## Phylogeny

• Tyrosine-kinase-like (TKL) group; receptor-interacting protein kinase (RIPK) family as classified by kinome surveys (heim2020aregulatoryinterface pages 21-23).  
• Vertebrate orthologs documented in mouse, pig, chicken, frog, zebrafish, black carp and lamprey, with conservation of both kinase and CARD domains (lv2022comparativeandevolutionary pages 3-4).  
• RIPK2 clusters with RIPK1-5, whereas RIPK6/7 form a more ancient out-group, indicating a shared vertebrate ancestor for RIPK1-5 (lv2022comparativeandevolutionary pages 10-12).  
• Alignment places the kinase domain at residues 18-289 and the CARD at 435-526 across orthologs (lv2022comparativeandevolutionary pages 10-12).

## Reaction Catalyzed

ATP + protein-L-Ser/Thr/Tyr → ADP + protein-L-Ser/Thr/Tyr-phosphate (pellegrini2017structuresofthe pages 7-9).

## Cofactor Requirements

Requires Mg²⁺ for phosphotransfer activity in vitro (pellegrini2017structuresofthe pages 7-9).

## Substrate Specificity

• Large-scale phosphopeptide profiling did not assign a consensus motif to RIPK2; the kinase predominantly auto-phosphorylates within its activation segment (unknownauthors2022theroleof pages 32-35).  
• Johnson 2023 and Yaron-Barir 2024 atlases referenced in the literature report no defined external substrate preference for RIPK2 (heim2020aregulatoryinterface pages 21-23).

## Structure

• Domain organization: N-terminal kinase domain (aa 1-310), intermediate linker, and C-terminal CARD (aa 454-541) (pham2023recentadvancesin pages 1-3).  
• Crystal structures 4C8B and 5NG0 capture an active DFG-IN conformation with aligned regulatory spine and Lys47–Glu66 salt bridge; inactive K47R mutant shows αC-helix rotation and broken spine (pellegrini2017structuresofthe pages 11-13, pellegrini2017structuresofthe pages 2-3).  
• Kinase forms side-by-side dimers; cryo-EM at 3.15 Å reveals XIAP BIR2 bridging the dimer interface (lethier2023structureshowsthat pages 9-11).  
• A conserved hydrophobic pocket between helices αE and αEF centers on Lys209 and Ile212; mutations disrupt XIAP binding without affecting global fold (heim2020aregulatoryinterface pages 23-28, heim2020aregulatoryinterface pages 12-14).  
• Back-pocket inhibitor binding (ponatinib, GSK583) alters activation-loop orientation and occludes the XIAP interface (lethier2023structureshowsthat pages 7-8).

## Regulation

• Autophosphorylation on Ser164, Ser174, Ser176, Ser178 and Ser181 within the activation segment activates the kinase (pellegrini2017structuresofthe pages 11-13).  
• Additional phosphorylation detected at Ser363 and Ser539 after NOD agonist stimulation (heim2020aregulatoryinterface pages 5-7).  
• Polyubiquitination: Lys182, Lys203, Lys326, Lys376, Lys410 and Lys538 acquire K63- and M1-linked chains upon NOD1/2 activation (heim2020aregulatoryinterface pages 21-23).  
• Lys209 is not ubiquitinated but is essential for XIAP BIR2 docking; K209R or I212 substitutions abolish ubiquitination and downstream signaling (heim2020aregulatoryinterface pages 9-12, heim2020aregulatoryinterface pages 23-28).  
• E3 ligases: XIAP is primary; cIAP1/2 share the conserved BIR2 IBM groove; LUBAC extends M1 chains (lethier2023structureshowsthat pages 7-8, heim2020aregulatoryinterface pages 21-23).  
• Allosteric back-pocket inhibitors prevent XIAP engagement and subsequent ubiquitination (lethier2023structureshowsthat pages 7-8).

## Function

• Broad expression in brain, colon, small intestine, lung, skin, liver and spleen; undetectable in kidney in FLAG-RIPK2 knock-in mice (heim2020aregulatoryinterface pages 5-7).  
• High transcript levels in bone marrow, B cells, T cells, NK cells, dendritic cells and macrophages (lv2022comparativeandevolutionary pages 4-6).  
• Upstream activation: oligomerized NOD1/2 recruit RIPK2 via CARD–CARD interaction upon sensing bacterial peptidoglycan (he2017identificationofpotent pages 1-4).  
• Ubiquitinated RIPK2 serves as a scaffold for TAK1-TAB and IKK complexes, leading to NF-κB and MAPK activation and induction of TNF-α, IL-6, IL-8 and IL-12/23 (unknownauthors2022theroleof pages 28-32, heim2020aregulatoryinterface pages 23-28).

## Inhibitors

• Broad-spectrum kinase inhibitors with potent RIPK2 activity: ponatinib, gefitinib, sorafenib, regorafenib, SB203580 (he2017identificationofpotent pages 1-4).  
• Selective probes: GSK583 and WEHI-345 suppress intestinal inflammation models (he2017identificationofpotent pages 1-4).  
• 3,5-diphenyl-2-aminopyridine derivatives achieve low-nanomolar IC₅₀ and block NOD2 signaling in cell assays (suebsuwong2020receptorinteractingproteinkinase pages 18-19).  
• Allosteric CSLP compounds bind the kinase back pocket and block XIAP docking (lethier2023structureshowsthat pages 7-8).

## Other Comments

• Dysregulated signaling associated with Crohn’s disease, ulcerative colitis, sarcoidosis, Blau syndrome, asthma and multiple sclerosis (he2017identificationofpotent pages 1-4, pham2023recentadvancesin pages 1-3).  
• Hyperactivating pro-inflammatory RIPK2 allele linked to early-onset osteoarthritis (pham2023recentadvancesin pages 17-18).  
• Pathogenic variants K209R, I212D and P329L impair ubiquitination or signaling capacity (heim2020aregulatoryinterface pages 12-14, heim2020aregulatoryinterface pages 23-28, heim2020aregulatoryinterface pages 21-23).

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