**Beneficial effects of the direct AMP-Kinase activator PXL770 in *in vitro* and *in vivo* models of X-Linked Adrenoleukodystrophy**

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## Supplementary Material

### Supplementary Methods

#### *In vitro*

AMPK and ACC phosphorylation

Protein extraction was performed as follow: the cells were washed with cold Tris-buffered saline (20 mM Trizma base and 137 mM NaCl, pH 7.5) and lysed in 1X SDS sample-loading buffer (62.5 mM Trizma base, 2% [w/v] SDS,10% glycerol), and after sonication and centrifugation at 15,000 g for 5 min, the supernatant was used for the immunoblot assay. The protein concentration of samples was determined with the detergent compatible protein assay reagent (Bio-Rad) using BSA as the standard. The sample was boiled for 3 min with 0.1 volumes of 10% β-mercaptoethanol and 0.5% bromphenol blue mix. Then, 40 µg of total cellular protein was resolved by electrophoresis on 8 or 12% polyacrylamide gels, electrotransferred and blocked with Tween 20-containing Tris-buffered saline (TBST; 10 mM Trizma base, pH 7.4, 1% Tween 20, and 150 mM NaCl) with 5% skim milk. After incubation with antibodies at 4°C overnight, the membranes were then washed with TBST and incubated with horseradish peroxidase-conjugated anti-rabbit or mouse IgG for 1H at room temperature. The membranes were detected by autoradiography using ECL-plus (Amersham Biosciences) after washing with TBST buffer.

mRNA levels/ RT-qPCR

The primers sequences used are listed in the supplementary table 1.

***In vivo***

*Microscopy analysis*

Tissues were prepared as follows. The mice were deeply anesthetized using an inhalation mixture of isoflurane: $N_2O$:$O_2$ (2:68:30). The chest cavity was opened at the level of diaphragm, retracted anteriorly and held using an artery forceps. An 18 G blunt needle connected to a peristaltic pump with a normal saline reservoir was introduced into the aortic arch through the atrium. Saline injection was begun, and the vena cava nicked to allow blood washout. When the effluent ran clear, saline was replaced by the fixative (2.5% glutardialdehyde in a 4% solution of paraformaldehyde in PBS). Injection of the fixative was stopped after rigor set in.

The body of the mouse was placed prone on a dissection board. The dorsal skin was removed to expose the gluteus muscles bilaterally. The sciatic nerves were located using previously developed methods [3] and stored in the fixative solution until processed.

**References**


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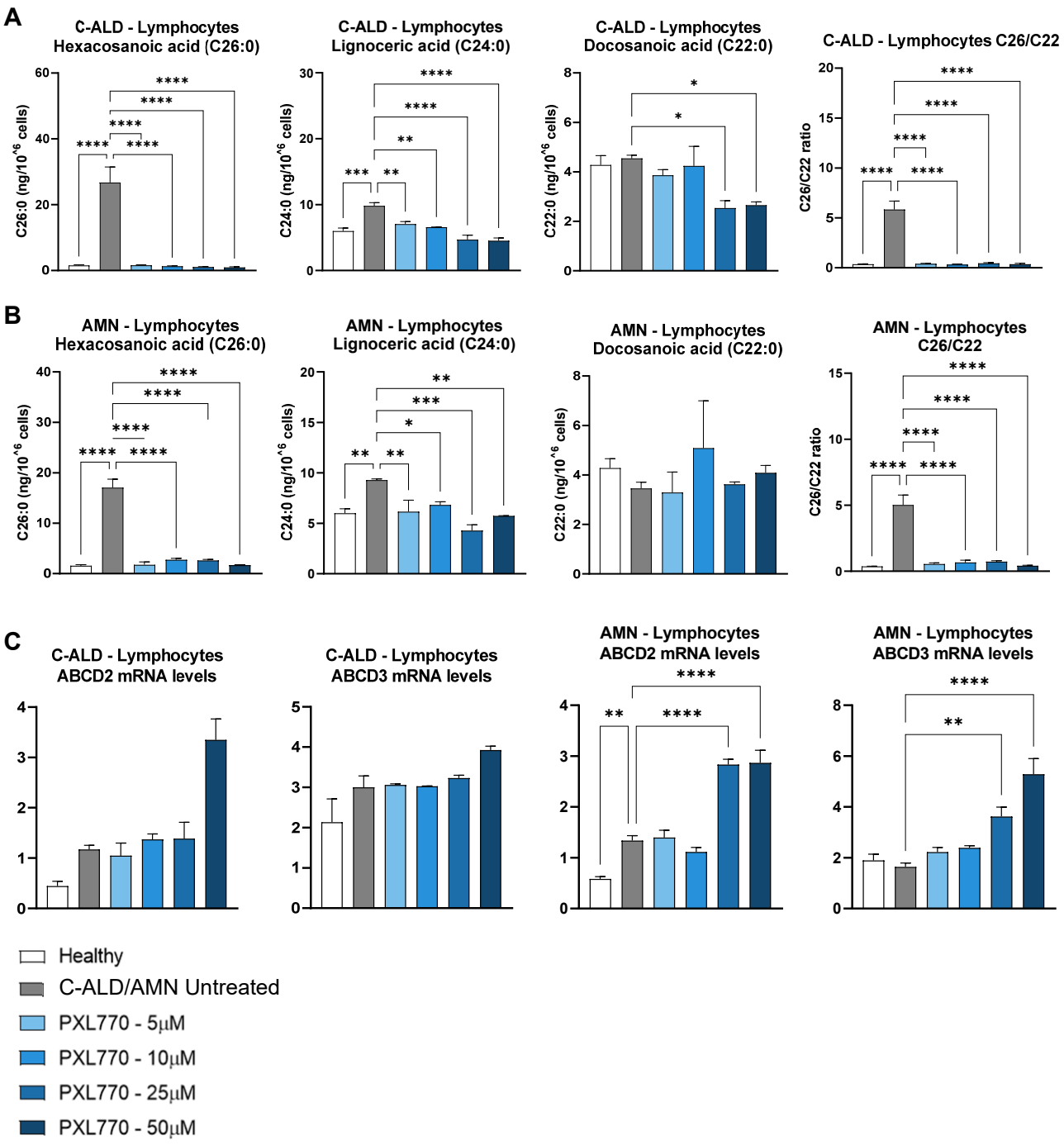
2. Hellmuth, C., M. Weber, B. Koletzko, and W. Peissner, Nonesterified fatty acid determination for functional lipidomics: comprehensive ultrahigh performance liquid chromatography-tandem mass spectrometry quantitation, qualification, and parameter prediction. Anal Chem 2012;84(3):1483-90.
3. Bala, U., K.-L. Tan, K.-H. Ling, and P.-S. Cheah, Harvesting the maximum length of sciatic nerve from adult mice: a step-by-step approach. BMC Research Notes 2014;7(1):714.
4. Pujol, A., C. Hindelang, N.l. Callizot, U. Bartsch, M. Schachner, and J.L. Mandel, Late onset neurological phenotype of the X-ALD gene inactivation in mice: a mouse model for adrenomyeloneuropathy. Human Molecular Genetics 2002;11(5):499-505.

**Supplementary Tables**

| | *Gene* | *Forward* | *Reverse* |
|---|---|---|---|
| **Human** | **ABCD2** | GATAACTGGTCCCAATGGTTG | TCCCGAAGACTTCCAAGAGA |
| | **ABCD3** | ACCCCTCTCAGTCTGCAGTATTG | TGATACATGGTAACCCCTCCTTGT |
| | **NF-kB** | TGAACCGAAACTCTGGCAGCTG | CATCAGCTTGCGAAAAGGAGCC |
| | **CCL5** | CCTGCTGCTTTGCCTACATTGC | ACACACTTGGCGGTTCTTTCGG |
| | **CCR3** | TACTCCCTGGTGTTCACTGTGG | ACGAGGAAGAGCAGGTCCGAAA |
| | **NOS2** | GCTCTACACCTCCAATGTGACC | CTGCCGAGATTTGAGCCTCATG |
| | **RPL27** | TGGACAAAACTGTCGTCAATAAGG | AGAACCACTTGTTCTTGCCTGTC |
| **Mouse** | **Abcd2** | CCATAGCAAGCGTGGAGGTAAC | CACTTCGCCCGCTGGTGTAATT |
| | **Abcd3** | CTGGGAACACTGAGAGACCAAG | CTTCTCGCTCAAGGATGTGACC |
| | **Nos2** | GAGACAGGGAAGTCTGAAGCAC | CCAGCAGTAGTTGCTCCTCTTC |
| | **Ccl5** | CCTGCTGCTTTGCCTACCTCTC | ACACACTTGGCGGTTCCTTCGA |
| | **NfkB** | TCCTGTTCGAGTCTCCATGCAG | GGTCTCATAGGTCCTTTTGCGC |
| | **Ccr2** | GCTGTGTTTGCCTCTCTACCAG | CAAGTAGAGGCAGGATCAGGCT |
| | **Ccr4** | GGACTAGGTCTGTGCAAGATCG | TGCCTTCAAGGAGAATACCGCG |
| | **Ccr7** | AGAGGCTCAAGACCATGACGGA | TCCAGGACTTGGCTTCGCTGTA |
| | **Il1β** | TGGACCTTCCAGGATGAGGACA | GTTCATCTCGGAGCCTGTAGTG |
| | **Tnfα** | GGTGCCTATGTCTCAGCCTCTT | GCCATAGAACTGATGAGAGGGAG |
| | **Il6rα** | TGCAGTTCCAGCTTCGATACCG | TGCTTCACTCCTCGCAAGGCAT |
| | **Rpl27** | ACATTGACGATGGCACCTC | ACATTGACGATGGCACCTC |

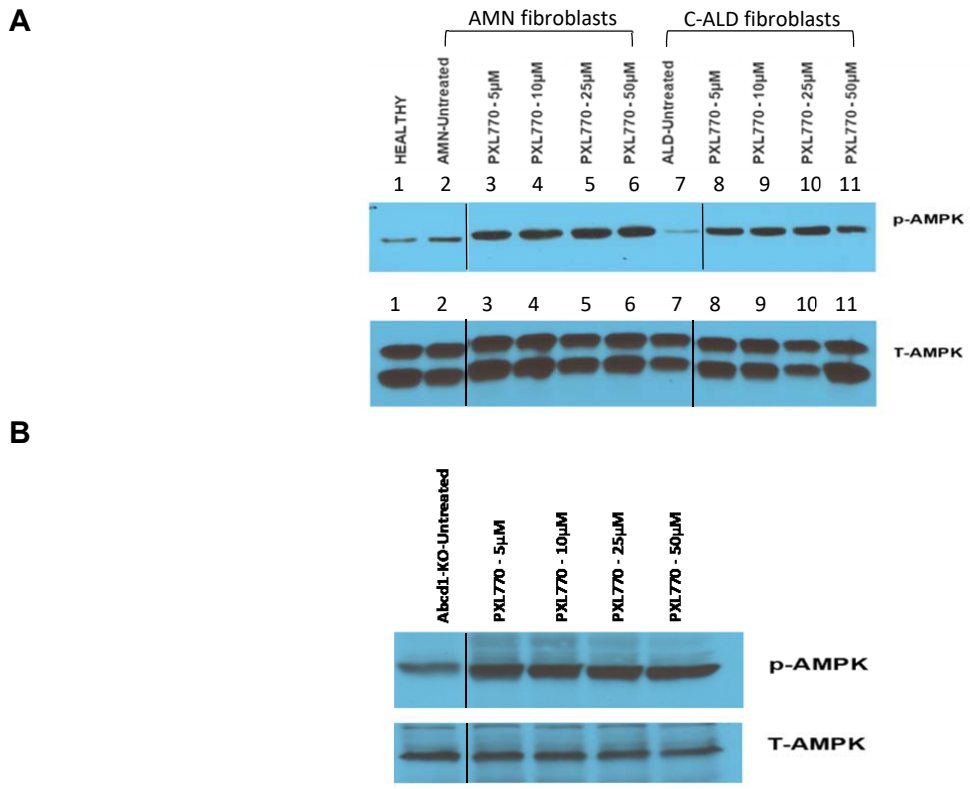
**Supplementary Table 1** : primers sequences used in the article.

## Supplementary Figures




**Supplementary Figure 1 : PXL770 treatment improve VLCFA markers in C-ALD and AMN patients lymphocytes.** Lymphocytes were isolated from **A)** C-ALD and **B)** AMN patients and were exposed for 7 days to PXL770 at 5, 10, 25 and 50 µM. VLCFA levels were analyzed by LC-MS after extraction of total lipids from pelleted cells. Data are mean ±SEM n=3 replicates. **C)** Lymphocytes were isolated from C-ALD/AMN patients and were exposed

for 72 hours to the treatment and mRNA levels were evaluated by RT-qPCR, normalized by RLP27 expression (no unit). Data are mean ±SEM, n=3 replicates. * p≤ 0.05, ** p≤0.01, *** p≤0.001, **** p≤0.0001 (Dunnett's or Kruskal Wallis test vs untreated).




**Supplementary Figure 2 : PXL770 treatment induces AMPK and ACC phosphorylation.** Phosphorylated AMPK (p-AMPK) and total AMPK (T-AMPK; subunits α1 α2) levels measured by western blot (representative images) in **A)** AMN (2-6) and C-ALD (7-11) fibroblasts and **B)** *Abcd1* KO glial cells treated for 72 hours with PXL770 at 5, 10, 25 and 50 µM. 40 µg of proteins were loaded on a 8-12% polyacrylamide gel. Lines indicate where images were edited to remove unrelated experimental conditions. Tracks were numbered in **A)** to help identify the tracks.