Fig. S1. Changes in signalling in response to AZD8055 and Rapamycin.

(A) AZD8055 fully inhibits mTOR signalling in resistant (CO115) and sensitive (COLO205) cell lines. CO115 and COLO205 cells were treated with increasing doses of AZD8055 for 24 hours. Whole cell lysates were fractionated by SDS-PAGE and immunoblotted with the indicated antibodies

(B) AZD8055 and Rapamycin causes strong dephosphorylation of 4EBP1 in SW620 cells. SW620 cells were treated for 1, 2 or 4 hours with 1μM AZD8055 or 1μM Rapamycin. Whole cell lysates were fractionated by SDS-PAGE and immunoblotted with the indicated antibodies.

In each case results are taken from a single experiment; identical results were obtained in n=3 experiments.

Fig. S2. Validation of pRL-IRES-FL as a reporter for cap-dependent translation.

(A-C) SW620 cells were transfected with a dual renilla/firefly luciferase construct, pRL-IRES-FL, together with either an empty vector control (EV), HA-eIF4E (eIF4E) or 4EBP1 R13A/F113A-Myc/His ($4EBP1^{AA}$). Lysates were prepared 24hr later and either used in a dual luciferase assay **(A & B)** or subjected to SDS-PAGE and immunoblotted with the indicated antibodies **(C)**. In **(A)** both the separate cap-dependent Renilla and IRES-dependent Firefly values are shown whilst in (B) the cap/IRES ratio is shown.

(D) Parental SW620 or SW620:8055R cells were left untreated or treated with 2μM AZD8055 for 24 hours. Samples were prepared and the abundance of the firefly and renilla mRNAs was assessed by (q)PCR and normalized to YWHA, a stable mRNA control (left panels). In parallel, cells treated identically were assayed for firefly and renilla using the duaa luciferase assay. Results are taken from a single experiment; identical results were obtained in n=3 experiments

Fig. S3. AZD8055-resistant SW620 cells are cross-resistant to other mTOR kinase inhibitors but not to cytoxic chemotherapy drugs.

SW620 and SW620:8055R cells were treated with increasing concentrations of (A) PP242, (B) WYE-125132, (C) Doxorubicin, (D) Paclitaxel or (E) Etoposide for 24 hours and cell proliferation was assayed by [^{3}H]thymidine incorporation. Results are the mean±CoV for three biological replicates from a single experiment; identical results were obtained in n=3 experiments.

Fig. S4. Response of AZD8055-resistant SW620 cells to a mixed PI3K/mTOR inhibitor (PI-103), a selective PI3K inhibitor (ZSTK474) or Rapamycin.

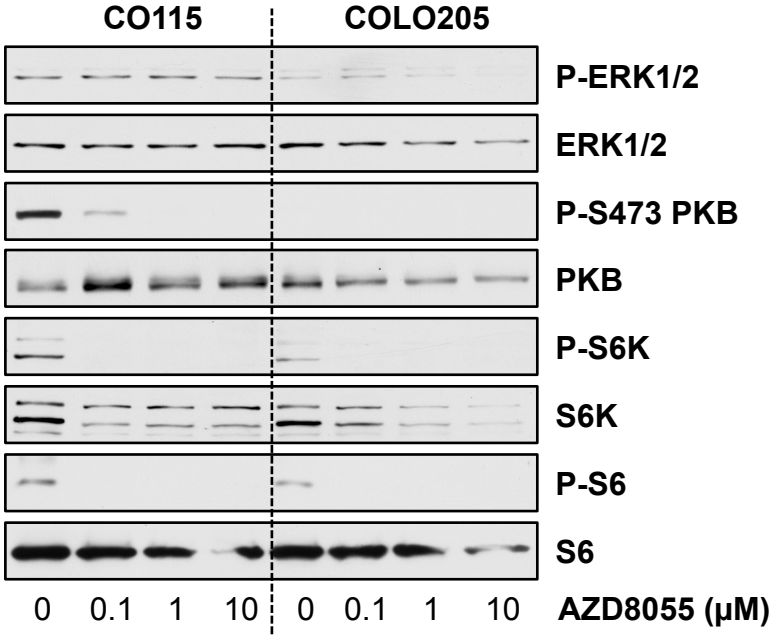
SW620 and SW620:8055R cells were treated with increasing concentrations of (A) PI103, (B) ZSTK474 or (C) Rapamycin for 24 hours and cell proliferation was assayed by [^{3}H]thymidine incorporation. Results are the mean±CoV for three biological replicates from a single experiment; identical results were obtained in n=3 experiments.

Fig. S5. AZD8055-dependent loss of cyclin D1 (CCND1) is rescued by eIF4E expression.

HEK293 TR cells, HEK293 TO-eIF4E cells or HEK293 TO-4EBP1^{AA} cells were treated over time course of 6 days with tetracycline to induce the expression of eIF4E (TO-eIF4E) or 4EBP1 R13A/F113A (TO-4EBP1^{AA}). Cells were then either treated with DMSO or AZD8055 for the final 24hrs and cell lysates were prepared, fractionated by SDS-PAGE and immunoblotted for the indicated antibodies (CCND1 = cyclin D1).

Supp Figure 1

A



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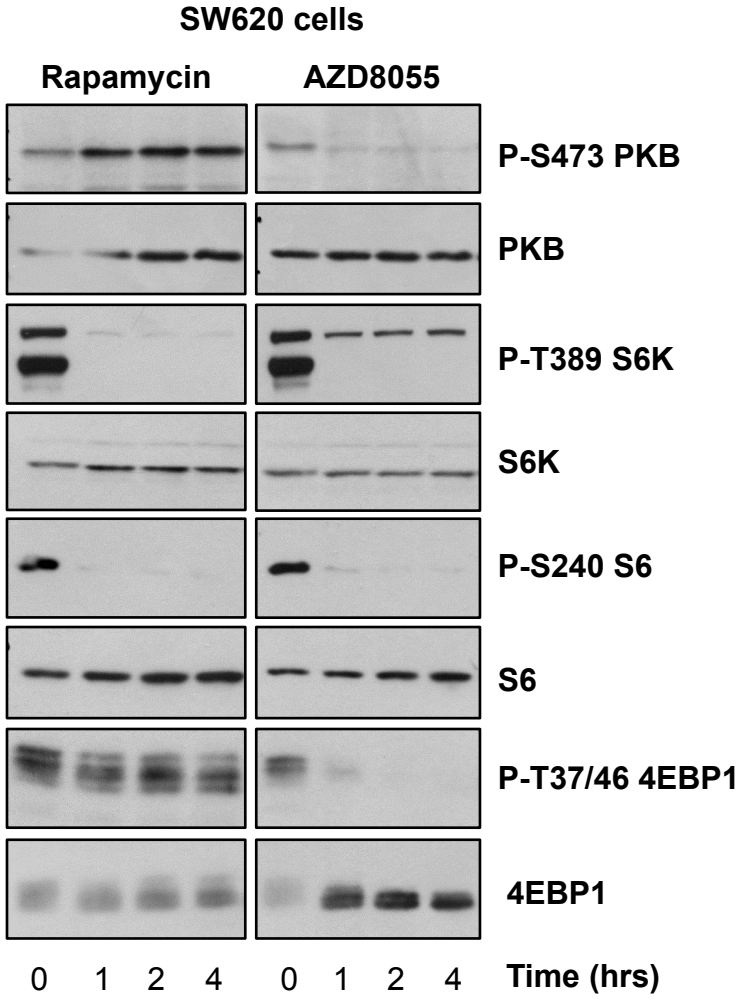
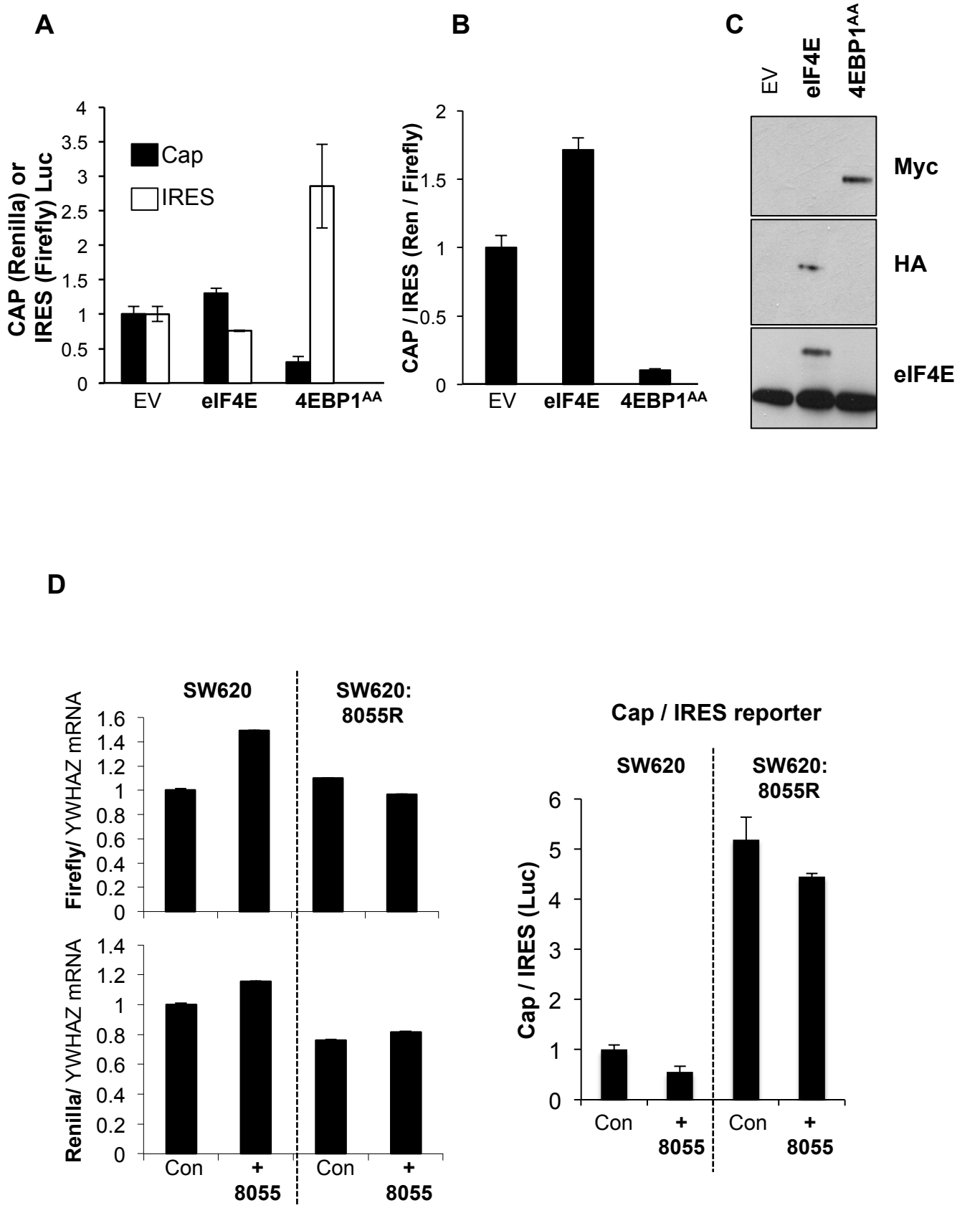
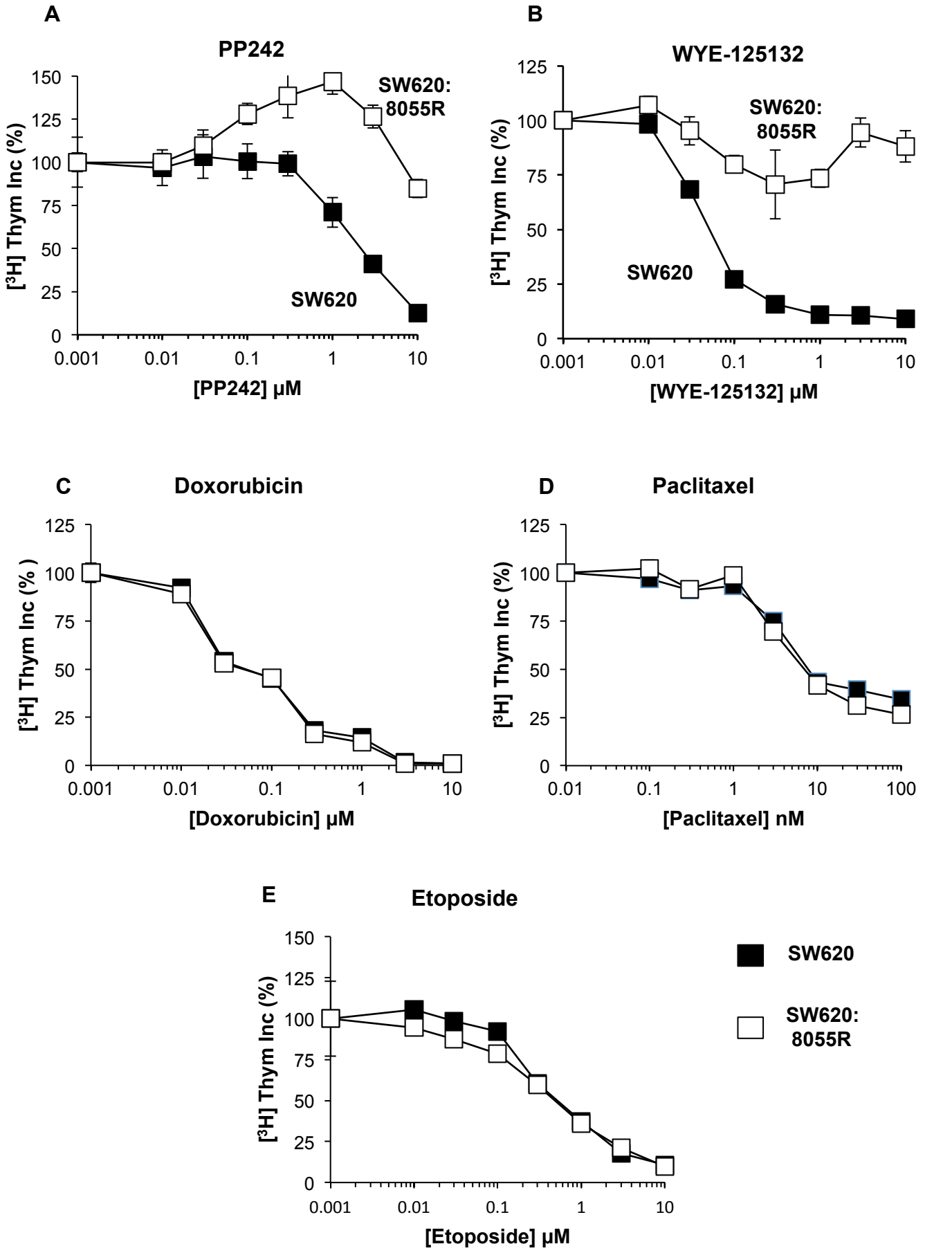


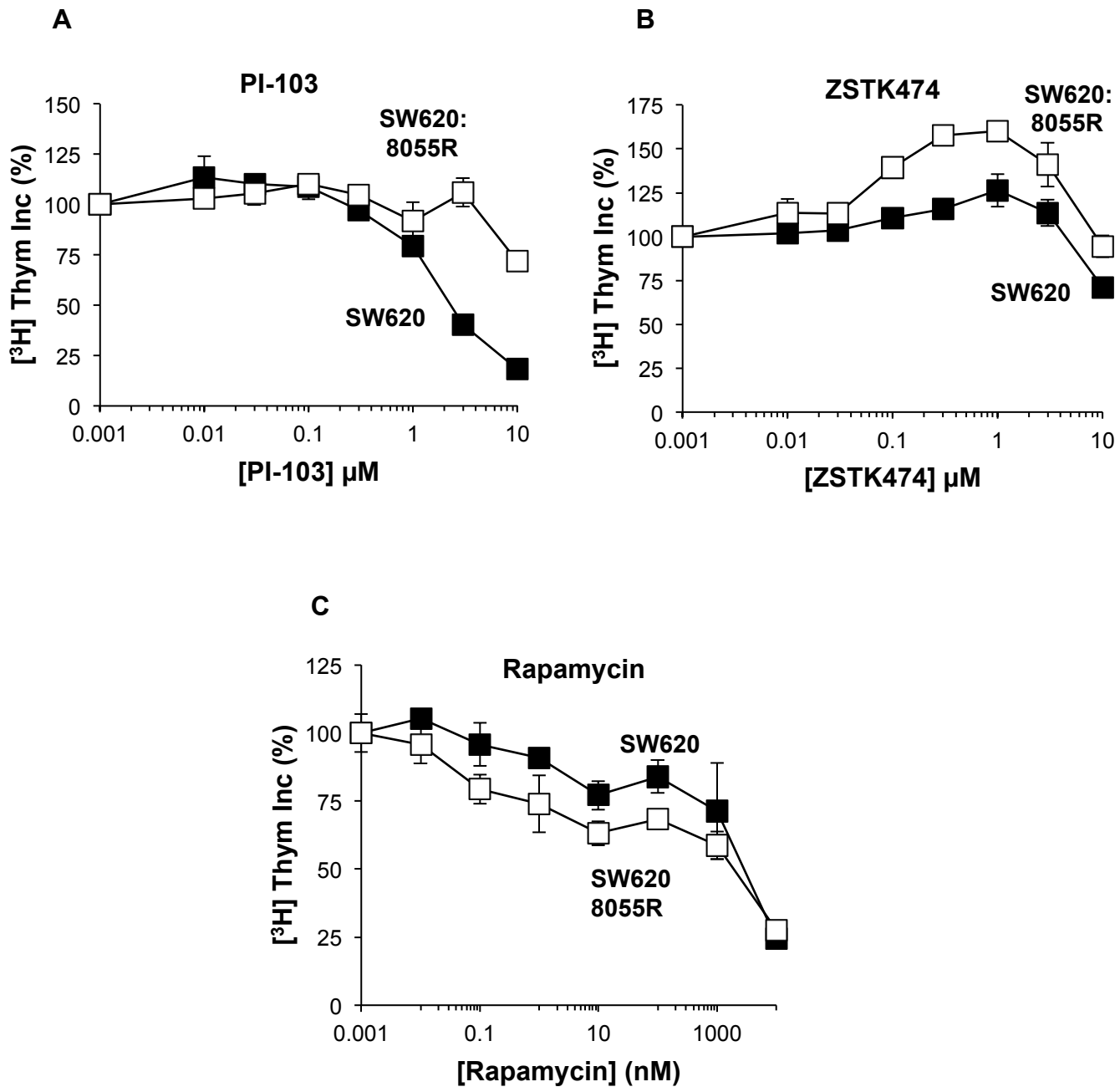
Supp Figure 2



Supp Figure 3



Supp Figure 4



Supp Figure 5