Acetylation-defective mutants of Pparγ are associated with decreased lipid synthesis in breast cancer cells

Supplementary Material

Total sequence coverage with two digests:

435/475 = 92 % coverage

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  1 MVDTEMPFWP TNFGISSVDL
 21 XVMEDHSHSF DIKPFTTVDF SSISAPHYED IPFTRADPMV ADYKYDLKLQ
 71 EYQSAIKVEP ASPPYYSEKT QLYNRPHEEP SNSLMAIECR VCGDKASGFH
121 YGVHACEGCK GFFRRTIRLK LIYDRCDLNC RIHKKSRNKC QYCRFQKCLA
171 VGMSHNAIRF GRMPQAEKEK LLAEISSDID QLNPESADLR ALAKHLYDSY
221 IKSFPLTKAK ARAILTGKTT DKSPFVIYDM NSLMMGEDKI KFKHITPLQE
271 QSKEVAIRIF QGCQFRSVEA VQEITEYAKN IPGFINLDLN DQVTLLKYGV
321 HEIIYTMLAS LMNKDGVLIS EGQGFMTREF LKSLRKPFGD FMEPKFEFAV
371 KFNALELDDS DLAIFIAVII LSGDRPGLLN VKPIEDIQDN LLQALELQLK
421 LNHPESSQLF AKVLQKMTDL RQIVTEHVQL LHVIKKTETD MSLHPLLQEI
471 YKDLY
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blue letters: only trypsin data
red letters: only chymotrypsin data
green letters: sequences from both trypsin and chymo digests

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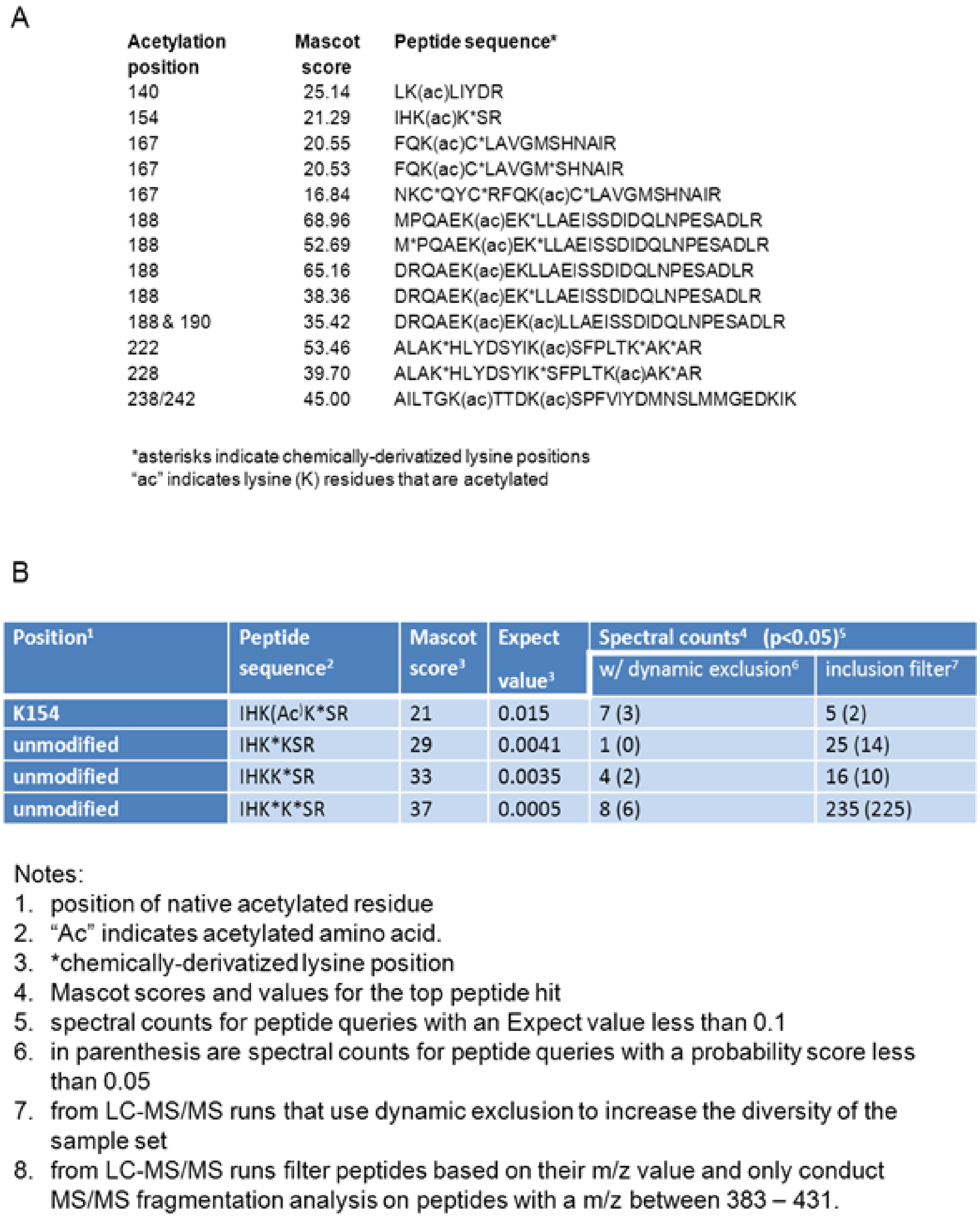
  1  MVDTEMPFWP TNFGISSVDL
 21  SVMEDHSHSF DIKPFTTVDF SSISAPHYED IPFTRADPMV ADYKYDLKLQ
 71  EYQSAIKVEP ASPPYYSEKT QLYNRPHEEP SNSLMAIECR VCGDKASGFH
121  YGVHACEGCK GFFRRTIRLK LIYDRCDLNC RIHKKSRNKC QYCRFQKCLA
171  VGMSHNAIRF GRMPQAEKEK LLAEISSDID QLNPESADLR ALAKHLYDSY
221  IKSFPLTKAK ARAILTGKTT DKSPFVIYDM NSLMMGEDKI KFKHITPLQE
271  QSKEVAIRIF QGCQFRSVEA VQEITEYAKN IPGFINLDLN DQVTLLKYGV
321  HEIIYTMLAS LMNKDGVLIS EGQGFMTREF LKSLRKPFGD FMEPKFEFAV
371  KFNALELDDS DLAIFIAVII LSGDRPGLLN VKPIEDIQDN LLQALELQLK
421  LNHPESSQLF AKVLQKMTDL RQIVTEHVQL LHVIKKTETD MSLHPLLQEI
471  YKDLY
```

Red letters: Sequence coverage of derivatized lysine

mapping of Pparγ1

Figure S1: The sequence coverage with different digestion. Mass spectrometry was conducted of immune-precipitated Pparγ in transfected HEK293 cells. Analysis totally covered >96% of the protein. (A) Chymotrypsin and trypsin mapping and sequence coverage of Pparγ1. (B) Sequence coverage of derivatized lysine mapping of Pparγ1.



A

Acetylation position	Mascot score	Peptide sequence*
140	25.14	LK(ac)LIYDR
154	21.29	IHK(ac)K*SR
167	20.55	FQK(ac)C*LAVGMSHNAIR
167	20.53	FQK(ac)C*LAVGM*SHNAIR
167	16.84	NKC*QYC*RFQK(ac)C*LAVGMSHNAIR
188	68.96	MPQAEK(ac)EK*LLAEISSDIDQLNPESADLR
188	52.69	M*PQAEK(ac)EK*LLAEISSDIDQLNPESADLR
188	65.16	DRQAEK(ac)EKLLAEISSDIDQLNPESADLR
188	38.36	DRQAEK(ac)EK*LLAEISSDIDQLNPESADLR
188 & 190	35.42	DRQAEK(ac)EK(ac)LLAEISSDIDQLNPESADLR
222	53.46	ALAK*HLYDSYIK(ac)SFPLTK*AK*AR
228	39.70	ALAK*HLYDSYIK*SFPLTK(ac)AK*AR
238/242	45.00	AILTGK(ac)TTDK(ac)SPFVIYDMNSLMMGEDKIK

*asterisks indicate chemically-derivatized lysine positions
"ac" indicates lysine (K) residues that are acetylated

B

Position[1]	Peptide sequence[2]	Mascot score[3]	Expect value[3]	Spectral counts[4] (p<0.05)[5]	
				w/ dynamic exclusion[6]	inclusion filter[7]
K154	IHK(Ac)K*SR	21	0.015	7 (3)	5 (2)
unmodified	IHK*KSR	29	0.0041	1 (0)	25 (14)
unmodified	IHKK*SR	33	0.0035	4 (2)	16 (10)
unmodified	IHK*K*SR	37	0.0005	8 (6)	235 (225)

Notes:
1. position of native acetylated residue
2. "Ac" indicates acetylated amino acid.
3. *chemically-derivatized lysine position
4. Mascot scores and values for the top peptide hit
5. spectral counts for peptide queries with an Expect value less than 0.1
6. in parenthesis are spectral counts for peptide queries with a probability score less than 0.05
7. from LC-MS/MS runs that use dynamic exclusion to increase the diversity of the sample set
8. from LC-MS/MS runs filter peptides based on their m/z value and only conduct MS/MS fragmentation analysis on peptides with a m/z between 383 – 431.

Figure S2: Peptide mapping the basic region of Pparγ (a.a. positions 152-157). (A) Mass spectrometry identified nine acetylated lysine residues by a chemical-derivatization protocol. (B) LC-MS/MS analysis of derivatized Pparγ after trypsin digestion. The spectral counts and Mascot scores for acetylated peptides were shown.