PNAS

Supplementary Information for

A solution to the long-standing problem of actin expression and purification

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This PDF file includes:

Other supplementary materials for this manuscript include the following:

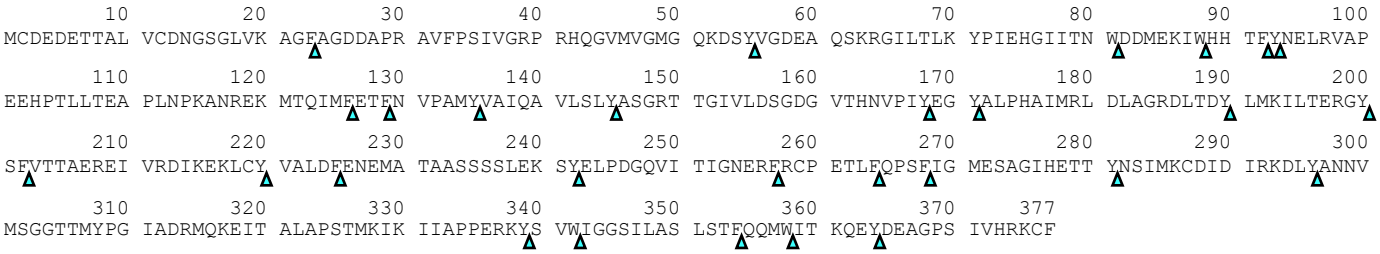


Fig. S1. Prediction of high-specificity chymotrypsin cleavage sites in skeletal α-actin (UniProt P68133)

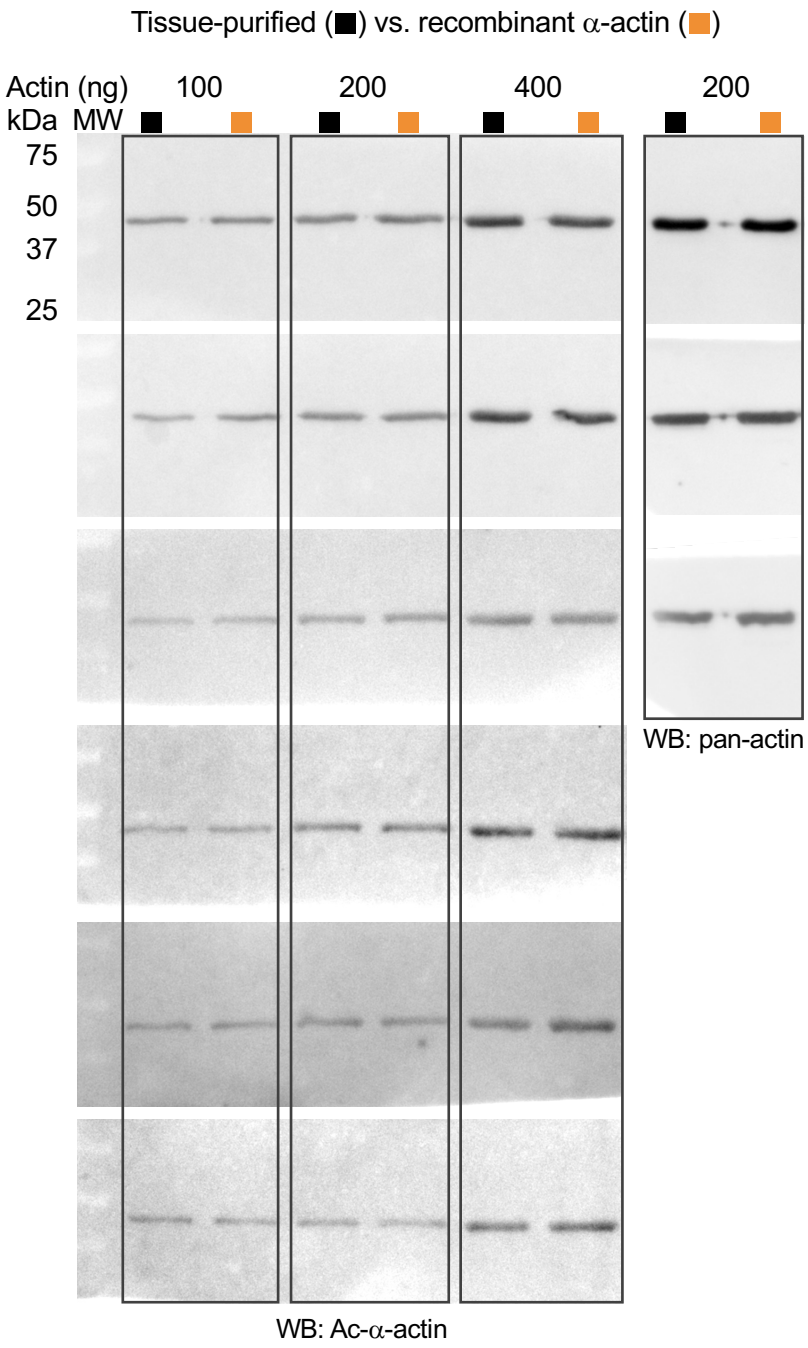


Fig. S2. Western blots of tissue-purified (endogenous) and recombinant α-actin. Left, Western blots using α-actin Nt-acetylation specific antibody. Right, Western blots using pan-actin antibody.

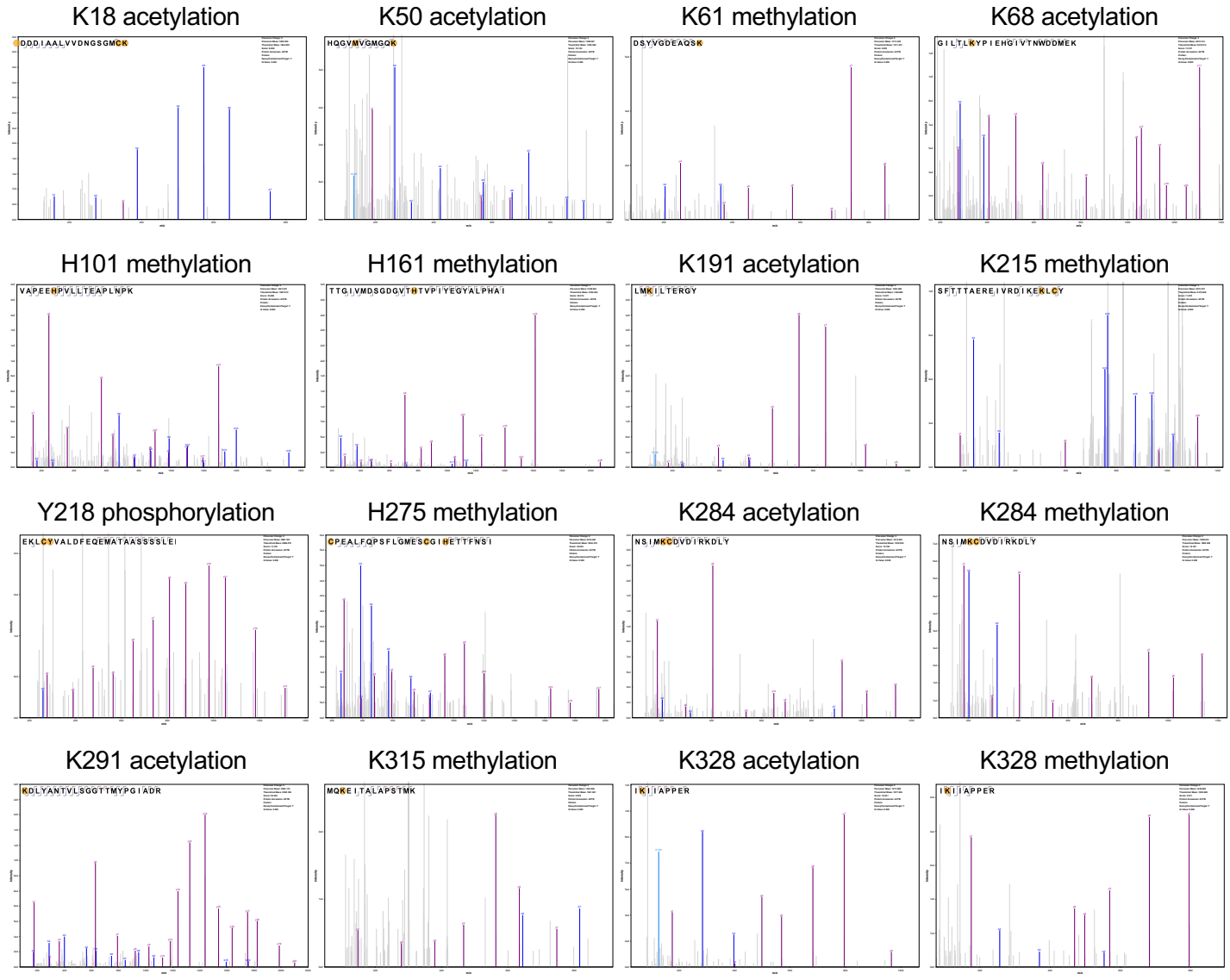


Fig. S3 (part 1 of 2). Annotated MS2 spectra of PTM peptides of endogenous β-actin with an FDR <1%. Modifications occurring during sample preparation for proteomics analysis, such as carbamylation and carbamidomethylation, are shown but not named. The title of each MS2 spectrum refers to common PTMs occurring in cells, such as phosphorylation, methylation and acetylation. See also Fig. 4*B* for MS2 spectra of Nt-acetylated and H73-methylated peptides.

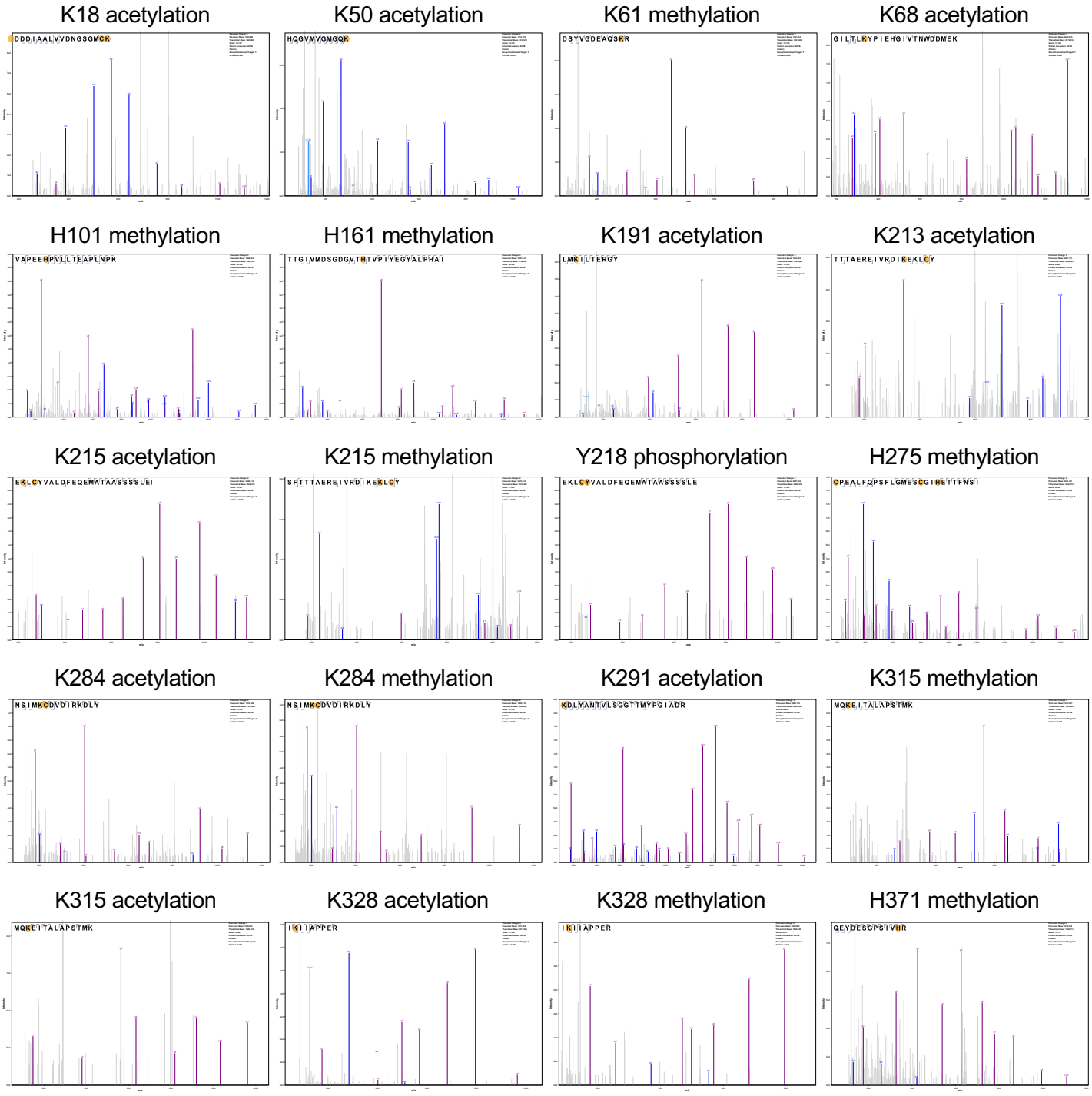


Fig. S3 (part 2 of 2). Annotated MS2 spectra of PTM peptides of recombinant β-actin.

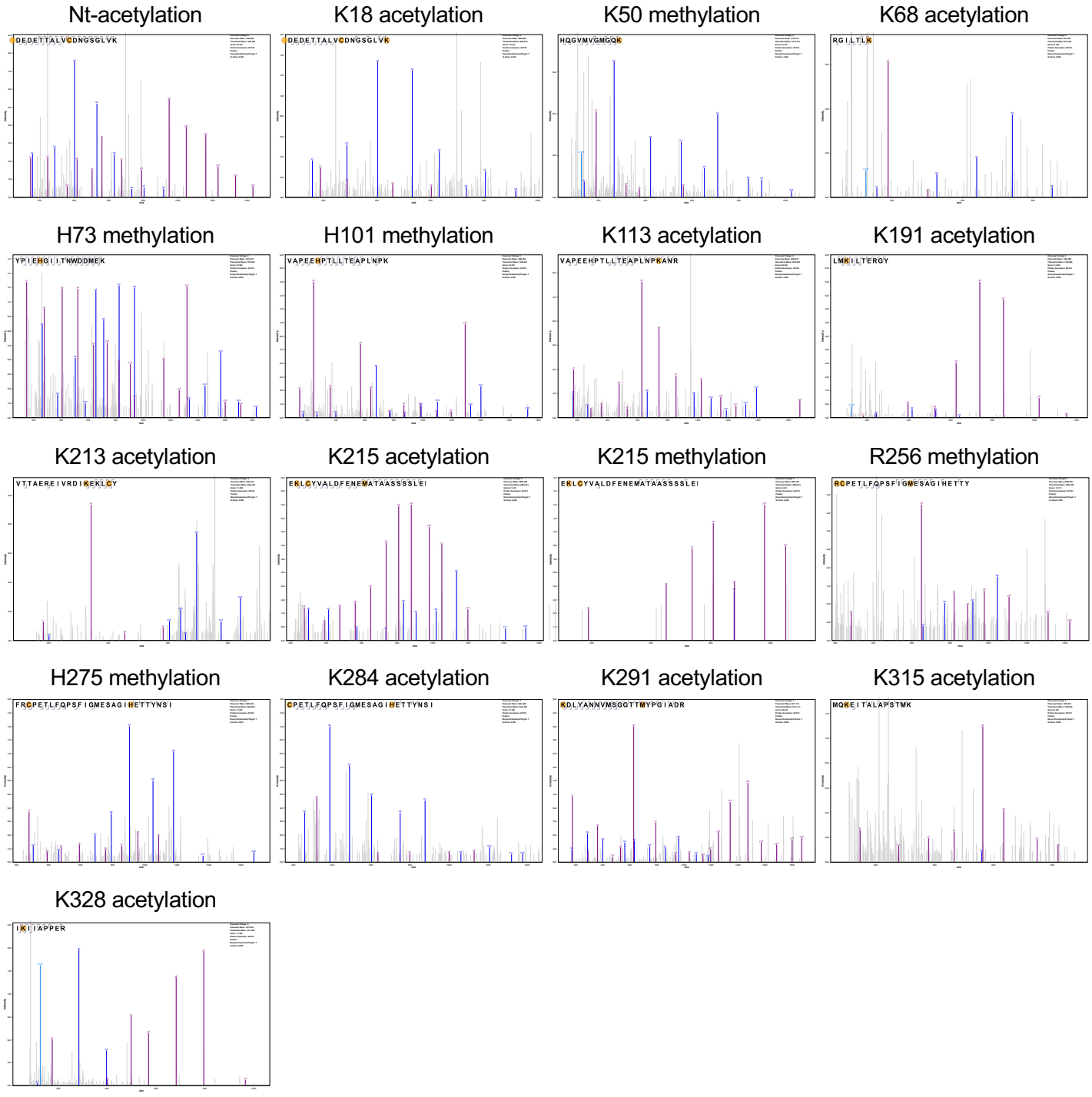


Fig. S4 (part 1 of 2). Annotated MS2 spectra of PTM peptides of tissue-purified α-actin with an FDR <1%. Modifications occurring during sample preparation for proteomics analysis, such as carbamylation and carbamidomethylation, are shown but not named. The title of each MS2 spectrum refers to common PTMs occurring in cells, such as phosphorylation, methylation and acetylation.

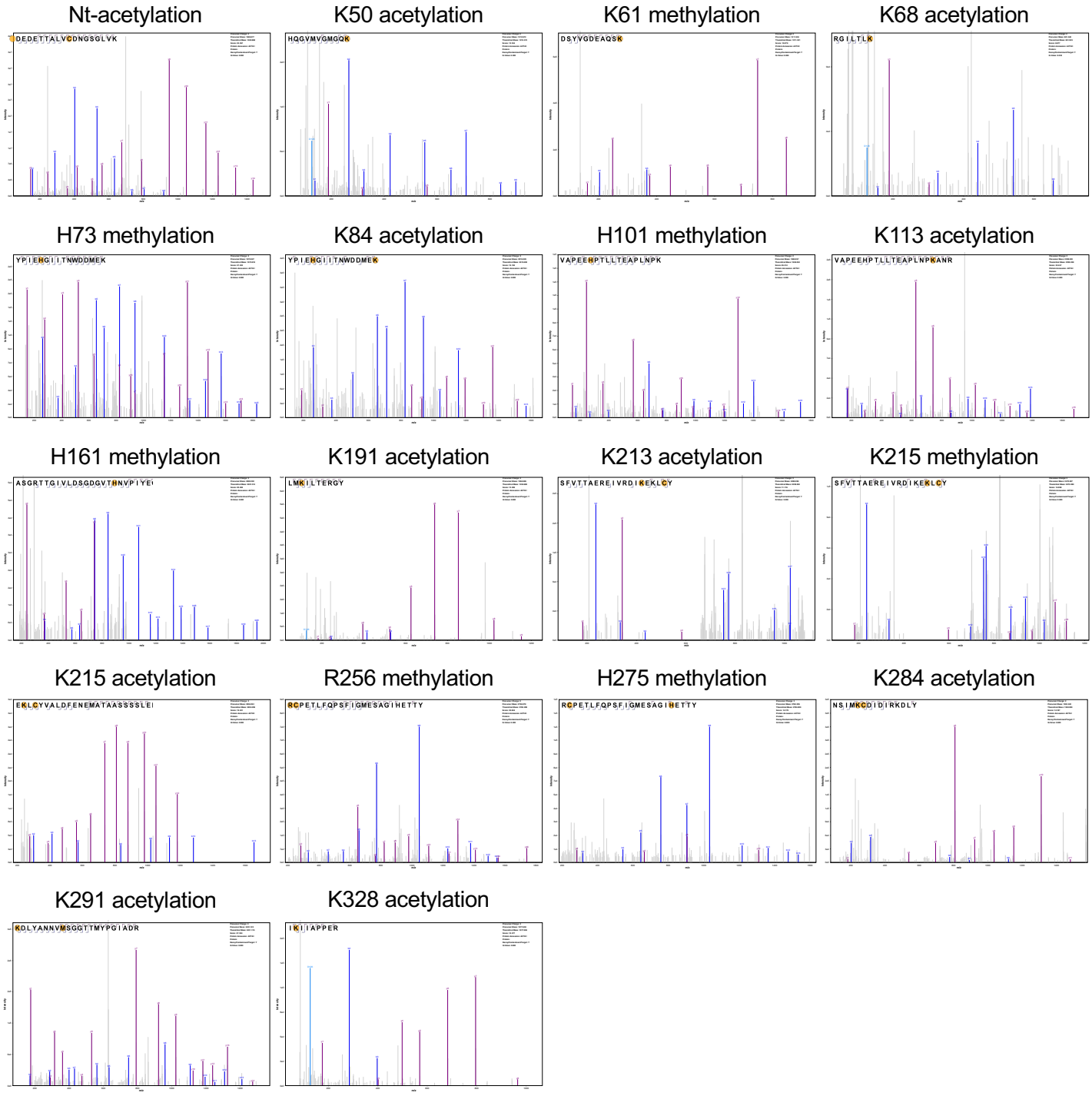
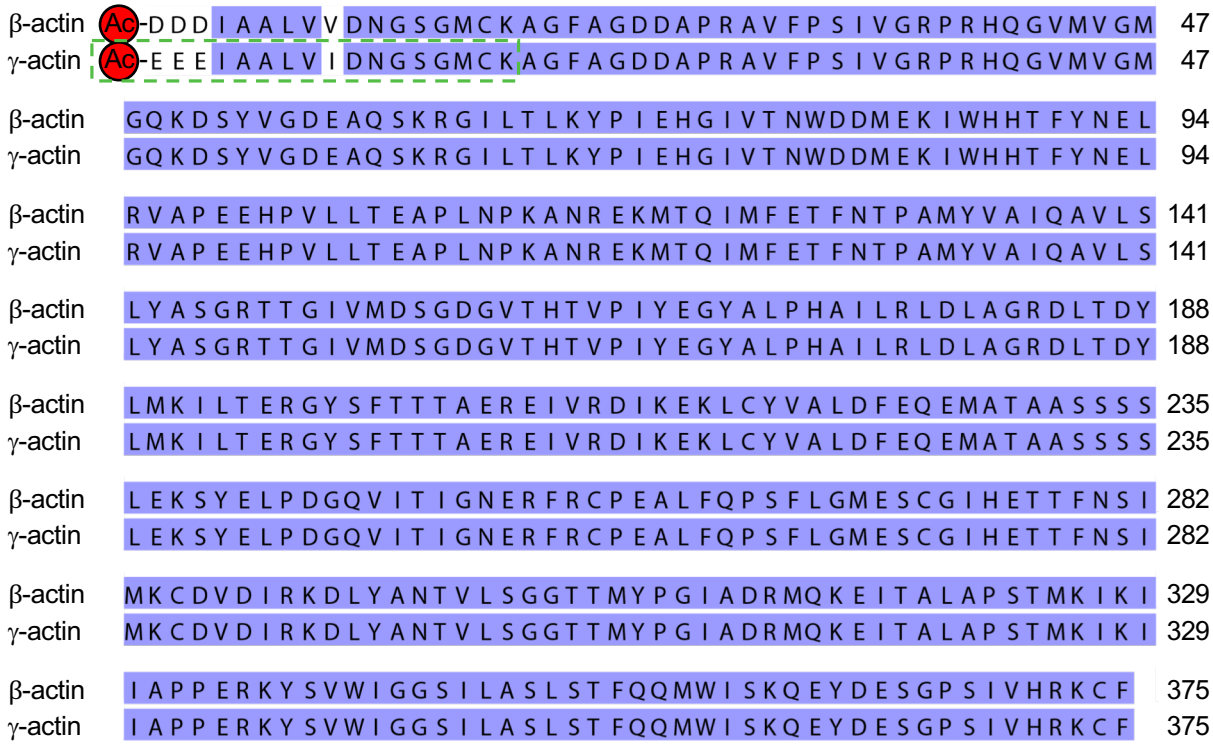


Fig. S4 (part 2 of 2). Annotated MS2 spectra of PTM peptides of recombinant α-actin.



Nt-acetylated peptide (amino acids 2-18) of endogenous γ-actin
purified from untransfected Expi293F cells

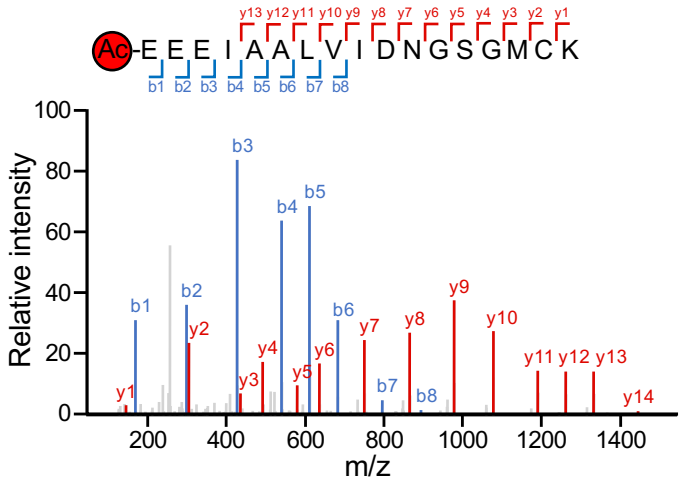


Fig. S5. Sequence alignment of cytoplasmic β- and γ-actin (top). A dashed green box highlights the Nt-acetylated N-terminal peptide (amino acids 2-18) of endogenous γ-actin whose MS2 spectrum is shown at the bottom. Note that endogenous β- and γ-actin co-purify from untransfected Expi293F cells.

Table S1. Published actin expression methods and their limitations.

Expression system	Reference	Limitations					
		Folding concerns or insolubility	Missing demonstration of native PTMs	Missing demonstration of endogenous actin removal	Issues with tag removal	Missing demonstration of native-like polymerization	Low yields
E. coli	(1)	x	x			x	x
	(2)	x	x			x	x
	(3)		x		x	x	x
S. cerevisiae	(4, 5)		x	Unreliable		x	x
	(6, 7)		x			x	x
P. pastoris	(8, 9)			x	x		x
Insect cells	(10)		x	x		x	
	(11)		x	x		x	
	(12)		x	x	x	x	x
	(13)	x	x	x		x	x
	(14)		x	x	x	x	x
	(15)		x	x			
	(16)		x	x	x	x	x
	(17)		x	x	x	x	
D. discoideum	(18)		Missing evidence	Missing evidence	x		
Synthetic	(19)		x			x	x
	(20)		x			x	x
Expi293F	(21)				x		x

Table S2. Primers used in this study

Primer name	Primer sequence	Purpose
ACTA1_HisFLAGtev_for1	acgatgacaagactagtgagaacctgtatttccaggacgaagacgagaccaccgccctc	Cloning His-FLAG-TEV α-actin
ACTA1_HisFLAGtev_for2	atcaccacagtggagattacaaggatgacgatgacaagactagtgagaacctgtatttc	Cloning His-FLAG-TEV α-actin
ACTA1_HisFLAGtev_for3_Sal1	ttcgtcgacgccaccatggcgcatcaccatcatcaccacagtggagattacaaggatgac	Cloning His-FLAG-TEV α-actin
ACTA1_stop_rev_BamH1	ttcggatccctagaagcatttgcggtggacgatg	Cloning His-FLAG-TEV α-actin
ACTB_TEV_Spe1_for	ttcactagtgagaacctgtatttccaggacgatgatattgctgctctggttgtc	Cloning His-FLAG-TEV β-actin
ACTB_stop_BamH1_rev	ttcggatccttagaagcacttgcggtgcacaatg	Cloning His-FLAG-TEV β-actin
ACTG2_for_Spe1TEV	ttcactagtgagaacctgtatttccaggaagaggagaccaccgcgc	Cloning His-FLAG-TEV γ-actin
ACTG2_stop_Bcl1_rev	ttctgatcactagaagcacttcctgtggacaatgg	Cloning His-FLAG-TEV γ-actin
hNat78s_Nde1_for	cttcatatgagcctggctgagttgaccc	Cloning His-Naa80Δ
hNat308_Xho1_rev	cttctcgagtcagatgtctttttccatccagaatatggg	Cloning His-Naa80Δ
Intein_His_Nde1_rev	cttcatatggtgatgatggtgatgaccagcattctgtacaacaacctgg	Cloning His-Naa80Δ
Intein_Nhe1_for	cttgctagcacaaatcctggtgtatccg	Cloning His-Naa80Δ
MBPG4G6_Pst1_for	ttcctgcagatgaaaatcgaagaaggtaaactggtaatctgg	Cloning His-MBP-G4G6
MBPG4G6_Hind3_rev	ttcaagctttcaggcagccagctcagc	Cloning His-MBP-G4G6
G4s_Xho1	ttcctcgaggccgcccagcacgg	Cloning His-MBP-G4G6
G4G6_Sal1_rev	ttcgtcgactcaggcagccagctcagc	Cloning His-MBP-G4G6

Table S3. Antibodies used in this study

Antibody	Dilution	Description	Source	Cat #
Primary antibodies				
Pan-actin	1:5000	Rabbit monoclonal recombinant anti-actin Ig	Abcam	ab179467
α-Actin	1:1000	Mouse monoclonal anti-alpha skeletal muscle actin Ig	Abcam	ab28052
β-Actin	1:5000	Mouse monoclonal anti-human actin beta Ig	Bio-Rad	MCA5775GA
Ac-α-actin	1:2000	Biotinylated monoclonal anti-alpha skeletal actin Ig	Novus Biologicals	NBP1-97723B
Ac-β-actin	1:5000	Mouse monoclonal anti-beta actin Ig	Abcam	ab6276
Secondary antibodies				
Anti-rabbit IgG-HRP	1:10000	Anti-rabbit HRP-linked Ig	Cell Signaling Technology	7074S
Anti-mouse IgG-HRP	1:10000	Anti-mouse HRP-linked Ig	Cell Signaling Technology	7076S
Streptavidin-HRP	1:5000	HRP-conjugated streptavidin	ThermoFisher Scientific	N100

Other supplementary materials for this manuscript not included in this file:

Dataset S1 (separate Microsoft Excel file). Complete list of MS peptides observed for recombinant β-actin and endogenous β/γ-actin. See below for a description of the dataset.

Dataset S2 (separate Microsoft Excel file). Complete list of MS peptides observed for tissue-purified and recombinant α-actin. See below for a description of the dataset.

For both **Dataset S1** and **S2**:
Rows correspond to actin peptides observed by MS, including the specific actin sample, digestion enzyme, and MS2 scan number. The full sequence displays the base amino acid sequence and the location of all modifications, while the essential sequence omits common fixed and variable modifications that occur as a result of sample preparation for MS analysis. The score, Q value, PEP, and PEP Q value are measures of confidence in the peptide assignment. PSM (peptide spectral match) count reports the number of times a given peptide was observed. More information about peptide assignment statistics can be found at the MetaMorpheus website (https://github.com/smith-chem-wisc/MetaMorpheus). Note that for β-actin (Dataset S1) the residue numbers in column X must be increased by one to match the conventional actin numbering. Sheets two and three of each dataset report the PTM occupancy of endogenous and recombinant actin, sorted by amino acid number. For each PTM, the occupancy is defined as the number of peptides (PSM count) containing the PTM divided by the total number of peptides that cover the residue site. The remaining sheets compile all the peptides covering PTM-containing residues.

SI Appendix references:

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