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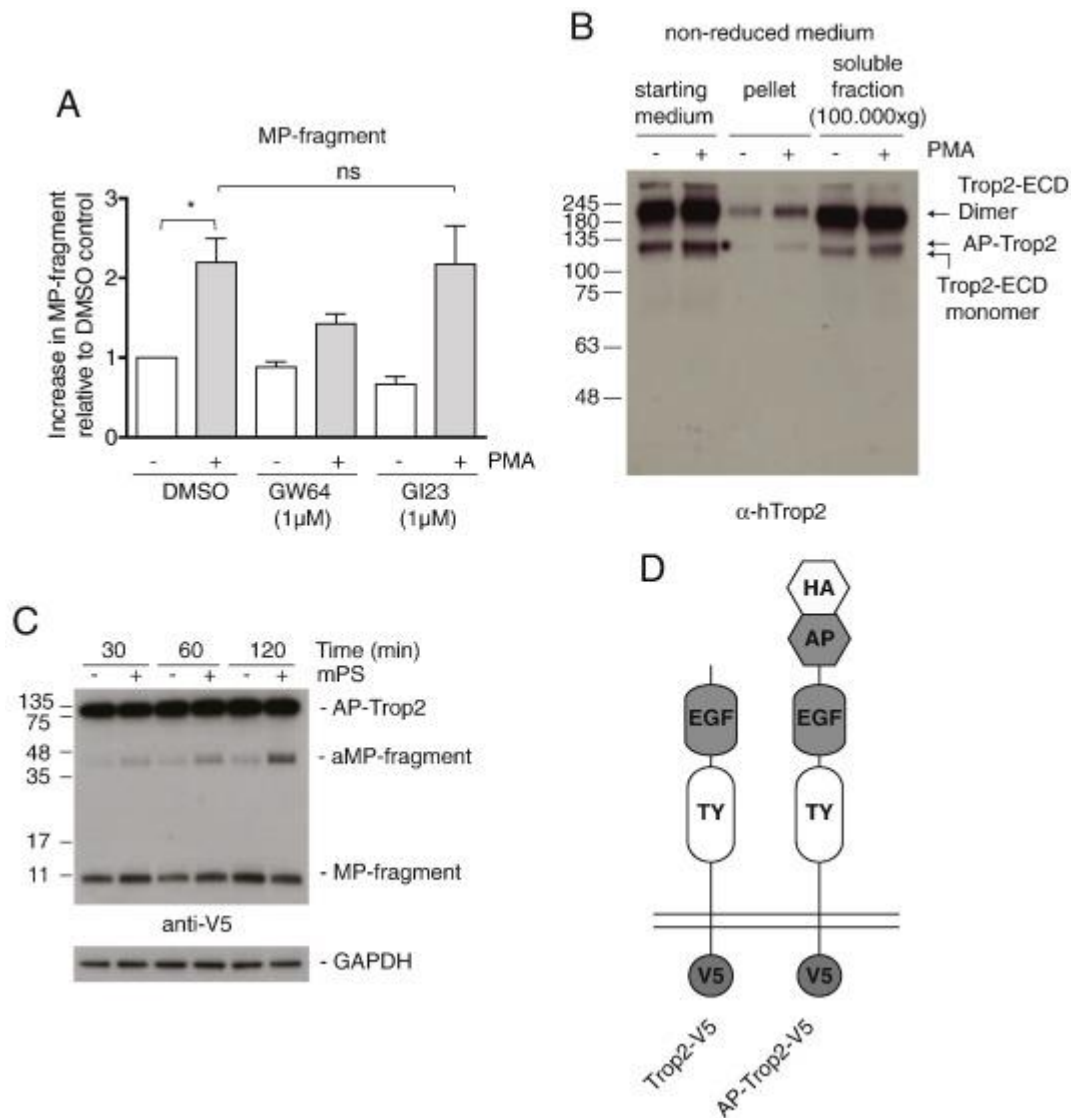


Fig. S1. TROP2 expression constructs and analysis of TROP2 levels using TROP2 antibody in non-reducing conditions.

(A) Increase in 11 kDa MP fragment in cell lysates in the presence or absence of 1  $\mu$ M GW64 or GI23. Data were normalised to GAPDH loading control. N = 3 blots. (B) Analysis of AP-TROP2 levels in medium following ultracentrifugation using anti TROP2 antibody shows that AP-TROP2-ECD dimers are enriched in the soluble fraction. (C) Analysis of aMP fragment production in response to mPS treatment over time from the shedding data presented in [Fig. 4A](#). by Western blotting using V5 antibody. (D) Schematic representation of TROP2 and AP-TROP2 expression constructs.

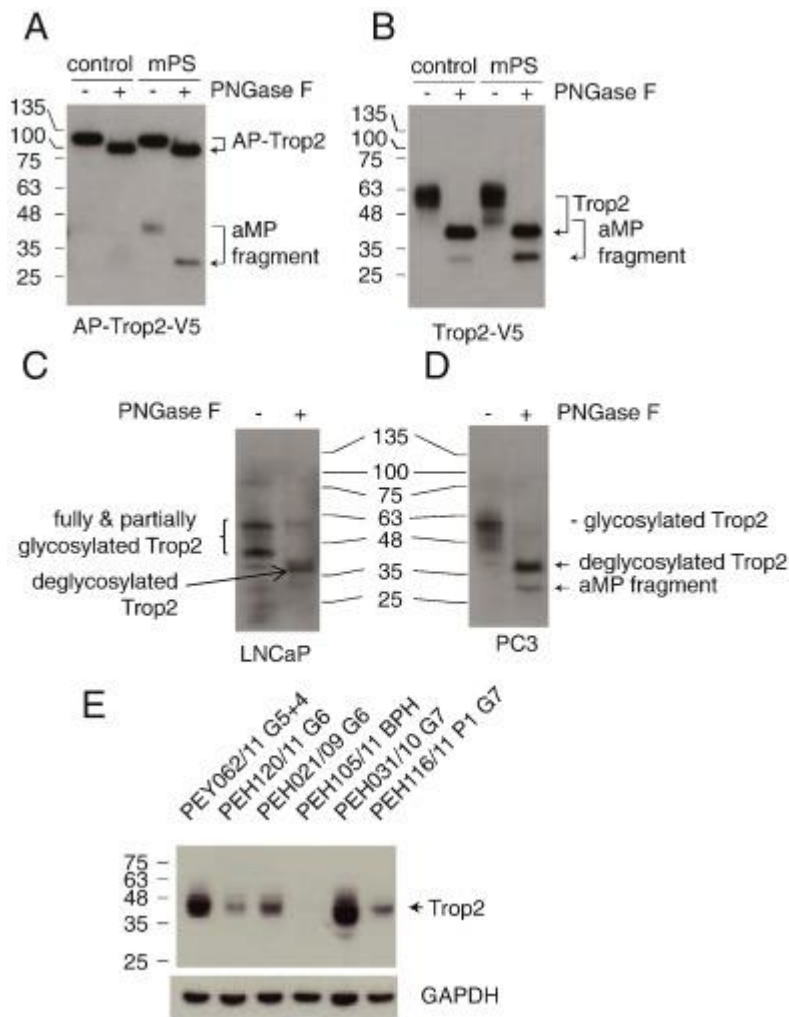


Fig. S2. Comparison of aMP-fragments in AP-TROP2, TROP2-V5 and PC3 cells following deglycosylation.

(A) AP-TROP2 cells were stimulated with mPS for 3 h prior to deglycosylation with PNGase F and analysed with V5-epitope antibody for the aMP fragment. Deglycosylation of the aMP fragment reduces its molecular mass to 32 kDa. (B) The same experiment described in A but performed with TROP2-V5 expressing cells shows induction of aMP fragment formation with mPS, showing an identical molecular weight after deglycosylation with PNGaseF. (C) LNCaP cell lysates contain fully and partially glycosylated TROP2 but lack the aMP fragment. (D) PC3 cell lysates are positive for aMP TROP2 fragment. (E) TROP2 is expressed in prostate cancer stem cells but not in benign hyperplasia (BPH sample). Samples in this blot represent glycosylated TROP2. Gleason score is indicated above and GAPDH loading control is shown below.