Fluorescent biosensor of CDK5 kinase activity in glioblastoma cell extracts and living cells

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SUPPORTING INFORMATION – Supplementary Figures

* Figure S1 – Fluorescence emission of Peptide Biosensors over time

Relative fluorescence intensity of 200 nM Cy5-labelled peptide biosensors in PBS buffer in the absence of any kinase (λ exc: 649 nm/ λ em: 670 nm)



* Figure S2 – Response of CDKACT5 to CDK5 inhibitors

Relative fluorescence intensity of 200nM Cy5-labelled CDKACT5-ER104 incubated with 100nM CDK5/p25 and different concentrations of CDK5 inhibitors (a) Roscovitine, (b) Dinaciclib and (c) AT7519. Curve fitting and IC50 determination based on decrease of maximal biosensor response at the endpoint of the assay (100% corresponds to biosensor fluorescence without inhibitor after 50min incubation) (d) Roscovitine, (e) Dinaciclib, (f) AT7519



* Figure S3

Relative fluorescence intensity of 200nM Cy5-labelled CDKACT5-ER104 incubated with different concentrations of CDK5 inhibitors in the absence of CDK5/p25 (a) Roscovitine, (b) Dinaciclib and (c) AT7519 (d) DMSO



* Figure S4 – Response of CDKACT5 biosensor to U87 extracts

Relative fluorescence intensity of 200 nM Cy5-labelled CDKACT5-ER104 to different concentrations of U87 cell extracts



* Figure S5 – Response of CDKACT5 biosensor to U87 extracts

Relative fluorescence intensity of different concentrations of (a) Cy5-labelled CDKACT5-ER104 incubated with 10 μ g U87 cell extracts (b) Dylight 650-labelled CDKACT5-ER104 incubated with 10 μ g U87 cell extracts (c) Cy5-labelled ER104 substrate incubated with 10 μ g U87 cell extracts



* Figure S6 – Response of CDKACT5 to inhibition of CDK5 in U87 cell extracts

Relative fluorescence intensity of 150 nM Cy5-labelled CDKACT5-ER104 was monitored upon incubation with 10 μ g U87 cell extracts +/- different concentrations of (a) Dinaciclib (b) Roscovitine (c) AT7519 (CDK2/4/5/9 inhibitor), (d) or DMSO



* Figure S7 – Dose-dependent response of CDKACT5 to other kinase inhibitors

Fluorescence of 150 nM CDKACT5-Cy5 was monitored upon incubation with 10 µg U87 cell extracts +/- different concentrations of PD-0332991 (CDK4/6 inhibitor), RO3306 (CDK1 inhibitor), PP2 (Src inhibitor), or U0126 (MEK1/MEK2 - MAPK inhibitor).



* Figure S8 – Specific CDK5 activity in different cell lines

(A) Fluorescence of 150 nM CDKACT5-Cy5 was monitored for 60minutes upon incubation with 10 μ g total protein from different cell lines (B) Relative maximal fluorescence of CDKACT5-Cy5 (C) Relative CDK5/actin levels in 10 μ g total protein from different cell lysates (D) Specific activity of CDK5 in each cell line defined as relative activity shown in (B) over relative CDK5/actin levels shown in (D).



* Figure S9 – Confocal images from the different z planes of a representative U87 cell internalized with Pep-CDKACT5- Cy5



* Figure S10 – Colocalization of Pep-CDKACT5- Cy5 with CDK5 and p25/p35 in U87 cells



* Figure S11 – Internalization and fluorescence emission of Pep-CDKACT5-Cy5 and Pep1-Cy5 following treatment by CDK5 inhibitors

(A) Fluorescence of Pep-CDKACT5-Cy5 in U87 cells following 24h treatment with 10 μ M dinaciclib or roscovitine. (B) Fluorescence of Pep1-Cy5 in U87 cells following 24h treatment with 10 μ M dinaciclib or roscovitine. Left panels correspond to Hoechst staining of nuclei; right panels to fluorescence emission in the Cy5 filter. Mean and standard deviation values corresponding to Cy5/Hoechst fluorescence for mock-treated and dinaciclib- or roscovitine-treated U87 cells are summarized in the table below. The relative ratio of mock-treated cells was fixed at 100%.



MOCK	Dinaciclib	Roscovitine	
100	42	22	Mean
28	13	12	SE
11	8	6	Nb of cells





МОСК	Dinaciclib	Roscovitine	
100	91	74	Mean
28	42	40	SE
34	28	35	Nb of cells

* Figure S12 –Pep-CDKACT5- ER104-Cy5 and CDKACT5-ER104mut-Cy5 fluorescence in U87 cells

Relative fluorescence of Pep-CDKACT5-ER105-Cy5 and the Pep-CDKACT5-ER104mutant-Cy5 over time in U87 cells following addition of 10 µM ionimycin to promote CDK5 activation. Micrographs of Pep-CDKACT5-ER104-Cy5 (blue) and Pep-CDKACT5-ER104mut-Cy5 (red) fluorescence in U87 cells prior to and at different time points following addition of ionomycin reveal progressive increase in fluorescence of the CDKACT5-Cy5 biosensor, in particular at the cell membrane in contrast to the mutant which does not respond.





Pep-CDKACT5-ER104mut-Cy5

