SUPPLEMENTARY

METHODS

Surface Plasmon Resonance

All the material needed, except hK2, was purchased from GE Healthcare and used according to the guidelines from the manufacturer. Shortly, immobilisation of antigen on a CM4-2 research grade chip was done using amine coupling with 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide and N-hydroxysuccinimide mixture. A solution containing 2.96 µg/mL of the antigen hK2 (stock solution of hK2 diluted in 10 mM sodium acetate buffert pH 3.8, provided from the University of Turku, Finland) was immobilized in one of the flow cells of the chip with another used as a blank. An immobilization of 688 response units (RU) was achieved. Five different concentrations (100, 50, 25, 12.5 and 6.25 nM) diluted in HSP-buffer were flown over the immobilized antigen and in the blank with a rate of 30µL/min. The association phase of the antibodies was followed for 4-5 min and the dissociation phase was allowed to take place for 480 min. The Langmuir 1:1 binding model was used for the kinetic analysis in the BiaEval software (GE Healthcare).

Tumor volume

Range of tumor volumes for treatment group 1,2,4,5,6 was at study start as follows

Group	Tumor size range (mm^3)	Comment
A.	~200-500	one at ~1000 mm^3
B.	~100-400	one at ~1000 mm^3
D.	~100-400	
E.	~20−170	Smaller range
F.	~170−470	two at 650-700 mm^3

Table S1 Tumor volume at study start in treatment groups A, B, D, E, F

The relative smaller size of the xenografts in the unlabeled hu11B6 group (group E) contributes to its higher average median survival as well as the larger xenografts within the NaCl group contributes to its lower average. If the mice with the largest xenografts are removed from the data set, the median survival of the NaCl group then becomes 34 d (*n* = 12) and the average median survival for all the control groups become 37 ± 6 d. The relatively smaller size of the xenografts in the unlabeled hu11B6 group also contributes to a fast increasing tumor burden compared to the other control groups not most likely not associated with the antibody. The limits on the mouse's metabolism and the general condition of the mice will influence tumor growth. Small tumors are less of a burden on the animal. This will influence the results.

Macrodosimetry

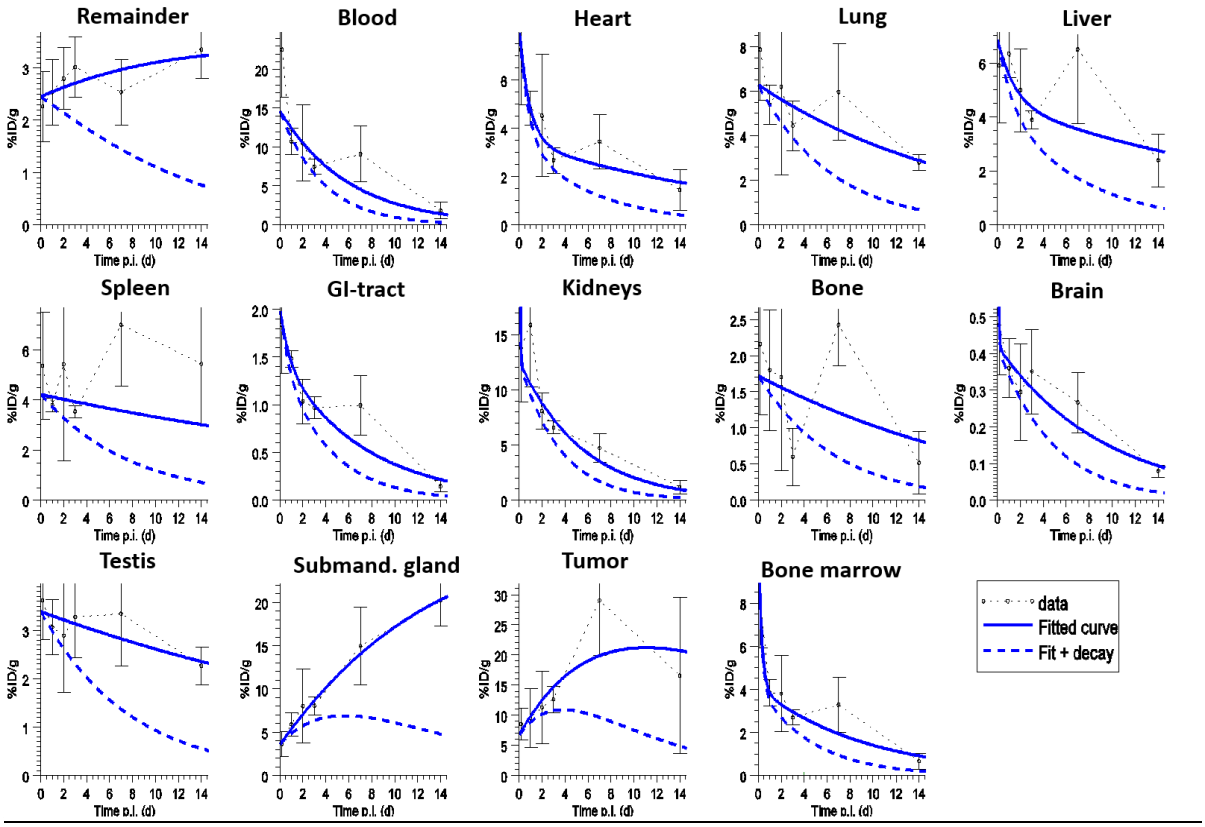


Figure S1 Bi-exponential fit for biokinetic of ^{177}Lu-hu11B6. The solid blue lines represent the bi-exponential fits to the biodistribution data for each organ and for xenografts, and the blue dashed line are those fits together with the physical decay of ^{177}Lu.

RESULTS

Binding Affinity

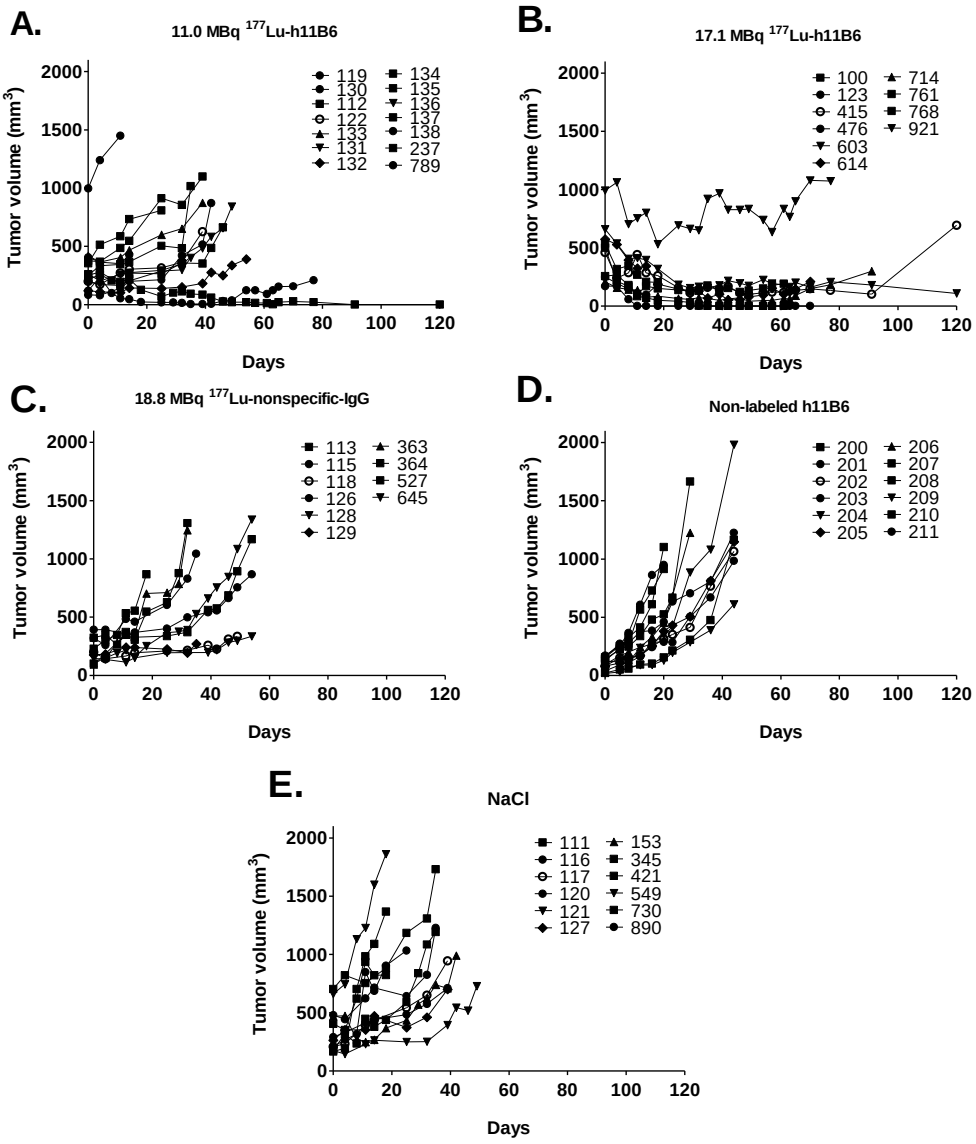
The binding affinity was measured with surface plasmon resonance (SPR) on a Biacore 3000 system. The measured affinity was high for both hu11B6 and conjugated hu11B6 and in the same range as its murine predecessor, m11B6, see table s2. However, their affinity constants (KD) are significantly different. Association to the antigen was fast with rate constants (kon) in the 10^5 M^{-1} s^{-1} range (n = 15–18 per antibody). Slow dissociation gives a high affinity constant (KD) in the 10^{-10} M range for, m11B6, hu11B6 and conjugated hu11B6. The slow dissociation process was at the technical limitation, the range of detection, for the instrument. This has previously also been seen with the murine predecessor. The affinity constant do not differ significantly between the naked humanized antibody and the conjugated counterpart (P = 0.18).

Antibody	Mean K_{off} (10^{-6} s^{-1})	Mean k_{on} (10^5 $M^{-1}s^{-1}$)	Mean K_D (10^{-11} M)
m11B6	3.4 ± 2.1	2.48 ± 0.85	19 ± 15
hu11B6	6.7 ± 0.4	1.17 ± 0.38	65 ± 25
DTPA-hu11B6	12.7 ± 9.1	1.82 ± 0.54	93 ± 78

Table S2. Affinity measurement with SPR on a Biacore 3000 instrument of the murine 11B6, m11B6, humanized 11B6, hu11B6 and conjugated DTPA-hu11B6. Values are presented as mean ± SD.

Therapeutic efficacy

The change in tumor volume after injection of ^{177}Lu-hu11B6 was measured with caliper in the



different treatment groups (A, B, D, E, F), see figure S2.

Figure S2. Tumor volume for treatment groups A (Fig S2A), B (Fig S2B), D (Fig S2C), E (Fig S2D), F (Fig S2E). Numbers refer to individual animals.

SA-β-gal activity staining

Representative images of the senescence-associated beta-galactosidase (SA-β-gal) activity staining, in whole tissue sections and in representative regions of the SA-β-gal activity staining in those sections are found in figure S3. SA-β-gal is a marker that indicates senescence.

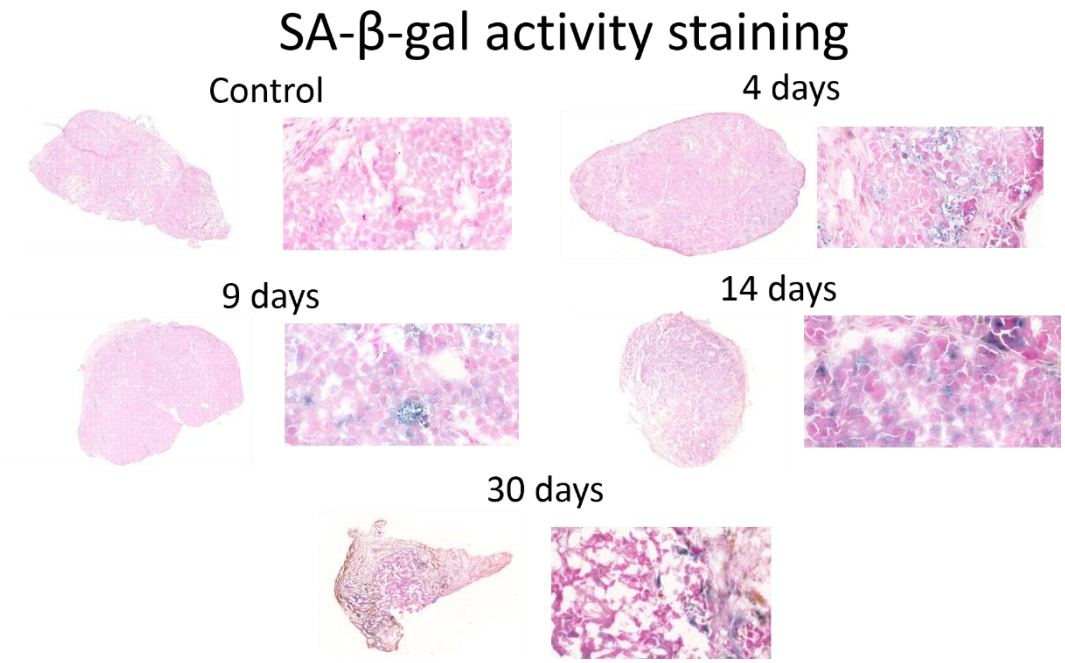


Figure S3. SA-β-gal activity staining at 4, 9, 14, and 30 days and in non-treated controls.

Macrodosimetry

Table S3. Absorbed doses (Gy/MBq)

Organ	Self-dose	Total absorbed dose
Remainder	0,558997	0,570639
Blood	0,927212	1,00992
Heart	0,547646	0,703304
Lung	0,35358	0,489218
Liver	0,568322	0,599658
Spleen	0,485101	0,535783
GI-tract	0,205869	0,263797
Kidney	0,76803	0,803415
Bone	0,10975	0,247238

Brain	0,00956298	0,0281627
Testis	0,590491	0,643859
Submandibular glands	1,88242	1,92817
Tumor	2,93992	2,95676
Bone marrow	0,318705	0,381134