

SUPPLEMENTARY FIGURE LEGENDS

Supp. Fig. 1. Infection with *L. major* does not induce apoptosis. P-MEFs were incubated under control conditions or treated with *L. major* or 10 ng/ml tumour necrosis factor- α (TNF α) in presence of 10 μ g/ml cycloheximide (TNF α treatment only) for the indicated duration. Caspase activity was measured by DEVDase as described previously (51). Values correspond to the rate of Ac-DEVD-7-amino-4-trifluoromethyl coumarin (AFC) peptide substrate hydrolysis and represent means \pm standard deviation of two independent experiments performed in triplicate. Increased apoptotic caspase activation was not detected in cells incubated for either 12 or 24 hours with *L. major*. As expected, treatment with TNF α induced a dramatic increase in caspase activity leading to apoptosis. These data provide strong evidence that prolonged exposure to *L. major* does not significantly induce apoptosis.

Supp. Fig. 2. The phosphorylation of p130Cas is increased in the B14V cells. p130Cas expressed in PTP-PEST cells (B14V and B11WT) was immunoprecipitated, separated by SDS-PAGE and its level of tyrosine phosphorylation was revealed by immunoblotting using an anti-phosphotyrosine antibody (4G10). Cells expressing PTP-PEST (B11WT) showed decreased levels of phosphorylation of p130Cas as compared to cells lacking PTP-PEST (B14V).

Supp. Fig. 3. Forms of p130Cas found in control and *L. major*-infected B14V fibroblasts interact with the SH2 domain of Crk in vitro. Serum-starved B14V and B11WT cells were incubated with or without *L. major* for 15 min, rinsed with ice-cold PBS and lysed in mRIPA (50mM Tris pH 7.4, 150 mM NaCl, 0.1% SDS, 0.5% sodium deoxycholate, 1% NP40, 10 mM NaF, 1 mM Na₃VO₄, Complete protease inhibitor). Cell extracts were centrifuged at 16000 \times g for 10 min at 4 $^{\circ}$ C, and the protein concentration was determined by Bradford assay. Cleared protein lysates (1 mg) were incubated for 2h at 4 $^{\circ}$ C with GST fusion proteins (GST or GST fused to the SH2 domain of Crk (GST-SH2^[Crk]), purified as described (19)) immobilized on glutathione sepharose beads (GE Healthcare). Beads were then washed three times with mRIPA, boiled in SDS-sample buffer and analyzed by immunoblotting. p130Cas is expressed similarly in B14V and B11WT cells, and *L. major* infection induces the appearance of a smaller form of p130Cas in both cell lines (right panel). The two forms of p130Cas protein interact with Crk SH2 exclusively in the absence of PTP-PEST (left panel). Filled arrowheads correspond to the long form of p130Cas and empty arrowheads point to the smaller form. The values on the right correspond to molecular weights in kDa.

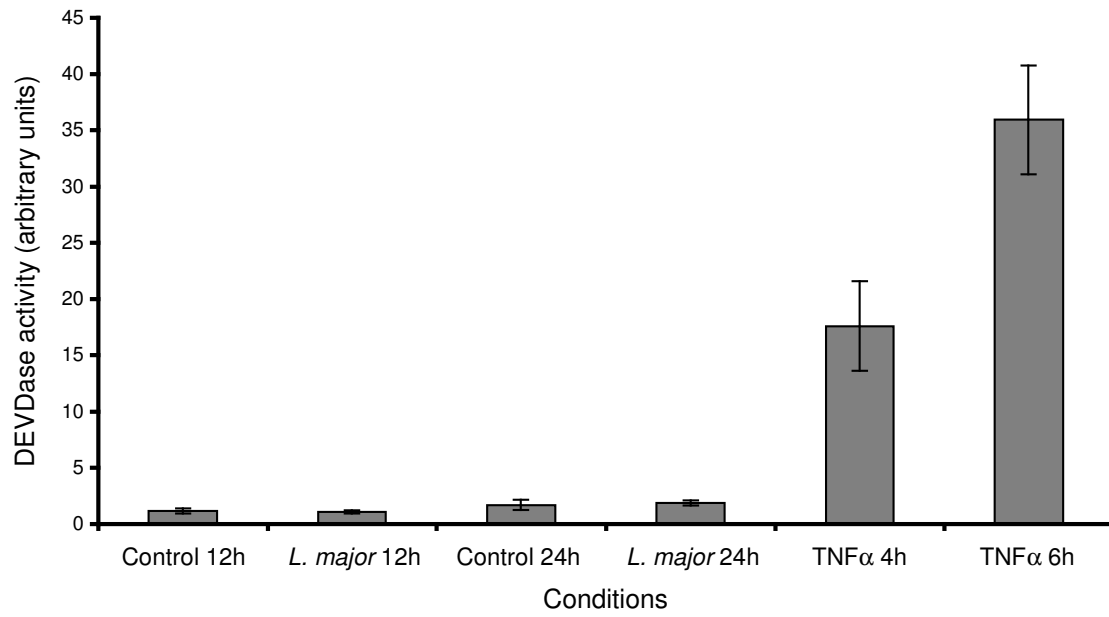
Supp. Fig. 4. *L. major*, but neither *L. tarentolae* nor *L. braziliensis* infection leads to rapid degradation of p130Cas and PTP-PEST. B11WT fibroblasts were incubated for the indicated time with the indicated *Leishmania* species at a ratio of 1:20. Protein lysates were harvested and analyzed by immunoblotting for p130Cas and PTP-PEST. Cellular p130Cas and PTP-PEST proteins were degraded within 15 min of incubation with *L. major*, whereas both proteins remained intact in cells incubated with *L. tarentolae* or *L. braziliensis* for 1h and 2h. Filled arrowheads identify intact proteins, and empty arrowheads point to cleavage products. The values on the right correspond to molecular weights in kDa.

Supp. Fig. 5. Infection with *L. major* does not induce the degradation of several proteins expressed in fibroblast-cells. P-MEFs were incubated with either *L. major*^{WT} or *L. major*^{gp63-/-} for 30 min at a ratio of 1:20 (cells:parasites). Protein extracts were analysed by immunoblotting for p130Cas, TC-PTP, integrin- β 1, STAT5, Shc, focal adhesion kinase (FAK), paxillin, I κ B α , AKT and PTP-1B. IB, immunoblot.

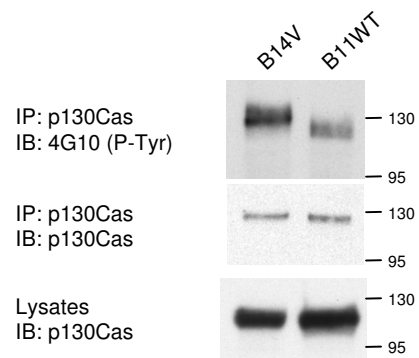
Supp. Fig. 6. Recombinant GP63 cleaves GST-PTP-PEST. GST-PTP-PEST was isolated from transfected *PTP-PEST*^{-/-} cells and incubated under control conditions or with 0.2 μ g or 1 μ g of purified recombinant GP63 (REGP63) for 30 min. Samples were analysed by immunoblotting for PTP-PEST (2530 antibody). The values on the right correspond to molecular weights in kDa.

Supp. Fig. 7. Expression of PTP-PEST promotes anisomycin-induced p38 MAP kinase phosphorylation. PTP-PEST^{-/-} parental cells and rescued clones were serum-starved (0.05% FBS DMEM, 16h) and stimulated with 50 ng/ml anisomycin in serum-free DMEM for the indicated time. Protein lysates were harvested and the level of phosphorylation of p38 was measured by immunoblotting using phospho-specific antibodies against phospho-thr180/tyr182 p38 MAP kinase. Total input of p38 as well as the expression level of PTP-PEST in each clone was also verified. Following treatment with anisomycin, PTP-PEST deficient cells displayed increased p38 phosphorylation. p38 was also activated in response to anisomycin in cells in which the empty vector was re-introduced (B14V). Importantly, all clones expressing PTP-PEST (B11WT, B118WT and B119WT) displayed increased phosphorylation of p38 following 15 min and 30 min treatment with anisomycin as compared to cells lacking PTP-PEST. These results strongly suggest that expression of PTP-PEST enhances the activation of p38 in anisomycin-treated cells.

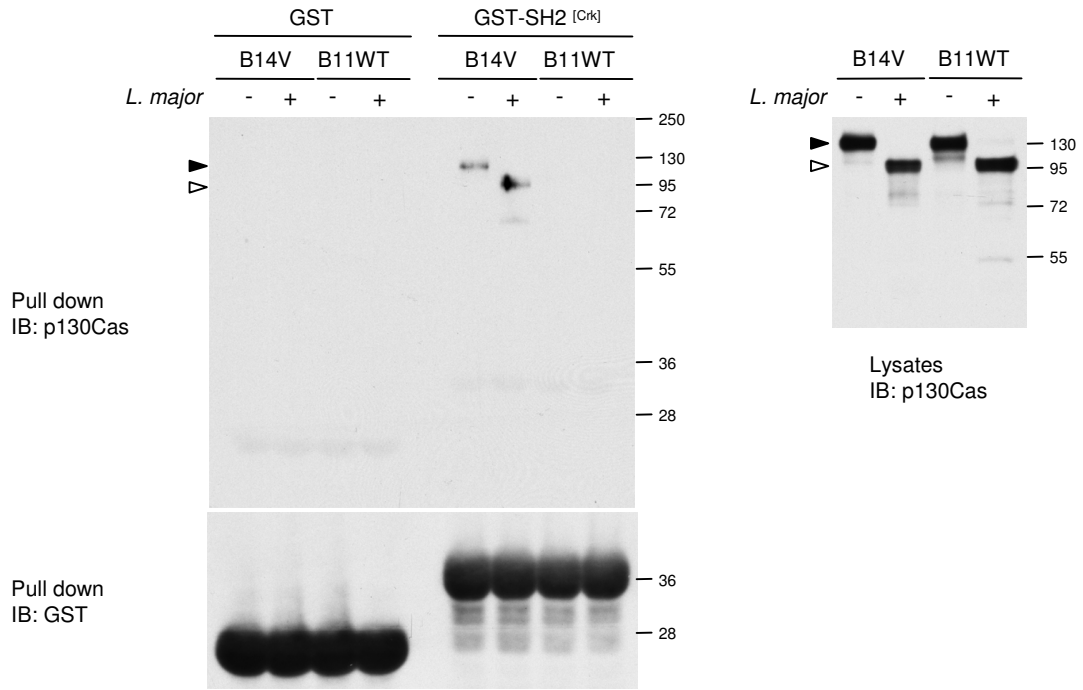
Supp. Fig. 8. Infection with *L. major* induces cleavage of TC-PTP near its C-terminus. (A) Schema illustrating the structural organisation of TC-PTP (TC45). The N-terminal catalytic domain (phosphatase) is followed by a nuclear localization signal (NLS). Localization of epitopes recognized by various antibodies (12A3, 6F7, 3E2 and 10B4) is shown. (B) P-MEFs were incubated with either *L. major*^{WT} or *L. major*^{gp63^{-/-}} for 30 min at a ratio of 1:20 (cells:parasites). The stability of TC-PTP was evaluated by immunoblotting of cellular lysates using different monoclonal antibodies specific for TC-PTP (12A3, 6F7, 10B4) (B). Although TC-PTP of P-MEFs infected with *L. major*^{WT} was cleaved, this PTP remained intact in cells exposed to *L. major*^{gp63^{-/-}}. These results imply that GP63 is necessary for parasite-mediated TC-PTP cleavage. Antibodies that are directed against the N-terminus segment of TC-PTP (12A3, 6F7, 3E2) all recognized a degradation product slightly smaller than the intact form (Fig. 5 D, Supplementary Fig. 1 and 2 B). In contrast, even following a long exposure, antibodies recognizing the C-terminus of TC-PTP (10B4) were not able to detect the same cleavage product (B). Together, these results suggest that the parasitic protease GP63 actively participates in the targeted cleavage of TC-PTP, a process that likely occurs within the C-terminal half of the enzyme. IB, immunoblot.



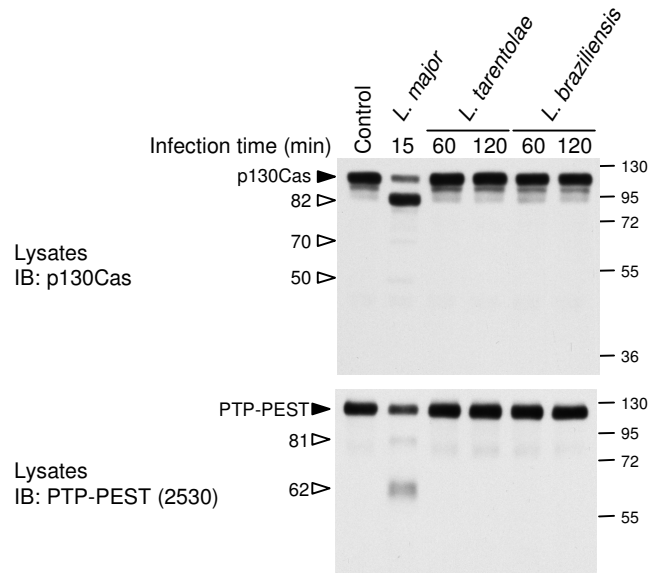
Supp. Fig. 1



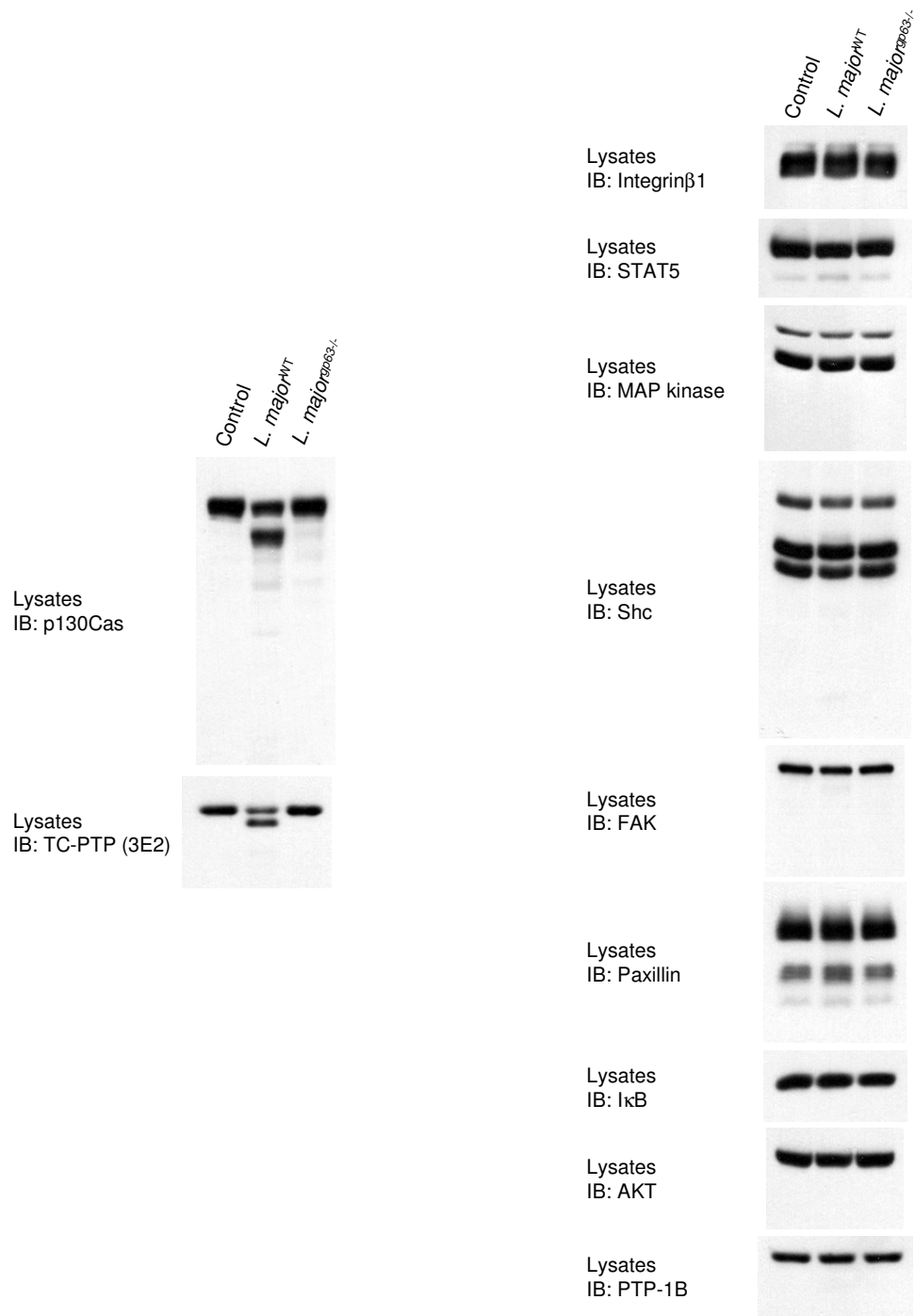
Supp. Fig. 2



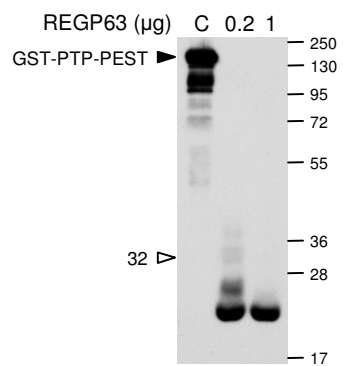
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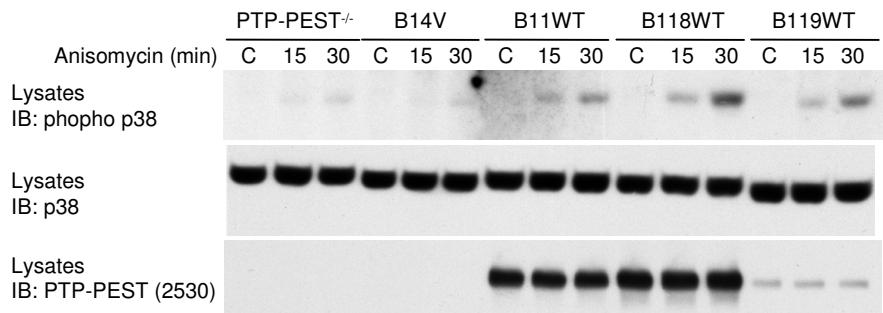
Supp. Fig. 4



Supp. Fig. 5

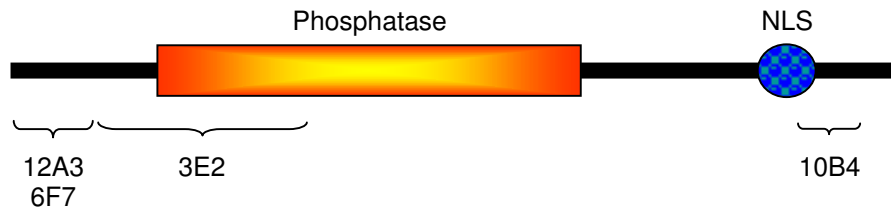


Supp. Fig. 6



Supp. Fig. 7

A.



B.

