Figure	Cell type	% Apical polarization ± SEM
1a	Ctrl	80 ± 2.08
	AT1	12 ± 1.26
	ErbB2	21 ± 1.31
	RasV12	0
2a	Ctrl	85 ± 2.27
	T4-2 Vehicle	15 ± 2.88
	T4-2 U0126	75 ± 2.55
2d	Ctrl	77 ± 5.80
	ErbB2 Vehicle	20 ± 2.08
	ErbB2 U0126	67 ± 4.84

Supplementary Table. Apical polarization.

Percentage of 3D colonies showing apical polarized GM130.



(a) MCF7 cells were transduced with a lentiviral scramble (shctrl) or a  $\alpha$ 5 integrin knockdown constructs (sh $\alpha$ 5) and analyzed for  $\alpha$ 5 integrin and Lamin B1 (loading control) expression by immunoblot. (b)  $\alpha$ 5 integrin was ectopically expressed in MCF10A cells and analyzed by immunoblot in Ctrl and  $\alpha$ 5 integrin (+ $\alpha$ 5 integrin) over-expressing MCF10A cells. (c) Representative pictures of control (Ctrl) and  $\alpha$ 5 integrin (+ $\alpha$ 5 integrin) over-expressing MCF10A cells. (c) cells. Bottom panels: fluorescent pictures of control and  $\alpha$ 5-integrin over-expressing MCF10A cells in 2D. Scale bar, 200µm, at the higher magnification, scale bar, 25µm.



(a) MCF10ARasV12 cells treated with DMSO (RasV12) or U0126 were grown in soft agar for 16 days. Phase contrast images of tumor colonies and the percentage of tumor colonies greater than 30 μm in diameter are shown. Scale bar: 60 μm (\*\*,p<0.01) (b) MCF10A (Ctrl), MCF10AT1 (AT1), MCF10A-ErbB2 (ErbB2) and MCF10A-RasV12 (RasV12) cells were analyzed by immunoblot for the expression of Brg1 and LaminB1 (loading control). (c) BRM mRNA was

analyzed from HMT-3522 S1 (Ctrl) and T4-2 cells treated with DMSO (Vehicle) or with the MEK inhibitor, U0126, grown in 3D rBM for 16 days (normalized to 18S) (\*p<0.05). (d) Schematic showing results of ChIP-seq experiments, part of the Encyclopedia of DNA Elements Project (ENCODE, via UCSC genome browser hg19). Using this strategy, c-Myc (red) was found to bind to the core promoter region of *SMARCA2* (which encodes the BRM protein).



(a) The area of the 3D colonies formed by the Ras and the Ras+B cells is shown on the y axis
(n>50 acini per sample; \*p<0.05). (b-c) Ras and Ras+B cells were inoculated subcutaneously into the mammary fatpad of Balb/c nu/nu mice. (b) Representative pictures of three tumors/type.</li>
(c) The weight of the tumors is reported on the y axis (n=10, \*\*\*, p< 0.001).</li>



MCF10A cells were transduced with a lentiviral scramble (Scrm) and a BRG1 silencing vector (BRGi). Left panels: cells were analyzed for BRM, BRG1,  $\alpha$ 5 integrin by immunoblotting. Right panel: protein expression was normalized to the loading control, LaminB1. Results are the mean ± S.E.M. of 3 separate experiments (\*\*\*, p<0.001).



(a) Representative phase contrast pictures of Scrambled (Ctrl) and Brm shRNA (BRMi) MCF10A cells grown in a 3D rBM for 16 days. (b) Colony area of cells in panel (a). (c) Representative immunoblot of total cellular BRM levels and LaminB1 (loading control) levels in Scrambled (Ctrl) and BRM shRNA alternative sequences. (d) Cells in (c) were grown in 3D, photographed and stained for  $\beta$ 4 integrin; nuclei were stained with DAPI. White dashed circles indicate cleared lumens; asterisks indicate absence of a lumen. (e) The percentage of colonies with a filled lumen is shown on the y axis. Results are the mean ± S.E.M. of 3 separate experiments (\*\*\*, p< 0.001). Scale bar, 25µm.



MCF7 cells were transduced with a shRNA Scrambled (Ctrl) or BRM (BRMi) knockdown constructs. (a) Phase contrast images and (b) percentage of tumor colonies greater than 30  $\mu$ m in diameter embedded within soft agar is shown. Scale bar, 60  $\mu$ m. (c) mRNA of BRM,  $\alpha$ 5 integrin and C/EBP $\beta$  from MCF7 Ctrl and BRMi was analyzed by RT-PCR and normalized to 18S.



MCF10A cells were transduced with a retroviral control and a tetracycline inducible retroviral ATPase dead BRM mutant (K749R). Left panels: levels of BRM, BRG1 and LaminB1 were analyzed by immunoblotting after tetracycline treatment for 48 hr. Right panel: Protein expression was normalized to the loading control, LaminB1. Results are the mean  $\pm$  S.E.M. of 3 separate experiments (\*,p<0.05).



(a) MCF10A (Ctrl) and ErbB2 cells treated with DMSO (Vehicle) or with the MEK inhibitor, U0126, were grown in 3D rBM for 16 days, and analyzed for C/EBP $\beta$  mRNA expression (normalized to 18S) (\*\*,p<0.01). (b) HMT-3522 S1 (Ctrl) and T4-2 cells treated with DMSO (Vehicle) or with the MEK inhibitor, U0126, were grown in 3D rBM for 16 days, and analyzed for C/EBP $\beta$  mRNA expression (normalized to 18S) (\*\*\*,p<0.001).



(a) BRM was ectopically expressed in MCF10A cells. MCF10A (Ctrl) and MCF10A+BRM (Ctrl+B) cells were analyzed for BRM,  $\alpha$ 5 integrin and  $\alpha$ 2 integrin protein expression and normalized to the loading control, Lamin B1. (b) Cells in panel (a) were grown in 3D rBM and stained for  $\beta$ 4 integrin (red), GM130 (red); nuclei were visualized using DAPI (blue). Scale bar, 25µm.

**Supplemetary Materials and Methods** 

## shRNA sequences

BRM/SMARCA2

Seq#2

Seq#3

GGAGGTGCTACGACACTTCTGAACATTCAAGAGATGTTCATAAGTGTCTTAGCACCTCCT Sigma (TRCN0000020332) CCGGCCAAACCTGTAGTGAGCGATTCTTACAGGTTTGGTTTTT C/EBPβ (Sigma TRCN0000007441)

CCGGCCTGCCTTTAAATCCATGGAACTCGAGTTCCATGGATTTAAAGGCAGGTTTTT

ITGA5 (α5 integrin Sigma TRCN0000029653)

CCGGCTCCTATATGTGACCAGAGTTCTCGAGAACTCTGGTCACATATAGGAGTTTT

# Real Time PCR primer sequence

18S, forward, 5'- CGGCTACCACATCCAAGGAA-3', reverse 5'-GCTGGAATTACCGCGGGCT-3'; BRM (SMARCA2) 5'- AGCAGCCAGATGAGTGACCT-3',5'- TCTCTTCGGTTTCCTGCCTA-3',  $\alpha$ 5 integrin (ITGA5) 5'-AGCCTCAGAAGGAGGAGGAGGAC-3', 5'-GGTTAATGGGTGATTGGTG-3',  $\alpha$ 2 integrin (ITGA2) 5'-AGCCACCAAATTAGCAGGTG-3', 5'-TGTGGTCCATCTGCATCCTA-3', C/EBP $\beta$  5'- GAAAGCTAGGTCGTGGGTCA-3', 5'-TCATAACTCCGGTCCCTCTG-3', c-Myc 5'-GGCACTTTGCACTGGAACTT-3', 5'-AGGCTGCTGGTTTTCCACTA-3'.

#### **ChIP primers**

 $\alpha$ 5 integrin promoter, forward 5'- CCCAGTCTAACCCAGTCCAG-3', reverse 5'-CCCGCTCTTCCCTGTCCT-3', C/EBP $\beta$  promoter, forward 5'- CCTCTCGCTCCCAATCCC-3', reverse 5'- TTCTCCTGAGCCCGGTTATT-3'.

#### Antibodies

Primary antibodies: C/EBPβ (sc-150), Iamin B1 (sc-30264)(Santa Cruz Biotechnology, Santa Cruz, CA);  $\alpha$ 3 integrin (MAB1952),  $\alpha$ 5 integrin (ab1928) (Millipore, Billerica, MA, USA);  $\alpha$ v integrin (MAB1953Z, Chemicon);, fibronectin (610077) (Millipore); β4 integrin, clone 3E1 (ATCC);  $\alpha$ 2 integrin (555669) GM130 (610822), Ki-67 (610968) (BD Bioscience); Laminin-5  $\alpha$ 3 chain specific, clone BM165 (M.P. Marinkovich, (64); Brm (D. Reisman, (65) and 6889, Cell Signaling); β-catenin (C2206, Sigma); cleaved-Caspase 3 (9661, Cell Signaling, Danvers, MA, USA); β1-integrin, clone AIIB2 (C. Damsky, (38). Secondary antibodies: Alexa 488/546 antigoat, mouse, rabbit, and rat antibodies (Invitrogen, San Diego, CA, USA). ECL Horseradish peroxidase conjugated anti-goat, mouse, rabbit and rat antibodies (GE Healthcare Biosciences, Pittsburgh, PA, USA).