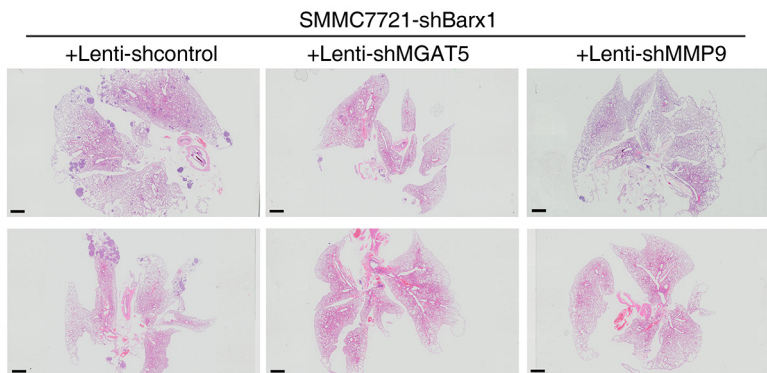


## Loss of Barx1 promotes hepatocellular carcinoma metastasis through up-regulating MGAT5 and MMP9 expression and indicates poor prognosis

### SUPPLEMENTARY MATERIALS



**Supplementary Figure 1: Representative HE staining images of the gross observation of lung metastasis from the indicated groups.** Scale bars represent 1 mm.

**Supplementary Table 1: List of genes differentially expressed in SMMC7721-shBarx1 versus SMMC7721-shcontrol cells using a human metastasis PCR array.**

See Supplementary File 1

Supplementary Table 2: Primer sequences used in the study

Primer name	Primer sequences	Enzyme
Primers for MGAT5 promoter construct:		
(-1530/+58)MGAT5 sense:	5'-TATAGGTACCAGAGTATCTGTTACATAT-3'	KpnI
(-871/+58)MGAT5 sense:	5'-TATAGGTACCTATGCTCAGGTCCTACTT-3'	KpnI
(-351/+58)MGAT5 sense:	5'-TATAGGTACCTGATGGTGATATGACTCC-3'	KpnI
antisense:	5'-TATACTCGAGCTGGTCTCACTCTGGCTC-3'	XhoI
Primers for MGAT5 promoter site-directed mutagenesis:		
Barx1 binding site 1 mutation sense:	5'-TTCATGACCCcgcgAGCATATATA-3'	
Barx1 binding site 1 mutation antisense:	5'-TATATATGCTcgcgGGGTCATGAA-3'	
Barx1 binding site 2 mutation sense:	5'-ATCTGGCTGCcgcgATATTGAAAC-3'	
Barx1 binding site 2 mutation antisense:	5'-GTTTCAATATcgcgGCAGCCAGAT-3'	
Primers used for ChIP in the MGAT5 promoter:		
Distant region sense:	5'-TGTAGAGATGCATTCTGT-3'	
Distant region antisense:	5'-CCAGTTCAGAGACATGCA-3'	
Barx1 binding site 1 sense:	5'-TAAGAGGTAGATAGTAAA-3'	
Barx1 binding site 1 antisense:	5'-ACTGATCATCTAGGCATG-3'	
Barx1 binding site 2 sense:	5'-CTATGCTCAGGTCCTACT-3'	
Barx1 binding site 2 antisense:	5'-GCATGCTGTAGCACAAGA-3'	
Primers for MMP9 promoter construct:		
(-1280/+139)MMP9 sense:	5'-TATAGGTACCTGGCATAAGTGTGATAAT-3'	KpnI
(-1016/+139)MMP9 sense:	5'-TATAGGTACCCATGGCTCACTGTATCT-3'	KpnI
(-468/+139)MMP9 sense:	5'-TATAGGTACCGGATATCTGACCTGGAG-3'	KpnI
(-152/+139)MMP9 sense:	5'-TATAGGTACCTCTCATGCTGGTGCTGCC-3'	KpnI
antisense:	5'-TATACTCGAGGGTGAGATTGGTTCTCAG-3'	XhoI
Primers for MMP9 promoter site-directed mutagenesis:		
Barx1 binding site 1 mutation sense:	5'-GCTCCTATAGcgcgTTTTCCCCCA-3'	
Barx1 binding site 1 mutation antisense:	5'-TGGGGGAAAACcgcgCTATAGGAGC-3'	
Barx1 binding site 2 mutation sense:	5'-GGCCTTAGTAcgcgAAACCAATCA-3'	
Barx1 binding site 2 mutation antisense:	5'-TGATTGGTTTcgcgTACTAAGGCC-3'	

(Continued)

Primer name	Primer sequences	Enzyme
Barx1 binding site 3 mutation sense:	5'-CAGGGGGATC <del>cgcg</del> GTTTCAGAAA-3'	
Barx1 binding site 3 mutation antisense:	5'-TTTCTGAAAC <del>cgcg</del> GATCCCCCTG-3'	
Primers used for ChIP in the MMP9 promoter:		
Distant region sense:	5'-TAGCATAGCAGTGAACAC-3'	
Distant region antisense:	5'-ATCTCAGCTCTGCCACTG-3'	
Barx1 binding site 1 sense:	5'-TTGGCATAAGTGTGATAA-3'	
Barx1 binding site 1 antisense:	5'-TCTAAGCTGCAGTGTAAC-3'	
Barx1 binding site 2 sense:	5'-GCAGTGACATAATCATGG-3'	
Barx1 binding site 2 antisense:	5'-CACTGTAGGTTGTAAGTC-3'	
Barx1 binding site 3 sense:	5'-AGGATATCTGACCTGGGA-3'	
Barx1 binding site 3 antisense:	5'-CTTGTGGGAACTGTATGA-3'	