Supplemental Data

Lysine acetylation regulates the RNA binding, subcellular localization and inclusion formation of FUS

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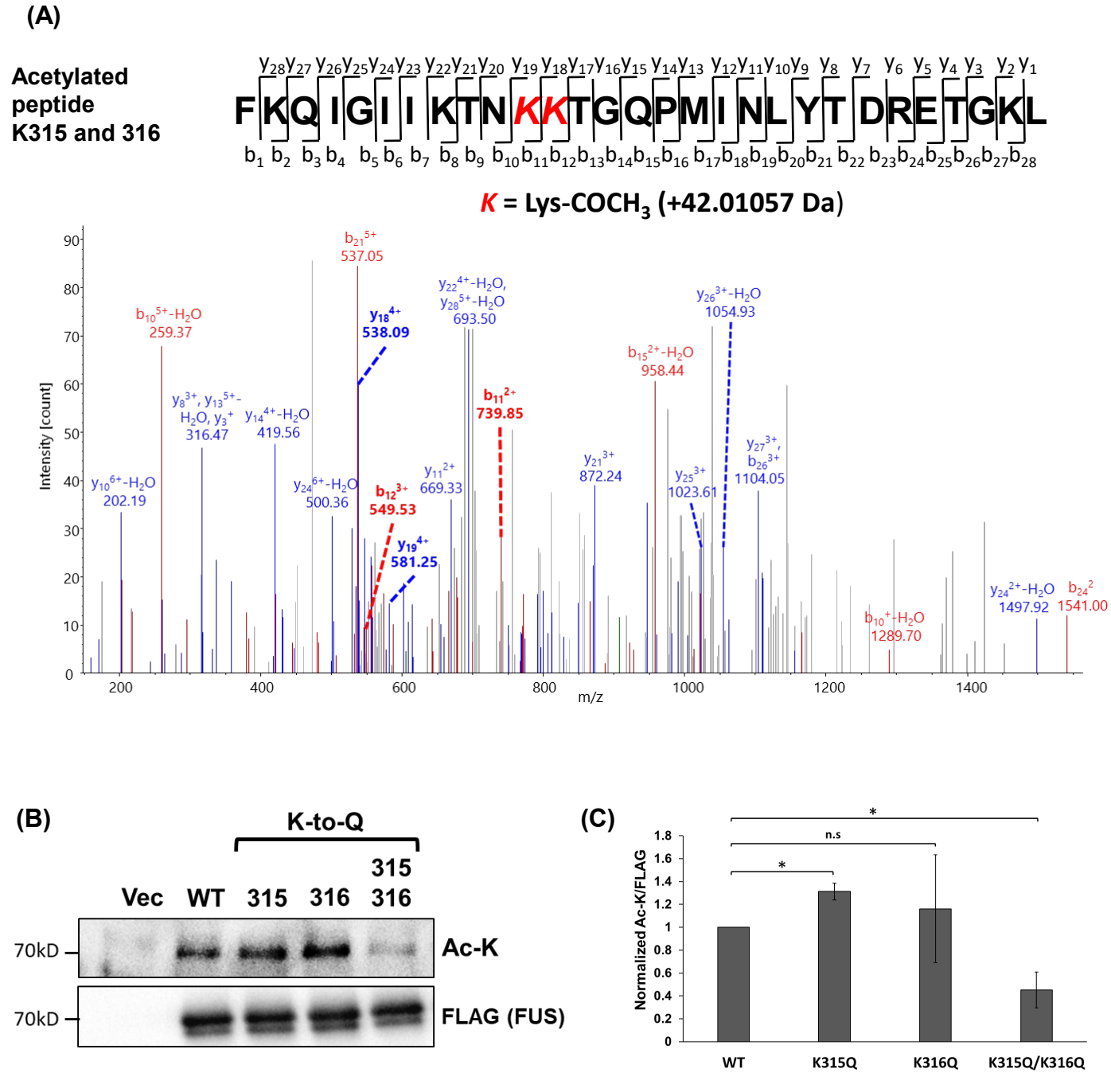
Supplemental Figure S1. Mass spectrometric identification of K315/K316 acetylation. The MS/MS spectrum of the di-acetylated peptide ^{305}FKQIGIIKTNKKTGQPMINLYTDRETGKL^{333}. The series of matched b and y ions are shown in blue and red, respectively. The b11, b12, y18 and y19 ions supporting the K315 and K316 acetylation sites are labeled in bold.

Supplemental Figure S2. FUS acetylation mimicking mutant inclusions co-localize with the stress granule marker G3BP1. Co-localization of endogenous G3BP1 with EGFP-tagged FUS in N2A cells. The co-localization of FUS and G3BP1 is shown by arrows. Scale bars, 10μm.

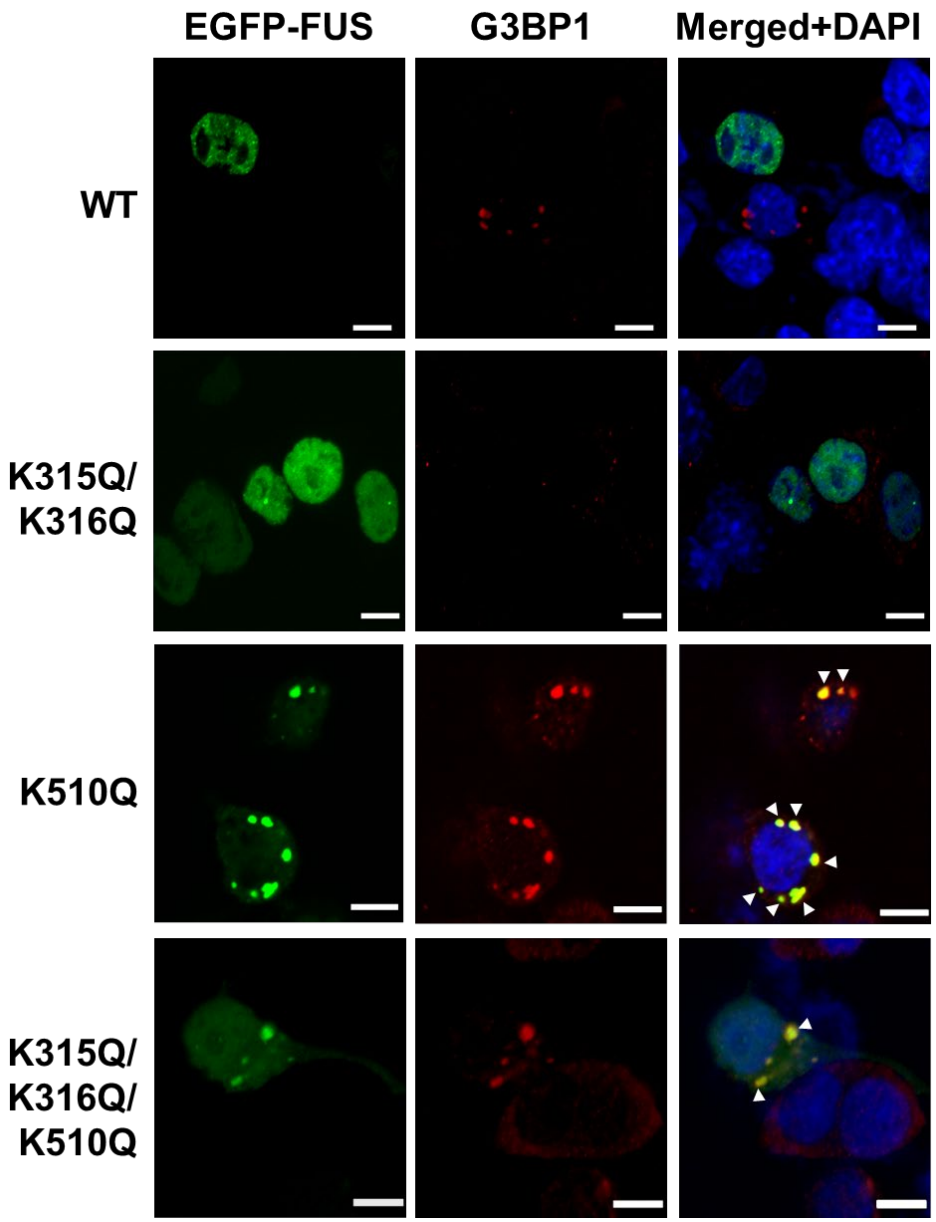
Supplemental figure S3. Characterization of the anti-K510-acetylated FUS antibody. (A) HEK293T, N2A, and N2A-ΔFUS (FUS knockout) cells were treated with deacetylase inhibitor (DACi) cocktail (30 mM nicotinamide, 50 mM sodium butyrate and 3 μM Trichostatin-A), as indicated. Immunoblotting was performed using the indicated antibodies. (B) N2A ΔFUS cells were transfected with 3×FLAG-tagged WT, K510R, or K510Q FUS or vector control with or without DACi cocktail as indicated. Immunoblotting was performed using the indicated antibodies.

Supplemental figure S4. Interaction screening of FUS with lysine deacetylases. HEK293T cells were transfected with 3×FLAG-HDAC 1-11 (A) or 3×FLAG SIRT 1-7 (B). After 48hrs, endogenous FUS immunoprecipitation was performed, followed by immunoblotting using the indicated antibodies. The 3×FLAG-ROA1 (hnRNPA1) construct was used as a positive control.

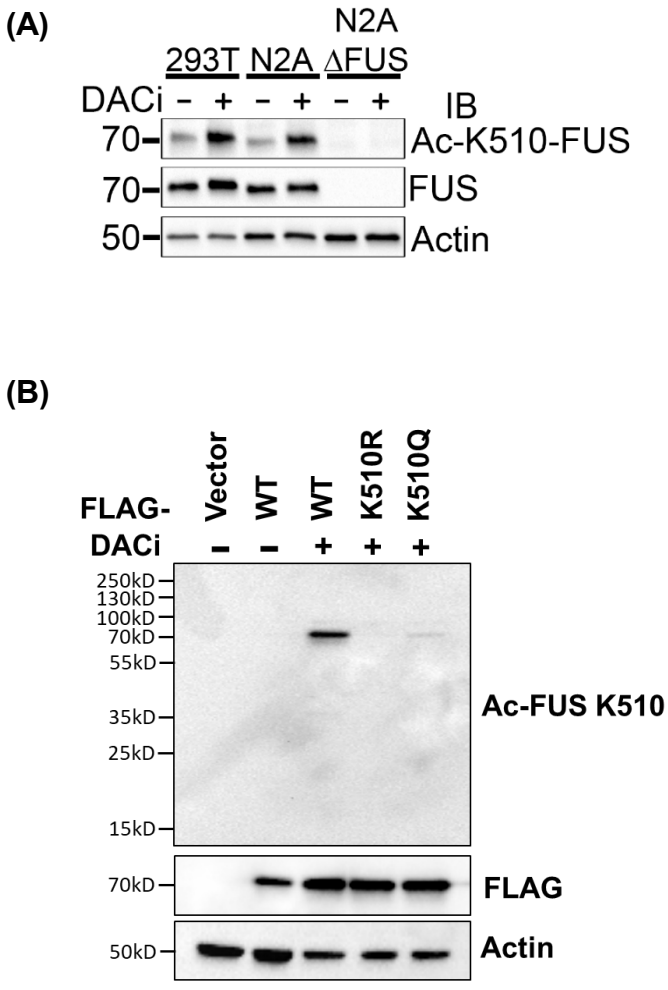
Supplemental Figure S1



Supplemental Figure S2



Supplemental Figure S3: Acetylated FUS-K510 specific antibody characterization



Supplemental Figure S4