# Long non-coding RNA AK058003, as a precursor of miR-15a, interacts with HuR to inhibit the expression of γ-synuclein in hepatocellular carcinoma cells

## Supplementary Materials

**Supplementary Table S1: miRNA binding sites of SNCG by starBase v2.0**

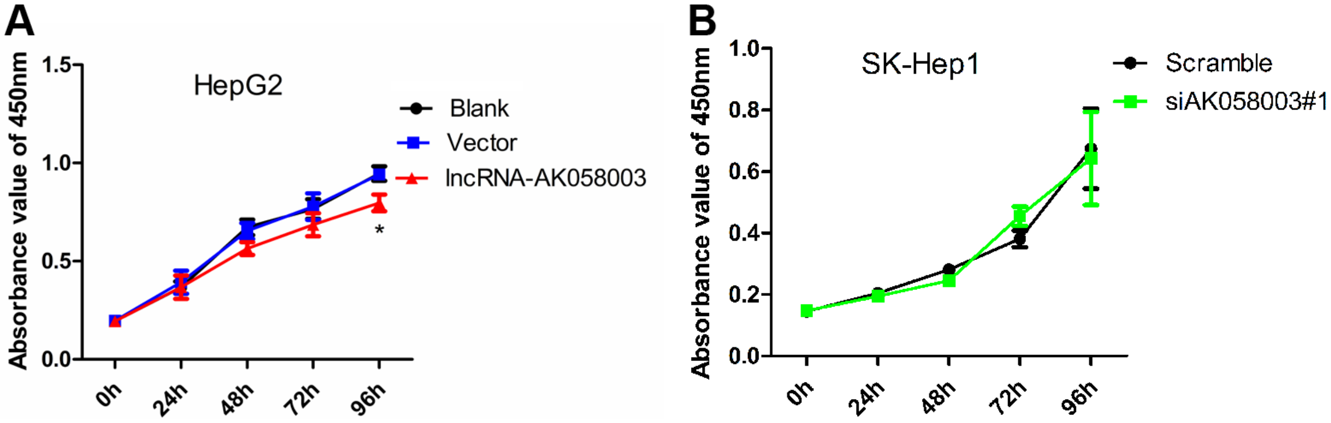
| Position | Seq-target | Name |
|---|---|---|
| 42 | TGGCAGTGTCTTAGCTGGTTGT | hsa-miR-34a-5p |
| 62 | ACAGCAGGCACAGACAGGCAGT | hsa-miR-214-3p |
| 62 | AGCAGCATTGTACAGGGCTATCA | hsa-miR-107 |
| 42 | AGGCAGTGTAGTTAGCTGATTGC | hsa-miR-34c-5p |
| 62,77 | TAGCAGCACGTAAATATTGGCG | hsa-miR-16-5p |
| 62,77 | TAGCAGCACATAATGGTTTGTG | hsa-miR-15a-5p |
| 42 | AGGTTGTCCGTGGTGAGTTCGCA | hsa-miR-323b-5p |
| 62,77 | TAGCAGCACAGAAATATTGGC | hsa-miR-195-5p |
| 42,62,77 | CAGCAGCACACTGTGGTTTGT | hsa-miR-497-5p |
| 62 | TTCACAGTGGCTAAGTTCCGC | hsa-miR-27a-3p |
| 62 | AGCAGCATTGTACAGGGCTATGA | hsa-miR-103a-3p |
| 39 | TAAGGCACGCGGTGAATGCC | hsa-miR-124-3p |
| 62,77 | TAGCAGCACATCATGGTTTACA | hsa-miR-15b-5p |
| 42 | TGGCAGTGTATTGTTAGCTGGT | hsa-miR-449a |
| 42 | AGGCAGTGTATTGTTAGCTGGC | hsa-miR-449b-5p |
| 62 | TTCACAGTGGCTAAGTTCTGC | hsa-miR-27b-3p |
| 62,77 | CAGCAGCAATTCATGTTTTGAA | hsa-miR-424-5p |

## Supplementary Table S2: The sequence of siRNA

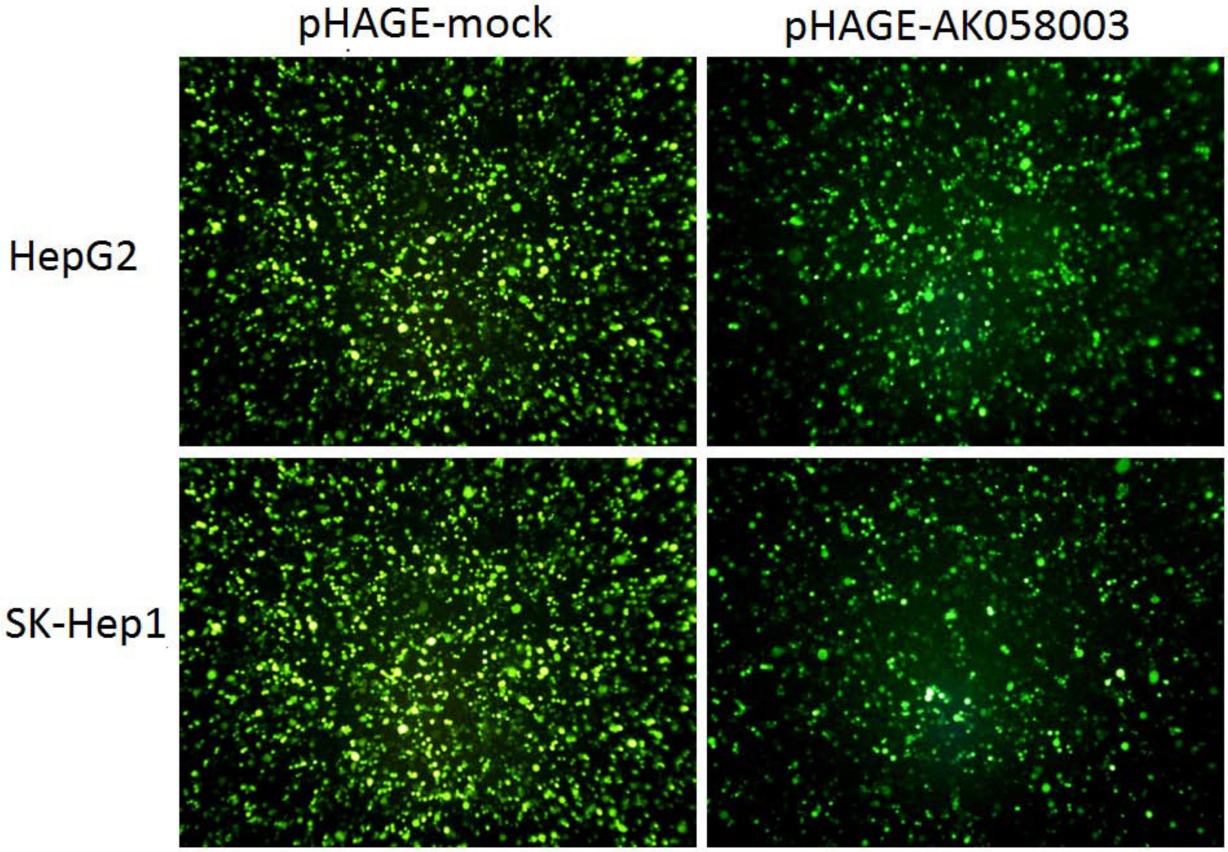
| Gene symble | Sequence |
|---|---|
| lncRNA-AK058003-si1 | |
| sense | 5′-CCACCAGUUACCUGCAAUATT dTdT-3′ |
| antisense | 3′-dTdT TTGGUGGUCAAUGGACGUUAU-5′ |
| lncRNA-AK058003-si2 | |
| sense | 5′-GGAACAAAGAUGGUUUCUATT dTdT-3′ |
| antisense | 3′-dTdT TTCCUUGUUUCUACCAAAGAU-5′ |
| SNCG-si1 | |
| sense | 5′-GGUGAGGCAUCCAAAGAGA dTdT-3′ |
| antisense | 3′-dTdT CCACUCCGUAGGUUUCUCU-5′ |
| SNCG-si2 | |
| sense | 5′-GACCAAGGAGAAUGUUGUA dTdT -3′ |
| antisense | 3′-dTdT CUGGUUCCUCUUACAACAU-5′ |
| SNCG-si3 | |
| sense | 5′- UGACGGAAGCAGCUGAGAA dTdT -3′ |
| antisense | 3′-dTdT ACUGCCUUCGUCGACUCUU-5′ |

## Supplementary Table S3: Genes and primer sequences

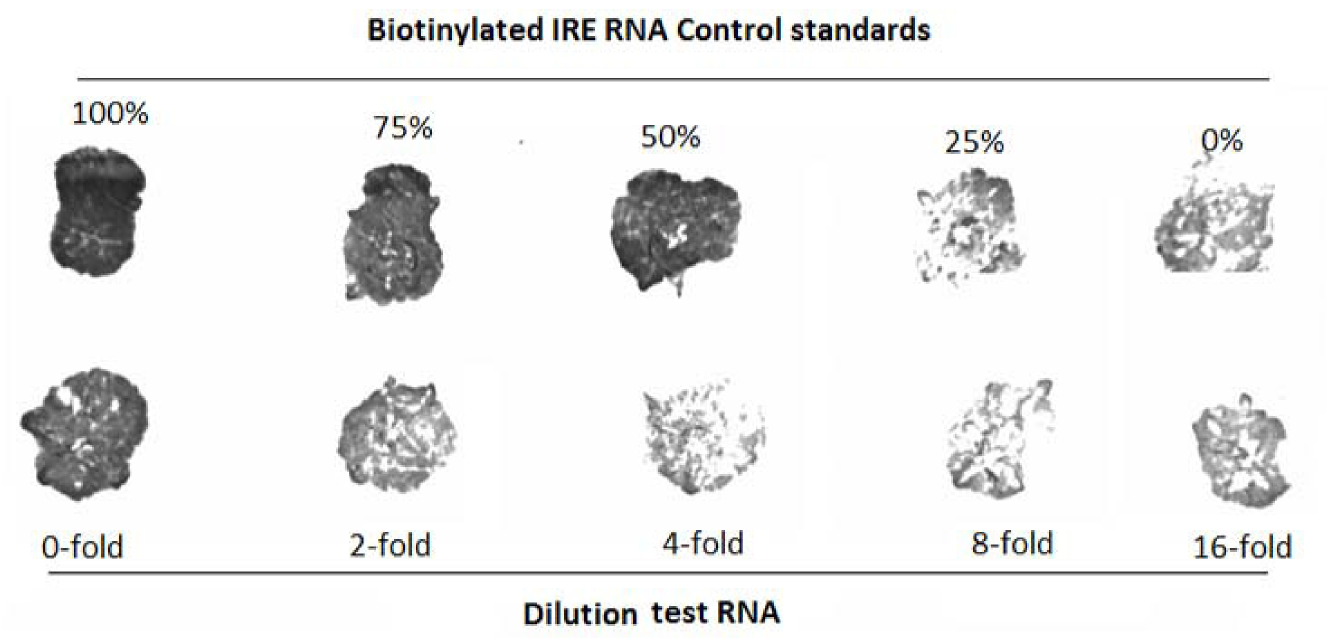
| Gene symbol | Sequence(from 5′ to 3′) |
|---|---|
| LncRNA-AK058003 | F: GGGAACAAAGATGGTTTCTACGT |
| | R: ACTGGTTCATAGTTAGGCTGGAT |
| SNCG | F: CGTGAGCGAGGCTGTGGTGA |
| | R:TCCTCTGCCACTTCCTCTTTCT |
| HuR | F:TCCGGCTGGTGCATTTTCAT |
| | R:AGTCGCGGATCACTTTCACA |
| MiR-15a | F:TGCGCTAGCAGCACATAATGGT |
| | R:CCAGTGCAGGGTCCGAGGTATT |
| | Loop:GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACCACAAAC |
| GAPDH | F: AGAAGGCTGGGGCTCATTG |
| | R: AGGGGCCATCCACAGTCTTC |
| β-actin | F:AGCGAGCATCCCCCAAAGTT |
| | R:GGGCACGAAGGCTCATCATT |
| U6 | F:CTCGCTTCGGCAGCACA |
| | R:AACGCTTCACGAATTTGCGT |
| LncRNA-AK058003 fragement | |
| 1-1197nt | F:GGCATGGTGGCTCATGCCTGTACTC |
| 421-1197nt | F:GGTTTCTACGTGCTTCCAGAG |
| 776-1197nt | F:CTCAAGTGACTGAGGTGGG |
| 1197-1176nt | R:GAGACGGAGTCTCGCCCTTTC |
| Aantisence | F:GAGACGGAGTCTCGCCCTTTC |
| | R:GGCATGGTGGCTCATGCCTGTAC |



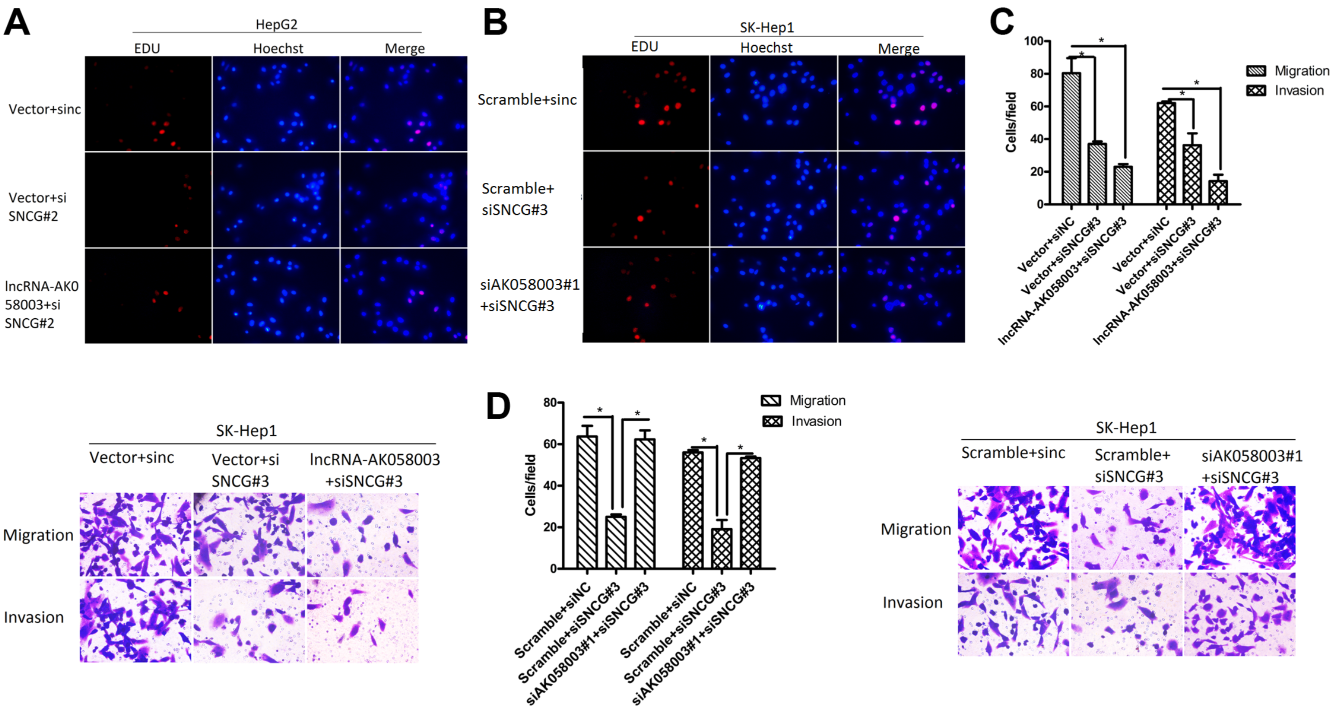
**Supplementary Figure S1:** (**A**) CCK8 assays showed that cell proliferation was suppressed by lncRNA-AK058003 overexpression in HepG2 cells. (**B**) The proliferation curves were not significantly different in lncRNA-AK058003 down-regulated SK-HeP1 cells. $^*p < 0.05$.



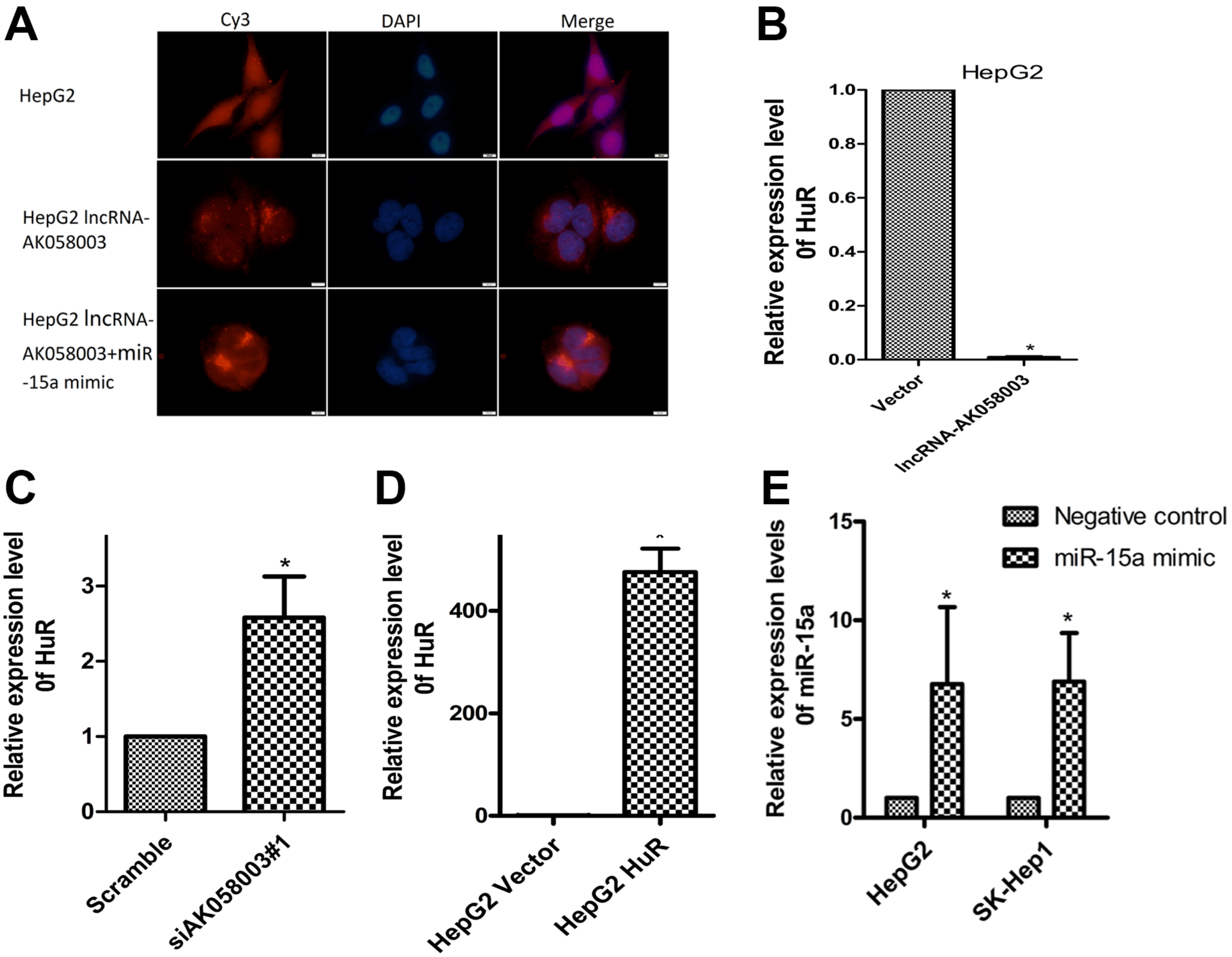
**Supplementary Figure S2: The infection efficiency of the recombinant lentivirus (pHAGE) containing human lncRNA-AK058003 gene groups or pHAGE-mock groups in HepG2 and SK-HeP1 cells were determined by fluorescent microscopy.** Original magnification, ×100. Data based on three independent experiments.



**Supplementary Figure S3: Biotinylated RNA efficiency by dot blotting analysis is shown.** A series of concentrations of biotinylated IRE RNA acted as standards.



**Supplementary Figure S4:** (**A**) LncRNA-AK058003 and siSNCG co-transfection in HepG2 cells and (**B**) siAK058003#1 and siSNCG co-transfection in SK-HeP1 cell lines were seeded into 96 well plates and cell proliferation was assessed by EdU immunofluorescence staining. Original magnification, ×400. The barplot on the right shows the percentage of EdU positive nuclei. (**C**, **D**) Transwell migration and invasion assays of SK-Hep1 cells were determined after co-transfection of lncRNA-AK058003 + siSNCG or siAK058003 + siSNCG. The graph on the left shows the positive cells per field . Original magnification, ×400. Data are shown as the mean ± S.E.M. based on three independent experiments.*$p < 0.05$, **$p < 0.01$,***$p < 0.001$.



**Supplementary Figure S5:** (**A**) RNA FISH detection of lncRNA-AK058003. Fixed HepG2 cells with DAPI-stained nuclei (blue) were probed simultaneously for lncRNA-AK058003. Original magnification, ×1000. (**B**, **C**) The expression of HuR had a negative correlation with lncRNA-AK058003. (**D**) qRT-PCR analysis shows the overexpressed level of HuR. (**E**) The expression of miR-15a in HCC cells after transfected miR-15a mimics. Data are shown as the mean ± S.E.M. based on three independent experiments.*$p < 0.05$, **$p < 0.01$,***$p < 0.001$.