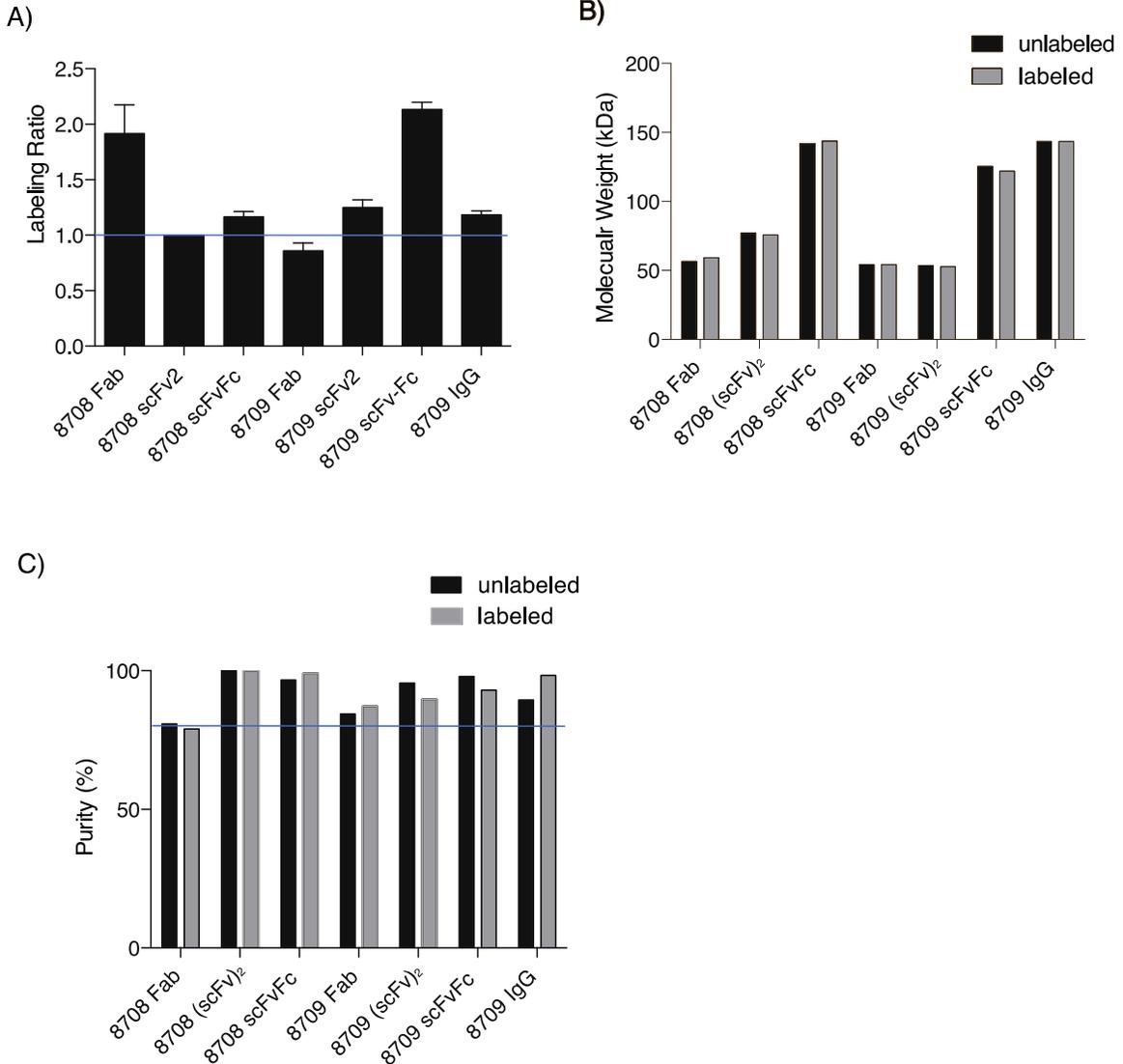
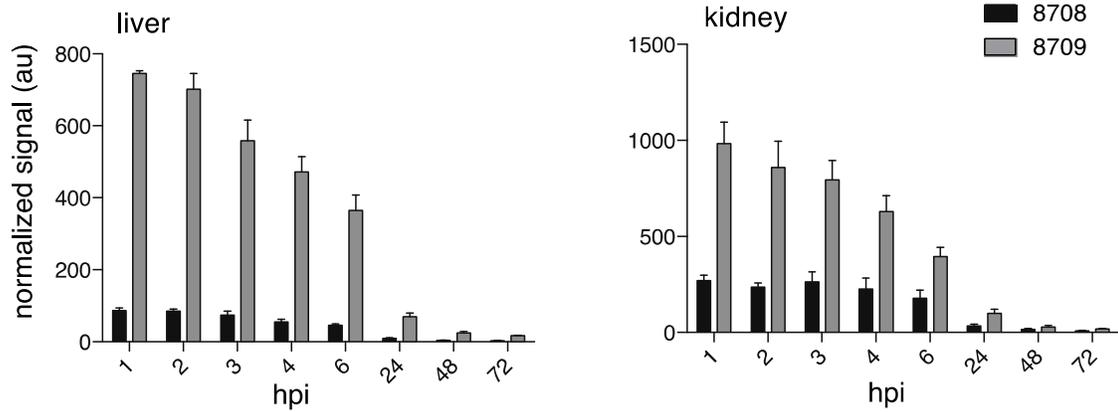


Supplementary Figures

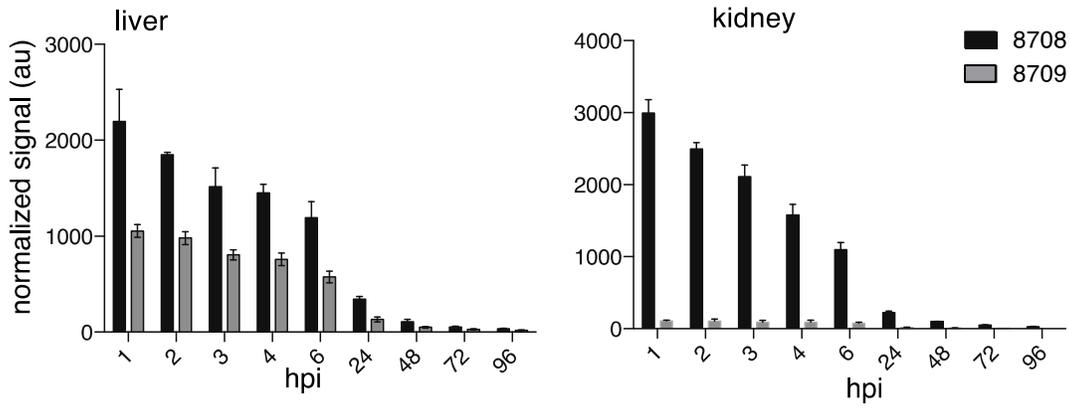


Supplementary Figure 1: Characterization of 8708 and 8709 fragments. A) Labeling ratios of IRDye800CW-labeled 8708 and 8709 fragments. B) Observed molecular weights of unlabeled and IRDye800CW-labeled 8708 and 8709 fragments using capillary electrophoresis. C) Purity of unlabeled and IRDye800CW-labeled 8708 and 8709 fragments using capillary electrophoresis.

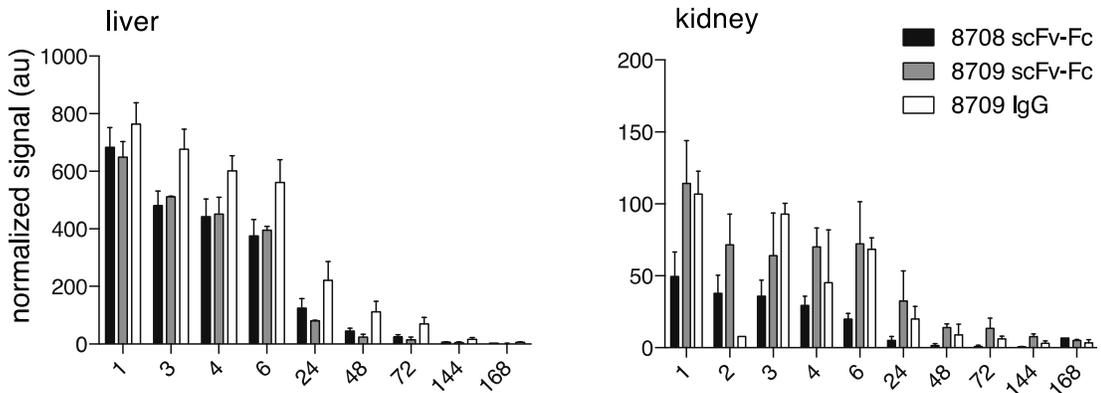
A Fab



B scFv2

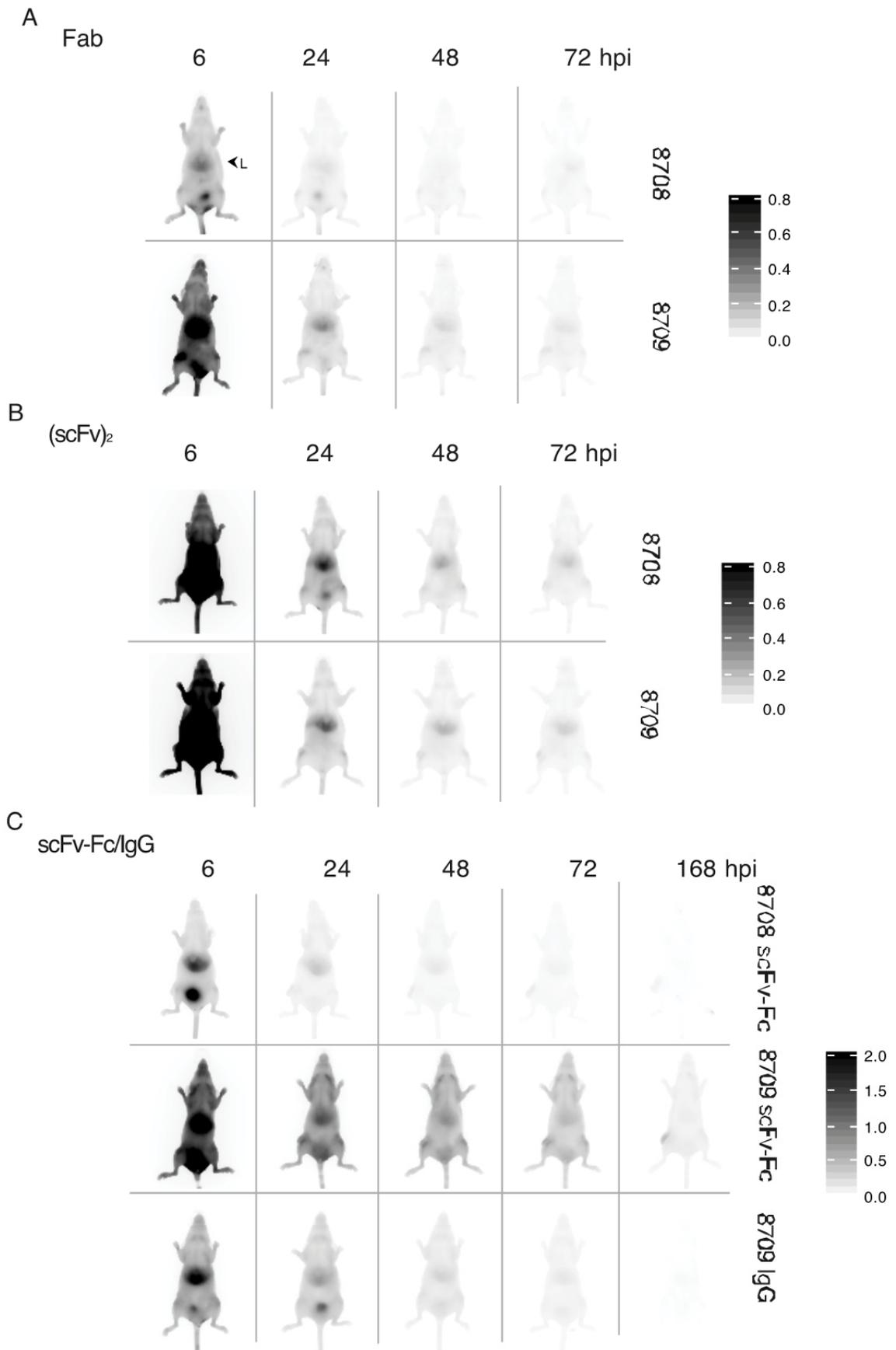


C scFv-Fc/IgG

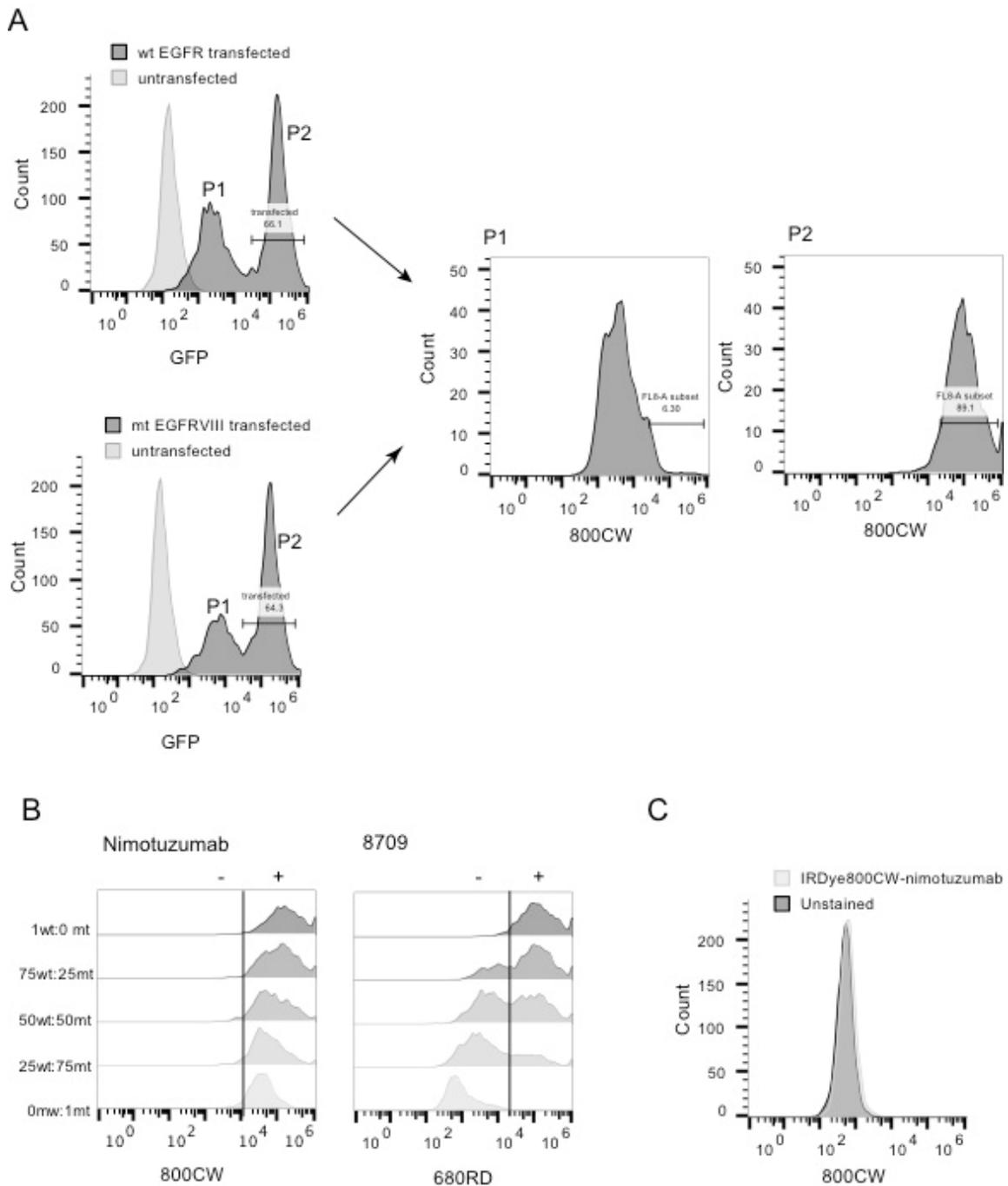


Supplementary Figure 2: Near infrared fluorescent image analysis of the liver and kidney. IRDye800CW-labeled fragments: A) Fab, B) (scFv)₂, and C) scFv-Fc and IgG were injected into A-431 xenograft bearing mice, and imaged at time, shown at various

hours post injection (hpi). Data represents the mean of the normalized signal of three different regions in the liver or kidney of interest of at least mice \pm SEM.



Supplementary Figure 3: Near infrared images of IRDye800CW-labeled 8708 and 8709 fragments in A-431 xenografted mice in the ventral position. Images shown were taken at 6, 24, 48, and 72 hours post injection (hpi) for the Fab and (scFv)₂ with an additional point at 168 hpi for the scFv-Fc and IgG. Near infrared images of the 8708 and 8709 A) Fab fragments, B) (scFv)₂ fragments, and C) scFv-Fc and 8709 IgG. The scale bars are shown on the right. L = liver.



Supplementary Figure 4: Flow cytometry analysis of wildtype EGFR and mutant EGFR $vIII$. HEK293T cells were transfected with a plasmid expressing EGFR-GFP or EGFR $vIII$ -GFP mutant. A) Flow cytometry analysis showed that there were two populations in the FITC (GFP) channel in cells transfected with either wildtype (wt)

EGFR-GFP or EGFR ν III-GFP mutant (mt). The two populations (P1 and P2) from cells transfected with wildtype EGFR-GFP or mutant EGFR ν III-GFP were tested for binding to IRDye800CW-nimotuzumab, which binds EGFR and EGFR ν III. IRDye800CW-Nimotuzumab (800CW) stained the P1 cell population stronger than P1. B) The P2 cell population was analyzed for binding to IRDye800CW-nimotuzumab or IRDye680RD-8709 scFv-Fc. The percent of cells in the positive gate (+) were calculated and reported in supplementary table 1. C) HEK293T cells unstained and stained with IRDye800CW-nimotuzumab.

Supplementary Table 1: Percent positive cells expressing wildtype EGFR (Wt) and mutant EGFR ν III (MtvIII) stained with IRDye800CW-nimotuzumab or IRDye680-8709 scFv-Fc

EGFR ratio		% cells		n
Wt	MtvIII	IRDye800CW-nimotuzumab \pm SD	IRDye680RD-8709 scFv-Fc \pm SD	
1	0	93.3 \pm 1.8	90.1 \pm 3.6	8
75	25	99.0 \pm 0.4	72.1 \pm 6.2	8
50	50	95.7 \pm 0.6	51.2 \pm 5.7	8
25	75	97.7 \pm 0.3	31.1 \pm 7.1	8
0	1	98.3 \pm 0.2	8.3 \pm 5.4	8

n represents the number of replicates