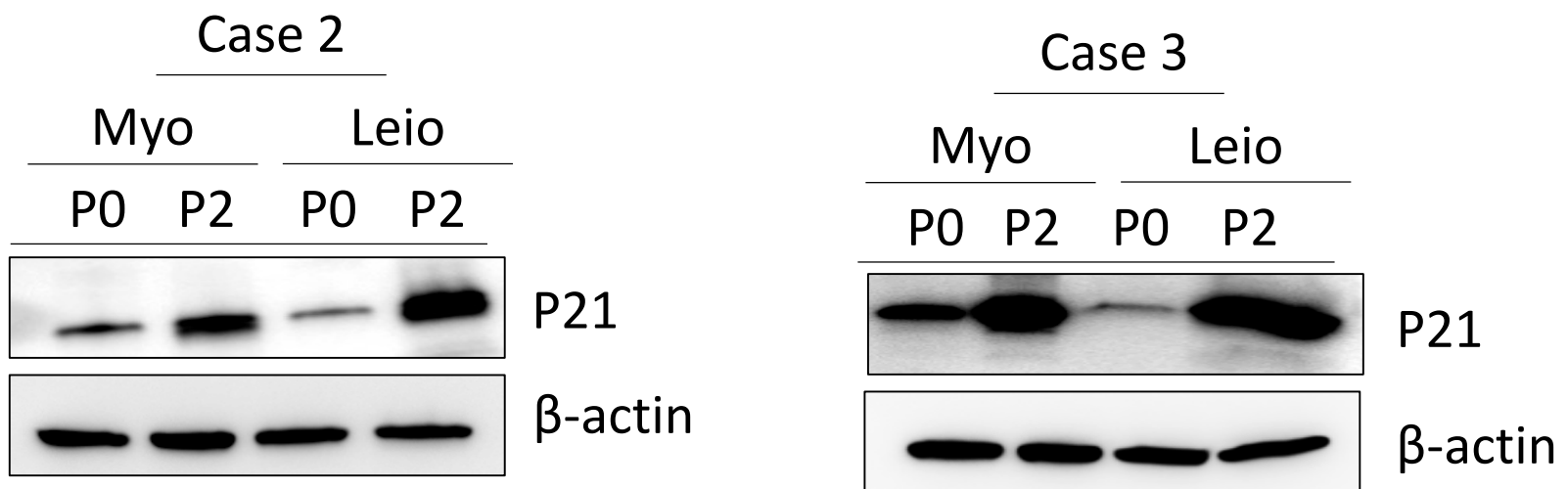
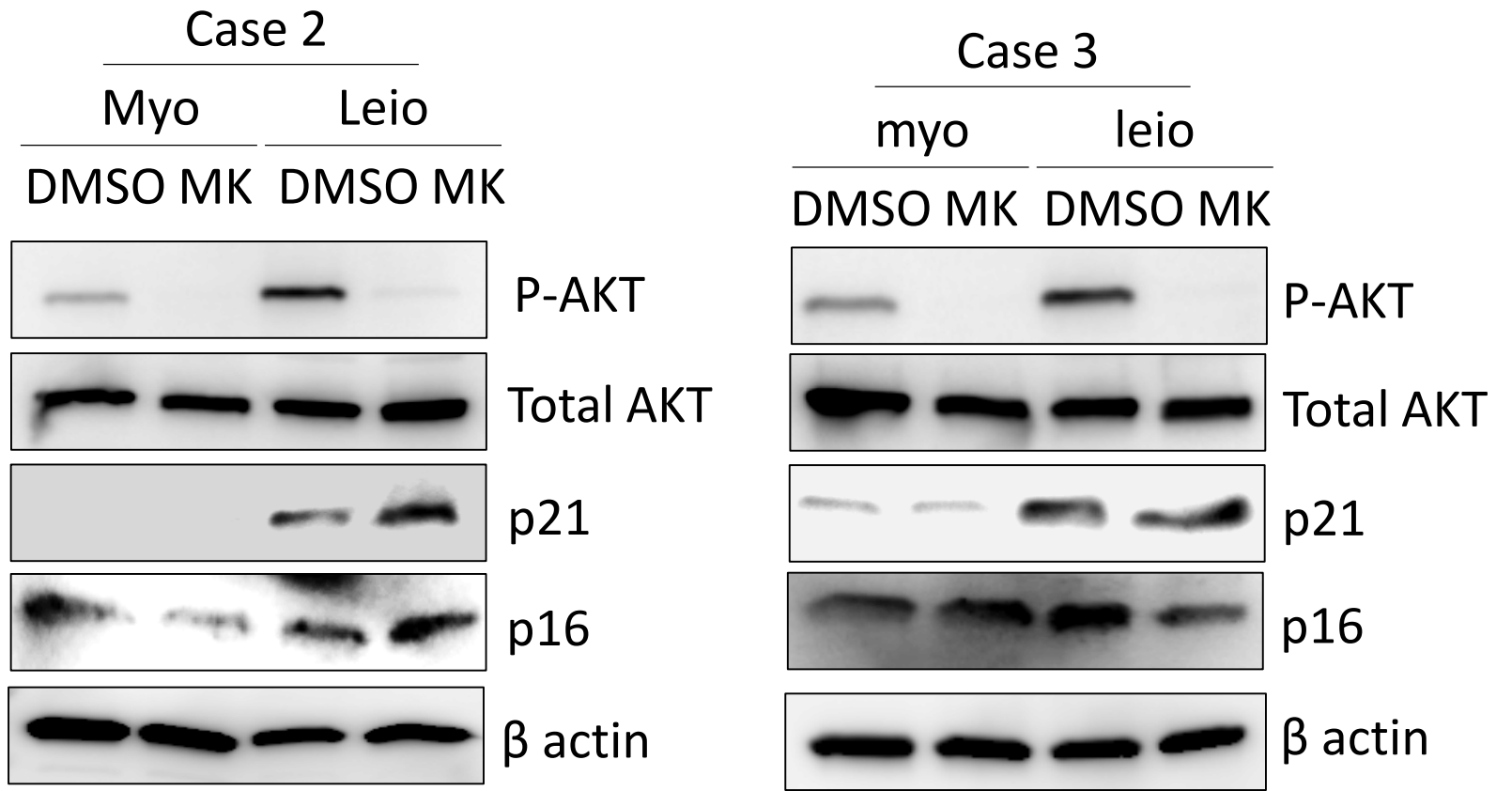


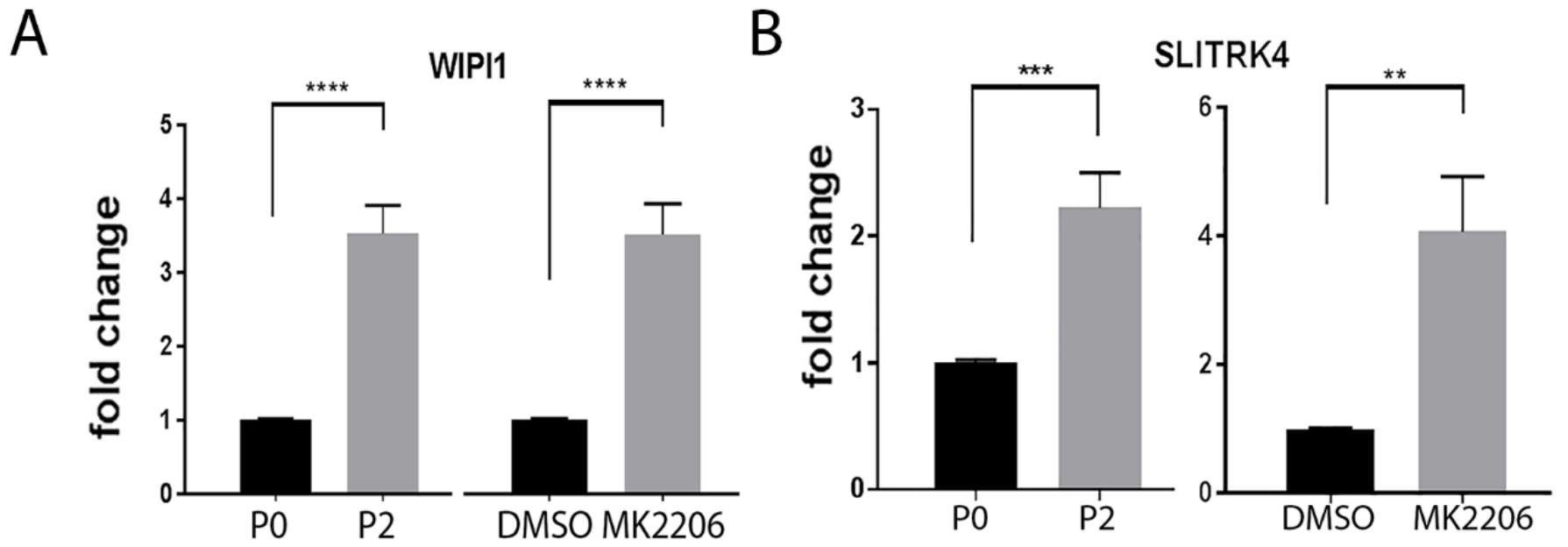
Supplementary Figure 1. SmGm2 media can maintain the smooth muscle cell populations during long-term culture. Immunofluorescence staining for desmin (green) demonstrated the minimal loss of smooth muscle cells in primary culture of myometrial (myo) and leiomyoma (leio) cells after two passages (P2) in SmGm2 medium (upper panel). In contrast, cells maintained in DMEM/F12 medium resulted in significant loss of smooth muscle cells (lower panel). A counterstain of DAPI (blue) highlighted all nuclei in culture. Cells outlined with white dotted lines were enlarged and shown at the bottom left corners of the corresponding images.



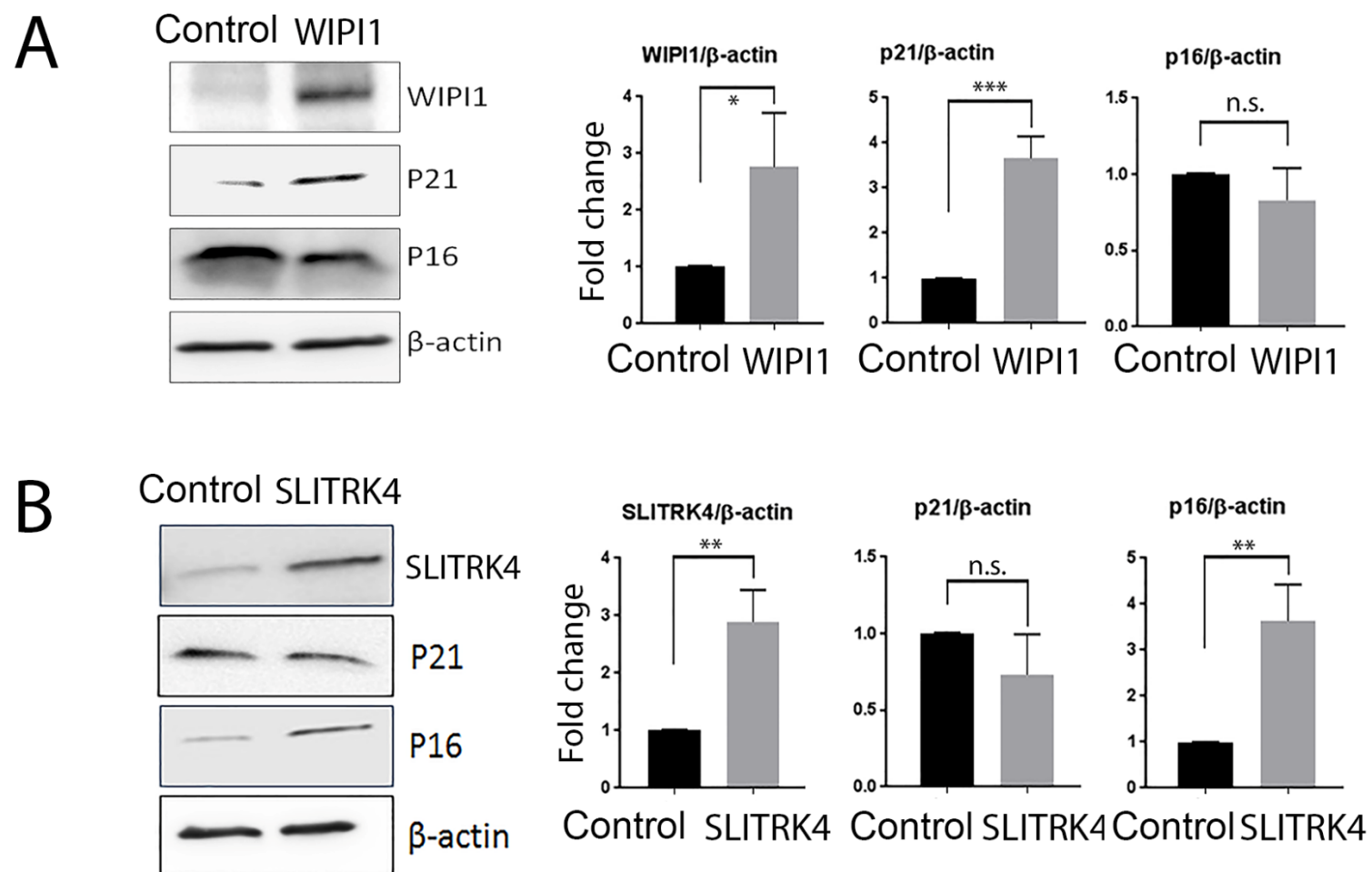
Supplementary Figure 2. Western blot analysis of p21 in myometrium (Myo) and leiomyoma (Leio) cells from P0 and P2. β -actin was used as a loading control.



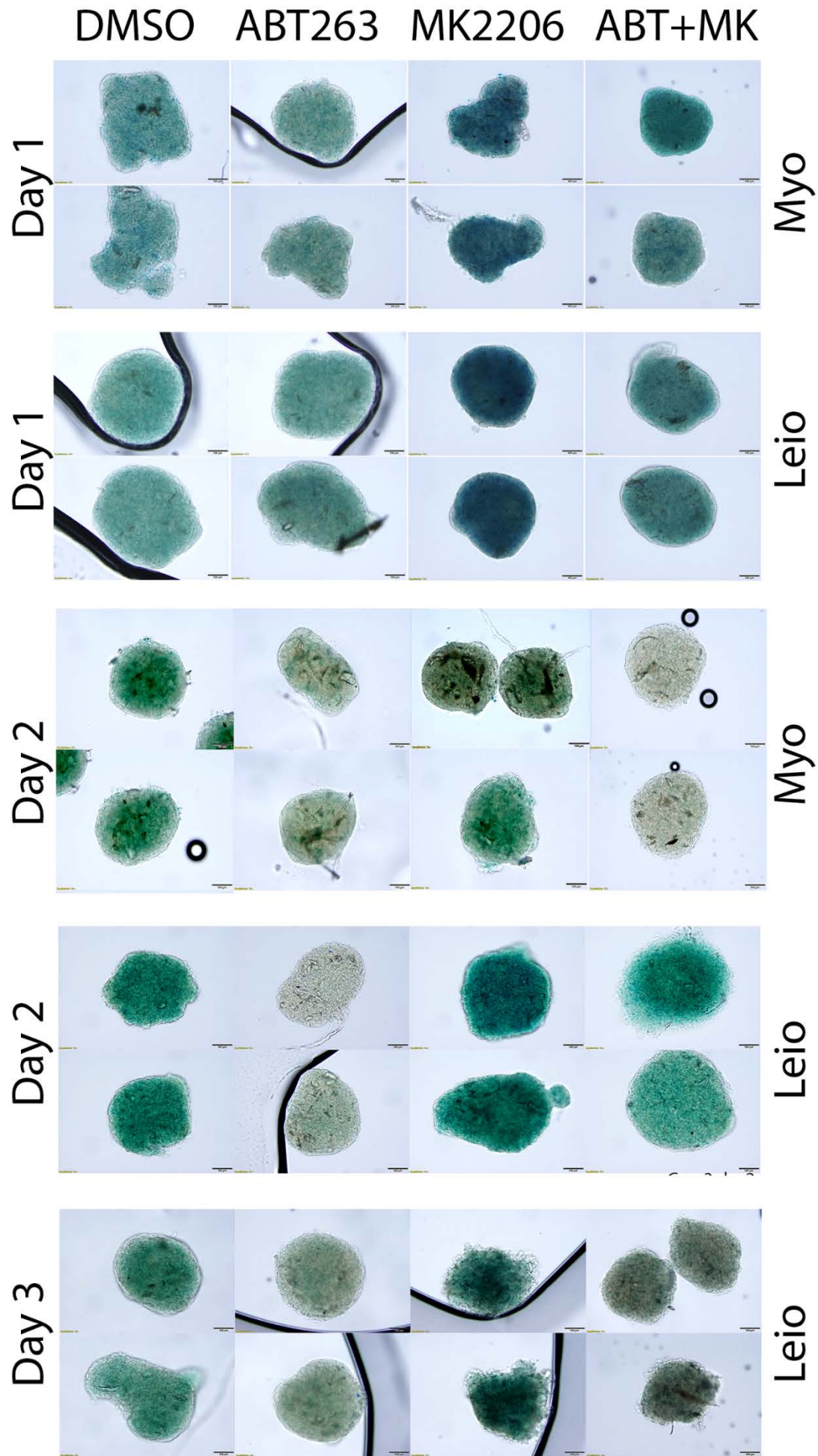
Supplementary Figure 3. Western blots analysis of the selected protein products in leiomyoma (Leio) and myometrial (Myo) cells in control (DMSO) and AKT inhibitor (MK2206) treatment.



Supplementary Figure 4. RT-PCR analysis of WIPI1 (A) and SLITRK4 (B) expression in replication senescent (P0 vs. P2) and MK2206 treated leiomyoma cells. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.



Supplementary Figure 5. Western blots of P16 and P21 expression in primary leiomyoma cells with overexpression of WIP1 and SLITRK4. The band density (right) was quantified (n=3) and expressed as the means \pm SD. N.s.: no significant change. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.



Supplementary Figure 6. The relative levels of senescent cells (intensity of green color) detected by SA- β -gal staining in leiomyoma (Leio) and myometrium (Myo) spheroids (duplicated) cells treated by control (DMSO), ABT263, MK2206, and combined ABT263+MK2206 from day 1 to day 3.

Supplementary Tables 1-4

Suppl Table 1 General information for uterine leiomyomas used in this study

Deidenti fy No.	Age (yrs.)	Tumor Size (cm) up to 13	endometrial phase	ethnicity	HMG2 by IHC	MED12 mutation	FH by IHC
Pt805	41	cm	secretory	African American	Negative		Positive
PT818	48	12 cm	proliferative	African American	Negative		Positive
PT822	48	6.6 cm	secretory	Asian	Negative		Positive
Pt826	53	6 cm	weakly proliferative	Caucasian	Negative	c.131G>A	Positive
Pt845	48	9.5 cm	secretory	African American	Negative		Positive
Pt875	55	8.5 cm	proliferative	African American	Negative	c.130 G>C p.G44R	Positive
PT894	44		weakly proliferative	African American	Negative		Positive
PT957	48	4.5 cm	secretory	Caucasian	Negative		Positive
PT961	37	7.8 cm	proliferative	Hispanic/Latina	Negative		Positive
PT963	44	10 cm	proliferative	Caucasian	Positive		Positive
PT965	40	6.5 cm	inactive to weakly proliferative	unknown	Negative	c.131G>A	Positive
PT971	35	10.2 cm	proliferative	African American	Negative		Positive
PT975	40	14.5 cm	proliferative	Caucasian	Negative		Positive
PT1013	46	7 cm	secretory	unknown	Negative	c.130G>A	Positive
PT1018	40	4 cm	basal	Caucasian	Negative	c.131G>T	Positive
PT1019	43		proliferative	African American	Negative	c.131G>A	Positive
PT1021	43	9 cm	Inactive	unknown	Positive		Positive
PT1022	44	25 cm	proliferative	Caucasian	Negative	c.131G>A	Positive
PT1025	46	10 cm	weakly proliferative	unknown	Negative		Positive
PT1031	43	5.5 cm	Weakly proliferative to early secretory	African American	Negative	c.131G>A	Positive
PT950	44	6.5 cm	proliferative	African American	Positive		Positive
PT976	50	18 cm	secretory	African American	Negative	c.131G>A	Positive
PT983	44	6 cm	inactive	Caucasian	Negative	c.130G>C	Positive
PT999	46	17 cm	inactive	African American	Negative		Positive
PT1007	48	6.5 cm	secretory	African American	Negative		Positive
PT1008	47	5 cm	inactive	African American	Negative	c.131G>A	Positive
PT1028	45	8.5 cm	weakly proliferative	Caucasian	Negative		Positive
PT1038	49	6.1 cm	secretory	Caucasian	Negative	c.131G>A	Positive

Suppl Table 2 Antibody information

Antibody	Vendor	Catalog number	Concentration
WIP1	Cell Signaling	12124S	1:100
SLITRK4	Invitrogen	PA5-20481	1:100
PAKT	Cell Signaling	12694	1:200
P21	Dako	M7202	1:50
P16	BIOCARE	ACR3007C	1:50
Ki67	Dako	M7240	1:100
ER	Thermo	RM-9101S	1:200
PR	Dako	M3569	1:1600
Smooth muscle actin	Dako	M0851	1:100
HMGA2	Biocheck,inc	59170AP	1:500
FH	Santa Cruz	sc-100743	1:200

Suppl Table 3 Primers for RT-PCR

Gene	Primers F	primers R
SGIP1	CCT GTT GCA GCA GCA TTT AC	CTG GGC AGA CAC CTG ATA TTT
FAM108C1	GCA ACA TCT TCT CCT ACG ACT AC	CCC TAC ATT CAG CTC TCA TTC C
WIPI1	CCA ATT CTT ACC TGG CCT ATC C	GGT AGT TGG TAG CAG CCA TAA A
SLITRK4	GCC AGT GCC TCT GTC TAT TT	GCC ACT GAC ACC TAT GCT ATT

Suppl Table 4 significantly dysregulated genes in several pathways

Hypoxia	Inflammation	P53 path	Estrogen Response	AKT path	DNA repair
LOX	DCBLD2	Gene	TFPI2	Gene	Gene
KDELR3	AXL	IRAK1	PRDX4	TRIB3	DFNA5
P4HA2	TIMP1	SEC61A1	CCND1	AP2M1	SEC61A1
TGFBI	SLC31A1	RNF19B	MGST1	ARPC3	DAD1
STC2	SELS	S100A4	TST	CALR	ERCC2
GBE1	PLAUR	MKNK2	GLRX	PIN1	BOLA2
GLRX	ADORA2B	TOB1	PFKP	HSP90B1	GUK1
PFKP	NLRP3	PPM1D	PAPSS2	ACTR2	DGUOK
LXN	CCL20	FOXO3	ATOX1	PRKAG1	GTF2H5
GAPDH	NAMPT	BTG1	MYOF	CFL1	RPA3
ALDOC	MYC	NDRG1	HSPB8	ACTR3	POLR2G
ALDOA	CSF3	NOTCH1	PDLIM1	ARHGDI1	NME1
S100A4	RGS16	STOM	TOB1	MAP3K7	ERCC1
MAP3K1	NDP	PPP1R15A	ISG20	ACACA	TAF10
SLC2A3	TNFRSF1B	DDIT4	TFAP2C	CLTC	RDBP
CDKN1C	PTGER4	RGS16	CYP26B1	PPP1CA	SDCBP
FOXO3	PTPRE	RALGDS	CKB	MAPKAP1	ADA
BTG1	BST2	PTPRE	ID2	PTEN	ELL
NDRG1	NFKB1	UPP1	FOXC1	DAPP1	STX3
TGFB3	CD14	ZBTB16	PGR	NCK1	
ISG20	HBEGF	TM4SF1	CXCL12	CXCR4	
ETS1	KLF6	IER5	KLF4	CDKN1B	
FOSL2	IL6	HBEGF	SOD2	MKNK2	
PPP1R15A	CCL2	KLF4	JUNB		
TIPARP	ICAM1	TSC22D1	ZFP36		
DDIT4	BTG2	JUN	FOS		
MAFF	RGS1	BTG2	PCP4		
NFIL3	IRF1	TXNIP			
PFKFB3	IRAK2	ATF3			
PIM1	IL1B	FOS			
STC1	CALCRL				
JUN	IL8				
KLF6	SELE				
IL6	NFKBIA				
ATF3	CX3CL1				
TNFAIP3					
CXCR7					
EFNA1					
ZFP36					
FOS					

