Text S1

Part 1. Details on curve fitting of FRAP data

The EGFP FRAP data can be fitted with a single exponential curve, whereas the actin FRAP data (WT, G13R, and R62D) can only be fitted accurately with double exponential function. Details are provided below.

Single exponential fit:

Let: $F(t) = a - b\exp\left(-\frac{t}{t_1}\right)$

$F(0) = F_0$ and $F(\infty) = F_e$

Thus: $F(t) = F_e - (F_e - F_0)\exp\left(-\frac{t}{t_1}\right)$

For EGFP: $F_0 = 0.0314;\quad F_e = 0.9999;\quad t_1 = 1.0444$

Double exponential fit:

Let: $F(t) = a - b\exp\left(-\frac{t}{\tau_1}\right) - c\exp\left(-\frac{t}{\tau_2}\right)$

$F(0) = F_0$ and $F(\infty) = F_e$

Thus: $F(t) = F_e - (F_e - F_0)\exp\left(-\frac{t}{\tau_1}\right) - c\left(\exp\left(-\frac{t}{\tau_2}\right) - \exp\left(-\frac{t}{\tau_1}\right)\right)$

For WT G-actin: $F_0 = 0.0960;\quad F_e = 0.8797;\quad c = 0.4161;\quad t_1 = 1.5112;\quad t_2 = 44.0287$

For G13R: $F_0 = 0.1041;\quad F_e = 1.0019;\quad c = 0.4637;\quad t_1 = 1.4744;\quad t_2 = 69.3191$

For R62D: $F_0 = 0.1021;\quad F_e = 1.0002;\quad c = 0.4506;\quad t_1 = 1.5724;\quad t_2 = 70.2468$

Part 2. Models for binding competition between G-actin and PH domains for association with the inositol lipids PI(4,5)P_2 and PI(3,4,5)P_3

First, we will consider competition models in which either G-actin or a PH domain can form a scaffold with PIP_2 (PIP_3) but not both. Fig. S6 (A and B) shows four such models: schemes a1 and a2 (schemes b1 and b2) correspond to interactions between G-actin and PIP_3 (PIP_2), respectively; schemes a1 and b2 (schemes a2 and b1) involve binding between PH_{Akt} and PIP_3 (PH_{PLC} and PIP_2), respectively. For all schemes, we assume that the concentrations of unbound G-actin and PH domains are in abundance and quickly equilibrate to a constant value. However, the expression of PH domain is different between measurements so that in correlation plots of total G-actin (bound plus unbound) versus total PH (bound plus unbound), the concentration of the unbound PH is an independent variable.

Chemical equations for the schemes in Fig. S6 (A and B) are given in Table S1 below:

Table S1

Scheme a1	Scheme a2	Scheme b1	Scheme b2
$PIP_3 \xrightarrow{k_1} PIP_2$;	$PIP_3 \xrightarrow{k_1} PIP_2$;	$PIP_3 \xrightarrow{k_1} PIP_2$;	$PIP_3 \xrightarrow{k_1} PIP_2$;
$PIP_3 \xleftarrow{k_{-1}} PIP_2$;	$PIP_3 \xleftarrow{k_{-1}} PIP_2$;	$PIP_3 \xleftarrow{k_{-1}} PIP_2$;	$PIP_3 \xleftarrow{k_{-1}} PIP_2$;
$PIP_3 + G_{actin} \xrightarrow{k_2} [PIP_3 \cdot G_{actin}]$;	$PIP_3 + G_{actin} \xrightarrow{k_2} [PIP_3 \cdot G_{actin}]$;	$PIP_2 + G_{actin} \xrightarrow{k_6} [PIP_2 \cdot G_{actin}]$;	$PIP_2 + G_{actin} \xrightarrow{k_6} [PIP_2 \cdot G_{actin}]$;
$PIP_3 + G_{actin} \xleftarrow{k_{-2}} [PIP_3 \cdot G_{actin}]$;	$PIP_3 + G_{actin} \xleftarrow{k_{-2}} [PIP_3 \cdot G_{actin}]$;	$PIP_2 + G_{actin} \xleftarrow{k_{-6}} [PIP_2 \cdot G_{actin}]$;	$PIP_2 + G_{actin} \xleftarrow{k_{-6}} [PIP_2 \cdot G_{actin}]$;
$PH_{Akt} + PIP_3 \xrightarrow{k_3} [PH_{Akt} \cdot PIP_3]$;	$PH_{PLC} + PIP_2 \xrightarrow{k_5} [PH_{PLC} \cdot PIP_2]$;	$PH_{PLC} + PIP_2 \xrightarrow{k_5} [PH_{PLC} \cdot PIP_2]$;	$PH_{Akt} + PIP_3 \xrightarrow{k_3} [PH_{Akt} \cdot PIP_3]$;
$PH_{Akt} + PIP_3 \xleftarrow{k_{-3}} [PH_{Akt} \cdot PIP_3]$.	$PH_{PLC} + PIP_2 \xleftarrow{k_{-5}} [PH_{PLC} \cdot PIP_2]$.	$PH_{PLC} + PIP_2 \xleftarrow{k_{-5}} [PH_{PLC} \cdot PIP_2]$.	$PH_{Akt} + PIP_3 \xleftarrow{k_{-3}} [PH_{Akt} \cdot PIP_3]$.

The corresponding rate laws in steady-state approximation are given in Table S2 below:

Table S2

Scheme a1	Scheme a2	Scheme b1	Scheme b2
$k_1 [PIP_3] = k_{-1} [PIP_2]$	$k_1 [PIP_3] = k_{-1} [PIP_2]$	$k_1 [PIP_3] = k_{-1} [PIP_2]$	$k_1 [PIP_3] = k_{-1} [PIP_2]$
$k_{-2} [PIP_3 \cdot G_{actin}] = k_2 [PIP_3] \cdot [G_{actin}]$	$k_{-2} [PIP_3 \cdot G_{actin}] = k_2 [PIP_3] \cdot [G_{actin}]$	$k_{-6} [PIP_2 \cdot G_{actin}] = k_6 [PIP_2] \cdot [G_{actin}]$	$k_{-6} [PIP_2 \cdot G_{actin}] = k_6 [PIP_2] \cdot [G_{actin}]$
$k_{-3} [PH_{Akt} \cdot PIP_3] = k_3 [PH_{Akt}] \cdot [PIP_3]$	$k_{-5} [PH_{PLC} \cdot PIP_2] = k_5 [PH_{PLC}] \cdot [PIP_2]$	$k_{-5} [PH_{PLC} \cdot PIP_2] = k_5 [PH_{PLC}] \cdot [PIP_2]$	$k_{-3} [PH_{Akt} \cdot PIP_3] = k_3 [PH_{Akt}] \cdot [PIP_3]$

For Scheme a1, the conservation of the total concentration of inositol lipids implies:

$$[PIP]_{sh1}^{total} = [PIP_2] + [PIP_3] + [PIP_3 \cdot G_{actin}] + [PH_{Akt} \cdot PIP_3]. \quad (1)$$

The total concentration of fluorescently tagged G_{actin} and PH_{Akt} measured for the correlation plots:

$$[G_{actin}]^{total} = [G_{actin}] + [PIP_3 \cdot G_{actin}], \quad (2)$$

$$[PH_{Akt}]^{total} = [PH_{Akt}] + [PH_{Akt} \cdot PIP_3]. \quad (3)$$

Using equations 1–3 and the rate lows in Table S2, we find that

$$[PIP_3] = \frac{[PIP]_{sh1}^{total}}{1 + k_1/k_{-1} + k_2[G_{actin}]/k_{-2} + k_3[PH_{Akt}]/k_{-3}}, \quad (4)$$

$$[G_{actin}]^{total} = [G_{actin}] + \frac{k_2[G_{actin}][PIP]_{sh1}^{total}/k_{-2}}{1 + k_1/k_{-1} + k_2[G_{actin}]/k_{-2} + k_3[PH_{Akt}]/k_{-3}}, \quad (5)$$

$$[PH_{Akt}]^{total} = [PH_{Akt}] + \frac{k_3[PH_{Akt}][PIP]_{sh1}^{total}/k_{-3}}{1 + k_1/k_{-1} + k_2[G_{actin}]/k_{-2} + k_3[PH_{Akt}]/k_{-3}}. \quad (6)$$

From equations 5 and 6, we can find the rate of change of the measured quantities with respect to the change of the PH_{Akt} concentration:

$$\frac{\partial[G_{actin}]^{total}}{\partial[PH_{Akt}]} = \frac{-k_2k_3[G_{actin}][PIP]^{total}_{sh1}/k_{-2}k_{-3}}{\left(1+\frac{k_1}{k_{-1}}+\frac{k_2[G_{actin}]}{k_{-2}}+\frac{k_3[PH_{Akt}]}{k_{-3}}\right)^2}, \tag{7}$$

$$\frac{\partial[PH_{Akt}]^{total}}{\partial[PH_{Akt}]} = 1+\frac{k_3[PIP]^{total}_{sh1}/k_{-3}}{1+\frac{k_1}{k_{-1}}+\frac{k_2[G_{actin}]}{k_{-2}}+\frac{k_3[PH_{Akt}]}{k_{-3}}} - \frac{k_3^2[PH_{Akt}][PIP]^{total}_{sh1}/k_{-3}^2}{\left(1+\frac{k_1}{k_{-1}}+\frac{k_2[G_{actin}]}{k_{-2}}+\frac{k_3[PH_{Akt}]}{k_{-3}}\right)^2}. \tag{8}$$

Finally, the tangent to the correlation curve of $[G_{actin}]^{total}$ versus $[PH_{Akt}]^{total}$ is

$$\frac{\partial[G_{actin}]^{total}}{\partial[PH_{Akt}]^{total}} = \frac{-1}{1+\frac{(k_{-1}+k_1)k_{-2}}{k_{-1}k_2[G_{actin}]}+\frac{k_{-2}k_{-3}/k_2k_3}{[G_{actin}][PIP]^{total}_{sh1}}\left(1+\frac{k_1}{k_{-1}}+\frac{k_2}{k_{-2}}[G_{actin}]+\frac{k_3}{k_{-3}}[PH_{Akt}]\right)^2} \geq -1 \tag{9}$$

Using analogous calculations for Scheme a2, for which

$$[PIP]^{total}_{sh2} = [PIP_2]+[PIP_3]+[PIP_3\cdot G_{actin}]+[PH_{PLC}\cdot PIP_2], \tag{10}$$

$$[G_{actin}]^{total} = [G_{actin}]+[PIP_3\cdot G_{actin}], \tag{11}$$

$$[PH_{PLC}]^{total} = [PH_{PLC}]+[PH_{PLC}\cdot PIP_2], \tag{12}$$

we find that

$$\frac{\partial[G_{actin}]^{total}}{\partial[PH_{PLC}]^{total}} = \frac{-1}{1+\frac{(k_{-1}+k_1)k_{-2}}{k_{-1}k_2[G_{actin}]}+\frac{k_{-1}k_{-2}k_{-5}/k_1k_2k_5}{[G_{actin}][PIP]^{total}_{sh2}}\left(1+\frac{k_1}{k_{-1}}+\frac{k_2}{k_{-2}}[G_{actin}]+\frac{k_1k_5}{k_{-1}k_{-5}}[PH_{PLC}]\right)^2} \geq -1 \tag{13}$$

For Scheme b1:

$$[PIP]^{total}_{sh3} = [PIP_2]+[PIP_3]+[PIP_2\cdot G_{actin}]+[PH_{PLC}\cdot PIP_2], \tag{14}$$

$$[G_{actin}]^{total} = [G_{actin}]+[PIP_2\cdot G_{actin}], \tag{15}$$

$$[PH_{PLC}]^{total} = [PH_{PLC}]+[PH_{PLC}\cdot PIP_2], \tag{16}$$

and

$$\frac{\partial[G_{actin}]^{total}}{\partial[PH_{PLC}]^{total}} = \frac{-1}{1+\frac{(k_{-1}+k_1)k_{-6}}{k_1k_6[G_{actin}]}+\frac{k_{-5}k_{-6}/k_5k_6}{[G_{actin}][PIP]^{total}_{sh3}}\left(1+\frac{k_{-1}}{k_1}+\frac{k_6}{k_{-6}}[G_{actin}]+\frac{k_5}{k_{-5}}[PH_{PLC}]\right)^2} \geq -1 \tag{17}$$

Finally, for Scheme b2:

$$[PIP]^{total}_{sh4} = [PIP_2]+[PIP_3]+[PIP_2\cdot G_{actin}]+[PH_{Akt}\cdot PIP_3], \tag{18}$$

$$[G_{actin}]^{total} = [G_{actin}]+[PIP_2\cdot G_{actin}], \tag{19}$$

$$[PH_{Akt}]^{total} = [PH_{Akt}]+[PH_{Akt}\cdot PIP_3], \tag{20}$$

so that

$$\frac{\partial\left[G_{actin}\right]^{total}}{\partial\left[PH_{Akt}\right]^{total}}=\frac{-1}{1+\frac{\left(k_{-1}+k_1\right)k_{-6}}{k_1k_6\left[G_{actin}\right]}+\frac{k_1k_{-3}k_{-6}/k_{-1}k_3k_6}{\left[G_{actin}\right]\left[PIP\right]_{sh4}^{total}}\left(1+\frac{k_{-1}}{k_1}+\frac{k_6}{k_{-6}}\left[G_{actin}\right]+\frac{k_{-1}k_3}{k_1k_{-3}}\left[PH_{Akt}\right]\right)^2}\quad\geq\quad -1 \tag{21}$$

Equations (9), (13), (17), and (21) show that, in all four schemes, the slope of the correlation curve $\left[G_{actin}\right]^{total}$ versus $\left[PH\right]^{total}$ is between –1 and 0, which is consistent with the data for $\left[G_{actin}\right]^{total}$ versus $\left[PH_{PLC}\right]^{total}$ but not for $\left[G_{actin}\right]^{total}$ versus $\left[PH_{Akt}\right]^{total}$. This result argues against the original assumption that both G_{actin} and PH_{Akt} cannot form (even transiently) a scafold with PIP_3. Thus, to achieve an agreement with the experimental measurements, we

1) rule out schemes b1 and b2, i.e., we rule out the direct interaction between G_{actin} and PIP_2, and
2) modify scheme 1a by adding transitions between $PIP_3\cdot G_{actin}$ and $PH_{Akt}\cdot PIP_3$ through $PH_{Akt}\cdot PIP_3\cdot G_{actin}$, as shown in Fig. S6 C.

Chemical equations for the schemes in Fig. S6 C are given in Table S3 below:

Table S3

Scheme c1		Scheme c2
$PIP_3 \xrightarrow{k_1} PIP_2$;	$PIP_3 \xleftarrow{k_{-1}} PIP_2$;	$PIP_3 \xrightarrow{k_1} PIP_2$; $PIP_3 \xleftarrow{k_{-1}} PIP_2$;
$PIP_3 + G_{actin} \xrightarrow{k_2} [PIP_3\cdot G_{actin}]$;	$PIP_3 + G_{actin} \xleftarrow{k_{-2}} [PIP_3\cdot G_{actin}]$;	$PIP_3 + G_{actin} \xrightarrow{k_2} [PIP_3\cdot G_{actin}]$;
$PH_{Akt} + PIP_3 \xrightarrow{k_3} [PH_{Akt}\cdot PIP_3]$;	$PH_{Akt} + PIP_3 \xleftarrow{k_{-3}} [PH_{Akt}\cdot PIP_3]$;	$PIP_3 + G_{actin} \xleftarrow{k_{-2}} [PIP_3\cdot G_{actin}]$;
$PH_{Akt} + [PIP_3\cdot G_{acin}] \xrightarrow{k_7} [PH_{Akt}\cdot PIP_3\cdot G_{actin}]$;	$PH_{Akt} + [PIP_3\cdot G_{actin}] \xleftarrow{k_{-7}} [PH_{Akt}\cdot PIP_3\cdot G_{actin}]$;	$PH_{PLC} + PIP_2 \xrightarrow{k_5} [PH_{PLC}\cdot PIP_2]$;
$[PH_{Akt}\cdot PIP_3] + G_{actin} \xrightarrow{k_8} [PH_{Akt}\cdot PIP_3\cdot G_{actin}]$;	$[PH_{Akt}\cdot PIP_3] + G_{actin} \xleftarrow{k_{-8}} [PH_{Akt}\cdot PIP_3\cdot G_{actin}]$.	$PH_{PLC} + PIP_2 \xleftarrow{k_{-5}} [PH_{PLC}\cdot PIP_2]$.

The corresponding rate laws in steady-state approximation are given in Table S4 below:

Table 4

Scheme c1	Scheme c2
$k_1\left[PIP_3\right]=k_{-1}\left[PIP_2\right]$	$k_1\left[PIP_3\right]=k_{-1}\left[PIP_2\right]$
$k_{-2}\left[PIP_3\cdot G_{actin}\right]+k_{-3}\left[PH_{Akt}\cdot PIP_3\right]=k_2\left[PIP_3\right]\cdot\left[G_{actin}\right]+k_3\left[PH_{Akt}\right]\cdot\left[PIP_3\right]$	$k_{-2}\left[PIP_3\cdot G_{actin}\right]=k_2\left[PIP_3\right]\cdot\left[G_{actin}\right]$
$k_{-2}\left[PIP_3\cdot G_{actin}\right]+k_7\left[PH_{Akt}\right]\cdot\left[PIP_3\cdot G_{actin}\right]=k_2\left[PIP_3\right]\cdot\left[G_{actin}\right]+k_{-7}\left[PH_{Akt}\cdot PIP_3\cdot G_{actin}\right]$	$k_{-5}\left[PH_{PLC}\cdot PIP_2\right]=k_5\left[PH_{PLC}\right]\cdot\left[PIP_2\right]$
$k_{-3}\left[PH_{Akt}\cdot PIP_3\right]+k_8\left[PIP_3\cdot PH_{Akt}\right]\cdot\left[G_{actin}\right]=k_3\left[PH_{Akt}\right]\cdot\left[PIP_3\right]+k_{-8}\left[PH_{Akt}\cdot PIP_3\cdot G_{actin}\right]$	

Using symbolic solver (in MATLAB and Mathematica) for the equations in Table S4, we find the explicit expressions for $\left[G_{actin}\right]^{total}$ and $\left[PH_{PLC}\right]^{total}$. With these solutions, we use the MATLAB least square optimization to fit the correlation data (Fig. 5, C and D; and Fig. S6 E).