

Supporting Information

Mapping post-translational modifications of *de novo* purine biosynthetic enzymes: Implications for pathway regulation

Chunliang Liu,^{1†§} Giselle M. Knudsen,^{2†‡} Anthony M. Pedley,^{1†} Jingxuan He,¹ Jared L. Johnson,³ Tomer M. Yaron,^{3,4} Lewis C. Cantley,^{3,5} Stephen J. Benkovic^{1*}

¹ Department of Chemistry, The Pennsylvania State University, University Park, PA 16802, USA

² University of California San Francisco Mass Spectrometry Facility, Department of Pharmaceutical Chemistry, San Francisco, CA 94158, USA

³ Meyer Cancer Center, Department of Medicine, Weill Cornell Medical College, New York, NY 10065, USA

⁴ Institute for Computational Biomedicine, Department of Physiology and Biophysics, Weill Cornell Medical College, New York, NY 10065, USA

⁵ Department of Medicine, Beth Israel Deaconess Medical Center, Boston, MA 02215, USA; Department of Systems Biology, Harvard Medical School, Boston, MA 02115, USA

† Contributed equally to this manuscript

§ Current Address: Boragen, Inc., Durham, NC 27709, USA

‡ Current Address: Alaunus Biosciences, Inc., San Francisco, CA 94107, USA

* Corresponding Author: Stephen J. Benkovic, 414 Wartik Laboratory, The Pennsylvania State University, University Park, PA 16802, USA. Email: sjb1@psu.edu. Phone: 1-814-865-2882

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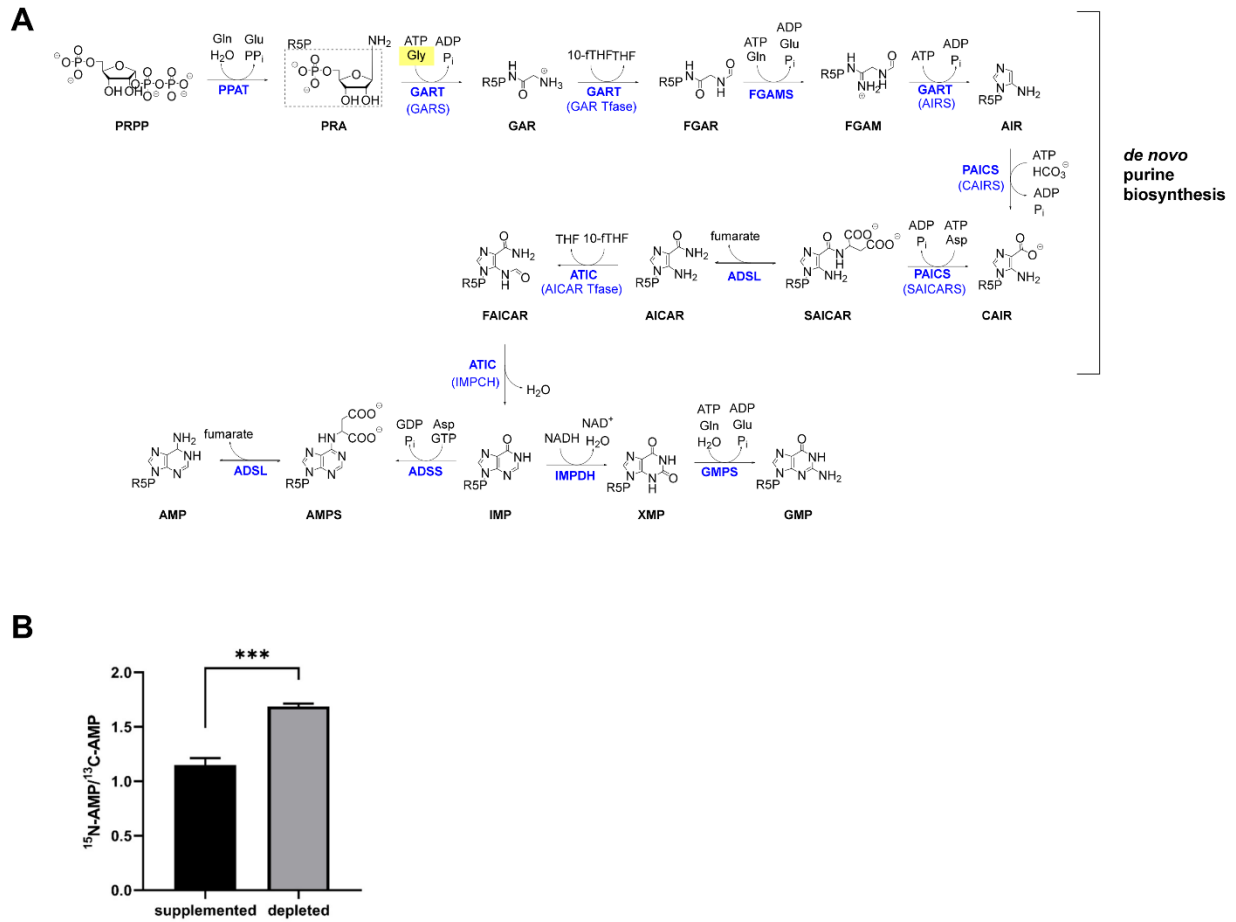


Figure S1. The *de novo* purine biosynthetic pathway is activated in 293T cells upon purine-depletion. (A) The *de novo* purine biosynthetic pathway converts phosphoribosyl pyrophosphate (PRPP) into inosine 5' monophosphate (IMP) in 10 steps catalyzed by six human enzymes (blue). The second step of the pathway utilizes glycine (yellow highlight) for generation of glycinamide ribonucleotide (GAR) and is the site of ^{15}N -glycine incorporation for metabolic flux experiments. (B) ^{15}N -glycine incorporation into AMP in the presence (supplemented, black bar) or absence (depleted, gray bar) of purines. The ^{15}N -glycine pulse was added for 4 h, and the degree of incorporation ($^{15}\text{N-AMP}/^{13}\text{C-AMP}$) was determined by mass spectrometry. Data represent mean $\pm$ standard deviation, $N = 4$ independent experiments, *** p value = 0.0002 as determined by an unpaired t-test.

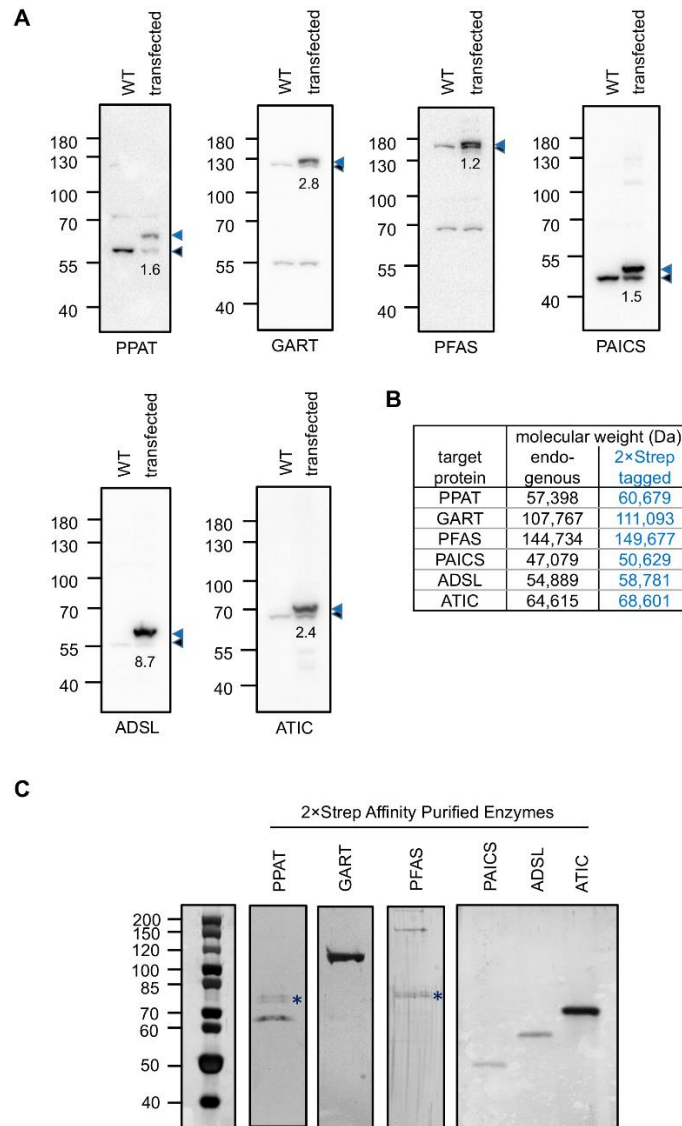


Figure S2. Expression and purification of recombinant *de novo* purine biosynthetic enzymes. (A) 293T cells were transfected with plasmids encoding 2xStrep tagged enzymes for 48 h and the degree of transient expression of the 2xStrep tagged enzyme (blue marker) compared to endogenous (black marker) was determined by Western blot and corresponded roughly to the calculated molecular weights in (B). Fold change values were based on the band intensity of the 2xStrep tagged enzyme relative to the band intensity corresponding to the endogenous protein. These values are reported under the endogenous protein band in the transfected sample lane. Untransfected (wild-type, WT) 293T cell lysate was also analyzed to help identify the band corresponding to endogenous protein. For PPAT and PFAS, 40 μ g of protein lysate was loaded in each lane; expression levels of all other proteins were determined with 30 μ g of protein lysate. Rabbit polyclonal antibodies used included: PPAT (LifeSpan Biosciences, cat no: LS-C80815), GART (Bethyl Laboratories, cat no: A304-311A), PFAS (Bethyl Laboratories, cat no: A304-220A), PAICS (Bethyl Laboratories, cat no: A304-546A), ADSL (Bethyl Laboratories, cat no: A304-778A), and ATIC (Bethyl Laboratories, cat no: A304-271A). (C) Affinity purified 2xStrep enzymes from 293T cells with MagStrep “type3” XT beads was analyzed by SDS-PAGE and detected by silver staining. Eluted protein samples predominantly show the expression of the 2xStrep tagged enzyme and corresponds to the calculated molecular weight. Asterisks denote background protein from the affinity resin.

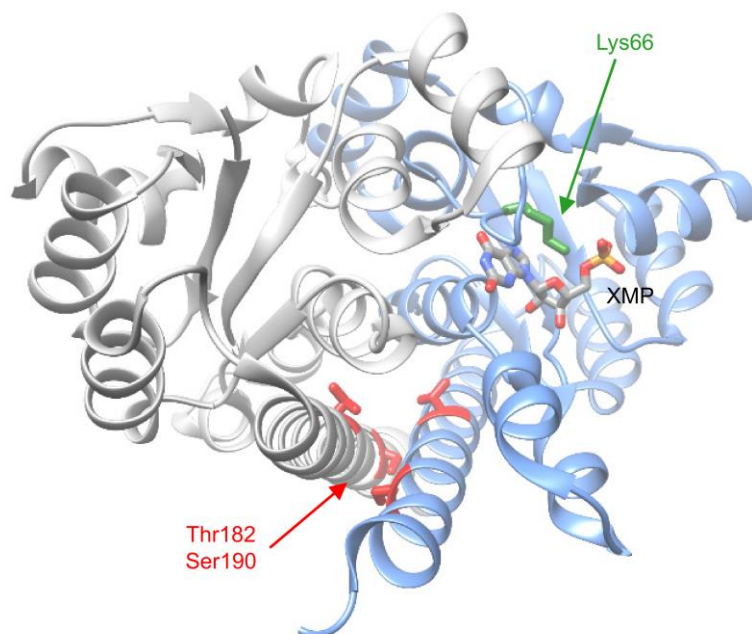


Figure S3. Structural model of human ATIC with identified sites of modification. The IMP cyclohydrolase domain of human ATIC showing the interface of two monomers (gray and light blue). At the interface are residues Thr182 and Ser190 (red), both shown to be phosphorylated. These phosphorylation events might impair proper dimer formation. Lys66 (green) was shown to be ubiquitinated, which could alter substrate (FAICAR, XMP) binding. PDB ID: 1PKX.

A

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purD      --MKVLVINGGREHALAWKAAQSPLVETVVFVAPGNAGTALEPALQNVAIGVTDIPALLD 58
GARS      MAARVLIIGSGGREHTLAWKLAQSHHVQVVLVAPGNAGTACSEKISNTAISISDHTALAQ 60
          :*:**:*****:*** ** *:**:***** . :*.**.:* ** :

purD      FAQNEKIDLTIVGPEAPLVKGVVDTFRAAGLKIFGPTAGAAQLEGSKAFTKDFLARHKIP 118
GARS      FCKEKKIEFVVVGPEAPLAAGIVGNLRSAGVQCFGPTAEAAQLESSKRFKAEFMDRHGIP 120
          *:**:**:**:*****. *:**:**:**: ***** *****.* ** *:**: ** **

purD      TAEYQNFTEVEPALAYLREKGA-PIVIKADGLAAGKGVIVAMTLEEEAAVHDMLAGNAF 177
GARS      TAQWKAFTKPEEACSFILSADFPALVVKASGLAAGKGVIVAKSKEEACKAVQEIMQECAF 180
          **:**: *: * * : : . . :*:**.*****: ** *:**: :**

purD      GDAGHRIVIEEFLDGEEASFIVMVDGEHVLPMATSQDHRVGDKDTGPNTGGMGAYSPAP 237
GARS      GAAGETIVIEELLDGEEVSLCFTDGKTVAPMPPAQDHRKRLLEGDGGPNTGGMGAYCPAP 240
          * ** . *****:*****.* : :*:*: * ** :*****: : * *****.***

purD      VVTDDVHQRTMERIIWPTVKGMAAEGNTYTGFYAGLMIDKQGNPKVIEFNCRFGDPETQ 297
GARS      QVSNDDLLKIKDTVLQRTVDGMQEGTPYTGILYAGIMLTKN-GPKVLEFNCRFGDPECQ 299
          *:**: : : : **.* ** *:**:*:*:*:*:*:*:*:*:*:*:*:*:*:

purD      PIMLRMKSDDLVELCLAACESKLDEKTSEWDE-RASLGVMMAAGGYPGDYRTGDVIHGLPL 356
GARS      VILPLLKSDLYEVIQSTLDGLLCTSLPVWLENHTALTVMASKGYPGDYTKGVEITGFPE 359
          *: :**** *: : : . * * :*: *****: ***** . * * *:

purD      EEVAGGKVFHAGTKLADDEQVVTNGGRVLCVTALGHTVAEAQKRAYALMTDIHWDDCFR 416
GARS      AQALGLEVFHAGTALKN-GKVVTTHGGRVLAVTAIRENLISALEEAKKGLAAIKFEGAIYR 418
          :. * :***** * : :*:**:*****.***: .. .* :.* : :*:**: :

purD      KDIGWRAIEREQN 429
GARS      KDVGFRATIAFLQ- 430
          **:**:*** *

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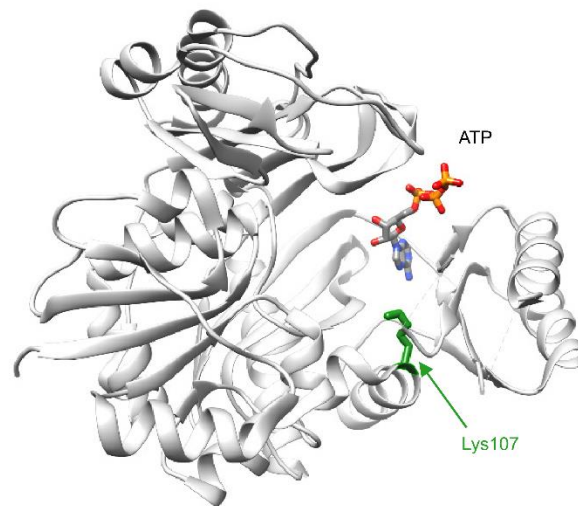
B

Figure S4. Structural model of GART with identified ubiquitination site (Lys107). (A) Structural alignment of *E.coli* PurD homolog to human GAR synthetase (GARS). The yellow highlighted lysine residue corresponds to Lys107 (human) and Lys105 (*E.coli*), a noted ubiquitination site in the lid region of ATP grasp family proteins. (B) Human GARS domain showing the proximity of Lys107 (green, site of identified ubiquitination) to the ATP binding site. PDB ID: 2QK4.

Table S1. 2×Strep-tagged protein construct sequences. The amino acid sequence (prepared by ExPASy ProtParam tool) is colored such that the blue amino acids correspond to the amino acid sequence of the target protein, gray and underlined for the spacer region between the target protein and 2×Strep tag (orange).

target protein	amino acid sequence of 2×Strep tagged construct
PPAT	10 20 30 40 50 60 MELEELGIRE ECGVFGCIAS GEWPTQLDVP HVITLGLVGL QHRGQESAGI VTSDGSSVPT
	70 80 90 100 110 120 FKSHKGMGLV NHVFTEDNLK KLYVSNLGIG HTRYATTGKC ELENCPQFVV ETLHGKIAVA
	130 140 150 160 170 180 HNGELVNAAR LRKKLLRHGI GLSTSSDSEM ITQLLAYTPP QEQDDTPDWV ARIKNLMKEA
	190 200 210 220 230 240 PTAYSLIMH RDVIYAVRDP YGNRPLCIGR LIPVSDINDK EKKTSETEGW VVSSESCSFL
	250 260 270 280 290 300 SIGARYYREV LPGEIVEISR HNVQTLDIIS RSEGNPVAFC IFEYVYFARP DSMFEDQMVY
	310 320 330 340 350 360 TVRYRCGQQL AIEAPVDADL VSTVPESATP AALAYAGKCG LPYVEVLCKN RYVGRTFIQP
	370 380 390 400 410 420 NMRLRQLGVA KKFGVLSDNF KGKRIVLVDD SIVRGNTISP IIKLLKESGA KEVHIRVASP
	430 440 450 460 470 480 PIKYPFCMGI NIPTKEELIA NKPEFDHLAE YLGANSVVYL SVEGLVSSVQ EGIKFKKQKE
	490 500 510 520 530 540 KKHDIMIQUEN GNGLECFEKS GHCTACLTGK YPVELEWVPG SAWSHPQFEK GGGSGGGSGG
	550 SAWSHPQFEK
GART	10 20 30 40 50 60 MAARVLIIGS GGREHTLAWK LAQSHHVQV LVAPGNAGTA CSEKISNTAI SISDHTALAQ
	70 80 90 100 110 120 FCKEKKIEFV VVGPEAPLAA GIVGNLRSAG VQCFGPTAEA AQLESSKRFA KEFMDRHGIP
	130 140 150 160 170 180 TAQWKAFTKP EEACSFILSA DFPALVVKAS GLAAGKGVIV AKSKEEACKA VQEIMQEKAQ
	190 200 210 220 230 240 GAAGETIVIE ELLDGEEVSC LCFTDGKTVA PMPPAQDHRK LLEGDGGPNT GGMGAYCPAP
	250 260 270 280 290 300 QVSNLLLLKI KDTVLRQRTVD GMQQEGTPYT GILYAGIMLT KNGPKVLEFN CRFGDPECQV
	310 320 330 340 350 360

	ILPLKSDLY EVIQSTLDGL LCTSLPVWLE NHTALTVMMA SKGYPGDYTK GVEITGFPEA 370 380 390 400 410 420 QALGLEVFHA GTALKNGKVV THGGRVLAVT AIRENLISAL EEAKKGAAI KFEGAIYRKD 430 440 450 460 470 480 VGFRAlAFIQ QPRSLTYKES GVDIAAGNML VKKIQLAKA TSRSGCKVDL GGFAGLFDLK 490 500 510 520 530 540 AAGFKDPLLA SGTDGVGTKL KIAQLCNKHD TIGQDLVAMC VNDILAQGAE PLFFLDYFSC 550 560 570 580 590 600 GKLDLSVTEA VVAGIAKACG KAGCALLGGE TAEMPDMYPP GEYDLAGFAV GAMERDQKLP 610 620 630 640 650 660 HLERITEGDV VVGIASSGLH SNGFSLVRKI VAKSSLQYSS PAPDGCQDQT LGDLLLTPTTR 670 680 690 700 710 720 IYSHSLLPVL RSGHVKAFAH ITGGGLENI PRVLPEKLG V DLDQQTWRIP RVFSWLQQEG 730 740 750 760 770 780 HLSEEMART FNCGVGAVLV VSKEQTEQIL RDIQQHKEEA WVIGSVVARA EGSPRVKVKNN 790 800 810 820 830 840 LIESMQINGS VLKNGSLTNH FSFEKKKARV AVLISGTGSN LQALIDSTRE PNSSAQIDIV 850 860 870 880 890 900 ISNKAAGVAGL DKAERAGIPT RVINHKLKYN RVEFDSAIDL VLEEFSDIV CLAGFMRILS 910 920 930 940 950 960 GPFVQKWNGK MLNIHPSLLP SFGKSNAHEQ ALETGVTVTG CTVHFVAEDV DAGQIILQEA 970 980 990 1000 1010 1020 VPVKGRTVA TLSERVKLAE HKIFPAALQL VASGTVQLGE NGKICWVKEE LEGSAW ^{SH} PQ 1030 1040 FEKGGGSGGG SGGSAW ^{SH} PQ FEK
PFAS	10 20 30 40 50 60 MSPVLHIFYVR PSGHEGAAPG HTRRKLGKQL PELQGVETEL CYNVNWTAAE LPSAEETKKL 70 80 90 100 110 120 MWLFGCPLLL DDVARESWLL PGSNDLLLEV GPRLNFTPT STNIVSVCRA TGLGPVDRVE 130 140 150 160 170 180 TTRRYRLSFA HPPSAEVEAI ALATLHDRMT EQHFPHPIS FSPESMPEPL NGPINILGEG 190 200 210 220 230 240 RLALEKANQE LGLALDSWDL DFYTKRFQEL QRNPSTVEAF DLAQSNSEHS RHWFFKGQLH 250 260 270 280 290 300 VDGQKLVSIL FESIMSTQES SNPNNVLKFC DNSSAIQGKE VRFLRPEDPT RPSRFQQQQG 310 320 330 340 350 360 LRHVFTAET HNFPTGVCPF SGATTGTGGR IRDVQCTGRG AHVVAGTAGY CFGNLHIPGY 370 380 390 400 410 420 NLPWEDPSFQ YPGNFARPLE VAIEASNGAS DYGNKFGEFV LAGFARSLGL QLPDQQRREW 430 440 450 460 470 480 IKPIMFSGGI GSMEADHISK EAPEPGMEVV KVGGPVYRIG VGGGAASSVQ VQGDNTSDLD 490 500 510 520 530 540 FGAVQRGDPE MEQKMNRVIR ACVEAPKGNP ICSLHDQAG GNGNVLKELS DPAGAIYTS

	550 560 570 580 590 600 RFQLGDPTLN ALEIWGAEYQ ESNALLLRSP NRDFLTHVSA RERCPACFVG TITGDRRIVL
	610 620 630 640 650 660 VDDRECPVRR NGQGDAPPTP LPTPVDELE WVLGKMPRKE FFLQRKPPML QPLALPPGLS
	670 680 690 700 710 720 VHQALERVLR LPVASKRYL TNKVDRSVGG LVAQQQCVGP LQTPLADVAV VALSHEELIG
	730 740 750 760 770 780 AATALGEQPV KSLLDPKVAA RLAVAEALTN LVFALVTDLR DVKCSGNWMW AAKLPGEGAA
	790 800 810 820 830 840 LADACEAMVA VMAALGVAVD GGKDSLMAA RVGTETVRAP GSLVISAYAV CPDITATVTP
	850 860 870 880 890 900 DLKHPEGRGH LLYVALSPGQ HRLGGTALAQ CFSQLGEHPP DLDLPENLVR AFSITQGLLK
	910 920 930 940 950 960 DRLLCSGHDV SDGGLVTCLL EMAFAGNCGL QVDVPVPRVD VLSVLFAEEP GLVLEVQEPD
	970 980 990 1000 1010 1020 LAQVLKRYRD AGLHCLELGH TGEAGPHAMV RVSVNGAVVL EEPVGELRAL WEETSQQLDR
	1030 1040 1050 1060 1070 1080 LQAEPRCVAE EERGLRERMG PSYCLPPTFP KASVPREPGG PSPRVAILRE EGSNGDREMA
	1090 1100 1110 1120 1130 1140 DAFHLAGFEV WDVTMQDLCS GAIGLDTFRG VAFVGGSFYA DVLGSAKGWA AAVTFHPRAG
	1150 1160 1170 1180 1190 1200 AELRRFRKRP DTFSLGVCNG CQLLALLGWV GGDPNEDAAE MGPDSQPARP GLLLRHNLGS
	1210 1220 1230 1240 1250 1260 RYESRWASVR VGPGPALMLR GMEGAVLPVW SAHGEYVAF SSPELQAQIE ARGLAPLHWA
	1270 1280 1290 1300 1310 1320 DDDGNPTEQY PLNPNGSPGG VAGICSCDGR HLAVMPHPER AVRPWQAWAR PPPFDTLTTS
	1330 1340 1350 1360 1370 1380 PWLQLFINAR NWTLEGSCRI LQSTVPRARD PPVATGSAWS HPQFEKGGGS GGGSGGSAWS
	HPQFEK
PAICS	10 20 30 40 50 60 MATAEVLNIG KKLYEGKTKE VYELLDSPGK VLLQSKDQIT AGNAARKNHL EGKAAISNKI
	70 80 90 100 110 120 TSCIFQLLQE AGIKTAFTRK CGETAFIAPQ CEMPIEWVC RRIATGSFLK RNPGVKEGYK
	130 140 150 160 170 180 FYPKVELFF KDDANNDPQW SEEQLIAAKF CFAGLLIGQT EVDIMSHATQ AIFEILEKSW
	190 200 210 220 230 240 LPQNCTLVDM KIEFGVDVTT KEIVLADVID NDSWRLWPSG DRSQQKDKQS YRDLKEVTPE
	250 260 270 280 290 300 GLQMVKKNFE WVAERVELLL KSESQCRVVV LMGSTSDLGH CEKIKKACGN FGIPCELRVT
	310 320 330 340 350 360 SAHKGPDETL RIKAEYEGDG IPTVFVAVAG RSNGLGPVMS GNTAYPVISC PPLTPDWGVQ

	370 380 390 400 410 420 DVWSSRLRPS GLGCSTVLSP EGSAQFAAQI FGLSNHLVWS KLRASILNTW ISLKQADKKI
	430 440 450 460 RECNLPPVAT GSAWSHPQFE KGGGSGGGSG GSAWSHPQFE K
ADSL	10 20 30 40 50 60 MAAGGDHGSP DSYRSPLASR YASPEMCFVF SDRYKFRTWR QLWLWLAEAE QTLGLPITDE 70 80 90 100 110 120 QIQEMKSNLE NIDFKMAAEE EKRLRHDMVA HVHTFGHCCP KAAGIIHLGA TSCYVGDNTE 130 140 150 160 170 180 LIILRNALDL LLPKLARVIS RLADFAKERA SLPTLGFTHF QPAQLTTVGK RCCLWIQDLC 190 200 210 220 230 240 MDLQNLKVR DDLRFRGVKG TTGTQASFLQ LFEGDDHKVE QLDKMVTEKA GFKRAFIITG 250 260 270 280 290 300 QTYTRKVDIE VLSVLASLGA SVHKICTDIR LLANLKEMEE PFEKQQIGSS AMPYKRNPMP 310 320 330 340 350 360 SERCCSLARH LMTLVMDPLQ TASVQWFERT LDDSANRRIC LAEAFILTADT ILNTLQNISE 370 380 390 400 410 420 GLVVYPKVE RRIRQELPFM ATENIIMAMV KAGGSRQDCH EKIRVLSQQA ASVVKQEGGD 430 440 450 460 470 480 NDLIERIQVD AYFSPHSQL DHLLDPSSFT GRASQQVQRF LEEEVYPLLK PYESVMKVKA 490 500 510 520 ELCLARDPPV ATGSAWSHPQ FEKGGGSGGG SGGSWSHPQ FEK
ATIC	10 20 30 40 50 60 MASAWSHPQF EKGSGSGGGS GGSWSHPQF EKSGRIRSVM APGQLALFSV SDKTGLVEFA 70 80 90 100 110 120 RNLTAAGLNL VASGGTAKAL RDAGLAVRDV SELTGFPPEML GGRVKTLPVA VHAGILARNI 130 140 150 160 170 180 PEDNADMARL DFNLRVAC NLYPFVKTV SPGVTVVEAV EQIDIGGVTL LRAAAKNHAR 190 200 210 220 230 240 VTVVCEPEDY VVVSTEMQSS ESKDTSLETR RQLALKAFTH TAQYDEAISD YFRKQYSGV 250 260 270 280 290 300 SQMPRLRYGMN PHQTPAQLYT LQPKLPITVL NGAPGFINLC DALNAWQLVK ELKEALGIPA 310 320 330 340 350 360 AASFHVSPA GAAVGIPSE DEAKVCMVYD LYKTLTPISA AYARARGADR MSSFGDFVAL 370 380 390 400 410 420 SDVCDVPTAK IISREVSDGI IAPGYEEAL TILSKKKNGN YCVLQMDQSY KPDENEVRTL 430 440 450 460 470 480 FGLHLSQKRN NGVVDKSLFS NVVTKNKDLF ESALRDLIVA TIAVKYTQSN SVCYAKNGQV 490 500 510 520 530 540 IGIGAGQQSR IHCTRLAGDK ANYWWLRHHP QVLSMKFKTG VKRAEISNAI DQYVTGTIGE 550 560 570 580 590 600 DEDLIKWKAL FEEVPELLE AEKKEWVEKL TEVSISSDAF FPFDRNDVRA KRSGVAYIAA 610 620 630 PSGSAADKVV IEACDELGII LAHTNLRLFH H

Table S5. Comparison of phosphorylated residues between selected studies.

target protein	res. no.	this study	Olsen, J. et al. 2010 [†]	Mertins, P. et al. 2013 [‡]
PPAT	259	X		
	356		X	
	397	X		
GART	46		X	
	48		X	
	51		X	
	53		X	
	121		X	
	128	X		
	390		X	
	398	X		
	440	X		
	491	X		
	498	X		
	546		X	
	548		X	
	714			X
	730		X	
	796			X
	802			X
	900		X	
	973	X		
PFAS	83	X		
	128	X		
	215	X		X
	216	X		
	261	X		
	290	X		
	530		X	
	538		X	
	539		X	
	569		X	X
	576	X		
	619	X	X	
	623	X	X	
	826	X		
	857	X		
	873	X		
	893	X		
	1062	X	X	
PAICS	18	X		
	27	X		X
	35	X		
	53		X	
	62			X
	107			X
target protein	res. no.	this study	Olsen, J. et al. 2010 [†]	Mertins, P. et al. 2013 [‡]
PAICS (cont'd)	238	X		X
	274			X
	276			X
	300	X		
	323	X		
	409	X		
	412	X		
ADSL	9		X	X
	12		X	
	15		X	X
	21	X		
	114	X		
	239		X	
	242		X	
	243		X	
	244		X	
	253		X	
	257		X	
	261		X	
	407	X		
	412	X		
	434	X		
ATIC	25		X	
	34		X	
	37		X	
	52	X		
	55	X		
	67	X		
	143		X	
	155		X	
	156		X	
	160		X	
	161		X	
	163		X	
	180	X		
	182	X		
	190	X		
	215	X		
	264	X		
	269	X		
	322	X		
	346	X		
	387	X		
	413	X		
	422	X		

[†] Olsen, J. V.; Vermeulen, M.; Santamaria, A.; Kumar, C.; Miller, M. L.; Jensen, L. J.; Gnad, F.; Cox, J.; Jensen, T. S.; Nigg, E. A.; Brunak, S.; Mann, M., Quantitative phosphoproteomics reveals widespread full phosphorylation site occupancy during mitosis. *Sci Signal* **2010**, 3 (104), ra3.

[‡] only included data from 'high' proteome analysis coverage from Mertins, P.; Qiao, J. W.; Patel, J.; Udeshi, N. D.; Clauser, K. R.; Mani, D. R.; Burgess, M. W.; Gillette, M. A.; Jaffe, J. D.; Carr, S. A., Integrated proteomic analysis of post-translational modifications by serial enrichment. *Nat Methods* **2013**, 10 (7), 634-7.

Table S6. Comparison of ubiquitinated residues between selected studies.

target protein	res. no.	this study	Kim, W. <i>et al.</i> 2011 [†]	Mertins, P. <i>et al.</i> 2013 [‡]
PPAT	62		X	X
	80		X	
	99			X
	349			X
	381		X	X
	403		X	X
	411		X	X
GART	28			X
	66		X	
	107	X	X	X
	111		X	X
	156		X	
	219		X	
	251		X	X
	281		X	
	285			X
	350		X	
	375		X	
	378	X	X	
	404		X	
	411		X	
	438		X	
	459			X
	467			X
	485		X	X
	557			X
	598			X
	697		X	X
	852			X
PFAS	205		X	
	422		X	X
	451		X	
	494		X	X
	507			X
	527			X
	646			X
	683		X	X
	737		X	
	843			X
	900		X	
PAICS	11	X	X	X
	19			X
	30			X
	36			X
	45		X	
	53			X
	56		X	
	62		X	

target protein	res. no.	this study	Kim, W. <i>et al.</i> 2011 [†]	Mertins, P. <i>et al.</i> 2013 [‡]
PAICS (cont'd)	79		X	
	80			X
	100		X	
	110			X
	116			X
	136		X	
	142		X	
	157		X	
	235			X
	247			X
	261		X	
	272		X	
	273		X	
	286			X
	304			X
	330		X	
	339		X	
	414			X
	440		X	
ADSL	75		X	X
	134	X		X
	170	X		X
	218			X
	243		X	
	264	X		
	276			X
	290		X	
	298		X	
	391	X		
	415			X
	429		X	
	479			X
ATIC	39	X		
	66	X	X	X
	177			X
	199			X
	254			X
	266			X
	331	X		
	356		X	
	397		X	X
	406		X	
	437			X
	461	X		X
	477	X		
	507		X	
	524		X	X

[†] Kim, W.; Bennett, E. J.; Huttlin, E. L.; Guo, A.; Li, J.; Possemato, A.; Sowa, M. E.; Rad, R.; Rush, J.; Comb, M. J.; Harper, J. W.; Gygi, S. P., Systematic and quantitative assessment of the ubiquitin-modified proteome. *Mol Cell* **2011**, *44* (2), 325-40.

[‡] only included data from 'high' proteome analysis coverage from Mertins, P.; Qiao, J. W.; Patel, J.; Udeshi, N. D.; Clauser, K. R.; Mani, D. R.; Burgess, M. W.; Gillette, M. A.; Jaffe, J. D.; Carr, S. A., Integrated proteomic analysis of post-translational modifications by serial enrichment. *Nat Methods* **2013**, *10* (7), 634-7.

Table S7. Comparison of acetylated residues between selected studies.

target protein	res. no.	this study	Choudhary, C. <i>et al.</i> 2009 [†]	Mertins, P. <i>et al.</i> 2013 [‡]
PPAT	65	X		
	81	X	X	X
	99			X
	349	X		
	371	X		
	372	X		X
GART	28			X
	107	X		
	251	X		
	350		X	
	378	X		
	438	X		
	452	X		
	459	X		
	499	X		
	852	X		X
	906	X		
PAICS	11	X		
	19	X		
	30	X		X
	36	X		
	53	X		
	59	X		
	79		X	
	80	X		
	110			X
	246	X		X
	247	X		X
	261			X
	273		X	
	283	X		X
	285			X

target protein	res. no.	this study	Choudhary, C. <i>et al.</i> 2009 [†]	Mertins, P. <i>et al.</i> 2013 [‡]
PAICS (cont'd)	286	X		
	304	X		
	309		X	
	311		X	
	313	X		
ADSL	82			X
	134	X		
	147	X	X	
	170	X		
	187	X		
	199	X		
	264	X		
	295	X	X	X
	391	X		
	402	X		
	415	X		
ATIC	66	X		
	164	X		
	199	X	X	
	285	X		
	294	X		
	331	X		
	356		X	X
	357		X	
	372	X		
	389	X		
	397	X		
	408	X		
	437	X		
	461	X		
	477	X		

[†] Choudhary, C.; Kumar, C.; Gnad, F.; Nielsen, M. L.; Rehman, M.; Walther, T. C.; Olsen, J. V.; Mann, M., Lysine acetylation targets protein complexes and co-regulates major cellular functions. *Science* **2009**, 325 (5942), 834-40.

[‡] only included data from 'high' proteome analysis coverage from Mertins, P.; Qiao, J. W.; Patel, J.; Udeshi, N. D.; Clauser, K. R.; Mani, D. R.; Burgess, M. W.; Gillette, M. A.; Jaffe, J. D.; Carr, S. A., Integrated proteomic analysis of post-translational modifications by serial enrichment. *Nat Methods* **2013**, 10 (7), 634-7.