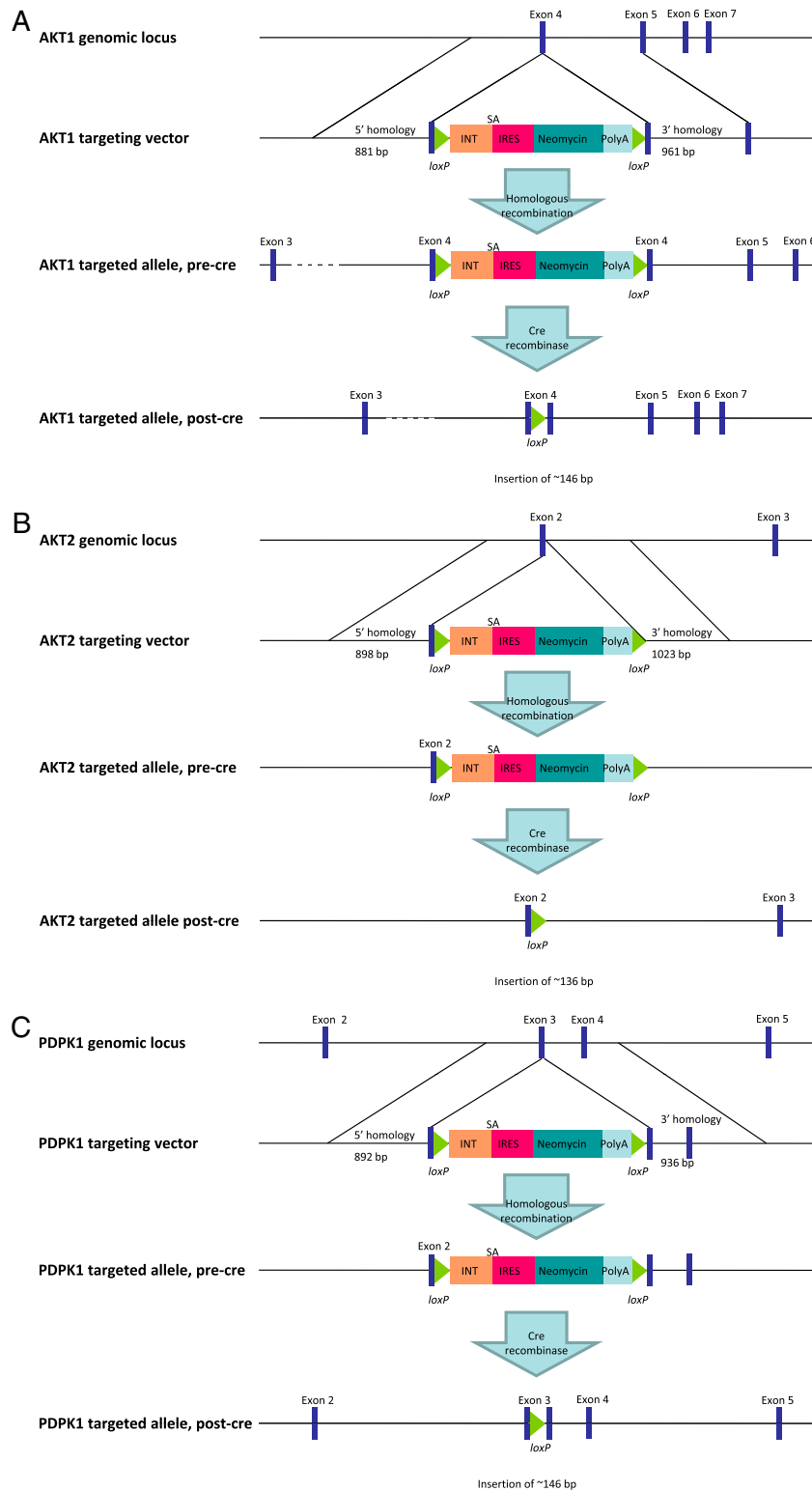
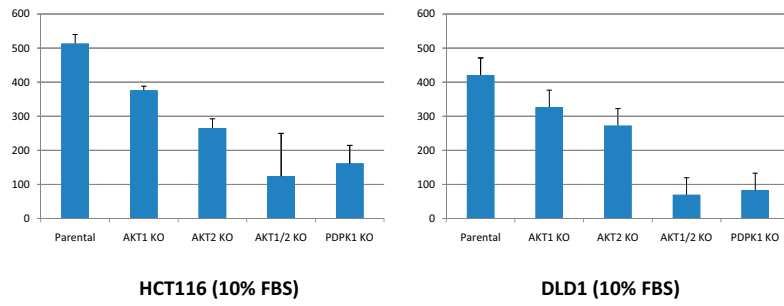


# Supporting Information

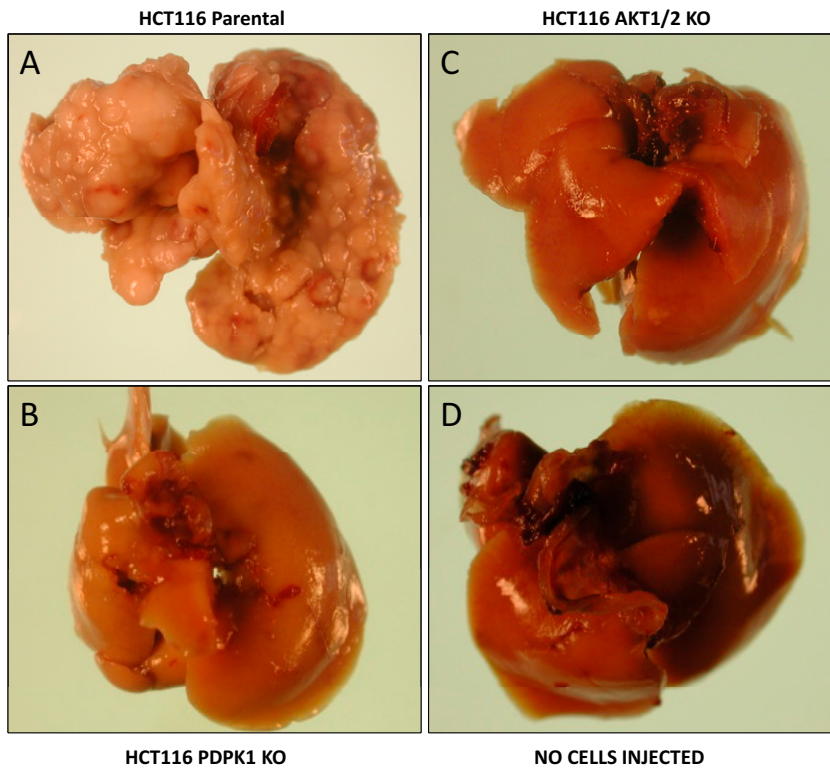
Ericson et al. 10.1073/pnas.0914018107



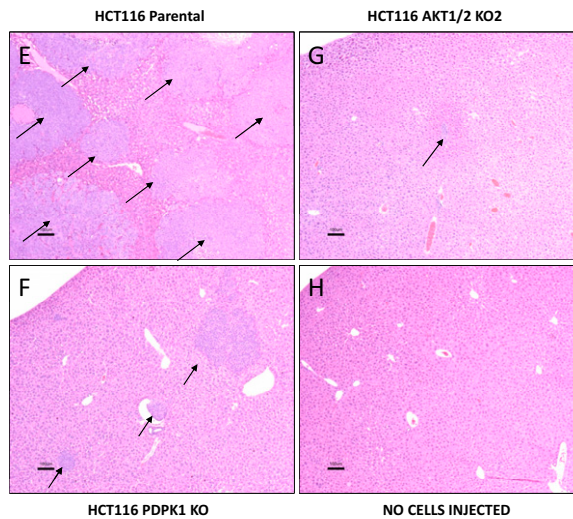
**Fig. S1.** Targeting strategy. The genetic loci for *AKT1*, *AKT2*, and *PDPK1* are shown along with the targeting constructs and the targeted allele before and after Cre-mediated excision of the vector sequences. INT, synthetic intron; SA, splice acceptor site; IRES, internal ribosomal entry site; Neomycin, neomycin resistance gene. (A) *AKT1* targeting vector. (B) *AKT2* targeting vector. (C) *PDPK1* targeting vector.



**Fig. S2.** Colony formation in normal growth media. Approximately 1,500 cells of the indicated cell lines were seeded in T25 flasks, cultured for 11–12 days in medium containing 10% FBS, and then stained and counted.



**Fig. S3.** Tumor foci visible on the liver surface 5 weeks after injection of the indicated cells.



**Fig. S4.** H&E-stained sections of the livers shown in Fig. S3. The arrows indicate areas containing cancer cells.



**Table S1. Tumorigenicity following intrasplenic injection**

Mouse ID	Cell type	Lung		Liver				Spleen			
		Histology (Number of foci)	Median	Visible on surface	Median*	Histology**	Median	Human DNA, pg	Median	Human DNA, pg	Median
H6-1	HCT116	10	1.5	>200	>200	TNTC	TNTC	14300	19800	22600	20000
H6-2	Parental	1		>200		TNTC		20700		13500	
H6-3		N/D		>200		TNTC		26800		18700	
H6-4		2		>200		TNTC		19800		20000	
H6-5		1		>200		TNTC		15600		23800	
F6-1	HCT116	0	0	0	0	0	0	0.1	7.7	97.8	9800
F6-2	AKT1 /	0		0		0		1.5		9800	
F6-3	AKT2 KO1	0		0		2		9.3		15745	
F6-4		0		0		4		11.6		10600	
F6-5		0		0		0		7.7		6520	
G6-1	HCT116	0	0	0	0	0	0	0.3	0.3	166	166
G6-2	AKT1 /	0		0		0		8.4		1800	
G6-3	AKT2 KO2	0		0		0		0.3		0.1	
G6-4		0		1		0		0.1		1.2	
G6-5		0		0		1		0.3		889	
B6-1	HCT116	0	0	3	3	10	10	81.4	81.4	5240	5240
B6-2	PDPK1 KO1	0		4		22		565		6820	
B6-3		0		0		8		40.0		6110	
B6-4		0		3		19		183		5160	
B6-5		0		0		4		1.9		4100	
C6-1	HCT116	0	1	1	1	6	6	168	80.9	9250	6880
C6-2	PDPK1 KO2	2		3		9		2380		6880	
C6-3		0		0		8		40.3		3940	
C6-4		1		2		6		10.0		7870	
C6-5		2		0		4		80.9		6330	
I6-1	HCT116	7	1.5	1	33	9	41.5	20.3	5650	69.5	14400
I6-2	AKT1 KO1	3		7		10		71.4		2560	
I6-3		0		30		66		5650		28800	
I6-4		0		43		51		13400		10800	
I6-5		0		33		32		7550		21400	
I6-6		0		55		52		1830		11100	
I6-7		11		96		TNTC		16300		14400	
J6-1	HCT116	0	0	6	3	21	6	3600	88.7	6680	14400
J6-2	AKT1 KO2	0		0		1		88.7		12900	
J6-3		0		3		6		4.7		16000	
J6-4		6		22		16		1200		14400	
J6-5		0		0		6		82.0		18500	
D6-1	HCT116	0	0	74	74	72	57	7320	1430	22900	10600
D6-2	AKT2 KO1	0		81		57		1270		11300	
D6-3		0		23		20		21.5		10600	
D6-4		0		21		12		1430		7830	
D6-5		0		94		131		3160		10400	
E6-1	HCT116	0	0	>200	>200	TNTC	TNTC	5600	5600	9160	8770
E6-2	AKT2 KO2	0		>200		TNTC		4910		8650	
E6-3		0		24		21		548		8170	
E6-4		1		>200		TNTC		7720		8770	
E6-5		N/D		>200		TNTC		10400		11300	
E7-1	No cells injected	0	0	0	0	0	0	0.1	0.1	0.1	0.0
E7-2		0		0		0		0.0		0.0	
A7-1	DLD1	0	0	0	10	0	7	1.4	175	5 070	5 340
A7-2	Parental	0		23		13		198		4 290	
A7-3		5		27		7		612		6 540	
A7-5		0		10		7		175		9 230	
A7-6		1		0		5		3.6		5 340	
N7-1	DLD1 AKT1 /	0	0	0	0	0	0	1.0	2.0	4 670	4 940
N7-2	AKT2 KO1	0		0		0		3.8		4 940	
N7-3		0		0		0		0.9		4 080	
N7-4		0		0		0		2.0		6 000	
N7-5		0		0		0		2.4		5 520	

**Table S1. Cont.**

Mouse ID	Cell type	Lung		Liver					Spleen		
		Histology (Number of foci)	Median	Visible on surface	Median*	Histology**	Median	Human DNA, pg	Median	Human DNA, pg	Median
O7-1	DLD1 AKT1 /	0	0	0	0	0	0	1.1	0.3	3 327	642
O7-2	AKT2 KO2	0		0		0		0.1		50.7	
O7-3		0		0		0		0.4		807	
O7-4		0		0		0		0.3		642	
O7-5		0		0		0		0.1		419	
H7-1	DLD1	0	0	0	0	0	0	0.1	0.2	2 020	2 020
H7-2	PDPK1 KO1	0		1		1		0.1		1 110	
H7-3		0		0		0		0.2		185	
H7-4		0		0		0		0.7		8 010	
H7-5		0		1		1		0.4		6 200	
I7-1	DLD1	0	0	0	0	0	0	0.2	0.2	5 400	4 340
I7-2	PDPK1 KO2	0		0		0		0.2		4 020	
I7-3		0		0		0		0.1		6 300	
I7-4		0		0		0		0.2		4 340	
I7-5		0		0		0		0.1		1 620	
F7-1	DLD1	0	0	15	18	8	7	195	40.6	5 320	5 320
F7-2	AKT1 KO1	1		9		4		0.5		4 710	
F7-3		0		23		7		600		2 680	
F7-4		0		19		16		0.6		6 050	
F7-5		3		18		6		40.6		5 680	
G7-1	DLD1	0	0	7	7	1	7	19.6	14.3	11 200	9 030
G7-2	AKT1 KO2	0		5		7		14.3		8 310	
G7-3		3		7		3		0.5		9 030	
G7-4		0		11		9		251		9 560	
G7-5		0		13		12		6.8		5 630	
J7-1	DLD1	0	0	0	0	0	1	0.5	0.4	7 340	5 840
J7-2	AKT2 KO1	0		3		1		0.2		2 740	
J7-4		0		2		2		0.2		6 360	
J7-5		0		0		1		0.5		6 010	
J7-7		0		0		1		1.0		4 530	
J7-8		0		0		0		0.3		5 680	
K7-1	DLD1	0	0	0	4	0	0	1.6	2.4	7 550	7 220
K7-2	AKT2 KO2	0		4		3		2.7		7 150	
K7-3		0		4		0		4.3		5 130	
K7-4		0		5		0		2.0		7 220	
K7-5		0		0		1		2.4		7 360	
E7-1	No cells	0	0	0	0	0	0	0.1	0.1	0.1	0.0
E7-2	injected	0		0		0		0.0		0.0	

\*\*In the indicated specimens, there were at least 200 foci but the actual number was difficult to count because of adjoining lesions (see Fig. S3).

\* The number of foci present in a total of five sections, one from each of five lobes of the liver. TNTC: Too numerous to count.

**Table S2. Primers used in Fig. 1**

Wild-type PDPK1	Forward	ACCACCAGCCAGCTGTATGAC
	Reverse	TCGCATATTCTCTGGAGGTTG
pSEPT inserted in PDPK1	Forward	ACCACCAGCCAGCTGTATGAC
	Reverse	AAGCCTGCCACACCTCAAGC
Wild-type AKT1	Forward	AGGCTCCCCTCAACAACCTTC
	Reverse	CTCCTCCTCCTCTGCTTCT
pSEPT inserted in AKT1	Forward	CCTCGCAGACAGCGAATTA
	Reverse	ATGGCACCTTCATTGGCTAC
Wild-type AKT2	Forward	GGTGCCTCCTGCATGTCC
	Reverse	CCTCTCGGTCTTCATCAGC
pSEPT inserted in AKT2	Forward	GGTGCCTCCTGCATGTCC
	Reverse	AAGCCTGCCACACCTCAAGC