OMTM, Volume *17*

Supplemental Information

In Vivo Myoblasts Tracking Using the Sodium Iodide Symporter Gene Expression in Dogs

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Supplemental information

Figures S1 to S6, table and supplemental materials and methods.

Supplemental figures

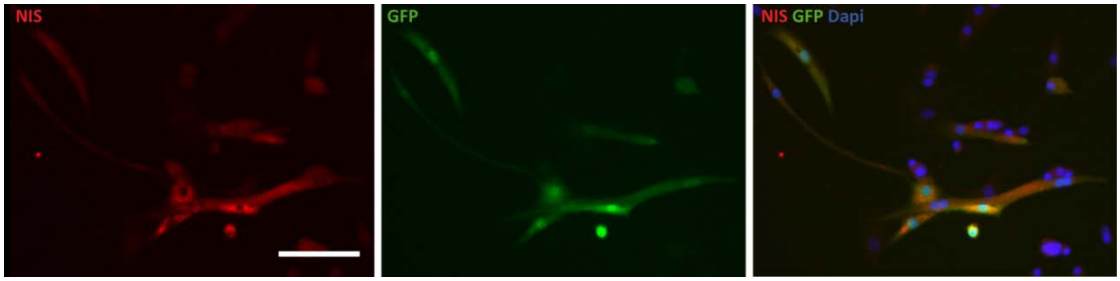


Figure S1. Transduced myoblasts display cNIS and GFP expression.

Expression of both cNIS and GFP proteins was observed in transduced canine myoblasts. Immunostaining was performed with primary antibody targeting cNIS protein and secondary antibody Alexa Fluor 647. GFP expression was directly observed without immunostaining. Scale bar: 100 µm.

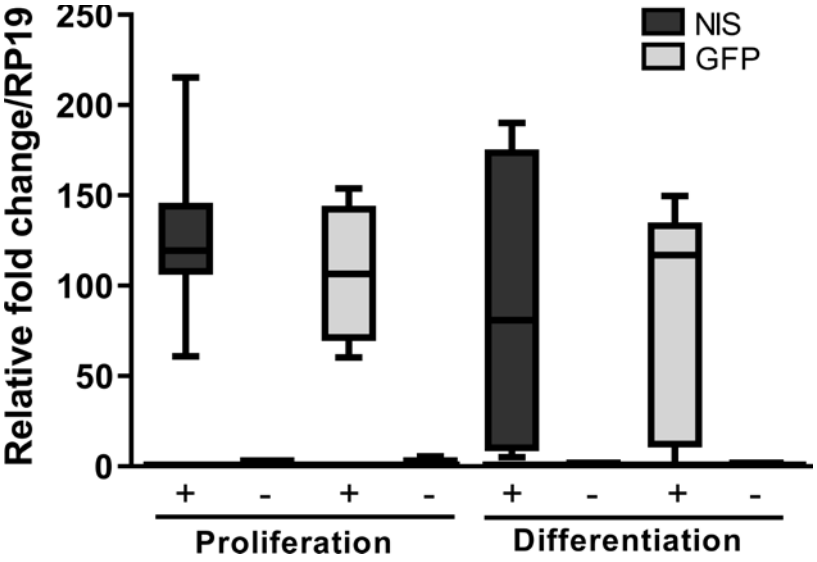


Figure S2. cNIS and GFP expression levels in primary canine myoblasts.

qPCR from $cNIS^+$ and $cNIS^-$ myoblasts RNAs showing the expression of cNIS (dark grey) and GFP (pale grey) transcripts in proliferation versus differentiation states. Plots of proliferating cells correspond to a pool of three independent samples at passage P6, P7 and P8. qPCR from both myoblasts in proliferation or myotubes in differentiation media showed expression of cNIS and GFP cDNAs with no significant differences between transcripts level in the two different conditions. qPCR was repeated three times and each sample was tested in triplicates. Statistical analysis was performed with GraphPrism using one-way Anova test.

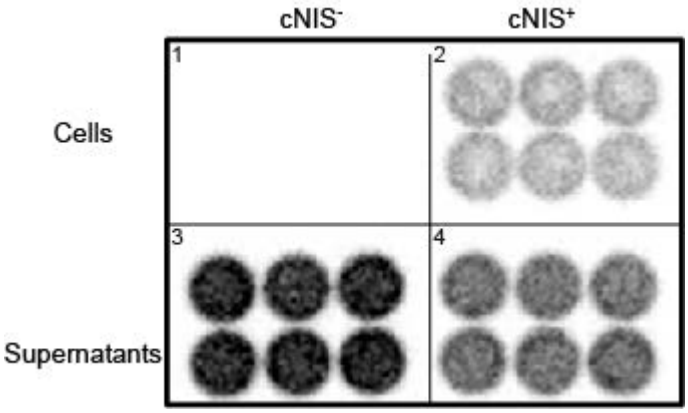


Figure S3. Scintigraphy image acquisition of cNIS$^+$/GFP$^+$ canine myoblasts *in vitro*.

SPECT/CT imaging of 6-wells plates of cNIS$^-$ (1) and cNIS$^+$ myoblasts (2) and their supernatant (3 and 4 respectively) incubated for 1 hour with 185 kBq of $^{99m}TcO_4^-$. No signal was detected for control (NIS$^-$) plate whose supernatant contained the full amount of radioactivity (3). NIS$^+$ myoblasts with substantial tracer uptake and the non-incorporated $^{99m}TcO_4^-$ in their supernatant (4). Representative image of 6 wells plates with six replicates.

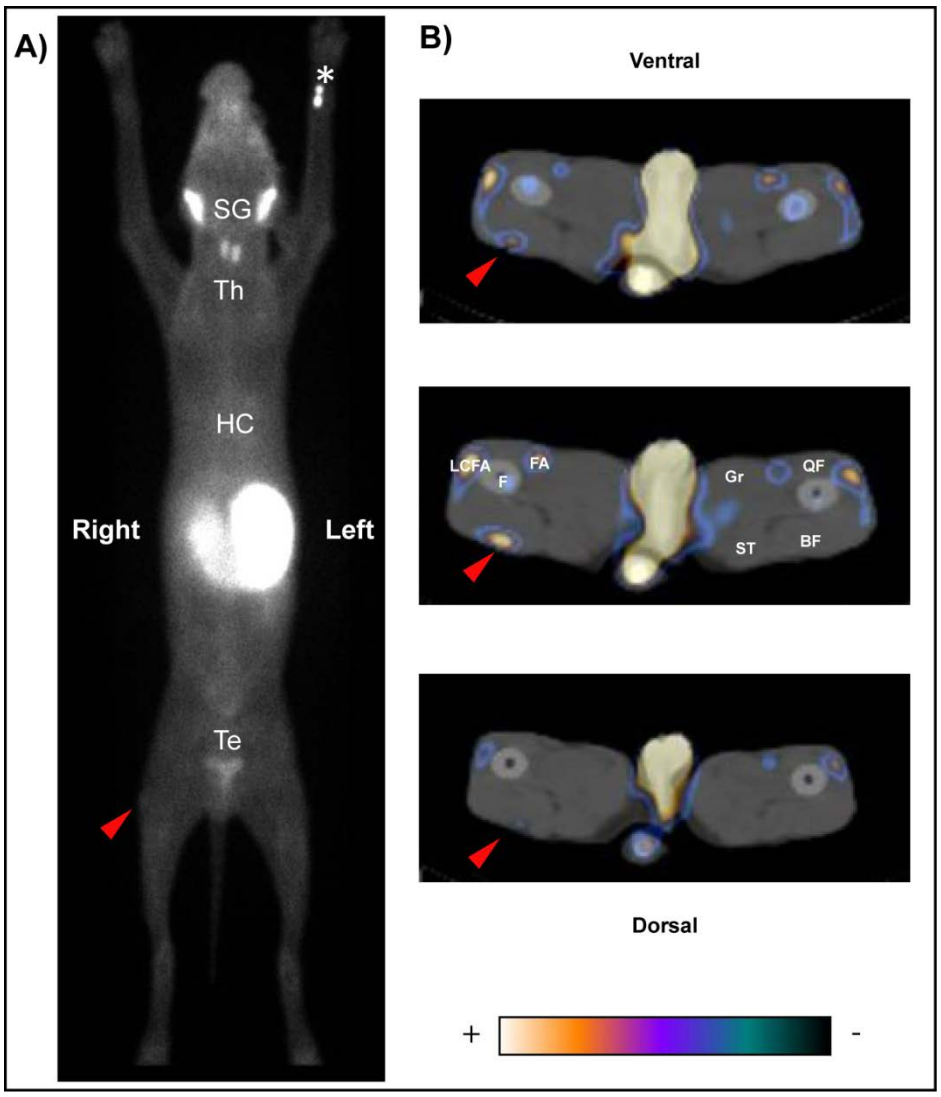


Figure S4. *In vivo* visualization of allogeneic cNIS$^+$ canine injected myoblasts.

SPECT/CT acquisitions at 48h post cell-injections (third injected dog, Jedi). (**a**) Posterior view of the planar whole body acquisition (1 hour post $^{99m}TcO_4^-$ injection). The signal seen at the level of the left carpus (* on the image) corresponds to the venous catheter through which the $^{99m}TcO_4^-$ was injected. No clear signal originating from any of the six injection sites was observed, except a slight spot at the level of the right thigh (indicated by a red arrowhead). (**b**) Axial slices of the SPECT/CT acquisition of the pelvic limbs (around mid-thigh) at the level of the active uptake site, the upper slice corresponding to the more proximal image, and the lower one to the more distal. Lesser emitting spots were visible symmetrically in both thighs, and were originating from the femur (F), and the vasculature (femoral artery, FA, lateral circumflex femoral artery, LCFA). A focus of increased uptake (indicated by a red arrowhead) was observed unilaterally, located in the right *biceps femoris* (rBF) muscle in a central and superficial region, compatible with the injection site (3 x 10^6 $cNIS^+/GFP^+$ myoblasts). No signal originating from the contralateral BF (lowest dose, 5 x 10^4 $cNIS^+/GFP^+$ myoblasts) was seen, nor from the other (intermediated doses) injected muscles. Gr: *gracilis* muscle, QF: *quadriceps femoris* muscle, ST: *semi-tendinous* muscle.

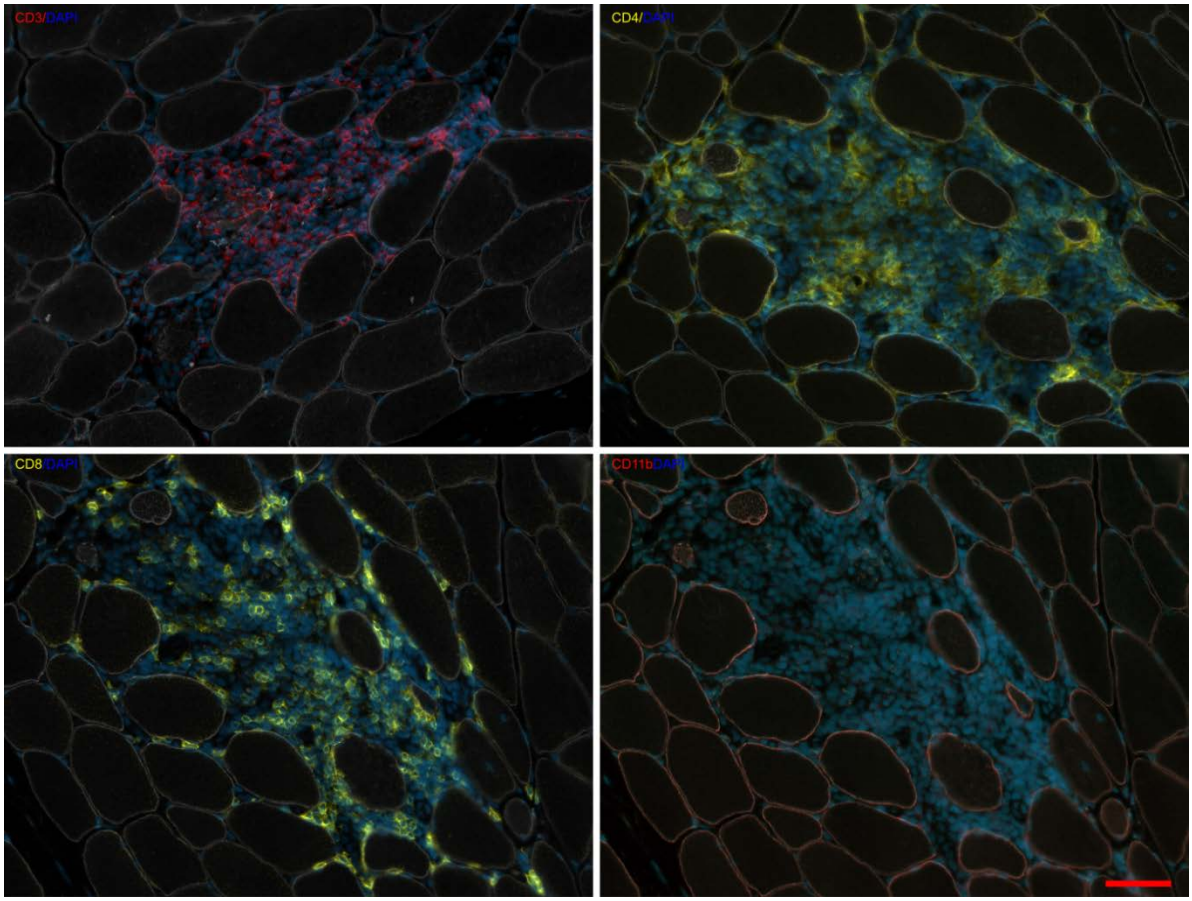


Figure S5. Infiltrates of cells in injected muscles.

The right BF muscle injected with 3 x 10^6 NIS^+ myoblasts and harvested after the last SPECT/CT, 1 month post cell injection. Immunostaining against CD3 (upper left), CD4 (upper right), CD8 (lower left) and CD11b (lower right) proteins. Nuclei DAPI staining in blue. Scale bar: 50µm.

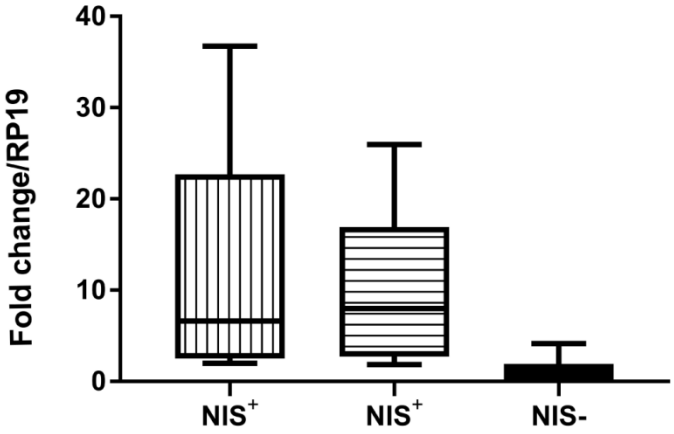


Figure S6. cNIS transcript expression in injected muscle.

qPCR from injected muscles' RNAs showing the expression of cNIS transcripts. Two quantities of $cNIS^{+:}$ corresponding to 20 x 10^6 NIS^+ myoblasts (vertical lines) and 3 x 10^6 NIS^+ myoblasts (horizontal lines) and $cNIS^-$ (black). Plots correspond to a pool of three independent samples. Relative fold change of cDNA relative to RP19 housekeeping gene. qPCR was repeated three times and each sample was tested in triplicates. No significant difference is observed between conditions. Statistical analysis performed with GraphPrism using one-way Anova test.

Supplemental table

Table of primers

cNIS cloning For	GGGGACAACTTTGTACAAAAAAGTTGGCATGGCCGCCGTCGAG
cNIS cloning Rev	GGGGACAACTTTGTACAAGAAAGTTGGGTATCAGAGGTCTGTCTCCCGAA
RPS19 For	CCTTCCTCAAAAAGTCTGGG
RPS19 Rev	GTTCTCATCGTAGGGAGCAAG
cNIS For	CTACCGCTACGGCTTCAAGT
cNIS Rev	TCCAGGTACTGGTAGGTGCT
GFP For	ATGTTGTGGCGGATCTTGAAG
GFP Rev	CAACAGCCACAACCTTATCATG

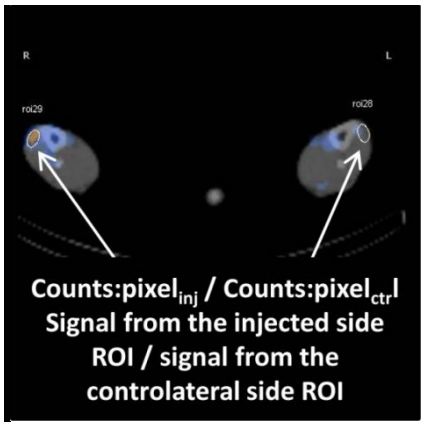
Supplemental Materials & Methods

Immunostaining S1

Transduced myoblasts were fixed with 2 % PFA for 10 min at room temperature and permeabilized with 0.5 % Triton X100 for 45 min. Cells were stained with mouse anti-NIS Ab, clone 2-2 (1/200 ; Millipore) and rabbit anti-GFP (1/300) in PBS + 2 % BSA overnight at 4 °C, then and anti-mouse Ab coupled to Alexa Fluor 647 (1/500) and anti-rabbit Alexa Fluor 488 (1/500) in PBS + 2 % BSA for 1 hour at room temperature. Nuclear staining was performed with DAPI 300 nM (Sigma Aldrich).

SPECT/CT analysis of cells *in vitro*

cNIS^{+} myoblasts or control myoblasts were seeded at a concentration of 5,000 cells/cm^{2} five days before imaging. Two 6-wells plates were imaged in parallel with dogs. Cells were treated with 185 kBq of $^{99m}TcO_4^-$ for 1 hour, then rinsed with PBS and supernatants were placed in a new empty 6-wells plate to be imaged too. To assess the minimal number of cells detected by the gamma camera, a plate was seeded with three different concentrations of cells: 10^6, 10^5, and 5 x 10^4 and 185 kBq of $^{99m}TcO_4^-$ were added to cells for 1 hour, then rinsed with PBS and supernatants were placed in a new plate to be imaged.



Region of interest delineation method

Selected ROI around the identified signal spot (on the left) and the symmetric ROI set on the contralateral limb (right). For imaging analysis and quantification, the ratio of the injected side signal spot (counts/pixel) is divided by the contralateral side signal (counts/pixel).