

## Supplementary Materials

### Small molecule inhibitors targeting Gα<sub>i2</sub> protein attenuate migration of cancer cells

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## Supplementary materials

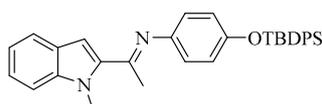
### Chemistry

**Materials.** Anhydrous solvents and other reagents were purchased either from Sigma or VWR and were used without further purification. Analtech silica gel plates (60 F254) were utilized for analytical TLC, and Analtech preparative TLC plates (UV254, 2000  $\mu\text{m}$ ) were used for purification. Silica gel (200–400 mesh) was used in column chromatography. TLC plates were visualized using UV light, anisaldehyde, and/or iodine stains. NMR spectra were obtained on a Varian-Gemini 400 MHz and Bruker Ascend™ 500 and 700 MHz magnetic resonance spectrometer.  $^1\text{H}$  NMR spectra were recorded in parts per million (ppm) relative to the residual peaks of  $\text{CHCl}_3$  (7.24 ppm) in  $\text{CDCl}_3$  or  $\text{CHD}_2\text{OD}$  (4.78 ppm) in  $\text{CD}_3\text{OD}$  or  $\text{DMSO-}d_5$  (2.49 ppm) in  $\text{DMSO-}d_6$ . MestReNova (version 11.0) was used to process the original “fid” files. High-resolution mass spectra were gathered with the assistance of the Georgia Institute of Technology mass spectrometry facility (Atlanta, GA). *N*-methyl-2-acetylindole (**1**)[1,2], 1-(3-hydroxy-1-methylindol-2-yl)ethanone (**2**)[3], 4-((*tert*-butyldiphenylsilyl)oxy)aniline (**5**)[4], and 4-((*tert*-butyldimethylsilyl)oxy)aniline (**7**)[5] were synthesized adapting literature protocols.

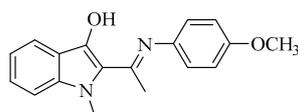
General Procedure for Preparation of *Ketimines* (*Method A*). A solution of the corresponding methylketones **1-4** (1 mmol), corresponding amines **5-7** (1.2 mmol) and *p*-toluenesulfonic acid monohydrate (5 mol%) in anhydrous toluene (5 mL) was heated under reflux with a Dean-Stark trap for 10 hour, then cooled and neutralized by adding saturated aqueous  $\text{NaHCO}_3$  solution and then organic layer was separated. The aqueous layer was further extracted with ethyl acetate (20 mL) and then the combined organic layers was washed with water (10 mL), brine (10 mL), dried over anhydrous  $\text{Na}_2\text{SO}_4$ , filtered and evaporated to dryness. The residue was purified by preparative chromatography with a hexane-ethyl acetate mixture as mobile phase to give the ketimine compounds **8-11**.

General Procedure for silyl deprotection (*Method B*). Silyl protected ketimine compounds **8**, **10**, and **11** were dissolved in 2:1 MeOH-THF and then  $\text{CsF}$  (2 equiv.) was added to the solution and the resultant solution was stirred for 1 h. The reaction was quenched by adding water and was extracted with ethyl acetate (20 mL) and the

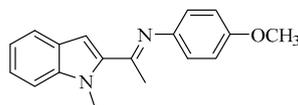
aqueous layer was separated. Ethyl acetate layer was washed with brine (10 mL), dried over anhydrous Na<sub>2</sub>SO<sub>4</sub>, filtered, and concentrated to dryness. The residue was purified by preparative chromatography with a hexane-ethyl acetate mixture as mobile phase to give the target molecules **12-14**.



*(E)*-*N*-(4-((*tert*-butyldiphenylsilyloxy)phenyl)-1-(1-methyl-1*H*-indol-2-yl)ethan-1-imine **8**. Method A; purified by preparative chromatography using 5% ethyl acetate-hexane mixture as mobile phase. Yellow oil; yield: 15%. <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 7.75 – 7.66 (m, 4H), 7.64 – 7.55 (m, 1H), 7.45 – 7.31 (m, 6H), 7.28 (t, *J* = 7.6 Hz, 1H), 7.23 – 7.18 (m, 1H), 7.13 – 7.05 (m, 1H), 6.97 (s, 1H), 6.78 – 6.69 (m, 2H), 6.57 (d, *J* = 8.3 Hz, 2H), 4.14 – 4.00 (s, 3H), 2.23 (s, 3H), 1.09 (s, 9H).

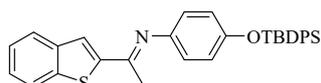


*(E)*-2-(1-((4-methoxyphenyl)imino)ethyl)-1-methyl-1*H*-indol-3-ol **9a**. Method A; purified by preparative chromatography using 40% ethyl acetate-hexane mixture as mobile phase. Orange solid; yield: 15%. <sup>1</sup>H NMR (700 MHz, CDCl<sub>3</sub>-*d*) δ 7.81 (d, *J* = 7.7 Hz, 1H), 7.40 (t, *J* = 7.6 Hz, 1H), 7.09 (dd, *J* = 13.1, 8.2 Hz, 3H), 6.94 (t, *J* = 7.3 Hz, 1H), 6.89 (d, *J* = 8.4 Hz, 2H), 3.80 (s, 3H), 3.49 (s, 3H), 2.40 (s, 3H). <sup>13</sup>C NMR (176 MHz, CDCl<sub>3</sub>) δ 177.58, 157.76, 150.15, 149.89, 131.60, 131.45, 126.43, 123.41, 123.17, 121.53, 118.80, 114.70, 114.66, 110.93, 55.72, 35.93, 17.07. HRMS (EI) *m/z* Calcd. for C<sub>18</sub>H<sub>18</sub>O<sub>2</sub>N<sub>2</sub> [M]<sup>+</sup>: 294.1371, found 294.1368.

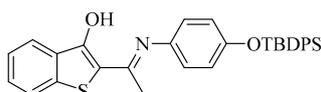


*(E)*-*N*-(4-methoxyphenyl)-1-(1-methyl-1*H* indole-2-yl)ethane-1-imine **9b**. Method A; purified by preparative chromatography using 10% ethyl acetate-hexane mixture as mobile phase. Yellow solid; yield 30%. <sup>1</sup>H NMR (400 MHz, Chloroform-*d*) δ 7.64 (dq, *J* = 7.9, 0.8 Hz, 1H), 7.37 (dq, *J* = 8.4, 0.9 Hz, 1H), 7.30 (ddd, *J* = 8.2, 6.9, 1.1 Hz, 1H), 7.11

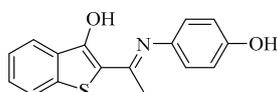
(ddt,  $J = 7.7, 6.9, 0.9$  Hz, 1H), 7.00 (d,  $J = 0.8$  Hz, 1H), 6.96 – 6.85 (m, 2H), 6.82 – 6.69 (m, 2H), 4.15 (s, 3H), 3.81 (s, 3H), 2.30 (s, 3H).  $^{13}\text{C}$  NMR (176 MHz,  $\text{CDCl}_3$ )  $\delta$  160.4, 156.2, 144.4, 140.3, 137.8, 135.1, 126.6, 125.4, 124.1, 121.7, 120.2, 114.70, 111.3, 110.2, 107.5, 55.7, 33.1, 18.8. HRMS (EI)  $m/z$  Calcd. for  $\text{C}_{18}\text{H}_{19}\text{ON}_2$   $[\text{M} + \text{H}]^+$ : 279.1492, found 279.1493.



(*E*)-1-(benzo[*b*]thiophen-2-yl)-*N*-(4-((*tert*-butyldiphenylsilyl)oxy)phenyl)ethan-1-imine **10**. Method A; purified by preparative chromatography using 10% ethyl acetate-hexane mixture as mobile phase. Yellow solid; yield: 28%.  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  7.82 – 7.74 (m, 2H), 7.74 – 7.68 (m, 4H), 7.63 (d,  $J = 0.9$  Hz, 1H), 7.44 – 7.38 (m, 2H), 7.38 – 7.29 (m, 6H), 6.77 – 6.72 (m, 2H), 6.61 – 6.56 (m, 2H), 2.26 (s, 3H), 1.09 (s, 9H).

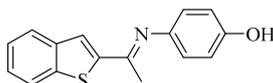


(*E*)-2-(1-(4-((*tert*-butyldiphenylsilyl)oxy)phenyl)imino)ethylbenzo[*b*]thiophene-3-ol **11**. Method A; purified by preparative chromatography using 10% ethyl acetate-hexane mixture as mobile phase. Yellow solid; yield: 6%.  $^1\text{H}$  NMR (700 MHz, Chloroform-*d*)  $\delta$  7.96 (dd,  $J = 7.9, 3.3$  Hz, 1H), 7.57 (dd,  $J = 7.9, 2.8$  Hz, 1H), 7.52 (t,  $J = 7.6$  Hz, 1H), 7.48 – 7.43 (m, 1H), 7.40 (q,  $J = 8.1, 5.5$  Hz, 1H), 7.30 – 7.26 (m, 1H), 7.07 – 7.02 (m, 2H), 6.86 – 6.82 (m, 2H), 2.36 – 2.20 (m, 3H), 1.03 – 0.91 (m, 8H), 0.28 – 0.12 (m, 5H).

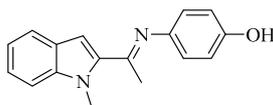


(*E*)-2-(1-(4-hydroxyphenyl)imino)ethylbenzo[*b*]thiophen-3-ol **12**. Method B; purified by preparative chromatography using 40% ethyl acetate-hexane mixture as mobile phase. Yellow solid; yield: 84%.  $^1\text{H}$  NMR (700 MHz,  $\text{MeOH-}d_4$ )  $\delta$  7.83 (d,  $J = 7.8$  Hz, 1H), 7.60 (dd,  $J = 8.0, 2.8$  Hz, 1H), 7.45 (t,  $J = 7.6$  Hz, 1H), 7.25 (t,  $J = 7.0$  Hz, 1H), 7.12 – 7.04 (m, 2H), 6.80 (t,  $J = 5.3$  Hz, 2H), 2.29 (d,  $J = 2.9$  Hz, 3H).  $^{13}\text{C}$  NMR (176 MHz,  $\text{CDCl}_3$ )  $\delta$  182.17, 161.85, 159.57, 154.99, 142.34, 134.80, 131.31, 130.04, 127.03, 125.36,

124.28, 123.59, 116.49, 56.21, 19.48, 14.35. HRMS (ESI)  $m/z$  Calcd. for  $C_{16}H_{14}O_2NS$   $[M+H]^+$ : 284.0740, found 284.0738.



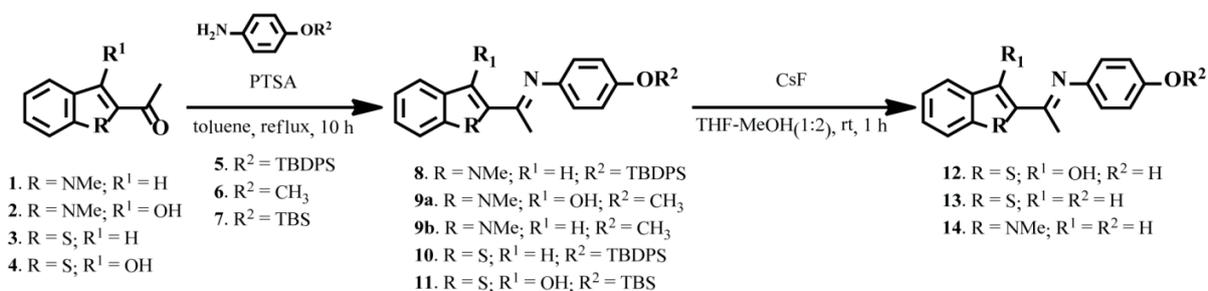
(*E*)-4-((1-(benzo[*b*]thiophen-2-yl)ethylidene)amino)phenol **13**. Method B; purified by preparative chromatography using 30% ethyl acetate-hexane mixture as mobile phase. Yellow solid; yield: 65%.  $^1H$  NMR (700 MHz,  $CDCl_3$ )  $\delta$  7.79 (dd,  $J = 23.2, 7.7$  Hz, 2H), 7.65 (s, 1H), 7.34 (dt,  $J = 18.0, 7.3$  Hz, 2H), 6.93 (d,  $J = 8.0$  Hz, 2H), 6.72 (d,  $J = 8.0$  Hz, 2H), 2.32 (s, 3H).  $^{13}C$  NMR (176 MHz,  $CDCl_3$ )  $\delta$  160.91, 153.26, 146.99, 142.83, 141.22, 139.81, 125.95, 125.29, 124.57, 124.43, 122.58, 121.59, 115.82, 63.24, 52.91, 17.15, 8.05. HRMS (ESI)  $m/z$  Calcd. for  $C_{16}H_{14}ONS$   $[M+H]^+$ : 268.0791, found 268.0790.



(*E*)-4-((1-(1-methyl-1*H*-indol-2-yl)ethylidene)amino)phenol **14**. Method B; purified by preparative chromatography using 20% ethyl acetate-hexane mixture as mobile phase. Brown solid; yield: 53%.  $^1H$  NMR (700 MHz,  $CDCl_3$ )  $\delta$  7.64 (d,  $J = 7.9$  Hz, 1H), 7.37 (d,  $J = 8.3$  Hz, 1H), 7.29 (t,  $J = 7.7$  Hz, 1H), 7.11 (t,  $J = 7.4$  Hz, 1H), 7.00 (s, 1H), 6.83 (d,  $J = 8.1$  Hz, 2H), 6.70 (d,  $J = 8.1$  Hz, 2H), 4.14 (s, 3H), 2.29 (s, 3H).  $^{13}C$  NMR (176 MHz,  $CDCl_3$ )  $\delta$  160.58, 151.93, 144.53, 140.15, 137.82, 126.60, 124.11, 121.74, 121.17, 120.20, 115.90, 110.22, 107.56, 33.16, 18.85. HRMS (ESI)  $m/z$  Calcd. for  $C_{17}H_{17}ON_2$   $[M+H]^+$ : 265.1330, found 265.1335.

## Supplementary Figures

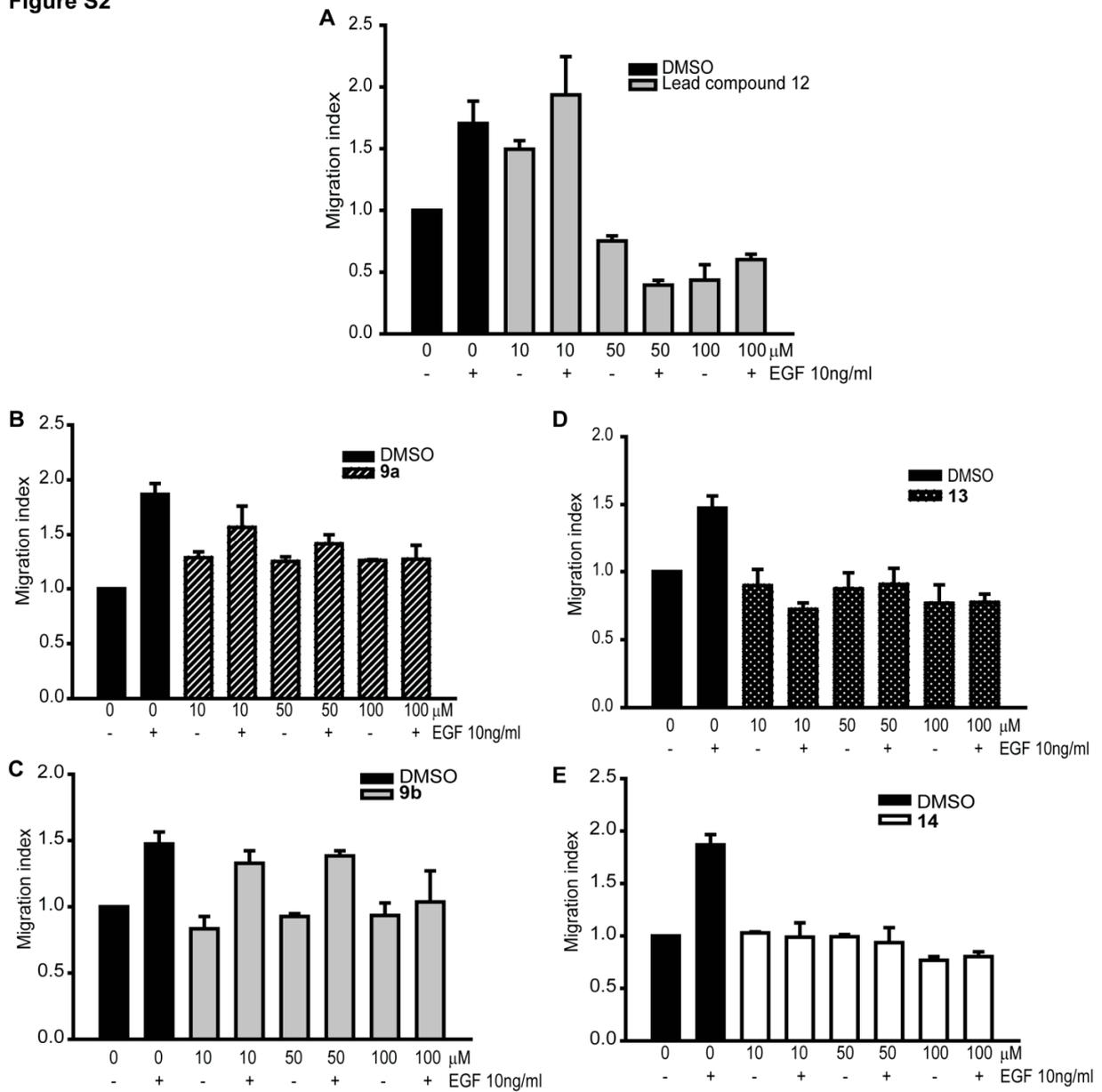
Figure S1



### Synthesis of ketimines 9a-b, 12-14

**Fig. S1. Synthesis of ketimines 9a-b, 12-14.** Ketimines **9-11** were synthesized from the corresponding methylketones **1-4** and anisidine (**6**) or *O*-silyl-protected *p*-hydroxyaniline (**5** and **7**) using catalytic amount of *p*-toluenesulfonic acid and toluene as solvent. The reactions were performed in Dean-Stark apparatus to remove water. Silyl deprotection of compounds **8**, **10**, and **11** were performed by using CsF in methanol-THF mixture to give the final target molecules **12-14**.

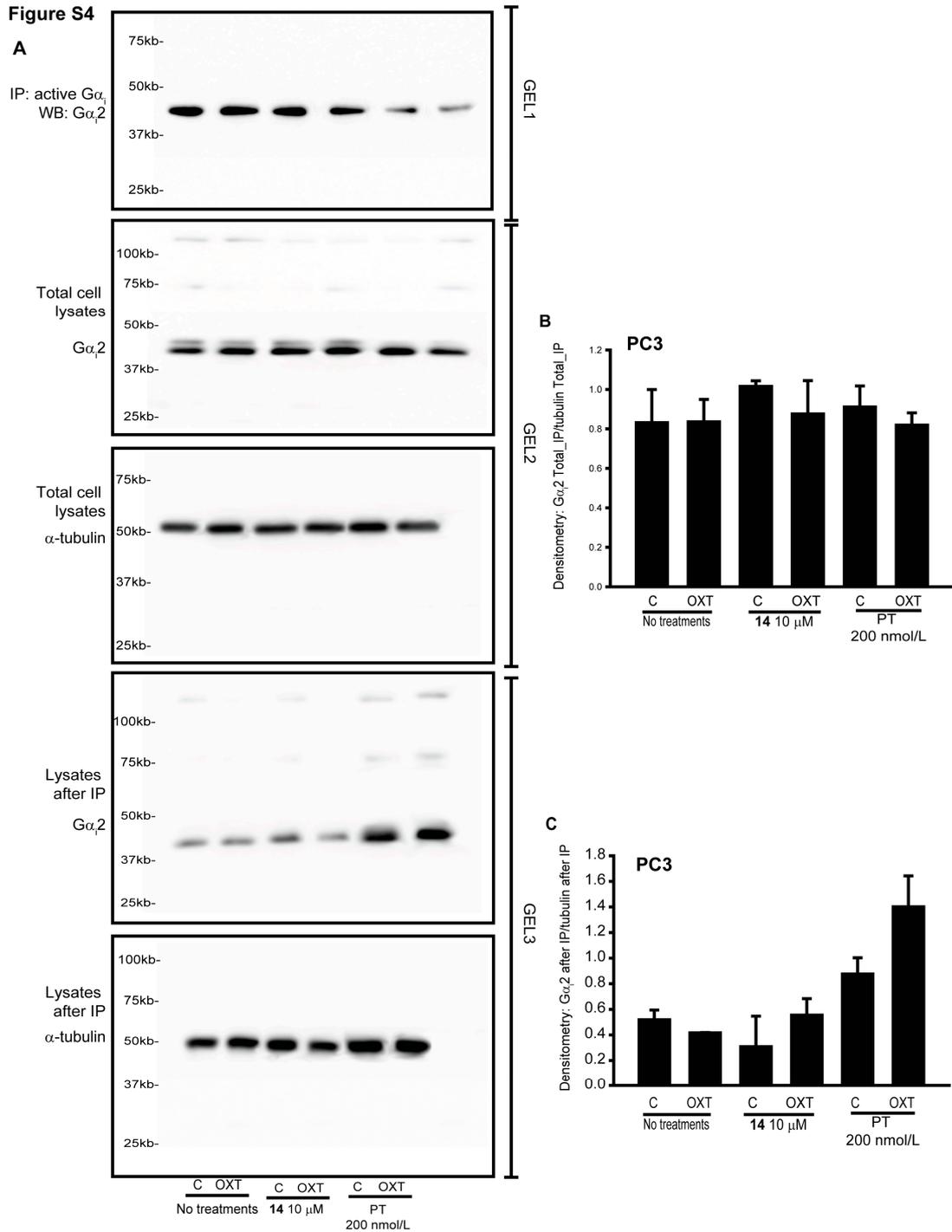
Figure S2



**Fig. S2. Optimization of the dosage of the small molecule inhibitors, using PC3 cell lines.** A. Transwell migration assay of PC3 cells treated with different concentrations (10, 50, 100  $\mu\text{M}$ ) of compound **12**, in presence and absence of EGF treatments (10 ng/ml). Results are expressed as migration index. Each bar represents

mean  $\pm$  SEM of two different inserts of one experiment. **B.** Transwell migration assay of PC3 cells treated with different concentrations (10, 50, 100  $\mu$ M) of compound **9a** with or without EGF treatments (10 ng/ml). **C.** Transwell migration assay of PC3 cells treated with different concentrations (10, 50, 100 $\mu$ M) of compound **9b** with or without EGF treatments (10 ng/ml). **D.** Transwell migration assay of PC3 cells treated with different concentrations (10, 50, 100 $\mu$ M) of compound **13** with or without EGF treatments (10ng/ml). **E.** Transwell migration assay of PC3 cells treated with different concentrations (10, 50, 100  $\mu$ M) of compound **14** with or without EGF treatments (10ng/ml).

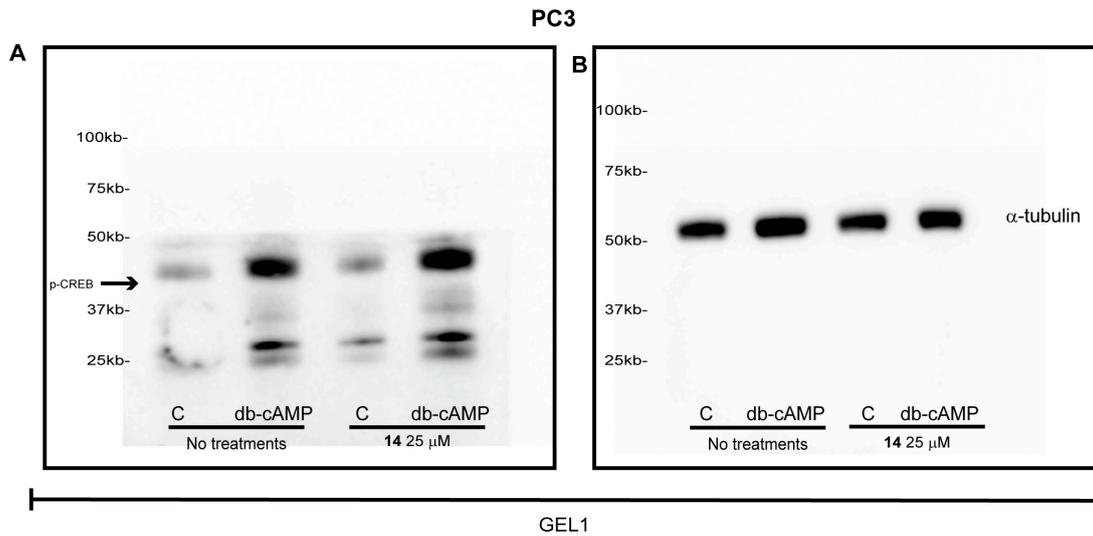




**Fig. S4. Full Western blot images, related to the representative data of the Immunoprecipitation experiments. A.** Full blots of the Immunoprecipitation experiment. The densitometry of the first panel, that is the IP of active  $G\alpha_i$  and western

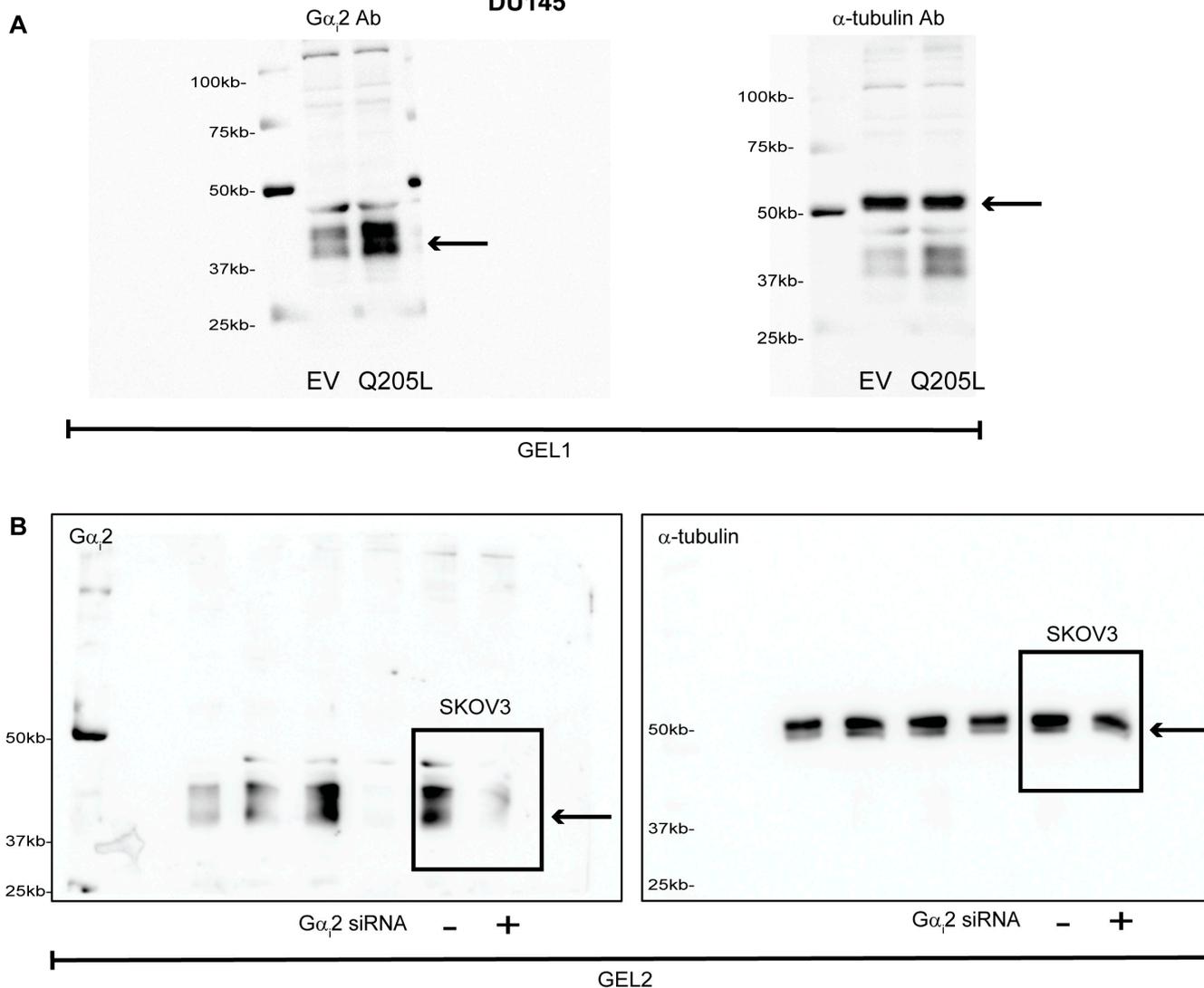
blot for  $G\alpha_2$  experiment is presented in Fig. 4B (right panel), as average of three different experiments ( $G\alpha_2$  IP/ $G\alpha_2$  Total Cell Lysate), in the main manuscript. **B.** Densitometric analysis of  $G\alpha_2$  protein expression, using total cell lysates, was performed. **C.** Densitometric analysis of  $G\alpha_2$  protein expression, using lysates after IP, was performed. In all the experiments the molecular weight markers are not coming up because the  $G\alpha_2$  antibody (AbCam) used is very specific and it does not interfere with it.

Figure S5



**Fig. S5. Full Western blot images, related to the representative data of the levels of p-CREB (A) and  $\alpha$ -tubulin (B), after treatments with compound 14, in presence of db-cAMP.** The densitometric analysis of this experiment is presented in Fig.4A in the main manuscript. In these experiments the molecular weight markers are not coming up because the p-CREB antibody used is very specific and it does not interfere with it.

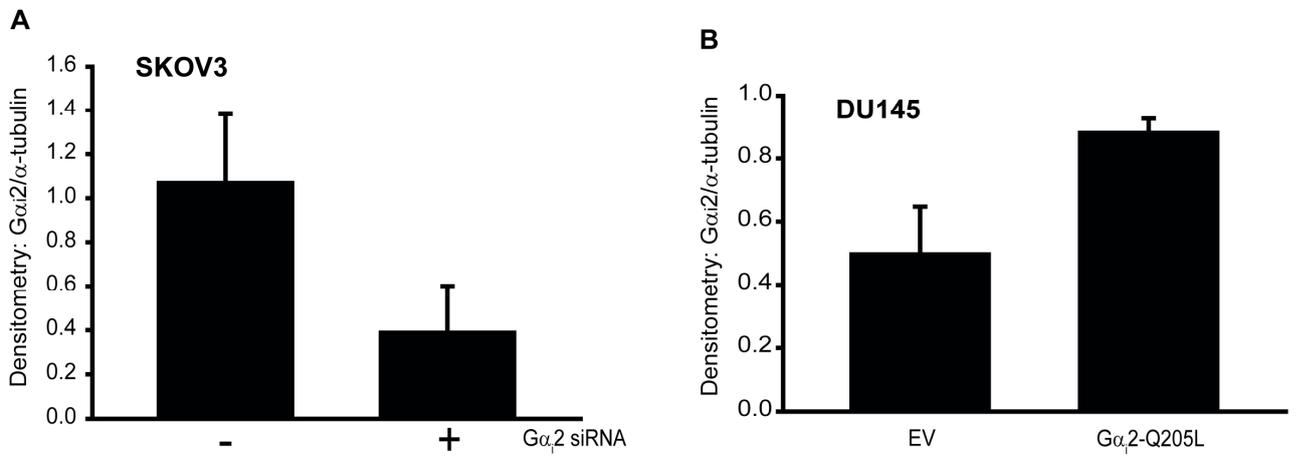
Figure S6



**Fig. S6. Full Western blot images, related to the representative data of the overexpression and knockdown of Gα<sub>i</sub>2 protein experiments in DU145 (Gα<sub>i</sub>2-Q205L), and SKOV3. A.** DU145 cell lines were transiently transfected with EV and Gα<sub>i</sub>2-

Q205L plasmids and used for further assays. Western blot analysis was performed to confirm the overexpression. **B.** SKOV3 cells were transiently transfected with Control and Gα<sub>i</sub>2 siRNAs and used for further assays. Western blot analysis was performed to confirm the knockdown.

**Figure S7**



**Fig. S7. Densitometric analysis of Western blot images: A.** Densitometric analysis of SKOV3 cells, transfected with Control and G $\alpha$ <sub>i</sub>2 siRNAs, was performed. **B.** Densitometric analysis of DU145 overexpressing EV and G $\alpha$ <sub>i</sub>2-Q205L was performed.

## References

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