

Codon-Specific Divergence of FSP1 and ACSL4 Senescence Co-expression Decoupling in KRAS-Driven Cancers: Convergent Dual Escape in Colorectal Cancer, Enzyme-Selective Escape in Pancreatic Adenocarcinoma

Cumhur Gokhan Ekmekci

cgekmekci2@yahoo.com

Independent Researcher <https://orcid.org/0000-0002-0967-172X>

Research Article

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Abstract

Background. KRAS-mutant cancers upregulate FSP1 (AIFM2) through NRF2 and MAPK activation, conferring ferroptosis resistance parallel to ACSL4. Both enzymes connect to the ferro-aging senescence programme in wild-type tissue. Whether KRAS mutation disrupts their senescence co-regulatory connections, and whether the pattern holds across cancer types and codon variants, has not been examined.

Methods. Spearman co-expression correlations between FSP1 or ACSL4 and seven senescence genes were computed in KRAS codon-stratified TCGA-COAD (n = 397; WT n = 337, G12D n = 53, G12V n = 38, G13D n = 30) and TCGA-PAAD (n = 151; WT n = 66, G12D n = 39, G12V n = 26, G12R n = 20). BH-FDR correction was applied within each variant group. The Orphan State Score (OSS) was defined as mean expression / (FDR-significant senescence edges + 0.5).

Results. In TCGA-COAD, both FSP1 and ACSL4 lose all FDR-significant senescence co-expression in every KRAS-mutant group (OSS ~1.1–1.3 in WT vs ~9.2–9.6 in mutant groups). The decoupling is convergent: both enzymes lose senescence co-regulation simultaneously. In TCGA-PAAD, the pattern diverges by codon. FSP1 decouples in G12D (OSS 8.35, 0 edges) while ACSL4 retains three senescence edges (OSS 1.47). G12V and G12R show the inverse: ACSL4 decouples while FSP1 retains partial or full senescence co-expression. The FSP1–KEAP1 ferroptosis co-expression edge is preserved in WT, G12D and G12V in both cohorts.

Conclusions. KRAS mutation disrupts FSP1 and ACSL4 senescence co-expression in both CRC and PAAD, but the enzyme specificity and codon selectivity differ by cancer type. CRC shows convergent dual escape; PAAD shows codon-matched enzyme-selective escape. PAAD G12D may represent a selective FSP1 inhibition opportunity, consistent with published organoid data. All findings are correlational and require functional validation.

1. Introduction

KRAS mutations occur in ~ 40% of colorectal cancers (CRC) and ~ 92% of pancreatic ductal adenocarcinomas (PAAD) [1, 2]. Ferroptosis – iron-dependent cell death driven by phospholipid peroxidation [18, 19] – is a key vulnerability in KRAS-mutant cancers. KRAS mutations actively build ferroptosis resistance through NRF2 transcriptional activation and MAPK signalling [15]; both the GPX4–glutathione arm and the FSP1–CoQ10 arm are upregulated as a result [3, 4, 5, 6]. The three dominant PAAD codon variants – G12D (~ 35%), G12V (~ 30%) and G12R (~ 15%) – have distinct downstream signalling properties [17]. G12D drives robust EGFR/MAPK/NRF2 activation; G12R has impaired ERK/MAPK transcriptional output and weaker SOS/NF1-mediated MAPK signalling; G12V sits between them [7, 8, 9]. G12R patients have longer overall survival than G12D in large real-world PDAC cohorts, likely reflecting these mechanistic differences [10].

The FSP1–CoQ10–NAD(P)H pathway, identified in 2019, uses NAD(P)H to regenerate ubiquinol; ubiquinol then traps lipid peroxyl radicals at the plasma membrane independently of glutathione [4, 5, 22]. KRAS-mutant cells specifically upregulate FSP1 through MAPK and NRF2; FSP1 inhibition is required alongside ferroptosis induction for effective cell killing in LsL-KRAS^{G12V} PDAC organoids [6]. ACSL4 drives the peroxidation substrate supply that GPX4 detoxifies [11]; its co-expression with senescence-associated genes (LMNB1, MKI67, SERPINE1, IL6) links ferroptosis biology to the ferro-aging senescence programme [12, 21, 24]. Liu et al. showed that ACSL4-driven sub-lethal lipid peroxidation activates cellular senescence in primate tissues, and vitamin C alleviates this process by inhibiting ACSL4 [12]. SLC7A11/xCT also sustains redox homeostasis in oncogenic RAS transformation [20].

In KRAS-mutant CRC, ACSL4 progressively loses co-expression with senescence genes across codon variants, a state termed the ACSL4 Orphan State, which tracks both the ferroptosis/NRF2 Network Disruption Score and DepMap ferroptosis sensitivity [13, 14, 23, 25]. This was interpreted as dual-programme escape: simultaneous loss of ferroptotic and senescence co-regulation. Whether FSP1 undergoes the same decoupling, and whether the pattern extends to PAAD with its codon-specific signalling differences, are the questions this study addresses.

2. Materials and Methods

2.1 Datasets and KRAS stratification

RNA-sequencing data (log2 TPM, STAR-Counts) were obtained from the GDC portal for TCGA-COAD (n = 397; WT n = 337, G12D n = 53, G12V n = 38, G13D n = 30) and TCGA-PAAD (n = 159 total; n = 151 with KRAS annotation; WT n = 66, G12D n = 39, G12V n = 26, G12R n = 20). KRAS variants were extracted from per-sample aliquot MAF files.

PAAD WT caveat. Given PAAD's ~ 92% KRAS mutation frequency, the 66 tumours classified as WT likely include cases with low-VAF mutations or non-standard-codon mutations below MAF detection sensitivity. This group is an operational reference, not a confirmed KRAS-WT population.

2.2 Co-expression analysis

Spearman correlations were computed between FSP1 (AIFM2) or ACSL4 and seven senescence genes (CDKN1A, CDKN2A, LMNB1, MKI67, SERPINE1, IL6, TP53) and seven ferroptosis/NRF2 genes (GPX4, SLC7A11, SLC3A2, FTH1, PTGS2, NFE2L2, KEAP1) within each variant group. BH-FDR was applied within each variant × cohort combination. An edge was counted at FDR < 0.05; correlation direction is reported separately. OSS = mean expression (log2 TPM) / (FDR-significant senescence edges + 0.5). Differential expression between WT and mutant groups was tested with Wilcoxon rank-sum tests, BH-FDR-corrected. Analyses were conducted in Python 3.10 (scipy 1.11, pandas 2.0, statsmodels 0.14). TCGA data are publicly available at <https://portal.gdc.cancer.gov>; scripts are available on request.

3. Results

3.1 FSP1 transcript is selectively elevated in PAAD G12D

FSP1 expression differs by KRAS codon in PAAD (Wilcoxon vs WT: G12D $p = 0.034$, G12V $p = 0.11$, G12R $p = 0.10$) but not in CRC (all $p > 0.30$). The nominally significant elevation in PAAD G12D (mean 4.18 vs 3.79 log2 TPM) is consistent with FSP1 upregulation through MAPK and NRF2 downstream of KRAS G12D [6].

3.2 CRC: convergent senescence decoupling

In TCGA-COAD WT, FSP1 shows three FDR-significant positive senescence edges (LMNB1 $r = 0.227$, MKI67 $r = 0.212$, TP53 $r = 0.143$; all $FDR < 0.03$) and ACSL4 retains four (LMNB1, MKI67, SERPINE1, IL6). In every KRAS-mutant group, both enzymes lose all FDR-significant senescence edges (Tables 1–2). FSP1 OSS rises from 1.33 in WT to 9.18–9.35 across mutant groups; ACSL4 follows the same trajectory (1.07 → 9.39–9.64). The ACSL4 OSS tracks the NDS severity hierarchy (G12D < G12V < G13D); it mirrors the Network Disruption Score hierarchy [13]. The FSP1 OSS is near-uniform, with FSP1 senescence co-regulation collapsing uniformly at KRAS mutation onset, with no gradient across codon severity. The FSP1–KEAP1 ferroptosis edge is preserved in WT ($r = 0.504$), G12D ($r = 0.392$) and G12V ($r = 0.517$; all $FDR < 0.03$), borderline in G13D ($r = 0.347$, $FDR = 0.060$; Table 4).

Table 1

FSP1 Orphan State Score. † $FDR < 0.05$, positive correlations only; direction noted separately. ‡OSS = mean log2 TPM / (FDR-sig edges + 0.5). §PAAD WT is an operational reference. ¶G12R $n = 20$; provisional. №Two positive edges (LMNB1, MKI67) count toward OSS; TP53 $r = -0.570$ is inverse, excluded.

Cohort	Variant	n	FSP1 senescence edges†	Mean FSP1 log2 TPM	FSP1 OSS‡
TCGA-COAD	WT	337	3 (LMNB1, MKI67, TP53)	4.67	1.33
TCGA-COAD	G12D	53	0	4.59	9.18
TCGA-COAD	G12V	38	0	4.63	9.25
TCGA-COAD	G13D	30	0	4.68	9.35
TCGA-PAAD§	WT	66	6 (CDKN1A, CDKN2A, LMNB1, MKI67, SERPINE1, TP53)	3.79	0.58
TCGA-PAAD	G12D	39	0	4.18	8.35
TCGA-PAAD	G12V	26	1 (MKI67 only)	4.08	2.72
TCGA-PAAD	G12R¶	20	3№ (LMNB1, MKI67; TP53 inverse)	4.19	1.20

Table 2
ACSL4 Orphan State Score. Symbols as in Table 1.

Cohort	Variant	n	ACSL4 senescence edges†	Mean ACSL4 log2 TPM	ACSL4 OSS‡
TCGA-COAD	WT	337	4 (LMNB1, MKI67, SERPINE1, IL6)	4.83	1.07
TCGA-COAD	G12D	53	0	4.69	9.39
TCGA-COAD	G12V	38	0	4.82	9.64
TCGA-COAD	G13D	30	0	4.42	8.85
TCGA-PAAD§	WT	66	5 (CDKN1A, CDKN2A, LMNB1, MKI67, SERPINE1)	5.05	0.92
TCGA-PAAD	G12D	39	3 (CDKN1A, CDKN2A, SERPINE1)	5.14	1.47
TCGA-PAAD	G12V	26	0	5.34	10.67
TCGA-PAAD	G12R¶	20	0	5.10	10.20

3.3 PAAD: codon-specific enzyme-selective decoupling

PAAD WT retains six FDR-significant FSP1 senescence edges vs three in CRC WT. Whether this reflects genuine tissue differences or partial contamination of the PAAD WT group with low-VAF KRAS-mutant tumours cannot be determined from these data. In PAAD G12D, FSP1 loses all senescence co-expression (OSS 8.35) while ACSL4 retains three edges (OSS 1.47). In G12V, ACSL4 decouples (OSS 10.67) while FSP1 retains one positive senescence edge (MKI67 $r = 0.706$, FDR = 0.001; OSS 2.72). In G12R, ACSL4 decouples (OSS 10.20) while FSP1 retains two positive senescence edges (LMNB1 $r = 0.680$, MKI67 $r = 0.605$). A third G12R edge — TP53 $r = -0.570$, FDR = 0.030 — is an inverse correlation, not pro-senescence co-regulation; it is listed in Table 4 but excluded from the retention count. G12R findings are provisional given $n = 20$ (power ~ 50% at $r = 0.50$). The FSP1–KEAP1 ferroptosis edge is preserved in PAAD WT, G12D and G12V (all FDR < 0.05) but non-significant in G12R ($r = 0.152$, FDR = 0.732), consistent with G12R's impaired ERK/MAPK transcriptional output [7, 8].

Table 3

Codon × enzyme decoupling matrix. OSS values in parentheses. **Therapeutic implications are computational hypotheses only. ¶G12R n = 20; provisional.

Cohort	Variant	FSP1 decoupled?	ACSL4 decoupled?	Pattern	Hypothesis**
CRC	G12D	Yes (9.18)	Yes (9.39)	Convergent	Both enzymes candidate targets
CRC	G12V	Yes (9.25)	Yes (9.64)	Convergent	Both enzymes candidate targets
CRC	G13D	Yes (9.35)	Yes (8.85)	Convergent	Both enzymes candidate targets
PAAD	G12D	Yes (8.35)	No (1.47)	FSP1-selective	FSP1 as candidate over ACSL4
PAAD	G12V	Partial (2.72)	Yes (10.67)	ACSL4-selective	ACSL4 pathway as candidate
PAAD	G12R¶	No (1.20)	Yes (10.20)	ACSL4-selective	ACSL4 pathway as candidate

3.4 Significant FSP1 co-expression edges

Table 4 lists all FDR-significant FSP1 co-expression edges. Direction is stated for each entry; all WT edges and most mutant retained edges are positive. The only inverse entry is PAAD G12R TP53 ($r = -0.570$), which is excluded from the retention count.

Table 4

FDR-significant FSP1 co-expression edges. (+) = positive; (-) = inverse. ¶G12R provisional.

Cohort	Variant	Gene	r	FDR	Category
TCGA-COAD	WT	LMNB1	0.227	< 0.001	Senescence (+)
TCGA-COAD	WT	MKI67	0.212	0.0004	Senescence (+)
TCGA-COAD	WT	TP53	0.143	0.021	Senescence (+)
TCGA-COAD	WT	KEAP1	0.504	< 0.001	Ferroptosis (+)
TCGA-COAD	G12D	KEAP1	0.392	0.026	Ferroptosis (+)
TCGA-COAD	G12V	KEAP1	0.517	0.013	Ferroptosis (+)
TCGA-COAD	G13D	KEAP1	0.347	0.060	Ferroptosis (borderline)
TCGA-PAAD	WT	LMNB1	0.416	0.004	Senescence (+)
TCGA-PAAD	WT	MKI67	0.382	0.007	Senescence (+)
TCGA-PAAD	WT	CDKN1A	0.329	0.024	Senescence (+)
TCGA-PAAD	WT	CDKN2A	0.309	0.031	Senescence (+)
TCGA-PAAD	WT	SERPINE1	0.303	0.031	Senescence (+)
TCGA-PAAD	WT	TP53	0.276	0.044	Senescence (+)
TCGA-PAAD	WT	KEAP1	0.283	0.043	Ferroptosis (+)
TCGA-PAAD	G12D	KEAP1	0.474	0.032	Ferroptosis (+)
TCGA-PAAD	G12V	MKI67	0.706	0.001	Senescence (+) partial
TCGA-PAAD	G12V	KEAP1	0.534	0.035	Ferroptosis (+)
TCGA-PAAD	G12R¶	LMNB1	0.680	0.013	Senescence (+) provisional

Cohort	Variant	Gene	r	FDR	Category
TCGA-PAAD	G12R	MKI67	0.605	0.022	Senescence (+) provisional
TCGA-PAAD	G12R	TP53	-0.570	0.030	Senescence (-) inverse – excluded from retention

4. Discussion

Two findings emerge. In CRC, FSP1 and ACSL4 both decouple from senescence co-expression in every KRAS-mutant group. The FSP1 decoupling does not add an independent mechanism to the dual-programme escape model proposed for ACSL4 [13]; it confirms the same model with a second enzyme. One distinction holds: the ACSL4 OSS tracks the NDS severity hierarchy; it mirrors the Network Disruption Score. The FSP1 OSS is near-uniform, with FSP1 senescence co-regulation collapsing uniformly at KRAS mutation onset, with no gradient across codon severity.

In PAAD, the two enzymes diverge by codon. FSP1 decouples in G12D while ACSL4 is retained; G12V and G12R drive the inverse. This divergence is consistent with the codon-specific signalling hierarchy in PDAC: G12D engages the EGFR/MAPK/NRF2 axis most robustly [7, 8, 9], and Müller et al. demonstrated FSP1 upregulation and ferroptosis resistance specifically in the LsL-KRAS^{G12D} PDAC model [6]. The reduced ERK/MAPK transcriptional output of G12R [7, 8] is consistent with the weaker NRF2–FSP1 activation that may account for the provisionally retained FSP1 senescence co-expression in that group.

The PAAD G12D finding has a direct therapeutic implication as a hypothesis: FSP1 is the selectively decoupled enzyme in this allele, and FSP1 inhibition synergises with ferroptosis induction in KRAS G12D PDAC organoids [6]. For G12V and G12R, where ACSL4 rather than FSP1 decouples, ferroptosis strategies acting on the ACSL4 lipid substrate supply may be better matched to the network state. Isogenic G12D/G12V/G12R PDAC lines with DCPIP FSP1 enzymatic activity and SA-β-gal/p21 senescence measurements are needed to test these inferences.

Three limitations are critical. First, the PAAD WT group is an operational reference, not a confirmed KRAS-WT population. Second, G12R n = 20 is underpowered; its retained FSP1 edges are provisional. Third, all conclusions are bulk RNA-seq correlations from primary tumours; co-expression loss does not establish that enzymatic activity is altered.

5. Conclusions

FSP1 and ACSL4 both decouple from senescence co-expression in KRAS-mutant CRC and PAAD, but with distinct codon and enzyme selectivity. CRC shows convergent dual escape; PAAD shows codon-matched enzyme-selective escape. The G12D-specific FSP1 Orphan State in PAAD, aligned with the KRAS G12D–NRF2–FSP1 axis, identifies FSP1 as a codon-matched candidate in this subset. All evidence is transcriptomic.

Declarations

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Competing interests: None.

Author contributions: C.G.E. conceived the study, performed all analyses and wrote the manuscript.

Data availability: TCGA data are available at <https://portal.gdc.cancer.gov>. Scripts available on request.

AI disclosure: AI-assisted tools (Claude, Anthropic) were used for language editing. All scientific content was designed, performed and verified by the author.

References

1. Hobbs GA, Der CJ, Rossman KL (2016) RAS isoforms and mutations in cancer at a glance. *J Cell Sci* 129(7):1287–1292
2. Waters AM, Der CJ, KRAS (2018) The critical driver and therapeutic target for pancreatic cancer. *Cold Spring Harb Perspect Med* 8(9):a031435
3. Dixon SJ, Lemberg KM, Lamprecht MR et al (2012) Ferroptosis: an iron-dependent form of nonapoptotic cell death. *Cell* 149(5):1060–1072
4. Bersuker K, Hendricks JM, Li Z et al (2019) The CoQ oxidoreductase FSP1 acts parallel to GPX4 to inhibit ferroptosis. *Nature* 575(7784):688–692
5. Doll S, Freitas FP, Shah R et al (2019) FSP1 is a glutathione-independent ferroptosis suppressor. *Nature* 575(7784):693–698
6. Müller F, Lim JKM, Bebbler CM et al (2023) Elevated FSP1 protects KRAS-mutated cells from ferroptosis during tumor initiation. *Cell Death Differ* 30(2):442–456
7. Neumann LM, Arrazola AM, Wanjiru C et al (2026) KRASG12R-mutant pancreatic cancer features limited ERK/MAPK transcriptional activity. *Cancer Res.* 10.1158/0008-5472.CAN-25-2630
8. Kamgar M, Hobbs GA, Davidson R et al (2025) Mutant and wild-type RAS crosstalk in KRASG12R pancreatic ductal adenocarcinoma. *Cancer Res*
9. Ardalan B, Ciner A, Baca Y et al (2025) Distinct molecular and clinical features of KRAS codon 12 variants in pancreatic adenocarcinoma. *Clin Cancer Res* 31(6):1132–1141
10. Ardalan B, Baca Y, Hinton A et al (2023) KRAS G12D and survival outcome in pancreatic adenocarcinoma. *J Clin Oncol* 41(16suppl):4145
11. Doll S, Proneth B, Tyurina YY et al (2017) ACSL4 dictates ferroptosis sensitivity by shaping cellular lipid composition. *Nat Chem Biol* 13(1):91–98
12. Liu L, Zheng Z, You W et al (2026) Vitamin C inhibits ACSL4 to alleviate ferro-aging in primates. *Cell Metab* 38(4):673–693

13. Ekmekci CG (2026) Oncogenic KRAS decouples ACSL4 from the ferro-aging senescence programme in colorectal cancer. Preprint. Research Square. rs-11317331 (DOI pending). Submitted to Biogerontology
14. Ekmekci CG (2026) Oncogenic KRAS rewires the ferroptosis/NRF2 co-expression network in colorectal cancer. Preprint. Research Square. rs-11319422 (DOI pending). Submitted to Computational Biology and Chemistry
15. DeNicola GM, Karreth FA, Humpton TJ et al (2011) Oncogene-induced Nrf2 transcription promotes ROS detoxification and tumorigenesis. *Nature* 475(7354):106–109
16. Koppula P, Zhang Y, Zhuang L, Gan B (2018) Amino acid transporter SLC7A11/xCT at the crossroads of redox homeostasis and nutrient dependency. *Cancer Commun* 38(1):12
17. Kim M, Lee JS, Seo J et al (2025) Clinical and molecular characteristics of KRAS codon-specific mutations in advanced pancreatic ductal adenocarcinoma. *Int J Mol Sci* 26(22):10908
18. Stockwell BR, Friedmann Angeli JP, Bayir H et al (2017) Ferroptosis: a regulated cell death nexus. *Cell* 171(2):273–285
19. Lei G, Zhuang L, Gan B (2022) Targeting ferroptosis as a vulnerability in cancer. *Nat Rev Cancer* 22(7):381–396
20. Lim JKM, Delaidelli A, Minaker SW et al (2019) Cystine/glutamate antiporter xCT facilitates oncogenic RAS transformation. *Proc Natl Acad Sci USA* 116(19):9433–9442
21. Gao S, Qi L, Bai W et al (2026) Ferro-aging: a novel paradigm. *Free Radic Biol Med* 253:817–829
22. Nakamura T, Mishima E, Yamada N et al (2023) Integrated chemical and genetic screens unveil FSP1 mechanisms. *Nat Struct Mol Biol* 30(10):1806–1815
23. Tsherniak A, Vazquez F, Montgomery PG et al (2017) Defining Cancer Dependency Map *Cell* 170(3):564–576
24. Sun N, Wang J, Qin J et al (2024) Oncogenic RTKs sensitize cancer cells to ferroptosis via c-Myc and ACSL4. *Cell Death Dis* 15(11):1–11
25. Ekmekci CG (2026) Selective NRF2 activation and co-expression network disruption in KRAS-mutant colorectal cancer. Submitted to Computational Biology and Chemistry

Figures

FSP1 Mirrors ACSL4 Senescence Decoupling in KRAS-Mutant CRC TCGA-COAD | n=397 | Spearman | BH-FDR

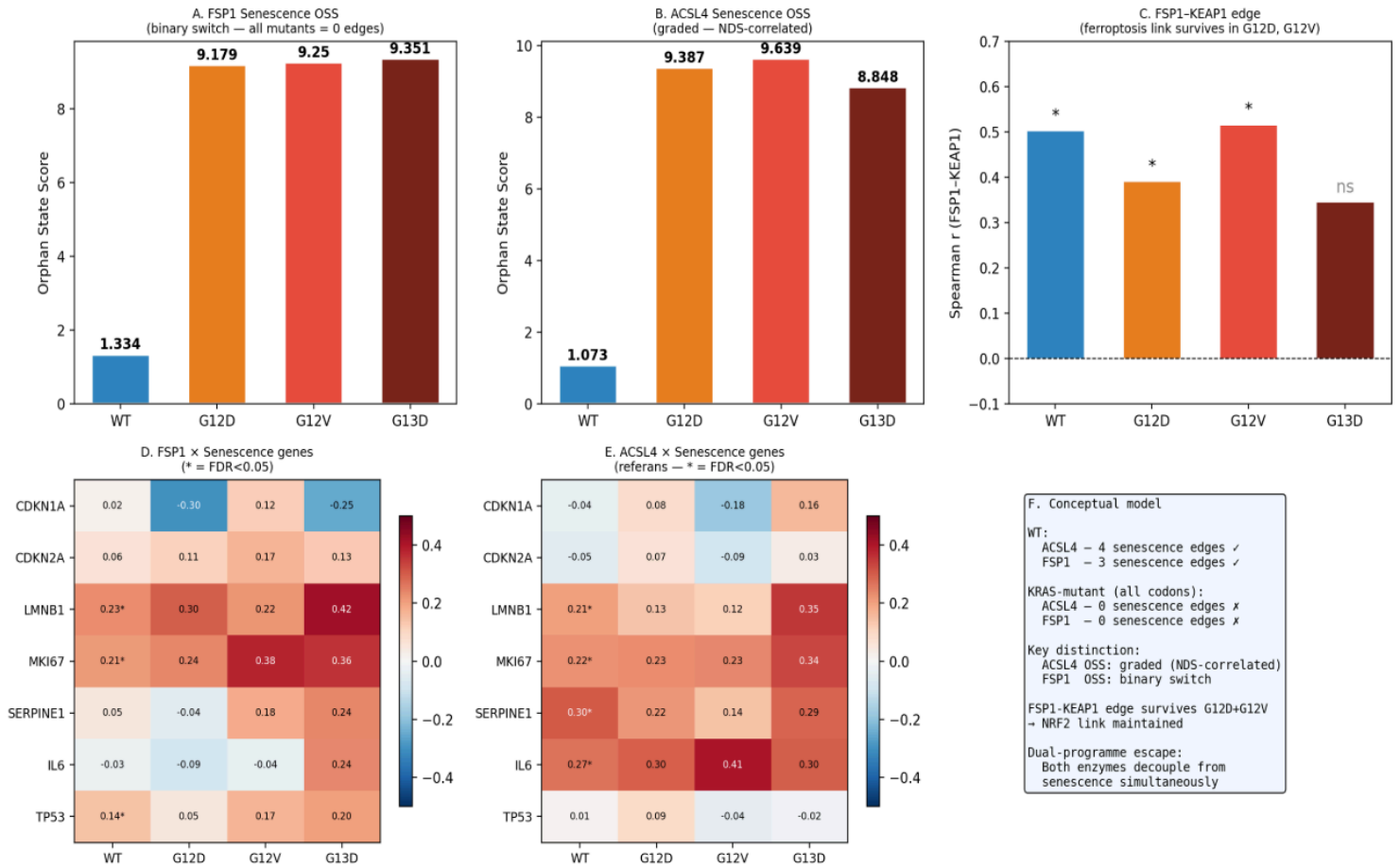


Figure 1

FSP1 (AIFM2) mirrors ACSL4 in senescence co-expression decoupling in KRAS-mutant CRC. (A) FSP1 OSS; (B) ACSL4 OSS; (C) FSP1-KEAP1 Spearman r; * FDR<0.05. (D-E) heatmaps of senescence co-expression r values. (F) conceptual model. TCGA-COAD n=397; BH-FDR.

FSP1 Senescence Orphan State: CRC vs PAAD TCGA-COAD (n=397) | TCGA-PAAD (n=159)

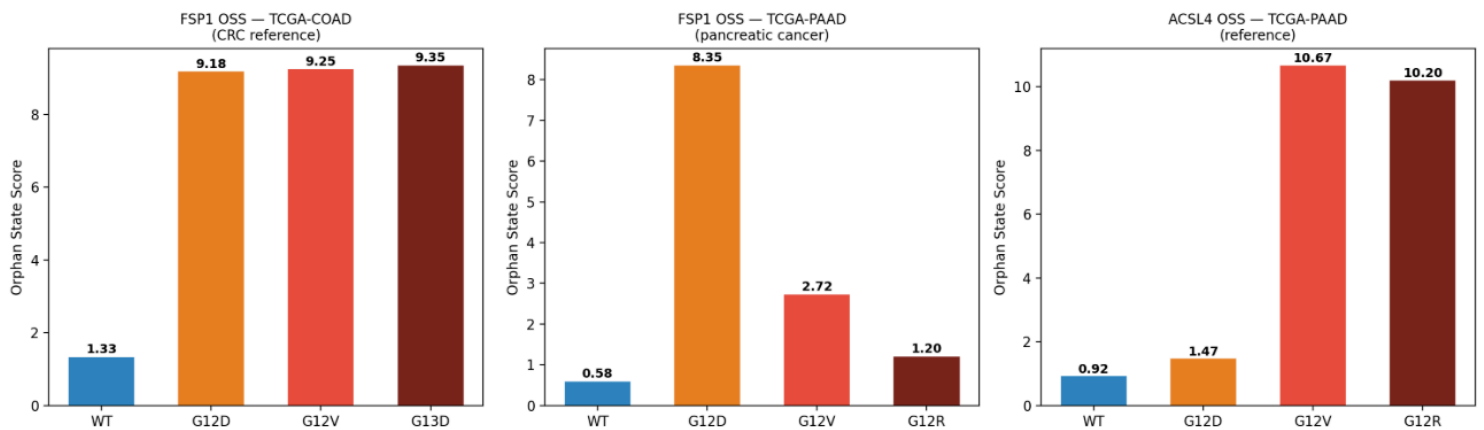


Figure 2

FSP1 and ACSL4 OSS: CRC vs PAAD. Left: FSP1 OSS in CRC (all mutant groups uniform). Centre: FSP1 OSS in PAAD (G12D selectively elevated). Right: ACSL4 OSS in PAAD (inverse pattern). TCGA-COAD n=397; TCGA-PAAD n=151.