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Supplemental Information

Temporary Reduction of Membrane CD4 with the Antioxidant MnTBAP Is Sufficient to Prevent Immune Responses Induced by Gene Transfer

Sylvie Da Rocha, Jérémy Bigot, Fanny Onodi, Jérémie Cosette, Guillaume Corre, Jérôme Poupiot, David Fenard, Bernard Gjata, Anne Galy, and Thi My Anh Neildez-Nguyen

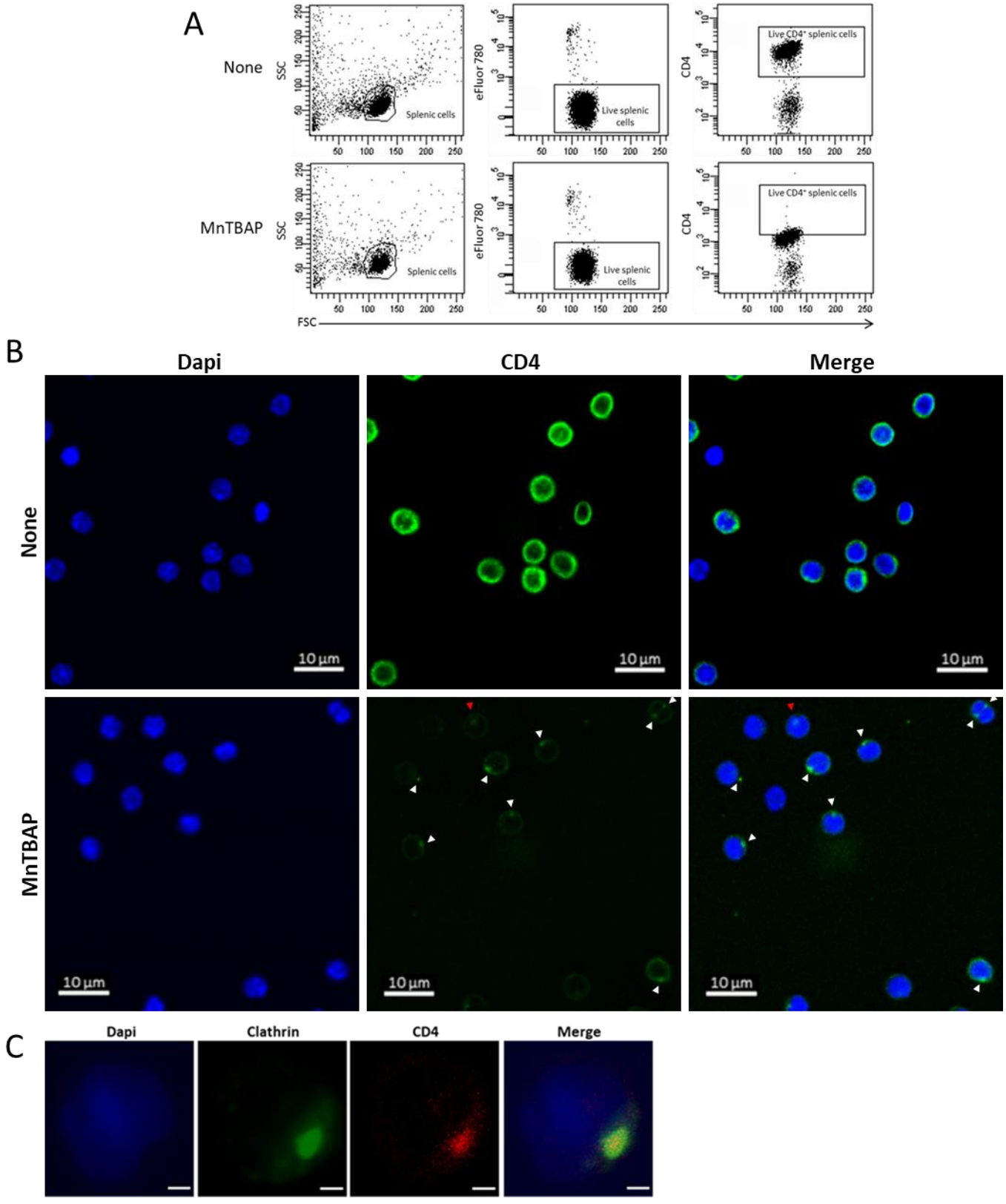

Figure S1. MnTBAP induces disappearance of CD4 from the cell surface and its internalization through clathrin-coated pits. (A) Flow cytometry analysis of $CD4^+$ T cells isolated by negative selection from mouse spleen cell suspensions, and treated for 2 hours in complete medium with or without MnTBAP at 400 µM. Dot plots show gated splenic cells (left panels) according to Forward Scatter (FSC)/Side Scatter (SSC) parameters; live splenic cells (middle panels) according to FSC and the viability dye, eFluor 780; and live $CD4^+$ splenic cells (right panels) according to FSC

and CD4 expression. One representative experiment out of at least six is shown. **(B)** Internalization of CD4 by treatment of $CD4^+$ T cells with MnTBAP. Panels show DAPI nuclear staining (left panels), CD4 staining (middle panels) and merged images (right panels). The green fluorescence (CD4-APC) shows CD4 localized to the cell surface in non-treated cells (None), but internalized into vesicles in 2 hours MnTBAP-treated cells (MnTBAP). Arrows show CD4 in vesicular structures. Scale bar, 10 µm. **(C)** High magnification images of colocalization of CD4 and clathrin observed after treatment of murine splenic $CD4^+$ T cell for 2 h with MnTBAP. Panels show DAPI nuclear staining (blue, left panel), clathrin (green, second panel) and CD4 (red, third panel) immunostaining, and merged image (fourth panel). Scale bar, 1 µm.

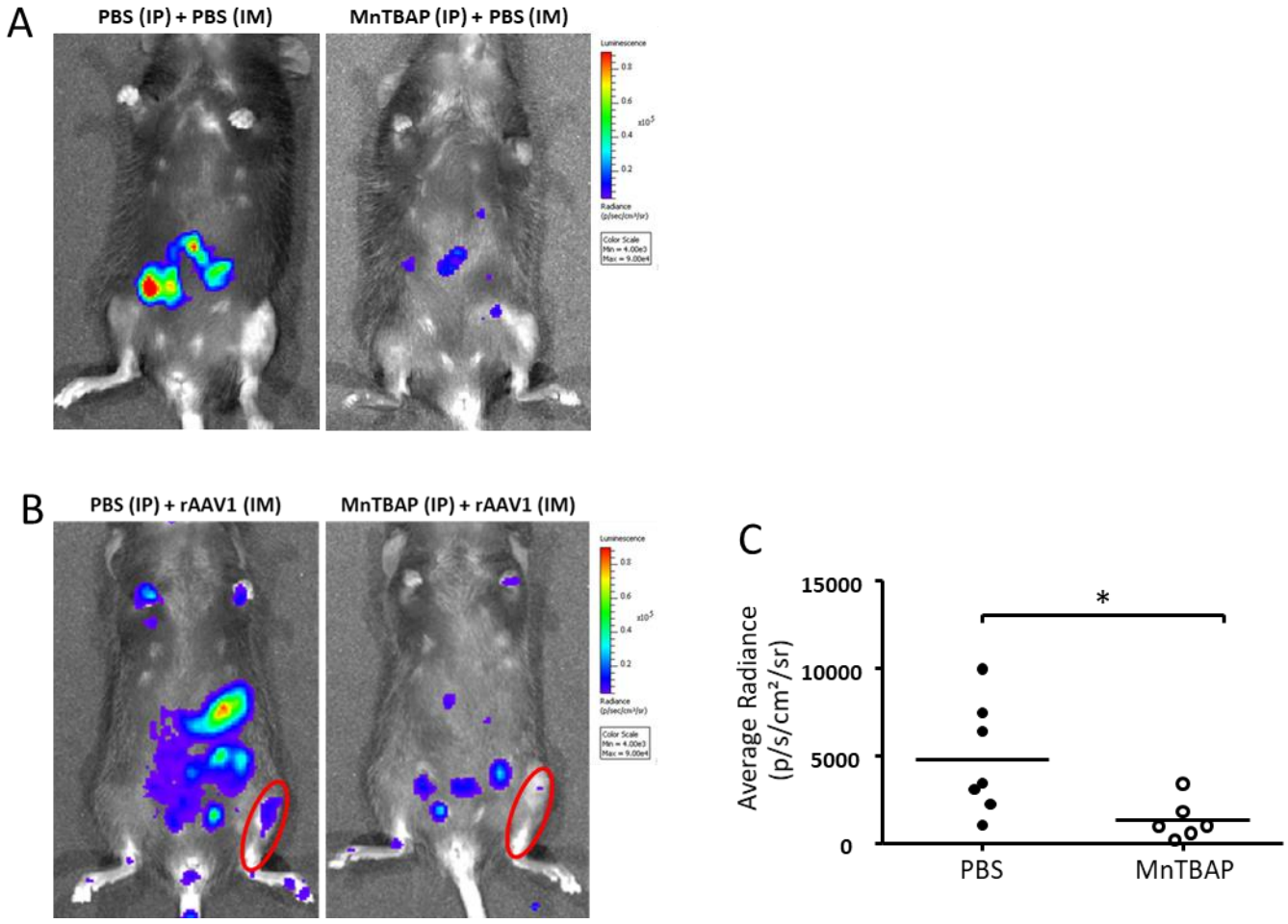

Figure S2. MnTBAP reduces inflammation induced by AAV-mediated gene transfer. Mice were i.p. injected with MnTBAP or with an equivalent volume of PBS, daily during five days. Two hours post first injection of MnTBAP, mice were administrated with PBS or rAAV1_CMV_SGCA_HY vector in the left TA. Four days post vector/PBS injection, mice were intravenously injected with 1.5 mg L-012, a chemical agent that reacts with ROS to produce light. Mice were placed in an *in vivo* imaging system. **(A)** Representative images of ROS detection in PBS- or MnTBAP-treated mice administrated with PBS in the left TA. Light emission, defined as radiance, was identified using the indicated pseudo-color scale. **(B)** Representative images of ROS detection in PBS- or MnTBAP-treated mice administrated with rAAV1 in the left TA. **(C)** Quantification of the average radiance of a Region Of Interest (ROI) drawn around the rAAV1 injected left inferior limb of each mouse (see Figure A). PBS-treated mice exhibit a significantly higher average radiance and therefore higher inflammation level than MnTBAP-treated mice. (n=2, 3-4 mice per group). All statistical analyses were performed using Mann-Whitney test. *$P < 0.05$.