Spexin alleviates insulin resistance and inhibits hepatic gluconeogenesis via the FoxO1/PGC-1α pathway in high-fat-diet-induced rats and insulin-resistant cells

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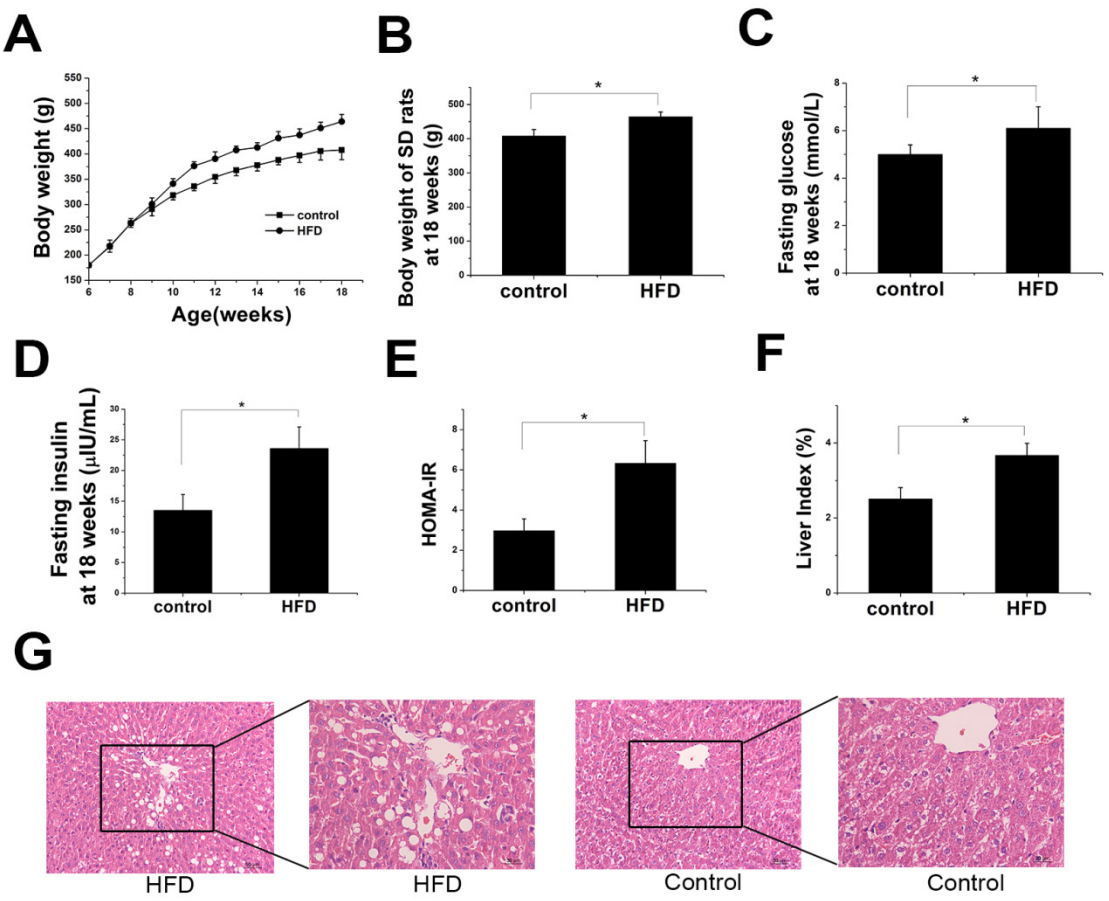
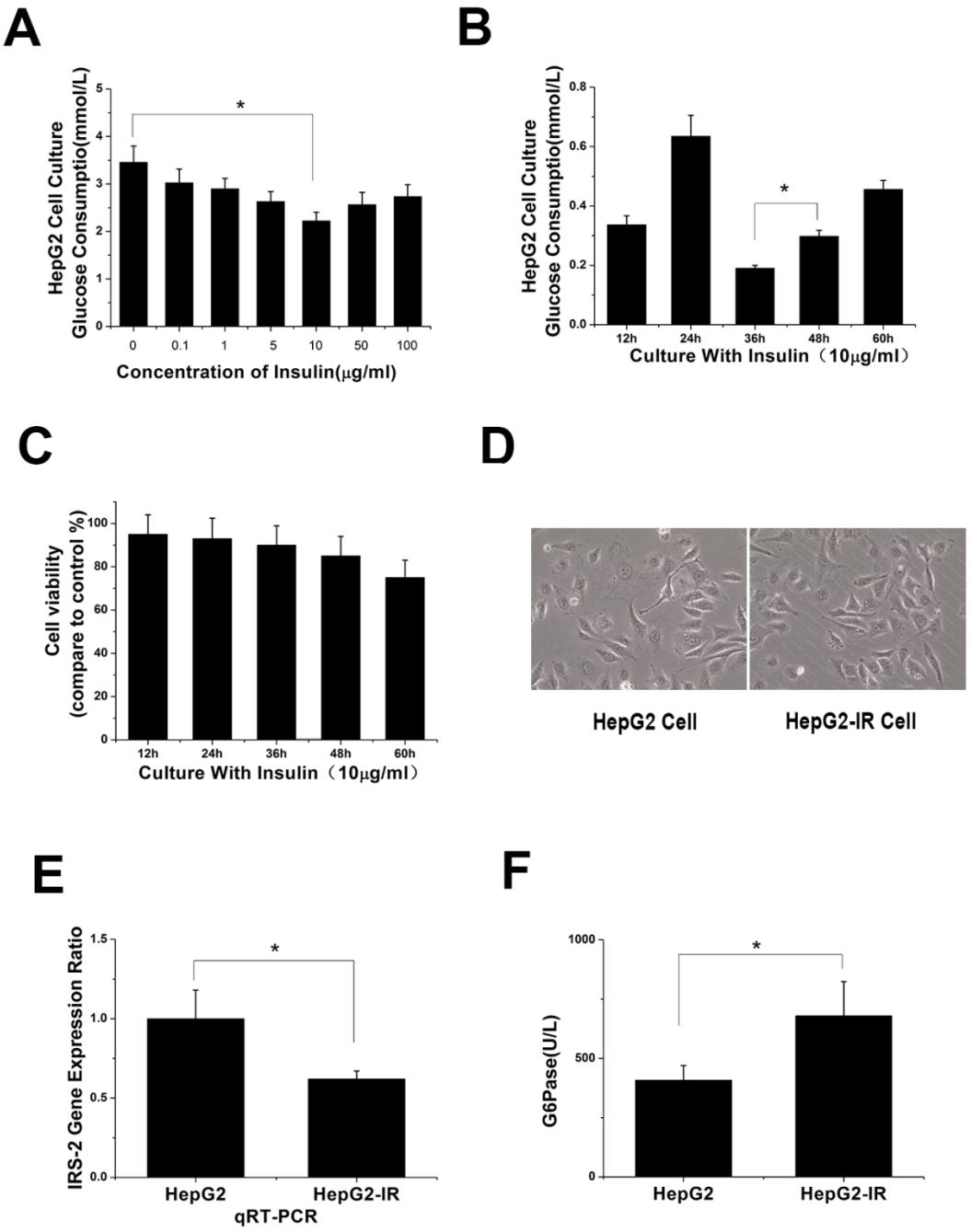
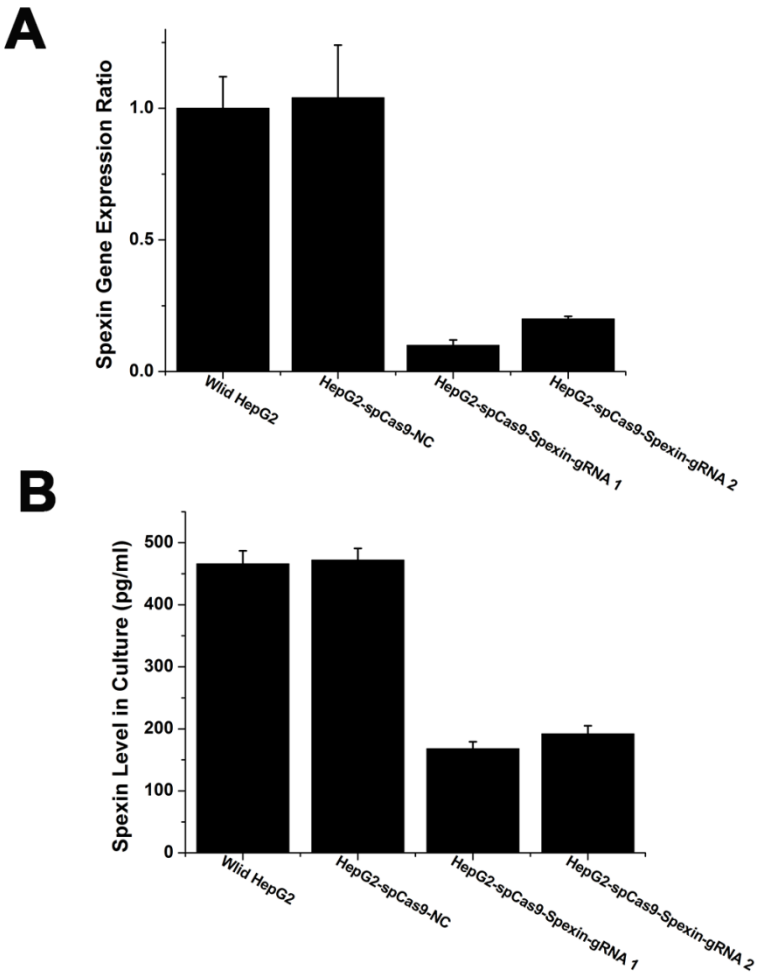


Supplementary Figure 1. Comparison of body weight, blood parameters, and HOMA-IR in HFD-induced SD rats and control rats at 18 weeks old. (A) The changes of body weight from 0 to 18 weeks in HFD-induced rats and control group. (B) Body weight in HFD group was significantly higher than control group at 18 weeks (* $P < 0.05$). (C) Fasting plasma glucose in HFD group was significantly higher than control group at 18 weeks (* $P < 0.05$). (D) Fasting insulin level in HFD group was significantly higher than control group at 18 weeks (* $P < 0.05$). (E) HOMA-IR in HFD group was significantly higher than control group at 18 weeks (* $P < 0.05$). (F) The liver index in HFD group was significantly higher than that in control group (* $P < 0.05$). (G) Liver morphology observed by H&E staining in HFD group and control group. Liver cells in the HFD group showed cell enlargement, diffused hepatic steatosis, and obvious fat infiltration. In control group, the liver cells showed a compact structure, clear edge, and normal size, with a large and round cell nucleus, even cytoplasm, and no lipid droplets.



Supplementary Figure 2. HepG2 cells cultured with 10 μg/ml insulin for 36 h could establish a stable and successful insulin resistant model of HepG2 cells. (A) Different concentrations of insulin treated HepG2 cells for 36 h, and glucose consumption in the culture of 10 μg/ml insulin group was the smallest. (B) HepG2 cells were cultured with 10 μg/ml insulin for 12 h, 24 h, 36 h, 48 h, and 60 h. Glucose consumption in the supernatant in 36 h group was the smallest. (C) No significant difference in cell viability was detected by CCK-8 assay after 36 h treatment of high

concentration of insulin. (D) No difference in morphology of HepG2 cells and HepG2-IR cells was observed under the optical microscope. (E) Expression of IRS-2 mRNA detected by qRT-PCR showed a significant decrease in HepG2-IR cells (* $P < 0.05$). (F) Expression of G-6-Pase in HepG2-IR cell culture was significantly increased detected by ELISA (* $P < 0.05$).



Supplementary Figure 3. CRISPR/Cas9-mediated disruption of spexin expression in HepG2 cells. (A) Compared with the blank group and the HepG2-spCas9-NC group, the cell expression of spexin mRNA in HepG2-spCas9-Spexin-gRNA group was significantly lower (* $P < 0.05$). (B) The protein expression of spexin in cell culture was detected by ELISA. The level of spexin expression in HepG2-spCas9-Spexin-gRNA culture was significantly lower than that in blank group and HepG2-spCas9-NC group (* $P < 0.05$).

Supplementary Table 1A. Primer sequences for qRT-PCR.

Gene Name Primer	Species	Primer Sequence（5-3）
PGC-1α	Human	F:AGGCAAGCAAGCAGGTCT R:GTCATCAAACAGGCCATCC
	Rat	F: GGAGCTGGATGGCTTGGGACAT R: TTCGCAGGCTCATTGTTGTAC
FoxO1	Human	F: TGGACATGCTCAGCAGACATC R: TTGGGTCAGGCGGTTCA
	Rat	F: CAGCAAATCAAGTTATGGAGGA R: TATCATTGTGGGGAGGAGAGTC
PEPCK	Human	F: GGTTCCCTGGGTGCATGAAA R: CACGTAGGGTGAATCCGTCAG
	Rat	F: GAGATCATCTCCTTCGGAAGCG R: TTAGTTATGCCCAGGATCAGCATG
G-6-Pase	Human	F: GACCTCAGGAATGCCTTCTACG R: AGTCAGTATCCAAAACCCACCAG
	Rat	F: AACGTCTGTCTGTCCCGGATCTAC R: ACCTCTGGAGGCTGGCATTG
GAPDH	Human	F: ATGGGGAAGGTGAAGGTCG R: GGGGTCATTGATGGCAACAATA
β-actin	Rat	F: ACCCACACTGTGCCCATCTATG R: AATGTCACGCACGATTTCCCT