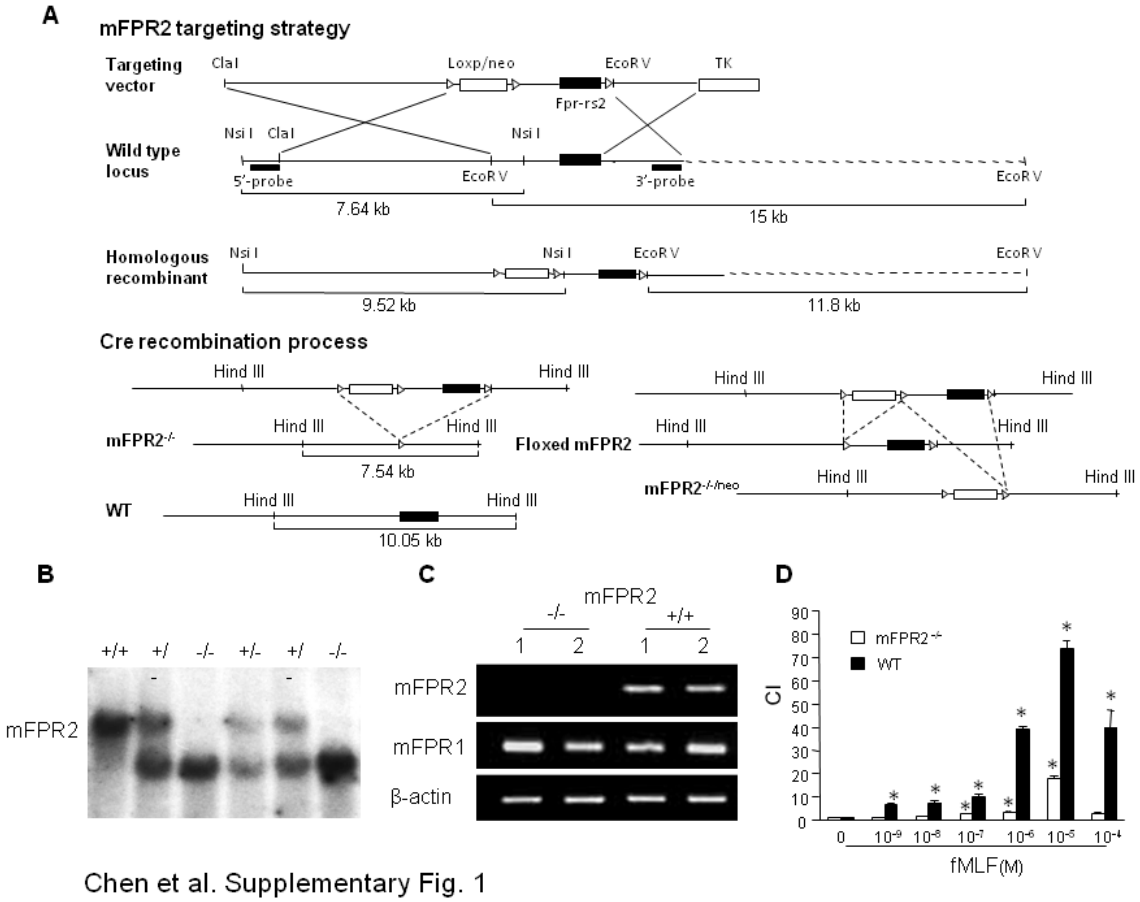
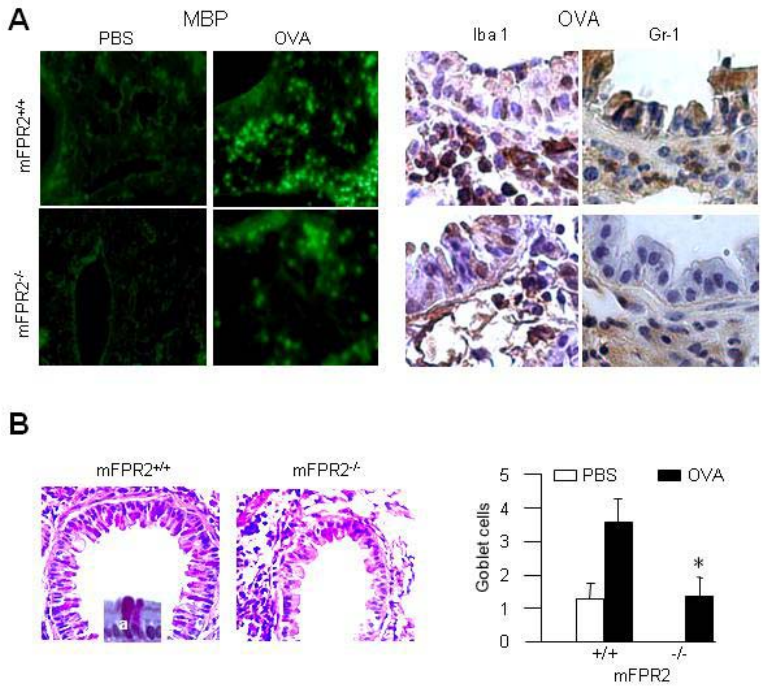


Chen et al. Supplementary Fig. 1

Figure S1. Generation of mFPR2-deficient mice. A: Cre-loxp strategy was used to deplete mouse (m) FPR2 gene (*Fpr-rs2*). Mouse *Fpr-rs2* targeting vector was constructed by flanking *Fpr-rs2* with a floxed *neo* cassette and a loxP site (triangles), and introduced into 129/Svj ES cells by electroporation. Correct homologous recombinants were screened by Southern hybridization with an internal (3'-) and an external (5'-) probe. Schematic representation of the Cre recombination processes. Homozygous *EIIaCre* mice were crossed with homozygous $FPR2^{neoloxp}$ mice to obtain mice with mosaic Cre-ecombination patterns, which were mated with wild type C57BL/6 mice and screened for offspring that inherited an allele with either neo excision (Floxed mFPR2) or total excision (both neo and *Fpr-rs2*) ($mFPR2^{+/-}$). $mFPR2^{+/-}$ mice thus obtained were backcrossed with wild type C57BL/6 mice for 5 generations before being used in the study. neo: neomycin resistance cassette; TK, thymidine kinase cassette. **B:** Mouse *Fpr2* gene was identified by Southern blot of genomic DNA after Hind III digestion. $mFPR2^{+/+}$ (WT) mouse DNA shows one

band at 10.1 Kb. Mouse DNA with disrupted mFPR2 shows a band at 7.5 Kb, while mFPR2$^{+/-}$ mouse DNA shows both 10 and 7.5 Kb bands. **C:** For detection of mFPR2 mRNA, total RNA isolated from BMCs was utilized for RT-PCR. The expression of mFPR1 (Primers: sense: 5'-CAT GAA CAA GTC TGC AGT GAACCT- 3'; antisense: 5'-AGG TTT ATG TCT ATT ACA GTA TAT-3') and β-actin mRNA was used as a control. **D:** Migration of bone marrow cells (BMCs) from WT and mFPR2$^{-/-}$ littermates in response to the mFPR1 and mFPR2 agonist fMLF. The results were expressed as CI representing fold increase in cell migration in response to fMLF over the base-line migration (to medium). * indicates significantly increased BMC migration in response to fMLF compared to baseline migration (to medium) ($P < 0.01$)**.** All mice used were 8 week female littermates.



Chen et al. Supplementary Fig. 2

Figure S2. Inflammatory cell infiltration and PAS positive goblet cells in the lung.

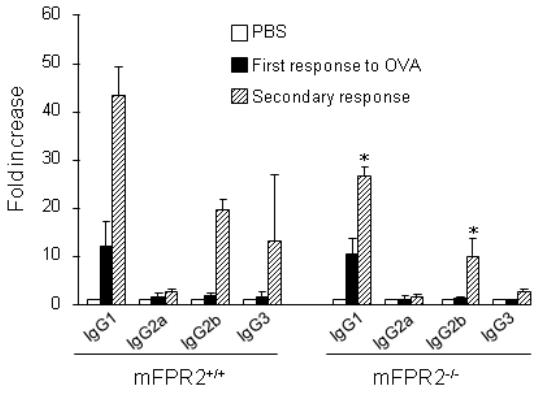
A: Immunofluorescence staining showing MBP^+ eosinophils (Green), immunohistochemical staining showing lba 1^+ macrophages and Gr-1^+ neutrophils in lung tissues of mice after OVA/Alum immunization and aerosol OVA challenge. **B.** PAS staining of airway epithelia to show goblet cells, 400 ×; Inset a: an amplified PAS cell pasitive bronchial region of OVA-treated WT mouse, 1000 ×. PAS positive goblet cell proliferation were scored and * indicates significantly reduced severity in $mFPR2^{-/-}$ mice as compared with WT littermates ($p < 0.01$)**.** All mice used were female littermates at the age of 8 weeks.

A

Mean±SEM

	WT			mFPR2$^{-/-}$		
	Naïve (Pg/ml)	OVA (Pg/ml)	Fold increase	Naïve (Pg/ml)	OVA (Pg/ml)	Fold increase
IL-4	17.78±0.53	77.52±4.17	4.36±0.23	18.30±0.62	60.26±5.18	3.29±0.26*
IL-5	28.41±2.08	931.54±59.9	32.79±2.11	75.61±5.26	1099.91±42.69	14.48±0.55*
IL-13	736.91±63.39	1204.77±75.68	1.64±0.10	1202.64±55.77	1115.88±70.34	0.93±0.05*
IFN-γ	4027.46±336.13	4059.89±393.76	1.04±0.05	4063.52±388.69	4180.02±296.83	1.03±0.02

B



Chen et al. Supplementary Fig. 3

Figure S3. Cytokine production by slenocytes and immunoglobulins in mouse sera.

A: Mice were immunized with injection of OVA/Alum by I.P. on day 1 and 10. Twenty four hours after the second OVA/Alum I.P. injection, splenocytes suspended at 5×10^6 cells/ml, were incubated in vitro with 200 μg/ml OVA in RPMI 1640 with 10% FCS at 37°C for 4 days. The supernatants were collected to measure the levels of IL-4, IL-5, IL-13 and IFN-γ. * indicates significantly reduced production of cytokines by splenocytes from mFPR2$^{-/-}$ mice as compared with WT littermates ($P < 0.05$). **B:** Mice were I.P. immunized with OVA on day 1 and 14. The sera were collected on day 10 and day 21 for measurement of primary (First) and secondary anti-OVA immunoglobulins. * indicates significantly reduced Ig levels in the sera of mFPR2$^{-/-}$mice as compared to WT littermates ($P < 0.05$). All mice used were 8 week old female littermates.

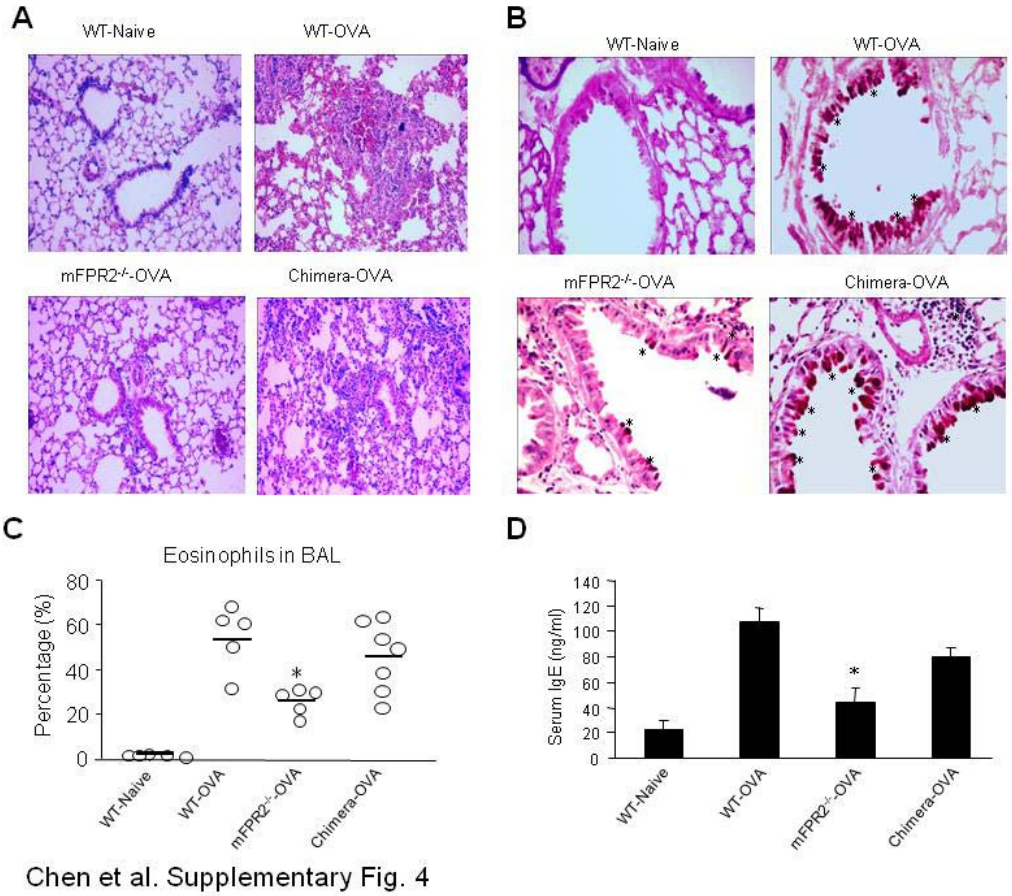
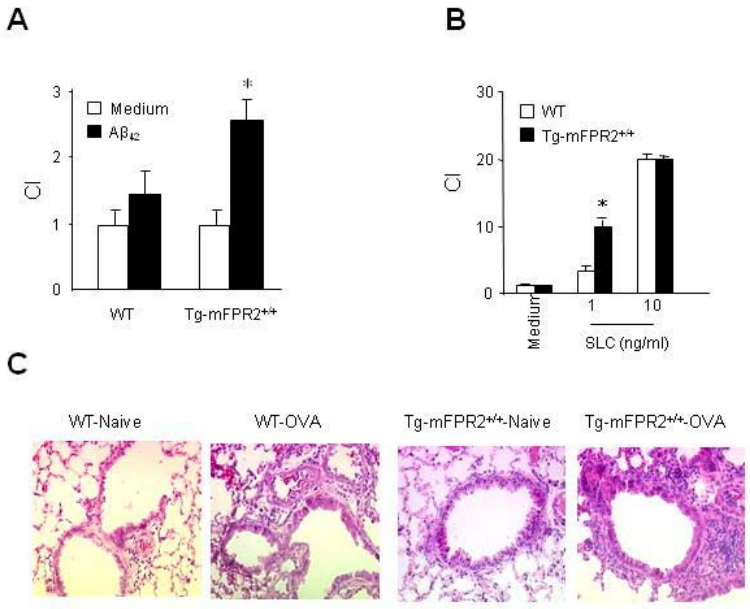
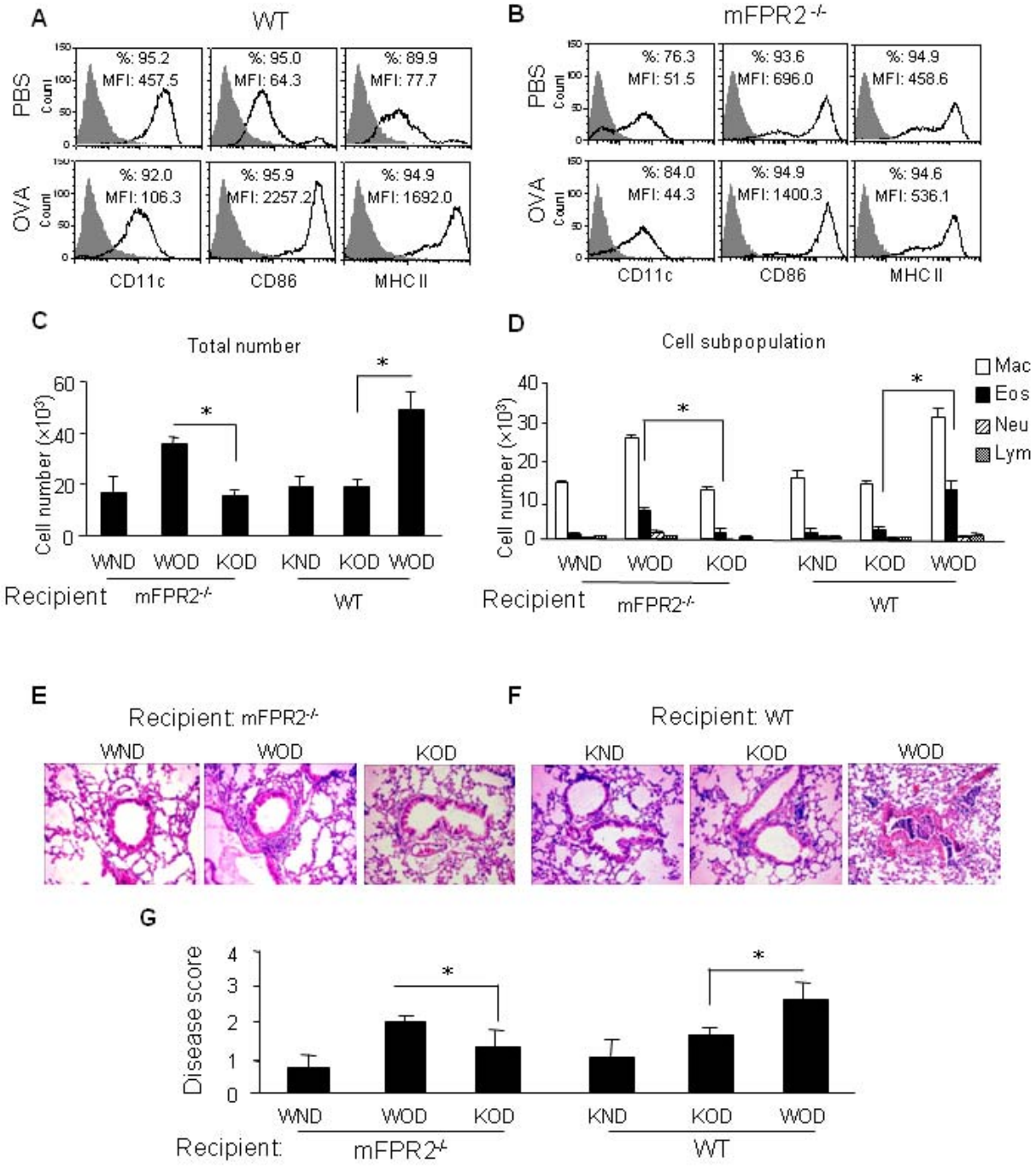


Figure S4. Substantial restoration of airway inflammation and immune responses after OVA/Alum immunization and OVA challenge in mFPR2$^{-/-}$ mice transplanted with WT bone marrow (Chimera). A: Substantial restoration of airway leukocyte infiltration (× 200, H&E) and **B:** Substantial restoration of airway mucus production by goblet cells in Chimera mice (× 400, PAS). * indicate the PAS^{+} cells. **C:** Restoration of eosinophil infiltration in BAL and **D:** Total serum IgE level in Chimera mice. * indicates significantly reduced BAL eosinophils **(C)** or serum IgE level **(D)** in mFPR2$^{-/-}$ mice as compared with WT littermates and Chimera mice ($p < 0.05$)**.**



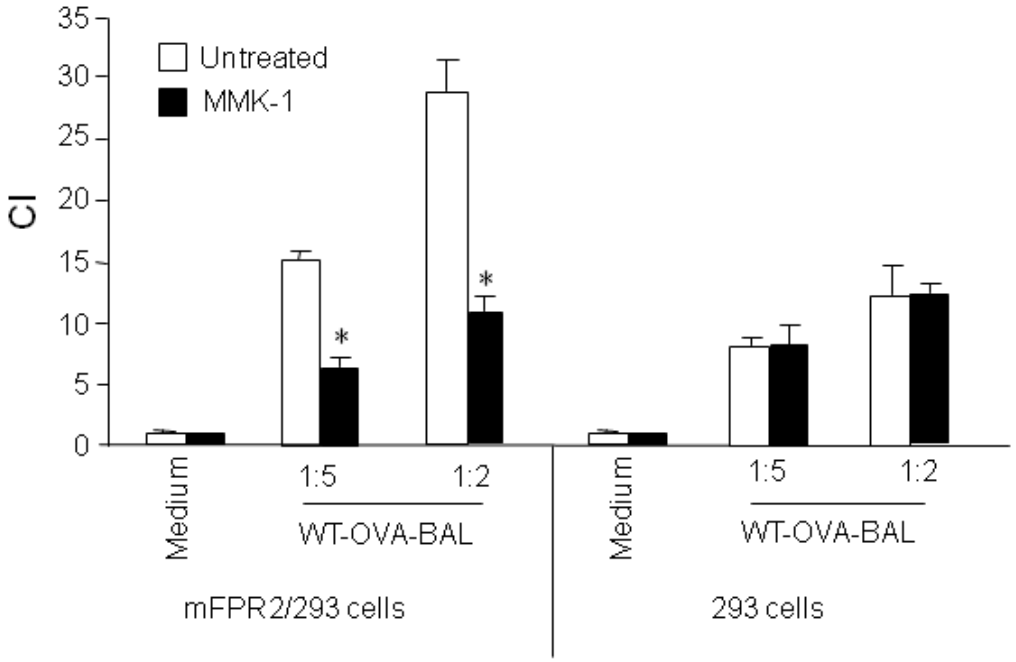
Chen et al. Supplementary Fig. 5

Figure S5. Increased airway inflammation induced by OVA immunization and challenge in mFPR2 transgenic (Tg-mFPR2$^{+/+}$) FVB mice. A: Migration of bone marrow cells (BMCs) from WT and Tg-mFPR2$^{+/+}$littermates in response to mFPR2 agonist Aβ_{42}. The results were expressed as CI representing fold increase in cell migration in response to the Aβ_{42} over the base-line migration (to medium). * indicates significantly increased migration of BMC from Tg-mFPR2$^{+/+}$ mice as compared to WT littermates ($P < 0.05$)**. B:** BM nucleated cells cultured in presence of GM-CSF and IL-4 for 6 days were stimulated by LPS for 24 h as mature dendritic cells (mDCs). The migration of mDCs from WT and Tg-mFPR2$^{+/+}$ littermates was examined in response to CCR7 agonists CCL21/SLC. The results were expressed as CI representing fold increase in cell migration in response to SLC over the base-line migration (to medium). * indicates significantly increased mDC migration from Tg-mFPR2$^{+/+}$ mice as compared to WT littermates ($P < 0.05$)**. C:** Increased severity of allergic airway inflammation in Tg-mFPR2$^{-/-}$ mice (× 200, H&E). All mice used were 8 week male littermates.



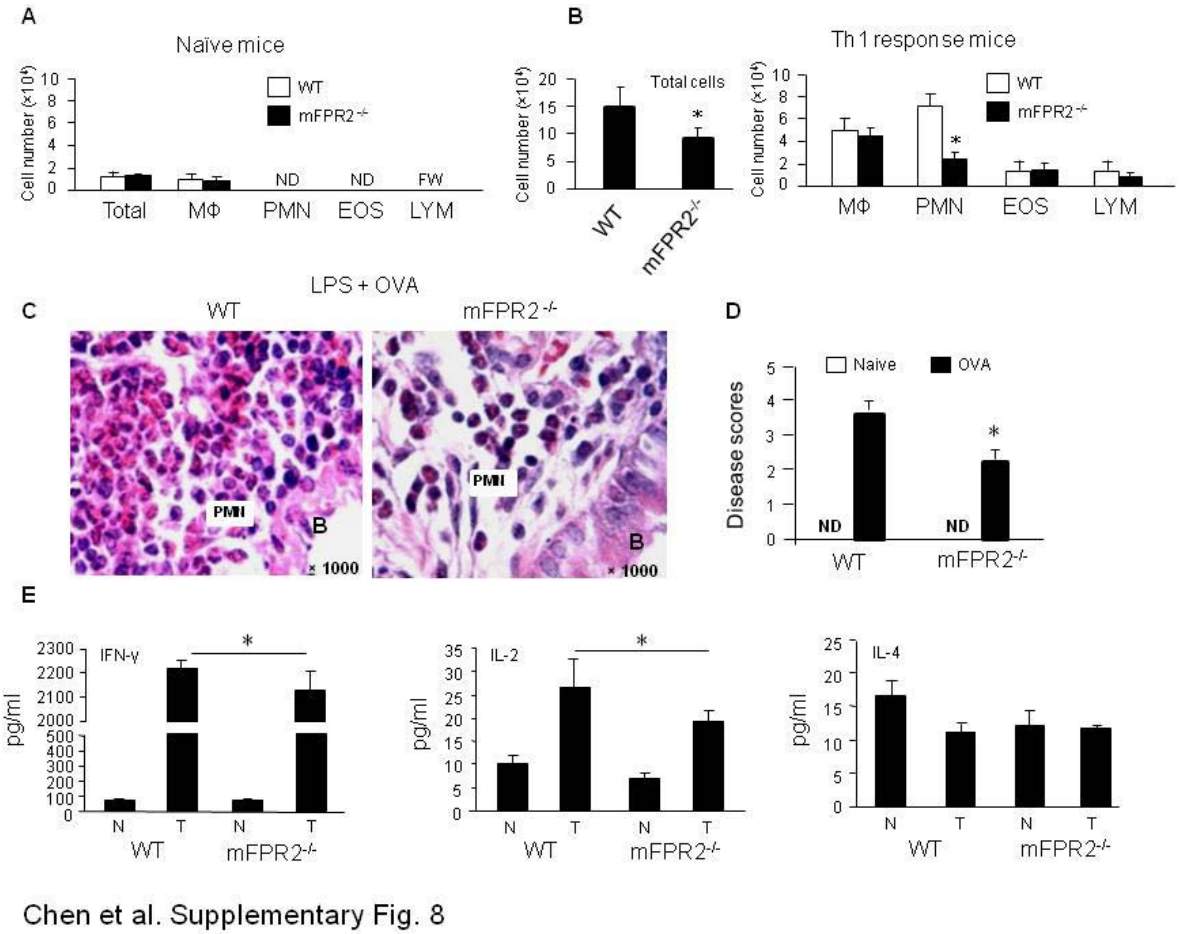
Chen et al. Supplementary Fig. 6

Figure S6. Substantial restoration of airway inflammation after transfer of OVA-pulsed WT DCs into mFPR2$^{-/-}$ mice. A, B: BM nucleated cells from WT and mFPR2$^{-/-}$ mice were cultured in presence of GM-CSF and IL-4 for 9 days, then were stimulated with OVA (200 μg/ml) or PBS for 48 hours. The expression of CD11c, CD86, and MHC II was analyzed by FACS. **C, D:** BM nucleated cells from WT and mFPR2$^{-/-}$ mice were cultured in presence of GM-CSF and IL-4 for 9 days, then were stimulated with OVA (200 μg/ml) (WT DC = WOD, mFPR2$^{-/-}$ DC = KOD) or PBS (WT DC = WND, mFPR2$^{-/-}$ DC = KND) for 48 hours. WODs were intranasally transferred into mFPR2$^{-/-}$ mice and WT mice (5 mice/each group), WNDs into mFPR2$^{-/-}$ mice (5 mice/group) as controls. KODs were also intranasally transferred into WT mice and mFPR2$^{-/-}$ mice (5 mice/each group) and KND into WT mice (5 mice/group) as controls. Ten days after DC transfer, animals were challenged with 100 μg OVA intranasally (i.n.) for 4 consecutive days. On day 15, mice were sacrificed and leukocytes exudating into BAL were determined. * indicates significantly increased total cells **(C)** and eosinophils **(D)** in BAL of mice received OVA pulsed DCs from WT mice (WOD) as compared to mice receiving OVA-pulsed DCs from mFPR2$^{-/-}$ mice ($P < 0.05$). **E, F:** After intranasal transfer of OVA-pulsed DCs and OVA challenge, the mice were sacrificed and lung tissues were harvested. Histology was performed to demonstrate infiltration of inflammatory cells in the perivascular and Peribronchial regions of the lung tissues (H&E, 400 ×). **G:** The severity of lung inflammation was scored and * indicates significantly increased severity in mice transferred with OVA-pulsed DCs from WT mice (WOD) as compared with the mice transferred with OVA-pulsed DCs from mFPR2$^{-/-}$ mice (KOD) ($p < 0.05$)**.** Transfer of DCs with or without OVA pulse from mFPR2$^{-/-}$ mice did not increase airway inflammation in WT or mFPR2$^{-/-}$ mice. Mice used were 8 week old females.



Chen et al. Supplementary Fig. 7

Figure S7. Desensitization of mFPR2/293 cell migration to BAL from WT mice with airway inflammation by defined mFPR2 agonist peptide MMK-1. Parental 293 and mFPR2/293 cells were pre-treated with a defined mFPR2 ligand MMK-1 at 10^{-5} M for 60 min at 37^{o}C. The cells were then examined for migration to BAL from WT mice with airway inflammation. The results were expressed as CI representing fold increase in cell migration in response to the BAL over the baseline migration (to medium). * indicates significantly reduced cell migration of mFPR2/293 cells pre-treated with MMK-1 as compared to untreated mFPR2/293 cells ($P < 0.05$). MMK-1 did not affect the migration of parental 293 cells to inflammatory BAL.



Chen et al. Supplementary Fig. 8

Figure S8. Reduction of a Th1 immune response in mFPR2$^{-/-}$ mice.

Mice were immunized with LPS (100 μg) and OVA (100 μg)/mouse intranasally on day 0, 1 and 2. On day 14, 15, 18 and 19, mice were challenged with OVA (25 ug/50 ul PBS/mouse) intranasally. On day 21, mice were euthanized and BAL and lung were harvested. **A:** Total and differential counts of leukocytes contained in the BAL from naïve mice. MΦ: macrophages, EOS: eosinophils, PMN: neutrophils, LYM: lymphocytes, ND: not detected, FW: few cells. **B:** Total and differential counts of leukocytes contained in the BAL from LPS/OVA treated mice. * indicates significantly reduced total cells and PMN in the BAL of mFPR2$^{-/-}$ mice as compared to WT mice ($P < 0.05$). **C:** Reduced PMN infiltration in the lung of mFPR2$^{-/-}$ mice as compared to WT mice. B: Bronchiol **D:** The severity of lung inflammation was scored and * indicates significantly reduced severity in mFPR2$^{-/-}$ mice ($p < 0.05$), ND: not detected**. E:** Mice were injected i.p. with 2 mg OVA and 25 μg *E. coli* LPS/mouse. Spleens were harvested 4 day later and 1×10^6 splenocytes were plated in

triplicate in 12-well plates in 1000 µl RPMI-1640 supplemented with 10% FBS in the presence of 200 µg/ml OVA for 4 days. The supernatants were then harvested and assayed for IFN-γ, IL-2 and IL-4 by ELISA. * indicates significantly reduced IFN-γ, IL-2 in the supernatants from mFPR2$^{-/-}$ mice as compared to WT mice. N: naïve, T: LPS/OVA treatment. Mice used were 8 week old females.