Supplemental information

m6A demethylase FTO suppresses pancreatic cancer tumorigenesis by demethylating \( PJA2 \) and inhibiting Wnt signaling

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Material S1

Sequences for all the siRNAs

| Primers | Sequences (5'to3') |
|---------|--------------------|
| FTO (Human) siRNA-1  | GAAACUGAGGCCUCUUUGAATT |
| FTO (Human) siRNA-2  | GUACUUUGCUAGAAUUUCATT |
| FTO (Human) siRNA-3  | CACGAAUUUGCCCGAACAUUTT |
| PJA2 (human) siRNA-100 | GAUGAAUGGUAGUACGATT |
| PJA2 (human) siRNA-122 | GCACAUUUCGGAUUUCUTT |
| PJA2 (human) siRNA-429 | GUUCACAGGAAAAUUCCUTT |
| Negative control (NC) | UUCUCGAACGUGUCAGGUTT |

Plasmids construction

The human FTO gene (GenBank Accession No. NM_001080432) was subcloned into pEZ-M90 vector (GeneCopoeia) to generate a Puromycin-tagged fusion protein. The plasmids with site-directed mutants were constructed using Stratagene's QuikChange II site-directed mutagenesis kit. The following pairs of primers were used:

hFTO_H231A/D233A_F: 5'-GAAAAATGCGAGTGAAGTGGTGGACAGGTCAGCGGTGGCAGTGTACAGTTATAGCTGTGAAGGCCCTGAAGAGGAAAGTGAGGATGACTCTC
hFTO_H231A/D233A_R: 5'-ATGCACTATTGCTGCGCCACAAAGATTACGTAACCGAGGCTGAGTGAAGGCCCTGAAGAGGAAAGTGAGGATGACTCTC

The wild-type human FTO gene sequence used for construction:

ATGAAGCGCACCCCGACTGGCGAGGACAGGAGCCGAAGCTAAGAATGGCAGTGAGCTGGGCTCATGCTGAAAATCTGGTGGACAGGTCAGCGGTGGCAGTGTACAGTTATAGCTGTGAAGGCCCTGAAGAGGAAAGTGAGGATGACTCTC

The following pairs of primers were used:

hFTO_H231A/D233A_F: 5'-GAAAAATGCGAGTGAAGTGGTGGACAGGTCAGCGGTGGCAGTGTACAGTTATAGCTGTGAAGGCCCTGAAGAGGAAAGTGAGGATGACTCTC
hFTO_H231A/D233A_R: 5'-ATGCACTATTGCTGCGCCACAAAGATTACGTAACCGAGGCTGAGTGAAGGCCCTGAAGAGGAAAGTGAGGATGACTCTC

The wild-type human FTO gene sequence used for construction:

ATGAAGCGCACCCCGACTGGCGAGGACAGGAGCCGAAGCTAAGAATGGCAGTGAGCTGGGCTCATGCTGAAAATCTGGTGGACAGGTCAGCGGTGGCAGTGTACAGTTATAGCTGTGAAGGCCCTGAAGAGGAAAGTGAGGATGACTCTC
CGAGTTTGAGTGGCTGAGGCAGTTTTGGTTTCAAGGCAATCGATACAGAAA
GTGCACTGACTGGTGGTGTAACCCATGGCTCAAACCTGGAAGCATGTGGA
AGAAGATGAGGGTTGTGACAAATGCTGTCATGGAAGTTAAGAAGAGAG
GGGCTCCTCCGTGGAACAAAAAGGAATGAAATCTTTGACTGCGCCATCTTGCTCG
CTACTGCAAGCCAGAACACTGAGAGAGAATGGCATGCCAGGTGCCAGTC
ACGAATTGCCCCGAACATTACCTGCTGATCGAGCAGCCAGAATGTCGGCCATA
CTGGGAAAAGATGATGCTTCGACTGCTGCGTGTTTGACCTCACAGACAT
CGTTTCAGAACTCAGAGGTTCAGCTTCTGGAAGCAAAACCCTAG

Material S2 All statistic information in this study

| Relative m6A RNA modification in total RNA | Position | Comparison | p-Value |
|-------------------------------------------|----------|------------|---------|
| Figure 1B                                 | PAAD tissues-vs-para-tumor tissues | 0.0314 |
| Figure 1C                                 | SW1990-vs-HPDE6-C7 | 0.0279 |
| Figure 1C                                 | AsPC-1-vs-HPDE6-C7 | 0.0033 |
| Figure 1C                                 | PANC-1-vs-HPDE6-C7 | 0.0004 |
| Figure 1C                                 | BxPC-3-vs-HPDE6-C7 | 0.0009 |
| Figure 1C                                 | Capan-2-vs-HPDE6-C7 | 0.0183 |
| Figure 3G                                 | siFTO-1-vs-NC in SW1990 cell | 0.0026 |
| Figure 3G                                 | siFTO-2-vs-NC in SW1990 cell | 0.0003 |
| Figure 3G                                 | siFTO-3-vs-NC in SW1990 cell | 0.0028 |
| Figure 3H                                 | FTO-mut-vs-NC in BxPC-3 cell | 0.9331 |
| Figure 3H                                 | FTO-WT-vs-NC in BxPC-3 cell | 0.0024 |

| Relative PJA2 m6A level | Position | Comparison | p-Value |
|-------------------------|----------|------------|---------|
| Figure 6H                | FTO-mut-vs-NC in BxPC-3 cell | 0.8714 |
| Figure 6H                | FTO-WT-vs-NC in BxPC-3 cell | <0.0001 |
| Figure 6H                | siFTO-1-vs-NC in BxPC-3 cell | 0.0013 |
| Figure 6H                | siFTO-2-vs-NC in BxPC-3 cell | 0.0001 |
| Figure 6H                | siFTO-3-vs-NC in BxPC-3 cell | 0.0061 |
| Figure 6I                | FTO-mut-vs-NC in SW1990 cell | 0.3530 |
| Figure 6I                | FTO-WT-vs-NC in SW1990 cell | 0.0010 |
| Figure 6I                | siFTO-1-vs-NC in SW1990 cell | <0.0001 |
| Figure 6I                | siFTO-2-vs-NC in SW1990 cell | <0.0001 |
| Figure 6I                | siFTO-3-vs-NC in SW1990 cell | 0.0005 |

| Relative mRNA level of FTO | Position | Comparison | p-Value |
|----------------------------|----------|------------|---------|
| Figure 3A                  | SW1990-vs-HPDE6-C7 | 0.0447 |
| Figure 3A                  | AsPC-1-vs-HPDE6-C7 | 0.0015 |
| Figure 3A                  | PANC-1-vs-HPDE6-C7 | 0.0006 |
| Figure 3A                  | BxPC-3-vs-HPDE6-C7 | 0.0004 |
Relative protein level of FTO

| Position | Comparison | p-Value |
|----------|------------|---------|
| Figure 3B | SW1990-vs-HPDE6-C7 | 0.0077 |
| Figure 3B | AsPC-1-vs-HPDE6-C7 | 0.0007 |
| Figure 3B | PANC-1-vs-HPDE6-C7 | 0.0006 |
| Figure 3B | BxPC-3-vs-HPDE6-C7 | 0.0002 |
| Figure 3B | Capan-2-vs-HPDE6-C7 | 0.0008 |

FTO expression in PAAD based on individual patient cancer stages

| Position | Comparison | p-Value |
|----------|------------|---------|
| Figure 4C | StageII-vs-StageI | 0.0002 |
| Figure 4C | StageIII-vs-StageI | 0.048 |
| Figure 4C | StageIV-vs-StageI | 0.0181 |

FTO expression in PAAD based on nodal metastasis status

| Position | Comparison | p-Value |
|----------|------------|---------|
| Figure 4D | N1-vs-N0 | <0.0001 |
| Figure 4D | N2-vs-N0 | <0.0001 |

Relative mRNA level of PJA2

| Position | Comparison | p-Value |
|----------|------------|---------|
| Figure 7B | SW1990-vs-HPDE6-C7 | 0.0022 |
| Figure 7B | AsPC-1-vs-HPDE6-C7 | 0.0002 |
| Figure 7B | PANC-1-vs-HPDE6-C7 | 0.0002 |
| Figure 7B | BxPC-3-vs-HPDE6-C7 | 0.0001 |
| Figure 7B | Capan-2-vs-HPDE6-C7 | 0.0007 |
| Figure 6C | FTO-mut-vs-NC in BxPC-3 cell | 0.8260 |
| Figure 6C | FTO-WT-vs-NC in BxPC-3 cell | <0.0001 |
| Figure 6C | siFTO-1-vs-NC in BxPC-3 cell | 0.0002 |
| Figure 6C | siFTO-2-vs-NC in BxPC-3 cell | <0.0001 |
| Figure 6C | siFTO-3-vs-NC in BxPC-3 cell | 0.0001 |
| Figure 6D | FTO-mut-vs-NC in SW1990 cell | 0.5013 |
| Figure | Comparison | p-Value |
|--------|------------|---------|
| 6D     | FTO-WT-vs-NC in SW1990 cell | <0.0001 |
| 6D     | siFTO-1-vs-NC in SW1990 cell | 0.0002  |
| 6D     | siFTO-2-vs-NC in SW1990 cell | <0.0001 |
| 6D     | siFTO-3-vs-NC in SW1990 cell | 0.0026  |
| 7D     | siPJA2-429-vs-NC in BxPC-3 cell | <0.0001 |
| 7D     | siPJA2-122-vs-NC in BxPC-3 cell | <0.0001 |
| 7D     | siPJA2-100-vs-NC in BxPC-3 cell | <0.0001 |
| S3A    | siPJA2-429-vs-NC in SW1990 cell | <0.0001 |
| S3A    | siPJA2-122-vs-NC in SW1990 cell | 0.0002  |
| S3A    | siPJA2-100-vs-NC in SW1990 cell | 0.0004  |
| 6J     | siYTHDF1-vs-NC in BxPC-3 cell | 0.2112  |
| 6J     | siYTHDF2-vs-NC in BxPC-3 cell | 0.0011  |
| 6K     | siYTHDF1-vs-NC in SW1990 cell | 0.8554  |
| 6K     | siYTHDF2-vs-NC in SW1990 cell | 0.0011  |

**Relative protein level of PJA2**

| Position | Comparison | p-Value |
|----------|------------|---------|
| 7C       | SW1990-vs-HPDE6-C7 | 0.0012  |
| 7C       | AsPC-1-vs-HPDE6-C7 | 0.0015  |
| 7C       | PANC-1-vs-HPDE6-C7 | 0.0002  |
| 7C       | BxPC-3-vs-HPDE6-C7 | <0.0001 |
| 7C       | Capan-2-vs-HPDE6-C7 | 0.0001  |
| 6E       | FTO-mut-vs-NC in BxPC-3 cell | 0.2109  |
| 6E       | FTO-WT-vs-NC in BxPC-3 cell | 0.0001  |
| 6E       | siFTO-1-vs-NC in BxPC-3 cell | <0.0001 |
| 6E       | siFTO-2-vs-NC in BxPC-3 cell | 0.0009  |
| 6E       | siFTO-3-vs-NC in BxPC-3 cell | 0.0070  |
| 6F       | FTO-mut-vs-NC in SW1990 cell | 0.6458  |
| 6F       | FTO-WT-vs-NC in SW1990 cell | <0.0001 |
| 6F       | siFTO-1-vs-NC in SW1990 cell | <0.0001 |
| 6F       | siFTO-2-vs-NC in SW1990 cell | <0.0001 |
| 6F       | siFTO-3-vs-NC in SW1990 cell | <0.0001 |
| 6L       | siYTHDF1-vs-NC in BxPC-3 cell | 0.9532  |
| 6L       | siYTHDF2-vs-NC in BxPC-3 cell | <0.0001 |
| 6M       | siYTHDF1-vs-NC in SW1990 cell | 0.9472  |
| 6M       | siYTHDF2-vs-NC in SW1990 cell | 0.0006  |

**Remaining mRNA level of PJA2**

| Position | Comparison | p-Value |
|----------|------------|---------|
| 6N       | siYTHDF2-vs-NC in BxPC-3 cell | <0.0001 |
| 6N       | FTO-mut-vs-NC in BxPC-3 cell | 0.7527  |
| 6N       | FTO-WT-vs-NC in BxPC-3 cell | 0.0010  |
| 6N       | siFTO-2-vs-NC in BxPC-3 cell | 0.0016  |
| 6O       | siYTHDF2-vs-NC in SW1990 cell | <0.0001 |
### Relative mRNA level in BxPC-3 cell

| Position | Gene      | Comparison                  | p-Value |
|----------|-----------|----------------------------|---------|
| Figure 8E | CTNNB1    | FTO-WT-vs-Vector            | 0.0003  |
| Figure 8E | WNT5A     | FTO-WT-vs-Vector            | <0.0001 |
| Figure 8E | LEF1      | FTO-WT-vs-Vector            | 0.0011  |
| Figure 8E | GSK3B     | FTO-WT-vs-Vector            | 0.3943  |
| Figure 8E | AXIN1     | FTO-WT-vs-Vector            | <0.0001 |
| Figure 8E | WIF1      | FTO-WT-vs-Vector            | <0.0001 |
| Figure 8E | CTNNB1    | FTO-WT + siPJA2-429-vs-FTO-WT + siCtrl | 0.0007  |
| Figure 8E | WNT5A     | FTO-WT + siPJA2-429-vs-FTO-WT + siCtrl | <0.0001 |
| Figure 8E | LEF1      | FTO-WT + siPJA2-429-vs-FTO-WT + siCtrl | <0.0001 |
| Figure 8E | GSK3B     | FTO-WT + siPJA2-429-vs-FTO-WT + siCtrl | 0.6561  |
| Figure 8E | AXIN1     | FTO-WT + siPJA2-429-vs-FTO-WT + siCtrl | 0.0019  |
| Figure 8E | WIF1      | FTO-WT + siPJA2-429-vs-FTO-WT + siCtrl | <0.0001 |

### Phase Object Confluence

| Position | Comparison                  | p-Value |
|----------|----------------------------|---------|
| Figure 5A | FTO-mut-vs-NC in BxPC-3 cell | >0.9999 |
| Figure 5A | FTO-WT-vs-NC in BxPC-3 cell  | 0.0236  |
| Figure 5A | siFTO-1-vs-NC in BxPC-3 cell | 0.0022  |
| Figure 5A | siFTO-2-vs-NC in BxPC-3 cell | 0.0007  |
| Figure 5A | siFTO-3-vs-NC in BxPC-3 cell | 0.0033  |
| Figure S2A | FTO-mut-vs-NC in SW1990 cell | 0.9983  |
| Figure S2A | FTO-WT-vs-NC in SW1990 cell  | 0.0010  |
| Figure S2A | siFTO-1-vs-NC in SW1990 cell | 0.0001  |
| Figure S2A | siFTO-2-vs-NC in SW1990 cell | <0.0001 |
| Figure S2A | siFTO-3-vs-NC in SW1990 cell | 0.0012  |
| Figure 7E  | siPJA2-429-vs-NC in BxPC-3 cell | <0.0001 |
| Figure 7E  | siPJA2-122-vs-NC in BxPC-3 cell | <0.0001 |
| Figure 7E  | siPJA2-100-vs-NC in BxPC-3 cell | <0.0001 |
| Figure S3B | siPJA2-429-vs-NC in SW1990 cell | 0.0009  |
| Figure S3B | siPJA2-122-vs-NC in SW1990 cell | <0.0001 |
| Figure S3B | siPJA2-100-vs-NC in SW1990 cell | 0.0021  |
| Figure 8A  | FTO-WT-vs-Vector             | 0.0004  |
| Figure 8A  | FTO-WT + siCtrl-vs-FTO-WT    | 0.9937  |
| Figure 8A  | FTO-WT + siPJA2-429-vs-FTO-WT + siCtrl | 0.0131 |

### Absorbance of OD 450 nm

| Position | Comparison                  | p-Value |
|----------|----------------------------|---------|
| Figure 5A | FTO-mut-vs-NC in BxPC-3 cell | 0.3149  |
Figure 5A  FTO-WT-vs-NC in BxPC-3 cell  0.0007
Figure 5A  siFTO-1-vs-NC in BxPC-3 cell  0.0183
Figure 5A  siFTO-2-vs-NC in BxPC-3 cell  0.0006
Figure 5A  siFTO-3-vs-NC in BxPC-3 cell  0.0873
Figure S2A  FTO-mut-vs-NC in SW1990 cell  0.4730
Figure S2A  FTO-WT-vs-NC in SW1990 cell  0.0045
Figure S2A  siFTO-1-vs-NC in SW1990 cell  0.0013
Figure S2A  siFTO-2-vs-NC in SW1990 cell  0.0011
Figure S2A  siFTO-3-vs-NC in SW1990 cell  0.0207
Figure 7E  siPJA2-429-vs-NC in BxPC-3 cell  0.0138
Figure 7E  siPJA2-122-vs-NC in BxPC-3 cell  0.0011
Figure 7E  siPJA2-100-vs-NC in BxPC-3 cell  0.0319
Figure S3B  siPJA2-429-vs-NC in SW1990 cell  0.0020
Figure S3B  siPJA2-122-vs-NC in SW1990 cell  0.0027
Figure S3B  siPJA2-100-vs-NC in SW1990 cell  0.0336
Figure 8A  FTO-WT-vs-Vector  0.0003
Figure 8A  FTO-WT+siCtrl-vs-FTO-WT  0.8603
Figure 8A  FTO-WT+siPJA2-429-vs-FTO-WT+siCtrl  0.0005

| Colony forming efficiency % |
|-----------------------------|
| Position  | Comparison  | p-Value  |
| Figure 5B | FTO-mut-vs-NC in BxPC-3 cell | 0.8512 |
| Figure 5B | FTO-WT-vs-NC in BxPC-3 cell | 0.0003 |
| Figure 5B | siFTO-1-vs-NC in BxPC-3 cell | 0.0004 |
| Figure 5B | siFTO-2-vs-NC in BxPC-3 cell | <0.0001 |
| Figure 5B | siFTO-3-vs-NC in BxPC-3 cell | <0.0001 |
| Figure S2B | FTO-mut-vs-NC in SW1990 cell | >0.9999 |
| Figure S2B | FTO-WT-vs-NC in SW1990 cell | 0.0004 |
| Figure S2B | siFTO-1-vs-NC in SW1990 cell | <0.0001 |
| Figure S2B | siFTO-2-vs-NC in SW1990 cell | <0.0001 |
| Figure S2B | siFTO-3-vs-NC in SW1990 cell | 0.0002 |
| Figure 7F | siPJA2-429-vs-NC in BxPC-3 cell | 0.0004 |
| Figure 7F | siPJA2-122-vs-NC in BxPC-3 cell | 0.0018 |
| Figure 7F | siPJA2-100-vs-NC in BxPC-3 cell | 0.0018 |
| Figure S3C | siPJA2-429-vs-NC in SW1990 cell | <0.0001 |
| Figure S3C | siPJA2-122-vs-NC in SW1990 cell | 0.0004 |
| Figure S3C | siPJA2-100-vs-NC in SW1990 cell | 0.0013 |
| Figure 8B | FTO-WT-vs-Vector | <0.0001 |
| Figure 8B | FTO-WT+siCtrl-vs-FTO-WT | 0.3486 |
| Figure 8B | FTO-WT+siPJA2-429-vs-FTO-WT+siCtrl | <0.0001 |

| Wound Width |
|-------------|
| Position  | Comparison  | p-Value  |
| Figure 5C | FTO-mut-vs-NC in BxPC-3 cell | 0.9985 |
| Figure 5C | FTO-WT-vs-NC in BxPC-3 cell | 0.0177 |
| Figure 5C | siFTO-1-vs-NC in BxPC-3 cell | 0.0001 |
| Figure 5C | siFTO-2-vs-NC in BxPC-3 cell | <0.0001 |
| Figure 5C | siFTO-3-vs-NC in BxPC-3 cell | 0.0025 |
| Figure S2C | FTO-mut-vs-NC in SW1990 cell | 0.9991 |
| Figure S2C | FTO-WT-vs-NC in SW1990 cell | 0.0069 |
| Figure S2C | siFTO-1-vs-NC in SW1990 cell | 0.0294 |
| Figure S2C | siFTO-2-vs-NC in SW1990 cell | 0.0183 |
| Figure S2C | siFTO-3-vs-NC in SW1990 cell | 0.0981 |
| Figure 7G | siPJA2-429-vs-NC in BxPC-3 cell | <0.0001 |
| Figure 7G | siPJA2-122-vs-NC in BxPC-3 cell | <0.0001 |
| Figure 7G | siPJA2-100-vs-NC in BxPC-3 cell | <0.0001 |
| Figure S3D | siPJA2-429-vs-NC in SW1990 cell | <0.0001 |
| Figure S3D | siPJA2-122-vs-NC in SW1990 cell | 0.0015 |
| Figure S3D | siPJA2-100-vs-NC in SW1990 cell | 0.0001 |
| Figure 8C | FTO-WT-vs-Vector | 0.0076 |
| Figure 8C | FTO-WT+siCtrl-vs-FTO-WT | 0.9695 |
| Figure 8C | FTO-WT+siPJA2-429-vs-FTO-WT+siCtrl | 0.0015 |

Transwell invasion results

| Position | Comparison | p-Value |
|----------|------------|---------|
| Figure 5D | FTO-mut-vs-NC in BxPC-3 cell | 0.9895 |
| Figure 5D | FTO-WT-vs-NC in BxPC-3 cell | 0.0003 |
| Figure 5D | siFTO-1-vs-NC in BxPC-3 cell | 0.0004 |
| Figure 5D | siFTO-2-vs-NC in BxPC-3 cell | 0.0001 |
| Figure 5D | siFTO-3-vs-NC in BxPC-3 cell | 0.0035 |
| Figure S2D | FTO-mut-vs-NC in SW1990 cell | 0.5773 |
| Figure S2D | FTO-WT-vs-NC in SW1990 cell | 0.0012 |
| Figure S2D | siFTO-1-vs-NC in SW1990 cell | 0.0003 |
| Figure S2D | siFTO-2-vs-NC in SW1990 cell | 0.0001 |
| Figure S2D | siFTO-3-vs-NC in SW1990 cell | 0.0002 |
| Figure 7H | siPJA2-429-vs-NC in BxPC-3 cell | 0.0005 |
| Figure 7H | siPJA2-122-vs-NC in BxPC-3 cell | 0.0003 |
| Figure 7H | siPJA2-100-vs-NC in BxPC-3 cell | 0.0069 |
| Figure S3E | siPJA2-429-vs-NC in SW1990 cell | 0.0008 |
| Figure S3E | siPJA2-122-vs-NC in SW1990 cell | 0.0029 |
| Figure S3E | siPJA2-100-vs-NC in SW1990 cell | 0.0036 |
| Figure 8D | FTO-WT-vs-Vector | 0.0054 |
| Figure 8D | FTO-WT+siCtrl-vs-FTO-WT | 0.4033 |
| Figure 8D | FTO-WT+siPJA2-429-vs-FTO-WT+siCtrl | 0.0018 |

Table S1 cBioPortal analysis of the top 20 genes significantly positively or negatively related to FTO in PAAD

| Positively correlation | Negatively correlation |
|------------------------|------------------------|
|                        |                        |

Figures 5C, Figure S2C, Figure 7G, Figure S3D, Figure 8C, Figure S2D, Figure 7H, Figure 8D, Figure 8C, Figure S3D, Figure 7H, Figure 8D, Figure S3D, Figure 8D, and Table S1.
| Gene     | Pearson's Correlation | p-Value  | Gene     | Pearson's Correlation | p-Value  |
|----------|-----------------------|----------|----------|-----------------------|----------|
| NEK1     | 0.78                  | 1.69E-38 | TRIM11   | -0.73372              | 1.59E-31 |
| BBS9     | 0.77                  | 1.14E-36 | SIGIRR   | -0.72754              | 8.91E-31 |
| AKT3     | 0.76                  | 1.66E-34 | RASSF7   | -0.71859              | 9.96E-30 |
| CYP2U1   | 0.74                  | 8.61E-33 | YDJC     | -0.71014              | 8.94E-29 |
| PJA2     | 0.74                  | 2.1E-32  | ARRD1C1  | -0.70755              | 1.72E-28 |
| DNAJC18  | 0.73                  | 4.95E-31 | ESRRA    | -0.701                | 8.78E-28 |
| MPDZ     | 0.73                  | 6.75E-31 | POLD4    | -0.69942              | 1.29E-27 |
| MYH10    | 0.73                  | 1.07E-30 | ANO9     | -0.6968               | 2.44E-27 |
| ZYG11B   | 0.73                  | 1.22E-30 | LPAR2    | -0.68748              | 2.21E-26 |
| FAM168A  | 0.72                  | 6.9E-30  | SIRT7    | -0.68645              | 2.81E-26 |
| TENM3    | 0.72                  | 7.41E-30 | NDOR1    | -0.68432              | 4.59E-26 |
| PLEKHM3  | 0.72                  | 1.11E-29 | VPS37B   | -0.68073              | 1.04E-25 |
| PLXNC1   | 0.72                  | 1.26E-29 | EBP      | -0.67842              | 1.74E-25 |
| PKD2     | 0.71                  | 6.27E-29 | GSDMD    | -0.67796              | 1.93E-25 |
| DSYTK    | 0.71                  | 1.25E-28 | POLR2H   | -0.67475              | 3.94E-25 |
| WDR47    | 0.71                  | 2.77E-28 | CIB1     | -0.6747               | 3.99E-25 |
| PEG3     | 0.70                  | 5.06E-28 | BIK      | -0.67327              | 5.46E-25 |
| TBCEL    | 0.70                  | 5.72E-28 | TNIP2    | -0.67296              | 5.85E-25 |
| MBD5     | 0.70                  | 7.99E-28 | NUDT8    | -0.6715               | 8.04E-25 |
| MAP1A    | 0.70                  | 1.22E-27 | ZDHHHC12 | -0.67006              | 1.1E-24  |

Table S2 LinkedOmics analysis of the top 20 genes significantly positively or negatively related to FTO in PAAD

| Gene       | Pearson's Correlation | p-Value  | Gene     | Pearson's Correlation | p-Value  |
|------------|-----------------------|----------|----------|-----------------------|----------|
| NEK1       | 0.79                  | 6.7E-40  | TRIM11   | -0.75011              | 1.96E-33 |
| BBS9       | 0.78                  | 2.2E-37  | SIGIRR   | -0.73969              | 4.29E-32 |
| CYP2U1     | 0.77                  | 9.11E-36 | ARRD1C1  | -0.72265              | 4.88E-30 |
| AKT3       | 0.76                  | 2.37E-34 | EBP      | -0.72036              | 9E-30    |
| PJA2       | 0.75                  | 9.5E-34  | NUDT8    | -0.71752              | 1.9E-29  |
| MYH10      | 0.75                  | 1.6E-33  | ZDHHHC12 | -0.71301              | 6.12E-29 |
| PLEKHM3    | 0.74                  | 1.19E-32 | YDJC     | -0.71284              | 6.39E-29 |
| ZYG11B     | 0.74                  | 3.16E-32 | NDOR1    | -0.70702              | 2.79E-28 |
| KATNAL1    | 0.74                  | 1.46E-31 | RASSF7   | -0.70256              | 8.43E-28 |
| WDR47      | 0.73                  | 1.97E-31 | ESRRRA   | -0.69954              | 1.76E-27 |
| MPDZ       | 0.73                  | 4.5E-31  | PLEKHM3  | -0.69802              | 2.54E-27 |
| MBD5       | 0.73                  | 5.36E-31 | EFCAB4A  | -0.69469              | 5.64E-27 |
| LOC653653  | 0.73                  | 8.56E-31 | MXD3     | -0.6919               | 1.09E-26 |
| SERINC1    | 0.73                  | 9.85E-31 | GIYD2    | -0.69167              | 1.15E-26 |
| ZNF25      | 0.72                  | 2.77E-30 | CIB1     | -0.69059              | 1.48E-26 |
| UHRF1BP1L  | 0.72                  | 2.82E-30 | POLR2H   | -0.69013              | 1.65E-26 |
| Gene   | Pearson's Correlation | p-Value | Gene   | Pearson's Correlation | p-Value |
|--------|------------------------|---------|--------|------------------------|---------|
| WDR47  | 0.83                   | <0.05   | COMTD1 | -0.5                   | <0.05   |
| ZNF25  | 0.83                   | <0.05   | NUDT8  | -0.47                  | <0.05   |
| ZYG11B | 0.81                   | <0.05   | TPRN   | -0.46                  | <0.05   |
| ZFP90  | 0.81                   | <0.05   | EFCAB4A| -0.45                  | <0.05   |
| AKT3   | 0.81                   | <0.05   | LPAR2  | -0.45                  | <0.05   |
| RNF11  | 0.8                    | <0.05   | ADAT3  | -0.44                  | <0.05   |
| PLDN   | 0.8                    | <0.05   | STAP2  | -0.44                  | <0.05   |
| KIDINS220 | 0.8                   | <0.05   | TMEM52 | -0.43                  | <0.05   |
| PIGK   | 0.79                   | <0.05   | LOC100271831 | -0.42     | <0.05   |
| TRPC1  | 0.79                   | <0.05   | TCIRG1 | -0.42                  | <0.05   |
| EID1   | 0.79                   | <0.05   | RASSF7 | -0.42                  | <0.05   |
| GLG1   | 0.79                   | <0.05   | YDJC   | -0.42                  | <0.05   |
| PLEKHM3| 0.78                   | <0.05   | LENG9  | -0.42                  | <0.05   |
| PJA2   | 0.78                   | <0.05   | KLK1   | -0.41                  | <0.05   |
| KATNAL1| 0.78                   | <0.05   | SPINK1 | -0.41                  | <0.05   |
| UBQLN2 | 0.78                   | <0.05   | PLEKJ1 | -0.41                  | <0.05   |
| CBX1   | 0.78                   | <0.05   | MPST   | -0.4                   | <0.05   |
| SPIN1  | 0.78                   | <0.05   | SLC25A10 | -0.4               | <0.05   |
| DSTYK  | 0.78                   | <0.05   | FAM195A| -0.4                   | <0.05   |
| UHRF1BP1L | 0.78           | <0.05   | ANO9   | -0.4                   | <0.05   |

Table S3 UALCAN analysis of the top 20 genes significantly positively or negatively related to FTO in PAAD

| Antibody            | Application | Dilution fold | Company           | Catalog number |
|---------------------|-------------|---------------|-------------------|----------------|
| rabbit anti-FTO     | WB          | 1:1000        | Abcam             | ab126605       |
| rabbit anti-FTO     | IHC         | 1:500         | Abcam             | ab126605       |
| rabbit anti-YTHDF1  | WB          | 1:1000        | Affinity Biosciences | AF0462         |
| rabbit anti-YTHDF2  | WB          | 1:1000        | Abcam             | ab220163       |
| rabbit anti-PJA2    | WB          | 1:500         | Affinity Biosciences | DF4021         |
| rabbit anti-β-catenin| WB        | 1:1000        | Affinity Biosciences | AF6266         |
| rabbit anti-Wnt5a   | WB          | 1:1000        | Affinity Biosciences | DF6856         |
| rabbit anti-LEF1    | WB          | 1:1000        | Abcam             | ab137872       |
| rabbit anti-GSK-3β  | WB          | 1:5000        | Abcam             | ab32391        |
| rabbit anti-pS9-GSK-3β| WB       | 1:10000       | Abcam             | ab75814        |
| Primer name     | Primer sequence (from 5' to 3') | Table S5 Primer sequences used in this study |
|----------------|--------------------------------|---------------------------------------------|
| METTL3 FORWARD | TTGTCTCAAACCCTCCGTAGT         |                                             |
| METTL3 REVERSE | CCAGATCAGAGAGTGGTAG           |                                             |
| METTL14 FORWARD| GAGTGTGTTTACGAAAAATGGG         |                                             |
| METTL14 REVERSE| CCGTCTGGCTACCGGTTCA           |                                             |
| WTAP FORWARD   | ACTGGCCTAAGAGAGTCGAAG         |                                             |
| WTAP REVERSE   | GTTGCTAGTCGCAATTACAAGGA       |                                             |
| VIRMA FORWARD  | AAGTGGCCCTGTGTTTGAGT          |                                             |
| VIRMA REVERSE  | ACCAGACCATAGATTACCCTCGT      |                                             |
| RBMX FORWARD   | TACTCAAGCAGCAGGAGTGG          |                                             |
| RBMX REVERSE   | AGGGTACCACCCTTTCCATAG         |                                             |
| RBM15 FORWARD  | GGCTGCTAGAGAGGTGGG           |                                             |
| RBM15 REVERSE  | CCGCTACTGCTCAATGTCGCCGACTG    |                                             |
| RBM15B FORWARD | ATCTTTCAAGAGTCGCTACGG         |                                             |
| ZC3H13 FORWARD | GATCAAGTAGACGCTAGGAGAC        |                                             |
| ZC3H13 REVERSE | CTCTGCTGATGGTGCTCATATCGA      |                                             |
| FTO FORWARD    | GCTGCTATTCCGAGGACCTG          |                                             |
| FTO REVERSE    | AGCCTTGATTACCAATGAGG          |                                             |
| ALKBH5 FORWARD | GCAAGGTAGAGAGGCGGCAATCC       |                                             |
| ALKBH5 REVERSE | GTCCACCCTGCTGCTGTTGACTA       |                                             |
| YTHDF1 FORWARD | ACCTGTCAGTCATTACCG           |                                             |
| YTHDF1 REVERSE | TGGTGAGGTATTGCCGAGGAATCGGAG   |                                             |
| YTHDF2 FORWARD | CTTAGTGCGGAGCCATGAGT          |                                             |
| YTHDF2 REVERSE | TCTGCTGATCCACCTTACGT          |                                             |
| AKT3 FORWARD   | AATGGACAGAAGCTATCCAGG         |                                             |
| AKT3 REVERSE   | TGATGGGTTGAGAGGCATCC          |                                             |
| PJA2 FORWARD   | CAGCAGCAATGGAGCCAAGAAT         |                                             |
| PJA2 REVERSE   | TGCTTGAATTGTGTGATACCTCT       |                                             |
| PLEKHM3 FORWARD| GTGGGCGATGAGTACTCAG           |                                             |
| PLEKHM3 REVERSE| GTGGTCCTCTGCTGGACCATAA        |                                             |
| ZYG11B FORWARD | GAGGAGGCGCTCCTCTATTCC         |                                             |
| ZYG11B REVERSE | GCATCTGGTGCCCCCTAAAA          |                                             |
| WDR47 FORWARD  | CTCTGCTGACCAATCATGCC          |                                             |
| WDR47 REVERSE  | AGAAGCCTCAGCTTTCTGTAAT        |                                             |
| NUDT8 FORWARD  | CAGTTTCCAGCGGTAAGT            |                                             |
| NUDT8 REVERSE  | CACGTTGCGAAGTACTGGGA          |                                             |
YDJC  FORWARD   TTTCTTGGAAGATGGGATTC
YDJC  REVERSE  GGAAGCAGCTTAGTTGGGC
RASSF7  FORWARD TCTGTGGGTCTCAGAGCAG
RASSF7  REVERSE TGCGCTCAGGACAAACTG
CTNNB1  FORWARD CATCTACACAGTGTGATGCT
CTNNB1  REVERSE GCAAGTTTGTCACTTCTCAGGGA
WNT5A  FORWARD GCACTATCAATTCCAGTGACATC
WNT5A  REVERSE TCACCGCGTATGTGAGGGC
WIF1  FORWARD CACAGAGAATGCCAGCTTCT
WIF1  REVERSE GATCTGCCCATGATGCCCTTTATC
LEF1  FORWARD TGCCAAATATGAAATACGCACCCCA
LEF1  REVERSE GAGAAAGTGCTCGTCAGTCT
AXIN1  FORWARD TGGAAGCCCTGTTACATC
AXIN1  REVERSE GGACACGATGCCATTGTTATC
GSK3B  FORWARD GCAGCATGAAAGTTAGCAGA
GSK3B  REVERSE GGCGACCAGTTCTCGGAATC
GAPDH  FORWARD AAGGGCTCGGGGCTATTG
GAPDH  REVERSE AGGGGCATCCACAGTCTTC
HPRT1  FORWARD CCTGGCGCTGTAGTCTGAT
HPRT1  REVERSE AGACGTTCCAGTCCCTCATAA
Figure S1 The impact of FTO and PJA2 expression on the OS of PAAD patients in different stages separately. (A,B) A Kaplan-Meier plot and Log-rank test analyzed the survival data of PAAD patients stage I and II from TCGA separately. Red and black represent the FTO high and low expression groups separately. (C,D) A Kaplan-Meier plot and Log-rank test analyzed the survival data of PAAD patients stage I and II from TCGA separately. Red and black represent the PJA2 high and low expression groups separately. As sample number in stage III and IV was very low for meaningful analysis, only stage I and II cases were evaluated. PAAD, pancreatic adenocarcinoma; TCGA, The Cancer Genome Atlas.
Figure S2 Decreased FTO expression promotes proliferation, migration, and invasion of pancreatic cancer cell SW1990 in vitro. (A,B) IncuCyte Zoom live cell imaging system and clonogenic cell survival assays detected proliferation of SW1990 cells after siRNAs knockdown (siFTO-1, siFTO-2 and siFTO-3) or overexpression of FTO. Simultaneously, CCK8 assay was performed to assess the proliferation potential of these cells at 48 h. (C) Wound healing migration assays revealed the effect of FTO on the migration ability of SW1990 cells. The gap was measured at 0, 24, 48, 72, and 96 h after the scratch was performed. (D) Transwell invasion assays revealed the effect of FTO on the invasion ability of SW1990 cells. CCK8, Cell Counting Kit-8; FTO-WT, wild-type FTO; FTO-mut, loss-of-function mutated FTO; NC, negative control; siRNAs, small interference RNAs. *P < 0.05; **P < 0.01; ***P < 0.001; ns, no statistical significance by unpaired Student’s t-test.
Figure S3 PJA2 inhibits proliferation, migration, and invasion of pancreatic cancer cell SW1990 *in vitro*. (A) RT-qPCR and WB confirmed that PJA2 expression was significantly reduced after siRNAs (siPJA2-429, siPJA2-122, and siPJA2-100) transfection in SW1990 cells. (B,C) IncuCyte Zoom live cell imaging system and clonogenic cell survival assays showed the proliferation of SW1990 cells after PJA2 knockdown. Simultaneously, CCK8 kit was used to assess the proliferation potential of SW1990 cells after PJA2 knockdown at 48 h. (D) Wound healing migration assays revealed the effect of PJA2 on the migration ability of SW1990 cells. The gap was measured at 0, 24, 48, 72, and 96 h after the scratches were performed. (E) Transwell invasion assays revealed the effect of PJA2 on the invasion ability of SW1990 cells. CCK8, Cell Counting Kit-8; NC, negative control; siRNAs, small interference RNAs. *P < 0.05; **P < 0.01; ***P < 0.001; ns, no statistical significance by unpaired Student’s *t*-test.