Supporting Information

Curcumin inhibits liquid–liquid phase separation of fused in sarcoma and attenuates the sequestration of pyruvate kinase to restore cellular metabolism

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Supplementary methods.

CCK-8.

10^4 cells/well were seeded in 96-well plates and incubated with different concentrations (0, 2.5, 5, 10, 20 μM) of curcumin for 24 hours. Then 10 μL of the CCK8 (Elabscience, E-CK-A362) was added to each well. The absorbance of each well was evaluated at a wavelength of 450 nm after incubation of one hour.

Cellular thermal shift assay.

293T-FUS were incubated with 10 μM curcumin and a same volume of vehicle DMSO and resuspend with PBS. The cell suspension mixtures were divided into 8 parts and then heated at a temperature gradient of 49, 52, 55, 58, 61, 64, 67, and 70 ℃ for 3min respectively. After heated the cell lysate were obtained by repeating freeze-thawed, and target proteins were detected by western blot.

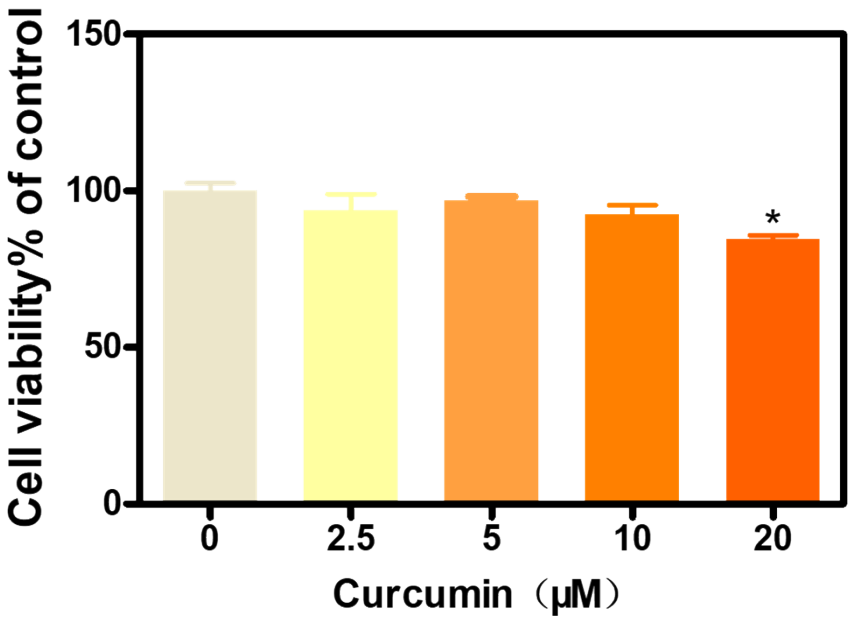

Fig S1. CCK-8 assay of 293T-FUS treated with different concentration of curcumin. The values were illustrated as the mean ± SD. * $P < 0.05$.

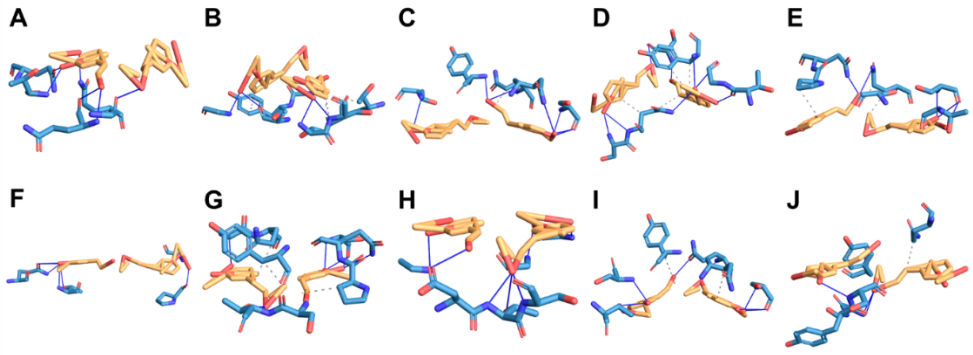

Fig S2. Curcumin docked on FUS LC. FUS LC: fused in sarcoma low-complexity domain

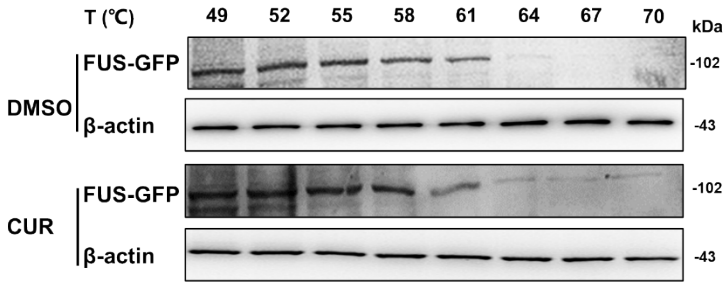

Fig S3. Cellular thermal shift assay results with curcumin treatment.

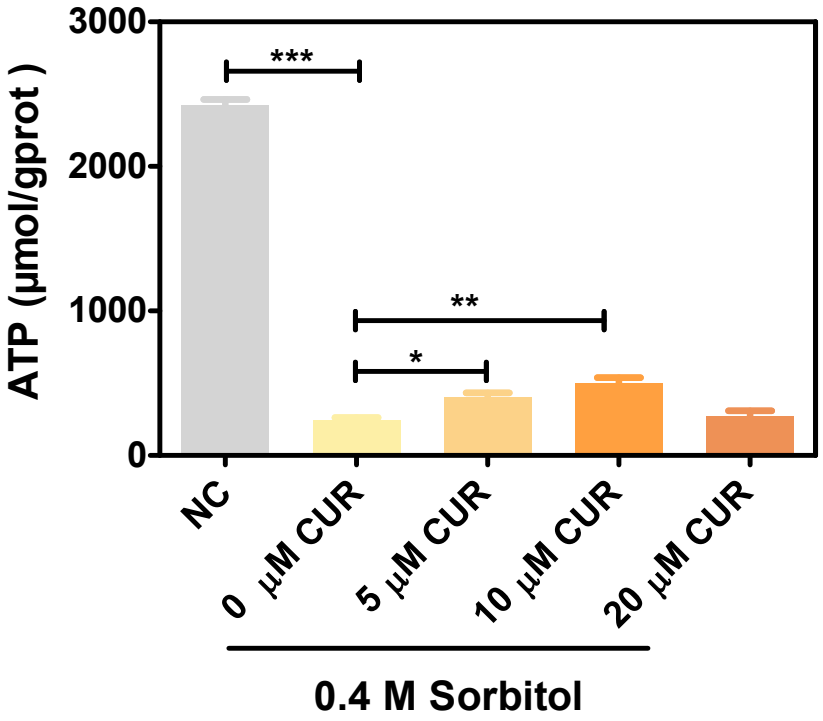

Fig S4. Increased ATP in CUR group. The values were illustrated as the mean ± SD. * $P < 0.05$, ** $P < 0.01$.

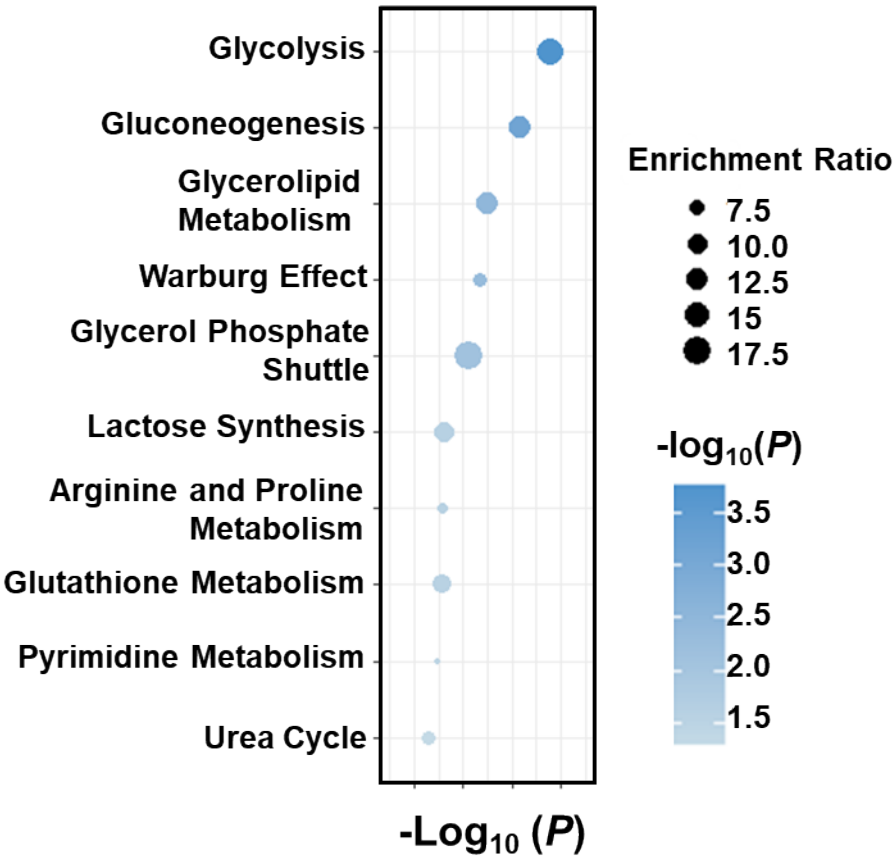

Fig S5. Pathway enrichment of Sorbitol and NC group.

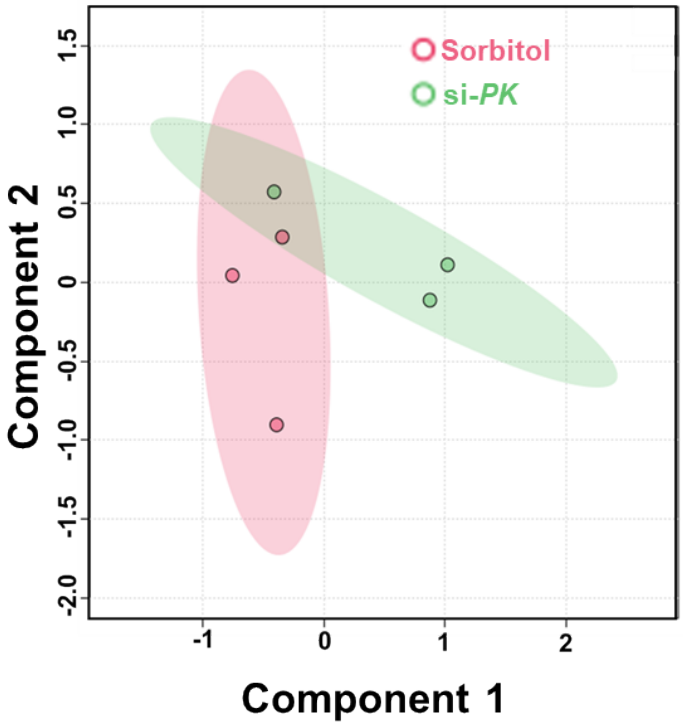

Fig S6. PCA-2D score plot of Sorbitol and si-*PK* group. PCA: principal component analysis

	FUS (10% PEG 8000) (%)	FUS + CUR (10% PEG 8000) (%)
Helix	13.26	16.53
β-sheet	28.52	26.67
β-turn	40.27	18.40
Random coil	13.20	38.40

Table S1. The percentage of protein secondary structure.

T(K)	K_a ($\times 10^4$ M^{-1})	N	ΔH ($\times 10^6$ cal/mol)	ΔS ($\times 10^3$ cal/mol/deg)
310	0.04 ± 0.364	3.58 ± 0.0471	2.797 ± 0.299	9.04

Table S2. Binding constant (K_a), stoichiometric number (N), entropy (ΔS), and enthalpy (ΔH) parameters for the interaction of curcumin with FUS.