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Supplemental information

The subunits of IL-12, originating from two distinct cells, can functionally synergize to protect against pathogen dissemination *in vivo*

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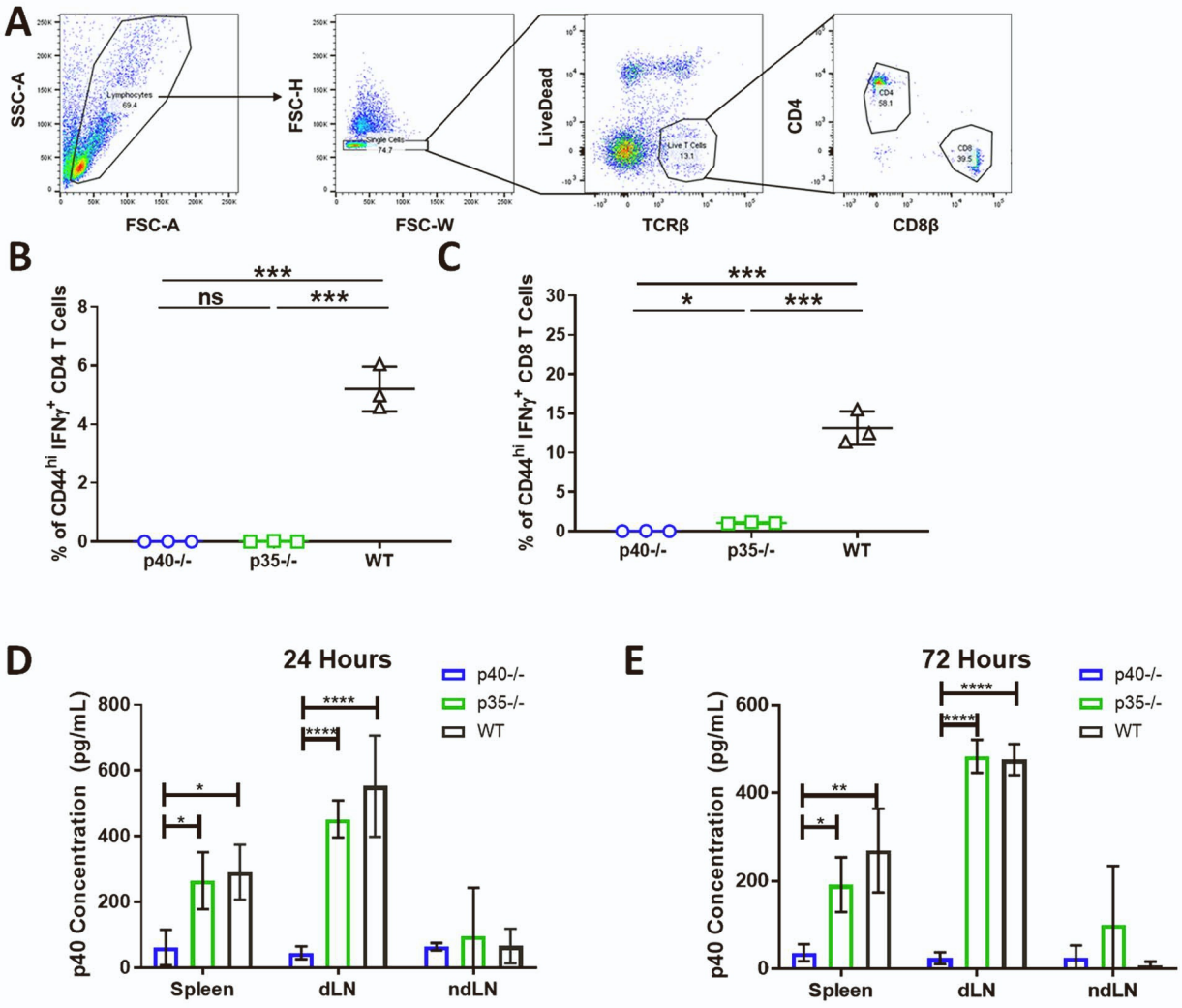

Figure S1. Supplemental Data for Figure 1

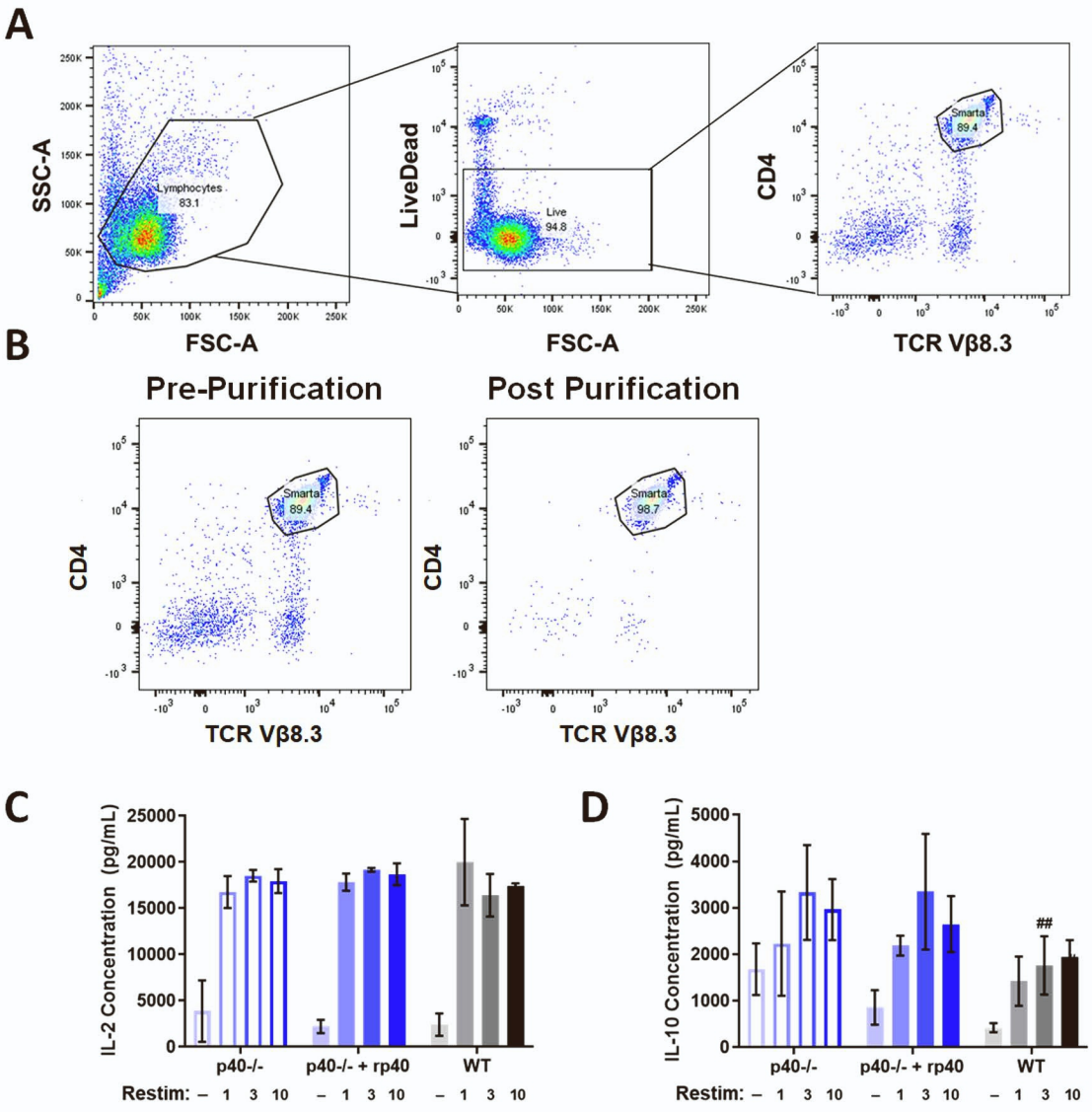

Figure S2. Supplemental Data for Figure 2

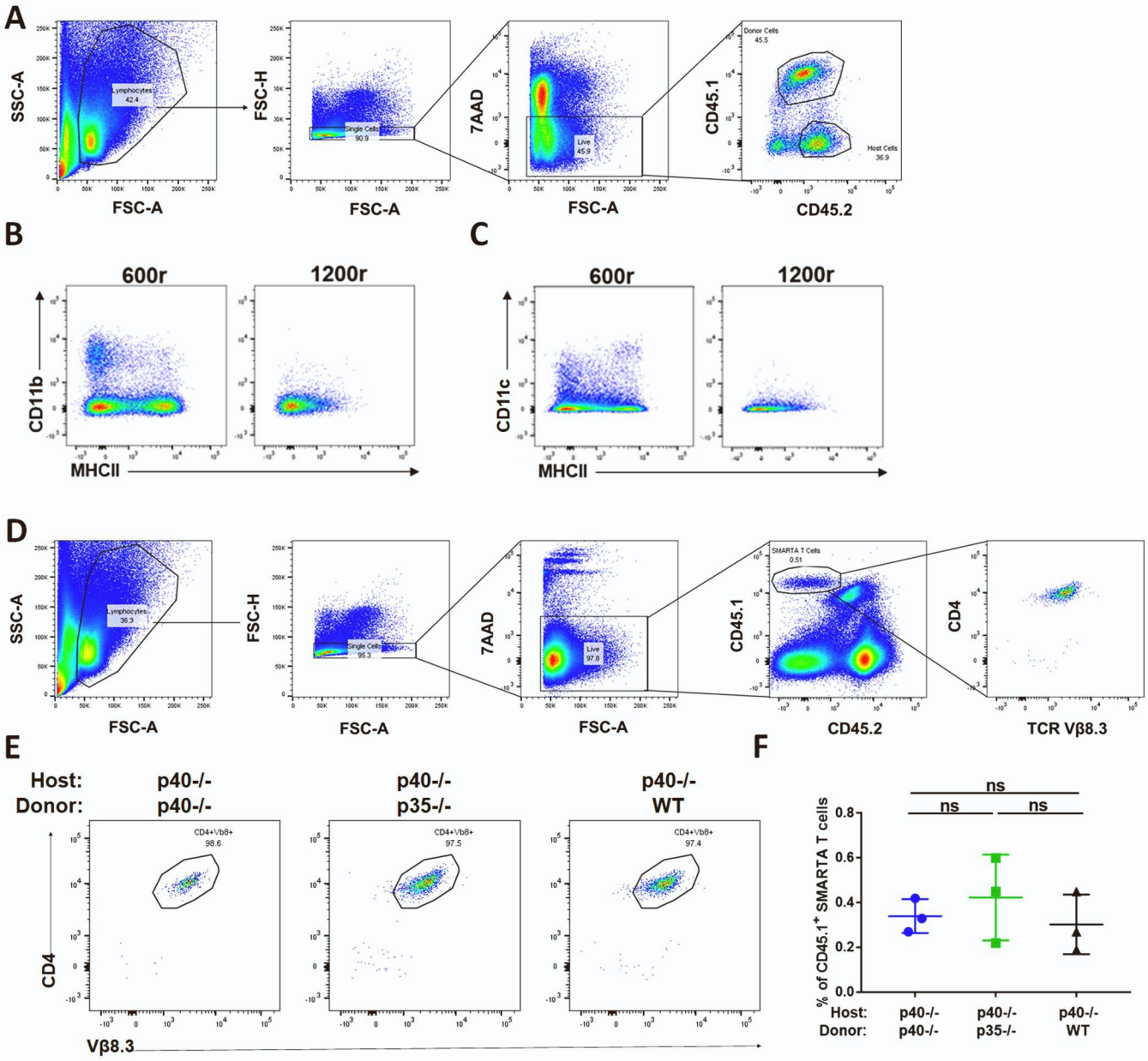

Figure S3. Supplemental Data for Figure 3

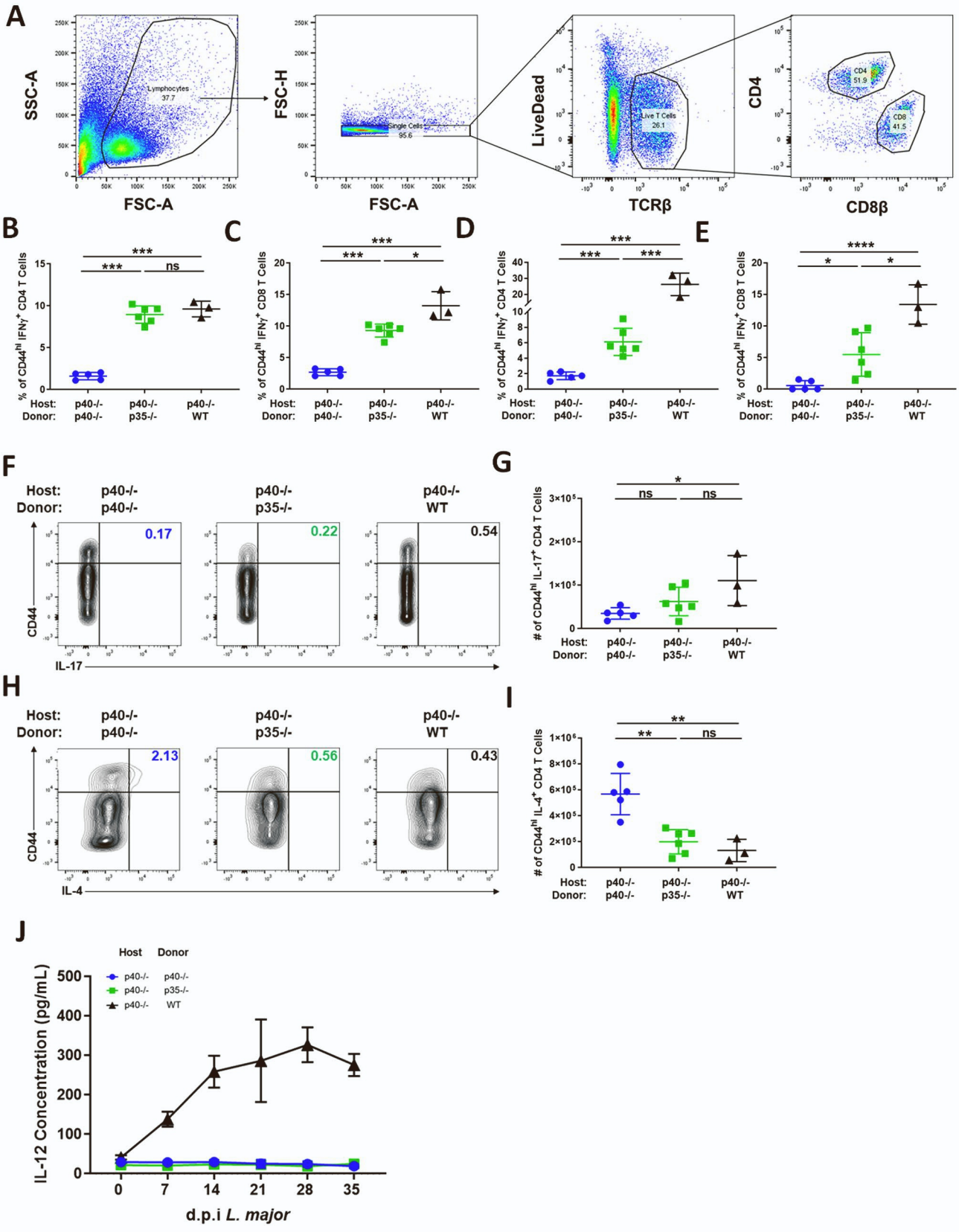

Figure S4. Supplemental Data for Figure 4

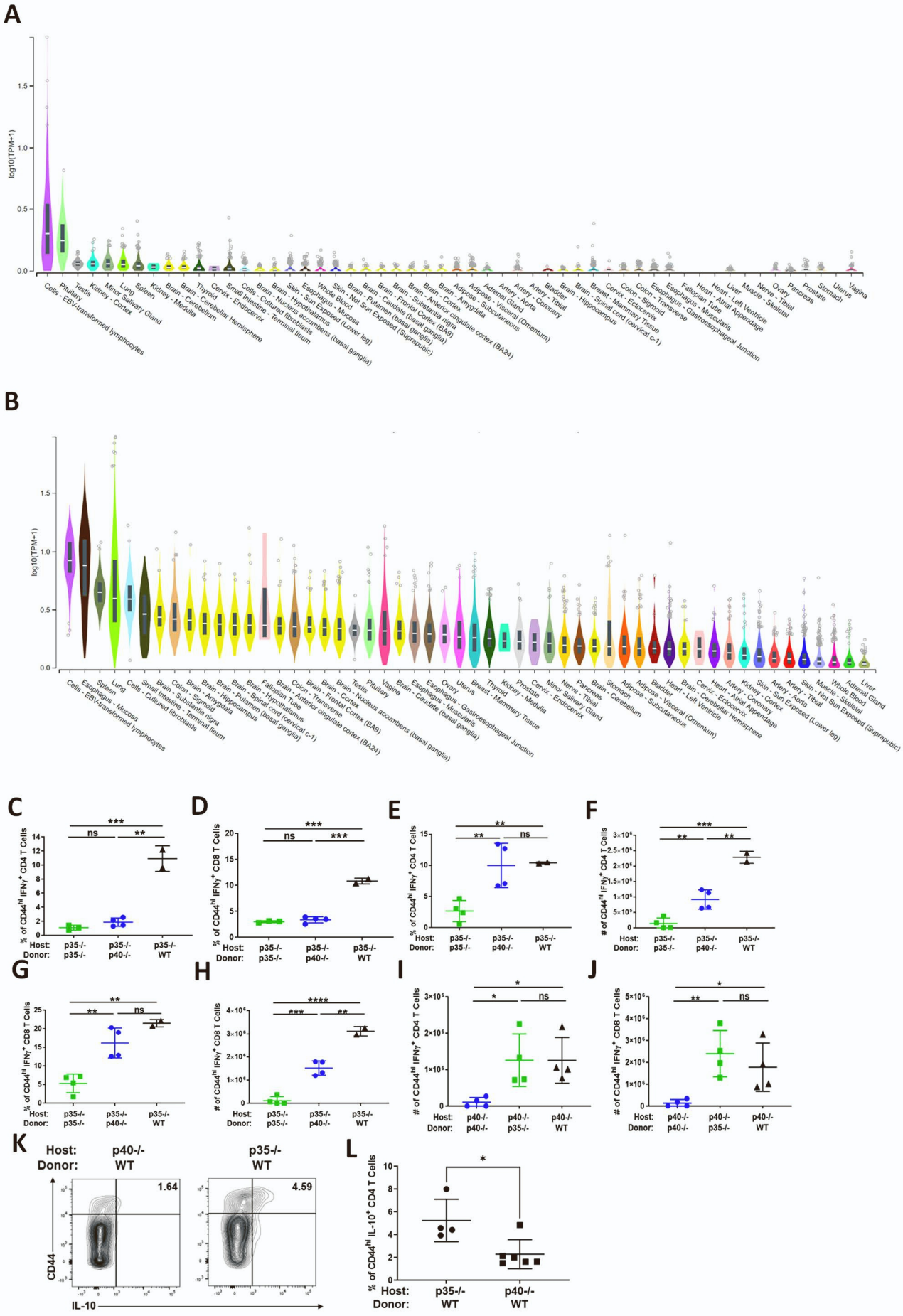

Figure S5. Supplemental Data for Figure 5

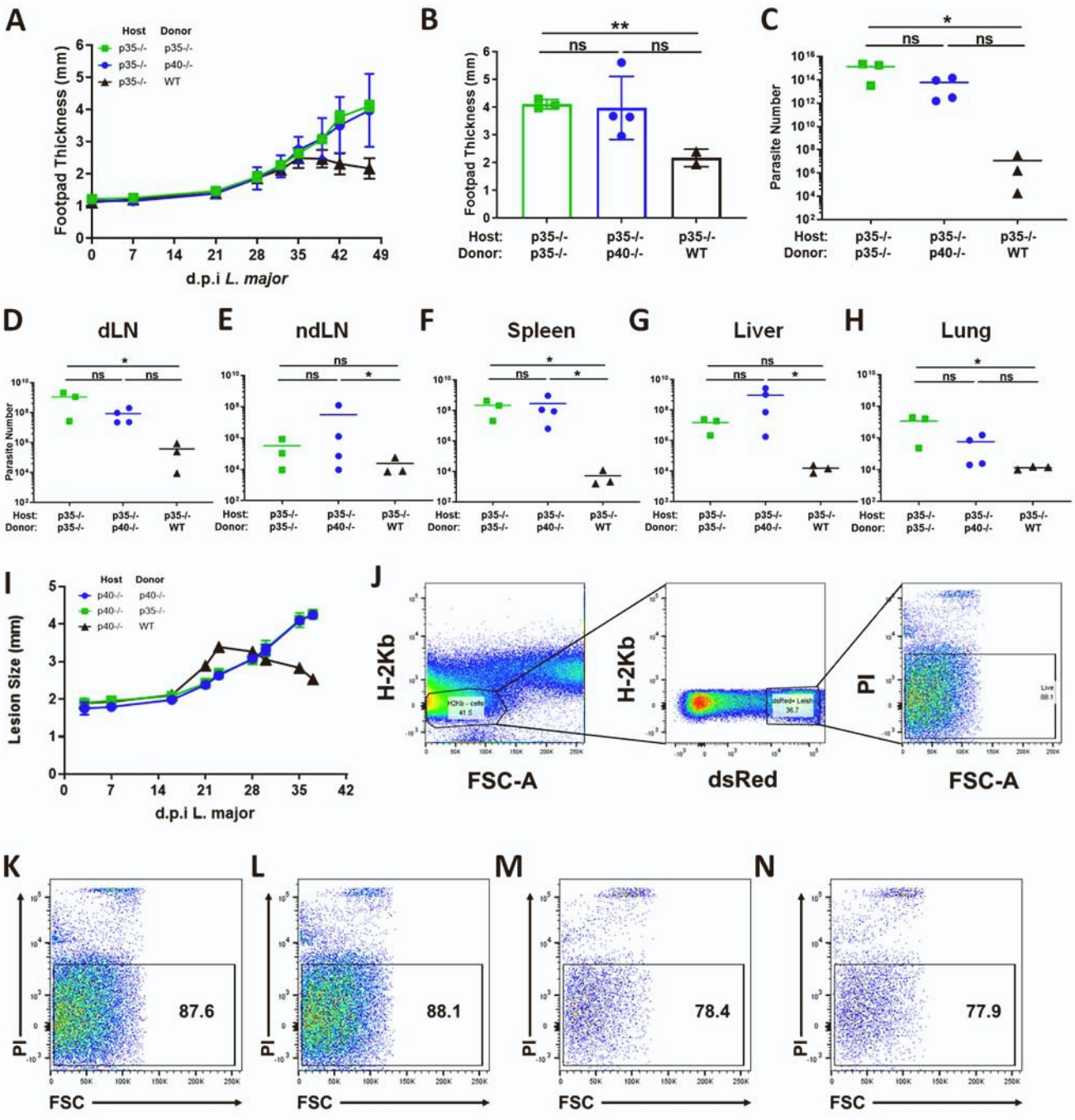

Figure S6. Supplemental Data for Figure 6

Supplemental Information

Figure S1. T cell differentiation is disrupted in p40-/- and p35-/- mice. Related to Figure 1.

(**A**) Gating strategy used for Figure 1D-G

(**B**) Percentage of $CD44^{hi}$ $IFN\gamma^{+}$ $CD4^{+}$ T cells in the spleen of p40-/- (open circles, blue), p35-/- (open squares, green) and WT (open triangles, black) animals measured 28 days after infection (unpaired t test, n=3)

(**C**) Same as in (**B**) but $CD8^{+}$ T cells(unpaired t test, n=3)

(**D-E**) Cells from the spleen, draining lymph node (dLN) and non-draining lymph node (ndLN) were harvested 28 days after infection and incubated *in vitro*. Concentration of p40 in supernatants by tissues collected from p40-/- (blue), p35-/- (green) and WT (black, shaded) after (**D**) 24 and (**E**) 72 hours. (unpaired t test, n=3)

Data from this figure are representative of two independent experiments where the n indicates biological replicates. (B-E) are displayed as mean ± SEM. Significance is indicated as follows: $p<0.05$ = * ; $p<0.01$ = ** ; $p<0.001$ = *** ; $p<0.0001$ = ****. Anything not marked is considered not statistically significant

Figure S2. Purification of SMARTA T cells and cytokine production in response to rp40 administration, related to Figure 2

(**A**) Gating strategy used to verify purity of SMARTA-tg T cells in Figure 2.

(**B**) Purity of SMARTA-tg T cells after Dynabead Magnetic Sorting

(**C-D**) Splenocytes harvested 5 days after challenge were normalized to SMARTA T cell number and re-stimulated in vitro with GP_{61-80} for 48h. Re-stimulation doses are in µg. Concentration of (**C**) IL-2, and (**D**) IL-10 in supernatant from splenocytes of SMARTA T cells transferred into p40-/- animals treated with PBS (left, blue open bars) p40-/- animals given 1µg recombinant p40 (middle, blue closed bars) or WT animals (right, gray bars)

Data from this figure are representative of one independent experiment where the n indicates biological replicates. (C-D) are displayed as mean ± SEM. Significance is indicated as follows: $p<0.05$ = * ; $p<0.01$ = ** ; $p<0.001$ = *** ; $p<0.0001$ = ****. Anything not marked is considered not statistically significant.

Figure S3. SMARTA T cell adoptive transfer into reconstituted p40-/- host chimeric animals, related to Figure 3

(**A-C**) Bone marrow chimeras were generated by reconstituting $CD45.2^{+}$ p40-/- mice that were irradiated with either one or two doses of 600rad with bone marrow cells from $CD45.1^{+}CD45.2^{+}$ WT donor mice. Chimeric mice were analyzed for host cell depletion after a 6 week reconstitution period.

(**A**) Gating strategy for Supplemental Figure 2C-D

(**B**) $CD45.2^{+}$ host cell expression of $CD11b^{+}$ and $MHCII^{+}$ in chimeric animals that were irradiated with 600r (left) versus 1200r (right)

(**C**) $CD45.2^{+}$ host cell expression of CD11c and MHCII in chimeric animals that were irradiated with 600r (left)

versus 1200r (right)

(**D**) Gating strategy for Figure 2B

(**E**) Frequency of $CD4^+V\beta8.3^+$ T cells in Donor SMARTA gates shown in Figure 2B

(**F**) Frequencies of transferred SMARTA T cells in spleens of recipient chimeras 5 days after challenge (n=3).

Data from this figure are representative of one independent experiment where n indicates the number of biological replicates. (F) is displayed as mean ± SEM. Significance is indicated as follows: $p<0.05$ = * ; $p<0.01$ = ** ; $p<0.001$ = *** ; $p<0.0001$ = ****. Anything not marked is considered not statistically significant.

Figure S4. Cytokine responses by T cells in the presence or absence of two-cell IL-12, related to Figure 4

(**A**) Gating strategy used for Figure 3

(**B**) Percentage of $CD44^{hi}$ $IFN\gamma^+$ CD4+ T cells in the spleen of p40-/- host chimeras reconstituted with p40-/- (circles, blue), p35-/- (squares, green) or WT (triangles, black) donor bone marrow 35 days after infection (unpaired t test, p40-/-→p40-/- n =4; p35-/-→p40-/- n=6; WT→p40-/- n=3)

(**C**) Same as in (**B**) but $CD8^+$ T Cells (unpaired t test, n=3-6)

(**D**) Percentage of $CD44^{hi}$ $IFN\gamma^+$ CD4+ T cells in lymphocytes harvested from the footpad lesion of p40-/- host chimeras reconstituted with p40-/- (circles, blue) p35-/- (squares, green) or WT (triangles, black) donor bone marrow 35 days after infection (unpaired t test, p40-/-→p40-/- n =4; p35-/-→p40-/- n=6; WT→p40-/- n=3).

(**E**) Same as in (**D**) but CD8 T cells (unpaired t test, p40-/-→p40-/- n =4; p35-/-→p40-/- n=6; WT→p40-/- n=3).

(**F**) Percentage and (**G**) Number of $CD44^{hi}$ IL-17^+ CD4 T cells in the spleen of p40-/- host chimeras reconstituted with p40-/- (circles, blue), p35-/- (squares, green) or WT (triangles, black) donor bone marrow 35 days after infection (unpaired t test, p40-/-→p40-/- n =4; p35-/-→p40-/- n=6; WT→p40-/- n=3)

(**H**) Percentage and (**I**) Number of $CD44^{hi}$ IL-4^+ CD4 T cells in the spleen of p40-/- host chimeras reconstituted with p40-/- (circles, blue), p35-/- (squares, green) or WT (triangles, black) donor bone marrow 35 days after infection (unpaired t test, p40-/-→p40-/- n =4; p35-/-→p40-/- n=6; WT→p40-/- n=3)

(**J**) Infected animals were bled prior to infection (d0), and at d7, d14, d21, d28 and d35 post infection. Serum IL-12 levels for p40-/- host chimeras reconstituted with p40-/- (circles, blue) p35-/- (squares, green), and WT (triangles, black) animals were measured by ELISA (p40-/-→p40-/- n =4; p35-/-→p40-/- n=6; WT→p40-/- n=3).

Data from this figure are representative of two independent experiments where the n indicates the number of biological replicates. (B-E, G, I-J) are displayed as mean ± SEM. Significance is indicated as follows: $p<0.05$ = * ; $p<0.01$ = ** ; $p<0.001$ = *** ; $p<0.0001$ = ****. Anything not marked is considered not statistically significant.

Figure S5. Hematopoietic cell expression of p40 is critical for two-cell IL-12 formation, related to Figure 5

(**A**) GTEx Portal analysis for expression levels of p40 throughout different tissues

(**B**) GTEx Portal analysis for expression levels of p35 throughout different tissues

(**C-D**) Bone marrow chimeras were generated by reconstituting $CD45.2^+$ p35-/- mice irradiated with 1200rad with bone marrow cells from $CD45.1^+$ p40-/-, $CD45.2^+$ p35-/- or $CD45.1^+CD45.2^+$ WT donor mice. Chimeric mice were

infected in the footpad dermis with 10^6 dsRed *L. major* parasites following reconstitution. 35 days after infection, tissues were harvested and restimulated for intracellular cytokine staining and flow cytometry analysis. Gating strategy used for these panels is shown in Supplemental Figure 3A

(**C**) Percentage of $CD44^{hi}$ $IFN\gamma^+$ $CD4^+$ T cells in the spleen of 1200r p35-/- host chimeras reconstituted with p35-/- (squares, green), p40-/- (circles, blue) or WT (triangles, black) donor bone marrow 35 days after infection (unpaired t test, p35-/-→p35-/- n=3; p40-/-→p35-/- n=4; WT→p35-/- n=2)

(**D**) Same as in (**C**) but CD8 T cells (unpaired t test, p35-/-→p35-/- n=3; p40-/-→p35-/- n=4; WT→p35-/- n=2)

(**E-H**) Bone marrow chimeras were generated by reconstituting $CD45.2^+$ p35-/- mice irradiated with 600rad with bone marrow cells from $CD45.1^+$ p40-/-, $CD45.2^+$ p35-/- or $CD45.1^+CD45.2^+$ WT donor mice. Chimeric mice were infected in the footpad dermis with 10^6 dsRed *L. major* parasites following reconstitution. 35 days after infection, tissues were harvested and restimulated for intracellular cytokine staining and flow cytometry analysis. Gating strategy used for these panels is shown in Supplemental Figure 3A

(**E**) Percentage and (**F**) Number of $CD44^{hi}$ $IFN\gamma^+$ $CD4^+$ T cells in the spleen of 600r p35-/- host chimeras reconstituted with p35-/- (squares, green), p40-/- (circles, blue) or WT (triangles, black) donor bone marrow 35 days after infection (unpaired t test, p35-/-→p35-/- n=3; p40-/-→p35-/- n=4; WT→p35-/- n=2)

(**G**) Same as in (**E**) but $CD8^+$ T cells (unpaired t test, p35-/-→p35-/- n=3; p40-/-→p35-/- n=4; WT→p35-/- n=2)

(**H**) Same as in (**F**) but $CD8^+$ T cells (unpaired t test, p35-/-→p35-/- n=3; p40-/-→p35-/- n=4; WT→p35-/- n=2)

(**I**) Number of $CD44^{hi}$ $IFN\gamma^+$ $CD4^+$ T cells in the spleen of 600r p40-/- host chimeras reconstituted with p40-/- (circles, blue), p35-/- (squares, green) or WT (triangles, black) donor bone marrow 35 days after infection (unpaired t test, n=4)

(**J**) Same as in (**I**) but $CD8^+$ T cells (unpaired t test, n=4)

(**K-L**) Percentage of $CD44^{hi}$ IL-10^+ CD4+ T cells in the spleen of p35-/- or p40-/- host chimeras reconstituted with WT bone marrow 35 days after infection (unpaired t test, n=4-6).

Data from this figure is representative of two independent experiments where the n refers to the number of biological replicates. (C-J, L) are displayed as mean ± SEM. Significance is indicated as follows: $p<0.05$ = * ; $p<0.01$ = ** ; $p<0.001$ = *** ; $p<0.0001$ = ****. Anything not marked is considered not statistically significant.

Figure S6. Control of *L. major* parasite burden in p35-/- host chimeric animals, related to Figure 6

(**A-H**) Bone marrow chimeras were generated by reconstituting irradiated $CD45.2^+$ p35-/- mice with bone marrow cells from $CD45.1^+$ p40-/-, $CD45.2^+$ p35-/- or $CD45.1^+CD45.2^+$ WT donor mice. Chimeric mice were infected in the footpad dermis with 10^6 dsRed *L. major* parasites following reconstitution.

(**A**) Biweekly measurement of footpad thickness at the midline following establishment of visible lesion for p35-/- host chimeras reconstituted with p35-/- (squares, green), p40-/- (circles, blue) or WT (triangles, black) through the duration of infection (p35-/-→p35-/- n=3; p40-/-→p35-/- n=4; WT→p35-/- n=2)

(**B**) Footpad thickness at the midline of p35-/- host chimeras reconstituted with p35-/- (squares, green), p40-/- (circles, blue) or WT (triangles, black) 35 days post infection (p35-/-→p35-/- n=3; p40-/-→p35-/- n=4; WT→p35-/- n=2)

(**C-H**) dsRed *L. major* parasite quantified by limiting dilution from (**C**) footpad lesion, (**D**) draining popliteal lymph node (dLN), (**E**) non-draining popliteal lymph node (ndLN), (**F**) spleen, (**G**) liver and (**H**) lung tissue of p35-/- host chimeras reconstituted with p35-/- (squares, green), p40-/- (circles, blue) or WT (triangles, black) 35 days post infection (unpaired t test, p35-/-→p35-/- n=3; p40-/-→p35-/- n=4; WT→p35-/- n=2)

(**I**) Bone marrow chimeras infected with 10^6 WT *L. major* parasite for comparison to chimeras infected with dsRed *L. major* parasite in Figure 6A. (n=4)

(**J-N**) p40-/- animals were infected with dsRed *L. major* in the footpad dermis. Following 22 days, tissues were harvested and stained for the presence of live, dsRed parasites via flow cytometry. (**J**) Gating strategy to differentiate live and dead dsRed *L. major* in different tissues during infection. Cells were gated on $H2Kb^-$, small cells that were $dsRed^+$ and viability measured by PI staining. The proportion of live dsRed+ parasites in the (**K**) lymph node, (**L**) spleen, (**M**) lung and (**N**) liver is shown.

Data from this figure is representative of two independent experiments where the n refers to the number of biological replicates. (A-I) are displayed as mean ± SEM. Significance is indicated as follows: $p<0.05$ = * ; $p<0.01$ = ** ; $p<0.001$ = *** ; $p<0.0001$ = ****. Anything not marked is considered not statistically significant.