

Supplementary Information for:

Radiosynthesis and Preclinical Evaluation of [⁶⁸Ga]Ga-NOTA-Folate for PET Imaging of Folate Receptor β -Positive Macrophages

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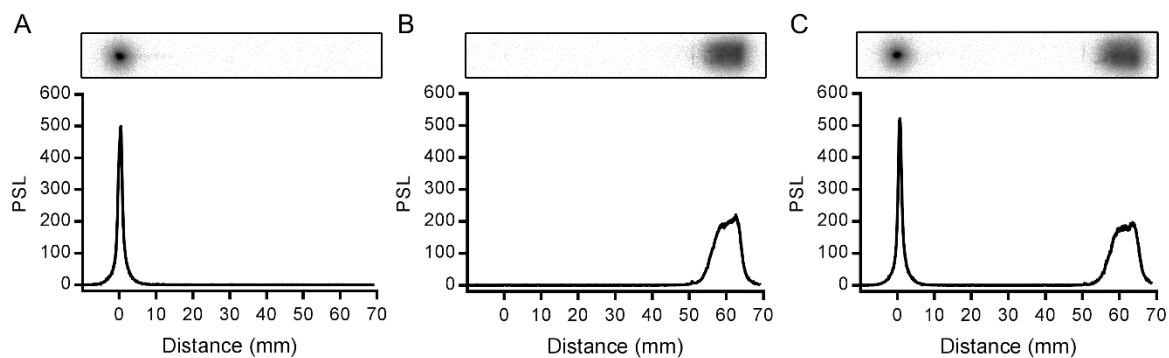


Figure S1. Representative autoradiographs (upper panels) and chromatograms (lower panels) of instant thin-layer silica gel (iTLC-SG) strips developed with 50 mM citric acid for (A) ^{68}Ga -FOL, (B) unbound ^{68}Ga in the reaction mixture without NOTA-folate precursor, and (C) co-application of ^{68}Ga -FOL and ^{68}Ga .

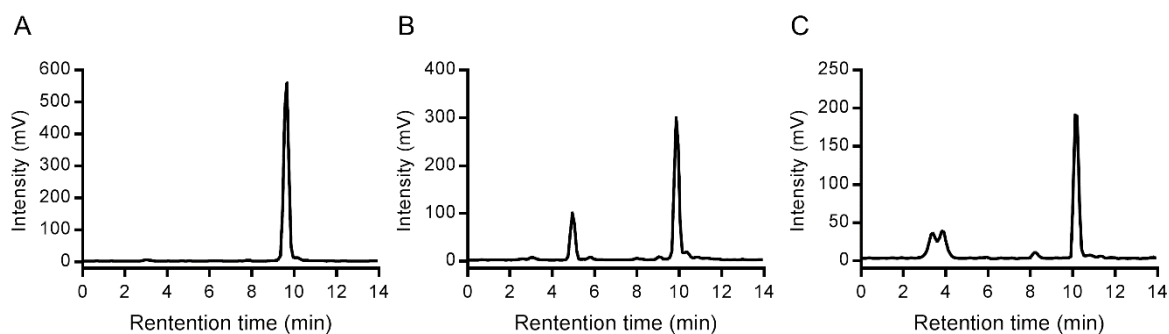


Figure S2. Representative radio-HPLC chromatograms of (A) ^{68}Ga -FOL standard, (B) rat plasma 60 minutes after ^{68}Ga -FOL injection, and (C) mouse plasma 60 minutes after ^{68}Ga -FOL injection.

Table S1. *Ex vivo* biodistribution of ⁶⁸Ga-FOL 60 minutes post-injection in mice, expressed as standardized uptake values (means ± SD)

Tissue	LDLR ^{-/-} ApoB ^{100/100} atherosclerotic mice (n = 4)	LDLR ^{-/-} ApoB ^{100/100} atherosclerotic mice, blocking (n = 4)	C67BL/6JRj control mice (n = 6)
Aorta	0.75 ± 0.12	0.09 ± 0.03 (<i>P</i> = 0.001) ^a	0.41 ± 0.10 (<i>P</i> = 0.004) ^b
Brown adipose tissue	0.29 ± 0.04	0.06 ± 0.02 (<i>P</i> = 0.006) ^a	0.31 ± 0.05 (<i>P</i> = 0.51) ^b
Blood	0.23 ± 0.09	0.29 ± 0.10 (<i>P</i> = 0.48) ^a	0.25 ± 0.14 (<i>P</i> = 0.84) ^b
Bone (skull)	0.13 ± 0.05	0.06 ± 0.02 (<i>P</i> = 0.04) ^a	0.16 ± 0.04 (<i>P</i> = 0.42) ^b
Bone + marrow (femur)	0.24 ± 0.18	0.07 ± 0.01 (<i>P</i> = 0.17) ^a	0.18 ± 0.04 (<i>P</i> = 0.57) ^b
Brain	0.08 ± 0.01	0.02 ± 0.00 (<i>P</i> = 0.001) ^a	0.06 ± 0.01 (<i>P</i> = 0.02) ^b
Heart	0.20 ± 0.02	0.05 ± 0.02 (<i>P</i> < 0.0001) ^a	0.24 ± 0.03 (<i>P</i> = 0.04) ^b
Intestine, small (empty)	0.44 ± 0.06	0.42 ± 0.10 (<i>P</i> = 0.84) ^a	0.47 ± 0.27 (<i>P</i> = 0.76) ^b
Intestine, large (empty)	0.73 ± 0.14	0.19 ± 0.06 (<i>P</i> = 0.003) ^a	0.66 ± 0.08 (<i>P</i> = 0.38) ^b
Kidneys	22.30 ± 3.28	2.65 ± 1.80 (<i>P</i> = 0.0002) ^a	20.27 ± 5.48 (<i>P</i> = 0.49) ^b
Lungs	0.46 ± 0.03	0.19 ± 0.04 (<i>P</i> < 0.0001) ^a	0.39 ± 0.06 (<i>P</i> = 0.05) ^b
Liver	1.04 ± 0.43	0.33 ± 0.13 (<i>P</i> = 0.04) ^a	0.76 ± 0.16 (<i>P</i> = 0.29) ^b
Lymph node	1.37 ± 0.57	0.14 ± 0.02 (<i>P</i> = 0.02) ^a	4.07 ± 0.73 (<i>P</i> = 0.0002) ^b
Muscle	0.20 ± 0.02	0.05 ± 0.01 (<i>P</i> < 0.0001) ^a	0.13 ± 0.02 (<i>P</i> = 0.0003) ^b
Pancreas	0.43 ± 0.06	0.07 ± 0.02 (<i>P</i> = 0.0007) ^a	0.37 ± 0.03 (<i>P</i> = 0.17) ^b
Plasma	0.40 ± 0.15	0.48 ± 0.13 (<i>P</i> = 0.44) ^a	0.34 ± 0.15 (<i>P</i> = 0.61) ^b
Spleen	0.23 ± 0.07	0.15 ± 0.05 (<i>P</i> = 0.11) ^a	0.28 ± 0.13 (<i>P</i> = 0.42) ^b
Stomach (empty)	0.78 ± 0.14	0.25 ± 0.05 (<i>P</i> = 0.002) ^a	0.65 ± 0.12 (<i>P</i> = 0.17) ^b
Thymus	0.42 ± 0.13	0.06 ± 0.01 (<i>P</i> = 0.01) ^a	0.33 ± 0.08 (<i>P</i> = 0.25) ^b
White adipose tissue	0.22 ± 0.16	0.03 ± 0.01 (<i>P</i> = 0.10) ^a	0.30 ± 0.08 (<i>P</i> = 0.40) ^b

The blocking study was performed by injecting a 100-fold molar excess of folate glucosamine 1 minute before ⁶⁸Ga-FOL.

^a Difference between LDLR^{-/-}ApoB^{100/100} atherosclerotic mice vs. atherosclerotic mice + blocking, as assessed by independent-samples *t*-test.

^b Difference between LDLR^{-/-}ApoB^{100/100} atherosclerotic vs. C57BL//6JRj control mice, as assessed by independent-samples *t*-test.

Table S2. Human dose equivalent estimates (mSv/MBq) extrapolated from rat PET data

Organ	Organ doses
Adrenals	0.0243
Brain	0.0043
Esophagus	0.0121
Eyes	0.0110
Gallbladder wall	0.0139
Left colon	0.0140
Small intestine	0.0180
Stomach wall	0.0131
Right colon	0.0184
Rectum	0.0134
Heart wall	0.0078
Kidneys	0.1420
Liver	0.0118
Lungs	0.0069
Pancreas	0.0135
Prostate	0.0134
Salivary glands	0.0119
Red marrow	0.0102
Osteogenic cells	0.0096
Spleen	0.0173
Testes	0.0119
Thymus	0.0120
Thyroid	0.0113
Urinary bladder wall	0.0132
Total body	0.0130
Effective dose	0.0105