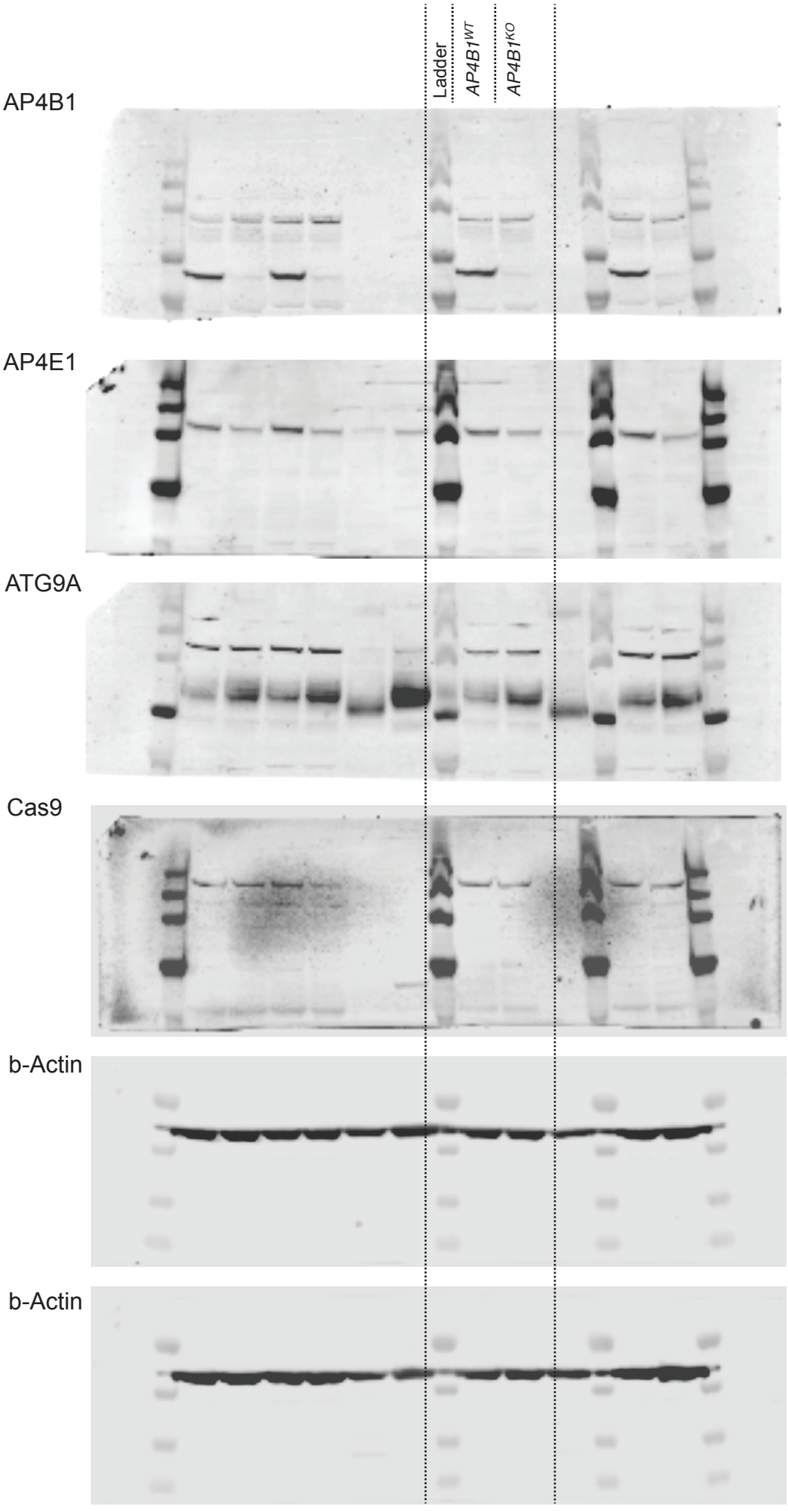
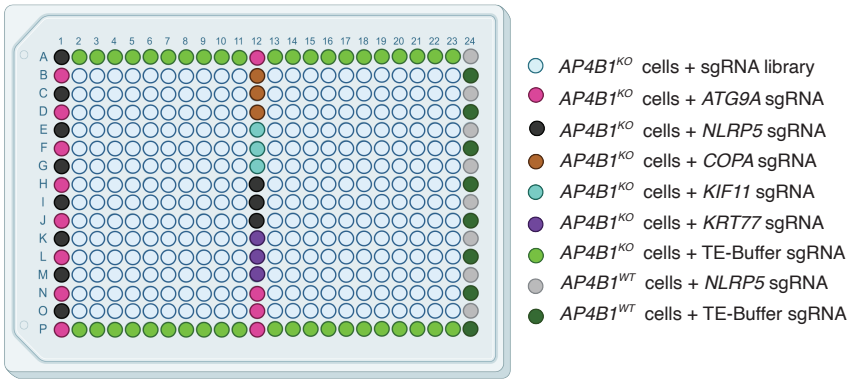
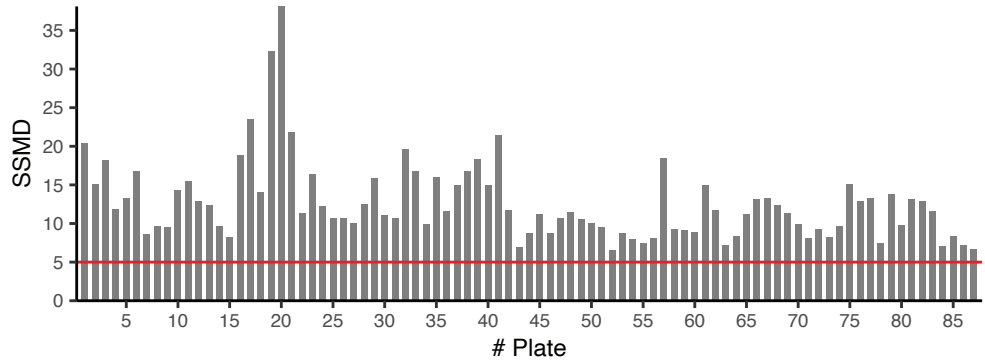

Ladder
AP4B1WT
AP4B1KO
AP4B1
AP4E1
ATG9A
Cas9
b-Actin
b-Actin

Supplementary Figure 1: Uncut Western Blots (*Figure 1B)*

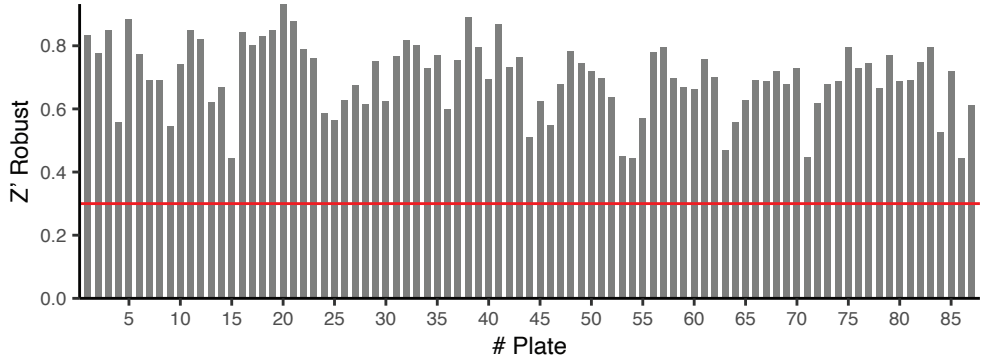
A


1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24
A
B
C
D
E
F
G
H
I
J
K
L
M
N
O
P
AP4B1KO cells + sgRNA library
AP4B1KO cells + ATG9A sgRNA
AP4B1KO cells + NLRP5 sgRNA
AP4B1KO cells + COPA sgRNA
AP4B1KO cells + KIF11 sgRNA
AP4B1KO cells + KRT77 sgRNA
AP4B1KO cells + TE-Buffer sgRNA
AP4B1WT cells + NLRP5 sgRNA
AP4B1WT cells + TE-Buffer sgRNA

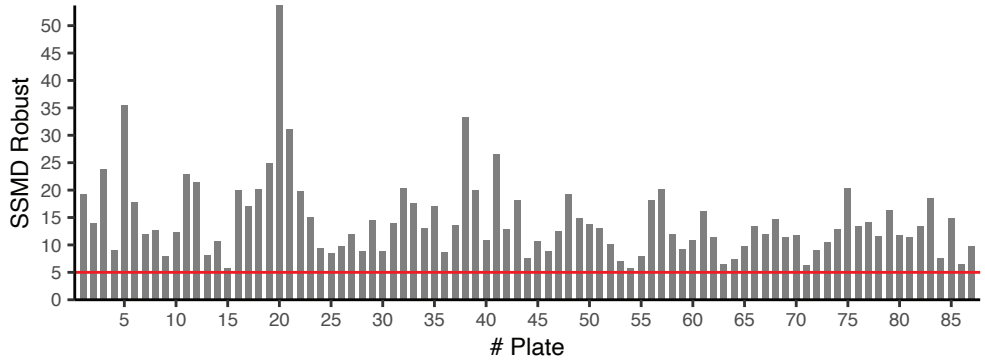
B


SSMD
0
5
10
15
20
25
30
35
5
10
15
20
25
30
35
40
45
50
55
60
65
70
75
80
85
Plate

C


Z' Robust
0.0
0.2
0.4
0.6
0.8
5
10
15
20
25
30
35
40
45
50
55
60
65
70
75
80
85
Plate

D


SSMD Robust
0
5
10
15
20
25
30
35
40
45
50
5
10
15
20
25
30
35
40
45
50
55
60
65
70
75
80
85
Plate

Supplementary Figure 2: Primary Screen Plate Design and Quality Control Metrics

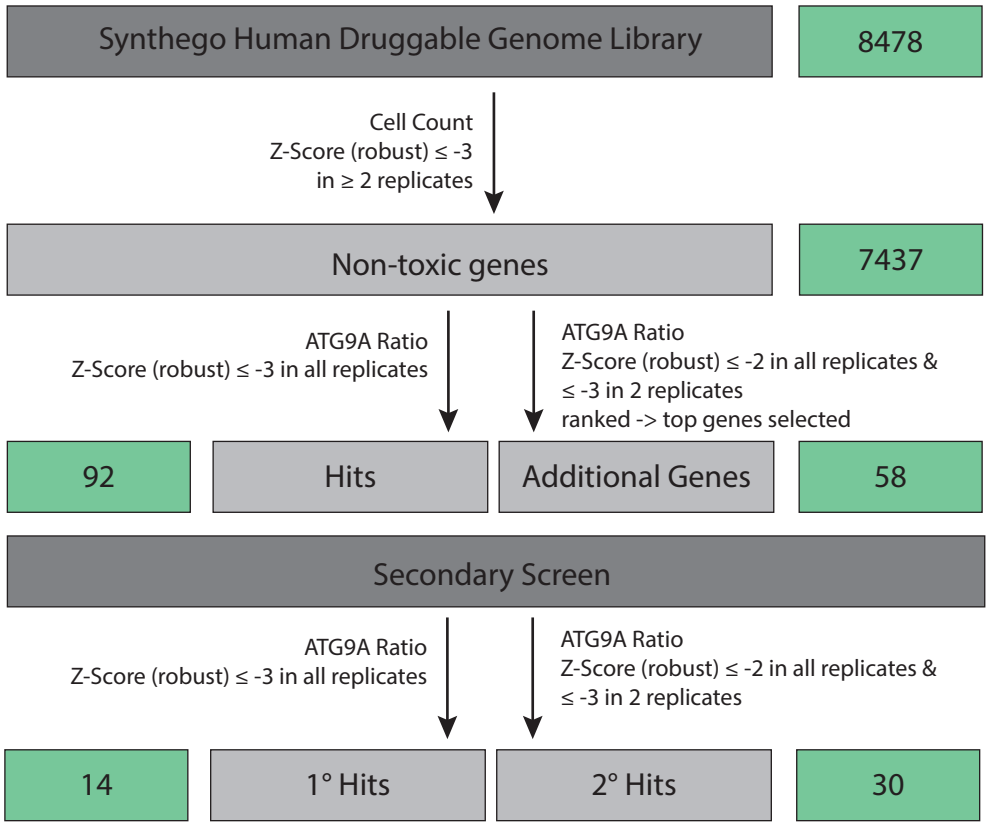
A) Plate layout for primary screen. Positive controls included *AP4B1*^{KO} cells transfected with sgRNAs targeting *ATG9A*, and *AP4B1*^{WT} cells treated with TE-buffer or transfected with sgRNAs targeting the non-essential gene *NLRP5*. Negative controls comprised *AP4B1*^{KO} cells treated with TE-buffer or transfected with sgRNAs targeting non-essential genes *NLRP5* or *KRT77*. To assess transfection and knockout efficiency, cell death-inducing sgRNAs targeting essential genes *COPA* and *KIF11* were used. This figure was partially created in BioRender. Kim, H. (2026) https://BioRender.com/hiargyc.

B) Strictly standardized mean difference (SSMD) for all 87 screened plates demonstrated robust assay performance. Horizontal line indicates predefined quality control threshold (SSMD > 5).

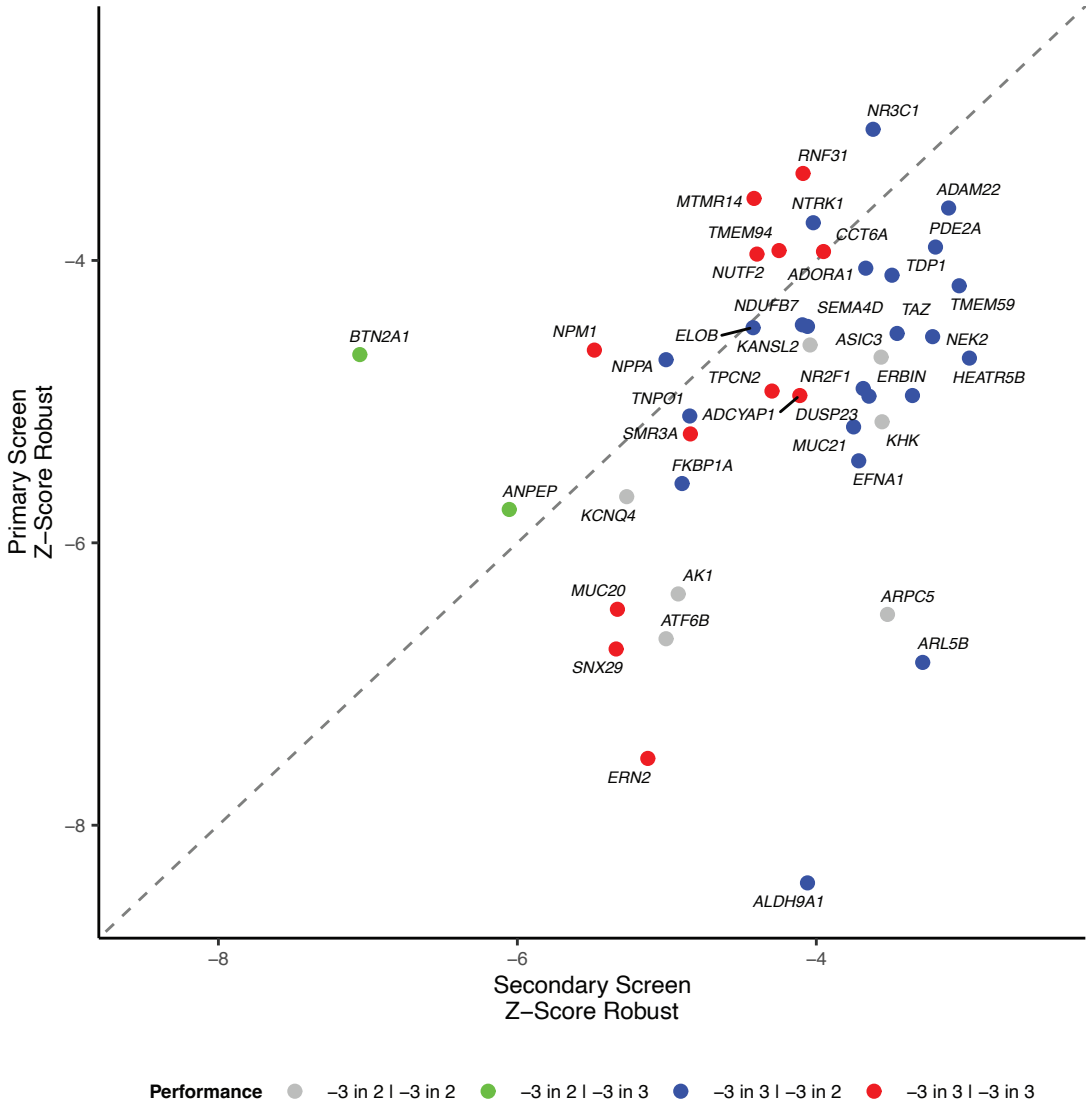
C) Z' robust for all 87 screened plates. Horizontal line indicates predefined quality control threshold (Z' > 0.3).

D) SSMD robust for all 87 screened plates. Horizontal line represents predefined quality control threshold (SSMD robust > 5).

A


Synthego Human Druggable Genome Library
8478
Cell Count
Z-Score (robust) ≤ -3
in ≥ 2 replicates
Non-toxic genes
7437
ATG9A Ratio
Z-Score (robust) ≤ -3 in all replicates
ATG9A Ratio
Z-Score (robust) ≤ -2 in all replicates &
≤ -3 in 2 replicates
ranked -> top genes selected
92
Hits
Additional Genes
58
Secondary Screen
ATG9A Ratio
Z-Score (robust) ≤ -3 in all replicates
ATG9A Ratio
Z-Score (robust) ≤ -2 in all replicates &
≤ -3 in 2 replicates
14
1° Hits
2° Hits
30

B

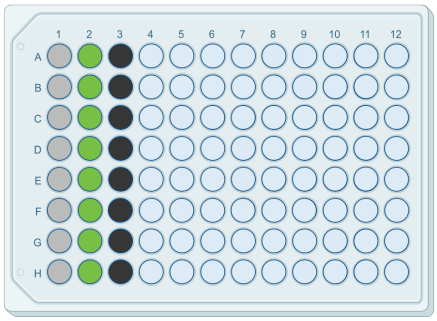

Primary Screen
Z–Score Robust
Secondary Screen
Z–Score Robust
−4
−6
−8
NR3C1
RNF31
ADAM22
MTMR14
NTRK1
PDE2A
TMEM94
CCT6A
TDP1
NUTF2
ADORA1
TMEM59
NDUFB7
SEMA4D
TAZ
BTN2A1
NPM1
ELOB
KANSL2
ASIC3
NEK2
NPPA
TPCN2
NR2F1
ERBIN
HEATR5B
TNPO1
ADCYAP1
DUSP23
SMR3A
KHK
MUC21
FKBP1A
EFNA1
ANPEP
KCNQ4
AK1
MUC20
ATF6B
ARPC5
ARL5B
SNX29
ERN2
ALDH9A1
Performance
–3 in 2 I –3 in 2
–3 in 2 I –3 in 3
–3 in 3 I –3 in 2
–3 in 3 I –3 in 3

Supplementary Figure 3: Overview of Primary and Secondary Screens and Comparison of Hit Genes

A) Schematic overview of hit identification strategy for the primary and secondary screen. The 'Synthego Human Druggable Genome' sgRNA library targeted 8,478 genes. Toxic sgRNAs were excluded based on reduction of cell count by at least 3 standard deviations in two or more replicates, resulting in 7,437 non-toxic genes. Non-toxic genes were ranked according to ATG9A ratio reduction. A total of 92 genes reduced ATG9A ratio with a z-score robust < -3 in all replicates and were classified as hits. For secondary screening, additional 58 genes were selected from the top of the list of genes with a z-score robust < -3 in two replicates and < -2 in the third replicate, ranked by their mean ATG9A ratio across all replicates.

B) Comparison of confirmed hits from the secondary screen with primary screen results. Data are presented as mean z-scores robust across all replicates in the respective screen. Colors indicate if z-score robust of < -3 was achieved in only two replicates or across all three replicates.

A



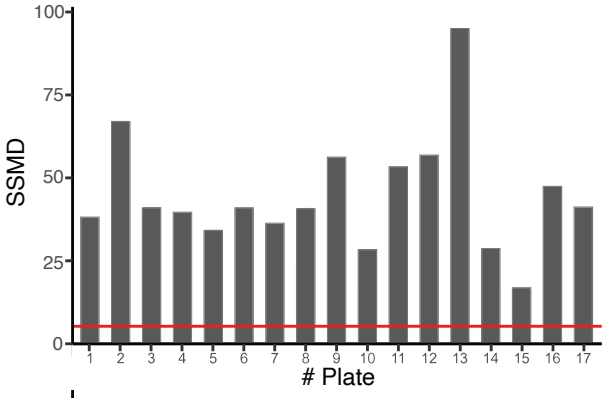
AP4B1^{WT} cells control

AP4B1^{KO} cells control

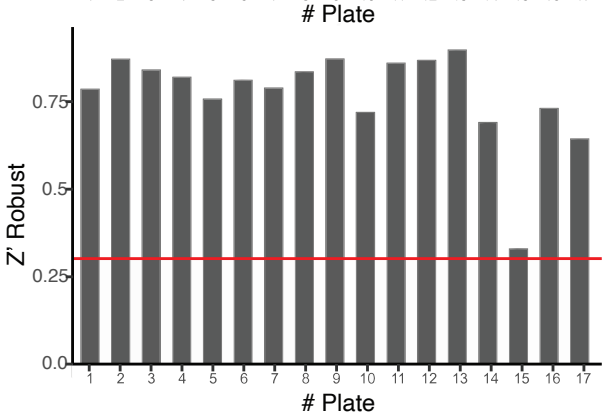
AP4B1^{KO} cells + *NLRP5* sgRNA

AP4B1^{KO} cells + assay sgRNA

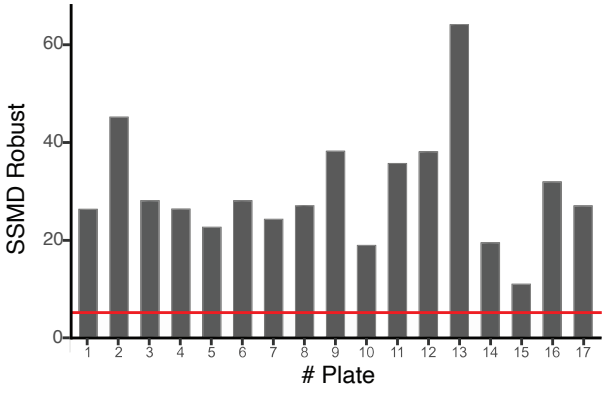
B



C



D



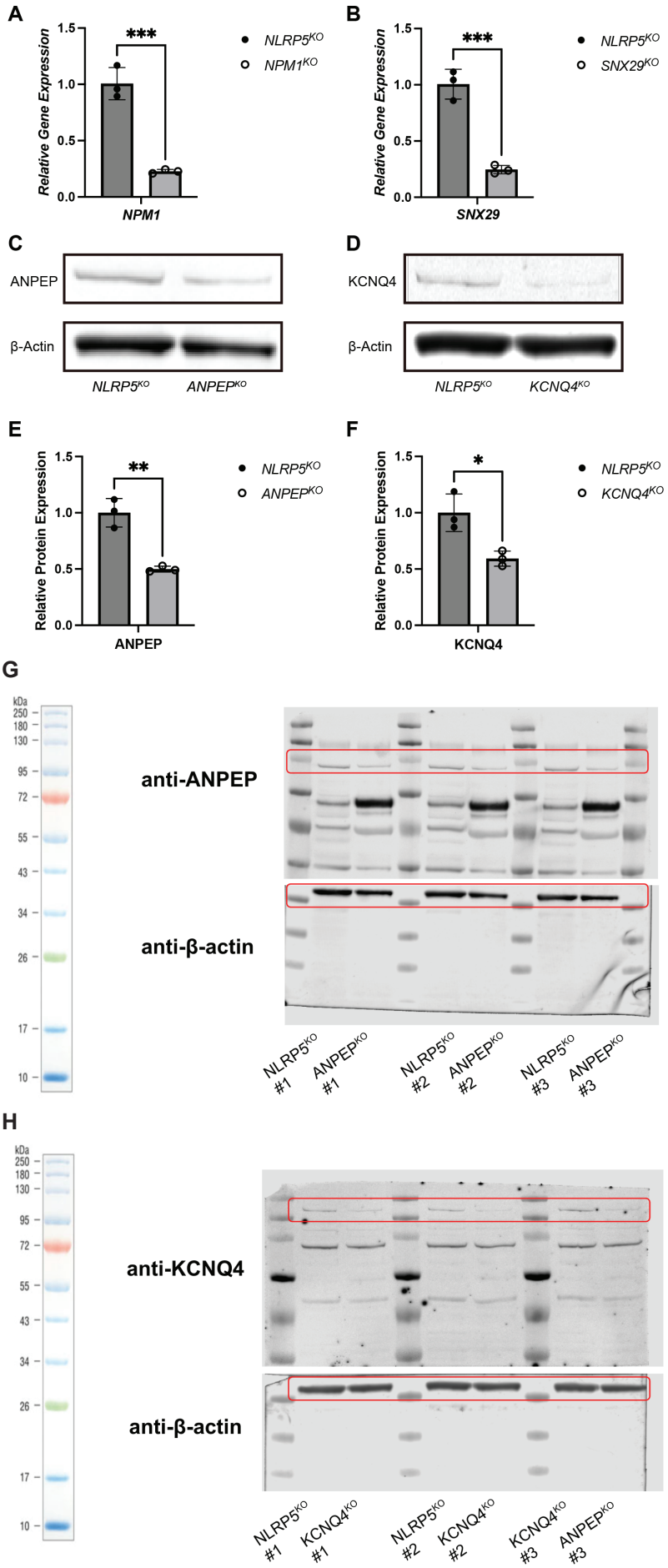
Supplementary Figure 4: Validation Experiments Plate Design and Quality Control Metrics

A) Plate layout for validation experiments. Untransfected *AP4B1*^{WT} cells served as positive control. Negative controls consisted of *AP4B1*^{KO} cells untransfected or with transfection of sgRNAs targeting the non-essential gene *NLRP5*. This figure was partially created in BioRender. Kim, H. (2026) https://BioRender.com/7ig5l8j.

B) Strictly standardized mean difference (SSMD) for all 17 plates of the validation experiments. Horizontal line indicates the predefined quality control threshold (SSMD > 5).

C) Z'-factor robust for all 17 plates of the validation experiments. The horizontal line indicates the predefined quality control threshold (Z' > 0.3).

D) SSMD robust for all 17 plates of the validation experiments. Horizontal line represents predefined quality control threshold (SSMD robust > 5).


A
Relative Gene Expression
1.5
1.0
0.5
0.0
***
NPM1
NLRP5KO
NPM1KO
B
Relative Gene Expression
1.5
1.0
0.5
0.0
***
SNX29
NLRP5KO
SNX29KO
C
ANPEP
β-Actin
NLRP5KO
ANPEPKO
D
KCNQ4
β-Actin
NLRP5KO
KCNQ4KO
E
Relative Protein Expression
1.5
1.0
0.5
0.0
**
ANPEP
NLRP5KO
ANPEPKO
F
Relative Protein Expression
1.5
1.0
0.5
0.0
*
KCNQ4
NLRP5KO
KCNQ4KO
G
kDa
250
180
130
95
72
55
43
34
26
17
10
anti-ANPEP
anti-β-actin
NLRP5KO #1
ANPEPKO #1
NLRP5KO #2
ANPEPKO #2
NLRP5KO #3
ANPEPKO #3
H
kDa
250
180
130
95
72
55
43
34
26
17
10
anti-KCNQ4
anti-β-actin
NLRP5KO #1
KCNQ4KO #1
NLRP5KO #2
KCNQ4KO #2
KCNQ4KO #3
ANPEPKO #3

Supplementary Figure 5: Validation of knockout efficiency for selected hit genes.

(A–B) Quantitative RT–PCR analysis demonstrating efficient depletion of (A) NPM1 and (B) SNX29 transcripts following gene knockout. Expression levels were normalized to RPL32 and are shown relative to control (NLRP5 knockout).

(C–D) Representative Western blot analyses showing reduced protein levels of (C) ANPEP and (D) KCNQ4 in corresponding knockout conditions compared to NLRP5 control, with β-actin as loading control.

(E–F) Densitometric quantification of (E) ANPEP and (F) KCNQ4 protein levels from Western blots in (C–D), normalized to β-actin and expressed relative to control.

(G–H) Full-length Western blots for (G) ANPEP (G) and (H) KCNQ4, including replicate samples, confirming specificity and consistency of protein depletion.

All data represent three independent biological replicates. Statistical significance is indicated as $p < 0.05$ (), $p < 0.01$ (), and $p < 0.001$ ().