Supplemental data

Tables

Table I Sequence of oligopeptides used as substrates with corresponding protein names.

Oligopeptide sequence	Protein name
[H]**SYSMDHF** RWGRPVGRRRRPVRVYP[OH]*	ACTH (aa 138-161) (P01189)
[H]**SESSSKS** RWGRPVGRRRRPVRVYP[OH] *	High mobility group protein A1 (P17096)
[H]**MLGPEGG** RWGRPVGRRRRPVRVYP [OH]*	hnRNP F; (P52597)
[H]**MLDPEGG** RWGRPVGRRRRPVRVYP [OH]*	hnRNP F; G3D mutant
[H]**MLLPEGG** RWGRPVGRRRRPVRVYP [OH]*	hnRNP F; G3L mutant
[H]**MLRPEGG** RWGRPVGRRRRPVRVYP [OH]*	hnRNP F; G3R mutant
[H]**MIGPEGG** RWGRPVGRRRRPVRVYP [OH]*	hnRNP F; L2I mutant
[H]**MFGPEGG** RWGRPVGRRRRPVRVYP [OH]*	hnRNP F; L2F mutant
[H]**MLGTEGG** RWGRPVGRRRRPVRVYP[OH]*	hnRNP H; (P31943)
[H]**MLALISR** RWGRPVGRRRRPVRVYP [OH]*	hARL8b; (Q9NVJ2)
[H]**MLGTGPA** RWGRPVGRRRRPVRVYP [OH]*	mTOR; (P42345)
[H]**MKEEVKG** RWGRPVGRRRRPVRVYP[OH]*	Kinesin KIF4A (Q95239)
[H]**MDELFPL** RWGRPVGRRRRPVRVYP [OH]*	NF-κB/p65 (Q04206)
[H]**DDIAAL** RWGRPVGRRRRPVRVYP[OH]*	β-actin (NP_001092)

The custom made peptides were obtained from BioGenes or Sigma Genosys.
* Bold indicates amino acids found in corresponding protein while underlines indicate the residues used to identify the peptides in the text.

Table II Primer sequences used to mutate GST-hNaa50p

hNaa50p mutants	Sequence 5' – 3'
R84A	GTCTGGCACCTTACGCAAGGCTAGGAATAGG
Y124F	GTCGGCAATTGACTTCTTCAGGAAGTTTGGCTTTGAG
K34R	CCAGTCAGCTACAATGACAGGTTCTACAAGGATGTGCTG
K37R	GACAAGTTCTACAGGGATGTGCTGGAGGTTGGC
K140R	CAAAGAAGAACTACTATAGGAGGATAGAGCCCGC

Table III Potential human *in vivo* substrates for hNaa50p mediated acetylation

Protein name	Protein ID code	N-terminal protein sequence
EF-hand calcium-binding domain-containing protein 2	Q5VUJ9	MLGP GQVRLRPR
Protein kinase-like protein SgK071	Q8NE28	MLGP GSNRRRPT
Putative uncharacterized protein C1orf180	Q8NAE3	MLGP NAQVTVVV
Dihydrodipicolinate synthase-like, mitochondrial precursor	Q86XE5	MLGP QVWSSVRQ
Synaptopodin	Q8N3V7	MLGP HLPPPPLA
Taste receptor type 1 member 3 precursor (Sweet taste receptor T1R3)	Q7RTX0	MLGP AVLGLSLW
Alkaline phosphatase, placental type precursor (PLAP-1)	P05187	MLGP CMLLLLLL
Olfactory receptor 52I2	Q8NH67	MLGP AYNHTMET
Olfactory receptor 52I1 (Olfactory receptor OR11-13)	Q8NGK6	MLGP AYNHTMET
Heterogeneous nuclear ribonucleoprotein F (hnRNP F)	**P52597**	**MLGP EGGEGFV**
Calcium-dependent secretion activator 1 (CAPS-1)	Q9ULU8	MLDP SSSEEESD
Calcium-dependent secretion activator 2 (CAPS-2)	Q86UW7	MLDP SSSEEESD
TIMELESS-interacting protein	Q9BVW5	MLEP QENGVIDL
Putative forkhead box protein ENSP00000381211	A8MTJ6	MLSP ERRDQPRS
Transmembrane protein C9orf144B	Q6ZU69	MLSP TFVLWEVG
RUN and SH3 domain-containing protein 1 (Nesca)	Q9BVN2	MLSP QRALLCNL
Armadillo repeat-containing protein 2	Q8NEN0	MLSP NDKMLGKL
Rho GTPase-activating protein 22	Q7Z5H3	MLSP KIRQARRA
Zinc finger protein 671	Q8TAW3	MLSP VSRDASDA
Atlastin-3	Q6DD88	MLSP QRVAAAAS
p53 and DNA damage-regulated protein 1	Q9NUG6	MLSP EAERVLRY
Olfactory receptor 5M10 (Olfactory receptor OR11-207)	Q6IEU7	MLSP NHTIVTEF
Sulfotransferase family cytosolic 1B member 1	O43704	MLSP KDILRKDL
Uncharacterized protein C11orf49	Q9H6J7	MLSP ERLALPDY
Uncharacterized protein MGC35361	Q8N0U4	MLAP CSGWELGC
Thrombospondin-4 precursor	P35443	MLAP RGAAVLLL
Leucine-rich repeat and transmembrane domain protein 2 precursor	Q8N967	MLAP GSSPGQRG
Kanadaptin (Lung cancer oncogene 3 protein - HLC-3)	Q9BWU0	MLAP LRNAPGRE
Uncharacterized protein FLJ37543 precursor	Q2M2E5	MLAP LFLCCLRN
Putative transcript Y 12 protein	Q9BZ98	MIDP ETRHKAFL
Retrotransposon-like protein 1	A6NKG5	MIEP SEDSFETM
Chloride anion exchanger (Solute carrier family 26 member 3)	P40879	MIEP FGNQYIVA
C-type lectin domain family 2 member A	Q6UVW9	MINP ELRDGRAD
Zinc finger protein 221	Q9UK13	MISP SLELLHSG
TRAF3-interacting modulator (TRAF3-interacting protein 3)	Q9Y228	MISP DPRPSPGL
Alkaline phosphatase, tissue-nonspecific isozyme precursor	P05186	MISP FLVLAIGT
Beta-2-glycoprotein 1 precursor (Beta-2-glycoprotein I)	P02749	MISP VLILFSSF
Putative BMP-2-inducible kinase-like protein	Q5H9B9	MIAP SPKSSEEE
Zinc finger BED domain-containing protein 5	Q49AG3	MIAP LLCILSYN

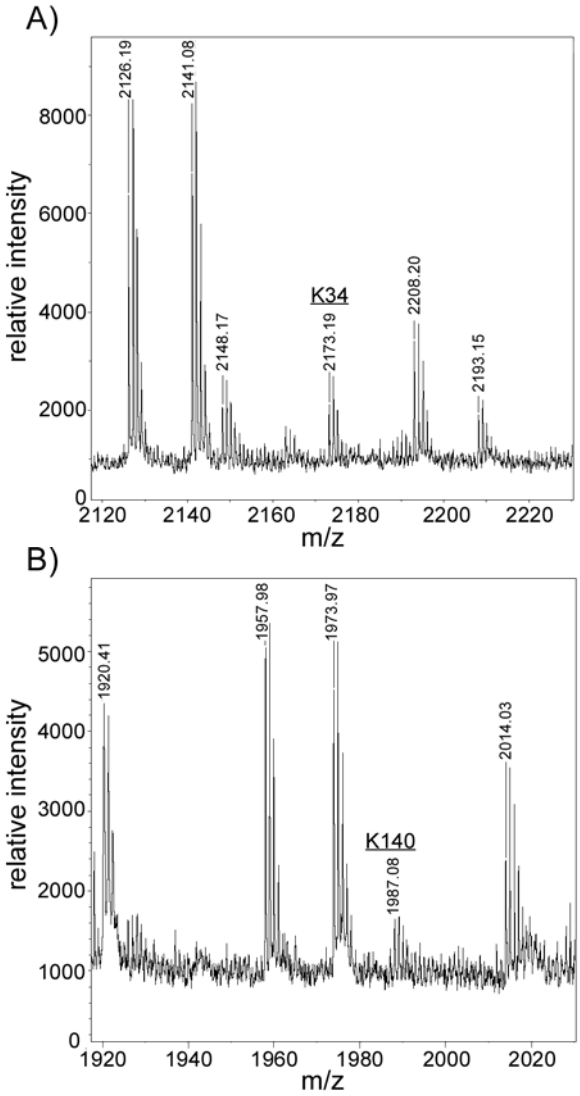
Supplemental Figures

Figure S1. MALDI-MS spectra of immunoprecipitated Xpress-hNaa50p from transfected HeLa cells.
Xpress-hNaa50p was isolated from transfected HeLa cells by immunoprecipitation with anti-Xpress antibody. ***A.*** MALDI-MS spectra (mass range 2.100- 2.200) showing a peptide with m/z of 2173.2 corresponding to acetylated lysine 34. ***B.*** MALDI-MS spectra (mass range 1.190- 2.050) showing a peptide with m/z of 1987.1 corresponding to acetylated lysine 140.

Figure S2. MALDI-MS and MS/MS spectra of autoacetylated GST-hNaa50p.
A. MALDI-MS spectrum of Lys-C produced peptides of recombinant GST-hNaa50p. Monoisotopic peaks are labelled with their respective *m/z* ratio. The +42 Da MALDI-molecular ions of *m/z* 1552.769, 1986.980 and 2173.072, are indicated with B**,** C and D, respectively, which also refer to the MS/MS spectra shown in the figure. The identities of the acetylated lysines present in the peptides are indicated. ***B***, ***C*** and ***D***. Tandem mass spectra of the m/z 1552.769, 1986.980 and 2173.072 ions, respectively, indicate the observed fragmentation pattern and the sequence ion assignments. Predicted ions (b and y) are shown at top of the figures. The acetylated lysines are displayed as 'acK' in the amino acid sequences.

Figure S3. Knockdown of human NatA with siRNA targeting hNAA15p also reduces protein levels of hNaa50p.
WB analyses using anti-hNaa50p, anti-hNaa15p and anti-tubulin antibodies. si-non-targeting represent cell lysate from HeLa cells transfected with a non-targeting siRNA, lane 2: Cell lysate from HeLa cells transfected with si-*NAA15*, lane 3: Cell lysate from HeLa cells transfected with si-h*NAA50*.

Figure S1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m/z
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2141.08
2148.17
K34
2173.19
2208.20
2193.15
B)
relative intensity
m/z
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1957.98
1973.97
K140
1987.08
2014.03

Figure S2

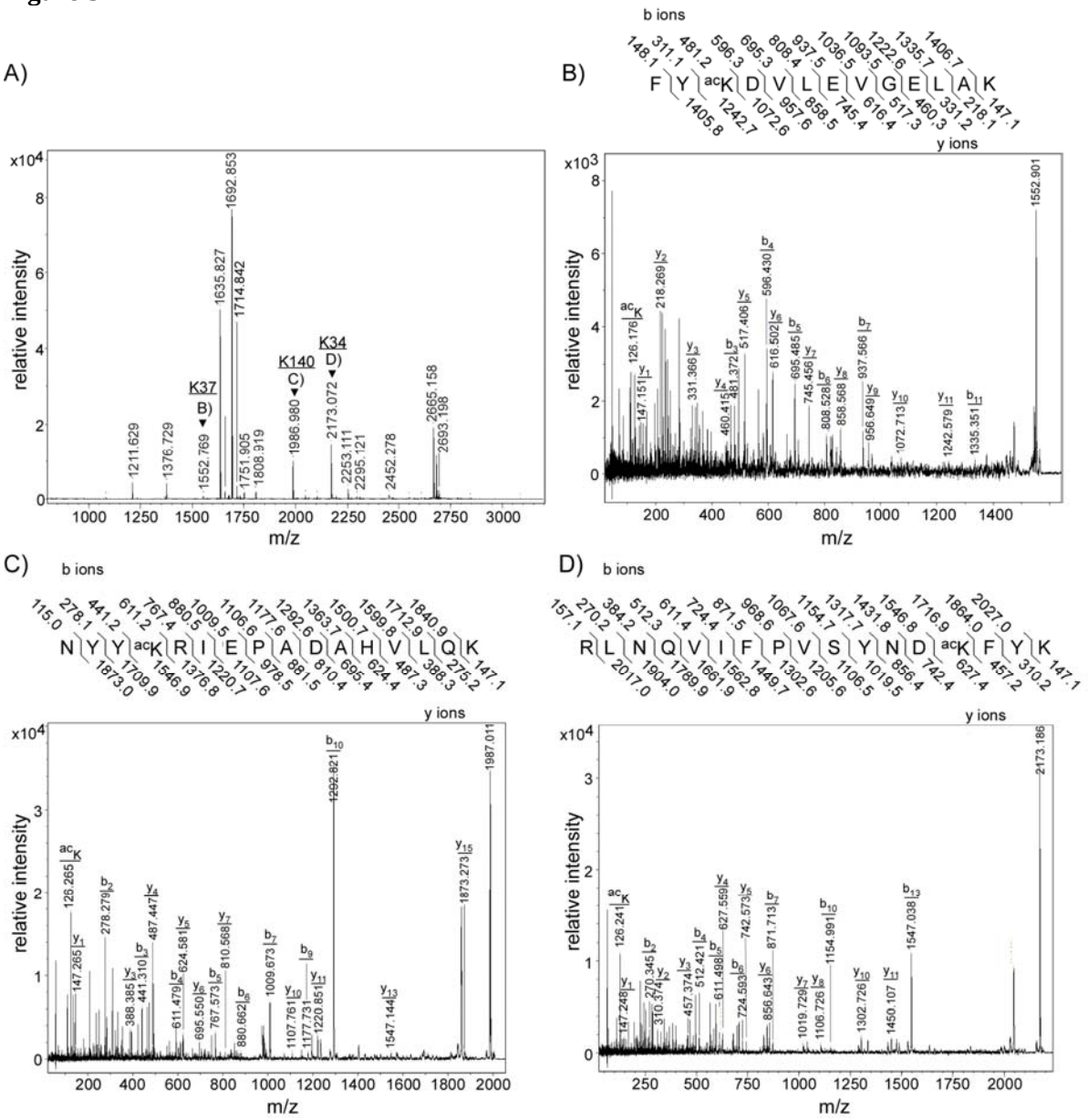
A)
B)
C)
D)
b ions
y ions
relative intensity
m/z
K37
B)
K140
C)
K34
D)
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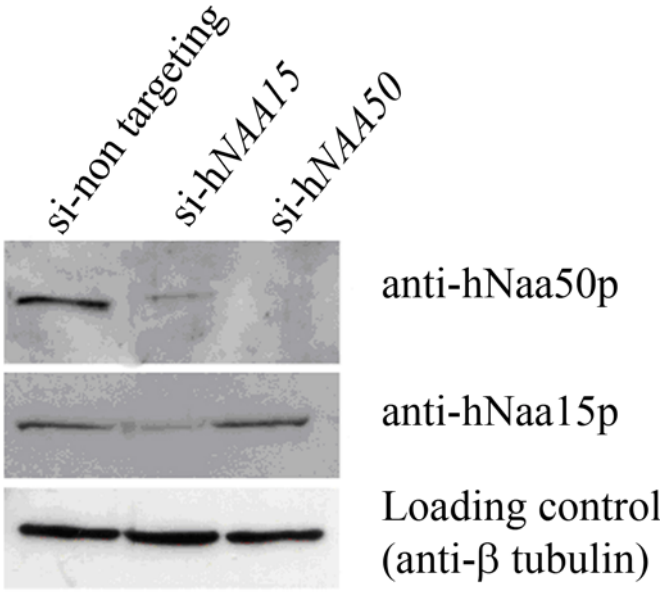
Figure S3


si-non targeting
si-hNAA15
si-hNAA50
anti-hNaa50p
anti-hNaa15p
Loading control
(anti-β tubulin)