Supplementary Figures

Figure S1. MCT-1 expression enhances the PI3K/AKT signaling. (**a**) MCF-10A cells starved in serum-free medium for 24 h were treated with LY294002 (PI3K inhibitor) for 4 h before serum activation for 30 min. LY suppressed the MCT-1-enhanced AKT phosphorylation (ser473). The increased PTEN phosphorylation (ser380) suggested that MCT-1 inhibited PTEN activity (lanes 5 and 6). (**b**) MCF-7 cells starved for 24 h were incubated with wortmannin for 4 h and then activated by serum/insulin for 30 min. Wortmannin effectively suppressed MCT-1-stimulated AKT phosphorylation (ser473). (**c**) MCF-10A cells starved for 24 h were then treated with LY, PP2 or UO for 4 h before activation with EGF (+EGF) or serum for 30 min. The MCT-1-induced AKT phosphorylation was well inhibited by LY and partly suppressed by PP2 but not by a MEK inhibitor, UO. (**d**) The MDA-MB-231 cells were cultured with 200 μM cycloheximide for the indicated time intervals. The residual PTEN protein was examined after block of protein biosynthesis. (**e**) PTEN degradation rate and PTEN half-life were evaluated in MDA-MB-468 cells with or without increasing MCT-1 expression.

Fig. S1

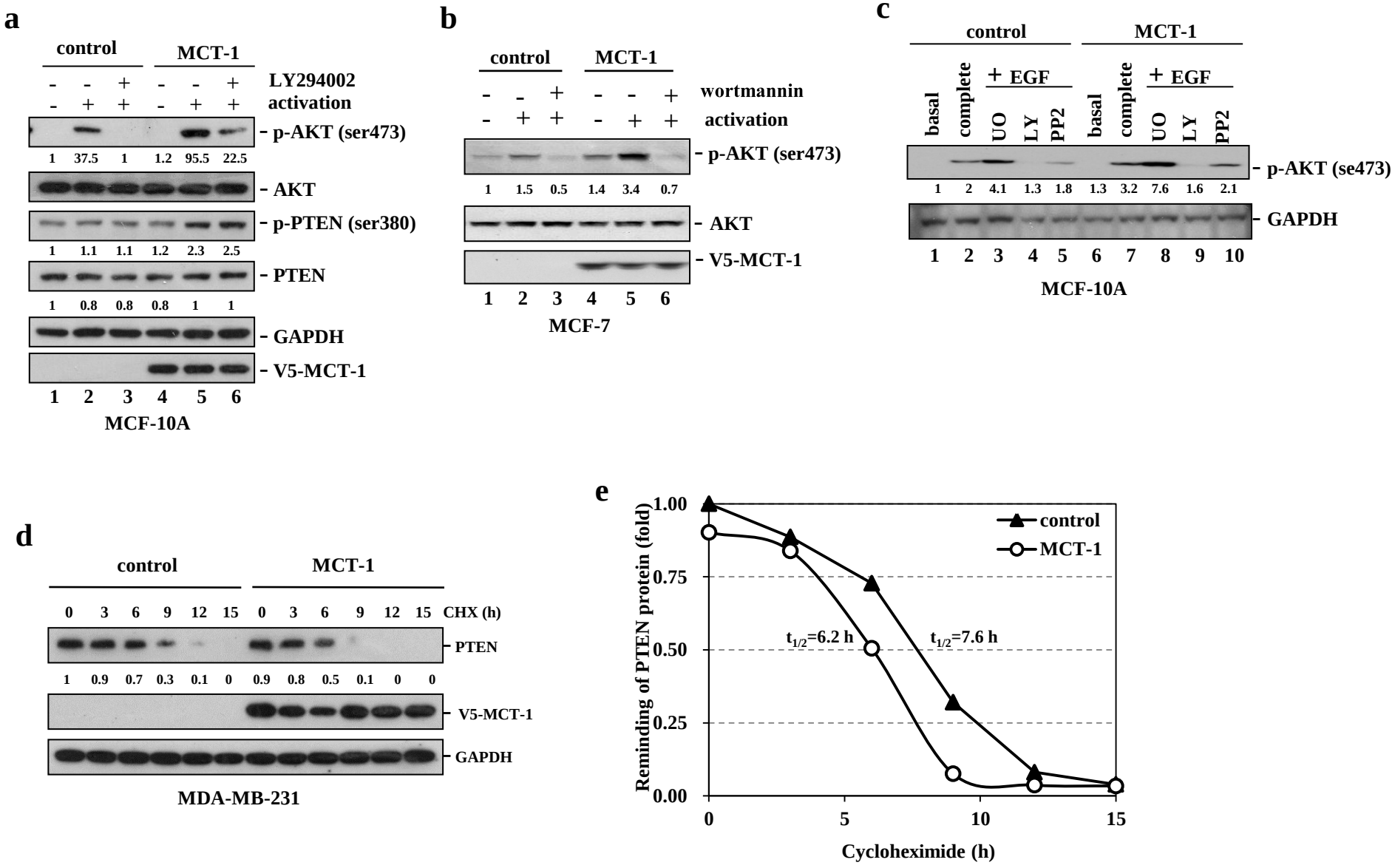

a
control
MCT-1
LY294002
activation
p-AKT (ser473)
1 37.5 1 1.2 95.5 22.5
AKT
p-PTEN (ser380)
1 1.1 1.1 1.2 2.3 2.5
PTEN
1 0.8 0.8 0.8 1 1
GAPDH
V5-MCT-1
1 2 3 4 5 6
MCF-10A
b
control
MCT-1
wortmannin
activation
p-AKT (ser473)
1 1.5 0.5 1.4 3.4 0.7
AKT
V5-MCT-1
1 2 3 4 5 6
MCF-7
c
control
MCT-1
basal
complete
+ EGF
UO
LY
PP2
p-AKT (se473)
1 2 4.1 1.3 1.8 1.3 3.2 7.6 1.6 2.1
GAPDH
1 2 3 4 5 6 7 8 9 10
MCF-10A
d
control
MCT-1
0 3 6 9 12 15 0 3 6 9 12 15 CHX (h)
PTEN
1 0.9 0.7 0.3 0.1 0 0.9 0.8 0.5 0.1 0 0
V5-MCT-1
GAPDH
MDA-MB-231
e
Reminding of PTEN protein (fold)
1.00
0.75
0.50
0.25
0.00
control
MCT-1
t1/2=6.2 h
t1/2=7.6 h
0
5
10
15
Cycloheximide (h)

Figure S2. MCT-1 expression and PTEN deficiency synergistically promote spindle multipolarity and multinuclearity. MCF-10A cells with the indicated expression condition of MCT-1 and PTEN were examined. (**a**) Cells were exposed to nocodazole for 24 h and cultured in the nocodazole-free medium for 1 h. Spindle aster (NuMA staining, red), microtubule structure (α-tubulin staining, green) and nuclear DNA (DAPI, blue) were assessed. (**b**) Cell populations with multiple spindle arrays were quantified. The numbers of cells scored in each cellular context were indicated. (**c**) Cells were exposed to nocodazole for 24 h and released for 1 h. Mitotic regulators p190B, NuMA and p-histone H3 were more expressed in the MCT-1/-PTEN cells than the other cohorts. (**d**) Time-lapse microscopy was conducted. The control/-PTEN cell entered mitosis and divided into two daughter cells. Abortive abscission of the MCT-1/-PTEN cell was observed.

Fig. S2

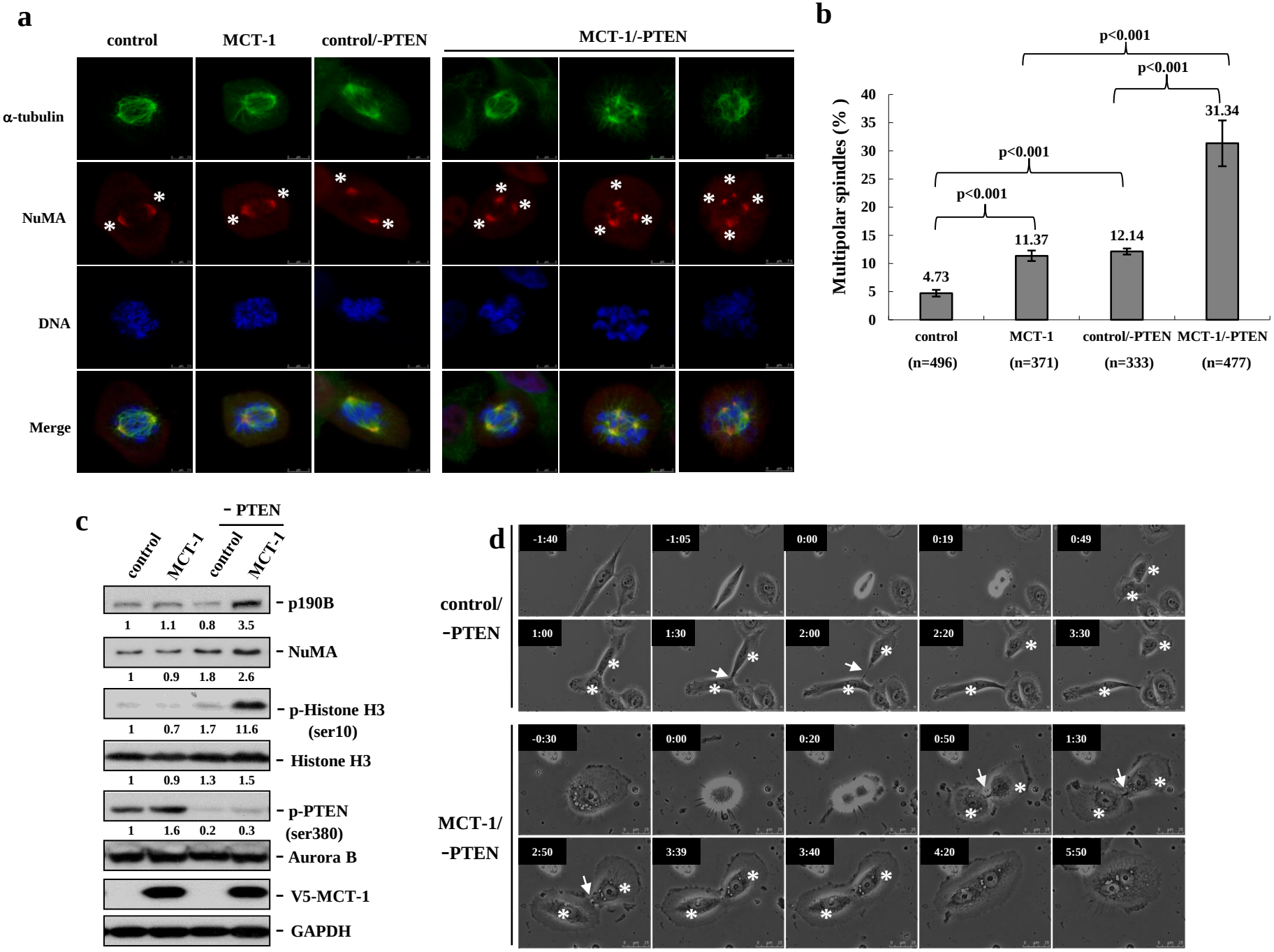

a
control
MCT-1
control/-PTEN
MCT-1/-PTEN
α-tubulin
NuMA
DNA
Merge
b
p<0.001
p<0.001
p<0.001
p<0.001
Multipolar spindles (%)
40
35
30
25
20
15
10
5
0
4.73
11.37
12.14
31.34
control
MCT-1
control/-PTEN
MCT-1/-PTEN
(n=496)
(n=371)
(n=333)
(n=477)
c
- PTEN
control
MCT-1
control
MCT-1
- p190B
1 1.1 0.8 3.5
- NuMA
1 0.9 1.8 2.6
- p-Histone H3 (ser10)
1 0.7 1.7 11.6
- Histone H3
1 0.9 1.3 1.5
- p-PTEN (ser380)
1 1.6 0.2 0.3
- Aurora B
- V5-MCT-1
- GAPDH
d
control/ -PTEN
MCT-1/ -PTEN
-1:40
-1:05
0:00
0:19
0:49
1:00
1:30
2:00
2:20
3:30
-0:30
0:00
0:20
0:50
1:30
2:50
3:39
3:40
4:20
5:50

Figure S3. MCT-1 regulates *p190B* gene expression. MDA-MB-468 cells were examined. (**a**) The mRNA levels of *MCT-1*, *p190B* and *p190A* in the vector control and MCT-1-overexpressing cells were analyzed by Q-RT-PCR. Expression levels of *p190B* but not *p190A* was stimulated by ectopic MCT-1 expression. (**b**) The *MCT-1*, *p190B* and *p190A* mRNA levels were analyzed in the cells with (shMCT-1) or without (MOCK) MCT-1 knockdown. Knockdown of MCT-1 suppressed *p190B* gene expression.

Fig. S3

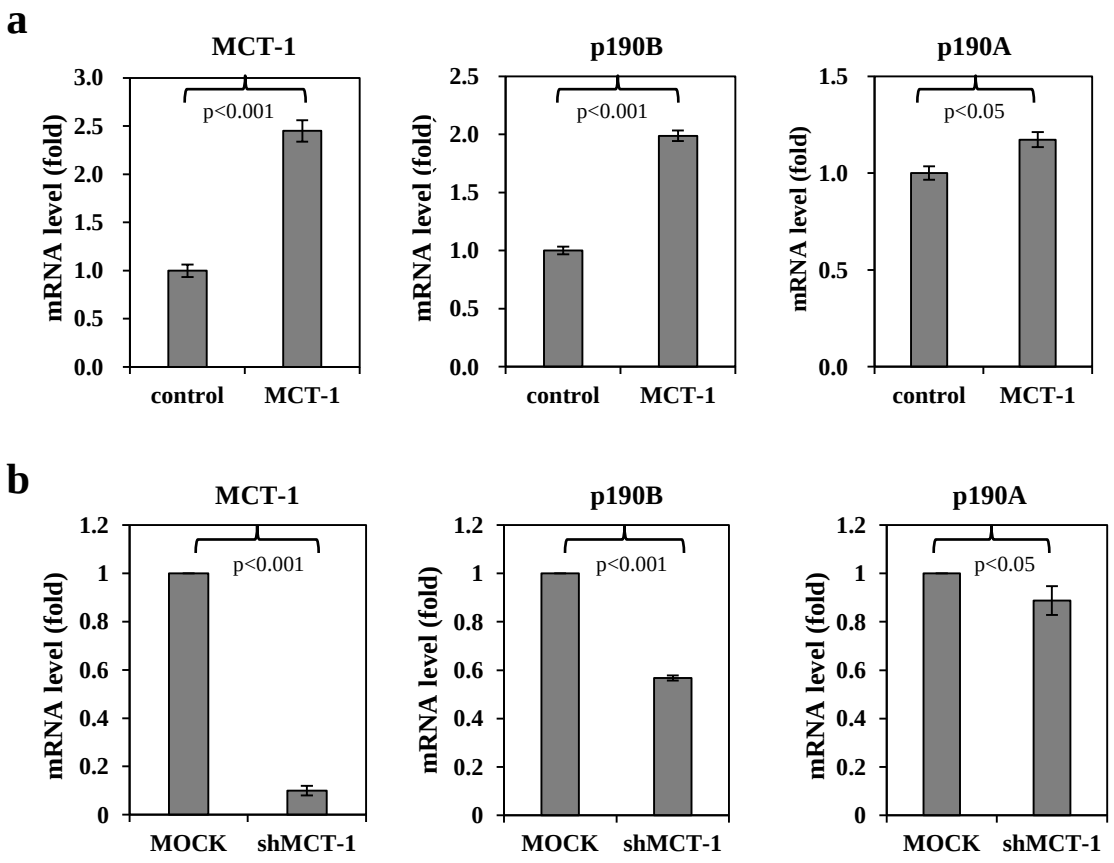

a
MCT-1
p190B
p190A
mRNA level (fold)
p<0.001
p<0.001
p<0.05
control
MCT-1
b
MCT-1
p190B
p190A
mRNA level (fold)
p<0.001
p<0.001
p<0.05
MOCK
shMCT-1

Figure S4. PTEN loss and MCT-1 activation synergistically promotes multinucleation. **(a)** MCF-10A cells with the indicated expression condition of MCT-1 and PTEN were reactivated for 30 min after serum starvation for 24 h. The active Src (tyr416) and p190B (p-tyr) levels were induced and the active RhoA (RhoA-GTP) was suppressed in MCT-1/-PTEN cells. **(b)** Multinuclear frequencies and numbers of cells scored in each group were indicated.

Fig. S4

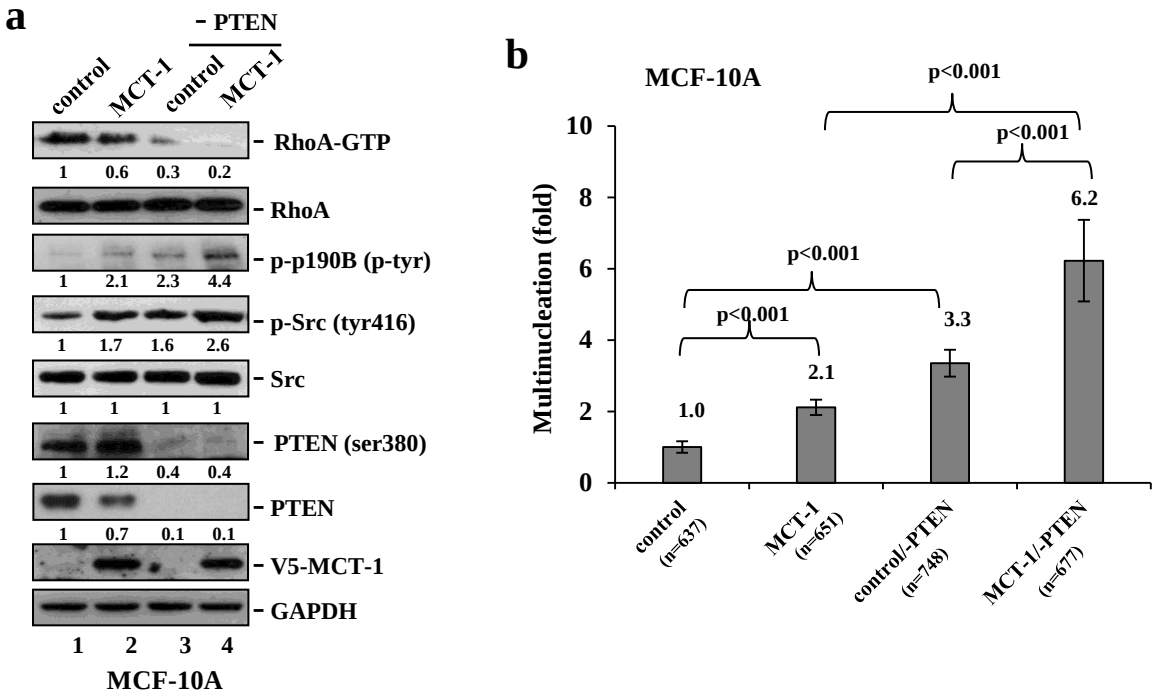

a
- PTEN
control
MCT-1
control
MCT-1
- RhoA-GTP
1 0.6 0.3 0.2
- RhoA
- p-p190B (p-tyr)
1 2.1 2.3 4.4
- p-Src (tyr416)
1 1.7 1.6 2.6
- Src
1 1 1 1
- PTEN (ser380)
1 1.2 0.4 0.4
- PTEN
1 0.7 0.1 0.1
- V5-MCT-1
- GAPDH
1 2 3 4
MCF-10A
b
MCF-10A
Multinucleation (fold)
p<0.001
p<0.001
p<0.001
p<0.001
1.0
2.1
3.3
6.2
control (n=637)
MCT-1 (n=651)
control/-PTEN (n=748)
MCT-1/-PTEN (n=677)

Figure S5. Overexpressing MCT-1 deregulates G2/M mitotic checkpoint. (**a**) After treatment with nocodazole or taxol, cell cycle profiling was examined by flow cytometry. The ectopic MCT-1 cells arrested at the G2/M stage were less than the control cells upon microtubule disruption. **(b)** The G2/G1 ratio upon mitotic arrest was analyzed. Reduced G2/G1 ratio may correspond to the G2/M checkpoint impaired in the ectopic MCT-1 cells.

Fig. S5

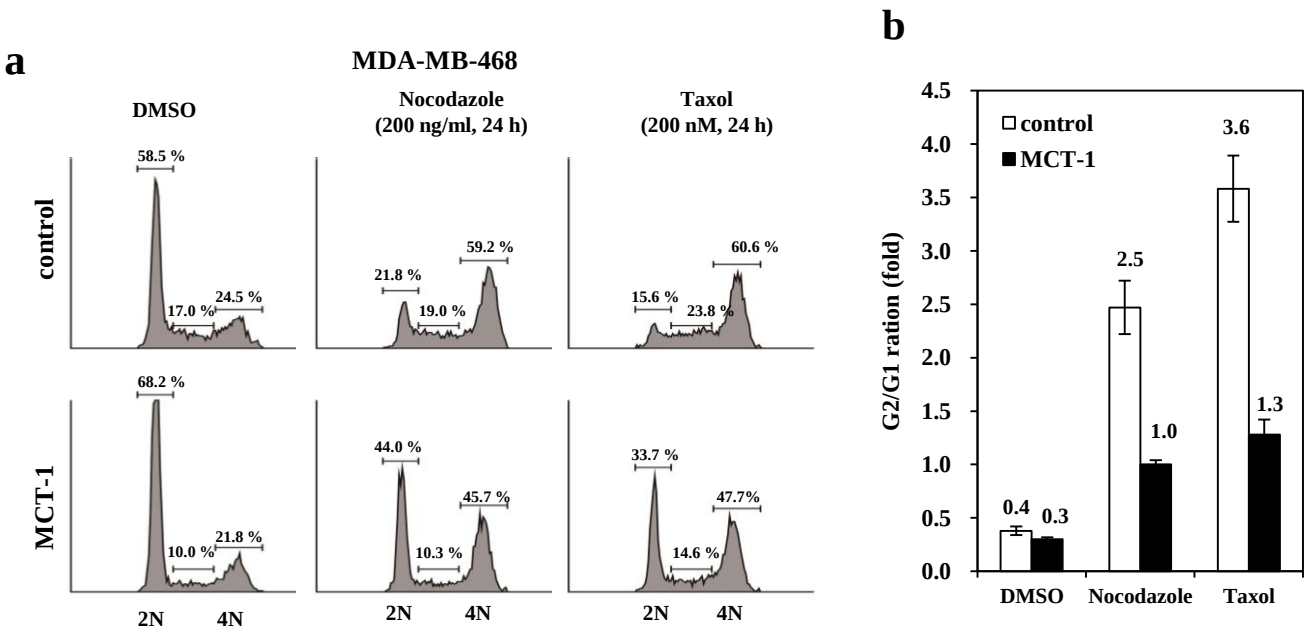
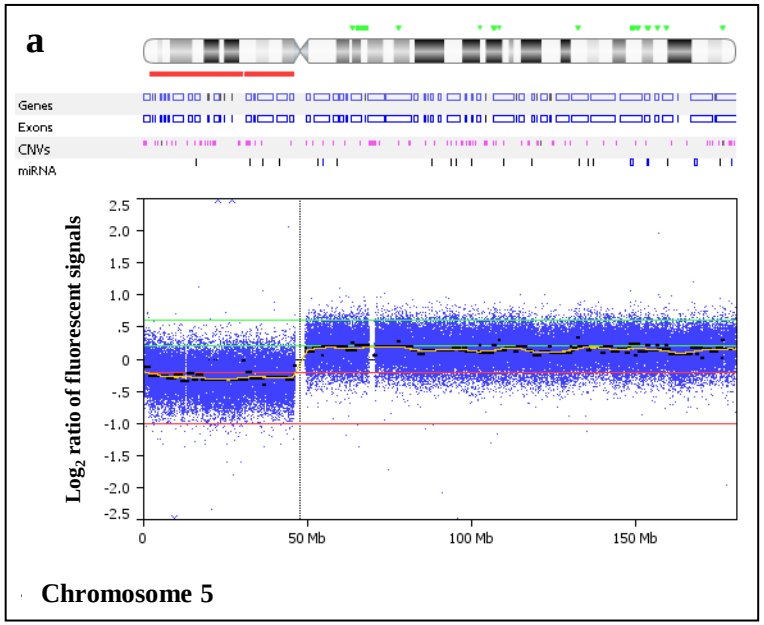
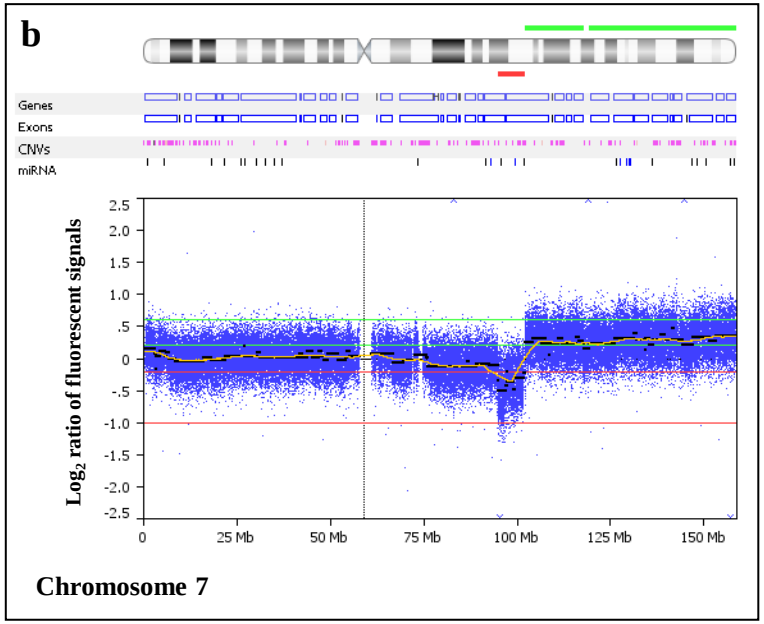
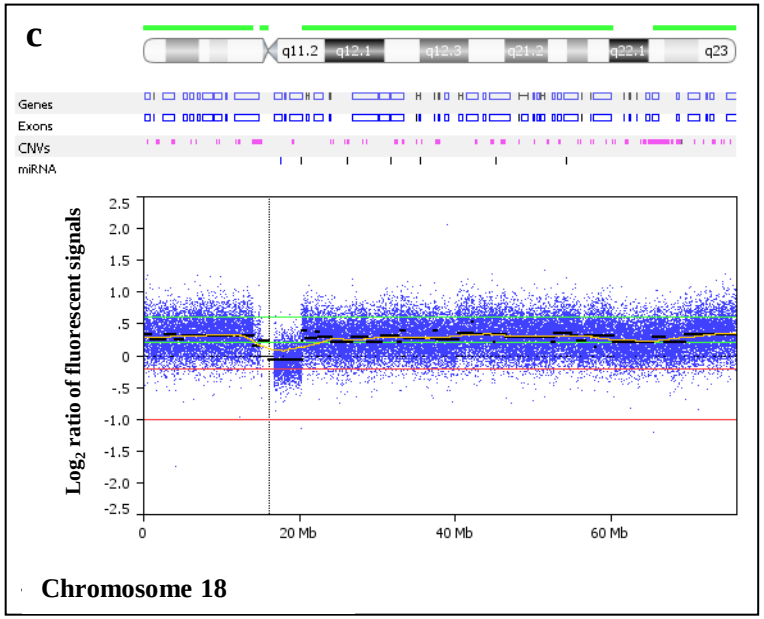

a
MDA-MB-468
DMSO
Nocodazole
(200 ng/ml, 24 h)
Taxol
(200 nM, 24 h)
control
MCT-1
58.5 %
17.0 %
24.5 %
21.8 %
19.0 %
59.2 %
15.6 %
23.8 %
60.6 %
68.2 %
10.0 %
21.8 %
44.0 %
10.3 %
45.7 %
33.7 %
14.6 %
47.7%
2N
4N
b
G2/G1 ration (fold)
control
MCT-1
0.4
0.3
2.5
1.0
3.6
1.3
DMSO
Nocodazole
Taxol

Figure S6. Chromosomal abnormalities induced by the overexpression of MCT-1. For the whole genome comparison between the MCT-1 overexpression (test) and control (reference) MCF-10A cells, their genomic DNA samples were individually labeled with Cy3 and Cy5. (a) Chromosome 5p showed a long region with a deletion score of -0.3. (b) Chromosome 7q revealed only a short region with a deletion score of -0.4, followed by a long region with an amplification score of +0.3. (c) Chromosome 18 contained a large area with amplification scores greater than the 0.2 cutoff.

Fig. S6


a
Genes
Exons
CNVs
miRNA
Log2 ratio of fluorescent signals
2.5
2.0
1.5
1.0
.5
0
-.5
-1.0
-1.5
-2.0
-2.5
0
50 Mb
100 Mb
150 Mb
Chromosome 5


b
Genes
Exons
CNVs
miRNA
Log2 ratio of fluorescent signals
2.5
2.0
1.5
1.0
.5
0
-.5
-1.0
-1.5
-2.0
-2.5
0
25 Mb
50 Mb
75 Mb
100 Mb
125 Mb
150 Mb
Chromosome 7


c
q11.2
q12.1
q12.3
q21.2
q22.1
q23
Genes
Exons
CNVs
miRNA
Log2 ratio of fluorescent signals
2.5
2.0
1.5
1.0
.5
0
-.5
-1.0
-1.5
-2.0
-2.5
0
20 Mb
40 Mb
60 Mb
Chromosome 18