Mizoguchi et al.:

A Novel Coculture System for Assessing Respiratory Sensitizing Potential by IL-4 in T Cells

Supplementary Data

Tab. S1: Properties of chemical sensitizers used in this study

Sensitizers	Catalog number	Purity (%)	Molecular weight	Specific gravity	Concentration (mM) of 1% solution
OXA	E0753	≥ 90	217.22		46.0
FA	F8775	36.5-38	30.03	1.09	333.0
DNCB	138630	97	202.55		49.4
OPA	P1378	≥ 97	134.13		74.5
HDI	H0324	> 98	168.20	1.05	59.5
TMA	B4600	97	192.13		52.0

Sensitizers OXA, FA, DNCB, OPA, and TMA were purchased from Sigma-Aldrich. HDI was purchased from Tokyo Chemical Industry Co. Ltd. The above information was obtained from individual data sheets of Sigma-Aldrich and Tokyo Chemical Industry.

Tab. S2: Immature DCs and naive $CD4^+$ T cells were obtained from seven different donors (donor A – G) and used in each experiment, and CD14-ML cell lines were established from three different donors (donor A – C) and used in Fig. 3 – Fig. 4 and Supplementary Fig. 4 – Fig. 7

	DC	$CD4^+$T		DC	$CD4^+$T		DC	$CD4^+$T
Fig. S2B Exp. 1	G	A	Fig. S2B Exp. 2	A	B	Fig. S2B Exp. 3	D	A
Fig. S2C Exp. 1	A	D	Fig. S2C Exp. 2	A	F	Fig. S2C EXP. 3	B	A
Fig. S2D Exp. 1	D	A	Fig. S2D Exp. 2	D	A	Fig. S2D Exp. 3	B	A
Fig. S5B Exp. 1	A		Fig. S5B Exp. 2	B		Fig. S5B Exp. 3	A	
Fig. S5C Exp. 1	C		Fig. S5C Exp. 2	C		Fig. S5C Exp. 3	C	
Fig. S5D Exp. 1	A		Fig. S5D Exp. 2	C		Fig. S5D Exp. 3	A	
Fig. S7B Exp. 1	A	B	Fig. S7B Exp. 2	B	A	Fig. S7B Exp. 3	B	F
Fig. S7C Exp. 1	A	G	Fig. S7C Exp. 2	C	A	Fig. S7C Exp. 3	C	A
Fig. S7D Exp. 1	A	F	Fig. S7D Exp. 2	C	A	Fig. S7D Exp. 3	A	G

	DC	$CD4^+$T
Fig. S1B	A	E
Fig. S1C	A	D
Fig. S3B	A	D
Fig. S4B	A	
Fig. S4C	A	
Fig. S4D	B	
Fig. S6B	B	
Fig. S6C	A	
Fig. S6D	C	

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Tab. S3: Primers used in this study

Name	Direction	Sequence 5' to 3'
CD69	forward	CCTGTGTGCTGTAATGAATGTGGTC
	reverse	GGCTGTCTGATGGCATTGAGAA
IFN-γ	forward	CTTTAAAGATGACCAGAGCATCCAA
	reverse	GGCGACAGTTCAGCCATCAC
IL-4	forward	CTGTGCACCGAGTTGACCGTA
	reverse	AGCTGCTTGTGCCTGTGGAA
c-Fos	forward	AAAGCATCCATGTGTGGACTCAA
	reverse	AGGCCTGGCTCAACATGCTA
T-bet	forward	CCGTGACTGCCTACCAGAATG
	reverse	AACAGGATACTGGTTGGGTAGGA
GATA-3	forward	ACCACAACCACACTCTGGAGGA
	reverse	TCGGTTTCTGGTCTGGATGCCT
IL-2	forward	CCCAGGGACTTAATCAGCAATATCA
	reverse	GGTTGCTGTCTCATCAGCATATTCA
CD86	forward	CTGTAACTCCAGCTCTGCTCCGTA
	reverse	GCCCATAAGTGTGCTCTGAAGTGA
CD80	forward	ATTATAAAGGCCAGCGCCAGAAC
	reverse	GGACAAATTCTACTTCCAGCAGCAC
OX40L	forward	CAGTGCACATGCAGGCCTAAGTA
	reverse	GAAATATCCCTGTGTGGTTGCAGA
TSLPR	forward	GGTGACGTGTTCTGACCTGTCCTA
	reverse	TTCTCGGCATCCAAGCCTTC
IL-7Rα	forward	ATCGCAGCACTCACTGACCTGT
	reverse	TCAGGCACTTTACCTCCACGAG
IL-17RB	forward	ACAAACGCGAGCTTCAGTGGTG
	reverse	ATGCAGTCGCTGCCACAAGTAG
ST2	forward	CTCTGTTTCCAGTAATCGGAGCC
	reverse	GCAGCCAAGAACTGAGTGCCTT
HPRT	forward	GGCAGTATAATCCAAAGATGGTCAA
	reverse	GTCAAGGGCATATCCTACAACAAAC

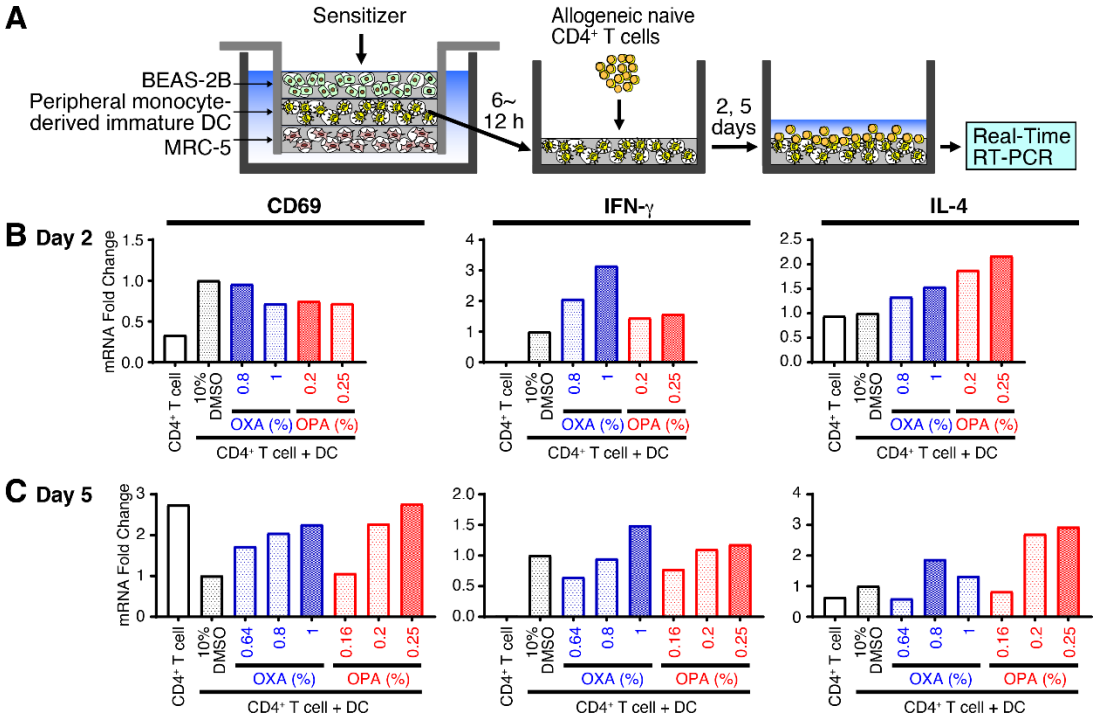
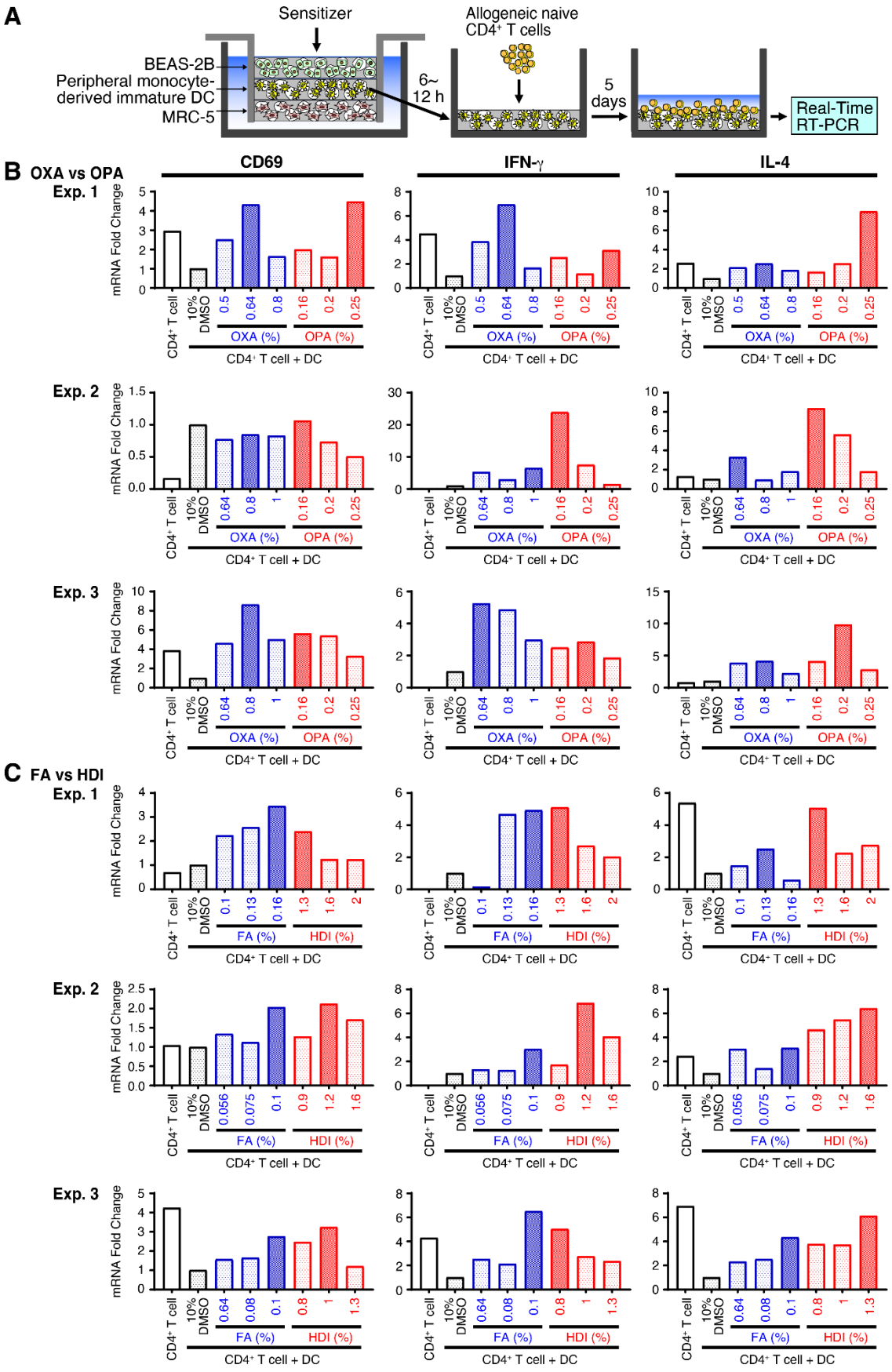


Fig. S1: Early mRNA upregulation of IFN-γ by OXA and late mRNA upregulation of IL-4 by OPA in the DC/T cell coculture system with peripheral monocyte-derived immature DCs and allogeneic naïve CD4⁺ T cells

Known skin and respiratory chemical sensitizers, OXA and OPA, were applied to the DC/T cell coculture system with peripheral monocyte-derived immature DCs and allogeneic naïve CD4⁺ T cells (A). The concentration of DMSO in the aliquot was 10% when sensitizers were added onto the top of the scaffold, and the same DMSO concentrations were used in the control. After incubation for 2 (B) and 5 (C) days, total RNA was prepared from CD4⁺ T cells stimulated with chemical sensitizer-treated DCs and subjected to real-time RT-PCR analysis to examine the expression of CD69, IFN-γ, and IL-4 together with HPRT. Each mRNA expression was normalized to HPRT mRNA expression and relative mRNA fold change to control vehicle (DMSO solution) was calculated in each concentration. The column showing the highest relative mRNA fold change for each chemical was filled in blue or red. Similar results were obtained in two independent experiments.


A
Sensitizer
Allogeneic naive CD4+ T cells
BEAS-2B
Peripheral monocyte-derived immature DC
MRC-5
6~12 h
5 days
Real-Time RT-PCR
B OXA vs OPA
CD69
IFN-γ
IL-4
Exp. 1
Exp. 2
Exp. 3
mRNA Fold Change
CD4+ T cell
10% DMSO
OXA (%)
OPA (%)
CD4+ T cell + DC
C FA vs HDI
FA (%)
HDI (%)

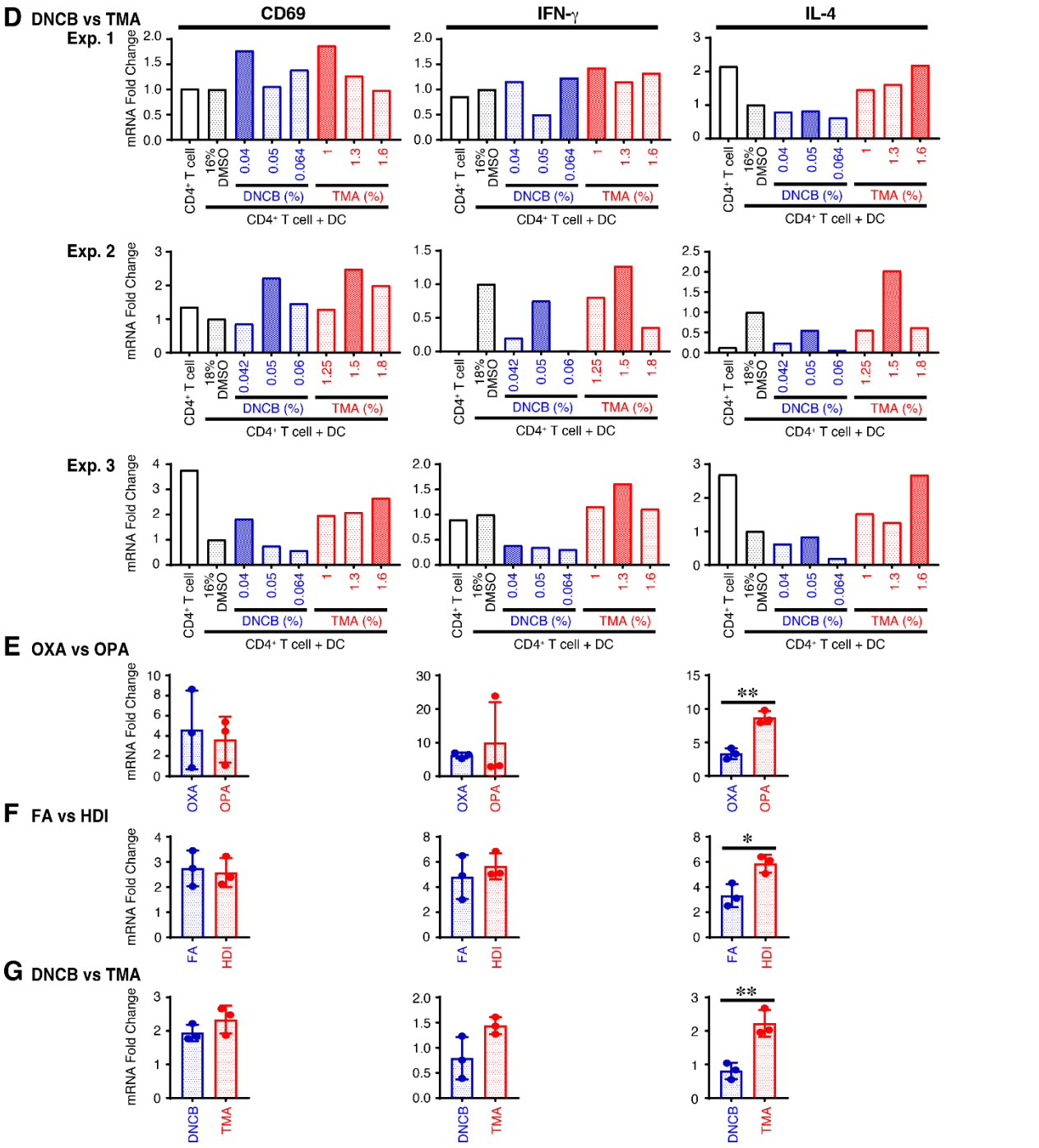


Fig. S2: Selective mRNA upregulation of IL-4 by three typical respiratory sensitizers in the DC/T coculture system with peripheral monocyte-derived immature DCs and allogeneic naïve CD4⁺ T cells

Three independent experiments corresponding to Fig. 2 are shown. Three sets of typical skin and respiratory chemical sensitizers, OXA and OPA (B), FA and HDI (C), and DNCB and TMA (D) were applied to the DC/T cell coculture system with peripheral monocyte-derived immature DCs and allogeneic naïve CD4⁺ T cells (A). The concentration of DMSO in the aliquot was 10% (OVA, OPA, FA, and HDI) or 16% or 18% (DNCB, and TMA) when sensitizers were added onto the top of the scaffold, and the same DMSO concentrations were used in the control. After incubation for 5 days, total RNA was extracted from CD4⁺ T cells stimulated with chemical sensitizer-treated DCs and subjected to real-time RT-PCR analysis to examine the expression of CD69, IFN-γ, and IL-4 normalized to HPRT. Relative mRNA fold change to control vehicle (DMSO solution) was calculated in each concentration of chemicals. The column showing the highest relative mRNA fold change for each chemical was filled in blue or red. The difference in the highest relative mRNA fold change to control vehicle (DMSO solution) between skin and respiratory sensitizers in each pair set was statistically analyzed using an unpaired two-tailed Student's *t*-test (E-G). Data are shown as the mean ± SD of three independent experiments. **P* < 0.05; ***P* < 0.01.

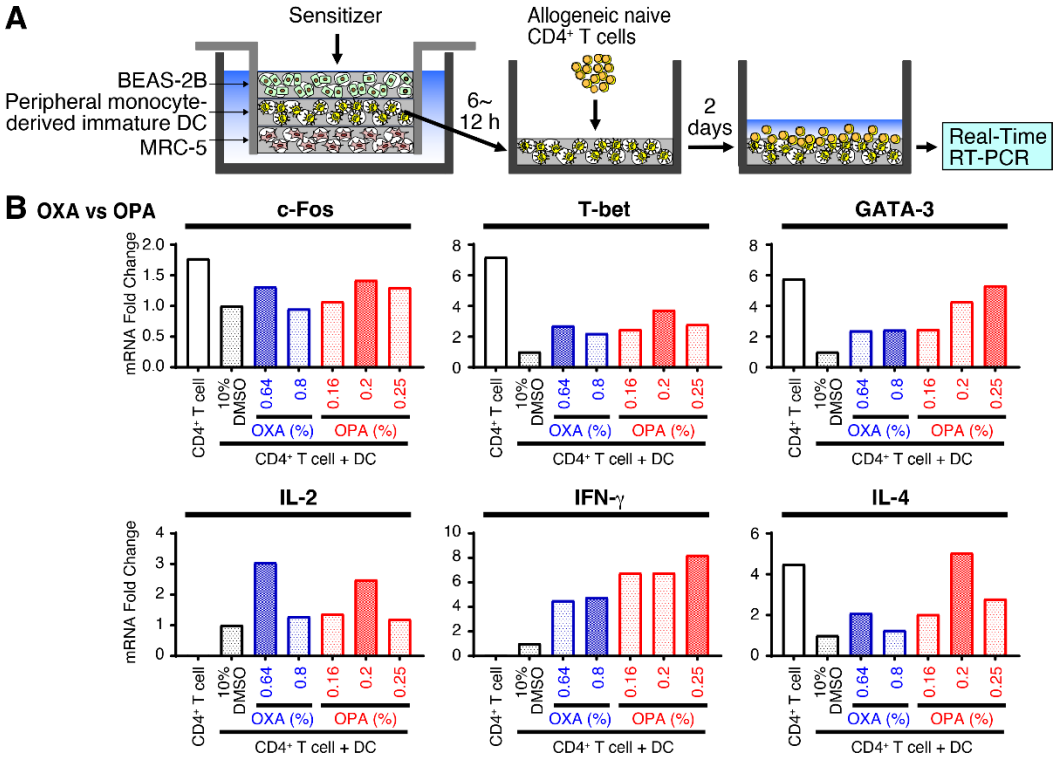


Fig. S3: Selective mRNA upregulation of GATA-3 by OPA in the DC/T cell coculture system with peripheral monocyte-derived immature DCs and allogeneic naïve CD4⁺ T cells

Typical skin and respiratory chemical sensitizers, OXA and OPA, were applied to the DC/T cell coculture system with peripheral monocyte-derived immature DCs and allogeneic naïve CD4⁺ T cells (A). The concentration of DMSO in the aliquot was 10% when sensitizers were added onto the top of the scaffold, and the same DMSO concentrations were used in the control. After incubation for 2 days, total RNA was extracted from CD4⁺ T cells stimulated with chemical sensitizer-treated DCs and subjected to real-time RT-PCR analysis to examine expression of the transcription factors c-Fos, T-bet, and GATA-3 together with their respective target cytokines IL-2, IFN-γ, and IL-4, respectively, and HPRT (B). mRNA expression was normalized to HPRT mRNA expression and relative mRNA fold change to control vehicle (DMSO solution) was calculated. The column showing the highest relative mRNA fold change for each chemical was filled in blue or red. Similar results were obtained in two independent experiments.

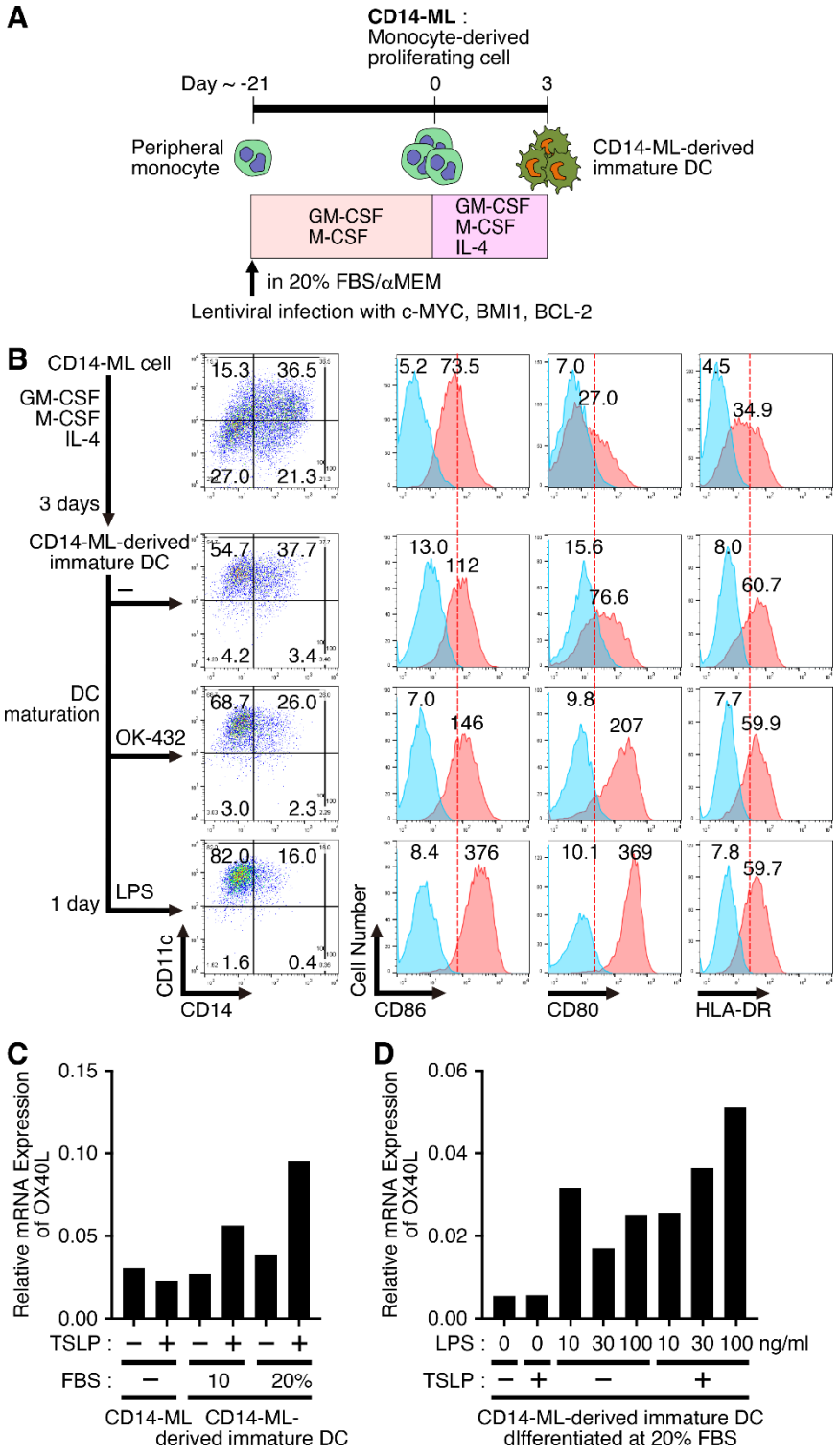
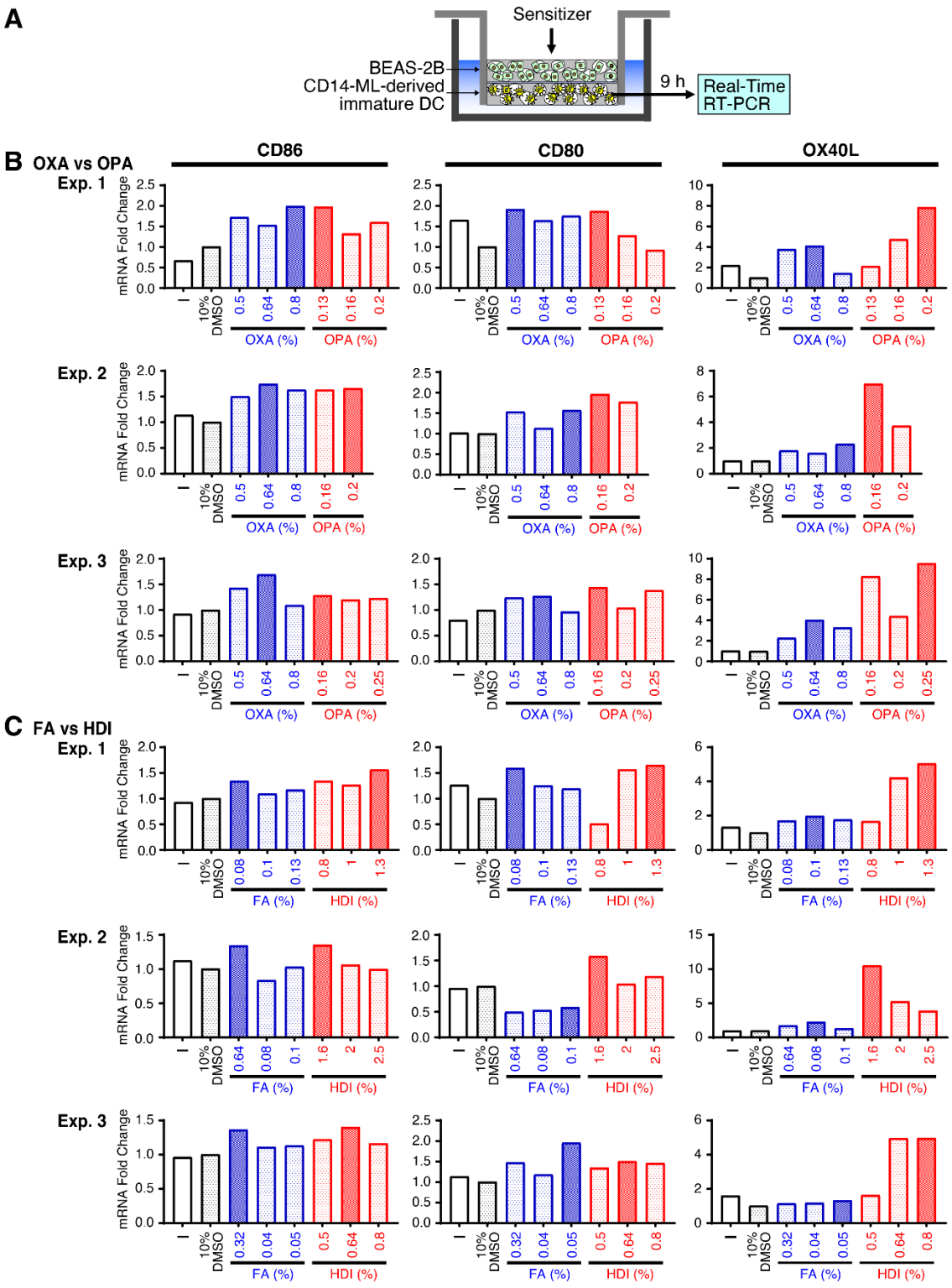


Fig. S4: Cell surface expression of CD14, CD11c, CD86, CD80, and HLA-DR and mRNA expression of OX40L in CD14-ML cell lines after differentiation into DCs and their maturation

CD14-ML cell lines were established by infection of peripheral CD14$^+$ monocytes with lentivirus expressing c-MYC, BIM1, and BCL-2 and then cultured in αMEM medium containing GM-CSF and M-CSF (A). Outgrowing CD14-ML cells were further differentiated into immature DCs by GM-CSF, M-CSF, and IL-4 and then stimulated with OK-432 (5 μg/mL) or LPS (100 ng/mL) for 1 day to induce DC maturation followed by cell surface analysis with flow cytometry (B). Differentiation of CD14-ML cells to immature DCs was performed in the presence of 10% or 20% FBS and then stimulated with and without TSLP (100 ng/mL) in the presence or absence of varying concentrations of LPS as indicated (C,D). mRNA expression of OX40L and HPRT was determined by real-time RT-PCR analysis. OX40L mRNA expression was normalized to HPRT mRNA expression and relative mRNA expression of OX40L are shown. Similar results were obtained in two independent experiments.


A
Sensitizer
BEAS-2B
CD14-ML-derived
immature DC
9 h
Real-Time
RT-PCR
B OXA vs OPA
CD86
CD80
OX40L
Exp. 1
Exp. 2
Exp. 3
mRNA Fold Change
–
10% DMSO
OXA (%)
OPA (%)
C FA vs HDI
FA (%)
HDI (%)

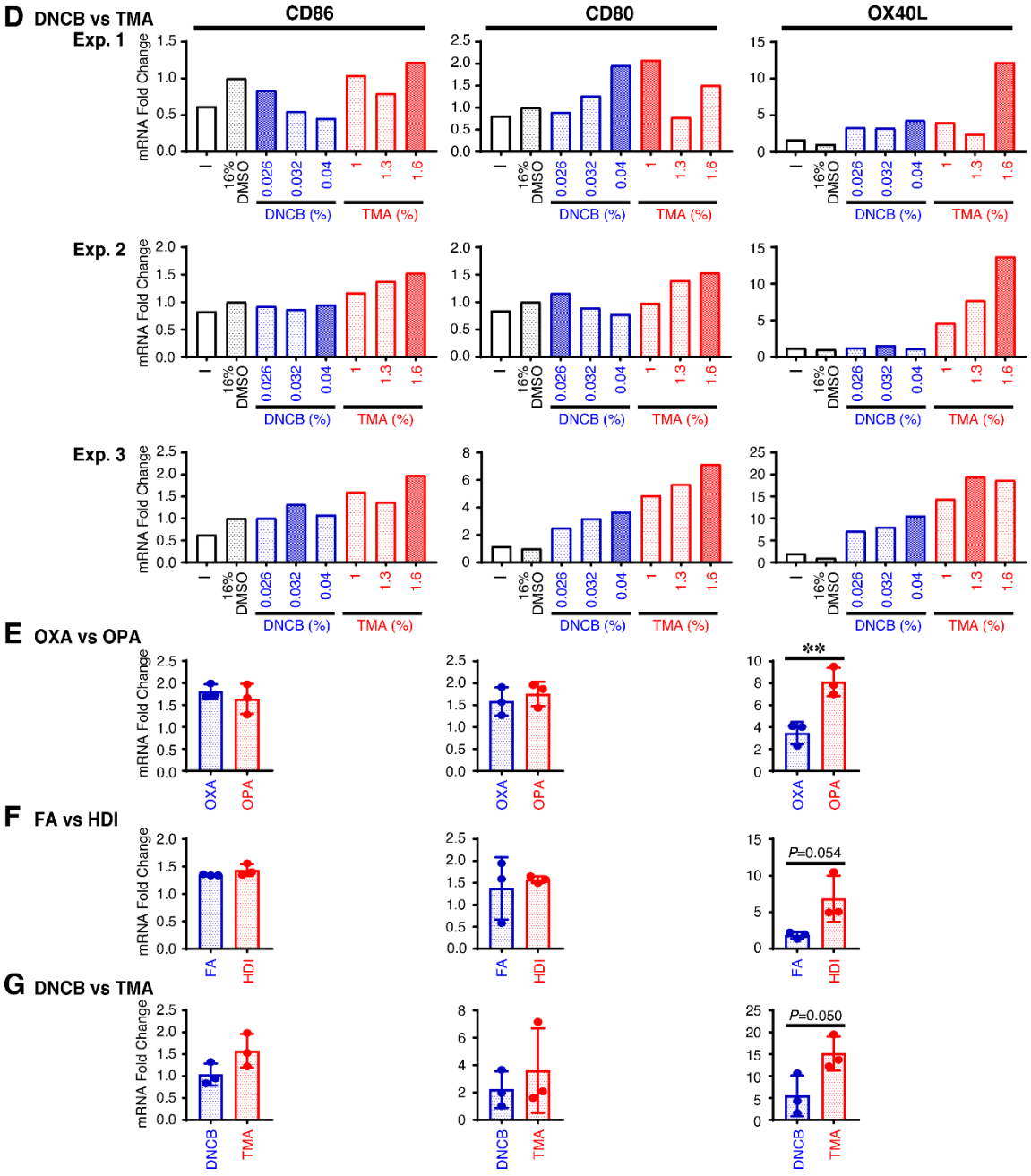


Fig. S5: Selective mRNA upregulation of OX40L by three typical respiratory sensitizers in the DC coculture system with CD14-ML-derived immature DCs

Three independent experiments corresponding to Fig. 3 are shown. Three sets of typical skin and respiratory sensitizers, OXA and OPA (B), FA and HDI (C), and DNCB and TMA (D) were applied to the DC coculture system with CD14-ML-derived immature DCs (A). The concentration of DMSO in the aliquot was 10% (OVA, OPA, FA, and HDI) or 16% (DNCB, and TMA) when sensitizers were added onto the top of the scaffold, and the same DMSO concentrations were used in the control. After incubation for 9 h, total RNA was extracted from the DC scaffold stimulated with chemical sensitizers and subjected to real-time RT-PCR analysis to examine the expression of CD86, CD80, and OX40L together with HPRT. Each mRNA expression was normalized to HPRT mRNA expression and relative mRNA fold change to control vehicle (DMSO solution) was calculated in each concentration of chemicals. The column showing the highest relative mRNA fold change for each chemical was filled in blue or red. The difference in the highest relative mRNA fold change to control vehicle (DMSO solution) between skin and respiratory sensitizers in each pair set was statistically analyzed using the unpaired two-tailed Student's *t*-test (E-G). Data are shown as the mean ± SD of three independent experiments. $^{**}P < 0.01$.

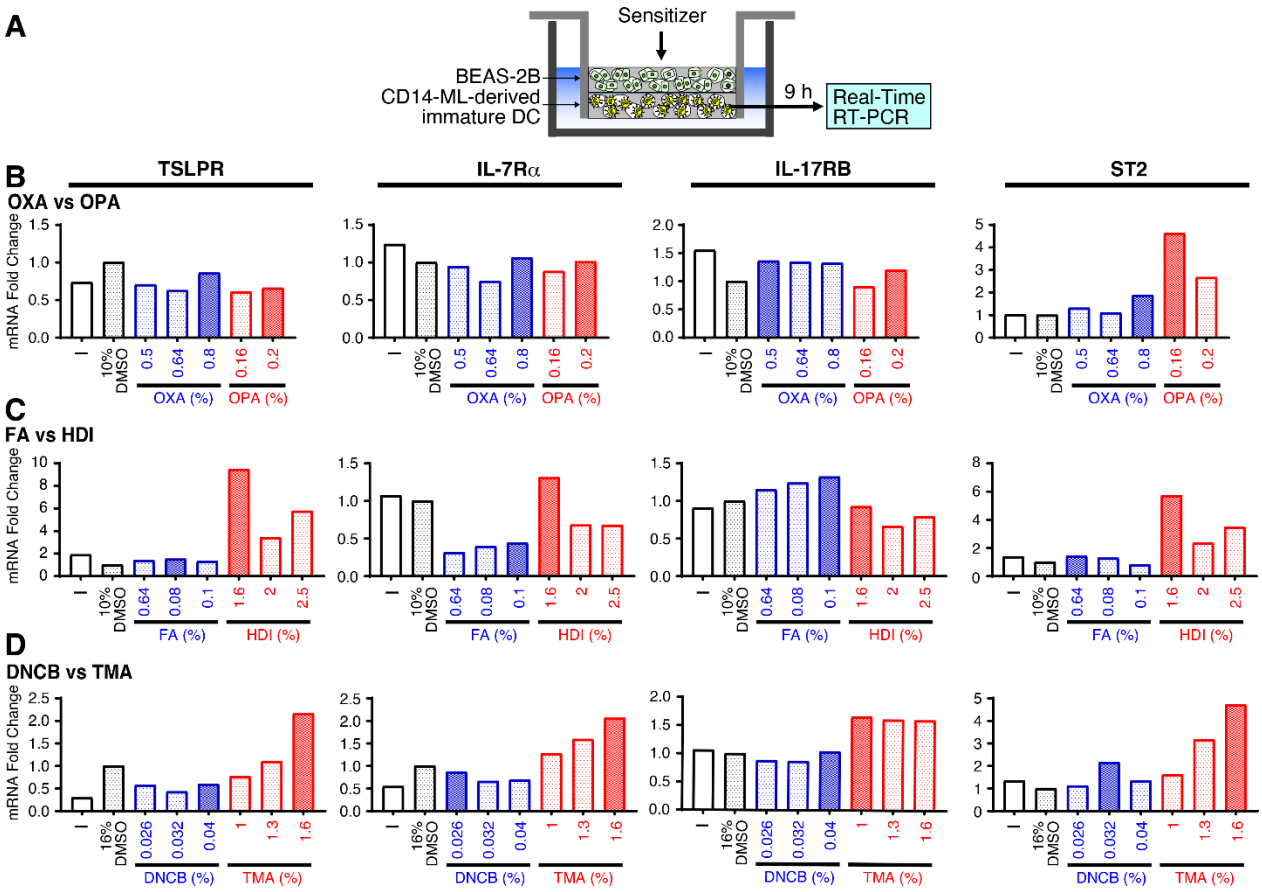
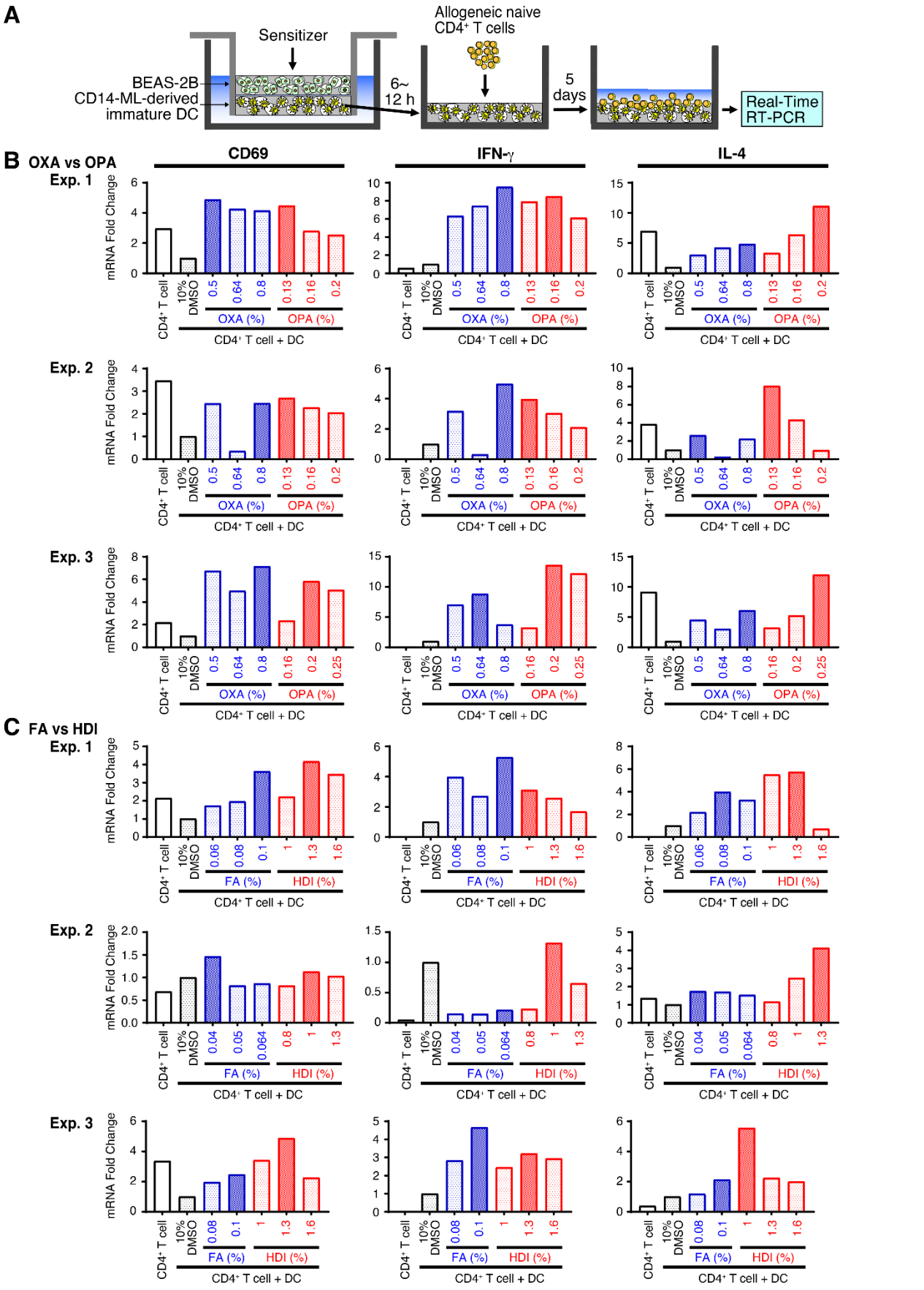


Fig. S6: Sensitizer-dependent selective mRNA upregulation of TSLPR, IL-7Rα, and ST2 by three typical respiratory sensitizers in the DC coculture system with CD14-ML-derived immature DCs

Three sets of typical skin and respiratory sensitizers, OXA and OPA (B), FA and HDI (C), and DNCB and TMA (D) were applied to the DC co-culture system with CD14-ML-derived immature DCs (A). The concentration of DMSO in the aliquot was 10% (OVA, OPA, FA, and HDI) or 16% (DNCB, and TMA) when sensitizers were added onto the top of the scaffold, and the same DMSO concentrations were used in the control. After incubation for 9 h, total RNA was extracted from the DC scaffold stimulated with chemical sensitizers and subjected to real-time RT-PCR analysis to examine the expression of TSLPR, IL-7Rα, IL-17RB, and ST2 together with HPRT. Each mRNA expression was normalized to HPRT mRNA expression and relative mRNA fold change to control vehicle (DMSO solution) was calculated in each concentration of chemicals The column showing the highest relative mRNA fold change for each chemical was filled in blue or red. Similar results were obtained in two independent experiments.


A
Sensitizer
Allogeneic naive CD4+ T cells
BEAS-2B
CD14-ML-derived immature DC
6~12 h
5 days
Real-Time RT-PCR
B OXA vs OPA
CD69
IFN-γ
IL-4
Exp. 1
Exp. 2
Exp. 3
mRNA Fold Change
CD4+ T cell
10% DMSO
OXA (%)
OPA (%)
CD4+ T cell + DC
C FA vs HDI
FA (%)
HDI (%)

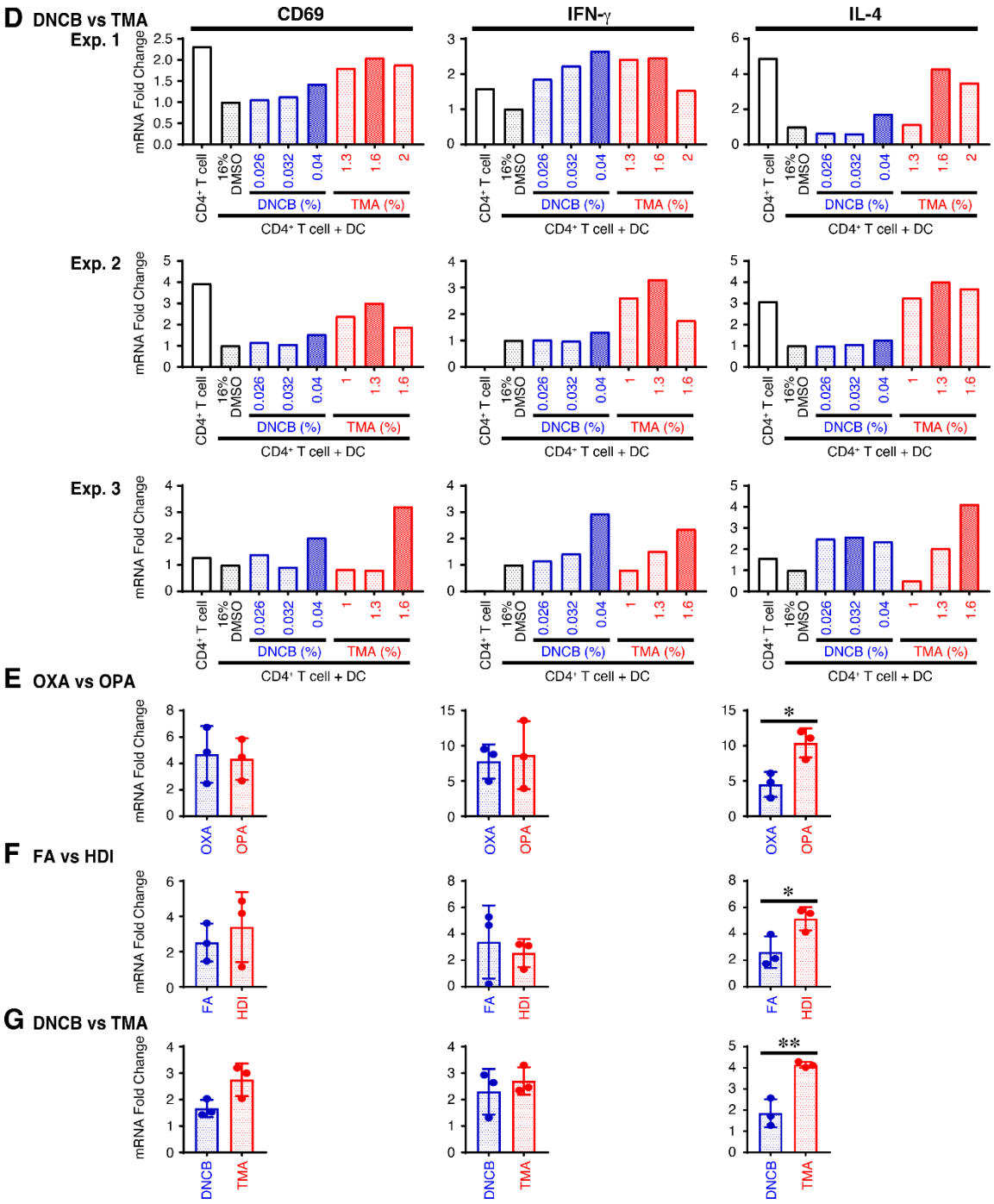


Fig. S7: Selective mRNA upregulation of IL-4 by three typical respiratory sensitizers in the DC/T coculture system with CD14-ML-derived immature DCs and allogeneic naïve CD4⁺ T cells

Three replicate experiments corresponding to Fig. 4 are shown. Three sets of typical skin and respiratory chemical sensitizers, OXA and OPA (B), FA and HDI (C), and DNCB and TMA (D) were applied to the DC/T cell coculture system with CD14-ML-derived immature DCs and allogeneic naïve CD4⁺ T cells (A). The concentration of DMSO in the aliquot was 10% (OVA, OPA, FA, and HDI) or 16% (DNCB, and TMA) when sensitizers were added onto the top of the scaffold, and the same DMSO concentrations were used in the control. After incubation for 5 days, total RNA was extracted from CD4⁺ T cells stimulated with chemical sensitizer-treated DCs and subjected to real-time RT-PCR analysis to examine the expression of CD69, IFN-γ, and IL-4 together with HPRT. Each mRNA expression was normalized to HPRT mRNA expression and relative mRNA fold change to control vehicle (DMSO solution) was calculated in each concentration of chemicals. The column showing the highest relative mRNA fold change for each chemical was filled in blue or red. The difference in the highest relative mRNA fold change to control vehicle (DMSO solution) between skin and respiratory sensitizers in each pair set was statistically analyzed using the unpaired two-tailed Student's *t*-test (E-G). Data are shown as the mean ± SD of three independent experiments. **P* < 0.05; ***P* < 0.01.