

Supplementary Information

# Preclinical Evaluation of a Novel Monoclonal Antibody H6-11 for Prostate Cancer Imaging

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## Table of contents

Table S1. Immunostaining of tissue array of prostate tumor with H6-11 .....	S2
Table S2. Summary of monoclonal antibody H6-11 reactivity with human prostate tumor tissues .....	S5
Figure S1. SDS-PAGE and autoradiography of <sup>125</sup> I-H6-11.....	S6
Figure S2. <i>In vitro</i> autoradiography of <sup>125</sup> I-H6-11 with tumor tissue.....	S7
Figure S3. Identification of H6-11 antigen epitopes via radioactivity binding measurements with human prostate cancer cell line PC-3.....	S8
Figure S4. TLC of Zr-89-H6-11 and SDS-PAGE autoradiography. ....	S9
Figure S5. Immunoreactivity of unmodified and p-SCN-Bz-DFO modified H6-11 <b>SError!</b> <b>Bookmark not defined.</b>	
Figure S6. Amino acid sequence for the CDRs region of H6-11 hybridoma cell line. ....	S11

**Table S1. Immunostaining of tissue array of prostate tumor with H6-11**

No	Sex	Age	Organ	Pathology diagnosis	Grade	Stage	TNM	Type	Staining Scores
1	M	72	Prostate	Adenocarcinoma	1	II	T2N0M0	Malignant	+
2	M	64	Prostate	Adenocarcinoma	1	I	T1N0M0	Malignant	+++
3	M	60	Prostate	Adenocarcinoma	1	IV	T4N1M1c	Malignant	++
4	M	66	Prostate	Adenocarcinoma	1	IV	T3N1M1c	Malignant	++
5	M	65	Prostate	Adenocarcinoma	1	II	T2N0M0	Malignant	++
6	M	75	Prostate	Adenocarcinoma	1	IV	T2N1M1c	Malignant	++
7	M	71	Prostate	Adenocarcinoma	2-3	II	T2N0M0	Malignant	+++
8	M	78	Prostate	Adenocarcinoma	2	IV	T3N2M1	Malignant	+++
9	M	74	Prostate	Adenocarcinoma	2	IV	T4N1M1c	Malignant	+
10	M	69	Prostate	Adenocarcinoma	2	III	T3N0M0	Malignant	++
11	M	75	Prostate	Adenocarcinoma	2	IV	T4N1M1	Malignant	++
12	M	69	Prostate	Adenocarcinoma	2	II	T2N0M0	Malignant	+++
13	M	73	Prostate	Adenocarcinoma	2	IV	T3N1M1c	Malignant	+++
14	M	56	Prostate	Adenocarcinoma	2-3	II	T2N0M0	Malignant	+++
15	M	73	Prostate	Adenocarcinoma	2	II	T2N0M0	Malignant	+++
16	M	70	Prostate	Adenocarcinoma	2	III	T3N0M0	Malignant	++
17	M	20	Prostate	Adenocarcinoma	1-2	III	T3N0M0	Malignant	++
18	M	61	Prostate	Adenocarcinoma	1-2	III	T3N1M0	Malignant	++
19	M	73	Prostate	Adenocarcinoma	2	III	T3N0M0	Malignant	+++

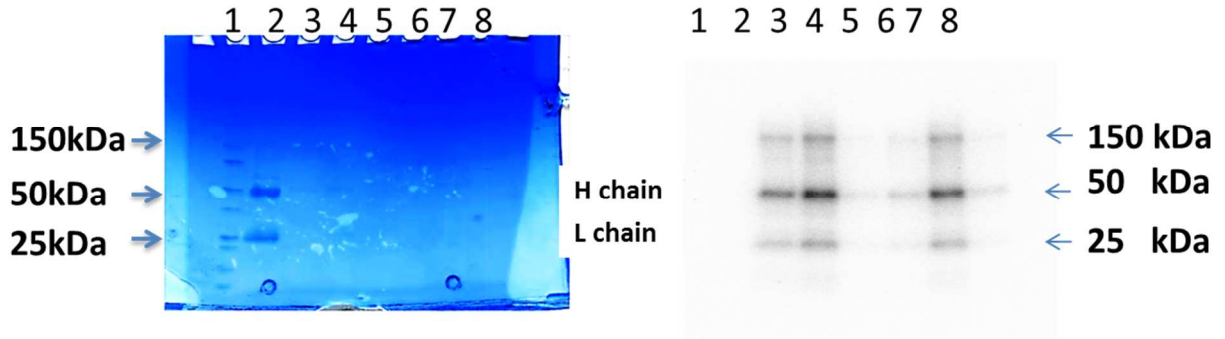
							0		
20	M	82	Prostate	Adenocarcinoma	2	II	T2N0M0	Malignant	++
21	M	75	Prostate	Adenocarcinoma	3	IV	T4N1M1	Malignant	+
22	M	78	Prostate	Adenocarcinoma	3	IV	T4N1M1b	Malignant	+-
23	M	60	Prostate	Adenocarcinoma	2	IV	T3N1M1b	Malignant	+
24	M	73	Prostate	Adenocarcinoma	2	IV	T3N1M1b	Malignant	+++
25	M	62	Prostate	Adenocarcinoma	2	IV	T3N1M1b	Malignant	+
26	M	51	Prostate	Adenocarcinoma	2	II	T2N0M0	Malignant	++
27	M	62	Prostate	Adenocarcinoma	3	II	T2N0M0	Malignant	+++
28	M	60	Prostate	Adenocarcinoma	2	IV	T3N1M0	Malignant	+++
29	M	68	Prostate	Adenocarcinoma	2	II	T2N0M0	Malignant	++
30	M	64	Prostate	Adenocarcinoma	3	IV	T3N0M1b	Malignant	+
31	M	66	Prostate	Adenocarcinoma	3	II	T2N0M0	Malignant	++
32	M	87	Prostate	Adenocarcinoma	3	II	T2N0M0	Malignant	+
33	M	81	Prostate	Adenocarcinoma	3	III	T3aNO M0	Malignant	+++
34	M	80	Prostate	Adenocarcinoma	3	IV	T4N1M1c	Malignant	++
35	M	76	Prostate	Adenocarcinoma	3	IV	T3N1M1b	Malignant	+-
36	M	73	Prostate	Adenocarcinoma	3	IV	T4N1M1c	Malignant	+
37	M	63	Prostate	Adenocarcinoma	3	IV	T2N1M1b	Malignant	++
38	M	67	Prostate	Adenocarcinoma	3	II	T2N0M0	Malignant	++
39	M	65	Prostate	Adenocarcinoma	3	IV	T2N1M1	Malignant	++
40	M	64	Prostate	Adenocarcinoma	3	II	T2N0M0	Malignant	++
41	M	35	Prostate	Normal prostate tissue	-	-	-	Normal*	+-
42	M	43	Prostate	Normal prostate tissue	-	-	-	Normal*	+-

43	M	19	Prostate	Normal prostate tissue	-	-	-	Normal*	+-
44	M	46	Prostate	Normal prostate tissue	-	-	-	Normal*	+-
45	M	40	Prostate	Normal prostate tissue	-	-	-	Normal*	+-
46	M	28	Prostate	Normal prostate tissue	-	-	-	Normal*	+-
47	M	33	Prostate	Normal prostate tissue	-	-	-	Normal*	+-
48	M	37	Prostate	Normal prostate tissue	-	-	-	Normal*	+-
-	F	55	liver	Hepatocellular liver cancer (tissue marker)	3		T3N0M0	Malignant	+++

\*Epithelia from normal prostate glands are weak positive staining with mAb H6-11, but not from fibromuscular stroma.

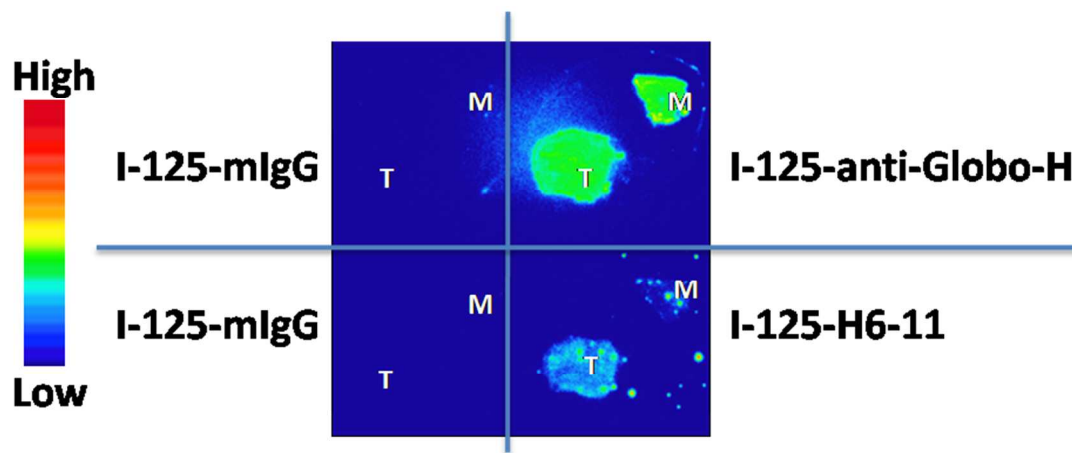
**Table S2. Summary of monoclonal antibody H6-11 reactivity with human prostate tumor tissues**

Scoring grade	-	±	+	++	+++	Total
Prostate tumor Case (%)	0 (0%)	2 (5%)	8 (20%)	18 (45%)	12 (30%)	40 (100%)
			38 (95%)			



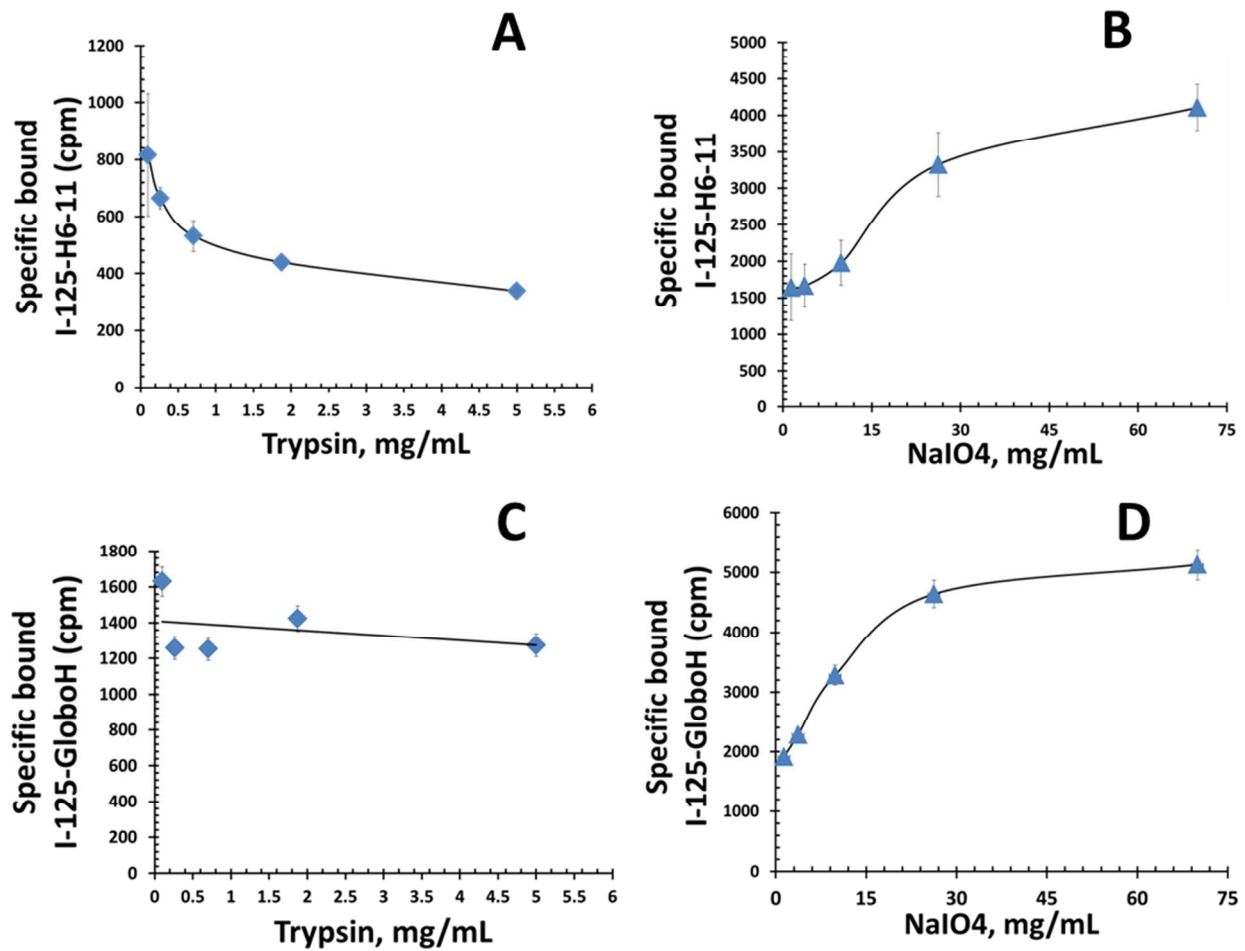
**Figure S1. SDS-PAGE (left) and autoradiography (right) of  $^{125}\text{I}$ -H6-11.**

Lane1: standard protein marker; Lane 2: unlabeled IgG; Lanes 3-5:  $^{125}\text{I}$  labeled H6-11 sample1 elution 1-3; Lane 6-8:  $^{125}\text{I}$  labeled H6-11 sample2 elution 1-3. Autoradiography from  $^{125}\text{I}$  labeled H6-11, two samples, both showed full length IgG (150 KDa), and heavy chain (50 KDa) and light chain (25 KDa) bands indicating successful iodination.



**Figure S2. *In vitro* autoradiography of  $^{125}\text{I}$ -H6-11 with tumor tissue.**

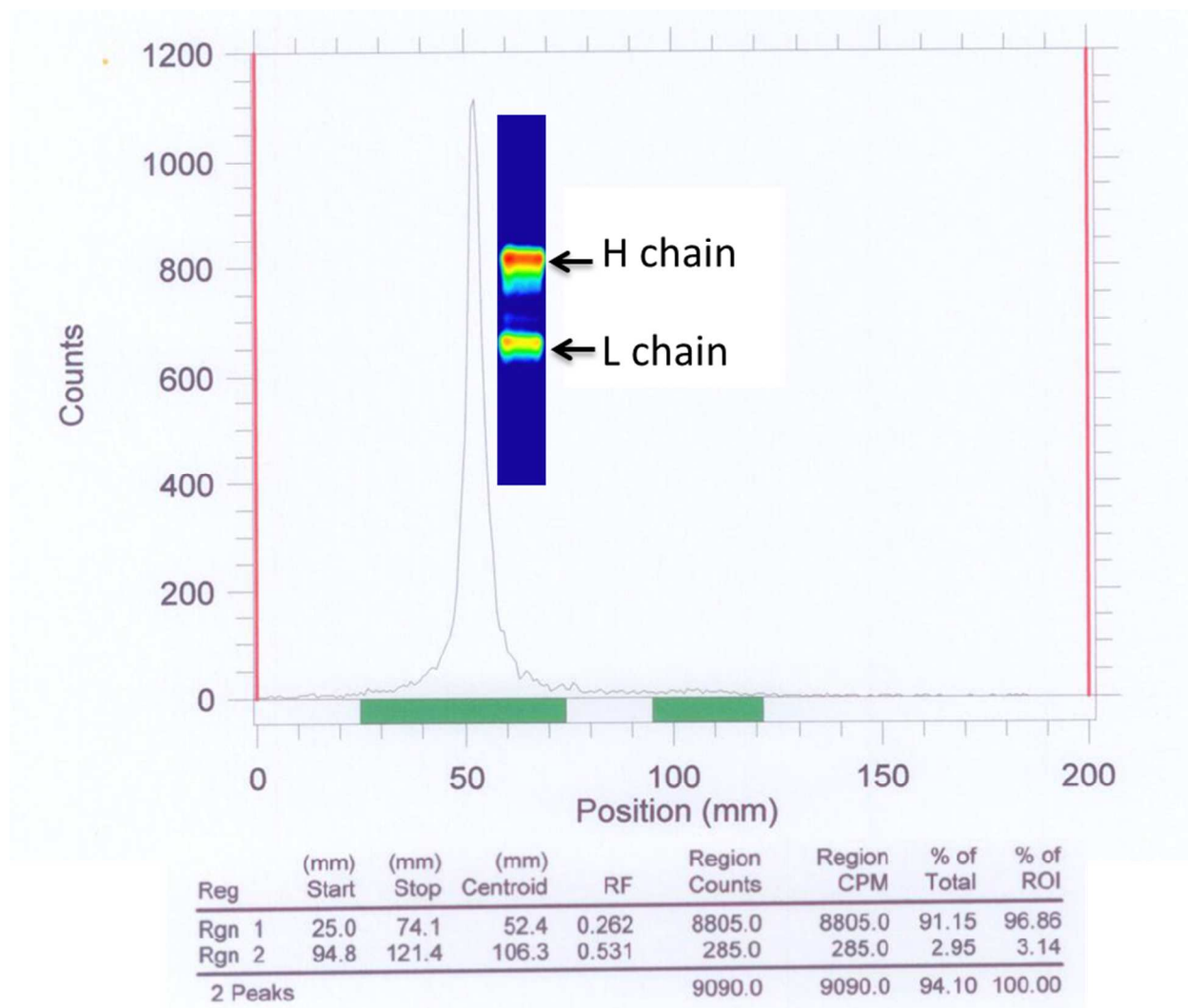
1  $\mu\text{Ci}/\text{mL}$   $^{125}\text{I}$  labeled mouse IgG, H6-11 and anti-Globo-H antibodies were incubated with tissue slides collected from PC-3 implanted tumor (T) or muscle (M). After incubation and washing the slides three times with PBS-TW-20, the slides were directly placed in the FLA-7000 imager to process for autoradiography.  $^{125}\text{I}$ -mIgG has no any detectable interaction with either tumor or muscle tissues.  $^{125}\text{I}$ -Globo-H reacted with both the tumor and muscle tissues evenly.  $^{125}\text{I}$ -H6-11 reacted strongly to tumor collected from PC-3 xenograft mouse.



**Figure S3. Identification of H6-11 antigen epitopes via radioactivity binding measurements with human cancer cells.**

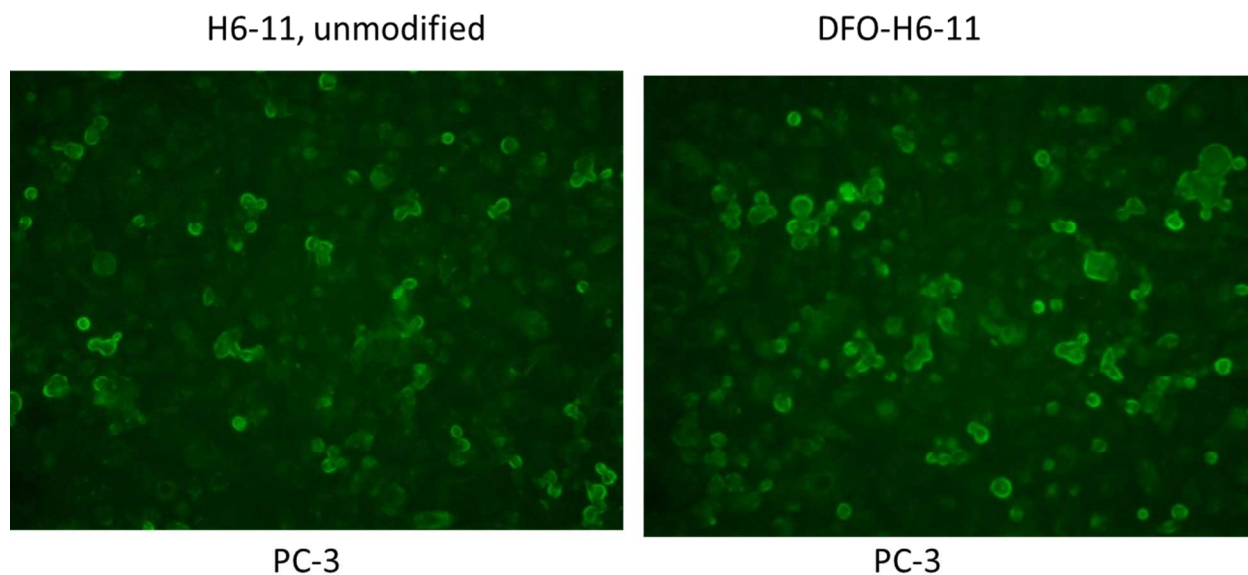
**Panel A:** For PC-3 cells, increasing the concentration of trypsin decreased the  $^{125}\text{I}$ -H6-11 binding. **Panel B:** For PC-3 cells, increasing the concentration of periodate increased the  $^{125}\text{I}$ -H6-11 binding. **Panel C:** For MCF-7, increasing the concentration of trypsin barely changed the  $^{125}\text{I}$ -anti-Globo-H binding. **Panel D:** For MCF-7, increasing the concentration of periodate increased the  $^{125}\text{I}$ -anti-Globo-H binding.





**Figure S4. TLC of  $^{89}\text{Zr}$ -H6-11 and SDS-PAGE autoradiography.**

The  $^{89}\text{Zr}$  labeled H6-11 after purification on the Pierce Zeba desalting column was quantified by TLC. The major peak (96.86%) was  $^{89}\text{Zr}$ -H6-11 at position 50 mm (baseline), while the free  $^{89}\text{Zr}$  peak (3.14%) is at 100 mm position. The inserted image is from the SDS-PAGE and autoradiography of the major peak. Both the heavy and light chains are radiolabeled.



**Figure S5. Immunofluorescence of unmodified H6-11 and *p*-SCN-Bz-DFO modified H6-11.**

PC-3 cell was immunofluorescence stained with 5  $\mu\text{g}/\text{mL}$  H6-11 (left) and *p*-SCN-Bz-DFO modified H6-11 (abridge: DFO-H6-11) (right). No significant immunoreactivity changes were observed between *p*-SCN-Bz-DFO modified H6-11 and unmodified H6-11.

**Heavy chain: Amino acids sequence (135 AA)**

**Leader sequence-FR1-CDR1-FR2-CDR2-FR3-CDR3-FR4**  
**MAVWVWTLFLMAAAQSIQAQIQLVQSGPELKKPGETVKISCKASGYTF**  
**TDYSMHVWKQAPGKGLKWMGWINTETGEPTYADDFKGRFAFSLETS**  
**STAYLQINNLIKNETATYFCARSRRYDDYWGQGTTLVSS**

**Light chain: Amino acids sequence (133 AA)**

**Leader sequence-FR1-CDR1-FR2-CDR2-FR3-CDR3-FR4**  
**MDSQAQVLMLLLLWVSGTCGDIVMSQSPSSLAVSVGEKVTMSCKSSQSL**  
**LYSSNQKNYLAWYQQKPGQSPKLLIYWASTRESGVPDRFTGSGSGTDFTL**  
**TISSVKAEDLAVYYCQQYYSYPYTFGGGTKLEIK**

**Figure S6. Amino acid sequence for the CDRs region of H6-11 hybridoma cell line.**

The amino acid sequences are translated from cDNA sequencings. The predicted binding sites (including heavy chain and light chain) from CDRs were blue coded.