

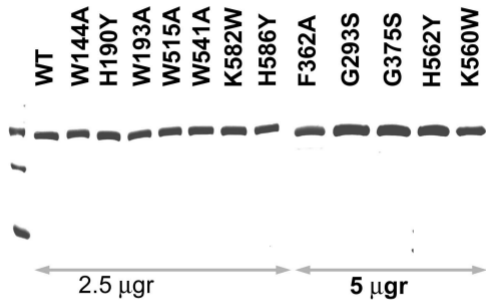
### **Legends for supplemental figures**

**Fig. S1:** Purified receptors (WT and twelve mutants). 2.5 µgr or 5 µgr of each LDL-R variant was subject to SDS-PAGE electrophoresis on a 10% gel and visualized with Coomassie staining. The first lane contains molecular weight markers (75 kDa, 50 kDa and 25 kDa).

**Fig. S2:** Scatchard plot analysis of all twelve mutants and WT. Mutants were characterized in sets (a,b,c,d), taking along a WT control for each set. For each mutant (in black) also superimposed is WT (in grey) permitting visual assessment of LDL affinity loss (decreased slope) or LDL affinity gain (increased slope) compared to WT. The slopes were calculated using non-linear regression of the specific binding curves using a one-binding site model. 'Bound/Free' is defined as bound I<sup>125</sup>-LDL [(fmol/well)/ free I<sup>125</sup>-LDL [nM]. a) WT, H190Y, H562Y, H586Y; b) WT, F362A, G293S; c) WT, G375S, K560W, K582W; d) WT, W144A, W193A, W515A, W541A.

**Fig. S3:** Hydrodynamic characterization of WT LDL-R. Size exclusion chromatography of WT LDL-R was carried out at pH 8 (green trace), 6.3 (red trace) and 5.5 (blue trace) as well as pH 5.5 in presence of 20 mM EDTA (black trace).

**Figure S1:**



**Figure S2:**

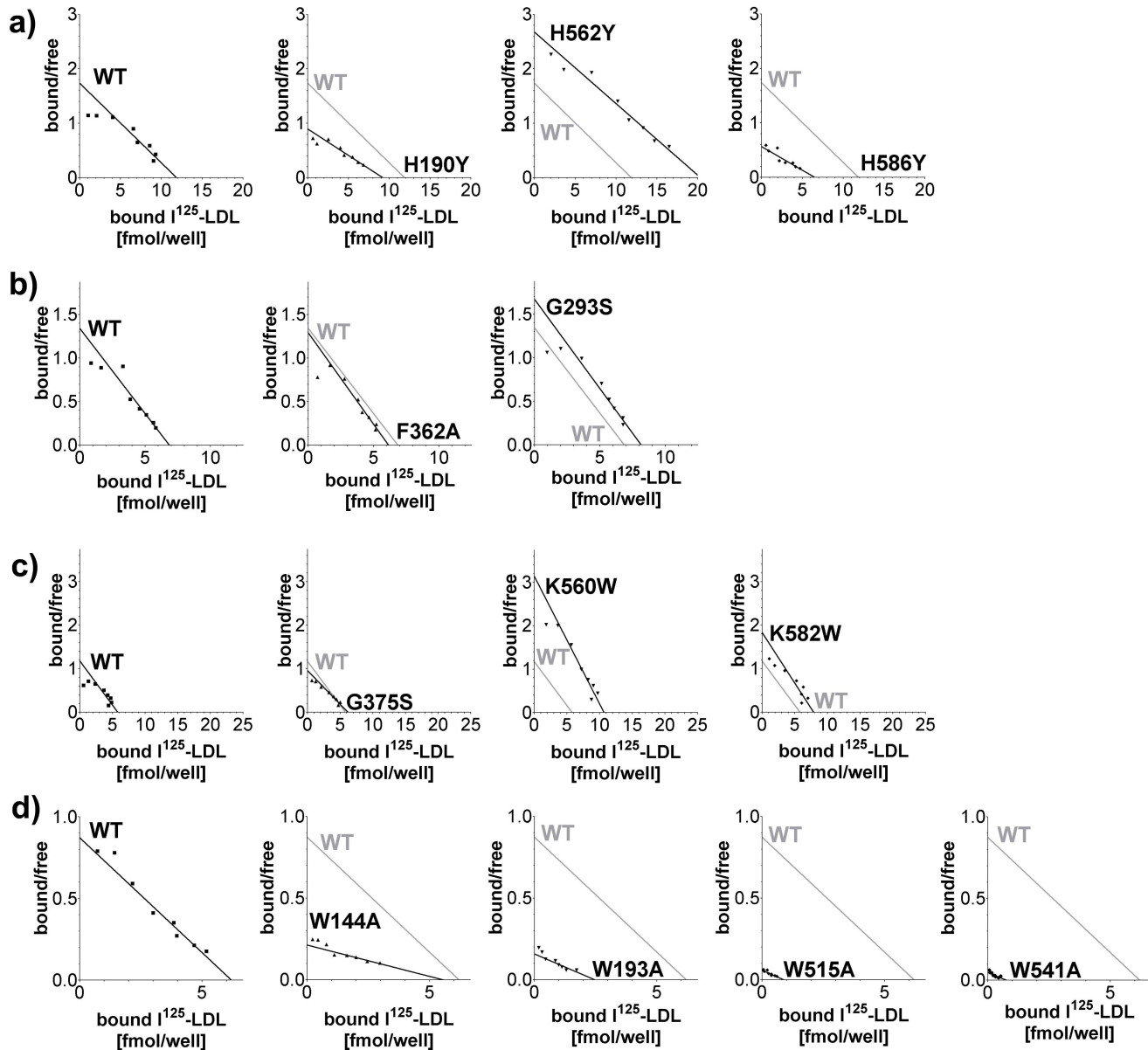
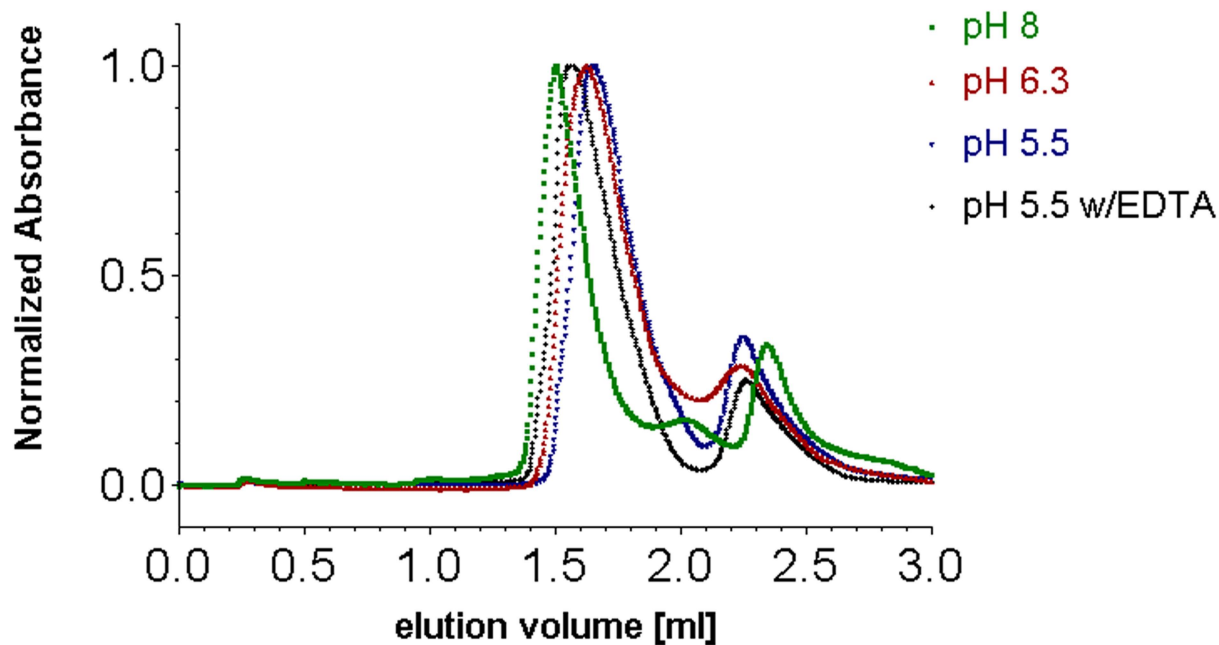


Figure S3:

# LDL-R WT



**Supplemental Table 1:**

	<b>Total (W+B) [cpm]</b>	<b>% release pH 5</b>	<b>% release pH 8</b>	<b>% specific release</b>
<b>WT</b>	4202.5 (SD 111.2)	62.2 (SEM 1.1)	16.0 (SEM 0.1)	46.2 (SEM 1.1)
<b>W144A</b>	2571.1 (SD 205.7)	63.7 (SEM 1.1)	15.8 (SEM 0.3)	47.9 (SEM 1.1)
<b>K560W</b>	5902.6 (SD 141.9)	63.0 (SEM 0.6)	10.3 (SEM 0.1)	52.7 (SEM 0.6)
<b>K582W</b>	5131.8 (SD 237.5)	62.7 (SEM 0.6)	14.0 (SEM 0.1)	48.7 (SEM 0.6)
<b>WT</b>	4299.4 (SD 108.6)	63.1 (SEM 0.6)	16.9 (SEM 0.2)	46.2 (SEM 0.6)
<b>H190Y</b>	2449.5 (SD 112.0)	41.7 (SEM 0.7)	19.7 (SEM 0.5)	22.0 (SEM 0.9)
<b>H562Y</b>	5467.7 (SD 199.4)	45.2 (SEM 0.1)	8.5 (SEM 0.3)	36.6 (SEM 0.3)
<b>H586Y</b>	1551.9 (SD 47.3)	23.4 (SEM 0.2)	12.2 (SEM 0.1)	11.3 (SEM 0.3)
<b>WT</b>	2478.6 (SD 99.5)	61.7 (SEM 0.4)	20.1 (SEM 1.1)	41.6 (SEM 1.2)
<b>G293S</b>	2797.2 (SD 90.8)	63.0 (SEM 0.8)	20.4 (SEM 0.3)	42.6 (SEM 0.9)
<b>F362A</b>	2102.3 (SD 79.9)	58.2 (SEM 1.6)	20.5 (SEM 0.9)	37.7 (SEM 1.8)
<b>G375S</b>	2306.6 (SD 87.7)	42.4 (SEM 1.8)	16.9 (SEM 0.1)	25.6 (SEM 1.8)
<b>WT</b>	3422.1 (SD 127.5)	64.4 (SEM 1.1)	20.5 (SEM 0.3)	43.9 (SEM 1.2)
<b>W193A</b>	2883.7 (SD 205.1)	49.2 (SEM 0.7)	22.4 (SEM 0.8)	26.8 (SEM 1.0)
<b>W515A</b>	574.9 (SD 47.1)	41.6 (SEM 1.4)	19.1 (SEM 0.6)	22.5 (SEM 1.5)
<b>W541A</b>	448.0 (SD 59.9)	40.2 (SEM 2.4)	20.4 (SEM 0.6)	19.8 (SEM 2.5)

Total = well [cpm] + buffer [cpm] (mean over 8 values i.e. 2.5', 5', 10', 20' at pH 5 as well as pH 8);

% release at pH 5, release seen after 20 minutes incubation at pH 5 at 4°C

% release at pH 8, release seen after 20 minutes incubation at pH 8 at 4°C

% acid specific release = (% release at pH 5) – (% release at pH 8)

Samples were counted for 5 minutes, but the values are reported in cpm.

SD, standard deviation; SEM, standard error of the mean