Supplemental Data

Plasma Protein Binding

For the evaluation of plasma protein binding, (*R*)-[^{18}F]YH134 (10 μL, 3−5 MBq) was incubated with 150 μL of plasma at room temperature for 10 min. The mixture was afterward transferred to an Amicon ultra-0.5 centrifugal filter unit (MerckMillipore, UFC501096) with 300 μL of ice-cold PBS inside and vortexed briefly. The samples were centrifuged at 15 000g at 4 °C for 10 min. The procedure was repeated with additional 100 μL of ice-cold PBS three times. Afterward, the filters were inverted and centrifuged at 1000g at 4 °C for 2 min to obtain plasma protein fraction. The radioactivity in the filtrate, filter, and protein ($A_{protein}$) were measured by the gamma counter separately. The total radioactivity (A_{total}) was defined by the sum of the radioactive signals collected above. The plasma protein binding was calculated as (1- $A_{protein}/A_{total}$), and the measurement was carried out in triplicate.

In vitro autoradiography study

Based on the published process for (*R*)-[^{18}F]YH149 (*18*), *in vitro* autoradiography was performed with 3% fatty-acid free bovine serum albumin (BSA) in an aqueous buffer containing 30 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES), 0.56 mM $MgCl_2$, 110 mM NaCl, 5 mM KCl and 3.3 mM $CaCl_2$, pH 7.4. Brain slices were incubated with 13 nM (*R*)-[^{18}F]YH134 for 30 min. For blocking experiments, the incubation solution contained 10 μM covalent MAGL inhibitor (SAR127303 or PF-06809247) or the non-radiolabelled (*R*)-YH134 in addition to the tracer. We furthermore used brain slices from MAGL knockout and the corresponding wild-type mice to evaluate the off-target binding of (*R*)-[^{18}F]YH134 *in vitro*. After incubation and washing, the brain sections were exposed to a phosphor imaging plate (Fuji, Dielsdorf, Switzerland), which was scanned in a BAS5000 reader (Fuji). The images were generated using the AIDA software (version 4.50.010; Raytest Isotopenmessgeräte GmbH).

PET experiments and biodistribution after dissection of MAGL knockout and wild-type mice

MAGL knockout (n = 4) or wild-type (n = 4) mice were anesthetized with isoflurane 2.5-4.5% in oxygen/air (1/1) for PET/CT (Super Argus; Sedecal; axial field of view, 4.8 cm; spatial resolution 1.6–1.7 mm full width at half maximum) (*26*). The radiotracer was injected into a tail vein (5.61-

12.63 MBq, 2.68-14.34 nmol/kg) and data acquisition was started 1 min post-injection (p.i.) and lasted for 90 min. The PET data were reconstructed by 2D Fourier rebinning/ordered-subset expectation maximization (FORE/OSEM) with 2 iterations and 16 subsets, correcting for singlets and randoms but not attenuation. Data were re-binned in user-defined time frames (2 × 60 s, 3 × 120 s, 1 × 210 s, 1 × 300 s, 1 × 450 s, 1 × 750 s, 3 × 900 s). The voxel size was 0.3875 × 0.3875 × 0.775 (axial) mm^3. The regions of interest (ROI) were defined on a mouse MRI T2 (W. Schiffer) template in PMOD (v4.201; PMOD Technologies, Fällanden, Switzerland) to generate the corresponding time-activity curves (TAC) from the PET data.

Tracer biodistribution was furthermore investigated after dissection. Awake MAGL knockout and wild-type mice (n = 4 for each group) were injected intravenously with (*R*)-[^{18}F]YH134 (4.32-11.85 MBq, 2.13-2.51 nmol/kg), and euthanized under isoflurane (5%) anesthesia by decapitation at 30 min p.i. The organs of interest were dissected, weighed and radioactivity was measured in a gamma-counter (Wizard, Perkin Elmer). PET and dissection data are reported as standardized uptake value (SUV) and the percentage normalized injected dose per gram of tissue (%ID/g), respectively. Mean values are shown with standard deviations (SD).

In vivo radiometabolite analyses and tracer plasma-to-whole blood ratio

The radiometabolite analyses were carried out in C57BL/6 mice and Wistar rats. All samples were analyzed by radio-ultra-performance liquid chromatography (UPLC). The mice were injected with 38-81 MBq (*R*)-[^{18}F]YH134 (n = 2). The brain and plasma samples were collected after euthanasia of the mice under isoflurane anaesthesia at 45- and 90-min p.i. The rats were assigned to a baseline (n = 2) and a blockade (n = 2) group. The blockade groups were pretreated with compound **3** (Figure 1; 10 mg/kg dissolved in 30% *N*-methyl-2-pyrrolidone and 70% β-cyclodextrin) 1 min before tracer administration to investigate whether the blocker affected tracer plasma/whole blood distribution and metabolism. At time zero, 148-154 MBq (*R*)-[^{18}F]YH134 was injected into a tail vein. Blood (~ 500 µL) was collected from the artery of the anaesthetized (2.5- 4% isoflurane in oxygen/air 1/1) rat at different time points (5, 15, 30, 45, 60, 90, 100 min p.i.), separated into an aliquot for radiometabolite analysis and an aliquot for the measurement of plasma-to-whole blood radioactivity ratio. Three rats (2 baseline at 60 and 90 min p.i., 1 blocking at 60 min p.i.) were euthanized. Brains were dissected and homogenized. Acetonitrile (1 mL) was added to the brain homogenate and the suspension was vortexed for 1 min followed by centrifugation at 5000 g for

5 min. The supernatants were collected and filtered through a syringe filter (0.45 μm, Minisart SRP 4, Sartorius Stedim Biotech) and analyzed by radio UPLC. The proteins of the supernatant plasma were precipitated by addition of an equal volume of acetonitrile and centrifuged at 5000 g for 5 min. The supernatants of plasma were collected, filtered and analyzed by analytical radio UPLC.

Rat PET scans without an arterial input function

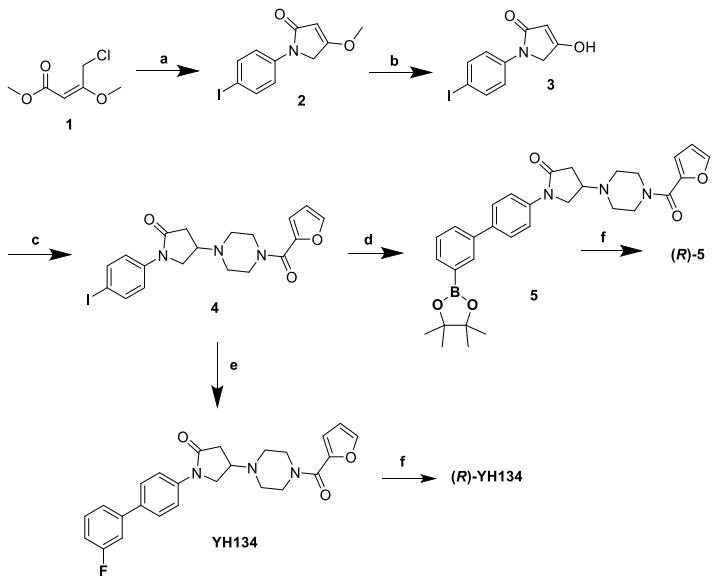
As described in the previous section, PET scans were conducted in anesthetized male Wistar rats (301-355 g) with 8.33-47.90 MBq (*R*)-[^{18}F]YH134 (0.40-4.48 nmol/kg). Data acquisition started at 1 min pre-injection and lasted for 90 min. In the blockade conditions, the animals were pretreated by tail-vein injection with 2 mg/kg compound **1** (dissolved in 5% dimethyl sulfoxide, 5% cremophor and 90% saline) 60 min before tracer administration, or 1 mg/kg compound **2** (dissolved in 30% *N*-methyl-2-pyrrolidone and 70% β-cyclodextrin), or various doses of compound **3** (1, 3, 5, or 10 mg/kg dissolved in 30% *N*-methyl-2-pyrrolidone and 70% β-cyclodextrin), or 3 mg/kg compound **4** (dissolved in 30% *N*-methyl-2-pyrrolidone and 70% β-cyclodextrin) 1 min before tracer administration. The injection time point for compound 1 is based on the reference 19. In which, the MAGL inhibition by compound 1 reached maximum at 0.5, 1- and 2-hour post-injection. As for compounds 2 to 4, we did not test its max. MAGL inhibition in vivo and injected 1 min before the tracer injection according to our standard protocol. PET data was reconstructed with the user-defined time frames as described above. Blood from the rats receiving compound **3** (1, 3, 5, 10 mg/kg, n = 1) was collected at 100 min p.i. after completing the PET scans, and plasma was separated by centrifugation and stored at -80 °C before being analyzed by LC-MS/MS to determine the concentration of compound **3** in plasma.

Rat PET scans with an arterial input function

PET kinetics with an arterial input function were determined in male Wistar rats (344-427 g; Table S1). For the arterial input function (IF), blood activity was determined from an arteriovenous shunt simultaneously to the acquisition of the PET data (*27*). Blood from either the cannulated tail artery (minimally invasive protocol; Table S1) or a femoral artery (invasive protocol) was guided through a Twilite coincidence counter (Swisstrace, Menzingen, Switzerland), a peristaltic pump (200-300 μL/min), and transferred back to a cannulated lateral tail vein (minimally invasive protocol) or a

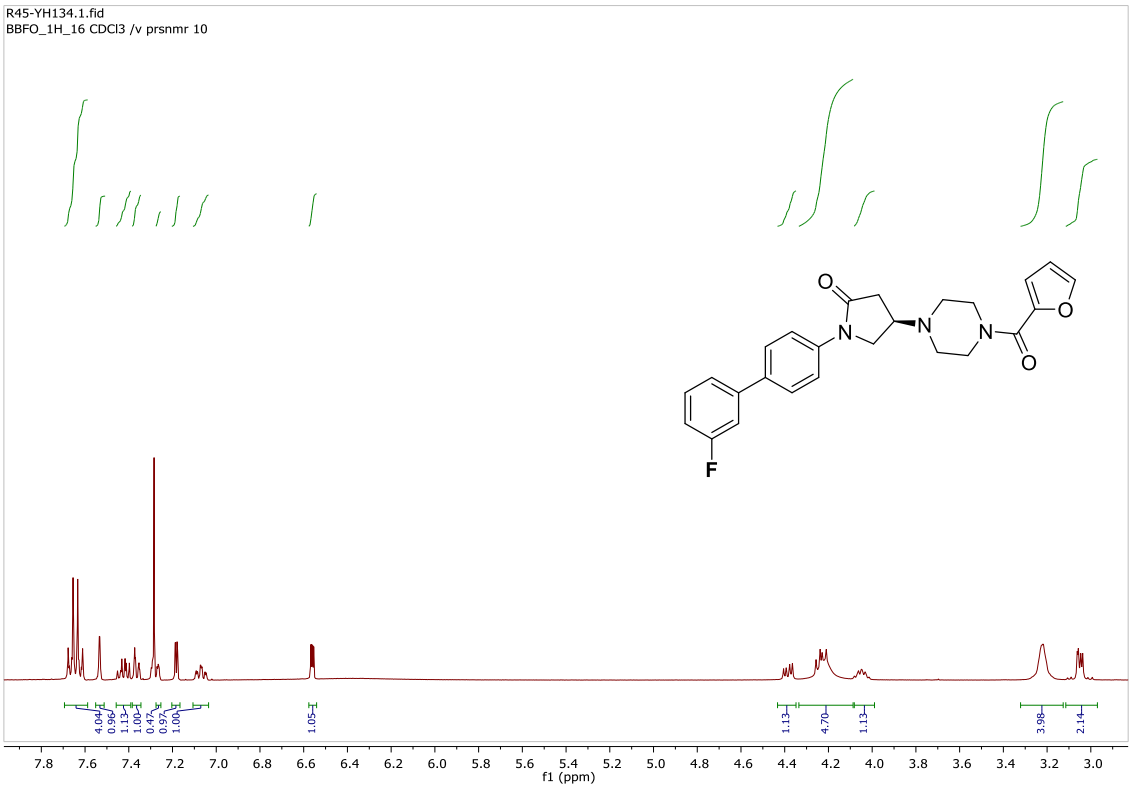
femoral vein (invasive protocol). The blood coincidences were monitored at 1 s intervals. The animal was treated with either vehicle (30% *N*-methyl-2-pyrrolidone and 70% β-cyclodextrin) or compound **3** at 1, 2, 4, 5, 8, 10 mg/kg or compound **2** at 1, 2 or 3 mg/kg (Table S1) dissolved in above-mentioned vehicle *via* a separate, cannulated tail vein, followed by the injection of radiotracer to the same tail vein 1 min later.

Synthetic procedures, ^{1}H, ^{13}C nuclear magnetic resonance and high-resolution mass spectrometry of (*R*)-YH134 and the precursor for ^{18}F-radiolabeling.



Scheme S1. Synthetic procedure of (*R*)-YH134 and corresponding precursor. Reagents and conditions: a) triethylamine, tetrabutylammonium iodide, CH_3CN/toluene, 50 °C, 4-iodoaniline, overnight, 32%; b) conc. hydrochloric acid, CH_3CN, 50 °C, 4-5 h, 87%; c) sodium cyanoborohydride, acetic acid, anhydrous THF, r.t, furan-2-yl(piperazin-1-yl)methanone, overnight, 55%; d) 1,3-phenyldiboronic acid, potassium acetate, [1,1′-bis(diphenylphosphino)ferrocene]dichloropalladium(II), anhydrous DMF, 80 °C, 4 h, 23%; e) cesium carbonate, *tris*(dibenzylideneacetone)dipalladium(0), SPhos, anhydrous dioxane, 3-fluorobenzeneboronic acid, overnight, 69%; f) chiral supercritical fluid chromatography separation.

(*R*)-YH134: ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.66 – 7.62 (m, 2H), 7.61 – 7.58 (m, 2H), 7.52 – 7.50 (m, 1H), 7.44 – 7.32 (m, 2H), 7.28 – 7.23 (m overlapped with chloroform-*d*, 1H), 7.16 (dd, *J* = 3.6, 0.9 Hz, 1H), 7.08 – 7.01 (m, 1H), 6.54 (dd, *J* = 3.5, 1.8 Hz, 1H), 4.36 (dd, *J* = 11.2, 5.0 Hz, 1H), 4.25 – 4.13 (m, 5H), 4.08 – 3.97 (m, 1H), 3.31 – 3.10 (m, 4H), 3.06 – 3.00 (m, 2H). ^{13}C NMR (101 MHz, Chloroform-*d*) δ 168.97, 163.37 (d, $J_{C\text{-}F}$ = 245.6 Hz), 158.89, 146.90, 144.72, 142.34, 137.53, 137.26, 130.53 (d, $J_{C\text{-}F}$ = 8.7 Hz), 127.95, 122.71, 120.70, 118.86, 114.47 (d, $J_{C\text{-}F}$ = 20.7 Hz), 113.96 (d, $J_{C\text{-}F}$ = 21.7 Hz), 112.13, 57.94, 49.62, 49.54, 33.95. ^{19}F NMR (376 MHz, Chloroform-*d*) δ -112.80. HRMS (ESI) calculated for $C_{25}H_{24}FN_3NaO_3^+$ [M + Na]$^+$ 456.1694 *m/z*, found 456.1699 *m/z*.



(*R*)-Precursor: ^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.08 – 7.98 (m, 1H), 7.82 – 7.74 (m, 1H), 7.70 – 7.60 (m, 5H), 7.51 – 7.41 (m, 2H), 7.03 (d, *J* = 3.4 Hz, 1H), 6.49 (dd, *J* = 3.4, 1.8 Hz, 1H), 4.06 – 3.75 (m, 6H), 3.33 – 3.24 (m, 1H), 2.79 (dd, *J* = 16.7, 8.1 Hz, 1H), 2.71 – 2.54 (m, 5H), 1.36 (s, 12H). ^{13}C NMR (101 MHz, Chloroform-*d*) δ 171.71, 159.03, 147.81, 143.79, 139.64, 137.93, 137.66, 133.71, 133.20, 129.78, 128.25, 127.65, 120.26, 116.79, 111.40, 83.92, 57.23, 52.25, 50.59, 37.30, 29.02, 24.90. HRMS (ESI) calculated for $C_{31}H_{37}BN_3O_5^+$ $[M + H]^+$ 542.2821 *m/z*, found 542.2828 *m/z*.

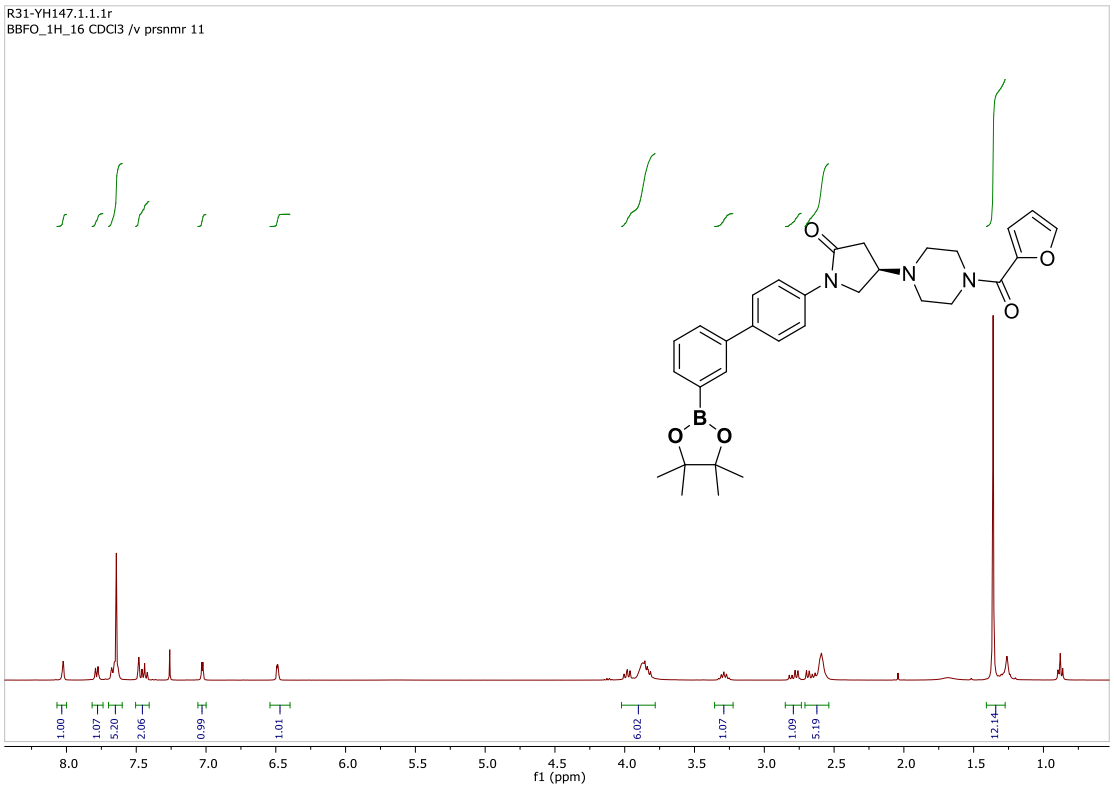


Table 1. Details of PET scans with an IF.

Treatment [a]	Dose (mg/kg)	Body weight (g) [b]	Tracer dose (MBq)	Tracer dose (nmol/kg)	Arterio-venous shunt [c]	Comment	Included in study
Vehicle	-	344.3	23.70	1.08	min inv.	-	yes
	-	360.2	31.83	1.10	min inv.	-	yes
	-	399.1	29.66	1.03	min inv.	-	yes
Compound **2**	1	371.9	39.75	1.55	invasive	-	yes
	1	385.7	36.41	0.92	invasive	-	yes
	1	389.0	40.12	1.25	invasive	-	yes
	2	372.8	10.97	0.93	min inv.	-	yes
	3	347.4	26.83	0.47	min inv.	-	yes
	1	n.r.	n.r.	n.r.	invasive	Woke up during scan	no
	1	n.r.	n.r.	n.r.	min inv.	Shunt clocked	no
	1	n.r.	n.r.	n.r.	min inv.	Vein damaged	no
	2	n.r.	n.r.	n.r.	min inv.	Shunt clocked	no
Compound **3**	1	355.3	35.69	1.48	min inv.	-	yes
	2	382.2	38.82	0.57	min inv.	-	yes
	4	427.6	34.80	0.67	min inv.	-	yes
	5	394.5	34.35	0.53	min inv.	-	yes
	8	375.0	35.67	2.12	min inv.	-	yes
	10	353.9	28.10	0.45	min inv.	-	yes
	10	378.3	33.24	2.10	min inv.	-	yes
	4	n.r.	n.r.	n.r.	min inv.	High blood activity fluctuations	no

[a] Vehicle as indicated in the text. [b] n.r., not relevant as not included in the study. [c] Invasive and minimally invasive (min inv.) arteriovenous shunts, see main manuscript, Methods section.

Table 2. CEREP screen of (*R*)-YH134 over 50 receptors/enzymes.

Target	Assay type	Species	Inhibition (%)
α_{2A} Adrenergic receptor	Binding	Human	4
β_1 Adrenergic receptor	Binding	Human	2
β_2 Adrenergic receptor	Binding	Human	23
Angiotensin 1 Receptor	Binding	Human	13
$GABA_A$ (Benzodiazapine binding site)	Binding	Rat	-14
Cholecystokinin 1 receptor	Binding	Human	38
Dopamine D_1 receptor	Binding	Human	-13
Glycine receptor (strychine insensitive)	Binding	Rat	-4
Muscarinic acetylcholine receptor M_1	Binding	Human	7
Muscarinic acetylcholine receptor M_2	Binding	Human	5
μ-Opioid receptor	Binding	Human	19
Phencyclidine receptor	Binding	Rat	-9
Serotonin 1A receptor	Binding	Human	12
Ca^{2+} channel (diltiazem site)	Binding	Rat	8
GABA receptor (Cl^- chanel)	Binding	Rat	25
Xanthine oxidase	Enzymatic	Bovine	4
HIV-1 protease	Enzymatic	HIV-1	12
Norepinephrine transporter	Binding	Human	-18
Acetylcholinesterase	Enzymatic	Human	2
5-HT_3 receptor	Binding	Human	5
Serotonin transporter	Binding	Human	-9
Adenosine A_1 receptor	Binding	Human	5
Glucocorticoid receptor	Binding	Human	-6
5-HT_{2A} serotonin receptor	Binding	Human	16

Matrix metallopeptidase 9	Enzymatic	Human	-7
Peroxisome proliferator-activated receptor gamma	Binding	Human	8
Zeta-chain-associated protein kinase 70	Enzymatic	Human	-1
Histamine H_1 Receptor	Binding	Human	24
Androgen receptor	Binding	Human	-5
Nicotinic acetylcholine receptor	Binding	Human	-2
Histamine H_2 receptor	Binding	Human	-7
Dopamine D_2 receptor (short)	Binding	Human	13
Histamine H_3 Receptor	Binding	Human	8
Prostaglandin F receptor	Binding	Human	21
α_{1A} Adrenergic receptor	Binding	Human	4
Glycogen synthase kinase-3α	Enzymatic	Human	-13
Glycogen synthase kinase-3β	Enzymatic	Human	-8
Cyclin-dependent kinase 2	Enzymatic	Human	-9
Nicotine receptor (α-BGTX insensitive type)	Binding	Human	5
Tyrosine-protein kinase ABL1	Enzymatic	Human	-9
Angiotensin-converting enzyme	Enzymatic	Human	-20
Phosphodiesterase 3B	Enzymatic	Human	9
Phosphodiesterase 4D	Enzymatic	Human	3
Estrogen Receptors Alpha	Binding	Human	0
Cyclooxygenase-2	Enzymatic	Human	-23
κ-Opioid receptor	Binding	Human	31
Cannabinoid 1 receptor	Binding	Human	-4
Monoamine oxydase A	Enzymatic	Human	1

Table 3. *Ex vivo* biodistribution of (*R*)-[^{18}F]YH134 at 30 min post-injection (n = 4)[a].

Organ	Wild-type	MAGL knockout	% Difference[b]	*p*[c]
spleen	2.58 ± 0.26	2.45 ± 0.15		0.4523
liver	8.35 ± 0.48	7.7 ± 0.62		0.1480
kidney	5.17 ± 0.57	5.23 ± 0.56		0.8842
lungs	3.28 ± 0.65	3.6 ± 0.38		0.4224
bone	4.22 ± 0.3	4.91 ± 0.32	16	0.0205
heart	2.53 ± 0.17	2.06 ± 0.17	-19	0.0078
brain	1.75 ± 0.24	1.31 ± 0.16	-25	0.0225
gall bladder	27.16 ± 12.99	51.8 ± 29.86		0.1809
blood	1.14 ± 0.09	1.21 ± 0.1		0.2951
stomach w/o cont	4.54 ± 1.74	6.62 ± 5.17		0.4733
s.intest. w.cont.	23.56 ± 2.27	31.1 ± 6.45		0.0697
urine	90.47 ± 57.24	112.11 ± 50.21		0.5904
WAT (white fat)	2.31 ± 0.78	1.88 ± 0.35		0.3551
BAT (brown fat)	8.79 ± 1.94	4.7 ± 0.98	-46	0.0093
muscle	1.14 ± 0.15	1.1 ± 0.12		0.7051
pancreas	2.58 ± 0.5	2.43 ± 0.3		0.6284
skin	1.3 ± 0.11	1.05 ± 0.03	-19	0.0052
adrenal gland	5.78 ± 1.17	3.42 ± 0.54	-41	0.0105
brain/blood ratio	1.53 ± 0.13	1.07 ± 0.05	-30	0.0007

[a] Data are expressed as the percentage of injected dose per gram tissue (%ID/g; mean ± SD; n = 4). [b] (%ID/g(wild-type)-%ID/g(knockout))/%ID/g(wild-type) calculated if $p < 0.05$. [c] Values for the wild-type *vs*. MAGL knockout group at 30 min after injection as determined by a Student's t test (homoscedastic, two-tailed), without post-hoc correction for multiple comparisons. s.intest. w.cont., small intestines with content.

Table 4. The percentages of (*R*)-[^{18}F]YH134 in various samples from *in vivo* metabolism.

	Animal	Time (min)	Sample	Percentage of (*R*)-[^{18}F]YH134
vehicle	Wild-type mouse	45	brain	100%
		45	plasma	41%
		90	brain	100%
		90	plasma	0
vehicle	Wistar rat	5	plasma	100%
		15	plasma	100%
		30	plasma	100%
		60	brain	100%
		60	plasma	100%
		90	brain	100%
		90	plasma	Not detectable
10 mg/kg compound **3**	Wistar rat	5	plasma	100%
		30	plasma	100%
		60	brain	100%
		60	plasma	100%

Table 5. IC_{50} values and apical efflux ratios from P-glycoprotein (P-gp) of Compounds **2** and **3**.

Compound	MAGL IC_{50} (nM)	P-gp efflux ratio
(*R*)-YH134	Mouse: 6.7; 16.0	Mouse: 1.05; human:1.13
2	Mouse: 9.5; 34.5; rat: 70.8	Mouse: 1.91
3	Mouse: 7.7; 4.7; 5.5; rat: 10.6	Mouse: 7.65

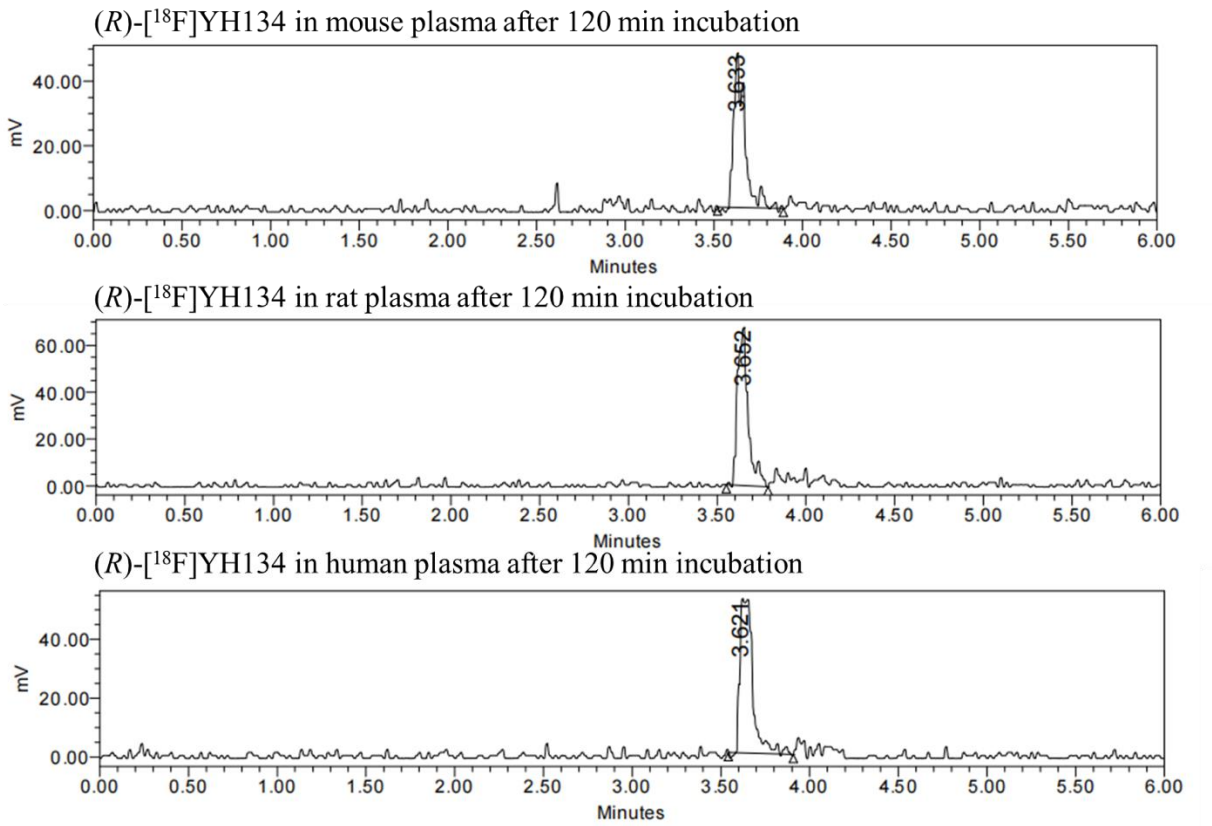


Figure 1. *In vitro* stability of (*R*)-[^{18}F]YH134 in mouse, rat or human serum up to 120 min incubation at 37 °C.

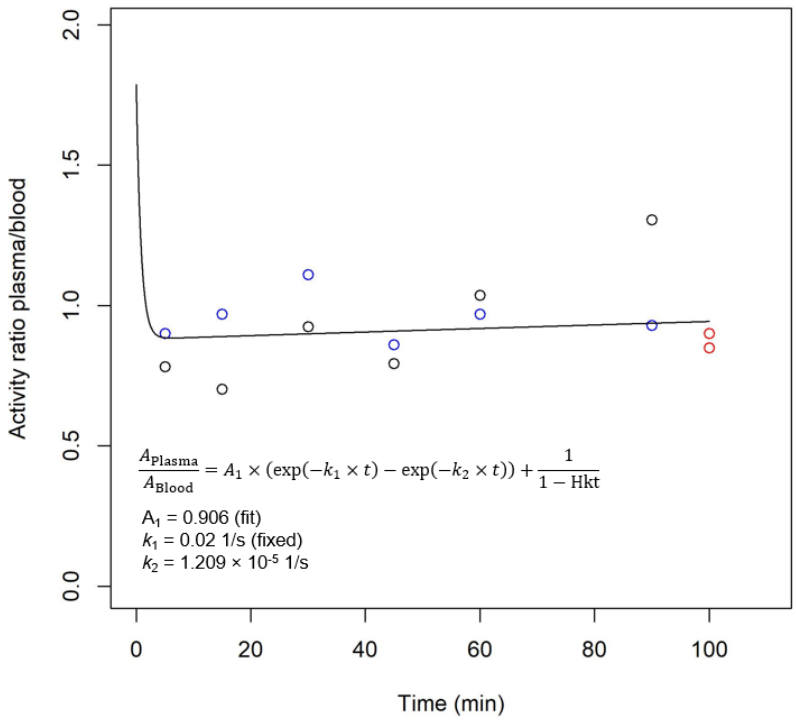


Figure 2. Plasma-to-whole-blood distribution of (*R*)-[^{18}F]YH134 in Wistar rats. Black symbols, baseline conditions; blue and red symbols, 10 mg/kg and 4 mg/kg, respectively, **3** i.v. 1 min before tracer injection. Black line, simulated activity ratio between plasma and blood, assuming a hematocrit (Hkt) of 0.44 (DOI: 10.1080/13685538.2017.1350156) and a k_1 (initial decrease from distribution in plasma only (1/(1-Hkt)) to the first measured ratios (at 5 min p.i.). Model function and fit parameters, see Figure insert. The model function is not following a mechanistic model, it is chosen as a simple function to describe the data. No effect of blockers was assumed for the simulation and further data analysis.

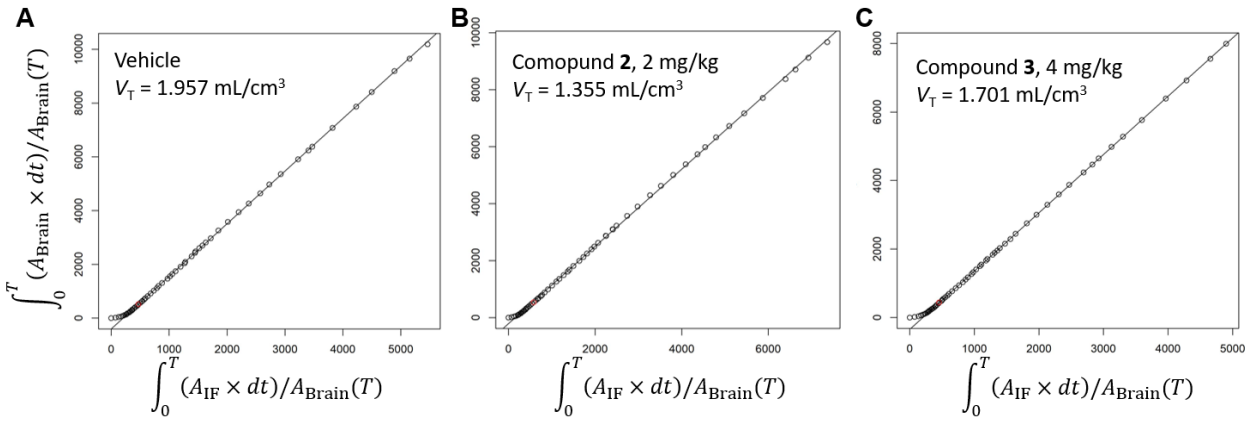


Figure 3. Representative Logan plots under baseline and blocking conditions. A) Vehicle; B) After injection of a MAGL inhibitor distributing to the brain, as indicated; C) After injection of a MAGL inhibitor not distributing to the brain, as indicated. Black straight line, linear regression; red symbol, first data point included in the linear regression (at 7.5 min p.i.). V_T (slope of the black line) for the data shown are indicated in the plots. A_{Brain} and A_{IF}, activity in brain and input function, respectively. *T*, image time points. The input function was generated from the blood coincidences after correction for the plasma-to-whole blood ratios (Figure 2). *A* in kBq/cm^3, time in s.

Dose (mg/kg)	Measured exposure (ng/mL after 1.5h)	Average conc. (nM, back-calc.)	Average conc. Free (nM)	calc. %TO periphery
1	8.1*	77.7*	10.4*	49.6*
3	4.99	47.9	6.4	37.7
5	10.6	101.7	13.6	56.3
10	28	268.7	36	77.3

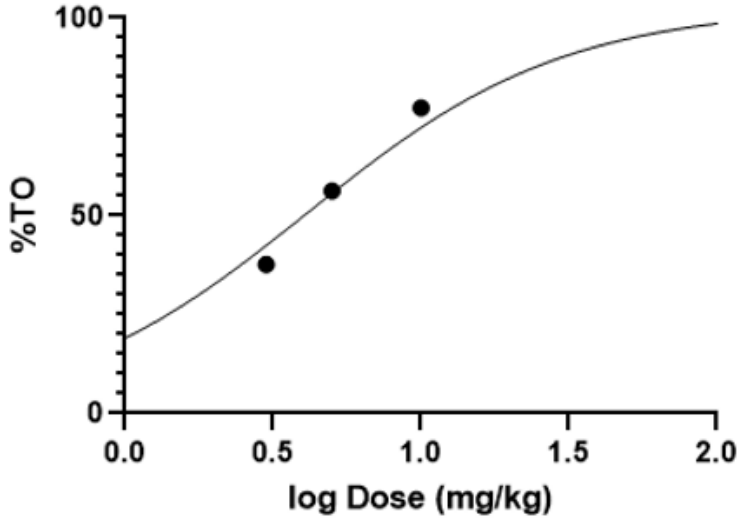


Figure 4. The plasma concentrations of compound **3** were measured at the end of the experiment (100 min p.i.). The averaged concentration was back-calculated based on a previously determined concentration-time profile after i.v. administration and estimated to be 5-fold higher than the concentration determined at 100 min p.i.The averaged free concentration of compound **3** was calculated based on the experimentally-determined free fraction in rat plasma (13.4%) and IC_{50} value in rats (10.6 nM) of compound **3**. %TO stands for percentage of target occupancy which was generated in Prism. *The value after 1 mg/kg i.v. administration was considered an outlier and excluded in the analysis.

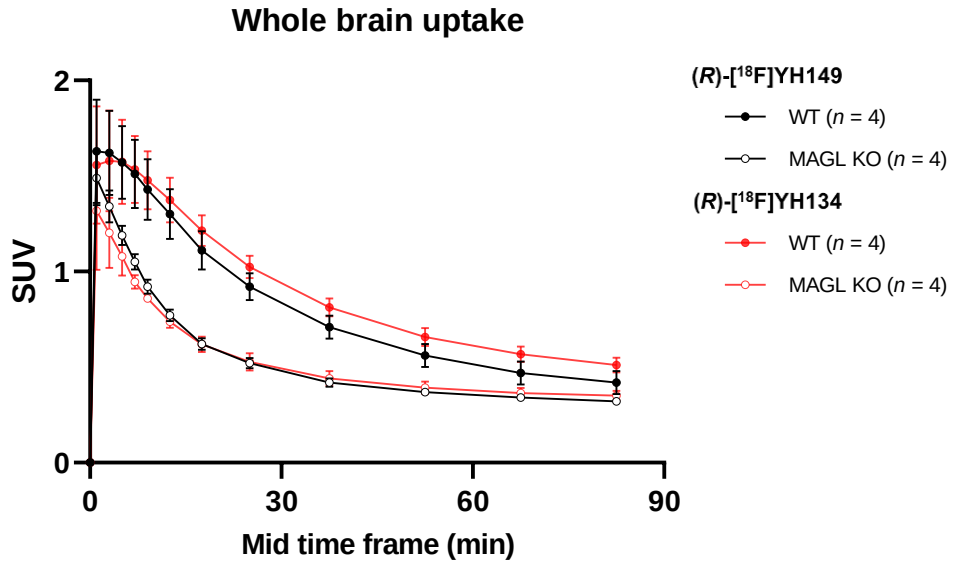


Figure 5. The whole brain time-activity curves (TACs) of (*R*)-[^{18}F]YH134 and (*R*)-[^{18}F]YH149. Data of (*R*)-[^{18}F]YH149 were previously published by He *et al* (18).