Supplementary material

Title: *Ganoderma lucidum* Combined with the EGFR Tyrosine Kinase Inhibitor, Erlotinib Synergize to Reduce Inflammatory Breast Cancer Progression

Supplementary Figures

Supplementary Figure S1

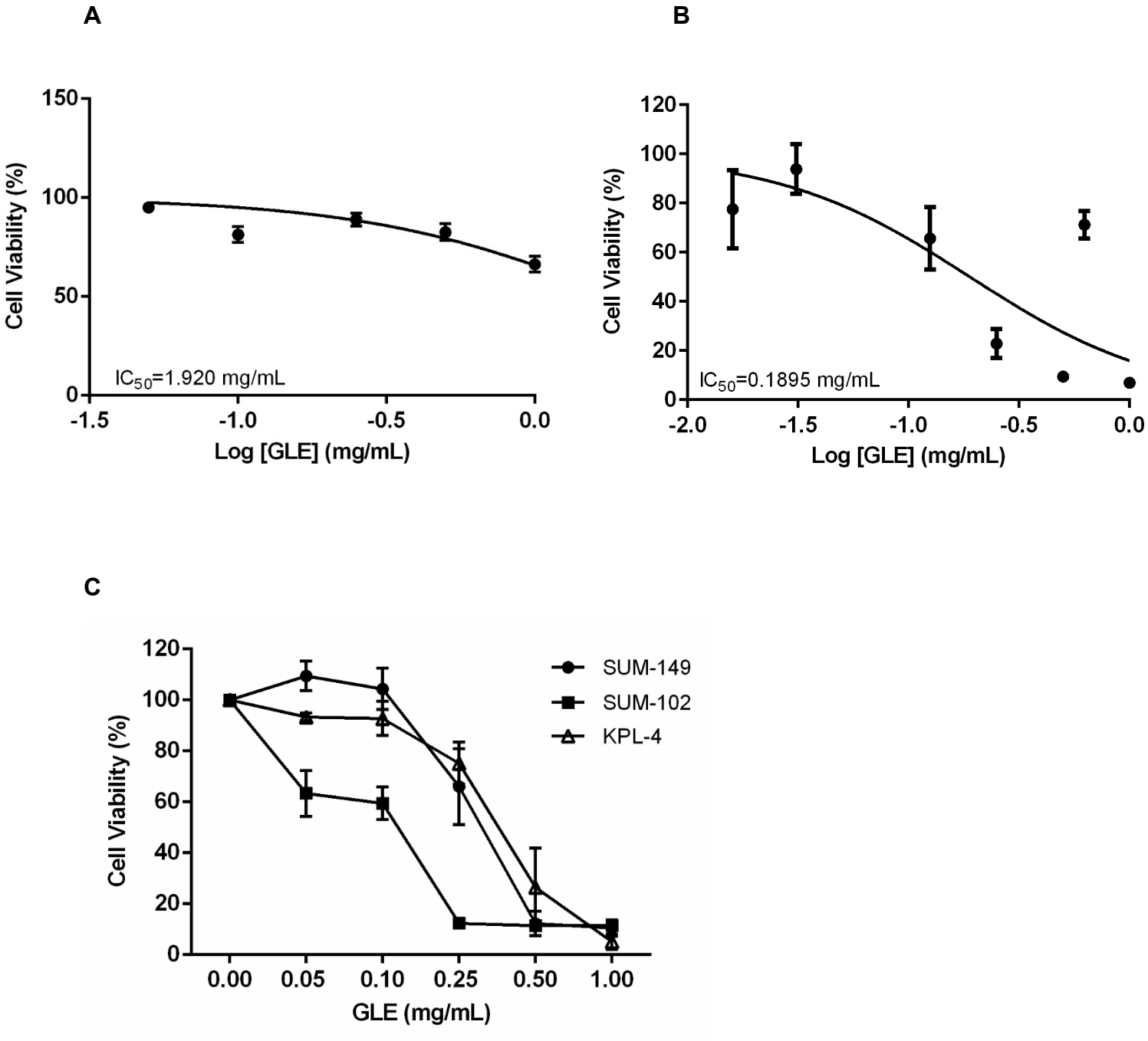
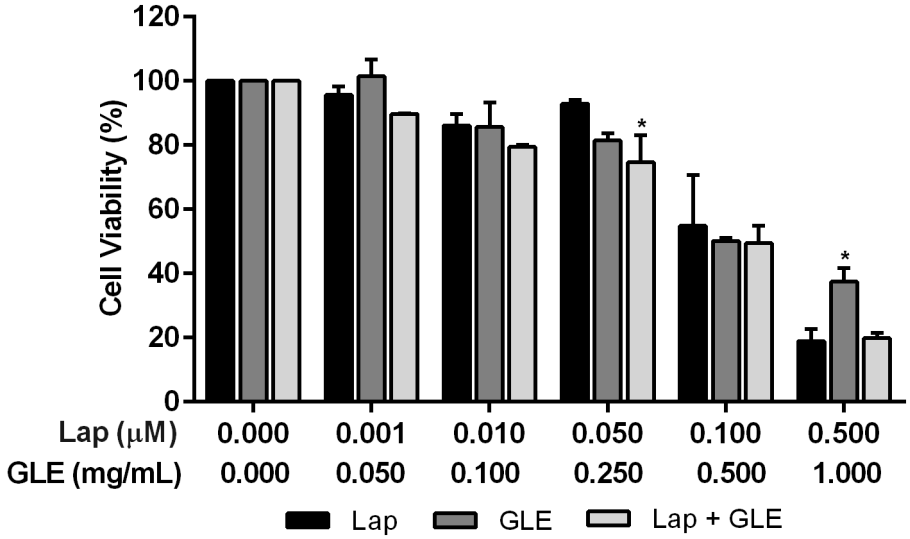


Fig S1. *GLE inhibits cell viability of HER-2 overexpressing breast cancer cells.* A, B**.** MDA-IBC-3 and KPL-4 cells were treated with increasing concentrations of GLE (0 - 1.5 mg/mL) for 72h. Viable cells were fixed with cold methanol and nuclei were stained with 0.4% PI. Cell viability was calculated as the percentage of surviving cells after treatment as measured by differences in fluorescence units between treated and untreated wells. IC_{50} was obtained from dose response curve fittings using the non-linear regression function of GraphPad Prism®. C. SUM-102, SUM-149 and KPL-4 cells were treated in duplicate for 72h with increasing concentrations of GLE ranging from (0 - 1.0 mg/mL) for washout experiments. The treatment was then removed and the cells were washed with sterile 1X PBS and incubated for an additional 72h with fresh media. After the incubation period assessment for viable cells was performed. All experiments were repeated at least three times.

Supplementary Figure S2

A



B

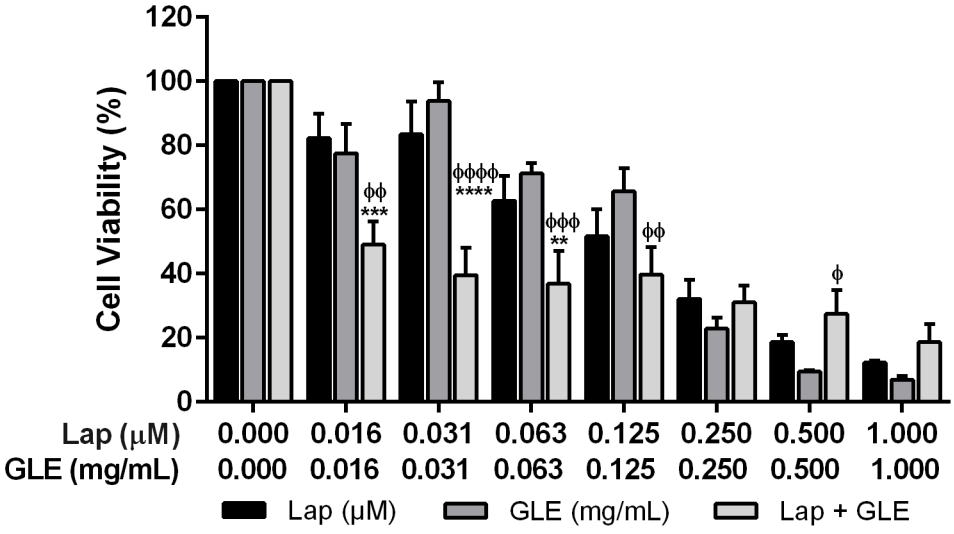


Fig. S2. *Effects of Lapatinib and/or GLE in HER-2 overexpressing breast cancer cells.* A, B. MDA-IBC-3 and KPL-4 cells were treated with increasing concentrations of Lapatinib (Lap), GLE or Lapatinib/GLE for 72h. Cell viability was calculated as the percentage of surviving cells after treatment as measured by differences in fluorescence units between treated and untreated wells. Significance against: (*) Lapatinib, (ϕ) GLE. All experiments were repeated at least three times. $P \leq 0.05$.

Supplementary Figure S3

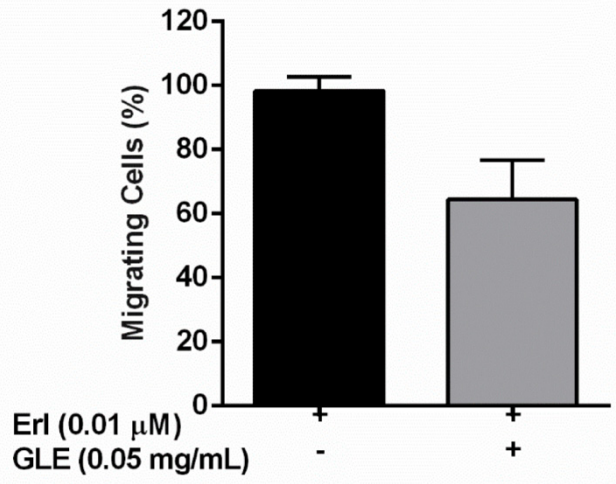


Fig. S3. *Effects of lower doses of an Erlotinib/GLE combination in motility capacity of SUM-149 cells.* Cell migration capacity of SUM-149 cells was measured using FluoroBlok™ Cell Culture Inserts. SUM-149 cells were seeded in duplicate into the top chambers with serum free medium and treated with 0.01 μM Erlotinib (Erl), or the combination with 0.05 mg/mLGLE. Cells were allowed to migrate toward 10% FBS medium. After 72h, cells on the surface were removed with a cotton swab. The cells attached to the bottom surface of the membrane were fixed in cold methanol and stained with 0.4% PI. Ten micrographs were captured per transwell, and migrating cells were quantified using ImageJ software (NIH). Data were calculated as the percentage of migrating cells after treatment. All experiments were repeated at least three times.