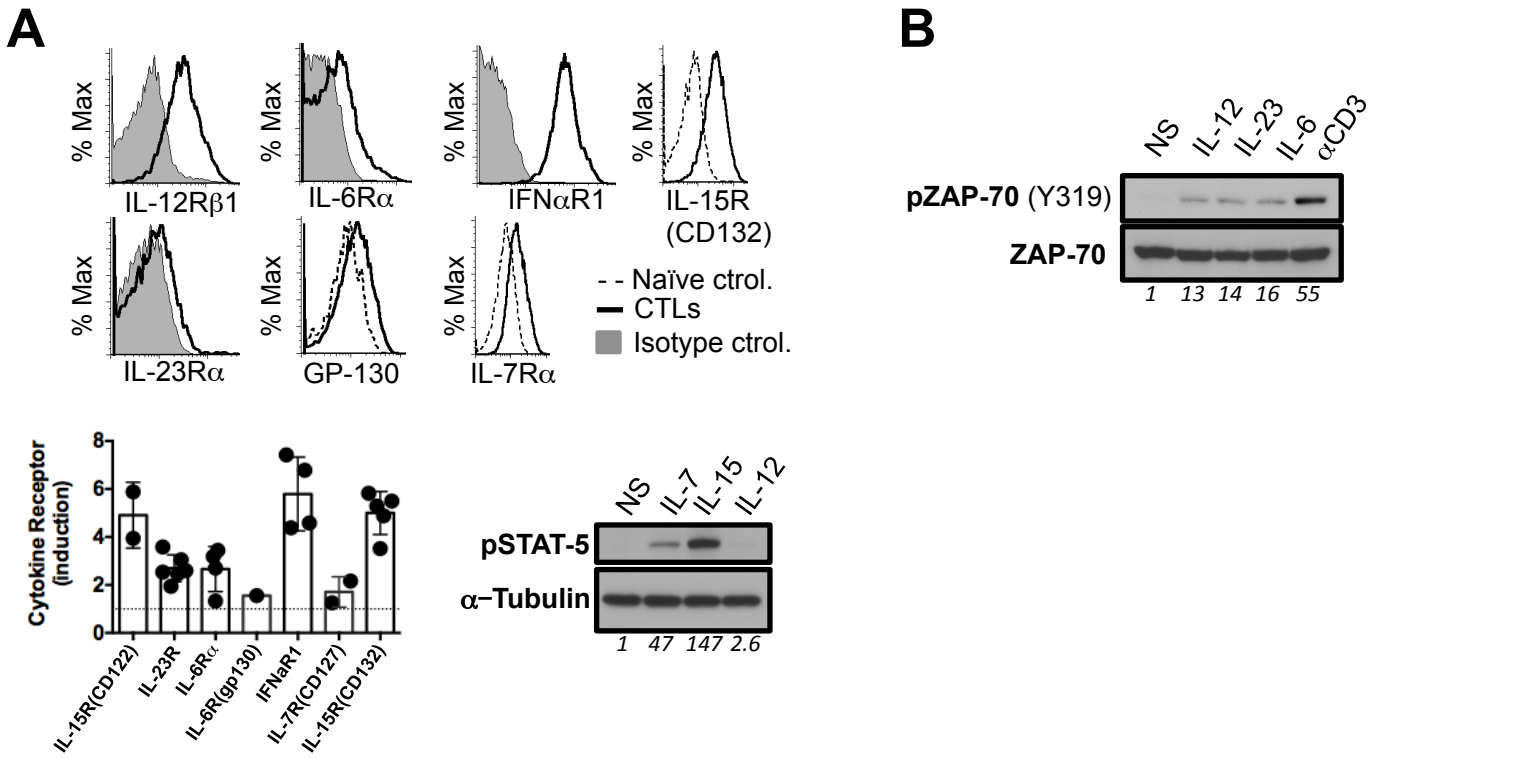
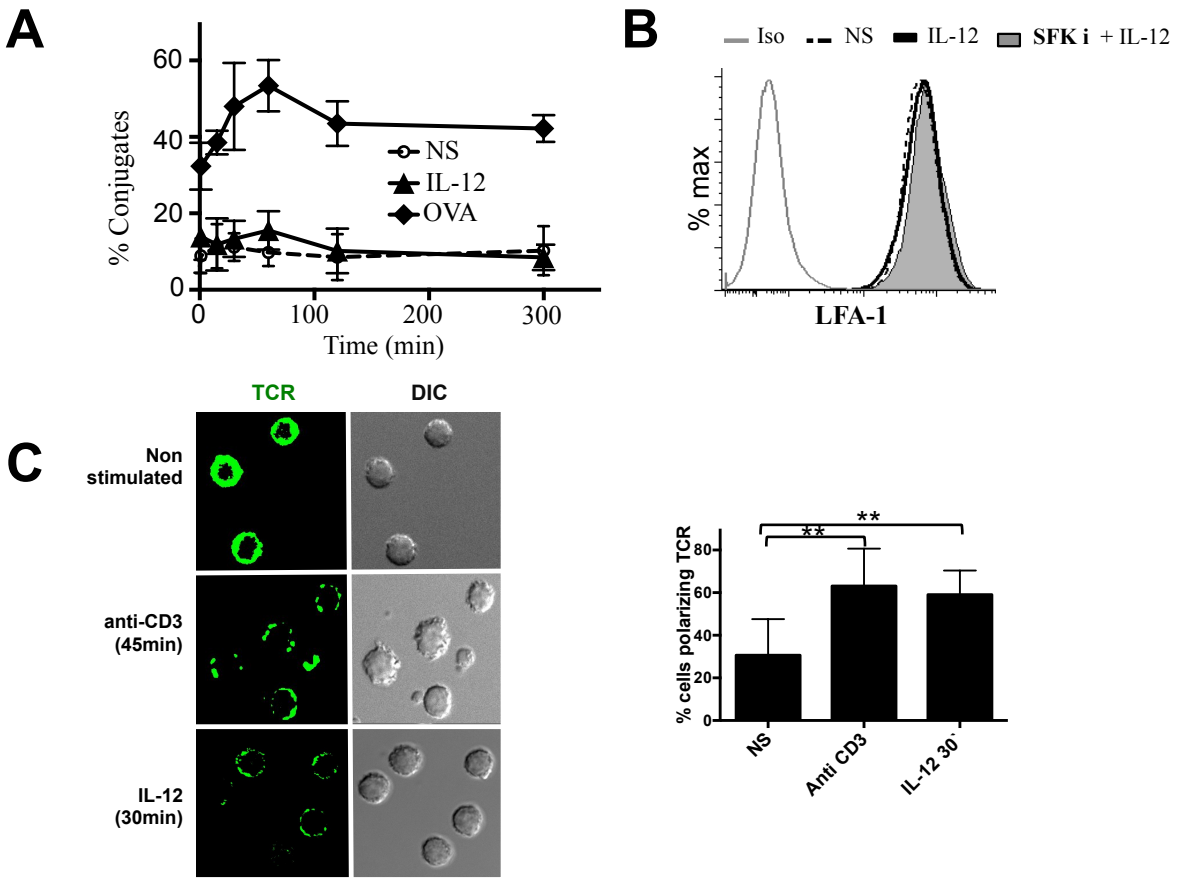
Supplemental Fig. 1
related to Figure 1



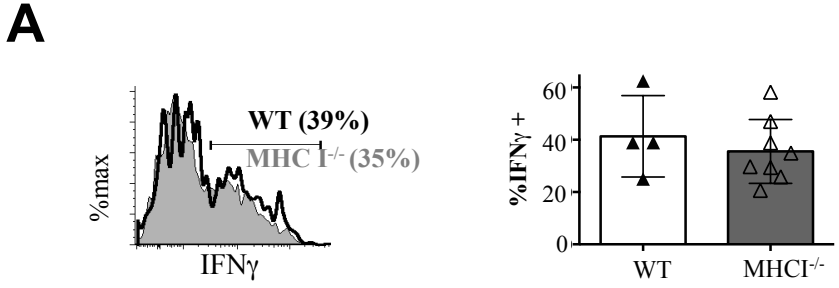
Supplemental Fig. 1. Pro-inflammatory cytokines induce the phosphorylation of ZAP-70 in Effector CD8 T cells. Day 3 polyclonal CTLs generated upon stimulation of anti-CD3/28 were assayed for surface expression of the indicated cytokine receptor subunits by flow cytometry (top histograms and bar graph) or stimulated with 10 ng/mL of the indicated cytokines for 15 minutes followed by immunoblot with antobodies specific for pSTAT-5 (Y694) or B) pZAP-70 (Y319). Tubulin and ZAP-70 loading controls were determined in the same ge l(A) or after stripping (B)Bar graph indicates induction of the subunit of the cytokine receptor indicated relative to control.

Supplemental Fig. 2 related to Figure 3



Supplemental Fig. 2. Bystander activation does not influence conjugate formation or LFA-1 expression. However, IL-12 stimulation enhances TCR/CD3 clustering (A) Day 3 CTLs were mixed 1:1 with congenically marked APCs pulsed with OVAp as a positive control or not pulsed in the presence of IL-12 or in the absence of IL-12 (NS). % Conjugates were determined by flow cytometry as described in (Teixeiro et al., 2009). (B) CTLs were cultured as in figure 3D before assessing LFA-1 expression by flow cytometry. Graphs show mean±SD with data from 3 independent experiments. (C) Day 3 CTLs were stimulated for 15-30 minutes with 20 ng/mL IL-12 or 10ug/mL anti-CD3 (followed by crosslinking with goat anti-Hamster-Alexa 488). Clustering of CD3 was assessed by confocal microscopy relative to positive and negative controls as described in (Teixeiro et al. Immunity 2004). **p<0.01.

Supplemental Fig. 3 related to Figure 6



Supplemental Fig. 3. In vivo bystander activation of OT-I memory is independent of MHC I (A) $5x10^4$ congenically marked OT-1 memory T-cells (CD8-enriched, $CD45.2^+$, $CD44^{hi}$) from ≥ 120 days VSV-OVA infected chimeric mice generated as in Figure 6 were adoptively transferred into MHC I + sufficient (WT) or deficient (MHC I -/-) hosts and subsequently re-challenged with (1 x 10^5 CFU) *Listeria monocytogenes*. 16 hours later cells were harvested from lymph nodes and spleens and *de novo* intracellular IFNγ synthesis was measured over 3h. by flow cytometry. All graphs show mean ±SD for frequency of IFNγ positive bystander activated memory OT-I cells. Data is representative of at least 3 independent experiments with n= 3-4 mice per group.