



Phosphorylation of signaling molecules AKT and the MAP kinases, ERK-1/2, and IFN- γ production via activating receptor, Ly49D stimulation is increased in Dok-1/Dok-2-deficient (DKO) NK cells.

(A) As described in the Figure 6 of the manuscript for NKp46 triggering, here the splenocytes were stimulated with plate – bound anti-Ly49D mAb at the indicated concentrations during 4 – 5 hours. IFN- γ NK production was tested by flow cytometry gating on CD11b⁺ NK cells (CD3⁻ NKp46⁺) from WT (white squares, dotted line) and DKO (black squares) mice. Representative data from 3 independent experiments (n= 7 - 8 mice each genotype/ experiment; mean \pm SD). **(B)** Following stimulation by Ly49D mAb-cross-linking (3 min at 37°C), splenocytes from WT (white squares, dotted line) or DKO (black squares) mice were extracellular stained for CD3, CD122, CD49b (DX5), NKp46 and CD11b, fixed/permeabilized and indirectly intracellular stained for phospho Ser473-Akt or phospho ERK1/2 (# 4058 and 4377, Cell Signaling Technology). The results shown represent mean MFI \pm SD gating on the CD11b⁺ NK cell population (supplemental Figure 3). Representative data from 2 independent experiments (n= 6 - 7 mice each genotype/experiment). ***P* < 0.01 and **P* < 0.05 (Mann-Whitney test).