Supplemental Materials

Molecular Biology of the Cell

Liao et al.

Supplementary Figure Legends

Figure S1. Chemoattractant-mediated TORC2 phosphorylation of PKBR1.

Quantified PKBR1 phosphorylation data from Figure 1D. Cells at 0 hr of development are equivalently responsive to folate and cAMP. Cells were stimulated with 10 μM cAMP or 50 μM folate. Normalized TORC2 phosphorylation of PKBR1 is expressed as relative intensity.

Figure S2. Secondary activation of TORC2 phosphorylation of PKBR1 following folate stimulation is caused by increases in cAMP.

Quantified PKBR1 phosphorylation data from Figure 2. WT and *aca*-null cells were stimulated with 50 μM folate. Normalized TORC2 phosphorylation of PKBR1 is expressed as relative intensity.

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Figure S3. Adaptation of TORC2 activation to cAMP and folate.

A. Quantified PKBR1 and AKT phosphorylation data from Figure 3A,B. Cells were treated with varying concentrations of cAMP and samples collected after 30 sec. TORC2 phosphorylation of PKBR1 and AKT is expressed as relative intensity (solid lines). Following adaptation, cells were secondarily stimulated with 10 μM cAMP. The inverse sensitivity for normalized TORC2 phosphorylation of PKBR1 following an initial

stimulus (see Figure 3B) is expressed as a dose response decrease in relative intensity (open circles).

B. Quantified PKBR1 and AKT phosphorylation data from Figure 3D,E. Cells were treated with varying concentrations of folate and samples collected after 30 sec. TORC2 phosphorylation of PKBR1 and AKT is expressed as relative intensity (solid lines). Following adaptation, cells were secondarily stimulated with 50 μM folate. The inverse sensitivity for normalized TORC2 phosphorylation of PKBR1 following an initial stimulus (see Figure 3E) is expressed as a dose response decrease in relative intensity (open circles).

Figure S4. TORC2 phosphorylation of AKT and PKBR1 does not cross-adapt to different chemoattractants.

A. Quantified PKBR1 phosphorylation data from Figure 4A. Cells stimulated with saturating doses of cAMP remain responsive to folate. Cells were stimulated with 10 μM cAMP (solid lines) and then stimulated with either 10 μM cAMP or 50 μM folate at 60 sec (open circles). Normalized TORC2 phosphorylation of PKBR1 is expressed as relative intensity.

B. Quantified PKBR1 phosphorylation data from Figure 4B. Cells stimulated with saturating doses of folate remain responsive to cAMP. Cells were stimulated with 50 μM folate (solid lines) and then stimulated with either 50 μM folate or 10 μM cAMP at 60 sec (open circles). Normalized TORC2 phosphorylation of PKBR1 is expressed as relative intensity.

C. Quantified PKBR1 phosphorylation data from Figure 4C. Cells stimulated with sub-saturating doses of cAMP remain responsive to sub-saturating doses folate. Cells were stimulated with 15 nM cAMP (solid lines) and then stimulated with either 15 nM cAMP or 70 nM folate at 75 sec (open circles). Normalized TORC2 phosphorylation of PKBR1 is expressed as relative intensity.

D. Quantified PKBR1 phosphorylation data from Figure 4D. Cells stimulated with sub-saturating doses of folate remain responsive to sub-saturating doses cAMP. Cells were stimulated with 70 nM folate (solid lines) and then stimulated with either 70 nM folate or 15 nM cAMP at 60 sec (open circles). Normalized TORC2 phosphorylation of PKBR1 is expressed as relative intensity.

Figure S5. RasC activation does not cross-adapt to different chemoattractants.

A. Quantified RasC-GTP data from Figure 6A. Cells stimulated with saturating doses of cAMP remain responsive to folate. Cells were stimulated with 10 μM cAMP (solid squares) and then stimulated with either 10 μM cAMP (open circles) or 50 μM folate (solid triangle) at 75 sec. Normalized RasC-GTP levels are expressed as relative intensity.

B. Quantified RasC-GTP data from Figure 6B. Cells stimulated with saturating doses of folate remain responsive to cAMP. Cells were stimulated with 50 μM folate (solid squares) and then stimulated with either 50 μM folate (solid triangles) or 10 μM cAMP (open circle) at 75 sec Normalized RasC-GTP levels are expressed as relative intensity.

Figure S6. TORC2 and RasC responses to folate and cAMP are non-additive.

A. Quantified PKBR1 phosphorylation data from Figure 7A. Maximal TORC2 phosphorylations are non-additive in response to a mixture of saturated cAMP and folate. Cells were stimulated either with 10 μM cAMP (closed boxes), 50 μM folate (closed triangles), or 10 μM cAMP + 50 μM folate (open circle, dashed line). Normalized TORC2 phosphorylation of PKBR1 is expressed as relative intensity.

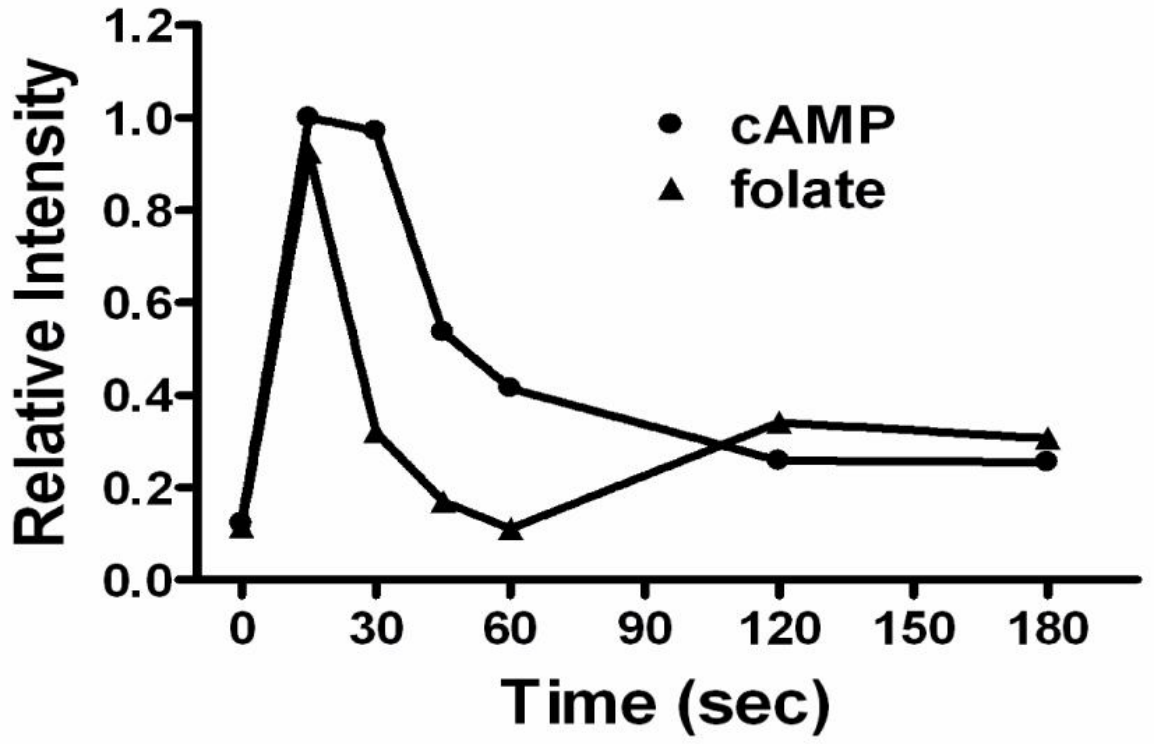
B. Quantified RasC-GTP data from Figure 7B. RasC activation is non-additive in response to a mixture of saturated cAMP an folate. Cells were stimulated either with 10 μM cAMP (closed boxes), 50 μM folate (closed triangles), or 10 μM cAMP + 50 μM folate (open circle, dashed line). Normalized RasC-GTP levels are expressed as relative intensity.

Figure S7. Very rapid de-adaptation of TORC2 to cAMP and folate.

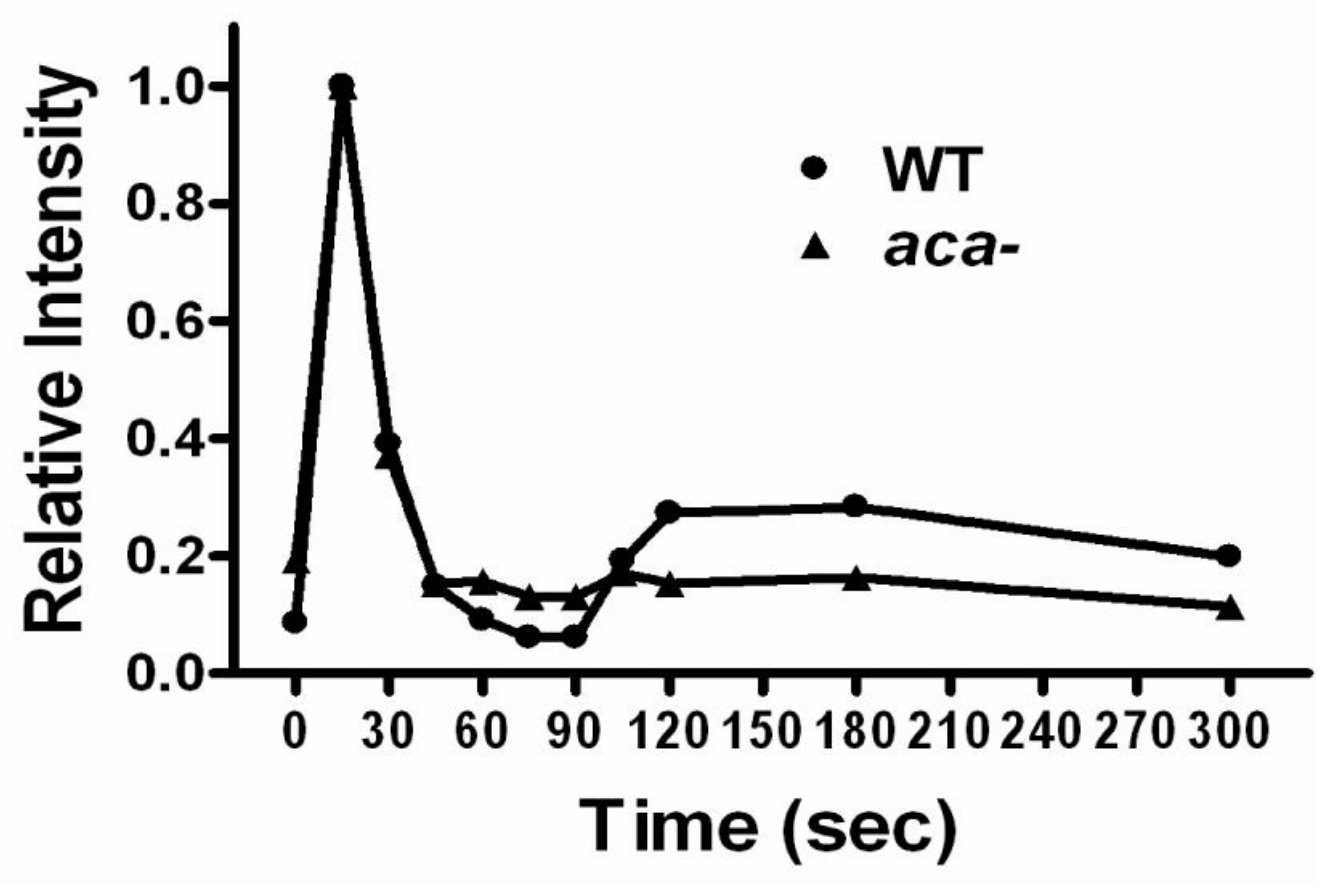
A. Quantified PKBR1 phosphorylation data from Figure 8A. Cells become rapidly (<2 min) re-responsive to cAMP. Cells were stimulated with 100 nM cAMP and TORC2 phosphorylation of AKT and PKBR1 was assayed by immunoblot at the times indicated. At 1 min, cells were diluted 10x into buffer, to reduce cAMP to ~10 nM. Cells were either maintained without additional cAMP (solid line) or stimulated one time with 100 nM cAMP at each of the single times indicated (open circles). Samples were assayed at +15 sec. Normalized TORC2 phosphorylation of PKBR1 is expressed as relative intensity.

B. Quantified PKBR1 phosphorylation data from Figure 8B. Cells become rapidly (<1 min) re-responsive to folate. Cells were stimulated with 70 nM folate and TORC2

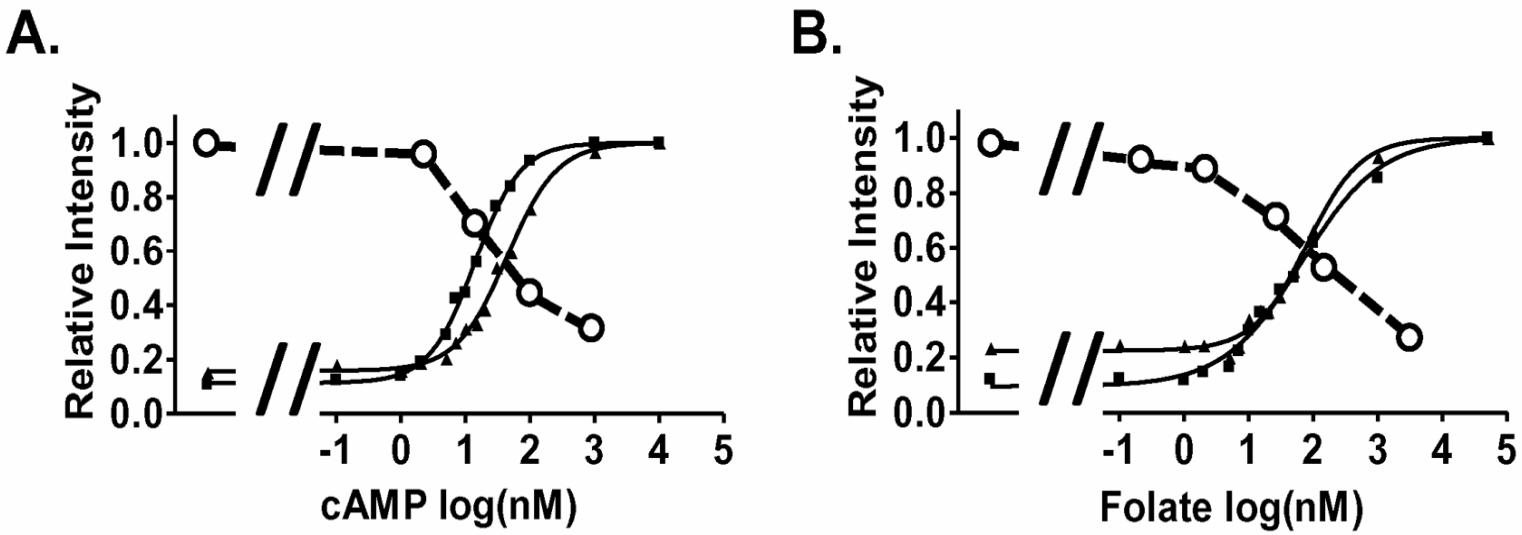
phosphorylation of AKT and PKBR1 was assayed by immunoblot at the times indicated. At 1 min, cells were diluted 10x into buffer, to reduce folate to ~7 nM. Cells were either maintained without additional folate (solid line) or stimulated one time with 70 nM folate at each of the single times indicated (open circles). Samples were assayed at +15 sec. Normalized TORC2 phosphorylation of PKBR1 is expressed as relative intensity.



Supplemental Figure S1



Supplemental Figure S2

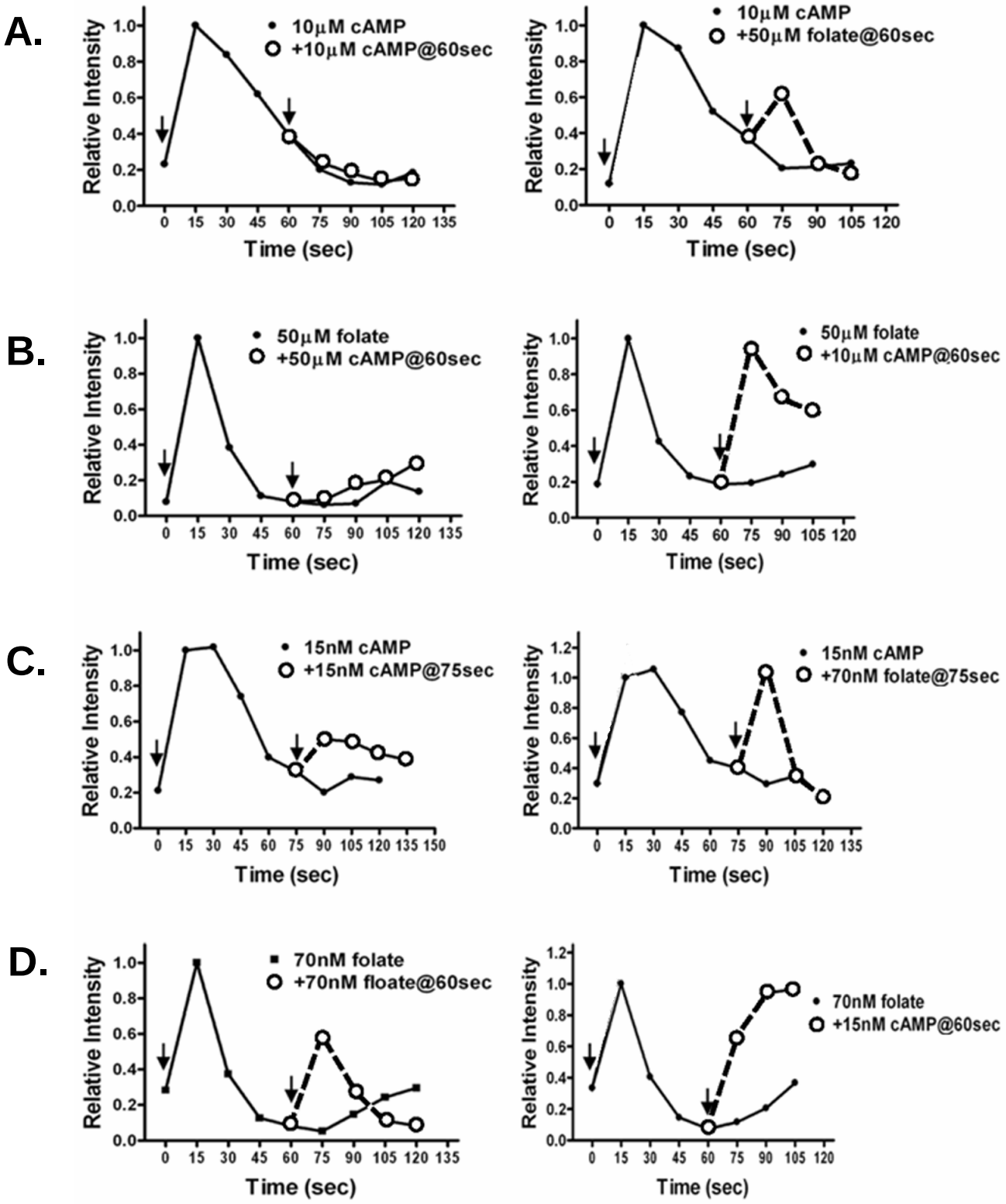


■ p-PKBR1, primary stimulus

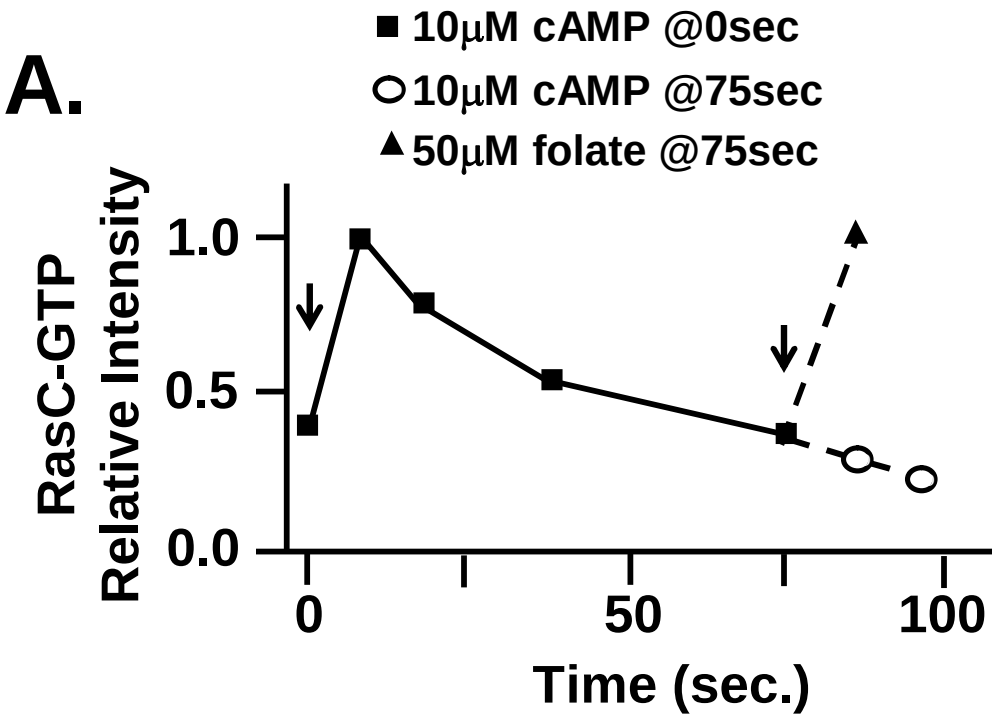
▲ p-AKT, primary stimulus

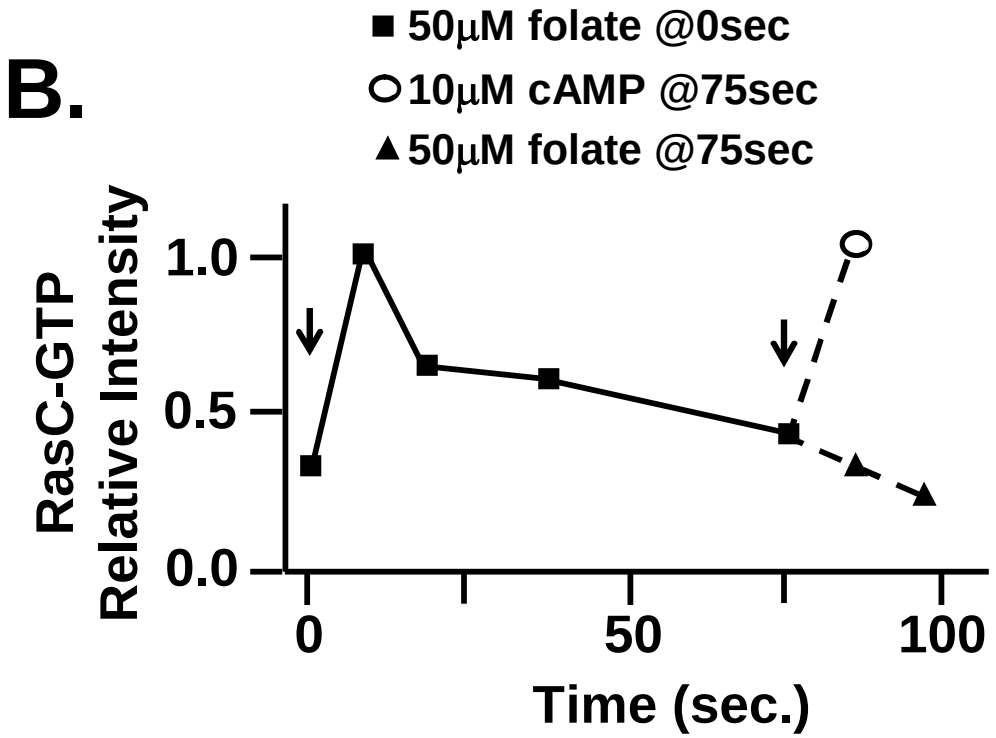
○ p-PKBR1, secondary stimulus

Supplemental Figure S3



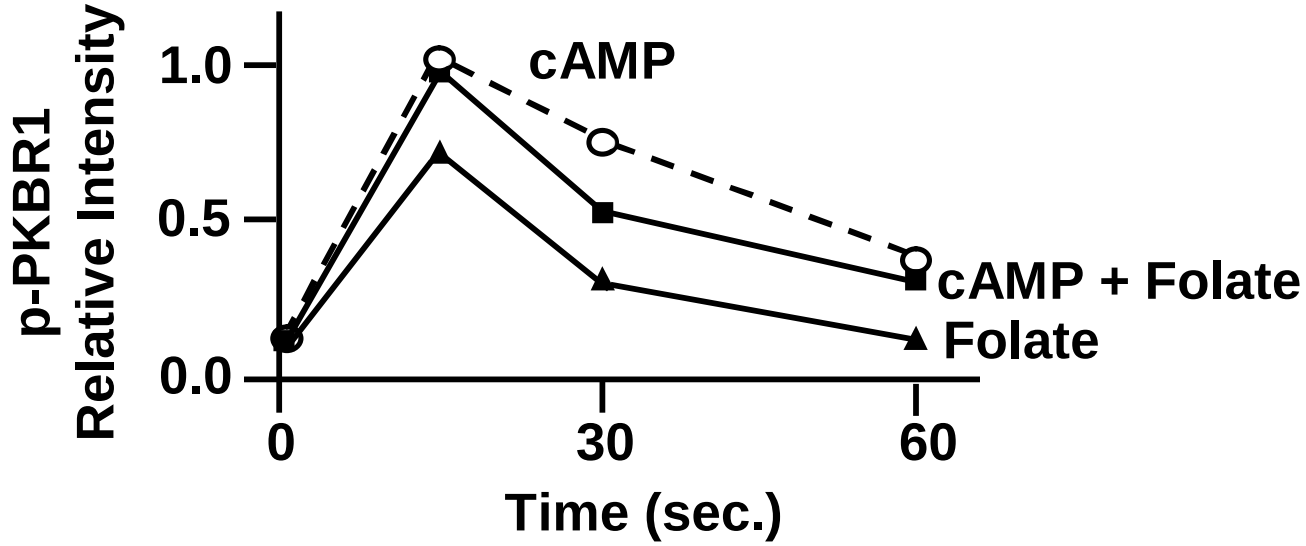
Supplemental Figure S4





Supplemental Figure S5

A.



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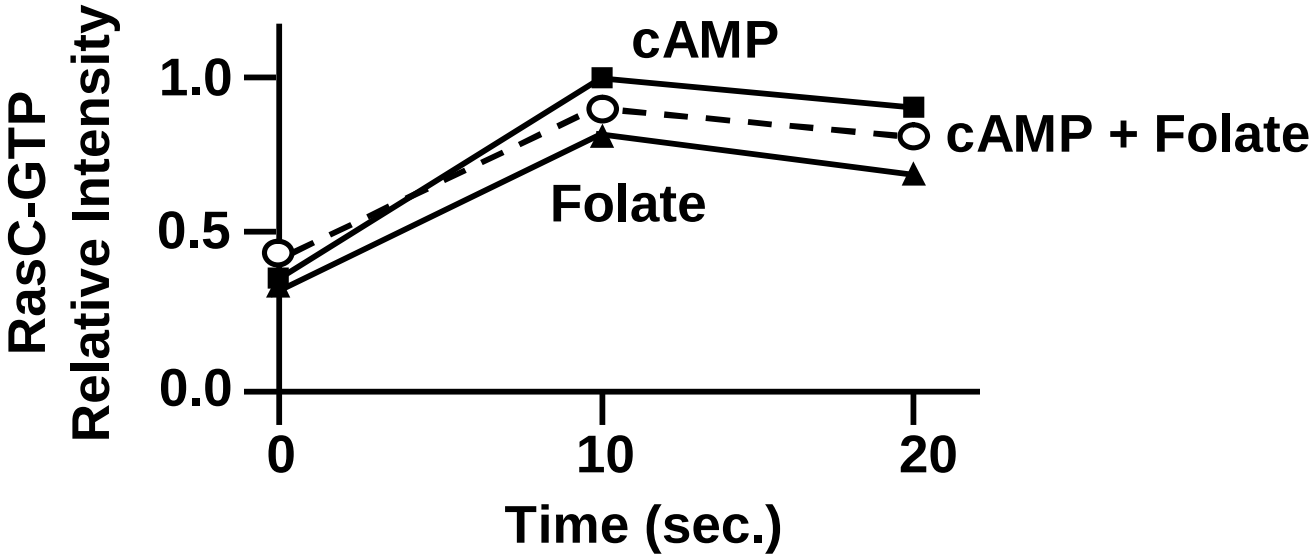
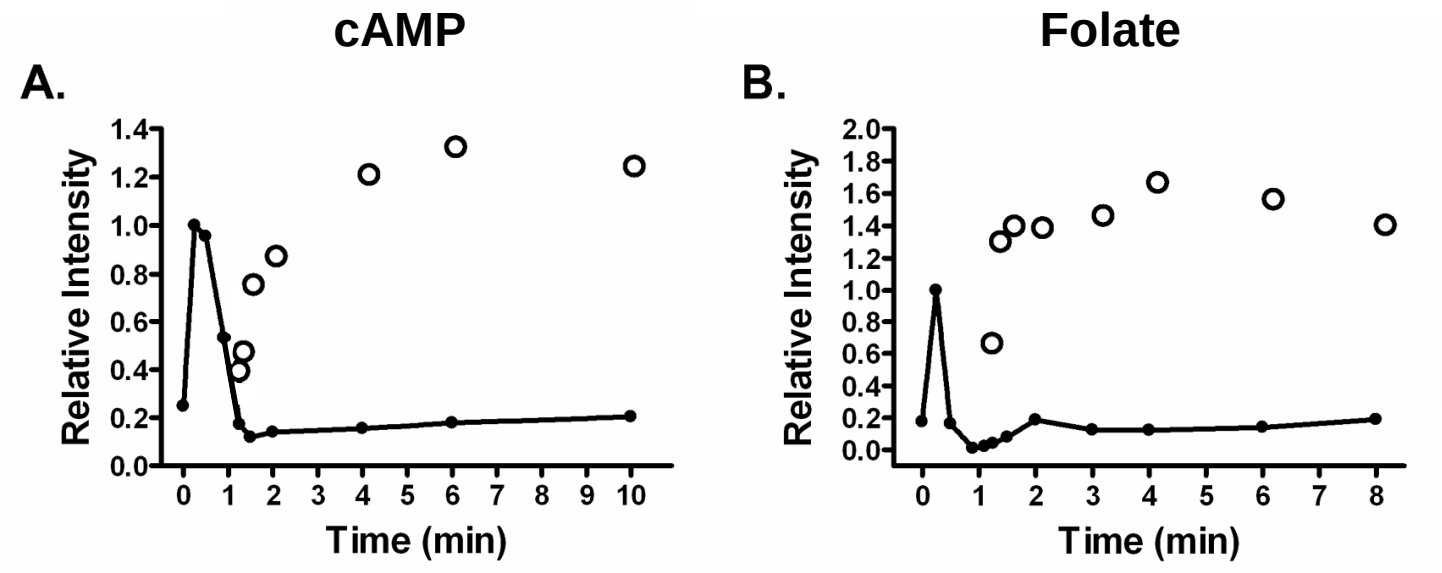


Supplemental Figure S6



Supplemental Figure S7