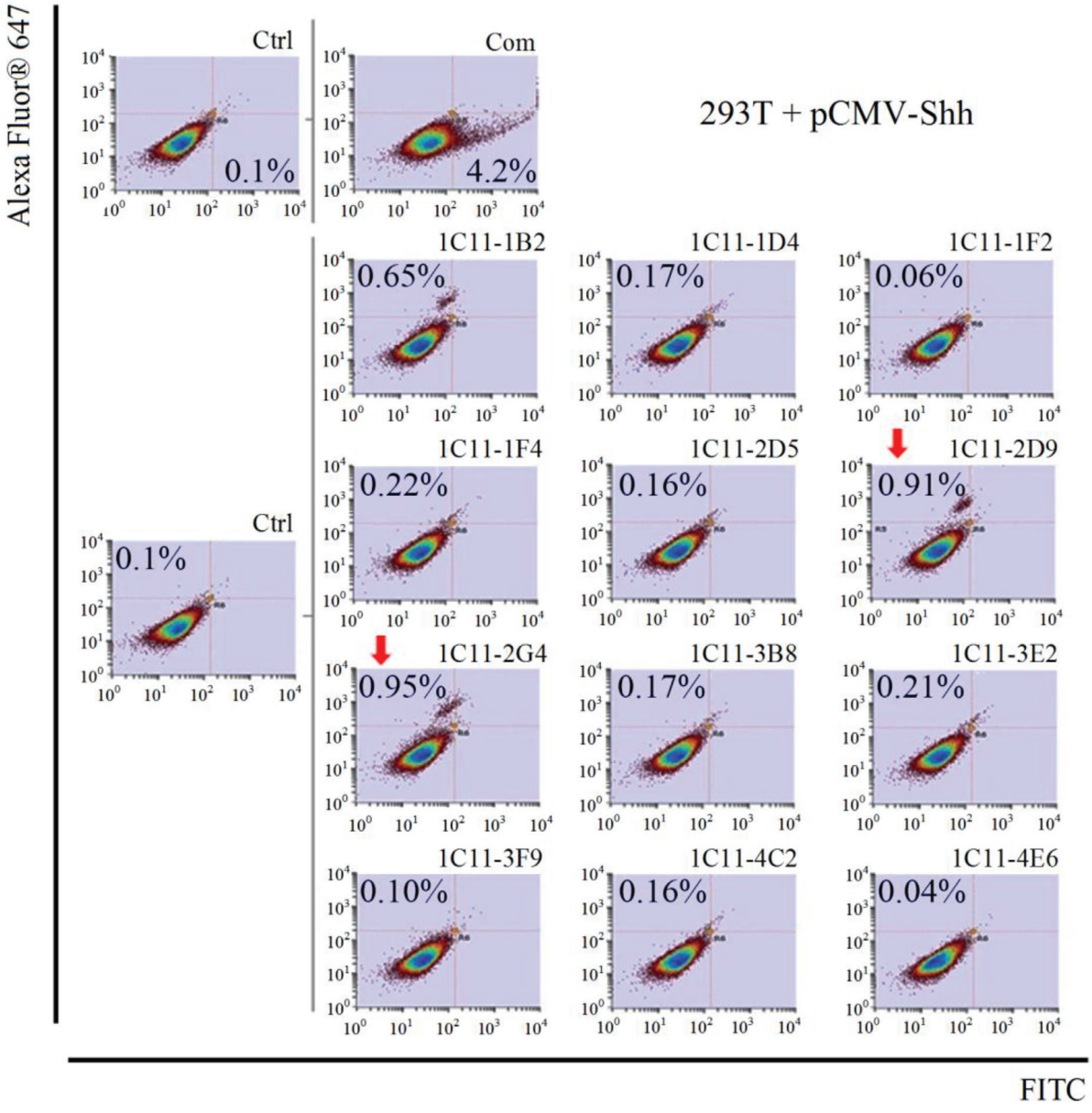
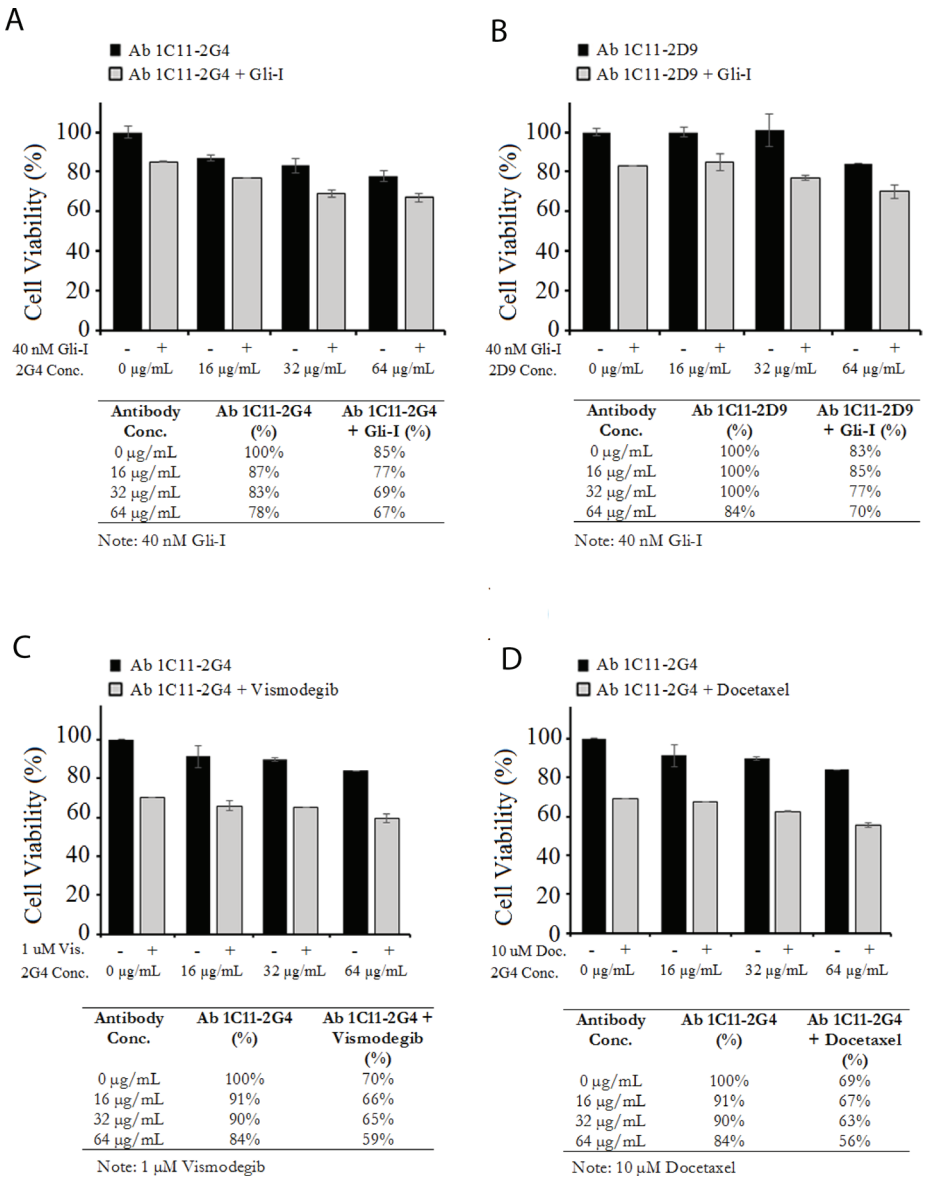
Preclinical characterization of therapeutic antibodies targeted at the carboxy-terminus of Sonic hedgehog

SUPPLEMENTARY MATERIALS



Supplementary Figure 1: Screening of candidate therapeutic anti-Shh antibodies directed at the C-terminal of the Sonic Hedgehog protein. Flow cytometric evaluation of Shh recognition performed with clonal supernatants incubated with 293T cells transfected with exogenous Shh. Twelve sub-clones of 1C11 were tested and 2D9 and 2G4 detected the highest percentage of cell-surface Shh compared with the other clones. The commercial (Com) antibody was used as a positive control. The secondary antibody alone incubated with cells was run as a negative control (Ctrl.).



Antibody Conc.	Ab 1C11-2G4 (%)	Ab 1C11-2G4 + Gli-I (%)
0 µg/mL	100%	85%
16 µg/mL	87%	77%
32 µg/mL	83%	69%
64 µg/mL	78%	67%

Note: 40 nM Gli-I

Antibody Conc.	Ab 1C11-2D9 (%)	Ab 1C11-2D9 + Gli-I (%)
0 µg/mL	100%	83%
16 µg/mL	100%	85%
32 µg/mL	100%	77%
64 µg/mL	84%	70%

Note: 40 nM Gli-I

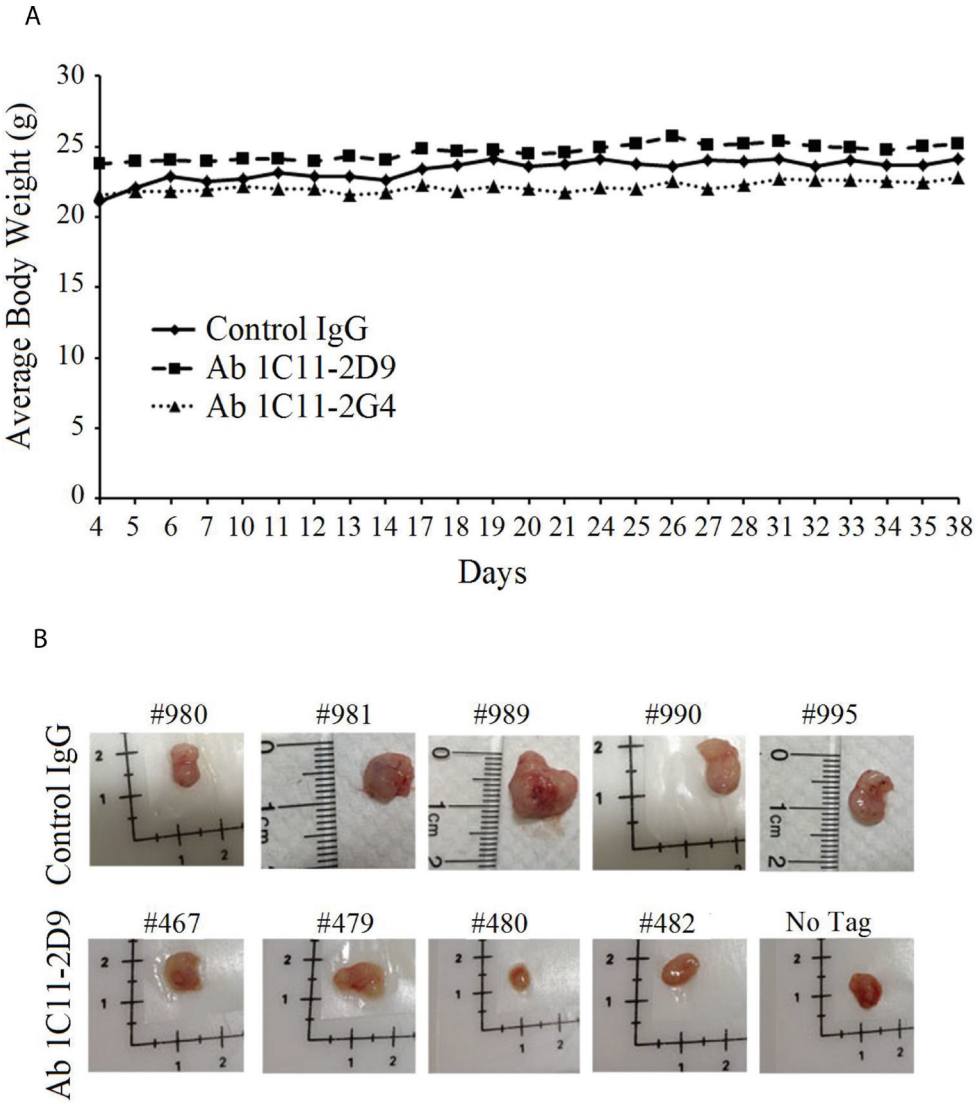
Antibody Conc.	Ab 1C11-2G4 (%)	Ab 1C11-2G4 + Vismodegib (%)
0 µg/mL	100%	70%
16 µg/mL	91%	66%
32 µg/mL	90%	65%
64 µg/mL	84%	59%

Note: 1 µM Vismodegib

Antibody Conc.	Ab 1C11-2G4 (%)	Ab 1C11-2G4 + Docetaxel (%)
0 µg/mL	100%	69%
16 µg/mL	91%	67%
32 µg/mL	90%	63%
64 µg/mL	84%	56%

Note: 10 µM Docetaxel

Supplementary Figure 2: Combinatorial regimens of C-terminal directed anti-Shh along with targeted therapies or chemotherapy result in increased efficacy. NSCLC A549 cells were treated with indicated doses of C-term anti-Shh antibodies and combined with targeted therapies [Ab 1C11-2G4 + Gli-I (**A**) or Ab 1C11-2D9 + Gli-I (**B**) or Ab 1C11-2G4 + Vismodegib (**C**)] or chemotherapy [Ab 1C11-2G4 + Docetaxel (**D**)] in triplicate for 96 hours. The cell viability/ATP content was measured using CellTiter-Glo Luminescent Cell Viability Assay.



Supplementary Figure 3: *in vivo* studies of C-term Shh Ab 1C11-2G4 and Ab 1C11-2D9 in lung NSCLC. (**A**) Average body weight of all mice treated with Ab 1C11-2G4, Ab 1C11-2D9 or control IgG over the course of the study (38 days). (**B**) Images of tumors collected from control IgG antibody-treated or Ab 1C11-2D9 antibody-treated mice.