Tissue-specific effects of saposin A and saposin B on glycosphingolipid degradation in mutant mice

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Supplemental Figure Legends

Figure S1. Knock-in mutations on saposin A and saposin B by homologous recombination. (A) Targeting construct. The mutation in saposin A and saposin B (Cys→Phe) located on exon 4 and exon 7, respectively. Individual saposin was encoded by the exons under dotted line. The mutation destroys one of three disulfide bridges on the saposin, which leads to deficiency of saposin A and saposin B protein. The neo gene was removed by recombination on loxP sites through cross breeding of saposin AB heterozygous and Zp3-Cre transgenic mice. B, BamH I; Bg, Bgl II; H, Hind III; E, EcoR I; P, Pvu II; C, Cla I; K, Kpn I; Xh, Xho I. *, mutation site. (B) Targeted ES cells were confirmed by Southern blot using 3'probe. The clones were digested by Dra I/Bgl II that generated 11.7 kb fragment for WT and 5.6 kb for recombinant allele. (C) The mutations were confirmed by direct sequencing of PCR product generated from recombinant ES clone. Non-targeted ES clone was used as WT control. (D) PCR genotyping of saposin AB mice after Cre recombination using primers B-F and B-R across loxP site. The PCR product from wild type was 200 bp, and from AB-/- without neo was 307 bp.

Figure S2. Alcian blue staining on AB-/- mice. (A) WT mouse spinal cord. (B) AB-/- mouse spinal cord. (C) AB-/- mouse brainstem. (D) WT mouse kidney. (E) AB-/- mouse kidney. (F) AB-/- mouse white matter of cerebellum. AB-/- mice showed sulfatide (blue) storage in brain, spinal cord and kidney tubules.

Figure S3. Sulfatide species levels in 10-14 weeks group by LC/MS analysis. (Upper panels) Major Sulfatide species in brain is C18:0 and C24:1 for NFA sulfatide and C18:0, C22:0, C24:1 and C24:0 for HFA sulfatide. C18:0 and C20:0 NFA and HFA sulfatides were accumulated in B-/- and AB-/- brain. (Middle panels) Long acyl chain HFA sulfatides (C20:0, C22:0, C24:0) were major species accumulated in B-/- and AB-/- mice kidneys. (Bottom panels) Accumulated sulfatide species in AB-/- mice livers included both short and long acyl chain (C16:0 to C24:0). A-/- and B-/- mice in 10-14 weeks group were at terminal stage. The ages of mice in 10-14

weeks group are: A-/- mice, 10-13 weeks; B-/- mice, 14 weeks, AB-/- mice, 12-14 weeks; and WT mice, 14 weeks. The amount of sulfatides were normalized by mg tissue weight (n=3 mice).

Figure S4. GalCer species levels in 10-14 weeks group by LC/MS analysis. In comparison to A-/- mice, AB-/- mice had decreased GalCer level in the brain and kidney. Major accumulated GalCer species were C24:1 and C18:0 in A-/- mice brains, and C16:0, C20:0, C22 and C24:0 in A-/- mice kidneys. GalCer was separated for GluCer by LC/MS analysis. Tissue samples are as described in Figure S3.

Figure S5. LacCer species levels in 10-14 weeks group by LC/MS analysis. Major accumulated LacCer species were C18:0 in the AB-/- brain, C16:0, C18:0 and C24:0 in the A-/-, B-/- and AB-/- kidney, and long acyl chain species (C22:0, C24:1 and C24:0) in the A-/- and AB-/- liver and spleen. Tissue samples are as described in Figure S3.

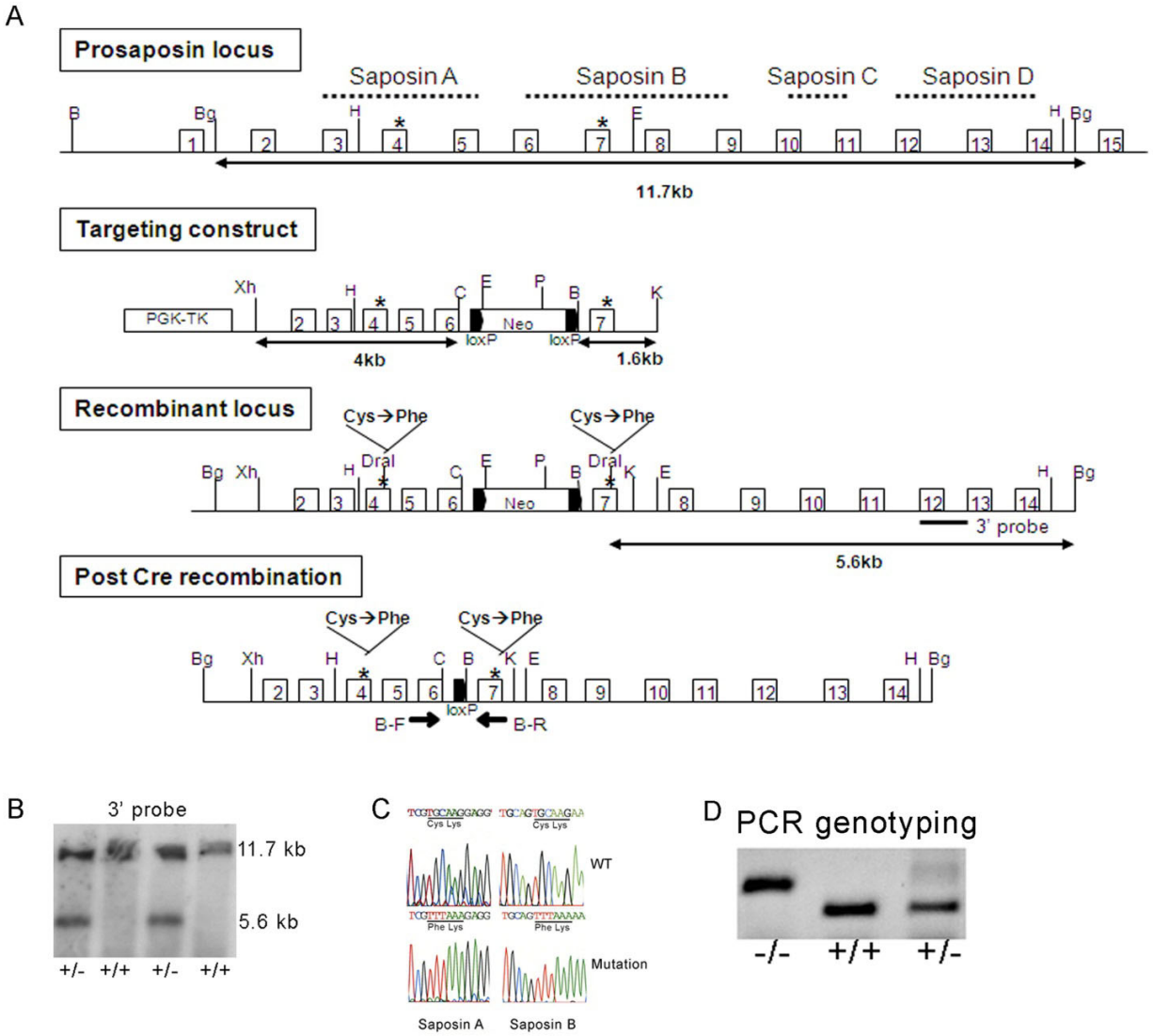
Figure S6. Lactosylceramide (LacCer) levels in liver by LC/MS analysis. Total LacCer levels increased with age in AB-/- and A-/- mice livers (n=3 mice).

Figure S7. Glucosylceramide (GluCer) levels in 10-14 weeks group by LC/MS analysis. (A) Total GluCer levels were increased in AB-/- mice brains and A-/- and AB-/- livers at 10-14 weeks of age. GluCer levels were differ between the A-/- and the B-/- and AB-/- kidney, and not altered in the spleen of those mutant mice. Brain GluCer was separated from GalCer by LC/MS. (B) GluCer species.C18:0 is the dominant in the brain and C20:0 to 24:0 are the major GluCer species in the spleen and liver. GluCer was separated from GalCer by LC/MS analysis. Tissue samples are as described in Figure S3.

Figure S8. Ceramide levels in 10-14 weeks group by LC/MS analysis. (A) Total ceramide levels in A-/- and AB-/- mice brains were reduced compared to WT. A-/- mice had significantly increased ceramide levels in the liver and spleen. Ceramide levels were not altered in the kidney of those mutants. (B) Ceramide species. C18:0 is major ceramide species in the brain. C16:0, C24:0 and C24:1 are the major species in kidney, liver and spleen. Tissue samples are as described in Figure S3.

Figure S9. TLC analyses of GSLs. (A) Globotriaosylceramide (Gb3) levels were increased in AB-/- liver and kidney. LacCer accumulation was in AB-/- mice livers and sulfatide was enhanced in AB-/- mice kidneys (n=2 mice). (B) Gangliosides in AB-/- mice brains were at same levels as that in the WT mice. WT and AB-/- mice samples were from 14 weeks old mice (n=3 mice).

Figure S1


A
Prosaposin locus
Saposin A
Saposin B
Saposin C
Saposin D
11.7kb
Targeting construct
PGK-TK
Neo
loxP
loxP
4kb
1.6kb
Recombinant locus
Cys→Phe
Cys→Phe
DraI
DraI
3' probe
5.6kb
Post Cre recombination
Cys→Phe
Cys→Phe
loxP
B-F
B-R
B
3' probe
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Saposin B
D
PCR genotyping
-/- +/+ +/-

Figure S2

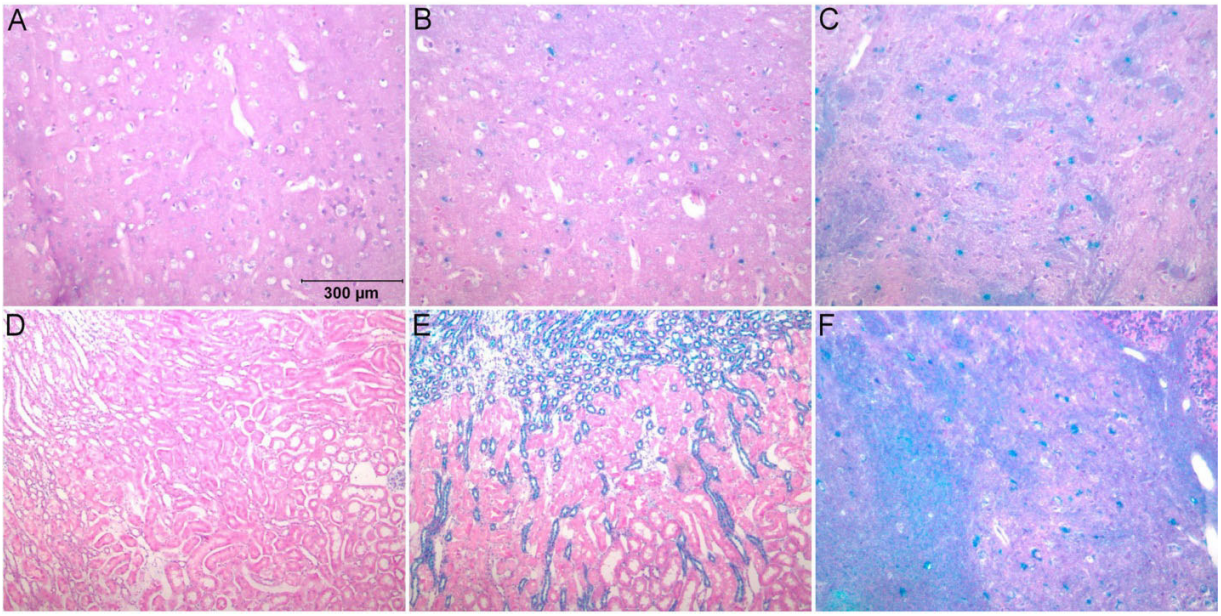


Figure S3

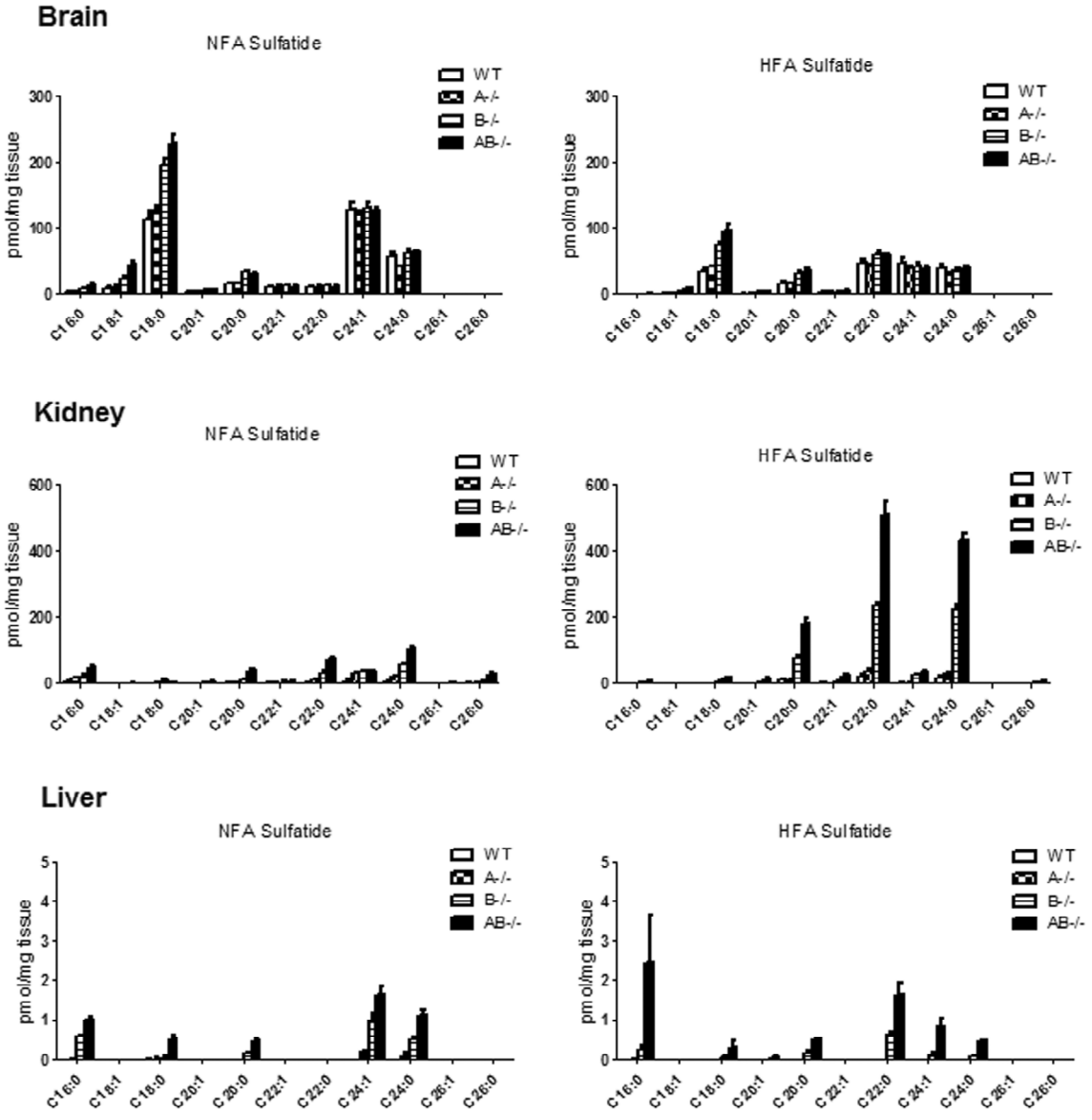
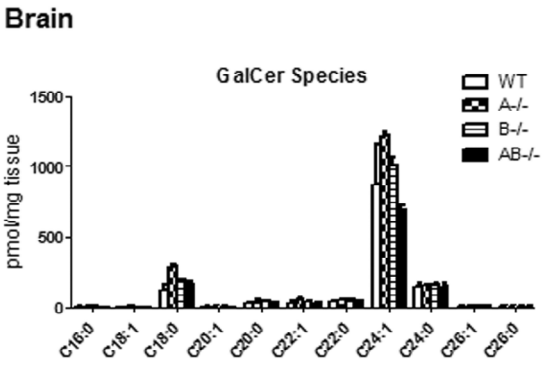

Brain
NFA Sulfatide
HFA Sulfatide
Kidney
NFA Sulfatide
HFA Sulfatide
Liver
NFA Sulfatide
HFA Sulfatide
WT
A-/-
B-/-
AB-/-
pmol/mg tissue
C16:0
C18:1
C18:0
C20:1
C20:0
C22:1
C22:0
C24:1
C24:0
C26:1
C26:0

Figure S4



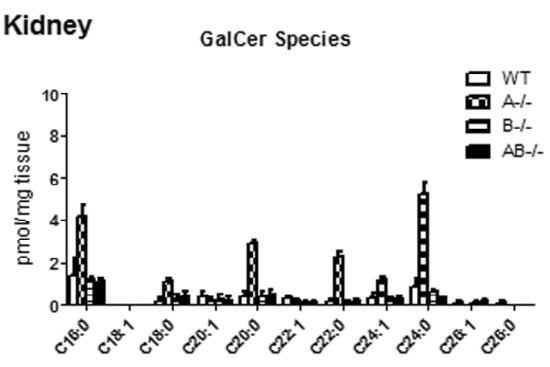
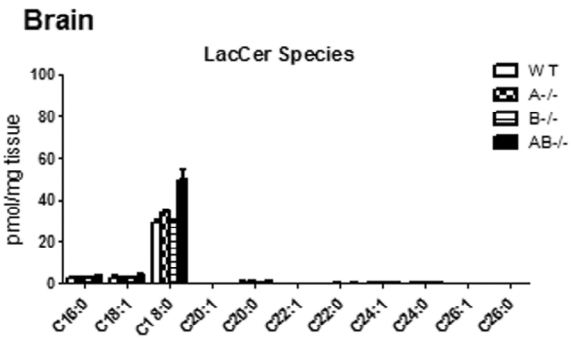
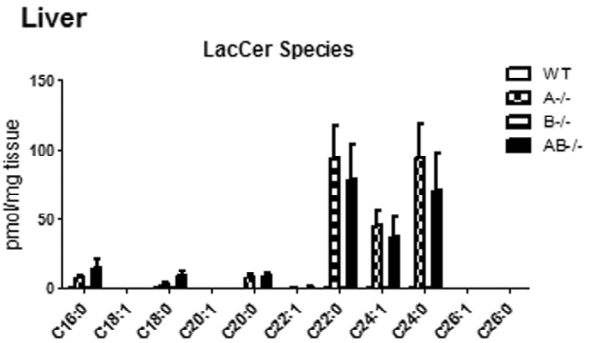
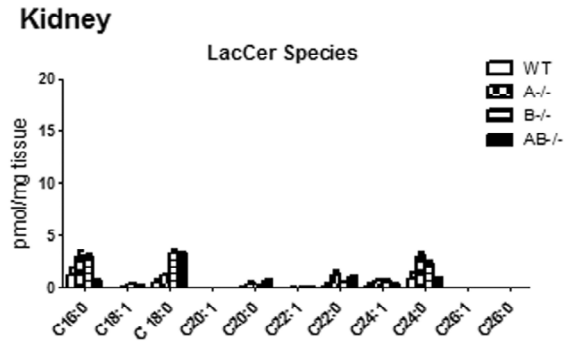


Figure S5







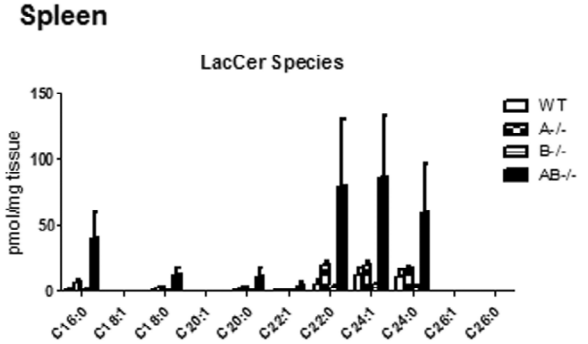


Figure S6

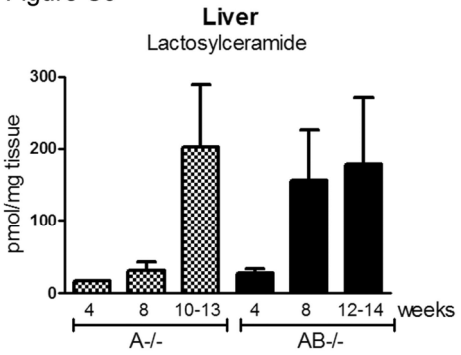
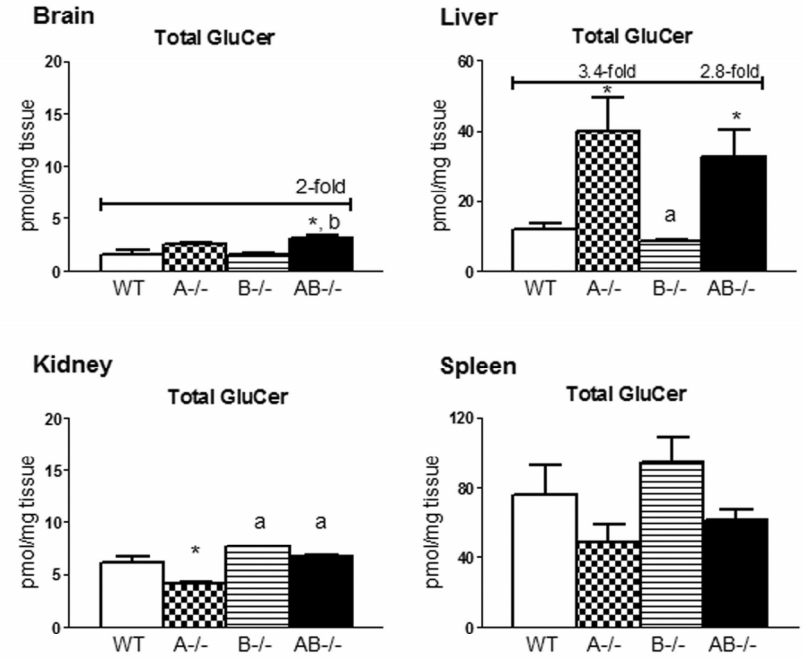


Figure S7

A


Brain
Total GluCer
pmol/mg tissue
2-fold
*, b
WT A-/- B-/- AB-/-
Liver
Total GluCer
3.4-fold
2.8-fold
*
a
Kidney
Total GluCer
a
Spleen
Total GluCer

B

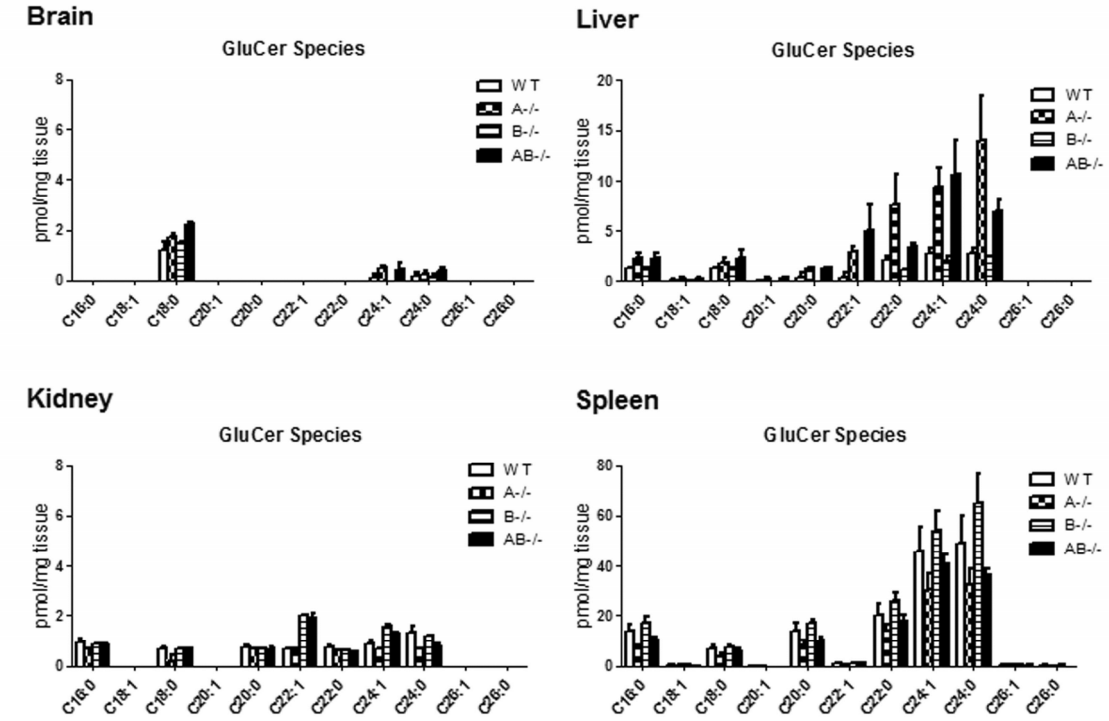

Brain
GluCer Species
pmol/mg tissue
WT
A-/-
B-/-
AB-/-
C16:0 C18:1 C18:0 C20:1 C20:0 C22:1 C22:0 C24:1 C24:0 C26:1 C26:0
Liver
Kidney
Spleen

Figure S8

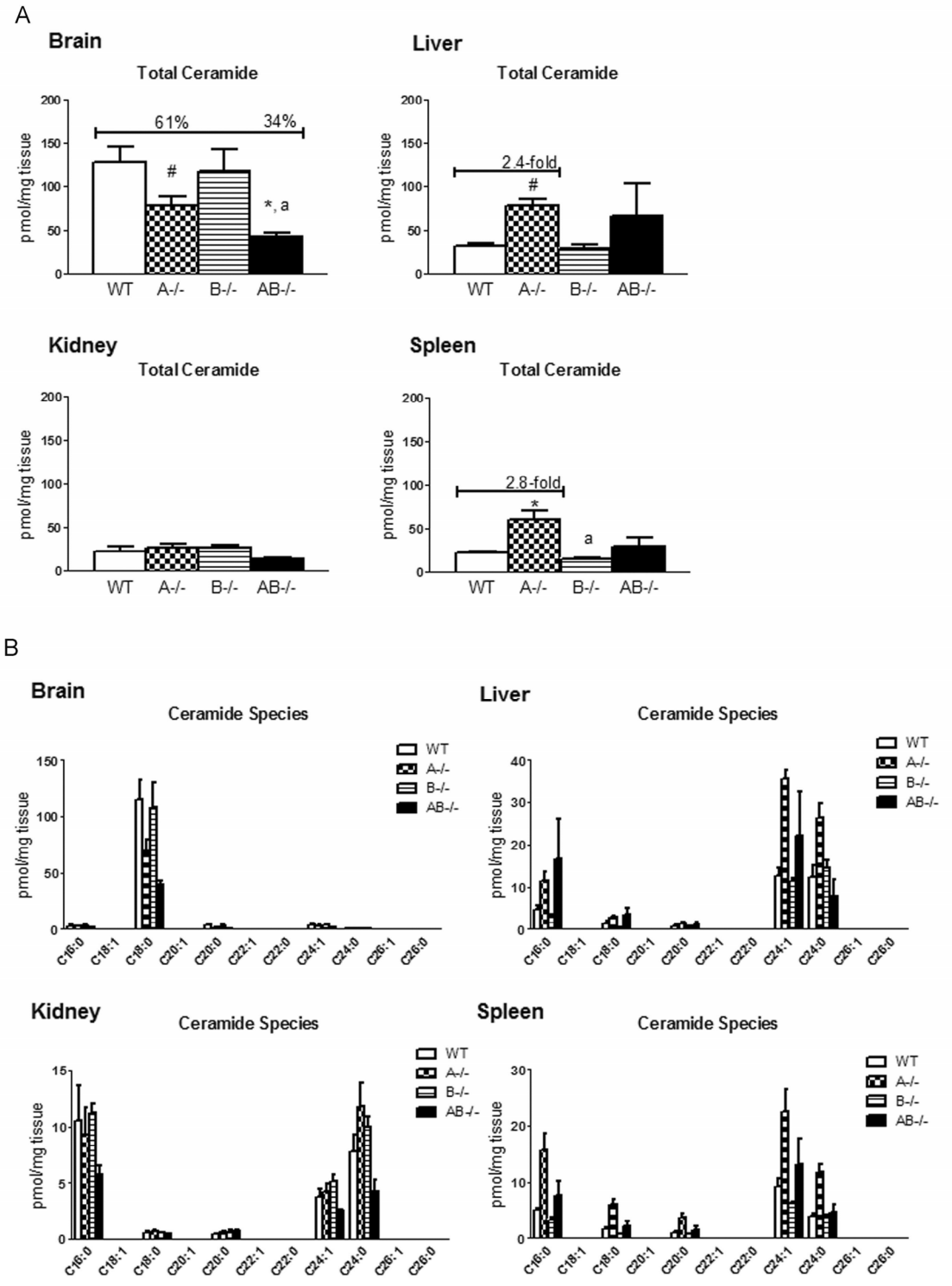
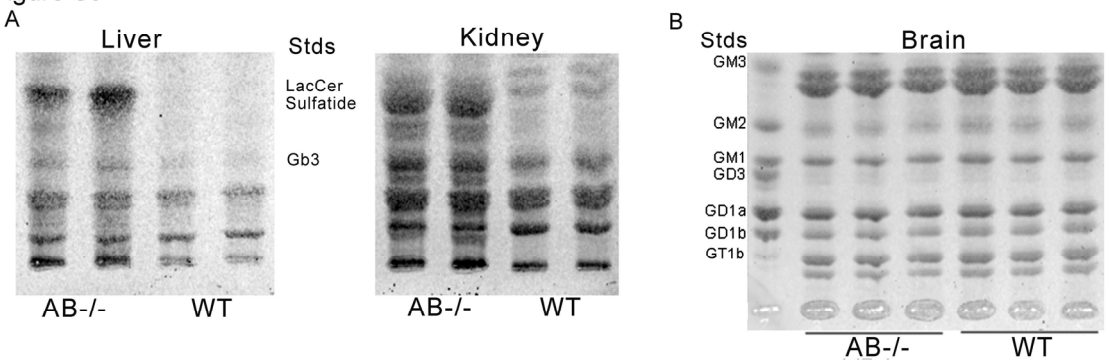

A
Brain
Total Ceramide
pmol/mg tissue
61%
34%
#
*, a
WT
A-/-
B-/-
AB-/-
Liver
Total Ceramide
2.4-fold
#
Kidney
Total Ceramide
Spleen
Total Ceramide
2.8-fold
*
a
B
Brain
Ceramide Species
Liver
Ceramide Species
Kidney
Ceramide Species
Spleen
Ceramide Species
C16:0
C18:1
C18:0
C20:1
C20:0
C22:1
C22:0
C24:1
C24:0
C26:1
C26:0

Figure S9


A
Liver
Stds
LacCer
Sulfatide
Gb3
Kidney
AB-/-
WT
AB-/-
WT
B
Stds
Brain
GM3
GM2
GM1
GD3
GD1a
GD1b
GT1b
AB-/-
WT