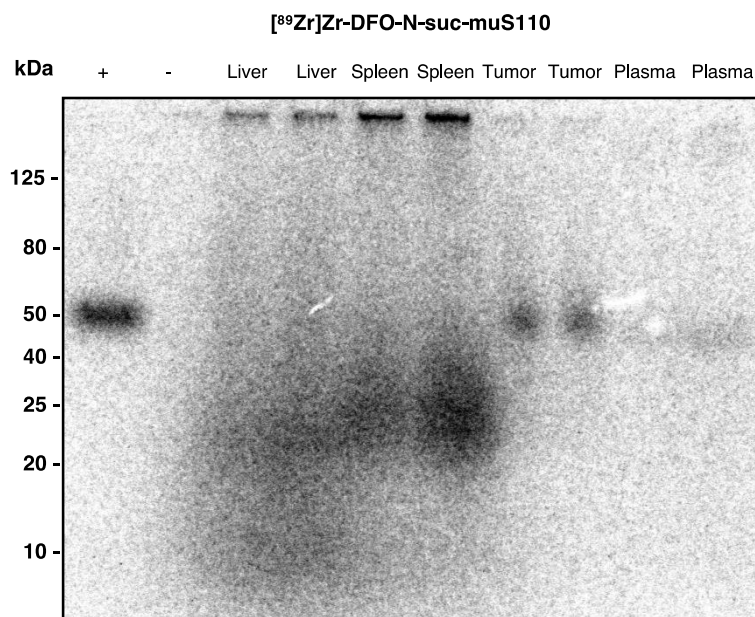
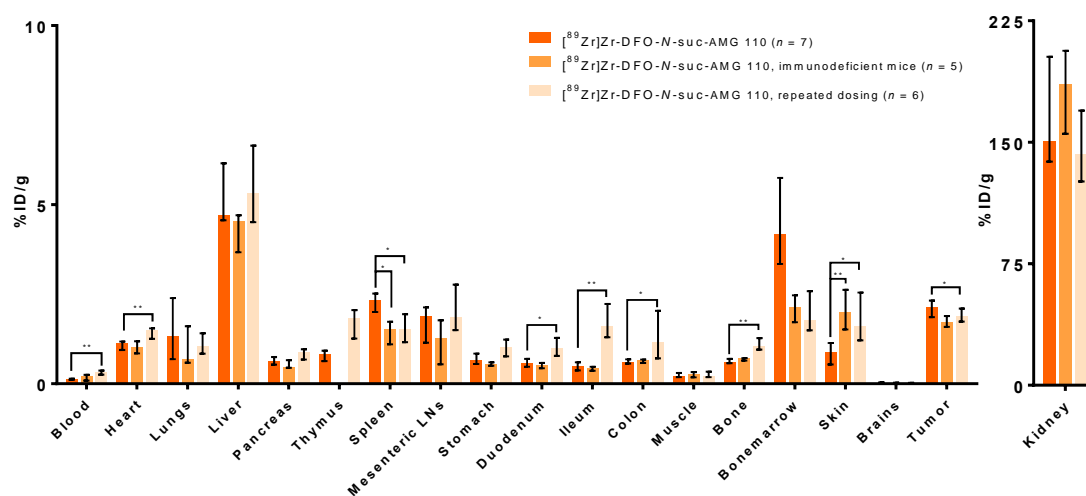


Supplemental figure 1. Evaluation and quality control and validation of $[^{89}\text{Zr}]\text{Zr-DFO-N-suc-BiTE}$ molecules. **A**, Radiochemical signal of size exclusion high performance liquid chromatography chromatogram of $[^{89}\text{Zr}]\text{Zr-DFO-N-suc-BiTE}$ antibody molecules. BiTE elutes after 15 minutes. Free ^{89}Zr elutes after 24 minutes. **B**, Binding of DFO-N-suc-BiTE antibody molecules tested on murine CD3-positive T-cells and B16/F10 murine EpCAM-positive cells. Functionality assessed by cell-based assays with T-cells: Tumor

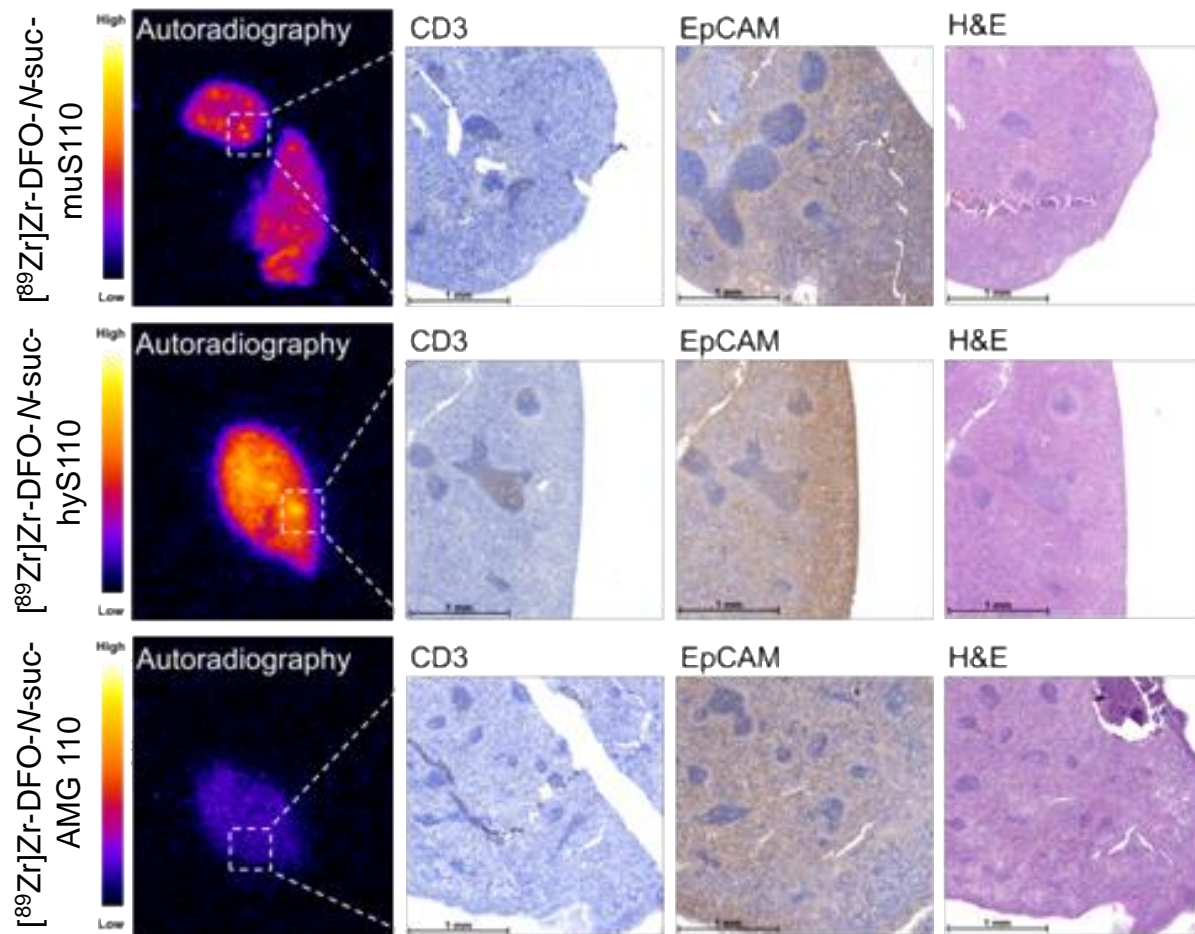
cells incubated at a ratio of 10:1. The read-outs are cytotoxicity (**C**) and T-cell activation by CD69 and CD25 expression (**D**). Data are mean \pm SD ($n = 2$).



Supplemental figure 2. Integrity of 10 μ g [⁸⁹Zr]Zr-DFO-*N*-suc-muS110 in 4T1-tumor bearing immunocompetent mice, 24 hours pi, by SDS-PAGE autoradiography. Lysates of liver spleen and tumor were loaded, as well as plasma of corresponding mice. + = [⁸⁹Zr]Zr-DFO-*N*-suc-muS110, - = empty lane.

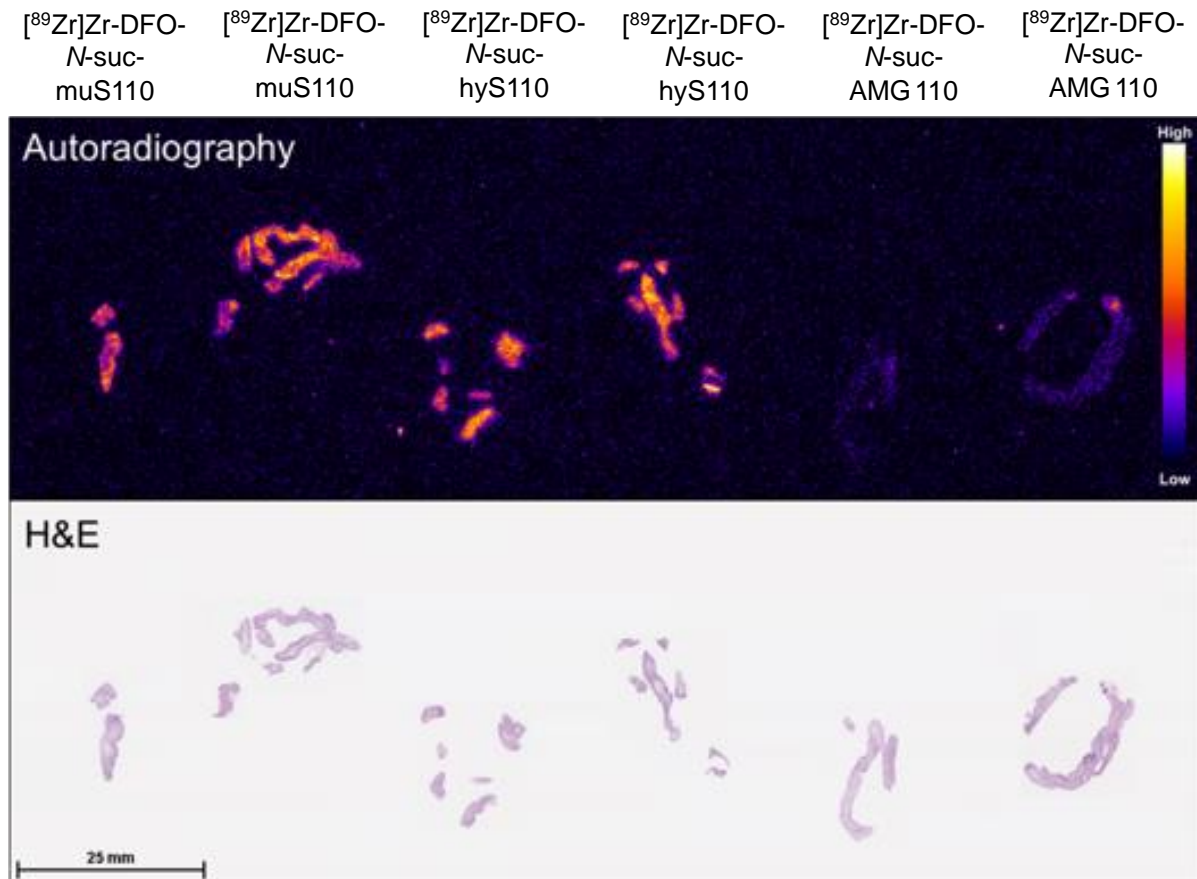


Supplemental figure 3. *Ex-vivo* biodistribution 24 hours after injection of 10 μg $[^{89}\text{Zr}]\text{Zr-DFO-N-suc-AMG 110}$ of immunocompetent mice ($n = 7$), immunodeficient mice ($n = 5$) and repeated mus110 administration mice ($n = 6$), 24 hours pi. Data is presented as median with interquartile range; *: $P \leq 0.05$, **: $P \leq 0.01$.

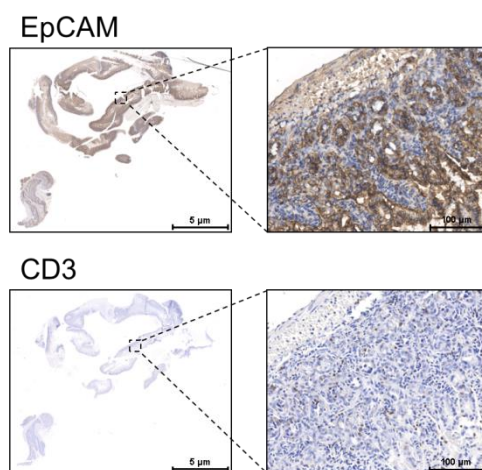


Supplemental figure 4. Microscopic analysis of the spleen from immunocompetent mice 24 hours after injection of 10 µg , $[^{89}\text{Zr}]\text{Zr-DFO-}N\text{-suc-BiTE}$ molecule. From left to right, autoradiography followed by zoomed in CD3 and EpCAM IHC and H&E. From top to bottom, $[^{89}\text{Zr}]\text{Zr-DFO-}N\text{-suc-muS110}$, $[^{89}\text{Zr}]\text{Zr-DFO-}N\text{-suc-hyS110}$ and $[^{89}\text{Zr}]\text{Zr-DFO-}N\text{-suc-AMG 110}$.

A



B



Supplemental figure 5. *Ex-vivo* macro and microscopic analysis of the duodenum from immunocompetent mice 24 hours after injection of 10 μg $[^{89}\text{Zr}]\text{Zr-DFO-}N\text{-suc-BiTE}$ molecule. **A**, top panel shows an overview of the autoradiography signal. Below the

corresponding H&E stained tissue. **B**, EpCAM and CD3 staining of the duodenum showing high uniform presence of EpCAM-positive and CD3-positive cells.

Supplemental Table 1. % ID/g values from *ex-vivo* biodistribution of ^{89}Zr -muS110 ($n = 7$), ^{89}Zr -AMG 110 ($n = 7$) and ^{89}Zr -hyS110 ($n = 7$) in 4T1-tumor bearing immunocompetent mice 24 hours pi. Differences between either ^{89}Zr -AMG 110 and ^{89}Zr -hyS110 versus ^{89}Zr -muS110 were tested with a Bonferroni-corrected Mann-Whitney U test. P -values ≤ 0.05 are shown.

Tissue	^{89}Zr Zr-DFO- <i>N</i> -suc-muS110	^{89}Zr Zr-DFO- <i>N</i> -suc-AMG 110	Difference versus-muS110 (P)	^{89}Zr Zr-DFO- <i>N</i> -suc-hyS110	Difference versus muS110 (P)
Blood	0.27 (0.23 to 0.29)	0.12 (0.11 to 0.13)	0.001	0.44 (0.34 to 0.48)	0.044
Heart	1.10 (0.98 to 1.14)	1.13 (1.02 to 1.16)	0.001	1.78 (1.64 to 1.95)	0.001
Lung	1.51 (1.21 to 1.67)	1.33 (0.76 to 1.93)	n.s.	1.48 (1.16 to 1.80)	n.s.
Liver	9.18 (8.66 to 11.65)	4.71 (4.58 to 5.69)	0.002	10.94 (10.71 to 12.26)	n.s.
Pancreas	1.54 (1.19 to 1.67)	0.63 (0.56 to 0.74)	0.001	1.13 (1.01 to 1.20)	n.s.
Thymus	1.83 (1.78 to 2.26)	0.84 (0.70 to 0.90)	0.001	2.48 (2.17 to 2.69)	n.s.
Spleen	6.89 (6.74 to 8.29)	2.33 (2.18 to 2.43)	0.002	9.14 (8.72 to 10.66)	n.s.
Mesenteric LNs	3.02 (2.80 to 4.16)	1.90 (1.20 to 2.04)	0.001	6.78 (5.70 to 8.50)	0.002
Stomach	1.74 (1.55 to 2.08)	0.65 (0.57 to 0.80)	0.001	2.01 (1.81 to 2.34)	n.s.
Duodenum	3.66 (3.33 to 4.43)	0.58 (0.49 to 0.66)	0.001	2.01 (1.67 to 2.44)	0.001
Ileum	2.76 (2.45 to 3.63)	0.49 (0.40 to 0.59)	0.001	1.87 (1.82 to 2.25)	0.04
Colon	1.00 (0.87 to 1.24)	0.61 (0.58 to 0.65)	0.008	1.14 (1.04 to 1.51)	n.s.
Muscle	0.25 (0.19 to 0.27)	0.22 (0.19 to 0.26)	n.s.	0.39 (0.36 to 0.48)	0.008
Bone	0.96 (0.95 to 1.10)	0.60 (0.59 to 0.66)	0.001	0.70 (0.57 to 1.07)	n.s.
Bone marrow	3.41 (2.43 to 7.07)	4.17 (3.76 to 4.96)	n.s.	3.86 (2.84 to 7.81)	n.s.
Skin	0.93 (0.70 to 1.22)	0.88 (0.60 to 1.11)	n.s.	1.17 (1.12 to 1.30)	n.s.
Brain	0.02 (0.02 to 0.03)	0.02 (0.02 to 0.03)	n.s.	0.02 (0.02 to 0.04)	n.s.
Tumor	1.96 (1.39 to 2.61)	2.13 (1.92 to 2.21)	n.s.	2.66 (2.51 to 3.18)	n.s.
Kidney	58.24 (57.07 to 67.49)	150.57 (143.26 to 182.62)	0.001	72.30 (70.53 to 81.45)	n.s.

Supplemental Table 2. % ID/g values from *ex-vivo* biodistribution of [⁸⁹Zr]Zr-DFO-*N*-suc-muS110 (*n* = 4) and [⁸⁹Zr]Zr-DFO-*N*-suc-AMG 110 (*n* = 5) in 4T1-tumor bearing immunodeficient mice 24 hours pi. Differences between [⁸⁹Zr]Zr-DFO-*N*-suc-muS110 and [⁸⁹Zr]Zr-DFO-*N*-suc-AMG 110 and were tested with a Mann-Whitney U test. *P*-values ≤ 0.05 are shown.

Tissue	[⁸⁹ Zr]Zr-DFO- <i>N</i> -suc-muS110	[⁸⁹ Zr]Zr-DFO- <i>N</i> -suc-AMG 110	Difference (<i>P</i>)
Blood	0.13 (0.10 to 0.18)	0.20 (0.09 to 0.24)	n.s.
Heart	0.71 (0.60 to 0.80)	1.03 (0.94 to 1.18)	n.s.
Lung	2.05 (1.32 to 2.71)	0.69 (0.60 to 1.50)	n.s.
Liver	5.79 (4.57 to 6.80)	4.54 (4.06 to 4.65)	n.s.
Pancreas	1.06 (0.96 to 1.10)	0.46 (0.45 to 0.57)	0.032
Spleen	3.37 (2.62 to 3.76)	1.51 (1.19 to 1.55)	n.s.
Mesenteric LNs	1.67 (1.24 to 2.07)	1.52 (1.23 to 1.77)	n.s.
Stomach	0.92 (0.83 to 0.97)	0.55 (0.50 to 0.56)	0.016
Duodenum	1.61 (1.48 to 1.73)	0.52 (0.44 to 0.56)	0.016
Ileum	1.31 (1.22 to 1.48)	0.42 (0.41 to 0.46)	0.016
Colon	0.70 (0.63 to 0.74)	0.64 (0.59 to 0.66)	n.s.
Muscle	0.22 (0.19 to 0.25)	0.27 (0.21 to 0.31)	n.s.
Bone	0.82 (0.73 to 0.84)	0.70 (0.67 to 0.71)	n.s.
Bone marrow	1.53 (1.32 to 1.60)	2.14 (1.78 to 2.46)	n.s.
Skin	1.86 (1.66 to 2.04)	1.98 (1.59 to 2.47)	n.s.
Brain	0.01 (0.01 to 0.02)	0.02 (0.02 to 0.03)	n.s.
Tumor	1.46 (1.27 to 1.58)	1.72 (1.64 to 1.74)	n.s.
Kidney	46.43 (39.41 to 53.17)	185.91 (174.76 to 192.56)	0.016

Supplemental Table 3. % ID/g values from *ex-vivo* biodistribution of [⁸⁹Zr]Zr-DFO-*N*-suc-muS110 (*n* = 7) and [⁸⁹Zr]Zr-DFO-*N*-suc-AMG 110 (*n* = 6) in 4T1-tumor bearing immunocompetent mice 24 hours pi, after receiving 10 µg muS110 for 5 days. Differences between [⁸⁹Zr]Zr-DFO-*N*-suc-muS110 and [⁸⁹Zr]Zr-DFO-*N*-suc-AMG 110 and were tested with a Mann-Whitney U test. *P*-values ≤ 0.05 are shown.

Tissue	[⁸⁹ Zr]Zr-DFO- <i>N</i> -suc-muS110	[⁸⁹ Zr]Zr-DFO- <i>N</i> -suc-AMG 110	Difference (<i>P</i>)
Blood	0.33 (0.24 to 0.39)	0.31 (0.27 to 0.35)	n.s.
Heart	0.76 (0.71 to 0.95)	1.51 (1.36 to 1.54)	0.001
Lung	0.69 (0.66 to 0.83)	1.05 (0.87 to 1.33)	0.041
Liver	7.28 (5.56 to 8.60)	5.33 (4.65 to 6.40)	n.s.
Pancreas	0.73 (0.51 to 0.90)	0.88 (0.72 to 0.95)	n.s.
Thymus	2.02 (1.51 to 2.21)	1.83 (1.47 to 1.95)	n.s.
Spleen	4.15 (3.92 to 5.07)	1.53 (1.26 to 1.83)	0.001
Mesenteric LNs	1.89 (1.70 to 2.38)	1.87 (1.53 to 2.57)	n.s.
Stomach	0.85 (0.83 to 1.02)	1.02 (0.86 to 1.17)	n.s.
Duodenum	1.46 (1.34 to 1.72)	1.00 (0.87 to 1.15)	0.022
Ileum	1.24 (1.02 to 1.45)	1.62 (1.34 to 2.02)	n.s.
Colon	0.64 (0.61 to 0.75)	1.17 (0.82 to 1.37)	0.022
Muscle	0.18 (0.15 to 0.23)	0.22 (0.19 to 0.29)	n.s.
Bone	1.07 (0.95 to 1.23)	1.06 (0.99 to 1.21)	n.s.
Bone marrow	1.76 (1.40 to 2.07)	1.78 (1.58 to 1.99)	n.s.
Skin	0.92 (0.79 to 1.12)	1.60 (1.33 to 2.25)	0.014
Brain	0.02 (0.02 to 0.03)	0.02 (0.02 to 0.02)	n.s.
Tumor	1.38 (1.09 to 1.43)	1.89 (1.82 to 2.00)	0.001
Kidney	65.01 (52.31 to 67.98)	142.74 (137.14 to 157.45)	0.001

Supplemental Table 4. % ID/g values from ex-vivo biodistribution comparing 10 µg [⁸⁹Zr]Zr-DFO-*N*-suc-muS110 to immunocompetent mice (*n* = 7), immunodeficient mice (*n* = 4) and repeated muS110 administration mice (*n* = 7), 24 hours pi. Differences between either the immunodeficient mice and the repeated muS110 administration group versus the immunocompetent mice were tested with a Bonferroni-corrected Mann-Whitney U test. *P*-values ≤ 0.05 are shown.

Tissue	[⁸⁹ Zr]Zr-DFO- <i>N</i> -suc-muS110 (immunocompetent)	[⁸⁹ Zr]Zr-DFO- <i>N</i> -suc-muS110 (Immunodeficient)	Difference versus immunocompetent (<i>P</i>)	[⁸⁹ Zr]Zr-DFO- <i>N</i> -suc-muS110 (repeated dosing)	Difference versus immunocompetent (<i>P</i>)
Blood	0.27 (0.23 to 0.29)	0.13 (0.10 to 0.18)	n.s.	0.33 (0.24 to 0.39)	n.s.
Heart	1.10 (0.98 to 1.14)	0.71 (0.60 to 0.80)	0.024	0.76 (0.71 to 0.95)	n.s.
Lung	1.51 (1.21 to 1.67)	2.05 (1.32 to 2.71)	n.s.	0.69 (0.66 to 0.83)	0.002
Liver	9.18 (8.66 to 11.65)	5.79 (4.57 to 6.80)	0.024	7.28 (5.56 to 8.60)	n.s.
Pancreas	1.54 (1.19 to 1.67)	1.06 (0.96 to 1.10)	n.s.	0.73 (0.51 to 0.90)	0.004
Spleen	6.89 (6.74 to 8.29)	1.67 (1.24 to 2.07)	0.012	4.15 (3.92 to 5.07)	0.001
Mesenteric LNs	3.02 (2.80 to 4.16)	0.92 (0.83 to 0.97)	0.012	1.89 (1.70 to 2.38)	0.001
Stomach	1.74 (1.55 to 2.08)	1.61 (1.48 to 1.73)	0.024	0.85 (0.83 to 1.02)	0.004
Duodenum	3.66 (3.33 to 4.43)	1.31 (1.22 to 1.48)	0.012	1.46 (1.34 to 1.72)	0.001
Ileum	2.76 (2.45 to 3.63)	0.70 (0.63 to 0.74)	0.012	1.24 (1.02 to 1.45)	0.001
Colon	1.00 (0.87 to 1.24)	0.22 (0.19 to 0.25)	n.s.	0.64 (0.61 to 0.75)	0.022
Muscle	0.25 (0.19 to 0.27)	0.82 (0.73 to 0.84)	n.s.	0.18 (0.15 to 0.23)	n.s.
Bone	0.96 (0.95 to 1.10)	1.53 (1.32 to 1.60)	n.s.	1.07 (0.95 to 1.23)	n.s.
Bone marrow	3.41 (2.43 to 7.07)	1.86 (1.66 to 2.04)	0.012	1.76 (1.40 to 2.07)	0.014
Skin	0.93 (0.70 to 1.22)	0.01 (0.01 to 0.02)	0.024	0.92 (0.79 to 1.12)	n.s.
Brain	0.02 (0.02 to 0.03)	1.46 (1.27 to 1.58)	n.s.	0.02 (0.02 to 0.03)	n.s.
Tumor	1.96 (1.39 to 2.61)	46.43 (39.41 to 53.17)	n.s.	1.38 (1.09 to 1.43)	n.s.
Kidney	58.24 (57.07 to 67.49)	0.13 (0.10 to 0.18)	0.048	65.01 (52.31 to 67.98)	n.s.

Supplemental Table 5. % ID/g values from ex-vivo biodistribution comparing 10 µg [⁸⁹Zr]Zr-DFO-*N*-suc-AMG 110 to immunocompetent mice (*n* = 7), immunodeficient mice (*n* = 4) and repeated mus110 administration mice (*n* = 6), 24 hours pi. Differences between either the immunodeficient mice and the repeated muS110 administration group versus the immunocompetent mice were tested with a Bonferroni-corrected Mann-Whitney U test. *P*-values ≤ 0.05 are shown.

Tissue	[⁸⁹ Zr]Zr-DFO- <i>N</i> -suc-AMG 110 (immunocompetent)	[⁸⁹ Zr]Zr-DFO- <i>N</i> -suc-AMG 110 (Immunodeficient)	Difference versus immunocompetent (<i>P</i>)	[⁸⁹ Zr]Zr-DFO- <i>N</i> -suc-AMG 110 (repeated dosing)	Difference versus immunocompetent (<i>P</i>)
Blood	0.12 (0.11 to 0.13)	0.20 (0.09 to 0.24)	n.s.	0.31 (0.27 to 0.35)	0.002
Heart	1.13 (1.02 to 1.16)	1.03 (0.94 to 1.18)	n.s.	1.51 (1.36 to 1.54)	0.044
Lung	1.33 (0.76 to 1.93)	0.69 (0.60 to 1.50)	n.s.	1.05 (0.87 to 1.33)	n.s.
Liver	4.71 (4.58 to 5.69)	4.54 (4.06 to 4.65)	n.s.	5.33 (4.65 to 6.40)	n.s.
Pancreas	0.63 (0.56 to 0.74)	0.46 (0.45 to 0.57)	n.s.	0.88 (0.72 to 0.95)	n.s.
Spleen	2.33 (2.18 to 2.43)	1.51 (1.19 to 1.55)	0.017	1.53 (1.26 to 1.83)	0.017
Mesenteric LNs	1.90 (1.20 to 2.04)	1.52 (1.23 to 1.77)	n.s.	1.87 (1.53 to 2.57)	n.s.
Stomach	0.65 (0.57 to 0.80)	0.55 (0.50 to 0.56)	n.s.	1.02 (0.86 to 1.17)	n.s.
Duodenum	0.58 (0.49 to 0.66)	0.52 (0.44 to 0.56)	n.s.	1.00 (0.87 to 1.15)	0.028
Ileum	0.49 (0.40 to 0.59)	0.42 (0.41 to 0.46)	n.s.	1.62 (1.34 to 2.02)	0.002
Colon	0.61 (0.58 to 0.65)	0.64 (0.59 to 0.66)	n.s.	1.17 (0.82 to 1.37)	0.016
Muscle	0.22 (0.19 to 0.26)	0.27 (0.21 to 0.31)	n.s.	0.22 (0.19 to 0.29)	n.s.
Bone	0.60 (0.59 to 0.66)	0.70 (0.67 to 0.71)	n.s.	1.06 (0.99 to 1.21)	0.002
Bone marrow	4.17 (3.76 to 4.96)	2.14 (1.78 to 2.46)	n.s.	1.78 (1.58 to 1.99)	n.s.
Skin	0.88 (0.60 to 1.11)	1.98 (1.59 to 2.47)	0.005	1.60 (1.33 to 2.25)	0.022
Brain	0.02 (0.02 to 0.03)	0.02 (0.02 to 0.03)	n.s.	0.02 (0.02 to 0.02)	n.s.
Tumor	2.13 (1.92 to 2.21)	1.72 (1.64 to 1.74)	0.034	1.89 (1.82 to 2.00)	n.s.
Kidney	150.57 (143.26 to 182.62)	185.91 (174.76 to 192.56)	n.s.	142.74 (137.14 to 157.45)	n.s.