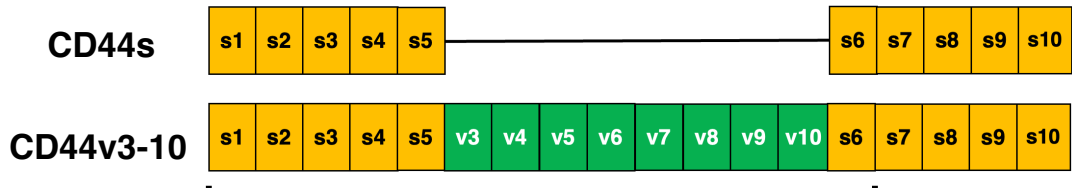
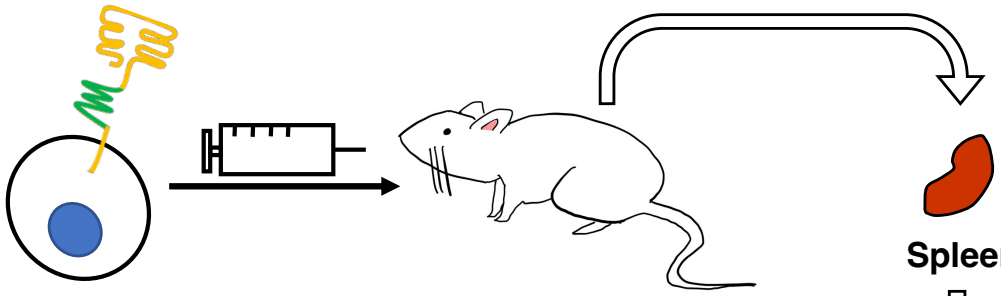
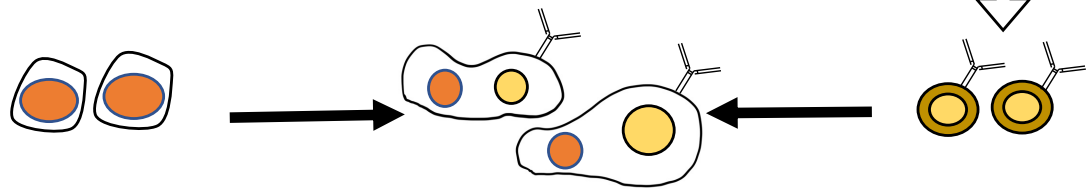
1. Immunization of CHO/CD44v3-10 cells



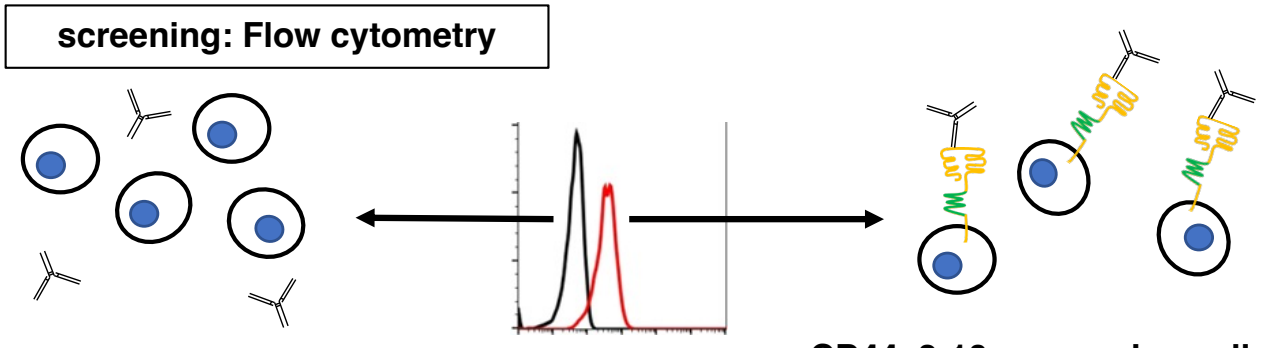
CHO/CD44v3-10
CD44v3-10-expressing CHO cells



2. Hybridomas production

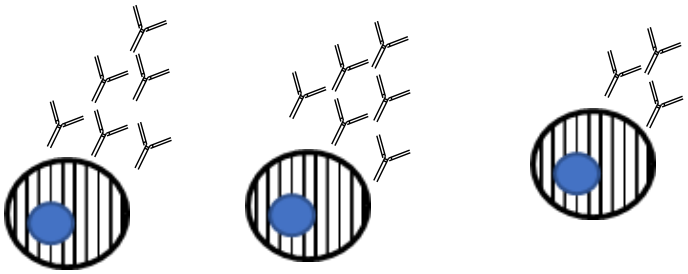


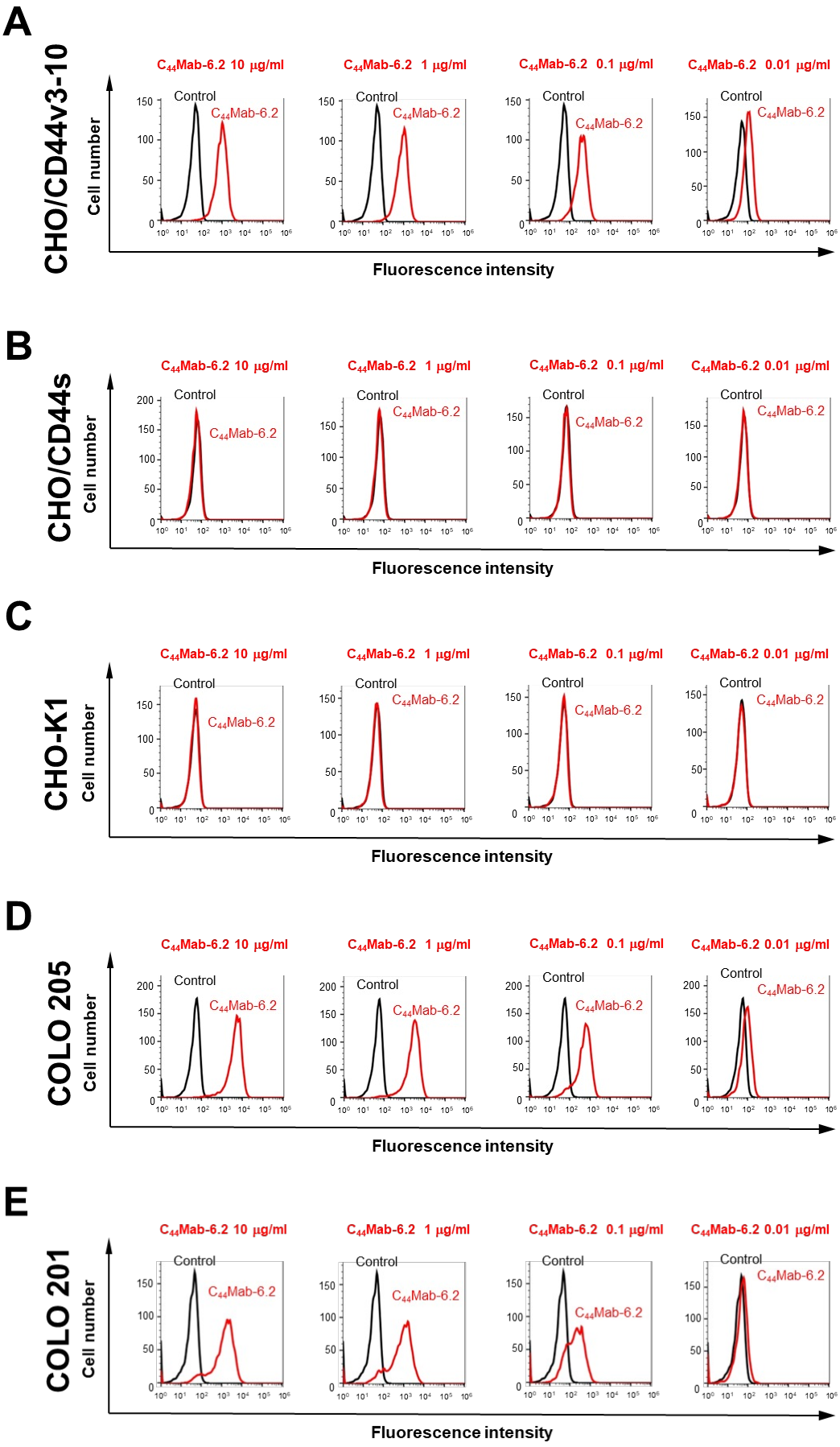
3. Screening of surpernatants by flow cytometry



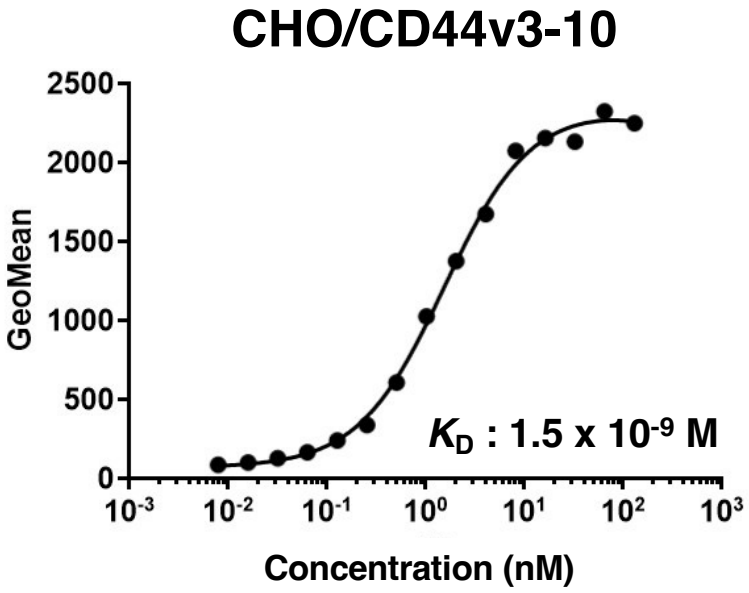
4. Cloning of hybridomas

Establishment of anti-CD44 mAb-producing clones

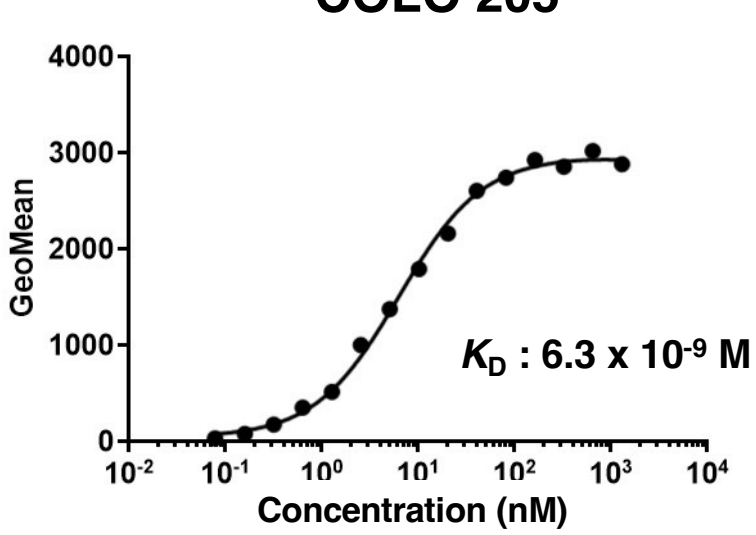



A
CHO/CD44v3-10
B
CHO/CD44s
C
CHO-K1
D
COLO 205
E
COLO 201
C44Mab-6.2 10 μg/ml
C44Mab-6.2 1 μg/ml
C44Mab-6.2 0.1 μg/ml
C44Mab-6.2 0.01 μg/ml
Control
C44Mab-6.2
Cell number
Fluorescence intensity

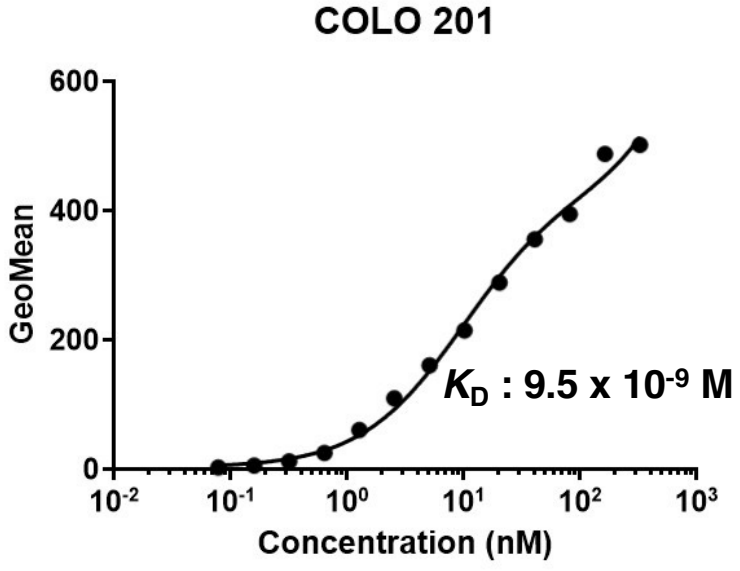
A

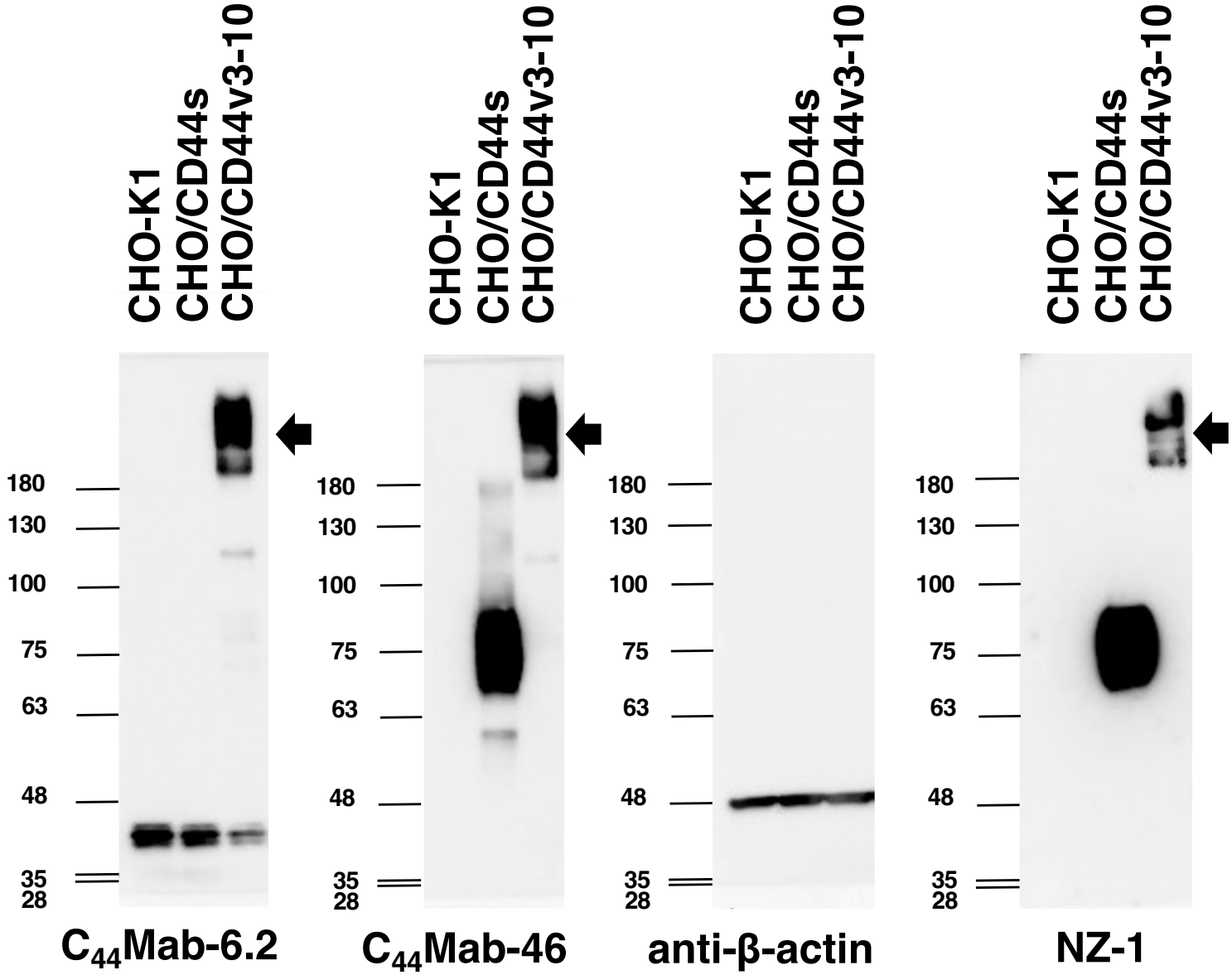

CHO/CD44v3-10
GeoMean
2500
2000
1500
1000
500
0
10^{-3} 10^{-2} 10^{-1} 10^{0} 10^{1} 10^{2} 10^{3}
Concentration (nM)
K_D : 1.5 x 10^{-9} M

B

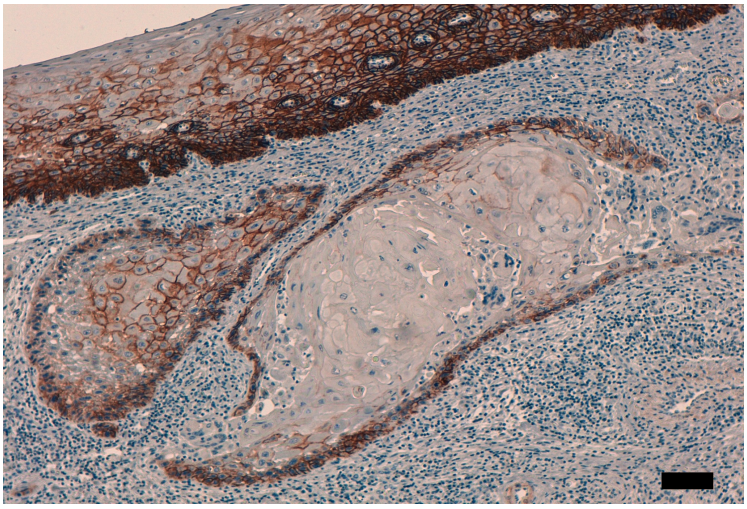

COLO 205
GeoMean
4000
3000
2000
1000
0
10^{-2} 10^{-1} 10^{0} 10^{1} 10^{2} 10^{3} 10^{4}
Concentration (nM)
K_D : 6.3 x 10^{-9} M

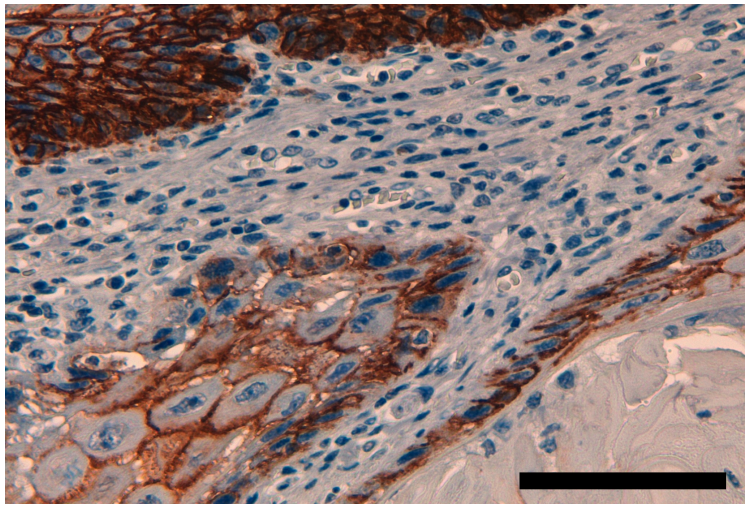
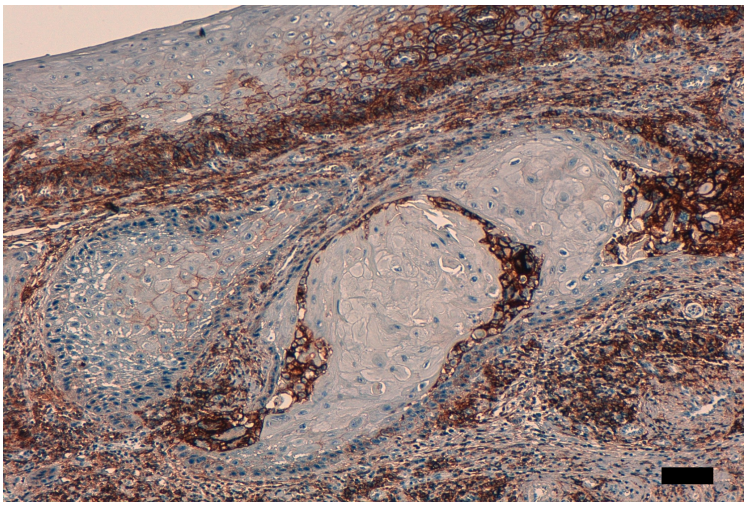
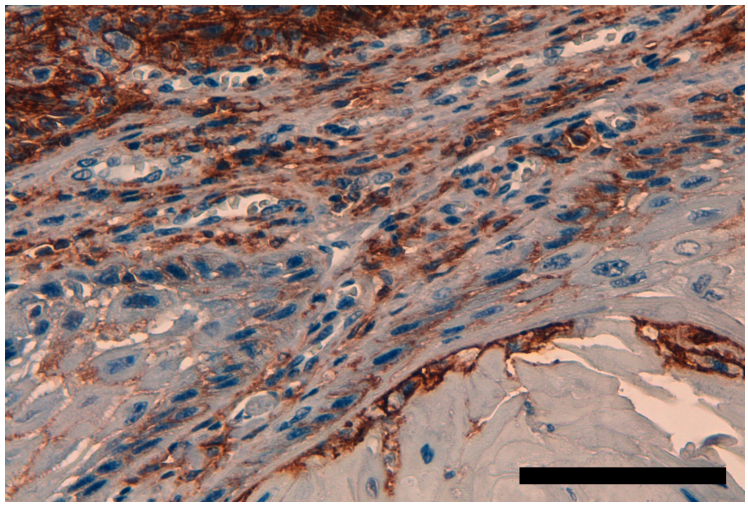
C


COLO 201
GeoMean
600
400
200
0
10^{-2} 10^{-1} 10^{0} 10^{1} 10^{2} 10^{3}
Concentration (nM)
K_D : 9.5 x 10^{-9} M


CHO-K1
CHO/CD44s
CHO/CD44v3-10
180
130
100
75
63
48
35
28
C44Mab-6.2
C44Mab-46
anti-β-actin
NZ-1

C_{44}Mab-6.2, 5 μg/mL





C_{44}Mab-46, 1 μg