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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 5

Supplementary Figure 6