Supplementary materials for

Human forebrain organoid-based multi-omics analyses of *PCCB* as a schizophrenia associated gene linked to GABAergic pathways

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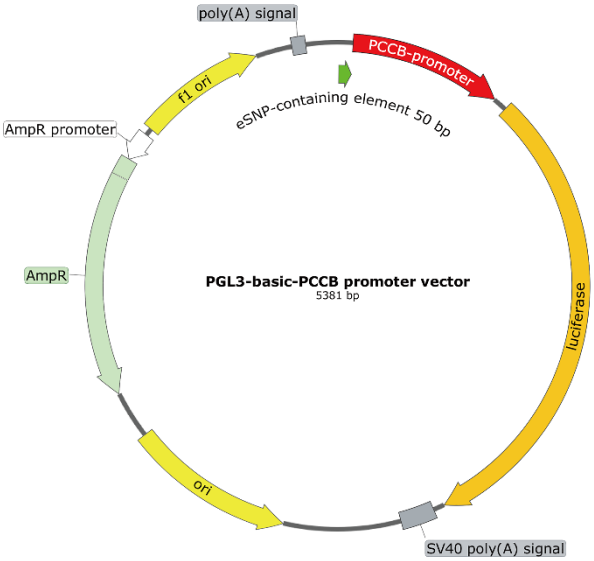
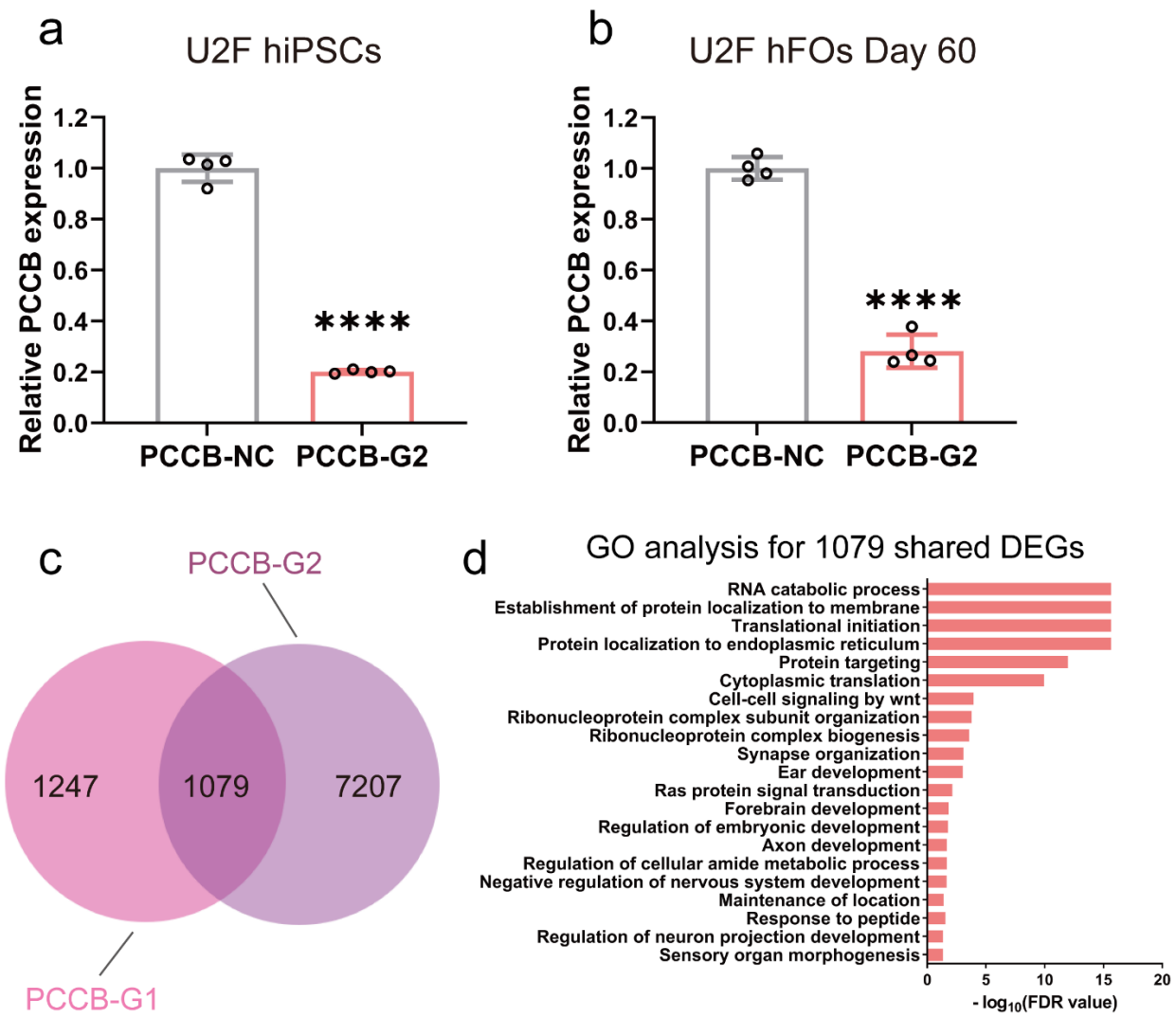
This supplementary file includes:

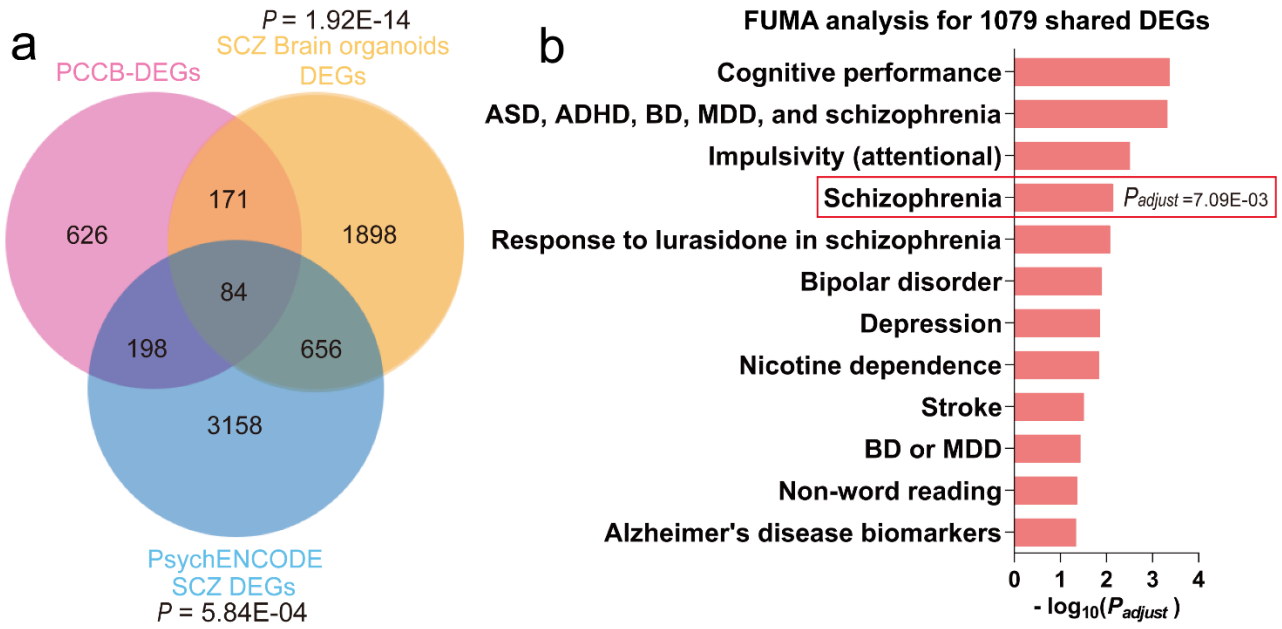


Supplementary Fig. S1. Scheme of luciferase reporter vector construction.

About 50 bp DNA sequence flanking the *PCCB* eSNP was synthesized and cloned into the pGL3-basic luciferase reporter vector. The *PCCB* promoter sequence (~600 bp) was cloned into downstream of the eSNP-containing DNA fragment.

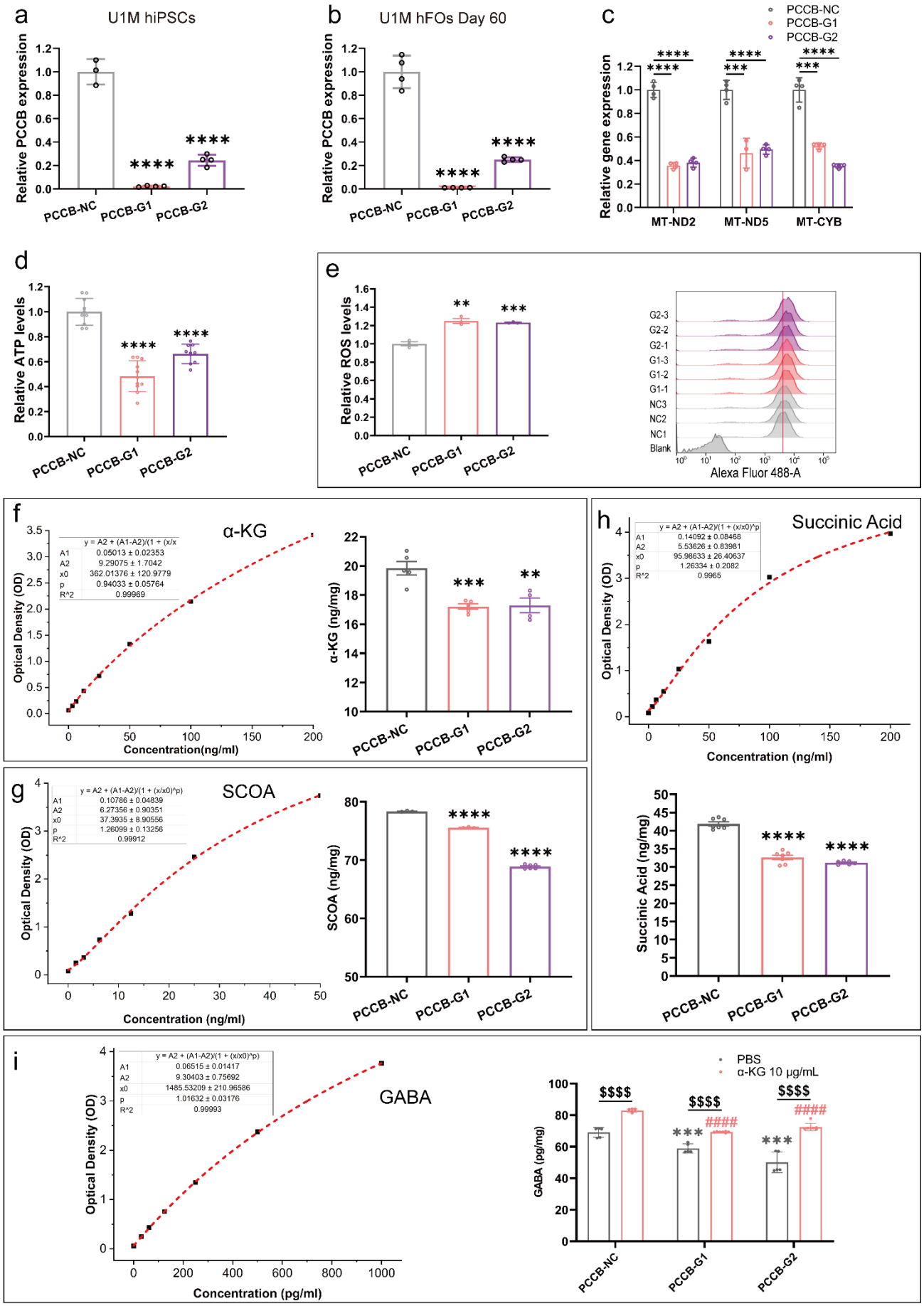


Supplementary Fig. S2. Effects of *PCCB* knockdown by *PCCB*-G2 gRNA in U2F hFOs. **a, b** RT-qPCR analysis for *PCCB* expression in U2F hiPSCs (**a**) and hFOs (**b**) after *PCCB* knockdown by *PCCB*-G2 gRNA. At least three technical replicates per group were used for the RT-qPCR analysis. Data are shown as Mean ± SD. Unpaired two-tailed t-test, ****$P < 0.0001$. **c** Overlap between DEGs identified in *PCCB*-G1 and *PCCB*-G2 hFOs. **d** GO enrichment analysis for 1079 shared DEGs between *PCCB*-G1 and *PCCB*-G2 hFOs. Source data underlying a-b are provided as a Source Data file.



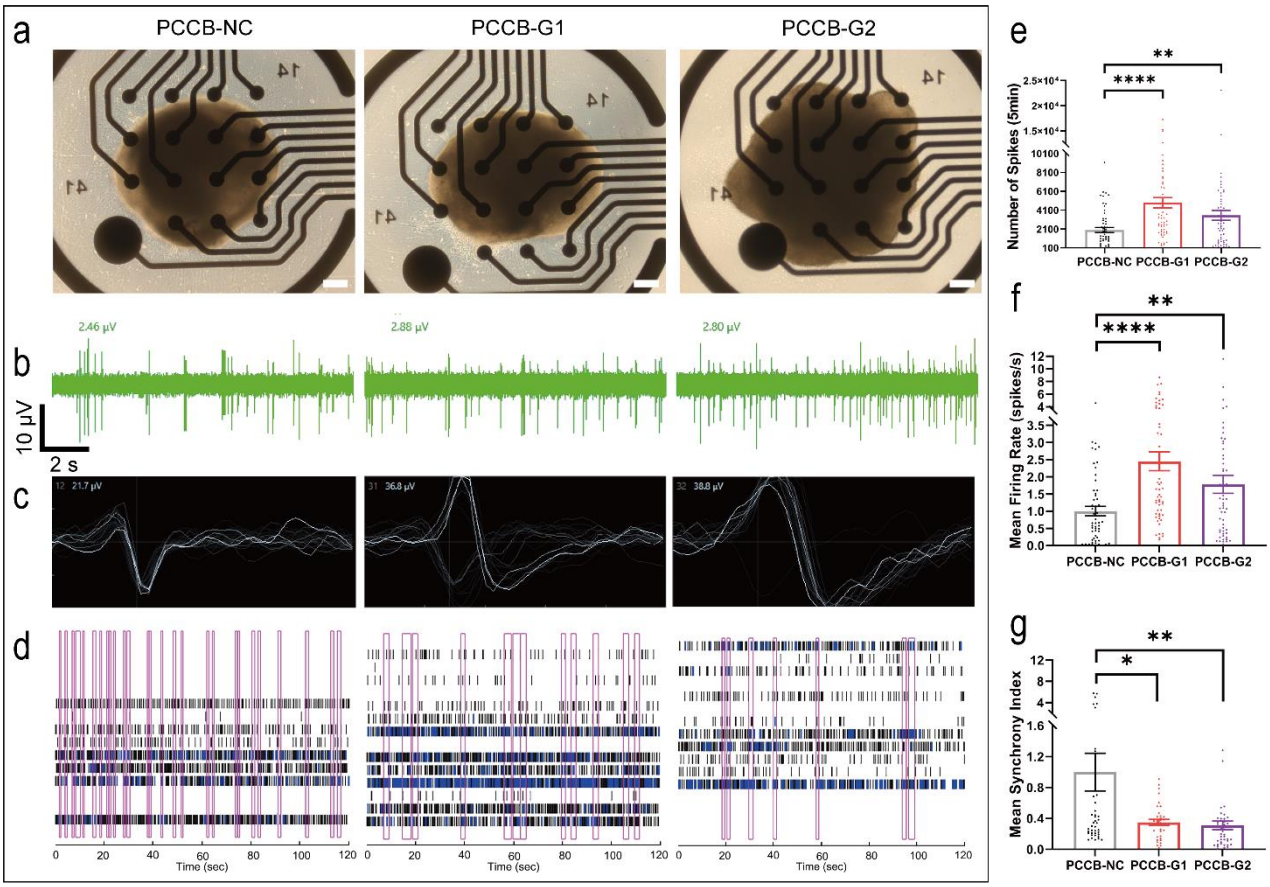
Supplementary Fig. S3. Enrichment of 1079 *PCCB* knockdown-induced DEGs with SCZ-related genes.

a 1079 *PCCB* knockdown-induced DEGs were significantly overlapped with genes dysregulated in PsychENCODE SCZ brains and SCZ patient-derived cerebral organoids. **b** 1079 *PCCB* knockdown-induced DEGs were significantly overlapped with genes reported in SCZ GWAS from the FUMA analysis. Only items related to brain diseases were displayed. *P* values are calculated by the hypergeometric test, a *P* value less than 0.05 is considered statistically significant.


a
U1M hiPSCs
Relative PCCB expression
PCCB-NC
PCCB-G1
PCCB-G2
b
U1M hFOs Day 60
c
PCCB-NC
PCCB-G1
PCCB-G2
Relative gene expression
MT-ND2
MT-ND5
MT-CYB
d
Relative ATP levels
e
Relative ROS levels
G2-3
G2-2
G2-1
G1-3
G1-2
G1-1
NC3
NC2
NC1
Blank
Alexa Fluor 488-A
f
α-KG
Optical Density (OD)
Concentration(ng/ml)
α-KG (ng/mg)
h
Succinic Acid
Concentration (ng/ml)
Succinic Acid (ng/mg)
g
SCOA
Concentration (ng/ml)
SCOA (ng/mg)
i
GABA
Concentration (pg/ml)
PBS
α-KG 10 μg/mL
GABA (pg/mg)

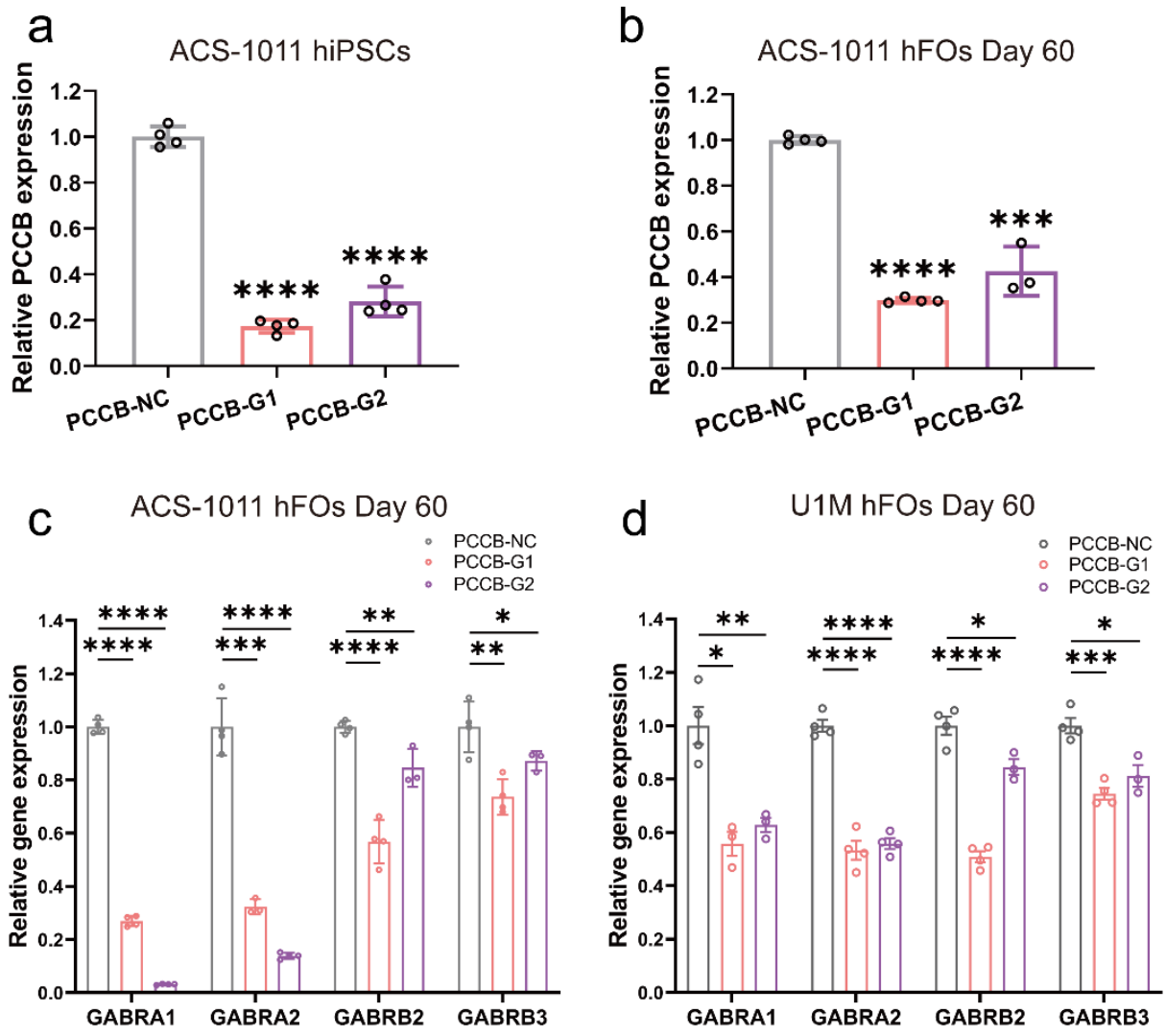
Supplementary Fig. S4. *PCCB* knockdown leads to mitochondrial dysfunction and decreased GABA levels in U1M hFOs.

a, **b** RT-qPCR analysis for *PCCB* expression in U1M hiPSCs (**a**) and hFOs (**b**) after *PCCB* knockdown by *PCCB*-G1 and *PCCB*-G2 gRNA. **c** RT-qPCR analysis for mitochondrial genes (*MT-ND2*, *MT-ND5*, and *MT-CYB*) in U1M hFOs after *PCCB* knockdown. At least three technical replicates per group were used for the RT-qPCR analysis. Data are shown as Mean ± SD. **d, e** Decreased ATP synthesis (**d**) and increased ROS content (**e**) were observed in *PCCB* knockdown hFOs. **f-h** ELISA analysis confirmed the reduction of α-KG (**f**), SOCA (**g**), and succinic acid (**h**) in *PCCB* knockdown hFOs. **i** Adding α-KG (10 μg/ml) into the culture medium of *PCCB* knockdown hFOs restored the GABA levels. At least three biological replicates were used in each group. Data are shown as Mean ± SEM. Unpaired two tailed t-test, $^{**}P < 0.01$, $^{***}P < 0.001$, $^{****}P < 0.0001$, $^{\#\#\#\#}P < 0.0001$, $^{\$\$\$\$}P < 0.0001$. Source data underlying a-i are provided as a Source Data file.



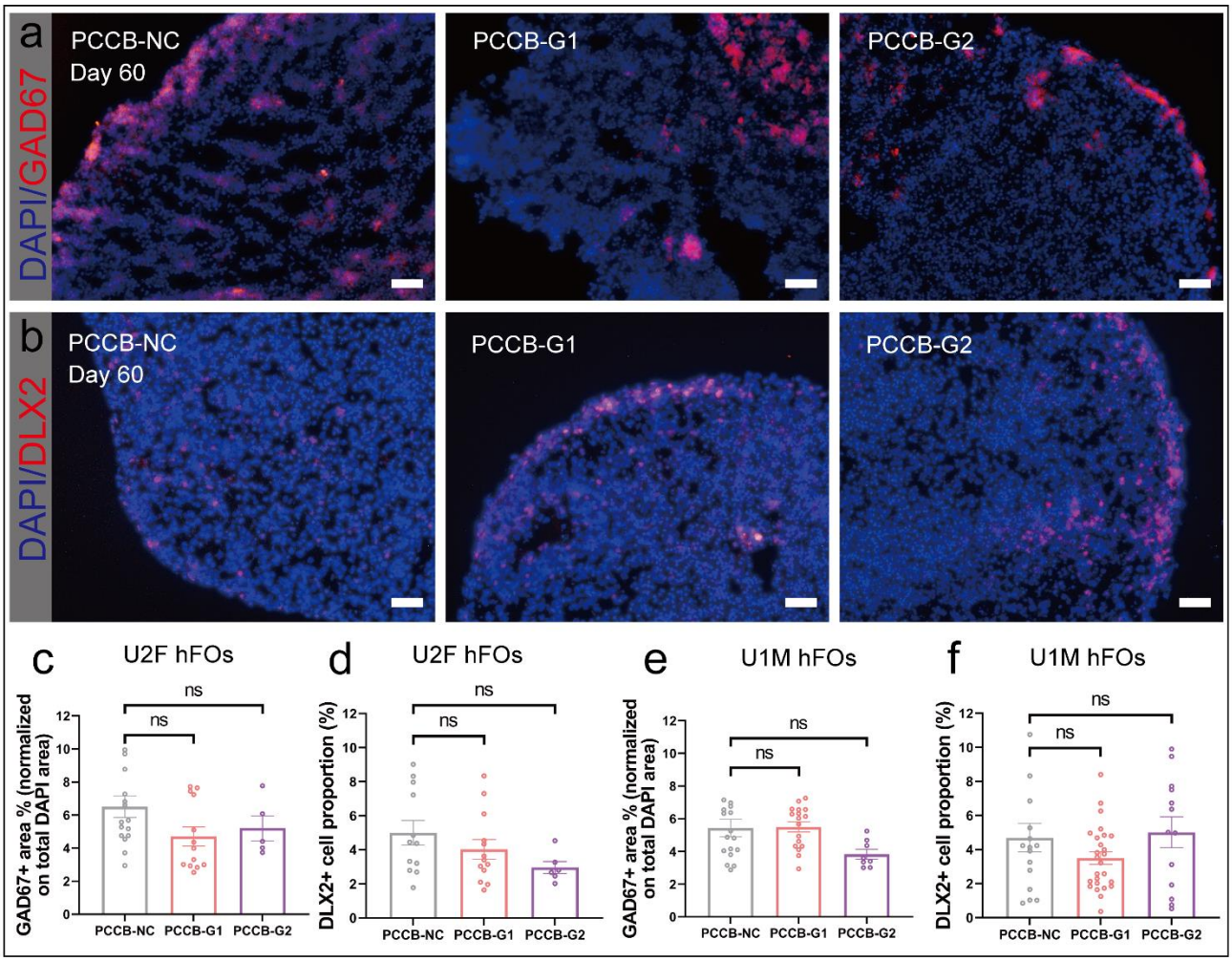
Supplementary Fig. S5. MEA assay after *PCCB* knockdown in U1M hFOs.

a Bright field of U1M hFOs cultured in 24-well MEA plate. Scale bar, 100 μm. **b** Representative burst traces for individual electrode recorded in U1M hFOs. **c** Schematic diagram of a single unit event. **d** Raster plot of synchronized burst activity. Each pink box represents a synchronized burst. **e-g** *PCCB* knockdown led to increased number of spikes (**e**) and mean neuron firing rate (**f**) but reduced synchronized burst index in U1M hFOs (**g**). Data are shown as Mean ± SEM (averaged 8 biological replicates in each group). The unpaired two-tailed t-test was used to assess difference between the *PCCB*-NC and *PCCB*-G1 or *PCCB*-G2 group. **P* < 0.05, ***P* < 0.01, *****P* < 0.0001. Source data underlying e-g are provided as a Source Data file.



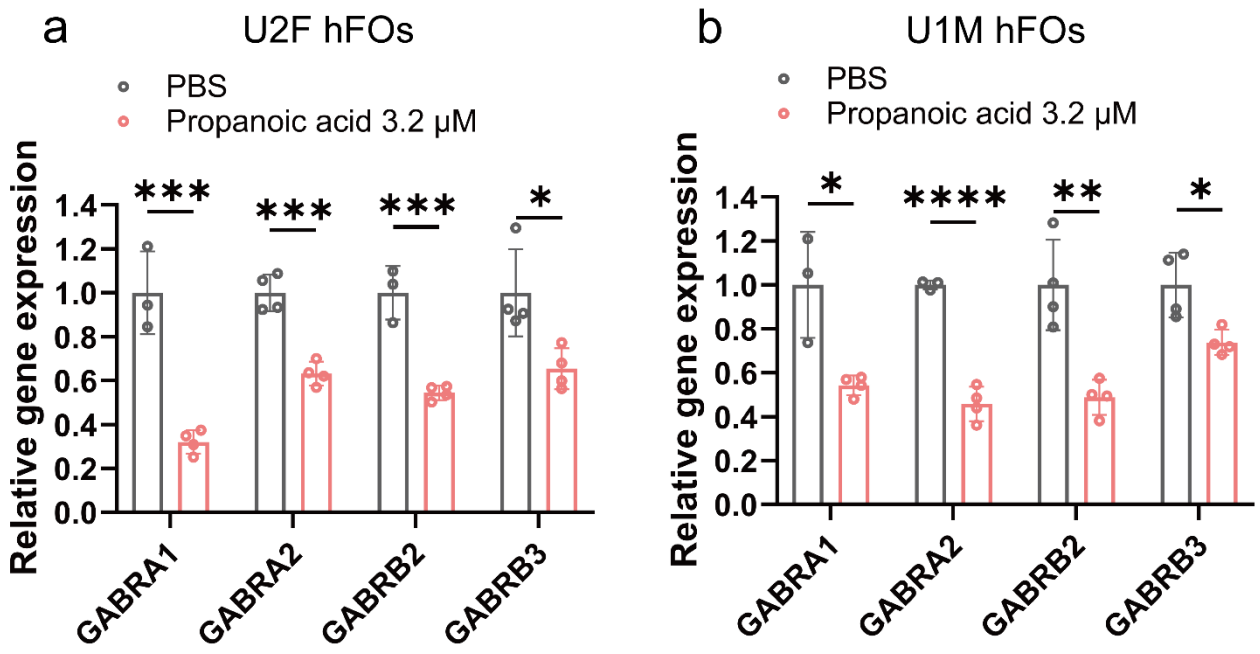
Supplementary Fig. S6. Expression of GABA receptor genes in ACS-1011 and U1M hFOs.

a, **b** RT-qPCR analysis for *PCCB* expression in ACS-1011 hiPSCs (**a**) and hFOs (**b**) after *PCCB* knockdown by *PCCB*-G1 and *PCCB*-G2 gRNA. **c, d** RT-qPCR analysis for GABA receptor genes (*GABRA1*, *GABRA2*, *GABRB2*, and *GABRB3*) in *PCCB* knockdown ACS-1011 hFOs (**c**) and U1M hFOs (**d**). RT-qPCR analysis was conducted in at least three technical replicates. Data are shown as Mean ± SD. The unpaired two-tailed t-test was used to assess difference between the *PCCB*-NC and *PCCB*-G1 or *PCCB*-G2 group. **P* < 0.05, ***P* < 0.01, ****P* < 0.001, *****P* < 0.0001. Source data underlying a-d are provided as a Source Data file.



Supplementary Fig. S7. Immunostaining assay confirmed the existence of GABAergic neurons in hFOs.

a, b, Immunostaining assay was used to detect cells expressing the GABAergic neuron markers GAD67 (**a**) and DLX2 (**b**) in hFOs (day 60). Antibodies are Mouse-anti-GAD67 (Abcam, ab26116) and Mouse-anti-DLX2 (Santa Cruz, sc-393879). Scale bar, 50 μm. **c-f** Proportions of GAD67+ areas and DLX2+ cells in U2F (**c-d**) or U1M (**e-f**) hFOs were blindly quantified from 4 organoids (at least 5 organoid sections) in each group. Data are shown as Mean ± SEM. The unpaired two-tailed t-test was used to assess difference between the *PCCB*-NC and *PCCB*-G1 or *PCCB*-G2 group, ns, non-significance. Source data underlying c-f are provided as a Source Data file.



Supplementary Fig. S8. Effects of propanoic acid exposure on expression of GABA receptor genes in U1M and U2F hFOs.

a, **b** U1M and U2F hFOs were exposed to propanoic acid (3.2 μM) or PBS for 14 days. RT-qPCR was then used to detect expression of GABA receptor genes (*GABRA1*, *GABRA2*, *GABRB2*, and *GABRB3*) in U2F hFOs (**a**) and U1M hFOs (**b**). At least three technical replicates were used for the RT-qPCR analysis. Data are shown as Mean ± SD. Unpaired two tailed t-test, **P* < 0.05, ***P* < 0.01, ****P* < 0.001, *****P* < 0.0001. Source data underlying a-b are provided as a Source Data file.