

## **Supplemental Material**

Identification of a Genetic Locus on Chromosome 11 that Regulates Leukocyte Infiltration in Mouse Carotid Artery

Elaine M. Smolock<sup>1</sup>, Dietrich E. Machleder<sup>1</sup>, Vyacheslav A. Korshunov<sup>1</sup> and Bradford C. Berk<sup>1\*</sup>

<sup>1</sup>From the Aab Cardiovascular Research Institute and Department of Medicine, University of Rochester School of Medicine and Dentistry, Rochester, NY USA

\*To whom correspondence should be addressed:

Bradford C. Berk, MD, PhD  
University of Rochester  
Aab Cardiovascular Research Institute,  
601 Elmwood Ave, Box 679  
Rochester, NY 14642  
Phone: 585-273-1946  
Fax: 585-273-1497  
Email: Bradford\_Berk@urmc.rochester.edu

**Running title:** Genetics of Leukocyte infiltration in Mouse Carotid

**Supplemental Table SI.** List of SNPs differing between C3H/F and SJL mice in the chr11 *Im2* locus

Gene symbol	Name / description	Chromosome location
1700007J10Rik	RIKEN cDNA 1700007J10 gene	11:59539419-59553656
1700008A04Rik	RIKEN cDNA 1700008A04 gene	11:32718529-32724605
1810063I02Rik	RIKEN cDNA 1810063I02 gene	11:59735001-59735391
3010026O09Rik	RIKEN cDNA 3010026O09 gene	11:49987946-50013617
4930403D09Rik	RIKEN cDNA 4930403D09 gene	11:34199707-34208113
4930469K13Rik	RIKEN cDNA 4930469K13 gene	11:33873961-33998554
4930505A04Rik	RIKEN cDNA 4930505A04 gene	11:30326006-30371827
4930555O08Rik	RIKEN cDNA 4930555O08 gene	11:32689762-32690725
4933406G16Rik	RIKEN cDNA 4933406G16 gene	11:18965279-18975743
4933424L21Rik	RIKEN cDNA 4933424L21 gene	11:57437200-57462996
4933427E13Rik	RIKEN cDNA 4933427E13 gene	11:25226107-25267137
4933430M04Rik	RIKEN cDNA 4933430M04 gene	11:24381909-24469952
5133400J02Rik	RIKEN cDNA 5133400J02 gene	11:51003335-51033920
8430419K02Rik	RIKEN cDNA 8430419K02 gene	11:18745854-18774312
A230004M16Rik	RIKEN cDNA A230004M16 gene	11:41523844-41786619
Acyp2	acylphosphatase 2, muscle type	11:30405991-30549587
Adams2	a disintegrin-like and metallopeptidase (reprolysin type) with thrombospondin type 1 motif, 2	11:50415586-50621075
Adra1b	adrenergic receptor, alpha 1b	11:43588108-43714712
Aff4	AF4/FMR2 family, member 4	11:53164335-53235332
Aftph	aftphilin	11:20585087-20641592
Asb3	ankyrin repeat and SOCS box-containing 3	11:30854395-31002704
Atp10b	ATPase, class V, type 10B	11:42963379-43075787
BC049762	cDNA sequence BC049762	11:51067153-51076548
Bc111a	B cell CLL/lymphoma 11A (zinc finger protein)	11:23978056-24074123
Btnl9	butyrophilin-like 9	11:48979087-49000661
Clqtnf2	Clq and tumor necrosis factor related protein 2	11:43287778-43305027
Canx	calnexin	11:50107969-50139175
Ccdc85a	coiled-coil domain containing 85A	11:28285685-28484324
Ccdc88a	coiled coil domain containing 88A	11:29273658-29410808
Ccdc99	coiled-coil domain containing 99	11:34622692-34647143
Ccnj1	cyclin J-like	11:43342286-43400499
Cdk13	cyclin-dependent kinase-like 3	11:51817723-51903286
Cdkn2aipnl	CDKN2A interacting protein N-terminal like	11:51781163-51790836
Chac2	ChaC, cation transport regulator 2	11:30876707-30886350
Clint1	clathrin interactor 1	11:45665553-45724127
Clk4	CDC like kinase 4	11:51075232-51095268
Col23a1	collagen, type XXIII, alpha 1	11:51103422-51397420
Cops3	COP9 (constitutive photomorphogenic) homolog, subunit 3 (Arabidopsis thaliana)	11:59631297-59653340
Cyfp2	cytoplasmic FMR1 interacting protein 2	11:46007357-46125796
D930048N14Rik	RIKEN cDNA D930048N14 gene	11:51464456-51471183
Dock2	dedicator of cyto-kinesis 2	11:34126815-34597394
Ebfl	early B cell factor 1	11:44430819-44821593
<b>Efemp1</b>	<b>epidermal growth factor-containing fibulin-like extracellular matrix protein 1</b>	<b>11:28753204-28826743</b>
Eml6	echinoderm microtubule associated protein like 6	11:29643048-29926033
F630206G17Rik	RIKEN cDNA F630206G17 gene	11:45621589-45654673
Fam114a2	family with sequence similarity 114, member A2	11:57296495-57332119
Fam196b	family with sequence similarity 196, member B	11:34214822-34322640
Fanc1	Fanconi anemia, complementation group L	11:26286135-26371876
Fbxw11	F-box and WD-40 domain protein 11	11:32542724-32646789
Fstl4	follistatin-like 4	11:52578136-53002040
Gabra1	gamma-aminobutyric acid (GABA) A receptor, subunit alpha 1	11:41944441-41996432
Gabra6	gamma-aminobutyric acid (GABA) A receptor, subunit alpha 6	11:42119939-42134574
Gabrb2	gamma-aminobutyric acid (GABA) A receptor, subunit beta 2	11:42233259-42442530
Gabrg2	gamma-aminobutyric acid (GABA) A receptor, subunit gamma 2	11:41723705-41814359
Galnt10	UDP-N-acetyl-alpha-D-galactosamine:polypeptide N-acetylglucosaminyltransferase 10	11:57458944-57601016
Gm12021	predicted gene 12021	11:18408040-18409943
Gm12022	predicted gene 12022	11:18367482-18393258
Gm12023	predicted gene 12023	11:18495251-18610225
Gm12068	predicted gene 12068	11:24326246-24622646
Gm12069	predicted gene 12069	11:25201263-25222786
Gm12082	predicted gene 12082	11:28651633-28715767
Gm12092	predicted gene 12092	11:29512324-29545509
Gm12093	predicted gene 12093	11:29588241-29589835
Gm12100	predicted gene 12100	11:30650832-30656147
Gm12111	predicted gene 12111	11:32803137-32826160

Gene symbol	Name / description	Chromosome location
Gm12128	predicted gene 12128	11:37472491-37486668
Gm12130	predicted gene 12130	11:38306832-38333515
Gm12132	predicted gene 12132	11:39821696-40151333
Gm12146	predicted gene 12146	11:43010926-43073286
Gm12147	predicted gene 12147	11:43073293-43124590
Gm12153	predicted gene 12153	11:43772591-43948489
Gm12160	predicted gene 12160	11:44974445-45027158
Gm12162	predicted gene 12162	11:45222544-45282006
Gm12665	predicted gene 12665	11:24803983-24826058
Gm12714	predicted gene 12714	11:59711876-59720821
Gm16641	predicted gene, 16641	11:50050511-50102341
Gm16953	predicted gene, 16953	11:51763384-52083016
Gm8098	predicted gene 8098	11:30167464-30180491
Gm9972	predicted gene 9972	11:42849248-42850780
Gnb211	guanine nucleotide binding protein (G protein), beta polypeptide 2 like 1	11:48613834-48619936
Gria1	glutamate receptor, ionotropic, AMPA1 (alpha 1)	11:56824889-57143746
Hmmr	hyaluronan mediated motility receptor (RHAMM)	11:40514897-40546924
Il12b	interleukin 12b	11:44213565-44227535
Kcnip1	Kv channel-interacting protein 1	11:33529339-33893152
Kif3a	kinesin family member 3A	11:53380881-53415469
Lsm11	U7 snRNP-specific Sm-like protein LSM11	11:45741771-45758437
Maml1	mastermind like 1 (Drosophila)	11:50069136-50105813
Mat2b	methionine adenosyltransferase II, beta	11:40492816-40508705
Med9	mediator of RNA polymerase II transcription, subunit 9 homolog (yeast)	11:59761708-59775707
Meis1	Meis homeobox 1	11:18780433-18918961
Mfap3	microfibrillar-associated protein 3	11:57332166-57347317
Mgat1	mannoside acetylglucosaminyltransferase 1	11:49057748-49076532
Mprp	myosin phosphatase Rho interacting protein	11:59474807-59594356
Mtif2	mitochondrial translational initiation factor 2	11:29426408-29445279
Nhp2	NHP2 ribonucleoprotein homolog (yeast)	11:51433237-51437216
Nlrp3	NLR family, pyrin domain containing 3	11:59355070-59380457
Nt5m	5',3'-nucleotidase, mitochondrial	11:59652949-59694470
Olf156	olfactory receptor 56	11:48864235-48948889
P4ha2	procollagen-proline, 2-oxoglutarate 4-dioxygenase (proline 4-hydroxylase), alpha II polypeptide	11:53913597-53945167
Pemt	phosphatidylethanolamine N-methyltransferase	11:59784116-59859991
<b>Pnpt1</b>	<b>polynucleotide nucleotidyltransferase 1</b>	<b>11:29030744-29061828</b>
Ppp3r1	protein phosphatase 3, regulatory subunit B, alpha isoform (calcineurin B, type I)	11:17059266-17100378
Psme4	proteasome (prosome, macropain) activator subunit 4	11:30671726-30780361
Pttg1	pituitary tumor-transforming gene 1	11:43233752-43239753
Rad50	RAD50 homolog (S. cerevisiae)	11:53463021-53520821
Rai1	retinoic acid induced 1	11:59918515-60012699
Ranbp17	RAN binding protein 17	11:33111795-33413746
Rars	arginyl-tRNA synthetase	11:35621883-35648008
Rmnd5b	required for meiotic nuclear division 5 homolog B (S. cerevisiae)	11:51437173-51449398
Rnf145	ring finger protein 145	11:44332466-44379022
Rtn4	reticulon 4	11:29592947-29644331
Sar1b	SAR1 gene homolog B (S. cerevisiae)	11:51577189-51605427
Sec24a	Sec24 related gene family, member A (S. cerevisiae)	11:51505766-51577136
Sgcd	sarcoglycan, delta (dystrophin-associated glycoprotein)	11:46709755-47802471
Sh3pxd2b	SH3 and PX domains 2B	11:32247820-32328173
Slit3	slit homolog 3 (Drosophila)	11:34934726-35522009
Slu7	SLU7 splicing factor homolog (S. cerevisiae)	11:43247246-43261483
Smek2	SMEK homolog 2, suppressor of mek1 (Dictyostelium)	11:29072890-29120797
Snap47	synaptosomal-associated protein, 47	11:59220636-59264688
Spnb2	spectrin beta 2	11:29999395-30168175
Spred2	sprouty-related, EVH1 domain containing 2	11:19824378-19924029
Sqstm1	sequestosome 1	11:50012868-50024329
Stc2	stanniocalcin 2	11:31257307-31270074
Stk10	serine/threonine kinase 10	11:32433305-32524587
Tcf7	transcription factor 7, T cell specific	11:52065873-52096516
Trim41	tripartite motif-containing 41	11:48619906-48630855
Ube2b	ubiquitin-conjugating enzyme E2B	11:51798999-51814264
Uqcrcq	ubiquinol-cytochrome c reductase, complex III subunit VII	11:53241424-53244333
Vrk2	vaccinia related kinase 2	11:26371322-26493999
Wdr92	WD repeat domain 92	11:17082110-17133799
Wnt3a	wingless-related MMTV integration site 3A	11:59061535-59104254
Wnt9a	wingless-type MMTV integration site 9A	11:59120430-59147054
Wwcl	WW, C2 and coiled-coil domain containing 1	11:35651902-35794029

<b>Gene symbol</b>	<b>Name / description</b>	<b>Chromosome location</b>
Zfp62	zinc finger protein 62	11:49016794-49032318
Zfp672	zinc finger protein 672	11:58128626-58136825
Zfp867	zinc finger protein 867	11:59274699-59285976
Zkscan17	zinc finger with KRAB and SCAN domains 17	11:59299022-59340253

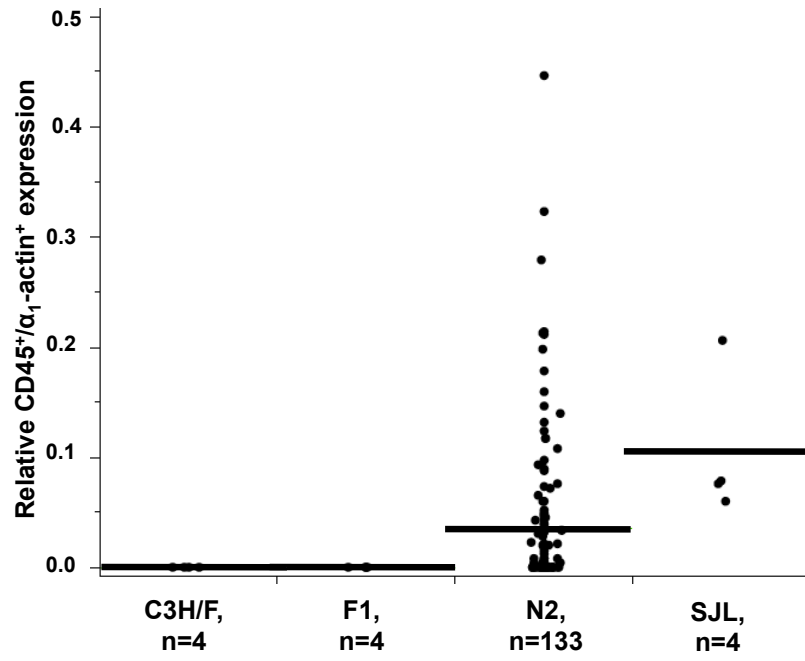
## Supplemental Figure Legends

**Supplemental Figure SI. CD45<sup>+</sup> expression normalized to  $\alpha_1$ -actin<sup>+</sup> in the C3H/FxSJL backcross. A.** Relative CD45<sup>+</sup> normalized to  $\alpha_1$ -actin<sup>+</sup> expression in the vascular wall of C3H/F, F1 and N2 progeny and SJL mice 14 days after injury. CD45<sup>+</sup> expression in the N2 progeny exhibited an intermediate phenotype.

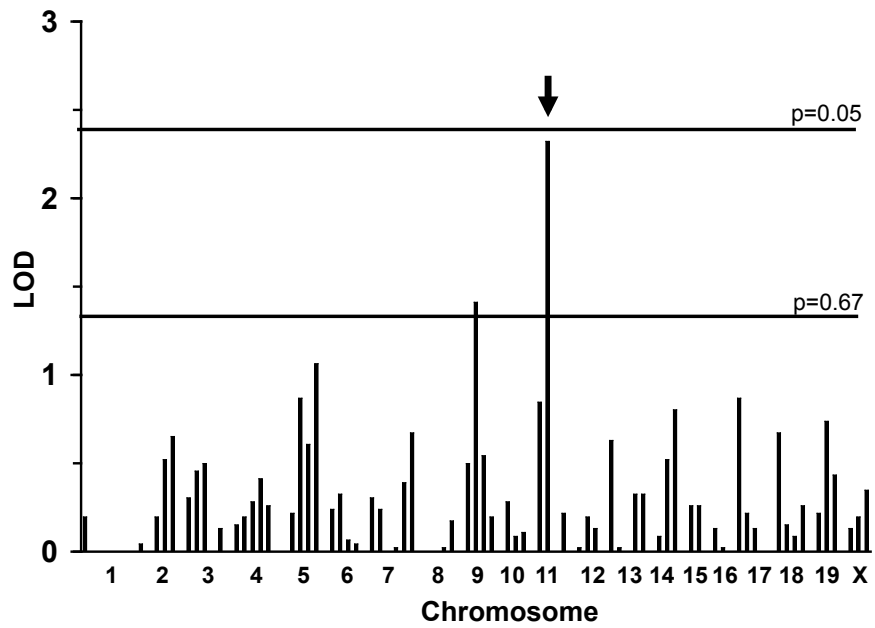
**Supplemental Figure SII. Quantitative trait linkage analysis for CD45<sup>+</sup> expression normalized to  $\alpha_1$ -actin<sup>+</sup>. A.** All N2 progeny were used for QTL analysis of the ration of CD45<sup>+</sup>/ $\alpha_1$ -actin<sup>+</sup> expression. A nearly significant QTL was found on chr11 (LOD 2.3). A nearly suggestive QTL was identified on chr9 (LOD 1.4).

**Supplemental Figure SIII. *Im2* congenic mice and morphometric analysis. A-C.** Lumen, intima+media, and external elastic lamina (EEL) were quantified in parental and congenic mice 14 days following partial carotid ligation. There were no significant differences in media (A) intima+media (B) of EEL (C) volumes among the C3H/F and congenic lines.

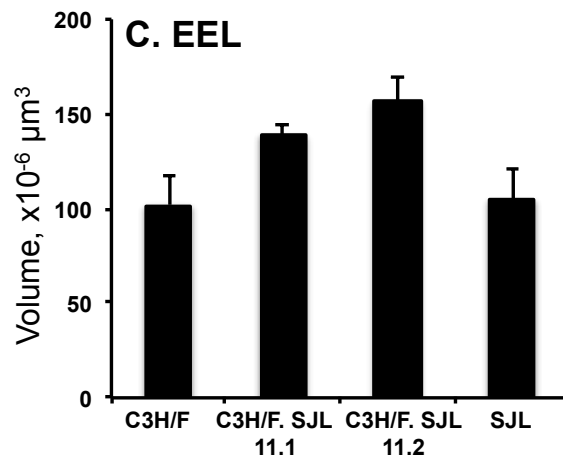
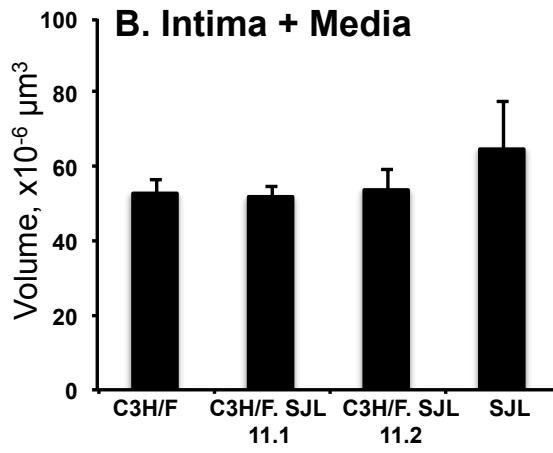
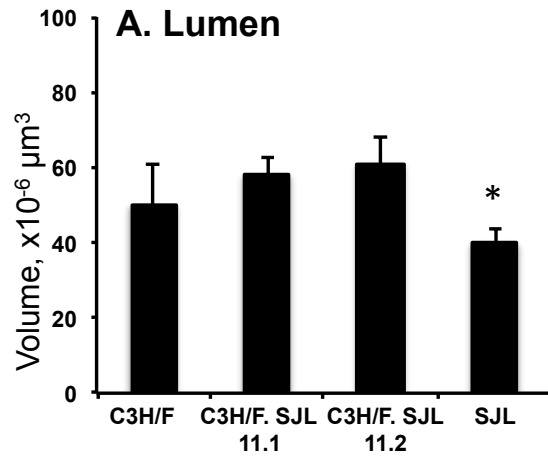
**Supplemental Figure SIV. CD45<sup>+</sup> expression normalized to  $\alpha_1$ -actin<sup>+</sup> in the congenic mice.** Sections from C3H/F.SJL.11.1 and C3H/F.SJL.11.2 where intima area was observed were stained for CD45<sup>+</sup> and  $\alpha_1$ -actin<sup>+</sup> 14 days after injury. CD45<sup>+</sup> expression normalized to  $\alpha_1$ -actin<sup>+</sup> was increased in C3H/F.SJL.11.1 but not in C3H/F.SJL.11.2 sections compared to C3H/F.



**SUPPLEMENTAL FIGURE S1**

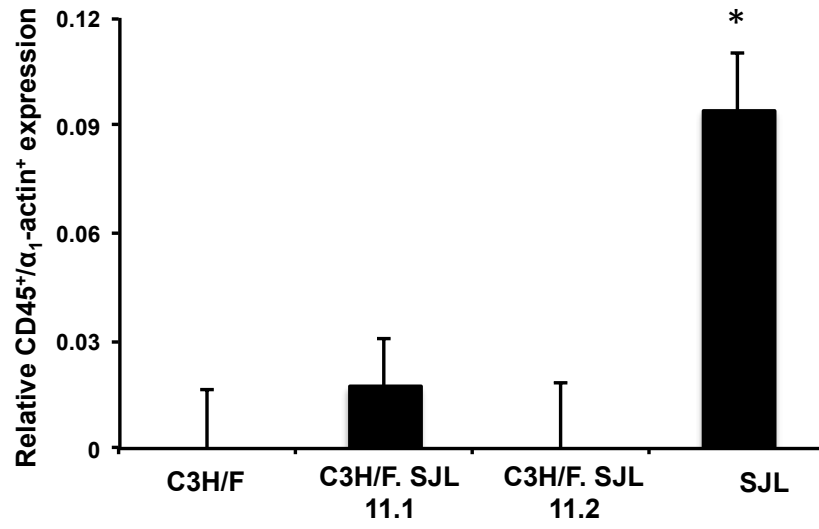


**SUPPLEMENTAL FIGURE SII**



**SUPPLEMENTAL FIGURE SIII**





**SUPPLEMENTAL FIGURE SIV**

## References for Materials and Methods

1. Korshunov VA, Berk BC. Genetic modifier loci linked to intima formation induced by low flow in the mouse carotid. *Arterioscler Thromb Vasc Biol.* 2009;29:47-53
2. Lande R, Thompson R. Efficiency of marker-assisted selection in the improvement of quantitative traits. *Genetics.* 1990;124:743-756
3. Korshunov VA, Berk BC. Flow-induced vascular remodeling in the mouse: A model for carotid intima-media thickening. *Arterioscler Thromb Vasc Biol.* 2003;23:2185-2191
4. Lander E, Kruglyak L. Genetic dissection of complex traits: Guidelines for interpreting and reporting linkage results. *Nat Genet.* 1995;11:241-247.