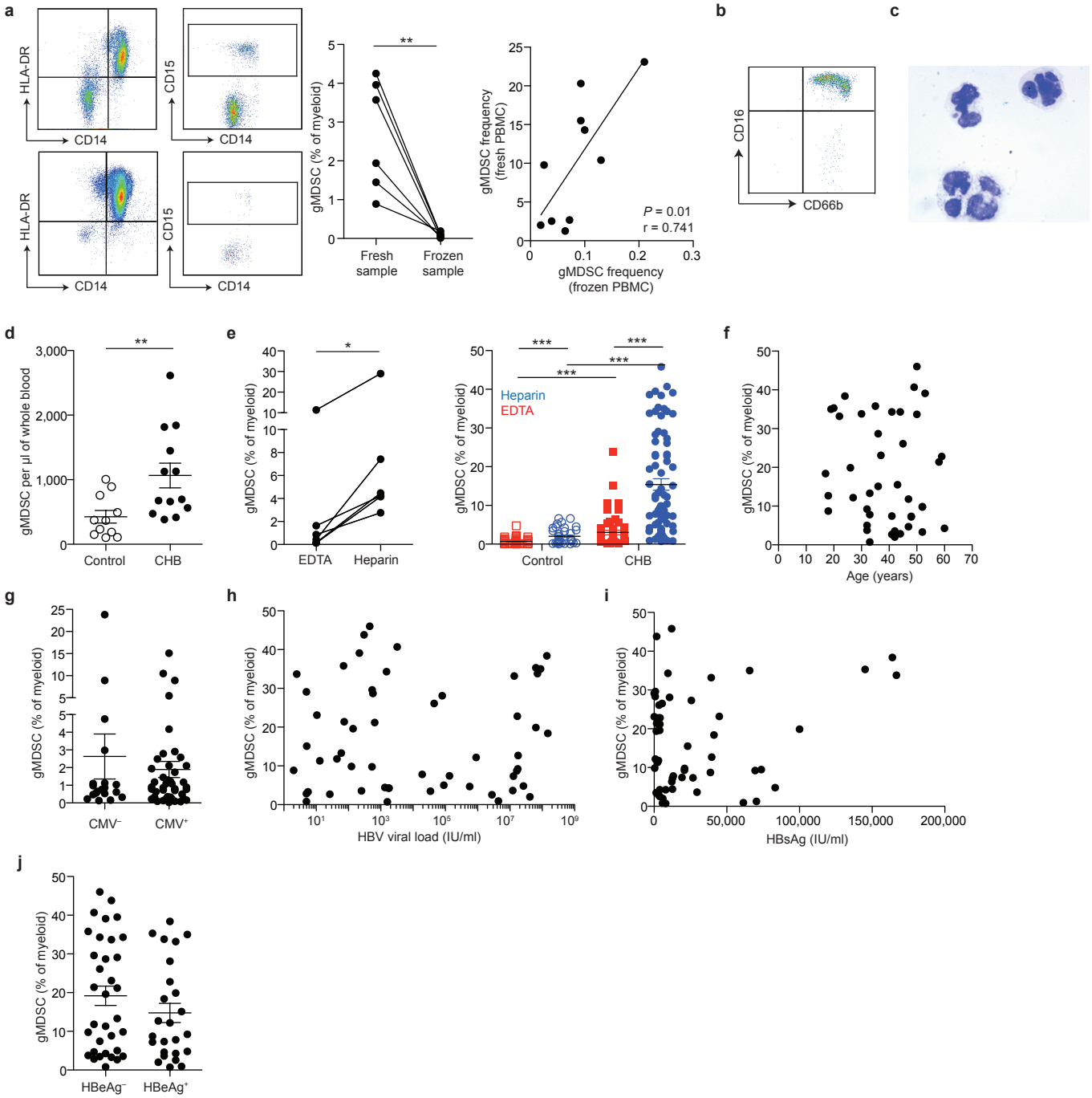
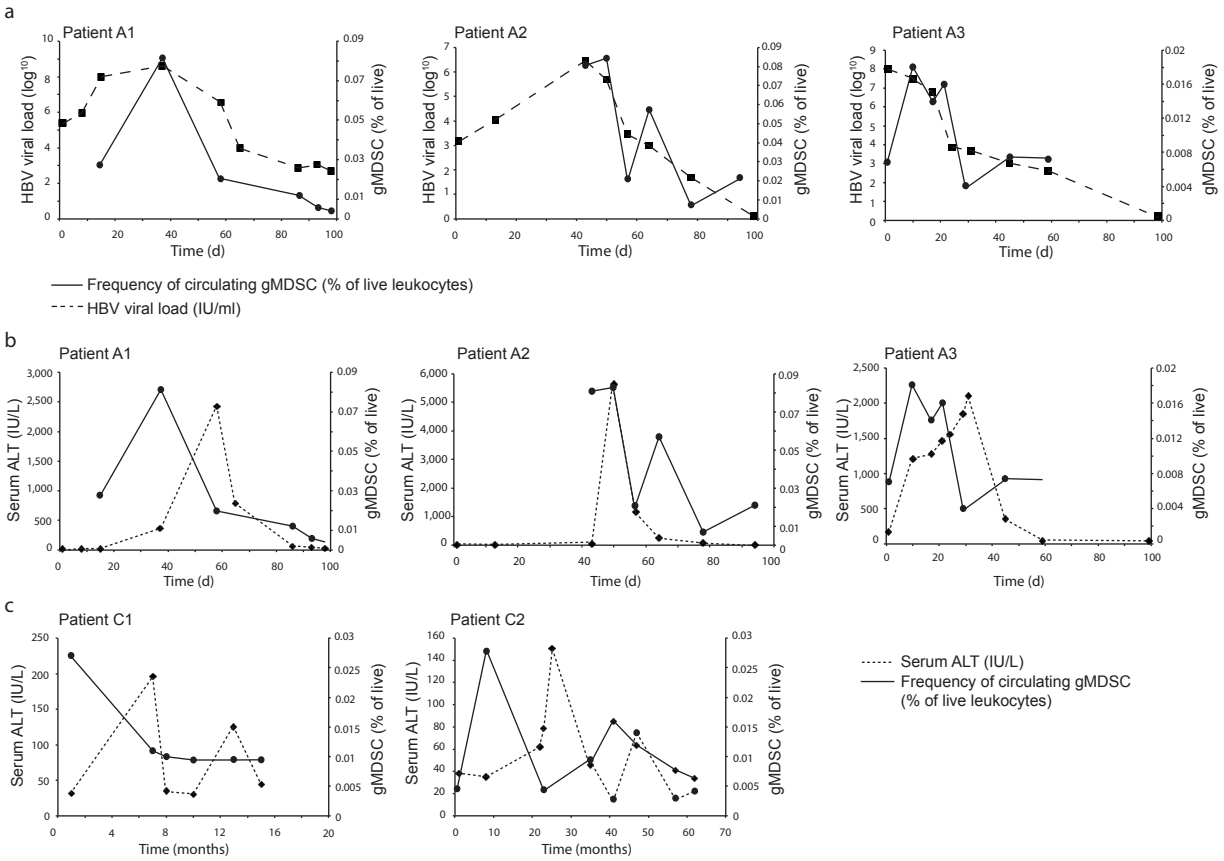
SUPPLEMENTARY FIGURE 1



Supplementary Figure 1: *Analysis of gMDSC frequencies in CHB cohorts*
a) PBMC from a small cohort of patients with CHB and healthy controls were stained for CD33, CD11b, HLA-DR, CD14 and CD15 and analyzed for gMDSC frequencies (as shown in **Fig. 1a**) when freshly isolated (upper-panel) or post-freezing (lower-panel) in FBS supplemented with 10% DMSO (24hr, –80 °C; comparison sample plots, paired frequencies, and correlation between frequencies). **b**) Representative FACS plot depicting co-expression of CD16 and CD66b on gMDSC. **c**) Haematoxylin-eosin staining of flow cytometric-isolated gMDSC. **d**) Absolute number of gMDSC, assessed using BD-TruCount tubes from whole, heparinised blood (n = 11, healthy controls; n = 13, CHB). **e**) gMDSC frequencies from isolated PBMC samples collected in the two different anti-coagulants, EDTA or heparin (n = 1, healthy control; n = 4, CHB) and cumulative data from the study cohorts, one collected in EDTA (n = 55, healthy controls; n = 54, CHB) and the other in heparin (n = 44, healthy controls; n = 84, CHB). Cross-sectional analysis of study participants, gMDSC frequencies were classified (where relevant) by: **f**) age (years), **g**) CMV serostatus, **h**) HBV viral load (IU/ml), **i**) circulating HBsAg (IU/ml) and **j**) presence of HBeAg. Error bars represent the mean ± SEM for the cohorts indicated; * p<0.05; ** p<0.01; *** p<0.001; **a, e** paired *t* test; **d, g, j** unpaired *t* test; **a, f, h–i** Pearson product-moment correlation coefficient; **e** one way ANOVA (Tukey's multiple comparisons test).

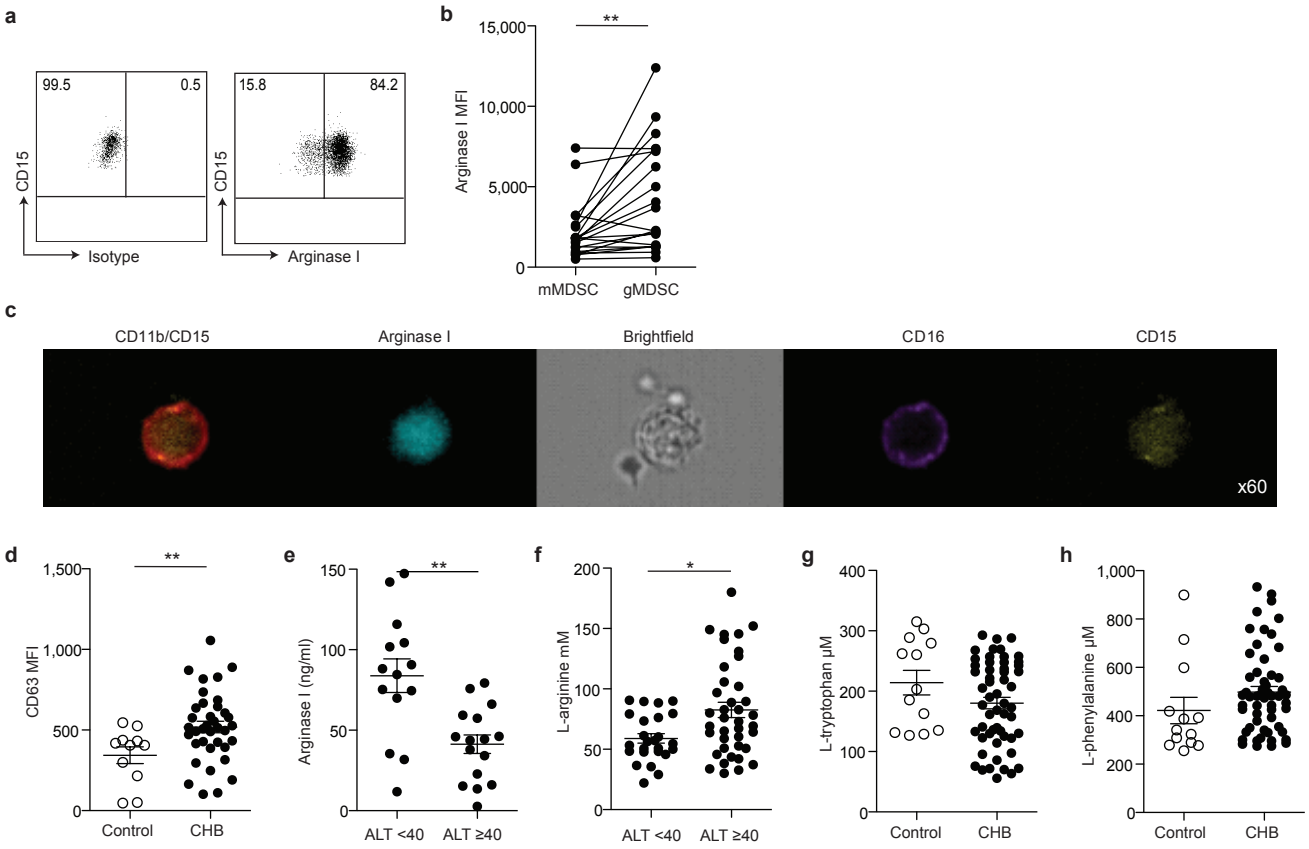
SUPPLEMENTARY FIGURE 2



Supplementary Figure 2: *Temporal changes in gMDSC in flares of acute and chronic HBV infection as a percentage of live leukocytes*

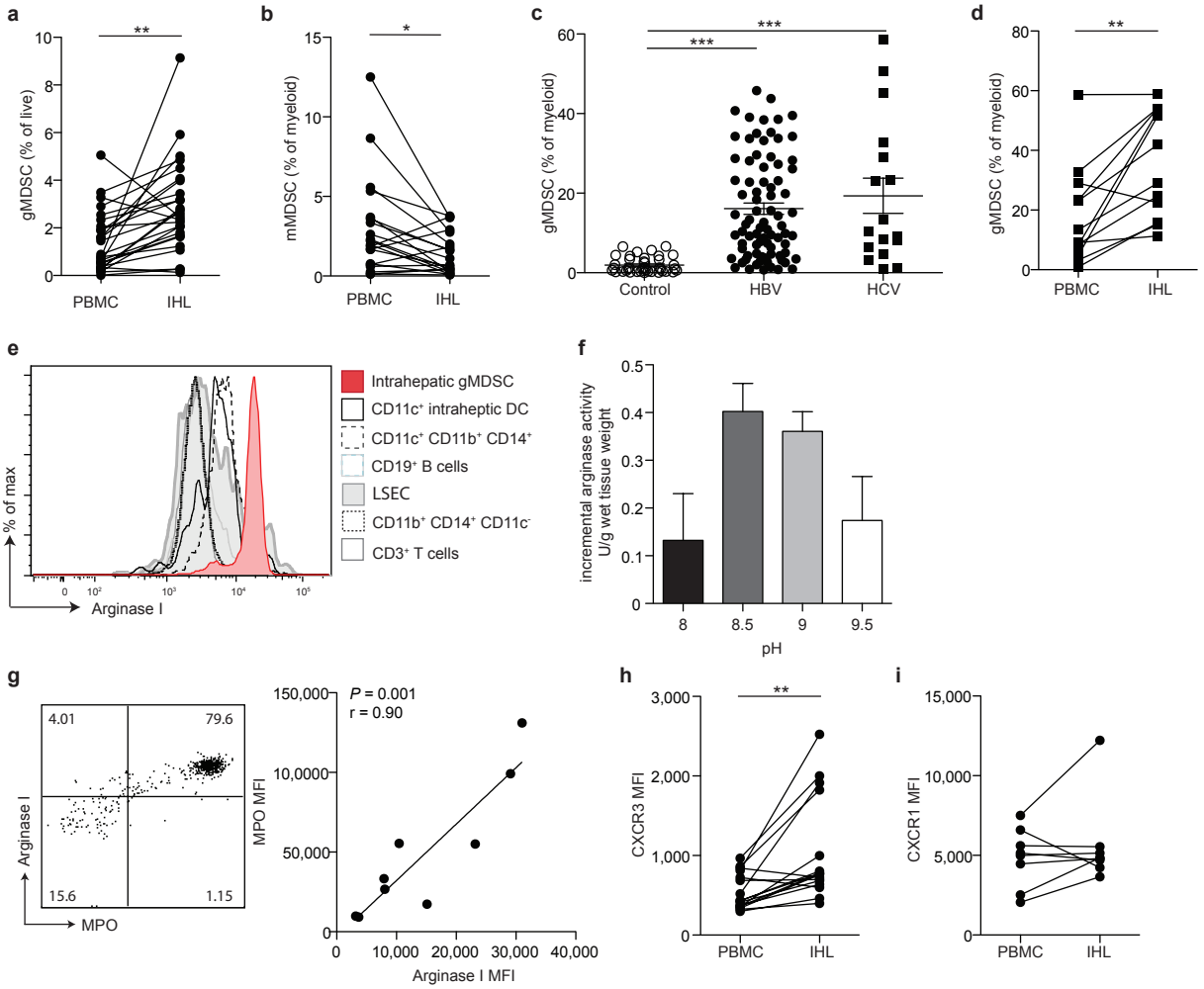
gMDSC were quantified as a percentage of live leukocytes from cryopreserved, longitudinal samples as detailed in **Fig. 2**. Frequencies were plotted according to **a**) HBV viral load (IU/ml) in acute HBV infection and throughout hepatic flares as measured by to serum ALT (IU/L) increases >2000 in **b**) acute HBV infection and **c**) throughout spontaneous chronic HBeAg$^-$ disease.

SUPPLEMENTARY FIGURE 3



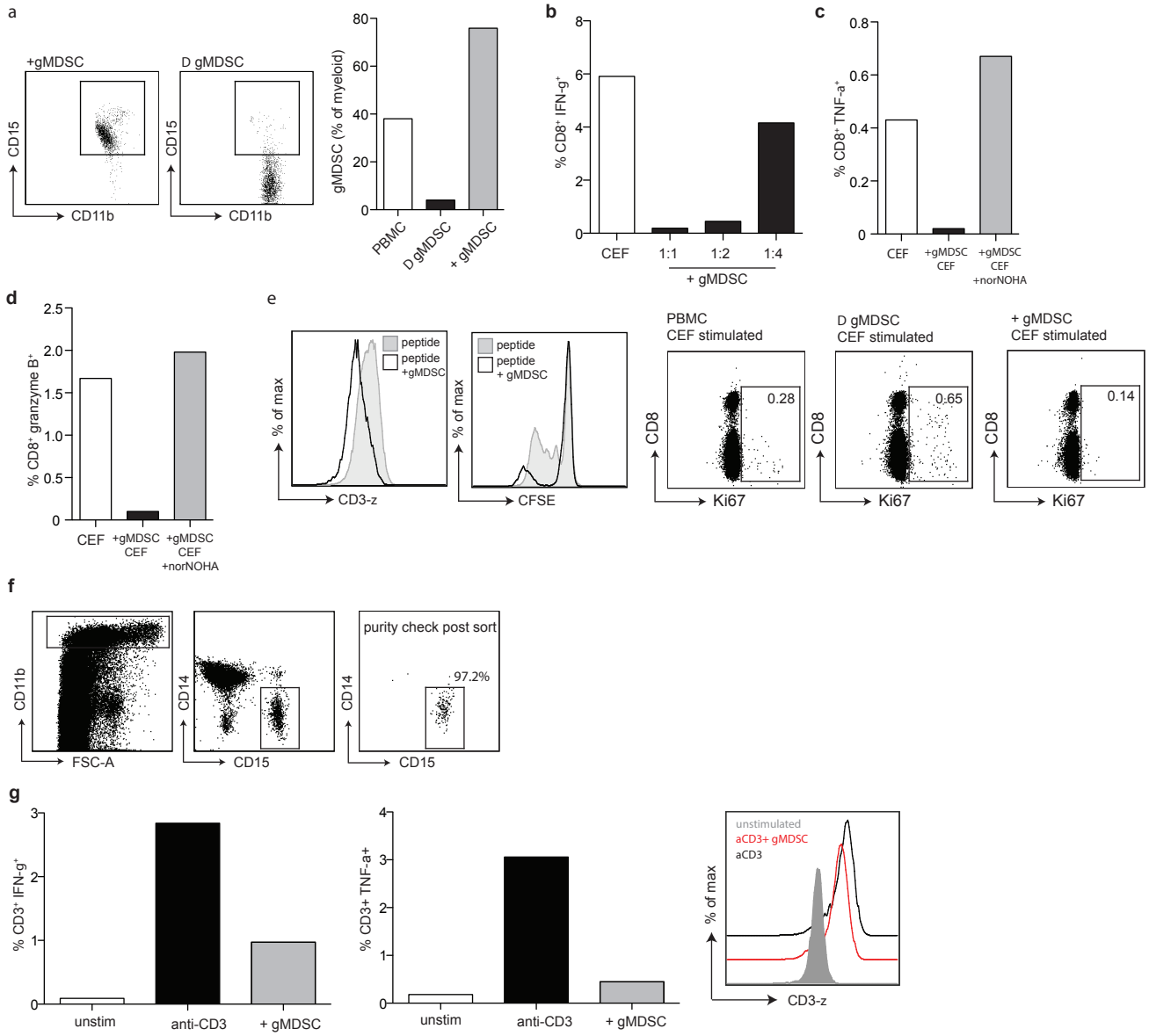
Supplementary Figure 3: *Arginase I granules in MDSC and serum amino acids*
a) Representative FACS plots depicting intracellular arginase I staining of gMDSC compared to matched isotype control (gated on live, singlet, $CD11b^{high}CD33^{+}HLA\text{-}DR^{-}CD14^{-}CD15^{+}$). **b**) gMDSC expression (mean fluorescence intensity (MFI)) of arginase I from peripheral mMDSC and gMDSC (n = 21, CHB). **c**) Representative ImageStream ISX (60x objective), cells were stained for $CD11b^{high}CD15^{+}CD16^{+}$ and intracellular arginase I. **d**) Cumulative cell surface CD63 expression (MFI) on gMDSC (n = 10, healthy controls; n = 36, CHB). **e**) Cumulative data: arginase I concentrations (ng/ml) by ELISA classified by serum ALT (IU/L) in subjects with CHB. Tandem high-performance liquid chromatography mass spectrometry analysis of serum **f**) L-arginine concentrations in CHB, cross-sectional anlaysis according to serum ALT (IU/L) and **g**) L-tryptophan (n = 13, healthy controls; n = 56, CHB) and **h**) L-phenylalanine (n = 13, healthy controls; n = 56, CHB) concentrations (µM). Error bars represent the mean ± SEM for the cohorts indicated; ** p<0.01; **b** paired *t* test; **d–h** unpaired *t* test.

SUPPLEMENTARY FIGURE 4



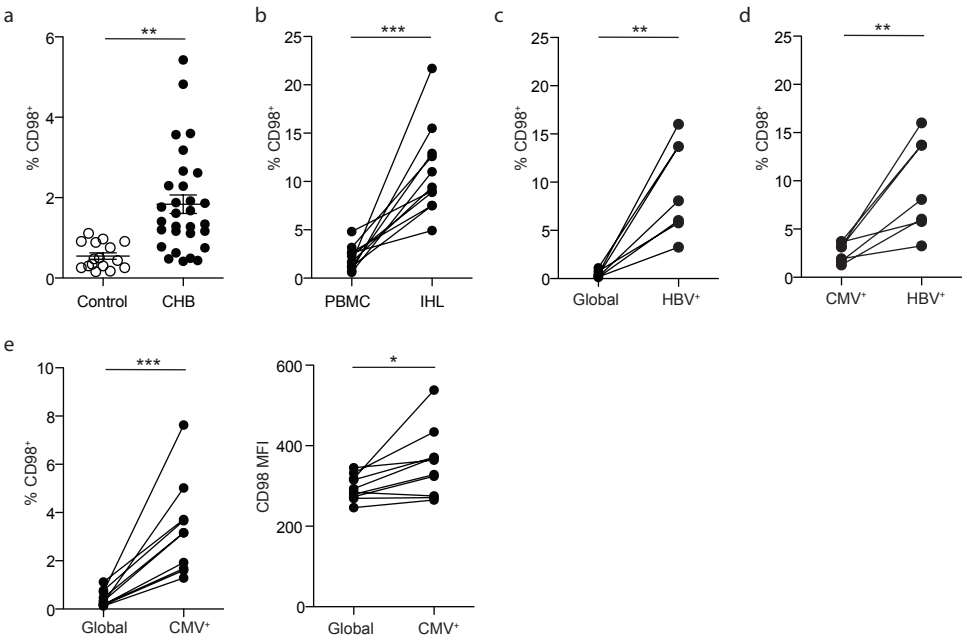
Supplementary Figure 4: *The accumulation of arginase⁺ gMDSC in the liver*
a) Cumulative data: peripheral (PBMC) compared to intrahepatic (IHL) frequencies of gMDSC presented as a percentage of live leukocytes (n = 36, paired CHB). **b**) Cumulative data: peripheral and intrahepatic mMDSC frequencies represented as a percentage of myeloid cells ($CD11b^{high}CD33^{+}$). **c**) Cumulative data: peripheral gMDSC frequencies in an extended cohort of study participants (n = 44, healthy controls; n = 84, CHB; n = 17, chronic HCV infected subjects). **d**) Cumulative data comparing peripheral and intrahepatic frequencies of gMDSC in chronic HCV infection (n = 12). **e**) Analysis of intracellular arginase I expression (mean fluorescence intensity (MFI)) in various intrahepatic cellular fractions. Subpopulations identified as: $CD3^{-}CD19^{+}$ B cells, $CD3^{+}$ T cells, $CD11c^{+}$ intrahepatic dendritic cells, $CD11b^{+}CD14^{+}$ monocytes, $CD11b^{+}CD14^{+}CD11c^{-}$ monocytes, $CD3^{-}CD11c^{-}CD49d^{+}$ liver sinusoidal endothelial cells (LSEC). **f**) Arginase I specific enzymatic activity at increasing pH in healthy liver tissue. **g**) Representative example of gMDSC arginase I co-staining with MPO and correlative analysis of gMDSC MPO and arginase I expression. Cumulative expression (MFI) on paired peripheral and intrahepatic gMDSC of **h**) CXCR3 (n = 16, CHB) and **i**) CXCR1 (n = 8, CHB). Error bars represent the mean ± SEM for the cohorts indicated; * p<0.05; ** p<0.01; *** p<0.001; **a–b, d, h–i** paired *t* test; **c** unpaired *t* test; **g** Pearson product-moment correlation coefficient.

SUPPLEMENTARY FIGURE 5



Supplementary Figure 5: *Purified gMDSC inhibit expansion of functional T cells*
a) Sample purity of gMDSC and PBMC depleted of gMDSC (Δ gMDSC) using sequential magnetic bead isolation ($CD14^{-}CD15^{+}$). **b**) $CD8^{+}$ T cell IFN–g response to 0.5 µg/ml CEF peptide stimulation after co-culture with reducing gMDSC:PBMC ratios. Representative examples depicting **c**) $CD8^{+}$ TNF–a and **d**) $CD8^{+}$ cytotoxicity (granzyme-B accumulation) in the presence or absence of gMDSC and arginase I inhibitor, nor-NOHA. **e**) CD3–z and CFSE dilution of $CD3^{+}$ T cells at d5 after stimulation with an HLA-A2-restricted CMV pp65 peptide (NLVPMVATV) ± gMDSC enrichment; intracellular Ki67 staining of $CD3^{+}$ T cells stimulated with CEF upon depletion (Δ gMDSC) or enrichment of gMDSC (+ gMDSC). Flow cytometric isolation of a pure gMDSC; **f**) gating strategy used on the FACSAria to isolate a 97% pure population of gMDSC ($CD33^{+}CD11b^{high}CD14^{-}CD15^{+}$) from PBMC. **g**) IFN–g and TNF–a response and CD3–z expression from $CD3^{+}$ T cell population following stimulation with plate-bound anti-CD3 with or without readdition of flow cytometric purified gMDSC (effector:target ratio: 1:2).

SUPPLEMENTARY FIGURE 6



Supplementary Figure 6: *Differential CD98 expression on T cells in CHB*
Cumulative data depicting expression (percentage) of CD98 on $CD3^+$ T cells from **a**) 16 healthy controls and 30 subjects with CHB; **b**) paired PBMC and IHL samples (n = 11, paired CHB); **c**) paired global and HBV–specific $CD8^+$ T cells (identified using HLA-A2 restricted dextramers, described in **Online Methods**), **d**) HBV– and CMV–specific $CD8^+$ T cells (n = 7, CHB) and **e**) global and CMV–specific $CD8^+$ T cells (n = 10, CHB). Error bars represent the mean ± SEM for the cohorts indicated; * $p<0.05$; ** $p<0.01$; *** $p<0.001$; **a** unpaired *t* test, **b–e** paired *t* test.

Supplementary Table 1: Study participant details

Heparin	**Age (years)** median (range)	**Sex (%)** male : female	**ALT IU/L** median (range)	**HBV DNA IU/ml** median (range)	**HBsAg IU/ml** median (range)	**HBeAg (%)** pos : neg
CHB (n = 84)	39 (17 - 61)	62 : 38	47 (10 - 311)	$1.2x10^8$ (blq - $1.5x10^9$)	$0.9x10^4$ (26 - $1.6x10^6$)	37 : 63
Healthy control (n = 44)	35 (24 - 64)	45 : 55	n/a	n/a	n/a	n/a

EDTA	**Age (years)** median (range)	**Sex (%)** male : female	**ALT IU/L** median (range)	**HBV DNA IU/ml** median (range)	**HBsAg IU/ml** median (range)	**HBeAg (%)** pos : neg
CHB (n = 54)	38 (19 - 72)	61 : 39	31 (10 - 586)	$9.3x10^4$ (blq - $7x10^7$)	1805 (42.6 - $8.3x10^4$)	13 : 87
Healthy control (n = 55)	31 (21 - 85)	45 : 55	n/a	n/a	n/a	n/a

* CHB = subjects with chronic hepatitis B; ALT = serum alanine transaminase; blq = below the level of quantification; n/a = not applicable

Supplementary Table 2: Full details of all directly-conjugated antibodies

Marker	Clone	Flurochrome	Supplier	Dilution	Catalogue No.
CD11b	ICRF44	PECy7	eBioscience	2:100	25-0118-42
HLA-DR	L243	eFluor450	eBioscience	3:100	9048-9952-025
HLA-DR	G46.6	HorizonV500	BD Bioscience	2:100	561224
CD33	WM53	AlexaFluor700	eBioscience	3:100	56-0338-41
CD16	3G8	APCCy7	BD Bioscience	2:100	557758
CD63	HSC6	PE	BD Bioscience	2:100	561925
CD15	HI98	APC	BD Bioscience	7:100	551376
CD14	M5E2	HorizonV500	BD Bioscience	2:100	561391
CD66b	G10F5	PerCPCy5.5	Biolegend	2:100	305107
CXCR1 (CD181)	8F1/CXCR1	FITC	Biolegend	2:100	320605
CCR2 (CD192)	K036C2	PE	Biolegend	2:100	357205
CXCR3 (CD183)	IC6	PerCPCy5.5	BD Bioscience	2:100	560832
CXCR4 (CD184)	12G5	PE	Biolegend	2:100	306505
CD3ε	UCHT1	PECy7	eBioscience	1:100	25-0038-42
CD8	OKT8	AlexaFluor700	eBioscience	1:200	56-0086-73
CD4	RPA-T4	APC-eFluor780	eBioscience	1:200	47-0049-42
Granzyme B	GB11	FITC	Biolegend	1:100	515403
CD19	HIB19	HorizonV500	BD Bioscience	1:100	561125
CD98	MEM-108	FITC	Biolegend	2:100	315603
CD71	CY1G4	APCCy7	Biolegend	2:100	334109
Live/dead	na	Blue (UV)	Invitrogen	2:1000	L-23105
Arginase I	6589922	FITC	R&D Systems	5:100	IC5868F/IC8026G
MPO	MPO455-8E6	PE	eBioscience	1:400	12-1299-41
CD3-ζ	6B10.2	PE	eBioscience	1:200	12-2479-80
IFN-γ	B27	HorizonV450	BD Bioscience	2:100	560371
Ki67	B56	PE	BD Bioscience	5:100	556027
TNF-α	6401.111	APC	Biolegend	1:200	502912

* Antibodies used for intracellular staining are in *italics*