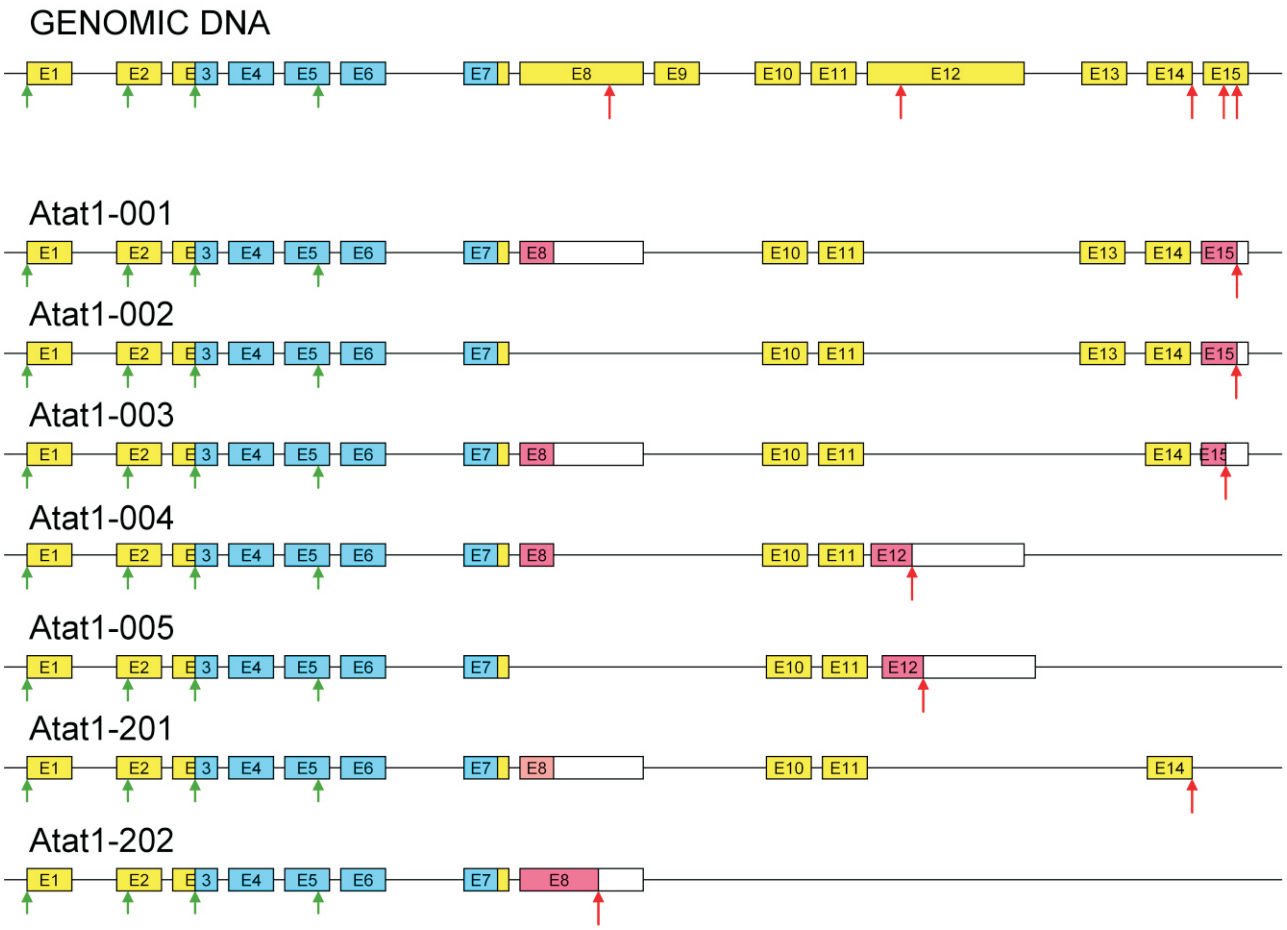
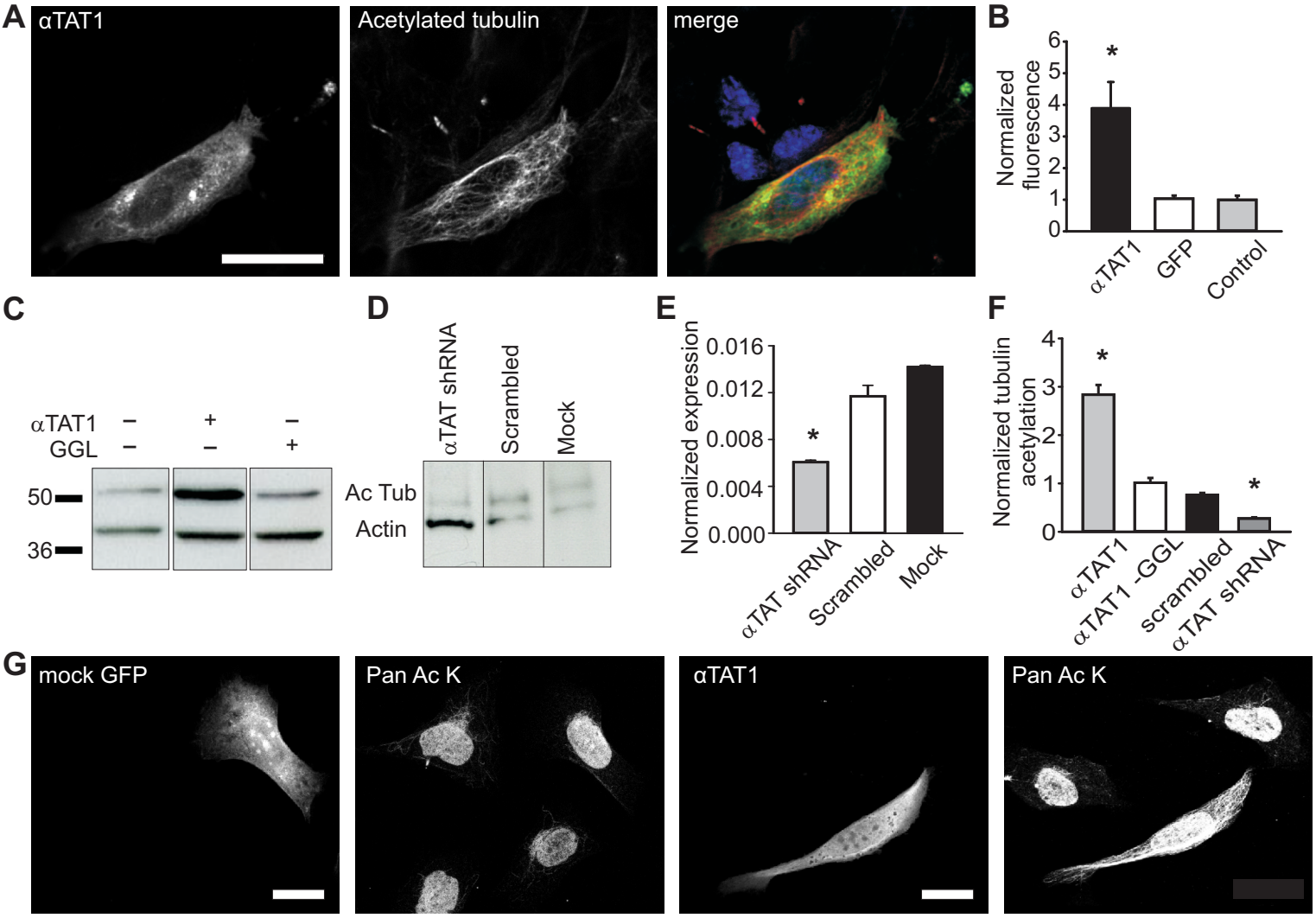
Supplementary Figure S1



Supplementary figure S1. **αTAT1 splice variants.** The mouse Atat1 gene (ENSMUSG00000024426) is located on chromosome 17 and it consists of 15 exons. 18 transcripts have been predicted (source: ENSEMBL database). Most do not result in a recognizable protein product or they are targeted by nonsense mediated decay (source: ENSEMBL database). For five of the protein-coding transcripts we generated specific primers to test them by qRT-PCR. All of the tested transcripts contain the N-terminus (exons 1-2) and acetyl-transferase domain (exons 3-7). However, their C-termini differ. Transcripts atat1-001 and atat1-002 contain exons 10, 11, 13, 14 and 15 with transcript atat1-001 containing also a part of exon 8. The same part of exon 8 is present also in the transcript atat1-201 that also contains exons 10, 11 and 14. Transcript atat1-005 is identical to atat1-201 except it does not contain exon 14, but 12. Finally, transcript atat1-202 is unique since it contains a longer version of exon 8 that harbors a stop codon.

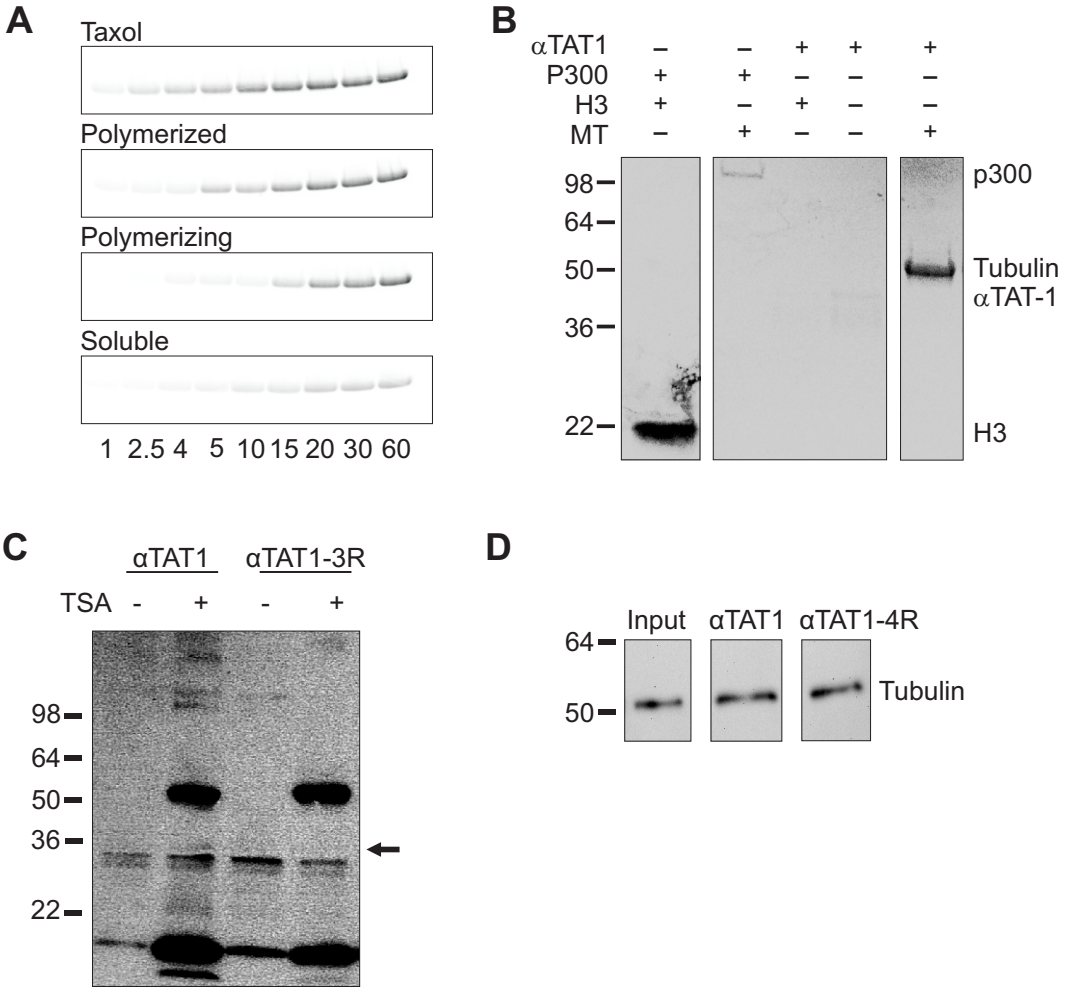
The image is a schematic representation of Atat1 transcripts. Exons are marked in yellow with a letter “E” and a number. Blue color indicates the acetylatransferase domain. Pink color indicates truncated exons. Start and stop codons are marked with green and red arrows, respectively.

Supplementary Figure S2



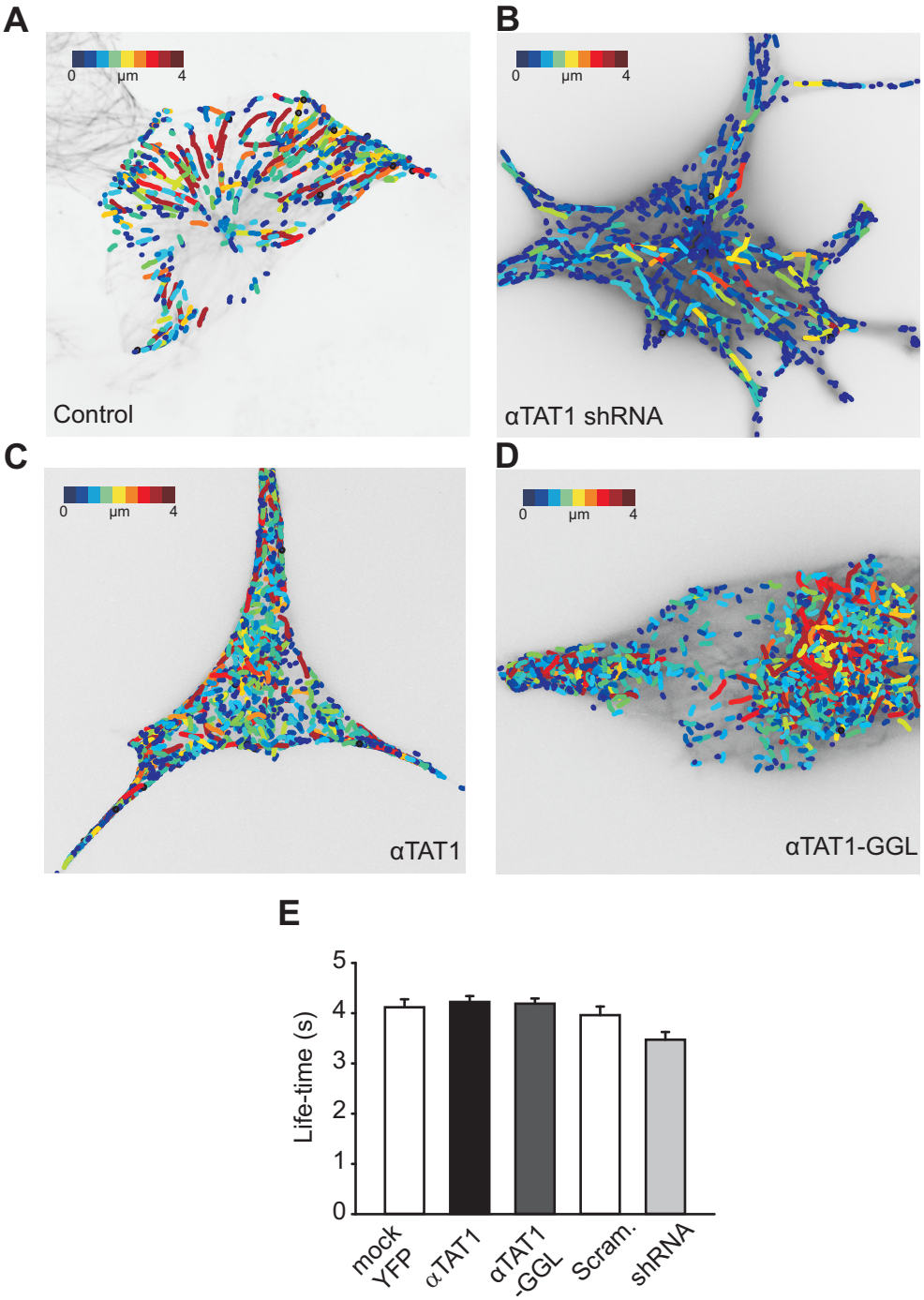
Supplementary figure S2. αTAT1 acetylates microtubules. (A and B) CHO cells were transiently transfected with YFP-αTAT1 or mock GFP and stained with 6-11b-1 acetylated tubulin antibody (A). Quantification (B) shows average fluorescent intensity ($p<0.015$, $n=20$). (C to E) Immunoblots of whole cell extracts with 6-11b-1. The αTAT1-GGL mutant is catalytically inactive whereas overexpression of αTAT1 increases tubulin acetylation (C). shRNA targeted against αTAT1 reduced endogenous tubulin acetylation (D) and expression levels of αTAT1 in NIH3T3 cells (E) measured by qRT-PCR ($p<0.01$, $n=3$). Quantification of acetylated tubulin levels (F) shows higher acetylation with αTAT1 over expression and 70% reduction with shRNA ($p<0.01$ in both cases, $n>3$). (G) . CHO cells transfected with YFP-αTAT1 (left 2 panels) or mock GFP (right 2 panels) and stained with pan specific anti-acetyl lysine antibody. Scale bars, 20μm.

Supplementary Figure S3



Supplementary figure S3. αTAT1 mediated acetylation in vitro. (A) Stable and polymerized microtubules are the preferred substrate for αTAT1. Example of autoradiography for analysis displayed in Figure 2C. Reactions were stopped by adding SDS loading buffer at times indicated below (in minutes). (B) αTAT1 specifically acetylates microtubules in vitro. Autoradiography of [^{14}C]acetyl incorporation. αTAT1 readily acetylates microtubules (MT), but does not acetylate histones (H3). In the reaction with histones and without tubulin a band at 37kDa is visible that corresponds to αTAT1 itself. The histone acetyl-transferase P300/CBP strongly acetylates histones, but does not influence microtubule acetylation. (C) αTAT1-3R (we originally discovered 3 lysine residues to be acetylated; K56R, K210R, K221R, see also Supplementary table S1) acetylation is undetectable. Acetyl-lysine immunoblot of 3T3 cells treated or not treated with trichostatin A (TSA, 5μM) for 4 hours. Additional band (arrow) that corresponds to αTAT1 itself is absent in αTAT1-3R over expression. (D) αTAT1-4R interacts with purified microtubules. Experiment was performed as in Figure 2D.

Supplementary Figure 4



Supplementary figure S4. αTAT1 affects displacement but not lifetime of EB3 particles. (A - E) αTAT1 knock-down decreases and over expression increases the displacement of EB3 particles. Live cell images with a color-coded overlay representing EB3 particle displacement. NIH3T3 cells were transfected with RFP-EB3 and mock YFP (A), αTAT1 shRNA (B), YFP-αTAT1 (C), or YFP-αTAT1-GGL (D) respectively. (E) Quantification of EB3 particle life-time reveals no significant difference between groups (n=20).

Supplementary videos:

video1.avi EB3 particle growth. NIH3T3 cells were transfected with RFP-EB3 and mock YFP and imaged 24 hours later using an Ultraview Vox Spinning disk microscope (PerkinElmer) with a 100x objective.

Supplementary table S1. Identification of acetylated lysine residues on α-tubulin and αTAT1[1].

Protein	Acetylation sites	Region
α-tubulin	K40	Lumen
αTAT1	K56	Ac domain – motif C
αTAT1	K146	Ac domain – motif A
αTAT1	K210	C-terminus
αTAT1	K221	C-terminus

[1] Initially we found 3 lysine sites on αTAT1 that can be acetylated (K56, K210, K221). Subsequently, we also identified the 4th site (K146). We were not able to find any further sites, although we leave the possibility that other sites might be present open. When we had identified only three sites we performed all experiments on cells and observed an effect (the 3R mutant showed roughly 50% less acetylation of microtubules). We also found that the 3R mutant cannot be detected on a western blot against pan acetylated lysine antibody after the cells have been treated with TSA (Supplementary figure S3C). Upon identification of the 4th site we repeated the experiments in cells and found a stronger effect, as presented in the Fig. 4E-F.