Shen et al., http://www.jcb.org/cgi/content/full/jcb.200810183/DC1

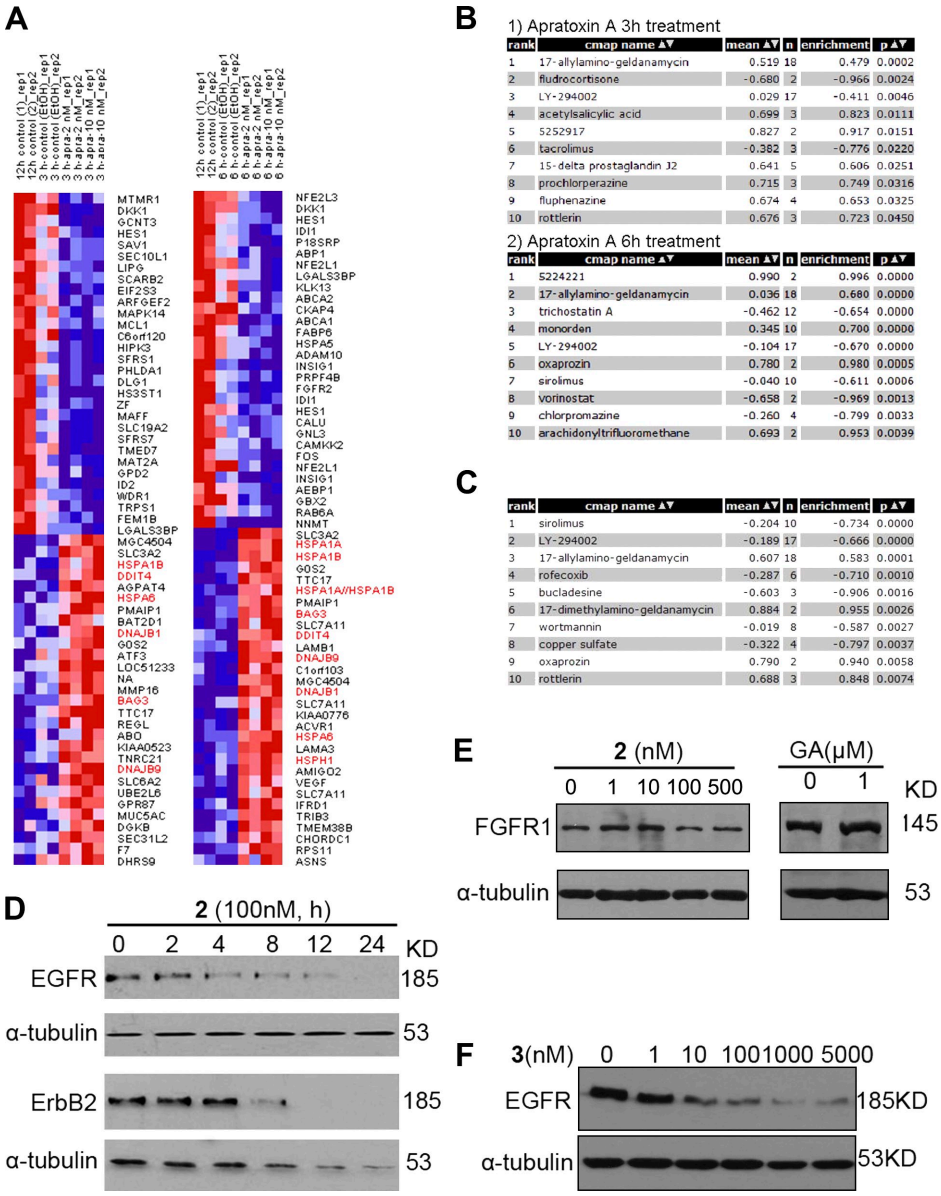

Figure S1. **Apratoxin A and its analogue inhibit Hsp90 signaling pathway.** (A) Gene expression signatures of apratoxin A treatment were defined by retrospective mining gene expression profiles of HT-29 cells treated with apratoxin A at 2 and 10 nM for 3 and 6 h as compared with vehicle-treated cells. The top 30 class neighbor signal to noise ratio markers were shown by row-normalized heat maps. Heat shock–responsive genes are marked in red. (B) The expression profiles of 17-AAG treatment show enrichment of apratoxin A signatures at 3 and 6 h of treatment. The combined results were shown for the indicated time point ordered by their combined p-values. The mean column shows the mean value of the connectivity scores for all instances of the indicated drug in the connectivity map, minus indicates an inverse relationship in their action mechanisms, and the p-values are from the permutation testing of the connectivity map instances. (C) The apratoxin A signatures excluding those heat shock–responsive genes still showed high similarity to those of Hsp90 inhibitors as in A. (D) Oz-apraA promotes the degradation of Hsp90 client proteins in a time-dependent manner. A549 and MDA-MB-453 cells were treated with 100 nM oz-apraA for the indicated times. The cell lysates were separated by SDS-PAGE. The blots were probed with anti-EGFR and anti-ErbB2 antibodies. Anti–α-tubulin was used as a loading control. (E) Oz-apraA does not promote non-Hsp90 client protein degradation. B16 cells were treated for 24 h with oz-apraA for the indicated concentrations or with 1 μM GA. The cell lysates were separated by SDS-PAGE. The blots were probed with anti-FGFR1 antibody. Anti–α-tubulin was used as a loading control. (F) Bio–oz-apraA decreases the protein levels of EGFR. HeLa cells were treated with the indicated concentration of bio–oz-apraA for 24 h and lysed for Western blotting. Anti–α-tubulin was used as a loading control.

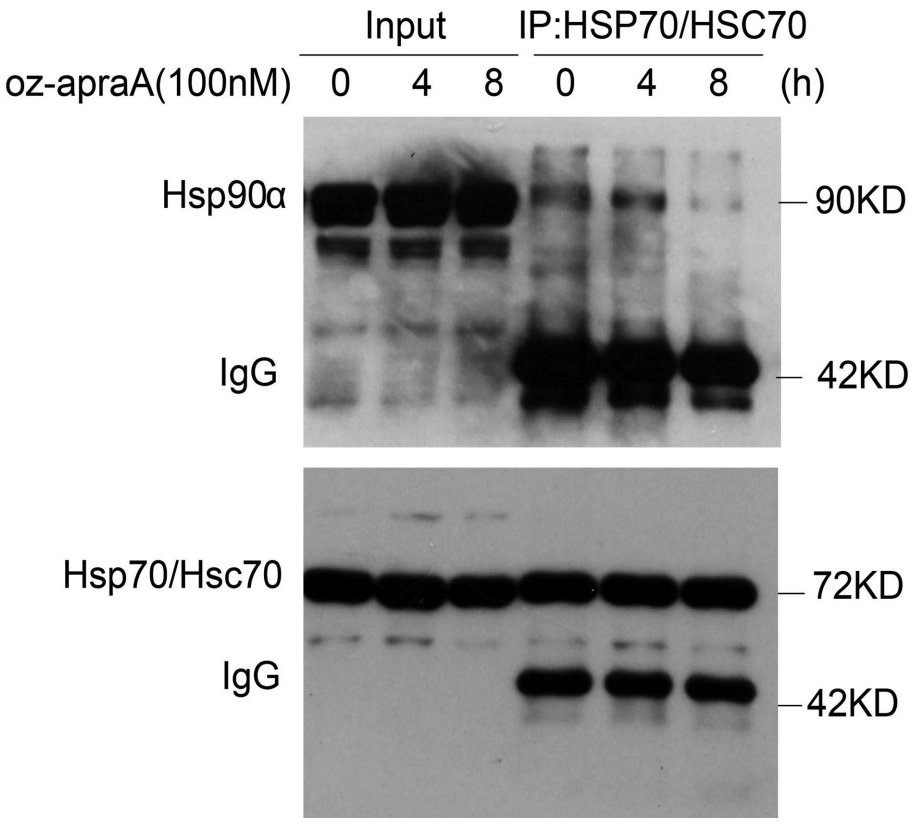

Figure S2. **The effect of oz-apraA on the interaction of Hsp70 and Hsp90.** HeLa cells were cultured with or without 100 nM oz-apraA for the indicated periods of time. Cell lysates were incubated with anti-Hsp70/Hsc70 antibodies followed by precipitation with protein A/G agarose beads. The precipitates were washed five times, and products were separated on SDS-PAGE and analyzed by Western blotting using the indicated antibodies.

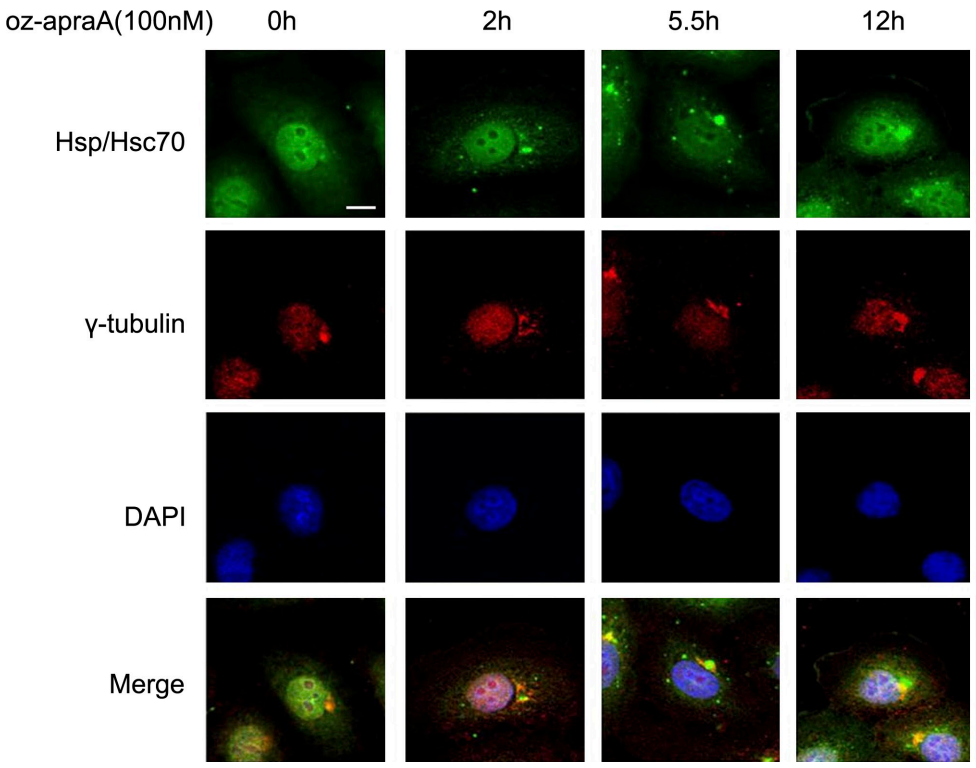

Figure S3. **Oz-apraA promotes aggresome formation.** HeLa cells were treated with 100 nM oz-apraA for the indicated periods of time followed by immunostaining with anti-Hsp70/Hsc70 (green), anti–γ-tubulin (red), and DAPI (blue) as indicated. Superimposed confocal images (merge) demonstrate the colocalization of Hsp70/Hsc70 with aggresome markers (γ-tubulin). Bar, 10 μm.

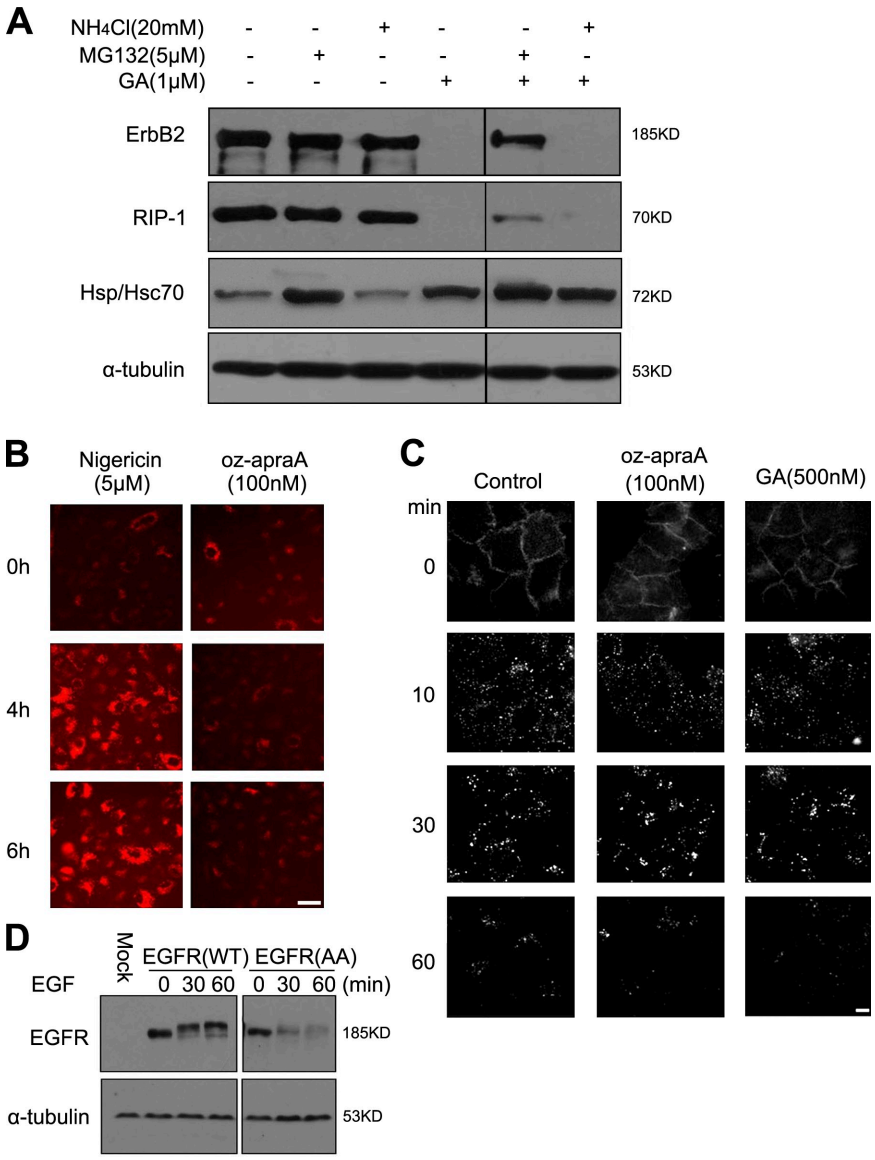

Figure S4. **Oz-apraA does not modulate endocytosis and does not increase the amount of lysosomes.** (A) GA induces Hsp90 client protein degradation through MG132-sensitive proteasome pathway. MDA-MB-453 cells were pretreated with 5 μM MG132 or 20 mM ammonium chloride (NH_4Cl) for 2 h followed by treating with 1 μM GA for 24 h. The cells were lysed, and the cell lysates were separated by SDS-PAGE. The Western blot was probed with anti-RIP1, anti-ErbB2, and anti-Hsp/Hsc70 antibodies. Anti–α-tubulin was used as a loading control. Black lines indicate that intervening lanes have been spliced out. (B) Oz-apraA does not increase the number of acidic lysosomes. HeLa cells were treated with 5 μM nigericin or 100 nM oz-apraA for 12 h followed by staining with acidic lysosomal stain lysotracker. The cells were fixed, and representative confocal images were shown. Bar, 50 μm. (C) Oz-apraA and GA do not directly affect the EGF-stimulated EGFR endocytosis. HeLa cells were preincubated with 100 nM oz-apraA or 500 nM GA for 2 h in a serum-free medium after stimulation with 100 ng/ml rhodamine-labeled EGF for 1 h on ice. Rhodamine-labeled EGF endocytosis was monitored for the indicated chasing time. Bar, 15 μm. (D) EGFR site-directed mutation does not abrogate EGFR(AA) degradation upon stimulation with EGF. HEK293 cells were transiently transfected with EGFR(wt) or EGFR(AA) for 24 h and treated with 100 ng/ml EGF to induce endocytosis of EGFR. Cell lysates were collected to detect the degradation of EGFR level by Western blotting. Anti–α-tubulin was used as a loading control.

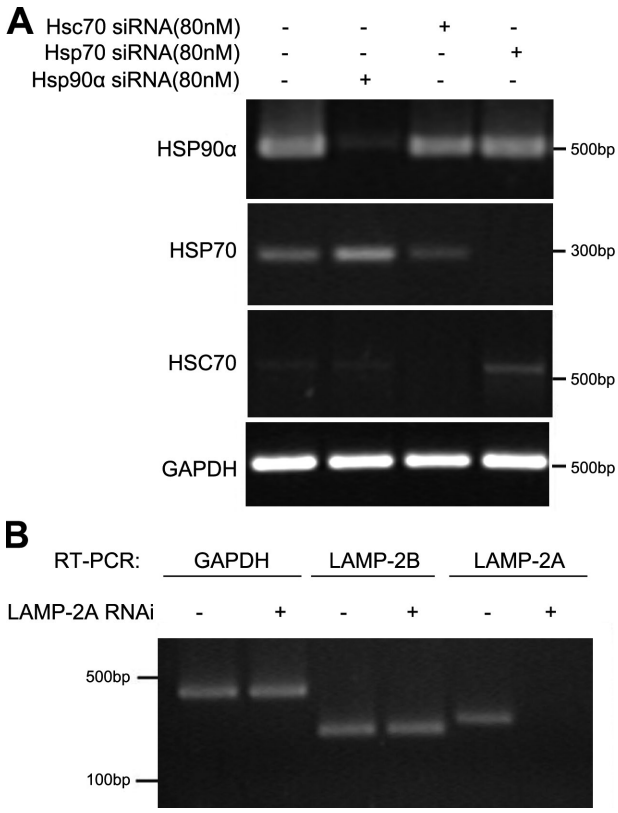

Figure S5. **Knockdown efficiency of the siRNA experiments.** (A and B) RT-PCR was used to detect the RNAi efficiency on Hsp proteins (A), LAMP-2A (B), and LAMP-2B.

Table S1. **Class neighbor gene signatures of apratoxin A (3-h treatment)**

Probe set ID	GenBank	Gene symbol	Title
Down-regulated genes			
214975_s_at	AK001816	MTMR1	Myotubularin-related protein 1
204602_at	NM_012242	DKK1	Dickkopf homologue 1 (*X. laevis*)
219508_at	NM_004751	GCNT3	Glucosaminyl (*N*-acetyl) transferase 3 mucin type
203395_s_at	NM_005524	HES1	Hairy and enhancer of split 1 (*Drosophila*)
218276_s_at	NM_021818	SAV1	Salvador homologue 1 (*Drosophila*)
218748_s_at	NM_006544	SEC10L1	SEC10-like 1 (*S. cerevisiae*)
219181_at	NM_006033	LIPG	Lipase endothelial
201647_s_at	NM_005506	SCARB2	Scavenger receptor class B member 2
205321_at	NM_001415	EIF2S3	Eukaryotic translation initiation factor 2 subunit 3-γ 52 kD
215931_s_at	AV657604	ARFGEF2	ADP ribosylation factor guanine nucleotide exchange factor 2 (brefeldin A inhibited)
210449_x_at	AF100544	MAPK14	Mitogen-activated protein kinase 14
200796_s_at	BF594446	MCL1	Myeloid cell leukemia sequence 1 (BCL2 related)
221786_at	BF197222	PHF10	Plant homeodomain finger protein 10
210148_at	AF305239	HIPK3	Homeodomain-interacting protein kinase 3
201742_x_at	NM_006924	SFRS1	Splicing factor arginine/serine-rich 1 (splicing factor 2, alternate splicing factor)
217997_at	AI795908	PHLDA1	Pleckstrin homology–like domain family A member 1
217208_s_at	AL121981	DLG1	Discs large (*Drosophila*) homologue 1
205466_s_at	NM_005114	HS3ST1	Heparan sulfate (glucosamine) 3-*O*-sulfotransferase 1
202979_s_at	NM_021212	ZF	Host cell factor–binding transcription factor Zhangfei
36711_at	4848734_RC	MAFF	V-maf musculoaponeurotic fibrosarcoma oncogene homologue F (avian)
209681_at	AF153330	SLC19A2	Solute carrier family 19 (thiamine transporter) member 2
201129_at	NM_006276	SFRS7	Splicing factor arginine/serine-rich 7 35 kD
209404_s_at	AF151867	CGI-109	CGI-109 protein
200769_s_at	NM_005911	MAT2A	Methionine adenosyltransferase II-α
211613_s_at	U79250	GPD2	Glycerol-3-phosphate dehydrogenase 2 (mitochondrial)
201565_s_at	NM_002166	ID2	Inhibitor of DNA-binding 2 dominant-negative helix loop helix protein
210935_s_at	AF274954	WDR1	WD repeat domain 1
218502_s_at	NM_014112	TRPS1	Trichorhinophalangeal syndrome I
212367_at	AI799061	FEM1B	Fem-1 homologue b (*C. elegans*)
200923_at	NM_005567	LGALS3BP	Lectin galactoside-binding soluble 3 binding protein
Up-regulated genes			
219270_at	NM_024111	MGC4504	Hypothetical protein MGC4504
200924_s_at	NM_002394	SLC3A2	Solute carrier family 3 (activators of dibasic and neutral amino acid transport) member 2
202581_at	NM_005346	HspA1A	Heat shock 70-kD protein 1A
202887_s_at	NM_019058	RTP801	HIF-1–responsive RTP801
219693_at	NM_020133	LPAAT-δ	Lysophosphatidic acid acyltransferase-δ
213418_at	NM_002155	HspA6	Heat shock 70-kD protein 6 (Hsp70B)
204285_s_at	AI857639	PMAIP1	Phorbol-12-myristate-13-acetate–induced protein 1
214052_x_at	AW301305	XTP2	HbxAg-transactivated protein 2
200666_s_at	NM_006145	DNAJB1	DnaJ (Hsp40) homologue subfamily B member 1
213524_s_at	NM_015714	G0S2	Putative lymphocyte G0/G1 switch gene
202672_s_at	NM_001674	ATF3	Activating transcription factor 3
221108_at	NM_016449	LOC51233	Hypothetical protein LOC51233
215272_at	R59977	TADA3L	Transcriptional adaptor 3 (NGG1 homologue, yeast)–like
207013_s_at	AB009303	MMP16	Matrix metalloproteinase 16 (membrane inserted)
217911_s_at	NM_004281	BAG3	BCL2-associated athanogene 3
218972_at	NM_018259	FLJ10890	Hypothetical protein FLJ10890
207778_at	NM_006508	REGL	Regenerating islet-derived–like, pancreatic stone protein–like, pancreatic thread protein–like (rat)
216929_x_at	U15197	ABO	ABO blood group (transferase A, α-1-3-*N*-acetylgalactosaminyltransferase; transferase B, α-1-3-galactosyltransferase)
213157_s_at	BF115148	KIAA0523	KIAA0523 protein
216382_s_at	U80756	NA	NA
202842_s_at	AL080081	DNAJB9	DnaJ (Hsp40) homologue subfamily B member 9
210353_s_at	M65105	SLC6A2	Solute carrier family 6 (neurotransmitter transporter, noradrenalin) member 2
201649_at	NM_004223	UBE2L6	Ubiquitin-conjugating enzyme E2L 6
219936_s_at	NM_023915	GPR87	G protein–coupled receptor 87
217187_at	Z34282	NA	NA
216307_at	AB018261	DGKB	Diacylglycerol kinase-β 90 kD
209889_at	AF274863	SEC31B-1	Secretory pathway component Sec31B-1
207300_s_at	NM_000131	F7	Coagulation factor VII (serum prothrombin conversion accelerator)
219799_s_at	NM_005771	RDHL	NAD phosphate–dependent retinol dehydrogenase/reductase

NA, not applicable. Class neighbor gene signatures of apratoxin A treatment versus vehicle treatment. Apratoxin A gene expression raw data were obtained from the National Center for Biotechnology Information's Gene Expression Omnibus (http://www.ncbi.nlm.nih.gov/geo/; accession no. GSE2742). Raw data were processed by robust multiarray average and normalized using quantile normalization. For marker gene selection, we used the signal to noise statistic to rank the genes that correlated with apratoxin A–treated versus vehicle-treated cell distinction. Signal to noise ratio = $(\mu_0 - \mu_1)/(\sigma_0 + \sigma_1)$, where μ and σ represent the mean and the standard deviation of the expression, respectively, for each class with performing 1,000 permutation (Golub et al., 1999).

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Table S2. **Class neighbor gene signatures of apratoxin A (6-h treatment)**

Probe set ID	GenBank	Gene symbol	Title
Down-regulated genes			
204702_s_at	NM_004289	NFE2L3	Nuclear factor (erythroid-derived 2)–like 3
204602_at	NM_012242	DKK1	Dickkopf homologue 1 (*X. laevis*)
203395_s_at	NM_005524	HES1	Hairy and enhancer of split 1 (*Drosophila*)
204615_x_at	NM_004508	IDI1	Isopentenyl-diphosphate-δ isomerase
217608_at	AW408767	NA	Similar to RIKEN cDNA A930021C24
203559_s_at	NM_001091	ABP1	Amiloride-binding protein 1 (amine oxidase [copper containing])
200758_s_at	AI361227	NFE2L1	Nuclear factor (erythroid-derived 2)–like 1
200923_at	NM_005567	LGALS3BP	Lectin galactoside-binding soluble 3–binding protein
216670_at	AL050220	KLK13	Kallikrein 13
210100_s_at	AF327657	ABCA2	ATP-binding cassette subfamily A (ABC1) member 2
200999_s_at	NM_006825	CKAP4	Cytoskeleton-associated protein 4
215869_at	AK022254	ABCA1	ATP-binding cassette subfamily A (ABC1) member 1
210445_at	U19869	FABP6	Fatty acid–binding protein 6 ileal (gastrotropin)
211936_at	AF216292	HspA5	Heat shock 70-kD protein 5 (glucose-regulated protein, 78 kD)
202603_at	N51370	ADAM10	A disintegrin and metalloproteinase domain 10
201627_s_at	NM_005542	INSIG1	Insulin-induced gene 1
202126_at	AA156948	PRPF4B	PRP4 pre-mRNA processing factor 4 homologue B (yeast)
211401_s_at	AB030078	FGFR2	Fibroblast growth factor receptor 2 (bacteria-expressed kinase, keratinocyte growth factor receptor, craniofacial dysostosis 1, Crouzon syndrome, Pfeiffer syndrome, Jackson-Weiss syndrome)
208881_x_at	BC005247	IDI1	Isopentenyl-diphosphate-δ isomerase
203394_s_at	BE973687	HES1	Hairy and enhancer of split 1 (*Drosophila*)
214845_s_at	AF257659	CALU	Calumenin
217850_at	NM_014366	NS	Nucleostemin
210787_s_at	AF140507	CAMKK2	Calcium/calmodulin-dependent protein kinase kinase 2-β
209189_at	BC004490	FOS	V-fos Finkel-Biskis-Jinkins murine osteosarcoma viral oncogene homologue
214179_s_at	H93013	NFE2L1	Nuclear factor (erythroid-derived 2)–like 1
201625_s_at	BE300521	INSIG1	Insulin-induced gene 1
201792_at	NM_001129	AEBP1	Adipocyte enhancer–binding protein 1
210560_at	AF118452	GBX2	Gastrulation brain homeo box 2
201045_s_at	BF513857	RAB6A	RAB6A member rat sarcoma oncogene family
202238_s_at	NM_006169	NNMT	Nicotinamide *N*-methyltransferase
Up-regulated genes			
200924_s_at	NM_002394	SLC3A2	Solute carrier family 3 (activators of dibasic and neutral amino acid transport) member 2
202581_at	NM_005346	HspA1A	Heat shock 70-kD protein 1A
200799_at	NM_005345	HspA1A	Heat shock 70-kD protein 1A
213524_s_at	NM_015714	G0S2	Putative lymphocyte G0/G1 switch gene
218972_at	NM_018259	FLJ10890	Hypothetical protein FLJ10890
200800_s_at	NM_005345	HspA1A	Heat shock 70-kD protein 1A
204285_s_at	AI857639	PMAIP1	Phorbol-12-myristate-13-acetate–induced protein 1
217911_s_at	NM_004281	BAG3	BCL2-associated athanogene 3
217678_at	AA488687	NA	*Homo sapiens*–transcribed sequence with moderate similarity to protein reference no. NP_060312.1 (*H. sapiens*) hypothetical protein FLJ20489 (*H. sapiens*)
202887_s_at	NM_019058	RTP801	HIF-1–responsive RTP801
201505_at	NM_002291	LAMB1	laminin β1
202842_s_at	AL080081	DNAJB9	DnaJ (Hsp40) homologue subfamily B member 9
220235_s_at	NM_018372	FLJ11269	Hypothetical protein FLJ11269
219270_at	NM_024111	MGC4504	Hypothetical protein MGC4504
200666_s_at	NM_006145	DNAJB1	DnaJ (Hsp40) homologue subfamily B member 1
209921_at	AB040875	SLC7A11	Solute carrier family 7 (cationic amino acid transporter, y+ system) member 11
212633_at	AL132776	KIAA0776	KIAA0776 protein
203935_at	NM_001105	ACVR1	Activin A receptor type I
213418_at	NM_002155	HspA6	Heat shock 70-kD protein 6 (Hsp70B)
203726_s_at	NM_000227	LAMA3	Laminin α3
206976_s_at	NM_006644	HspH1	Heat shock 105-kD/110-kD protein 1
222108_at	AC004010	AMIGO2	Amphoterin-induced gene 2
210512_s_at	AF022375	VEGF	Vascular endothelial growth factor
207528_s_at	NM_014331	SLC7A11	Solute carrier family 7 (cationic amino acid transporter, y+ system) member 11
202146_at	AA747426	IFRD1	Interferon-related developmental regulator 1
218145_at	NM_021158	C20orf97	Chromosome 20 open reading frame 97
218772_x_at	NM_018112	FLJ10493	Hypothetical protein FLJ10493
218566_s_at	NM_012124	CHORDC1	Cysteine- and histidine-rich domain containing zinc-binding protein 1
213350_at	BF680255	RPS11	Ribosomal protein S11
205047_s_at	NM_001673	ASNS	Asparagine synthetase

NA, not applicable. Class neighbor gene signatures of apratoxin A treatment versus vehicle treatment. Apratoxin A gene expression raw data were obtained from the National Center for Biotechnology Information's Gene Expression Omnibus (http://www.ncbi.nlm.nih.gov/geo/; accession no. GSE2742). Raw data were processed by robust multiarray average and normalized using quantile normalization. For marker gene selection, we used the signal to noise statistic to rank the genes that correlated with apratoxin A–treated versus vehicle-treated cell distinction. Signal to noise ratio = $(\mu_0 - \mu_1)/(\sigma_0 + \sigma_1)$, where μ and σ represent the mean and the standard deviation of the expression, respectively, for each class with performing 1,000 permutation (Golub et al., 1999).

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Table S3. **Del-2 somatic mutation in human NSCLC cancers**

Exon	Mutation type	Nucleotide change	Amino acids change	Histological type	Reference
19	Microindel	c.2250_2277delinsGAGAAGCAAG	p.Thr751_Ile759delins ArgSerLys	Adenosquamous	Kang et al., 2007
19	Microindel	c.2252_2276delinsA	p.Thr751_Ile759delinsAsn	ADC	Soung et al., 2005
19	Microindel	c.2252_2276delinsG	p.Thr751_Ile759delinsSer	ND	Kosaka et al., 2004
19	Microdeletion	c.2253_2276del	p.Ser752_Ile759del	ND	Tam et al., 2006
19	Microdeletion	c.2253_2276del	p.Ser752_Ile759del	ND	Eberhard et al., 2005
19	Microdeletion	c.2253_2276del	p.Ser752_Ile759del	ND	Kosaka et al., 2004
19	Microdeletion	c.2254_2277del	p.Ser752_Ile759del	ADC	Paez et al., 2004
19	Missense	c.2266A>G	p.Asn756Asp	ADC	Tomizawa et al., 2005
19	Duplication	c.2268_2270dupCAA	p.Asn756_Lys757insAsn	NSCLC	Endo et al., 2005
19	Missense	c.2273A>G	p.Glu758Gly	ND	Shih et al., 2006

NSCLC, nonsmall cell lung cancer; ADC, adenocarcinoma.

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