Electronic Supplementary Material (ESI) for Nanoscale Horizons.

Supplementary Information

Gene-editing by CRISPR-Cas9 in combination with anthracycline therapy via tumor microenvironment-switchable, EGFR-targeted, and the nucleus-directed nanoparticles for head and neck cancer suppression

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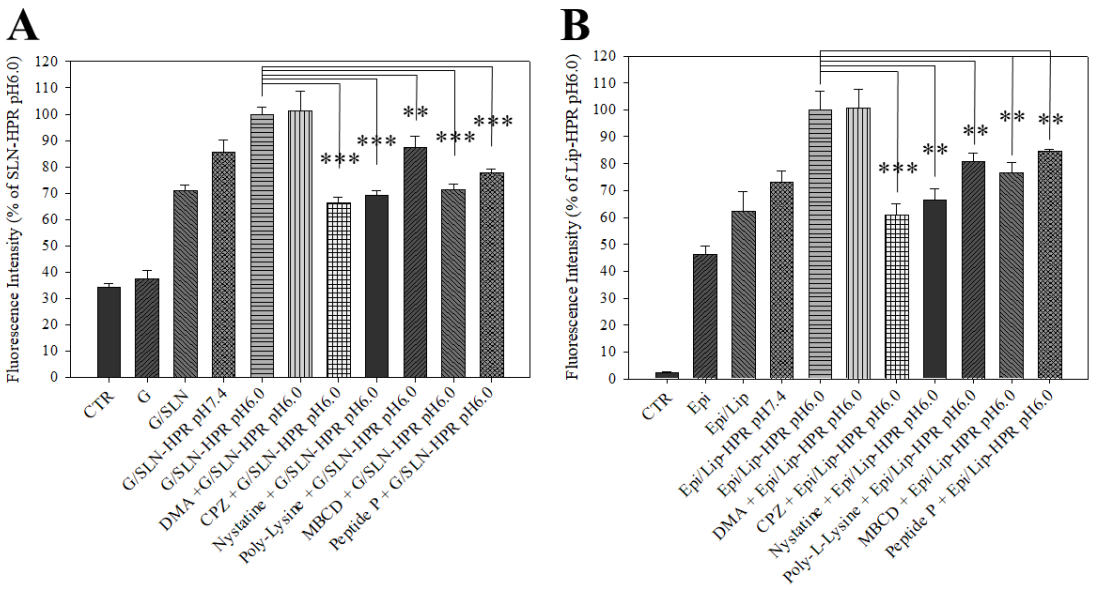


Fig. S1. Identification of the endocytosis or fusion pathways of GFP-plasmid/SLN-HPR and Epi/Lip-HPR in SAS cells. The cells were pretreated with different endocytosis inhibitors for 1 h and then treated with (A) GFP-plasmid/SLN-HPR or (B) Epi/Lip-HPR for another 3 h. Fluorescence intensity was detected by flow cytometry. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

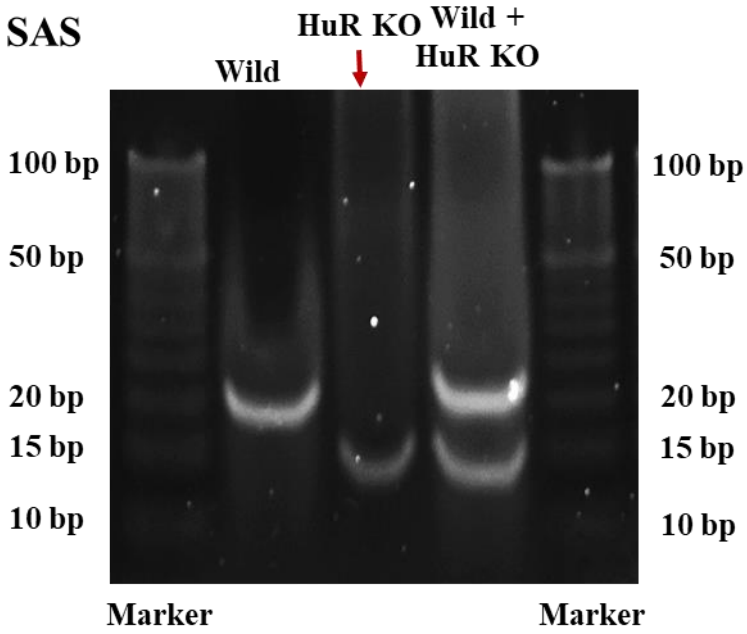


Fig. S2. Verification of HuR knockout in SAS cells. The cells were treated with HuR CRISPR/SLN-HPR. DNA from each clone was extracted and run on a 12% acrylamide gel. The representative image of DNA electrophoresis is shown.

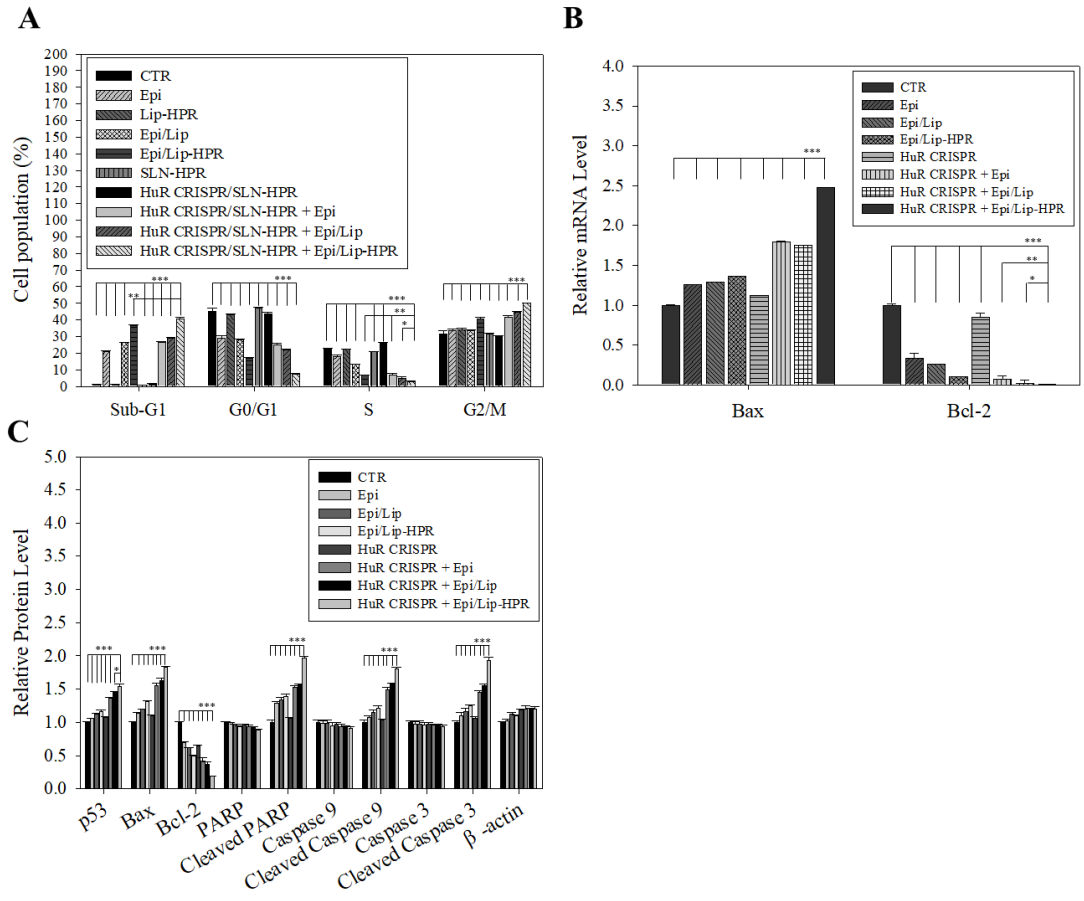


Fig. S3. Effect of different formulations on cell cycle distribution, mRNA expressions, and quantification of the Western blot analysis of apoptosis-associated pathway in SAS cells. (A) The relative population percentages of the cell cycle distribution; (B) mRNA expression levels of Bax and Bcl-2, as determined by RT-PCR; (C) Quantification of Western blot results by ImageJ. *p <0.05; ** p <0.01; *** p <0.001.

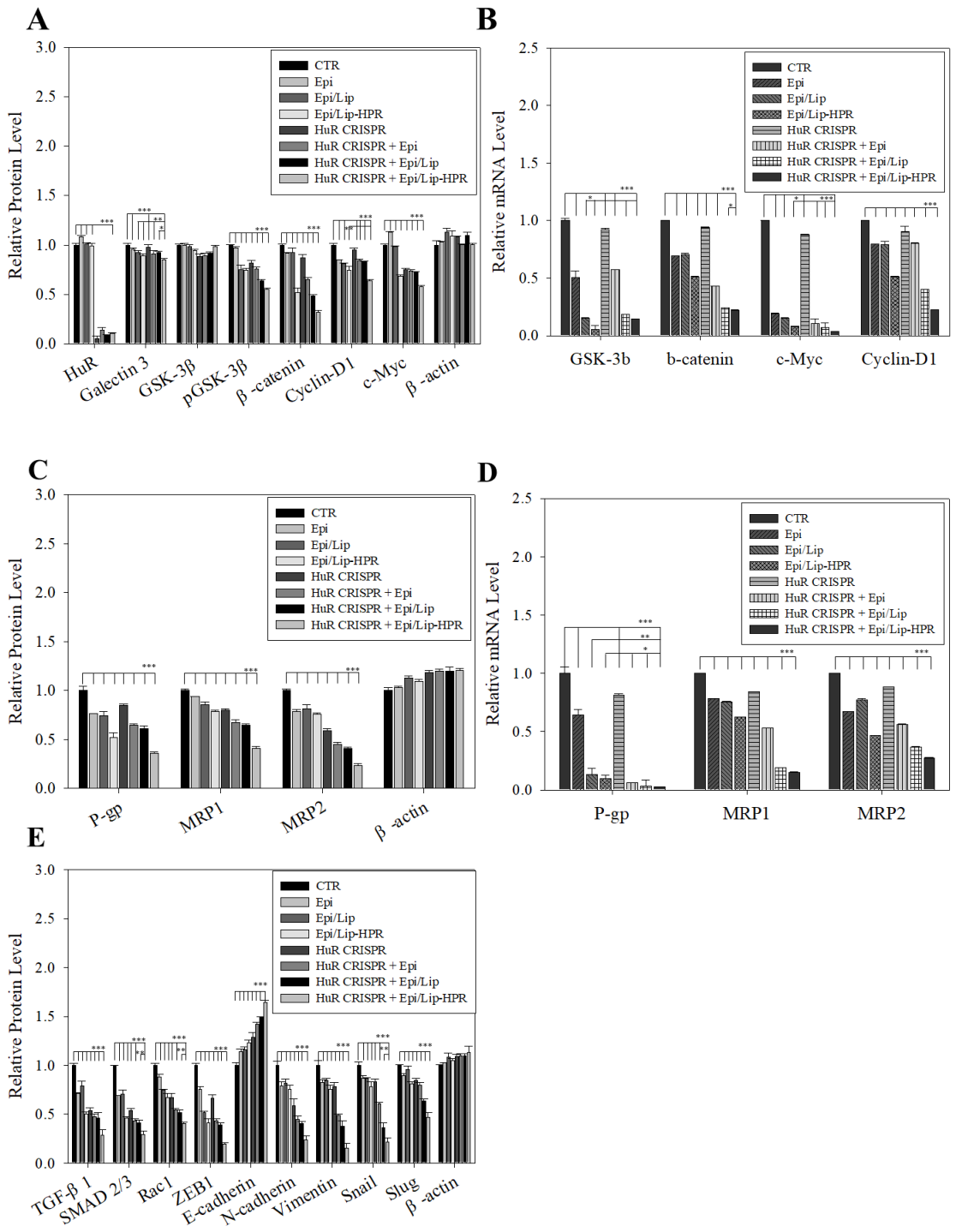
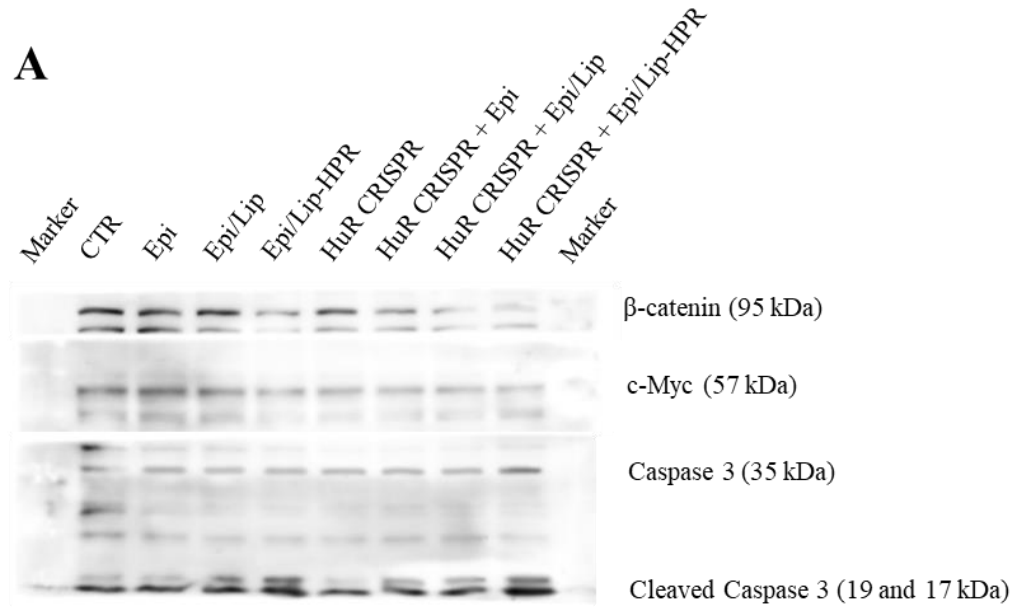
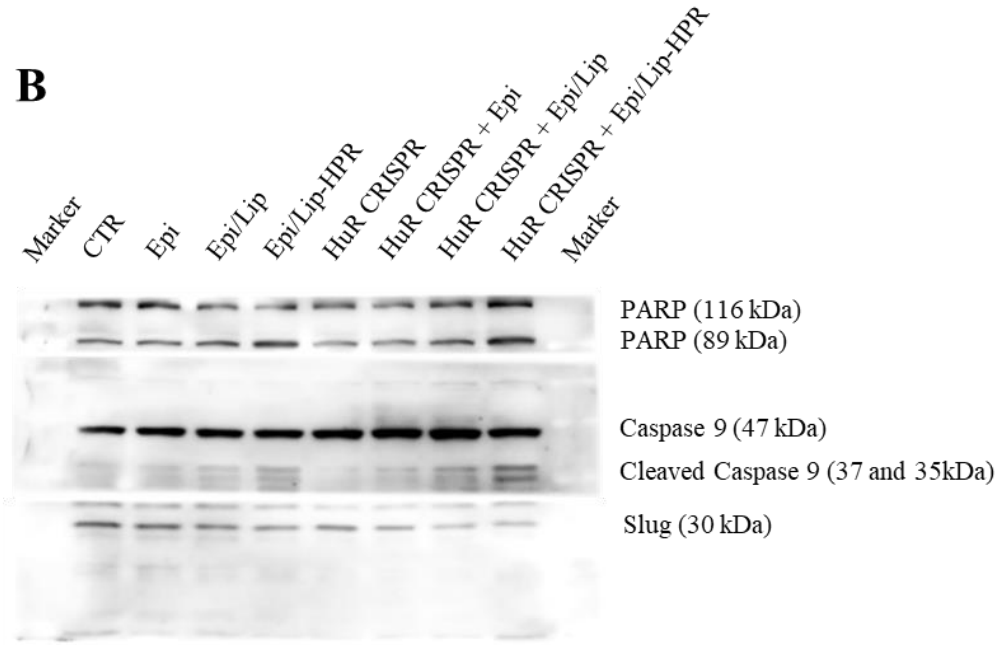
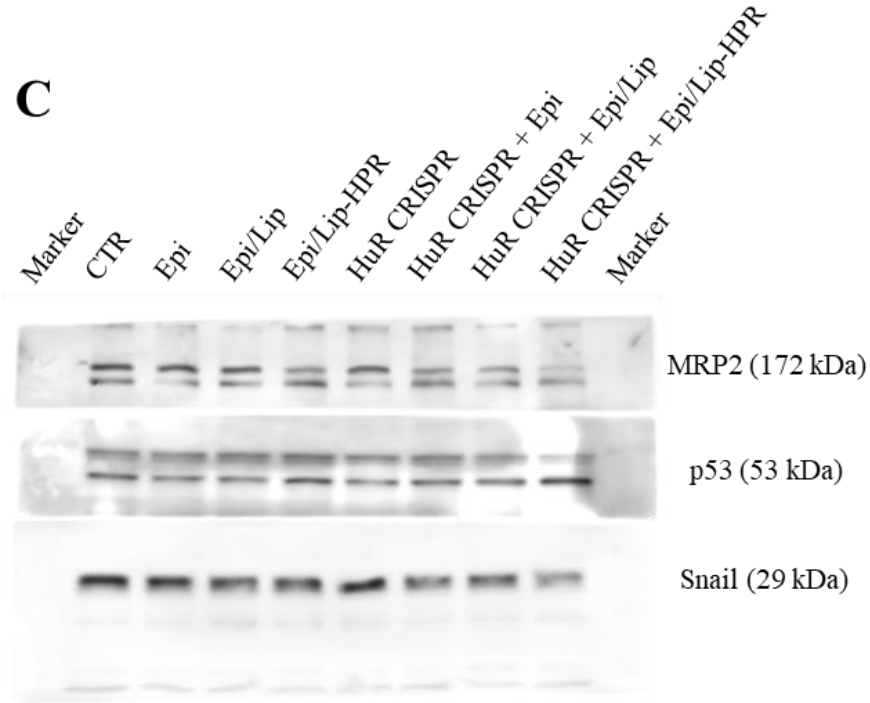
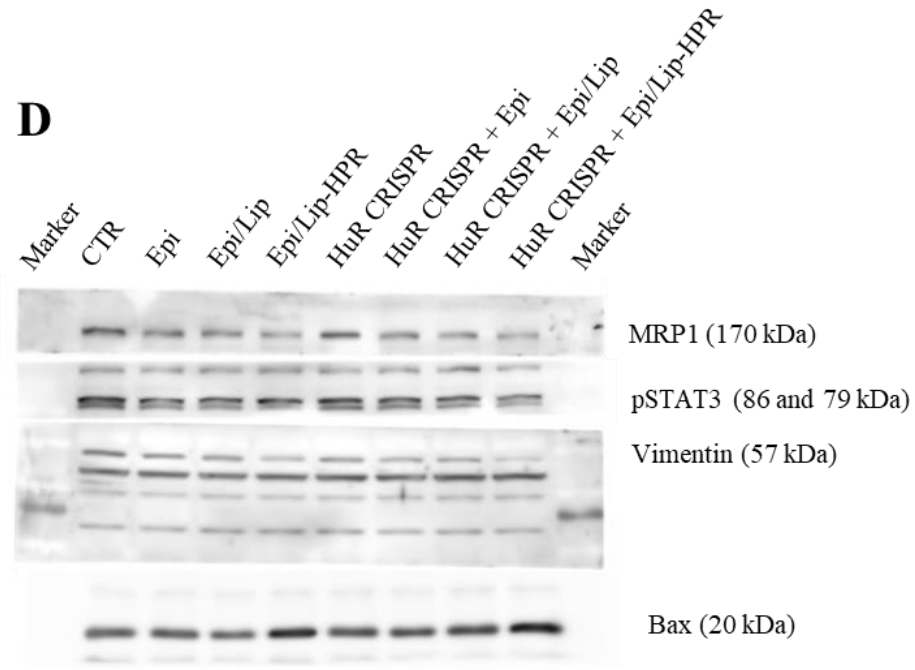


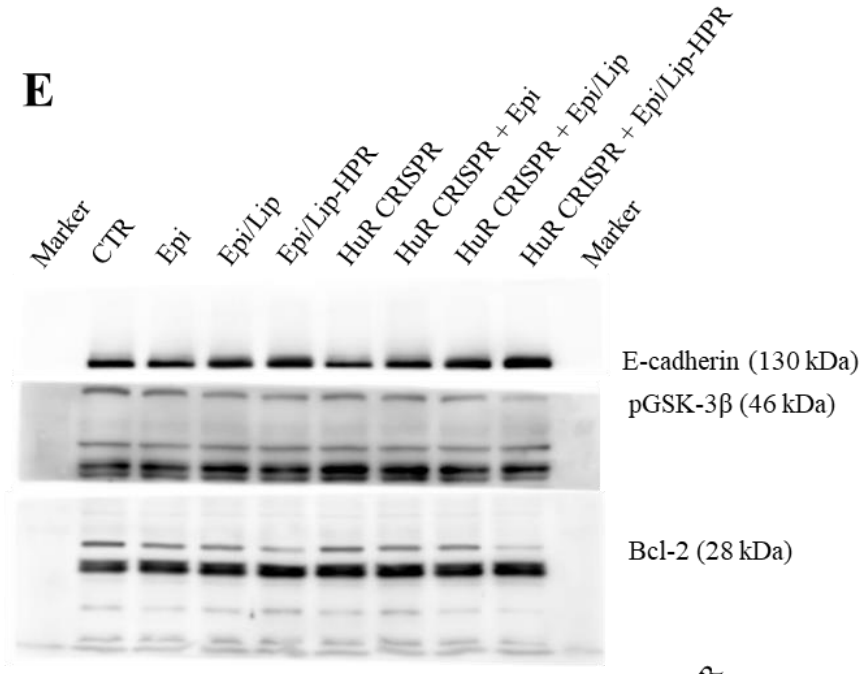
Fig. S4. Effect of different treatments for 48 h on the levels of mRNA and proteins of various pathways in SAS cells. (A) Protein and (B) mRNA expression levels of the HuR/Wnt-activated pathway. (C) Protein and (D) mRNA expression levels of the MDR pathway. (E) Protein levels of the EMT pathway. (A, C, E) Western blot quantification was analyzed by ImageJ and (B, D) mRNA expression levels were determined by quantitative real-time PCR. *p <0.05; ** p <0.01; *** p <0.001.

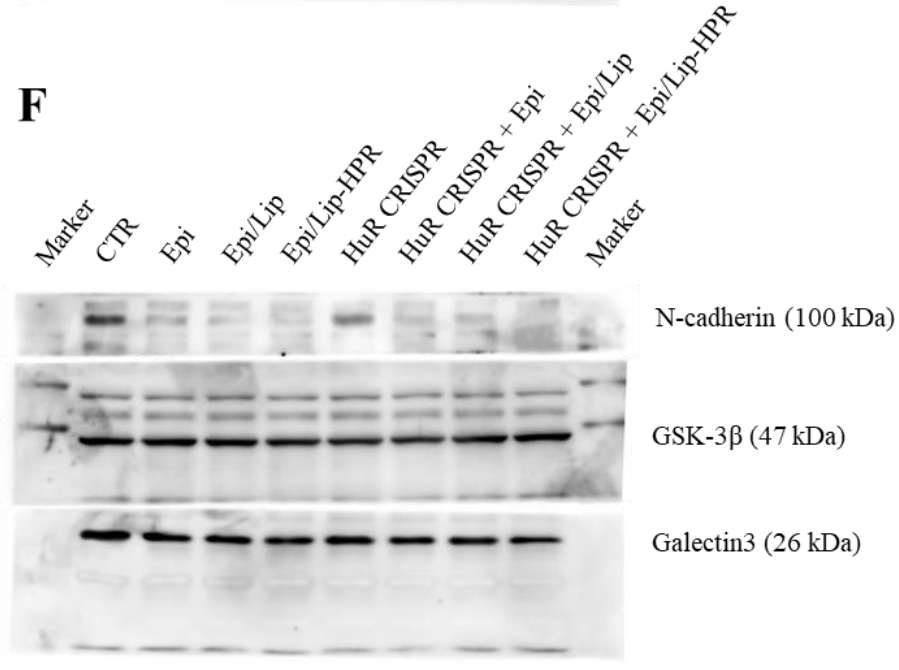

A
Marker
CTR
Epi
Epi/Lip
Epi/Lip-HPR
HuR CRISPR
HuR CRISPR + Epi
HuR CRISPR + Epi/Lip
HuR CRISPR + Epi/Lip-HPR
Marker
β-catenin (95 kDa)
c-Myc (57 kDa)
Caspase 3 (35 kDa)
Cleaved Caspase 3 (19 and 17 kDa)

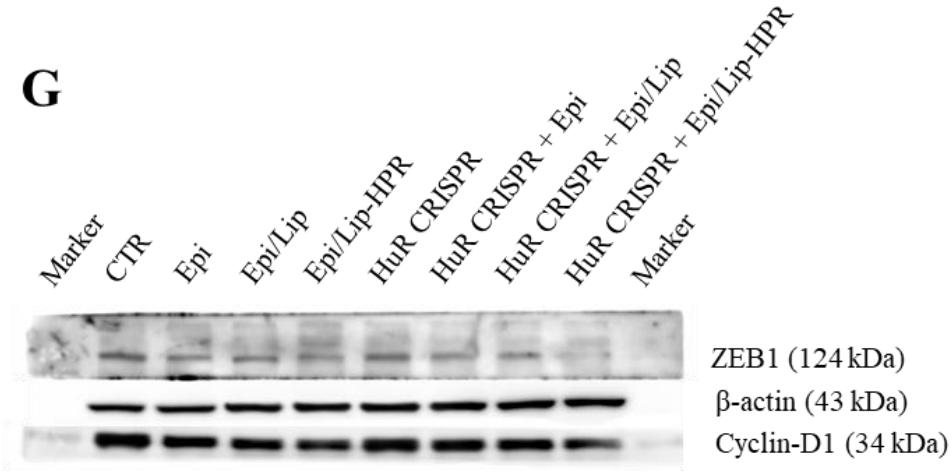

B
Marker
CTR
Epi
Epi/Lip
Epi/Lip-HPR
HuR CRISPR
HuR CRISPR + Epi
HuR CRISPR + Epi/Lip
HuR CRISPR + Epi/Lip-HPR
Marker
PARP (116 kDa)
PARP (89 kDa)
Caspase 9 (47 kDa)
Cleaved Caspase 9 (37 and 35kDa)
Slug (30 kDa)

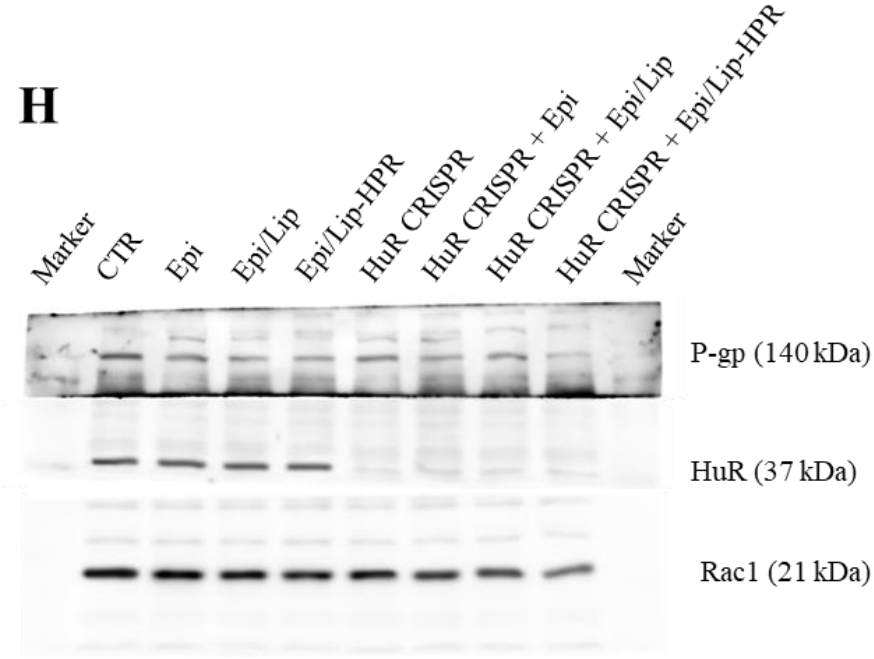

C
Marker
CTR
Epi
Epi/Lip
Epi/Lip-HPR
HuR CRISPR
HuR CRISPR + Epi
HuR CRISPR + Epi/Lip
HuR CRISPR + Epi/Lip-HPR
Marker
MRP2 (172 kDa)
p53 (53 kDa)
Snail (29 kDa)

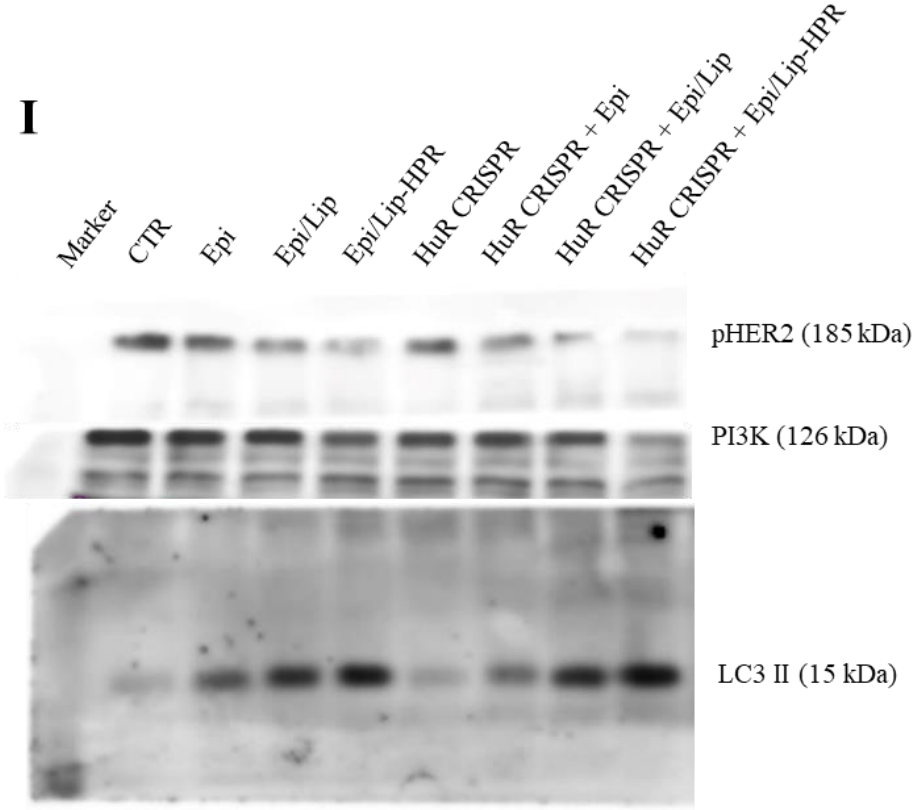

D
Marker
CTR
Epi
Epi/Lip
Epi/Lip-HPR
HuR CRISPR
HuR CRISPR + Epi
HuR CRISPR + Epi/Lip
HuR CRISPR + Epi/Lip-HPR
Marker
MRP1 (170 kDa)
pSTAT3 (86 and 79 kDa)
Vimentin (57 kDa)
Bax (20 kDa)

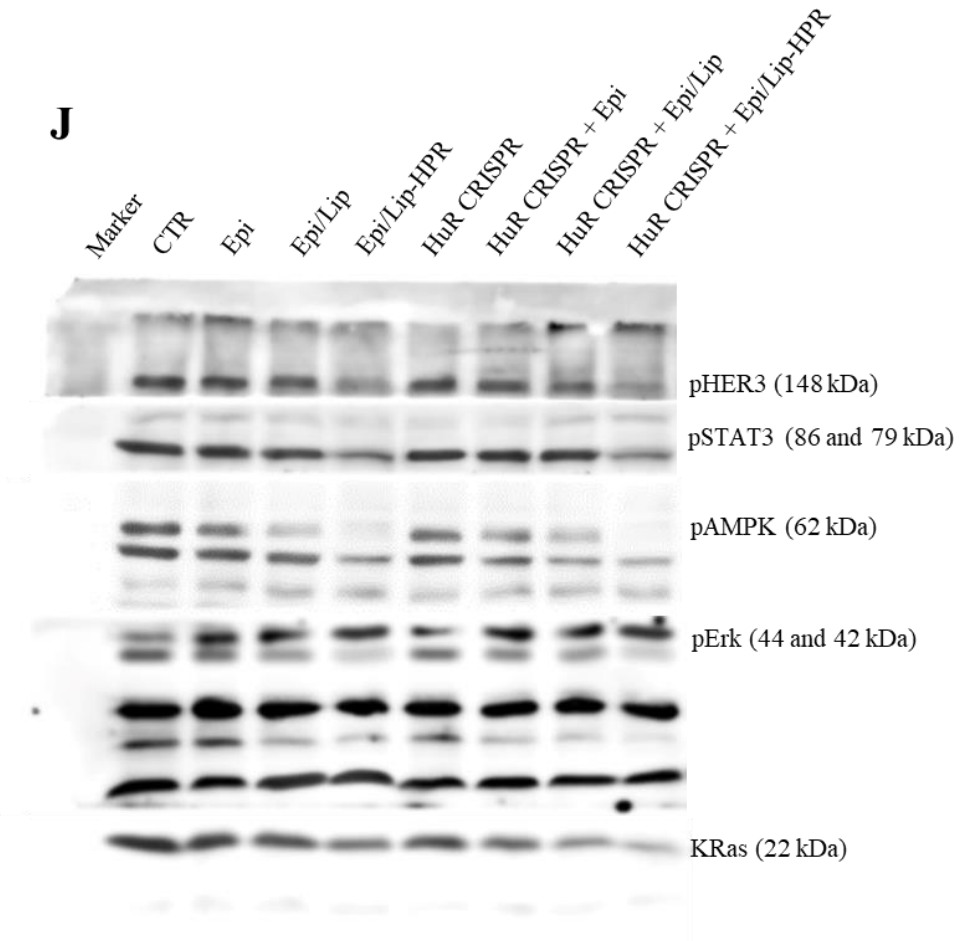

E
Marker
CTR
Epi
Epi/Lip
Epi/Lip-HPR
HuR CRISPR
HuR CRISPR + Epi
HuR CRISPR + Epi/Lip
HuR CRISPR + Epi/Lip-HPR
Marker
E-cadherin (130 kDa)
pGSK-3β (46 kDa)
Bcl-2 (28 kDa)

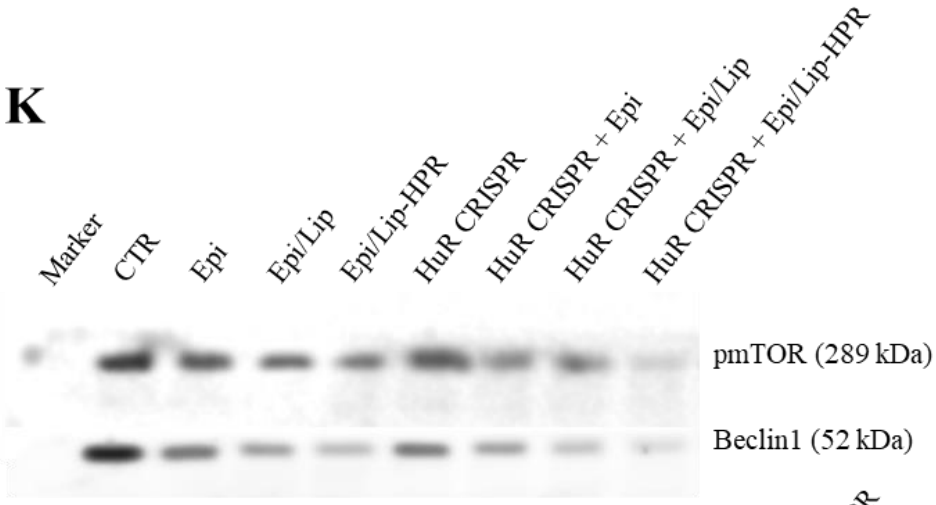

F
Marker
CTR
Epi
Epi/Lip
Epi/Lip-HPR
HuR CRISPR
HuR CRISPR + Epi
HuR CRISPR + Epi/Lip
HuR CRISPR + Epi/Lip-HPR
Marker
N-cadherin (100 kDa)
GSK-3β (47 kDa)
Galectin3 (26 kDa)

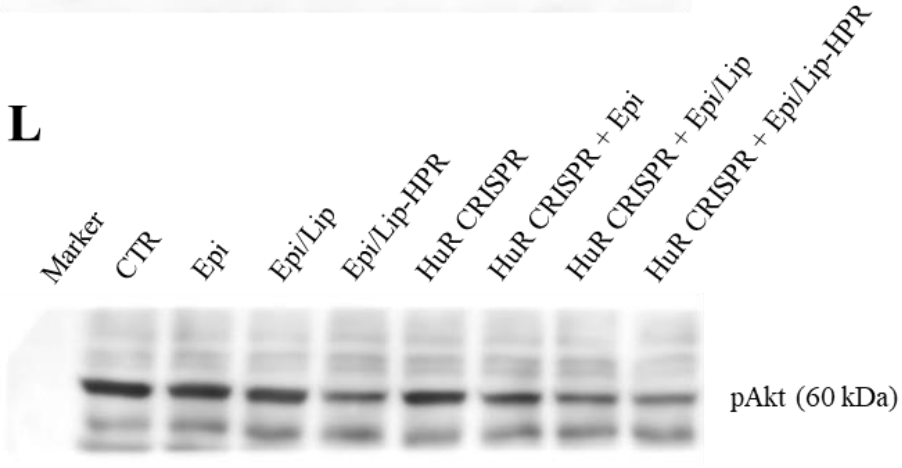

G
Marker
CTR
Epi
Epi/Lip
Epi/Lip-HPR
HuR CRISPR
HuR CRISPR + Epi
HuR CRISPR + Epi/Lip
HuR CRISPR + Epi/Lip-HPR
Marker
ZEB1 (124 kDa)
β-actin (43 kDa)
Cyclin-D1 (34 kDa)


H
Marker
CTR
Epi
Epi/Lip
Epi/Lip-HPR
HuR CRISPR
HuR CRISPR + Epi
HuR CRISPR + Epi/Lip
HuR CRISPR + Epi/Lip-HPR
Marker
P-gp (140 kDa)
HuR (37 kDa)
Rac1 (21 kDa)


I
Marker
CTR
Epi
Epi/Lip
Epi/Lip-HPR
HuR CRISPR
HuR CRISPR + Epi
HuR CRISPR + Epi/Lip
HuR CRISPR + Epi/Lip-HPR
pHER2 (185 kDa)
PI3K (126 kDa)
LC3 II (15 kDa)


J
Marker
CTR
Epi
Epi/Lip
Epi/Lip-HPR
HuR CRISPR
HuR CRISPR + Epi
HuR CRISPR + Epi/Lip
HuR CRISPR + Epi/Lip-HPR
pHER3 (148 kDa)
pSTAT3 (86 and 79 kDa)
pAMPK (62 kDa)
pErk (44 and 42 kDa)
KRas (22 kDa)


K
Marker
CTR
Epi
Epi/Lip
Epi/Lip-HPR
HuR CRISPR
HuR CRISPR + Epi
HuR CRISPR + Epi/Lip
HuR CRISPR + Epi/Lip-HPR
pmTOR (289 kDa)
Beclin1 (52 kDa)


L
Marker
CTR
Epi
Epi/Lip
Epi/Lip-HPR
HuR CRISPR
HuR CRISPR + Epi
HuR CRISPR + Epi/Lip
HuR CRISPR + Epi/Lip-HPR
pAkt (60 kDa)

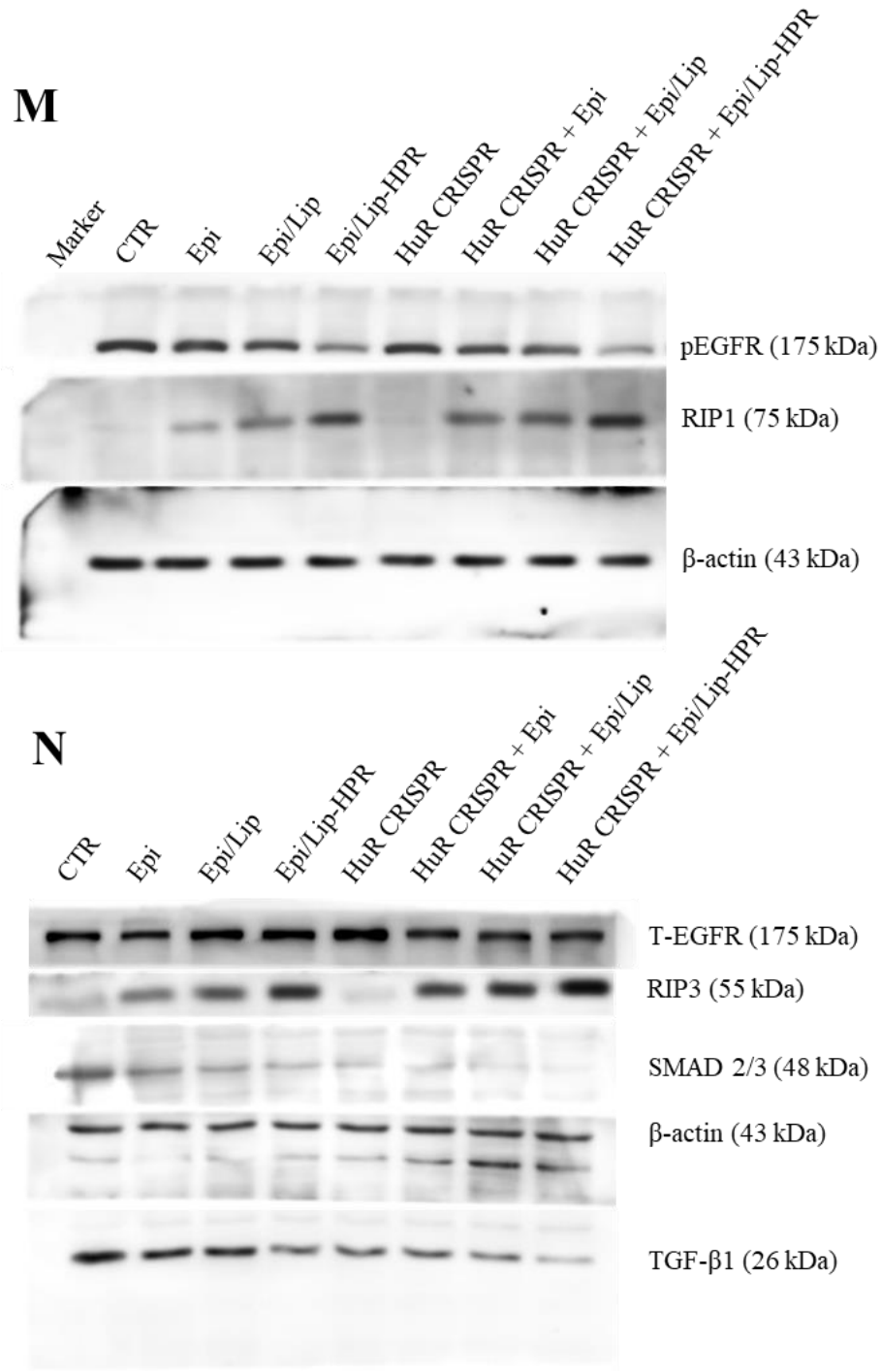


Fig. S5. Raw images of proteins of various pathways by western blot assay in SAS cells. Specifically, after the blot was transferred from the gel to the membrane, the membrane was cut into multiple bands of the appropriate size according to the molecular weight of the respective protein. Then, the membrane was agitated with blocking buffer at room temperature for 1 h, washed by Tris-buffered saline with 0.1% Tween 20 (TBST) for 3 times and soaked into the primary antibody of various proteins at 4°C overnight. Hence, a piece of membrane can be incubated with different primary antibodies at the same time. The membrane was washed by TBST for 3 times, and incubated with horseradish peroxidase-conjugated immunoglobulin G (Jackson ImmunoResearch Inc., PA, USA) at 4°C for 1 h. Finally, the blots were washed again, and visualized with enhanced chemiluminescence kits (Millipore, Billerica, MA, USA). The images were taken by Luminescence Imaging System (Amersham ™ Imager 600; GE Healthcare, Chicago, USA).