SUPPLEMENTARY MATERIAL

Pivotal role of protein tyrosine phosphatase 1B (PTP1B) in the macrophage response to pro-inflammatory and anti-inflammatory challenge

Short Title: *PTP1B modulation of macrophage responses*

[1]Paqui G. Través*, [1,2]Virginia Pardo*, [1]María Pimentel-Santillana*, [1,2]Águeda González-Rodríguez, [1]Marina Mojena, [3]Daniel Rico, [1]Yenny Montenegro, [1]Carmela Calés, [1,4]Paloma Martín-Sanz, [1,2]Angela M. Valverde# and [1,4]Lisardo Boscá#

[1]Instituto de Investigaciones Biomédicas Alberto Sols (Centro Mixto CSIC-UAM), Arturo Duperier 4, 28029 Madrid, Spain.

[2]Centro de Investigación Biomédica en Red de Diabetes y Enfermedades Metabólicas Asociadas (Ciberdem), ISCIII; Spain

[3]Structural Biology and Biocomputing Programme, Spanish National Cancer Research Center (CNIO), ISCIII, Madrid, Spain.

[4]Centro de Investigación Biomédica en Red de Enfermedades Hepáticas y Digestivas (Ciberehd), ISCIII; Spain

*Contributed equally to the work

#Address for correspondence:

Lisardo Boscá. IIB-Alberto Sols. Arturo Duperier 4. 28029 Madrid. Spain

Fax: 34 914972747; e-mail: lbosca @iib.uam.es

and

Angela M Valverde. IIB-Alberto Sols. Arturo Duperier 4. 28029 Madrid. Spain

Fax: 34 915854401; e-mail: avalverde @iib.uam.es

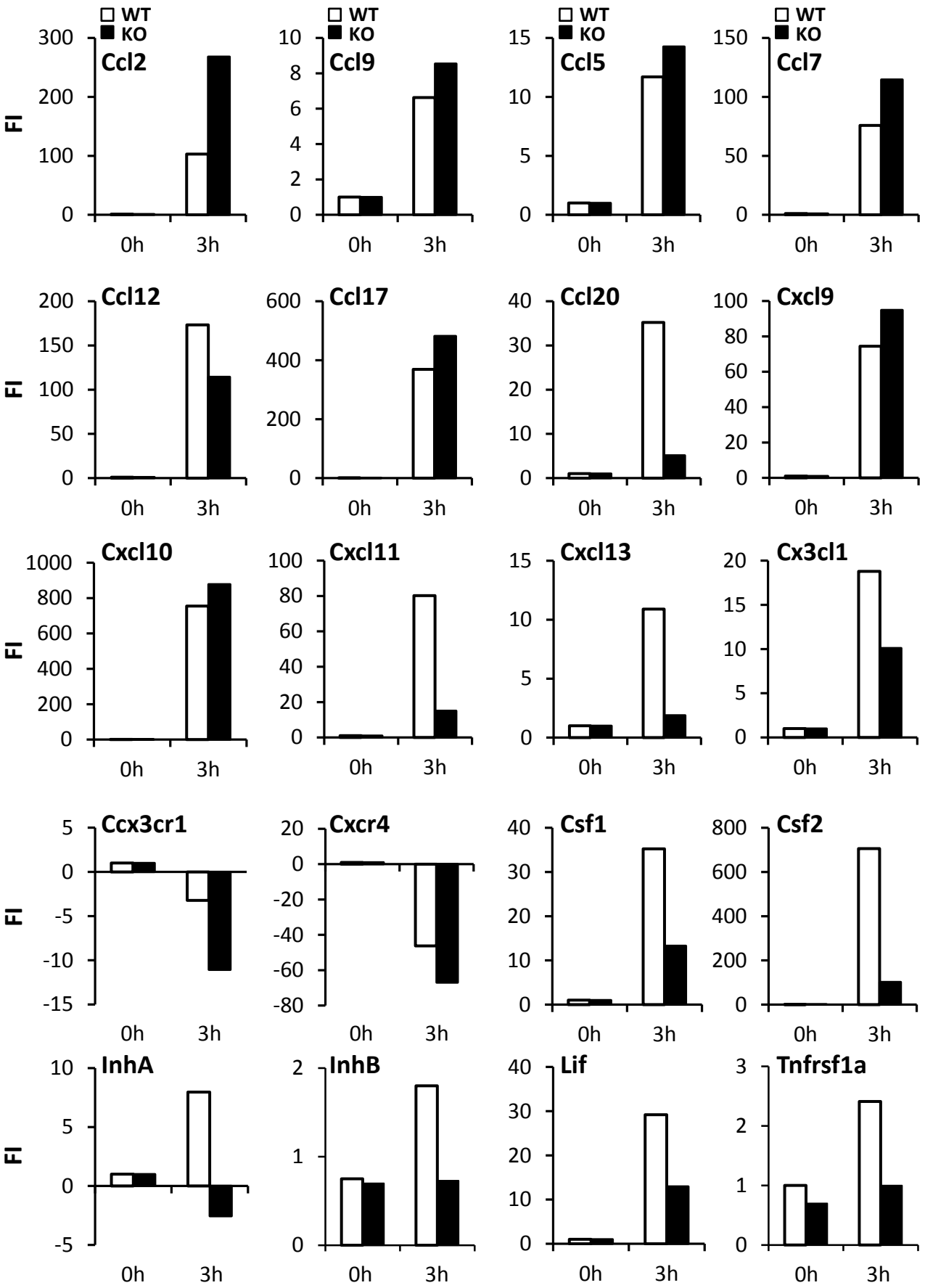
Supplementary Figures:

Figure S1. ***RT-PCR mini-array analysis of the response to LPS in PTP1B-deficient macrophages.*** Macrophages from WT and PTP1B KO mice were challenged for the indicated periods of time with 200 ng/ml of LPS and the levels of mRNA were determined using a 96-well Superarray test for 'Chemokines and receptors'. Samples were processed as indicated by the supplier and a selection of the genes was represented. Results show the mean of three experiments combining the macrophages of at least 4 animals in each experiment.

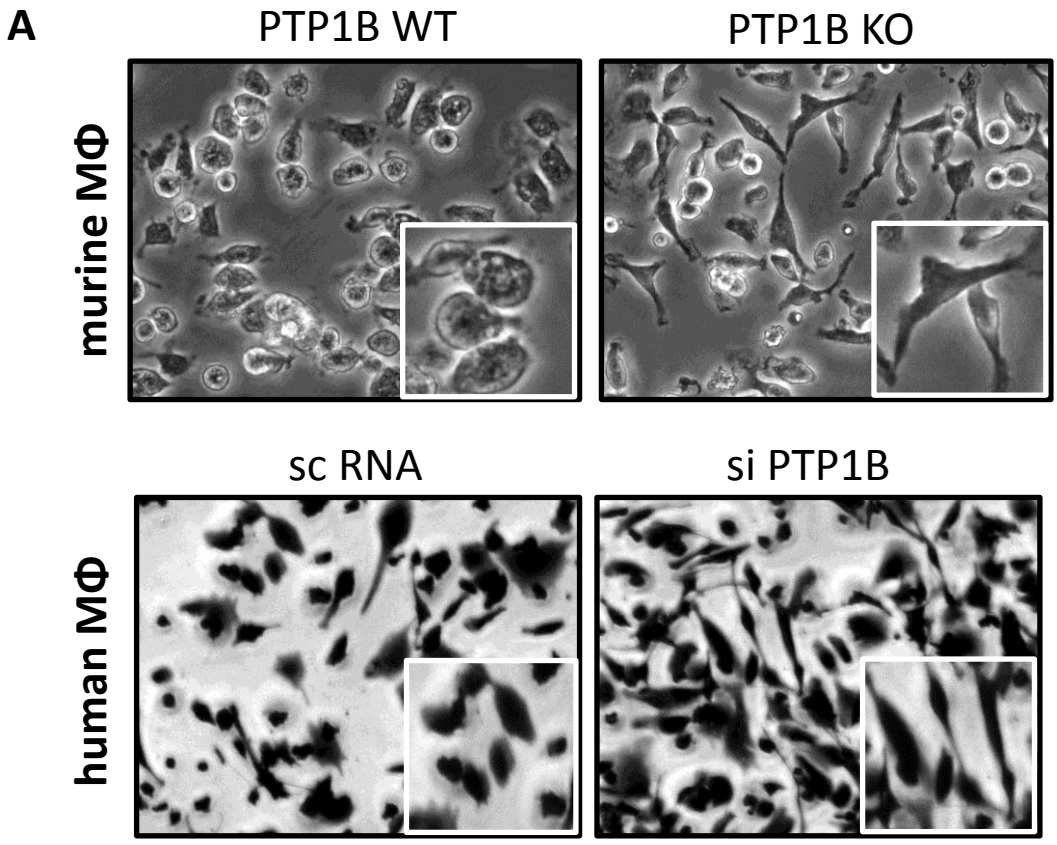
Figure S2. ***Deficiency of PTP1B or silencing of PTP1B with specific siRNA induces morphological changes in murine and human macrophages (A), and effect of peroxovanadate (POV) on the response to LPS+IFNγ of human macrophages (B,C).*** Peritoneal macrophages from WT or PTP1B KO mice or human macrophages treated with a mixture of sc- or siRNA oligonucleotides to silence PTP1B were plated as indicated in Methods and clear field images (murine cells) or after staining with crystal violet (human) were obtained. Insets show representative morphologies of the corresponding cells (A). Human monocytes were differentiated into macrophages and treated with a fresh solution of POV or the same mixture lacking vanadate (vehicle). After 10 min cells were challenged with LPS+IFNγ as in Fig. 5 and samples were collected to determine the protein levels of PTP1B, IκBα, IκBβ and the cytosolic/nuclear distribution of p65 at the indicated times (B). To evaluate the effect of PTP inhibition by POV on cell viability, macrophages were incubated with the indicated stimuli (as in Fig. 5) and viability was determined as the population negative for PI and annexin V staining. z-VAD was used at 50 μM (C). Results show a representative blot out of three (B) or the mean of three experiments (C).

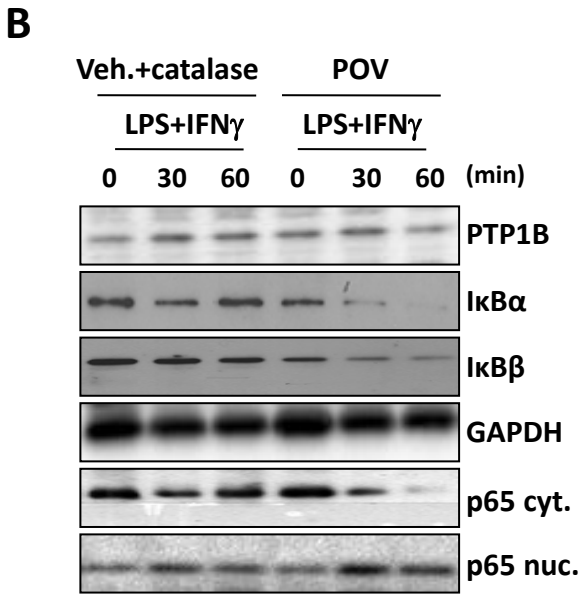
Figure S3. ***Analysis of animal viability and measurement of TNF-α serum levels in mice submitted to bone marrow transplantation (Tx) and treated with clodronate liposomes.*** WT recipient mice (8-10 per group) were transplanted with bone marrow from WT or PTP1B KO donors. Two weeks later, mice were i.p. injected 200 μl of clodronate liposomes, and 12 weeks after transplantation mice were injected LPS/D-GalN. Kaplan-Meier curves for survival after LPS/D-Gal administration (A). The serum levels of TNF-α were determined at 1h after LPS/D-GalN challenge (B). The replenishment of PTP1B-deficient macrophages in the liver and blood after bone marrow transplantation was determined by RT-PCR after finishing the LPS/D-GalN protocol (C). The effectiveness of clodronate treatment was evaluated at 48h after administration of the clodronate-liposomes in liver sections stained with F4/80-FITC, laminin and DAPI; white bar= 100 μm (D). Results show the mean±SD of the TNF-α measurements (B). *P<0.05 *vs.* the same condition in animals transplanted with WT bone marrow, or three

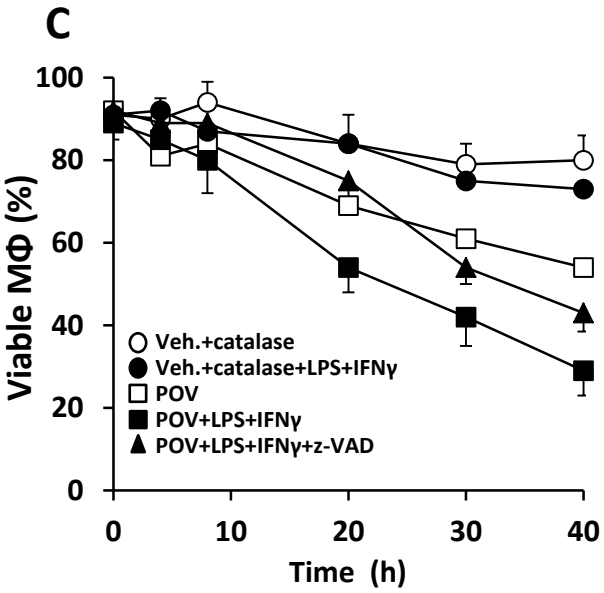
representative animals of each group after bone marrow transplantation. The blood from a heterozygote animal was used as a control (C).



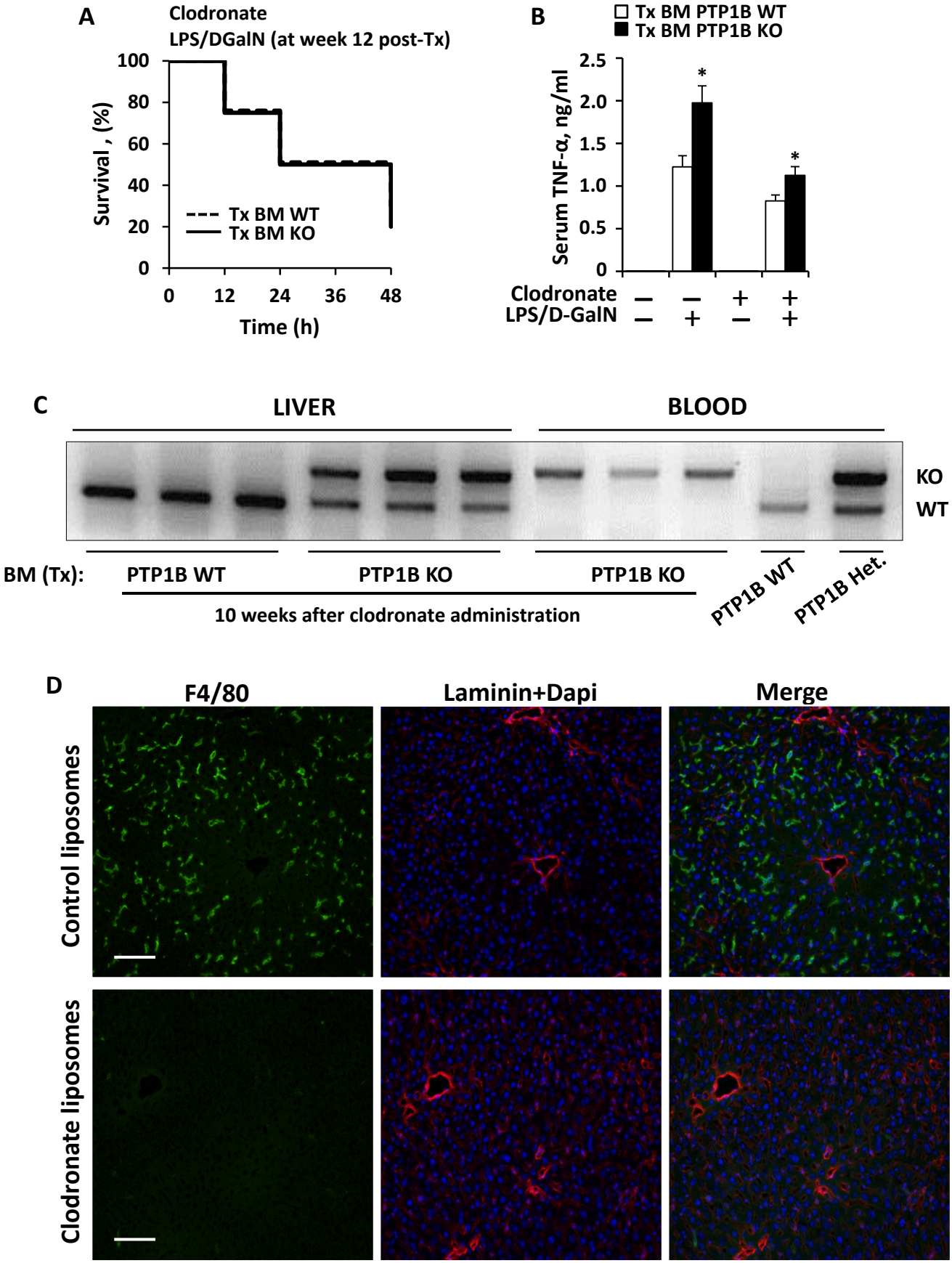
Supplementary Fig. S1







Supplementary Fig. S2



Supplementary Fig. S3