

Development of 5B1, an anti-CA19.9 monoclonal antibody, as a near-infrared fluorescent probe for intraoperative imaging of pancreatic

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BACKGROUND

Pancreatic ductal adenocarcinoma (PDAC) ranks fourth as a cause of cancer mortality, yet our ability to properly diagnose, accurately stage, and effectively treat PDAC is lacking. For patients diagnosed with PDAC, resection offers the best chance of survival. However, for those 20% of patients who qualify for resection, the 5-year survival rate is only 25% due to the high incidence of undiscovered metastases. Along with identifying metastases, properly defining the margin between tumor and healthy tissue is also a determinant of the success of resection. The intraoperative use of near-infrared fluorescence (NIRF) imaging agents to facilitate the identification of previously undetected metastases and to define resection could improve outcomes in patients. Monoclonal antibodies are one promising platform for the development of NIRF imaging agents due to their high-specificity and low background in healthy tissues. CA19.9 is a well-established biomarker for PDAC- overexpression of CA19.9 is a key event in invasion and metastasis of many tumors. CA19.9 is especially attractive as target for immunoNIRF imaging because it is the most highly expressed tumor antigen in most cases of PDAC and is minimally expressed in healthy pancreas. 5B1, a fully human, anti-CA19.9 antibody has already shown promise **both** as a platform for development of a positron emission tomography agent (**Figure 1**).¹ Based on previous studies we investigated 5B1's potential as a NIRF imaging agent for intraoperative applications

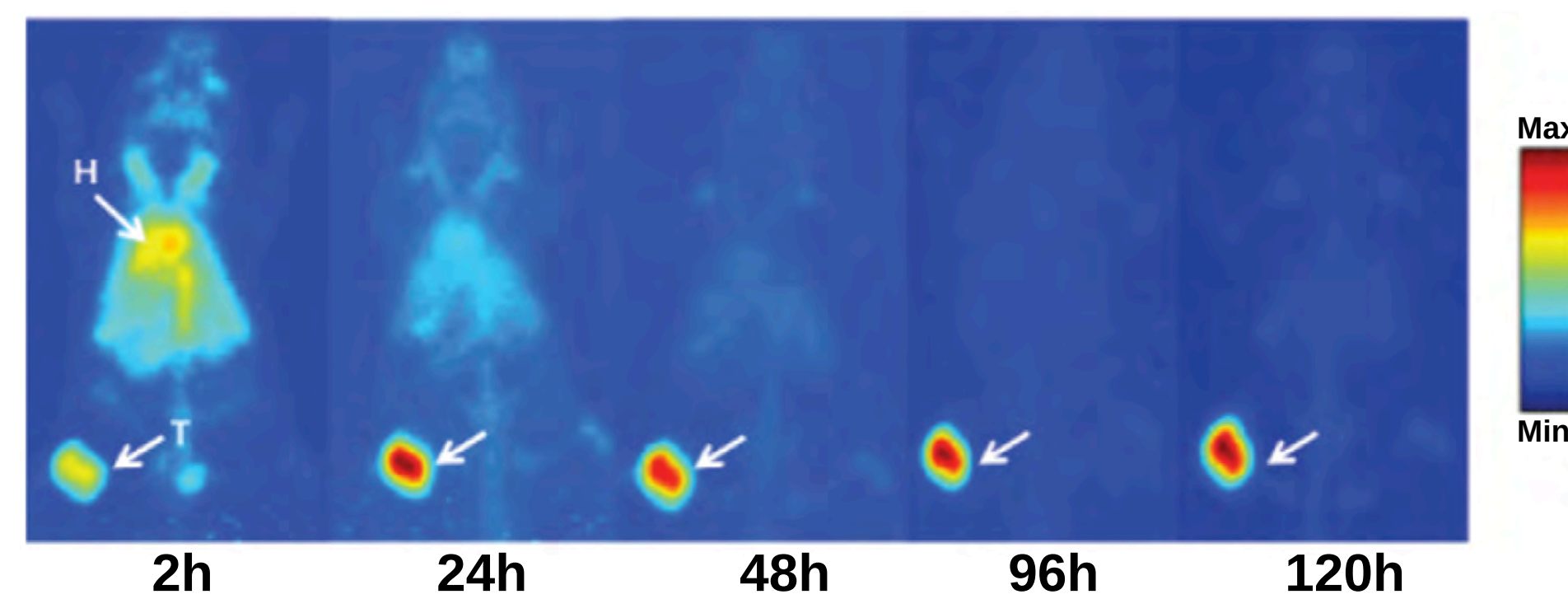


Figure 1. Serial PET maximum intensity projection images acquired from 2-120 h with ⁸⁹Zr-5B1 in mice with BxPC3 tumors, demonstrating high tumor uptake with clearance of non-specifically bound tracer as early as 24 h.¹

OBJECTIVES

- Conjugate two NIRF dyes (IRDye650 and IRDye800CW) to 5B1 and an non-specific IgG (IRDye800CW only)
- Evaluate the constructs *in vitro* using cell-based binding assays
- Evaluate the constructs potential for NIRF-imaging and NIRF image-guided resection *in vivo* using multiple CA19.9 positive and negative cell lines
- Determine optimal construct for further pre-clinical evaluation

MATERIALS AND METHODS

- 5B1 was functionalized with the *N*-hydroxysuccinimide esters (NHS) of IRDye650 and IRDye800CW
- Each construct was characterized using cell-based *in vitro* binding experiments and compared to fluorescent IgG
- Each construct was evaluated *in vivo* in subcutaneous and orthotopic pancreas xenograft models
 - Serial fluorescent imaging (1-6 days) was performed to determine *in vivo* behavior and optimal **time** imaging times
 - Dose-optimization and image-guided surgery were performed at 144 hours
 - The two constructs were compared at optimal dosing in a bilateral subcutaneous xenograft model

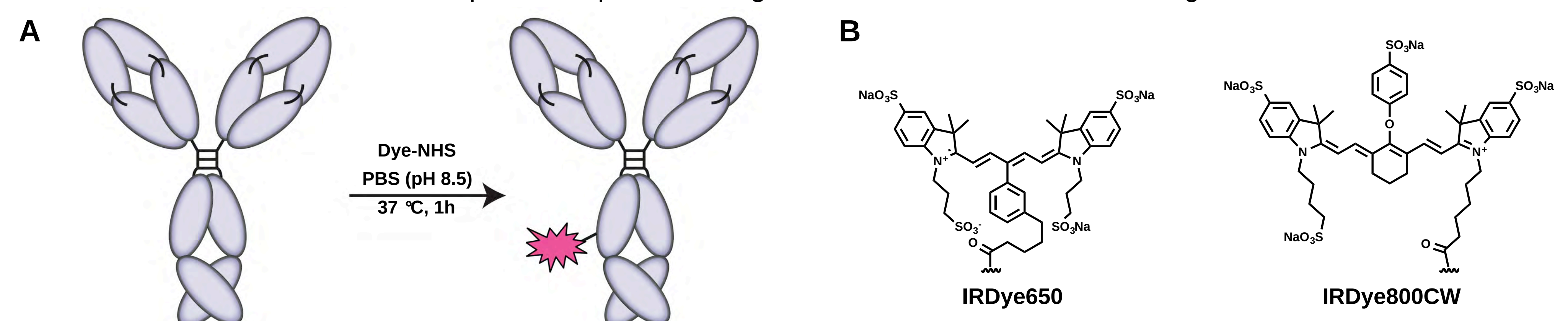


Figure 2. The conditions for the conjugation of the NHS-ester two NIRF dyes (A) and the molecular structure of the two NIRF dyes (B).

RESULTS

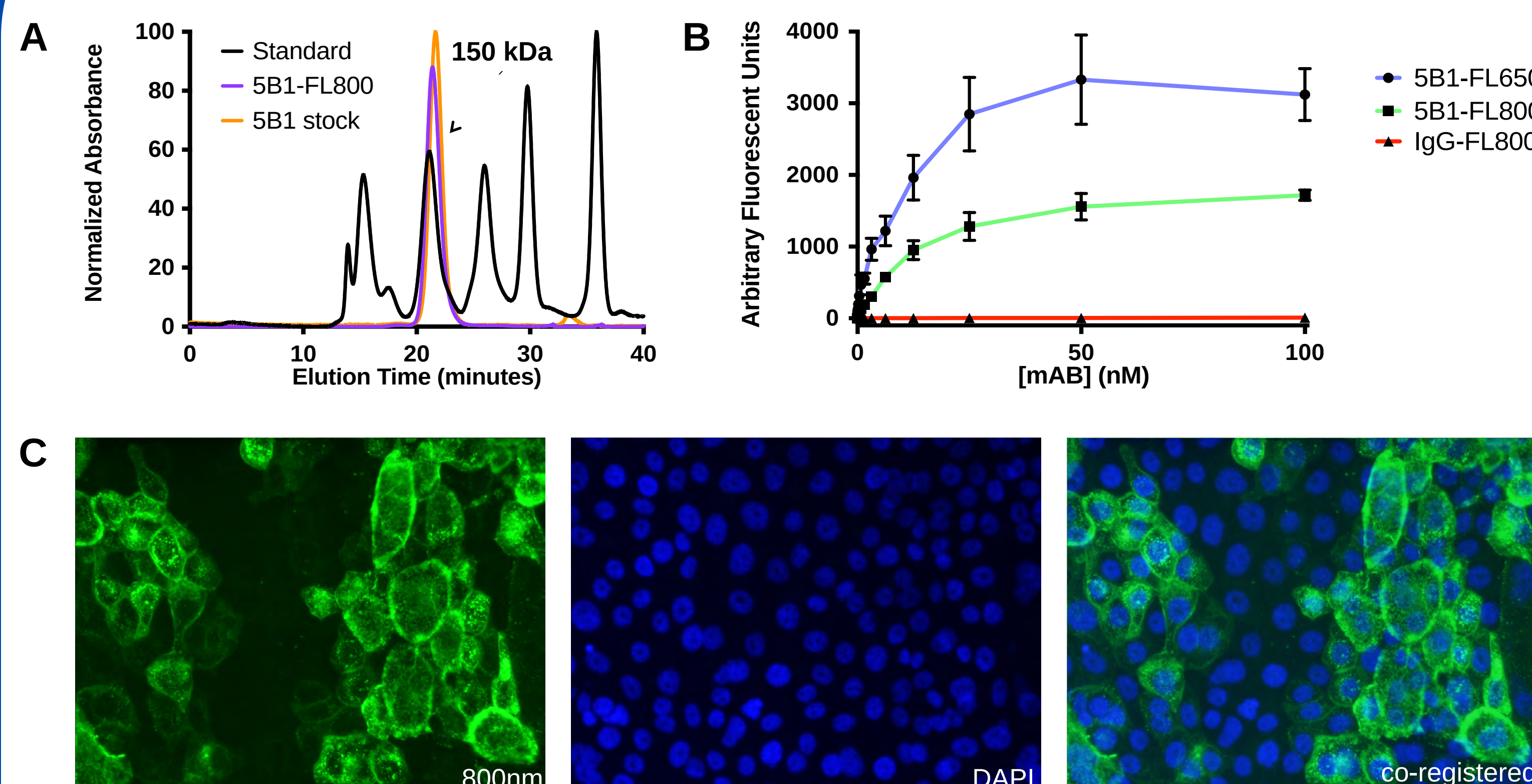


Figure 3. Dye-conjugated antibodies (5B1-FL800 is shown) were characterized by gel filtration chromatography and compared to the 5B1 stock and MW standard (A). The affinity of the constructs was determined with BxPC3 cells in a 96-well plate binding assay (B) and their ability to bind to CA19.9-positive cells (BxPC3) confirmed via fluorescence microscopy on an 8-well chamber slide (C)- the results are shown for 5B1-FL800. The results of the *in vitro* characterization are summarized for all three constructs (D). A pilot imaging study was performed in athymic nude mice with subcutaneous BxPC3 xenograft to evaluate the ability of the 5B1 to bind CA19.9 *in vivo* (E). Results of a serial imaging study (24h-144h post injection) with 5B1-FL800 (Ex. 750 / Em. 800) are shown.

Construct	Dye:mAB	Purity	K _d (nM)
5B1-FL650	~ 4.5	>98%	7.9 ± 1.4
5B1-FL800	~ 2.9	>98%	14.3 ± 1.3
IgG-FL800	~ 1.8	>98%	--

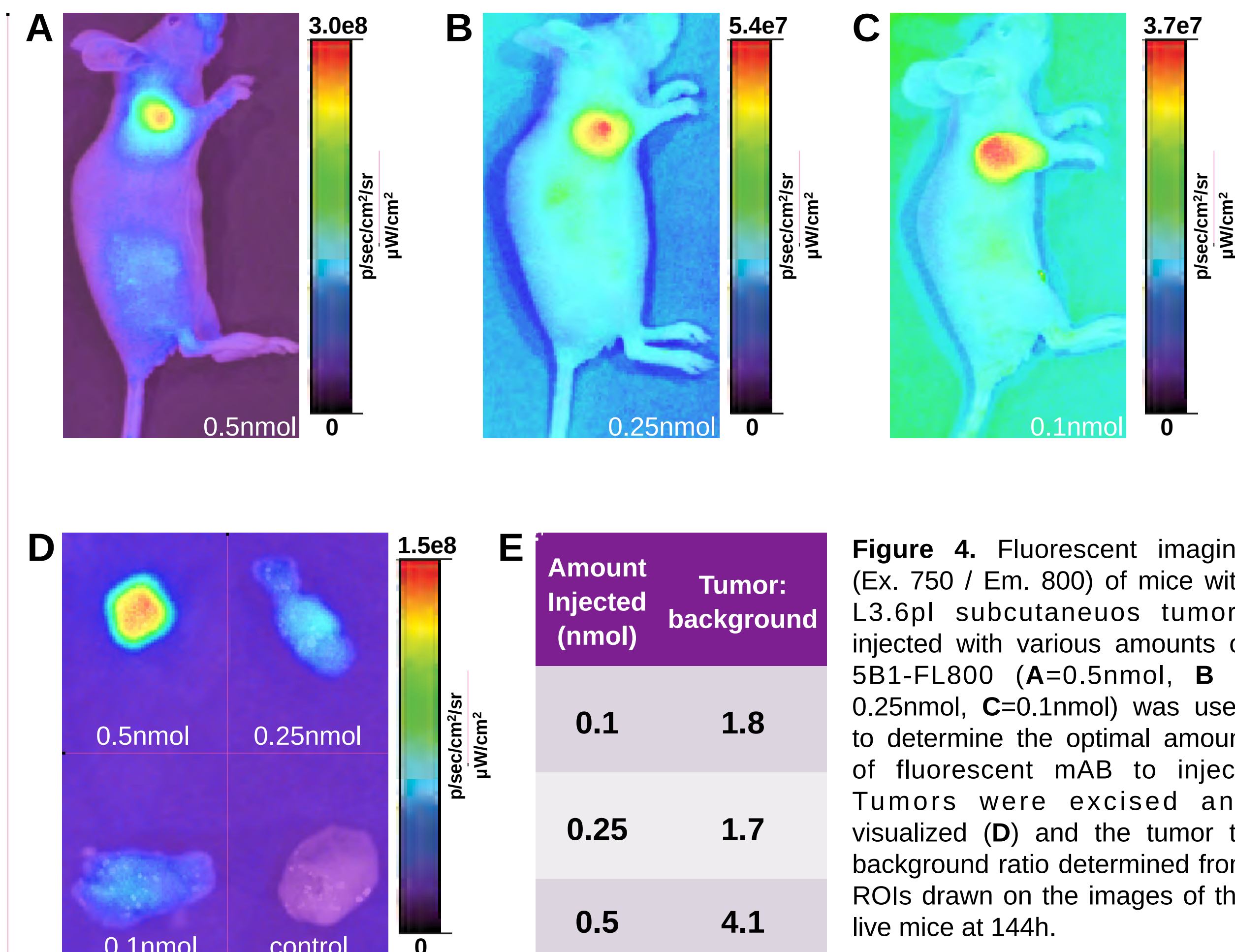
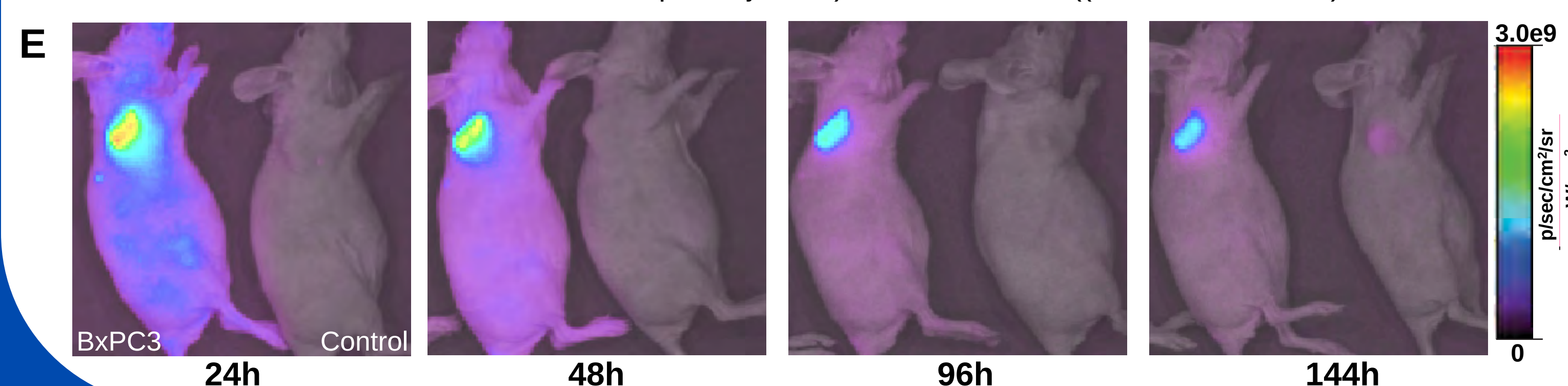


Figure 4. Fluorescent imaging (Ex. 750 / Em. 800) of mice with L3.6pl subcutaneous tumors injected with various amounts of 5B1-FL800 (A=0.5nmol, B = 0.25nmol, C=0.1nmol) was used to determine the optimal amount of fluorescent mAb to inject. Tumors were excised and visualized (D) and the tumor to background ratio determined from ROIs drawn on the images of the live mice at 144h.

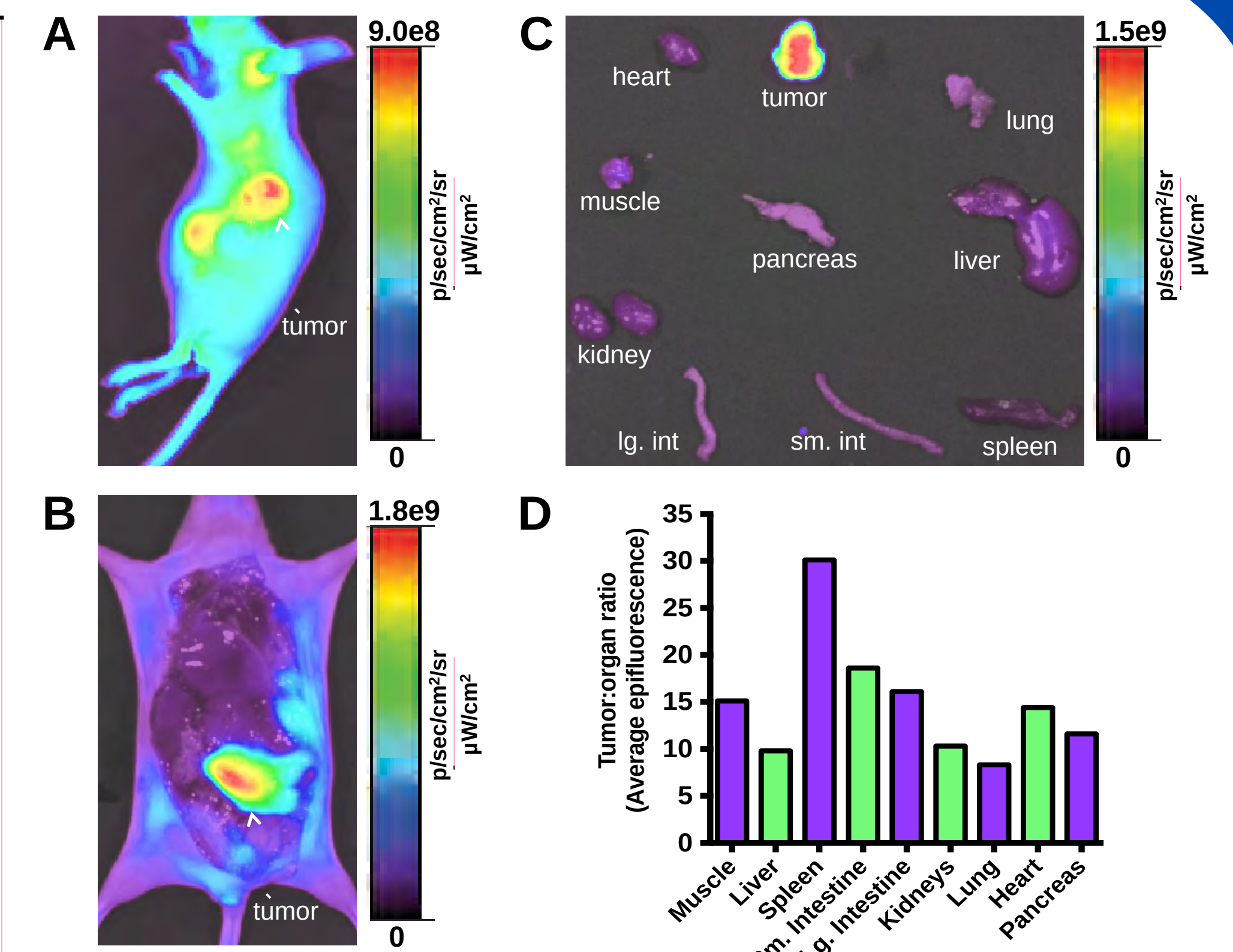


Figure 5. Fluorescent images (Ex. 640 / Em. 680) of mice with L3.6pl orthotopic pancreas tumors demonstrate the potential of 5B1-FL650 (0.5 nmol) for use in NIRF-guided surgery. Images of the flank (A) and open carcass (B) show excellent tumor delineation. The excised organs (C) are also pictured and the tumor:organ epifluorescence is quantified (D).

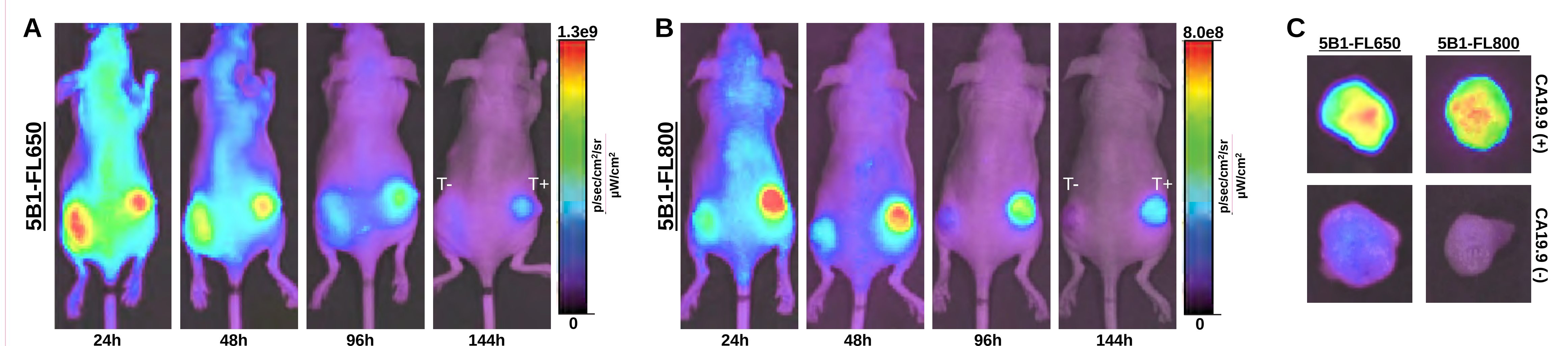


Figure 6. Serial fluorescent images (24-144h post injection) were obtained of mice with bilateral CA19.9-positive (T+, right flank) and CA19.9-negative (T-, left flank) subcutaneous xenografts that were administered (A) 5B1-FL650 (0.5 nmol, Ex. 640 / Em. 680) or (B) 5B1-FL800 (0.5 nmol, Ex. 750 / Em. 800) via the lateral tail vein. Faster clearance and higher contrast of the fluorescent 5B1 labeled with IRDye800 was observed in the images and excised tumors (C) further confirmed that 5B1-FL800 provides greater potential for use in NIRF-imaging due increased tumor:background signal.

CONCLUSIONS

IRDye800CW and IRDye650 were conjugated to the anti-CA19.9, fully-human mAB, 5B1, which was successfully used for *in vivo* NIRF imaging in murine models of PDAC. 5B1 demonstrated exceptional potential for use in delineation of pancreatic tumor margins, NIRF guided-resection, and identification of metastases that could escape detection by other imaging techniques.

ACKNOWLEDGEMENTS and REFERENCES

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1. Viola-Villegas *et al.* J Nucl Med. 2013 Nov;54(11):1876-82.