## Phylogeny

ULK3 belongs to the Unc-51-like kinase (ULK) family within the serine/threonine protein kinase group of the human kinome (preuss2020nucleotidebindingevolutionary pages 13-13).  
Phylogenetic analyses group ULK3 with the STK36/Fused subfamily, sharing 38 % identity with human STK36 and 37 % identity with the Drosophila fused (dFu) kinase across the catalytic domain (maloverjan2010identificationofa pages 4-6, maloverjan2010identificationofa pages 3-4).  
Within the ULK family, ULK3 diverges from ULK1/2/4, reflecting a distinct evolutionary branch linked to Hedgehog signaling rather than canonical autophagy (karmacharya2023smallmoleculeinhibitors pages 2-5).

## Reaction Catalyzed

ATP + [protein]-Ser/Thr → ADP + [protein]-O-phospho-Ser/Thr (maloverjan2010identificationofa pages 4-6).

## Cofactor Requirements

Catalytic activity requires divalent cations, with in-vitro assays demonstrating dependence on Mg²⁺ or Mn²⁺ (kasak2018characterizationofprotein pages 7-12).

## Substrate Specificity

ULK3 phosphorylates GLI transcription factors, exhibiting highest activity toward GLI2 and lower activity toward GLI1 and GLI3 (maloverjan2010identificationofa pages 6-9).  
On GLI1, phosphorylation is confined to the N-terminal residues 1–426 and the C-terminal region, whereas the central segment 426–754 is not modified (maloverjan2010identificationofa pages 9-10).  
A global consensus phosphorylation motif for ULK3 has not been reported in the available literature (maloverjan2010identificationofa pages 9-10).

## Structure

ULK3 is a 472-residue protein comprising an N-terminal serine/threonine kinase domain (14–270), a microtubule-interacting and trafficking (MIT) domain (279–353) and a C-terminal regulatory tail (354–472) (maloverjan2010identificationofa pages 4-6).  
The kinase domain contains canonical VAIK (Lys44), HRD and DFG motifs that form the catalytic core (kasak2018characterizationofprotein pages 7-12).  
A second essential catalytic lysine, Lys139, is required for activity; the K139R mutation abolishes autophosphorylation and substrate phosphorylation (maloverjan2010identificationofa pages 4-6).  
Homology modeling shows that at least six autophosphorylated serines are surface-exposed around the active site (kasak2018characterizationofprotein pages 14-16).  
No experimental crystal or cryo-EM structure has been solved; inhibitor docking and structural inferences rely on homology models of the kinase domain (kasak2018characterizationofprotein pages 23-26).

## Regulation

ULK3 autophosphorylates at Ser22 and Ser55 within the kinase domain and at Ser300, Ser350, Ser384 and Ser464 in the C-terminal tail (kasak2018characterizationofprotein pages 18-22, maloverjan2010dualfunctionof pages 7-8).  
Autophosphorylation of Ser22/Ser55 is dispensable for catalysis, whereas phosphorylation by an unidentified kinase at Ser176 and at Ser467/468 abolishes activity (kasak2018characterizationofprotein pages 7-12).  
In the absence of Sonic Hedgehog ligand, Suppressor of Fused (SUFU) binds the kinase domain of ULK3 and completely blocks autophosphorylation and substrate phosphorylation (maloverjan2010dualfunctionof pages 6-7).  
SHH stimulation dissociates the SUFU-ULK3 complex, enabling ULK3 autophosphorylation and subsequent phosphorylation of GLI2 (maloverjan2010dualfunctionof pages 10-12).  
SUFU-bound ULK3 also recruits PKA, GSK3β and CK1 to promote proteolytic processing of GLI2 into its repressor form, reflecting a kinase-independent scaffolding function (maloverjan2010dualfunctionof pages 10-12).  
The small-molecule SU6668 binds the ATP pocket and acts as a mixed-type inhibitor, increasing Km and decreasing Vmax for ATP (kasak2018characterizationofprotein pages 18-22).

## Function

qRT-PCR analyses show highest ULK3 mRNA levels in fetal brain and in adult hippocampus, cerebellum, olfactory bulb and optic nerve (maloverjan2010identificationofa pages 4-6).  
Active ULK3 enhances GLI1 and GLI2 transcriptional activity and drives nuclear localization of GLI1, thereby positively regulating Hedgehog signaling (maloverjan2010identificationofa pages 6-9).  
Partial ULK3 knockdown paradoxically increases GLI1 induction by SHH, highlighting its dual positive and negative roles in pathway modulation (maloverjan2010dualfunctionof pages 10-12).  
ULK3 is up-regulated in cancer-associated fibroblasts under metabolic stress, where it is essential for autophagy-driven pro-tumorigenic conversion (karmacharya2023smallmoleculeinhibitors pages 2-5).  
Verified protein interactors include SUFU via the kinase domain and GLI1-3 via both kinase and MIT regions (maloverjan2010dualfunctionof pages 6-7, maloverjan2010identificationofa pages 6-9).

## Inhibitors

SU6668 photo-crosslinks to Ile43 in the ATP pocket and inhibits ULK3 kinase activity in a mixed competitive manner (kasak2018characterizationofprotein pages 23-26).  
A photoreactive analogue, SUX, covalently binds the same site and suppresses catalytic activity (kasak2018characterizationofprotein pages 14-16).  
SU6668 reduces SHH-induced GLI expression in cell-based assays through ULK3 inhibition (montagnani2019roleofprotein pages 7-8).

## Other Comments

Elevated ULK3 activity in cancer-associated fibroblasts identifies the kinase as a potential therapeutic target for modulating the tumor micro-environment (karmacharya2023smallmoleculeinhibitors pages 2-5).  
No recurrent disease-linked mutations in ULK3 have been reported in the cited literature (maloverjan2010identificationofa pages 6-9).

References

1. (maloverjan2010identificationofa pages 4-6): Alla Maloverjan, M. Piirsoo, Piret Michelson, P. Kogerman, and T. Osterlund. Identification of a novel serine/threonine kinase ulk3 as a positive regulator of hedgehog pathway. Experimental cell research, 316 4:627-37, Feb 2010. URL: https://doi.org/10.1016/j.yexcr.2009.10.018, doi:10.1016/j.yexcr.2009.10.018. This article has 107 citations and is from a peer-reviewed journal.
2. (maloverjan2010identificationofa pages 9-10): Alla Maloverjan, M. Piirsoo, Piret Michelson, P. Kogerman, and T. Osterlund. Identification of a novel serine/threonine kinase ulk3 as a positive regulator of hedgehog pathway. Experimental cell research, 316 4:627-37, Feb 2010. URL: https://doi.org/10.1016/j.yexcr.2009.10.018, doi:10.1016/j.yexcr.2009.10.018. This article has 107 citations and is from a peer-reviewed journal.
3. (karmacharya2023smallmoleculeinhibitors pages 2-5): Ujjwala Karmacharya and Jongjon Jung. Small molecule inhibitors for unc-51-like autophagy-activating kinase targeting autophagy in cancer. International Journal of Molecular Sciences, Jan 2023. URL: https://doi.org/10.3390/ijms24020953, doi:10.3390/ijms24020953. This article has 19 citations and is from a peer-reviewed journal.
4. (kasak2018characterizationofprotein pages 14-16): Lagle Kasak, Mihkel Näks, Priit Eek, Alla Piirsoo, Rohit Bhadoria, Pavel Starkov, Merilin Saarma, Sergo Kasvandik, and Marko Piirsoo. Characterization of protein kinase ulk3 regulation by phosphorylation and inhibition by small molecule su6668. Biochemistry, 57:5456-5465, Aug 2018. URL: https://doi.org/10.1021/acs.biochem.8b00356, doi:10.1021/acs.biochem.8b00356. This article has 11 citations and is from a peer-reviewed journal.
5. (kasak2018characterizationofprotein pages 23-26): Lagle Kasak, Mihkel Näks, Priit Eek, Alla Piirsoo, Rohit Bhadoria, Pavel Starkov, Merilin Saarma, Sergo Kasvandik, and Marko Piirsoo. Characterization of protein kinase ulk3 regulation by phosphorylation and inhibition by small molecule su6668. Biochemistry, 57:5456-5465, Aug 2018. URL: https://doi.org/10.1021/acs.biochem.8b00356, doi:10.1021/acs.biochem.8b00356. This article has 11 citations and is from a peer-reviewed journal.
6. (kasak2018characterizationofprotein pages 7-12): Lagle Kasak, Mihkel Näks, Priit Eek, Alla Piirsoo, Rohit Bhadoria, Pavel Starkov, Merilin Saarma, Sergo Kasvandik, and Marko Piirsoo. Characterization of protein kinase ulk3 regulation by phosphorylation and inhibition by small molecule su6668. Biochemistry, 57:5456-5465, Aug 2018. URL: https://doi.org/10.1021/acs.biochem.8b00356, doi:10.1021/acs.biochem.8b00356. This article has 11 citations and is from a peer-reviewed journal.
7. (maloverjan2010dualfunctionof pages 10-12): Alla Maloverjan, M. Piirsoo, L. Kasak, Lauri Peil, Torben Østerlund, and P. Kogerman. Dual function of unc-51-like kinase 3 (ulk3) in the sonic hedgehog signaling pathway\*. The Journal of Biological Chemistry, 285:30079-30090, Jul 2010. URL: https://doi.org/10.1074/jbc.m110.133991, doi:10.1074/jbc.m110.133991. This article has 63 citations.
8. (maloverjan2010dualfunctionof pages 6-7): Alla Maloverjan, M. Piirsoo, L. Kasak, Lauri Peil, Torben Østerlund, and P. Kogerman. Dual function of unc-51-like kinase 3 (ulk3) in the sonic hedgehog signaling pathway\*. The Journal of Biological Chemistry, 285:30079-30090, Jul 2010. URL: https://doi.org/10.1074/jbc.m110.133991, doi:10.1074/jbc.m110.133991. This article has 63 citations.
9. (maloverjan2010identificationofa pages 6-9): Alla Maloverjan, M. Piirsoo, Piret Michelson, P. Kogerman, and T. Osterlund. Identification of a novel serine/threonine kinase ulk3 as a positive regulator of hedgehog pathway. Experimental cell research, 316 4:627-37, Feb 2010. URL: https://doi.org/10.1016/j.yexcr.2009.10.018, doi:10.1016/j.yexcr.2009.10.018. This article has 107 citations and is from a peer-reviewed journal.
10. (montagnani2019roleofprotein pages 7-8): Valentina Montagnani and B. Stecca. Role of protein kinases in hedgehog pathway control and implications for cancer therapy. Cancers, Mar 2019. URL: https://doi.org/10.3390/cancers11040449, doi:10.3390/cancers11040449. This article has 54 citations and is from a peer-reviewed journal.
11. (kasak2018characterizationofprotein pages 18-22): Lagle Kasak, Mihkel Näks, Priit Eek, Alla Piirsoo, Rohit Bhadoria, Pavel Starkov, Merilin Saarma, Sergo Kasvandik, and Marko Piirsoo. Characterization of protein kinase ulk3 regulation by phosphorylation and inhibition by small molecule su6668. Biochemistry, 57:5456-5465, Aug 2018. URL: https://doi.org/10.1021/acs.biochem.8b00356, doi:10.1021/acs.biochem.8b00356. This article has 11 citations and is from a peer-reviewed journal.
12. (maloverjan2010dualfunctionof pages 7-8): Alla Maloverjan, M. Piirsoo, L. Kasak, Lauri Peil, Torben Østerlund, and P. Kogerman. Dual function of unc-51-like kinase 3 (ulk3) in the sonic hedgehog signaling pathway\*. The Journal of Biological Chemistry, 285:30079-30090, Jul 2010. URL: https://doi.org/10.1074/jbc.m110.133991, doi:10.1074/jbc.m110.133991. This article has 63 citations.
13. (maloverjan2010identificationofa pages 3-4): Alla Maloverjan, M. Piirsoo, Piret Michelson, P. Kogerman, and T. Osterlund. Identification of a novel serine/threonine kinase ulk3 as a positive regulator of hedgehog pathway. Experimental cell research, 316 4:627-37, Feb 2010. URL: https://doi.org/10.1016/j.yexcr.2009.10.018, doi:10.1016/j.yexcr.2009.10.018. This article has 107 citations and is from a peer-reviewed journal.
14. (preuss2020nucleotidebindingevolutionary pages 13-13): Franziska Preuss, Deep Chatterjee, Sebastian Mathea, Safal Shrestha, Jonathan St-Germain, Manipa Saha, Natarajan Kannan, Brian Raught, Robert Rottapel, and Stefan Knapp. Nucleotide binding, evolutionary insights, and interaction partners of the pseudokinase unc-51-like kinase 4. Structure, 28:1184-1196.e6, Nov 2020. URL: https://doi.org/10.1016/j.str.2020.07.016, doi:10.1016/j.str.2020.07.016. This article has 27 citations and is from a domain leading peer-reviewed journal.