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A Bromodomain and Extraterminal Protein Inhibitor OTX015 Suppresses T Helper Cell Proliferation and Differentiation

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Supplementary Fig 1. Human $CD4^+$ T cells sorting panel. A single-cell suspension of live human $CD4^+$ T cells were sorted for $CD3^+/CD4^+$ double-positive cells, then $CXCR3^+CCR4^-CCR6^-$ were sorted as Th1 cells, $CXCR3^+CCR4^-CCR6^+$ as IFN-γ & IL-17 double positive cells and $CXCR3^-CCR4^+CCR6^+$ as Th17 cells.

Supplementary Fig 2. Effects of OTX015 concentrations and treatment durations on murine $CD4^+$ T cells. Murine $CD4^+$ T cells were isolated and treated with different concentrations of OTX015 for different durations. (A) Viability change of murine Th1 or Th17 cells induced from $CD4^+$ T cells by different concentrations of OTX015 for 96 hours (n = 2). (B) Viability of murine Th1 or Th17 cells induced from $CD4^+$ T cells treated with 50 or 100 nM OTX015 for different durations of time (n = 2). (C) Proliferation change of murine Th1 or Th17 cells induced from $CD4^+$ T cells treated with different concentrations of OTX015 for 96 hours (n = 2). (D) Proliferation change of murine Th1 or Th17 cells induced from $CD4^+$ T cells treated with 50 or 100 nM OTX015 for different durations of time (n = 2). (E) Frequencies of IFN-γ- or IL-17–expressing cells in murine Th1 or Th17 cells induced from $CD4^+$ T cells treated with different concentrations of OTX015 for 96 hours (n = 2). (F) Frequencies of IFN-γ– or IL-17–expressing cells in murine Th1 or Th17 cells induced from $CD4^+$ T cells treated with 50 or 100 nM OTX015 for different duration of time (n = 2). (G) There was no difference in viability between control and 50 nM OTX015 treated group in murine Th1/Th17 induced from naïve $CD4^+$ T cell or in stimulated murine memory $CD4^+$ T cells (n =5 for Th1/Th17 cells and n =4 for memory $CD4^+$ T cells). Data are shown as means ± SD, * $P < 0.05$ ** $P < 0.01$.

Supplementary Fig 3. Effects of JQ1 and OTX015 on murine $CD4^+$ T cell subsets. Murine $CD4^+$ T cells were isolated and polarized into Th1 or Th17. Cells were treated with or without JQ1 or OTX015 at 50 and 100 nM for 3 days. (A) Frequencies of IFN-γ– or IL-17–expressing cells in murine Th1 or Th17

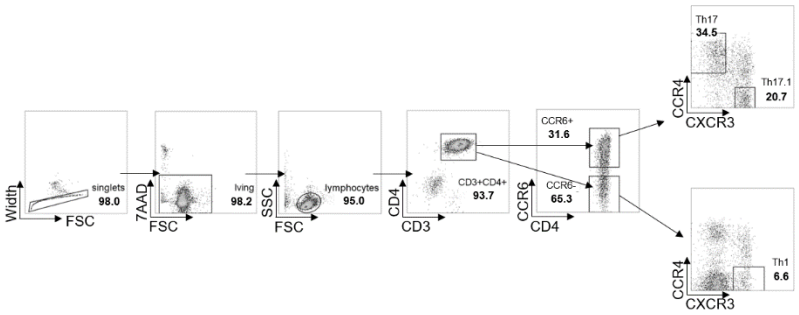
induced from $CD4^+$ T cells (n =4). (B) Suppression of proliferation in murine Th1 or Th17 induced from $CD4^+$ T cells (n = 4). (C) Viability of murine Th1 or Th17 induced from CD4+ T cell (n = 4). Data are shown as means ± SD.

Supplementary Fig 4. Viability of human $CD4^+$ T cells treated with OTX015. Human $CD4^+$ T cells were isolated and treated with different concentrations of OTX015 for 5 days (n = 12). Data are shown as means ± SD

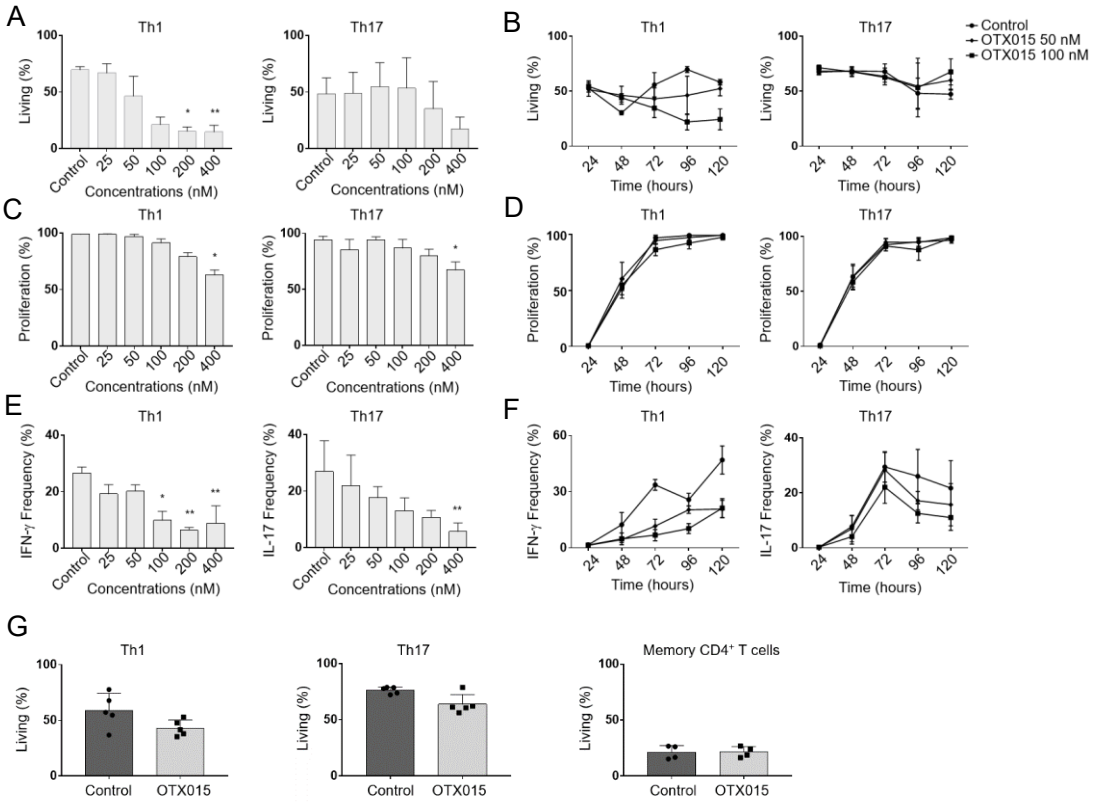
Supplementary Fig 5. Equivalent effects of JQ1 and OTX015 on human $CD4^+$ T cells. (A - C) JQ1 and OTX015 equivalently affected the frequencies of IL-17 single-positive cells (A), IFN-γ single-positive cells (B) and IFN-γ/IL-17 double-positive cells (C) in the stimulated human $CD4^+$ T cells (n =6). (D) JQ1 and OTX015 equivalently affected the proliferation of the stimulated human $CD4^+$ T cells (n =6). (E) JQ1 and OTX015 equivalently affected the viability of the stimulated human $CD4^+$ T cells (n =6). Data are shown as means ± SD

Supplementary Fig 6. Viability of different human T cell subsets treated with OTX015. Viability change of human naïve, central memory and effector memory T cells treated with different concentrations of OTX015 on day 5
Data are shown as means ± SD, ** $P < 0.01$ *** $P < 0.005$ **** $P < 0.001$.

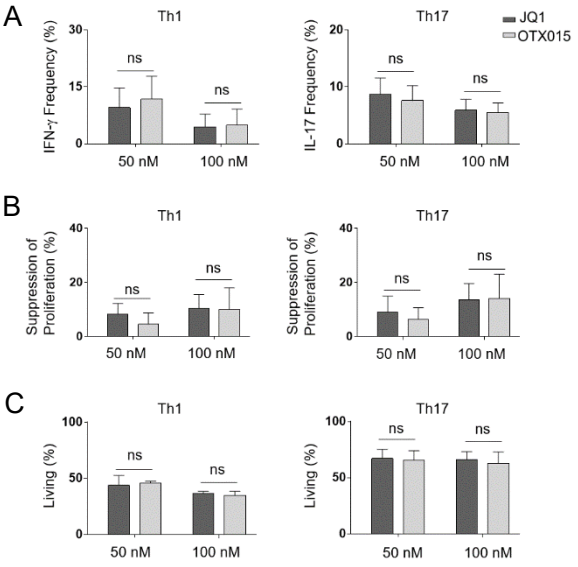
Supplementary Figure 1



Supplementary Figure 2



Supplementary Figure 3



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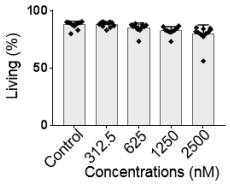
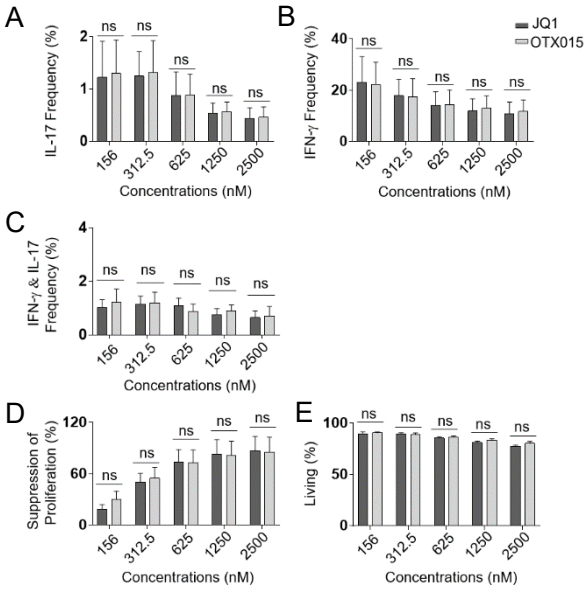


Supplementary Figure 5



Supplementary Figure 6