Expanded View Figures

		351	371	
Hs 309 PRTIGAELMELVRNT Bt 307 PRTIGAELMELVRNT X1 328 PKTIAAELIEMVRNT Dr 401 PLTIGAELTELVRNT Dm 397 ESSDVYQIVDALRNT Sp 85	GLSHELCRVAIGTIVGHIQAS GLSHELCRVAIGVVVGHIQAS NLSYELSRVAIGVIVGHIQAS GLSCELSRVAVGVVVGRLQGA NLSFDLSCEALRVVLTSLEQL	E VPAS-SPVMEQVLLSLVEGK VPAS-SPVMEQVLLSLVEGK VPST-ASAMEQILVSLVESK LPGA-QVQLEQVLQSLA YNGAINPYLEAVAVHVTGKV		EVIFADLARRKDDAQ EVIFADLARRKDDAQ EVIFADLARRKDDAQ QVIFGDLARHKDDAQ QVIFGDLARHREDSQ QYLFAQLADCKNDTE
Hs 398 QRSWALYEDEGVIRCY Bt 396 QRSWALYEDEGVIRCY X1 417 QRSWALYEDEGVIRCY Dr 486 QRSWALYEDENVILCY Dm 485 QRTWALYEDEDHALIACY Dm 485 QRTWALYEDEEDIIQF Sp 85	H1-3 H2-1 LEELLHILTDADPEVCKKMCK LEELLHILTDADPEVCKKMCK LEELLLITDADPEVCKKMCK LEELLQILTDADAEVCRKMCR LEELVEILINADESISCYEMS	H2-2 RNEFES <mark>VLALVAYYQMEHRA</mark> RNEFESVLALVAYYQMEHRV RNQFEAVLSLVAYYQMEHRV VNEFEPVLSLVSYYQMEHRV CDQYQMFINLVQYYQME CFFFLKEQQ	H2-3 SLRLLLLKCFGAMCSL - D SLRLLLLKCFGAMCSL - D SLRLLLLKCFGAMCNL - D SLRLWLLKVFGAMCGL - D SIKRLLLKTFTAACHL - D TLKFIII - VFQSEIQE - N	AAIISTLVSSVLPVE AAIISTLVSSVLPVE ASIISALVNSVLPME AALISTLLNSVLTME YIIVDILLTSVLPLE EYCLRLLASTGFIPV
H3-2 Hs 487 LARDMQTDTQDF Bt 485 LARDMQTDTQDF X1 506 LALDMQTHTQF Dr 575 LARDLQTDTDFF Dm 574 IVEDMKTHFSNL Sp 125 LIKAMKQFKDRSENVA Sc 185 IIDEMKQIS	516 IQKLCYSALILAMVFSMGE AV IQKLCYSALILAMVFSMGE AV IQKMCYSALVLAMIFSMGE - PL IEKMCYSALVLAMIFSMGE - PL IEKMCYSALLMAMVFSTGE - QI DRFKQLVKMLTIIFSLGQ-PM IFTSFRYALFLVYYICRSR-RL TVLMDLLFQIMKYCKC	H4-1 PYALYEHLGTPFAQFLLNIV PYALYEHLGTPFAQFLLSIV PYHLYEHLNSQFVQFLLEVI PUHYEHLNAGFLQFLLDVI PVNQDYLGVHFASFLLEIV SPTDLVAIDEYFLVNLFKTS VIANLQIVDDFFVYYMMESM	EDGLPLD - TTEQLPDLCV EDGLPLD - TTEQLPDLCV EAGLPSD - ITEQLPDLFI EDGLPTD - STEQLPDLFI EGNNPEVLVDMVI EEAWNDE DMDSFGMCV RSDTMDD MENNAEF	NLLLALNLHLP NLLLALNLHLP NVILAFNLHIS NVLLAFNLHIS ALILAFNQQFS LTLLSINEQFMLVRLAS KLLLALNEQYMM-FAKE
H5-1 Hs 567 AADQNVIMAALSKHAN Bt 565 APDQNVIMAALSKHAN X1 586 VPANNIIMKTLVKQSN Dr 655 VPDSNIVMQTLMKRDN Dm 650 EHTYNVIIEGMQNLPS Sp 214 KGSFEIANGIMDLLSS Sc 262 YDIE <mark>N</mark> KVYKYLINGSV	H5-2 H5-2	- PVRIFKHEPQPPHSVLFFL - PVRIFKHEPQPPHSILFFL - PVRIFKHEPQPPHSILFFL - PVSIFKHQPQPPNSVLFIL - PVCVFSHAPAAPHAVVFFL - PTRLLKHPNEHMNTVLRMF PRSRMLILFQL PFLQIMMCKII	← "LRD/DUF 20VFGSPATAAIFYH 2DVFASPTTAAIFYH 2DVFASATAAIFYH 2DVFASADSAQIFYR 2DVFSSADSAQIFYR IDIFSHPDTAGMFYT FLLFTTPATYEIFYT YLILTPRGDYSPMNFFYT	H6-2 TDMMALIDITVRHIA TDMMALIDITVRHIA TDMMALIDITVRHIA SDLMVLIDITVRQIS NDLNVLIDITVRQIS NDLNVLIDIFIREIN NDLRVLIDVLIRELQ R645E
Hs 649 DLSPG-DKLEMEYLS Bt 647 DLSPG-DKLEMEYLS X1 668 DLSPG-DKLEMEYLS Dr 737 DLEPG-DRMRAQYLS Dm 732 DLDAG-STTPCYLE Sp 292 NIPDELSDLEYAYLS Sc 337 NISEDEEVLENTLE	53 LMHAIVTTPYLQRHRLPD- LMHAVVSTPYLQRHRLPD- LMHAIVSTPYLQRHRLPD- LMHAIVSTPYLQRHRLAD- SLMLSVMRCSEYQEHRHLAD- LCRRILRNTNYQE QHRKHD- QVLIPLLENTQVRHPPHYKTKC VLIPLLENTQVRHPHYKTKC VLIPLLENTQUSXTHYRKD	H7-2 LQAILRRILNEE TSPQC LQATLRRILAEE ASPQC LHGILQRILSEE ASPHC LRAALQRILGEE SC LMKIFTRIFCEE ECS IVDAVNNVLISHSKSSNMEE LNKLLNYLSTLDNICVDSPA	H7-3 QMDRMIVREMCKEFLVLG QMDRMIVQEMCKEFPVLG QMDKIIIREIYKEFPQM GAAERSIVLQIYEEFPDU GASDQQLVREIANEFPQL DARTTDVAIRVL-EVPWL ALHEHQVTVALSRKCLQQI	EAPS EAPS PQAS SS KA QEMKSLGTA PWLETPSTP

Figure EV1. Sequence alignment of SPIN90 from diverse species.

Conserved residues are indicated by colored boxes (green: hydrophobic; yellow: hydrophilic; red: basic; cyan: acidic). The highly conserved segment among WDS proteins, the "leucine-rich domain" (LRD)/DUF2013 segment, is indicated below the sequences, along with residues mutated in this study. Truncation sites are indicated with dashed lines. Alpha helices in the SPIN90 structure are shown above the sequence as rectangles. Hs, *H. sapiens*; Sc, *S. cerevisiae*; Sp, *S. pombe*; Bt, *B. taurus*; Dm, *D. melanogaster*; XI, *X. laevis*; Dr, *D. rerio.*

A Surface conservation



B Contacts to SPIN90



Figure EV2. The SPIN90 contact surface overlaps with a conserved patch of residues on Arp2/3 complex.

- A Surface representation of the "front side" of Arp2/3 from the apo Arp2/3 complex structure (1k8k; Robinson *et al*, 2001) showing residue conservation based on analysis with the ConSurf server (Ashkenazy *et al*, 2016). Residues are colored according to conservation, with the following order, going from most to least conserved: dark magenta > pink > light pink > gray > light cyan > cyan.
- B Surface representation of Arp2/3 complex (1k8k) showing residues (green) that are within 6 Å of SPIN90 in the co-complex structure.



Figure EV3. The H5-1/2 loop is flipped toward the C-terminus of the ARM domain in Arp2/3-bound SPIN90.

- A Cα trace of SPIN90 (351–722) superposed onto the structure of SPIN90 (269–722) from the cocomplex structure. His580 is shown in stick representation. H5-1/2 is flipped away from the C-terminus in the inactive 351–722 structure and toward it in the co-complex structure.
- B Zoomed in view from panel (A) showing the difference in conformation of the H5-1/2 loop in the inactive (351–722, sticks with gray carbon atoms) versus active (269–722, sticks with cyan carbon atoms) SPIN90 structures. Positive (green) or negative (red) F_o-F_c electron density maps are contoured at $\pm 2.0 \sigma$ and were generated from the co-complex reflection file plus a PDB file in which H5-1/2 loop (residues 577–585) of the co-complex structure was replaced with the same residues from chain A in the 351–722 structure.



Figure EV4. SPIN90 binds to the same surface of Arp2/3 complex as actin filaments.

- A Surface representation of the "front side" of Arp2/3 from SPIN90 co-complex structure showing residues that are within 5 Å of SPIN90 (red) or between 5 and 8 Å
- (pink). Side chains were modeled onto the co-complex structure before generating the surface.B Surface representation of the "front side" of Arp2/3 from the branch junction model colored as described in (A) (Rouiller *et al*, 2008), except close contacts between the complex and actin filaments are colored.