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Supplementary Information for

Uniquely-Human CHRFAM7A Gene Increases the Hematopoietic Stem Cell Reservoir in Mice and Amplifies their Inflammatory Response

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This PDF file includes:

(1) Figs. S1 to S10
(2) Tables S1 to S4
(3) Methodological details

Other supplementary materials for this manuscript include the following:

None

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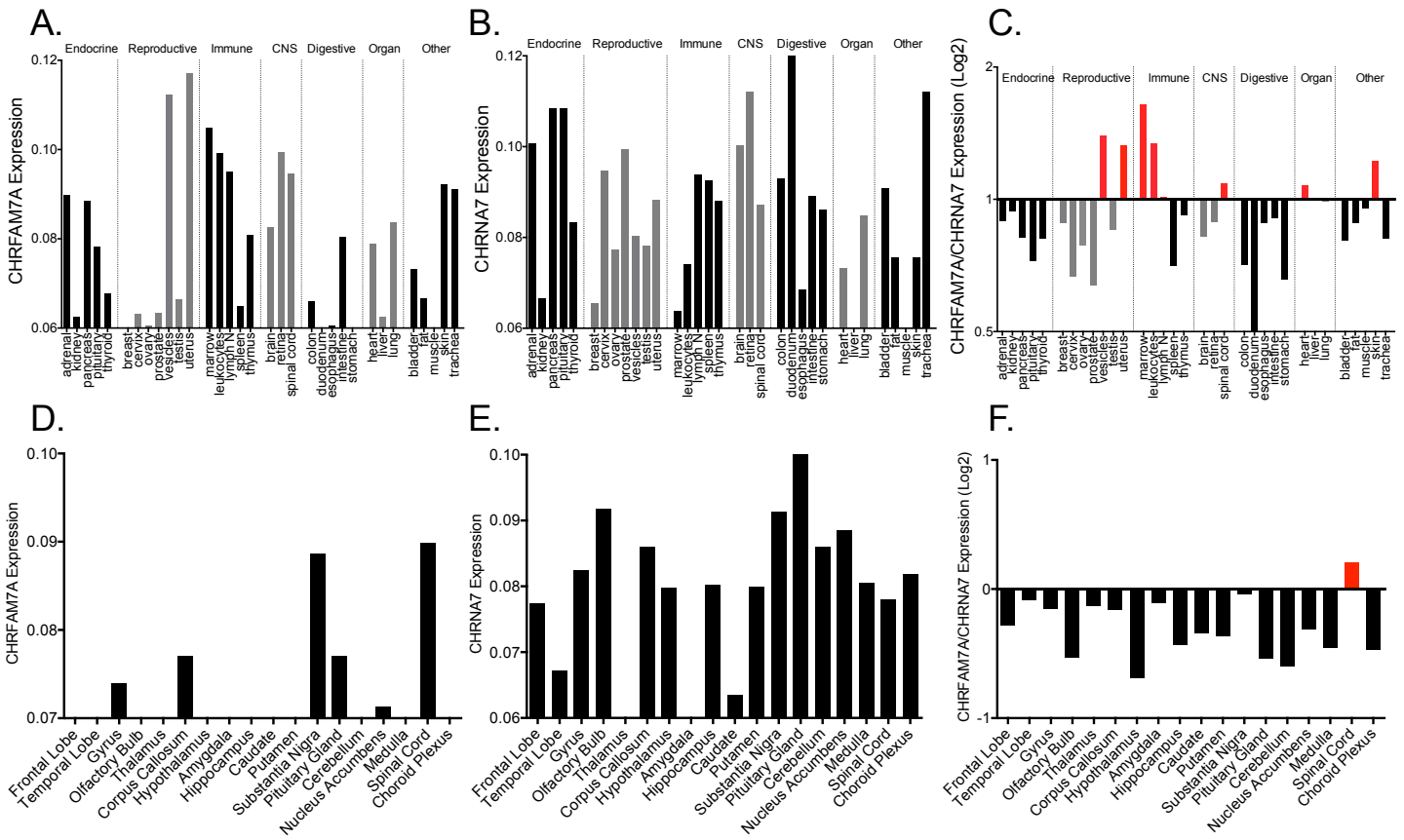


Figure S1 Distribution of CHRFAM7A in human tissues Quantitative PCR was used to measure the expression of the CHRFAM7A gene (**Panel A and D**) and the parental CHRNA7 gene (**Panel B and E**) in a commercial array of human biopsied peripheral tissues and brain, with the ratio of each calculated (**Panel C and F**). qPCR was performed to assess CHRFAM7A and CHRNA7 gene expression in 48 well plates of cDNAs in Origene Tissue Scan Arrays that were prepared from either normal human peripheral tissues (Cat #HMRT101) or human brain (Cat #HBRT 102). Characteristics of these specimens are available online with details regarding quality control of the RNA isolation and cDNA preparations (http://www.origene.com/qPCR/Tissue-qPCR-Arrays.aspx). The original de-identified tissues were collected from accredited medical institutions in the United States using Institutional Review Board (IRB) approved protocols, selected by board-certified pathologists and then deposited into the Origene tissue biorepository along with any of the available clinical data supporting pathologic diagnoses. The quantity of cDNA was compared to a plate validated with GAPDH primers provided by Origene and in our laboratories the Ct value of GAPDH gene expression in each well was determined in our laboratories to be highly consistent (average 23.51 cycles +/- 0.13 (SEM, N=47) and 22.04 cycles +/- 0.14 (SEM, N=24)). The individual (ΔCt) values from each well were then used to calculate relative gene expression in each biopsy as noted by the array manufacturer and results expressed as the reciprocal to enable tissue-to-tissue comparison and graphical representation.

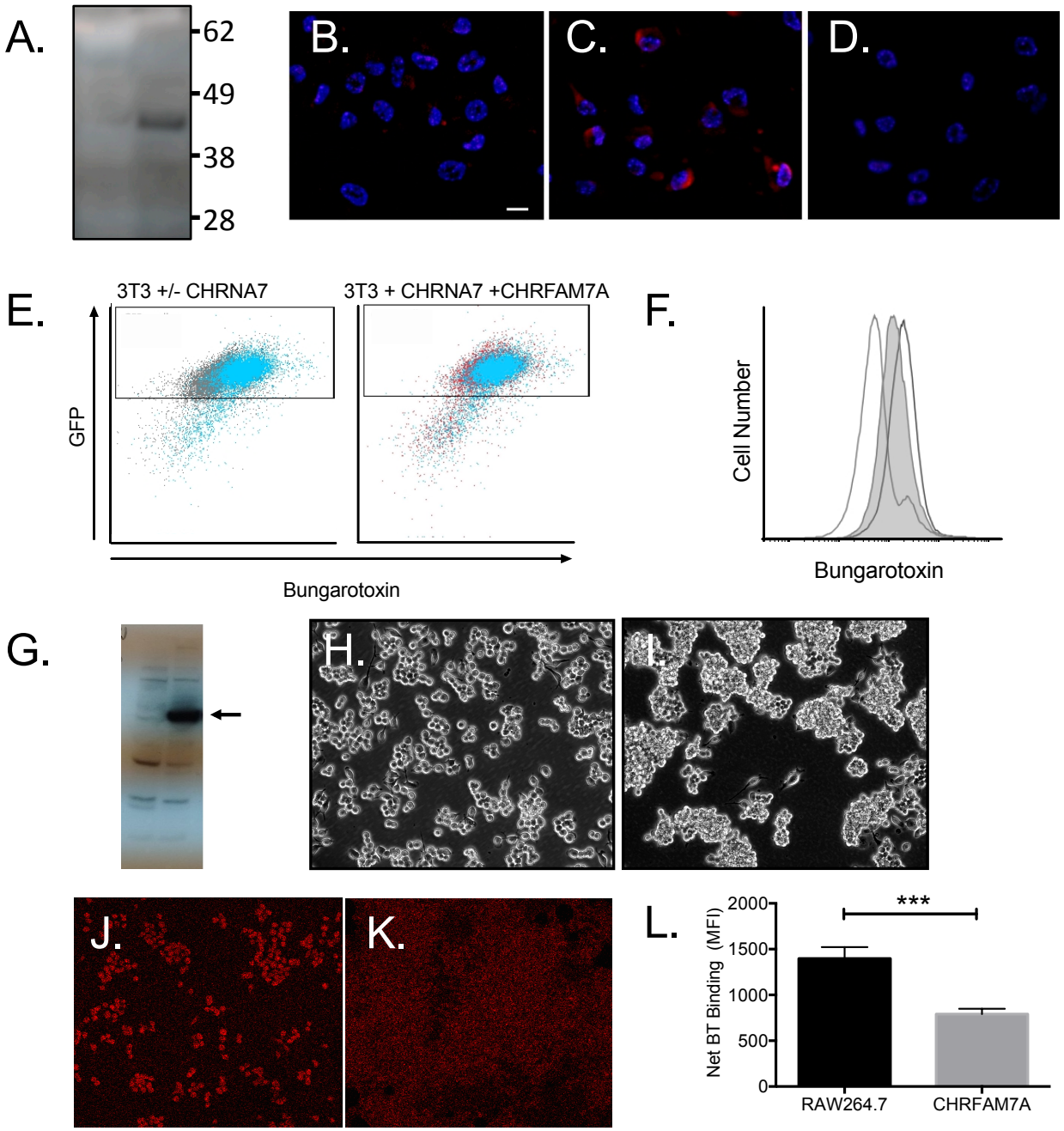


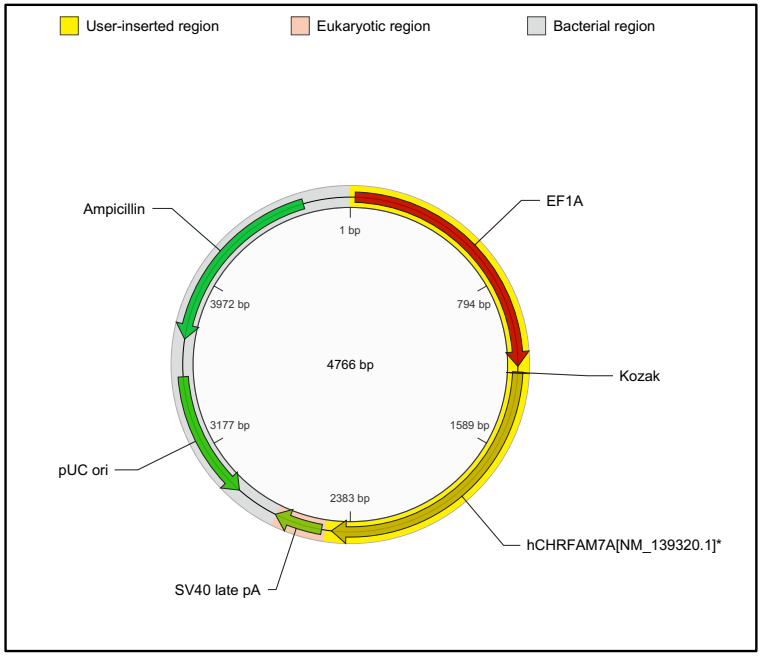
Figure S2. Human CHRFAM7A is expressed as a functional protein in mice. Before creating a transgenic mouse, we verified that the CHRFAM7A construct encoded a human CHRFAM7A that is expressed and had activity in mouse cells. To that end, mouse Balb C 3T3 fibroblasts were transiently transfected with CHRFAM7A transcript 1 and cell membranes immunoblotted with an anti CHRFAM7A antibody (**Panel A).** These cells do not bind bungarotoxin, a ligand for α7AChR (**Panel B**) unless stable transfectants express CHRNA7, the gene encoding α7nAChR (**Panel C**). Transient transfection of these stable CHRNA7-transfected cells with CHRFAM7A plasmid eliminates bungarotoxin binding (**Panel D**) that can be quantified by flow cytometry (**Panel E**) comparing bungarotoxin binding in wild type Balb C 3T3 fibroblasts (black dots) to that of cells that express α7nAChR (blue dots) which are decreased with CHRFAM7Atransduction and illustrated in the histogram of (**Panel F**). To ensure that CHRFAM7A was equally active on endogenous mouse α7nAChR, RAW 264.7 macrophages that express endogenous CHRNA7 were stably transduced with CHRFAM7A and shown by immunoblotting to express the CHRFAM7A protein (**Panel G**). As shown in (**Panels H and I**), these transfected cells have the same morphological phenotype as described for human THP1 monocytes (Costantini et al Mol. Med. 2015, 21:323-36. doi:10.2119/molmed.2015.00018. PMID: 25860877). As expected these cells bind bungarotoxin because they express endogenous α7nAChR (**Panel J**) unless transfected with CHRFAM7A (**Panel K**). This bungarotoxin binding can also be quantified by fluorescence spectroscopy of bungarotoxin binding with an Omega plate reader of cells plated in dishes (**Panel L**).

A.

Vector Summary

Vector ID	VB160506-1004nde
Vector Name (official)	pRP[Exp]-EF1A>hCHRFAM7A[NM_139320.1]*
Date Created (Pacific Time)	2016-05-05
Size	4766 bp
Vector Type	Regular plasmid gene expression vector
Inserted Promoter	EF1A
Inserted ORF	hCHRFAM7A[NM_139320.1]*
Inserted Marker	No marker
Copy Number	High
Bacterial Resistance	Ampicillin
Cloning Host	Stbl3

B.



C.

Vector Components

Component Name	Nucleotide Position	Full Name	Description
EF1A	22-1200	EF1A	Component entered by user
Kozak	1225-1230	Kozak	Component entered by user
hCHRFAM7A[NM_139320.1]*	1231-2469	hCHRFAM7A[NM_139320.1]*	Component entered by user
SV40 late pA	2514-2735	SV40 polyadenation signal	Allows transcription termination and polyadenylation of mRNA.
pUC ori (c)	3519-2931	pUC origin of replication	Permits high-copy replication and maintenance in E. coli.
Ampicillin (c)	4550-3690	Ampicillin resistance gene	Allows selection of the plasmid in E. coli.

D.

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   1 CAACTTTGTA TAGAAAAGTT GGGCTCCGGT GCCCGTCAGT GGGCAGAGCG CACATCGCCC
  61 ACAGTCCCCG AGAAGTTGGG GGGAGGGGTC GGCAATTGAA CCGGTGCCTA GAGAAGGTGG
 121 CGCGGGGTAA ACTGGGAAAG TGATGTCGTG TACTGGCTCC GCCTTTTTCC CGAGGGTGGG
 181 GGAGAACCGT ATATAAGTGC AGTAGTCGCC GTGAACGTTC TTTTTCGCAA CGGGTTTGCC
 241 GCCAGAACAC AGGTAAGTGC CGTGTGTGGT TCCCGCGGGC CTGGCCTCTT TACGGGTTAT
 301 GGCCCTTGCG TGCCTTGAAT TACTTCCACC TGGCTGCAGT ACGTGATTCT TGATCCCGAG
 361 CTTCGGGTTG GAAGTGGGTG GGAGAGTTCG AGGCCTTGCG CTTAAGGAGC CCCTTCGCCT
 421 CGTGCTTGAG TTGAGGCCTG GCCTGGGCGC TGGGGCCGCC GCGTGCGAAT CTGGTGGCAC
 481 CTTCGCGCCT GTCTCGCTGC TTTCGATAAG TCTCTAGCCA TTTAAAATTT TTGATGACCT
 541 GCTGCGACGC TTTTTTTCTG GCAAGATAGT CTTGTAAATG CGGGCCAAGA TCTGCACACT
 601 GGTATTTCGG TTTTTGGGGC CGCGGGCGGC GACGGGGCCC GTGCGTCCCA GCGCACATGT
 661 TCGGCGAGGC GGGGCCTGCG AGCGCGGCCA CCGAGAATCG GACGGGGGTA GTCTCAAGCT
 721 GGCCGGCCTG CTCTGGTGCC TGGTCTCGCG CCGCCGTGTA TCGCCCCGCC CTGGGCGGCA
 781 AGGCTGGCCC GGTCGGCACC AGTTGCGTGA GCGGAAAGAT GGCCGCTTCC CGGCCCTGCT
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 901 CAAAGGAAAA GGGCCTTTCC GTCCTCAGCC GTCGCTTCAT GTGACTCCAC GGAGTACCGG
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1021 GGGGAGGGGT TTTATGCGAT GGAGTTTCCC CACACTGAGT GGGTGGAGAC TGAAGTTAGG
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1861 ATGATCATCG TGGGCCTCTC GGTGGTGGTG ACGGTGATCG TGCTGCAGTA CCACCACCAC
1921 GACCCCGACG GGGGCAAGAT GCCCAAGTGG ACCAGAGTCA TCCTTCTGAA CTGGTGCGCG
1981 TGGTTCCTGC GAATGAAGAG GCCCGGGGAG GACAAGGTGC GCCCGGCCTG CCAGCACAAG
2041 CAGCGGCGCT GCAGCCTGGC CAGTGTGGAG ATGAGCGCCG TGGCGCCGCC GCCCGCCAGC
2101 AACGGGAACC TGCTGTACAT CGGCTTCCGC GGCCTGGACG GCGTGCACTG TGTCCCGACC
2161 CCCGACTCTG GGGTAGTGTG TGGCCGCATG GCCTGCTCCC CCACGCACGA TGAGCACCTC
2221 CTGCCAGGTG GGCAACCCCC CGAGGGGGAC CCGGACTTGG CCAAGATCCT GGAGGAGGTC
2281 CGCTACATTG CCAACCGCTT CCGCTGCCAG GACGAAAGCG AGGCGGTCTG CAGCGAGTGG
2341 AAGTTCGCCG CCTGTGTGGT GGACCGCCTG TGCCTCATGG CCTTCTCGGT CTTCACCATC
2401 ATCTGCACCA TCGGCATCCT GATGTCGGCT CCCAACTTCG TGGAGGCCGT GTCCAAAGAC
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2521 GATAAGATAC ATTGATGAGT TTGGACAAAC CACAACTAGA ATGCAGTGAA AAAAATGCTT
2581 TATTTGTGAA ATTTGTGATG CTATTGCTTT ATTTGTAACC ATTATAAGCT GCAATAAACA
2641 AGTTAACAAC AACAATTGCA TTCATTTTAT GTTTCAGGTT CAGGGGGAGG TGTGGGAGGT
2701 TTTTTAAAGC AAGTAAAACC TCTACAAATG TGGTAGCGGC CGCGGCGCTC TTCCGCTTCC
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2821 AAGGCGGTAA TACGGTTATC CACAGAATCA GGGGATAACG CAGGAAAGAA CATGTGAGCA
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3181 TGTGTGCACG AACCCCCCGT TCAGCCCGAC CGCTGCGCCT TATCCGGTAA CTATCGTCTT
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3301 AGCAGAGCGA GGTATGTAGG CGGTGCTACA GAGTTCTTGA AGTGGTGGCC TAACTACGGC
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4201 ACTGTCATGC CATCCGTAAG ATGCTTTTCT GTGACTGGTG AGTACTCAAC CAAGTCATTC
4261 TGAGAATAGT GTATGCGGCG ACCGAGTTGC TCTTGCCCGG CGTCAATACG GGATAATACC
4321 GCGCCACATA GCAGAACTTT AAAAGTGCTC ATCATTGGAA AACGTTCTTC GGGGCGAAAA
4381 CTCTCAAGGA TCTTACCGCT GTTGAGATCC AGTTCGATGT AACCCACTCG TGCACCCAAC
4441 TGATCTTCAG CATCTTTTAC TTTCACCAGC GTTTCTGGGT GAGCAAAAAC AGGAAGGCAA
4501 AATGCCGCAA AAAAGGGAAT AAGGGCGACA CGGAAATGTT GAATACTCAT ACTCTTCCTT
4561 TTTCAATATT ATTGAAGCAT TTATCAGGGT TATTGTCTCA TGAGCGGATA CATATTTGAA
4621 TGTATTTAGA AAAATAAACA AATAGGGGTT CCGCGCACAT TTCCCCGAAA AGTGCCACCT
4681 GACGTCTAAG AAACCATTAT TATCATGACA TTAACCTATA AAAATAGGCG TATCACGAGG
4741 CCCTTTCGTC GGCGCGCCGC GGCCGC
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Figure S3. Strategy designing CHRFAM7A mice. The vector selected to create transgenic mice (**Panels A and B**) was designed to contain 6 elements including the promoter, a Kozak consensus sequence for translation, the CHRFAM7A sequence corresponding to transcript 1, the late SV40 polyadenylation sequence to enhance translation and the pUC ori and ampicillin resistance for plasmid manufacture **(Panel C)** with the specific sequences shown in **Panel D**

A.

Product			
Name of Injected DNA	**pRP[Exp]-EF1A>hCHRFAM7A[NM_139320.1]***		
Mouse Strain	**C57BL/6xC57BL/6**		
Date of Birth	**08-23-2016**		
Description	**Total: 82 pups** **pRP[Exp]-EF1A>hCHRFAM7A[NM_139320.1]*: 1-82**		
PCR-Positive Pups	♂	**4**	**pRP[Exp]-EF1A>hCHRFAM7A[NM_139320.1]*: 21, 57, 62 and 76**
	♀	**1**	**pRP[Exp]-EF1A>hCHRFAM7A[NM_139320.1]*: 81**

B.

1. Primers used:

Transgene PCR primer F1: TGGCACTTGATGTAATTCTCCTTGG

Transgene PCR primer R1: CACATCAAAGGGAAACCAGCG

Internal control PCR primer F: CTATCAGGGATACTCCTCTTTGCC

Internal control PCR primer R: GATACAGGAATGACAAGCTCATGGT

Annealing Temp: 60 ℃

Expected PCR product:

Transgene PCR product size: 357 bp

Internal control PCR product size: 507 bp

C.

2. Primers used:

Transgene PCR primer F2: CGCTACATTGCCAACCGCTT

Transgene PCR primer R2: CACACCTCCCCCTGAACCTGAA

Internal control PCR primer F: GCAGAAGAGGACAGATACATTCAT

Internal control PCR primer R: CCTACTGAAGAATCTATCCCACAG

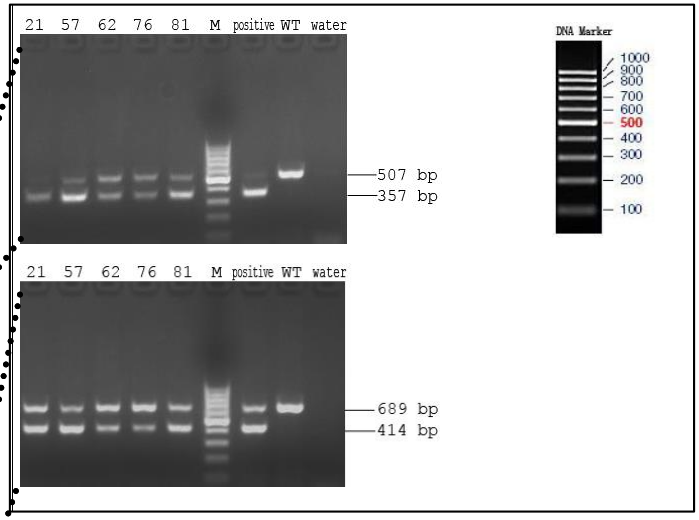
Annealing Temp: 60 ℃

Expected PCR product:

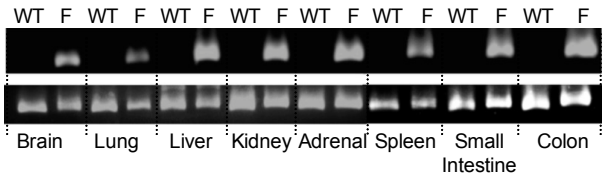
Transgene PCR product size: 414 bp

Internal control PCR product size: 689 bp

D.



E.



F.

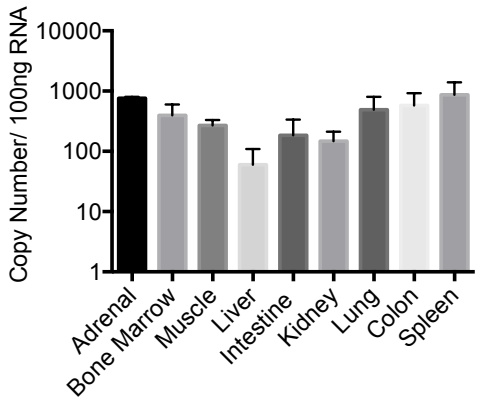


Figure S4. Characterization of CHRFAM7A transgenic mice. The vector to create the CHRFAM7A gene (**Panel A**) led to the the generation of 82 progeny of which 5 were found transgenic using two sets of probes (**Panels B and C**) capable of identifying the correct amplicon by PCR (**Panel D**). Tissue distribution of F0 progeny (**Panel E**) confirmed the wide distribution in transgenic tissues (WT, wild type; F, CHRFAM7A transgenic mice) that were also confirmed by quantitative PCR to be relatively homogeneous (**Panel F**). Mouse 62 (Line 3) and mouse 76 (Line 4) generated stable transgenic progeny in later generations.

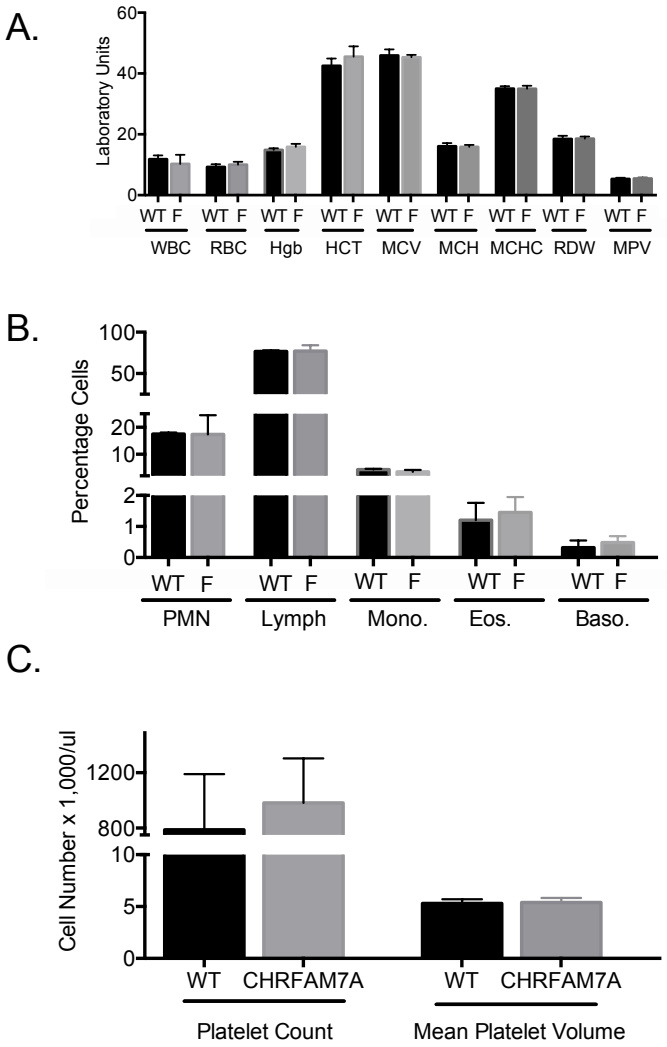
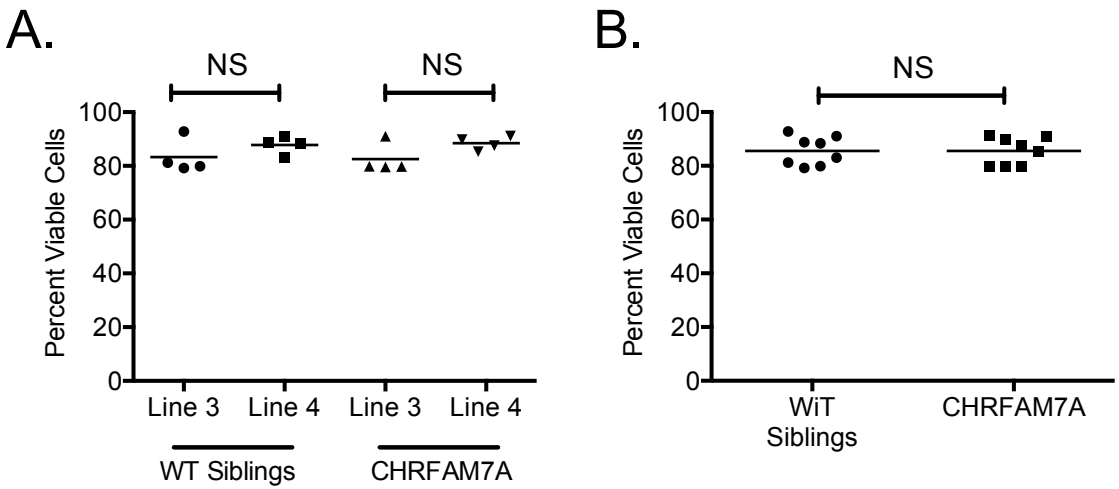


Figure S5 Transgenic mice are indistinguishable from sibling wild type mice. Hemograms from 13 animals were submitted for analyses and showed there were no differences in circulating white blood cells (WBC), red blood cells (RBC), hemoglobin (Hbg), hematocrit (HCT), (MCV),(MCH), (MCHC), (RDW), (MPV) (**Panel A**). Similarly, as shown in **Panel B**, these cell analyses failed to reveal differences in polymorphonuclear cells (PMN), lymphocytes (Lmph.), Monocytes (Mono.), eosinophils (Eos.) or basophils (Baso.) . Finally, the mice had no difference in platelet count or mean platelet volume (MPV, **Panel C**).



S6 There are no differences in bone marrow cell viability after colony formation in MethoCult for 7 days. Sibling wild type and transgenic bone marrow cells from each line of CHRFAM7A transgenic mice have the same viability regardless of their source (**Panel A**). There are also no differences between cells from CHRFAM7A transgenic mice and their wild type control counterparts (**Panel B**).

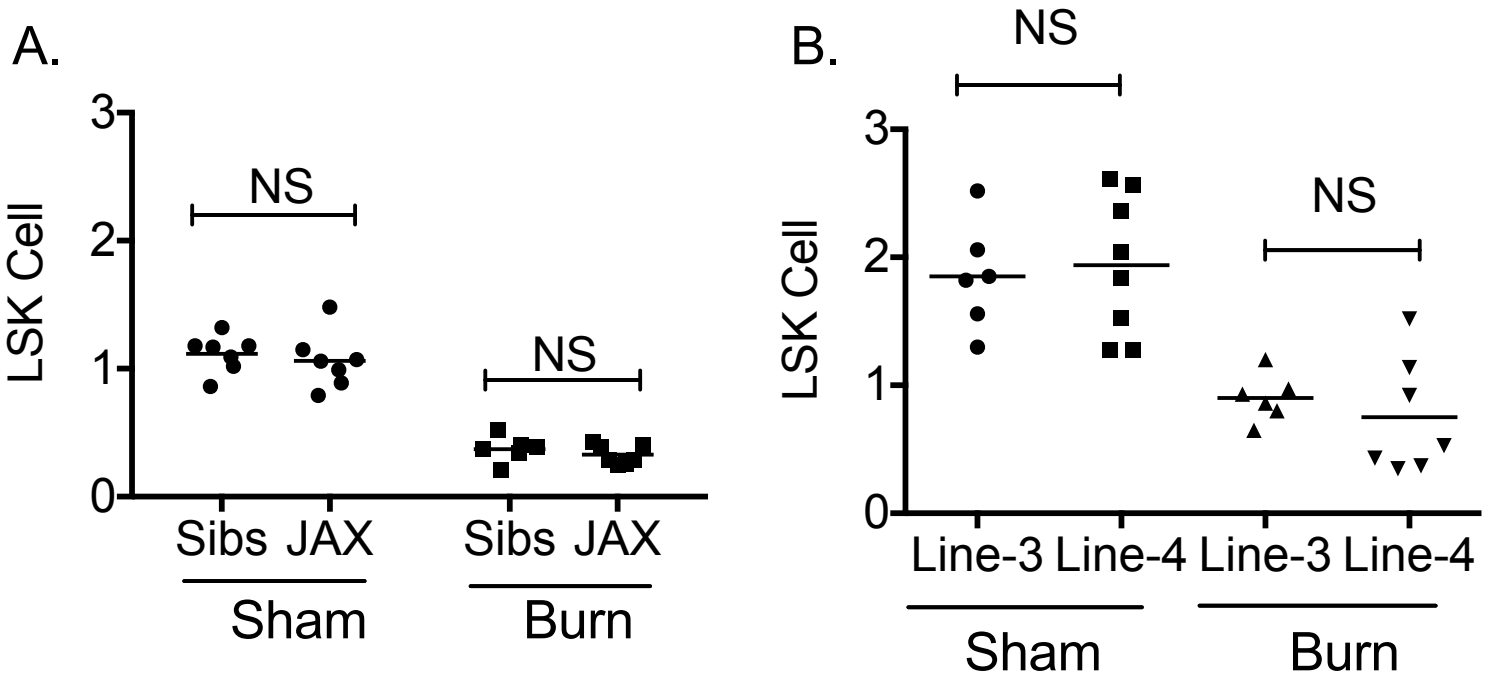


Figure S7 Comparing responses of wild type sibling and commercial C57/Bl6 mice and the different lines of transgenic mice. The KSL number in bone marrow of male wild type (non-transgenic) siblings (Sibs) and age-matched male commercial wild type (Jackson) C57/Bl6 mice (**Panel A**) show they are not different before or after thermal injury (burn). Similarly the percentage of LSK cells in the two lines of CHRFAM7A mice or their respective response to cutaneous thermal injury is not different (**Panel B**). Both lines show the same differences in LSK and LK (**Panel C and D**) but SL number is not different (**Panel E**). In all instances, there are no differences between the two transgenic lines (**Panel C-E**).

Figure S8 CD34+ cells are decreased in human burns

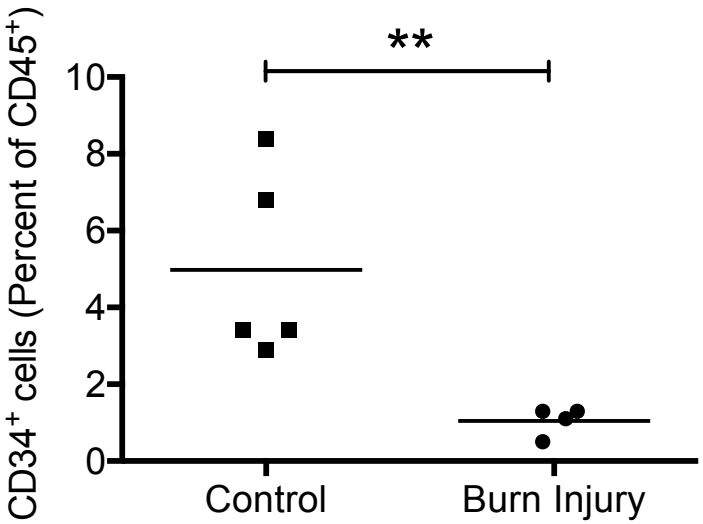


Figure S8. CD34$^+$ cells in human blood are decreased after thermal injury Blood was analyzed from three healthy male ages 24, 30 and 33 and two healthy non-pregnant female volunteers (23 and 24 yo) who donated blood after informed consent. These results were compared to those of two male burn patients aged 24 and 37 and two female aged 62-67 with 12-25% total surface area burns. The University of California San Diego Institutional Review Board approved study participants, protocols, and consent forms and study participants provided written informed consent to participate.

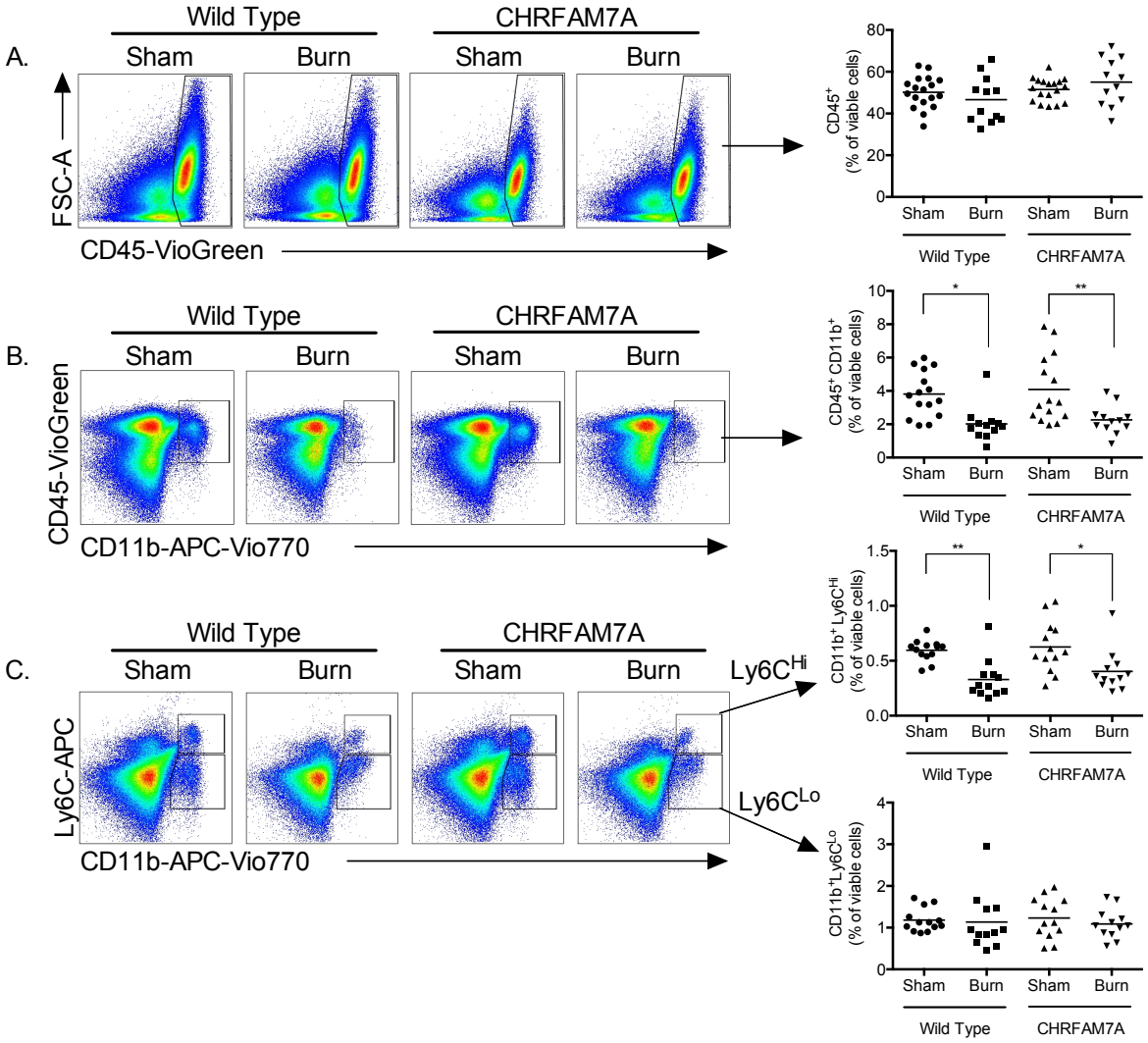


Figure S9. Flow cytometric analysis of mouse spleen after burn injury. Splenic reservoir of myeloid cells was identified after enzymatic digestion and exclusion of debris, doublets and non-viable cells. The subsets were evaluated included (A) pan leucocytes CD45+; (B) monocytes (CD45+CD11b+) and, (C) inflammatory/patrolling monocytes that are CD11b+Ly6CHi and CD11b+Ly6CHi/Lo. Lines represent mean. Statistical analysis between the groups was done using one-way ANOVA. **P*< 0.05, ***P*< 0.01.

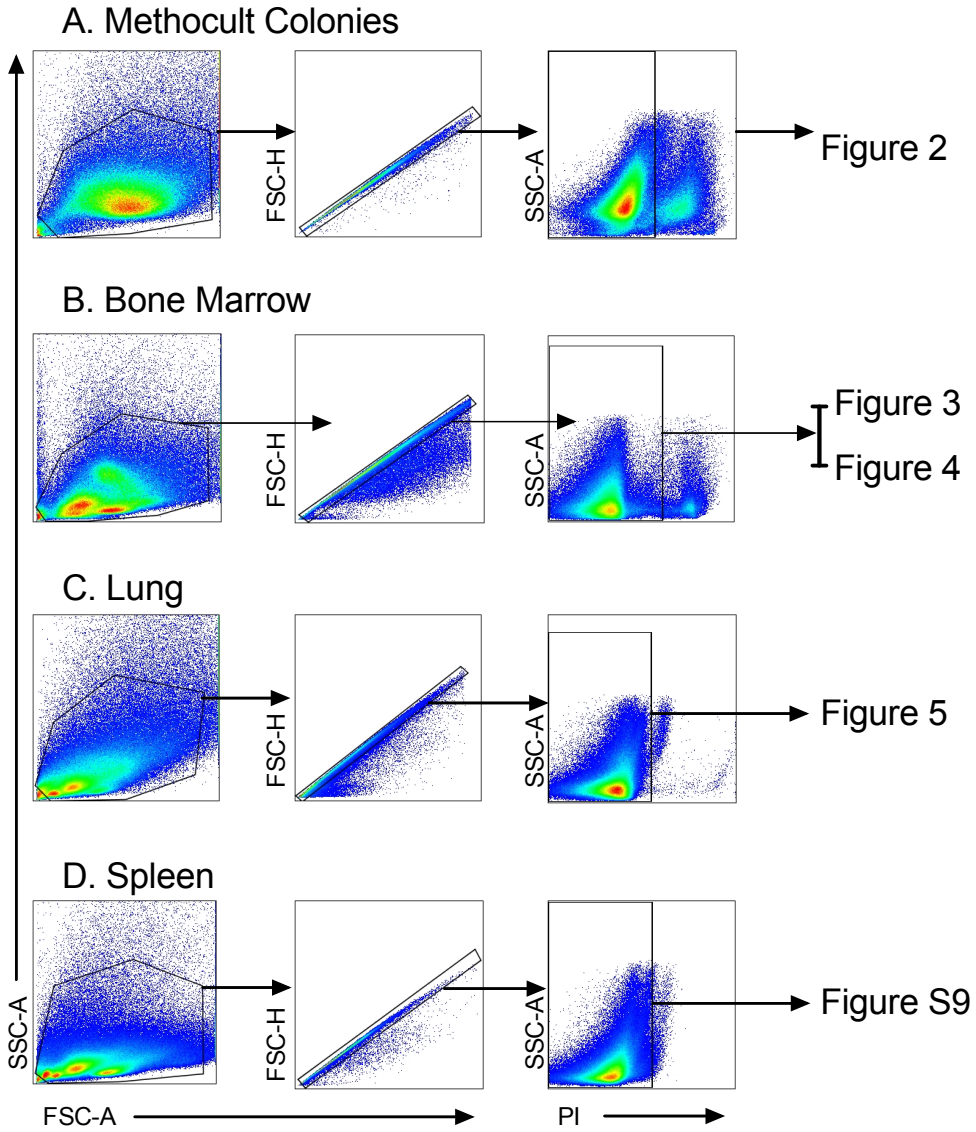


Figure S10. Gating strategies for flow cytometry of bone marrow cells differentiated in GM-CSF containing Methocult, spleen and lung myeloid cells. Seven days after culture in Methocult™ containing GM-CSF (**Panel A**), cells were processed for flow cytometry as described in Methods and each of the indicated gates for total cells, single cells and PI-excluding (viable) cells used for subsequent analyses. Similarly gating for cells obtained from spleen (**Panel B**) and lung (**Panel C**) was used to prepare them for analyses by flow cytometry as described in the text. In lung (**Panel C**), additional $CD45^+$ sub-gating (see Figure 5D) was added to isolate alveolar macrophages and dendritic cells while $CD11c^+$ gating (Figure 5G) was added to detect and measure interstitial macrophages.

Table S1 Antibody Panel for Differentiating Bone Marrow Colonies

Antibody	Probe	Catalog Number (Miltenyi Biotec)	Quantity
anti-GR1	VioBlue	# 130-102-233	2ul
anti-CD45	VioGreen	# 130-110-803	0.5ul
anti-Ly6G	FITC	# 130-107-912	2ul
anti-F4/80	PE	# 130-102-422	2ul
anti-CD11c	PE-Vio770	# 130-110-840	0.5ul
anti-Ly6C	APC	# 130-102-341	2ul
anti-CD11b	APC-Vio770	# 130-109-288	2ul
Propidium Iodide.		# 130-093-233	
Compensation	beads	#130-104-693	

Table S2 Antibody Panel for the Detection of Bone Marrow HSCs

Antibody	Probe	Catalog Number (Miltenyi Biotec)	Quantity
anti-lineage	Biotin	# 130-092-613	5ul
anti-Biotin	FITC	# 130-098-796	5ul
anti-CD117 (c-kit)	APC	# 130-102-492	5ul
anti-Sca1	PE	# 130-116-489	2ul
Propidium Iodide.		# 130-093-233	
FcR blocking		# 130-092-575	
Compensation	beads	#130-104-693	

Table S3 Antibody Panel to Detect Lung and Spleen Myeloid Lineage Cells

Antigen	Probe	Catalog Number (Miltenyi Biotec)	Quantity
anti-CD24	VioBlue	# 130-110-831	1ul
anti-CD45	VioGreen	# 130-110-803	0.5ul
anti-SiglecF	FITC	# 130-112-336	1ul
anti-CD103	PE	# 130-111-685	1ul
anti-CD11c	PE-Vio770	# 130-110-840	0.5ul
anti-CD64	APC	# 130-103-809	2ul
anti-CD11b	APC-Vio770	# 130-109-288	2ul
Propidium Iodide.		# 130-093-233	
Compensation	beads	#130-104-693	

Table S4 Antibody Panel for the Detection of Lung and Spleen Myeloid, Alveolar Macrophage and Dendritic Cells

Antigen	Probe	Catalog Number (Miltenyi Biotec)	Quantity
anti-Gr1	VioBlue	# 130-102-233	2ul
anti-MHCII	VioGreen	# 130-108-363	2ul
anti-F4/80	PE	# 130-102-422	2ul
anti-CD11c	PE-Vio770	# 130-110-840	0.5ul
anti-Ly6C	APC	# 130-102-341	2ul
anti-CD11b	APC-Vio770	# 130-109-288	2ul
Propidium Iodide.		# 130-093-233	
Compensation	beads	#130-104-693	

Supplementary Information Text

Methodological Details

Gene expression in normal human tissues: Two strategies were used to assess gene expression in normal tissues. First we mined (August 2018) CHRFAM7A and CHRNA7 expression in human tissues using data in the Human Protein Atlas (https://www.proteinatlas.org) as reported in Gentree (http://gentree.ioz.ac.cn), a database comparing species-specific gene assembly (see text). Second, we used qRT-PCR to assess CHRFAM7A and CHRHNA7 gene expression in 48 well plates of cDNAs in Origene Tissue Scan Arrays that were prepared from either normal human peripheral tissues (Cat #HMRT101) or human brain (Cat #HBRT 102). Characteristics of these specimens are available online with details regarding quality control of the RNA isolation and cDNA preparations (http://www.origene.com/qPCR/Tissue-qPCR-Arrays.aspx). The original de-identified tissues were collected from accredited medical institutions in the United States using Institutional Review Board (IRB) approved protocols, selected by board-certified pathologists and then deposited into the Origene tissue biorepository along with clinical data. The quantity of cDNA was compared to a plate validated with GAPDH primers provided by Origene and in our laboratories the Ct value of GAPDH gene expression in each well was determined in our laboratories to be highly consistent (average 23.51 cycles +/- 0.13 (SEM, N=47) and 22.04 cycles +/- 0.14 (SEM, N=24)). The individual (ΔCt) values from each well were then used to calculate relative gene expression in each biopsy as noted by the array manufacturer and results expressed as the reciprocal to enable tissue-to-tissue comparison and graphical representation.

Recruitment of human volunteers for blood donation Healthy male and non-pregnant and non-menopausal female volunteers between the ages of 20 and 65 were targeted for recruitment through a combination of mailings to student and faculty directories at UC San Diego, flyer postings on, and off, campus, and word of mouth. Informed consent was obtained and venous blood collected by peripheral venipuncture in BD Vaccutainer® blood collection tubes containing EDTA (BD Biosciences, Franklin Lakes, New Jersey) at the UCSD Medical Center by their Laboratories Blood Drawing Services. Blood was collected from burn patients in a similar fashion after informed consent and the University of California San Diego Institutional Review Board approved the consent forms, specimen collection protocols and provided guidelines for study procedures.

Isolation of $CD14^+$, $CD15^+$ and $CD3^+$ cells from blood: To isolate $CD3^+$ lymphocytes, $CD14^+$ myeloid precursor cells and $CD15^+$ granulocytes from human blood, 15-20 ml. was collected into two 10 ml blood collection tubes (BD Vacutainer K2 EDTA, catalog number 366643), inverted to mix and processed immediately using whole blood magnetic microbeads conjugated with antibodies directed to human CD3 (Cat #130-090-874), CD14 (Cat#130-090-879), and CD15 (Cat#130-091-058) and separated using an autoMACS Pro Separator (Miltenyi Biotec) according to manufacturers recommendations. Isolated cells were then collected by centrifugation for RNA isolation, cDNA synthesis and PCR as described below.

Preparation of mRNA from $CD3^+$, $CD14^+$ and $CD15^+$ cells for synthesis of cDNA. The pellets of $CD3^+$, $CD14^+$ and $CD15^+$cells obtained from affinity purification of cells were processed for extraction with the μMACS mRNA Isolation Kit (Miltenyi #130-075-201) per the manufacturer's protocol. Mechanical shearing of the isolated cells was performed using a 20-gauge needle through a 3-mL syringe with 1mL Lysis/Binding Buffer. Samples were then centrifuged at 13,000g for 3 minutes in order to reduce foam present in the sample. The cell lysate was then placed on a MACS Column matrix previously hydrated with lysis buffer in the MACS Separator and Oligo(dT) Microbeads were added for mRNA hybridization. cDNA synthesis was performed directly in the column using a thermoMACS Separator and the μMACS One-step cDNA kit (Miltenyi #130-091-902) following the manufacturer's protocol. Equilibration Buffer was added onto the column matrix and the kit's resuspended enzyme mix was added in their Resuspension Buffer to the column. Samples were incubated for 1 hour at 42°C and their cDNA Release Solution was added. Once samples were further incubated for 10 minutes at 42°C, the synthesized cDNA was eluted twice with cDNA Elution Buffer.

Design and Generation of transgenic mice CHRFAM7A transgenic mice were generated by contract (TGMB-160506-ACH-01-B) with Cyagen Inc. (Santa Clara, CA). A vector was designed to express CHRFAM7A under control of the Elongation Factor 1A (EF1A) promoter to generate near-ubiquitous tissue expression (Supplemental Data, Figure S1). The full-length CHRFAM7A open reading frame used (Variant 1: NM_139320.1) differs from the shorter CHRFAM7A Variant 2 (NM_148911) in that it includes the 5'-30bp sequence that encodes a 90 amino acid amino terminus that includes a 27aa unique FAM7A/ULK sequence (NH2-MQKYCIYQHF QFQLLIQHLW IAANCDI). From a total of 82 pups generated, four male and female mice were supplied expressing the CHRFAM7A gene (Supplemental Data, Figure 2) and delivered to the animal quarantine facilities of the UC San Diego School of Medicine. There, genotyping was

confirmed by PCR and lines propagated as part of the UC San Diego Animal core facility. Of the four mouse lines obtained, two derived from male founder mice #62 and #76 and termed either pRP[Exp]-EF1A>hCHRFAM7A[NM_139320.1-L3 (Line 3) or pRP[Exp]-EF1A>hCHRFAM7A[NM_139320.1-L4 (Line 4) respectively, were successfully expanded in a C57/BL6 mouse background. All of the experiments described here were carried out on both Line 3 and Line 4 mouse lines to ensure that any differences could be attributed to CHRFAM7A and not its insertion. Hemograms of blood collected from sibling and transgenic mice were obtained using fresh blood submitted to and processed at UC San Diego School of Medicine core laboratories.

Collection, extraction and processing of transgenic tissues for RT-PCR: Tissues were collected from either male wild type C57/Bl6 that were purchased from Jackson Laboratories, generated as non-transgenic siblings in the course of breeding or CHRFAM7A transgenic mice of Lines 3 or 4. Freshly collected bone marrow cells were washed once with PBS, centrifuged at 1200 rpm for 5 minutes and lysed in 1 ml trizol reagent followed by total RNA purification according manufacture instructions (Direct-zol™ RNA Miniprep Plus Zymo Research) including the DNAase treatment. Solid mouse tissues were initially homogenized using Lysing Matrix D tubes and FastPrep-120 homogenizer (MP Biomedical, cat#6913-100) followed by lysis in 1 ml trizol reagent and RNA extraction as described above.

Collection and processing of bone marrow harvest from mice. For both Methocult^{TM} cultures and direct flow cytometry, both femurs and tibias from donor mice were harvested by flushing with either 5 mL of Iscove's Modified Dulbecco's Medium (IMDM) containing 2% FBS in syringe or phosphate buffered saline with a 30G needle. The cell suspension was transferred through a 70 μm strainer and into a sterile 50 mL tube, diluted to 25 ml and centrifuged at 1200 rpm for 10 minutes before re-suspending and counting in the appropriate buffer for analyses.

Collection, extraction and processing of tissues for immunoblotting. Tissue extracts and 10×10^6 bone marrow cell were prepared by extraction in RIPA buffer (50 mM Tris–HCl, pH 7.5, 150 mM NaCl, 2 mM EDTA, 1% Triton X-100, protease and phosphatase inhibitors, 5 mM DTT and 0.2 mM PMSF) and sonicated for 15 seconds each with 40 seconds intervals on ice. The protein was extracted over a 30 min. incubation on ice following bench centrifugation for 15min at 13000 rpm. Protein concentrations were measured using the bicinchoninic acid (BCA) protein assay (Pierce, cat#23225) and a microplate reader.

CHRFAM7A immunoblotting. Samples containing 50 µg of protein lysate were suspended in the sodium dodecyl sulfate (SDS) sample buffer (Novex, cat#NP0008) and boiled at 100ºC for 5 min. After separation by SDS-PAGE using 4-12% tris-glycine polyacrylamide gradient gel, proteins were transferred to nitrocellulose membranes (Novex, cat#LC2005). The membranes were pre-incubated for 1 h with blocking buffer 5% BSA in Tris-buffered saline (TBS)/TWEEN 20 for 1 h at room temperature, before overnight incubation at 4ºC with primary rabbit anti-CHRFAM7A (1ug/ml) or rabbit anti- β –actin (CST, cat#Rb4967L, 1:1000). The following day, membranes were washed and incubated for 1 h at room temperature with an anti-rabbit IgG secondary antibody conjugated with horseradish peroxidase (CST, cat#7074S, 1:1000) in 5% BSA/TBST. After washing the membranes, the Supersignal West Femto Chemiluminescent Kit (cat#34095, ThermoFisher Sci.) was applied, and binding detected with CL-XPosure film (ThermoFisher Sci.).

Antibodies to CHRFAM7A: Antibodies to CHRFAM7A were raised in rabbits by contract for our laboratories with Genway Inc. (Poway, California) using a synthetic peptide antigen conjugated to keyhole limpet hemocyanin. The primary sequence of the peptide antigen was derived from the predicted amino terminus of CHRFAM7A and the sole sequence (NH2-MQKYC IYQHF QFQLL IQHLW IAANC DIADER-COOH) that distinguishes CHHRFAM7A from all known forms of the human α7 nicotinic acetylcholine receptor. BLAST comparisons of this sequence against human, mouse and rat protein database (https://blast.ncbi.nlm.nih.gov/Blast.cgi) identified 100% cross reactivity with the four known CHRFAM7A transcripts encoding (1) isoform X3 (XP_011506795.1, (2) XP_011520457.1), (3) isoform 1 (NP_647536.1) and (4) isoform X1 (XP_011506794.1). The IgG obtained from both rabbits after the second boost immunization were affinity purified on a peptide affinity column at Genway, shown to recognize the antigen by ELISA (OD 1.6 at 1ug/ml) and two lots (160115A and 160115B) provided to our laboratories as frozen aliquots of 1mg/ml. Antibodies from lot 2 were used at the concentrations indicated in the figure legends.

Plasmids: In the experiments reported here, we used two expression plasmids, both built into the bi-cistronic pLVX-IRES-ZsGreen1 lentivirus plasmid (Clontech cat#632187) to encode (1) pLVX-Vector (GFP alone) (2) pLVX-CHRFAM7A for stable transfection of CHRFAM7A and GFP in RAW264.7, Balb/c-3T3 and THP1 cells, and (3) pLVX-CHRNA7 for stable transfection of human □7nAChR and GFP in Balb/c-3T3 cells. To engineer the CHRFAM7A plasmids, a pCMV6-Entry plasmid encoding full-length CHRFAM7A (variant 1, NM_139320.1) was purchased from Origene (Cat#: RC215588). This full-length CHRFAM7A (Variant 1: NM_139320.1) differs a shorter

CHRFAM7A (Variant 2: NM_148911) in that it encodes a 90 amino acid amino terminus containing the unique FAM7A sequence (NH2-MQKYCIYQHF QFQLLIQHLW IAANCDI) The CHRFAM7A open reading frame was amplified by PCR using (forward) 5'-aGTCCTCGAGATGcaaaaatattgcatct-3' and (reverse) 5'-attcGGATCCTTACGCAAAGTCTTTGGACACGGC-3' primers. The PCR product was purified and cloned into the pLVX-IRES-ZsGreen1 at XhoI and BamHI restriction sites and its identity confirmed by DNA sequencing at Eton Bioscience Inc.

To engineer the CHRNA7 plasmid, the human CHRNA7 (variant 1, NM_000746) open reading frame was amplified from a pCMV6-Entry plasmid (Cat# SC124074, Origene) using PCR. This shorter full-length CHRNA7 (Variant 1: NM_000746) differs from Variant 2 (NM_001190455) in that this alternative transcript lacks a GKATASPPSTPPWDPGHIPGASVRPAPGP sequence located at His[18] of the longer □7nAChR subunit.

Cells in culture: Stock BALB/c 3T3 (ATCC #CCL-163) and RAW 264.7 (ATCC #TIB-71) cell lines were originally purchased from American Tissue Culture Cell Repository (Manassas, VA) expanded upon arrival and maintained in liquid phase of liquid nitrogen in the cell repositories of UC San Diego Department of Surgery. RAW 264.7 cells were maintained in DMEM cultured media containing 10% FCS, 0.1 mM MEM Non-Essential Amino Acids, 1 mM MEM Sodium Pyruvate, 2 mM L-Glutamine, Penicillin/streptomycin, allowed to grow to 90% confluence and split 1:5 two times per week. BALB/C 3T3 were maintained in DMEM cultured media containing 10% FCS, 0.1 mM MEM Non-Essential Amino Acids, 1 mM MEM Sodium Pyruvate, 2 mM L-Glutamine, Penicillin/streptomycin, allowed to grow to 90% confluence and split 1:5 two times per week. No cell type was used beyond 15 passages after which new cells were thawed, expanded and used for further experiments.

Stable transfection of RAW264.7 and Balb/c-3T3 cells: pLVX-CHRFAM7A-IRES-ZsGreen1, pLVX-CHRNA7-IRES-ZsGreen1 and pLVX-IRES-ZsGreen1 lentiviral plasmids were packaged into lentivirus using the Lenti-X Single Shot protocol and reagents from Clontech, (cat# 631275) and the virus produced and released into supernatant of HEK293T cells over 48 hours was harvested, filtered and used to infect target cells. Briefly, particles were added to target cells, incubated overnight, washed the following day and allowed to recover. Transduction of RAW264.7 cells was enhanced by the inclusion of 6ug/ml Polybrene and a 90 minute centrifugation of cells at 2500rpm at 33C. Transfected GFP positive cells were detected by fluorescence microscopy 2-5

days later and as cells neared confluence contract fluorescence activated cell sorting (FACS) was performed at the Center for AIDS Research (UC San Diego) detecting ZsGreen1 expression to isolate stable transfectants. Stable expression was monitored weekly to ensure retention of GFP expression in >85% cells (as measured by flow cytometry) and sorting FACS repeated if necessary. Transfected cells were expanded and aliquots frozen in liquid nitrogen for retrieval or further propagated for experiments up to twenty generations or if GFP expression decreased below 80%. Transgene expression in transfectants was confirmed by RT-PCR. Cells infected with pLVX-IRES-ZsGreen1 and no insert were used as "vector" controls.

Transient transfection of RAW264.7 and CHRNA7-transfected Balb/c-3T3 cells We used a pCMV-Entry-MycDDK mammalian expression vector that encodes CHRFAM7A (Variant 1, NM_139320.1) to transiently express CHRFAM7A in RAW264.7 cells that express endogenous CHRNA7 or Balb/c-3T3 that are stable transfectants engineered to express hCHRNA7 (variant1) by transfection overnight using lipofectamine 3000 according to instructions provided by the manufacturer (Invitrogen). The transiently transfected cells were allowed to recover for 48 hours, the transfection confirmed by RT-PCR of an aliquot of cells and the remaining cells used in experiments within the next four days.

Cell extraction Lysates from total cells were prepared using 5×10^6 cells. Briefly, the cells were collected by centrifugation for 5 min at 1000 rpm and 4 °C. The pellets were re-suspended in 150 µl of RIPA buffer (50 mM Tris–HCl, pH 7.5, 150 mM NaCl, 2 mM EDTA, 1% Triton X-100, protease and phosphatase inhibitors, 5 mM DTT and 0.2 mM PMSF) and the protein was extracted by means of 30min incubation on ice following bench centrifugation for 15min at 12000 rpm.

Preparation of cell membrane fractions. 20 million CHRFAM7A-expressing Balb/c-3T3 were used for membrane fraction preparation. The cells were washed twice with cold PBS, scraped into 10ml PBS and centrifuged for 10min at 1000 rpm. After aspiration of PBS the cells were re-suspended in 500ul of ice-cold homogenization buffer (0.25M sucrose, 0.1M K2HPO4 (pH 7.4), 1mM EDTA containing protease and phosphatase inhibitors (ThermoFisher, cat#78426 & 1860932)). The cells were sonicated in the homogenization buffer using medium speed three times for 15 sec each with 40 sec intervals on ice. The samples were centrifuged at 2000 rpm for 10 min at 4 °C, and the membrane containing supernatant was recovered and re-centrifuged in a TL-100 ultracentrifuge (Beckman Coulter, Inc., CA) at 60,000 rpm for 50 min at 4 °C. The pellet containing

cell membranes was re-suspended in a RIPA buffer (50 mM Tris–HCl, pH 7.5, 150 mM NaCl, 2 mM EDTA, 1% Triton X-100, protease and phosphatase inhibitors, 0.2 mM PMSF).

α-bungarotoxin binding to transduced Balb/c3T3 and RAW264.7 cells To observe bungarotoxin binding to 3T3 and RAW264.7 cells, 2 x 10^4 3T3 cells or 5 x 10^4 RAW264.7 cells were plated onto collagen-1 coated coverslips placed into a 35mm well and 2ml of culture media. The following day, attached cells were washed and either a PBS solution of α-Bungarotoxin (BT linked to tetramethylrhodamine (Sigma, 1μg/ml) was added either directly to 3T3 cells or after a 4% PFA 10 minute fixation for RAW264.7 cells. Thirty minutes later, cells were washed three times in PBS for 5 minutes and counterstained with Hoechst stain. Image-matched photographs of fluorescence staining were taken using either an Olympus FSX100 inverted microscope equipped for fluorescence or an Olympus FV1000 confocal microscope, as indicated in legends. Differences in fluorescent BT binding were also confirmed by measuring cell bound fluorescence with an Omega plate reader equipped with 544/590nm excitation/emission filters. In this instance, 5 x 10^3 cells were plated in 96 well plates and α-Bungarotoxin binding measured after normalization of cell density by total protein measured after RIPA lysis and BCA protein assay as described in Immunoblotting section above.

Detection and quantification of bungarotoxin binding by flow cytometry To assess BT binding in Balb/c3T3 cells, vector (GFP) and CHRNA7 transfectants were transiently transfected with either pLV-vector or pLV-CHRFAM7A plasmids so that they either expressed GFP alone, CHRNA7 alone, or CHRFAM7A and CHRNA7. These double transiently transfected cells were allowed to recover in 6 well plates for 48 hours, trypsinized, washed once with PBS and incubated in the dark and at room temperature with PBS containing 1μg/ml Alexa Fluor® 647 α-bungarotoxin (ThermoFisher) for 30 minutes. The cells were then washed three times with PBS and BT binding measured using an Accuri Sciences flow cytometer and analyzed with FlowJo software gating GFP positive (transduced) cells. Same procedure was applied to assess α-bungarotoxin binding to RAW264.7 cells expressing CHRFAM7A and Balb/c3T3 expressing CHRFAM7A, CHRNA7 or vector.

Isolation of RNA from cultured cells and preparation of cDNA for PCR and q-PCR Total RNA was prepared from cell lysates using the RNeasy kit (Qiagen, San Diego CA) and quantified using a Nanodrop Spectrophotometer. One μg of the total RNA was reversed transcribed using

iScript cDNA synthesis kit (BioRad, San Diego CA) in a 20 µl reaction as described by the manufacturer and 1 µl was used for RT-PCR or real-time qPCR analyses.

PCR for human CHRFAM7A, human and mouse CHRNA7 and mouse GAPDH. Real-time qPCR was performed using a BioRad iCycler Real-time PCR Detection System and a 25 µl reaction that contained 12.5 µl 2x Origene SensiMix SYBR Master Mix #QP100008, 1.0 ul of each primer (10 uM), cDNA corresponding to 1ug of the original mRNA and the remaining volume water. PCR cycling conditions for CHRFAM7A, human CHRNA7, mouse GAPDH, human GAPDH were 95°C for 10 minutes followed by 45 cycles of 94°C for 20 seconds, 60°C for 20 seconds, and 72°C for 25 seconds, followed by 71 cycles of 60.0°C for 30 seconds. Primers for PCR included CHRFAM7A (F) 5'-ATAGCTGCAAACTGCGATA-3' and (R) 5'-CAGCGTACATCGATGTAGCAG-3'; human CHRNA7 (F) 5'-ACATGCGCTGCTCGCCGGGA-3'and (R) 5'-GATTGTAGTTCTTGACCAGCT-3′; mouse CHRNA7 (F) 5'-CGTGGGCCTCTCTGTAGTGG-3 and (R) 5'-CTGGAGTTGGGGCACAGTGC-3′; rat/mouse GAPDH QuantiTect#QT01658692 and human GAPDH (F) 5'-CATGAGAAGTATGACAACAGCCT-3'and (R) 5'-AGTCCTTCCACGATACCAAAGT-3'.

Relative gene expression was calculated by either comparing the thermal cycle number (Ct) to that of GAPDH or to a standard curve generated with plasmid and expressed as either a reciprocal of dCt, plasmid copy number or fold increase using the ΔΔCt method. For mouse CHRNA7 cDNA was generated using iScript RT supermix (BioRad, cat#1708840) according manufacture instructions. RT-PCR was then performed on Thermal Cycler (BioRad) using Platinum SuperFi Green PCR 2X master mix (Invitrogen). Cycle conditions were: 95°C for 2 min, followed by 40cycles of 95°C for 20 sec, 56.5°C for 30 sec and 72°C for 60 sec, and final single step of 72°C for 5 min. PCR products were run on 2% agarose gel.

Animal Model of Cutaneous Thermal Injury All animal studies were conducted in accordance with NIH Guide for the Care and Use of Laboratory Animals (8th edition) and pre-approved by the University of California San Diego's Animal Subjects (IACUC) Committee. CHRFAM7A transgenic lines were propagated as heterozygotes and sibling non-transgenic and CHRFAM7A-transgenic mice obtained through the UC San Diego breeding core once identified as transgenic by tail clip analyses (Transnetyx, Memphis, TN). Only male mice were used in the studies reported here because female mice have differential sensitivity to thermal injury. Where indicated, age-matched C57bl/6 mice were also purchased from Jackson Laboratories and analyzed in crossover

studies. The day before cutaneous thermal injury, the dorsal fur of mice was clipped and mice fasted overnight with water, ad libitum. The following day, animals received buprenorphine for pain control and a template exposing an $8cm^2$ surface area of the backs of shaved mice was used to localize the effects of a 7 second exposure to steam (96-99°C). Animals then received a subcutaneous injection of 1ml normal saline (s.c.) and allowed to recover.

Colony formation of bone marrow-derived cells in GM MethoCult™ To evaluate the growth and differentiation of hematopoietic progenitor cells in vitro colony formation of bone marrow cells was assessed in Methocult™ according to manufacturers (Stem Cell Technologies) recommendations. Briefly, after determining the total cell number that were recovered from bone marrow, cells were diluted to 10^5 cells/ml in media supplemented with 2% FBS and 0.3ml added to 3ml of granulocyte/macrophage (GM) MethoCult™ media. After distribution of 1.1 ml of this solution into three 35mm dishes, the cells were incubated 7 days within a 150mm dish containing a 4^{th} 35mm dish and water and kept at 37°C, in 5% CO2 with ≥ 95% humidity. Representative colonies that had formed by 7days of culture were photographed and then directly counted manually using a NIKON tissue culture microscope.

Flow Cytometry of bone marrow colonies differentiating in GM MethoCult™ Bone marrow cells from wild type, sibling (non-transgenic) or transgenic mice were analyzed after 7 days of differentiation in GM Methocult™ in vitro. After counting colonies, wells were gently scraped of colonies from the plate surface, the cells were washed twice with washing buffer (PBS containing 0.5% BSA) and then stained for multipanel flow cytometry (MACS Miltenyi Biotec). FcR blocking reagent (MACS Miltenyi Biotec ,cat # 130-092-575) was added to 5 x 10^5 cells harvested from Methocult™ cultures and incubated for 10 min at 4°C. Staining was then performed according to the manufacturers' recommendations using a 50ul final volume for 20 minutes on ice and in the dark. A "myeloid cell panel" of antibodies capable of detecting monocytes, macrophages, granulocytes and dendritic cells consisted of anti-Gr1, anti-CD45, anti-Ly6G, anti-F4/80, anti-CD11c, anti-Ly6c, anti-CD11b and propidium iodide to cells to assess cell viability (Table S1). The cells were then washed once with 1ml washing buffer and resuspended in 200ul fresh buffer for analysis by flow cytometry. Compensation settings were established using MACS Comp bead kits.

Flow cytometry of hematopoietic stem cells in bone marrow Fresh bone marrow cells were collected from tibia and femur of donor mice, triturated, filtered though 70um cell strainer (Falcon)

and washed twice with a phosphate buffered saline washing buffer containing 0.5% bovine serum albumin. 10^6 bone marrow cells/ml were stained for analyses in a multi-panel flow cytometer (MACS Miltenyi Biotec) using an antibody panel capable of detecting c-kit$^+$ and Sca1$^+$ cells in a lineage-negative (KSL$^-$) HSC population (Table S2)

Flow cytometry of lung and spleen myeloid cells Lung or spleen tissues harvested form control and transgenic mice were dissociated into single-cell suspensions using an enzymatic digestion and a mechanical gentleMACS dissociator according to the manufacturer's instructions (Lung Dissociation Kit cat # 130-095-927; Spleen Dissociation Kit cat # 130-095-926, MACS Miltenyi Biotec). Splenic single-cell suspensions were submitted to myeloid cell immunostaining using the antibody panel of Table S1 used for Methocult colonies (above). To further enable the detection of lung alveolar macrophages (AM) and dendritic cells (DC), flow cytometry panel was adapted as described in Table S3.

To further specify the myeloid subsets of cells in lung the second panel was composed of anti-Gr1, anti-MHCII, anti-F4/80, anti-CD11c, anti-Ly6C and anti-CD11b (Table S4). The cells were washed once with 1ml washing buffer and re-suspended in 200ul fresh buffer for analysis by flow cytometry with compensation settings set by MACS Comp bead kits.

Statistical analyses All data and calculations were performed using Graphpad Prism software (V6.0) including ANOVA and t-tests where appropriate as indicated in Figure Legends.