

Supplementary Material (Figures)

Targeted beta therapy of prostate cancer with ^{177}Lu -labelled Miltuximab[®] antibody against glypican-1 (GPC-1)

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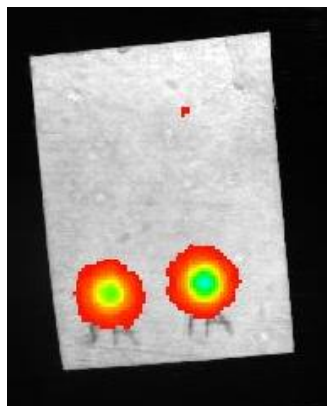
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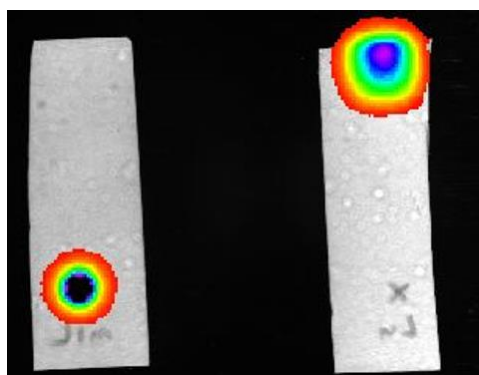
Australian Prostate Cancer Research Centre - Queensland, Institute of Health and Biomedical Innovation, School of Biomedical Sciences, Queensland University of Technology, Princess Alexandra Hospital, Translational Research Institute, 37 Kent Street, Woolloongabba 4102, Queensland, Australia.

a



[⁸⁹Zr]Zr-DFO-Miltuximab®

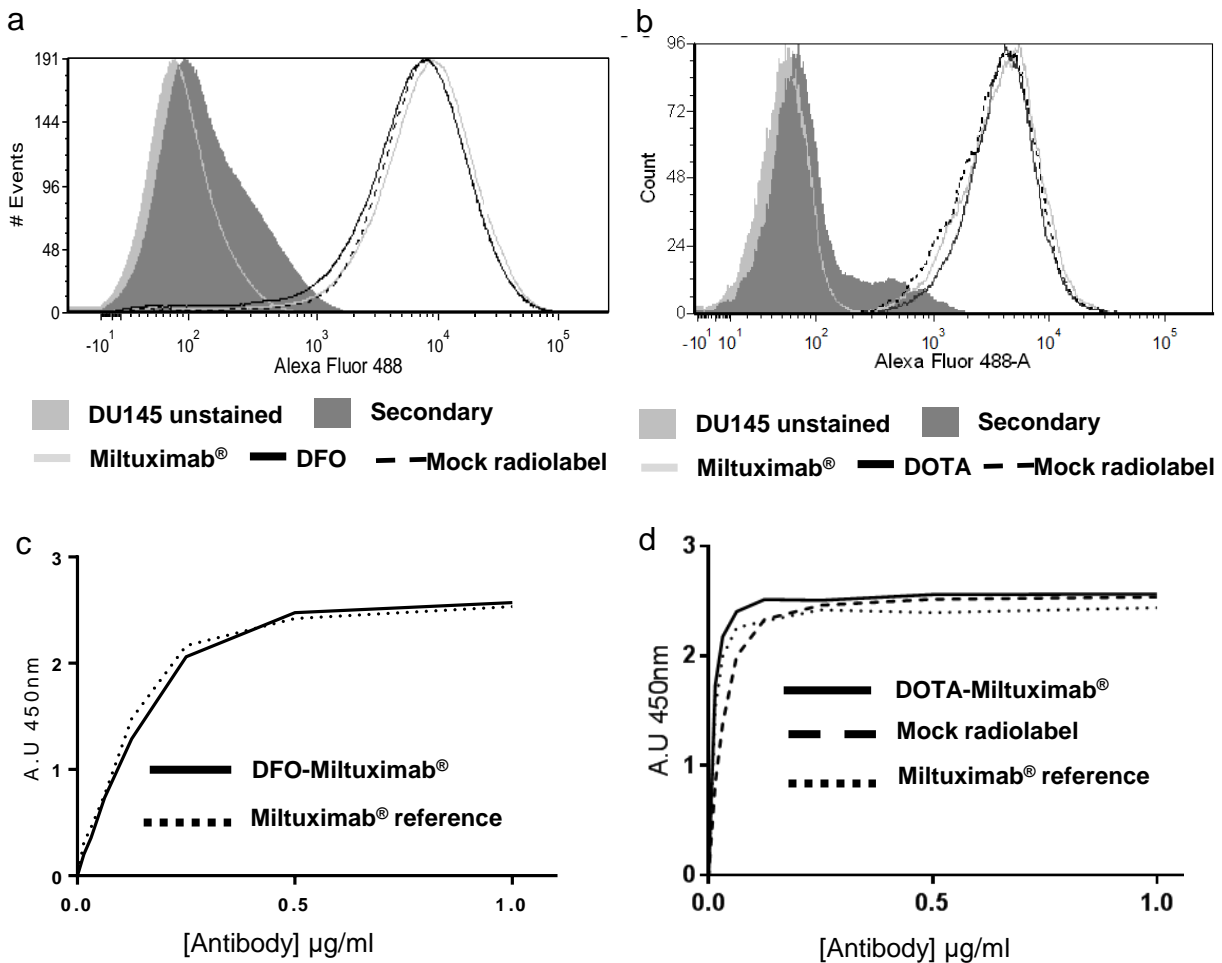
b



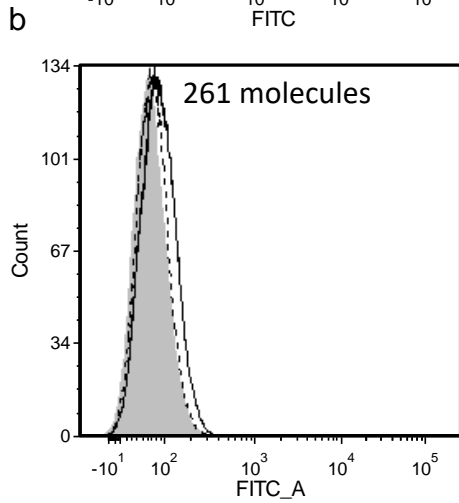
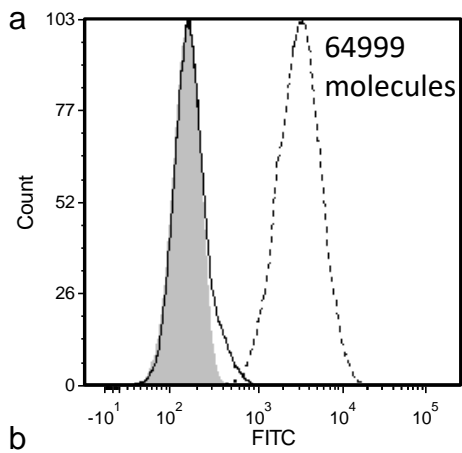
[¹⁷⁷Lu]Lu-DOTA-Miltuximab®

[¹⁷⁷Lu]Lu-DTPA

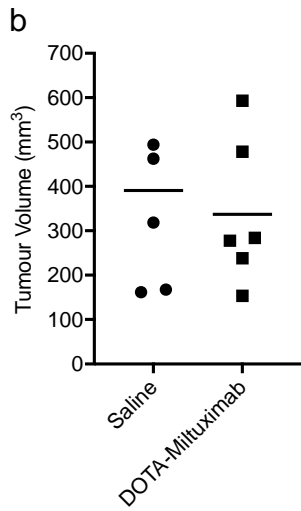
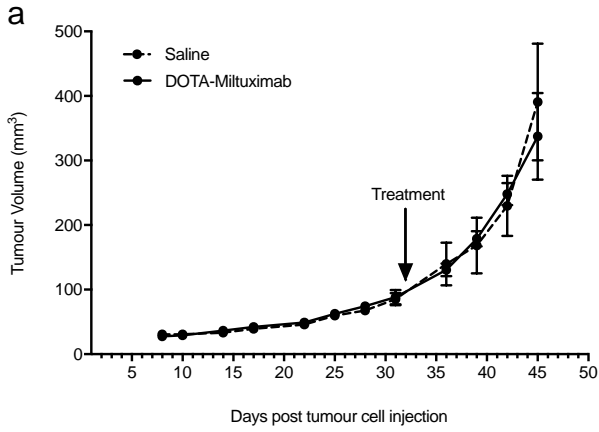
Supplementary Fig 1. a Radio-TLC of purified antibody conjugates showing successful radiolabelling of DFO-Miltuximab® with ⁸⁹Zirconium. **b** Radio-TLC of purified antibody conjugates showing successful radiolabelling of DOTA-Miltuximab® with ¹⁷⁷Lutetium.



Supplementary Fig. 2 Immunoreactivity of **a,c** DFO-Miltuximab[®] and **b,d** DOTA-Miltuximab[®] to cell surface GPC-1 on DU-145 cells measured via flow cytometry (**a,b**) and ELISA (**c,d**). For some tests, antibody was “mock” radiolabelled, i.e. treated in the same way as the radiolabelled antibody, but without the addition of radiolabel, to estimate the effect of radiolabelling conditions on immunoreactivity.



Supplementary Fig. 3 Quantitative flow cytometry analysis of DU-145 cells using MIL-38 binding for quantification. MIL-38 was used with the QIFIKIT antigen density analysis kit to determine GPC-1 density on the cell surface of **a.** prostate cancer cell line DU-145 and **b.** GPC-1 negative lymphoma cell line Raji.

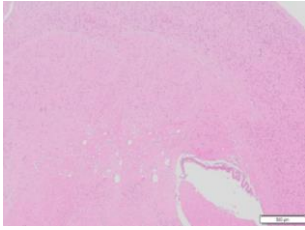


Supplementary Fig 4. No effect of DOTA-Miltuximab[®] alone on *in vivo* tumour growth.

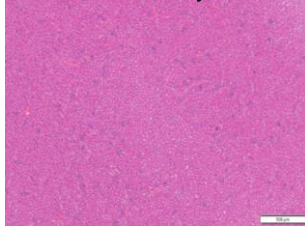
DU-145-RFP-Luc cells (5×10^6) in matrigel were injected subcutaneously into the right flank of BALB/c/nude mice. When tumours reached $\sim 100 \text{mm}^3$, mice were injected with saline (n=6) or DOTA-Miltuximab[®] (n=6) (80ug) intravenously. All mice were euthanised approximately 2 weeks thereafter. **a** Mean weekly mouse tumour volume. **d** Individual mouse tumour volume at endpoint. Data expressed as Mean \pm SEM and statistical analysis performed using an unpaired t test. *p<0.05

a

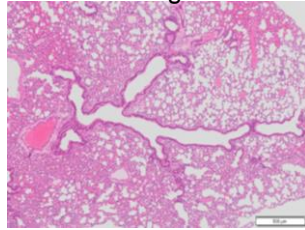
Brain



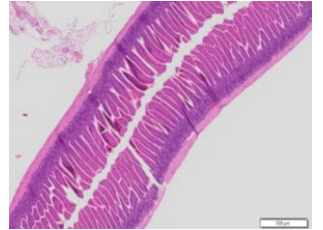
Kidney



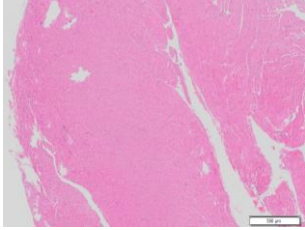
Lung



Small intestine



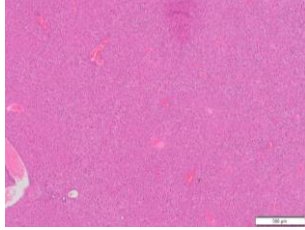
Heart



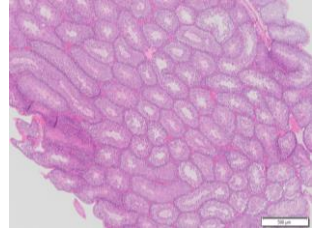
Spleen



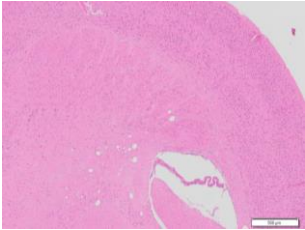
Liver



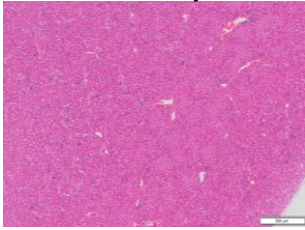
Testis

**b**

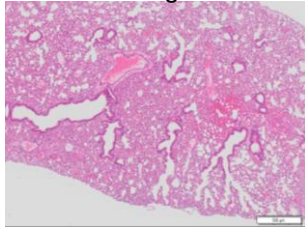
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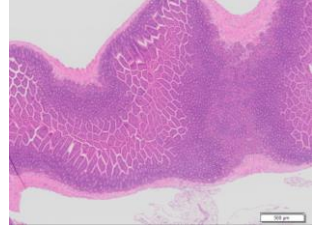
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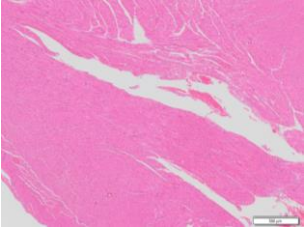
Lung



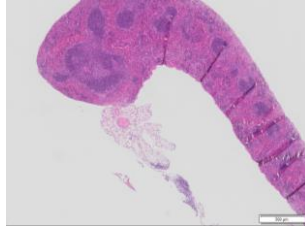
Small intestine



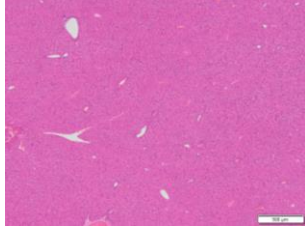
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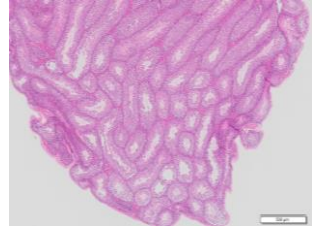
Spleen



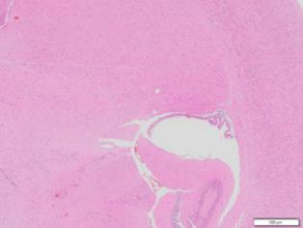
Liver



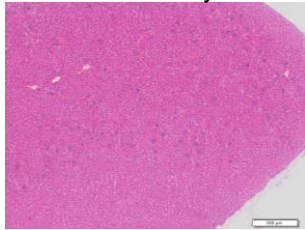
Testis

**c**

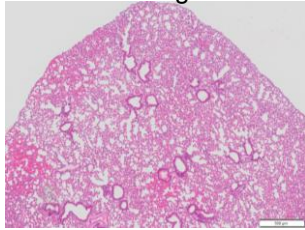
Brain



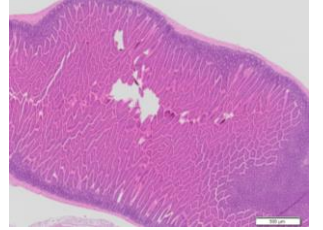
Kidney



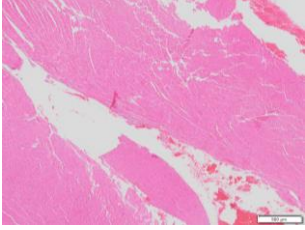
Lung



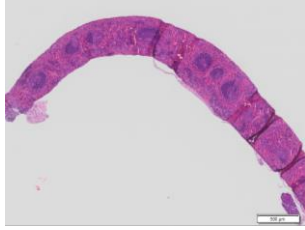
Small intestine



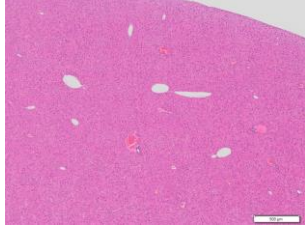
Heart



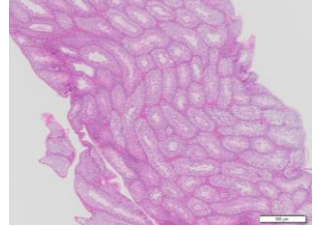
Spleen

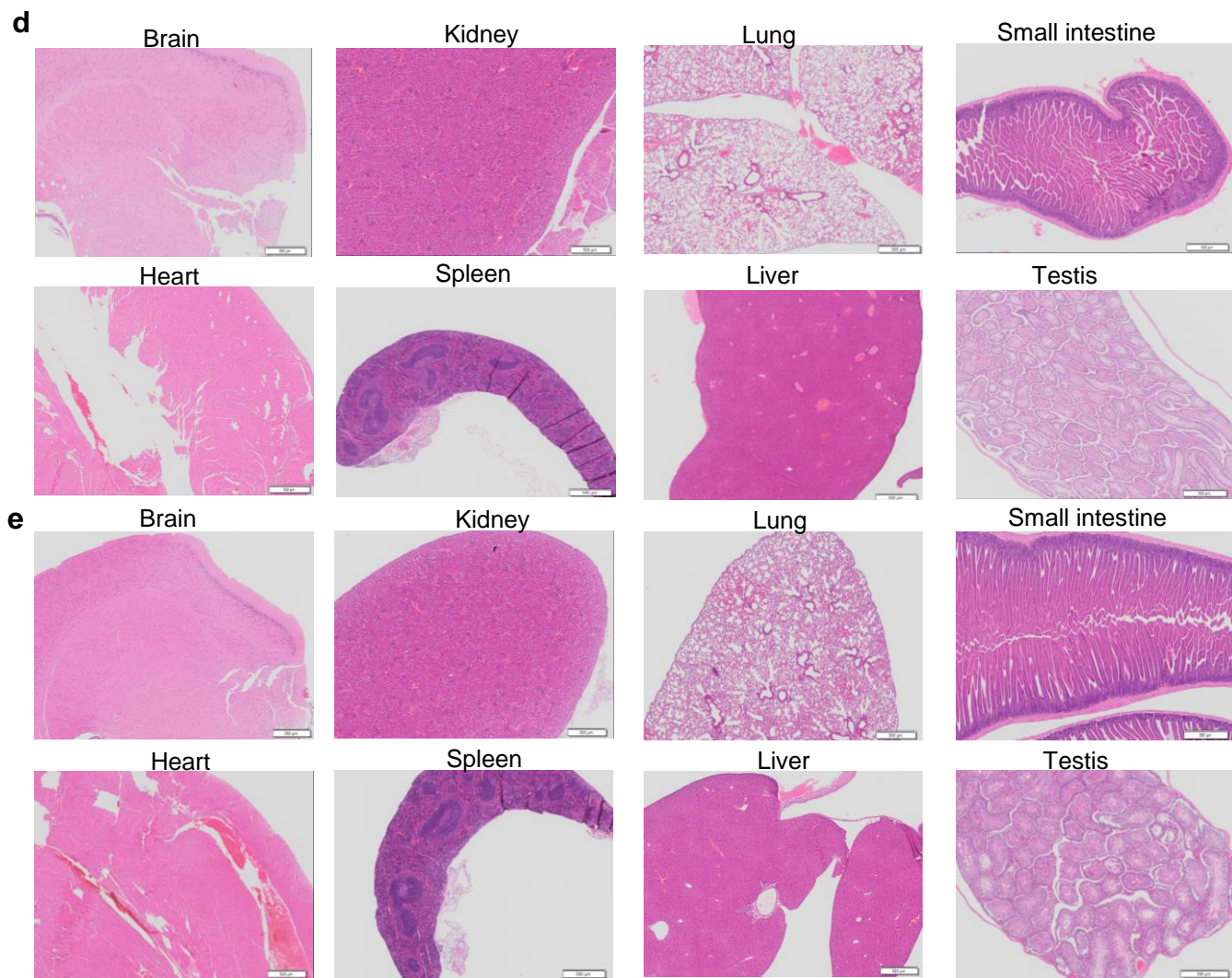


Liver

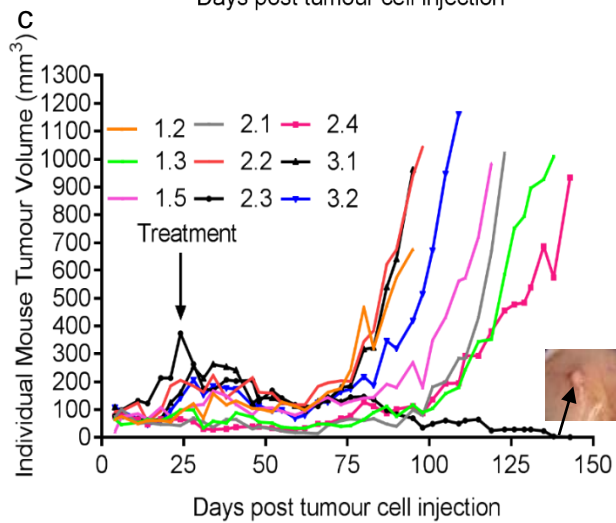
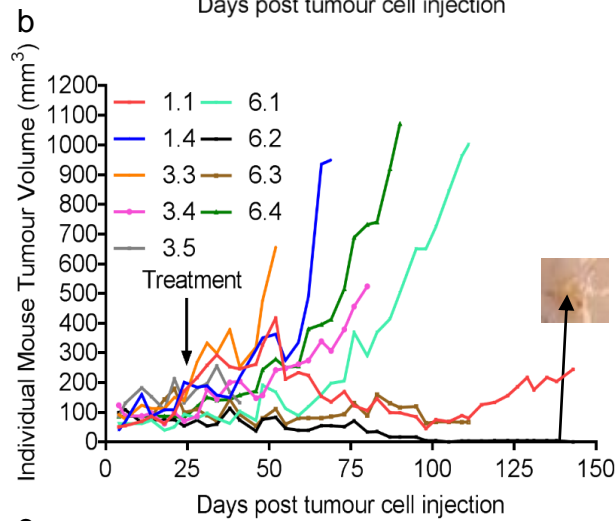
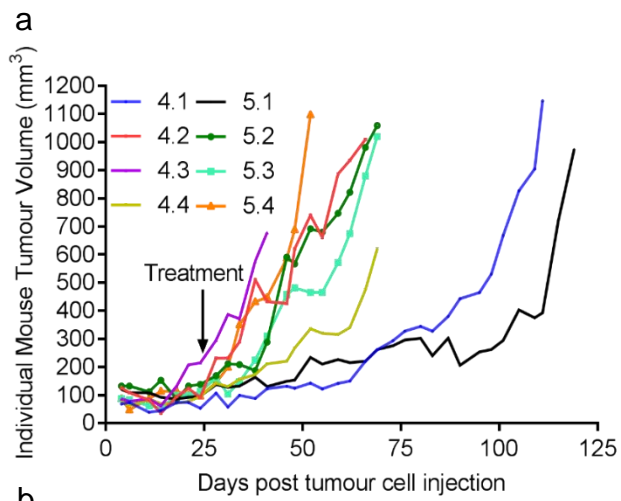


Testis





Supplementary Fig. 5 Representative H&E staining of the mouse brain, heart, lung, liver, kidney, spleen, small intestine and testis tissue **a** 3 days, **b** 5 days, **c** 7 days and **d** 27 days post 6MBq [^{177}Lu]Lu-DOTA-Miltuximab[®] treatment or **e** 27 days post DOTA-Miltuximab[®] treatment.



Supplementary Fig. 6 Individual weekly mouse tumour volumes of mice treated with **a** DOTA-Miltuximab[®] (n=8) **b** 3MBq [¹⁷⁷Lu]Lu-DOTA-Miltuximab[®] (n=9) or **c** 10MBq [¹⁷⁷Lu]Lu-DOTA-Miltuximab[®] (n=9) treated DU-145 xenograft mice.