Cancer Cell, Volume *33*

Supplemental Information

Complex Interplay between Epitope Specificity and Isotype Dictates the Biological Activity of Anti-human CD40 Antibodies

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Table S1. Related to Figure 2.

	ChiLob 7/4 Fab:CD40 Complex
Data collection	
Space group	P 3_1 2 1
Cell dimensions	
a, b, c (Å)	158.75 158.75 96.62
a, b, g (°)	90 90 120
Resolution (Å)	51.96 - 3.00 (3.08-3.00)
R_{sym} or R_{merge}	0.157 (1.445)
I / sI	13.0 (32.9)
Completeness (%)	100 (98.4)
Redundancy	13.7 (11.4)
Refinement	
Resolution (Å)	3.00
No. reflections	27540
R_{work} / R_{free}	0.190 / 0.237
No. atoms	
Protein	4091
Ligand/ion	0
Water	23
B-factors	
Protein	87.03
Ligand/ion	0
Water	78.14
R.m.s. deviations	
Bond lengths (Å)	0.0169
Bond angles (°)	2.135

Data Collection and Refinement Statistics of ChiLob 7/4 Fab':CD40 Complex (PDB ID 6FAX). High resolution shell statistics shown in parenthesis. 5% of reflections were used for calculation of Rfree.

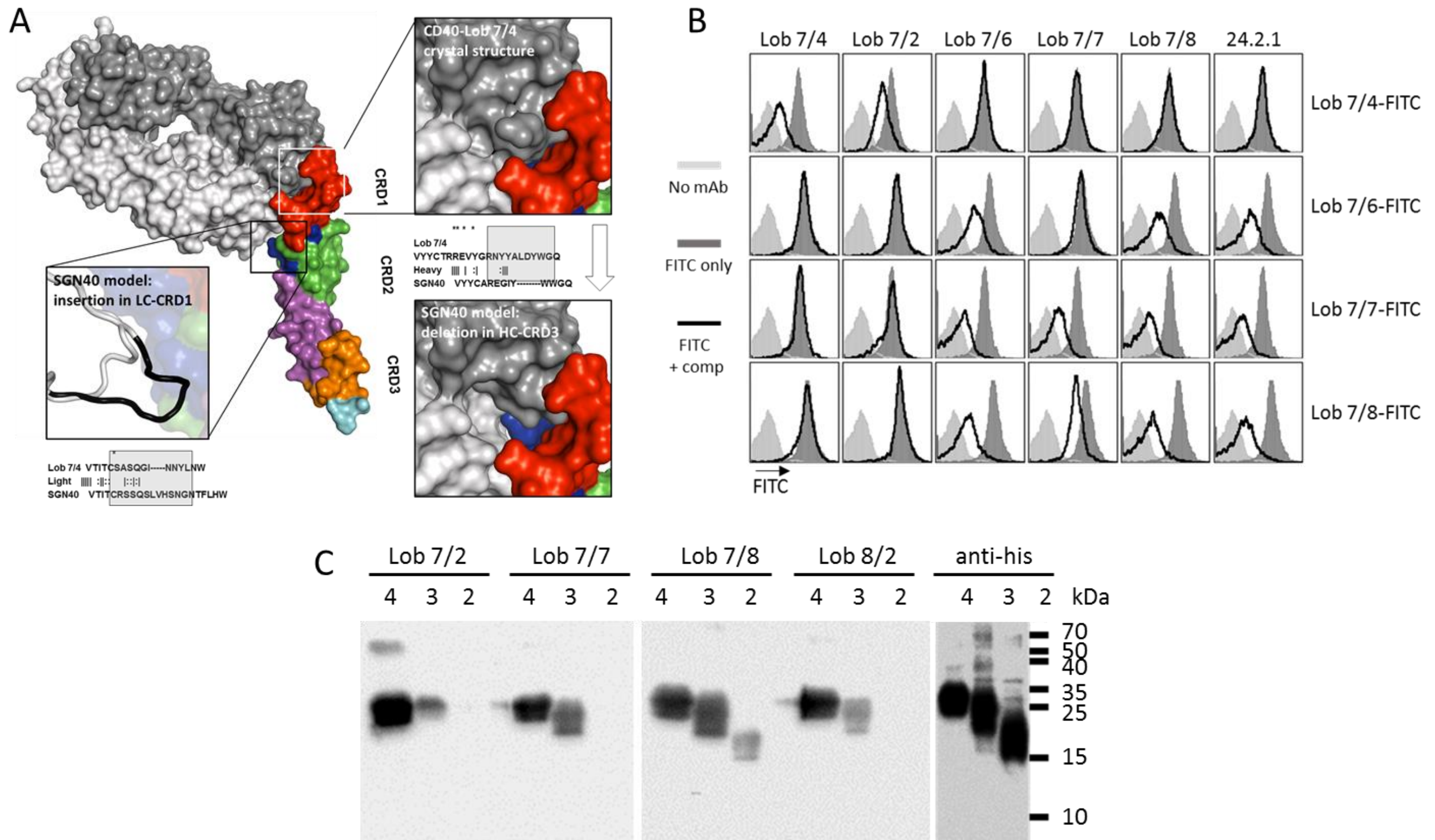

Figure S1. Epitope mapping of hCD40 mAb. Related to Figure 3. (A) Modelling of SGN40 binding onto the ChiLob 7/4:CD40 crystal structure. Surface view of the structure of the CD40:ChiLob 7/4 complex with CRD regions of CD40 coloured and mapped as in Figure 2 with various insets highlighting key parts of the interaction. Below are models produced to show the predicted structural differences apparent in the SGN40 and ChiLob 7/4 Fab' based on loop length changes in the heavy chain CRD3 and the light chain CRD1. Panels: Top right panel highlights the binding of ChiLob 7/4 to CD40 in the crystal structure. Note: The LC CDR3 of ChiLob 7/4 binds into a pocket on the CRD1-B2 of CD40 and the light chain CRD1 of ChiLob 7/4 binds in a pocket of CD40 CDR1-A1. Below is shown the sequence alignment between the HC CDR3 of ChiLob 7/4 and SGN40 with the key binding residues in the structure of ChiLob 7/4 starred. Below (bottom right) shows how the 8 amino-acid deletion in the SGN40 HC would prevent it making the same interaction with the pocket of CRD1-B2 of

CD40 as ChiLob 7/4. The bottom left panel shows a modelled representation of how the 5 amino-acid insertion of the SGN40 LC CDR1 (bottom loop, black) would impair binding in this region. Below is the sequence alignment between the LC CDR1 of ChiLob 7/4 and SGN40 with the key binding residue starred. (B) hCD40Tg mouse B cells were incubated with FITC-labelled Lob 7/4, Lob 7/6, Lob 7/7 or Lob 7/8 as indicated in the absence or presence of a 10-fold excess of competitor (comp) mAb indicated above each plot. Cell labelling was assessed by flow cytometry; light grey, unlabelled cells; dark grey, FITC-labelled mAb alone; black line, FITC-labelled mAb + competitor. (C) C-terminally His-tagged CD40 proteins consisting of 4, 3, or 2 CRD domains (4 = full length; 3 = CRD1 deleted; 2 = CRD1 and 2 deleted) were analysed by Western blotting with the mAb indicated above each plot used for detection. A composite image from multiple blots is shown.

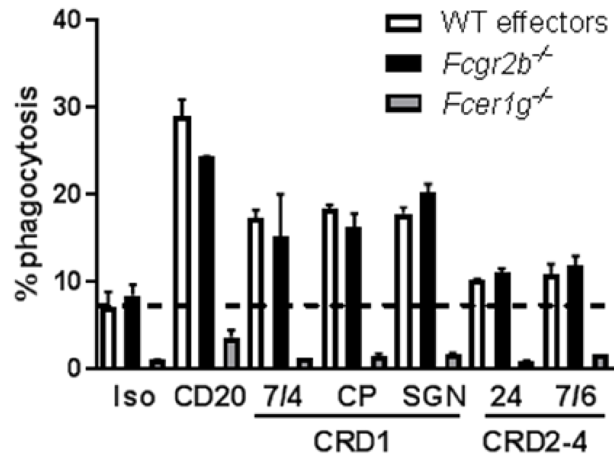

Figure S2. anti-hCD40 mAb-mediated phagocytosis. Related to Figure 4. Purified hCD40Tg mouse splenic B cells were opsonised with the indicated hCD40 mAb, mouse CD20 mAb, or isotype control, all m2a isotype, before incubation with bone marrow derived mouse macrophages from WT, *Fcgr2b*$^{-/-}$ or *Fcer1g*$^{-/-}$ mice. Phagocytosis was measured by flow cytometry as described in STAR Methods. Results (mean ± SEM) from one of 2 independent experiments are shown, * $p<0.05$ versus isotype control for WT cells.

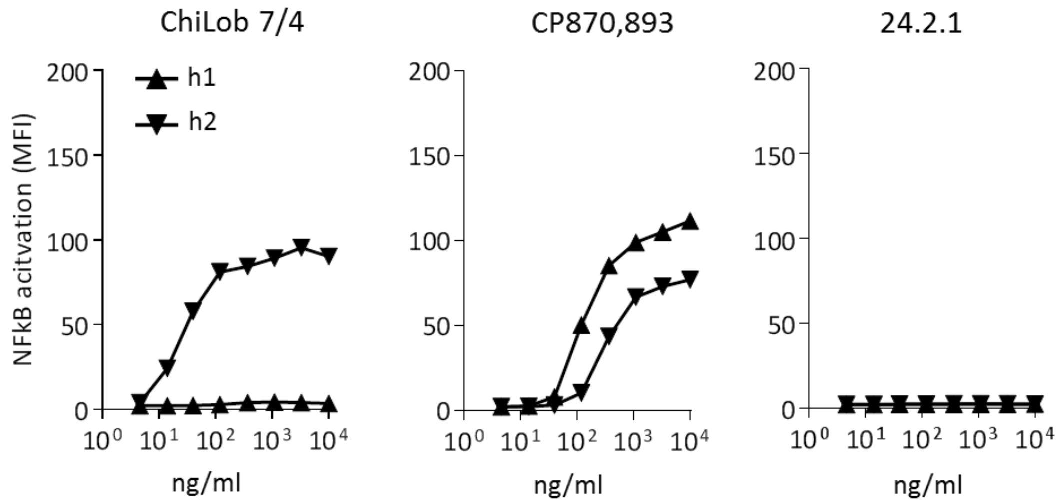

Figure S3. NF- κ B activation by anti-hCD40 mAb. Related to Figure 5. NF-κB/Jurkat/GFP reporter cells stably transfected with hCD40 were incubated with increasing concentrations of the indicated anti-hCD40 mAb of h1 or h2 isotypes for 8 hours, and then the level of NF- κ B activation was assessed by GFP quantification using flow cytometry. Mean ± SEM of triplicates, data representative of at least 2 independent experiments.