A humanized yeast-based toolkit for monitoring phosphatidylinositol 3-kinase activity at both single cell and population levels

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SUPPLEMENTARY MATERIAL

SUPPLEMENTARY TABLES

Table S1. Yeast strains used in this work.

Strain name	Genotype	From
BY4741	*MATa; his3Δ1; leu2Δ0; met15Δ0; ura3Δ0*	EUROSCARF WGD collection
YPH499	*MATa; ade2-10; trp1-63; leu2-1; ura3-52; his3-Δ20; lys2-801*	[1]
1784	*MATα ura3-52 leu 2-3,112 trp1-1 his4 CanR*	[2]
VCY1	Isogenic to 1784, *cdc10-11*	[3]
VMY8	Isogenic to BY4741, *SUR7-GFP::URA3*	[4]
VMY9	*MATα lsp1Δ::kanMX4 pil1Δ::kanMX4 ura3Δ0 his3Δ1 leu2Δ0 SUR7-GFP::URA3*	[4]

Table S2. Plasmids generated and used in this work

Plasmid	Description	Source/Refererence
YCpLG	*CEN*, *LEU2*,*GAL1* promoter, empty vector.	[5]
YCpLG-PI3Kα	Expresses wild type p110α cDNA from the *GAL1* promoter	[6]
YCpLG-PI3Kα-CAAX	Expresses p110α-CAAX from the *GAL1* promoter	[6]
YCpLG-Myr-PI3Kα	Expresses Myr-p110α from the *GAL1* promoter	[6]
YCpLG-PI3Kα(K802R)	Expresses kinase-dead p110α(K802R) from the *GAL1* promoter	[6]
pLA10	Expresses Cdc10-GFP from its own *CDC10* promoter	[3]
YCpLG-Cdc10-p110α	Expresses Cdc10-p110α from the *GAL1* promoter	This work
YCpLG-Cdc10-GFP-p110α	Expresses Cdc10-GFP-p110α from the *GAL1* promoter	This work
YCpLG-Cdc10-p110α(K802R)	Expresses Cdc10-p110α(K802R) from the *GAL1* promoter	This work
YCpLG-Cdc10-GFP-p110α(K802R)	Expresses Cdc10-GFP-p110α(K802R) from the *GAL1* promoter	This work
YCpLG-*cdc10-11*-p110α	Expresses Cdc10(G179D)-p110α from the *GAL1* promoter	This work
YCpLG-*cdc10-11*-p110α(K802R)	Expresses Cdc10(G179D)-p110α(K802R) from the *GAL1* promoter	This work
YCpLG-*cdc10-11*-GFP-p110α	Expresses Cdc10(G179D)-GFP-p110α from the *GAL1* promoter	This work

YCpLG-*cdc10-11*-GFP-p110α(K802R)	Expresses Cdc10(G179D)-GFP-p110α(K802R) from the *GAL1* promoter	This work
YCpLG-Cdc10-p110α-(C2/4KA)	Expresses Cdc10-p110α-(C2/4KA) from the *GAL1* promoter	This work
YCpLG-Pil1-p110α	Expresses Pil1-p110α from the *GAL1* promoter	This work
YCpLG-Pil1-p110α(K802R)	Expresses Pil1-p110α(K802R) from the *GAL1* promoter	This work
YCpLG-Pil1-STOP	Pil1-STOP-p110α. Expresses Pil1 from the *GAL1* promoter	This work
YCpLG-Pil1-p110α(C2/4KA)	Expresses Pil1-p110α-(C2/4KA) from the *GAL1* promoter	This work
pESC-TRP	*2*μ, *TRP1*, *GAL1/GAL10* bidirectional promoter, empty vector.	Agilent Technologies
pRS426-GFP2XPH(PLCδ)	*2*μ, *URA3*, GFP-2×PH(PLCδ) fluorescent reporter for PtdIns4,5P_2	[7]
mCherry-Akt3-pYES3	Expresses mCherry-Akt3 from the *GAL1* promoter	I. Rodríguez-Escudero & Teresa Fernández-Acero, to be published elsewhere
pJMCS-DM23	GFP-2×PH (PLCδ)-mCherryPH(Akt3) double marker in pESC-TRP	This work
pJMCS-DM20	GFP-2×PH (PLCδ) in pESC-TRP	This work
pGNG1	GFP expression reporter Grow N'Glow™ system	MoBiTec
pEG202-GAL4	LexA-GAL4 positive control fusion for two-hybrid system	MoBiTec
pJMCS-LG3	Expresses the LexA-GAL4-PH(Akt3) fusion	This work

Table S3. Oligonucleotides used in this work.

Name	Sequence
Cdc10-Fw	(5’-CGGGATCCATGGACCCTCTCAGCTCAGTACAGC-3’)
Cdc10-Rv	(5’-CGGGATCCGGCACCGGCTCCAGCGCCTGCACCAGCTCCACGTTGAATGGCGTTGCTAG-3’)
Cdc10-GFP-Rv	(5’-CGGGATCCGGCACCGGCTCCAGCGCCTGCACCAGCTCCTTTGTATAGTTCATCCATGCC-3’)
Pil1-Fw	(5’-CGGGATCCATGCACAGAACTTACTCTTTA-3’)
Pil1-Rv	(5’-CGGGATCCGGCACCGGCTCCAGCGCCTGCACCAGCTCCAGCTGTTGTTTGTTGGGGA-3’)
Cdc10-G179D-Fw	(5’-CAAATGTTATACCAGTTATTGACAAGTCGGATACATTGACTTTA-3’)
Cdc10-G179D-Rv	(5’-TAAAGTCAATGTATCCGACTTGTCAATAACTGGTATAACATTTG-3’).
STOP-p110α-Fw	(5’-AGCCGGTGCCTGATCCGCCACC-3’)
STOP-p110α-Rv	(5’-GTGGCGGATCAGGCACCGGCTC-3’)
GFP-Fw	(5’-GACTAGTATGGGTAAAGGTGTAGAAC-3’)
PH(PLCδ) Rv	(5’-GAAGATCTCCCCTCGAGGTCGACTA-3’)
Cherry-Fw	(5’-CGGGATCCATGGTGAGCAAGGGCGAG-3’)
PH (Akt3)-Rv	(5’-CTAGCTAGCTTACAAAATAACTTTCCCAAAAG-3’)
Insert-NotI-Fw	(5’-AAAGAGAGCGGCCGCTAAAATGAATCGTAGATACTGAAAAACCCCG-3’)
Insert-NotI-Rv	(5’-ATTTTAGCGGCCGCTCTCTTTTTTTGGGTTTGGTGGGGTATCTTCA-3’)
NotI-PH(Akt3)-Fw	(5’-AAGGAAAAAAGCGGCCGCATGAGCGATGTTACCATTGTG-3’)
NotI-PH(Akt3)-RV	(5’-AAGGAAAAAAGCGGCCGCTTACAAAATAACTTTCCCAAAAGT-3’)

SUPPLEMENTARY FIGURES

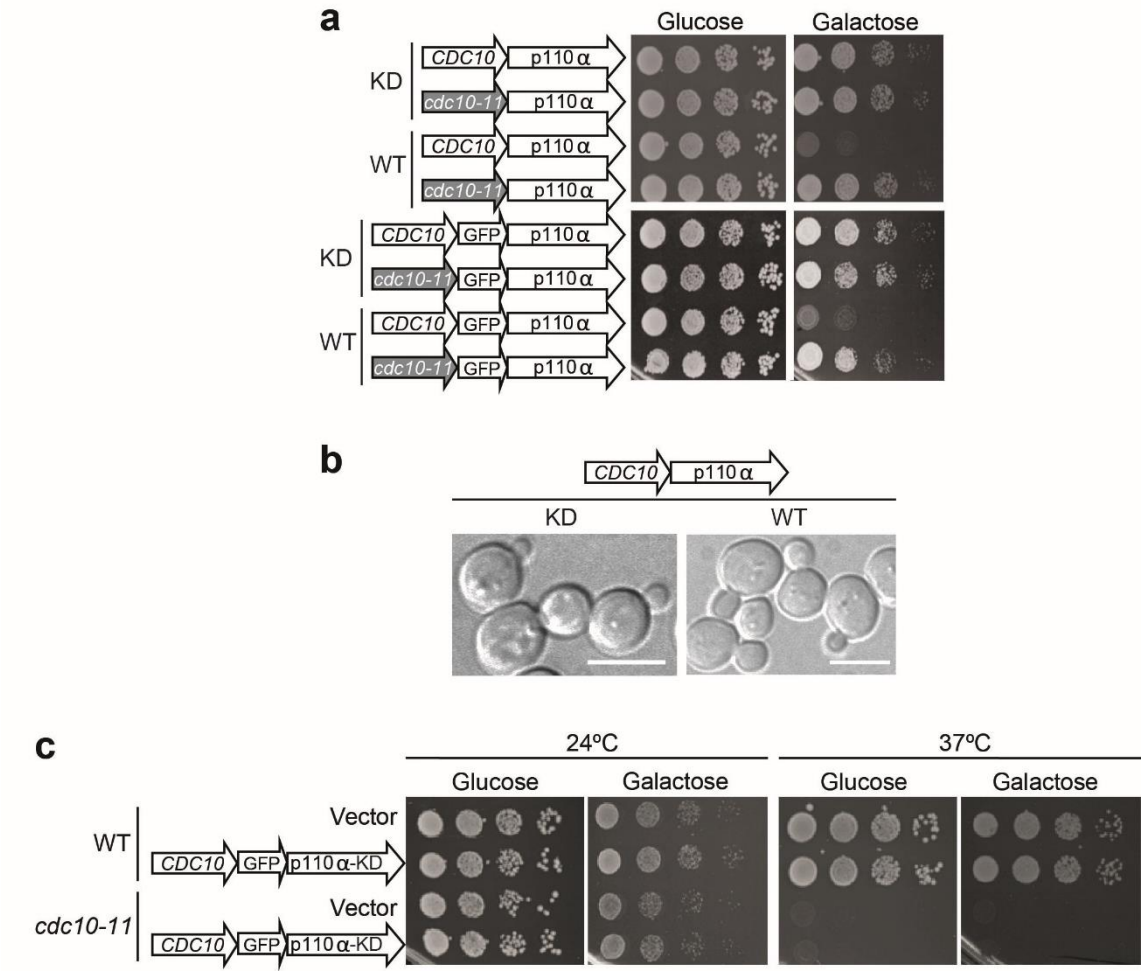

Figure S1. (a) Ten-fold serial dilutions of YPH499 yeast cells transformants bearing the plasmids YCpLG-Cdc10-p110α(K802R), YCpLG-*cdc10-11*-p110α(K802R), YCpLG-Cdc10-p110α, YCpLG-*cdc10-11*-p110α, YCpLG-Cdc10-GFP-p110α(K802R), YCpLG-*cdc10-11*-GFP-p110α(K802R), YCpLG-Cdc10-GFP-p110α, YCpLG-*cdc10-11*-GFP-p110α and cultured at 24°C on SD (Glucose) or SG (Galactose) agar plates. The expressed constructs are indicated on the sketches at the left. (b) Differential Interference Contrast (DIC) microscopy images of YPH499 cells expressing kinase-dead Cdc10-p110α(K802R) (KD, left) or wild type Cdc10-p110α (WT, right). (c) Ten-fold serial dilutions of wild type 1784 and the isogenic *cdc10-11* mutant VCY1 transformed with either empty vector (YCpLG) or the YCpLG-Cdc10-p110α(K802R) plasmid cultured in SD (Glucose) or SG (Galactose) at the indicated temperatures.

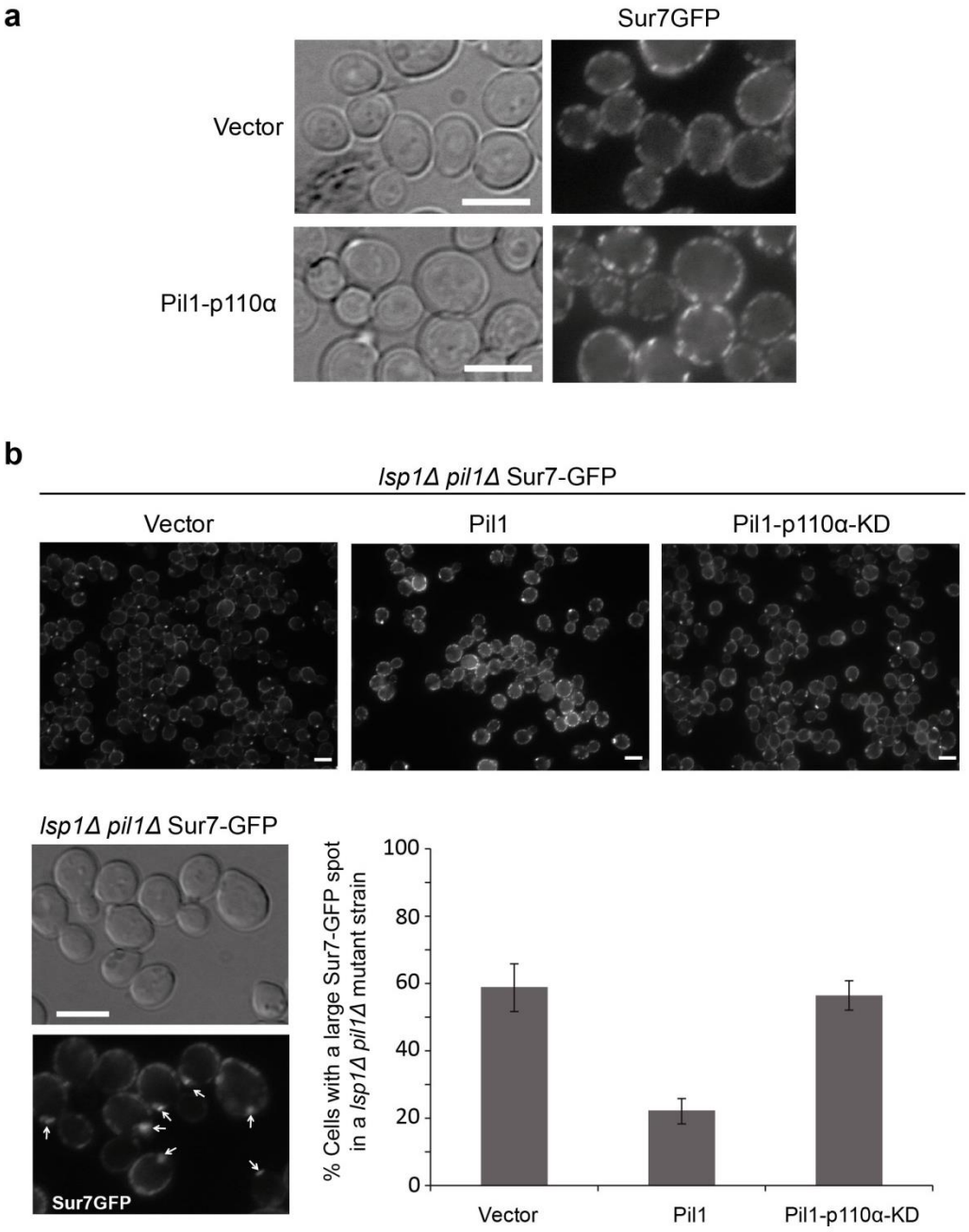

Figure S2. (a) Bright field and the corresponding GFP fluorescence microscopy images of representative cells of a Sur7-GFP tagged strain (VMY8), transformed with the empty vector YCpLG as a control or the plasmid YCpLG-Pil1-p110α. (b) On the upper side, GFP fluorescence microscopy images of a representative field of a *lsp1*Δ *pil1*Δ Sur7-GFP strain (VMY9) transformed with the empty vector YCpLG (Vector) or plasmids YCpLG-Pil1-STOP (Pil1) and YCpLG-Pil1-p110α(K802R) (Pil1-p110α-KD). On the lower side, at the left, representative field showing VMY9 cells typically displaying a large Sur7-GFP spot, marked by arrows. At the right side, a graph indicating the percentage of cells (n=30) with such spot in cultures expressing the same plasmids than in (a). The results correspond to three biological replicates and error bars represent SD.

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