Structural mapping techniques distinguish the surfaces of fibrillar 1N3R and 1N4R human tau

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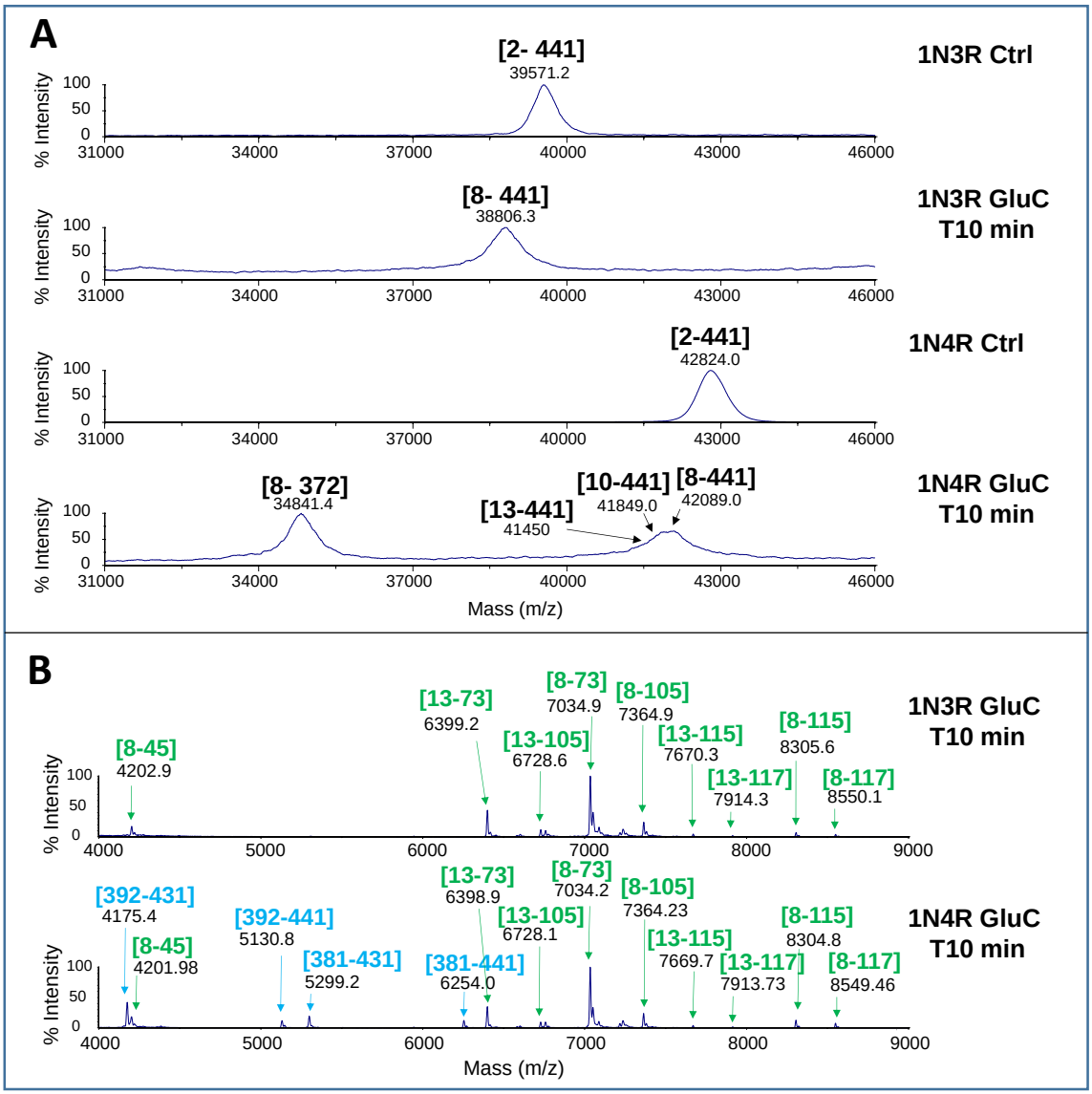

Figure S1: MALDI-MS spectra of proteolytic peptides obtained after 10 min GluC digestion of 1N3R and 1N4R htau fibrils. Proteolytic products of high mass (**A**) and in the mass range of 4000 to 9000 Da (**B**) were detected in the positive linear mode using sinapinic acid as matrix. The experimental average MH^+ masses and the corresponding amino acid sequences of GluC proteolytic peptides are indicated in bold (2N4R numbering is used). Htau N-terminal GluC fragments are in green. Htau C-terminal GluC fragments are labeled in cyan.

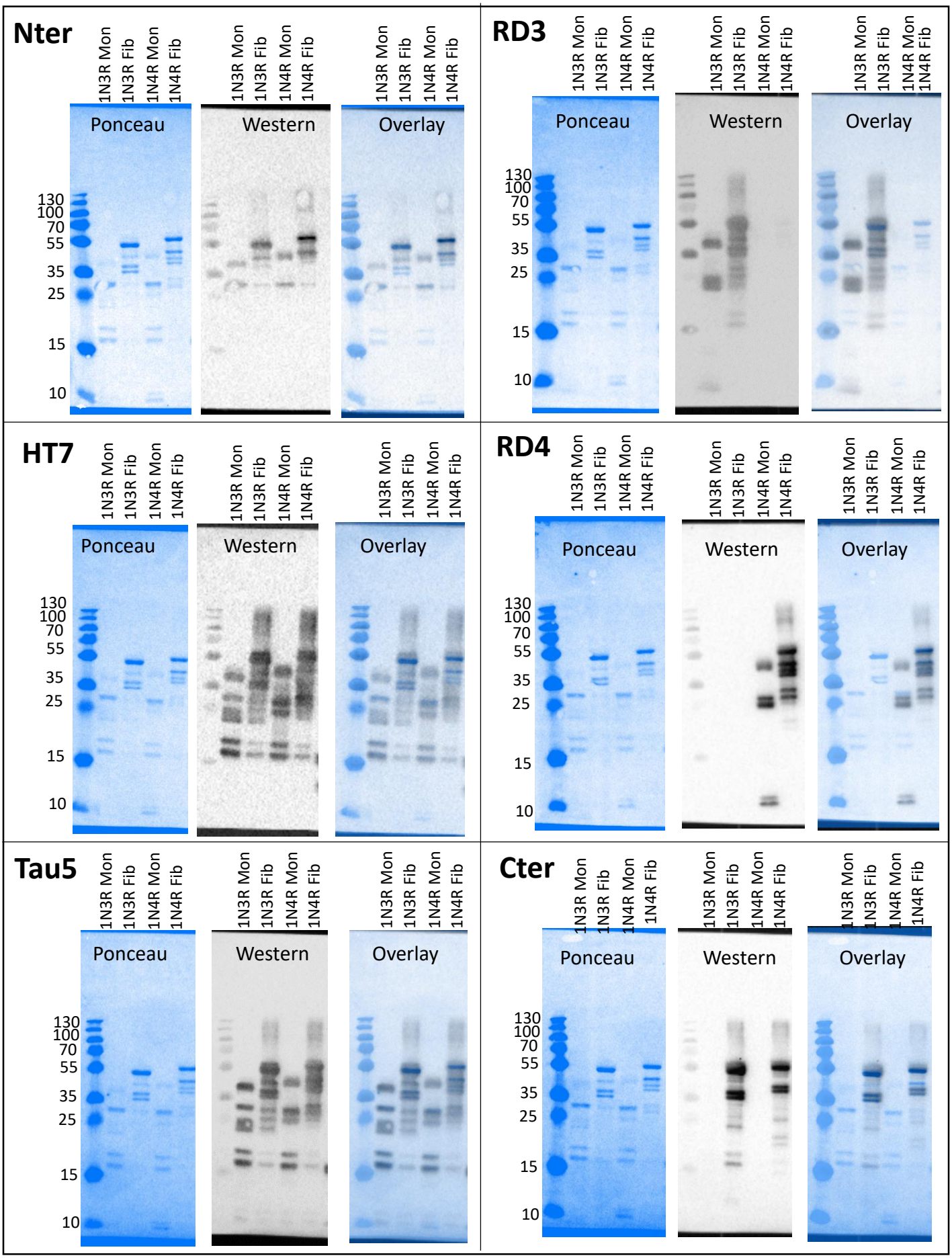

Figure S2: SDS-PAGE gels and western-blots corresponding to 45 min GluC digestion of 1N3R and 1N4R monomers (Mon) and fibrils (Fib) in support of data presented in Figure 1 i, 1 j). To be able to averlay the polypeptides bands vizualized by protein staining and western blotting, the nitrocellulose membranes were stained with Ponceau red (colored artificially in blue using the Chemidoc MP Imaging System, BioRad, to gain contrast) before immunodetection with the indicated antibodies. For each antibody and gel the overlay is presented .

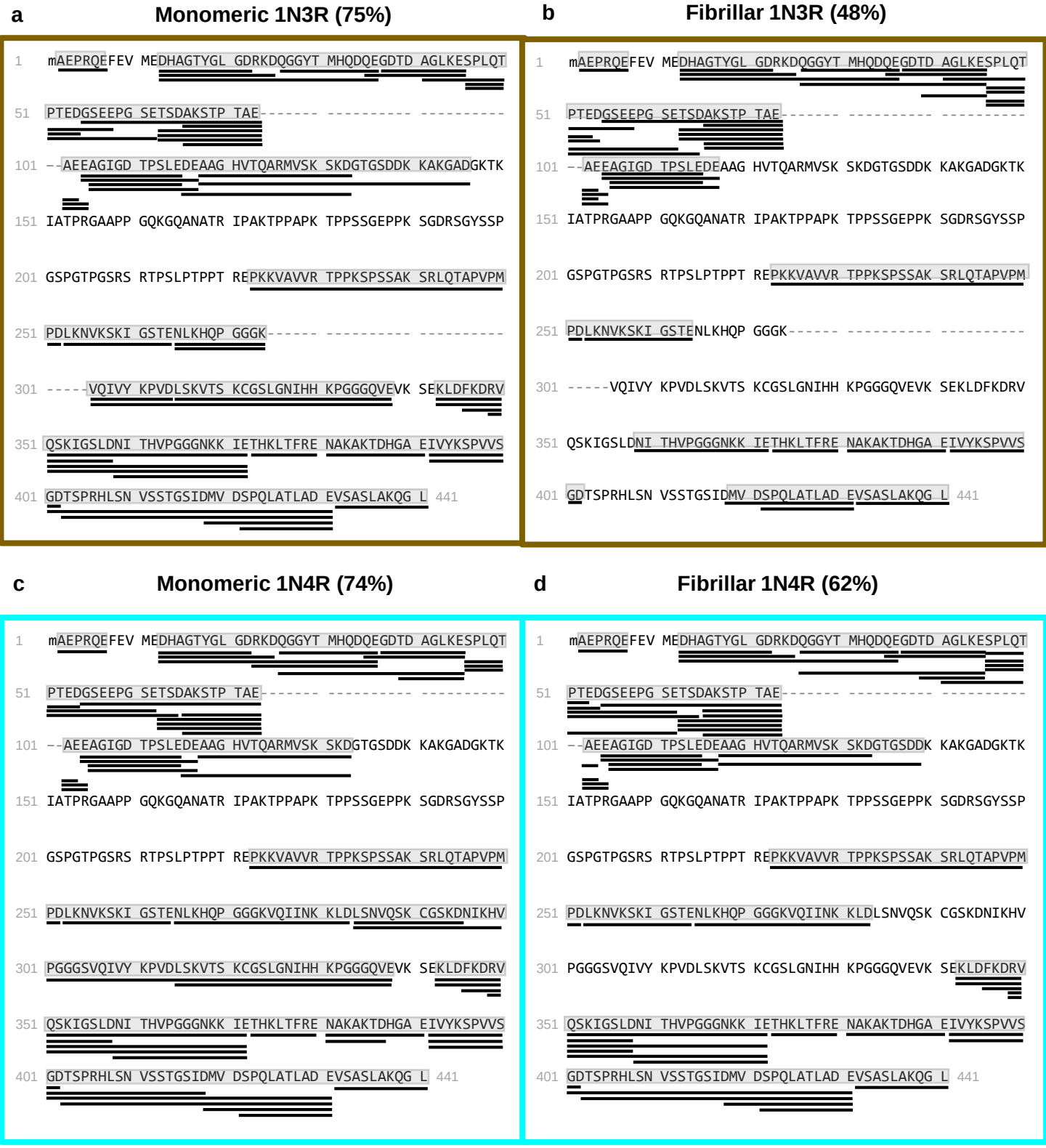

Figure S3. Sequence coverage of htau 1N3R (a,b) and 1N4R (c,d) monomers (a,c) and fibrils (c,d) obtained after GluC digestion in-solution. Sequence coverage (in grey) was obtained using MASCOT MS/MS ions search with the parameters : Glu-C as enzyme; Oxidation (M) as variable modification; peptide mass tolerance of 40 ppm; fragment mass tolerance of 0.05 Da ; 5 missed cleavages. Only peptides identified automatically with a MASCOT score of at least 20 and/or checked manually, in at least two out of three replicas were considered.

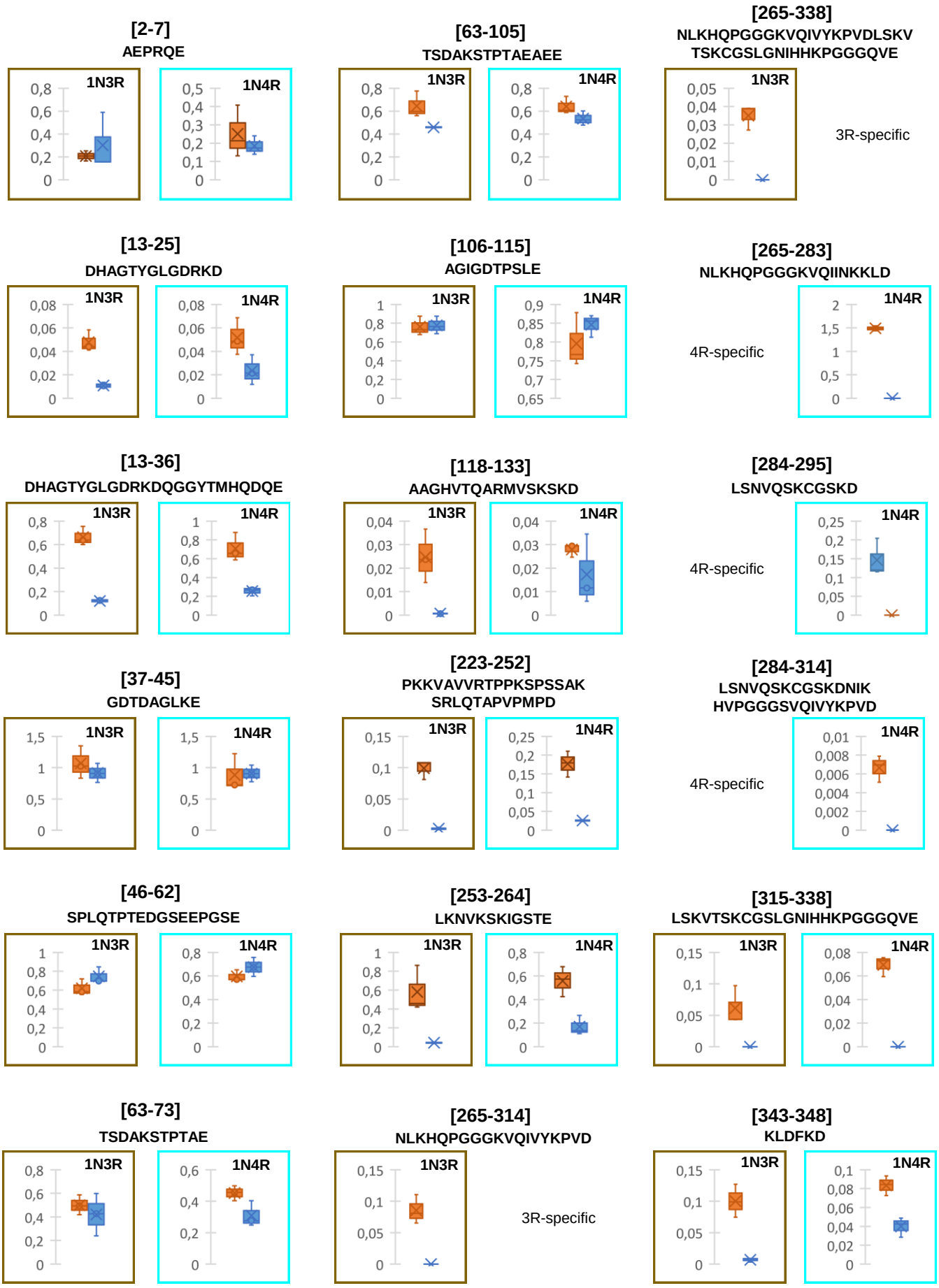

Figure S4a Changes in Enzyme Accessibility upon htau 1N3R and 1N4R assembly into fibrils. GluC was used for digestion in solution. The boxplots represent the normalized intensities of each GluC peptide from amino acid residues 2 to 348 for both htau 1N3R (brown) and htau 1N4R (cyan) monomers (orange) and fibrils (blue) for n=3 experiments. The average is represented by an X and the median by the bar.

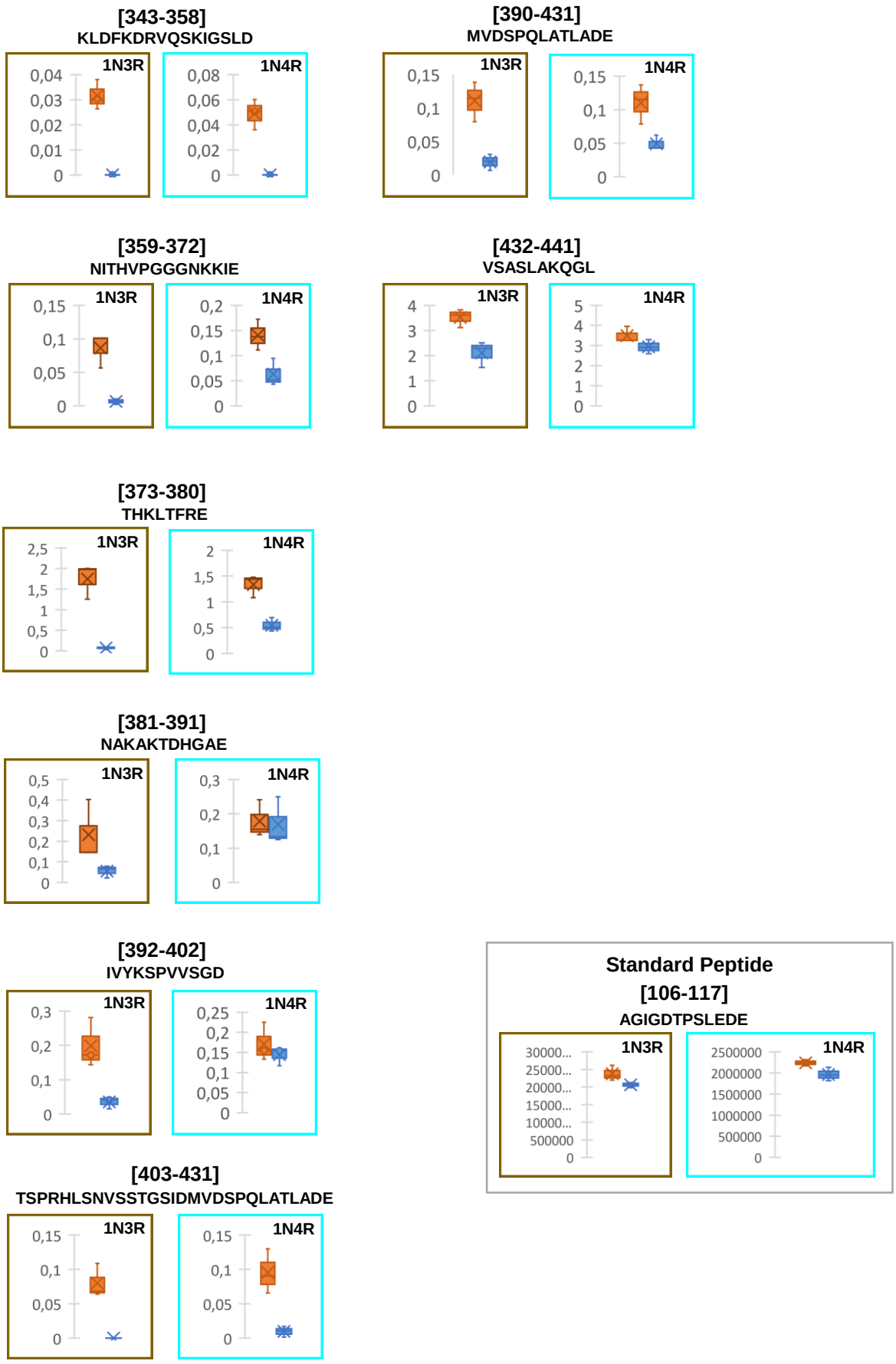

Figure S4b Changes in Enzyme Accessibility upon tau 1N3R and 1N4R assembly into fibrils. GluC was used for digestion in solution. The boxplots represent the normalized intensities of each GluC peptide from amino acid residues 359 to 441 for both htau 1N3R (brown) and htau 1N4R (cyan) monomers (orange) and fibrils (blue) for n=3 experiments. The intensity of the standard peptide used for normalization is represented. The average is represented by an X and the median by the bar.

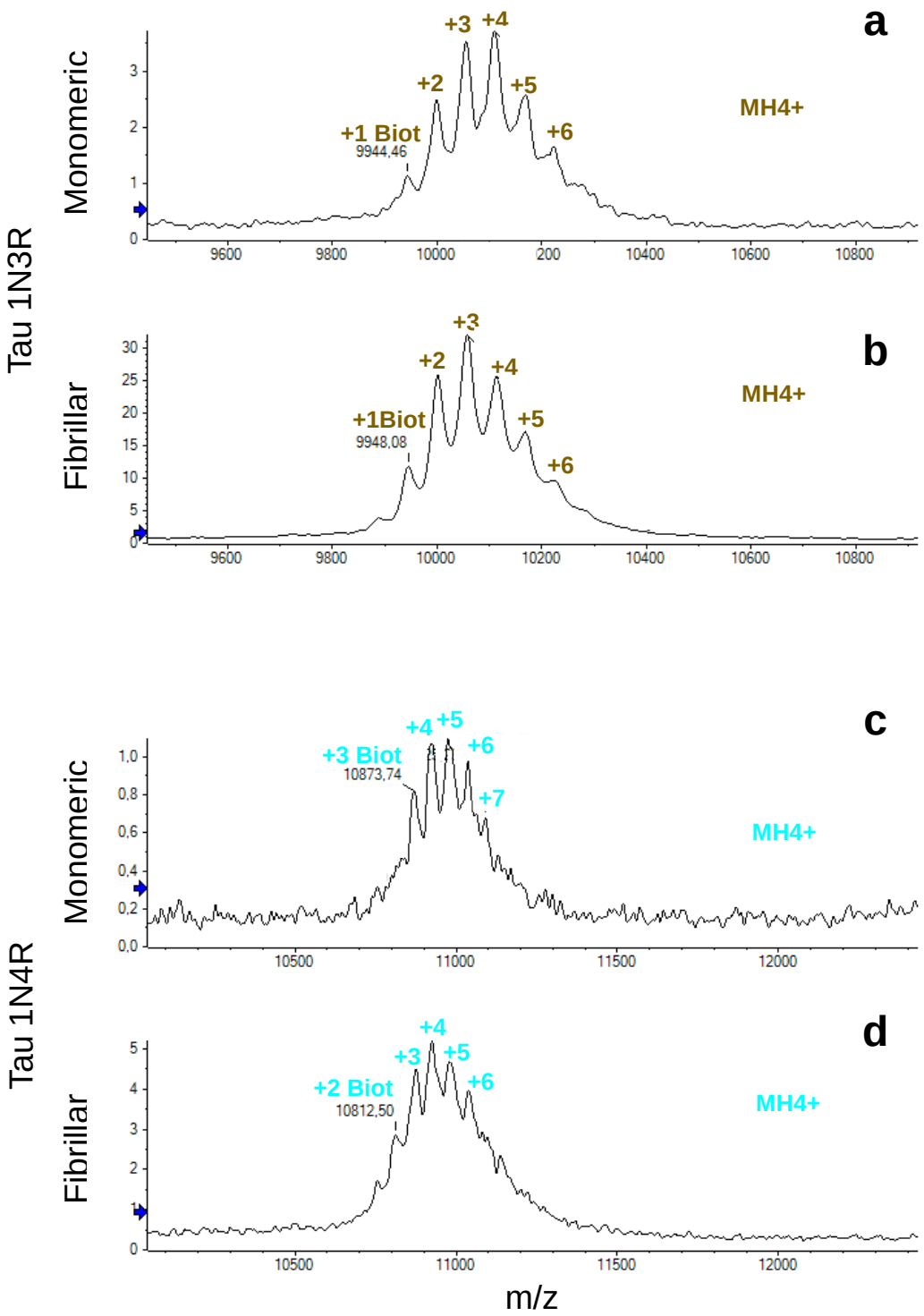

Figure S5. Biotinylation Efficiency of htau 1N3R and 1N4R in monomeric and fibrillar forms.
MALDI-MS spectra from the top to bottom of the tetra-charged ions of biotinylated 1N3R htau monomers (a) and fibrils (b), and 1N4R htau monomers (c) and fibrils (d). The m/z value of one tetra-charged biotinylated ion is indicated.

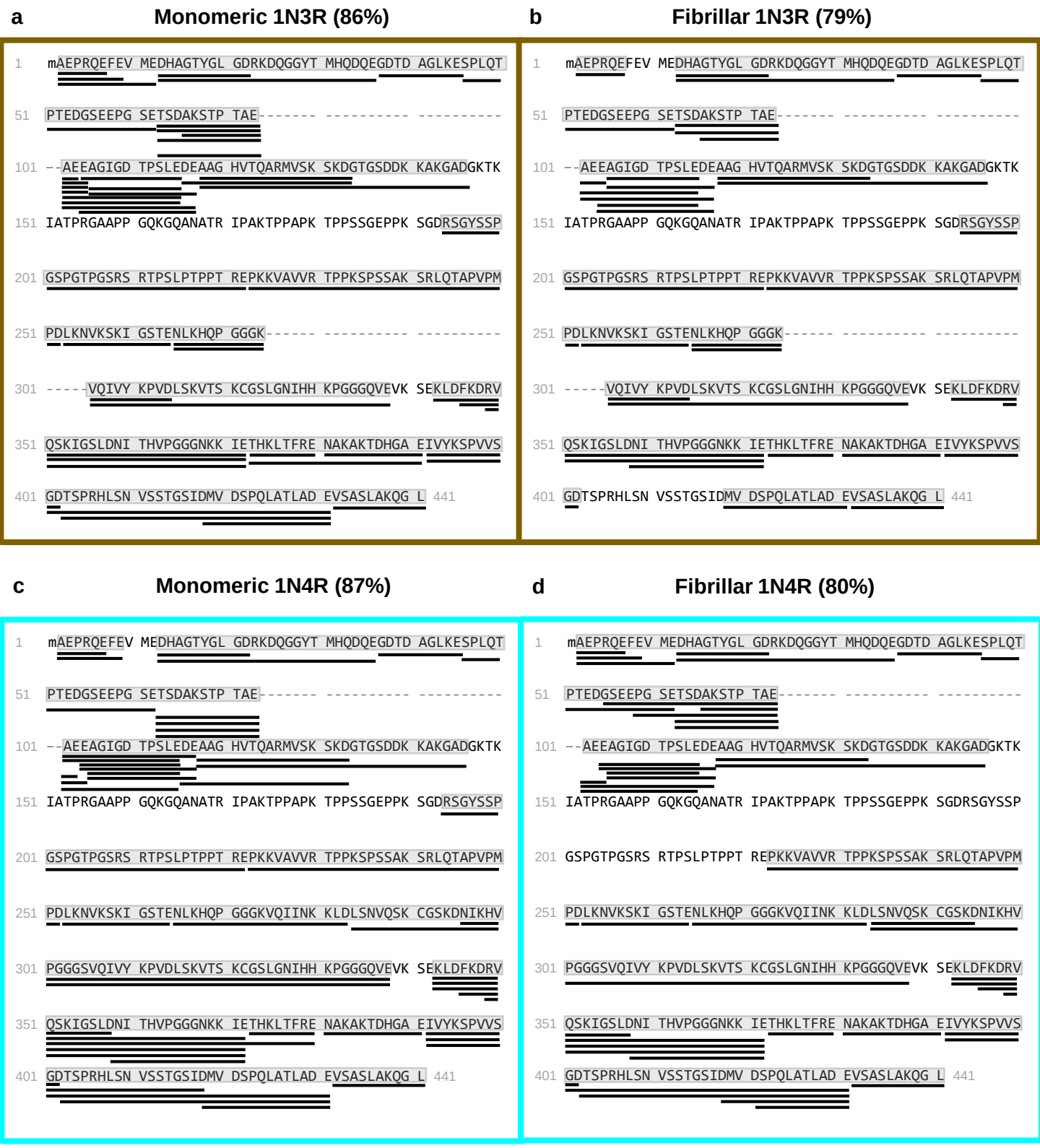

Figure S6. Sequence coverage of htau 1N3R (a,b) and 1N4R (c,d) monomers (a,c) and fibrils (c,d) obtained after GluC in-gel digestion. Sequence coverage (in grey) was otained using MASCOT MS/MS ions search with the parameters : Glu-C as enzyme; Carbamidomethylation (C) as fixed modification; Oxidation (M) as variable modification; peptide mass tolerance of 40 ppm; fragment mass tolerance of 0.05 Da ; 5 missed cleavages. Only peptides identified automatically with a MASCOT score of at least 20 and/or checked manually, in at least two out of three replicas were considered.

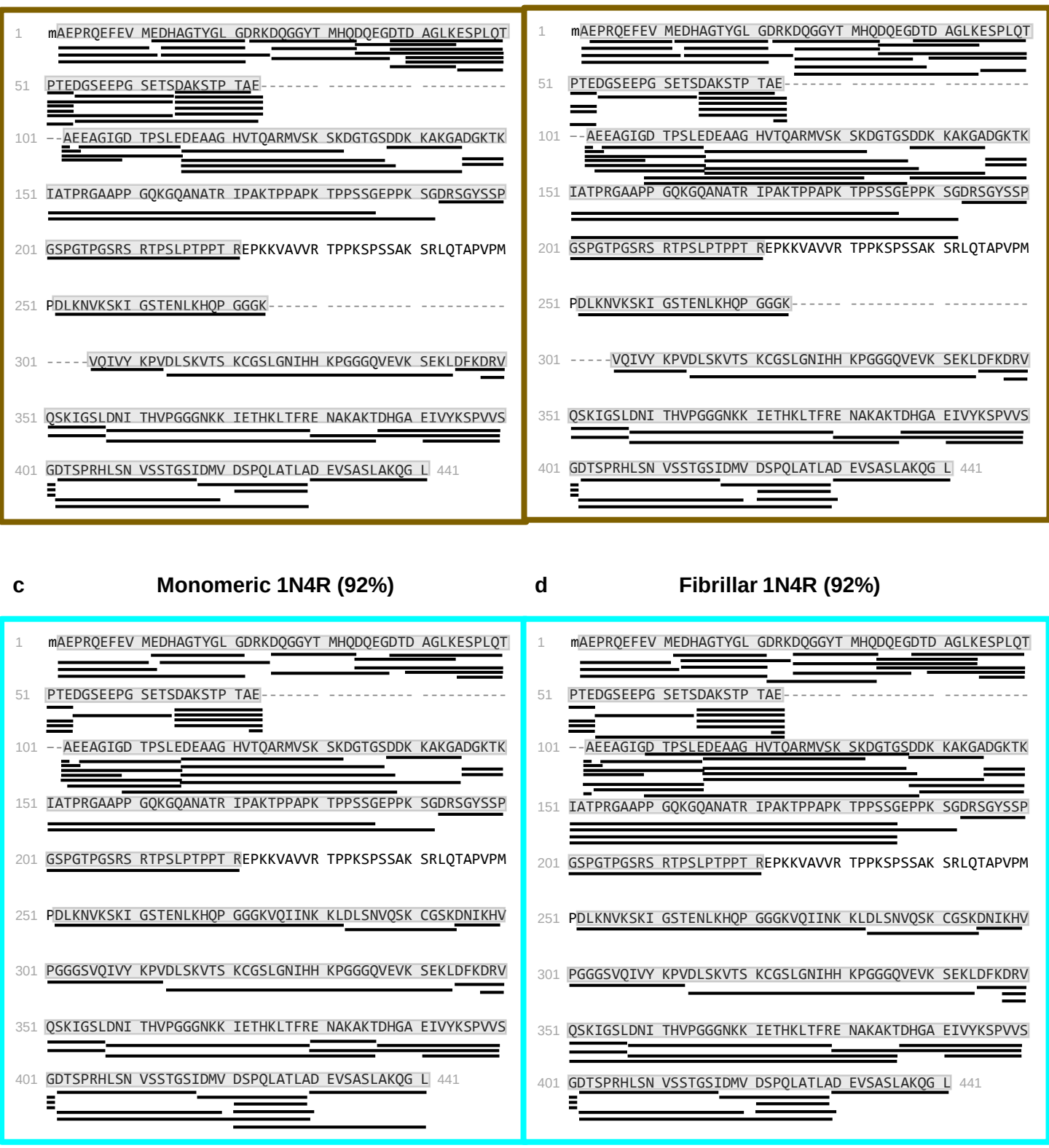

Figure S7. Sequence coverage of control htau 1N3R (a,b) and 1N4R (c,d) monomers (a,c) and fibrils (c,d) obtained after AspN in-gel digestion. Sequence coverage (in grey) was obtained using MASCOT MS/MS ions search with the parameters : AspN and semi-AspN as enzyme; Carbamidomethylation (C) as fixed modification; Oxidation (M) as variable modification; peptide mass tolerance of 40 ppm; fragment mass tolerance of 0.05 Da ; 5 missed cleavages. Only peptides identified automatically with a MASCOT score of at least 20 and/or checked manually, in at least two out of three replicates were considered.

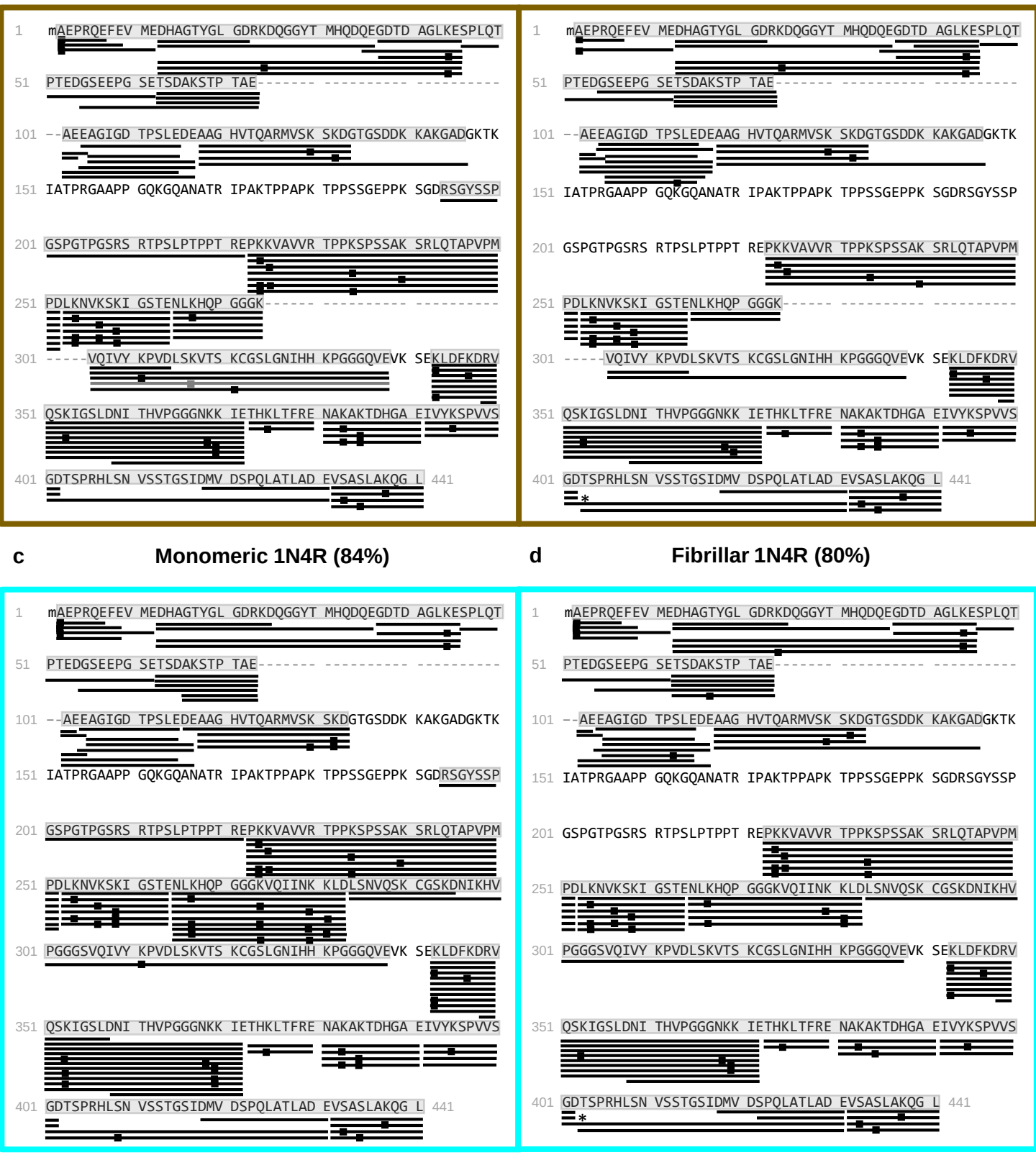

Figure S8. Sequence coverage of biotinylated htau 1N3R (a,b) and 1N4R (c,d) monomers (a,c) and fibrils (c,d) obtained after in-gel GluC digestion. Sequence coverage (in grey) was obtained using MASCOT MS/MS ions search with the parameters : Glu-C as enzyme; Carbamidomethylation (C) as fixed modification; biotin (C,S,T,Y, K, and protein N-ter), Oxidation (M) as variable modification; peptide mass tolerance of 40 ppm; fragment mass tolerance of 0.05 Da ; 5 missed cleavages. Only peptides identified automatically with a MASCOT score of at least 20 and/or checked manually, in at least two out of three replicates were considered.

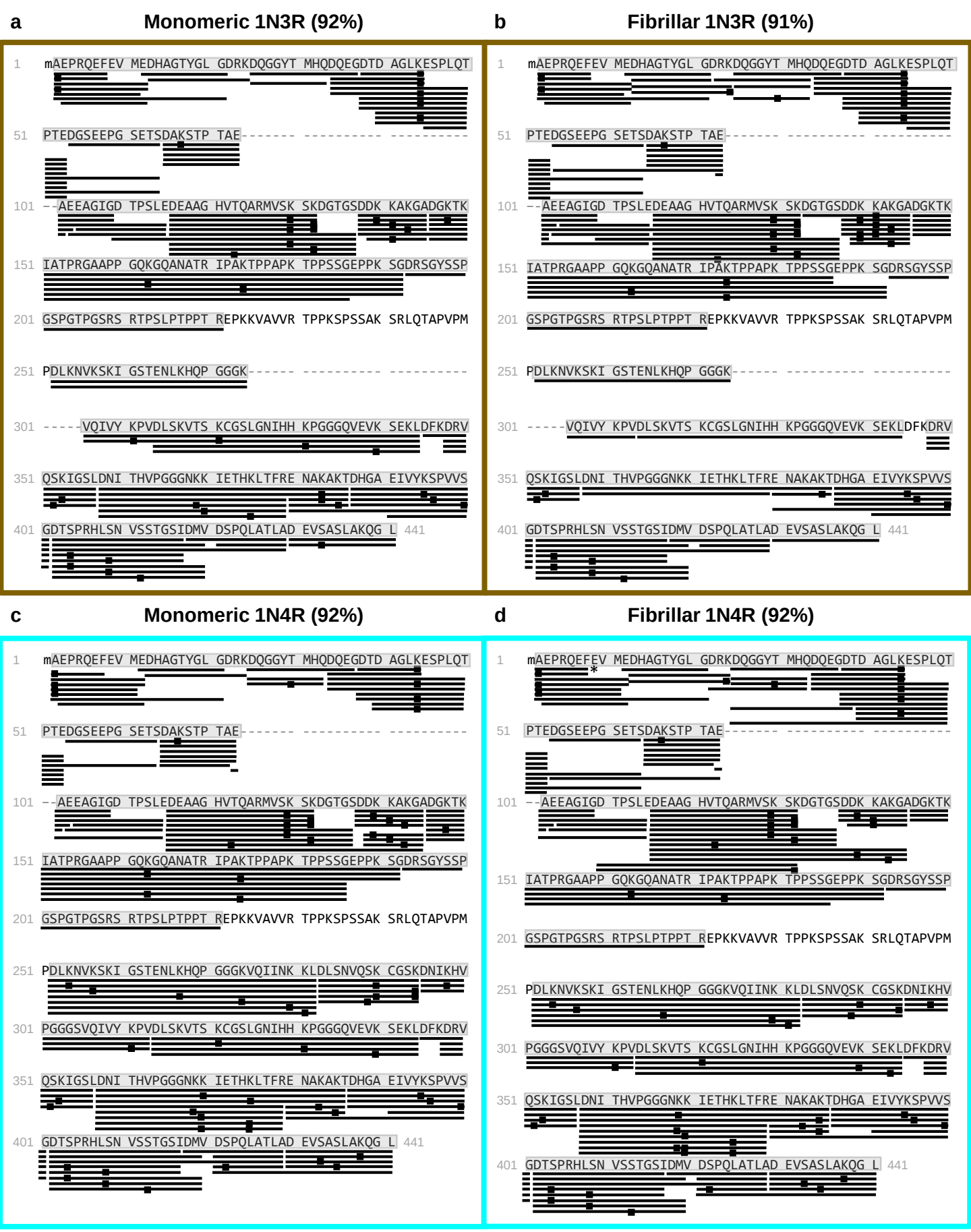

Figure S9. Sequence coverage of biotinylated htau 1N3R (a,b) and 1N4R (c,d) monomers (a,c) and fibrils (c,d) obtained after in-gel AspN digestion. Sequence coverage (in grey) was obtained using MASCOT MS/MS ions search with the parameters : AsN and semi-AspN as enzyme; Carbamidomethylation (C) as fixed modification; biotin (C,S,T,Y, K, and protein N-ter), Oxidation (M) as variable modification; peptide mass tolerance of 40 ppm; fragment mass tolerance of 0.05 Da ; 5 missed cleavages. Only peptides identified automatically with a MASCOT score of at least 20 and/or checked manually, in at least two out of three replicates were considered.

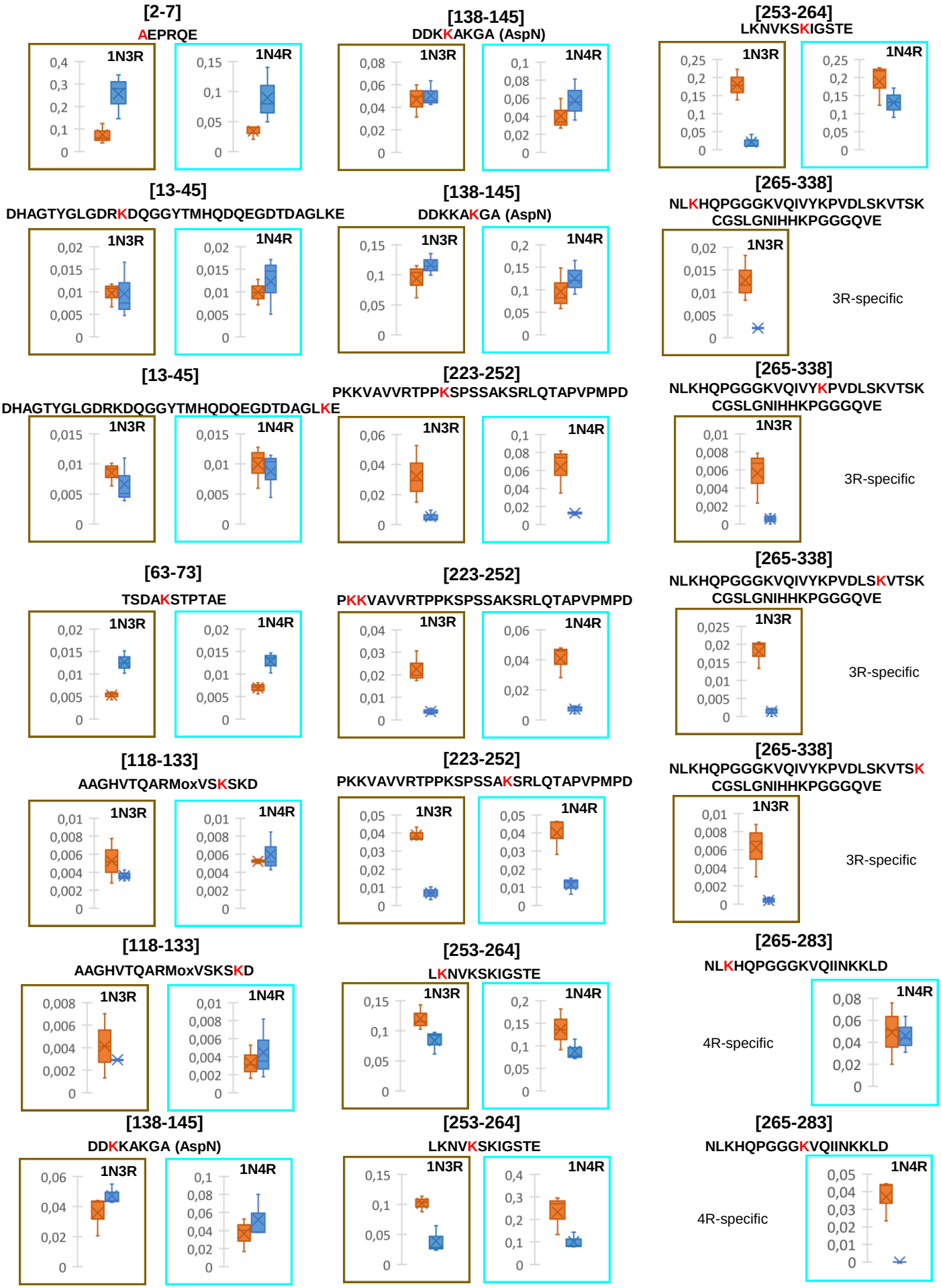

Figure S10a Changes in surface labelling upon htau 1N3R and 1N4R assembly into fibrils. GluC was used for in-gel digestion, or AspN when indicated. The boxplots represent the normalized intensities of each peptide from amino acid 2 to 338 for both 1N3R (brown) and 1N4R (cyan) htau monomers (orange) and fibrils (blue) for n=3 experiments. The intensity of the standard peptide used fore normalization is represented. The average is represented by an X and the median by the bar.

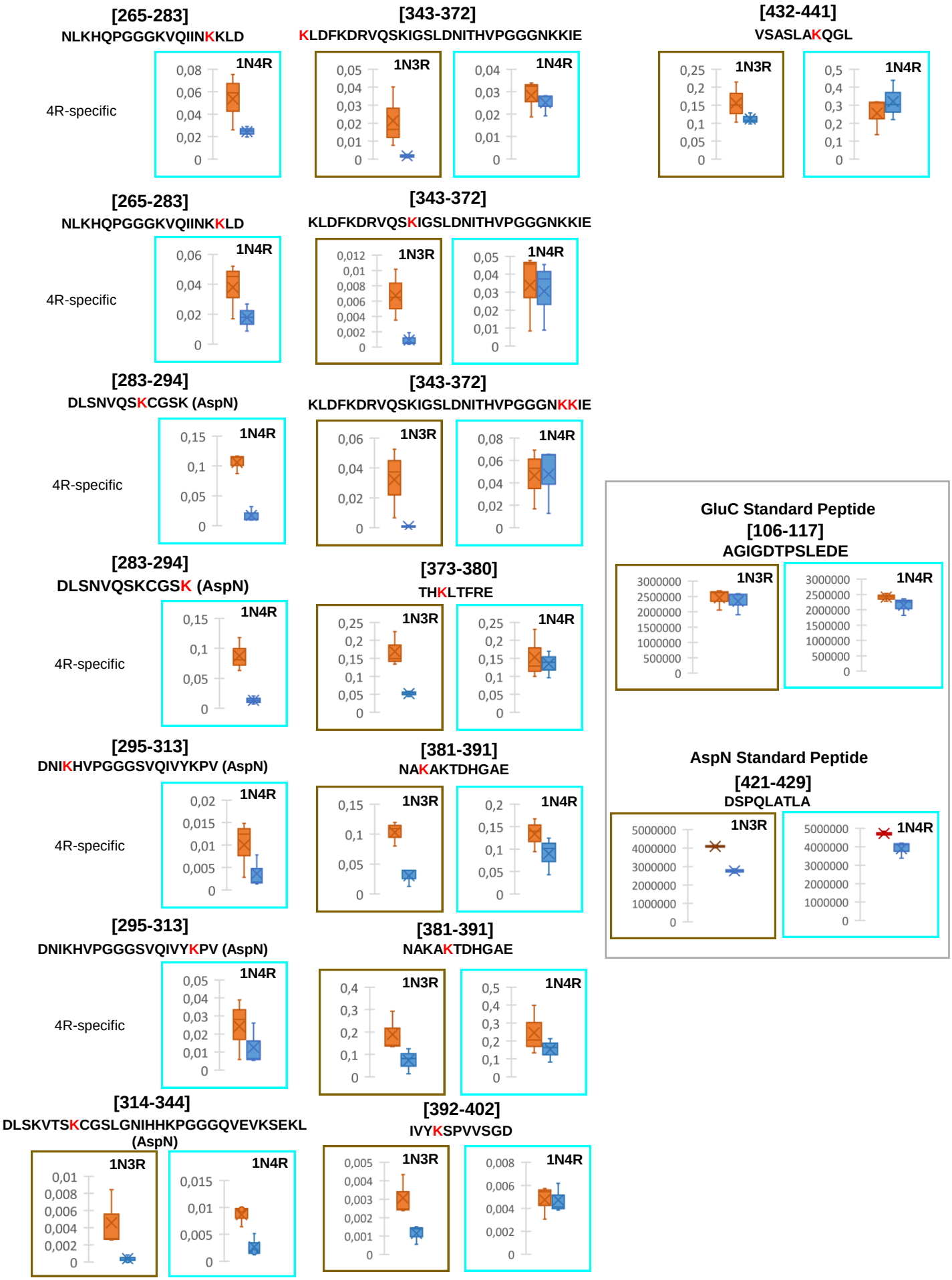

Figure S10b Changes in surface labelling upon htau 1N3R and 1N4R assembly into fibrils. GluC was used for in-gel digestion, or AspN when indicated. The boxplots represent the normalized intensities of each peptide from amino acid 265 to 441 for both 1N3R (brown) and 1N4R (cyan) htau monomers (orange) and fibrils (blue) for n=3 experiments. The intensity of the standard peptide used fore normalization is represented. The average is represented by an X and the median by the bar.

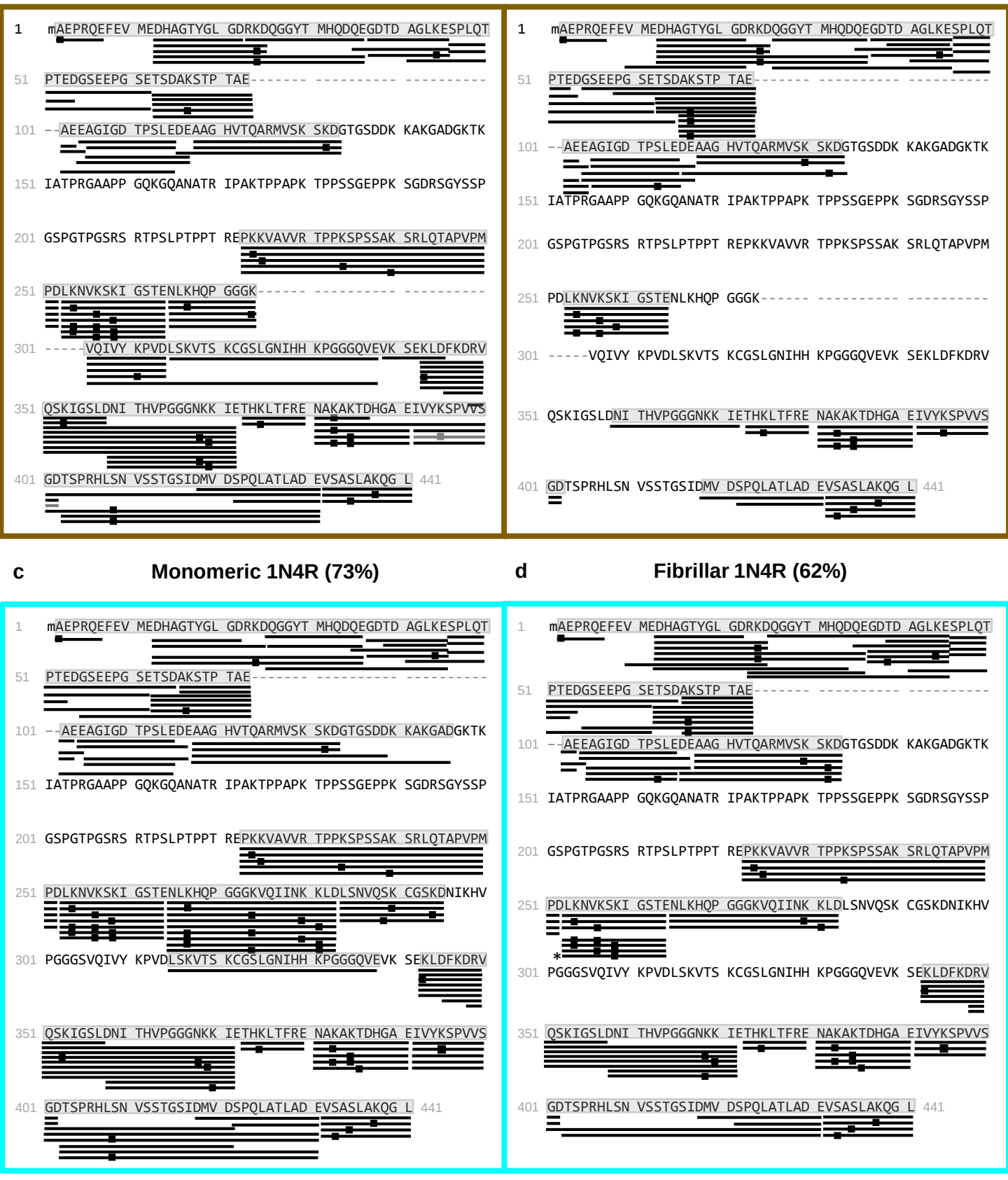

Figure S11. Sequence coverage of biotinylated htau 1N3R (a,b) and 1N4R (c,d) monomers (a,c) and fibrils (c,d) obtained by GluC digestion in solution . Sequence coverage (in grey) was obtained using MASCOT MS/MS ions search with the parameters : Glu-C as enzyme; Carbamidomethylation (C) as fixed modification; biotin (C,S,T,Y, K, and protein N-ter), Oxidation (M) as variable modification; peptide mass tolerance of 40 ppm; fragment mass tolerance of 0.05 Da ; 5 missed cleavages. Only peptides identified automatically with a MASCOT score of at least 20 and/or checked manually, in at least two out of three replicas were considered.

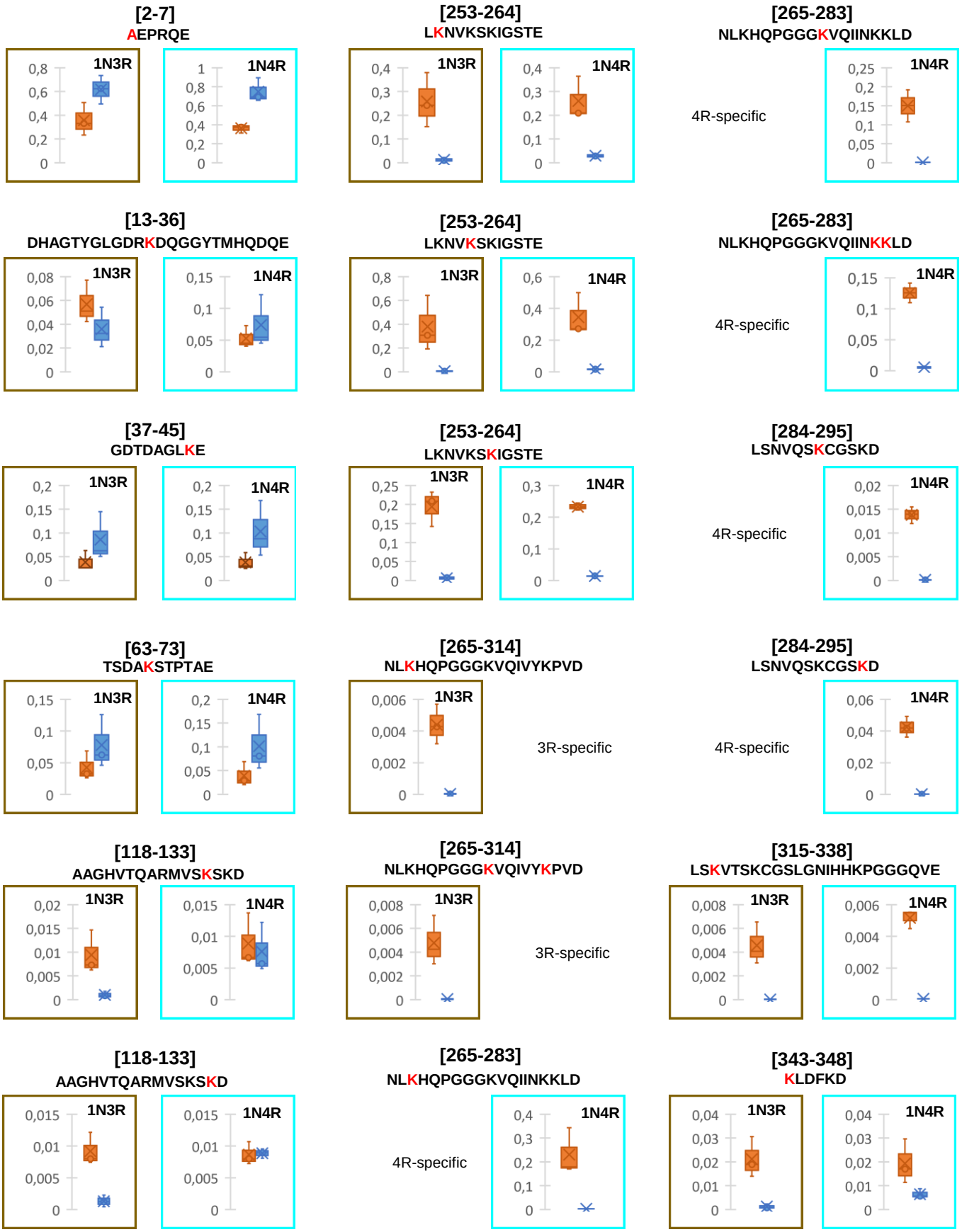

Figure S12a Changes in enzyme accessibility and surface labelling upon htau 1N3R and 1N4R assembly into fibrils. GluC was used for in-solution digestion. The boxplots represent the normalized intensities of each peptide from amino acid 2 to 348 for both 1N3R (brown) and 1N4R (cyan) htau monomers (orange) and fibrils (blue) for n=3 experiments. The intensity of the standard peptide used fore normalization is represented. The average is represented by an X and the median by the bar.

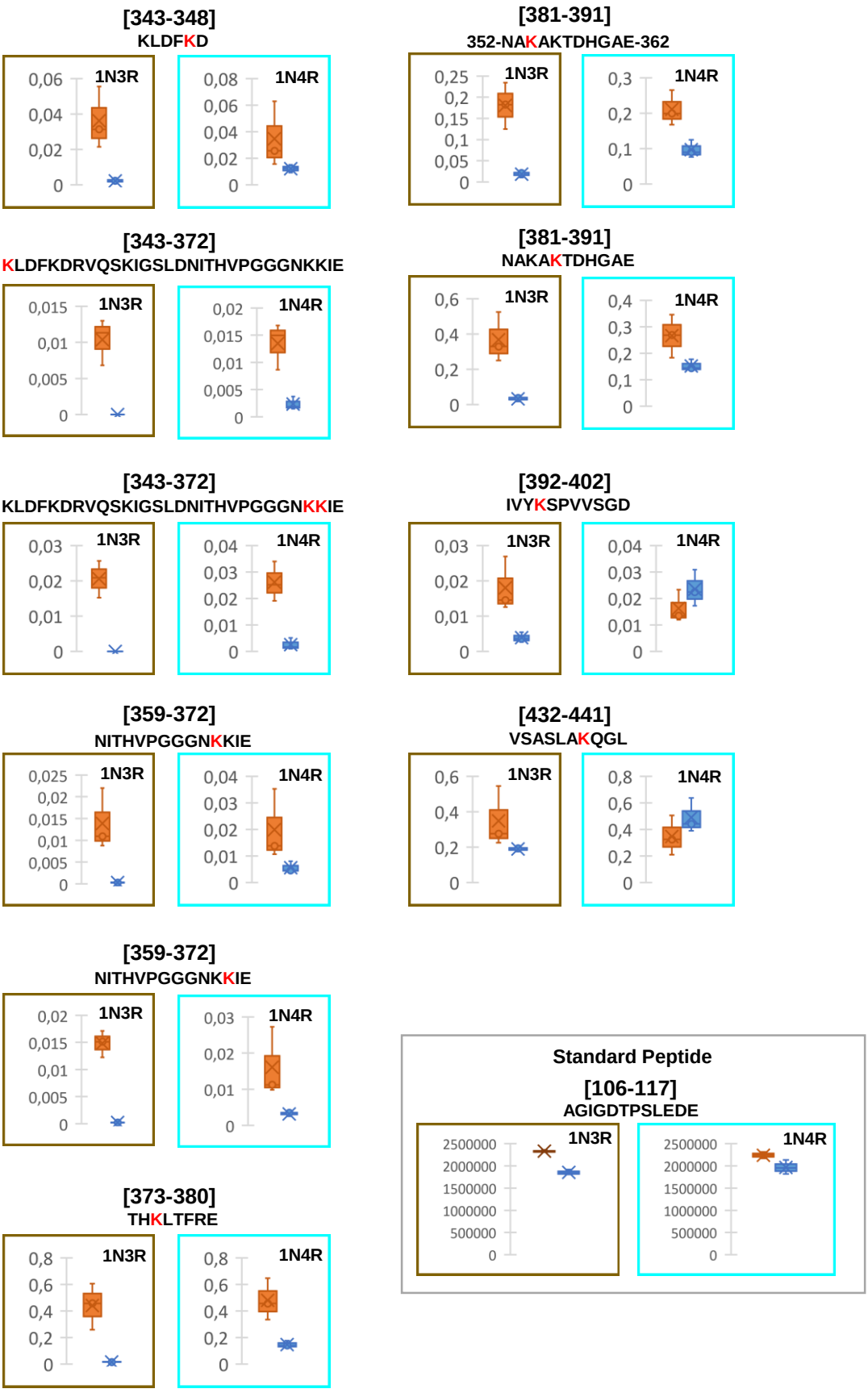

Figure S12b Changes in enzyme accessibility and surface labelling upon htau 1N3R and 1N4R assembly into fibrils. GluC was used for in-solution digestion. The boxplots represent the normalized intensities of each peptide from amino acid 343 to 441 for both 1N3R (brown) and 1N4R (cyan) htau monomers (orange) and fibrils (blue) for n=3 experiments. The intensity of the standard peptide used fore normalization is represented. The average is represented by an X and the median by the bar.