

Western blot analysis of phosphorilated and not proteins of CD4 and CD8 T cells from WT and Ldlr -/- (KO) mice. Samples were loaded on the same gel and after the transfer the nitrocellulose membranes were cut at the molecular weight of the protein of interest. All the proteins investigated in this figure (ZAP, Lck, NFAT and b-actin) were acquired on different segments of the same membrane, therefore the b-actin is the same for all CD4 data and all CD8 data.

In the next slide, two images of the same membrane are provided:

- Image 1 has been acquired under the chemiluminescent channel (for HRP and AP chemiluminescent substrate), used for band quantification and representation of the abundance of the selected proteins;
- Image 2 has been acquired in the visible channel (exitation at 685 for protein detection), that allows to appreciate molecular weights (first line)

pZAP (MW 70 kDa)

SDS gel 10% - loaded 25 µg protein/sample

#Supplemental Figure 4J

Image 1

CD8

WT

0 5'

KO

0 5'

75 kDa →

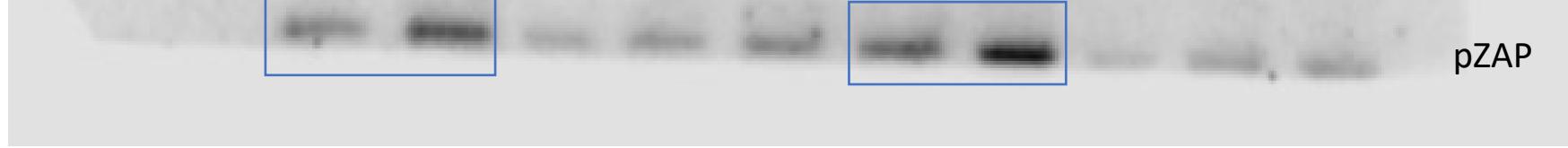
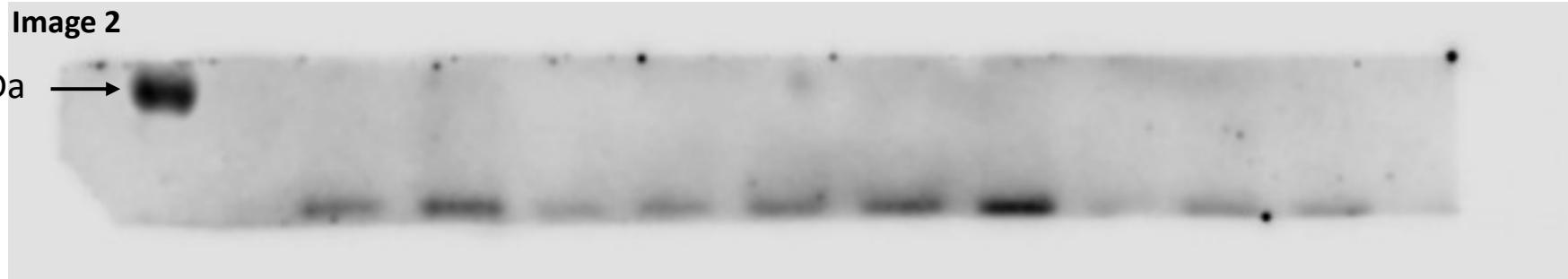


Image 2

75 kDa →



pLCK (MW 56 kDa)
SDS gel 10% - loaded 25 µg protein/sample

#Supplemental Figure 4J

Image 1

CD8

WT

0 5'

KO

0 5'

pLCK

50 kDa →

Image 2

50 kDa →

β -actin (MW 46 kDa)– CD8
SDS gel 10% - loaded 25 μ g protein/sample

#Supplemental Figure 4J

Image 1

CD8

WT

0 5'

50 kDa →

KO

0 5'

β -actin

Image 2

50 kDa →

pZAP (MW 70 kDa)

SDS gel 10% - loaded 25 µg protein/sample

#Supplemental Figure 4L

Image 1

CD4

WT

0 5'

KO

0 5'

75 kDa →

pZAP

Image 2

75 kDa →

pLCK (MW 56 kDa)
SDS gel 10% - loaded 25 µg protein/sample

#Supplemental Figure 4L

Image 1

CD4

WT

0 5'

KO

0 5'

50 kDa →

pLCK

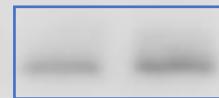
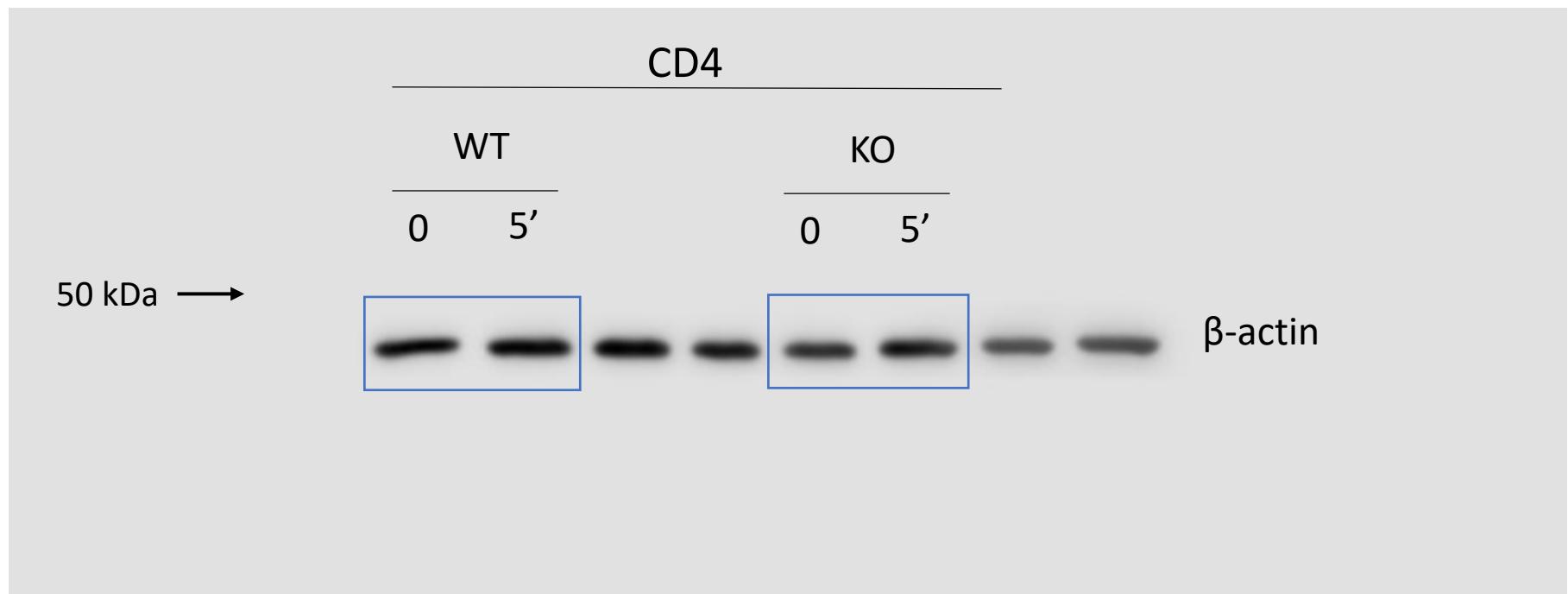


Image 2

50 kDa →

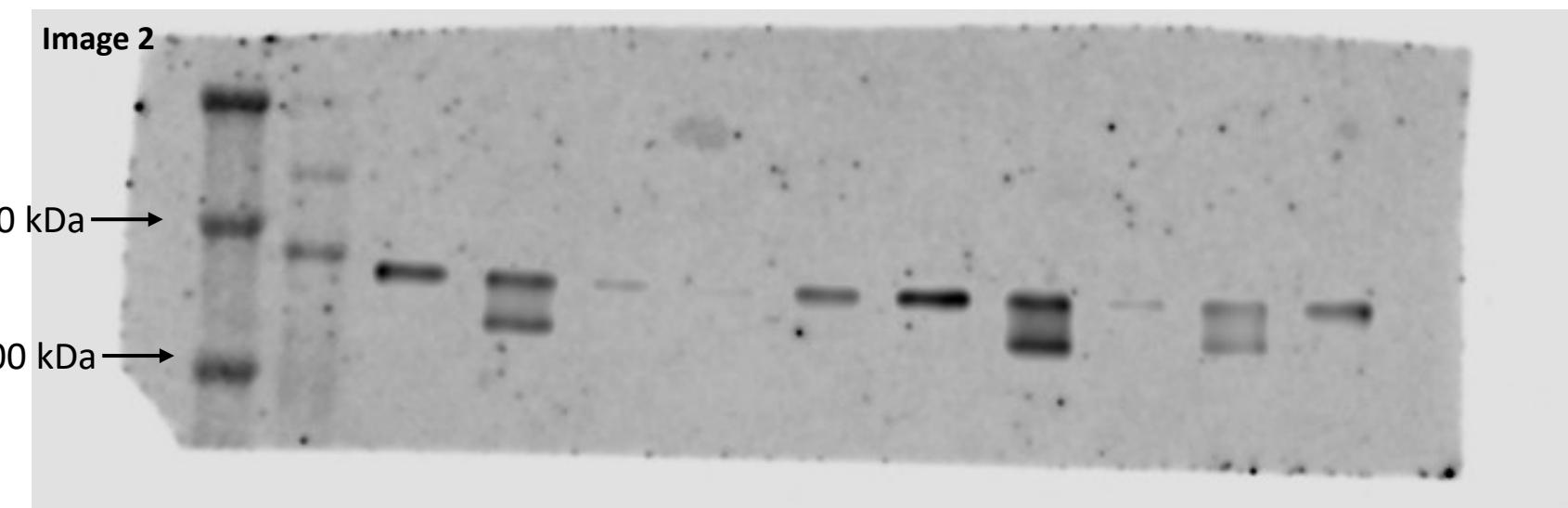
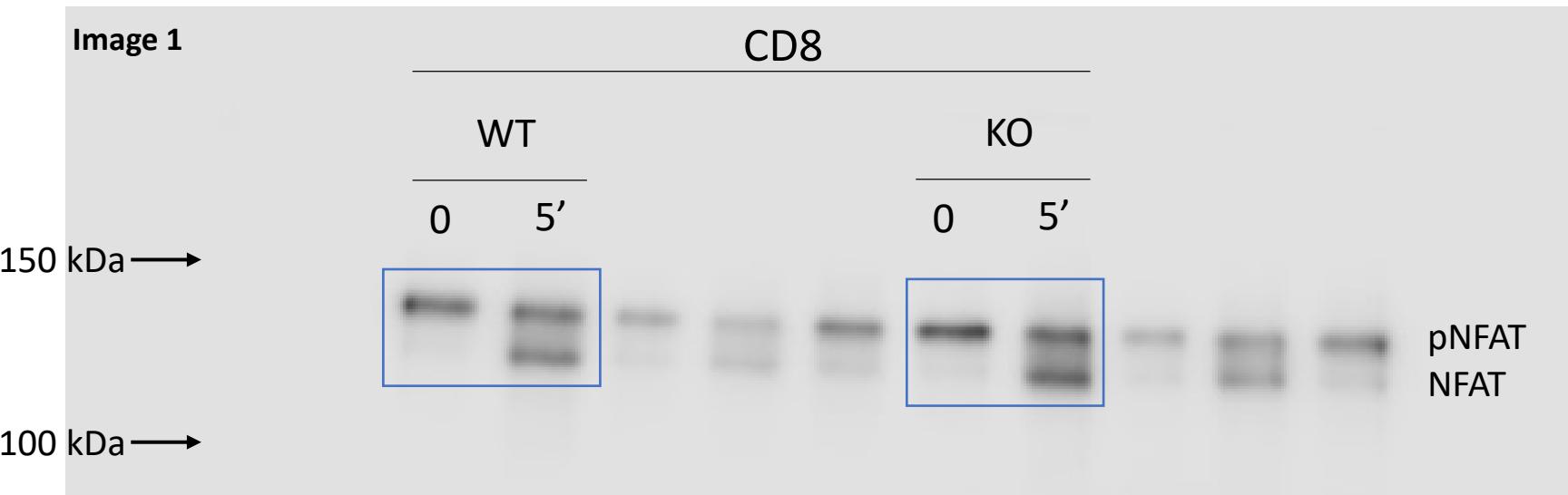
β -actin (MW 46 kDa)– CD8
SDS gel 10% - loaded 25 μ g protein/sample

#Supplemental Figure 4L



pNFAT/NFAT (MW 140 kDa)
SDS gel 10% - loaded 25 µg protein/sample

#Supplemental Figure 4N



β -actin (MW 46 kDa)– CD8
SDS gel 10% - loaded 25 μ g protein/sample

#Supplemental Figure 4N

Image 1

CD8

WT

0 5'

50 kDa →

KO

0 5'

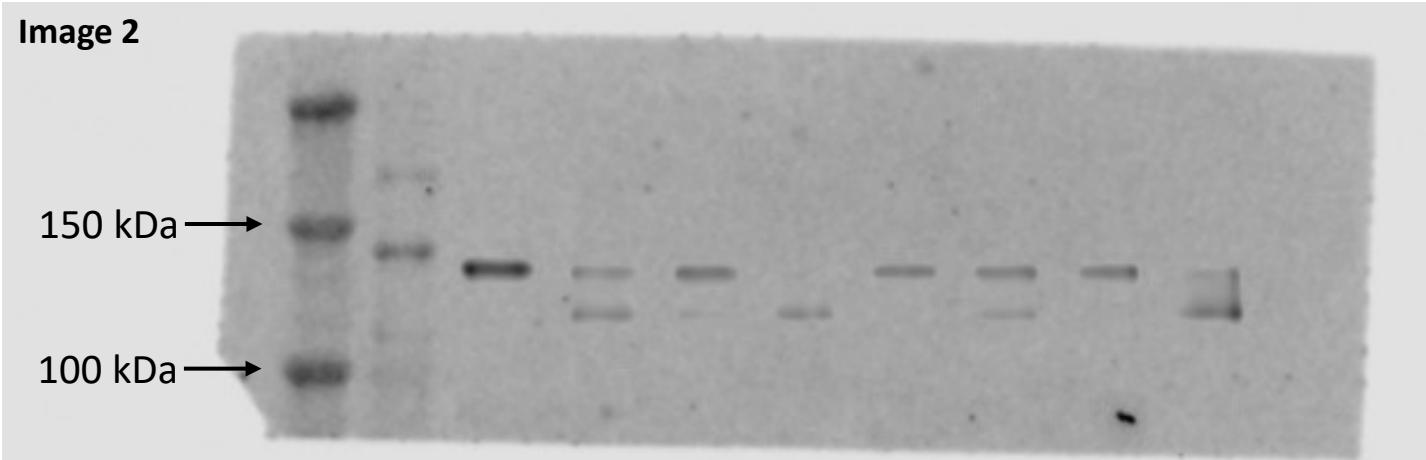
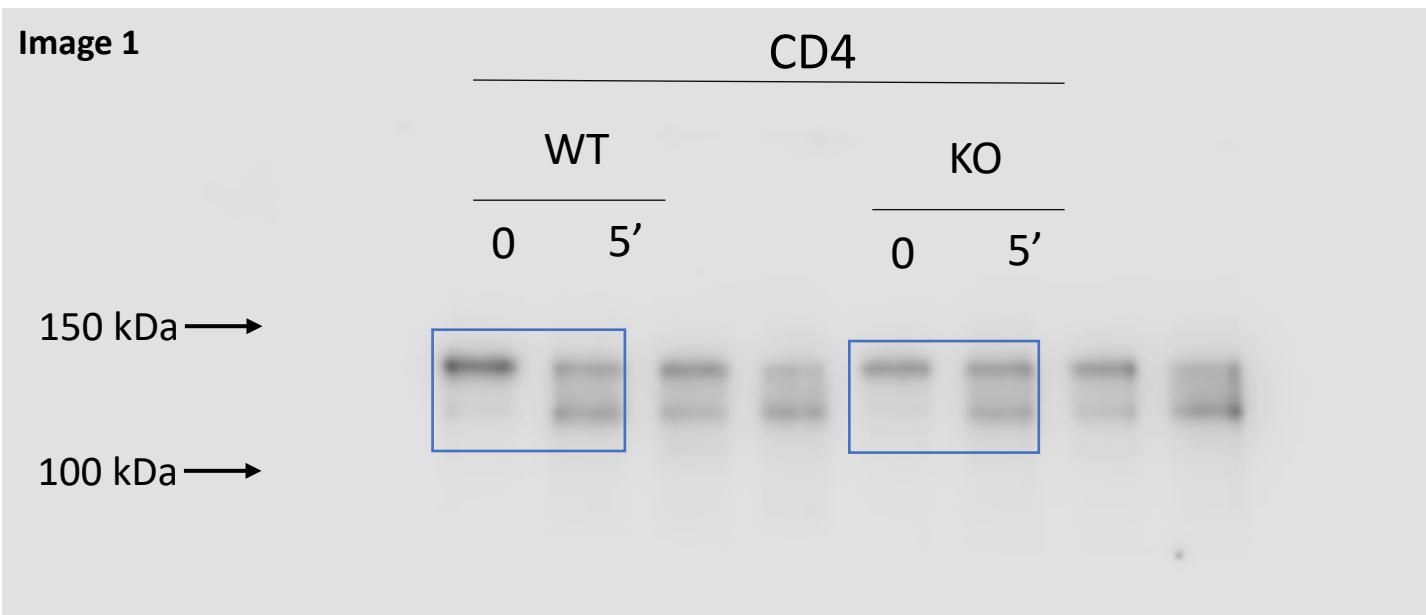
β -actin

Image 2

50 kDa →

pNFAT/NFAT (MW 140 kDa)
SDS gel 10% - loaded 25 µg protein/sample

#Supplemental Figure 4O



β -actin (MW 46 kDa)– CD8
SDS gel 10% - loaded 25 μ g protein/sample

#Supplemental Figure 4O

