```
CLUSTAL O(1.2.3) multiple sequence alignment

                                                              ↓
UbiX        ----------------------------------------MQSLPASLTGQLFMKRLIVG
Pad1        MLLFPRRTNIAFFKTTGIFANFPLLGRTITTSPSFLTHKLSKEVTRASTSPPRPKRIVVA
AroY-B      --------------------------------------------------------MKLIIG
                                                              ::::.

UbiX        ISGASGAIYGVRLLQVLRDVTDIETHLVMSQAARQTLSLETDFSLREVQALADVTHDARD
Pad1        ITGATGVALGIRLLQVLKEL-SVETHLVISKWGAATMKYETDWEPHDVAALATKTYSVRD
AroY-B      MTGATGAPLGVALLQALRDMPEVETHLVMSKWAKTTIELETPWTAREVAALADFSHSPAD
            ::**:*.   *: ***.*::: .:*****:*: .  *:. ** :   ::* ***  ::.  *

UbiX        IAASISSGSFQTLGMVILPCSIKTLSGIVHSYTDGLLTRAADVVLKERRPLVLCVRETPL
Pad1        VSACISSGSFQHDGMIVVPCSMKSLAAIRIGFTEDLITRAADVSIKENRKLLLVTRETPL
AroY-B      QAATISSGSFRTDGMIVIPCSMKTLAGIRAGYAEGLVGRAADVVLKEGRKLVLVPREMPL
             :* ******:   **::***:*:*:.*   .:::.*: ***** :** * *:*   ** **

UbiX        HLGHLRLMTQAAEIGAVIMPPVPAFYHRPQSLDDVINQTVNRVLDQFAITLPEDLFARWQ
Pad1        SSIHLENMLSLCRAGVIIFPPVPAFYTRPKSLHDLLEQSVGRILDCFGIH--ADTFPRWE
AroY-B      STIHLENMLALSRMGVAMVPPMPAYYNHPETVDDITNHIVTRVLDQFGLD--YHKARRWN
             **. *    .. *. :.**:**:* :*:::.*: :: * *:** *.:    .   **:

UbiX        GA-----------
Pad1        GIKSK--------
AroY-B      GLRTAEQFAQEIE
            *
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FIG S1 Sequence alignment of Pad1, AroY-B and UbiX. Asterisks indicate fully conserved amino acid residues, whereas colons indicate residues with strongly similar properties. Protein sequences were derived from *S. cerevisiae* (reference strain S288C) Pad1 (NP_010827), *K. pneumoniae* AroY-B (AAY57854) and *E. coli* K-12 (AKK15681). The arrow marks the position of the deletion of the first 54 amino acid residues in mutant Pad1$^{\Delta 1\text{-}54}$.

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CLUSTAL O(1.2.4) multiple sequence alignment

KpAroY-Ciso    --MTAPIQDLRDAIALLQQHDNQYLETDHPVDPNAELAGVYRHIGAGGTVKRPTRIGPAM
AnFdc1         MSAQPAHLCFRSFVEALKV-DNDLVEINTPIDPNLE--AAA------ITRRVCETNDKAP
ScFdc1         MRKLNPALEFRDFIQVLKD-EDDLIEITEEIDPNLE--VGA------IMRKAYESHLPAP
KpAroY-C       ----MAFDDLRSFLQALDD-QGQLLKISEEVNAEPDLAAAA------NATGRIGDGAPAL
EcUbiD         -MDAMKYNDLRDFLTLLEQ-QGELKRITLPVDPHLE--ITE------IADRTLRAGGPAL
                     :*. : *. ::. . :: .:                        *

KpAroY-Ciso    MFNNIKGYPHSRIL---------VGMHASRQRAALLLGCEASQLALEVGKAV-----KKP
AnFdc1         LFNNLIGMKNGLFRILGAPGSLRKSSADRYGRLARHLALPPTASMREILDKMLSASDMPP
ScFdc1         LFKNLKGASKDLFSILGCPAGLRSKEKGDHGRIAHHLGLDPKTTIKEIIDYLLECKEKEP
KpAroY-C       WFDNIRGFNDARVT---------MNTIGSWQNHAISLGLPPNTPVKK-----------Q
EcUbiD         LFENPKGYSMPVL----------CNLFGTPKRVAMGMGQEDVSALREVGKLLAFLKEPEP
               *.* *     .                  . *  :.            :

KpAroY-Ciso    VAP----------------VVVPASSAPCQEQIFLADDPDFDLRTLLPAHTNTPIDAGP
AnFdc1         IPP----------------TIVP--TGPCKENSL--DDSEFDLTEL-PVPLIHKSDGGK
ScFdc1         LPP----------------ITVPVSSAPCKTHIL--SEEKIHLQSL-PTPYLHVSDGGK
KpAroY-C       IDEFIRRWDNFP------VTPE-RRANPAWAENTV--DGDDINLFDILPLFRLNDGDGGF
EcUbiD         PKGFRDLFDKLPQFKQVLNMPTKRLRGAPCQQKIV--SGDDVDLNRI-PIMTCWPEDAAP
                                   :  .   ...*  :  *        *..

                                          ↓
KpAroY-Ciso    FF-CLGLALASDPV---DASLTDVTIHRLCVQGRDELSMFLAAGRHIEVFRQ--KAEAAG
AnFdc1         YIQTYGMHIVQSP----DGTWTNWSIARAMVHDKNHLTGLVIPPQHIWQIHQMWKKEGR-
ScFdc1         YLQTYGMWILQTP----DKKWTNWSIARGMVVDDKHITGLVIKPQHIRQIADSWAAIGKA
KpAroY-C       YL-DKACVVSRDPLDKDNFGKQNVGIYRMEVKGKRKLGLQPVPMHDIAL--HLHKAEERG
EcUbiD         LI-TWGLTVTRGP----HKERQNLGIYRQQLIGKNKLIMRWLSHRGGALDYQEWCAAHPG
                :  . :  *      .   :  * *  :.  .:        :      .

KpAroY-Ciso    KPLPITINMGLDPAIYIGACFEAPTTPFGYNELGVAGALRQRPVELVQGVSVPEKAIARA
AnFdc1         SDVPWALAFGVPPAAIMASSMP---IPDGVTEAGYVGAMTGSSLELVKCDTNDLYVPATS
ScFdc1         NEIPFALCFGVPPAAILVSSMP---IPEGVSESDYVGAILGESVPVVKCETNDLMVPATS
KpAroY-C       EDLPIAITLGNDPIITLMGATP---LKYDQSEYEMAGALRESPYPIATAPLTGFDVPWGS
EcUbiD         ERFPVSVALGADPATILGAVTP---VPDTLSEYAFAGLLRGTKTEVVKCISNDLEVPASA
               . .*  :: :*  *   : .      .*   .* :     :.          . :

                              ↓              ↓
KpAroY-Ciso    EIVIEGELLPGVRVREDQHTNSGHAMPEFPGYC-GGANPSLPVIKVKAVTMRNNAILQTL
AnFdc1         EIVLEGTLSISETGPE-------GPFGEMHGYIFPGDTHLGAKYKVNRITYRNNAIMPMS
ScFdc1         EMVFEGTLSLTDTHLE-------GPFGEMHGYVFKSQGHPCPLYTVKAMSYRDNAILPVS
KpAroY-C       EVILEGVIEGRKREIE-------GPFGEFTGHYSGGR--NMTVVRIDKVSYRSKPIFESL
EcUbiD         EIVLEGYIEQGETAPE-------GPYGDHTGYYNEVD--SFPVFTVTHITQREDAIYHST
               *:::** :      *         :  *:          : :: *.. *

KpAroY-Ciso    VG--PGEEHTTLAGLPTEASIWNAVEAAIPGFLQNVYAHTAGGGKFL-------GILQVK
AnFdc1         SCGRLTDETHTMIGSLAAAEIRKLCQQNDLPI-TDAFAPFESQVTWVALRVDTEKLRAMK
ScFdc1         NPGLCTDETHTLIGSLVATEAKELAIESGLPI-LDAFMPYEAQALWLILKVDLKGLQALK
KpAroY-C       YLGMPWTEIDYLMGPATCVPLYQQLKAEFPEV-QAVNA--MYTHGLL-------AIISTK
EcUbiD         YTGRPPDEPAVL-GVALNEVFVPILQKQFPEI-VDFYLPPEGCSYRL-------AVVTIK
                      *   :  *            :  *      :             :    .

KpAroY-Ciso    KRQPADEGRQGQAALLALATYSELKNIILVDEDVDIFDSDDILWAMTTRMQGDVSITTIP
AnFdc1         TTSEGFRKRVGDVVFNHKAGYTIHR-LVLVGDDIDVYEGKDVLWAFSTRCRPGMDETLFE
ScFdc1         TTPEEFCKKVGDIYFRTKVGFIVHE-IILVADDIDIFNFKEVIWAYVTRHTPVADQMAFD
KpAroY-C       KRYGGFARAVGLRAMTTPHGLGYVKMVIMVDEDVDPFNLPQVMWALSSKVNPAGDLVQLP
EcUbiD         KQYAGHAKRVMMGVWSFLRQFMYTKFVIVCDDDVNARDWNDVIWAITTRMDPARDTVLVE
               .         . :::  :*:: : :::** ::           :       .

KpAroY-Ciso    GIRGHQLDPSQTPEYSPSIRGNGISCKTIFDCTVPWALKSH--FERAPFADVDPRPFA--
AnFdc1         DVRGFPLIPYMGHGNG----PAHRGGKVVSDALMPTEYTTGRNWEAADFNQSYPEDLKQK
ScFdc1         DVTSFPLAPFVSQSSR---SKTMKGGKCVTNCIFRQQYERSFDYITCNFEKGYPKGLVDK
KpAroY-C       NMSVLELDPGSS-------PAGITDKLIIDATTPVAPDLRGHYSQPVQD--LP-ETKAW
EcUbiD         NTPIDYLDFASP--------VSGLGSKMGLDATNKWPGETQREWGRPIKK--DP-DVVAH
                .   *           *    * :.                  :          *

KpAroY-Ciso    -PEYFARLEK-NQGSAK
AnFdc1         VLDNWTKMGFSN-----
ScFdc1         VNENWKRYGYK------
KpAroY-C       AEKLTA---MLANRK--
EcUbiD         IDAIWDELAIFNNGKSA
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FIG S2 Sequence alignment of Fdc1, UbiD, AroY-C and AroY-C^{iso}. Asterisks indicate fully conserved amino acid residues, whereas colons indicate residues with strongly similar properties. Protein sequences were derived from *S. cerevisiae* S288C (NP_010828) and *A. niger* Fdc1 (XP_001390534), *K. pneumoniae* AroY-C (AAY57855) and AroY-C^{iso} (AB479384), and *E. coli* K-12 UbiD (NP_418285). Arrows mark the position of conserved residues (R173, E277 and E282 in *A. niger* Fdc1) involved in polar interactions with the cofactor, as described in K.A. Payne, M.D. White, K. Fisher, B. Khara, S.S. Bailey, D. Parker, N.J. Rattray, D.K. Trivedi, R. Goodacre, R. Beveridge, P. Barran, S.E. Rigby, N.S. Scrutton, S. Hay, and D. Leys, Nature 522:497-501, 2015.