

Supplemental information on:

T cell CX3CR1 mediates excess atherosclerotic inflammation in renal impairment

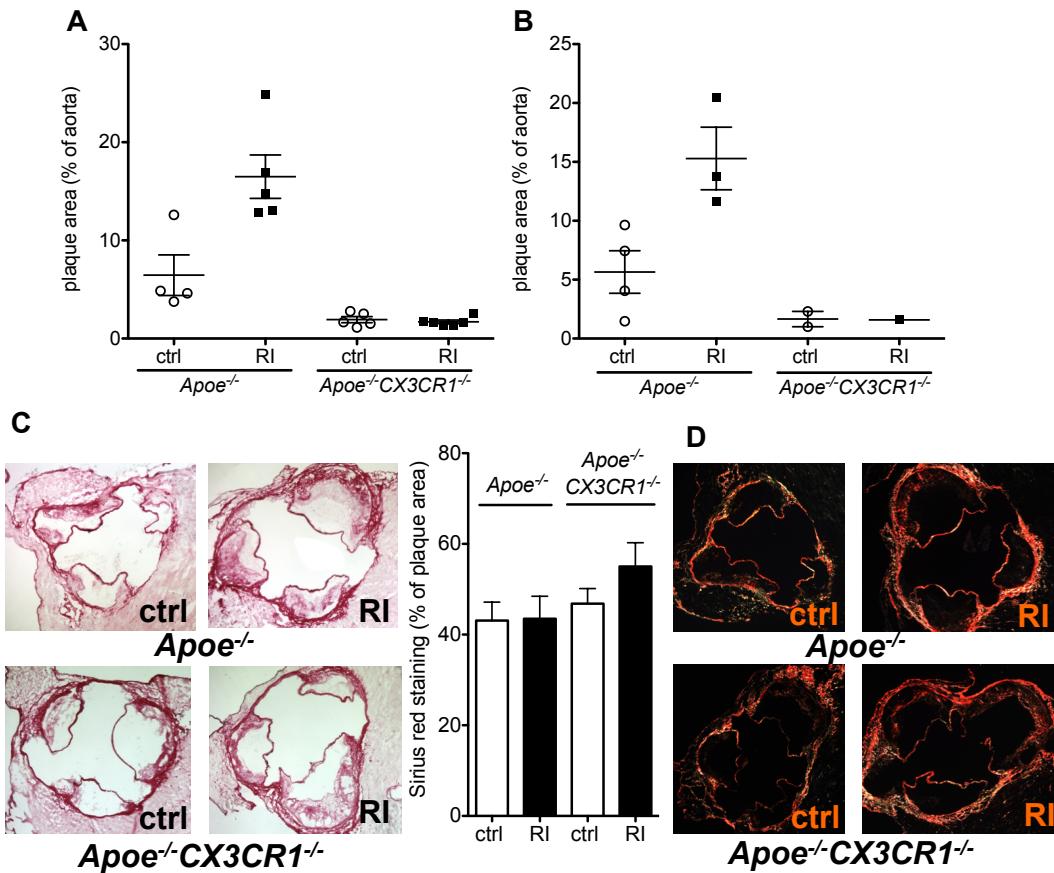
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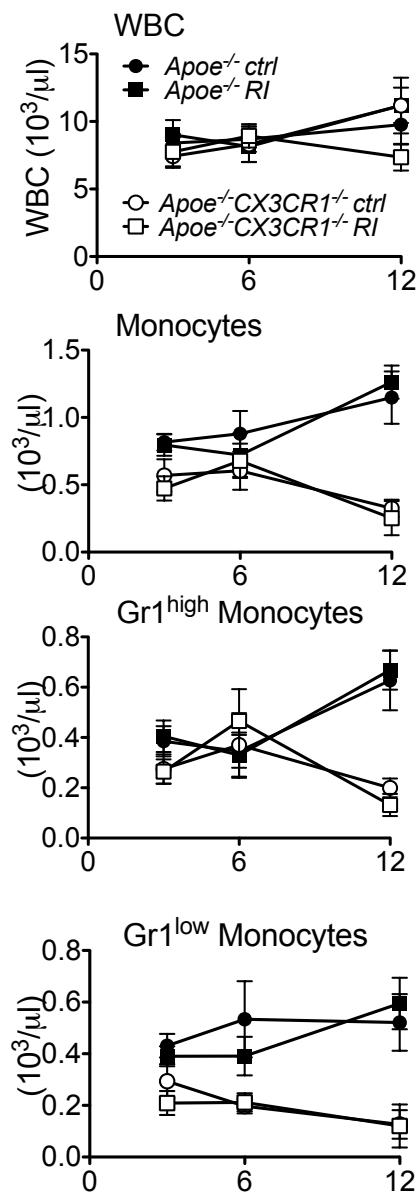
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Supplemental figure 1



Supplemental figure 1: Atherosclerotic aortic lesion characterization in *Apoe*^{-/-} and *CX3CR1*^{-/-}*Apoe*^{-/-} mice

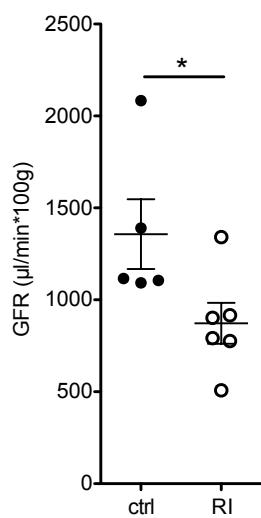
Atherosclerotic lesions were analyzed in wt *Apoe*^{-/-} and *CX3CR1*^{-/-}*Apoe*^{-/-} mice after unilateral nephrectomy (RI) or control surgery (ctrl) and 12 weeks of high fat diet (A,B). Aortic en face lesion size was assessed after lipid-staining for the total vessel for female (A) and male (B) mice separately. (C,D) Collagen contents was assessed after Picro-Sirius Red stain in translucent images (C, n=5-7 mice per group). Polarized light was used to assess collagen structure (D).



Supplemental figure 2: Peripheral blood monocyte dynamics in Apoe $^{-/-}$ and CX3CR1 $^{-/-}$ Apoe $^{-/-}$ mice

Blood monocytes were analyzed during the course of high fat diet in wt Apoe $^{-/-}$ and CX3CR1 $^{-/-}$ Apoe $^{-/-}$ mice after unilateral nephrectomy (RI) or control surgery (ctrl) (A-D) Peripheral blood total leukocytes (A), monocytes (B), Gr 1^{high} (C) and Gr 1^{low} (D) monocyte counts in after 3, 6 and 12 weeks high fat diet.

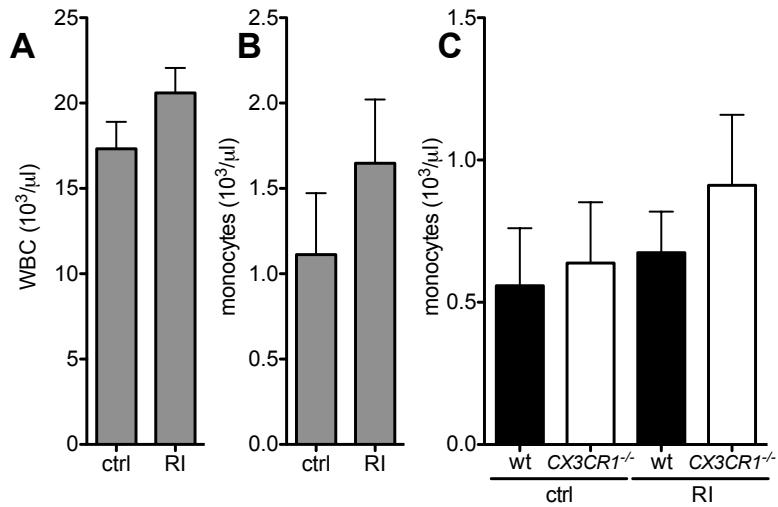
Supplemental figure 3



Supplemental figure 3: Glomerular filtration rate is significantly reduced by unilateral nephrectomy in $LDLr^{-/-}$ mice

Transcutaneous measurement of FITC sinistrin clearance using NIC kidney device five to six weeks after unilateral nephrectomy (RI) or control surgery (ctrl) (n=5-6 $LDLr^{-/-}$ mice).

Supplemental figure 4



Supplemental figure 4: Circulating leukocyte and monocyte counts and subtypes in $LDLr^{-/-}$ mice

(A-C) Total white blood count (A), blood monocytes (B) and absolute monocyte counts per genotype (C) in blood at 6 weeks high fat diet in mixed bone marrow chimeric wt and $CX3CR1^{-/-}LDLr^{-/-}$ mice (n=5-7).

Suppl. tables:

Suppl. table 1: Characteristics of *Apoe*^{-/-} mice after 12 weeks high fat diet

BM genotype	control		RI		p-value		p-value	
	wt	<i>CX3CRI</i>^{-/-}	wt	<i>CX3CRI</i>^{-/-}	wt	<i>CX3CRI</i>^{-/-}	ctrl	RI
Body (g)	30±2(7)	27±2(7)	30±2(7)	24±1 (7)	ANOVA not significant			
Spleen (g)	0.18±0(7)	0.19±0(7)	0.20±0(7)	0.21±0(7)	ANOVA not significant			
Kidney (g)	0.24±0(7)	0.19±0(7)	0.34±0(7)	0.24±0(7)	<0.05	n.sig.	n.sig.	<0.05
Rel. kidney w.	0.8±0(7)	0.7±0(7)	1.1±0(7)	1.0±0(7)	<0.001	<0.001	n.sig.	n.sig.
Urea (mmol/l)	11±0(7)	10±1(7)	12±1(4)	14±1(5)	ANOVA not significant			
Ca ²⁺ (mmol/l)	2.4±0(7)	2.2±0(5)	2.4±0(5)	2.4±0(5)	ANOVA not significant			
Pho. (mmol/l)	3.2±0(7)	2.8±0(5)	3.0±0(5)	2.8±0(5)	ANOVA not significant			
Leukocytes (10 ³ /μl)	10±1.5 (6)	12±1.5(7)	11±1.3(6)	8±1.0(7)	ANOVA not significant			
Monocytes (10 ³ /μl)	1.3±1(5)	0.7±0 (5)	1.4±0 (5)	0.5±0 (5)	ANOVA not significant			
Thrombocytes (10 ³ /μl)	591±120 (4)	421±113 (7)	685±126 (4)	598±841 (7)	ANOVA not significant			
Erythrocytes (10 ⁶ /μl)	9.6±0 (4)	9.6±0 (7)	8.9±0 (4)	8.9±0 (7)	ANOVA not significant			
Cholesterol (mmol/l)	32±5 (6)	34±4 (4)	34±4 (7)	35±3 (5)	ANOVA not significant			
Triglycerides (mmol/l)	0.8±0(5)	0.9±0(4)	1.2±0(4)	1.1±0 (5)	ANOVA not significant			

Values are means ± SEM (n). P values are given for Bonferroni's test if ANOVA of all four groups was significant. Relative weight is in % of body weight. Control: mice after control surgery, RI: mice after unilateral nephrectomy.

Suppl. table 2: Characteristics of *LDLr*^{-/-} mice with renal impairment with wt and CX3CR1 bone marrow after 10 weeks high fat diet

BM genotype	wt	<i>CX3CRI</i> ^{-/-}
Body (g)	20±1 (12)	21±1 (11)
Kidney (g)	0.18±0(5)	0.22±0(6)
Rel. kidney weight	0.9±0(5)	0.9±0(6)
Creatinine (μmol/l)	33±3 (7)	28±2 (7)
Urea (mmol/l)	15±1(7)	14±1(7)
Ca ²⁺ (mmol/l)	2.4±0(7)	2.4±0(6)
Phosphorous (mmol/l)	2.5±0(7)	2.6±0(6)
Leukocytes (10 ³ /μl)	12±2 (11)	14±3(11)
Monocytes (10 ³ /μl)	1.1±0.2(10)	1.1±0.2 (11)
Thrombocytes (10 ³ /μl)	491±53 (11)	507±160 (10)
Erythrocytes (10 ⁶ /μl)	7.2±0 (11)	7.4±0 (11)
Cholesterol	37±5 (7)	33±4 (7)
Triglycerides	5.5±0 (7)	4.7±0 (6)

Values are means ±SEM (n). No significant differences were observed (Student's T tests).

Suppl. table 3: Characteristics of *LDLr*^{-/-} mice transplanted with 50% wt and 50% CX3CR1 bone marrow after 10 weeks high fat diet

Renal surgery	control	RI	p-value
Body (g)	23±1 (7)	22±2 (4)	not significant
Kidney (g)	0.17±0 (7)	0.22±0 (4)	not significant
Rel. kidney weight	0.8±0 (7)	1.0±0 (4)	*p=0.03
Creatinine (μmol/l)	21±3 (5)	25±2 (4)	*p=0.02
Urea (mmol/l)	8.9±1 (5)	12.6±1 (4)	**p< 0.01
Ca ²⁺ (mmol/l)	2.4±0 (5)	2.5±0(4)	not significant
Phosphorous (mmol/l)	2.2±0 (6)	2.2±0(4)	not significant
Leukocytes (10 ³ /μl)	8.6±1 (7)	9.6±2 (4)	not significant
Monocytes (10 ³ /μl)	0.8±0.1(7)	0.9±0.1 (4)	not significant
Thrombocytes (10 ³ /μl)	596±76 (7)	752±1147 (4)	not significant
Erythrocytes (10 ⁶ /μl)	9.1±0 (7)	9.7±1 (4)	not significant
Cholesterol	39±4 (6)	40±8 (4)	not significant
Triglycerides	4.9±0 (5)	3.9±0 (3)	not significant

Values are means ±SEM (n). P values are given for student's T tests if significant. Relative weight is in % of body weight. Control: mice after control surgery, RI: mice after unilateral nephrectomy.