Title Page

Preclinical evaluation and first-in-human study of Al^{18}F-FAP-NUR for PET imaging cancer-associated fibroblasts

Running title: Preclinical evaluation and first-in-human imaging of Al^{18}F-FAP-NUR

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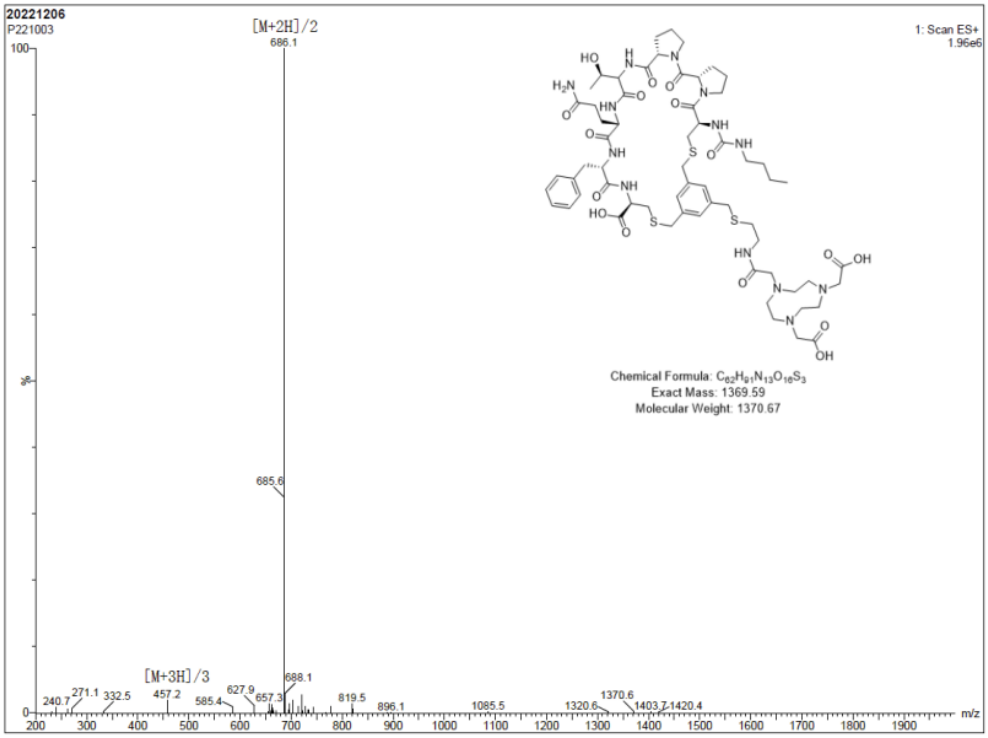
Supporting information

The mass spectrum of NOTA-FAP-NUR



Radio HPLC

The identification and radiochemical purity of Al^{18}F-FAP-NUR were determined using an Analytical-HPLC system (U3000, Thermo Fisher Scientific, USA) equipped with a UV detector (280 nm) and a radio-HPLC detector FC3600 (Eckert & Ziegler Medical). Analysis was performed on an InertSustainAQ-C18 analytic column (4.6 * 150 mm, 5 μm; Shimadzu, Japan). A small aliquot (3.7 MBq) of Al^{18}F-FAP-NUR was quantified by radio-HPLC.

A linear gradient elution was performed at a flow rate of 1 mL/min for 10 min with solvent A:B ratios ranging from 95:5 to 1:99. From 10 to 15 minutes, ratios were reversed, going from 1:99 to 95:5 and the flow rate remained at 1 mL/min. The elution continued until the method was completed at 17 minutes.

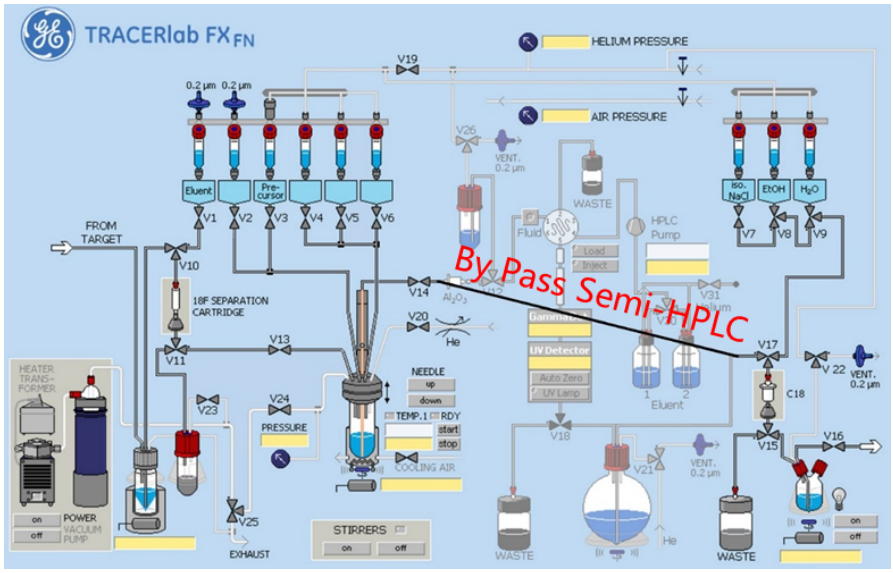


Fig. S1 Schematic representation of Al^{18}F-FAP-NUR synthesis on a modified GE TRACERlab FxFN platform

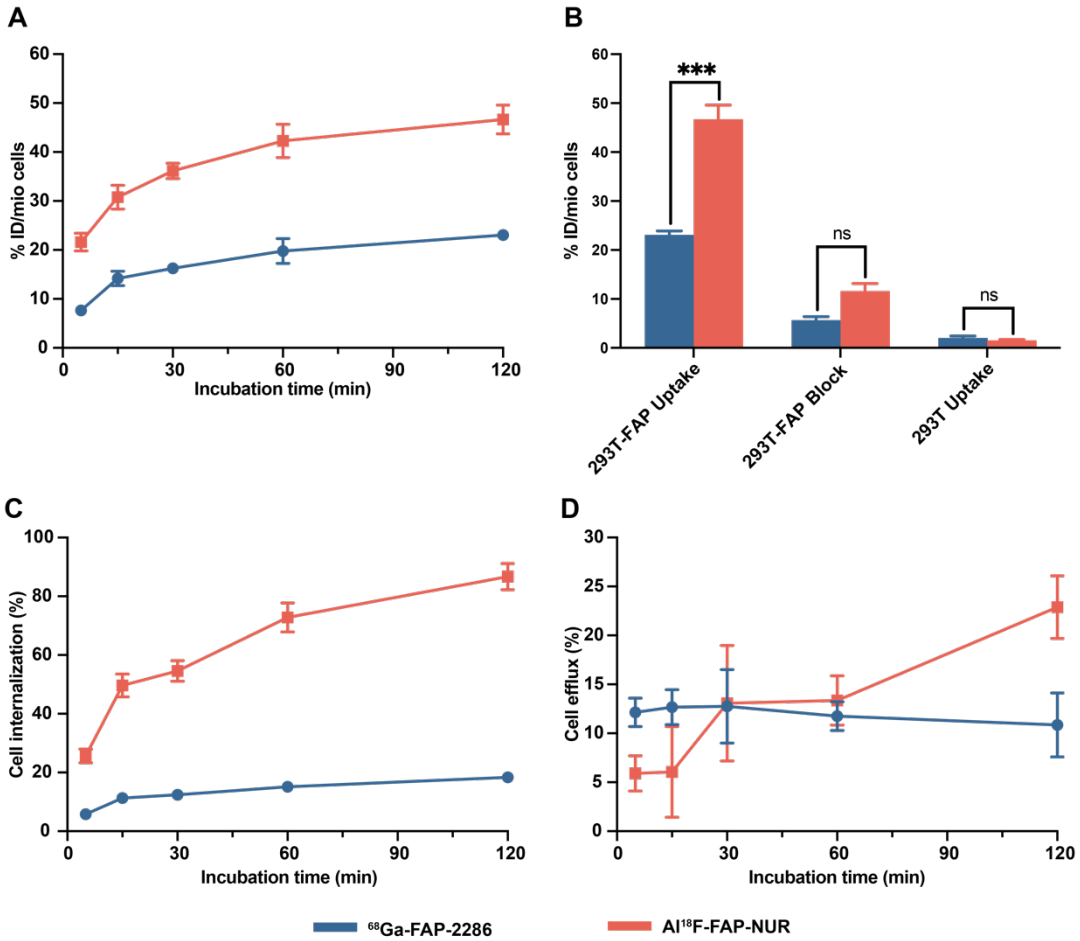


Fig. S2 Cellular uptake of ^{68}Ga-FAP-2286 and Al^{18}F-FAP-NUR. **A** Uptake in 293T-FAP cells after incubation for 5-120 min. **B** Binding in 293T-FAP cells and 293T cells after 60 min of incubation, with and without blocking using DOTA-FAP-2286 as a competitor. **C** Internalization into 293T-FAP cells after incubation for 5-120 min. **D** Efflux kinetics after 60 min of incubation of 293T-FAP cells with

radiolabeled compounds, followed by incubation with compound-free medium for 5-120 min. Data were represented as mean ± *SD* (n = 4/group). *, $P<0.05$; **, $P<0.01$; ***, $P<0.001$; ****, $P<0.0001$

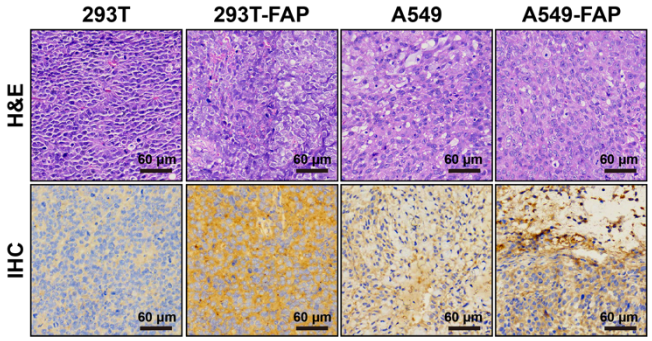


Fig. S3 H&E and IHC results of the tumor of 293T, 293T-FAP, A549, and A549-FAP xenografts. Scale bar, 60 μm

	Radioactivity (CPM)		
Radiopharmaceuticals	1	2	3
^{68}Ga-FAP-2286			
PBS	1585131	1590420	1598017
n-octanol	3912	3863	3861
Al^{18}F-FAP-NUR			
PBS	3504290	3646318	3801410
n-octanol	10885	15283	13183

Table S1 The partition coefficient (LogP) of Al^{18}F-FAP-NUR and ^{68}Ga-FAP-2286, which was -2.45 ± 0.07 (n = 3) and -2.61 ± 0.01 (n = 3), respectively