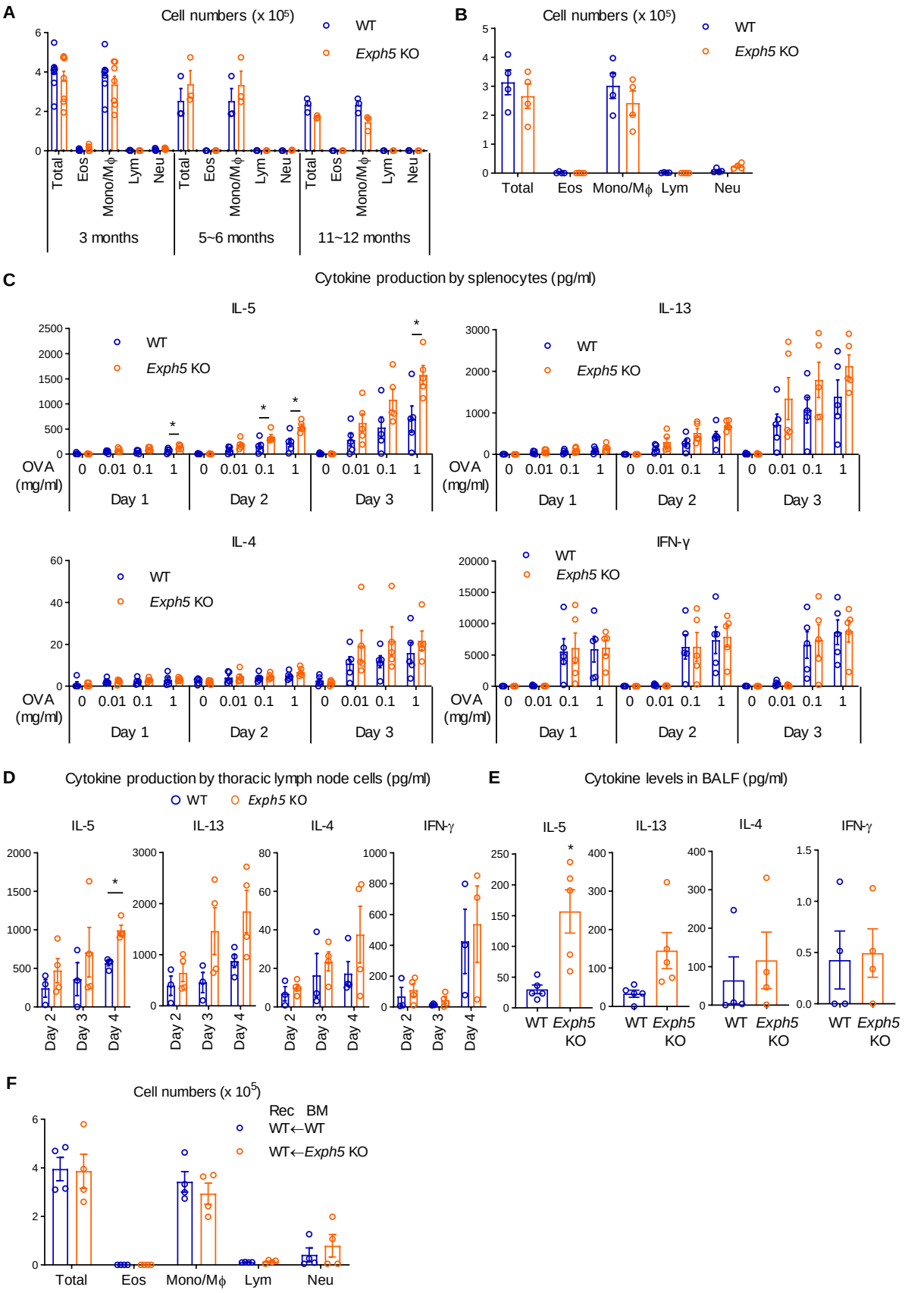
A
Cell numbers (x 10⁵)
WT
Exph5 KO
Total
Eos
Mono/Mϕ
Lym
Neu
3 months
5~6 months
11~12 months
B
Cell numbers (x 10⁵)
C
Cytokine production by splenocytes (pg/ml)
IL-5
IL-13
IL-4
IFN-γ
OVA (mg/ml)
0 0.01 0.1 1
Day 1
Day 2
Day 3
D
Cytokine production by thoracic lymph node cells (pg/ml)
Day 2
Day 3
Day 4
E
Cytokine levels in BALF (pg/ml)
WT Exph5 KO
F
Cell numbers (x 10⁵)
Rec BM
WT←WT
WT←Exph5 KO

Figure S1. Basal characteristics of *Exph5* KO mice in eosinophilic lung inflammation. (A) Cell numbers in BALF obtained from naïve untreated mice at the indicated ages (n=7 for 3-month old mice, and n=3 for others). (B) Cell numbers in BALF from OVA-sensitized mice in the absence of OVA aerosol challenge. (n=4 mice). (C) Time and dose-dependent cytokine production in response to ex vivo OVA re-stimulation by splenocytes obtained from OVA-sensitized mice before OVA aerosol challenges. Data for OVA 1 mg/ml on day 3 are identical to Figure 1B. (n=5 combined from 3 independent experiments) (D) Time-dependent cytokine production in response to ex vivo OVA re-stimulation by lung lymph node cells obtained from mice 24 h after the final OVA aerosol challenge. Data on day 4 are same to Figure 1C. (n=3-4 gathered from 2 independent experiments) (E) Cytokine levels in BALF after 3-day OVA aerosol challenges. (n=5 from 3 independent experiments) (F) Cell numbers in BALF of BM chimera mice without OVA aerosol challenges. BM from WT mice or exophilin5 (*Exph5*) KO mice were transferred into recipient WT mice. 6 weeks later, mice were sensitized with OVA/alum twice, and then received saline aerosol challenges for 3 days. 24 h after the final saline challenge, BALF was collected from each mouse, and cell numbers in BALF were determined. Data were obtained from 4 mice combined from 2 independent experiments with similar results. $*p < 0.05$; unpaired t-test.

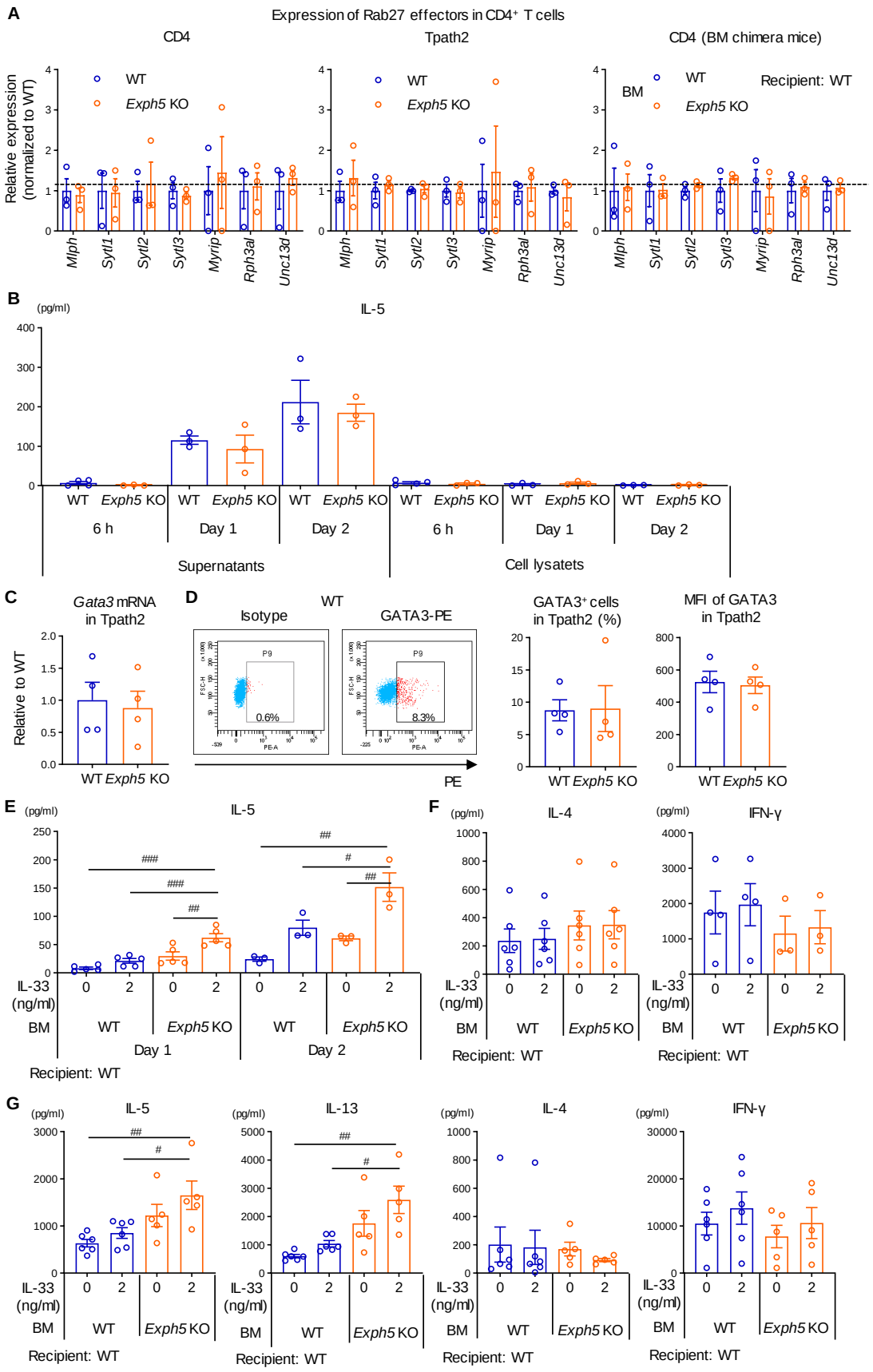
A
Expression of Rab27 effectors in CD4+ T cells
CD4
Tpath2
CD4 (BM chimera mice)
Relative expression (normalized to WT)
WT
Exph5 KO
BM
Recipient: WT
Mlph
Sytl1
Sytl2
Sytl3
Myrip
Rph3al
Unc13d
B
(pg/ml)
IL-5
WT Exph5 KO
6 h
Day 1
Day 2
Supernatants
Cell lysatets
C
Gata3 mRNA in Tpath2
Relative to WT
D
WT
Isotype
GATA3-PE
0.6%
8.3%
PE
GATA3+ cells in Tpath2 (%)
MFI of GATA3 in Tpath2
E
IL-5
IL-33 (ng/ml)
Day 1
Day 2
Recipient: WT
F
IL-4
IFN-γ
G
IL-5
IL-13
IL-4
IFN-γ

Figure S2. Effects of exophilin-5 deficiency on characteristics of CD4$^+$ T cells. (A) mRNA levels of Rab27 effector (other than exophilin-5) in the indicated splenic T cells (n=3). *Rph3al*, *Sytl4/Gph*, and *Sytl5* were undetected in T cells from either WT mice or *Exph5* KO mice. (B) Time course of IL-5 levels in the culture supernatants and in lysates of pathogenic Th2 cells. At the indicated time points, culture plates were first centrifuged at 500g for 5 min at 4℃, and then supernatants and cell lysates were harvested. IL-5 levels in supernatants and in cell lysates were determined by ELISA. (C, D) Levels of *Gata3*mRNA (C) and of GATA3 proteins (D) in pathogenic Th2 cells obtained from WT and *Exph5* KO mice (n=4). (D) (Left panel) A dot plot representative of WT mice (n=4) is shown. Percentages (Middle) and mean fluorescence intensity (MFI, right) of GATA3 positive cells in pathogenic Th2 cells were determined by FACS analysis. (E, F) Time-dependent IL-5 secretion (E), and IL-4 and IFN-γ secretion after 2-day culture (F) by TCR-stimulated CD4$^+$ T cells with or without IL-33 (2 ng/ml). CD4$^+$ T cells obtained from WT recipient mice transplanted with WT BM or *Exph5* KO BM were cultured with anti-CD3e Ab stimulation ± IL-33 (2 ng/ml) for the indicated durations. Then, cytokine concentrations in the supernatants were determined. (n=3-6 gathered from at least 2 independent experiments with similar results). (G) Cytokine production upon TCR and IL-33 stimulation by lung CD4$^+$ T cells obtained from OVA-sensitized WT mice transplanted with either WT BM or *Exph5* KO BM cells. CD4$^+$ T cells were treated as described in E and F for 1 day. Data were obtained from n=5-6 mice gathered from 3 independent experiments. Data represent the mean ± SEM. #$p < 0.05$, ##$p<0.01$; one-way ANOVA with Tukey's post hoc test.

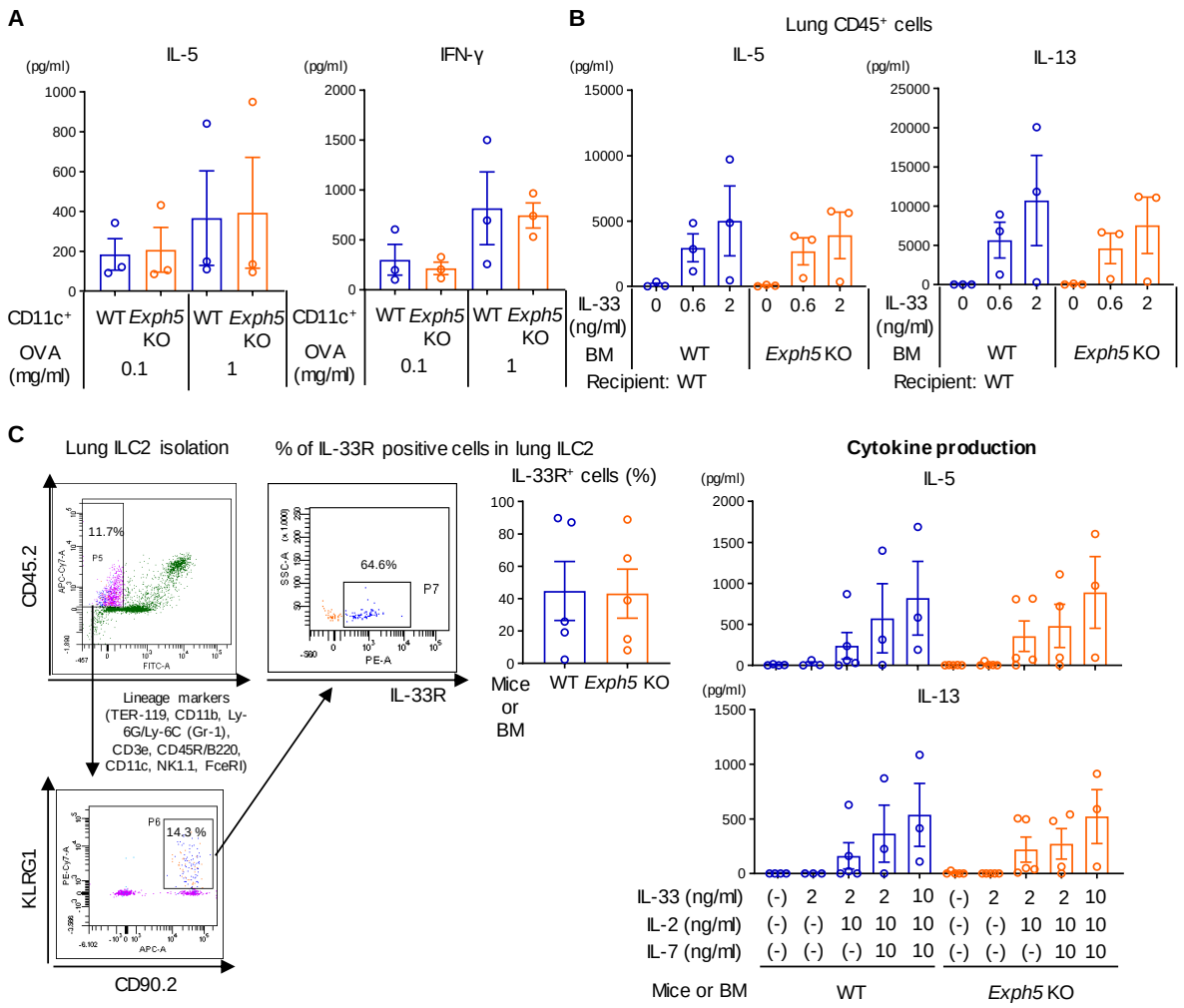

Figure S3. Effects of exophilin-5 deficiency on immune cells other than CD4$^+$ T cells. (A) Th-polarizing capacity of CD11c$^+$ splenic DCs. CD11c$^+$ splenic DCs and CD4$^+$ T cells were isolated as described in the "Methods" section. Purified CD11c$^+$ DCs (0.75×10^5 cells) and CD4$^+$ T cells (3×10^5 cells) were co-cultured with 0.1 or 1 mg/ml of OVA in 150 µl of complete RPMI1640 media per well of a 96-well plate for 3 days. Then, supernatants were collected for measurements of IL-5 and IFN-γ by ELISA. Data represent the mean ± SEM from 3 independent experiments. (B) IL-5 and IL-13 production by CD45$^+$ lung leukocytes in response to IL-33 without OVA antigen. Sorted CD45$^+$ lung leukocytes were cultured at 6×10^5 cells/well in a 96-well plate in the presence or absence of IL-33 (2 ng/ml) for 3 days. Then, IL-5 and IL-13 levels in the supernatants were determined by ELISA. (n=3 from 1 cohort representative of 2 independent cohorts with similar results) (C) IL-5 and IL-13 production by lung ILC2 in response to IL-33 (± IL-2 ± IL-7) after 3-day culture. (Left panel) Sorting strategy of lung ILC2. (Middle panel) % of IL-33R$^+$ cells in ILC2. (Right panel) Cytokines levels in the supernatants after 3-day culture with the indicated cytokines (n=3-5 for the indicated stimulation obtained from total n=5 experiments with similar results; 2 experiments using OVA-sensitized mice and 3 experiments using OVA-sensitized WT mice transplanted WT BM or *Exph5* KO BM).

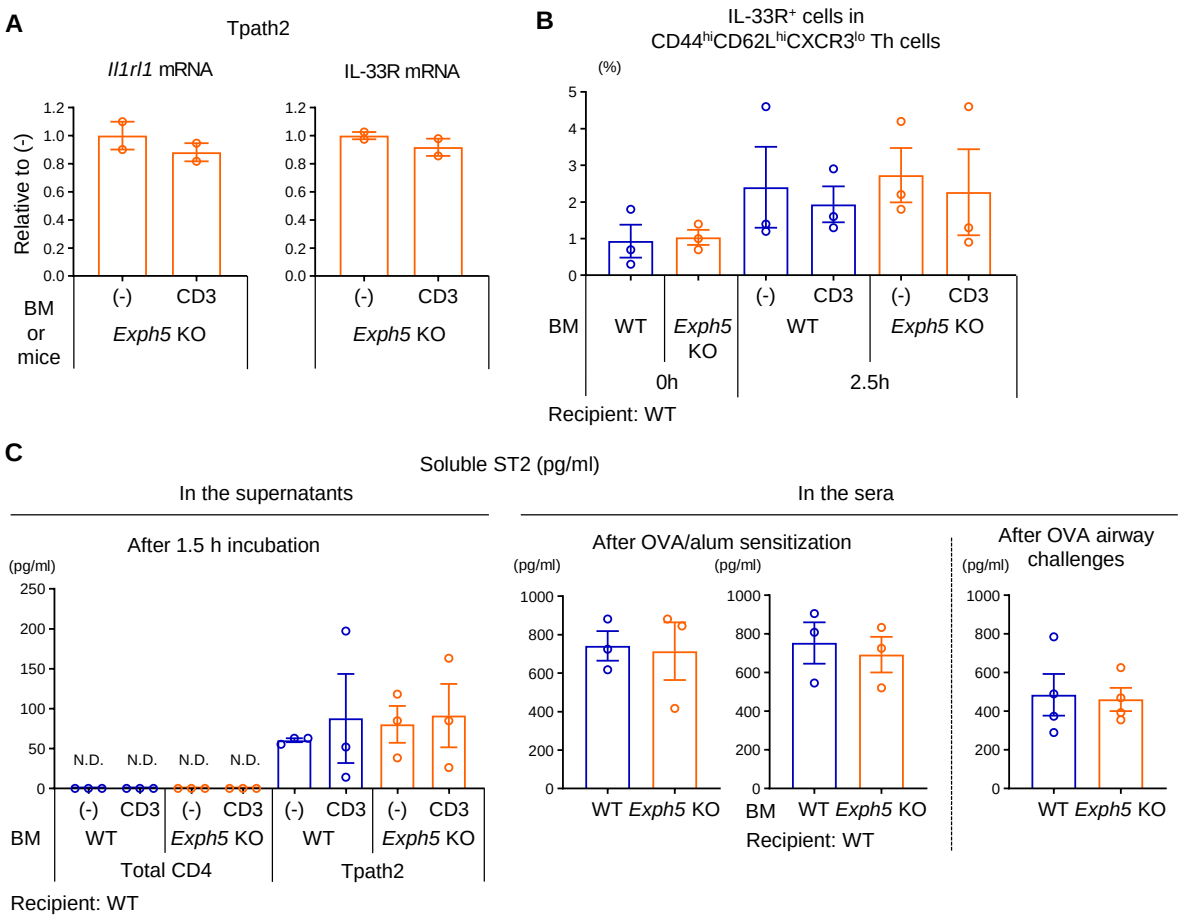

Figure S4. Effects of exophilin-5 deficiency on IL-33R mRNA levels in pathogenic Th2 cells and soluble ST2 production by T cells or in the whole body. (A) Levels of whole *Il1rl1* transcripts and transcripts specific for cell surface transmembrane IL-33 receptor (IL-33R) in pathogenic Th2 cells. After 1.5 h culture in the presence or absence of anti-CD3ε Ab, RNA was extracted from pathogenic Th2 cells, and the levels of the indicated mRNAs were determined using the specific primers. Total *Il1rl1* transcripts and IL-33R transcripts showed similar tendency, suggesting that amount of total *Il1rl1*transcripts mainly reflected the amounts of IL-33R transcripts. Data represent mean ± half range obtained from n=2 combined from one experiment using OVA-sensitized *Exph5* KO mice and one experiment using WT recipient mice transplanted with *Exph5* KO BM. (B) Changes in cell surface IL-33R expressions in $CD4^{+}CD44^{hi}CD62L^{hi}CXCR3^{lo}$ cells were determined as described in Figure 6B. (n=3) (C) Soluble ST2 levels in the indicated samples. Soluble ST2 levels in the supernatants of $CD4^{+}$ T cells or pathogenic Th2 cells obtained after 1.5 h culture (left panel), and in the sera obtained from OVA-sensitized mice (middle left panel), from OVA-sensitized WT recipient mice transplanted with WT BM or *Exph5* KO BM (middle right panel), or from mice received 3-day OVA aerosol challenges (right panel) were determined by ELISA (n=3-4). The lower limit of this assay was 31.25 pg/ml. N.D., not detectable.

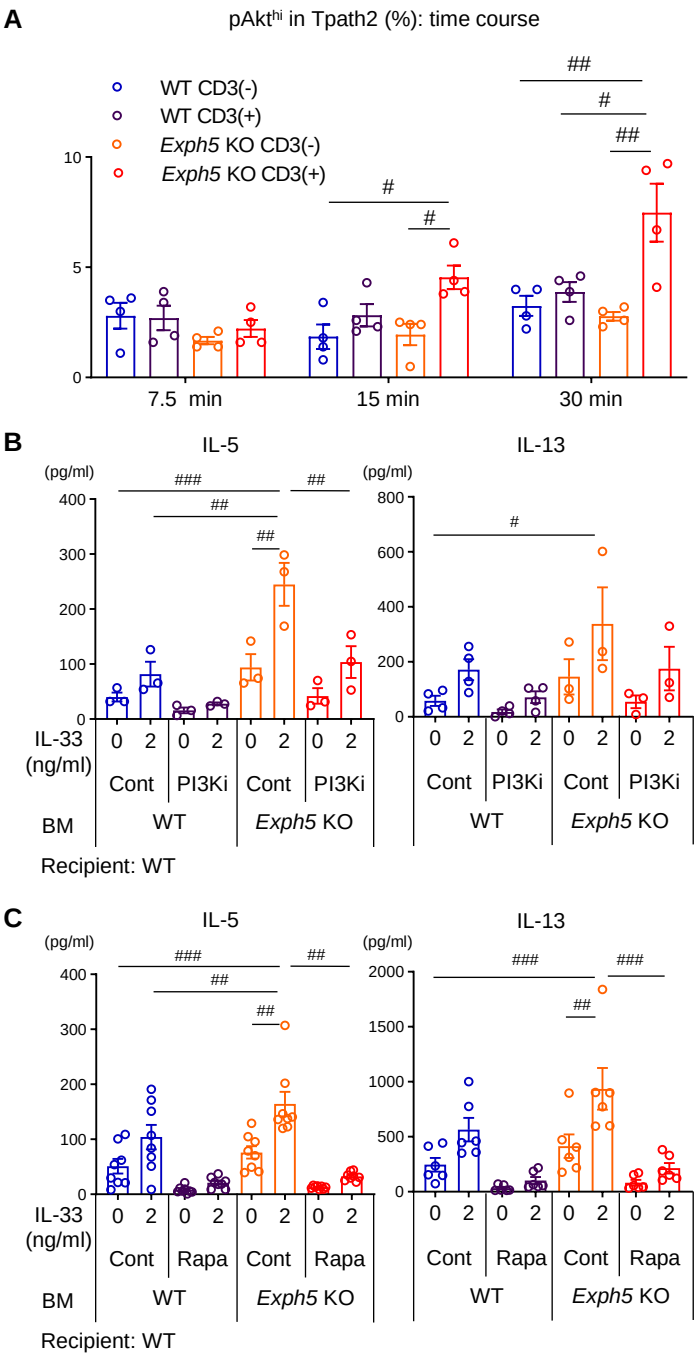

Figure S5. Exophilin-5 deficiency enhances PI3K/Akt/mTOR pathway and its downstream IL-33-dependent IL-5/IL-13 production in CD4$^+$ T cells. (A) Percentages of pAkt^{hi} cells in CD44^{hi}CD62L^{lo}CXCR3^{lo} pathogenic Th2 cells after anti-CD3ε Ab stimulation for the indicated durations. Splenic CD4$^+$ T cells obtained from OVA-sensitized WT mice or *Exph5* KO mice were first stained for cell surface molecules, and were then cultured on anti-CD3ε Ab-coated plates. At the indicated time points, cells were harvested, fixed, permeabilized, and stained with anti-pAkt-PE Ab. Data at 30 min are identical to Figure 8A. (B, C) Effects of the PI3K inhibitor wortmannin (PI3Ki, 100 nM) (B) and the mTOR inhibitor Rapamycin (Rapa, 10 nM) (C) on IL-5 and IL-13 production by CD4$^+$ T cells. After 10 min pre-incubation ± the indicated inhibitors, CD4$^+$ T cells were cultured as described in Figure 8C and D for 2 days. Then, cytokine levels in the supernatants were measured by ELISA. CD4$^+$ T cells were obtained from OVA-sensitized BM chimera mice in B and C. Data were obtained from n=4 independent experiments in A, n=3-4 mice in B, and n=6-8 mice in C, gathered from at least 2 independent experiments. #p < 0.05, ##p<0.01, ###p < 0.001; one-way ANOVA with Tukey's post hoc test.

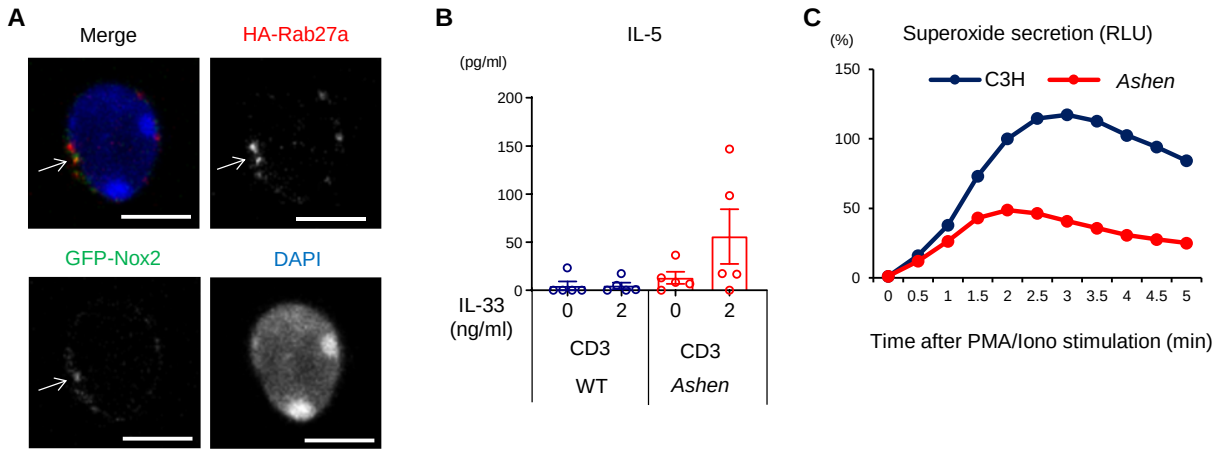

Figure S6. Rab27a deficiency demonstrates similar phenotypes as *Exph5* KO deficiency does in CD4$^+$ T cells. (A) Colocalization of Rab27a and Nox2 in CD4$^+$ Th cells. CD4$^+$ T cells were transfected with HA-Rab27a and GFP-Nox2 plasmids, and were then stained for each protein. Red: HA-Rab27a, Green: Nox2, Blue: DAPI. White scale bar: 5 μm. (B, C) IL-5 production in response to IL-33 (B) and extracellular superoxide secretion by CD4$^+$ T cells from WT C3H mice and Rab27a deficient *ashen* mice (C) were determined. As same as exophilin5 deficiency, Rab27a deficiency tended to increase IL-33 sensitivity and to decrease extracellular superoxide secretion by CD4$^+$ T cells. Data represent the mean ± SEM from n=5 mice combined from 2 independent experiments (Left panel) or 1 representative of 2 different experiments with similar results (Right panel).

Supplemental Table 1. List of antibodies used for FACS analysis

Antibodies (Clone)	Source	Identifier
anti-mouse CD16/CD32 (93)	eBioscience	14-0161-85
anti-mouse CD16/CD32 (2.4G2)	BD Biosicences	553142
anti-mouse CD11c PE (HL3)	BD Biosicences	557401
anti-mouse CD11c FITC (HL3)	BD Biosicences	561045
anti-mouse CD4 FITC (RM4-4)	BD Biosicences	553055
anti-mouse CD3e PE-Cy7 (145-2C11)	BD Biosicences	552774
anti-mouse CD45 FITC (30-F11)	BD Biosicences	553080
anti-mouse CD44 APC-Cy7 (IM7)	BD Biosicences	560568
anti-mouse CD62L PE (MEL-14)	BD Biosicences	561918
anti-mouse CD62L PE-Cy7 (MEL-14)	eBiosciences	25-0621-81
anti-mouse CXCR3 FITC (CXCR3-173)	eBiosciences	11-1831-82
anti-mouse CXCR3 APC (CXCR3-173)	BD Biosicences	562266
anti-mouse CXCR3 PE (CXCR3-173)	BioLegend	126506
anti-mouse CD326 PE (G8.8)	BD Biosicences	563477
anti-mouse CD326 APC (G8.8)	BD Biosicences	563478
anti-mouse CD49b PE (DX5)	BD Biosicences	553858
anti-mouse CD117 APC (2B8)	BD Biosicences	553356
anti-mouse Siglec-F PE (E50-2440)	BD Biosicences	552126
anti-mouse CD45.2 APC-Cy7 (104)	BD Biosicences	560694
anti-mouse CD90.2 APC (53-2.1)	BD Biosicences	561974
anti-mouse NK-1.1 FITC (PK136)	eBiosciences	11-5941-81
anti-mouse KLRG1 PE-Cy7	BioLegend	138415
anti-mouse IL-33R PE (RMST2-2)	eBioscience	12-9335-82
anti-mouse IL-33R PE (DIH9)	Biolegend	145304
anti-mouse FcεRIα FITC (MAR-1)	eBiosciences	11-5898-81
anti-mouse phosphorylated (p) Akt PE (M89-61)	BD Biosicences	561671
anti-mouse GATA3 PE (TWAJ)	eBiosciences	12-9966-41
Biotin anti-mouse Lineage Panel	BioLegend	133307
Streptavidin FITC	eBiosciences	11-4317-87

Supplemental Table 2. The primer sequences used in the present study

Gene (mouse)	Primer	Sequence
Rplp0/36B4	Forward	ggccctgcactctcgctttc
	Reverse	tgccaggacgcgcttgt
18S	Forward	cgccgctagaggtgaaattc
	Reverse	ttggcaaatgctttcgctc
Exph5	Forward	atgagggaggagagcggtat
	Reverse	cagcttgttgtccaaatcgtc
Cybb	Forward	ctggtgtggttggggctgaatgtc
	Reverse	cagagccagtgctgacccaaggagt
Il1rl1	Forward	tgacggccaccagatcattcacag
	Reverse	gccaaagcaagctgaacaggcaatac
IL-33R	Forward	gcatgataaggcacaccataa
	Reverse	atcgtagagcttgccatcgt
Gata3	Forward	ccgaaaccggaagatgtcta
	Reverse	agatgtggctcagggatgac
Mlph	Forward	acaacagctgtgccctctct
	Reverse	tcaaaagaaccactgtctacgc
Sytl1	Forward	cgcctcagccttggtaca
	Reverse	gaaggccatccacccact
Sytl2	Forward	agtcgccagaagacaagagc
	Reverse	tccatccaatccacttcagtc
Sytl3	Forward	aagacctacctgctgcctga
	Reverse	tcacttctccgaggaacacc
Myrip	Forward	taccggtgacaccaaggact
	Reverse	tgtgtgtggagtcctgctttag
Rph3al	Forward	caccatcttcagcagtggaa
	Reverse	gcctctctgctctctggatg
Unc13d	Forward	tgctacatgaacaccaacctg
	Reverse	gtgggtccacaacagagtca

Supplemental Table 3. List of antibodies used for immunoblotting and immunostaining

Antibodies	Host, clonality	Source	Identifier
(Primary antibodies)			
For immunoprecipitation			
Anti-FLAG M2 Affinity Gel	Rabbit polyclonal	Sigma-Aldrich	A2220
For immunoblotting			
HA(hemagglutinin)	Rabbit polyclonal	MBL	561
FLAG	Rabbit polyclonal	Sigma-Aldrich	F7425
GFP	Rabbit polyclonal	MBL	598
For immunostaining			
Nox2	Rabbit polyclonal	Abcam	ab80508
HA (3F10)	Rat monoclonal	Roche Diagnostics GmbH	11867423001
(Secondary antibodies)			
For immunoblotting			
Peroxidase AffiniPure goat anti-mouse IgG (H+L)	Goat polyclonal	Jackson ImmunoResearch Laboratoies	115-035-166
Peroxidase AffiniPure donkey anti-rabbit IgG (H+L)	Donkey Polyclonal	Jackson ImmunoResearch Laboratoies	711-035-152
For immunostaining			
Alexa Fluor 568 goat anti-rat IgG (H+L)	Goat polyclonal	Thermo Fisher Scientific	A11077
Alexa Fluor 488 goat anti-rabbit IgG (H+L)	Goat polyclonal	Thermo Fisher Scientific	A11034

Supplemental methods

Isolation of lung ILC2s

Lung ILC2s were isolated following the published protocol (1) with slight modification. In brief, ILC2-enriched fraction was purified by density gradient centrifugation using Percoll (GE Healthcare) from lung single cell suspensions pooled from 2-4 mice. ILC2-enriched fraction was first stained with biotinylated lineage marker antibodies (Biolegend, catalog 133307) which consist of anti-mouse TER-119/Erythroid Cells, anti-mouse CD11b, anti-mouse Ly-6G/Ly-6C (Gr-1), anti-mouse CD3e and anti-mouse CD45R/B220. Then, cells positive for lineages markers above were removed using streptavidin-conjugated microbeads (IMag Streptavidin Particles, BD Pharmingen, catalog 557812), and the remaining cells were stained with FITC-conjugated streptavidin, anti-mouse NK1.1, anti-mouse FceRI, and anti-mouse CD11c, APC-Cy7-conjugated-CD45.2, APC-conjugated anti-mouse CD90.2, PE-Cy7-conjugated KLRG1, and PE-conjugated anti-mouse ST2 mAbs. FITC-negative $CD45.2^{+}CD90.2^{+}KLRG1^{+}$ cells were sorted as lung ILC2, and were cultured at 2,000 cells/well in 200 ul of culture media with the indicated cytokines in a 96-well plate for 3 days.

Culture of mouse splenic $CD4^{+}$ T cells and $CD11c^{+}$ DCs

Purified $CD11c^{+}$ DCs (0.75×10^5 cells) and $CD4^{+}$ T cells (3×10^5 cells) were co-cultured with 0.1 or 1 mg/ml of OVA in 150 μl of complete RPMI1640 media per well in a 96 well plate for 3 days and supernatant was collected for cytokine analysis by ELISA.

Supplemental references

1. Moro K, Ealey KN, Kabata H, Koyasu S. Isolation and analysis of group 2 innate lymphoid cells in mice. *Nat Protoc.* 2015;10(5):792-806.