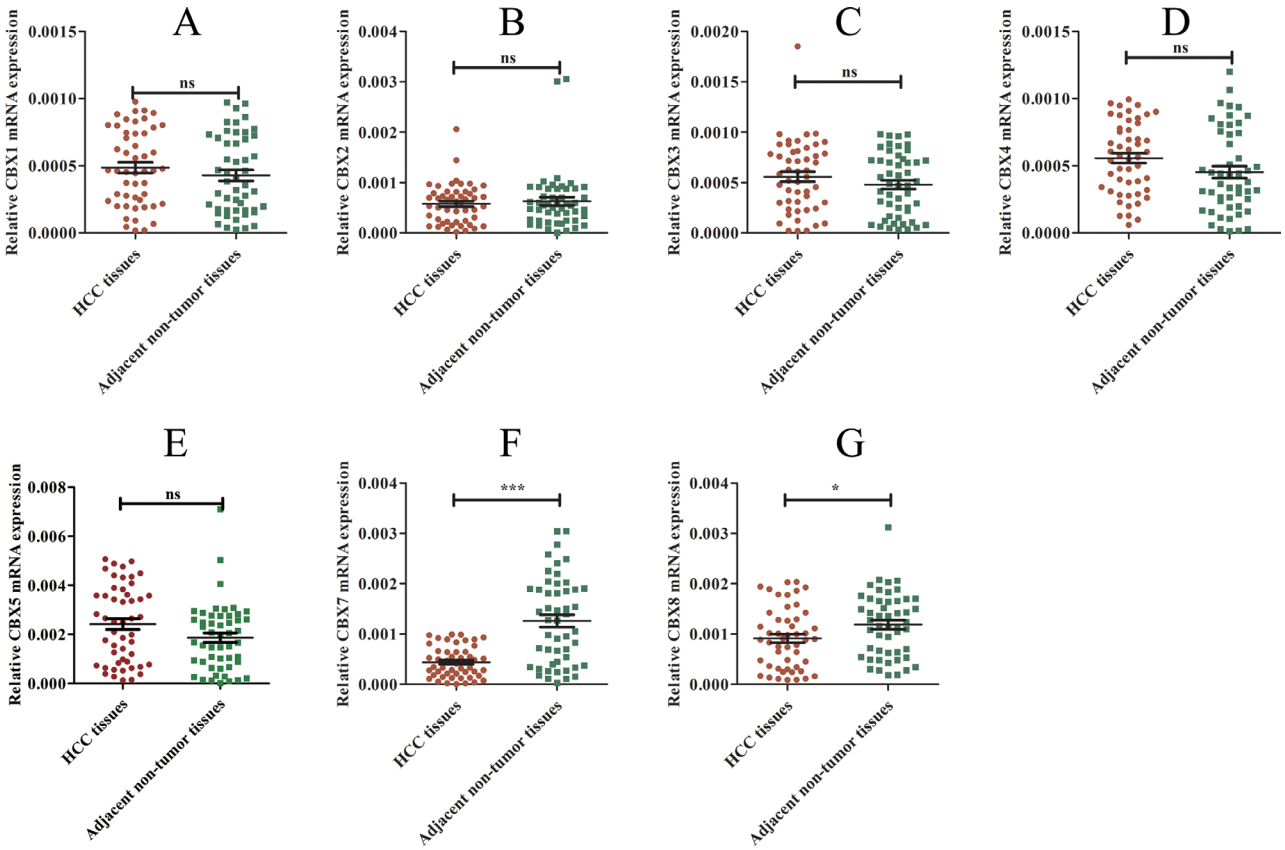
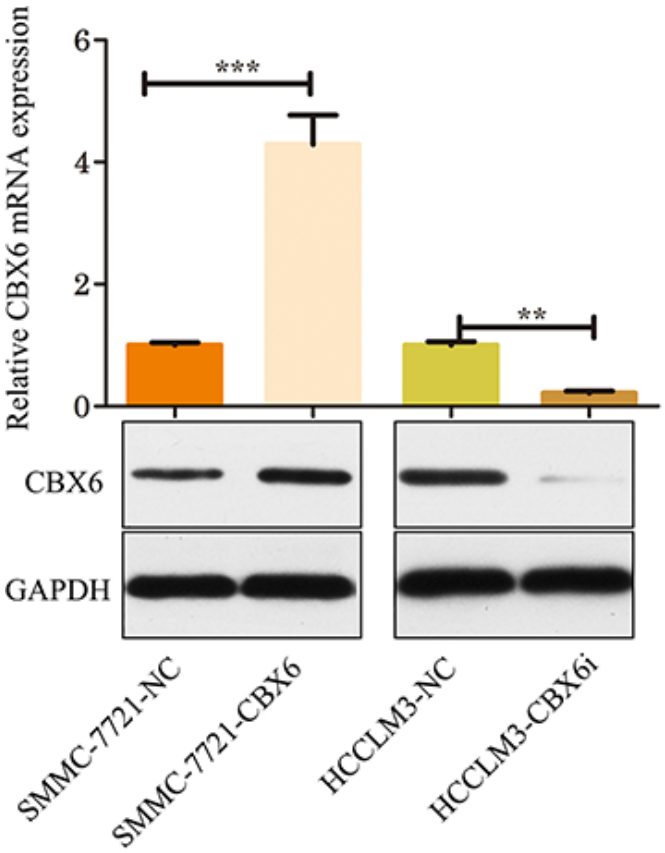
# CBX6 overexpression contributes to tumor progression and is predictive of a poor prognosis in hepatocellular carcinoma

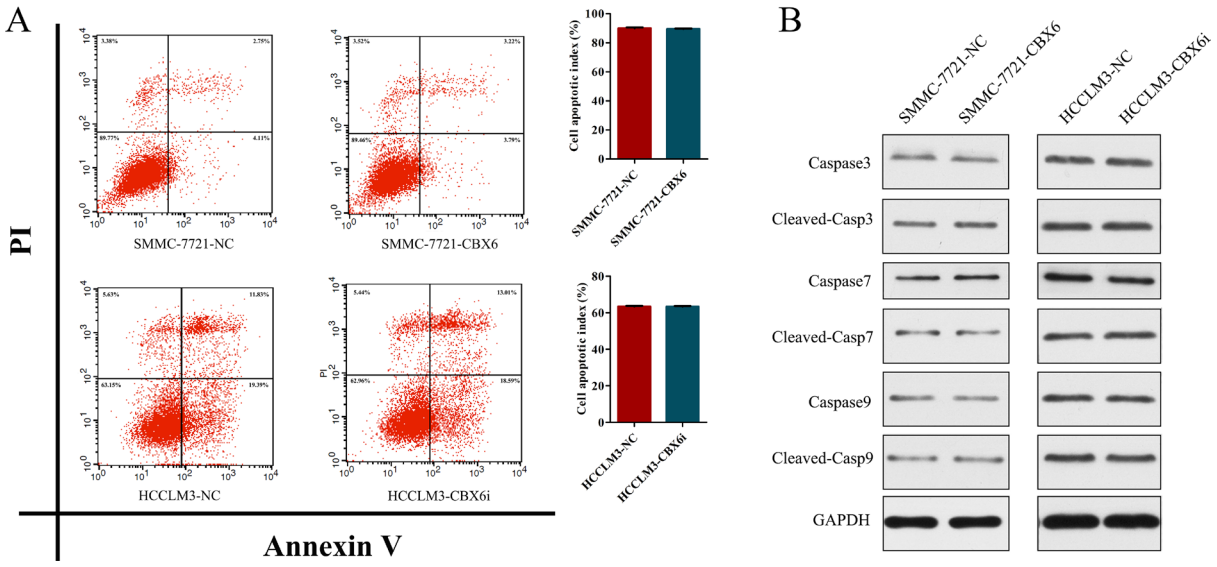
## Supplementary Materials



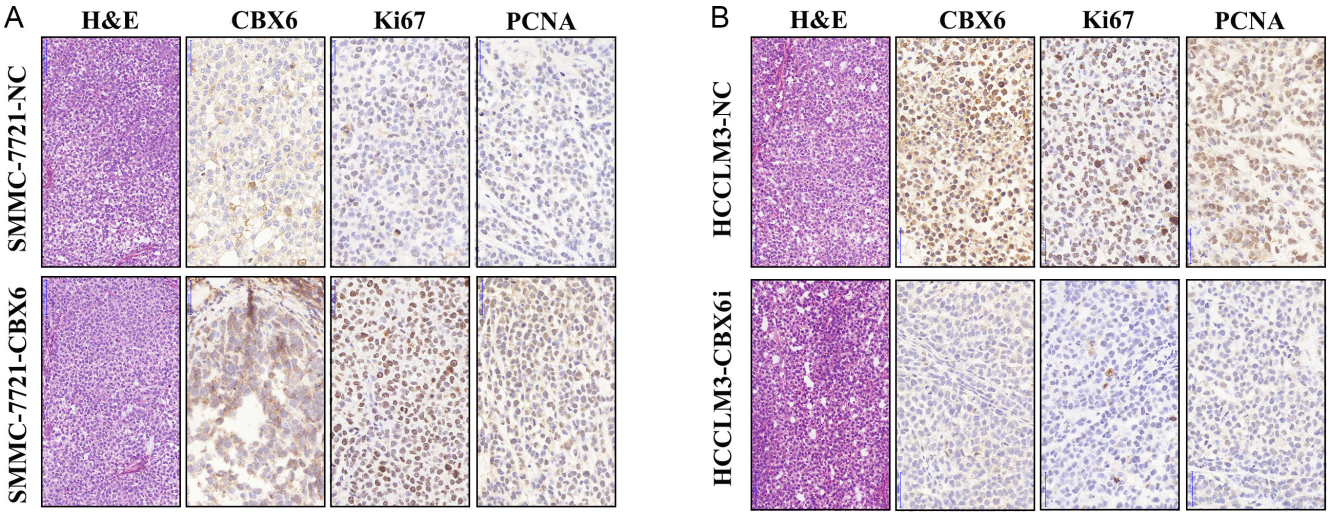

**Supplementary Figure 1: CBX family protein expression was measured in HCC samples.** (**A**–**G**) CBX1-5, CBX7 and CBX8 expression in 50 pairs of HCC tissues and matched adjacent non-tumor tissueswas measured via real-time qPCR.



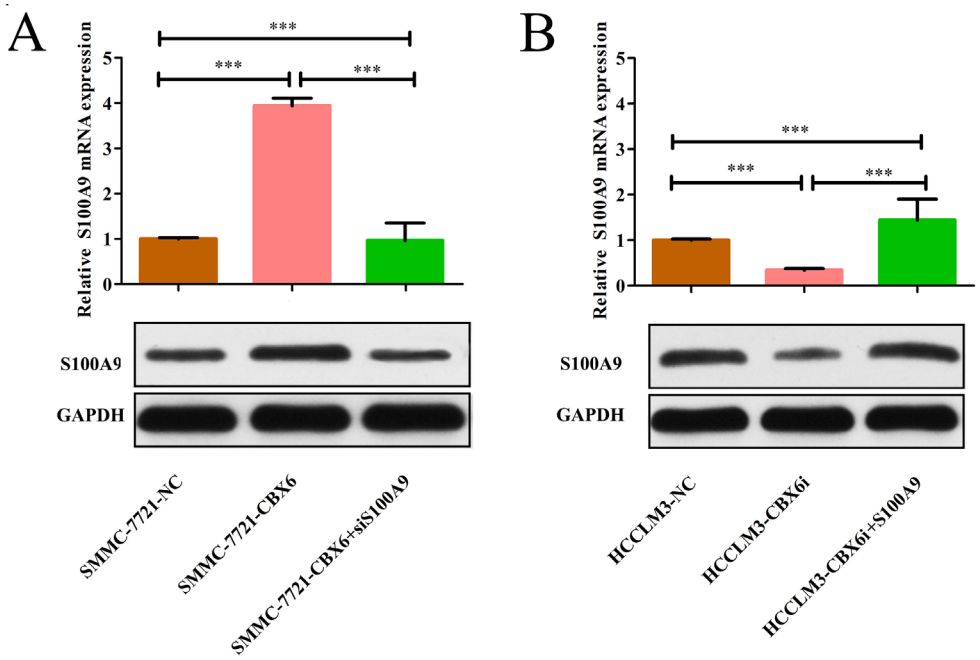

**Supplementary Figure 2: CBX6 was stably overexpressed in SMMC-7721 cells and knocked down in LM3 cells.** qRT-PCR and Western blotting were performed to assess CBX6 overexpression in SMMC-7721 cells and CBX6 knockdown in LM3 cells (*$p < 0.05$;**$p < 0.01$; ***$p < 0.001$).




**Supplementary Figure 3: Anti-apoptotic effects of PON3 were not observed in HCC cells.** (**A**) Flow cytometry analysis of apoptosis using annexin V-FITC and PI staining in CBX6-overexpression and knockdown cells. (**B**) Westernblot analysis of the expression of the apoptosis-related proteins caspase-3, 7, 9 and their respective cleavage fragments in CBX6-overexpression and knockdown cells. GAPDH was used as an internal control.




**Supplementary Figure 4: CBX6 promotes HCC tumor growth *in vivo*.** (**A**) H&E-stained paraffin-embedded sections obtained from the xenografts. IHC staining shows that Ki67 and PCNA expression levels were enhanced in the SMMC-7721-CBX6 group compared with the SMMC-7721-NC group. (**B**) H&E-stained paraffin-embedded sections obtained from the xenografts. IHC staining shows that Ki67 and PCNA expression levels weredecreased in the HCCLM3-CBX6igroup compared with the HCCLM31-NC group.




**Supplementary Figure 5: S100A9 plays a critical role in mediating CBX6 function.** (**A**) S100A9 mRNA and protein levels in SMMC7721 cells overexpressing S100A9 that were transduced with siS100A9 or a control vector were detected by qRT-PCR and westernblotting. (**B**) S100A9 mRNA and protein levels in HCCLM3 CBX6-knockdown cells that were transduced with siS100A9 or a control vector were detected by qRT-PCR and westernblotting (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$).

**Supplemental Table 1: Univariable analysis for recurrence free survival (RFS) and overall survival (OS)**

| Variable | RFS | | | OS | | |
|---|---|---|---|---|---|---|
| | Hazard ratio | 95% CI | *P* value | Hazard ratio | 95% CI | *P* value |
| **Age**, years, ≥ 55 vs. < 55 | 1.182 | 0.879–1.591 | 0.268 | 1.072 | 0.790–1.456 | 0.655 |
| **Sex**, male vs. female | 1.139 | 0.729–1.778 | 0.568 | 1.178 | 0.746–1.860 | 0.482 |
| **HBsAg**, positive vs. negative | 1.990 | 1.191–3.327 | 0.009 | 2.067 | 1.196–3.572 | 0.009 |
| **HBeAg**, positive vs. negative | 1.633 | 1.173–2.272 | 0.004 | 1.692 | 1.211–2.364 | 0.002 |
| **AFP**, μg/L ≥ 20 vs. < 20 | 1.447 | 1.059-1.977 | 0.020 | 1.363 | 0.992–1.872 | 0.056 |
| **Edmondson-Steiner classification**, III-IV vs. I-II | 1.578 | 0.930–2.677 | 0.091 | 1.312 | 0.784–2.196 | 0.300 |
| **Tumor diameter**, cm, ≥ 5 vs. < 5 | 1.462 | 1.085–1.970 | 0.013 | 1.442 | 1.061–1.961 | 0.019 |
| **Tumor number**, multiple vs. solitary | 1.237 | 0.873–1.751 | 0.231 | 1.336 | 0.937–1.905 | 0.109 |
| **Vascular invasion**, present vs. absent | 1.566 | 1.152–2.129 | 0.004 | 1.337 | 0.979–1.826 | 0.068 |
| **CBX6 expression**, high vs. low | 1.493 | 1.115–2.000 | 0.007 | 1.626 | 1.202–2.200 | 0.002 |

**Abbreviation**: HBsAg, hepatitis B surface antigen; HBeAg, hepatitis B e antigen; AFP, alpha-fetoprotein.

**Supplementary Table 2: Primer sequences for real-time PCR**

| | |
|---|---|
| CBX1, forward | CTTGCAGAATTGGGTGTGTG |
| CBX1, reverse | CCTCCCTCCCCCAAATAATA |
| CBX2, forward | GAGCTCCATCGTGCACTACA |
| CBX2, reverse | CTTTCTGCGTCCTCACCTTC |
| CBX3, forward | TTGGCAGTTTAGGACCTGCT |
| CBX3, reverse | TGTTCTTCCTGGCTTTTGCT |
| CBX4, forward | GTGAAATGGAGAGGCTGGTC |
| CBX4, reverse | TCTTCCGATATCCCATCAGC |
| CBX5, forward | CAGGGTTTCTGGTGTGGTCT |
| CBX5, reverse | AGCAGAAGTGGGGAGTGCTA |
| CBX6, forward | AGATGTCACCCTGCTCCAAT |
| CBX6, reverse | AGCCACCTTCTCGAAATCCT |
| CBX7, forward | GCTGGTTTTCCCCTTTTCTC |
| CBX7, reverse | AAGCCTCTCTGAAGGGGAAG |
| CBX8, forward | GGTCGCAGAAGTACAGCACA |
| CBX8, reverse | TGGCTCCTCGGCTTTGACAGAGT |
| S100A9, forward | TCGTAAGTTTTGGCCTTTGC |
| S100A9, reverse | TGGGTGCCCCAGCTTCACAGA |
| GAPDH, forward | AGGTGAAGGTCGGAGTCAAC |
| GAPDH, reverse | CGCTCCTGGAAGATGGTGAT |