Supplementary Information

A p38α Selective Chemosensor for use in Unfractionated Cell Lysates

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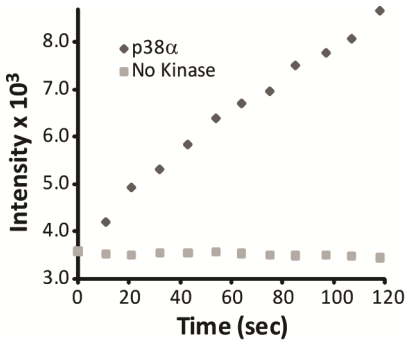
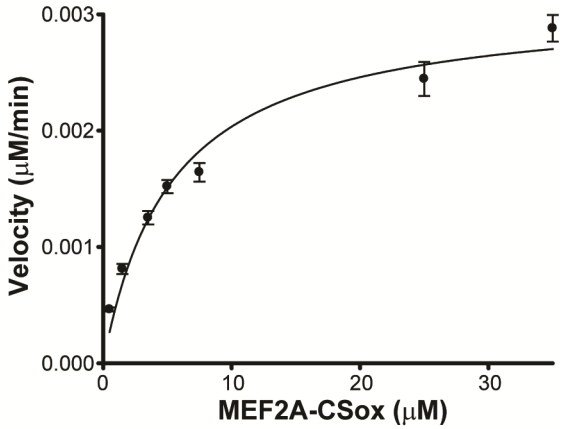


Supplementary Figure S1. MEF2A-CSox is a substrate for p38α. Initial phosphorylation reactions with and without the addition of kinase (15 nM) indicated that MEF2A-CSox (1 μM) was a substrate for p38α.

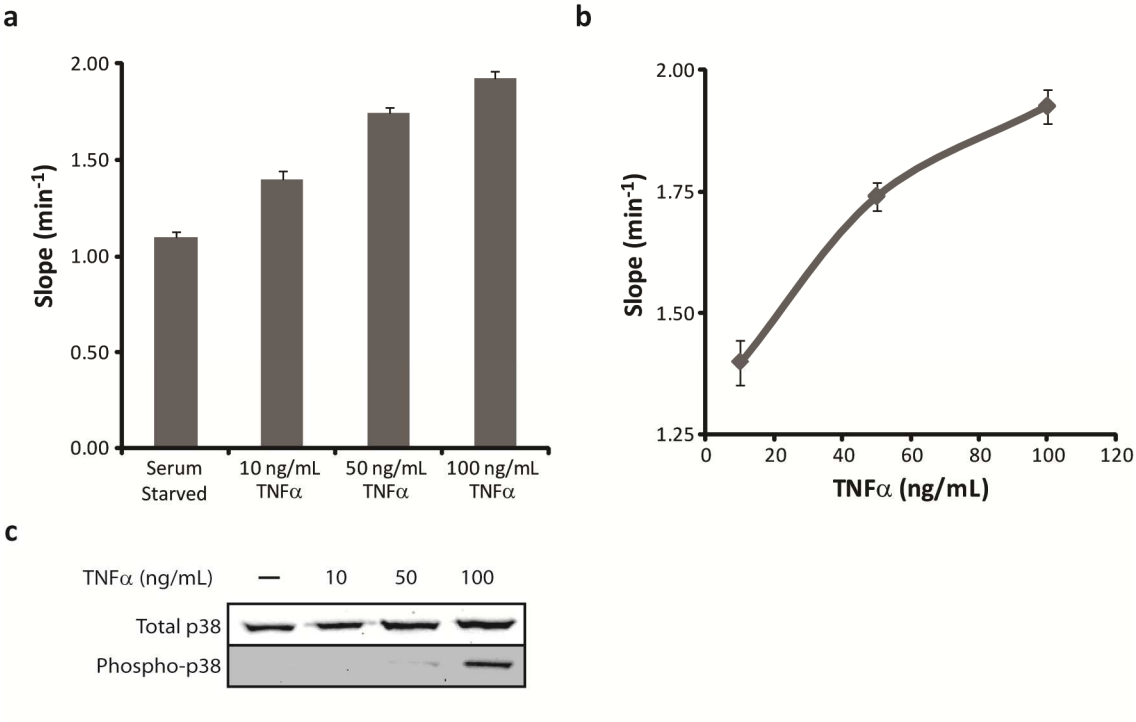
a



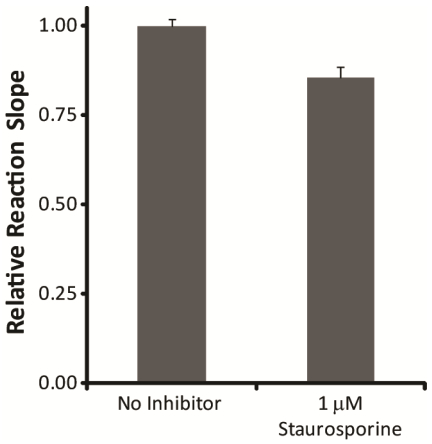
b

Kinase	K_M (μM)	k_{cat} (min^{-1})	Catalytic Efficiency
p38α	1.3	74	17
p38β	5.3	18	1.0

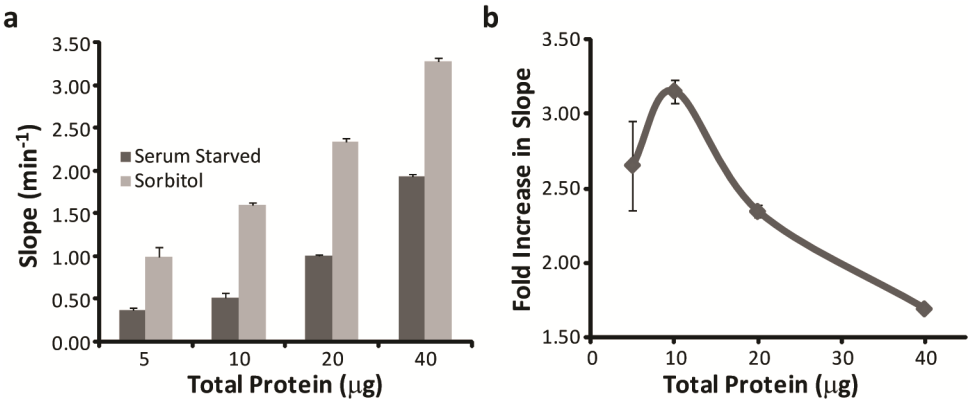
Supplementary Figure S2. Kinetic parameters of MEF2A-CSox for p38β. **a**) A direct fit of a velocity versus MEF2A-CSox concentration plot using the Briggs-Haldane equation (1 ng p38β). **b**) Kinetic parameters of MEF2A-CSox for the indicated kinases, demonstrating that MEF2A-CSox is a more efficient substrate for p38α. Catalytic efficiency was determined using relative k_{cat}/K_M values.



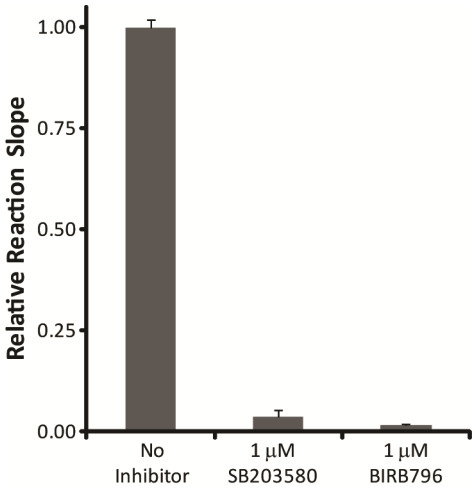
Supplementary Figure S3. MEF2A-CSox phosphorylation varies with TNFα stimulation. **a**) Fluorescence slopes of reactions conducted without staurosporine using 1 μM substrate and 5 μg of HeLa lysates derived from cells stimulated for 10 min with the indicated amount of TNFα. Assays were also performed with lysates from untreated (serum starved) cells. **b**) The relationship between MEF2A-CSox phosphorylation and the amount of TNFα used for stimulation. **c**) A western blot of the indicated lysates with the corresponding antibodies, demonstrating increased phosphorylation of p38 with increasing amounts of TNFα.



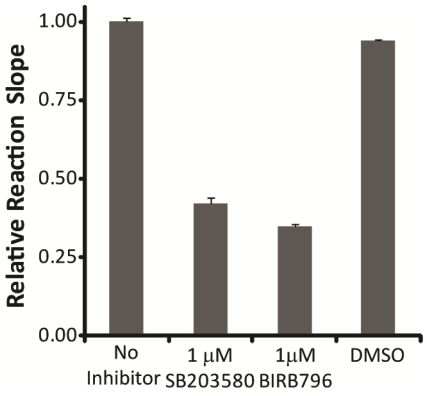
Supplementary Figure S4. Staurosporine does not significantly alter p38α activity (*1*). Reactions were performed using 1 nM recombinant p38α and 1 μM substrate.



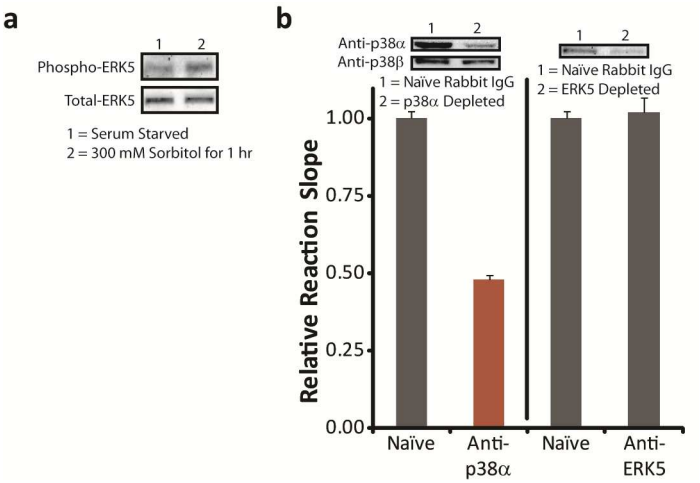
Supplementary Figure S5. The effect of the amount of lysate used in the assay on the rate of phosphorylation of MEF2A-CSox. **a**) Reactions conducted with 1 μM substrate and the indicated amount of serum starved and sorbitol-stimulated HeLa lysates. **b**) The fold change in reaction slope between sorbitol-stimulated and serum starved lysates with respect to the amount of lysate used in the assay. The assay is most sensitive when 10 μg of lysate is used. Presumably the sensitivity of the assay decreases with increasing concentrations of lysate due to the increasing amounts of off-target kinases. All reactions contained 1 μM staurosporine.



Supplementary Figure S6. The addition of 1 μM SB203580 or BIRB796 completely abolishes recombinant p38α activity. Reactions were conducted with 1 μM substrate and 1 nM recombinant enzyme. Slopes for assays containing the slow-binding inhibitor BIRB796 were determined after incubation of the entire reaction at 30 ^{o}C for 30 min prior to data collection, after which fluorescence increases were linear with respect to time.



Supplementary Figure S7. The inhibitor solvent, DMSO, does not influence the phosphorylation of MEF2A-CSox. Assays were conducted using 1 μM MEF2A-CSox and 10 μg of sorbitol-stimulated HeLa lysates with the indicated inhibitor. All reactions contained 1 μM staurosporine.



Supplementary Figure S8. The related ERK5 kinase does not significantly contribute to the phosphorylation of MEF2A-CSox (1 μM) in sorbitol-stimulated HeLa cell lysates. **a**) Western blot analysis of sorbitol-stimulated lysates demonstrating activation of ERK5. **b**) Immunodepletion of p38α leads to a dramatic decrease in MEF2A-CSox phosphorylation (left) while depletion of ERK5 demonstrated no significant loss of signal (right). Values were background subtracted using the activity remaining in the input lysates after addition of 1 μM SB203580. Western blots of the respective lysates demonstrating depletion of the target kinase are shown in the insets of each panel. Note that p38β levels remain unchanged, indicating that the remaining MEF2A-CSox activity is likely due to residual p38α (~30% remaining by gel densitometry). All reactions contained 1 μM staurosporine.

	Inhibitor		
Kinase	SB203580	BIRB796	Staurosporine
p38α	12	0.37	>10,000
p38β	70	1,500	>10,000
p38γ	1,500	19	42
JNK1	1,100	>10,000	220
JNK2	130	7.3	>10,000
JNK3	35	110	110
ERK1	>10,000	>10,000	8,400
ERK2	>10,000	>10,000	7,300
ERK5	>10,000	>10,000	1,600

K_D in nM

Supplementary Table S1. Reported dissociation constants of SB203580, BIRB796, and staurosporine for the indicated kinase are given (*1*).

References

1. Karaman, M. W., Herrgard, S., Treiber, D. K., Gallant, P., Atteridge, C. E., Campbell, B. T., Chan, K. W., Ciceri, P., Davis, M. I., Edeen, P. T., Faraoni, R., Floyd, M., Hunt, J. P., Lockhart, D. J., Milanov, Z. V., Morrison, M. J., Pallares, G., Patel, H. K., Pritchard, S., Wodicka, L. M., and Zarrinkar, P. P. (2008) A quantitative analysis of kinase inhibitor selectivity, *Nat. Biotechnol. 26*, 127-132.