

The virus-induced protein APOBEC3G inhibits anoikis by activation of Akt kinase in pancreatic cancer cells

Jia Wu¹, Tian-Hui Pan¹, Song Xu¹, Li-Tao Jia^{1,3}, Lin-Lin Zhu¹, Jian-Shan Mao¹, Yong-Liang Zhu^{1,2} & Jian-Ting Cai¹

¹ Laboratory of Gastroenterology, Second Affiliated Hospital of Zhejiang University, School of Medicine, Hangzhou, China; ² Cancer Institute and Education Ministry Key Laboratory of Cancer Prevention and Intervention, Zhejiang University School of Medicine, Hangzhou, China; ³ Department of Gastroenterology, Tongde Hospital of Zhejiang Province, Hangzhou, China.

Corresponding author: Dr. Yong-Liang Zhu, Laboratory of Gastroenterology, Second Affiliated Hospital of Zhejiang University School of Medicine, Hangzhou, Zhejiang Province, 310009, China. Tel: 86-571-87767061, Fax: 86-571-87214404, Email: drylzhu@163.com, or Dr. Jian-Ting Cai, Email: jtcai@live.cn.

Supplementary Table S1 Differently expressed genes in the global cDNA microarray

Gene Symbol	Ratio	Gene Symbol	Ratio	Gene Symbol	Ratio
ANKRD22	20.528	TMCC1	3.156	CCDC69	2.524
KRT20	9.074	SDR16C5	3.098	TNFRSF10D	2.524
CCL26	8.972	CXorf50B	3.098	SEPP1	2.523
TXNIP	8.891	CCDC69	3.095	TNFSF9	2.514
G0S2	8.336	EREG	3.063	PDZK1	2.507
CSF2	8.315	ANXA6	3.061	CDH17	2.505
APOBEC3G	6.756	ATP8A1	3.059	RAB26	2.501
EGR2	6.693	CARD16	3.023	JUNB	2.497
ARRDC4	6.185	LGALS7 /// LGALS7B	3.02	ABLIM3	2.493
PCK1	6.039	PLAC1	3.016	MLF1	2.488
LYZ	5.533	TMEM178	3.011	C7orf68	2.487
IL8	5.444	PTGR1	3.006	LYZ	2.487
EGR3	5.148	NMI	2.995	BCHE	2.483
MLF1	5.139	RGS2	2.99	SECTM1	2.479
ADM	5.069	LRCH3	2.989	VWDE	2.47
IGFBP3	4.937	SCN3A	2.981	FXYD2	2.458
CCL20	4.881	C3	2.961	PRSS1 /// TRY6	2.456
SERPINI1	4.776	SOD2	2.957	CD58	2.456
LPXN	4.67	SYCP2	2.937	HBA1 /// HBA2	2.455
LY96	4.663	ZFP36	2.928	PROCR	2.454
FOS	4.627	GMFG	2.91	OTUB2	2.451
UBE2L6	4.503	TBC1D8B	2.904	APOBEC3F	2.446
PSMB9	4.464	BHLHE41	2.874	LIF	2.428
FXYD2	4.403	GEM	2.859	ISG20	2.411
TBC1D4	4.374	TIGD7	2.853	ABAT	2.408
GBP1	4.248	HLA-DQB1	2.852	NOD2	2.408
TRIM31	4.247	SRPX	2.846	CTSH	2.407
SERPINA1	4.243	CD163L1	2.838	C17orf60	2.399
CCDC153	4.229	TIMP3	2.797	FBXO2	2.399
SLC5A3	4.213	EGR1	2.786	LOC728730	2.384
SGK1	4.131	HLA-DQB1	2.766	SLC5A3	2.38
HCP5	4.107	CXCL6	2.762	ZFAND2A	2.38
SERPINA1	4.088	GBP2	2.757	AREGB	2.374
KIAA1128	4.058	C10orf116	2.752	GRTP1	2.363
LEMD1	4.048	PRRG4	2.751	HOXA13	2.359
EGR4	3.962	SNAI2	2.748	MTRF1L	2.358
SERPINE2	3.859	WFDC3	2.74	DKK3	2.355
TFF2	3.833	TFPI	2.727	STXBP6	2.353
PRAP1	3.771	ECM1	2.714	C1orf161	2.353
FABP1	3.62	CTSS	2.709	DIRAS2	2.35
MAGEB6	3.608	FAM13A	2.704	CD44	2.342
CXCL1	3.599	CARD16 /// CASP1	2.7	LY75	2.339
CXCL2	3.579	TUBB2B	2.697	PTPRM	2.339
PPP4R4	3.561	DUSP1	2.691	DDIT3 /// NR1H3	2.337
GBP3	3.52	PER3	2.677	FGD5	2.337
TMEM154	3.487	SYTL4	2.676	JUN	2.334
ADSSL1	3.485	CCND2	2.672	BDH2	2.333
AREG	3.472	DKK3	2.666	MAGEB2	2.329
GABBR1 /// UBD	3.441	SLC20A1	2.665	SAT1	2.327
HSPA6	3.402	EAF2	2.661	JUN	2.322
LRRC70	3.401	TRIM34 /// TRIM6-TRIM34	2.657	CD58	2.321
ANKRD37	3.392	RASGEF1A	2.651	PSMB8	2.321
GDF15	3.374	JUN	2.643	ISG20	2.317
GIMAP2	3.372	CDKN1A	2.635	ZNF226	2.316
FHL1	3.369	TCAM1	2.635	BHLHE41	2.315
DHRS2	3.343	TMEM45B	2.634	ATP2B4	2.313
EGR1	3.337	ITPR1	2.628	NFKBIZ	2.311
KL	3.332	TNNT1	2.603	CST6	2.305
DDIT4	3.315	EGR1	2.597	NRP2	2.304
CREBZF	3.31	NAGS	2.592	EFNA4	2.302
ALPK1	3.259	CYP3A5	2.585	OAS1	2.3
EPAS1	3.248	HECA	2.581	TMEM45B	2.299
EHF	3.243	GCOM1	2.575	MXD1	2.288
DUSP2	3.236	BEAN	2.573	SLC22A4	2.278
CSPG5	3.231	PRSS2	2.565	AK7	2.277
ATP6V0A4	3.228	HCN1	2.555	ABAT	2.268
SRGN	3.189	PRSS1	2.536	IGF2 /// INS-IGF2	2.26

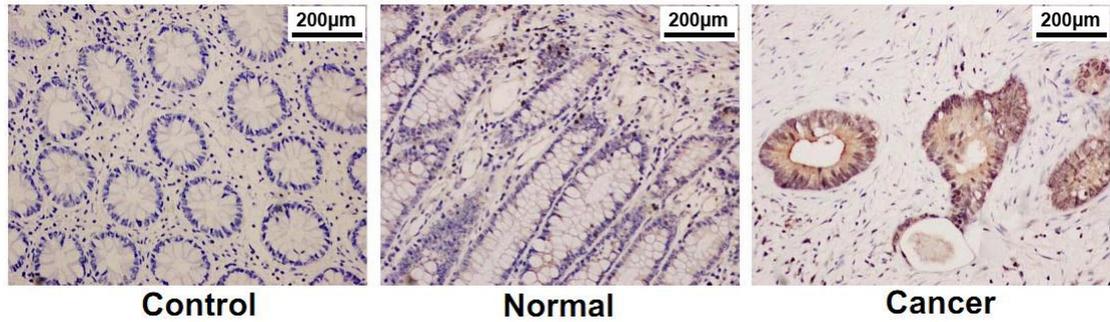
Gene Symbol	Ratio	Gene Symbol	Ratio	Gene Symbol	Ratio
C10orf125	2.256	PKIB	2.045	POF1B	0.387
PSD2	2.253	APOC2 /// APOC4	2.044	ITPR2	0.383
ZFP14	2.234	PPIC	2.041	CNNM2	0.372
CAMK2N1	2.233	BIRC3	2.041	SLC25A31	0.37
JAKMIP3	2.228	SPINK7	2.039	HIST1H1E	0.33
BCL3	2.226	ZNF818P	2.034	PCDH9	0.33
TNFAIP3	2.224	CD58	2.033	ITPR2	0.321
FAM113A	2.222	IL8	2.032	FAM43A	0.319
TGM2	2.22	EPAG	2.029	AMOTL2	0.318
WSB1	2.215	FGFR3	2.023	THBS1	0.315
HEMGN	2.215	CXCL3	2.022	SDPR	0.302
GDF15	2.213	SLC2A1	2.018	SEMA6A	0.29
JUN	2.211	GPR160	2.011	MIRHG1	0.273
PRSS3	2.206	ARRB1	2.01	LPCAT1	0.26
KLF11	2.202	ERAP1	2.008	SEMA6A	0.26
SPAG4	2.201	CHD2	2.007	ABCA13	0.155
TFPI	2.2	PTK6	2.005	SDPR	0.117
PEX13	2.2	HLA-DQB1	2.		
FAM13A	2.195	FOXP1	0.499		
BTG2	2.184	SOX2OT	0.498		
COL6A1	2.181	HIST1H1B	0.496		
PAQR5	2.181	PPL	0.495		
ASPHD2	2.169	ASNS	0.495		
WWTR1	2.164	SPATA1	0.495		
C1orf58	2.164	WNT11	0.491		
DOCK8	2.162	JUB	0.491		
CDC2L6	2.162	HIST3H2A	0.49		
E2F3	2.161	IGF1R	0.488		
TFPI	2.147	TMPO	0.488		
DAZAP2	2.145	GALNT7	0.486		
DUSP5	2.136	SFRS2B	0.484		
SMOC2	2.133	MATR3	0.482		
RNF183	2.127	HMGB3	0.48		
SLC5A3	2.126	PHF17	0.48		
VWA5A	2.125	CYFIP2	0.479		
TIMP3	2.121	SFRS2B	0.479		
GIMAP6	2.121	TMEM133	0.47		
CSRNP1	2.119	COL2A1	0.463		
TRPC1	2.118	RPS20	0.461		
TFPI	2.115	ALDH1A2	0.459		
SORBS1	2.111	ILF3	0.457		
TAF5	2.109	ELOVL6	0.453		
SFRS11	2.108	HIST1H2AC	0.452		
CAB39L	2.106	ADAL	0.452		
CAMK2N1	2.103	NCRNA00153	0.45		
S100A2	2.101	ZNF124	0.449		
HGD	2.1	KIAA1659	0.449		
TSPAN8	2.099	SOX2	0.449		
AP1S2	2.098	GPR177	0.448		
FAM114A1	2.097	KCNE3	0.446		
BOLA1	2.097	CHM	0.443		
DNAJC12	2.096	FRAS1	0.439		
DKK3	2.093	DENND1A	0.438		
OTUB2	2.087	RPL27A	0.437		
CEACAM1	2.077	KRTAP3-3	0.437		
AKR1C1	2.073	SYCP3	0.433		
RRAS	2.072	ROR1	0.43		
WDR5B	2.072	ELOVL6	0.426		
NAV2	2.071	MX2	0.423		
IFIH1	2.066	RPL13	0.422		
RAB26	2.065	MTMR1	0.418		
PLAU	2.064	FILIP1	0.417		
SLC6A1	2.062	NUP62	0.41		
PDK4	2.061	KCNQ5	0.409		
CALB2	2.055	IL18	0.407		
PMS2L1	2.052	SHANK2	0.402		
STXBP6	2.049	RIN2	0.39		

Supplementary Table S2 Basic information on the pancreatic cancer patients (n=54)

Variable	Data
Age,y	
Mean(SD)	61.3 (8.2)
Range	36-77
Sex	
Male	30
Female	24
CA199,U/mL	
<=37	9
>37	41
Tumor size, cm	
<=2	6
>2	45
Location	
Head of the pancreas	39
Body and tail of pancreas	15
TNM stage	
I	15
II	10
III	27
IV	2
Differentiation grade	
Poorly deferentiation	14
Moderately differentiation	37
Well differentiation	22
Pathological type	
Adenocarcinoma	49
Adenosquamous carcinoma	2
Mucinous cystadenocarcinoma	3

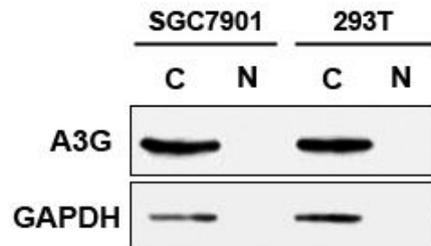
Supplementary Table S3 Primers for site-directed mutagenesis of *A3G*

Sites	Primers
T32A	top 5'-ATCCTTTCTCGTCGGAATGCCGTCTGGCTGTGCTACGAA-3' bottom 5'-TTCGTAGCACAGCCAGACGGCATTCCGACGAGAAAGGAT-3'
T32D	top 5'-ATCCTTTCTCGTCGGAATGACGTCTGGCTGTGCTACGAA-3' bottom 5'-TTCGTAGCACAGCCAGACGTCATTCCGACGAGAAAGGAT-3'
T32E	top 5'-ATCCTTTCTCGTCGGAATGAAGTCTGGCTGTGCTACGAA-3' bottom 5'-TTCGTAGCACAGCCAGACTTCATTCCGACGAGAAAGGAT-3'
T218A	top 5'-GTCAGAGGACGGCATGAGGCTTACCTGTGTTATGAGGTG-3' bottom 5'-CACCTCATAACACAGGTAAGCCTCATGCCGTCCTCTGAC-3'
T218D	top 5'-GTCAGAGGACGGCATGAGGATTACCTGTGTTATGAGGTG-3' bottom 5'-CACCTCATAACACAGGTAATCCTCATGCCGTCCTCTGAC-3'
T218E	top 5'-GTCAGAGGACGGCATGAGGAATACCTGTGTTATGAGGTG-3' bottom 5'-CACCTCATAACACAGGTATTCCTCATGCCGTCCTCTGAC-3'



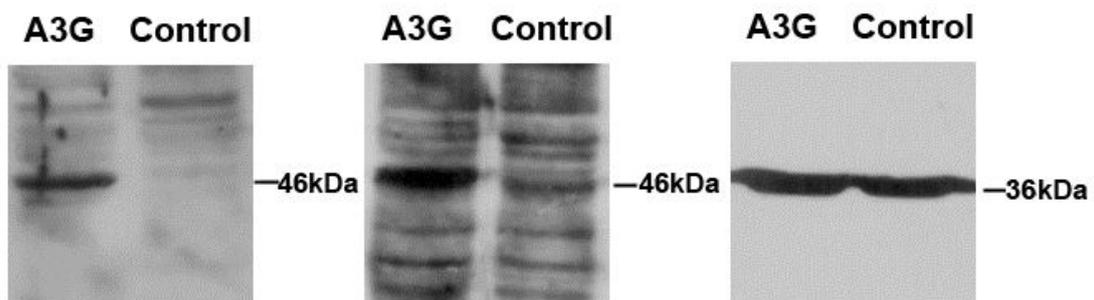
Supplementary Figure S1 Immunohistochemical staining

Slides from 23 matched human colon cancer and para-cancerous tissues were detected by immunohistochemical staining.

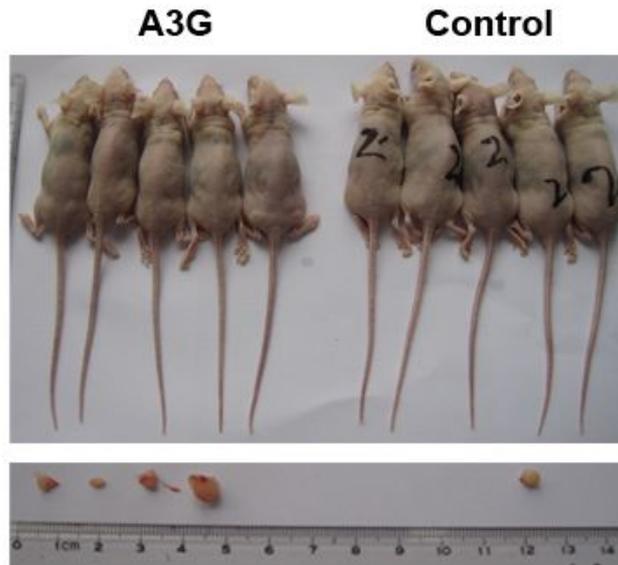


Supplementary Figure S2 A3G distributed in cytoplasm

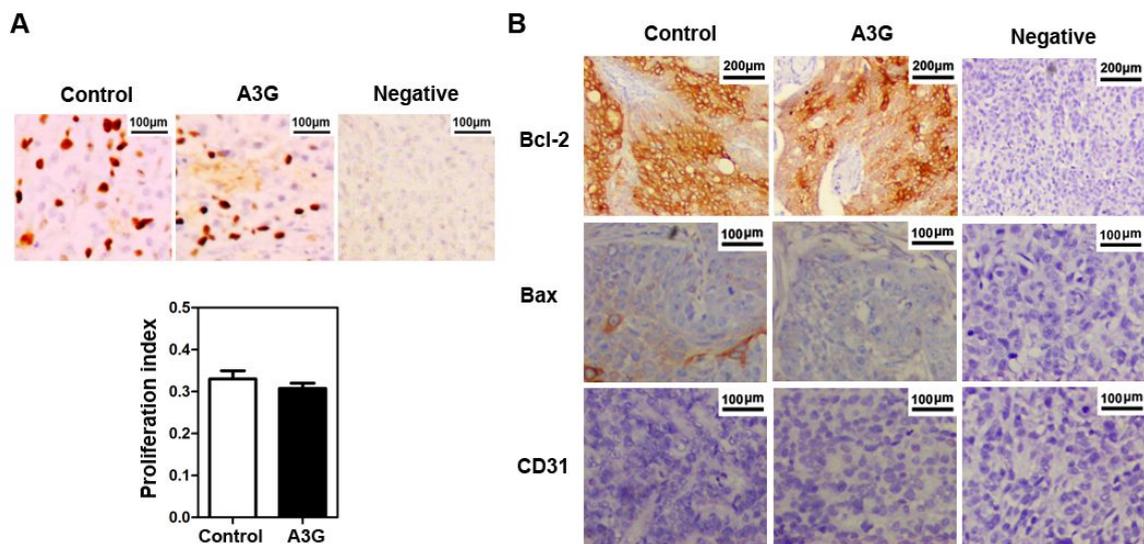
A3G was transfected into SGC7901 and 293T cells respectively, nucleus and cytoplasm were separated and subsequently detected by western blot (C: Cytoplasm; N: Nucleus). GAPDH was used as a loading control.



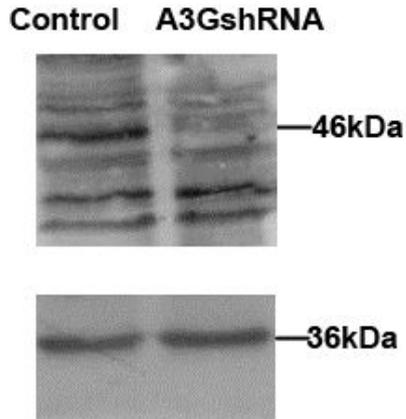
Supplementary Figure S3 Full length western immunoblots as presented in Figure 2C. The left immunoblot represents HA-tag staining. The middle immunoblot represents A3G staining. The right immunoblot represents GAPDH staining.



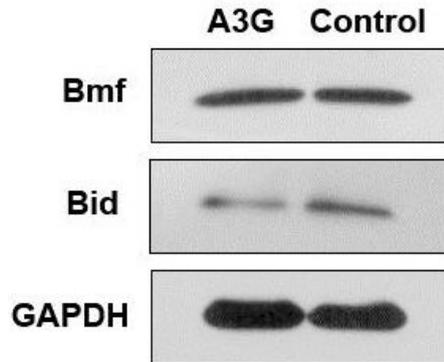
Supplementary Figure S4 Compared with the control, 1×10^5 quantity of the A3G-expressing group could form more xenograft tumor in nude mice after 4 weeks. Two groups' photograph of tumors is shown.



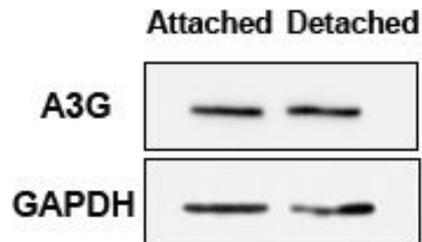
Supplementary Figure S5 Immunostaining analysis of Ki67, Bcl-2, Bax and CD31 of xenograft tumors respectively generated by A3G-expressing cells and control cells in nude mice for 4 weeks. **A.** Percentage of Ki67-positive cells. $P > 0.05$, χ^2 test; **B.** Detection of Bcl-2, Bax and CD31 in A3G-expressing group.



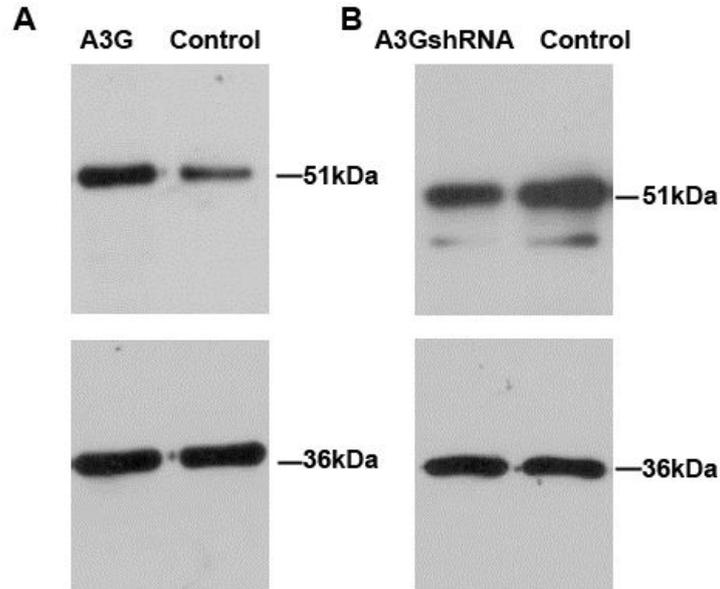
Supplementary Figure S6 Full length western immunoblots as presented in Figure 3B. The top immunoblot represents A3G staining. The bottom immunoblots represent GAPDH staining.



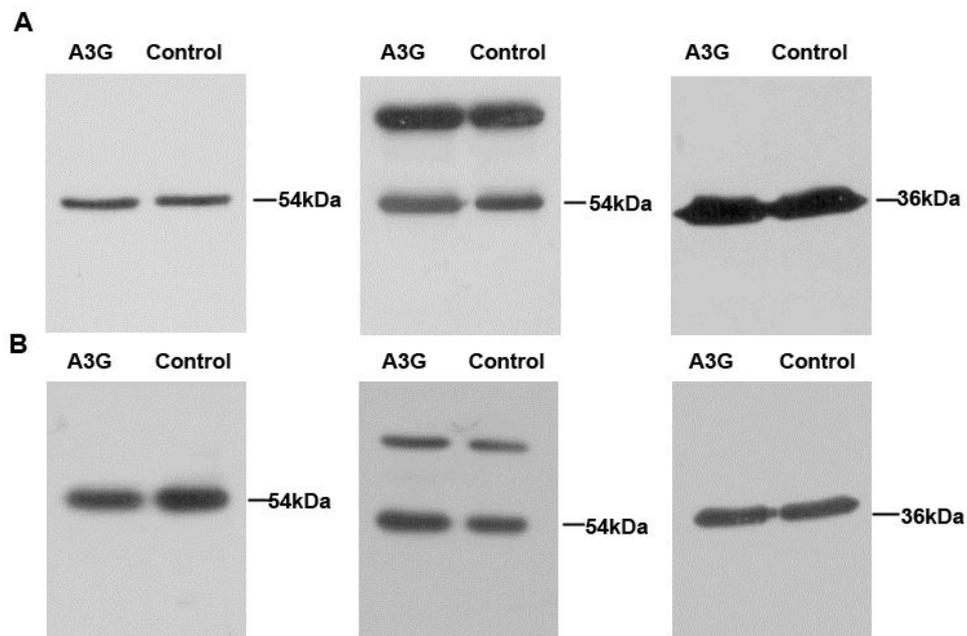
Supplementary Figure S7 **Detection of Bmf and Bid.** Western blot showed that Bid was down-regulated in stable A3G expressing cells, and Bmf was not changed. All data are repeated for three times. GAPDH was used as a loading control.



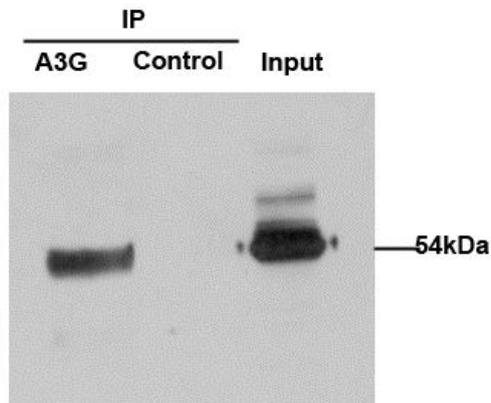
Supplementary Figure S8 Detection of A3G in attached and detached cells. GAPDH was used as a loading control.



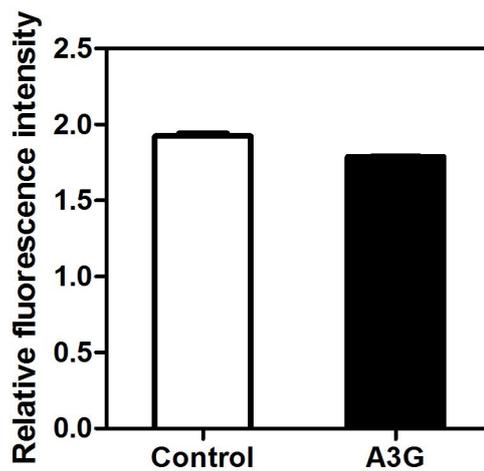
Supplementary Figure S9 Full length western immunoblots as presented in Figure 4B. The A top represents phospho-GSK3 β staining and the A bottom represents GAPDH staining for overexpression of A3G. The B top represents phospho-GSK3 β staining and the B bottom represents GAPDH staining for knockdown of A3G.



Supplementary Figure S10 Full length western immunoblots as presented in Figure 4E. The A left represents PTEN staining, A middle represents phosphor-PTEN staining and A right represents GAPDH staining in BxPC3 cells. The B left represents PTEN staining, B middle represents phosphor-PTEN staining and B right represents GAPDH staining in SGC7901 cells.



Supplementary Figure S11 Full length western immunoblots as presented in Figure 4F. The immunoblot represents PTEN staining.



Supplementary Figure S12 Up-regulation of A3G not affects the NF- κ B activity. A3G was co-transfected into 293T cells with the NF- κ B/pGL2 luciferase reporter plasmid, and luciferase activity was assayed 24 h later (* P <0.05, ** P <0.01; Student's t -test). All data are represented as the means \pm SEM of triplicate experiments.