Supporting information for
The use of PET imaging for prognostic integrin α₂β₁ 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 $^{68}$Ga-DOTA-A2B1, $^{68}$Ga-DOTA-A2B1-Block and $^{18}$F-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 α2 antibody demonstrated that after A549 cells were inoculated into a living animal, integrin α2β1 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 $^{68}\text{Ga-DOTA-A2B1}$, and red bars, n = 5 for $^{18}\text{F-FDG}$; 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 $^{18}$F-FDG tracers. (A) Representative autoradiographs of the lung after injection of $^{18}$F-FDG. Arrows indicate tumor lesions. Autoradiographs acquired from 40-μm tissue slices 60 min after injection of $^{18}$F-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 $^{68}$Ga-DOTA-A2B1 and $^{18}$F-FDG in animals with osseous tumors (blue bars, n = 5 for $^{68}$Ga-DOTA-A2B1, and red bars, n = 5 for $^{18}$F-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 (68Ga)-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 (68Ga)-DOTA-A2B1 Injection

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<th>No.</th>
<th>Items</th>
<th>Release Limit</th>
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<td>01</td>
<td>Appearance</td>
<td>Clear, particulate free</td>
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<tr>
<td>02</td>
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</tr>
<tr>
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<td>$\geq 91%$</td>
<td>RCP: 100%</td>
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<tr>
<td>05</td>
<td>Impurity</td>
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<tr>
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<td>Chemical identity (API)</td>
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<td>RRT = 1.37</td>
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<tr>
<td>07</td>
<td>Radiochemical impurity (68Ga(III) ion)</td>
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<td>Radiochemical impurity (68Ga in colloidal form)</td>
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<tr>
<td>09</td>
<td>Radionuclidic identity (68Ga)</td>
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<td>$T_{1/2} = 66.66$ min</td>
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<td>Strength</td>
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<td>Radionuclidic Purity</td>
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<td>Radionuclidic impurity (Retain the preparation to be examined for at least 48 h)</td>
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<td>Sterility</td>
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