

Supplemental Materials

Molecular Biology of the Cell

Nepi et al.

Supplemental Materials

Supplemental Figure 1. Point mutations in conserved residues in the C-terminus of the Fab1 kinase domain result in dominant-active alleles. A)

Single point mutations in the C-terminal kinase domain exhibit a fragmented vacuole phenotype as compared to wild-type Fab1 indicative of elevated PI(3,5)P₂ levels. Increased vacuole fragmentation is quantified as an increase in the number of vacuoles per cell. >100 cells counted per strain, error bars represent S.E.M. N=3 independent experiments. **B)** The C-terminal Fab1 kinase domain is conserved across multiple phyla. ClustalOmega alignment of Fab1 from the species indicated. **Yellow:** dominant-active mutations that are loosely conserved among species. **Green:** residues strongly conserved across all Fab1.

Supplemental Figure 2. The CCR domain and kinase region of Fab1 are conserved in *C. thermophilum*. ClustalOmega alignment of the CCR domain and kinase region of *C. thermophilum* Fab1 compared to *S. cerevisiae*. **A)** Alignment of CCR domains. **B)** Alignment of kinase regions.

Supplemental Figure 3. A screen for dominant active alleles of Fab1 identifies point mutations in the CCR domain. A)

Twenty-one dominant active mutants were recovered from a directed gapped-plasmid screen of Fab1. **B)** Single point mutations in the CCR domain exhibit a fragmented vacuole phenotype as compared to wild-type Fab1 indicative of elevated PI(3,5)P₂ levels. Increased vacuole fragmentation quantified as an increase in the number of vacuoles per cell. >100 cells counted per strain, error bars represent S.E.M. N=3 independent experiments.

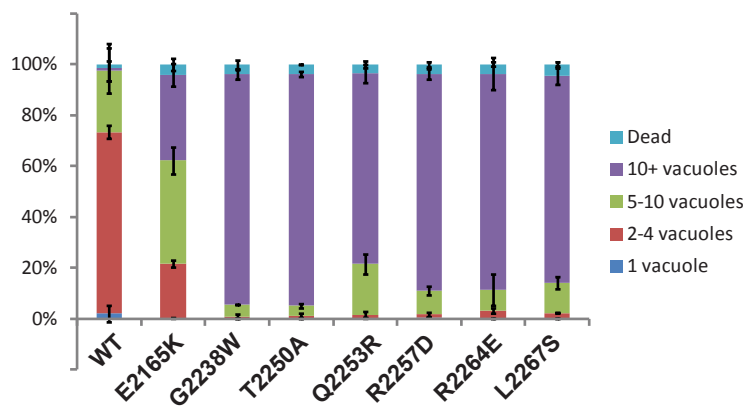
Table S1 Yeast strains used in this study

Strain	Genotype	Source
LWY2054	<i>MATa leu2,3-112 ura3-52 his3-Δ200 trp1-Δ901 lys2-801 suc2-Δ9 vac7Δ::HIS3</i>	Bonangelino et al., 2002
LWY2055	<i>MATa, leu2,3-112, ura3-52, his3-Δ200, trp1-Δ901, lys2-801, suc2-Δ9, fab1Δ::LEU2</i>	Bonangelino et al., 2002
LWY5055	<i>MATa trp1-901 leu2-3,112 ura3-52 his 3-200 gal4delta gal80delta GAL2-ADE2 LYS2::GAL1-HIS3 met2::GAL7-lacZ</i>	James et al., 1996
LWY7235	<i>MATa, leu2,3-112, ura3-52, his3-Δ200, trp1-Δ901, lys2-801, suc2-Δ9</i>	Bonangelino et al., 1997
LWY8812	<i>MATα, leu2,3-112, ura3-52, his-Δ200, trp1-Δ901, lys2-801, suc2-Δ9, fig4Δ::TRP1, FAB1-3xGFP::KAN</i>	Jin et al., 2008
LWY6474	<i>MATa, leu2, 3-112, ura3-52, his3-Δ200, trp1-Δ901, lys2-801, suc2-Δ9, fig4Δ::TRP1</i>	Duex et al., 2006a
LWY7016	<i>MATa, leu2, 3-112, ura3-52, trp1-Δ901, lys2-801, suc2-Δ9, Fab1-T2250A::KAN, fig4Δ::TRP1</i>	This study

Table S2 Plasmids used in this study:

Plasmid	Description	Source
pRS416-Fab1	CEN URA3	Jin et al., 2008
pRS416-Fab1-E2165K	CEN URA3	This study
pRS416-Fab1-G2238W	CEN URA3	This study
pRS416-Fab1-T2250A	CEN URA3	This study
pRS416-Fab1-Q2253R	CEN URA3	This study
pRS416-Fab1-R2257D	CEN URA3	This study
pRS416-Fab1-R2264E	CEN URA3	This study
pRS416-Fab1-L2267S	CEN URA3	This study
pRS416-Fab1-K1171R -H1192R	CEN URA3	This study
pRS416-Fab1-Q1419R	CEN URA3	This study
pRS416-Fab1-D1486N	CEN URA3	This study
pRS416-Fab1-T1491A	CEN URA3	This study
pRS416-Fab1-T1491M-T1553A	CEN URA3	This study
pRS416-Fab1-E1524K	CEN URA3	This study
pRS416-Fab1-D1568G	CEN URA3	This study
pGBD-(C1)	2 μ TRP1	James et al.,
pGAD (C1)	2 μ LEU2	James et al.,
pGBD-(C1)-Fab1-Kinase (1723-2278)	2 μ TRP1	This study
pGAD-(C1)-Fab1-Kinase (1723-2278)	2 μ LEU2	This study
pGAD-(C1)-Fab1-Kinase-E2165K	2 μ LEU2	This study
pGAD-(C1)-Fab1-Kinase-G2238W	2 μ LEU2	This study
pGAD-(C1)-Fab1-Kinase-T2250A	2 μ LEU2	This study
pGAD-(C1)-Fab1-Kinase-Q2253R	2 μ LEU2	This study
pGAD-(C1)-Fab1-Kinase-R2257D	2 μ LEU2	This study
pGAD-(C1)-Fab1-Kinase-R2264E	2 μ LEU2	This study
pGAD-(C1)-Fab1-Kinase-L2267S	2 μ LEU2	This study
pGBD-(C1)-CCR (1181-1585)	2 μ TRP1	This study
pGAD (C1)-CCR (1181-1585)	2 μ LEU2	This study
pGBD-(C1)-CCR-H1192R	2 μ TRP1	This study
pGBD-(C1)-CCR-Q1419R	2 μ TRP1	This study
pGBD-(C1)-CCR-D1486N	2 μ TRP1	This study
pGBD-(C1)-CCR-T1491A	2 μ TRP1	This study
pGBD-(C1)-CCR-T1553A	2 μ TRP1	This study
pGBD-(C1)-CCR-E1524K	2 μ TRP1	This study
pGBD-(C1)-CCR-D1568N	2 μ TRP1	This study
pGAD-(C1)-Vac14	2 μ LEU2	Jin et al., 2008
pGBD-(C1)-Vac14	2 μ TRP1	Jin et al., 2008
pGBD-(C1)-Fig4	2 μ TRP1	Jin et al., 2008
pGBD-(C1)-Vac7	2 μ TRP1	Jin et al., 2008
pGBD-(C1)-Atg18	2 μ TRP1	Jin et al., 2008
pGBD-(C1)-Fab1 (538-1917)	2 μ TRP1	This study
pGBD-(C1)-Fab1 (1373-1917)	2 μ TRP1	This study
pGBD-(C1)-Fab1 (538-1085)	2 μ TRP1	Jin et al., 2008
pQlinkMBP-Kinase_GST-CCR	Amp ^r	This study
pRS415-Fig4-4XMyc	CEN LEU2	Jin et al., 2008
pRS416-Fab1-TAP	CEN URA3	Jin et al., 2008

A)



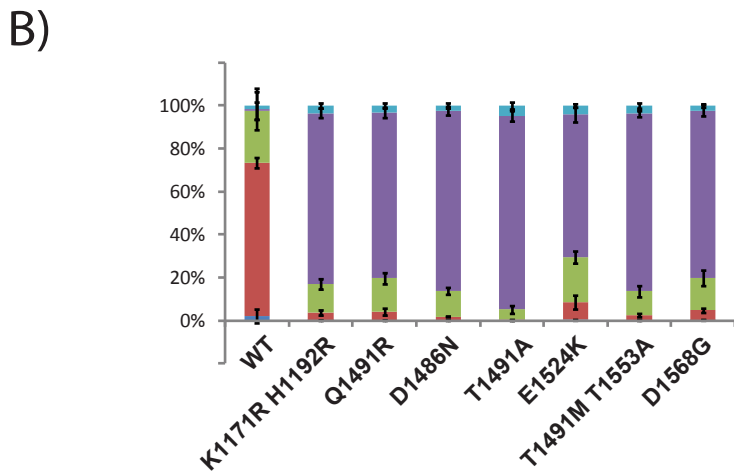
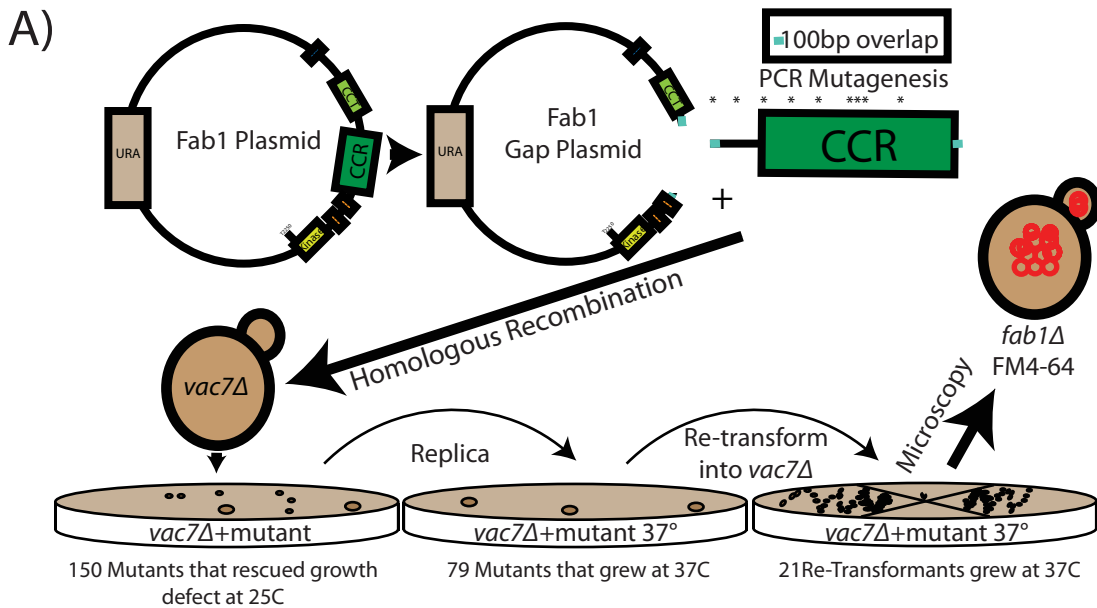
B)

Species	Sequence	Position
H_sapiens	DNPLYIRSHSKAVLRITSIHSDSHFLSSHLIIDYSLLVGRDDTSNELVVGIIDYIRTFD	2044
M_musculus	DNPLYIRSHSKSELRTSIHSDAHFLSSHLIIDYSLLVGRDDTSNELVVGIIDYIRTFD	1998
G_gallus	DNPLYIRSHCKAVLRASIHSDSQFLSSHLIIDYSLLVGRDDTSNELVVGIIDYIRTFD	2064
D_rerio	DNPLYIRSHCKAILRAAIHSDALFLSSHLIIDYSLLVGRDDSTDELVVGIIDYIRTFD	2029
X_tropicalis	DNPLYIRSHCKSVLRASIHSDSHFLSSHLIIDYSLLVGRDDTTDELVVGIIDYIRTFD	324
C_elegans	NQQLYVLPHSKAMNQAIISNDSHFLSSQYIMDYSLLVGVDDNGELIILGIVDMRYYTLD	1321
D_melanogaster	SKPLYVLSHSTVLRDAIQRDSSFLEKNLVMYSLLVGLDKKNGVVLVGIIDYIRTFD	1756
A_thaliana	TSPIFVGSKAKRLLERAVWNTSFLASIHVMDYSLLVGVDEERNELVGIIDFMRQYTD	1677
O_sativa	TSPIFVGNKAKRLLERAVWNTAFLASIGVMDYSLLVGVDEKKEHLMVGIIDFMRQYTD	1635
Z_mays	TSPIFVGNKAKRLLERAVWNTSFLASIDVMDYSLLVGVDEKRHELMVGIIDFIRQYTD	1746
S_pombe	ESPIFVSEQLKSLHSLWNTDTLFLSKLNIMDYSLVIGIDYTKKELYVGIIDFIRTYTD	1877
Y_lipolytica	ESPLFVREHAKLLRSTLYNDTLFLAKMNVMDYSLVIGIDSEEKVLVVGIIDIIRTFD	1986
C_albicans	ESPVFVGGQSKKLLRGCLFNDSFSLSAMVMDYSLVIGIDSSKKLYLGIIDWLRTFD	2570
C_thermophilum	ESPLFAREHSKRLLRASVFNDFLFLAKQDVMYSLMVAVDEAKKELVVGIIDCIRTYTD	2485
K_lactis	ESPIFVREYDKKLLRASLWNTDTLFLAKMNVMDYSLVGVVDNQTHSLTVGIIDCIRTFD	2001
S_cerevisiae	ESPIHVREYDKKLLRASVWNTDTLFLAKMNVMDYSLVIGIDNEGYTLTVGIIDFIRTFD	2224
	. . . * . . . : * : ** : : * * * * : . * * * : * : * : * *	

Species	Sequence	Position
H_sapiens	KKLEMVVKSTGILGGQ--GKMPTVVPELYRTVFCEAMDKYFMVDPDHWHTGLGLNC----	2098
M_musculus	KKLEMVVKSTGILGGQ--GKMPTVVPELYRTVFCEAMDKYFMVDPDHWHTGLDLNC----	2052
G_gallus	KKLEMVVKSTGILGGQ--GKMPTVVPELYRTVFCEAMDKYFMVDPDHWHTGLGLNC----	2118
D_rerio	KKLEMVVKSTGILGGQ--GKMPTVVPELYRTVFCEAMDKYFMVDPDHWHTGLGLNC----	2083
X_tropicalis	KKLEMVVKSTGILGGQ--GKMPTVVPELYRTVFCEAMDKYFMVDPDHWHTGLGGNC----	378
C_elegans	KKLESWVKIVAIPTA---HLPTILPEMYCAIFSEAIIDSYFPVDPQWTGLGIRSIRSY--	1375
D_melanogaster	KRVESIIGKSGILGGK--GKDPTVVNPERYKQAFIDAMDRYFETVDPDRWEGLSKV----	1809
A_thaliana	KHLETWVKTSGLLGGPK--NSTPTVIFPQYKKEFRKAMTAYFMVDPDQWSPAAVVPSSNS	1736
O_sativa	KHLETWVKTSGLLGGPK--NVAPTVIIPKQYKMEFRKAMSTYFVVPDQWSPPAVVPSPKQG	1694
Z_mays	KHLETWVKASGILGGPK--NVSPTVIFPKQYKKEFRKAMSAYFVVPDQWSPVVPVPSKQA	1805
S_pombe	KKLESWVKEKGLVGR---GPEPTVIFPKQYKMEFRKAMDCYILASQDFETGEGFKFCE--	1932
Y_lipolytica	KKLESWVKERGLVGG--AKEPTVVIPRQYKMEFREAMEYRIMVPE-----	2031
C_albicans	KKVENWVKGNLIDGNKRKGDPTIVIPKQYRIMEFREAMEYRIMEAPDIWYEGSK-----	2624
C_thermophilum	KQLESWIKQRGLAGS--RNRPTVIFPKKEYSIFRVAMAK-----	2523
K_lactis	KKLESWVKEKGLVGGST--KEPTVVIPRQYKMEFREAMDRYIMEVDPDPWYQDTET----	2054
S_cerevisiae	KKLESWVKEKGLVGGASVIKQPTVVIPRQYKMEFREAMEYRIMEVDPDPWYREGN-----	2278
	* : * : * : * * : . * * * * :	

Supplemental Figure S1

A)	Yeast_CCR	-----		
	C.thermo_CCR	AFYSDMVAKYETRILSVSPFVRFVTPQPYLLMKAREQERRLEYLKRRLRDQDQIQEPDQPKP		1246
	Yeast_CCR	-----SSSQNLGG		1188
	C.thermo_CCR	EEFQLIKPEMIHQMGQKAPRKIMEVIHAVHDAEYDKALHNYLTQTQKWETYLQNCCLDLFD		1305
		... :*		
	Yeast_CCR	TGSHQSITVLYSMVSTKTATPCVGPQIVTIDYF-----WSDSISIGQFIENNVGTA		1239
	C.thermo_CCR	PYAHQNIIVLVSSVCTAATQIPCEVPALMAIEFYNQHPDEGGFLDQDFTLGGYIEDICDTA		1365
		:**.*.** * :..* * ** * :::**:: *.*::**::**:: **		
	Yeast_CCR	RYPCQQ-GCNGLYLDHYRSYVHSGKVDVLEKFKQT---RLP-----KLKDIILT		1285
	C.thermo_CCR	NYVCTANNCGRKMIEHHRFTVHDNARLPIILKPSQWPENFPEKQPERGGDDEGTGICM		1425
		. * * * * :*:**::** :..: ::: :.*		
	Yeast_CCR	WSYCKKCGTSTPILQISEKTWNHNSFGKYLEVMFWSYKDSVTGIGKCPHDFTKDHVKYFGY		1345
	C.thermo_CCR	WNYCKVCDKHFGMLPMSVSTWKYSFGKYLELSEFWNTGLRLHPETGCPHDHKKDHVRYFYY		1485
		*.*** * . : : * .**::*****: ** . : *****.***:*** *		
	Yeast_CCR	--NDLVVRLEYSDELVEHELITPPRKIKWKPHIDIKLKVELYKILEKINNFYGSVLSRLE		1403
	C.thermo_CCR	TYLDIAVKVHYDPIIDLYELIIVPRKRITWKVDIDLKLNDFVTKAEERWNRFINSVKARLK		1545
		.::.*. :::*:**.* :*:** **.*:*** : : * * : *.* ** :**:		
	Yeast_CCR	RIKLDSTKDKVLSGQAKI IELKSNATEEQKMLQDLDTFYADSPCQHLPLNLVIKSLY		1463
	C.thermo_CCR	SIKIDSVLPEKAEDCKAEVDRLLEKAEEDHKEMIKVLQAYMNSKYYEIIIPFNVFRQML		1605
		:: :*. . :*: : * .** *:* * : : . * * * : :*:**:::		
	Yeast_CCR	DKAVNWNSTFAIFAKSYLPSETDISRITAKQLKFLY----- 1500		
	C.thermo_CCR	ERATEWDSAFTQFESKFLS-DKDVRQLTLIQLKMFSDHEKD 1646		
		::*.:**:*:* * . :.* :*: :*: *****		
B)	Yeast_Kinase_Region	--EKSLLMKTLNSFWADRSAYLWKPLVYPTCPSEHIFTSDVIREDEPSSLIAFCLSTS		1879
	C.thermo_Kinase_Region	KHQKKS LMDRLANFWAERSASSWPQLEYPVNMGDVMFSDVIVREDEPSSLVAYAMSLN		2191
		:* . ** . * :***:*** * * ** . :*: : *****:*****:*.:* . *		
	Yeast_Kinase_Region	DYRNKMMNLNVQQQQQQQTAEAAPAKTGGNSGGTQTQGDPSVNI SPSVSTTSHNKGRDSE		1939
	C.thermo_Kinase_Region	DYKEKLAAIRRDLRMS-----NFGETDVSGDSLE-----		2220
		::: :. : : . * * * :**		
	Yeast_Kinase_Region	ISSLVTTKEGLNLTPIEGARDRTPQESQTHSQANLDTLQLEKIMTKKTATHLRYQFEE		1999
	C.thermo_Kinase_Region	-----DNMGT-----PVDPEAAKERARKIDMELEKTLRSTGTHVKYQFVH		2261
		: * * : : : . ***** : :*.**::*** .		
	Yeast_Kinase_Region	GLTVMSCKIFFTEHFDVFRKICDCQENFIQSLSRCVKWDSNGGKSGSGLFKTLDDRIFIK		2059
	C.thermo_Kinase_Region	GTAKMMCKIFFAEQFDALRRCGAADRFVSELSRCLKWDSKGGTKSVFLKTLDDRFLVK		2321
		* : * *****:*:**:.* * . :*: :*****:*****:***: * *****:.*		
	Yeast_Kinase_Region	ELSHAELEAFIKFAPSYFEYMAQAMFHDLPPTLAKVFGFYQIQVKSSISSSKSYKMDVII		2119
	C.thermo_Kinase_Region	SLSVPETQSFLKAPDYFNIMAEALFHELPSVIAKMLGFFRVHIKNPVTN-TDIKLDLLV		2380
		.** * :*:**.* ** :*:**.***.:**:*:**:***:*. :. . *.*:::		
	Yeast_Kinase_Region	MENLFYEKKTTRIFDLKGSMRNRHVEQTGKANEVLLDENMVEYIYESPIHVREYDKLLR		2179
	C.thermo_Kinase_Region	MENLFYDRTPSRFTDLKGSMRNRRIQSTGERDEVLLDENMVEYIYESPLFAREHSKRLLR		2440
		*****:.. :* *****:***:***: :*****:*****:***:*.**::***		
	Yeast_Kinase_Region	ASVWNDFLFLAKMNVMDYSLVIGIDNEGTYLTVGIIIDFIRFTWDKKLESVWKEKGLVGG		2239
	C.thermo_Kinase_Region	ASVFNDTLFLAKQDVMYSLMVAVDEAKKELVVGII DCIRTYTWDKQLESWIKQRGLAGG		2500
		:** :*****:..*: * .***** ***:***:***:***:***:*** **		
	Yeast_Kinase_Region	ASVIKQPTVVTPRQYKKRFREAMERYILMVPDPWYWEGN	2278	
	C.thermo_Kinase_Region	SR--NRPTVTSKEYKSRFRVAMAK-----	2523	
		: :***:*.**.* ** *		



Supplemental Figure S3