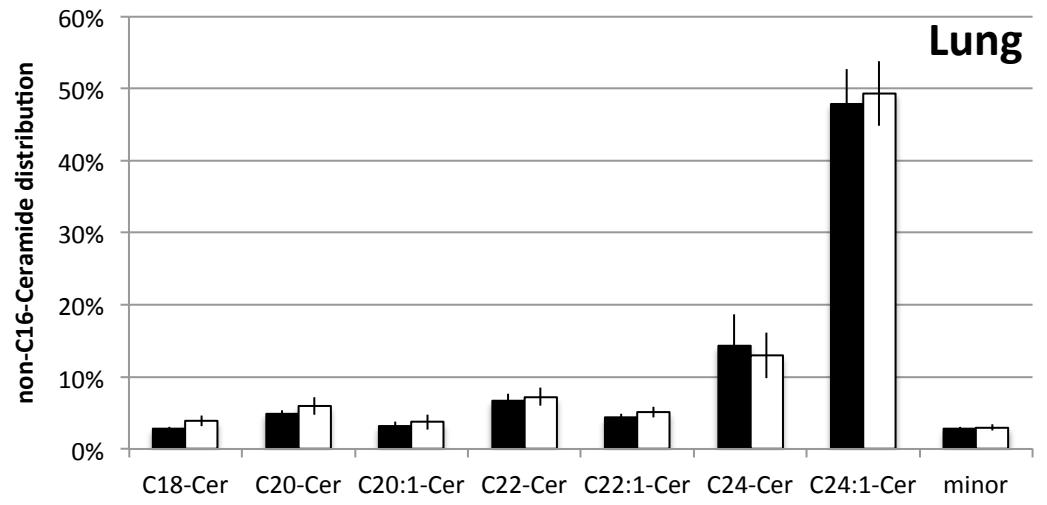
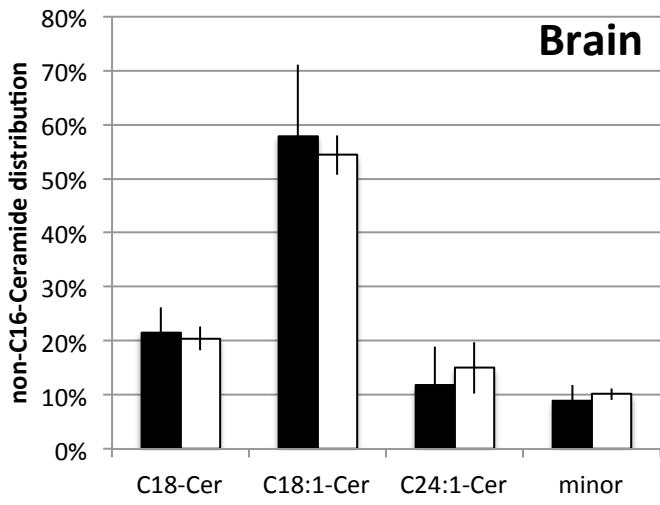
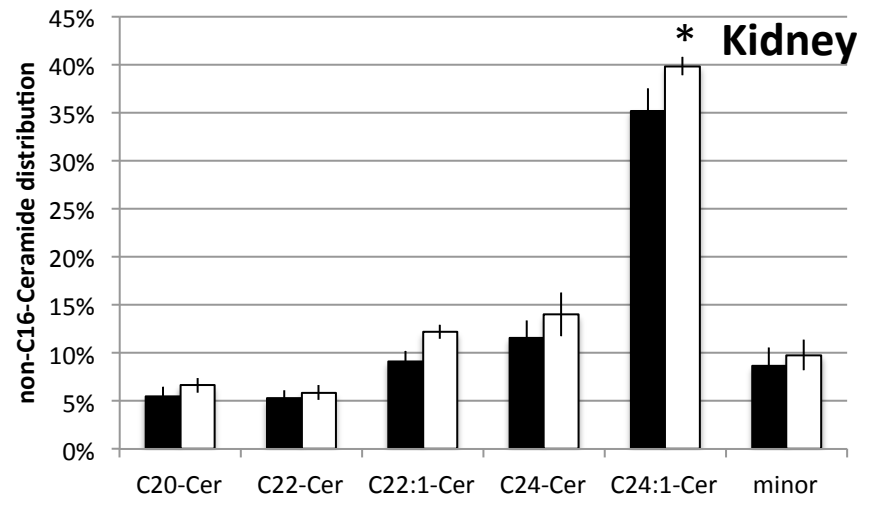
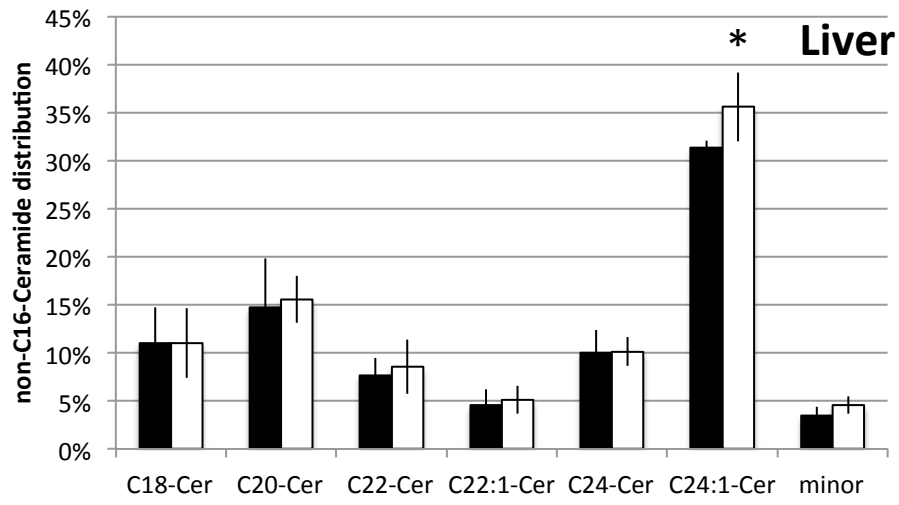


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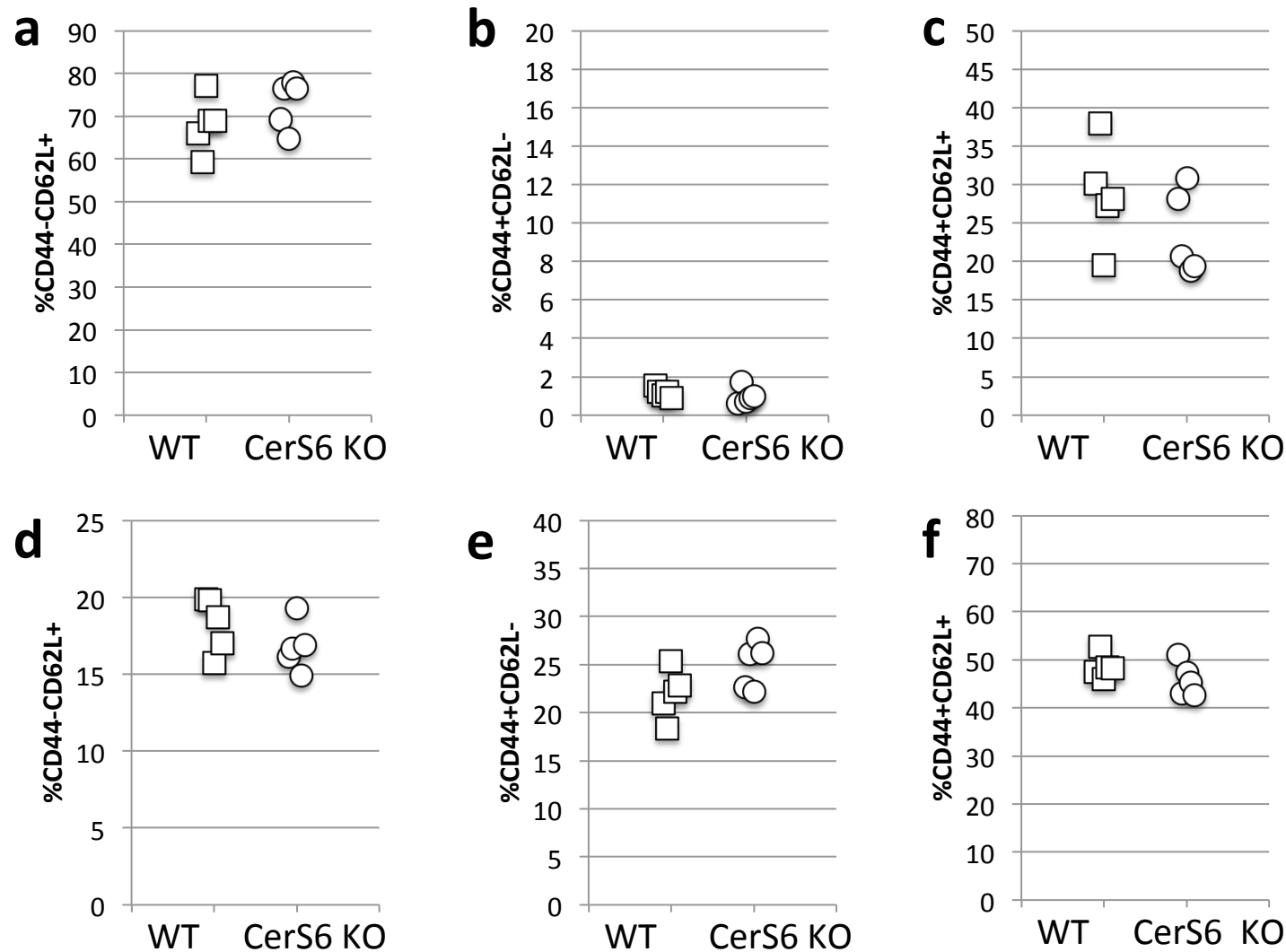
Adoptive Transfer of Ceramide Synthase 6 Deficient Splenocytes Reduces the Development of Colitis

**Authors:**

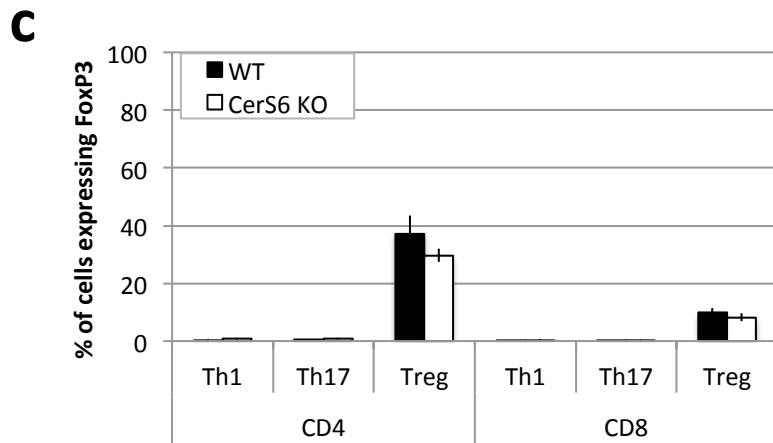
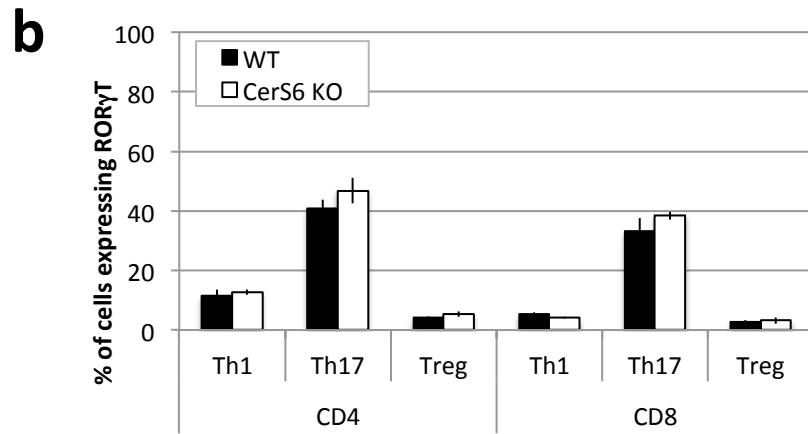
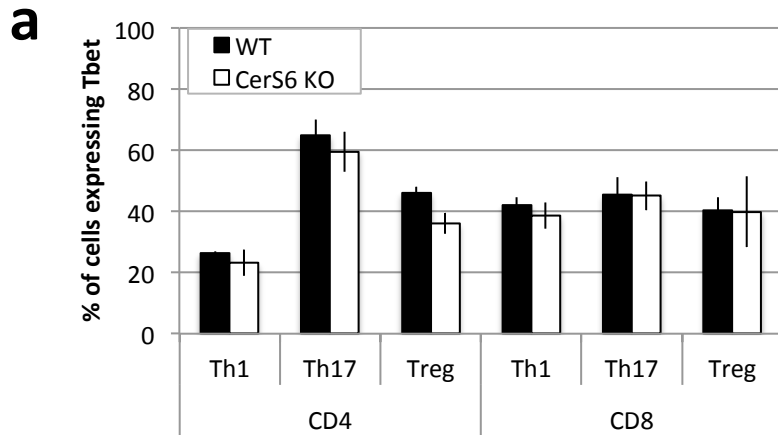
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**Figure S1. Ceramide distribution.** The percentage of non-C<sub>16</sub>-ceramides in various tissues is shown. Ceramide species representing less than 2% of the total were grouped and are shown as “minor”. A two-sample t test was used to calculate statistical significance. n=4-5. The C<sub>16</sub>-ceramides of this set of data is shown in Fig. 1b of the manuscript. Closed bars are wild type and open bars CerS6 KO mice.



**Figure S2. Splenocytes memory phenotype.** Naïve splenocytes were isolated from wild type and CerS6-deficient littermates, processed, counted and surface stained with antibodies against CD3, CD4, CD8, CD44 and CD62L. Populations were gated on CD3/CD4 (a-c) or CD3/CD8 (d-f). No differences were found in between wild type and CerS6 deficient naïve T cells (CD44-CD62L+) (a, d), effector memory T cells CD44+CD62L- (b, e) and or double positive CD44+CD62L+ (c, f). N=5. Similar results were observed in an independent experiment.



**Figure S3. Transcription factor expression following polarization.** Naïve splenocytes were isolated from 2 wild type and 2 CerS6-deficient littermates, processed, counted and 4 million cells of each culture polarized as described in the materials and methods. On day 4, cells were surface stained with antibodies against CD3, CD4, CD8, permeabilized and intracellularly stained for the transcription factors Tbet (a), ROR $\gamma$ T (b) or FoxP3 (c). Expression of ROR $\gamma$ T and FoxP3 was preferential in cells that had been polarized towards the T17 and Treg lineage, respectively. No significant differences were observed between wild type and CerS6-deficient cells. Similar results were observed in an independent repeat of this experiment.

	<b>C14</b>	<b>C16</b>	<b>C18</b>	<b>C18:1</b>	<b>C20</b>	<b>C20:1</b>	<b>C20:4</b>	<b>C22</b>	<b>C22:1</b>	<b>C24</b>	<b>C24:1</b>	<b>C26</b>	<b>C26:1</b>
<b>Liver (WT)</b>	15.9 ± 4.0	230.9 ± 61.1	137.2 ± 46.3	12.9 ± 4.6	183.8 ± 63.1	11.4 ± 2.0	13.4 ± 9.7	95.0 ± 23.4	57.0 ± 20.3	125.3 ± 28.7	391.1 ± 9.6	3.2 ± 0.7	2.4 ± 0.3
<b>Liver (CerS6 KO)</b>	8.5± 3.5*	111.2 ± 20.1**	132.5 ± 47.6	14.4 ± 4.5	205.4 ± 35.0	14.3 ± 2.2	18.6 ± 8.0	112.5 ± 41.9	69.8 ± 19.4	134.3 ± 20.3	461.9 ± 53.0*	4.8 ± 2.5	3.4 ± 1.4
<b>Kidney (WT)</b>	10.3± 0.7***	317.5± 55.6***	24.1 ± 6.8	24.0 ± 5.2	70.6 ± 12.8	37.2 ± 8.7	7.8 ± 3.4	67.4 ± 11.2	117.3 ± 14.3	148.9 ± 22.5	452.1 ± 30.2	4.5 ± 1.2	3.3 ± 0.3
<b>Kidney (CerS6 KO)</b>	5.2 ± 0.9	156.0 ± 18.8	33.2 ± 8.3	29.8 ± 5.4	88.5 ± 10.4	46.2 ± 10.6	6.5 ± 2.9	78.4 ± 10.7	162.6 ± 9.7**	186.6 ± 30.3	531.4 ± 13.0**	5.5 ± 2.7	3.9 ± 0.4
<b>Brain (WT)</b>	3.5 ± 1.7	37.1 ± 13.7	354.5 ± 90.6	1122.9 ± 259	29.6 ± 10.3	38.1 ± 4.5	6.6 ± 3.1	22.7 ± 11.3	16.0 ± 4.1	26.0 ± 20.6	297.6 ± 140	1.3 ± 0.7	3.3 ± 2.7
<b>Brain (CerS6 KO)</b>	4.3 ± 0.2	29.7 ± 6.0	395.2 ± 42.1	1053.0 ± 71.6	34.9 ± 2.3	48.0 ± 2.1	8.7 ± 4.4	28.2 ± 5.6	16.4 ± 3.6	22.8 ± 4.7	291.2 ± 92.5	1.0 ± 0.2	2.8 ± 0.8
<b>Lung (WT)</b>	1.9 ± 0.4	55.1 ± 7.4	11.9 ± 1.2	5.6 ± 0.9	20.7 ± 1.9	13.3 ± 2.6	0.3 ± 0.0	28.1 ± 3.9	18.4 ± 2.0	60.1 ± 18.5	201.1 ± 20.5	1.7 ± 0.5	2.3 ± 0.5
<b>Lung (CerS6 KO)</b>	1.2 ± 0.2*	42.5 ± 4.3*	18.6 ± 3.6	8.4 ± 2.2	28.7 ± 6.1	18.0 ± 5.2	34.9 ± 0.2	28.1 ± 5.9	24.6 ± 3.6	62.5 ± 15.2	237.6 ± 21.3	1.9 ± 0.9	2.4 ± 0.5
<b>Splenocytes (WT)</b>	0.6 ± 0.3	14.7 ± 1.7	5.8 ± 0.1	0.7 ± 0.1	4.1 ± 0.4	0.4 ± 0.0	0.1 ± 0.1	5.7 ± 1.1	1.2 ± 0.5	8.6 ± 3.7	23.7 ± 6.0	0.8 ± 0.0	0.4 ± 0.2
<b>Splenocytes (CerS6 KO)</b>	0.5 ± 0.2	8.6 ± 1.2	7.6 ± 1.7	0.8 ± 0.2	4.3 ± 1.4	0.3 ± 0.0	BDL	8.1 ± 2.3	1.4 ± 0.0	12.5 ± 2.6	26.8 ± 0.6	0.7 ± 0.4	0.3 ± 0.1

**Supplementary Table 1. Distribution of ceramide species in tissues and splenocytes.** Tissue or splenocytes from 4-5 mice were analyzed by LC-MS. For tissue data is expressed as pmol ceramide/mg protein (\*1000). For splenocytes, data is expressed as pmol ceramide /5 million cells. BDL=below detectable levels. \*p<0.05, \*\*p<0.005, \*\*\*p<0.0005.