LYSOSOMAL ACID LIPASE DEFICIENCY IMPAIRS REGULATION OF ABCA1 AND FORMATION OF HIGH DENSITY LIPOPROTEINS IN CHOLESTERYL ESTER STORAGE DISEASE*

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Running title: Impaired ABCA1 expression in Cholesteryl Ester Storage Disease

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Supplemental Data

Supplemental Figure Legends:

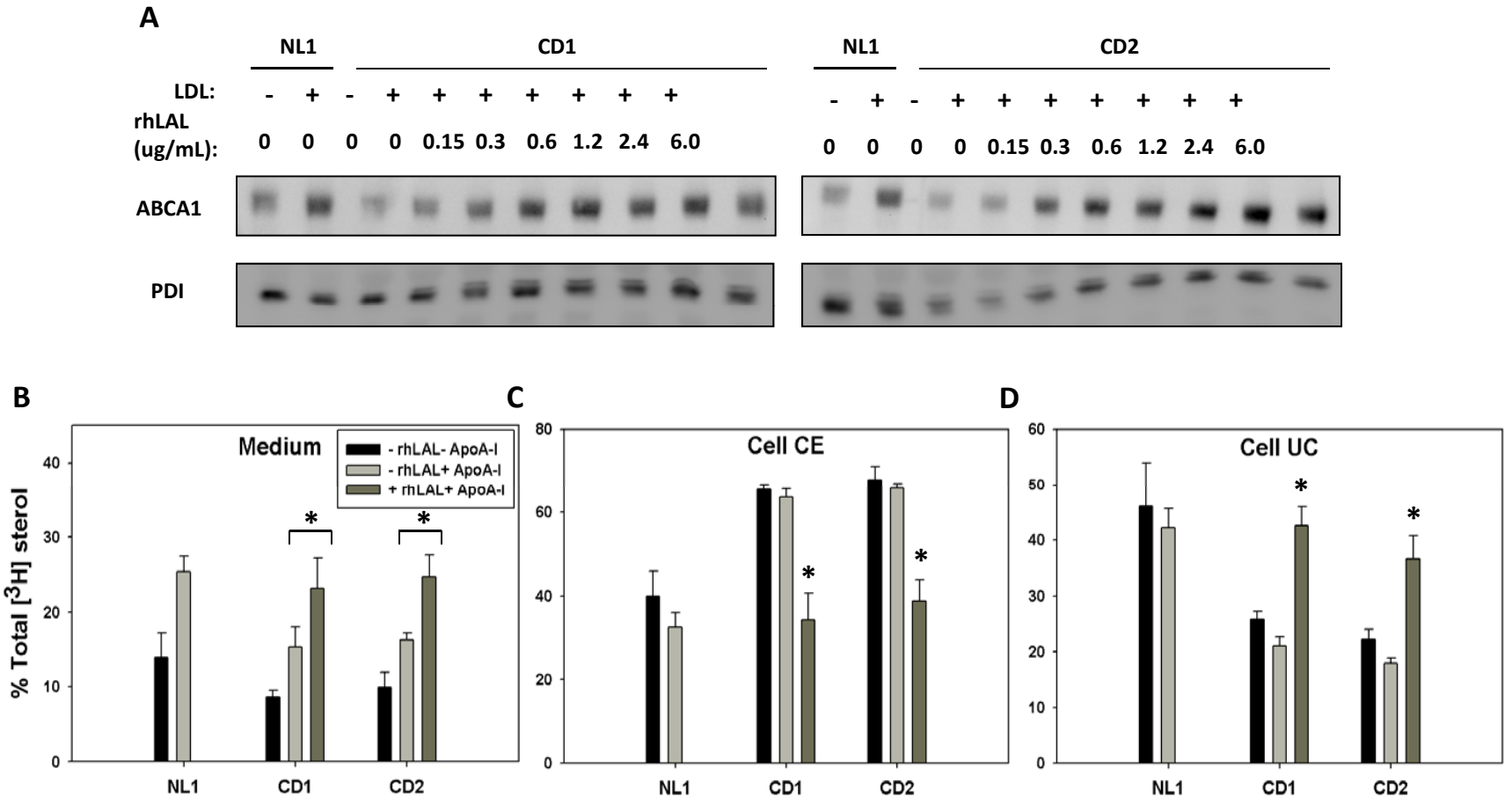
Supplemental Figure 1. Recombinant human LAL rescues ABCA1 expression and apoA-I-mediated cholesterol efflux in CESD fibroblasts. Normal (NL1) and CESD (CD1, CD2) fibroblasts were grown to confluence in DMEM containing 5% LPDS. Recombinant human LAL (rhLAL) was added to CD1 and CD2 fibroblasts at the concentrations indicated (μg/ml medium) (**A**) or 1.2 μg/ml rhLAL (**B,C,D**) for 24 hours. Cell monolayers were then incubated in DMEM with (+) or without (-) 50 μg/ml LDL (**A**) or 50 μg/ml [^{3}H]cholesteryl-linoleate LDL (**B,C,D**) for 24 h. ABCA1 protein in cell lysates was analyzed by western blot (**A**) as described in Figure 1. Cells were incubated with 10 μg/ml apoA-I for 24 h and [^{3}H]cholesterol in the medium and cell lipid extracts was determined as in Figure 2. Results indicate the percent of total [^{3}H]cholesterol in the medium (**B**), cell CE (**C**) or cell UC (**D**), mean ± SD of triplicates, and are representative of 3 experiments with similar results. *, values significantly greater (**B,C**) or lower (**D**) than non-conditioned medium-treated CESD cells, $p < 0.05$.

Supplemental Figure 2. Reduced α-HDL particle formation by CESD fibroblasts is rescued following treatment with recombinant human LAL. Fibroblasts were treated as in Supplemental figure 1 with or without 1.2 μg/ml recombinant human LAL (rhLAL or no addition, NA). Efflux medium following a subsequent 24 h incubation with 10 μg/ml apoA-I was concentrated to 1/10 volume and 20 μl of each sample was run in the first dimension on 0.75 % agarose and, separated in the second dimension on a 5-23% gradient gel, and analyzed by western blot using a rabbit polyclonal antibody to human apoA-I. Boxed areas represent α-HDL particles. Blots are representative of 2 experiments with similar results.

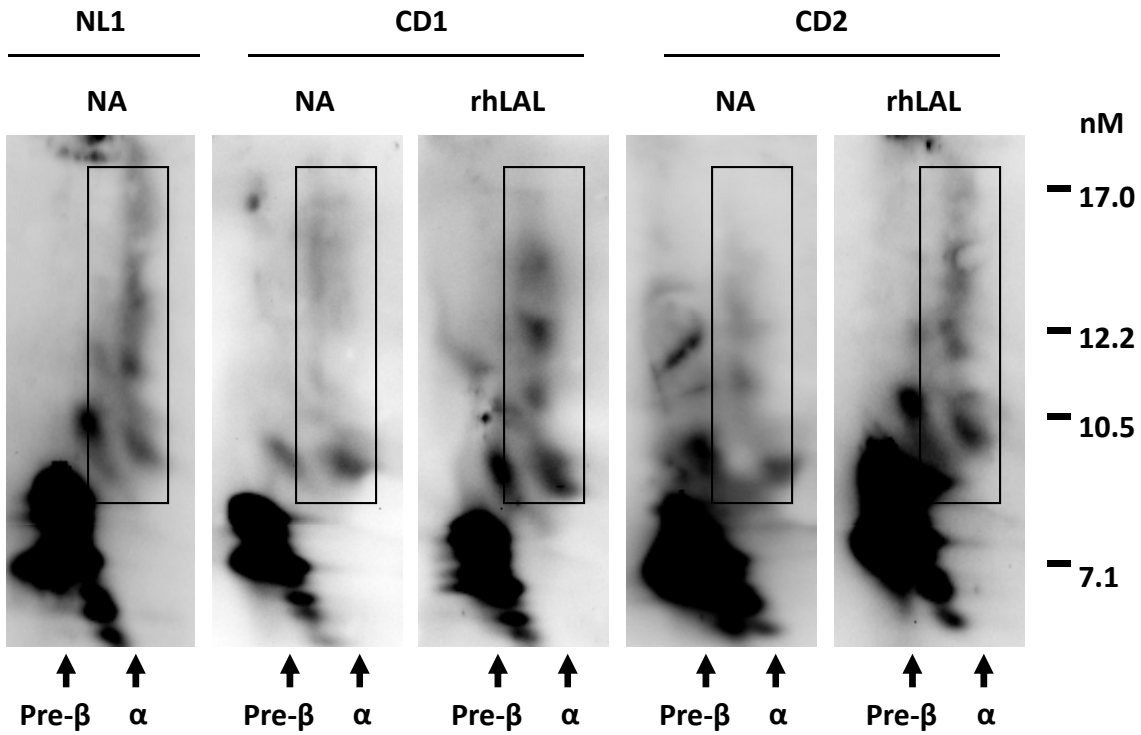
Supplemental Figure 3. 27-HC formation following treatment with recombinant human LAL. Normal (NL1) and CESD (CD1,CD2) fibroblasts were treated as in figure 5, either with (+rhLAL) or without treatment with recombinant human LAL. Lipids from cells and media were extracted and the pooled lipid fractions were separated by gas chromatography and the mass of 27-HC was quantified as described in Experimental Procedures. Results represent the difference

between LDL-loaded and non-LDL-loaded cells, mean ± SD of triplicates, and are representative of four experiments with similar results.

Supplemental Figure 1



Supplemental Figure 2



Supplemental Figure 3