cells

MDPI

Supplementary Materials

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Dictyostelium     6   DINVYSQTTIPNAMAELMDNHNKMEQISAYCKSLYA-NGDAAQAYEQTQGYAKNALLNVA  64
                      ++ +  +  IP     L D++  +E+++ YC++ Y  + D  +A E+T+ Y   +L +VA
H. sapiens        3   ELQMLLEEEIPGGRRALFDSYTNLERVADYCENNYIQSADKQRALEETKAYTTQSLASVA  62

Dictyostelium    65   YHIQTVGTHITSLLQLQTNEMEKLNIEIQTLTQRVRMIHDSTGTNVFSNQDAAKPYKSSL  124
                      Y I T+  ++  +L +Q +++ ++   I  ++Q V +  +             K   +S
H. sapiens       63   YLINTLANNVLQMLDIQASQLRRMESSINHISQTVDIHKEKVARREIGILTTNK──NTSR  120

                                                                  166 168 169
Dictyostelium   125   KNRKVDTEATKAPVKYVHKPISYGISASDINQNGVPPPLNHSNNSSANL  172
                       ++ +     + PV+Y+ KPI Y I   DI  +GV   L    S+ N+
H. sapiens      121   THKIIAPANLERPVRYIRKPIDYTI-LDDIG-HGVKWLLRFKVSTQNM  166
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Figure S1. Amino acid sequence comparison of *Dictyostelium* Abi with *Homo sapiens* Abi2. *Dictyostelium* Abi amino acid sequence was BLAST searched against *H. sapiens* which resulted in Abi2 as the nearest homolog with 24% identity and 49% similarity (E value=4-18). Conserved serine and threonine residue is indicated in red brackets.

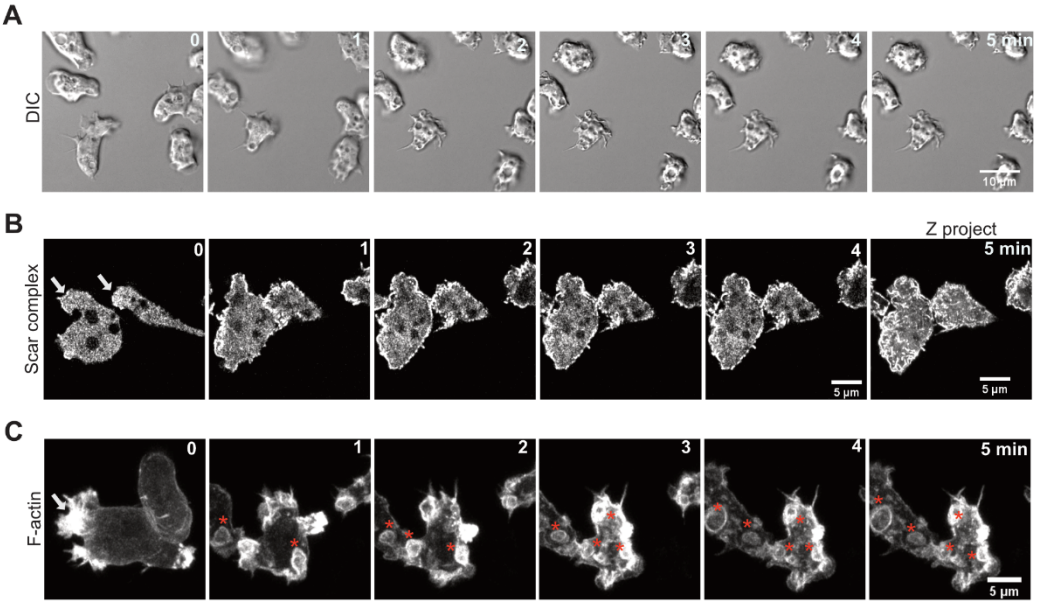

Figure S2. Effect of osmolarity on cell shape, Scar/WAVE and Actin localization. (**A**) Retraction of pseudopods and shrinkage of cells after addition of sorbitol (0.4 M). Cells in glass-bottom dishes were imaged by DIC microscopy at a frame interval of 2 s (Scale bar = 10 mm). (**B**) Distribution of the Scar/WAVE complex after sorbitol treatment. Cells expressing EGFP-Nap1, a marker of the Scar/WAVE complex, were imaged by AiryScan confocal microscopy, and 0.4 M sorbitol was added during imaging. Sorbitol inhib localization of the Scar/WAVE complex from pseudopodia and causes relocation to the cell cortex (Scale bar = 5 mm). (**C**) Distribution of actin after sorbitol treatment. Cells expressing lifeact-mRFPmars2 were imaged by AiryScan confocal microscopy. Actin relocates to macropinosomes/vesicles (*) after the addition of sorbitol (Scale bar = 5 mm).

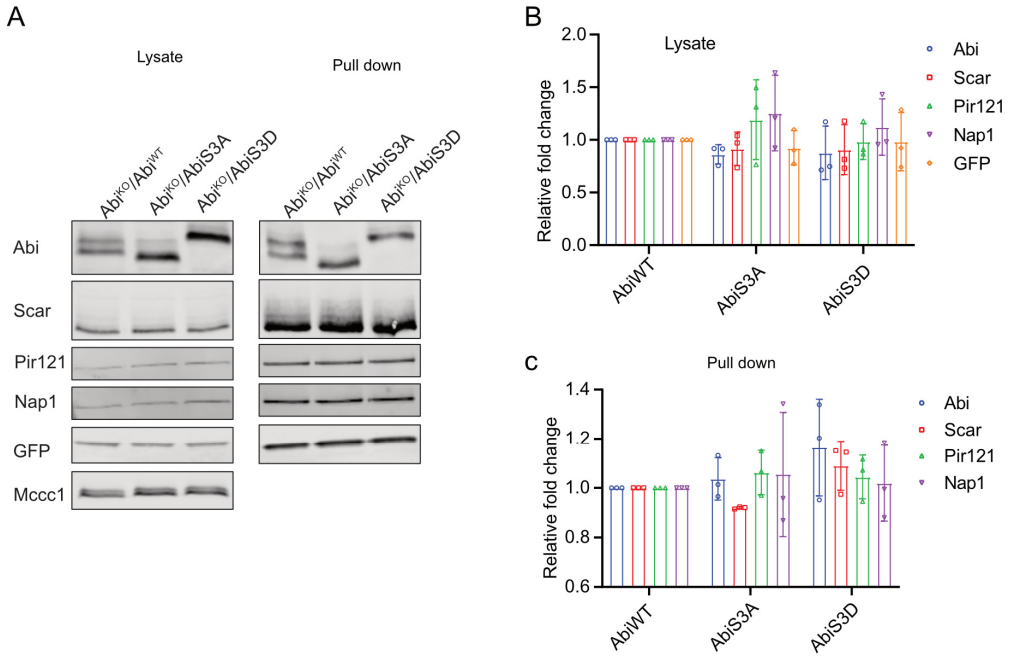

Figure S3. Expression pattern and effect of Abi^{WT}, Abi^{S3A} and Abi^{S3D} in the Scar/WAVE complex formation. (**A**) Abi^{WT}, Abi^{S3A} and Abi^{S3D} were co-expressed with HSPC300-eGFP in Abi- cells, and cell lysates were immunoprecipitated using GFP-TRAP. Lysate and pull-down samples were analyzed for the expression of Abi, Scar, Pir121, Nap1 and GFP by Western blotting. (**B**,**C**) Quantification of Western blots shows that Abi^{WT}, Abi^{S3A} and Abi^{S3D} formed stable complexes.

Video S1: Recruitment of the Scar/WAVE complex after folate treatment. eGFP-Nap1 cells were seeded on Lab-Tek II coverglass chambers and imaged with an AiryScan microscope. Folate (200 μM) was added to the cells undergoing imaging. Filmed at 1 frame/3 s, the movie shows 10 frames/second. Folate was added after frame 4 (after 12 s).

Video S2: Recruitment of the Scar/WAVE complex after cAMP treatment. eGFP-Nap1 cells were seeded on Lab-Tek II coverglass chambers and imaged by AiryScan confocal microscopy. cAMP (10 μM) was added to the cells undergoing imaging. Filmed at 1 frame/3 s, the movie shows 10 frames/second. cAMP was added after frame 4 (after 12 s).

Video S3: Change in cell morphology after sorbitol treatment. *Dictyostelium* cells were seeded on Lab-Tek II coverglass chambers and imaged by DIC microscopy (60x). Filmed at 1 frame/1 s, the movie shows 10 frames/second.

Video S4: Recruitment of the Scar/WAVE complex after sorbitol treatment. eGFP-Nap1 cells were seeded on Lab-Tek II coverglass chambers and imaged by AiryScan confocal microscopy. Sorbitol (0.4 M) was added to the cells undergoing imaging. Filmed at 1 frame/3 s, the movie shows 10 frames/second.

VideoS5: Recruitment of F-actin after sorbitol treatment. Life-act-eGFP expressing cells were seeded on Lab-Tek II coverglass chambers and imaged by AiryScan confocal microscopy. Sorbitol (0.4 M) was added to the cells undergoing imaging. Filmed at 1 frame/3 s, the movie shows 10 frames/second.

Video S6: Recruitment of the Scar/WAVE and Arp2/3 complexes after latrunculin treatment. eGFP-Nap1 cells expressing ArpC2-mRFPmars 2 were seeded on Lab-Tek II coverglass chambers and imaged by AiryScan confocal microscopy. LatrunculinA (5 μM) was added to the cells undergoing imaging. Filmed at 1 frame/3 s, the movie shows 5 frames/second. Latrunculin was added after frame 1 (after 1 min).

Video S7: Scar complex activation in Abi phosphomutants. Abi^{KO} cells co-expressing HSPC300-eGFP and Abi were allowed to migrate under agarose up folate gradient and Scar complex activation in pseudopods were observed by AiryScan confocal microscopy. Imaged at 1 frame/3 s, the movie shows 10 frames/second.

Video S8: Pseudopod formation in Abi phosphomutants. Abi^{KO} cells expressing Abi^{WT}, Abi^{S3A} and Abi^{S3D} were allowed to migrate under agarose up a folate gradient and observed by DIC microscopy. Filmed at 1 frame/2 s, the movie shows 10 frames/second.

Video S9: Scar complex activation in Abi phosphomutants. Scar-/Abi-/Pir121-eGFP cells expressing $Scar^{WT}/Abi^{WT}$, $Scar^{S8A}/Abi^{S3A}$ and $Scar^{S8D}/Abi^{S3D}$ were allowed to migrate under agarose up folate gradient and Scar complex activation in pseudopods were observed by AiryScan confocal microscopy. Imaged at 1 frame/3 s, the movie shows 10 frames/second.

Video S10: Pseudopod formation in Scar and Abi phosphomutants. Scar-/Abi- cells expressing $Scar^{WT}/Abi^{WT}$, $Scar^{S8A}/Abi^{S3A}$ and $Scar^{S8D}/Abi^{S3D}$ were allowed to migrate under agarose up a folate gradient and observed by DIC microscopy. Filmed at 1 frame/2 s, the movie shows 10 frames/second.

Table S1. Cell lines used in this study.

No.	Cell line	Given Name	Reference
1.	Ax3		dictyBase
2.	NC4		dictyBase
3.	Abi-	Ap5b	[1]
4.	eGFP-Nap1	-	[2]
5.	Scar-/Abi-	SP2	This study
6.	Scar-/Abi-/Pir121-eGFP	SP21	This study
7.	Pir121-	SP3	[3]
8.	Pir121-eGFP WT	SP7	[3]
9.	Pir121-eGFP A site	SP11	[3]

Table S2. List of primers.

Amplicon Name	Forward Primer (5′-3′)	Reverse Primer (5′-3′)
Abi 5′UTR	GAATTCGCCGGCG-GAGGGAAAAATTTTAGAGGA	GCATTTGGAATTGTGGTTT-GTGAATAAACG
Abi S166, 168, 169A	GTTCCACCACCATT-GAATCATGCAAATGCAG-CAGCTAATTTAACATCATCAAG	CTTGATGATGTTAAATT-AGCTGCTGCATTTGCATGAT-TCAATGGTGGTGGAAC
Abi S166, 168, 169D	GATAATGACGAC-GCTAATTTAACATCATCAAGTG	ATTAGCGTCGTCATTATCATGATTCAATGGTGGTGGAAC
Pir121 3′UTR	CAAAAAGATAAA-GCTTATCGCCAC-CACCCCCACCAATGTAA	TACGCAAACCGCCTCTGCCG-GAATTCTTGA-GAACTCCCTATCTTTATGATT
Blasticidin (BSR)	GCAGAAGCCATT-GCGATTGGT	ACCAATCG-CAATGGCTTCTGC
Abi 5′UTR	CCAATAGTAACAC-TATCTGCCAT	
Abi 3′UTR		TATTAAAGGATGGTGG-TATATTC

Table S3. Plasmids created and used in this study.

No.	Name of the Plasmid	Gene/s
1.	pSP287	Shuttle vector of Abi WT
2.	pSP318	Pir121eGFP knock in vector
3.	pSP384	Abi WT
4.	pSP396	Abi S166, 168, 169A (S3A)
5.	pSP427	Abi S166, 168, 169D (S3D)
6.	pSP428	Abi WT and HSPC300-eGFP
7.	pSP429	Abi S166, 168, 169A (S3A) and HSPC300-eGFP
8.	pSP430	Abi S166, 168, 169D (S3D) and HSPC300-eGFP
9.	pSP487	ScarWT and AbiWT
10.	pSP488	ScarS8A and AbiS3A
11.	pSP489	ScarS8D and AbiS3D

References

1. Pollitt, A.Y.; Insall, R.H. Abi mutants in Dictyostelium reveal specific roles for the SCAR/WAVE complex in cytokinesis. *Curr Biol* **2008**, *18*, 203-210, doi:10.1016/j.cub.2008.01.026.
2. Ura, S.; Pollitt, A.Y.; Veltman, D.M.; Morrice, N.A.; Machesky, L.M.; Insall, R.H. Pseudopod growth and evolution during cell movement is controlled through SCAR/WAVE dephosphorylation. *Curr Biol* **2012**, *22*, 553-561, doi:10.1016/j.cub.2012.02.020.
3. Schaks, M.; Singh, S.P.; Kage, F.; Thomason, P.; Klünemann, T.; Steffen, A.; Blankenfeldt, W.; Stradal, T.E.; Insall, R.H.; Rottner, K. Distinct Interaction Sites of Rac GTPase with WAVE Regulatory Complex Have Non-redundant Functions in Vivo. *Current Biology* **2018**, *28*, 3674-3684. e3676.