SUPPLEMENTAL DATA

Human Endotrophin as a Driver of Malignant Tumor Growth

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Supplemental Figure 1. Standard curves (**A, B**) show sensitivity and linear dose response to endotrophin concentrations. Comparison of a tissue stain (**C, D**) with antibody ETPmAb4 with and without antigen pre-adsorption to demonstrate specific antigen staining. **E-H:** Col6a3 expression in pre- and post-menopausal tumors, and different tumor subtypes. The data shown in Figure 1 (all patients) was subdivided into pre- (**E**) and post-menopausal breast cancer (**F**), or into estrogen receptor positive (**G**) and estrogen receptor negative subpopulations (**H**).

Supplemental Figure 2. (A) ZR-75-1 breast cancer cells (5 X 10^5 cells), n=3. **(B)** MCF-7 breast cancer cells (5 X 10^5 cells). n=3. **(C)** MDAMB-231 breast cancer cells (5 X 10^5 cells), n=3, were plated into 6-well plates and treated with endotrophin (0.1 ug/mL) three times (every other day). Total RNA was then extracted from each well. The EMT marker genes *Twist*, *Snail*, *Cdh2* and *Cdh1* were determined by qRT-PCR, then normalized to GAPDH. **(D)** MCF-7 cells (40,000 cells), n=4. **(E)** ZR-75-1 cells (40,000 cells), n=4. **(F)** MDAMB-453 cells (40,000 cells), n=4. **(G)** MDAMB-231 (40,000 cells), n=4, were plated into 24-well plates. When the cells reached 90% confluence, the monolayer was scratched with a 1 mL pipette tip to create 2 perpendicular straight lines across the center of the well. Cells were then treated with increasing concentrations of endotrophin (0.1 ug/mL). Images were obtained using a Nikon Cool Scope microscope (Nikon) after a 48 hr incubation. Migrating cell numbers were evaluated using ImageJ software. **(H)** MCF-7 cells (5 X 10^5 cells), n=4. **(I)** ZR-75-1 cells (5 X 10^5 cells), n=4. **(J)** MDAMB-453 cells (5 X 10^5 cells), n=4. **(K)** MDAMB-231 cells (5 X 10^5 cells), n=4, were plated in the top chamber of a trans-well plate. Endotrophin (0.1 ug/mL) was then added with or without

1% FBS in the lower chamber, then incubated for 16 hrs. Images were then obtained on a Nikon Cool Scope microscope (Nikon) after a 16 hr incubation. In all cases, data was represented as mean ± SEM, and statistical significance (***p<0.0001) was calculated using unpaired, two-tailed *t-test w/Holm-Sidak correction for multiple comparisons*.

Supplemental Figure 3. Screening for rabbit monoclonal antibodies. MCF-7 breast cancer cells (20,000 cells) were plated into a 96-well plate. Cells were then treated with 10 μm of cisplatin and 100 ng/mL of endotrophin. All 132 neutralized endotrophin antibodies were screened. Cell survival was measured using a CellTiter One Solution Cell Proliferation Assay.

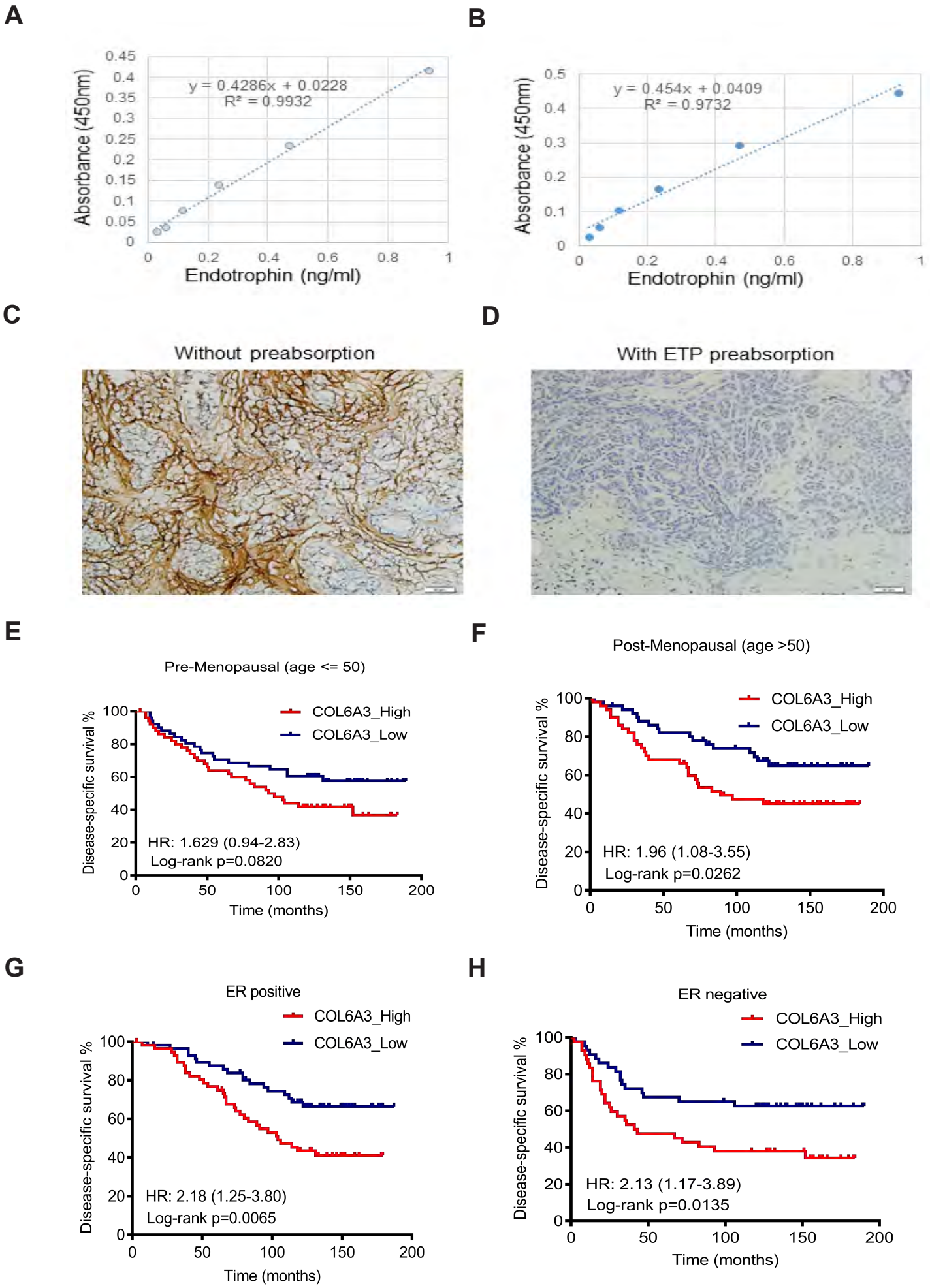
Supplemental Figure 4. SC macrophage cells (50,000 cells) were seeded at the top of a chamber. Then 100 ng/mL of endotrophin was added with 1% FBS in the bottom chamber. Next, 10 ug/mL of anti-ETP antibodies (#1, #2, #4, #6, #10, #11 and #72) were added to the bottom chamber, and incubated for 2 hr. Migrated cells were counted after 2 hr. Data was represented as mean ± SEM, n=4 and statistical significance (***P<0.0001) was calculated using unpaired, two-tailed *t-test w/Holm-Sidak correction for multiple comparisons*.

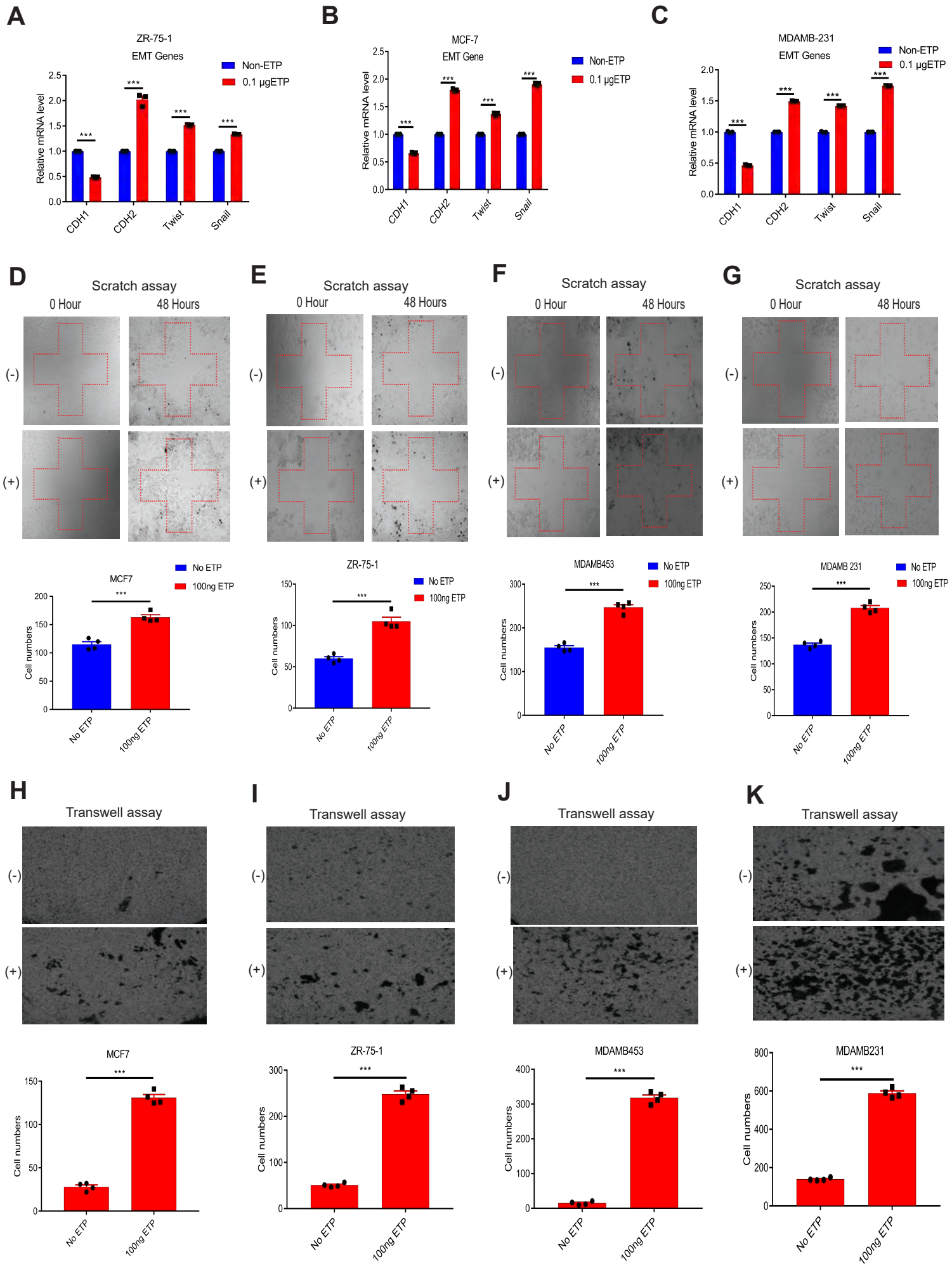
Supplemental Figure 5. (A) H&E staining and endomucin immunofluorescence staining from CTRL Ab and #4 Ab-treated ETP transfected MCF-7 tumors. Scale bars: 50μm. **(B)** Mac2 immunofluorescence staining for tumor tissues from CTRL Ab and #4 Ab-treatment ETP transfected MCF-7 tumors. Scale bars: 50μm. **(C)** E-CAD immunofluorescence

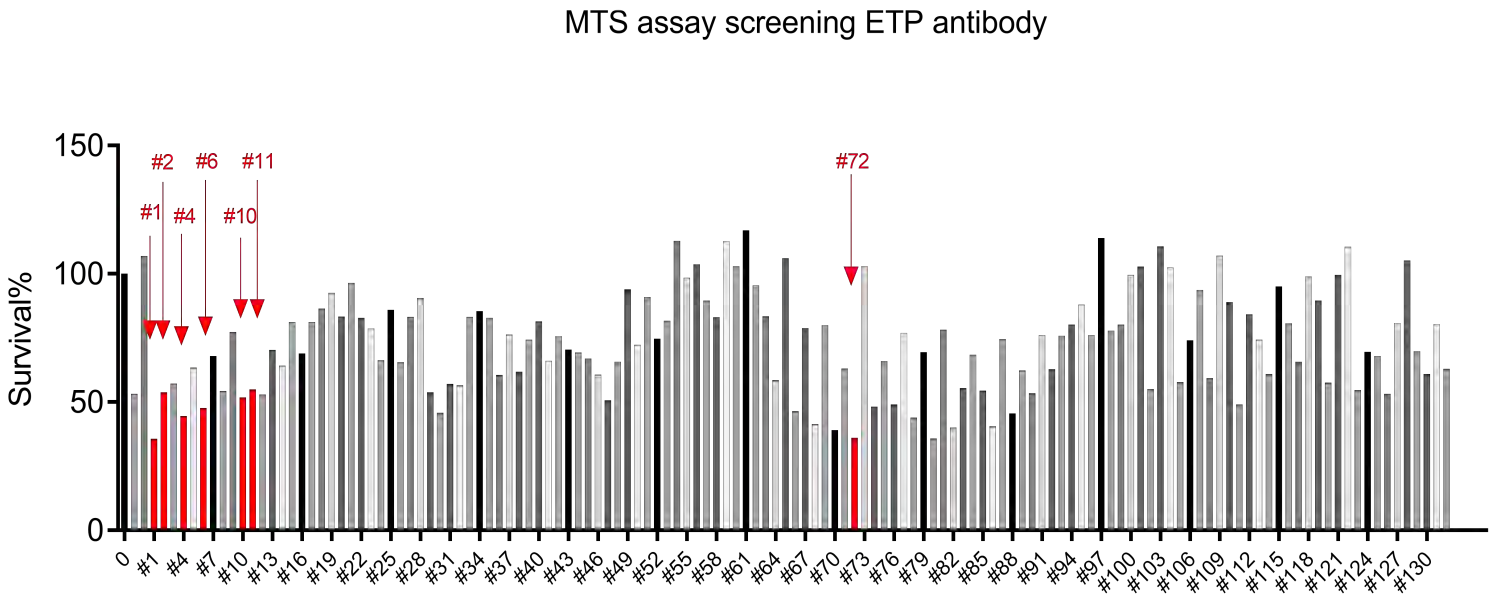
staining for tumor tissues from CTRL Ab and #4 Ab-treatment ETP transfected MCF-7 tumors. The last panel for each marker reflects a stain with DAPI. Scale bars: 50μm.

Supplemental Figure 6. (A) H&E staining and endomucin immunofluorescence staining from CTRL Ab and #4 Ab-treatment MDAMB-231 tumors. Scale bars: 50μm**. (B)** Mac2 immunofluorescence staining for tumor tissues from CTRL Ab and #4 Ab treatment MDAMB-231tumors. Scale bars: 50μm. **(C)** E-CAD immunofluorescence staining for tumor tissues from CTRL Ab and #4 Ab-treatment MDAMB-231 tumors. The last panel for each marker reflects a stain with DAPI. Scale bars: 50μm.

Supplemental Figure 7. Kinetics of the rabbit parental antibody ENTmAb4 (left) and the humanized version hENTmAb4 (right) were assessed using an Octet RED96.


A
y = 0.4286x + 0.0228
R² = 0.9932
Absorbance (450nm)
Endotrophin (ng/ml)
B
y = 0.454x + 0.0409
R² = 0.9732
Absorbance (450nm)
Endotrophin (ng/ml)
C
Without preabsorption
D
With ETP preabsorption
E
Pre-Menopausal (age <= 50)
COL6A3_High
COL6A3_Low
Disease-specific survival %
HR: 1.629 (0.94-2.83)
Log-rank p=0.0820
Time (months)
F
Post-Menopausal (age >50)
COL6A3_High
COL6A3_Low
Disease-specific survival %
HR: 1.96 (1.08-3.55)
Log-rank p=0.0262
Time (months)
G
ER positive
COL6A3_High
COL6A3_Low
Disease-specific survival %
HR: 2.18 (1.25-3.80)
Log-rank p=0.0065
Time (months)
H
ER negative
COL6A3_High
COL6A3_Low
Disease-specific survival %
HR: 2.13 (1.17-3.89)
Log-rank p=0.0135
Time (months)


A
ZR-75-1
EMT Genes
Non-ETP
0.1 μgETP
Relative mRNA level
CDH1
CDH2
Twist
Snail
B
MCF-7
EMT Gene
C
MDAMB-231
EMT Genes
D
Scratch assay
0 Hour
48 Hours
(-)
(+)
MCF7
No ETP
100ng ETP
Cell numbers
E
ZR-75-1
F
MDAMB453
G
MDAMB 231
H
Transwell assay
I
J
K
MDAMB231


MTS assay screening ETP antibody
Survival%
150
100
50
0
#1
#2
#4
#6
#10
#11
#72
0
#1
#4
#7
#10
#13
#16
#19
#22
#25
#28
#31
#34
#37
#40
#43
#46
#49
#52
#55
#58
#61
#64
#67
#70
#73
#76
#79
#82
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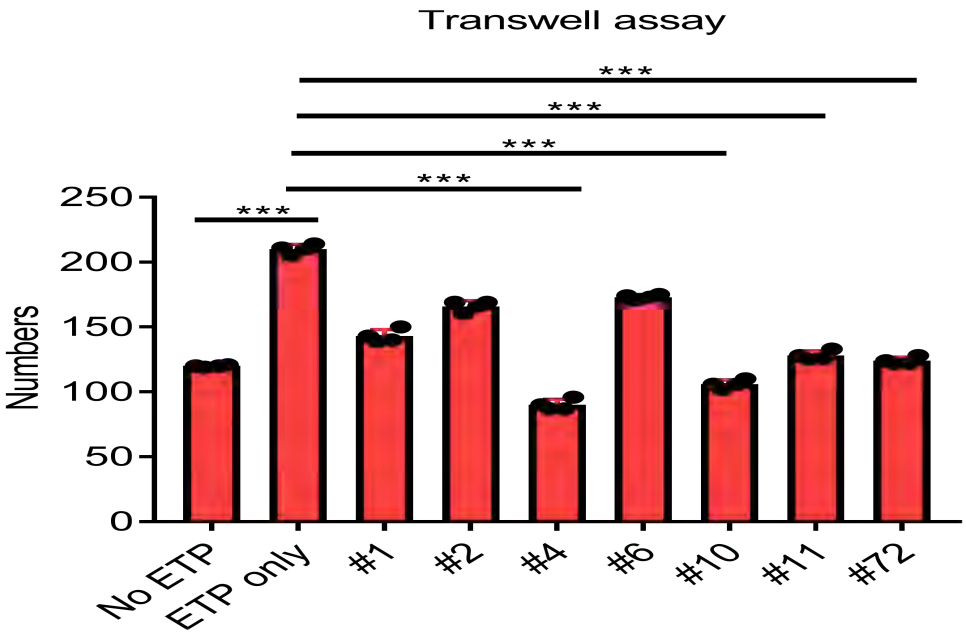
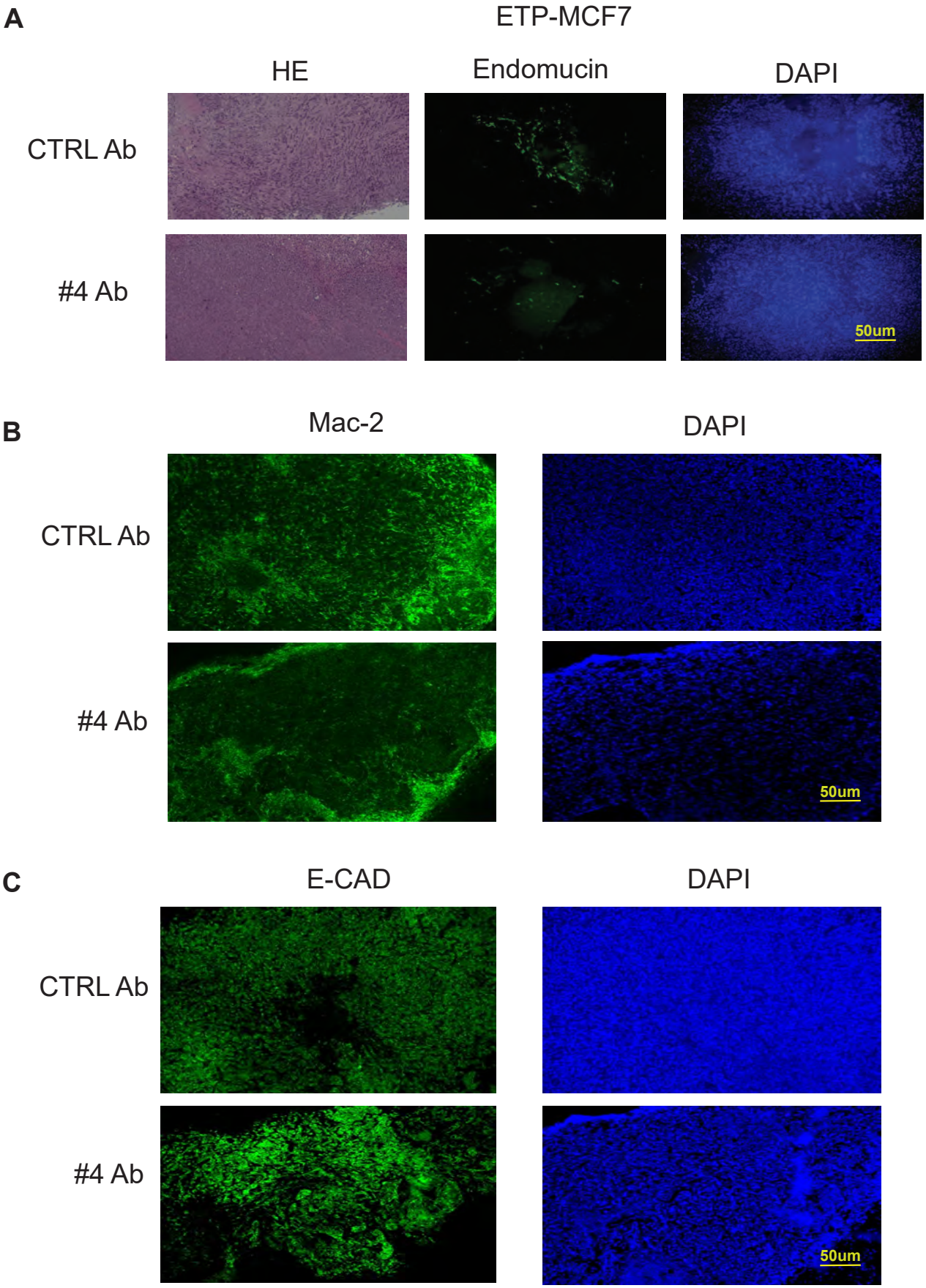
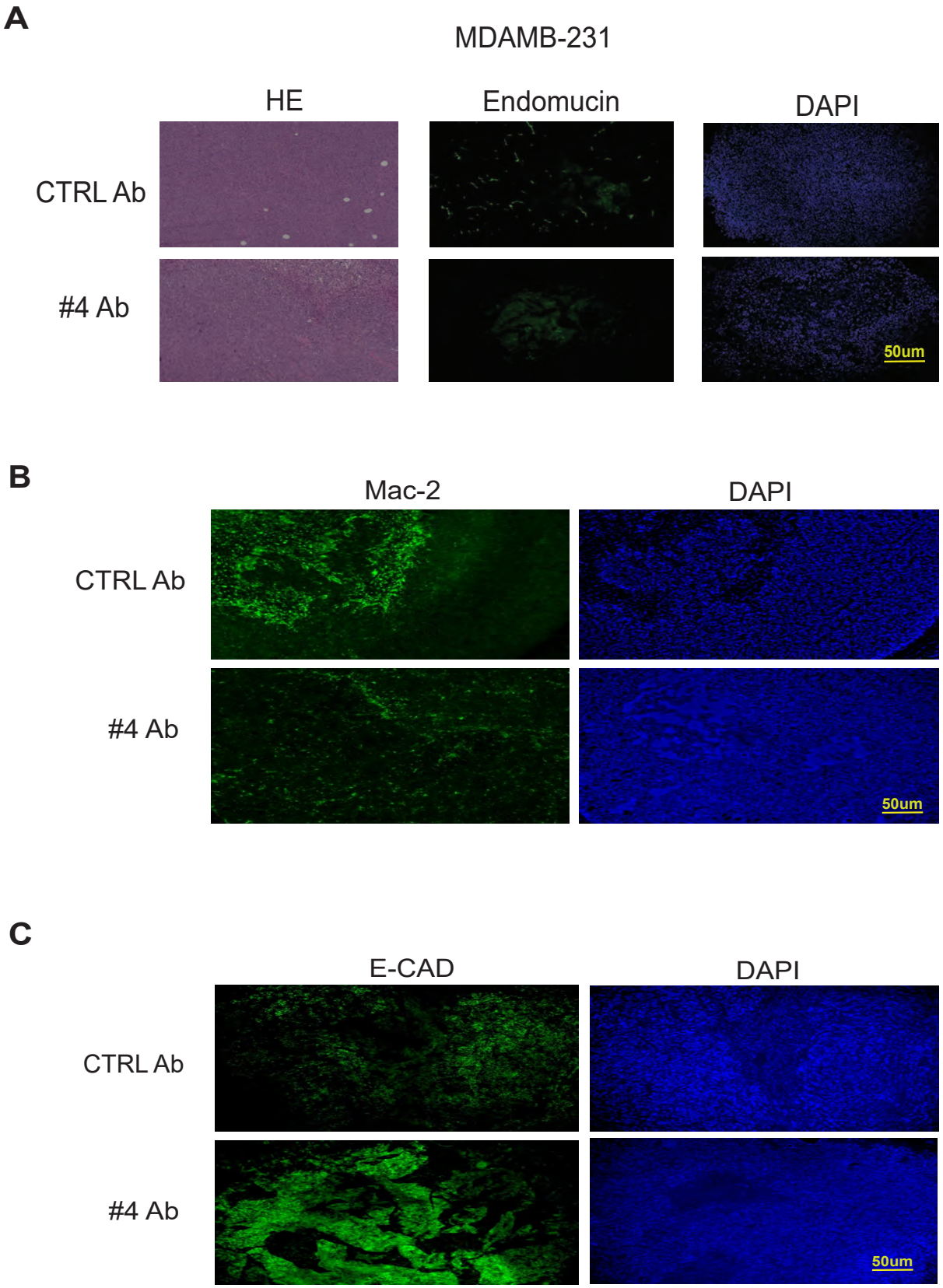
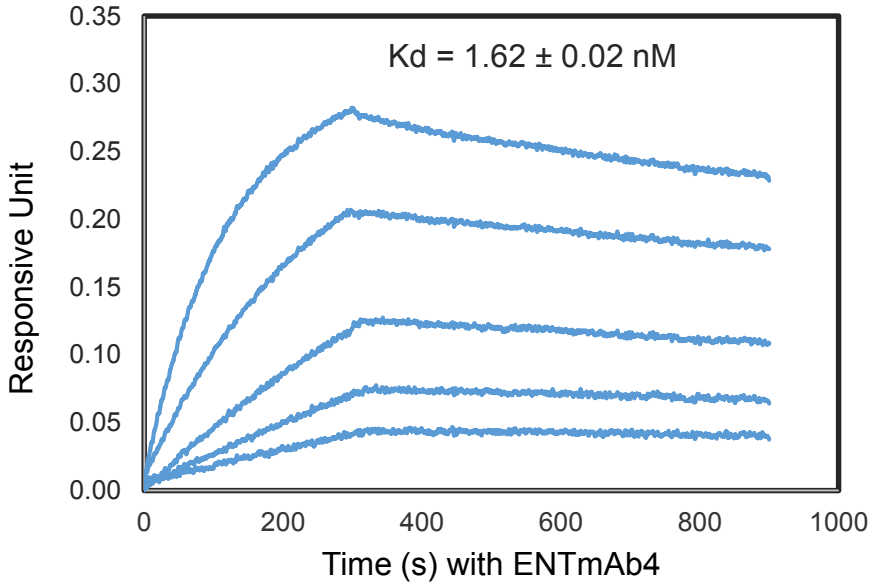
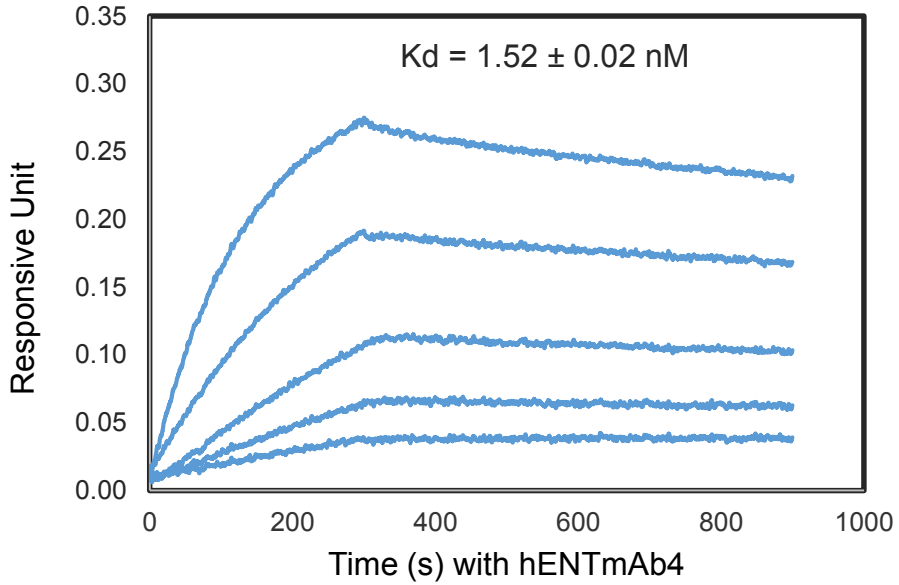
#130


Transwell assay
***
***
***
***
***
250
200
150
100
50
0
Numbers
No ETP
ETP only
#1
#2
#4
#6
#10
#11
#72


A
ETP-MCF7
HE
Endomucin
DAPI
CTRL Ab
#4 Ab
50um
B
Mac-2
DAPI
CTRL Ab
#4 Ab
50um
C
E-CAD
DAPI
CTRL Ab
#4 Ab
50um


A
MDAMB-231
HE
Endomucin
DAPI
CTRL Ab
#4 Ab
50um
B
Mac-2
DAPI
CTRL Ab
#4 Ab
50um
C
E-CAD
DAPI
CTRL Ab
#4 Ab
50um





Antibody	KD (M)	KD Error	kon(1/Ms)	kdis(1/s)	Full X^2	Full R^2
ENTmAb4	1.62E-09	1.70E-11	1.65E+05	2.67E-04	0.0654	0.9978
hENTmAb4	1.52E-09	1.51E-11	1.51E+05	2.30E-04	0.0414	0.9985