Supplemental Method

Srsf2^{P95H/+} CRISPR knockout pooled library screen

At the start of the screen, 130x10⁶ Cas9 expressing cells from each cell line (n=3 independently derived cell lines per genotype) were infected with the Brie CRISPR knockout pooled library (Virus aliquots provided by the Victorian Centre for Functional Genomics; Addgene #73633)³⁹. The number of cells infected was calculated by multiplying the number of guides in the library (78,637) by the desired number of cells for each guide at the start of the experiment (aiming for 500 copies per sgRNA), divided by the multiplicity of infection required (MOI=0.3). For each cell line, the 130x10⁶ cells were separated into four T75 flasks (Nunc[™]) and, in each spin-infection flask, 35x10⁶ cells were mixed with 122µL of concentrated lentivirus containing the Brie library and 35µL of polybrene (8µg/mL, Sigma-Aldrich) in 35mL of IMDM-Cas9 media. The cells/virus mix was then spun at 1,100g for 90 minutes and the cells were re-incubated immediately after the spin-infection. Approximately 4 hours later, the mixture was transferred into two T-175 flasks (Falcon) with the addition of 65mL of IMDM-Cas9 media and cells were re-incubated. After 48 hours, cells were counted and diluted to 8x10⁵ cells/mL before adding puromycin (0.5µg/mL; Merck). After 4 days of puromycin selection, the puromycin and blasticidin were washed out and the cells were cultured in IMDM with 10%FBS, 1% GM-CSF and 400nM 4-hydroxy tamoxifen (Merck Millipore). This timepoint is labelled day 0 of the screen. The tamoxifen was removed from the cells after 4 days and cells were then passaged at 2x10⁵/mL every 2 days or 1x10⁵/mL for a 3-day interval for a total of 18 days. A minimum of 40x10⁶ cells was maintained during each passage. On day 0, day 4, day 11 and day 18 of the screen, pellets of 40x10⁶ cells were collected for gDNA isolation and library preparation.

Data availability

All datasets related to this work are deposited in GEO

(https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE165506).

Supplemental Table

Supp Table 1 Datasets used in comparison of *SRSF2/Srsf2* mis-spliced genes.Supp Table 2 Mapped read counts and coverage of 24 libraries in *Srsf2* CRISPR screen.

Supplemental Figures

Supp Figure 1 Generation and characterization of Hoxb8 *R26*Cre-ERT2^{ki/+} GM-CSF and Cas9-blasticidin resistant Hoxb8 *R26*Cre-ERT2^{ki/+} GM-CSF cell lines. (A) A schematic diagram illustrating the generation of Hoxb8 *R26*Cre-ERT2^{ki/+} GM-CSF and Cas9-blasticidin resistant Hoxb8 *R26*Cre-ERT2^{ki/+} GM-CSF cell lines. (B) FACS plot of Hoxb8 GM-CSF cells stained with antibodies against CD11b and Gr-1. (C) Cas9 Hoxb8 GM-CSF *Srsf2*^{+/+} and *Srsf2*^{P95H/+} cell lines have similar proliferation rates (n=3 per genotype). (D) Cas9 Hoxb8 GM-CSF *Srsf2*^{P95H/+} cells achieve full recombination after 4 days of 400nM tamoxifen treatment and remain stably recombined after tamoxifen withdrawal. Cas9 *Srsf2*^{P95H/+} cell lines are: #243, #81 and #83. Cas9 *Srsf2*^{+/+} cell lines are #281, #144 and #145.

Supp Figure 2 Heatmap of top differentially expressed genes with absolute log fold change (absLogFC) >2 and FDR < 0.05 between Cas9 $Srsf2^{+/+}$ and $Srsf2^{P95H/+}$ cell lines (n=3).

Supp Figure 3 The sample count distribution and gini index of *Srsf2* CRISPR screen. (**A**) Sample count distribution of sgRNA of all 24 libraries showing unbiased representation of sgRNA. (**B**) The Gini index of sgRNA in 24 libraries indicating fewer clones of the sgRNA pool remain as the screen progresses. **Supp Figure 4** The correlation between all six individual cell lines across all timepoints of the screen. The color code and number in each box indicate the concordance between the two samples. The color scale is shown at the top right. A red color and number close to 1 means the two samples are near identical. In contrast, a blue color and number close to 0 means the two samples are not correlated.

Supp Figure 5 Depletion of the CEG2 core essential genes at day 4 of the CRISPR screen. The top 10 depleted CEG2 essential genes are labelled. *Hoxb8* and *Csf2a* are highlighted in green and marked in blue dots. Grey dots: all the sgRNA identified; yellow dots: sgRNA targeting CEG2 essential genes.

Supp Figure 6 CDK6 is a druggable target of Srsf2^{P95H} cell lines. (**A**) The top 30 genes that are exclusively negatively selected in the *Srsf2^{P95H/+}* cell lines at day 18. Genes are ranked by MaGeCK score from the lowest (the most negatively enriched) to highest. FDR<0.05 for *Srsf2^{+/+}* cell lines, FDR<0.01 for *Srsf2^{P95H/+}* cell lines. (**B**) The guide count for CDK2 sgRNA, and CDK4 sgRNA (**C**) in *Srsf2^{+/+}* and *Srsf2^{P95H/+}* cells show no depletion over the course of the screen. (**D**) *Srsf2^{P95H/+}* cell lines are not sensitive to inhibition of mitochondrial OXPHOS targeting agents, Tigecycline and Mubritinib (n=2 independent lines per genotype).

Supplemental files:

Dataset S1. Gene level (a) and transcript level (b) expression of the GMCSF cellsDataset S2. Splicing GMCSF cells (a) rMATS (b) PSI-sigma

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Dataset S3. CRISPR results (a) Counts (b)

SRSF2_Mageck_MLE_Screen_Results_reps_combined (c)

Combined_gene_results_annotated_MaGeCK

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Dataset description	Dataset URL	Database, license, and accessibility	Reference			
		information				
Human datasets						
mRNA splicing data from primary CMML (n=13; 3 with <i>SRSF2</i> mutation) and AML (n=9; 5 with <i>SRSF2</i> mutation) patients*	Dataset retrieved from table S1 in the supplement	Available in the supplement information table S1	(Kim et al., 2015)			
mRNA profile of MDS-L cells	https://www.ncbi.nlm.nih.gov	Publicly available at Gene Expression Omnibus	(Komeno et al., 2015)			
(in triplicate) transfected with	/geo/guery/acc.cgi?acc=GSE61052	(GEQ): GSE61052				
Srsf2 P95H shRNA construct	. goo. que . j/ uee egi : uee - e e e e e e e e e e e e e e e e e	Mis-spliced gene list available in the supplement information table S4				
mRNA profile of K562	https://www.ncbi.nlm.nih.gov	Publicly available at Gene Expression Omnibus	(Zhang et al., 2015)			
CRISPR cell clones (with	/geo/query/acc.cgi?acc=GSE71299	(GEO):				
wild-type or mutant SRSF2,		GSE71299				
n=4 per genotype)		Mis-spliced gene list available in the supplement				
		information table S1				
mRNA profile of primary MDS	Dataset retrieved from table S7 in	Available in the supplement information tables	(Qiu et al., 2016)			
bone marrow samples	the supplement	S7				
(n=115)						
mRNA profile of primary	Dataset retrieved from table SX-	Available in the supplement information tables	(Hurtado et al., 2018)			
CMML (n=20; 7 with SRSF2	XIV in the supplement	SX-XIV				
mutation) patients						
Murine datasets						
mRNA profiles of murine	https://www.ncbi.nlm.nih.gov	Publicly available at Gene Expression Omnibus	(Kim et al., 2015)			
model (MP/LSK cells)	/geo/query/acc.cgi?acc=GSE65349	(GEO): GSE65349				
expressing Srst2 WI and		Mis-spliced gene list available in the supplement				
mutants^		Information table S1	(1/			
mRNA profile of murine	http://trace.ddbj.nig.ac.jp	Publicly available at DNA Data Bank of Japan	(Kon et al., 2018)			
model (MP/LSK cells)	/DRASearch/submission?acc=	repository: DRAU06224				
expressing Srst2 vv i or	DRA006224	Mis-splicing gene list available in supplemental				
\$I\$IZP95H/+ *						
mRNA profile of murine	https://www.ncbi.nlm.nih.gov	Publicly available at Gene Expression Omnibus	(Smeets et al., 2018)			
model (sorted LK+ eYFP+	/geo/query/acc.cgi?acc=GSE99852	(GEO):				
cells) expressing Srsf2 WT or		GSE99852				
Srsf2 _{P95H/+}		Mis-splicing gene list available in supplemental				
		dataset 2				
mRNA profile of Hoxb8	N/A	Available in this manuscript	Published with this			
immortalised GM-CSF cell			manuscript			
lines expressing Srsf2 WT or						
Srsf2p95H/+						

*These datasets contain mis-splicing data from two different cell/disase types

Sample_name	Mappability (%)	Total_reads	Mapped_reads	Average Coverage (Copy number /sgRNA)				
WT_D0_S144	85.72347	38583293	33074936	420.6				
WT_D4_S144	85.87031	34396145	29536075	375.6				
WT_D11_S144	84.44593	32585779	27517364	349.9				
WT_D18_S144	87.12968	36646854	31930287	406.0				
WT_D0_S145	86.08064	30683490	26412546	335.9				
WT_D4_S145	86.90123	34345631	29846775	379.6				
WT_D11_S145	84.85053	32027437	27175449	345.6				
WT_D18_S145	86.29772	30632262	26434944	336.2				
WT_D0_S281	85.63385	33078080	28326034	360.2				
WT_D4_S281	86.1783	32858200	28316637	360.1				
WT_D11_S281	85.12764	30345497	25832405	328.5				
WT_D18_S281	86.19909	37822483	32602635	414.6				
P95H_D0_S81	84.96886	26147819	22217505	282.5				
P95H_D4_S81	84.81972	34788027	29507107	375.2				
P95H_D11_S81	84.13214	32715519	27524267	350.0				
P95H_D18_S81	86.06658	32080958	27610984	351.1				
P95H_D0_S83	85.048	32903333	27983628	355.9				
P95H_D4_S83	85.34779	28279733	24136126	306.9				
P95H_D11_S83	84.42695	30106447	25417955	323.2				
P95H_D18_S83	85.38084	28122359	24011106	305.3				
P95H_D0_S243	85.01022	36751029	31242132	397.3				
P95H_D4_S243	85.58722	36307614	31074678	395.2				
P95H_D11_S243	85.15725	32322150	27524653	350.0				
P95H_D18_S243	86.3542	28848293	24911713	316.8				

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P95H

WT

A. Sample count distribution



Sample

	Srsf2+/+											Srsf2 ^{P95H/+}												1		
Т	-	1.00	0.62	0.61	0.81	0.49	0.50	0.40	0.42	0.38	0.09	0.29	0.15	0.68	0.69	0.68	0.56	0.55	0.56	0.42	0.39	0.41	0.09	0.21	0.25	D0_S144
+/+0		0.62	1.00	0.63	0.48	0.82	0.49	0.25	0.71	0.39	0.07	0.49	0.15	0.67	0.68	0.66	0.53	0.52	0.54	0.40	0.37	0.40	0.12	0.21	0.25	D0_S145
		0.61	0.63	1.00	0.48	0.49	0.79	0.25	0.41	0.66	0.07	0.28	0.29	0.67	0.68	0.67	0.53	0.53	0.54	0.39	0.37	0.40	0.09	0.20	0.25	D0_S281
		0.81	0.48	0.48	1.00	0.58	0.58	0.76	0.36	0.34	0.47	0.34	0.21	0.55	0.55	0.53	0.66	0.63	0.65	0.48	0.41	0.36	0.26	0.35	0.30	D4_S144
		0.49	0.82	0.49	0.58	1.00	0.58	0.52	0.79	0.34	0.42	0.66	0.22	0.53	0.55	0.52	0.62	0.60	0.62	0.46	0.39	0.35	0.29	0.35	0.29	D4_S145
Srsf.		0.50	0.49	0.79	0.58	0.58	1.00	0.52	0.36	0.77	0.42	0.33	0.47	0.54	0.55	0.54	0.63	0.61	0.64	0.46	0.39	0.36	0.27	0.34	0.30	D4_S281
0,		0.40	0.25	0.25	0.76	0.52	0.52	1.00	0.20	0.18	0.88	0.28	0.17	0.28	0.28	0.27	0.55	0.52	0.56	0.27	0.24	0.21	0.11	0.27	0.26	D11_S144
		0.42	0.71	0.41	0.36	0.79	0.36	0.20	1.00	0.28	0.07	0.88	0.12	0.45	0.47	0.45	0.41	0.40	0.41	0.33	0.30	0.31	0.11	0.19	0.21	D11_S145
		0.38	0.39	0.66	0.34	0.34	0.77	0.18	0.28	1.00		0.21	0.62	0.42	0.43	0.42	0.38	0.38	0.39	0.29	0.29	0.30	0.11	0.19	0.22	D11_S281
		0.09	0.07	0.07	0.47	0.42	0.42	0.88	0.07		1.00	0.21	0.14	0.08	0.08	0.07	0.41	0.39	0.43	0.12	0.11	0.06	0.07	0.21	0.18	D18_S144
		0.29	0.49	0.28	0.34	0.66	0.33	0.28	0.88	0.21	0.21	1.00	0.13	0.31	0.32	0.31	0.38	0.37	0.36	0.32	0.26	0.22	0.17	0.24	0.19	D18_S145
		0.15	0.15	0.29	0.21	0.22	0.47	0.17	0.12	0.62	0.14	0.13	1.00	0.16	0.16	0.16	0.23	0.22	0.23	0.17	0.14	0.11	0.13	0.14	0.12	D18_S281
+	-	0.68	0.67	0.67	0.55	0.53	0.54	0.28	0.45	0.42		0.31	0.16	1.00	0.76	0.74	0.81	0.61	0.62	0.58	0.43	0.45	0.13	0.24	0.27	D0_S81
		0.69	0.68	0.68	0.55	0.55	0.55	0.28	0.47	0.43		0.32	0.16	0.76	1.00	0.75	0.63	0.82	0.63	0.48	0.60	0.46	0.13	0.31	0.29	D0_S83
		0.68	0.66	0.67	0.53	0.52	0.54	0.27	0.45	0.42	0.07	0.31	0.16	0.74	0.75	1.00	0.60	0.59	0.83	0.44	0.42	0.66	0.11	0.22	0.41	D0_S243
		0.56	0.53	0.53	0.66	0.62	0.63	0.55	0.41	0.38	0.41	0.38	0.23	0.81	0.63	0.60	1.00	0.72	0.70	0.79	0.49	0.40	0.40	0.45	0.32	D4_S81
+/+		0.55	0.52	0.53	0.63	0.60	0.61	0.52	0.40	0.38	0.39	0.37	0.22	0.61	0.82	0.59	0.72	1.00	0.69	0.58	0.79	0.40	0.35	0.58	0.32	D4_S83
f2 ^{P9{}		0.56	0.54	0.54	0.65	0.62	0.64	0.56	0.41	0.39	0.43	0.36	0.23	0.62	0.63	0.83	0.70	0.69	1.00	0.52	0.44	0.74	0.28	0.37	0.56	D4_S243
Srs		0.42	0.40	0.39	0.48	0.46	0.46	0.27	0.33	0.29	0.12	0.32	0.17	0.58	0.48	0.44	0.79	0.58	0.52	1.00	0.50	0.29	0.66	0.56	0.23	D11_S81
		0.39	0.37	0.37	0.41	0.39	0.39	0.24	0.30	0.29	0.11	0.26	0.14	0.43	0.60	0.42	0.49	0.79	0.44	0.50	1.00	0.28	0.31	0.79	0.21	D11_S83
	ľ	0.41	0.40	0.40	0.36	0.35	0.36	0.21	0.31	0.30		0.22	0.11	0.45	0.46	0.66	0.40	0.40	0.74	0.29	0.28	1.00	0.06	0.15	0.85	D11_S243
		0.09	0.12	0.09	0.26	0.29	0.27	0.11	0.11	0.11	0.07	0.17	0.13	0.13	0.13	0.11	0.40	0.35	0.28	0.66	0.31		1.00	0.58	0.11	D18_S81
		0.21	0.21	0.20	0.35	0.35	0.34	0.27	0.19	0.19	0.21	0.24	0.14	0.24	0.31	0.22	0.45	0.58	0.37	0.56	0.79	0.15	0.58	1.00	0.17	D18_S83
		0.25	0.25	0.25	0.30	0.29	0.30	0.26	0.21	0.22	0.18	0.19	0.12	0.27	0.29	0.41	0.32	0.32	0.56	0.23	0.21	0.85	0.11	0.17	1.00	D18_S243
	_ "	D0_S144	D0_S145	D0_S281	D4_S144	D4_S145	D4_S281	D11_S144	D11_S145	D11_S281	D18_S144	D18_S145	D18_S281	D0_S81	D0_S83	D0_S243	D4_S81	D4_S83	D4_S243	D11_S81	D11_S83	D11_S243	D18_S81	D18_S83	D18_S243	1

0.8 0.6

1

0.4 0.2 Supp figure 5 Xu et al., 2021





D. Mubritinib 150 P95H/+ IC50=0.93uM +/+ % cell viability 100 IC50=1.14uM 50 ÷. 0**+** 1000 10000 é 100 Concentration (nM)

Tigecycline

