FIGURE 1A

Heavy Chain

Variable region shown **in bold**
CDRs highlighted in yellow
Affinity maturation mutated residues underlined
Fc mutations (E→R and K→A) shown in red

```
        10         20         30         40         50         60
EVQLQESGPG LVKPSETLSL TCTVSGDSIT NGFWIWIRKP PGNKLEYMGY ISYSGSTYYN

        70         80         90        100        110        120
PSLKSRISIS RDTSKNQFSL KLSSVTAADT GVYYCAYRSY GRTPYYFDYW GQGTTLTVSS

       130        140        150        160        170        180
ASTKGPSVFP LAPSSKSTSG GTAALGCLVK DYFPEPVTVS WNSGALTSGV HTFPAVLQSS

       190        200        210        220        230        240
GLYSLSSVVT VPSSSLGTQT YICNVNHKPS NTKVDKKAEP KSCDKTHTCP PCPAPELLGG

       250        260        270        280        290        300
PSVFLFPPKP KDTLMISRTP EVTCVVVDVS HRDPEVKFNW YVDGVEVHNA KTKPREEQYN

       310        320        330        340        350        360
STYRVVSVLT VLHQDWLNGK EYKCRVSNKA LPAPIEKTIS KAKGQPREPQ VYTLPPSRDE

       370        380        390        400        410        420
LTKNQVSLTC LVKGFYPSDI AVEWESNGQP ENNYKTTPPV LDSDGSFFLY SKLTVDKSRW

       430        440        450
QQGNVFSCSV MHEALHNHYT QKSLSLSPGK
```

FIGURE 1B

Light Chain

Variable region shown **in bold**
CDRs highlighted in yellow
Affinity maturation mutated residues underlined

```
        10         20         30         40         50         60
DIVMTQSPSF LSASVGDRVT ITCKASSNLG HAVAWYQQKP GKSPKLLIYS ASNRYTGVPD

        70         80         90        100        110        120
RFSGSGSGTD FTLTISSLQP EDFADYFCQQ YDDYPYTFGG GTKLEIKRTV AAPSVFIFPP

       130        140        150        160        170        180
SDEQLKSGTA SVVCLLNNFY PREAKVQWKV DNALQSGNSQ ESVTEQDSKD STYSLSSTLT

       190        200        210
LSKADYEKHK VYACEVTHQG LSSPVTKSFN RGEC
```

FIGURE 1C

INX021 Sequences

Variable Heavy (SEQ ID NO. 1)
EVQLQESGPGLVKPSETLSLTCTVSGDSITNGFWIWIRKPPGNKLEYMGYISYSGSTYYNPSLKSRISISR
DTSKNQFSLKLSSVTAADTGVYYCAYRSYGRTPYYFDYWGQGTTLTVSS

Variable Light (SEQ ID NO. 2)
DIVMTQSPSFLSASVGDRVTITCKASSNLGHAVAWYQQKPGKSPKLLIYSASNRYTGVPDRFSGSGSGTDF
TLTISSLQPEDFADYFCQQYDDYPYTFGGGTKLEIK

Constant Heavy (SEQ ID NO. 3)
ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTV
PSSSLGTQTYICNVNHKPSNTKVDKKAEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEV
TCVVVDVSHRDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCAVSNKALPA
PIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSD
GSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

Constant Light (SEQ ID NO. 4)
RTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSST
LTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

INX021 Variable Heavy CDR1 (SEQ ID NO: 5)
GDSITNGFWI

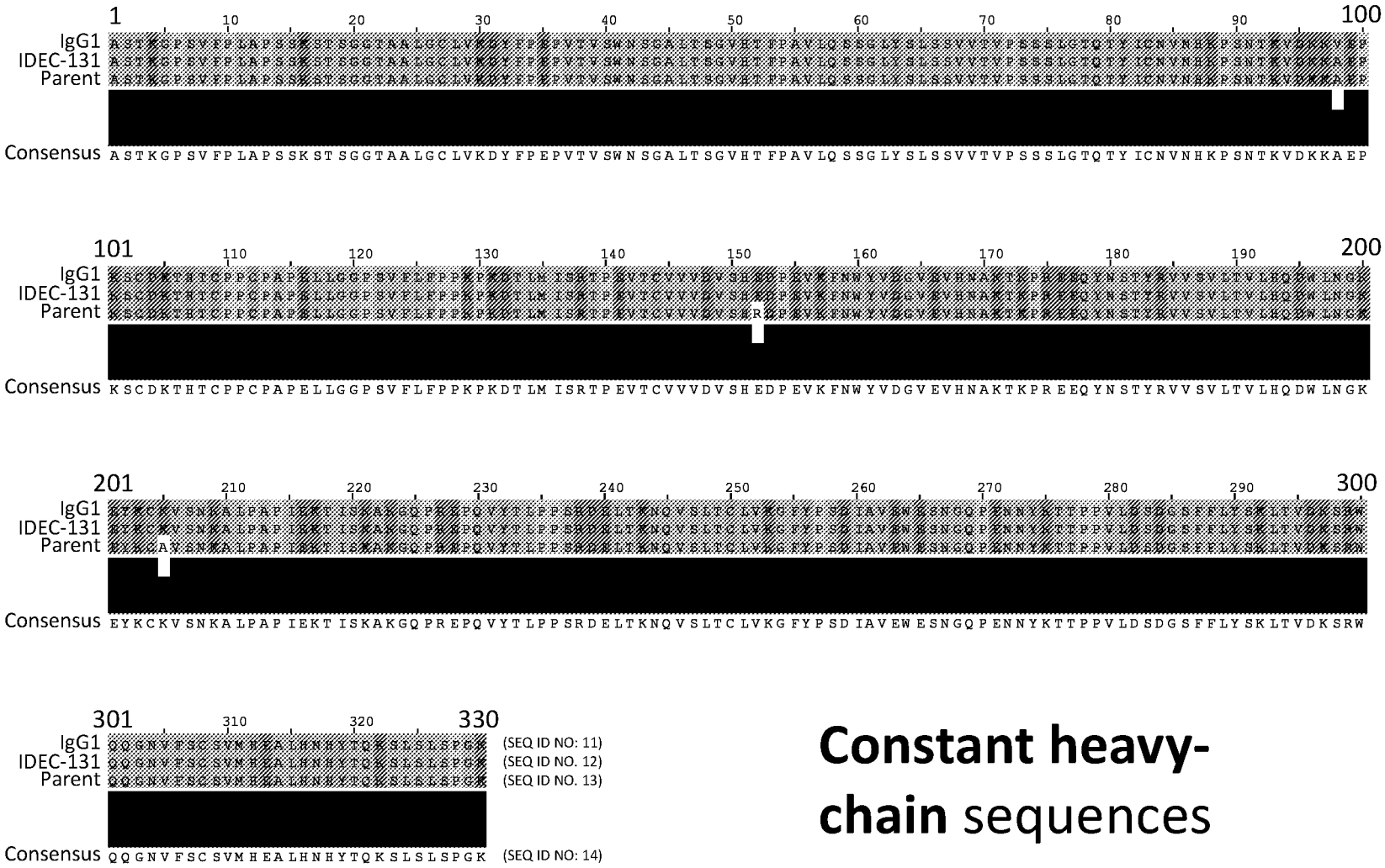
INX021 Variable Heavy CDR2 (SEQ ID NO: 6)
YISYSGSTY

INX021 Variable Heavy CDR3 (SEQ ID NO: 7)
YRSYGRTPYYFDY

INX021 Variable Light CDR1 (SEQ ID NO: 8)
KASSNLGHAVA

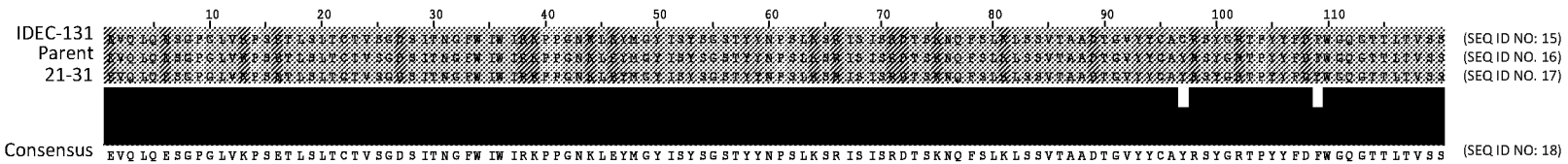
INX021 Variable Light CDR2 (SEQ ID NO: 9)
SASNRYT

INX021 Variable Light CDR3 (SEQ ID NO: 10)
QQYDDYPYT



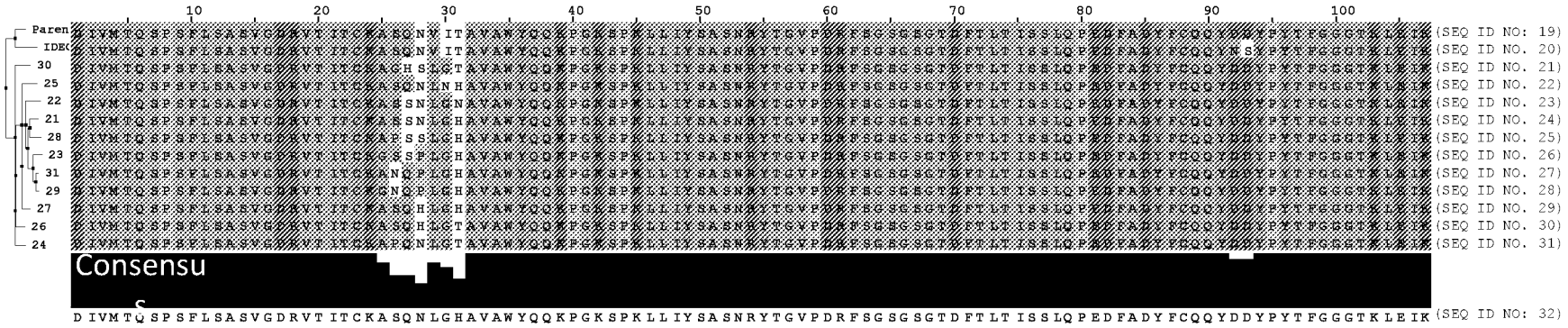
Constant heavy-chain sequences

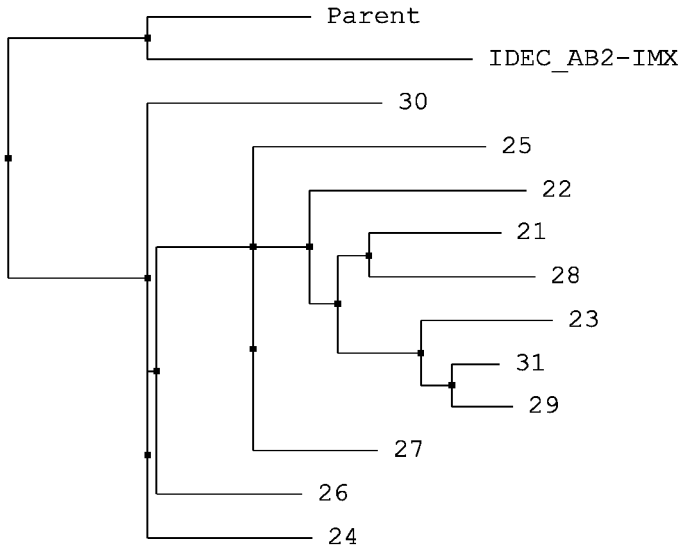
FIG. 2A



Variable heavy-chain sequences

FIG. 2B





Variable light-chain sequences

FIG. 2C

Improved potency: using IDEC epitope

FIG. 3

INX021 shows better binding to CD40L on T cells

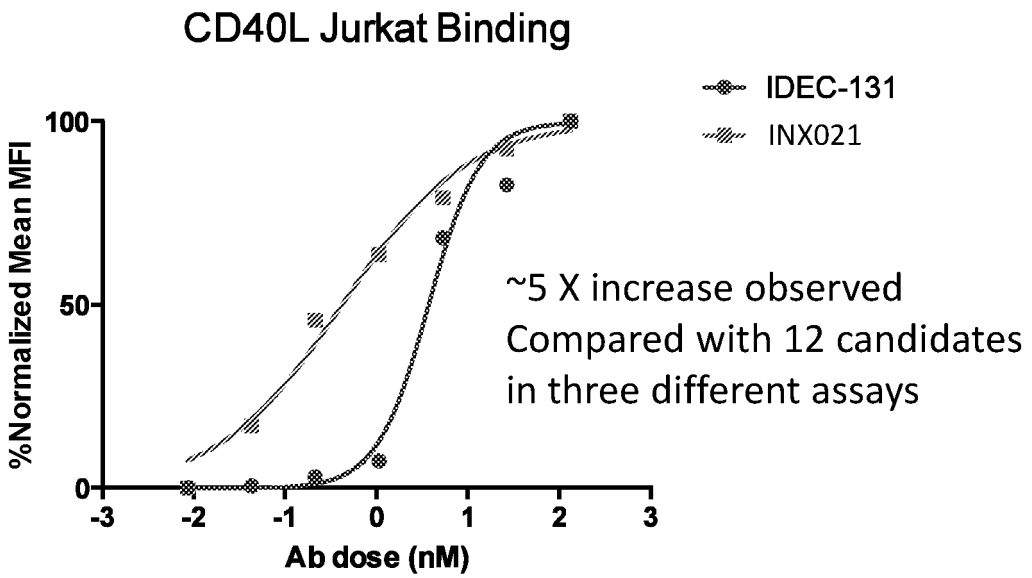
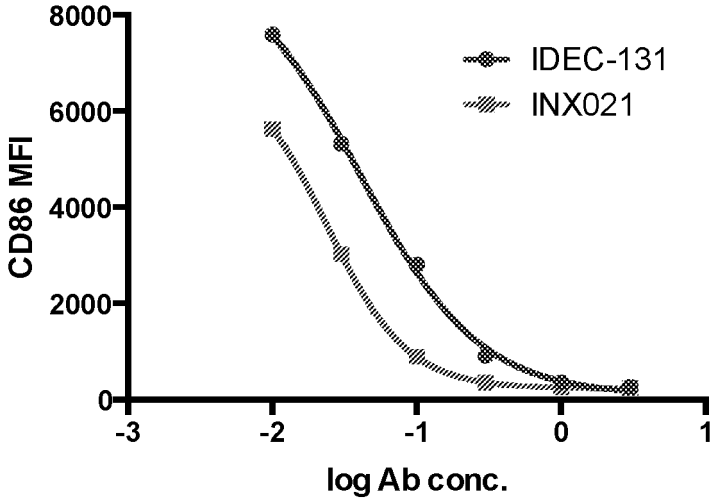


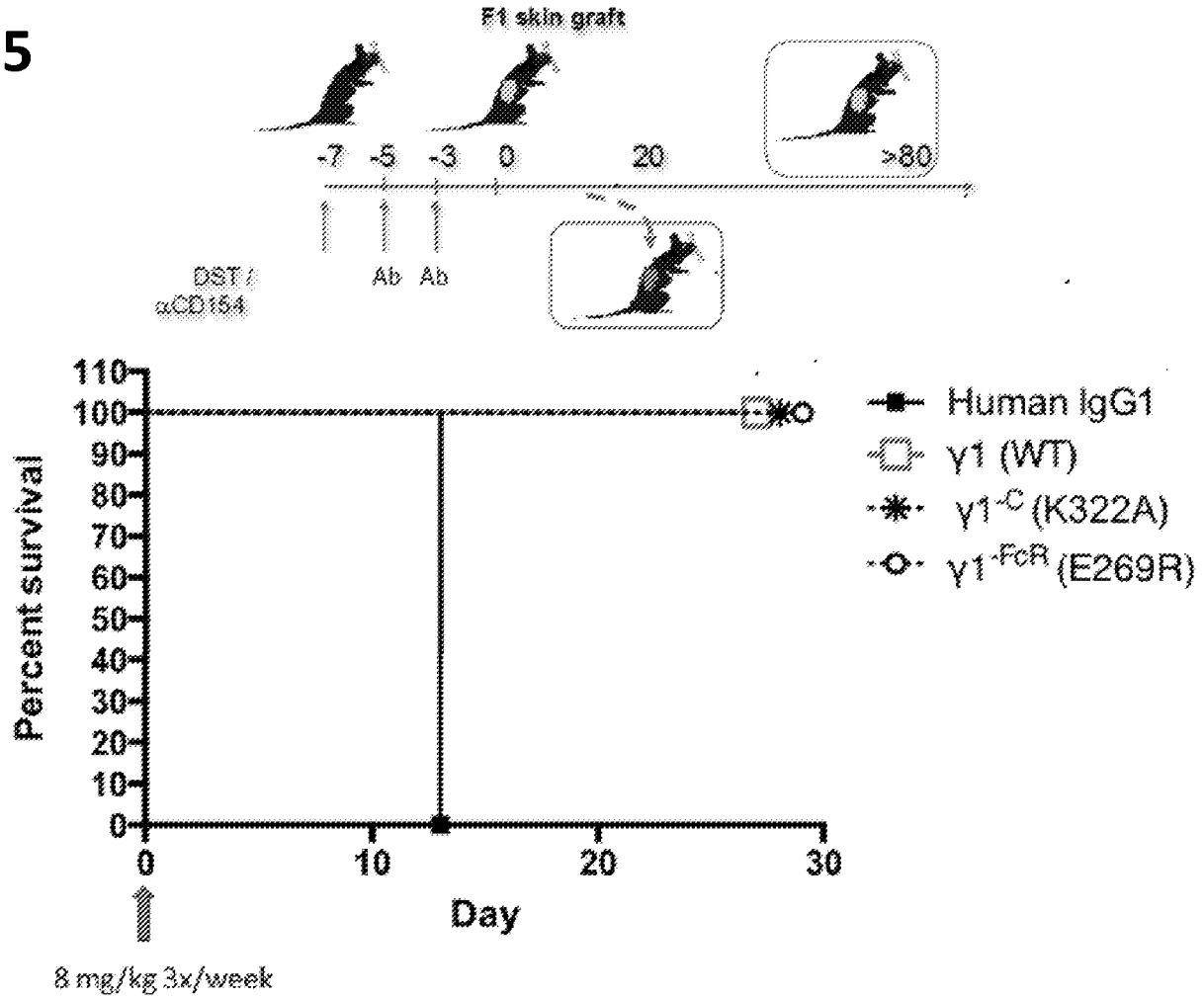
FIG. 4

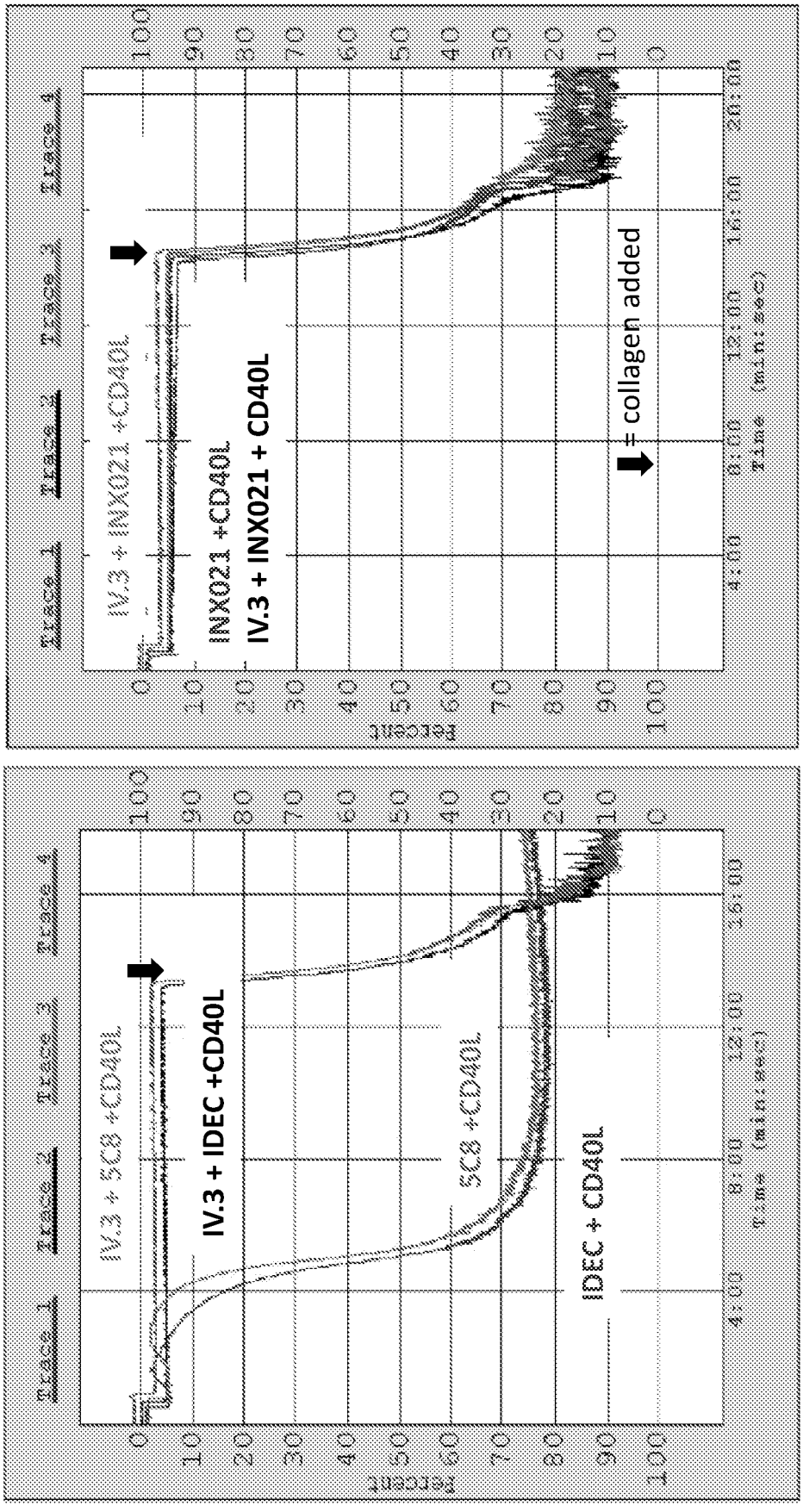
INX021 shows more potent inhibition of T cell CD40L-driven B cell activation



FcR mutants of hIgG anti-mCD40L induce tolerance

FIG. 5





Immune Complexes of FcR mutants did not induce isolated mouse and human platelet activation in vitro.

FIG. 6

Lack of platelet activities by FcR mutants of hIgG1 anti-huCD40L in vivo

FcR mutants did not induce thrombotic stress, reduced platelet number, or clots in the lung.

FIG. 7A

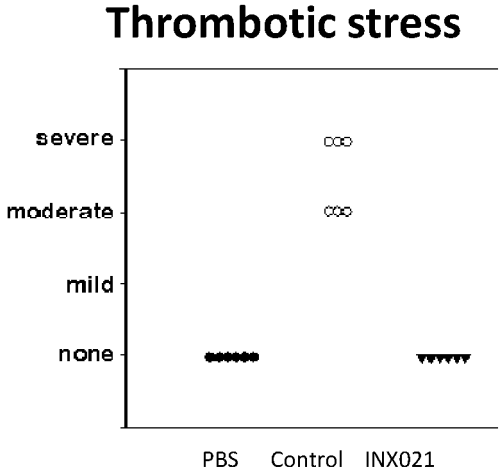


FIG. 7C

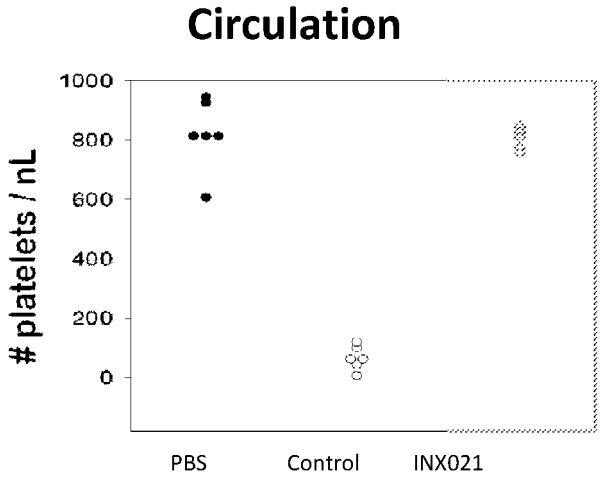


FIG. 7B

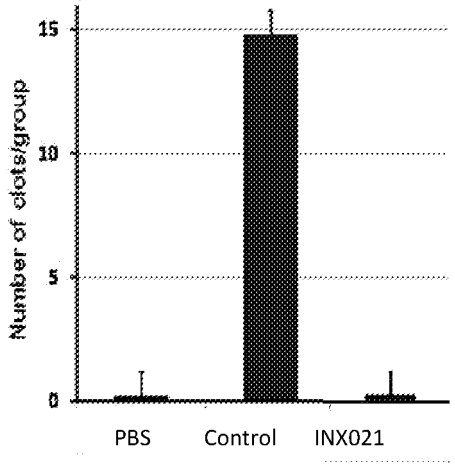
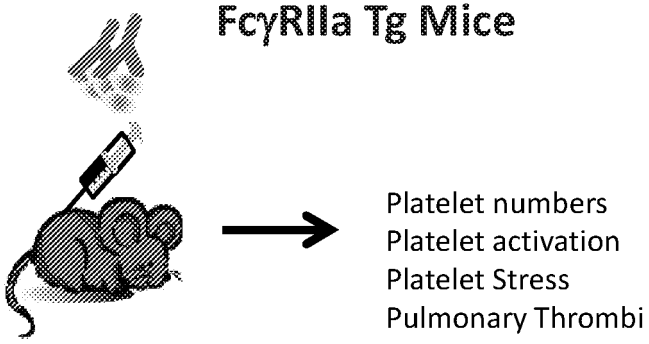


FIG. 7D



Activity of FcR/C1q mutant anti-mCD40L abs

FcR/C1q-binding mutants are still active in models of interest such as EAE, tolerance and immune response to MOG.

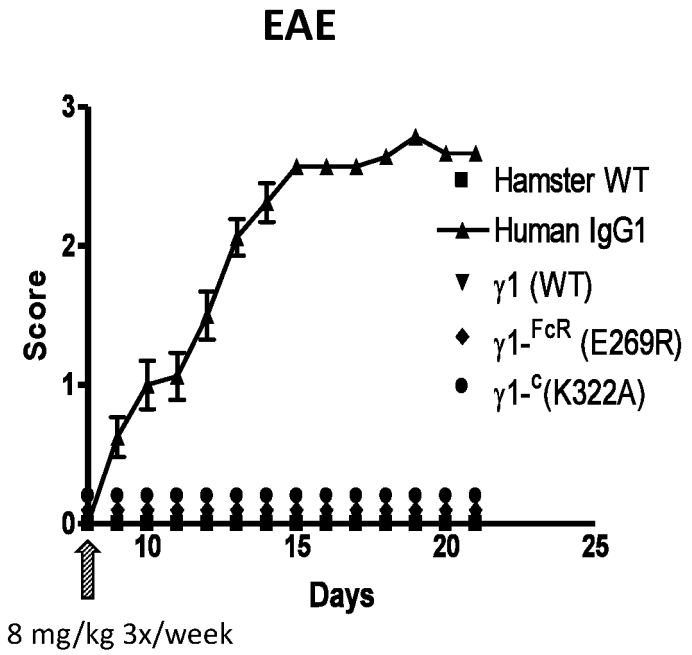


FIG. 8A

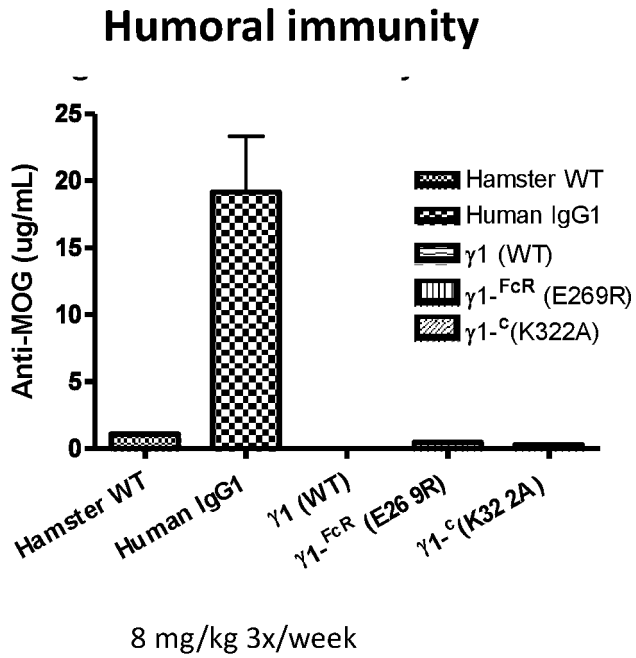


FIG. 8B

FIG. 9

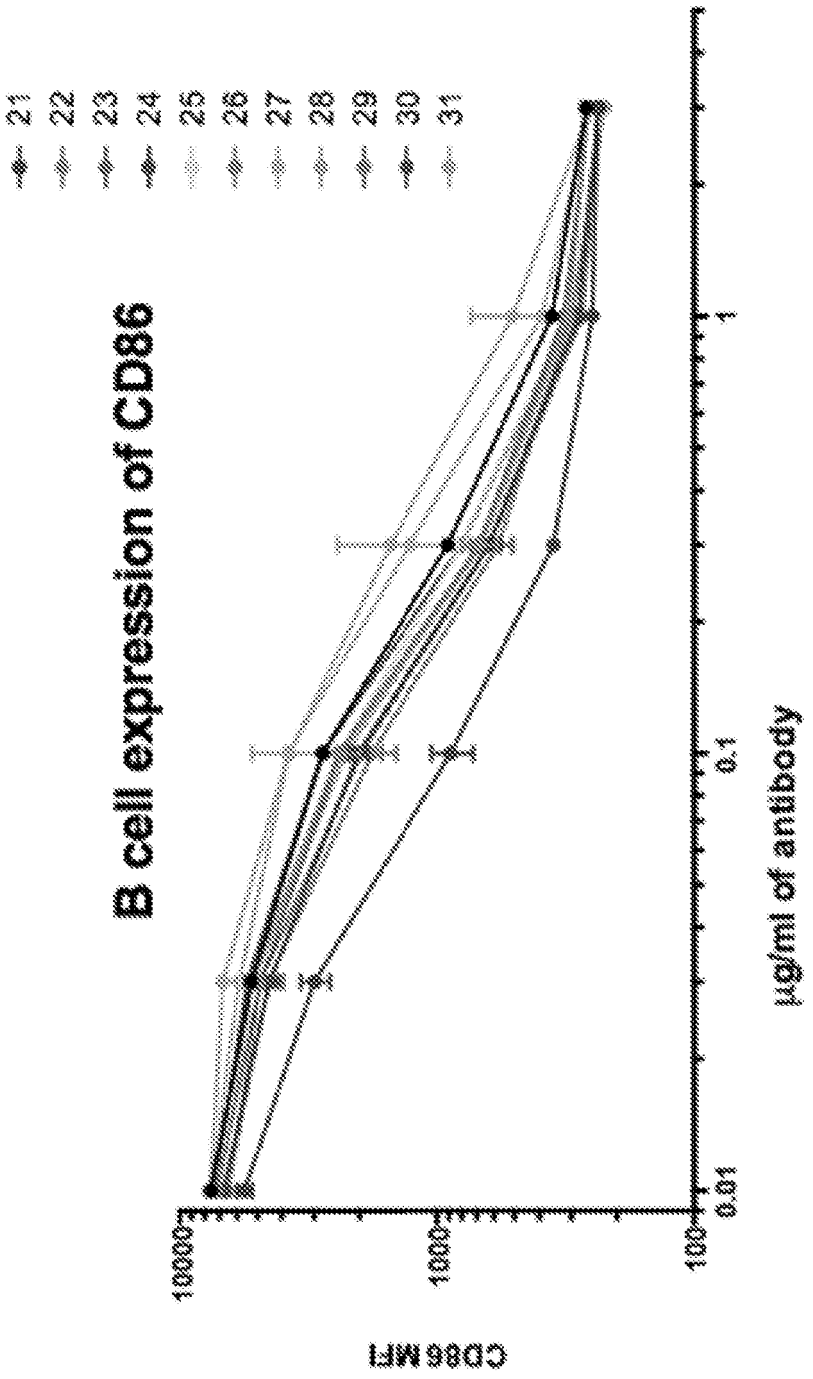

B cell expression of CD86
21
22
23
24
25
26
27
28
29
30
31
CD86 MFI
10000
1000
100
0.01
0.1
1
µg/ml of antibody

FIGURE 10A

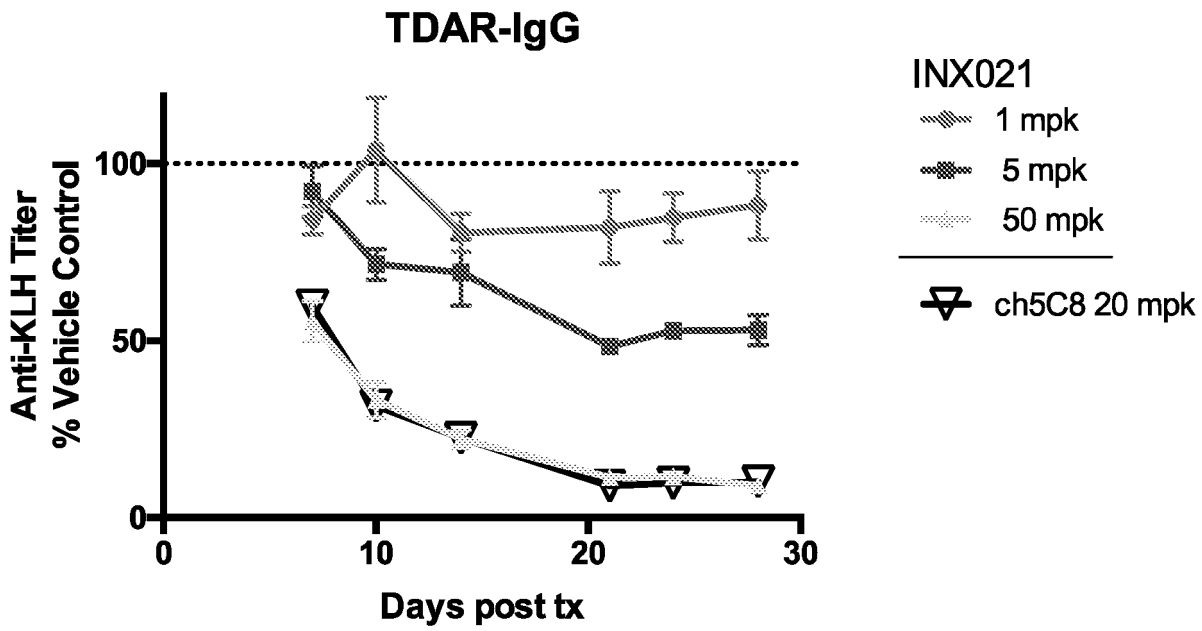
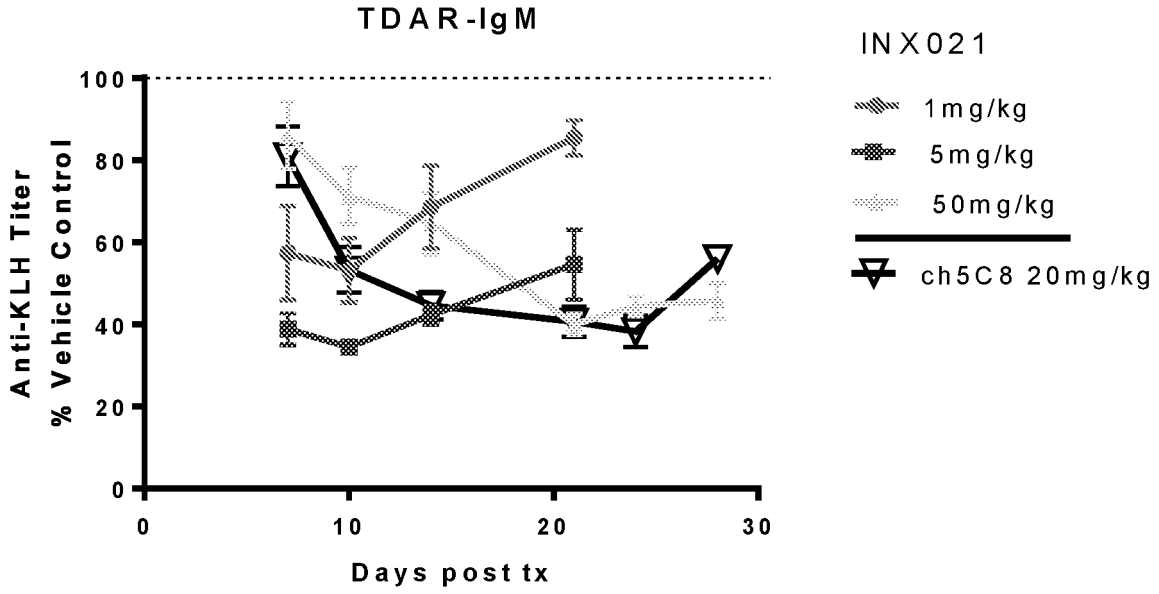
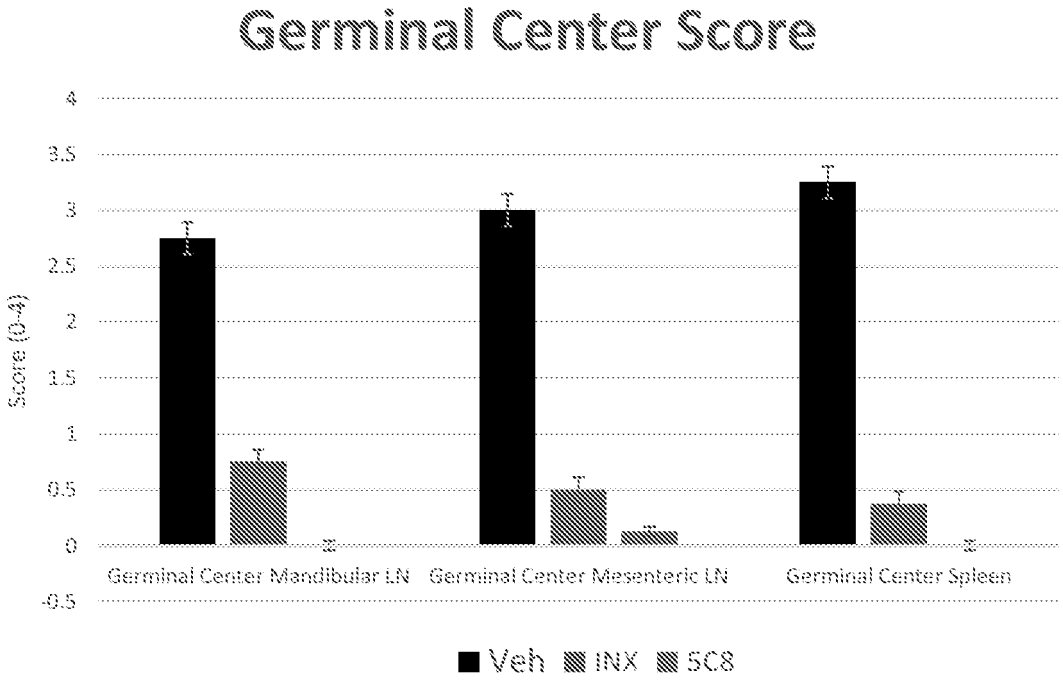


FIGURE 10B



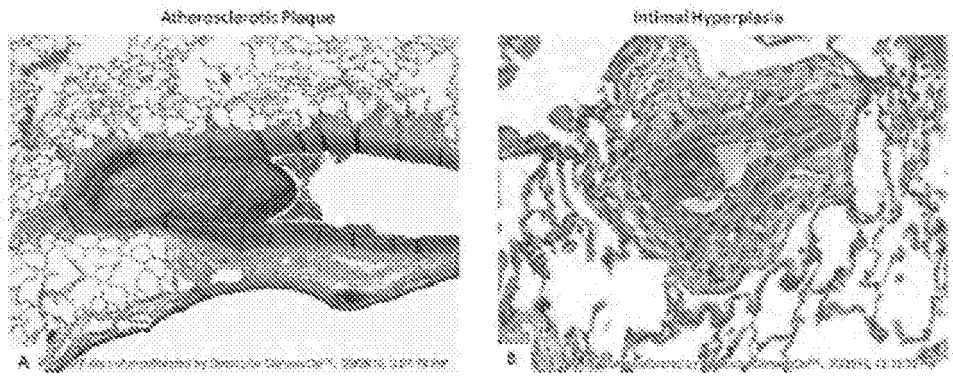
TDAR results: IgG TDAR (TOP) compared with saline controls (dotted line). IgM TDAR (bottom) compared with saline controls (dotted line).

FIGURE 11A

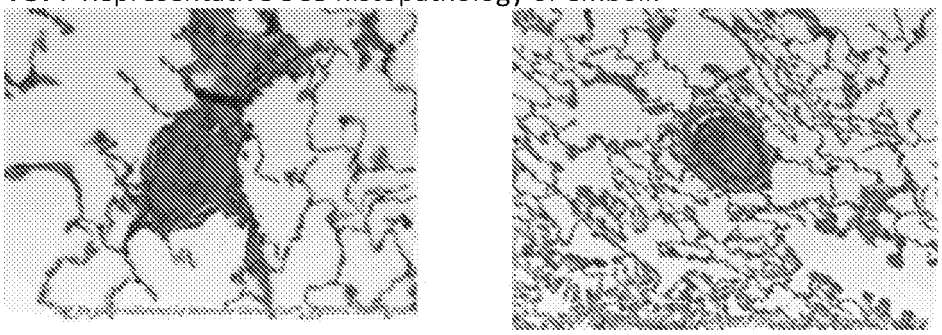


Germinal Center scores (above): Number and degree of cellularity of the germinal centers in spleen and LN of treated animals.

FIGURE 11B



TOP: Representative 5C8 histopathology of emboli.



Bottom: Representative histopathology of saline (left) and INX021 (right) emboli

FIGURE 12

Summary of lesion frequency compared with published UCB emboli data:

(CDP7657, an anti-CD40L antibody lacking an Fc domain, inhibits CD40L-dependent immune responses without thrombotic complications: an in vivo study Arthritis Res Ther. 2015; 17(1): 234.)

Dose Group	Animals affected (%)	Total lung sections examined	Lung sections affected (%)
Historic Controls			
Saline	7 (50%)	406	2.5%
Biogen/UCB Pegylated Fab			
Saline	1 (25%)	116	0.9%
Peg-Fab CDP765	3 (37.5%)	203	2.0%
Aglycosyl 5C8	3 (37.5%)	232	1.3%
hu5C8	5 (62.5%)	232	17.6%
ImmuNext INX021			
Vehicle	2 (50%)	174	1.7%
INX021	3 (37.5%)	232	3.4%
ch5C8	6 (75%)	232	3.8%