

Supplementary Information

Immunoproteasome subunit $\beta 5i$ /LMP7-deficiency in atherosclerosis

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Supplement tables

Supplement table S1: Body weight and lipid levels

	6 weeks control diet			24 weeks control diet		
	LDLR ^{-/-}	LDLR ^{-/-} LMP7 ^{-/-}	p value	LDLR ^{-/-}	LDLR ^{-/-} LMP7 ^{-/-}	p value
Body weight, g	26.6 ± 2.3	26.7 ± 1.7	0.967	30.7 ± 2.0	27.9 ± 3.5	0.034
Total cholesterol, mg/dl	286.7 ± 33.3	284.7 ± 54.7	0.920	290.2 ± 22.1	276.4 ± 31.1	0.245
HDL-C, mg/dl	90.9 ± 19.2	96.3 ± 7.4	0.4	128.1 ± 12.7	124.8 ± 19.8	0.818
Non-HDL-C, mg/dl	195.9 ± 19.2	188.4 ± 56.9	0.695	162.1 ± 53.4	151.6 ± 31.9	0.582
Triglycerides, mg/dl	164.9 ± 38.5	121.6 ± 48.2	0.034	94.4 ± 13.5	81.2 ± 20.0	0.083

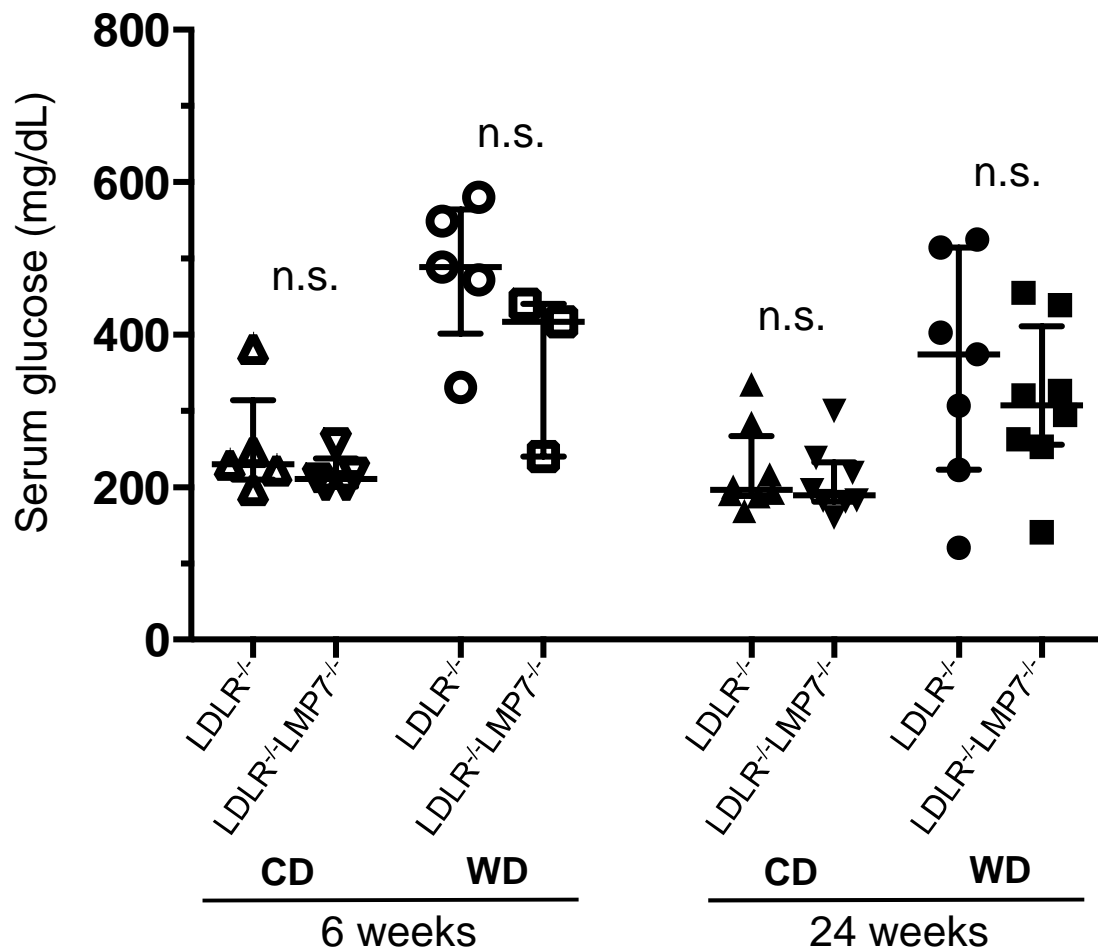
n=11 per group; mean ± SD. Statistical analysis using unpaired *t*-test (Welch test for unequal variances). HDL-C, high-density lipoprotein cholesterol.

Supplement Table S2: Cardiac dimensions and function

	6 weeks control diet			6 weeks WD		
	LDLR ^{-/-}	LDLR ^{-/-} LMP7 ^{-/-}	p value	LDLR ^{-/-}	LDLR ^{-/-} LMP7 ^{-/-}	p value
n	5	5		4	3	
LVIDd, mm	4.0 ± 0.3	3.8 ± 0.3	0.256	3.9 ± 0.1	3.7 ± 0.2	0.286
LVIDs, mm	2.7 ± 0.4	2.6 ± 0.2	0.503	2.5 ± 0.1	2.5 ± 0.2	0.8
LVEF, %	60.9 ± 7.5	61.0 ± 3.1	0.98	65.1 ± 1.9	62.4 ± 4.3	0.4
FS, %	32.4 ± 5.2	32.3 ± 2.2	0.948	35.2 ± 1.4	33.1 ± 3.2	0.4
Stroke volume, µl	42.0 ± 4.2	37.3 ± 6.4	0.203	41.2 ± 3.3	36.3 ± 6.3	0.230

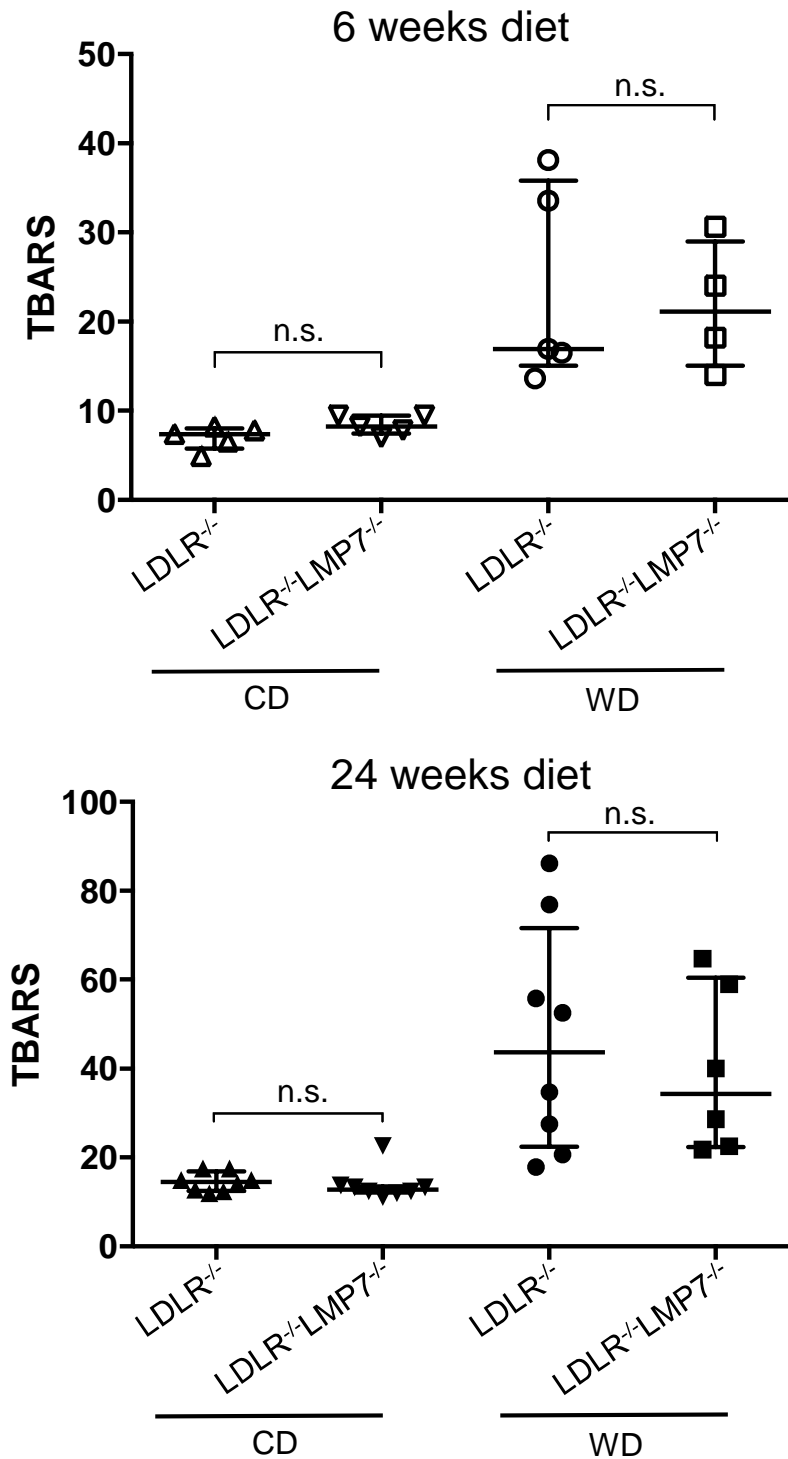
Mean ± SD. Statistical analysis using unpaired *t*-test or Mann-Whitney *U* test. WD, Western-type diet; LVIDd, end-diastolic left ventricular inner dimension; LVIDs, systolic left ventricular inner dimension; FS, fractional shortening; LVEF, left ventricular ejection fraction.

Supplement Figure S1



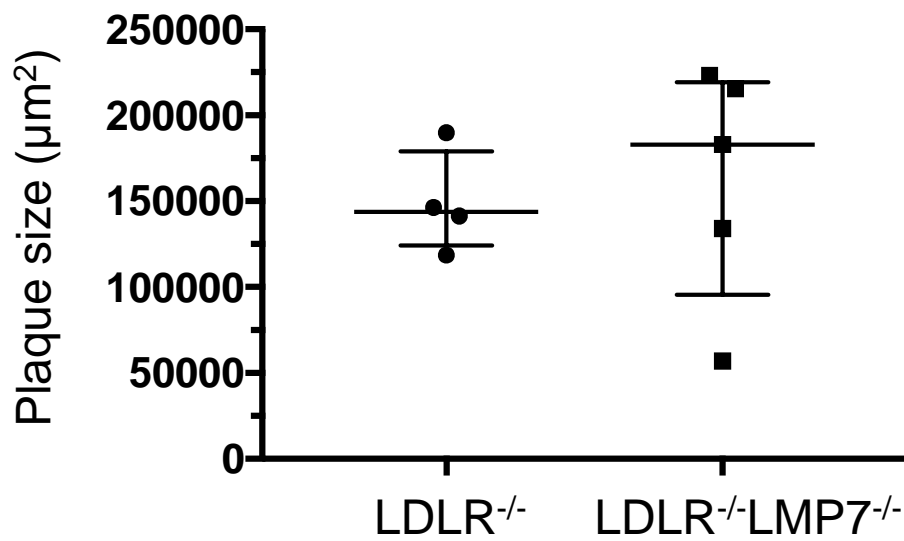
Supplement Figure S1. Serum glucose levels. LDLR^{-/-}LMP7^{-/-} and LDLR^{-/-} mice were fed a control (CD) or a high-fat Western-type diet (WD) for either 6 or 24 weeks. Data presented as mean \pm SEM. n.s.= statistically non-significant using unpaired *t*-test or Mann-Whitney *U* test.

Supplement Figure S2



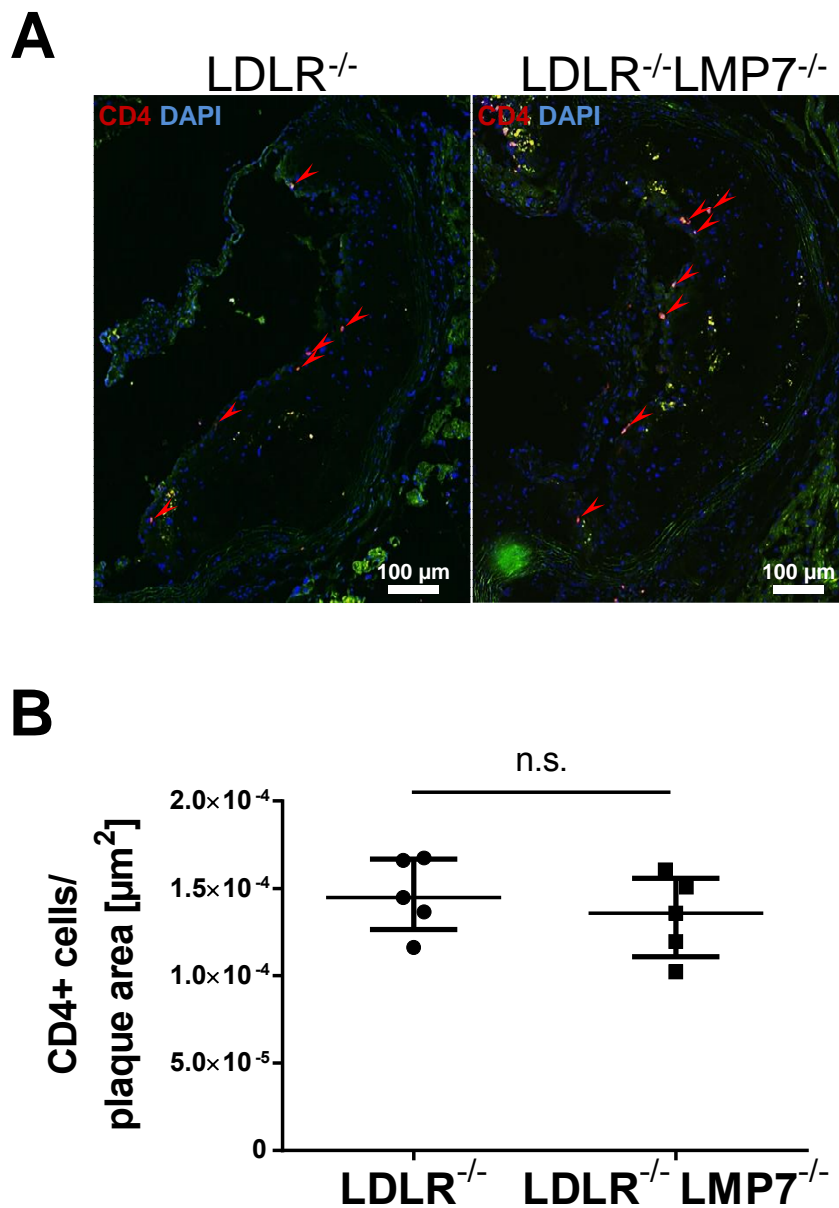
Supplement Figure S2. Serum levels of thiobarbituric acid reactive substances (TBARS). LDLR^{-/-}LMP7^{-/-} and LDLR^{-/-} mice were fed a control (CD) or a high-fat Western-type diet (WD) for either 6 or 24 weeks. Data are presented as individual values with median and interquartile ranges indicated. All comparisons between CD and WD fed mice were statistically significant ($p < 0.05$). n.s.= statistically not significant using unpaired t -test or Mann-Whitney U test.

Supplement Figure S3



Supplement Figure S3. Plaque size in the *truncus brachiocephalicus*. LDLR^{-/-} LMP7^{-/-} and LDLR^{-/-} mice were fed a high-fat Western-type diet for 24 weeks to induce advanced stage atherosclerosis. Data are presented as individual values with median and interquartile ranges indicated. n.s. = statistically non-significant using Mann-Whitney *U* test.

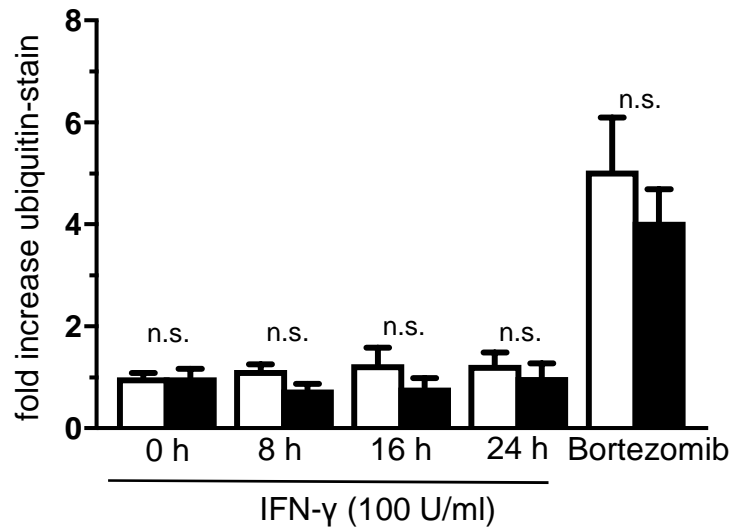
Supplement Figure S4



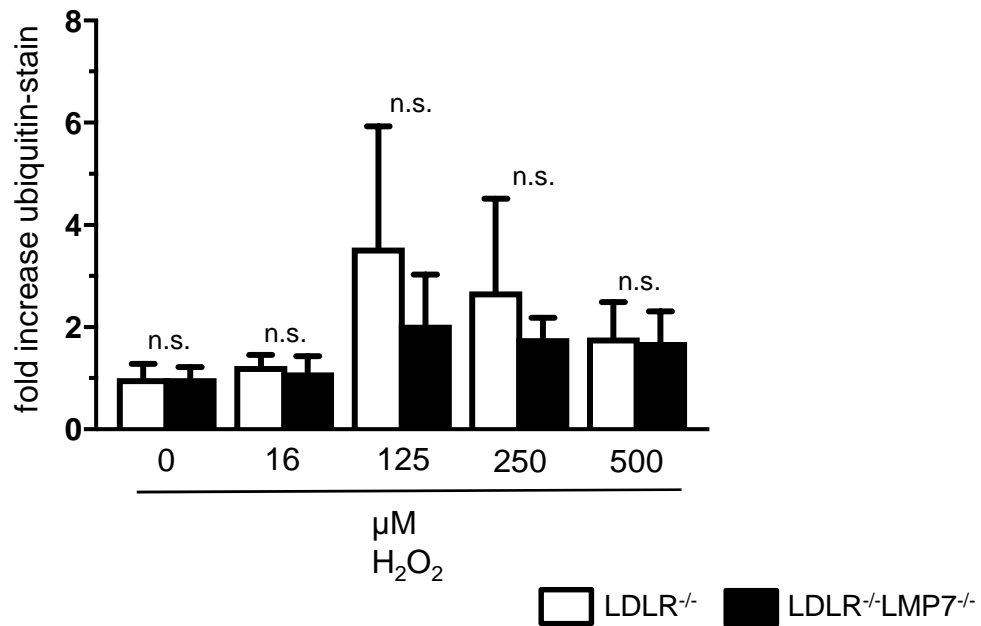
Supplement Figure S4: T cells (CD4) in atherosclerotic plaques of the aortic root. Aortic root cryosections from LDLR^{-/-} and LDLR^{-/-}LMP7^{-/-} mice fed a Western-type diet for 24 weeks were analyzed for the number of CD4-positive cells per plaque area. **A** Representative merged images showing anti-CD4 immunofluorescence in red (red arrowheads). Nuclei were stained with DAPI (blue). Autofluorescence in green. **B** Quantification of CD4-positive cells per plaque area. Data presented as individual values with median and interquartile ranges indicated. n=5 per group. n.s.= statistically non-significant using unpaired *t*-test.

Supplement Figure S5

A

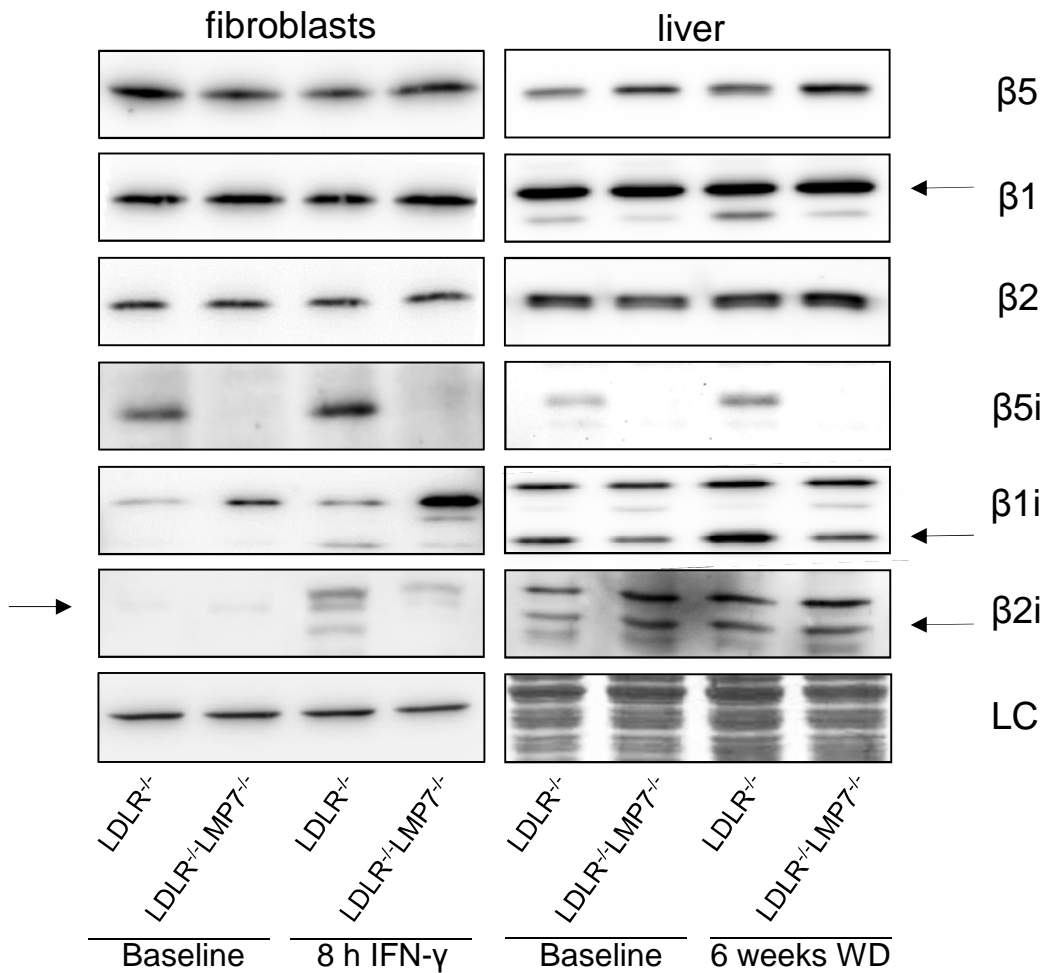


B



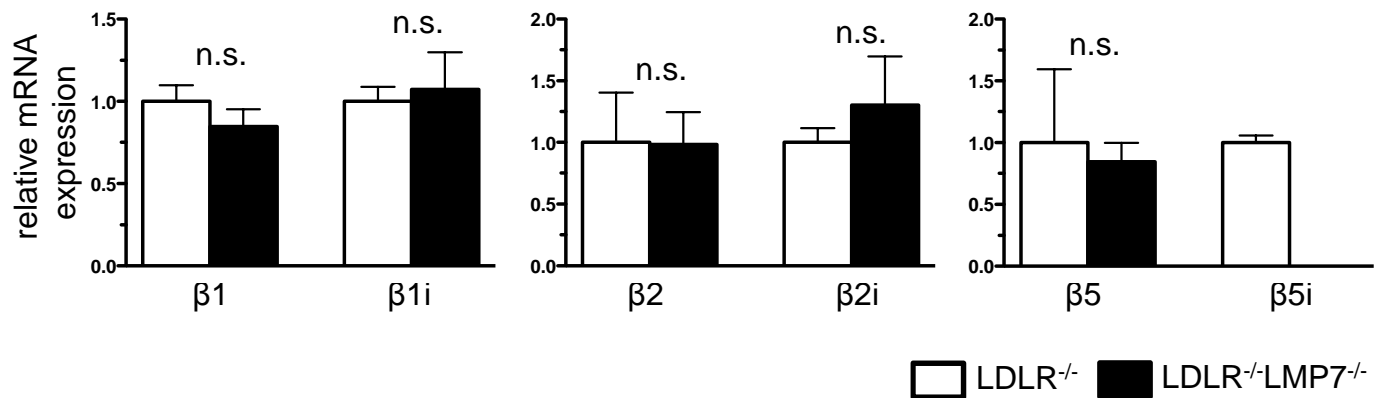
Supplement Figure S5. Densitometric analyses of anti-ubiquitin Western blots (Figure 2C and 2D). Bone marrow derived macrophages (BMDM) of LDLR^{-/-}LMP7^{-/-} and LDLR^{-/-} mice: **(A)** lysates of IFN-γ (100 U/ml) treated BMDM; Bortezomib (5 ng/ml) treated BMDM served as positive controls, n = 4 experiments; **(B)** lysates of BMDM after treatment with hydrogen peroxide (H₂O₂) over 1 hour; n = 3 experiments. Data are presented as mean ± SEM; n.s. = statistically non-significant using two-way ANOVA followed by Sidak's multiple comparisons test.

Supplement Figure S6



Supplement Figure S6. Impact of $\beta 5i/LMP7$ -deficiency on proteasome composition. Western blot analysis of standard proteasome ($\beta 1$, $\beta 2$, and $\beta 5$) and immunoproteasome ($\beta 1i$, $\beta 2i$, and $\beta 5i$) subunits expression in whole lysates of murine $LDLR^{-/-}LMP7^{-/-}$ and $LDLR^{-/-}$ fibroblasts before (baseline) and after treatment with IFN- γ (100 U/ml) for 8 hours (left panel) and of liver lysates of $LDLR^{-/-}LMP7^{-/-}$ and $LDLR^{-/-}$ mice. LC indicates loading controls, actin (for fibroblasts) and amidoblack (for liver).

Supplement Figure S7



Supplement Figure S7. Impact of $\beta 5i/LMP7$ -deficiency on proteasome composition. mRNA expression determined by quantitative real-time RT-PCR of standard proteasome ($\beta 1$, $\beta 2$, and $\beta 5$) and immunoproteasome ($\beta 1i$, $\beta 2i$, and $\beta 5i$) subunits of murine $LDLR^{-/-}LMP7^{-/-}$ and $LDLR^{-/-}$ bone marrow derived macrophages (BMDM); $LDLR^{-/-}LMP7^{-/-}$ columns show fold-change in mRNA expression levels relative to expression levels of BMDM of $LDLR^{-/-}$ mice; $n = 3$ experiments. Data are presented as mean \pm SEM; n.s. = statistically non-significant using Mann-Whitney U test.