

## Uncovering novel plasma membrane carboxylate transporters in the yeast *Cyberlindnera jadinii*

Sousa-Silva, M.<sup>1,2</sup>, Soares, P.<sup>1,2</sup>, Alves, J.<sup>1,2</sup>, Vieira, D.<sup>1,2</sup>, Casal, M.<sup>1,2</sup>, Soares-Silva, I.<sup>1,2\*</sup>

<sup>1</sup>Institute of Science and Innovation for Bio-Sustainability (IB-S), University of Minho, Portugal

<sup>2</sup>Centre of Molecular and Environmental Biology (CBMA), Department of Biology, University of Minho, Campus de Gualtar, 4710-057 Braga, Portugal

\*Corresponding author: Isabel Soares-Silva; [ijoao@bio.uminho.pt](mailto:ijoao@bio.uminho.pt)

## Supplementary material

**Table S1.** Identification of *C. jadinii* carboxylate transporter homologs of the distinct transporter families. For ScAto1 and ScJen1 homologs, homology search was performed by BlastP. Protein conserved domains were determined in the Conserved Domain Database (CDD) and the number of transmembrane segments (TMSs) was determined with TMHMM Server v.2.0.

Accession number	Query	Query cover	Identity	E-value	TMSs	ID gene
XP_020070445.1	NP_009936.1 Ady2p [ <i>S. cerevisiae</i> ]	98 %	56 %	2e <sup>-99</sup>	4	CjAto1
XP_020073179.1		96 %	57 %	2e <sup>-96</sup>	6	CjAto2
XP_020073031.1		91 %	55 %	2e <sup>-100</sup>	5	CjAto3
XP_020073178.1		98 %	57 %	4e <sup>-109</sup>	5	CjAto4
XP_020067765.1		94 %	41 %	6e <sup>-72</sup>	6	CjAto5
CEP23088.1	NP_012705.1 Jen1p [ <i>S. cerevisiae</i> ]	82 %	58 %	0	10	CjJen1
CEP21966.1		85 %	51 %	1e <sup>-175</sup>	11	CjJen2
CEP22358.1		77 %	40 %	1e <sup>-119</sup>	12	CjJen3
CEP21989.1		76 %	41 %	5e <sup>-116</sup>	12	CjJen4
CEP21602.1		67 %	43 %	1e <sup>-103</sup>	10	CjJen5
CEP25129.1		82 %	41 %	8e <sup>-127</sup>	12	CjJen6

Accession number	Domain designation	Accession	Description	E-value	TM Ss	ID gene
XP_020067635.1	MFS_MCT_SL C16	cd17352	Monocarboxylate transporter (MCT) family of the Major Facilitator Superfamily of transporters	2.27e <sup>-64</sup>	12	CjSlc16
XP_020068154.1	SLC5-6-like_sbd super family	cl00456	Solute carrier families 5 and 6-like; solute binding domain	3.25e <sup>-14</sup>	11	CjSlc5
XP_020068891.1	TDT super family	cl04176	Tellurite-resistance/Dicarboxylate Transporter (TDT) family	1.19e <sup>-83</sup>	9	CjTDT
XP_020069270.1	SLC13_perme ase	cd01115	Permease SLC13 (solute carrier 13); Sodium/dicarboxylate cotransporter NaDC-1	1.56e <sup>-98</sup>	12	CjSlc13-1
XP_020073044.1			Permease SLC13 (solute carrier 13); Sodium/dicarboxylate cotransporter NaDC-1	7.26e <sup>-103</sup>	12	CjSlc13-2

**Table S2.** Oligonucleotides used for strain construction, cloning and expression.

Name	Sequence
p416-CJAd1_fwd	GCAGGATCCATGTCAGACAAGGAAAGC
p416-CJAd1_rev	GCAGAATTCCTAGGAGTGCACCTGAGC
p416-CJAd2_fwd	GCAGGATCCATGTCTAGTATTAATGAG
p416-CJAd2_rev	GCAGAATTCTCACAAATGGCTAGCACC
p416-CJAd3_fwd	GCAGGATCCATGGCCGCAACGTTGAC
p416-CJAd3_rev	GCAGAATTCTCAGGCACGCTTTGCACC
p416-CJAd4_fwd	GCAGGATCCATGTCGGACAAGGAAAAC
p416-CJAd4_rev	GCAGAATTCTCAAGAACGCTTTGCACC
p416-CJjen1_fwd	GCA GGATCCATGCACAAACTGAAGAG
p416-CJjen1_rev	GCAGAATTCTCACTTCTTCTCCTGTGG
p416-CJjen2-new_Fwd	GCAGGATCCATGACTTCACCATTGCCT
p416-CJjen2-new_Rev	GCACTCGAGTCACTCACTTGAAGAGCC
p416-CJjen3_fwd	GCAGAATTCATGGCAATGTCTGATGTT
p416-CJjen3_rev	GCAGAATTCTCAGGTTTTTTCATTATG
p416-CJjen4-new_Fwd	GCAGGATCC ATGACTGCTGGGAGATAC
p416-CJjen4-new_Rev	GCACTCGAGCTAGTCTCTAGCAGATTC
p416-CJjen5_fwd	GCAGGATCCATGGACTGGGATGCTTTC
p416-CJjen5_rev	GCAGAATTCTCACTTTGGCTCTATCTT
Cut-Jen6_Fwd	GCCTCTAGAATGGGATTCAAGACGTAC
Cut-Jen6_Rev	GCCGAATTCTCATTTAACCTCAGAAAC
ct1.635_Fwd	GCCACTAGTATGACTGAAATCATCACT
ct1.635_Rev	GCCGTCGACTCAGAATTTACAAATTCT
ct2.154_Fwd	GCCACTAGTATGTTTGCAGAGACCGAG
ct2.154_Rev	GCCGTCGACTCACGAGTTGTCAGCACA
ct3.891_Fwd	GCCACTAGTATGACATCTGATGAGAAT
ct3.891_Rev	CCGTCGACTCAATTCCTTTCACTGTT
ct4.270_Fwd	GCCACTAGTATGAAATTCTCCCTCTCT
ct4.270_Rev	GCCGTCGACTTAACCGTGTAAGTTGC
ct8.044_fwd	GCCACTAGTATGAAGTTCTCCCATTCG
ct8.044_rev	GCCCTCGAGTCACATCCCTGTTAATCT
ct9.7765_fwd	GCCACTAGTATGTCGACCTCTTCTCTC
ct9.7765_rev	GCCGAATTCCTAAACTCTTGGTGCATG

**Table S3.** Parameters obtained with HHPred for 3D-model construction.

Protein templates	PDB Hit	Organism	E-value	Similarity	Identity	Score	Probability (%)
<b>ScAto1</b>	5YS3	<i>Citrobacter koseri</i>	$3.7e^{-32}$	0.566	35%	236.5	100.0
<b>EcSatP</b>	5YS3	<i>Citrobacter koseri</i>	$1.5e^{-36}$	1.498	92%	235.4	100.0
<b>CjAto2</b>	5YS3	<i>Citrobacter koseri</i>	$6.7e^{-33}$	0.538	32%	237.2	100.0
<b>CjAto5</b>	5YS3	<i>Citrobacter koseri</i>	$7e^{-35}$	0.525	26%	252.0	100.0

**Table S4.** Residues of Ato homologs presenting strong intramolecular interactions with lactate, succinate and citrate identified by molecular docking studies.

3D-Protein templates	Lactate	Succinate		Citrate
	(-1)	(-1)	(-2)	(-3)
<b>ScATO1</b>	R111; T238; N179; T222; T102; S106; E140; T209; N255	T238; N179; R111; T222; T102; Q133; S208; N145; N255; T209; E140	R111; N179; T238; T222; T209; S208; N145	N255; T209; S208; N89; N145; Q133; T238; H230; R111; N179
<b>CjATO2</b>	K244; S189; N127; N91; T203; I97; T99; Y158	N236; K244; S189; C185; T203; N91; T84; T99; D150; R214; Q219; S211; W144; Y158	S189; K244; N127; T203; W144; T99; Q219; R214; Y158	ND
<b>CjATO5</b>	N101; G220; Q103; T229; K230	E125; K221	K221	N137; S199; K198; N81; K221; Q103; T229; K230; G220

Note: ND - not determined.

**Table S5.** Average of the binding affinity values [kcal/mol] calculated with PyRx software for the docking of Ato proteins with the distinct charged substrates tested.

3D-Protein templates	Average of binding affinities (kcal/mol) at different binding sites						
	Lactate (-1)						
	S4			S3	S2	S1	
	a	b	c			a	b
<b>ScAto1</b>	-	-3.8	-	-4.1	-3.9	-3.3	-3.1
<b>EcSatP</b>	-3	-	-	-3.6	-3.8	-3	-
<b>CjAto2</b>	-3.4	-	-3.4	-4.2	-2.6	-3.4	-3.1
<b>CjAto5</b>	-3.2	-3.1	-	-3.3	-3.8	-3.4	-
	Succinate (-1)						
	S4			S3	S2	S1	
	a	b	c			a	b
<b>ScAto1</b>	-	-4.0	-	-5	-4.5	-3.9	-3.8

EcSatP	-3.6	-	-	-4.5	-4.6	-4.2	-
CjAto2	-4.2	-	-4.2	-4.7	-3.2	-3.9	-4
CjAto5	-3.8	-	-	-3.9	-4.7	-	-
	Succinate (-2)						
	S4			S3	S2	S1	
	a	b	c			a	b
ScAto1	-	-4.0	-	-5	-4.4	-3.7	-
EcSatP	-3.4	-	-	-4.4	-4.7	-4.3	-
CjAto2	-4	-	-4.1	-4.8	-3.1	-4.1	-4
CjAto5	-3.8	-	-	-3.9	-4.6	-	-
	Citrate (-3)						
	S4			S3	S2	S1	
	a	b	c			a	b
ScAto1	-	-5.0	-	-6.1	-3.7	-4.6	-
EcSatP	-4.2	-	-	-5	-4.3	-4.2	-
CjAto5	-4	-4	-	-3.5	-5.5	-4.5	-4.2

**Figure S1.** Multiple sequence alignment of Ato homologs from *Saccharomyces cerevisiae* (Ato1, Ato2, Ato3), *C. jadinii* (CjAto1-5) and the *Escherichia coli* SatP. The sequence alignment was built with ClustalOmega (<https://www.ebi.ac.uk/Tools/msa/clustalo/>). Localization of transmembrane segments (TMSs) was predicted by the PSI/TM-Coffee software (<http://tcoffee.crg.cat/apps/tcoffee/do:tmcoffee>). Grey background highlights the previously identified and characterized signature motifs of the AceTr family (Ribas et al., 2019). Blue rectangles indicate residues from the narrowest hydrophobic constriction site F98-Y155-L219 (refer to ScAto1p) (Qiu et al., 2018).

1<sup>st</sup>TMS

ScAto1 GRQKFLKSDLYQAFG-GTLNPGLAP-APVHKFANPAPLGLSAFALTTFVLMSFNARAQGI 115

ScAto2 GRQKFLRDDLFEAFG-GTLNPGLAP-APVHKFANPAPLGLSGFALTTFVLMSFNARAQGI 114

ScAto3 GSSTYRRRDLNLALDRGDGEEGNCAKYTPHQFANPVPGLGLASFSLSCVLVSLINANVRGV 112

CjAto1 GRTKVLRLSELWNAFG-GDLQPGIHA-PPPRRFANPAPLGLCGFALTTFVLMSNARAMGI 110

CjAto2 GRMKVRKSELWSAFG-GDLQPGVHA-QPQRKFANPVPGLCGFALTTLVLMSANARAMGI 97

CjAto3 GRTKVLRLSELWNAFG-GDLQPGIHA-TPPRRFANPAPLGLCAFALTTFVLMSVNARAMGI 104

CjAto4 GHQKVLRLSELWTAFG-GDLQPGVHA-PPQRLANPAPLGLCGFALTTFVLMSANARAMGV 106

CjAto5 GNQAFSKKDLFNAFA-GDLQPGLHA-TPHRPMGNPVPMLGTSFCICCFVSVLVNAQARGV 107

EcSatp -----MGNTKLANPAPLGLMGFSMTTILLNLHNVGYFA- 33

2<sup>nd</sup>TMS 3<sup>rd</sup>TMS

ScAto1 TVPNVVVGCAMFYGGLVQLIAGIWEIALENTFGGTALCSYGGFWLSFAAIYI-PWFGILE 174

ScAto2 TIPNVVVGCAMFYGGLVQLIAGIWEIALENTFGGTALCSFGGFWLSFGAIYI-PWFGILD 173

ScAto3 TDGKWALSFLMFFGGAIELFAGLLCFVIGDTYAMTVFSSFGGFWICYGYGLT-DTDNLVS 171

CjAto1 TVANVAVGPAFFYGGIIQLLSGMWEISLDNTFGGTVLSSYGGFWLSWAAIQI-DWFGIQR 169

CjAto2 RTPNVAVAPAFFYGGFAQILAGMWEIALENTFGSVVLTSYGCFWLSWAAIEI-DWFGIKA 156

CjAto3 TTPNIVVGLALFYGGFVQLLAGMWEIALDNTFGGTALSSYGGFWMSYAAIQI-DWFGIKS 163

CjAto4 TIPNAAVGAACFYGGLVQLLAGMWEISLDNTFGGTALSSYGGFWMSWAAIQI-DWFGIKK 165

CjAto5 TNAKVIASCALFFAGVVETISGLWCLVIENTFAATALGSFGGFWMGYAGLLI-DAFGITS 166

EcSatp -LDGIILAMGIFYGGIAQIFAGLLEYKKGNTEFGLTAFTSYGSFWLTLVAILLMPKLGLED 92

4<sup>th</sup>TMS 5<sup>th</sup>TMS

ScAto1 AYEDNESDLNNALGFYLLGWAIFFTGLTVCTMKSTVMFFLLFFILLALTFLLLSIGHFANR 234

ScAto2 AYKDKESDLGNALGFYLLGWALFTFGLSVCTMKSTIMFFALFFILLAVTFLLLSIANFTGE 233

ScAto3 GYTDP-TMLNNVIGFFLAGWTVFTFLMLMCTLKSTWGLFLLLTFLDLTFLLLCIGTFIDN 230

CjAto1 AYDDP-IMLNNGLGFLLGWVIFTLMVLICTVKSTVAFFSLFFFELEMTFLLLTIGEFTRS 228

CjAto2 AYDDP-IELENAIGFLLGWVIFTFLILLCTMKSTVAFFSMFFILEITFILLTVASFTRH 215

CjAto3 AYTDP-IELANAVSFFLLGWTIFTFMILLCTVKSTVSFFSLFFFELEITFLLLTIGDFTRR 222

CjAto4 AYDDP-IMLANAVGFLLGWTIFTFMVLVLCCTVKSTVAFFSLFFFLDITFLLLTIGEFTRK 224

CjAto5 SYSTT-EELGNALGFYLTAWTIFAFLMWLCTFKSTWPFFILFLIVVFLMCLAIGKYNDN 225

EcSatp A-----PNAQFLGVYLGWGVFTLFMFFGTLKGARVLQVFVFSLTVLVALLAIGNIAGN 146

6<sup>th</sup>TMS

ScAto1 LGVTRAGGVLGVVAFIAWYNAYAGVATKQNSYVLARFPFLPSTERVIF--- 283

ScAto2 VGVTRAGGVLGVIVAFIAWYNAYAGIATRQNSYIMVHPFALPSNDKVFF--- 282

ScAto3>NNLKMAGGYFGILSSCCGWYSLYCSVSVSPNSYLAFAHTMPNAP----- 275

CjAto1 VGVTRAAGVFGVITSFLGWYNALAGFATRENSYFVATAVPLPGAKRAQVHS- 279

CjAto2 VGCQRAGGVFGVITGFLAWYNAYAGIATKEISYFVPKPWPLPGASHL----- 262

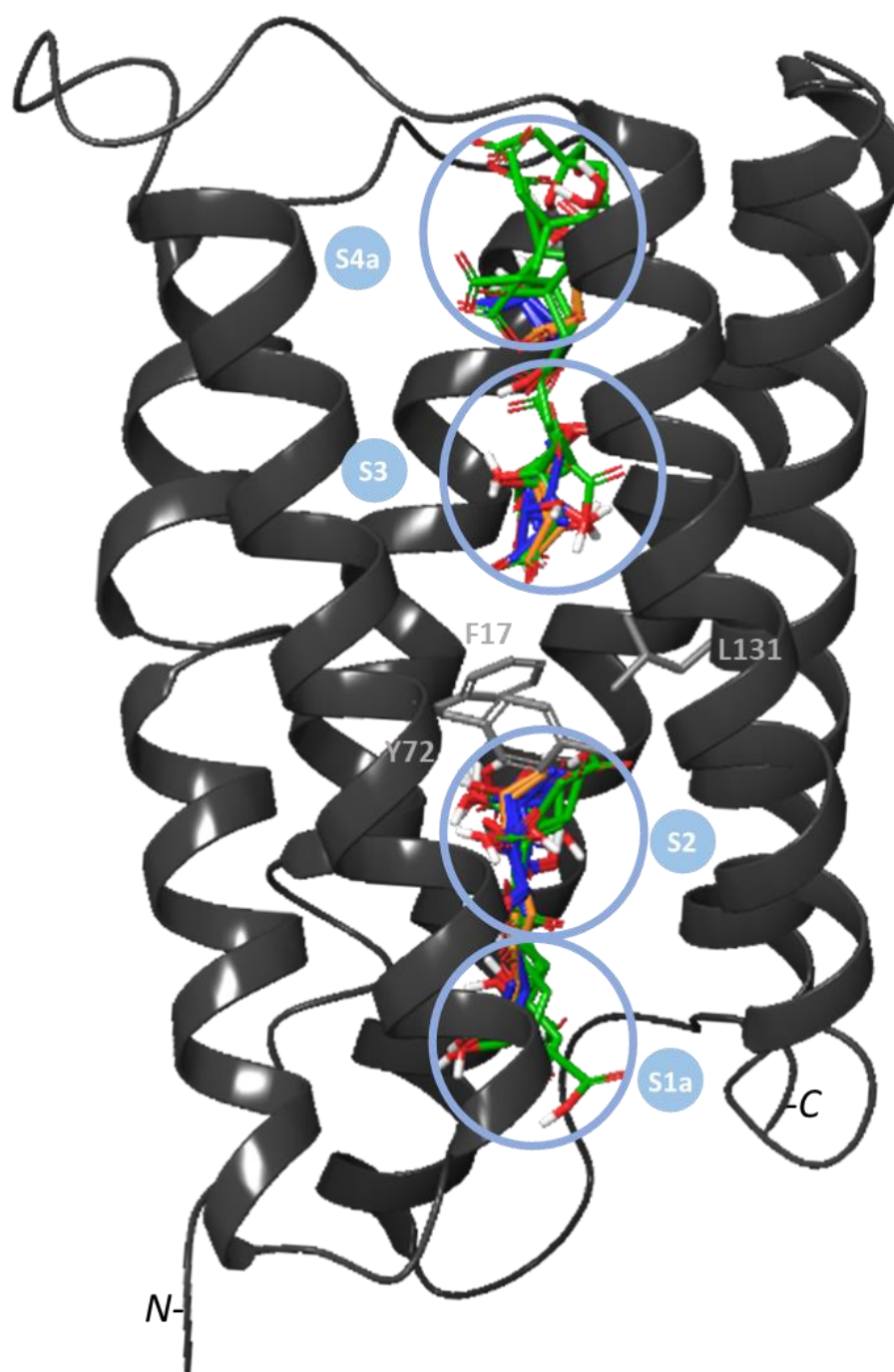
CjAto3 VGVTRAGGVFGVITAFIAWYNAFAGIATKENSYITIKAWPLPGAKRA----- 269

CjAto4 TGVSRAAGVFGVITSFIAWYNAFAGLATKENSYVVAIPLPLPGAKRS----- 271

CjAto5 TTATKAGGVFLGVATFVGFFIVYAGVADSSNSYLTIPASPMPHAPRV----- 272

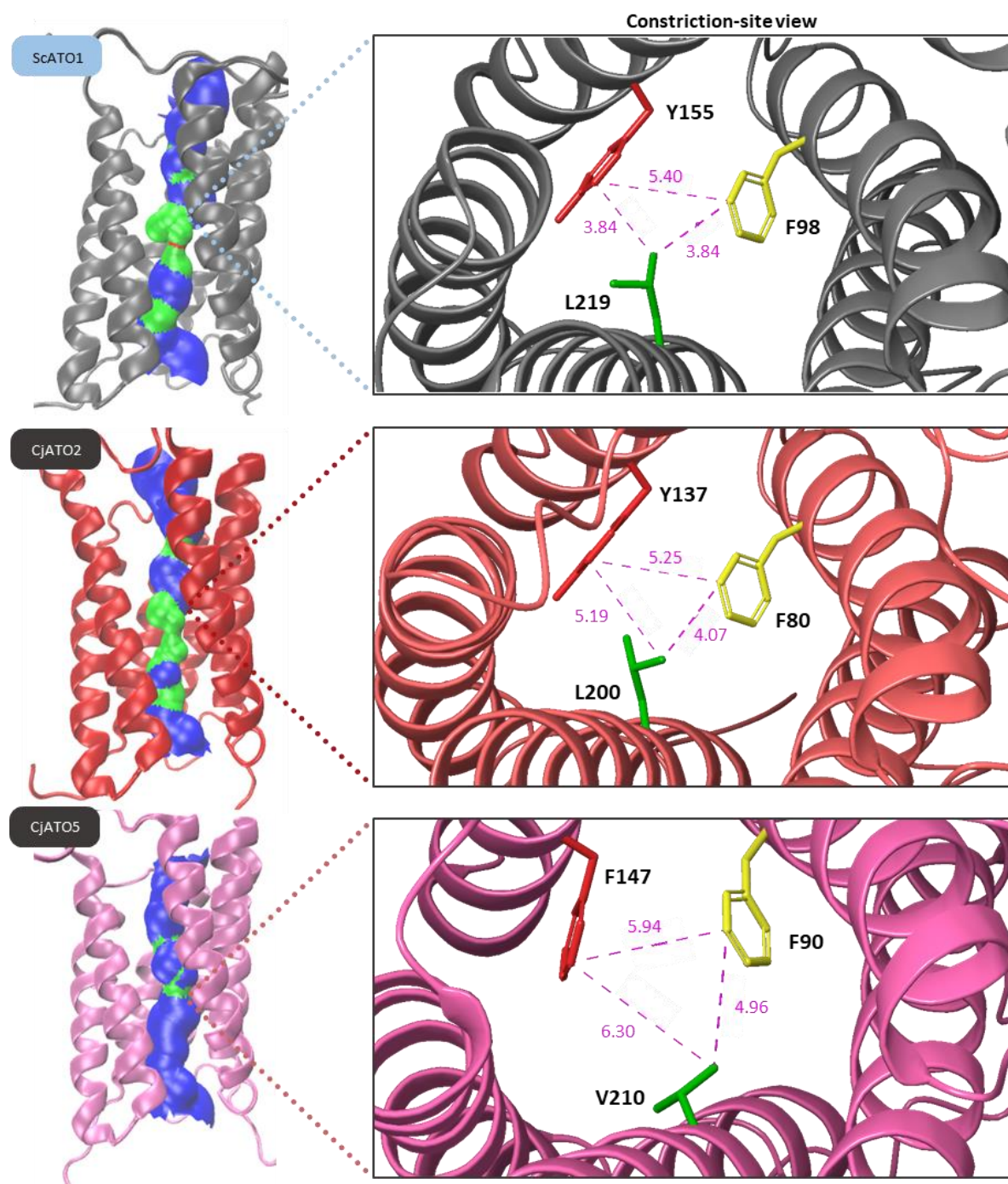
EcSatp AAIHFAGWIGLICGASAIYLAMGEVLNEQFGRTVL---PIGESH----- 188

**Figure S2.** Molecular docking of *Escherichia coli* Satp 3D-model, based on SatP\_Ck structure, with the substrates lactate (blue ligand), succinate (orange ligand) and citrate (green ligand). The four binding sites are depicted from S1 to S4 sites, including the localization of N- and C-terminal of the protein. The narrowest hydrophobic constriction of the anion pathway formed by F17, Y72 and L131 is also represented.





**Figure S3.** Predicted 3D structures of Ato proteins visualized with Maestro and HOLE softwares. On the left are the pore predictions: blue (larger aperture), green (intermediate pore size) and red (constricted pore). The top view of the narrowest hydrophobic constriction site of the anion pathway is also presented, including the predicted distances (Å) between represented residues.





**Figure S4.** Multiple sequence alignment of Jen homologs from *Saccharomyces cerevisiae*, *Candida albicans* (CaJen1-2), *Cluyveromyces lactis* (KlJen1-2) and *C. jadinii* (CjJen1-6). The sequence alignment was built with ClustalOmega (<https://www.ebi.ac.uk/Tools/msa/clustalo/>) and the localization of transmembrane segments (TMSs) predicted by the PSI/TM-Coffee (<http://tcoffee.crg.cat/apps/tcoffee/do:tmcoffee>). Grey background highlights the previously identified and characterized motifs of the SHS family for carboxylate transporter affinity (Soares-Silva et al., 2007; 2011).

1 <sup>st</sup> TMS						
ScJen1	ALTRFTSLLHIEF----	SWENVNPIELRKMTWQNNWYFMGYFAWLAAWAFFFCVSV	161	ScJen1	FLPILLIFWRLWPETKYFTKVLKARKLILSD---AVKANGGEPLPK--ANFKQKMVSMK	358
CaJen1	FATRLTLLDPLHYTHKKWYEVINPIGLKSMKSDWNWYFLGFAWALDAMDFFFCVSV	112	CaJen1	GLSLILIVWRLFTPESPDYIKMKIKKEKFNQQRKLEQEONGGVAVKEKFWQIKDISIL	316	
CaJen2	AITRVTSWVGWDELKQYSWHEVLNPFEPVEMNLHQWNNFFFLGFAWATWDALDFFVFTSL	95	CaJen2	GPPILIIWRFINPETSQYRQKERFDQGAQV---K-----NSKAAEFKS----QAK	282	
KlJen1	AITRFTTTELHHRV-----SMENINPIELRKMTLHNWNYFMGYAAWLCAWAFFFAVSV	154	KlJen1	WLPVALLIWLRLVWPETKYFTKVLKARQIMRDD----AIKNGGQPLPK--LSFKQKFAVNVK	352	
KlJen2	LATRIPTLFPFKASIREARKEYPINFPFALRSMNWLQTYQFIVGFLAWTWDALDFFAVSL	100	KlJen2	GPPVLFIARWLMLPESQHYVERVRLEKL-----E-----NDGKSQFWK----NAK	302	
CjJen1	SKTRFTSLTHLHLH-----AVGDLNPLPSLRMSWNNWNFFFMGFVAWFSASFDFFLTAV	94	CjJen1	CFFPFLICWRLVFPETRYFTRVLKARELIKQD---QIEAGV---YVK--PTLTKWGSVK	287	
CjJen2	SRTRFTTLGELHLD-----SLSDLNPLPALKEMTPRNWNFFFMGFIAWFSASFAFLTAV	111	CjJen2	GIPFVLIWRLYLPETTYFTFTRVLKVKKLKEE---AST-----SPKISKWAKTK	289	
CjJen3	ILSRVPTLFCIPGT-----SFQALNPVPALSALSRDNWNYFLMGYAAWTIDAFDFFCVSA	64	CjJen3	APPAILFVWRMFFPEHPHFTALKRVQREKAL-----AST---SEGKHQAASPEWQFFADLK	273	
CjJen4	IATRIPSLFTLPGT-----PVKSLNPAPAMRALSRSDWNYFMGYCAWVIDAFDFFCVSA	61	CjJen4	APPAILFMWRLMFPPEHPHFTVEHKRVQREKAL-----AEGNHAQAASPEWQFFYDLK	253	
CjJen5	-----MDWDAFDFVFMVSL	13	CjJen5	GPPKL-----KPFLLQQLLHM-----KNKNTSFSE---DAC	182	
CjJen6	TRERFTLLPSREQWE--VEKSHMNPFPGRLAMTWRNQWYFIIGMLAWTWDALDFFFAMSL	72	CjJen6	GPPVLFIWRAFLPETEEFLQQLKLM-----SNRKSFTFR---DAW	252	
: ** :						
2 <sup>nd</sup> TMS						
ScJen1	SVAPLAELYDRPKDITWGLGLVLFVR-----SAGAVIFGLWTDKSSR	204	ScJen1	RTVQKYWLLFAYLVLLVGVNYLTHASQDLLPTMLRAQLGLSKDAVTIVVVNTIGATCG	418	
CaJen1	AAPEIANTLNI SVTDVTWGTVLVLMIR-----SAGAVIFGIADSYFGR	155	CaJen1	VTFKTEWLLIFS YLVLLYAGWNFTTHGSQDLYVTMITKYVHVLGDKKTVIIVVSNIGGIIG	376	
CaJen2	NVSNIAEDLDSTVKDWSWGITLVLMIR-----TVGALIFGAIGDTYGR	138	CaJen2	KALNQYWLIIIVYLIFLMAGNFSSSHGSQDLYPTMLTKQYHYGKDKSTVVNVCANLALAG	342	
KlJen1	SKTFLATLYGKETKDIDWGLSLVLFVR-----SAGAIIFGIWTDNYSR	197	KlJen1	KTVSKYWLLFGYLLILLVGVNYLTHASQDLFPMLRAQLRFSEDAVTVAIVVCLGSITAG	412	
KlJen2	NMTNLAKDLDRPVKDISHAITLVLLLR-----VIGALIFGYLDTRYGR	143	KlJen2	LACSQYWLMSIYLVLIMAGNFSSSHGSQDLYPTMLTSQYQFSADASTVNSVANLGAITAG	362	
CjJen1	SGTYIAQSLDVSTADITWGLSAVLMVR-----SAGAVIFGLWTDNYSR	137	CjJen1	AMLKKDWLLFTYLVLLLAGNYLTHASQDMYPTMLRSQLEWSDAQTVIAIVVNLGATCG	347	
CjJen2	SGTVIAESLEVSTKIDITWGLSSVLMVR-----SAGAVIFGLWTDNYSR	154	CjJen2	TMFSKYWLLFTYLVLLLAGSNFLTHASQDMYPTMLRSQFGWSDAQTVIAIVVNLGGVIG	349	
CjJen3	CAPALAQALDRSVDITWGITVLVMTREYFAHIDQQLTDIFEISGLGAVIFGSLSDTYGR	124	CjJen3	SAMRHHLMFVYLVLYMSLMNFS SHASQDLMPTMLQNLGFEANDRTIIMVVINIGATFG	333	
CjJen4	CAPALAKAFDRSIHDITWGITVLVMTREYFAHIDQQLTDIFEISGLGAVIFGSLSDTYGR	104	CjJen4	KALSNHLMFVYLVLYMSLMNFS SHASQDLMPTMLQNLGFFSANQRTAIMVNLGAMVG	313	
CjJen5	NVSKLATDLDRSVKDISWGITVLVMTREYFAHIDQQLTDIFEISGLGAVIFGSLSDTYGR	56	CjJen5	KVFKEQWLKMIYLVLIMAGNFMSSHGSQDLYPTMLTVQLNYSNPRSTVTNSVANLALAG	242	
CjJen6	NMSNISLDRSVKDVSWGITVLVMTREYFAHIDQQLTDIFEISGLGAVIFGSLSDTYGR	115	CjJen6	NTFKQQWKMGLVLLIMAGNFMSSHGSQDLYPTMLTKQLGFSRDRSTVTNSVANLALAG	312	
* : : * : : *						
3 <sup>rd</sup> TMS		4 <sup>th</sup> TMS		5 <sup>th</sup> TMS		
ScJen1	KWPYITCLFLFVIAQLCTPWCDTYEKFLGVRWITGIAMG-----GI	245	ScJen1	GMIFGQFMEVTGRRRLGLLIAC TMGGCTYPAFMLRSEKAILGAGFMYFCVGVWGVLPI	478	
CaJen1	KWTYISIVTLFVVVEVGTFGVQYQQLGVRAIFGILMG-----AM	196	CaJen1	GIIMGQASELLGRRLTVVISI VCAGAFLYPSFFNPD--RNWPAYI FLNAFVGSF SVGPA	434	
CaJen2	KWPYIINLSCLMVIGITGVFTYFQQFLGLRALFGVAMG-----GL	179	CaJen2	GIVIAHLSTFIRGRTALLGNVIAGIMIFYWAFHP---MMITAFMQFGQGSWSVVP I	398	
KlJen1	KWPYITCLGLFLICQLCTPWAKTYTQFLGVRWISGIAMG-----GI	238	KlJen1	GMFFGQMLEITGRRVGLLLALIMAGCTYPAFMLKTS SSVLGA GFMLWFSILGVWGVLPI	472	
KlJen2	KYSFVLTMALIIVIGITGVFNVSFAFLGCRAIFGIIMGSVFGSAFLGCRAIFXIMGSV	203	KlJen2	GIIVAHASSFFGRFRFSII VCCIGGGAMLYPWGFWANKSGINAVFLQFVQGAWGVIPI	422	
CjJen1	KWPFITTAAMFCALQIGTGFCCKTYQCFMAVRAISGIAMG-----GT	178	CjJen1	GLIAGTFMEVTGRRLLAILICCVIGGCFVYPA YMMHNSAVLGGGFLFFAIVGVWGVLPI	407	
CjJen2	KWPFITATAAMFCVLQIGTGFCNTYTYQFL-----AG-----GT	186	CjJen2	SLVTGIVMEVLGRRLSILLCCVIGGSFIYPAIMLHTTSATLGC GGFMMFVLGVWGVIPI	409	
CjJen3	KPTYLAVMCLFCIEVGTGFVNQYQFLVVRAMFGICMG-----GC	165	CjJen3	GLCVGTISEYTGRRLLAVFVCTICSSALIIYPAFYTTDMAGLICGGFFMQFVVMGCWGVTSV	393	
CjJen4	KPTYLAVMALFSIIIEIGTGFGVQNYTQFLIVRLFGICMG-----GC	145	CjJen4	GLCVGTISEFTGRRLLAFSCAVGSSALIIYPAFFSTSI GGLMAGGFMQFVVMGCWGVTSV	373	
CjJen5	KWPLIVNLFCLVVIQIGTGFINTYAEFIVGRALFGVFMG-----SM	97	CjJen5	GMTFGHFGSGLGRRSGIIIC SILGACMIYPWAFVRN--SGINAGVFFLQFVQGAWGVIPI	301	
CjJen6	KWPLIVNLCILVIGITGVFTKYSEFLGVRALFGIFMG-----SM	156	CjJen6	GMVIGHFGSGFIRRAAIMVCCICGGAMIYPWAFVTG--NGINAGVFFLQFVQGAWGVIPI	371	
* : : * : : *						
3 <sup>rd</sup> TMS		4 <sup>th</sup> TMS		5 <sup>th</sup> TMS		
ScJen1	KWPYITCLFLFVIAQLCTPWCDTYEKFLGVRWITGIAMG-----GI	245	ScJen1	GMIFGQFMEVTGRRRLGLLIAC TMGGCTYPAFMLRSEKAILGAGFMYFCVGVWGVLPI	478	
CaJen1	KWTYISIVTLFVVVEVGTFGVQYQQLGVRAIFGILMG-----AM	196	CaJen1	GIIMGQASELLGRRLTVVISI VCAGAFLYPSFFNPD--RNWPAYI FLNAFVGSF SVGPA	434	
CaJen2	KWPYIINLSCLMVIGITGVFTYFQQFLGLRALFGVAMG-----GL	179	CaJen2	GIVIAHLSTFIRGRTALLGNVIAGIMIFYWAFHP---MMITAFMQFGQGSWSVVP I	398	
KlJen1	KWPYITCLGLFLICQLCTPWAKTYTQFLGVRWISGIAMG-----GI	238	KlJen1	GMFFGQMLEITGRRVGLLLALIMAGCTYPAFMLKTS SSVLGA GFMLWFSILGVWGVLPI	472	
KlJen2	KYSFVLTMALIIVIGITGVFNVSFAFLGCRAIFGIIMGSVFGSAFLGCRAIFXIMGSV	203	KlJen2	GIIVAHASSFFGRFRFSII VCCIGGGAMLYPWGFWANKSGINAVFLQFVQGAWGVIPI	422	
CjJen1	KWPFITTAAMFCALQIGTGFCCKTYQCFMAVRAISGIAMG-----GT	178	CjJen1	GLIAGTFMEVTGRRLLAILICCVIGGCFVYPA YMMHNSAVLGGGFLFFAIVGVWGVLPI	407	
CjJen2	KWPFITATAAMFCVLQIGTGFCNTYTYQFL-----AG-----GT	186	CjJen2	SLVTGIVMEVLGRRLSILLCCVIGGSFIYPAIMLHTTSATLGC GGFMMFVLGVWGVIPI	409	
CjJen3	KPTYLAVMCLFCIEVGTGFVNQYQFLVVRAMFGICMG-----GC	165	CjJen3	GLCVGTISEYTGRRLLAVFVCTICSSALIIYPAFYTTDMAGLICGGFFMQFVVMGCWGVTSV	393	
CjJen4	KPTYLAVMALFSIIIEIGTGFGVQNYTQFLIVRLFGICMG-----GC	145	CjJen4	GLCVGTISEFTGRRLLAFSCAVGSSALIIYPAFFSTSI GGLMAGGFMQFVVMGCWGVTSV	373	
CjJen5	KWPLIVNLFCLVVIQIGTGFINTYAEFIVGRALFGVFMG-----SM	97	CjJen5	GMTFGHFGSGLGRRSGIIIC SILGACMIYPWAFVRN--SGINAGVFFLQFVQGAWGVIPI	301	
CjJen6	KWPLIVNLCILVIGITGVFTKYSEFLGVRALFGIFMG-----SM	156	CjJen6	GMVIGHFGSGFIRRAAIMVCCICGGAMIYPWAFVTG--NGINAGVFFLQFVQGAWGVIPI	371	
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5 <sup>th</sup> TMS		6 <sup>th</sup> TMS		7 <sup>th</sup> TMS		
ScJen1	YGCASATAIEDAPVKARSFSLGFLFFSAYAMGFI FAIFYRAFGYF--RDDGKWLFWFSI	303	ScJen1	HIAELAPADARALVAGLSYQLGNLASAASSTIETQLADRYPLERD--ASGAVIKEDYAKVM	537	
CaJen1	YPIAMVTALEGQPIAARSVLISGLFLPGYCFGYIMAMVYRAFAGTYKEGEGWRS LWFWSG	256	CaJen1	YLELVNSTHRTLGLSGVAYQLGNLVSSASSTIEAKIGERFPLKQD----PGMFDYKVM	489	
CaJen2	FGICAAEALGDAPKARGVLSIGIFQEGYAFGYLLAVVFQRAIADTT--EKTWRSEVWFSA	237	CaJen2	HLSELSPPHFRSFGVSVYQLGNLVSSASSTIEATIEE-----QIHIDYKTM	445	
KlJen1	YACASATAIEDAPVKARSFSLGFLFFSAYAMGFI FAIFYRAFGLVNN--GENYKWLQVWFSI	297	KlJen1	HLSELSPPEARALVSGLAYQLGNLASAASVVIENDLADLYPIEWN--SAVKVTNKDYKVM	531	
KlJen2	FGVASXTALENAPNKAISILSGIFQEGYAFGXLLGVVFQRAIVDNS--PHGWRAIFWFS	261	KlJen2	HLTELAPTEFRALITGVAYQLGNMISASSTIEASIGERFPLE----GREDAYDYKVM	477	
CjJen1	YATAAATSMDDAPLKARSFSLGFLFFSAYAFGMI FAATFWRAFES--TKHSWKALFWFS	235	CjJen1	HLSELSPDARALVSGLAYQLGNLASSASSTIETRLAKWPLEWD--AEGNPKIDYAKTI	466	
CjJen2	YATAAATSLDAPLKARSFSLGFLFFSAYAFGMI FAATFWRAFES--THWTWALFWFS	243	CjJen2	HLSELSPDARALVSGLAYQLGNLASSASSTIETDLAQRWPLEWD--AEGNPKIDYAKTI	468	
CjJen3	YTTASATALESQPVGSRVLSIGIFLPGYNLGYILAVAFYRAFES--EHGWRALFWFS	222	CjJen3	YIMELSPNAPRALFGGLAYQLGNLASSASSTIEAEISEAFPLSDI----GPEVVDYARM	449	
CjJen4	YTTASATALESQPTTSRVLGIGIFLPGYNLGYILAVAFYRAFES--THGWRALFWFS	202	CjJen4	YIMELSPNAPRALFGGLAYQLGNLASSASSTIEAEISEAFPLSDI----GPEVVDYARM	429	
CjJen5	YGLASATAMEGLPTSARSFSLGVYQGYALGYLLGVVFQRAITDIT--THTWRS LWFWSA	155	CjJen5	HLSELSPPEFRAFVGVSVYQLGNLASSASSTIESTLGERYPDYDDNGDIEGVYDYARM	361	
CjJen6	YGLASATALEGLPTDARSFSLGVYQGYALGYLLAVIFQRAITDIT--EKGWRS LWFWSA	214	CjJen6	HLSELSPPEFRAFVGVSVYQLGNLASSASSTIETTIGERFPLYTETGERREGVYDYARM	431	
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Figure S6. Detailed phylogenetic tree of Jen homologs, with all nominations and bootstrap values.





**Figure S7.** Detailed phylogenetic tree of Slc5 homologs, with all nominations and bootstrap values.

