SUPPLEMENTARY METHODS

Promoter Methylation Analysis

Genomic DNA was extracted from ~200-300 mg of fresh-frozen primary hLSCC tissue and matching normal lung squamous cell tissue from 18 individuals (obtained from The Prince Charles Hospital and University if Queensland Thoracic Research Centre). High molecular-weight DNA was purified using the DNeasy Tissue Kit (Qiagen). Cleaned genomic DNA (1 μg) was bisulfite-converted using the EZ DNA Methylation Kit (Zymo Research), hybridized to multisample Infinium HumanMethylation27 v1.0 BeadChips (Illumina). Images were processed, data extracted and control probes checked using the BeadStudio Methylation Module (Illumina) using default settings. All CpGs located on the X-chromosome were removed to avoid gender-specific bias.

Percent methylation was calculated by measuring the intensity ratio of methylated to unmethylated DNA, giving a β-value between 0 (100% methylation) and 1 (0% methylation) (Supplementary Table S2). For each gene, the fold change was calculated for each individual by dividing the percent methylation in the tumor sample by the normal control. A gene with a fold change ≥1.5 was considered hypermethylated (i.e., ≥50% increase in methylation) in hLSCC compared to normal lung, whereas a gene with a fold change <1.5 was considered to have no change in methylation status. To evaluate the significance of any observed number of hypermethylation events n for each gene, we estimated the probability of obtaining the value n or more in random data drawn according to a null model (i.e., individuals are independent and the hypermethylation rate is uniformly distributed among genes). Statistical analyses were performed using R (1). The *P*-value for each gene was estimated based on binomial distribution.

Ectopic Expression of TSGs and Analysis of Tumor Suppressor Activity

TSG cDNAs (see Supplementary Table S10) were cloned, by PCR (using primers listed in Supplementary Table S9) followed by restriction enzyme digestion, into MSCV PIG (Puro-

IRES-GFP) (Addgene plasmid 18751). For some genes, a 3xFlag tag sequence was incorporated into the primers for cloning in-frame with the target gene. Murine stem cell viruses (MSCVs) carrying TSGs were packaged in 293T cells and used to infect NCI-H520 cells.

For Supplementary Fig. S8B, NCI-H520 cells were infected with retroviruses expressing TSGs for 24 hours, and then the virus was removed. Cells were then puromycin selected for 24 hours, and recovered in fresh medium without puromycin for 3 days. Cell extracts were prepared and subjected to immunoblot analysis using the antibodies against ANGPT1 (Strategic Diagnostics), CDK5R1, MYD88 (Cell Signaling Technology), PTGIS (Santa Cruz), SRSF9 (Abcam), SPOP (Abnova) and Flag-M2 (Sigma).

For the assay shown in Supplementary Fig. S8C, NCI-H520 cells ($5x10^3$) were plated in 6-well plates, infected with retroviruses at an MOI of 2, puromycin selected for 4 weeks and stained with crystal violet. For the assay shown in Supplementary Fig. S8D, NCI-H520 cells were first stably transfected with an *FRS2* expression vector (2) or empty vector (pEYFP-N1, Clontech) and then infected with retroviruses expressing TSGs. For the mouse tumorigenesis assays of Supplementary Fig. S8G, NCI-H520 cells were infected with retroviruses and 2 days later FACS sorted for GFP-positive cells. $2x10^6$ viable GFP-positive cells were injected subcutaneously into nude mice, and tumor dimensions were measured and calculated every 3-4 days [using the formula (length x width^2) x (π/4)].

SUPPLEMENTARY REFERENCES

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2. Tomasovic A, Traub S, Tikkanen R. Molecular networks in FGF signaling: flotillin-1 and cbl-associated protein compete for the binding to fibroblast growth factor receptor substrate 2. PLoS One 2012;7:e29739.

SUPPLEMENTARY FIGURE LEGENDS

Supplementary Figure S1. Target gene expression analysis, soft agar colony formation assays and tumor formation assays for the candidate TSG NIH 3T3 KD cell lines. **A**, qRT-PCR analysis monitoring shRNA-mediated knockdown efficiency for each candidate TSG. Values are given relative to expression of each gene following treatment with a non-silencing (NS) shRNA, which was set to 1. **B**, Soft agar colony formation assay. NIH 3T3 cells stably transduced with an shRNA directed against a candidate TSG, or as a control a NS shRNA, were analyzed for their ability to form colonies in soft agar. **C**, Soft agar colony formation assay as described in (**B**) using a second, unrelated shRNA against the same target gene. **D**, qRT-PCR analysis monitoring knockdown efficiency as described in (**A**) using a second, unrelated shRNA against the same target gene. **E**, Tumor formation assay using a second, unrelated shRNA against a subset of 17 TSGs. Data are represented as mean ± SD. *P<0.05 and **P<0.01.

Supplementary Figure S2. Down-regulation of candidate TSGs in hLSCC samples. qRT-PCR analysis monitoring expression of each candidate TSG in 27 hLSCC samples. Values were normalized to the expression of the candidate TSG in nine normal lung samples, the average of which was set to 1. The red line indicates 2-fold down-regulation. Asterisks indicate samples whose fold down-regulation vastly exceeds that shown in the graph. For each gene, samples have been re-ordered from least to most down-regulated. Data are represented as mean ± SD.

Supplementary Figure S3. Expression of candidate TSGs in human lung adenocarcinoma samples. qRT-PCR analysis monitoring expression of each candidate TSG in 10 human lung adenocarcinoma samples. Values were normalized to the expression of the candidate TSG in nine normal lung samples, the average of which was set to 1. The red line indicates 2-fold down-regulation. Data are represented as mean ± SD.

Supplementary Figure S4. TSG promoter hypermethylation in hLSCC samples. **A**, Left, Methylation heat map for TSGs showing hypomethylation (red) or hypermethylation (green) in the tumor sample. Genes that are significantly hypermethylated (defined as a ≥50% increase in methylation) in tumor samples (*P*-value < 0.05) are indicated by an asterisk. Right, color key. **B**. Percent of individuals with significant hypermethylation in nine candidate TSGs. **P*<0.05 and ***P*<0.01.

Supplementary Figure S5. Knockdown efficiency for human shRNAs, and confirmation of increased pFRS2 levels following knockdown of TSGs. **A**, qRT-PCR analysis monitoring shRNA-mediated knockdown efficiency for each TSG in SA cells. Values are given relative to expression of each gene following treatment with a NS shRNA, which was set to 1. **B**, qRT-PCR analysis monitoring knockdown efficiency as described in (**A**) using a second, unrelated shRNA against the same target gene. Data are represented as mean ± SD. **P*<0.05 and ***P*<0.01. **C**, (Top) Immunoblots monitoring phosphorylated FRS2-Y436 (pFRS2-Y436) and total FRS2 (tFRS2) in the SA KD cell lines using a second shRNA unrelated to that used in Fig. 3A. α-tubulin (TUBA) was monitored as a loading control. (Bottom) Quantification of the immunoblots. The red line indicates a two-fold increase in phospho-protein level relative to that observed in NS cells, which was set to 1.

Supplementary Figure S6. Confirmation that FGFR signaling is increased in SA and NIH 3T3 KD cell lines. **A**, Immunoblots monitoring phosphorylated FRS2-Y196 (pFRS2-Y196) or total FRS2 (tFRS2) in SA KD cell lines and NCI-H520 cells. α-tubulin (TUBA) was monitored as a loading control. **B**, (Left) Extracts from SA or NCI-H520 cells were immunoprecipitated with a control (IgG) or PLC- γ antibody, and the immunoprecipitate analyzed by immunoblotting with an antibody against phosphorylated tyrosine (pY). Input extracts were immunoblotted for total PLC-γ (tPLC-γ). The results confirm the specificity of the PLC- γ antibody. (Right)

Immunoprecipitate-immunoblot analysis monitoring phosphorylated PLC-γ and tPLC-γ levels in SA KD cell lines. **C**, Immunoblot monitoring pFRS2-Y196 or tFRS2 in SA KD cell lines using a second shRNA unrelated to that used in (**A**). **D**, Immunoprecipitate-immunoblot analysis monitoring phosphorylated PLC- γ and tPLC-γ levels in SA KD cell lines using a second shRNA unrelated to that used in (**B**). **E**, Immunoblots monitoring pFRS2-Y436 and tFRS2 in NIH 3T3 KD cell lines.

Supplementary Figure S7. Effect and specificity of TSG knockdown on FGFR1 expression. **A** and **B**, qRT-PCR analysis monitoring *FGFR1* expression levels in SA KD cell lines using two unrelated TSG shRNAs. Data are represented as mean ± SD. **P*<0.05 and ***P*<0.01. **C** and **D**, Immunoblot analysis monitoring tFGFR2, tFGFR3, tFGFR4, tEGFR and tIR levels in SA KD cell lines using two unrelated TSG shRNAs. α-tubulin (TUBA) was monitored as a loading control.

Supplementary Figure S8. Ectopic expression of TSGs that encode repressors of FGFR signaling reduces proliferation of NCI-H520 cells. **A**, qRT-PCR analysis monitoring the expression level of TSGs following ectopic expression in NCI-H520 cells. Values are given relative to expression of the TSG in NCI-H520 cells expressing vector alone, which was set to 1. **B**, Immunoblot analysis monitoring tumor suppressor protein levels in NCI-H520 cells expressing either the TSG or vector. Blots were probed using an antibody against the endogenous protein or against a Flag tag for epitope-tagged proteins. β-actin (ACTB) was monitored as a loading control. **C**, Colony formation assay measuring proliferation of NCI-H520 cells expressing a TSG relative to that obtained with empty vector, which was set to 100%. **D**, Colony formation assay measuring proliferation of NCI-H520 cells expressing a TSG in the presence or absence of ectopic FRS2 expression. White bars, proliferation of NCI-H520 cells expressing a TSG relative to that obtained with empty vector, which was set to 1. Gray bars, fold

increase in the proliferation of NCI-H520 cells co-expressing a TSG and FRS2 relative to that obtained upon expression of the TSG and vector control. **E**, Immunoblots monitoring phosphorylated (p) and total (t) FRS2 in NCI-H520 cells expressing a representative TSG or empty vector. α-tubulin (TUBA) was monitored as a loading control. (Bottom) Quantification of the immunoblots. The red line indicates a two-fold decrease in phospho-protein level relative to that observed in vector control cells, which was set to 1. **F**, Colony formation assay measuring proliferation of SA/HRAS cells expressing a TSG relative to that obtained with empty vector. **G**, Tumor formation in mice injected with NCI-H520 cells expressing a TSG or empty vector. Data are represented as mean ± SD. *P<0.05 and **P<0.01.

Supplementary Fig. S9. Control experiments related to Figure 4. **A**, Immunoblot measuring pFRS2 and tFRS2 in tumors formed from SA KD cell lines. α-tubulin (TUBA) was monitored as a loading control. **B**, Soft agar assay measuring colony formation of SA KD cell lines relative to that obtained with the NS shRNA, which was set to 1. Data are represented as mean ± SD. *P<0.05 and **P<0.01. **C**, Tumor formation in mice injected with SA KD cell lines; tumors were photographed at various time points following injection. **D**, Immunoblot measuring pFRS2 and tFRS2 in tumors formed from SA KD cell lines using a second shRNA unrelated to that used in (**A**).

Supplementary Figure S10. Ponatinib sensitivity of SA, SA/HRAS, SA KD and SA/FGFR1 cells. **A,** Percentage of surviving SA or SA/HRAS cells following treatment with ponatinib in liquid culture. Briefly, $5x10^3$ SA or SA/HRAS cells were plated in a 96-well plate, and ponatinib (0, 31.3, 62.5, 125, 250, 500, 1000, 2000, 4000 or 8000 nM) was added. After 3 days, the percentage of surviving cells was monitored by Presto Blue (Invitrogen). The results show that the ponatinib sensitivity of HRAS-transformed SA cells was similar to that of parental SA cells. **B**, Soft agar assay measuring colony formation of SA KD cells, generated using a second shRNA

unrelated to that used in Fig. 5A, treated with varying concentrations of ponatinib. Colony number was normalized to that obtained in the absence of ponatinib, which was set to 100%. **C**, Soft agar assay measuring colony formation of SA KD cells, generated using a second shRNA unrelated to that used in Fig. 5B, treated with 125 nM ponatinib, normalized as described in (**B**). **D,** Soft agar assay measuring colony formation of SA cells overexpressing FGFR1 or, as a control, SA/HRAS cells, treated with varying concentrations of ponatinib. Colony number was normalized to that obtained in the absence of ponatinib, which was set to 100%. **E**. Colony formation assay measuring viability of SA KD cells, generated using a second shRNA unrelated to that used in Fig. 5C, expressing an FRS2 shRNA relative to that obtained with an NS shRNA. Viability was normalized to that obtained in NS shRNA-expressing cells, which was set to 1. Data are represented as mean ± SD. **P*<0.05 and ***P*<0.01.

Supplementary Figure S11. Substantial down-regulation of *DAPP1*, *MYD88* and *STK11* in A427 cells. qRT-PCR analysis monitoring expression of *DAPP1*, *MYD88* and *STK11* in A427 cells relative to HBECs. Data are represented as mean ± SD. (error bars are too small to be visualized. **P*<0.05 and ***P*<0.01. The results show that *DAPP1*, *MYD88* and *STK11* are substantially down-regulated in A427 cells.

Supplementary Figure S12. Proliferation of single and multiple knockdown SA cells. $5x10^3$ SA cells transduced with a single shRNA or combinations of shRNAs against DAPP1, MYD88, STK11 were plated in 96-well plates, and the percentage of surviving cells was monitored by Presto Blue. Data are represented as mean ± SD. The results show that TSG knockdown increased the proliferation rate of SA cells and this effect was greater with multiple compared to single knockdowns.

Supplementary Figure S13. Instability of an engineered truncated SH3BP2 protein. **A**, Immunoblot analysis monitoring the level of SH3BP2 in NCI-H520 cells expressing vector, full-length SH3BP2 or an engineered truncated SH3BP2 in which exon 10 has been deleted. **B**, qRT-PCR analysis monitoring expression of *SH3BP2* exon 10 and *SH3BP2* exons 7-8 (total *SH3BP2*) in NCI-H520 cells expressing full-length or truncated SH3BP2. Data are represented as mean ± SD. **P*<0.05 and ***P*<0.01.

Supplementary Figure S14. Growth of tumors derived by SRSF9 knockdown is dependent on FGFR signaling. **A**, Tumor formation assay in mice (n=3 per group) injected with SA cells lines co-expressing one of two SRSF9 shRNAs and one of two FRS2 shRNAs. Tumor volume was measured at 6 weeks. **B**, Tumor formation assay in mice (n=3 per group) injected with SRSF9 SA KD cells and treated with either vehicle or ponatinib. Ponatinib was formulated in aqueous 25 mM citrate buffer (pH 2.75) and mice were gavaged orally with 100 μl at a dose of 30 mg/kg every other day, starting 14 days after injection of SRSF9 SA KD cells. Tumor volume was measured at 6 weeks. **C**, Knockdown efficiencies of FRS2 (left) and SRSF9 (right) shRNAs in SA KD cells. Expression was normalized to that obtained with a NS shRNA, which was set to 1. Data are represented as mean ± SEM. **P*<0.05 and ***P*<0.01. For *SRSF9* expression, differences between SRSF9/NS and SRSF9/FRS2 double KD cells were not significant (*P*>0.05).

Supplementary Table S1. Summary of Oncomine data analysis querying whether candidate TSGs are down-regulated in cancer versus normal tissue.

Gene	Bladder	Brain&CNS	Breast	Cervical	Colorectal	Esophageal	Gastric	Head&Neck	Kidney	Leukemia	Liver	Lung	Lymphoma	Melanoma	Myeloma	Other	Ovarian	Pancreatic	Prostate	Sarcoma
ANGPT1	●	●	●		●			●	●	●		●	●	●		●	●	●	●	●
CDK5R1		●	●	●		●		●		●		●	●							
DAPP1		●	●			●				●		●	●	●		●			●	●
DDX52		●								●			●		●					
DNAJC12	●	●	●		●				●	●	●			●	●	●				●
FLNA	●	●	●		●				●	●		●	●						●	●
FPR3					●					●		●			●					
GAPVD1			●			●						●				●				
GZMA		●			●					●		●	●	●					●	
IGF2R		●	●							●										
MAP1A	●	●	●						●			●	●		●	●	●	●	●	●
MYD88								●		●		●								
NAA38		●			●					●								●		
NME4	●	●	●							●					●	●				
NUP205			●							●			●							
ORC1		●	●							●		●								
PIGH		●	●					●								●	●	●		●
PKD1L3																				
PTGIS	●		●	●	●	●		●	●	●	●	●		●		●	●	●	●	●
PTPN4		●	●						●			●	●							●
SDF2L1								●			●							●		●
SEMA3B		●	●		●		●		●	●		●		●	●	●				●
SRSF9		●	●			●														
SPAST		●	●						●	●				●						
SPOP	●		●									●								
STK11									●	●		●				●				
TXNRD1	●	●	●							●		●	●				●		●	
ZNF22		●	●							●		●	●	●	●			●	●	

● indicates one or more reports of significant down-regulation of the gene ($P < 0.05$, fold change > 2) in cancer versus normal tissue.

Supplementary Table S2. Intensity ratios of methylated to unmethylated DNA for TSG promoters in paired primary hLSCC (LC) and normal lung squamous cell (NL) samples from 18 individuals. Beta-values range from 0 (100% methylation) to 1 (0% methylation).

See the accompanying Excel file.

Supplementary Table S3. Summary of immunoblot results of Figure 3B and C.

	Increase in pFRS2 levels	Increase in pERK levels	Increase in pFGFR1 levels	Increase in tFGFR1 levels	Step at which the TSG represses FGFR signaling
ANGPT1	●	●	●	●	Total FGFR1 levels
CDK5R1	●	●	–	–	FGFR1-independent FRS2 activation
DAPP1	●	●	●	–	FGFR1 phosphorylation
DDX52	●	●	–	–	FGFR1-independent FRS2 activation
FLNA	●	●	●	●	Total FGFR1 levels
FPR3	●	●	●	–	FGFR1 phosphorylation
GAPVD1	●	●	●	●	Total FGFR1 levels
IGF2R	–	●	NT	NT	
NAA38	–	●	NT	NT	
MAP1A	–	●	NT	NT	
MYD88	●	●	●	●	Total FGFR1 levels
NME4	●	●	●	–	FGFR1 phosphorylation
PIGH	–	●	NT	NT	
PKD1L3	–	–	NT	NT	
PTGIS	●	●	●	●	Total FGFR1 levels
PTPN4	●	●	●		FGFR1 phosphorylation
SDF2L1	●	●	–	–	FGFR1-independent FRS2 activation
SEMA3B	–	●	NT	NT	
SRSF9	●	●	●	●	Total FGFR1 levels
SPAST	●	●	●	●	Total FGFR1 levels
SPOP	●	●	–	–	FGFR1-independent FRS2 activation
STK11	●	●	–	–	FGFR1-independent FRS2 activation
TXNRD1	●	●	–	–	FGFR1-independent FRS2 activation
ZNF22	–	●	NT	NT	

● , increase in signal; –, no increase in signal; NT, not tested.

Supplementary Table S4. List of candidate genes, obtained from the RNA-Seq analysis, whose splicing is significantly altered upon SRSF9 knockdown.

Gene symbol	Gene name	Isoform_ID	log2 fold change	FPKM_isoform level (SRSF9 shRNA)	FPKM isoform level (NS shRNA)
EIF3C	eukaryotic translation initiation factor 3, subunit C	TCONS_00027843	-2.37834	3.84557	0.739619
		TCONS_00027842	∞	0	4.46266
RDM1	RAD52 motif 1	TCONS_00033289	-2.74613	0.896831	0.133673
LPIN2	lipin 2	TCONS_00035660	-12.4935	0.72986	0.000126565
		TCONS_00035661	∞	0	0.480218
		TCONS_00035662	-∞	0.215162	0
		TCONS_00035663	0.765454	1.41165	2.39966
ISYNA1	inositol-3-phosphate synthase 1	TCONS_00039566	-∞	1.74542	0
BCL2L11	BCL2-like 11 (apoptosis facilitator)	TCONS_00042593	-5.04712	0.972931	0.029427
		TCONS_00042596	2.19213	0.128717	0.58821
PLK1S1	polo-like kinase 1 substrate 1	TCONS_00047382	-∞	0.77293	0
		TCONS_00047383	∞	0	0.677097
		TCONS_00047390	∞	0	0.88007
SLC26A6	solute carrier family 26, member 6	TCONS_00055045	-∞	1.98335	0
		TCONS_00055046	∞	0	4.5935
SH3BP2	SH3-domain binding protein 2	TCONS_00057162	-0.811013	2.37631	1.35445
		TCONS_00057165	∞	0	1.64538
APC	adenomatous polyposis coli	TCONS_00061273	0.114483	3.53617	3.82821
PPT2	palmitoyl-protein thioesterase 2	TCONS_00064884	-0.646425	9.02312	5.76453
		TCONS_00064888	0.704032	4.38923	7.15028

Supplementary Table S5. Summary of NCBI SKY/MFISH & CGH data for candidate TSGs.

Gene	Chromosome location	Bladder	Brain&CNS	Breast	Cervical	Colorectal	Kidney	Leukemia	Liver	Lung	Lymphoma	Melanoma	Myeloma	Ovarian	Pancreatic	Prostate	Sarcoma
ANGPT1	8q22.3-q23		●	●		●	●	●		●	●	●		●			
CDK5R1	17q11.2	●	●	●	●	●	●	●		●	●			●	●	●	
DAPP1	4q25-q27	●	●	●	●	●	●		●	●	●	●	●	●	●	●	●
DDX52	17q21.1	●	●	●	●	●	●	●		●	●			●	●	●	
DNAJC12	10q22.1	●	●	●	●	●	●		●	●	●	●		●	●	●	●
FLNA	Xq28		●	●	●	●	●	●		●	●	●		●	●	●	●
FPR3	19q13.3-q13.4	●	●	●	●	●	●	●		●		●	●	●	●	●	●
GAPVD1	9q33.3	●	●	●	●	●	●	●	●	●	●			●	●	●	●
GZMA	5q11-q12		●	●	●	●	●	●		●	●	●		●		●	●
IGF2R	6q26	●	●	●	●	●	●	●		●	●	●		●	●	●	●
MAP1A	15q13-qter	●	●	●	●	●	●	●	●	●	●	●		●	●	●	
MYD88	3p22	●	●	●	●	●	●		●	●	●	●		●	●	●	●
NAA38	7q31.1-q31.3		●	●	●		●	●		●	●			●	●	●	
NME4	16p13.3	●	●	●	●	●	●	●		●	●	●		●	●	●	●
NUP205	7q33		●	●	●	●	●	●		●	●			●	●	●	●
ORC1	1p32		●	●	●	●	●			●	●	●		●	●	●	●
PIGH	14q11-q24	●	●	●	●	●	●				●		●	●	●	●	●
PKD1L3	16q22.2	●	●	●	●	●	●	●		●	●	●	●	●	●	●	●
PTGIS	20q13.13		●	●	●	●	●	●						●		●	●
PTPN4	2q14.2	●	●	●	●	●	●	●		●	●	●		●		●	●
SDF2L1	22q11.21	●	●	●	●	●	●	●		●		●	●	●	●	●	●
SEMA3B	3p21.3	●	●	●	●	●	●		●	●	●	●		●	●	●	●
SRSF9	12q24.31	●	●	●	●	●	●	●	●	●	●	●		●	●	●	●
SPAST	2p24-p21	●	●	●	●	●	●	●		●	●	●		●	●	●	●
SPOP	17q21.33	●	●	●	●	●	●	●		●	●			●	●	●	
STK11	19p13.3	●	●	●	●	●	●	●		●				●	●	●	●
TXNRD1	12q23-q24.1	●	●	●	●		●	●	●	●	●	●		●	●	●	●
ZNF22	10q11	●	●	●	●	●	●	●	●	●	●	●		●	●	●	●

● indicates a reported deletion of the gene in the cancer type.

Supplementary Table S6. Summary of COSMIC database analysis querying candidate TSGs for loss of heterozygosity, homozygous deletions and recurrent mutations.

Gene	Loss of heterozygosity frequency (%)	Homozygous deletion frequency (%)	Number of recurrent mutations found*
ANGPT1	2-20	0	0
CDK5R1	20-50	0	0
DAPP1	20-50	0	0
DDX52	20-50	0	0
DNAJC12	20-50	>0 – 0.2	1
FLNA	>50	>0 – 0.2	0
FPR3	20-50	0	3
GAPVD1	20-50	0	2
GZMA	20-50	0	0
IGF2R	20-50	0	3
MAP1A	20-50	0	2
MYD88	20-50	>0 – 0.2	8
NAA38	2-20	0	0
NME4	20-50	0	0
NUP205	2-20	0	3
ORC1	2-20	0	0
PIGH	20-50	0	0
PKD1L3	20-50	0	0
PTGIS	2-20	0	0
PTPN4	2-20	0	1
SDF2L1	20-50	0	0
SEMA3B	20-50	>0 – 0.2	0
SRSF9	2-20	0	0
SPAST	2-20	>0 – 0.2	0
SPOP	20-50	0	13
STK11	20-50	>0 – 0.2	35
TXNRD1	2-20	0	1
ZNF22	20-50	0	0

*A “recurrent mutation” is defined as a non-synonymous amino acid substitution occurring in ≥2 individuals.

Supplementary Table S7. Summary of The Cancer Genome Atlas Lung Squamous Cell Carcinoma project database analysis querying for DNA promoter methylation frequency.

Gene	**Promoter methylation (%)**
ANGPT1	19
CDK5R1	85
DAPP1	11
DDX52	88
DNAJC12	100
FLNA	1
FPR3	5
GAPVD1	3
GZMA	3
IGF2R	0
MAP1A	94
MYD88	0
NAA38	0
NME4	0
NUP205	0
ORC1	0
PIGH	86
PKD1L3	N/A
PTGIS	40
PTPN4	0
SDF2L1	0
SEMA3B	99
SRSF9	0
SPAST	0
SPOP	0
STK11	0
TXNRD1	0
ZNF22	97

High promoter methylation frequency is defined as ≥40%. N/A indicates the gene was not present on the HumanMethylation27 BeadChip used for the analysis.

Supplementary Table S8. List of catalog numbers for shRNAs obtained from Open Biosystems/Thermo Scientific.

Gene	Catalog number for 1st shRNA	Catalog number for 2nd shRNA
Angpt1 (mouse)	RMM1766-96738501	RMM1766-96739079
ANGPT1 (human)	RHS4430-101135646	
Cdk5r1 (mouse)	RMM1766-96739750	RMM1766-96738443
CDK5R1 (human)	RHS4430-101164622	
Cr1l (mouse)	RMM1766-97042824	RMM3981-98063631
Dapp1 (mouse)	RMM1766-96738885	RMM1766-96880910
DAPP1 (human)	RHS4430-101165994	
Ddx52 (mouse)	RHS1764-9687389	RMM1766-96889298
DDX52 (human)	RHS4430-100988582	
Dnajc12 (mouse)	RMM1766-9106520	RMM1766-96743200
Flna (mouse)	RMM1766-96741212	RMM1766-9352033
FLNA (human)	RHS4430-101098236	
Fpr3 (mouse)	RMM1766-96746297	RMM1766-98468326
FPR3 (human)	RHS4430-101064277	
FRS2 (human)	RHS3979-9628906	RHS3979-9628905
Gapvd1 (mouse)	RMM1766-9336338	RMM1766-9336322
GAPVD1 (human)	RHS4430-100991657	
Gzma (mouse)	RMM1766-96745254	RMM1766-9106902
Igf2r (mouse)	RMM1766-97044218	RMM1766-98467508
IGF2R (human)	RHS4430-101064905	
Map1a (mouse)	RMM1766-9341607	RMM1766-96874116
MAP1A (human)	RHS4430-101028258	
Myd88 (mouse)	RMM1766-97042719	RMM3981-97065530
MYD88 (human)	RHS4430-101135026	
Naa38 (mouse)	RMM1766-97044512	RMM1766-96881489
NAA38 (human)	RHS4430-100991439	
Nme4 (mouse)	RMM1766-96740301	RMM1766-96740215
NME4 (human)	RHS4430-101163431	
Nup205 (mouse)	RMM1766-96873936	RMM1766-9355153
Orc1 (mouse)	RMM1766-96744068	RMM1766-96744205
Pigh (mouse)	RMM1766-96891459	RMM1766-96737813
PIGH (human)	RHS4430-101162323	
Pkd1l3 (mouse)	RMM1766-9353837	RMM1766-9354529
PKD1L3 (human)	RHS3979-99217795	
Prl7a2 (mouse)	RMM1766-96744814	RMM3981-98069754
Ptgis (mouse)	RMM1766-97042638	RMM1766-96738598
PTGIS (human)	RHS4430-101129208	
Ptpn4 (mouse)	RMM1766-96740179	RMM1766-96740341
PTPN4 (human)	RHS4430-101131499	
Sdf2l1 (mouse)	RMM1766-96743359	RMM3981-99012961
SDF2L1 (human)	RHS4430-100988030	
Sema3b (mouse)	RMM1766-97042710	RMM3981-9621999
SEMA3B (human)	RHS4430-101135304	
Srsf9 (mouse)	RMM1766-9106052	RMM3981-97059906
SRSF9 (human)	RHS4430-101103063	RHS3979-9575538
SH3BP2 (human)	RHS4430-200261800	RHS4430-200263770
Slfn4 (mouse)	RMM1766-96878856	RMM1766-97043261
Spast (mouse)	RMM1766-96737853	RMM1766-96871825

SPAST (human)	RHS4430-101104106	
Spop (mouse)	RMM1766-97044276	RMM3981-98497970
SPOP (human)	RHS4430-101025486	
Stk11 (mouse)	RMM1766-96740219	RMM3981-9591552
STK11 (human)	RHS4430-101030527	
Txnrd1 (mouse)	RMM1766-97042622	RMM1766-97042953
TXNRD1 (human)	RHS4430-101127479	
Wap (mouse)	RMM1766-97043220	RMM1766-97044227
Zfp422 (mouse)	RMM1766-9336451	RMM1766-96739222
ZNF22 (human)	RHS4430-101133524	

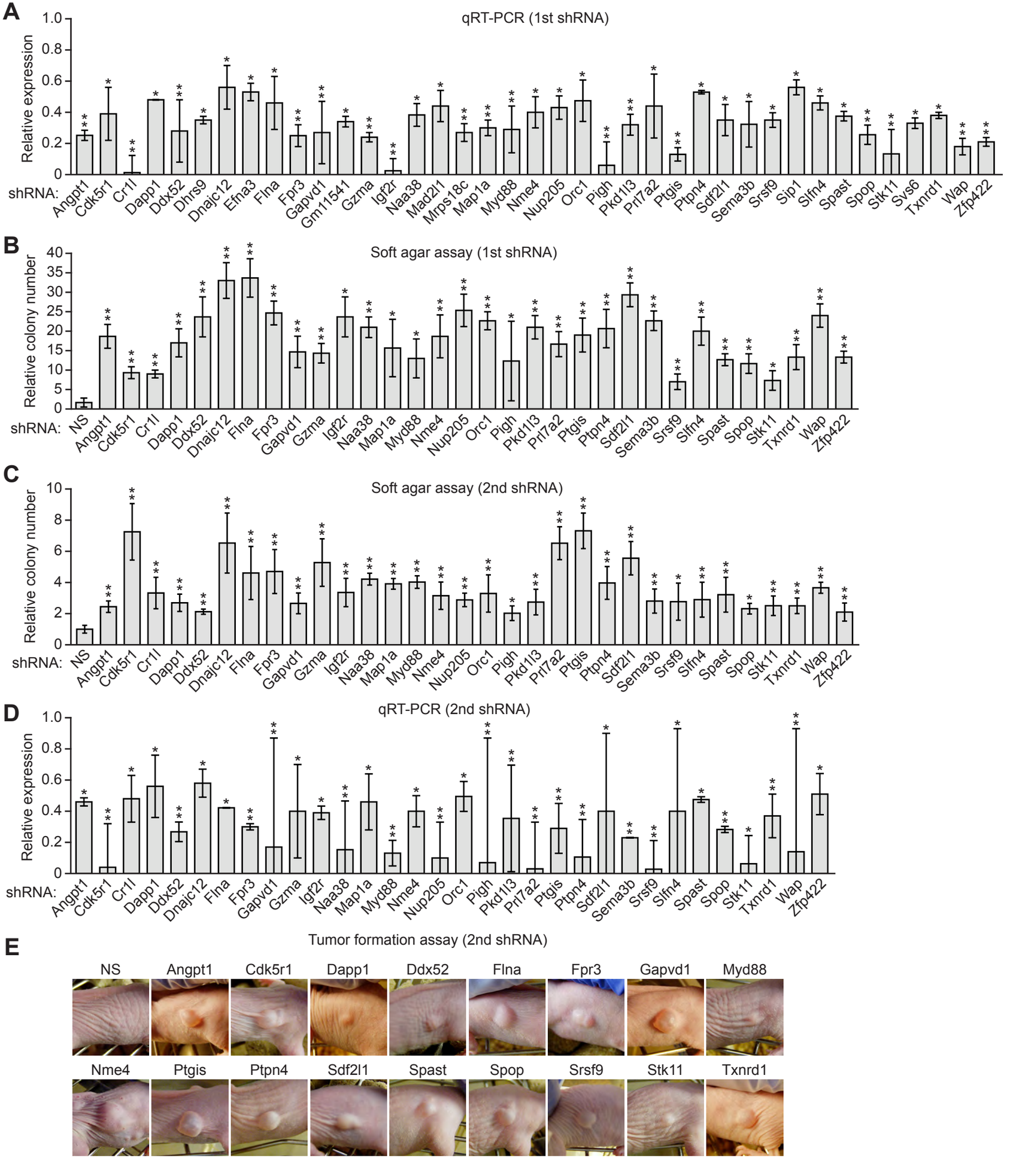
Supplementary Table S9. List of primers used for quantitative real-time RT-PCR and for cloning TSGs.

Gene	Forward primer (5' → 3')	Reverse primer (5' → 3')
qRT-PCR		
Angpt1 (mouse)	GTGCCGATTTCAGCACGAAG	CCATGATTTTGTCCCGCAGT
ANGPT1 (human)	GGACAGCAGGAAAACAGAGC	GGGCACATTTGCACATACAG
Cdk5r1 (mouse)	TCATGAGCTCCAAGATGCTG	CCTGACCGCTCTCATTCTTC
CDK5R1 (human)	TGCTGACATGCCTGTACCTC	TTGATGACAGAGAGGCAACG
Cr1l (mouse)	TGTCCGCCTTCAGTCTCTGC	TGTTCTCATTTCACGTTGCTGCT
Dapp1 (mouse)	TGTGCAAAGACGGGAGTTGAA	TCGGACGGTATCTTCTCCTTGA
DAPP1 (human)	CTCTGTGCAAAGACCGGAGT	CCCTTGGTTGAGCTGTTTTC
Ddx52 (mouse)	CACAGTCCACAGCTTCAGAGCA	TCCCCTATTCCCTGCTCTTCC
DDX52 (human)	TGCTAGCAAGAGGGATTGATT	CCCTTATTCCCTGCTCTTCC
Dnajc12 (mouse)	TTCCCAGAGGAGGGCATCTC	GTTCTGAGGGAGCGTCACCA
DNAJC12 (human)	TCTGGAATGTCACCCAGACA	TCCTTTGCCTTCTGCAGTTT
Flna (mouse)	AAGGCCTGGGGCTAAGCAAG	GCCCATGTGTTTCACCAGGA
FLNA (human)	CCAGAAGAGCAGCTTCACAG	ACGTGCTTCACCAGGATCTC
Fpr3 (mouse)	TCTGTGTCCCCTGAATCTGGA	TCCCAGCACACCAAGGAAGA
FPR3 (human)	GGGACTCTGGATTTTCACCA	GTCACCCCAGAATGCAAAGT
FRS2 (human)	CCCGATATCCCTCATTTGGA	TTTTCCGCTCTTCTTGCACA
Gapvd1 (mouse)	TGGCCAACGAGGACTCTGTC	CTCCACGGCTGCTGTGAACT
GAPVD1 (human)	GCGGATGACTTTGTTCCTGT	TGTGAACTGCATCCACCAAT
Gzma (mouse)	GTTGACTGCTGCCCACTGTA	TGGTTCCTGGTTTCACATCA
GZMA (human)	CCTCCGAGGTGGAAGAGACT	TTTCAAGGCCAAAGGAAGTG
Igf2r (mouse)	AGAAGAAGCTCGGGCGTGTC	CTTGCCCGTCCTTGCCTAGT
IGF2R (human)	CCGACTGCCAGTACCTCTTC	GTTCTGACAGCCCCTTGTG
Map1a (mouse)	TGGAAATGACCCTGCCAATG	TGCTGCTGTTGCTCGTGTGT
MAP1A (human)	TCCAAAGGCCTAGTCAATGG	CGGAAGAAGTCAAGGTCAGC
Myd88 (mouse)	TCCCAGTATCCTGCGGTTCA	TCTGGCTCCGCATCAGTCTC
MYD88 (human)	GCACATGGGCACATACAGAC	TAGCTGTTCCTGGGAGCTGT
Naa38 (mouse)	CATGAGCGGGTGTTCAGCTC	AGGCTCTGCTCGGATGTTCC
NAA38 (human)	CAGCTCTTCACAGGGGGTAG	CTGCTCGAATATTCCCCAAA
Nme4 (mouse)	GGACAATCAGGGGCGACTTC	ACCACCATCTGCCCAGTTCA
NME4 (human)	GACTTCAGCGTCCACATCAG	CTGGAACCACAGCTGGATCT
Nup205 (mouse)	CGGAGAGTCGCTGCAAAAGA	TTCCTGGACAGCCTCAGCAG
NUP205 (human)	TTTATTCTTTGGCGCCATCT	GATTGGTTTCTGAGGCGAAG
Orc1 (mouse)	CCGTCGGTCAGGACTAGAGGA	ACTGGGCTCCACCAGAAGGA
ORC1 (human)	GTCGATCAGGACTGGAGGAA	CCATGGTCTCTGACATGGTG
Pigh (mouse)	TACTGAAGGAGCCGGGGAAG	GCTGTGGCTTTCTGGTGTGC
PIGH (human)	CAGTGGAACCACATGGGATA	TCTCCTGGCAGCTCCTGTAT
Pkd1l3 (mouse)	CAATCCTGGGCTCCCTTTTG	GGTGCAGGCGGATTCCTAAC
PKD1L3 (human)	TGCAGCATCTCTGACTACCG	GGACTGGGTCTAAAGCGAAA
Prl7a2 (mouse)	CAAAACTTGCAGAGCTTTTTGA	CGGTGTATTCCACATTTCCTG
Ptgis (mouse)	AACCAGTGCCTGGGGAAGAG	CTGGCTGCATCAGACCGAAG
PTGIS (human)	ACTGCCTGGGGAGGAGTTAT	GATCTCCACATCTGCGTTGA
Ptpn4 (mouse)	CCTGGCCTGACCATGGAGTA	CAATGAGACACATGGCAGTTTCC
PTPN4 (human)	GAGCCATGATGATCCAAACA	CAAAGCCTTCTTCATAAACTTTCA
Sdf2l1 (mouse)	AACCTGCACACGCACCACTT	TTGCCCAGAACATCGGACTG
SDF2L1 (human	GCACCTCTGTGTTCCTGTCA	TTCCACGTATTGTGCGTGTT
Sema3b (mouse)	GCATGTGCAGTGGACCTTCC	CAACCGCGACGCAAAGATAC
SEMA3B (human)	GGACCCAGGAAGGATAGAGG	GGCTGCGAAAGATGGTAAAG
Srsf9 (mouse)	CGGAATGGGGATGGTTGAAT	CAGACCGCGACCGTGAGTAG
SRSF9 (human)	ATATGCCCTGCGTAAACTGG	AGCTGGTGCTTCTCTCAGGA

SRSF9 (human)	TGTGGAGTTCCCCAGGACTT	CAGCTTCTCGCATGTGATCC
SH3BP2 exon 10	CACTGCCCAACTCGGTCT	CTTTCCACTTCGCAGGACTC
SH3BP2 exons 7-8	ACGACGATGAGGATGACTCC	ACTGGGGGTGGTGGGTAAG
Slfn4 (mouse)	GAATGGGTGAAGCGCCAGAT	TCTTGCAAGCCCAGATCCAA
Spast (mouse)	TGCAATCTGCTGGAGATGACAG	TTTGGTAAGGACACATATACCCGTTT
SPAST (human)	CACTGGGTCCTATCCGAGAA	GCTGACGCTGCGTTTTATTT
Spop (mouse)	CTGACCTCCACAGCGCAGAT	GGAAAGGGCACTGTGCTGAA
SPOP (human)	GCCCTCTGCAGTAACCTGTC	TTGACTTCCACCCAGAGGTC
Stk11 (mouse)	GCAGCAAGGTGAAGCCAGAA	CCAACGTCCCGAAGTGAGTG
STK11 (human)	CATGACTGTGGTGCCGTACT	TGTGACTGGCCTCCTCTTCT
Txnrd1 (mouse)	AGCAGCTGGACAGCACCATC	TCTTGGCAACAGCATCCACA
TXNRD1 (human)	AGTAGGTCCACATGCACACG	TTTGATCAAGTTCAAGCACAAAA
Wap (mouse)	CCAGCGACCGTGAGTGTTCT	TCCAGGAGTGAAGGGTCTTGC
Zfp422 (mouse)	CAGCCAAGGAAAAGCCTATG	TTCTCGTCCACGTTCCTTCT
ZNF22 (human)	CTCTCATCTGAGGCAGCACA	GAGACCAGCCACAGACTTCC
Cloning		
ANGPT1	GGAAGATCTTCCATGACAGTTTTCCTTTCCTTTG	CCGCTCGAGCGGTCAAAAATCTAAAGGTCGAATC
CDK5R1	CCGCTCGAGCGGATGGGCACGGTGCTGTCCCT	CCGCTCGAGCGGTCACCGATCCAGGCCTAGGA
DAPP1	CCGCTCGAGCGGATGGGCAGAGCAGAACTTCTA	CCGCTCGAGCGGCTATTTAAAGATGAACGACCGAG
DDX52	GGAAGATCTATGGACTACAAAGACCATGACGGTGATTATAAAGATCATGACATCGATTACAAGGATGACGATGACAAGATGGACGTCCACGATCTCTT	CCGCTCGAGCGGTTAACTTTTGTCTTCAAGAGCTA
FPR3	GAAGATCTATGGACTACAAAGACCATGACGGTGATTATAAAGATCATGACATCGATTACAAGGATGACGATGACAAGATGGAAACCAACTTCTCCATT	CCGCTCGAGCGGTCACATTGCTTGTAACTCCGT
MYD88	GGAAGATCTTCCGAATTCCCGGGATATCATGC	GGAAGATCTTCCTCAGGGCAGGGACAAGGCC
PTGIS	GGAAGATCTTCCATGGCTTGGGCCGCGCTC	GGAAGATCTTCCTCATGGGCGGATGCGGTAG
SDF2L1	GGAAGATCTTCCATGTGGAGCGCGGGCCGC	CCGCTCGAGCGGTCAGAGTTCATCGTGACCTGC
SRSF9	CCGCTCGAGATGGACTACAAAGACCATGACGGTGATTATAAAGATCATGACATCGATTACAAGGATGACGATGACAAGATGTCGGGCTGGGCGGAC	CCGGAATTCTCAGTAGGGCCTGAAAGGAG
SPOP	GAAGATCTATGGACTACAAAGACCATGACGGTGATTATAAAGATCATGACATCGATTACAAGGATGACGATGACAAGATGTCAAGGGTTCCAAGTCC	CCGCTCGAGCGGTTAGGATTGCTTCAGGCGTTT
STK11	GGAAGATCTATGGACTACAAAGACCATGACGGTGATTATAAAGATCATGACATCGATTACAAGGATGACGATGACAAGATGGAGGTGGTGGACCCG	CCGCTCGAGCGGTCACTGCTGCTTGCAGGCC

Supplementary Table S10. List of the source and catalog numbers for human cDNAs used to clone TSGs.

Gene	Source	Catalog number
ANGPT1	Open Biosystems	EHS1001-99608061
CDK5R1	Open Biosystems	MHS1010-58341
DAPP1	Open Biosystems	MHS1010-7429735
DDX52	Open Biosystems	MHS1011-9199110
FPR3	Open Biosystems	MHS1010-98684799
MYD88	Open Biosystems	MHS1010-73828
PTGIS	Open Biosystems	MHS1010-98052790
PTPN4	Open Biosystems	MHS1010-73632
SDF2L1	Open Biosystems	MHS4426-99240278
SRSF9	Open Biosystems	MHS1010-98052451
SPAST	MGC collection	6441742
SPOP	Open Biosystems	MHS1010-57540
STK11	Open Biosystems	MHS1011-62254
TXNRD1	Open Biosystems	MHS1010-58057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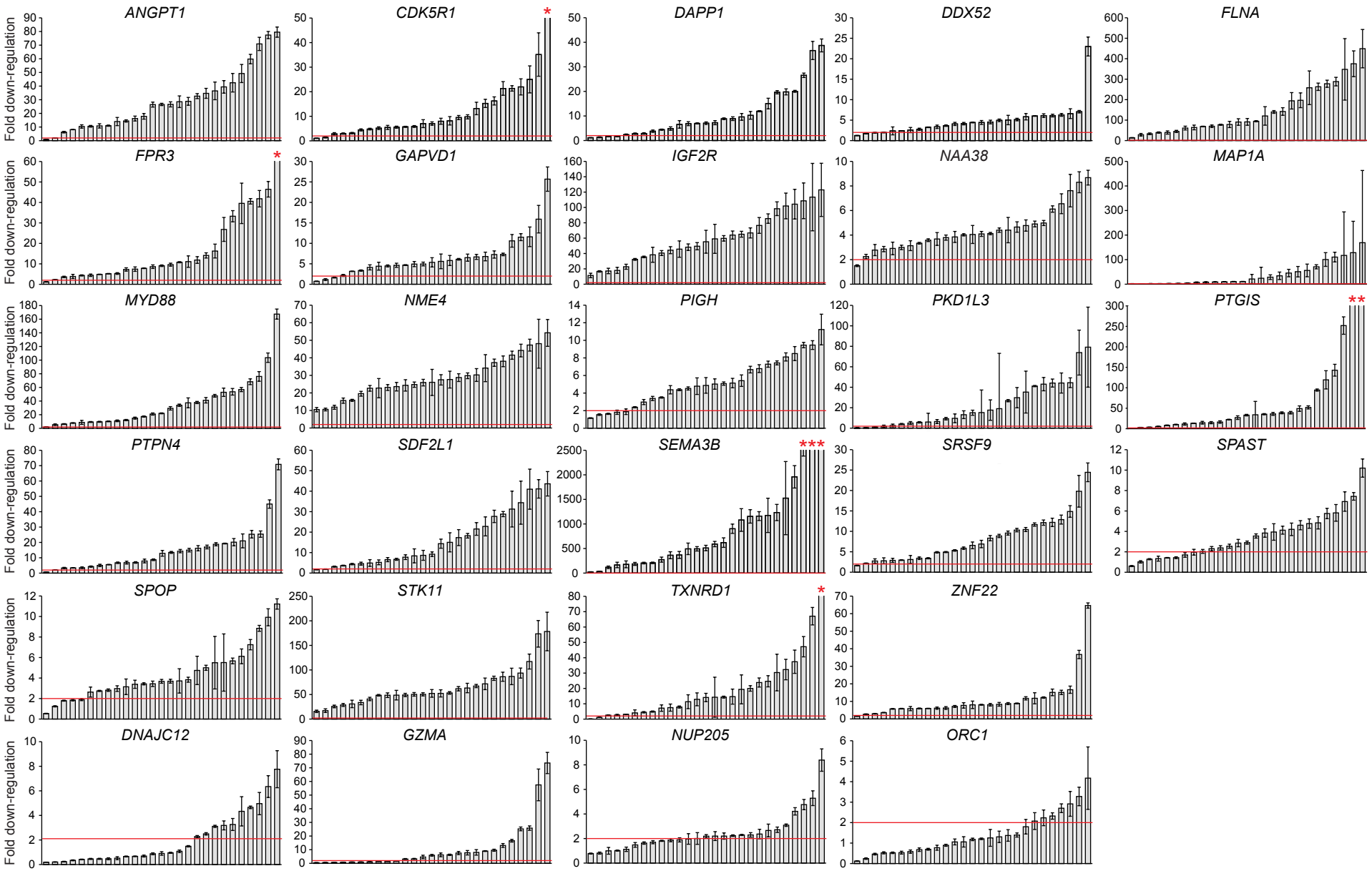
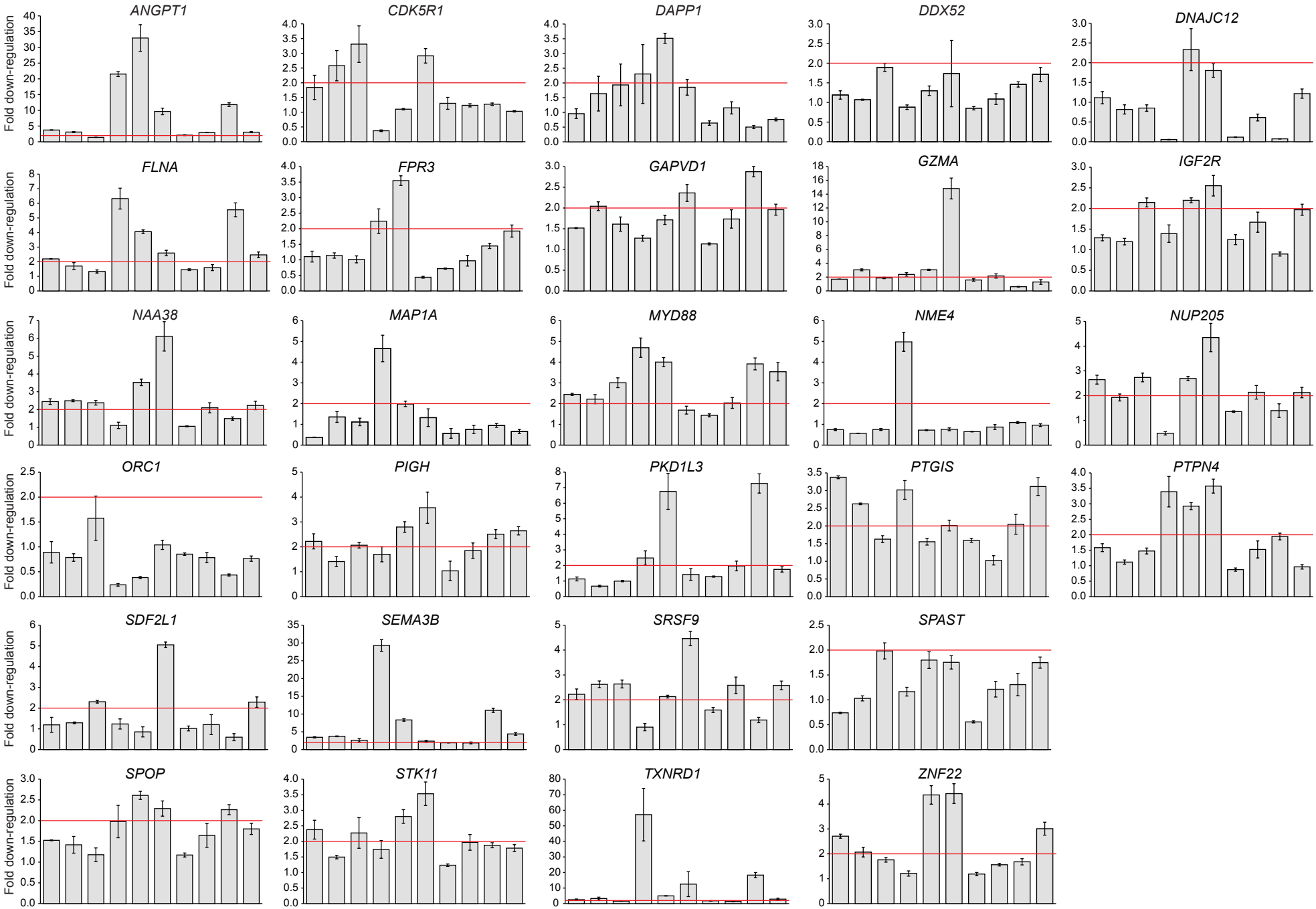
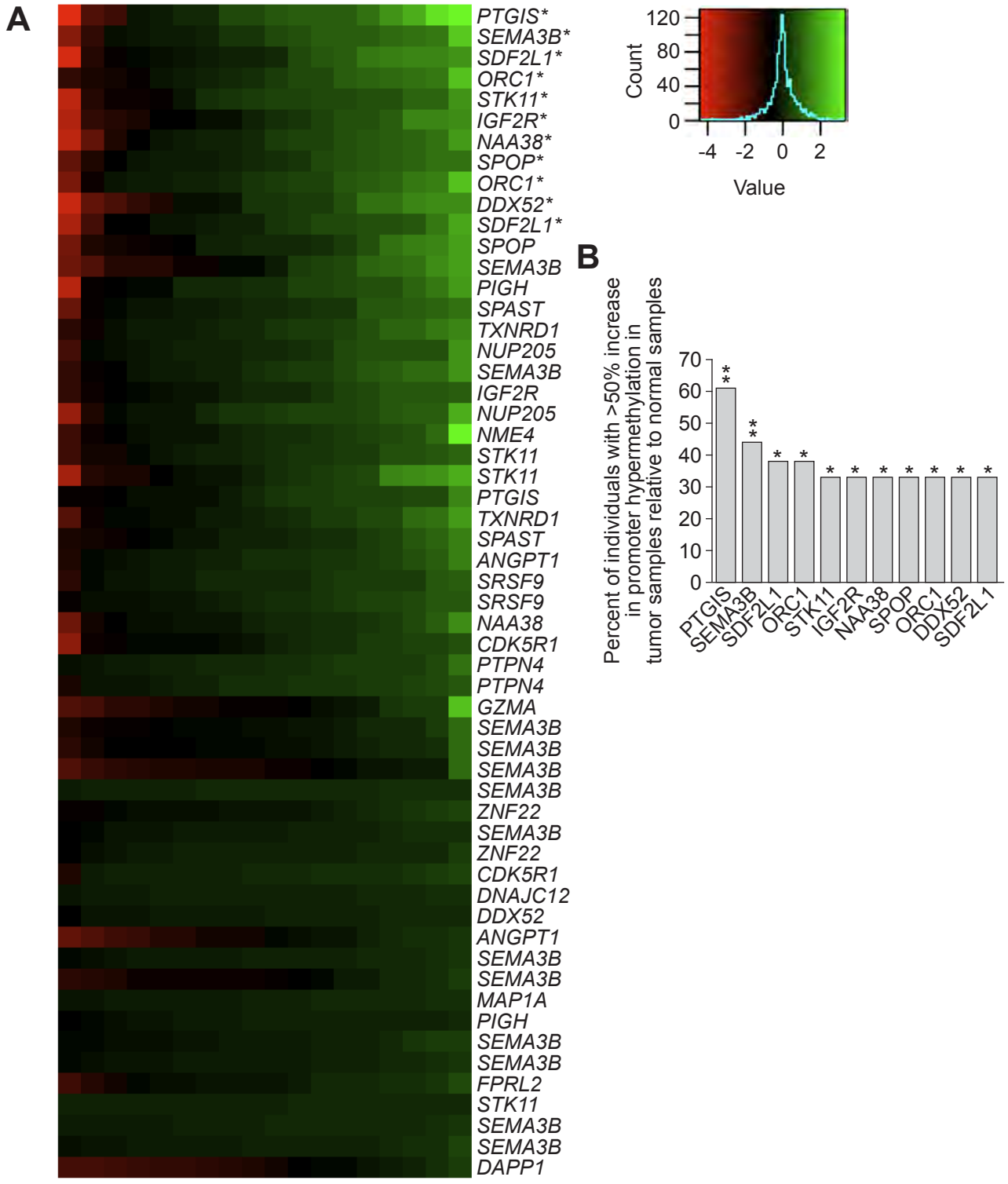
qRT-PCR (1st shRNA)
Relative expression
shRNA:
Angpt1 Cdk5r1 Cr1l Dapp1 Ddx52 Dhrs9 Dnajc12 Efna3 Flna Fpr3 Gapvd1 Gm11541 Gzma Igf2r Naa38 Mad2l1 Mrps18c Map1a Myd88 Nme4 Nup205 Orc1 Pigh Pkd1l3 Prl7a2 Ptgis Ptpn4 Sdf2l1 Sema3b Srsf9 Sip1 Slfn4 Spast Spop Stk11 Svs6 Txnrd1 Wap Zfp422
B
Soft agar assay (1st shRNA)
Relative colony number
shRNA:
NS Angpt1 Cdk5r1 Cr1l Dapp1 Ddx52 Dnajc12 Flna Fpr3 Gapvd1 Gzma Igf2r Naa38 Map1a Myd88 Nme4 Nup205 Orc1 Pigh Pkd1l3 Prl7a2 Ptgis Ptpn4 Sdf2l1 Sema3b Srsf9 Slfn4 Spast Spop Stk11 Txnrd1 Wap Zfp422
C
Soft agar assay (2nd shRNA)
Relative colony number
shRNA:
NS Angpt1 Cdk5r1 Cr1l Dapp1 Ddx52 Dnajc12 Flna Fpr3 Gapvd1 Gzma Igf2r Naa38 Map1a Myd88 Nme4 Nup205 Orc1 Pigh Pkd1l3 Prl7a2 Ptgis Ptpn4 Sdf2l1 Sema3b Srsf9 Slfn4 Spast Spop Stk11 Txnrd1 Wap Zfp422
D
qRT-PCR (2nd shRNA)
Relative expression
shRNA:
Angpt1 Cdk5r1 Cr1l Dapp1 Ddx52 Dnajc12 Flna Fpr3 Gapvd1 Gzma Igf2r Naa38 Map1a Myd88 Nme4 Nup205 Orc1 Pigh Pkd1l3 Prl7a2 Ptgis Ptpn4 Sdf2l1 Sema3b Srsf9 Slfn4 Spast Spop Stk11 Txnrd1 Wap Zfp422
E
Tumor formation assay (2nd shRNA)
NS Angpt1 Cdk5r1 Dapp1 Ddx52 Flna Fpr3 Gapvd1 Myd88
Nme4 Ptgis Ptpn4 Sdf2l1 Spast Spop Srsf9 Stk11 Txnrd1

Supplementary Fig. S2



Supplementary Fig. S3

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ORC1*
STK11*
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SPOP*
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SPOP
SEMA3B
PIGH
SPAST
TXNRD1
NUP205
SEMA3B
IGF2R
NUP205
NME4
STK11
STK11
PTGIS
TXNRD1
SPAST
ANGPT1
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SRSF9
NAA38
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SEMA3B
SEMA3B
SEMA3B
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DDX52
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SEMA3B
SEMA3B
MAP1A
PIGH
SEMA3B
SEMA3B
FPRL2
STK11
SEMA3B
SEMA3B
DAPP1
120
80
40
0
Count
-4
-2
0
2
Value
B
Percent of individuals with >50% increase in promoter hypermethylation in tumor samples relative to normal samples
70
60
50
40
30
20
10
0
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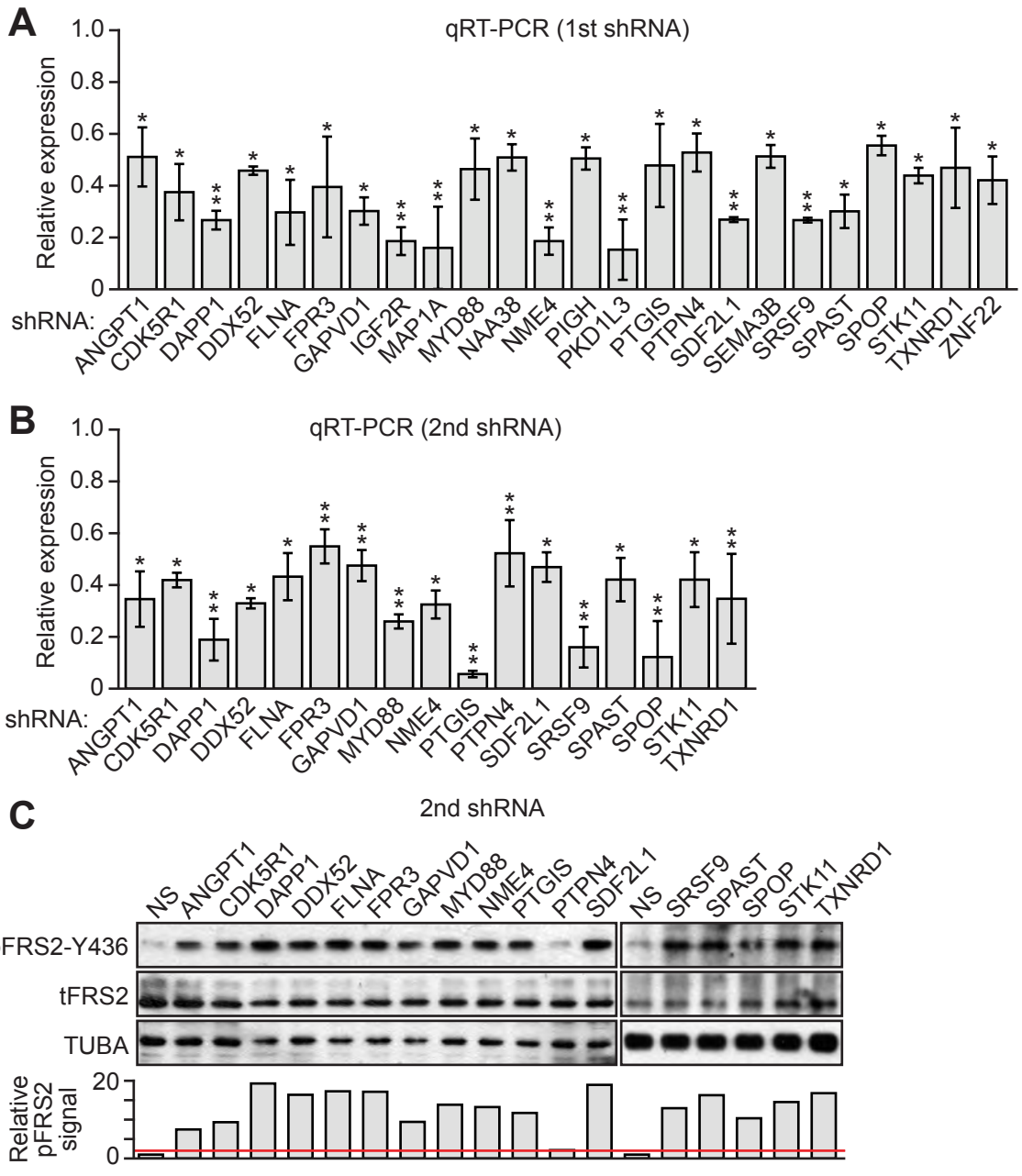
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Relative expression
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0.8
0.6
0.4
0.2
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shRNA:
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DAPP1
DDX52
FLNA
FPR3
GAPVD1
IGF2R
MAP1A
MYD88
NAA38
NME4
PIGH
PKD1L3
PTGIS
PTPN4
SDF2L1
SEMA3B
SRSF9
SPAST
SPOP
STK11
TXNRD1
ZNF22
B
qRT-PCR (2nd shRNA)
Relative expression
1.0
0.8
0.6
0.4
0.2
0
shRNA:
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CDK5R1
DAPP1
DDX52
FLNA
FPR3
GAPVD1
MYD88
NME4
PTGIS
PTPN4
SDF2L1
SRSF9
SPAST
SPOP
STK11
TXNRD1
C
2nd shRNA
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DAPP1
DDX52
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FPR3
GAPVD1
MYD88
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PTGIS
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SDF2L1
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SPOP
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tFRS2
TUBA
Relative pFRS2 signal
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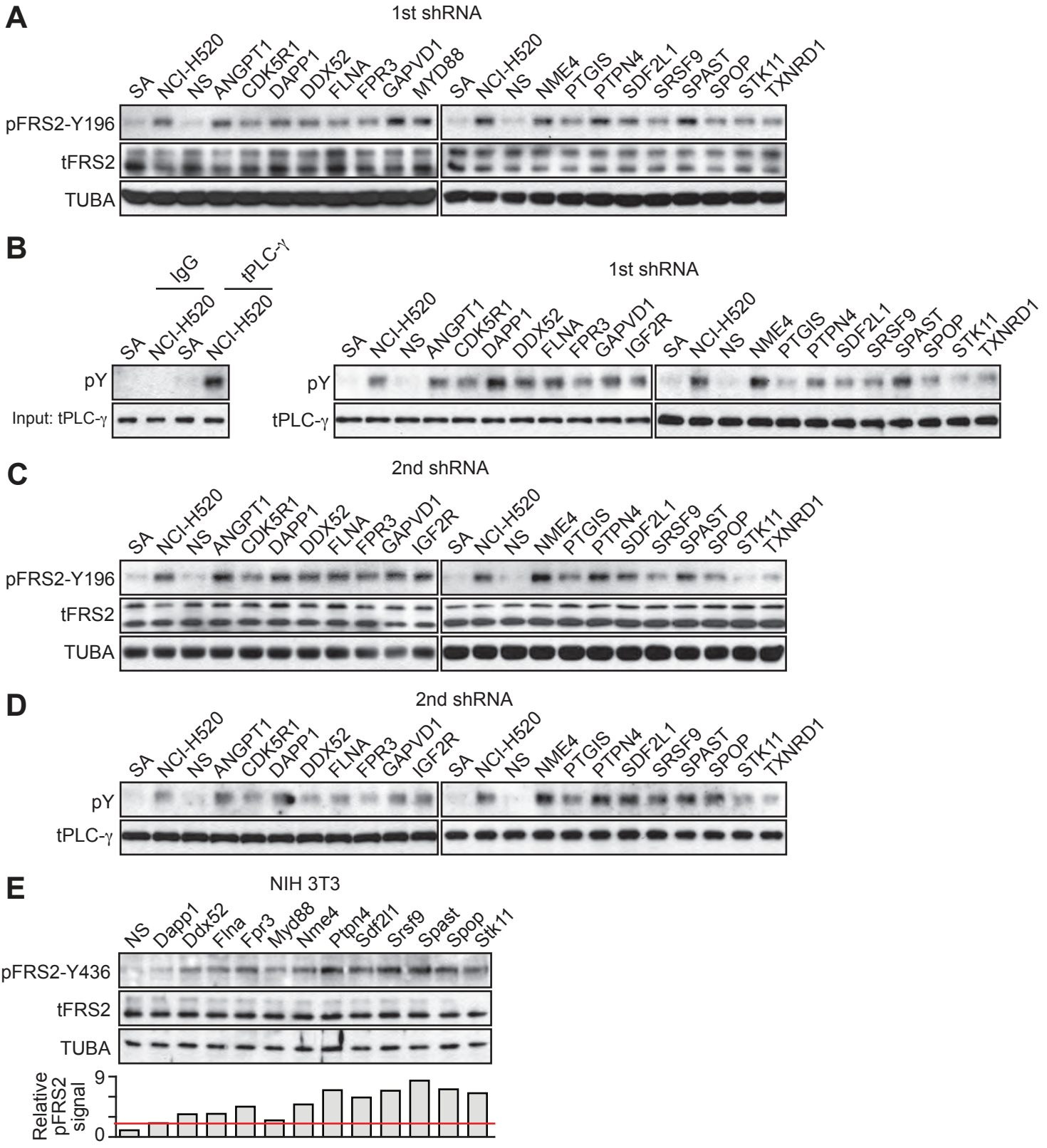
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PTGIS
PTPN4
SDF2L1
SRSF9
SPAST
SPOP
STK11
TXNRD1
pFRS2-Y196
tFRS2
TUBA
B
IgG
tPLC-γ
1st shRNA
pY
Input: tPLC-γ
tPLC-γ
C
2nd shRNA
IGF2R
D
2nd shRNA
E
NIH 3T3
NS
Dapp1
Ddx52
Flna
Fpr3
Myd88
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Spop
Stk11
pFRS2-Y436
Relative pFRS2 signal
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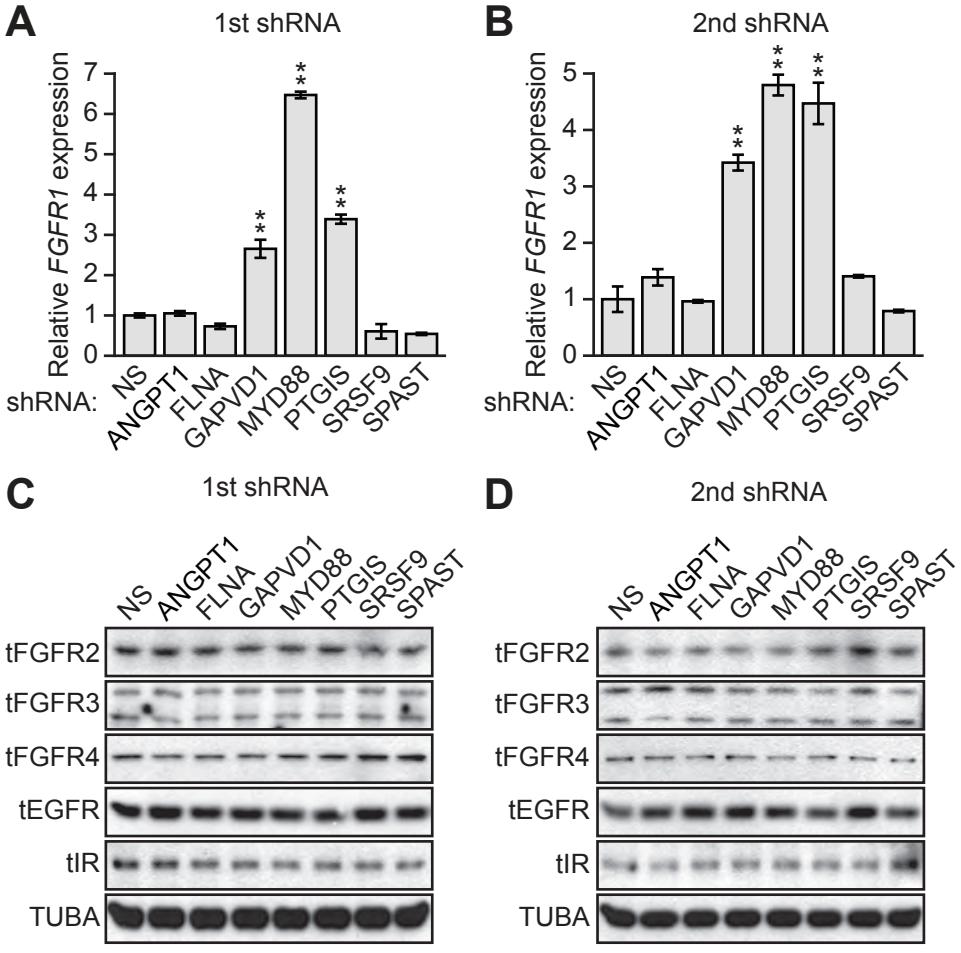
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3
4
5
6
7
**
**
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FLNA
GAPVD1
MYD88
PTGIS
SRSF9
SPAST
B
2nd shRNA
Relative FGFR1 expression
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1
2
3
4
5
**
**
**
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GAPVD1
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PTGIS
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C
1st shRNA
NS
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FLNA
GAPVD1
MYD88
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tFGFR3
tFGFR4
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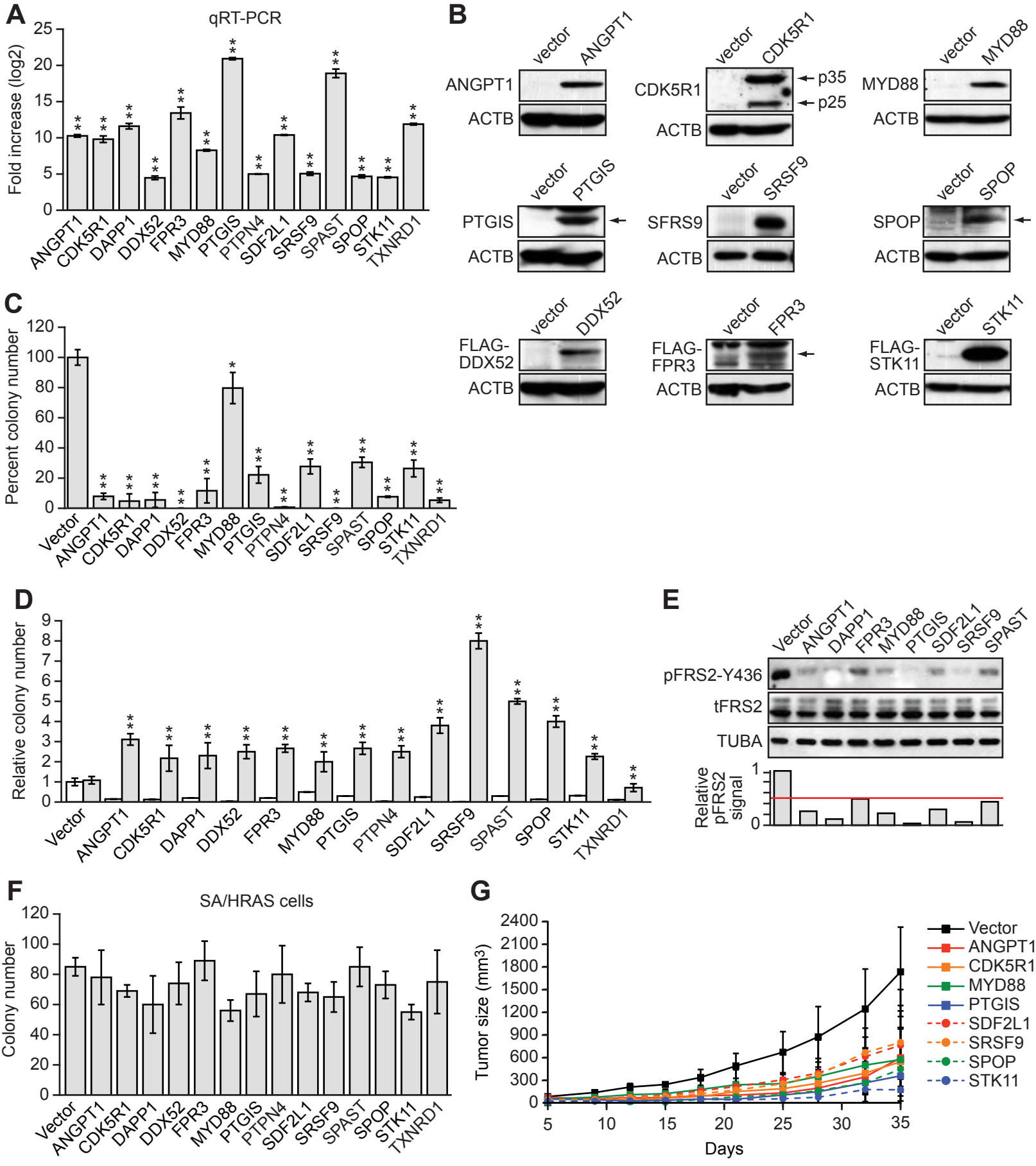
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SRSF9
SPAST
SPOP
STK11
TXNRD1
B
vector
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ACTB
CDK5R1
p35
p25
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PTGIS
SFRS9
SRSF9
SPOP
FLAG-DDX52
DDX52
FLAG-FPR3
FPR3
FLAG-STK11
STK11
C
Percent colony number
Vector
D
Relative colony number
E
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TUBA
Relative pFRS2 signal
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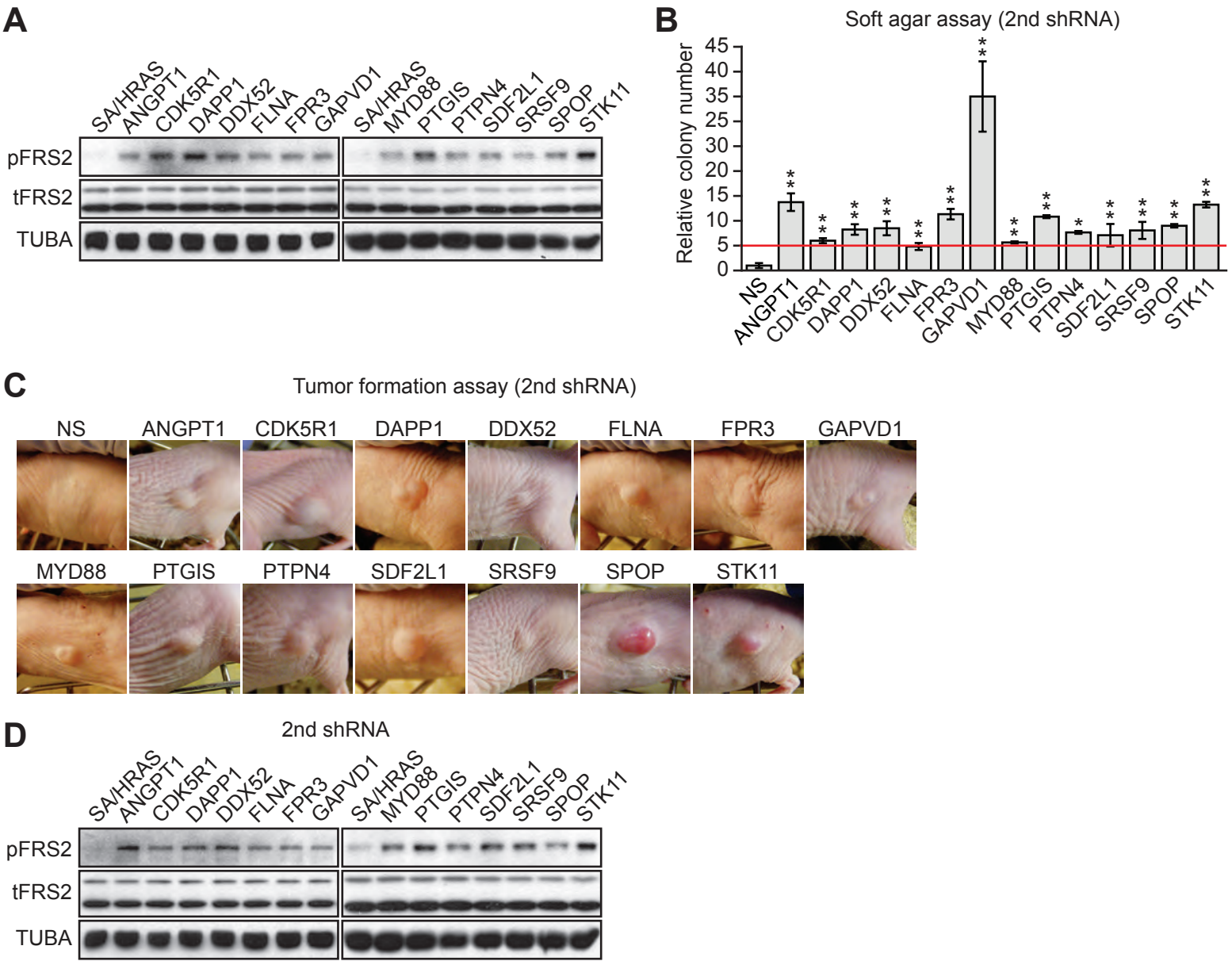
SA/HRAS cells
Colony number
G
Tumor size (mm3)
Days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SA/HRAS
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SDF2L1
SRSF9
SPOP
STK11
pFRS2
tFRS2
TUBA
B
Soft agar assay (2nd shRNA)
Relative colony number
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35
30
25
20
15
10
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ANGPT1
CDK5R1
DAPP1
DDX52
FLNA
FPR3
GAPVD1
MYD88
PTGIS
PTPN4
SDF2L1
SRSF9
SPOP
STK11
C
Tumor formation assay (2nd shRNA)
NS
ANGPT1
CDK5R1
DAPP1
DDX52
FLNA
FPR3
GAPVD1
MYD88
PTGIS
PTPN4
SDF2L1
SRSF9
SPOP
STK11
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FPR3
GAPVD1
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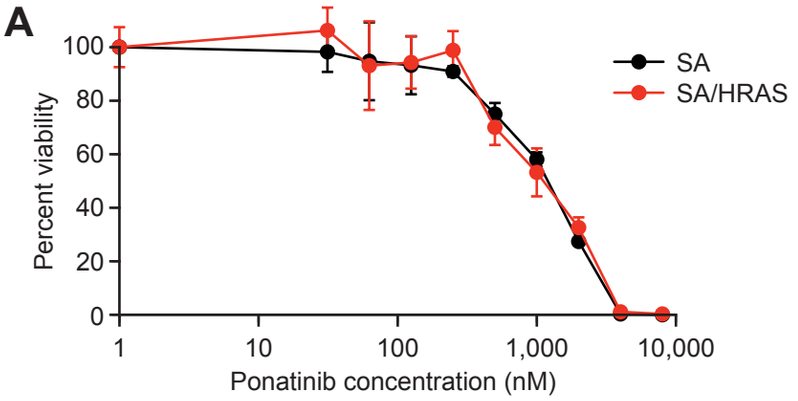
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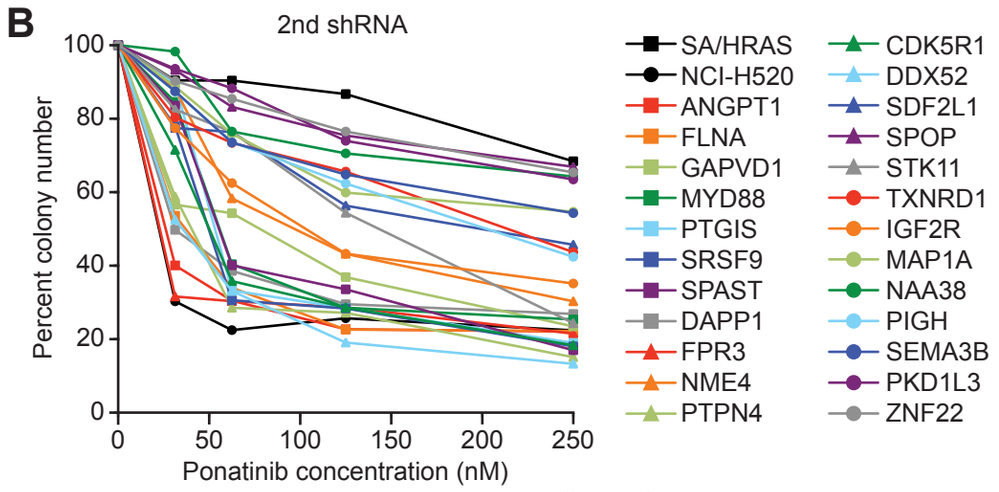
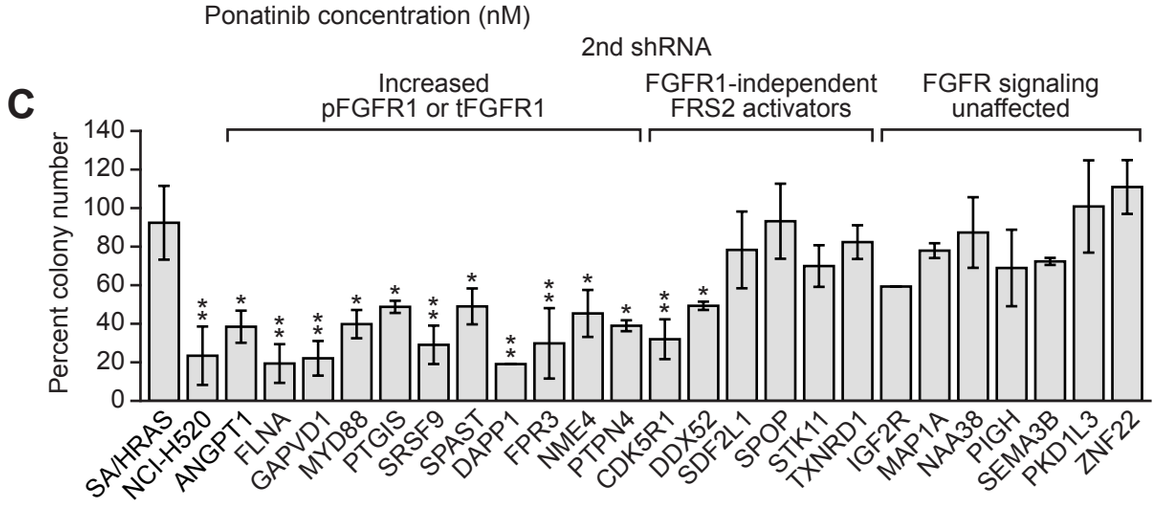
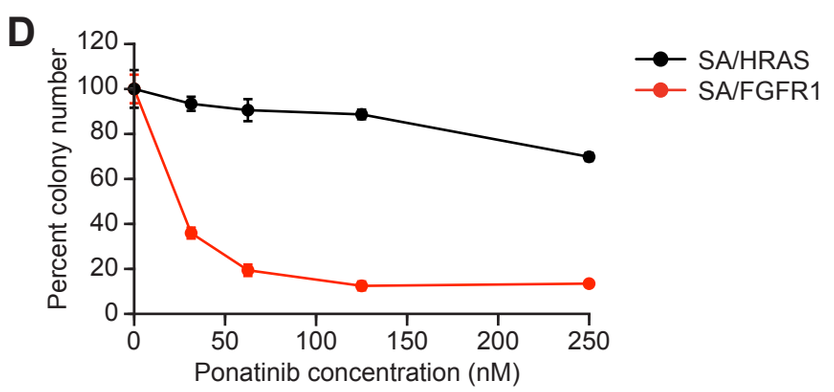
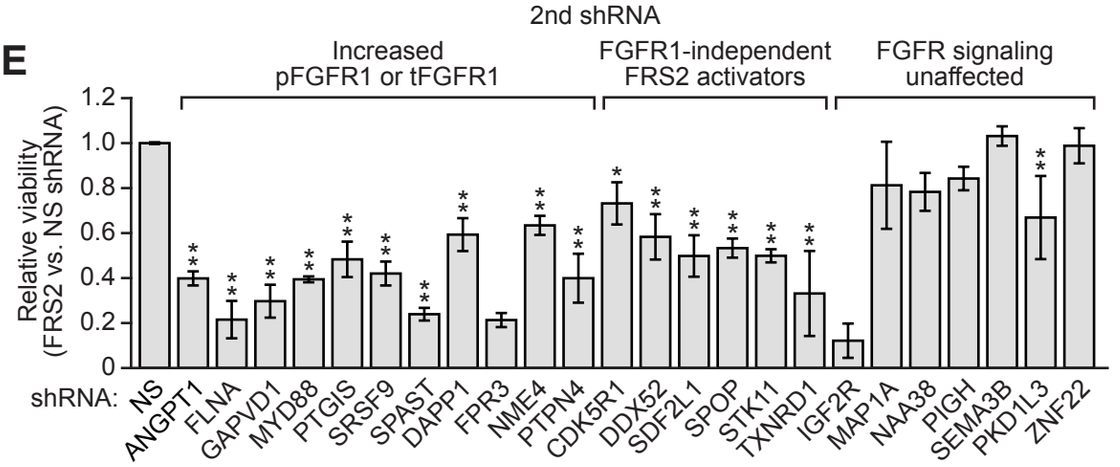
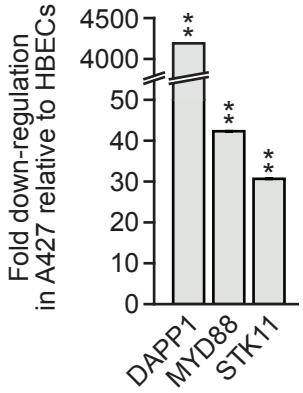
Percent viability
Ponatinib concentration (nM)
SA
SA/HRAS

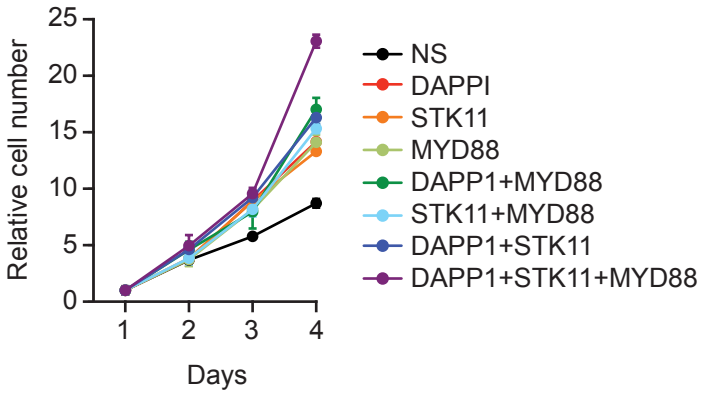

B
2nd shRNA
Percent colony number
Ponatinib concentration (nM)
SA/HRAS
NCI-H520
ANGPT1
FLNA
GAPVD1
MYD88
PTGIS
SRSF9
SPAST
DAPP1
FPR3
NME4
PTPN4
CDK5R1
DDX52
SDF2L1
SPOP
STK11
TXNRD1
IGF2R
MAP1A
NAA38
PIGH
SEMA3B
PKD1L3
ZNF22

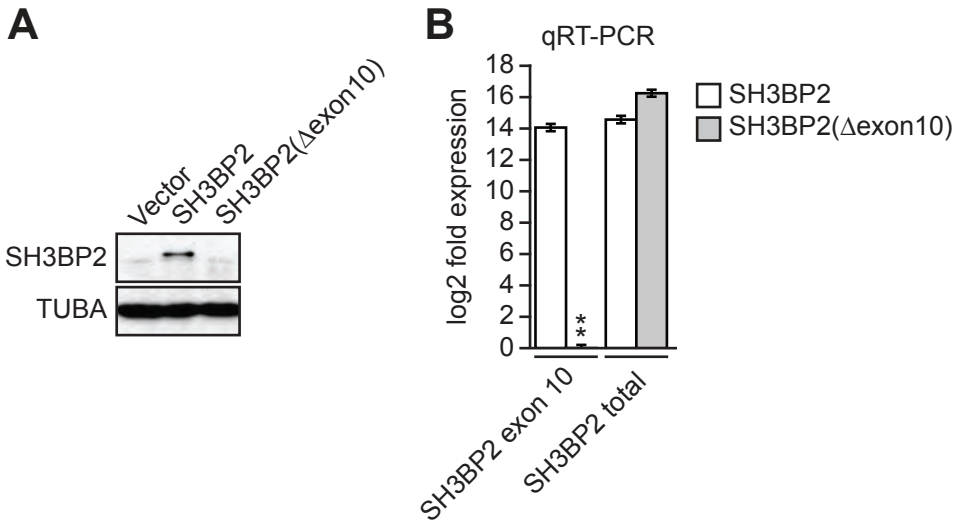

C
2nd shRNA
Increased pFGFR1 or tFGFR1
FGFR1-independent FRS2 activators
FGFR signaling unaffected
Percent colony number
SA/HRAS
NCI-H520
ANGPT1
FLNA
GAPVD1
MYD88
PTGIS
SRSF9
SPAST
DAPP1
FPR3
NME4
PTPN4
CDK5R1
DDX52
SDF2L1
SPOP
STK11
TXNRD1
IGF2R
MAP1A
NAA38
PIGH
SEMA3B
PKD1L3
ZNF22

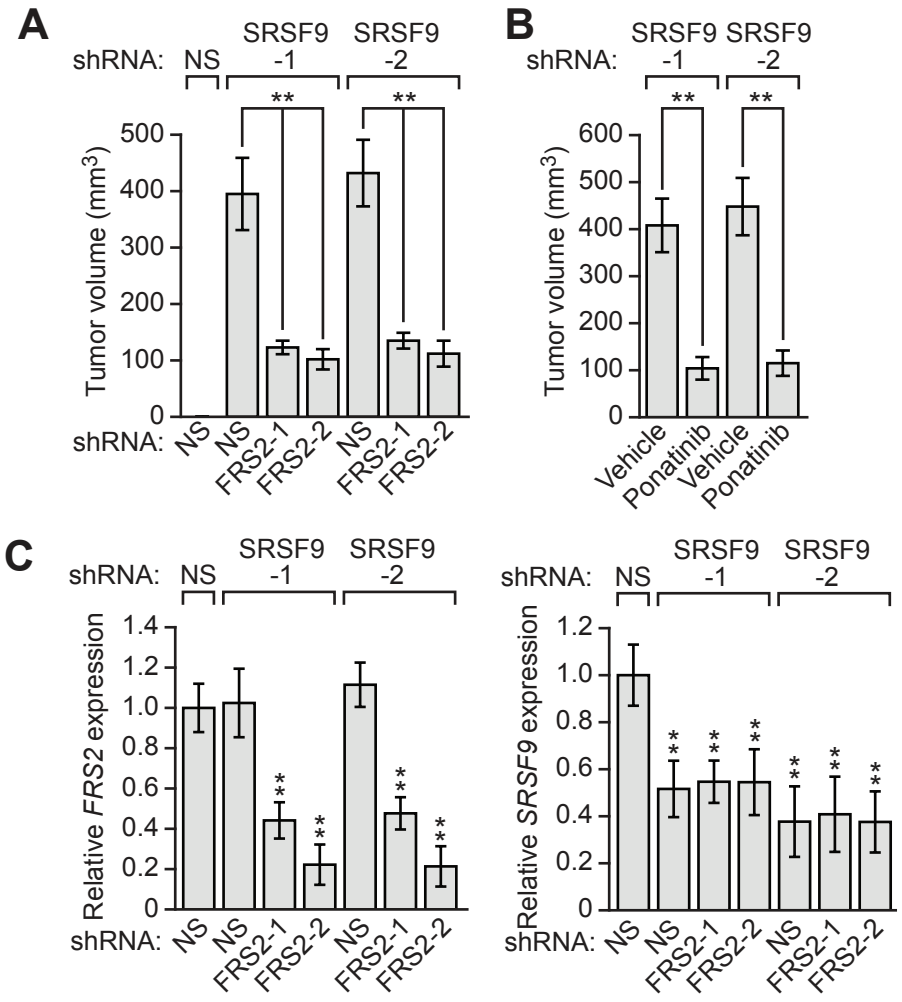

D
Percent colony number
Ponatinib concentration (nM)
SA/HRAS
SA/FGFR1


E
2nd shRNA
Increased pFGFR1 or tFGFR1
FGFR1-independent FRS2 activators
FGFR signaling unaffected
Relative viability (FRS2 vs. NS shRNA)
shRNA:
NS
ANGPT1
FLNA
GAPVD1
MYD88
PTGIS
SRSF9
SPAST
DAPP1
FPR3
NME4
PTPN4
CDK5R1
DDX52
SDF2L1
SPOP
STK11
TXNRD1
IGF2R
MAP1A
NAA38
PIGH
SEMA3B
PKD1L3
ZNF22


Fold down-regulation
in A427 relative to HBECs
4500
4000
50
40
30
20
10
0
**
**
**
DAPP1
MYD88
STK11


Relative cell number
25
20
15
10
5
0
1
2
3
4
Days
NS
DAPPI
STK11
MYD88
DAPP1+MYD88
STK11+MYD88
DAPP1+STK11
DAPP1+STK11+MYD88


A
Vector
SH3BP2
SH3BP2(Δexon10)
SH3BP2
TUBA
B
qRT-PCR
log2 fold expression
18
16
14
12
10
8
6
4
2
0
**
SH3BP2
SH3BP2(Δexon10)
SH3BP2 exon 10
SH3BP2 total


A
shRNA: NS
SRSF9 -1
SRSF9 -2
**
**
Tumor volume (mm3)
500
400
300
200
100
0
shRNA: NS NS FRS2-1 FRS2-2 NS FRS2-1 FRS2-2
B
shRNA:
SRSF9 -1
SRSF9 -2
**
**
Tumor volume (mm3)
600
500
400
300
200
100
0
Vehicle
Ponatinib
Vehicle
Ponatinib
C
shRNA: NS
SRSF9 -1
SRSF9 -2
Relative FRS2 expression
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0
**
**
**
shRNA: NS NS FRS2-1 FRS2-2 NS FRS2-1 FRS2-2
shRNA: NS
SRSF9 -1
SRSF9 -2
Relative SRSF9 expression
1.2
1.0
0.8
0.6
0.4
0.2
0
**
**
**
**
**
**
shRNA: NS NS FRS2-1 FRS2-2 NS FRS2-1 FRS2-2