



Gating strategy for phospho-epitope detection in NK cells upon activating receptor triggering.

(A) Following stimulation by Ab-cross-linking, splenocytes were extracellularly stained for CD3, CD122, CD49b (DX5), NKp46 and CD11b, fixed/permeabilized and indirectly intracellularly stained for phospho-Ser473-Akt or phospho-ERK1/2 (# 4058 and 4377, Cell Signaling Technology) as described previously (Firaguay and Nunès, 2009). (B) Representative histograms showing profiles of p-AKT (Ser473) of CD11b^{HIGH} NKp46⁺ NK cells untreated (NT, grey) or after Ly49D crosslinking (2 μg/ml) for 3 min at 37°C (red) from WT and DKO mice. Values represent the Mean Fluorescence Intensity (MFI) of p-AKT staining.