Figure 1A-C

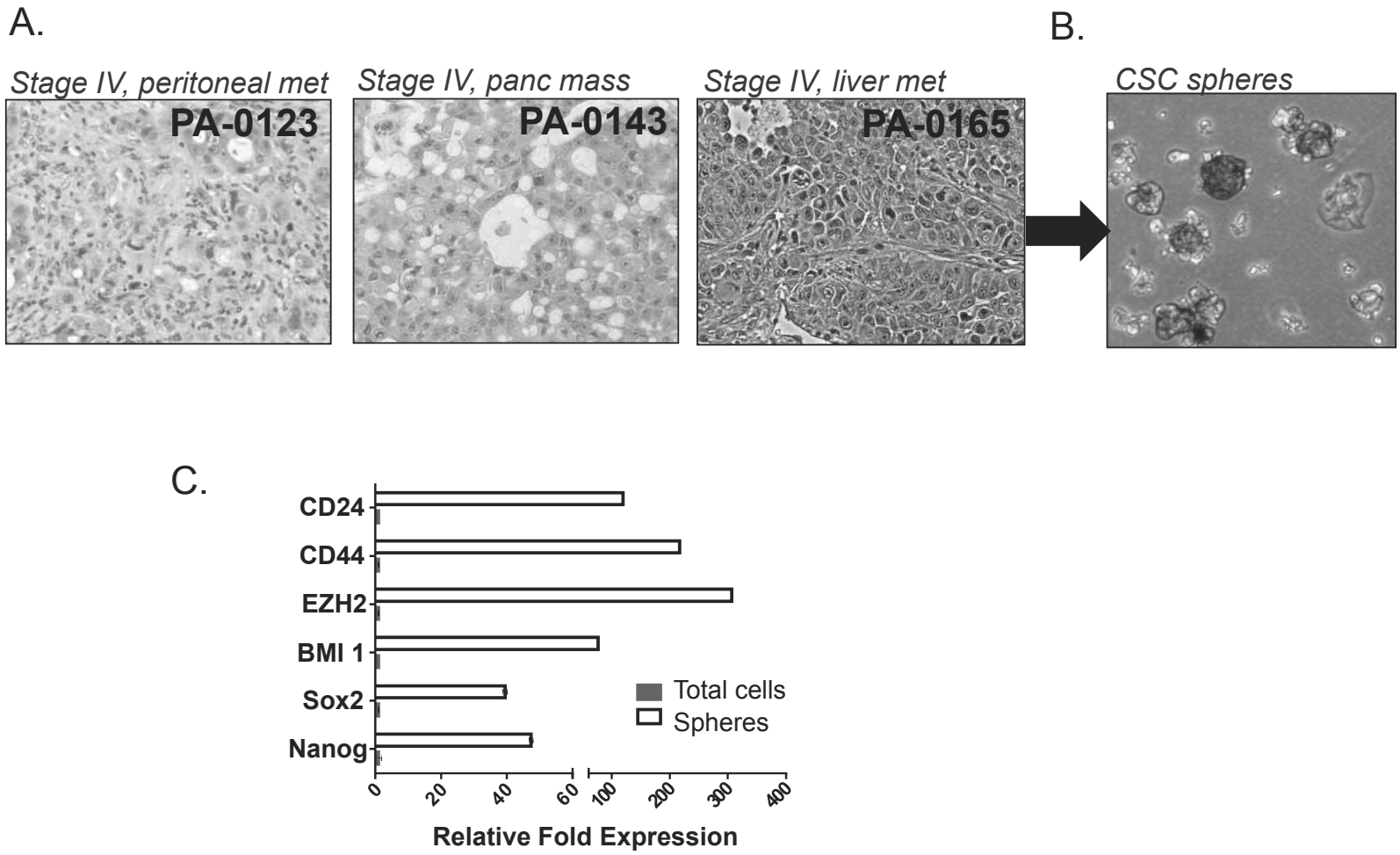

A.
B.
Stage IV, peritoneal met
PA-0123
Stage IV, panc mass
PA-0143
Stage IV, liver met
PA-0165
CSC spheres
C.
CD24
CD44
EZH2
BMI 1
Sox2
Nanog
Total cells
Spheres
0
20
40
60
100
200
300
400
Relative Fold Expression

Figure 2

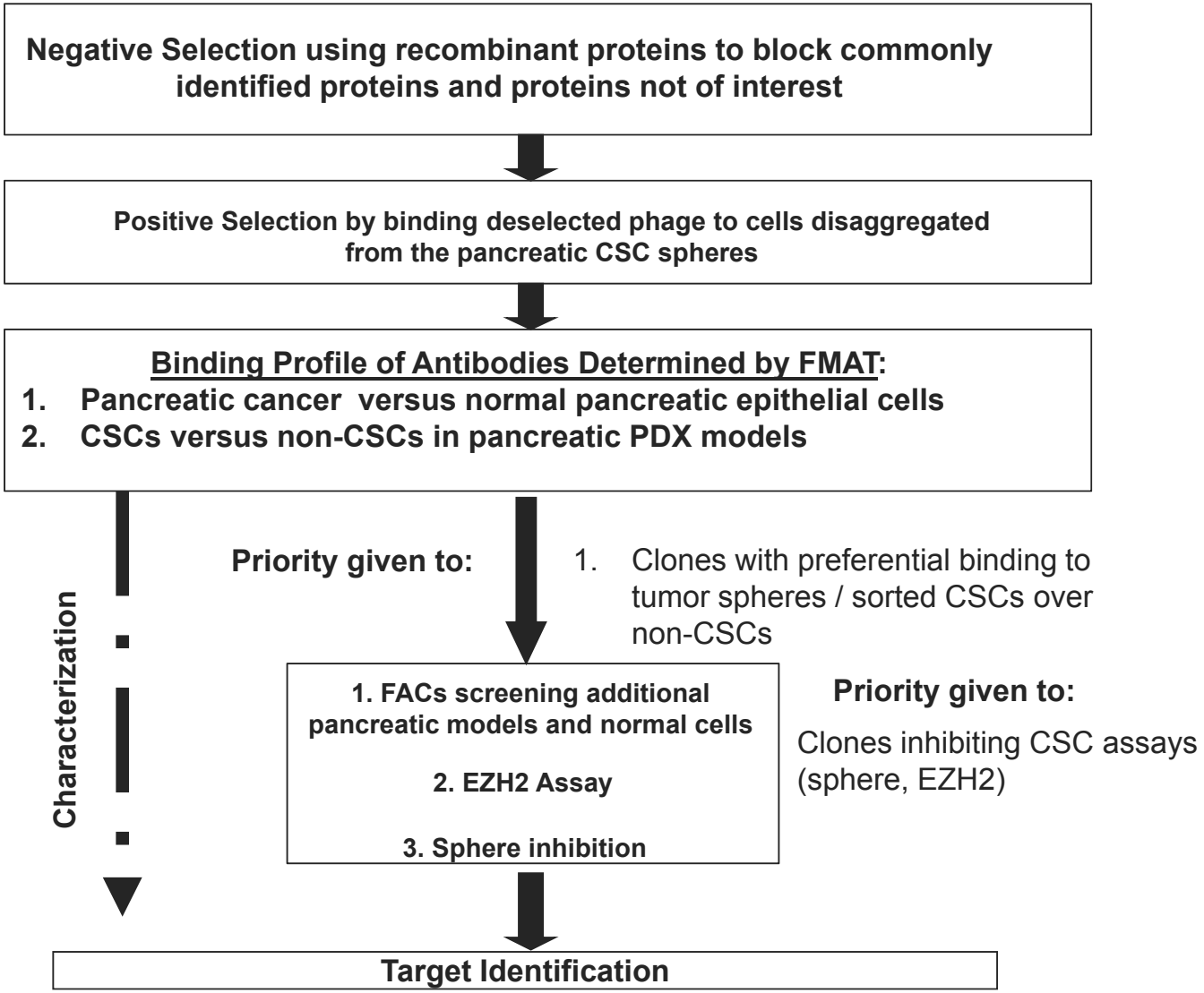
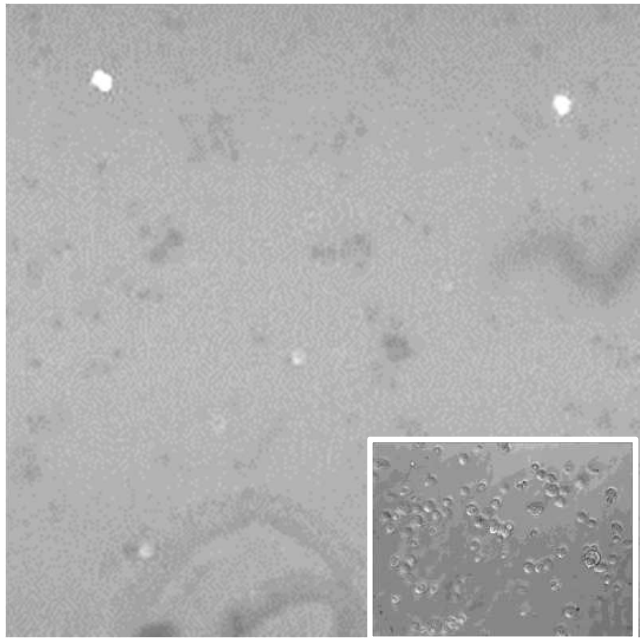

Negative Selection using recombinant proteins to block commonly identified proteins and proteins not of interest
Positive Selection by binding deselected phage to cells disaggregated from the pancreatic CSC spheres
Binding Profile of Antibodies Determined by FMAT:
1. Pancreatic cancer versus normal pancreatic epithelial cells
2. CSCs versus non-CSCs in pancreatic PDX models
Characterization
Priority given to:
1. Clones with preferential binding to tumor spheres / sorted CSCs over non-CSCs
1. FACs screening additional pancreatic models and normal cells
2. EZH2 Assay
3. Sphere inhibition
Priority given to:
Clones inhibiting CSC assays (sphere, EZH2)
Target Identification

Figure 3A

Normal Pancreatic Epithelial Cells



Sorted Pancreatic CSCs

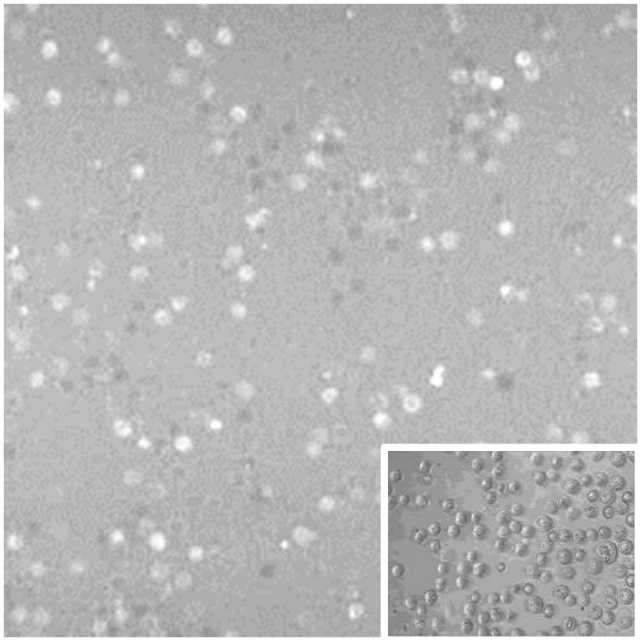


Figure 3B

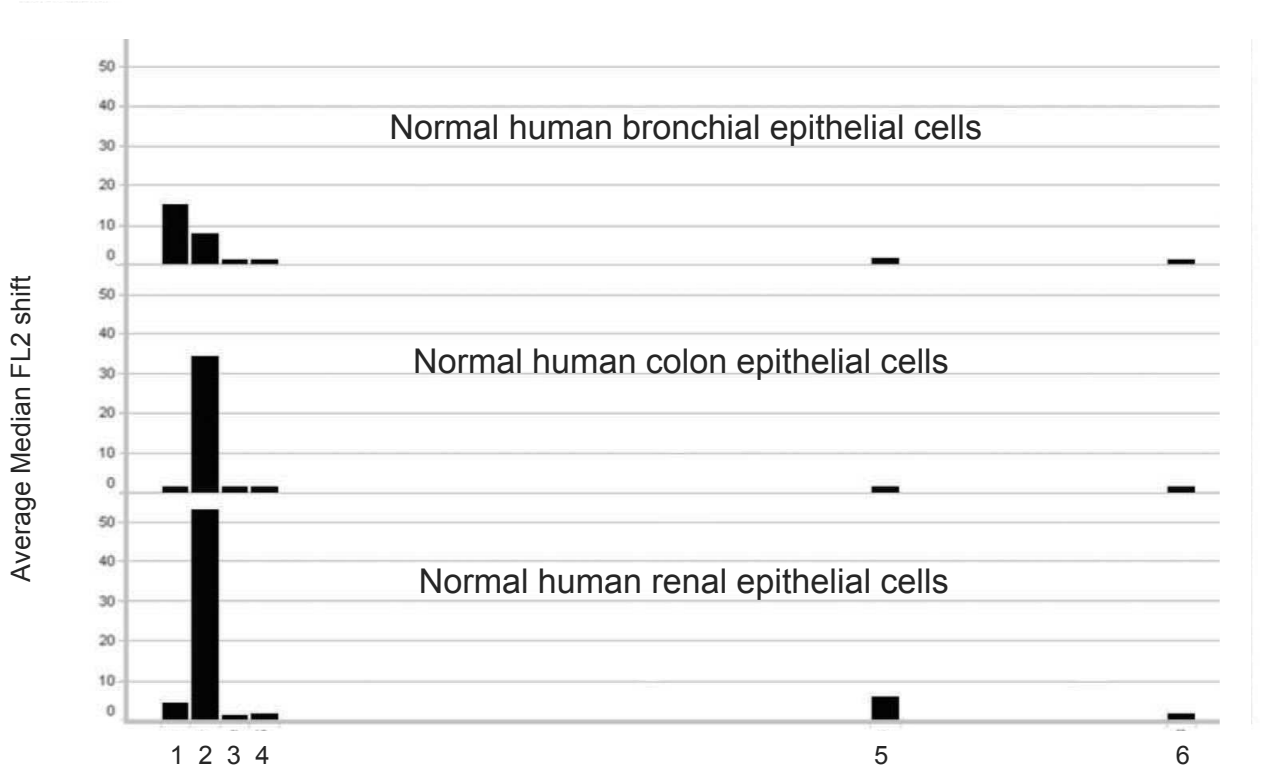
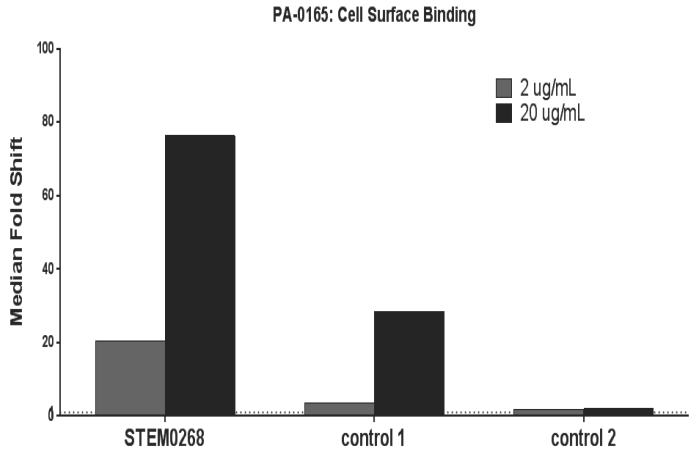
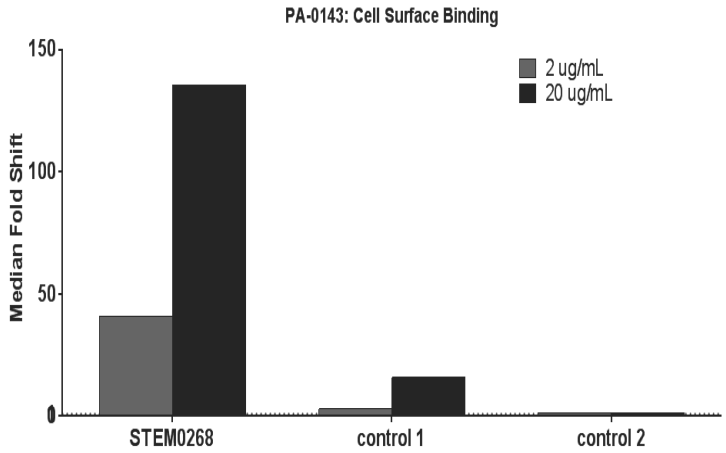

Average Median FL2 shift
Normal human bronchial epithelial cells
Normal human colon epithelial cells
Normal human renal epithelial cells
50
40
30
20
10
0
1 2 3 4
5
6

Figure 4

A.


PA-0165: Cell Surface Binding
Median Fold Shift
100
80
60
40
20
0
2 ug/mL
20 ug/mL
STEM0268
control 1
control 2

B.


PA-0143: Cell Surface Binding
Median Fold Shift
150
100
50
0
2 ug/mL
20 ug/mL
STEM0268
control 1
control 2

C.

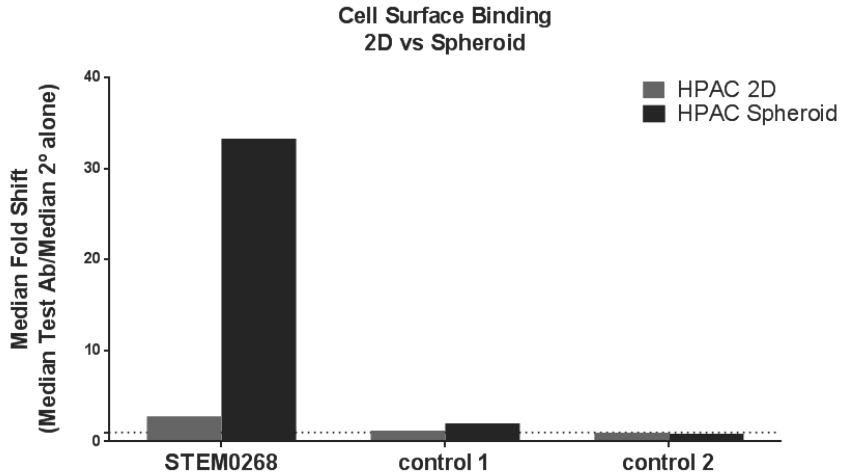
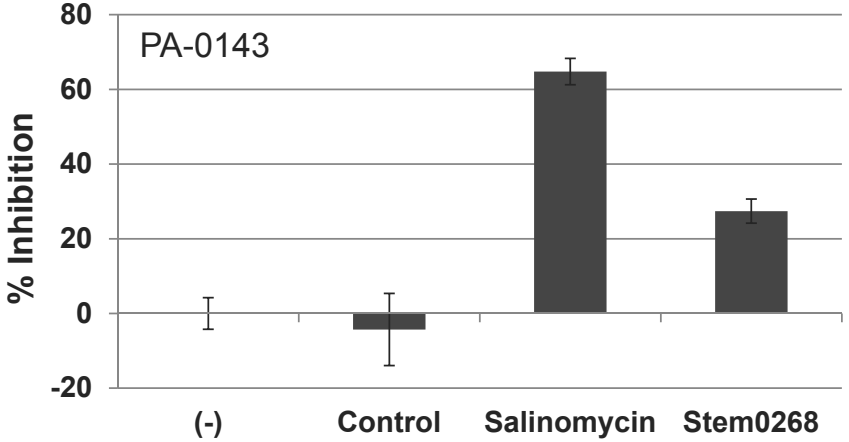
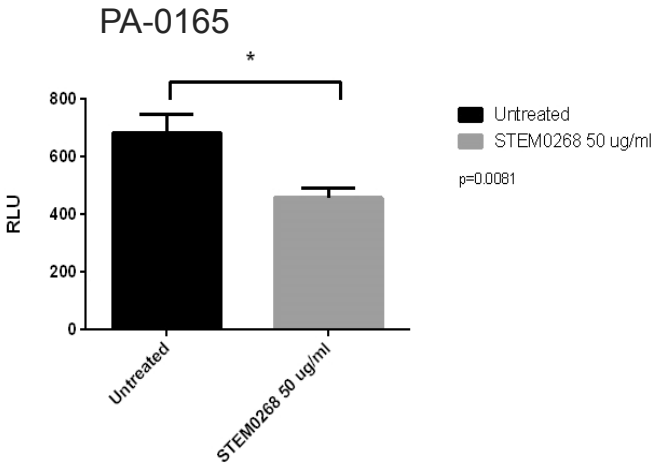

Cell Surface Binding
2D vs Spheroid
Median Fold Shift
(Median Test Ab/Median 2° alone)
40
30
20
10
0
HPAC 2D
HPAC Spheroid
STEM0268
control 1
control 2

Figure 5 A-C

A.



B.



C.

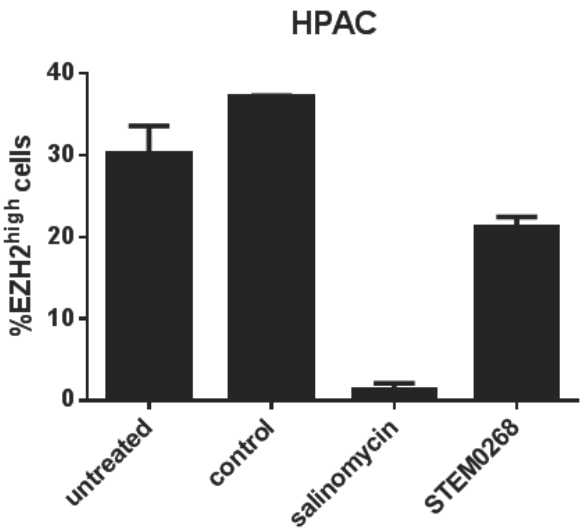


Figure 6 A-C

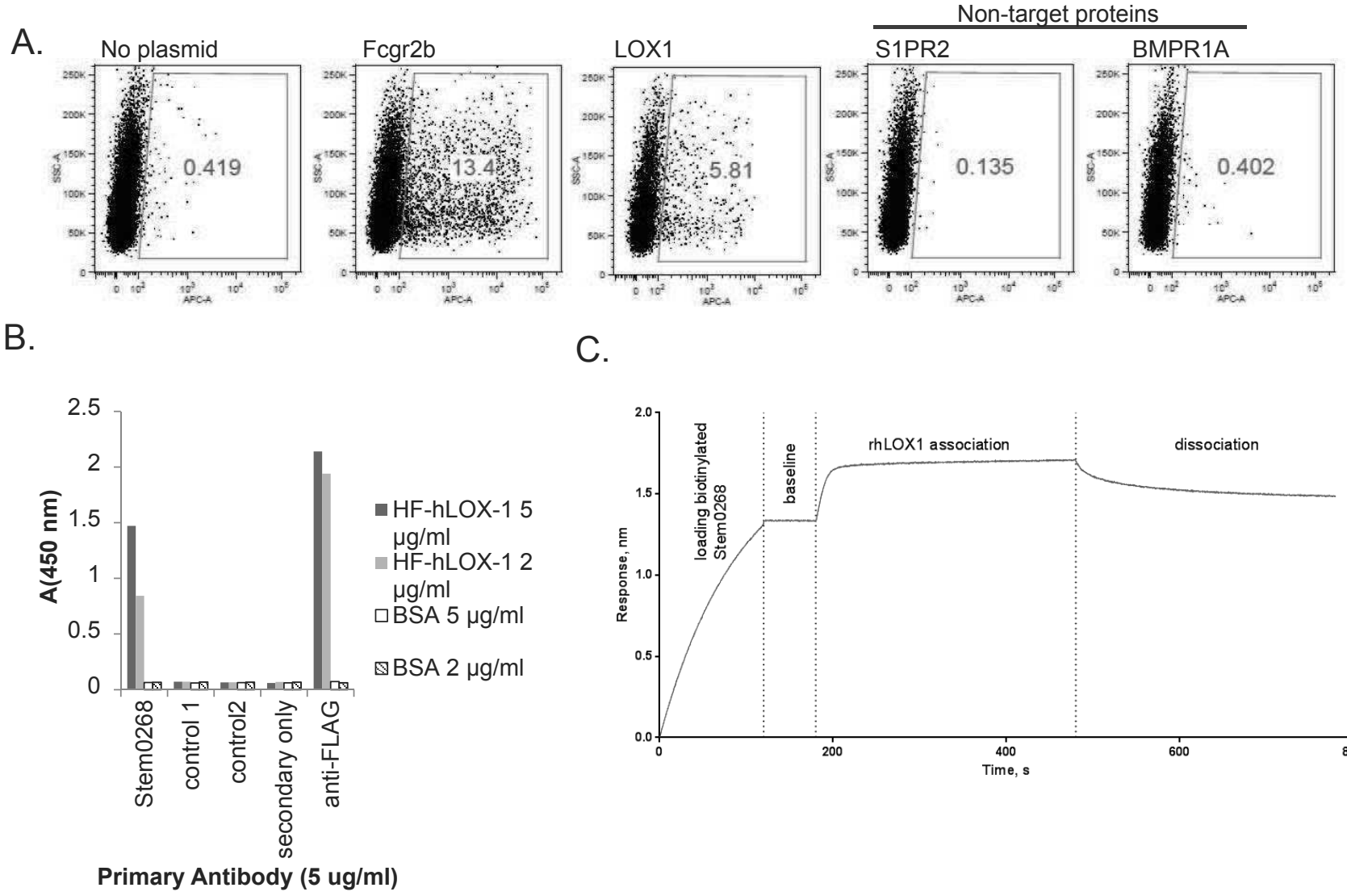
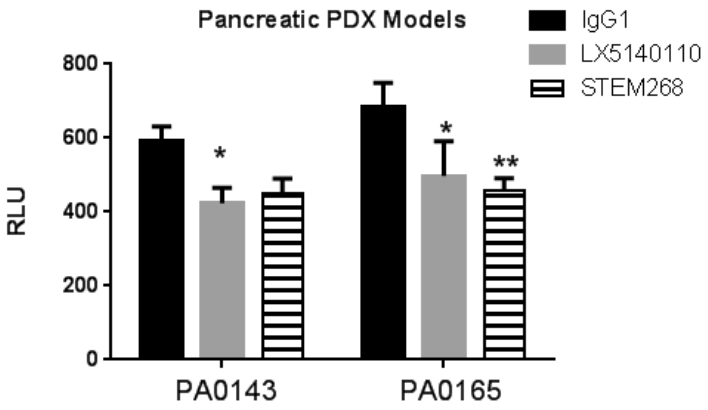
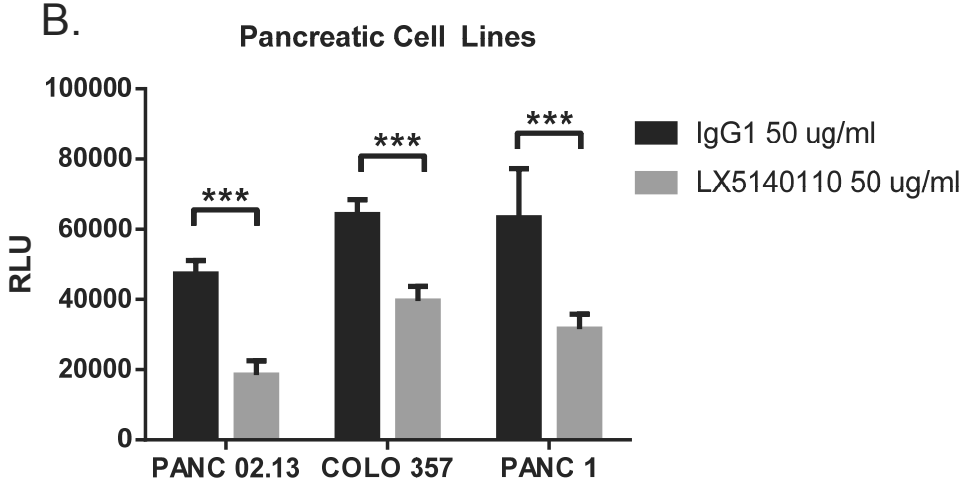

A.
Non-target proteins
No plasmid
Fcgr2b
LOX1
S1PR2
BMPR1A
0.419
13.4
5.81
0.135
0.402
SSC-A
APC-A
B.
A(450 nm)
HF-hLOX-1 5 µg/ml
HF-hLOX-1 2 µg/ml
BSA 5 µg/ml
BSA 2 µg/ml
Stem0268
control 1
control2
secondary only
anti-FLAG
Primary Antibody (5 ug/ml)
C.
loading biotinylated Stem0268
baseline
rhLOX1 association
dissociation
Response, nm
Time, s

Figure 7 A-C

A.



B.



C.

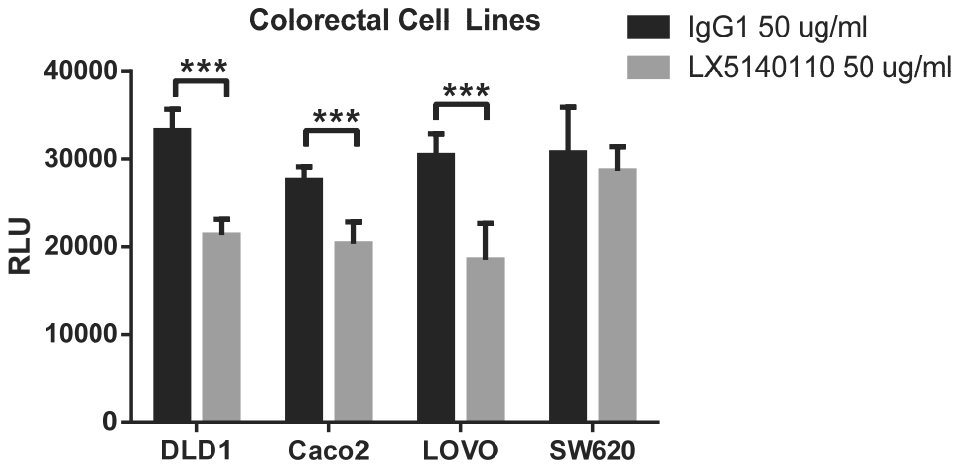
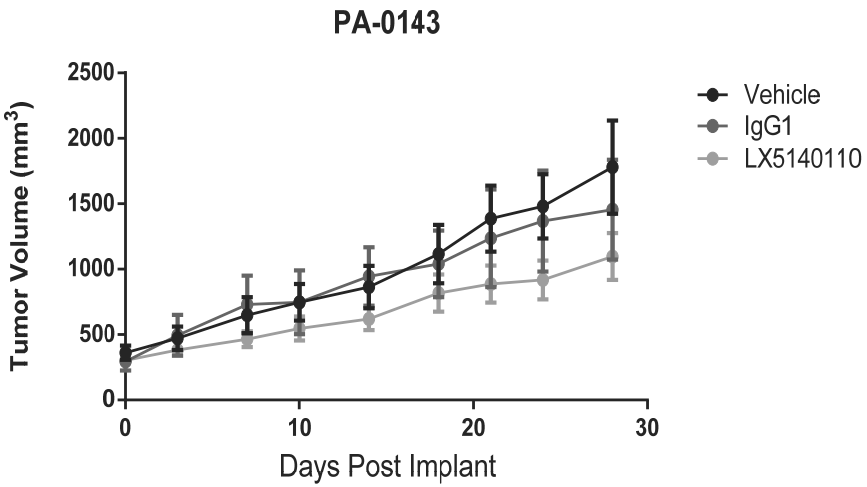


Figure 8 A-B

A.



B.

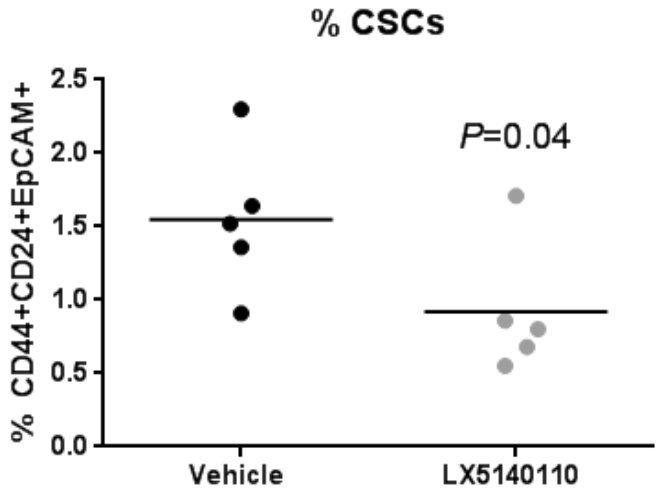


Figure 9 A-B

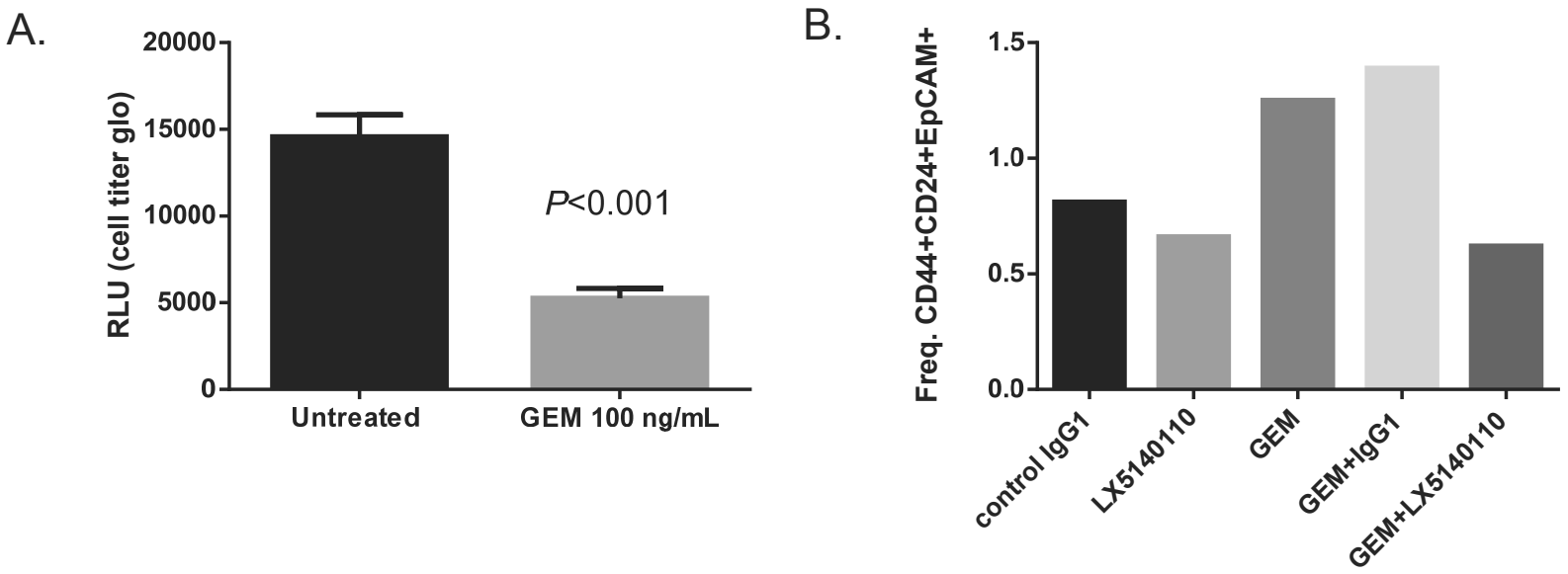

A.
RLU (cell titer glo)
20000
15000
10000
5000
0
P<0.001
Untreated
GEM 100 ng/mL
B.
Freq. CD44+CD24+EpCAM+
1.5
1.0
0.5
0.0
control IgG1
LX5140110
GEM
GEM+IgG1
GEM+LX5140110

Figure 10 A-B

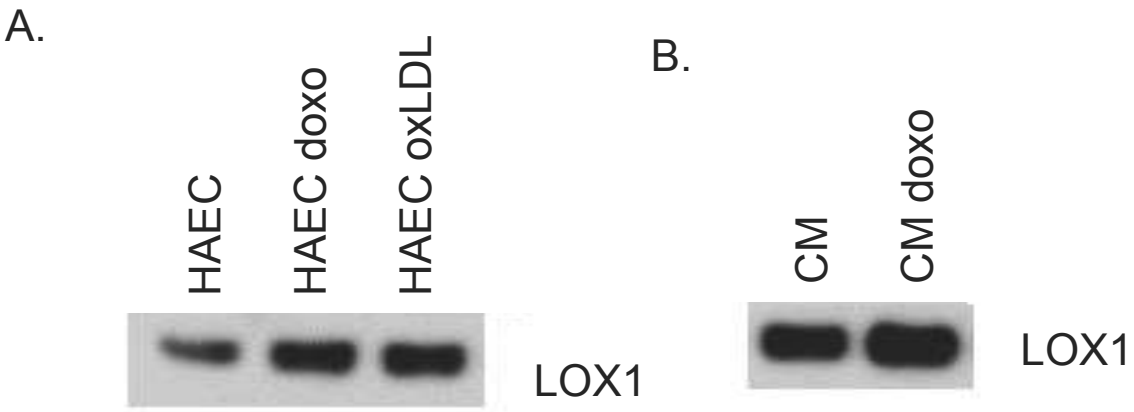
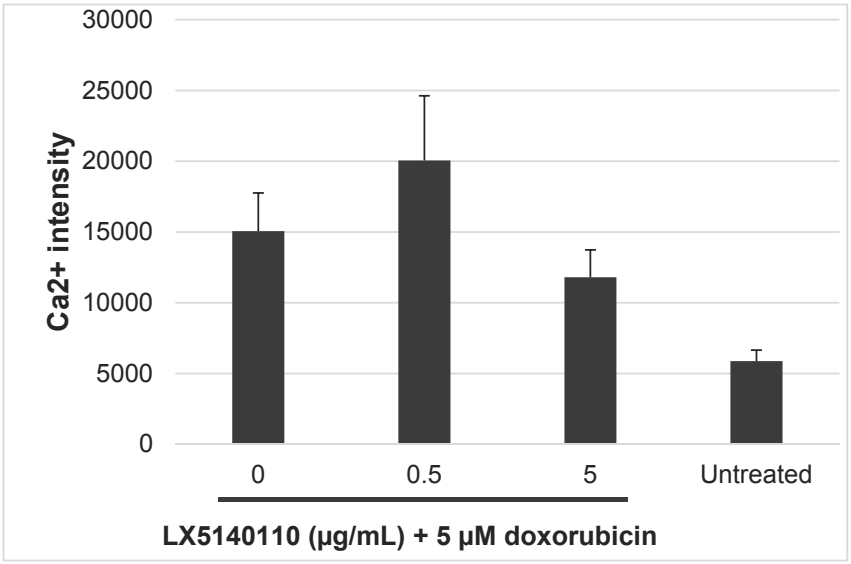


Figure 11 A-B

A.



B.

