

**Supplementary material**

**Resveratrol is a class IA phosphoinositide 3-kinase inhibitor**

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Running title: resveratrol inhibits PI 3-kinase

### Supplementary figure legends

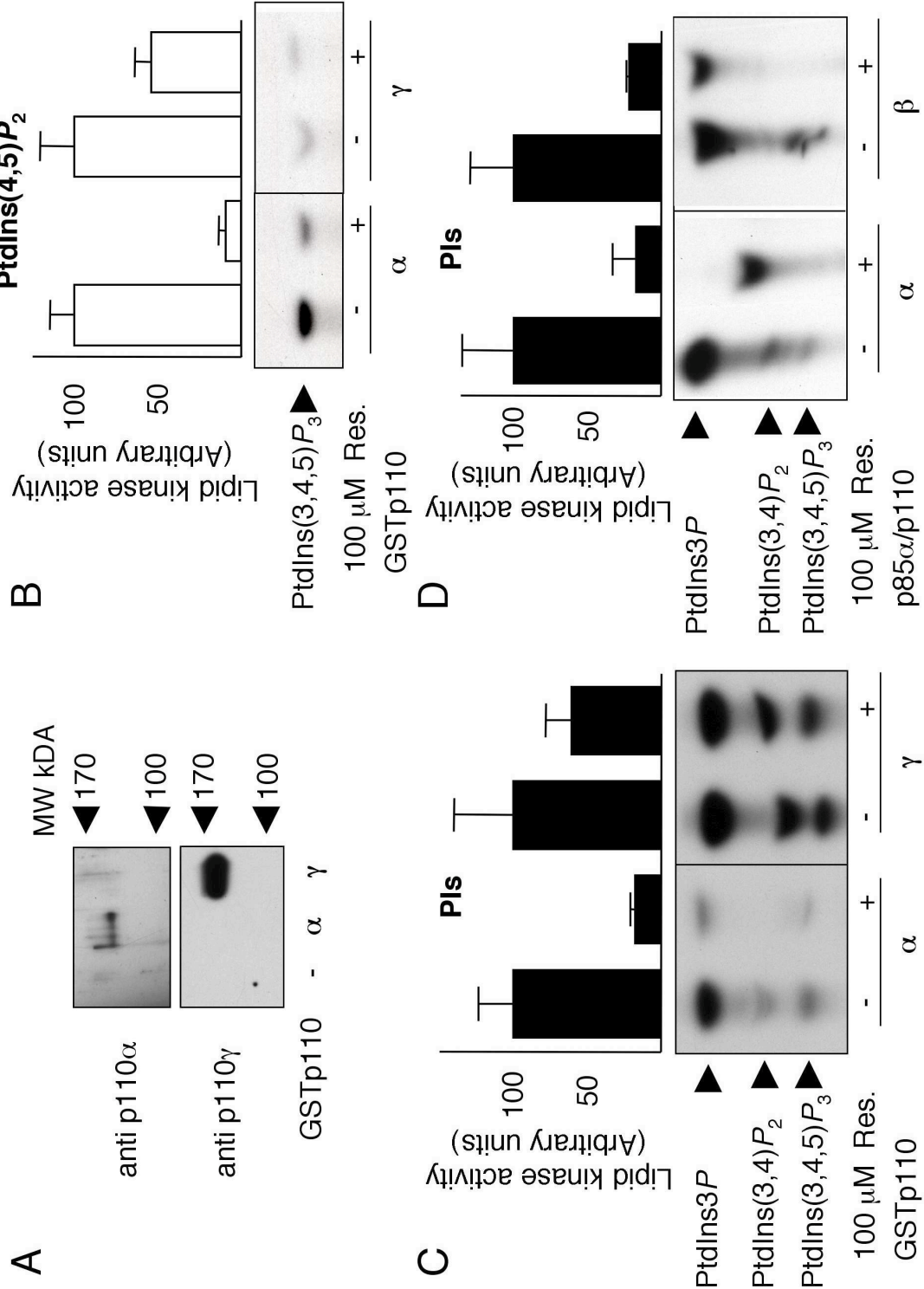
**Supplementary figure 1.** *Resveratrol inhibits recombinant class IA, but not class IB, PI3K lipid kinase activity.* (A) Overexpressed GST-p110 $\alpha$  and GST-p110 $\gamma$  were detected with isoform-specific antibodies. (B) Inhibition by resveratrol of class IA p85 $\alpha$ /GST-p110 $\alpha$ , and class IB GST-p110 $\gamma$  in lipid kinase assays using PtdIns(4,5)P<sub>2</sub> as substrate. (C) Inhibition by resveratrol of p85 $\alpha$ /GST-p110 $\alpha$ , and class IB GST-p110 $\gamma$  in lipid kinase assays using PIs as substrate. (D) Inhibition by resveratrol of p85 $\alpha$ /p110 $\alpha$  and p85 $\alpha$ /p110 $\beta$  in lipid kinase assays using PIs as substrate. For B, C, D, representative thin layer chromatograms are shown on the bottom and assays for each experimental condition were performed at least three times, activity in the absence of resveratrol was normalised at 100%. In all diagrams, resveratrol-inhibited activities are significantly different from the reactions carried out in the absence of inhibitor (unpaired Student's t test, p<0.05) with GST-p110 $\gamma$  retaining a >50% activity and only 10-20% in the case of class IA PI3Ks.

**Supplementary figure 2.** *Resveratrol inhibits recombinant class IA, but not class IB, PI3K protein kinase activity.* (A) Gels used for autoradiograms as in figure 5 were stained with coomassie blue to visualise p85 $\alpha$ /GST-p110 $\alpha$  (left gel) and GST-p110 $\gamma$  (right gel). (B) Inhibition of p85 $\alpha$ /p110 $\alpha$  and p85 $\alpha$ /p110 $\beta$  autophosphorylation activities by 100  $\mu$ M resveratrol (R) and 50  $\mu$ M LY294002 (LY). As previously reported, p85 $\alpha$ /p110 $\alpha$  autophosphorylates on the p110 $\alpha$  subunit [1], while p85 $\alpha$ /p110 $\beta$  autophosphorylation takes place on both subunits [2]. % activity refers to the incorporated radioactivity into p85 $\alpha$  (for p85 $\alpha$ /p110 $\alpha$ ) or the incorporated radioactivity into p85 $\alpha$  and p110 $\beta$  (for p85 $\alpha$ /p110 $\beta$ ) relative to the autophosphorylation reaction without inhibitor

### References to supplementary material

- 1 Dhand, R., Hiles, I., Panayotou, G., Roche, S., Fry, M. J., Gout, I., Totty, N. F., Truong, O., Vicendo, P., Yonezawa, K. and et al. (1994) PI 3-kinase is a dual specificity enzyme: autoregulation by an intrinsic protein-serine kinase activity. *Embo J* **13**, 522-33.
- 2 Czupalla, C., Culo, M., Muller, E. C., Brock, C., Reusch, H. P., Spicher, K., Krause, E. and Nurnberg, B. (2003) Identification and characterization of the autophosphorylation sites of phosphoinositide 3-kinase isoforms beta and gamma. *J Biol Chem* **278**, 11536-45.

# Supplementary figure 1



# Supplementary figure 2

