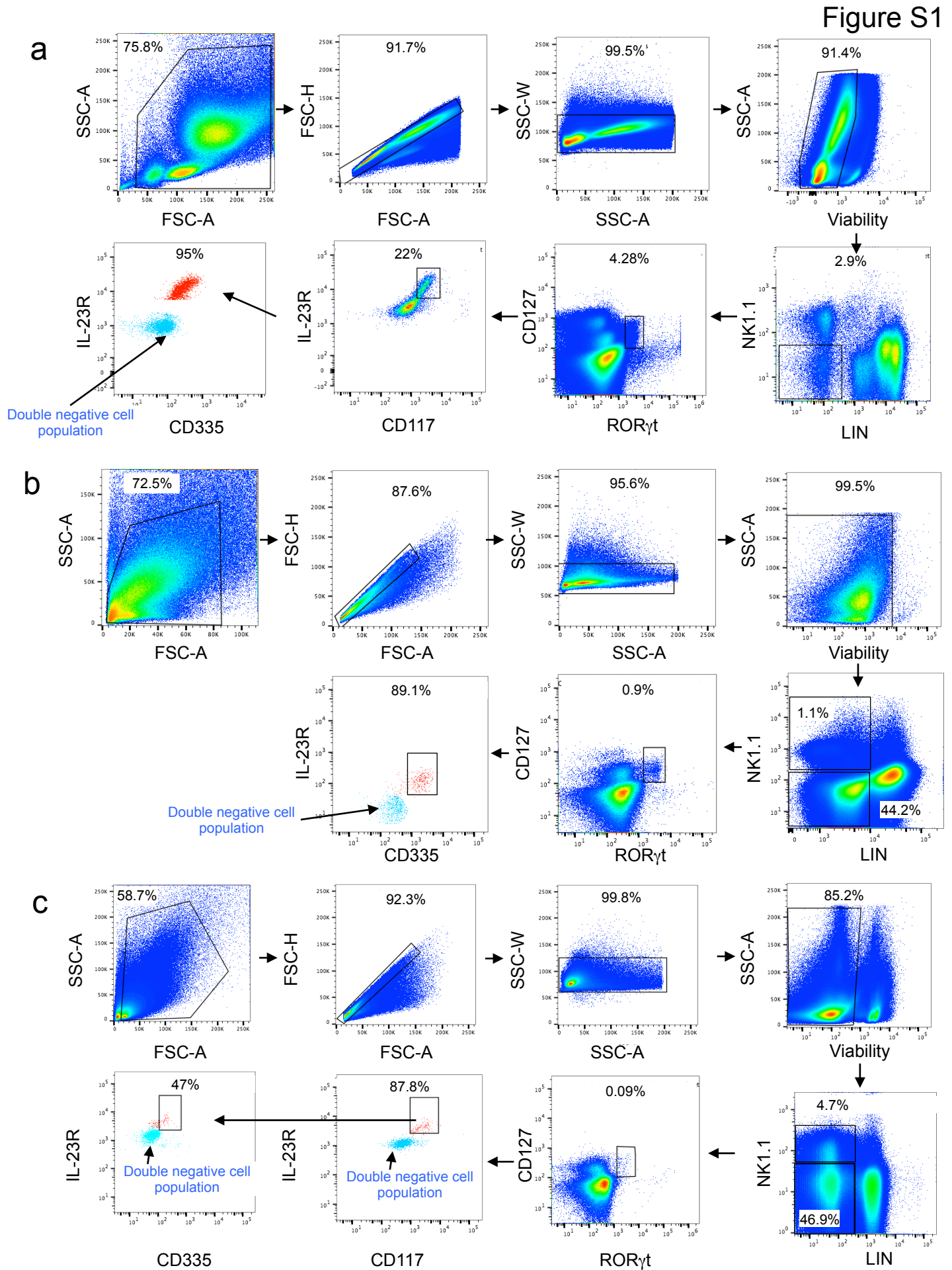
Figure S1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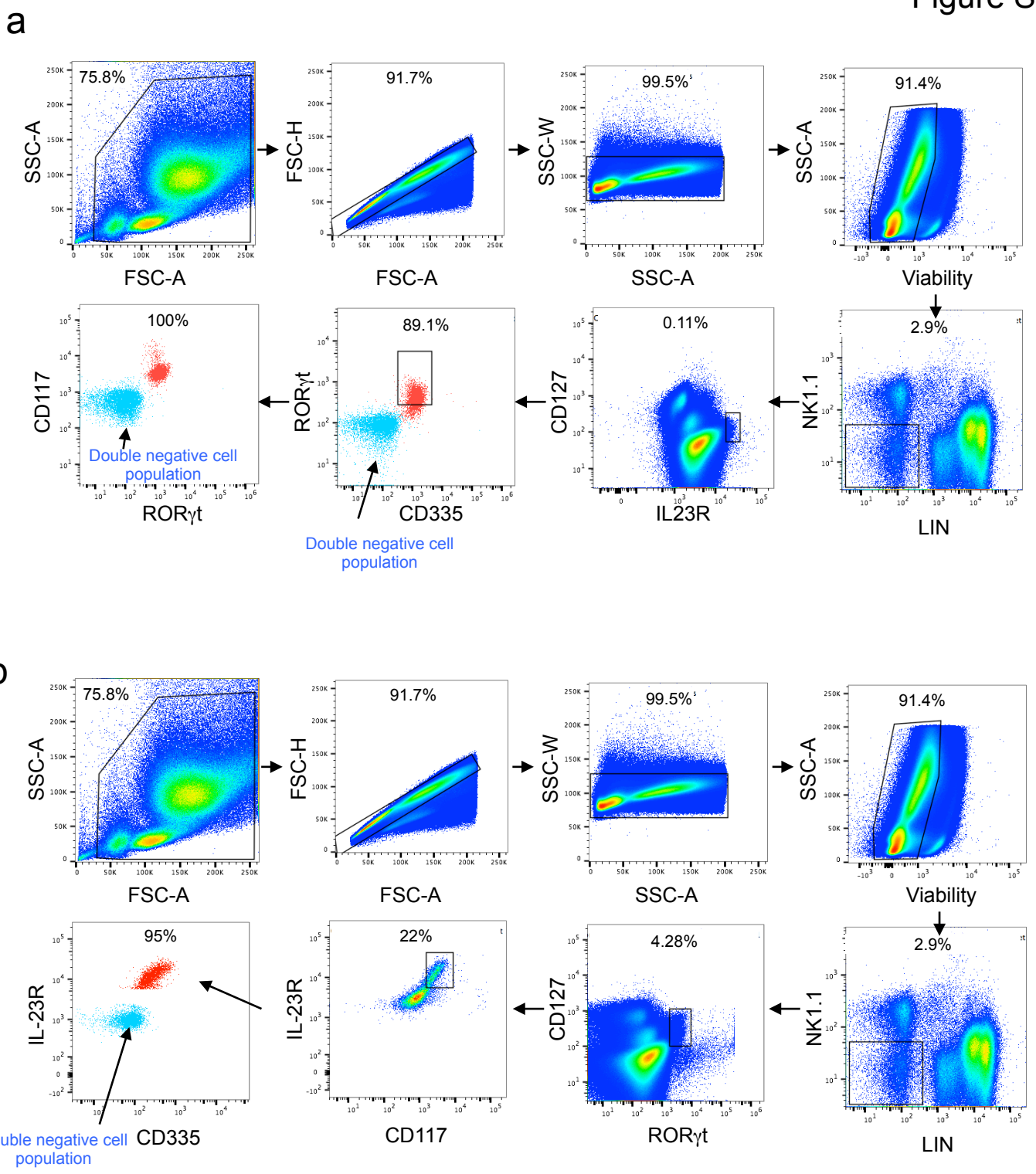
75.8%
SSC-A
FSC-A
91.7%
FSC-H
FSC-A
99.5%
SSC-W
SSC-A
91.4%
SSC-A
Viability
2.9%
NK1.1
LIN
4.28%
CD127
RORγt
22%
IL-23R
CD117
95%
IL-23R
CD335
Double negative cell population
b
72.5%
SSC-A
FSC-A
87.6%
FSC-H
FSC-A
95.6%
SSC-W
SSC-A
99.5%
SSC-A
Viability
1.1%
44.2%
NK1.1
LIN
0.9%
CD127
RORγt
89.1%
IL-23R
CD335
Double negative cell population
c
58.7%
SSC-A
FSC-A
92.3%
FSC-H
FSC-A
99.8%
SSC-W
SSC-A
85.2%
SSC-A
Viability
4.7%
46.9%
NK1.1
LIN
0.09%
CD127
RORγt
87.8%
IL-23R
CD117
Double negative cell population
47%
IL-23R
CD335
Double negative cell population

Figure S2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SSC-A
FSC-A
91.7%
FSC-H
FSC-A
99.5%
SSC-W
SSC-A
91.4%
SSC-A
Viability
2.9%
NK1.1
LIN
0.11%
CD127
IL23R
89.1%
RORγt
CD335
Double negative cell population
100%
CD117
RORγt
Double negative cell population
b
75.8%
SSC-A
FSC-A
91.7%
FSC-H
FSC-A
99.5%
SSC-W
SSC-A
91.4%
SSC-A
Viability
2.9%
NK1.1
LIN
4.28%
CD127
RORγt
22%
IL-23R
CD117
95%
IL-23R
Double negative cell population
CD335

Figure S3

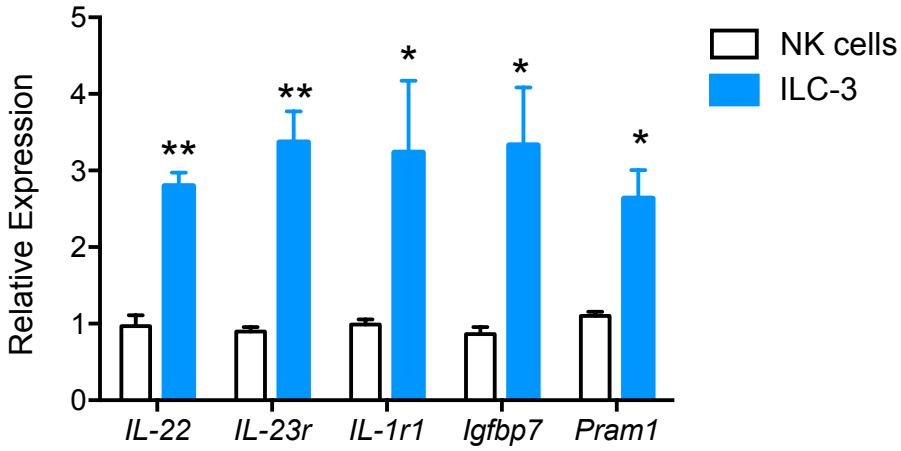
Relative Expression
5
4
3
2
1
0
**
**
*
*
*
IL-22
IL-23r
IL-1r1
Igfbp7
Pram1
NK cells
ILC-3

Figure S4

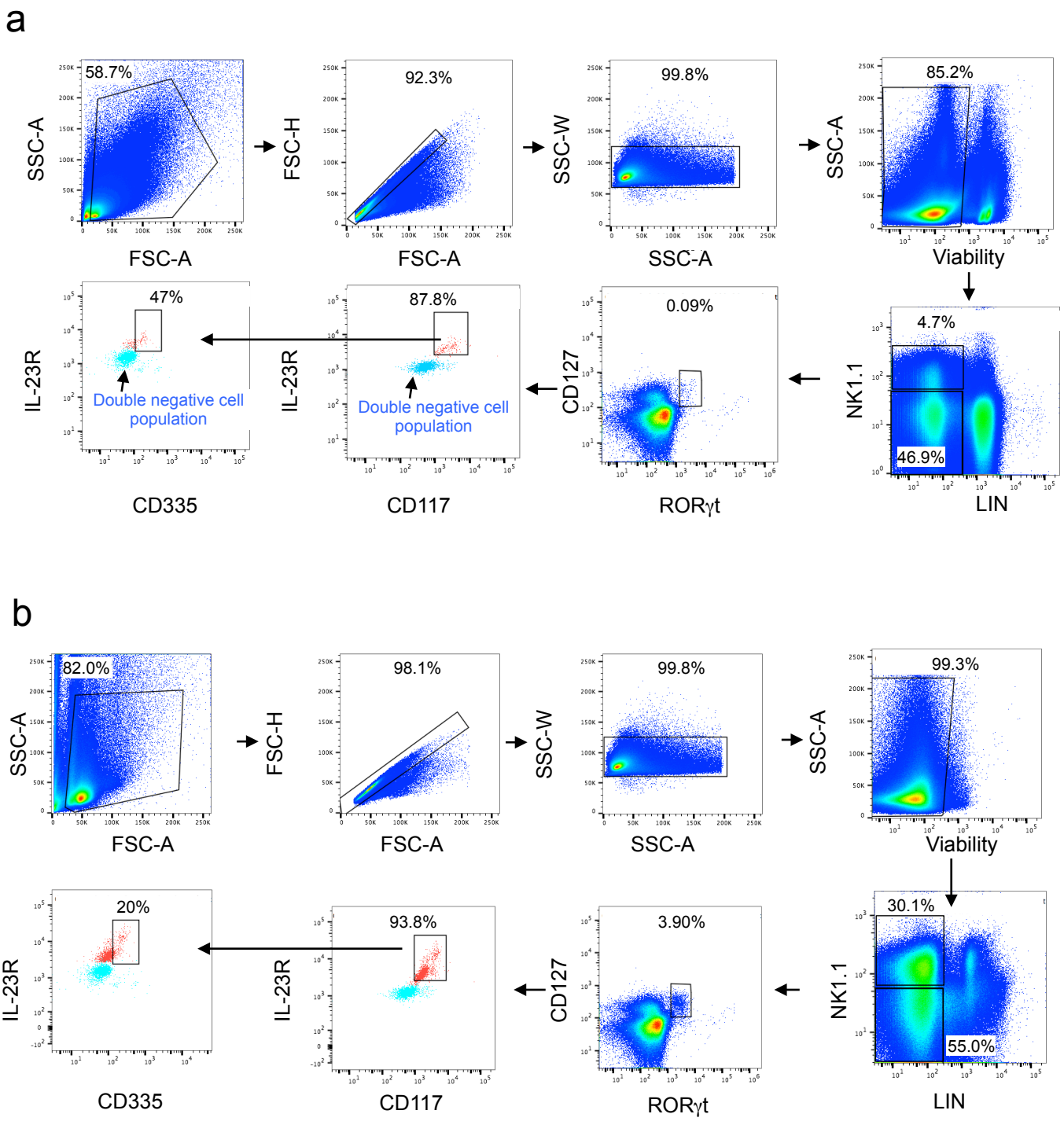
a
58.7%
SSC-A
FSC-A
92.3%
FSC-H
FSC-A
99.8%
SSC-W
SSC-A
85.2%
SSC-A
Viability
4.7%
NK1.1
46.9%
LIN
0.09%
CD127
RORγt
87.8%
IL-23R
Double negative cell population
CD117
47%
IL-23R
Double negative cell population
CD335
b
82.0%
SSC-A
FSC-A
98.1%
FSC-H
FSC-A
99.8%
SSC-W
SSC-A
99.3%
SSC-A
Viability
30.1%
NK1.1
55.0%
LIN
3.90%
CD127
RORγt
93.8%
IL-23R
CD117
20%
IL-23R
CD335

Figure S5

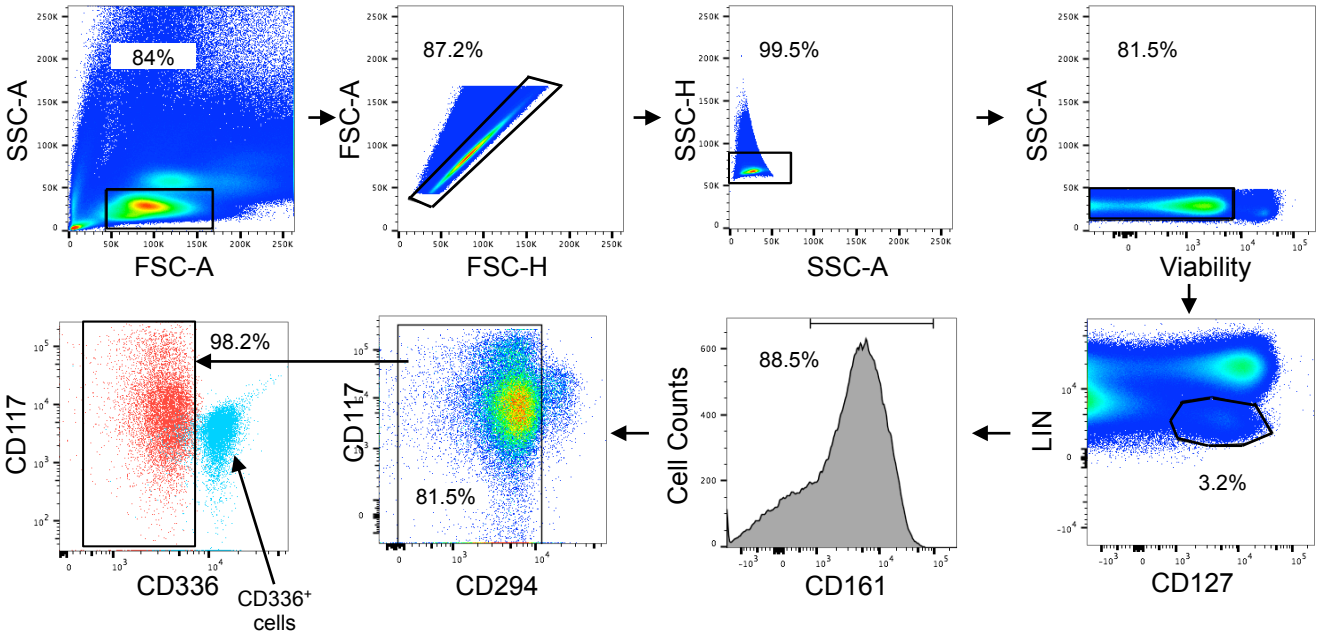
84%
SSC-A
FSC-A
87.2%
FSC-A
FSC-H
99.5%
SSC-H
SSC-A
81.5%
SSC-A
Viability
LIN
3.2%
CD127
88.5%
Cell Counts
CD161
CD117
81.5%
CD294
98.2%
CD117
CD336
CD336+ cells

Figure S6

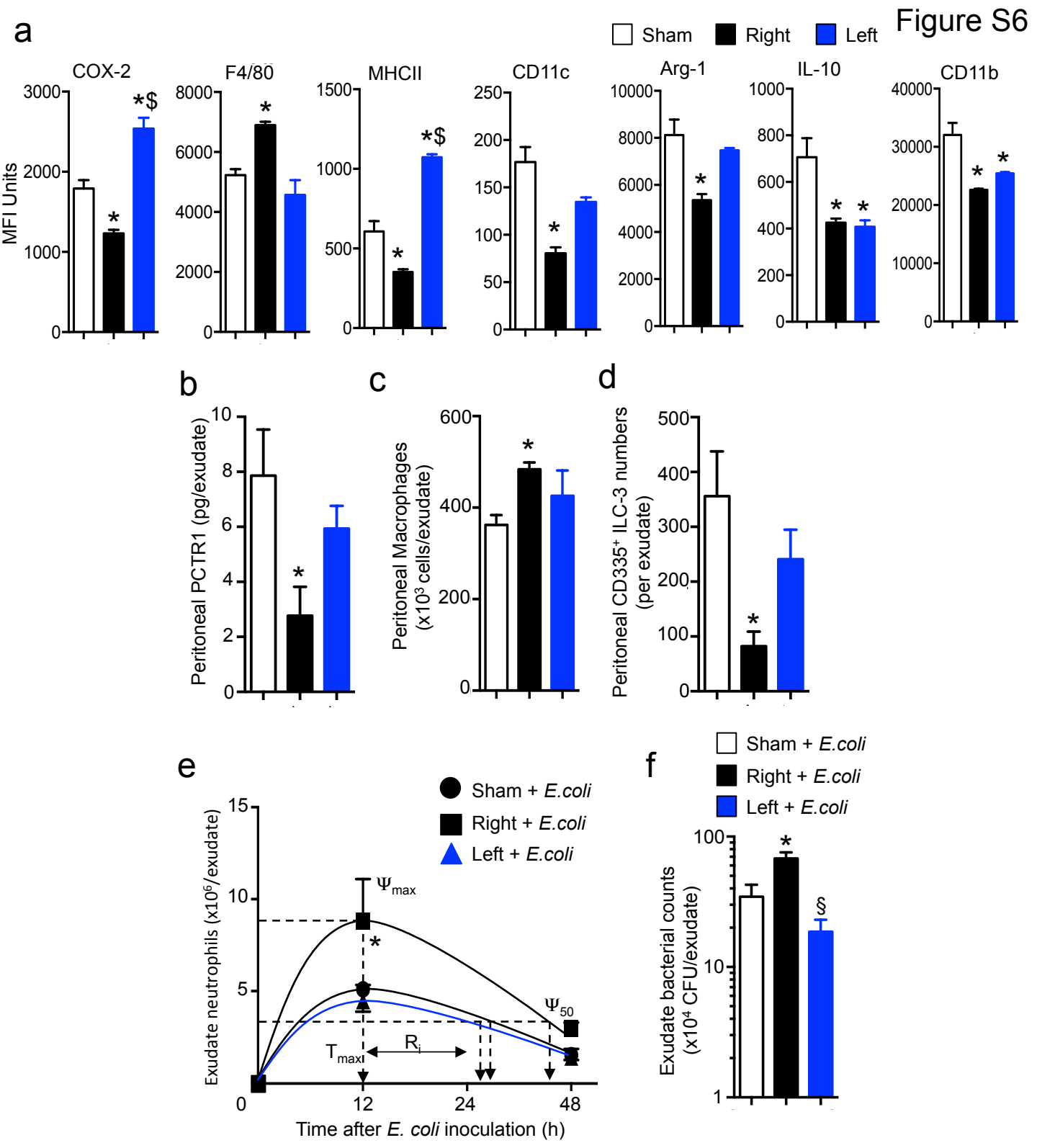
a
Sham
Right
Left
COX-2
F4/80
MHCII
CD11c
Arg-1
IL-10
CD11b
MFI Units
b
Peritoneal PCTR1 (pg/exudate)
c
Peritoneal Macrophages (x10³ cells/exudate)
d
Peritoneal CD335⁺ ILC-3 numbers (per exudate)
e
Sham + E.coli
Right + E.coli
Left + E.coli
Exudate neutrophils (x10⁶/exudate)
Ψmax
Ψ50
Tmax
Ri
Time after E. coli inoculation (h)
f
Sham + E.coli
Right + E.coli
Left + E.coli
Exudate bacterial counts (x10⁴ CFU/exudate)

Figure S1: Identification of peritoneal ILC-3 and comparison to intestinal and splenic ILC-3 populations. Related to Figure 2

a) Peritoneal cells were collected by lavaging the peritoneum with 4 ml of PBS without Ca^{2+} and Mg^{2+}. Cells were then incubated with live/dead fixable dye, then with anti-CD32/CD18 antibodies prior to incubation with fluorescently labelled antibodies. Depicted is the gating strategy employed to identify the ILC-3 population with percentage values denoting the proportion of cells within the gate from the parent cell population. The staining was then evaluated using a BD FACS Aria flow cytometer and FlowJo software. ILC-3 were then identified using the following gating strategy: after gating for live, Lineage negative, NK1.1 low cells, cells were gated for RoRγt positive, CD127 positivity, then for CD117 positive IL-23R positive cells and finally the expression of CD335 was determined. A double negative population, denoted in blue was included in the last two panels for reference. Results are representative of n = 7 mice

b) Mouse intestines were collected from male FvB mice (6-8 weeks of age) and lamina propria lymphocytes were isolated following tissue digestion and Percoll isolation. Cells were then incubated with live/dead fixable dye, then with anti-CD32/CD18 antibodies prior to incubation with fluorescently labelled antibodies. Depicted is the gating strategy employed to identify the ILC-3 population with percentage values denoting the proportion of cells within the gate from the parent cell population. The staining was then evaluated using a BD FACS Aria flow cytometer and FlowJo software. ILC-3 were then identified using the following gating strategy: after gating for live, Lineage negative, NK1.1 low cells, cells were gated for RoRγt positive, CD127 positivity, then for CD335 positive IL-23R positive cells. In the last panel we also included a double negative population, denoted in blue for reference. Results are representative of n = 3 mice.

c) Mouse spleens were collected from male FvB mice (6-8 weeks of age) and gently dispersed using a 70 μm cells strainer. (c) Cells were then stained as in panel **a.** Results are representative of n = 6 mice.

Figure S2: Gating strategy employed for the sorting of peritoneal ILC-3: comparison with conventional ILC-3 gating strategy. Related to Figure 2
Peritoneal lavages were collected from male FvB mice (6-8 weeks of age) and stained as in Figure S1a. (a) ILC-3 were then identified as Lineage negative, NK1.1 low cells, IL-23R and CD127 double positive cells were then gated for expression of CD335 expression. For validation purposes we also assessed the expression of RoRγt and CD117 in these cells were >89% of the cells were found to express these molecules (last two panels). (b) For comparison the conventional gating strategy outlined in Figure S1a. Results are representative of n = 6 mice. A double negative population, denoted in blue was included for reference.

Figure S3: Upregulation if ILC-3 associated genes in peritoneal ILC-3 in comparison with peritoneal NK-cells. Related to Figure 2
Peritoneal lavages were collected from male FvB mice (6-8 weeks of age) and stained as in Figure 1a and ILC-3 sorted as outlined in Figure 2b, were as NK cells were sorted as live/dead negative, Lineage negative and NK1.1 positive cells. RNA was extracted using an RNAeasy Micro Kit (Qiagen), reverse transcribed using a Qunatitect Reverse Transcription Kit (Qiagen) and the gene expression was assessed using Realtime qPCR. Results are mean ± sem. n = 4 mice.

Figure S4. Mice that underwent vagotomy of the left nerve display essentially identical host responses to peritoneal infections to sham mice. Related to Figure 2
Mice were subjected to unilateral vagotomy of the right or left vagus or sham surgery, after 7 days (a-d) peritoneal lavaged were obtained and (a) expression of macrophage phenotypic markers assessed using flow cytometry, (b) peritoneal PCTR1 levels assessed using lipid mediator metabololipidomics (c) peritoneal macrophage and (d) CD335+ ILC-3 counts assessed using flow cytometry and light microscopy. (e-f) Mice were inoculated with *E.coli* ($1x10^6$ CFU/mouse) and exudates collected at the indicated time intervals (e) Exudate neutrophil counts and resolution indices (f) exudate bacterial loads were determined at the 12h interval. Results are mean ± sem of representative of n= 5 mice per group from two distinct experiments.

Figure S5: Enrichment of splenic ILC-3 population for sorting and adoptive transfer experiments. Related to Figure 6

Spleens were collected from male FvB mice (6-8 weeks of age) and gently dispersed using a 70 µm cells strainer. (a) Cells were then stained as in Figure S2a or (d) enriched using a mouse NK cell enrichment kit (Stemcell) following manufacturer's instructions and then incubated with a viability dye and fluorescently conjugated antibodies as in Figure S2a**.** Results are representative of n = 6 mice.

Figure S6: Identification and sorting of human peripheral blood ILC-3. Related to Figure 6

Human peripheral blood mononuclear cells were isolated from healthy volunteers, cell were enriched using a negative selection approach with a human NK cell enrichment kit (Stemcell) and stained with the denoted fluorescently labelled antibodies along with a live/dead fixable dye. ILC-3 were identified by gating on live, Lineage negative, CD127 high cells, from these the CD161 expressing cells were identified and then the CD294 negative, CD117 positive cells were gated as ILC-3. The expression of CD336 was also determined in this cell population. For reference a CD336 positive cell population was also included in the last panel and denoted in blue. Percentage values denote the subset of cells gated from the parent population. Results are representative 4 healthy volunteers.

Supplemental Table 1. Temporal regulation of macrophage phenotypic markers in lavages from mice subjected to vagotomy. Related to Figure 2.

Macrophage markers	Sham			Day 1			Day 3			Day 7		
F4/80	5231	±	231	3248	±	143*	3321	±	270*	6899	±	129
CD11b	32062	±	2380	18173	±	648*	14927	±	880**	22605	±	251*
COX-2	1791	±	122	1629	±	121	1078	±	41*	1232	±	50**
TGFβ	1840	±	74	1313	±	58*	932	±	36*	1415	±	71*
Arg-1	8121	±	756	6034	±	297*	5004	±	151*	5356	±	295*
MHCII	607	±	75	291	±	23**	349	±	101*	352	±	21**
CD11c	177	±	18	88	±	16*	49	±	4**	81	±	7**
IL-10	707	±	94	423	±	19*	326	±	23**	426	±	20**

Mice were subjected to vagotomy procedure. Peritoneal lavages were collected at indicated time point and the expression of macrophage phenotypic markers assessed using flow cytometry and expressed as Mean Fluorescent Intensity (MFI). Results are mean ± s.e.m.; n = 4 mice per groups. * $p < 0.05$ and ** $p < 0.01$ *vs* macrophages from Sham mice.

Supplemental Table 2. Temporal lipid mediator profile from isolated peritoneal macrophages from mice subjected to vagotomy procedure. Related to Figure 2.

			Peritoneal macrophages Lipid mediators levels (pg/1x10^6 macrophages)								
DHA bioactive metabolome	**Q1**	**Q3**	**Sham**			**Day 1**			**Day 7**		
RvD1	375	233	5.8	±	0.5	1.1	±	0.3 ##	6.5	±	1.0
RvD2	375	141	0.5	±	0.4	0.1	±	0.1	0.5	±	0.1
RvD3	375	147	0.2	±	0.1	0.4	±	0.1	0.2	±	0.1
RvD4	375	101	0.1	±	0.1		*			*	
RvD5	359	199	2.2	±	0.6	1.1	±	0.3	2.7	±	0.8
RvD6	359	101	0.2	±	0.1		*			*	
17R-RvD1	375	141	0.1	±	0.1		*		0.1	±	0.1
17R-RvD3	375	147	0.5	±	0.2	0.9	±	0.2	0.8	±	0.1
PD1	359	153	5.1	±	1.1	1.9	±	0.7#	3.0	±	1.5
17R-PD1	359	153	3.9	±	0.9	1.7	±	0.5#	2.5	±	1.4
10S,17S-diHDHA	359	153	24.3	±	7.2	8.8	±	2.3#	12.0	±	4.5
PCTR1	650	231	10.3	±	3.8	2.6	±	0.5#	3.5	±	2.2
PCTR2	521	231	0.2	±	0.1		*			*	
PCTR3	464	231	0.2	±	0.1	0.4	±	0.1		*	
MaR1	359	221	1.8	±	0.5	1.4	±	0.6	1.0	±	0.7
7S,14S-diHDHA	359	221	1.3	±	0.3	0.7	±	0.3	0.5	±	0.4
4S,14S-diHDHA	359	101	3.0	±	1.4	0.5	±	0.2#	1.0	±	0.7
EPA bioactive metabolome											
RvE1	349	161	0.3	±	0.1	0.3	±	0.1	0.4	±	0.1
RvE2	333	159	0.1	±	0.1	0.2	±	0.1	0.1	±	0.1
RvE3	333	201	0.3	±	0.1		*		0.1	±	0.1
AA bioactive metabolome											
LXA_4	351	217		*			*			*	
LXB_4	351	221	0.7	±	0.3	0.6	±	0.2	0.5	±	0.1
5S,15S-diHETE	335	235	38.9	±	8.8	20.1	±	6.3#	17.1	±	5.4#
15R-LXA_4	351	217	0.3	±	0.3		*		1.0	±	0.9
15R-LXB_4	351	221	5.0	±	0.4	5.8	±	0.6	4.8	±	0.4
LTB_4	335	195	3.9	±	1.0	1.3	±	0.3#	1.8	±	0.9
5S,12S-diHETE	335	195	3.5	±	0.6	1.0	±	0.5##	1.4	±	0.8#
20-OH-LTB_4	351	195		*			*			*	
LTC_4	626	189	60.2	±	7.3	30.7	±	12.2	43.0	±	11.4
LTD_4	497	189	0.3	±	0.1	0.9	±	0.3	0.1	±	0.1
LTE_4	440	189	0.7	±	0.1	0.3	±	0.4	0.8	±	0.2
PGD_2	351	189	3.8	±	0.2	2.9	±	0.7	3.4	±	1.0
PGE_2	351	189	93.6	±	28.0	19.0	±	5.3#	34.2	±	17.1#
$PGF_{2\alpha}$	353	193	10.3	±	2.4	3.1	±	0.8#	7.1	±	0.9
TxB_2	369	169	87.1	±	12.7	25.7	±	8.3##	32.6	±	12.9#

Mice were subjected to right vagotomy. Peritoneal lavages were collected at indicated time point, peritoneal macrophages isolated and LM levels were assessed using LM-metabololipidomics. Results are mean ± s.e.m.; n = 3 mice per group. *, below limit, limit ≈ 0.1 pg. #p<0.05, ##p<0.01 *vs* Sham mice

Supplemental Table 3. PCTR1 redresses the altered peritoneal macrophage phenotype in vagotomized mice. Related to Figure 4.

Macrophage markers	Sham	Vagotomy + Vehicle	Vagotomy + PCTR1
F4/80	7430 ± 883	10063 ± 649$^{\#}$	7068 ± 485††
CD11b	32889 ± 2517	40366 ± 1185$^{\#\#}$	29631 ± 2387††
COX-2	2384 ± 300	2735 ± 322	2929 ± 189
TGFβ	6633 ± 98	5881 ± 151$^{\#\#}$	6816 ± 662
Arg-1	1195 ± 104	798 ± 103$^{\#\#}$	1106 ± 102†
MHCII	41447 ± 5507	25982 ± 5172$^{\#}$	37835 ± 4347†
Tim 4	14463 ± 899	18362 ± 904$^{\#\#}$	14678 ± 1061††
iNOS	2611 ± 367	1823 ± 294	2891 ± 272††
IL10	529 ± 98	493 ± 99	651 ± 92
CD11c	408 ± 35	260 ± 29$^{\#}$	458 ± 41††

Mice were subjected to unilateral vagotomy or sham surgery, after 7 days vagotomised mice were administered vehicle (saline containing 0.01% EtOH) or PCTR1 (75ng/mouse) via i.p. injection. Sixteen hours later peritoneal cells were collected and macrophage phenotypic marker expression was assessed using flow cytometry and expressed as MFI. Results are mean ± s.e.m; n = 5-6 mice per group. $^{\#}$ $p < 0.05$ and $^{\#\#}$ $p < 0.01$ *vs* Sham and † $p < 0.05$ and †† $p < 0.01$ *vs* Vagotomy + Vehicle.

Supplemental Table 4. PCTR1 rectifies the dysregulated peritoneal LM profiles in vagotomised mice. Related to Figure 4.

			Lipid mediators levels (pg/lavage)		
DHA bioactive metabolome	**Q1**	**Q3**	**Sham**	**Vagotomy + Vehicle**	**Vagotomy + PCTR1**
RvD1	375	215	1.8 ± 1.3	0.8 ± 0.5	0.7 ± 0.5
RvD2	375	175	2.6 ± 1.3	2.5 ± 1.4	3.3 ± 0.7
RvD3	375	191	*	*	0.2 ± 0.1$^{\#\#}$
RvD5	359	199	3.1 ± 0.8	6.6 ± 5.8	7.4 ± 3.5
RvD6	359	101	22.6 ± 18.9	18.6 ± 12.9	10.9 ± 6.8
17R-RvD1	375	215	0.1 ± 0.1	0.1 ± 0.1	0.2 ± 0.1
17R-RvD3	375	191	*	*	*
PD1	359	153	5.9 ± 1.8	4.9 ± 4.1	8.8 ± 2.0
17R-PD1	359	153	*	*	*
PCTR1	650	231	21.3 ± 4.0	9.7 ± 3.0$^{\#}$	19.7 ± 3.1†
PCTR2	521	231	12.4 ± 1.6	11.8 ± 2.5	4.4 ± 1.1$^{\#\#\#,\dagger\dagger}$
PCTR3	464	231	18.6 ± 4.6	15.2 ± 5.2	74.5 ± 6.7$^{\#\#\#,\dagger\dagger\dagger}$
MaR1	359	250	0.1 ± 0.1	0.4 ± 0.3	0.1 ± 0.1
EPA bioactive metabolome					
RvE1	349	161	10.3 ± 5.9	3.1 ± 0.9	6.1 ± 1.7
RvE2	333	253	2.6 ± 0.8	3.8 ± 2.1	2.9 ± 1.0
RvE3	333	245	1.7 ± 1.0	0.6 ± 0.3	4.0 ± 1.6†
AA bioacive metabolome					
LXA_4	351	115	3.0 ± 2.2	1.9 ± 1.1	0.5 ± 0.2
LXB_4	351	221	5.8 ± 3.7	2.3 ± 1.2	2.8 ± 0.8
5S,15S-diHETE	335	235	3.1 ± 0.7	3.9 ± 2.2	4.0 ± 1.4
15R- LXA_4	351	115	0.4 ± 0.2	0.1 ± 0.1	1.4 ± 0.5†
15R-LXB_4	351	221	*	*	*
LTB_4	335	195	5.6 ± 0.5	11.7 ± 2.4$^{\#}$	5.9 ± 0.6†
LTC_4	626	189	132.0 ± 36.2	44.4 ± 13.9$^{\#}$	43.3 ± 13.7$^{\#}$
LTD_4	497	189	4.3 ± 1.5	3.5 ± 2.2	6.2 ± 2.6
LTE_4	440	189	33.0 ± 12.3	23.2 ± 7.1	28.7 ± 9.8
PGD_2	351	189	20.7 ± 7.1	52.1 ± 22.3	16.3 ± 4.8
PGE_2	351	189	128.8 ± 46.8	164.4 ± 84.3	119.6 ± 34.5
$PGF_{2\alpha}$	353	193	4.0 ± 0.9	8.4 ± 1.9$^{\#}$	4.0 ± 0.5†
TxB_2	369	169	81.9 ± 17.1	201.2 ± 63.2$^{\#}$	52.5 ± 19.9†

Mice were subjected to unilateral vagotomy or sham surgery, after 7 days vagotomised mice were administered vehicle (saline containing 0.01% EtOH) or PCTR1 (75ng/mouse) via i.p. injection. Sixteen hours later peritoneal lavages were collected and LM profiles assessed using LM-metabololipidomics. Results are expressed as pg/lavage; mean ± s.e.m.; n = 5-6 mice per groups. $^{\#}$ $p < 0.05$, $^{\#\#}$ $p < 0.01$ and $^{\#\#\#}$ $p < 0.001$ *vs* Sham and † $p < 0.05$, †† $p < 0.01$ and ††† $p < 0.001$ *vs* Vagotomy + Vehicle; * = below limit, limit ≈ 0.1 pg. Q1, M-H (parent ion); and Q3, diagnostic ion in the MS-MS (daughter ion).

Supplemental Table 5. Temporal lipid mediator profile in lavages from mice subjected to vagotomy procedure Related to Figure 5.

Macrophage markers	Sham			Vagotomy			Vagotomy + Carbacol		
F4/80	5231	±	231	6899	±	129 #	7713	±	1199
CD11b	32062	±	2380	22605	±	251 #	24220	±	737
COX-2	1791	±	122	1232	±	50 ##	2693	±	233 **
TGFβ	1840	±	74	1415	±	71 #	1678	±	75
Arg-1	8121	±	756	5356	±	295 #	10726	±	957
MHCII	607	±	75	352	±	21 ##	1286	±	69 **
CD11c	177	±	18	81	±	7 #	157	±	7 *
IL-10	707	±	94	426	±	20 #	600	±	73

Mice were subject to vagotomy or sham procedure. Mice were then given Vehicle(PBS) or Carbachol 0.1mg/Kg/day for 7 days and peritoneal lavages were collected and the expression of macrophage phenotypic markers assessed using flow cytometry and expressed as Mean Fluorescent Intensity (MFI). Results are mean ± s.e.m.; n = 4 mice per groups. # $p < 0.05$ and ## $p < 0.01$ *vs* macrophages from Sham mice. * $p < 0.05$ and ** $p < 0.01$ *vs* macrophages from vagotomized mice

Supplemental Table 6. ILC-3 depletion alters peritoneal lipid mediator profile and macrophage phenotype. Related to Figure 5.

a

			Lipid mediators levels (pg/lavage)					
DHA bioactive metabolome	**Q1**	**Q3**	**Isotype**			**Anti-CD90.2**		
RvD1	375	215	6.1	±	3.5	12.5	±	7.6
RvD2	375	175	70.5	±	46.4	151.2	±	89.3
RvD3	375	191	3.1	±	1.5	6.5	±	3.8
RvD5	359	199	131.1	±	100.2	264.4	±	152.8
RvD6	359	101	1.7	±	1.0	3.2	±	1.8
AT-RvD1	375	215	12.9	±	8.2	21.1	±	12.4
AT-RvD3	375	191	5.6	±	3.6	15.4	±	9.2
PD1	359	153	15.5	±	4.8	28.4	±	15.5
AT-PD1	359	153	13.6	±	3.7	18.3	±	9.1
PCTR1	650	231	13.2	±	3.5	2.9	±	$0.9^{\#}$
PCTR2	521	231	7.5	±	1.9	1.0	±	$0.4^{\#\#}$
PCTR3	464	231	2.6	±	0.5	0.2	±	$0.1^{\#\#}$
MaR1	359	250	3.2	±	2.6		*	
EPA bioactive metabolome								
RvE1	349	161	6.8	±	1.8	15.7	±	7.3
RvE2	333	253	19.9	±	12.7	37.6	±	20.6
RvE3	333	245	5.7	±	3.8	6.9	±	4.0
AA bioacive metabolome								
LXA_4	351	115	2.9	±	2.1	7.3	±	4.6
LXB_4	351	221	16.1	±	9.4	36.2	±	21.9
5S,15S-diHETE	335	235	123.2	±	73.3	230.0	±	133.5
AT- LXA_4	351	115	6.2	±	3.5	10.3	±	5.4
AT-LXB_4	351	221	1.3	±	0.8	2.1	±	1.3
LTB_4	335	195	3.2	±	1.9	2.5	±	1.6
LTC_4	626	189	36.7	±	18.4	7.8	±	2.9
LTD_4	497	189	14.9	±	4.5	2.9	±	1.5
LTE_4	440	189	11.3	±	6.5	12.9	±	6.6
PGD_2	351	189	59.1	±	29.7	64.5	±	34.3
PGE_2	351	189	369.3	±	192.9	532.9	±	337.8
$PGF_{2\alpha}$	353	193	32.8	±	16.2	31.2	±	16.0
TxB_2	369	169	120.2	±	43.0	113.5	±	54.1

$Rag1^{-/-}$ mice were administered an anti-CD90.2 (250µg/mouse) or Isotype control (250µg/mouse) antibody. Peritoneal lavages were collected after 3 days and LM levels were assessed using LM-metabololipidomics. Results are expressed as pg/lavage; mean ± s.e.m.; n = 4 per group. $^{\#}$ $p < 0.05$, $^{\#\#}$ $p < 0.01$ *vs* Isotype mice; * = below limit, limit ≈ 0.1 pg. Q1, M-H (parent ion); and Q3, diagnostic ion in the MS-MS (daughter ion).

b

Macrophage markers	Isotype			Anti-CD90.2		
F4/80	34601	±	1330	41457	±	2113$^{\#}$
CD11b	33357	±	3762	25995	±	1963
COX-2	386	±	31	478	±	42
TGFβ	1601	±	475	768	±	236
Arg-1	232	±	25	184	±	6
MHCII	575	±	40	460	±	48
Tim 4	13666	±	664	16769	±	503$^{\#\#}$
iNOS	307	±	40	222	±	12$^{\#}$

Rag1$^{-/-}$ mice were administered an anti-CD90.2 (250µg/mouse) or Isotype control (250µg/mouse) antibody. Peritoneal cells were collected after 3 days and macrophage markers were assessed using flow cytometry and expressed as Mean Fluorescent Intensity (MFI). Results are mean ± s.e.m.; n = 4 per group; $^{\#}$ $p < 0.05$ and $^{\#\#}$ $p < 0.01$ *vs* Isotype.

Supplemental Methods

Bacterial Phagocytosis and Loads

To assess **bacterial phagocytosis** in peritoneal exudate leukocytes, exudate cells were incubated with PerCP-Cy5.5-conjugated anti-mouse CD11b antibody (30 min, 4^{o}C, in staining solution), then fixed and permeabilized using BD Perm/Wash™ Buffer (BD Biosciences) following manufacturer's instructions and incubated with FITC-conjugated anti-*E. coli* antibody (GenTex, 30 min, 4^{o}C, in BD Perm/Wash™ Buffer). **Bacterial counts** in peripheral blood and inflammatory exudates were determined by overnight cultures (37^{o}C) of serially diluted samples on LB agar plates. In select experiments mice were given PCTR1 75ng/mouse or vehicle (Saline containing 0.01% EtOH) *via i.p.* injection 16h prior to E. coli inoculation, then lavages were collected at determined time intervals and processed as outlined above.

Exudate Acethylcholine

Acetylcholine concetrations were determined using a commercially available ELISA (Abcam) following manufacturer's instructions. Briefly total levels of acetylcholine and choline were determined in peritoneal lavages by converting the acetylcholine to choline and measuring total choline amounts. The choline levels were then determined separately and the levels of acetylcholine levels were ascertained by subtracting the total choline levels from the free choline levels.

ILC3 transfer experiments

In determined experiment human ILC3 (1x10^{3} cells/200µl) or mouse ILC3 (5x10^{2} cells/200µl) isolated using the antibody combinations and sorting strategies described in Figures S4 and S6) were incubated with baicalin (10µM; lipoxygenase inhibitor) or vehicle (37^{o}C, 20 min, saline) then with DHA (1µM) and ACh (1nM, 45 min, 37^{o}C). These

incubations were then injected to FvB mice (male, 6-8 weeks of age) 7 day after unilateral vagotomy. After 3h mice were inoculated with *E. coli* ($1x10^6$ CFU/mice) and cell composition, bacterial phagocytosis, and bacterial counts were established at determined intervals as outlined above.

Antibody mediated depletion of ILC3s

In select experiments *Rag1*$^{-/-}$ (male, 6-8 weeks, Jackson Laboratories, fed lab diet containing essential fatty acids as from supplier) were injected with an anti-CD90.2 (250 µg/mouse; clone: 30H12, BioXcell;) or Isotype control (250 µg/mouse; BioXcell) antibody. 3 days later peritoneal lavages were obtained at 0 h or peritonitis was initiated by i.p. injection with *E. coli* (serotype O6:K2:H1; $1x10^5$ colony forming units CFU/mouse). Cell composition, bacterial phagocytosis, and bacterial counts were established at determined intervals as above.

Realtime PCR.

Human and mouse ILC3 sorted using the antibody combinations outlined above and in Figure S3 were centrifuged and resuspended in RTL Buffer. RNA was isolated using Qiagen RNEasy Micro kits following manufacturers instructions, cDNA was obtained using Superscript III reverse transcriptase (Invitrogen) and the expression of human GAPDH and 15-LOX type 1 was investigated using QuantiTect Primer Assays, RT2 SYBR® Green Mastermix, and Stratagene real-time PCR machine (model Mx 3005; Stratagene). Expression of mouse *Chrm1, Chrm2, Chrm3, Chrm4, Chrm5, Chrma4 Chrma7, Chrmb4, Il-22, Il-23r, Il-1r1, Igfbp7, Pram1* and *GAPDH* was assessed as described above.

Confocal Staining

Omenta were surgically removed from mice; these were placed immediately in 4% paraformaldehyde and kept for 15 min at room temperature. After fixing, the omenta were washed twice in PBS and 0.1% Tween 20 buffer for 2h at room temperature then in blocking solution (PBS containing 10% goat serum, 5% bovine serum albumin and 2 mM EDTA) for 4h at room temperature. Tissues were then incubated in blocking solution with anti-choline acetyltransferase (ChAt; Abcam, cat no: ab6168) overnight at 4^{o}C. The tissues were then washed in PBS and incubated with donkey anti-rabbit Alexa Fluor-594 conjugated antibody (Biolegend; cat no: 406418) for 7h at 4^{o}C. Tissues were washed in PBS and then incubated in permeabilization buffer (eBioscience) with BV421-anti-F4/80 (clone: BM8), Alexa Fluor-anti-CD3 (clone: 17A2) and Alexa Fluor-647-anti-RORγt (clone: Q21-559; BD Bioscience) overnight at 4^{o}C. The omenta were then washed and mounted in Mowiol® (10-98 Mw ~61,000; Sigma Aldrich) and imaged using a Zeiss LSM 710 confocal microscope, images were processed using Zen 2009 and ImageJ software.