FIG. 1

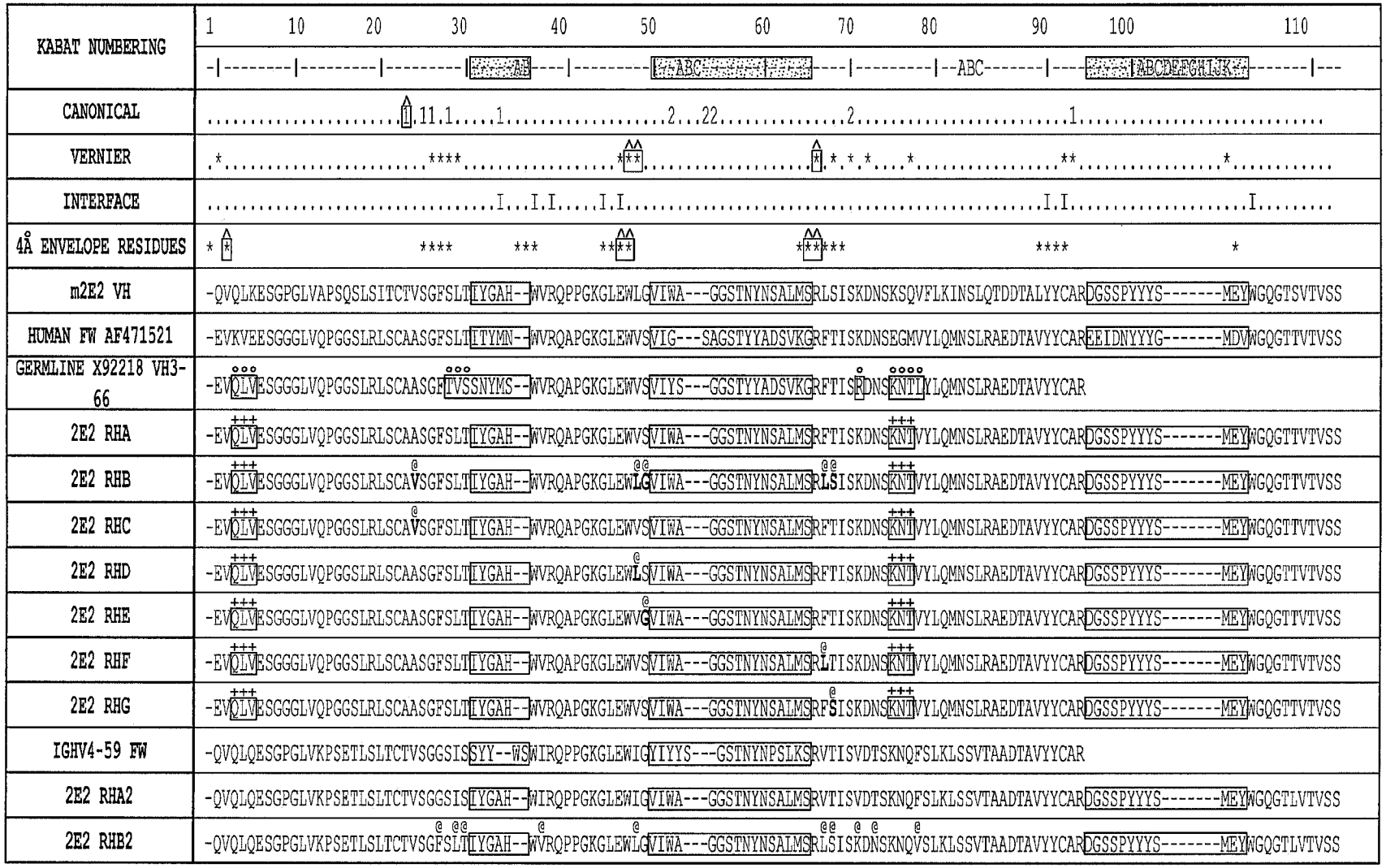

KABAT NUMBERING
CANONICAL
VERNIER
INTERFACE
4Å ENVELOPE RESIDUES
m2E2 VH
HUMAN FW AF471521
GERMLINE X92218 VH3-66
2E2 RHA
2E2 RHB
2E2 RHC
2E2 RHD
2E2 RHE
2E2 RHF
2E2 RHG
IGHV4-59 FW
2E2 RHA2
2E2 RHB2

FIG. 2

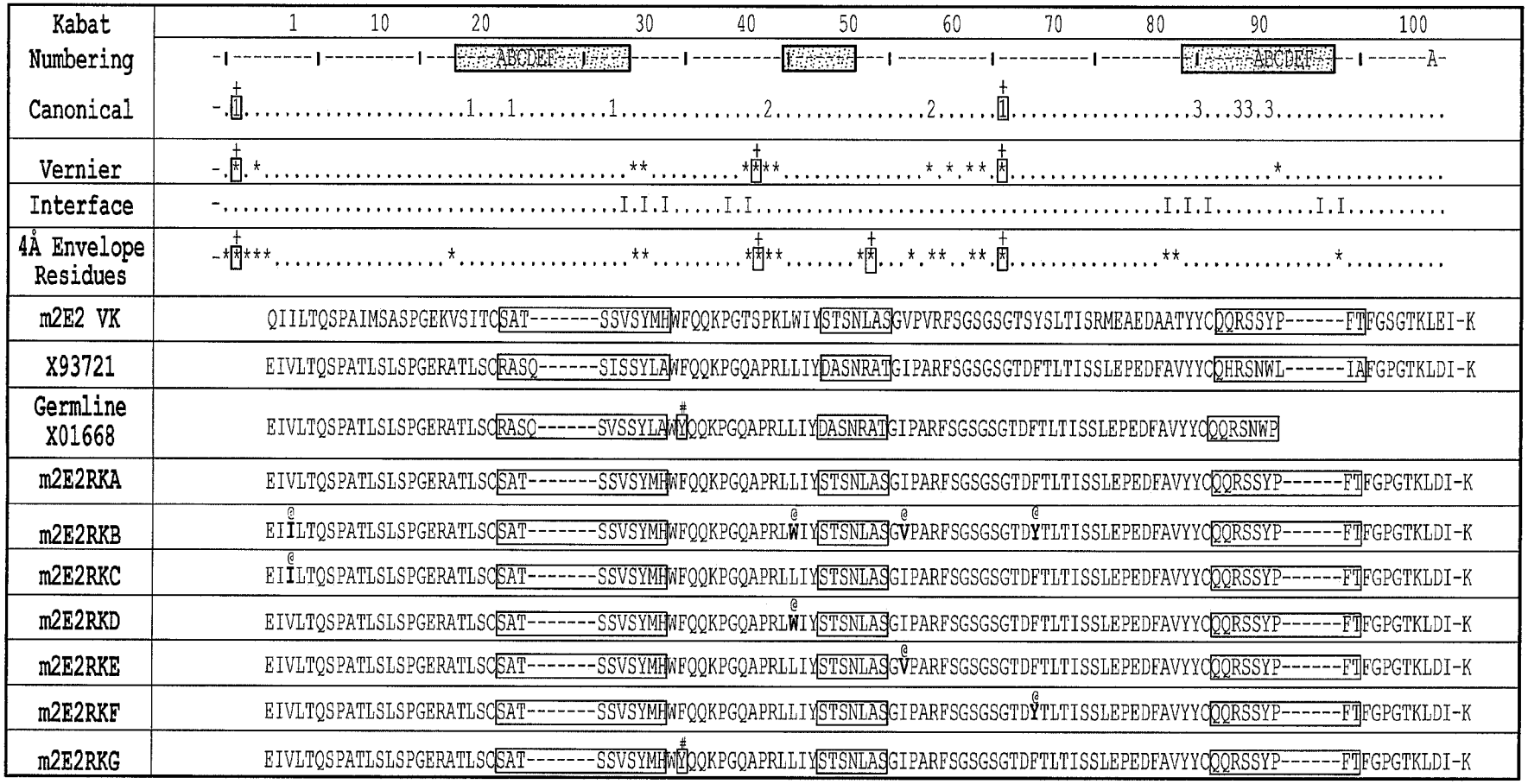

Kabat Numbering
Canonical
Vernier
Interface
4Å Envelope Residues
m2E2 VK
X93721
Germline X01668
m2E2RKA
m2E2RKB
m2E2RKC
m2E2RKD
m2E2RKE
m2E2RKF
m2E2RKG

FIG. 3

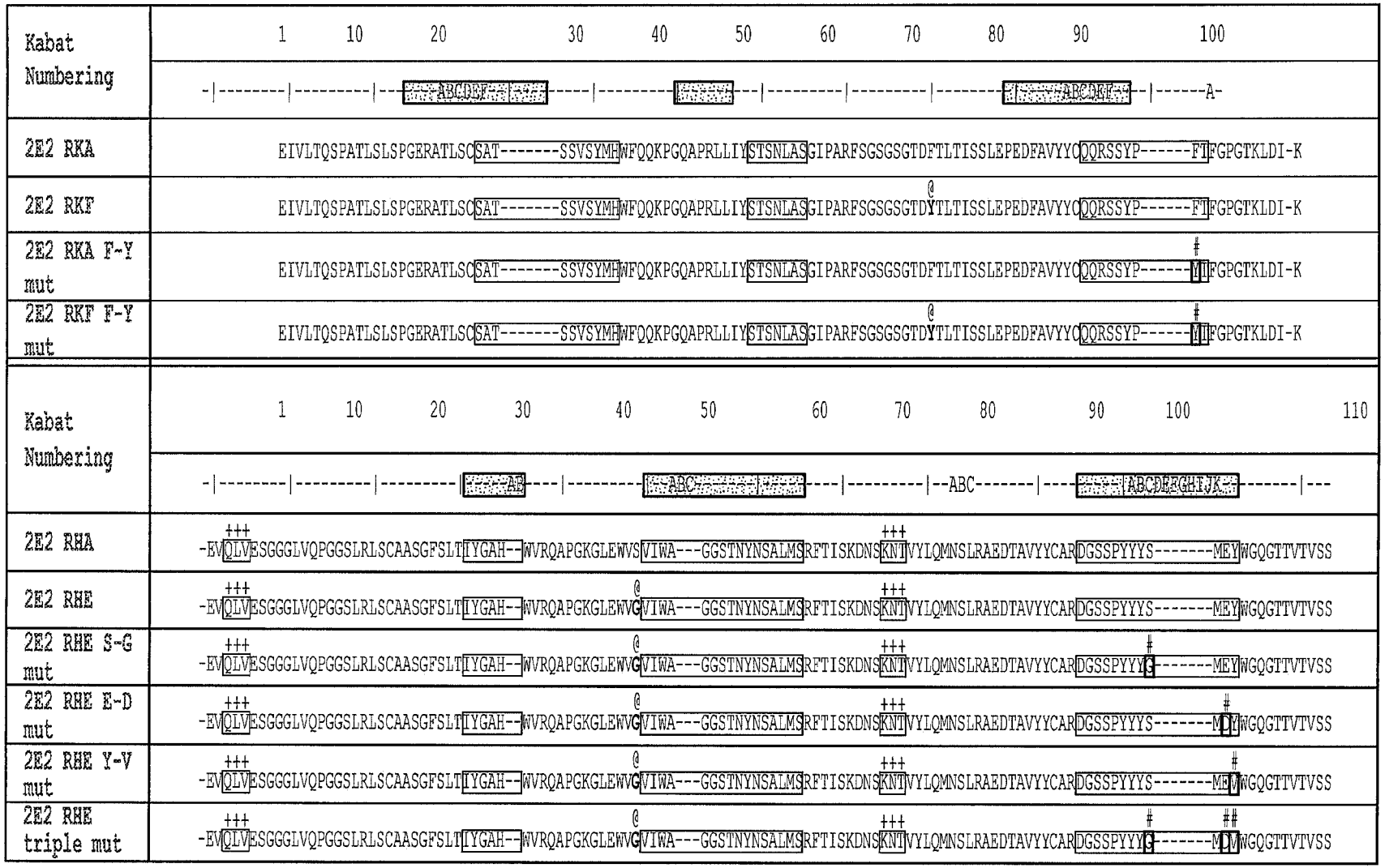

Kabat Numbering
1 10 20 30 40 50 60 70 80 90 100
2E2 RKA
EIVLTQSPATLSLSPGERATLSCSAT-------SSVSYMHWFQQKPGQAPRLLIYSTSNLASGIPARFSGSGSGTDFTLTISSLEPEDFAVYYCQQRSSYP------FTFGPGTKLDI-K
2E2 RKF
EIVLTQSPATLSLSPGERATLSCSAT-------SSVSYMHWFQQKPGQAPRLLIYSTSNLASGIPARFSGSGSGTDYTLTISSLEPEDFAVYYCQQRSSYP------FTFGPGTKLDI-K
2E2 RKA F-Y mut
EIVLTQSPATLSLSPGERATLSCSAT-------SSVSYMHWFQQKPGQAPRLLIYSTSNLASGIPARFSGSGSGTDFTLTISSLEPEDFAVYYCQQRSSYP------MFFGPGTKLDI-K
2E2 RKF F-Y mut
EIVLTQSPATLSLSPGERATLSCSAT-------SSVSYMHWFQQKPGQAPRLLIYSTSNLASGIPARFSGSGSGTDYTLTISSLEPEDFAVYYCQQRSSYP------MFFGPGTKLDI-K
Kabat Numbering
1 10 20 30 40 50 60 70 80 90 100 110
2E2 RHA
-EVQLVESGGGLVQPGGSLRLSCAASGFSLTIYGAH--WVRQAPGKGLEWVSVIWA---GGSTNYNSALMSRFTISKDNSKNTVYLQMNSLRAEDTAVYYCARDGSSPYYYS-------MEYWGQGTTVTVSS
2E2 RHE
-EVQLVESGGGLVQPGGSLRLSCAASGFSLTIYGAH--WVRQAPGKGLEWVGVIWA---GGSTNYNSALMSRFTISKDNSKNTVYLQMNSLRAEDTAVYYCARDGSSPYYYS-------MEYWGQGTTVTVSS
2E2 RHE S-G mut
-EVQLVESGGGLVQPGGSLRLSCAASGFSLTIYGAH--WVRQAPGKGLEWVGVIWA---GGSTNYNSALMSRFTISKDNSKNTVYLQMNSLRAEDTAVYYCARDGSSPYYYG-------MEYWGQGTTVTVSS
2E2 RHE E-D mut
-EVQLVESGGGLVQPGGSLRLSCAASGFSLTIYGAH--WVRQAPGKGLEWVGVIWA---GGSTNYNSALMSRFTISKDNSKNTVYLQMNSLRAEDTAVYYCARDGSSPYYYS-------MDYWGQGTTVTVSS
2E2 RHE Y-V mut
-EVQLVESGGGLVQPGGSLRLSCAASGFSLTIYGAH--WVRQAPGKGLEWVGVIWA---GGSTNYNSALMSRFTISKDNSKNTVYLQMNSLRAEDTAVYYCARDGSSPYYYS-------MEVWGQGTTVTVSS
2E2 RHE triple mut
-EVQLVESGGGLVQPGGSLRLSCAASGFSLTIYGAH--WVRQAPGKGLEWVGVIWA---GGSTNYNSALMSRFTISKDNSKNTVYLQMNSLRAEDTAVYYCARDGSSPYYYG-------MDVWGQGTTVTVSS

FIG. 4

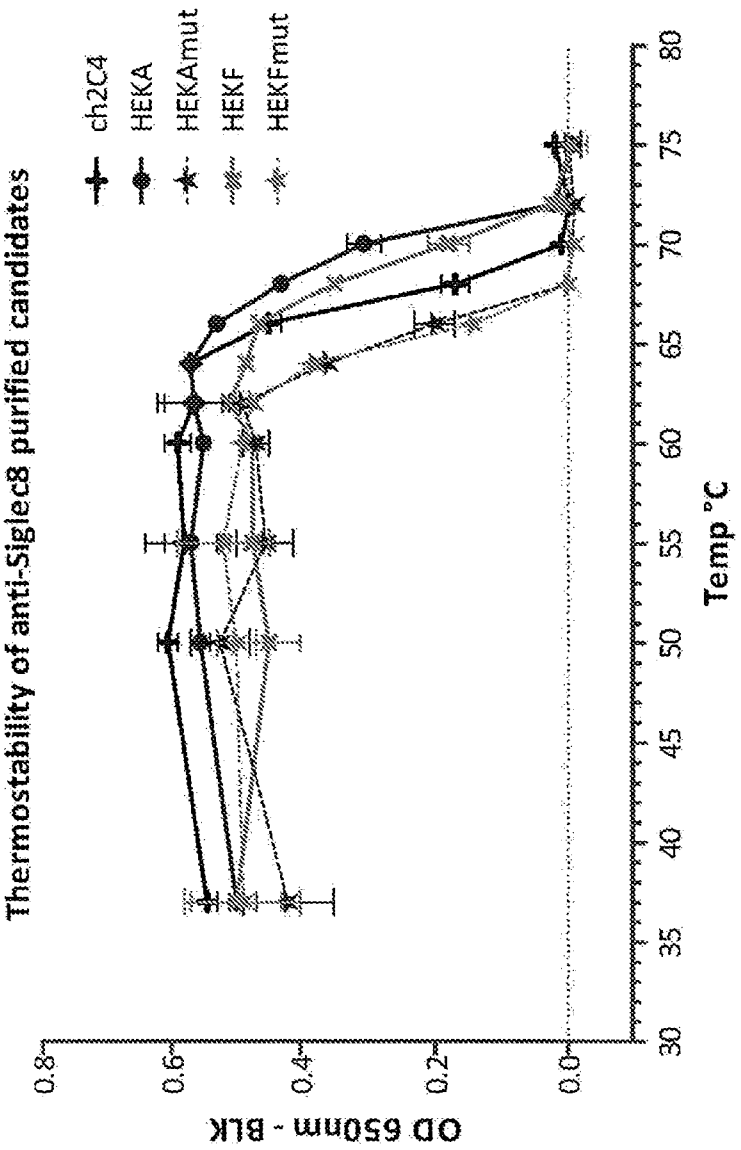

Thermostability of anti-Siglec8 purified candidates
ch2C4
HEKA
HEKAmut
HEKF
HEKFmut
OD 650nm - BLK
0.8
0.6
0.4
0.2
0.0
30
35
40
45
50
55
60
65
70
75
80
Temp °C

FIG. 5

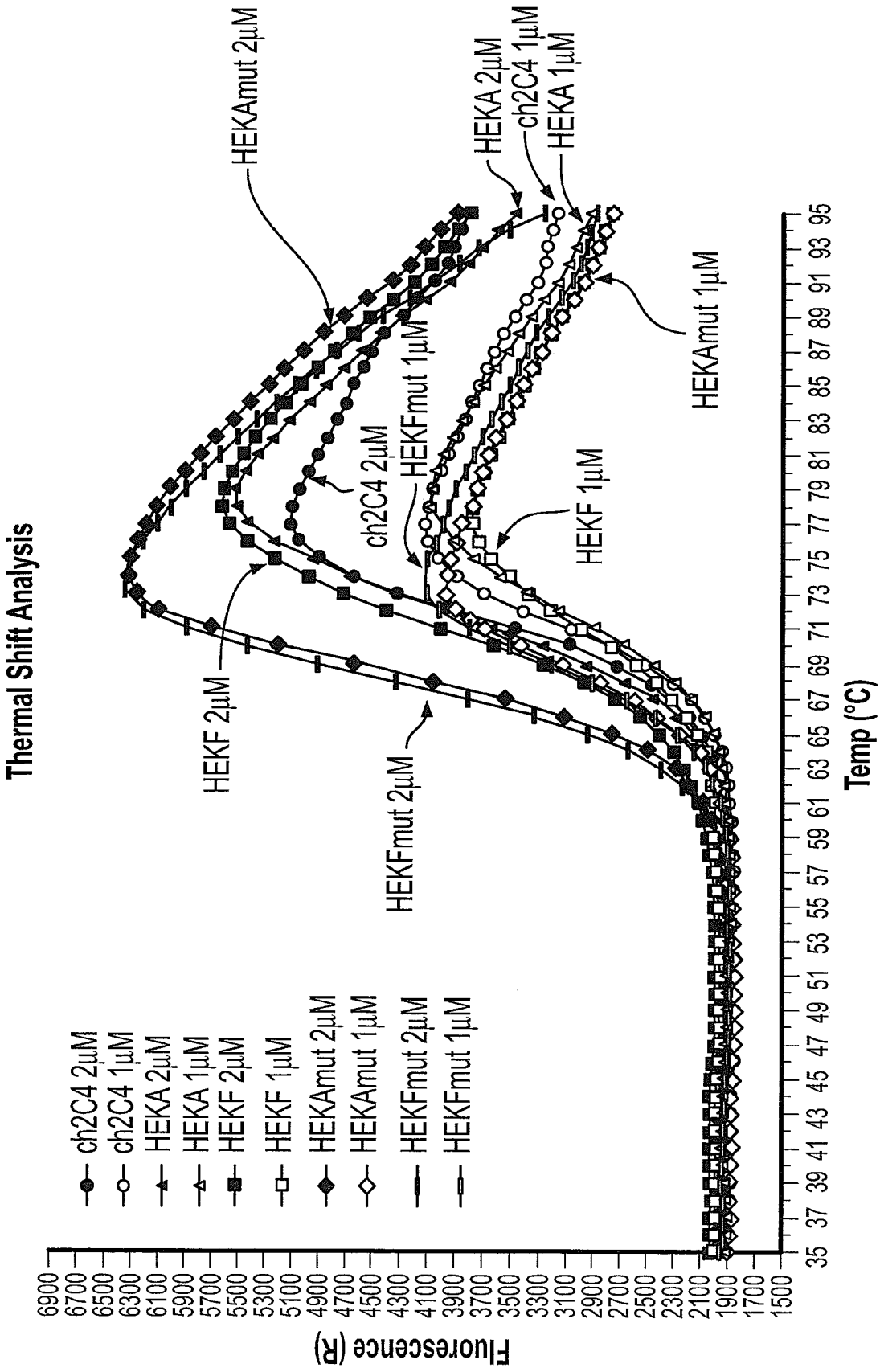

Thermal Shift Analysis
Fluorescence (R)
Temp (°C)
ch2C4 2µM
ch2C4 1µM
HEKA 2µM
HEKA 1µM
HEKF 2µM
HEKF 1µM
HEKAmut 2µM
HEKAmut 1µM
HEKFmut 2µM
HEKFmut 1µM
HEKAmut 2µM
HEKA 2µM
ch2C4 1µM
HEKA 1µM
HEKF 2µM
ch2C4 2µM
HEKFmut 1µM
HEKF 1µM
HEKFmut 2µM
HEKAmut 1µM

FIG. 6

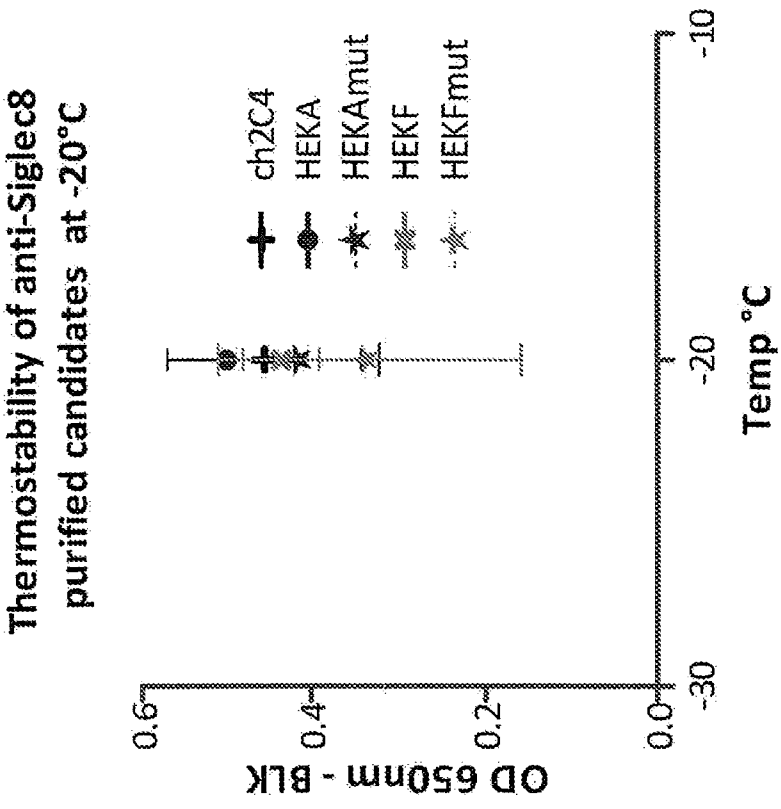

Thermostability of anti-Siglec8
purified candidates at -20°C
OD 650nm - BLK
0.6
0.4
0.2
0.0
-30
-20
-10
Temp °C
ch2C4
HEKA
HEKAmut
HEKF
HEKFmut

FIG. 7

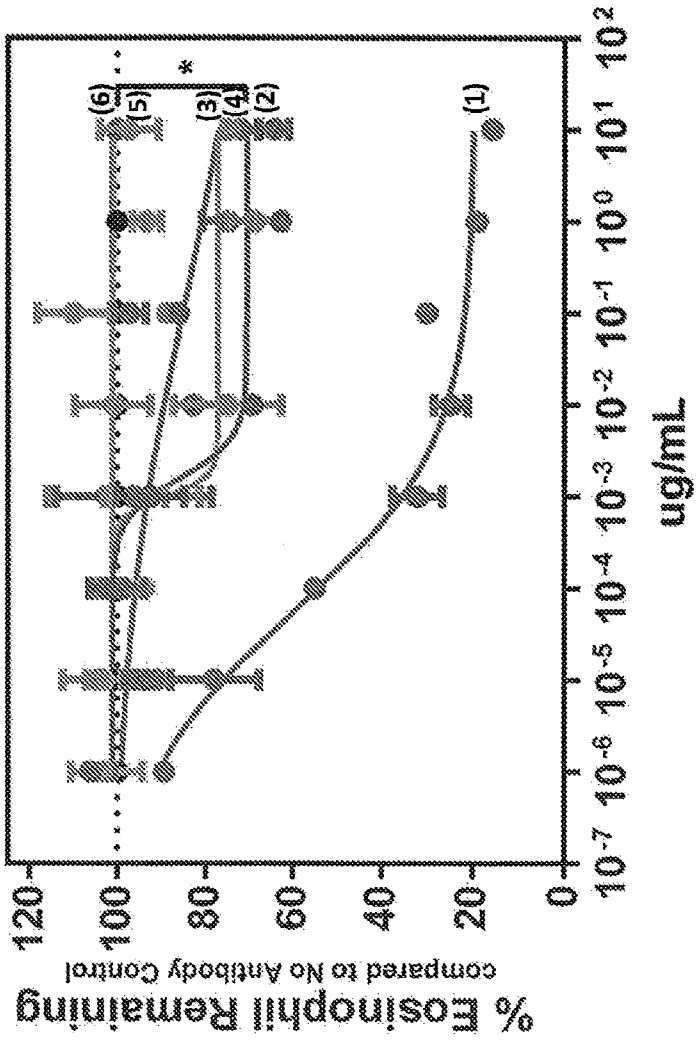

(1) Low-fucose Chimeric 2E2 IgG1
(2) Fucosylated Chimeric 2E2 IgG1
(3) Chimeric 2E2 IgG4
(4) Mouse 2E2 IgG1
(5) Isotype control IgG1
(6) No antibody
% Eosinophil Remaining
compared to No Antibody Control
120
100
80
60
40
20
0
*
(6)
(5)
(3)
(4)
(2)
(1)
10^{-7} 10^{-6} 10^{-5} 10^{-4} 10^{-3} 10^{-2} 10^{-1} 10^{0} 10^{1} 10^{2}
ug/mL

FIG. 8A

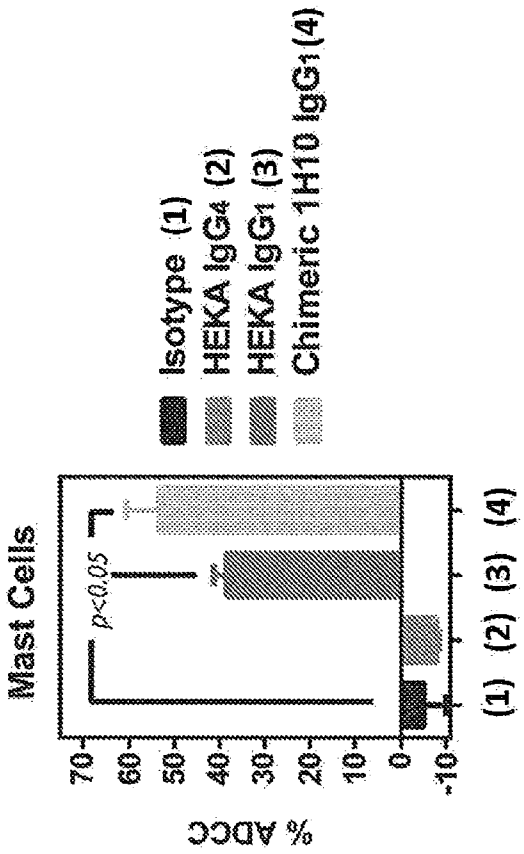

Mast Cells
p<0.05
% ADCC
70
60
50
40
30
20
10
0
-10
(1) (2) (3) (4)
Isotype (1)
HEKA IgG4 (2)
HEKA IgG1 (3)
Chimeric 1H10 IgG1 (4)

FIG. 8B

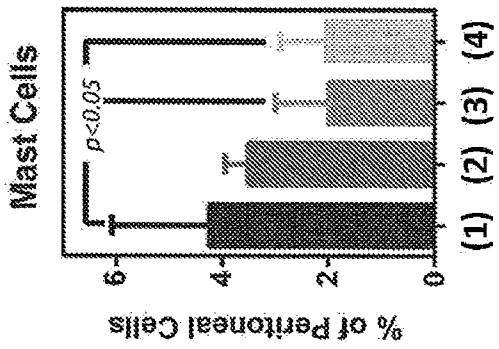
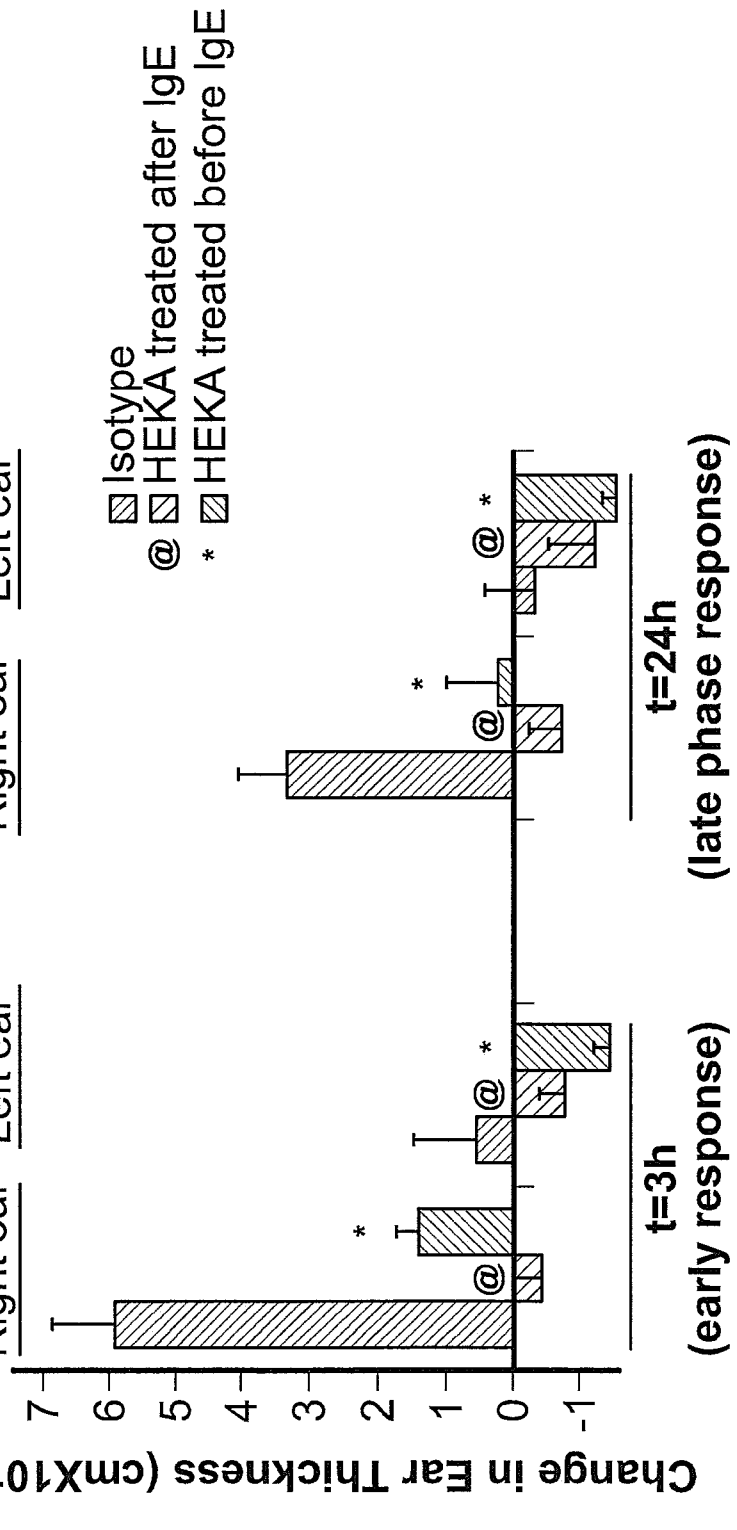

Mast Cells
p<0.05
% of Peritoneal Cells
6
4
2
0
(1) (2) (3) (4)
Isotype (1)
HEKA IgG4 (2)
HEKA IgG1 (3)
1C3 (4)

FIG. 9


Change in Ear Thickness (cmX10-3)
7
6
5
4
3
2
1
0
-1
IgE
Right ear
PBS
Left ear
IgE
Right ear
PBS
Left ear
Isotype
@ HEKA treated after IgE
* HEKA treated before IgE
t=3h
(early response)
t=24h
(late phase response)

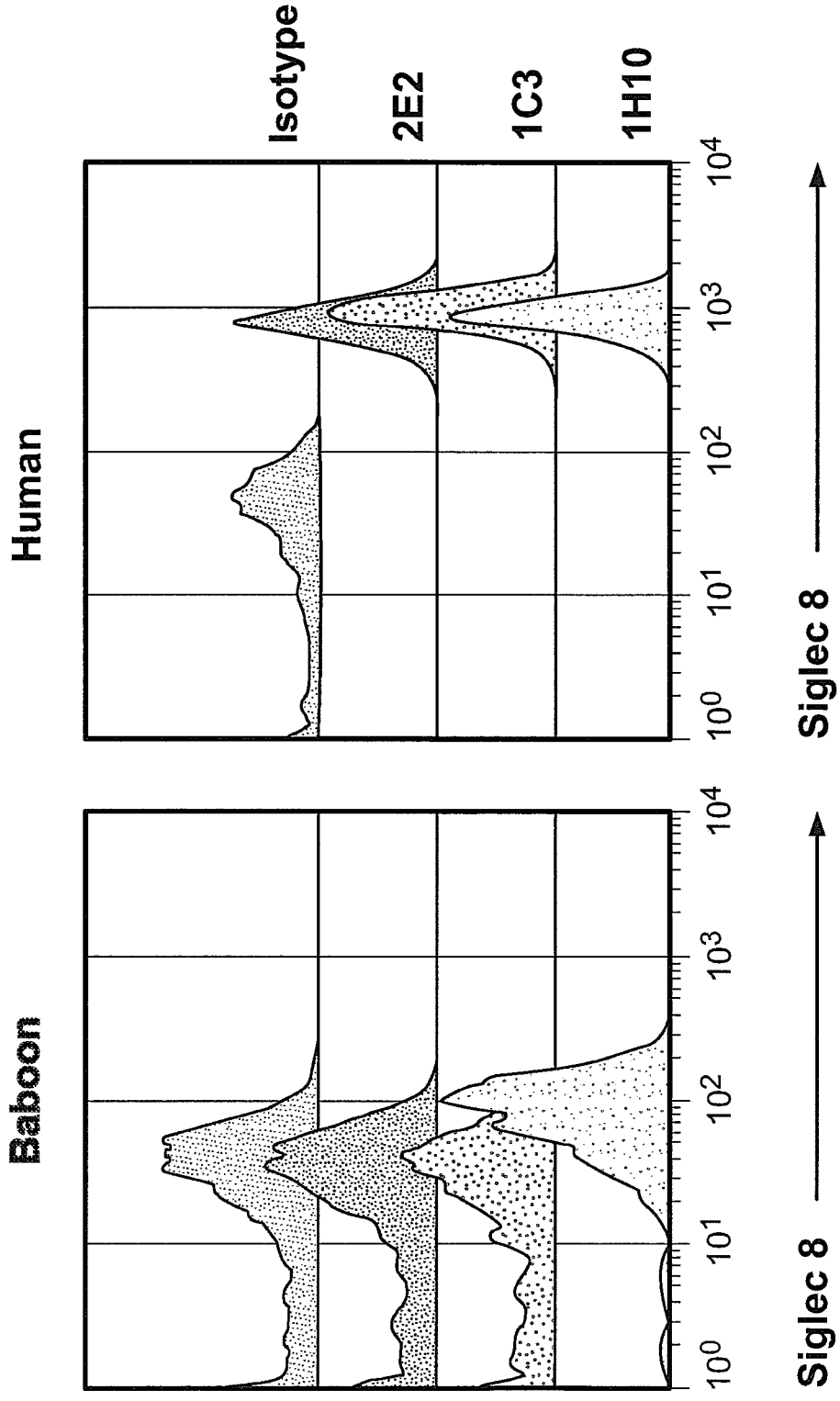

Human
Baboon
Isotype
2E2
1C3
1H10
10^0
10^1
10^2
10^3
10^4
Siglec 8
Siglec 8

FIG. 10