

A stabilized abdominal imaging platform for intravital interrogation of preclinical omental metastatic niche models

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Abstract

The omentum is a highly specialized immunological and metabolic tissue that frequently acts as a permissive niche for metastatic dissemination within the peritoneal cavity. Despite its central role in metastatic progression and therapeutic response, real-time interrogation of the omental microenvironment in preclinical *in vivo* models remains technically challenging because of respiratory motion, tissue drift, and the fragility of abdominal tissues. Current methodologies largely rely on endpoint analyses or invasive tissue externalization procedures that disrupt physiological tissue dynamics and limit longitudinal investigation.

Here, we present a mechanically stabilized abdominal imaging platform that enables high-resolution longitudinal intravital imaging of the omentum in live mice. The system combines a custom abdominal imaging window with a lift-support stabilization structure that mechanically isolates the omentum from respiratory motion while preserving tissue architecture and physiological immune-cell behavior. Using fluorescent reporters, we demonstrate stable, long-term visualization of tumor progression, immune cell recruitment, and treatment-induced remodeling within the omental niche. As a *proof-of-concept* application, the platform was validated using an omentum-tropic model of peritoneal ovarian cancer dissemination, enabling longitudinal monitoring of metastatic engraftment and spatiotemporal immune dynamics during locoregional immunotherapy. Importantly, imaging through the stabilized window preserved lymphocyte motility significantly better than conventional tissue externalization approaches, thereby supporting the physiological relevance of the system.

Overall, this platform provides a robust, broadly adaptable framework for studying immune and metastatic processes in the peritoneal cavity and may facilitate preclinical evaluation of intraperitoneal therapies, cell-based immunotherapies, and microenvironment-targeted interventions.

INTRODUCTION

Peritoneal dissemination represents a major clinical challenge across multiple malignancies, including ovarian, gastric, colorectal, pancreatic, and appendiceal cancers (1). Once tumor cells colonize the peritoneal cavity, disease progression becomes difficult to control because of the emergence of spatially distributed metastatic niches that support tumor growth, immune evasion, and resistance to therapy (2, 3). Among these niches, the omentum plays a particularly important role due to its unique anatomical and immunological properties (4).

The omentum is a highly vascularized adipose-rich tissue containing specialized immune aggregates known as milky spots, which contribute to immune surveillance, tissue repair, and leukocyte trafficking under physiological conditions (5–8). However, during tumor dissemination, this tissue undergoes profound functional and structural remodeling and becomes a permissive microenvironment that supports metastatic engraftment and progression (9, 10). Interactions among stromal cells, adipocytes, vascular structures, resident immune populations, and infiltrating tumor cells generate a complex ecosystem that actively shapes disease evolution and therapeutic response (4, 11, 12).

Increasing evidence indicates that the omental microenvironment exerts a central role in regulating metastatic fitness, local immune suppression, and treatment resistance (13, 14). Consequently, understanding the spatial and temporal dynamics of this compartment has become increasingly relevant for the development of locoregional therapeutic strategies, including intraperitoneal chemotherapy, adoptive cell therapy, immunomodulatory approaches, and nanoparticle-based drug delivery systems.

Despite this growing interest, studying the omentum *in vivo* remains technically challenging. Conventional imaging modalities, such as computed tomography and positron emission tomography, provide information on tumor burden and anatomical distribution but lack the spatial resolution required to investigate cellular interactions within the metastatic niche (15). Histological and endpoint analyses provide static snapshots that fail to capture dynamic biological processes such as immune cell migration, treatment-induced remodeling, or early metastatic colonization (16). Although intravital microscopy has emerged as a powerful approach for interrogating tissue biology at cellular resolution (17), its implementation within the peritoneal cavity is hindered by respiratory motion, tissue drift, and the intrinsic mobility and fragility of abdominal organs (18, 19).

Previous approaches to imaging the omentum have often relied on extensive tissue externalization procedures requiring large laparotomies and prolonged mechanical exposure of abdominal tissues (16, 19, 20). While these methods can provide transient optical access, they substantially alter tissue physiology, compromise vascular perfusion, perturb immune-cell behavior, and preclude longitudinal follow-up studies. To address these limitations, we developed a mechanically stabilized abdominal imaging platform specifically designed for longitudinal intravital interrogation of the omental microenvironment. The system combines a custom-designed abdominal imaging window with a dedicated lift-support stabilization structure that isolates the omentum from respiratory motion while preserving tissue integrity and physiological dynamics. Using fluorescent tumor models, transgenic reporter mice, *in vivo* antibody labeling, and adoptive immunotherapy approaches, we demonstrate that this platform enables stable long-term visualization of metastatic progression, immune-cell recruitment, and treatment-associated remodeling within the omental niche.

As a *proof-of-concept* application, we validated the system using an omentotropic model of ovarian cancer dissemination, chosen for its robust, reproducible metastatic colonization of the omentum. However, the platform is broadly adaptable to multiple models of peritoneal metastasis and locoregional therapeutic interventions (21–23). By enabling physiologically preserved longitudinal imaging of the omental microenvironment, this approach provides a versatile framework for studying tumor–immune dynamics within metastatic niches *in vivo*.

RESULTS

Design and 3D fabrication of an Abdominal Imaging Window for artifact-free intravital microscopy

Intravital microscopy of the peritoneal cavity is often limited by natural tissue drift and pronounced breathing artifacts, which distort image acquisition and compromise data quality. Previous imaging procedures developed in our lab to study the omentum required externalization of the tissue via an extensive laparotomy. This highly invasive approach was restricted to endpoint procedures because the surgical trauma prevented wound closure and animal survival (16, 19, 20).

To overcome these limitations and enable longitudinal observation, we developed a custom 3D-printed Abdominal Imaging Window (AIW) together with a complementary support structure (FIGURE 1A). Our functional assessment confirmed that this two-part system provides a highly stable mechanical interface between the animal and the microscope. The materials selected for the final design successfully combined biocompatibility and optical clarity in the imaging window, while the robust matte-finished support platform effectively minimized stray reflections during high-intensity laser imaging. This structural design proved highly effective in elevating and stabilizing the omentum externally without compromising tissue integrity.

The use of biocompatible plastics and 3D printing allows cheaper production, quicker implementation by other labs, and compatibility with other imaging approaches as compared with titanium based AIWs.

Optimized surgical protocol enables minimally invasive, high-resolution in vivo imaging

To limit invasiveness and ensure longitudinal viability, we evaluated the efficacy of a refined surgical and postoperative protocol designed to reduce trauma and maximize survival. By employing a tightly restricted laparotomy and a specialized purse-string suture technique, the AIW was securely anchored to the abdominal wall without inducing peritoneal disruption, as illustrated step by step in FIGURE 2A. The correct positioning of the window within the lift support structure and the elevation of the abdomen are shown in FIGURE 2B. Crucially, integration of environmental controls within our customized imaging platform, especially thermal regulation and anesthesia delivery, effectively prevented hypothermia and surgical stress (FIGURE 2C). Combined with our targeted analgesic and antibiotic regimen, this optimized setup resulted in excellent postoperative recovery and maintained the physiological stability of the mice. These conditions proved essential for acquiring high-quality intravital data over extended periods, validating the platform's suitability for longitudinal survival studies.

The AIW system eliminates respiratory artifacts and enables longitudinal monitoring of tumor progression in the ovarian PCa model

To quantify the reduction in breathing-induced drift, we performed two-hour imaging sessions using CD2-RFP mice and tracked lymphocyte displacement along the Z axis with and without the lift-support structure. The analysis demonstrated that the absence of lift support significantly increased Z-plane movement ($p < 0.0001$), indicating greater respiratory drift that compromised image clarity and increased

motion variance (FIGURE 3A). These data validate our imaging system as a robust tool for optimized intravital microscopy.

Following this technical validation, we evaluated whether AIW implantation interfered with natural omental tumor growth. We surgically implanted the AIW in a cohort of four CD2-RFP mice and immediately administered via *i.p.* 2.5×10^6 ID8.OVA-Vegf/GFP cells. This approach enabled real-time observation of tumor cell migration to the omentum within the first hour after administration, followed by successful engraftment and subsequent tumor growth over seven days, with tumor volume increasing consistently across all operated animals.

To monitor longitudinal tumor progression, three additional groups of four mice were inoculated *i.p.* with the same dose of ID8.OVA-Vegf/GFP cells. Surgeries and imaging sessions were scheduled to observe the cohorts from days 7 to 14, 14 to 21, and 21 to 24 post-inoculation. Consistent with initial observations, mice within the same experimental groups exhibited uniform increases in tumor volume over time. Three-dimensional analysis of these tumors revealed progressive increases in tumor volume, area, and immune cell recruitment across the observed specimens (FIGURE 3B). Representative intravital images from the early post-implantation period documented the arrival and engraftment of tumor cells in the omentum from 1 hour to 7 days after inoculation (FIGURE 3C), whereas later time points showed progressive tumor expansion and lymphocyte accumulation at the tumor site (FIGURE 3D). ID8.OVA-Vegf/GFP tumors conserved their characteristic acinar-like morphology that we had previously evidenced by intravital imaging of explanted omental tissue (**SUPPLEMENTARY VIDEO 1–3**).

In Vivo imaging through the AIW preserves physiological lymphocyte motility

We next investigated whether this new *in vivo* imaging approach improved lymphocyte motility compared with the *ex vivo* externalization techniques used in our previous studies. AIWs were implanted in two CD2-RFP mice, and imaging was performed one day after surgery. This cohort was compared with two CD2-RFP mice whose omentum were externalized by laparotomy, stretched over a glass-bottom imaging surface, and secured with a coverslip. After one hour of imaging, we analyzed lymphocyte track displacement and speed within three milky spots per mouse (FIGURE 4A). The analysis indicated that lymphocytes observed through the AIW traveled significantly longer distances (FIGURE 4B) and at higher speeds than those in the externalized omentum group ($p < 0.0001$) (FIGURE 4C). Independent repetitions of this experiment yielded reproducible data (**SUPPLEMENTARY VIDEO 4 and 5**). These findings suggest that the AIW better preserves proper oxygenation, blood flow, and temperature within the omentum, conditions that may be lost or impaired during tissue externalization for imaging.

Multiparametric in vivo characterization of immune populations within the omental microenvironment

To characterize immune populations responding to tumor growth, we used fluorescent antibodies to label lymphoid and myeloid cells in C57BL/6 mice. Mice were inoculated *i.p.* with 2.5×10^6 ID8.OVA-Vegf/GFP cells one week prior to AIW implantation. Before imaging, mice received an *i.v.* injection of 50 μ L of our fluorescent antibody panel. This multiplexed mixture successfully delineated several enriched populations within the tumor microenvironment, including lymphoid (FIGURE 5A) and myeloid (FIGURE 5B) populations. Although signal intensity varied for certain markers, likely due to low antigen expression or suboptimal fluorophore brightness, this experiment demonstrates that the AIW effectively delivers and visualizes exogenous fluorescent markers within the target tissue.

Longitudinal assessment of locoregional immunotherapy dynamics

Previous studies in models of peritoneal carcinomatosis demonstrated that *i.p.* adoptive cell therapy achieves superior therapeutic efficacy compared with *i.v.* administration, although the mechanisms underlying this benefit have remained poorly characterized *in vivo*. Because the AIW enables longitudinal visualization of immune-cell trafficking within the omentum, we sought to directly evaluate the homing dynamics of transferred lymphocytes following locoregional versus systemic administration. To this end, mice bearing ID8.OVA-Vegf/GFP tumors received OT-I-CD2/RFP lymphocytes either *i.p.* or *i.v.* one week after tumor inoculation and AIW implantation. Imaging acquisition started immediately after treatment and continued over a 48-hour period to monitor lymphocyte recruitment to the omental metastatic niche. Longitudinal intravital imaging revealed marked differences between administration routes. Intraperitoneally delivered OT-I cells rapidly accumulated within the omentum, with detectable infiltration observed within the first hour after administration. In contrast, *i.v.* administered lymphocytes failed to efficiently localize to the tissue during the observation period (FIGURE 6B and **SUPPLEMENTARY VIDEO 6**). These findings provide direct *in vivo* evidence that locoregional administration enhances early lymphocyte homing to the omental metastatic niche, potentially explaining the superior efficacy previously observed for intraperitoneal adoptive immunotherapy in peritoneal carcinomatosis models.

DISCUSSION

The omentum represents a central immunological and metastatic niche within the peritoneal cavity, yet its dynamic biology remains incompletely understood due to technical challenges in imaging abdominal tissues *in vivo* (24). Here, we describe a mechanically stabilized abdominal imaging platform that enables longitudinal and physiologically preserved interrogation of the omental microenvironment at high spatial and temporal resolution.

A major strength of this system lies in its ability to mechanically decouple the imaging field from respiratory motion while maintaining tissue integrity. Abdominal tissues are inherently difficult to image due to continuous motion from respiration, intestinal motility, and tissue deformation. Although intravital microscopy has transformed the study of multiple organs, implementation within the peritoneal cavity has remained limited because stable optical access is difficult to achieve without extensive tissue

manipulation (25). Previous methodologies often relied on large laparotomies and tissue externalization procedures that substantially perturb tissue physiology. While these approaches provide temporary optical access, they may compromise vascular perfusion, alter oxygenation, disrupt tissue hydration, and induce inflammatory stress responses capable of modifying immune-cell behavior (26, 27). Our comparative analyses revealed markedly reduced lymphocyte motility in externalized tissues, suggesting that conventional methodologies may underestimate the true dynamic nature of immune interactions within the omentum.

Importantly, imaging through the stabilized abdominal window preserved significantly greater lymphocyte displacement and migration speed, supporting the physiological relevance of the platform. These findings highlight the importance of preserving native tissue architecture and mechanical stability when interrogating dynamic immune processes *in vivo*. Beyond mechanical stabilization, the platform enables longitudinal visualization of metastatic progression within the same animal over extended periods of time. This represents a substantial advantage over endpoint histological analyses, which provide only static representations of highly dynamic biological processes. Through longitudinal imaging, we were able to visualize early tumor-cell arrival, metastatic engraftment, progressive expansion, immune-cell recruitment, and treatment-induced remodeling within the omental niche.

The system additionally proved compatible with multiple complementary imaging approaches, including fluorescent reporter models, adoptive cell transfer strategies, and *in vivo* antibody labeling. This flexibility substantially broadens the applicability of the platform and creates opportunities for future studies incorporating biosensors, multiphoton microscopy, vascular permeability analyses, stromal remodeling studies, macrophage dynamics, and nanoparticle biodistribution approaches. The plastic nature of our AIW allows easier shareability, better cost effectiveness, and can allow multimodal imaging, which is unfeasible with titanium-based AIWs that cannot allow the isolation of the omental tissue.

From a translational perspective, the platform offers important advantages for studying locoregional therapeutic strategies targeting peritoneal malignancies. Intraperitoneal drug delivery has gained increasing interest because it enables high local therapeutic concentrations while limiting systemic toxicities. However, understanding how therapeutic agents dynamically interact with the metastatic microenvironment has remained difficult because of the absence of longitudinal imaging tools. Our adoptive cell transfer experiments directly visualized differential recruitment dynamics according to delivery route and demonstrated that intraperitoneally administered lymphocytes efficiently localized to the omental niche, whereas *i.v.* delivered cells displayed limited accumulation. These findings emphasize the importance of locoregional administration for overcoming the anatomical and biological barriers associated with peritoneal metastatic disease (28). Although ovarian cancer dissemination was used here as a *proof-of-concept* model because of its strong tropism for the omentum, the platform itself is not disease-restricted. The system is broadly adaptable to multiple models of peritoneal dissemination, including gastric, colorectal, pancreatic, and mesothelial malignancies, as well as inflammatory and regenerative conditions affecting the peritoneal cavity.

Several limitations should nevertheless be acknowledged. The surgical implantation procedure remains technically demanding and introduces some variability regarding the amount and positioning of exposed omental tissue (20). Although the stabilization system markedly reduces motion artifacts, minor drift can still occur during prolonged acquisitions. Additionally, imaging depth and signal intensity remain constrained by optical limitations and fluorophore performance. Finally, because the platform focuses specifically on the omentum, metastatic events occurring in other regions of the peritoneal cavity may not be simultaneously visualized.

Despite these limitations, this work establishes a versatile and broadly adaptable framework for physiologically preserved intravital interrogation of the omental metastatic niche. By enabling longitudinal visualization of tumor–immune interactions within the peritoneal cavity, this platform may facilitate mechanistic studies of metastatic progression and accelerate preclinical evaluation of microenvironment-targeted therapeutic strategies.

METHODS

Ethics information

All procedures involving mice were approved by the Universidad de Navarra Ethics Committee under protocol number 100/24. The experimental procedures followed the ARRIVE guidelines. In addition, all methods were performed in accordance with the relevant guidelines and regulations.

Animals

All mice were housed in the animal facility at the Cima Universidad de Navarra (Pamplona, Spain). Female C57BL/6 CD2-RFP mice (strain: Tg(CD2-dsRED)#Kio; 6–8 weeks old), originally obtained from Dr. Mark Coles's laboratory (University of York, UK), were used to establish the peritoneal carcinomatosis models (17322904). For exogenous antibody labeling, female wild-type C57BL/6 mice (6–8 weeks old; Envigo, Inotiv, Barcelona, Spain) were used. To obtain red fluorescent OT-I cells, CD2-RFP mice were crossed with OT-I mice (Jackson Laboratory, CA, USA). CD2-RFP × OT-I mice were euthanized by CO₂ asphyxiation. Immediately after euthanasia, the spleen was collected and mechanically dissociated through a 70 µm nylon cell strainer (FALCON, Corning Incorporated, NY, USA). The resulting cell suspension was resuspended in Roswell Park Memorial Institute (RPMI) medium (Life Technologies Limited, Paisley, UK), centrifuged, and treated with ACK lysis buffer (Gibco, Life Technologies Limited, Paisley, UK) for 1 minute to eliminate erythrocytes and residual tissue debris. Splenocytes were then counted and resuspended in phosphate-buffered saline (PBS, Life Technologies Limited, Paisley, UK).

Mice were socially housed in groups of 5 to 6 per cage under standard pathogen-free conditions (24 °C, 50–60% relative humidity, 12-hour light/dark cycle) with ad libitum access to food and water.

Cell Culture

ID8.OVA-Vegf/GFP cells were obtained from Dr. George Coukos (Ludwig Cancer Research, Switzerland). These cells were cultured in high-glucose Dulbecco's modified Eagle's medium (DMEM, Invitrogen, Carlsbad, CA, USA) supplemented with 4% fetal bovine serum (FBS, Gibco, Thermo Fisher Scientific), a mixture of 100 U/mL penicillin and 100 U/mL streptomycin (Gibco, Thermo Fisher Scientific, Life Technologies Corporation, NY, USA), 4mM L-Glutamine () and a solution containing 5 µg/mL insulin, 5 µg/mL transferrin, and 5 ng/mL sodium selenite (Thermo Fisher Scientific, Life Technologies Corporation, NY, USA). ID8.OVA-Vegf/GFP was maintained in a humidified incubator at 37 °C and 5% CO₂.

Abdominal Window and Insulation System Design

A custom-designed abdominal window and insulated bed system was developed to enable high-resolution, stable, and reproducible *in vivo* imaging of the omentum. The system was specifically engineered to allow anatomical isolation of the omentum, minimize respiratory motion artifacts, and facilitate long-term optical access for multiphoton microscopy.

The abdominal window consisted of a circular structure with external flanges designed to secure the skin and abdominal wall around the incision site. A peripheral groove was incorporated to accommodate a standard 12 mm circular glass coverslip (GL100 #1 Glass Coverslip, APJ Trading Co, Shanghai, China), thereby providing a stable and optically flat surface for imaging. Centrally, a longitudinal pin traversed the diameter of the window and served as a mechanical support to elevate the omentum above the peritoneal cavity.

The window was designed to interface with a custom insulated bed through a lateral notch, allowing fixed and reproducible mounting. The insulated bed itself consisted of a main horizontal platform (210 × 150 mm) on which the mouse was positioned, together with a lifting mechanism composed of two lateral arms connected to a movable platform supporting the abdominal window. Controlled elevation of the platform was achieved by adjusting threaded screws located underneath the base, enabling fine-tuning of the window height relative to both the animal and the microscope stage.

Additional design features included an anterior clip to secure the isoflurane anesthesia tubing and a compartment beneath the mouse platform to house a flexible heating mat, which maintained the animal's physiological temperature during imaging.

The designs are freely available for download in the supplementary data (**SUPPLEMENTARY DIGITAL DATA**).

Window and Insulation System Production

All components were designed using Autodesk Fusion 360 and manufactured through a combination of stereolithography (SLA) and fused deposition modeling (FDM) 3D-printing techniques. The abdominal window was fabricated using biocompatible BioMed Clear resin (Formlabs®), whereas the insulated platform, lifting structures, support arms, and window housing were produced using black PLA filament.

Mechanical adjustment elements such as screws, nuts, and springs were incorporated into the lifting system using off-the-shelf components.

Post-processing steps included resin curing under UV light, mechanical cleaning, and sterilization of all components before *in vivo* use.

Surgical Procedure

Mice were anesthetized with 2.5% isoflurane (IsoVet, Piramal Critical Care B.V., Voorschoten, Netherlands). The abdominal area was shaved, and the skin was sterilized with topical povidone-iodine (Betadine®, MEDA Pharma Ltd., Madrid, Spain). A 1.5 cm incision was made through the skin and peritoneum to expose the organs, and a purse-string suture consisting of 8 stitches (Vicryl 11 mm, ETHICON Inc., Madrid, Spain) was placed around the wound. The abdominal window was inserted into the opening, and the suture was tightened and double-tied to secure the device. The omentum was then gently positioned on top of the window's mast to elevate it above the rest of the peritoneal cavity. A 12 mm coverslip (GL100 #1 Glass Coverslip, APJ Trading Co, Shanghai, China) was used to seal the window and was attached using 6 drops of surgical glue (Vetbond Tissue Adhesive, 3M, Neuss, Germany) applied to the designated peripheral groove. For postoperative care, mice received 3 doses of buprenorphine (Bupaq, VetViva Richter GmbH., Wels, Austria) at 0.3 mg/kg, and enrofloxacin (Kariflox, Karizoo Laboratories Ltd., Barcelona, Spain) was administered in the drinking water at 25 mg/kg.

For mice requiring omentum externalization, animals were maintained under isoflurane anesthesia. The abdomen was opened via a longitudinal incision along the linea alba and a transverse incision along the rib cage to expose the viscera. Using surgical sutures, the xiphoid process was tied and gently pulled to reduce respiratory movement. Mice were placed in a lateral decubitus position, the omentum was carefully exteriorized from the peritoneal cavity and extended onto a glass-bottom imaging surface, and a glass coverslip was then placed over the omentum to stabilize it. During imaging sessions, mice were covered with a heated pad (DAGA, Tenacta Group, Bergamo, Italy), and body temperature was monitored using a PhysioSuite rectal thermometer (Kent Scientific Corporation, CT, USA) to ensure that it remained close to 37 °C. After imaging, the mice were euthanized because of the severity of the surgical procedure.

Adoptive Cell Therapy Treatment

C57BL/6 mice (Envigo, Inotiv, Barcelona, Spain) were divided into two groups of six and inoculated with ID8.OVA-Vegf/GFP cells four days before window implantation surgery. The following day, mice were treated with OT-I splenocytes immediately after cell isolation and counting. A total of 2.5×10^6 cells in 300 µL PBS was injected either intraperitoneally (*i.p.*) or intravenously (*i.v.*).

Fluorescent Antibody Panels

To visualize tumor-infiltrating cell populations, fluorescently labeled antibodies (BioLegend, CA, USA) were administered *i.v.* at a 1:20 dilution. The lymphoid panel consisted of antibodies against CD45R

conjugated to BV421 and CD8 conjugated to PE. Similarly, the myeloid panel comprised a mixture of antibodies against F4/80 conjugated to BV421, Ly6G conjugated to PE, and MHC II conjugated to AF647.

Imaging Acquisition Setup & Analysis

Imaging was performed using an inverted Zeiss LSM 880 confocal microscope (Carl Zeiss, Oberkochen, Germany) equipped with a 25× immersion objective (NA 0.8). The system featured five acquisition channels: three high-sensitivity gallium arsenide phosphide (GaAsP) detectors and two conventional photomultiplier tubes (PMTs). The setup used laser lines at 405, 488, 543, and 633 nm. Fluorescence emission was collected using an LSM BiG 1935 – 176 Green-Red filter (Carl Zeiss MicroImaging GmbH, Jena, Germany). To adapt the inverted microscope to an upright configuration, an InversterScope (LSM Tech, PA, USA) objective inverter was used to position the objective above the imaging platform. During acquisition, mice were maintained under general anesthesia (2.5% isoflurane) using a SomnoFlo anesthetic delivery system (Kent Scientific Corporation, CT, USA). Immersol (Carl Zeiss Jena GmbH, Oberkochen, Germany), a low-fluorescence, halogen-free, water-based solution with a refractive index of 1.334, was used as immersion fluid. To track the same regions of interest longitudinally, the vasculature and the position of tumors relative to lipid structures were used as anatomical landmarks.

Images were acquired using ZEN Black software (Carl Zeiss, Oberkochen, Germany). To evaluate the mechanical stability of the abdominal window system, tissue movement was analyzed using Imaris Viewer software (Oxford Instruments, Abingdon, UK). Quality parameters were assessed to quantify the isolation from respiratory interference and to track cell motility, whereas volume and area measurements were used to monitor tumor progression. Spider-plots were generated using the cell motility data with the help of AI tools.

Statistical Analysis

For statistical analysis of the imaging data, we used GraphPad Prism version 8 software (GraphPad Software, Boston, MA, USA). Data normality was assessed using the Shapiro-Wilk test. Differences between two independent groups were analyzed using an unpaired t-test for normally distributed data, whereas the Mann-Whitney U test was applied to nonparametric data. For multiple comparisons involving three or more experimental groups, one-way ANOVA or the nonparametric Kruskal-Wallis test was used, depending on data normality. Statistical significance was set at $p < 0.05$.

Declarations

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AUTHOR CONTRIBUTION

F.A. and A.T. designed the experiments. R.G-F., N. L-A., D.M-L. performed the experiments and processed the samples. D.R-G performed bioinformatic analyses. I.M. contributed to the main conceptual idea of the study. F.A. and P.B. performed all the statistical analyses and analyzed the data. R.G-F., A.T. and F.A. wrote the manuscript. All authors critically reviewed the manuscript for important intellectual content and gave final approval of the manuscript.

COMPETING INTEREST STATEMENT

Ignacio Melero has received research funding from AstraZeneca, BMS, Highlights Therapeutics, Alligator, Pfizer, Genmab, and Roche. He has also received honoraria for speaking engagements from MSD and serves as a consultant or advisory board member for BMS, Roche, AstraZeneca, Genmab, Pharmamar, F-Star, Bioncotech, Bayer, Numab, Pieris, Gossamer, Alligator, and Merck Serono. All other authors declare that they have no competing interests.

Data availability

The datasets used and/or analysed during the current study available from the corresponding author on reasonable request.

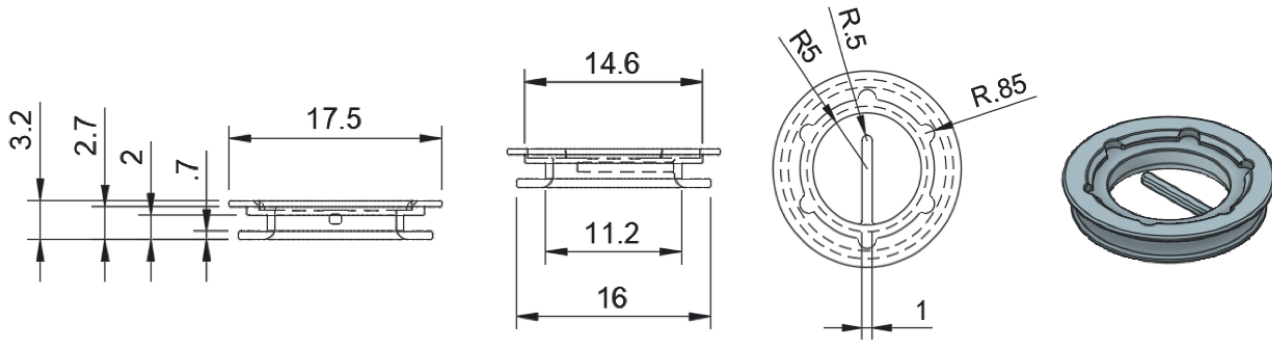
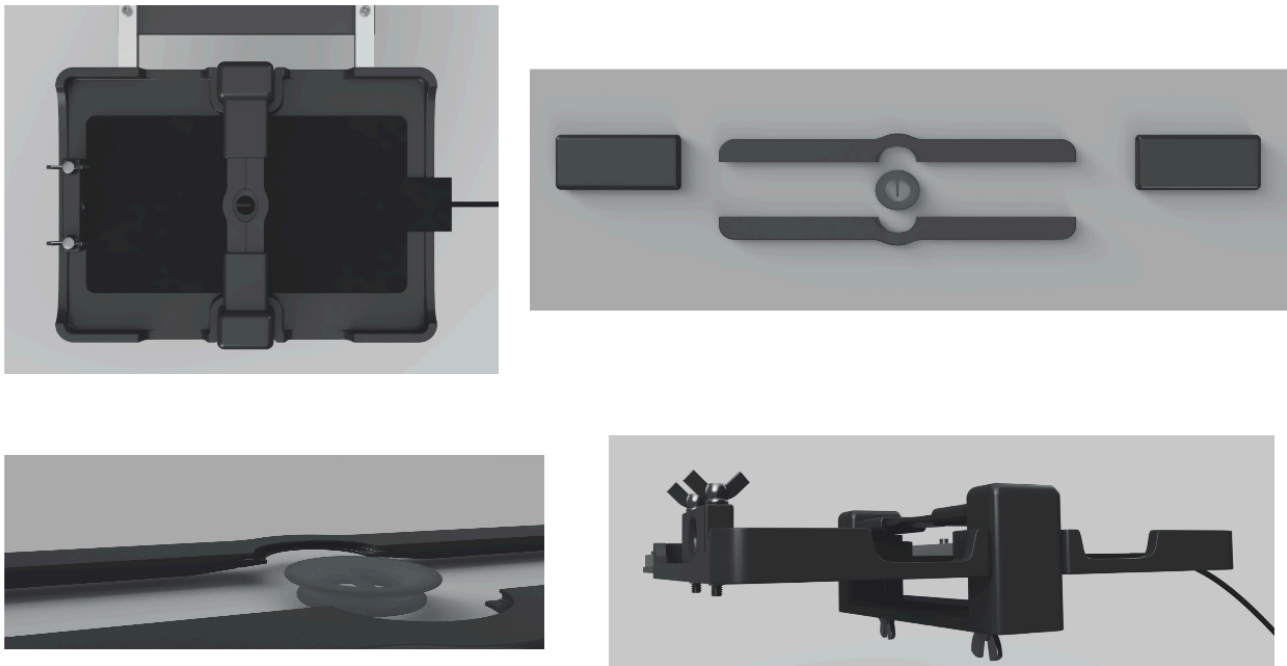
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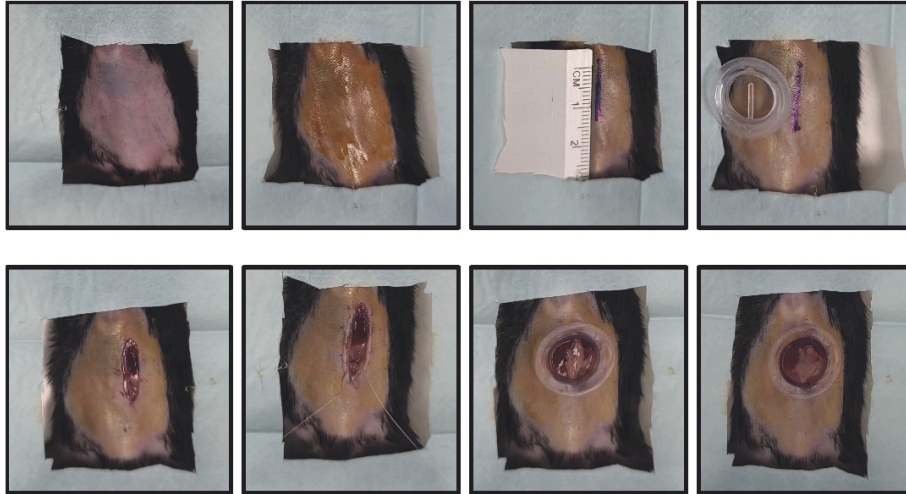
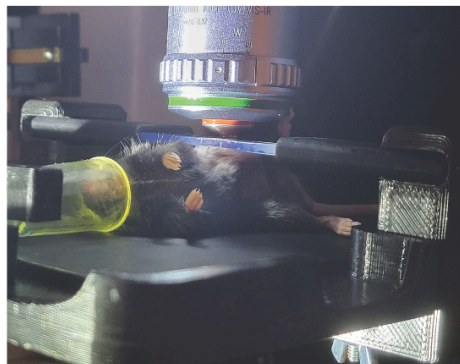
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Figures

A**B****FIGURE 1****Figure 1**

Design of the abdominal imaging window and support platform. (A) Schematic representation of the circular abdominal imaging window (AIW), highlighting the dimensions of the external flanges for skin attachment, the peripheral grooves, and the central longitudinal pin designed to support and elevate the omentum. (B) Three-dimensional renderings of the support structure for the window, showing the individual components that clamp the window and secure the mouse to the platform.

A**B****C****FIGURE 2****Figure 2**

Surgical implantation and intravital imaging setup. (A) Sequential images showing abdominal disinfection, laparotomy, purse-string suture placement, window implantation, and omentum exposure. (B) Top view of the abdominal window mounted within the support structure, and side view of the abdomen with the platform support elevated. (C) *In vivo* imaging setup for confocal microscopy. The

platform stabilizes the mouse and secures the isoflurane delivery nose cone while the objective is positioned above the imaging window.

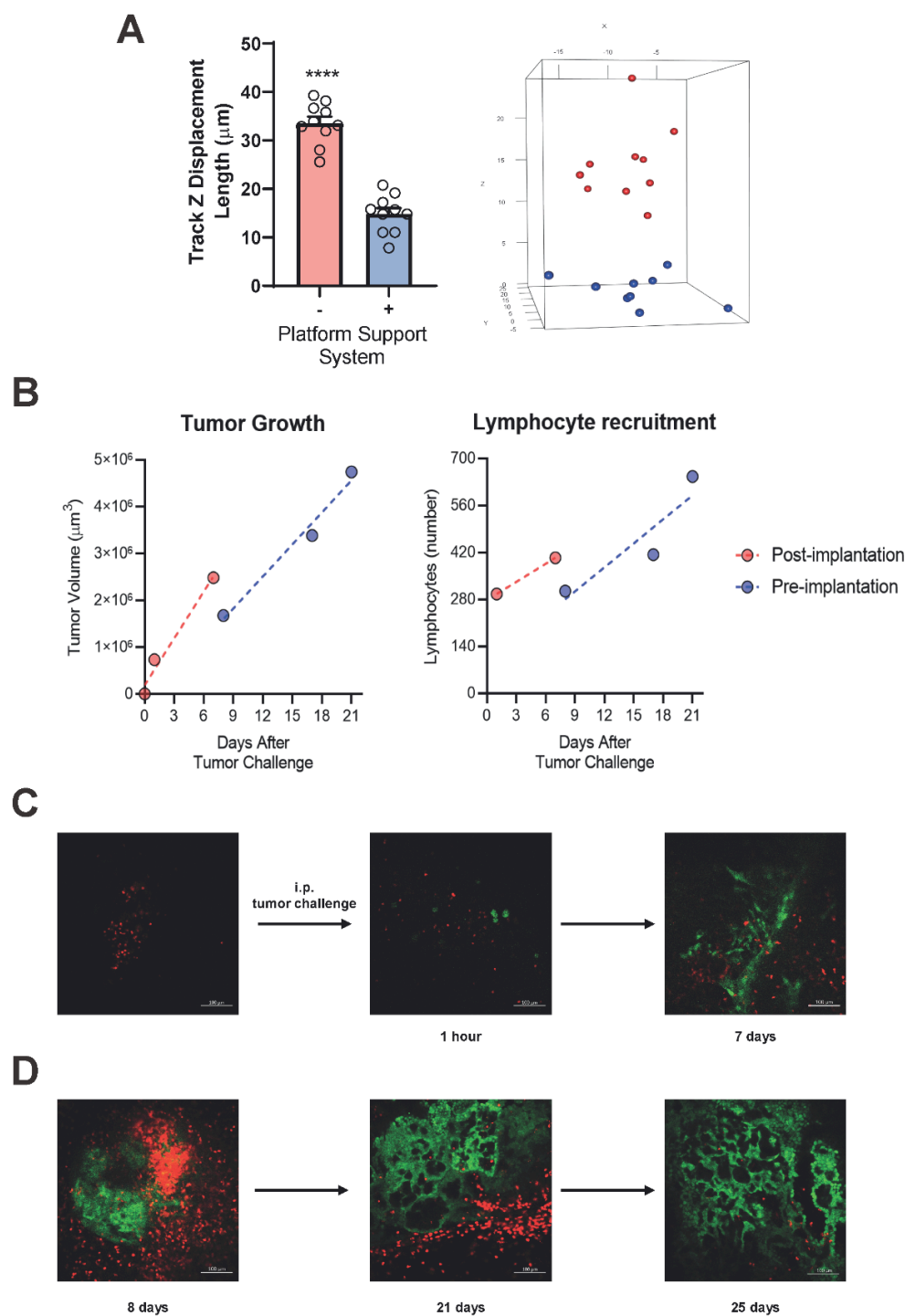


FIGURE 3

Figure 3

Longitudinal intravital imaging of omental tumor progression. (A) Lymphocyte displacement in the z plane in the presence or absence of the support platform, used to assess breathing-related motion

artifacts. Displacement (μm) recorded during a 2-hour imaging session is shown as both a graph and a three-dimensional representation ($p < 0.0001$). **(B)** Tumor volume progression and lymphocyte recruitment in two experimental settings. The red dashed line represents the post-implantation group, in which tumor inoculation was performed after surgical window placement and evaluated during the first 7 days after challenge. The blue dashed line represents the pre-implantation group, in which the window was placed after tumor challenge and imaging was performed from day 7 to day 25 after inoculation. **(C)** Representative intravital confocal images from the post-implantation group showing the arrival of tumor cells (green) in the omentum from 1 hour to 7 days after intraperitoneal inoculation. **(D)** Representative intravital confocal images at days 8, 21, and 25 after inoculation, showing tumor spread within the omentum (green) and lymphocyte recruitment to the tumor site (red).

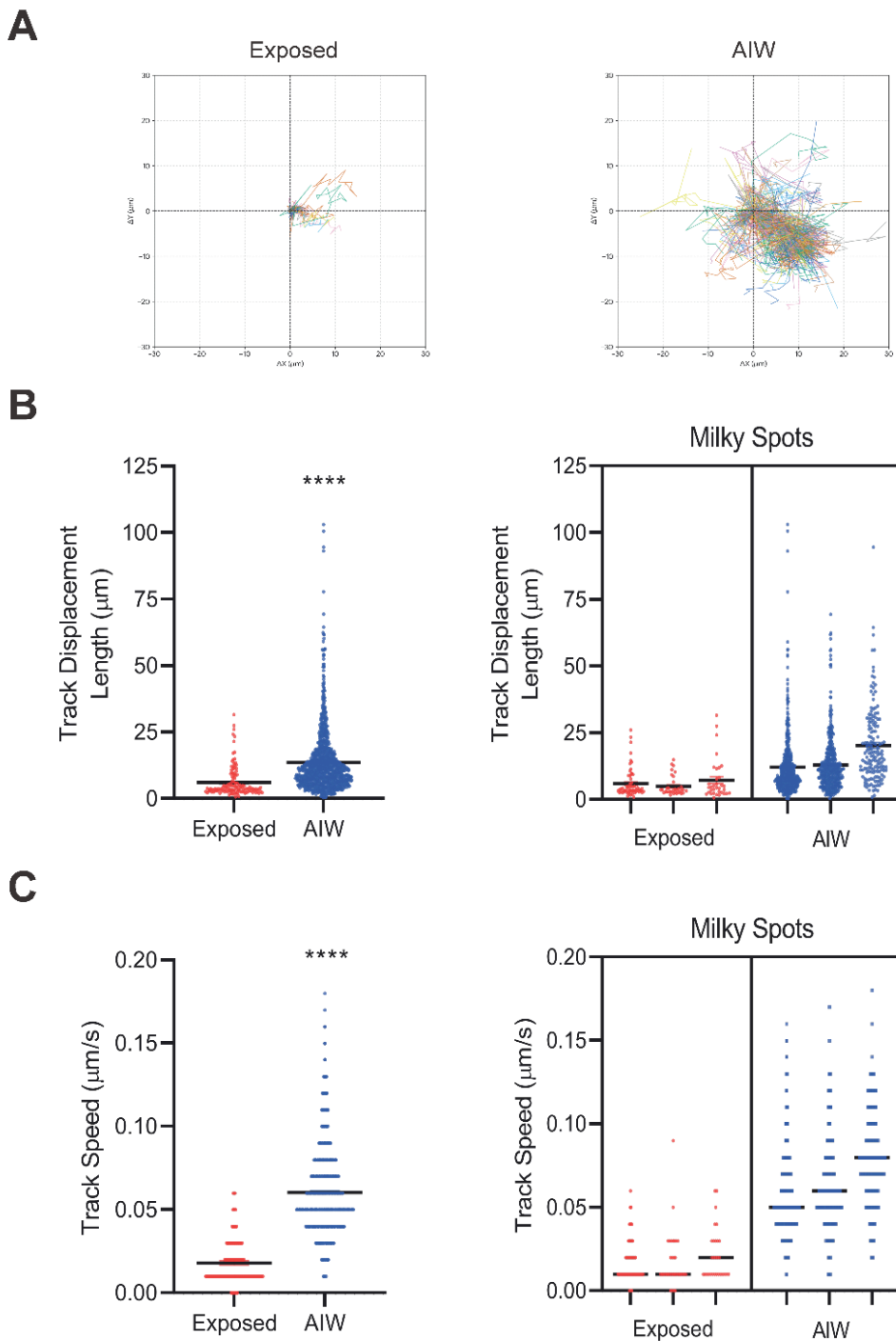


FIGURE 4

Figure 4

Comparison of lymphocyte motility in externalized versus window-imaged omenta. (A) Spider-plots displaying the range of movement (μm) of lymphocytes measured in one FOV of each experimental group. (B) Lymphocyte track displacement in the omentum (μm). Data compare externalized omenta (red) with omenta imaged through the abdominal imaging window (AIW, blue), shown both as pooled values and values stratified by individual milky spots. Lymphocytes displayed significantly greater

displacement when imaged through the AIW ($p < 0.0001$). (C) Mean lymphocyte migration speed ($\mu\text{m/s}$). Red bars indicate externalized omenta and blue bars indicate omenta imaged through the AIW. Lymphocytes migrated significantly faster when observed through the AIW ($p < 0.0001$).

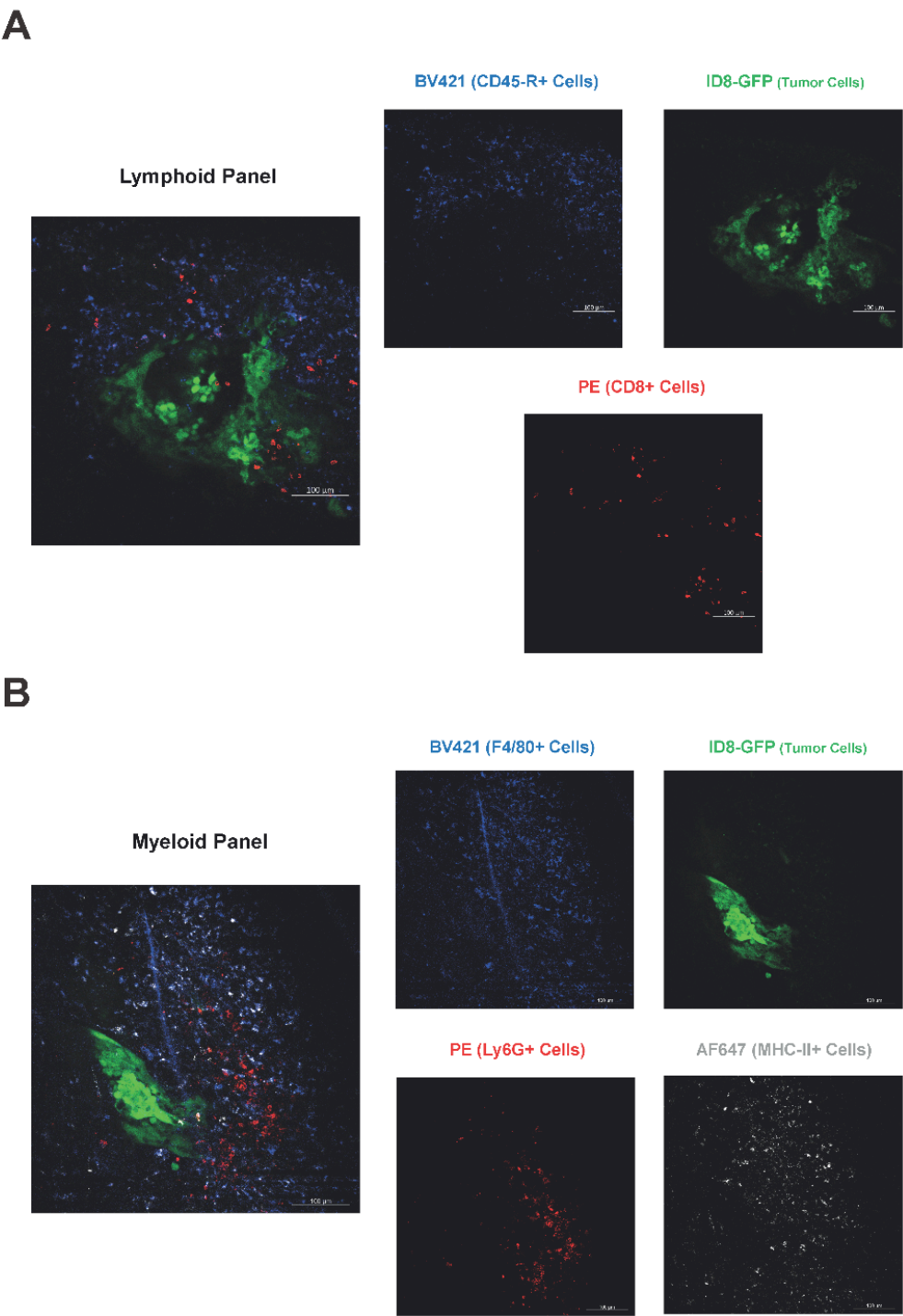


FIGURE 5

Figure 5

***In vivo* multiparametric labeling of lymphoid and myeloid populations.** (A) *In vivo* labeling of lymphoid cell populations. A representative image shows CD45+ cells (blue, BV421), ID8.OVA-Vegf/GFP tumor cells (green, GFP) and CD8+ cells (red, PE. (B) *In vivo* labeling of myeloid cell populations. A representative image shows F4/80+ cells (blue, BV421), ID8.OVA-Vegf/GFP tumor cells (green, GFP), Ly6G+ cells (red, PE), and MHC-II+ cells (white, AF647).

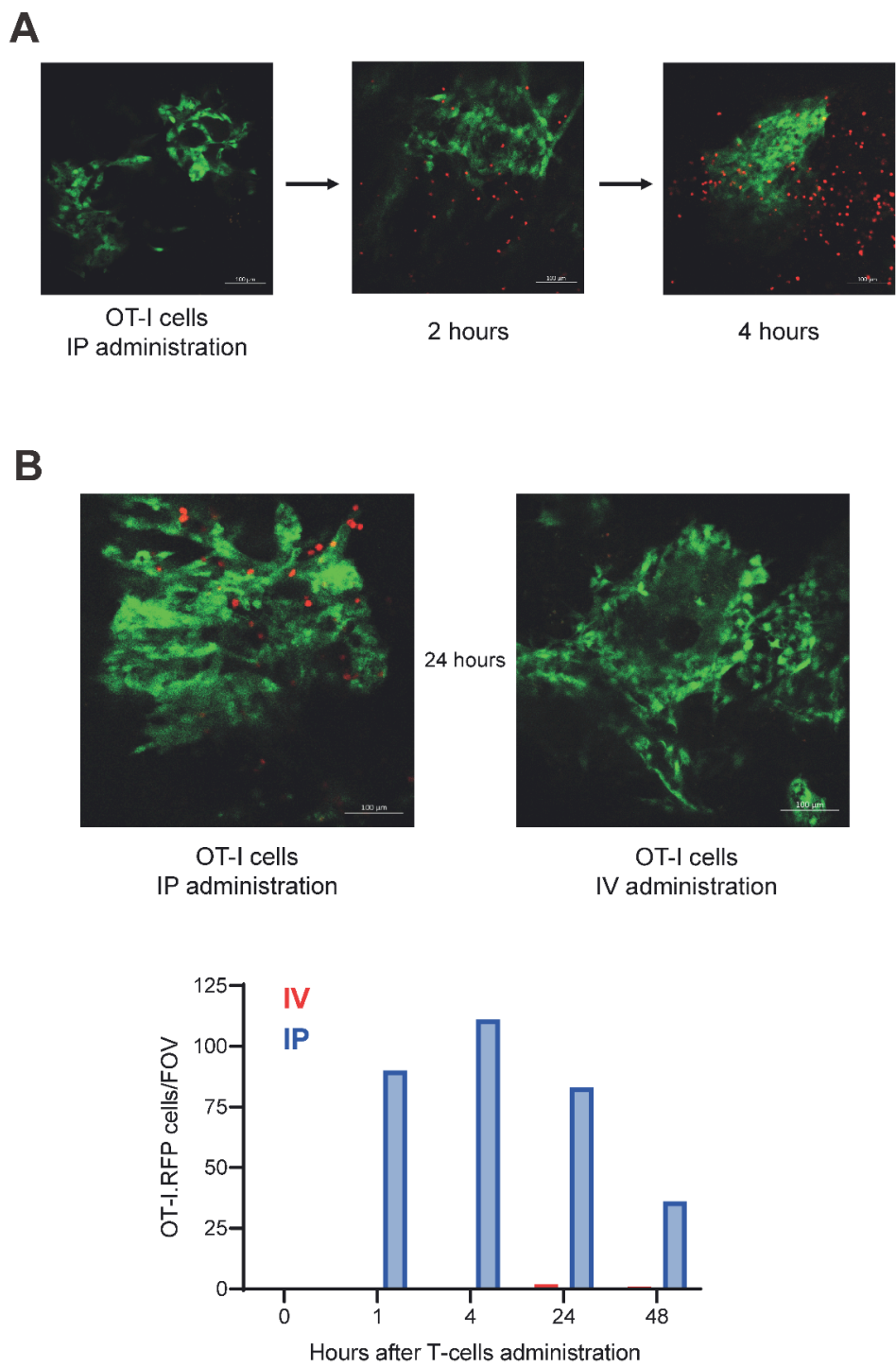


FIGURE 6

Figure 6

Dynamic assessment of adoptively transferred T-cell homing to the omentum via *i.p.* (A) Time-course tracking of *i.p.* administered OT-I cells at 0, 2, and 4 hours after inoculation. ID8.OVA-Vegf/GFP tumor cells are shown in green and OT-I RFP lymphocytes in red. (B) Comparison of recruitment efficiency between delivery routes. Upper panels show representative images obtained 24 hours after inoculation, comparing omental infiltration of OT-I cells after *i.p.* versus *i.v.* administration. The lower panel shows the quantification of OT-I lymphocytes per FOV at 0, 1, 4, 24, and 48 hours after treatment. Blue bars represent mice treated by *i.p.* injection, whereas red bars represent mice treated by systemic *i.v.* administration.