

Table S1. Summary of Akt shRNA oligos and constructs

Target	Construct #	Selection	Target sequence^a
Akt1	252	Pur	5'-TGACCATGAACGAGITTA-3'
Akt1	253	Pur	5'-GTGGACCACTGTCATCGAA-3'
Akt2	254	Pur	5'-CCTGGAGGCCACGGTACTT-3'
Akt2	255	Pur	5'-TGACTTCGACTATCTAAA-3'
Akt3	259	Pur	5'-GAATTGTAGTCCAACCTCA-3'
Akt3	260	Pur	5'-GCACTTTGGAAAGTTAT-3'
Akt3	261	Hyg	5'-GCACTTTGGAAAGTTAT-3'
Akt12	256	Pur	5'-GCTACTACGCCATGAAGAT-3'
Akt12	257	Pur	5'-AGGTGCTGGAGGACAATGA-3'
Akt13	258	Pur	5'-CTACAACCAGGACCATGAG-3'
Akt13	253 + 261	Pur + Hyg	253 and 261 target sequences
Akt23	255 + 261	Pur + Hyg	255 and 261 target sequences
Akt123	257 + 261	Pur + Hyg	257 and 261 target sequences

Hyg, hygromycin; Pur, puromycin.

^aSense sequence in the target.

Table S2. Summary of Akt KD efficiency and effect on xenograft tumor growth for various PC3-Akt shRNA clones

Target/construct	Clone	Treatment	% Akt1 ^a	% Akt2 ^a	% Akt3 ^a	n ^b	P ^c	R ^d	% TGI (d14) ^e	DRS ^f	% TGI (DRS) ^g
Akt1											
252	2	Dox-	100	100	100	8	6	0			
		Dox+	21.9 ± 1.1	93.8 ± 10.2	151 ± 58.3	8	8	0	-14	>21	
252	9	Dox-	100	100	100	8	5	0			
		Dox+	22.3 ± 2.3	122.7 ± 11.3	120.3 ± 8.6	8	2	1	72	21	81 ^g
252	10	Dox-	100	100	100	8	8	0			
		Dox+	11.2 ± 2.6	89.1 ± 13.9	106.8 ± 3.2	8	5	0	52	20	58 ^g
253	17	Dox-	100	100	100	8	7	0			
		Dox+	11.6 ± 1.2	80.1 ± 9.7	106.1 ± 4.3	8	2	3	97 ^g	13	97 ^g
253	25	Dox-	100	100	100	8	5	1			
		Dox+	13.9 ± 5	82.3 ± 1.3	92.9 ± 5.3	8	7	0	26	>21	
253	29	Dox-	100	100	100	10	10	0			
		Dox+	5.9 ± 0.1	70.3 ± 5.6	98 ± 1	10	8	0	56 ^g	5	56 ^g
Akt2											
255	4	Dox-	100	100	100	8	7	0			
		Dox+	88 ± 16.5	11.6 ± 2.4	116.4 ± 32	8	5	2	-7	>21	
255	23	Dox-	100	100	100	10	10	0			
		Dox+	105 ± 6.1	10.4 ± 1.3	106.3 ± 8	10	9	0	22	>21	
Akt3											
260	6	Dox-	100	100	100	8	6	1			
		Dox+	101 ± 4	95.5 ± 10.5	10 ± 4	8	4	1	45	>21	
Akt12											
257	4	Dox-	100	100	100	8	3	2			
		Dox+	5.7 ± 0.9	13.2 ± 1.1	129.6 ± 11.3	8	0	5	137 ^g	14	137 ^g
257	6	Dox-	100	100	100	8	7	0			
		Dox+	6.6 ± 0.3	12.3 ± 1.6	128.9 ± 18.3	8	1	4	80 ^g	11	90 ^g
Akt13											
253 + 261	2	Dox-	100	100	100	8	8	0			
		Dox+	5.1 ± 2.0	66 ± 2.6	18 ± 3.7	8	1	3	102 ^g	7	120 ^g
Akt23											
255 + 261	8	Dox-	100	100	100	8	6	0			
		Dox+	96.8 ± 18.8	6.3 ± 0.3	9.9 ± 2.1	8	0	0	82 ^g	7	93 ^g
Akt123											
257 + 261	10	Dox-	100	100	100	10	8	2			
		Dox+	1.2 ± 0.3	9.5 ± 0.7	4.5 ± 2.1	10	0	6	116 ^g	6	135 ^g
257 + 261	12	Dox-	100	100	100	8	6	0			
		Dox+	9 ± 3.6	21.7 ± 2.9	12.7 ± 0.9	8	0	5	116 ^g	7	110 ^g
257 + 261	12	Dox-	100	100	100	10	5	0			
		Dox+	9 ± 3.6	21.7 ± 2.9	12.7 ± 0.9	10	1	4	106 ^g	10	129 ^g
EGFP											
310	3	Dox-	100	100	100	8	6	1			
		Dox+	93.7 ± 13.2	95 ± 6.4	87.3 ± 4.7	8	7	0	-90	>21	

TGI, tumor growth inhibition; DRS, days to reach significance.

^aPercentage of message level after 72 h of Dox treatment compared with untreated control determined by real-time quantitative RT-PCR (Taqman). Data represent mean ± SEM of at least three independent experiments.

^bNumber of tumors analyzed in each cohort.

^cNumber of tumors progressed by day 14, defined by tumor volume more than twofold of the initial size at the start of treatment.

^dNumber of tumors regressed by day 14, defined by tumor volume <50% of the initial size at the start of treatment.

^ePercentage of tumor growth inhibition at day 14 or the first day when significant difference was achieved, calculated as % TGI = % [Vc(dx - d0) - Vt(dx - d0)]/Vc(dx - d0) × 100, in which Vc(dx - d0) is the difference in mean tumor volume of the control cohort (Vc) between the day of analysis (dx) and the day when treatment started (d0), and Vt(dx - d0) is the difference in mean tumor volume of the treated cohort (Vt) between the day of analysis and the day when treatment started. % TGI >100 indicates tumor regression.

^fThe number of days taken after treatment before significant difference between the control and the treatment group is achieved.

^gP < 0.05 compared with the sucrose vehicle-treated group, determined by Student's *t* test.