

## Supplementary Materials

# Fluoroquinolones Suppress TGF- $\beta$ and PMA-Induced MMP-9 Production in Cancer Cells: Implications in Repurposing Quinolone Antibiotics for Cancer Treatment

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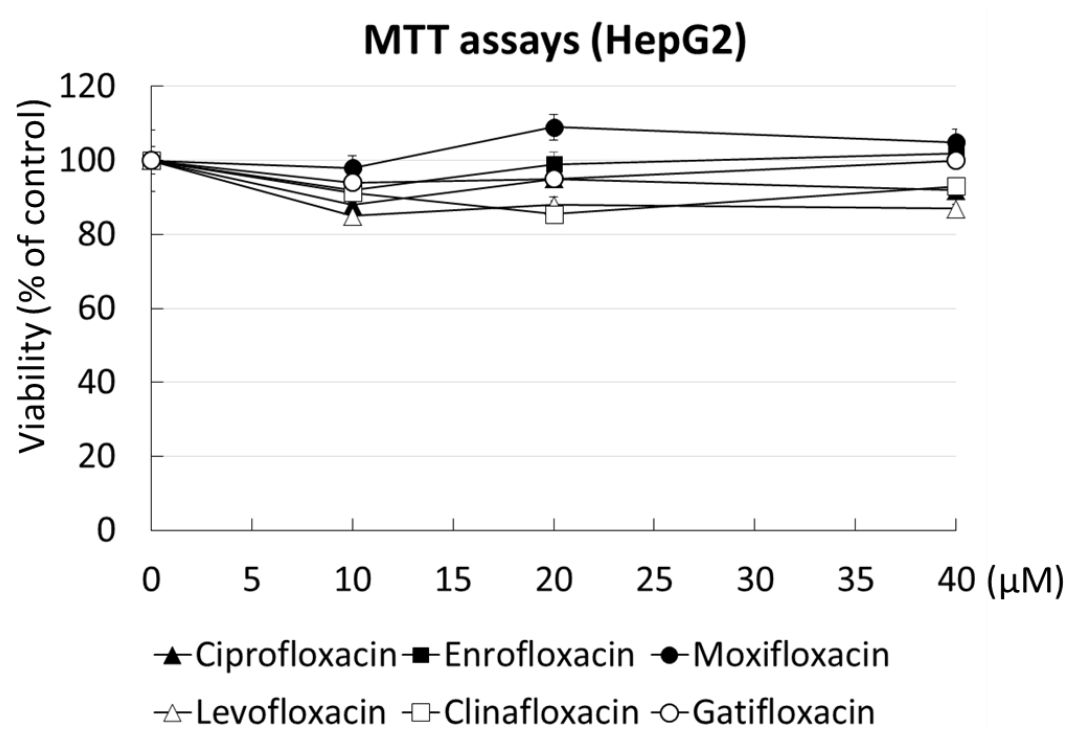
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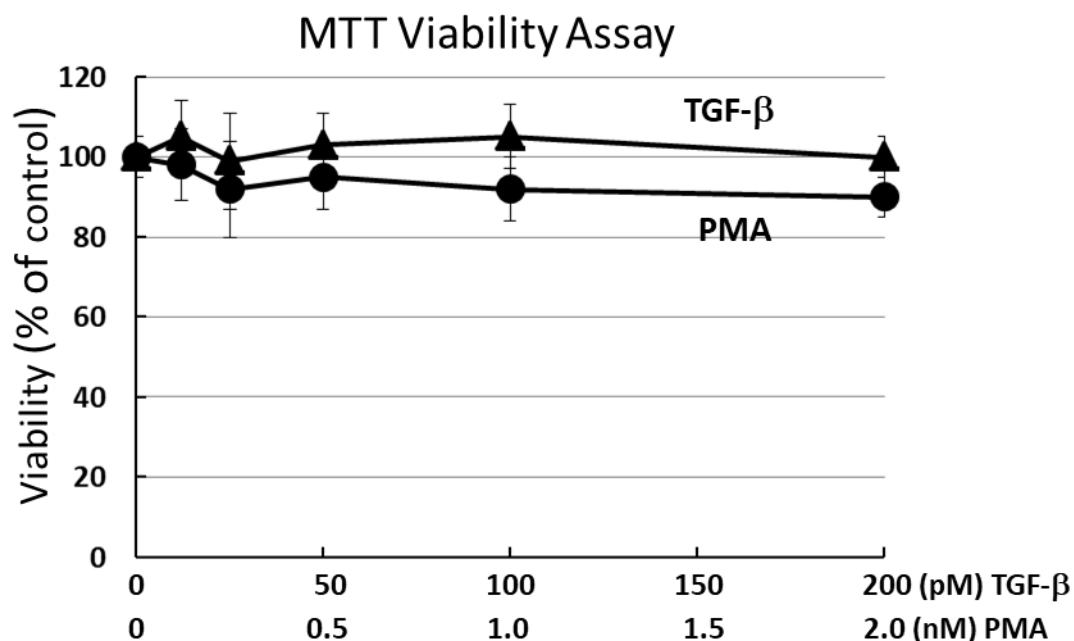
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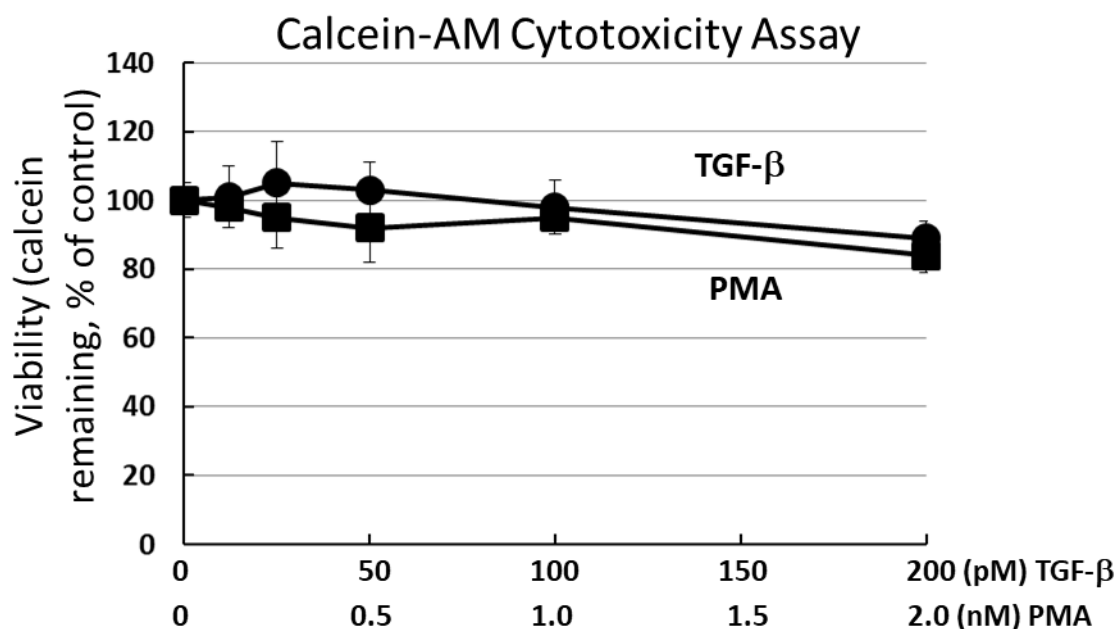
**Figure S1.** Cytotoxicity effects of fluoroquinolones in HepG2 cells.



**Figure S2.** Cytotoxicity effects of TGF- $\beta$  and PMA in A549 cells.

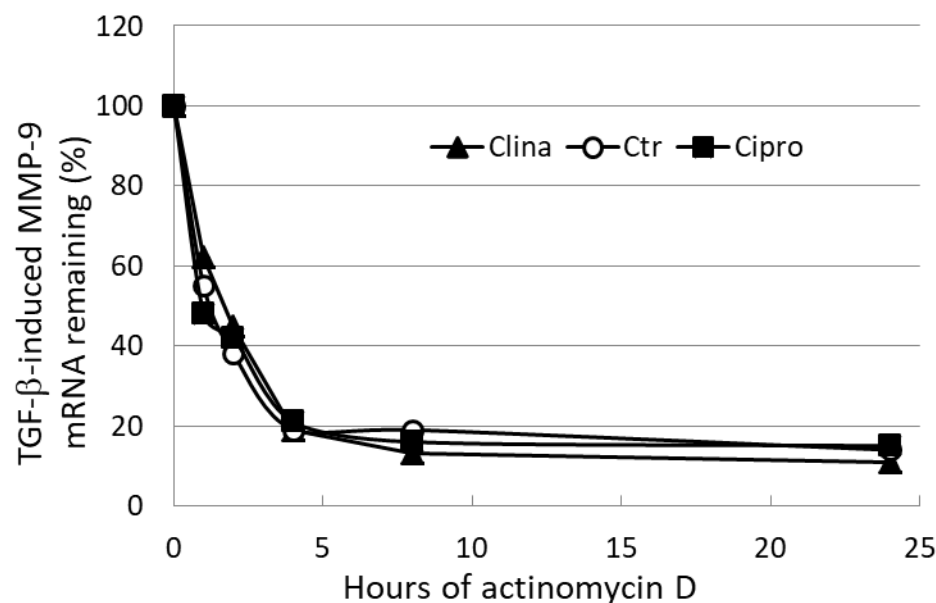


(A) HepG2 cells ( $1 \times 10^4$ /well in a 96-well plate) were treated with 0–200 pM of TGF- $\beta$  or 0–2nM of PMA for 48 h. Cell viability of cells was determined by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay. Each treatment group was normalized to each un-treated control. The data (mean  $\pm$  standard deviation, SD) are representative of three independent experiments; error bars indicate SD.



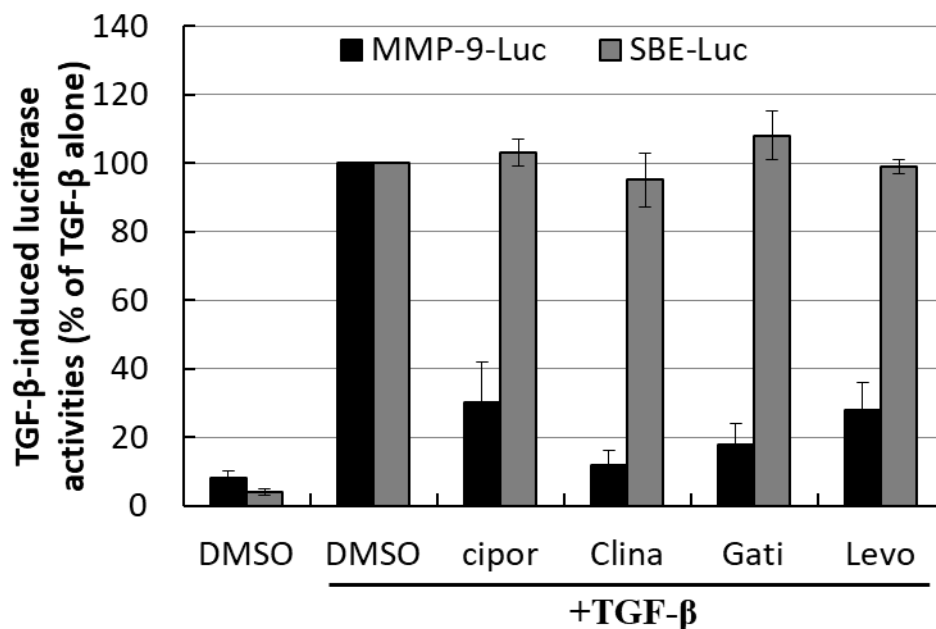
(B) Fluorometric assays on A549 cells. Dose-response relation to increasing concentrations of TGF- $\beta$  or PMA on calcein-AM loaded A549. Fluorescent signal was read 45 min after TGF- $\beta$  or PMA treatment. For each concentration, the fluorescence of eight wells was measured.

**Figure S3.** Effects of fluoroquinolones in stability of TGF- $\beta$ -stimulated MMP-9 mRNA in HepG2 cells.



Ciprofloxacin and clinafloxacin did not change TGF- $\beta$ -induced MMP-9 mRNA stability. mRNA degradation was quantified by real time RT-PCR at various times after addition of TGF- $\beta$  + actinomycin D, TGF- $\beta$  + actinomycin D + ciprofloxacin, or clinafloxacin. Addition of actinomycin D induced a rapid degradation of MMP-9 mRNA, which was not changed in the presence of ciprofloxacin, or clinafloxacin. Cells were stimulated with 200 pM TGF- $\beta$  for 24 h, medium was replaced, and 20  $\mu$ M ciprofloxacin, clinafloxacin, or DMSO as vehicle control were added for 1 h before actinomycin D chase.

**Figure S4.** Cytotoxicity effects of fluoroquinolones in HepG2 cells.



Ciprofloxacin, clinafloxacin, gatifloxacin, and levofloxacin blocked the activation of MMP-9 responsive element (MMP-9-Luc) without affecting TGF- $\beta$ -responsive elements activity (SBE4-Luc).



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