Animal Model Preparation. Five human-sized, age-matched, male, healthy Wisconsin Miniature Swine^{TM} were included average age (range) of 15.3 (14-17) months and average (range) baseline weight 63 kg (56 – 76). No other inclusion or exclusion criteria were applied. No control cohort was enrolled as each animal served as its own control by the virtue of undergoing imaging and tissue sampling both at baseline and at endpoint (post fibrosis induction). No randomization was performed. Sample size was decided based on previously published literature on evaluation of liver fibrosis in swine.*(46)*

Animals underwent PET/MRI imaging followed by same-day ultrasound-guided core-needle liver biopsy to establish baseline histologic and imaging characteristics of the liver. A few days after baseline imaging/biopsy (to allow for recovery), gradually increasing volumes of ethanol were added to the animals' daily diets, starting from 40 mL of ethanol on day 1 to 280 mL on day 7, which was maintained as part of the diet until the study endpoint. To increase animal compliance with oral ethanol intake, ethanol was mixed with a sweetened electrolyte solution to a total volume of 2800 mL.

Liver Embolization. At 8 – 9 days after initiation of oral ethanol intake, animals underwent image-guided transarterial liver embolization as follows: under general anesthesia, through a percutaneous femoral arterial access, the common hepatic artery was catheterized under real-time fluoroscopic guidance. The gastroduodenal artery was coil-embolized to prevent non-target embolization and stomach/bowel necrosis. A microcatheter was advanced and positioned at the proximal proper hepatic artery, through which 30 mL of an emulsion of 1:3 (by volume) ethanol:ethiodized oil (Lipiodol, Guerbet, France) was gradually administered. The volume of embolic emulsion was based on the maximum tolerated dose demonstrated on previous studies.*(26)* Catheter position was monitored and maintained by real-time fluoroscopy. Following

liver embolization, animals continued oral alcohol intake for a total of 8 weeks as aforementioned, after which they underwent endpoint PET/MRI followed by necropsy and harvesting of the liver for histologic analysis.

Radiotracer (FAPI) Production. To a solution of 50 µg of FAPI-46 precursor (SOFIE, Dulles, VA, USA) in 100 µL ultra-trace water were added 0.35 mL of 0.07 M sodium ascorbate and 0.45 mL of 1.5 M sodium acetate in ultra-trace water. The mixture was transferred into a 10 mL sealed reaction vial. The ^{68}Ge/^{68}Ga generator (GalliaPharm, Eckert & Ziegler, Radiopharma, Berlin, Germany) was eluted with 5 mL of 0.1 N HCl using a syringe pump at the rate of 2 mL/min. The eluate was passed through a sterile filter and a silicon-coated low-metal-releasing needle into the reaction vial. The reaction vial was heated by a heating block at 97° C for 20 min. After cooling for 5 min, 1 mL of 1.5 M sodium acetate was added. The crude product was sterile filtrated with a 0.22 µm Millipore filter into a final product vial. Product radiochemical purity was $> 95\%$.

Image Acquisition. Radiotracer production is detailed in the Supplemental Data section. Images were acquired under general anesthesia in the supine position on a simultaneous whole-body PET/MRI scanner (Signa PET/MR, GE Healthcare, Chicago, IL, USA), using a 16-channel anterior array torso coil and a 14-channel posterior coil embedded at the isocenter of the scanner. Continuous dynamic PET data with the field of view centered over the liver were acquired for a total of 92 minutes, where FAPI was administered intravenously 1 minute after the initiation of data acquisitions. Average (range) administered radiotracer activity was 4.3 (3.5 – 4.6) MBq/kg body weight. Simultaneous MRI images were obtained during this period, including two-point Dixon images for fat-water separation for MRI-based attenuation correction, T1-weighted fat-saturated images before and after intravenous administration of contrast (0.05 mmol/kg, gadoxetate disodium, Bayer Healthcare, NJ, USA) for anatomic localization.

Tissue Procurement and Histologic Evaluation. Baseline percutaneous liver core biopsies were performed under general anesthesia and real-time ultrasound guidance by a fellowship-trained abdominal radiologist or a fellowship-trained interventional radiologist. Core samples were obtained from each liver lobe, fixed in 10% formalin for 24-48 hours, and stored in 70% ethanol thereafter until embedded in paraffin. After endpoint imaging, animals were euthanized, and livers were harvested. Liver lobe surfaces were marked with ink to preserve the landmarks needed for imaging-histology co-localization and correlation. Harvested livers were sectioned using a "bread-loafing" technique in the axial (transverse) plane to resemble the axial slices on PET/MRI as accurately as possible. Liver slices were also first fixed in 10% formalin for 24-48 hours, and then stored in 70% ethanol thereafter. Using anatomic landmarks, liver areas corresponding to the ROIs on post-fibrosis images were identified, and wedge sections of the identified regions were cut out and embedded in paraffin.

Baseline and endpoint embedded tissues were processed with Masson's Trichrome and Picrosirius red stains. Automated immunohistochemistry was performed on the Ventana Discovery Ultra BioMarker Platform (Ventena Medical Systems). Deparaffinization was carried out on the instrument, as was heat-induced epitope retrieval with cell conditioner 1 buffer (Ventana #950-224), an EDTA based buffer pH 8.4, for 48 minutes at 95 °C. The primary antibody was the anti-FAP monoclonal antibody (SP325, ab240989, Abcam, plc) diluted 1:100 in Renaissance Background Reducer Diluent (BioCare Medical #PD905) and incubated for 40 minutes at 37 °C. Slices were rinsed with reaction buffer (Ventana #950-300), incubated with Discovery OmniMap anti-rabbit horseradish peroxidase (Ventana #760-4311) for 16 minutes at 37 °C, and then rinsed with reaction buffer. Discovery ChromoMap DAB detection kit (Ventana #760-159) was used for visualization. For negative control, slides were incubated with reaction buffer (Ventana #950-300)

without primary antibody (results not shown). Slides were removed from the instrument, counterstained with Harris hematoxylin (1:5) for 45 seconds, rinsed with dH_2O, dehydrated by oven drying and dipping in xylene.

Histologic review and analysis were carried out by a board-certified, fellowship-trained hepatobiliary pathologist who was blinded to all imaging results and the timepoints of tissue procurement (i.e., baseline versus endpoint). Two histologic standards were used as reference: the METAVIR fibrosis score and collagen proportionate area (CPA). The METAVIR score is an ordinal five-point scale (F0 to F4) used for human liver fibrosis staging. This scale was slightly modified for the purposes of this swine study (Figure 2). In humans, F0 is absence of fibrosis and F4 is end-stage fibrosis/cirrhosis. However, given that normal swine liver has thin, organized bands of fibrosis at baseline, F0 and F1 were grouped into one category that was assigned to normal swine liver with expected uniform thin bands of fibrosis, herein F0/F1. F2 was assigned to presence of slightly thickened bands of fibrosis and/or rare septa, F3 to thickened bands of fibrosis with numerous septa without cirrhosis, and F4 to cirrhosis.

The second utilized histologic measure of liver fibrosis, CPA, is measured as the proportion of collagen deposition area relative to the total tissue area (reported in %) on Picrosirius red-stained slides. CPA is validated as a predictor of outcomes in patients with various chronic liver diseases and can sub-classify cirrhosis and predict decompensation. *(29-35)* Picrosirius red-stained slides were scanned at the 20× magnification of using the Vectra Multispectral Imaging System (PerkinElmer, Inc. Hopkinton, MA, USA). Images were analyzed using the InForm software (version 2.4, PerkinElmer, Inc. Hopkinton, MA, USA) to quantify the Picrosirius red staining in five regions within each liver section. Each region's CPA was calculated as following:

$$\text{Region's CPA} = \text{region's stained area} \div \text{region's total tissue area} \times 100\%$$

The CPA measurements at the five regions in each section were averaged to yield a per-section CPA. CPA analysis was performed only for endpoint wedge sections and not for baseline tissues due to the relatively small amount of tissue procured through core needle biopsy at baseline.

Statistical Analysis. Liver FAPI uptake time-activity curves were generated by plotting the pooled ROI SUVs against time for different stages of fibrosis. Box-and-whisker plots were generated to compare liver FAPI uptake and CPA across different histologic stages of liver fibrosis, using Kruskal-Wallis rank sum test. Linear regression was used to correlate liver FAPI with CPA, using the Pearson correlation coefficient. To account for the correlation among repeated measurements and their non-monotone change over time, a linear mixed effects model was used with pig- and ROI-specific random effects and piecewise linear time trend. $P<0.05$ was considered statistically significant. A post-hoc power analysis for testing F2 versus F0/F1, and F3/F4 versus F0/F1 stages under the current sample size was performed. All analyses were performed using R Statistical Software (Version 4.02; Foundation for Statistical Computing, Vienna, Austria).