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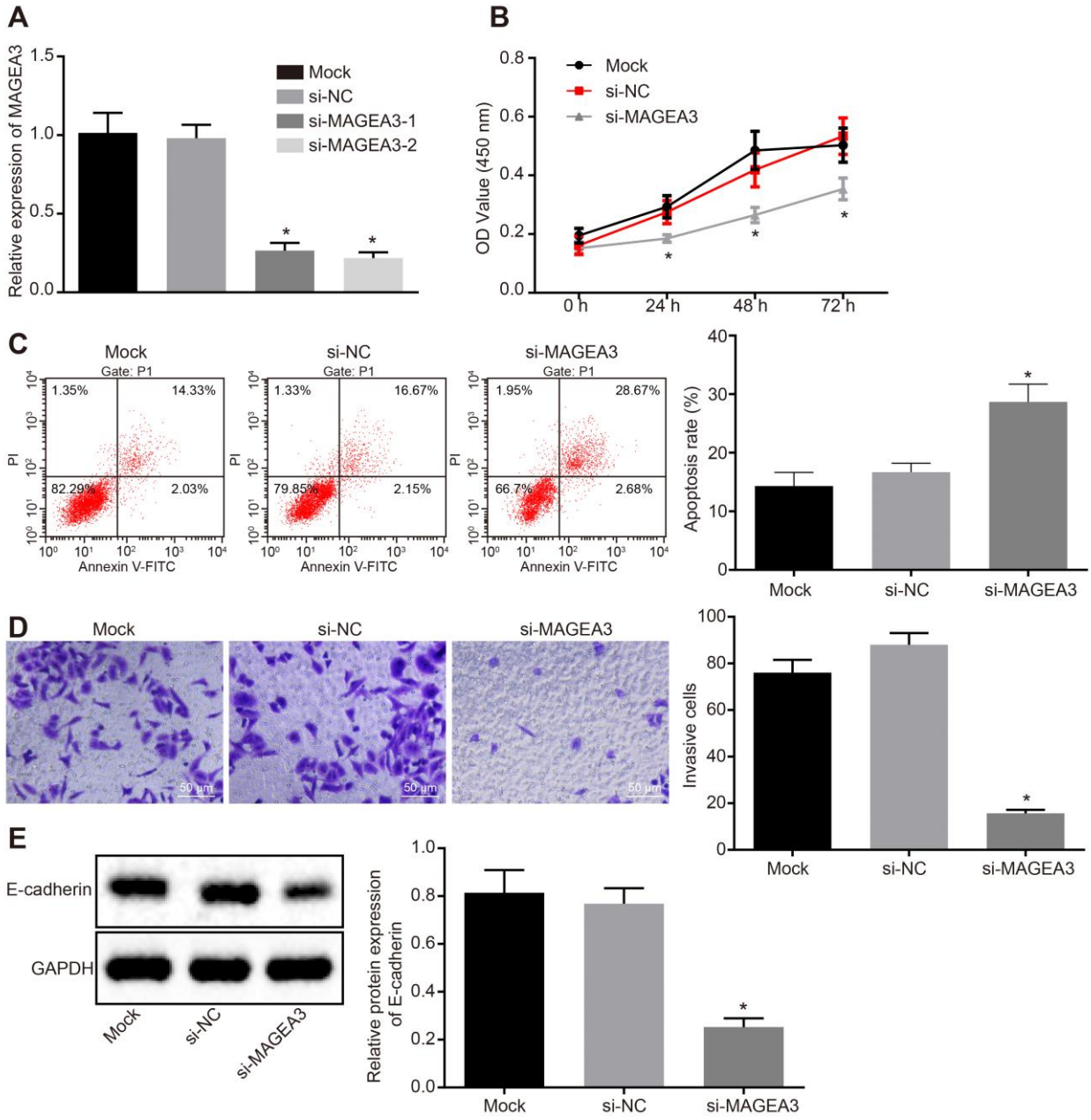
Supplemental Information

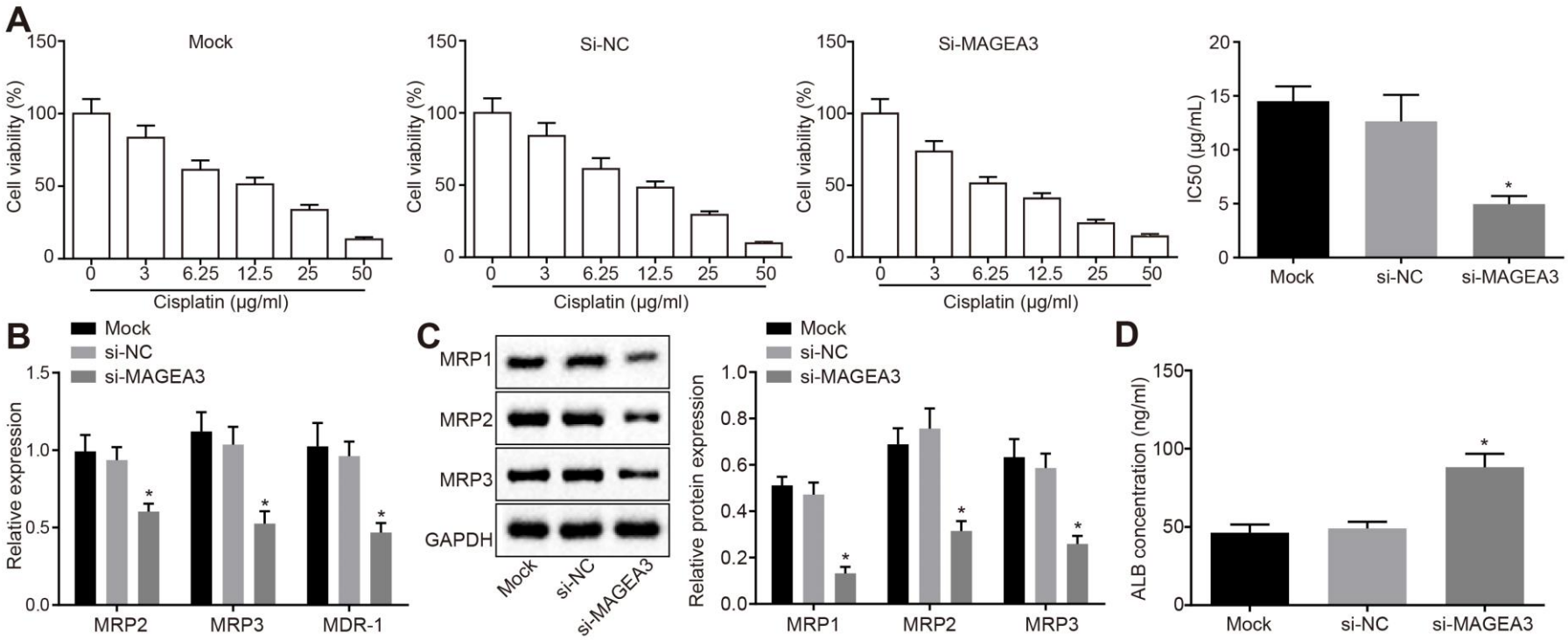
LINC01234/MicroRNA-31-5p/MAGEA3 Axis Mediates the Proliferation and Chemoresistance of Hepatocellular Carcinoma Cells

Yunhao Chen, Hui Zhao, Haibo Li, Xiao Feng, Hui Tang, Jianwen Zhang, Binsheng Fu, and Chunhui Qiu

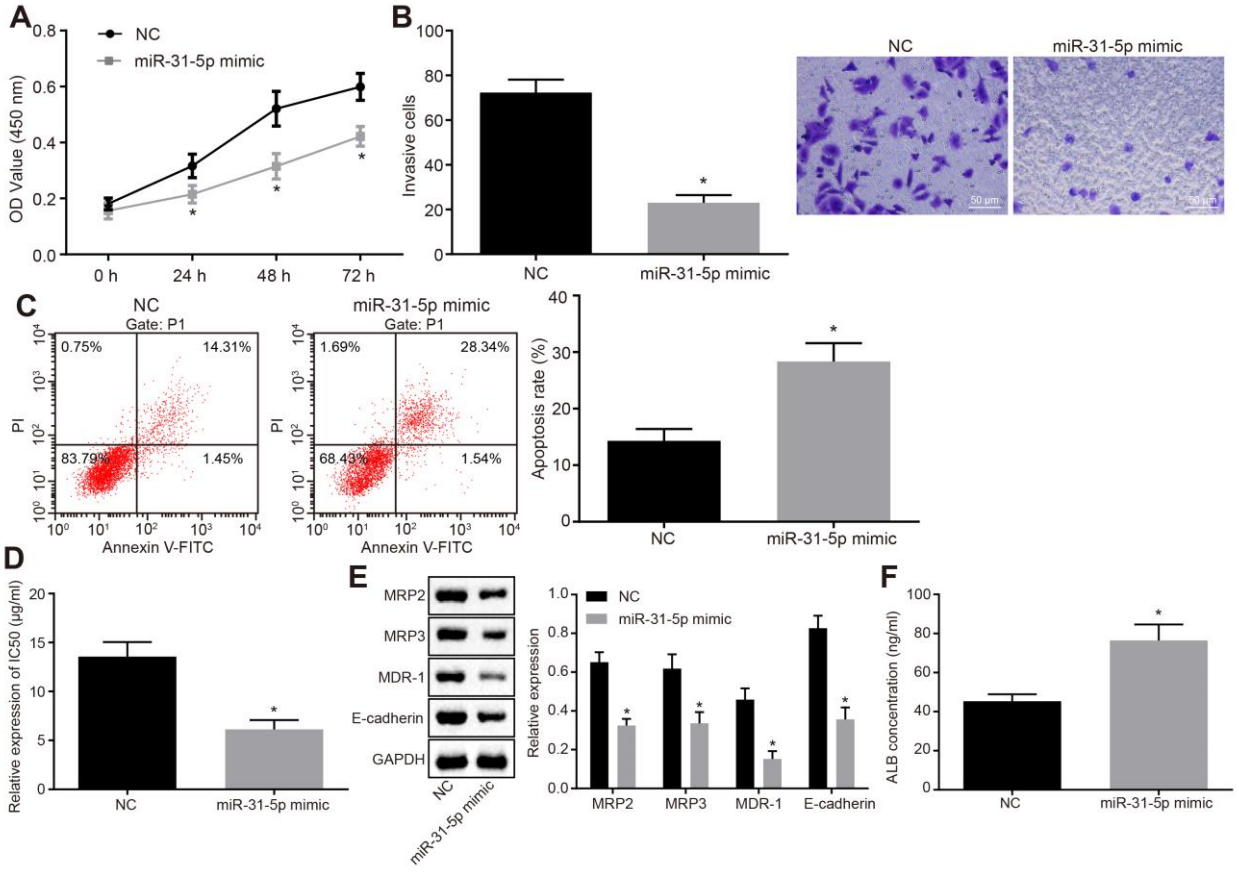


Supplementary Figure 1. Depleted MAGEA3 represses the proliferation and invasion but potentiates the apoptosis of Huh7 cells. A, transfection efficiency of siRNAs targeting MAGEA3 (si-MAGEA3-1, si-MAGEA3-2, si-MAGEA3-3) in Huh7 cells determined using RT-qPCR; B-D, viability (B), apoptosis (C) and invasion (× 200; D) of Huh7 cells in response to MAGEA3 depletion, as measured using MTT assay, flow cytometry and Transwell assay, respectively; E, protein expression of E-cadherin in Huh7 cells in response to MAGEA3 depletion measured using Western blot analysis. Measurement data were expressed as mean ± standard deviation. Data in panel B were analyzed using repeated-measures ANOVA along with Tuckey's post hoc test and data among groups in other panels were compared using one-way NAOVA together with Tuckey's

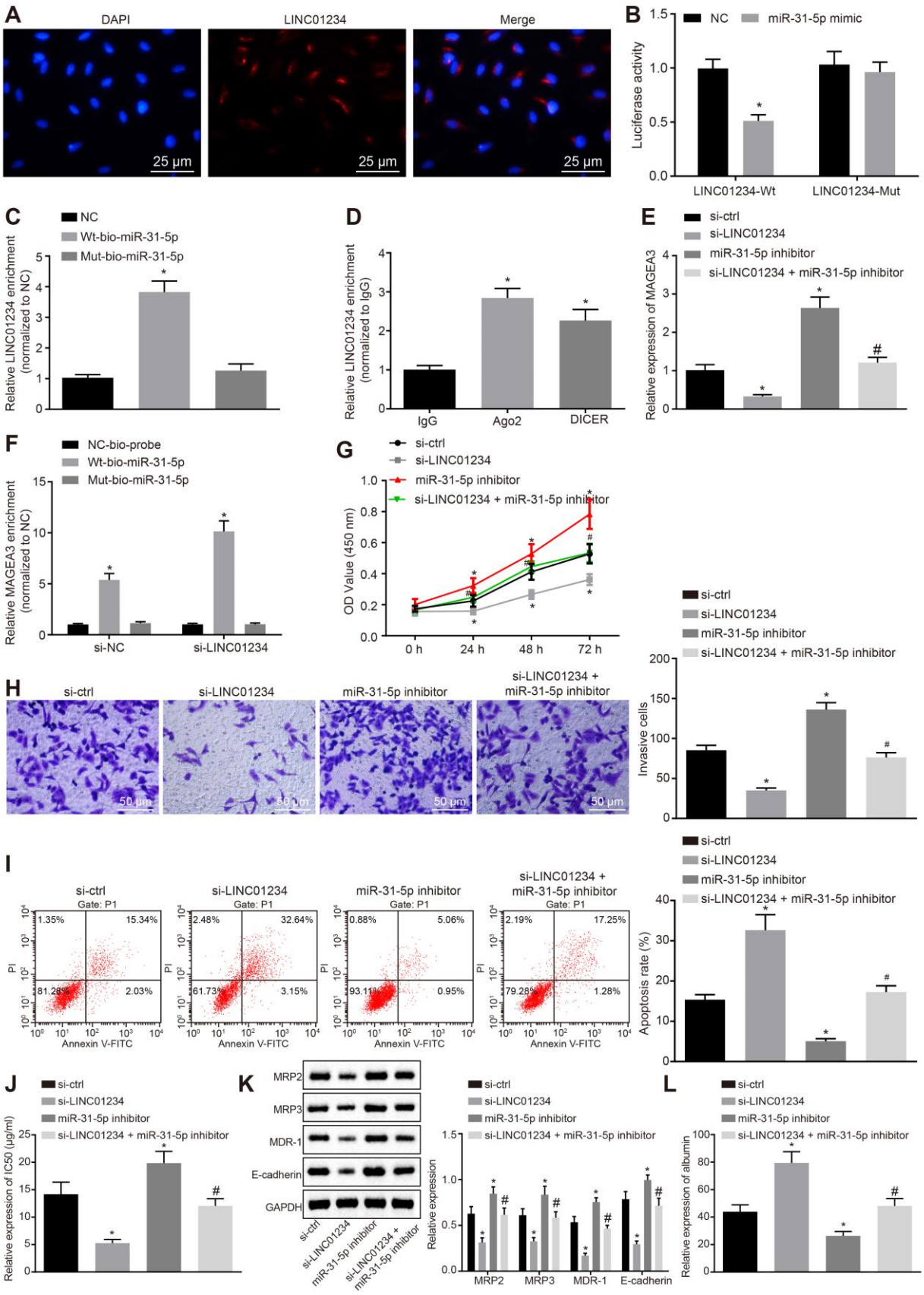
post hoc test. The experiment was repeated three times. *$p < 0.05$ vs. mock- or si-NC-transfected Huh7 cells.



Supplementary Figure 2. MAGEA3 silencing reduces chemoresistance of Huh7 cells to cisplatin. A, CCK-8 assay showing viability and the IC_{50} of Huh7 cells transfected with mock, si-NC or si-MAGEA3 upon treatment with cisplatin at different concentrations; B, the mRNA levels of MRP2, MRP3 and MDR1 in Huh7 cells following silencing of MAGEA3 determined by RT-qPCR; C, the protein levels of MRP2, MRP3, and MDR1 in Huh7 cells transfected with si-MAGEA3 measured by Western blot analysis. D, ALB content in the supernatant of Huh7 cells in response to si-MAGEA3 transfection detected by ELISA. Measurement data were expressed as mean ± standard deviation. The comparisons among multiple groups were assessed with one-way ANOVA, followed by Tukey's post hoc test. Each experiment was repeated three times. * $p < 0.05$ *vs.* the mock- or si-NC-transfected Huh7 cells.



Supplementary Figure 3. miR-31-5p dampens Huh7 cell proliferation and reduces chemoresistance to cisplatin. A-C, viability (A), invasion (×200; B) and cisplatin-induced apoptosis (C) of Huh7 cells in response to miR-31-5p mimic transfection using MTT assay, Transwell assay and flow cytometry, respectively; D, IC_{50} value of Huh7 cells in response to miR-31-5p mimic transfection; E, protein expression of MRP2, MRP3, MDR-1, and E-cadherin in Huh7 cells in response to miR-31-5p mimic transfection measured by Western blot analysis; F, content of ALB in the supernatant of Huh7 cells in response to miR-31-5p mimic transfection detected using ELISA. Measurement data were expressed as mean ± standard deviation. The comparison between two groups was analyzed by independent sample *t*-test and the comparisons among multiple groups by one-way ANOVA, followed by Tukey's post hoc test. Data in panel C were analyzed using repeated-measures ANOVA with Tuckey's post hoc test. Each experiment was repeated three times. * $p < 0.05$ vs. NC-transfected Huh7 cells.



Supplementary Figure 4. Reduction of LINC01234, results in suppression of HCC progression *via* miR-31-5p-mediated inhibition of MAGEA3. A, the subcellular localization of LINC01234 in Huh7 cells detected using FISH (× 400); B, the luciferase activity of LINC01234 in Huh7 cells

upon miR-31-5p mimic transfection detected by dual-luciferase reporter gene assay; C, the binding between LINC01234 and miR-31-5p in Huh7 cells detected by RNA pull-down assay; D, the binding between LINC01234 and Ago2 or DICER in Huh7 cells detected by RIP assay; E, MAGEA3 expression in Huh7 cells measured using RT-qPCR; F, binding of miR-31-5p to MAGEA3 in Huh7 cells detected using RNA pull-down; G-I, the viability (G), invasion (× 200; H) and cisplatin-induced apoptosis (I) of Huh7 cells upon silence of LINC01234 and/or miR-31-5p evaluated by MTT assay, Transwell assay (× 200) and flow cytometry, respectively; J, IC_{50} value of Huh7 cells upon silence of LINC01234 and/or miR-31-5p; K, protein expression of MRP2, MRP3, MDR-1, and E-cadherin in Huh7 cells determined using Western blot analysis; L, content of ALB in the supernatant of Huh7 cells detected by ELISA. Measurement data were expressed as mean ± standard deviation. Differences among groups at different points of time were assessed with repeated-measures ANOVA. The comparison between two groups was analyzed by independent sample *t*-test and the comparisons among multiple groups by one-way ANOVA, followed by Tukey's post hoc test. Each experiment was repeated three times. **p* < 0.05 *vs.* the IgG immunoprecipitates or the NC- or si-ctrl-transfected cells; #*p* < 0.05 *vs.* the si-LINC01234-transfected cells.