Table S1. GM3S loss inhibits cystogenesis in *jck* mice

Gender	GM3S genotype	No of animals	K/BW ratio (%)	Cystic volume (%BW)	BUN (mg/dl)
Male	+/+	9	9.28 ± 0.32	4.36 ± 0.29	51.56 ± 2.84
Male	**+/-**	23	10.11 ± 0.51	4.36 ± 0.43	64.73 ± 7.44
Male	-/-	13	5.71 ± 0.51*†	2.05 ± 0.62*†	34.77 ± 2.74*†
Female	+/+	13	7.86 ± 0.53	2.63 ± 0.33	50.40 ± 6.10
Female	+/-	33	8.69 ± 0.43	3.10 ± 0.35	46.78 ± 2.76
Female	-/-	11	5.91 ± 0.53*†	1.54 ± 0.39†	36.14 ± 3.68*†

* $P<0.05$ compared to gender-matched GM3S+/+ animals
† $P<0.05$ compared to gender-matched GM3S+/- animals
Values are mean +/- standard error
64 day old animals were analyzed

Table S2. Sphk1 loss exacerbates cystogenesis in *jck* mice

Gender	Sphk1 genotype	No of animals	K/BW ratio (%)	Cystic volume (%BW)	BUN (mg/dl)
Male	+/+	13	5.41 ± 0.26	1.38 ± 0.14	41.69 ± 4.33
Male	**+/-**	25	6.89 ± 0.38*	2.51 ± 0.27*	47.94 ± 4.37
Male	-/-	23	8.54 ± 0.57*†	3.44 ± 0.39*	65.08 ± 8.99
Female	+/+	20	3.57 ± 0.13	0.59 ± 0.05	29.92 ± 1.34
Female	+/-	27	5.12 ± 0.26*	1.36 ± 0.13*	32.29 ± 1.62
Female	-/-	26	6.37 ± 0.43*†	2.03 ± 0.26*†	35.67 ± 3.38

* $P<0.05$ compared to gender-matched Sphk1+/+ animals
† $P<0.05$ compared to gender-matched Sphk1+/- animals
Values are mean +/- standard error
50 day old animals were analyzed

Legends to Supplemental Figures

Figure S1. **Altered sphingolipid metabolism in GM3S-/- mice.** LC-MS analysis of GSL and SL levels from adult male C57BL/6 mice with normal GM3S activity (GM3S+/+), or homozygous for the GM3S mutation (GM3S-/-). *, $P<0.05$ compared to wild-type.

Figure S2. Quantification of western blots from GM3S+/+, +/-, and -/- animals. The western blots presented in Figure 2E-G were scanned using an Epson Expression 1680 scanner (Epson Corp., Long Beach, CA) in transparency mode. Greyscale images were quantified using ImageJ software (Rasband, W.S., ImageJ, U. S. National Institutes of Health, Bethesda, Maryland, USA, http://imagej.nih.gov/ij/, 1997-2011). Data are expressed as densitometric units normalized to the densitometric units of GAPDH.

Figure S3. Reduced β-catenin and c-Myc expression in cystic epithelia from GM3S-/- mice. Immunofluorescent detection of β-catenin (upper panels) and c-Myc (lower panels) in cystic kidneys from 64 day old male Jck mice carrying mutations in the GM3S gene. Animals lacking GM3S (GM3S-/-) show reduced staining of β-catenin and c-Myc in the cystic epithelia compared to wild-type (GM3S+/+) or heterozygous (GM3S+/-) animals. Positive staining is shown in green; blue shows nuclear counterstaining (DAPI).

Figure S4. Altered SL metabolism in Sphk1-/- mice. LC-MS analysis of GSL and SL levels from adult male C57BL/6 mice with normal Sphk1 activity (Sphk1+/+) or homozygous for the Sphk1 mutation (Sphk1-/-). *, $P<0.05$ compared to wild-type.

Figure S5. Quantification of western blots from Sphk1+/+, +/-, and -/- animals. The western blots presented in Figure 3E-G were quantified as described in Figure S2.

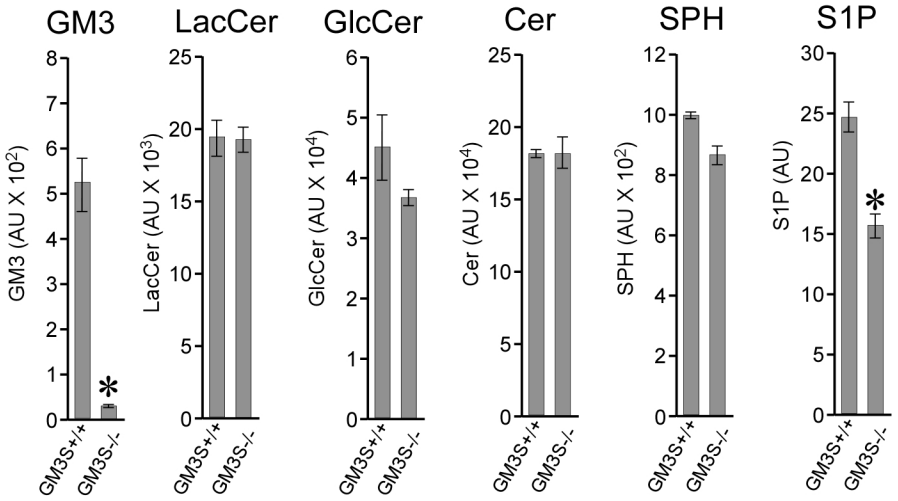
GM3
LacCer
GlcCer
Cer
SPH
S1P
GM3 (AU X 10^2)
LacCer (AU X 10^3)
GlcCer (AU X 10^4)
Cer (AU X 10^4)
SPH (AU X 10^2)
S1P (AU)
GM3S+/+
GM3S-/-
*

Figure S1

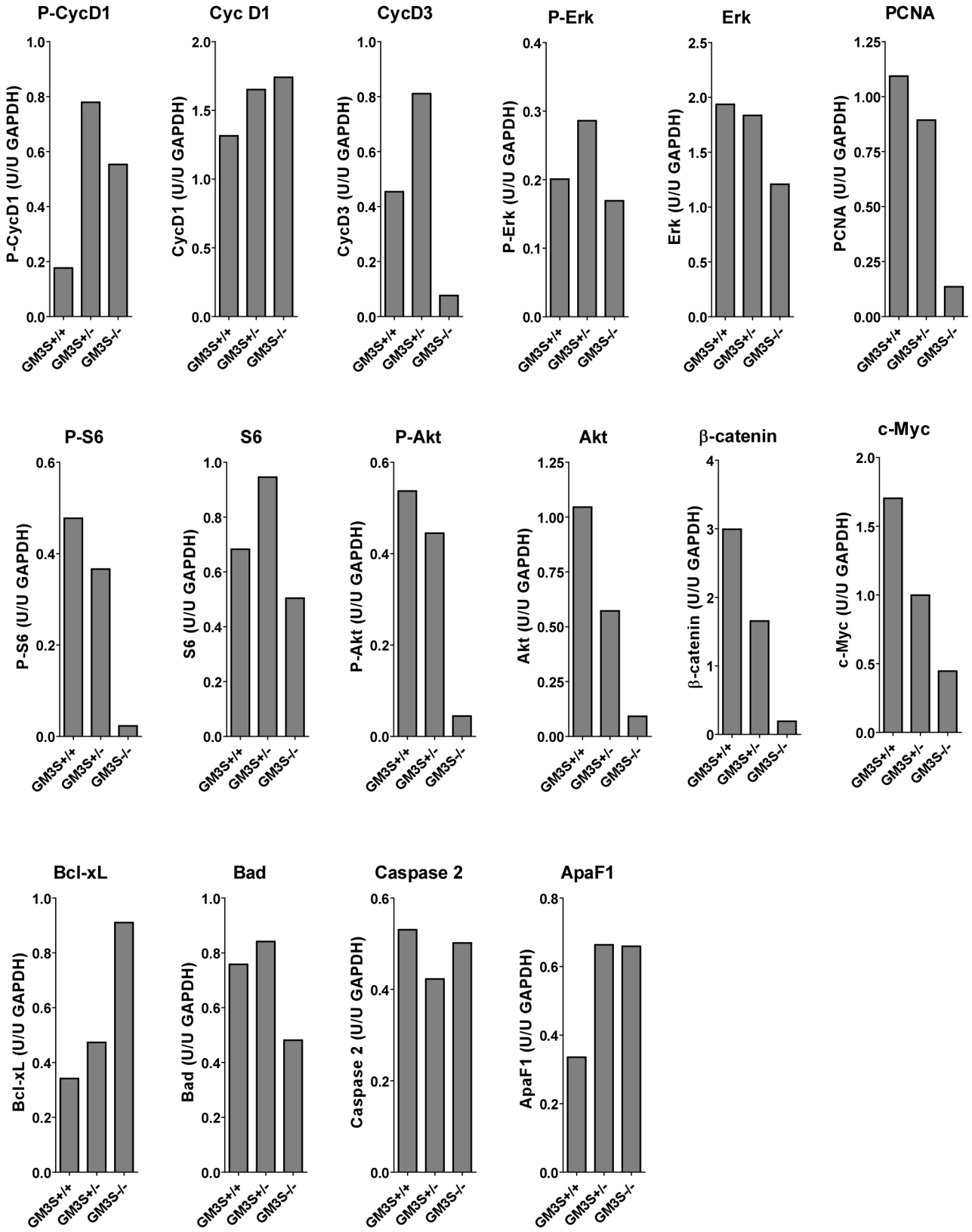
P-CycD1
P-CycD1 (U/U GAPDH)
Cyc D1
CycD1 (U/U GAPDH)
CycD3
CycD3 (U/U GAPDH)
P-Erk
P-Erk (U/U GAPDH)
Erk
Erk (U/U GAPDH)
PCNA
PCNA (U/U GAPDH)
P-S6
P-S6 (U/U GAPDH)
S6
S6 (U/U GAPDH)
P-Akt
P-Akt (U/U GAPDH)
Akt
Akt (U/U GAPDH)
β-catenin
β-catenin (U/U GAPDH)
c-Myc
c-Myc (U/U GAPDH)
Bcl-xL
Bcl-xL (U/U GAPDH)
Bad
Bad (U/U GAPDH)
Caspase 2
Caspase 2 (U/U GAPDH)
ApaF1
ApaF1 (U/U GAPDH)
GM3S+/+
GM3S+/-
GM3S-/-

Figure S2

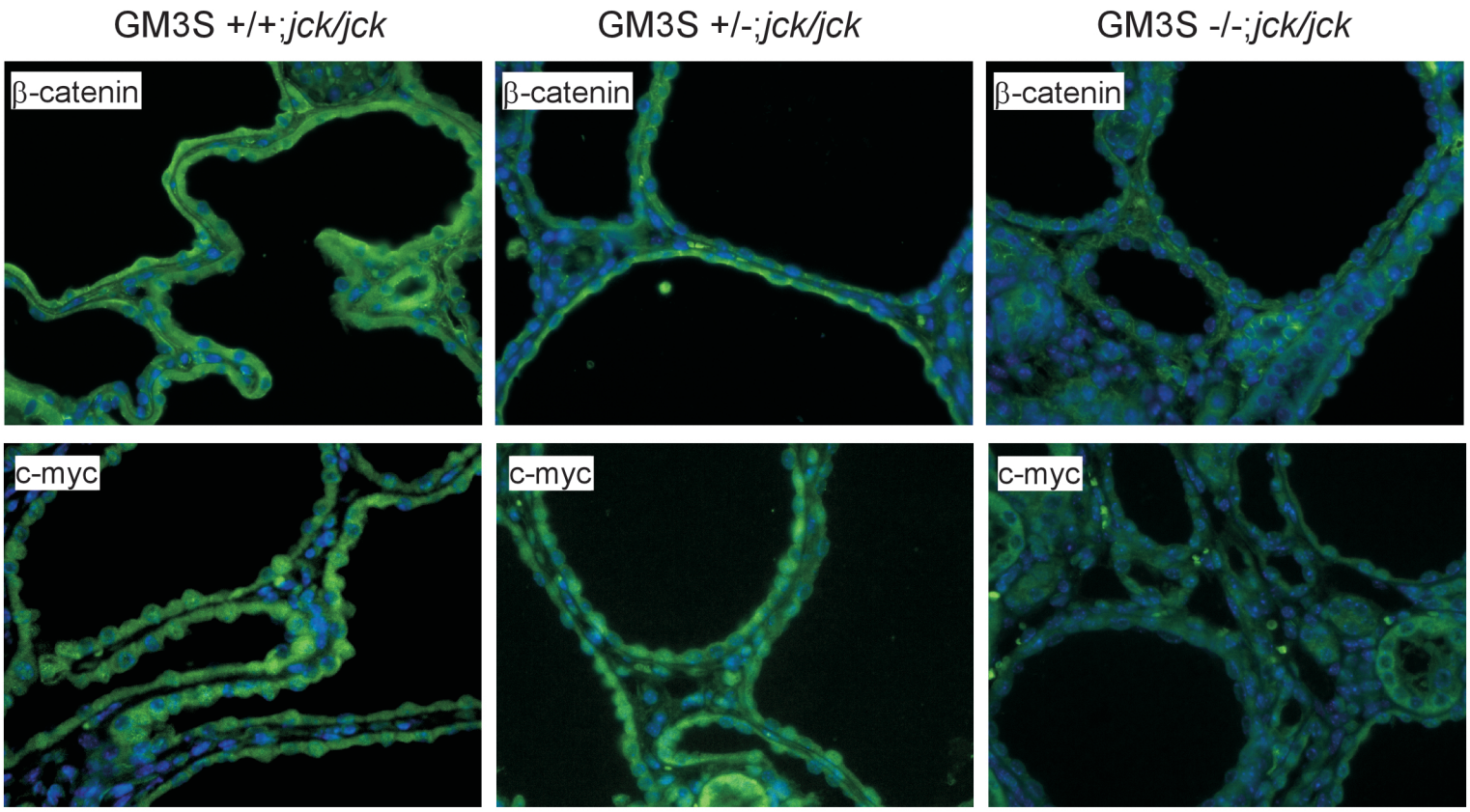
GM3S +/+;*jck/jck*
GM3S +/-;*jck/jck*
GM3S -/-;*jck/jck*
β-catenin
β-catenin
β-catenin
c-myc
c-myc
c-myc

Figure S3

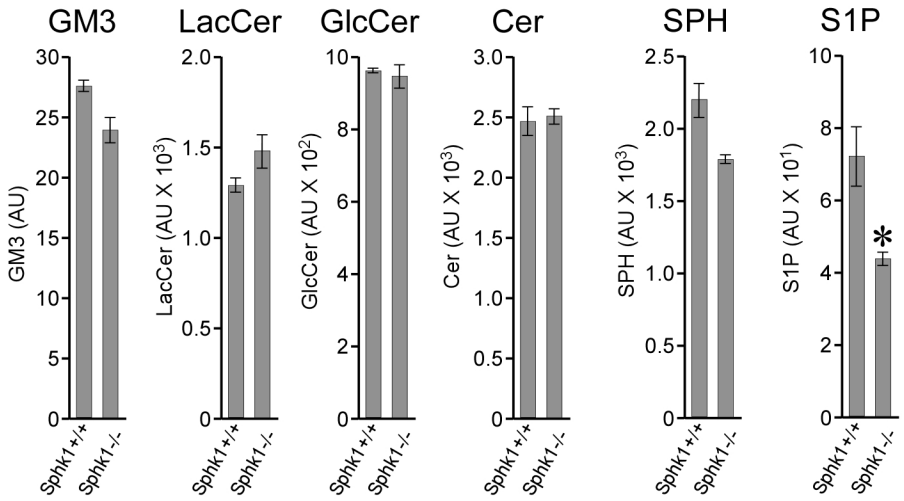
GM3
LacCer
GlcCer
Cer
SPH
S1P
GM3 (AU)
LacCer (AU X 10^3)
GlcCer (AU X 10^2)
Cer (AU X 10^3)
SPH (AU X 10^3)
S1P (AU X 10^1)
Sphk1+/+
Sphk1-/-
*

Figure S4

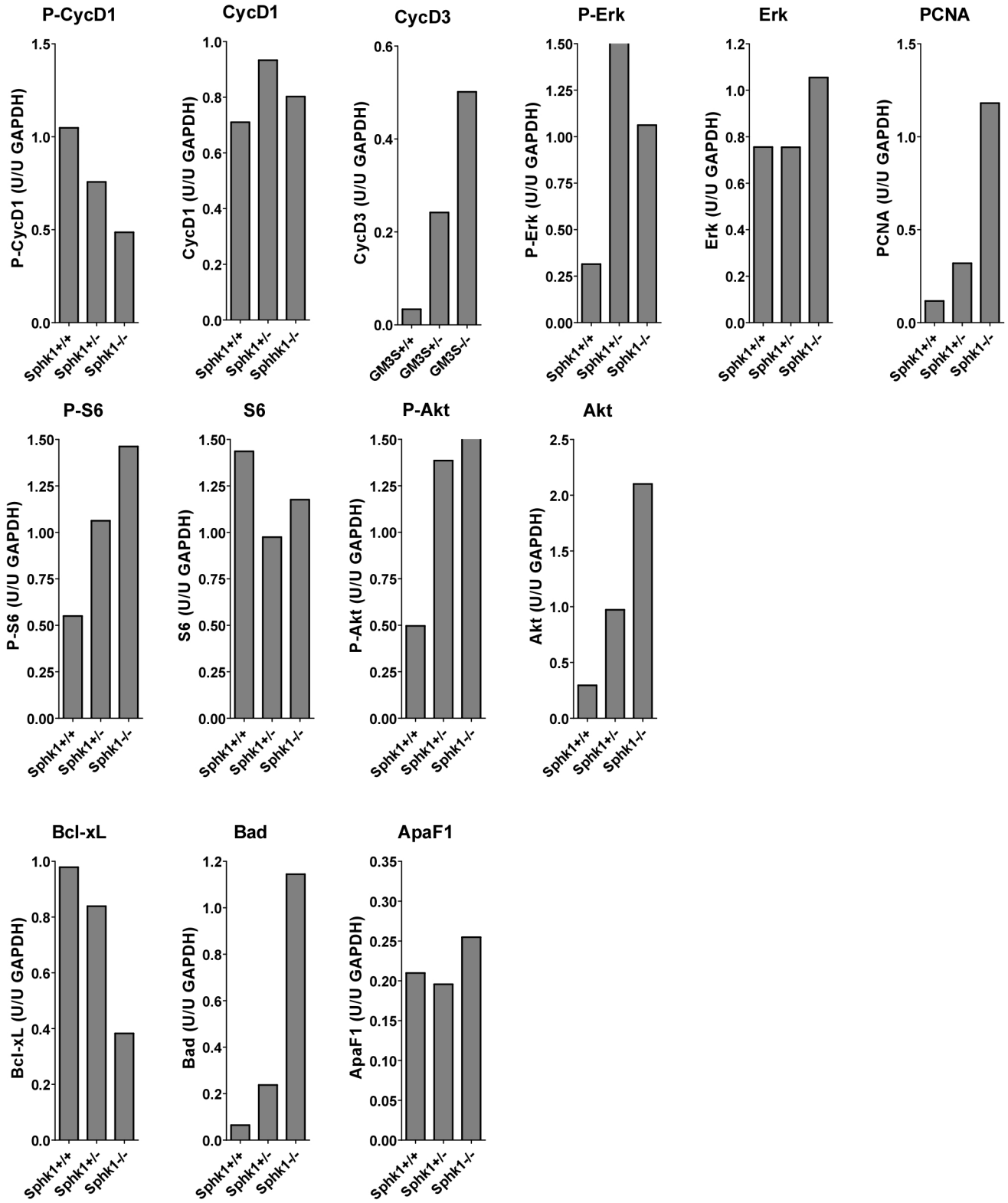
P-CycD1
P-CycD1 (U/U GAPDH)
1.5
1.0
0.5
0.0
Sphk1+/+
Sphk1+/-
Sphk1-/-
CycD1
CycD1 (U/U GAPDH)
1.0
0.8
0.6
0.4
0.2
0.0
Sphk1+/+
Sphk1+/-
Sphhk1-/-
CycD3
CycD3 (U/U GAPDH)
0.6
0.4
0.2
0.0
GM3S+/+
GM3S+/-
GM3S-/-
P-Erk
P-Erk (U/U GAPDH)
1.50
1.25
1.00
0.75
0.50
0.25
0.00
Sphk1+/+
Sphk1+/-
Sphk1-/-
Erk
Erk (U/U GAPDH)
1.2
1.0
0.8
0.6
0.4
0.2
0.0
Sphk1+/+
Sphk1+/-
Sphk1-/-
PCNA
PCNA (U/U GAPDH)
1.5
1.0
0.5
0.0
Sphk1+/+
Sphk1+/-
Sphk1-/-
P-S6
P-S6 (U/U GAPDH)
1.50
1.25
1.00
0.75
0.50
0.25
0.00
Sphk1+/+
Sphk1+/-
Sphk1-/-
S6
S6 (U/U GAPDH)
1.50
1.25
1.00
0.75
0.50
0.25
0.00
Sphk1+/+
Sphk1+/-
Sphk1-/-
P-Akt
P-Akt (U/U GAPDH)
1.50
1.25
1.00
0.75
0.50
0.25
0.00
Sphk1+/+
Sphk1+/-
Sphk1-/-
Akt
Akt (U/U GAPDH)
2.5
2.0
1.5
1.0
0.5
0.0
Sphk1+/+
Sphk1+/-
Sphk1-/-
Bcl-xL
Bcl-xL (U/U GAPDH)
1.0
0.8
0.6
0.4
0.2
0.0
Sphk1+/+
Sphk1+/-
Sphk1-/-
Bad
Bad (U/U GAPDH)
1.2
1.0
0.8
0.6
0.4
0.2
0.0
Sphk1+/+
Sphk1+/-
Sphk1-/-
ApaF1
ApaF1 (U/U GAPDH)
0.35
0.30
0.25
0.20
0.15
0.10
0.05
0.00
Sphk1+/+
Sphk1+/-
Sphk1-/-

Figure S5