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## Supplemental Data

### **A thyroid hormone based strategy for correcting the biochemical abnormality in X-linked adrenoleukodystrophy (X-ALD)**

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**Supplemental Table 1.** Serum C26-LPC levels. For the short-term 7 day and 28 day experiments, the control mice were grouped together for statistical analysis due to high variability in C26-LPC levels in the control cohorts. The means of the two control groups were identical ( $106.5 \pm 12.5$  for 7 day and  $104.5 \pm 16.3$  for 28 day). Values represent mean  $\pm$  SEM. Statistical analysis is performed with pairwise two-tailed student t-tests with comparison to the control *Abcd1*(y/-) group and the exact P value is reported. In addition, statistical significance is determined by ANOVA analysis with Dunnett's post-test with comparisons to the control *Abcd1*(y/-) group in each experiment (NS = not significant P value  $> 0.05$ , \*P  $\leq 0.05$ , \*\*P  $< 0.01$ ).

	Duration	N	C26-LPC (ng/ $\mu$ L)	t-test P value	ANOVA analysis	Figure
Wild type (C57BL6/J)	N/A	4	$5.8 \pm 0.9$	-	-	2, 4, 6, 7
Control <i>Abcd1</i> (y/-)	7 day/28 day	11	$106.5 \pm 9.5$	-	-	2, 3
Hypothyroid	8 weeks	12	$117.5 \pm 5.7$	0.28	NS	2
Hyperthyroid (1 mg/kg ip)	7 day	6	$55.7 \pm 4.5$	0.002	**	2, 3
Sobetirome (0.1 mg/kg ip)	7 day	6	$67.3 \pm 11.7$	0.03	*	3
Sobetirome (1.0 mg/kg ip)	7 day	6	$52.5 \pm 6.1$	0.002	**	3
Hyperthyroid (1 mg/kg ip)	28 day	6	$74.3 \pm 8.5$	0.04	*	4
Sobetirome (0.1 mg/kg ip)	28 day	4	$68.2 \pm 5.8$	0.04	*	4
Sobetirome (1.0 mg/kg ip)	28 day	4	$71.1 \pm 4.7$	0.05	NS	4
Control juvenile <i>Abcd1</i> (y/-) (chow)	12 weeks	7	$165.4 \pm 10.7$	-	-	6
Hyperthyroid juvenile (4 mg/kg chow)	12 weeks	4	$62.3 \pm 5.5$	$<0.0001$	**	6
Sobetirome juvenile (0.4 mg/kg chow)	12 weeks	3	$83.0 \pm 4.1$	0.0013	**	6
Sobetirome juvenile (2.0 mg/kg chow)	11 weeks	5	$56.2 \pm 6.3$	$<0.0001$	**	6
Sobetirome juvenile (0.4 mg/kg chow)	18 weeks	2	$83.2 \pm 11.0$	0.007	**	6
Control adult <i>Abcd1</i> (y/-) (chow)	12 weeks	4	$135.7 \pm 7.1$	-	-	7
Hyperthyroid adult (4 mg/kg chow)	12 weeks	6	$36.5 \pm 3.7$	$<0.0001$	**	7
Sobetirome adult (0.4 mg/kg chow)	12 weeks	5	$81.6 \pm 8.2$	0.002	**	7
Sobetirome adult (2.0 mg/kg chow)	11 weeks	7	$44.5 \pm 2.6$	$<0.0001$	**	7

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23 **Supplemental Table 2.** Adrenal gland total VLCFA levels. Values represent mean  $\pm$  SEM. Statistical analysis is performed with pairwise two-  
 24 tailed student t-tests with comparison to the control *Abcd1*(y/-) group and the exact P value is reported. In addition, statistical significance is  
 25 determined by ANOVA analysis with Dunnett's post-test with comparisons to the control *Abcd1*(y/-) group in each experiment (NS = not  
 26 significant P value > 0.05, \*P  $\leq$  0.05, \*\*P < 0.01).

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	Duration	N	C22 (ng/gland)	C26 (ng/gland)	C26/C22	t test P value	ANOVA analysis (C26/C22)	Figure
Wild type (C57BL6/J)	N/A	6	99.1 $\pm$ 21.7	3.7 $\pm$ 0.8	0.04 $\pm$ 0.01	-	-	2, 4, 6, 7
Control <i>Abcd1</i> (y/-)	7 day	6	89.3 $\pm$ 14.2	94.6 $\pm$ 15.6	1.06 $\pm$ 0.05	-	-	2, 3
Hypothyroid	8 weeks	11	242.3 $\pm$ 15.7	356.5 $\pm$ 28.4	1.46 $\pm$ 0.05	<0.0001	**	2
Hyperthyroid (1 mg/kg ip)	7 day	5	138.9 $\pm$ 62.0	114.8 $\pm$ 61.9	0.74 $\pm$ 0.09	0.009	*	2, 3
Sobetirome (0.1 mg/kg ip)	7 day	5	76.0 $\pm$ 17.2	70.2 $\pm$ 18.8	0.91 $\pm$ 0.11	0.24	NS	3
Sobetirome (1.0 mg/kg ip)	7 day	3	120.0 $\pm$ 18.4	92.6 $\pm$ 12.3	0.79 $\pm$ 0.13	0.05	NS	3
Control <i>Abcd1</i> (y/-)	28 day	5	139.4 $\pm$ 33.8	155 $\pm$ 39.1	1.12 $\pm$ 0.04	-	-	4
Hyperthyroid (1 mg/kg ip)	28 day	6	416.5 $\pm$ 39.9	328.1 $\pm$ 44.3	0.78 $\pm$ 0.05	0.0006	**	4
Sobetirome (0.1 mg/kg ip)	28 day	5	249.9 $\pm$ 40.7	231.7 $\pm$ 38.5	0.93 $\pm$ 0.06	0.03	NS	4
Sobetirome (1.0 mg/kg ip)	28 day	4	242.1 $\pm$ 64.7	223.8 $\pm$ 44.4	0.88 $\pm$ 0.04	0.006	*	4
Control juvenile <i>Abcd1</i> (y/-) (chow)	12 weeks	9	65.8 $\pm$ 13.9	89.9 $\pm$ 16.0	1.55 $\pm$ 0.14	-	-	6
Hyperthyroid juvenile (4 mg/kg chow)	12 weeks	5	246.2 $\pm$ 21.7	100.4 $\pm$ 15.0	0.42 $\pm$ 0.07	0.0001	**	6
Sobetirome juvenile (0.4 mg/kg chow)	12 weeks	5	225.8 $\pm$ 10.4	167.7 $\pm$ 11.9	0.74 $\pm$ 0.02	0.002	**	6
Sobetirome juvenile (2.0 mg/kg chow)	11 weeks	5	287.8 $\pm$ 13.8	127.5 $\pm$ 13.5	0.44 $\pm$ 0.04	0.0001	**	6
Sobetirome juvenile (0.4 mg/kg chow)	18 weeks	2	222.0 $\pm$ 4.4	137.6 $\pm$ 2.7	0.62 $\pm$ 0.01	0.02	**	6
Control adult <i>Abcd1</i> (y/-) (chow)	12 weeks	4	116.4 $\pm$ 6.1	134.9 $\pm$ 3.4	1.17 $\pm$ 0.04	-	-	7
Hyperthyroid adult (4 mg/kg chow)	12 weeks	6	344.9 $\pm$ 56.5	186.1 $\pm$ 22.1	0.56 $\pm$ 0.04	<0.0001	**	7
Sobetirome adult (0.4 mg/kg chow)	12 weeks	5	146.9 $\pm$ 37.0	171.4 $\pm$ 13.7	0.94 $\pm$ 0.05	0.02	**	7
Sobetirome adult (2.0 mg/kg chow)	11 weeks	7	302.1 $\pm$ 27.5	187.5 $\pm$ 12.2	0.63 $\pm$ 0.04	<0.0001	**	7

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31 **Supplemental Table 3.** Brain total VLCFA levels. Values represent mean  $\pm$  SEM. Statistical analysis is performed with pairwise two-tailed  
 32 student t-tests with comparison to the control *Abcd1(y/-)* group and the exact P value is reported. In addition, statistical significance is determined  
 33 by ANOVA analysis with Dunnett's post-test with comparisons to the control *Abcd1(y/-)* group in each experiment (NS = not significant P value >  
 34 0.05, \*P  $\leq$  0.05, \*\*P < 0.01).

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	Duration	N	C22 (ng/mg tissue)	C26 (ng/mg tissue)	C26/C22	t-test P value	ANOVA analysis (C26/C22)	Figure
Wild type (C57BL6/J)	N/A	4	105.6 $\pm$ 12.1	1.7 $\pm$ 0.6	0.016 $\pm$ 0.006	-	-	2, 4, 6, 7
Control <i>Abcd1(y/-)</i>	7 day	6	153.4 $\pm$ 6.8	30.0 $\pm$ 1.2	0.196 $\pm$ 0.004	-	-	2, 3
Hypothyroid	8 weeks	13	129.7 $\pm$ 3.3	26.5 $\pm$ 1.4	0.204 $\pm$ 0.009	0.56	NS	2
Hyperthyroid (1 mg/kg ip)	7 day	6	150.8 $\pm$ 7.2	28.8 $\pm$ 1.7	0.190 $\pm$ 0.004	0.29	NS	2, 3
Sobetirome (0.1 mg/kg ip)	7 day	6	158.0 $\pm$ 7.8	29.4 $\pm$ 1.1	0.187 $\pm$ 0.003	0.09	NS	3
Sobetirome (1.0 mg/kg ip)	7 day	6	151.1 $\pm$ 5.7	28.3 $\pm$ 0.6	0.188 $\pm$ 0.004	0.14	NS	3
Control <i>Abcd1(y/-)</i>	28 day	5	118.3 $\pm$ 10.4	23.6 $\pm$ 2.3	0.199 $\pm$ 0.011	-	-	4
Hyperthyroid (1 mg/kg ip)	28 day	6	118.5 $\pm$ 5.9	22.7 $\pm$ 2.6	0.189 $\pm$ 0.017	0.64	NS	4
Sobetirome (0.1 mg/kg ip)	28 day	5	113.2 $\pm$ 6.6	23.7 $\pm$ 1.8	0.209 $\pm$ 0.005	0.46	NS	4
Sobetirome (1.0 mg/kg ip)	28 day	4	125.6 $\pm$ 0.9	24.3 $\pm$ 2.0	0.194 $\pm$ 0.150	0.77	NS	4
Control juvenile <i>Abcd1(y/-)</i> (chow)	12 weeks	9	174.2 $\pm$ 9.4	31.6 $\pm$ 1.5	0.182 $\pm$ 0.002	-	-	6
Hyperthyroid juvenile (4 mg/kg chow)	12 weeks	5	189.2 $\pm$ 9.1	32.8 $\pm$ 1.3	0.174 $\pm$ 0.002	0.03	NS	6
Sobetirome juvenile (0.4 mg/kg chow)	12 weeks	5	203.8 $\pm$ 8.9	31.5 $\pm$ 0.9	0.155 $\pm$ 0.003	<0.0001	**	6
Sobetirome juvenile (2.0 mg/kg chow)	11 weeks	5	198.2 $\pm$ 10.3	31.8 $\pm$ 1.5	0.161 $\pm$ 0.003	<0.0001	**	6
Sobetirome juvenile (0.4 mg/kg chow)	18 weeks	2	193.9 $\pm$ 5.4	29.1 $\pm$ 0.9	0.150 $\pm$ 0.001	<0.0001	**	6
Control adult <i>Abcd1(y/-)</i> (chow)	12 weeks	4	168.9 $\pm$ 3.0	35.3 $\pm$ 1.7	0.209 $\pm$ 0.006	-	-	7
Hyperthyroid adult (4 mg/kg chow)	12 weeks	6	157.9 $\pm$ 5.3	30.6 $\pm$ 0.8	0.194 $\pm$ 0.004	0.09	NS	7
Sobetirome adult (0.4 mg/kg chow)	12 weeks	5	162.8 $\pm$ 4.8	28.7 $\pm$ 1.3	0.176 $\pm$ 0.003	0.01	**	7
Sobetirome adult (2.0 mg/kg chow)	11 weeks	7	170.5 $\pm$ 1.8	31.5 $\pm$ 0.6	0.184 $\pm$ 0.002	0.002	**	7

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37 **Supplemental Table 4.** Brain C26-LPC levels. Values represent mean  $\pm$  SEM. Statistical analysis is performed with pairwise two-tailed student t-  
38 tests with comparison to the control *Abcd1(y/-)* group and the exact P value is reported. In addition, statistical significance is determined by  
39 ANOVA analysis with Dunnett's post-test with comparisons to the control *Abcd1(y/-)* group in each experiment (NS = not significant P value >  
40 0.05, \*P  $\leq$  0.05, \*\*P < 0.01). The C26-LPC values in the brain were not determined for the short-term 28 day experiments.  
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	Duration	N	C26-LPC (ng/mg protein)	t-test P value	ANOVA analysis (C26-LPC)	C26-LPC/C22	t-test P value	ANOVA analysis (C26- LPC)	Figure
Wild type (C57BL6/J)	N/A	4	5.0 $\pm$ 0.3	-	-	0.0011 $\pm$ 0.0001	-	-	2, 4, 6, 7
Control <i>Abcd1(y/-)</i>	7 day	6	23.0 $\pm$ 4.4	-	-	0.0183 $\pm$ 0.0044	-	-	2, 3
Hypothyroid	8 weeks	13	32.8 $\pm$ 1.5	0.02	*	0.0280 $\pm$ 0.0014	0.02	*	2
Hyperthyroid (1 mg/kg ip)	7 day	6	23.2 $\pm$ 1.9	0.96	NS	0.0180 $\pm$ 0.0014	0.96	NS	2, 3
Sobetirome (0.1 mg/kg ip)	7 day	6	28.9 $\pm$ 4.6	0.38	NS	0.0220 $\pm$ 0.0036	0.52	NS	3
Sobetirome (1.0 mg/kg ip)	7 day	6	33.8 $\pm$ 7.0	0.22	NS	0.0248 $\pm$ 0.0049	0.34	NS	3
Control juvenile <i>Abcd1(y/-)</i> (chow)	12 weeks	9	24.0 $\pm$ 0.6	-	-	0.0100 $\pm$ 0.0003	-	-	6
Hyperthyroid juvenile (4 mg/kg chow)	12 weeks	5	18.2 $\pm$ 1.1	0.0002	**	0.0090 $\pm$ 0.0006	0.17	NS	6
Sobetirome juvenile (0.4 mg/kg chow)	12 weeks	5	20.9 $\pm$ 0.3	0.002	*	0.0073 $\pm$ 0.0006	0.0002	**	6
Sobetirome juvenile (2.0 mg/kg chow)	11 weeks	5	19.9 $\pm$ 1.2	0.004	**	0.0073 $\pm$ 0.0004	0.0001	**	6
Sobetirome juvenile (0.4 mg/kg chow)	18 weeks	2	19.0 $\pm$ 0.5	0.003	**	0.0071 $\pm$ 0.0001	0.004	**	6
Control adult <i>Abcd1(y/-)</i> (chow)	12 weeks	4	31.6 $\pm$ 0.9	-	-	0.0127 $\pm$ 0.0004	-	-	7
Hyperthyroid adult (4 mg/kg chow)	12 weeks	6	26.6 $\pm$ 0.3	0.0003	**	0.0116 $\pm$ 0.0004	0.07	NS	7
Sobetirome adult (0.4 mg/kg chow)	12 weeks	5	25.9 $\pm$ 1.2	0.009	**	0.0109 $\pm$ 0.0003	0.008	**	7
Sobetirome adult (2.0 mg/kg chow)	11 weeks	7	27.3 $\pm$ 0.7	0.005	**	0.0112 $\pm$ 0.0003	0.01	**	7

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42 **Supplemental Table 5.** Testes total VLCFA levels. Values represent mean  $\pm$  SEM. Statistical analysis is performed with pairwise two-tailed  
 43 student t-tests with comparison to the control *Abcd1(y/-)* group and the exact P value is reported. In addition, statistical significance is determined  
 44 by ANOVA analysis with Dunnett's post-test with comparisons to the control *Abcd1(y/-)* group in each experiment (NS = not significant P value >  
 45 0.05, \*P  $\leq$  0.05, \*\*P < 0.01).  
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	Duration	N	C22 (ng/mg tissue)	C26 (ng/mg tissue)	C26/C22	t-test P value	ANOVA analysis	Figure
Wild type (C57BL6/J)	N/A	4	15.7 $\pm$ 0.5	0.16 $\pm$ 0.03	0.010 $\pm$ 0.002	-	-	6, 7
Control juvenile <i>Abcd1(y/-)</i> (chow)	12 weeks	5	15.6 $\pm$ 0.3	2.22 $\pm$ 0.05	0.143 $\pm$ 0.005	-	-	6, 7
Sobetirome juvenile (0.4 mg/kg chow)	12 weeks	4	17.4 $\pm$ 0.7	1.04 $\pm$ 0.19	0.059 $\pm$ 0.007	<0.0001	**	6
Sobetirome juvenile (2.0 mg/kg chow)	11 weeks	4	16.7 $\pm$ 1.3	0.91 $\pm$ 0.15	0.054 $\pm$ 0.006	<0.0001	**	6
Sobetirome juvenile (0.4 mg/kg chow)	18 weeks	2	17.8 $\pm$ 0.3	1.16 $\pm$ 0.02	0.065 $\pm$ 0.002	0.0004	**	6
Sobetirome adult (0.4 mg/kg chow)	12 weeks	5	15.5 $\pm$ 1.1	1.19 $\pm$ 0.17	0.076 $\pm$ 0.008	0.0001	**	7
Sobetirome adult (2.0 mg/kg chow)	11 weeks	7	16.5 $\pm$ 0.9	1.13 $\pm$ 0.14	0.067 $\pm$ 0.005	<0.0001	**	7

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49 **Supplemental Table 6.** Sobetirome levels in blood and brain. Values represent mean  $\pm$  SEM (N). Sobetirome was measured in serum and brain  
 50 samples from the terminus of the 28 Day i.p. dosing and 12 week chow dosing experiments using LC-MS/MS following previously published  
 51 methodology (37) with the following modifications. The serum sample (25  $\mu$ l) was extracted with 125  $\mu$ l acetonitrile and an extracted standard  
 52 curve ranging from 1 – 2000 ng/ml was prepared in control serum. The brain homogenate (125  $\mu$ l, 200 mg tissue/ml) was extracted with 500  $\mu$ l of  
 53 acetonitrile and an extracted standard curve ranging from 1 – 100 ng/g tissue was prepared in control brain homogenate. \*Values are outside of  
 54 standard curve.

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	<b>28 Day 0.1 mg/kg sobetirome i.p.</b>	<b>28 Day 1.0 mg/kg sobetirome i.p.</b>	<b>12 week 0.4 mg/kg sobetirome chow</b>	<b>12 week 2 mg/kg sobetirome chow</b>
Brain (ng/g tissue)	1.1 $\pm$ 0.7 (4)	4.5 $\pm$ 3.0 (3)	0.3 $\pm$ 0.1* (3)	4.0 $\pm$ 0.7 (11)
Serum (ng/ml)	19.0 $\pm$ 3.8 (5)	102.2 $\pm$ 12.0 (5)	13.3 $\pm$ 1.7 (7)	87.0 $\pm$ 13.6 (14)
Brain/Serum	0.09 $\pm$ 0.07 (4)	0.04 $\pm$ 0.02 (3)	0.02 $\pm$ 0.01 (3)	0.07 $\pm$ 0.01 (11)

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