**SUPPLEMENTARY MATERIAL**

**Supplementary figure 1.** Pairwise sequence alignment of human POLR3A and yeast RPC1.

**Supplementary figure 2.** Multiple sequence alignment of human POLR3A with orthologues showing the extent of conservation and residues contributing to the intermolecular interactions within the DNA-dependent RNA polymerase III complex.

**Supplementary figure 3.** Structural and functional impact of the identified disease-associated missense *POLR3A* variants.

**Supplementary table 1.** Exome sequencing data output.

**Supplementary table 2.** List of variants in affected subjects WRS001 and WRS002 retained after WES data filtering.

**Supplementary table 3.** *POLR3A* targeted sequencing output.

**Supplementary figure 1.** **Pairwise sequence alignment of human POLR3A and yeast RPC1.** The alignment has been used for modeling human POLR3A based on the structure of yeast RPC1, and shows the considerable conservation between the two orthologues (invariant residues are indicated by asterisks, conserved residues by colons, and semi-conserved residues by periods). Residues of yeast RPC1 that are engaged in intermolecular interactions (either with other subunits or nucleic acid chains) within the RNA polymerase III elongation complex (PDB ID: 5FJ8) are marked by blue letters on top of the alignment. These letters denote the interactors using the same annotation reported in the PDB structure. The identities of the yeast and human orthologues corresponding to the chains of the complex participating to the intermolecular interactions follow: chain B, RPC2, POLR3B; chain C, RPAC1, POLR1C; chain D, RPC9, CRCP; chain E, RPABC1, POLR2E; chain F, RPABC2, POLR2F; chain G, RPC8, POLR3H; chain H, RPABC3, POLR2H; chain I, RPC10, POLR3K; chain K, RPAC2, POLR1D; chain O, RPC3, POLR3C; chain P, RPC6, POLR3F. The chains referring to nucleic acid interactors include R (RNA), S (non-template DNA) and T (template DNA). POLR3A and RPC1 share 49% amino acid identity, which increases to 54% when amino acid identity is calculated exclusively for residues involved in intermolecular interactions. Residues affected by disease-associated missense mutations are highlighted in cyan together with the identified amino acid substitution.

**GGGG G BBB B BBBB B B G O**

POLR3A KEQFRETDVAKKISHICFGMKSPEEMRQQAHIQVVSKNLYSQDNQHAPLLYGVLDHRMGT

5fj8-A KEVVVS-ETPKRIKGLEFSALSAADIVAQSEVEVSTRDLFDLEKDRAPKANGALDPKMGV

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**B B O O**

POLR3A SEKDRPCETCGKNLADCLGHYGYIDLELPCFHVGYFRAVIGILQMICKTCCHIMLSQEEK

5fj8-A SSSSLECATCHGNLASCHGHFGHLKLALPVFHIGYFKATIQILQGICKNCSAILLSETDK

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**O O E R RO OO O SS O S**

POLR3A KQFLDYLKRPGLTYLQKRGLKKKISDKCRKKNICHHCGAFNGTVKKCGLLKIIHEKYKTN

5fj8-A RQFLHELRRPGVDNLRRMGILKKILDQCKKQRRCLHCGALNGVVKKAAALKIIHDTFRWV

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**R O O O O OO P OOOO**

POLR3A KKVVDPIVSNFLQSFETAIEHNKEVEPLLGRAQENLNPLVVLNLFKRIPAEDVPLLLMNP

5fj8-A GKKSAPEKDIWVGEWKEVLAHNPELERYVKRCMDDLNPLKTLNLFKQIKSADCELLGIDA

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**O B TB OO**

POLR3A --EAGKPSDLILTRLLVPPLCIRPSVVSDLKSGTNEDDLTMKLTEIIFLNDVIKKHRISG

5fj8-A TVPSGRPETYIWRYLPAPPVCIRPSVMMQDSPASNEDDLTVKLTEIVWTSSLIKAGLDKG

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**R**

**O OO OO B R B B R B BB**

POLR3A AKTQMIMEDWDFLQLQCALYINSELSGIPLN------MAPKKWTRGFVQRLKGKQGRFRG

5fj8-A ISINNMMEHWDYLQLTVAMYINSDSVNPAMLPGSSNGGGKVKPIRGFCQRLKGKQGRFRG

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**R K**

**B BBBBBB B B B BB BB B FF**

POLR3A NLSGKRVDFSGRTVISPDPNLRIDEVAVPVHVAKILTFPEKVNKANINFLRKLVQNGPEV

5fj8-A NLSGKRVDFSGRTVISPDPNLSIDEVAVPDRVAKVLTYPEKVTRYNRHKLQELIVNGPNV

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**B B TR B B**

POLR3A HPGANFIQQRHTQMKRFLKYGNREKMAQELKYGDIVERHLIDGDVVLFNRQPSLHKLSIM

5fj8-A HPGANYLLKRNEDARRNLRYGDRMKLAKNLQIGDVVERHLEDGDVVLFNRQPSLHRLSIL

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**T**

**K BB B B TB BB B FBFF FB B**

POLR3A AHLARVKPHRTFRFNECVCTPYNADFDGDEMNLHLPQTEEAKAEALVLMGTKANLVTPRN

5fj8-A SHYAKIRPWRTFRLNECVCTPYNADFDGDEMNLHVPQTEEARAEAINLMGVKNNLLTPKS

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**K**

**B HHH CKC KK KK HHHHHH H**

POLR3A GEPLIAAIQDFLTGAYLLTLKDTFFDRAKACQIIASILVGKDEKIKVRLPPPTILKPVTL

5fj8-A GEPIIAATQDFITGSYLISHKDSFYDRATLTQLLSMMS---DGIEHFDIPPPAIMKPYYL

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**H HH H**

POLR3A WTGKQIFSVILRPSDDNPVRANLRTKGKQY-----CGKGEDLCANDSYVTIQNSELMSGS

5fj8-A WTGKQVFSLLIKPNHNSPVVINLDAKNKVFVPPKSKSLPNEMSQNDGFVIIRGSQILSGV

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**K BB BB B**

POLR3A MDKGTLGSGSKNNIFYILLRDWGQNEAADAMSRLARLAPVYLSNRGFSIGIGDVTPGQGL

5fj8-A MDKSVLGDGKKHSVFYTILRDYGPQEAANAMNRMAKLCARFLGNRGFSIGINDVTPADDL

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**H**

POLR3A LKAKYELLNAGYKKCDEYIEALNTGKLQQQPGCTAEETLEALILKELSVIRDHAGSACLR

5fj8-A KQKKEELVEIAYHKCDELITLFNKGELETQPGCNEEQTLEAKIGGLLSKVREEVGDVCIN

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**HH B BB B B B B**

POLR3A ELDKSNSPLTMALCGSKGSFINISQMIACVGQQAISGSRVPDGFENRSLPHFEKHSKLPA

5fj8-A ELDNWNAPLIMATCGSKGSTLNVSQMVAVVGQQIISGNRVPDGFQDRSLPHFPKNSKTPQ

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**R**

**B BB BB B B B BB B R B BB FF**

POLR3A AKGFVANSFYSGLTPTEFFFHTMAGREGLVDTAVKTAETGYMQRRLVKSLEDLCSQYDLT

5fj8-A SKGFVRNSFFSGLSPPEFLFHAISGREGLVDTAVKTAETGYMSRRLMKSLEDLSCQYDNT

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**G903R**

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**E E E H**

POLR3A VRSSTGDIIQFIYGGDGLDPAAMEGKDEPLEFKRVLDNIKAVFPCPSEPALSKNELILTT

5fj8-A VRTSANGIVQFTYGGDGLDPLEMEGNAQPVNFNRSWDHAYNITFNNQDKGLLPYAIMETA

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POLR3A ESIMKK-----------------SEFLC--------CQDSFLQEIKKFIKGVSEKIKKTR

5fj8-A NEILGPLEERLVRYDNSGCLVKREDLNKAEYVDQYDAERDFYHSLREYINGKATALANLR

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**HH H H**

POLR3A DKYGINDNGT-----------------TEPRVLYQLDRITPTQVEKFLETCRDKYMRAQM

5fj8-A KSRGMLGLLEPPAKELQGIDPDETVPDNVKTSVSQLYRISEKSVRKFLEIALFKYRKARL

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**R1069Q**

**F B B** | **S**

POLR3A EPGSAVGALCAQSIGEPGTQMTLKTFHFAGVASMNITLGVPRIKEIINASKAISTPIITA

5fj8-A EPGTAIGAIGAQSIGEP----------------MNVTLGVPRIKEIINASKVISTPIINA

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**K1131R**

**IIII I** | **E**

POLR3A QLDKDDDADYARLVKGRIEKTLLGEISEYIEEVFLPDDCFILVKLSLERIRLLRLEVNAE

5fj8-A VLVNDNDERAARVVKGRVEKTLLSDVAFYVQDVYKDNLSFIQVRIDLGTIDKLQLELTIE

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**E II IB I**

POLR3A TVRYSICT-SKLRVKPGDVAVHGEAV--VCVTPRENSKSSMYYVLQFLKEDLPKVVVQGI

5fj8-A DIAVAITRASKLKIQASDVNIIGKDRIAINVF---PSENDVFYRMQQLRRALPDVVVKGL

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**E EE E E EEE**

POLR3A PEVSRAVIHIDEQSGKEKYKLLVEGDNLRAVMATHGVKGTRTTSNNTYEVEKTLGIEAAR

5fj8-A PDISRAVINIRDDGKRE---LLVEGYGLRDVMCTDGVIGSRTTTNHVLEVFSVLGIEAAR

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**D1292N**

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**E E E RS R**

POLR3A TTIINEIQYTMVNHGMSIDRRHVMLLSDLMTYKGEVLGITRFGLAKMKESVLMLASFEKT

5fj8-A YSIIREINYTMSNHGMSVDPRHIQLLGDVMTYKGEVLGITRFGLSKMRDSVLQLASFEKT

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**G1335R**

| **F**

**B B** | **B B B FFGFG G G GGG DD D**

POLR3A ADHLFDAAYFGQKDSVCGVSECIIMGIPMNIGTGLFKLLHKAD-RDPNPPKRPLIFDTNE

5fj8-A TDHLFDAAFYMKKDAVEGVSECIILGQTMSIGTGSFKVVKGTNISEKDLVPKRCLFESLS

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**DD**

POLR3A FHIPL

5fj8-A NEAAL

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**Supplementary figure 2. Multiple sequence alignment of human POLR3A with orthologues showing the extent of conservation and residues contributing to the intermolecular interactions within the DNA-dependent RNA polymerase III complex.** Residues of yeast RPC1 engaged in intermolecular interactions with other subunits of the RNA polymerase III elongation complex were identified in the PDB structure 5FJ8 and mapped on the human POLR3A based on the sequence alignment reported in Supplementary figure 1. Conservation of the alignment is shown at the bottom (invariant residues are indicated by an asterisk, conserved residues by a colon, and semi-conserved residues by a period). Interactions with other subunits are reported with blue letters above the alignment (the involved subunit is indicated using the same single letter annotation of molecular chains of PDB structure 5FJ8; see legend of supplementary figure 1 for correspondence between chains and proteins/nucleic acids within the complex). Vertebrate orthologues share >80% amino acid identity in residues involved in intermolecular interactions. Residues affected by disease-associated missense mutations are highlighted in cyan together with the identified amino acid substitution.

**interaction GGGG G BBB B BBBB B B G O**

H. sapiens MVKEQFRETDVAKKISHICFGMKSPEEMRQQAHIQVVSKNLYSQ-DNQHAPLLYGVLDHR

M. musculus MVKEQFRETDVAKKISHICFGMKSPEEMRQQAHIQVVSKNLYSQ-DNNHAPLLYGVLDHR

O. anatinus -----------------ICFGMKSPEEMRQQAHIQVVSKNLYSQ-DNHHSPLLHGVLDHR

G. gallus MVKEQFRESDVAKKISHICFGMKSPEEMRQQAHIQVVSKNLYSQ-DNHHAPLQYGVLDHR

X. tropicalis -MKEQFRETDVAKKISHICFGVKSAEQMRQQAHIQIVSKSLYSQ-DNNHTPLAFGVLDHR

D. rerio MVKEQFRETDVARKISHICFGMKSSEQMRQQAHIQVVSKNLYSQ-DTSHKPLQYGVLDHR

H. comes MVKEQFRETDVAKKISHICFGMKSADQMRQQAHIQVVSKNLYSQ-DTKHSPLPYGVLDHR

D. melanogaster MPKEQFRASALNKKISHVQFGISGADEIQQEALVRIISKNLY---QAQRQPVPYGVLDRR

C. briggsae MGKEQFREADKALKVVGVKFAPGDCEFMRQTAHVPIFNNKLYEDVGGKLQPASYGPLDPR

V. vinifera --KEPYIEDVGPRKIESIRFALFSESEISKAAEVQVWRGVYY---DANRKPIENGLLDPR

D. carota --KQPYIEDVGPRRIKSITFSTFSEADIFKAAEVQVYKDKYY---DEKRKPVEGGLLDPR

E. histolytica -------------KVCDIQFTMFSQEEMNRESEFEVTEHFLFQ--PNTRKPNPNGVLDRR

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**interaction B B O O**

H. sapiens MGTSEKDRPCETCGKNLADCLGHYGYIDLELPCFHVGYFRAVIGILQMICKTCCHIMLSQ

M. musculus MGTSEKDRPCETCGKNLADCLGHYGYIDLELPCFHVGYFRAVIGILQMICKTCCHIMLSQ

O. anatinus MGTSEKDRPCETCGKNLADCLGHYGYIDLELPCFHVGYFKAVIGVLQMICKTCCHIMLSS

G. gallus MGTSEKDRPCETCGKNLADCLGHYGYIDLELPCFHVGYFKAVIGILQMICKTCCRIMLSV

X. tropicalis MGTSEKDRHCQTCGKNLAECLGHYGYLDLELPCFHVGYFKAVIGILQMICKTCSRILLNA

D. rerio MGTSEKDRPCETCGKNLADCLGHYGYLDLELPCFHIGYFKAIVGILQMICKTCSHILLTK

H. comes MGTSEKDRPCLTCGKNLADCLGHYGYLDLELPCFHVGYFKAIIGILQMVCKTCSSIMLSK

D. melanogaster MGISTKDAMCETCGQGLNECIGHFGYLDLALPVFHIGHFRSTINILQMICKVCAHVMLKP

C. briggsae MGVSTKTGICATCGLGLTDCVGHFGYFDLDVPVFHIGFFKLTIQLLQCICKNCSSILLTP

V. vinifera MGPANKNGTCATCLGNFRDCPGHCGYLTLALPVYNVGYLSTIVDILKCICKSCSRVLLDE

D. carota MGPTSKNGFCATCDGSFRDCPGHYGCLTLSVPVYNVGYLGTIVDILKCICKSCASILLPE

E. histolytica LGVCDSLSVCETCGKRIEECSGHFAVINLELPVFHIGFFRPIIQVLQCVCKNCNRILLTN

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**interaction O O E R RO OO O SS**

H. sapiens EEKKQFLDYLKRPGLTYLQKRGLKKKISDKC------RKK-NICHHCGAFNGTVKKC--G

M. musculus EEKQQFLDFLKRPGLTYLQKRGLKKKISDKC------RKK-STCHYCGAFNGTVKKC--G

O. anatinus EERKQFLDYLKRPGLTYLQKRGLKKKVSDKC------KKK-NTCHHCGAFNGTVKKC--G

G. gallus EEKKQFLDYLKRPGLTYLQKRGLKKKVSEKC------RKK-NTCPYCGAFNGTVKKC--G

X. tropicalis EERKQFLDFLKRPGLTYLQKRGLKKKISDKC------RKK-TVCLYCGAYNGSVKKC--G

D. rerio EEKLQFLDYLRRPGLAYLQKRGLKKKISDKC------RKK-TVCLNCNAFNGSVKKC--G

H. comes EDKLQFMDMLKRPNLPYLQKRGLKKKISDKC------RKR-SVCLNCSAFNGTVKKC--G

D. melanogaster EDRQLYEKKLHNPNFSYLGKKALHVQMLAKA------KKV-TKCPHCGSPNGGVKKGP-G

C. briggsae EQHRVFSRQVMNPNIDYLHRKALHKRIVAAC------KKS-STCTQCGLKNGTVKKAVGA

V. vinifera KASKDYLKKMRSQKMEALKKAELMKKIVQKCTAMASSKKA-VKCSRCGYMNGIVKKAV-S

D. carota KEYADYLKKMRK-DIDPLKKNELFKRIVKKCTAMASSKKA-VKCLKCGYINGTVKRA---

E. histolytica EERDFYLRRMRNKKTDTVARTYLVRALIAQC------RKHYNSCPHCNAINGSIKKY--G

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**interaction O S R O O O O OO P**

H. sapiens LLKIIHEKYKTNKKVVDPIVSNFLQSFETAIEHNKEVEPLLGR--AQENLNPLVVLNLFK

M. musculus LLKIIHEKYKTNKKVVDPIVSNFLQSFETAIEHNKEVEPLLGR--AQENLNPLVVLNLFK

O. anatinus LLKIIHEKYKTNKKVVDPVVSNFLQSFETAIEYNKEVEPLLGR--AQENLNPLVVLNLFK

G. gallus LLKIIHEKYKTNKKVVDPIVSTFLQSFETAIEHNKEVEPLLGR--AQENLNPLVVLNLFK

X. tropicalis LLKIIHEKYKTTKKVVDPMVSHFLQSFDTAIEHNKEVESLLGR--AQENLNPLIVLNLLR

D. rerio LLKIIHEKYKSTKKVVDPAVSDFLQSFDVAIEHNKEVESLLTR--AQENLNPLVVLNLFR

H. comes LLKIIHEKYKTTKKVVEPFVSDFLQSFDTAIEHNKLMEPLLAR--AQENLNPLVVLSIFM

D. melanogaster LLKILHDPYKGRK--MDSLFTSNMNEMLRSTQTNRDLNSTLGNYSTAEELTPLMVLDLFE

C. briggsae VLKIAF---------ASPVPADELGKYQTVFNANQEIGDHVKK-MKFTLLNPLFVQKLFA

V. vinifera VLGIIHDRSK--------IADGSLEECSSAISHTKESKASF---SVVDILNPVKVLSLFK

D. carota GLGVEHDRSK--------VTDSSLEESRSAISHLKDSTGS----KSPSIINPDRVLSLFK

E. histolytica NLRLVHDKYHRDP--TITEMNEFYDGFEESIKLDKDINAFINN--AQEDLDPRKVLSIFR

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**interaction O OO O B TB**

H. sapiens RIPAEDVPLLLMNP-EAGKPSDLILTRLLVPPLCIRPSVVSDLKSGTNEDDLTMKLTEII

M. musculus RIPAEDVPLLLMNP-ESGKPSDLILTRLLVPPLCIRPSVVSDLKSGTNEDDLTMKLTEII

O. anatinus RIPAEDIPLLLMNP-EAGKPSDLILTRLLVPPLCIRPSVVSDLKSGTNEDDLTMKLTEII

G. gallus RIPAEDIPLLLMNP-EAGKPSDLILTRLLVPPLCIRPSVVSDLKSGTNEDDLTMKLTEII

X. tropicalis QIPAEDIPLLLMNP-EAGKPSDLILTRLLVPPLCIRPSVVSDLKSGTNEDDLTMKLTEII

D. rerio RIPDKDVPLLLMNP-ESGKPADLILTRLLVPPLCIRPSVVSDLKSGTNEDDLTMKLTEII

H. comes RIPQEDVPLLLMNP-EAGKPADLILTRLLVPPLCIRPSVVSDLKSGTNEDDLTMKLTEII

D. melanogaster QIPQRDVALLGMCS-HDAHPKHLIVTRVFVPPACIRPSVLSEVKAGTTEDDLTMKQSEIL

C. briggsae NIKEADIPVLMVRSGEEKHPNDLLLTRMPVPPVCIRPSVVSEVKSGTTEDDVTMKLMEIM

V. vinifera RMMDEDCDLLNL----AERPEKLVLTNIGVPPIAIRPSVFMDGGTQSNENDVTERLKRII

D. carota KIQDKVCDLLYL----SDRPEKFLITSIAVPPVPIRPSVFVDGGQMSNENDISERLKLII

E. histolytica SLIREDIETMDFDP-DNAPPENMILTSVPVPPIAIRPSVAMDGGNASNEDDLTTQLKEIM

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**interaction OO O OO OO B R B B**

H. sapiens FLNDVIKKHRISGAKTQMIMEDWDFLQLQCALYINSELSGIPLNMAPKKWTRGFVQRLKG

M. musculus FLNDVIKKHRISGAKTQMIMEDWDFLQLQCALYINSELSGIPLNMAPKKWTRGFVQRLKG

O. anatinus FLNDVIKKHRISGAKTQMIMEDWDFLQLQCALYINSELSGIPLNMAPKKWTRGFVQRLKG

G. gallus FLNDVIKKHRISGAKTQMIMEDWDFLQLQCALYINSELSGIPLNMAPKKWTRGFVQRLKG

X. tropicalis FLNDVIKKHRITGAKTQMIMEDWDFLQLQCALYINSELSGIPMNMAPKKWTRGFVQRLKG

D. rerio FLNDVIKKHRMTGAKTQMIMEDWDFLQLQCALYINSELSGIPLNMAPKKWTRGFVQRLKG

H. comes FLNDVIKKHRMTGAKTQMIMEDWDFLQLQCALYINSELSGIPLNMAPKKWTRGFVQRLKG

D. melanogaster LINDVIQRHMATGGKIELIHEDWDFLQLHVALYFHSEISGIPINMAPKKTTRGIVQRLKG

C. briggsae LTNDVLKKHKRDGAPAKTLFETWEHLQIQCALYINSEMSGLPPDMQPKRAMRAFTQRLKG

V. vinifera QANASLHQELQDTGSTSKCLAGWDYLQIEVAQYINSDVRGVPLAMQAARPLSGFVQRLKG

D. carota QTNATLHQNLTTASYAPKALELWPLLQVEVARYINSDVRGVPLSMQESKPLSGFVQRLKG

E. histolytica ECNDRIKEMIKRGASPYQYYDSWDQLTVAVSMFINSELPGCDDK---GKPLRAICQRLKG

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**interaction R R K**

**R B BB B BBBBBB B B B BB BB B FF**

H. sapiens KQGRFRGNLSGKRVDFSGRTVISPDPNLRIDEVAVPVHVAKILTFPEKVNKANINFLRKL

M. musculus KQGRFRGNLSGKRVDFSGRTVISPDPNLRIDEVAVPVHVAKILTFPEKVNKANINFLRKL

O. anatinus KQGRFRGNLSGKRVDFSGRTVISPDPNLRIDEVAVPVHVAKILTFPEKVNKANISFMRKL

G. gallus KQGRFRGNLSGKRVDFSGRTVISPDPNLRIDEVAVPIHVAKILTFPEKVNKANINFMRKL

X. tropicalis KQGRFRGNLSGKRVDFSGRTVISPDPNLRIDEVAVPVHVAKILTFPEKVNQANMQLMRKL

D. rerio KQGRFRGNLSGKRVDFSGRTVISPDPNLRIDEVAVPVHVAKILTYPEKVNKANIELMRKL

H. comes KQGRFRGNLSGKRVDFSGRTVISPDPNLRIDEVAVPVHVAKILTYPERVNKANLELMRKL

D. melanogaster KQGRFRCNLSGKRVDFSGRTVISPDPNLMINQVGVPVRVAKILTYPERVNPANIRHMREL

C. briggsae KQGRFRGNLCGKRVDFSGRTVISPDPNLRIDQVGVPVHVAMTLTFPEIVNASNIDKMRKL

V. vinifera KQGRFRGNLSGKRVEYTGRTVISPDPNLKITEVAIPILMAKILSYPERVSHHNIEKLRQC

D. carota KQGRFRGNLSGKRVEYTGRTVISPDPNLKITEVAIPILMAQILTYPERVSSHNIEKLRQC

E. histolytica KHGRFRGNLLGKRVDFSGRTVISPDPNLDIEQIGVPKLMAVKLTYPERVTKHNIDKLRQL

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**interaction B B TR**

H. sapiens VQNGPEVHPGANFIQQRHTQMKRFL--KYGNREKMAQELKYGDIVERHLIDGDVVLFNRQ

M. musculus VRNGPDVHPGANFIQQRHMQMKRFL--KYGNREKMAQELKFGDIVERHLIDGDVVLFNRQ

O. anatinus VRNGPEVHPGANFIQQRHMQMKRFL--KYGNREKMAQELKYGDIVERHLIDGDVVLFNRQ

G. gallus VRNGPDVHPGANFIQQRHTQMKRFL--KYGNREKMAQELKFGDIVERHLIDGDIVLFNRQ

X. tropicalis VQNGPDVHPGANFIQQRHTQMKRFL--KYGNREKIAQELRFGDIVERHLIDGDIVLFNRQ

D. rerio VRNGPEVHPGANFIQQRHMQMKRFL--KYGNREKMAQELKFGDVVERHMIDGDIVLFNRQ

H. comes VRNGPDVHPGANFIQNRRNQMKRFL--KYGNREKIAQELRFGDIVERHMIDGDIVLFNRQ

D. melanogaster VRNGPSMHPGANYVQQRGSSFKKYL--AYGNREKVAQELKCGDVVERHLRDGDIVLFNRQ

C. briggsae IVNGSDVHPGANYLVDKKTGHKKLL--KYGNREELAKNLRLGDTVERHLDDNDVVLFNRQ

V. vinifera ILNGPFKYPGAKLIRYPDGSM-RSL--MFSGRKRFADELKYGYIVERHLEDGDVVLFNRQ

D. carota VRNGCYKYPGANFIRMADGTL-MVL--KFPSRVRLADELKFGQIVERHLEDGDIILFNRQ

E. histolytica VINGSNNYPGCNSIIGGSDGLRRSMCIKLEMREKFAKDLKIGDIVERHLEDNDIVLFNRQ

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**interaction T**

**B B K BB B B TB BB B FBFF FB**

H. sapiens PSLHKLSIMAHLARVKPHRTFRFNECVCTPYNADFDGDEMNLHLPQTEEAKAEALVLMGT

M. musculus PSLHKLSIMAHLARVKPHRTFRFNECVCTPYNADFDGDEMNLHLPQTEEAKAEALVLMGT

O. anatinus PSLHKLSIMAHLARVKPHRTFRFNECVCTPYNADFDGDEMNLHLPQTEEAKAEALVLMGT

G. gallus PSLHKLSIMAHIARVKPHRTFRFNECVCTPYNADFDGDEMNLHLPQTEEAKAEALVLMGT

X. tropicalis PSLHKLSIMAHIARVKPHRTFRFNECVCTPYNADFDGDEMNLHLPQTEEAKAEAYVLMGT

D. rerio PSLHKLSIMAHIARVKPHRTFRFNECVCTPYNADFDGDEMNLHLPQTEEAKAEALVLMGT

H. comes PSLHKLSIMAHIARVKPHRTFRFNECVCTPYNADFDGDEMNLHLPQTEEAKAEALILMGT

D. melanogaster PSLHKMSIMCHRAKVQPQRTFRFNECACTPYNADFDGDEMNLHLPQTEEARAEALILMGN

C. briggsae PSLHKISIMSHRAKVMPGRTFRFNECACTPYNADFDGDEMNLHLPQTYEAKAEASELMNV

V. vinifera PSLHRMSIMCHRARIMPWRTLRFNESVCNPYNADFDGDEMNMHVPQTEEARTEALMLMGV

D. carota PSLHRMSIMCHRARIMPWRTLRFNESVCNPYNADFDGDEMNMHVPQTEEARTEALMLMGV

E. histolytica PSLHRISIMCHRAKILPWRTLRFNECVCTPYNADFDGDEMNLHVPQTEEARAECLMLMHS

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**interaction K**

**B B HHH CKC KK KK**

H. sapiens KANLVTPRNGEPLIAAIQDFLTGAYLLTLKDTFFDRAKACQIIASIL-VGKDEKIKVRLP

M. musculus KANLVTPRNGEPLIAAIQDFLTGAYLLTLKDTFFDRAKACQIIASIL-VGKDEKIKVRLP

O. anatinus KANLVTPRNGEPLIAAIQDFLTGAYLLTLKDTFFDRAKTCQIIASIL-VGKDEKIKVRLP

G. gallus KANLVTPRNGEPLIAAIQDFLTGAYLLTLKDTFFDRAKACQIIASIL-VGKDEKIKVRLP

X. tropicalis KANLVTPRNGEPLIAAIQDFLTAAYLLTLKDTFFDRAKACQIIASIL-VGKDEKIKVRLP

D. rerio KANLVTPRNGEPLIAAIQDFLTGAYLLTLKDTFFDRTKACQIVASIL-VGKDEKIKISLP

H. comes KANLVTPRNGEPLIAAIQDFLTAGYLLTLKDTFFDRSKACQIVASIL-VGKDEKVKIALP

D. melanogaster QSNLVTPKNGEILIAATQDFITGGYLLTQKEVFLTKEEAMQLAACFL-ANEDSTMHIKLP

C. briggsae KNNLITPRSGEPLVAAIQDFITGGYLLTHKDTFLPRAEVYRFAAALIDASAKKQSKIRIP

V. vinifera QNNLCTPKNGEILVASTQDFLTSSFLITRKDTFYDRAAFSLMCSYMG----DGMDLVDLP

D. carota QNNLCTPKNGEILVASTQDFLTSSFLITRKDTFYDRASFSLMCCYMG----DAMDHIDLP

E. histolytica AKNLQTPRTGQMIIAETQDFLTTSYLLTSKDFVIEEEDMMQWACWFH----NAGTEIDLP

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**interaction HHHHHH H H HH**

H. sapiens PPTILKPVTLWTGKQIFSVILRPSDDNPVRANLRTKGKQYCGKG--EDLCANDSYVTIQN

M. musculus PPTILKPVTLWTGKQIFSVILRPSDDNPVRANLRTKGKQYCGKG--EDLCVNDSYVTIQN

O. anatinus PPTILKPVTLWTGKQVFSLILRPSDASPVRANLRTKGKQYCGRG--EDLCVNDSYVTIQN

G. gallus PPAILKPVTLWTGKQVFSSILKPSDDCPVKANLRTKGKQYCGKG--EDLCYNDSYVTIQN

X. tropicalis PPAILKPVTLWTGKQVFSLILQPSHDNPVRANLRTKGKQYCGKG--EDLCHNDSYVTIQN

D. rerio HPAILKPIRLWTGKQIFSLILKPSKSCPVMANLRTKGKQYCGKG--EDLCANDSFVVIHN

H. comes RPSIVKPMALWTGKQIFSLILKPSKDCPVKANLRTKGKQYCGKG--EDLCHNDSFVVIHN

D. melanogaster PPALLKPRRLWTGKQMFSLLMRPNDDSQVRLNMVNKGRNYTRN---KDLCSNDSWIHIRN

C. briggsae PPAIRRPVELWTGKQLIELIIRPDKGSQVSLNLTAKNKSYSGN---LELCSKDSYVIIRN

V. vinifera TPAIIKPVELWTGKQLFNVLLRPHANVRVYLNLTVMEKTYNKRRGKETMCPSDGFVYFRN

D. carota TPAVIKPIELWTGKQLFSVLLRPHANMRVYLTLTVKEKSYTSG---ETMCQSDGFVYFRN

E. histolytica TPAYLKPKKLWTGKQLYGLALKPNKHEGVNISLDVPSKLYSKEG--KWMCVKDGWICFKN

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**interaction H K BB BB B**

H. sapiens SELMSGSMDKGTLGSGSKNNIFYILLRDWGQNEAADAMSRLARLAPVYLSNRGFSIGIGD

M. musculus SELMCGSMDKGTLGSGSKNNIFYILLRDWGQNEAADAMSRLARLAPVYLSNRGFSIGIGD

O. anatinus SELMSGSMDKGTLGSGSKNNIFYILLRDWGQLEAADAMSRLARLAPVYLSNRGFSIGIGD

G. gallus SELMSGSMDKGTLGSGSKNNIFYILLRDWGQVEAADAMSRLARLAPVYLSNRGFSIGIGD

X. tropicalis SELMCGSLDKGTLGSGSKNNIFYILLRDWGQVEAADAMSRLARLAPVFLSNRGFSIGIGD

D. rerio SELMCGSMDKGTLGSGSKNNIFYILLRDWGQQEAADAMSRLARLAPVYLSNRGFSIGIGD

H. comes SELMCGSMDKSTLGSGSKNNIFYILLRDWGQLEAANAMSRLARLAPVYLSNRGFSIGIGD

D. melanogaster SELMCGVMDKATMGSGTKQCIFYLLLRDFGESHATKAMWRLARIASYFLQNRGFSFGISD

C. briggsae SVLLAGVLDKSLLGSSSKVNIFYMLMRDYGEDAAVDAMWRLARMAPVFLSNRGFSIGIGD

V. vinifera SELISGQLGKATLGNGNKDGLFSVLLRDYNAHAAAACMNRLAKLSARWIGNHGFSIGIDD

D. carota SELVSGQLGKATLGNGNKDGMYSILLRDYKAHAASVCMNRLAKLSARWIGNHGFSIGIDD

E. histolytica SQLLSGQIEKSIIGSGNKTGLFHLILRDYGTLESAKIMAHISRFCARFLGDYGFSIGISD

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**interaction**

H. sapiens VTPGQGLLKAKYELLNAGYKKCDEYIEALNTGKLQQQPGCTAEETLEALILKELSVIRDH

M. musculus VTPGQGLLKAKYELLNAGYKKCDEYIEALNTGKLQQQPGCTAEETLEALILKELSVIRDH

O. anatinus VTPGQGLLKAKYELLNAGYKKCDEYIEALNTGKLQQQPGCTAEETLEALILKELSVIRDH

G. gallus VTPGQGLLKAKYELLHAGYKKCDEYIEALNTGKLQQQPGCTAEETLEALILKELSVIRDH

X. tropicalis VTPGQGLLKAKQDLLDAGYKKCDEYIEALNTGKLQPQPGCSAEETLEALILRELSVIRDH

D. rerio VTPGQGLLKAKQELLDAGYEKCDEYIDALKTGRLQQQPGCTAEETLEALILKELSVIRDH

H. comes VTPGQGLLKAKQDLLDDGYRKCDEYIEALKTGKLQQQPGCTAEETLEALILRELSVIRDR

D. melanogaster VTPSKKLLQHKELLLNNGYAKCNEYIELLKAGTLQCQPGCTPEETLESVMLRELSAIREQ

C. briggsae VRPSERLLQEKGQLVDNGYEKCAQYIRELEEGKLKAQPGCTEEETLEAIILRELSTIRDH

V. vinifera VQPGGLLNDQKSKRIEEGYENCHELIQQYNKGKLKLQPGCNAAQTLEAEITGVLNKIRET

D. carota VQPGDLLNKDKEGKITDGNVQCDELINKYNNGKIELLAGCDAAQTLETLIAKVLNDIRGE

E. histolytica VTPSADLTQMKEELVSEGYKKCDQFIEDYKSGKQEVQAGSTPEQTLEALLNNELSQIRER

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**interaction H HH B BB B B B B**

H. sapiens AGSACLRELDKSNSPLTMALCGSKGSFINISQMIACVGQQAISGSRVPDGFENRSLPHFE

M. musculus AGSACLRELDKSNSPLTMALCGSKGSFINISQMIACVGQQAISGSRVPDGFENRSLPHFE

O. anatinus AGSACLRELDKSNSPLVMAVCGSKGSFINISQMIACVGQQAISGSRVPDGFENRSLPHFE

G. gallus AGSACLRELDKSNSPLIMALCGSKGSFINISQMIACVGQQAISGSRVPDGFENRSLPHFE

X. tropicalis AGSACLRELDKSNSPLIMALCGSKGSFINISQMIACVGQQAISGSRVPDGFENRSLPHFE

D. rerio AGSACLRELDKSNSPLIMALCGSKGSFINISQMIACVGQQAISGSRVPDGFENRSLPHFQ

H. comes AGSACLRELDKSNSPLIMALCGSKGSFINISQMIACVGQQAISGSRVPDGFENRSLPHFE

D. melanogaster AAKTCFAELHPTNSALIMALSGSKGSNINISQMIACVGQQAISGKRVPNGFENRALPHFE

C. briggsae AGQVCLRNLSKYNAPLTMAVCGSKGSFINISQMIACVGQQAISGHRPPDGFEERSLPHFE

V. vinifera TANVCMEELHWRNSPLIMSQCGSKGSPINISQMIACVGQQSVGGRRAPDGFIDRTLPHFP

D. carota AAKLCMKSLHWRNSPLIMSQCGSKGSPINISQMVACVGQQSVSGGRAPNGFLDRSLPHFH

E. histolytica AGKRCIEELKSDNSPLVMALSGSKGSVINISQMIACVGQQTVNGGRVSDGFISRSLPHFA

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**interaction R**

**B BB BB B B B BB B R B BB**

H. sapiens KHSKLPAAKGFVANSFYSGLTPTEFFFHTMAGREGLVDTAVKTAETGYMQRRLVKSLEDL

M. musculus KHSKLPAAKGFVANSFYSGLTPTEFFFHTMAGREGLVDTAVKTAETGYMQRRLVKSLEDL

O. anatinus KHSKLPAAKGFVANSFYSGLTPTEFFFHTMAGREGLVDTAVKTAETGYMQRRLVKSLEDL

G. gallus KHSKLPAAKGFVANSFYSGLTPTEFFFHTMAGREGLVDTAVKTAETGYMQRRLVKSLEDL

X. tropicalis KHSKLPAAKGFVANSFYSGLTPTEFFFHTMAGREGLVDTAVKTAETGYMQRRLVKSLEDL

D. rerio KHSKLPAAKGFVADSFYSGLTPTEFFFHTMAGREGLVDTAVKTAETGYMQRRLVKSLEDL

H. comes KHSKLPAAKGFVADSFYSGLTPTEFFFHTMAGREGLVDTAVKTAETGYMQRRLVKSLEDL

D. melanogaster RHSAIPAARGFVQNSFYSGLTPTEFFFHTMAGREGLVDTAVKTAETGYLQRRLVKCLEDL

C. briggsae RKKKTPEAKGFVANSFYSGLTPTEFFFHTMGGREGLVDTAVKTAETGYMQRRLVKCLEDL

V. vinifera RKSKTPDAKGFVASSFYTGLTATEFFFHTMGGREGLVDTAVKTADTGYMSRRLMKALEDL

D. carota KNSKIPAAKGFVGNSFYSGLTATEFFFHTMGGREGLVDTAVKTADTGYISRRLMKALEDL

E. histolytica LHSRTPQAKGFVKNSFFTGLNATEFFFHTMGGREGLVDSAVKTAETGYMQRRLMKALEDL

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**G903R**

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**interaction FF E E E H**

H. sapiens CSQYDLTVRSSTGDIIQFIYGGDGLDPAAMEGKDE-P-LEFKRVLDNIKAVFPCPSEPAL

M. musculus CSQYDLTVRSSTGDIIQFIYGGDGLDPAAMEGKDE-P-LEFKRVLDNIKAVYPCQTEPAL

O. anatinus CSQYDLTVRSSTGDIIQFIYGGDGLDPAAMEGKDE-P-LEFKRVLDNIKAVFPCQSEPAL

G. gallus CSQYDLTVRSSTGDIIQFIYGGDGLDPAAMEGKDE-P-LEFKRVLDNIRAVYPCRSEPAL

X. tropicalis CSQYDLTVRSSTGDIIQFIYGGDGLDPAAMEGKDE-P-LEFKRVLENVKAVYPCSEEPAL

D. rerio CSQYDLTVRSSTGDIIQFIYGGDGLDPAAMEGKDE-P-LEFKRVLDNIRAVYSCTGEPAL

H. comes CSQYDLTVRSSTGDIIQFIYGGDGLDPADMEGKDE-P-LEFKRVLDNIRAVYTCAHEPTL

D. melanogaster VVHYDGTVRNAVNEMVDTIYGGDGLDPVSMETRNK-P-VDLVHQYDNLRAQHPQGKDRPL

C. briggsae CASYDGTVRSSVGDVIEFVFGEDGLDPSMMEAKDG-SVVDFTHVLEHAKNIQTKK-EKPI

V. vinifera SIQYDETVRNANGSIVQFLYGDDGMDPARMEGKDGFP-LNFNRLFLKVKATCPAGENASL

D. carota AVHYDKTVRNASGCIVQFTYGDDGMDPSQMEEKGGLP-LNFDRLLLKVKAACPPEEHIGM

E. histolytica HVHYDGTVRSASLTVIEFKYGDDGLDPLKVESDKY-P-INLESVLSNLQQNQKPD-DEYI

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**interaction**

H. sapiens SKNELILTTESIMKKSEFL---CCQDSFLQEIKKF-IKGVSEKIK---------------

M. musculus SKNDLTLTAEAIMKKNEFL---CCQDSFLQEIKTF-IKGVSEKIK---------------

O. anatinus SKNELVLTTESIMKKNEFL---CCQDSFLQEIKKF-IKVVSEKIK---------------

G. gallus SKNELVLTSESIMKKNEFL---CCRDSFLQEIKKF-IKGVSEKIK---------------

X. tropicalis SKNELILTSESIMKKTEFL---CCQDSYLQEIKKF-VTGVSERIK---------------

D. rerio SRNELLLTSDSIMKRKDFL---CCKDTFLEEIRKF-IKTVSEKVK---------------

H. comes SKGELVLASDTIMKKADFV---CCRNSFLEEIKKF-IRGFSERIK---------------

D. melanogaster NAEEMSEALETLLRTPEFA---EARDDFKLDVRNH-INTVSKRIG---------------

C. briggsae PAEKLDEVLKTEV-QKQFK---EKYVHFADQMKDYLLQTEIKKSKKWQGIQTHCAEHESA

V. vinifera SALQIEETVKR-LKEHNTSAE-GCSDAFKTNLSGF-LEECKEKFK---------------

D. carota PSSEIVKVVDETLTKHVMASEGDCSKAFNKSLSEF-LKACAERSN---------------

E. histolytica TPESFDKEYNAIISQTKIV-----SELWKQQIETF-LKEKKEEMT---------------

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**interaction HH H H F**

H. sapiens --KTRDKYGINDN------GTTEPRVLYQLDRITPTQVEKFLETCRDKYMRAQMEPGSAV

M. musculus --KTRDKYGINDN------GTTEPRVLYQLDRITPTQIEKFLETCRDKYMRAQMEPGSAV

O. anatinus --KTRDKYGINDN------GTTEPRVLYQLDRITPTQLEKFLETCRDKYMRAQMEPGSAV

G. gallus --KTRDKYGINDN------GTTEPRVLYQLDRITPTQLEKFLETCRDKYMRAQMEPGSAV

X. tropicalis --KTRDKYGINDN------GTTEPRVLYQLDRITPTQLEKFLETCRDKYMRAQMEPGSAV

D. rerio --KTRDKYGINDN------GTTEPKVLYQLDRITPTQLEKFLETCRDKYMRAQMEPGSAV

H. comes --KTRDKYGINDN------GTSEPKVLYQLDRITPTQLKKFLETCRDKYMRAQMEPGSAV

D. melanogaster --QLQKRYEK------------CIDLCHQIECLTTEQLLQFVRRINDRYNRAVTEPGTAV

C. briggsae NAKTREECAKCTN------LDTYRTSLLKNSCLTRSQLLSFIQLCYYKIARAITEPGTAV

V. vinifera --NTREALGLHGEHVGEENLDIQEKFAKNISGITSKQLQVFLDTCISRYQLKRIEAGTAI

D. carota --NTRVALKLEKE-LRSEDFDNLENVAKNISGITHQQLQVFMETCISRYHQKVVEGGTAI

E. histolytica --EIYNHY-------------NQENAQHGLCGITIQTLKDFISICHEKGLRSVCEAGTPV

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**R1069Q**

**interaction** **B B** | **S**

H. sapiens GALCAQSIGEPGTQMTLKTFHFAGVASMNITLGVPRIKEIINASKAISTPIITAQLDKDD

M. musculus GALCAQSIGEPGTQMTLKTFHFAGVASMNITLGVPRIKEIINASKAISTPIITAQLDKDD

O. anatinus GALCAQSIGEPGTQMTLKTFHFAGVASMNITLGVPRIKEIINASKAISTPIITAQLDSDD

G. gallus GALCAQSIGEPGTQMTLKTFHFAGVASMNITLGVPRIKEIINASKAISTPIITAQLDKDD

X. tropicalis GALCAQSIGEPGTQMTLKTFHFAGVASMNITLGVPRIKEIINASKNISTPIITAHLDIDD

D. rerio GALCAQSIGEPGTQMTLKTFHFAGVASMNITLGVPRIKEIINASKNISTPIISANLDTND

H. comes GALCAQSIGEPGTQMTLKTFHFAGVASMNITLGVPRIKEIINASKNISTPIITAHLDVED

D. melanogaster GAIAAQSIGEPGTQMTLKTFHFAGVASMNITQGVPRIVEIINATKTISTPIITAELENCH

C. briggsae GAIAATSIGEPSTQMTLKTFHFAGVASMNITQGVPRIKEIINAVKTISTPIITASLLDPY

V. vinifera GAIGAHSIGEPGTQMTLKTFHFAGVASMNVTLGVPRIKEIINGAKRISTPIITAALECNN

D. carota GAIGAHSIGEPGTQMTLKTFHFAGVASMNVTQGVPRIKEIINAAKNISTPIITAKLEFSD

E. histolytica GALAGQSMGEPSTQMVLKTFHFAGVASMNIALGVPRIKEIINASKKIQTPIVTAKLVNSQ

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**K1131R**

**interaction** **IIII I** | **E E**

H. sapiens DADYARLVKGRIEKTLLGEISEYIEEVFLPDDCFILVKLSLERIRLLRLEVNAETVRYSI

M. musculus DADYARLVKGRIEKTLLGEISEYIEEVFLPDDCFILVKLSLERIRLLRLEVNAETVRYSI

O. anatinus DSDFARLVKGRIEKTLLGEISEYIEEVFLPDDCFILVKLSLERIRLLRLEVNAETVRYSI

G. gallus DPDFARLVKGRIEKTLLGEISEYIEEVFLPDDCFILVKLSLERIRLLRLEVNAETVRYSI

X. tropicalis DADFARLVKGRIEKTLLGEISEYIEEVFLPDDCFLLVKLSLERIRLLRLEVNAETVRYSI

D. rerio DPDFARLVKGRIEKTLLGEISEYIEEVFLPDDTFILVKLSLDRIRLLRLEVNAETVRYSI

H. comes DADFARLVKGRIEKTLLGEISEYIEEVFLPDDCFILVKLSLERIRLLRLEVNAETVRYSI

D. melanogaster SMEFARQVKARIEKTTLAELSSYVEVVCGPYSCYLAIGVDMARIKLLGLHIDLDTIVFSI

C. briggsae DESLARRVKARIEKTTLGEICDYIEEVYLPDDYFLLVKLNSKRIRLLQLEVSLESISYAI

V. vinifera NVKTARMVKGRIERTTLGQVAKSIKIVLTSRLALIAVALDMEGIQASQLSIDSNIVRESI

D. carota SLVSARLVKGRIEKTVLGQVAKSIKIVMASRSASVTVTLDMDVIQASELCINAHTVKESI

E. histolytica DVTSARIVKGRIEATRLKEIAKSVKIVFKPAEAYISVKLDFETIKQLELECTIEKVQDVL

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**interaction II IB I**

H. sapiens CTS-KL--RVKPGDV-AVHGEAVVCVTPRENSKSSMYYVLQFLKEDLPKVVVQGIPEVSR

M. musculus CTS-KL--RVKPGDV-AVHGEAVVCVTPRENSKSSMYYVLQFLKEDLPKVVVQGIPEVSR

O. anatinus CMS-KL--RVKPGDV-AVHGEAVLCVTPRENSKSSMYYVLQFLKEDLPKVVVQGIPEVSR

G. gallus CIS-KL--RVKPGDV-AVHGEAVVCVTPRENSKSSMYYVLQSLKEELPKVVVQGIPEVSR

X. tropicalis CMS-KL--RVKPGDI-AVHGEAVLCVTPRENSKSSMYYVLQSLKEDLPKVVVQGIPEVAR

D. rerio CTS-KL--RVKPGDV-QVHGEAVVCVSPRENSKSSMYYVLQSLKQDLPKVVVQGIPEVSR

H. comes CMS-KL--RVKPGDI-AVHGEAVVCVSPRENNKSSMYYVMQSLKAELPKVVVQGIPEVAR

D. melanogaster LKS-RM--RVKPTQVEVVASQSRIVVRVEATRTSTINAELARLALSLQNVVVAGLPNINR

C. briggsae VTS-KVCPMMRGCKI-ISHGKTMMAIRPPSTSKLSKTMTMQMLKYSLGNVVVKGISSVNR

V. vinifera LRNRRI--KLKQQHI-KVLDAGKLEVHP-QGDRSTIHFELHALKNLLPTVVVKGIETVER

D. carota LSTPKI--KLKEQNI-HVLDARKLEVIP-TSDRSKLHFELHRLKDRLPTVVVRGINSIKR

E. histolytica LNYRGL--KLKDNQV-IIRNKQKLRVYV-DKSSTSLLFALNSLRNQLLEVIVCGIENTGR

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**interaction E EE E E EEE E**

H. sapiens AVIHIDEQSGKEKYKLLVEGDNLRAVMATHGVKGTRTTSNNTYEVEKTLGIEAARTTIIN

M. musculus AVIHIDEQSGKEKFKLLVEGDNLRAVMATHGVKGTRTTSNNTYEVEKTLGIEAARTTIIN

O. anatinus AVIHIDEQSGKEKYKLLVEGDNLRAVMATHGVKGTKTTSNNTYEVEKTLGIEAARTTIIN

G. gallus AVIHIDEQSGKEKYKLLVEGDNLRAVMATHGVKGTKTSSNNTYEVEKTLGIEAARTTIIN

X. tropicalis AVIHIDEQSGKEKYKLLVEGDNLRSVMATHGVKGSRTTSNNTYEVEKTLGIEAARSTIIN

D. rerio AVIHIDEQSGKQKFKLLVEGDNMRSVMATHGVNGSKTTSNNTYEVEKTLGIEAARSTIIN

H. comes AVIHIDEQSSKRKYKLLVEGDNLRAVMATHGVNGSRTTSNNTYEVEKTLGIEAARSTIIN

D. melanogaster AVIAVDDARQPPTYKLCIEGYGLRDVIATYGVVGKRTRSNNICEIYQTLGIEAARTIIMS

C. briggsae CVIHADEKKG-DCYSLLVEGTDFRSVLSSVGVDPKKTNFNNALVVAEVLGIEAARTCIIN

V. vinifera AVINKDNK---VKYNLLVEGTGLQTVMGTEGVIGRETTSNHIIEVQQTLGIEAARKCIIN

D. carota AVIKECDGKK-GTYELAVEGTGLDAVMGTEGVDGPKTTSNHIMEVQKTLGIEAARKCIIL

E. histolytica AVINKNK----GSFELLVEGSELLKVMGTPGVIGEETTSNHIAVVEKVLGIEAARSTIIS

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**D1292N**

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**interaction E E RS R B**

H. sapiens EIQYTMVNHGMSIDRRHVMLLSDLMTYKGEVLGITRFGLAKMKESVLMLASFEKTADHLF

M. musculus EIQYTMVNHGMSIDRRHVMLLSDLMTYKGEVLGITRFGLAKMKESVLMLASFEKTADHLF

O. anatinus EIQYTMVNHGMSIDRRHVMLLSDLMTYKGEVLGITRFGLAKMKESVLMLASFEKTADHLF

G. gallus EIQYTMVNHGMSIDRRHVMLLSDLMTYKGEVLGITRFGLAKMKESVLMLASFEKTADHLF

X. tropicalis EIQYTMVNHGMSIDRRHVMLLADLMTYKGEVLGITRFGLAKMKESVLMLASFEKTADHLF

D. rerio EIQYTMVNHGMSIDRRHVMLLADLMSYKGEILGITRFGLAKMKESVLMLASFEKTADHLF

H. comes EIQYTMVNHGMSIDRRHVMLLADLMSYKGEILGITRFGLAKMKESVLMLASFEKTADHLF

D. melanogaster EITEVMEGHGMSVDWRHIMLLASQMTARGEVLGITRHGLAKMRESVFNLASFEKTADHLF

C. briggsae EIIATMDAHGIGLDRRHVMLLADVMTYRGEVLGITRNGLVKMKDSVLLLASFEKTMDHLF

V. vinifera EIQYTMASHGMSIDIRHMMLLADLMTFRGEVLGITRFGIQKMDKSVLMLASFEKTADHLF

D. carota EIQKTMKD--MSIDVRHMMLLADLMTYKGEVLGITRHGIQKMKDSVLMLASFEKTSDHLF

E. histolytica EIKNVMDAYGLSIDVRHLLLLSDLMTFKGVILGITRYGISKMKDSVFTFASFERTNDHLF

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**G1335R**

**interaction** | **F**

**B** | **B B B FFGFG G G GGG DD D DD**

H. sapiens DAAYFGQKDSVCGVSECIIMGIPMNIGTGLFKLLHKADRDPNPPKRPLIFDTNEFHIPLV

M. musculus DAAYFGQKDSVCGVSECIIMGIPMNIGTGLFKLLHKANRDPKPPRRPLIFDTHEFHIPLV

O. anatinus DAAYFGQKDSVCGVSECIIMGIPMNIGTGLFKLLHKADRDPNPPERPLVFDTSEFHIPIV

G. gallus DAAYFGQKDSVCGVSECIIMGIPMNIGTGLFKLLHKADKESTPPRRPLIFDNNEFHIPIV

X. tropicalis DAAYFGQKDSVCGVSECIIMGIPMNIGTGLFKLLHKADKDPSPPRRPLIFDHEDFHHPFL

D. rerio DAAYFGQKDSVCGVSECIIMGIPMNIGTGLFKLLHRSNREADPPRRPLLFDSPDFHIPM-

H. comes DAAYFGQKDSVCGVSECIIMGIPMNIGTGLFKLLQKADRAPSLTKRPLLFDNADFHIPLA

D. melanogaster DAAYYGQTDAINGVSERIILGMPACIGTGIFKLLQQHEDKQVPPIEPTI-----------

C. briggsae EAAFFSQKDVIHGVSECIIMGTPMTVGTGTFKLMQKYEKKALLKKNTPIFERPELAITL-

V. vinifera NASVSGRDDKIEGVSECIIMGIPMQLGTGILKVRQRLQQVP-------------------

D. carota NASVNGRVDKIEGVSECIIMGIPMQIGTGMLKVMQR------------------------

E. histolytica DAAVHSRKDEIKGVSESIIVGNQVGLGTGLMQILYDPPKP--------------------

:\*: .: \* : \*\*\*\* \*\*:\* :\*\*\* :::

**interaction**

H. sapiens T

M. musculus T

O. anatinus T

G. gallus T

X. tropicalis -

D. rerio -

H. comes T

D. melanogaster -

C. briggsae -

V. vinifera -

D. carota -

E. histolytica -

**Supplementary figure 3. Structural and functional impact of the identified disease-associated missense *POLR3A* variants.** Detailed view around the sites affected by variants mapped on the homology model of the POLR3A subunit within the yeast Pol III elongation complex. For clarity, only the nearest protein/nucleic acid chains to the POLR3A subunit are shown. (A) Gly903. The non-conservative Gly903Arg amino acid substitution is predicted to affect intermolecular interactions with the POLR2E subunit. (B) Asp1292. The Asp1292Asn change is expected to affect POLR3A/POLR2E interactions. (C) Arg1069. The Arg1069Gln substitution is predicted to affect interactions with the POLR2E subunit. (D) Lys1131. It can be noticed that Lys1131 is engaged in multiple interactions with surrounding residues in proximity to the region of binding of POLR3K and close to a region where the latter subunit starts to be disordered. This feature suggests that the changes accompanying the Lys1131Arg change might locally affect the structures of POLR3A and POLR3K in their region of mutual interaction, impairing the formation of the complex. (E) Gly1335. The Gly1335Arg variant is predicted to affect the interactions of POLR3A with POLR3B.

F:\---PAPERS---\---SOTTOMESSI-ACCETTATI---\---POLR3A_Raoul_SUBMITTED\---REVISED MANUSCRIPT---\Emanuele Bellacchio\Fig S3A+B.tif

F:\---PAPERS---\---SOTTOMESSI-ACCETTATI---\---POLR3A_Raoul_SUBMITTED\---REVISED MANUSCRIPT---\Emanuele Bellacchio\Fig S3 C+D+E.tif

**Supplementary table 1. Exome sequencing data output.**

|  |  |  |
| --- | --- | --- |
| Patients | WRS001 | WRS002 |
| Target region coverage with depth >=20x | 94.6% | 89.1% |
| Average sequencing depth on target | 100x | 96x |
| Total variants | 90573 | 71982 |
| Allele frequency <1%a | 5734 | 4351 |
| Coding, splicing (+/-6) and/or clinically-associated variantsb | 1069 | 724 |
| Predicted *de novo* variants | 6 (5 genes)c | NA |
| Predicted homozygous variants | 2c | 2c |
| Predicted compound heterozygous variants | 10 (5 genes)c | 14 (7 genes)c |

a Minor allele frequency is referred to 1000Genomes project Phase1 and Phase3, ESP6500 database, and dbSNP142.

b Variants annotated in HGMD and/or ClinVar.

c See Supplemental Table S3.

Supplementary table 2. List of variants in affected subjects WRS001 and WRS002 retained after WES data filtering.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Patient | Zygosity | Genomic position | Gene | Nucleotide change | Aminoacid change | rs ID |
| WRS001 | *de novo* | Chr3:11372811 | *ATG7* | c.679-3C>T | / | . |
| Chr11:116969114 | *SIK3* | c.16\_18delGCG | p.A6del | . |
| Chr17:40831575 | *CCR10* | c.1085A>G | p.N362S | . |
| Chr5:32379253 | *ZFR* | c.2803C>T | p.R935\* | . |
| Chr2:196728936 | *DNAH7* | c.7443A>C | p.= | rs201418733 |
| Chr2:196728930 | *DNAH7* | c.7449G>A | p.= | . |
| Homozygous | Chr11:114398627 | *NXPE1* | c.408-5\_408-4insT | / | rs76450715 |
| Chr17:1410315 | *INPP5K* | c.507C>T | p.= | rs145033682 |
| Compound heterozygous | Chr1:169495255 | *F5* | c.5600G>T | p.G1867V | rs756675239 |
| Chr1:169509739 | *F5* | c.4589A>C | p.E1530A | rs6007 |
| Chr1:152276023 | *FLG* | c.11339G>A | p.R3780K | rs367989347 |
| Chr1:152284044 | *FLG* | c.3318G>C | p.W1106C | rs753041653 |
| Chr10:79744964 | *POLR3A* | c.3206G>A | p.R1069Q | rs778985686 |
| Chr10:79769277 | *POLR3A* | c.1909+18G>A | / | rs267608677 |
| Chr9:139340132 | *SEC16A* | c.6832C>T | p.P2278S | rs761962148 |
| Chr9:139369091 | *SEC16A* | c.2977G>A | p.A993T | rs11788702 |
| Chr19:57954790 | *ZNF749* | c.274A>G | p.I92V | rs745942628 |
| Chr19:57954822 | *ZNF749* | c.306C>T | p.= | rs368642630 |
|  |  |  |  |  |  |  |
|  |  |  |  |  |  |  |
| WRS002 | Homozygous | Chr6:32497959 | *HLA-DRB5* | c.42\_43insTT | p.L15fs | rs745343465 |
| Chr19:49216607 | *MAMSTR* | c.1165C>T | p. P389S | rs73057542 |
| Compound heterozygous | Chr14:105405287 | *AHNAK2* | c.16501G>A | p.V5501M | rs201885720 |
| Chr14:105409105 | *AHNAK2* | c.12683A>G | p.K4228R | rs200965863 |
| Chr10:99006061 | *ARHGAP19* | c.961C>T | p.H321Y | rs145032100 |
| Chr10:98994996 | *ARHGAP19* | c.1262G>A | p.R421H | . |
| Chr19:19765990 | *ATP13A1* | c.1593C>T | p.= | rs145435578 |
| Chr19:19770591 | *ATP13A1* | c.496G>A | p.G166R | rs144224381 |
| Chr5:41186186 | *C6* | c.712A>C | p.N238H | rs115613673 |
| Chr5:41195939 | *C6* | c.542C>T | p.T181I | rs114609505 |
| Chr3:156697059 | *LEKR1* | c.822G>C | p.M274I | rs200658547 |
| Chr3:156660356 | *LEKR1* | c.598A>G | p.R200G | . |
| Chr2:219495516 | *PLCD4* | c.1263C>T | p.= | rs199832756 |
| Chr2:219492952 | *PLCD4* | c.973C>T | p.R325W | rs61733653 |
| Chr1:152084350 | *TCHH* | c.1343A>G | p.K448R | rs777935551 |
| Chr1:152084175 | *TCHH* | c.1500\_1517dupGGAGAGGCGCGAGCAGCA | p.E501\_Q506dup | . |

## Supplementary table 3. *POLR3A* targeted sequencing output.

|  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Patients | WRS005 | WRS006 (father) | WRS006 (mother) | WRS007 | WRS008 | WRS009 | WRS010 (father) | WRS010 (mother) | WRS011 | WRS012 |
| Target region coverage with depth ≥20 | 100% | 100% | 100% | 100% | 100% | 100% | 100% | 100% | 100% | 100% |
| Average sequencing depth | 430 | 310 | 593 | 287 | 324 | 277 | 273 | 327 | 384 | 408 |
| Total *POLR3A* variantsa | 4 | 2a | 0 | 1 | 2 | 2 | 1 | 0 | 1 | 1 |
| *POLR3A* variants with minor allele frequency ≤1%b | 4 | 1 | 0 | 1 | 2 | 2 | 1 | 0 | 1 | 1 |

a One variant with MAF >1% was not further studied

b Exac/GnomAD