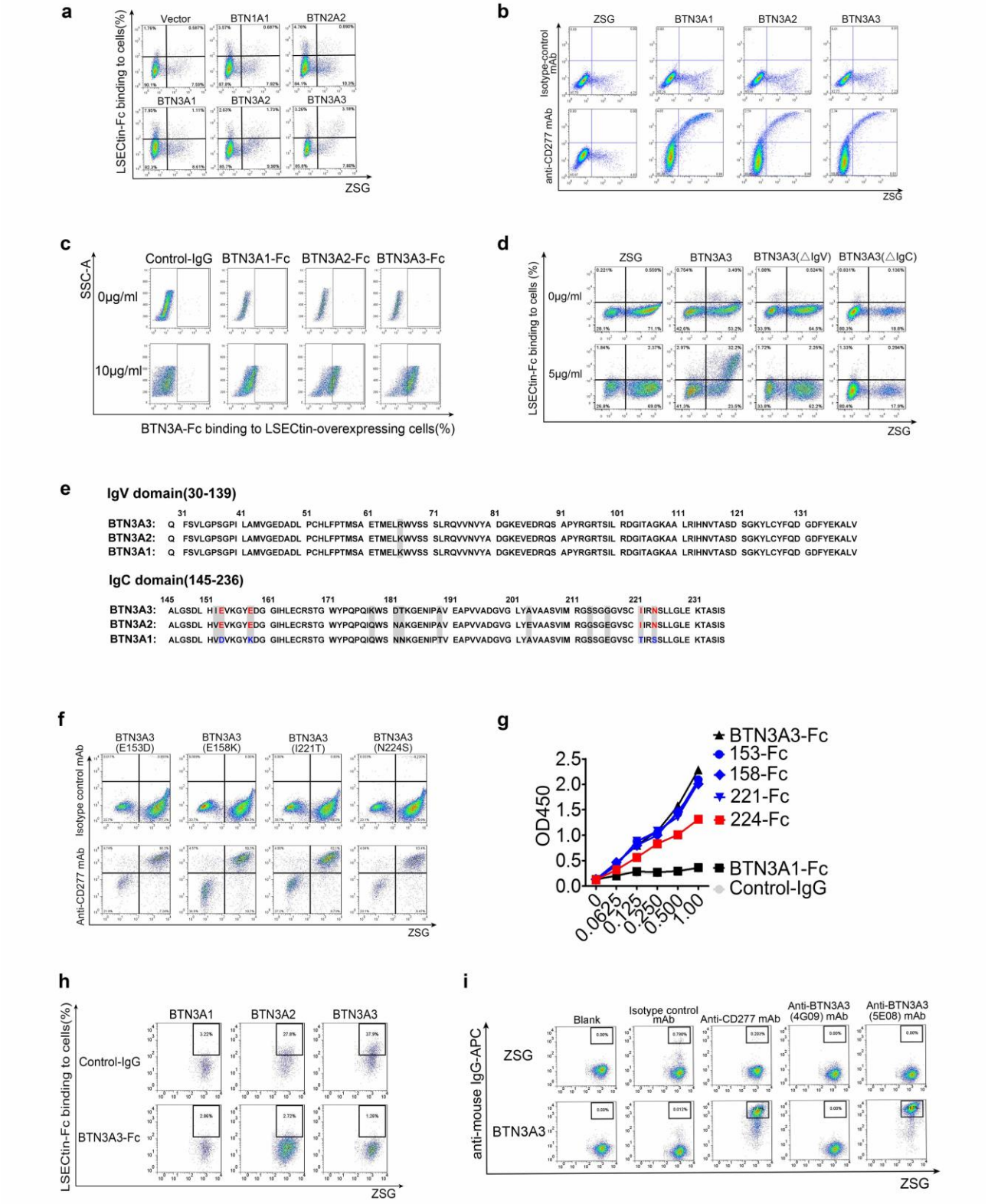
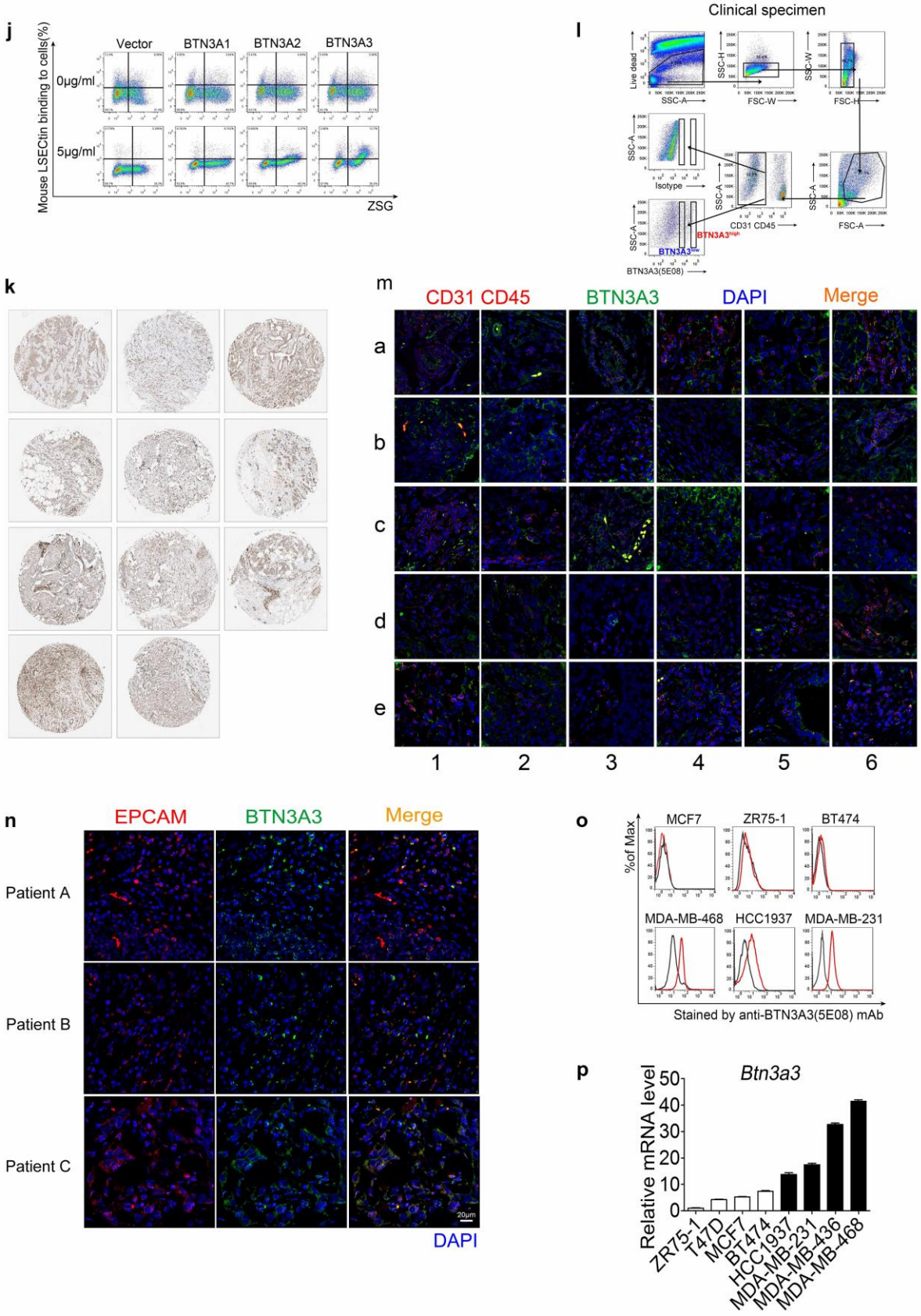
Supplementary information, Fig. S3




j
Vector
BTN3A1
BTN3A2
BTN3A3
0μg/ml
5μg/ml
Mouse LSECtin binding to cells(%)
ZSG
l
Clinical specimen
Live dead
SSC-A
SSC-H
FSC-W
SSC-W
FSC-H
Isotype
CD31 CD45
FSC-A
BTN3A3high
BTN3A3low
BTN3A3(5E08)
k
m
CD31 CD45
BTN3A3
DAPI
Merge
a
b
c
d
e
1
2
3
4
5
6
n
EPCAM
BTN3A3
Merge
Patient A
Patient B
Patient C
20μm
DAPI
o
MCF7
ZR75-1
BT474
MDA-MB-468
HCC1937
MDA-MB-231
%of Max
Stained by anti-BTN3A3(5E08) mAb
p
Btn3a3
Relative mRNA level
0
10
20
30
40
50
ZR75-1
T47D
MCF7
BT474
HCC1937
MDA-MB-231
MDA-MB-436
MDA-MB-468

Supplementary information, Fig. S3. LSECtin can interact with BTN3A3 expressed on human breast cancer cells

(a) HEK293 cells transfected with BTNs were stained with LSECtin-Fc recombinant protein at 2 μg/ml. One of three experiments is shown.
(b) HEK293 cells transfected with BTN3As were stained with anti-CD277 mAb (eBioBT3.1) or isotype control mAb. One of two experiments is shown.
(c) Stable HEK293 cell lines expressing human LSECtin were recognized by recombinant BTN3A-Fc or control-IgG at 5 μg/ml. One of two experiments is shown.
(d) Binding of LSECtin-Fc recombinant protein at 2 μg/ml to HEK293 cells transfected with BTN3A3 deletions. One of two experiments is shown.
(e) Protein sequence encoded by BTN3As and comparison of the BTN3A Ig-V domain and the Ig-C domain.
(f) HEK293 cells transfected with BTN3A3 mutants were stained with anti-CD277 mAb (eBioBT3.1) or isotype control mAb. One of two experiments is shown.
(g) LSECtin-his recombinant protein bound to BTN3A3-Fc, BTN3A3(E153D)-Fc("153-Fc"), BTN3A3(E158K)-Fc("158-Fc"), and BTN3A3(I221T)-Fc("221-Fc") recombinant protein but bound to BTN3A3(N224S)-Fc("224-Fc") weakly. One of three experiments is shown.
(h) The interaction between LSECtin-Fc recombinant protein at 1 μg/ml and BTN3A3 expressed in the HEK293 cells was blocked by BTN3A3-Fc but not by control-IgG at 10 μg/ml. One of three experiments is shown.
(i) HEK293 cells stably transfected with BTN3A3 were stained by anti-BTN3A3 mAb. Isotype control mAb was used as a negative control. A commercial anti-CD277 mAb was used as positive control. One of three experiments is shown.
(j) Binding of mouse LSECtin-Fc recombinant protein at 5 μg/ml to HEK293 cells transfected with BTN3As. One of two experiments is shown.
(k) Tissue array of BTN3A3 expression in breast cancer tissues. All immunohistochemically stained sections were downloaded from the publicly available Human Protein Atlas database (www.proteinatlas.org) and scanned using an automated slide-scanning system at 20× magnification.
(l) Gating strategy for the identification of breast cancer cells in clinical tumor specimens.
(m) Immunofluorescent staining of tumor tissues representing different types of breast cancer for CD31 (red), CD45 (red) or BTN3A3 (green). A high proportion of BTN3A3-positive cells are CD31-negative and CD45-negative cells (scale bar=20 μm). Details regarding the tumor pathology are presented in Supplementary information, Table S4.
(n) Immunofluorescent staining of tumor tissues representing breast cancer for EPCAM (red) or BTN3A3 (green). A high proportion of BTN3A3-positive cells are EPCAM-positive cells (scale bar=20 μm). Details regarding the tumor pathology are presented in Supplementary information, Table S2.

(o) BTN3A3 expression in different breast cancer cell lines. Breast cancer cell lines were stained with isotype control mAb or with anti-BTN3A3 (5E08) mAb. One of three experiments is shown.
(p) Real-time PCR analysis showing the expression of BTN3A3 relative to that of the housekeeping gene *β-actin* in breast cancer cell lines. One of three experiments is shown.