SUPPLEMENTARY INFORMATION

THE SH3 DOMAIN IS REQUIRED FOR JAM-A BINDING TO THE THIRD PDZ DOMAIN OF ZO-1*

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*Running title: Structure of the ZO-1 PDZ3-SH3-GUK domains

FIGURE LEGENDS

SUPPLEMENTARY FIGURE S1. *PSG asymmetric unit.* PSG crystallized in the P3₂ space group and contains 4 molecules in the asymmetric unit. Two of the four molecules are missing the GUK domain that could not be modeled. Molecules are represented as a ribbon diagram and colored by chain.

SUPPLEMENTARY FIGURE S2. *Electrostatic surface potential of the PSG core module.* The GUK domain presents a patch of basic residues (blue color) while the PDZ3 domain exhibits a negatively charged surface (red color) on the same face of the protein.

SUPPLEMENTARY FIGURE S3. Sequence alignment of mammalian JAM proteins. Sequences of mammalian JAM proteins have been aligned using Clustal W. Sequences corresponding to the 12mer cytoplasmic tail of JAM are boxed in grey. The highly conserved Phe -8 is highlighted in blue and Phe -2 and Ser -3 in red.

SUPPLEMENTARY FIGURE S4. *NMR characterization of JAM-A_P6 binding to PSG.* (A) 1D NMR spectrum of JAM-A_P6. (B) WaterLOGSY of *JAM-A_P6.* (C) WaterLOGSY of JAM-A_P6 in the presence of PSG. (D) WaterLOGSY of JAM-A_P6 in the presence of PDZ3. Experimental conditions were 300 μ M JAM-A_P6 and +/- 10 μ M PSG or PDZ3 in 100 mM PO₄/pH 7.2 at 25°C. Resonances corresponding to the sidechain of Phe2 are boxed in grey.

SUPPLEMENTARY FIGURE S5. *Titration of PSG with (A) JAM-A_P6 and (B) JAM-A_P12*. The intensity corresponds that to the phenylalanine signal at 7.2 ppm.

SUPPLEMENTARY FIGURE S1



SUPPLEMENTARY FIGURE S2



SUPPLEMENTARY FIGURE S3:

sp	Q9XT56	JAM-A BOVIN	MGTKAKVGSTELLLFT-SMILCSLALGRGAVOTYEPVVRVPENNPAK	46
sp	Q2WGK2	JAM-A FELCA	MGTEARAGRRQLLVFT-SVVLSSLALGRGAVYTSEPDVRVPEDKPAK	46
sp	Q9Y624	JAM-A HUMAN	MGTKAQVERKLLCLFILAILLCSLALGSVTVHSSEPEVRIPENNPVK	47
sp	088792	JAM-A_MOUSE	MGTEGKAGRKLLFLFT-SMILGSLVQGKGSVYTAQSDVQVPENESIK	46
sp	Q9JHY1	JAM-A RAT	MGTEGKAGSKLLFLFT-SMILGSLVQGKGSVYSPQTAVQVPENDSVK	46
sp	P57087	JAM-B_HUMAN	MARRSRHRLLLLLLRYLVVALGYHKAYGFSAPKDQ-QVVTAVEYQEAI	47
tr	A4IFI9	JAM-B_BOVIN	MARRSRHHLLLLLLRYLVVALDYHKAYGFSASKDH-QVVTAIEYQEAI	47
sp	Q9JI59	JAM-B_MOUSE	MARSPQGLLMLLLLHYLIVALDYHKANGFSASKDHRQEVTVIEFQEAI	48
sp	Q9BX67	JAM-C_HUMAN	MALRRPPRLRLCARLPDFFLLLLFRGCLIGAVNLKSSNRTPVVQEFESVE	50
tr	A7YW22	JAM-C BOVIN	MALRRPSLVLLLLLVRGCLIGAVNLKSSNRTPVVQEFESVE	42
sp	Q9D8B7	JAM-C_MOUSE	MALSRRLRLYARLPDFFLLLLFRGCMIEAVNLKSSNRNPVVHEFESVE	50
sp	Q68FQ2	JAM-C RAT	MALSRRLRLRLCARLPDFFLLLLFRGCVIEAVNLKSSNRNPVVHEFESVE	50
		_	: *.	
sp	Q9XT56	JAM-A_BOVIN	LSCSYSGFSSPRVEWKFTHGDIRGLVCYNNKITASYENRVTFSDTG-I	93
sp	Q2WGK2	JAM-A_FELCA	LSCSYSGFSNPRVEWKFAHGDITSLVCYKNKITASYADRVTFSHSG-I	93
sp	Q9Y624	JAM-A_HUMAN	LSCAYSGFSSPRVEWKFDQGDTTRLVCYNNKITASYEDRVTFLPTG-I	94
sp	088792	JAM-A_MOUSE	LTCTYSGFSSPRVEWKFVQGSTTALVCYNSQITAPYADRVTFSSSG-I	93
sp	Q9JHY1	JAM-A_RAT	LPCIYSGFSSPRVEWKFVQGSTTALVCYNNQITVPYADRVTFSSSG-I	93
sp	P57087	JAM-B_HUMAN	LACKTPKKTVSS-RLEWKKLGRS-VSFVYYQQTLQGDFKNRAEMIDFN-I	94
tr	A41F19	JAM-B_BOVIN	LACKYPKKTVSS-RLEWKKLGRG-VSFVYYQQALQGDFKDRAEMIDFS-1	94
sp	0000002	JAM-B_MOUSE	LACKTPRKTTSS-RLEWRKVGQG-VSLVYYQQALQGDFRDRAEMIDFN-1	95
sp		JAM-C_HOMAN		100
TI	A/IWZZ	JAM-C_BOVIN	LSCIITDSQTNDPRIEWKKIQDEQTTIVFFDNKIQGDLTDRAELLGKTSL	92
sp		JAM-C_MOUSE	LSCIITDSQTSDPRIEWKKIQDGQTTIVIFDNKIQGDLAGRTDVFGKTSL	100
sp	Q00rQ2	JAM-C_RAT	* * * **** * · · *	100
sp	Q9XT56	JAM-A_BOVIN	TFHSVTRKDTGMYTCMVSDEGGNTYGEVTVQLIVLVPPSKPTINVPSS	141
sp	Q2WGK2	JAM-A_FELCA	TFHSVTRKDTGTYTCMVSDDGGNTYGEVSVQLTVLVPPSKPTVHIPSS	141
sp	Q9Y624	JAM-A HUMAN	TFKSVTREDTGTYTCMVSEEGGNSYGEVKVKLIVLVPPSKPTVNIPSS	142
sp	088792	JAM-A_MOUSE	TFSSVTRKDNGEYTCMVSEEGGQNYGEVSIHLTVLVPPSKPTISVPSS	141
sp	Q9JHY1	JAM-A_RAT	TFSSVTRKDNGEYTCMVSEDGGQNYGEVSIHLTVLVPPSKPTVSIPSS	141
sp	P57087	JAM-B_HUMAN	$\verb RIKNVTRSDAGKYRCEVSAPSEQGQNLEEDTVTLEVLVAPAVPSCEVPSS $	144
tr	A4IFI9	JAM-B_BOVIN	$\verb RIKNVTRNDAGKYRCEVSAPSEQGQNLEEDTVTLEVLVAPAVPSCEVPNS $	144
sp	Q9JI59	JAM-B_MOUSE	$\verb RIKNVTRSDAGEYRCEVSAPTEQGQNLQEDKVMLEVLVAPAVPACEVPTS $	145
sp	Q9BX67	JAM-C_HUMAN	KIWNVTRRDSALYRCEVVARNDR-KEIDEIVIELTVQVKPVTPVCRVPKA	149
tr	A7YW22	JAM-C_BOVIN	KIWNVTRKDSALYRCEVVARNDR-KEIDEIVIELTVQVKPVAPVCRVPRA	141
sp	Q9D8B7	JAM-C_MOUSE	RIWNVTRSDSAIYRCEVVALNDR-KEVDEITIELIVQVKPVTPVCRIPAA	149
sp	Q68FQ2	JAM-C_RAT	RIWNVTRSDSAIYRCEVVALNDR-KEVDELTIELIVQVKPVAPVCRVPKA	149
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sp	Q9X156	JAM-A_BOVIN	VTIGTRAVLTCSERDGSPPSEYKWFKDGVEMP-LEPKSNRAFSNSSYTLN	190
sp	QZWGKZ	JAM-A_FELCA	ATIGSRAVLTCSEKDGSPPSEIIWFKDGVRMP-LEPKGNRAFSNSSISLN	190
sp	000702	JAM-A_HOMAN	ATIGNRAVLTCSEQDGSPPSEITWFKDGIVMP-TNPKSTRAFSNSSIVLN	191
sp		TAM-A PAT	VIIGNAAVLICSENDGSFFSEISWERDGISHLIADAAKIAAFINSSEIID	101
an	257087	JAM-R HIMAN	ALSCTWUFLPCODKFCNDADEVTWFKDCTPLL_FNDBLCSOSTNSSYTMM	193
5P +r		JAM-B BOWIN	ALBGIVVELKCODKEGNPAPETIWFKDGIKLL-ENFKLGSOTMSSTIMM	193
en		JAM-B MOUSE	MERGIVVELRCQDREGNPAPETIWFRNGVELS-ENFRLGSQTINSSTIMN	193
an	098867	JAM-C HUMAN	VPUCKMATLHCOESECHPRPHYSWYRNDVPLP_TDSRANPFRNSSEHLN	198
5p +r	A7VW22	JAM-C BOWIN	VPVGKMATERCQESEGRERERTISWIRNDVFEF=TDSRAWERERKISSFILM	190
sn	090887	JAM-C MOUSE	VPVGKTATLOCOESEGVPRPHVSWVRNDVPLP-TDSRANPRFONSSEHVN	198
sp	068F02	JAM-C RAT	VPVGKIATLOCOESEGYPRPYVSWYRNDVPLP-TDSRANPRFONSSFHVN	198
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sp	Q9XT56	JAM-A_BOVIN	$\label{eq:construction} QKTGELIFDPVSASDTGDFTCQAQNGYASPVKSDTVHMDAVELNVGGIVA$	240
sp	Q2WGK2	JAM-A_FELCA	EKTGELVFDPVSAWDTGEYTCEAQNGYGMPMRSEAVRMEAAELNVGGIVA	240
sp	Q9¥624	JAM-A_HUMAN	PTTGELVFDPLSASDTGEYSCEARNGYGTPMTSNAVRMEAVERNVGVIVA	241
sp	088792	JAM-A_MOUSE	PKSGDLIFDPVTAFDSGEYYCQAQNGYGTAMRSEAAHMDAVELNVGGIVA	241
sp	Q9JHY1	JAM-A_RAT	PKSGDLVFDPVSAFDSGEYYCEAQNGYGTAMRSEAVRMEAVELNVGGIVA	241
sp	P57087	JAM-B_HUMAN	TKTGTLQFNTVSKLDTGEYSCEARNSVG-YRRCPGKRMQVDDLNISGIIA	242
tr	A41F19	JAM-B_BOVIN	PKSGTLQFNTVSKMDSGEYSCEARNSVG-HRRCPGKRMQVDDLNISGIIA	242
sp	097123	JAM-B_MOUSE	TKSGILQFNMISKMDSGEYYCEARNSVG-HRRCPGKRMQVDVLNISGIIA	242
sp		JAM-C_HOMAN	SETGTLVFTAVHKDDSGQYYCIASNDAG-SARCEEQEMEVIDLNIGGIIG	247
CI CD		JAM-C_BOVIN	PEIGILVESAVINEDSGUICIASNDAG-SARCELQDMEVIDLNIGGIIG	239
ap	068203	JAM-C RAT	SETGIEVINAVIRODOGUICIAONDAG-AARCEGUDEVIDENIAGIIG SETGTIVESAVHKEDSGOVVCIASNDAG-AARCEGUDEVIDENIAGIIG	241
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sp	Q9XT56	JAM-A_BOVIN	AVFVTLILLGALIFGIWFAYSRGYFDRAKKGTS-NKKVIYSQPNAR	285
sp	Q2WGK2	JAM-A_FELCA	AVLVTLILLGFLILGIWFAYRRGYFDRTKKGTS-SKKVIYSQPAAR	285
sp	Q9Y624	JAM-A_HUMAN	AVLVTLILLGILVFGIWFAYSRGHFDRTKKGTS-SKKVIYSQPSAR	286
sp	088792	JAM-A_MOUSE	AVLVTLILLGLLIFGVWFAYSRGYFETTKKGTAPGKKVIYSQPSTR	287
sp	Q9JHY1	JAM-A_RAT	AVLVTLILLGLLIFGIWFAYSRGYFERTKKGTAPGKKVIYSQPSAR	287
sp	P57087	JAM-B_HUMAN	AVVVVALVISVCGLGVCYAQRKGYFSKETSFQKSNSSSKATTM	285
tr	A4IFI9	JAM-B_BOVIN	AVVIVALVISVCGLGVCYAQRKGYFSKETSFQKSSSASKATTM	285
sp	Q9JI59	JAM-B_MOUSE	TVVVVArVISVCGLGTCYAQRKGYFSKETSFQKGSPASKVTTM	285
sp	USBX67	JAM-C_HUMAN	GVLVVLAVLALITLGICCAYRRGYFINNKQDGESYKNPGKPDGVNYIRTD	291
tr	A/YW22	JAM-C BOVIN	GVLVVLTVLALITGGICCAYRRGYFISHDRSGESYKNPGKPDGVNYIRTD	∠89 207
sp		JAM-C RAM	GVLVVLIVLAVITMGICCAYRRGCFISSKQDGESYKSPGKHDGVNYIRTS	29/
зp	1 20 0 I QZ	OAN-C_RAT	* * * * * * * *	231
sp	Q9XT56	JAM-A_BOVIN	S D G E F R Q T S S F L V 298	
sp	Q2WGK2	JAM-A_FELCA	SEGEFRQTS <mark>SF</mark> LV 298	
sp	Q9Y624	JAM-A_HUMAN	SEGEFKQTS <mark>SF</mark> LV 299	
sp	088792	JAM-A_MOUSE	SEGEFKQTSSFLV 300	
sp	Q9JHY1	JAM-A_RAT	SEGEFKQTSSFLV 300	
sp	P57087	JAM-B_HUMAN	SENDFKHTKSFII 298	
tr	A4IFI9	JAM-B_BOVIN	SENDFKHTKSFII 298	
sp	Q9JI59	JAM-B_MOUSE	SENDFKHTKSFII 298	
sp	Q9BX67	JAM-C_HUMAN	EEGDFRHKSSFVI 310	
τr	A/YW22	JAM-C_BOVIN	EEGUFRHKSSFVI 302 FFCDFPHKSSFVI 310	
<i>a</i>	000007			
sp	Q9D8B7	JAM-C_MOUSE	EEGDERHKSSEVI 310	
sp sp	Q9D8B7 Q68FQ2	JAM-C_MOUSE	$\mathbf{E} = \mathbf{E} \mathbf{G} \mathbf{D} \mathbf{F} \mathbf{R} \mathbf{H} \mathbf{K} \mathbf{S} \mathbf{S} \mathbf{F} \mathbf{V} \mathbf{I} $ 310	



SUPPLEMENTARY FIGURE S5

