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Supplemental Data

c-Met Inhibitor Synergizes with Tumor Necrosis Factor–Related Apoptosis-Induced Ligand to Induce Papillary Thyroid Carcinoma Cell Death

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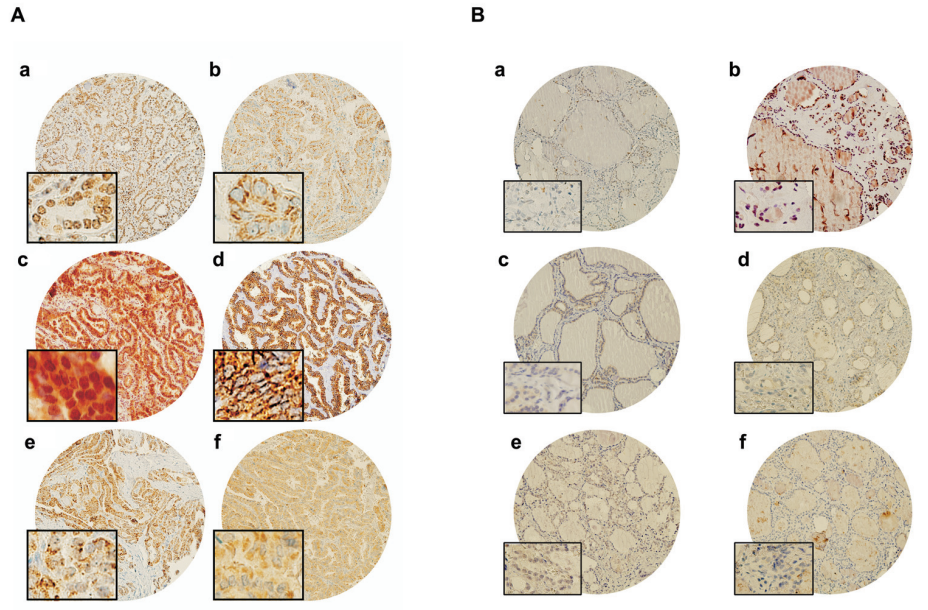
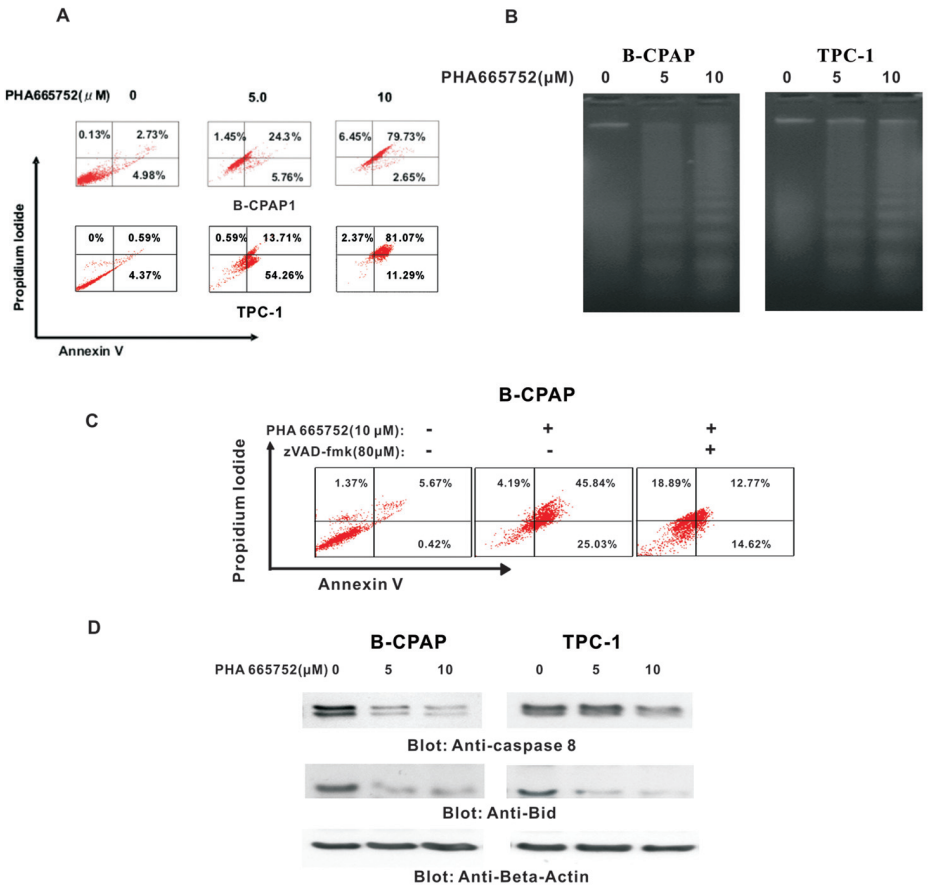
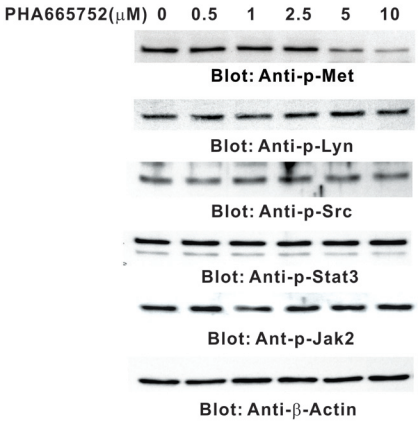


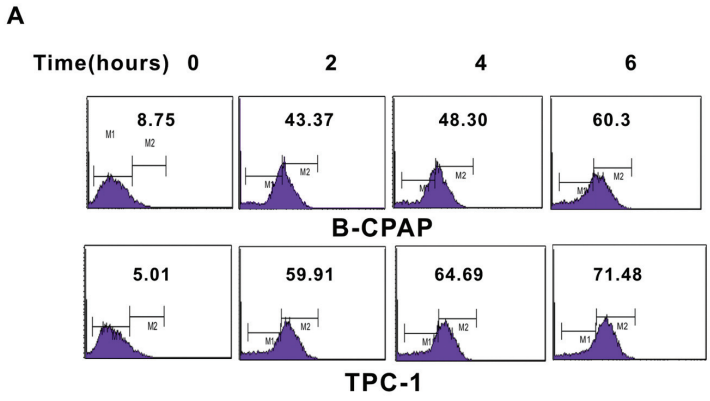
Supplementary Figure 1. Tissue microarray based immunohistochemical analysis of p-Met, TRAIL-R2 or DR5, p-AKT, BCL-XL, XIAP and HGF in PTC patients neoplastic thyroid tissue and non-neoplastic normal thyroid tissue. A: The Array spot showing high expression of p-Met (a) DR5 (b), p-AKT(c), Bcl-XL (d), XIAP (e) and HGF (f) in in PTC patients neoplastic thyroid tissue. B: The Array spot showing lower expression level of p-Met (a) DR5 (b), p-AKT(c), Bcl-XL (d), XIAP (e) and HGF in non-neoplastic normal thyroid tissue. 20 X/0.70 objective on an Olympus BX 51 microscope. (Olympus America Inc, Center Valley, PA, USA. with the inset showing a 40X 0.85 aperture magnified view of the same.

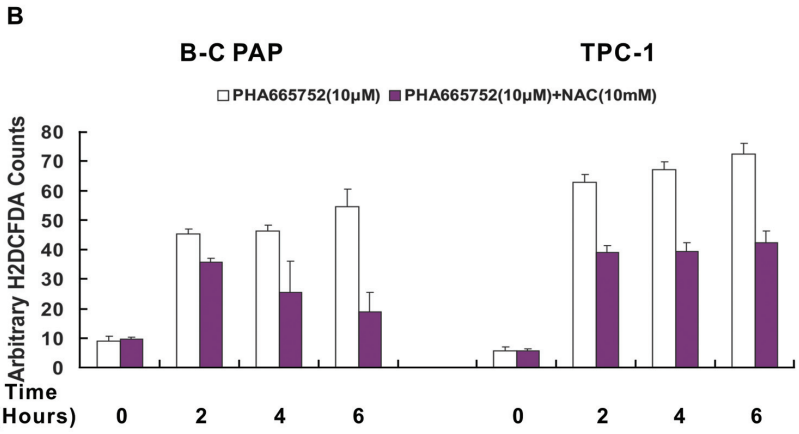


Supplementary Figure 2. A: PHA 665752-induced apoptosis detected by Annexin V/propidium iodide dual staining. B-CPAP and TPC-1 cells were treated with various doses of PHA 665752 (as indicated) for 24 h and cells were subsequentlt stained with fluorescein-conjugated Annexin V and propidium iodide and analyzed by flow cytometry. B: PHA 665752-induced apoptosis detected by DNA laddering. B-CPAP and TPC-1 cells were treated with PHA 665752 as indicated for 24 h, and DNA was extracted and separated by electrophoresis on 1.2% agarose gel. C: Effect of zVAD/fmk on PHA665752-induced apoptosis detected by Annexin V/propidium iodide dual staining. B-CPAP cells were pretreated with 80uM zVAD/fmk for 2 h and subsequently treated with 10 uM PHA665752 for 24 h, and then cell were stained with fluorescein-conjugated Annexin V and propidium iodide and analyzed by flow cytometry. D: PHA665752-induced activation of caspase 8 and cleavage of Bid. B-CPAP and TPC-1 cells were treated with various doses of PHA665752 as indicated for 24 h. Cells were lysed and equal amounts of proteins were separated by SDS-PAGE, transferred to PVDF membrane and immunoblotted with antibodies against caspase 8 and Bid. The blots were probed with an antibody against beta-actin for equal loading.

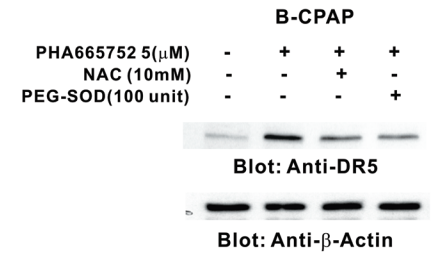


Supplementary Figure 3. PHA665752 treatment did not affect phosphorylaton of Lyn, stat3, Jak2 and Src. B-CPAP cells were treated with 0, 0.5, 1. 2.5, 5 and 10 μM PHA665752 for 4 h. After cell lysis, equal amounts of proteins were separated by SDS-PAGE, transferred to immobilon membrane, and immunoblotted with antibodies against p-Met, p-Lyn, p-stat3, p-Jak2, p-Src and β-actin.





Supplementary Figure 4. A: Increase of ROS generation in PHA665752 treated PTC cells. B-CPAP and TPC-1 cells were treated with PHA665752 10μM for 0, 2, 4 and 6 h respectively, then loaded with 10μM H2DCFDA and incubated at 37°C for 45 min. After washing with PBS, cells were re-suspended in PBS and immediately analyzed using flow cytometry for intracellular accumulation of ROS. B: B-CPAP and TPC-1 cells were pre-treated with 10mM NAC for two hours followed by treatment with 10μM PHA665752 for indicated time periods, then loaded with 10μM H2DCFDA and incubated at 37°C for 45 min. After washing with PBS, cells were re-suspended in PBS and immediately analyzed using flow cytometry for intracellular accumulation of ROS.



Supplementary Figure 5. B-CPAP cells were pre-treated with 10mM NAC and 100 units PEG-SOD for 2 h, followed by 5 uM PHA665752 treatment for 24 h. After cell lysis, equal amounts of proteins were separated by SDS-PAGE, transferred to immobilon membrane, and immunoblotted with antibodies against DR5 and β-actin.

Supplementary Table 1 Antibodies used for tissue microarray immunohistochemical analysis.

Antibody	Clone	Company	Source	Dilution*	Antigen Retrieval	Subcellular Localization	Detection System
p-MET	Polyclonal	Invitrogen	Rabbit	1:300	pH6, PC	Nuclear	EnVision+
DR5	TNFRSF10B	R&D	Goat	1:1000	pH6, PC	Cytoplasmic	EnVision+
HGF	Polyclonal	SCBT	Rabbit	1:1000	pH9, PC	Cytoplasmic	EnVision+
P110α	C73F8	Cell signalling	Rabbit	1:100	pH9, MW	Cytoplasmic	EnVision+
p-AKT	Ser 473	Cell signalling	Rabbit	Predilute	pH9, MW	Nuclear/Cytoplasmic	EnVision+
PTEN	6H2.1	Cascade	Mouse	1:300	pH9, MW	Cytoplasmic	Envision+
XIAP	48	BD Transduction	Mouse monoclonal	1:300	pH9, MW	Cytoplasmic	EnVision+
BCLXL	54H6	Cell Signalling	Rabbit polyclonal	1:800	pH9, MW	Cytoplasmic	EnVision+

*-Over night incubation
PC: Pressure cooker
MW: Microwave
P110α: Phosphatidylinositol 3 kinase catalytic subunit-110α