

## Appendix

### Supporting Information

#### Figure legends

**Supporting Figure 1. (A)** MIN6 cells preincubated in KRBH with 2 mM glucose as in *Fig. 1* were stimulated with 20 mM glucose for the indicated times. Endogenous B-Raf and C-Raf were immunoprecipitated and assayed as in *Fig. 1*. C-Raf activity was the same as background. **(B)** Hela cells were preincubated in free-serum DMEM for 6 h prior to stimulation with 100 nM phorbol myristic acid for 10 min. Endogenous C-Raf was immunoprecipitated from lysates and assayed as in *Fig. 1*. **(C)** MIN6 cells preincubated as in *Fig. 1* were stimulated with 20 mM glucose for 10 min. Endogenous C-Raf and B-Raf immunoprecipitates were immunoblotted with antibodies for the indicated Raf phosphorylation sites. **(D)** MIN6 cells were transiently transfected with the indicated siRNA oligonucleotides. After 2 d, cells were preincubated in DMEM without glucose for 4 h and then stimulated with 20 mM glucose for 1 h. Immunoblotting was with the indicated antibodies. Lower panel, pERK1/2 was quantified with ImageJ software and is expressed as fold change. **(E)** MIN6 were pretreated with 200 nM FK506 or vehicle for 20 min prior to the addition of 20 ng/ml EGF or 100 nM PMA for 10 min. Upper panels, immunoblots are shown. Lower panel, B-Raf was immunoprecipitated and assayed. **(F)** C3H10T1/2 cells were transfected with the indicated siRNAs for 2 d and placed in serum-free medium for 6 h before stimulation with 20 ng/ml EGF for indicated times. Immunoblots are shown.

**Supporting Figure 2. (A)** MIN6 cells expressing Myc-C-Raf were preincubated and stimulated with glucose for the indicated times as in *Fig. 1*. Myc-Raf was immunoprecipitated and co-precipitating proteins were detected by immuno blotting. **(B)** Diagram of calcineurin constructs

used in these studies. *Act* refers to a constitutively active fragment. *In* refers to a catalytically inactive mutant. **(C)** Flag-tagged Wild-type and inactive calcineurin were expressed in 293 cells. After 2 d, B-Raf was immunoprecipitated and the precipitates and lysates were immunoblotted with the indicated antibodies.

**Supporting Figure 3.** Flag-tagged wild-type or inactive calcineurin were expressed without or with HA-B-Raf in 293 cells. After 2 d, cells were harvested and HA immunoprecipitates were immunoblotted with HA and Flag antibodies.

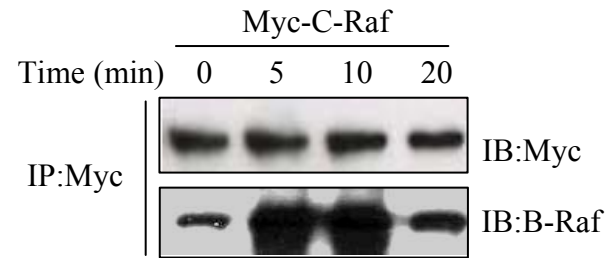
**Supporting Figure 4. (4)** GST-B-Raf 1-440 was phosphorylated with pERK1 and [<sup>32</sup>P]ATP in vitro. The <sup>32</sup>P-labeled fusion proteins were repurified on GST-beads and incubated with or without calcineurin and calmodulin. The proteins were separated by SDS-PAGE and visualized by autoradiography, upper panel. Coomassie blue stain, lower panel.

**Supporting Figure 5.** C-Raf (**A**) or B-Raf (**B**) immunoprecipitates were immunoblotted with the indicated antibodies. **(C)** MIN6 cells preincubated as in *Fig. 1* were pretreated with 200 nM FK506 or okadaic acid (OA) for 20 min and then stimulated with 20 mM glucose for 10 min.

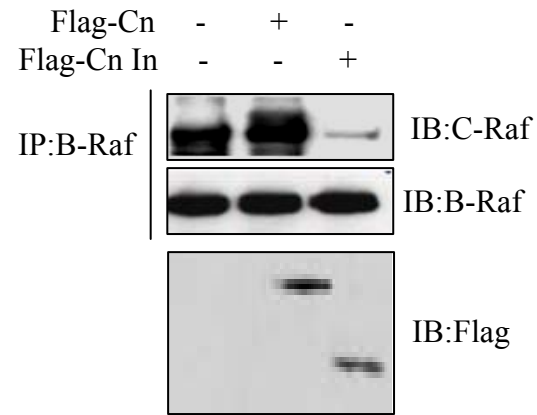


**Fig. S2**

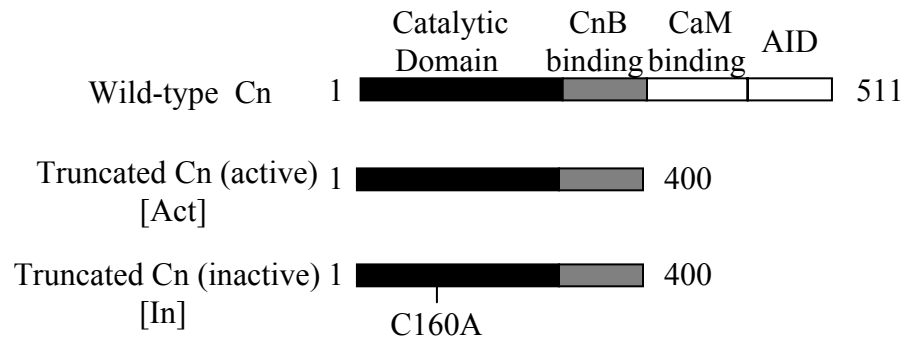
**A**



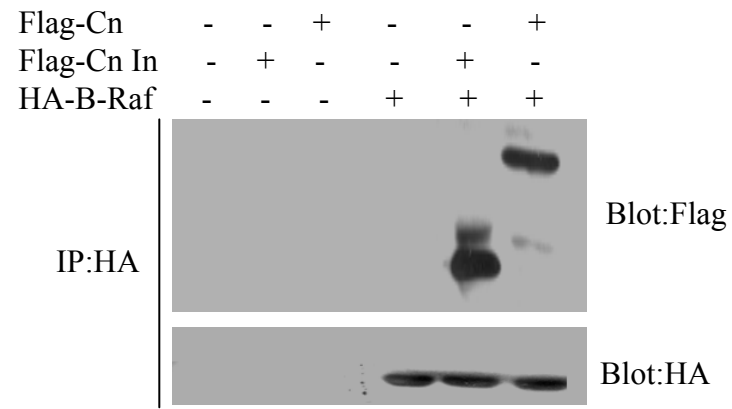
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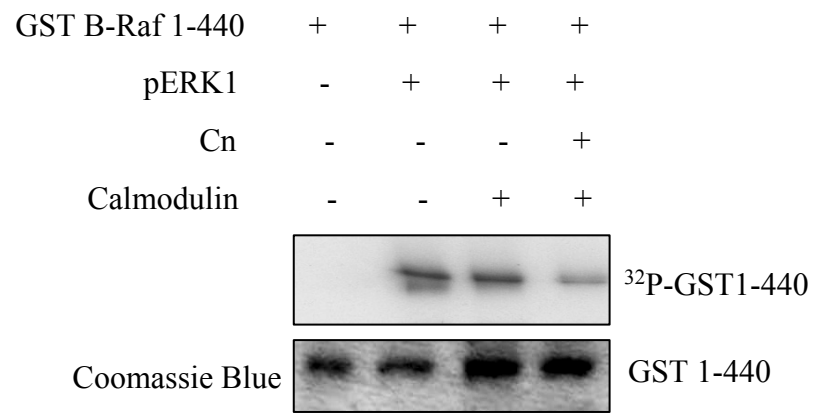
**B**



**Fig. S3**

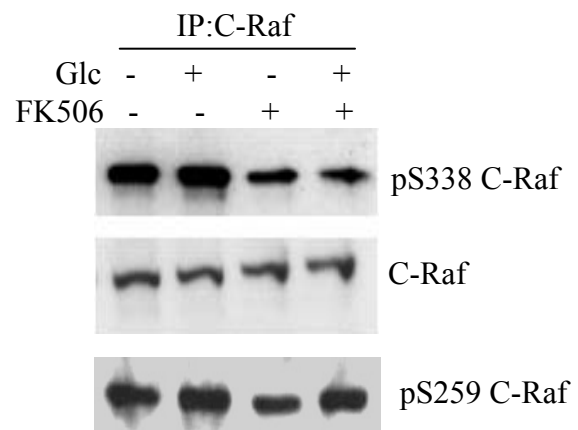


**Fig. S4**

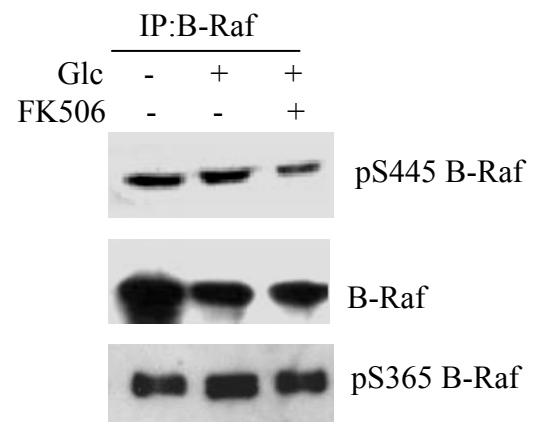


**Fig. S5**

**A**



**B**



**C**

