

## Supporting Information

# Robust and Quantitative Assay Platform for Multiplexed, High Throughput Screening of Protein Kinase Inhibitors

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## **Materials.**

Sulfuric acid (H<sub>2</sub>SO<sub>4</sub>) was purchased from Samchun chemical (Seoul, Korea). Titanium(IV) butoxide (Ti(BuO)<sub>4</sub>) was purchased from Sigma-Aldrich (MO, USA). 7-Hydroxycoumarin-3-carboxylic acid was purchased from Anaspec (California, USA). 5-(and-6)-Carboxytetramethylrhodamine was purchased from Invitrogen (Massachusetts, USA).

## **Preparation of TiO<sub>2</sub> decorated GO (TiO<sub>2</sub>-GO).**

3ml of 1 mg/ml of GO was dissolved in 150 ml EtOH and the suspension was heated to 80 °C with stirring. 160 μL of Ti(BuO)<sub>4</sub> and 120 μL of H<sub>2</sub>SO<sub>4</sub> was prepared in 4 mL of EtOH, which was injected in the mixture. The final solution was stirred at 80 °C for 12 h. Then, the reaction mixture was cooled down and the TiO<sub>2</sub>-GO was collected by centrifugation, followed by washing with EtOH and water.

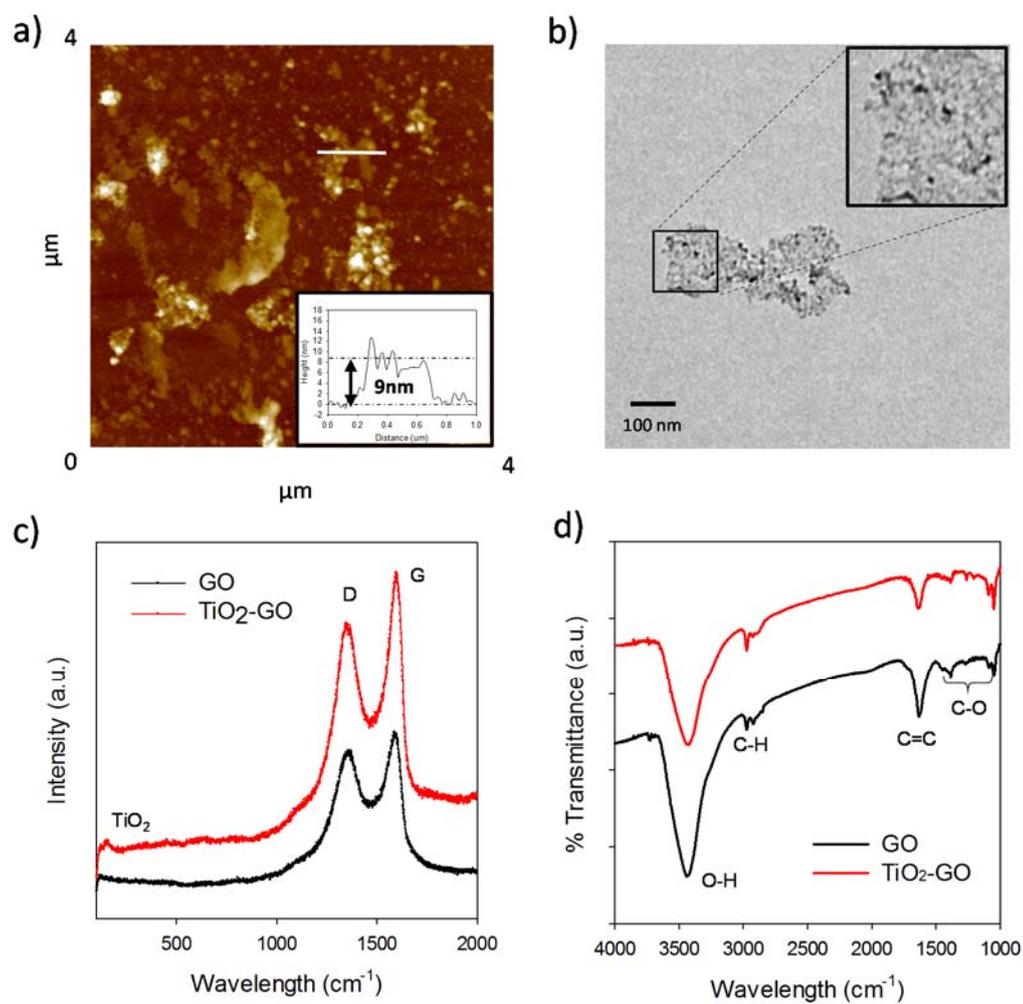
## **Protein kinase A assay using TiO<sub>2</sub>-GO.**

For phosphorylation by kinase, 20 pmol of dye conjugated substrate peptide and 200 pmol of ATP was mixed with corresponding kinase in Tris-HCl buffer (pH 7.4 buffer containing 40 mM Tris and 20 mM MgCl<sub>2</sub>). After incubation at 30 °C, 0.5 μg of TiO<sub>2</sub>-GO pre-dispersed in 50% ACN and 0.1% TFA was added to the reaction mixture. Fluorescence intensity was measured at 450 nm.

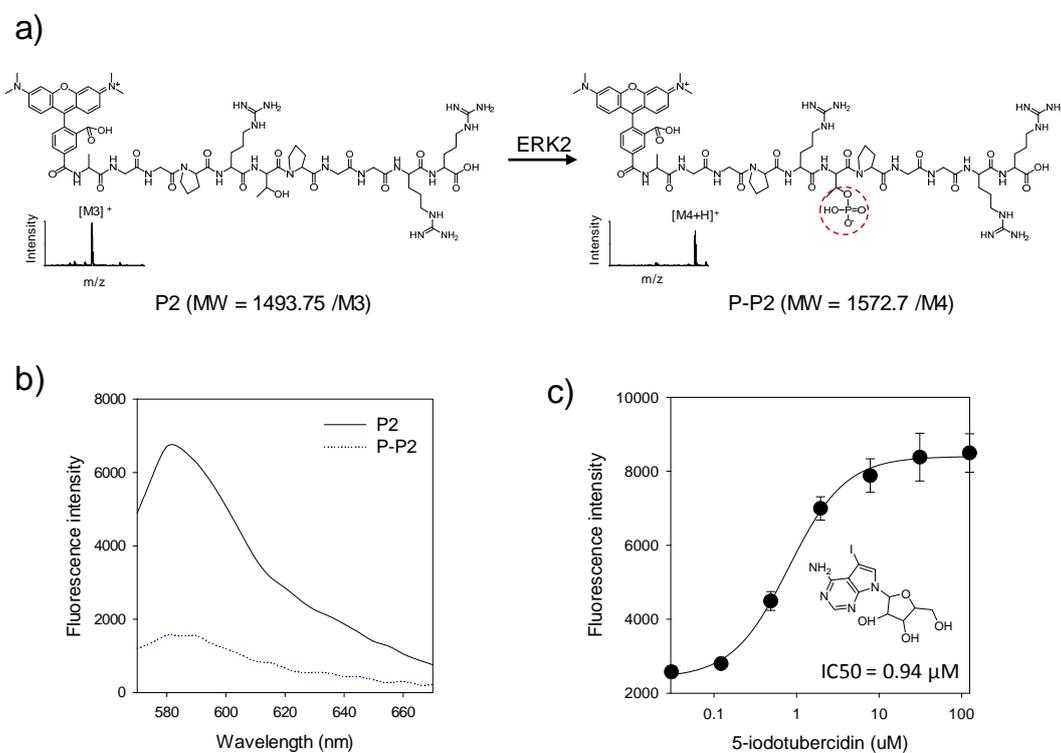
## **Multiplex Protein kinase inhibition assay.**

For multiplex inhibition assay using H-89 and 5-iodotubercidin, each 40 μM of inhibitors was prepared in kinase mixture containing 20 U/ml of PKA and 1 μM of ERK2. Then, each 20 pmol of coumarin-PKA substrate and TAMRA-ERK2 substrate was mixed with 400 pmol of ATP in Tris-HCl buffer (pH 7.4 buffer containing 40 mM Tris and 20 mM MgCl<sub>2</sub>), which

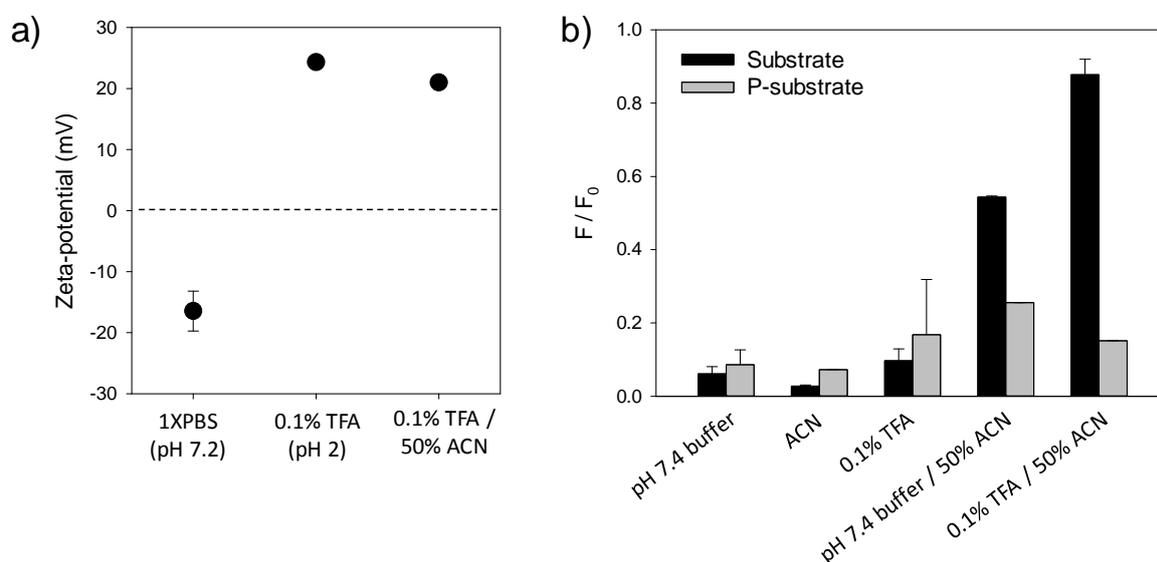
was added to kinase/inhibitor mixture. After incubation at 30 °C, for 1 hr, 1.5 µg of TiO<sub>2</sub>-GO pre-dispersed in 50% ACN and 0.1% TFA was added to the reaction mixture. Fluorescence intensity was measured at ex360/em450 nm and ex550 nm/em585nm for coumarin and TAMRA, respectively.



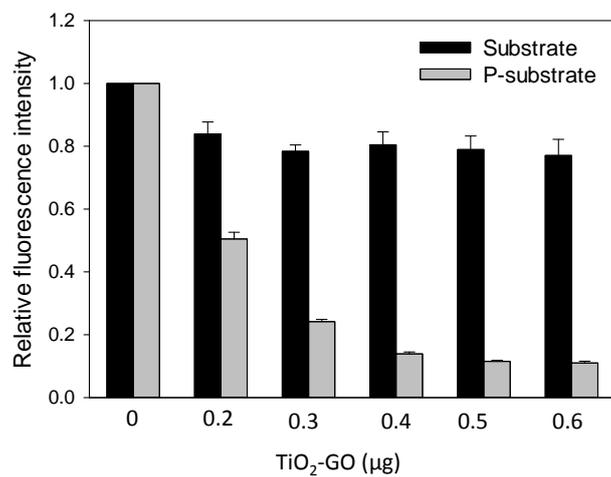
**Figure S1.** Characterization of TiO<sub>2</sub>-GO. a) AFM image and height profile (inset), b) TEM image of TiO<sub>2</sub>-GO. c) Raman spectrum of TiO<sub>2</sub>-GO showing identified peaks with GO and TiO<sub>2</sub> nanocrystal. d) FT-IR spectrum of TiO<sub>2</sub>-GO confirming characteristic peaks of oxygen functional groups in GO.



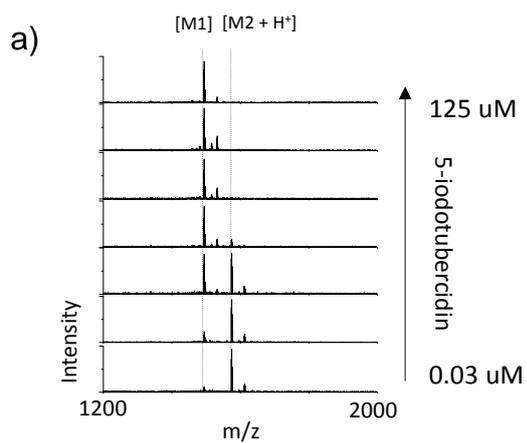
**Figure S2.** a) Chemical structure of TAMRA conjugated ERK2 substrate peptide before and after ERK2 reaction. The each mass spectrum represented the successful phosphorylation of substrate peptide by ERK2. b) Selective fluorescence quenching of dye conjugated to phosphorylated peptide in the presence of 0.5  $\mu$ g of TiO<sub>2</sub>-GO. c) ERK2 inhibition curve and IC<sub>50</sub> using 5-iodotubercidin.



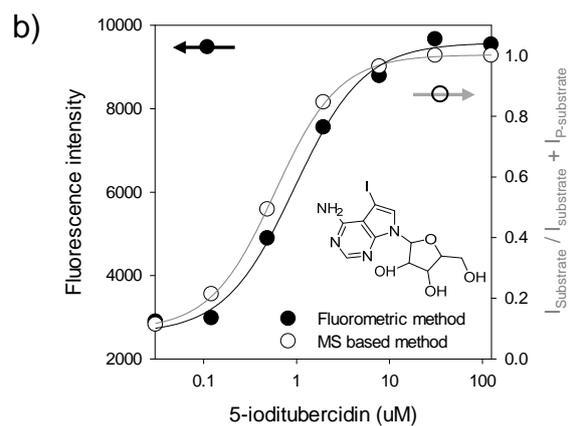
**Figure S3.** a) pH dependent zeta potential of TiO<sub>2</sub>-GO dispersion. At acidic condition, TiO<sub>2</sub>-GO carried a positive charge. b) Relative fluorescence intensity of dye conjugated peptides in various combination of solvent. The maximum intensities were measured at ex 390 / em 450 nm except for 0.1% TFA and TFA/ACN. In case of TFA containing solvent, the maximum signal was measured at ex 360 /em 450 nm ( $F_0$  : fluorescence of peptide with TiO<sub>2</sub>-GO 0  $\mu$ g,  $F$  : fluorescence of peptides with TiO<sub>2</sub>-GO 0.5  $\mu$ g).



**Figure S4.** a) Fluorescence intensities of Coumarin-PKA substrate (Substrate) and phosphorylated substrate (P-substrate) in the presence of various amounts of TiO<sub>2</sub>-GO in TFA/ACN.



M1 : TAMRA-ERK2 substrate  
 M2 : phosphorylated TAMRA-ERK2 substrate

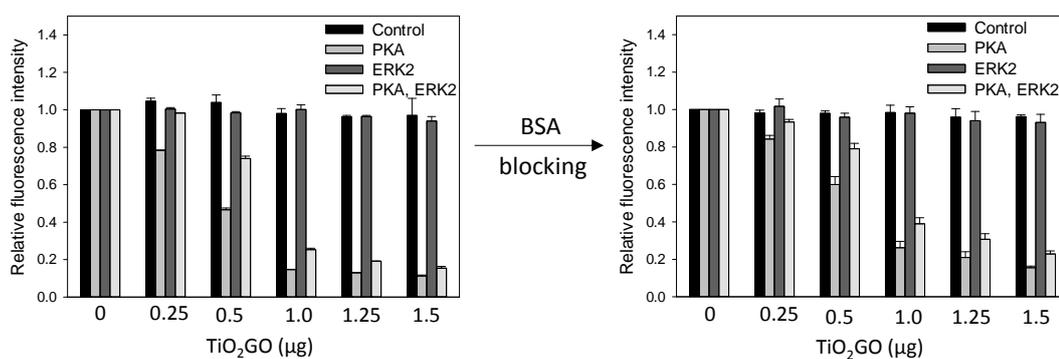


#### IC<sub>50</sub> value

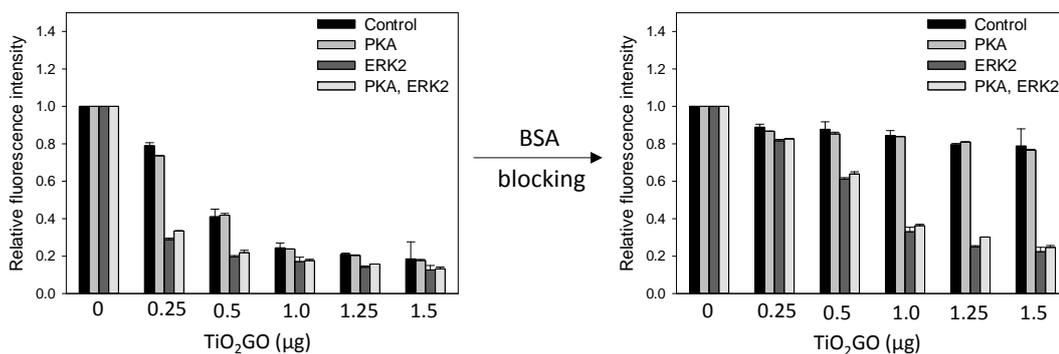
- Fluorometric method : 0.94  $\mu$ M
- MS based method : 0.58  $\mu$ M

**Figure S5.** Inhibition assay of ERK2 using 5-iodotubercidin using MALDI-ToF MS based method. a) The MALDI-ToF MS spectra of kinase reaction mixtures containing various amounts of 5-iodotubercidin. b) ERK2 inhibition curve and IC<sub>50</sub> obtained from fluorescent method using TiO<sub>2</sub>-GO and MALDI-ToF MS based method.

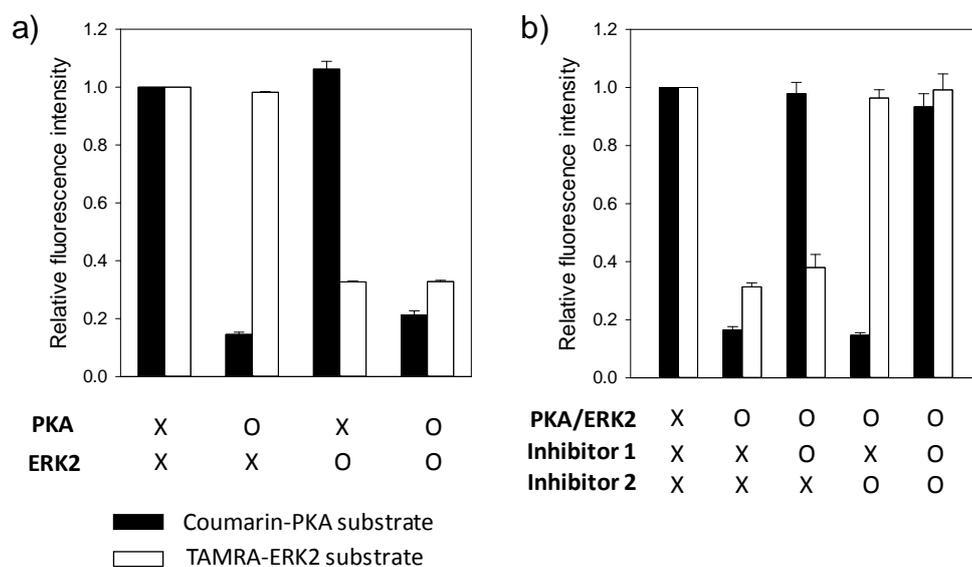
a) Analysis of coumarin-PKA substrate (ex 360 / em 450 nm)



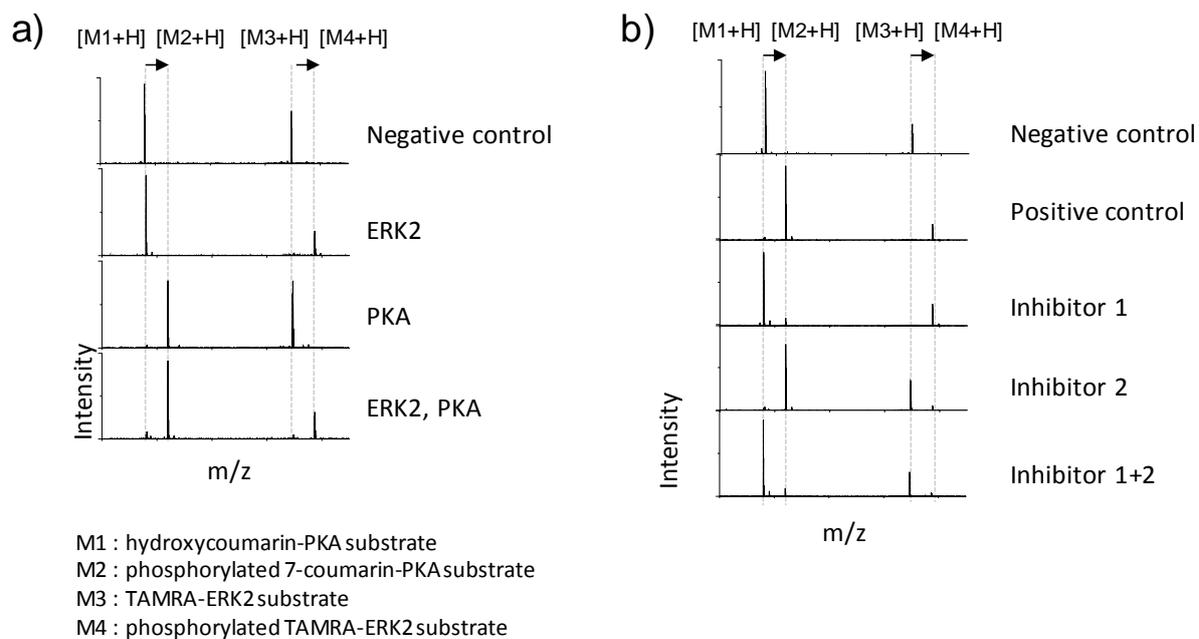
b) Analysis of TAMRA-ERK2 substrate (ex 550 / em 585nm)



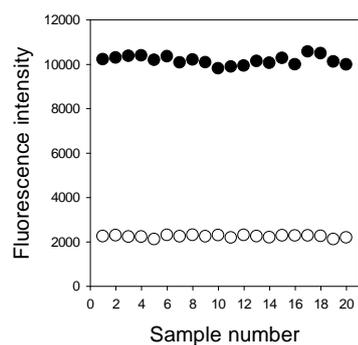
**Figure S6.** Relative fluorescence intensity of a) Coumarin-PKA substrate and b) TAMRA-ERK2 substrate pre-incubated with various combinations of two kinases (PKA, ERK2), in the presence of TiO<sub>2</sub>-GO and/or BSA in TFA/ACN. BSA was a great help to prevent adsorption of substrate peptide in the presence of high concentration of TiO<sub>2</sub>-GO.



**Figure S7.** Multiplex inhibition assay of PKA and ERK2 in a homogeneous solution using TiO<sub>2</sub>-GO based fluorescence analysis. a) Peptide sequence specific phosphorylation: Relative fluorescence intensity ( $F_{\text{Substrate}} = 1$ ) of two kinase substrate mixture with kinases. b) Inhibitor specific inhibition of kinase-mediated phosphorylation: Relative fluorescence intensity ( $F_{\text{Substrate}} = 1$ ) kinase reaction mixtures with each specific inhibitor. Inhibitor 1: H-89/ PKA inhibitor, Inhibitor 2: 5-iodotubercidin/ERK2 inhibitor.

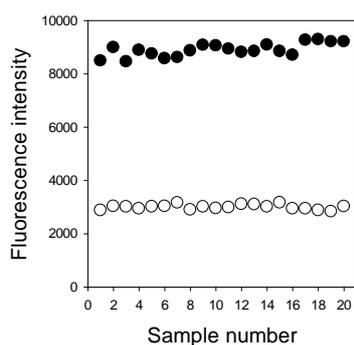


**Figure S8.** MALDI-TOF mass spectra of two kinds of peptides in a solution for a) phosphorylation with corresponding protein kinase, b) inhibition of phosphorylation by specific inhibitors in the presence of both PKA and ERK2.



**PKA (Z' factor = 0.90)**

- Negative control  
Mean = 8900.25 / STD = 251.80
- Positive control  
Mean = 2995.75 / STD = 91.45

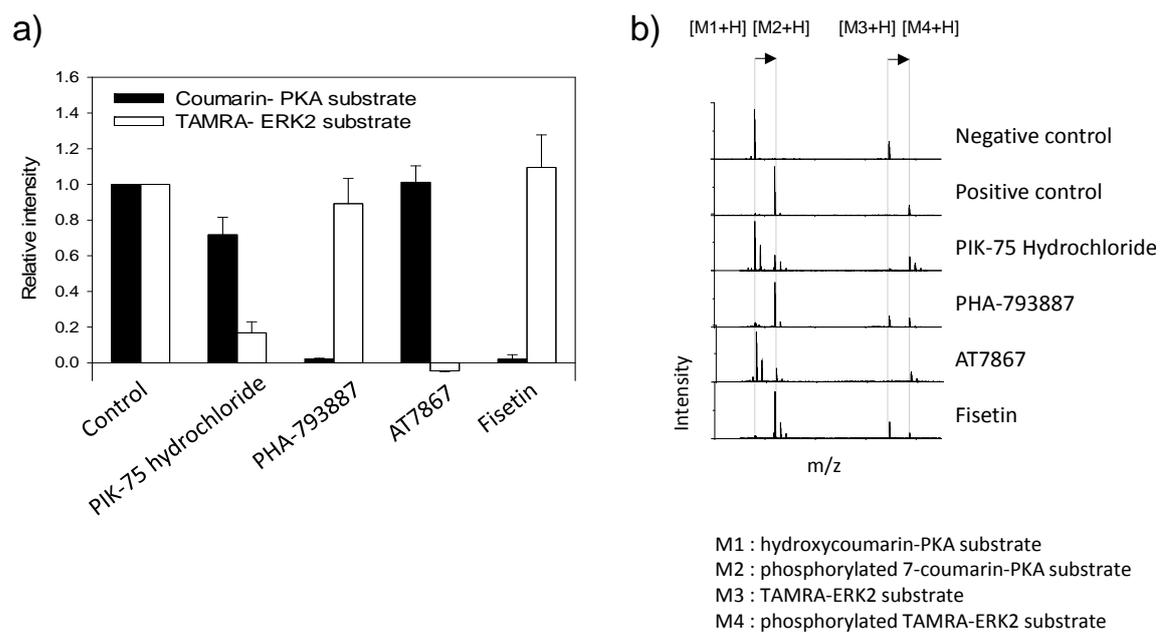


**ERK2 (Z' factor = 0.82)**

- Negative control  
Mean = 8900.25 / STD = 251.80
- Positive control  
Mean = 2995.75 / STD = 91.45

**Figure S9.** Z<sup>2</sup>-factor calculated for multiplex protein kinase (PKA and ERK2) inhibitor screening from each control assay using TiO<sub>2</sub>-GO based platform (20 samples).

STD=standard deviation.



**Figure S10.** a) Relative fluorescence intensity and b) MALDI-TOF mass spectra for inhibition of phosphorylation of each peptides by selected compounds from multiplexed screening assay.