
Soluble TNFRp75 regulates host protective immunity against *Mycobacterium tuberculosis*.

Roanne Keeton¹, Nasiema Allie¹, Ivy Dambuza¹, Brian Abel¹, Nai-Jen Hsu¹, Boipelo Sebesho¹, Philippa Randall¹, Patricia Burger¹, Elizabeth Fick¹, Valerie Quesniaux³, Bernhard Ryffel³ and Muazzam Jacobs^{1,2}

INSTITUTIONAL AFFILIATIONS

¹ Division of Immunology, Department of Clinical Laboratory Sciences and the Institute of Infectious Disease and Molecular Medicine, University of Cape Town, South Africa

² National Health Laboratory Service, South Africa

³ University of Orleans and CNRS UMR6218, Molecular Immunology and Embryology, 45071 Orleans, France

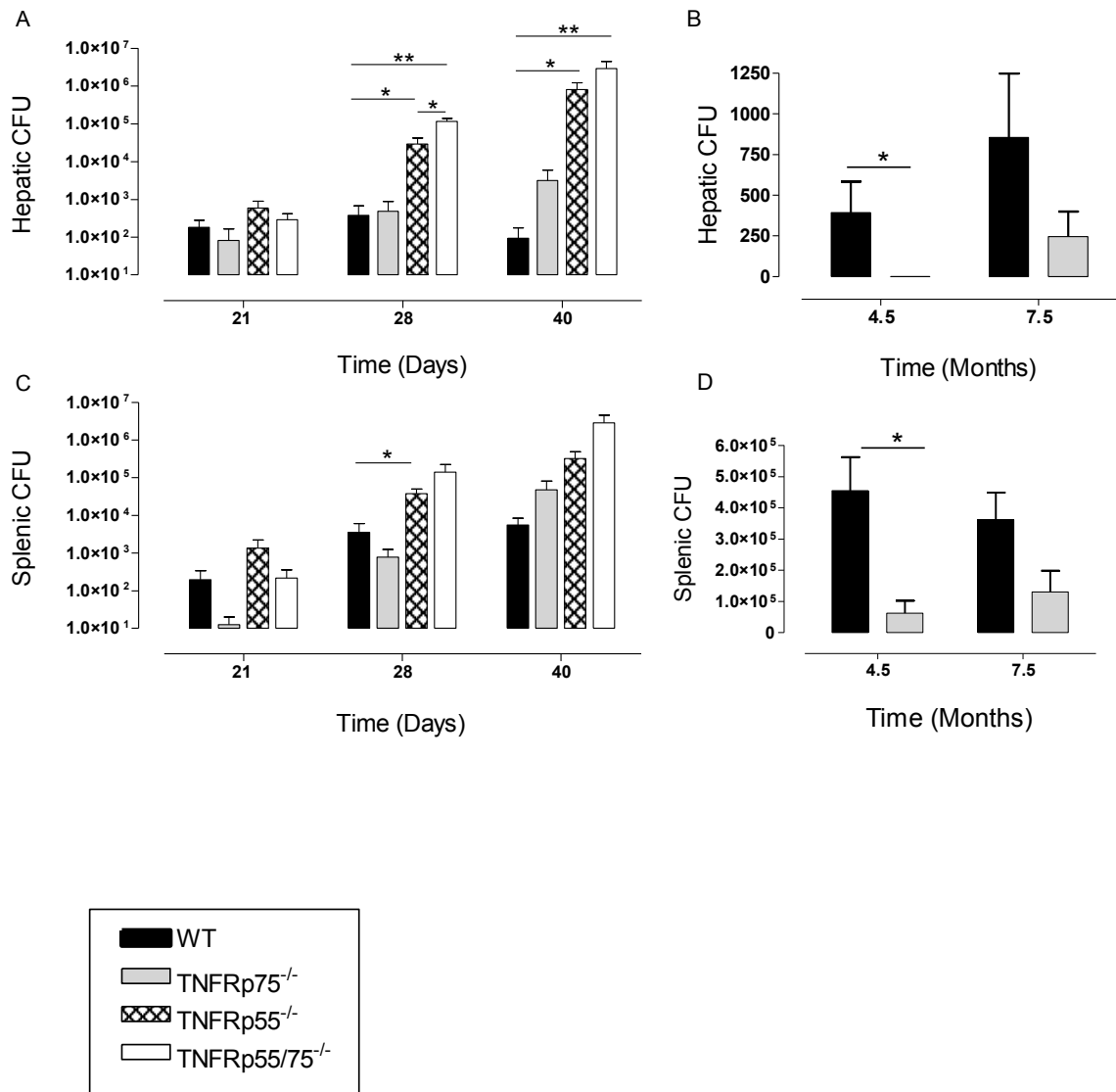
The authors have declared that no conflict of interest exists.

CORRESPONDING AUTHOR

Muazzam Jacobs
Room S1.33, Level 1, Werner Beit South
Institute of Infectious Disease and Molecular Medicine
Division of Immunology,
Health Sciences Faculty
University of Cape Town
Anzio Road 7925 Observatory, Cape Town
South Africa.
Email: Muazzam.Jacobs@uct.ac.za
Tel: +27 21 406 60 78 or +27 21 406 61 47

Supplementary Figures

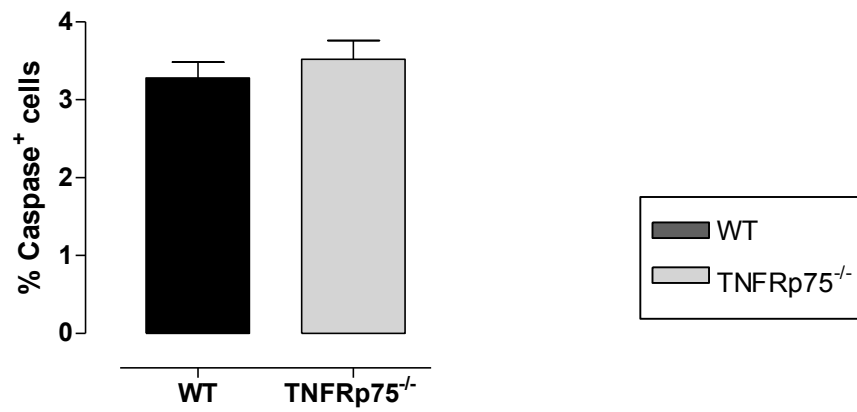
1
2
3
4
5
6



7
8
9
10
11
12

Figure 1

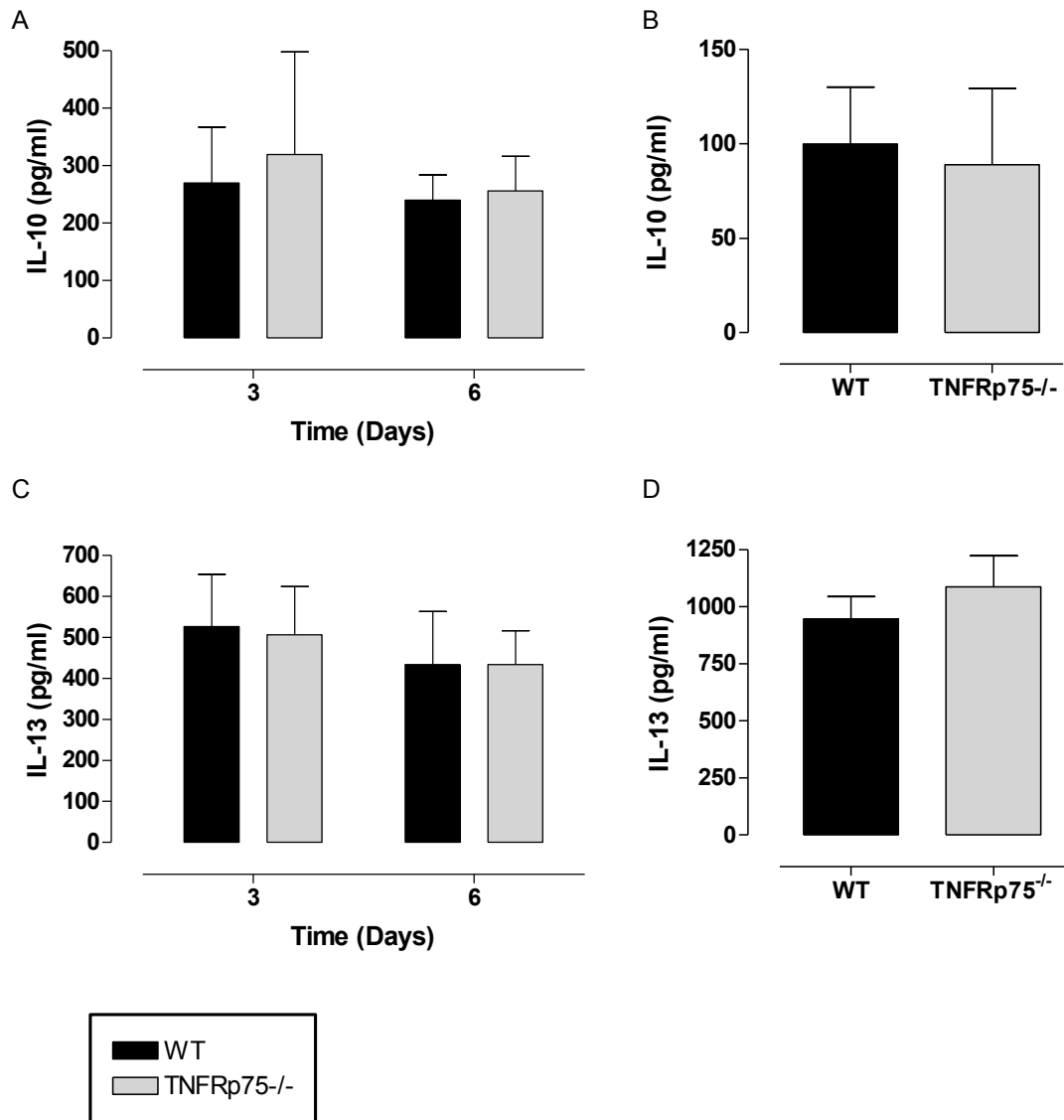
13
14
15
16
17
18
19
20



21
22
23
24
25
26
27
28
29
30
31
32

Figure 2

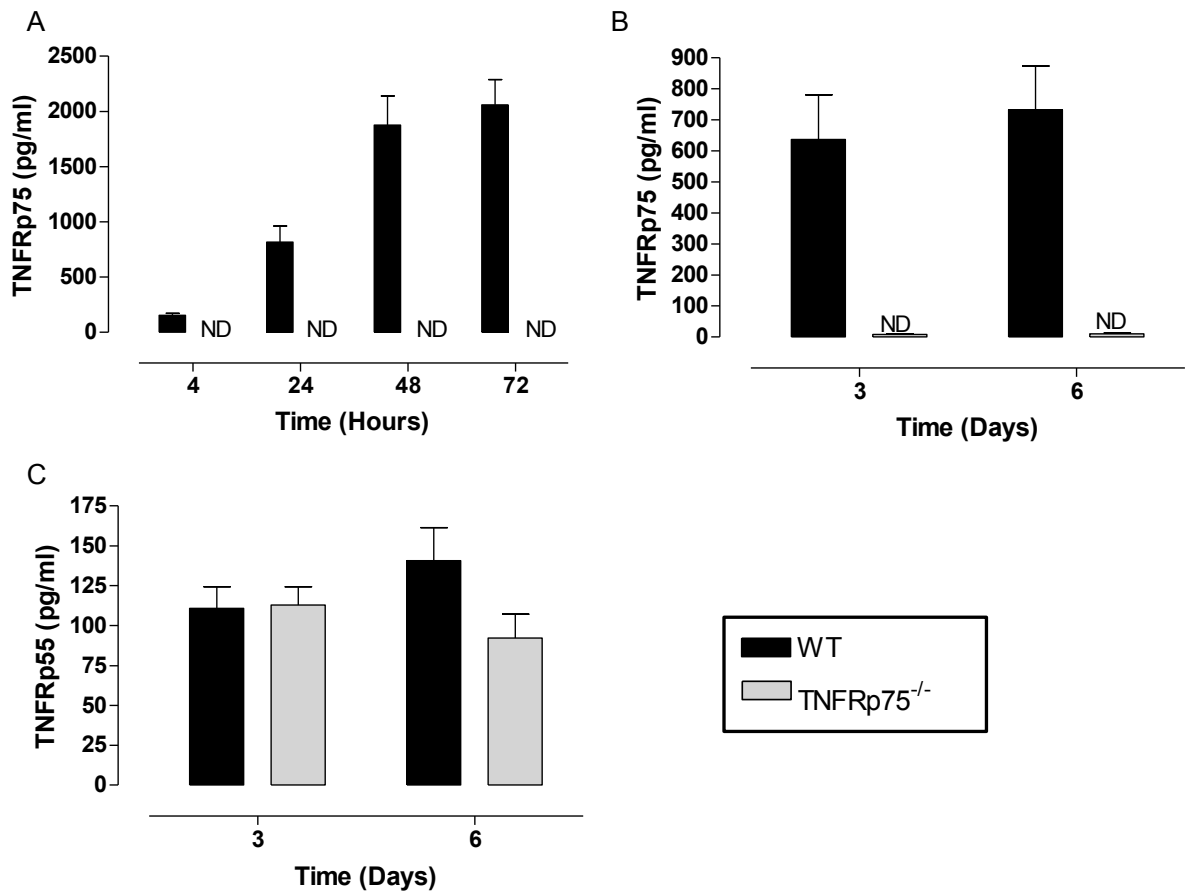
33
34
35
36



37
38
39
40
41
42

Figure 3

43
44
45
46
47



48
49
50
51
52
53
54
55
56

Figure 4

57

SUPPLEMENTARY FIGURE LEGENDS

58 **Figure 1: Enhanced control of dissemination in TNFRp75^{-/-} mice during chronic *M.***
59 ***tuberculosis* infection.** WT- , TNFRp75^{-/-} , TNFRp55^{-/-} and TNFRp55/75^{-/-} mice were
60 infected with 50-100 CFU *M. tuberculosis*. Extra-pulmonary bacilli burdens were determined
61 in the liver (A and B) and spleen (B and D) during acute and chronic infection by colony
62 enumeration assay. The data points are the mean ± SEM of the CFU of 4 mice per time
63 point. Significant differences (*p<0.05, ** p<0.01) were determined using the ANOVA test.
64 The data are representative of three similar experiments.

65 **Figure 2: Dendritic cell apoptosis are equivalent in WT and TNFRp75^{-/-} mice during**
66 **acute *M. tuberculosis* infection.** WT and TNFRp75^{-/-} mice were infected at 50-100 CFU
67 with *M. tuberculosis* and lungs harvested at 14 days post-infection. The percentage of
68 pulmonary CD11c⁺ cells expressing Caspase 3 was analysed by flowcytometry. The data
69 points represent the mean ± SEM of 5 mice/group. The data are representative of two similar
70 experiments.

71 **Figure 3: T_H2 cytokine production.** WT- and TNFRp75^{-/-} mice were infected with 50-100
72 CFU *M. tuberculosis*. IL10 (A and B) and IL13 (C and D) measured during acute infection in
73 BALF and at 6 months, during chronic infection in lung homogenates by ELISA. The data
74 points are the mean ± SEM of 4-5 mice/group and are representative of one of two similar
75 experiments. Significant differences (*p<0.05, ** p<0.01) were determined using ANOVA.
76 (ND= not detectable).

77

78 **Figure 4: *M. tuberculosis* induces TNFRp75 shedding.** WT- and TNFRp75^{-/-} bone
79 marrow derived dendritic cells were infected with *M. tuberculosis* at an MOI of 5:1 and
80 soluble TNFRp75 (A) was measured by ELISA. WT- and TNFRp75^{-/-} mice were infected with
81 50-100 CFU *M. tuberculosis* and soluble TNFRp75 (B) or TNFRp55 (C) measured in BALF
82 by ELISA. The data points are the mean ± SEM of quadruplicate experiments and are
83 representative of one of two experiments. (ND= not detectable).