**Generation of human induced pluripotent stem cell-derived liver buds with chemically defined and animal origin-free media**

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**Supplementary Note**

Detailed clarification of interpretation associated to Fig 7c;

ALB: PHH differ from Conv and CD-AOF while not differ from Liver. Liver do not differ from any other sample.

TTR: No significant difference among all samples.

OTC: No significant difference among all samples.

AAT: Liver differ from other 3 samples.

RBP4: PHH differ from other 3 samples.

FGA: PHH differ from other 3 samples.

F7: No significant difference among all samples.

ASGR1: PHH differ from Conv and CD-AOF while not differ from Liver. Liver do not differ from any other sample.

GLUT2: No significant difference among all samples.

APOA1: PHH and Liver differ from Conv and CD-AOF. PHH vs Liver or Conv vs CD-AOF show no significant difference.

SCD: PHH and Liver differ from Conv and CD-AOF. PHH vs Liver or Conv vs CD-AOF show no significant difference.

FAH: PHH differ from Liver.

TAT: Liver differ from other 3 samples.

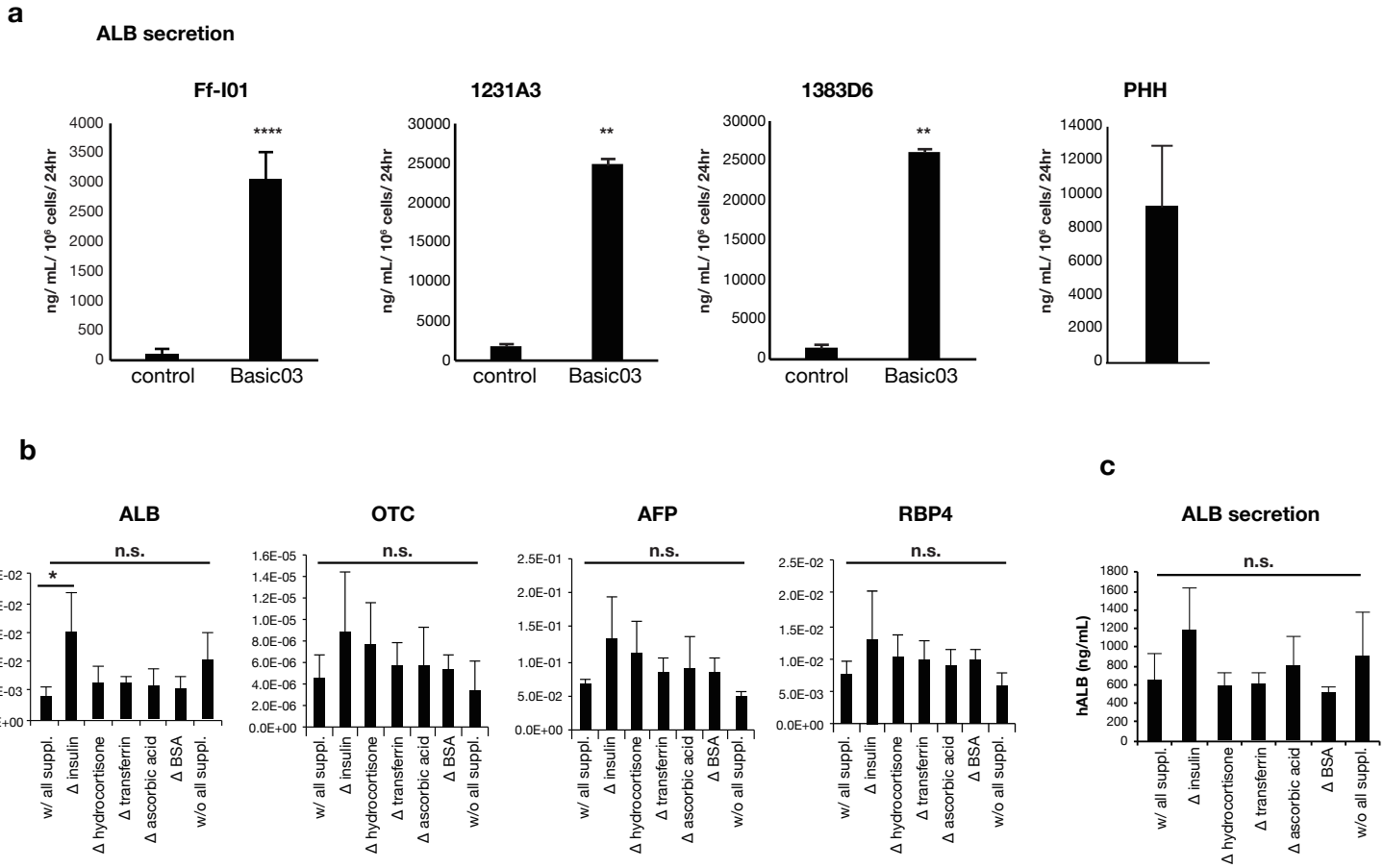
**Supplementary Figure Legends**

**Figure S1. Hepatic and liver bud function using defined medium.** (a) immature hepatocytes (IHs) derived with the chemically defined, animal origin-free (CD-AOF) or the conventional medium were evaluated at the MH stage in several iPSC lines. HCM (without EGF) was used to differentiate IHs to MHs in both conditions. IHs derived using the CD-AOF medium outperformed when evaluated at the MH stage even same media were used to differentiate the IHs to MHs. (b, c) Evaluation of supplements in Single Quotes kit in mature hepatocytes (MHs). The gene expression (c), and albumin secretion (c) were comparable between the MHs differentiated in Dulbecco's modified Eagle medium without any supplements and those differentiated in DMEM with supplements.

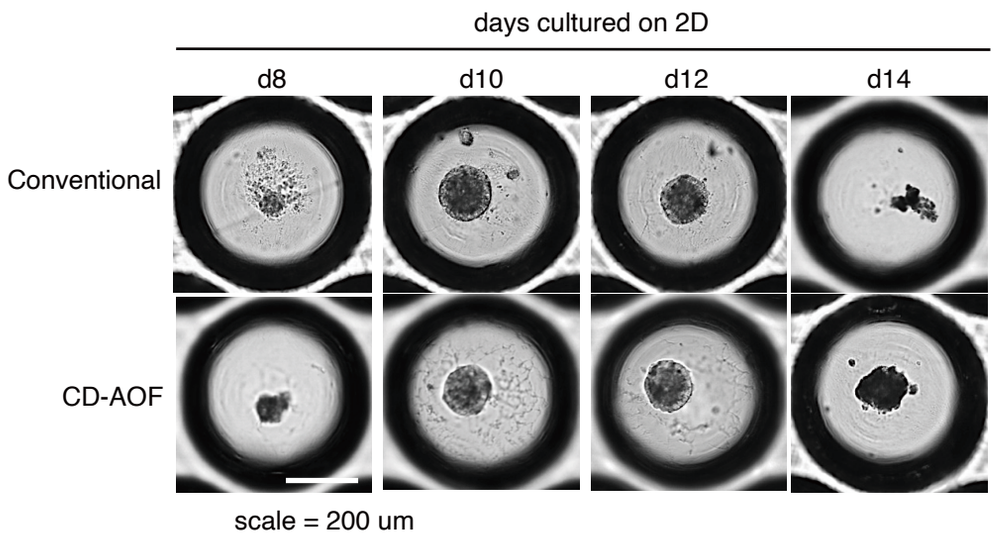
**Figure S2. Examination of the timing to generate LBs after the DE stage.** We examined the HE timing to generate LBs after DE. Four days after the DE stage (10 days from the iPSC stage) was selected for further evaluation.

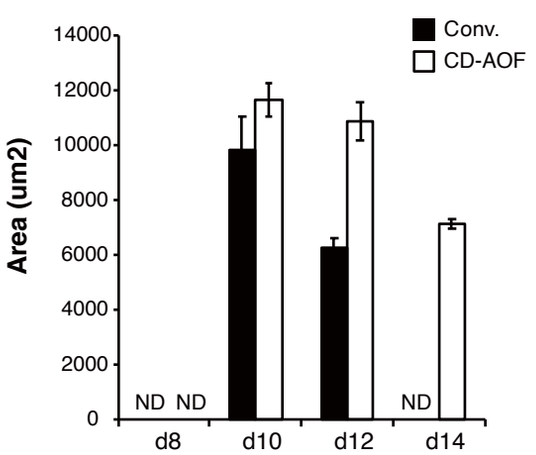
**Figure S3. Generation of neural crest cells using defined medium.** (a) Morphological representation of neural crest cells differentiated using medium supplemented with AS400 or B27. Scale bar, 200 μm. (b) Flow cytometric analysis of CD271 expression showing no significant difference between the two conditions.

Figure S1


a
ALB secretion
Ff-I01
1231A3
1383D6
PHH
ng/ mL/ 10⁶ cells/ 24hr
control
Basic03
****
**
b
ALB
OTC
AFP
RBP4
n.s.
*
w/ all suppl.
Δ insulin
Δ hydrocortisone
Δ transferrin
Δ ascorbic acid
Δ BSA
w/o all suppl.
c
ALB secretion
hALB (ng/mL)

# Figure S2


days cultured on 2D
d8
d10
d12
d14
Conventional
CD-AOF
scale = 200 um


Conv.
CD-AOF
Area (um2)
14000
12000
10000
8000
6000
4000
2000
0
ND ND
ND
d8
d10
d12
d14

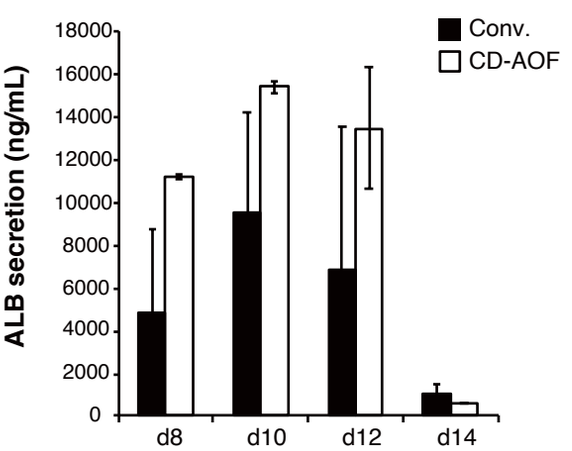

Conv.
CD-AOF
ALB secretion (ng/mL)
18000
16000
14000
12000
10000
8000
6000
4000
2000
0
d8
d10
d12
d14

Figure S3

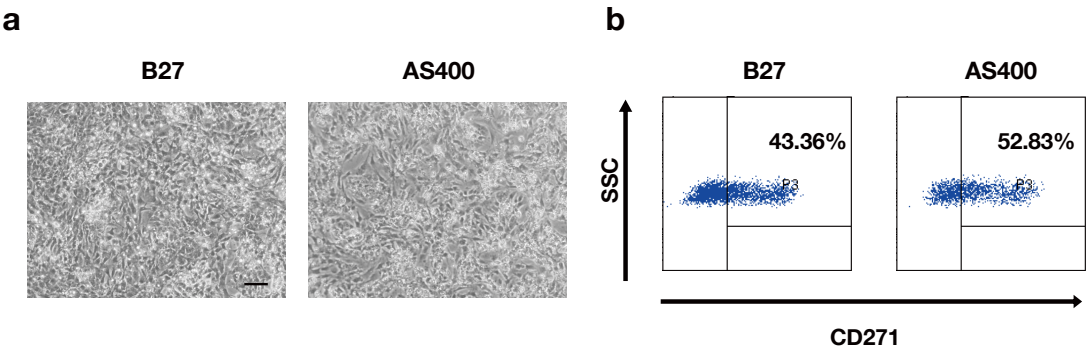


**Table S1. Number of residual undifferentiated induced pluripotent stem cells in differentiated cell populations**

| | Colony number (colonies /cm$^2$) | N |
|---|---|---|
| Conventional Media | 0 | 31 |
| CD-AOF media | 0 | 12 |

**Table S2.** Primers and probes used for qPCR in this study

| | Forward | Reverse | Probe No. |
|---|---|---|---|
| POU5F1 (OCT3/4) | CTTCGCAAGCCCTCATTTC | GAGAAGGCGAAATCCGAAG | 60 |
| CXCR4 | ATTGGGATCAGCATCGACTC | CAAACTCACACCCTTGCTTG | 79 |
| SOX17 | ACGCCGAGTTGAGCAAGA | TCTGCCTCCTCCACGAAG | 61 |
| HNF4A | TCAGACCCTGAGCCACCT | AGCAACGGACAGATGTGTGA | 27 |
| AFP | TCCTTGTAAGTGGCTTCTTGAAC | TGTACTGCAGAGATAAGTTTAGCTGAC | 61 |
| ALB | AATGTTGCCAAGCTGCTGA | CTTCCCTTCATCCCGAAGTT | 27 |
| OTC | CACCTTCAGGCAGCTACTCC | TGGTACCATTCTCTTTGGCATA | 53 |
| RBP4 | CCAGAAGCGCAGAAGATTG | TTTCTTTCTGATCTGCCATCG | 17 |
| ALCAM | CAGTTCCTGCCGTCTGCT | CTGAATTTACAGTATACCATCCAAGG | 34 |
| CD73 | GCACTATCTGGTTCACCGTGT | CCTTCCACACCATTATCAAATTC | 48 |
| PDGFRB | CATCTGCAAAACCACCATTG | GAGACGTTGATGGATGACACC | 10 |
| PECAM1 (CD31) | CAGAGAGACCGGCTGTGG | CATTGTTCCCGGTTTCCA | 46 |
| CDH5 (CD144) | CATGAGCCTCTGCATCTTCC | GCAGTCCAACGGAACAGAA | 30 |
| KDR | GAACATTTGGGAAATCTCTTGC | CGGAAGAACAATGTAGTCTTTGC | 18 |
| APOA1 | CCTTGGGAAAACAGCTAAACC | CCAGAACTCCTGGGTCACA | 39 |
| SCD | CCTAGAAGCTGAGAAACTGGTGA | ACATCATCAGCAAGCCAGGT | 82 |
| F7 | CCTTCTGCTTGGGCTTCA | GGCATGTCCCGTGTTTCT | 30 |
| ASGR1 | TGACCACCATCAGCTCAGAA | ACAGACAACCACAAGCAGCA | 8 |