**Supplementary Figure Legends**

**Supplementary Figure S1.**

Combined inhibition of BET and WEE1 produces synergistic cytotoxicity in NSCLC cell lines. Dose-response curves of the viability of 5 NSCLC cell lines, A549, H1299, H1975, H460, and H520, treated with JQ1 and AZD1775 (**A**) or AZD5153 and AZD1775 (**B**); cell viability was measured using MTT cell-proliferation assay. Circles: mean values (*n* = 4/group); bars: SD.

**Supplementary Figure S2.**

(**A**) Dose-response curves of the viability of H1299 cells transfected BRD4 siRNA or non-target (NT) siRNA followed by AZD1775 administration. Circles: mean values (*n* = 4/group); bars: SD. \**P* < 0.05, Welch *t* test. (**B**) Western blotting results showing the efficiency of BRD4 knockdown through RNA interference. The images of BRD4 and actin bands originate from Supplementary Figure S4C. (**C**) Dose-response curves of the viability of non-tumorigenic 293T cells treated with JQ1 and AZD1775; cell viability was measured using MTT cell-proliferation assay. Circles: mean values (*n* = 4/group); bars: SD. CI, Combination index; Fa, Fraction affected.

**Supplementary Figure S3.**

Combined inhibition of BET and WEE1 induces apoptosis. (**A**) Representative images of flow cytometry for apoptosis in A549 cells treated with indicated compounds (at 1.0 μmol/L) or vehicle for 72 h. Bar charts showing percentages of apoptotic cells among H1299 (**B**), and H1975 (**C**) cells after 72 h treatment with JQ1, AZD1775, or their combination. Apoptotic-cell percentages were determined using flow cytometry and Annexin V/PI double staining. Boxes: mean values (*n* = 3/group); error bars: SD. \**P* < 0.05, Welch *t* test. (**D**) Western blotting analysis of cleaved PARP and cleaved caspase-3 in A549 cells treated with indicated compounds or vehicle for 72 h.

**Supplementary Figure S4.**

Combined inhibition of BET and WEE1 induces DNA double-strand breaks. Western blotting analysis of γH2AX in cells treated with JQ1 and AZD1775 (**A**) or AZD5153 and AZD1775 (**B**) for 24 h. (**C**) Western blotting analysis of γH2AX in cells transfected BRD4 siRNA or non-target (NT) siRNA followed by AZD1775 administration.

**Supplementary Figure S5.**

Quantitative RT-PCR analysis of HR pathway-related genes in A549 cells treated for 24 h with 1.0 μmol/L JQ1 (**A**) or AZD5153 (**B**). Boxes: mean values (*n* = 3/group); error bars: SD. Quantitative RT-PCR (**C**) and Western blotting (**D**) analyses of NHEJ pathway-related genes and proteins in A549 cells treated for 24 h with 1.0 μmol/L indicated compounds or vehicle. Boxes: mean values (*n* = 3/group); error bars: SD.

**Supplementary Figure S6.**

BET inhibitors repress the expression of NHEJ pathway-related genes. Quantitative RT-PCR analysis of NHEJ pathway-related genes in H1299 (**A**, **C**) and H1975 cells (**B**, **D**) treated for 24 h with 1.0 μmol/L JQ1 and AZD1775 (**A**, **B**) or AZD5153 and AZD1775 (**C**, **D**). Boxes: mean values (*n* = 3/group); error bars: SD.

**Supplementary Figure S7.**

BET inhibition diminishes NHEJ activity. Representative images of flow cytometry for NHEJ reporter assay using EJ5-GFP reporter.

**Supplementary Figure S8.**

Combined inhibition of BET and WEE1 promotes mitotic entry. Western blotting analysis of pHH3 in cells treated with JQ1 and AZD1775 (**A**) or AZD5153 and AZD1775 (**B**) for 24 h. (**A**) The actin bands of H1299 and H460/H520 are shared with Figure 2A and Supplementary Figure S4A, respectively. These results originate from the same experiments. (**B**) The actin bands of H1975 are shared with Supplement Figure S4B. These results originated from the same experiments.

**Supplementary Figure S9.**

BET inhibition represses MYT1 expression. (**A**) Quantitative RT-PCR analysis of G2-M checkpoint-related genes in A549 cells treated with 1.0 μmol/L AZD5153 or vehicle for 24 h. Boxes: mean values (*n* = 3/group); error bars: SD. (**B**) Quantitative RT-PCR analysis of *MYT1* in H1975 cells treated for 24 h with the indicated compounds (at 1.0 μmol/L) or vehicle. Boxes: mean values (*n* = 3/group); error bars: SD. (**C**) Western blotting analysis of MYT1 in cells treated with AZD5153 for 24 h. The actin bands of A549 are shared with Supplementary Figure S5D, because these data originate from the same experiment.

**Supplementary Figure S10.**

Combined inhibition of BET and WEE1 does not induce significant body weight loss in a mouse xenograft model of non-small cell lung cancer. Body weight of mice treated with vehicle, JQ1 (50 mg/kg), AZD1775 (20 mg/kg), or the drug combination. Circles: mean values of % body weight change (*n* = 4/group); bars: SD.