**Supplemental Figures**

**Supplementary Figure 1**. TCR mediated FasL upregulation and secretion in $CD4^+$ T cell subsets. (**a**) Activated T cells derived from the indicated sorted, activated and IL-2 expanded subsets of $CD4^+$ T cells were stimulated for 6H with anti-CD3. FasL secreted in the supernatant was measured by ELISA. Data is representative of 5 different experiments. (**b**) anti-CD3 stimulated cells were also subjected to quantitative RT-PCR analysis to assess upregulation of FasL mRNA. (**c**) SKW6.4 cells were treated with the supernatants obtained from (**a**) and assayed for FasL induced apoptosis after 6H incubation by annexin/PI. Data is an average of at least 3 independent experiments**.** P value =0.0286, student T test in (**b**) and Mann Whitney test in (**c**).

**Supplementary Figure 2.** RICD in activated mouse $CD4^+$ subsets. (**a**) Naïve and memory subsets were sorted on the basis of surface CD44 and CD62L which is indicated on pre-sorted as well as cells activated in CD3/28 followed by expansion in IL2. (**b**) Fas levels were measured in activated WT B6 and B6 *Lpr* $CD4^+$ subsets. Filled histograms indicate staining with isotype control. (**c**) Activated cells from WT or *Lpr* were also tested for apoptosis through RICD by stimulating with the indicated concentrations of platebound anti-mouse CD3 or FasL-LZ (**d**). The apoptosis assays are an average of 5 independent experiments.

**Supplementary Figure 3.** Levels of anti-apoptotic and survival pathway factors are comparable within the various human $CD4^+$ T cell subsets. (**a**) Intracellular staining for Bcl-2 and $Bcl_{XL}$ in activated T cells derived from the indicated subsets is shown, with shaded histogram indicating isotype control staining. (**b**) Immunoblot analysis of the AKT survival pathway was performed on activated cells derived from the indicated subsets with actin as loading control.

**Supplementary Figure 4.** Effect of PI3-K inhibitor (LY294002) on Fas induced apoptosis. (**a**) Activated and IL-2 expanded human naïve and memory T cell subsets pretreated with LY or DMSO (solvent control) were stimulated with indicated

concentrations of bivalent anti-Fas antibody for 6-8H. Cell death was determined with annexin/PI staining. (**b**) Effect of LY treatment on cell viability is indicated. All experiments are an average of 2 different experiments and error bars are +/- s.e.m.

**Supplementary Figure 5**. **Human memory $CD4^+$ T cells form an efficient DISC when stimulated with uncrosslinked anti-Fas antibodies**. Immunoblot analysis of DISC components in naïve and memory human $CD4^+$ T cells after stimulating (30 minutes) with either uncrosslinked (-XL) or crosslinked (+XL) anti-Fas antibody and immunoprecipitating with protein A/G beads as indicated. Higher exposure of caspase-8 specific bands is shown in the inset below. Bands marked with an asterisk represent non-specific immunoglobulin fragments and are prominent in unstimulated lanes as a result of addition of immunoprecipitating anti-Fas antibodies directly to the cell lysates. (b) Densitometry analysis of recruited DISC components normalized to immunoprecipitated Fas is shown. Lower panel compares caspase-8 cleavage as a ratio of unprocessed vs. cleaved caspase-8 in naïve and memory cells.

**Supplementary Figure 6**. Kinetics of DISC formation in human $CD4^+$ T cell subsets. DISC component immunoblot analysis of sorted and 2 week expanded $T_{CM}$ and $T_{EM}$ cells were performed after immunoprecipitating with crosslinked anti-Fas antibodies for the time (in minutes) indicated.

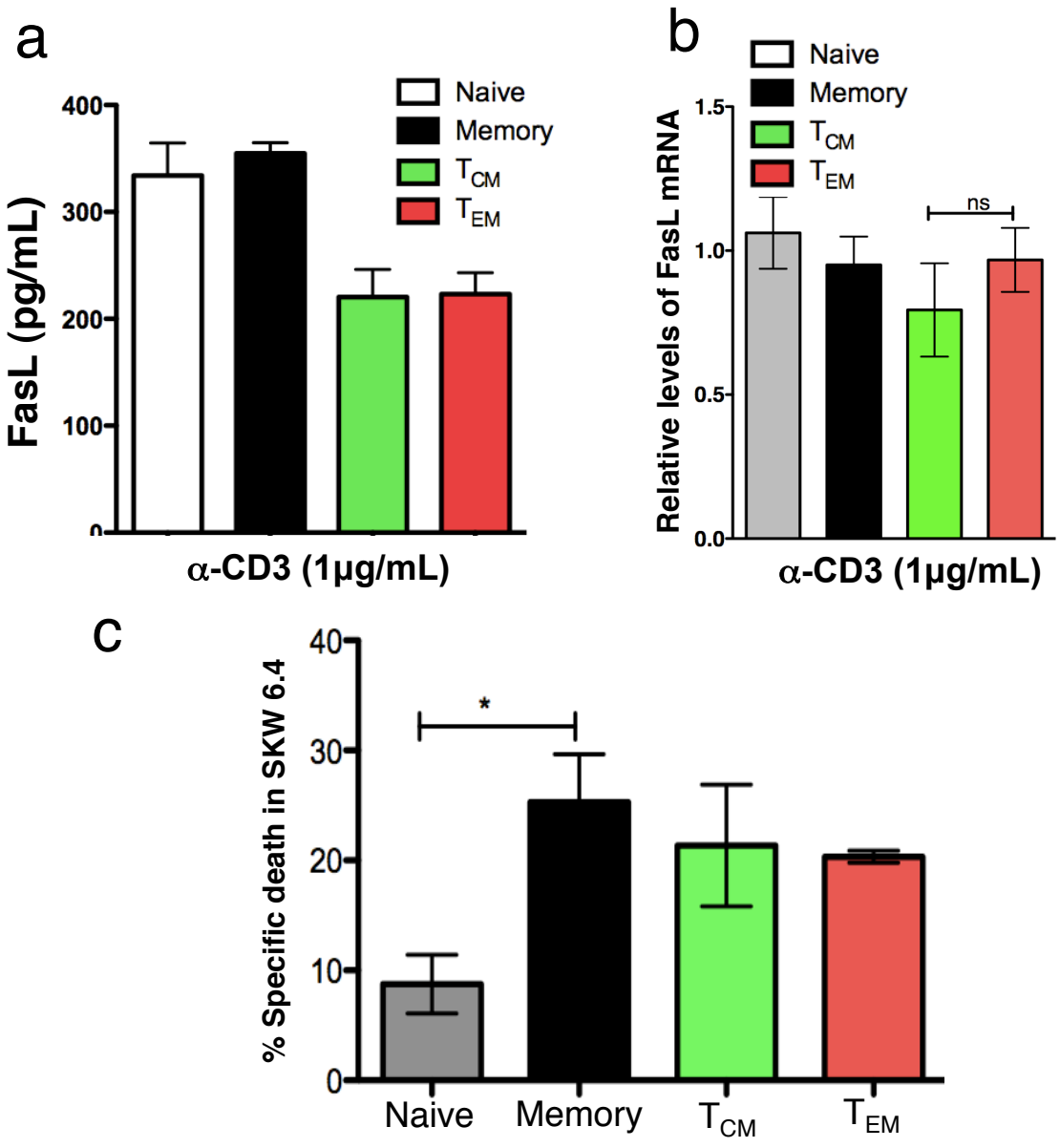
**Supplementary Figure 7**. c-FLIP recruitment in Fas DISC. Recruitment of long and short forms of c-FLIP to the DISC is indicated in the different human $CD4^+$ T cell subsets. Immunoprecipitation with crosslinked anti-Fas antibodies was performed on $CD4^+$ subsets that were activated and cultured for 2 weeks. Lysates were immunoblotted as loading control and the data is representative of 2 independent experiments.

**Supplementary Figure 8.** Active caspase-8 and PARP cleavage in human naïve and memory cells. Lysates from activated naïve or memory T cells stimulated with crosslinked and uncrosslinked anti-Fas antibodies were subjected to immunoblot analysis

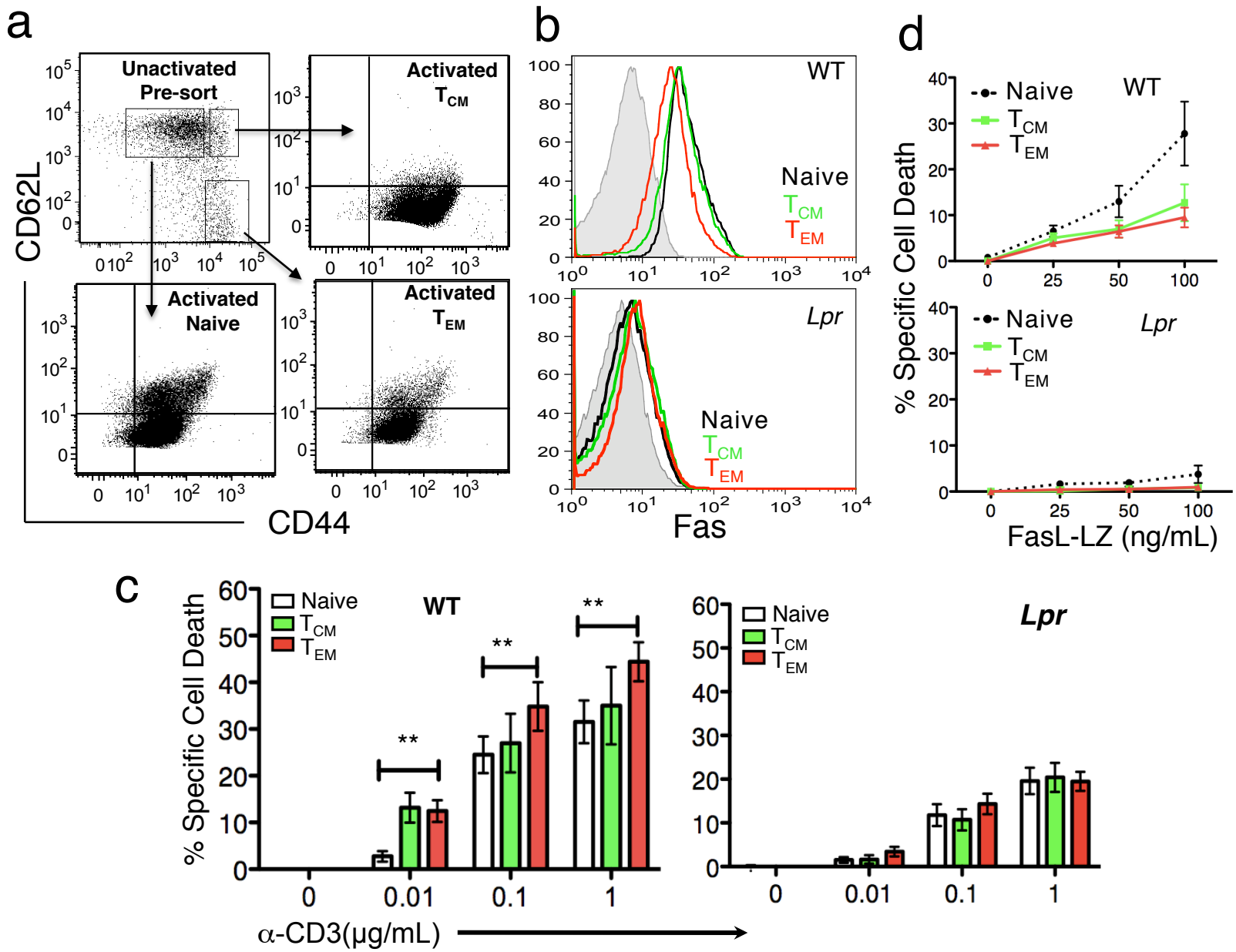
to detect PARP cleavage as well as p18 fragment of cleaved caspase-8. Data is representative of at least 2 different experiments.

**Supplementary Figure 9**. Effect of 2-Bromopalmitate on FasL-induced cell death. (**a**) Activated and one week IL-2 expanded human naïve and memory T cells were stained with filipin to detect cholesterol content of the cell membrane. (**b**) Cultured naïve or memory CD4 T cells were treated with 2-bromopalmitate for 16H prior to treatment with recombinant FasL-LZ and subjected to multiparameter flow cytometry for determination of cell death. Data is an average of 2 different experiments.

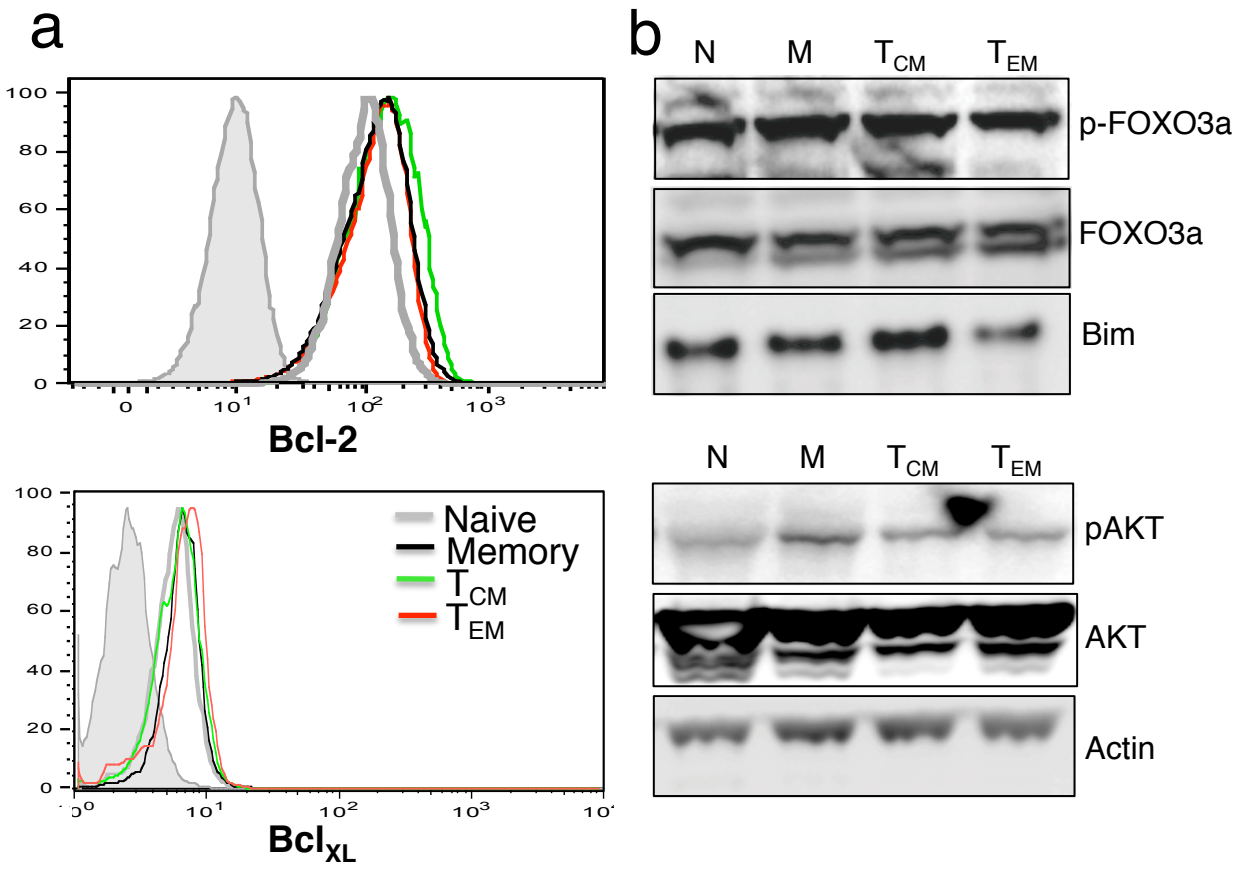
Supplementary Figure 1



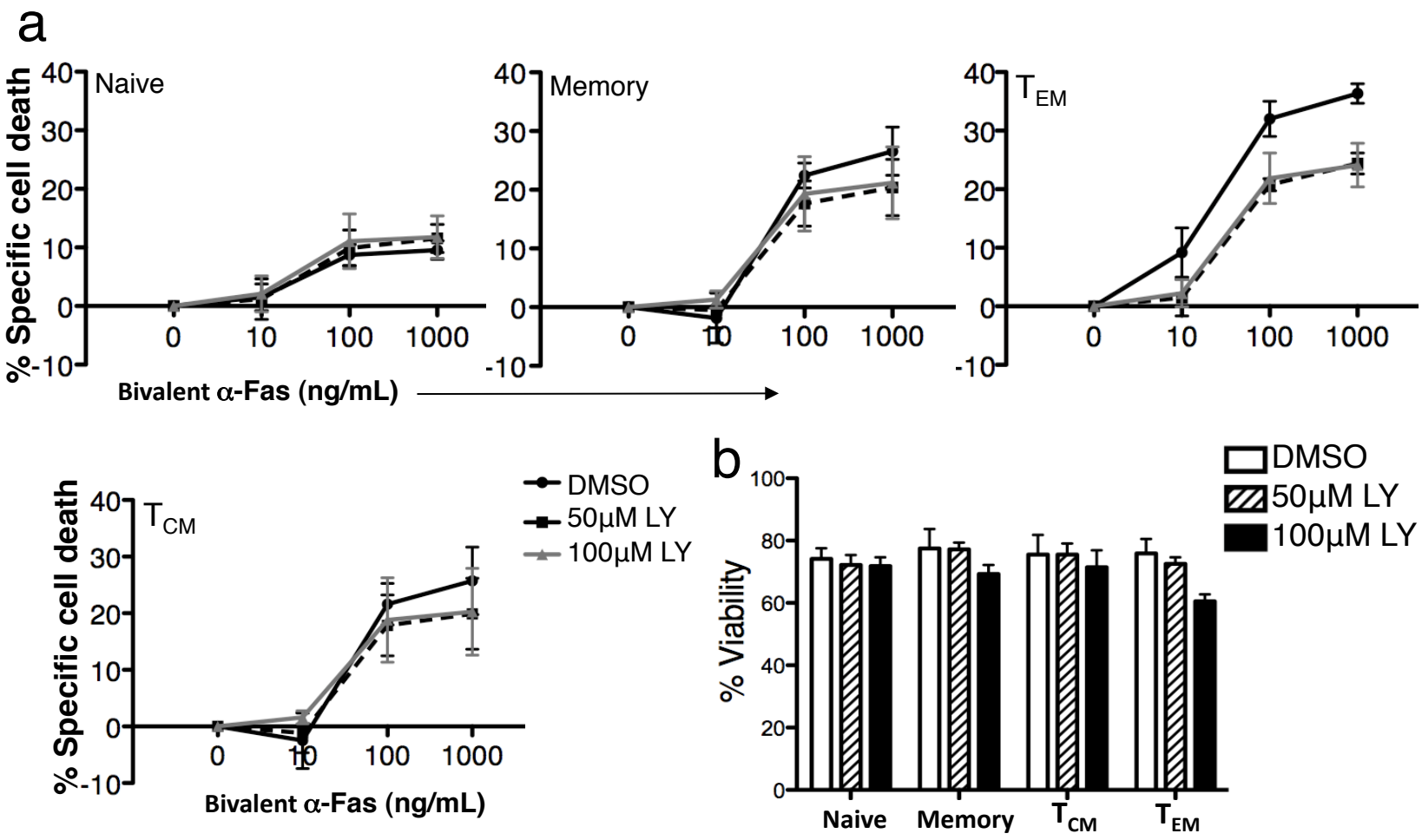
# Supplementary Figure 2



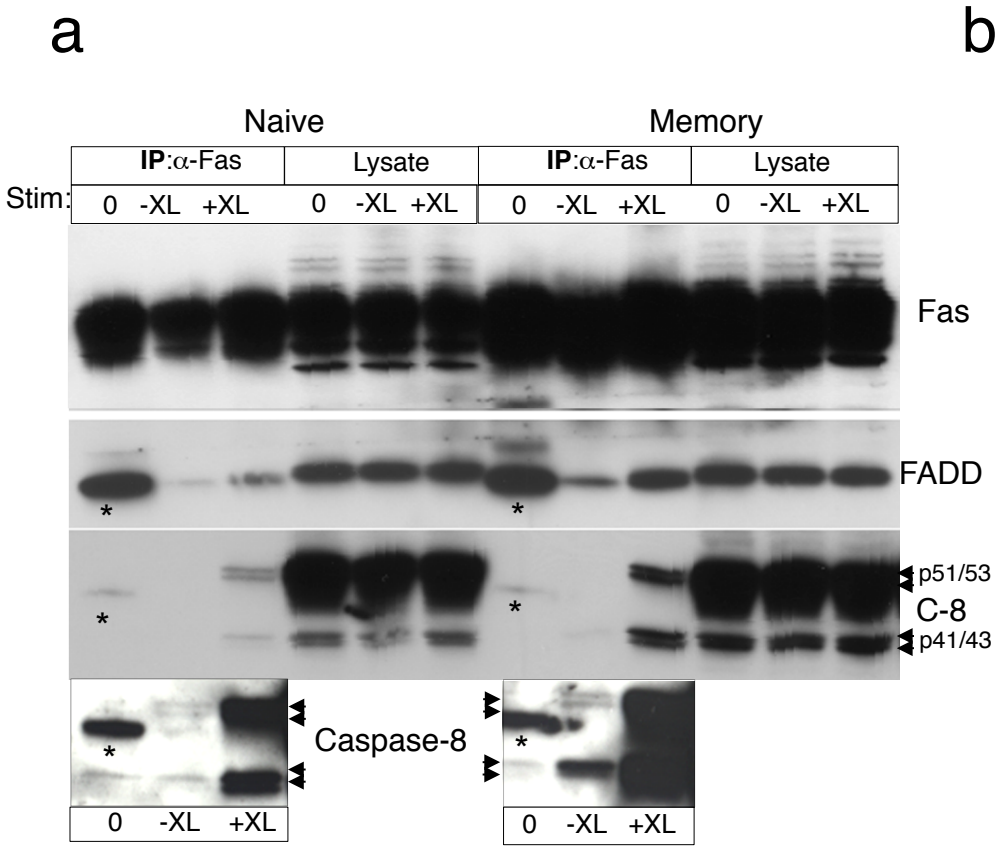
Supplementary Figure 3

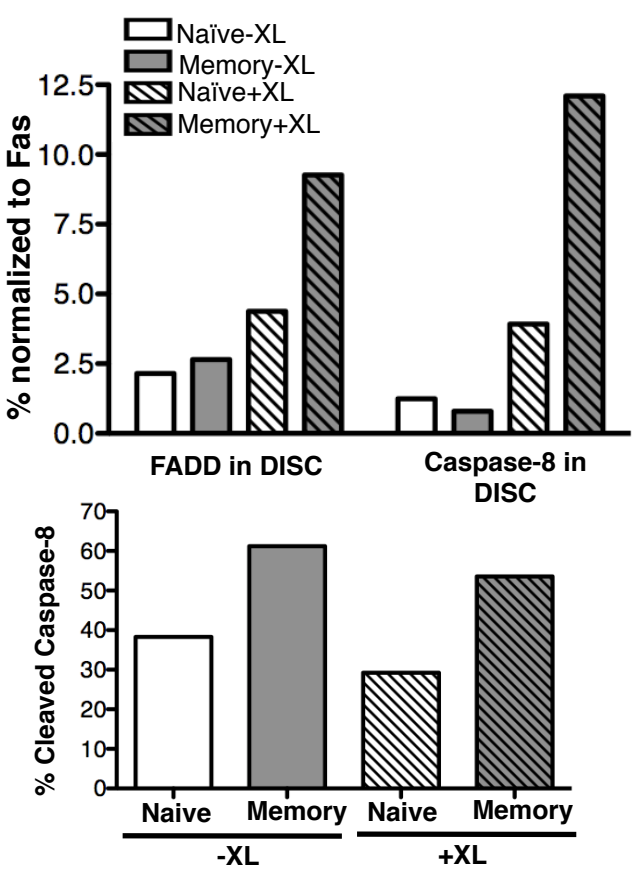


Supplementary Figure 4

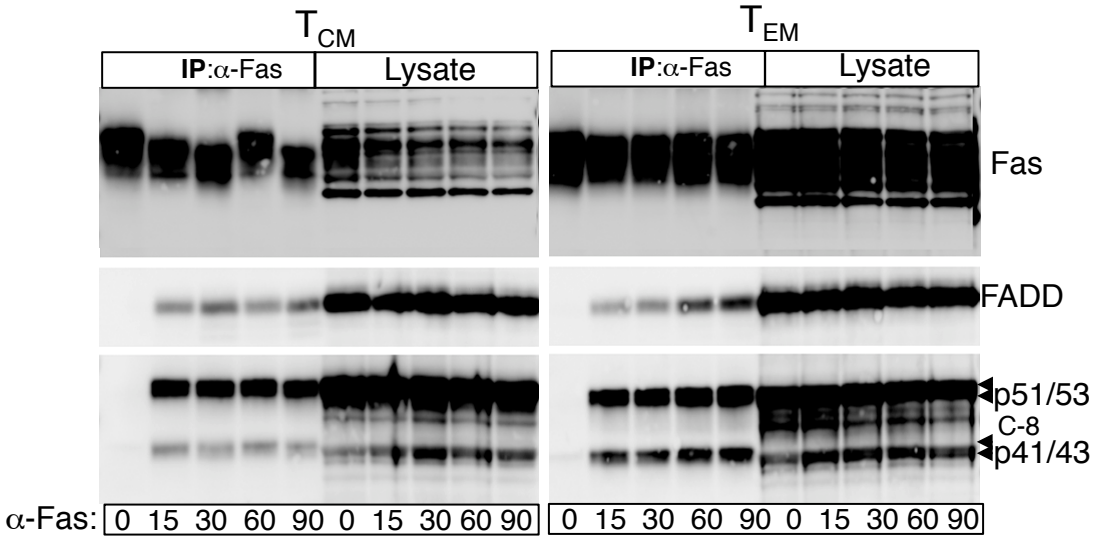


# Supplementary Figure 5

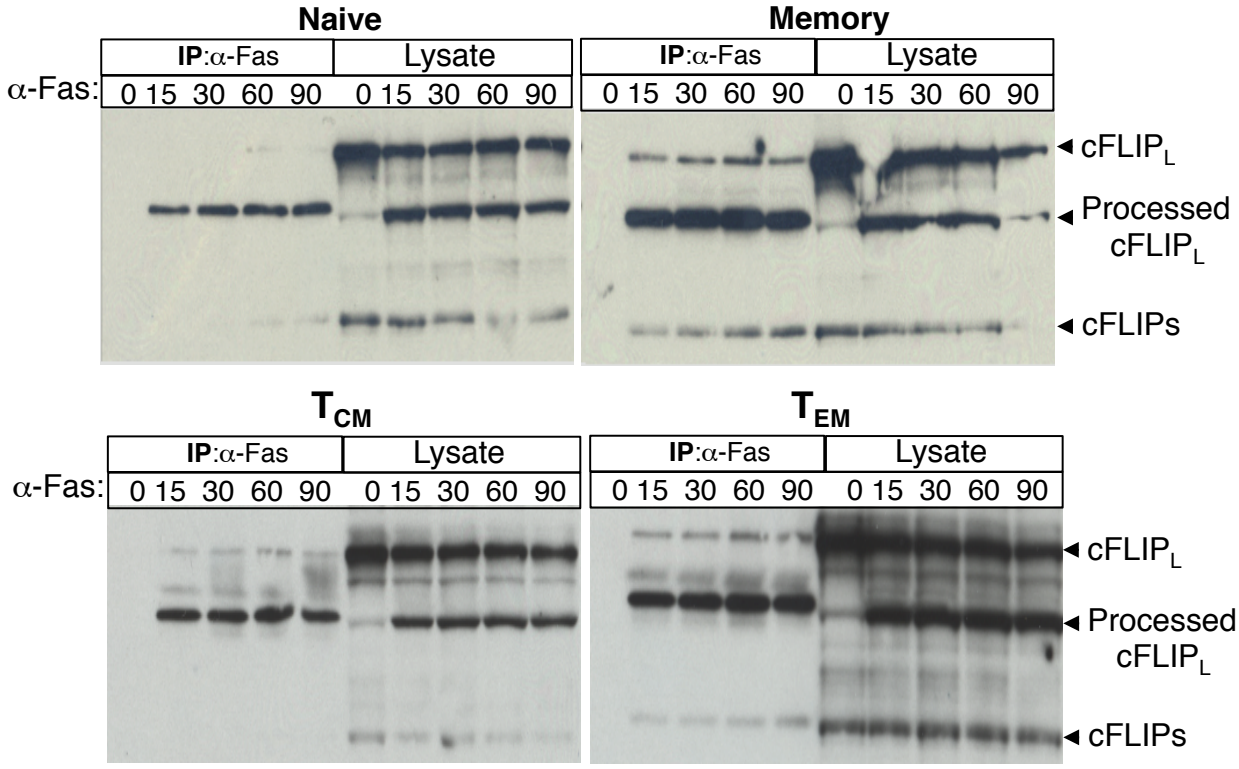


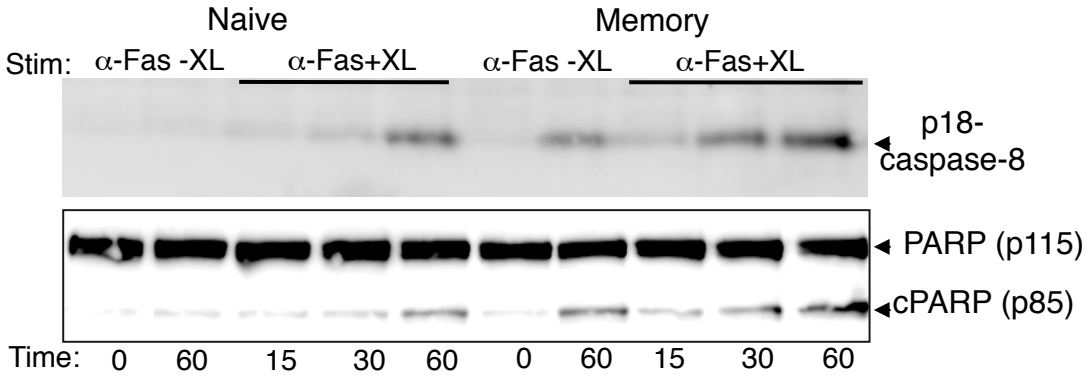
# Supplementary Figure 6



# Supplementary Figure 7



# Supplementary Figure 8



Supplementary Figure 9