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Supplemental information

Addition of BTK inhibitor orelabrutinib to rituximab improved anti-tumor effects in B cell lymphoma

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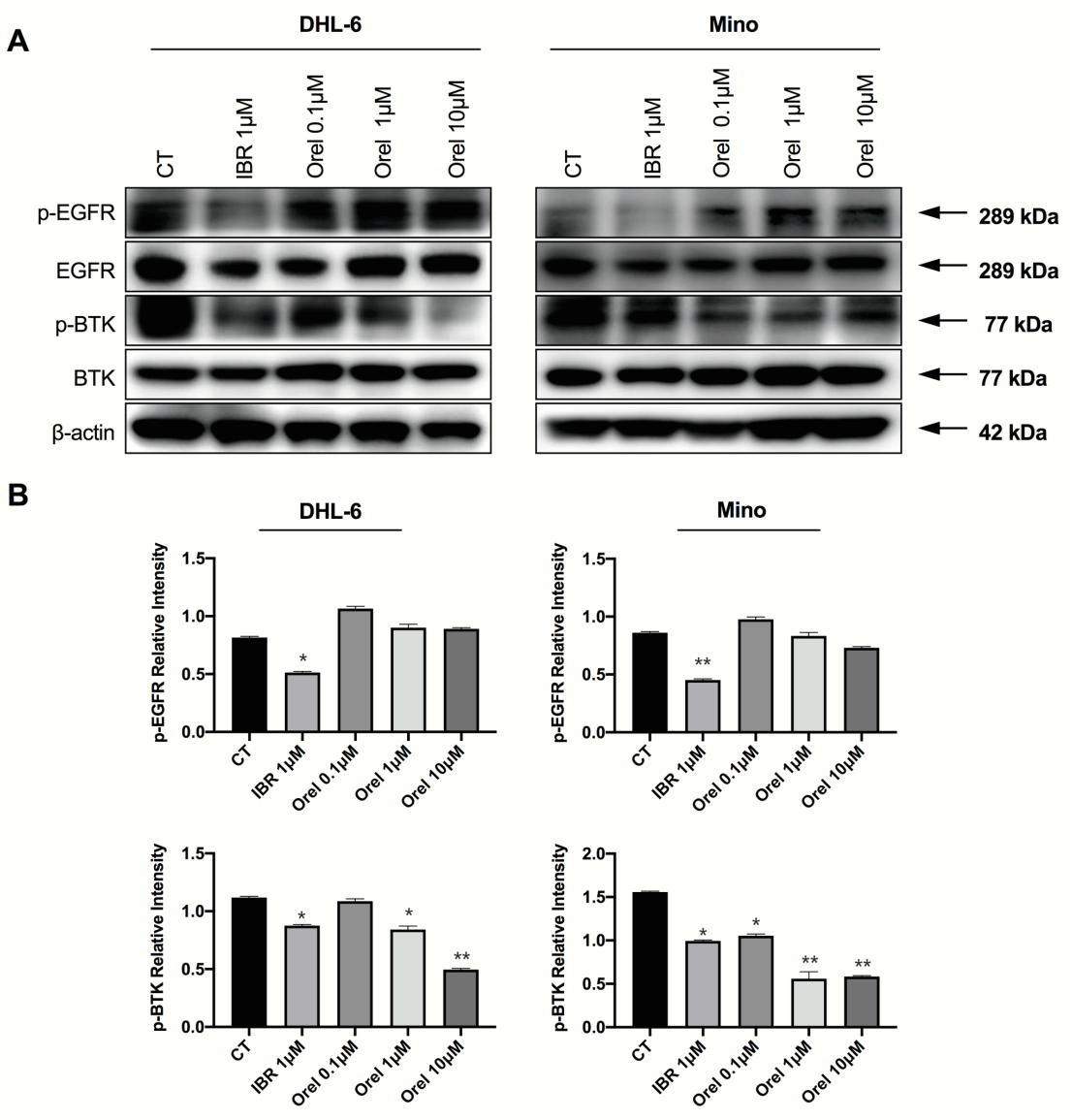

Fig S1 **The effects of BTK inhibitors on the target of EGFR. A.** Phosphorylation of EGFR and BTK in DHL-6 and Mino cells was assessed after the treatment of DMSO, IBR or Orel for 1h. **B.** Western blots bands were quantified from three biologically repeated tests. * $p < 0.05$, ** $p < 0.01$compared with control group.

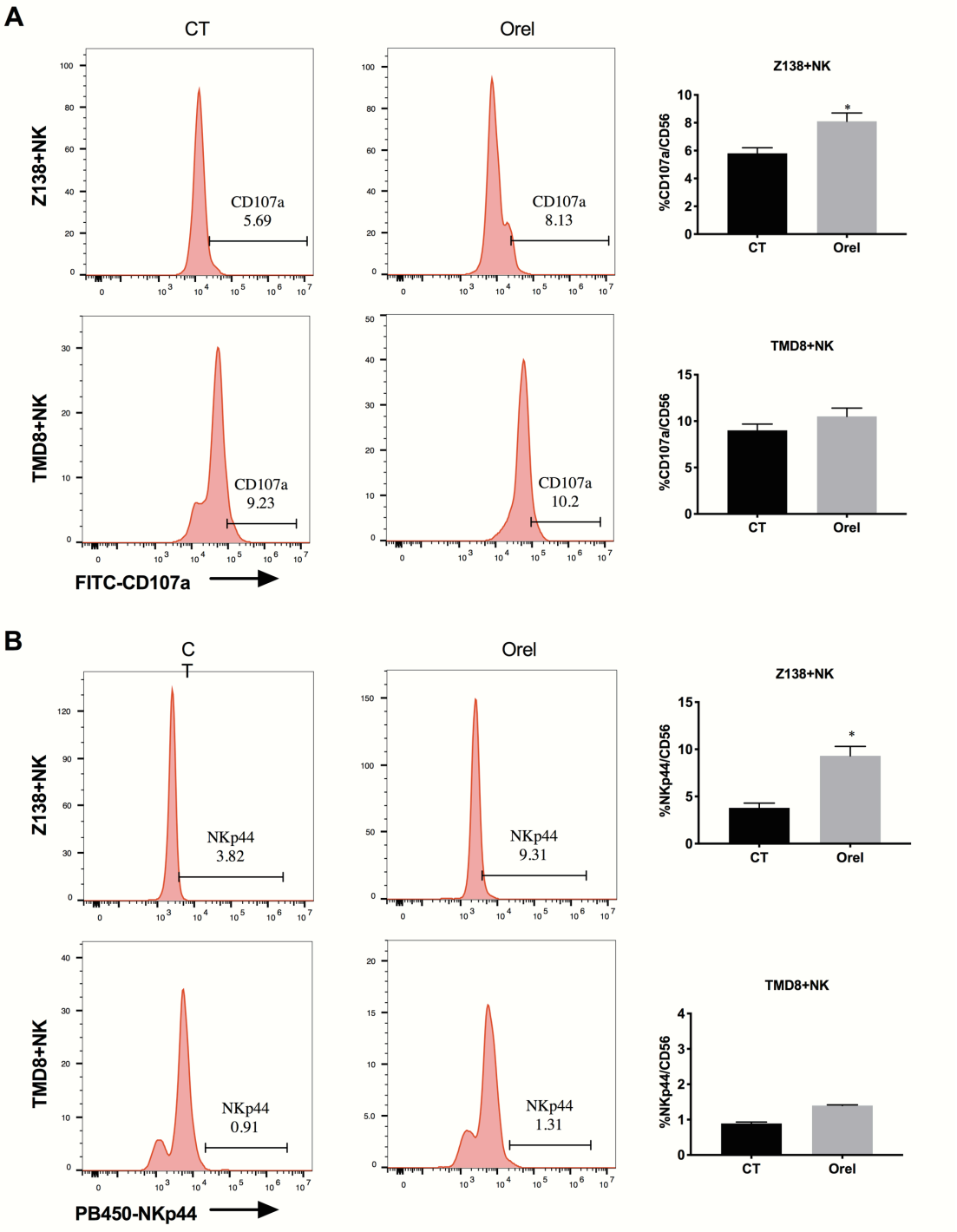

Fig S2 **Orelabrutinib enhanced the activity of NK cells in coculture system with B cell lymphomas.** NK cells obtained from healthy individuals were cocultured with Z138 and TMD8 cells for 4 hours with different treatments. **A&B.** The activity of NK cells was presented as the percentage of CD56+CD107a+ cells and CD56+NKp44 cells. Each cell line was biologically tested for at least 3 times. * $p < 0.05$

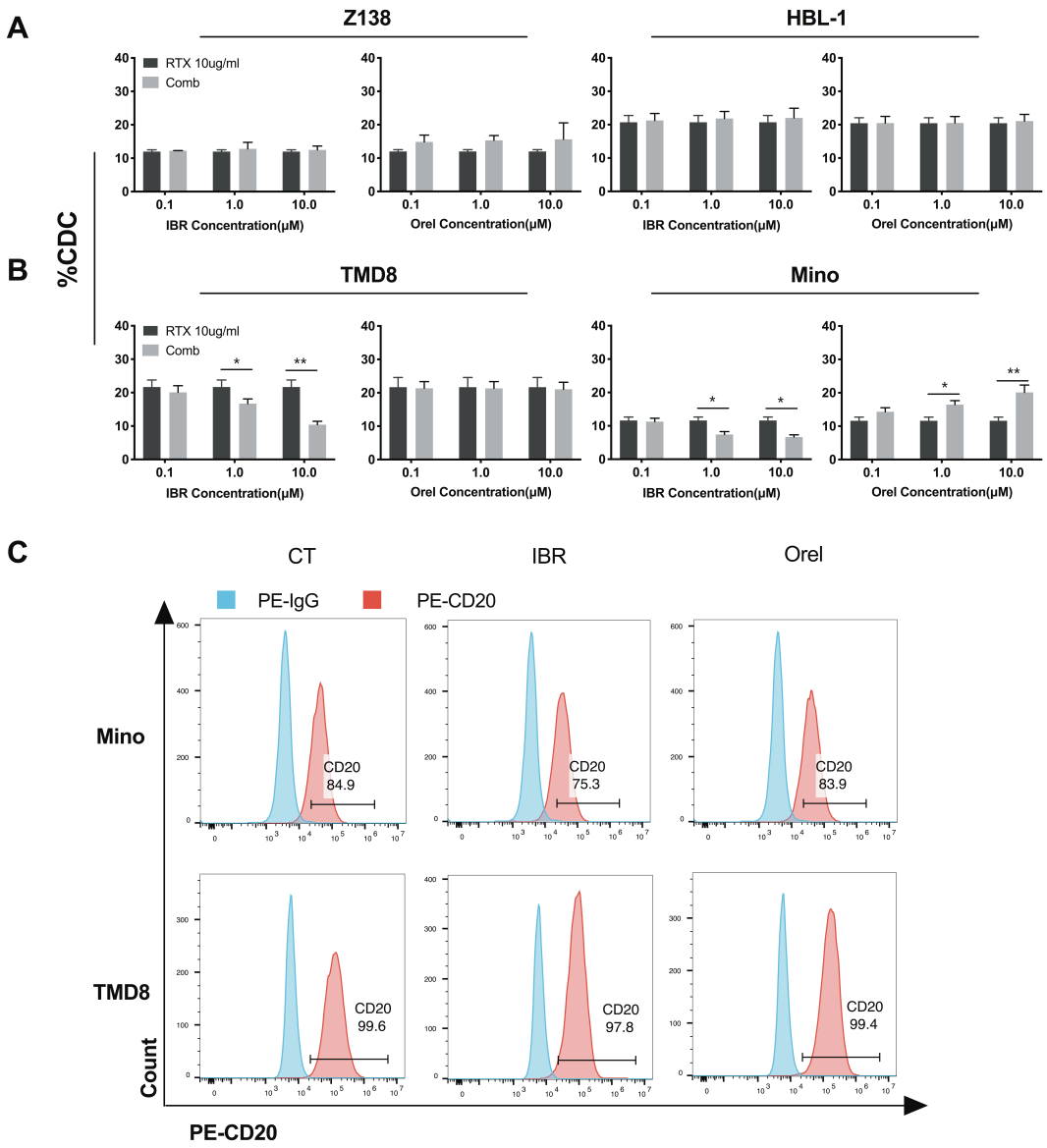

Fig S3 **Ibrutinib but not Orelabrutinib inhibited complement-dependent cytotoxicity (CDC) slightly in specific B cell lymphoma cell lines.** Cells (Z138, HBL-1, TMD8, Mino) were pre-treated with indicated concentration of ibrutinib or Orelabrutinib for 1 h, then rituximab (10mg/mL) or PBS was added, and 10 min later, 20% pooled human AB serum was added. After incubation at 37°C for 4 h, cell death was measured with flow cytometry. **A.** In Z138 and HBL-1 cell lines, both ibrutinib and Orelabrutinib had no significant effects on CDC of rituximab. **B&C.** While in Mino and TMD8 cell lines, ibrutinib but not Orelabrutinib inhibited CDC, and in Mino cells, Orelabrutinib enhanced CDC compared with rituximab alone, which is similar with CD20 modulation of these two BTK inhibitors.

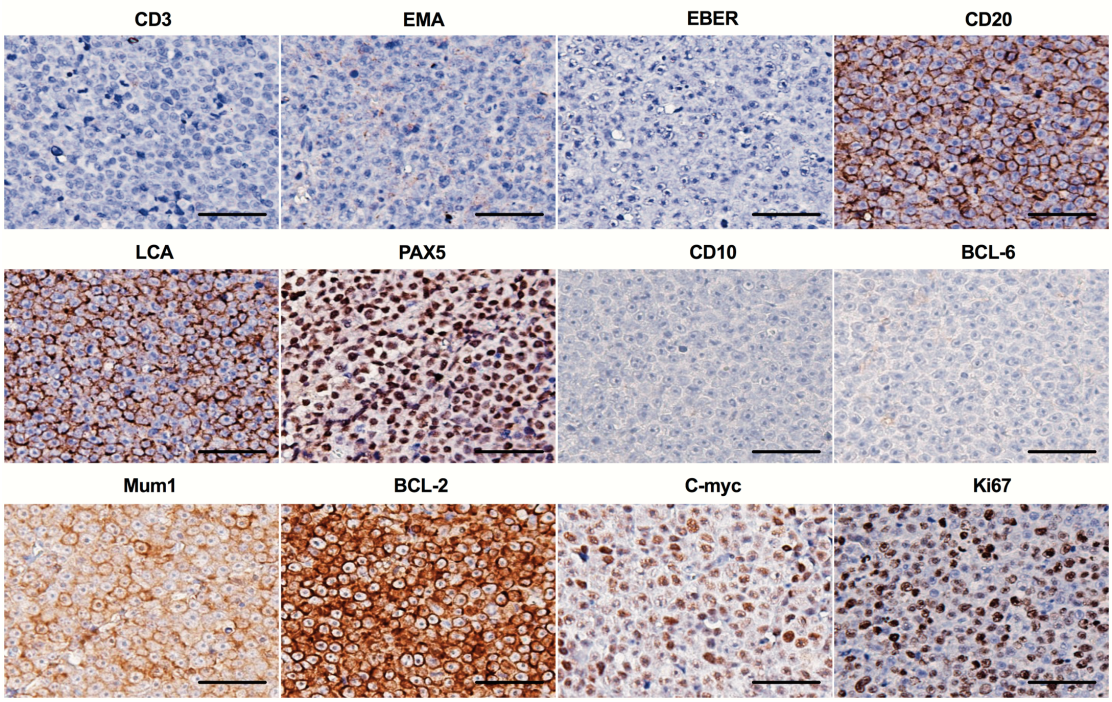

Fig S4 **The verification of DLBCL patient-derived tumor xenograft by IHC.** Immunohistochemical results showed the tumor: CD3(-), EMA (-), EBER (-), CD20(+), LCA (+), PAX5(+), CD10(-), Bcl-6(-), Mum1(+), Bcl-2(+90%), C-myc (+30%), Ki67(+70%), which is matched with the pathological type of the derived patient (Non-GCB DLBCL). Scale bar: 50 μm.

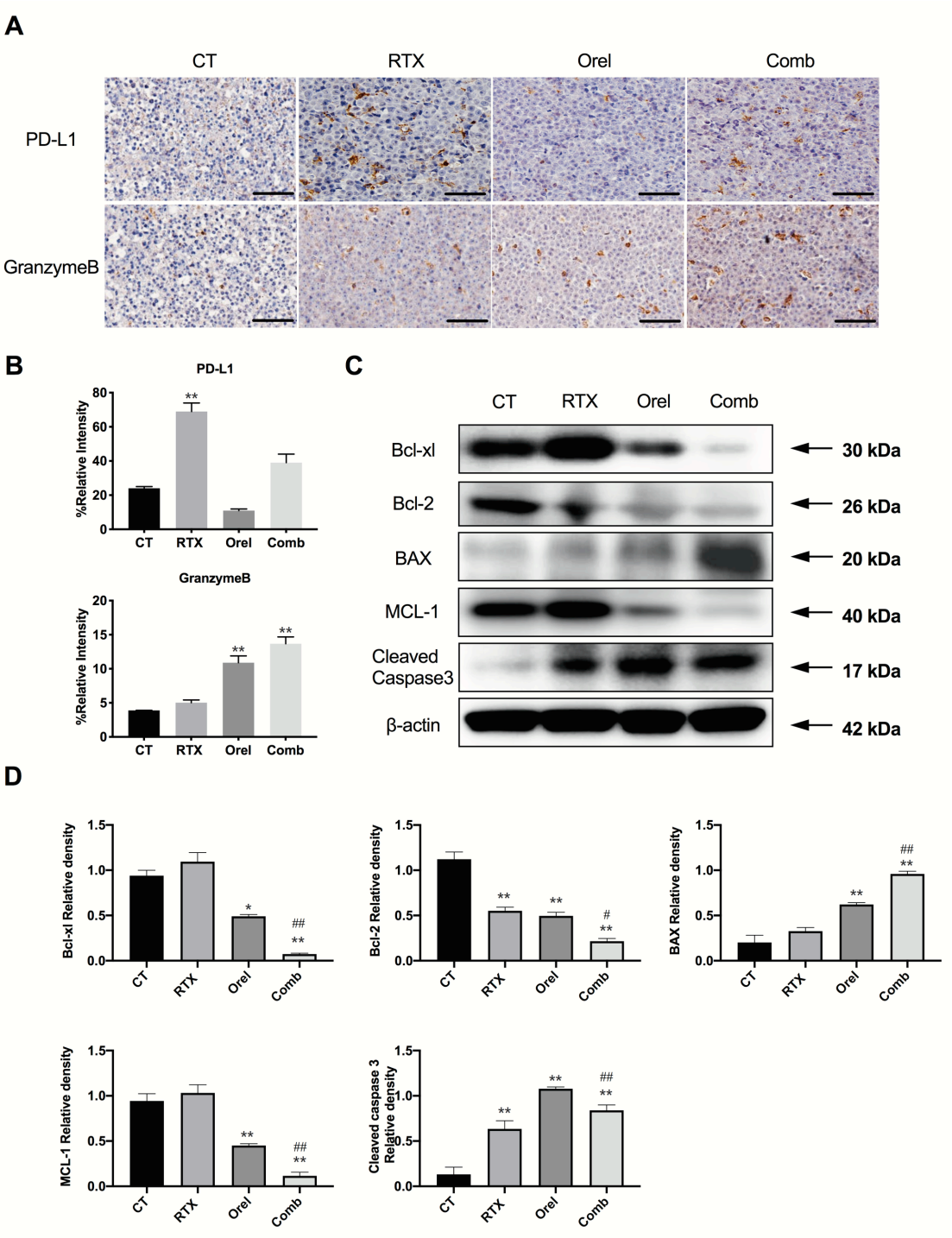

Figure S5 **Assessment of tumor tissues from PDX model. A&B.** PD-L1 and Granzyme B expression were assessed by IHC from PDX model. Scal bar 60 μm. **C.** To further verify the combined effects on inducing apoptosis of tumor cells, western blotting of apoptosis-related protein was performed including pro-apoptotic proteins (BAX and Cleaved Caspase3) and anti-apoptotic proteins (Bcl-2, Bcl-xl and MCL-1). **D.** The relative intensity of apoptosis associated proteins from three biologically repeated experiments were quantified by comparing to the β-actin band. Values

present as percentages of control group in mean ± SD, ** $p < 0.01$ compared with control group. # $p < 0.05$ compared with rituximab group, ## $p < 0.01$ compared with rituximab group.