Supplementary Figure

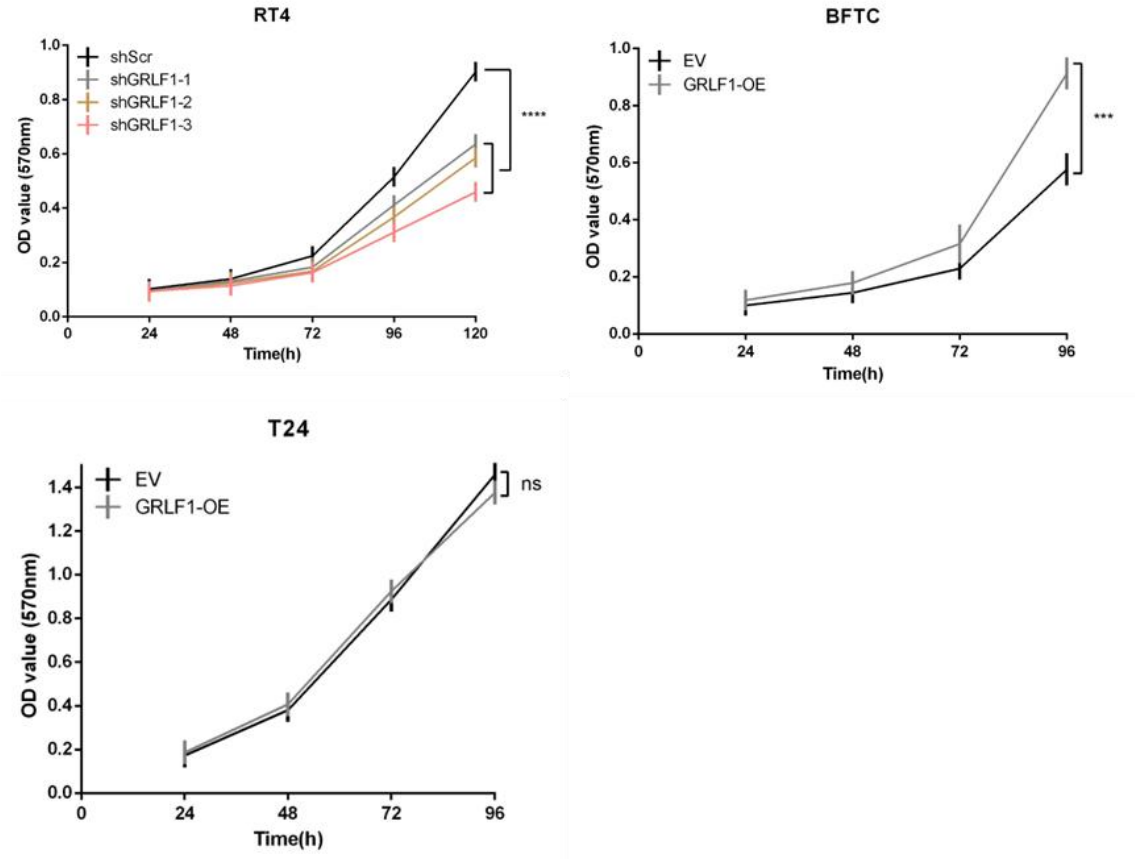

Figure S1. GRLF1 influences cell proliferation of bladder cancer in MTT assay. Cell proliferation is decreased in RT4 cells with GRLF1 knockdown compared to scramble control (shScr) and increased in BFTC cells with GRLF1 overexpression (OE) compared to empty control (EV). Cell proliferation in T24 EV and GRLF1 OE cells shows no significant difference. Data are presented as mean ± SD (n=3, ns: $P > 0.05$, *** $p < 0.001$, **** $p < 0.0001$, Two-Way ANOVA).

Method

MTT assay

Cell viability was quantified using an MTT assay. Cells were digested and transferred into 15 ml sterile tubes. Count cell number three times and dilute 100,000 cells in 10 ml of culture medium. Mix the cell suspension thoroughly and seed the cells 100 μl per well into a 96-well plate with 6 replicates. The cells were repeatedly seeded in 5 plates, and cell viability was detected at 24, 48, 72, 96, and 120-hour time points. Add 20 μl of 5 mg/ml MTT solution to each well for detection. One set of wells was included with MTT but without cells (control). Incubate the plate at 37°C for 4 hours. After that, aspirate the medium carefully and add 100 μl MTT lysis buffer into each well. Wrap the plate in foil and shake on an orbital shaker for 10 minutes. The absorption of samples was measured at 590 nm wavelength in a Spectrophotometer.