cells

MDPI

Supplementary data

Investigation and Distinction of Energy Metabolism in Proliferating Hepatocytes and Hepatocellular Carcinoma Cells

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Table S1. Gene specific primers purchased from Qiagen (Hilden, Germany) for RT-qPCR analyses.

Article	Gene name	Article number
Hs_BDH1_1_SG QuantiTect® Primer Assay	*BDH1*	QT01673525
Hs_CDH1_1_SG QuantiTect Primer Assay	*E-Cadherin*	QT00080143
Hs_CYP3A4_1_SG QuantiTect Primer Assay	*CYP3A4*	QT00067396
Hs_FOXO1_1_SG QuantiTect® Primer Assay	*FOXO1*	QT00044247
Hs_GAPDH_vb.1_SG QuantiTect® Primer Assay	*GAPDH*	QT02504278
Hs_GCK_1_SG QuantiTect Primer Assay	*GCK/HK4*	QT00000812
Hs_GSK3A_1_SG QuantiTect® Primer Assay	*GSK3A*	QT00075306
Hs_GSK3B_1_SG QuantiTect® Primer Assay	*GSK3B*	QT00057134
Hs_GUSB_1_SG QuantiTect® Primer Assay	*GUSB*	QT00046046
Hs_HIF1A_1_SG QuantiTect® Primer Assay	*HIF1A*	QT00083664
Hs_HK1_1_SG QuantiTect Primer Assay	*HK1*	QT00045514
Hs_HK2_1_SG QuantiTect Primer Assay	*HK2*	QT00013209
Hs_HMGCL_1_SG QuantiTect® Primer Assay	*HMGCL*	QT00088921
Hs_HNF4A_1_SG QuantiTect Primer Assay	*HNF4A*	QT00019411
Hs_LDHA_1_SG QuantiTect® Primer Assay	*LDHA*	QT00001687
Hs_MYC_1_SG QuantiTect Primer Assay	*c-MYC*	QT00035406
Hs_PKLR_1_SG QuantiTect Primer Assay	*PKL*	QT00016156
Hs_PKM_1_SG QuantiTect Primer Assay	*PKM*	QT00028875
Hs_RRN18S_1_SG QuantiTect Primer Assay	*RRN18S*	QT00199367
Hs_SLC2A1_1_SG QuantiTect Primer Assay	*GLUT1*	QT00068957
Hs_SLC2A2_2_SG QuantiTect Primer Assay	*GLUT2*	QT01008399

Primers were solved in 1.1 ml TE Buffer.

Table S2. Primary antibodies used for Western blot analyses.

Target	article	company	Article number	Protein concentration [µg]	Positive control	Gel concentration [%]
BDH1	BDH1 Monoclonal Antibody (1A5)	Thermo Fisher Scientific, Waltham, US	MA5-15594	20	kidney (mouse)	15
c-MYC	c-Myc (D84C12) Rabbit mAb	Cell Signaling Technology, Masachusetts, US	5605S	25	Brain (mouse)	12
FOXO1	Fox01 (**L**27) Rabbit Ab	Cell Signaling Technology, Masachusetts, US	9454S	30	heart (mouse)	12
GCK	Recombinant Anti-Glucokinase antibody [EPR10374]	Abcam, Cambridge, GB	ab155962	10	PHH (human)	12
GLUT1	Glut1 (D3J3A) Rabbit mAb	Cell Signaling Technology, Masachusetts, US	12939S	15	Gallbladder (human)	12
GLUT2	Recombinant Anti-Glucose Transporter GLUT2 antibody [EPR16550]	Abcam, Cambridge, GB	ab192599	15	Gallbladder (human)	12
GSK3A	GSK-3α (D80E6) Rabbit mAb #4337	Cell Signaling Technology, Masachusetts, US	4337S	15	Brain (mouse)	15
GSK3B	GSK-3β (3D10) Mouse mAb #9832	Cell Signaling Technology, Masachusetts, US	9832S	15	Brain (mouse)	15
HIF1A	HIF-1α (D2**U**3T) Rabbit mAb	Cell Signaling Technology, Masachusetts, US	14179S	25	HepG2 in hypoxie	8
HK1	Hexokinase I (C35C4) Rabbit mAb	Cell Signaling Technology, Masachusetts, US	2024S	20	lung (mouse)	12
HK2	Hexokinase II (C64G5) Rabbit mAb	Cell Signaling Technology, Masachusetts, US	2867S	30	HepG2	12
HMGCL	HMGCL Polyclonal Antibody	Thermo Fisher Scientific, Waltham, US	PA5-21996	30	Lung (mouse)	15
LDHA	LDHA (C4B5) Rabbit mAb #3582	Cell Signaling Technology, Masachusetts, US	3582S	25	THP-1	15
PKL	Recombinant Anti-PKLR antibody [EPR11093]	Abcam, Cambridge, GB	ab171744	10	PHH (human)	12
PKM	PKM1/2 (C103A3) Rabbit mAb	Cell Signaling Technology, Masachusetts, US	3190S	15	Gallbladder (human)	15

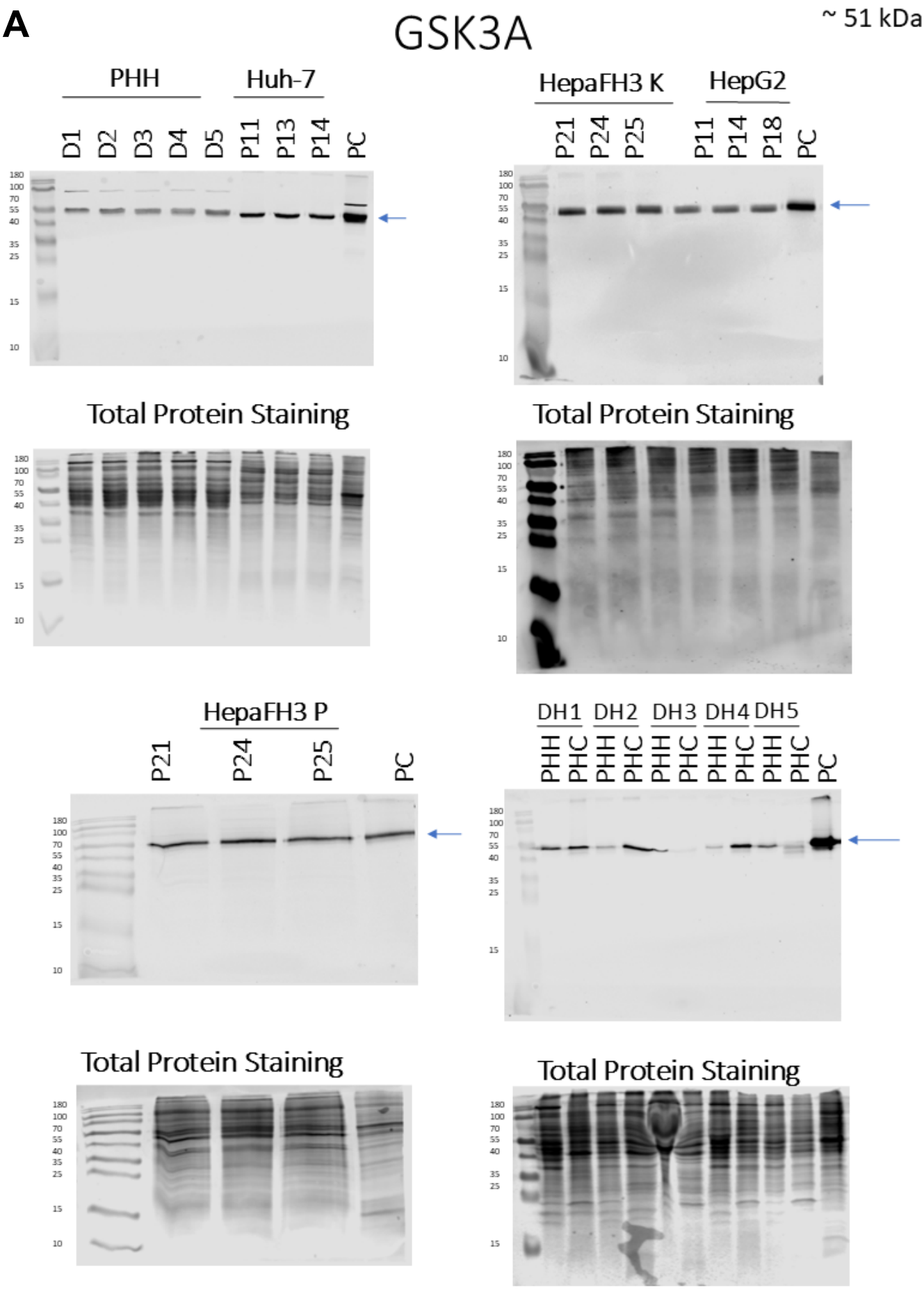
All antibodies were diluted 1:1,000 in Intercept Blocking Buffer with 0.1 % TWEEN except from HMGCL 1:3,000.

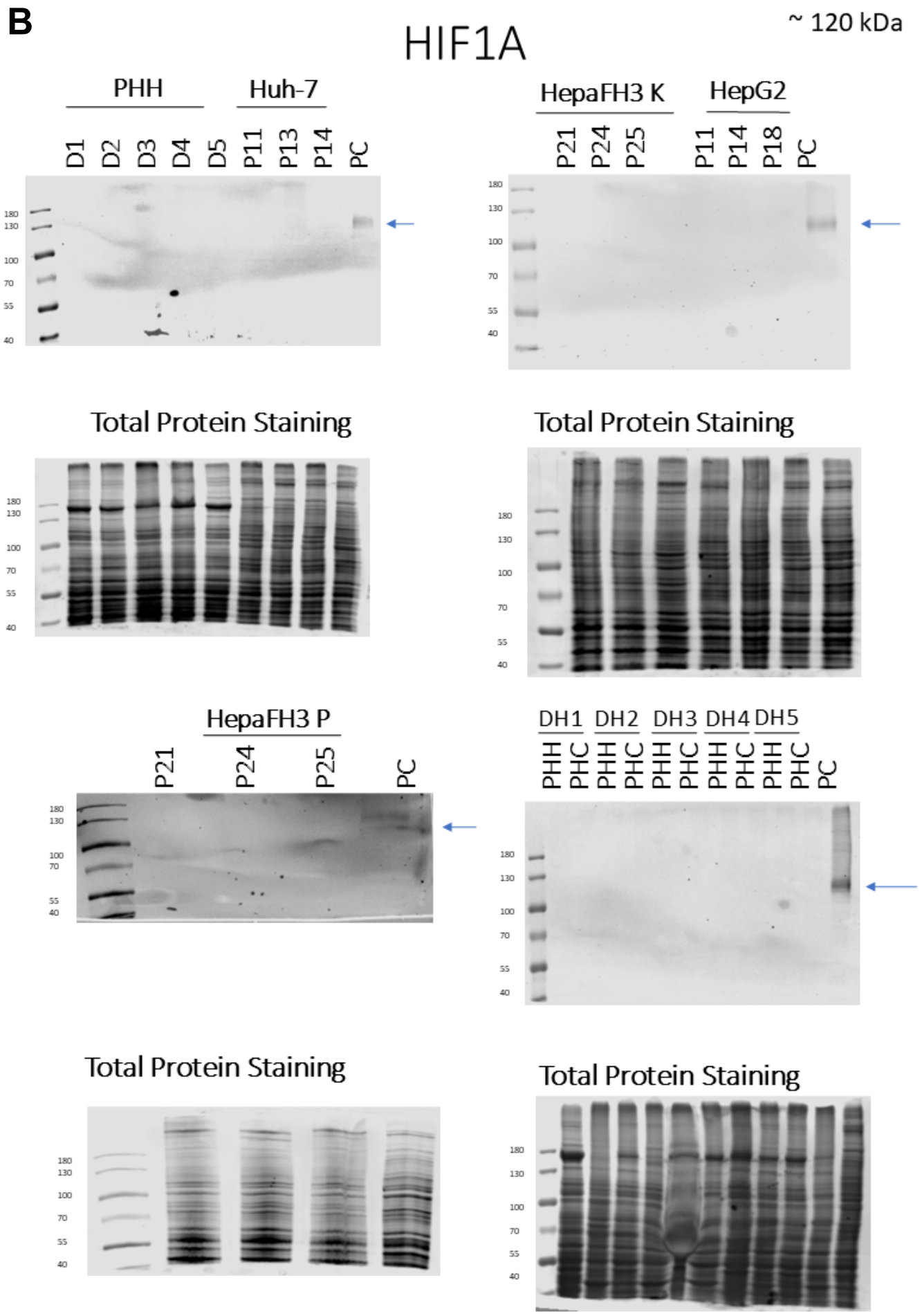
Table S3. Secondary antibodies used for Western blot analyses.

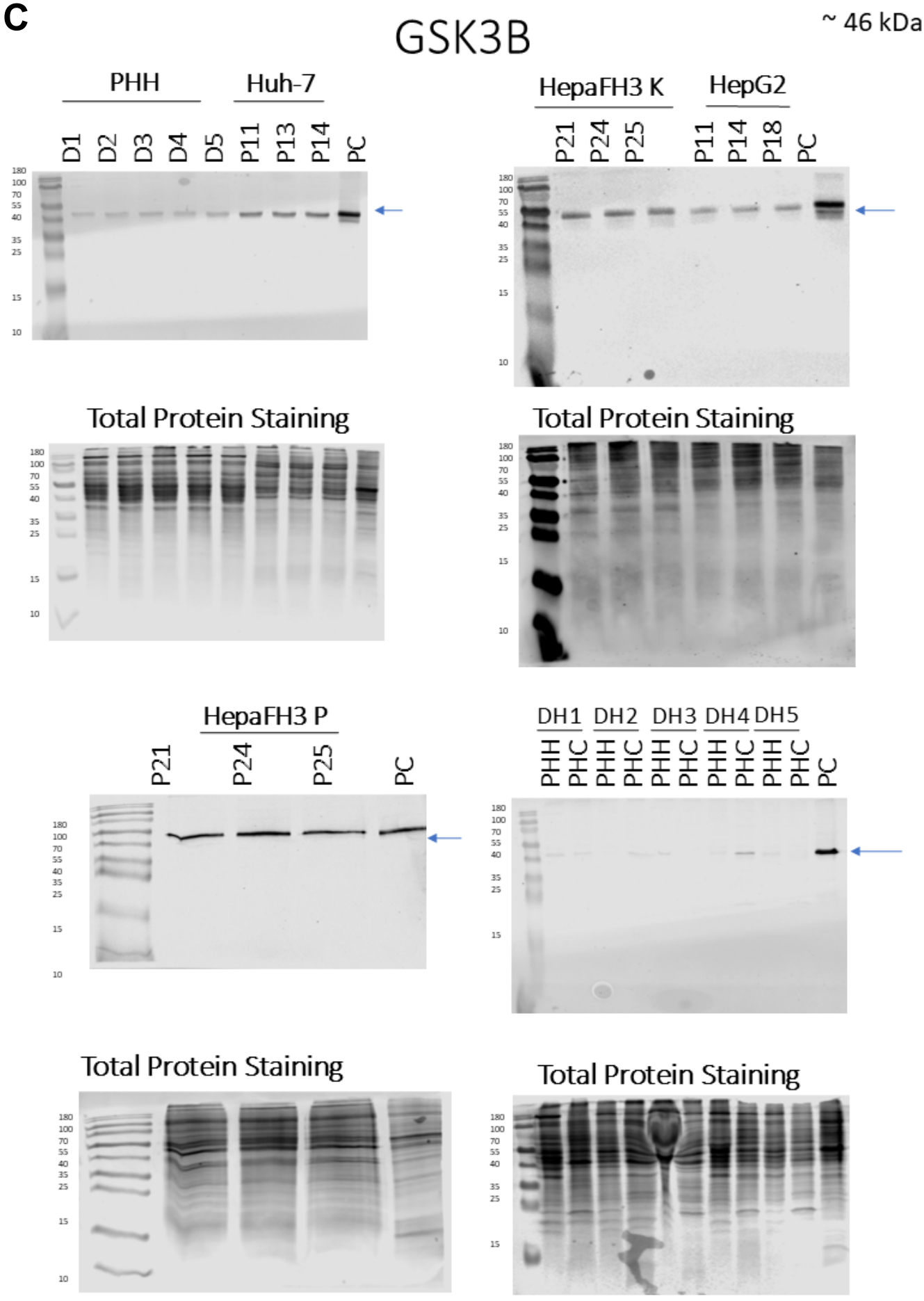
article	company	Article number

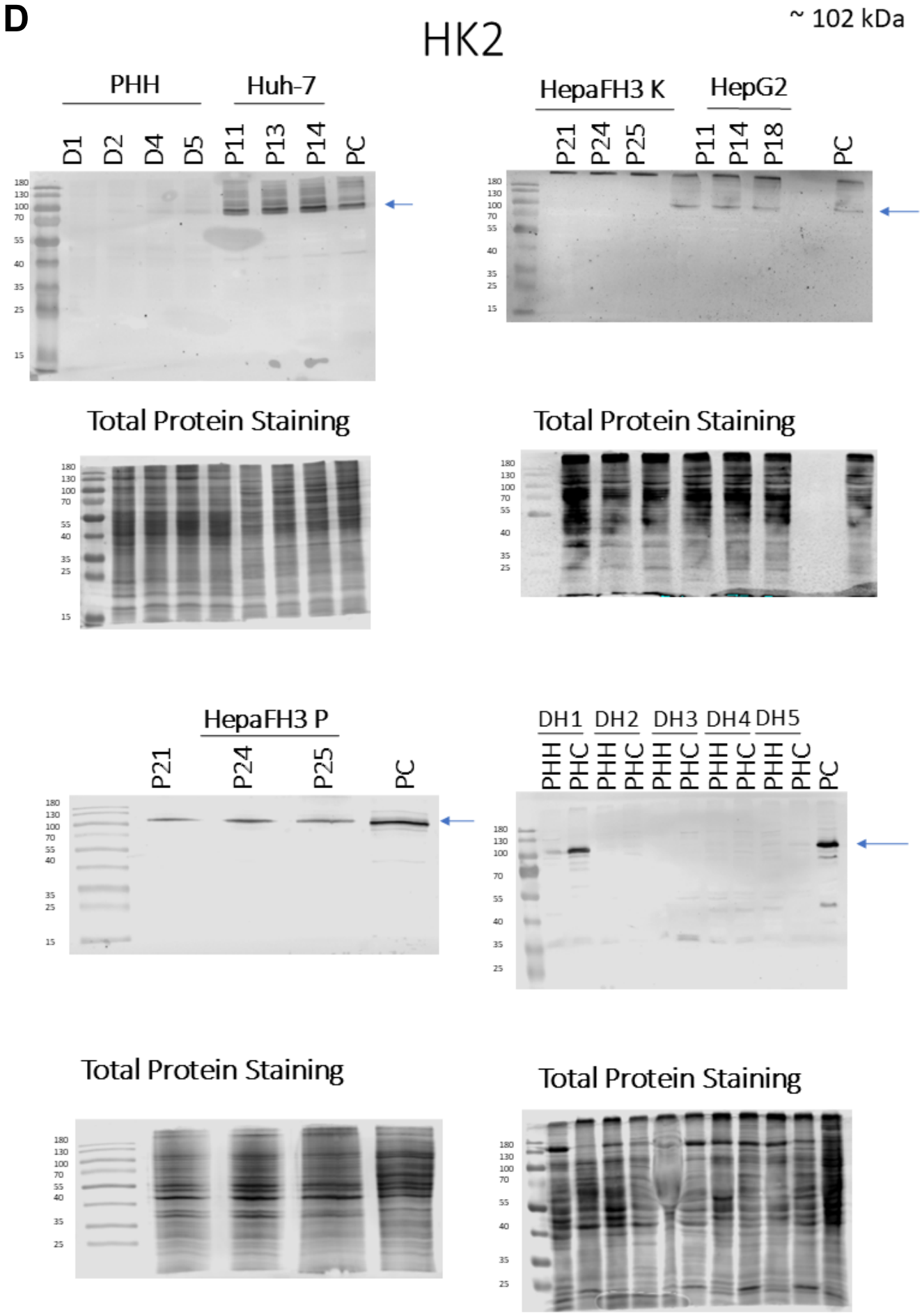
IRDye® 800CW Goat anti-Mouse IgG Secondary Antibody	Li-Cor Biosciences, Lincoln, US	926-32210
IRDye® 800CW Goat anti-Rabbit IgG Secondary Antibody	Li-Cor Biosciences, Lincoln, US	926-32211

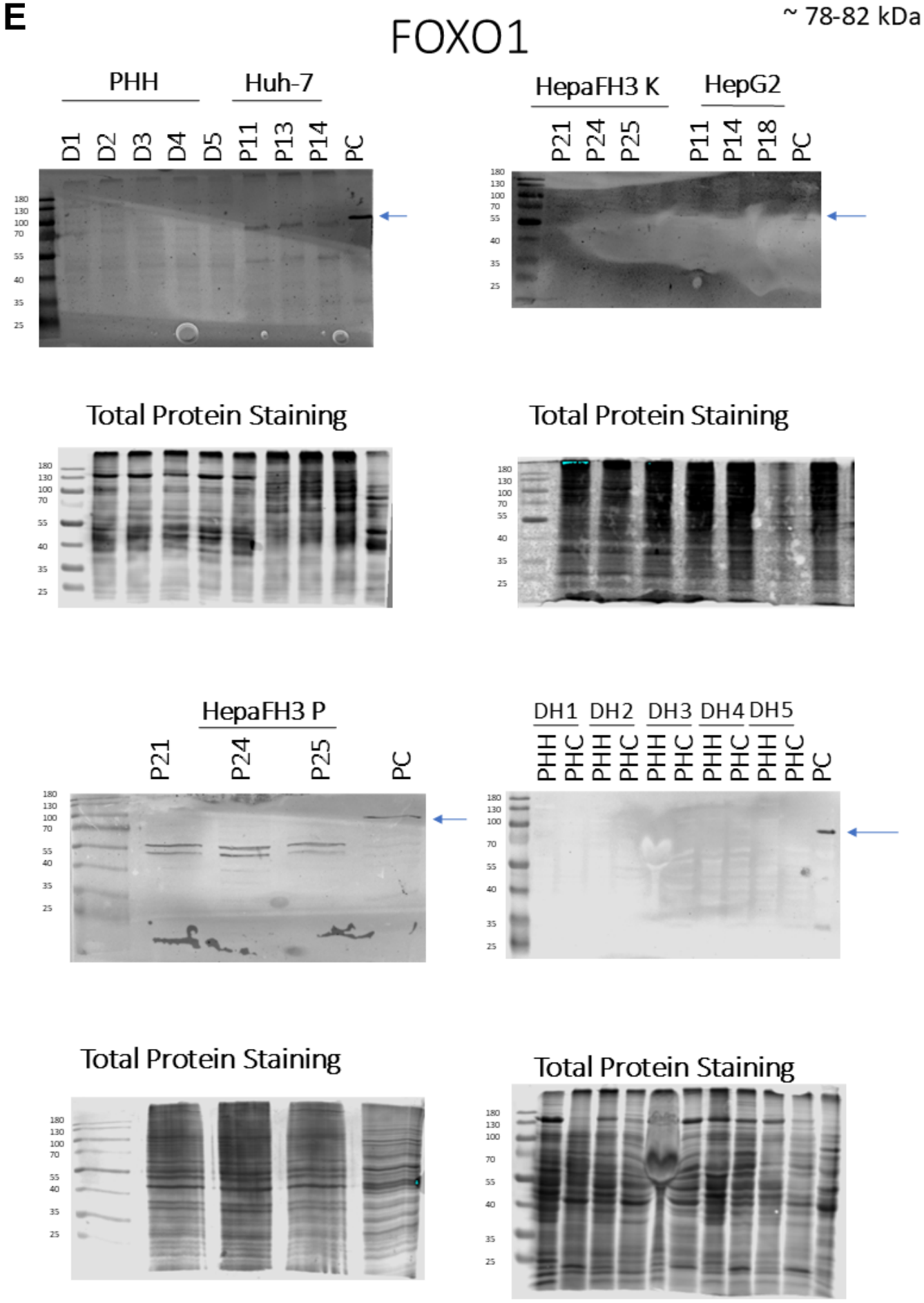
Antibodies were diluted 1:10,000 in Intercept Blocking Buffer with 0.1 % Tween.

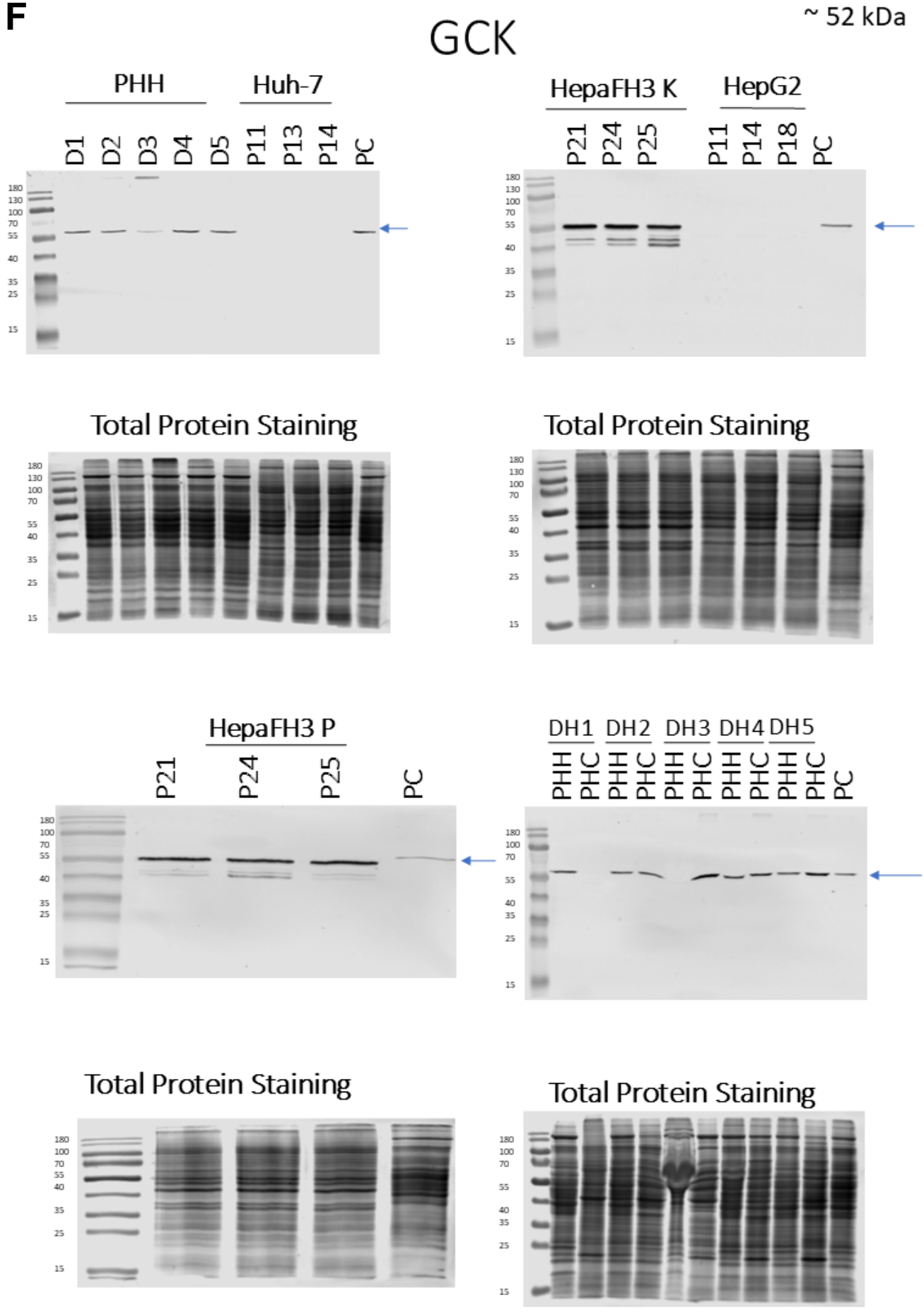

A
GSK3A
~ 51 kDa
PHH
Huh-7
D1
D2
D3
D4
D5
P11
P13
P14
PC
HepaFH3 K
HepG2
P21
P24
P25
P11
P14
P18
PC
Total Protein Staining
Total Protein Staining
HepaFH3 P
P21
P24
P25
PC
DH1
DH2
DH3
DH4
DH5
PHH
PHC
PC
Total Protein Staining
Total Protein Staining

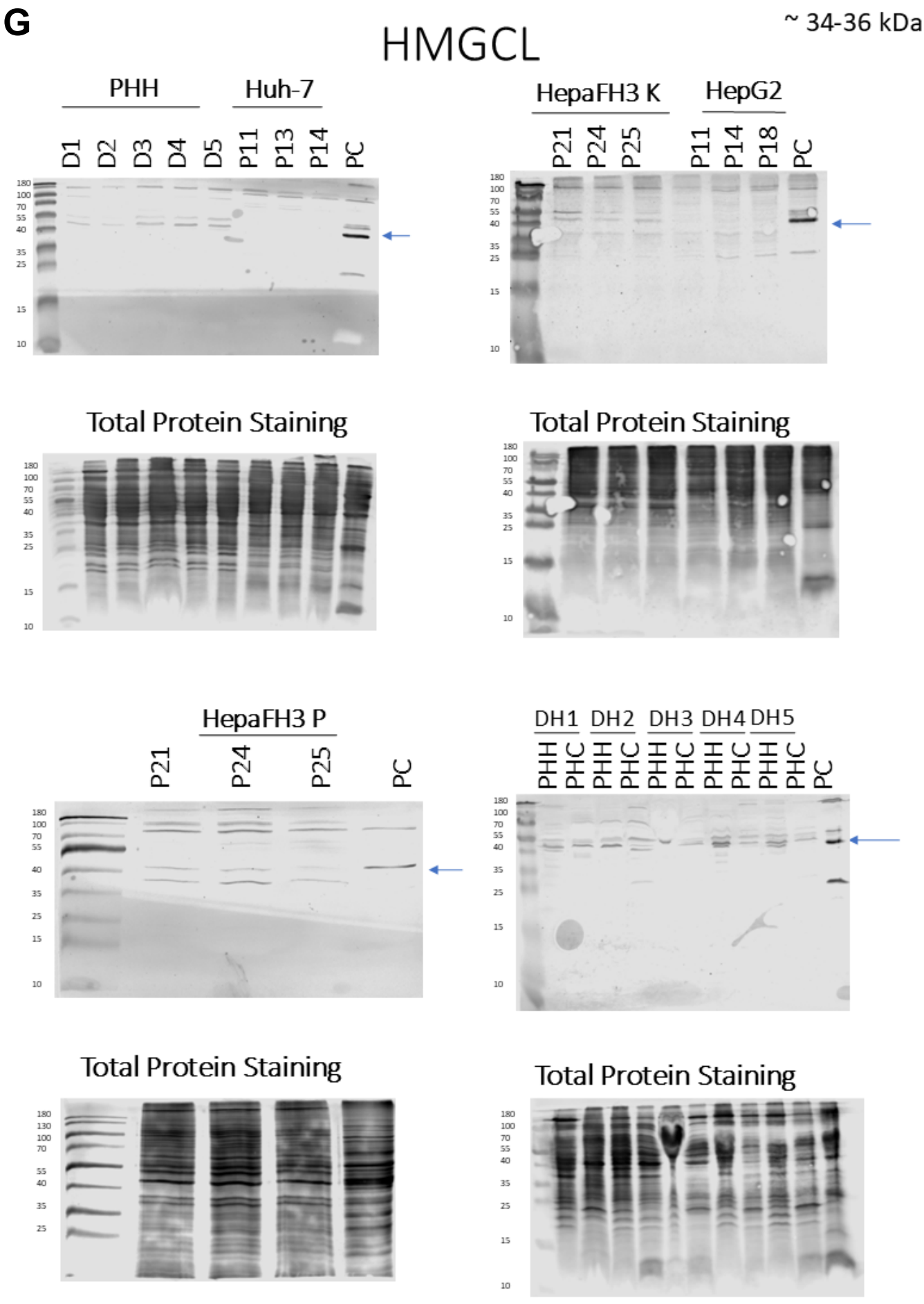

B
HIF1A
~ 120 kDa
PHH
Huh-7
D1
D2
D3
D4
D5
P11
P13
P14
PC
HepaFH3 K
HepG2
P21
P24
P25
P11
P14
P18
PC
180
130
100
70
55
40
Total Protein Staining
HepaFH3 P
P21
P24
P25
PC
DH1
DH2
DH3
DH4
DH5
PHH
PHC
PC

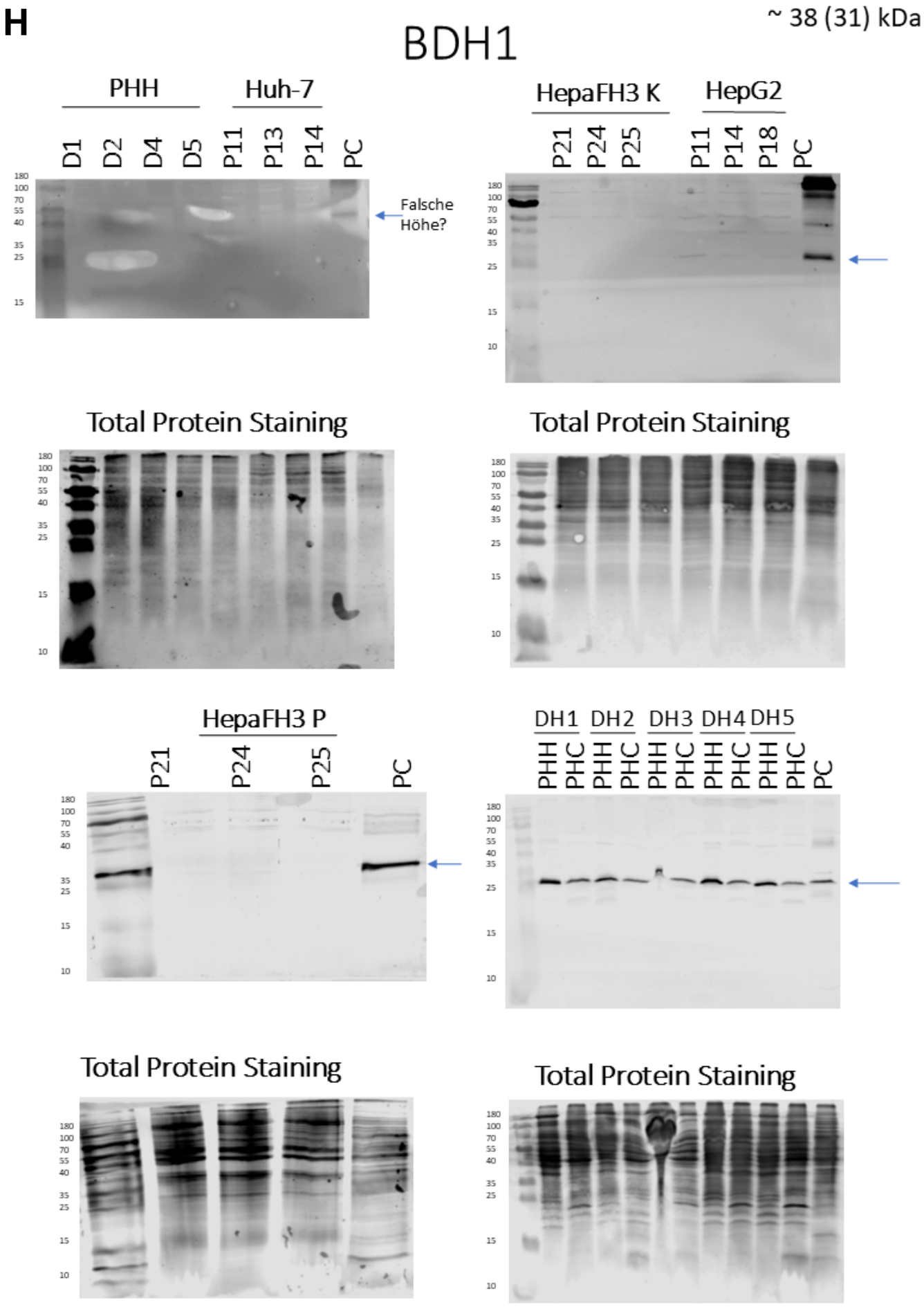

C
GSK3B
~ 46 kDa
PHH
Huh-7
D1
D2
D3
D4
D5
P11
P13
P14
PC
HepaFH3 K
HepG2
P21
P24
P25
P11
P14
P18
PC
Total Protein Staining
Total Protein Staining
HepaFH3 P
P21
P24
P25
PC
DH1
DH2
DH3
DH4
DH5
PHH
PHC
PC
Total Protein Staining
Total Protein Staining

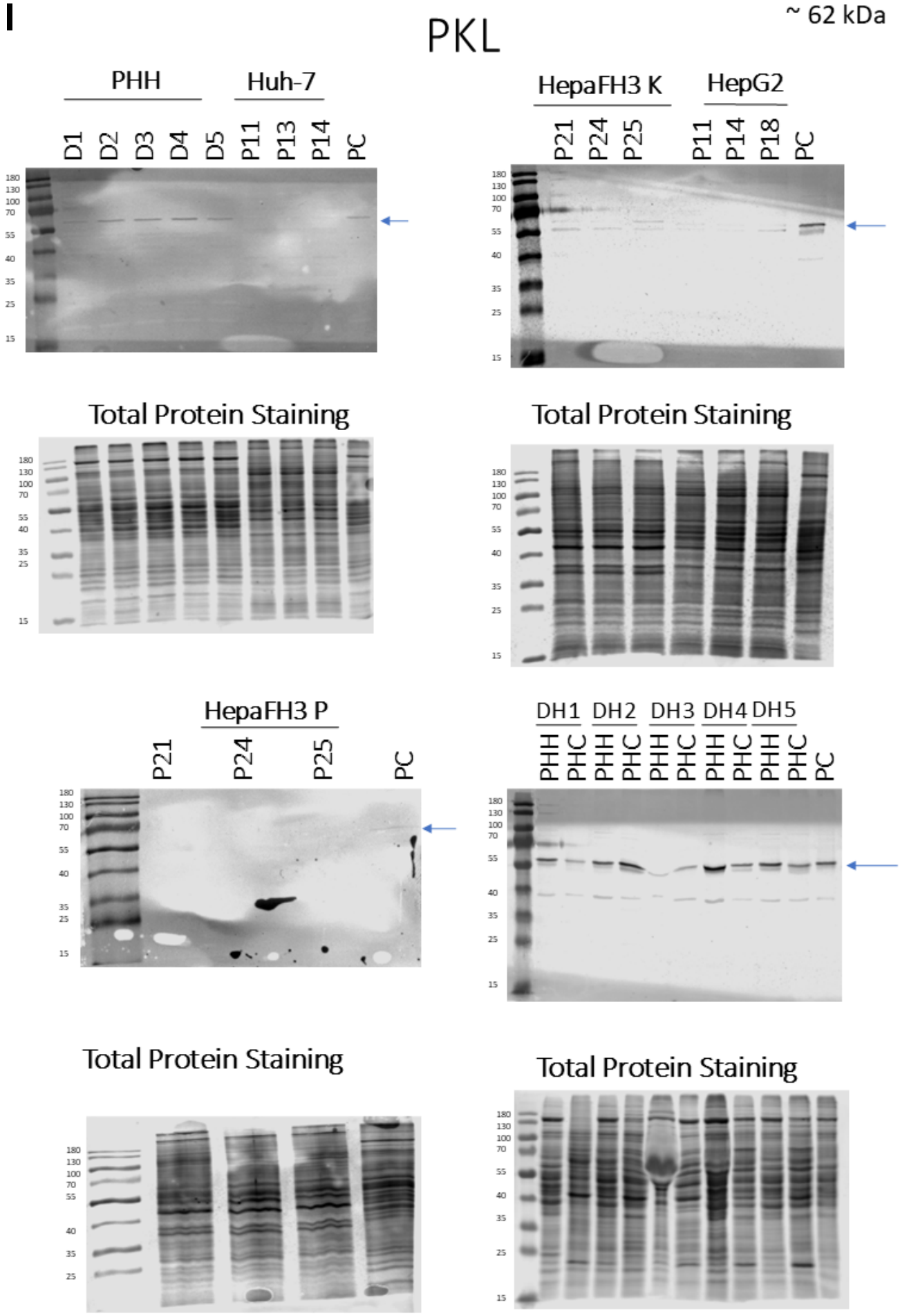

D
HK2
~ 102 kDa
PHH
Huh-7
D1
D2
D4
D5
P11
P13
P14
PC
HepaFH3 K
HepG2
P21
P24
P25
P11
P14
P18
PC
Total Protein Staining
Total Protein Staining
HepaFH3 P
P21
P24
P25
PC
DH1
DH2
DH3
DH4
DH5
PHH
PHC
PC
Total Protein Staining
Total Protein Staining

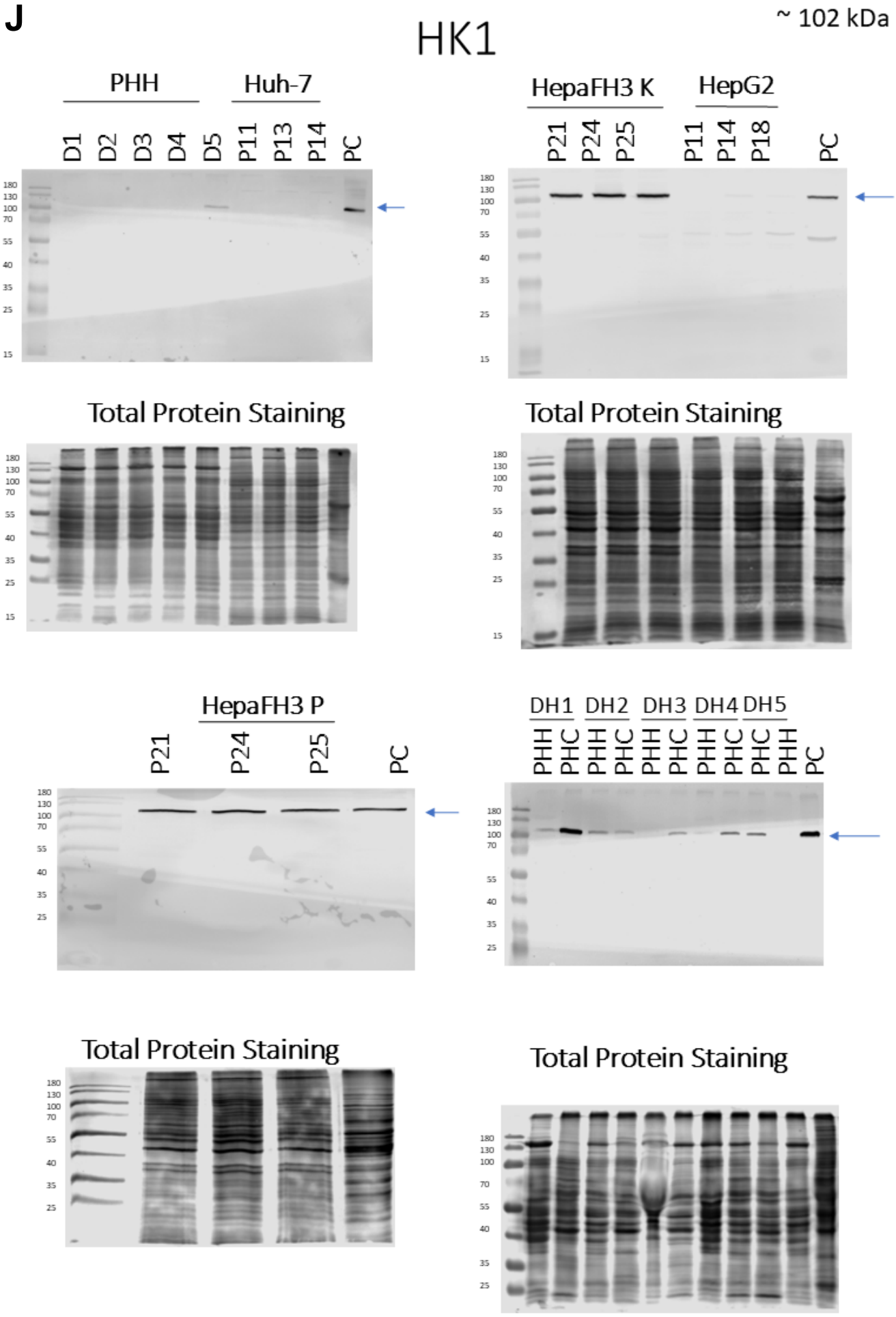

E
FOXO1
~ 78-82 kDa
PHH
Huh-7
D1
D2
D3
D4
D5
P11
P13
P14
PC
HepaFH3 K
HepG2
P21
P24
P25
P11
P14
P18
PC
180
130
100
70
55
40
35
25
Total Protein Staining
Total Protein Staining
HepaFH3 P
P21
P24
P25
PC
DH1
DH2
DH3
DH4
DH5
PHH
PHC
Total Protein Staining
Total Protein Staining


F
GCK
~ 52 kDa
PHH
Huh-7
D1
D2
D3
D4
D5
P11
P13
P14
PC
HepaFH3 K
HepG2
P21
P24
P25
P11
P14
P18
PC
Total Protein Staining
Total Protein Staining
HepaFH3 P
P21
P24
P25
PC
DH1
DH2
DH3
DH4
DH5
PHH
PHC
PC
Total Protein Staining
Total Protein Staining


G
HMGCL
~ 34-36 kDa
PHH
Huh-7
D1
D2
D3
D4
D5
P11
P13
P14
PC
HepaFH3 K
HepG2
P21
P24
P25
P11
P14
P18
PC
Total Protein Staining
Total Protein Staining
HepaFH3 P
P21
P24
P25
PC
DH1
DH2
DH3
DH4
DH5
PHH
PHC
PC
Total Protein Staining
Total Protein Staining


H
BDH1
~ 38 (31) kDa
PHH
Huh-7
D1
D2
D4
D5
P11
P13
P14
PC
Falsche Höhe?
HepaFH3 K
HepG2
P21
P24
P25
P11
P14
P18
PC
Total Protein Staining
Total Protein Staining
HepaFH3 P
P21
P24
P25
PC
DH1
DH2
DH3
DH4
DH5
PHH
PHC
Total Protein Staining
Total Protein Staining


I
PKL
~ 62 kDa
PHH
Huh-7
D1
D2
D3
D4
D5
P11
P13
P14
PC
HepaFH3 K
HepG2
P21
P24
P25
P11
P14
P18
PC
Total Protein Staining
Total Protein Staining
HepaFH3 P
P21
P24
P25
PC
DH1
DH2
DH3
DH4
DH5
PHH
PHC
PC
Total Protein Staining
Total Protein Staining


J
HK1
~ 102 kDa
PHH
Huh-7
D1
D2
D3
D4
D5
P11
P13
P14
PC
HepaFH3 K
HepG2
P21
P24
P25
P11
P14
P18
PC
Total Protein Staining
Total Protein Staining
HepaFH3 P
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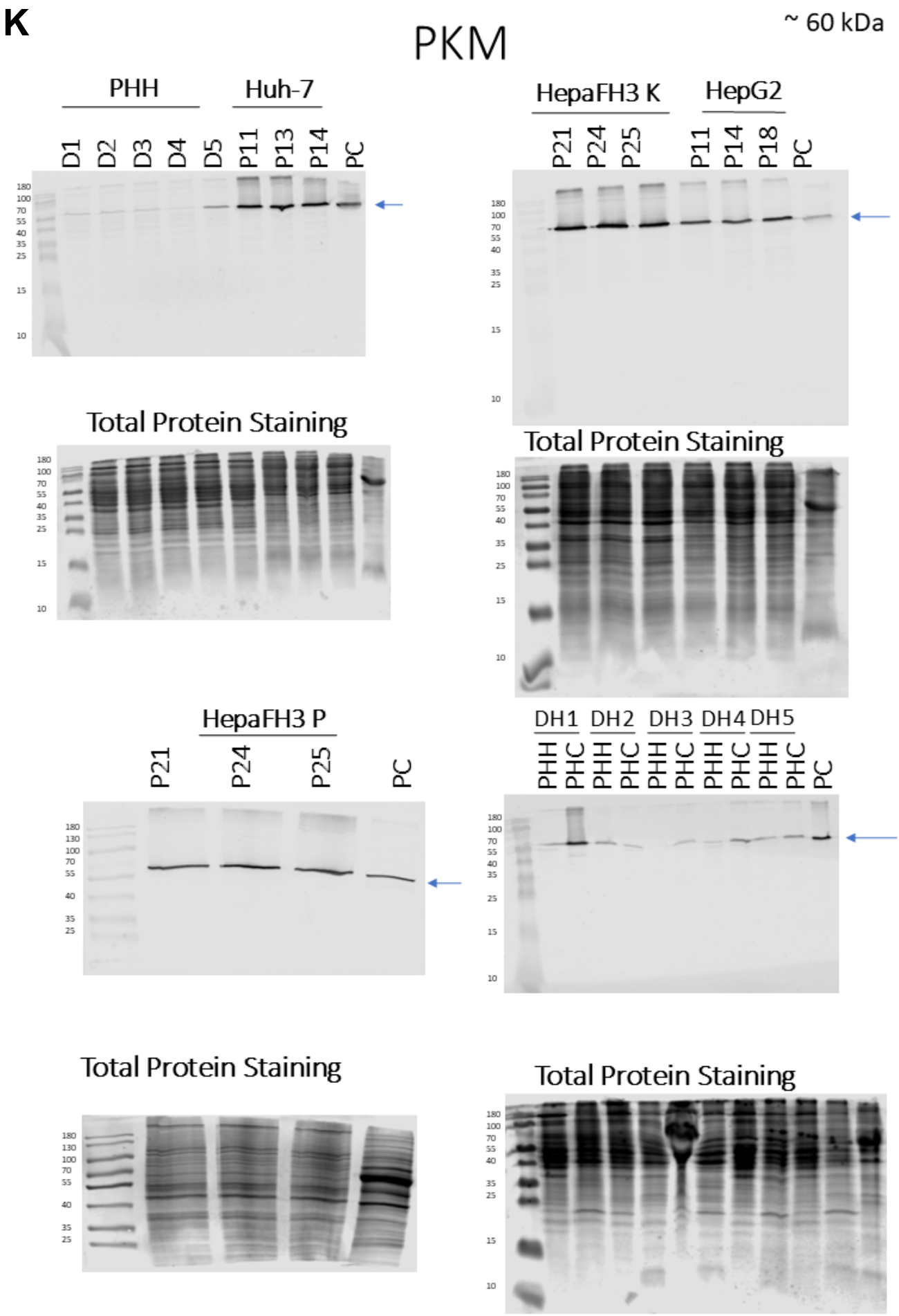
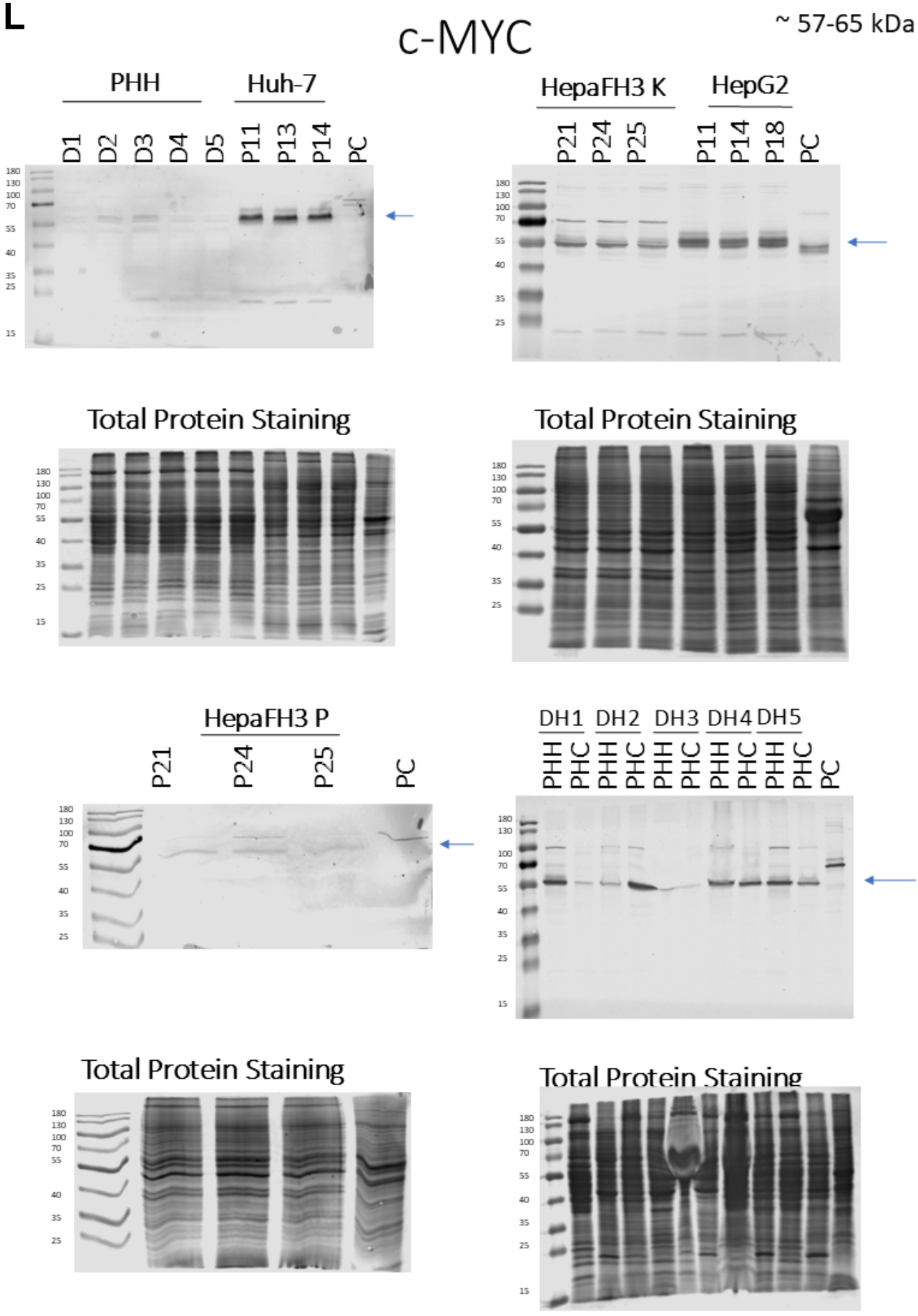
P24
P25
PC
DH1
DH2
DH3
DH4
DH5
PHH
PHC
PC
Total Protein Staining
Total Protein Staining


K
PKM
~ 60 kDa
PHH
Huh-7
D1
D2
D3
D4
D5
P11
P13
P14
PC
HepaFH3 K
HepG2
P21
P24
P25
P11
P14
P18
PC
Total Protein Staining
Total Protein Staining
HepaFH3 P
P21
P24
P25
PC
DH1
DH2
DH3
DH4
DH5
PHH
PHC
PC
Total Protein Staining
Total Protein Staining


L
c-MYC
~ 57-65 kDa
PHH
Huh-7
D1
D2
D3
D4
D5
P11
P13
P14
PC
HepaFH3 K
HepG2
P21
P24
P25
P11
P14
P18
PC
Total Protein Staining
Total Protein Staining
HepaFH3 P
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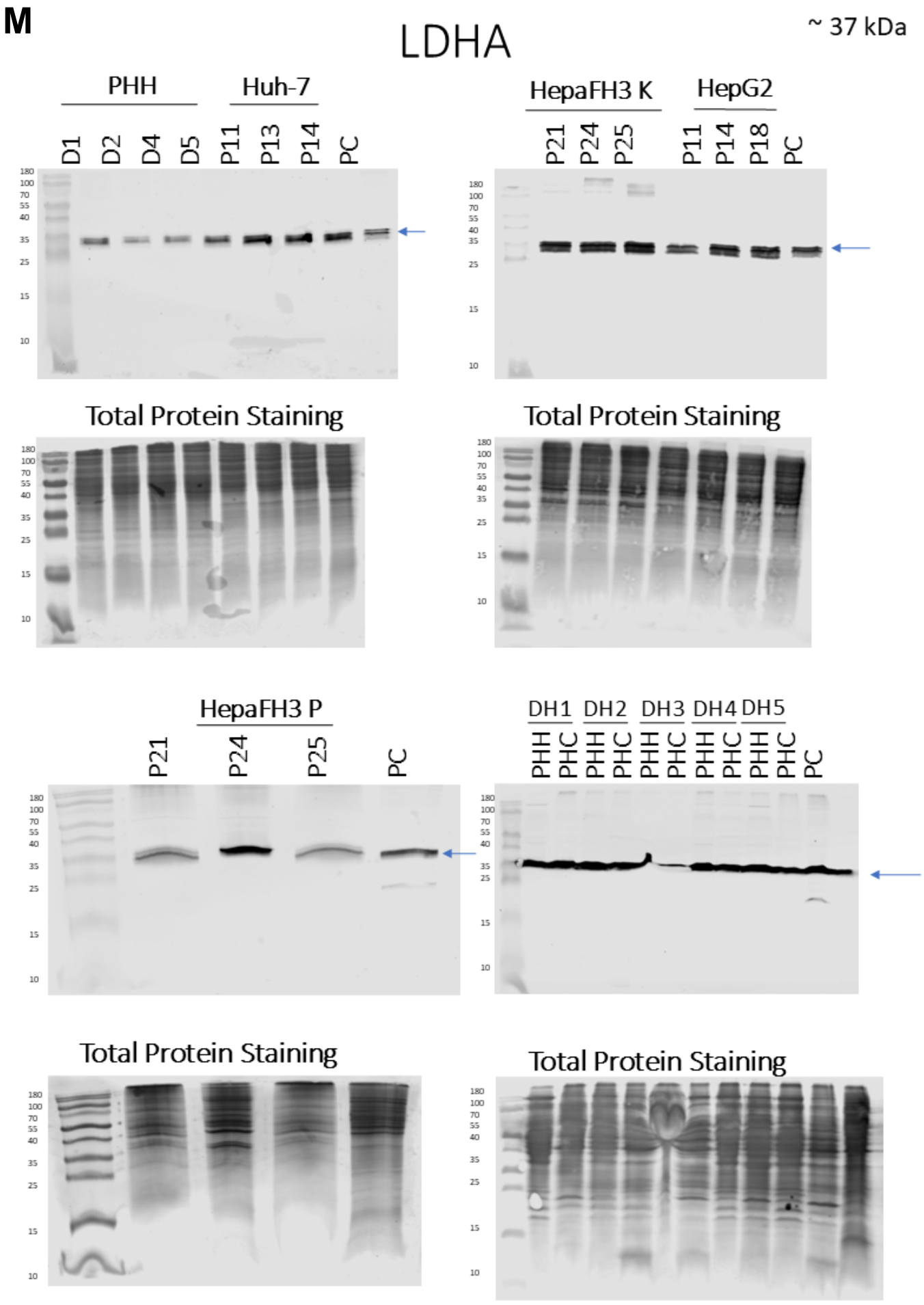
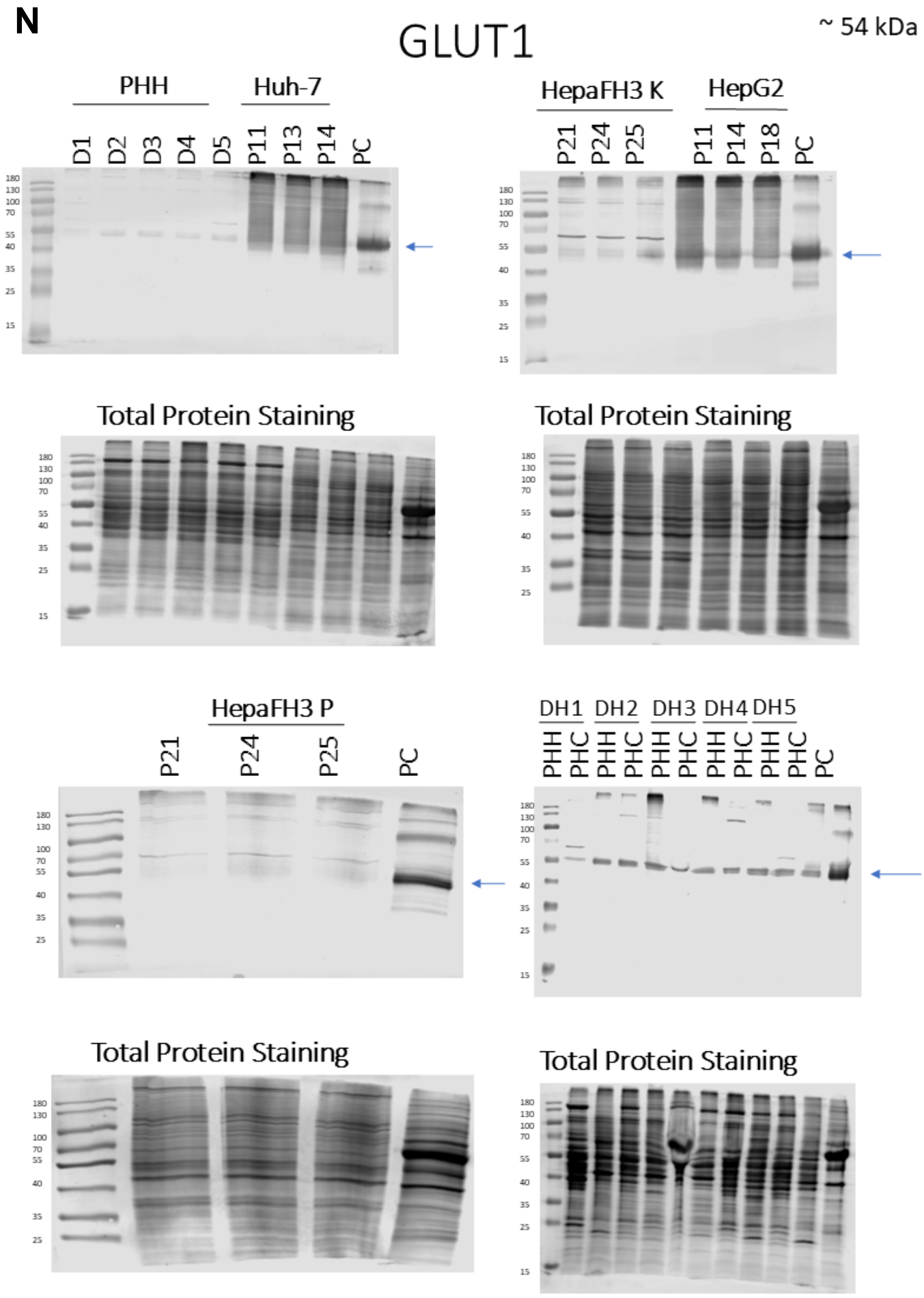
DH1
DH2
DH3
DH4
DH5
PHH
PHC
PC
Total Protein Staining
Total Protein Staining


M
LDHA
~ 37 kDa
PHH
Huh-7
D1
D2
D4
D5
P11
P13
P14
PC
HepaFH3 K
HepG2
P21
P24
P25
P11
P14
P18
PC
180
100
70
55
40
35
25
15
10
Total Protein Staining
Total Protein Staining
HepaFH3 P
P21
P24
P25
PC
DH1
DH2
DH3
DH4
DH5
PHH
PHC
PC
Total Protein Staining
Total Protein Staining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GLUT1
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PHH
Huh-7
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D2
D3
D4
D5
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P13
P14
PC
HepaFH3 K
HepG2
P21
P24
P25
P11
P14
P18
PC
Total Protein Staining
Total Protein Staining
HepaFH3 P
P21
P24
P25
PC
DH1
DH2
DH3
DH4
DH5
PHH
PHC
PC
Total Protein Staining
Total Protein Staining

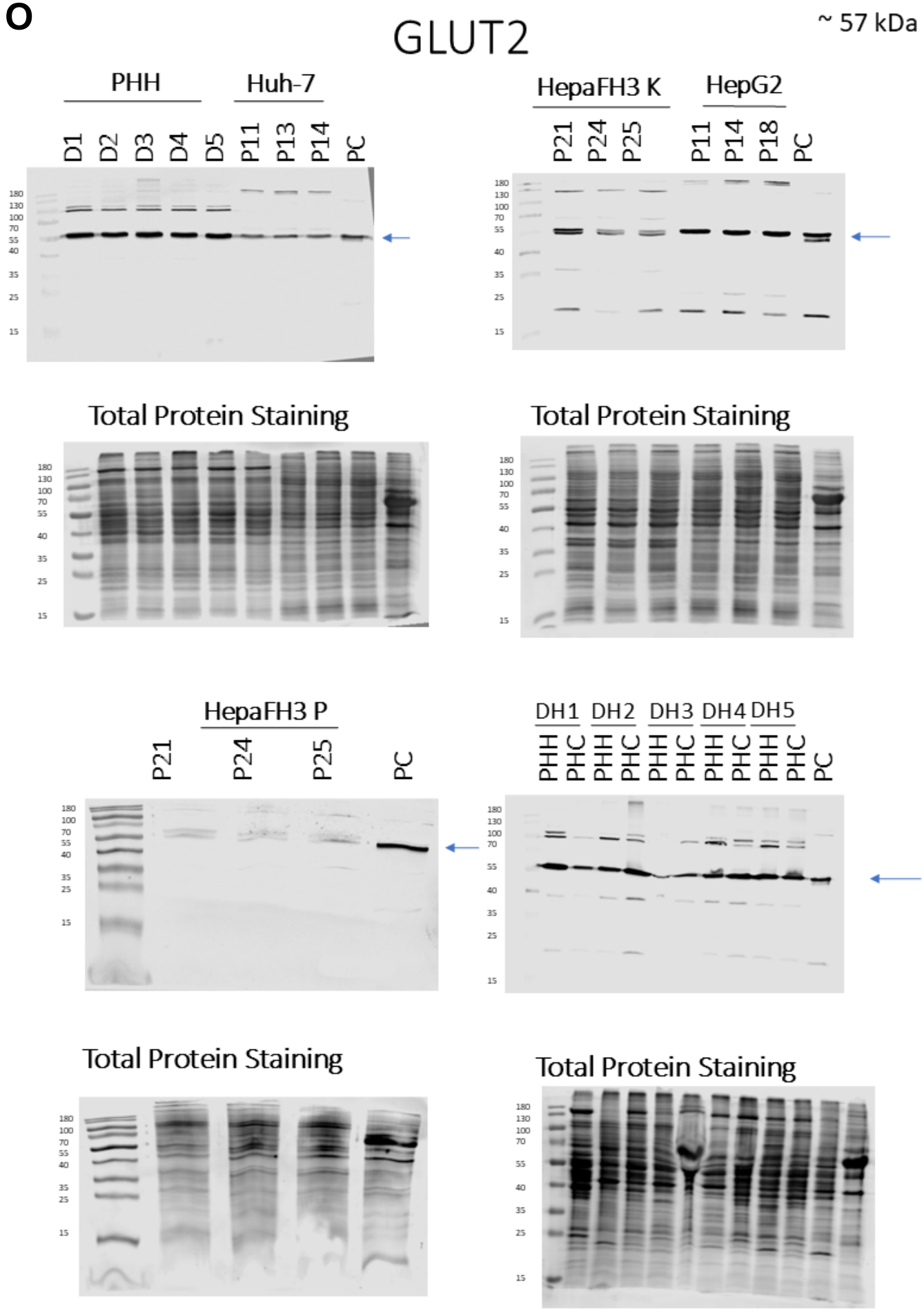
o
GLUT2
~ 57 kDa
PHH
Huh-7
D1
D2
D3
D4
D5
P11
P13
P14
PC
HepaFH3 K
HepG2
P21
P24
P25
P11
P14
P18
PC
Total Protein Staining
Total Protein Staining
HepaFH3 P
P21
P24
P25
PC
DH1
DH2
DH3
DH4
DH5
PHH
PHC
PC
Total Protein Staining
Total Protein Staining

Figure S1. Expression of various proteins in primary hepatic cells and hepatic cell lines. Western blot analyses were performed to assess the expression of (A) GSK3A, (B) HIF1A, (C) GSK3B, (D) HK2, (E) FOXO1, (F) GCK, (G) HMGCL, (H) BDH1, (I) PKL, (J) HK1, (K) PKM, (L) c-MYC, (M) LDHA, (N) GLUT1 and (O) GLUT2 in protein lysates from non-HCC primary human hepatocytes (non-HCC-PHHs, N = 5), HCC-derived PHHs (HCC-PHHs, N = 5), primary human hepatoma cells (PHCs, N = 5), Upcyte® hepatocytes HepaFH3 cells in confluent (HepaFH3 C, N = 3), and proliferating (HepaFH3 P, N = 3) states. Hepatoma cell lines HepG2 (N = 3) and Huh7 (N = 3 as well as a positive control (PC) were included for comparison. Arrows indicate the specific protein bands. Total protein staining is shown below each blot and was used for normalization. The positive control was also used for normalization purposes. Abbreviations: BDH1, 3-hydroxybutyrate dehydrogenase 1; C, confluent; c-MYC, MYC proto-oncogene; FOXO1, forkhead box O1; GCK, glucokinase; GLUT1, glucose transporter type 1; GLUT2, glucose transporter type 2; GSK3A, glycogen synthase kinase 3 alpha; GSK3B, glycogen synthase kinase 3 beta; HCC, hepatocellular carcinoma; HIF1A, hypoxia inducible factor 1 alpha; HK1, hexokinase 1; HK2, hexokinase 2; HMGCL, 3-hydroxymethyl-3-methylglutaryl-CoA lyase; LDHA, lactate dehydrogenase A; P, proliferating; PHC, primary human hepatoma cells; PHH, primary human hepatocytes; PKL, pyruvate kinase L; PKM, pyruvate kinase M1/2.

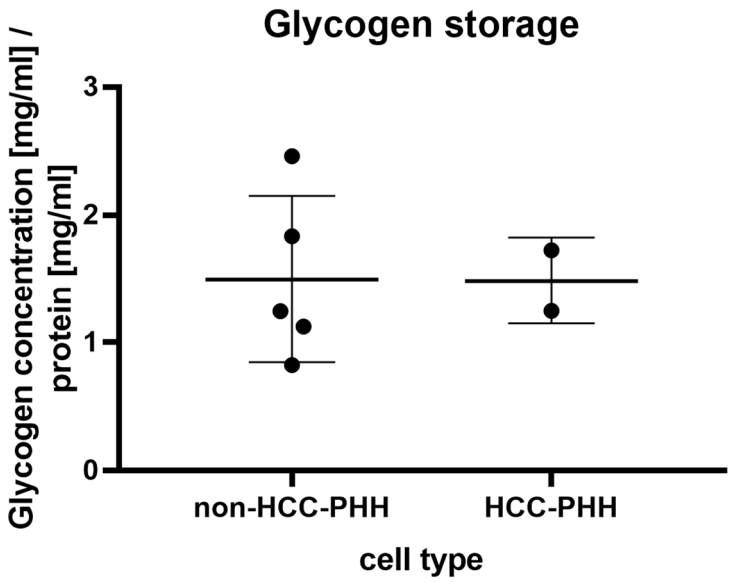

Figure S2. Glycogen levels in PHHs from non-HCC and HCC donors. Glycogen was quantified in cell lysates of non-HCC-PHHs (N = 5) and HCC-PHHs (N = 2). Values were normalized to total protein content. Abbreviations: HCC, hepatocellular carcinoma; PHH, primary human hepatocytes.

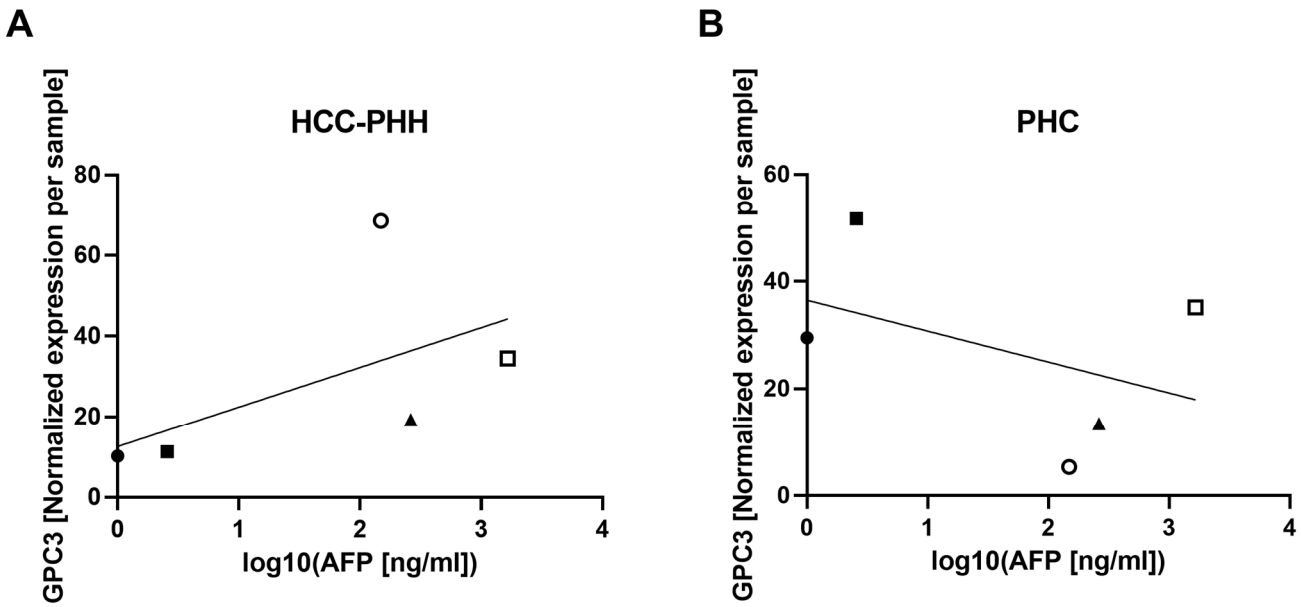

Figure S3. *GPC3* gene expression and AFP serum levels in HCC-PHHs and corresponding PHCs.

GPC3 mRNA expression was quantified in HCC-PHHs (N = 4) and matched PHCs (N = 4) from individual donors. Preoperative serum AFP levels were measured in the same HCC donors. Pearson correlation analyses were performed between *GPC3* expression and AFP levels after log transformation of AFP values. (A) Correlation between *GPC3* expression in HCC-PHHs and log-transformed AFP levels (Pearson's r = 0.559, p = 0.3272). (B) Correlation between *GPC3* expression in PHCs and log-transformed AFP levels (Pearson's r = -0.4346, p = 0.4646). The data represent the individual donor values and are indicated of various symbols. Abbreviations: AFP, α-fetoprotein; *GPC3*, glypican-3; HCC, hepatocellular carcinoma; PHCs, primary human hepatoma cells; PHHs, primary human hepatocytes.