## Phylogeny

– Orthologs are conserved from Saccharomyces cerevisiae Vps34, Drosophila melanogaster Vps34 and Arabidopsis thaliana AtVps34 to Mus musculus Pik3c3, demonstrating a pan-eukaryotic lineage (bilanges2019pi3kisoformsin pages 24-25).  
– PIK3C3 is the sole member of the class III PI3K group and represents the primordial branch within the PI3K/PI4K superfamily (bilanges2019pi3kisoformsin pages 11-12).  
– Evolutionary segregation from class I and II PI3Ks underlies distinct catalytic and regulatory properties (burke2023beyondpi3kstargeting pages 22-23).  
– The VPS15 regulatory pseudokinase partnership is conserved from yeast to mammals (backer2016theintricateregulation pages 2-3).

## Reaction Catalyzed

– ATP + phosphatidyl-1-D-myo-inositol → ADP + phosphatidyl-1-D-myo-inositol-3-phosphate (PI(3)P) (backer2016theintricateregulation pages 1-2).

## Cofactor Requirements

– Catalysis requires divalent cations, with Mg²⁺ (and Mn²⁺ in vitro) supporting phosphotransfer (cook2025structuralpathwayfor pages 6-8).

## Substrate Specificity

– Exclusively phosphorylates phosphatidylinositol to generate PI(3)P; no activity toward PI(4)P or PI(4,5)P₂ (burke2023beyondpi3kstargeting pages 22-23).  
– Substrate recognition is lipid-based; no peptide consensus motif has been defined (bilanges2019pi3kisoformsin pages 11-12).

## Structure

– Domain organisation: N-terminal C2 (membrane docking), central helical scaffold, C-terminal kinase domain capped by an autoinhibitory α-helix (bilanges2019pi3kisoformsin pages 11-12).  
– Assembles into V-shaped tetramers with VPS15, Beclin-1 and ATG14 (complex I) or UVRAG (complex II) (backer2016theintricateregulation pages 2-3).  
– Catalytic residues Lys833 and Asp950 occupy the conserved HRD/DFG motifs in cryo-EM structures (cook2025structuralpathwayfor pages 6-8).  
– A basic membrane-binding patch (Arg561, Arg566, Lys567, Lys568) plus helix Kα12 insert into bilayers to orient the active site (cook2025structuralpathwayfor pages 4-6).  
– The C-terminal helix occludes the ATP pocket in solution and is displaced upon membrane engagement (bilanges2019pi3kisoformsin pages 11-12).  
– High-resolution structures of PI3KC3 complexes are deposited as PDB 5DFZ, 6XIO and 6SEE (li2019ampkandautophagy. pages 305-309).  
– An adjacent hydrophobic cavity in the ATP pocket underpins inhibitor selectivity (burke2023beyondpi3kstargeting pages 24-25).

## Regulation

– ULK1 phosphorylates Ser249, enhancing PI3KC3-C1 activity (licheva2022phosphoregulationofthe pages 11-12).  
– CDK1/5 phosphorylate Thr159, weakening Beclin-1 binding and inhibiting autophagy (licheva2022phosphoregulationofthe pages 9-10).  
– PRKD1 phosphorylates Thr677, promoting catalytic activity (licheva2022phosphoregulationofthe pages 9-10).  
– Cul3-KLHL20 ubiquitinates Vps34 during prolonged starvation, driving degradation (backer2016theintricateregulation pages 12-13).  
– USP10/USP13 deubiquitinate Beclin-1, stabilising the complex (backer2016theintricateregulation pages 12-13).  
– VPS15 clamps the activation loop; GTP-loaded RAB1A triggers a 140° rotation of the kinase domain to relieve inhibition (cook2025structuralpathwayfor pages 3-4).  
– NRBF2 and AMBRA1 positively modulate complex I (bilanges2019pi3kisoformsin pages 11-12).  
– Rubicon binds complex II and suppresses activity (bilanges2019pi3kisoformsin pages 11-12).  
– The ATG14 BATS domain senses high membrane curvature, amplifying catalysis on phagophores (bilanges2019pi3kisoformsin pages 11-12).  
– Nutrient-responsive kinases mTORC1 and AMPK modify complex components to couple autophagy with metabolic status (bilanges2019pi3kisoformsin pages 12-14).

## Function

– Ubiquitous expression; complete knockout causes embryonic lethality at E6.5–E8.5 (bilanges2019pi3kisoformsin pages 14-15).  
– Complex I nucleates autophagosomes at the endoplasmic reticulum via PI(3)P generation (bilanges2019pi3kisoformsin pages 11-12).  
– Complex II governs autophagosome maturation and endosome-lysosome trafficking (backer2016theintricateregulation pages 1-2).  
– PI(3)P recruits FYVE/PX-domain effectors (e.g., DFCP1, WIPI proteins) to coordinate membrane dynamics (backer2016theintricateregulation pages 2-3).  
– Generates midbody PI(3)P to recruit FYVE-CENT, TTC19 and KIF13A for ESCRT-mediated abscission (backer2016theintricateregulation pages 13-14).  
– Lysosomal PI(3)P attracts PLD1, protrudin and FYCO1, facilitating amino-acid-dependent mTORC1 activation (bilanges2019pi3kisoformsin pages 14-15).  
– Endosomal PI(3)P activates SGK3 and positions PTEN, influencing downstream kinase signalling (bilanges2019pi3kisoformsin pages 31-33).  
– Supports macropinocytosis, phagocytosis and endosomal sorting (bilanges2019pi3kisoformsin pages 11-12).

## Inhibitors

– SAR405: pyrimidinone, K\_D 1.5 nM, cellular IC₅₀ 27 nM; blocks autophagy and synergises with everolimus in renal carcinoma cells (pasquier2016autophagyinhibitors pages 6-8).  
– VPS34-IN1: bis-aminopyrimidine, IC₅₀ 25 nM; highly selective (pasquier2016autophagyinhibitors pages 6-8).  
– PIK-III: bis-aminopyrimidine, IC₅₀ 18 nM; potent autophagy inhibitor (pasquier2016autophagyinhibitors pages 6-8).  
– Compound 31: orally bioavailable; 50 % target inhibition at 0.37 µM in rodents (pasquier2016autophagyinhibitors pages 6-8).  
– SB02024 and related molecules exploit the hydrophobic cavity adjacent to the P-loop for nanomolar potency (burke2023beyondpi3kstargeting pages 24-25).

## Other Comments

– Germline PIK3C3 mutations cause monogenic diseases, highlighting dosage sensitivity (burke2023beyondpi3kstargeting pages 24-25).  
– Systemic inhibition carries on-target toxicity risks due to essential housekeeping functions (burke2023beyondpi3kstargeting pages 24-25).  
– Heterozygous kinase-dead knock-in mice display improved insulin sensitivity and protection from diet-induced steatosis (bilanges2019pi3kisoformsin pages 14-15).  
– VPS15 mutations that destabilise the complex impair secretion pathways (backer2016theintricateregulation pages 12-13).

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