Supplemental Figures

Supplementary Figure Legends

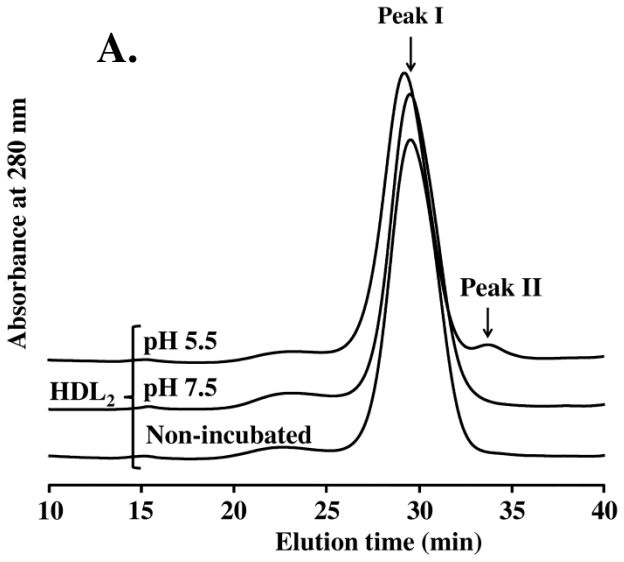
Figure S1. Effect of pH on the size of HDL_2. (A) SEC Profile of HDL_2 at different pH. HDL_2 (1 mg/ml) was incubated in either 20 mM MES (pH 5.5) or 20 mM Tris (pH 7.5) for 24 h after which HDL samples were centrifuged at 10 000 g for 10 min. Aliquots of the supernatants (50 µl) were injected into the Superose HR6 column and eluted with PBS buffer (pH 7.4). Main fractions are labeled as Peaks I and II. $nHDL_2$ = non-incubated HDL_2. Each profile is representative of three to four independent experiments. **(B)** HDL_3 or **(C)** HDL_2 (1 mg/ml) was incubated at pH 5.5 for 24 h and aliquots of nHDL and acidic pH-treated HDL (10 µg) were loaded on 0.6% agarose gel using the Paragon electrophoresis system and proteins were transferred from the agarose gel to the polyvinylidene difluoride (PVDF) membrane by pressure blotting. ApoA-I was identified using a monoclonal anti-human apoA-I antibody.

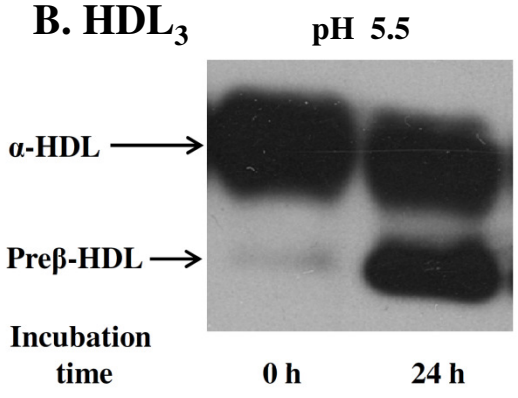
Figure S2. Effect of pH on structure of LDL. (A) SEC Profile of LDL at different pH. LDL (1 mg/ml) was incubated in either 20 mM MES (pH 5.5) or 20 mM Tris (pH 7.5) for 24 h after which LDL samples were centrifuged at 10 000 g for 10 min. Aliquots of supernatant (100 µl) were injected into Superose HR6 column and eluted with PBS buffer (pH 7.4). **(B)** Far-UV CD analysis of LDL. LDL (1 mg/ml) was incubated in 20 mM MES (pH 5.5) or 20 mM Tris (pH 7.5) for 30 min, after which aliquots of samples were diluted to 50 µg/ml for CD measurement as described in the Methods. nLDL= non-incubated LDL. Data are representative of at least two independent experiments.

Figure S3. Time-dependent effect of acidic pH on HDL_2. (A) SEC Profile of HDL_2 at different incubation times. HDL_2 (1 mg/ml) was incubated in 20 mM MES (pH 5.5) for the indicated times. Aliquots of the supernatants (50 µl) were analyzed by SEC. Each profile is representative of at least two independent experiments. **(B)** Time-dependent effect of acidic pH on preβ-HDL level. HDL_2 (1 mg/ml) was incubated for various times at pH 7.5 or pH 5.5 after which α-HDL and preβ-HDL contents were measured using 2-dimensional crossed immunoelectrophoresis. The amounts of preβ-HDL are expressed as a percentage of the sum of the preβ- and α-mobile areas.

Figure S4. Effect of acidic pH on HDL_3 size and preβ-HDL formation as a function of HDL_3 concentration. (A) SEC Profile of HDL_3 at different concentrations. HDL_3 at different concentrations (0.1-2 mg /ml) was incubated in 20 mM MES (pH 5.5) for 24 h after which the samples were analyzed by SEC. Aliquots of the supernatants (50 µl for HDL_3 at concentration 0.5-2 mg/ml and 100 µl for HDL_3 at concentration below 0.25 mg/ml) were injected into Superose HR6 column and eluted with PBS buffer (pH 7.4). Main fractions are labeled as Peaks I and II. Non-incubated HDL_3 (1 mg/ml) served as a control. Each profile is representative of at least two independent experiments. **(B)** Formation of acidic pH-induced preβ-HDL at low concentration. HDL_3 (0.1-0.25 mg /ml) was incubated in 20 mM MES (pH 5.5) for 24 h after which α-HDL and preβ-HDL contents were measured using 2-dimensional crossed immunoelectrophoresis. The amounts of preβ-HDL are expressed as a percentage of the sum of the preβ- and α-mobile areas.

Figure S1


A.
Peak I
Peak II
Absorbance at 280 nm
pH 5.5
HDL2
pH 7.5
Non-incubated
10
15
20
25
30
35
40
Elution time (min)


B. HDL3
pH 5.5
α-HDL
Preβ-HDL
Incubation time
0 h
24 h

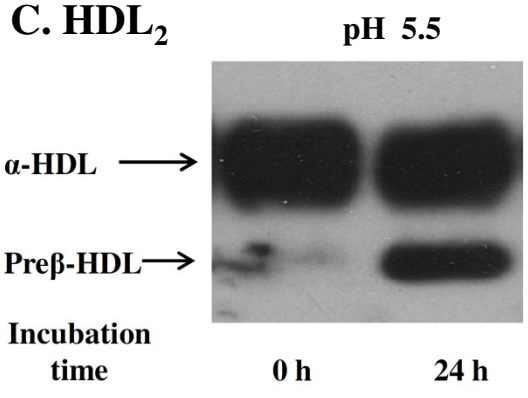

C. HDL2
pH 5.5
α-HDL
Preβ-HDL
Incubation time
0 h
24 h

Figure S2

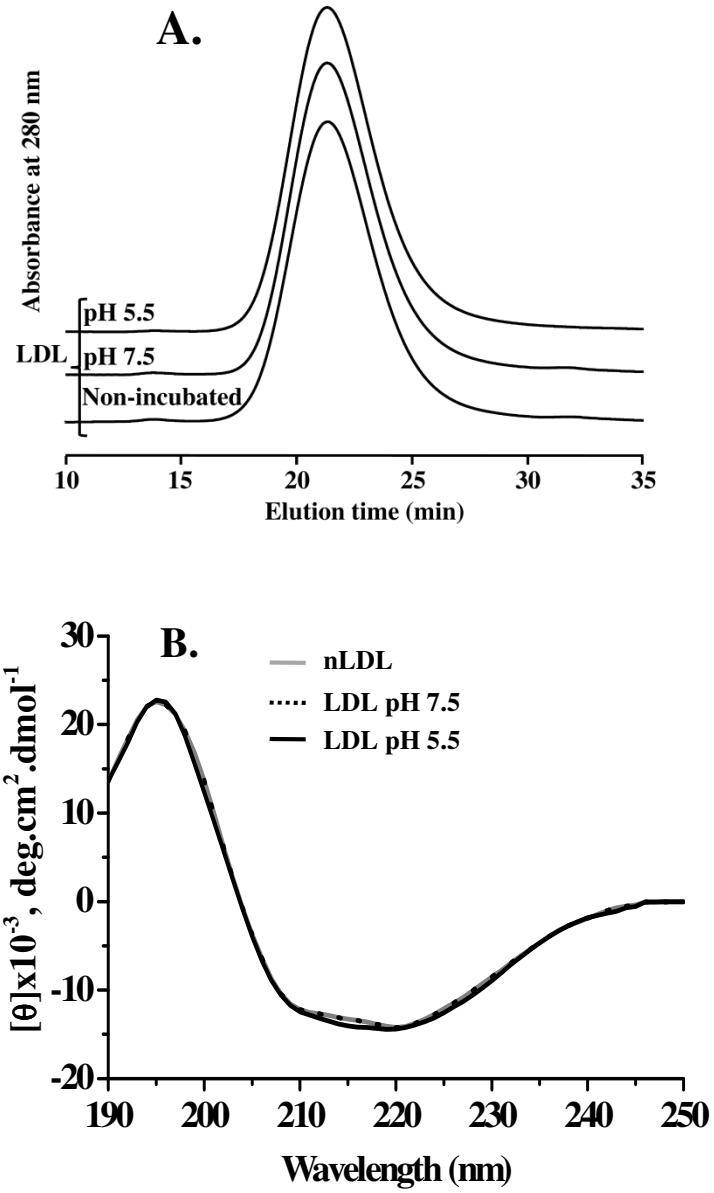

A.
Absorbance at 280 nm
LDL
pH 5.5
pH 7.5
Non-incubated
10
15
20
25
30
35
Elution time (min)
B.
nLDL
LDL pH 7.5
LDL pH 5.5
[θ]x10-3, deg.cm2.dmol-1
30
20
10
0
-10
-20
190
200
210
220
230
240
250
Wavelength (nm)

Figure S3

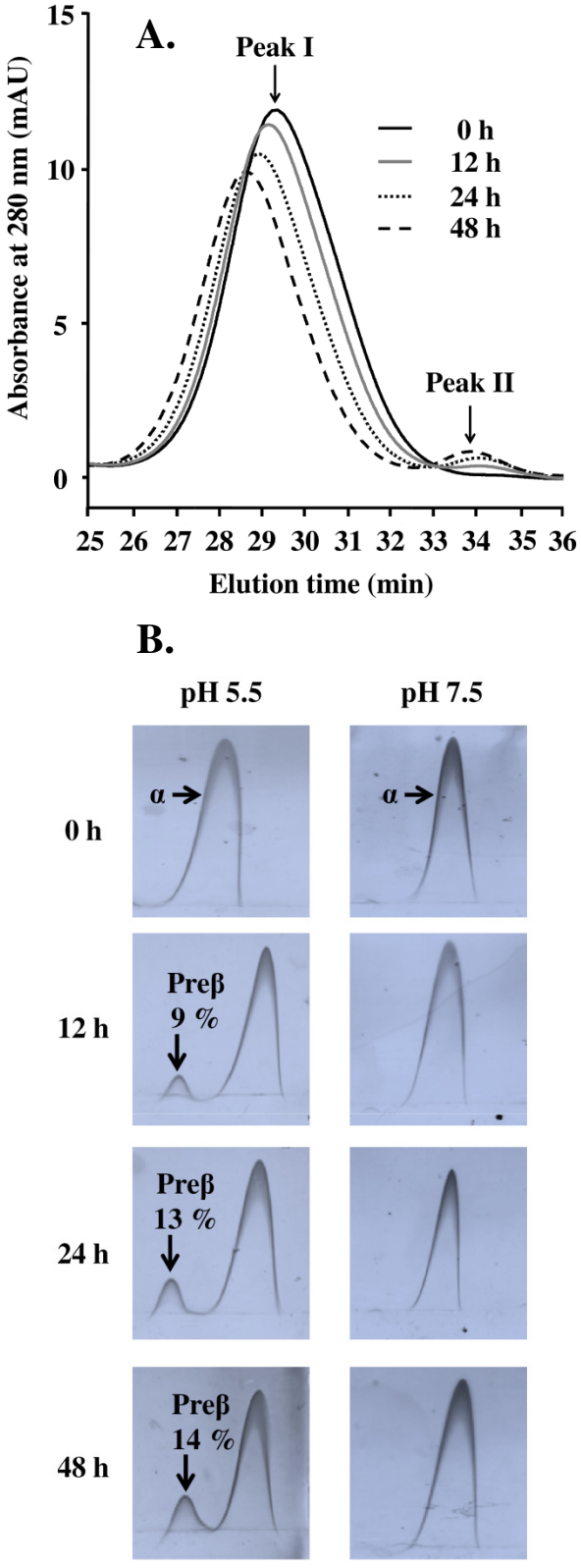
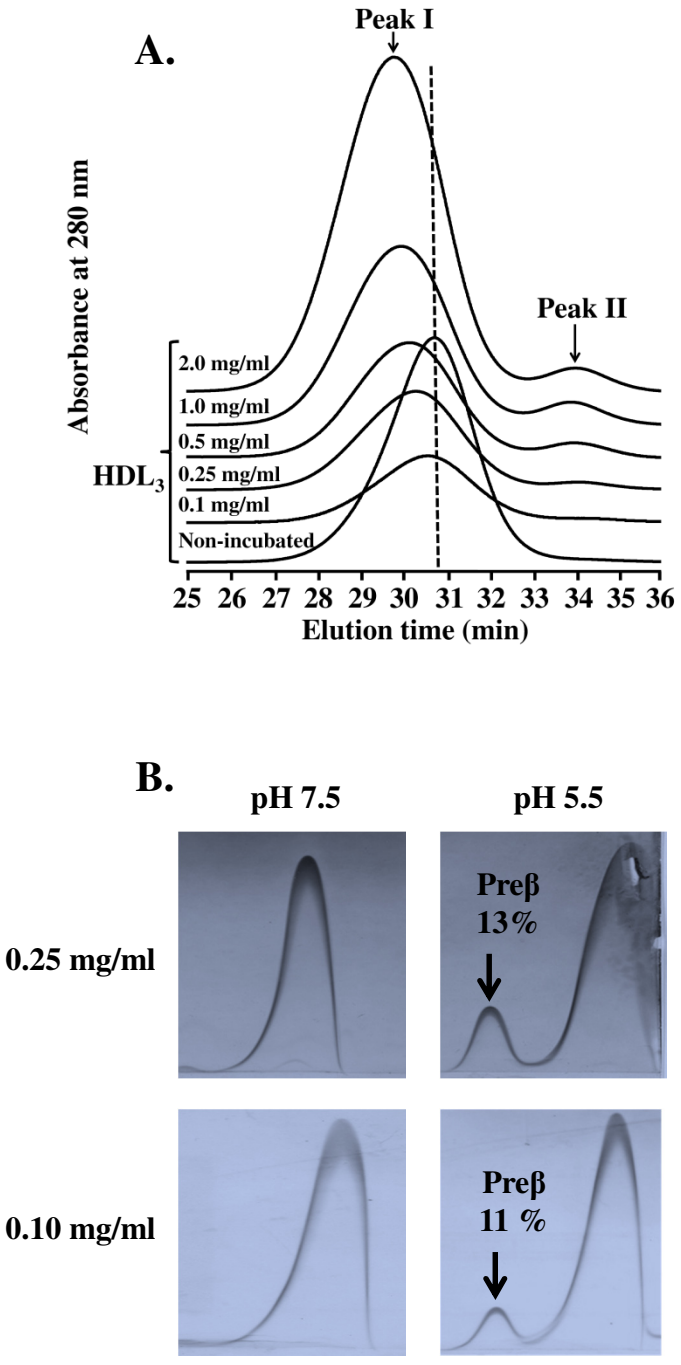

A.
Peak I
Peak II
0 h
12 h
24 h
48 h
Absorbance at 280 nm (mAU)
15
10
5
0
25 26 27 28 29 30 31 32 33 34 35 36
Elution time (min)
B.
pH 5.5
pH 7.5
0 h
12 h
24 h
48 h
α
Preβ
9 %
Preβ
13 %
Preβ
14 %

Figure S4


A.
Peak I
Peak II
Absorbance at 280 nm
2.0 mg/ml
1.0 mg/ml
0.5 mg/ml
HDL3
0.25 mg/ml
0.1 mg/ml
Non-incubated
25 26 27 28 29 30 31 32 33 34 35 36
Elution time (min)
B.
pH 7.5
pH 5.5
0.25 mg/ml
Preβ
13%
0.10 mg/ml
Preβ
11 %