Supplemental Figures

Curcumin Inhibition of TGFβ signaling in bone metastatic breast cancer cells and the possible role of oxidative metabolites

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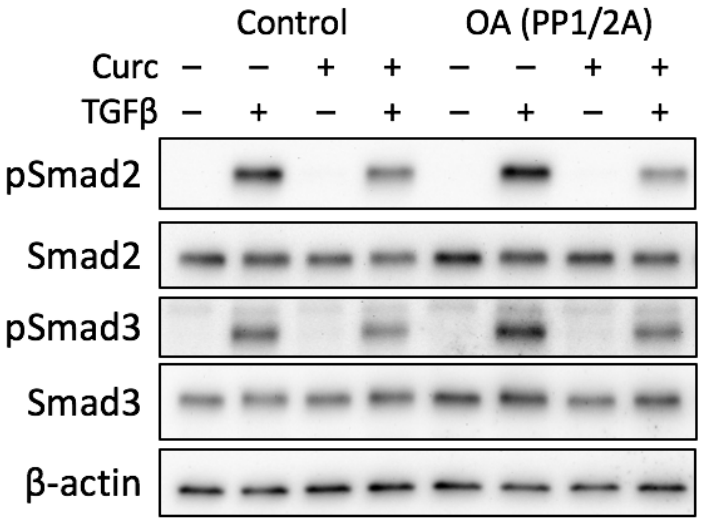

Figure S1: Effect of phosphatase inhibition on TGFβ-stimulated pSmad2/3 levels in control vs. curcumin-treated cells. Effect of okadaic acid pre-treatment (OA, 10 nM, 1 hour) on curcumin (30 μM, 16 h pre-treatment) inhibition of TGFβ-stimulated Smad2/3 phosphorylation in MDA-SA cells.

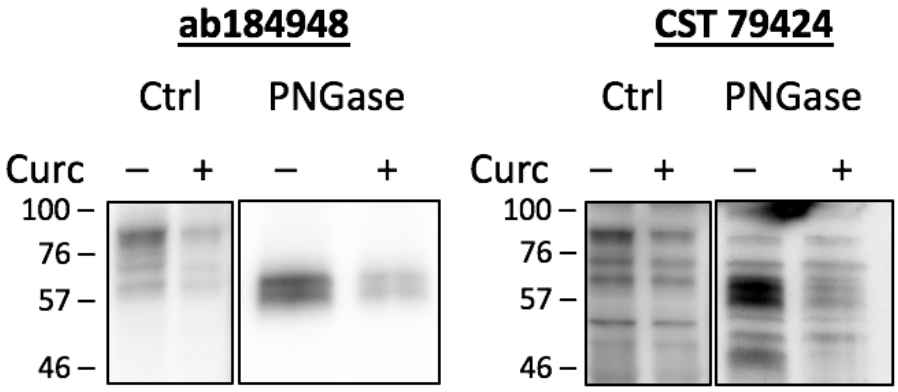

Figure S2. Western analysis of curcumin effects on TGFβR2. Effects of curcumin (30 μM for 4h) on TGFβR2 protein levels were determined by Western analysis using antibodies directed against two different TGFβR2 epitopes [80] (extracellular domain [ab184948], and intracellular domain [CST 79424]), including treatment of samples with PNGase F to document collapse of bands into an anticipated 60 kD protein [51] following removal of N-linked glycosylation.

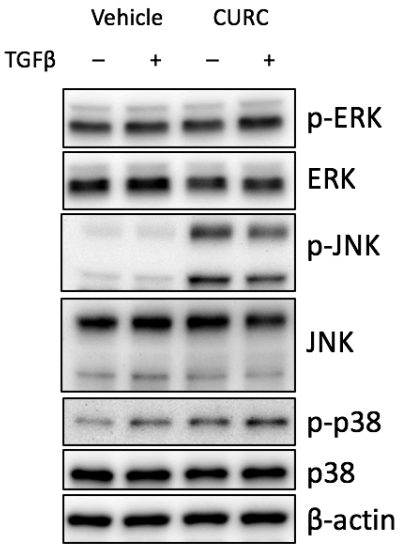

Figure S3. Curcumin effects on MAPK signaling pathways in MDA-SA cells. Western blot analysis of MDA-SA cells following curcumin treatment (30 μM, 16h pre-treatment) and/or TGFβ stimulation (1h). Representative of at least 3 experiments.

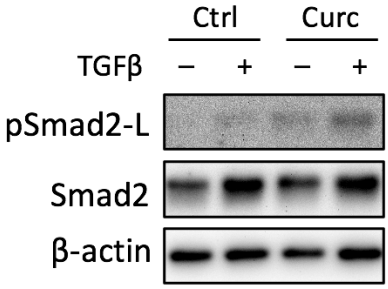

Figure S4: Early phosphorylation of Smad2 linker region. Western blot analysis of Smad2 linker region phosphorylation in MDA-SA cells following 4h of curcumin (30 μM) pretreatment followed by 1h of concurrent TGFβ. Representative of at least 3 experiments.

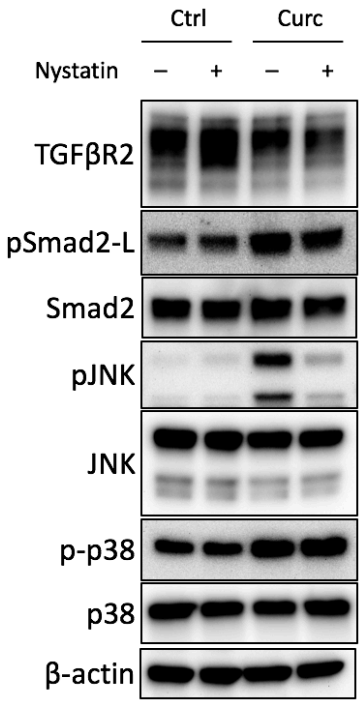

Figure S5. Nystatin increases TGFβR2 while inhibiting JNK activation and Smad2-L phosphorylation. MDA-SA cells were pre-treated with nystatin (50 μg/mL) for 1h followed by curcumin (30 μM) for 4 hours. Representative of n=2 blots.

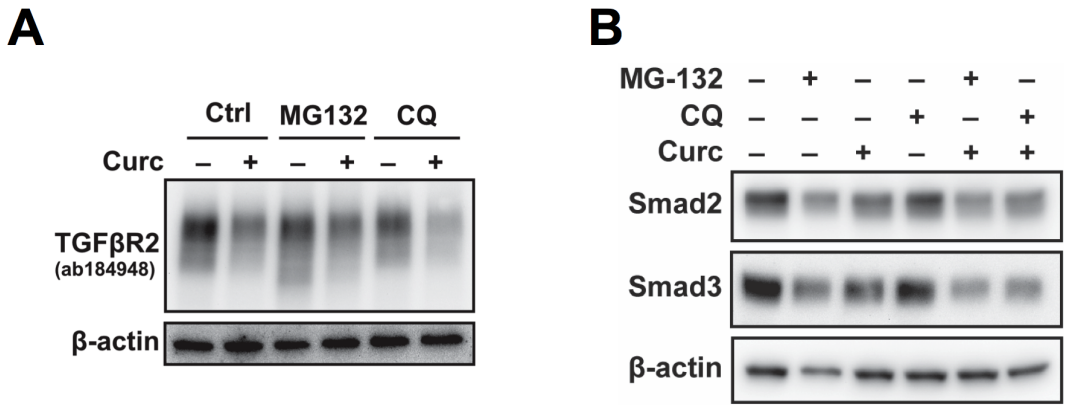

Figure S6. Effect of degradative pathway inhibitors on curcumin-induced decreases in TGFβR2 and R-Smads. Effect of lysosomal inhibitor chloroquine (CQ, 40 μM) or proteasomal inhibitor MG-132 (10 μM) on curcumin-mediated decreases in **A)** TGFβR2 (2 h pre-treatment, followed by 4h concurrent curcumin) or **B)** Smad2/3 protein levels (3h pre-treatment, followed by 16h concurrent curcumin).

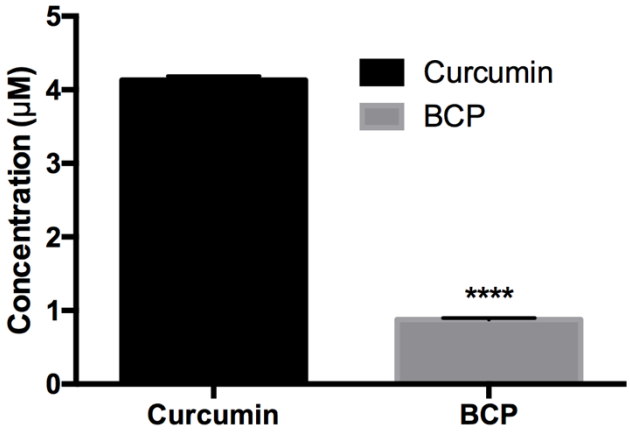

Figure S7: Oxidation of curcumin by MDA-SA cells. MDA-SA cells were incubated with aglycone curcumin (10 μM) for 4 hours at 37°C in media. Curcumin and its stable end product of oxidative metabolism, bicyclopentadione (BCP) were analyzed by LC/MS (n=8/group). Significance tested with a paired t-test. **** $p < 0.0001$.