

## Supplementary File

Article Title: Molecular Imaging of Fibroblast Activation Protein after Myocardial Infarction using the Novel Radiotracer [<sup>68</sup>Ga]MHLL1

Journal Name: Theranostics

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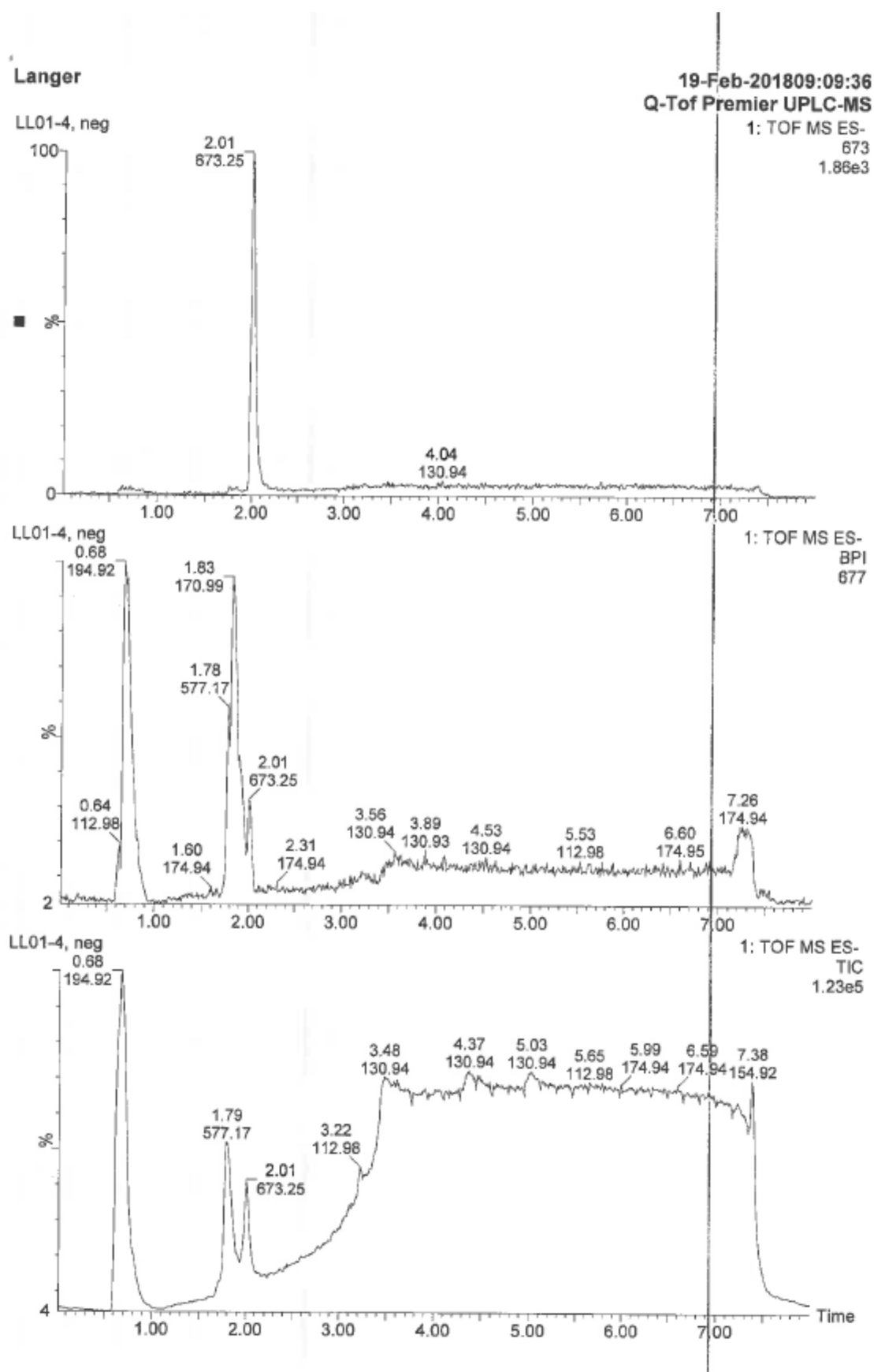
§ contributed equally

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MHHAB050.30.fid  
Langer, MHH / Klinik fuer Nuklearmedizin MH B05 12mg in DMSO-d6 at 298.0K, 27.02.2017 Koertje  
1H-1d nach den Wochenend-Messungen  
LL01-2

Chemical structure of the molecule is shown, with atoms numbered 1 through 47. The structure is a complex molecule, likely a nucleoside or nucleotide derivative, featuring a purine-like ring system, a thiazolidine ring, and a cytosine-like base. The numbering indicates the positions of various protons and carbons, which are correlated with the peaks in the spectrum.

Figure S2: UPLC-MS Mass data: MHLL1



# Elemental Composition Report

Page 1

## Single Mass Analysis

Tolerance = 10.0 PPM / DBE: min = -1.5, max = 50.0

Selected filters: None

Monoisotopic Mass, Even Electron Ions

3264 formula(e) evaluated with 43 results within limits (up to 50 closest results for each mass)

Elements Used:

C: 0-55 H: 0-80 N: 0-8 O: 0-8 Na: 0-1 S: 0-2

Langer

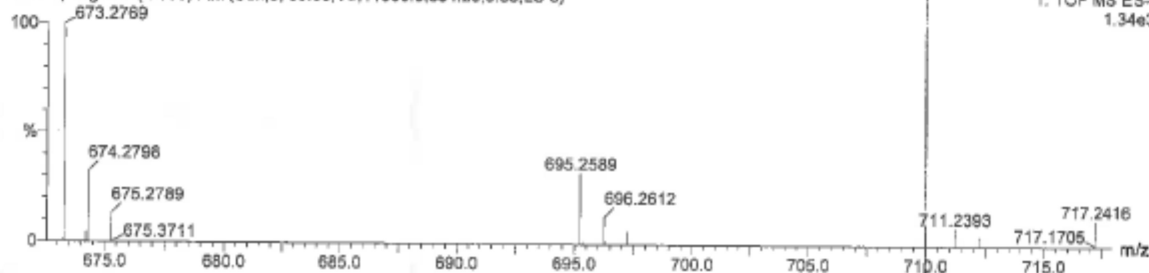
Q-ToF Premier UPLC-MS

LL01-4, neg 215 (2.033) AM (Cen, 5, 65.00, Ar, 11000.0, 554.26, 0.55, LS 5)

19-Feb-2018 09:09:36

1: TOF MS ES-

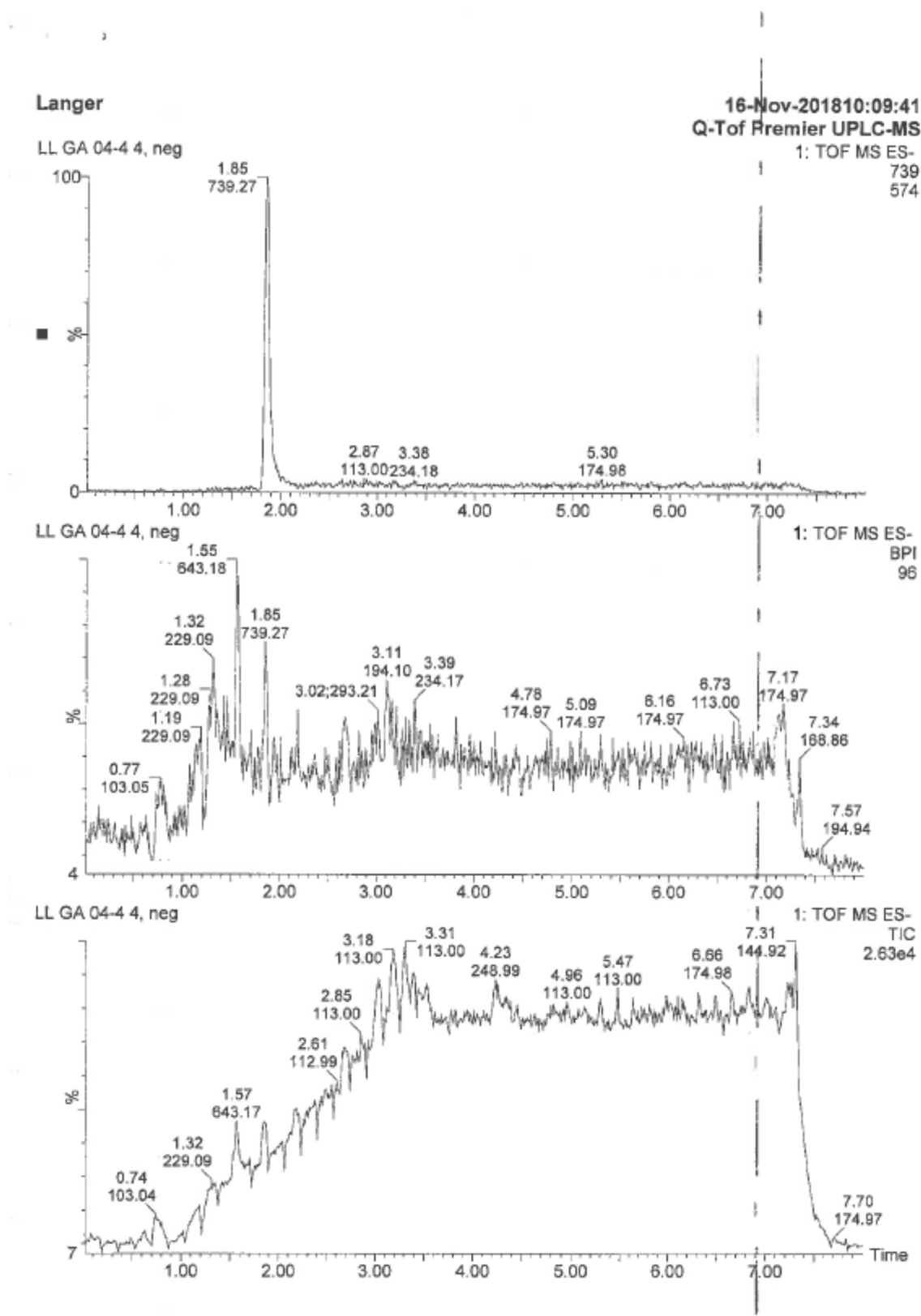
1.34e3



Minimum: -1.5  
Maximum: 5.0 10.0 50.0

Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Formula
673.2769	673.2770	-0.1	-0.1	17.5	34.4	C38 H45 N2 O5 S2
	673.2768	0.1	0.1	14.5	5.5	C30 H41 N8 O8 S
	673.2775	-0.6	-0.9	23.5	26.8	C38 H37 N6 O6
	673.2776	-0.7	-1.0	26.5	51.7	C46 H41 O3 S
	673.2777	-0.8	-1.2	19.5	26.2	C40 H42 O8 Na
	673.2778	-0.9	-1.3	6.5	4.5	C25 H46 N8 O8 Na S2
	673.2759	1.0	1.5	19.5	32.2	C37 H42 N6 O Na S2
	673.2783	-1.4	-2.1	22.5	41.2	C39 H41 N6 O S2
	673.2784	-1.5	-2.2	15.5	10.5	C33 H42 N6 O6 Na S
	673.2752	1.7	2.5	23.5	42.2	C44 H42 O3 Na S
	673.2786	-1.7	-2.5	18.5	43.4	C41 H46 O3 Na S2
	673.2751	1.8	2.7	20.5	22.5	C36 H38 N6 O6 Na
	673.2750	1.9	2.8	27.5	41.1	C42 H37 N6 O S
	673.2791	-2.2	-3.3	24.5	33.9	C41 H38 N4 O4 Na
	673.2746	2.3	3.4	14.5	26.1	C36 H46 N2 O5 Na S2
	673.2744	2.5	3.7	11.5	2.7	C28 H42 N8 O8 Na S
	673.2743	2.6	3.9	31.5	58.5	C49 H37 O3
	673.2743	2.6	3.9	18.5	24.3	C34 H41 N8 O3 S2
	673.2801	-3.2	-4.8	22.5	32.2	C42 H41 O8
	673.2736	3.3	4.9	22.5	32.9	C41 H41 N2 O5 S
	673.2802	-3.3	-4.9	9.5	7.6	C27 H45 N8 O8 S2
	673.2734	3.5	5.2	19.5	17.6	C33 H37 N8 O8
	673.2804	-3.5	-5.2	29.5	42.3	C42 H34 N8 Na
	673.2730	3.9	5.8	13.5	19.3	C33 H45 N4 O7 S2
	673.2808	-3.9	-5.8	18.5	16.1	C35 H41 N6 O6 S
	673.2810	-4.1	-6.1	21.5	53.5	C43 H45 O3 S2
	673.2811	-4.2	-6.2	14.5	16.9	C37 H46 O8 Na S
	673.2726	4.3	6.4	24.5	32.8	C40 H38 N6 O Na S
	673.2815	-4.6	-6.8	27.5	40.5	C43 H37 N4 O4
	673.2818	-4.9	-7.3	10.5	11.7	C30 H46 N6 O6 Na S2
	673.2719	5.0	7.4	28.5	50.5	C47 H38 O3 Na
	673.2719	5.0	7.4	15.5	17.4	C32 H42 N8 O3 Na S2
	673.2716	5.3	7.9	32.5	50.3	C45 H33 N6 O
	673.2824	-5.5	-8.2	19.5	23.8	C38 H42 N4 O4 Na S
	673.2712	5.7	8.5	19.5	25.3	C39 H42 N2 O5 Na S
	673.2711	5.8	8.6	26.5	64.5	C45 H41 N2 S2
	673.2828	-5.9	-8.8	32.5	49.4	C44 H33 N8
	673.2710	5.9	8.9	16.5	16.1	C31 H38 N8 O8 Na
	673.2709	6.0	8.9	23.5	24.5	C37 H37 N8 O3 S
	673.2831	-6.2	-9.2	28.5	49.5	C46 H38 N2 O2 Na
	673.2706	6.3	9.4	10.5	13.5	C31 H46 N4 O7 Na S2
	673.2835	-6.6	-9.8	17.5	24.2	C39 H45 O8 S

Figure S3:UPLC-MS Mass data: [<sup>nat</sup>Ga]MHLL1



## Elemental Composition Report

Page 1

## Single Mass Analysis

Tolerance = 20.0 PPM / DBE: min = -0.5, max = 50.0

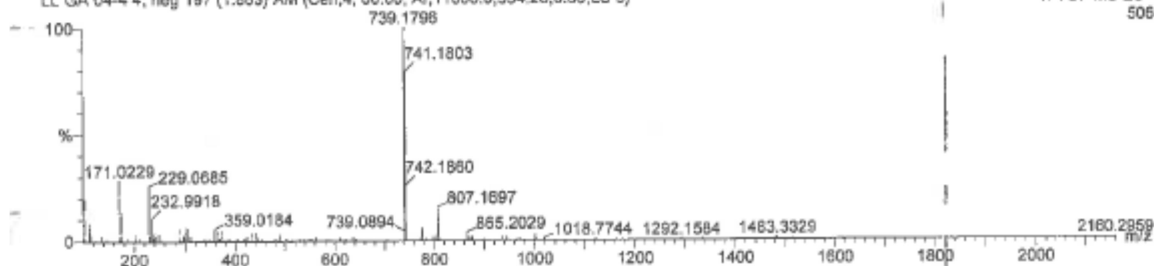
Selected filters: None

## Monoisotopic Mass, Even Electron Ions

7550 formula(e) evaluated with 162 results within limits (all results (up to 1000) for each mass)

Elements Used:

C: 0-55 H: 0-100 N: 0-9 O: 0-12 Na: 0-1 S: 0-1 Ga: 0-1

Larger Q-ToF Premier UPLC-MS  
LL GA 04-4 4, neg 197 (1.863) AM (Cen, 4, 80.00, Ar, 11000.0, 554.26, 0.55, LS 5)16-Nov-2018 10:09:41  
1: TOF MS ES-  
506Minimum: -0.5  
Maximum: 5.0 20.0 50.0

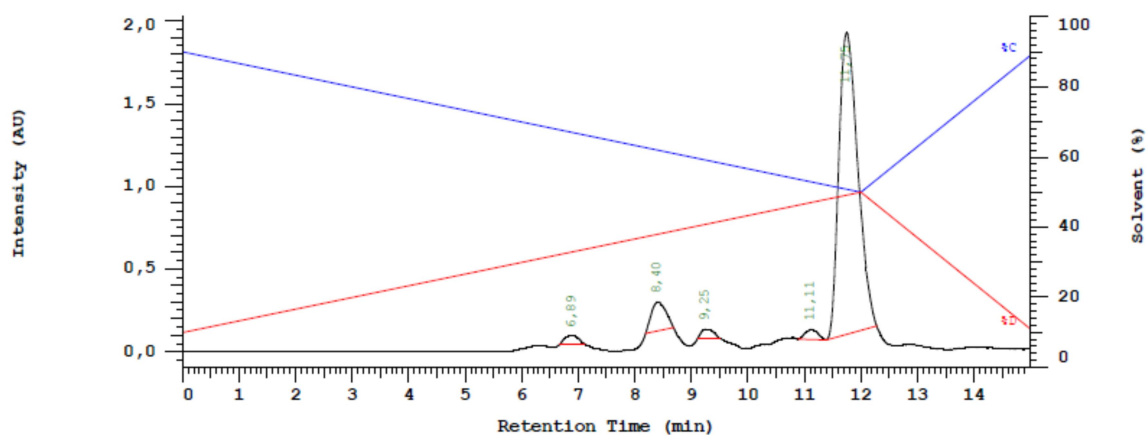
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Formula
739.1796	739.1796	0.0	0.0	25.5	1.5	C38 H34 N6 O6 Ga
739.1797	739.1797	-0.1	-0.1	28.5	5.9	C46 H38 O3 S Ga
739.1798	739.1798	-0.2	-0.3	21.5	1.5	C40 H39 O8 Na Ga
739.1798	739.1798	-0.2	-0.3	21.5	129.7	C34 H32 N6 O10 Na S Ga
739.1792	739.1792	0.4	0.5	12.5	0.3	C32 H43 N2 O10 Na S Ga
739.1791	739.1791	0.5	0.7	25.5	141.9	C41 H32 O12 Na
739.1791	739.1791	0.5	0.7	32.5	122.8	C47 H31 O7 S
739.1789	739.1789	0.7	0.9	29.5	143.8	C39 H27 N6 O10
739.1789	739.1789	0.7	0.9	16.5	0.3	C30 H38 N8 O8 S Ga
739.1804	739.1804	-0.8	-1.1	37.5	124.9	C48 H27 N4 O3 S
739.1805	739.1805	-0.9	-1.2	17.5	0.0	C33 H39 N6 O6 Na S Ga
739.1805	739.1805	-0.9	-1.2	30.5	143.0	C42 H28 N4 O8 Na
739.1786	739.1786	1.0	1.4	43.5	140.6	C54 H24 N2 O Na
739.1782	739.1782	1.4	1.9	20.5	0.6	C37 H38 N2 O10 Ga
739.1782	739.1782	1.4	1.9	20.5	131.2	C31 H31 N8 O12 S
739.1780	739.1780	1.6	2.2	34.5	125.8	C46 H28 N4 O3 Na S
739.1812	739.1812	-1.6	-2.2	26.5	2.9	C41 H35 N4 O4 Na Ga
739.1816	739.1816	-2.0	-2.7	28.5	139.8	C43 H31 O12
739.1776	739.1776	2.0	2.7	11.5	1.2	C29 H42 N4 O12 S Ga
739.1816	739.1816	-2.0	-2.7	15.5	0.1	C34 H42 N2 O10 S Ga
739.1818	739.1818	-2.2	-3.0	35.5	144.3	C43 H24 N8 O4 Na
739.1773	739.1773	2.3	3.1	25.5	4.2	C44 H39 O3 Na S Ga
739.1772	739.1772	2.4	3.2	22.5	0.8	C36 H35 N6 O6 Na Ga
739.1820	739.1820	-2.4	-3.2	38.5	125.6	C51 H28 N2 O Na S
739.1771	739.1771	2.5	3.4	29.5	4.1	C42 H34 N6 O S Ga
739.1822	739.1822	-2.6	-3.5	24.5	127.3	C36 H31 N6 O10 S
739.1822	739.1822	-2.6	-3.5	24.5	2.6	C42 H38 O8 Ga
739.1770	739.1770	2.6	3.5	42.5	140.2	C51 H23 N4 O3 Ga
739.1825	739.1825	-2.9	-3.9	31.5	4.7	C42 H31 N8 Na Ga
739.1825	739.1825	-2.9	-3.9	20.5	125.5	C38 H36 O12 Na S
739.1766	739.1766	3.0	4.1	29.5	124.0	C45 H32 O7 Na S
739.1765	739.1765	3.1	4.2	13.5	1.2	C28 H39 N8 O8 Na S Ga
739.1765	739.1765	3.1	4.2	26.5	146.3	C37 H28 N6 O10 Na
739.1764	739.1764	3.2	4.3	33.5	125.6	C43 H27 N6 O5 S
739.1764	739.1764	3.2	4.3	33.5	9.1	C49 H34 O3 Ga
739.1829	739.1829	-3.3	-4.5	20.5	0.3	C35 H38 N6 O6 S Ga
739.1829	739.1829	-3.3	-4.5	33.5	141.2	C44 H27 N4 O8
739.1830	739.1830	-3.4	-4.6	13.5	1.2	C29 H39 N6 O11 Na Ga
739.1832	739.1832	-3.6	-4.9	16.5	0.4	C37 H43 O8 Na S Ga
739.1758	739.1758	3.8	5.1	17.5	134.5	C29 H32 N8 O12 Na S
739.1758	739.1758	3.8	5.1	17.5	0.3	C35 H39 N2 O10 Na Ga
739.1757	739.1757	3.9	5.3	24.5	2.6	C41 H38 N2 O5 S Ga

### Figure S4: Radiochromatogram of [<sup>68</sup>Ga]MHLL1

For all HPLC chromatograms following methods were used:

10% Methanol: 90% 70 mM phosphate buffer (pH = 2.5) to 50% Methanol: 50% 70 mM phosphate buffer (pH = 2.5) in 13 minutes leading to a radiotracer retention time of 11.75 minutes (column: Phenomenex Luna C18(2), 150x4.6 mm, 5 µM, 100 Å).

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No.	RT	Area	Name	Conc 1	BC
1	6,89	434101		1,813	BB
2	8,40	1517564		6,336	BB
3	9,25	417535		1,743	BB
4	11,11	475353		1,985	BB
5	11,75	21105390		88,123	BB
		23949943		100,000	

### Figure S5: Radiochromatogram from efflux studies (60 minutes)

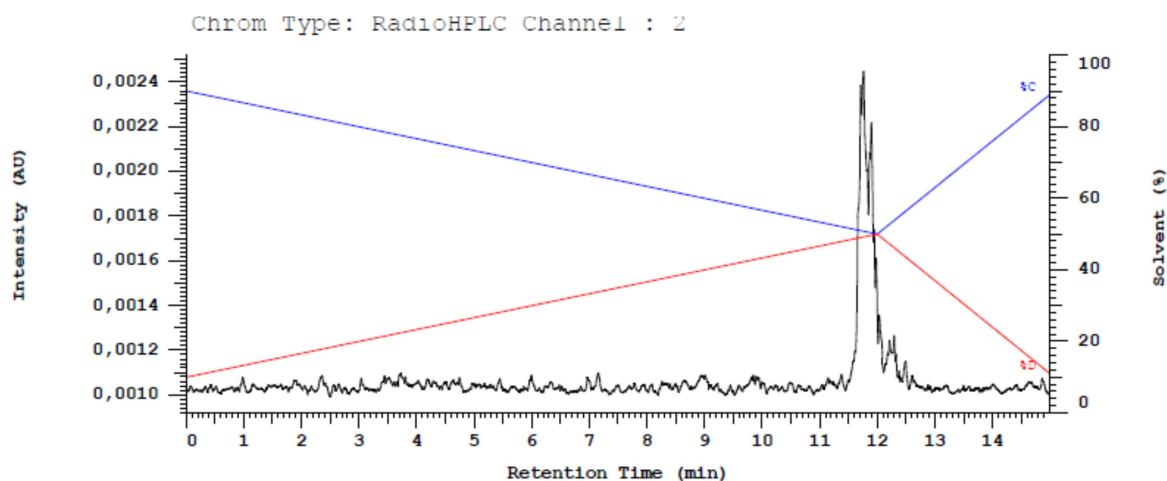


Figure S6: Radiochromatogram from biodistribution (urine) of [<sup>68</sup>Ga]MHLL1 in healthy mice.

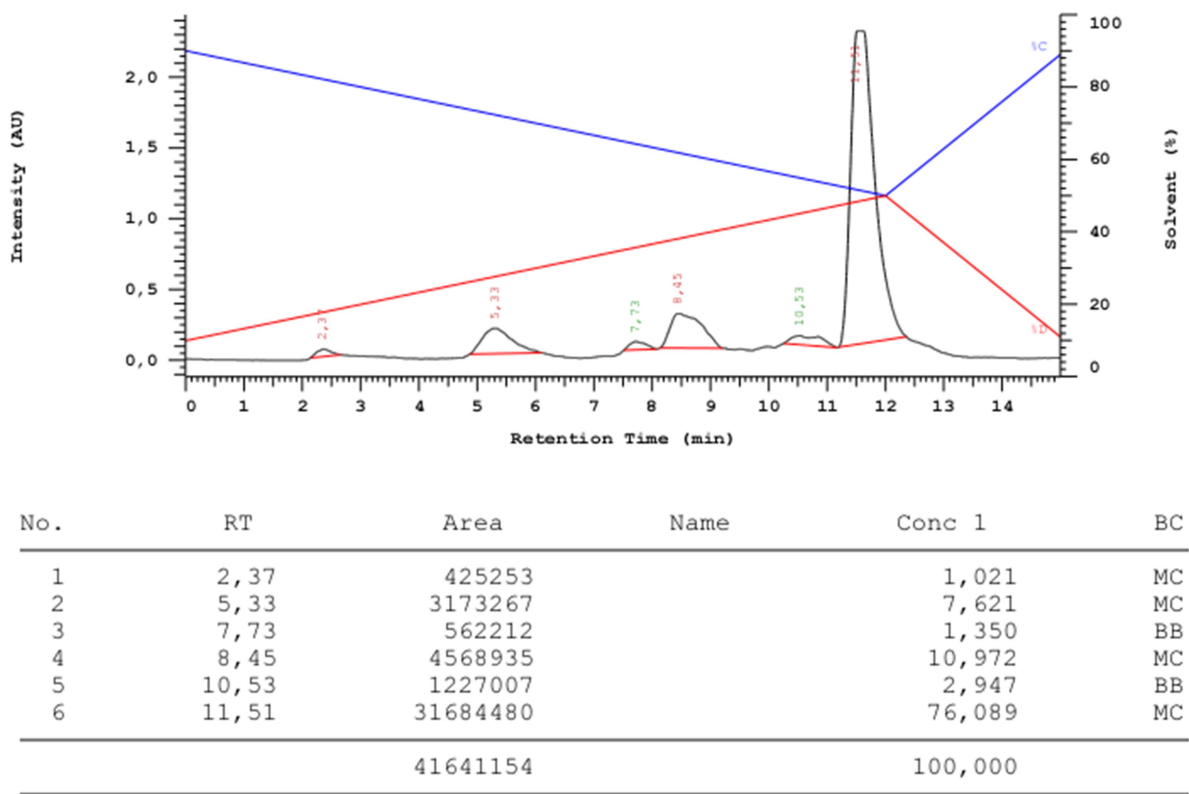
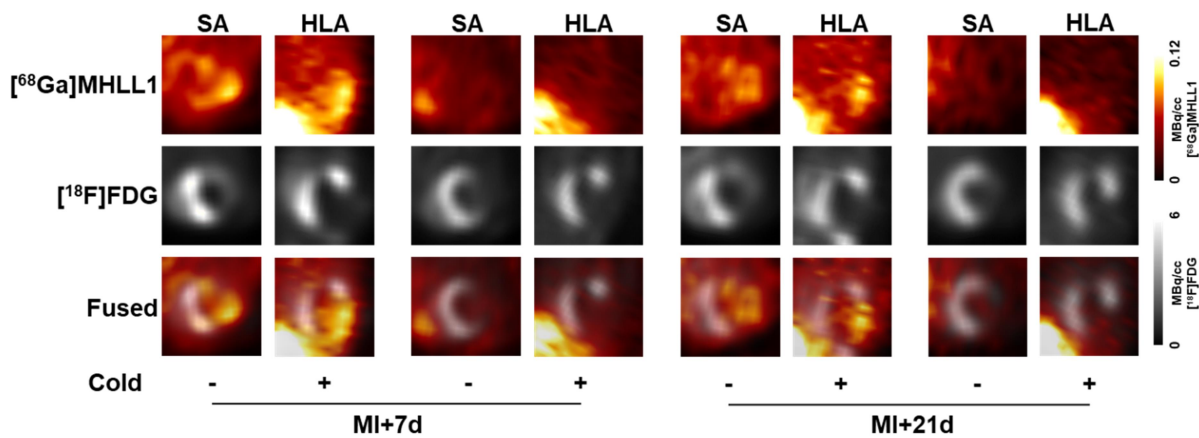


Figure S7: Representative cardiac axis images of [<sup>68</sup>Ga]MHLL1 (colourscale) and [<sup>18</sup>F]FDG (greyscale) display accumulation of the fibroblast activation protein-targeted radiotracer in the non-viable infarct region. Blocking with excess unlabeled MHLL1 (1mg/kg) lowers accumulation in the infarct and remote myocardial regions without affecting the liver signal. SA, short axis; HLA, horizontal long axis.



**Figure S8: (A)** Time activity curves for whole body distribution of [ $^{68}\text{Ga}$ ]MHLL1 after myocardial infarction display rapid accumulation in liver and clearance through the renal system. Blood activity is reduced **(B)** Time activity curves of infarct and remote non-infarct myocardium display deviation between the regions from 15 – 60 min after tracer injection. Images display average activity concentration  $\pm$  standard deviation over the image acquisition for  $n=3$  mice at each timepoint. The stable separation of the regions and the heart guided the selection of static imaging timepoint at 50 – 60 min after tracer injection.

