

Supplementary material and methods

### **Bone marrow cell isolation and human DCs**

Bone marrow cells were collected from femur and tibia of C57 BL/6 mice as described previously [41].

Cells were plated at  $4.10^6$  cells/mL in low Petri dishes and cultured at 37°C, 5% CO<sub>2</sub> in 10mL RPMI-1640 medium containing 10%FCS, 10mM HEPES, 1mM pyruvate, 2mM l-glutamine, 100U/mL penicillin, streptomycin (all from PAN BioTech), 1% non-essential amino acids (GIBCO), and 50 μM 2β-mercaptoethanol. For BMDCs and BMMs differentiation, 5ng/mL of GM-CSF (J558L cell supernatant) and 20ng/mL of M-CSF (L929 cell supernatant) were added respectively. For tolerogenic DCs, 1,25 dihydroxyvitamin D<sub>3</sub> 1,25(OH)<sub>2</sub>D<sub>3</sub> (Sigma) was added every 2 days at  $10^{-8}$ M. Neutrophils were purified using the Neutrophils isolation Kit (Miltenyi biotech).

For humanDCs, Cytapheresis products were obtained from Centre Atlantic Transfusion Department (EFS-CA). An enriched DCs fraction was obtained by gradient centrifugation with Percol® (Healthcare) and cells were differentiated for 6 days in RPMI 1640 (Dutscher) medium supplemented with 10% FCS (Dutscher), 66ng/mL GM-CSF(Miltenyi Biotec) and 25ng/mL IL-4 (Miltenyi Biotec). At day 6, cells were collected and used for *in vitro* stimulation.

### **Immunofluorescence assays**

Freshly collected tumors were incubated overnight at 4°C in 4% paraformaldehyde, 10% sucrose and then immersed in 30% sucrose for 4 h. Tumor tissue was snap frozen in OCT compound. Eight μm-thick cryosections were incubated 10 min in PBS 0.1% Triton X100 and blocked for 30 min with Fc-blocking antibody 2.4 G2 (BD Biosciences), washed in PBS 0.1% Tween 20 with 1% BSA and incubated overnight at 4°C with PE-conjugated anti-Ly-6G (clone1A8), (eBioscience), Alexa-488-conjugated anti-CD68 (clone FA-11, eBioscience) and APC-conjugated anti-CD3ε (clone 145-2C11, eBioscience). For *in vitro* infections, cells were fixed, with 4% paraformaldehyde for 30 min at room temperature, washed in PBS and permeabilized in PBS supplemented with 0,2% Triton X-100 for 20 min at room temperature. Tachyzoites were then labeled with primary (infection serum) and secondary antibodies (Alexa 488 Goat anti-rabbit IgG, Thermo Fisher Scientific). For non-adherent cells, cytospin were performed (1000rpm, 5min) prior to immunofluorescence staining. Images were captured with a confocal Leica TCS SP8 microscope using Leica LAS AF software, or Olympus IX73 microscope using CellSens Dimension software.

### **qPCR detection**

*N. caninum* qPCR was performed on 250ng of genomic DNA in a total volume of 20 $\mu$ l containing 10 $\mu$ L of GoTaq® Probe qPCR Master Mix (Promega), 0.5 $\mu$ M of two primers (5'-TCCAATCCTGTAACGTGTTGCT-3'; 5'-CACAAACAAAAAGGAGCCTTGCT-3', Eurofins Genomics) specific for the *nc5* gene of *N. caninum*, and 180nM of the probe 5'-CTGCGCCCAACAAC-3' modified with 5'-FAM and 3'-TAMRA as reporter and quencher, respectively [42].

### Electron microscopy

For scanning and transmission electron microscopy, cells were cultured in 24-well plates and were collected at various time points following the addition of *N. caninum* (MOI 5). Adherent cells were trypsinized (Pan Biotech), washed in PBS and fixed in 0.1M phosphate buffer (pH 7.2) containing 4% paraformaldehyde and 1% glutaraldehyde. Next, cells were prepared as previously described [43].

### Pathologic examination

Hematein phloxin saffran stained slides were scanned by using NanoZoomer (Hamamatsu, Hamamatsu City, Japan). Based on morphology analysis, total and necrotic tumor areas were delineated by a pathologist on the digitalized slides and then analyzed using ImageJ software. Results are expressed in percentage of necrosis in tumor tissue.

### Construction of the NC1-IL15hRec

*Plasmid construction:* The plasmid pUC8 CAT/GFP was used to construct the recombinants NC1-IL15hRec. pUC8 CAT/GFP-IL-15hRec is a pUC8 plasmid in which the sequence encoding the complex IL-15/IL-15R $\alpha$  sushi (IL-15hRec) including the N-terminal signal sequence and the prodomain motif of MIC5 is cloned in the expression cassette between PmeI and NotI sites. pUC8 contains two expression cassettes. One was designed to express a CAT-GFP fusion protein to allow drug selection of stably transfected parasites, the second was designed to express proteins of interest. The expression of CAT-GFP is driven by the promoter of the *T. gondii*  $\alpha$ -tubulin gene ( $\alpha$ TUB5) and the 3'untranslated region (3'UTR) of the *T. gondii* SAG1 gene. The expression of the protein of interest is driven by the promoter of the *T. gondii*  $\alpha$ -tubulin gene in which a five-repeat element was inserted upstream of the transcriptional start site (leading to promoter  $\alpha$ TUB8 [27]).

*Engineering of human recombinant cytokine IL-15 (IL-15hRec):* IL-15hRec results from the association of the human sushi domain of the human IL-15 receptor subunit alpha precursor (GenBank: U31628.1) with the human

mature protein IL-15 (GenBank: U14407.1) via a peptide linker as described [44]. All these sequences were also optimized for protozoan organism (<https://eu.idtdna.com/CodonOpt>). IL-15hRec was synthesized as preproprotein with an N-terminal signal peptide and a separate, cleavable prodomain motif of MIC5. It was purchased from GenScript.

*Production of human recombinant cytokine IL-15 (IL-15hRec) by N. caninum:* Transfections were performed with  $10^7$  *N. caninum* tachyzoites in 650 $\mu$ L of cytomix [45] containing 3mM ATP and 3mM glutathione and 50 $\mu$ g of purified plasmid DNA (the plasmids were purified using the Qiagen kit®) linearized with PciI. Electroporations were performed in disposable cuvettes with an electroporator Biorad (2000 V, 50 ohms, 25 mF). After electroporation, the parasites are kept in the hood for 15 min at room temperature and then transferred to a fresh culture of fibroblast monolayers. After 24h *N. caninum* are respectively subjected to 80 $\mu$ M of chloramphenicol selection. After 15 days of selection, the parasites are cloned by limiting dilution in the wells of a 96-well plate of HFF cells in the presence of selection agent and the clones are amplified.

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