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Supplemental Material to:

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Ca eine and the analog CGS 15943 inhibit cancer cell growth by targeting the phosphoinositide 3-kinase/Akt pathway

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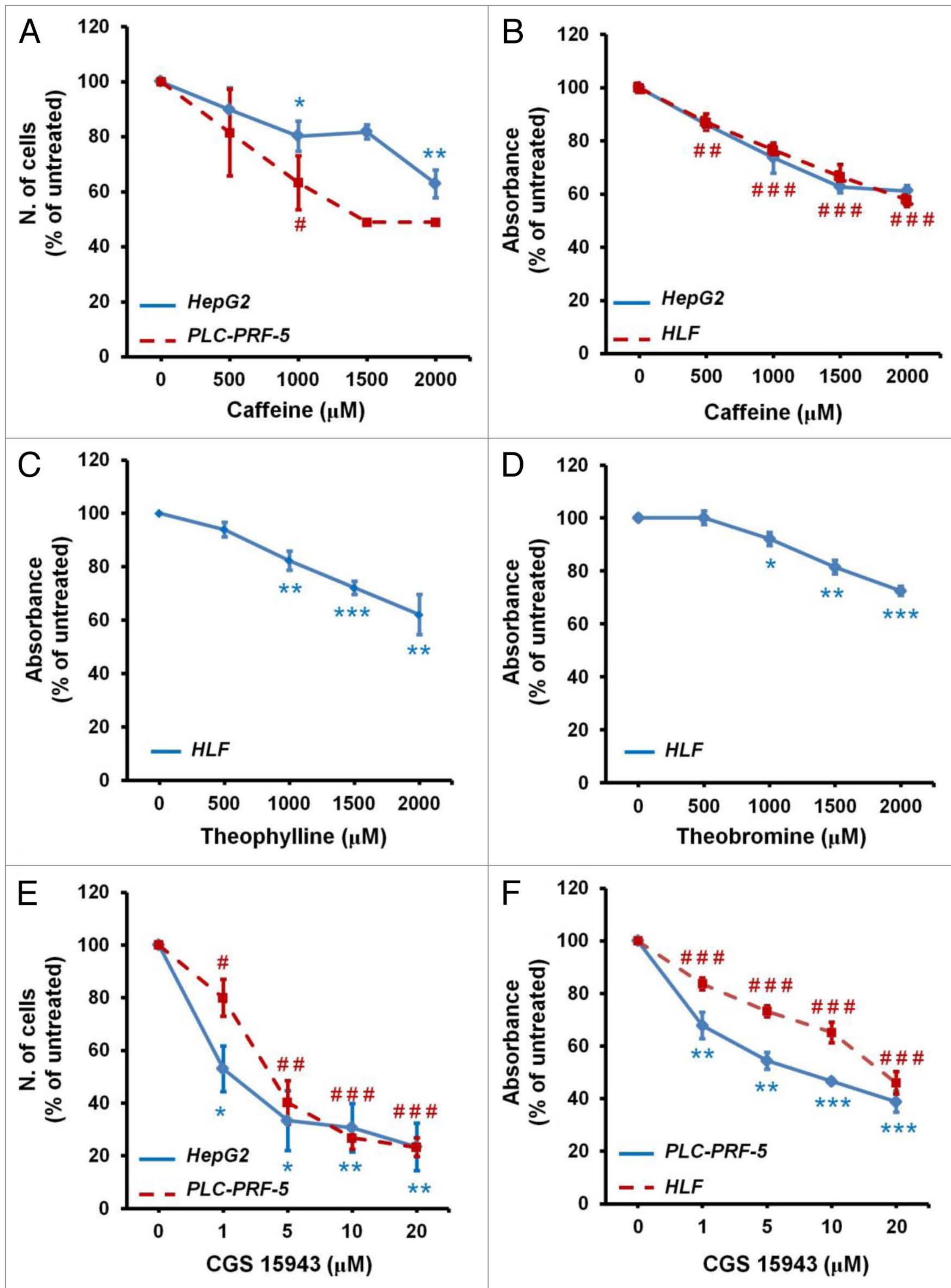

Figure S1. For figure legend, see next page.

Figure S1 (See previous page). In vitro activity of methyl xanthines and CGS 15943 in HCC cells. (**A**) HepG2 and PLC-PRF-5 cells were treated for 72 h with the indicated concentrations of caffeine in the presence of serum and cell proliferation was assessed by cell counting. Data are expressed as percentage of untreated cells and are means ± s.e.m. of $n = 2–5$ (HepG2) and $n = 1–3$ (PLC-PRF-5) independent experiments. HepG2: $^{*}P < 0.05$, $^{**}P < 0.01$, vs corresponding untreated cells as assessed by the Student *t* test (paired, one-tailed distribution). PLC-PRF-5: $^{\#}P < 0.05$ vs. corresponding untreated cells as assessed by the Student *t* test (paired, one-tailed distribution). (**B**) HepG2 and HLF cells were treated for 72 h with the indicated concentrations of caffeine in the presence of serum and cell viability was assessed by MTT assay. Data are expressed as percentage of untreated cells and are means ± s.e.m. of $n = 2$ (HepG2) and $n = 6$ (HLF) independent experiments. HLF: $^{\#\#}P < 0.01$, $^{\#\#\#}P < 0.001$ vs. corresponding untreated cells as assessed by the Student *t* test (paired, one-tailed distribution). (**C and D**) HLF cells were treated for 72 h with the indicated concentrations of theophylline (**C**) or theobromine (**D**) in the presence of serum and cell viability was assessed by MTT assay. Data are expressed as percentage of untreated cells and are means ± s.e.m. of $n = 3$ (**C**) and $n = 4$ (**D**) independent experiments. $^{*}P <0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ vs. corresponding untreated cells as assessed by the Student *t* test (paired, one-tailed distribution). (**E**) HepG2 and PLC-PRF-5 cells were treated for 72 h with the indicated concentrations of CGS 15943 in the presence of serum and cell proliferation was assessed by cell counting. Data are expressed as percentage of untreated cells and are means ± s.e.m. of $n = 3$ (HepG2) and $n = 3–4$ (PLC-PRF-5) independent experiments. HepG2: $^{*}P < 0.05$, $^{**}P < 0.01$, vs. corresponding untreated cells as assessed by the Student *t* test (paired, one-tailed distribution). PLC-PRF-5: $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, $^{\#\#\#}P < 0.001$ vs corresponding untreated cells as assessed by the Student *t* test (paired, one-tailed distribution). (**F**) PLC-PRF-5 and HLF cells were treated for 72 h with the indicated concentrations of CGS 15943 in the presence of serum and cell viability was assessed by MTT assay. Data are expressed as percentage of untreated cells and are means ± s.e.m. of $n = 3–5$ (PLC-PRF-5) and $n = 6$ (HLF) independent experiments. PLC-PRF-5: $^{**}P < 0.01$, $^{***}P < 0.001$; HLF: $^{\#\#\#}P < 0.001$ vs. corresponding untreated cells as assessed by the Student *t* test (paired, one-tailed distribution).

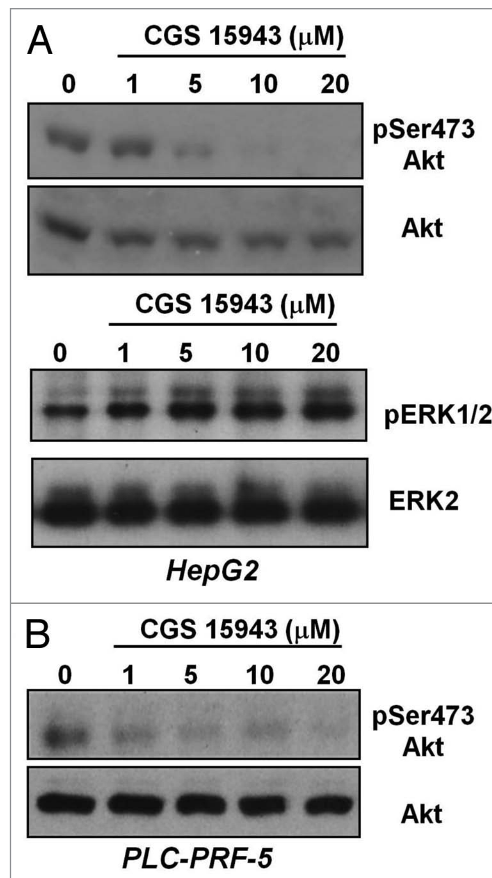

Figure S2. In vitro activity of CGS 15943 on Akt phosphorylation in HCC cells. HepG2 (**A**) and PLC-PRF-5 (**B**) cells were treated for 24 h with the indicated concentrations of CGS 15943 in the presence of serum. Akt activation was assessed by monitoring phosphorylation at its residue Ser473. In (**A**), phosphorylation of ERK1/2 was also assessed. Membranes were then stripped and re-incubated with anti-Akt or anti-ERK2.

A

Caffeine (μM)

0 500 1000 1500 2000

pSer473 Akt

ERK2

pThr308 Akt

ERK2

Sk-Hep-1

B

Theophylline (μM)

0 500 1000 1500 2000

pSer473 Akt

Akt

pERK1/2

ERK2

SK-Hep-1

C

Theobromine (μM)

0 500 1000 1500 2000

pSer473 Akt

Akt

pERK1/2

ERK2

SK-Hep-1

D

Theophylline (μM)

0 500 1000 1500 2000

pSer473 Akt

Akt

pERK1/2

ERK2

HLF

E

Theobromine (μM)

0 500 1000 1500 2000

pSer473 Akt

Akt

pERK1/2

ERK2

HLF

Figure S3. In vitro activity of methyl xanthines on Akt phosphorylation in HCC cells. (**A**) SK-Hep-1 cells were treated for 24 h with the indicated concentrations of caffeine in the presence of serum. Akt activation was assessed by monitoring phosphorylation at its residues Ser473 and Thr308. Equal loading was assessed using an anti-ERK2 antibody. (**B and C**) SK-Hep-1 cells were treated for 24 h with the indicated concentrations of theophylline (**B**) or theobromine (**C**) in the presence of serum. Akt activation was assessed by monitoring phosphorylation at its residues Ser473. Phosphorylation of ERK1/2 was also assessed. Membranes were then stripped and re-incubated with anti-Akt and anti-ERK2. (**D and E**) HLF cells were treated for 24 h with the indicated concentrations of theophylline (**D**) or theobromine (**E**) in the presence of serum. Akt activation was assessed by monitoring phosphorylation at its residues Ser473. Phosphorylation of ERK1/2 was also assessed. Membranes were then stripped and re-incubated with anti-Akt and anti-ERK2.

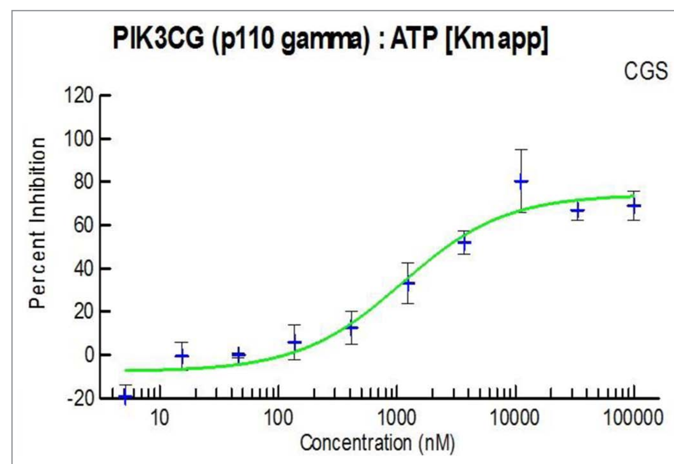

Figure S4. Analysis of CGS 15943 IC50 for p110γ as performed by Invitrogen-Life Technologies.

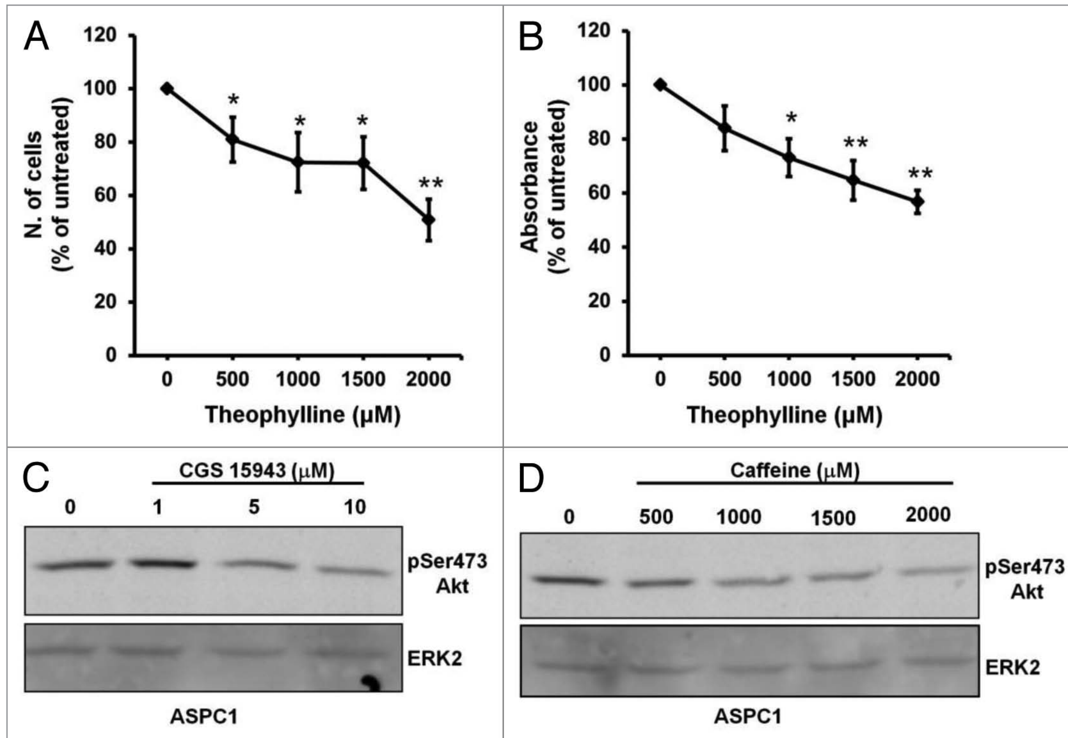

Figure S5. In vitro activity of methyl xanthines and CGS 15943 in ASPC1 cells. (**A and B**) ASPC1 cells were treated for 72 h with the indicated concentrations of theophylline in the presence of serum. Cell counting (**A**) and MTT assay (**B**) were performed to assess viability. Data are expressed as percentage of untreated cells and are means ± s.e.m. of $n = 5$ (**A**) and $n = 4$ (**B**) independent experiments. *$P < 0.05$, **$P < 0.01$ as assessed by the Student *t* test (paired, one-tailed distribution). (**C and D**) ASPC1 cells were treated for 24 h with the indicated concentrations of CGS 15943 (**C**) or caffeine (**D**) in the presence of serum. Akt activation was assessed by monitoring phosphorylation at its residues Ser473. Equal loading was assessed using an anti-ERK2 antibody.