Supplementary Figures Legends

Figure 1S. Evaluation of the toxicity of the PPIG3/DNA nanoparticles upon systemic administration in immunocompetent mice. **A)** Body weight measurement of adult mice upon treatment with the PPIG3/DNA nanoparticles. Over the total duration of the experiment (30 days) no major changes ($p > 0.1$) in relative body weight were observed between animals treated with the PPIG3/DNA nanoparticles (50 µg DNA complexed with 250 µg PPIG3, n = 6), with the PPIG3 dendrimers alone (250 µg PPIG3, n = 4), with the buffer alone (250 µl of 5% dextrose solution, n = 4) and the non-treated animals (n = 4). **B)** Serum transaminase level measurements in mice upon treatment with the PPIG3/DNA nanoparticles. At the time indicated in the histogram, blood samples were taken from mice described in *A* and serum prepared. Serum ALAT and ASAT transaminase activity levels were measured at the routine Biochemistry Department of CHU of Nantes University. Twenty four hours after the systemic administration, a statistically significant increases (* $p<0.05$) in serum ALAT and ASAT transaminase activity levels was found in mice treated with the PPIG3/DNA nanoparticles or with the PPIG3 alone as compared to the control groups. 72 hours after injection no statistically difference ($p > 0.1$) in serum ASAT and ALT transaminase activity levels was observed between all groups of animals. This lack of difference in all groups remained until the end of the experiment (30th day). **C)** Histology analysis of the liver, lungs, spleen and kidney tissues of mice upon treatment with the PPIG3/DNA nanoparticles. One mouse within the PPIG3/DNA-treated group described in *A* was sacrificed 5 days after the systemic administration of the PPIG3/DNA nanoparticles. The liver, lungs, spleen and kidney organs were processed for a histological examination after hemalin and phloxin tissues staining. Tissues sections were then observed by two independent pathologists of the Pathology Department of CHU of Nantes University. No microscopic changes in tissues structure or

inflammatory cell infiltration were detected in these tissues at the nominal magnification indicated in the figure or at x 400 magnification (data not shown).

Figure 2S. PPIG3/DNA nanoparticles uptake by the murine J774 A1 macrophage cell line. **A)** Determination of the percentage of macrophages that have captured the PPIG3/DNA nanoparticles in culture. J774 A1 cells ($5x10^5$ cells/wells) were seeded in 6 well plates overnight at 37°C in culture. The following day, the PPIG3/DNA nanoparticles were prepared by mixing first 50 µg of the plasmid DNA encoding the F-Luciferase gene (PGL3 vector, Promega France, Charbonnieres, France) with 50 nM of the YOYO-1 fluorescent DNA intercalant agent (Invitrogen, Paris, France) in 250 µl of 5% dextrose solution. After 10 minutes incubation, 250 µg of the PPIG3 nanoparticles was added to the tubes and the PPIG3/DNA nanoparticles left to assemble for 10 min. at room temperature. An aliquot of PPIG3/DNA nanoparticles corresponding to 4 µg of plasmid DNA was added to the J774 A1 cells and the plate placed in culture for 6 h. Nanoparticles not internalized by the macrophages were removed by washing the cell monolayers with cold PBS and the plate with fresh complete medium replaced in culture for a further 24 hours. Uptake of the PPIG3/DNA nanoparticles by the macrophages was then examined at the nominal magnification of x 400 using an inverted fluorescent microscope coupled to a digital camera controlled by the Cell^B analysis software (Olympus Soft Imaging Solutions GmbH, Münster, Germany). FITC and Nomarski images of the same field of view were superimposed and the number of cells with at least one apparent well-stained green dot (arrows) counted. The data are expressed as the mean +/- SEM of percentage of green positive determined from 10 optical fields of view from a representative experiment done in triplicate. Positive control includes the use of 12 µl of the cationic liposome LipoF (Lipofectamine, Invitrogen, Paris, France) complexed with 4 µg of Luciferase plasmid DNA according to the manufacturer's protocol (Invitrogen, Paris, France).

Incubation of macrophages with 4 µg of the Luciferase plasmid DNA alone (DNA) was also included to evaluate the efficacy of the two nanoparticles PPIG3 and LipoF carriers to promote efficient DNA uptake. A statistically significant difference (* $p< 0.05$) was found between the treatment of macrophages with the LipoF/DNA nanoparticles and the treatment with the PPIG3/DNA nanoparticles or the DNA alone. In addition a statistically significant difference (* $p< 0.05$) was also found between the treatment of macrophages with the PPIG3/DNA nanoparticles and the treatment with the DNA alone. **B)** PPIG3/DNA uptake by macrophages does not lead to gene expression in culture. 24 hours after treatment of macrophage as described in *A*, cells were washed once with PBS and cell lysates prepared using the Promega Cell lysis buffer (Promega France, Charbonnieres, France). 30µg of proteins lysate were incubated with 100 nM of Luciferin substrate (Promega France, Charbonnieres, France) and the luciferase activity in each tube was quantified using a luminometer. Values presented are the mean +/- SEM of a three independent experiments. A statistically significant difference (** $p< 0.01$) was found between treatment of macrophages with the LipoF/DNA nanoparticles and treatment with the PPIG3/DNA nanoparticles or the DNA alone. **C)** PPIG3/DNA nanoparticles uptake by the macrophages cells did not lead to cellular toxicity in culture. Macrophages were plated in triplicate into a 96 well plate ($5x10^4$ cells/wells) overnight in culture before the addition of either PPIG3/DNA nanoparticles (0.4 µg plasmid DNA complexed with 2.5 µg of PPIG3), LipoF/DNA nanoparticles (0.4 µg DNA complexed with 1.2 µl of Lipofectamine) or plasmid DNA alone (0.4 µg plasmid DNA). After 6 h in culture, nanoparticles not internalized by the macrophages were removed by washing the cell monolayers with cold PBS and the plate with fresh complete medium replaced in culture for a further 24 hours. Number of viable cells in each well was then quantified using the MTS assay according the manufacturer's protocol (Promega France, Charbonnieres, France). The absorbance was read at 490 nm and experiments conducted twice. The results

are expressed as a percentage +/- SEM of the control where the absorbance value of the untreated cells (NT) was normalized to 100 %. No statistically significant differences ($p > 0.1$) were observed between the macrophage treatments in this experiment.