Stem Cell Reports, Volume *5*

Supplemental Information

Sodium Iodide Symporter PET and BLI Noninvasively Reveal Mesoangioblast Survival in Dystrophic Mice

Bryan Holvoet, Mattia Quattrocelli, Sarah Belderbos, Lore Pollaris, Esther Wolfs, Olivier Gheysens, Rik Gijsbers, Jeroen Vanoirbeek, Catherine M. Verfaillie, Maurilio Sampaolesi, and Christophe M. Deroose

Supplemental Figures and Legends

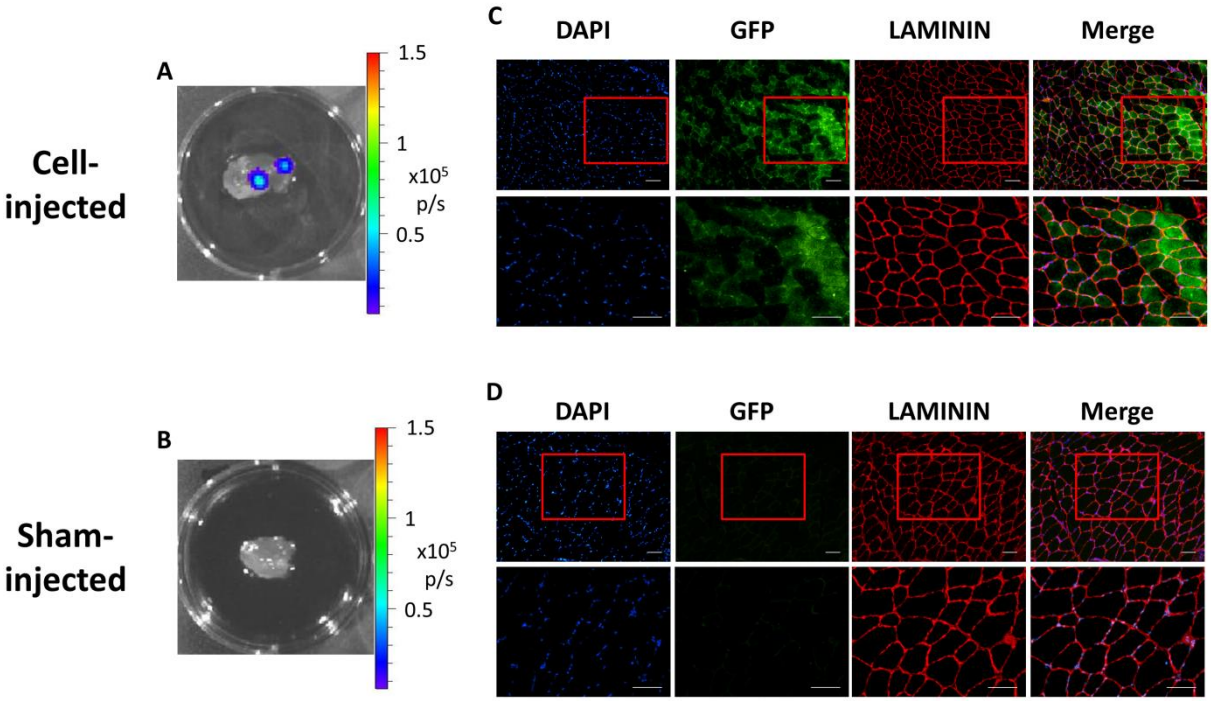

Fig. S1. Related to Fig. 6. Engraftment of mMABs in the gastrocnemius of nude mice with acute muscle damage. *Ex vivo* BLI indicated the presence of mMABs inside the gastrocnemius of cell-injected animals **(A)**, while a BLI signal is absent in sham-injected animals **(B)**. Histological assessment demonstrated engraftment of mMABs inside the gastrocnemius indicated via the presence of GFP^+ fibers surrounded by laminin **(C)**. No GFP^+ fibers could be observed in sham-injected animals **(D)**. Scale bar = 100 µm

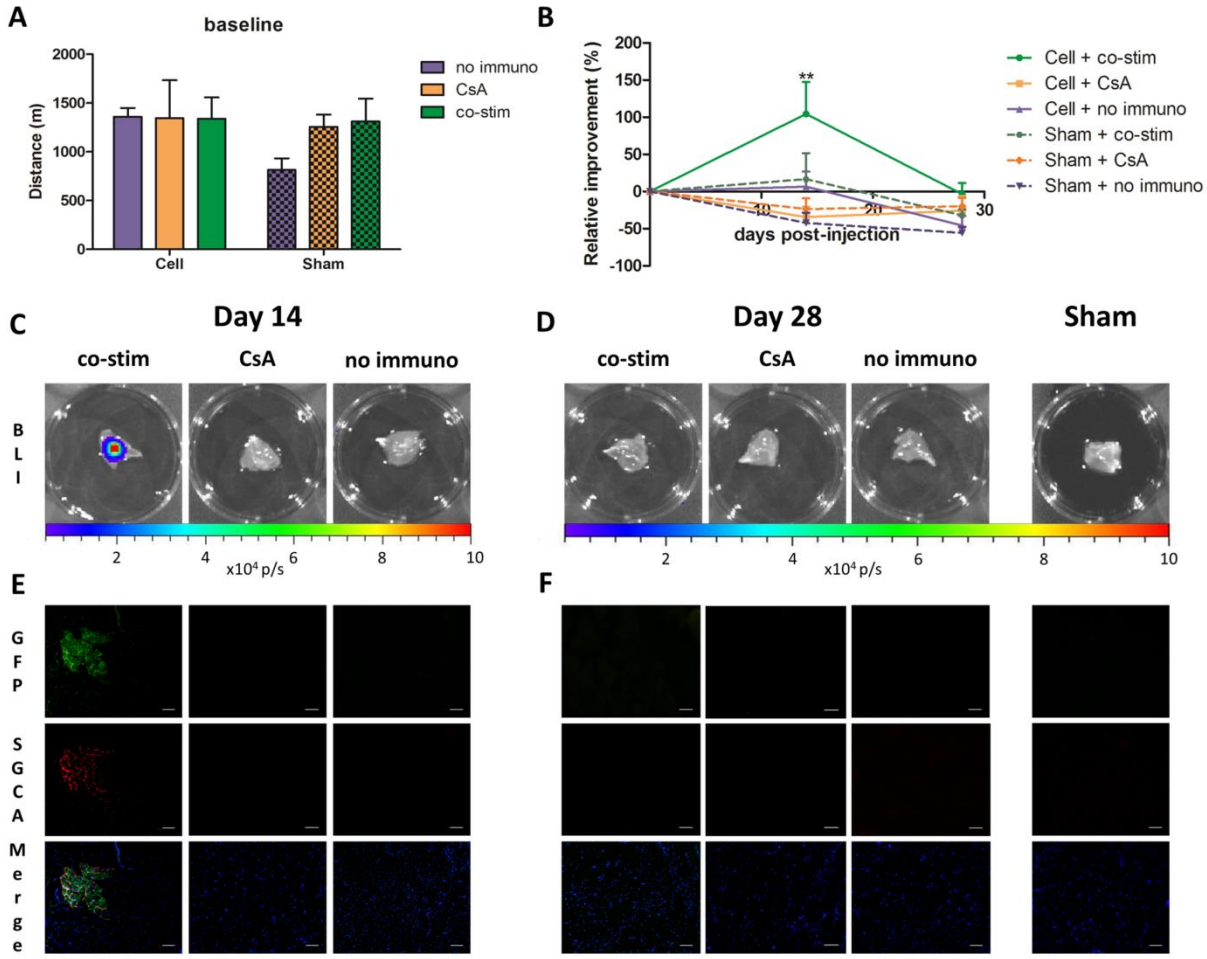

Fig. S2. Related to Fig. 5. Assessment of functional improvement of injected *Sgca*$^{-/-}$ mice. All animals were subjected to a treadmill exercise before cell injection to determine the baseline running distance. No difference in baseline running distance was observed between the different groups **(A)**. At day 14 post-injection, a significant relative improvement of running distance was observed in cell-injected animals with co-stim as immune suppressant compared to cell- and sham-injected animals (n≥5 IR) (**: $p < 0.01$). At day 28 post-injection, the functional relative improvement was lost in the cell-injected animals with co-stim. No significant differences were present between the groups **(**mean ± SEM) **(B)**. At day 14 post-injection, *ex vivo* BLI showed the presence of Fluc-hNIS^{+} mMABs inside the gastrocnemius of the cell-injected group receiving co-stim, while it was absent in the other cell-injected groups **(C)**. At day 28 post-injection, cells were lost in all cell-injected groups **(D)**. Histological assessment at day 14 demonstrated functionally integrated mMABs in the co-stim treated group via the presence of GFP^{+} fibers re-expressing SGCA. No GFP^{+} fibers could be retrieved in the other groups **(E)**. At day 28, no GFP^{+} fibers and SGCA could be retrieved **(F)**. scale bar = 100 µm

Supplemental Materials and Methods

Cell Culture

mMABs were isolated from the tibialis anterior muscle of healthy newborn C57/BL6 mice and sorted for alkaline phosphatase, were generated by our group (Sampaolesi et al., 2003). Cells were cultured in collagen (Sigma-Aldrich)-coated flasks (Nunc, Penfield, NY, USA).

Animals

Mice were sacrificed using Nembutal (60 mg/ml, Ceva, Libourne, France). The tibialis anterior, gastrocnemius and quadriceps of one hind limb were isolated and embedded in Tissue Tek OCT (Sakura, Alphen aan den Rijn, The Netherlands). The tissues were sliced in 10 µm cryosections using the CryoStar™ NX70 Cryostat (Prosan) with an environment temperature of -16°C and blade temperature of -22°C.

Immunohistochemistry

Samples were fixed with 4% paraformaldehyde (PFA (Kodak, Rochester, NY, USA) in FBS) for 10-15 min, rinsed and subsequently incubated with 0.2% triton (Triton x100, Sigma-Aldrich) in 1% bovine serum albumin (BSA in 1x PBS, Sigma-Aldrich) for 30 min. Samples were again rinsed and afterwards donkey serum (1/10 diluted in 1% BSA, VWR, Radnor, PA, USA) was added for 60 min. Next, samples were incubated with primary antibodies diluted in 1% BSA, either goat anti-GFP (1/500, # ab6673, Abcam, Cambridge, UK) and rabbit anti-laminin (1/400, #L9393, Sigma-Aldrich) or goat anti-GFP and mouse anti-SGCA (1/200, #A-SARC-CE (Leica Biosystems, Wetzlar, Germany), overnight at 4°C. The following day samples were rinsed and incubated with the secondary antibodies, either anti-goat 488 Alexa Fluorophore (#Z25002) and anti-rabbit 594 Alexa Fluorophore (#Z25307) or anti-goat 488 Alexa Fluorophore and anti-mouse 594 Alexa Fluorphore (#Z25007) (both 1/500 in 1% BSA, Life Technologies) for 1 hour at RT. Samples were rinsed and counterstained using Dapi (10 µg/mL in 1% BSA, Sigma-Aldrich). Afterwards, samples were analyzed with an Eclipse TI fluorescence inverted microscope (Nikon, Tokyo, Japan) using NIS Elements AR 4.11.01 software (Nikon).

Ex vivo bioluminescence

For *ex vivo* BLI scans, mice were placed in the flow chamber of IVIS Spectrum. Subsequently 126 mg/kg of D-luciferin was injected subcutaneously. Next, consecutive frames were acquired until the maximum signal intensity was reached. Afterwards, animals were sacrificed and muscles were harvested and placed in D-luciferin (15mg/mL) in the flow chamber of IVIS Spectrum.

Functional Tests

Treadmill

Treadmill analysis was performed on the Treadmill Simplex II (Colombus Instruments, Colombus, OH, USA) under a 10°-uphill oriented angle with a starting speed (n) of 10 m/min and an acceleration of 1 m/min^2. When the animal sat down on the grid for 2 seconds, the measurement was stopped. The final distance (d) run was calculated using following equation: $d = ((N+n)/2)*(N-n+1)$ with N = final speed. To calculate improvement, an individual baseline running distance was measured 3 days before injection. Afterwards, the obtained running distance at day 14 and day 28 of each mouse was subtracted with the corresponding baseline and normalized to baseline distance to achieve relative improvement (%).

Statistical Analysis

Data are presented as mean ± standard error of the mean (SEM). Two-way ANOVA statistical tests were performed with Bonferroni post-hoc test. P-values < 0.05 were considered statistically significant. Data were processed using GraphPad Prism version 5.00 for Windows.