Supplemental Data

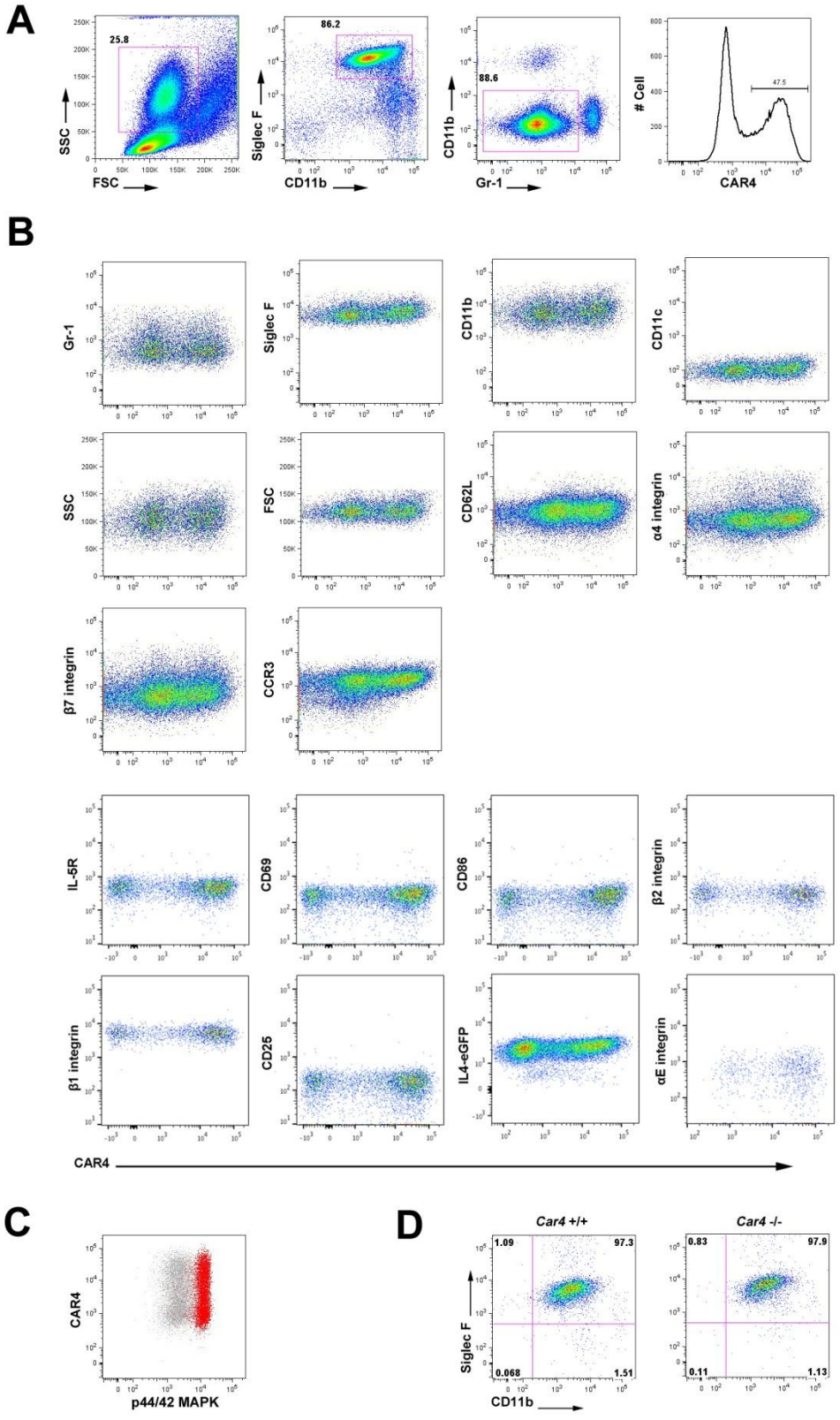

Figure S1. Bronchoalveolar eosinophil CAR4 expression in ovalbumin-induced asthma model and association with eosinophil lineage markers.
A, Mice were intranasally challenged with ovalbumin (OVA) after OVA/Alum sensitization. Bronchoalveolar (BALF) cells were extracted, and eosinophils were serially gated as SSC^{high}Siglec-$F^{+}CD11b^{+}GR\text{-}1^{+}CD11c^{-}$. A histogram of CAR4 surface expression of gated eosinophils is exhibited. B, Bronchoalveolar (BALF) eosinophils from *Aspergillus*-challenged mice were studied by FACS after serial eosinophil gating as SSC^{high}Siglec-$F^{+}CD11b^{+}$ $CD11c^{-}$. The double-plot between CAR4 and a panel of eosinophil surface markers and intracellular IL-4. C, Bone marrow–derived eosinophils were stimulated with media or 25 ng/mL eotaxin for 2 minutes. p44/42 MAPK activation was analyzed by FACS (media, grey; red, eotaxin, superimposed). D, Bone marrow from *Car4*$^{+/+}$ and *Car4*$^{-/-}$ mice were differentiated in vitro with IL-5 (10 ng/mL). When the culture matured at day 14, eosinophils from both genotypes were stained for the eosinophil markers Siglec-F and CD11b, which were double-plotted after FACS analysis.

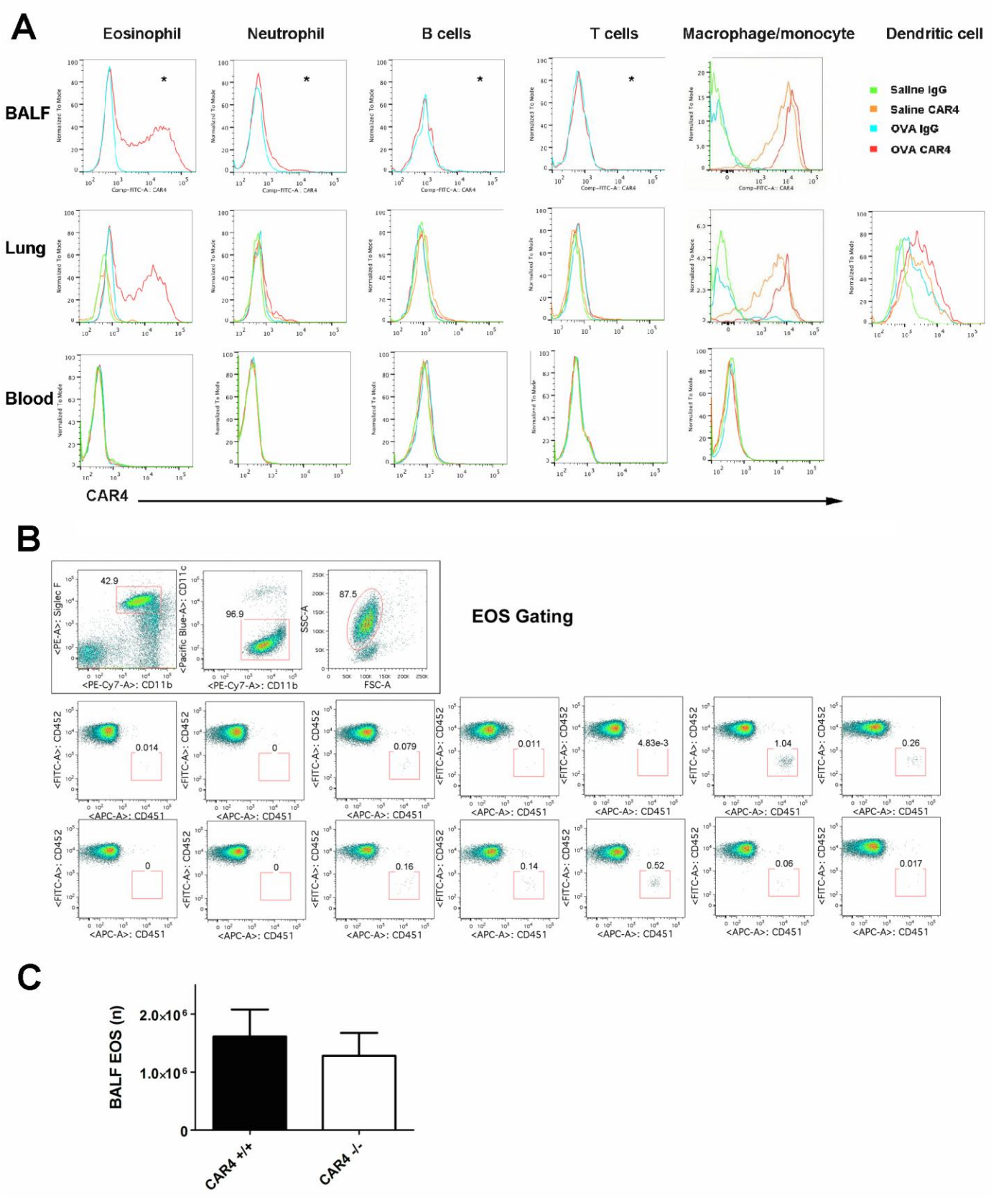
A
Eosinophil
Neutrophil
B cells
T cells
Macrophage/monocyte
Dendritic cell
BALF
Lung
Blood
Saline IgG
Saline CAR4
OVA IgG
OVA CAR4
CAR4
B
EOS Gating
42.9
96.9
87.5
0.014
0
0.079
0.011
4.83e-3
1.04
0.26
0
0
0.16
0.14
0.52
0.06
0.017
C
BALF EOS (n)
2.0x10^6
1.0x10^6
0
CAR4 +/+
CAR4 -/-

Figure S2. CAR4 differential expression on multiple leukocytes, eosinophil engraftment efficiency after bone marrow transplant and direct allergen challenge on *Car4*$^{+/+}$ and *Car4*$^{-/-}$ mice. A**,** Total WBC isolated from the OVA or saline -challenged lung, BALF and blood were co-stained with CAR4 and lineage specific markers (CD3, CD22, Siglec F, Gr-1, CD11c). After serial gating CAR4 expression histograms were shown on individual major leukocytes with Ig G control, saline vs. OVA challenge. * Saline histogram not available due to low physiological presence in specific compartment. B, Bone marrow transplant (BMT) was performed with *Car4*$^{+/+}$ and *Car4*$^{-/-}$ donors, with donors represented by the CD45.2 allele and recipients represented by the CD45.1 allele. Allergen challenge was initiated after CD11b^{+} cell engraftment reached > 99%. Bronchoalveolar (BALF) cells were FACS stained for CD45.1 and CD45.2 together with eosinophil (EOS) markers. After serial gating, total BALF eosinophil events were double-plotted for CD45.1 and CD45.2, indicating the presence of minimal residual native eosinophils by CD45.1 staining. C, *Car4*$^{+/+}$ and *Car4*$^{-/-}$ mice were directly subjected to *aspergillus* challenge, and bronchoalveolar (BALF) cells were extracted and stained for eosinophils (EOS). Total eosinophil levels were quantified by FACS enumeration.

Table S1. A cluster of 82 significant genes dysregulated in lung eosinophils following eosinophil activation

Gene symbol	Transcripts ID	Corrected p-value	Fold change
Chi3l3	10501020	0.020123405	42.52882
Car4	10379866	0.011772152	35.67207
Mmp12	10583056	0.012553694	32.577866
Retnla	10436095	0.012553694	28.131536
Cd34	10352905	3.74E-04	13.787505
Slc24a3	10476740	3.74E-04	9.795526
Enah	10360764	0.03067765	9.6138735
Egr2	10363735	0.019077016	9.051489
ENSMUSG00000075583	10429856	0.015867045	7.5380507
Qpct	10447056	0.011221767	6.8903117
Lipg	10459772	0.012553694	6.7715683
EG619945	10522494	0.027692037	6.647062
Hexb	10411373	0.03067765	6.6313777
Epx	10389654	0.0390082	5.7389402
Igsf2	10500656	0.010656019	5.6727343
Acp5	10591739	0.023440124	5.572733
Fbp1	10410007	0.015867045	5.088261
Gem	10503334	0.02661104	5.01466
Olfm4	10416689	0.038477343	5.014239
Acpp	10596303	0.026719332	5.0104766
Pdk3	10605711	0.04906303	4.9510646
Adam19|LOC100045780	10375402	0.023440124	4.7417
Il10ra	10593050	0.010656019	4.4473867
4930422I07Rik	10587655	0.04866087	4.3352757
Cd36	10528207	0.028912721	4.143904
Itgax	10557895	0.02661104	4.093909
Fbxl5	10529801	0.04605049	4.0551987
Atp10a	10553788	9.87E-04	3.9973352
Ccng2	10523297	0.019816801	3.9772398
LOC641050	10538890	0.038456343	3.941187
Gpr109a	10533720	0.011221767	3.838058
Adora2b	10376832	0.038456343	3.674053
H1f0	10425207	0.043288305	3.6016552
Dusp16	10548735	0.027166395	3.5922003
LOC100043256|LOC100045314	10394674	0.03067765	3.5826468
	10401931	0.04906303	3.5532143
BC005685	10401937	0.049180653	3.552429
Agl	10501699	0.023440124	3.4923165

Itgae	10378286	0.038477343	3.4668324
Atp6v0a1	10381187	0.011221767	3.4058828
Thex1	10578193	0.036152624	3.2520716
Bcl2l1	10488655	0.043288305	3.1740775
Dach1	10421950	0.047537882	3.0386393
Peg10	10536294	0.043288305	2.9797645
Rcn3	10563077	0.03067765	2.9610248
Lpin1	10399478	0.03376373	2.8765237
Dnajb9	10400137	0.030054986	2.7960322
Cxcl15	10523145	0.04510691	2.7402875
Hebp1	10548761	0.021281991	2.7362695
Syne2	10396608	0.043288305	2.7275488
Grn	10381588	0.02541297	2.718068
Tgm1	10420114	0.0390082	2.6799421
Lrp12	10428353	0.043288305	2.6363497
Creb5|9430076C15Rik	10538338	0.030080006	2.6045067
Ncoa7	10368534	0.038477343	2.5973206
Lrrk2	10426315	0.036152624	2.556557
Itgb5	10435305	0.028912721	2.5161908
Casd1	10536273	0.023440124	2.4850545
Lpp	10434806	0.029829124	2.4654164
	10439126	0.04906303	2.4514234
Clec4b2	10541599	0.03067765	2.3788
Fndc3a	10421488	0.02661104	2.3762236
Socs2	10372069	0.027239867	2.3307993
Pparg	10540897	0.03067765	2.2948604
Cpeb2	10521602	0.029829124	2.2172782
Ski	10519105	0.021464612	2.2114406
Mif|LOC100040259|LOC100044948	10590972	0.02661104	2.173588
Zmpste24	10516007	0.03929183	2.1373615
Btg3|EG654432|LOC100048453	10445875	0.026719332	2.1239157
Hmgb2	10518350	0.038477343	2.0664415
Hmgb2	10518352	0.03067765	2.0364242
	10407511	0.04510691	2.0323768
Hmgn2|LOC100041826|LOC100042405|LOC100044482|LOC637089	10403108	0.035860922	2.024345
Odc1	10394770	0.038456343	2.0198333
Oxct1	10422608	0.001760333	2.0116272
Btg3|EG654432|LOC100048453	10440419	0.029829124	2.0090618
Grina	10424781	0.03597291	2.006432
Uxs1	10354258	0.038456343	2.0024579
A230054D04Rik	10529758	0.036152624	-2.1741998

Hdc	10487238	0.04605049	-2.1786325
Ifitm3	10569017	0.021045567	-2.2407992
Ston2	10401891	0.03067765	-3.0209856

Table S2. Cluster of 37 significant genes dysregulated in the lung tissue following adoptive transfer of *Car4*$^{+/+}$ and *Car4*$^{-/-}$ bone marrow and subsequent allergen challenge

Gene symbol	Transcripts ID	Corrected p value	Fold change
Krt4	17322075	2.60E-05	4.634779
Car1	17404180	8.64E-04	3.8990877
Krt13	17269347	3.81E-06	2.6850803
Slc4a1	17270162	4.63E-04	2.6533484
Rhd	17419799	8.17E-04	1.9614452
Kel	17466360	0.001790935	1.8940217
Ctrb1	17513149	0.001304072	1.8876956
Spnb1	17282025	8.24E-05	1.7933112
MMCHR2_CTG5	17376441	2.00E-04	1.7366759
Scarna13|Mir3069	17283767	2.61E-05	1.6444792
Ngp	17522369	0.001230088	1.6360868
Lor	17407378	3.39E-05	1.6342173
Krtdap	17476557	1.52E-05	1.6080148
Defb4	17499594	8.65E-05	1.5839739
Acta1	17513995	0.001037918	1.5746803
Ank1	17500005	8.00E-04	1.5726726
Hemgn	17425028	0.002175863	1.569011
	17534980	9.02E-04	1.5477122
Lce1c|Lce1k	17399899	0.002330717	1.519077
Pnlip	17360751	0.001280171	1.5026282
Tgm3	17376191	2.09E-04	1.4853709
	17480566	0.002049547	1.4712522
Mt4	17503926	2.93E-04	1.4662353
	17512852	0.002534825	1.4587873
Slc10a6	17450319	1.66E-04	1.454301
	17366151	0.001186414	1.4481643
	17299313	1.65E-04	1.4293107
Rptn	17399981	5.88E-05	1.4224306
Ros1	17240750	3.50E-05	1.4189394
Mir1192	17358219	9.97E-04	1.4143491
Scarna3a|Mir1843b	17218565	4.88E-04	-1.449122
Tmc5	17482133	0.00205291	-1.7606255
A130040M12Rik	17267418	4.36E-04	-1.8176857
Nlrp1c	17265526	8.02E-04	-1.8641099
Ig V (Chr6)	17459403	8.03E-04	-1.9116696
MMCHR12_CTG7	17284645	5.18E-05	-2.2648532
IgK (Chr6)	17459341	0.002515408	-5.6372733