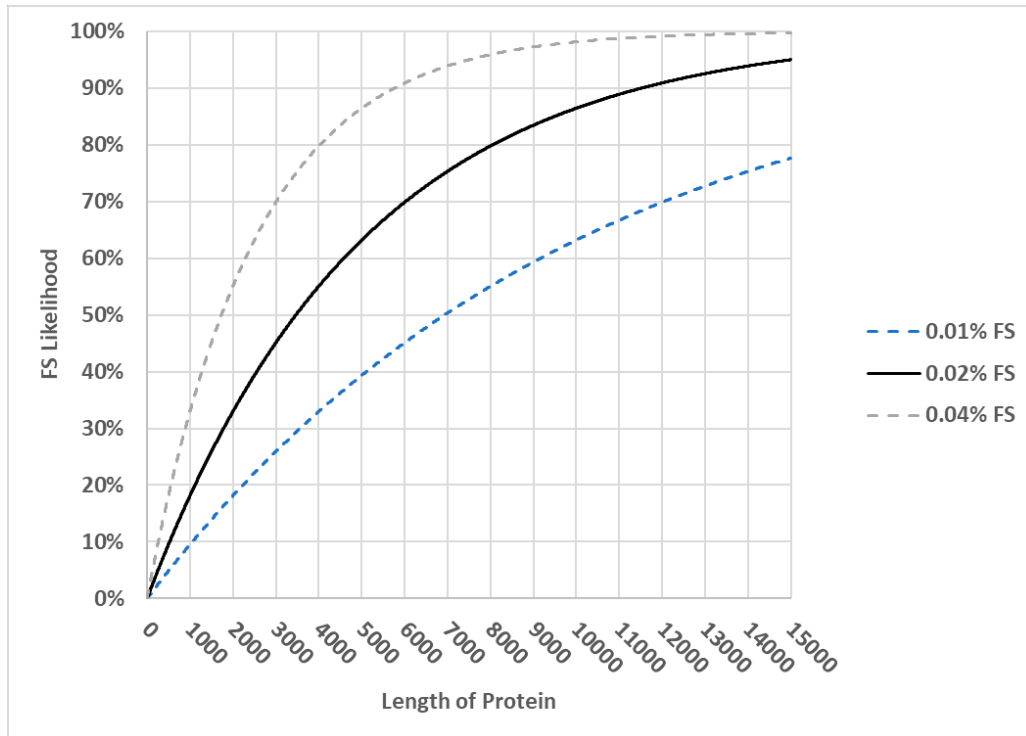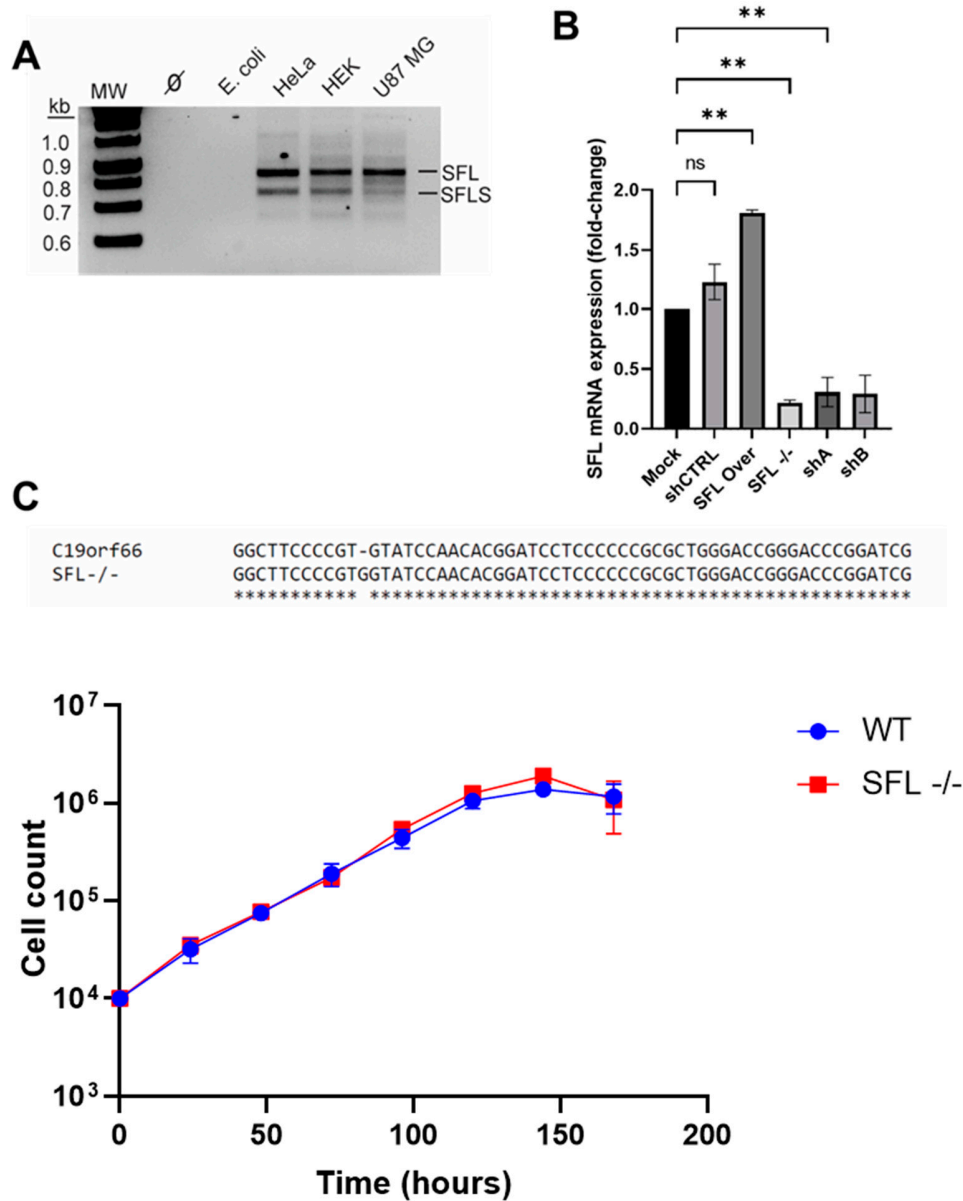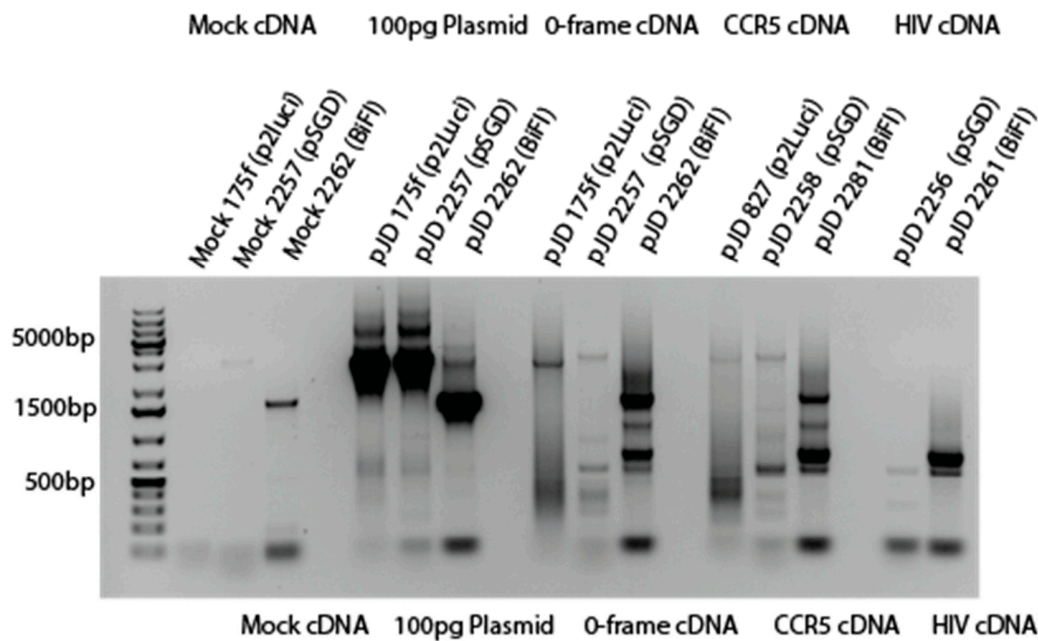**Supplemental Materials**

Supplemental Figure S1. Probability plot of the likelihood of a spontaneous ribosomal frameshift as a function of codon length. The plot was calculated with Excel using the following equation: P= 1-((1-rate of spontaneous frameshifting)^amino acid length).

A

kb
MW | Ø | E. coli | HeLa | HEK | U87 MG

1.0
0.9 — SFL
0.8 — SFLS
0.7
0.6

B

SFL mRNA expression (fold-change)

Mock | shCTRL | SFL Over | SFL -/- | shA | shB

C

```
C19orf66   GGCTTCCCCGT-GTATCCAACACGGATCCTCCCCCCGCGCTGGGACCGGGACCCGGATCG
SFL-/-     GGCTTCCCCGTGGTATCCAACACGGATCCTCCCCCCGCGCTGGGACCGGGACCCGGATCG
           **********  *************************************************
```
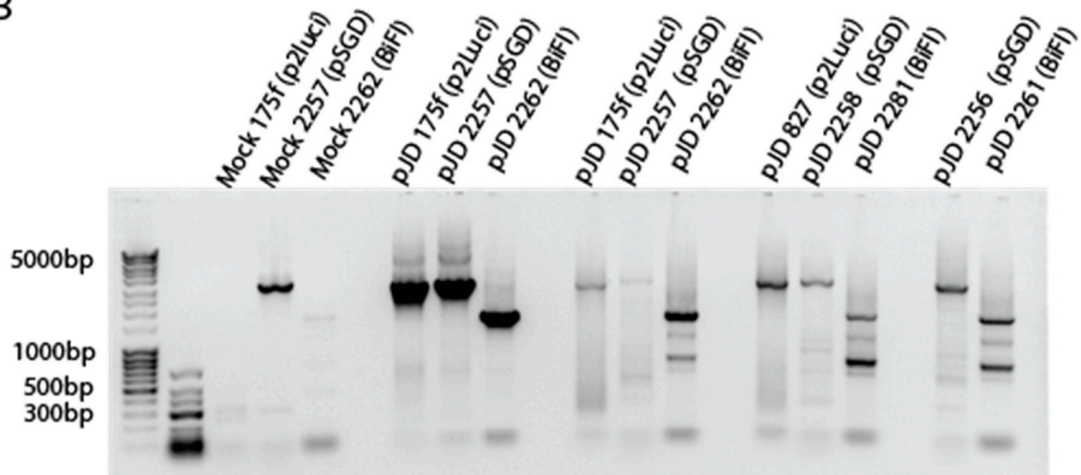
Supplemental Figure S2. Modifying SFL expression in the cell.

A. RT-PCR analysis of SFL in a blank sample (Ø), and RNA extracted from *E. coli*, HeLa, HEK293T and U87 MG cells. SFL denotes the full-length Shiftless mRNA while SFLS denotes a shorter Shiftless splice isoform. **B.** qRT-PCR analysis of SFL expression in HEK293T cells. **C.** Sequence validation of SFL$^{-/-}$ HEK293T cells. Yellow box indicates insertion of a G residue into the SFL coding sequence by CRISPR. **D.** SFL knockout does not alter cell growth. Growth curve of WT HEK293T cells (blue) and SFL-/- HEK293T cells (red).
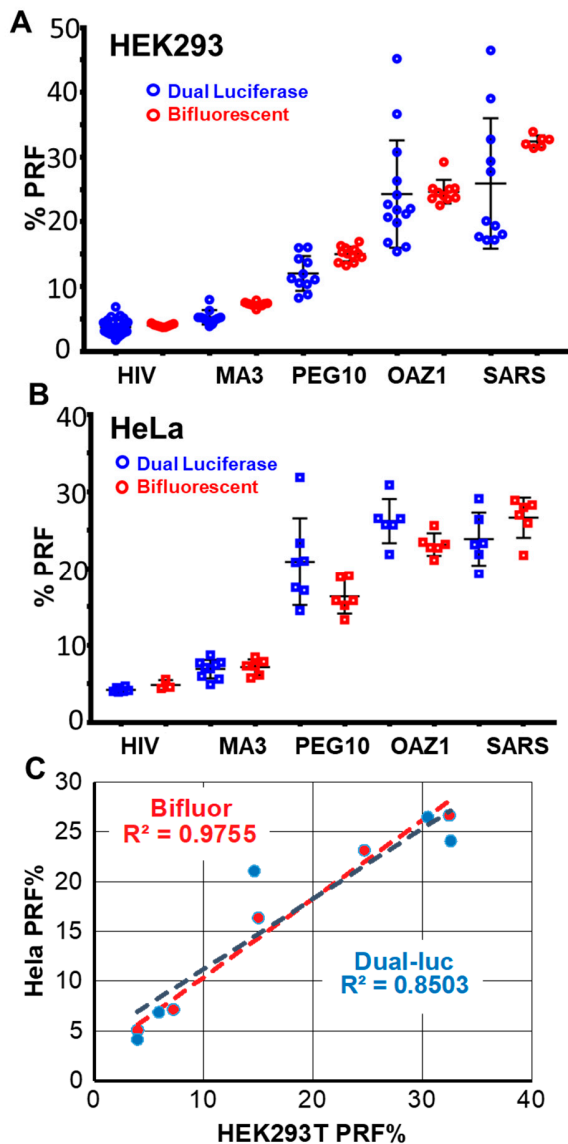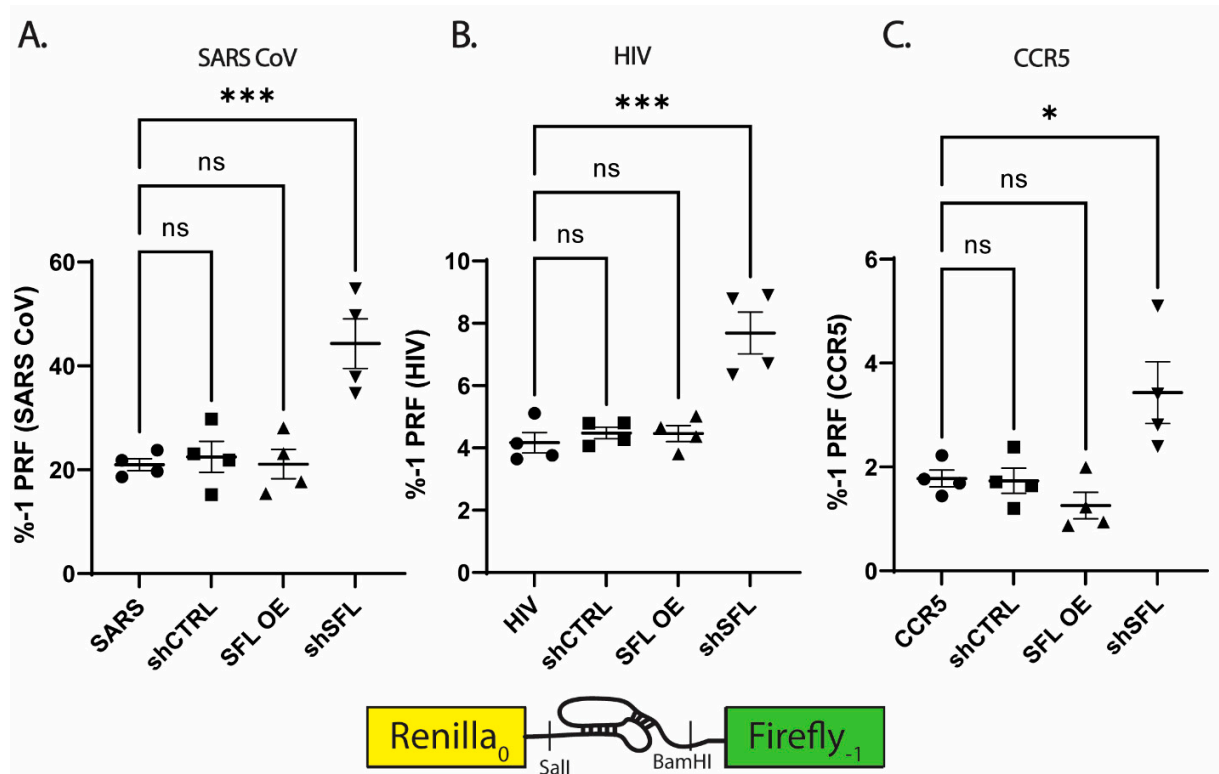
Supplemental Figure S3. Assessment of reporter splicing. RT-PCR analysis of plasmid DNA controls or mRNA extracted from HEK293T cells expressing first-generation dual luciferase (p2Luci), second-generation dual luciferase (pSGD), or bifluorescent (BiFl) reporters containing a 0-frame control, CCR5 -1 frameshift element, or HIV-1 -1 frameshift element and analyzed by gel electrophoresis. A and B denote two independent replicates.

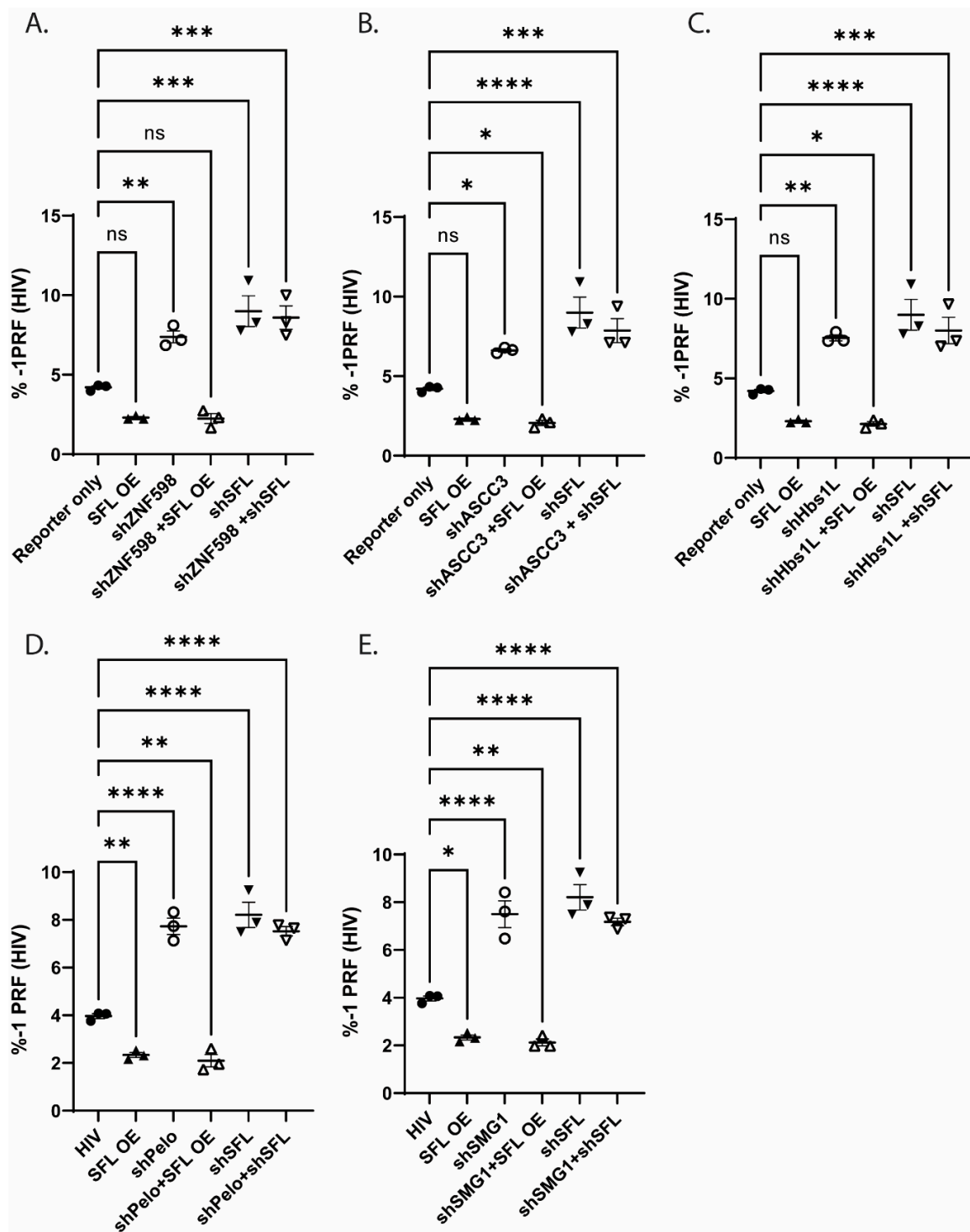**Supplemental Figure S4.** Comparison of translational recoding measured with dual luciferase and bifluorescent reporters.

A, B: -1 PRF (HIV, MA3, PEG10, SARS) and +1 PRF (OAZ1 were measured in HEK93 or HeLa cells as indicated. Each dot indicates three technical replicates of one independent biological replicate. Bars indicate standard error. **C:** Linear regression analyses of the data compared between the two assay systems.

Supplemental Figure S5. SFL overexpression or knockdown alters -1 PRF.

Frameshift efficiency of three translational recoding elements measured using dual luciferase reporters in HEK293T cells over or under-expressing SFL. (A) SARS-CoV -1 PRF signal, (B) HIV-1 -1 PRF signal, (C) CCR5 -1 PRF signal.

Supplemental Figure S6.

Frameshift efficiency of the HIV-1 -1 PRF signal in HEK293T cells over or under-expressing SFL in combination with shRNA knockdown of (A) ZNF598, (B) ASCC3, (C) Hbs1L, (D) Pelota, (E) SMG1.

A.

Effect of C19orf66 expression level on MESO patient survival

Expression Level
High expression (n=21)
Low/Medium-expression (n=64)

p = 0.028

B.

Effect of C19orf66 expression level on BLCA patient survival

Expression Level
High expression (n=100)
Low/Medium-expression (n=306)

p = 0.0082

C.

Effect of C19orf66 expression level on SKCM patient survival

p = 0.051

Expression Level
High expression (n=116)
Low/Medium-expression (n=343)

Supplemental Figure S7. Lower SFL expression decreases cancer patient survival.

Kaplan-Meyer survival plots comparing low and high-level Shiftless expression in (A) Lung mesothelioma, (B) Bladder urothelial carcinoma, and (C) Cutaneous melanoma.

	Normal			Cancer			Expression Change	
	Mean TPM	SD	N	Mean TPM	SD	N	Fold mean	% Change
Bladder (BLCA)	19.348	3.791429	9	12.09574	7.454498	432	0.625167	-37.4833
Breast	18.40608	6.584216	181	7.722593	5.202915	1253	0.419568	-58.0432
Colon	23.36609	9.238391	141	5.811777	4.246281	543	0.248727	-75.1273
Kidney (RCC)	20.41811	15.46794	28	8.765442	5.709652	615	0.429297	-57.0703
Liver	68.51388	24.48586	110	19.12129	11.57723	422	0.279086	-72.0914
Lung (MESO)	28.0186	10.74209	295	14.38403	6.311042	87	0.513374	-48.6626
Lung (LUAD)	28.0186	10.74209	295	8.378763	4.990853	598	0.299043	-70.0957
Prostate	30.51811	10.45481	100	6.631655	3.429536	556	0.217302	-78.2698
Skin (SKCM)	9.825529	3.824696	325	12.23792	7.540857	470	1.245522	24.55225
Average all							0.475232	-52.4768

Supplementary Table S1. Shiftless expression in common cancers.

Average transcripts per million reads (TPM) of Shiftless in Normal or cancerous tissues. Data mined from Genome Browser [1].

1. Lee, B.T.; Barber, G.P.; Benet-Pagès, A.; Casper, J.; Clawson, H.; DIekhans, M.; Fischer, C.; Gonzalez, J.N.; Hinrichs, A.S.; Lee, C.M.; et al. The UCSC Genome Browser Database: 2022 Update. *Nucleic Acids Res.* **2022**, *50*, doi:10.1093/nar/gkab959.