Supplementary Information

Inventory of Supplementary Information

Supplementary Figures and Figure Legends

Supplementary Figures and Figure Legends

Figure S1 The BTG3 C-terminal region between amino acids 147 and 252 mediates direct interaction with AKT *in vitro*. (**a**) Schematic diagrams depicting various BTG3 constructs. (**b**) GST pull-down assays. Experiments were conducted with recombinant GST-BTG3 proteins, either full-length (FL), d4 (amino acids 147-252 deleted), or d6 (amino acids 108-146 deleted), and 293T lysates expressing HA-AKT. Proteins pulled down were analyzed by western blotting using the indicated antibodies.

Figure S2 BTG3 does not impact on the membrane recruitment of the Grp1 PH domain. **(a)** Membrane localization of the GFP-fused GrP1 PH domain (GFP-PH-Grp1) was not affected by either full-length BTG3 (FL) or the BTG3-d4 mutant. Confocal microscopy was performed on 293T cells transfected with indicated constructs. The fluorescence intensity in the membrane as a fraction of the total is shown in **(b)**. n.s., not significant. **(c)** Western blots showing relative expression levels of the two BTG3 proteins used in (a).

Figure S3 BTG3 suppresses AKT-GSK3β-β-catenin signaling in PC3 cells. **(a)** Overexpression of BTG3 downregulates phosphorylation of AKT and GSK3β. Cells were transfected with myc-BTG3. Lysates were analyzed by western blotting using the indicated antibodies. **(b)** Altered expression of EMT markers by overexpression of BTG3. Numbers are from normalization to Actin. **(c)** The β-catenin/TCF reporter activity was inhibited by BTG3 overexpression. **(d)** Cell migration was reduced upon BTG3 expression, as assessed by transwell assay.

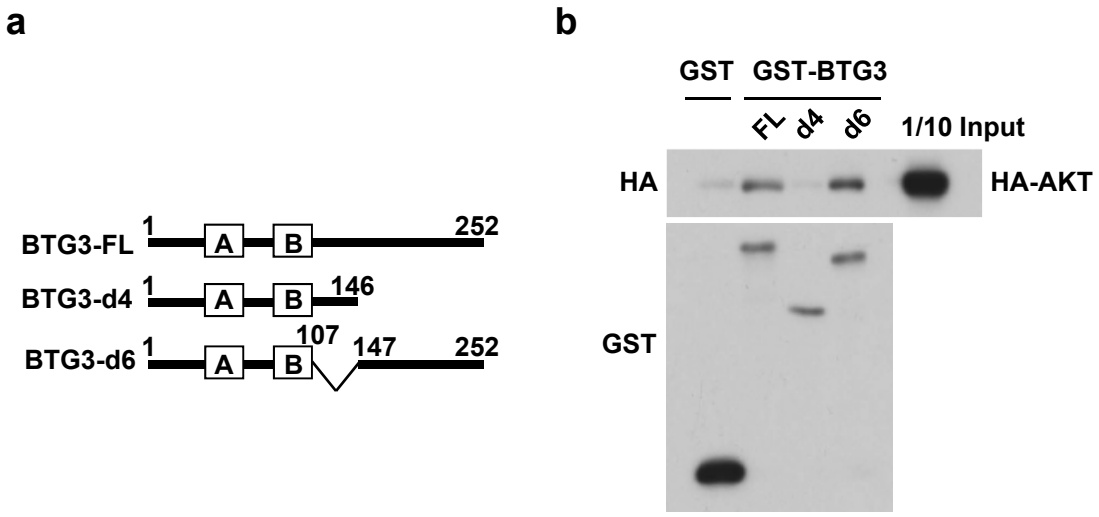
Figure S4 The C-terminal half of the BTG3 C5 peptide is responsible for suppression of AKT–GSK3β–β-catenin signaling. **(a)** Schematic representation of full-length BTG3 and the two C-terminal peptides C5-N and C5-C. **(b)** Amino acid sequences of

C5-N and C5-C. **(c)** C5-C but not C5-N competes with the His-tagged, full-length BTG3 protein for binding GST-AKT *in vitro*. An unrelated peptide HA was used as a control. **(d)** C5-C but not C5-N inhibits AKT in cells. 293T cells were transfected with the indicated peptide, and the resulting lysates were analyzed by immunoblotting using indicated antibodies. **(e)** Nuclear β-catenin in 293T cells was more effectively downregulated with the C5-C peptide than with the C5-N peptide. **(f)** β-Catenin/TCF activity was diminished in C5-C- but not C5-N-transfected cells. **(g)** ERK phosphorylation in 293T cells was not affected by either C5-C or C5-N.

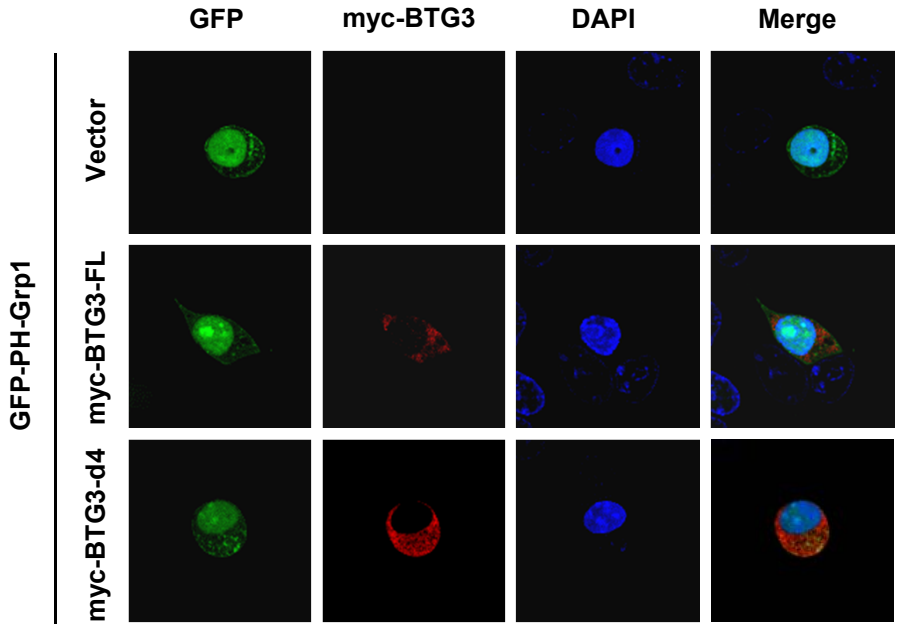
Figure S5 BTG3 suppresses growth of PC3 prostate cancer cells in 3D culture. **(a)** Characterization of PC3 Tet-On cells that express myc-tagged BTG3 upon the addition of doxycycline (+). TR, control cells that express only the tetracycline regulator. ovBTG3, Tet-On BTG3-overexpressing cells (clone #19). AKT T308 phosphorylation was downregulated in ovBTG3 cells. Leaky expression of BTG3 was observed before the addition of doxycycline, which may account for the decreased phospho-AKT T308. (**b, c**) BTG3 overexpression disrupted the polarized growth of PC3 cells (b), and caused reduced plating efficiency in 3D culture (c). The mean ± SD of three independent experiments is shown. *, $P < 0.05$ by Student's *t*-test.

Figure S6 BTG3 overexpression suppresses tumor growth. **(a)** BTG3 impedes the growth of xenograft tumors. PC3-TR or BTG3-overexpressing (ovBTG3) cells (clone #6) were injected s.c. into athymic (nude) mice as in Fig. 7, and tumors were removed 4 w after injection. Left: weekly comparison of the volume of control TR and ovBTG3 tumors. Right: mice with the xenograft tumors. Arrows on the left denote PC3 ovBT3 tumors; on the right, PC3 control TR tumors. *, $P<0.05$. **(b)** BTG3 expression in excised tumor. Tumor tissue extracts prepared from three different mice were analyzed by western blotting with the BTG3 antibody. Actin was used as loading control. #95, #97, and #99 denote mouse ID. **(c)** Representative hematoxylin

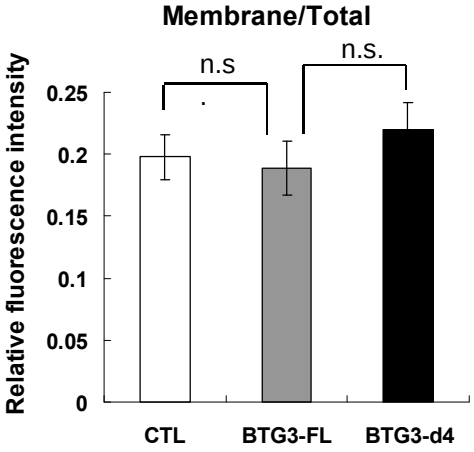
and eosin (H&E) staining of tissues from the TR (left) and the ovBTG3 (right) xenograft tumors. Original magnifications: 12.5 × (top) and 400 × (bottom). **(d, e)** IHC staining of tumor sections with anti-phospho-AKT Ser473 (d) and anti-β-catenin antibodies (e). Scale bar, 50 μm.


a
BTG3-FL
1
A
B
252
BTG3-d4
1
A
B
146
BTG3-d6
1
A
B
107
147
252
b
GST
GST-BTG3
FL
d4
d6
1/10 Input
HA
HA-AKT
GST

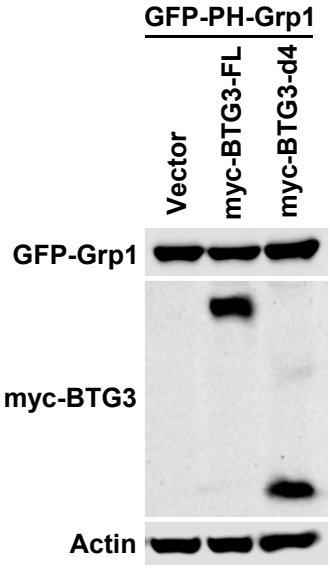
a


GFP
myc-BTG3
DAPI
Merge
GFP-PH-Grp1
Vector
myc-BTG3-FL
myc-BTG3-d4

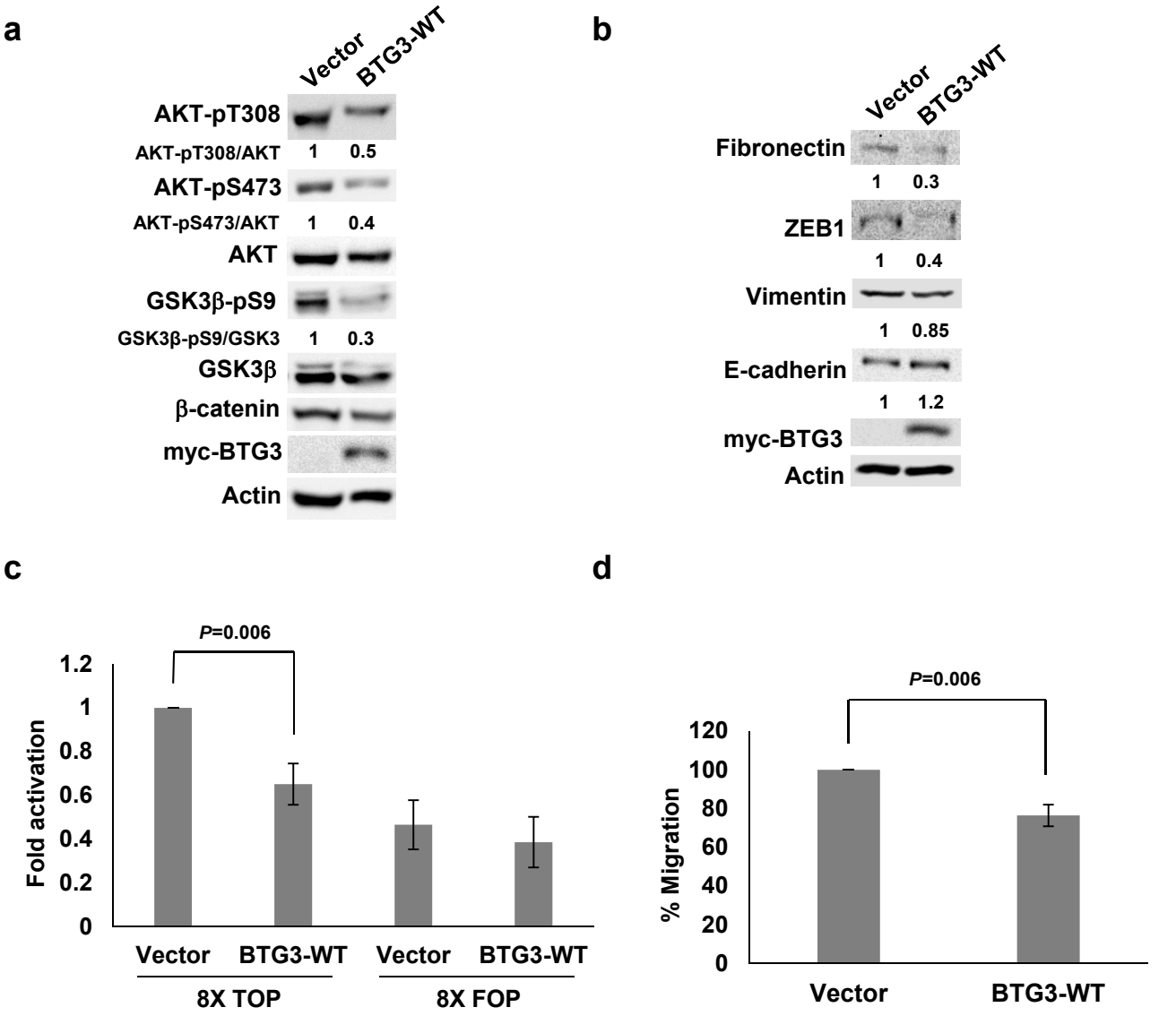
b

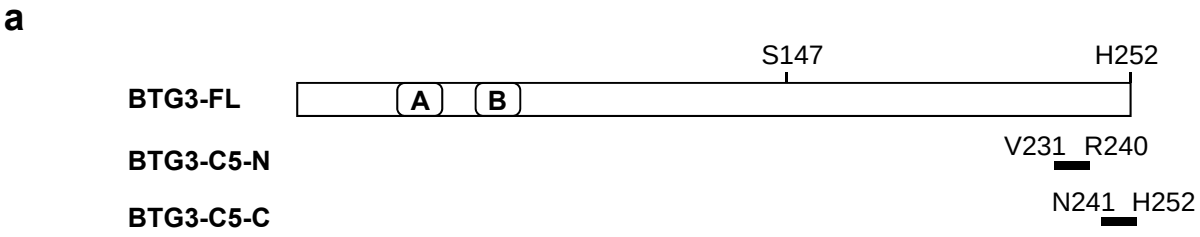

Membrane/Total
n.s
n.s.
Relative fluorescence intensity
0.25
0.2
0.15
0.1
0.05
0
CTL
BTG3-FL
BTG3-d4

c


GFP-PH-Grp1
Vector
myc-BTG3-FL
myc-BTG3-d4
GFP-Grp1
myc-BTG3
Actin

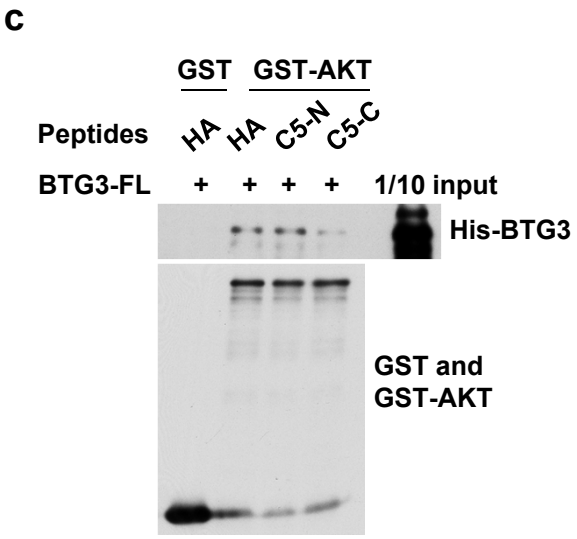
Cheng_FigS3

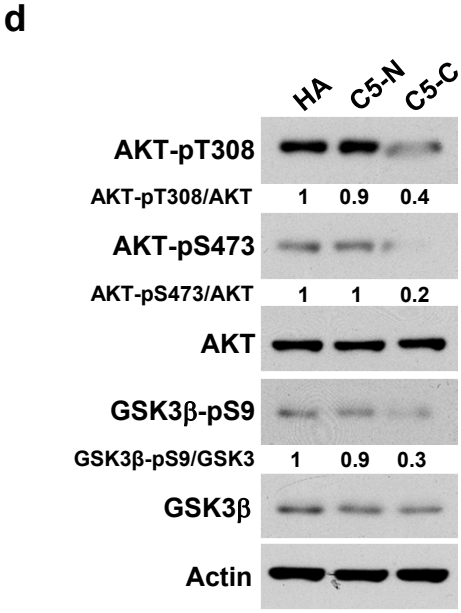

a
Vector
BTG3-WT
AKT-pT308
AKT-pT308/AKT 1 0.5
AKT-pS473
AKT-pS473/AKT 1 0.4
AKT
GSK3β-pS9
GSK3β-pS9/GSK3 1 0.3
GSK3β
β-catenin
myc-BTG3
Actin
b
Vector
BTG3-WT
Fibronectin
1 0.3
ZEB1
1 0.4
Vimentin
1 0.85
E-cadherin
1 1.2
myc-BTG3
Actin
c
P=0.006
Fold activation
1.2
1
0.8
0.6
0.4
0.2
0
Vector
BTG3-WT
Vector
BTG3-WT
8X TOP
8X FOP
d
P=0.006
% Migration
120
100
80
60
40
20
0
Vector
BTG3-WT

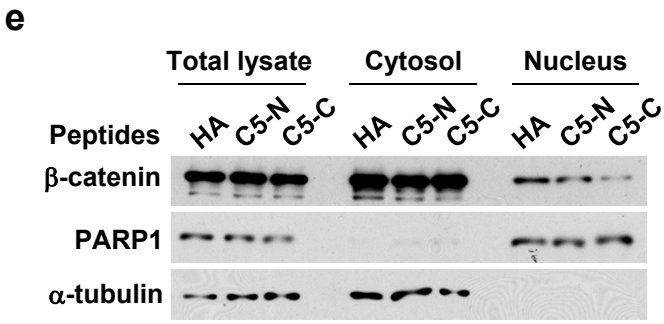


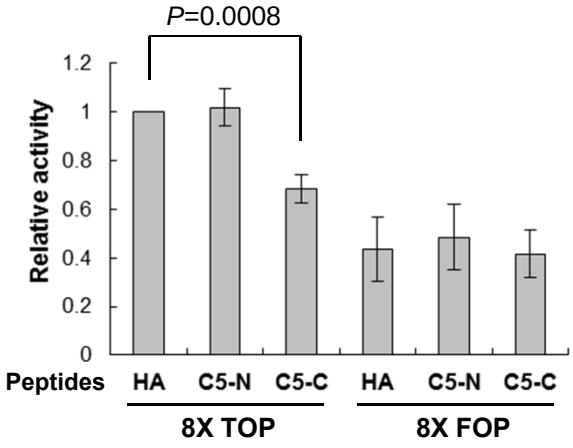
b

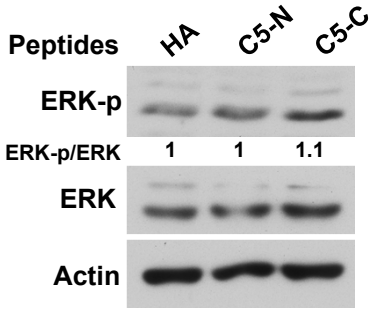
BTG3-C5-N	VPPPGMHCDR
BTG3-C5-C	NHWINPHMLAPH



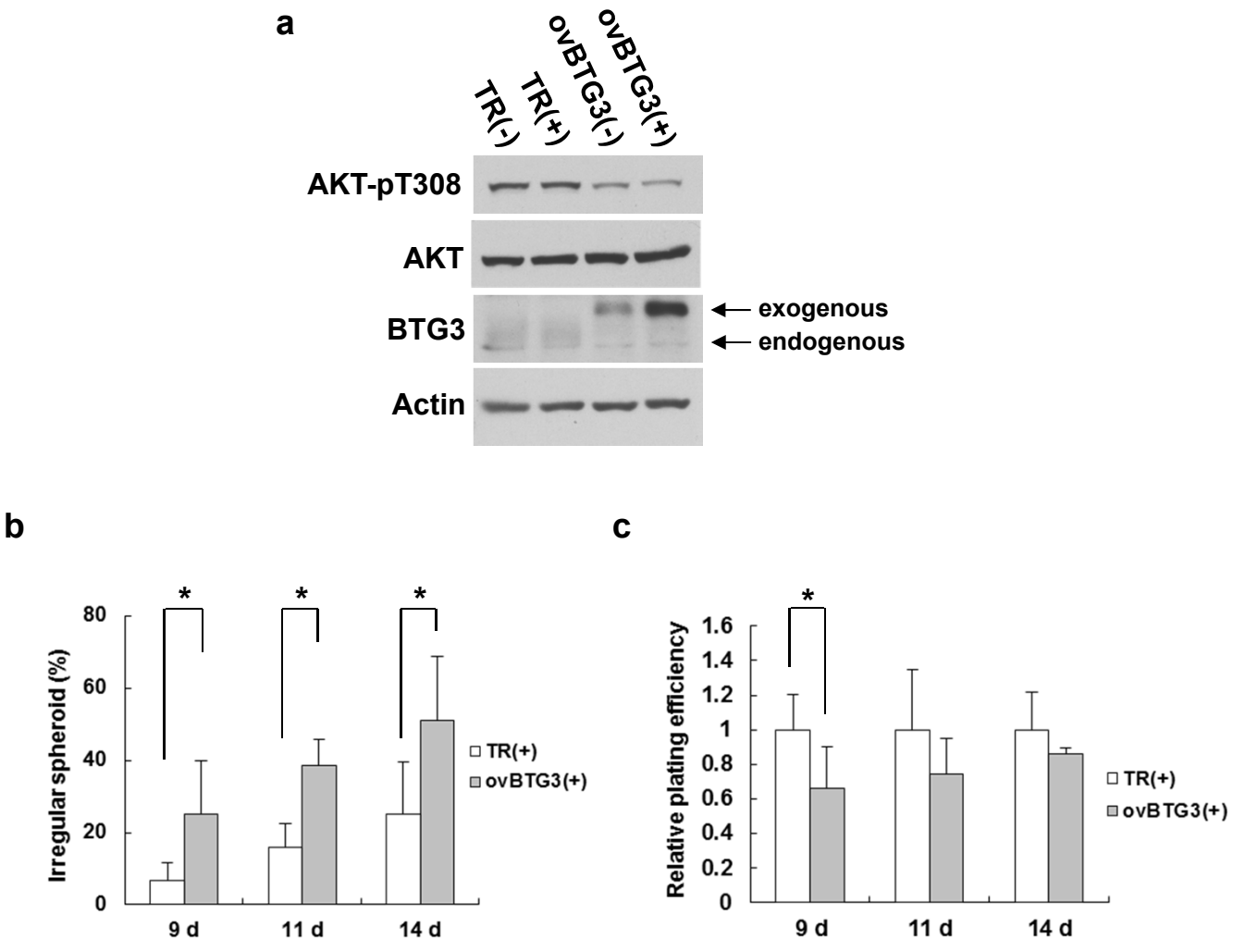




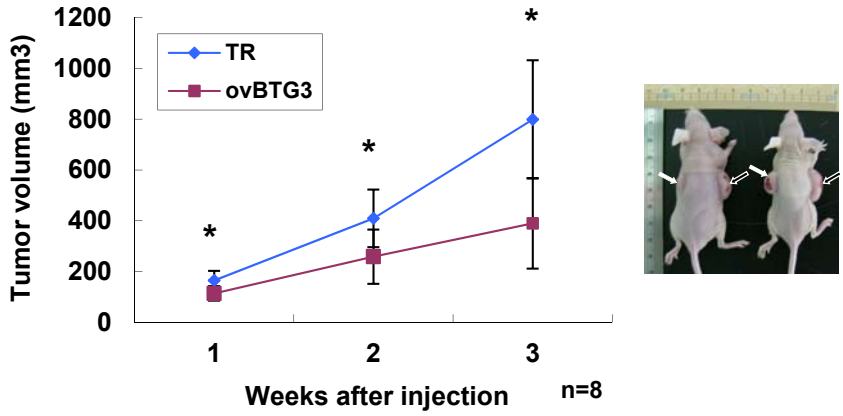




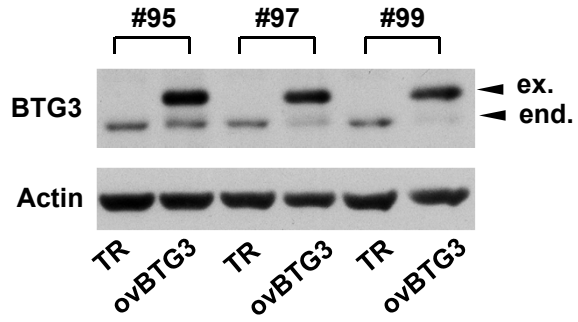
Cheng_FigS5


a
TR(-)
TR(+)
ovBTG3(-)
ovBTG3(+)
AKT-pT308
AKT
BTG3
exogenous
endogenous
Actin
b
Irregular spheroid (%)
80
60
40
20
0
*
*
*
9 d
11 d
14 d
TR(+)
ovBTG3(+)
c
Relative plating efficiency
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
*
9 d
11 d
14 d
TR(+)
ovBTG3(+)

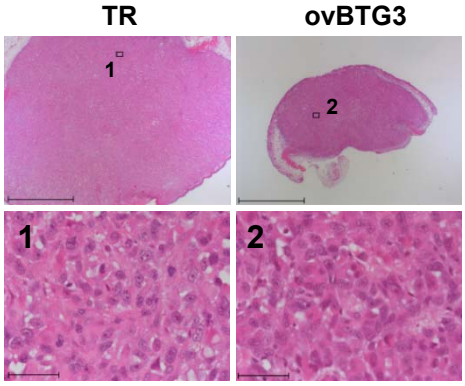
a



b

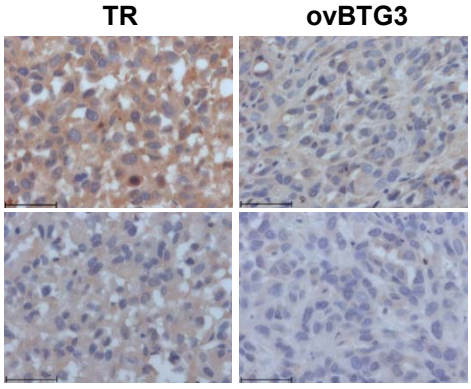


c



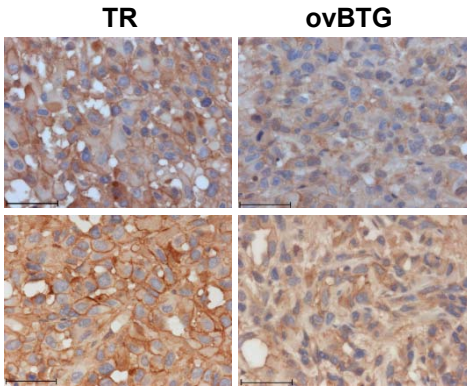
H&E

d



AKT-pS473

e



β-catenin