AUTOPHAGY
2026, VOL. 22, NO. 3, 632–634
https://doi.org/10.1080/15548627.2025.2609924

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AUTOPHAGIC PUNCTUM

TBK1 orchestrates autophagy and endo-lysosomal pathways in human neurons

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ABSTRACT

Haploinsufficiency of TBK1 causes familial ALS and frontotemporal dementia (FTD), yet the mechanisms by which TBK1 loss leads to neurodegeneration remain unclear. Using deep proteomics and phospho-proteomics, we demonstrate that TBK1 regulates select macroautophagy/autophagy factors, targeting cargo receptors and autophagy initiation factors, and also sustains the phosphorylation of the late endosomal marker RAB7A in stem cells and stem cell-derived excitatory neurons. We further uncovered novel TBK1-dependent phosphorylation sites in the key autophagy protein SQSTM1/p62. Loss of TBK1 function results in a cell-autonomous neurodegenerative phenotype characterized by impaired neurite outgrowth and lysosomal dysfunction.

ARTICLE HISTORY
Received 16 December 2025
Revised 19 December 2025
Accepted 22 December 2025

KEYWORDS
TBK1; selective autophagy; neurodegeneration; proteomics; lysosomes

TBK1 (TANK binding kinase 1) is a serine/threonine protein kinase in the IKK-related kinase family. TBK1 regulates the phosphorylation of selective autophagy cargo receptors, such as OPTN (optineurin), which target ubiquitinated cargos to phagophores for degradation. Besides autophagy, this ubiquitously expressed kinase plays roles in innate immunity, apoptosis, and cell proliferation, and has been implicated in autoimmune diseases, glaucoma, and neurodegeneration.

Whole-exome sequencing studies identified deleterious loss-of-function *TBK1* variants in familial amyotrophic lateral sclerosis (ALS), frontotemporal dementia (FTD), and combined ALS-FTD. Disease-associated coding variants span the length of the protein, including within its N-terminal kinase domain as well as its C-terminal coiled-coil protein-protein interaction domain. This strongly suggests that haploinsufficiency for TBK1 contributes to neuron dysfunction and loss. Intriguingly, variants in additional autophagy-associated genes, including *SQSTM1, OPTN, C9orf72, CHMP2B, and UBQLN2*, have been linked to rare forms of ALS.

Prior investigations of TBK1 have primarily utilized cell lines and mouse models, while the function of TBK1 in human neurons has not been comprehensively explored. Our study [1] examined the function of endogenous TBK1 in isogenic induced pluripotent stem cells (iPSCs) and iPSC-derived excitatory neurons under basal conditions, in the absence of lysosomal inhibitors and mitochondrial toxins, which are often employed to induce selective autophagy. Through unbiased proteomics, we defined the consequences of partial or complete loss of TBK1 function, or loss of OPTN function, on autophagy proteins.

What are the targets of TBK1 in neurons?

Although the amino acid consensus sequence of validated TBK1 substrates has not been unambiguously defined, we observed that loss of TBK1 function had the greatest effect on phospho-serine residues that were followed by leucine (SL or SSL motifs, and occasionally SI). Our proteome-wide analysis identified TBK1-dependent phosphorylation of established autophagy-associated substrates, such as serine 177 in OPTN, further validating our phospho-proteomic approach. Additionally, we identified novel TBK1 phosphorylation sites on known substrates and on proteins not previously associated with TBK1, thereby uncovering new potential TBK1 targets (Figure 1).

SQSTM1/p62 (sequestosome 1) is one of the most widely studied proteins involved in selective autophagy, and its levels are often used to estimate autophagic flux. Phosphorylation of serine 403 within its ubiquitin-binding domain (UBA) by CSNK2/CK2 (casein kinase 2), ULK1, and TBK1 regulates its interaction with ubiquitinated cargos. Our phospho-proteomics identified additional phospho-serine residues within an uncharacterized region between its LC3-interacting region (LIR) and UBA domains, and adjacent to its KEAP1-interacting region/KIR (AA 347–352). We found that the phosphorylation of S355 and adjacent S365/S366 was strongly dependent on TBK1 kinase activity in both stem cells and neurons. This opens the door for additional studies to determine whether phosphorylation in this unstructured region affects interactions of SQSTM1 with Atg8-family members or additional components of the autophagy machinery, ubiquitinated cargos, oligomerization, including the formation of filamentous biomolecular condensates, or other functions. Intriguingly, the closely related cargo receptor NBR1, which has been implicated in endosomal microautophagy, also shows a similar finding. Phosphorylation of an analogous C-terminal SL motif (S656) between its LIR domains is also dependent on TBK1. Phospho-specific antibodies developed against these novel sites in SQSTM1 and NBR1 could potentially serve to mark phase-separated SQSTM1/p62 bodies.

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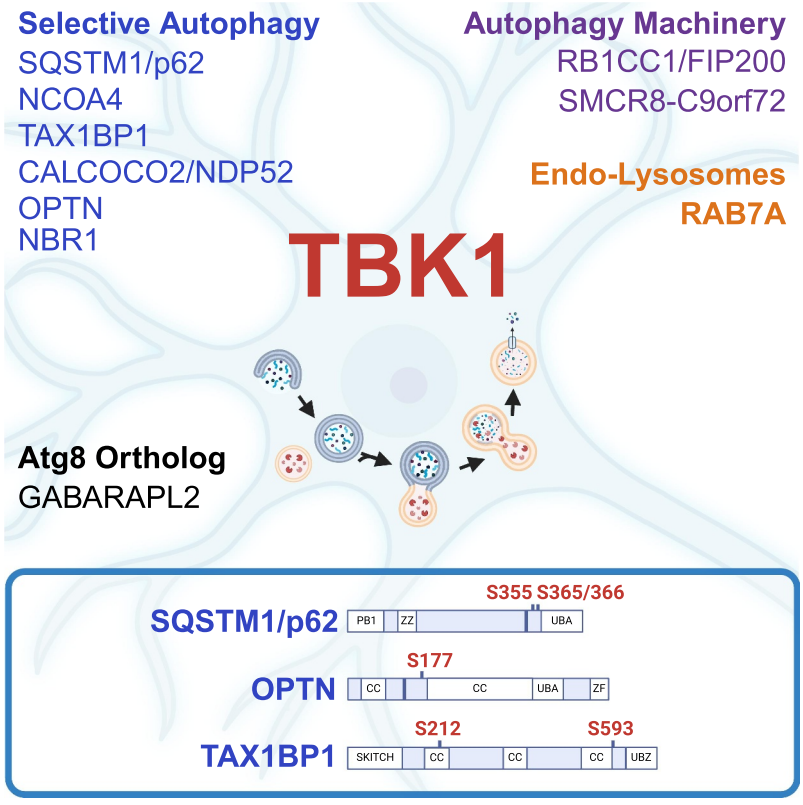

Figure 1. Schematic of TBK1's main targets in iPSC-derived excitatory neurons. In *TBK1* knockout neurons, phosphorylation of selective autophagy cargo receptors (SACRs), autophagy machinery proteins, an Atg8 ortholog, and endo-lysosomal proteins is altered. The bottom panel displays protein domains of representative SACRs, SQSTM1/p62, OPTN, and TAX1BP1, with select phospho-serines targeted by TBK1 shown in red.

The selective autophagy cargo receptors TAX1BP1 and NCOA4 regulate ferritinophagy. We identified TBK1-dependent phospho-serine residues in both TAX1BP1 and NCOA4, within its iron-sulfur cluster domain that are critical for sensing intracellular iron, in neurons. This suggests a potential mechanism by which TBK1 May regulate the lysosomal turnover of ferritin, which warrants further study.

Furthermore, we observed that loss of TBK1 affects the phosphorylation of additional autophagy factors, including RB1CC1/FIP200 and ATG9A. Among Atg8-family proteins, loss of TBK1 severely reduces the phosphorylation of GABARAPL2. This suggests GABARAPL2 May be more selective for TBK1-dependent autophagy, analogous to LC3B for canonical macroautophagy. In contrast, loss of OPTN does not generally phenocopy the effects of loss of TBK1 on the phospho-proteome.

What are the interacting partners of TBK1 in neurons?

We also sought to define interacting proteins of TBK1 in excitatory neurons. Using affinity purification coupled with mass spectrometry (AP-MS), we validated that endogenous TBK1 interacts with all three of its major adapter proteins, AZI2/NAP1, TANK, and TBKBP1/SINTBAD, in neurons. Furthermore, our global proteomic data revealed that the complete loss of TBK1 moderately reduces the abundance of these adapter proteins in neurons, without affecting the corresponding transcripts, suggesting that binding to TBK1 promotes their stability. Furthermore, we defined specific phospho-serine residues in AZI2, TANK, and TBKBP1 in neurons that are strongly dependent on the presence of TBK1. Additional studies will be necessary to understand the contributions of individual adapter proteins to selective autophagy, such as recruiting homodimers of TBK1 to certain cellular compartments or promoting the engagement of TBK1 with specific substrates.

How does loss of TBK1 function affect excitatory neurons?

We also asked whether the complete loss of TBK1 function is sufficient to affect neuron function. *TBK1* knockout neurons exhibit abnormal morphology, characterized by decreased length and complexity of neurites compared to isogenic control neurons. Additionally, loss of TBK1 is associated with dysregulation of lysosomal activity. Consistent with this finding, we observed that loss of TBK1 dramatically decreases the phosphorylation of the key late endosomal marker RAB7A in neurons.

Overall, our study provides a comprehensive view of how TBK1 regulates endo-lysosomal pathways and advances our understanding of how reduced TBK1 function affects protein homeostasis and contributes to neural dysfunction. Novel TBK1-dependent phosphorylation sites could serve as precise indicators of selective autophagy for guiding the development of therapeutic agents for ALS and other TBK1-associated diseases.

Acknowledgements

Figure created with BioRender.com.

Author contributions

CRediT: **Daniel A. Mordes:** Funding acquisition, Writing – original draft, Writing – review & editing; **Julie Smeyers:** Writing – review & editing.

Funding

Grant support to D.A.M. includes the NIH [NIA R01AG089849], Target ALS, Association for Frontotemporal Degeneration (AFTD). J.S. is a Target ALS Springboard Fellow.

Disclosure statement

No potential conflict of interest was reported by the author(s).

Data availability statement

Original data were not presented in this study. The reader is referred to the reference below.

Reference

[1] Smeyers J, Oses-Prieto JA, Yadanar L, Wang M, Iadarola M, Lu S, Wang KS, Watanabe TH, Debnath J, Burlingame AL, Mordes DA. Phospho-proteome profiling in human neurons reveals targets of TBK1 in ALS/FTD-associated autophagy networks. Cell Rep. 2025 Nov 25;44(11):116494. doi: 10.1016/j.celrep.2025.116494