=4) and YnMyr biological replicates (n=4) (log_2 values). The proteins shown in red were significantly enriched in the YnMyr samples. b) 257 substrates were identified by label-free quantification (LFQ) in HeLa cells. 108 proteins had an N-terminal MG motif, as required for *N*-myristoylation. Prediction of *N*-myristoylation was done by two online bioinformatic tools (the MYR predictor and the Myristoylator). Some proteins with no N-terminal MG motif are known to be palmitoylated or incorporate a GPI anchor. c) Log_2 LFQ enrichment was plotted for each protein significantly enriched in the YnMyr samples. Proteins shown in green have an N-terminal MG motif and proteins shown in black do not have an N-terminal MG motif.



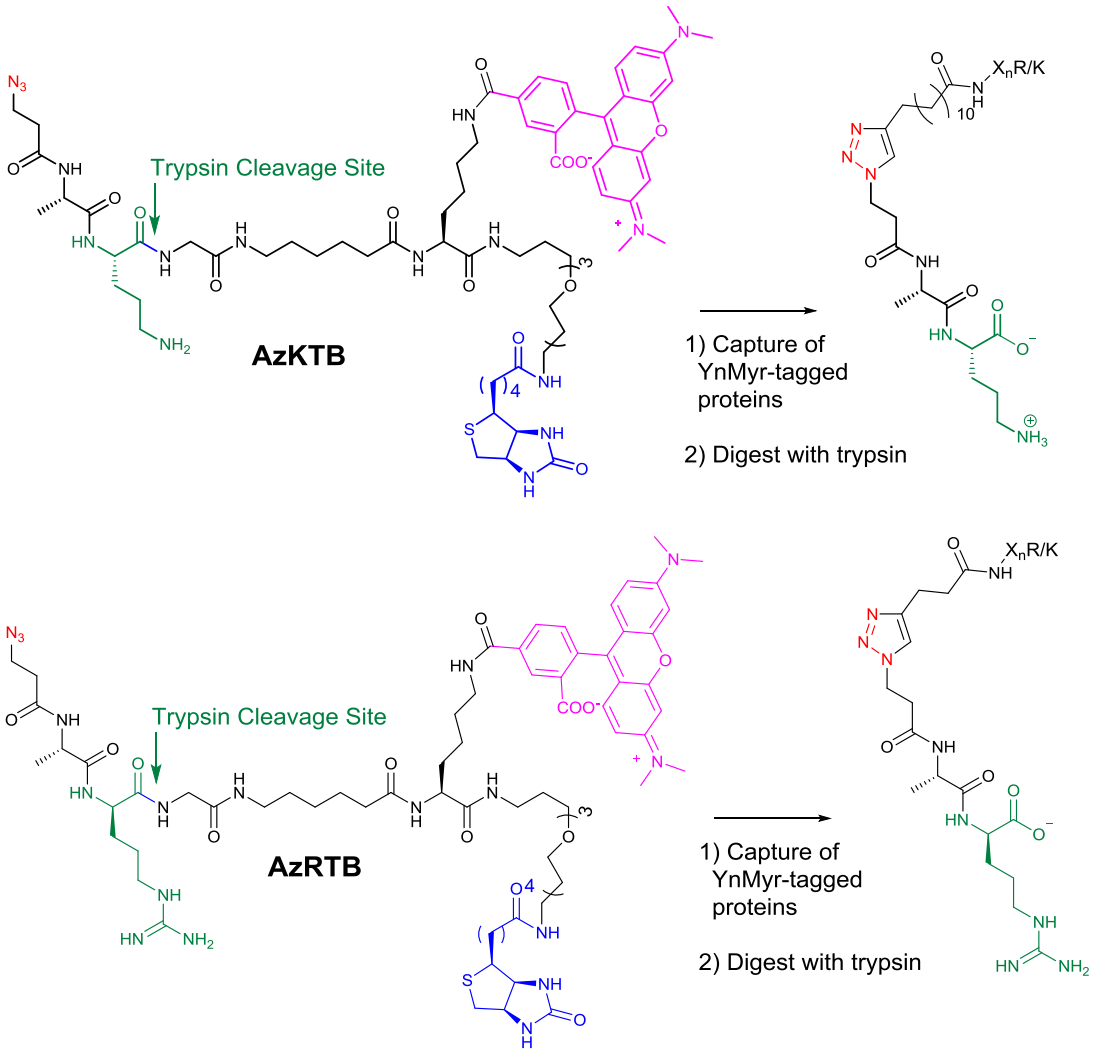
Supplementary Figure 3: Crystal structure of inhibitor 1 bound to HsNMT1. Top panel: Stereo view of initial electron density maps calculated at preliminary stages of refinement (in the absence of ligand) contoured at 2.5σ reveal regions of positive (green) and negative (red) density in the difference map (mF_O-dF_C). The ligand is shown in cylinder representation, coloured by atom; carbon (gray), oxygen (red), nitrogen (blue), sulphur (yellow) and chlorine (silver). **Lower panel:** Stereo view of the final, refined electron density map (2mF_O-dF_C) contoured at a level of 1 σ. The inhibitor and myristoyl-CoA is shown in thick cylinder representation, protein in thin cylinder and colored by atom, as above. The red spheres represent water molecules and the grey sphere represents a Mg atom.



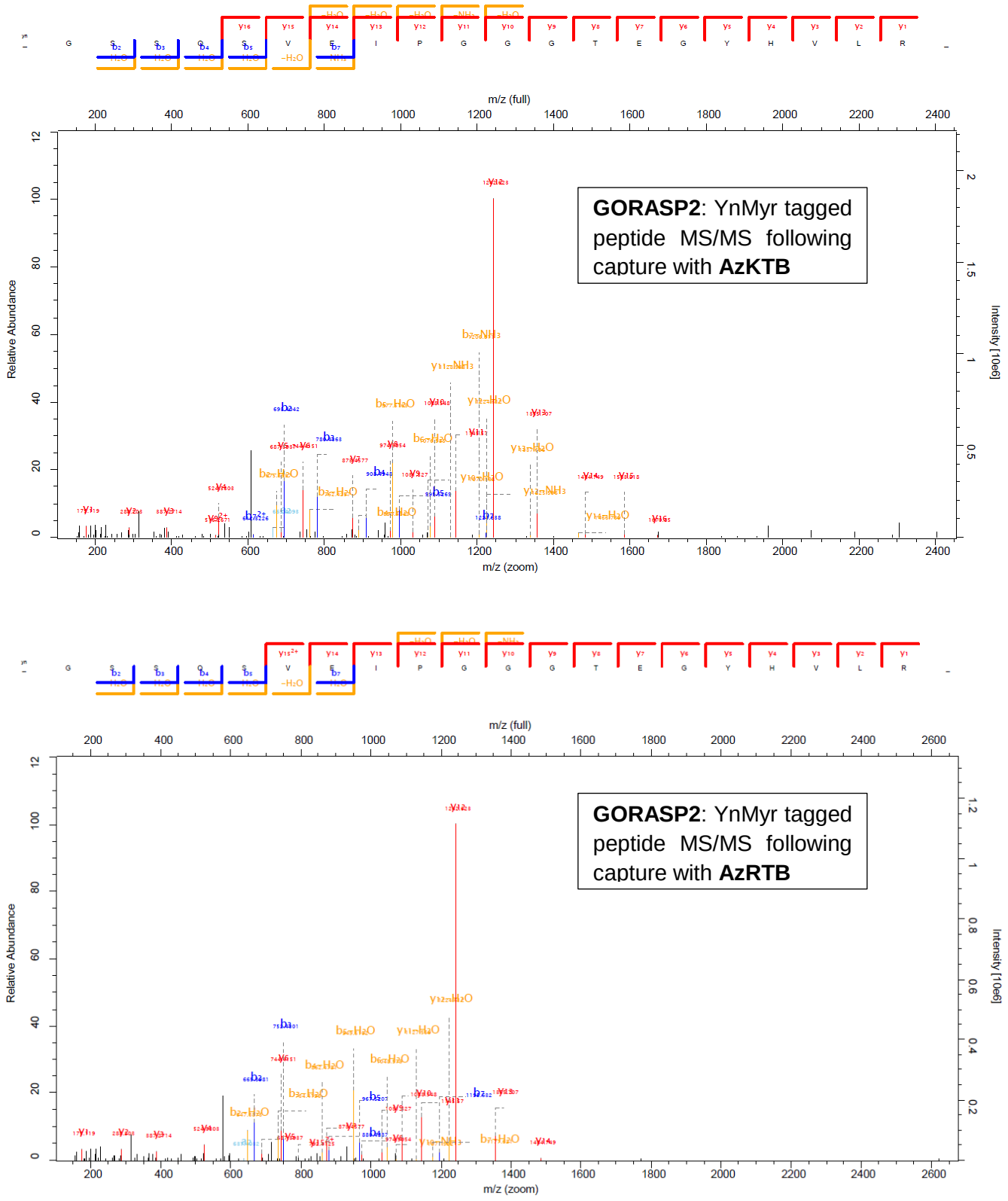
Supplementary Figure 4: quantitative analysis of whole proteome protein abundance following treatment with 1. The HeLa proteome was analyzed by 'spike-in' SILAC (see main text) following 24 hours treatment with 5 µM **1** relative to an untreated control. 1069 proteins were quantified with high confidence and fold change calculated in Perseus. Proteins with a statistically significant change in abundance are highlighted in red; see supplementary methods for experimental details and **Supplementary Data 5** for full dataset.



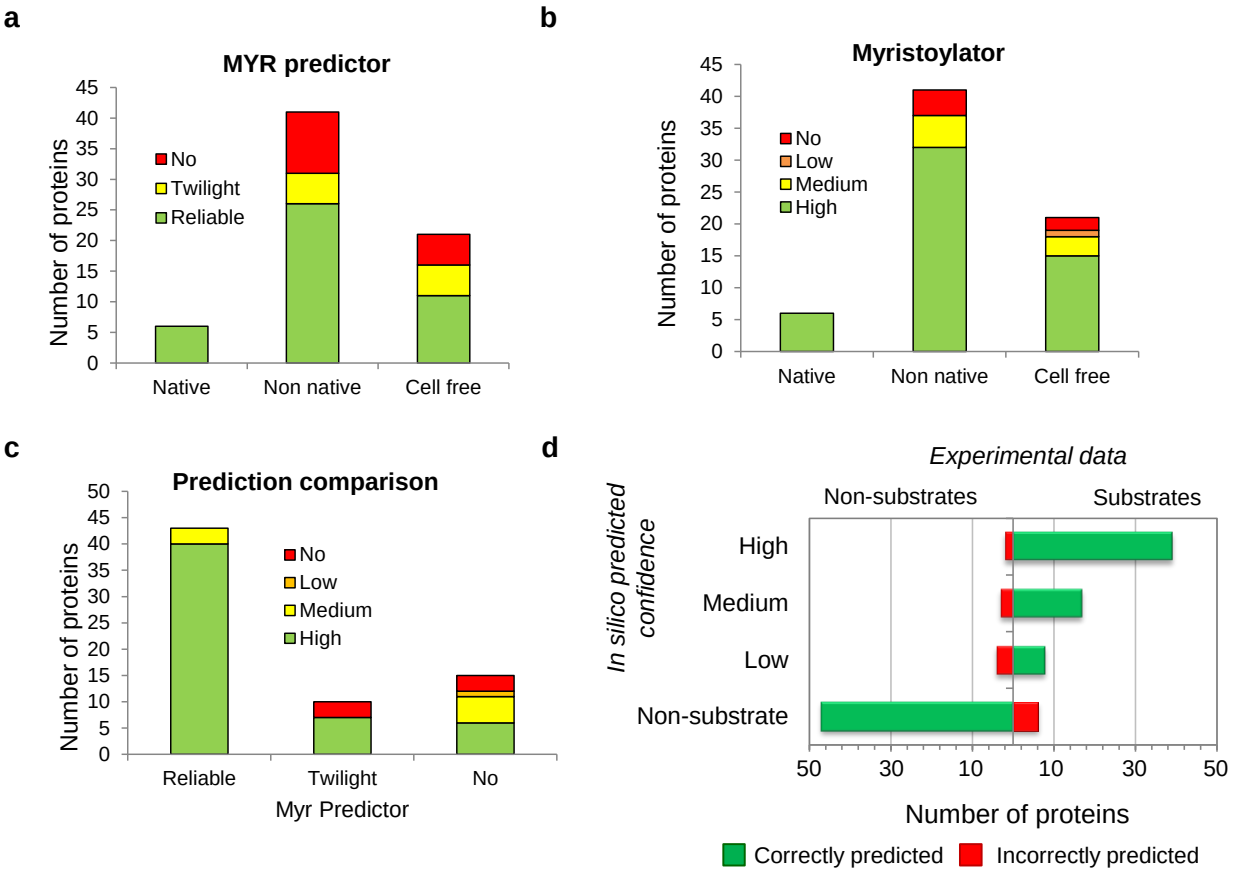
Supplementary Figure 5: Western blot analysis of novel NMT substrates. Proteins were analyzed by Western blot under the same conditions as in Fig. 2d (see main text). Proteins show a band shift to a higher apparent molecular weight on labeling consistent with their modification by CuAAC.



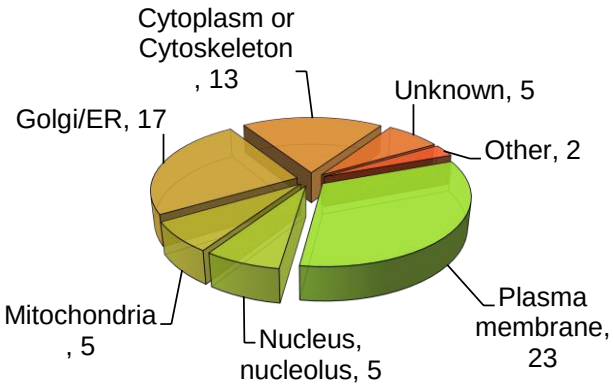
Supplementary Figure 6: **Generation of a tagged solution-phase peptide through on-bead tryptic digest using a) AzKTB or b) AzRTB.** X represents any amino acid except R and K, and n is the number of amino acid in the sequence (n>6 as the the minimum peptide length allowed for the MaxQuant search was seven amino acids)



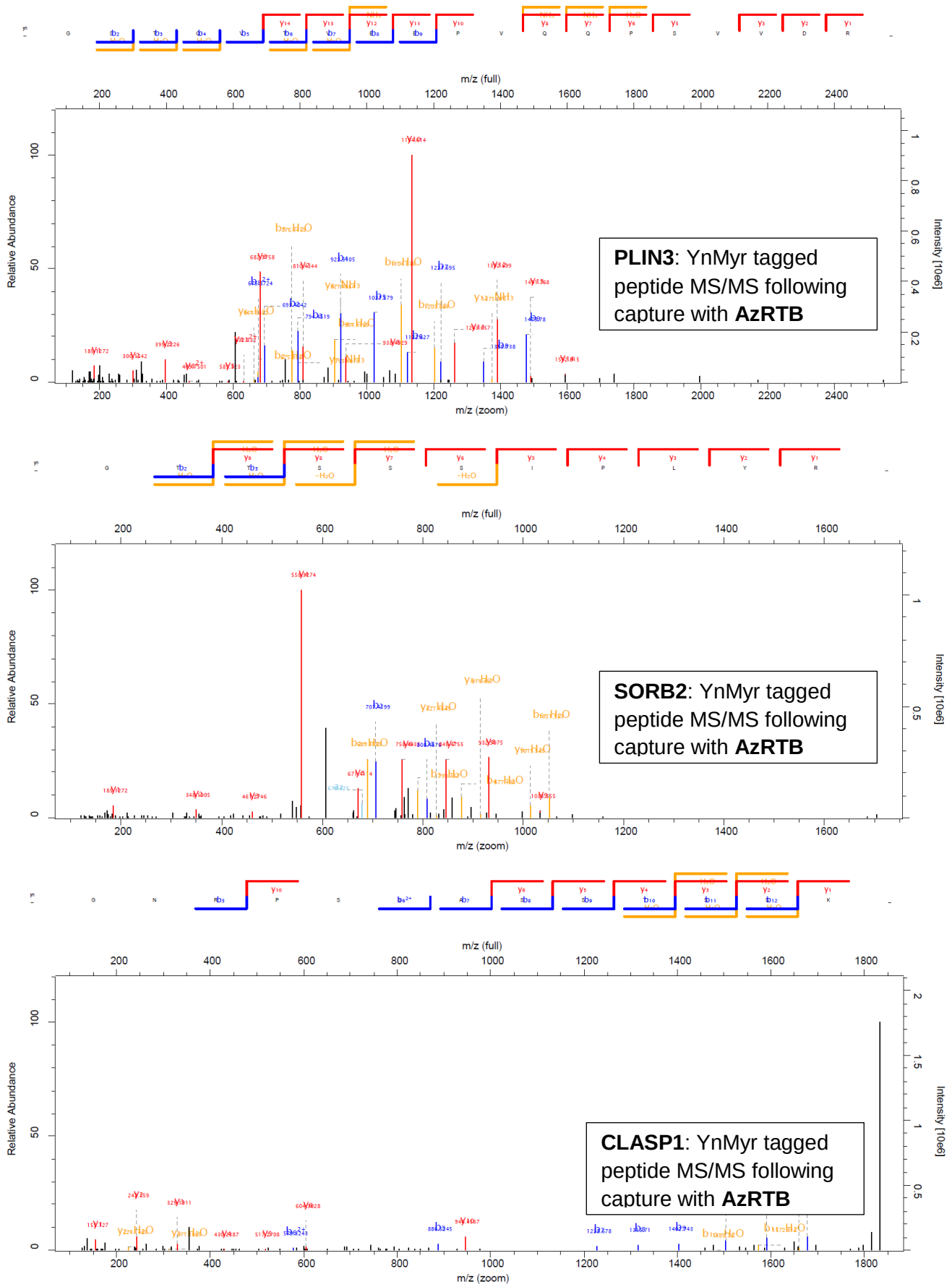
Supplementary Figure 7: Example of an MS/MS spectrum resulting from YnMyr tagging of the N-terminus of a co-translational NMT substrate. MS/MS spectra for the N-terminal **YnMyr**-tagged tryptic peptide derived from Golgi reassembly-stacking protein 2 (GORASP2) captured with **AzKTB** (top) or **AzRTB** (bottom). N-terminal tryptic peptide sequence is GSSQSVEIPGGGTEGYHVLR. Spectra were extracted from standard whole-proteome chemical proteomic analyses using **YnMyr** tagging of HeLa cells, ligation of proteins (CuAAC) to the appropriate capture reagent, affinity enrichment, on-bead digest and nanoLC-MS/MS analysis.



Supplementary Figure 8: Prediction of *N*-myristoylation for known NMT substrates (see Supplementary Table 1). Predictions by a) 'MYR predictor' or b) 'Myristoylator' are shown for proteins identified prior to the present study at native or non-native levels, or in a cell-free system (see Supplementary Table 1). 15 and 6 of these proteins could not be correctly predicted as NMT substrates by the MYR predictor and Myristoylator, respectively. c) The predictions were compared between predictors. 40 proteins predicted as reliable by MYR predictor were also predicted with the highest confidence by Myristoylator. 3 proteins were predicted not to be *N*-myristoylated by both predictors. The predictors disagree on more than 25 predictions. Low and medium predictions made by Myristoylator were considered to be equivalent to twilight prediction by MYR predictor. d) Proportion of bioinformatic predictions (high-medium-low confidence substrates, and non-substrates) validated experimentally in the present study (green) against proportion predicted incorrectly (red).



Supplementary Figure 9: Cellular localization for 70 NMT substrates identified in this study (Gene Ontology annotations).



Supplementary Figure 10: Example of MS/MS spectrum from YnMyr tagging of the N-terminus of post-translational NMT substrates. MS/MS spectra for the N-terminal **YnMyr**-tagged tryptic peptide derived from Perilipin-3 (PLIN3), Sorbin and SH3 domain-containing protein 2 (SORB2) and CLIP-associating protein 1 (CLASP1), labeled with **AzRTB** as described in Supplementary Fig. 7.

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ASPTFSSLLD VFLSTTVFLT LSTTCFLKYE AATVPPPPAA LAVFSAALLL EVLSLAVSIR
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421**0** 422**0** 423**0** 424**0** 425**0** 426**0**
LPEGPLPKGA GLKGHLPKVQ MPCLKMPKVA LKGPQVDVKG PKLDLKGPKA DVMTPVVEVS
427**0** 428**0** 429**0** 430**0** 431**0** 432**0**
LPSMEVDVEA PGAKLDSVRL EGDLSLADKD MTAKDSKFKM PKFKMPSFGV SAPGKSIEAS
433**0** 434**0** 435**0** 436**0** 437**0** 438**0**
LDVSALKVEA DVSLPSMQGD LKTTHLSIQP PSADLEVQAG QEDVKLPEGP VHEGAGLKGH
439**0** 440**0** 441**0** 442**0** 443**0** 444**0**
LPKLQMPSFK VPKVDLKGPQ IDVNVPKLDL KGPKVEVTSP NLDVSLPSME VDIQAPGAKL
445**0** 446**0** 447**0** 448**0** 449**0** 450**0**
DSTRLEGDLS LADKDVTAKD SKFKMPKFKM PSFGMLSPGK SIEVSVDVSA PKMEADMSIP
451**0** 452**0** 453**0** 454**0** 455**0** 456**0**
SMQGDLKTTD LRIQAPSADL EVQAGQVDLK LPEGHMPEVA GLKGHLPKVE MPSFKMPKVD
457**0** 458**0** 459**0** 460**0** 461**0** 462**0**
LKGPQVDVKG PKLDLKGPKA EVMAPDVEVS LPSVETDVQA PGSMLDGARL EGDLSLAHED
463**0** 464**0** 465**0** 466**0** 467**0** 468**0**
VAGKDSKFQG PKLSTSGFEW SSKKVSMSSS EIEGNVTFHE KTSTFPIVES VVHEGDLHDP
469**0** 470**0** 471**0** 472**0** 473**0** 474**0**
SRDGNLGLAV GEVGMDSKFK KLHFKVPKVS FSSTKTPKDS LVPGAKSSIG LSTIPLSSSE
475**0** 476**0** 477**0** 478**0** 479**0** 480**0**
CSSFELQQVS ACSEPSMQMP KVGFAGFPSS RLDLTGPHFE SSILSPCEDV TLTKYQVTVP
481**0** 482**0** 483**0** 484**0** 485**0** 486**0**
RAALAPELAL EIPSGSQADI PLPKTECSTD LQPPEGVPTS QAESHSGPLN SMIPVSLGQV
487**0** 488**0** 489**0** 490**0** 491**0** 492**0**
SFPKFYKPKF VFSVPQMAVP EGDLHAAVGA PVMSPLSPGE RVQCPLPSTQ LPSPGTCVSQ
493**0** 494**0** 495**0** 496**0** 497**0** 498**0**
GPEELVASLQ TSVVAPGEAP SEDADHEGKG SPLKMPKIKL PSFRWSPKKE TGPKVDPECS
499**0** 500**0** 501**0** 502**0** 503**0** 504**0**
VEDSKLSLVL DKDEVAPQSA IHMDLPPERD GEKGRSTKPG FAMPKLALPK MKASKSGVSL
505**0** 506**0** 507**0** 508**0** 509**0** 510**0**
PQRDVDPSLS SATAGGSFQD TEKASSDGGR GGLGATASAT GSEGVNLHRP QVHIPSLGFA
511**0** 512**0** 513**0** 514**0** 515**0** 516**0**
KPDLRSSKAK VEVSQPEADL PLPKHDLSTE GDSRGCGLGD VPVSQPCGEG IAPTPEDPLQ
517**0** 518**0** 519**0** 520**0** 521**0** 522**0**
PSCRKPDAEV LTVESPEEEA MTKYSQESWF KMPKFRMPSL RRSFRDRGGA GKLEVAQTQA
523**0** 524**0** 525**0** 526**0** 527**0** 528**0**
PAATGGEAAA KVKEFLVSGS NVEAAMSLQL PEADAEVTAS ESKSSTDILR CDLDSTGLKL
529**0** 530**0** 531**0** 532**0** 533**0** 534**0**
HLSTAGMTGD ELSTSEVRIH PSKGPLPFQM PGMRLPETQV LPGEIDETPL SKPGHDLASM
535**0** 536**0** 537**0** 538**0** 539**0** 540**0**
EDKTEKWSSQ PEGPLKLKAS STDMPSQISV VNVDQLWEDS VLTVKFPKLM VPRFSFPAPS
541**0** 542**0** 543**0** 544**0** 545**0** 546**0**
SEDDVFIPTV REVQCPEANI DTALCKESPG LWGASILKAG AGVPGEQPVD LNLPLEAPPI
547**0** 548**0** 549**0** 550**0** 551**0** 552**0**
SKVRVHIQGA QVESQEVTIH SIVTPEFVDL SVPRTFSTQI VRESEIPTSE IQTPSYGFSL
553**0** 554**0** 555**0** 556**0** 557**0** 558**0**
LKVKIPEPHT QARVYTTMTQ HSRTQEGTEE APIQATPGVD SISGDLQPDT GEPFEMISSS
559**0** 560**0** 561**0** 562**0** 563**0** 564**0**
VNVLGQQTLT FEVPSGHQLA DSCSDEEPAE ILEFPPDDSQ EATTPLADEG RAPKDKPESK
565**0** 566**0** 567**0** 568**0** 569**0** 570**0**
KSGLLWFWLP NIGFSSSVDE TGVDSKNDVQ RSAPIQTQPE ARPEAELPKK QEKAGWFRFP
571**0** 572**0** 573**0** 574**0** 575**0** 576**0**
KLGFSSSPTK KSKSTEDGAE LEEQKLQEET ITFFDARESF SPEEKEEGEL IGPVGTGLDS
577**0** 578**0** 579**0**
RVMVTSAART ELILPEQDRK ADDESKGSGL GPNEG

C2orf49	Q9BVC5

10 20 30 40 50 60
MAGDVGGRSC TDSELLLHPE LLSQEFLLLT LEQKNIAVET DVRVNKDSLT DLYVQHAIPL
70 80 90 100 110 120
PQRDLPKNRW GKMMEKKREQ HEIKNETKRS STVDGLRKRP LIVFDGSSTS TSIKVKKTEN
130 140 150 160 170 180
GDNDRLKPPP QASFTSNAFR KLSNSSSSVS PLILSSNLPV NNKTEHNNND AKQNHDLTHR
190 200 210 220 230
KSPSGPVKSP PLSPVGTTPV KLKRAAPKEE AEAMNNLKPP QAKRKIQHVT WP

CLASP1	Q7Z460;Q7Z460-3;Q7Z460-2

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        10         20         30         40         50         60
MEPRMESCLA QVLQKDVGKR LQVGQELIDY FSDKQKSADL EHDQTMLDKL VDGLATSWVN
        70         80         90        100        110        120
SSNYKVVLLG MDILSALVTR LQDRFKAQIG TVLPSLIDRL GDAKDSVREQ DQTLLLKIMD
       130        140        150        160        170        180
QAANPQYVWD RMLGGFKHKN FRTREGICLC LIATLNASGA QTLTLSKIVP HICNLLGDPN
       190        200        210        220        230        240
SQVRDAAINS LVEIYRHVGE RVRADLSKKG LPQSRLNVIF TKFDEVQKSG NMIQSANDKN
       250        260        270        280        290        300
FDDEDSVDGN RPSSASSTSS KAPPSSRRNV GMGTTRRLGS STLGSKSSAA KEGAGAVDEE
       310        320        330        340        350        360
DFIKAFDDVP VVQIYSSRDL EESINKIREI LSDDKHDWEQ RVNALKKIRS LLLAGAAEYD
       370        380        390        400        410        420
NFFQHLRLLD GAFKLSAKDL RSQVVREACI TLGHLSSVLG NKFDHGAEAI MPTIFNLIPN
       430        440        450        460        470        480
SAKIMATSGV VAVRLIIRHT HIPRLIPVIT SNCTSKSVAV RRRCFEFLDL LLQEWQTHSL
       490        500        510        520        530        540
ERHISVLAET IKKGIHDADS EARIEARKCY WGFHSHFSRE AEHLYHTLES SYQKALQSHL
       550        560        570        580        590        600
KNSDSIVSLP QSDRSSSSSQ ESLNRPLSAK RSPTGSTTSR ASTVSTKSVS TTGSLQRSRS
       610        620        630        640        650        660
DIDVNAAASA KSKVSSSSGT TPFSSAAALP PGSYASLGRI RTRRQSSGSA TNVASTPDNR
       670        680        690        700        710        720
GRSRAKVVSQ SQRSRSANPA GAGSRSSSPG KLLGSGYGGL TGGSSRGPPV TPSSEKRSKI
       730        740        750        760        770        780
PRSQGCSRET SPNRIGLARS SRIPRPSMSQ GCSRDTSRES SRDTSPARGF PPLDRFGLGQ
       790        800        810        820        830        840
PGRIPGSVNA MRVLSTSTDL EAAVADALKK PVRRRYEPYG MYSDDDANSD ASSVCSERSY
       850        860        870        880        890        900
GSRNGGIPHY LRQTEDVAEV LNHCASSNWS ERKEGLLGLQ NLLKSQRTLS RVELKRLCEI
       910        920        930        940        950        960
FTRMFADPHS KRVFSMFLET LVDFIIIHKD DLQDWLFVLL TQLLKKMGAD LLGSVQAKVQ
       970        980        990       1000       1010       1020
KALDVTRDSF PFDQQFNILM RFIVDQTQTP NLKVKVAILK YIESLARQMD PTDFVNSSET
      1030       1040       1050       1060       1070       1080
RLAVSRIITW TTEPKSSDVR KAAQIVLISL FELNTPEFTM LLGALPKTFQ DGATKLLHNH
      1090       1100       1110       1120       1130       1140
LKNSSNTSVG SPSNTIGRTP SRHTSSRTSP LTSPTNCSHG GLSPSRLWGW SADGLAKHPP
      1150       1160       1170       1180       1190       1200
PFSQPNSIPT APSHKALRRS YSPSMLDYDT ENLNSEEIYS SLRGVTEAIE KFSFRSQEDL
      1210       1220       1230       1240       1250       1260
NEPIKRDGKK ECDIVSRDGG AASPATEGRG GSEVEGGRTA LDNKTSLLNT QPPRAFPGPR
      1270       1280       1290       1300       1310       1320
ARDYNPYPYS DAINTYDKTA LKEAVFDDDM EQLRDVPIDH SDLVADLLKE LSNHNERVEE
      1330       1340       1350       1360       1370       1380
RKGALLELLK ITREDSLGVW EEHFKTILLL LLETLGDKDH SIRALALRVL REILRNQPAR
      1390       1400       1410       1420       1430       1440
FKNYAELTIM KTLEAHKDSH KEVVRAAEEA ASTLASSIHP EQCIKVLCPI IQTADYPINL
      1450       1460       1470       1480       1490       1500
AAIKMQTKVV ERIAKESLLQ LLVDIIPGLL QGYDNTESSV RKASVFCLVA IYSVIGEDLK
      1510       1520       1530
PHLAQLTGSK MKLLNLYIKR AQTTNSNSSS SSDVSTHS
```

CLASP2	O75122;O75122-2

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        10         20         30         40         50         60
MAMGDDKSFD DEESVDGNRP SSAASAFKVP APKTSGNPAN SARKPGSAGG PKVGGASKEG
        70         80         90        100        110        120
GAGAVDEDDF IKAFTDVPSI QIYSSRELEE TLNKIREILS DDKHDWDQRA NALKKIRSLL
       130        140        150        160        170        180
VAGAAQYDCF FQHLRLLDGA LKLSAKDLRS QVVREACITV AHLSTVLGNK FDHGAEAIVP
       190        200        210        220        230        240
```

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TLFNLVPNSA KVMATSGCAA IRFIIRHTHV PRLIPLITSN CTSKSVPVRR RSFEFLDLLL
       250        260        270        280        290        300
QEWQTHSLER HAAVLVETIK KGIHDADAEA RVEARKTYMG LRNHFPGEAE TLYNSLEPSY
       310        320        330        340        350        360
QKSLQTYLKS SGSVASLPQS DRSSSSSQES LNRPFSSKWS TANPSTVAGR VSAGSSKASS
       370        380        390        400        410        420
LPGSLQRSRS DIDVNAAAGA KAHHAAGQSV RRGRLGAGAL NAGSYASLED TSDKLDGTAS
       430        440        450        460        470        480
EDGRVRAKLS APLAGMGNAK ADSRGRSRTK MVSQSQPGSR SGSPGRVLTT TALSTVSSGV
       490        500        510        520        530        540
QRVLVNSASA QKRSKIPRSQ GCSREASPSR LSVARSSRIP RPSVSQGCSR EASRESSRDT
       550        560        570        580        590        600
SPVRSFQPLA SRHHSRSTGA LYAPEVYGAS GPGYGISQSS RLSSSVSAMR VLNTGSDVEE
       610        620        630        640        650        660
AVADALKKPA RRRYESYGMH SDDDANSDAS SACSERSYSS RNGSIPTYMR QTEDVAEVLN
       670        680        690        700        710        720
RCASSNWSER KEGLLGLQNL LKNQRTLSRV ELKRLCEIFT RMFADPHGKR VFSMFLETLV
       730        740        750        760        770        780
DFIQVHKDDL QDWLFVLLTQ LLKKMGADLL GSVQAKVQKA LDVTRESFPN DLQFNILMRF
       790        800        810        820        830        840
TVDQTQTPSL KVKVAILKYI ETLAKQMDPG DFINSSETRL AVSRVITWTT EPKSSDVRKA
       850        860        870        880        890        900
AQSVLISLFE LNTPEFTMLL GALPKTFQDG ATKLLHNHLR NTGNGTQSSM GSPLTRPTPR
       910        920        930        940        950        960
SPANWSSPLT SPTNTSQNTL SPSAFDYDTE NMNSEDIYSS LRGVTEAIQN FSFRSQEDMN
       970        980        990       1000       1010       1020
EPLKRDSKKD DGDSMCGGPG MSDPRAGGDA TDSSQTALDN KASLLHSMPT HSSPRSRDYN
      1030       1040       1050       1060       1070       1080
PYNYSDSISP FNKSALKEAM FDDDADQFPD DLSLDHSDLV AELLKELSNH NERVEERKIA
      1090       1100       1110       1120       1130       1140
LYELMKLTQE ESFSVWDEHF KTILLLLLET LGDKEPTIRA LALKVLREIL RHQPARFKNY
      1150       1160       1170       1180       1190       1200
AELTVMKTLE AHKDPHKEVV RSAEEAASVL ATSISPEQCI KVLCPIIQTA DYPINLAAIK
      1210       1220       1230       1240       1250       1260
MQTKVIERVS KETLNLLLPE IMPGLIQGYD NSESSVRKAC VFCLVAVHAV IGDELKPHLS
      1270       1280       1290
QLTGSKMKLL NLYIKRAQTG SGGADPTTDV SGQS
```

CUL4B	Q13620;Q13620-1;Q13620-3;Q13619;Q13619-2

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         10         20         30         40         50         60
MMSQSSGSGD GNDDEATTSK DGGFSSPSPS AAAAAQEVRS ATDGNTSTTP PTSAKKRKLN
         70         80         90        100        110        120
SSSSSSSNSS NEREDFDSTS SSSSTPPLQP RDSASPSTSS FCLGVSVAAS SHVPIQKKLR
        130        140        150        160        170        180
FEDTLEFVGF DAKMAEESSS SSSSSSPTAA TSQQQQLKNK SILISSVASV HHANGLAKSS
        190        200        210        220        230        240
TTVSSFANSK PGSAKKLVIK NFKDKPKLPE NYTDETWQKL KEAVEAIQNS TSIKYNLEEL
        250        260        270        280        290        300
YQAVENLCSY KISANLYKQL RQICEDHIKA QIHQFREDSL DSVLFLKKID RCWQNHCRQM
        310        320        330        340        350        360
IMIRSIFLFL DRTYVLQNSM LPSIWDMGLE LFRAHIISDQ KVQNKTIDGI LLLIERERNG
        370        380        390        400        410        420
EAIDRSLLRS LLSMLSDLQI YQDSFEQRFL EETNRLYAAE GQKLMQEREV PEYLHHVNKR
        430        440        450        460        470        480
LEEEADRLIT YLDQTTQKSL IATVEKQLLG EHLTAILQKG LNNLLDENRI QDLSLLYQLF
        490        500        510        520        530        540
SRVRGGVQVL LQQWIEYIKA FGSTIVINPE KDKTMVQELL DFKDKVDHII DICFLKNEKF
        550        560        570        580        590        600
INAMKEAFET FINKRPNKPA ELIAKYVDSK LRAGNKEATD EELEKMLDKI MIIFRFIYGK
        610        620        630        640        650        660
DVFEAFYKKD LAKRLLVGKS ASVDAEKSML SKLKHECGAA FTSKLEGMFK DMELSKDIMI
        670        680        690        700        710        720
QFKQYMQNQN VPGNIELTVN ILTMGYWPTY VPMEVHLPPE MVKLQEIFKT FYLGKHSGRK
```

```
       730        740        750        760        770        780
LQWQSTLGHC VLKAEFKEGK KELQVSLFQT LVLLMFNEGE EFSLEEIKQA TGIEDGELRR
       790        800        810        820        830        840
TLQSLACGKA RVLAKNPKGK DIEDGDKFIC NDDFKHKLFR IKINQIQMKE TVEEQASTTE
       850        860        870        880        890        900
RVFQDRQYQI DAAIVRIMKM RKTLSHNLLV SEVYNQLKFP VKPADLKKRI ESLIDRDYME
       910
RDKENPNQYN YIA
```

GSN	P06396;P06396-3;P06396-2;CON__Q3SX14

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        10         20         30         40         50         60
MAPHRPAPAL LCALSLALCA LSLPVRAATA SRGASQAGAP QGRVPEARPN SMVVEHPEFL
        70         80         90        100        110        120
KAGKEPGLQI WRVEKFDLVP VPTNLYGDFF TGDAYVILKT VQLRNGNLQY DLHYWLGNEC
       130        140        150        160        170        180
SQDESGAAAI FTVQLDDYLN GRAVQHREVQ GFESATFLGY FKSGLKYKKG GVASGFKHVV
       190        200        210        220        230        240
PNEVVVQRLF QVKGRRVVRA TEVPVSWESF NNGDCFILDL GNNIHQWCGS NSNRYERLKA
       250        260        270        280        290        300
TQVSKGIRDN ERSGRARVHV SEEGTEPEAM LQVLGPKPAL PAGTEDTAKE DAANRKLAKL
       310        320        330        340        350        360
YKVSNGAGTM SVSLVADENP FAQGALKSED CFILDHGKDG KIFVWKGKQA NTEERKAALK
       370        380        390        400        410        420
TASDFITKMD YPKQTQVSVL PEGGETPLFK QFFKNWRDPD QTDGLGLSYL SSHIANVERV
       430        440        450        460        470        480
PFDAATLHTS TAMAAQHGMD DDGTGQKQIW RIEGSNKVPV DPATYGQFYG GDSYIILYNY
       490        500        510        520        530        540
RHGGRQGQII YNWQGAQSTQ DEVAASAILT AQLDEELGGT PVQSRVVQGK EPAHLMSLFG
       550        560        570        580        590        600
GKPMIIYKGG TSREGGQTAP ASTRLFQVRA NSAGATRAVE VLPKAGALNS NDAFVLKTPS
       610        620        630        640        650        660
AAYLWVGTGA SEAEKTGAQE LLRVLRAQPV QVAEGSEPDG FWEALGGKAA YRTSPRLKDK
       670        680        690        700        710        720
KMDAHPPRLF ACSNKIGRFV IEEVPGELMQ EDLATDDVML LDTWDQVFVW VGKDSQEEEK
       730        740        750        760        770        780
TEALTSAKRY IETDPANRDR RTPITVVKQG FEPPSFVGWF LGWDDDYWSV DPLDRAMAEL
AA
```

ILF3	Q12906;Q12906-3;Q12906-2;Q12906-5;Q12906-7;Q12906-6;Q12906-4;Q96SI9;Q96SI9-2

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        10         20         30         40         50         60
MRPMRIFVND DRHVMAKHSS VYPTQEELEA VQNMVSHTER ALKAVSDWID EQEKGSSEQA
        70         80         90        100        110        120
ESDNMDVPPE DDSKEGAGEQ KTEHMTRTLR GVMRVGLVAK GLLLKGDLDL ELVLLCKEKP
       130        140        150        160        170        180
TTALLDKVAD NLAIQLAAVT EDKYEILQSV DDAAIVIKNT KEPPLSLTIH LTSPVVREEM
       190        200        210        220        230        240
EKVLAGETLS VNDPPDVLDR QKCLAALASL RHAKWFQARA NGLKSCVIVI RVLRDLCTRV
       250        260        270        280        290        300
PTWGPLRGWP LELLCEKSIG TANRPMGAGE ALRRVLECLA SGIVMPDGSG IYDPCEKEAT
       310        320        330        340        350        360
DAIGHLDRQQ REDITQSAQH ALRLAAFGQL HKVLGMDPLP SKMPKKPKNE NPVDYTVQIP
       370        380        390        400        410        420
PSTTYAITPM KRPMEEDGEE KSPSKKKKKI QKKEEKAEPP QAMNALMRLN QLKPGLQYKL
       430        440        450        460        470        480
VSQTGPVHAP IFTMSVEVDG NSFEASGPSK KTAKLHVAVK VLQDMGLPTG AEGRDSSKGE
       490        500        510        520        530        540
DSAEETEAKP AVVAPAPVVE AVSTPSAAFP SDATAEQGPI LTKHGKNPVM ELNEKRRGLK
       550        560        570        580        590        600
YELISETGGS HDKRFVMEVE VDGQKFQGAG SNKKVAKAYA ALAALEKLFP DTPLALDANK
       610        620        630        640        650        660
KKRAPVPVRG GPKFAAKPHN PGFGMGGPMH NEVPPPPNLR GRGRGGSIRG RGRGRGFGGA
```

```
       670        680        690        700        710        720
NHGGYMNAGA GYGSYGYGGN SATAGYSQFY SNGGHSGNAS GGGGGGGGGS SGYGSYYQGD
       730        740        750        760        770        780
NYNSPVPPKH AGKKQPHGGQ QKPSYGSGYQ SHQGQQQSYN QSPYSNYGPP QGKQKGYNHG
       790        800        810        820        830        840
QGSYSYSNSY NSPGGGGGSD YNYESKFNYS GSGGRSGGNS YGSGGASYNP GSHGGYGGGS
       850        860        870        880        890
GGGSSYQGKQ GGYSQSNYNS PGSGQNYSGP PSSYQSSQGG YGRNADHSMN YQYR
```

LRRFIP1	Q32MZ4;Q32MZ4-2;Q32MZ4-3

```
        10         20         30         40         50         60
MTSPAAAQSR EIDCLSPEAQ KLAEARLAAK RAARAEAREI RMKELERQQK EEDSERYSRR
        70         80         90        100        110        120
SRRNTSASDE DERMSVGSRG SLRVEERPEK DFTEKGSRNM PGLSAATLAS LGGTSSRRGS
       130        140        150        160        170        180
GDTSISIDTE ASIREIKELN ELKDQIQDVE GKYMQGLKEM KDSLAEVEEK YKKAMVSNAQ
       190        200        210        220        230        240
LDNEKTNFMY QVDTLKDMLL ELEEQLAESR RQYEEKNKEF EREKHAHSIL QFQFAEVKEA
       250        260        270        280        290        300
LKQREEMLEK HGIILNSEIA TNGETSDTLN NVGYQGPTKM TKEELNALKS TGDGTLGRAS
       310        320        330        340        350        360
EVEVKNEIVA NVGKREILHN TEKEQHTEDT VKDCVDIEVF PAGENTEDQK SSEDTAPFLG
       370        380        390        400        410        420
TLAGATYEEQ VQSQILESSS LPENTVQVES NEVMGAPDDR TRTPLEPSNC WSDLDGGNHT
       430        440        450        460        470        480
ENVGEAAVTQ VEEQAGTVAS CPLGHSDDTV YHDDKCMVEV PQELETSTGH SLEKEFTNQE
       490        500        510        520        530        540
AAEPKEVPAH STEVGRDHNE EEGEETGLRD EKPIKTEVPG SPAGTEGNCQ EATGPSTVDT
       550        560        570        580        590        600
QNEPLDMKEP DEEKSDQQGE ALDSSQKKTK NKKKKNKKKK SPVPVETLKD VKKELTYQNT
       610        620        630        640        650        660
DLSEIKEEEQ VKSTDRKSAV EAQNEVTENP KQKIAAESSE NVDCPENPKI KLDGKLDQEG
       670        680        690        700        710        720
DDVQTAAEEV LADGDTLDFE DDTVQSSGPR AGGEELDEGV AKDNAKIDGA TQSSPAEPKS
       730        740        750        760        770        780
EDADRCTLPE HESPSQDISD ACEAESTERC EMSEHPSQTV RKALDSNSLE NDDLSAPGRE
       790        800
PGHFNPESRE DTRGGNEKGK SKEDCTMS
```

PLIN3	O60664;O60664-4;O60664-3;O60664-2

```
        10         20         30         40         50         60
MSADGAEADG STQVTVEEPV QQPSVVDRVA SMPLISSTCD MVSAAYASTK ESYPHIKTVC
        70         80         90        100        110        120
DAAEKGVRTL TAAAVSGAQP ILSKLEPQIA SASEYAHRGL DKLEENLPIL QQPTEKVLAD
       130        140        150        160        170        180
TKELVSSKVS GAQEMVSSAK DTVATQLSEA VDATRGAVQS GVDKTKSVVT GGVQSVMGSR
       190        200        210        220        230        240
LGQMVLSGVD TVLGKSEEWA DNHLPLTDAE LARIATSLDG FDVASVQQQR QEQSYFVRLG
       250        260        270        280        290        300
SLSERLRQHA YEHSLGKLRA TKQRAQEALL QLSQVLSLME TVKQGVDQKL VEGQEKLHQM
       310        320        330        340        350        360
WLSWNQKQLQ GPEKEPPKPE QVESRALTMF RDIAQQLQAT CTSLGSSIQG LPTNVKDQVQ
       370        380        390        400        410        420
QARRQVEDLQ ATFSSIHSFQ DLSSSILAQS RERVASAREA LDHMVEYVAQ NTPVTWLVGP
       430
FAPGITEKAP EEKK
```

RBM15	Q96T37;Q96T37-3;Q96T37-2;Q8NDT2

```
        10         20         30         40         50         60
MRTAGRDPVP RRSPRWRRAV PLCETSAGRR VTQLRGDDLR RPATMKGKER SPVKAKRSRG
```

```
        70         80         90        100        110        120
GEDSTSRGER SKKLGGSGGS NGSSSGKTDS GGGSRRSLHL DKSSSRGGSR EYDTGGGSSS
       130        140        150        160        170        180
SRLHSYSSPS TKNSSGGGES RSSSRGGGGE SRSSGAASSA PGGGDGAEYK TLKISELGSQ
       190        200        210        220        230        240
LSDEAVEDGL FHEFKRFGDV SVKISHLSGS GSGDERVAFV NFRRPEDARA AKHARGRLVL
       250        260        270        280        290        300
YDRPLKIEAV YVSRRRSRSP LDKDTYPPSA SVVGASVGGH RHPPGGGGGQ RSLSPGGAAL
       310        320        330        340        350        360
GYRDYRLQQL ALGRLPPPPP PPLPRDLERE RDYPFYERVR PAYSLEPRVG AGAGAAPFRE
       370        380        390        400        410        420
VDEISPEDDQ RANRTLFLGN LDITVTESDL RRAFDRFGVI TEVDIKRPSR GQTSTYGFLK
       430        440        450        460        470        480
FENLDMSHRA KLAMSGKIII RNPIKIGYGK ATPTTRLWVG GLGPWVPLAA LAREFDRFGT
       490        500        510        520        530        540
IRTIDYRKGD SWAYIQYESL DAAHAAWTHM RGFPLGGPDR RLRVDFADTE HRYQQQYLQP
       550        560        570        580        590        600
LPLTHYELVT DAFGHRAPDP LRGARDRTPP LLYRDRDRDL YPDSDWVPPP PPVRERSTRT
       610        620        630        640        650        660
AATSVPAYEP LDSLDRRRDG WSLDRDRGDR DLPSSRDQPR KRRLPEESGG RHLDRSPESD
       670        680        690        700        710        720
RPRKRHCAPS PDRSPELSSS RDRYNSDNDR SSRLLLERPS PIRDRRGSLE KSQGDKRDRK
       730        740        750        760        770        780
NSASAERDRK HRTTAPTEGK SPLKKEDRSD GSAPSTSTAS SKLKSPSQKQ DGGTAPVASA
       790        800        810        820        830        840
SPKLCLAWQG MLLLKNSNFP SNMHLLQGDL QVASSLLVEG STGGKVAQLK ITQRLRLDQP
       850        860        870        880        890        900
KLDEVTRRIK VAGPNGYAIL LAVPGSSDSR SSSSSAASDT ATSTQRPLRN LVSYLKQKQA
       910        920        930        940        950        960
AGVISLPVGG NKDKENTGVL HAFPPCEFSQ QFLDSPAKAL AKSEEDYLVM IIVRGFGFQI
       970
GVRYENKKRE NLALTLL
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PYTYNAGLYN PPYSAQSHPA AKTQTYRPLS KSHSDNSPNA FKDASSPVPP PHVPPPVPPL
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RPRDRSSTEK HDWDPPDRKV DTRKFRSEPR SIFEYEPGKS SILQHERPAS LYQSSIDRSL
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        370        380        390        400        410        420
ARQNAEIWSS TEETVSPKIK SRSCDDLLND DCDSFPDPKV KSESMGSLLC EEDSKESCPM
        430        440        450        460        470        480
AWGSPYVPEV RSNGRSRIRH RSARNAPGFL KMYKKMHRIN RKDLMNSEVI CSVKSRILQY
        490        500        510        520        530        540
ESEQQHKDLL RAWSQCSTEE VPRDMVPTRI SEFEKLIQKS KSMPNLGDDM LSPVTLEPPQ
        550        560        570        580        590        600
NGLCPKRRFS IEYLLEEENQ SGPPARGRRG CQSNALVPIH IEVTSDEQPR AHVEFSDSDQ
        610        620        630        640        650        660
DGVVSDHSDY IHLEGSSFCS ESDFDHFSFT SSESFYGSSH HHHHHHHHHH RHLISSCKGR
        670        680        690        700        710        720
CPASYTRFTT MLKHERARHE NTEEPRRQEM DPGLSKLAFL VSPVPFRRKK NSAPKKQTEK
        730        740        750        760        770        780
AKCKASVFEA LDSALKDICD QIKAEKKRGS LPDNSILHRL ISELLPDVPE RNSSLRALRR
        790        800        810        820        830        840
SPLHQPLHPL PPDGAIHCPP YQNDCGRMPR SASFQDVDTA NSSCHHQDRG GALQDRESPR
        850        860        870        880        890        900
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970 980 990 1000 1010 1020
KGDRVILLKR VDQNWYEGKI PGTNRQGIFP VSYVEVVKKN TKGAEDYPDP PIPHSYSSDR
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IHSLSSNKPQ RPVFTHENIQ GGGEPFQALY NYTPRNEDEL ELRESDVIDV MEKCDDGWFV
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GTSRRTKFFG TFPGNYVKRL

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MASVYLEEGN LENAFVLYNK FITLFVEKLP NHRDYQQCAV PEKQDIMKKL KEIAFPRTDE
130 140 150 160 170 180
LKNDLLKKYN VEYQEYLQSK NKYKAEILKK LEHQRLIEAE RKRIAQMRQQ QLESEQFLFF
190 200 210 220 230 240
EDQLKKQELA RGQMRSQQTS GLSEQIDGSA LSCFSTHQNN SLLNVFADQP NKSDATNYAS
250 260 270 280 290 300
HSPPVNRALT PAATLSAVQN LVVEGLRCVV LPEDLCHKFL QLAESNTVRG IETCGILCGK
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LTHNEFTITH VIVPKQSAGP DYCDMENVEE LFNVQDQHDL LTLGWIHTHP TQTAFLSSVD
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LHTHCSYQLM LPEAIAIVCS PKHKDTGIFR LTNAGMLEVS ACKKKGFHPH TKEPRLFSIC
430
KHVLVKDIKI IVLDLR

Supplementary Figure 11: Protein sequences of post-translationally *N*-myristoylated proteins, including putative sites of modification. Below are listed the sequences (isoform 1, unless indicated otherwise) of proteins bearing PTMyr sites identified in this study (please refer to Supplementary Data 4), as reported in the UniProtKB/Swiss-Prot database. The sequences of the unique peptides detected in this study by LC-MS/MS are highlighted in yellow and grey (the two colors are used as needed to allow distinction between adjacent peptides), missed cleavage sites are underlined. The sequences of the YnMyr-modified peptides detected upon **AzKTB**/**AzRTB**-based enrichment are highlighted in cyan. Proteins are ordered by gene names and the Uniprot accession number is reported.

Fig. 2d

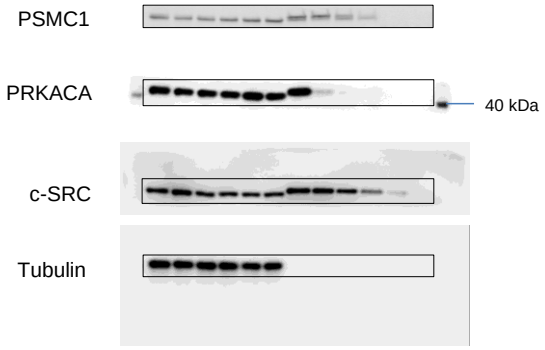


Fig. 4a

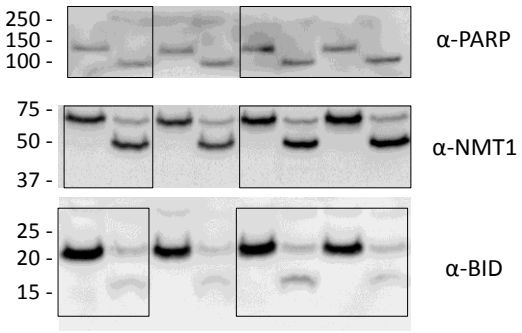


Fig. 4c

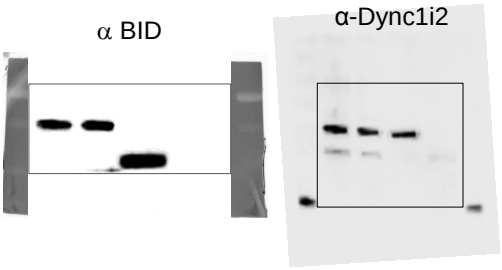
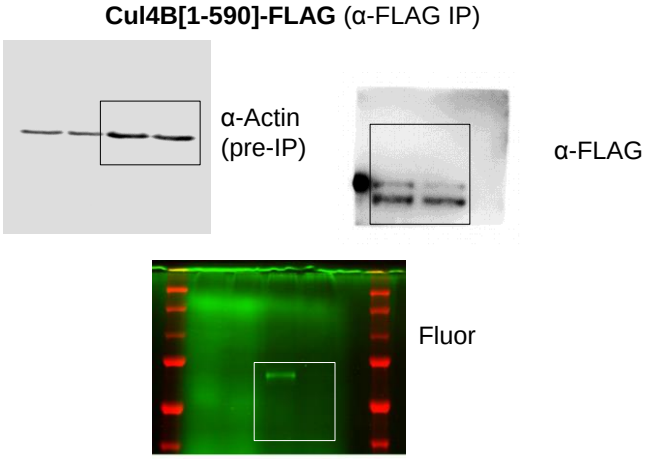
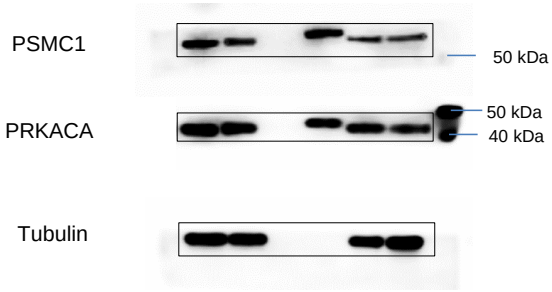


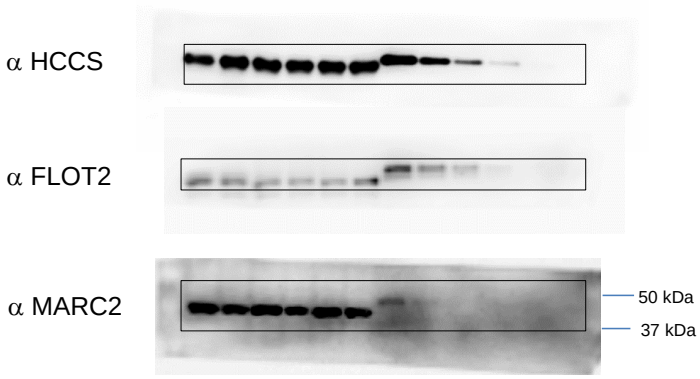
Fig. 4d



Supplementary Fig. 1g



Supplementary Fig. 5



Supplementary Figure 12: Full blots and gels relating to cropped sections shown in figures. Only cropped blots and gels are shown; in some cases, membranes were cut between specific molecular weight markers prior to blotting to maximize information recovered from an experiment, resulting in smaller sections shown in some figures.

Supplementary Table 1: Previous studies of *N*-myristoylated proteins expressed in HeLa.
Proteins with an N-terminal MG motif and shown to be *N*-myristoylated in a cell-free system or *E. coli* expression system (CF), a non-native protein construct in eukaryotic cells (NN) or native proteins in eukaryotic cells (N), typically via immunoprecipitation of a radiolabeled protein. Only proteins expressed in HeLa cells are reported.[3] A column indicates whether proteins have been predicted to be *N*-myristoylated by online predictors (**Myr predictor (MP)**: R= Reliable; T= twilight, N= No. **Myristoylator (Myr)**: H= high probability; M= medium probability; L= low probability; N= not myristoylated). A column indicates if the protein is palmitoylated (+: found in 1 study, ++: found in 2 studies; +++: found in 3 studies; ++++: found in 4 studies;[4] u: reported as palmitoylated in the UniProtKB/Swiss-Prot database).

Method	Protein name	Gene name	Protein IDs	Reference	Palm	MP	Myr
N	Annexin XIII	ANXA13	P27216	[5]		R	H
	Brain acid soluble protein 1	BASP1	P80723	[6]		R	H
	Golgi-associated plant pathogenesis-related protein 1	GLIPR2	Q9H4G4	[7]		R	H
	MARCKS	MARCKS	P29966	[8]		R	H
	cAMP dependent protein kinase alpha subunit	PRKACA	P17612	[9]		R	H
	Tyrosine protein kinase Src	SRC	P12931	[10]	+	R	H
NN	ABL1	ABL1	P00519	[11]		T	N
	A-kinase anchor protein 12	AKAP12	Q02952	[12]		R	H
	A-kinase anchor protein 7	AKAP7	O43687	[13]	u	R	H
	ADP ribosylation factor 1	ARF1	P84077	[14]	++	T	H
	ADP ribosylation factor 6	ARF6	P62330	[15]		R	H
	ADP-ribosylation factor-like 5B	ARL5B	Q96KC2	[16]		N	M
	Cyclin-Y	CCNY	Q8ND76	[17]	+++	R	H
	Coiled-coil-helix-coiled-coil-helix domain-containing protein 3, mitochondrial	CHCHD3	Q9NX63	[18]	+	R	H
	Charged multivesicular body protein 6	CHMP6	Q96FZ7	[19]		N	H
	Calcium and integrin-binding protein 1	CIB1	Q99828	[20]		R	H
	NADH cytochrome b5 reductase 3	CYB5R3	P00387	[21]	++	R	H
	Sphingolipid delta(4)-desaturase DES1	DEGS1	O15121	[22]		T	H
	Dual specificity protein phosphatase 22	DUSP22	Q9NRW4	[23]		N	H
	Formin-like protein 1	FMNL1	O95466	[24]		T	N
	Fibroblast growth factor receptor substrate 2 FRS2	FRS2	Q8WU20	[25]		R	H
	Fibroblast growth factor receptor substrate 3 FRS3	FRS3	O43559	[25]		R	H
	Tyrosine protein kinase Fyn	FYN	P06241	[26]	u; ++	R	H
	Guanine nucleotide-binding protein G(i) subunit alpha-1	GNAI1	P63096	[27]	u; ++	R	H
	Guanine nucleotide-binding protein G(i) subunit alpha-2	GNAI2	P04899	[27]	u; ++++	R	H
	Guanine nucleotide-binding protein G(k) subunit alpha	GNAI3	P08754	[27]	u; ++++	R	H
	Guanine nucleotide-binding protein G(o) subunit alpha	GNAO1	P09471	[27]	u; +++	R	H
	Guanine nucleotide-binding protein G(z) subunit alpha	GNAZ	P19086	[27]	u; +	R	H
	Neuron-specific calcium-binding protein hippocalcin	HPCA	P84074	[28]		R	H
	Hippocalcin-like protein 1	HPCAL1	P37235	[29]		R	H
	LanC-like protein 2	LANCL2	Q9NS86	[30]		N	H
	Tyrosine protein kinase Lyn	LYN	P07948	[31]	u; +	R	H
	Mitochondrial peptide methionine sulfoxide reductase	MSRA	Q9UJ68	[32]		R	H
	Neuronal calcium sensor 1	NCS1	P62166	[28]		R	H
	Nephrocystin-3	NPHP3	Q7Z494	[33]		T	H
	cGMP-dependent 3',5'-cyclic	PDE2A	O00408	[34]	u	N	N

	phosphodiesterase						
	Protein phosphatase 1A	PPM1A	P35813	[35]		N	H
	Protein phosphatase 1B	PPM1B	O75688	[35]		N	M
	Calcineurin B type 1	PPP3R1	P63098	[36]		R	H
	5'-AMP-activated protein kinase subunit beta-1	PRKAB1	Q9Y478	[37]		N	N
	5'-AMP-activated protein kinase subunit beta-2	PRKAB2	O43741	[38]		N	M
	26S protease regulatory subunit 4	PSMC1	P62191	[39]	++	R	H
	Raftlin	RFTN1	Q14699	[40]	u	N	H
	Protein-associating with the carboxyl-terminal domain of ezrin (PACE-1)	SCYL3	Q8IZE3	[41]		R	H
	TIR domain-containing adapter molecule 2	TICAM2	Q86XR7	[42]		R	M
	Tyrosine protein kinase Yes	YES1	P07946	[43]	u; ++	R	H
	E3 ubiquitin-protein ligase ZNRF2	ZNRF2	Q8NHG8	[44]		R	M
CF	Apoptosis-inducing factor 2	AIFM2	Q9BRQ8	[45]		R	H
	Ankyrin repeat and IBR domain-containing protein 1	ANKIB1	Q9P2G1	[45]		R	H
	ADP ribosylation factor-like protein 1	ARL1	P40616	[46]		R	H
	BTB/POZ domain-containing protein 7	BTBD7	Q9P203	[45]		R	M
	Calcineurin B homologous protein 1	CHP1	Q99653	[47]		R	H
	Calcineurin B homologous protein 3	CHP3	Q96BS2	[48]		R	H
	Dixin	DIXDC1	Q155Q3	[45]		T	N
	Dymeclin	DYM	Q7RTS9	[49]		R	H
	Formin-like protein 2	FMNL2	Q96PY5	[45]		T	H
	Formin-like protein 3 (FMNL3)	FMNL3	Q8IVF7	[45]		N	M
	Golgi reassembly-stacking protein 1 (GRASP65)	GORASP1	Q9BQQ3	[50]		R	H
	Uncharacterized protein KIAA1522	KIAA1522	Q9P206	[45]		N	H
	TLD domain-containing protein KIAA1609	KIAA1609	Q6P9B6	[45]		T	H
	Protein Lunapark	LNP	Q9C0E8	[45]		T	H
	E3 Ubiquitin-protein ligase MGRN1	MGRN1	O60291	[45]		R	H
	NADH dehydrogenase [ubiquinone] 1 beta subcomplex subunit 7	NDUFB7	P17568	[51]		N	L
	Phosphoinositide 3-kinase regulatory subunit 4 (PIK3R4)	PIK3R4	Q99570	[52]		T	H
	RING finger protein 11	RNF11	Q9Y3C5	[45]		N	M
	Serine incorporator 1	SERINC1	Q9NRX5	[45]		N	N
	Tescalin	TESC	Q96BS2	[48]		R	H
	Zinc finger ZZ-type and EF-hand domain-containing protein 1	ZZEF1	O43149	[45]		R	H

Supplementary Table 2: Known post-translationally *N*-myristoylated proteins.

Proteins shown to be *N*-myristoylated in systems where proteins were overexpressed in eukaryotic cells (NN) or were present at native expression levels (N). A column indicates if the new N-terminal sequences have been predicted to be *N*-myristoylated by online predictors (**Myr predictor**: R= Reliable; T= twilight. **Myristoylator**: H= high probability; M= medium probability; L= low probability; N= not myristoylated).

Method	Protein name	Gene name	Protein IDs	Ref.	New N-terminal sequence	Cleavage site	MP	Myr
N	BH3-interacting domain death agonist	BID	P55957	[53]	GNRSSHSRLGRIEADSE SQEDIIRNIARHL	G60	R	H
NN	Actin, cytoplasmic 1 (β-actin)	ACTB	P60709	[54]	GQVITIGNERFRCPEAL FQPSFLGMESCGI	G245	T	N
	Cell division control protein 6 homolog	CDC6	Q99741	[55]	GNRMTLSQEGAQDSFP LQQKILVCSLMLLI	G443	R	H
	Gelsolin	GSN	P06396	[54]	GLGLSYLSSHIANVERV PFDAATLHTSTAM	G404	R	M
	Huntingtin	HTT	P42858	[55]	GTQASSPISDSSQTTTE GPDSAVTPSDSSE	G551	R	H
	Microtubule-actin cross-linking factor 1, isoforms 1/2/3/5	MACF1	Q9UPN3	[55]	GSDASQLLHQAEVAQQ EFLEVKQRVNSGCV	G5087	R	H
	Induced myeloid leukemia cell differentiation protein	MCL1	Q07820	[55]	GSLPSTPPPAEEEEDEL YRQSLEIISRYLR	G158	R	H
	p21-activated protein kinase-2	PAK2	Q13177	[56]	GAAKSLDKQKKKTKMT DEEIMEKLRTIVSI	G213	T	H
	YTH domain family protein 2	YTHDF2	Q9Y5A9	[55]	GNGVGQSQAGSGSTP SEPHPVLEKLRSINN	G367	R	M

Supplementary Methods

1.1. General methods: Synthetic procedures

All reagents and solvents were purchased from Sigma-Aldrich, NovaBiochem UK or AGTC Bioproducts and used without further purification. Ultrapure water was obtained from MilliQ® Millipore water purification system. Moisture sensitive reactions were performed under nitrogen atmosphere using dried glassware and standard syringe/septa techniques.

Thin Layer Chromatography was performed on Merck pre-coated Silica plates (Aluminum oxide 60 F254, Merck). Spots were visualized by UV light (operating at 254 nm), and using the appropriate stain. Silica gel column chromatography was carried either by hand-made columns with Merck Silica 60Å, or using an Isolera (Biotage, UK) automated apparatus with fraction collector equipped with SNAP cartridges columns (Biotage, UK).

NMR spectra were recorded on 400MHz Bruker instruments and were referenced to residual solvent signals. Data are presented as follows: chemical shift, multiplicity (s = singlet, d = doublet, t = triplet, m = multiplet), coupling constant(s) in Hz and integration. High resolution mass spectrometry (HRMS) was performed on Waters LCT Premier Spectrometer.

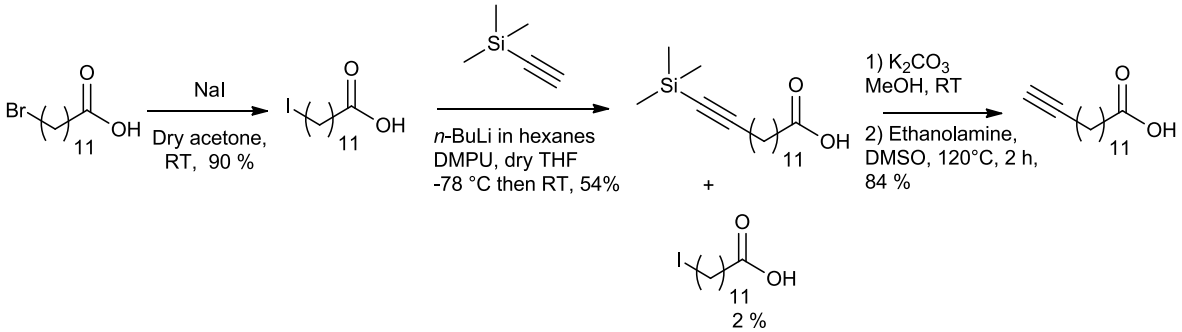
Analytical and semi-preparative RP-HPLC was carried out on a Waters 2767 system equipped with a photodiode array, a mass spectrometer and an X-Bridge C18 column (5 µM, 4.6 mM × 100 mM). The flow rate of 1.2 mL/min was used for the analytical mode and 20 mL/min were used for the preparative mode.

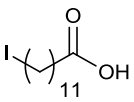
Freeze-drying was carried out using a freeze dryer Alpha 2-4 LD plus, Christ (Germany).

The syntheses of the peptide c-Src (N-terminal 8 amino acids from Gly2), Azidopropionic acid, the capture reagent AzTB (azido-tamra-biotin), the capture reagent AzKTB (azido-lysine-tamra-biotin) and inhibitor **1** have been described elsewhere. [1, 2, 57, 58]

1.2. Synthetic procedures

1.2.1. YnMyr synthesis

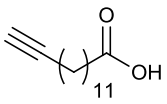




12-Iodododecanoic acid

To a solution of 12-bromododecanoic acid (1.00 g, 3.58 mmol, 1 eq) in dry acetone (20 mL) was added NaI (1.61 g, 10.74 mmol, 3eq) in one portion. The mixture was stirred at room temperature overnight. Completion of the reaction was checked by ^{1}H NMR. Water (200 mL) and DCM (100 mL) were added. The layers were separated and the aqueous layer was extracted with DCM (2 x 70 mL). The combined organic layers were washed with saturated aqueous sodium thiosulfate (100 mL), brine (100 mL), dried over $MgSO_4$ and concentrated under reduced pressure to yield a white solid (1.05 g,

90%). The crude product was used without further purification for the next reaction. **^{1}H NMR** (400 MHz, $CDCl_3$) δ = 3.17 (t, *J*=7.1, 2H), 2.33 (t, *J*=7.5, 2H), 1.84 – 1.75 (m, 2H), 1.60 (m, 2H), 1.27 (m, 14H).



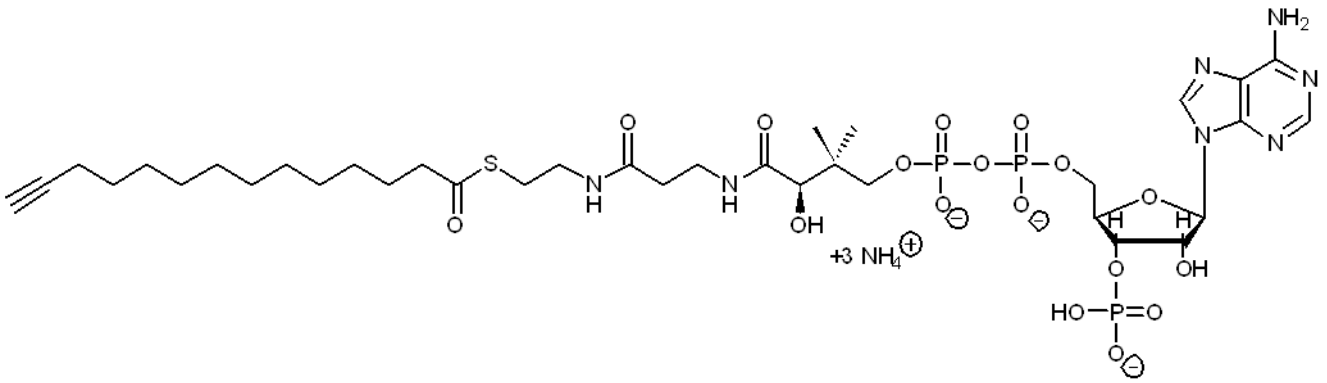
Tetradec-13-ynoic acid (YnMyr)

Under nitrogen atmosphere, a solution of TMS-acetylene (1.12 mL, 7.82 mmol, 2.5 eq) in dry THF (8 mL) was cooled down to -78 °C using a bath of acetone and dry ice. A solution of *n*-BuLi in hexanes (3.8 mL, 2.5 M solution, 9.39 mmol, 3.0 eq) was added drop wise. The clear reaction mixture was allowed to warm to room temperature for 10 minutes and then cooled to -78 °C. DMPU (7.9 mL, 21 eq) and a solution of 12-iododododecanoic acid (1.02 g, 3.13 mmol, 1.0 eq) in dry THF (8 mL) were added drop wise. The yellow reaction mixture was allowed to warm to room temperature and stirred overnight. The reaction mixture was cooled to -78 °C and quenched by the drop wise addition of saturated NH_4Cl (240 mL). Et_2O (100 mL) and water (50 mL) were added to the brown solution. The layers were separated and the aqueous layer was extracted with Et_2O (2 x 50 mL). The combined organic layers were washed with water (100 mL), brine (100 mL), dried over Na_2SO_4, filtered and concentrated under reduced pressure. The brown residue was purified by silica-gel chromatography (gradient: EtOAc: AcOH 99:0:1 to 95:4:1) to yield a white solid (500 mg, 54%). NMR analysis of the product showed that the product contained < 2% of 12-Iododododecanoic acid.

To a suspension of TMS-protected alkyne (490 mg, 1 eq, 1.65 mmol) in MeOH (10 mL) was added K_2CO_3 (457 mg, 3.31 mmol, 2 eq). The reaction mixture was stirred at room temperature overnight to give a clear reaction mixture. The mixture was concentrated under reduced pressure. The residue was taken up in 2N HCl (50 mL) and Et_2O (50 mL). The layers were separated and the aqueous layer was extracted with Et_2O (2 x 50 mL). The combined organic layers were washed with water (50 mL), brine (50 mL), dried over Na_2SO_4, filtered and concentrated under reduced pressure to yield an off-white solid (359 mg, 96%). NMR analysis of the product showed that the product contained < 2% of 12-Iododododecanoic acid.

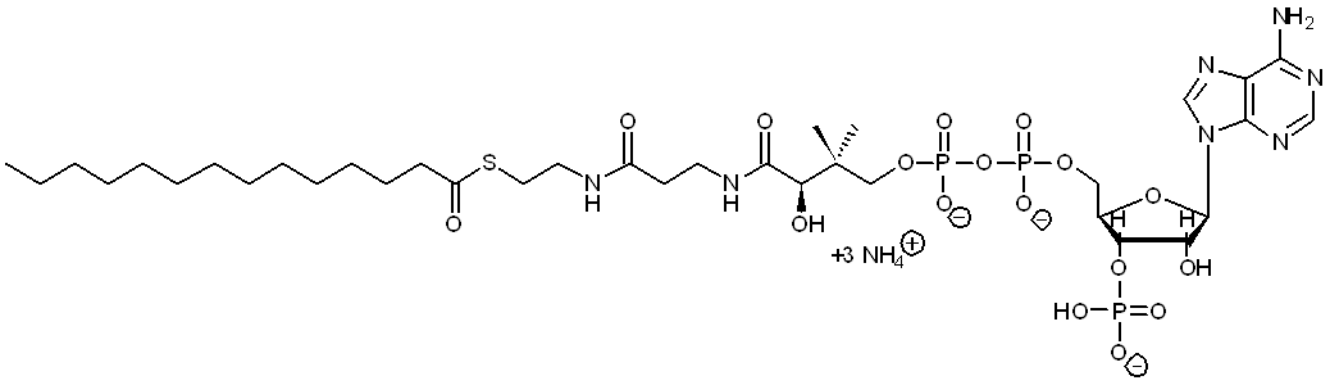
The white solid (YnMyr + impurity (< 2%; 12-iododododecanoic acid); 340 mg, 1.52 mmol) was dissolved in DMSO (500 µL) and ethanolamine (25 eq regarding 2% impurity, 0.76 mmol, 46 µL) was added in one portion. The reaction mixture was heated to 120 °C for 2 h and then allowed to cool down to room temperature. Et_2O (100 mL) and 2N HCl (100 mL) were added. The layers were separated and the aqueous layer was extracted with Et_2O (50 mL). The combined organic layers were washed with 0.5 HCl (50 mL), brine (50 mL), dried over $MgSO_4$ and concentrated under reduced pressure to yield a white solid (296 mg, 84%; overall yield = 37%). **^{1}H NMR** (400 MHz, $CDCl_3$) δ 2.33 (t, *J* = 7.5, 2H, C*H*$_2$CO$_2$H), 2.16 (dt, *J* = 2.6, 7.1, 2H, C*H*$_2$C≡CH), 1.92 (t, *J* = 2.6, 1H, C≡C*H*), 1.65 – 1.55 (m, 2H, C*H*$_2$CH$_2$CO$_2$H), 1.54 – 1.45 (m, 2H, C*H*$_2$CH$_2$C≡CH), 1.41 – 1.19 (m, 14H, 7xC*H*$_2$); **^{13}C NMR** (101 MHz, $CDCl_3$) δ 179.69 (C=O), 85.03(*C*≡CH), 68.02 (C≡*C*H), 34.14, 29.5, 29.45, 29.40, 29.2, 29.1, 29.0, 28.7, 28.5, 24.87, 18.60; **HRMS** (ESI, negative mode) found 223.1698 ([M - H]$^-$ requires 223.1699).

1.2.2. **YnMyrCoA synthesis**



To a suspension of tetradec-13-ynoic acid (YnMyr, 14.8 mg, 65 µmol, 2 eq) in dry THF (1.0 mL) was added a solution of 1,1'-carbonyl-diimidazole (12.7 mg, 78 µmol, 2.4 eq) in DCM (1.0 mL), under nitrogen atmosphere. The clear reaction mixture was stirred for 45 min at room temperature. The reaction mixture was concentrated under reduced pressure. The yellow residue was dissolved in dry THF (1.0 mL). Co-enzyme A hydrate from yeast (25 mg, 32 µmol, 1 eq) was dissolved in an aqueous solution of $NaHCO_3$ (0.5 M, 3.4 mL) and added to the solution of activated acid. The reaction mixture was stirred at room temperature for 3 h under nitrogen atmosphere. THF was removed under reduced pressure. The product was precipitated by adding 20% perchloric acid drop wise (1 mL). The white solid was pelleted by centrifugation and washed with 1% perchloric acid and acetone. The product was purified by semi preparative LC-MS over a gradient of MeOH in 25 mM ammonium bicarbonate pH 8 (50-98 %, 20 min), YnMyrCoA was obtained as a white lyophilized solid (22.2 mg, 66% yield). Analysis was consistent with NMR and MS analysis reported in the literature.[59]

1.2.3. **MyrCoA synthesis**



A similar procedure as YnMyrCoA was used to prepare MyrCoA. MyrCoA was obtained as a white lyophilized solid (10 mg, 32% yield). Analysis was consistent with NMR and MS analysis reported in the literature.[59]

1.2.4. **Capture reagents: AzKTB and AzRTB**

Biotin-PEG Novatag™ resin (0.47 mmol/g loading, 106.4 mg, 50.0 µmol, 1 eq) was swollen in DMF (2 mL, 30 min), Fmoc deprotected with 20% v/v piperidine in DMF (2 mL, 10 min × 3) and washed with DMF, DCM and DMF sequentially. Fmoc-Lysine(Mmt)-OH (96.1 mg, 3 eq), HATU (57 mg, 3 eq) and DIPEA (52.3 µL, 6 eq) were dissolved in DMF (1 mL), added to the deprotected resin and the reaction was shaken for 2h after which the procedure was repeated. All subsequent couplings, i.e. Fmoc-6-Ahx-OH, Fmoc-Gly-OH, Fmoc-Lys(Boc)-OH (Az-KTB) or Fmoc-Arg(Pbf)-OH (Az-RTB) , Fmoc-Ala-OH,

and Azidopropionic acid (all 5 eq) were performed using DIC/HOBt activation (5 eq each, 30 min x 2). Following the removal of Mmt protecting group with 1 % TFA in DCM (10 min x 4) and wash (DCM, DMF), TAMRA (43 mg, 2 eq) was activated for 10 min in DMF (1 mL) with DIC (15.7 mg, 2 eq) and HOAt (13.6 mg, 2 eq) and coupled to the peptidyl resin (2 h x 2). The crude product was then cleaved from the resin with 95 % TFA, 2.5 % water and 2.5 % triisopropylsilane (3 h) and precipitated with cold TBME. The solids were pelleted by centrifugation (15 min, 4300 rpm, 4 °C) and washed three times with TBME. The pelleted product was dried and purified by semi preparative LC-MS over a gradient of MeOH (0.1% FA) in water (0.1% FA) (2-98 %, 15 min), with detection over 100–600 nm. The product was obtained by lyophilisation as a bright pink amorphous solid (Az-KTB: 12 mg, 17 % yield; Az-RTB: 15 mg, 20 %). HRMS m/z (ESI), calculated for Az-KTB, $C_{71}H_{104}N_{16}O_{15}S$ ($[M + 2H]^{2+}$) 727.3873, found 727.3892; calculated for Az-RTB, $C_{71}H_{104}N_{18}O_{15}S$ ($[M + 2H]^{2+}$) 741.3904, found 741.3847.

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