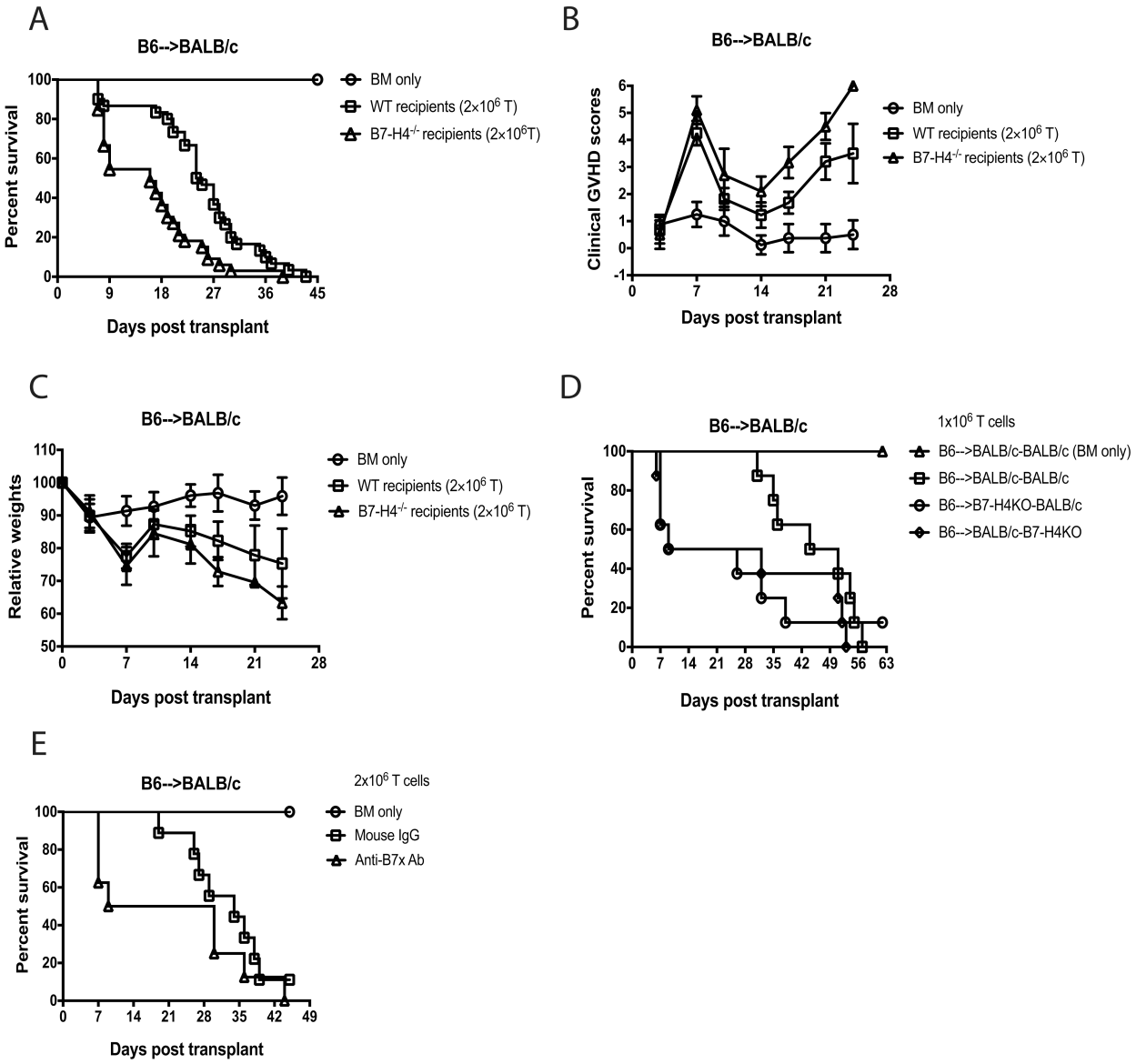

Figure S1. Absence of host B7-H4 expression accelerates GVHD lethality and B7-H4 expression on hematopoietic cells is critical for controlling acute GVHD. (A–C) Lethally irradiated WT BALB/c recipients or B7-H4$^{-/-}$ recipients were infused with 10^7 WT B6 BM cells alone or with 2×10^6 WT B6 purified T-cells. (**A**) Kaplan-Meier survival plot represents pooled data (n = 19–33 mice/group) from 3 independent experiments (BM + T-cells: WT versus B7-H4$^{-/-}$ recipients; $P = 0.0003$). (**B**) Transplanted mice were evaluated for clinical GVHD (n = 8–12/group). BM + T- cells: WT versus B7-H4$^{-/-}$ recipients, $P < 0.05$ on d10, d21, and d24; $P = 0.0053$ on d14; $P < 0.0006$ on d7 and d17. Data are representative of 3 independent experiments. (**C**) Relative weights of transplanted mice. Pooled data (n = 16–24/group) from 2 independent experiments (BM + T-cells: WT versus B7-H4$^{-/-}$ recipients; $P < 0.05$ on d21 and d24; $P = 0.0005$ on d17. (**D**) Lethally irradiated WT BALB/c recipients or B7-H4$^{-/-}$ recipients were infused with BM cells from B7-H4$^{-/-}$ or WT BALB/c mice, respectively, to create chimeras. We also created control chimeras (WT→WT). After 3 months, these chimeras were re-irradiated

and infused with allogeneic WT B6 BM cells with 1×10^6 WT B6 purified T-cells. Kaplan-Meier survival plot of transplanted mice (n = 8/group) is shown. WT→WT versus B7-H4$^{-/-}$→WT chimeras, $P = 0.0287$; WT→WT versus WT→ B7-H4$^{-/-}$ chimeras, $P = 0.078$; B7-H4$^{-/-}$→WT versus WT→ B7-H4$^{-/-}$ chimeras, $P = 0.915$. Data are representative of 2 independent experiments. (**E**) Lethally irradiated WT BALB/c recipients were infused with 10^7 WT B6 BM cells alone or with 2×10^6 WT B6 purified T-cells and treated with isotype-matched control antibody or anti-B7x mAb (clone 19D6; n = 8–9 mice/group). Kaplan-Meier survival plot of transplanted mice is shown (BM + T-cells: isotype control versus anti-B7x; $P = 0.305$). (B, C) Data represent mean ± SEM. *P* values were calculated by 2-tailed *t* test (B, C) or log-rank test (A, D, and E).

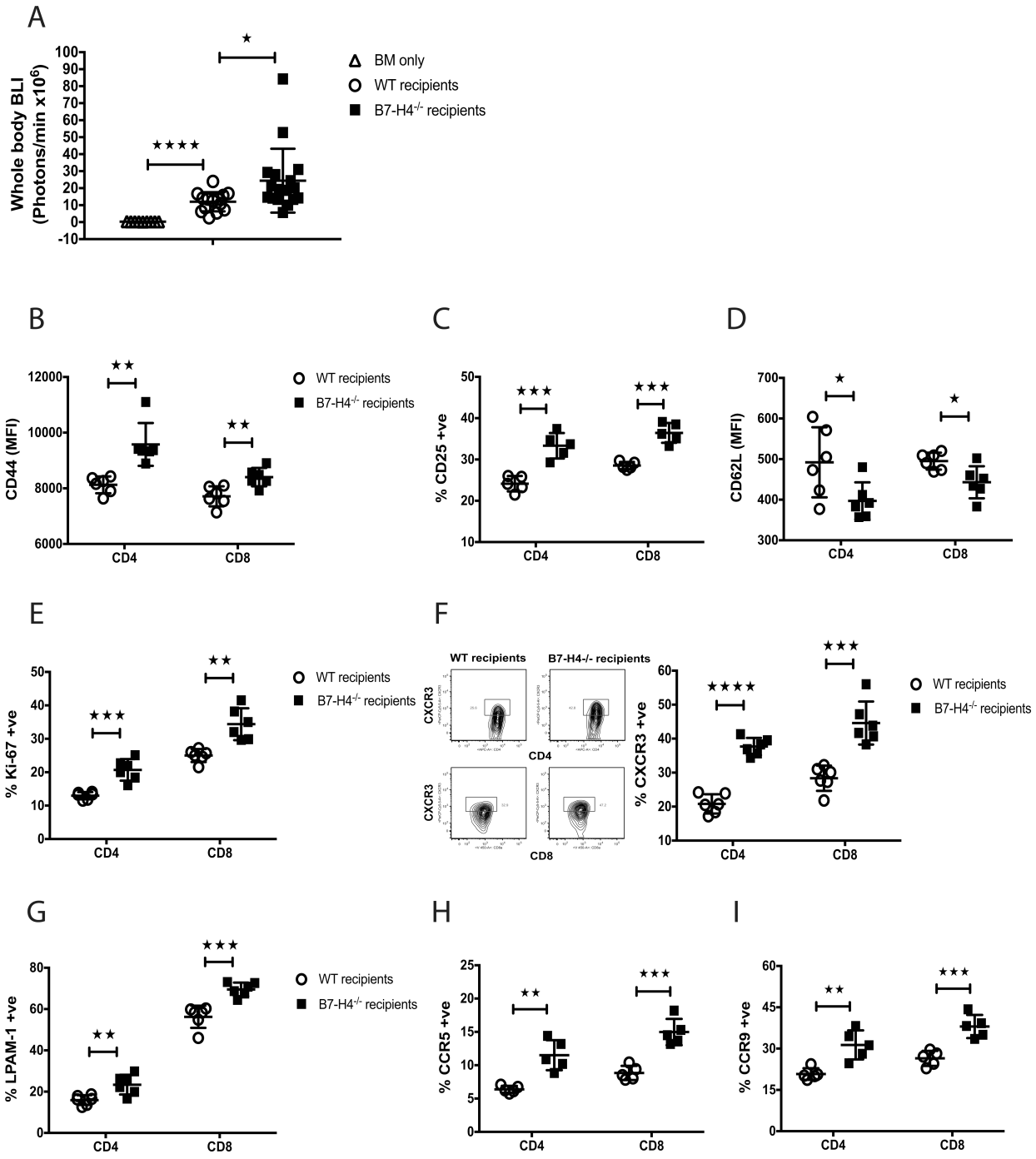

Figure S2. Absence of host B7-H4 expression increases donor T-cell activation and migration. (**A**) Lethally irradiated WT BALB/c recipients or B7-H4$^{-/-}$ recipients were infused with 10^7 WT B6 BM cells alone or with 1×10^6 B6 luciferase transgenic purified T-cells. On day 7 after BMT, mice were injected intraperitoneally with luciferin, and after 5 minutes, mice were imaged using a Xenogen IVIS imaging system. Pooled data were obtained from 2 independent experiments (n = 9–17 mice/group). (B–I) Lethally irradiated WT BALB/c recipients or B7-H4$^{-/-}$ recipients were infused with 10^7 WT B6 BM cells plus 1×10^6 WT B6 purified T-cells. Mice (n =

5–6/group) were sacrificed on day 7 after BMT, and donor CD4 and CD8 T-cells in spleen were analyzed by flow cytometry for the expression of CD44 (**B**), CD25 (**C**), CD62L (**D**), Ki-67 (**E**), CXCR3 (**F**), LPAM-1 (**G**), CCR5 (**H**), or CCR9 (**I**). MFI, mean fluorescence intensity. Data are representative of 2–3 independent experiments. Data represent mean ± SEM, and *P* values were calculated by 2-tailed *t* test. $*P < 0.05$, $**P < 0.01$, $***P < 0.001$, $****P < 0.0001$.

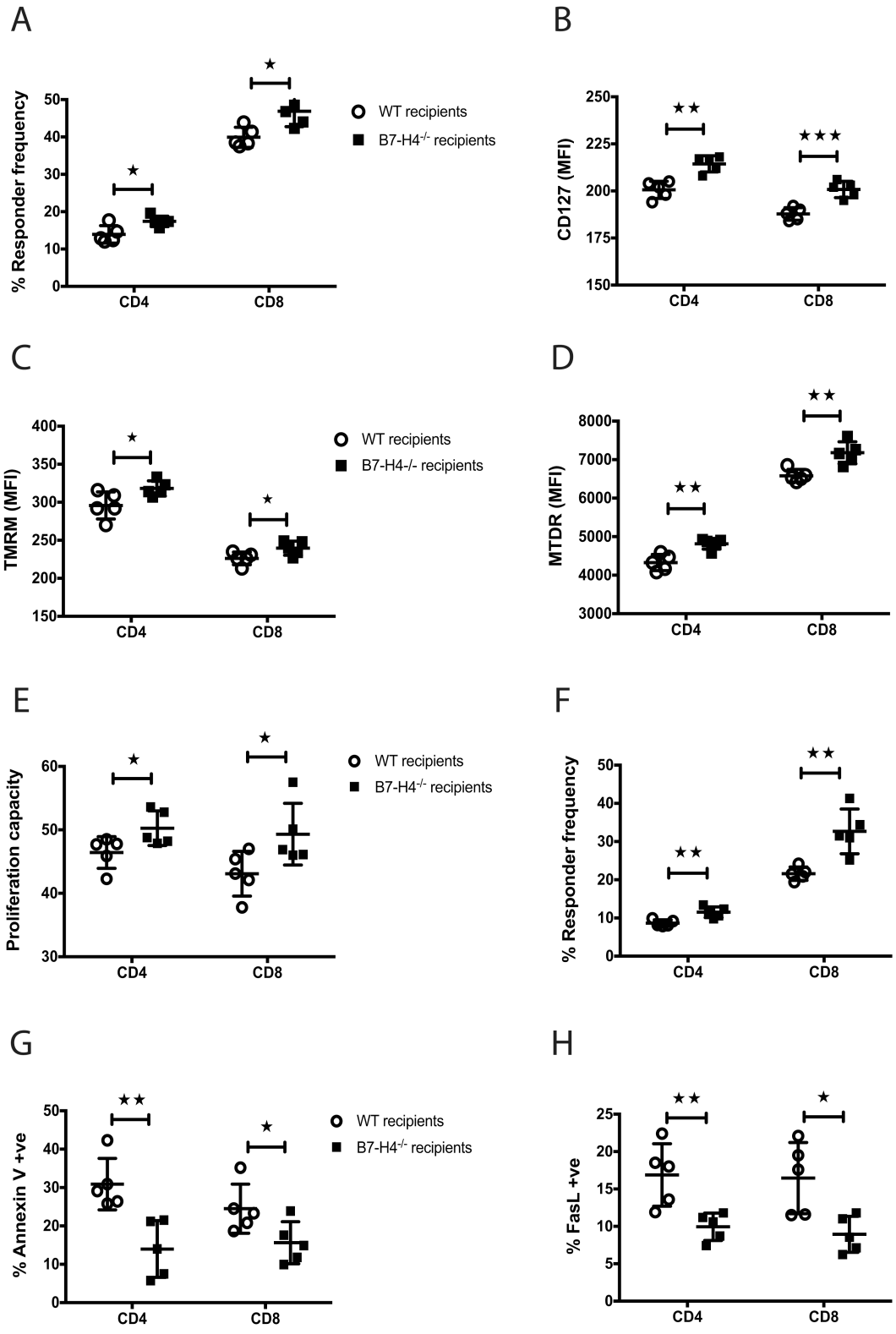

Figure S3. Absence of B7-H4 expression on host cells increases proliferation and survival of allogeneic donor T-cells. Lethally irradiated WT BALB/c recipients or B7-H4$^{-/-}$ recipients were

infused with 10^7 WT B6 BM cells plus 20×10^6 CFSE-labeled or 20×10^6 CTV-labeled WT B6 splenocytes. Mice were sacrificed on day 4 after BMT, and donor T-cells in spleens (A–D) and mLNs (E–H) were analyzed by flow cytometry. Cells were analyzed for CFSE dilution (**A, E, F**), TMRM (**C**), MTDR (**D**), Annexin V (**G**), or FasL (**H**) expression. (**B**) Lethally irradiated WT BALB/c recipients or B7-H4$^{-/-}$ recipients were infused with 10^7 WT B6 BM cells plus 2×10^6 WT B6 purified T-cells. Splenocytes were analyzed on day 4 to detect donor T-cells expressing CD127. (A–H) Data are representative of at least 5 mice per group from 2–3 independent experiments. MFI, mean fluorescence intensity. Data represent mean ± SEM, and *P* values were calculated by 2-tailed *t* test. $*P < 0.05$, $**P < 0.01$, $***P < 0.001$.

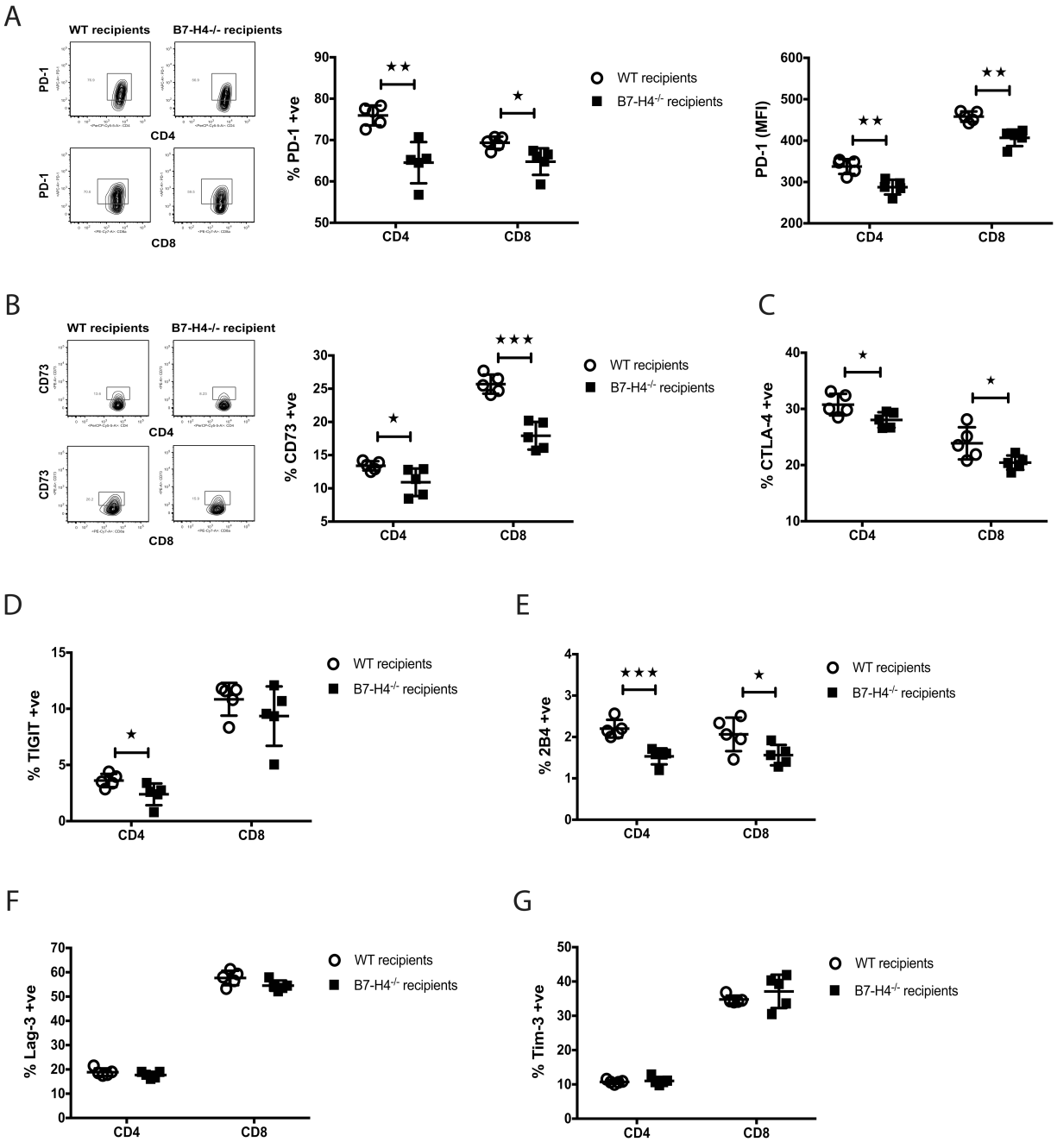

Figure S4. Increased proliferation of donor T-cells in B7-H4$^{-/-}$ recipients was associated with reduced expression of inhibitory receptors. Lethally irradiated WT BALB/c recipients or B7-H4$^{-/-}$ recipients were infused with 10^7 WT B6 BM cells plus 2×10^6 WT B6 purified T-cells. Mice (n = 5/group) were sacrificed on day 4 after BMT, and donor T-cells in spleen were analyzed by flow cytometry for the expression of PD-1 (**A**), CD73 (**B**), TIGIT (**D**), 2B4 (**E**), Lag-3 (**F**), Tim-3 (**G**), or intracellular expression of CTLA-4 (**C**). (A–G) Data are representative of 2 independent experiments. MFI, mean fluorescence intensity. Data represent mean ± SEM, and *P* values were calculated by 2-tailed *t* test. $*P < 0.05$, $**P < 0.01$, $***P < 0.001$.

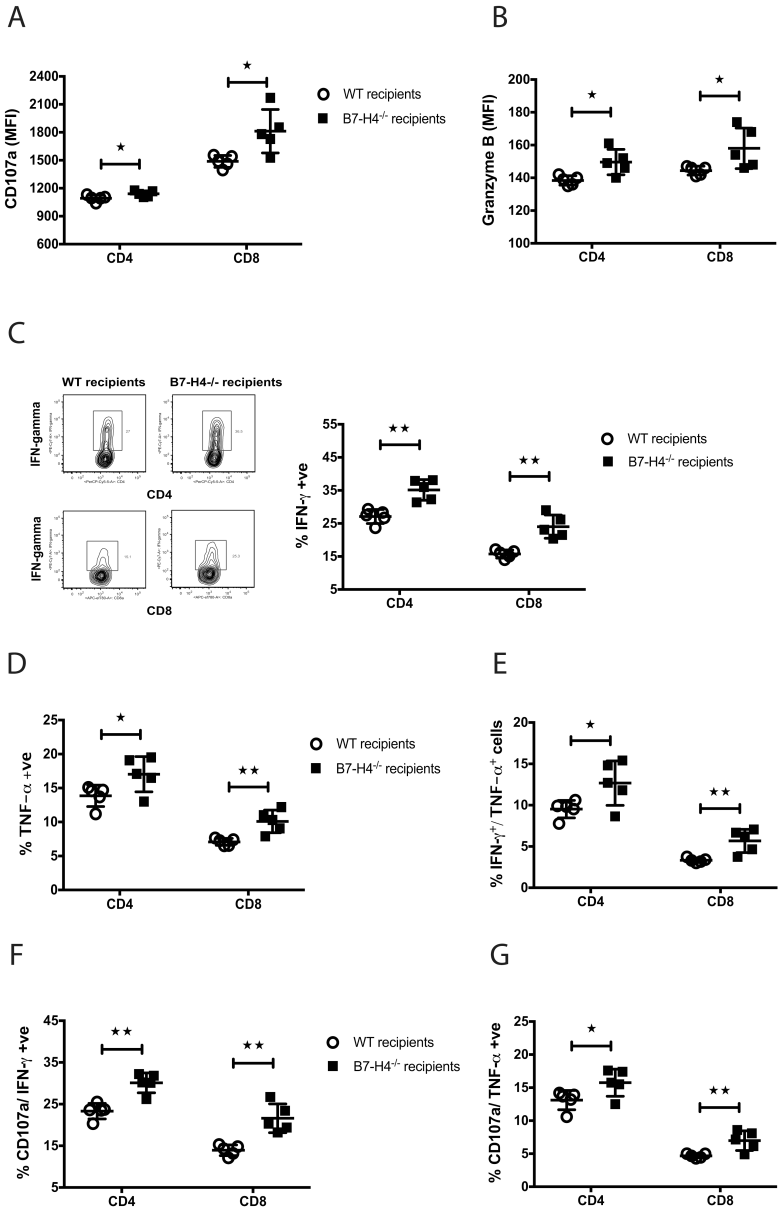

Figure S5. Absence of B7-H4 expression on host cells increases donor T-cell effector function that promote increased gut injury in recipients. Lethally irradiated WT BALB/c recipients or B7-H4$^{-/-}$ recipients were infused with 10^7 WT B6 BM cells plus 1×10^6 WT B6 purified T-cells. Mice (n = 10/group) were sacrificed on day 23 after BMT, and lymphocytes isolated from colon (2 colons were pooled to make 1 pooled sample and 5 pooled samples per group) were analyzed by flow cytometry. Donor CD4 and CD8 T-cells were analyzed for intracellular expression of CD107a (**A**), Granzyme B (**B**), IFN-γ (**C**), TNF-α (**D**), IFN-γ/TNF-α (**E**), CD107a/IFN-γ (**F**), or CD107a/TNF-α (**G**). MFI, mean fluorescence intensity. (A–G) Data are representative of 2 independent experiments. Data represent mean ± SEM. *P* values were calculated by 2-tailed *t* test. $*P < 0.05$, $**P < 0.01$.

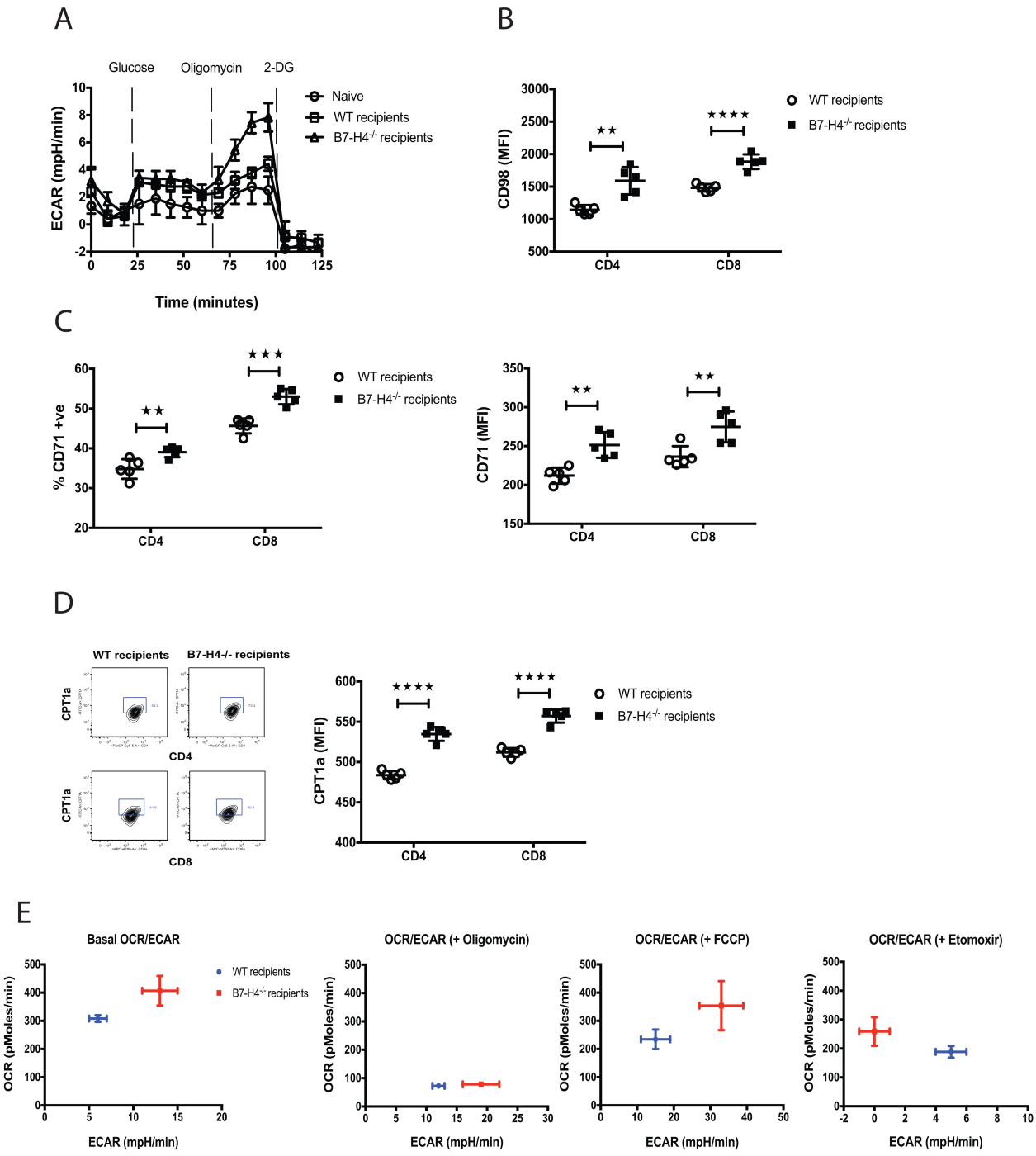

Figure S6. Metabolic alterations of donor T-cells in WT versus B7-H4$^{-/-}$ recipients. Lethally irradiated WT BALB/c recipients or B7-H4$^{-/-}$ recipients were infused with 10^7 WT B6 BM cells plus 20×10^6 WT B6 splenocytes. Mice were sacrificed on day 4 after BMT, and experiments were performed as described. (**A**) ECAR of purified donor T-cells was measured after addition of glucose, oligomycin, and 2-deoxyglucose (2-DG). T-cells from naïve WT B6 mice (n = 4) were included as control. Splenic donor T-cells were analyzed by flow cytometry for CD98 (**B**),

CD71 (**C**), or intracellular expression of CPT1a (**D**). MFI, mean fluorescence intensity. (**E**) OCR of purified donor T-cells was measured after addition of oligomycin, FCCP, etomoxir (Eto), and rotenone plus antimycin A. OCR versus ECAR data were analyzed. (A and E) Data are representative of 12 mice per group from 2–3 independent experiments. (B–D) Data are representative of 5 mice per group from 2–3 independent experiments. Data represent mean ± SEM, and *P* values were calculated by 2-tailed *t* test. $**P < 0.01$, $***P < 0.001$, $****P < 0.0001$.

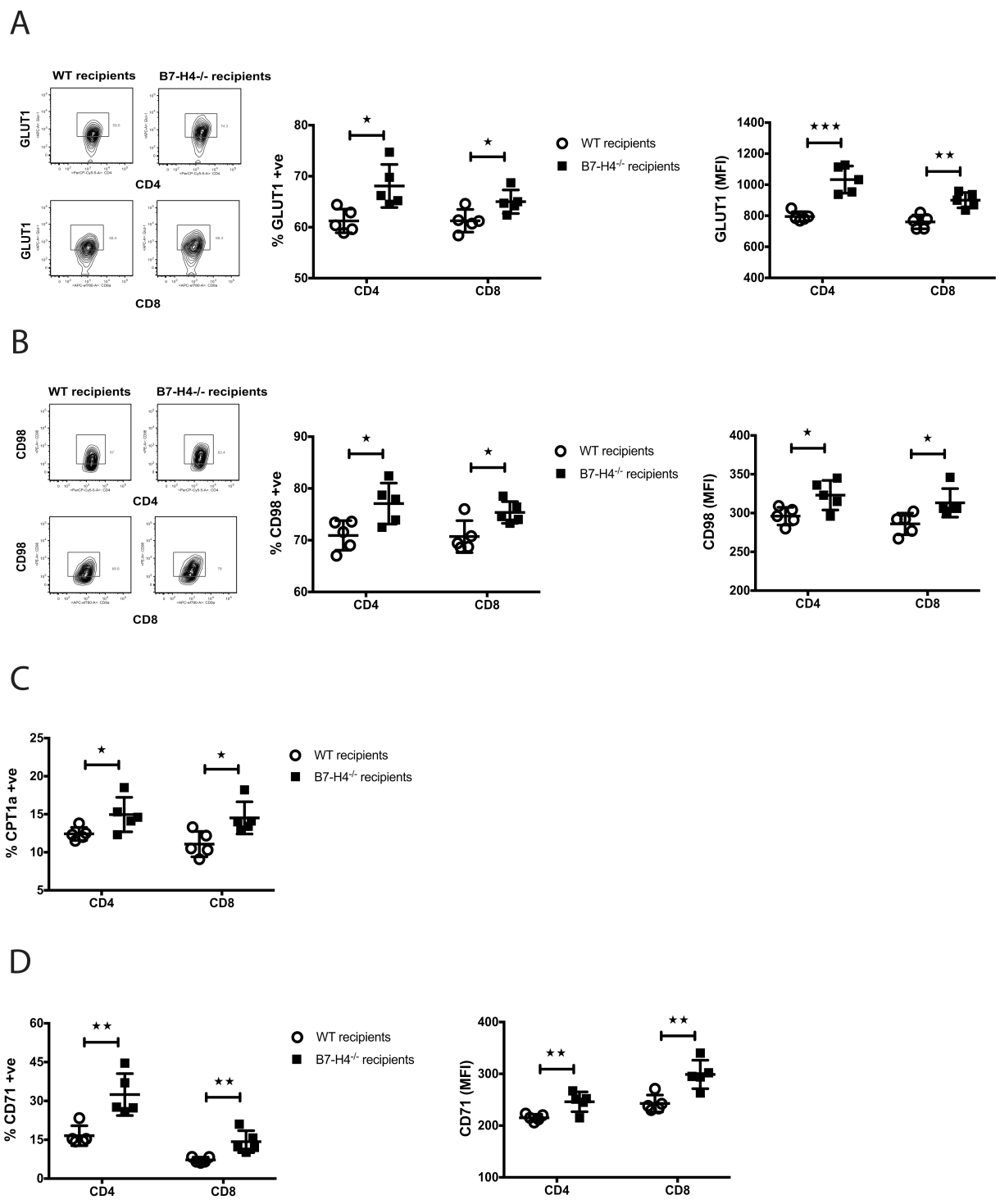

Figure S7. Metabolic alterations of donor T-cells in WT versus B7-H4$^{-/-}$ recipients. Lethally irradiated WT BALB/c recipients or B7-H4$^{-/-}$ recipients were infused with 10^7 WT B6 BM cells plus 1×10^6 WT B6 purified T-cells. Mice were sacrificed on day 23 after BMT (n = 10/group), and lymphocytes isolated from colon (2 colons were pooled to make 1 pooled sample and 5 pooled samples per group) were analyzed by flow cytometry. Donor CD4 and CD8 T-cells were

analyzed for intracellular expression of GLUT1 (**A**) or CPT1a (**C**). Donor T cells were also analyzed for CD98 (**B**), or CD71 (**D**) expression. (A–D) Data are representative of 2 independent experiments. Data represent mean ± SEM. *P* values were calculated by 2-tailed *t* test. $*P < 0.05$, $**P < 0.01$, $***P < 0.001$.

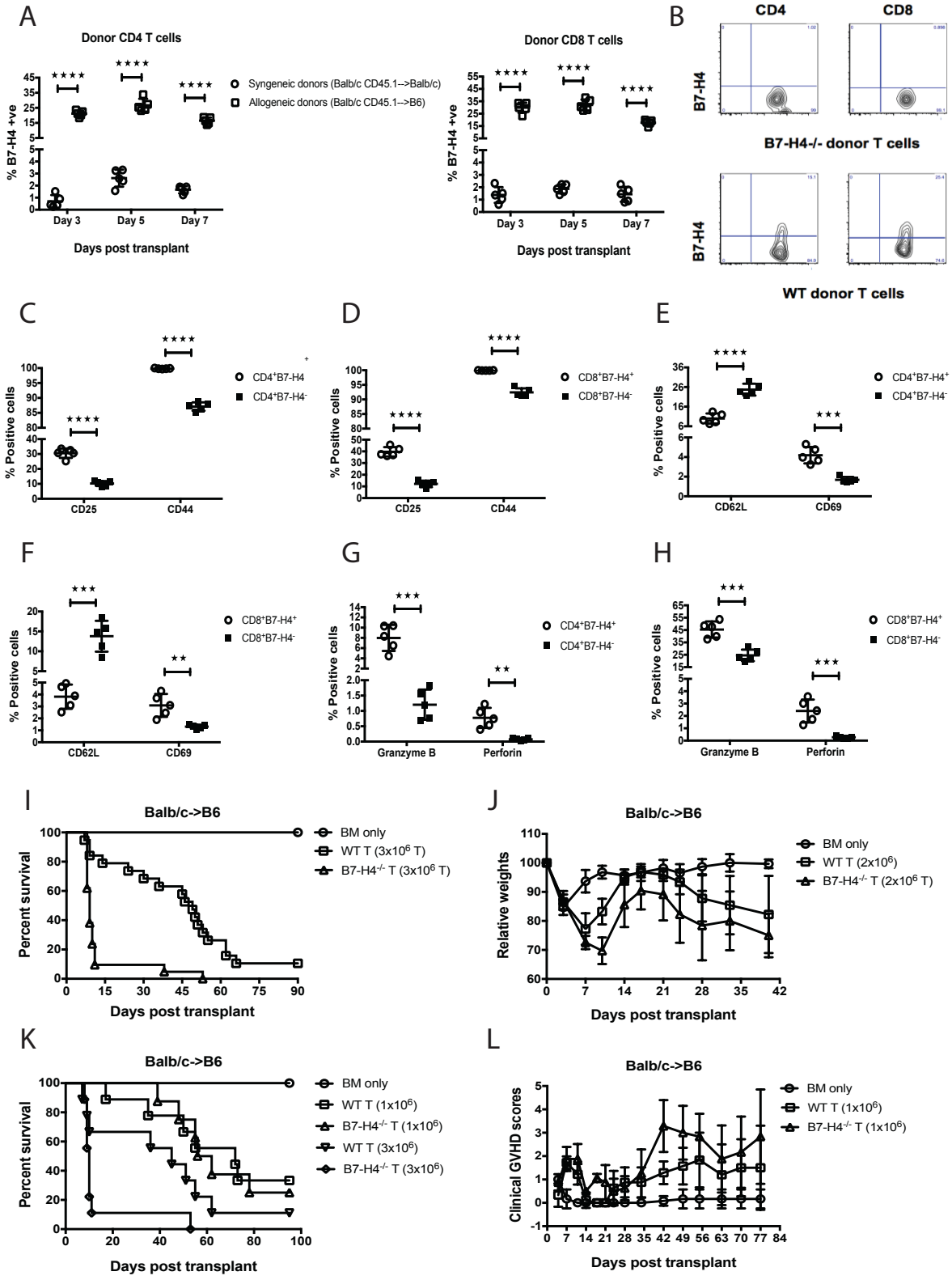

Figure S8. Absence of B7-H4 expression on donor T-cells lead to accelerated GVHD lethality. (**A**) Lethally irradiated WT BALB/c or B6 recipients were infused with 10^7 WT

BALB/c BM cells plus 2×10^6 BALB/c CD45.1 T-cells. Mice were sacrificed (n = 5/group/day) on day 3–7, and splenic donor T-cells were analyzed by flow cytometry for B7-H4 expression. Data were obtained from one experiment. **(B)** Lethally irradiated B6 recipients were infused with 10^7 WT BALB/c BM cells plus 2×10^6 WT BALB/c or B7-H4$^{-/-}$ T-cells. Mice were sacrificed on day 5, and splenic donor T-cells were analyzed by flow cytometry for B7-H4 expression as shown in Figure 5C. B7-H4 expression on WT donor T-cells were measured based on baseline expression of B7-H4 on B7-H4$^{-/-}$ donor T-cells. (**C–H**) Lethally irradiated B6 recipients were infused with 10^7 WT BALB/c BM cells plus 2×10^6 WT BALB/c T-cells. Mice were sacrificed (n = 5) on day 5. Splenic donor T-cells expressing B7-H4 and splenic donor T-cells negative for B7-H4 were analyzed for the expression of CD25, CD44, CD62L, CD69, Granzyme B, and Perforin. Data were obtained from 1 experiment. (**I**) Lethally irradiated B6 recipients were infused with 10^7 WT BALB/c BM cells alone (n = 14) or with 3×10^6 WT BALB/c (n = 19) or B7-H4$^{-/-}$ T-cells (n = 21). Kaplan-Meier survival plot represents pooled data from 2 independent experiments (BM + T-cells: recipients of WT versus B7-H4$^{-/-}$ donor T-cells, $P < 0.0001$). (**J**) Relative weights of transplanted mice. Lethally irradiated B6 recipients were infused with 10^7 WT BALB/c BM cells alone or with 2×10^6 WT BALB/c or B7-H4$^{-/-}$ T-cells (n = 8–9/group). Data are representative of 2 independent experiments (BM + T-cells: recipients of WT versus B7-H4$^{-/-}$ donor T-cells; $P < 0.05$ on d17 and d21, $P < 0.009$ on d14 and d24, $P < 0.0001$ on d10. (K and L) Lethally irradiated B6 recipients were infused with 10^7 WT BALB/c BM cells alone, or with 1×10^6 or 3×10^6 WT BALB/c or B7-H4$^{-/-}$ T-cells (n = 8–9/group). (**K**) Kaplan-Meier survival plot represents data from one experiment (BM + 3×10^6 T-cells: recipients of WT versus B7-H4$^{-/-}$ donor T-cells, $P = 0.04$). (**L**) Transplanted mice were evaluated for clinical GVHD. (A, C–H, J, and L) Data represent mean ± SEM. *P* values were calculated by 2-tailed *t* test (A, C–H, and J) or log-rank test (I and K). $**P < 0.01$, $***P < 0.001$, $****P < 0.0001$.

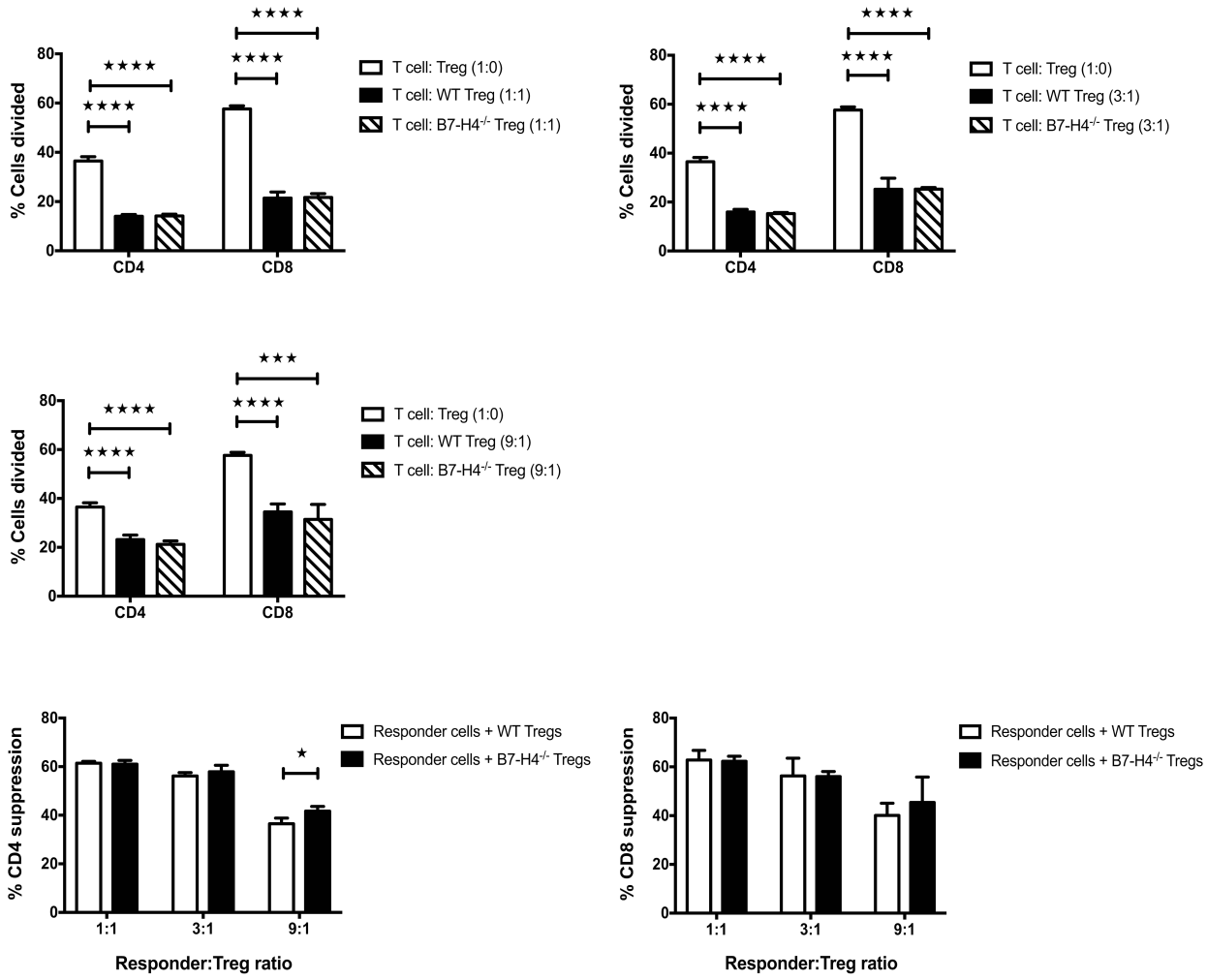

Figure S9. B7-H4$^{-/-}$ Tregs were as effective as WT Tregs in reducing CD3 driven T-cell proliferation in vitro. Tregs were isolated from naive WT BALB/c or B7-H4$^{-/-}$ mice. Naive B6 Ly5.2 mice splenocytes labeled with CFSE were used as responder cells. Responder cells were stimulated with anti-mCD3 Ab and were cultured with or without freshly isolated Tregs. T-cell proliferation was determined by CFSE dilution on day 4 using flow cytometry. *P* values were calculated by 2-tailed *t* test. $*P < 0.05$, $***P < 0.001$, $****P < 0.0001$. Data were obtained from 1 experiment.

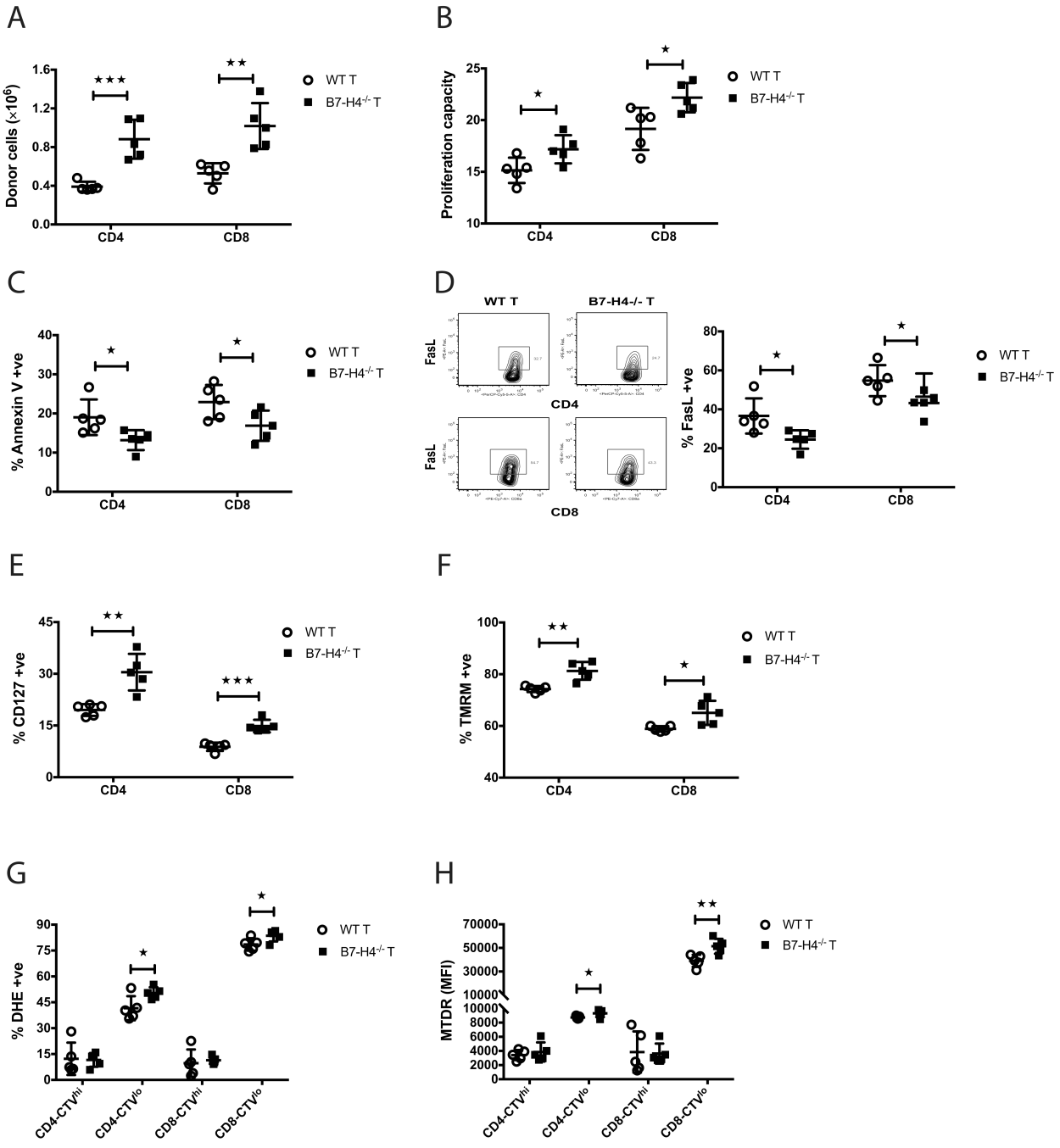

Figure S10. B7-H4$^{-/-}$ donor T-cells have increased proliferation and reduced apoptosis. Lethally irradiated B6 recipients were infused with 10^7 WT BALB/c BM cells plus 6×10^6 CFSE-labeled WT BALB/c or B7-H4$^{-/-}$ purified T-cells. Mice were sacrificed on day 5 after BMT, and splenoctes were analyzed by flow cytometry. Donor CD4 and CD8 T-cells were analyzed for total cell number (**A**), CFSE dilution (**B**), Annexin V (**C**), FasL (**D**), or TMRM (**F**) expression. (**E**) Lethally irradiated B6 recipients were infused with 10^7 WT BALB/c BM cells plus 2×10^6 WT BALB/c or B7-H4$^{-/-}$ purified T-cells. Mice were sacrificed on day 5 after BMT and donor T-cells in spleens were analyzed for the expression of CD127. (G and H) Lethally irradiated B6

recipients were infused with 10^7 WT BALB/c BM cells plus 6×10^6 CTV-labeled WT BALB/c or B7-H4$^{-/-}$ purified T-cells. Mice were sacrificed on day 5 after BMT, and splenocytes were analyzed by flow cytometry for the expression of DHE (**G**) or MTDR (**H**) in undivided (CTV^{hi}) and divided (CTV^{lo}) donor T-cells. (A–H) Data are representative of at least 5 mice per group from 2–3 independent experiments. MFI, mean fluorescence intensity. Data represent mean ± SEM, and *P* values were calculated by 2-tailed *t* test. $*P < 0.05$, $**P < 0.01$, $***P < 0.001$.

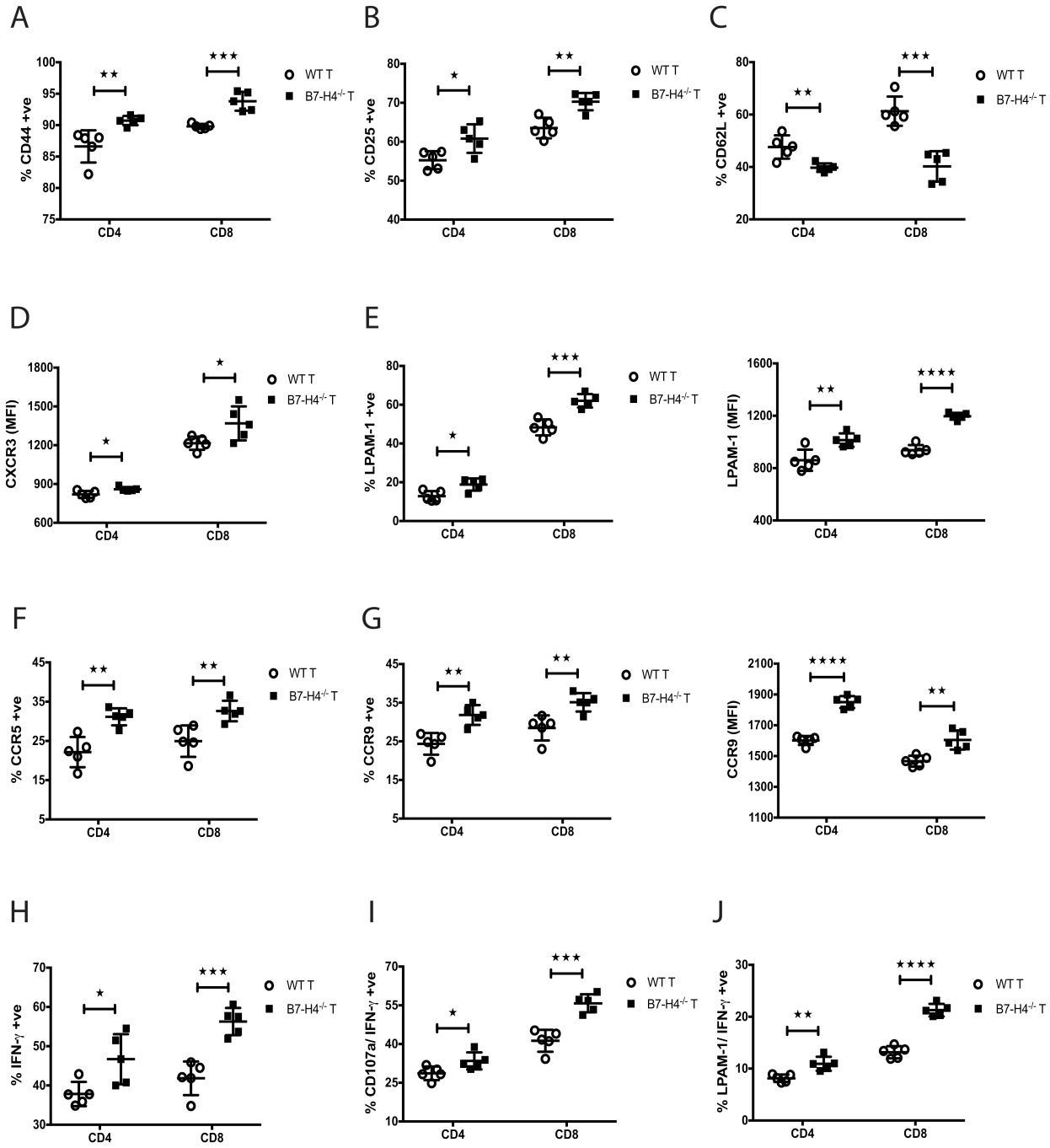

Figure S11. Increased Teffector function is central to B7-H4$^{-/-}$ donor T-cell mediated GVHD acceleration. Lethally irradiated B6 recipients were infused with 10^7 WT BALB/c BM cells plus 2×10^6 WT BALB/c or B7-H4$^{-/-}$ purified T-cells. Mice were sacrificed (n = 5–6/group) on day 7 after BMT, and donor CD4 and CD8 T-cells in spleen were analyzed by flow cytometry

for the surface expression of CD44 (**A**), CD25 (**B**), CD62L (**C**), CXCR3 (**D**), LPAM-1 (**E**), CCR5 (**F**), or CCR9 (**G**). Donor T-cells were also analyzed for the intracellular expression of IFN-γ (**H**), CD107a/IFN-γ (**I**), or LPAM-1/IFN-γ (**J**). MFI, mean fluorescence intensity. (A–J) Data are representative of 2 independent experiments. Data represent mean ± SEM. *P* values were calculated by 2-tailed *t* test. $*P < 0.05$, $**P < 0.01$, $***P < 0.001$, $****P < 0.0001$.

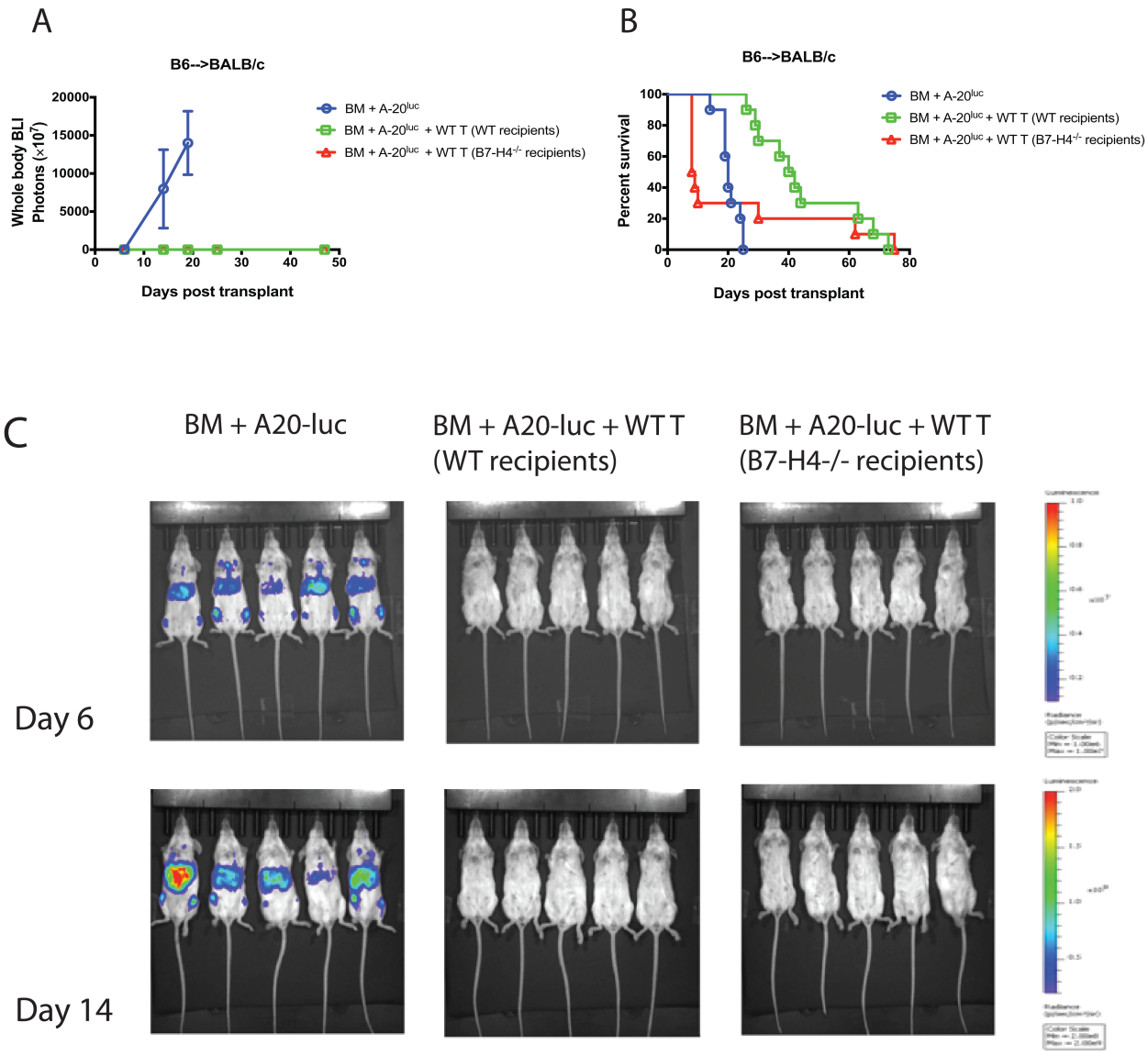

Figure S12. The GVL effect is retained in B7-H4$^{-/-}$ recipients of WT donor T-cells. Lethally irradiated WT BALB/c recipients (n = 10 mice) were infused with 10^7 T-cell depleted WT B6 BM cells plus 1×10^6 A20^{luc}-lymphoma cells, or lethally irradiated WT BALB/c or B7-H4$^{-/-}$ recipients (n = 10 mice per group) were infused with 10^7 T-cell depleted WT B6 BM cells plus 1×10^6 A20^{luc}-lymphoma cells along with 0.5×10^6 WT B6 purified T-cells on day 0. (**A**) Tumor growth was monitored by luciferase imaging on day 6, day 14, day 19, day 25, and day 47 after BMT. BLI, bioluminescence imaging. (**B**) Kaplan-Meier survival plot of transplanted mice is shown (BM + T-cells: WT versus B7-H4$^{-/-}$ recipients, $P = 0.016$). P value was calculated by wilcoxon test. (**C**) In vivo BLI of A20^{luc}-lymphoma cells on day 6 and day 14 after BMT. The scale to the right of the images describes the color map for the photon count. (A–C) Data were obtained from 1 experiment.