

Supporting information for

The use of PET imaging for prognostic integrin $\alpha_2\beta_1$ phenotyping to detect non-small cell lung cancer and monitor drug resistance responses

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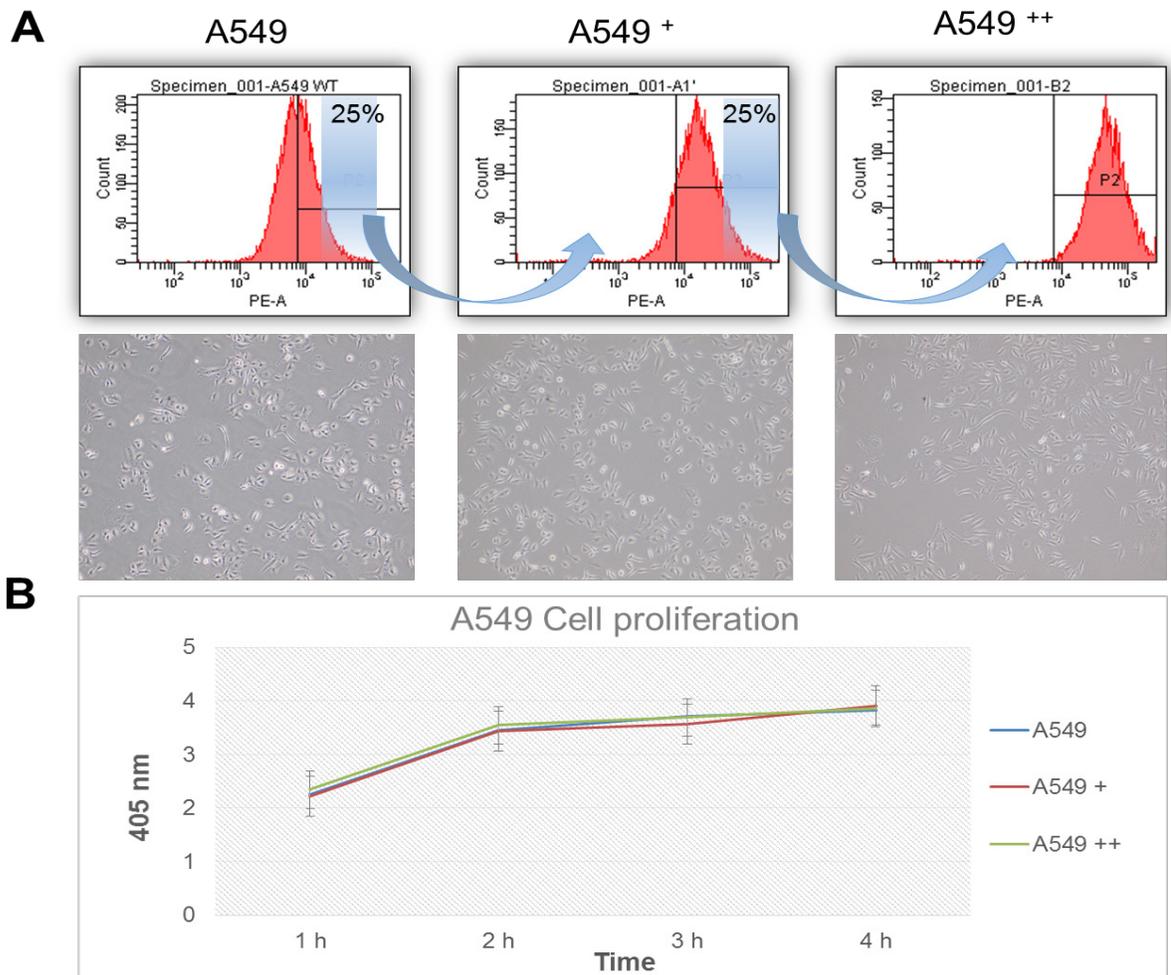


Figure S1. The integrin $\alpha_2\beta_1$ expression level and proliferation of A549- and A549-derived cell lines. (A) Sorting of cells that highly express integrin $\alpha_2\beta_1$ using FACS. The subpopulation of A549 cells showing strong integrin $\alpha_2\beta_1$ antibody (ab24697; ab30484, Abcam) staining (top 25%) were selected, collected and expanded for second-round selection by FACS. The subline from the first-round selection was designated A549⁺, and the subline from the next round of selection was designated A549⁺⁺. Experimental data were analyzed with Flowjo7.2.2 software. (B) The proliferation of the A549, A549⁺ and A549⁺⁺ cells was validated by CCK8 assay, which indicated no significant differences among these cell lines.



Figure S2. *In vitro* assessment of the stability of ^{68}Ga -DOTA-A2B1 in PBS (pH 7.4) and mouse serum at physiological temperature. After 1.5 h, the percentage of intact peptide probes remained greater than 90% in both conditions, as verified by radio-HPLC profiles.

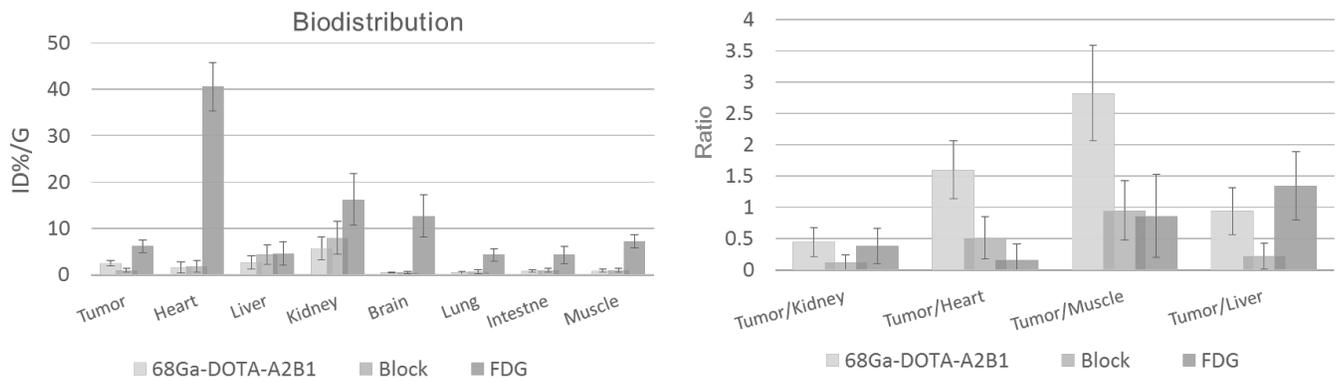


Figure S3. *Ex vivo* biodistribution data of the integrin tracer ⁶⁸Ga-DOTA-A2B1, ⁶⁸Ga-DOTA-A2B1-Block and ¹⁸F-FDG. After administration of the tracers, the tissues were collected, weighed, and counted; the results are presented as % ID/g ± SD (n = 3).

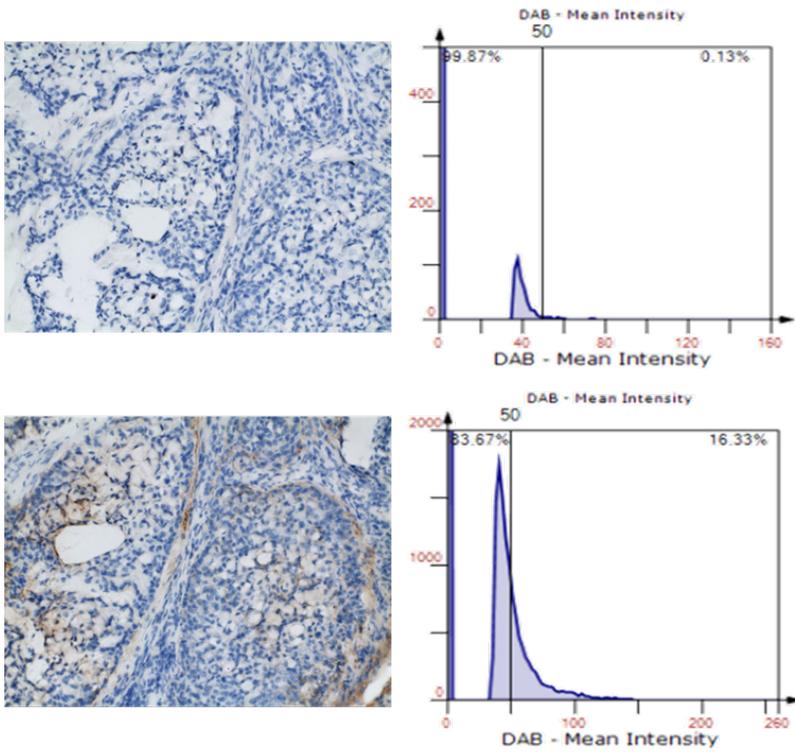


Figure S4. The histological results of dissected subcutaneous A549 tumors. Immunostaining of lung tumor tissue with an α -integrin α_2 antibody demonstrated that after A549 cells were inoculated into a living animal, integrin $\alpha_2\beta_1$ was still highly expressed in the xenografts.

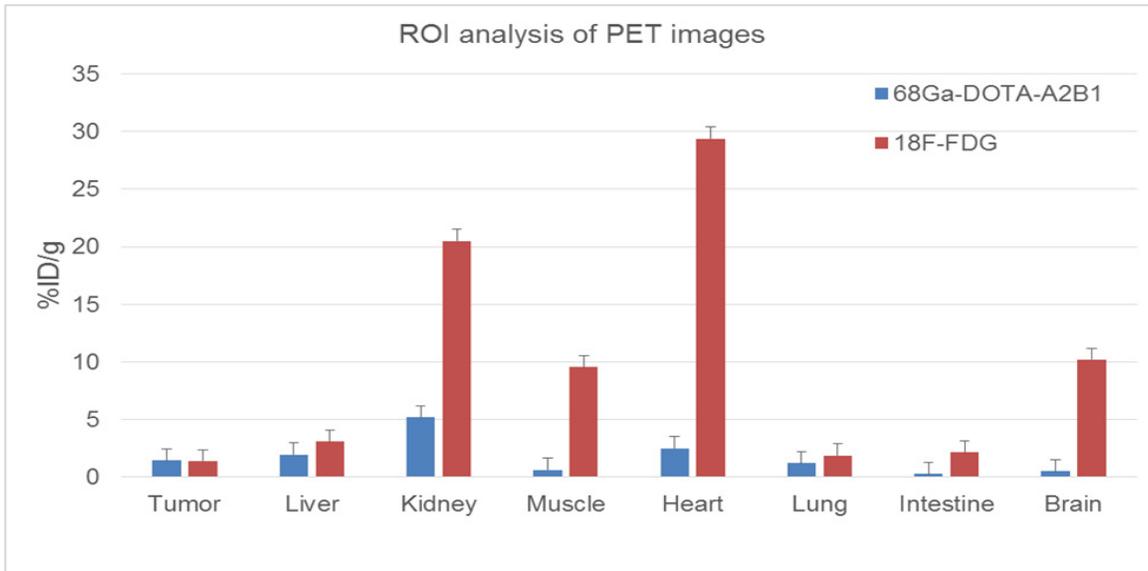


Figure S5. ROI analysis of PET images of the orthotopic A549 xenograft animal model. Blue bars, n = 5 for ⁶⁸Ga-DOTA-A2B1, and red bars, n = 5 for ¹⁸F-FDG; major organs were compared, and the data are reported as the means ± SEM. **p* < 0.001 compared with all organs.

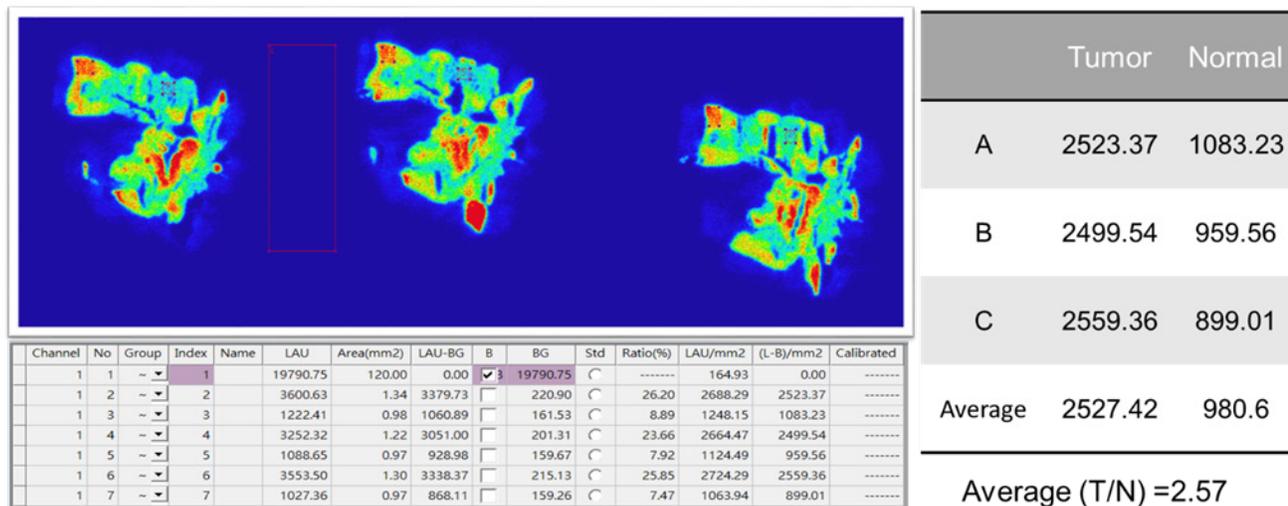


Figure S6. *Ex vivo* high-resolution autoradiography of the orthotopic lung cancer model after injection of ¹⁸F-FDG tracers. (A) Representative autoradiographs of the lung after injection of ¹⁸F-FDG. Arrows indicate tumor lesions. Autoradiographs acquired from 40- μ m tissue slices 60 min after injection of ¹⁸F-FDG radiotracer. (B) From the semi-quantitative results, the T/N ratio was calculated as 2.57.

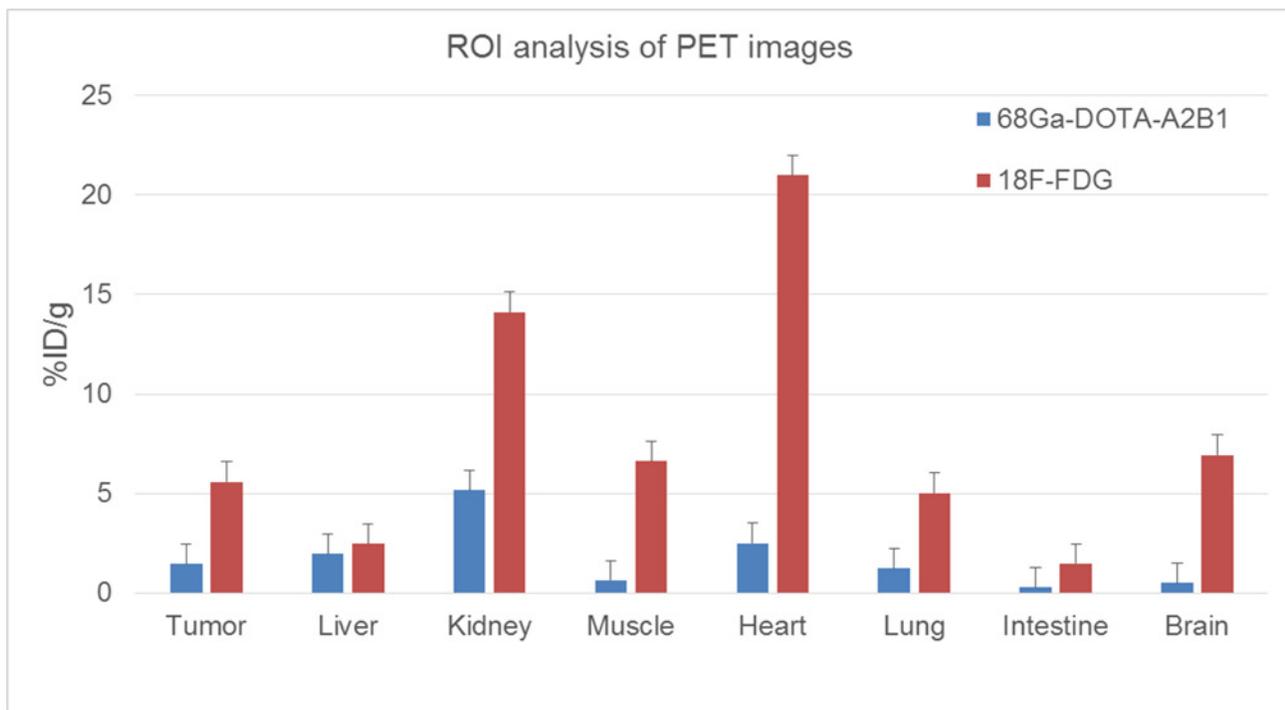


Figure S7. Comparison of uptake of intravenously injected ⁶⁸Ga-DOTA-A2B1 and ¹⁸F-FDG in animals with osseous tumors (blue bars, n = 5 for ⁶⁸Ga-DOTA-A2B1, and red bars, n = 5 for ¹⁸F-FDG) injected intravenously. Statistical significance was determined with a 2-tailed Student's *t*-test. For all graphs, data are represented as the means ± SEM. **p* < 0.001 compared with all organs.

Table S1. The Gallium (^{68}Ga)-labeled tracer should meet the “Quality Control Result (reference: European Pharmacopoeia 8th edition)” before release for any preclinical or clinical PET scan studies.

Quality Control Result
Gallium (^{68}Ga)-DOTA-A2B1 Injection

No.	Items	Release Limit	Result
01	Appearance	Clear, particulate free	Pass
02	Ethanol Content	$\leq 10\%$	Pass
03	pH	$4.0 < \text{pH} < 8.0$	pH = 6.5
04	Radiochemical purity	$\geq 91\%$	RCP: 100%
05	Impurity	$\leq 3.33 \mu\text{g/mL}$	Pass
06	Chemical identity (API)	$\text{RRT} = 1.3 \pm 0.13$	$\text{RRT} = 1.37$
07	Radiochemical impurity ($^{68}\text{Ga(III)}$ ion)	$\leq 2\%$	0%
08	Radiochemical impurity (^{68}Ga in colloidal form)	$\leq 3\%$	0%
09	Radionuclidic identity (^{68}Ga)	$62 \text{ min} \leq T_{1/2} \leq 74 \text{ min}$	$T_{1/2} = 66.66 \text{ min}$
10	Strength	$\geq 0.33 \text{ mCi/mL}$	15mCi/mL
11	Radionuclidic Purity	$\geq 99.9\%$ ub 0.511 MeV. 1.077MeV, 1.022MeV. 1.883 MeV and Compton scatter	Purity: 100%
12	Radionuclidic impurity (Retain the preparation to be examed for at least 48 h)	Radionuclidic Impurities $\leq 0.001\%$	0%
13	Bacterial endotoxin	$\leq 11.6 \text{ EU/mL}$	< 10 EU/mL
14	Sterility	Meet the requirements of the test	Pass