Chylomicronemia mutations yield new insights into interactions between lipoprotein lipase and GPIHBP1

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LEGENDS FOR SUPPLEMENTARY FIGURES

Figure S1. Western blot assay of LPL binding to GPIHBP1-expressing CHO-K1 cells. We tested the ability of LPL-V5, LPL-V5 (G409R), LPL-V5 (E410V) to bind to GPIHBP1. Both G409R and E410V render LPL susceptible to an endoproteolytic cleavage event that releases an ~28-kDa carboxyl-terminal fragment (containing the V5 tag). The medium from LPL-transfected cells (or medium alone) was incubated with CHO-K1 cells expressing S-protein–tagged GPIHBP1 at 4°C in the presence or absence of heparin (500 U/ml). After 2 h, cell extracts were prepared and western blots were performed. GPIHBP1 was detected with a rabbit antibody against the S-protein tag, and LPL was detected with a mouse antibody against the V5 tag. Antibodies against actin were used as a loading control. The relative amounts of LPL added to the cells (the binding cocktail) were assessed with an antibody against the V5 tag (top panel).

Figure S2. Effects of a furin inhibitor and a missense mutation in LPL's furin cleavage site (R297A) on LPL cleavage and LPL secretion from cells. CHO-K1 cells were transfected with LPL-V5 and mutant LPL-V5 constructs with the indicated substitutions in the presence or absence of the furin inhibitor CMK. LPL in the concentrated conditioned medium (M) and the cell pellet (CP) was detected with a mouse antibody against the V5 tag (green). Antibodies against actin were used as a loading control (red).

Figure S3. Experimental controls for the immunofluorescence microscopy assays shown in Figures 3, 4, 7, 8, and 10. Permeabilized and nonpermeabilized CHO-K1 cells that had been

transfected with GPIHBP1 alone, LPL-V5 alone, or ST-LPL-V5 alone were analyzed by immunocytochemistry. GPIHBP1 (red) was detected with a rabbit polyclonal antibody against GPIHBP1 (top) or a rat monoclonal antibody against GPIHBP1 (bottom); LPL (green) was detected with a mouse antibody against the V5 tag (top) or a rabbit antibody against the S-protein tag (bottom). Nuclei (blue) were stained with DAPI.

Figure S4. An enzyme-linked solid-phase immunoassay to assess binding of LPL-V5, ST-LPL-V5, LPL-V5 (G409R), or ST-LPL-V5 (G409R) to 96-well plates coated with a monoclonal antibody against LPL (5D2). Binding of LPL to antibody 5D2 was detected with horseradish peroxidase (HRP)–conjugated antibodies against the carboxyl-terminal V5 tag or the amino-terminal S-protein tag; HRP activity was revealed after color development with the SUB1 substrate.

Figure S5. Effects of a furin inhibitor and a missense mutation in LPL's furin cleavage site (R297A) on the cleavage of LPL-V5 (Δ50-289) and its secretion from cells. Western blots were performed on concentrated conditioned medium (M) and the cell pellet (CP) of cells expressing LPL-V5 (Δ50-289) in the presence or absence of the furin inhibitor CMK, and cells expressing LPL-V5 (Δ50-289;R297A). LPL was detected with a mouse antibody against the V5 tag (green). Antibodies against actin were used as a loading control (red).

Figure S6. Western blot assay of LPL binding to GPIHBP1-expressing CHO-K1 cells. Cells were incubated at 4°C for 2 h with medium alone or medium containing LPL-V5, LPL-V5 (R297A), LPL-V5 (Δ50-289), LPL-V5 (Δ50-289;R297A), and LPL-V5 (Δ50-297) in the presence or absence of heparin (500 U/ml). Western blots were performed on cell extracts and the binding cocktail. GPIHBP1 was detected with a rabbit antibody against the S-protein tag and LPL was detected with a mouse antibody against the V5 tag. Antibodies against actin were used as a loading control.

Figure S7. Immunoflourescence microscopy assay of LPL binding to GPIHBP1-transfected CHO-K1 cells. CHO-K1 cells that had been transfected with GPIHBP1 were mixed with cells that had been transfected with LPL-V5, LPL-V5 (R297A), LPL-V5 (Δ50-289), LPL-V5 (Δ50-289;R297A), LPL-V5 (Δ50-289;C418Y), or LPL-V5 (Δ50-289;E421K). The next day, permeabilized and nonpermeabilized cells were analyzed by immunocytochemistry. GPIHBP1 (red) was detected with a rabbit antibody against GPIHBP1; LPL (green) was detected with a mouse antibody against the V5 tag. Nuclei (blue) were stained with DAPI.

Figure S1

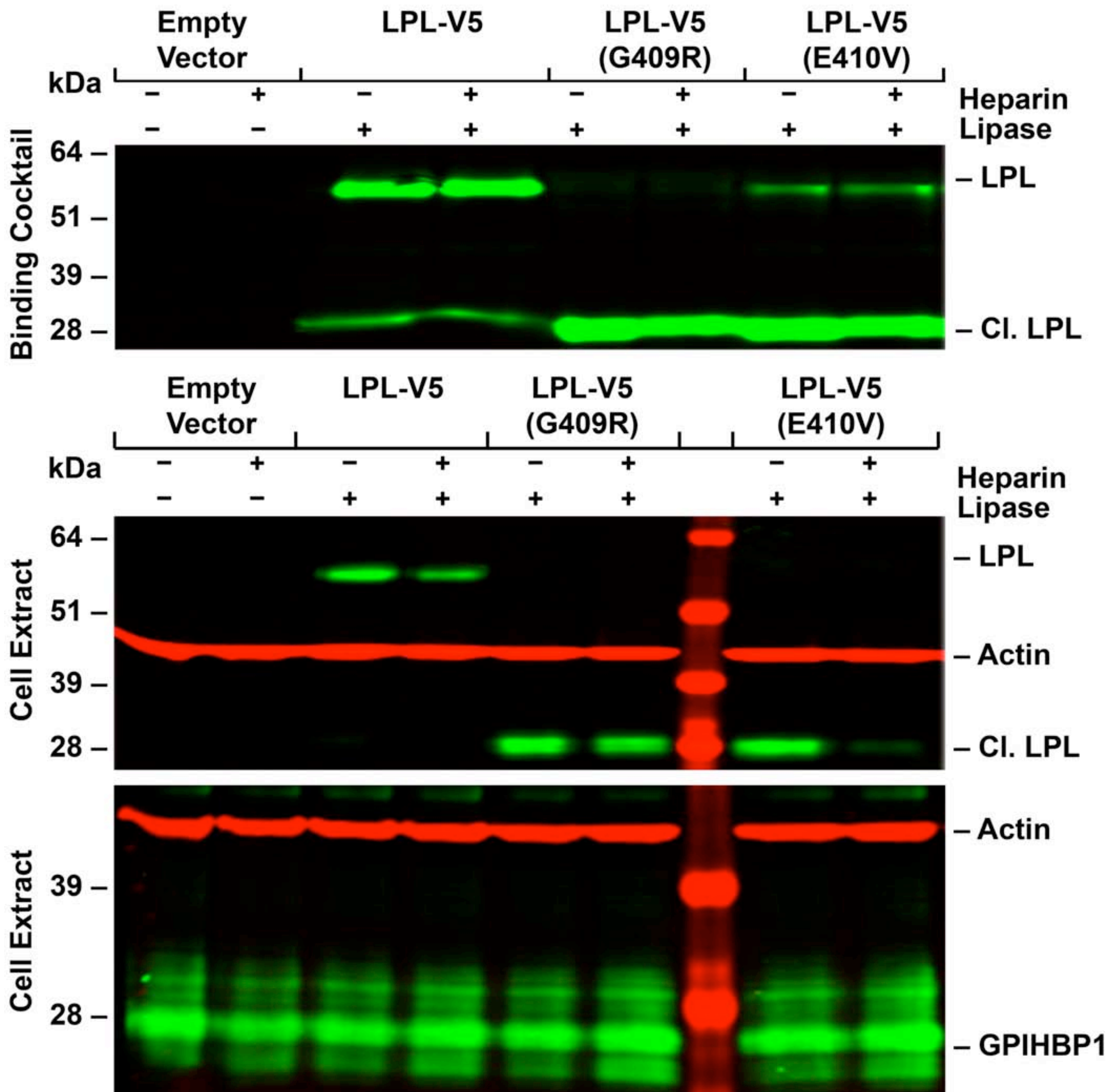


Figure S2

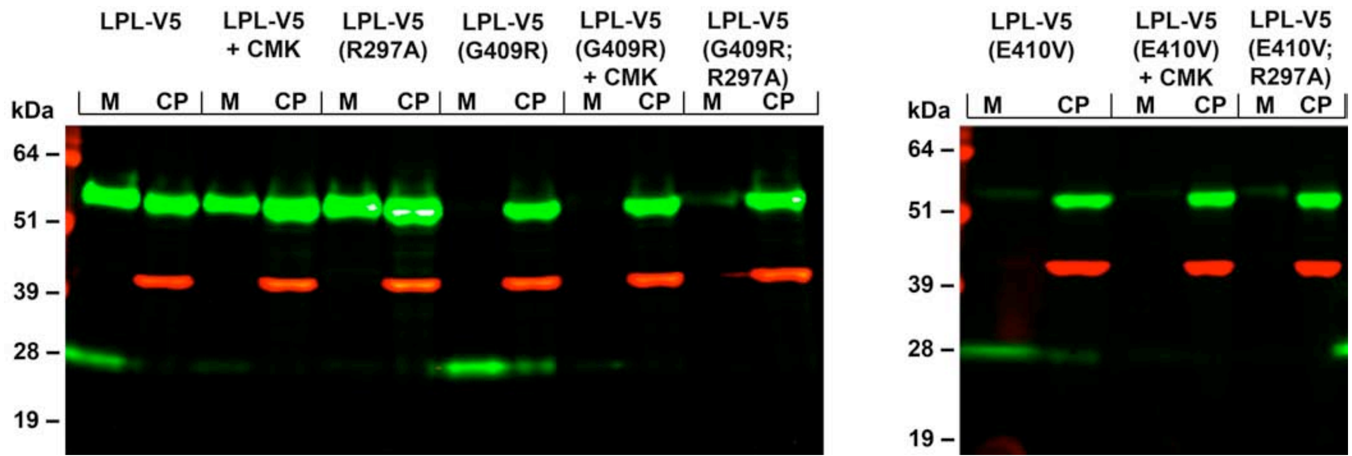
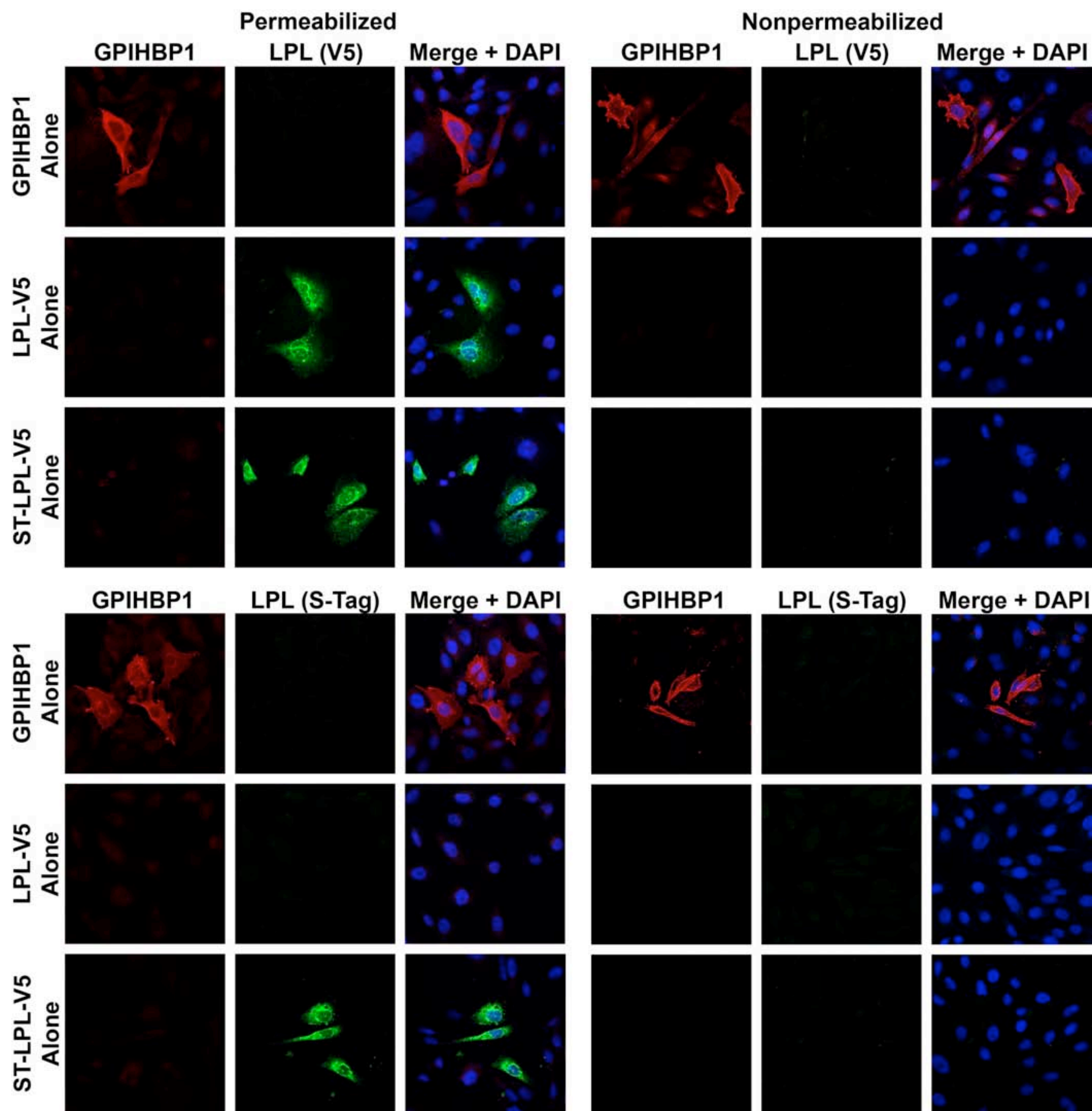


Figure S3



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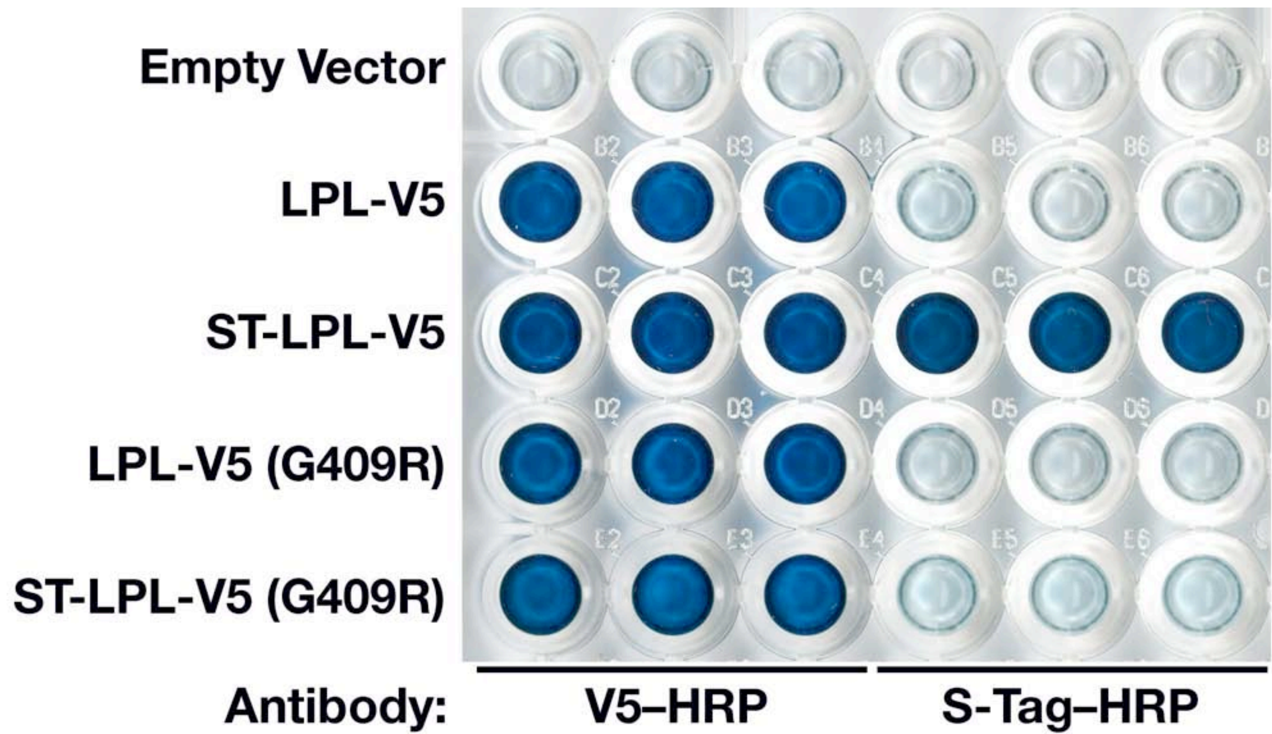

Figure S5

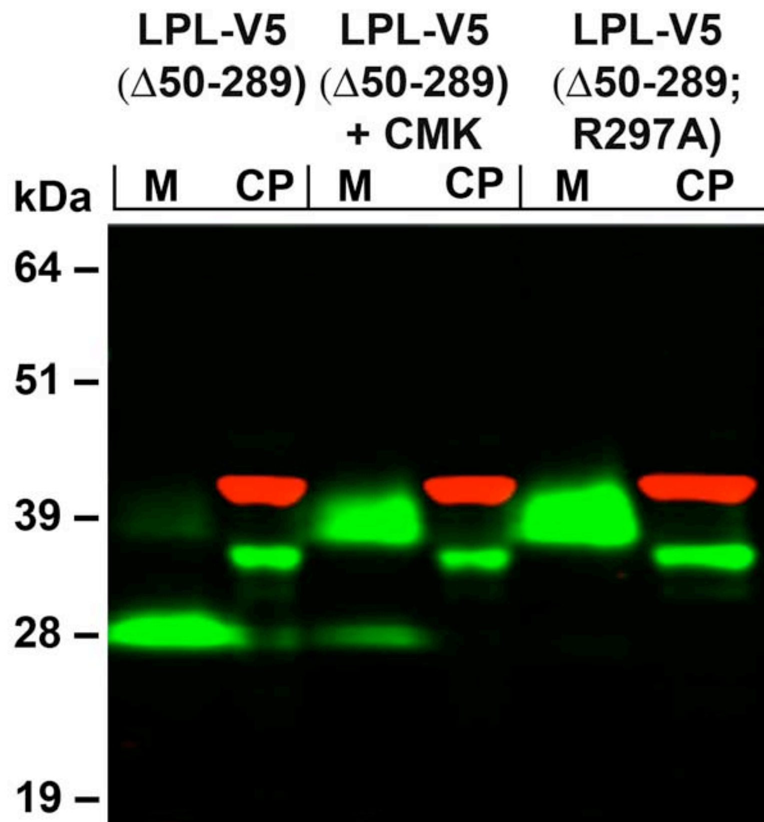

Figure S6

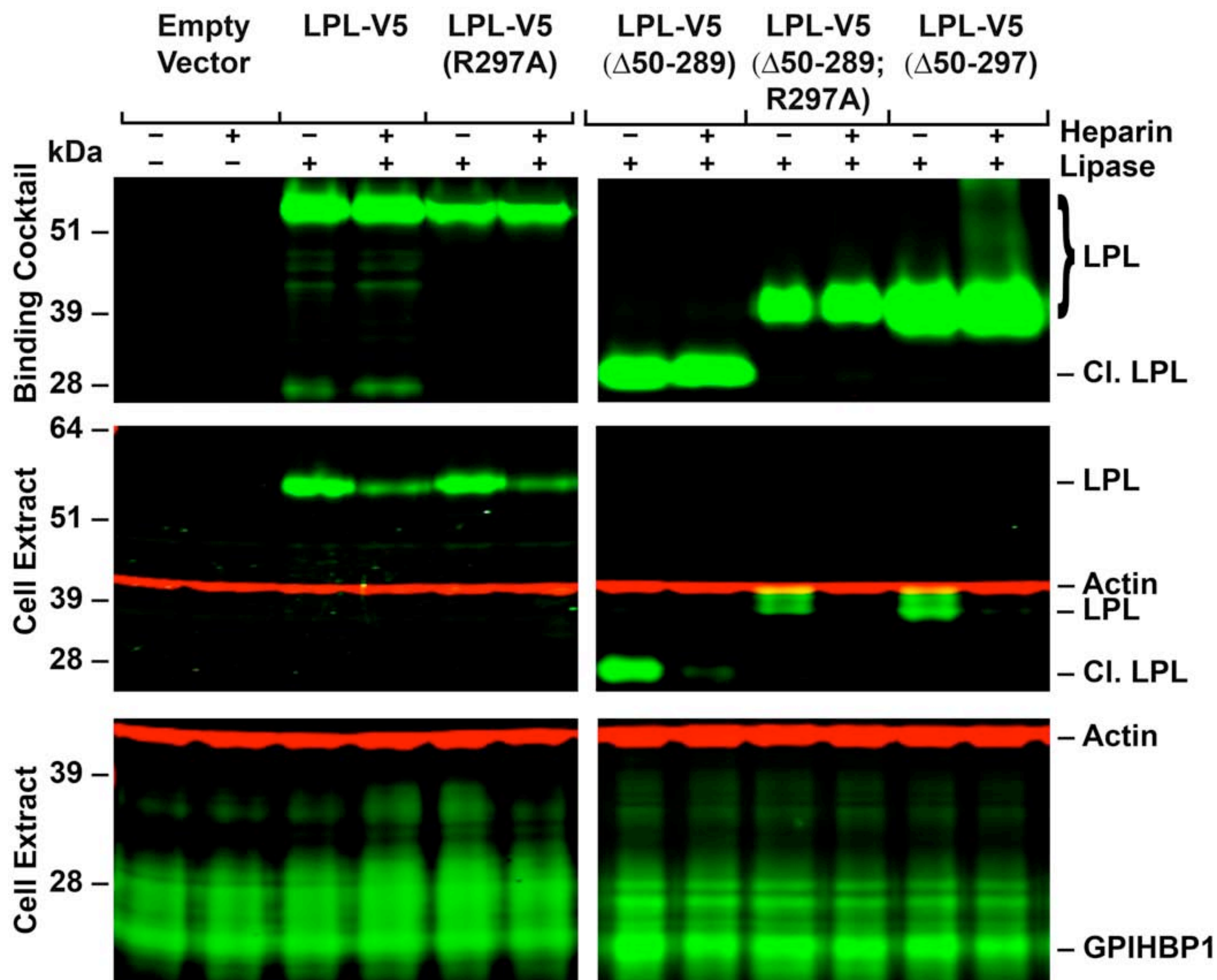

Figure S7