Supplementary

Table S1 No change in acute pancreatitis or inflammatory markers following ductal delivery of scAAV6 vector

Serum parameter	Day 1 post-injection			Day 3 post-injection		
	SO Ctrl (n=6)	scAAV6 (n=12)	P value [unpaired *t*-test]	SO Ctrl (n=6)	scAAV6 (n=12)	P value [unpaired *t*-test]
Amylase (U/dL)	1558±122	1666±230	0.21	1591±146	1700±274	0.29
Lipase (U/dL)	81.1±12.3	89.4±14.9	0.23	81.4±12.8	86.6±12.5	0.43
Crp (ng/mL)	198.9±27.9	223.1±43.4	0.17	199.4±32.9	215.0±40.0	0.39
Tnf-α (pg/mL)	2.48±0.36	2.74±0.45	0.21	2.46±0.39	2.76±0.62	0.23
Il-1β (pg/mL)	3.29±0.22	3.54±0.70	0.28	3.24±0.14	3.27±0.56	0.90

Data represented as means ± standard deviations (SDs). SO Ctrl, sham-operated control; Tap, trypsinogen activation peptide; Crp, C-reactive protein; Tnf-α, tumor necrosis factor-alpha

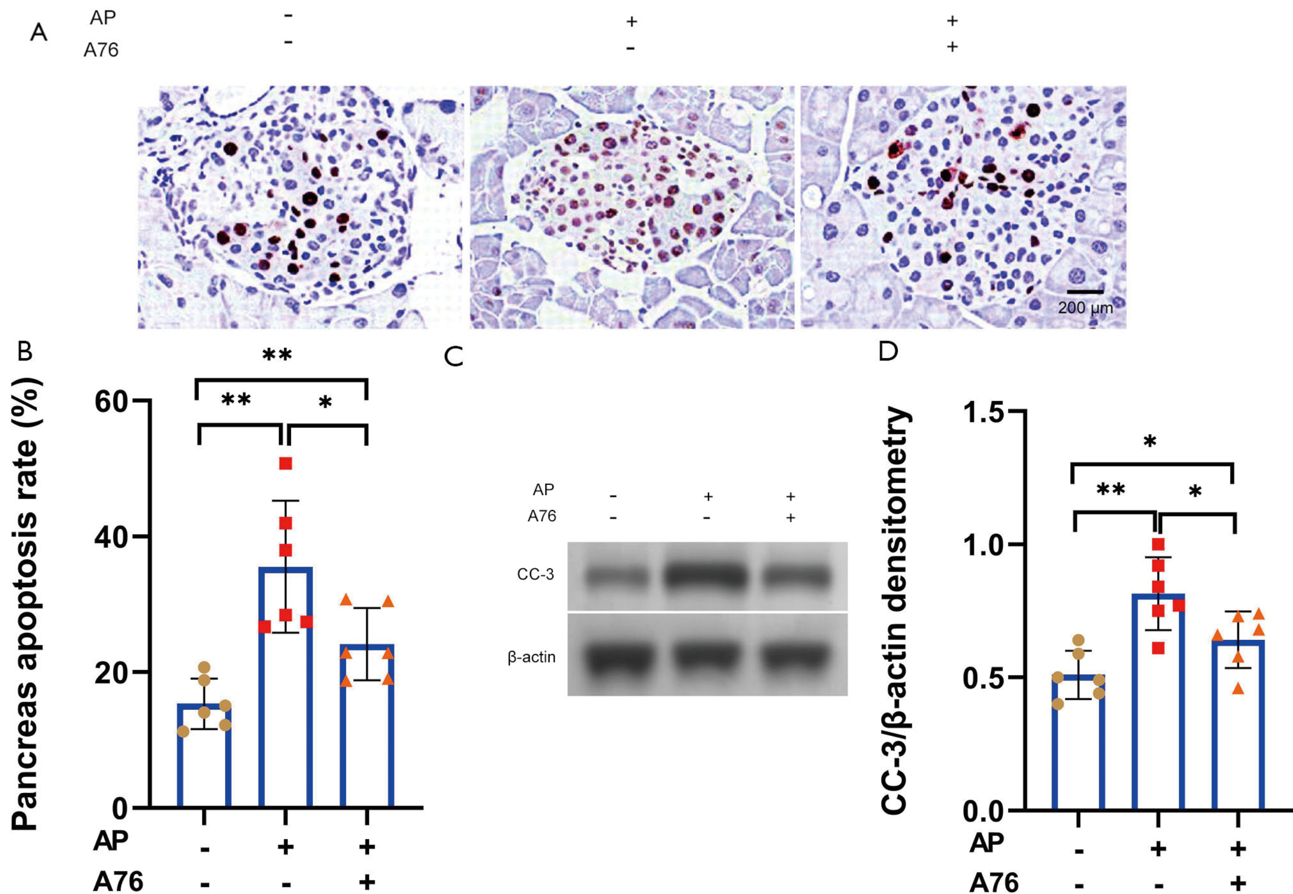

Figure S1 Enhancing Ampkα activity during acute pancreatitis reduces cell apoptosis. (A) Representative TUNEL staining measuring (B) pancreatic cell apoptosis in tissues from the treatment groups described in Figure 1. (C,D) Representative immunoblots and ImageJ densitometric quantitation of pancreatic cleaved caspase-3 (CC-3) expression. Data represented as means ± SDs. N=6 rats per cohort. *P<0.05, **P<0.01 (one-way ANOVA with Bonferroni post-hoc).

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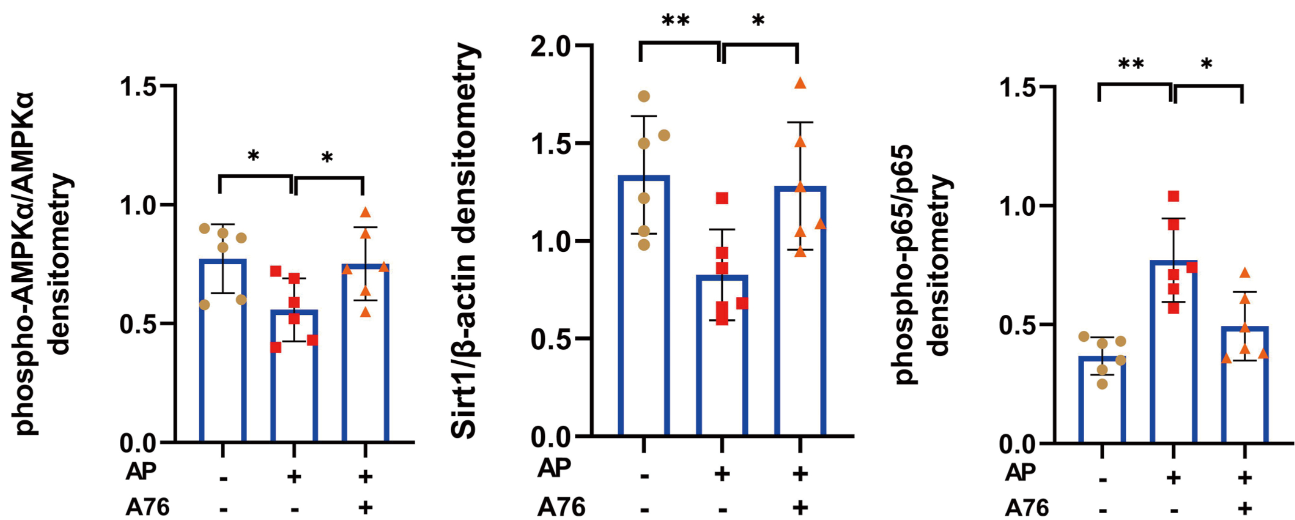

Figure S2 Densitometric analysis of Figure 1F immunoblots. (A-C) Quantitation of pancreatic Ampkα/Sirt1/NF-κB signalling protein expression by ImageJ densitometry. β-actin used as loading control. Data represented as means ± SDs. N=6 rats per cohort. *P<0.05, **P<0.01 (one-way ANOVA with Bonferroni post-hoc).

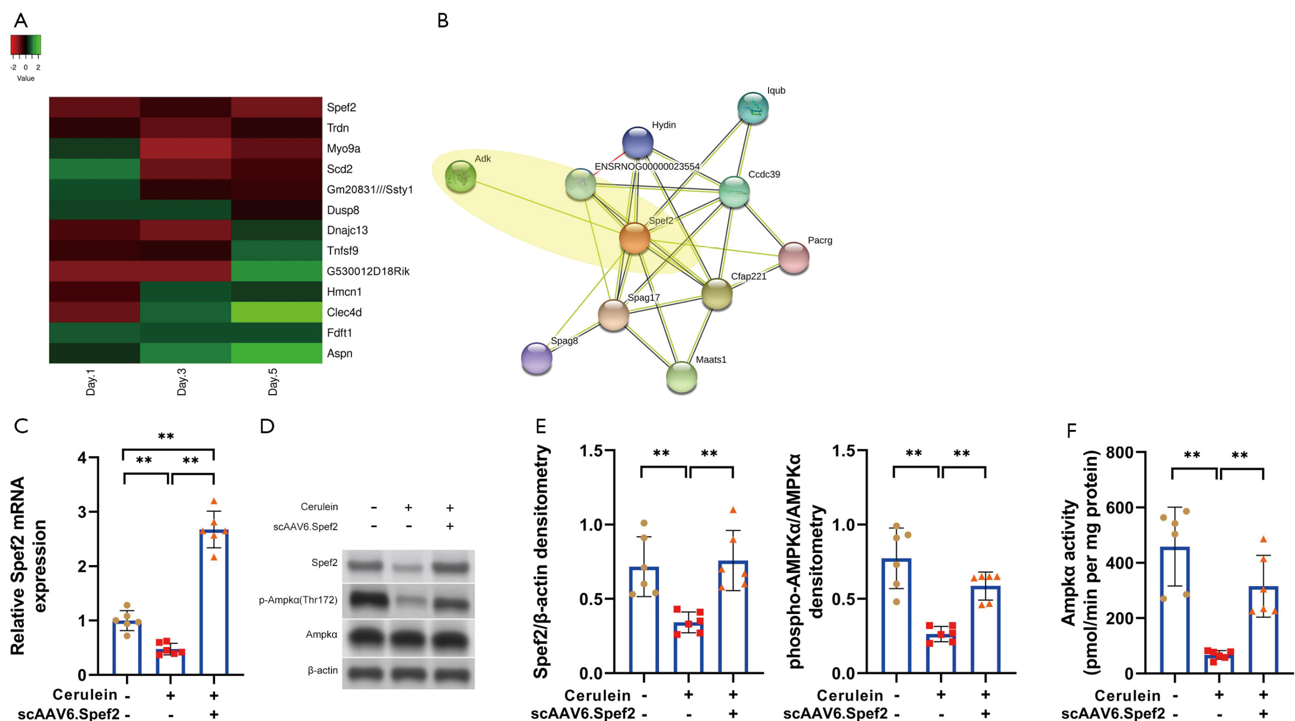

Figure S3 Spef2 overexpression promotes Ampkα phosphorylation and Ampkα activity in AR42J cells. (A) Heatmap of significantly dysregulated genes in microarray data derived from a murine model of cerulein-induced acute pancreatitis. (B) STRING protein-protein interaction analysis (medium confidence>0.040) reveals that rat Spef2 interacts with rat adenosine kinase (Adk). (C) qPCR of Spef2 mRNA expression in AR42J cells following adenoviral vector delivery of rat Spef2 (scAAV6.Spef2) or negative control (scAAV6.Ctrl). (D,E) Representative immunoblots and ImageJ densitometric quantitation of Spef2 expression and Ampkα^{Thr172} phosphorylation and (F) Ampkα activity assay in scAAV6.Ctrl AR42J cells and scAAV6.Spef2 AR42J cells. Data represented as means ± SDs. N=3 biological replicates ×2 technical replicates. **P<0.01 (one-way ANOVA with Bonferroni post-hoc).

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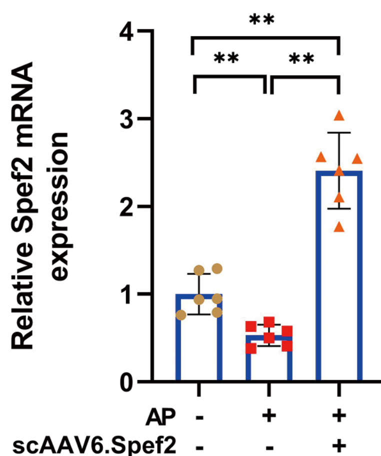

Figure S4 qPCR validation of Spef2 overexpression in scAAV6.Spef2 rats. Quantitation of pancreatic tissue *Spef2* mRNA expression by qPCR. *Gapdh* used as housekeeping control. Data represented as means ± SDs. N=6 rats per cohort. **P<0.01 (one-way ANOVA with Bonferroni post-hoc).

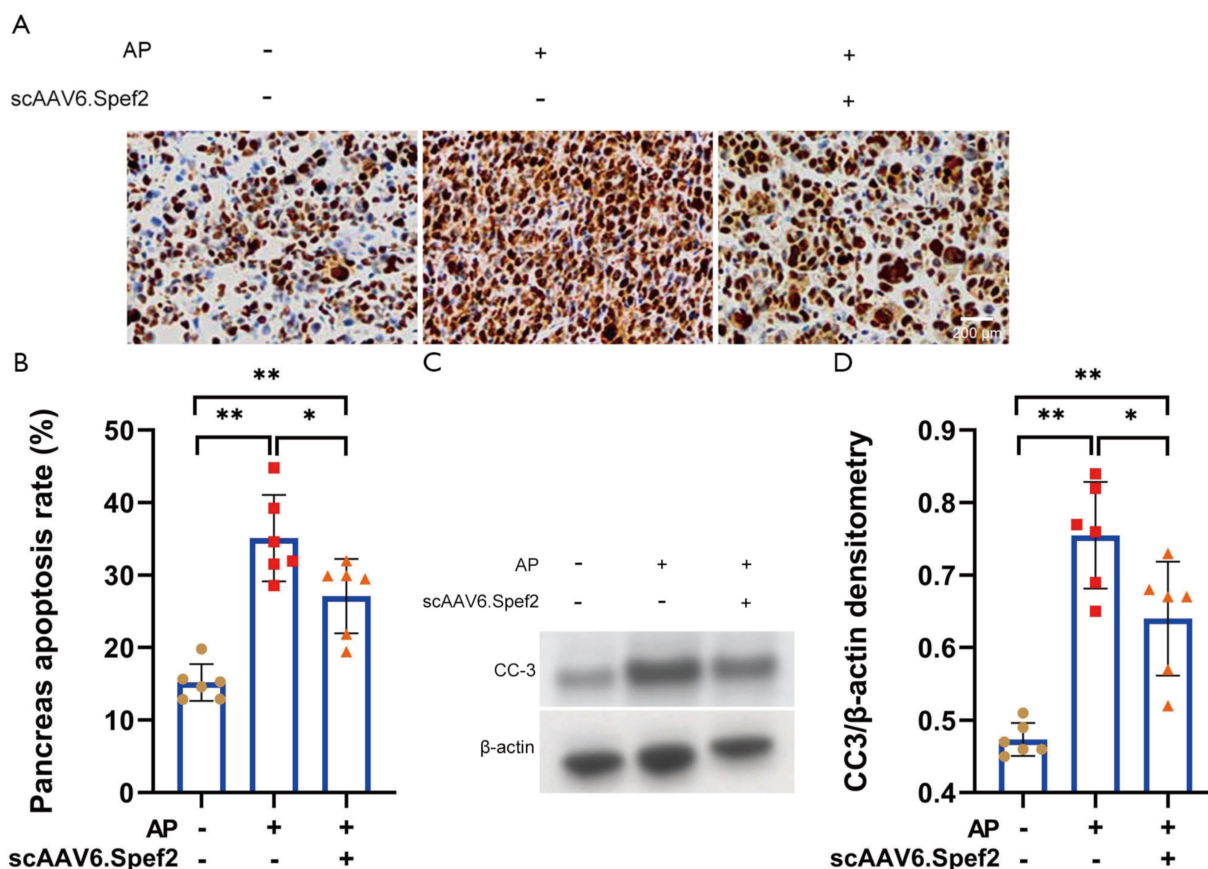

Figure S5 Spef2 overexpression during acute pancreatitis reduces cell apoptosis. (A) Representative TUNEL staining measuring (B) pancreatic cell apoptosis in tissues from the treatment groups described in Figure 2. (C,D) Representative immunoblots and ImageJ densitometric quantitation of pancreatic cleaved caspase-3 (CC-3) expression. Data represented as means ± SDs. N=6 rats per cohort. *P<0.05, **P<0.01 (one-way ANOVA with Bonferroni post-hoc).

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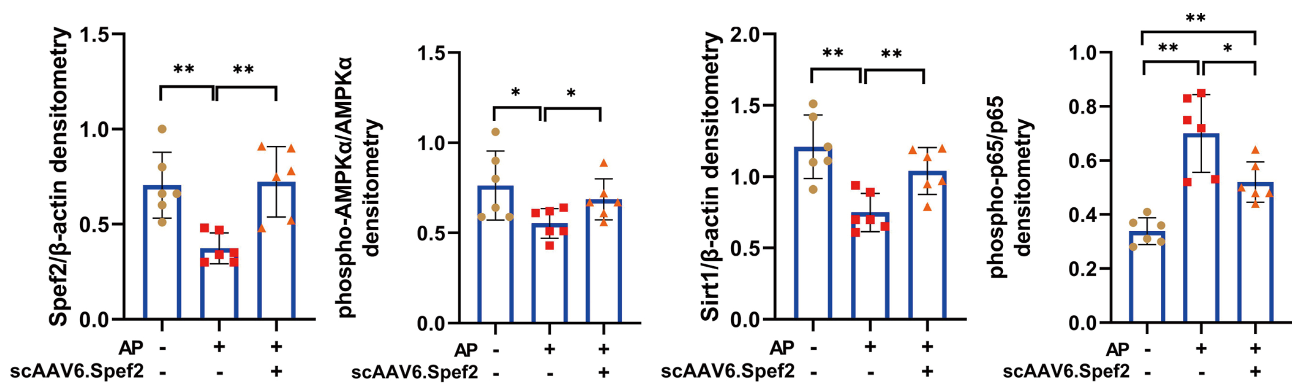

Figure S6 Densitometric analysis of Figure 2F immunoblots. Quantitation of pancreatic Spef2 and Ampkα/Sirt1/NF-κB signalling protein expression by ImageJ densitometry. β-actin used as loading control. Data represented as means ± SDs. N=6 rats per cohort. *P<0.05, **P<0.01 (one-way ANOVA with Bonferroni post-hoc).

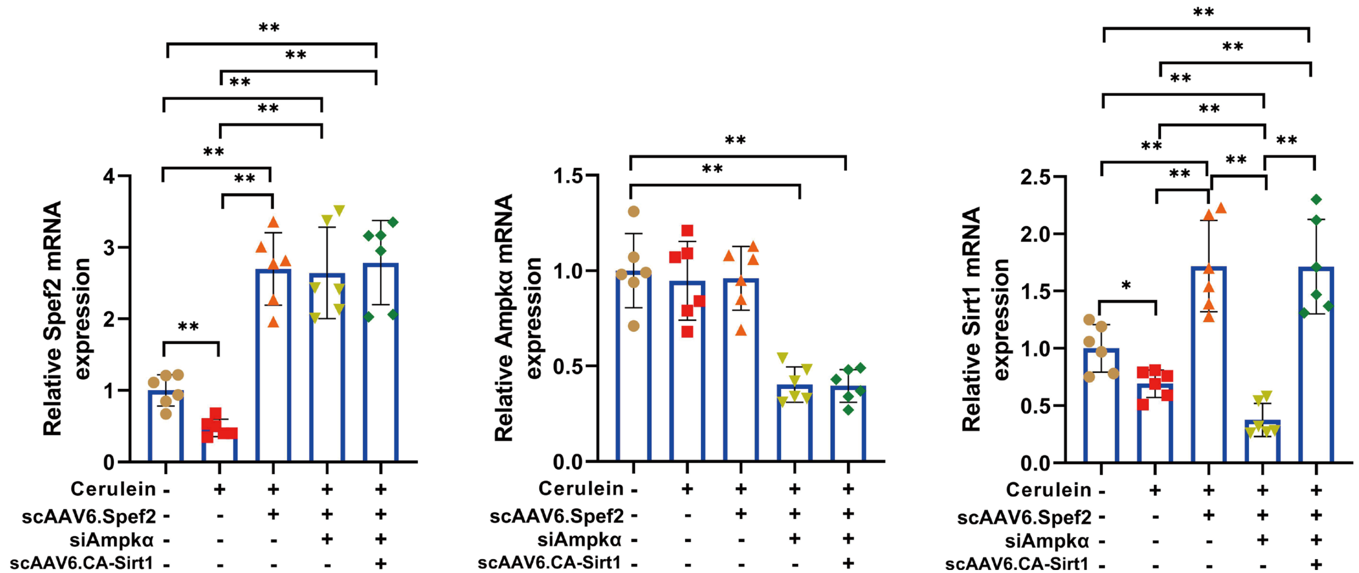

Figure S7 qPCR validation of gene overexpression and silencing in AR42J cells. Quantitation of AR42J cell *Spef2*, *Ampkα*, and *Sirt1* mRNA expression by qPCR. *Gapdh* used as housekeeping control. Data represented as means ± SDs. N=3 biological replicates ×2 technical replicates. *P<0.05, **P<0.01 (one-way ANOVA with Bonferroni post-hoc).

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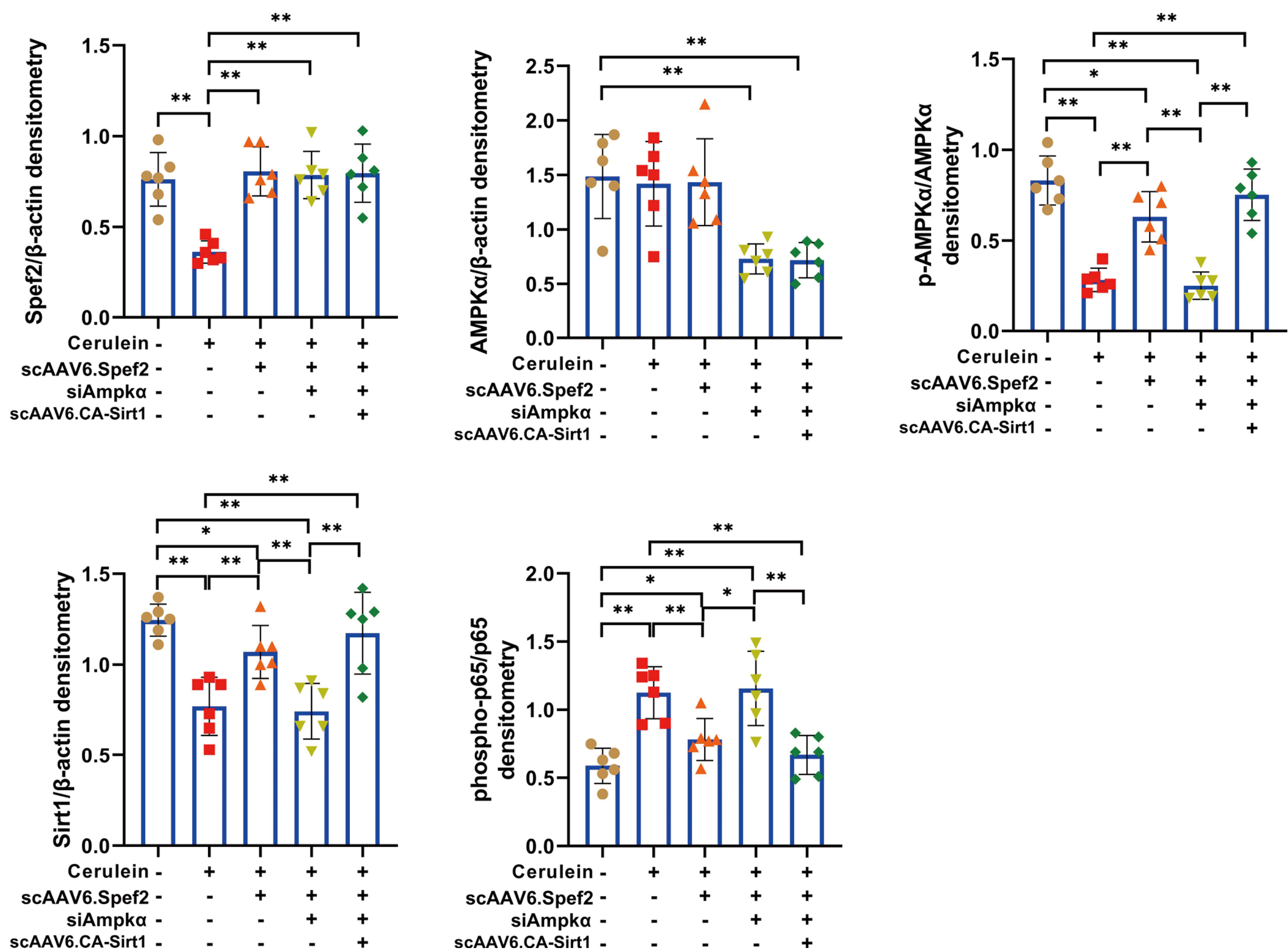

Figure S8 Densitometric analysis of Figure 3A immunoblots. Quantitation of AR42J cell Spef2 and Ampkα/Sirt1/NF-κB signalling protein expression by ImageJ densitometry. β-actin used as loading control. Data represented as means ± SDs. N=3 biological replicates × 2 technical replicates. *P<0.05, **P<0.01 (one-way ANOVA with Bonferroni post-hoc).

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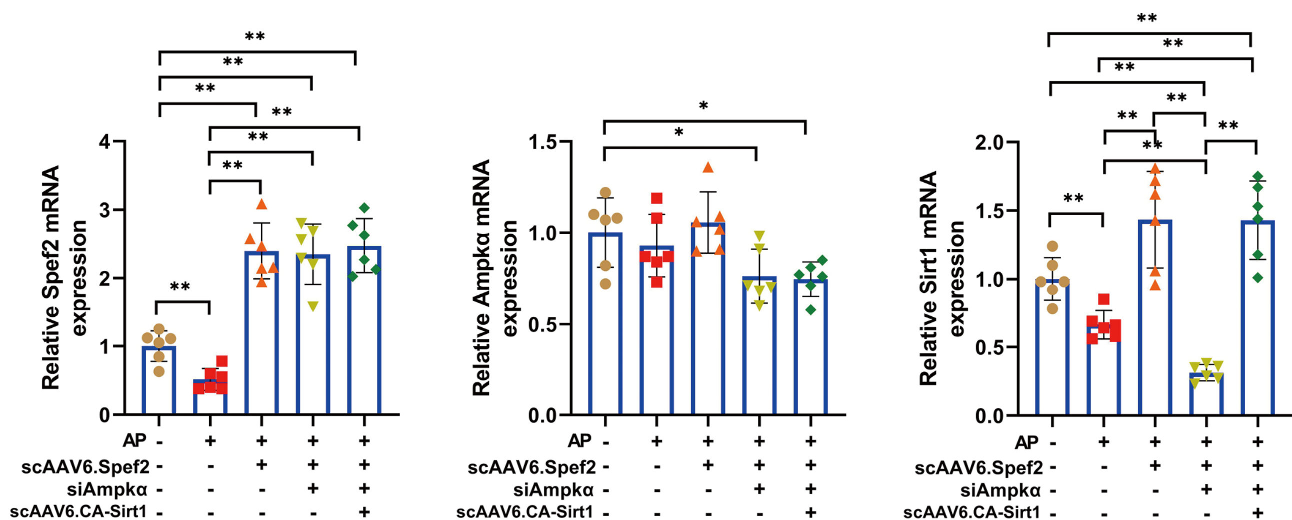

Figure S9 qPCR validation of gene overexpression and silencing in model rats. Quantitation of pancreatic tissue *Spef2*, *Ampkα*, and *Sirt1* mRNA expression by qPCR. *Gapdh* used as housekeeping control. Data represented as means ± SDs. N=6 rats per cohort. *P<0.05, **P<0.01 (one-way ANOVA with Bonferroni post-hoc).

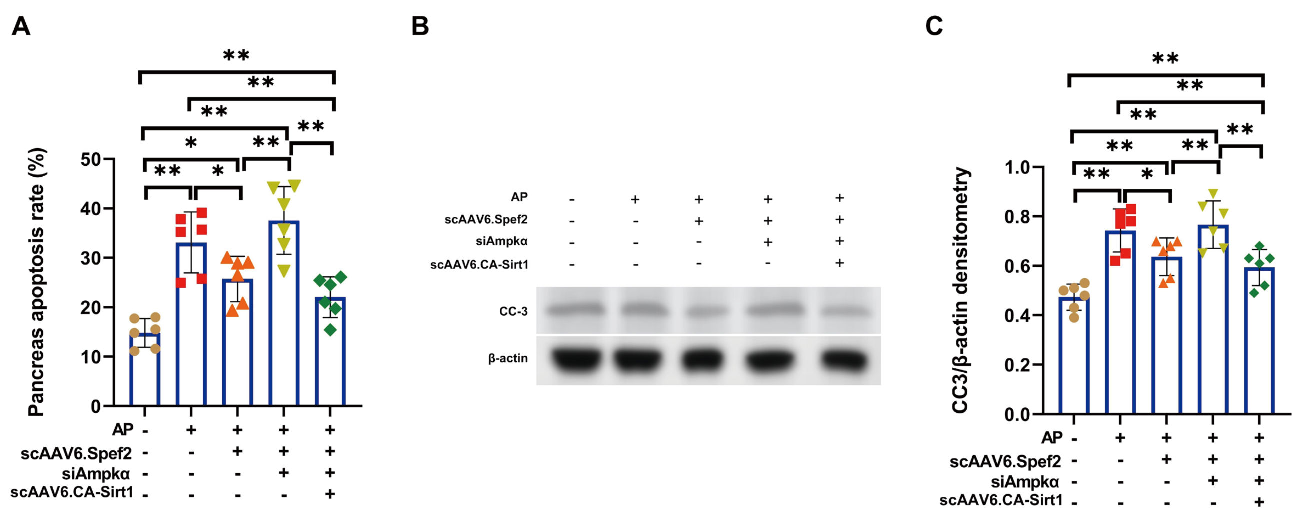

Figure S10 Spef2 overexpression during acute pancreatitis reduces cell apoptosis in a Sirt1/Ampk-dependent manner. (A) TUNEL staining-based assessment of pancreatic cell apoptosis in tissues from the treatment groups described in Figure 4. (B,C) Representative immunoblots and ImageJ densitometric quantitation of pancreatic cleaved caspase-3 (CC-3) expression. Data represented as means ± SDs. N=6 rats per cohort. *P<0.05, **P<0.01 (one-way ANOVA with Bonferroni post-hoc).

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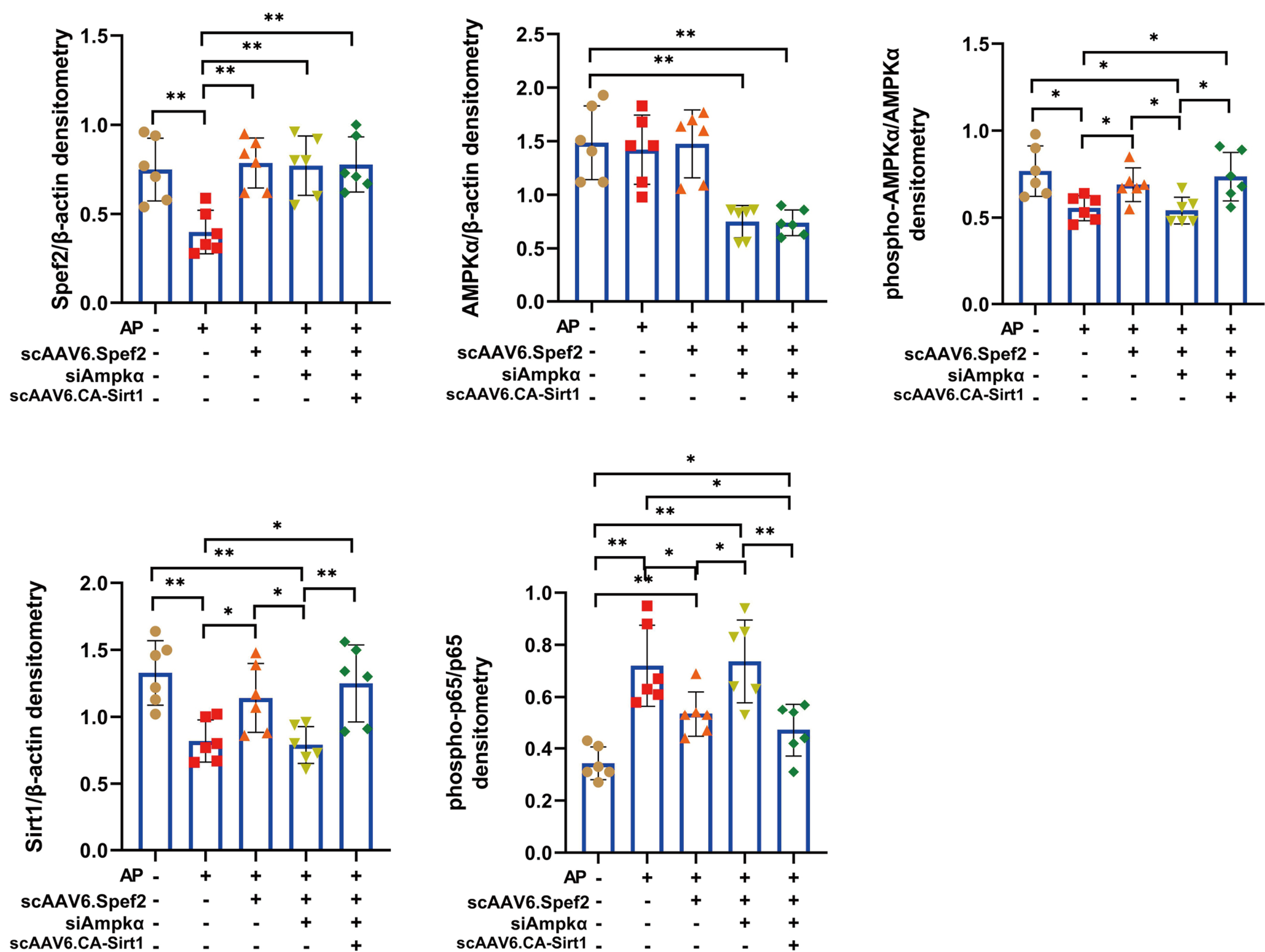

Figure S11 Densitometric analysis of Figure 4F immunoblots. Quantitation of pancreatic Spef2 and Ampkα/Sirt1/NF-κB signalling protein expression by ImageJ densitometry. β-actin used as loading control. Data represented as means ± SDs. N=6 rats per cohort. *P<0.05, **P<0.01 (one-way ANOVA with Bonferroni post-hoc).

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