Supplementary materials

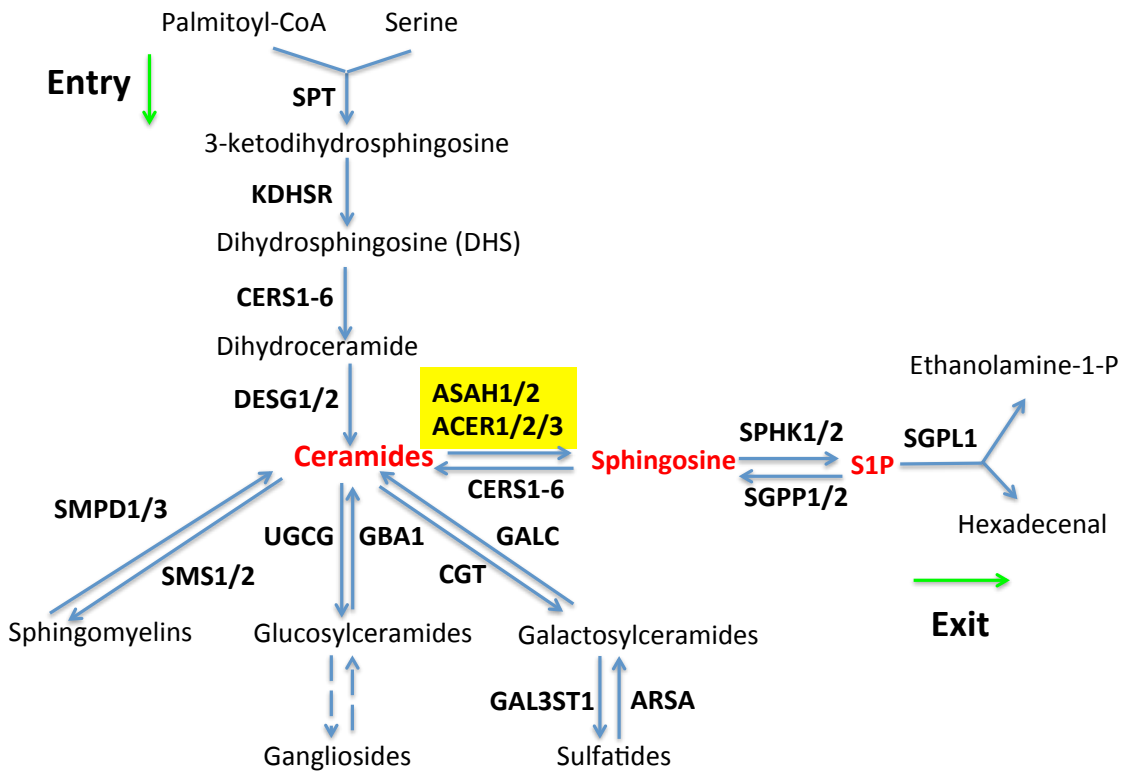

Figure S1 Metabolism of sphingolipids

S1P is formed from SPH by the action of the SPH kinases SPHK1 and SPHK2 and SPH is in turn derived from the hydrolysis of ceramides by the action of ceramidases encoded by 5 distinct genes *ASAH1*, *ASAH2*, *ACER1*, *ACER2*, and *ACER3*. S1P is cleaved into ethanolamine-1-phosphate and hexadecenal by the action of the S1P lyase SGPL1 or is dephosphorylated to form SPH by the action of the S1P phosphohydrolases SGPP1 and SGPP2.

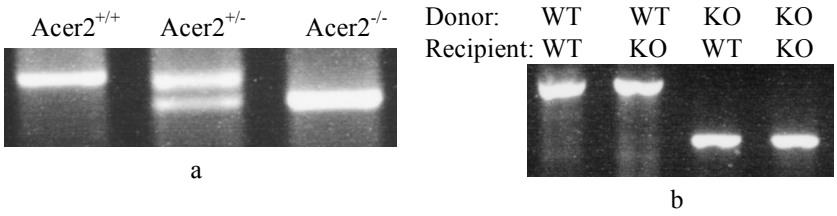

Figure S2 Genotyping of mice by PCR

a-b, tail biopsies (a) or blood samples (b) were subjected to PCR analysis for the genotypes as described under Materials and Methods. WT, *Acer2*$^{+/+}$ mice, and KO, *Acer2*$^{-/-}$ mice.

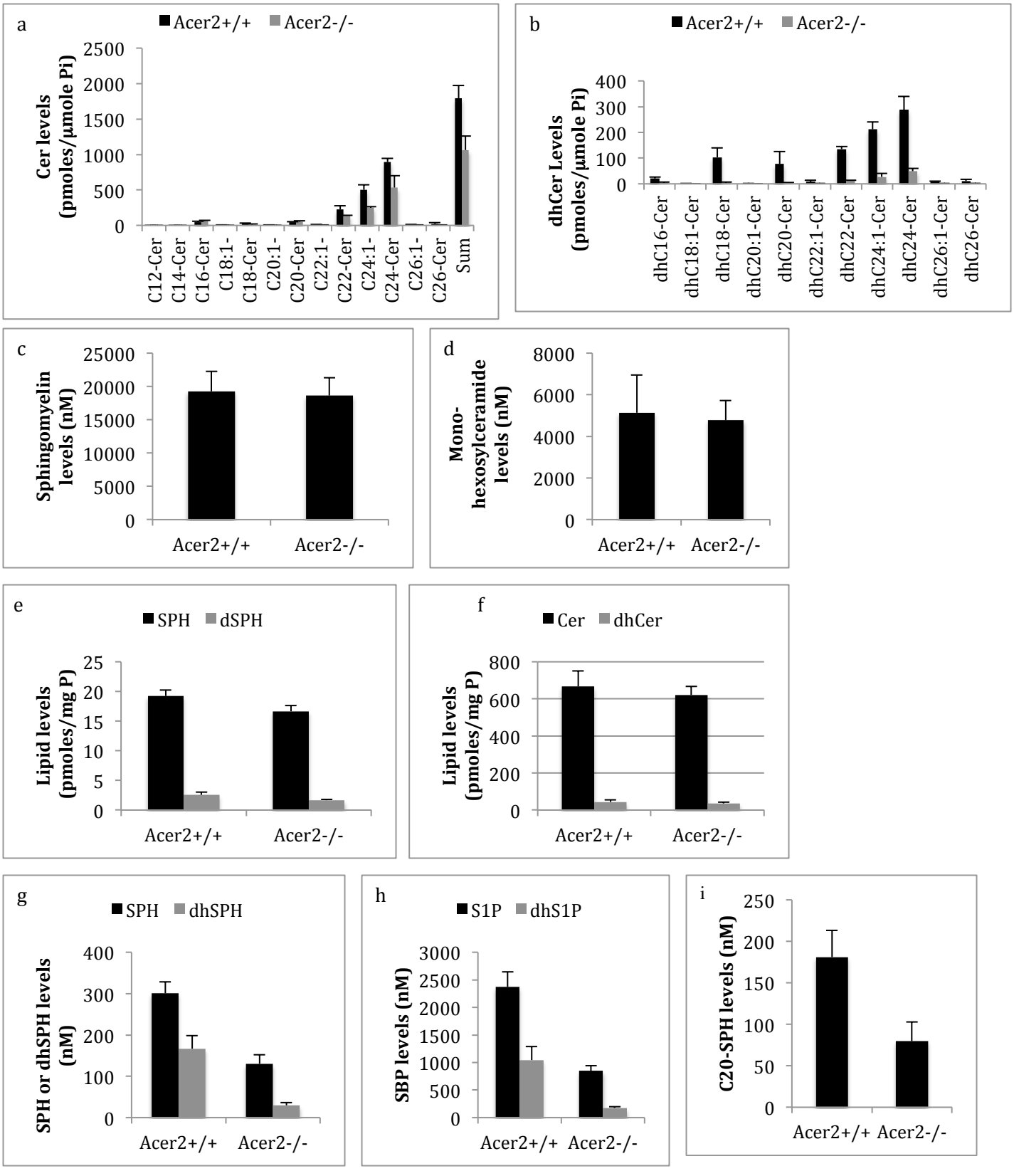

Figure S3 LC-MS/MS analysis of sphingolipids in mouse tissues

a-b, LC-MS/MS analysis of ceramides (Cer) (a) and dihydroceramides (dhCer) (b) in platelets from *Acer2*$^{+/+}$ or *Acer2*$^{-/-}$ mice.
c-d, LC-MS/MS analysis of sphingomyelins (c) and monohexosylceramides (d) in whole blood from *Acer2*$^{+/+}$ or *Acer2*$^{-/-}$ mice.
e-f, LC-MS/MS analysis of SPH/dhSPH (e) and ceramides/dihydroceramides (f) in thymus from *Acer2*$^{+/+}$ or *Acer2*$^{-/-}$ mice.
g-h, LC-MS/MS analysis of SPH/dhSPH (g) and S1P/dhS1P (h) in whole blood from *Acer2*$^{+/+}$ and *Acer2*$^{-/-}$ mice fasted overnight.
i, LC-MS/MS analysis of C20-SPH in whole blood from Acer2$^{+/+}$ and Acer2$^{-/-}$ mice. Data represent mean values ± SD, n=3 mice per genotype.

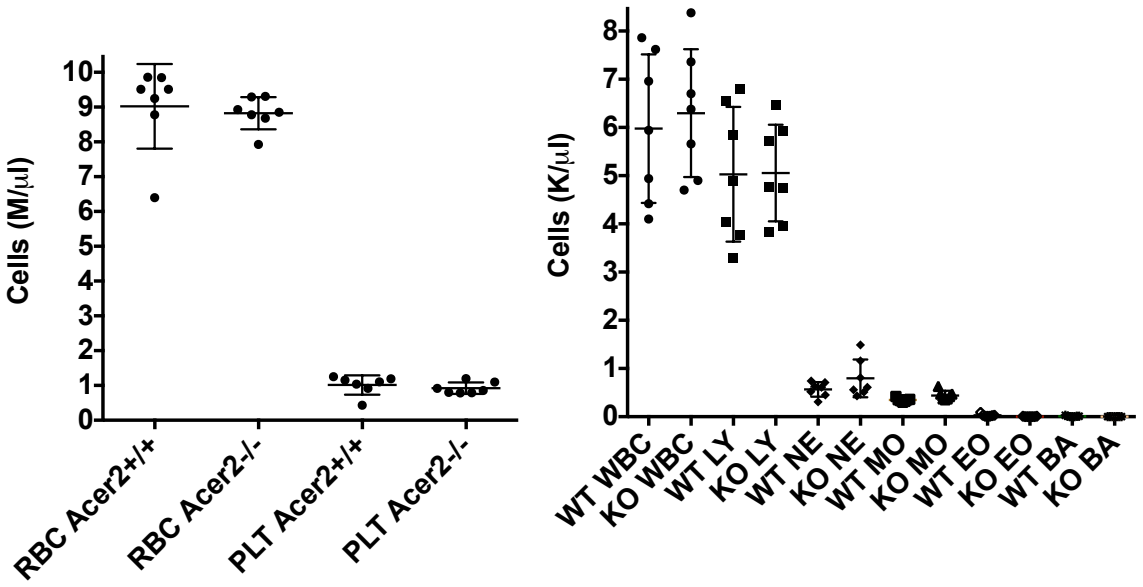

Figure S4 Complete blood count tests of *Acer2*$^{+/+}$ and *Acer2*$^{-/-}$ mice

Blood samples were collected from *Acer2*$^{+/+}$ or *Acer2*$^{-/-}$ mice at 6 weeks of age into EDTA-coated microcentrifuge and analyzed in duplicate for CBC on a Hemavet 950FS Multi Species Hematology System (Drew Scientific, CT) programmed with mouse hematology settings. Data represent mean values ± SD. M, million; K, thousand; RBC, red blood cells; PLT, platelets; WBC, white blood cells; Ly, lymphocytes; NE, neutrophils; MO, monocytes; EO, Eosinophils; BA, basophils; WT, Acer2$^{+/+}$; and KO, Acer2$^{-/-}$.

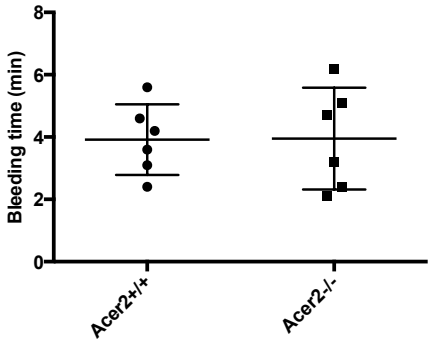

Figure S5 Hemostasis tests of *Acer2*$^{+/+}$ and *Acer2*$^{-/-}$ mice

Acer2$^{+/+}$ or *Acer2*$^{-/-}$ mice at 2 months of age were subjected to haemostasis tests as described (1). Briefly, mice were anesthetized and tails were immediately immersed in sterile saline pre-warmed at 37^{o}C after a 1.0 cm tail tip was nipped off. Bleeding time, namely time duration from tail clipping to bleeding termination, was recorded. Data represent mean values ± SD, n=6 mice per genotype.

References

1. Liu, Y., Jennings, N. L., Dart, A. M., and Du, X. J. (2012) Standardizing a simpler, more sensitive and accurate tail bleeding assay in mice. *World journal of experimental medicine* **2**, 30-36