

Supplementary Information

Combined Aberrant Expression of NDRG2 and LDHA Predicts Hepatocellular Carcinoma Prognosis and Mediates the Anti-tumor Effect of Gemcitabine

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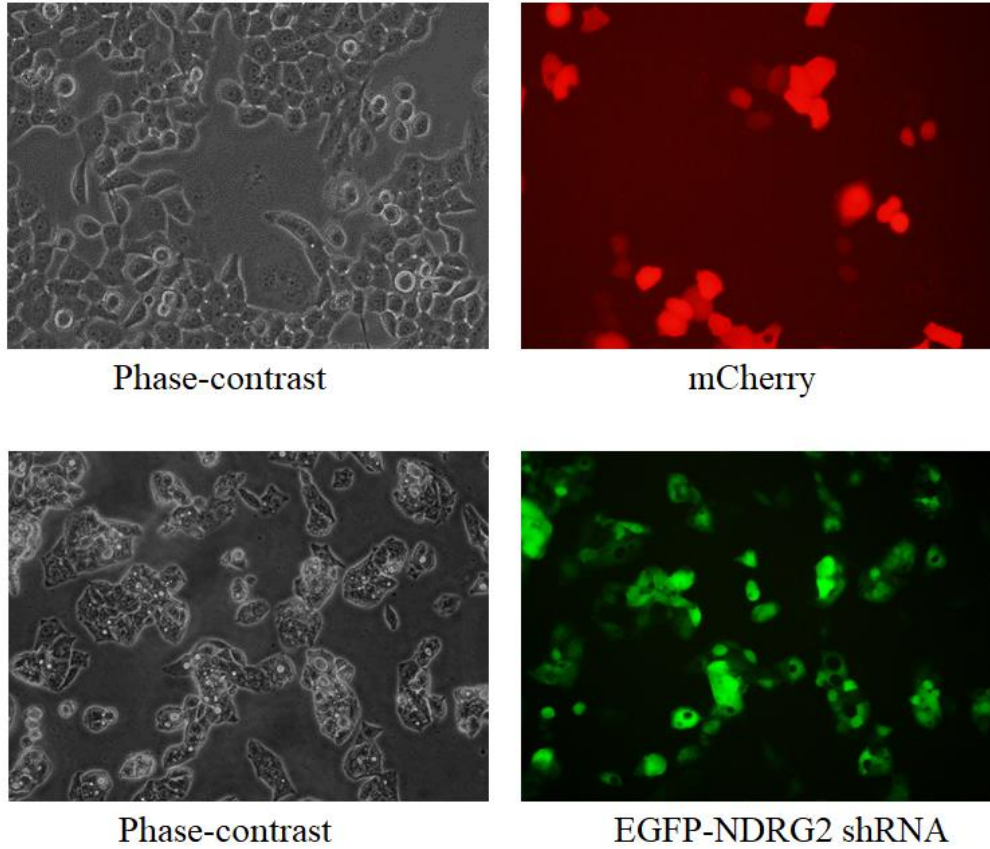
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Running title: NDRG2 inhibits LDHA expression and mediates the pharmacological effect of gemcitabine

Supplementary Data

Supplementary Figures and Figure legends

Supplementary Figure 1

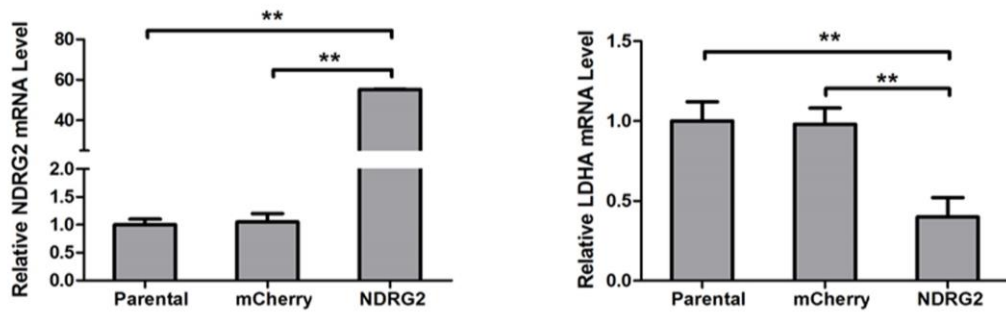


Supplementary Figure 1. Fluorescence microscopy shows the infection efficiency of HepG2 cells

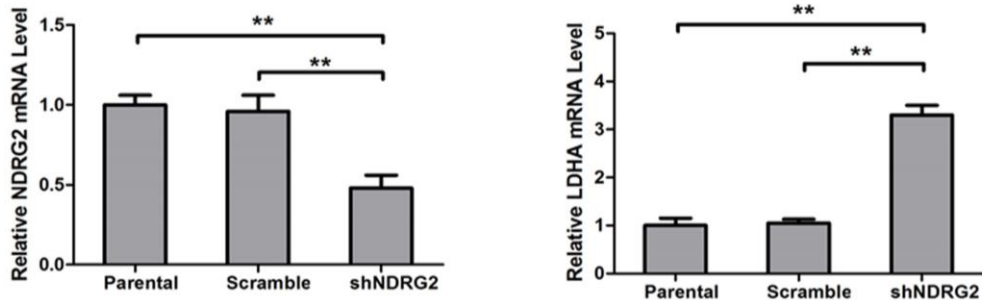
HepG2 cells were infected with Lenti-mCherry or Lenti-EGFP-NDRG2 shRNA for 48 h and then imaged with confocal fluorescence microscopy.

Supplementary Figure 2

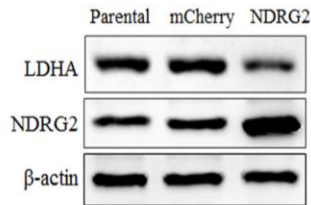
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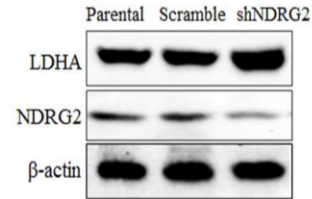
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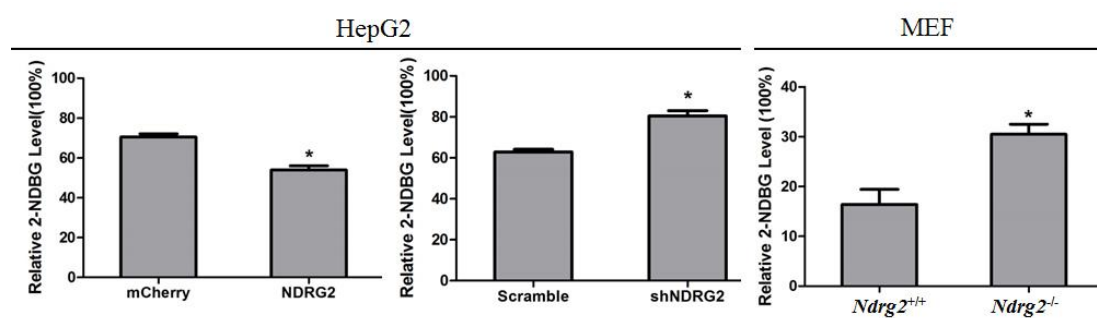
D



Supplementary Figure 2. NDRG2 inhibits LDHA expression in HuH-7 cells

(A) NDRG2 and LDHA mRNA levels in HuH-7 cells that were infected with lentivirus containing NDRG2 or mCherry, and β -actin served as an internal control to ensure equal loading. (B) NDRG2 and LDHA mRNA levels in HuH-7 cells that were infected with lentivirus containing NDRG2 shRNA or Scramble, and β -actin served as an internal control to ensure equal loading. (C) NDRG2 and LDHA protein levels in HuH-7 cells that were infected with lentivirus containing NDRG2 or mCherry, and β -actin served as an internal control to ensure equal loading. (D) NDRG2 and LDHA protein levels in HuH-7 cells that were infected with lentivirus containing NDRG2 shRNA or Scramble, and β -actin served as an internal control to ensure equal loading. All $**P < 0.01$.

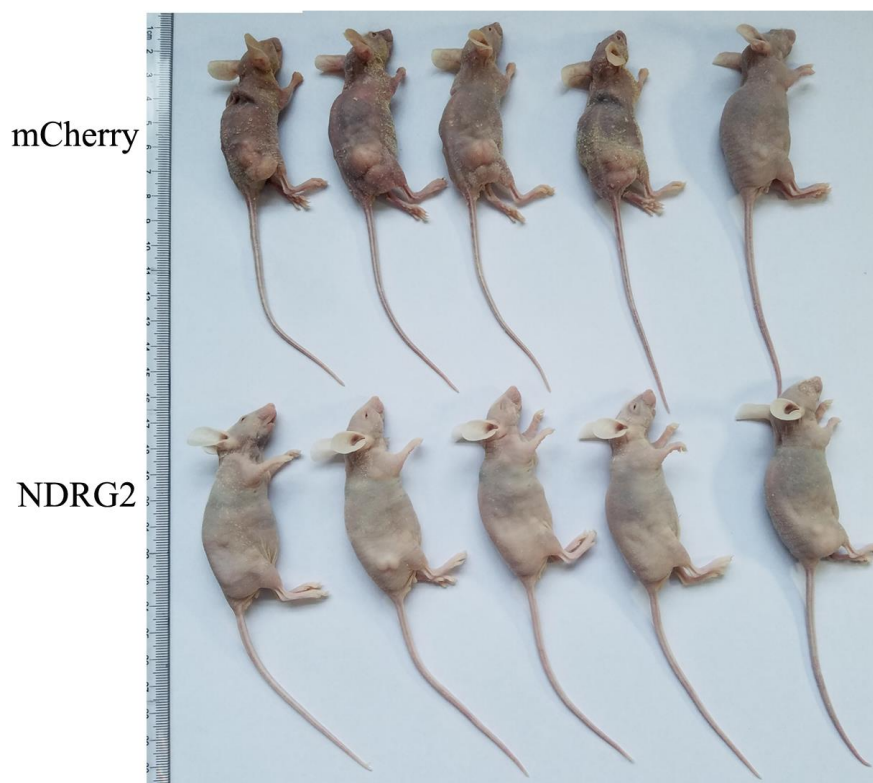
Supplementary Figure 3



Supplementary Figure 3. NDRG2 inhibits glucose uptake in HepG2 and MEF cells

Glucose uptake in HepG2 cells that were infected with lentivirus containing NDRG2 or mCherry (left), NDRG2 shRNA or Scramble (middle), and *Ndrg2* knockout Murine Embryonic Fibroblasts (MEF) cells (right). All **P*<0.05.

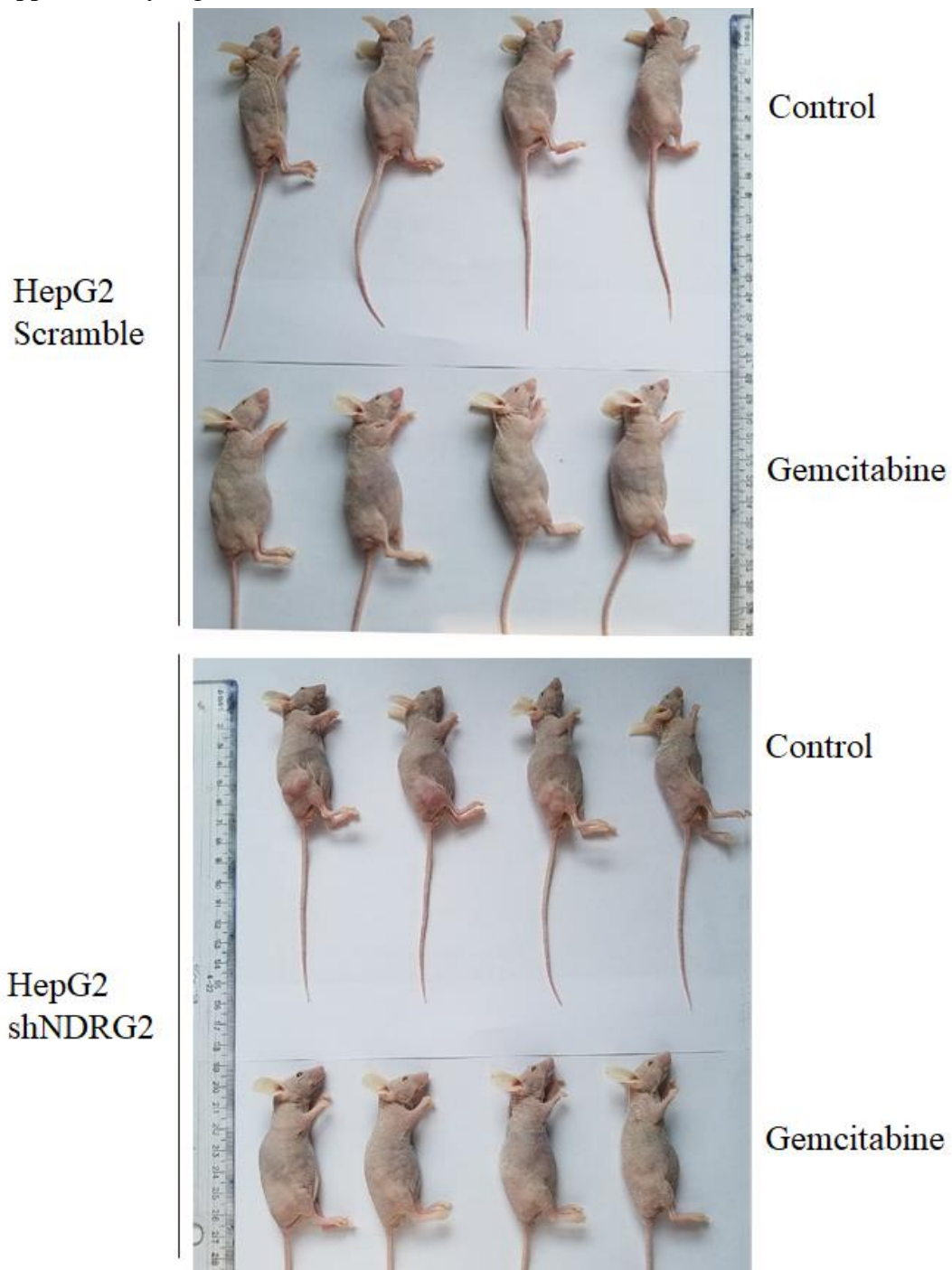
Supplementary Figure 4



Supplementary Figure 4. NDRG2 inhibits the growth and proliferation of HCC cells

Nude mice were injected subcutaneously in the right limb with 1×10^7 HepG2 cells or mCherry-overexpressing or NDRG2-overexpressing HepG2 cells respectively. Representative tumor formation was photographed 28 days after injection.

Supplementary Figure 5



Supplementary Figure 5. NDRG2 mediates gemcitabine inhibition of the growth of HCC cells

Nude mice were injected subcutaneously in the right limb with 1×10^7 HepG2 that were infected with lentivirus containing NDRG2 shRNA or Scramble. Once the tumor size reached an average of 700 mm^3 , the mice were treated with 80 mg/kg of gemcitabine diluted in saline on 8, 12 and 16 days post tumor inoculation (d.p.i.). Control mice received saline alone. Representative tumor formation was photographed after injection.

Supplementary Table

Supplementary Table 1. The primers used for quantitative real-time RT-PCR

Gene	Forward	Reverse
<i>NDRG2</i>	5'-GAGATATGCTCTTAACCACCCG-3'	5'-GCTGCCCAATCCATCCAA-3'
<i>LDHA</i>	5'-CTGGGAGTTCACCCATTAAGCT-3'	5'-CAGGCACACTGGAATCTCCAT-3'
<i>C-MYC</i>	5'-CAGTGGGCTGTGAGGAGGT-3'	5'-CAGGCTCCTGGCAAAGGT-3'
<i>β-ACTIN</i>	5'-CGCGAGAAGATGACCCAGAT-3'	5'-GTACGGCCAGAGGCGTACAG-3'
<i>Ndrp2</i>	5'-ACACCTTATGGCTCGGTCAC-3'	5'-TCTCTGCATATCCCCGAAC-3'
<i>Ldha</i>	5'-CAAAGACTACTGTGTAAGTGCAG-3'	5'-TGGACTGTACTTGACAATGTTGG-3'
<i>C-myc</i>	5'-GCTCGCCCAAATCCTGTA-3'	5'-TCCACAGACACCACATCAA-3'
<i>β-Actin</i>	5'-CGTGAAAAGATGACCCAGAT-3'	5'-GTACGACCAGAGGCATACAG-3'