Supplemental material

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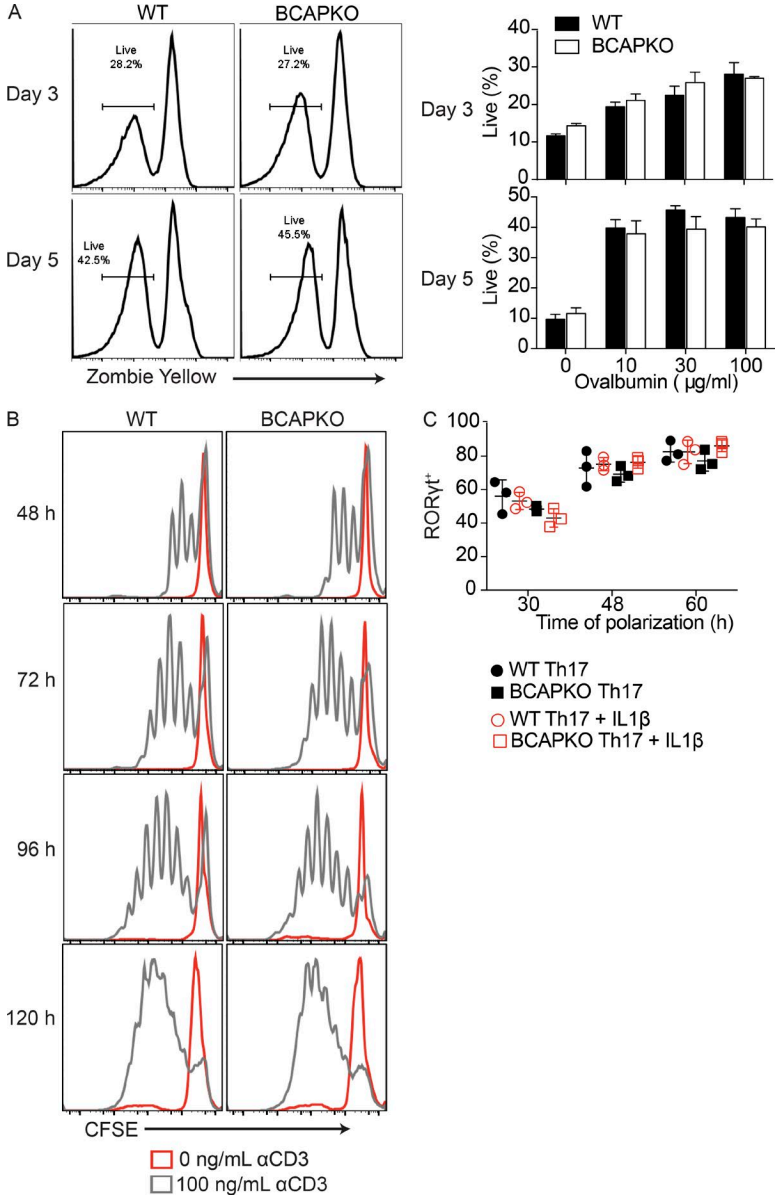

Figure S1. **BCAP deficiency does not affect CD4⁺ T cell survival during antigen-specific restimulation or proliferation in the absence of IL-1β or IL-18. (A)** Representative flow plots (left, 100 µg/ml OVA) and quantification (right) of CD4⁺ T cell survival after 3 d or 5 d of in vitro restimulation with WT DCs and indicated concentrations of OVA. Shown is the mean ± SEM; *n* = 4. Statistical analysis was performed with the two-tailed unpaired Student's *t* test. **(B)** Representative flow plots of WT and BCAPKO naive CD4⁺ T cells cultured with WT irradiated (12 Gy) B cells (1:2) and stimulated with indicated concentrations of αCD3. Cells were analyzed after the indicated time points to determine proliferation as determined by CFSE dilution. Data are representative of three independent experiments. **(C)** WT and BCAPKO naive CD4⁺ T cells were polarized to Th17 cells with or without IL-1β for the indicated time points, and RORγt expression was measured using flow cytometry. Shown is the mean ± SEM; *n* = 3. Statistical analysis was performed with the two-tailed unpaired Student's *t* test.

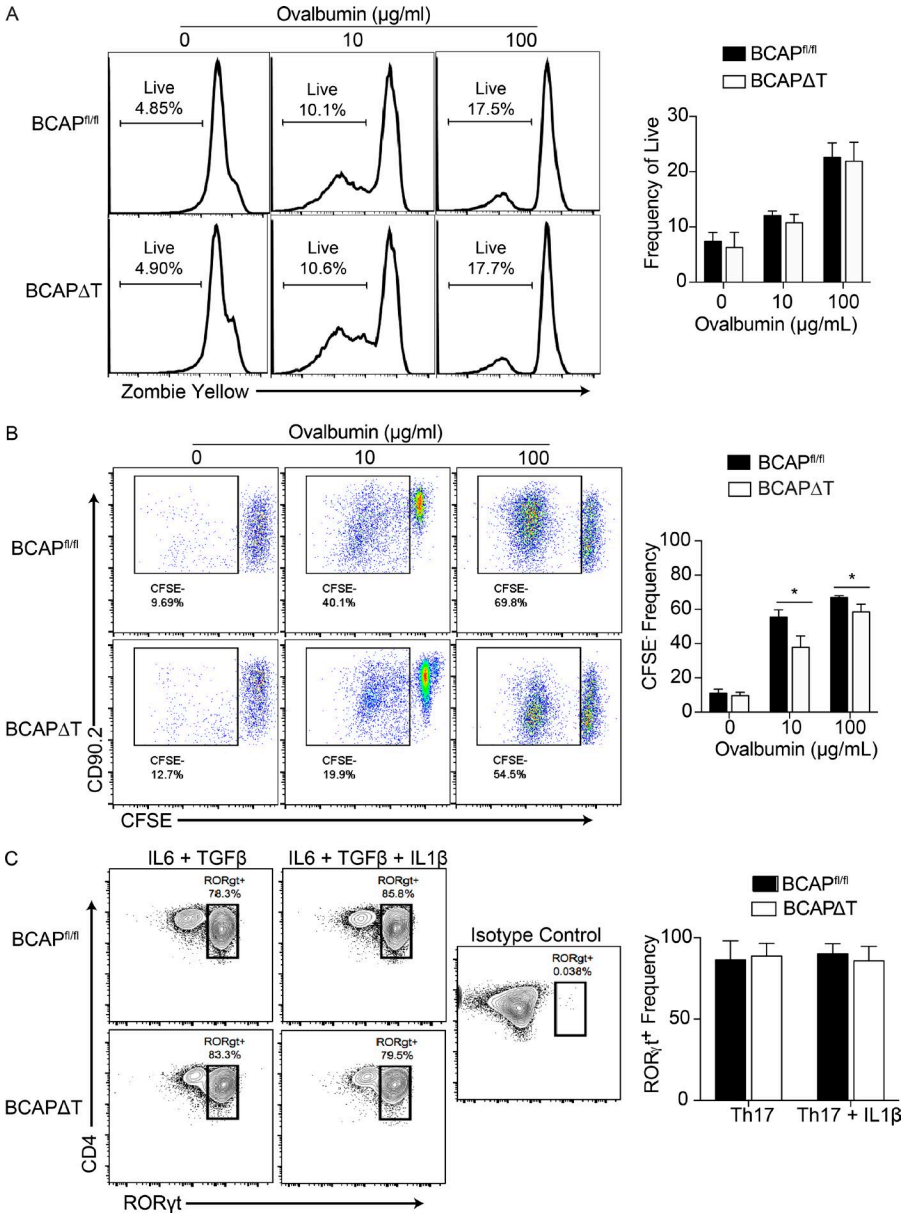

Figure S2. **T cell–specific BCAP deficiency does not affect T cell survival or RORγt expression.** Representative flow plots (left) and quantification (right) of CD4⁺ T cell survival (A) and CFSE dilution (B) after 3 d of in vitro restimulation with WT DCs and indicated concentrations of OVA. Shown is the mean ± SEM; $n = 4$. *, $P < 0.05$. Statistical analysis was performed with the two-tailed unpaired Student's t test. **(C)** Representative flow plots (left) and quantification (right) of RORγt expression of BCAP$^{fl/fl}$ and BCAPΔT naive CD4⁺ T cells polarized to Th17 cells with or without 10 ng/ml IL-1β for 5 d. Shown is the mean ± SEM; $n = 2$. Statistical analysis was performed with the two-tailed unpaired Student's t test.

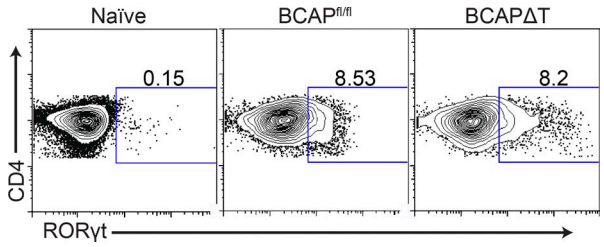

Figure S3. **BCAP deficiency does not affect RORγt expression in vivo.** Representative flow plots of RORγt expression in CD4⁺ T cells isolated from draining inguinal lymph nodes of MOG_{35-55}-immunized BCAP$^{fl/fl}$ and BCAPΔT mice 10 d after immunization. Data are representative of two independent experiments.

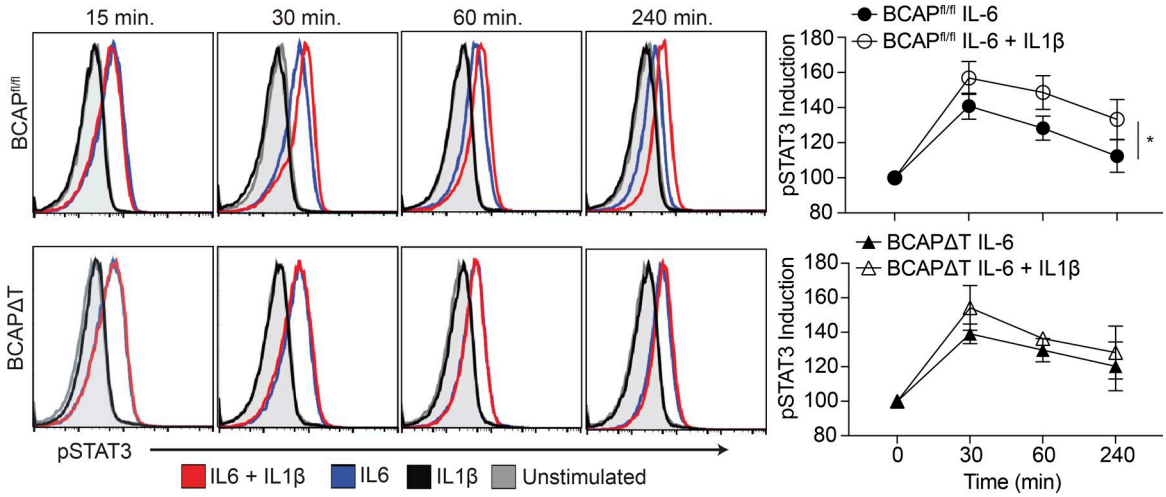

Figure S4. **BCAP is required for maximal IL-6–IL-1β synergism.** BCAP$^{fl/fl}$ and BCAPΔT CD4^{+}CD44^{hi} T cells were stimulated with 10 ng/ml IL-6 ± 2 ng/ml IL-1β for the indicated time. Cells were fixed to 2% PFA, permeabilized with 100% methanol, and probed for pSTAT3 (Y705). Top: A representative experiment. Bottom: Quantification of pSTAT3 induction from three independent experiments. Shown is the mean ± SEM. *, P < 0.05. Statistical analysis was performed with one-way ANOVA.

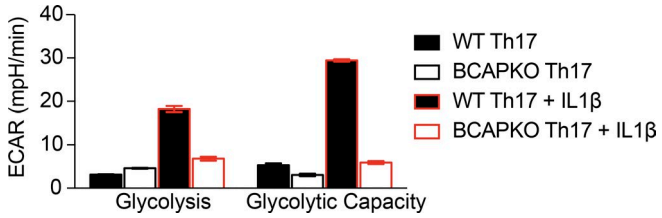

Figure S5. **IL-1β increases glycolysis through BCAP.** Naive WT and BCAPKO CD4^{+} T cells were polarized to Th17 cells for 48 h, and then stimulated with 10 ng/ml IL-1β for 12 h as indicated and analyzed using the glycolysis stress test on the Seahorse platform. Glycolytic rate was determined by the difference between the initial ECAR levels and the ECAR levels after the addition of glucose but before the addition of oligomycin. Glycolytic capacity was determined by the difference between the initial ECAR levels and the ECAR levels after the addition of oligomycin but before the addition of 2DG. Shown is the mean ± SD derived from five independent culture wells. Data are representative of three independent experiments.