Supplementary Table and Figures

Supplementary Table 1. Correlation between Bmi-1 and Mel-18 expression in invasive breast tumors. For relative comparison, + indicates that number of tumors that had a score of 4 or more, while – indicates the number of tumors that had a score of less than 4 as described in the main text (Materials and Methods). In absolute terms, - does not indicate that the tumor samples did not immunostain for the indicated protein.

		Bmi-1 expressing tumors		P value
		-	+	
Mel-18 expressing tumors	-	1	45	<0.0001
	+	9	6	

Supplementary Figure Legends

Fig. S1. Bmi-1 and Mel-18 immunostaining pattern in two different breast tumor samples in which normal and tumor tissues could be visualized side by side. Immunostaining was done as described in the main text (materials and methods).

Fig. S2. Mel-18 overexpression or Bmi-1 knockdown using Bmi-1 shRNA (Bmi-1 i#2) does not result in altered expression of p53 and pRb. However, overexpression of wild type Mel-18 but not the ring finger mutant of Mel-18 results in downregulation of Bmi-1 and Akt activity. Bmi-1 knockdown also results in downregulation of Akt activity.

Fig. S3. Regulation of Akt activity by Mel-18 and Bmi-1 in MCF7 depends on estradiol (E2) present in fetal calf serum (FCS). Charcoal stripped serum does not induce Akt activity, while regular FCS induces Akt activity in control cells but the induction of activated Akt is attenuated in Mel-18 overexpressing and Bmi-1 knockdown cells. * indicate a non-specific band.

Fig. S4. Bmi-1 regulates Akt activity via activation of PI3K pathway. Vector control or Bmi-1 overexpressing MCF10A cells (as indicated) were treated with PI3K inhibitors (LY294002 and Wortmannin) and analyzed for Akt activation as described in the main text (materials and methods). Bmi-1 induces Akt only in vehicle (DMSO) treated cells. Results also indicate that PI3K inhibitors do not modulate Bmi-1 expression.

Fig. S5. Restoration of Akt activity by overexpressing mAkt overcomes inhibition of anchorage-independent growth by knockdown of Bmi-1 expression. mAkt was overexpressed using a retroviral vector in MCF7 cells expressing Bmi-1 shRNA (Bmi-1 i). Control cells (Ctrl i), and Bmi-1 i, Ctrl i + mAkt and Bmi-1 i + mAkt cells were analyzed for the expression of activated Akt by western blot analysis (Fig. S5*A*) and colony formation using soft agar assay (Fig. S5*B*, and S5*C*) as described in the main text and Fig. 4 legend.

Fig. S1

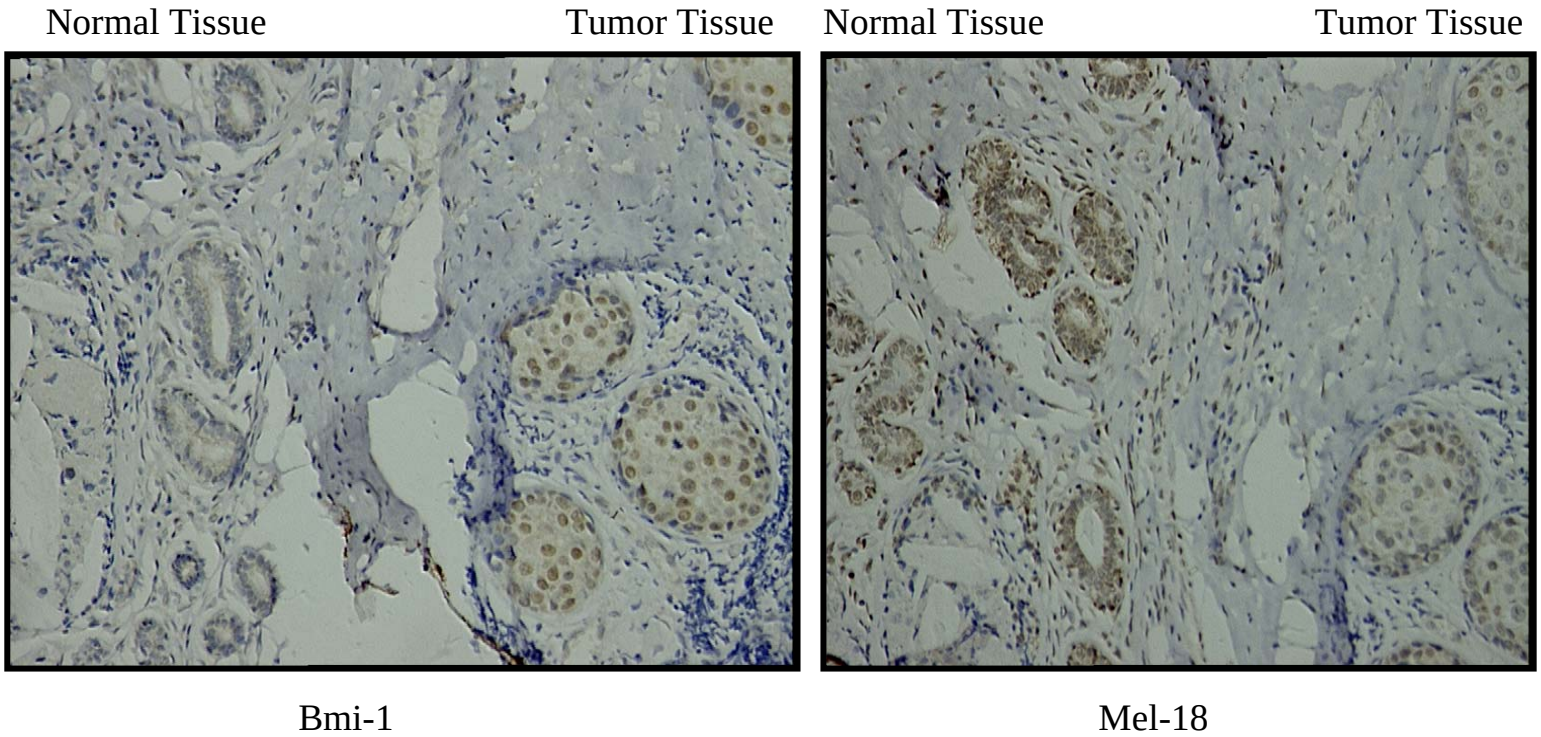

Normal Tissue
Tumor Tissue
Normal Tissue
Tumor Tissue
Bmi-1
Mel-18

Fig. S2

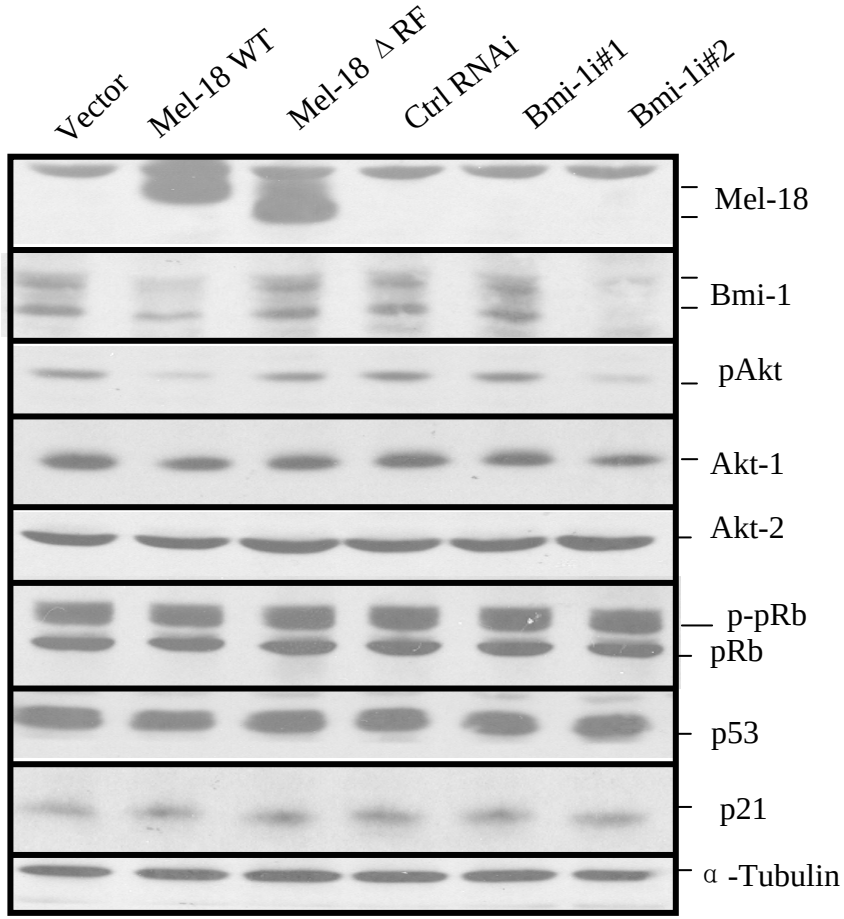

Vector
Mel-18 WT
Mel-18 ΔRF
Ctrl RNAi
Bmi-1i#1
Bmi-1i#2
Mel-18
Bmi-1
pAkt
Akt-1
Akt-2
p-pRb
pRb
p53
p21
α -Tubulin

Fig. S3

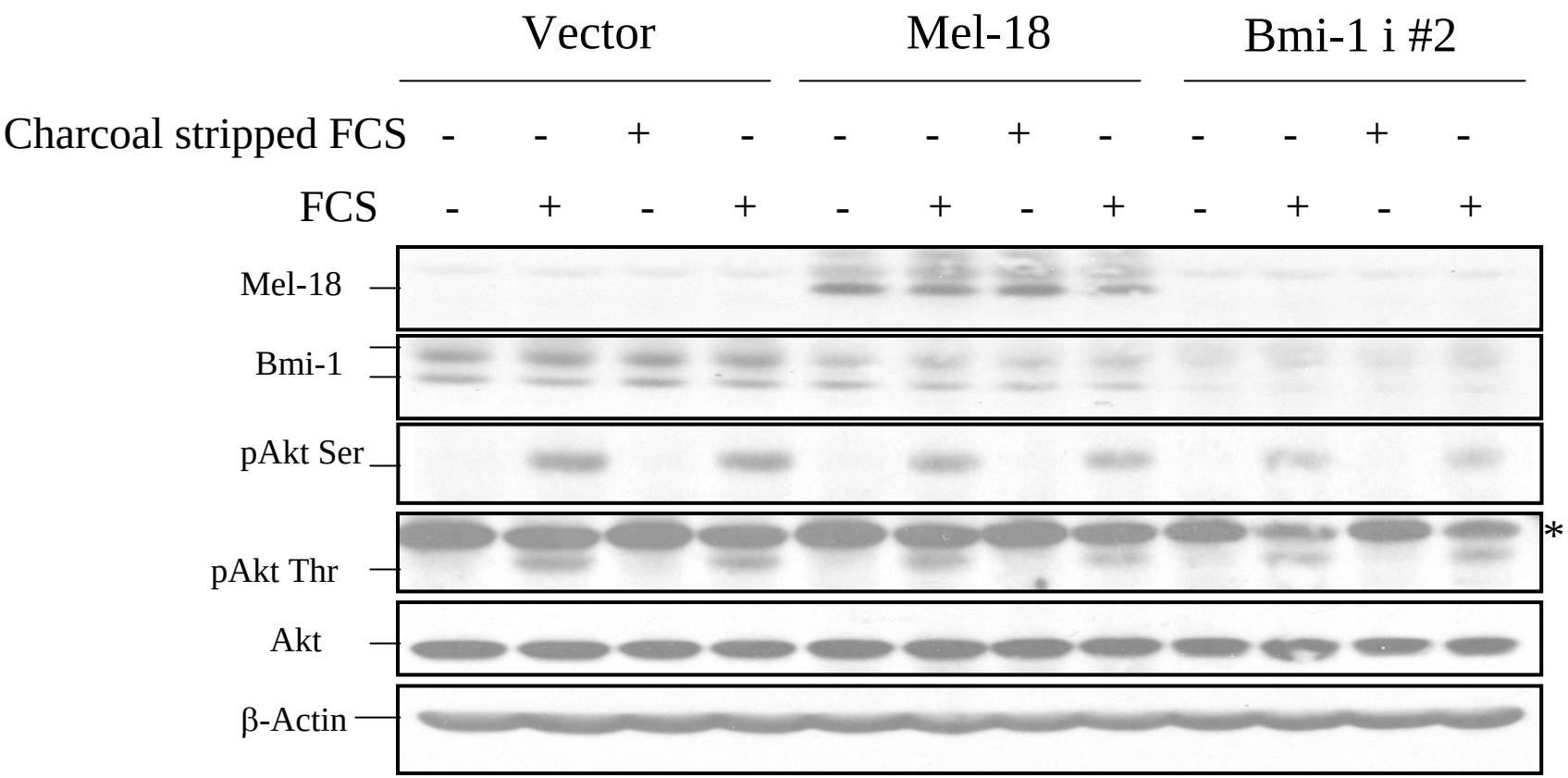

Vector
Mel-18
Bmi-1 i #2
Charcoal stripped FCS - - + - - - + - - - + -
FCS - + - + - + - + - + - +
Mel-18
Bmi-1
pAkt Ser
pAkt Thr
*
Akt
β-Actin

Fig. S4

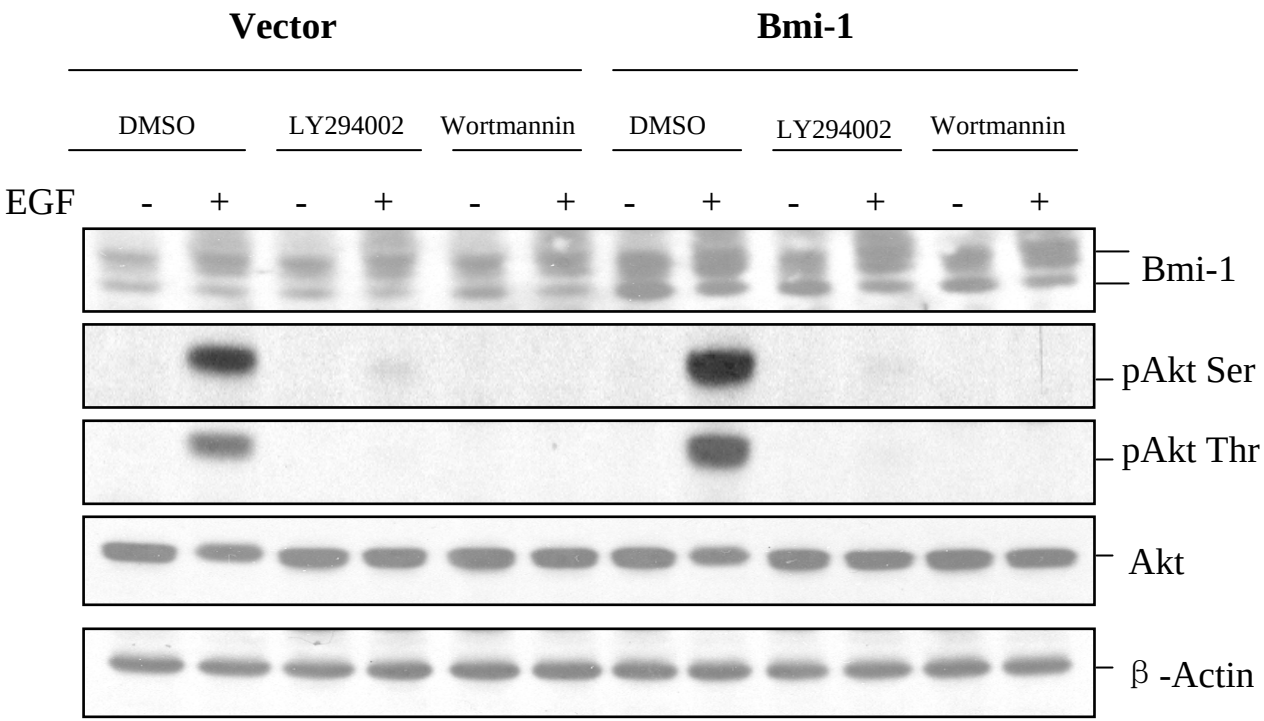
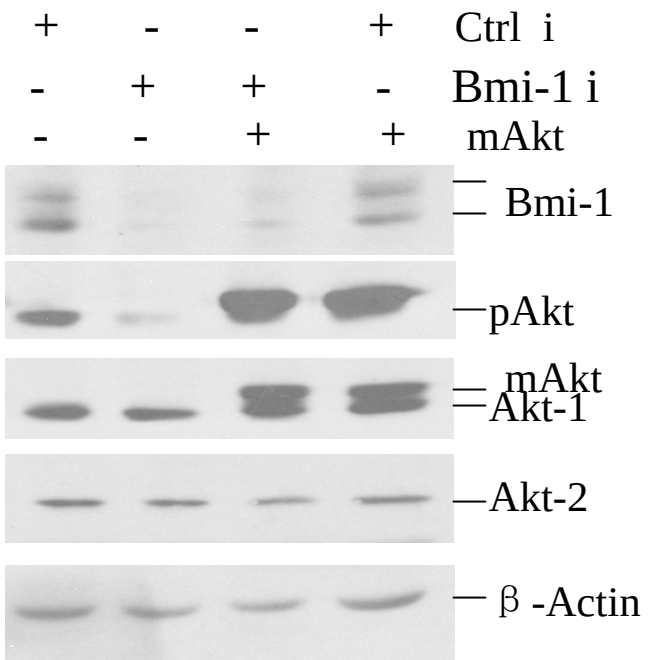
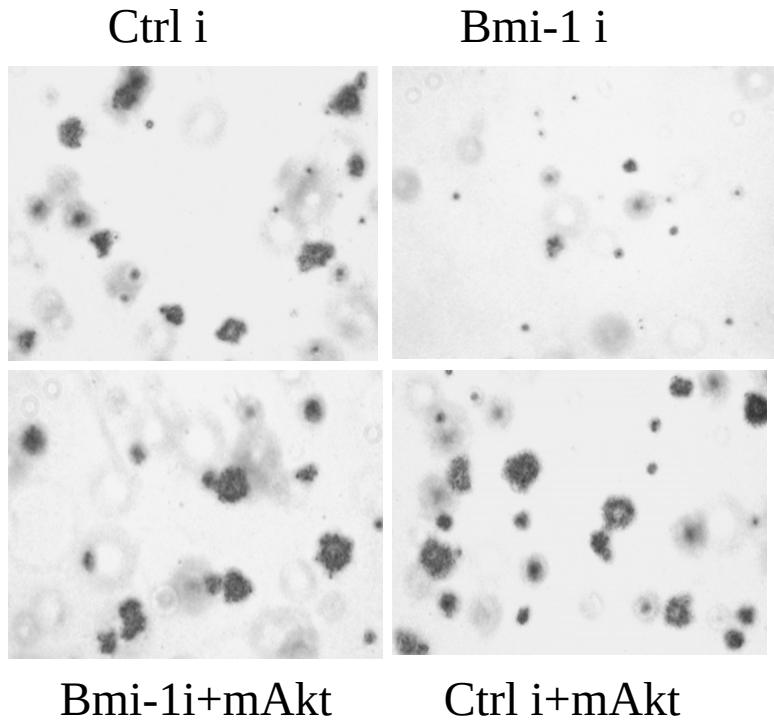

Vector
Bmi-1
DMSO
LY294002
Wortmannin
DMSO
LY294002
Wortmannin
EGF
- + - + - + - + - + - +
Bmi-1
pAkt Ser
pAkt Thr
Akt
β -Actin

Fig. S5

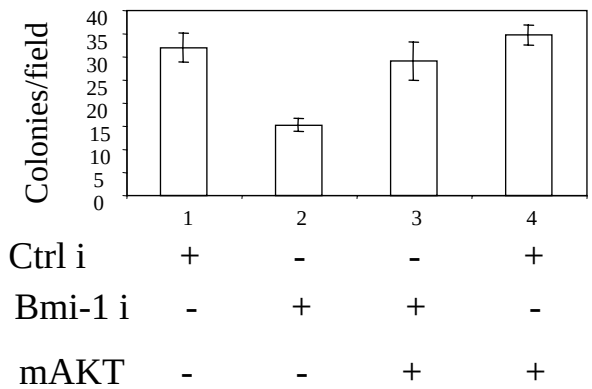
A.


+ - - + Ctrl i
- + + - Bmi-1 i
- - + + mAkt
Bmi-1
pAkt
mAkt
Akt-1
Akt-2
β -Actin

B.


Ctrl i
Bmi-1 i
Bmi-1i+mAkt
Ctrl i+mAkt

C.


Colonies/field
40
35
30
25
20
15
10
5
0
1
2
3
4
Ctrl i + - - +
Bmi-1 i - + + -
mAKT - - + +