Supplement 1

A

HLA-A*02
K_D: 190nM

MSLN
K_D: 30nM

ICAM-1
K_D: 39nM

B

ng mRNA:
1000
500
250
125
63
31
16
8
4
2
1
0.5
0.24
0.12
0

Anti-HLA-A*02

ng mRNA:
2000
1000
500
250
125
63
31
16
8
4
2
1
0.5
0.24
0

Anti-MSLN

ng mRNA:
1000
500
250
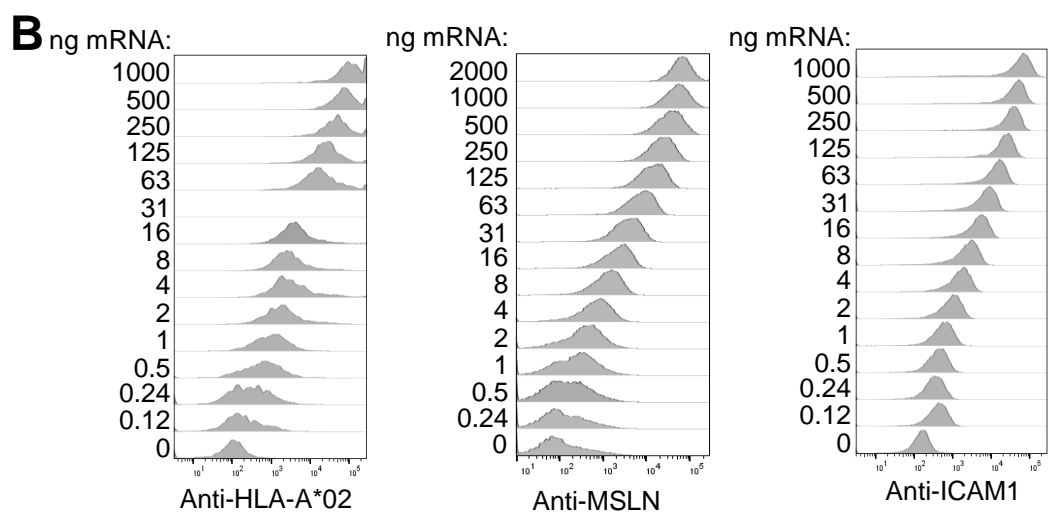125
63
31
16
8
4
2
1
0.5
0.24
0.12
0

Anti-ICAM1

C

- HLA-A*02
- MSLN
- ICAM1
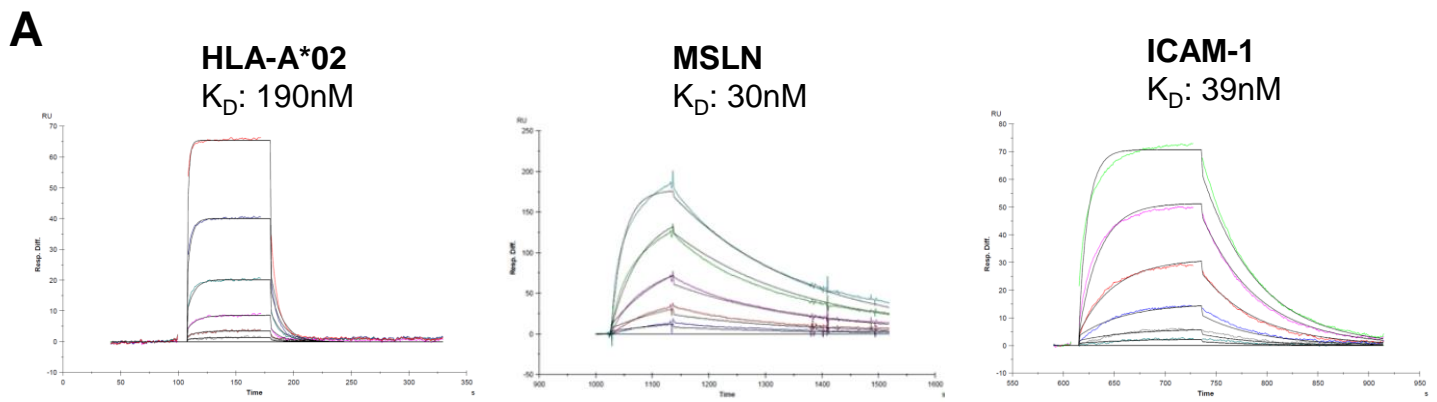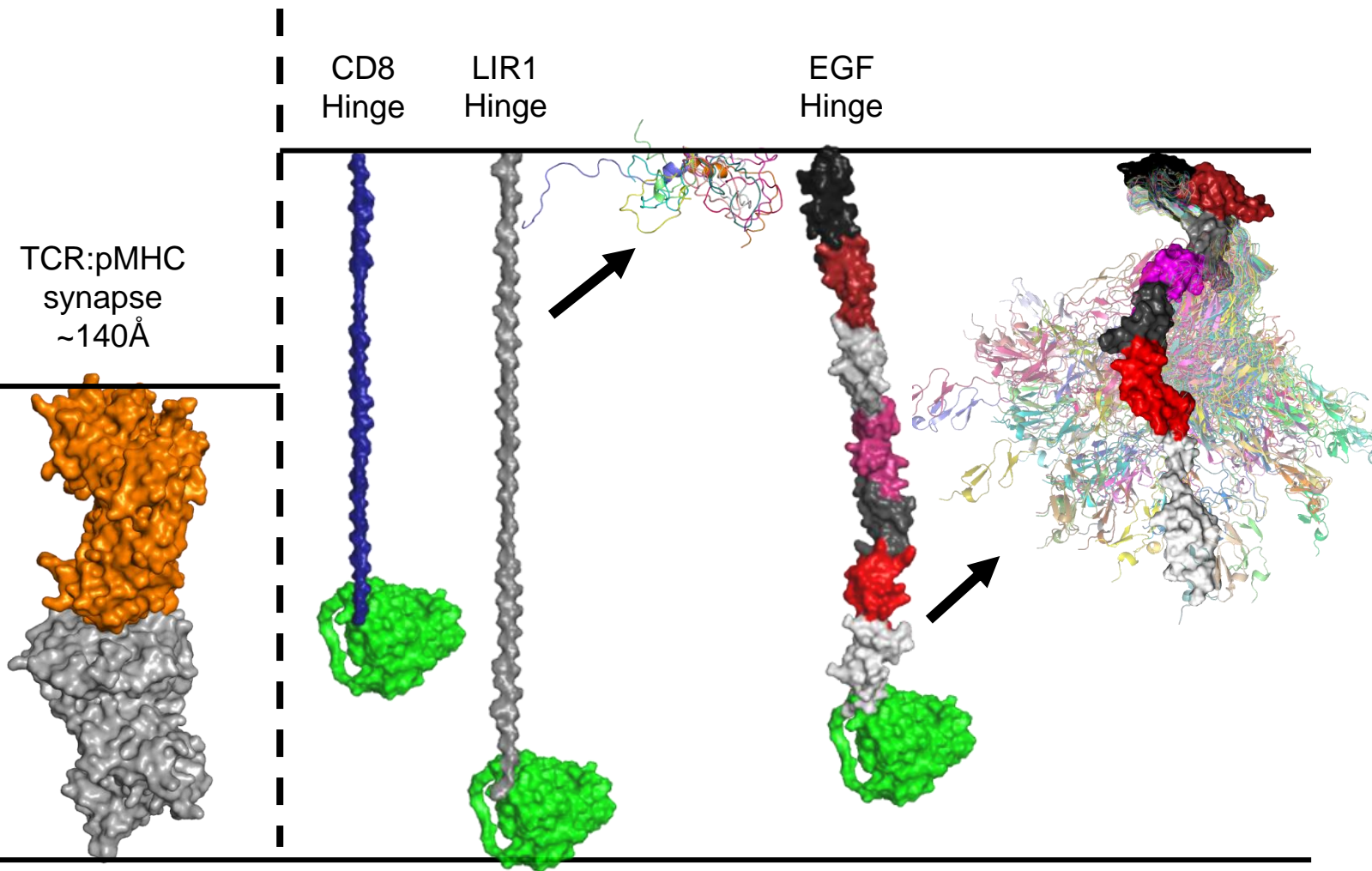
(A) scFv affinities for B-antigens shown in Figure 1. (B) mRNA titration of B-antigens on target cells. (C) Relative expression levels quantified by QIFI.

Supplement 2

CD8
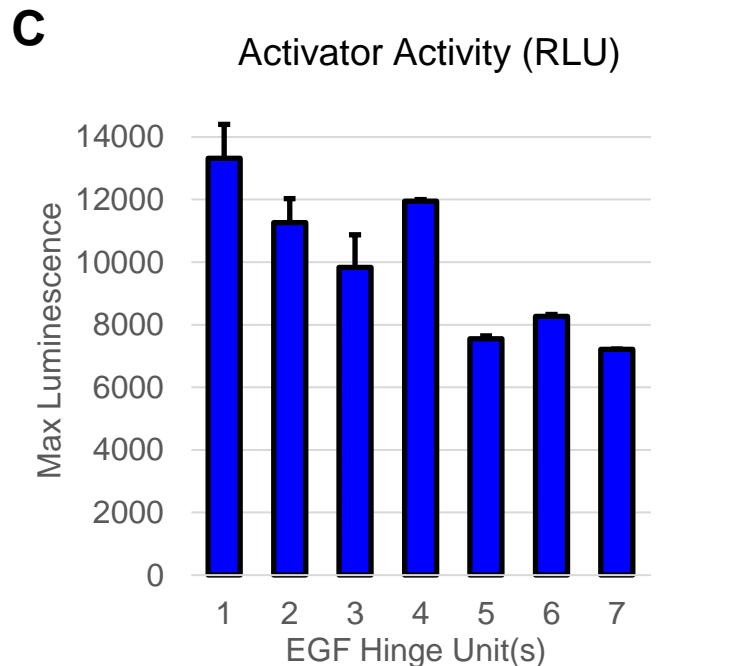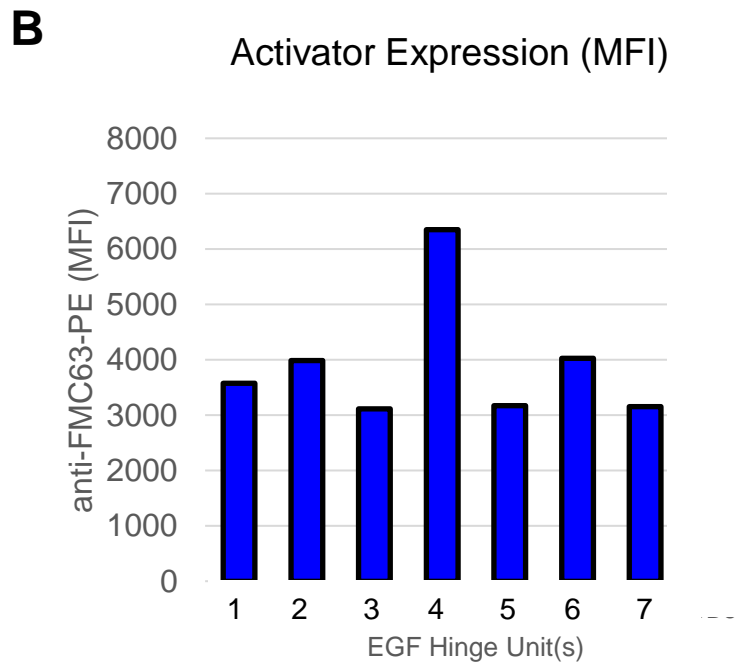Hinge

LIR1
Hinge

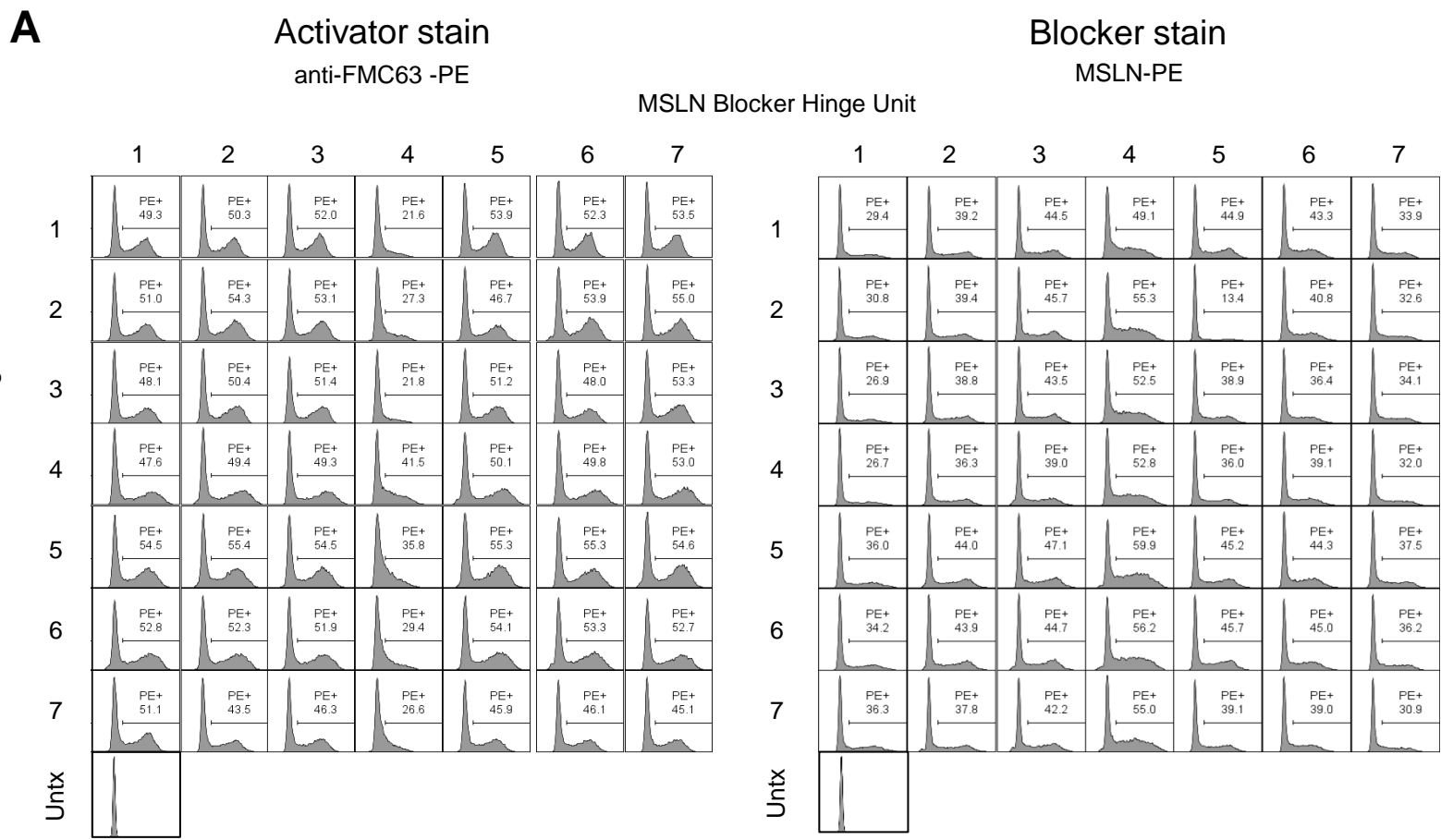EGF
Hinge

TCR:pMHC
synapse
~140Å

Comparison of Hinge Length and Flexibility relative to TCR:pMHC complex. 100 Monte Carlo
simulations for each hinge were overlayed to assess flexibility.

Supplement 3
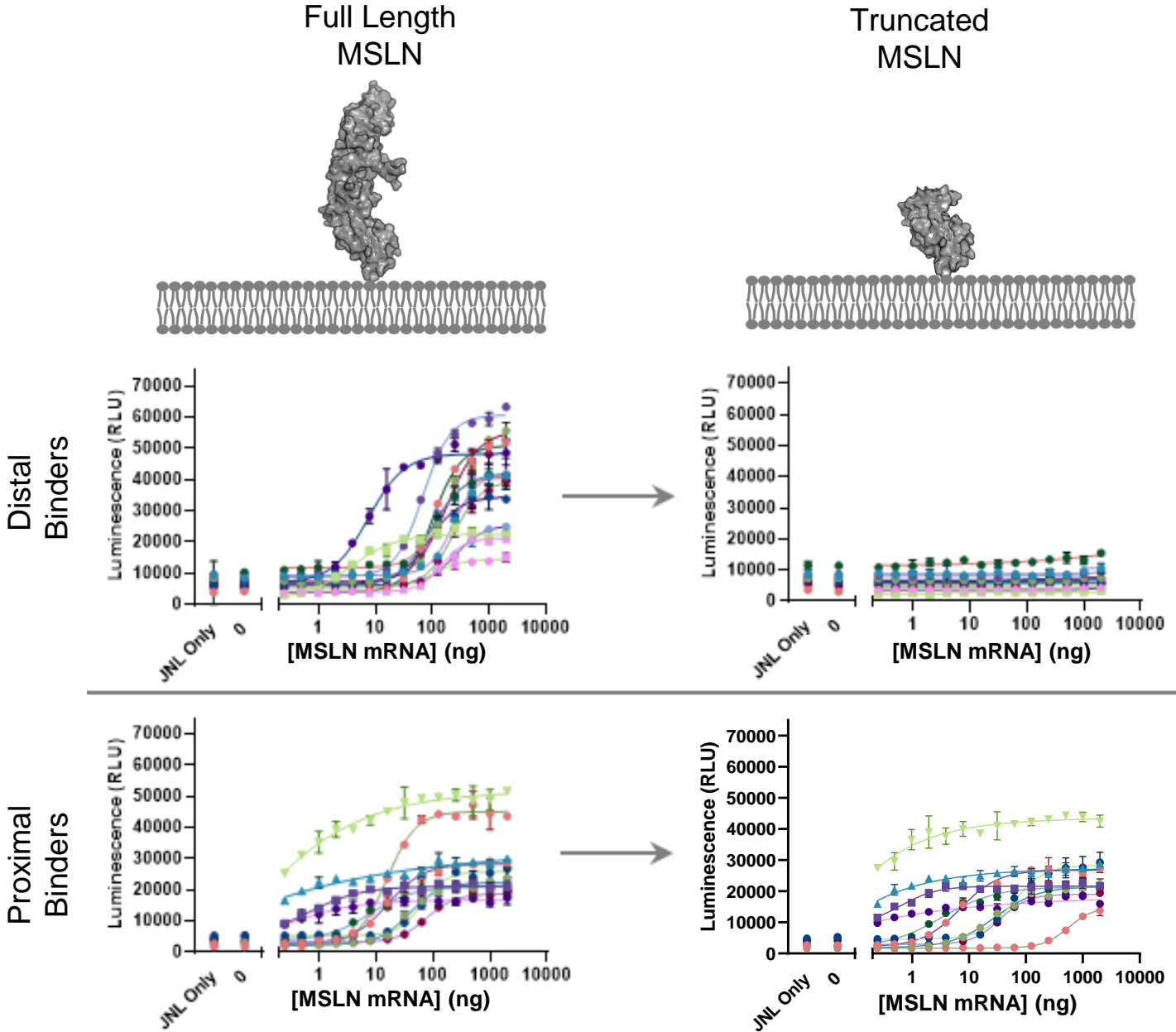
Functional assessment of MSLN CARs responding to full length or truncated MSLN. If a CAR did not respond to truncated form, it was classified as a distal binder

Supplement 4

A

Activator stain
anti-FMC63 -PE

Blocker stain
MSLN-PE

MSLN Blocker Hinge Unit

B

Activator Expression (MFI)


C

Activator Activity (RLU)

(A) Expression levels CD19 activator/MSLN blocker with EGF hinges. Untx, untransfected. (B) Relative expression levels of activator. (C) Max activation decreases as hinge length decreases independent of expression.

Supplement 5

A

R = 0.15

B

C
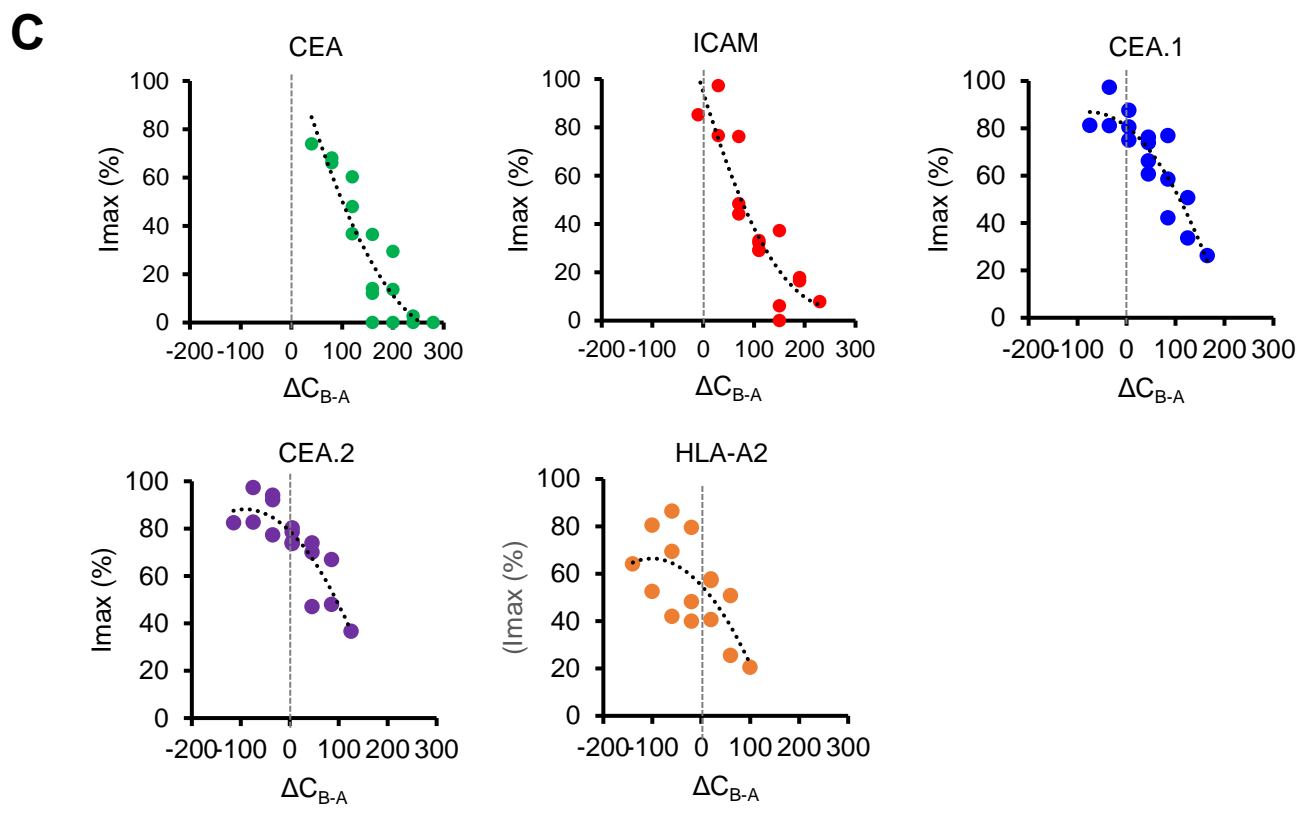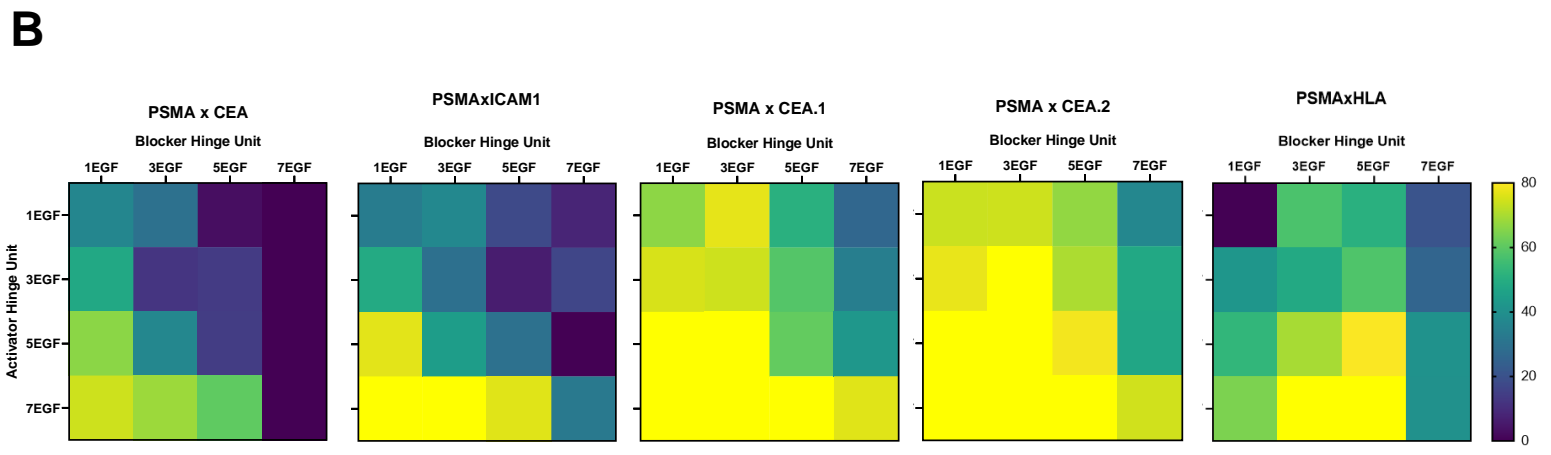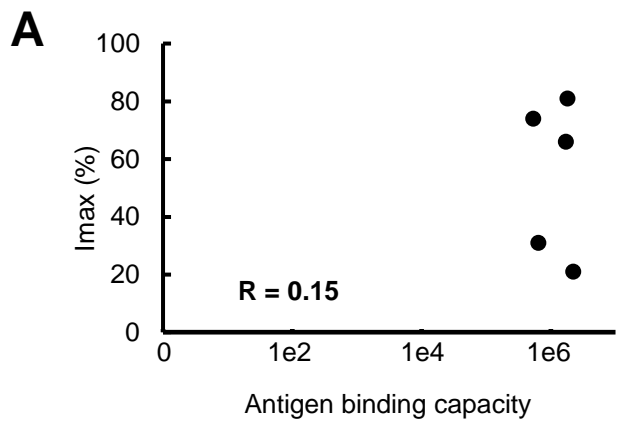
(A) Antigen density for FLAG-antigens exceeds 500k ABC and is uncorrelated with Imax. (B) Blocking profile when expanding the R_A or R_B with EGF modular hinges. (C) Plotting the Imax data from (B) versus total estimates of ΔC.

Supplement 6
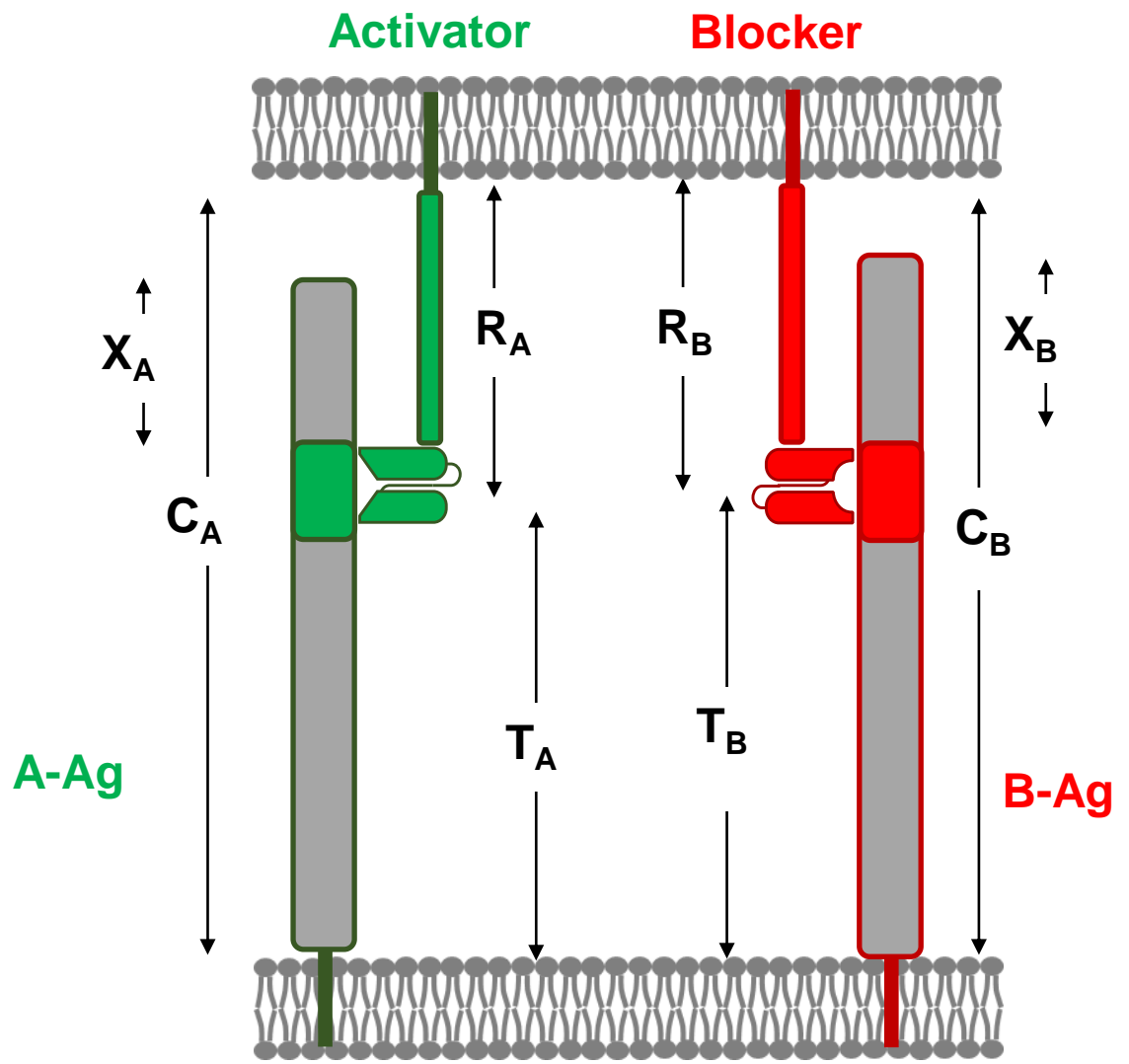
Full diagram of geometric parameters.