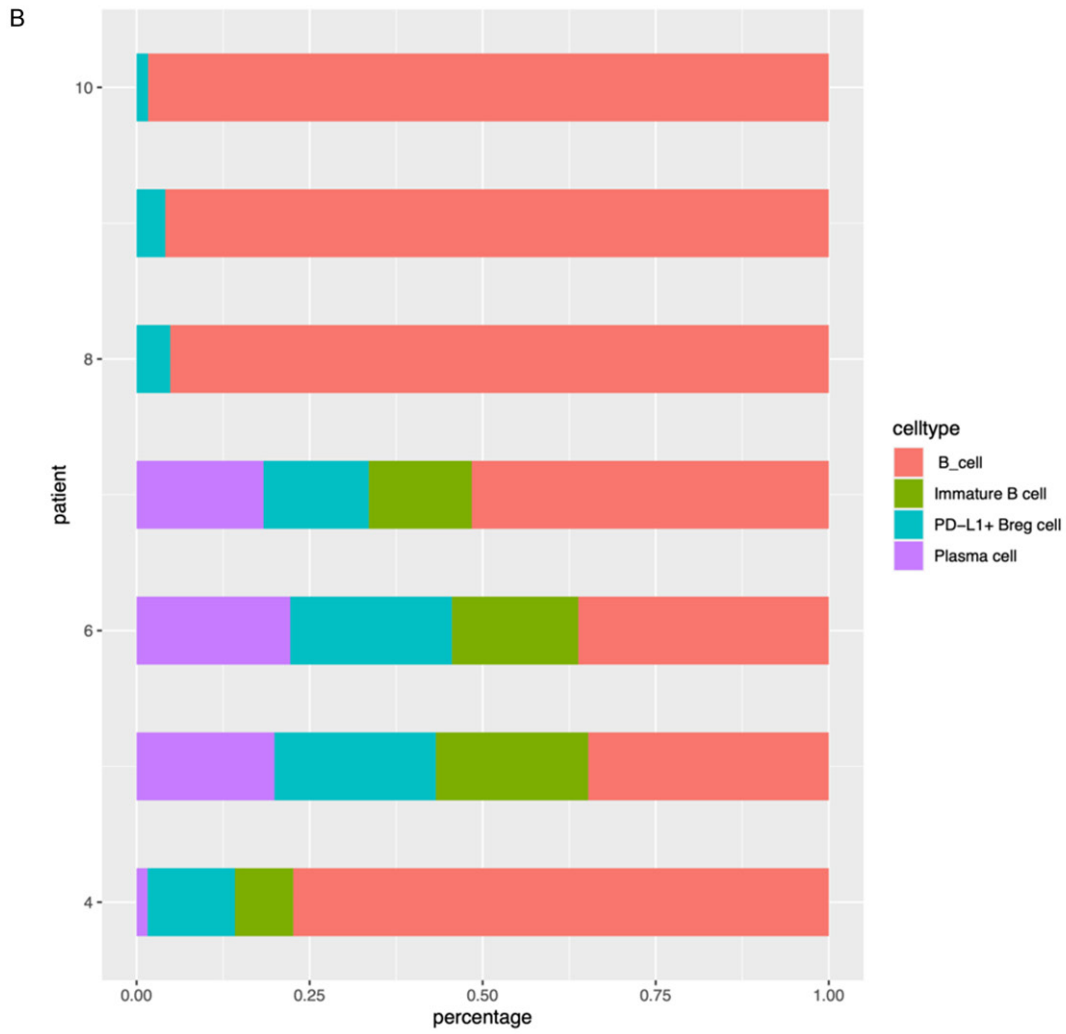
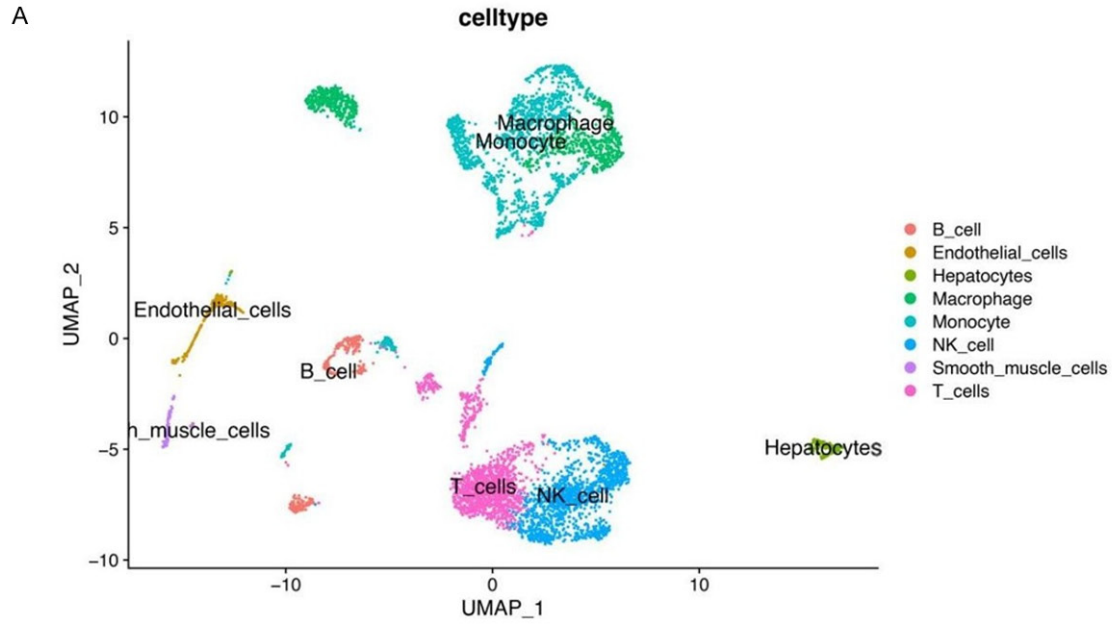


Macrophage regulate B cell PD-L1 in HCC

Table S1. Flow cytometry antibody used in our study

Antibody	Company	Clone
PE anti-human CD38 Antibody	Biolegend	HIT2
PerCP/Cyanine5.5 anti-human CD19 Antibody	Biolegend	HIB19
Brilliant Violet 510™ anti-human CD3 Antibody	Biolegend	OKT3
Brilliant Violet 421™ anti-human CD274 (B7-H1, PD-L1) Antibody	Biolegend	29E.2A3
Alexa Fluor® 700 anti-human CD45 Antibody	Biolegend	2D1
FITC anti-human CD163 Antibody	Biolegend	GHI/61
Human/Mouse CXCL12/SDF-1 PE-conjugated Antibody	Bio-techne	IC350P
FITC anti-human CD14 Antibody	Biolegend	HCD14

Macrophage regulate B cell PD-L1 in HCC



Macrophage regulate B cell PD-L1 in HCC

C

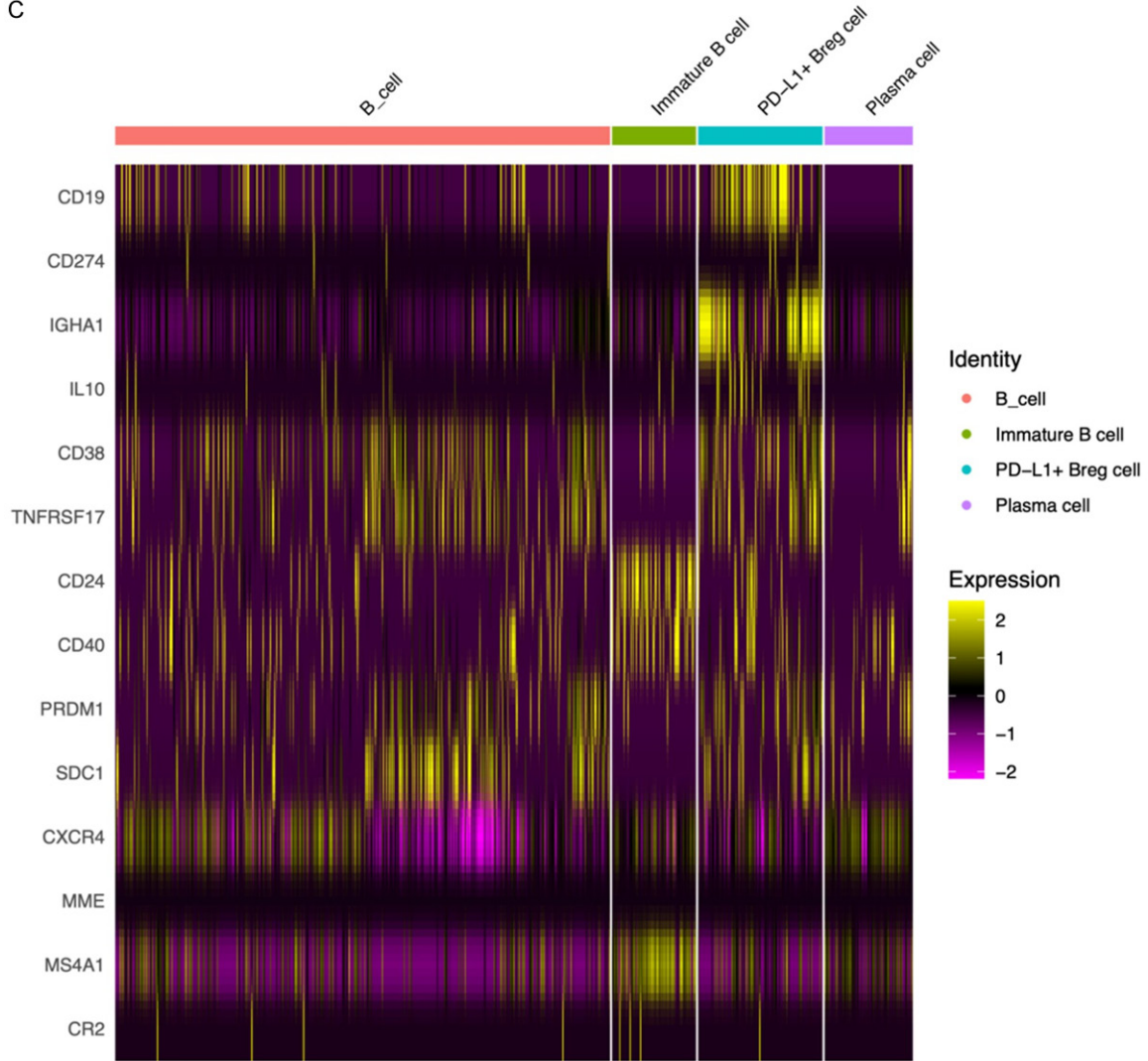


Figure S1. A. The UMAP plot of cells from one patient, representing eight distinct clusters. Each dot corresponds to one single cell. B. The percentage of four different subset of HCC infiltrating B cell in each sample. C. Heatmap of hallmark gene of PD-L1+Breg cell in different cluster of B cell.

Macrophage regulate B cell PD-L1 in HCC

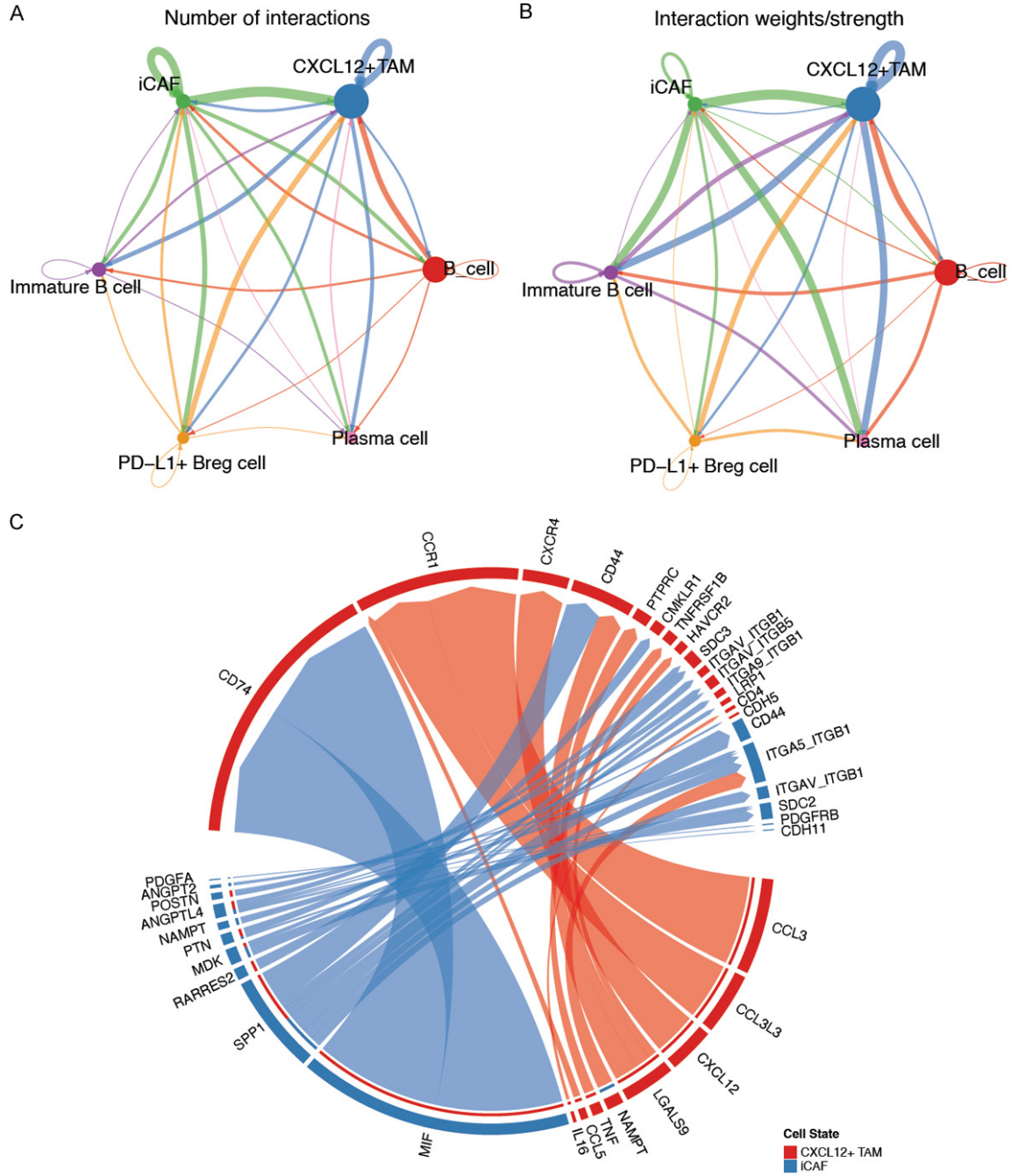


Figure S2. The MIF/CD74 axis plays an important role in iCAFs-CXCL12+ TAM interaction. A. A circle plot of the number of cellular communications in six identified types of cell populations in liver tissue. The width represents the number of cell interactions. B. A circle plot of the weights/strength of the number of cellular communications in six identified types of cell populations in liver tissue. The width represents the weights/strength of the interaction. C. A chord diagram of cell-to-cell contact interactions from CXCL12+ TAMs toward iCAF.

Macrophage regulate B cell PD-L1 in HCC

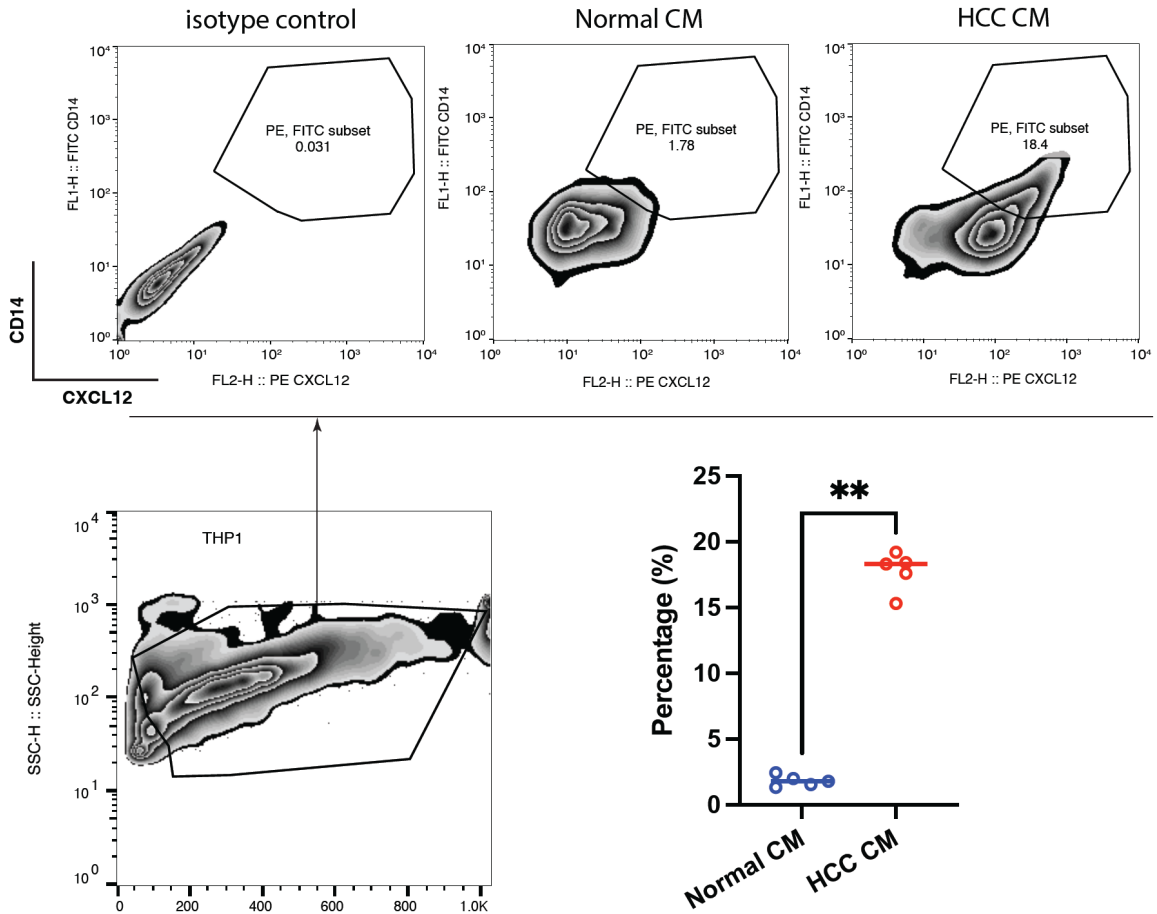


Figure S3. Determination and gating strategy of CXCL12 expression in macrophage (derived from THP1) induced by conditional medium of normal and HCC tissues respectively analyzed by flow cytometry.

Macrophage regulate B cell PD-L1 in HCC

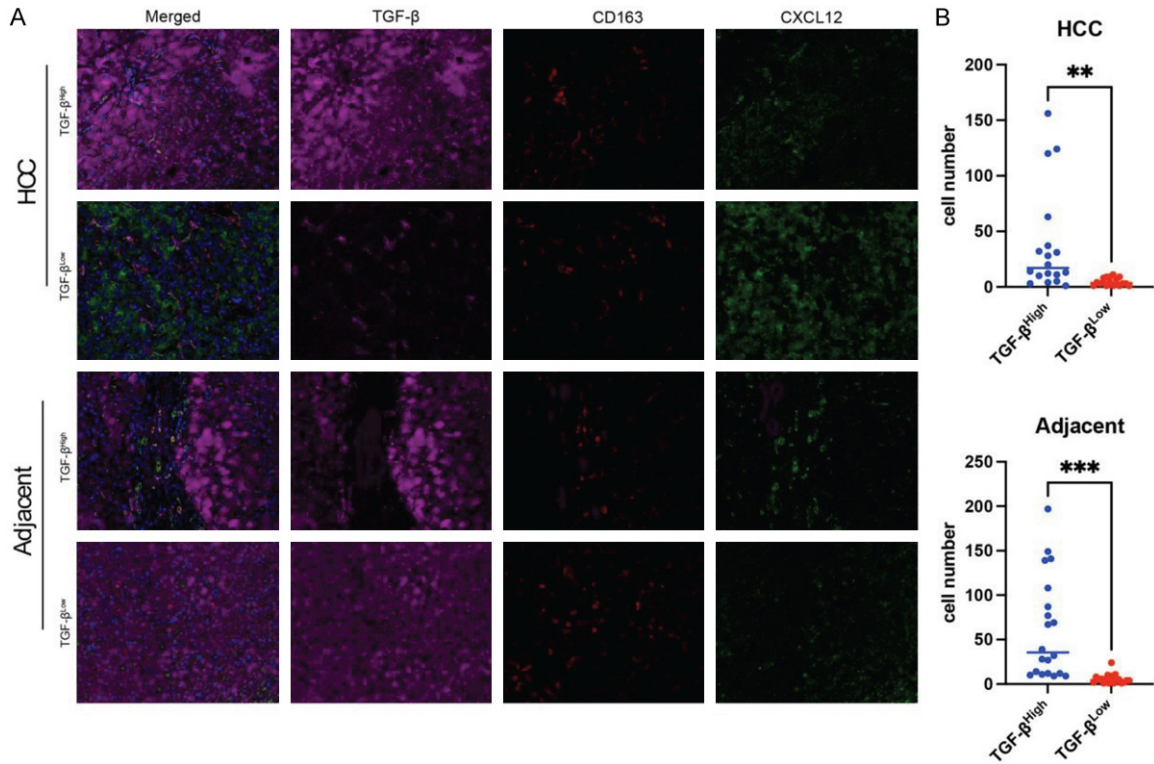


Figure S4. CXCL12+ TAMs aggregated and secreted more TGF- β in HCC than in adjacent tissues. A. Representative figures for multiple-color staining for TGF- β , CD163, and CXCL12 expression in human HCC and adjacent samples. B. Dot plots showing cell numbers of TGF- β high and TGF- β low in HCC and adjacent tissues. Red represents TGF- β low and blue represents TGF- β high. Data are presented as mean \pm SEM and were analyzed using Student's t-test (*** p <0.001, ** p <0.01).

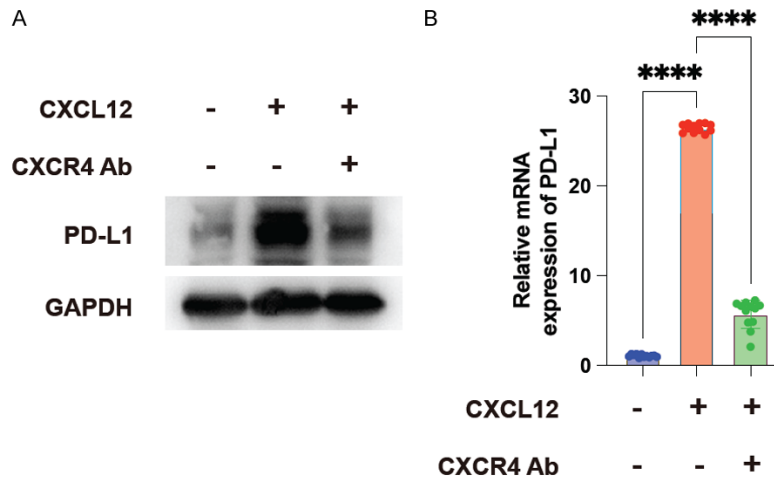


Figure S5. CXCL12-secreting TAMs regulate PD-L1+ Bregs through the CXCL12/CXCR4 axis. A. Western blot showing PD-L1 levels with CXCL12 treatment and CXCR4 antibody interference. GAPDH is used as a baseline reference. B. Bar graph depicting changes in PD-L1 mRNA after treatment with CXCL12 and blockage with CXCR4 antibody, indicating a significant change in expression (**** p <0.0001).

Macrophage regulate B cell PD-L1 in HCC

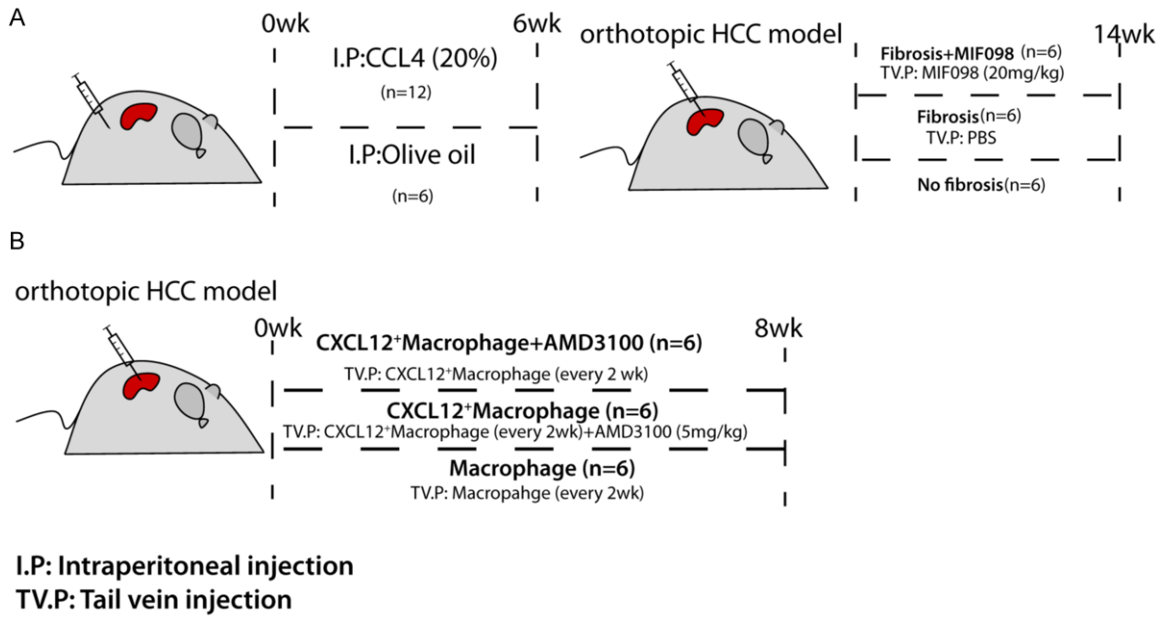


Figure S6. Experimental design of orthotopic HCC model.

Macrophage regulate B cell PD-L1 in HCC

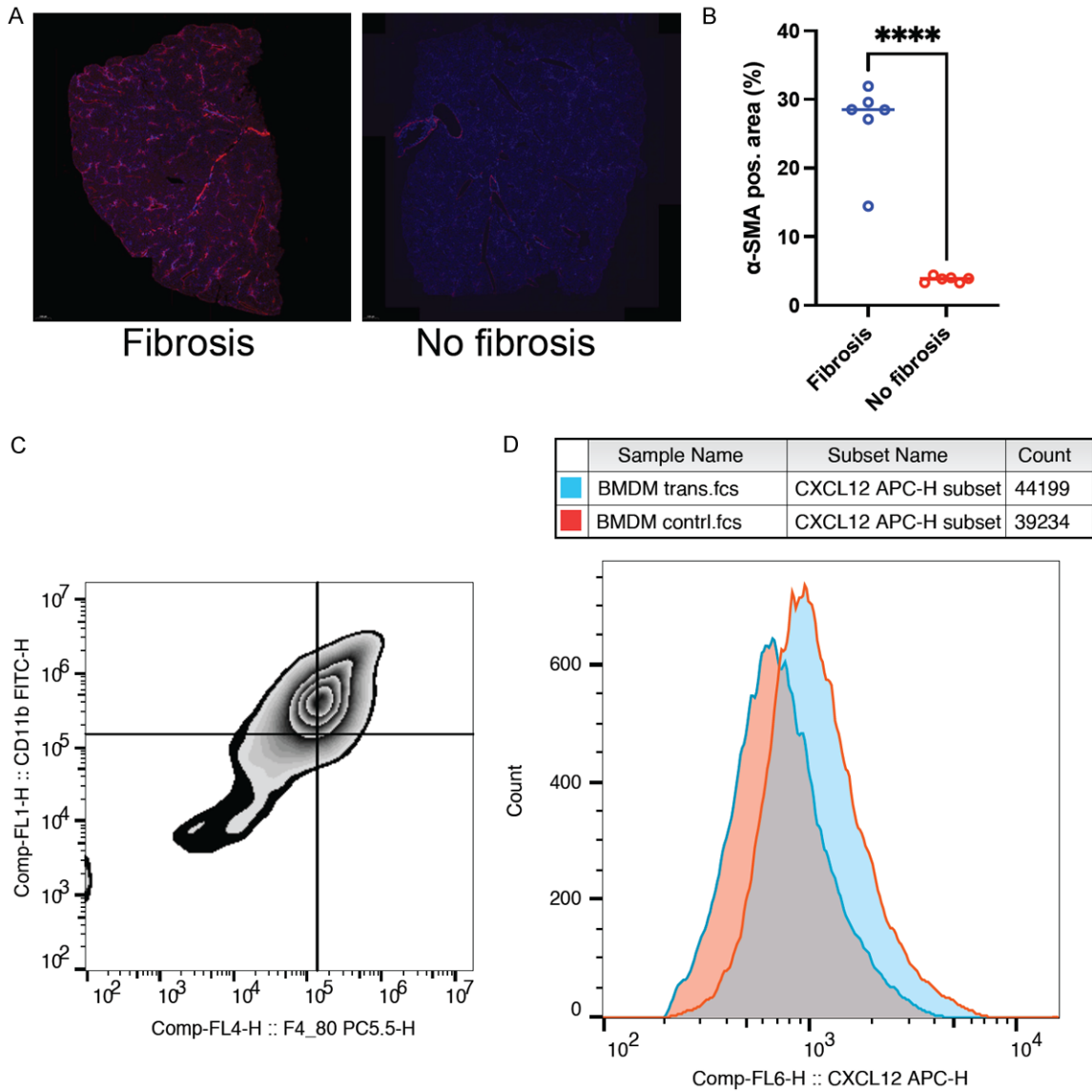


Figure S7. A. α -SMA staining of mouse liver tissues. B. Comparison of the ratio of α -SMA positive area in CCL4 treated and normal control mice. C, D. Expression of *cxcl12* in control and *cxcl12* transduced macrophage.

Macrophage regulate B cell PD-L1 in HCC

Table S2. Hallmarker gene of PD-L1+Breg cell compared with other cluster of B cells

gene	logFC	AveExpr	t	P.Value	adj.P.Val	B
IGHA1	2.1180681	-2.50E-16	13.12577205	1.57E-31	4.03E-27	59.58806589
IGHA2	2.081733459	2.77E-17	12.77894948	2.90E-30	3.73E-26	56.67683402
BRMS1L	1.038069146	-0.01047552	6.290636246	1.09E-09	7.26E-06	9.696991135
JCHAIN	1.193011828	-1.86E-16	6.284400325	1.13E-09	7.26E-06	9.662157137
KLHL14	1.184504147	-6.67E-18	6.233876275	1.51E-09	7.74E-06	9.380913387
PDIA4	1.145251994	-4.20E-18	6.002533022	5.49E-09	2.35E-05	8.115781413
CCNB2	1.127836447	-0.000921724	5.963406047	6.80E-09	2.50E-05	7.905531963
IL10	1.133417828	1.28E-17	5.933337701	8.02E-09	2.58E-05	7.744699378
ZNF341	1.027904135	-0.007511131	5.896213547	9.82E-09	2.80E-05	7.54701697
RP11-1212A22.4	0.96276259	-0.010208176	5.752493079	2.13E-08	5.48E-05	6.791077265
SLC16A6	0.989822565	-0.006082746	5.520923785	7.20E-08	0.000159251	5.60483825
DUSP5	1.060920955	1.36E-16	5.514720698	7.43E-08	0.000159251	5.573608867
DDOST	1.045721964	-1.21E-16	5.428056047	1.16E-07	0.000229504	5.140313712
CCR10	1.037789818	1.16E-16	5.382971473	1.46E-07	0.000257953	4.917142972
CD27	1.036736232	2.47E-16	5.376990497	1.50E-07	0.000257953	4.887652454
ITM2C	1.027955979	-1.09E-16	5.327213598	1.94E-07	0.000311007	4.643270638
VIMP	1.025529371	1.87E-16	5.313477583	2.07E-07	0.000313656	4.576165217
CDCA3	0.992486653	-3.97E-18	5.127317698	5.22E-07	0.000745517	3.680993445
MCM8	0.888118545	-0.008946227	5.102738637	5.88E-07	0.000792556	3.564805715
HARBI1	0.878116965	-0.00906989	5.093164944	6.16E-07	0.000792556	3.519677285
CLDN3	0.720644516	-0.017509292	4.993308133	9.98E-07	0.001222234	3.053248869
CRY1	0.955366963	3.42E-18	4.92008	1.41E-06	0.001653424	2.716191422
MYDGF	0.928595791	-2.54E-16	4.771803026	2.83E-06	0.002722143	2.046756668
LINC00092	0.746719312	-0.015251557	4.728451543	3.46E-06	0.002722143	1.854365931
RP11-16E12.1	0.746717627	-0.015228949	4.728451543	3.46E-06	0.002722143	1.854365931
TMTC2	0.746717161	-0.015222704	4.728451543	3.46E-06	0.002722143	1.854365931
POLE2	0.746713798	-0.015177601	4.728451543	3.46E-06	0.002722143	1.854365931
CRYBA4	0.746695248	-0.014928802	4.728451543	3.46E-06	0.002722143	1.854365931
RP13-58209.7	0.746688775	-0.014841977	4.728451543	3.46E-06	0.002722143	1.854365931
CD274	0.512658884	-0.003733405	2.679607594	3.46E-06	0.002722143	-5.409330346
SMOC1	0.746687263	-0.014821697	4.728451543	3.46E-06	0.002722143	1.854365931
C6orf52	0.746653948	-0.014374868	4.728451543	3.46E-06	0.002722143	1.854365931
CD226	0.73549686	-0.01469323	4.727869221	3.47E-06	0.002722143	1.851791944
AE000662.93	0.724587254	-0.01531844	4.726100883	3.49E-06	0.002722143	1.843977199
RP11-244H3.4	0.9091271	-0.000926225	4.712384533	3.72E-06	0.002754649	1.783446909
TANC2	0.688830532	-0.017184415	4.710663737	3.75E-06	0.002754649	1.775863772
TXNDC5	0.914058944	1.89E-16	4.691688539	4.09E-06	0.00292059	1.692403519
DNAJB11	0.899399822	4.91E-17	4.61117783	5.89E-06	0.004089521	1.341537608
GNS	0.898341958	2.79E-17	4.605378467	6.04E-06	0.004089521	1.316467791
EXPH5	0.605190314	-0.020583779	4.595652168	6.31E-06	0.004162521	1.274484051
SFT2D3	0.599017707	-0.020786415	4.581508481	6.73E-06	0.004242098	1.213570561
APTR	0.859175683	-0.003368799	4.567671898	7.16E-06	0.004242098	1.154138107
NEDD4L	0.797642911	-0.008717122	4.564832592	7.25E-06	0.004242098	1.141961792
PTPRG	0.849894889	-0.00420908	4.564655911	7.25E-06	0.004242098	1.141204318
HSP90B1	0.890870021	-4.65E-16	4.564456458	7.26E-06	0.004242098	1.140349244
RP11-455F5.5	0.795346522	-0.008807193	4.557777146	7.48E-06	0.004273227	1.111733242
PIIB	0.88825791	1.25E-16	4.550167099	7.74E-06	0.00432444	1.0791743
HBSIL	0.872828126	6.31E-18	4.465932262	1.12E-05	0.006140545	0.72196411
CD38	0.871927688	-1.44E-16	4.46102559	1.15E-05	0.006143326	0.70133701
TRAM1	0.86660986	-3.56E-16	4.432067744	1.30E-05	0.006829744	0.580007237
CHST12	0.86529408	1.33E-16	4.424908042	1.34E-05	0.006905166	0.550116098

Macrophage regulate B cell PD-L1 in HCC

P4HB	0.859310644	-147E-17	4.392375969	1.55E-05	0.007796376	0.414833453
UAP1	0.853745377	1.08E-16	4.362155891	1.76E-05	0.008711579	0.289953767
IGLV7-46	0.558034775	-0.021375212	4.350128316	1.86E-05	0.009000757	0.240463488
AL928768.3	0.843546753	3.46E-17	4.306871106	2.23E-05	0.010629427	0.0634706
DNAJC3	0.841687424	-5.54E-17	4.296805173	2.33E-05	0.01089296	0.022509075
SLC38A10	0.835080421	-5.82E-17	4.261068965	2.71E-05	0.012447749	-0.122226366
SDF2L1	0.831311722	-6.86E-17	4.240707221	2.95E-05	0.013326447	-0.204213496
BRCC3	0.825202246	-1.23E-17	4.207733078	3.39E-05	0.01482243	-0.336243794
PDGFD	0.766174473	-0.006231362	4.20547567	3.42E-05	0.01482243	-0.34524902
DERL3	0.82433913	2.15E-16	4.203078074	3.46E-05	0.01482243	-0.35480877
CTNNAL1	0.817116176	5.81E-17	4.16415581	4.07E-05	0.017143286	-0.509320371
TXNDC11	0.810008547	2.24E-16	4.12591199	4.76E-05	0.019753658	-0.659888025
AC011043.1	0.606663498	-0.017050895	4.099756994	5.30E-05	0.021643732	-0.762145632
STARD3	0.80268379	5.12E-17	4.086558363	5.60E-05	0.022487182	-0.813526838
SAMD3	0.640120018	-0.014532134	4.063124422	6.16E-05	0.024359788	-0.904387109
TP53INP2	0.712478338	-0.008636075	4.044219498	6.65E-05	0.025903592	-0.97734514
RRM2	0.788198231	1.72E-17	4.008904132	7.67E-05	0.029005306	-1.112814874
AARS	0.788179086	6.13E-18	4.00880165	7.67E-05	0.029005306	-1.113206438
CRB3	0.721255423	-0.007217996	4.004375824	7.81E-05	0.029098075	-1.130108114
SEC11C	0.785120838	-1.66E-17	3.992435898	8.19E-05	0.030090324	-1.175621322
CTD-3193K9.4	0.720592532	-0.006928716	3.976894481	8.72E-05	0.031570448	-1.234679382
ABCB9	0.780366653	1.63E-17	3.967014329	9.07E-05	0.032384433	-1.272116382
AQP3	0.778268829	-3.77E-17	3.955804444	9.48E-05	0.033399469	-1.314490125
POLA1	0.523294423	-0.020627922	3.946237166	9.85E-05	0.034225331	-1.350569135
HCG27	0.695265491	-0.008576369	3.937592989	0.000101939	0.034502028	-1.383099185
GLT8D1	0.774651669	7.14E-17	3.93648674	0.000102387	0.034502028	-1.387257606
TBC1D14	0.774221063	4.69E-17	3.934187966	0.000103324	0.034502028	-1.395895385
TYMS	0.768958415	2.02E-17	3.906109188	0.000115444	0.03805495	-1.501034753
WIPI1	0.767947113	-1.48E-17	3.900716694	0.00011792	0.03837938	-1.521148631
SLC38A5	0.765038285	7.47E-17	3.885212055	0.000125326	0.039964823	-1.578840389
RP5-1068E13.7	0.703566587	-0.006922695	3.884045093	0.0001259	0.039964823	-1.583174155
GPR155	0.762351397	-4.51E-18	3.870898144	0.000132551	0.041562874	-1.631916678
AL109761.5	0.709547509	-0.005609225	3.844391937	0.000146985	0.045533381	-1.729733016
RRAS	0.660596291	-0.010214189	3.839270216	0.00014994	0.045895889	-1.74856346
LETM2	0.754536449	-1.51E-17	3.82930737	0.00015585	0.04714375	-1.785127402
LMAN2	0.752286693	5.18E-19	3.817345767	0.000163237	0.048804135	-1.828912782