

Supplemental Figure S1 Clustal alignment of ROP2 and ROP8. Conserved subdomains characteristic of serine threonine kinases as defined by Hanks and Hunter are indicated. High homology exists across the entire protein with the exception of the substrate-binding region (within subdomain VIII).

Supplemental Figure S2 Clustal alignment of ROP2, ROP8 and ROP18 showing conserved interacting motifs between the N-terminal regulatory and the kinase core. The N-terminal subdomain is underlined. The conserved residues that make structurally conserved contacts between the kinase domain and the N-terminal extension are highlighted in different colors. A conserved aspartic acid highlighted in yellow located just before the start of α N1 (Asp200 on ROP2, Asp209 on ROP8, Asp180 on ROP18) interacts with a conserved triad on α F of the kinase domain – Gln392-Gln396-Arg399 on ROP2, Gln401-Gln405-Arg408 on ROP8 and Tyr373-Gln377-Lys380 on ROP18 (all also highlighted in yellow). Tucked between α N2 and β N is a conserved tryptophan (Trp238 in ROP2, Trp247 in ROP8 and Trp218 in ROP18, all highlighted in green) that extends its side chain into a hydrophobic pocket formed by a conserved quartet of amino acids in the beta lobe – Leu272-Val285-Val287-Phe358 in ROP2, Leu281-Val294-Val296-Phe367 in ROP8 and Tyr252-Val267-Val269-Phe338 in ROP18 (all highlighted in green as well). Highlighted in magenta is a conserved aspartate on α N2 (Asp230 on ROP2, Asp239 on ROP8 and Asp210 on ROP18) interacting with a conserved arginine on the beta sheet β 1 (Arg259 in ROP2, Arg268 in ROP8 and Arg239 in ROP18, also highlighted in magenta). In addition, the conserved beta strand β 1 is highlighted in cyan. These interacting motifs are also found in ROP4, ROP5 and ROP7 but not in ROP16 and ROP17.

Supplemental Figure S3 Conserved interacting motifs between the N-terminal extension and the kinase core shown on the structures of ROP2, ROP8 (in green) and ROP18. Red structural features including side chains are from ROP2. Green side chains including side chains are from ROP8. Yellow side chains including side chains are from ROP18. In addition, residue labels are italicized for ROP18 to distinguish them from the pseudokinase side chains. Blue structural features and side chains are from the N-terminal regulatory domain. A) A

conserved aspartic acid located just before the start of α N1 (blue) (Asp200 on ROP2 shown) interacts with a conserved triad on α F of the kinase domain – Gln392-Gln396-Arg399 on ROP2 (red), and Tyr373-Gln377-Lys380 on ROP18 (yellow). B) Tucked between α N2 and β N is a conserved tryptophan (blue) (Trp247 in ROP8 shown) that extends its side chain into a hydrophobic pocket formed by a conserved quartet of amino acids in the beta lobe – Leu272-Val285-Val287-Phe358 in ROP2, Leu281-Val294-Val296-Phe367 in ROP8 (green) and Tyr252-Val267-Val269-Phe338 in ROP18 (yellow). C) A conserved aspartate on α N2 (Asp230 in ROP2 shown in blue) interacts with a conserved arginine on the beta sheet β 1 (Arg259 in ROP2 shown in red, Arg239 in ROP18 in yellow).

Supplemental Figure S1

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ROP2 : -----MENCASVRSSSSCLIWLAFAAFVVSALGHVQQGAGVVRPRHWQNSEAAVSVRPPGGASPRPFHSPIEPVAFIDGHEHDEKHE : 80
ROP8 : MFSVLRNRNMRHGPEISSSSCLIWLAFAAFVVSALGHVQQGAGVVRPRHWQNSEAAVSVRPPGGASPRPFHSPIEPVAFIDGHEHDEKHE : 89

ROP2 : GSWLEQEAADVTPLPDSHTEAQTQSPSAFRRLRRLRFWRRGVTTGGSDAGEEAPQTPRPSLRTRILQYLRRVGTFFRRDI PAAALRFF : 169
ROP8 : GSWLEQEAADVTPLPDSHTEAQTQSPSAFRRLRRLRFWRRGVTTGGSDAGEEAPQTPRPSLRTRILQYLRRVGTFFRRDI PAAALRFF : 178

                                <----αN1---->      <----αN2---->  <---βN--->      <--
ROP2 : RRFRRVRQPVFPPDEF PEDVDTNPIYFRGTDPGDVVIEELFNRI PQANVRTTSEYMQSAADSLVSTSLWNTGQPFVSESELGERPRTL : 258
ROP8 : RRFRRVRQPVFPPDEF PEDVDTNPIYFRGTDPGDVVIEELFNRI PQANVRTTSEYMQSAADSLVSTSLWNTGQPFVSESELGERPRTL : 267
                                <-----N-terminal regulatory domain----->|<-----

-β1><β2> <--β3-->  <---β4--->      <-----αC----->      <--αD-->      <-β5>
ROP2 : RGTVLGQEDDPYAYLEATDQETGESFEVHVVPYFTERPPSNAIKQMKKEVLRRLRLLRGIKNQKQAKVHLRFIFPFDLVKDPQKKKMI R : 347
ROP8 : RGTVLGQEDDPYAYLEATDQETGESFEVHVVPYFTERPPSNAIKQMKKEVLRRLRLLRGIKNQKQAKVHLRFIFPFDLVKDPQKKKMI R : 356
--Hanks-I----->|<---Hanks-II----->|<-----Hanks-III----->|<---Hanks-IV----->|

                                <β6>      <-----αE----->  <-----αF----->
ROP2 : DERDMWVLSRFFLYPRMQSNLQTFGEVLLSHSSTHKSLVHHARLQTLQVIRLLASLHHYGLVHTYLRPVDIVLDQGGVFLTGFEHLV : 436
ROP8 : DERDMWVLSRFFLYPRMQSNLQTFGEVLLSHSSTHKSLVHHARLQTLQVIRLLASLHHYGLVHTYLRPVDIVLDQGGVFLTGFEHLV : 445
|<-----Hanks-V----->|<-----Hanks-VIa----->|<---Hanks-VIb-->|<---Hanks-VII

<-β7->                                <-β8->  <-----αG----->                                <----αH---->
ROP2 : RDGASAVSPTGRGFAPPEPTABR-MLEFGQHPTLMTAFDTMTLGLALYWIWICADLPNTDAAALGGSEWIFRSCKNIPOPVRALLEGF : 524
ROP8 : RDGARVVSIVSRGFEPPELEARRATI SYHRDRRTLMTSEDAWALGLVYWIWICADLPNTDAAALGGSEWIFRSCKNIPOPVRALLEGF : 534
->|<---Hanks-VIII----->|                                |<-----Hanks-IX----->|<---Hanks-X----->|<-----

                                <-----αI----->
ROP2 : LRYPKEDRLLPLQAMETPEYEQLRTELSAALPLYQTDGEPTRREGGAPPSGTSQPDEAGAAAVTAI : 590
ROP8 : LRYPKEDRLLPLQAMETPEYEQLRTELSAALPLYQTDGEPTRREGGAPPSGTSQPDEAGAAAVTAI : 600
-----Hanks-XI----->|

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Supplemental Figure S2

ROP2 -----MENCASVRSSSCLIWLAFAFFVSALGHVQOGAGVVRPRHWQNSEA 45
 ROP8 MFSVLRN-----RNMHRGPSIGSSSCLIWLAFAFFVSALGHVQOGAGVVRPRHWQNSEA 54
 ROP18 MFSVQRPLTRTVVRMGLATLLPKTACLAGLNVALVFLLF-QVQDGTGITLGPSKLDK 58

ROP2 AVSVRPPGGASPRPFHSPIEPVAFIDGHEDEDKHEGSWLEQEAEDVTPLPDSHTEAQTQ 105
 ROP8 AVSVRSPGGASPRPFHSPIEPVAFIDGHEDEDKHEGSWLEQEAEDVTPLPDSHTEAQTQ 114
 ROP18 PTSLDSQQHVADKRWLATVGHYKHLGAT-ESTRDVSLLEERAQHRVN-----AQETNQ 111

ROP2 SPSAFRRLTRRLRFWRRGVTGGSDAGEEAPQTPRPSLRTRILQYLRRVGTFFRRDIPAAA 165
 ROP8 SPSAFRRLTRRLRFWRRGVTGGSDAGEEAPQTPRPSLRTRILQYLRRVGTFFRRDIPAAA 174
 ROP18 RRTIFQRLNLLRRRERDGE-VSGSAADSSSRPRLSVRQRLAQLWRRAKSLFKRGIR--- 167

ROP2 LRFRRFRVRQPVFPDEFPEVDNPIYFRGTD**PGDVVIEELFNRIPOANVRTTSEYM** 225
 ROP8 LRFRRFRVRQPVFPDEFPEVDNPIYFRGTD**PGDVVIEELFNRIPOANVRTTSEYM** 234
 ROP18 -RYFPQGRNRQR-----SLRAQRRRSELVFEKAD**SGCVIGKRILAHMQEQIGQPOALEN** 220

ROP2 **QSAADSLVSTSLWNTGQPFVSE**ELGERPRTLVRGTVLQEDPYAYLEATDQETGESFEV 285
 ROP8 **QSAADSLVSTSLWNTGQPFVSE**ELGERPRTLVRGTVLQEDPYAYLEATDQETGESFEV 294
 ROP18 **SERLDRIILTVAANPPDVPKRFVSV**TGT**TRTLVRGAPLGS**GGFATVYEATDVETNEELAV 280

ROP2 HVPYFTERPPSNAIKQMKEEVLRRLRLLRGIKNQKQAKVHLRFIFPFDLVKDPQKKKMRV 345
 ROP8 HVPYFTERPPSNAIKQMKEEVLRRLRLLRGIKNQKQAKVHLRFIFPFDLVKDPQKKKMRV 354
 ROP18 KVFMSKEPTDETMLDLQRESSCYRNFSLAKTAKDAQESCRFMVPSDVVMLEGQPASTE 340

ROP2 RLDERDMWVLSRFLYPRMQSNLQTFGEVLLSHSSTHKS-LVHHARLQTLQVIRLLASL 404
 ROP8 RLDERDMWVLSRFLYPRMQSNLQTFGEVLLSHSSTHKS-LVHHARLQTLQVIRLLASL 413
 ROP18 VIGLTRWVPNYFLMMRAEADMSKVISWVFGDASVKNSEFGLVVRMYLSSQAIKLVANV 400

ROP2 HHYGLVHTYLRPVDIVLDQRGGVFLTGFELVRDQASAVSPIGRGFAPPETTAER-MLPF 463
 ROP8 HHYGLVHTYLRPVDIVLDQRGGVFLTGFELVRDQARVVSSVSRGFEPPELEARRATISY 473
 ROP18 QAQGIVHTDIKPANFLLKDGRLFLGDFGTYRINNSVGRAIGTPGYEPPEPFPQATGITY 460

ROP2 GQHHTLMTFAFDTWTLGLAIYWIWCADLPNTDDAALGGSEWIFRSCKNIPQVRRALLEG 523
 ROP8 HRDRRTLMTFSFDALGLVIYWIWCADLPITKDAALGGSEWIFRSCKNIPQVRRALLEG 533
 ROP18 -----TFPTDAWQLGITLYCIWCKERPTPADGIWD--YLFHADCPSTPELVQDLIRS 510

ROP2 FLRYPKEDRLLPLQAMETPEYEQLRTELSAALPLYQTDGEPTRREGGAPPSGTSQPDEAGA 583
 ROP8 FLRYPKEDRLLPLQAMETPEYEQLRTELSAALPLYQTDGEPTRREGGAPPSGTSQPDEAGA 593
 ROP18 LLNRDPQKRMLPLQALETAAFKEMDSVVKGAAQNFEQQEHLHTE----- 554

ROP2 AEAVTAI 590
 ROP8 AEAVTAI 600
 ROP18 -----

Supplemental Figure S3

