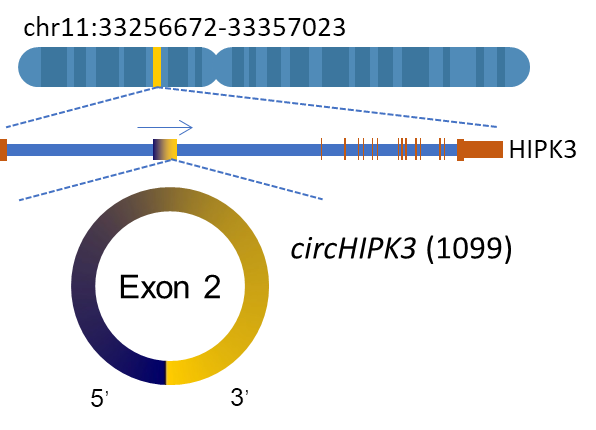
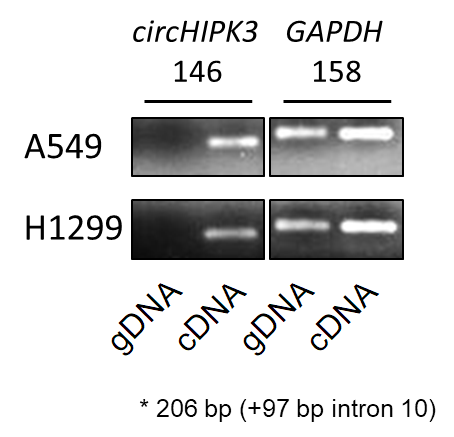
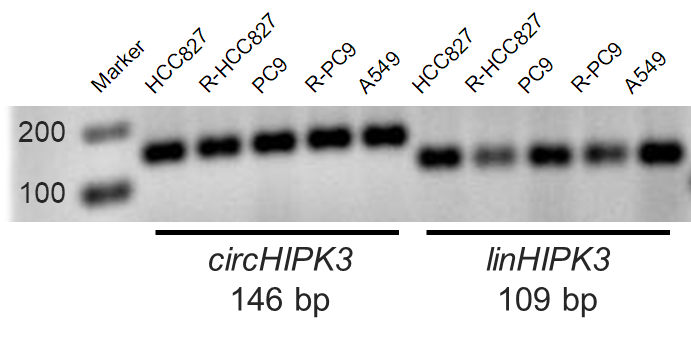
A

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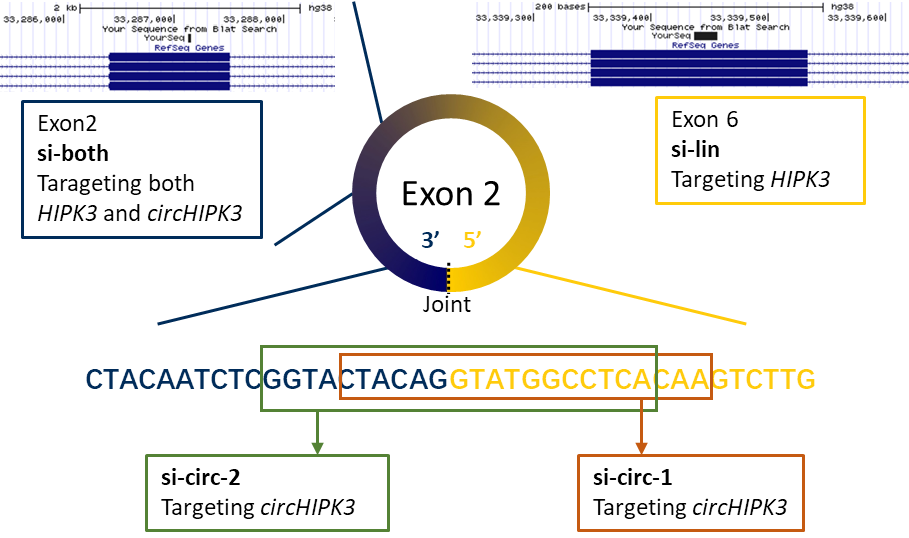


F

E

**Figure S1**. Biogenesis, expression and cellular distribution of *circHIPK3*. (**A**) Schematic of the biogenesis of *circHIPK3*. (**B**) Relative expression rate of *circHIPK3* in various cell lines by RT-PCR. (**C**) Expression of *circHIPK3* and *linHIPK3* in various cell lines with or without RNase R treatment by agarose gel electrophoresis. (**D**) Expression of *circHIPK3* and *GAPDH* in genomic DNA and cDNA by agarose gel electrophoresis. (**E**) Relative expression of *circHIPK3* in nucleus and cytoplasm in various cell lines by RT-PCR. (**F**) Relative expression of *linHIPK3* in nucleus and cytoplasm in various cell lines by RT-PCR.

A

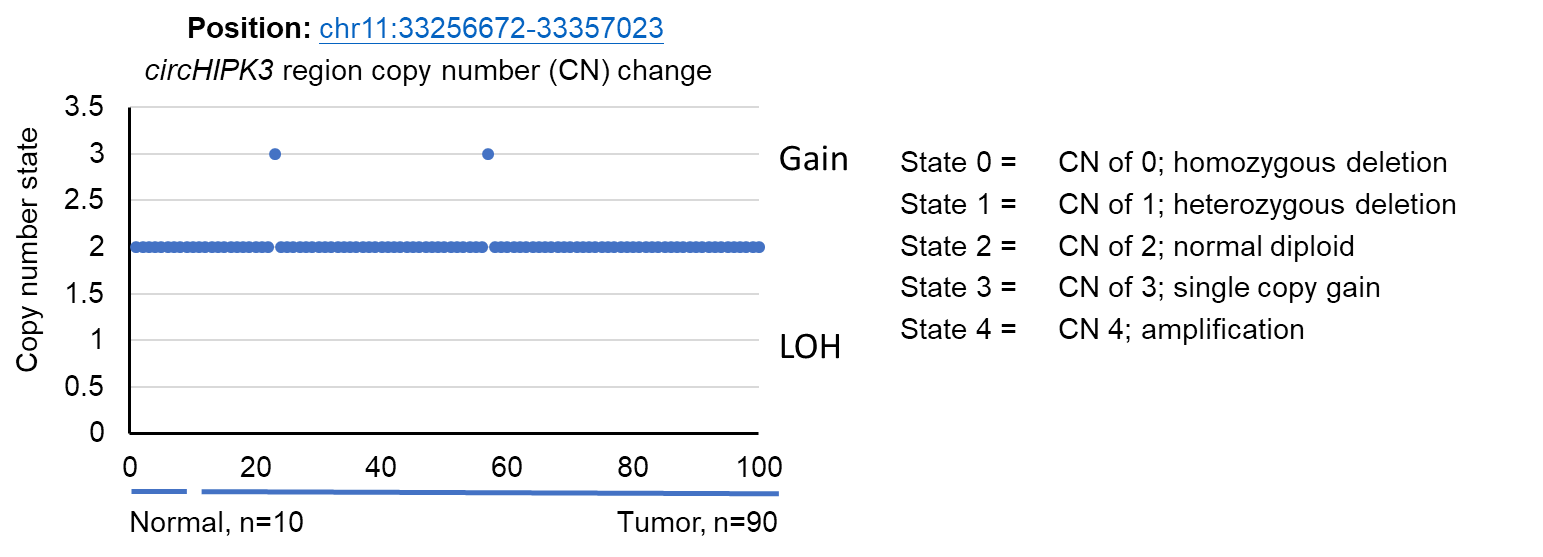




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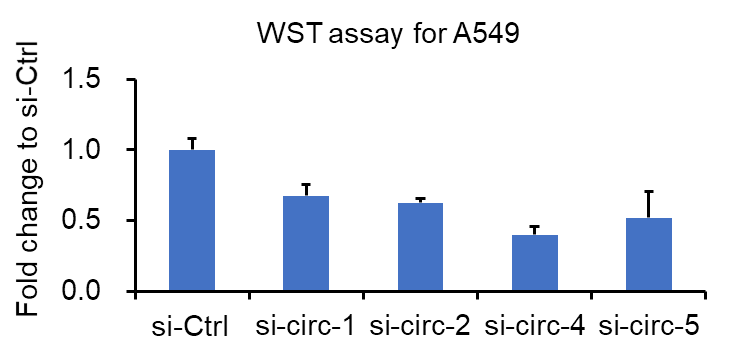


E

**Figure S2**. siRNA design, cellular distribution and epigenetic regulation of *circHIPK3*. (**A**) Schematic of siRNA designs. (**B, C**) Knockdown effect of 4 siRNAs in A549 and H838. (**D**) A549 was treated with TSA (150 nM), 5-AZA (2.5 μM) or both (TSA 150 nM, 5-AZA 2.5 μM) for 24 h. The expression of *circHIPK3* and *linHIPK3* were measured using RT-PCR. All data are presented as the means ± SD of at least 3 independent experiments. \* P < 0.05, \*\* P < 0.01. (**E**) *circHIPK3* (located at chr11:33256672-33357023) DNA copy number change in 90 lung adenocarcinomas and 10 normal lung tissues using SNP6.0, analyzed using Affymetrix Genotyping Console (GTC 4.1). 2 single copy gains were found.



A



C

B

**Figure S3**. Impact of *circHIPK3* and/or *linHIPK3* abrogation on cell viability and invasion. **(A)** Multiple NSCLC cell lines were treated with 5 siRNAs respectively for 120 h. Supplementary siRNAs were added every 48 h. WST-1 assay was carried out to illustrate the impact on cell viability. All data are presented as the means ± SD of at least 3 independent experiments. (**B**) In addition, we have designed another 2 new siRNAs which target *circHIPK3*, e.g. si-circ-4 (CAGGTATGGCCTCACAAGT) and si-circ-5 (CTACAGGTATGGCCTCACAA). WST indicated the cell proliferation was decreased by 50% upon treatment of these 2 new siRNA (together with si-circ-1 and si-circ-2) at 120 h, confirming that knockdown of *circHIPK3* decreased cell proliferation. (**C**) Invasion index of H1299 after treatment of si-Ctrl, si-circ-1, si-lin, si-both and si-circ-2. Only si-circ-2 treatment resulted in statistically significant impairment of invasion capacity.

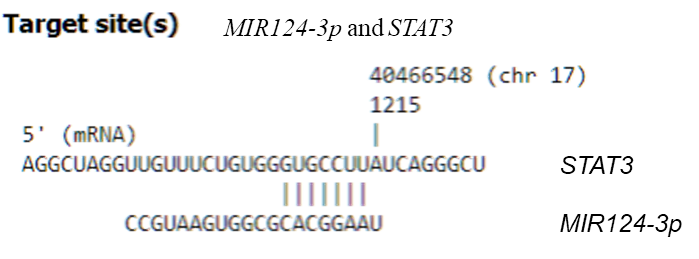


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B

**Figure S4**. Ratio of Autolysosome:Autophagosome for Figure 3B-G.

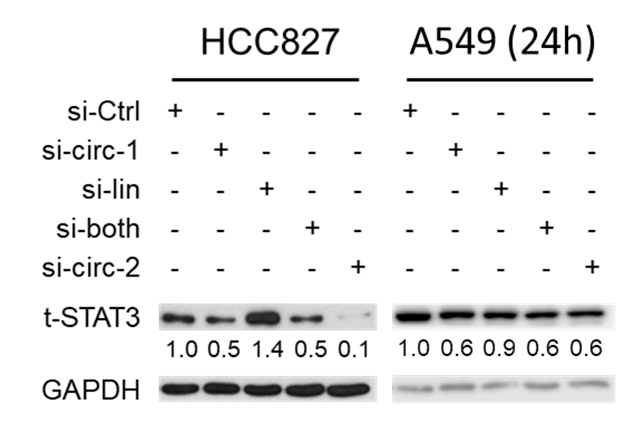
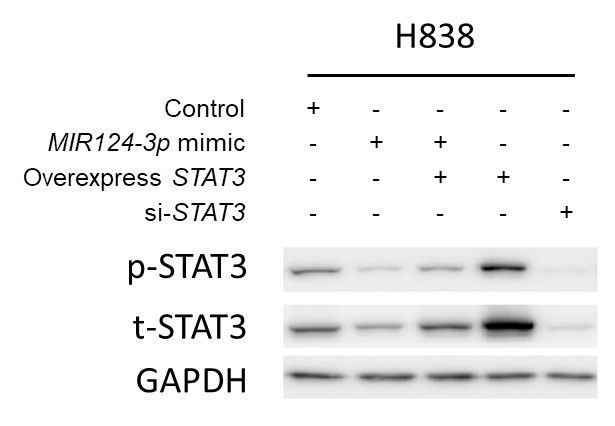
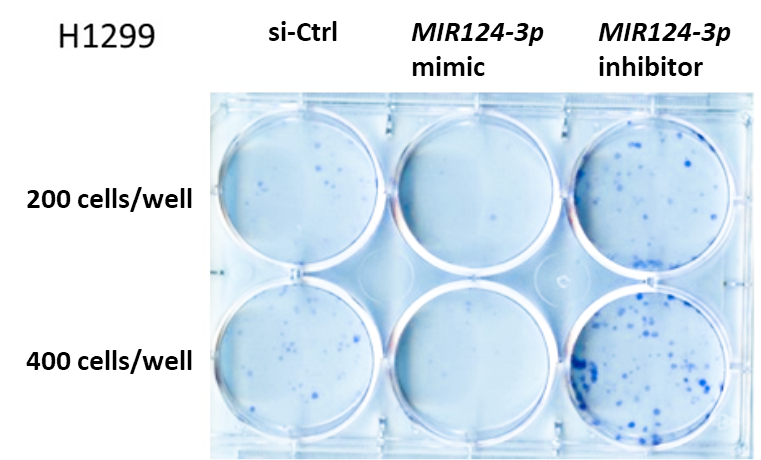


C

B

A

**Figure S5**. Predicted binding site for *MIR124-3p* and *STAT3* or *IL6R*. (**A**) Predicted binding site for *MIR124-3p* and *STAT3*. (**B**) Predicted binding site for *MIR124-3p* and *IL6R*. (**C**) H1299 was treated with both *circHIPK3* siRNAs for 48 h. Up regulation of *MIR124-3p* was observed.



**Figure S6**. Validation of *circHIPK3-MIR124-3p*-*IL6R*-STAT3 axis. (**A**) Western blot showing the LC3B protein changes after the treatment of *MIR124-3p* mimic. (**B**) Colony formation after the treatment of *MIR124-3p* mimic or inhibitor in H1299 cells. (**C**) Western blot showing STAT3 changed upon *MIR124-3p* mimic, *MIR124-3p* mimic+ over STAT3, over STAT3 and si-*STAT3* treatment on H838 cell lines. (**D**) A549 and HCC827 were treated with si-circ-1/2, si-lin and si-both for 24 h and 72 h respectively. Total STAT3 was downregulated upon silencing of *circHIPK3*. (**E**) The ratio of autolysosome:autophagosome for Fig. 4G and H. (**F)** p-STAT3 protein changes after si-Ctrl, si-circ-1, si-lin, si-both and si-circ-2 treatment at 72 h in H1299 cell line. Nuclear p-STAT3 was significantly decreased upon si-circ-1 and si-circ-2 treatment, while cytoplasmic p-STAT3 was not changed significantly; but both nuclear and cytoplasmic p-STAT3 were increased upon si-lin of HIPK3 treatment.

F

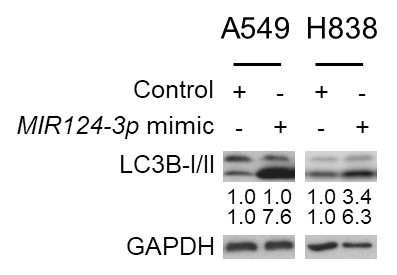
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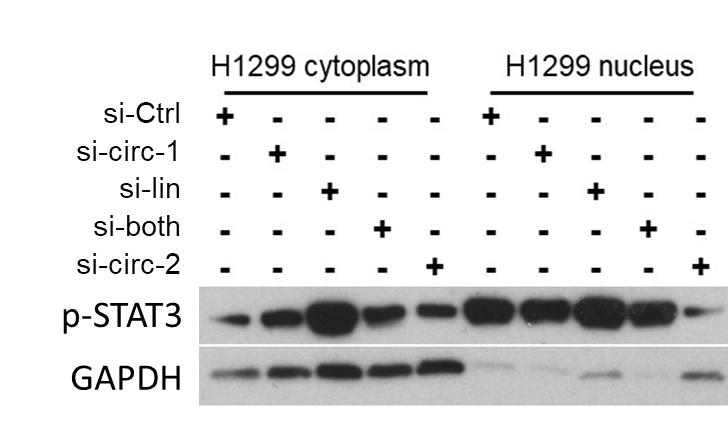
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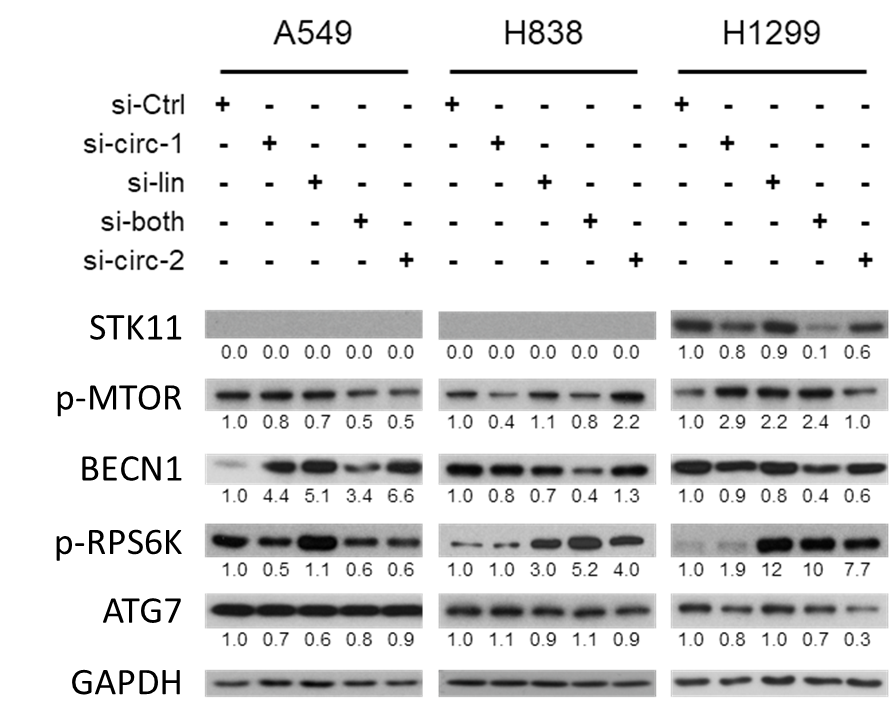
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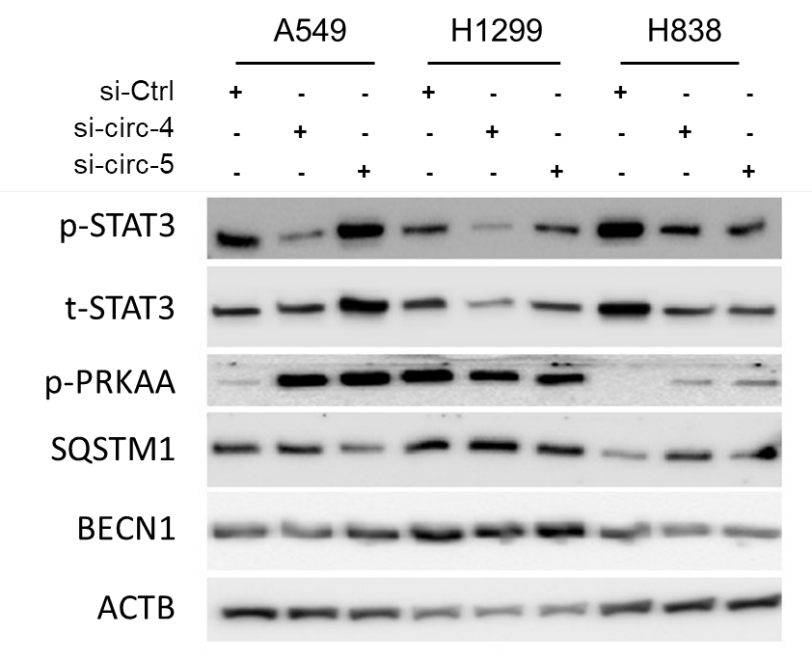




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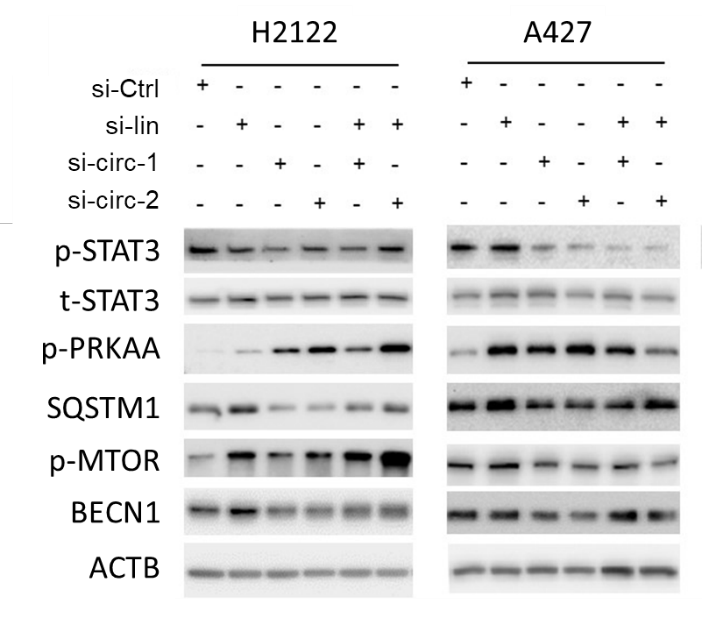


A



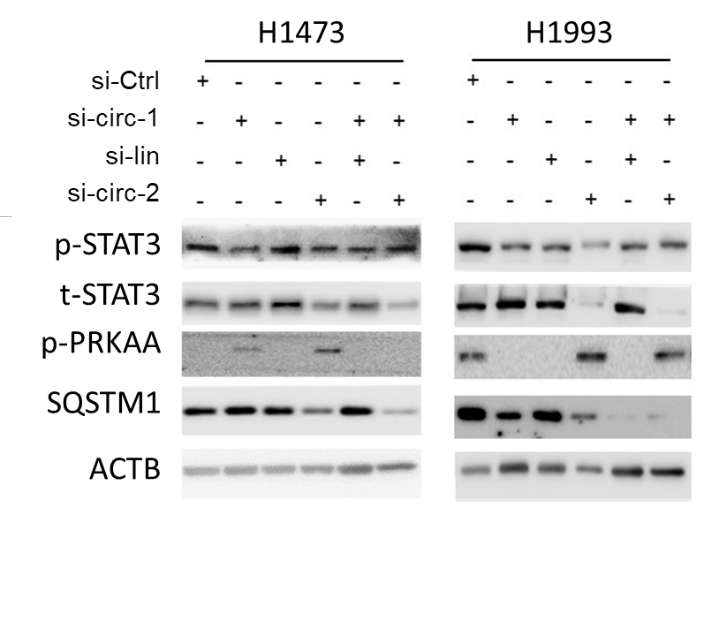
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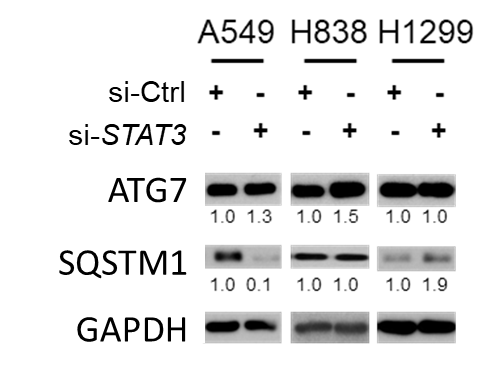
**Figure S7**. Traditional autophagy pathway proteins upon *circHIPK3* abrogation. (**A**) The ratio of autolysosome:autophagosome for Fig. 5C and D; (**B**) A549, H838 and H1299 were treated with 5 siRNAs respectively for 72 h before collecting whole cell protein. Upregulation of BECN1 was only observed in A549, downregulation of ATG7 was observed in H1299 upon silencing *circHIPK3.* (**C**) A549, H1299 and H838 treated with 2 siRNAs targeting circHIPK3 for 72 h before collecting whole cell protein. STAT3 and SQSTM1 were decreased, and p-PRKAA increased after *circHIPK3* knockdown by si-circ-4 and 5.



B

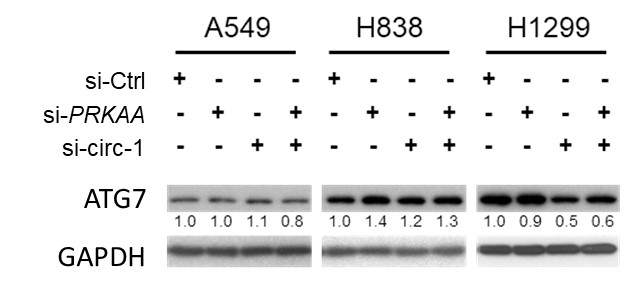
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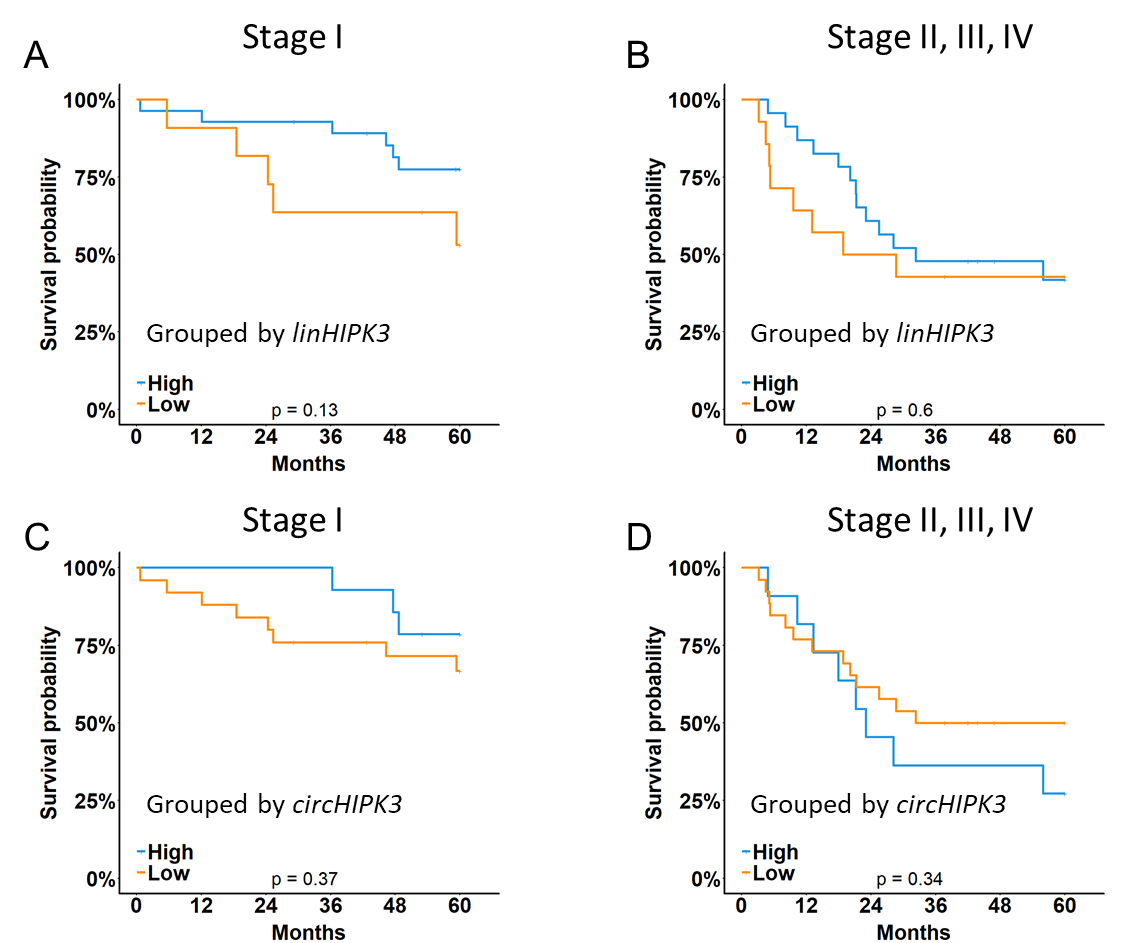


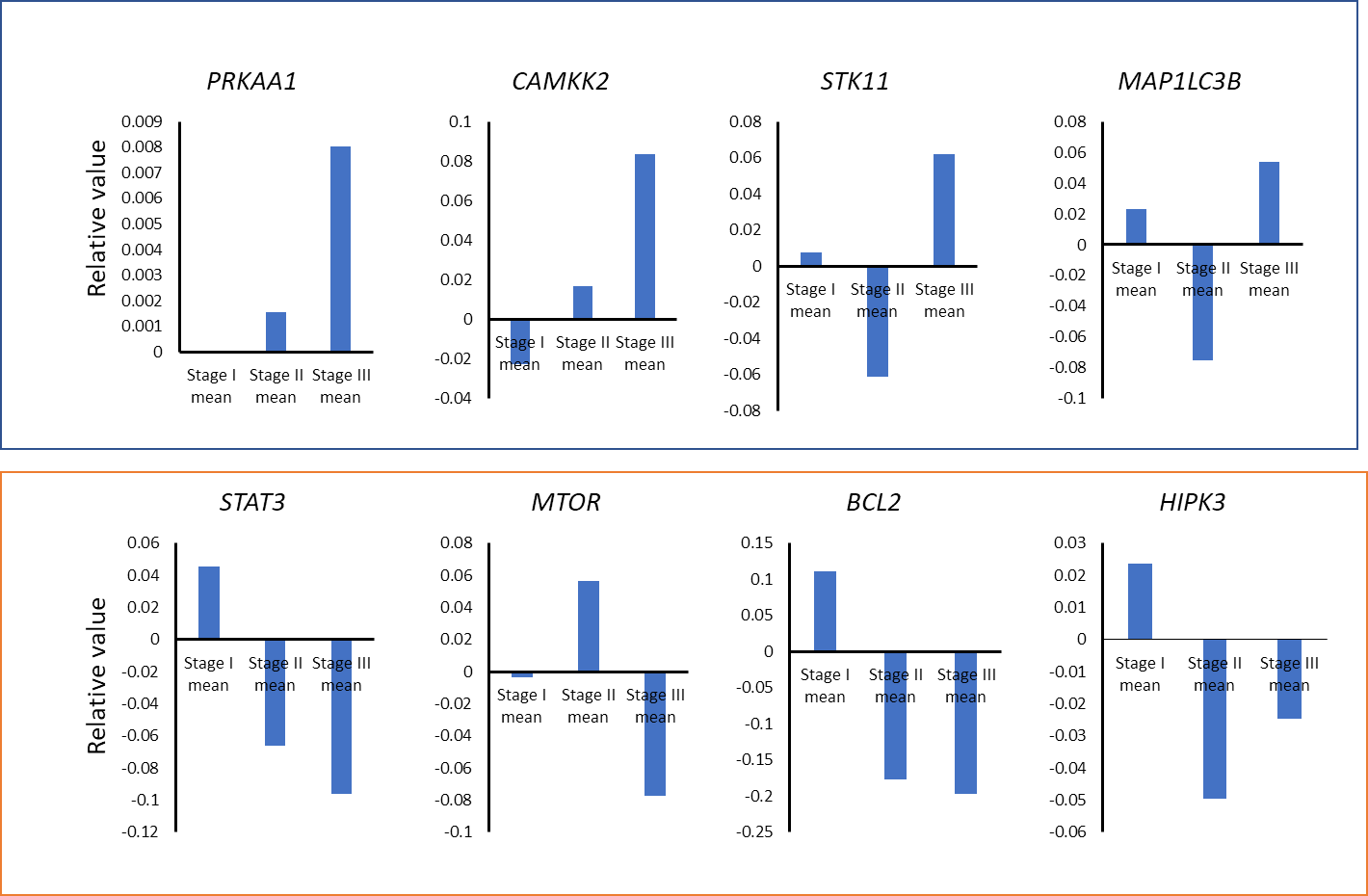
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**Figure S8**. Protein changed in more lung cancer cell lines with STK11 mutation. (**A** and **B**) STK11-deficient/mutated cell lines (H1437, H1993, H2122 and A-427) treated with 3 siRNAs targeting *linHIPK3* or *circHIPK3* and mix of si-lin and si-circ-1 or 2 respectively for 72 h before collecting whole cell protein. STAT3 and SQSTM1 were decreased, and p-PRKAA increased after *circHIPK3* knockdown by si-circ-1 and 2. (**C**). Silencing *STAT3* caused SQSTM1 degradation in A549 and didn’t regulate the expression of ATG7. (**D**) Silencing *PRKAA* didn’t regulate the expression of ATG7.





**Figure S9**. Overall survival of 76 lung cancer patients stratified by stage and autophagy related gene expression. (**A, B**) Patients were divided in to high and low groups according to the cutoff point of upper 2/3 quantile of linHIPK3 level. Neither stage I nor stage II, III and IV patients exhibit significant difference in survival. (**C, D**) Patients were divided in to high and low groups according to the cutoff point of upper 1/3 quantile of circHIPK3 level. Neither stage I nor stage II, III and IV patients exhibit significant difference in survival. (**E**) and (**F**) Autophagy related gene expressions in different stage based on 442 lung adenocarcinomas (Shedden data, Nature Med 2008, U133A array, mean center normalized data). Stage 1 n=276, Stage 2 n=104, Stge3 n=59.

E

F