# Spatial Transcriptomics Analysis of Zone-Dependent Hepatic Ischemia-Reperfusion Injury Murine Model

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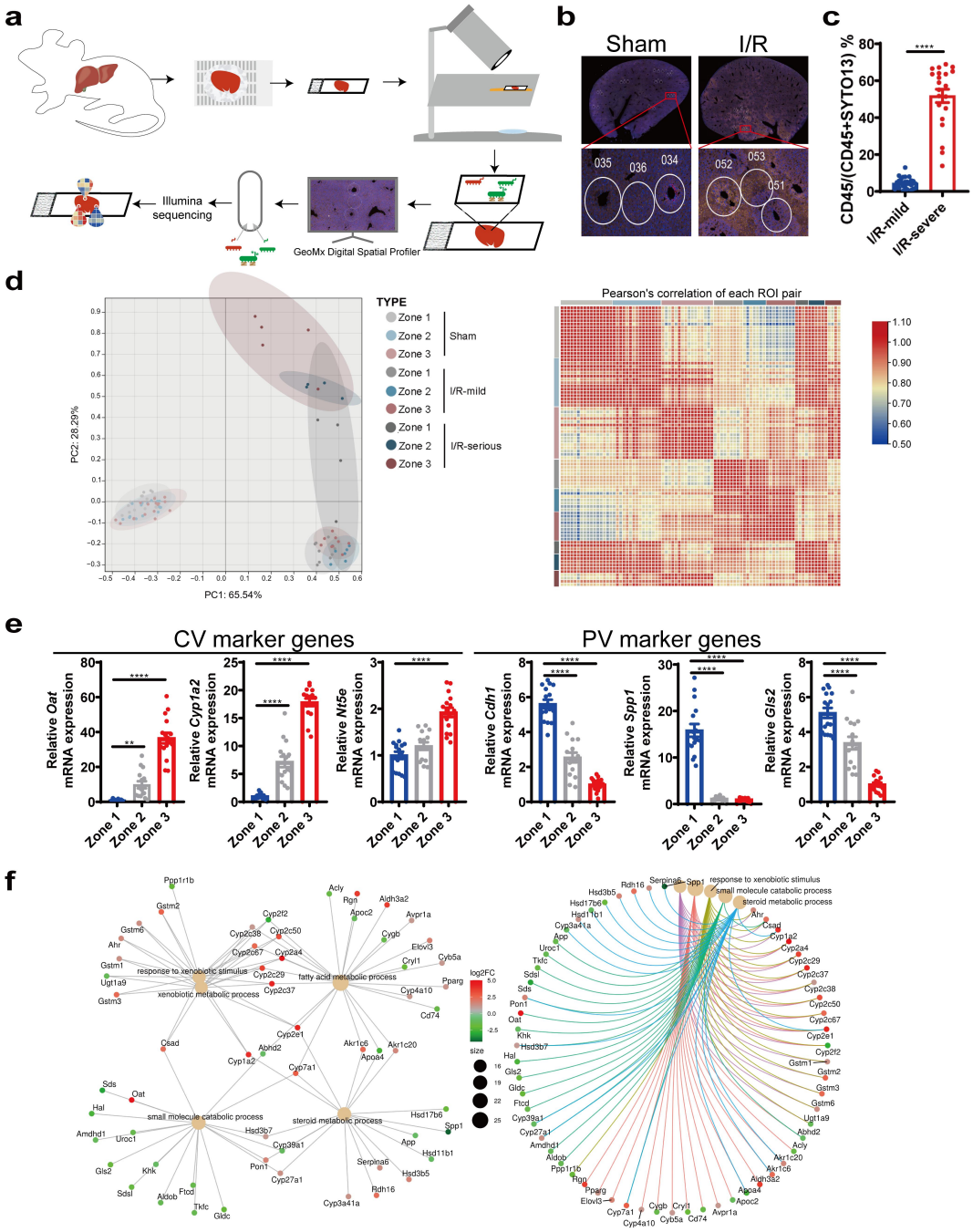
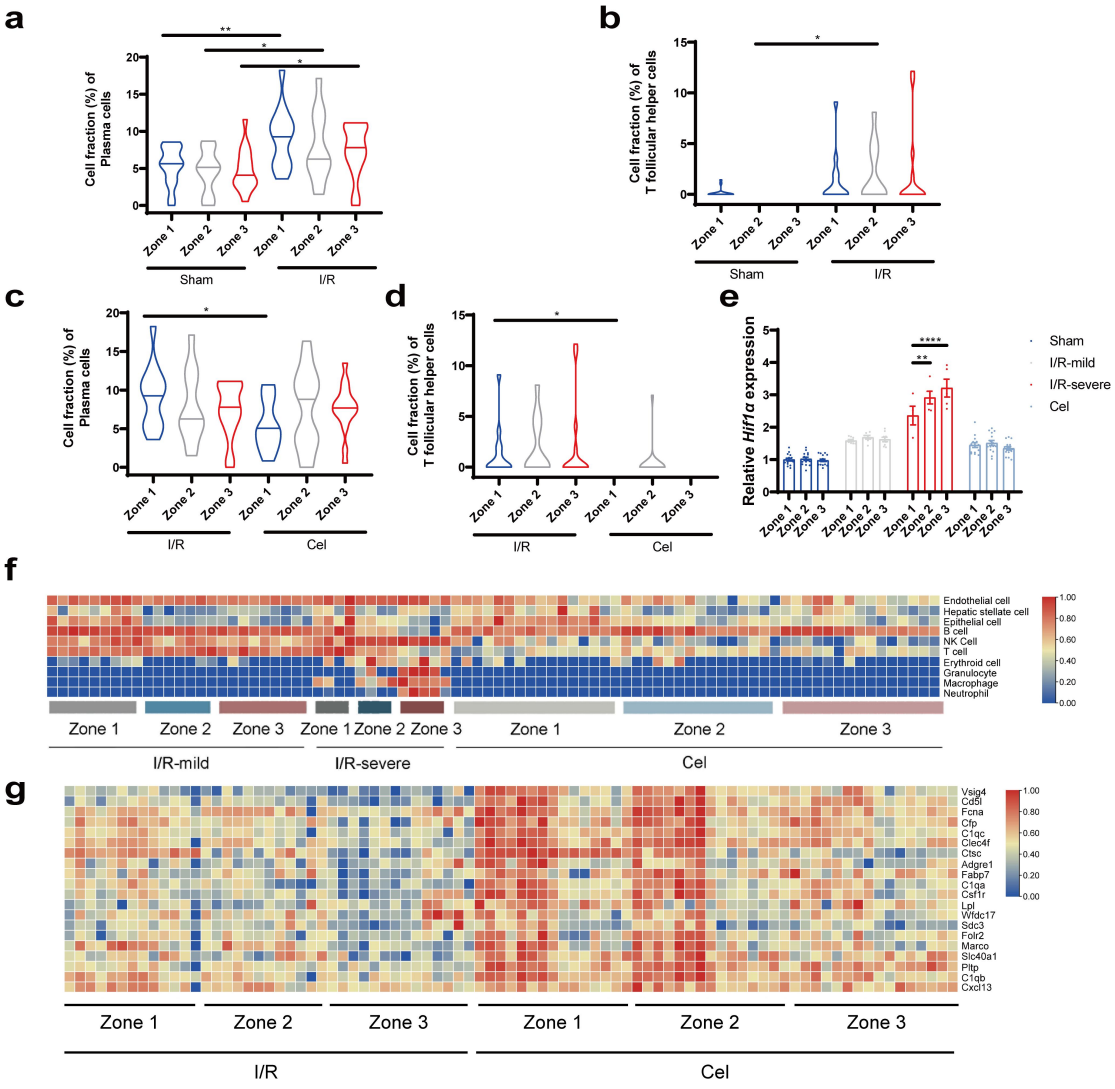



**Supplementary Fig 1. Hepatic zone-specific functional pathways and KCs abundance at the steady state.**

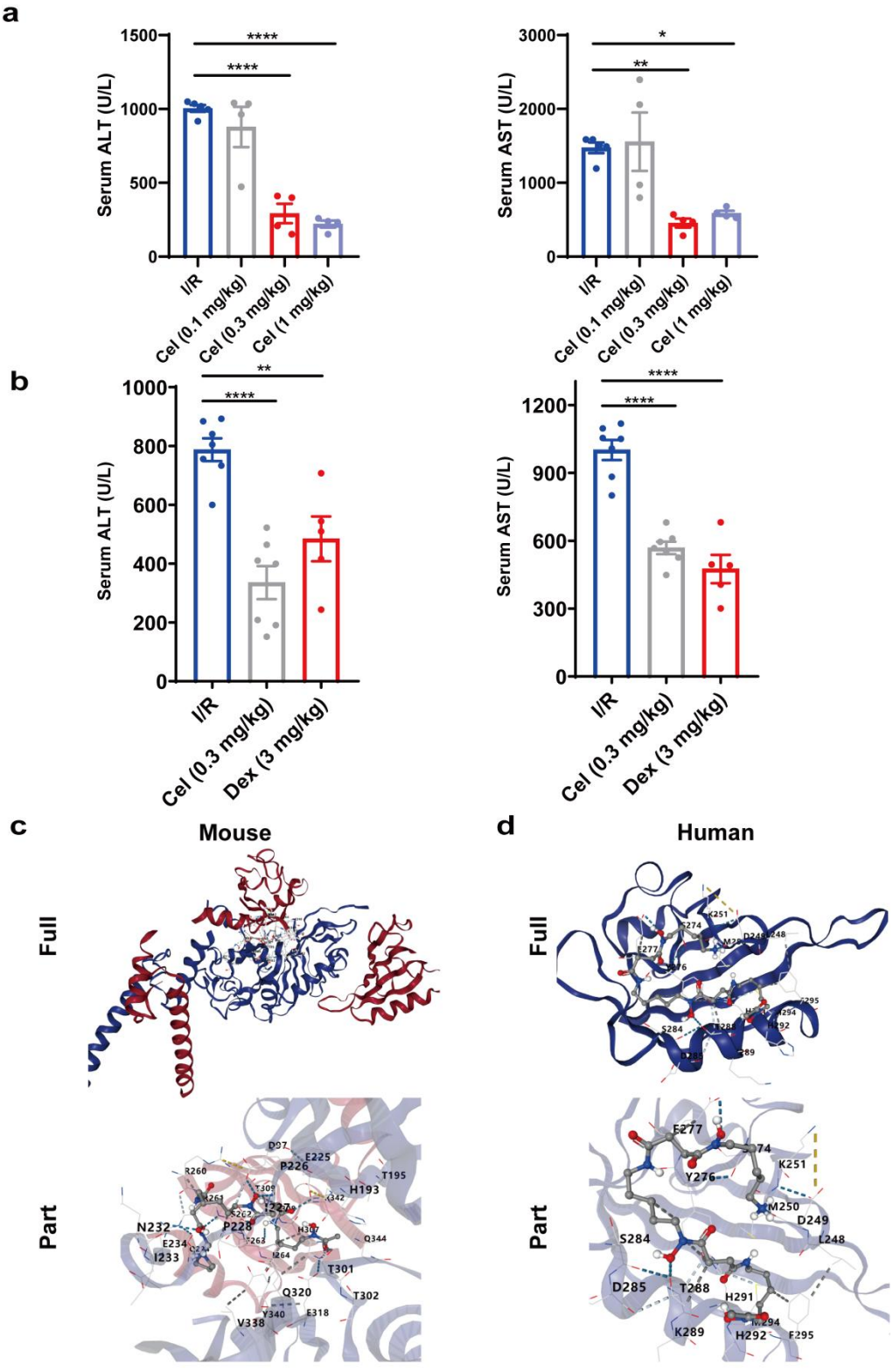
**a** Workflow of spatial transcriptomics. **b** Representative ROIs images of sham and I/R group. **c** CD45 positive rates calculated as CD45/(CD45+SYTO13) by ImageJ software (Student's t test, n=21-26, ****$p$ < 0.0001). **d** PCA and ROI correlation heatmap of each ROI pair. **e** Expression of representative CV and PV marker genes (One-way ANOVA, n=15-16, **$p$ < 0.01, ****$p$ < 0.0001). **f**

Zone 3-specific top GO and DEGs network. The node color represents $log_2$ fold change in gene expressions of zone 3 versus zone 1. Data were expressed as mean ± SEM.




**Supplementary Fig 2. The abundance of hepatic NPCs after I/R injury and celastrol treatment.**

**a-d** Cell fractions of plasma cells and Tfhs were calculated by Cibersort algorithm (Center lines indicate median value. Student's t test, n=11-16, *$p$ < 0.05, **$p$ < 0.01). **e** Relative HIF1α expression in each zone of different groups (Two-way ANOVA, n=15-16 for Sham and Cel group, and n=7-9 for I/R mild regions and n=4-5 for I/R severe regions, **$p$ < 0.01, ****$p$ < 0.0001, data were expressed as mean ± SEM). **f** The scaled proportion of NPCs in I/R and celastrol group. **g** The scaled Kupffer cells marker genes expression in I/R and celastrol group.


a
Serum ALT (U/L)
1500
1000
500
0
****
****
I/R
Cel (0.1 mg/kg)
Cel (0.3 mg/kg)
Cel (1 mg/kg)
Serum AST (U/L)
3000
2000
1000
0
*
**
b
Serum ALT (U/L)
1000
800
600
400
200
0
**
****
I/R
Cel (0.3 mg/kg)
Dex (3 mg/kg)
Serum AST (U/L)
1200
900
600
300
0
****
****
c
Mouse
Full
Part
d
Human
Full
Part
e
kDa
GAPDH
-42
-30
VEGFA
-30
-23

**Supplementary Fig 3. Celastrol reduced I/R injury and activated the hypoxia pathway.**

**a, b** Effect of one-week pre-treatment of celastrol or dexamethasone sodium phosphate on Serum transaminase levels after I/R injury (One-way ANOVA, n=4-7, *$p < 0.05$, **$p < 0.01$, ****$p < 0.0001$, data were expressed as mean ± SEM). **c, d** *In silico* molecular docking results of deferoxamine-mesylate with mouse HIF1α protein in **c** and human HIF1α protein in **d**. **e** Unprocessed gels of Fig. 7h.

## Supplementary Table 1: qPCR primer sequences

| Gene | Forward Primer (5'-3') | Reverse Primer (5'-3') |
|---|---|---|
| *Il-6* | TACCACTTCACAAGTCGGAGGC | CTGCAAGTGCATCATCGTTGTTC |
| *Il-1β* | TTGACGGACCCCAAAAGATG | AGGACAGCCCAGGTCAAAG |
| *Mcp-1* | ACTCACCTGCTGCTACTCATTCAC | GTATGTCTGGACCCATTCCTTCTT |
| *Gapdh* | AACTTTGGCATTGTGGAAGG | ACACATTGGGGGTAGGAACA |
| *Vegf* | GGCCTCCGAAACCATGAACT | CTGGGACCACTTGGCATGG |
| *Hif1α* | CCCATTCCTCATCCGTCAAATA | CCTGTGGTGACTTGTCCTTTAG |

## Supplementary Table 2: Antibody information

| Antibody | Company | Cat No. |
|---|---|---|
| Anti-VEGF | Bioss | Bs-1313R |
| Anti-GAPDH | Proteintech | 10494-1-AP |
| Anti-iNOS | Servicebio | GB11119 |
| Anti-CD163 | Servicebio | GB113751 |
| Anti-F4/80 | Servicebio | GB113373 |
| Anti-Rabbit IgG | Proteintech | SA00001-2 |

## Supplementary Table 3: Putative docking sites between celastrol/deferoxamine-mesylate and HIF1α

| Ligand | Protein | Binding Energy (kcal/mol) | Cavity Size ($Å^3$) | Center | | | Size | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | x | y | z | x | y | z |
| Celastrol | Mouse | -9.6 | 512 | -124 | -49 | 6 | 23 | 23 | 23 |
| | HIF1α | -9.4 | 548 | -112 | -61 | -31 | 23 | 23 | 23 |
| | | -8.8 | 3811 | -114 | -44 | 1 | 23 | 23 | 23 |
| | | -8.3 | 497 | -106 | -58 | -15 | 23 | 23 | 23 |
| | | -7.4 | 1595 | -95 | -34 | 14 | 23 | 23 | 23 |
| | Human | -7.3 | 44 | 19 | -17 | -35 | 23 | 23 | 23 |
| | HIF1α | -6.7 | 33 | 12 | -13 | -21 | 23 | 23 | 23 |
| | | -6.6 | 30 | 16 | 7 | -28 | 23 | 23 | 23 |
| | | -6.4 | 31 | 16 | 1 | -17 | 23 | 23 | 23 |
| | | -6.1 | 36 | 24 | 3 | -23 | 23 | 23 | 23 |
| Deferoxamine-mesylate | Mouse | -6.6 | 3811 | -114 | -44 | 1 | 38 | 38 | 38 |
| | HIF1α | -6.4 | 512 | -124 | -49 | 6 | 38 | 38 | 38 |
| | | -6.3 | 1595 | -95 | -34 | 14 | 38 | 38 | 38 |
| | | -6 | 548 | -112 | -61 | -31 | 38 | 38 | 38 |
| | | -5.5 | 497 | -106 | -58 | -15 | 38 | 38 | 38 |
| | Human | -4.7 | 30 | 16 | 7 | -28 | 38 | 38 | 38 |
| | HIF1α | -4.6 | 33 | 12 | -13 | -21 | 38 | 38 | 38 |
| | | -4.5 | 44 | 19 | -17 | -35 | 38 | 38 | 38 |
| | | -4.3 | 36 | 24 | 3 | -23 | 38 | 38 | 38 |
| | | -4.3 | 31 | 16 | 1 | -17 | 38 | 38 | 38 |