

## **Yeast display biopanning identifies human antibodies targeting**

### **glioblastoma stem-like cells**

Michael Zorniak<sup>1,2</sup>, Paul A. Clark<sup>2</sup>, Benjamin J. Umlauf<sup>3</sup>, Yongku Cho<sup>3</sup>, Eric V. Shusta\*<sup>3,4</sup>, & John S. Kuo\*<sup>1,2,4</sup>

<sup>1</sup>Neuroscience Training Program, <sup>2</sup>Department of Neurological Surgery, <sup>3</sup>Department of Chemical and Biological Engineering, <sup>4</sup>Carbone Cancer Center, University of Wisconsin School of Medicine and Public Health, Madison, WI, 53792-8660, USA. Correspondence and requests for materials should be addressed to E.V.S. (eshusta@wisc.edu) and J.S.K. (email: kuo@neurosurgery.wisc.edu).

### **Supplementary information**

**Supplementary Figure 1. Descriptive statistics of biopanning screening.** (a) Positive screening statistics against 22 GSC. (b) Negative screening statistics against hNSC, NHA, and 22T co-culture. (c) Re-positive screening statistics against 22 GSC. (d) Pie charts show increase in antibody clone diversity and selectivity after negative screening. scFv-9.1 is non-specific, and VH-9.7 is GSC-selective. Notably, by round 9 nearly half of the discovered clones are non-specific scFv-9.1. Negative screening rounds S.7, S.8, and S.9 show a steady rise in non-specific scFv-9.1 from 4.88% to 24.4 %, but still less than 45.8% observed in round 9 alone. (e) Antibody-dependent enrichment was determined by incubating yeast binders from round nine with GSC in the presence or absence of antibody expression-inducing media (galactose) or non-inducing media (glucose). Scale bar, 100  $\mu$ m.

**Supplementary Figure 2. Unique clones discovered by plasmid isolation, PCR and BstNI restriction enzyme digest revealing different patterns on DNA gel electrophoresis.** 62 unique scFv or VH clones were isolated out of 598 total yeast screened. Clones were isolated from round 6, 9, S.6, S.7, S.8, S.9, and RS.6. MW = Molecular Weight.

**Supplementary Figure 3. Unique yeast scFv and VH screened for GSC-selectivity.** (a) Examples of qualitative scoring of yeast scFv binding with various intensity, from 0 (no observed binding) to 3 (significant yeast binding). Yeast were seeded in 96 well plates, incubated for 2 hours at 4 °C and washed according to the biopanning protocol<sup>1</sup>. Scale bar, 50  $\mu$ m. (b) Heat map of 62 yeast scFv or VH clones binding 12 different cells lines: normal human astrocytes (NHA); human neural stem cells (hNSC); 12.1, 22, 33, 44, & 99 GSC; and 22, 33, 99, U251, & U87 serum cultured tumor lines. Negative control: anti-lysozyme D1.3. Positive control: anti-neural cell adhesion molecule (NCAM) scFv-J.

**Supplementary Figure 4. Monomeric affinity (K<sub>d</sub>) of VH-9.7 on 33 GSCs.** (a) VH-9.7 is produced largely as monomer. Ni-NTA purified VH-9.7 was analyzed by size exclusion chromatography. Elution fractions were collected and analyzed via western blotting for VH-9.7 against the c-Myc epitope tag. The major VH-9.7 peak (retention time = 13.1 min) corresponded to a size of 15.3 kDa, and the expected monomeric size of VH-9.7 is ~15 kDa. Arrowheads represent the elution times for molecular size standards. 97% of the VH-9.7 detected by western blotting was monomeric. (b) Affinity titration of monomeric VH-9.7 on 33 GSCs. Monomeric VH-9.7 was titrated against 33 GSCs and binding assessed by flow cytometry. Fitting to a standard biomolecular binding equilibrium model<sup>2</sup>, these data yielded a K<sub>d</sub> of 74.30 ± 9.85 nM. The results of four independent titrations are plotted as mean ± SEM. (c) Full-length immunoblot of Figure 3a elution fractions for VH-9.7 and scFv-4-4-20. Dashed boxes indicate location of image cropping.

**Supplementary Figure 5. VH-9.7 labels human cells in formalin fixed orthotopic mouse 44 GSC-derived orthotopic tumor xenografts.** (a) Dissociated brains from mouse xenografts were labeled with Ms-anti-c-Myc:AlexaFluor 647 (66 nM) and Gt-anti-Ms-IgG:AlexaFluor 488 (1:100) to serve as a negative control. (b) Addition of Ms-anti-human nuclei (HuNu) (1:100) and VH-9.7 (125 nM) to three 44 GSC-derived xenografts indicating human-specific labeling of VH-9.7 in quadrant 2 (Q2).

**Supplementary Figure 6.** Purified near-infrared VH-9.7 localizes to human 22 GSC orthotopic xenografts. Representative near-infrared fluorescent images captured from ex vivo coronal brain sections of orthotopic 22 GSC-derived tumors in mice. Tumor area was identified using H&E counterstaining. 22 GSC-derived xenografts have low expression of EGFR and the anti-EGFR antibody cetuximab was used as a control.

## **VH-9.7 IgBLAST (NCBI)**

IGHV1-46\*01, IGHD3-10\*01, & IGHJ4\*02

### **Plasmid Sequences**

Plasmids are available upon request as subject to a standard material transfer agreement.

### **Supplementary references**

- 1 Wang, X. X., Cho, Y. K. & Shusta, E. V. Mining a yeast library for brain endothelial cell-binding antibodies. *Nature methods* **4**, 143-145, doi:10.1038/nmeth993 (2007).
- 2 Tillotson, B. J., Cho, Y. K. & Shusta, E. V. Cells and cell lysates: a direct approach for engineering antibodies against membrane proteins using yeast surface display. *Methods* **60**, 27-37, doi:10.1016/j.ymeth.2012.03.010 (2013).

# Supplementary Figure 1

**a** Positive Screening Rounds Against 22 GSC

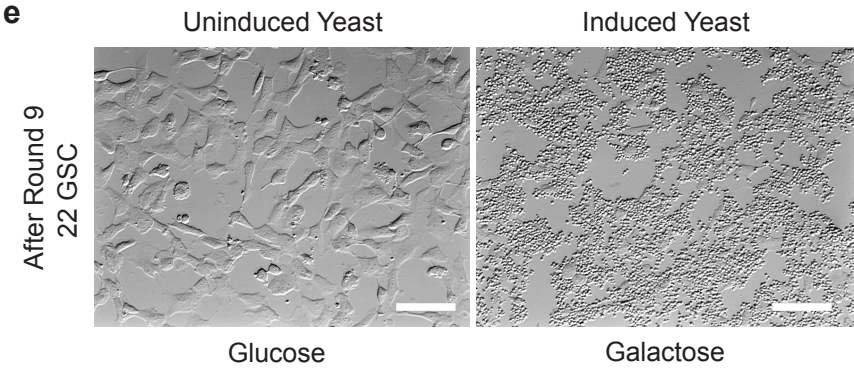
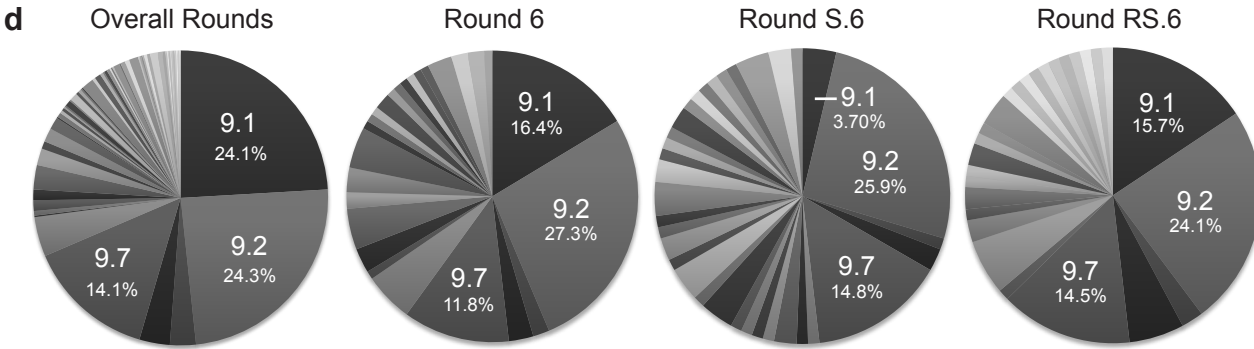
	1	2	3	4	5	6	7	8	9
Total no. yeast applied	$5 \times 10^9$	$2 \times 10^8$	$5 \times 10^7$	$5 \times 10^7$	$5 \times 10^7$	$5 \times 10^7$	$5 \times 10^7$	$5 \times 10^7$	$5 \times 10^7$
Yeast density (yeast/cm <sup>2</sup> )	$4 \times 10^7$	$4 \times 10^6$	$2 \times 10^6$	$2 \times 10^6$	$2 \times 10^6$	$2 \times 10^6$	$2 \times 10^6$	$2 \times 10^6$	$2 \times 10^6$
Surface area (cm <sup>2</sup> )	126	50.2	25.1	25.1	25.1	25.1	25.1	25.1	25.1
No. recovered yeast	ND	$2.58 \times 10^6$	$4.28 \times 10^5$	$9.05 \times 10^5$	ND	$2.58 \times 10^6$	$7.8 \times 10^6$	$5.86 \times 10^6$	$6.26 \times 10^6$
Recovery percentage	ND	1.3	0.86	1.8	ND	5.2	16	12	13
No. different binders/analyzed yeast	ND	ND	ND	ND	ND	26/110	ND	ND	15/201

**b** Negative Screening Rounds Against Co-Culture

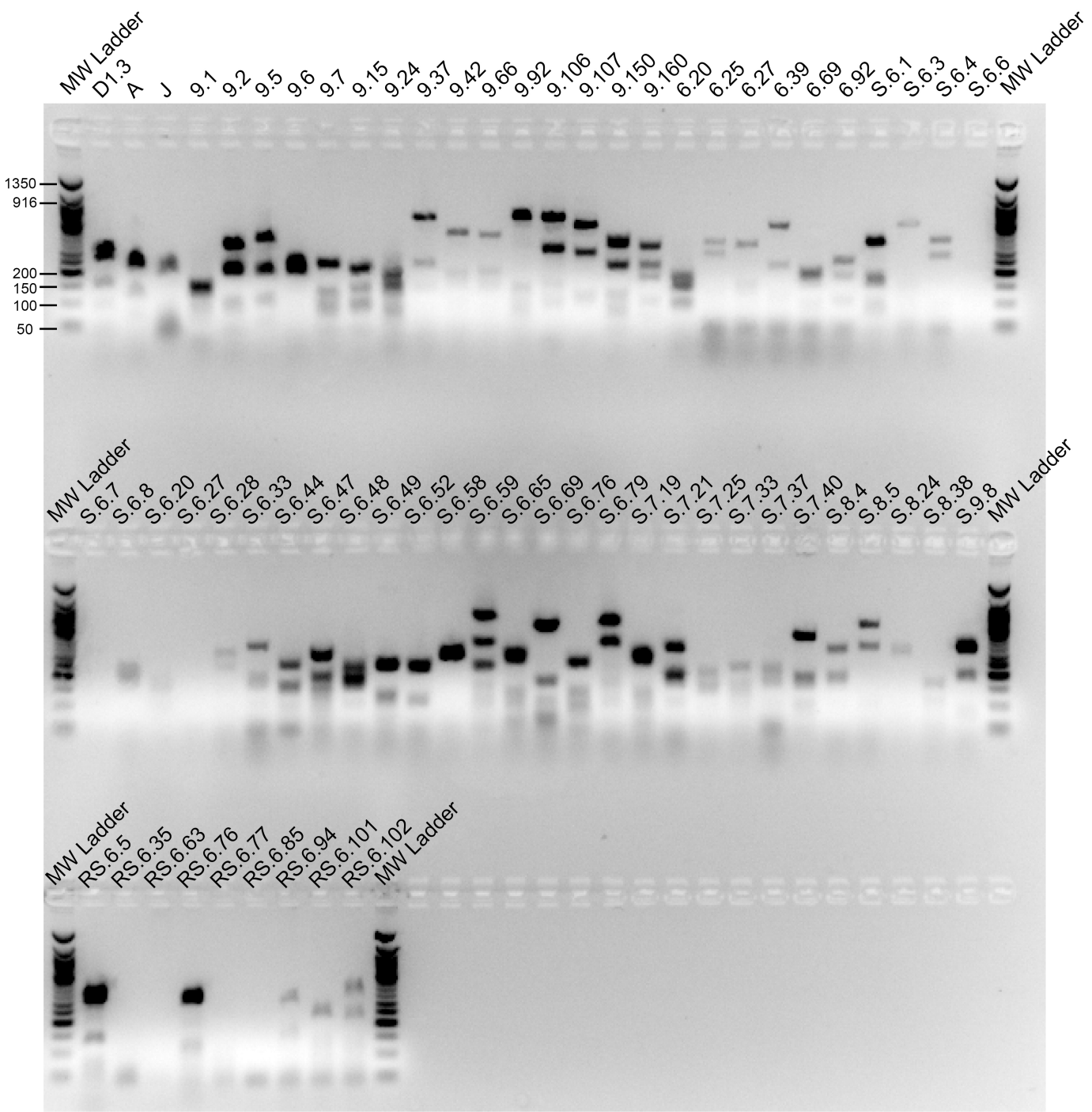
	S.6	S.7	S.8	S.9
Total no. yeast applied	$5 \times 10^6$	$5 \times 10^6$	$5 \times 10^6$	$5 \times 10^6$
Yeast density (yeast/cm <sup>2</sup> )	$2 \times 10^5$	$2 \times 10^5$	$2 \times 10^5$	$2 \times 10^5$
Surface area (cm <sup>2</sup> )	25.1	25.1	25.1	25.1
No. depleted yeast	$4.77 \times 10^6$	$4.62 \times 10^6$	$4.70 \times 10^6$	$4.66 \times 10^6$
Depletion percentage	95	93	94	93
No. different binders/analyzed yeast	34/81	19/41	12/41	11/41

**c** Re-Positive Screening Against 22 GSC

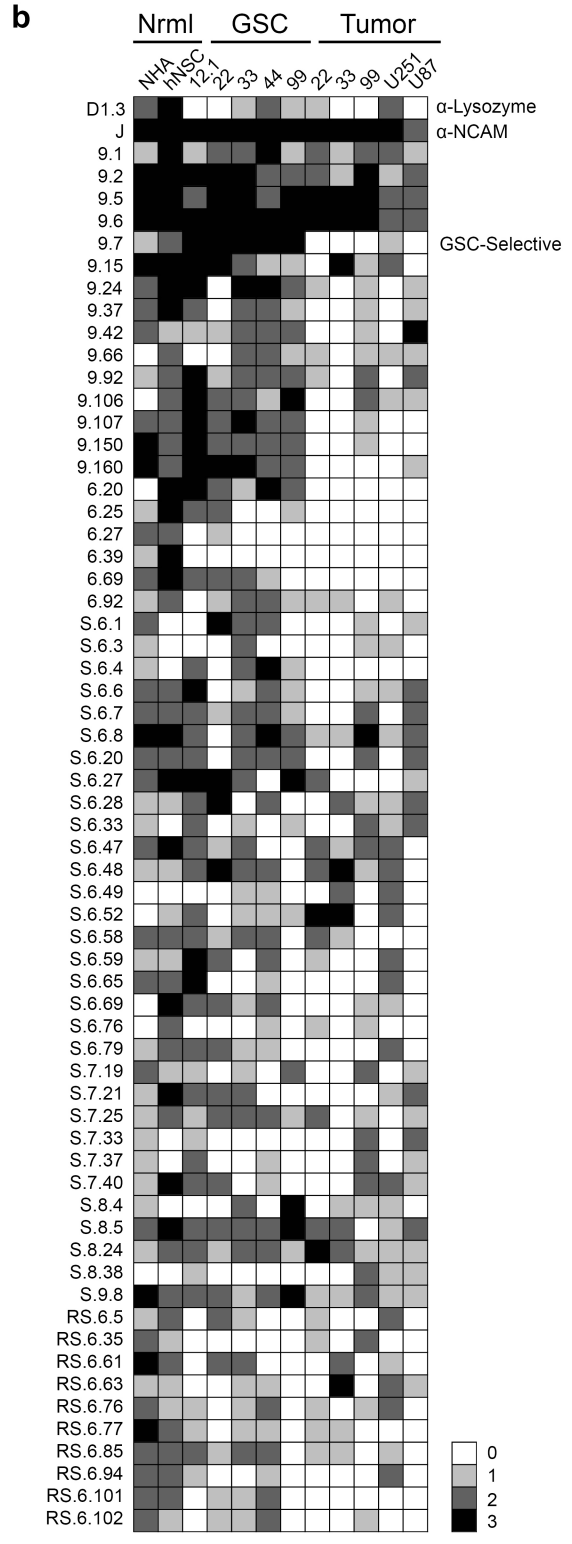
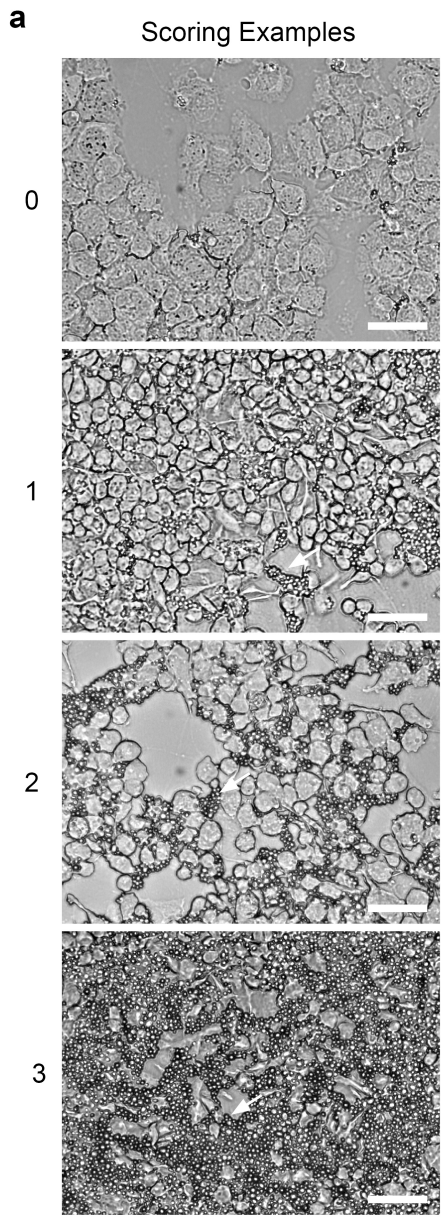
	RS.6
Total no. yeast applied	$5 \times 10^7$
Yeast density (yeast/cm <sup>2</sup> )	$2 \times 10^6$
Surface area (cm <sup>2</sup> )	25.1
No. recovered yeast	$2.62 \times 10^7$
Recovery percentage	52
No. different binders/analyzed yeast	27/83



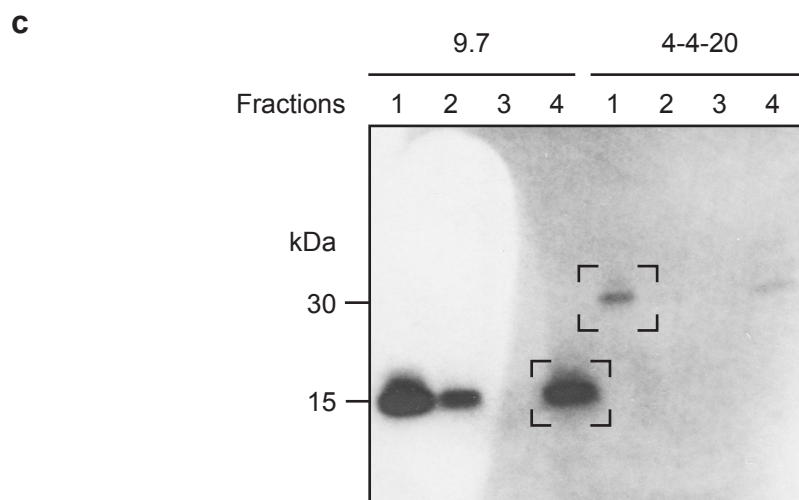
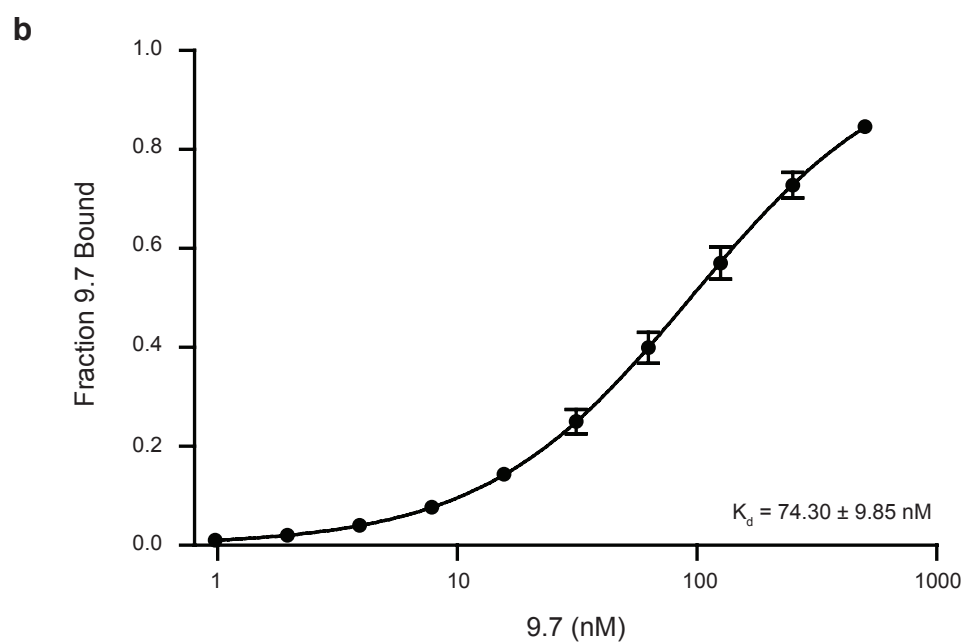
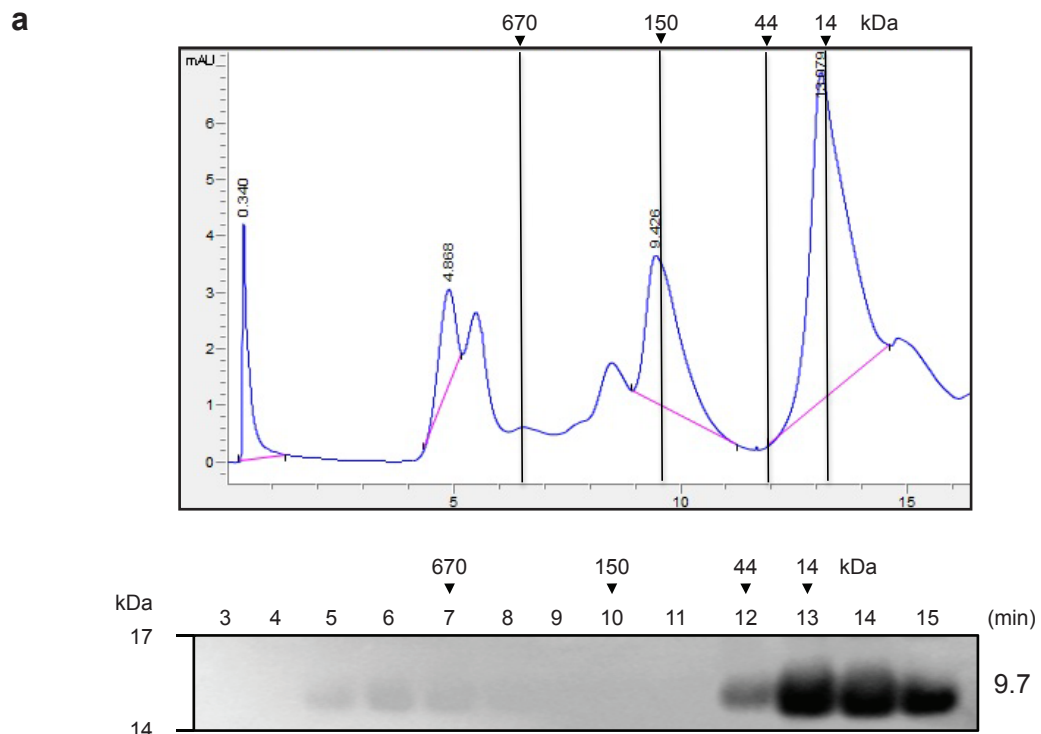
Supplementary Figure 2



**Supplementary Figure 3**

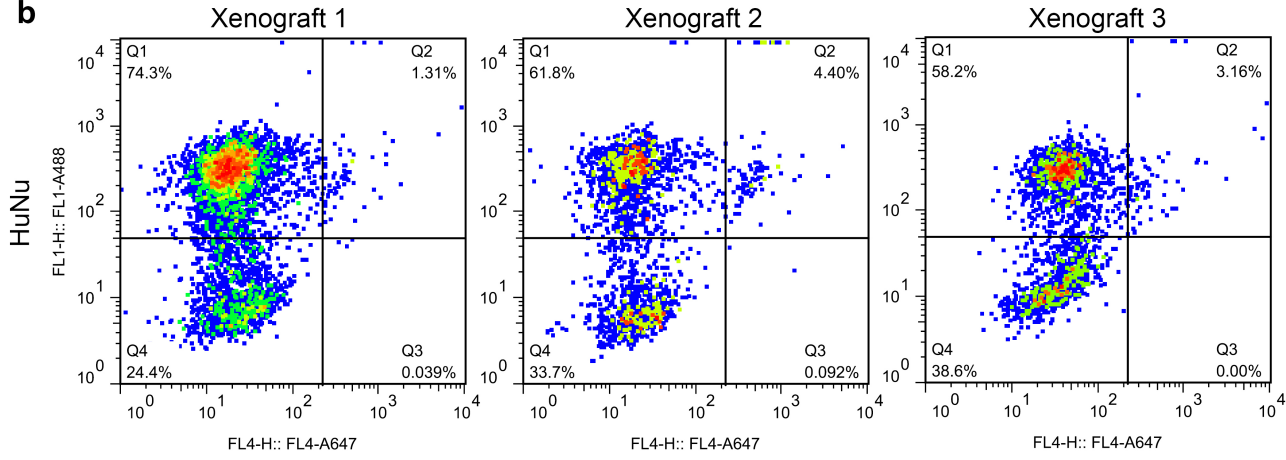
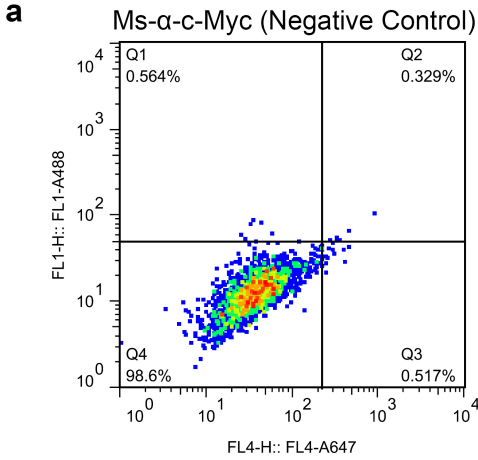


# Supplementary Figure 4



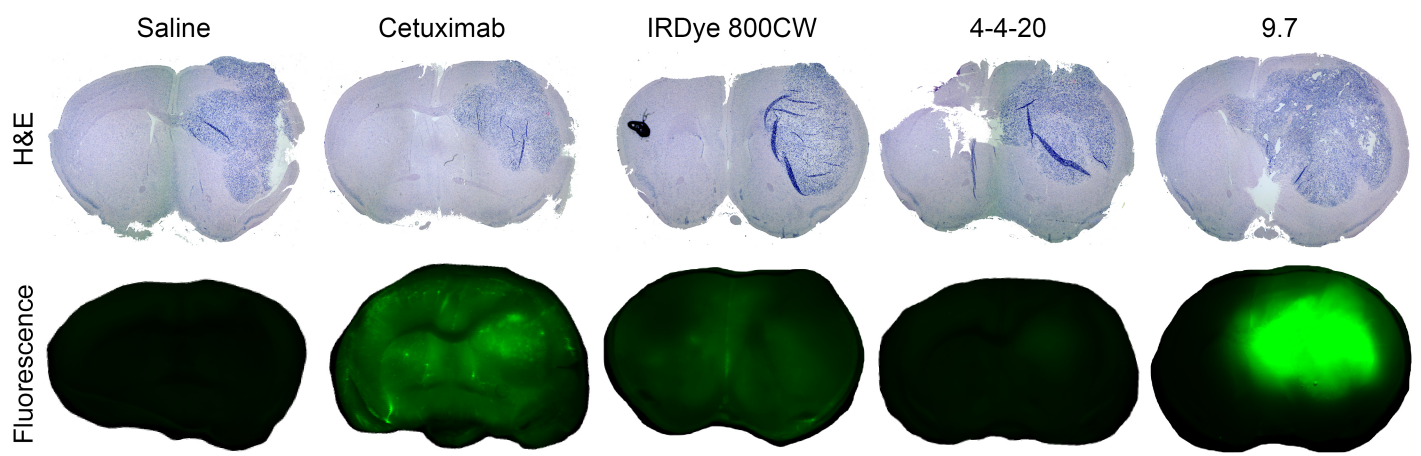


Supplementary Figure 5



Supplementary Figure 6

22 GSC Xenografts



**Supplementary Table 1. Yeast scFv clone discovery counts.**

#	Clone ID	Round 6	Round 9	Round S.6	Round S.7	Round S.8	Round S.9	Round RS.6	Total
1	9.1	18	92	3	2	6	10	13	144
2	9.2	30	42	21	12	11	9	20	145
3	9.5	2	10	1	0	0	2	2	17
4	9.6	3	4	2	1	3	2	5	20
5	9.7	13	23	12	4	11	9	12	84
6	9.15	6	13	1	0	2	4	0	26
7	9.24	0	1	0	0	0	0	0	1
8	9.37	0	3	0	0	0	0	0	3
9	9.42	1	3	0	2	0	1	0	7
10	9.66	3	1	1	1	0	1	0	7
11	9.92	5	4	2	1	2	1	1	16
12	9.106	2	2	1	1	0	0	5	11
13	9.107	0	1	1	2	1	0	0	5
14	9.150	3	1	1	2	0	0	2	9
15	9.160	5	1	1	0	0	0	1	8
16	6.20	1	0	0	0	0	0	0	1
17	6.25	1	0	0	0	0	0	2	3
18	6.27	1	0	0	0	0	0	1	2
19	6.39	2	0	0	0	0	0	0	2
20	6.69	1	0	0	0	0	0	0	1
21	6.92	1	0	0	0	0	0	0	1
22	S.6.1	1	0	3	0	0	0	0	4
23	S.6.3	0	0	1	0	0	0	0	1
24	S.6.4	1	0	3	0	0	0	1	5
25	S.6.6	1	0	1	0	0	0	0	2
26	S.6.7	0	0	2	0	0	0	0	2
27	S.6.8	0	0	1	0	0	0	0	1
28	S.6.20	0	0	1	0	0	0	0	1
29	S.6.27	0	0	3	5	0	0	0	8
30	S.6.28	0	0	2	0	0	0	0	2
31	S.6.33	1	0	1	0	0	0	2	4
32	S.6.47	0	0	1	0	0	0	1	2
33	S.6.48	0	0	1	0	0	0	0	1
34	S.6.49	0	0	2	0	0	0	0	2
35	S.6.52	0	0	1	0	0	0	1	2
36	S.6.58	0	0	1	0	0	0	0	1
37	S.6.59	0	0	1	0	0	0	0	1
38	S.6.65	0	0	1	0	0	0	0	1
39	S.6.69	0	0	1	0	0	0	3	4
40	S.6.76	0	0	1	0	0	0	0	1
41	S.6.79	0	0	3	0	0	0	0	3
42	S.7.19	0	0	2	1	0	0	0	3
43	S.7.21	3	0	1	1	0	0	0	6
44	S.7.25	0	0	0	1	0	0	0	1
45	S.7.33	0	0	0	1	0	0	0	1
46	S.7.37	0	0	0	1	0	0	0	1
47	S.7.40	0	0	0	1	0	0	0	1
48	S.8.4	0	0	0	0	1	0	1	2
49	S.8.5	0	0	0	1	1	0	0	2
50	S.8.24	2	0	0	1	1	1	0	5
51	S.8.38	2	0	0	0	1	0	0	3
52	S.9.8	1	0	0	0	0	1	0	2
53	RS.6.5	0	0	0	0	0	0	1	1
54	RS.6.35	0	0	0	0	0	0	1	1
55	RS.6.61	0	0	0	0	0	0	1	1
56	RS.6.63	0	0	0	0	0	0	1	1
57	RS.6.76	0	0	0	0	0	0	1	1
58	RS.6.77	0	0	0	0	0	0	1	1
59	RS.6.85	0	0	0	0	0	0	1	1
60	RS.6.94	0	0	0	0	0	0	1	1
61	RS.6.101	0	0	0	0	0	0	1	1
62	RS.6.102	0	0	0	0	0	0	1	1
Unique Clones		26	15	34	19	12	11	27	62
Total Analyzed		110	201	81	41	41	41	83	598