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Supplemental Figure Legends

Supplemental Figure 1 The distribution of hAtx-1[82Q] in Cos7 cells.

Cos7 cells are co-transfected with hAtx-1[82Q]-GFP (green) and either vector, NMNAT or Hsp70. Based on the distribution pattern, the cells can be classified into three categories. Type I (A-B), Atx-1[82Q] is distributed in nuclear and cytoplasmic aggregates (A). Type II (C-D), hAtx-1[82Q] is distributed in both aggregates and also diffusely. Type III, hAtx-1[82Q] is diffuse in nucleus and cytoplasm (E-F). Nuclei are labelled in blue with DAPI. Aggregate formation is specific for hAtx-1[82Q], as Cos7 cells transfected with pEGFP vector has a diffused cytoplasmic distribution pattern (G). (H) hAtx-1[2Q] also forms aggregates when expressed in the cell. (I) Quantification of number of cells based on the hAtx-1[82Q] distribution pattern. The effect of NMNAT on hAtx-1[2Q] is similar to its effect on hAtx-1[82Q] (J).

Supplemental Figure 2 Endogenous NMNAT is up-regulated when dAtx-1 or hAtx-1[82Q] is expressed.

Western blot analysis of 2 or 5 day-old flies that express dAtx-1, NMNAT or both dAtx-1 and NMNAT (A), or hAtx-1[82Q] (B) with *nervana-Gal4*. Blots are double-labeled for actin (red) and NMNAT (green). *Canton S* flies are used as controls. The total level of NMNAT is higher with dAtx-1 and NMNAT co-expression compared to expression of NMNAT alone, suggesting that endogenous NMNAT is upregulated upon expression of dAtx-1.

Supplemental Figure 3 (A-D) Overexpressed GFP neither affects NMNAT distribution (magenta) nor co-localizes with hAtx-1[82Q] aggregates. When overexpressed with

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nervana-GAL4, GFP (green) is distributed in the cytoplasm (B, D) and does not co-localize with hAtx-1[82Q] aggregates (arrows in C-D). Endogenous NMNAT distribution is primarily nuclear and unaltered by GFP expression (A). (E) Overexpression of NMNAT reduces the level of hAtx-1[82Q] aggregates. The fluorescence level of hAtx-1[82Q] was measured in 7 day old flies expressing hAtx-1[82Q] with pan-neuronal driver *nervana-GAL4*. Co-expression of NMNAT or inactive protein NMNAT-WR reduces the level of aggregates. Error bars: standard error of the mean. *P<0.05 (Student's T-test), n=6.

Supplemental Figure 4 The level of CS is not affected by adding chaperones.

At the end of the aggregation experiment, individual protein mixtures were taken from the spectrophotometer plates and resolved by SDS-PAGE. The proteins gels were stained with Coomassie Blue. The levels of CS remain the same among different conditions.

Supplemental Figure 5 The C-terminal region of NMNAT is required for chaperone activity.

(A) Diagram of full length and truncated NMNAT domain structure. NMNAT-ΔC deletes the C-terminal ATP binding domain (1-244). NMNAT-ΔN deletes the N-terminal catalytic motif (64-297). NMNAT-ΔCN deletes both the N-terminal and C-terminal domains (64-244). (B-D) Chaperone activity of truncated NMNAT proteins.

Supplemental Figure 6 NMNAT and Hsp70 act independently in the aggregation assay.

CS dimer (1 μM) was incubated in a spectrophotometer plate equilibrated at 43°C in 50 mM HEPES, pH7.0, with or without additional proteins (0 μM). (A) CS was incubated with either 2 μM of Hsp70, 2 μM of NMNAT or a mixture or 1 μM Hsp70 and

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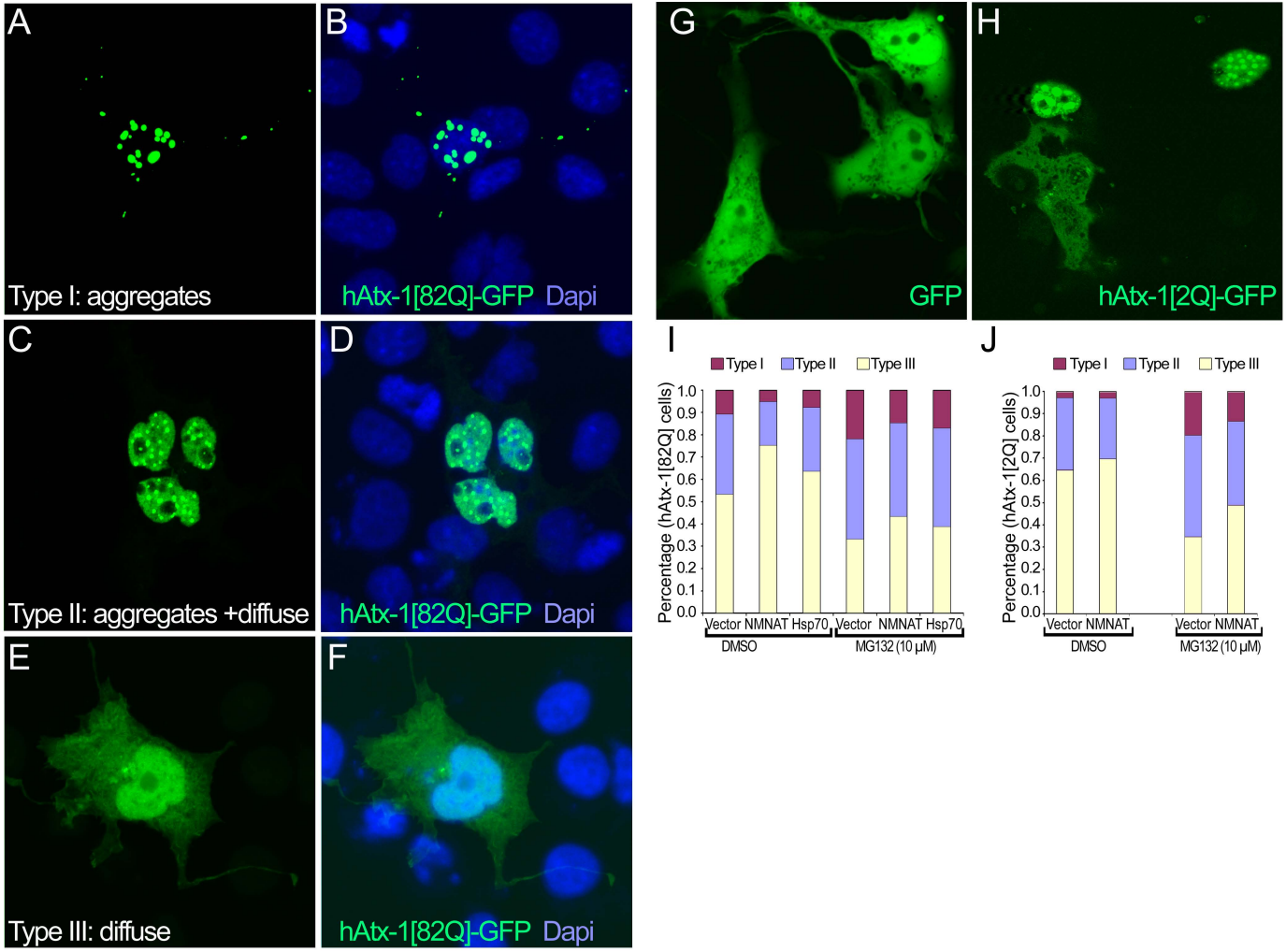
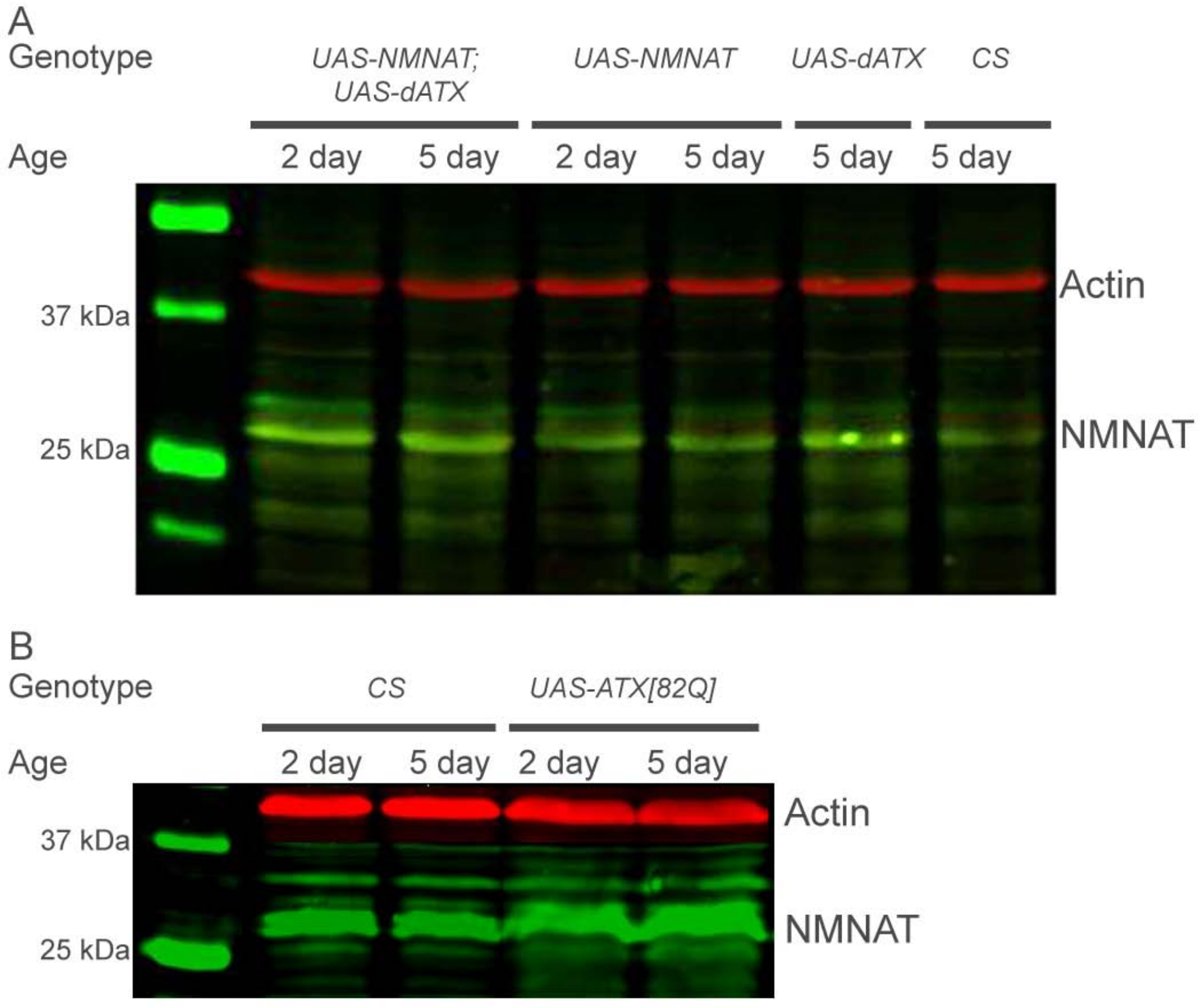
1 μM NMNAT. (B) CS was incubated with either 4 μM Hsp70, 4 μM of NMNAT-WR or a mixture of 2 μM Hsp70 and 2 μM NMNAT-WR. Aggregation of CS was monitored by measuring apparent light scattering (OD, A_{360}) every 20 s. No significant synergistic effect between Hsp70 and NMNAT proteins were observed.

Supplemental Figure 1



Supplemental Figure 2



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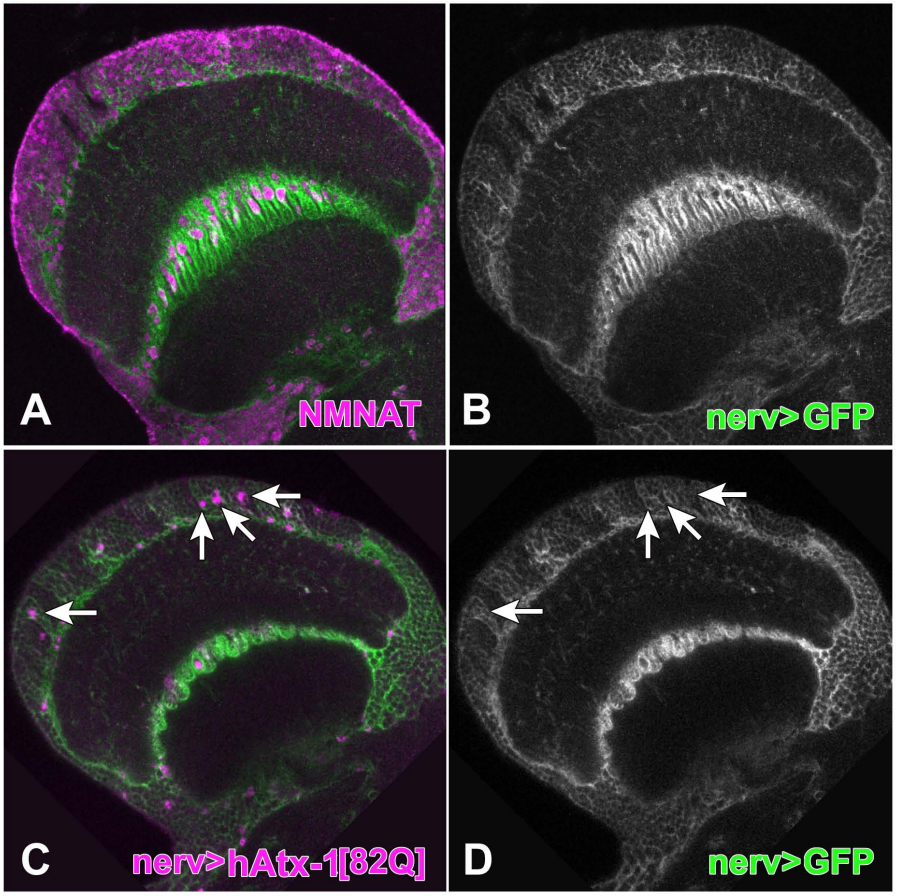

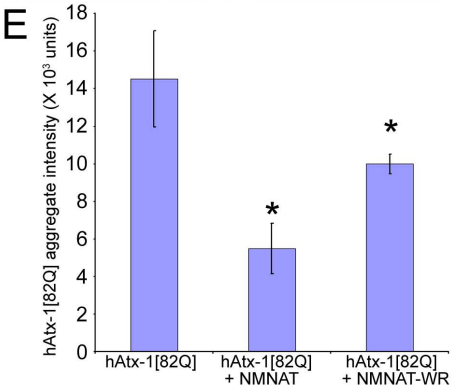

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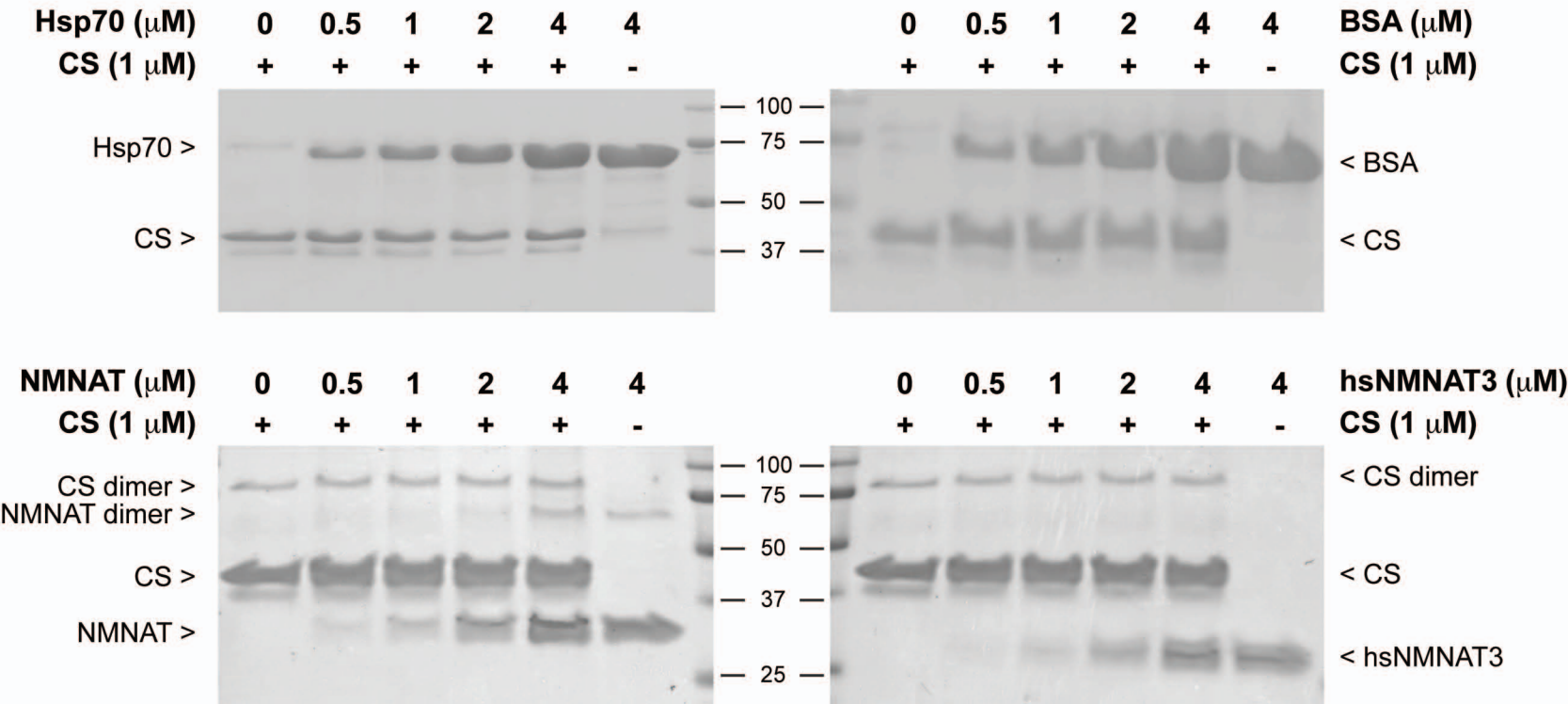
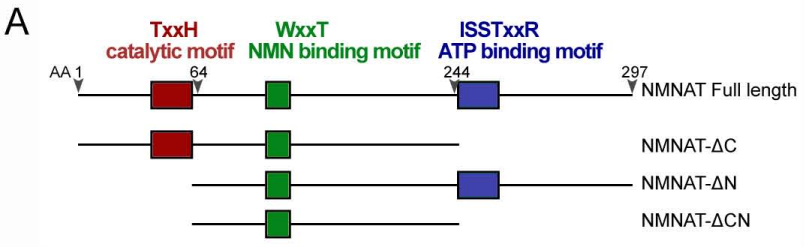
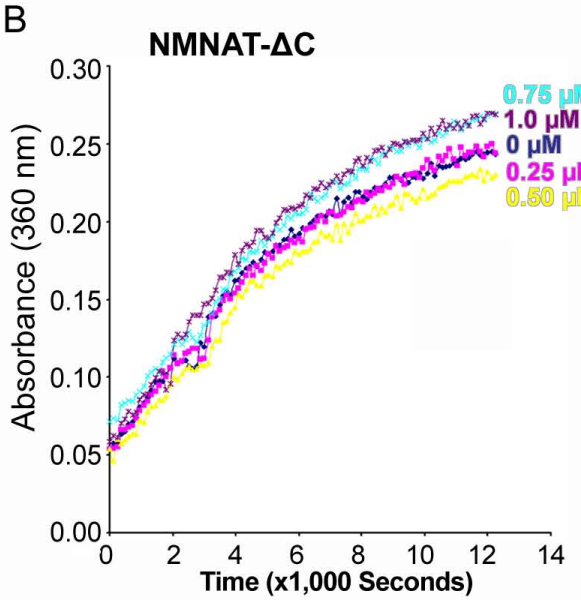
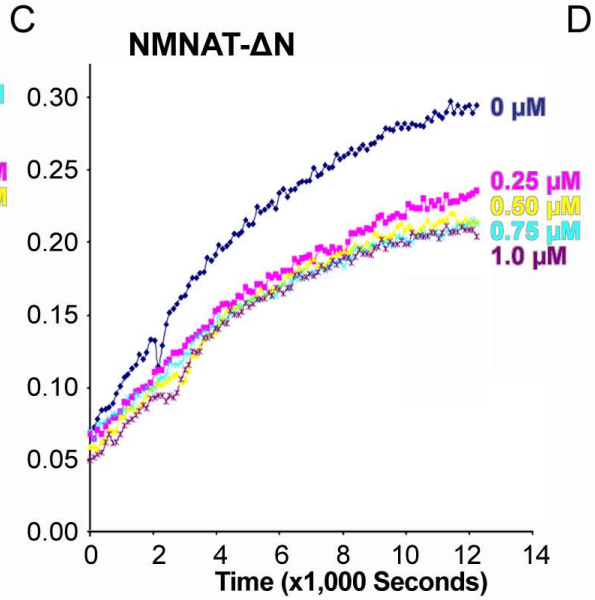
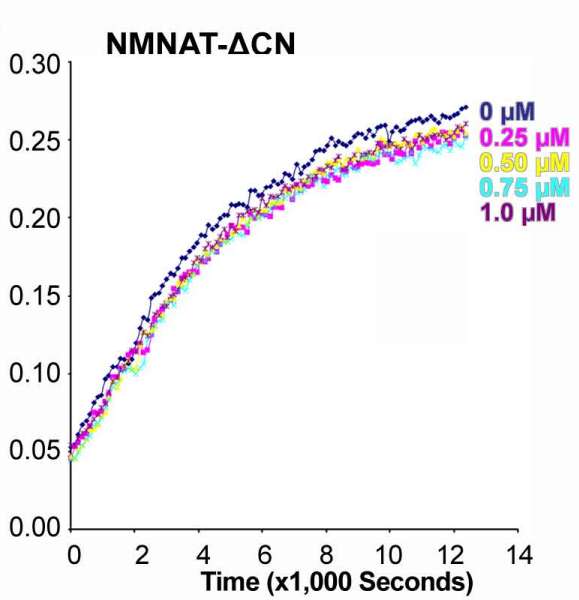


Supplemental Figure 5









Supplemental Figure 6