SUPPLEMENTAL TABLES AND FIGURES

Table I. Anti-atherogenic properties of the peptides *in vitro*

-, inactive, +, ++, +++, low, medium or high levels of activity

Peptide	Cholesterol efflux from macrophages	Inhibition of CD11b expression on monocytes	Inhibition of VCAM-1 expression on EC	Inhibition of LDL oxidation
ELK-2A2K2E[*]	+++	+	+	+
ELKA-CH2[*]	-	+++	+	++
ELK-2A[*]	-	-	+++	+
5A-CH1[*]	-	+	+	+++
5A-C1[*,†]	+	+	+++	++
ELK-2A2K2E /5A-C1 (1:1)[†]	+++	+++	+++	++
5A[*]	++	+	+	++

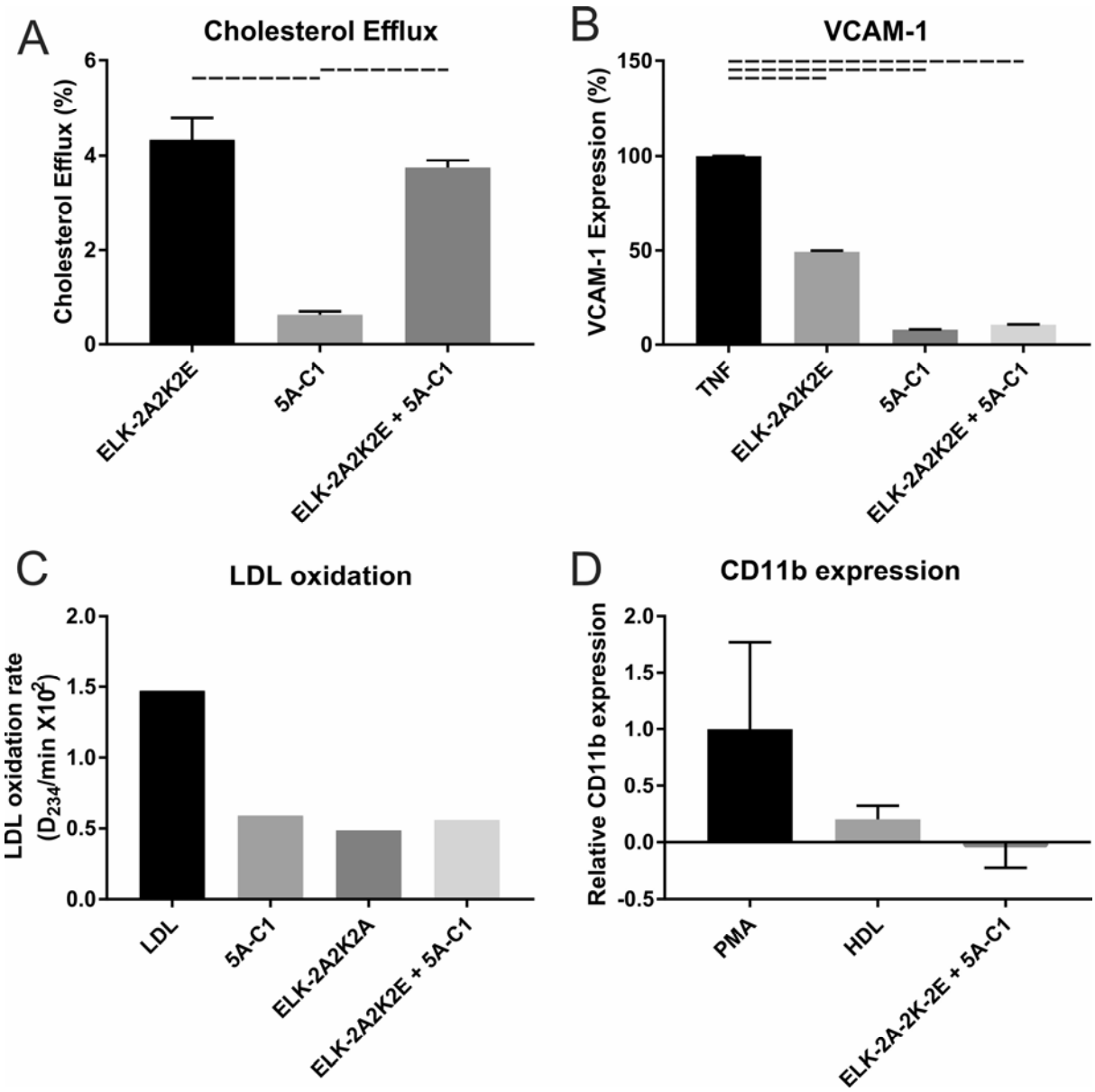
[*]Data derived from findings presented in reference 3.

[†]Data derived from findings presented in Supplemental Fig. I.

Table II. Effect of 4-weeks peptide treatment on plasma cytokine levels

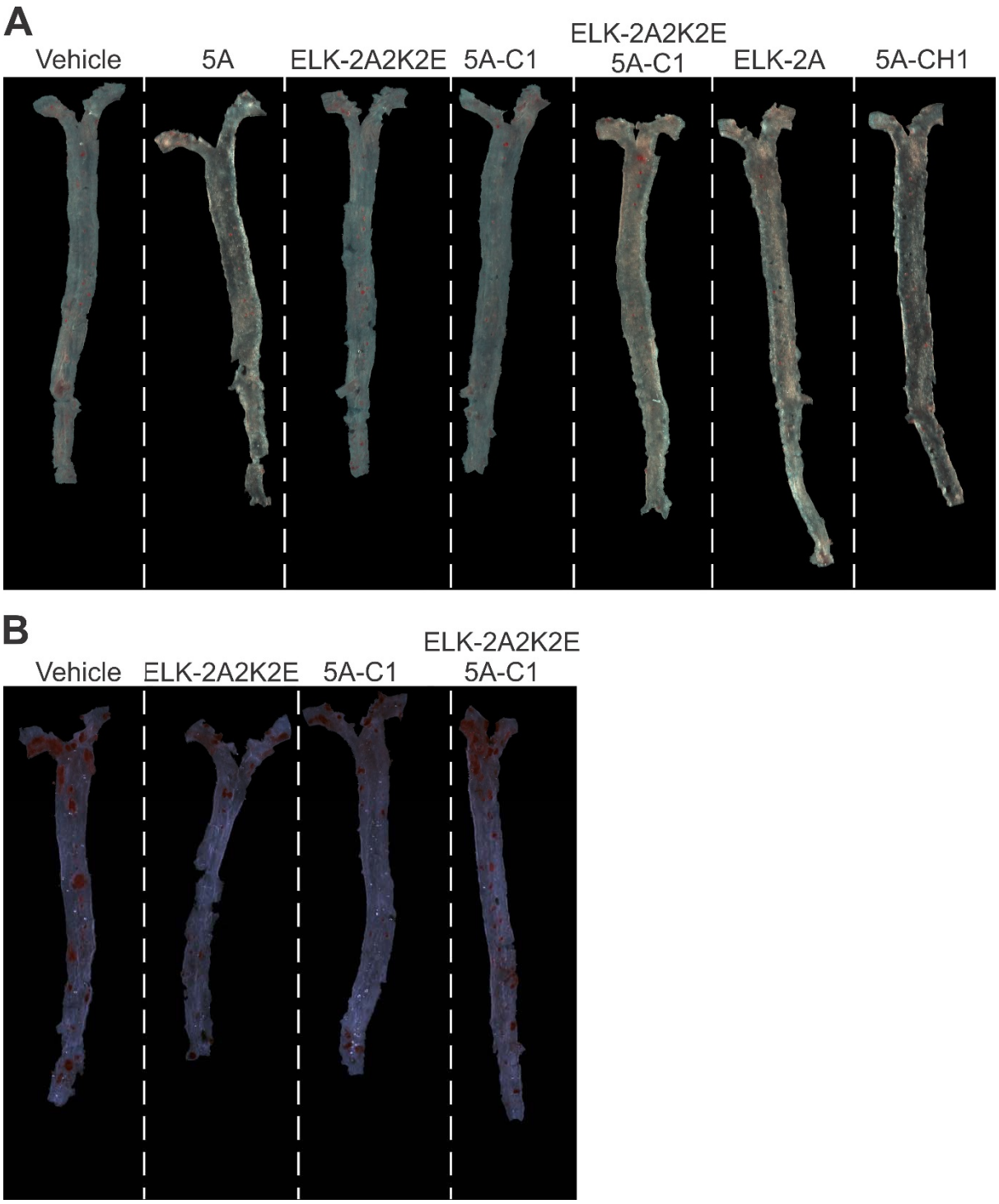
Cytokine	Vehicle	5A	ELK-2A2K2E	5A-C1	ELK-2A2K2E+5A-C1	ELKA-CH1	ELK-2A	5A-CH1
IL-23 (pg/ml)	53.22 ± 2.76	261.82 ± 69.08	116.82 ± 43.75	391.48 ± 178.68	103.22 ± 54.39	223.80 ± 66.17	342.77 ± 116.48	401.67 ± 133.16
IL-1α (pg/ml)	8.00 ± 1.00	15.75 ± 2.39	8.52 ± 1.32	10.22 ± 1.54	12.05 ± 3.42	9.88 ± 1.46	14.93 ± 2.05	11.98 ± 1.03
IFN-γ (pg/ml)	6.06 ± 0.65	15.75 ± 2.55	7.12 ± 1.05	17.91 ± 6.01	7.19 ± 2.49	13.59 ± 2.53	21.60 ± 5.50	16.33 ± 3.20
TNF-α (pg/ml)	10.48 ± 0.54	13.15 ± 1.96	10.74 ± 0.97	11.10 ± 0.95	32.87 ± 23.10	11.65 ± 1.20	16.07 ± 2.15	12.68 ± 1.90
MCP-1 (pg/ml)	12.95 ± 2.09	17.88 ± 3.41	12.31 ± 2.26	13.69 ± 2.34	27.91 ± 17.93	13.83 ± 1.95	19.77 ± 3.02	17.68 ± 3.32
IL-12p70 (pg/ml)	3.70 ± 0.22	9.81 ± 2.51	4.42 ± 0.67	5.84 ± 1.60	5.95 ± 1.99	8.19 ± 1.76	8.23 ± 1.72	9.21 ± 3.66
IL-1α (pg/ml)	30.29 ± 1.02	55.94 ± 12.44	50.61 ± 15.77	73.31 ± 26.09	71.72 ± 28.53	49.69 ± 14.81	71.41 ± 15.35	37.00 ± 3.05
IL-10 (pg/ml)	157.23 ± 19.58	355 ± 83.44	318.27 ± 75.18	249.77 ± 62.53	216.61 ± 57.69	240.98 ± 45.34	333.9 ± 76.13	300.21 ± 53.62
IL-6 (pg/ml)	4.65 ± 0.5	10.34 ± 2.37	8.84 ± 3.30	12.35 ± 5.65	14.62 ± 7.02	7.49 ± 2.47	11.06 ± 3.05	5.91 ± 0.69
IL-27 (pg/ml)	458.57 ± 124.20	1595.89 ± 531.88**	927.24 ± 369.21	1551.31 ± 658.89**	814.07 ± 333.50†	691.92 ± 177.07†	1370.75 ± 633.25**	837.27 ± 148.51†
IL-17A (pg/ml)	8.05 ± 1.54	27.00 ± 8.30	13.57 ± 3.51	75.64 ± 55.06	46.6 ± 25.81	20.81 ± 7.29	27.55 ± 6.16	14.67 ± 2.91
IFN-β (pg/ml)	725.56 ± 346.23	1398.61 ± 424.03*	1157.65 ± 458.75	1246.15 ± 409.40	1137.20 ± 680.83	1138.70 ± 431.56**	1609.33 ± 439.17	977.57 ± 283.20
GM-CSF (pg/ml)	10.72 ± 0.48	27.59 ± 7.98	20.70 ± 7.38	34.96 ± 13.69	24.22 ± 10.92	23.54 ± 6.85	29.27 ± 8.43	19.77 ± 3.61

Data shown as mean ± SEM. * p<0.05, ** p<0.01 *versus* vehicle. † p<0.05 *versus* 5A, ‡ p<0.05 *versus* 5A-C1



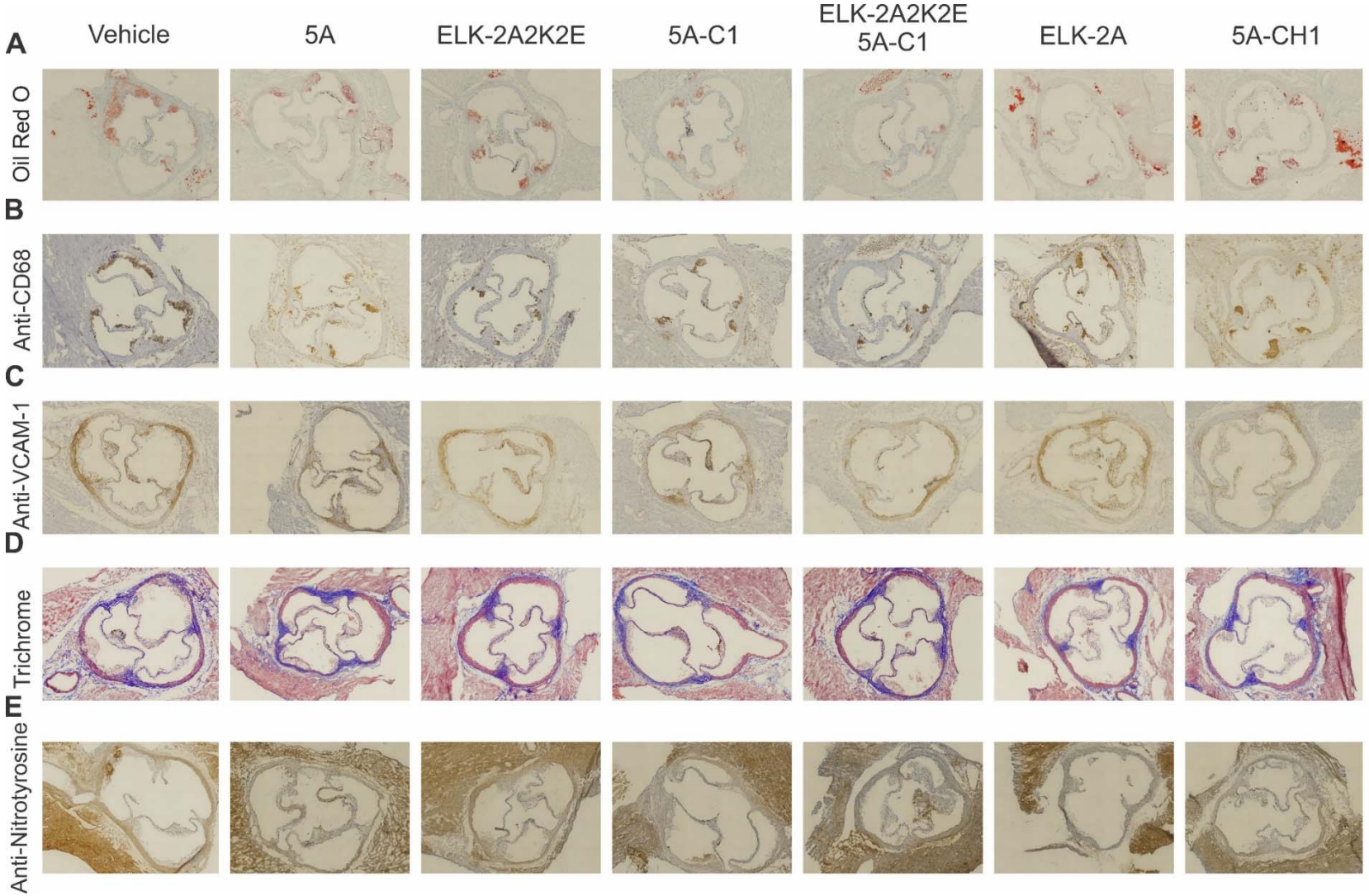
Supplemental Figure I. The effect of combination of the peptides on anti-atherogenic properties in vitro.

In "combination" the peptides were added at proportion 1:1 (w/w); each peptide was added at half the concentration compared to that when tested individually. **A** – Cholesterol efflux from RAW 264.7 cells. Peptide concentration 10 μg/ml; **B** – VCAM-1 expression in endothelial cells. Peptide concentration 0.75 mg/ml; **C** – LDL oxidation. Peptide concentration 100 μg/ml; **D** – CD11b expression in human monocytes. Peptide concentration 40 μg/ml. Methodology of each of the assay is described in the Materials and Method section. Dashed lines connect pairs with $p<0.01$.

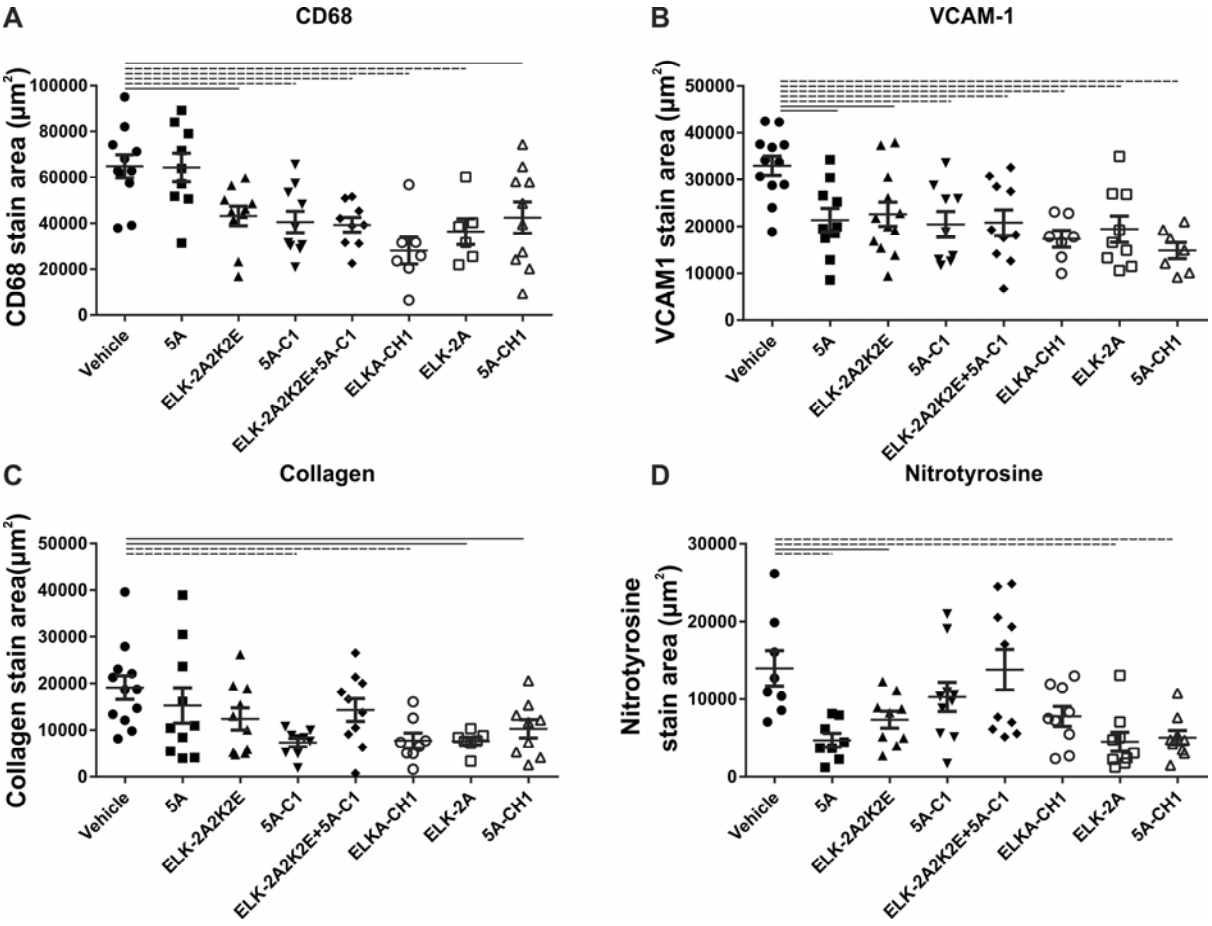


Supplemental Figure II. Representative *en face* images of the aortae of mice treated with the peptides.

A- Early lesions from 4 weeks study. B – Atherosclerotic lesions from 12 weeks study. The background around the vessels was replaced with uniform black to assist with visual assessment .

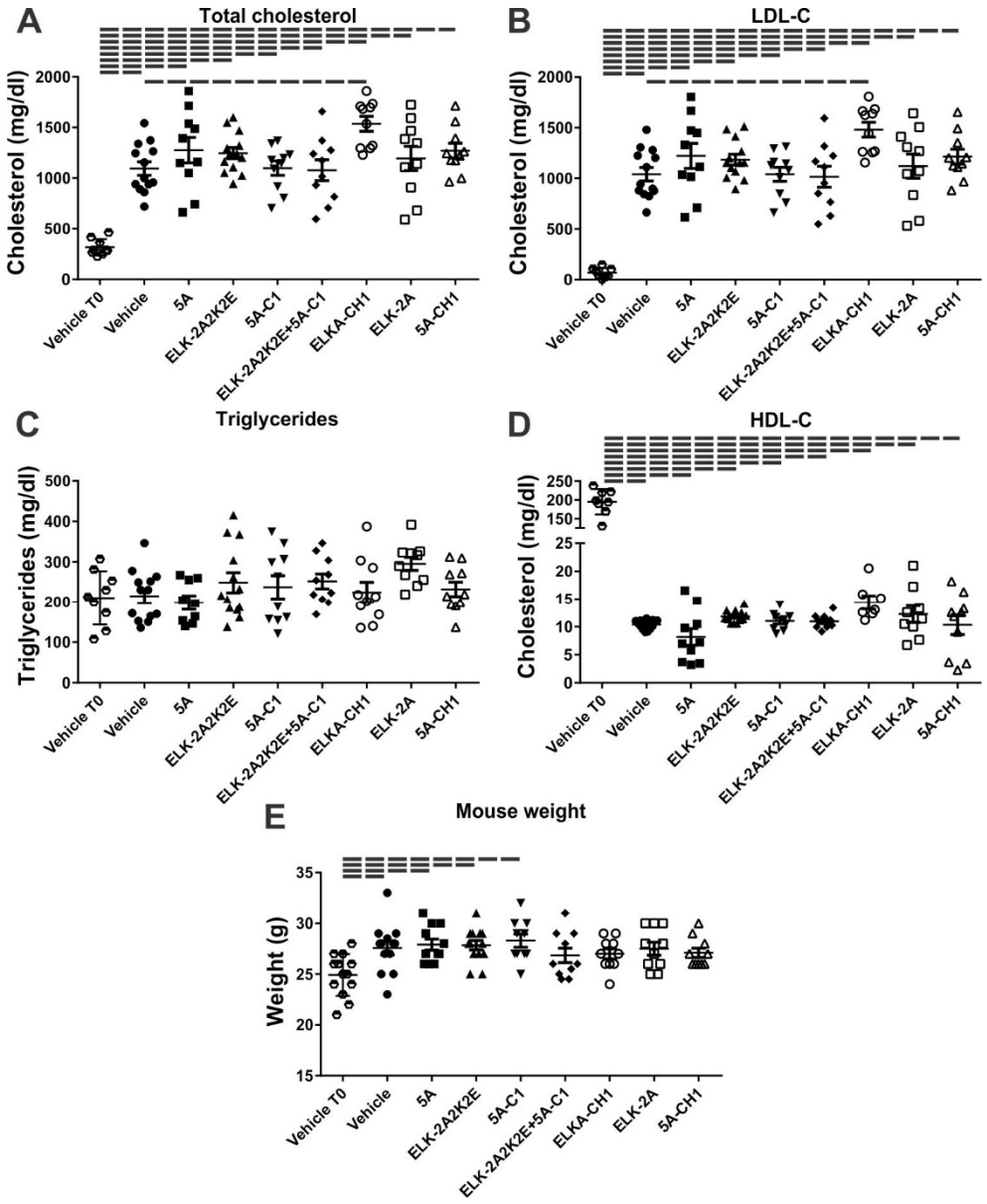


Supplemental Figure III. Morphology and immunostaining of aortic sinus sections after 4 weeks treatment with the peptides.



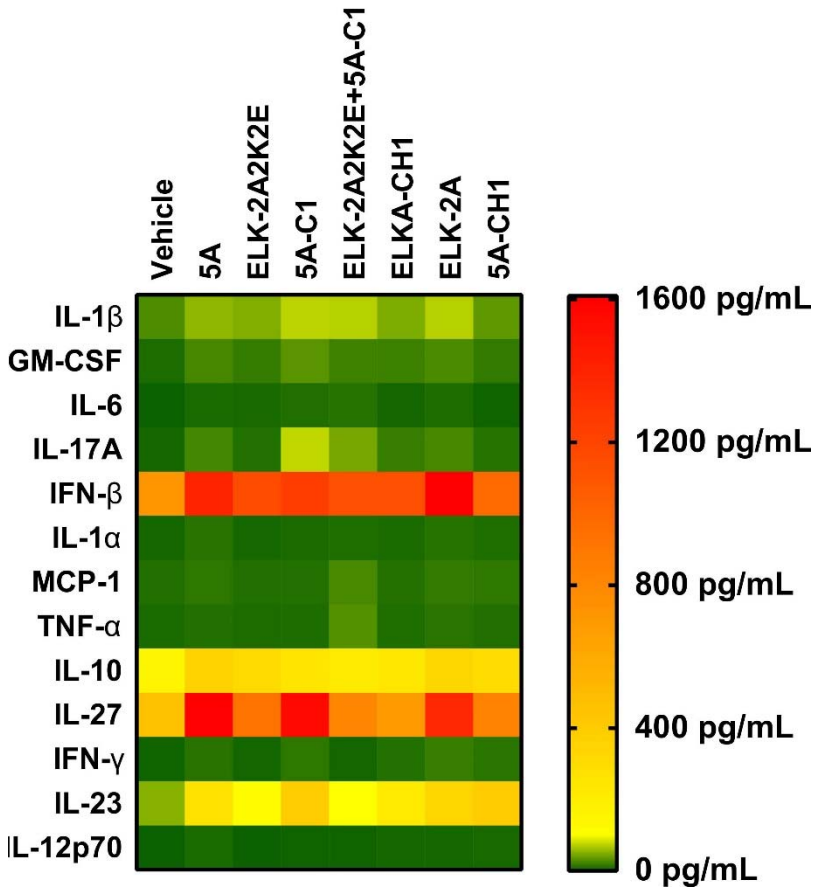
Supplemental Figure IV: Absolute values of the abundance of CD68+ cells (A), VCAM-1 (B), collagen (C) and nitrotyrosine (D) in early lesions; the effect of apoA-I mimetic peptides.

Solid lines connect pairs with $p<0.05$; dashed lines connect pairs with $p<0.01$



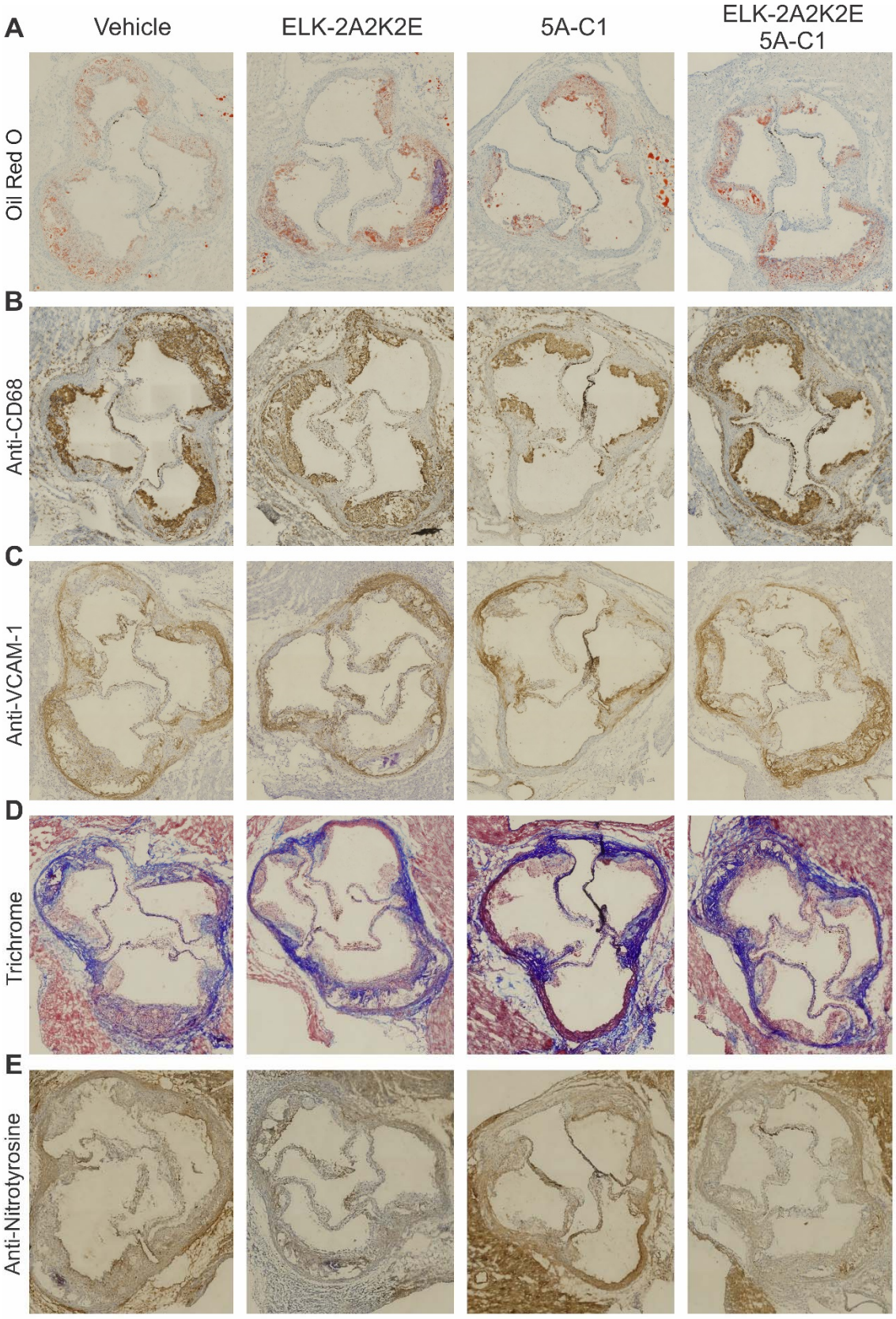
Supplemental Figure V. Plasma lipoprotein concentration and body weight after 4 weeks treatment with the peptides.

Effect of 4 week HFD feeding and peptide treatment on total cholesterol (**A**), LDL-C (**B**), triglycerides (**C**), HDL-C (**D**) and weight (**E**). Lipoprotein concentrations were determined as described in Materials and Methods section. Dashed lines connect pairs with $p<0.01$. "Vehicle T0" refers to time-point prior to commencement of HFD.

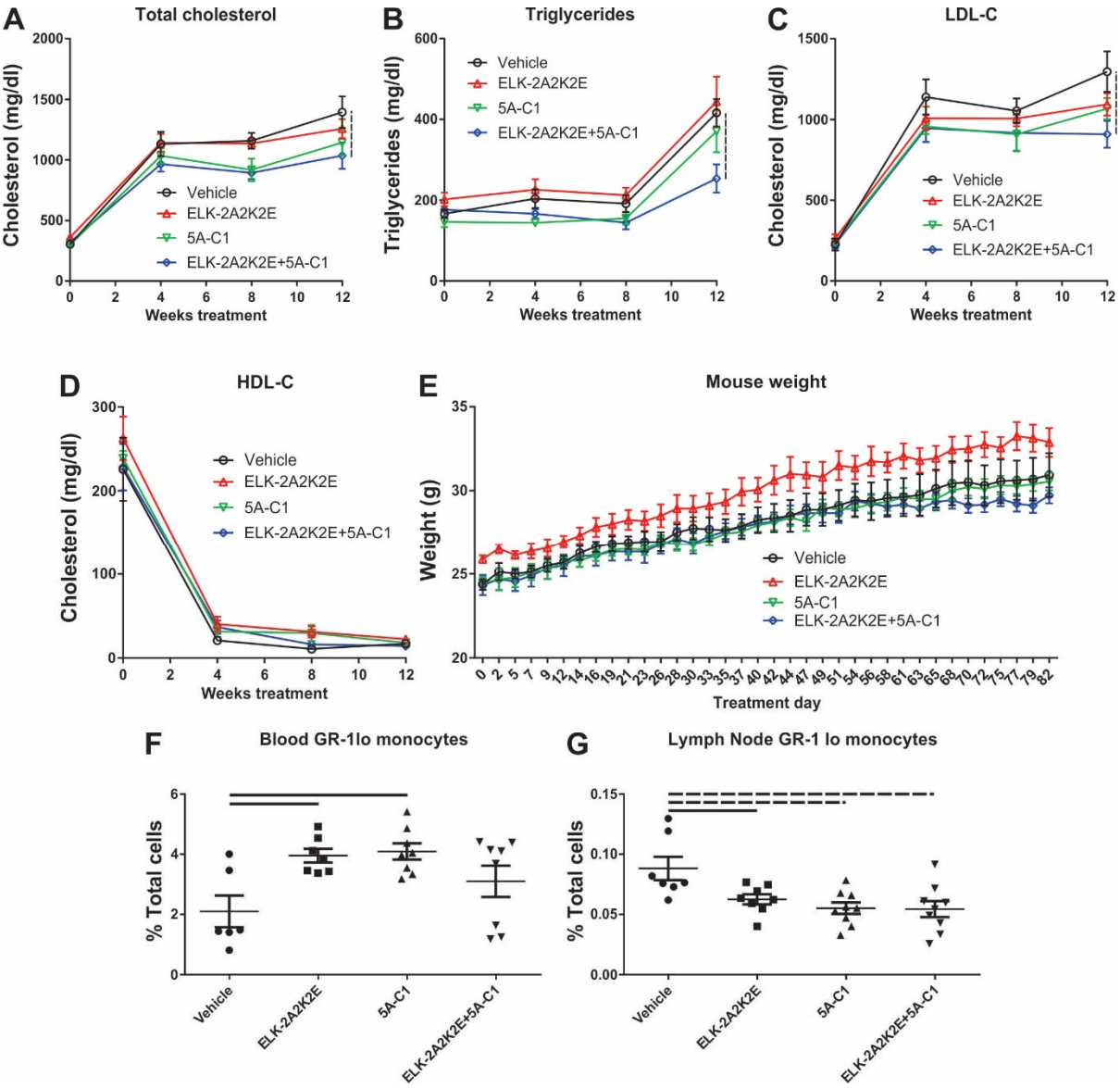


Supplemental Figure VI. Heat Diagram of the effect of 4-weeks peptide treatment on plasma cytokine levels.

This figure is representation of the data shown in Table II.

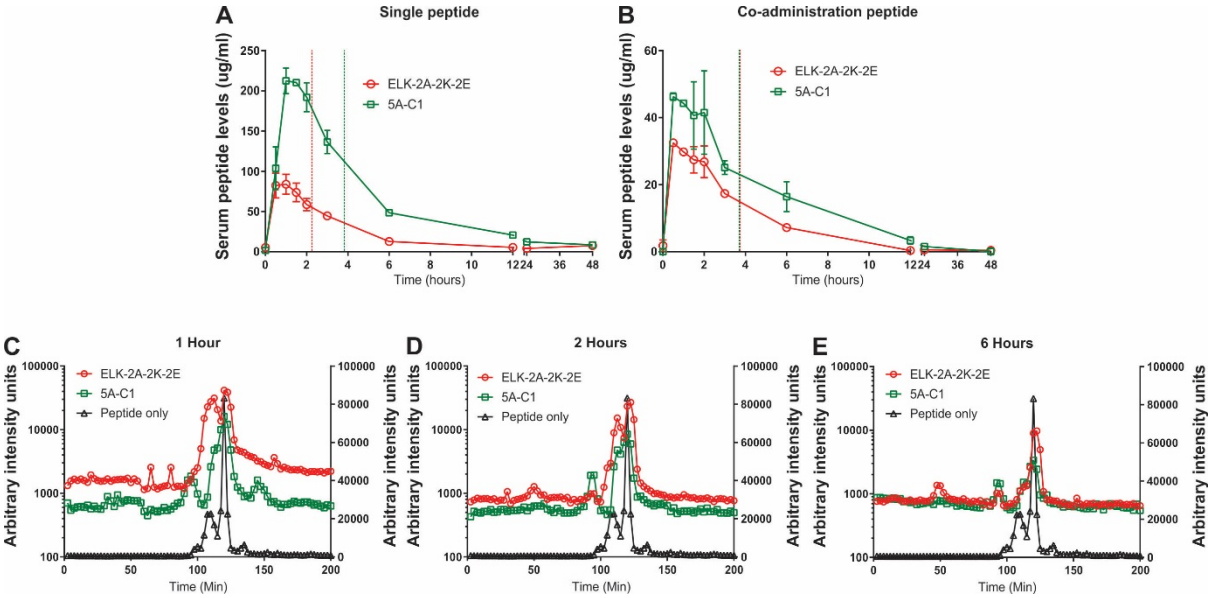


Supplemental Figure VII. Morphology and immunostaining of aortic sinus sections after 12 weeks treatment with the peptides.



Supplemental Figure VIII. Plasma lipoprotein concentration and body weight after 12 weeks HFD feeding and treatment with the peptides (A-E). Proportion of GR-Lo monocytes in blood and lymph nodes (F,G).

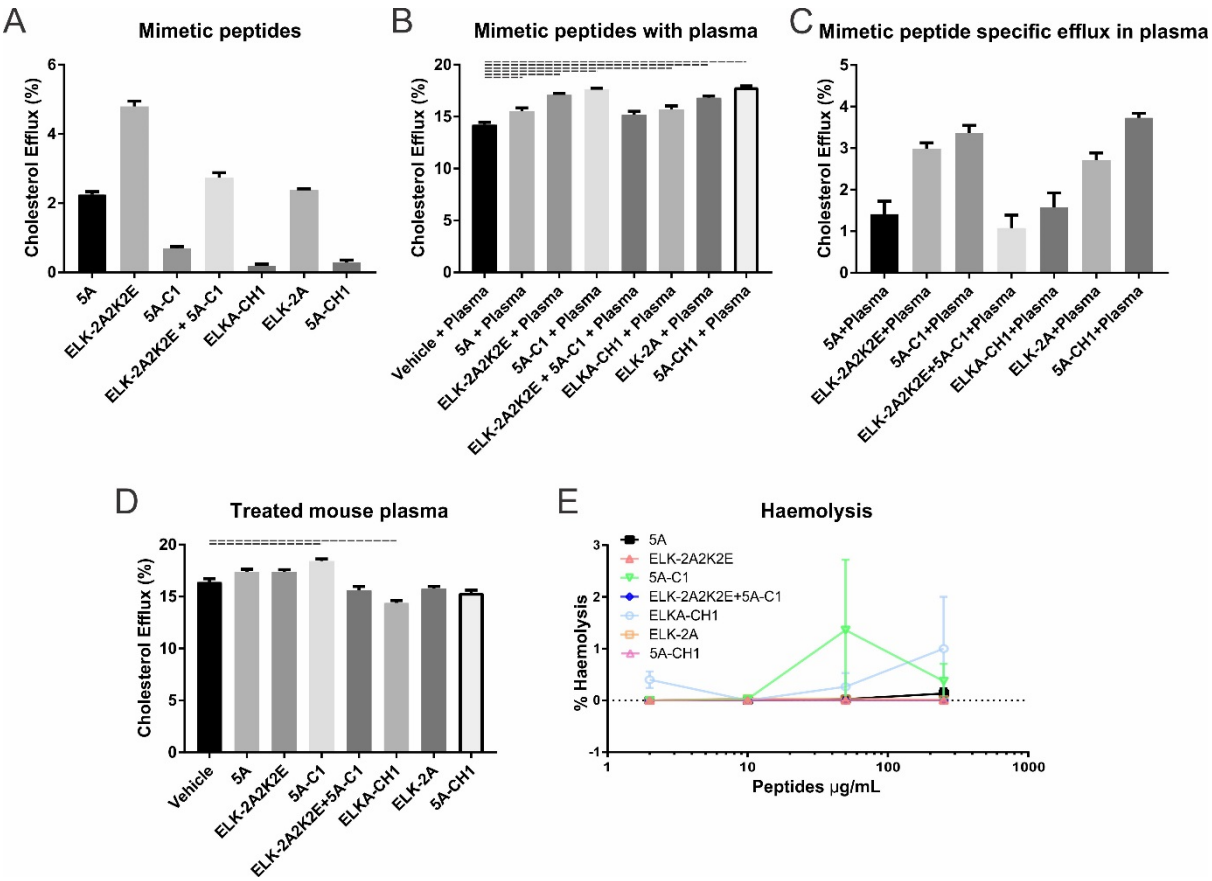
A – total cholesterol; **B** – LDL-C; **C** – triglycerides; **D** – HDL-C; **E** – body weight; **F** - GR1lo monocytes in blood; **G** – GR1lo monocytes in lymph nodes. Lipoprotein concentrations were determined and immune cells quantitated as described in Materials and Methods section. Solid lines connect pairs with $p<0.05$, dashed lines connect pairs with $p<0.01$. . "Vehicle T0" refers to time-point prior to commencement of HFD.



Supplemental Figure IX. Pharmacokinetics and biodistribution of the peptides

A, B - 1mg of fluorescently labelled 5A-C1 (green) and ELK-2A2K2E (red) were administered one at a time (A) or as 1:1 combination (B) via intraperitoneal injection to ApoE$^{-/-}$ mice fed HFD for 2 weeks. Vertical dashed lines indicate the calculated half-life of each peptide in plasma.

C-E – To elucidate peptide distribution in lipoprotein fractions plasma was separated by FPLC and individual fractions analysed for fluorescence. Fluorescently labelled peptide lipoprotein distribution one (**C**), two (**D**) and six (**E**) hours post injection are shown.



Supplemental Figure X. Functionality of the peptides in plasma.

A – Cholesterol efflux to the lipid-free peptides. **B** – Cholesterol efflux to the peptides added to mouse plasma in vitro and incubated for 1 h prior to the efflux assay. **C** – Values of cholesterol efflux to the peptides added to mouse plasma after subtraction of the efflux to plasma without peptides. **D** – Cholesterol efflux to plasma from mice treated with the peptides. **E** – Haemolysis following a 2 hour incubation of murine RBC with peptides.