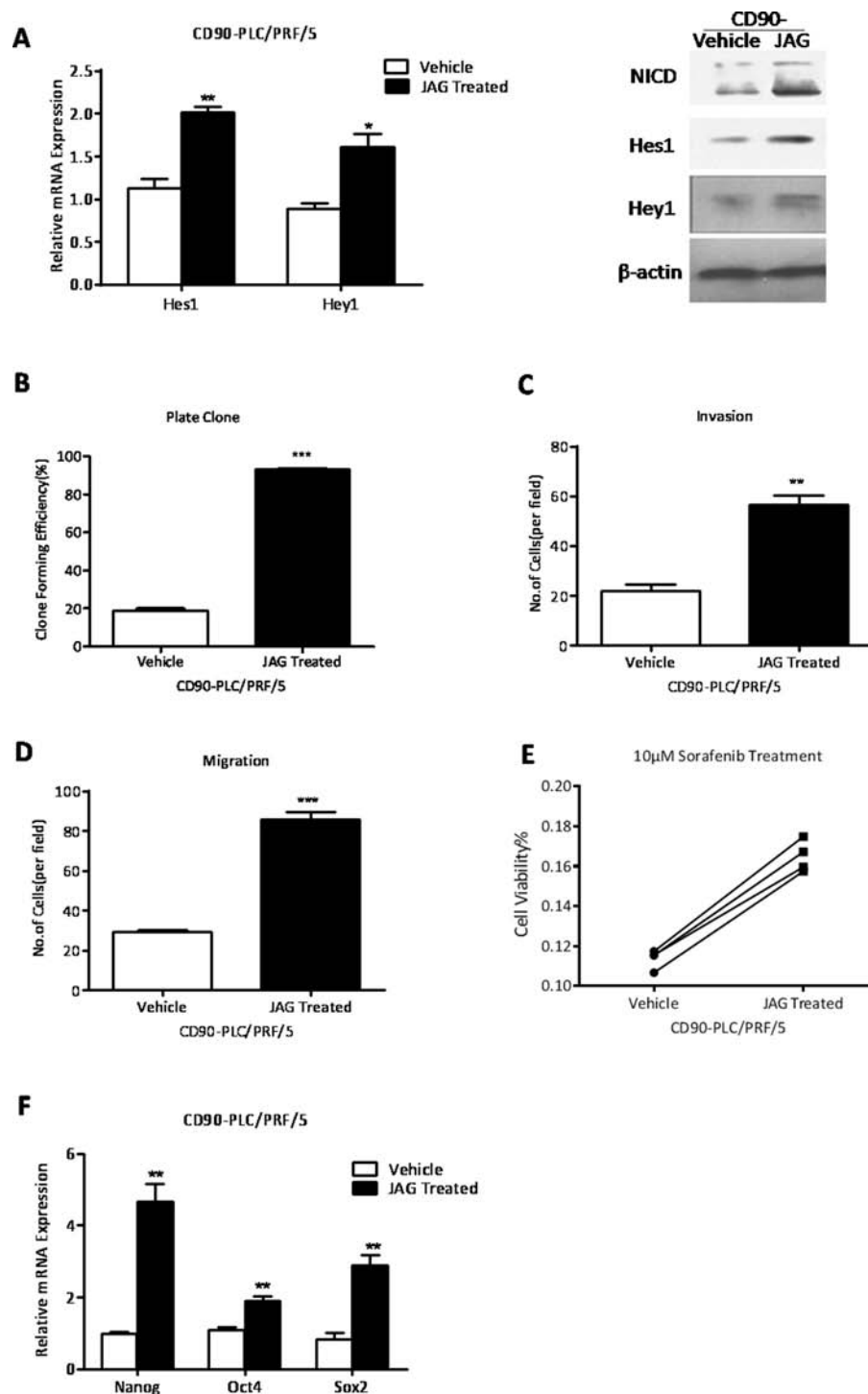
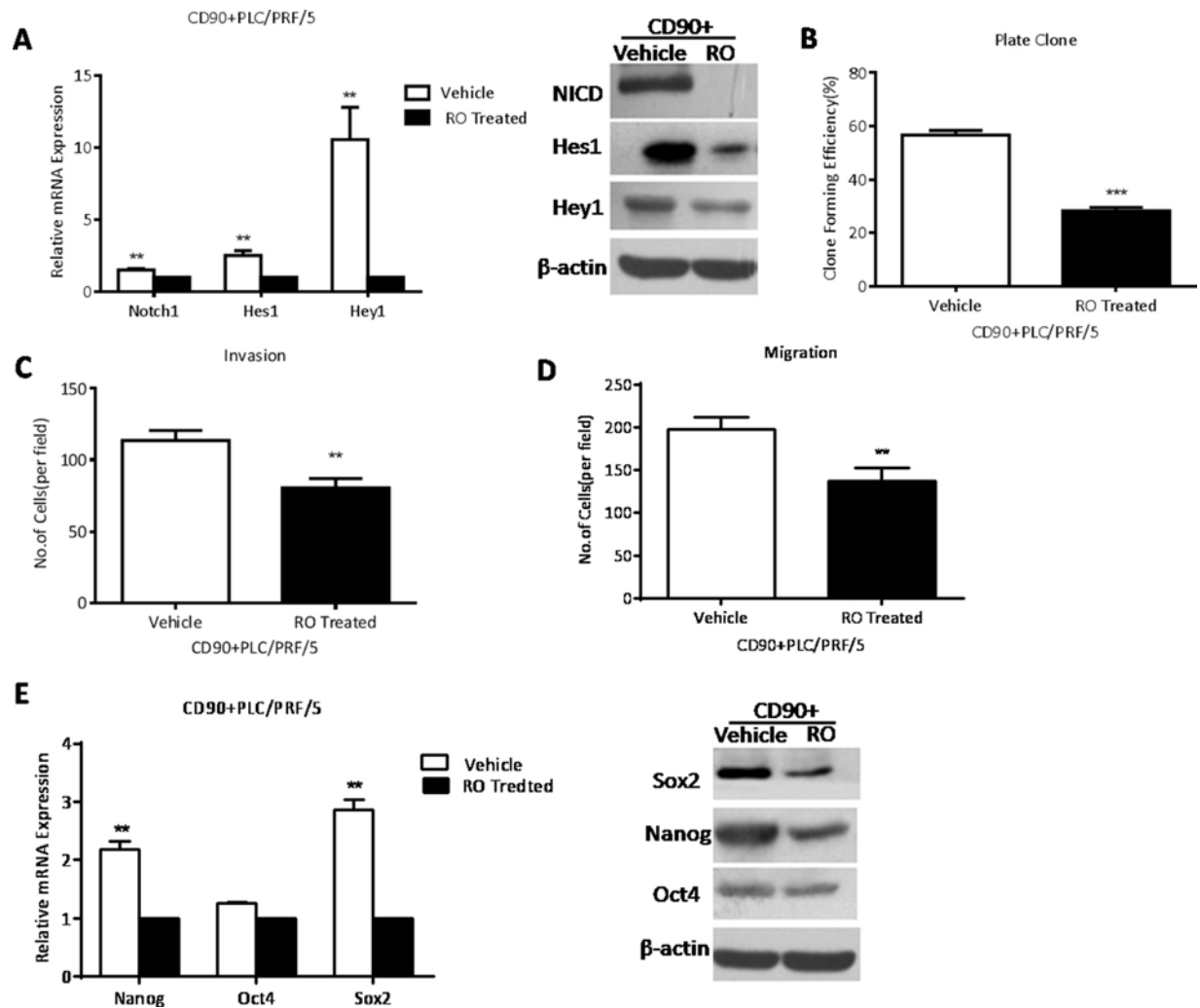


SUPPLEMENTARY FIGURES AND TABLES



Supplementary Figure S1: CD90⁺ cells possessed characteristics of cancer stem cells after Notch signaling activated by Jagged1. The core components of Notch signaling in CD90⁺ cells could be increased by human recombinant protein Jagged1 (Supplementary Fig. 1A). With the overexpression of NICD, we found the capacity of colony formation, invasion and migration in CD90⁺ cells was elevated following activation of Notch pathway (Supplementary Fig. 1B, 1C, 1D). Treated with 10 µM Sorafenib CD90⁺ cells displayed promoted viability following NICD overexpression (Supplementary Fig. 1E). In addition, Nanog, Sox2 and Oct4 were also enhanced in expression levels detected by qRT-PCR (Supplementary Fig. 1F).



Supplementary Figure S2: The inhibition of Notch pathway by RO4929097 attenuated cancer stem cell characteristics of CD90⁺ cells. RO4929097, as the γ -secretase inhibitor, could downregulate the Notch pathway in CD90⁺ cells as shown in Supplementary Fig. 2A. Accompanied with decreasing NICD, CD90⁺ cells showed presented the declining capacity of colony formation, migration and invasion (Supplementary Fig. 2B, 2C, 2D), and embryonic stem cell-associated markers (Nanog, Sox2) in CD90⁺ cells was also reduced after treated with RO4929097 (Supplementary Fig. 2E). However, there was no change in expression of Oct4.

Supplementary Table S1: Tumorigenic capacity of CD90⁺ cells from HCC cell lines

| Cell Lines | Phenotypes | No. of Injected Cells | Tumor Incidence* | Latency(days)** |
|------------|-------------------|-----------------------|------------------|-----------------|
| PLC/PRF/5 | CD90 ⁺ | 2×10 ³ | 2/3 | 20 |
| | | 1×10 ⁴ | 2/3 | 15 |
| | | 5×10 ⁴ | 3/3 | 7 |
| | CD90 ⁻ | 2×10 ³ | 0/3 | - |
| | | 1×10 ⁴ | 0/3 | - |
| | | 5×10 ⁴ | 0/3 | - |
| Huh-7 | CD90 ⁺ | 1×10 ³ | 0/3 | - |
| | | 2×10 ³ | 1/3 | 10 |
| | | 1×10 ⁴ | 3/3 | 8 |
| | CD90 ⁻ | 1×10 ³ | 0/3 | - |
| | | 2×10 ³ | 0/3 | - |
| | | 1×10 ⁴ | 0/3 | - |

*No. of mice with tumor formation/no. of mice injected.

**Approximate no. of days from cells injection to appearance of the tumor.

Supplementary Table S2: Tumorigenic capacity of CD90⁺/CD90⁻ cells from PLC/PRF/5 after the inhibition or activation of Notch pathway

| Phenotypes | No. of Injected Cells | Tumor Incidence* | Latency(days)** |
|---------------------------------|-----------------------|------------------|-----------------|
| CD90 ⁺ +Vehicle | 2×10 ³ | 0/3 | - |
| | 1×10 ⁴ | 0/3 | - |
| | 5×10 ⁴ | 1/3 | 36 |
| | 1×10 ⁵ | 3/3 | 28 |
| CD90 ⁺ +Lv-Notch1-si | 2×10 ³ | 0/3 | - |
| | 1×10 ⁴ | 0/3 | - |
| | 5×10 ⁴ | 0/3 | - |
| | 1×10 ⁵ | 0/3 | - |
| CD90 ⁻ +Vehicle | 1×10 ⁵ | 0/3 | - |
| | 2×10 ⁵ | 0/3 | - |
| | 5×10 ⁵ | 1/3 | 70 |
| | 1×10 ⁶ | 1/3 | 63 |
| CD90 ⁻ +Lv-NICD | 1×10 ⁵ | 2/3 | 67 |
| | 2×10 ⁵ | 2/3 | 56 |
| | 5×10 ⁵ | 2/3 | 44 |
| | 1×10 ⁶ | 2/3 | 37 |

*No. of mice with tumor formation/no. of mice injected.

**Approximate no. of days from cells injection to appearance of the tumor.

Supplementary Table S3: Primer sequences for quantitative RT-PCR analysis

| Gene | GENE ID. | Primer sequences (5'-3') | Product length |
|----------------|----------|---|----------------|
| Sox2 | 6657 | F:GCCCTGCAGTACAACCTCCAT R:GACTTGACCACCGAACCCAT | 110 |
| Oct4 | 5460 | F:CTTGAATCCCGAATGGAAAGGG R:GACTTGACCACCGAACCCAT | 111 |
| Nanog | 79923 | F:GTCCCAAAGGCAAACAACCC R:GCTGGGTGGAAGAGAACACA | 108 |
| CD90 | 7070 | F:ACTGCCGCCATGAGAATACC R:CTGGTGAAGTTGGTTCGGGA | 136 |
| MDR1 | 5243 | F:CCCATCATTGCAATAGCAGG R:GTTCAAACCTCTGCTCCTGA | 157 |
| GSTP1 | 2950 | F:TGGTGGACATGGTGAATGAC R:ATCTGGTCTCCACAATGAAG | 181 |
| LRP | 9961 | F:GGATGTCAAGACCGGAAAGGT R:TCTTTCTCCACGGACTTCGT | 80 |
| Hes1 | 3280 | F:GCTAAGGTGTTTGGAGGCT R:CCGCTGTTGCTGGTGTA | 122 |
| Notch1 | 4851 | F:CCGCAGTTGTGCTCCTGAA R:ACCTTGGCGGTCTCGTAGCT | 109 |
| Hey1 | 23462 | F:GTTTCGGCTCTAGGTTCCATGT CGTCGGCGCTTCTCAATTATTC | 88 |
| β -actin | 60 | F:GTTGCGTTACACCCTTTCTTG R:GACTGCTGTCACCTTCACCGT | 157 |
| CyclinD1 | 595 | F: GCTGCGAAGTGGAACCATC R: CCTCCTTCTGCACACATTTGAA | 135 |
| CyclinE1 | 898 | F:GCCAGCCTTGGGACAATAATG R:CTTGCACGTTGAGTTTGGGT | 104 |
| CDK2 | 1017 | F:GTACCTCCCCTGGATGAAGAT R:CGAAATCCGCTTGTTAGGGTC | 75 |
| CDK6 | 1021 | F:CCAGATGGCTCTAACCTCAGT R:AACTTCCACGAAAAAGAGGCTT | 152 |
| E2F1 | 1869 | F:ACGTGACGTGTCAGGACCT R:GATCGGGCCTTGTTTGCTCTT | 146 |

F: Forward; R: Reverse.