

Supplementary Table-1

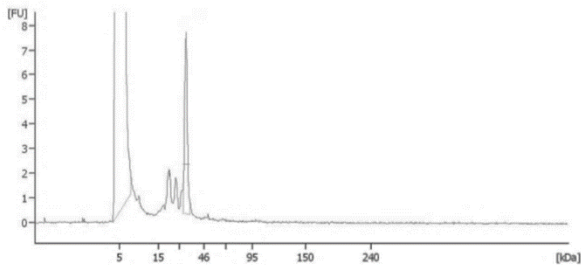
Protein sequence for anti-HER3 antibody fragments:

Anti-HER3 fragment	Amino acid sequence
scFv	SDIQMTQSPSSLSASVGDRVTITCRASQGISNYLAWYQQKPGKAPKLLIYAA SSLQSGVPSRFSGSRSGTDFLTISLQPEDFATYYCQQYGWLPLTFGQGTKV EIKGGGGSGGGGSGGGGSEISEVQLVESGGGLVQPGGSLRLSCAASGFTFSSY GMHWVRQAPGKGLEWVAVISYDGSNKYYADSVKGRFTISRDN SKNTLYLQ MNSLRAEDTAVYYCARTDPYSLGGYYFDYWGQGLTVTVSS
diabody	SDIQMTQSPSSLSASVGDRVTITCRASQGISNYLAWYQQKPGKAPKLLIYAA SSLQSGVPSRFSGSRSGTDFLTISLQPEDFATYYCQQYGWLPLTFGQGTKV EIKGGGGSEISEVQLVESGGGLVQPGGSLRLSCAASGFTFSSYGMHWVRQAP GKGLEWVAVISYDGSNKYYADSVKGRFTISRDN SKNTLYLQMNSLRAEDTA VYYCARTDPYSLGGYYFDYWGQGLTVTVSS
Fab	<p><i>Light Chain:</i> SDIQMTQSPSSLSASVGDRVTITCRASQGISNYLAWYQQKPGKAPKLLIYAA SSLQSGVPSRFSGSRSGTDFLTISLQPEDFATYYCQQYGWLPLTFGQGTKV EIKRTVAAPSVFIFPPSDSQLKSGTASVVCLLNNFYPREAKVQWKVDNALQS GNSQESVTEQDSKDYSLSTLTLSKADYEKHKVYACEVTHQGLSSPVTKS FNRGECGGSDYKDDDDK</p> <p><i>Heavy Chain:</i> EISEVQLVESGGGLVQPGGSLRLSCAASGFTFSSYGMHWVRQAPGKGLEWV AVISYDGSNKYYADSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCART DPYSLGGYYFDYWGQGLTVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLV KDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGTQTYI CNVNHKPSNTKVDKKEPKSCDKTHTGGSHHHHHH</p>

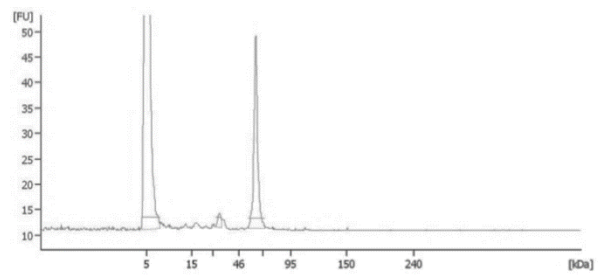
<p>scFv-C_{H3}</p>	<p>SDIQMTQSPSSLSASVGDRVTITCRASQGISNYLAWYQQKPGKAPKLLIYAA SSLQSGVPSRFSGSRSGTDFTLTISSLQPEDFATYYCQQYGWLPLTFGQGTKV EIKGGGGSGGGGSGGGGSEISEVQLVESGGGLVQPGGSLRLSCAASGFTFSSY GMHWVRQAPGKGLEWVAVISYDGSNKYYADSVKGRFTISRDN SKNTLYLQ MNSLRAEDTAVYYCARTDPYSLGGYYFDYWGQGLTVTVSSEPKSCDKTHTC PPCPGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPEN NYKTTTPVLDS DGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYT QKSLSLSPGK</p>
<p>scFv-Fc</p>	<p>SDIQMTQSPSSLSASVGDRVTITCRASQGISNYLAWYQQKPGKAPKLLIYAA SSLQSGVPSRFSGSRSGTDFTLTISSLQPEDFATYYCQQYGWLPLTFGQGTKV EIKGGGGSGGGGSGGGGSEISEVQLVESGGGLVQPGGSLRLSCAASGFTFSSY GMHWVRQAPGKGLEWVAVISYDGSNKYYADSVKGRFTISRDN SKNTLYLQ MNSLRAEDTAVYYCARTDPYSLGGYYFDYWGQGLTVTVSSEPKSCDKTHTC PPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYV DGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALP APIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWE SNGQPENNYKTTTPVLDS DGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALH NHYTQKSLSLSPGK</p>
<p>IgG</p>	<p><i>Light Chain:</i> SDIQMTQSPSSLSASVGDRVTITCRASQGISNYLAWYQQKPGKAPKLLIYAA SSLQSGVPSRFSGSRSGTDFTLTISSLQPEDFATYYCQQYGWLPLTFGQGTKV EIKRTVAAPS VFIFPPSDEQLKSGTASV VCLLN NFYPREAKVQWKVDNALQS GNSQESVTEQDSKDSTYLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKS FNRGEC</p> <p><i>Heavy Chain:</i> EISEVQLVESGGGLVQPGGSLRLSCAASGFTFSSYGMHWVRQAPGKGLEWV AVISYDGSNKYYADSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCART DPYSLGGYYFDYWGQGLTVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLV KD YFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYI CNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTL MISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRV VSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSR EEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDS DGSFFLYS KLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK</p>

Supplementary Figure S1

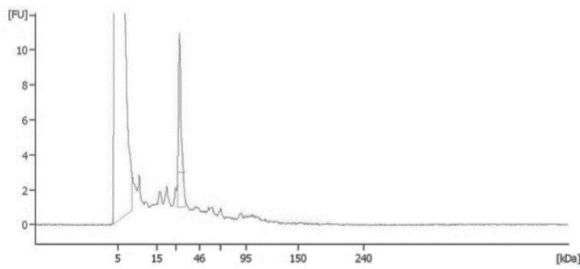
scFv



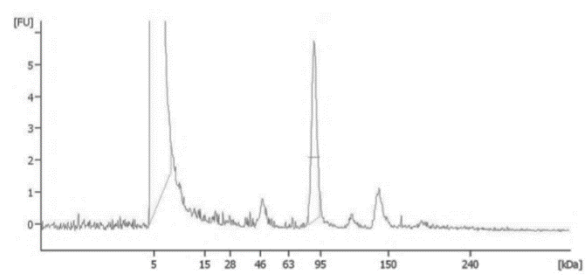
Fab



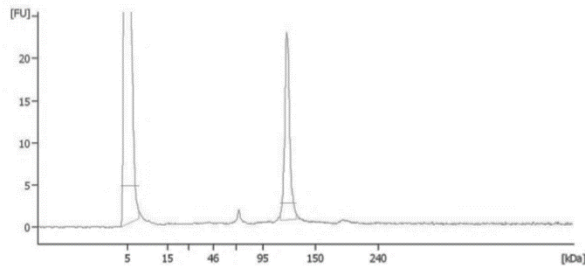
Diabody



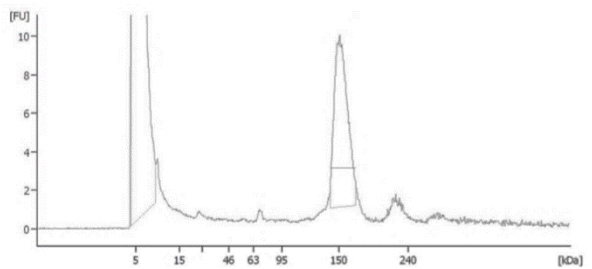
scFv-C_H3



scFv-Fc



IgG



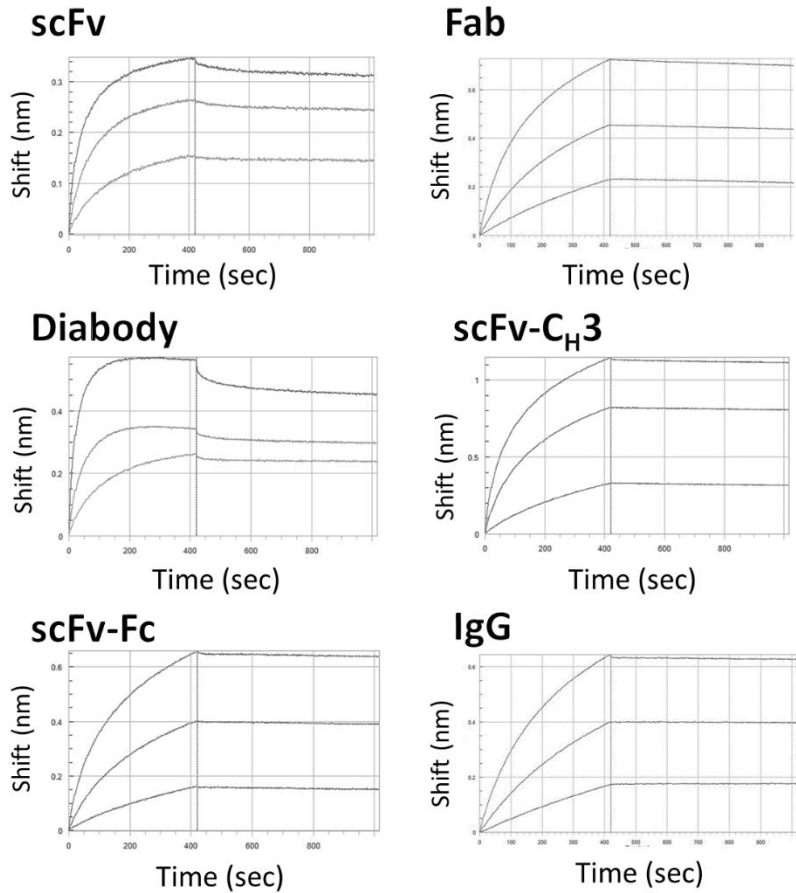
Anti-HER3 Construct	Observed size, kDa	Purity
scFv	32.6	75%
Fab	58.0	93%
Diabody	30.4 (60.8)*	87%
scFv-C _H 3	88.5	81%
scFv-Fc	120.7	96%
IgG	150.7	94%

*The size between brackets is the size of the dimer.

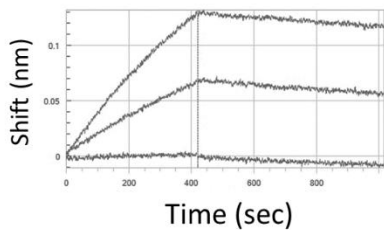
Supplementary Figure S1: Characterization of anti-HER3 antibody fragments using the Agilent Bioanalyzer 2100. Representative Bioanalyzer electropherograms of anti-HER3 scFv, diabody, Fab, scFv-C_H3, scFv-Fc, and IgG under non-reducing conditions. Molecular weight and purity of the IgG and antibody fragments (%) were calculated based on protein standards from an Agilent High Sensitivity Protein 250 kit and are summarized in the table.

Supplementary Figure S2

A

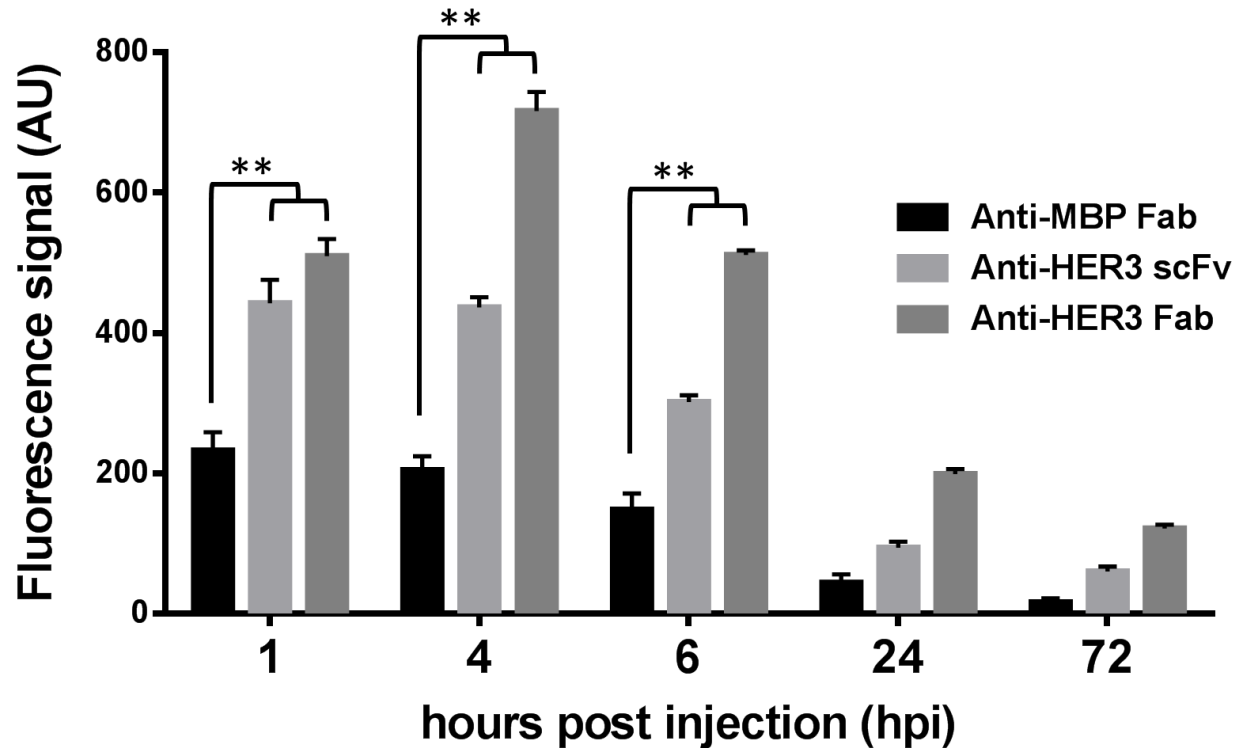


B



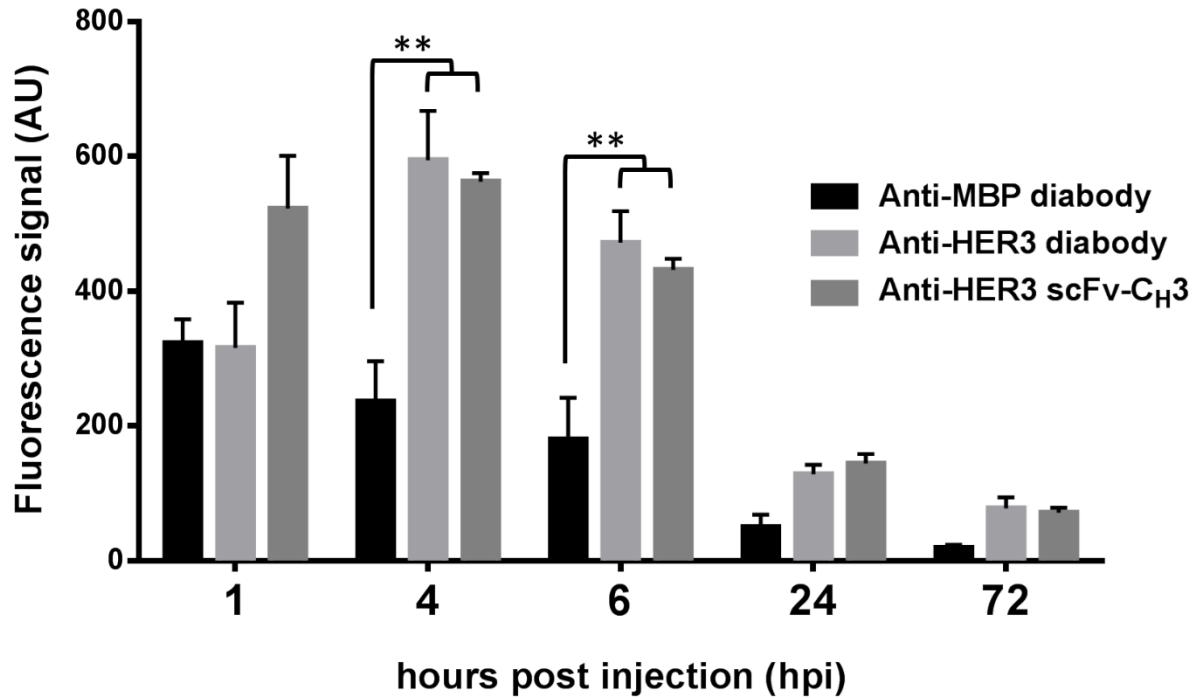
Supplementary Figure S2: Biolayer interferometry analysis of binding kinetics of anti-HER3 antibody fragments. **A)** Binding kinetics anti-HER3 scFv, diabody, Fab, scFv-CH₃, scFv-Fc, and IgG against recombinant human HER3 extracellular domain. Lines represent the three concentrations of target tested; 52.6, 131.6, and 263.2 nM **B)** Binding kinetics anti-HER3 IgG against recombinant mouse HER3 extracellular domain. Lines represent the three concentrations of target tested; 10.5, 131.6, and 263.2 nM

Supplementary Figure S3



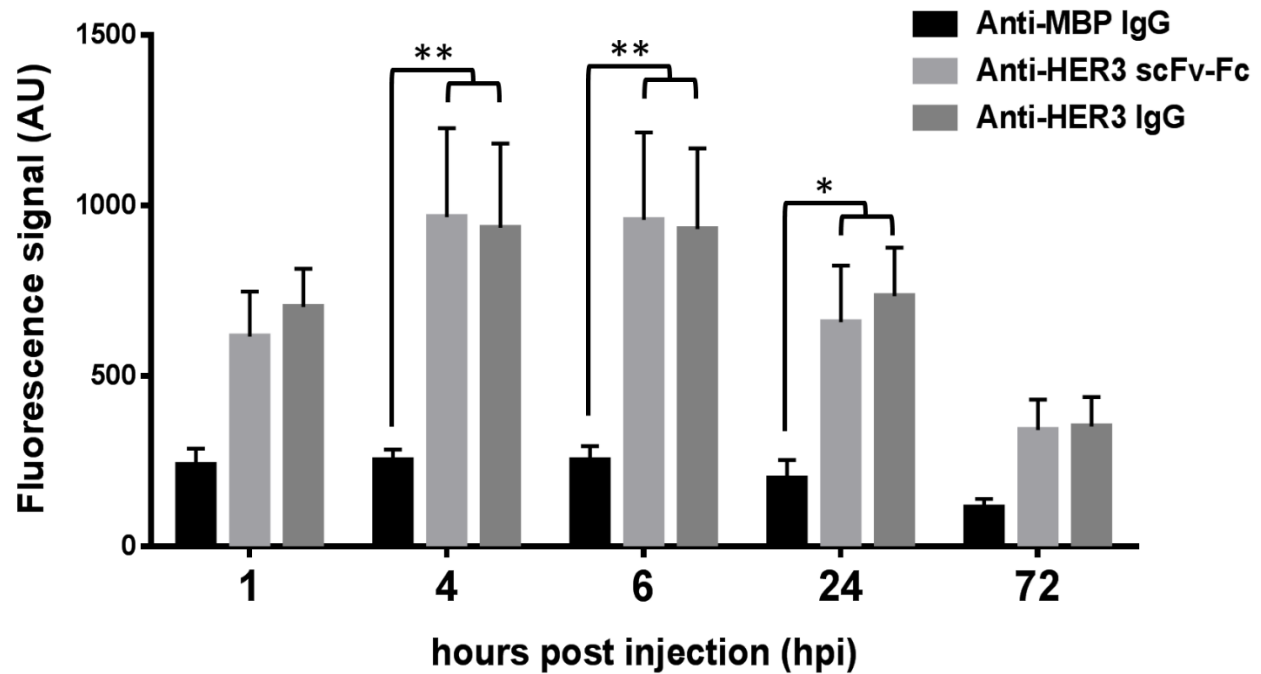
Supplementary Figure S3: Biodistribution analysis of IRDye800CW-labeled monovalent antibody fragments in mice bearing FaDu xenografts. Mean fluorescent signal (arbitrary units) for control anti-MBP Fab (black) anti-HER3 scFv (light gray), and Fab (dark gray) in FaDu xenografts at 1, 4, 6, 24 and 72 hours post injection (hpi). ** = p value < 0.01, AU represents arbitrary units. Error bars represent standard error of the mean.

Supplementary Figure S4



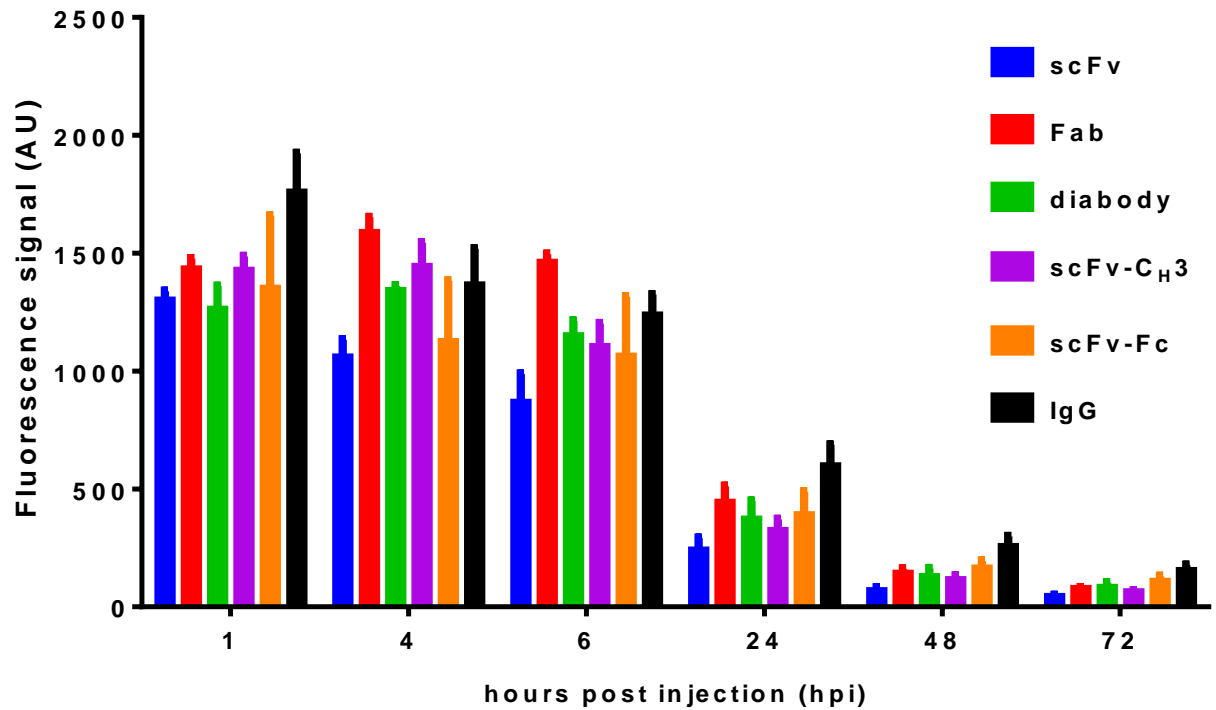
Supplementary Figure S4: Biodistribution analysis of IRDye800CW-labeled bivalent antibody fragments lacking Fc domain in mice bearing FaDu xenografts. Mean fluorescent signal (arbitrary units) for control anti-MBP diabody (black) anti-HER3 diabody (light gray), and scFv-CH₃ (dark gray) in FaDu xenografts at 1, 4, 6, 24 and 72 hours post injection (hpi). ** = p value < 0.01, AU represents arbitrary units. Error bars represent standard error of the mean.

Supplementary Figure S5



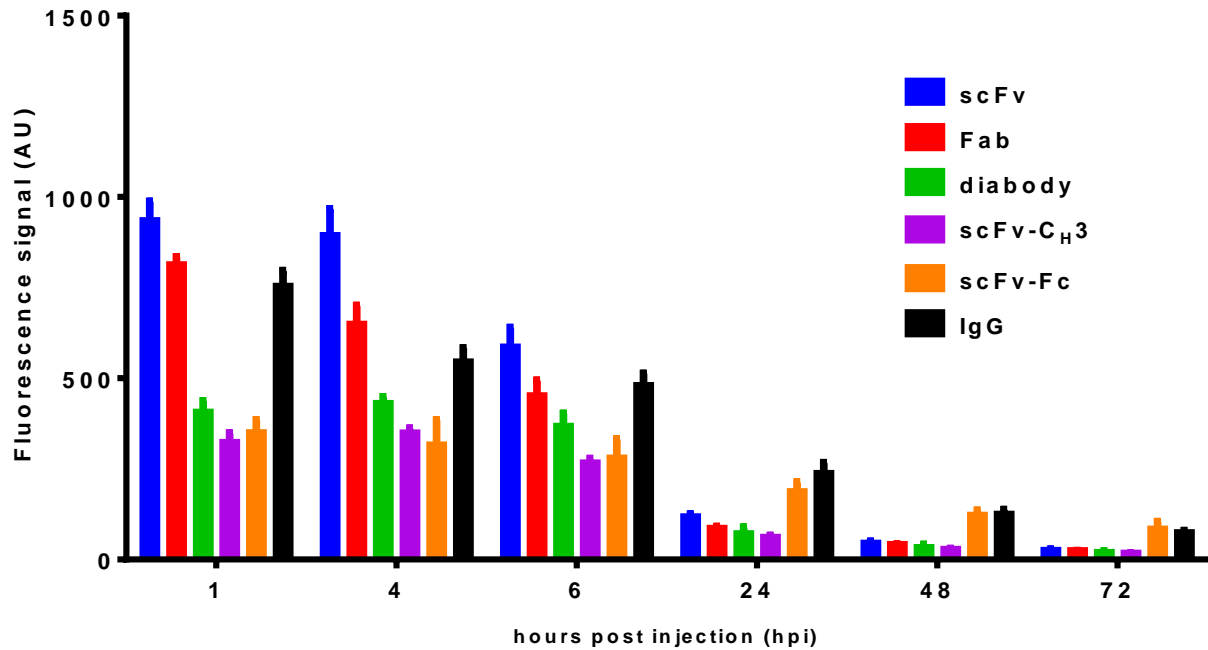
Supplementary Figure S5: Biodistribution analysis of IRDye800CW-labeled bivalent antibody and fragments containing Fc domain in mice bearing FaDu xenografts. Mean fluorescent signal (arbitrary units) for control anti-MBP IgG (black) anti-HER3 scFv-Fc (light gray), and IgG (dark gray) in FaDu xenografts at 1, 4, 6, 24 and 72 hours post injection (hpi). * = p value < 0.05, ** = p value < 0.01, AU represents arbitrary units. Error bars represent standard error of the mean.

Supplementary Figure S6



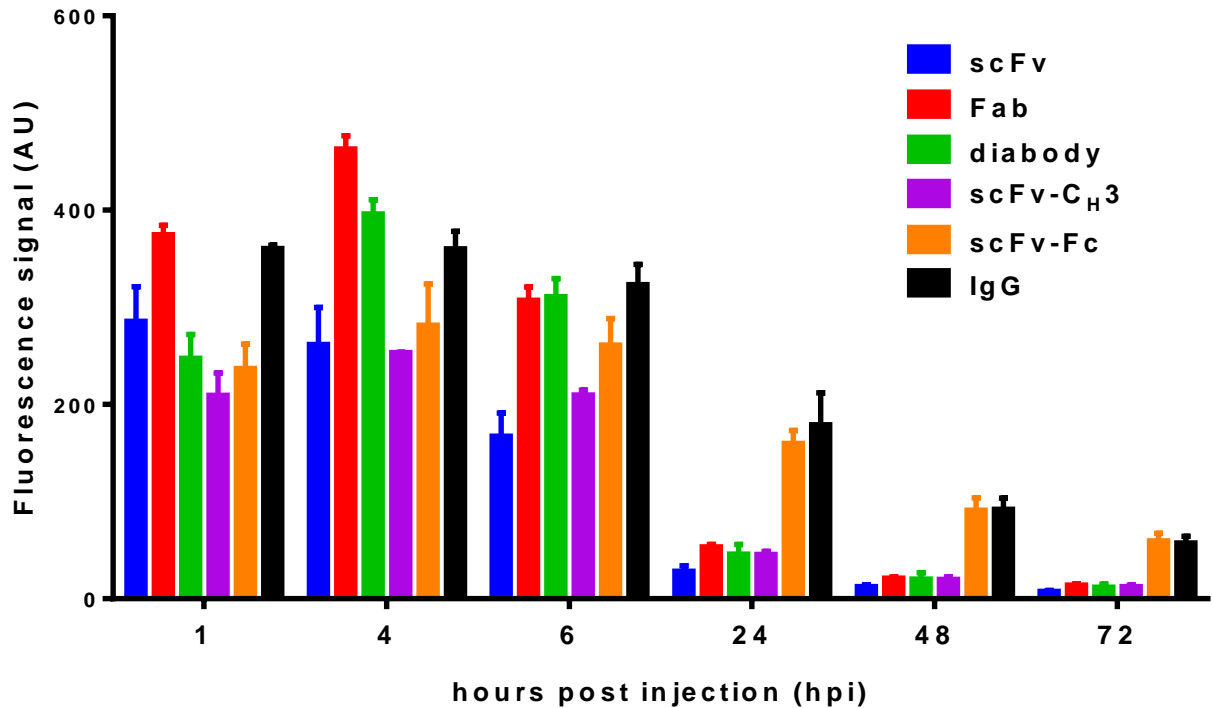
Supplementary Figure S6: Liver fluorescence of IRDye800CW-labeled anti-HER3 IgG and antibody fragments in mice bearing FaDu xenografts. Mean fluorescent signal (arbitrary units) for anti-HER3scFv (blue), Fab (red), diabody (green), scFv- C_{H3} (purple), scFv-Fc (orange) and IgG (black) in liver at 1, 4, 6, 24, 48 and 72 hours post injection (hpi). AU represents arbitrary units. Error bars represent standard error of the mean.

Supplementary Figure S7



Supplementary Figure S7: Kidney fluorescence of IRDye800CW-labeled anti-HER3 IgG and antibody fragments in mice bearing FaDu xenografts. Mean fluorescent signal (arbitrary units) for anti-HER3scFv (blue), Fab (red), diabody (green), scFv-C_H3 (purple), scFv-Fc (orange) and IgG (black) in kidney at 1, 4, 6, 24, 48 and 72 hours post injection (hpi). AU represents arbitrary units. Error bars represent standard error of the mean.

Supplementary Figure S8



Supplementary Figure S8: Contralateral site fluorescence of IRDye800CW-labeled anti-HER3 IgG and antibody fragments in mice bearing FaDu xenografts. Mean fluorescent signal (arbitrary units) for anti-HER3scFv (blue), Fab (red), diabody (green), scFv-CH₃ (purple), scFv-Fc (orange) and IgG (black) in contralateral site at 1, 4, 6, 24, 48 and 72 hours post injection (hpi). ** = p value < 0.01, ns = p value > 0.05, AU represents arbitrary units. Error bars represent standard error of the mean.