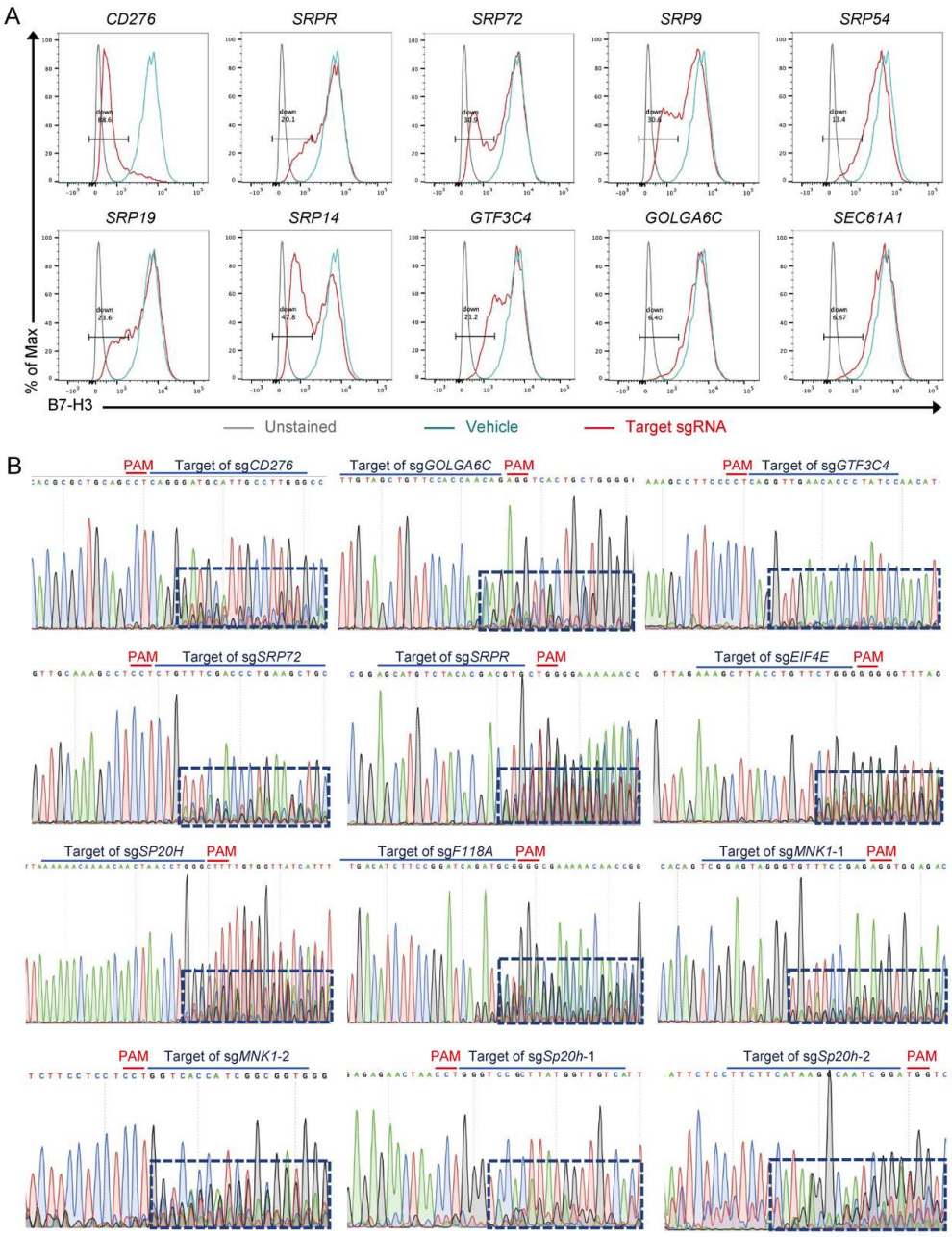


Figure S1. *In vitro* validation of positive regulators of B7-H3 by CRISPR/Cas9. (A) Flow cytometry analysis of cell-surface B7-H3 in A375 cells expressing Cas9 and a control sgRNA or sgRNA targeting indicated genes. (B) Sanger sequencing of gene knockout SK-OV-3 polyclones after targeting with the indicated sgRNAs.

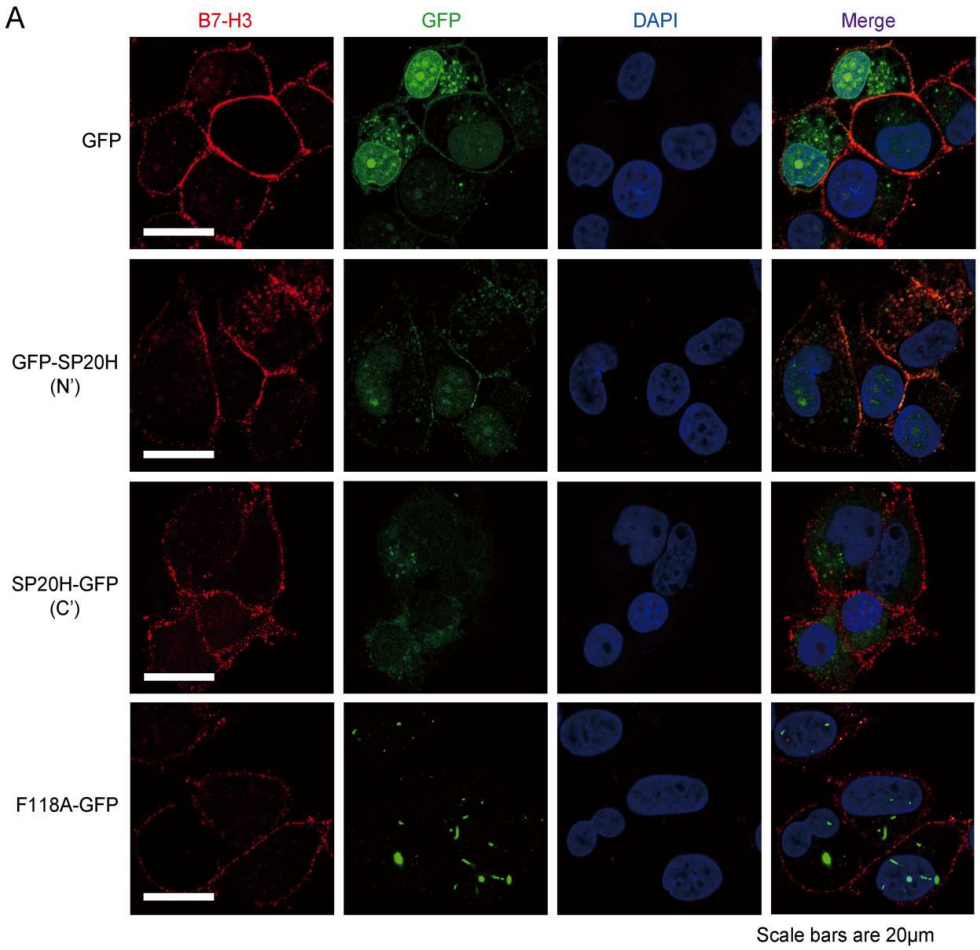
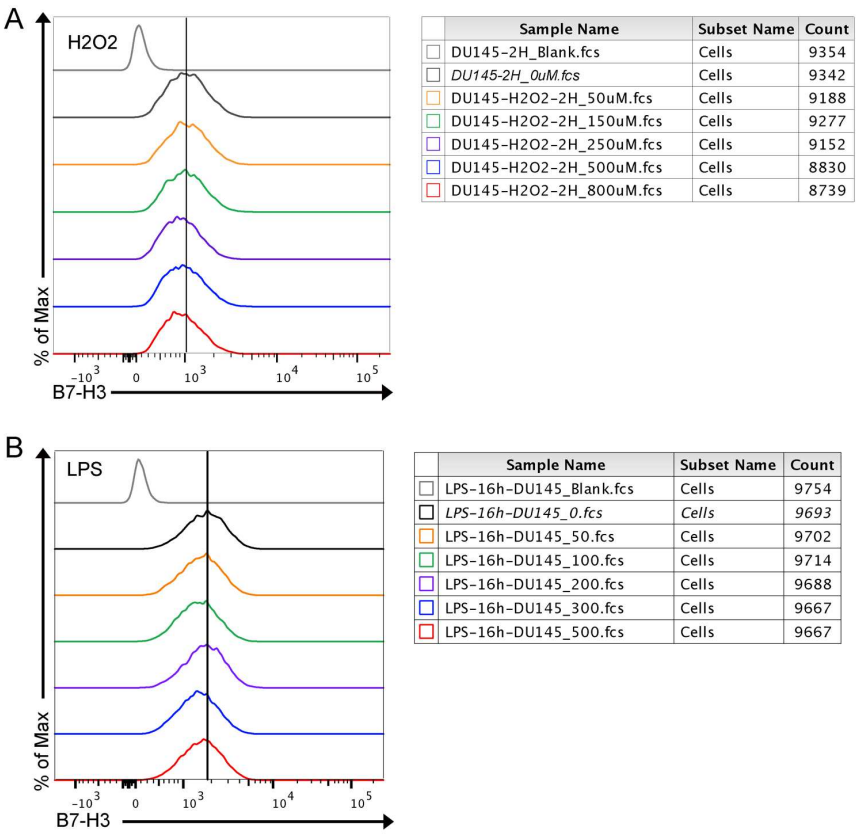


Figure S2. Cellular localization analysis of SP20H and F118A. (A) Co-localization of B7-H3 and SP20H, F118A in Hela cells were visualized by confocal immunofluorescent imaging. Red: Alexa Fluor 594–labeled B7-H3 staining; Green: GFP fusion proteins, N'/C': GFP fused to the N or C terminus of SP20H; Blue: DAPI indicates nuclei.



	Sample Name	Subset Name	Count
	DU145-2H_Blank.fcs	Cells	9354
	DU145-2H_0uM.fcs	Cells	9342
	DU145-H2O2-2H_50uM.fcs	Cells	9188
	DU145-H2O2-2H_150uM.fcs	Cells	9277
	DU145-H2O2-2H_250uM.fcs	Cells	9152
	DU145-H2O2-2H_500uM.fcs	Cells	8830
	DU145-H2O2-2H_800uM.fcs	Cells	8739

	Sample Name	Subset Name	Count
	LPS-16h-DU145_Blank.fcs	Cells	9754
	LPS-16h-DU145_0.fcs	*Cells*	*9693*
	LPS-16h-DU145_50.fcs	Cells	9702
	LPS-16h-DU145_100.fcs	Cells	9714
	LPS-16h-DU145_200.fcs	Cells	9688
	LPS-16h-DU145_300.fcs	Cells	9667
	LPS-16h-DU145_500.fcs	Cells	9667

Figure S3. Treatment with LPS and H_2O_2 have no significant effect on B7-H3 expression in cancer cells. (A-B) Histograms show the surface B7-H3 expression on DU145 cells after 16hr-treatment with different concentrations of LPS (0, 25, 50, 100, 125, 175, 200, 250, 300, 400, 500ng/ml), and H_2O_2 (0, 50, 150, 250, 500, 800 μM).

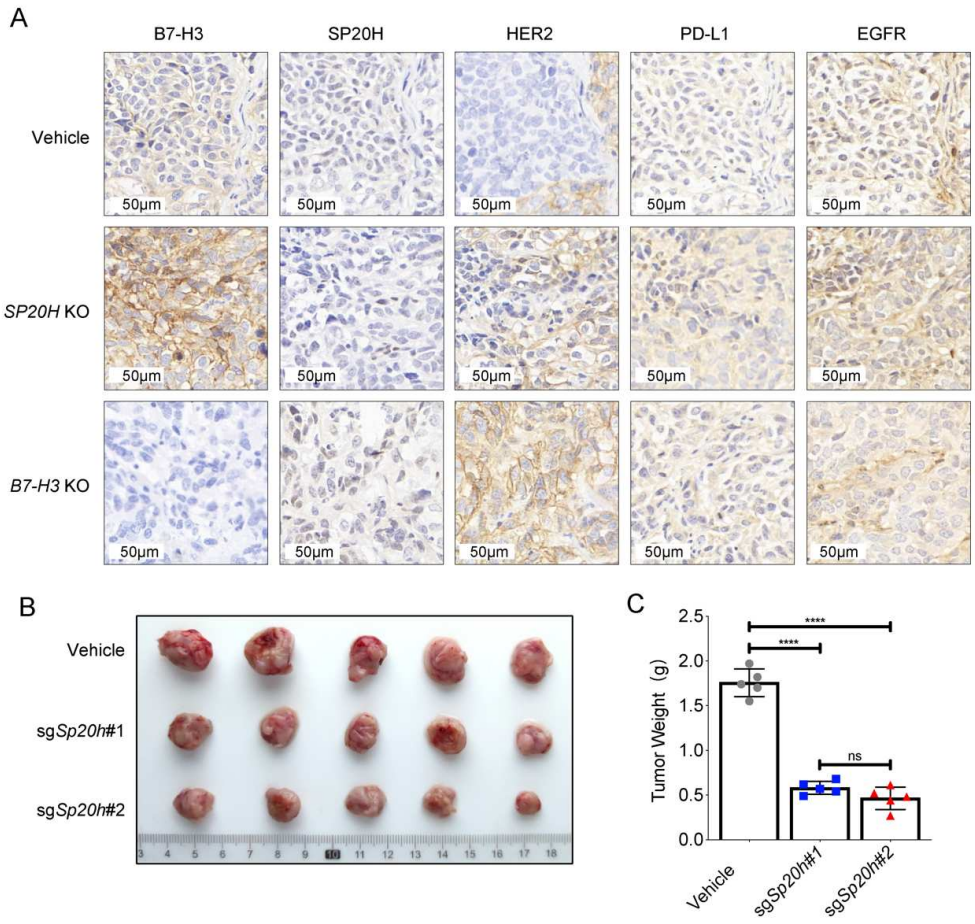


Figure S4. Effect of SP20H depletion on tumor surface marker expression and tumor growth. (A) Representation immunohistochemical stains of indicated markers on tumors generated after subcutaneous injection of control, *CD276*-, or *SP20H*-knockout SK-OV-3 cells in NOD-SCID mice (n=5). Brown: indicated antigens; Blue: Mayer hematoxylin nuclear counterstain. Scar bars are 50μm. (B) Pictures of tumors derived from control and *Sp20h*-KO 4T1 cells. (C) Weight of the harvested 4T1 tumors after 25 days growth (n=5; two-tailed Student's t-test; ****P <0.0001; ns, no significant).

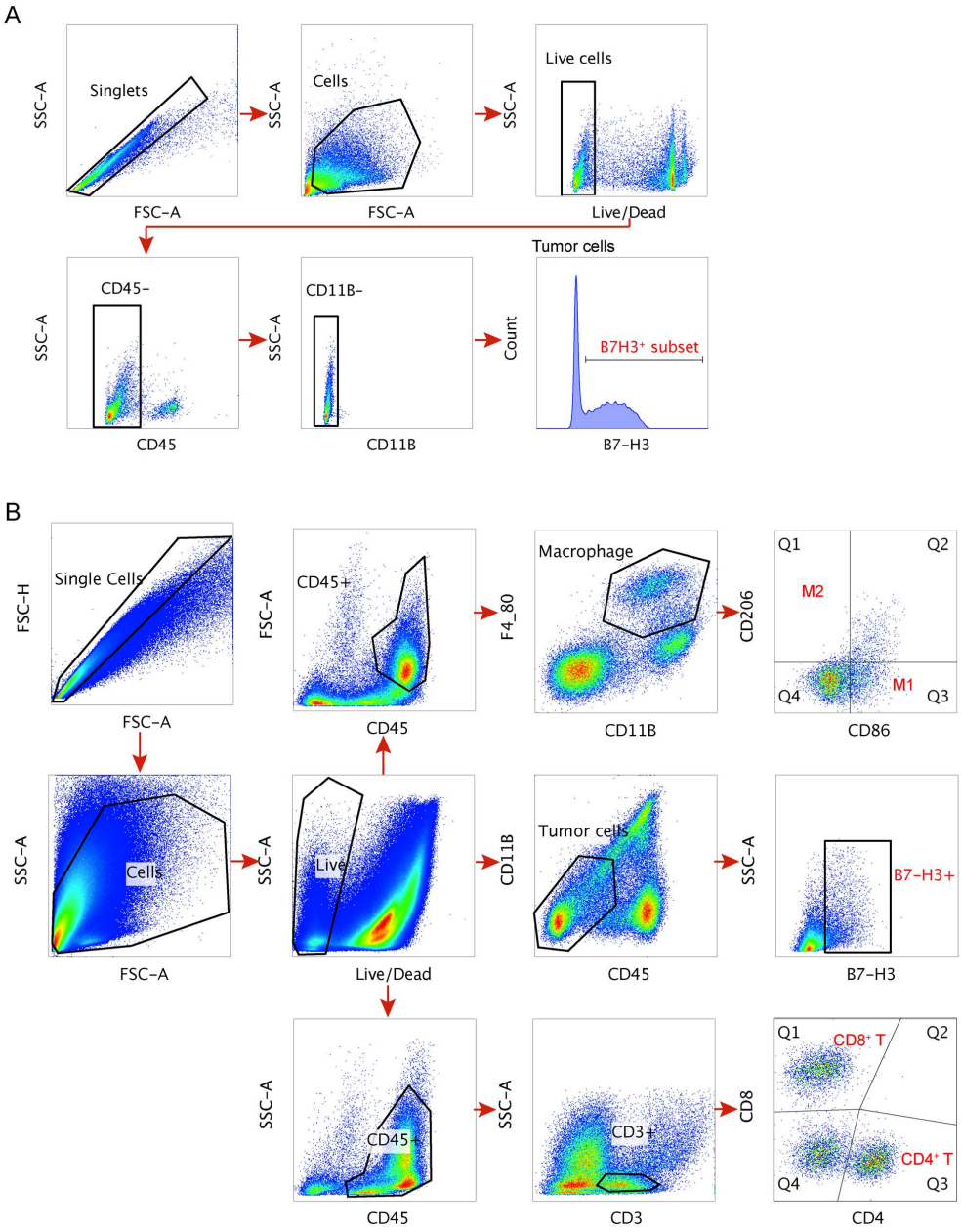


Figure S5. Representative gating strategies for flowcytometry analysis. (A) Gating strategy for figure 5D. (B) Gating strategy for figure 5F.

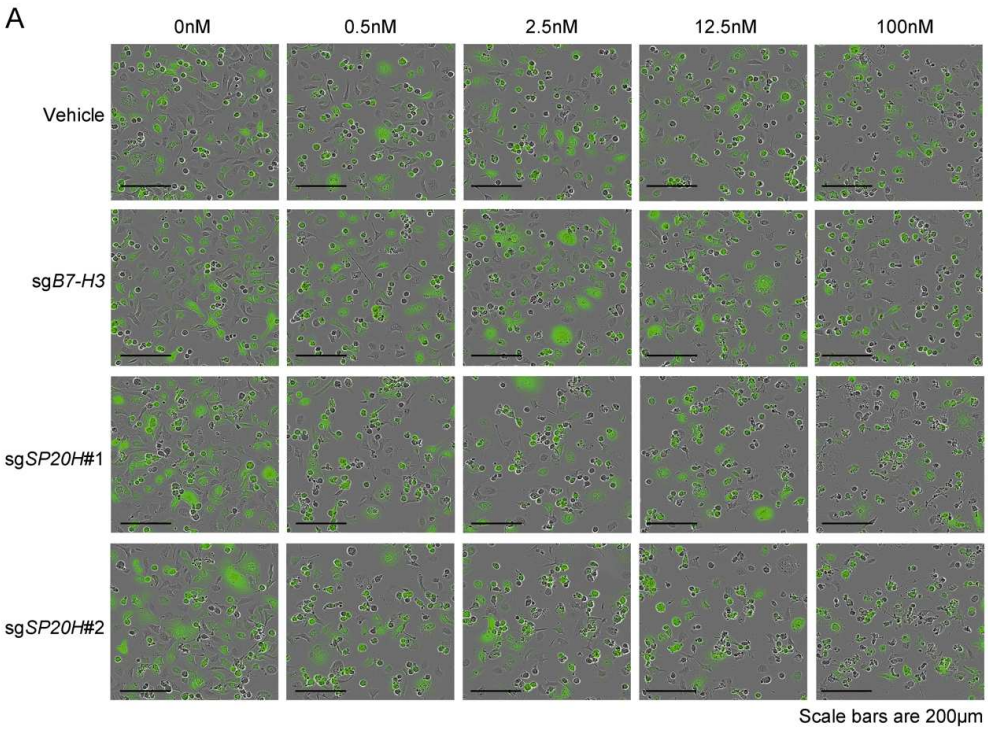


Figure S6. *In vitro* killing assay of αB7-H3-DM1 against SK-OV-3 cells with different expression of B7-H3. (A) Representation images of different concentration of αB7-H3-DM1 treated or untreated SK-OV-3-GFP cells from each group were shown.

Table S1. Sequence information of primers and sgRNAs	
sgRNA	**20nt targeting sequences**
sg*CD276*	CACAGGGCAACGCATCCCTG
sg*SP20H*-1	TGATAACCACAAATGGACCC
sg*SP20H*-2	GGATATTCTCTGATGCTCAG
sg*F118A*	CATCTTCCGGATCAGATCAT
sg*EIF4E-1*	AAACTTGGCAAGCAAACCTG
sg*EIF4E-2*	AAACTTGGCAAGCAAACCTG
sg*MNK1*-1	TCGGAGTAGGGTGTTTCGAG
sg*MNK1*-2	CCATCGCAGATGGTGACAGG
sg*SRP72*	GCAGCTTCAGGGTCGAACAG
sg*SRP9*	GATGCCGCAGTACCAGACCT
sg*SRPR*	AGCATGTCTACACGACGCTG
sg*SRP14*	TATAGACGCTGCCCGACGTC
sg*GTF3C4*	GGATAGGGTGTTCAACCCTG
sg*SRP19*	CCAATACAGAGGCAGAGTCC
sg*GOLGA6C*	TTGTAGCTGTTCCACCACAG
sg*Sp20h*-1	TGACAACCATAAGTGGACCC
sg*Sp20h*-2	TTCTTCATAAGGCAATCGGA
shRNA	**Sequences**
sh*SP20H*-F1	aattGCTTGTTATGCAAGAGACTCTCGAGAGTCTCTTGC ATAACAAGCTTTTTTTat
sh*SP20H*-R1	AAAAAAAGCTTGTTATGCAAGAGACTCTCGAGAGTC TCTTGCATAACAAGC
sh*SP20H*-F2	aattGCAACAAGCTTTAGAACTACTCGAGTAGTTCTAAA GCTTGTTGCTTTTTTTTat
sh*SP20H*-R2	AAAAAAAAGCAACAAGCTTTAGAACTACTCGAGTAG TTCTAAAGCTTGTTGC
sh*CD276*-F1	aattTTCAGCCTGGCACAGCTCAACCTCATCTGCTCGAG CAGATGAGGTTGAGCTGTGCCAGGCTGAATTTTTTTat
sh*CD276*-R1	AAAAAAATTCAGCCTGGCACAGCTCAACCTCATCTGC TCGAGCAGATGAGGTTGAGCTGTGCCAGGCTGAA
sh*CD276*-F2	aattTCGTGTGCTGGAGAAAGATCAAACAGAGCCTCGA GGCTCTGTTTGATCTTTCTCCAGCACACGATTTTTTTat
sh*CD276*-R2	AAAAAAATCGTGTGCTGGAGAAAGATCAAACAGAGC CTCGAGGCTCTGTTTGATCTTTCTCCAGCACACGA

sh*EIF4E*-F	aattCATATCCAGTTGTCTAGTACTCGAGTACTAGACAACTGGATATGTTTTTTTat
sh*EIF4E*-R	AAAAAAACATATCCAGTTGTCTAGTACTCGAGTACTAGACAACTGGATATG
Sramble shRNA-F	aattCCTAAGGTTAAGTCGCCCTCGCTCGAGCGAGGGCGACTTAACCTTAGGTTTTTTTat
Sramble shRNA-R	AAAAAAACCTAAGGTTAAGTCGCCCTCGCTCGAGCGAGGGCGACTTAACCTTAGG
Genome PCR	**Sequences (5'-3')**
CD276-F	CTGAGCCTGGCTTCAGCCTG
CD276-R	CAGAACACCTCAGCCTCAG
SP20H-F1	GTTGATCTCCTAGAAAAATCTCAGG
SP20H-R1	GCTTGTTATAGAGCAGTCTG
SP20H-F2	GAGAAGCTTGTTATGCAAGAGAC
SP20H-R2	TGCTATAGAAGGATCAAGACAGAG
F118A-F	CTCGTGGAGAAGCTGCATCG
F118A-R	AGGACCTTGGTCTTGTCCTTC
EIF4E-F1	TTCATTCATCTGTTGATGG
EIF4E-R1	AACTAATAAAGCATAATCACC
EIF4E-F2	ATATTGCTGTCATAGATGTG
EIF4E-R2	TGAACTCAAGACTCACAC
MNK1-F1	TGCTGCTTTGCCAACAGAG
MNK1-R1	TGTGGGGACAGGAGTGAG
MNK1-F2	ACCATCAGTGTAAACCTCC
MNK1-R2	CATCTGGCTCTAACTTGAC
GTF3C4-F	TGCCATCTCCTTGAGTAAATGC
GTF3C4-R	TGCAGTCTGTTGAGATTTGCC
SRP72-F	TGAATGTTTGGGTCATTTGG
SRP72-R	CTATGGAAAGCACAAATACTCC
SRP9-F	GCGTCGGTTGGCGACTC
SRP9-R	GCAGGGTCCATTTGTGTTCATTC
SRPR-F	GGCATTTTTGAGAGCATTCC
SRPR-R	CTGACCTTGGCAAGATTAGTAG
SRP14-F	CGTTACTCGAGACCTGTGC
SRP14-R	GAATGTGCTCAGTACAGG
SRP19-F	ACGTGGTTCTTCACTATTTTCC
SRP19-R	CTCTCCATGAGCAGTAGTCC

GOLGA6C-F	CTGTGCATCAAGAGGAGG
GOLGA6C-R	CTCCTGCTCCTGGAGTC
QPCR	**Primer sequences**
qh*CD276*-F	CCTGAGTCCCAGAGTCGG
qh*CD276*-R	CAGGGCTCCTGTGAGG
qh*SP20H*-F1	GACTTGTATATTGAAG
qh*SP20H*-R1	TGGTCTCGGAATCTGATCC
qh*SP20H*-F2	AGCGTACATAAGTTATCTC
qh*SP20H*-R2	ACAGATTTTCTTCCACTTG
qh*β actin*-F	CCTTCCTGGGCATGGAGTC
qh*β actin*-R	TGATCTTCATTGTGCTGGGTG