Construction of a human monoclonal antibody against bFGF for suppression of NSCLC

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Supplementary Methods

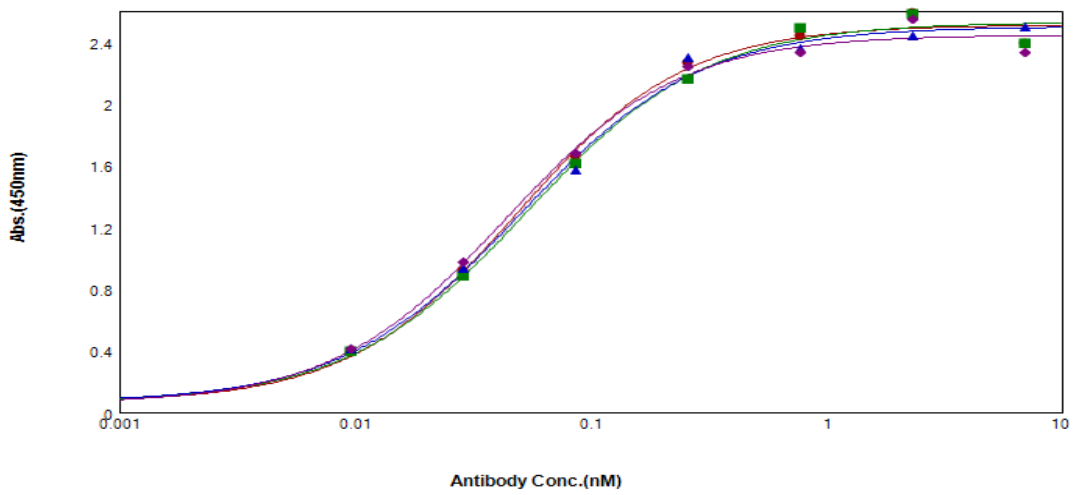
Purification and identification of humanized antibodies

The monoclonal antibodies were purified by Protein G chromatography from the cultural supernatant and then the antibodies were concentrated by centrifuge at 5000 g for 15 min at 4℃. The Molecular weight and structural integrity of the humanized antibodies(H1L1,H2L2,H3L3) was analyzed by SDS-PAGE. And the purity of the humanized antibodies was investigated by HPLC-SEC.

ELISA assay

The 96-well plates were coated with bFGF (40 ng/well)at 4℃ overnight and blocked with 5% non-fat milk and then the purified H3L3 were added in different concentration in each well and incubated for 1 h at 37℃. The HRP-conjugated goat anti-mouse or goat anti-human IgG was added and incubated for 45 min at 37℃. The plates were stained with DAB and the absorbance values at 450 nm (A450) were measured in an ELISA reader (BioTek, Highland Park, Winooski, VT, USA).

Supplementary Figures



● hAb H1L1 (hAb H1L1: MeanVal... vs Concentr...)
■ hAb H2L2 (hAb H2L2: MeanVal... vs Concentr...)
▲ hAb H3L3 (hAb H3L3: MeanVal... vs Concentr...)
◆ cAbE12 (cAb: MeanVal... vs Concentr...)

Curve Fit Results ▲

Curve Fit : 4-Parameter $y = D + \frac{A - D}{1 + \left(\frac{x}{C}\right)^B}$

	Parameter	Estimated Value	Std. Error	Confidence Interval
hAb H1L1 R^2 = 0.996 EC50 = 0.048	A	0.063	0.076	[-0.149, 0.275]
	B	1.215	0.139	[0.829, 1.601]
	C	0.048	0.005	[0.033, 0.064]
	D	2.510	0.054	[2.360, 2.660]
hAb H2L2 R^2 = 0.996 EC50 = 0.053	A	0.062	0.084	[-0.171, 0.296]
	B	1.141	0.143	[0.745, 1.536]
	C	0.053	0.007	[0.034, 0.072]
	D	2.527	0.063	[2.352, 2.702]
hAb H3L3 R^2 = 0.996 EC50 = 0.050	A	0.064	0.076	[-0.147, 0.275]
	B	1.143	0.130	[0.783, 1.502]
	C	0.050	0.006	[0.034, 0.066]
	D	2.499	0.056	[2.344, 2.654]
cAbE12 R^2 = 0.995 EC50 = 0.043	A	0.054	0.087	[-0.187, 0.295]
	B	1.183	0.155	[0.753, 1.613]
	C	0.043	0.006	[0.028, 0.059]
	D	2.445	0.060	[2.277, 2.613]

Figure S1. The antibody affinity comparison of H1L1, H2L2, H3L3 , cAb(E12) and calculate of concentration for 50% of maximal effect(EC50).

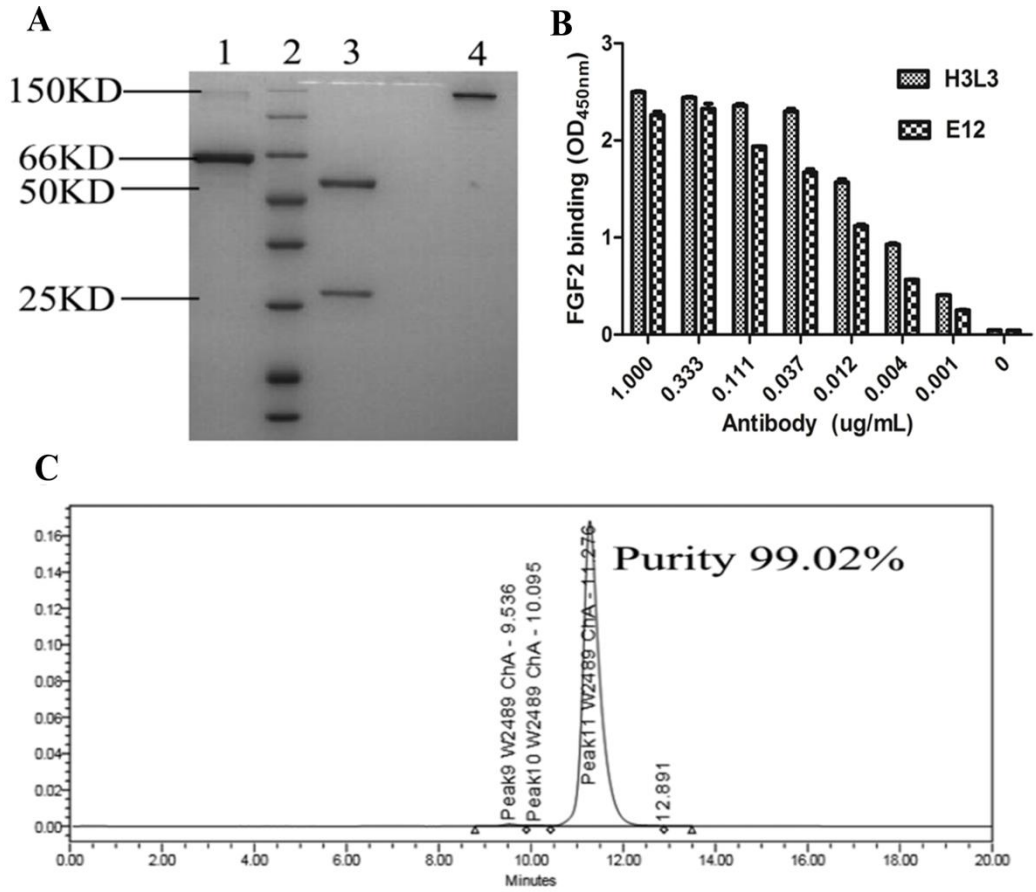


Figure S2. The purification, identification and functional analysis of H3L3. (A) SDS-PAGE of purified monocolonal antibody. Lane 1 was BSA sample ; Lane 2 was loaded with protein ladder; Lane 3 was loaded with reducing mAb H3L3; Lane 4 was loaded with non-reducing mAb H3L3. The samples were separated by using 12% gradient PAGE gels and stained with Coomassie Blue. (B)Analysis of purified H3L3 at 1mg/ml by SEC-HPLC. (C) Specific binding activity of H3L3 to bFGF compare to E12.

Table S1

Stress	H1L1			H2L2			H3L3		
	Agg%	Main%	Clip%	Agg%	Main%	Clip%	Agg%	Main%	Clip%
T=0	1.73	97.14	1.13	1.30	98.66	0.04	3.89	96.11	0
25℃ Day7	1.77	97.07	1.16	1.15	98.37	0.13	2.38	97.55	0.08
50℃ Day7	2.16	93.09	4.73	1.61	96.53	1.86	2.95	95.29	1.77
Low pH (3.5) Day7	2.48	94.91	2.61	12.01	86.45	1.49	10.54	87.76	1.70
High pH (10) Day7	1.38	97.64	0.98	2.05	97.82	0.13	2.39	97.55	0.06
F/T C6	1.81	97.25	0.94	1.45	98.52	0.03	2.35	97.61	0.05

Table S1.The stability of humanized antibody was tested at high or low pH, high or room temperature and freeze-thaw conditions after purification. F / T C6 represents repeated freezing and thawing six times.