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Supplemental Information

Targeting the *IGF1R* Pathway in Breast Cancer Using Antisense lncRNA-Mediated Promoter *cis* Competition

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Table S1. Oligonucleotide primers used for PCR

ID	Oligo Name	Oligo sequence	Product size
qPCR expression			
IGFIR	JH217	GAAGTCTGGCTCCGGAGGAGGGTC	180 bp
	JH218	ATGTGGAGGTAGCCCTCGATCAC	
IRAIN	JH248	CGACACATGGTCCAATCACTGTT	138 bp
	JH249	AGACTCCCCTAGGACTGCCATCT	
NM23	WX011	TTGAGCGTACCTCCATTGCGATC	370 bp
	WX012	TTTGCACTCTCCACAGAATCACT	
B-ACTIN	J880	CAGGTCATCACCATTGGCAATGAGC	135 bp
	J881	CGGATGTCCACGTCACACTTCATGA	
Targeting			
	SJ131	CTGGGGTAGAAAGATGACTGAA	
	JH583	ACGCATTTATTTATTTTGCAACAGC	
DNA methylation			
	SJ996	TYGTAYGTTTTGGGGAATYGGGTTT	204 bp
	SJ997	ACTAATAAACAAAAACCCCAACCTC	
	SJ998	GTGTTTTGGATTTGGGAAGGAGTT	279 bp
	SJ999	AAACAAAACCCAAATCTACCTAAAC	
Targeting vector			
	JH1094	CAGTGCAGGGGAAAGAATAG	422 bp
	JH485	TCAGTTTTTAGCCGGGAAGGT	
	SJ130	ACGCGTATATCTGGCCCGTACA	921 bp
	SJ131	CTGGGGTAGAAAGATGACTGAA	
ARM1	SJ016	TCAAAATTTTATCGATATTATCTGGCTATCACTC AGAACCT	777 bp
	SJ017	GCGTATATCTGGCCCGTACATCTTCGAAGATAA GTACGGTTTAGAAGACACG	
PCMV	SJ018	GATGTACGGGCCAGATATACGCG	659 bp
	SJ019	ACCGTGGGCTTGTACTCGGTCATTGTACAATTT CGATAAGCCAGTAAGCAGTG	
PURO	SJ020	ATGACCGAGTACAAGCCCACGGT	612 bp
	SJ021	GCCCTGGGGACGTCGTCGCGGGTGGCGAGGC GCACCGTGGGCTTGTACTCGGTCAT	
ARM2	JH986	GCAGAAGAGAGGGCGACACGACGCCGACCAC AACAAAACCAAGGC	2 kb
	SJ023	TTAAATCGACGCTAGCCCTCGGCTGTGACCTT CAGCGAGC	
PH1-1	SU47	CAGCCGAGGGCTAGCTTTAAGACCAATGACTT ACAAGGC	361 bp
	SJ138	CTAAACGTCTGGTAAGAAATGGGATCCAAGTG GTCTCATACAG	
gRNA1	SJ139	CCCATTTCTTACCAGACGTTTAGTTTTAGAGCT AGAAATAGCAAGTT	140 bp
	SJ140	AGGCCCTCTTCCTGCCCGACCTTGTCGACAAA AAAAGCACCGACTCGGTGCCA	

PU6	SJ141	GTGGCACCGAGTCGGTGCTTTTTTTGTCGACA AGGTCGGGCAGGAAGAGGGCCT	316 bp
	SJ142	CAGTGGGACGCGGACTGGGGCGGTGTTTCGT CCTTTCCACAAGA	
gRNA2	SJ143	CCGCCCCAGTCCGCGTCCCACTGTTTTAGAGC TAGAAATAGCAAGTT	119 bp
	SJ144	GCAAAAAGCAGAATCGAAGAATTCAAAAAAA GCACCGACTCGGTGCCAC	
	SU48	CCCCTACCCATTTAAATGAAGCAAAAAGCAGA ATCGAAGAATTC	
PH1-2	SU47	CAGCCGAGGGCTAGCTTTAAGACCAATGACTT ACAAGGC	361 bp
	SJ145	CTTACTAAGTCGTTTAGGTGGGGATCCAAGTG GTCTCATACAG	
PU6-1	SJ141	GTGGCACCGAGTCGGTGCTTTTTTTGTCGACA AGGTCGGGCAGGAAGAGGGCCT	316 bp
	SJ147	CGACCTTCCACAGATCTGGGCGGTGTTTCGTC CTTTCCACAAGA	
gRNA3	SJ146	CCCCACCTAAACGACTTAGTAAGTTTTAGAGC TAGAAATAGCAAGTT	145 bp
	SJ140	AGGCCCTCTTCCTGCCCGACCTTGTCGACAAA AAAAGCACCGACTCGGTGCCA	
PU6-2	SJ141	GTGGCACCGAGTCGGTGCTTTTTTTGTCGACA AGGTCGGGCAGGAAGAGGGCCT	316 bp
	SJ147	CGACCTTCCACAGATCTGGGCGGTGTTTCGTC CTTTCCACAAGA	
gRNA4	SJ148	CCGCCCAGATCTGTGGAAGGTCGTTTTAGAGC TAGAAATAGCAAGTT	119 bp
	SJ144	GCAAAAAGCAGAATCGAAGAATTCAAAAAAA GCACCGACTCGGTGCCAC	
RAT primers			
	JH513	CCTTTGTCCATGTGGTCAAGTT	
	JH400	CATCAGGTCCCTTCTACCATCC	
	JH745	ACGCATTTATTTATTTTGCAACAGCTGC	
	JH780	GTTTCCGCAGTAGCCGCTGAT	
siRNAs			
	IRAIN1	CCUCAUGCAGAGAACUUAATT	
		UUAAGUUCUCUGCAUGAGGTT	
	IRAIN2	CCAGUCACUUACGUAGCAATT	
		UUGCUACGUAAGUGACUGGTT	
	IRAIN3	CCGCAUGCACGCAUUUAUUTT	
		AAUAAAUGCGUGCAUGCGGTT	
	siCT	UUCUCCGAACGUGUCACGUTT	
		ACGUGACACGUUCGGAGAATT	

SUPPLEMENTARY FIGURE LEGENDS

Figure S1. ALIC targeting of the *IGF1R* and *IRAIN* locus.

A. CRISPR Cas9 *IRAIN*-gRNA targeting vector. Cas9: CRISPR Cas9; gRNA: Cas9 guiding RNAs that target the *IRAIN* promoter (sequences under the diagram); pEF1: the human EF-1a promoter; pH1: human H1 promoter; pU6: U6 promoter; T5: the TTTTT termination signal of RNA polymerase III.

B. Location of the four *IRAIN* Cas9 gRNAs. The *IRAIN* arm fragments was presented in green capitals. The *IRAIN* gRNA sequences were highlighted in yellow and PAM (NGG) in red in the *IRAIN* promoter region.

C. The *IRAIN* donor vector. pCMV: CMV promoter to transcribe the puromycin selection marker gene; pPGK: PGK promoter to transcribe the TK negative selection marker gene; TK+: the herpesvirus thymidine kinase gene for negative selection of the targeting clone cells; loxP: the locus of X-over P1 recombination site recognized by Cre to remove the Puro+ gene; Arm1, Arm2: the *IRAIN* arm sequences used for recombination.

Figure S2. Mapping of the IGF1R targeting locus by DNA sequencing.

A. The *IGF1R-IRAIN* targeting locus. The donor vector contains the IRAIN Arm2-Puro-pCMV-Arm1 insert. The Arm2-Arm1 fragments provide the donor sequences for homologous recombination after the Cas9-induced DNA break. Puro+: Puromycin selection marker used for selection. pCMV: CMV promoter. After homologous recombination and Cre recombinase treatment, the CMV promoter is inserted in front of the *IRAIN* lncRNA. Primers (SJ131 and JH583)

were used to amply the whole targeting locus containing the 5' flanking genomic DNA-Arm2-pCMV-Arm1-3' flanking genomic DNA. The PCR DNA was cloned into a pJet vector for sequencing.

B. Sequencing of the recombination sites in the *IGF1R* locus. The DNA fragment covering the whole targeting locus was sequenced. The joint sites of recombination were marked by red arrows.

Figure S3. IRAIN siRNA knockdown in ALIC targeted cells.

The pCMV-IRAIN knockin cells (ALIC) were transfected with IRAIN siRNAs (siIRAIN) or control siRNA (siCT). Cells were collected for gene analysis of *IRAIN* by quantitative PCR. Error bars represent the standard error of the average of three independent PCR reactions. a,b: $p<0.05$ as compared with the vector control (CTL); c: $p<0.05$ as compared with the siCT control.

Figure S4. The RAT-Seq assay.

Schematic diagram of the nuclear *in situ* lncRNA reverse transcription-associated trap (RAT) assay. After crosslinking to fix the lncRNA-chromatin structure, *IRAIN* lncRNA was *in situ* reverse transcribed into lncDNA in the nucleus with biotin-dCTP. The *IRAIN*-binding chromatin DNAs were sonicated into fragments and purified with streptavidin breads. After reversal of crosslink, lncDNA was extracted for DNA library construction and Illumina sequencing.

Figure S5. The *IRAIN* lncRNA binding targets.

Gene ontology analysis of *IRAIN* target genes from the nuclear *in situ* lncRNA reverse transcription-associated trap (RAT) assay.

Figure S6. Expression of target genes and cell growth in *IRAIN*-overexpressing cells.

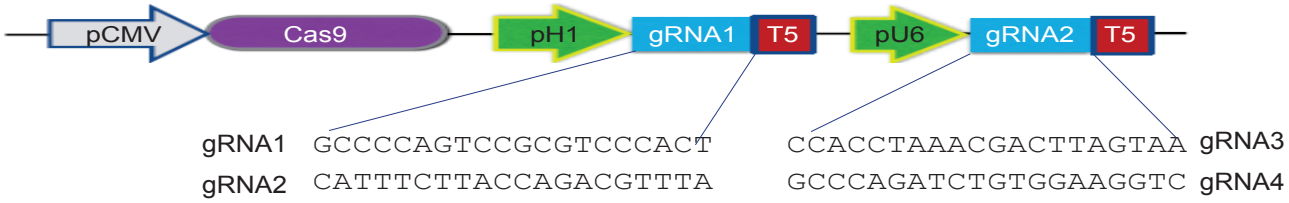
A. MDA-MB-231 tumor cells were infected with lentiviruses carrying the 5K *IRAIN* cDNA (IRAIN5K) or the copGFP vector control (Vector). After viral infection, cells were selected by puromycin and were collected for gene analysis of *IRAIN* target genes using quantitative PCR. The data shown are mean ± SD of three independent PCR reactions. * $p<0.05$, ** $p<0.01$ as compared with the vector control.

B. Cell proliferation. The *IRAIN*-overexpressing stable cells were collected for analysis of cell proliferation using the MTT assay. The cells that stably expressed the copGFP vector were used as the vector control (Vector). Cell growth was measured as the relative absorbance by setting 0 hour as 1. All experiments were performed in triplicate.

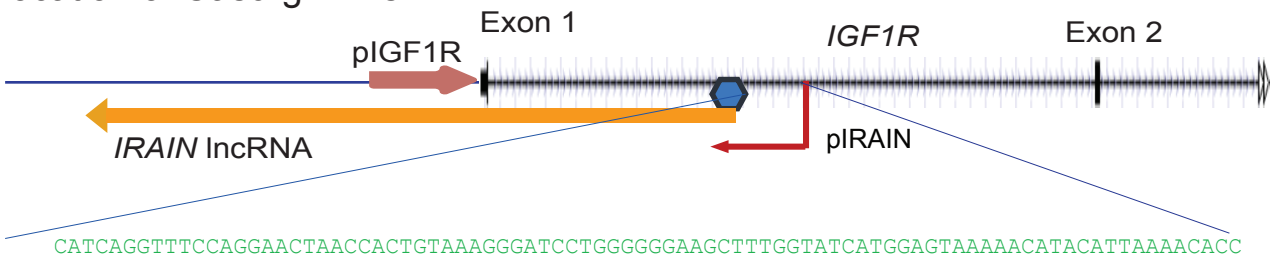
C. Cell cycle. The *IRAIN*-overexpressing stable cells and the vector control cells were collected at 24 hrs, 48 hrs, and 7 hrs for FACS analysis. Cell cycle phase distribution was determined by Cell Quest Pro software and was calculated as the relative value for each phase. No significance of phase distribution was detected between the *IRAIN*-overexpressing and vector control cells.

D. Cell migration. Cell migration was measured by scratch assay at 19 hrs following cell plating.

A. *IGF1R* ALIC gRNA vector



B. Location of Cas9-gRNAs



CATCAGGTTTCCAGGAACTAACCACTGTAAAGGGATCCTGGGGGGAAGCTTTGGTATCATGGAGTAAAAACATACATTAAAACACC
CCCTCCCTCCTGCCTGTGTTCTCAAAAATCGCCGTCTTGCACAAAGAATATCCTTCAAGAAGAACGCTCCAAAAGAAAAACAGCCT
ATTTTAAAATGCCCGACTCCTGCGTCGGCGAAGGCCATCCCTGCTGTTTTGAATTTCAGAACATTAAAAACACAAAACTAGAGAAG
CTATACAGTTCAACTGCATAACATAACAAAATGGATTATTTCTCCCGTGTCTTCTAAACCGTACTTATCttttaaagataaaaacc
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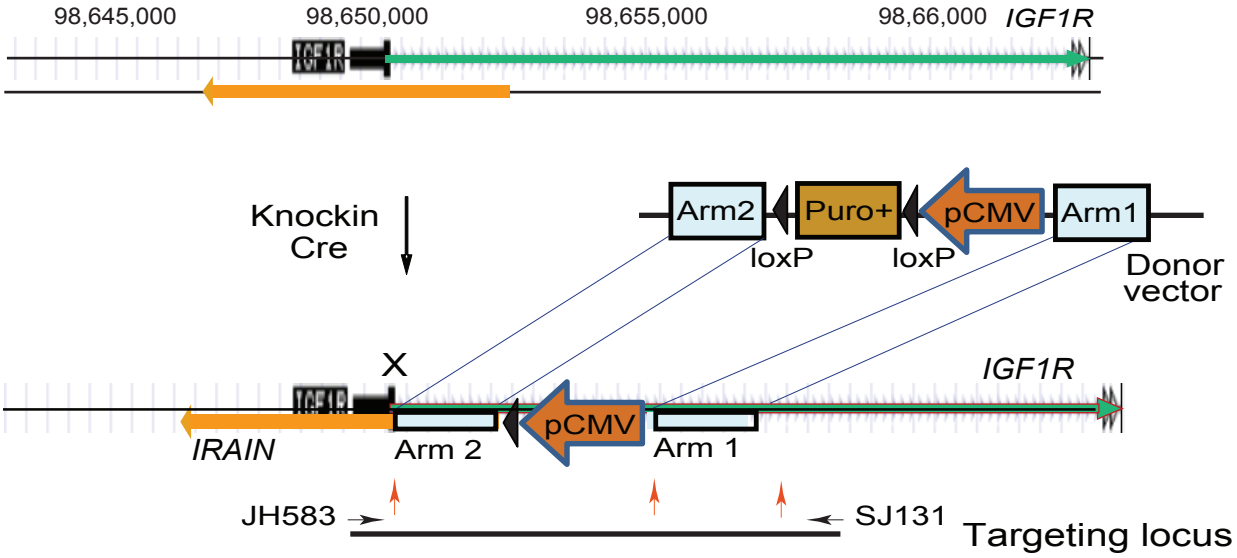
aaaacaaagagcaggagaGCCCAGATCTGTGGAAGGTCCGGCCCCAGTCCGCGTCCCACTCGGcagtaaacacgcgaaccacgatc
tgctctcgttccgtcgcggactCATTTCTTACCAGACGTTTAAGGatttgtttatagaaaCAGCCTTTCTGAATTGCCCcgGTGAT
GGGGCCATACAACCTCcgCTGCCTCCTCCTTCCCcgCCTCACTCGTGAAGGCTCAGTCGTGATTTTTTCAAAGTTAATCGGCACCA
CCTGCATACCCT

C. Donor vector

Figure S1. *IGF1R* ALIC Cas9-gRNA targeting and donor vectors

A. The *IGF1R-IRAIN* locus



B. Sequencing of the *IGF1R* targeting locus

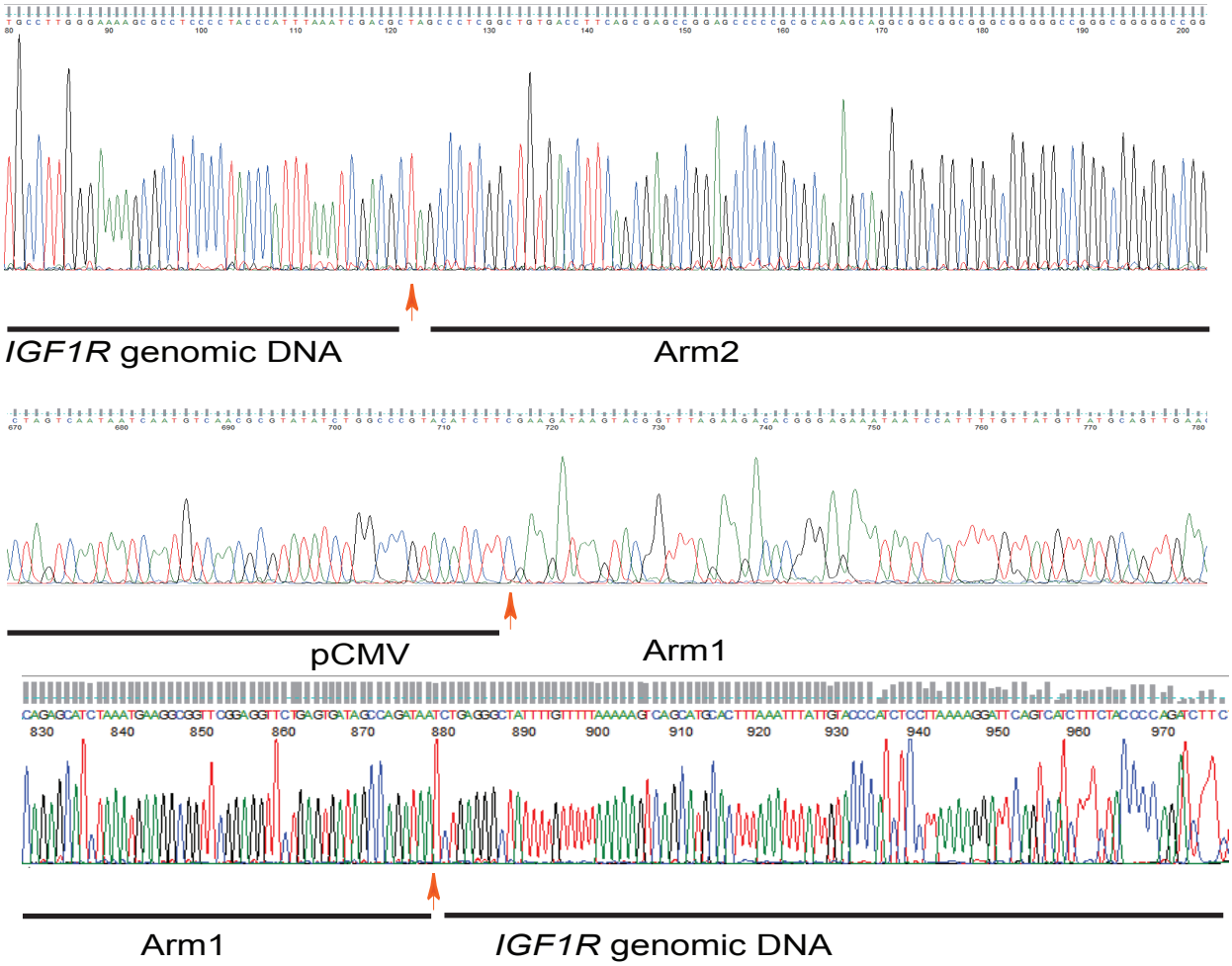


Figure S2. Sequencing of the targeting site of the *IGF1R* locus

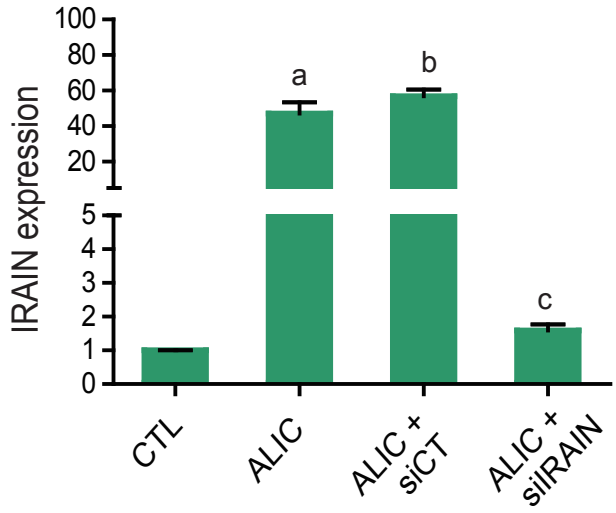
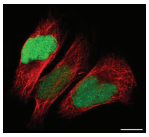
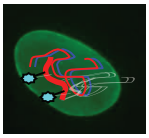


Figure S3. Knockdown of IRAIN lncRNA by siRNAs in ALIC targeted cells

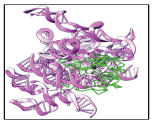
A. Nuclear *in situ* RAT assay



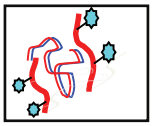
Cell fixation



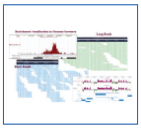
IRAIN lncRNA nulcear *in situ* RT
(biotin-*IRAIN* lncDNA)



Chromatin sonication



Streptavidin bead purification of *IRAIN* lncDNA
library construction



DNA sequencing
IRAIN target analysis

Figure S4. *IRAIN* lncRNA in situ RAT assay

A. *IRAIN* RAT-Seq targets

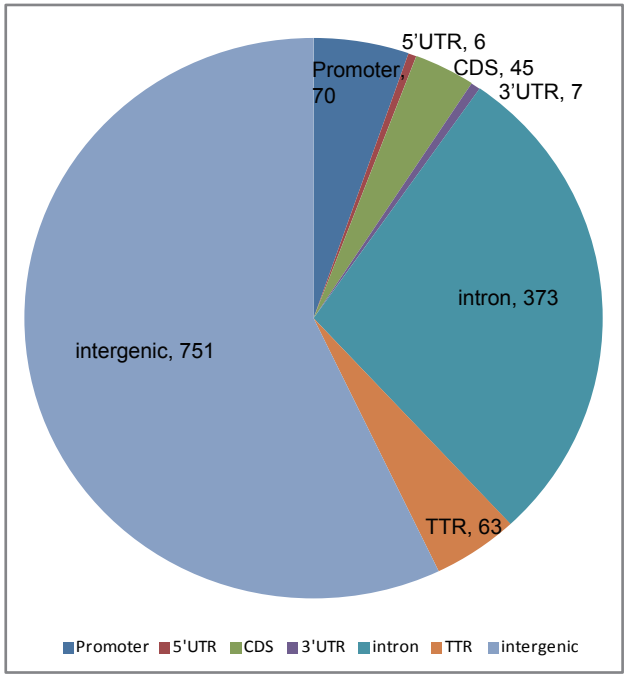
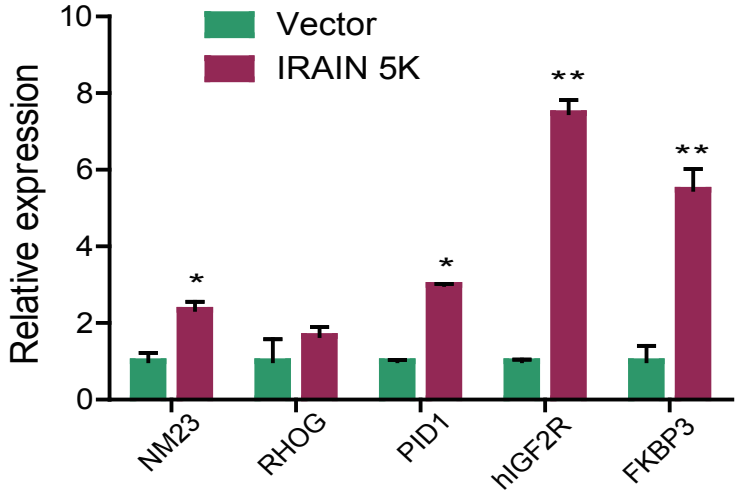
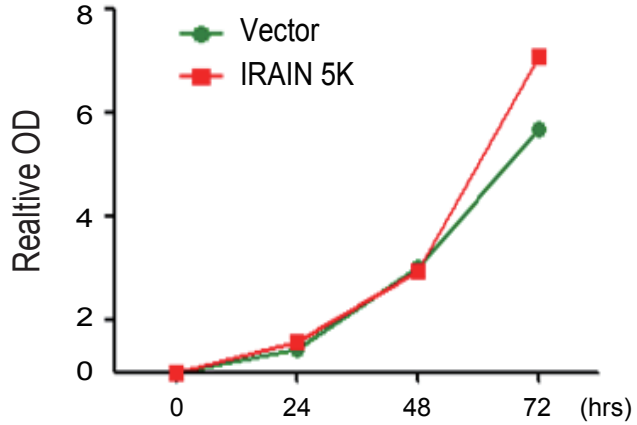


Figure S5. *IRAIN* RAT-Seq targets

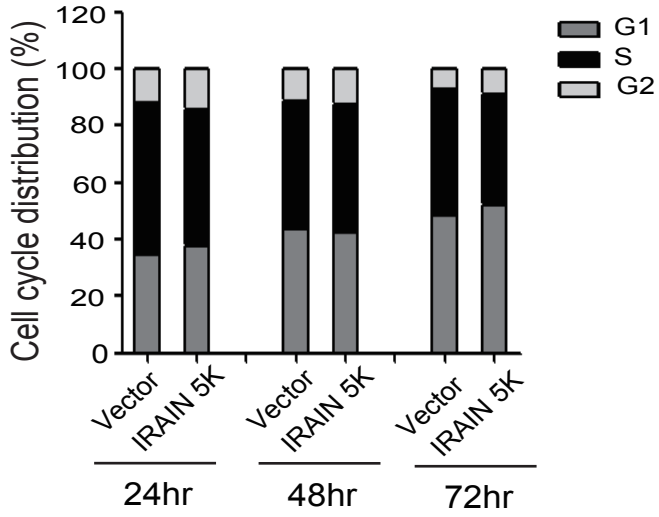
A. *IRAIN* target gene expression



B. Cell proliferation



C. Cell cycle



D. Cell migration

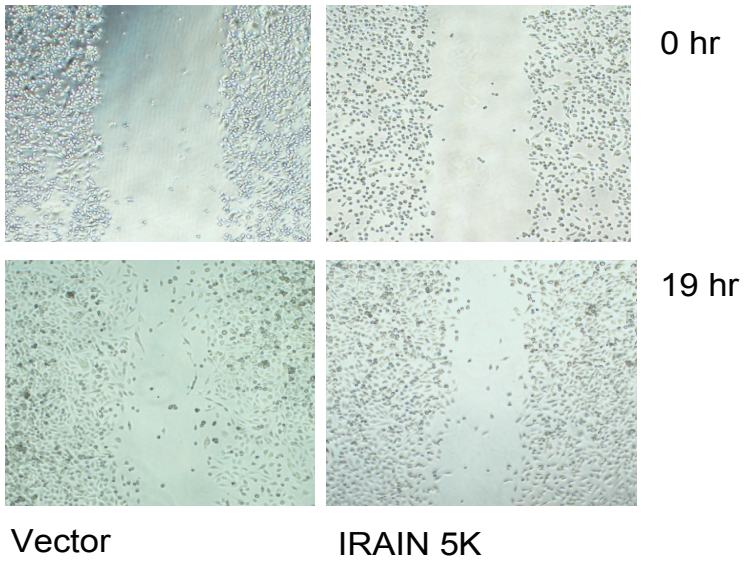


Figure S6. Target genes and cell growth in the *IRAIN trans*-overexpressing cells