Gao et al., http://www.jcb.org/cgi/content/full/jcb.201403129/DC1

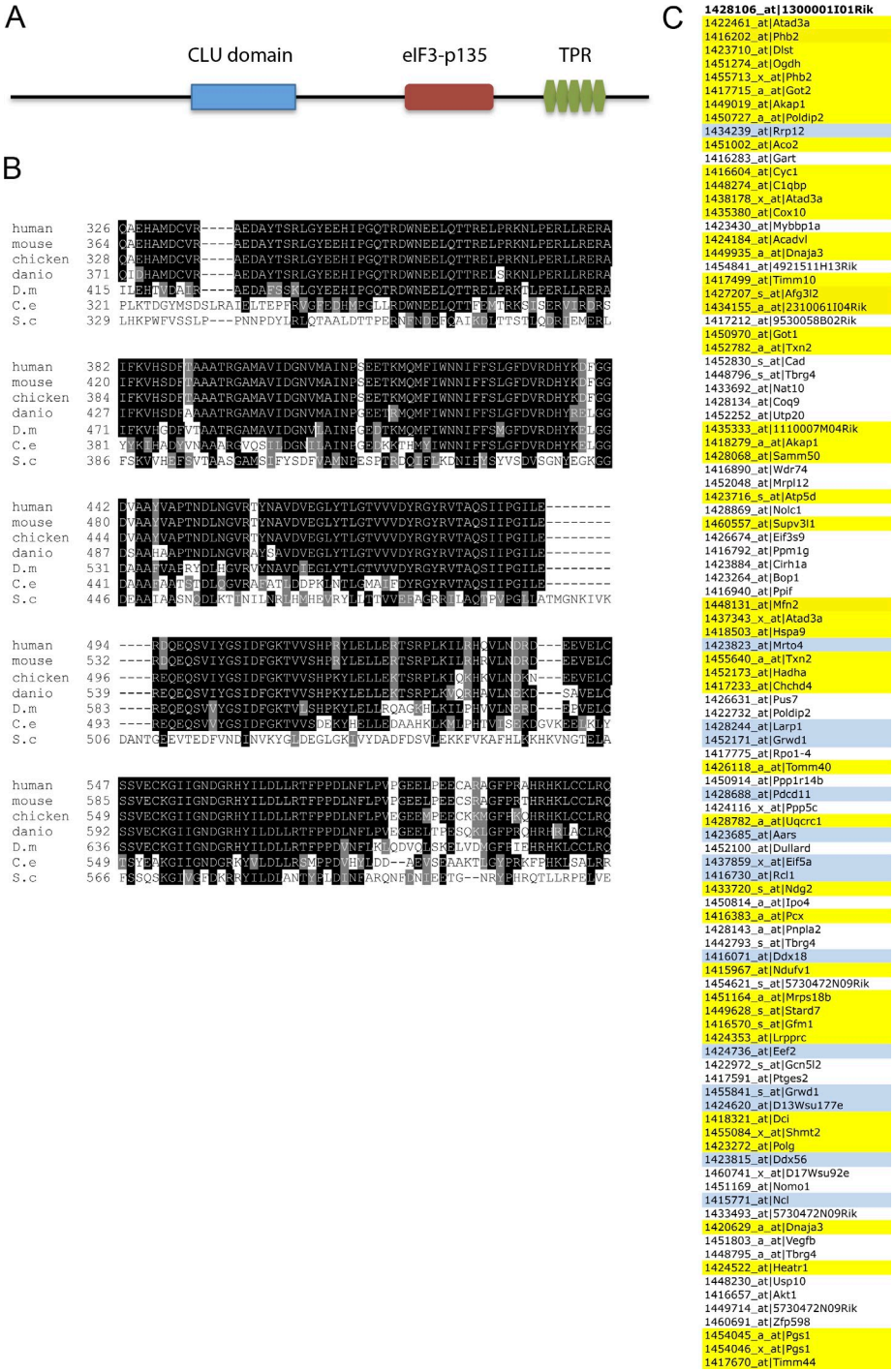

Figure S1. **Bioinformatic analysis of CLUH.** (A) Schematic representation of the human CLUH protein and its main domains, as annotated in Pfam. (B) Protein alignment of the Clu domain in different organisms. D.m, *Drosophila melanogaster*; C.e, *Caenorhabditis elegans*; S.c, *Saccharomyces cerevisiae*. Identical residues are highlighted in black, whereas residues with the same features are highlighted in gray. (C) List of the first 100 genes coregulated with mouse *Cluh*. Highlighted in yellow are genes encoding mitochondrial proteins, and in light blue are genes encoding proteins involved in ribosome biogenesis and translation.

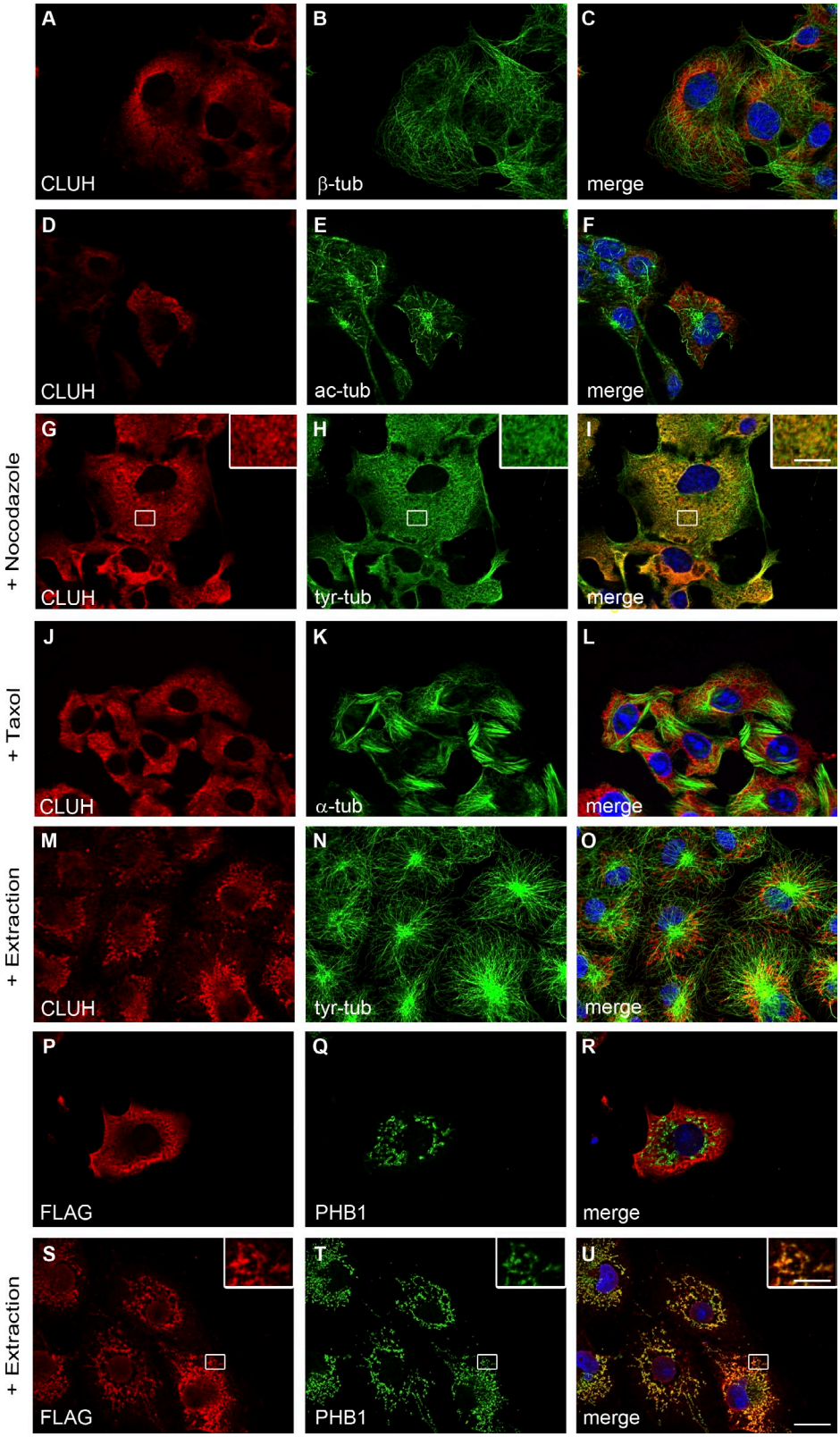

Figure S2. **Endogenous CLUH colocalizes with tyrosinated tubulin after MT disruption.** (A–F) Endogenous CLUH does not colocalize with β-tubulin or acetylated tubulin (ac-tub). (G–I) Colocalization of endogenous CLUH with tyrosinated tubulin (tyr-tub) is maintained after MT disruption with nocodazole (5 µM for 1 h). (J–L) CLUH does not colocalize to MT bundles after stabilization with taxol (10 µM taxol for 6 h). (M–O) After extraction with Triton X-100, endogenous CLUH does not decorate assembled MTs. (P–U) After extraction with Triton X-100, overexpressed CLUH-FLAG decorates mitochondria. Insets show magnifications of boxed regions. Bars: (main images) 20 µm; (insets) 5.5 µm.

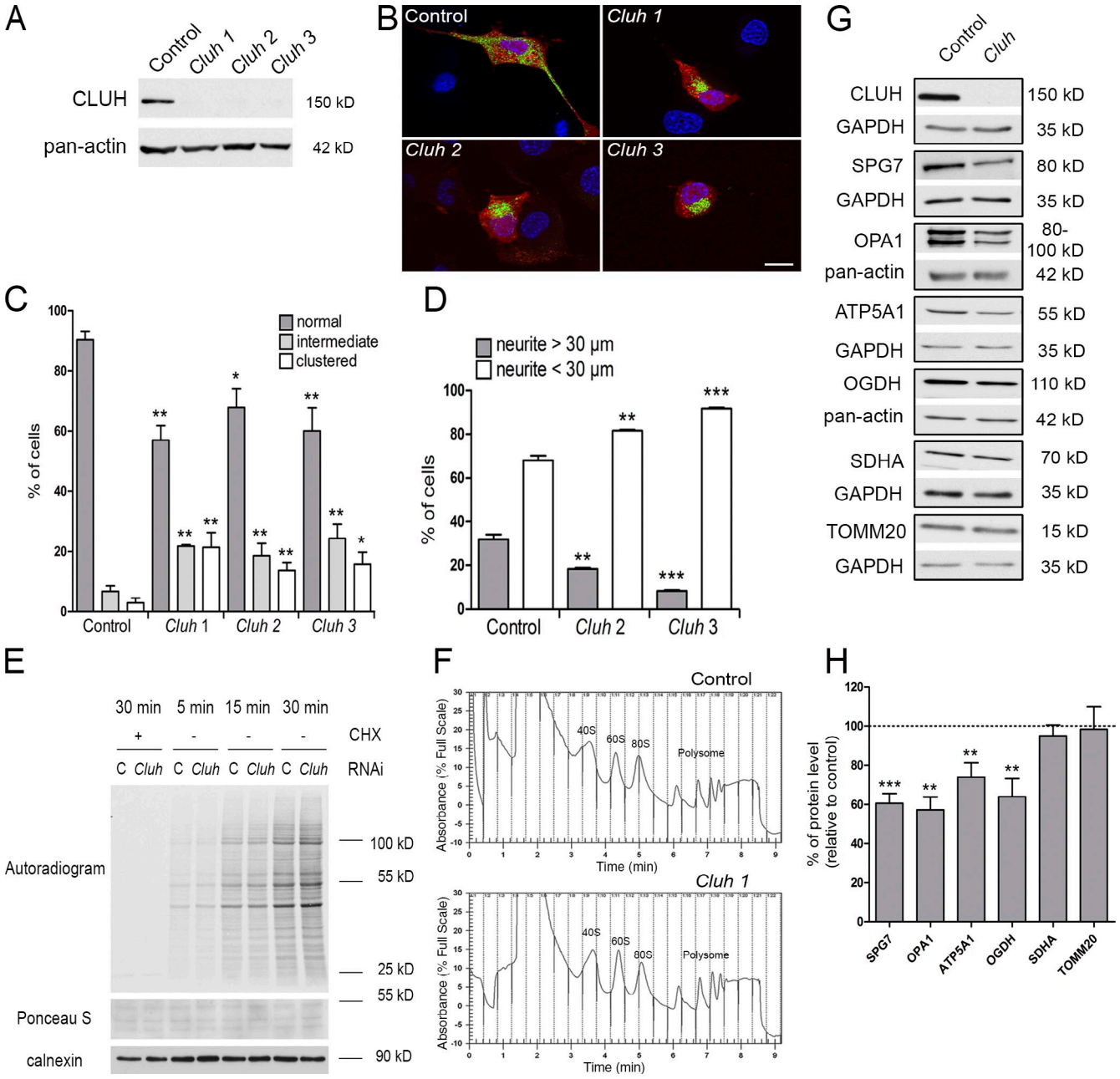

Figure S3. ***Cluh* down-regulation in NSC34 cells.** (A) Western blot showing efficient silencing of *Cluh* in NSC34 cells with three independent siRNAs. (B) NSC34 cells were transfected with *Cluh*-specific or control siRNAs. To label mitochondria and the cytosol, mito-GFP and cytosolic-mCherry were cotransfected, respectively. Bar, 20 µm. (C) Quantification of mitochondrial clustering phenotype in NSC34 cells. 200–300 cells were counted per experiment ($n = 3$). (D) Silencing of *Cluh* leads to shortening of neurites in NSC34 cells. (E) *Cluh* down-regulation does not result in obvious changes in protein synthesis in NSC34 cells. CHX, cycloheximide; C, control siRNA. (F) Polysome profiling of NSC34 cells depleted for CLUH does not show obvious difference compared with the control cells. This experiment has been completed once. (G and H) Western blot analysis and relative quantification show reduced steady-state levels of proteins encoded by selected CLUH target mRNAs ($n = 3–5$). Error bars show standard errors of the mean. ***, $P \leq 0.001$; **, $P \leq 0.01$; *, $P \leq 0.05$.

Table S1. **Library statistics used in this paper**

Label	No. of reads	No. aligned	Dupl removed	MapQ > 30	miRNA	rRNA	Other ncRNA
CLUH_S3	6.4	5.9	3.6	3.5	0.0	2.1	0.5
IgG_S5	4.2	3.7	1.8	1.6	0.0	0.8	0.4
Input_S1	6.7	6.1	4.2	4.0	0.0	1.8	0.6
CLUH_S9	5.3	4.8	3.1	3.0	0.0	1.4	0.4
IgG_S2	4.2	3.7	1.5	1.3	0.0	0.8	0.4
Input_S7	7.5	6.9	5.3	5.1	0.0	1.5	0.7
CLUH_S6	5.7	5.3	3.2	3.1	0.0	1.7	0.4
IgG_S8	5.1	4.7	2.9	2.7	0.0	1.0	0.4
Input_S4	6.6	6.0	4.4	4.2	0.0	1.7	0.6

All numbers are in millions. Dupl, duplicate; MapQ, mapping quality; rRNA, ribosomal RNA; ncRNA, noncoding RNA.

Table S2. **Primers for quantitative RT-PCR**

Primer	Sequence
ATP5A1 forward	5′-GATCCGCTGCCCAAACC-3′
ATP5A1 reverse	5′-GCCAATTCCAGCTTCATGGT-3′
SPG7 forward	5′-TCCCAGTTTCCTACAAGCGAA-3′
SPG7 reverse	5′-CCGTCATCCCCACAGAGTACA-3′.
GAPDH forward	5′-AATCCCATCACCATCTTCCA-3′
GAPDH reverse	5′-TGGACTCCACGACGTACTCA-3′
β-actin forward	5′-TCCCTGGAGAAGAGCTACGA-3′
β-actin reverse	5′-AGCACTGTGTTGGCGTACAG-3′
OGDH forward	5′-AAGACCAAAGCCGAACAGTTTTA-3′
OGDH reverse	5′-CGCCTCTCTCTGGGCCTTA-3′
GOT2 forward	5′-CACATCACCGACCAAATTGG-3′
GOT2 reverse	5′-AGCCGCTCCACCTGTTCA-3′
OPA1 forward	5′-CCCTTCATAGCCAGCGAAGA-3′
OPA1 reverse	5′-AGAGTGAGAAAACAGCAACTGAATCA-3′
TBP forward	5′-GCCCGAAACGCCGAATAT-3′
TBP reverse	5′-CGTGGCTCTTATCCTCATGA-3′
Cluh forward	5′-GGTAGCGGGCACGGTACA-3′
Cluh reverse	5′-GCATTGAGCACCCCAACAC-3′
Atp5a1 forward	5′-TGGGCCGTGTGGTTGAC-3′
Atp5a1 reverse	5′-CGTCTGCGGGTCTTGGAA-3′
Spg7 forward	5′-TGGTGGAAGTCTATCTGCATCCT-3′
Spg7 reverse	5′-CAACCTGCATCCGATCACATCA-3′
Got2 forward	5′-GGATGATAACGGAAAACCTTACGT-3′
Got2 reverse	5′-CCAAATTTTTTGCAGCAATCTG-3′
Ogdh forward	5′-TGGTGTCGGCGCTTCACT-3′
Ogdh reverse	5′-TGTCCTGAAGCCTTCCACAAG-3′
Opa1 forward	5′-CCTGTGAAGTCTGCCAATCCTT-3′
Opa1 reverse	5′-GAGTTTTTGGAGCGGTACGTTT-3′
Sdha forward	5′-GGCAGGCTCATCGGTGTT-3′
Sdha reverse	5′-CATATCGCAGAGATCTTCCATAC-3′
Pdha1 forward	5′-GAAATGTGACCTTCATCGGCT-3′
Pdha1 reverse	5′-TGATCCGCCTTTAGCTCCATC-3′
Tomm20 forward	5′-AGCTGGGCTTTCCAAGTTACC-3′
Tomm20 reverse	5′-ACCAAGCTGTATCTCTTCAAGGA-3′
Eif5a forward	5′-TTAGTGTTCCCCAGCCTCATG-3′
Eif5a reverse	5′-CAAATCTGGCTGGACGTCTCA-3′
Eif3d forward	5′-TGACTTTGACCTCCTGACTGTGA-3′
Eif3d reverse	5′-GAGTTGAAGGAGTTGCCTTCATC-3′
Polr2a forward	5′-GCCATGGGAGGACGAGAAG-3′
Polr2a reverse	5′-CTTCGCTGAATATACCCAGTCTCA-3′
Rpl13 forward	5′-AGCCCCACTTCCACAAGGA-3′
Rpl13 reverse	5′-TTGCGCCTGCGGATCT-3′
Gapdh forward	5′-AGGTCGGTGTGAACGGATTTG-3′
Gapdh reverse	5′-TGTAGACCATGTAGTTGAGGTCA-3′

Table S3 shows transcripts enriched with FC > 5 and P < 0.01 in CLUH-RIP versus IgG-RIP experiments and is provided online as an Excel (Microsoft) file.
Table S4 shows transcripts enriched with FC > 5 and P < 0.01 in CLUH-RIP versus HeLa input mRNA and is provide online as an Excel file.
Table S5 displays a comparison between the two types of analysis and is provide online as an Excel file.