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Supplementary figures:

Crosstalk between β-catenin and CCL2 drives migration of monocytes towards glioblastoma cells

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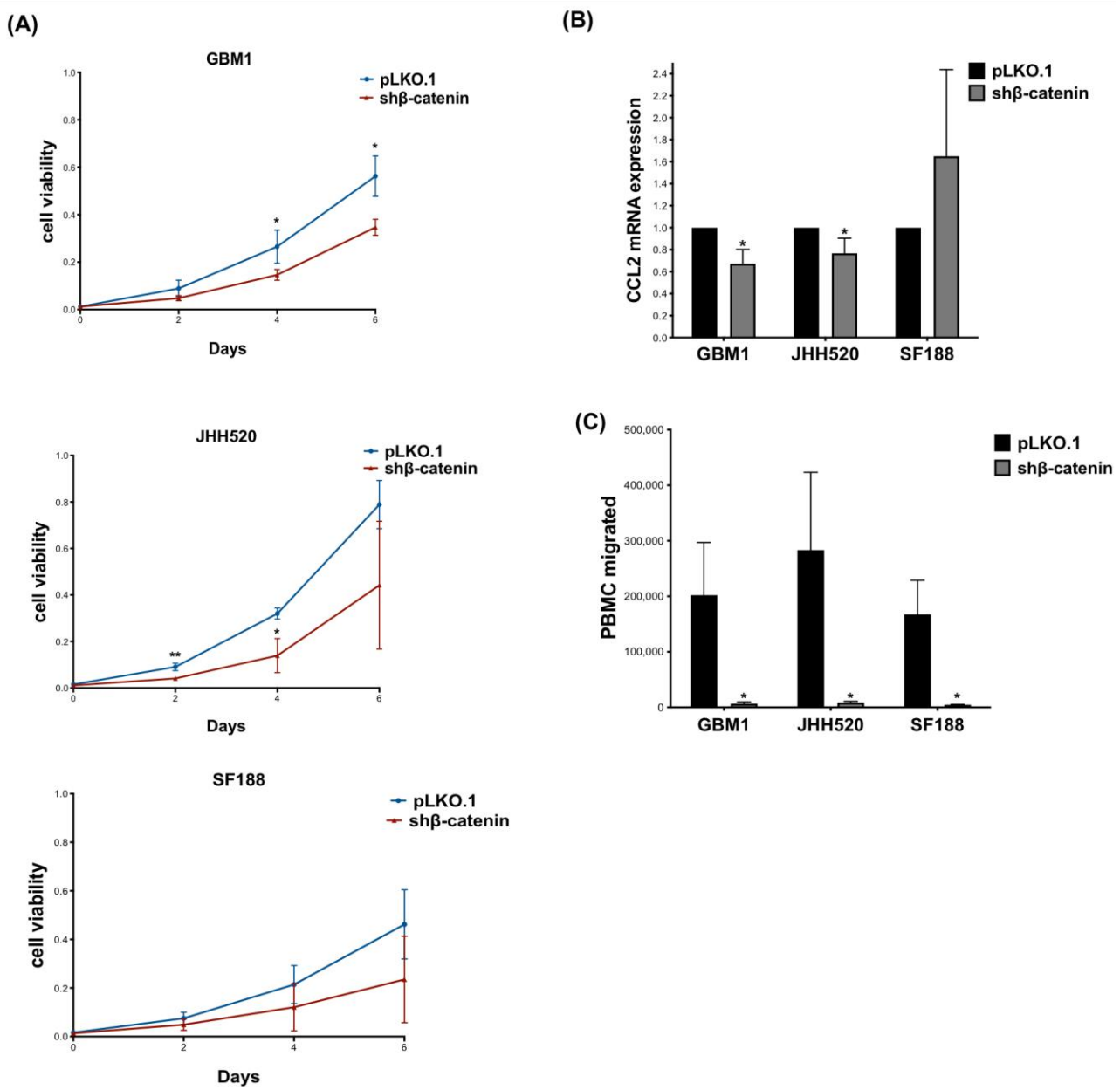
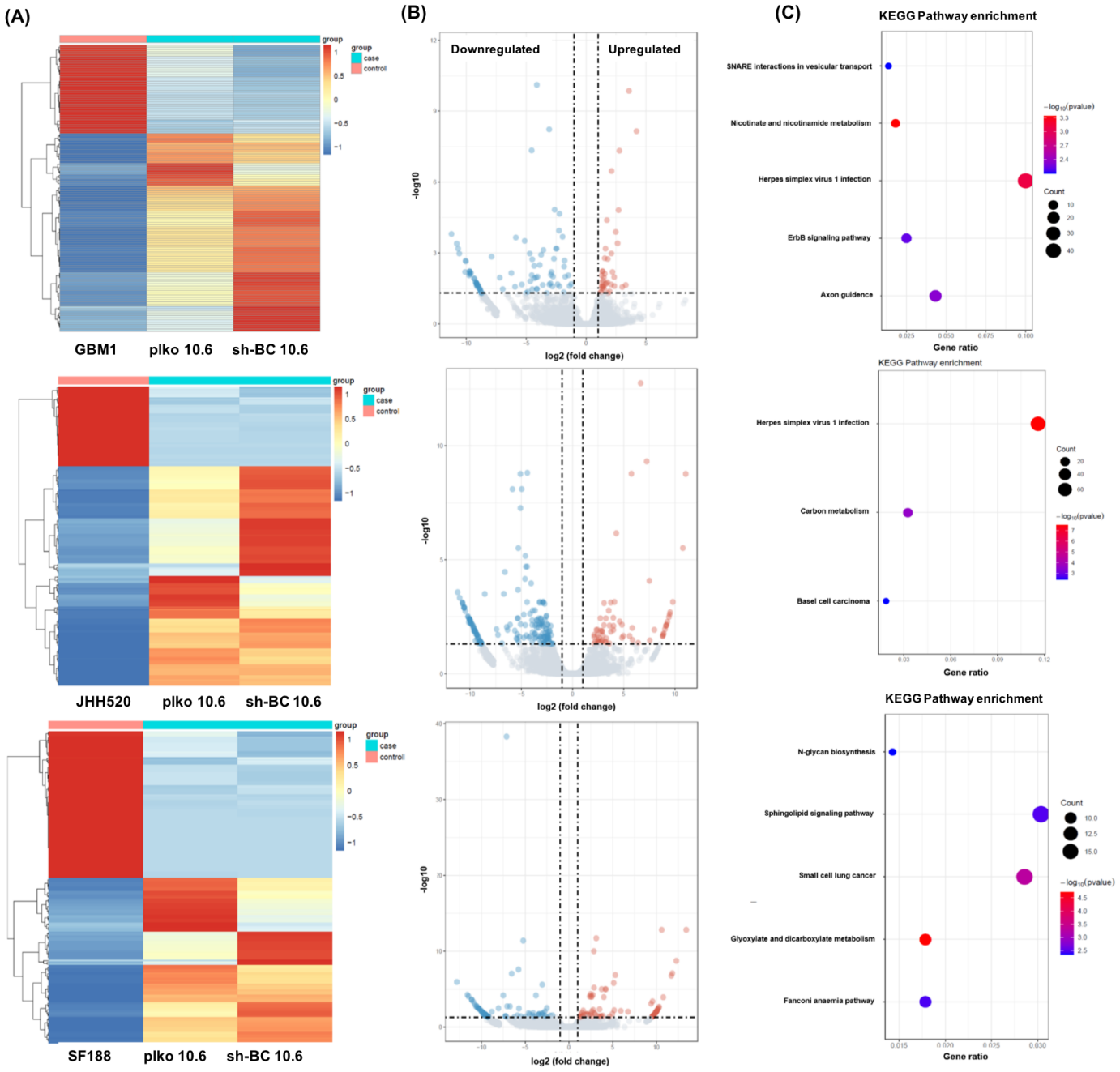
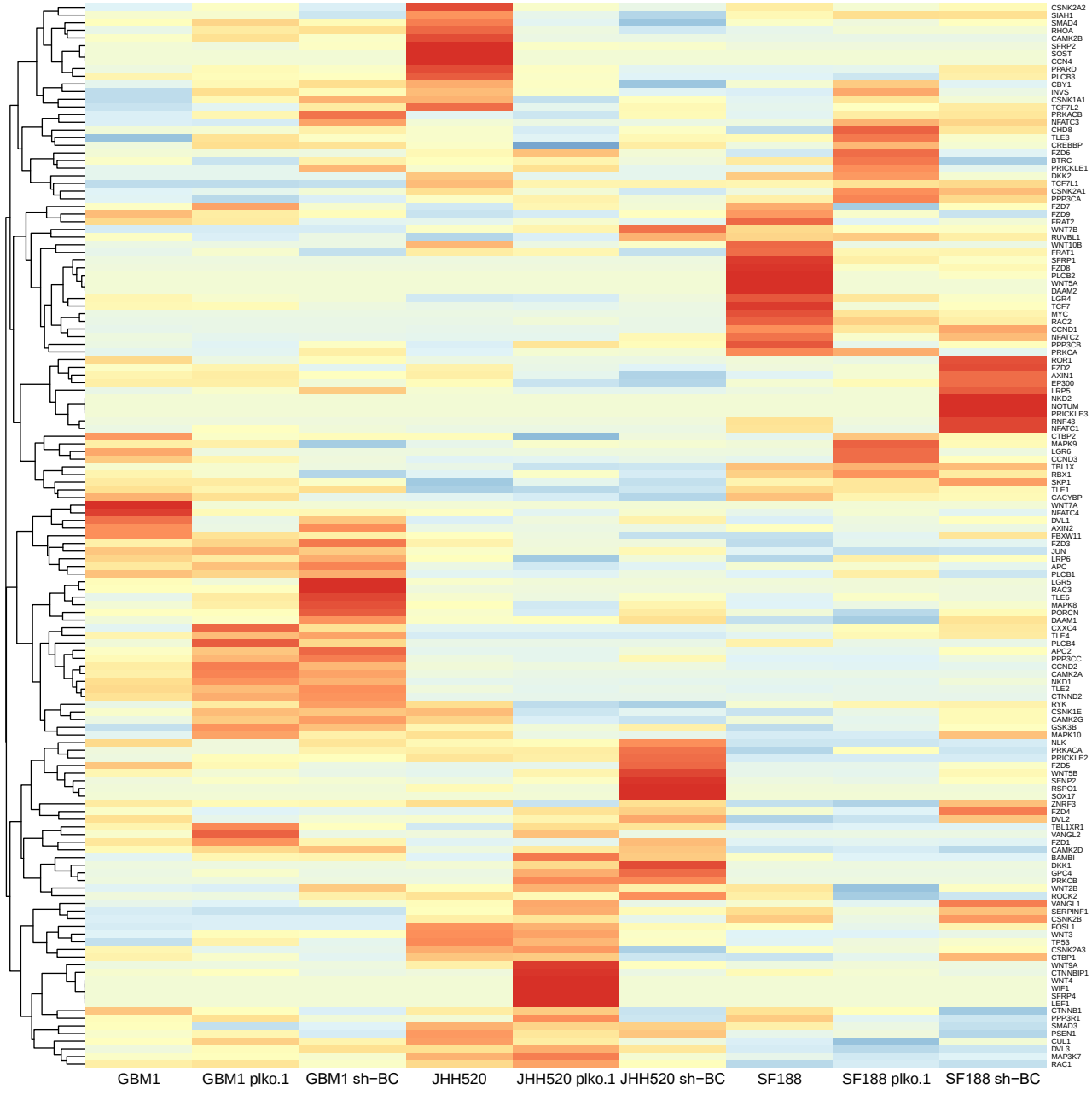


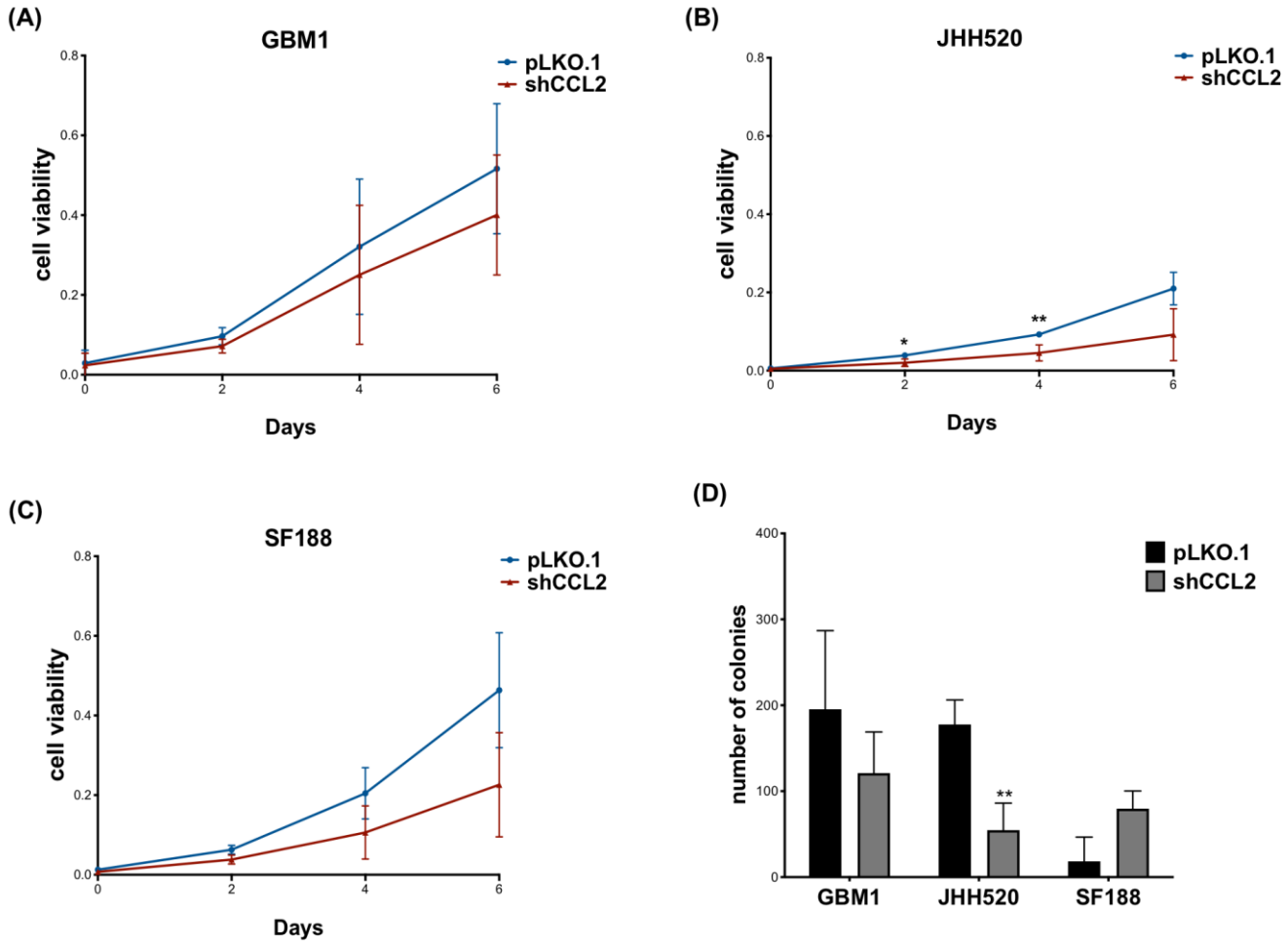
Supplementary Figure S1: β-catenin knockdown partially decreases cell viability and CCL2 mRNA expression in GBM cells. GBM cell lines (GBM1, JHH520, SF188) were transduced with lentiviral particles containing shβ-catenin plasmids: **(A)** Cell viability was partially reduced compared to control (pLKO.1) cells. Exponential growth curves were calculated for each condition. **(B)** The relative CCL2 mRNA expression levels were analysed by RT-qPCR in shβ-catenin cells and compared to controls (pLKO.1). **(C)** PBMC migration towards TCM of shβ-catenin GBM cells was significantly decreased compared to control (pLKO.1). Data are presented as mean ± SD (n=3). Statistical significance was calculated with unpaired t-test. * p ≤0.05 **p ≤0.01 ***p ≤0.001.



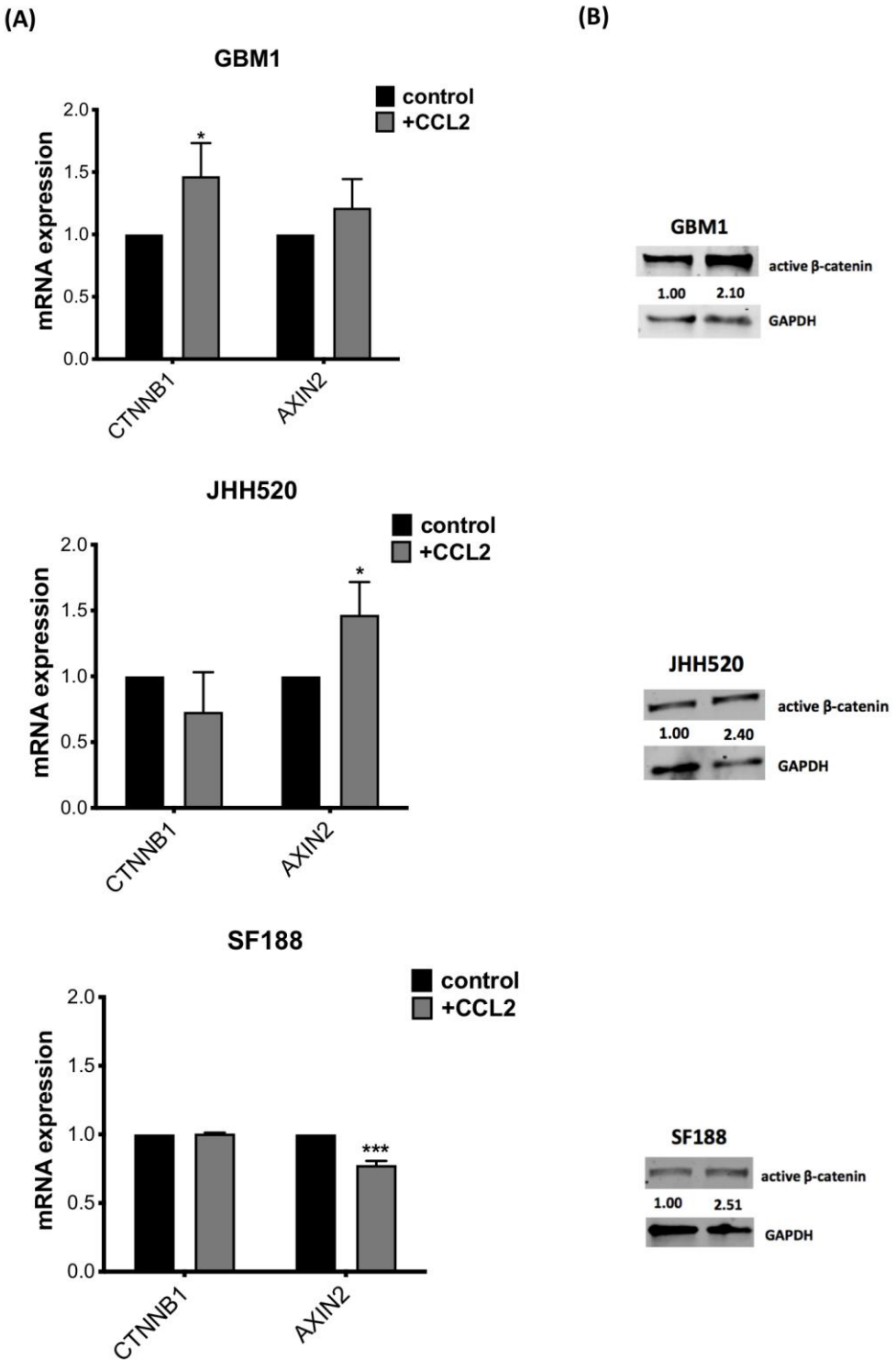
Supplementary Figure S2: Genome-wide gene expression analysis of three GBM cell lines: **(A)** heatmap showing variation of gene expression in control (pLKO.1) cells and shβ-catenin cells, **(B)** altered genes compared to the control cells (plKO.1), **(C)** KEGG associated pathways.



Supplementary Figure S3: A heat map shows changes in expression of genes involved in the Wnt/β-catenin pathway in three cell lines, control (pLKO.1) cells and shβ-catenin cells.



Supplementary Figure S4: CCL2 mediated cell viability and clonogenicity of GBM cells:
(A)–(C) Exponential growth curves representing cell viability in cell lines compared to control (pLKO.1) cells measured by MTT assay. **(D)** CCL2 suppression led to decreased clonogenicity of GBM1 and JHH520 while increasing clonogenicity of SF188 as detected by using a soft agar assay. Quantifications of three colony forming assays are shown. Data are presented as mean ± SD (n=3). Statistical significance was calculated with unpaired t-test. * p ≤0.05 **p ≤0.01 ***p≤ 0.001.



Supplementary Figure S5: Treatment with recombinant CCL2 alters β-catenin -activity of GBM cells: (**A**) The relative mRNA expression levels of β-catenin gene (*CTNNB1*) and target gene *AXIN2* were measured by RT-qPCR in CCL2-treated cells compared to control cells (PBS/BSA 0,1%). Cells were treated with 100 ng/ml recombinant CCL2 for 30 minutes. (**B**) Non-phospho- (active) β-catenin protein levels were increased after CCL2 treatment as assessed by immunoblotting. Data are presented as mean ± SD (n=3). Statistical significance was calculated with unpaired t-test. * $p \leq 0.05$ ** $p \leq 0.01$ *** $p \leq 0.001$.

Supplementary Table S1: Primer sequences used in RT qPCR

Primer	forward	reverse
β-actin	CCCAGCACAATGAAGATCAA	CGATCCACACGGAGTACTTG
CTNNB1	GGGCCTCAGAGAGCTGAGTA	TGAGCAGCATCAAACTGTGTAG
AXIN2	AGCCAAAGCGATCTACAAAAGG	GGTAGGCATTTTCCTCCATCAC
c-MYC	CCTTAATTAAAATGCCCCTCAACGTTAGCT	GGAATTCCATATGTTACGCACAAGAGTTCCGTA
SNAI1	GCTGCAGGACTCTAATCC	ATCTCCGGAGGTGGGATC
SNAI2	TGGTTGCTTCAAGGACACAT	GTTGCAGTGAGGGCAAGAA
ZEB1	AAGAATTCACAGTGGAG AGAAGCCA	CGTTTCTTGCAGTTTGGGCATT
SOX2	TGGACAGTTACGCGCACA	CGAGTAGGACATGCTGTA
CCL2	AGGTGACTGGGGCATTGAT	GCCTCCAGCATGAAAGTCTC