Supplementary Materials:

Partial Immunoblotting of 2D-Gels: A Novel Method to Identify Post-Translationally Modified Proteins Exemplified for the Myelin Acetylome

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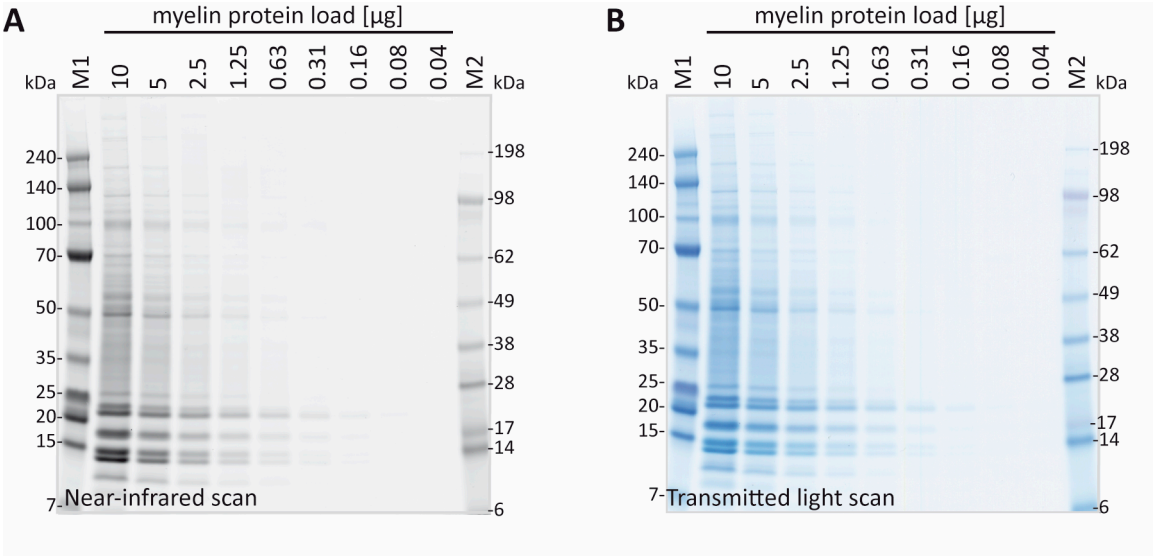

Figure S1. Detection sensitivity of different scanning methods. Serial dilutions of a myelin sample were separated in a 1D gel and stained with colloidal Coomassie (CCB). The gel was imaged by near-infrared detection (**A**) and by visible transmitted light scanning (**B**). Near-infrared detection was found to have a higher dynamic range and an approximately two-fold higher sensitivity.

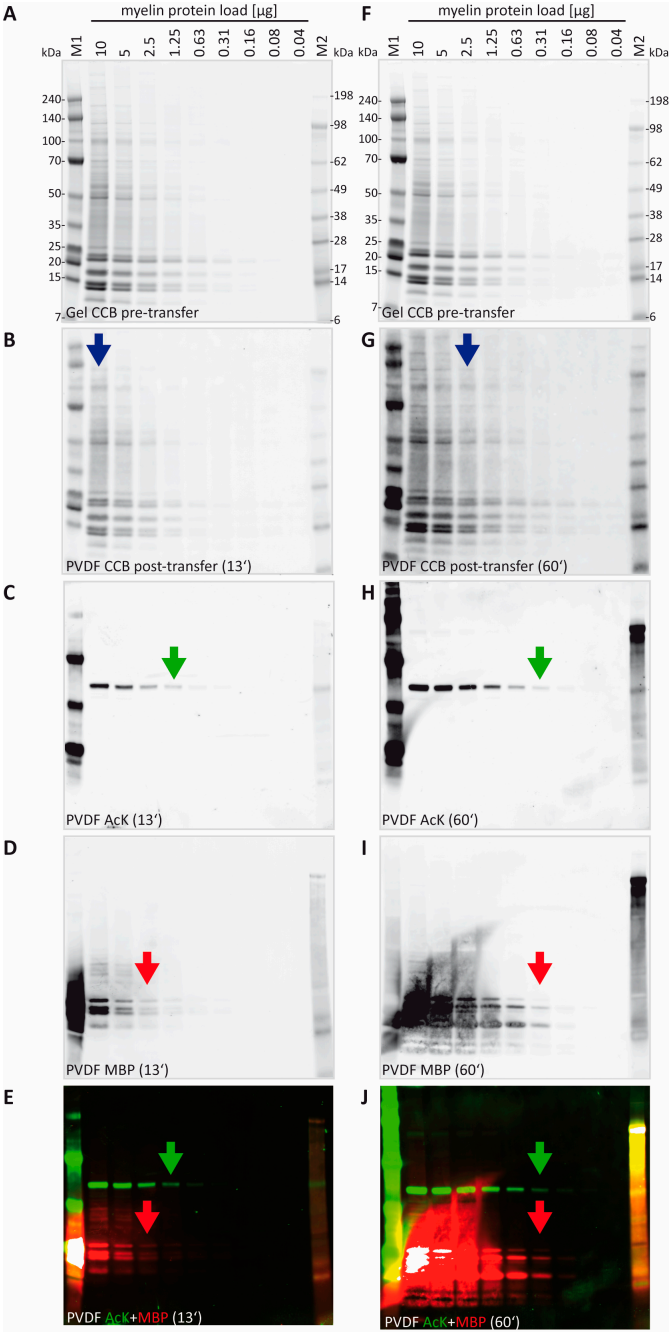

Figure S2. Estimation of transfer efficiency. (**A**,**F**) Serial dilutions of a myelin sample were separated in two parallel 1D gels and stained with colloidal Coomassie (CCB).(**B,G**) PVDF membrane with CCB stained proteins after partial transfer for 13 min **(B)** or standard transfer for 60 min **(G)**. Lanes indicated by blue arrows (10 µg protein in (**B**) and 2.5 µg protein in (**G**)) appeared similar in CCB staining intensity. (**C,H**) Acetylated proteins detected using an antibody specific for AcK (Immunechem). Lanes indicated by green arrows appeared similar in AcK immunofluorescence intensity (1.25 µg in (**C**) and 0.31 µg in (**H**)). Note that image acquisition and processing parameters were chosen for optimal display of the most prominent tubulin band, leaving other less intense signals (e.g. for acetylated MBP) undetected. (**D,I**) Myelin basic protein (MBP) detected using an antibody specific for MBP (BioLegend). Lanes indicated by red arrows appeared similar in MBP immunofluorescence intensity (2.5 µg in **D** and 0.31 µg in **I**). (**E,J)** False-colored overlay images of AcK (false colored in green) and MBP (false colored in red) immunodetections as shown separately in (**C,D**) or (**H,I**), respectively. White color indicates signal saturation in the respective lane. Protein standard used were M1: Dual color Protein Standard III (Serva) and M2: SeeBlue-Plus-2 (Thermo Fischer Scientific, Waltham, MA USA).

A

No.	Peptide	Sequence	[M+H]⁺ obs.	[M+H]⁺ calc.	ΔM (ppm)	Score	b-y	RMS (ppm)
1	39-68	DKTIGGGDDSFNTFFSETGAGKHVPRAVFV	3198.5425	3198.5490	-2,04	6.50	17	6.97
2	39-45	DKTIGGG	689.3450	689.3465	-2.16	6.12	8	10.30
3	33-45	DGQmPSDKTIGGG	1320.5703	1320.5736	-2.56	6.03	4	8.49

B

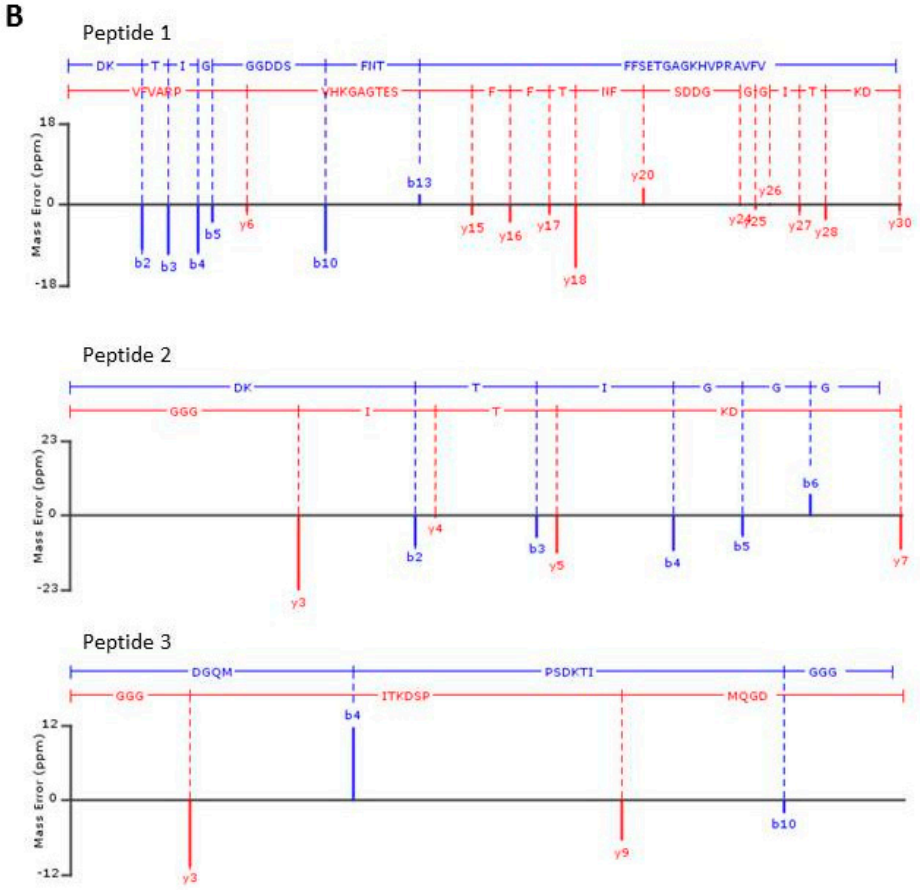

Figure S3. Confirmation of Lys-40 as acetylation site in α-tubulin by LC-MS/MS. (**A)** Table with identification details for the detected AcK^{40}-containing α-tubulin peptides. Columns show from left to right: number of peptide, numbering of amino acids according to the sequence of α-tubulin (UniProtKB accession P68369); peptide sequence (m, oxidized Met); observed and calculated mass of the singly protonated peptide; peptide mass deviation in ppm; PLGS score; number of b–y fragment ions; root mean square (RMS) fragment mass deviation in ppm. (**B**) Fragment ion mass spectra reconstructions on the basis of ion mobility-powered data-independent acquisition data ($HDMS^E$). Only b- and y-ions are labeled for the sake of clarity, and fragment ion mass deviation is shown. For peptide 1 also sequenced by MALDI-MS, acetylation was clearly assigned to K_{40} on the basis of the conclusive N-terminal ion series down to b2.