SUPPLEMENTAL MATERIAL:

Figure S1. Normal CTL effector function in *Def6*$^{-/-}$ OT-I CD8^{+} T cells.

A, Naïve WT B6 (CD45.2^{+}) mice received 5000 OT-I WT (CD45.1^{+}) or *Def6*$^{-/-}$ OT-I (CD45.1^{+}) CD8^{+} T cells. One day later, mice were challenged with 5 x 10^{6} Act-mOVA/K$^{b-/-}$ splenocytes. ***A,*** Data are presented as the frequency of expanded OT-I cells (CD45.1^{+} H-2K^{b}-OVA tetramer^{+}) generated in WT recipient mice (first and third panels from left) and % of specific killing of specific target cells pulsed with $OVA_{257-264}$ and control, irrelevant target cells (Ski9) (second and fourth panels from left). ***B***, *In vitro* killing of target cells by WT and KO OT-I CD8^{+} T cells at the indicated effector:target (E:T) ratios was evaluated by measuring ^{3}H retention in target cells, as described in Materials and Methods. Data are presented as percent ± SD of specific cytotoxicity of quadruplicate samples and are representative of three independent experiments. ***C,*** Cell surface expression of CD107a was analyzed in CD8^{+} OT-I recovered from immunized mice and restimulated *in vitro* with OVA peptide in the presence of Brefeldin A for 5 h.

Figure S2. Impaired secondary expansion of *Def6*$^{-/-}$ CD8^{+} T cells in response to LM-OVA infection is rescued by constitutive Cdc42 or NFAT1 activation.

Naive WT B6 (CD45.1/2^{+}) mice (n=5) received 50 WT OT-I (CD45.1^{+}) and 50 *Def6*$^{-/-}$ OT-I (CD45.2^{+}) CD8^{+} T cells. One day later, the mice were challenged with 5x10^{6} Act-mOVA/K$^{b-/-}$ splenocytes. On day 7, the mice were rechallenged with 1 x 10^{7} cfu of Act-A^{-} Lm-OVA followed by analysis on day 12. ***A,*** Representative FACS plots of WT and KO OT-I CD8^{+} T cell frequency in peripheral blood. ***B,*** Average ratio of WT to KO OT-I CD8^{+} T cells per mouse. The WT/KO ratio in non-immunized animals is set at 1. ***C,*** Average absolute numbers ± SEM of WT and KO OT-I CD8^{+} T cells in spleens from recipient mice on day 12. ***D***, Average expression ±

SEM of T-Bet and Eomes by WT and KO OT-I $CD8^+$ T cells 7 days after immunization. Data are representative of at least three independent experiments. ***E,*** Average frequency ± SEM of OT-I cells that expres KLRG-1 and CD127 in the peripheral blood. ***F,*** Average frequency ± SEM of WT and KO OT-I $CD8^+$ T cells producing IFN-γ alone (IFN-γ^+TNF-α^-) or both IFN-γ and TNF-α (IFN-γ^+TNF-α^+) among total splenocytes determined by ICS 12 days post-immunization. **G,** The $NFAT1^{CA}\Delta$dimer mutant protein can constitutively transactivate an NFAT-responsive reporter luciferase construct. Jurkat-TAg cells were cotransfected with an empty control vector (empty vector; 10 µg) or with an $NFAT^{CA}\Delta$dimer-expressing $NFAT^{CA}\Delta$dimer, together with NFAT-luciferase reporter (10 µg) plus a β-Gal (5 µg) reporter gene. Cells were left unstimulated for 16 hr and normalized luciferase activity (mean ± SD) was measured in triplicates. NFAT expression was detected using an anti-HA Ab, and actin immunoblotting served as a loading control. **H-I,** Constitutive Cdc42 or NFAT1 activation rescue secondary expansion of *Def6*$^{-/-}$ $CD8^+$ T cells. Retrogenic mice generation, adoptive transfer, priming with Act-mOVA/$K^{b-/-}$ splenocytes and challenge with LM-OVA were performed as in **Fig. 5**. Analysis was performed 5 days after LM-OVA challenge. ***H,*** Average frequency (mean ± SEM, n=4-5) of WT and KO OT-I $CD8^+$ T cells among peripheral blood $CD8^+$ cells. ***I***, Average ratio of WT to KO OT-I $CD8^+$ T cells. The ratio in non-immunized animals is set at 1.

Figure S3. *Def6* deficiency results in defective $CD8^+$ T cell response to *Listeria monocytogenes* infection.

Fifty naïve WT ($CD45.1^+$) and $Def6^{-/-}$ (KO; $CD45.2^+$) OT-I $CD8^+$ T cells were adoptively co-transferred into $CD45.1/2^+$ B6 recipient that were immunized one day later with 3000 cfu OVA-expressing *Listeria monocytogenes* (LM-OVA) followed by analysis at day 7 (primary), at day 49 (memory), and at day 54 (secondary) in mice that had received $1x10^7$ cfu OVA-expressing *Listeria monocytogenes* Act A deficient rechallenge 5 days earlier. Frequency of WT and KO

OT-I $CD8^+$ T cells in the blood from recipient mice (n=6) is shown. *P<0.05, ***P<0.0005 (two-tailed unpaired *t*-test).

Figure S4. Defective IL-2 production of $Def6^{-/-}$ $CD8^+$ T cells upon CD3/CD28 stimulation *in vitro*.

A, Purified peripheral $CD8^+$ T cells from WT and $Def6^{-/-}$ (KO) mice were stimulated with the indicated concentrations of plate-coated anti-CD3 plus soluble CD28 mAbs for 40 hours. **A**, [^{3}H]thymidine was added for the final 16 hours, and uptake was measured. **B,** $CD8^+$ T cells were stimulated for 24 hours as in **A**, and IL-2 production was measured by an ELISA. **C,** $CD8^+$ T cells from WT and KO mice were stimulated with the indicated concentrations of anti-CD3 plus 2.5μg/ml anti-CD28 mAbs for 16 hours, and analyzed by flow cytometry for CD25 and CD69 expression. Results are expressed as mean ± SD. Statistical differences were determined as in **Figure 1**. ***P<0.0005 (two-tailed unpaired *t*-test). Data are representative of three independent experiments.

Figure S1, Feau et al.

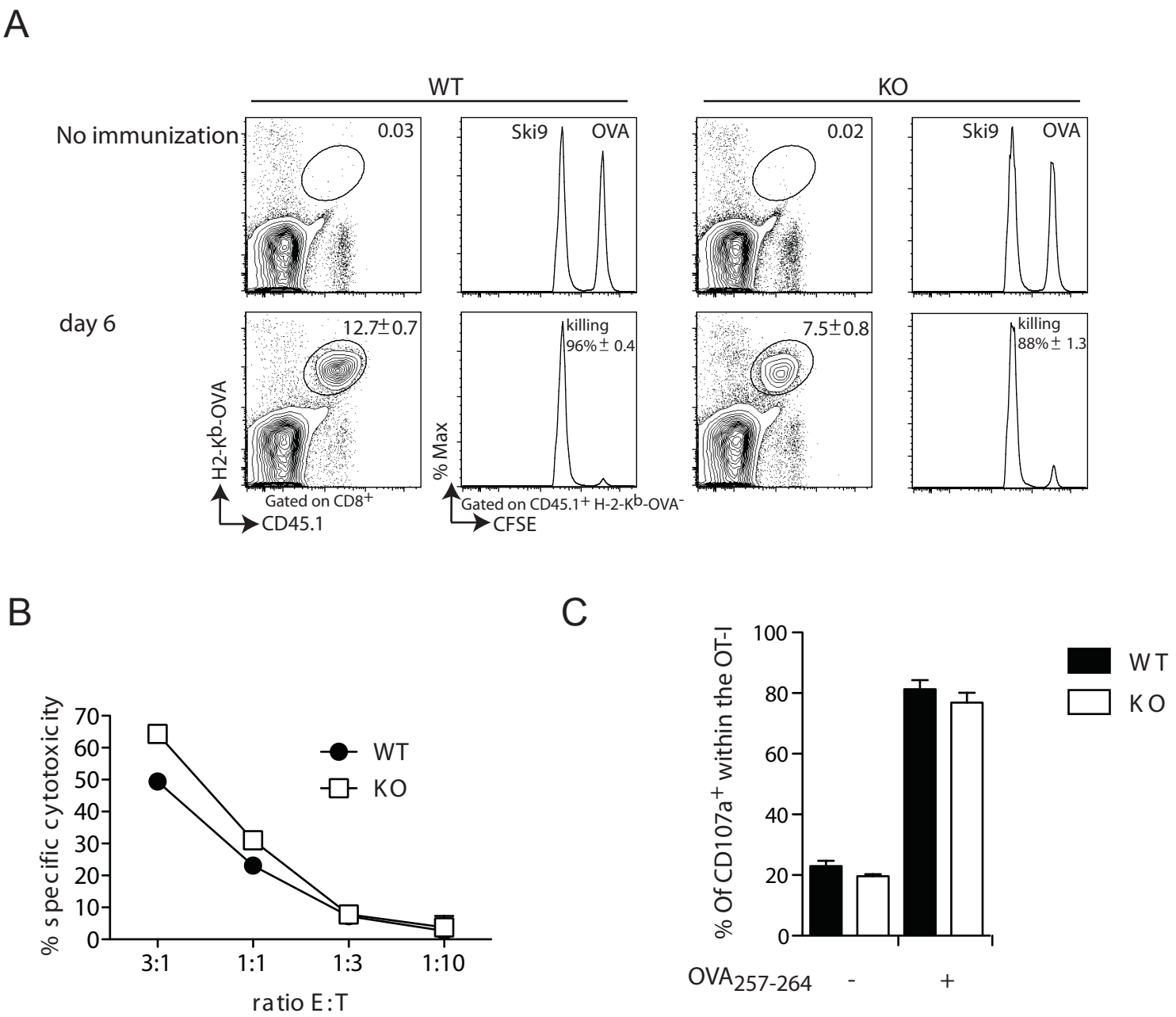

Figure S2, Feau et al.

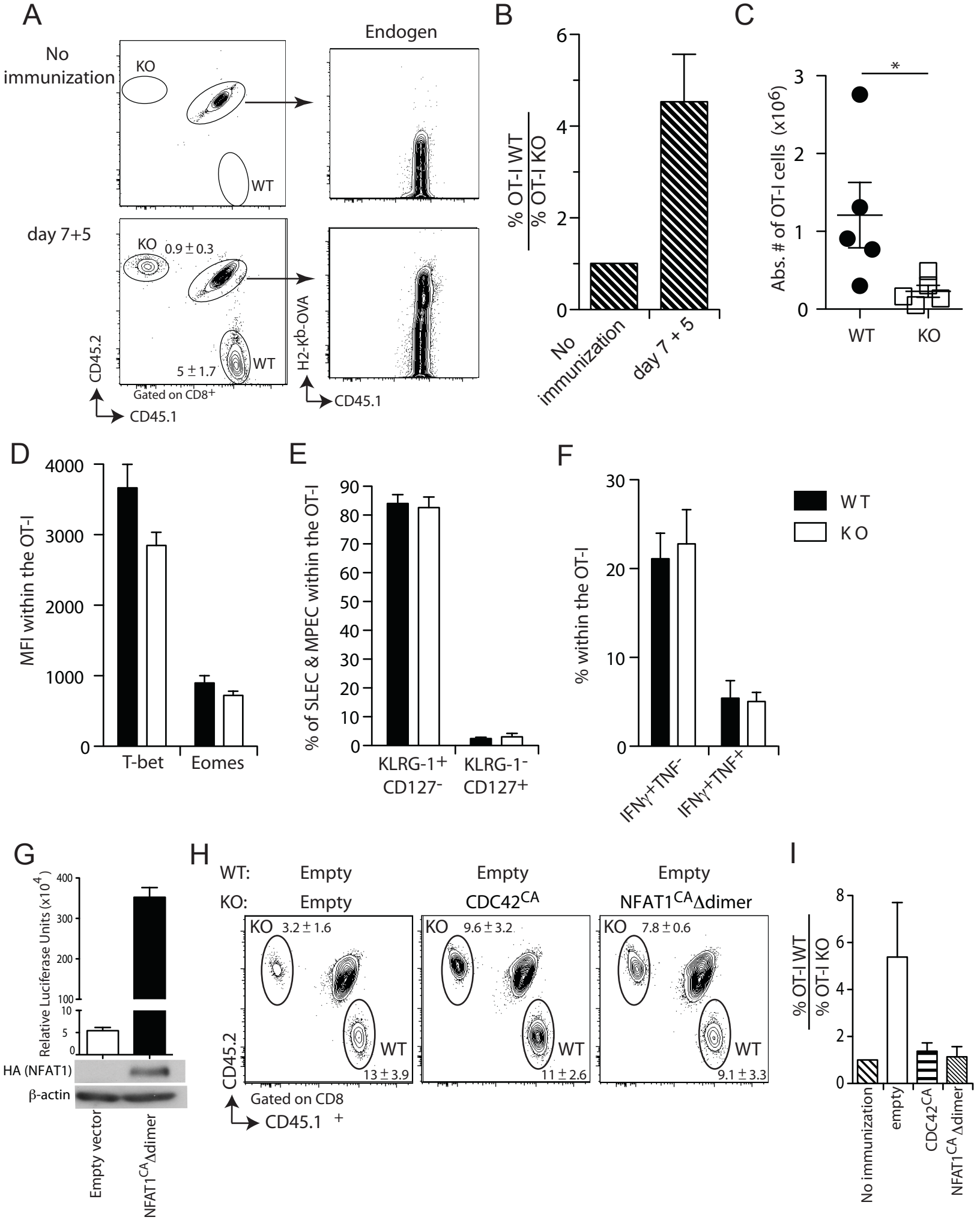

Figure S3, Feau et al.

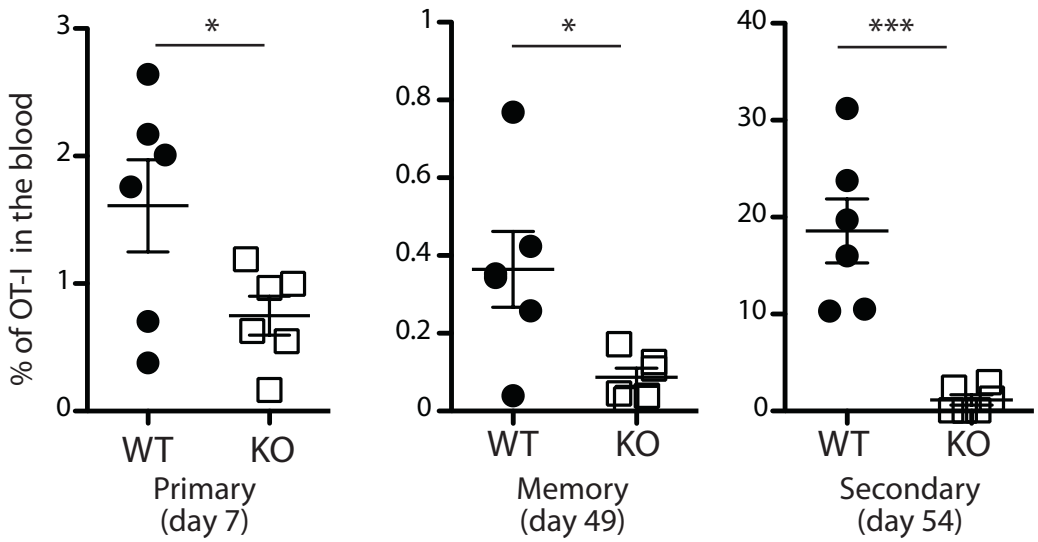

Figure S4, Feau et al.