Table S1. Simulation Parameters and Their Sampling Ranges, Related to Experimental Procedures

Name	Description	Sampling Range
C_A	Concentration of As (normalized by total number of binding sites on membrane)	0- 4L , where L = number of binding sites (linear)
C_B	Concentration of Bs (normalized by total number of binding sites on membrane)	0 – 4L (linear)
D_A	Membrane diffusion rate of A	0.01 – 100 (logarithmic)
D_B	Membrane diffusion rate of B	0.01 – 100 (logarithmic)
$R_{A \to A}$	Auto-regulation of A	0.1 – 1000 (logarithmic)
$R_{B \to A}$	Regulation of A by B	0.1 – 1000 (logarithmic)
$R_{B \to B}$	Auto-regulation of B	0.1 – 1000 (logarithmic)
$R_{A \to B}$	Regulation of B by A	0.1 – 1000 (logarithmic)

Table S2. Protein Domains and Their Sources, Related to Experimental Procedures

Domain	Description	Source
p110α	PI3 kinase	María Molina and Victor Cid (Universidad Complutense de Madrid, Spain)
PTEN	PIP_3 phosphatase	María Molina and Victor Cid
$CRIB_{Gic2}$	Binds Cdc42*	Genomic DNA PCR (Ozbudak et al., 2005) Forward primer: CACCTGCAAC AGCGATACTAGTGCAAGTATT ACCAATACTGGAAAC Reverse primer: CACCTGCCTT GCGCATTACTTATTTTCGTGC GATCTTGAAATGAGTTTGC
PH_{Akt}	Binds PIP_3	María Molina and Victor Cid
$2xPH_{PLC\delta}$	Binds PIP_2	Scott Emr, María Molina and Victor Cid
GAP_{Rga1}	GTPase activating factor for Cdc42*	Genomic DNA PCR (Tong et al., 2007) Forward primer: CACCTGCAACA GCGATAGCGCAGGCAGTGCTG GTTGGAAGTTTTTTTCATCTGCA AAGCCAC Reverse primer: CACCTGCCTTG CGCATTA CGTCAGGATATCTTT GTAGTTCCCAA
CAAX	Plasma membrane localization domain	Leon Chan (Weis lab, Berkeley, CA)
mCherry	Red fluorescent protein	
GFP	Green fluorescent protein	

Table S3. Strains Used in This Study, Related to Experimental Procedures

Strain	Description
YEF473**a**	**a** *trp1 leu2 ura3 his3 lys2*, haploid segregant of YEF473 (C276-4A x YPH500) from Erfei Bi (UPenn, Philadelphia, PA) (Bi and Pringle, 1996)
yJW8	YEF473**a** Δ*gal2*::Ø
yJW13	W303**a** Δ*gal2*::Nat^{R} *leu2 ura3 his3 trp1*

Table S4. Plasmids Used in This Study, Related to Experimental Procedures

Plasmid	Parent vector	Promoter	Domains
pJW166	pNH605	pCyc1	PH_{Akt} - mCherry
pJW481	pNH605	pIno4L	2xPH_{Akt} - 2xGFP
pJW459	pJW609	pSte5	$CRIB_{Gic2}$ - 4xmCherry
pJW226	pNH603	pCyc1	p110α - 2x$PH_{PLCδ}$
pJW227	pNH603	pCyc1	p110α
pJW484	pNH603	pIno4S	p110α - PH_{Akt}
pJW486	pNH603	pCyc1	p110α - PH_{Akt}
pJW487	pNH603	pCyc1	p110α – CAAX
pJW532	pNH603	pUra3	p110α - PH_{Akt}
pJW482	pNH603	pGal10	p110α - PH_{Akt}
pJW357	pSV606	pAdh1	PTEN K13E - $CRIB_{Gic2}$
pJW526	pJW609	pIno4S	PH_{Akt} - GAP_{Rga1}
pJW364	pJW609	pCyc1	PH_{Akt} - GAP_{Rga1}
pJW363	pJW609	pSte5	PH_{Akt} - GAP_{Rga1}
pJW241	pNH604	pGal10	PTEN K13E-CAAX
pJW243	pNH604	pGal10	PTEN K13E
pJW182	pSV606	pAdh1	Empty vector

Table S5. Plasmids Used in Specific Experiments in This Study, Related to Experimental Procedures. All experiments were performed in yeast strain yJW8 unless otherwise indicated.

Figure/panel	PIP_3 kinase	PIP_3 phosphatase	GAP	Reporter(s)
Figure 4C top*	pJW226	-	-	pJW166
Figure 4C bottom*	pJW227	-	-	pJW166
Figure 5A and 5B	pJW486	pJW357	pJW526	pJW459, pJW481
Figure 5C top	pJW486	-	-	pJW459, pJW481
Figure 5C bottom	pJW486	pJW357	pJW526	pJW459, pJW481
Figure 6A	Low: pJW484 Med: pJW486 High: pJW532	-	-	pJW459, pJW481
Figure 6B top	pJW487	-	-	pJW459, pJW481
Figure 6B bottom	pJW486	pJW357	pJW526	pJW459, pJW481
Figure 6C, left to right	pJW487	-	-	pJW459, pJW481
	pJW486	-	-	pJW459, pJW481
	pJW487	pJW357	-	pJW459, pJW481
	pJW486	-	pJW526	pJW459, pJW481
	pJW487	pJW357	pJW526	pJW459, pJW481
	pJW486	pJW357	-	pJW459, pJW481
	pJW486	pJW357	pJW526	pJW459, pJW481
Figure S4A top*	pJW226	pJW243	-	pJW166
Figure S4A bottom*	pJW226	pJW241	-	pJW166
Figure S5A	pJW486	-	-	pJW459, pJW481
Figure S5B	pJW486	pJW357	-	pJW459, pJW481
Figure S5C	pJW486	pJW357	pJW526	pJW459, pJW481
Figure S6A	pJW486	pJW357	pJW526	pJW459, pJW481
Figure S6C	pJW482			pJW459, pJW481, pJW182
Figure S6D	pJW482	pJW357	pJW526	pJW459, pJW481
Movies S1-S6	pJW486	pJW357	pJW526	pJW459, pJW481

* in yeast strain yJW13