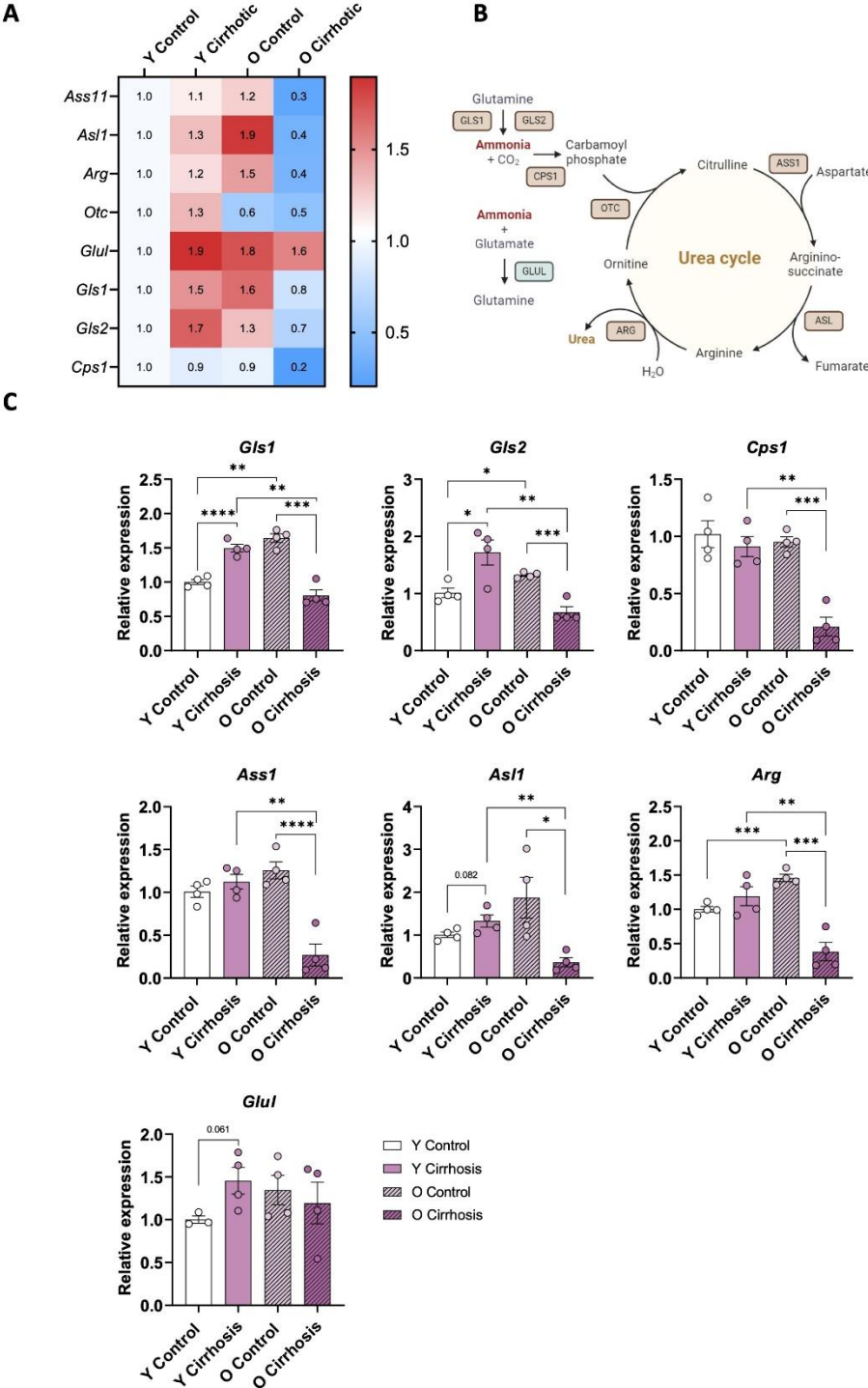


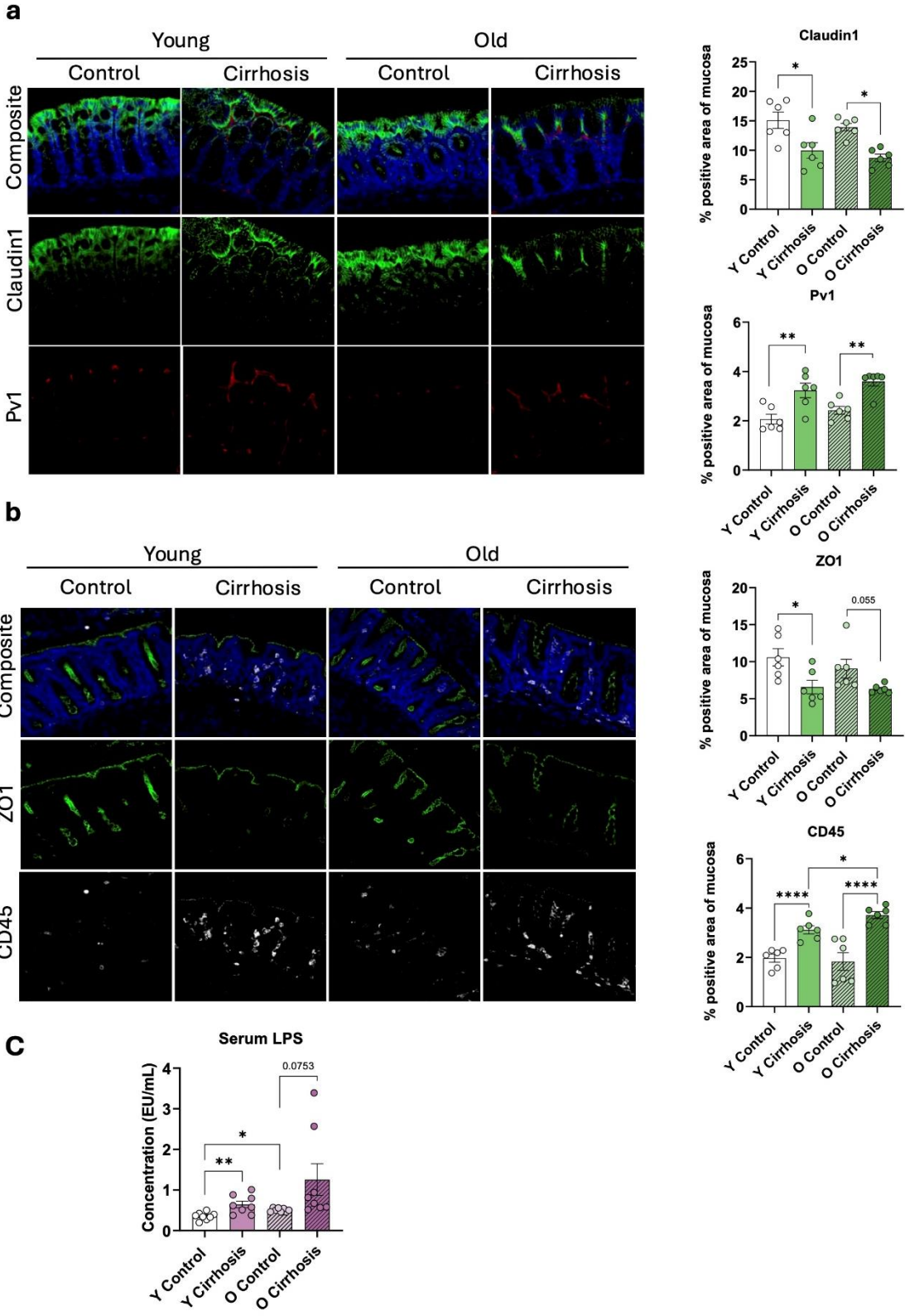
Aging Deteriorates Blood Brain Barrier Function and Polarizes Adaptive T Cell Expansion Contributing to Neurocognitive Damage in Experimental Cirrhosis

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SUPPLEMENTARY DATA

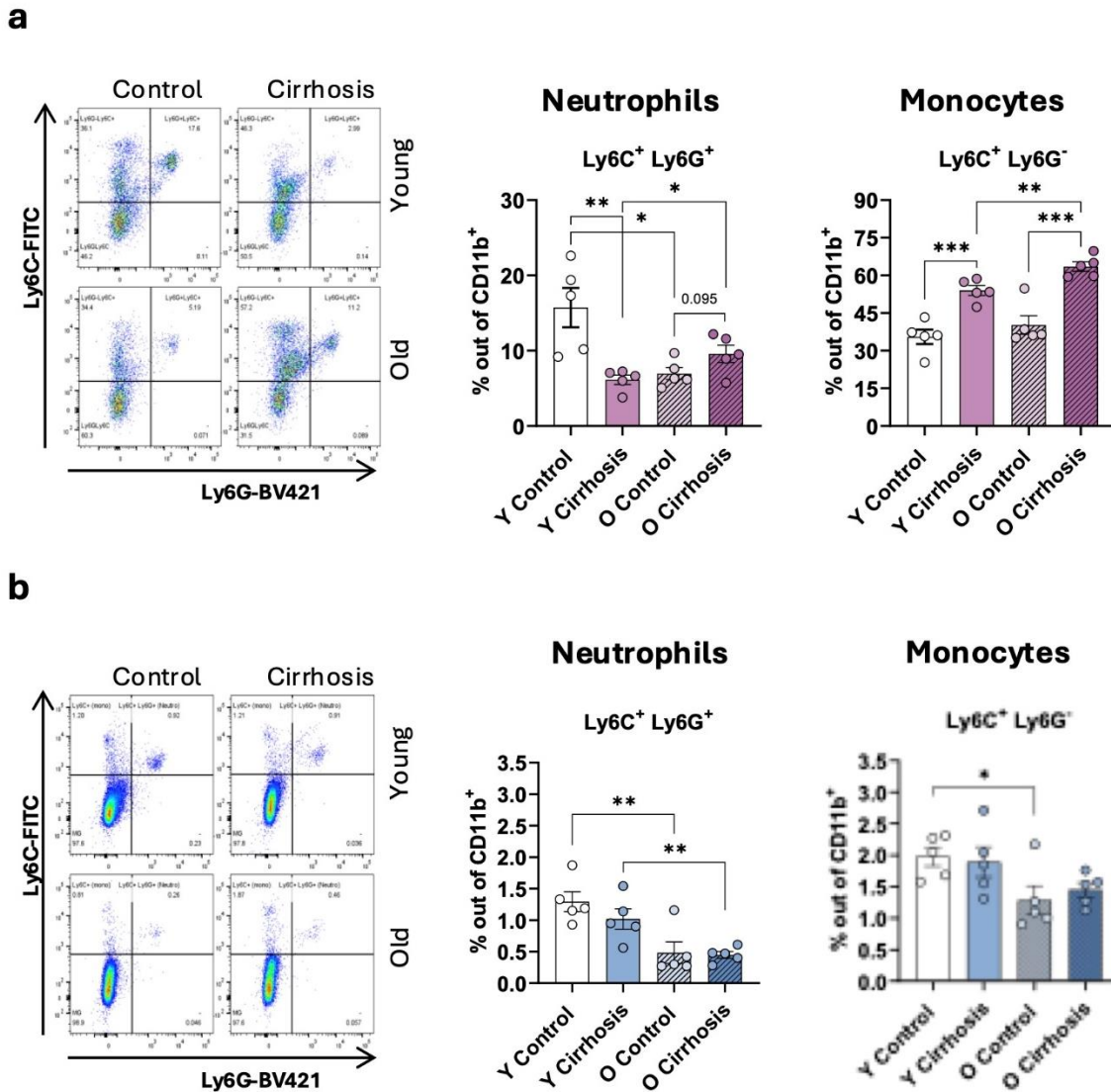


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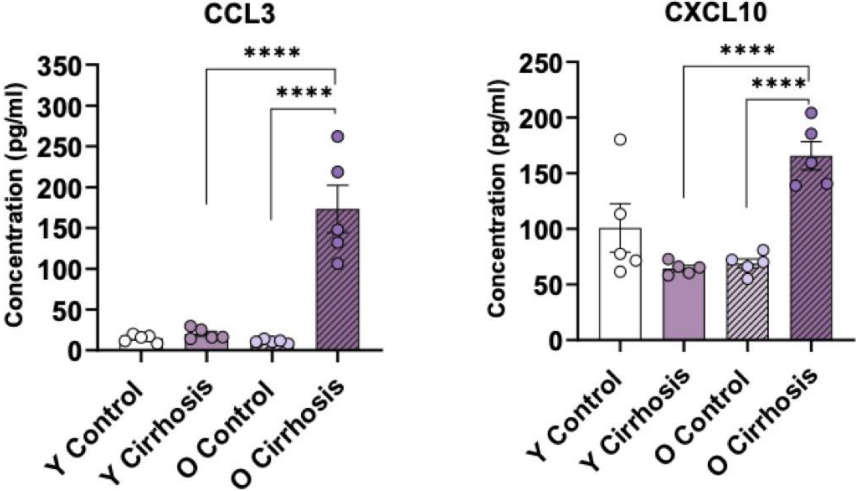
Supplementary Figure 2. Gut barrier integrity in young and old mice with cirrhosis. Representative Immunofluorescence of proteins involved in the integrity of the gut barrier in sections of paraffin-embedded mouse colon tissue. (A) Expression of Claudin1 and Pv1 (B) Expression of Zo1 and CD45. Expression was blindly measured by densitometry in user-specified regions of interest (ROIs) as percentage of positive area of mucosa. Area of mucosa was quantified using the DAPI channel using the ImageJ software. Mean \pm standard deviation is represented. (C) Quantification of endotoxin in serum as an indicative of gut permeability (8 animals/group). Abbreviations: Plasmalemma vesicle-associated protein (Pv1), Zonula Occludens1 (Zo1), Cluster of differentiation (CD), Lipopolysaccharide/Endotoxin (LPS), Young (Y) and Old (O). P values are indicated as follows (*) $p < 0.05$; (**) $p < 0.01$; (***) $p < 0.001$; and (****) $p < 0.0001$.



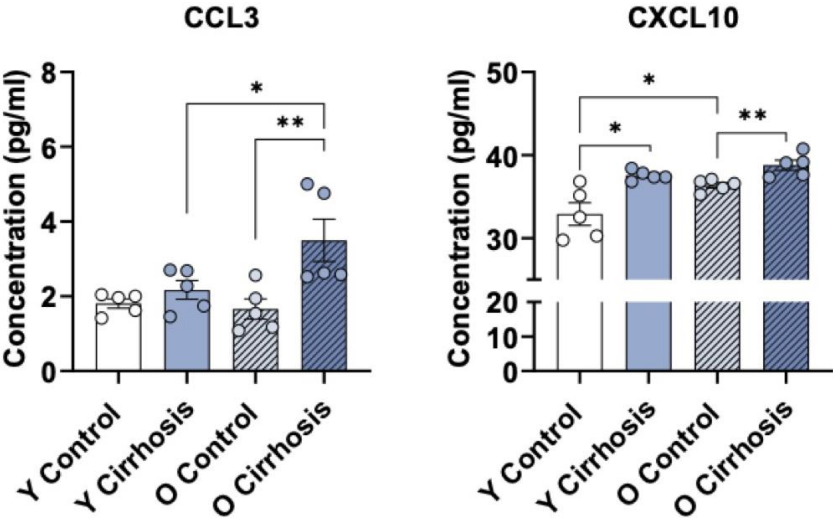
Supplementary Figure 3. Innate immune system alterations in brains and livers from young and old mice with cirrhosis. Representative dot plot images from flow cytometry analysis of CD11b⁺ cells of the liver (A) and the brain (B) expressing Ly6C alone for monocytes or in combination with Ly6G for neutrophils. Data is expressed as percentage of positive cells of the previous gate. Mean \pm standard deviation is represented (5 animals/group). Abbreviations: Cluster of differentiation (CD), Lymphocyte antigen 6 (Ly6), Young (Y) and Old (O). P values are indicated as follows (*) $p < 0.05$; (**) $p < 0.01$; (***) $p < 0.001$; and (****) $p < 0.0001$.

SUPPLEMENTARY DATA

a



b



Supplementary Figure 4. Chemokines involved in CD8⁺ cells recruitment in brains and livers from young and old mice with cirrhosis. CCL3 and CXCL10 levels in livers (A) and brain lysates (B) measured by a multi-analyte flow assay kit. Mean ± standard deviation is represented (5 animals/group). Abbreviations: C-C Motif Chemokine Ligand 3 (CCL3), C-X-C Motif Chemokine Ligand 10 (CXCL10), Young (Y) and Old (O). P values are indicated as follows (*) p < 0.05; (**) p < 0.01; (***) p < 0.001; and (****) p < 0.0001.

SUPPLEMENTARY DATA

Supplementary Table 1. Antibodies for flow cytometry.

PROTEIN	TARGET	DILUTION	DISTRIBUTOR	CONJUGATION
CD3	Mouse	1:200	Biolegend	PE
CD4	Mouse	1:200	Biolegend	FITC
CD8	Mouse	1:100	Biolegend	BV510
<u>LY6C</u>	<u>Mouse</u>	<u>1:200</u>	<u>Biolegend</u>	<u>FITC</u>
<u>LY6G</u>	<u>Mouse</u>	<u>1:200</u>	<u>Biolegend</u>	<u>BV521</u>
<u>CD11B</u>	<u>Mouse</u>	<u>1:200</u>	<u>Biolegend</u>	<u>PE-Cy7</u>
CD69	Mouse	1:100	Biolegend	APC
PD-1	Mouse	1:100	Biolegend	PE
IL-17	Mouse	1:50	Biolegend	APC
IL10	Mouse	1:50	Biolegend	PE-Cy7
IL-4	Mouse	1:50	Biolegend	BV421

Supplementary Table 2. Antibodies for histological analysis.

PROTEIN	TARGET	HOST	DILUTION	REFERENCE	TECHNIQUE
ASMA	Mouse	Rabbit	1:200	D4K9N	IHC
VIM	Mouse	Rabbit	1:100	D21H3	IHC
COL1A1	Mouse	Rabbit	1:300	AB765P	IHC
CD45	Mouse	Goat	1:300	AF114	IHC
CD3	Mouse	Rabbit	1:100	D7A6E	IHC
GFAP	Mouse	Goat	1:500	13-0300	IF
ZO1	Mouse	Rabbit	1:300	PA5-28858	IF
CLAUDIN1	Mouse	Rabbit	1:300	ab15098	IF
CD45	Mouse	Goat	1:200	AF114	IF
PV1	Mouse	Rat	1:200	550563	IF
CONJUGATION	TARGET	HOST	DILUTION	REFERENCE	TECHNIQUE
A488	Anti-goat	Donkey	1:300	ab150133	IF
A488	Anti-mouse	Donkey	1:300	406416	IF
A594	Anti-rat	Donkey	1:300	ab150156	IF
A647	Anti-goat	Donkey	1:300	A-21447	IF
BIOTIN	Anti-rabbit	Horse	1:250	BA-1100	IHC
BIOTIN	Anti-goat	Horse	1:250	BA-9200	IHC

SUPPLEMENTARY DATA

Supplementary Table 3. Neurological severity score (NSS)

TASKS	POINTS
INABILITY TO WALK ON A BEAM	
3 CM WIDE	1
2 CM WIDE	1
1 CM WIDE	1
INABILITY TO BALANCE ON A BEAM 0.5 CM WIDE FOR	
20 S	1
40 S	1
60 S	1
INABILITY TO BALANCE ON A ROUND STICK 0.5 CM IN DIAMETER FOR	
10S	1
INABILITY TO WALK STRAIGHT	1
INABILITY TO EXIT FROM A CIRCLE 30 CM IN DIAMETER WHEN LEFT IN ITS CENTER WITHIN	
20 S	1
40 S	1
60 S	1
TOTAL SCORE	11

Supplementary Table 4. Primers for qPCR.

GENE	TARGET	FORWARD PRIMER	REVERSE PRIMER
COL1A1	Mouse	actcgaacgggaatccat	gtgtccctactcagccg
TIMP1	Mouse	ccagaaccgcagtgaagagt	gaaacactgtgcacacccca
ACTA2	Mouse	gtcccagacatcagggagtaa	tcggatacttcagcgtcagga
MMP2	Mouse	cttggtgccaggaaagtgaa	ccgaggactatgaccgggata
ARG	Mouse	tggaagaaccacgggtctg	gcaccacactgactctcca
ASL1	Mouse	aagtggagccctgaagaacc	aaatccccagcccactcat
ASS1	Mouse	tccagtgcactctacgagga	caatctccactgtctgcga
CPS1	Mouse	cagcctacagcctcaactg	tgtccaattgtttgtaaccagtgt
GLUL	Mouse	gcaatggaattctcgtgccg	gcagccactgcttttccaa
GLS1	Mouse	tggaacatctgatcccagg	agcatgacaccatctgacgtt
GLS2	Mouse	atcttagccaggacacgctg	aggggagaaagagaacgact
ACTB	Mouse	atggctcgctcggtagacct	ttctccggtgggtggcgtga
MMP9	Mouse	gctgactacgataaggacggca	tagtggtgcaggcagagtagga
MMP12	Mouse	cacactcccaggaatcaagcc	ttggtgacacgacggaacagg

SUPPLEMENTARY DATA

Supplementary Table 5. TaqMan probes for brain regions.

GENE	TARGET	BRAIN AREA	REFERENCE
CRH	Mouse	PVN	Mm01293920_s1
NR3C1	Mouse	HIP	Mm00433832_m1
BDNF	Mouse	HIP	Mm04230607_s1
NTRK2	Mouse	HIP	Mm00435422_m1
18S	Mouse	Reference gene	Mm03928990_g1

Supplementary Table 6. Antibodies for Western blot.

PROTEIN	TARGET	HOST	DILUTION	REFERENCE	TECHNIQUE
ZO1	Mouse	Rabbit	1:1000	PA5-28858	WB
CLAUDIN1	Mouse	Rabbit	1:1000	ab15098	WB
CASP3-CLEAVED	Mouse	Rabbit	1:2000	9661	WB
GRANZYME B	Mouse	Rabbit	1:750	4275	WB
PERFORIN	Mouse	Rabbit	1:750	3693	WB
B-ACTIN	Mouse	Mouse	1:10000	A5441	WB
CONJUGATION	Target	Host	Dilution	Reference	Technique
HRP	Anti-rabbit	Goat	1:3000	31460	WB
HRP	Anti-mouse	Goat	1:5000	31430	WB