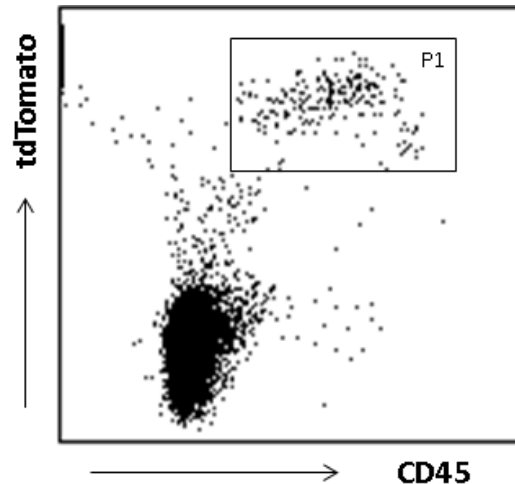


Supplementary figures and tables for:

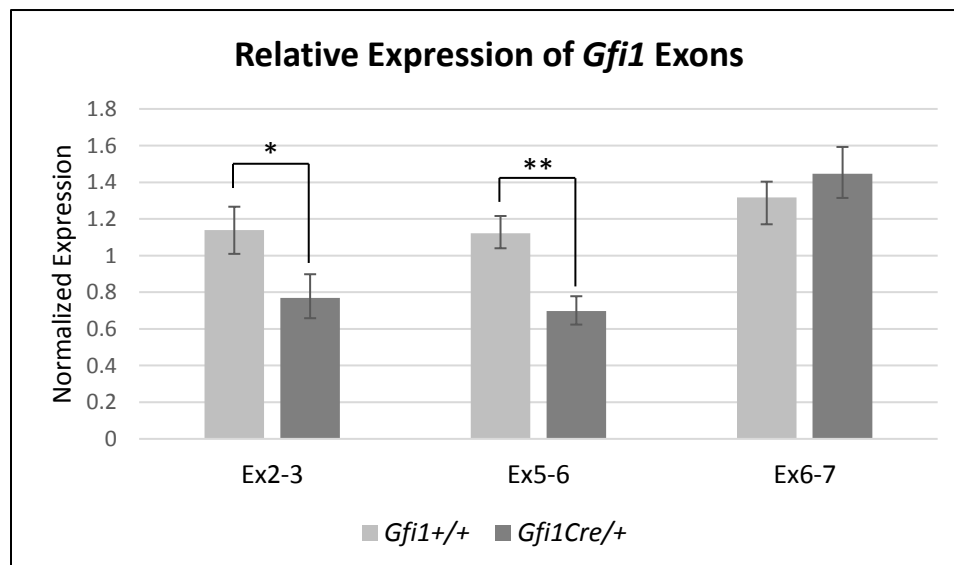
***Gfi1-Cre* mice have early onset progressive hearing loss and induce recombination in numerous inner ear non-hair cells**

Maggie Matern¹, Sarath Vijayakumar², Zachary Margulies¹, Beatrice Milon¹, Yang Song³, Ran Elkon⁴, Xiaoyu Zhang¹, Sherri M. Jones², and Ronna Hertzano*^{1,3,5}

Supplementary Figure 1 One representative FACS analysis of cochlear single cell suspensions from four day old *Gfi1^{Cre/+}; ROSA26^{CAG-tdTomato}* mice, analyzing expression of CD326 and CD45 in the tdTomato positive cell population. On average, 95.2% of CD45 positive cells were also positive for tdTomato (P1, immune cells).



Supplementary Figure 2 Quantitative comparative analysis of *Gfi1* transcript in *Gfi1^{+/+}* and *Gfi1^{Cre/+}* cochlea. RT-qPCR data showing relative expression levels of shared and unique exons between the wild-type and *Gfi1^{Cre}* knock-in alleles in the cochlea of newborn *Gfi1^{+/+}* and *Gfi1^{Cre/+}* littermates. A statistically significant ~30-40% decrease of *Gfi1* expression within regions substituted by Cre recombinase can be observed in *Gfi1^{Cre/+}* mice cochlea compared to wild-type: exons 2 to 3 (Ex2-3: fold change = 0.675, *p*-value = 0.022), and exons 5 to 6 (Ex5-6: fold change = 0.621, *p*-value = 0.005). No significant change in abundance was observed for a region shared between the two genotypes (Ex6-7: fold change = 1.099, *p*-value = 0.52). N=4. Error bars represent fold change values taking into account one standard deviation. Statistical significance was assessed using Welch's t-test.



Supplementary Table 1 List of genes encoding 521 transcripts enriched in HCs according to both Elkon et al. and Cai et al.^{1,32}

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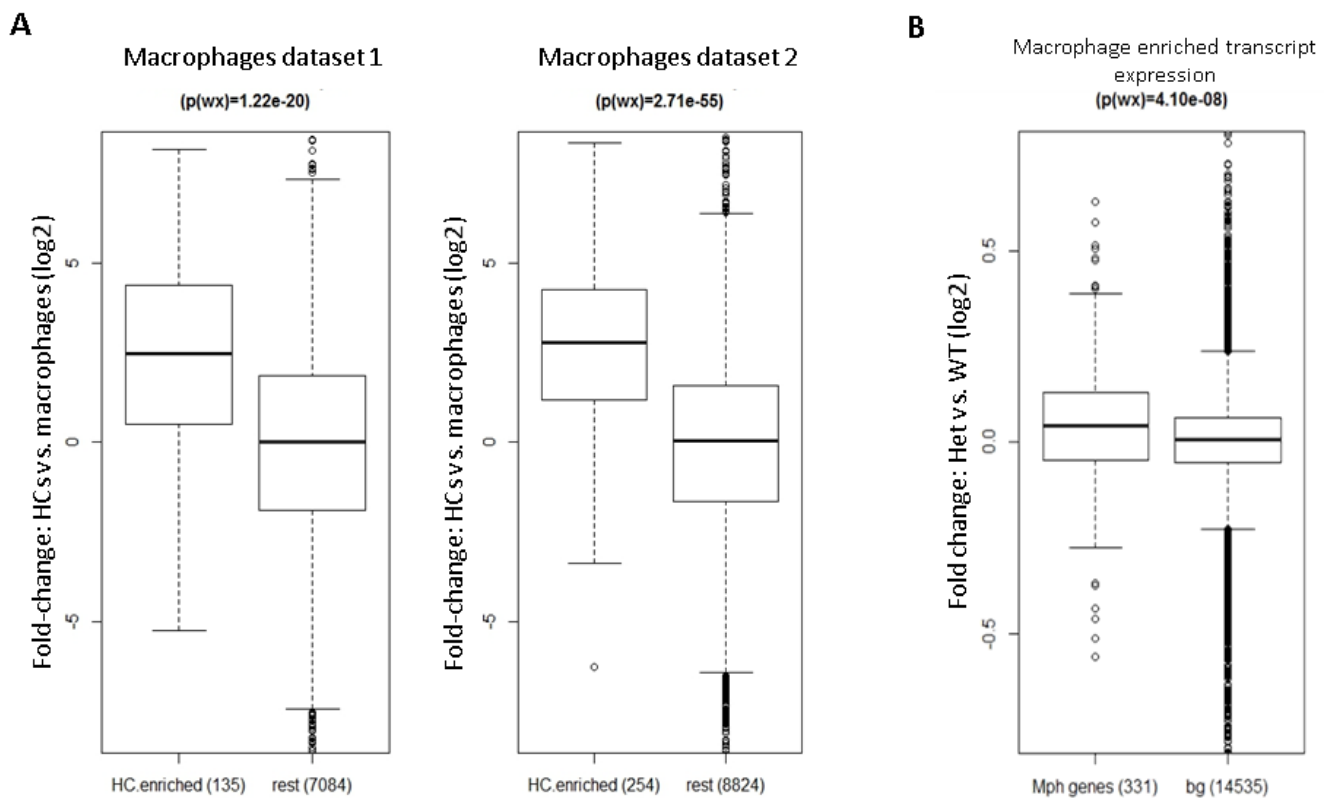
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Supplementary Table 2 Summary data for *Gfil*^{Cre/+} (HET1-3) and *Gfil*^{+/+} (WT1-3) RNA-seq samples.

Sample ID	Total reads	Total mapped reads	% mapped reads	% properly paired	Uniquely mapped reads	% exonic	% intronic	% intergenic	Total features	Features with coverage	Average RPKM
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HET2	47591802	43262912	90.9	93.54	41438699	93.07	4.55	2.38	43620	25218	6.042
HET3	45775884	41202266	90.01	95.29	39367365	93.45	4.12	2.43	43620	25126	6.312
WT1	38491984	33187310	86.22	94.52	31444234	91.33	4.79	3.88	43620	25675	8.193
WT2	40764300	36770716	90.2	95.61	35038788	93.08	4.46	2.46	43620	24908	6.736
WT3	42862376	38067548	88.81	95.33	36389984	91.86	5.14	2.99	43620	25205	6.458

Supplementary Figure 3 A. Expression of the set of 521 HC-enriched transcripts (used in the analysis shown in Figure 6C) is significantly elevated in HCs also when compared to macrophages (Mph). To address a possible concern that the downregulation of the HC-enriched transcripts in *Gfi1*^{Cre/+} cochlea could arise from changes in Mph gene expression, we further determined the relative expression levels of these 521 transcripts in two Mph datasets. For each gene that was expressed in both the HC dataset (based on intersection of Elkon et al., Cai et al.^{1,32}) and the Mph datasets (obtained from Avraham et al., Langlais et al.^{33,34}), HC to Mph fold-change of expression level was calculated. This resulted in a set of fold-change values for 7,219 and 9,078 genes expressed both in HCs and Mph. Of the 521 HC-enriched transcripts defined in Fig 6C, only 135 and 254 were expressed in the two Mph datasets, respectively. This analysis compares the distribution of fold-change values for these remaining HC-enriched transcripts vs. all other overlapping genes (*p*-values calculated using Wilcoxon's test.). Thus, the majority of the HC-enriched transcripts were not detected at all in Mph, and those that were detected (135 and 254) show significantly higher expression in HCs (*p*-values = 1.22E-20 and 2.71E-55 respectively). **B.** Based on the macrophage expression datasets, a set of 360 macrophage-enriched transcripts was defined. The expression of this set of genes is not decreased in the *Gfi1*^{Cre/+} mice cochlea.



Supplementary Table 3 List of genes encoding 360 transcripts enriched in macrophages according to both Avraham et al., 2016 and Langlais et al., 2016^{33,34}.

Ensembl ID	Gene symbol
ENSMUSG00000069892	9930111J21Rik2
ENSMUSG00000052760	A630001G21Rik
ENSMUSG00000023088	Abcc1
ENSMUSG00000020865	Abcc3
ENSMUSG00000044573	Acp1
ENSMUSG00000062825	Actg1
ENSMUSG00000025473	Adam8
ENSMUSG00000056413	Adap1
ENSMUSG00000039041	Adrm1
ENSMUSG00000011148	Adssl1
ENSMUSG00000027597	Ahcy
ENSMUSG00000062753	AI413582
ENSMUSG00000087107	AI662270
ENSMUSG00000024397	Aif1
ENSMUSG00000037860	Aim2
ENSMUSG00000024885	Aldh3b1
ENSMUSG00000060063	Alox5ap
ENSMUSG00000030649	Anapc15
ENSMUSG00000040701	Ap1g2
ENSMUSG00000022841	Ap2m1
ENSMUSG00000026786	Apbb1ip
ENSMUSG00000040613	Apobec1
ENSMUSG00000027255	Arfgap2
ENSMUSG00000048865	Arhgap30
ENSMUSG00000031389	Arhgap4
ENSMUSG00000030220	Arhgdib
ENSMUSG00000060216	Arrb2
ENSMUSG00000005470	Asf1b
ENSMUSG00000029752	Asns
ENSMUSG00000006057	Atp5g1
ENSMUSG00000038717	Atp5l
ENSMUSG00000033379	Atp6v0b
ENSMUSG00000022295	Atp6v1c1
ENSMUSG00000060131	Atp8b4
ENSMUSG00000054428	Atpif1
ENSMUSG00000060802	B2m
ENSMUSG00000059479	B3gnt8
ENSMUSG00000006731	B4galnt1

ENSMUSG00000004446	Bid
ENSMUSG000000061132	Blnk
ENSMUSG00000001999	Blvra
ENSMUSG000000040466	Blvrb
ENSMUSG000000021660	Btf3
ENSMUSG000000036887	C1qa
ENSMUSG000000036905	C1qb
ENSMUSG000000036896	C1qc
ENSMUSG000000040552	C3ar1
ENSMUSG000000049130	C5ar1
ENSMUSG000000056737	Capg
ENSMUSG000000070372	Capza1
ENSMUSG000000029836	Cbx3
ENSMUSG000000047810	Ccdc88b
ENSMUSG000000035385	Ccl2
ENSMUSG000000000982	Ccl3
ENSMUSG000000018930	Ccl4
ENSMUSG000000035373	Ccl7
ENSMUSG000000019122	Ccl9
ENSMUSG000000051439	Cd14
ENSMUSG000000021624	Cd180
ENSMUSG000000002944	Cd36
ENSMUSG000000030798	Cd37
ENSMUSG000000040747	Cd53
ENSMUSG000000025351	Cd63
ENSMUSG000000018774	Cd68
ENSMUSG000000024610	Cd74
ENSMUSG000000026437	Cdk18
ENSMUSG000000056501	Cebpb
ENSMUSG000000001128	Cfp
ENSMUSG000000090258	Churc1
ENSMUSG000000032436	Cmtm7
ENSMUSG000000030707	Coro1a
ENSMUSG000000028337	Coro2a
ENSMUSG000000091803	Cox16
ENSMUSG000000000088	Cox5a
ENSMUSG000000061518	Cox5b
ENSMUSG000000017778	Cox7c
ENSMUSG000000040713	Cregl
ENSMUSG000000006360	Crip1
ENSMUSG000000024621	Csf1r

ENSMUSG00000059326	Csf2ra
ENSMUSG00000071714	Csf2rb2
ENSMUSG00000005054	Cstb
ENSMUSG00000038642	Ctss
ENSMUSG00000016256	Ctsz
ENSMUSG00000052336	Cx3cr1
ENSMUSG00000018920	Cxcl16
ENSMUSG00000032872	Cyb5r4
ENSMUSG00000006519	Cyba
ENSMUSG00000015340	Cybb
ENSMUSG00000063694	Cycs
ENSMUSG00000018008	Cyth4
ENSMUSG00000026832	Cytip
ENSMUSG00000030347	D6Wsu163e
ENSMUSG00000001666	Ddt
ENSMUSG00000001674	Ddx18
ENSMUSG00000013973	Dedd
ENSMUSG00000017830	Dhx58
ENSMUSG00000019088	Dnase111
ENSMUSG00000003812	Dnase2a
ENSMUSG00000020143	Dock2
ENSMUSG00000052085	Dock8
ENSMUSG00000068335	Dok1
ENSMUSG00000022102	Dok2
ENSMUSG00000035711	Dok3
ENSMUSG00000053687	Dpep2
ENSMUSG00000020057	Dram1
ENSMUSG00000071644	Eef1g
ENSMUSG00000040659	Efhd2
ENSMUSG00000035150	Eif2s3x
ENSMUSG00000059796	Eif4a1
ENSMUSG00000021728	Emb
ENSMUSG00000040212	Emp3
ENSMUSG00000021996	Esd
ENSMUSG00000027533	Fabp5
ENSMUSG00000056069	Fam105a
ENSMUSG00000083012	Fam220a
ENSMUSG00000038274	Fau
ENSMUSG00000046865	Fbl
ENSMUSG00000058715	Fcer1g
ENSMUSG00000059498	Fcgr3

ENSMUSG00000059743	Fdps
ENSMUSG00000024742	Fen1
ENSMUSG00000024965	Fermt3
ENSMUSG00000037946	Fgd3
ENSMUSG00000055805	Fmnl1
ENSMUSG00000033386	Frrs1
ENSMUSG00000024661	Fth1
ENSMUSG00000050708	Ftl1
ENSMUSG00000009687	Fxyd5
ENSMUSG00000022148	Fyb
ENSMUSG00000057666	Gapdh
ENSMUSG00000058624	Gda
ENSMUSG00000031266	Gla
ENSMUSG00000021591	Glrx
ENSMUSG00000078862	Gm14326
ENSMUSG00000078866	Gm14420
ENSMUSG00000060791	Gmfg
ENSMUSG00000036246	Gmip
ENSMUSG00000006715	Gmnn
ENSMUSG00000027523	Gnas
ENSMUSG00000068523	Gng5
ENSMUSG00000052102	Gnpda1
ENSMUSG00000029816	Gpnmb
ENSMUSG00000021306	Gpr137b
ENSMUSG00000025156	Gps1
ENSMUSG00000034786	Gpsm3
ENSMUSG00000063856	Gpx1
ENSMUSG00000037894	H2afz
ENSMUSG00000061232	H2-K1
ENSMUSG00000067212	H2-T23
ENSMUSG00000060743	H3f3a
ENSMUSG00000027944	Hax1
ENSMUSG00000003283	Hck
ENSMUSG00000022831	Hcls1
ENSMUSG00000028800	Hdac1
ENSMUSG00000021665	Hexb
ENSMUSG00000025877	Hk3
ENSMUSG00000003038	Hmgn2
ENSMUSG00000035697	Hmha1
ENSMUSG00000005413	Hmox1
ENSMUSG00000026986	Hnmt

ENSMUSG00000029919	Hpgds
ENSMUSG00000015656	Hspa8
ENSMUSG00000027245	Hypk
ENSMUSG00000079017	Ifi2712a
ENSMUSG00000035004	Igsf6
ENSMUSG00000042349	Ikbke
ENSMUSG00000018654	Ikzf1
ENSMUSG00000032089	Il10ra
ENSMUSG00000022969	Il10rb
ENSMUSG00000002897	Il17ra
ENSMUSG00000030745	Il21r
ENSMUSG00000031304	Il2rg
ENSMUSG00000027947	Il6ra
ENSMUSG00000003882	Il7r
ENSMUSG00000062867	Impdh2
ENSMUSG00000029771	Irf5
ENSMUSG00000041515	Irf8
ENSMUSG00000030830	Itgal
ENSMUSG00000030786	Itgam
ENSMUSG00000000290	Itgb2
ENSMUSG00000028931	Kcnab2
ENSMUSG00000054342	Kcnn4
ENSMUSG00000018362	Kpna2
ENSMUSG00000055541	Lair1
ENSMUSG00000091512	Lamtor3
ENSMUSG00000028581	Laptm5
ENSMUSG00000040751	Lat2
ENSMUSG00000002699	Lcp2
ENSMUSG00000063229	Ldha
ENSMUSG00000050335	Lgals3
ENSMUSG00000033880	Lgals3bp
ENSMUSG00000024781	Lipa
ENSMUSG00000016239	Lonrf3
ENSMUSG00000015568	Lpl
ENSMUSG00000030263	Lrmp
ENSMUSG00000018819	Lsp1
ENSMUSG00000069516	Lyz2
ENSMUSG00000039208	Metrn1
ENSMUSG00000034854	Mfsd12
ENSMUSG00000033307	Mif
ENSMUSG00000012519	Mkl1

ENSMUSG00000025355	Mmp19
ENSMUSG00000003348	Mob3a
ENSMUSG00000039616	Mocos
ENSMUSG00000062270	Morf411
ENSMUSG00000023861	Mpc1
ENSMUSG00000046805	Mpeg1
ENSMUSG00000039640	Mrpl12
ENSMUSG00000024436	Mrps18b
ENSMUSG00000029918	Mrps33
ENSMUSG00000038880	Mrps34
ENSMUSG00000024677	Ms4a6b
ENSMUSG00000079419	Ms4a6c
ENSMUSG00000024672	Ms4a7
ENSMUSG00000025044	Msr1
ENSMUSG00000075705	Msrb1
ENSMUSG00000005667	Mthfd2
ENSMUSG00000024300	Myo1f
ENSMUSG00000020437	Myo1g
ENSMUSG00000061315	Naca
ENSMUSG00000002204	Napsa
ENSMUSG00000027698	Nceh1
ENSMUSG00000015950	Ncf1
ENSMUSG00000026480	Ncf2
ENSMUSG00000022488	Nckap11
ENSMUSG00000056234	Ncoa4
ENSMUSG00000020022	Ndufa12
ENSMUSG00000005510	Ndufs3
ENSMUSG00000047180	Neurl3
ENSMUSG00000058099	Nfam1
ENSMUSG00000032691	Nlrp3
ENSMUSG00000037601	Nme1
ENSMUSG00000056209	Npm3
ENSMUSG00000002108	Nr1h3
ENSMUSG00000026999	Nup35
ENSMUSG00000052776	Oas1a
ENSMUSG00000048779	P2ry6
ENSMUSG00000023249	Parp3
ENSMUSG00000022439	Parvg
ENSMUSG00000021496	Pcbd2
ENSMUSG00000027342	Pcna
ENSMUSG00000020553	Pctp

ENSMUSG00000011752	Pgam1
ENSMUSG00000062070	Pgk1
ENSMUSG00000038845	Phb
ENSMUSG00000025017	Pik3ap1
ENSMUSG00000039936	Pik3cd
ENSMUSG00000020901	Pik3r5
ENSMUSG00000021822	Plau
ENSMUSG00000046223	Plaur
ENSMUSG00000040061	Plcb2
ENSMUSG00000034330	Plcg2
ENSMUSG00000052160	Pld4
ENSMUSG00000020120	Plek
ENSMUSG00000031557	Plekha2
ENSMUSG00000026123	Plekha2
ENSMUSG00000028494	Plin2
ENSMUSG00000031146	Plp2
ENSMUSG00000021871	Pnp
ENSMUSG00000071866	Ppia
ENSMUSG00000004455	Ppp1cc
ENSMUSG00000045679	Pqlc3
ENSMUSG00000028691	Prdx1
ENSMUSG00000067713	Prkag1
ENSMUSG00000026979	Psd4
ENSMUSG00000036835	Psenen
ENSMUSG00000060073	Psma3
ENSMUSG00000068749	Psma5
ENSMUSG00000069744	Psemb3
ENSMUSG00000022193	Psemb5
ENSMUSG00000024338	Psemb8
ENSMUSG00000079197	Psmc2
ENSMUSG00000025429	Pstpip2
ENSMUSG00000056529	Ptafr
ENSMUSG00000043017	Ptgir
ENSMUSG00000004266	Ptpn6
ENSMUSG00000031506	Ptpn7
ENSMUSG00000026395	Ptprc
ENSMUSG00000030793	Pycard
ENSMUSG00000019832	Rab32
ENSMUSG00000033220	Rac2
ENSMUSG00000004952	Rasa4
ENSMUSG00000026430	Rassf5

ENSMUSG00000031167	Rbm3
ENSMUSG00000031387	Renbp
ENSMUSG00000026358	Rgs1
ENSMUSG00000030844	Rgs10
ENSMUSG00000031438	Rnf128
ENSMUSG00000030079	Ruvbl1
ENSMUSG00000019782	Rwdd1
ENSMUSG00000001020	S100a4
ENSMUSG00000001025	S100a6
ENSMUSG00000022876	Samsn1
ENSMUSG00000031101	Sash3
ENSMUSG00000078974	Sec61g
ENSMUSG00000028064	Sema4a
ENSMUSG00000074884	Serf2
ENSMUSG00000060147	Serpinb6a
ENSMUSG00000068856	Sf3b4
ENSMUSG00000028843	Sh3bgrl3
ENSMUSG00000054520	Sh3bp2
ENSMUSG00000059182	Skap2
ENSMUSG00000022372	Sla
ENSMUSG00000024737	Slc15a3
ENSMUSG00000025161	Slc16a3
ENSMUSG00000029175	Slc35f6
ENSMUSG00000032122	Slc37a2
ENSMUSG00000024327	Slc39a7
ENSMUSG00000034591	Slc41a2
ENSMUSG00000072620	Slfn2
ENSMUSG00000024217	Snrpc
ENSMUSG00000031662	Snx20
ENSMUSG00000029560	Snx8
ENSMUSG00000026222	Sp100
ENSMUSG00000070034	Sp110
ENSMUSG00000051457	Spn
ENSMUSG00000029304	Spp1
ENSMUSG00000024160	Spsb3
ENSMUSG00000034120	Srsf2
ENSMUSG00000032802	Srxn1
ENSMUSG00000028832	Stmn1
ENSMUSG00000020738	Sumo2
ENSMUSG00000021384	Susd3
ENSMUSG00000089847	Timm10b

ENSMUSG00000013701	Timm23
ENSMUSG00000033777	Tlr13
ENSMUSG00000091537	Tma7
ENSMUSG00000029390	Tmed2
ENSMUSG00000034947	Tmem106a
ENSMUSG00000038141	Tmem181a
ENSMUSG00000025521	Tmem192
ENSMUSG00000060538	Tmem219
ENSMUSG00000024401	Tnf
ENSMUSG00000028599	Tnfrsf1b
ENSMUSG00000045362	Tnfrsf26
ENSMUSG00000060126	Tpt1
ENSMUSG00000037318	Traf3ip3
ENSMUSG00000023992	Trem2
ENSMUSG00000018507	Trpv2
ENSMUSG00000041736	Tspo
ENSMUSG00000036918	Ttc7
ENSMUSG00000043091	Tuba1c
ENSMUSG00000073838	Tufm
ENSMUSG00000057130	Txn14a
ENSMUSG00000030579	Tyrobp
ENSMUSG00000061613	U2af1
ENSMUSG00000026956	Uap111
ENSMUSG00000090137	Uba52
ENSMUSG00000027078	Ube2l6
ENSMUSG00000060860	Ube2s
ENSMUSG00000057948	Unc13d
ENSMUSG00000036908	Unc93b1
ENSMUSG00000020163	Uqcr11
ENSMUSG00000034116	Vav1
ENSMUSG00000028452	Vcp
ENSMUSG00000064090	Vrk2
ENSMUSG00000051506	Wdfy4
ENSMUSG00000058486	Wdr91
ENSMUSG00000024066	Xdh

Supplementary Table 4 Gene-Ontology (GO) functional enrichment analysis demonstrating that the defined set of 360 macrophage enriched transcripts are significantly enriched for immune-system related functions.

Term	Count	P-Value	Fold Enrichment	FDR
GO:0006955: immune response	39	1.12E-13	4.18	1.88E-10
GO:0006954: inflammatory response	22	3.66E-09	4.94	6.15E-06
GO:0009611: response to wounding	26	2.34E-08	3.78	3.94E-05
GO:0006935: chemotaxis	19	3.86E-12	8.81	6.50E-09
GO:0048584: positive regulation of response to stimulus	17	8.45E-07	4.62	0.00142109
GO:0002709: regulation of T cell mediated immunity	7	8.47E-07	19.64	0.00142439
GO:0002703: regulation of leukocyte mediated immunity	11	1.05E-06	7.94	0.00177359
GO:0002819: regulation of adaptive immune response	10	1.33E-06	9.02	0.00223765
GO:0002706: regulation of lymphocyte mediated immunity	10	4.81E-06	7.77	0.00808705