

Supporting Information for:

Studying chemical-regulation of intracellular kinase activity by peptide microarray-based assay with gold nanoparticle probes

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Additional Experimental Section

Additional Figures: S1-S8

Additional References

Additional Experimental Section

Peptide Microarray Fabrication

Peptide microarrays were manufactured by the standard procedure using a SmartArrayer 48 system (Capitalbio Ltd., China).^{S1-S3} Peptides with desired concentration were spotted on aldehyde 3-D slides in 0.3 M PBS (pH 8.5, 0.2 M NaCl) with 20 $\mu\text{g}/\text{mL}$ BSA, following overnight incubation under vacuum at 30 °C. The slides were washed with 30 mL phosphate buffer (pH 7.5, 50 mM) containing 1% (w/v) BSA for 5 min (2 times), and then immersed in blocking buffer (pH 7.5, 50 mM PB, 0.15 M NaCl containing 1% w/v BSA and 0.1M ethanolamine) for 1 h to inactivate any free aldehyde groups. After incubation, the slides were washed with 30 mL washing buffer (pH 7.5, 20 mM tris, 0.15 M NaCl, 10 mM EDTA, 1 mM EGTA with 0.1% Triton X-100) for 10 min (3 times) and 30 mL kinase buffer (pH 7.5, 50 mM tris, 10 mM MgCl_2 , 1 mM DTT) for 10 min, respectively.

Recognition of phosphorylation and attachment of AuNP probes

After phosphorylated reaction, the arrays were incubated with anti-phosphoserine-biotin which was diluted to the desired concentration (20 $\mu\text{g}/\text{mL}$) with 200 μL of probe buffer (pH 7.5, 50 mM PB, 0.15 M NaCl, supplemented with 0.1% Tween-20 (v/v) and 1% BSA (w/v)). Following 1 h incubation at 37°C, a series of washing steps were applied to the arrays: (1) 30 mL PBS buffer with 1% Tween-20 for 5 min (3 times), (2) 30 mL PBS buffer for 5 min (3 times), and (3) 30 mL Milli-Q water for 3 min (18.2 $\text{M}\Omega \cdot \text{cm}$, 3 times). Subsequently, the slides were incubated with 200 μL AuNP probes (5×10^{-9} M in PBS-1) at 37 °C for 1 h. The slides were subjected

to a series of washing and drying steps as previously described, respectively.

Silver Enhancement

After labeling with AuNP probes, the slides were subjected to a series of washing and drying steps as previously described. Subsequently, 1 mL silver enhancer solution (solution A (AgNO_3) and solution B (hydroquinone) were mixed with the volume ratio of 1:1) were applied to each slide for 8 min followed by washing with water (3 times) and drying with centrifugation (200g for 40 s).

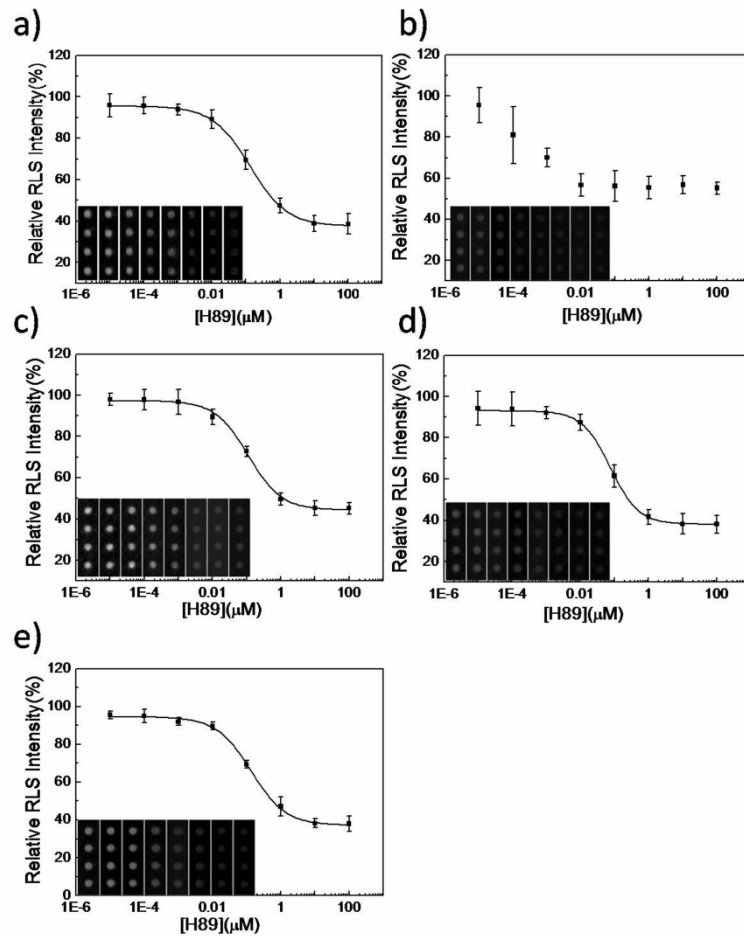


Fig. S1. RLS images (inset) and IC_{50} curves of H99 co-cultured with (a) SHG-44, (b) HeLa, (c) MCF-7, (d) SW620 and (e) PC-12 cells. The concentrations of H99 are 10^{-5} , 10^{-4} , 10^{-3} , 10^{-2} , 0.1, 1, 10 and 10^2 μM , respectively. The concentrations of substrate peptide in spotting solution, ATP in kinase buffer, cell lysate are 10 μM , 50 μM and 100 $\mu\text{g/mL}$, respectively. The signals have been normalized to the average RLS intensities obtained from control samples.

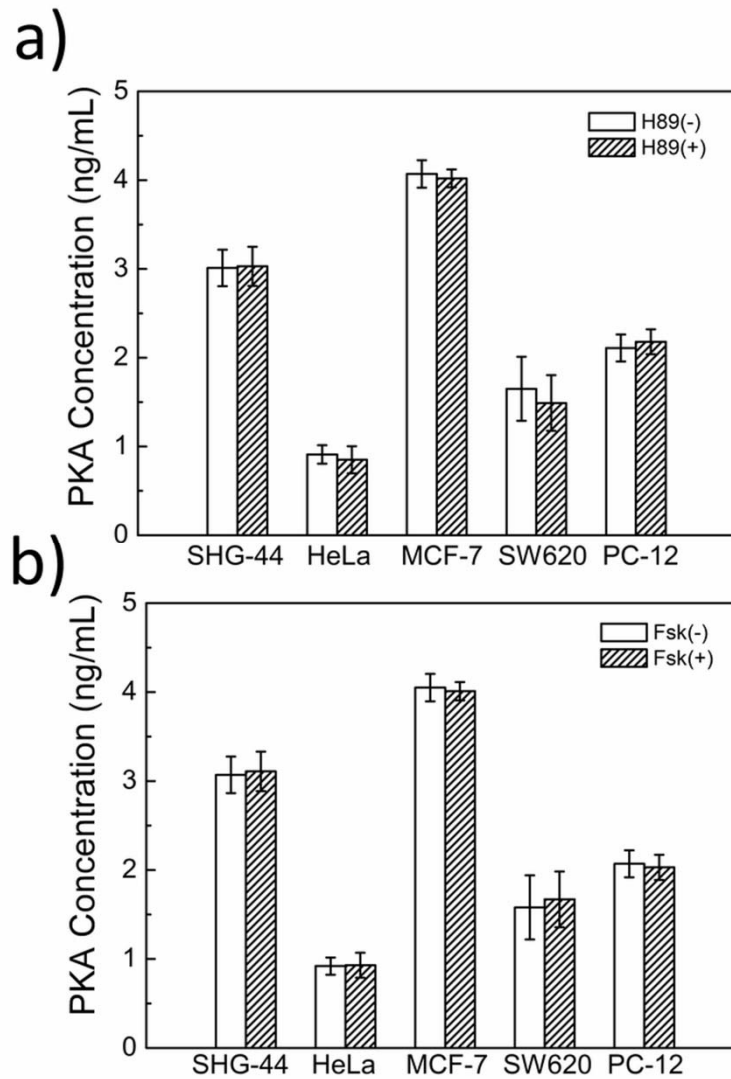


Fig. S2. The concentration of PKA in five cells (SHG-44, HeLa, MCF-7, SW620 and PC-12) co-cultured without or with (a) 10 μ M H89 and (b) 20 μ M Fsk. The concentration of cell lysate is 100 μ g/mL.

The concentrations of PKA were detected by commercial PKA ELISA kits (Biofine Ltd. Beijing, China). Error bars are standard deviations (n=3).

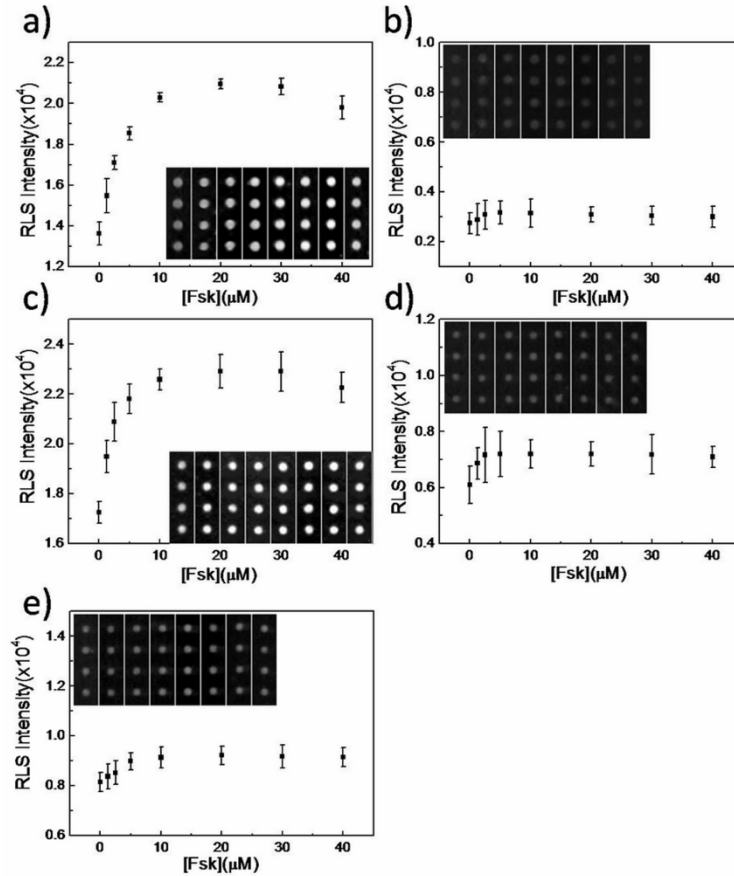


Fig. S3. RLS images (inset) and the curves of the integrated RLS intensity as a function of the concentration of Fsk co-cultured with (a) SHG-44, (b) HeLa, (c) MCF-7, (d) SW620 and (e) PC-12 cells. The concentrations of Fsk are 0, 1.25, 2.5, 5, 10, 20, 30, and 40 μM respectively. The concentrations of substrate peptide in spotting solution, ATP in kinase buffer, cell lysate are 10 μM , 50 μM and 100 $\mu\text{g/mL}$, respectively. The signals have been subtracted by the average RLS intensities of blank samples.

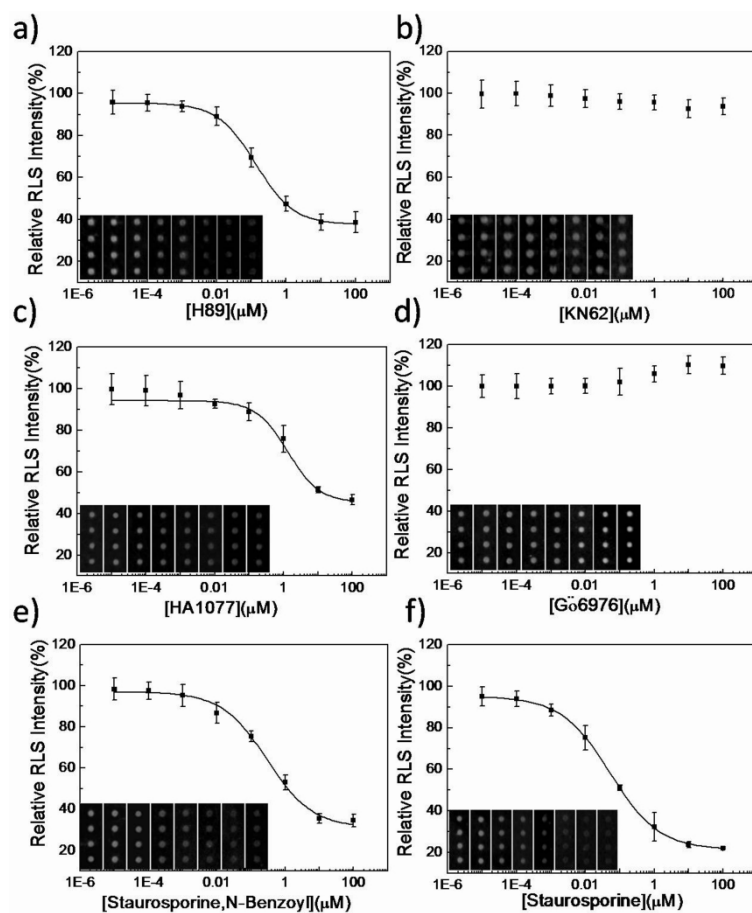


Fig. S4. RLS images (inset) and IC_{50} curves of (a) H89, (b) KN62, (c) HA1077, (d) Gö6976, (e) Staurosporine, N-Benzoyl and (f) Staurosporine co-cultured with SHG-44 cells. The concentration of inhibitors are 10^{-5} , 10^{-4} , 10^{-3} , 10^{-2} , 0.1, 1, 10 and 10^2 μM , respectively. The concentrations of substrate peptide in spotting solution, ATP in kinase buffer, SHG-44 cell lysate are 10 μM , 50 μM and 100 $\mu\text{g/mL}$, respectively. The signals have been normalized to the average RLS intensity obtained from control sample of SHG-44.

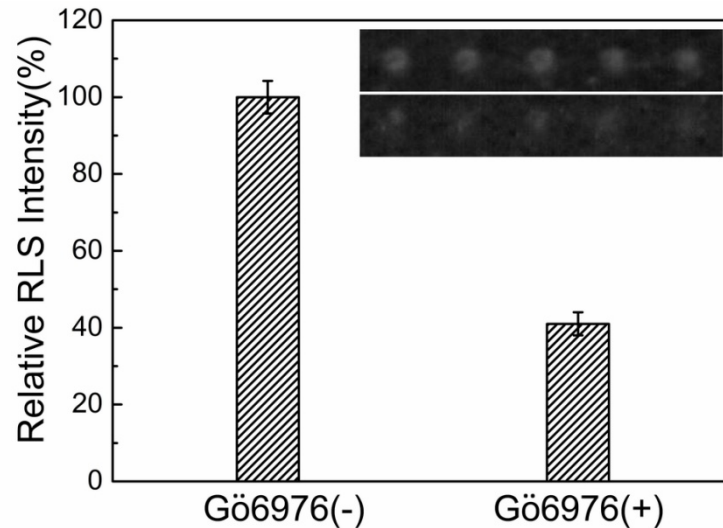


Fig. S5. RLS intensities and RLS images (inset) of SHG-44 cells co-cultured without or with Gö6976, respectively. The concentrations of PKC substrate peptide^{S4} (sequence: CRFARKGSLRQKNV) in the spotting solution, Gö6976, ATP in kinase buffer and SHG-44 cell lysate are 30 μ M, 1 μ M, 100 μ M and 200 μ g/mL, respectively.

SHG-44 cells were co-cultured with 1 μ M Gö6976 and lysated as described in experimental section (see experimental section 2.3 in the manuscript for details). Then, supernatants (cell lysates) were collected and diluted by desired volumes of PKC assay buffer (20 mM Tris-HCl, 10 mM MgCl₂, 0.5 mM CaCl₂, 0.1 mM ATP, 2 μ g/mL diacylglycerol (DAG), and 2 μ g/mL phosphatidylserine (PS), pH 7.5). Total proteins of cell lysates were determined using Bradford reagent. The concentration of cell lysate was defined by the amount of total proteins in the assay solution.

The relative RLS intensity of SHG-44 cells co-cultured with 1 μ M Gö6976 is 59% lower than that of normal cultured SHG-44 cells. This result suggests that intracellular PKC activity level in SHG-44 cell was significantly inhibited by co-culturing with Gö6976.

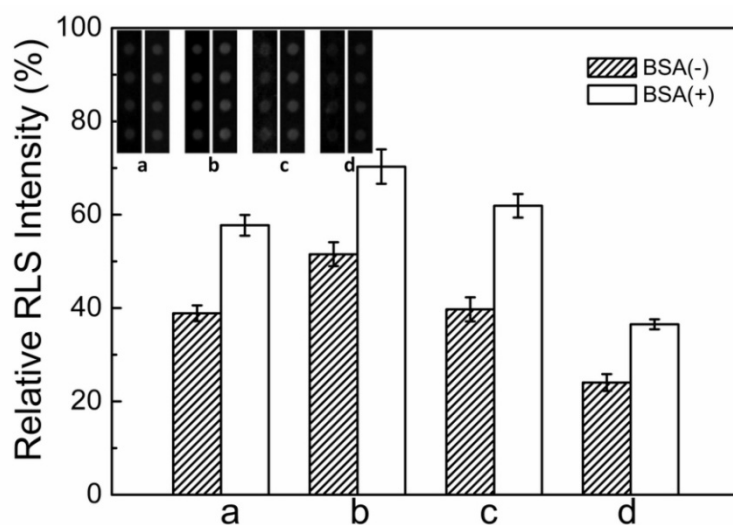


Fig. S6. RLS images (inset) and the relative RLS intensities of SHG-44 cells co-cultured with 10 μM (a) H89, (b) HA1077, (c) Staurosporine, N-Benzoyl and (d) Staurosporine in culture medium without or with 1.5 μM BSA, respectively. The concentrations of substrate peptide in the spotting solution, ATP in kinase buffer and cell lysate are 10 μM , 50 μM and 100 $\mu\text{g/mL}$, respectively.

The relative RLS intensity of inhibitors-stimulated SHG-44 cell lysate is increased by adding 1.5 μM BSA in the culture medium. The experimental result demonstrates that the inhibition efficiencies of inhibitors are decreased by adding BSA.

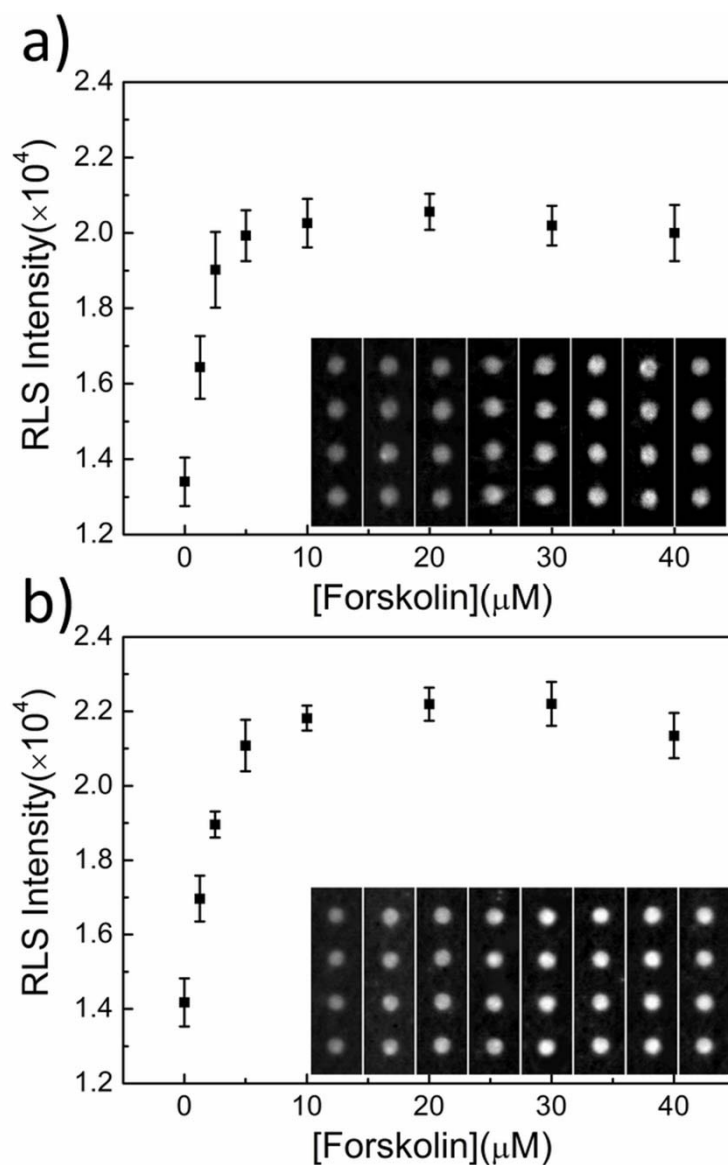


Fig. S7 RLS images (inset) and the curves of the integrated RLS intensity as a function of the concentration of Fsk co-cultured with (a) G2/M-phase arrested SHG-44 cells, (b) M/G1-phase arrested SHG-44 cells. The concentrations of Fsk are 0, 1.25, 2.5, 5, 10, 20, 30, and 40 μM respectively. The concentrations of substrate peptide in spotting solution, ATP in kinase buffer, cell lysate are 10 μM , 50 μM and 100 $\mu\text{g/mL}$, respectively. The signals have been subtracted by the average RLS intensities of blank samples.

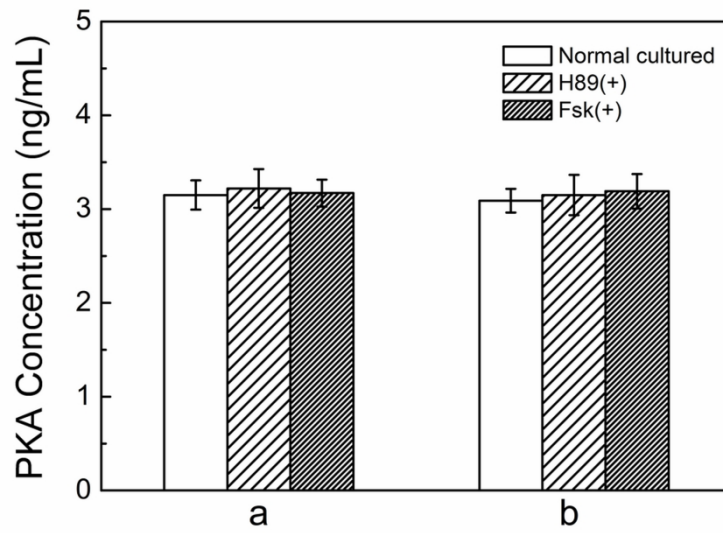


Fig. S8. The concentration of PKA in (a) G2/M-phase arrested SHG-44 cells, (b) M/G1-phase arrested SHG-44 cells co-cultured without or with 10 μ M H89 and 20 μ M Fsk. The concentration of cell lysate is 100 μ g/mL.

The concentrations of PKA were detected by commercial PKA ELISA kits (Biofine Ltd. Beijing, China). Error bars are standard deviations (n=3)

Additional References

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