Prostaglandin E$_2$ promotes Th1 differentiation via synergistic amplification of IL-12 signaling by cAMP and PI3-Kinase

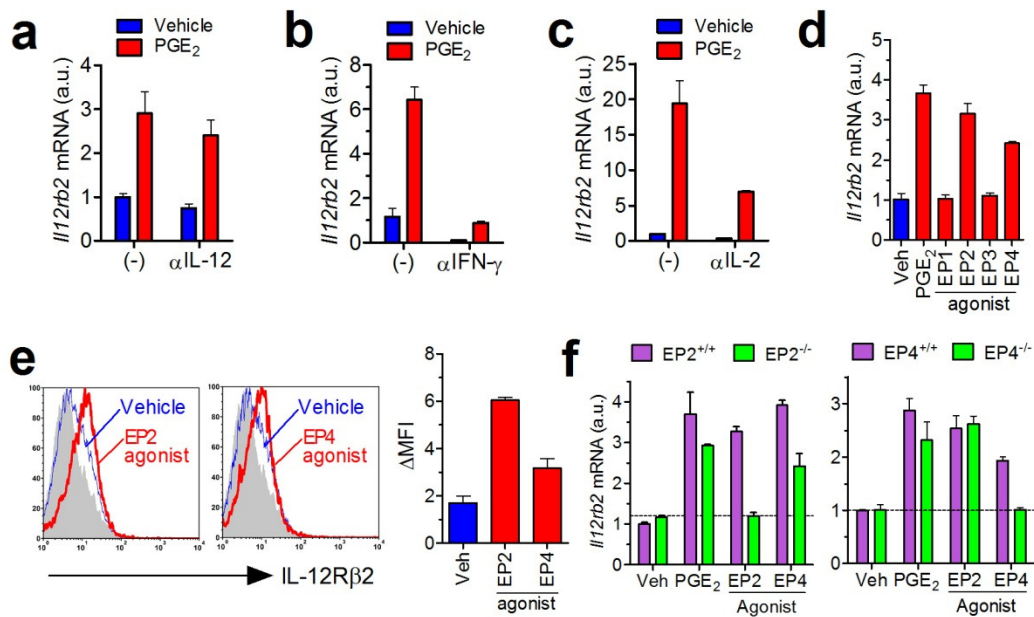Chengcan Yao, Takako Hirata, Kitipong Soontrapa, Xiaojun Ma, Hiroshi Takemori and

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Supplementary Figure S1. PGE$_2$–EP2/EP4 signaling induces IL-12Rβ2 expression in TCR-activated CD4$^+$ T cells. (**a,b**) Expression of *Il12rb2* mRNA in WT CD4$^+$ T cells activated for 24 h with anti-CD3 and anti-CD28 in the absence or presence of PGE$_2$ with or without anti-IL-12 (**a**) or anti-IFN-γ (**b**). (**c**) Expression of *Il12rb2* mRNA in IFN-γR1$^{-/-}$ CD4$^+$ T cells activated for 24 h with anti-CD3 and anti-CD28 in the absence or presence of PGE$_2$ with or without anti-IL-2. (**d**) Expression of *Il12rb2* mRNA in T cells activated with αCD3/CD28 in the absence or presence of PGE$_2$ or agonists selective to EP1 to EP4 for 24 h. (**e**) IL-12Rβ2 protein expression in T cells activated for 48 h with αCD3/CD28 in the absence or presence of PGE$_2$ or selective agonists to EP2 or EP4. (**f**) Expression of *Il12rb2* mRNA in WT, EP2$^{-/-}$ (left) and EP4$^{-/-}$ (right) T cells activated for 24 h with anti-CD3 and anti-CD28 in the absence or presence of PGE$_2$ or selective agonists to EP2 or EP4. Data shown as mean ± SEM are representative of two or more independent experiments with triplicates.

Supplementary Table S1: Primer sets used for real-time PCR.

Gene	Universal Probe Library, mouse*	Primer Sequence	
Cd69	#29	Forward	5'-AACGGAAAATAGCTCTTCACATCT-3'
		Reverse	5'-TGATGCTTCTCAAAATGTATACTGG-3'
Fosb	#71	Forward	5'-GTTCGCAGAGAGCGGAAC-3'
		Reverse	5'-GCCTTTTCCTCTTCAAGCTG-3'
Gapdh	/	Forward	5'-TGAACGGGAAGCTCACTGG-3'
		Reverse	5'-TCCACCACCCTGTTGCTGTA-3'
Icer	#4	Forward	5'-GCTGAGGCTGATGAAAAACA-3'
		Reverse	5'-GCCACACGATTTTCAAGACA-3'
Ifng	#21	Forward	5'-ATCTGGAGGAACTGGCAAAA-3'
		Reverse	5'-TTCAAGACTTCAAAGAGTCTGAGGTA -3'
Ifngr1	#69	Forward	5'-TCAAAAGAGTTCCTTATGTGCCTA-3'
		Reverse	5'-TACGAGGACGGAGAGCTGTT-3'
Il12rb2	#103	Forward	5'-GTGCCAGGATCCCTCTCTG -3'
		Reverse	5'-GAATGCAACTCTGGTTCTCCA -3'
Il2rb	#91	Forward	5'-AGCATGGGGGAGACCTTC-3'
		Reverse	5'-GGGGCTGAAGAAGGACAAG-3'
Nr4a2	#64	Forward	5'-TCAGAGCCCACGTCGATT-3'
		Reverse	5'-TAGTCAGGGTTTGCCTGGAA-3'
Ptger4	#77	Forward	5'-CCTAACCCCACCCTACAGGT-3'
		Reverse	5'-AGAAGGACGCGTTGACTCC-3'
Rn18s	#48	Forward	5'-GCAATTATTCCCCATGAACG-3'
		Reverse	5'-GGGACTTAATCAACGCAAGC-3'
Tbx21	#19	Forward	5'-TCAACCAGCACCAGACAGAG -3'
		Reverse	5'-AAACATCCTGTAATGGCTTGTG -3'

* Universal ProbeLibrary (Roche) were used together with the LightCycler Taqman Master kit (Roche). Otherwise, FastStart DNA Master^PLUS SYBR Green I kit (Roche) was used without Universal Probe Library.

Supplementary Table S2. Primer sets used for *Il12rb2* gene locus in ChIP assay.

Primer Set	Universal Probe Library, mouse*		Primer Sequence
A	/	Forward	5'-TTATGTCCTGCAGACAGGAGTG-3'
		Reverse	5'-CATCTGCAGGTAAATCAAAGCA-3'
B		Forward	5'-AGGGAAATTACTGGAATGATTTATGG-3'
		Reverse	5'-GACTGAGCAATTACTAGATTCTTGGA-3'
		Probe	5'-CTGCAGTCCAACTAACCCAACAATAGGTAC-3'
C	#69	Forward	5'-CAGCACGTCTTCTGTATAAACTGG-3'
		Reverse	5'-TGCACTGACTTGCTAACACATCT-3'
D		Forward	5'-GAGACTAGGATGGAAAATATAATCAA-3'
		Reverse	5'-TCTGGATGAAGCCTCTCTGA-3'
		Probe	5'-TTCTGCAATACTCATAGATTGGTGCCTAGC-3'
E		Forward	5'-GGACCTCACAGAGACAGAAACA-3'
		Reverse	5'-ACACCAAGCTAACAACCATAACATA-3'
		Probe	5'-ATGCAGAACTACCACAGACCCAAGCAGTC-3'
F	#110	Forward	5'-CGTCAGAAATGGAAACTGAGC-3'
		Reverse	5'-TGCCTGTGATGAAACTCAGC-3'
G	#98	Forward	5'-TGAGTGCCACTGTGGTTATGA-3'
		Reverse	5'-CCAACACGTCATCCAAAATG-3'
H	/	Forward	5'-AAATTTGGGCTTTTAAATATTTTGC-3'
		Reverse	5'-GGCCCACTTGAGCTATGGAG-3'
I	/	Forward	5'-GTCCACTTTCCGTTTTATATGTCC-3'
		Reverse	5'-GAACTTGCCCAGTGCAGGAG-3'
J	#109	Forward	5'-TCTGCCTGGACACTTGTCAT-3'
		Reverse	5'-GACGCAGAGAGGGATCCTG-3'
K	#82	Forward	5'-TGCCGTCACTGCTTTCAATA-3'
		Reverse	5'-TTCCAACAGCAAAGGAAACC-3'
L	#82	Forward	5'-TGCCGTCACTGCTTTCAATA-3'
		Reverse	5'-CAAATGCTGTTTCCCTGTGC-3'
M	/	Forward	5'-TAGTCCGTGTTACTGCCATCAA-3'
		Reverse	5'-GTAAATGACGCCTGATTAACTGC-3'
N	#32	Forward	5'-CTCCGTGGGACATCAGAATC-3'
		Reverse	5'-GCCCCTCATCTTCCCACT-3'
O	#50	Forward	5'-TCATGACGTCAGGTGACCTACTA-3'
		Reverse	5'-GCAGTTGATTATATACCCGAATTTCT-3'
P	#5	Forward	5'-TGCATTTCAAAAATATAAACCATCA-3'
		Reverse	5'-GATTTTGGCTTTGGGGATTT-3'

* Universal Probe Library (Roche) or specified probes were used together with the LightCycler Taqman Master kit (Roche). Otherwise, FastStart DNA Master^{PLUS} SYBR Green I kit (Roche) was used without Universal Probe Library.

Supplementary Table S3. Primer sets used for *Ifngr1* gene locus in ChIP assay.

Primer Set	Universal Probe Library, mouse*	Primer Sequence	
A	/	Forward	5'-CATGCCACTGCAGTAAAACTGTC-3'
A	/	Reverse	5'-CCAGTTTGGACTCTGTCTCTTCC-3'
B	#13	Forward	5'-ACGTGTCACTCCCTGATTGG-3'
B	#13	Reverse	5'-GCCGAGGGTGGTTCTCTACT-3'
C	/	Forward	5'-TTCTGAAATGTGTGACGTGAGG-3'
C	/	Reverse	5'-GCTCATCGACCCTTAACAAAAA-3'
D	/	Forward	5'-CTATTCCTTCCCCCTTAATCTCC-3'
D	/	Reverse	5'-CTGTGCGCATGCTTCCTAAG-3'
E		Forward	5'-CCCACACGCACACGCATG-3'
E		Reverse	5'-CCACTGAGGAAGAGGCACTGTA-3'
E		Probe	5'-CGCACGCACGTCTGATACTGTAATCTCATT-3'
F	#16	Forward	5'-CCTCAGTGGATACAAGGTCCA-3'
F	#16	Reverse	5'-GGATACCCAAGATATAGCTATGCAG-3'
G	/	Forward	5'-CGGAAACACTTAACTCCCAAGC-3'
G	/	Reverse	5'-CCATTTCTGAGGAAGGATCTGG-3'
H	#64	Forward	5'-CACTTCATAATCTGCTTCCTGGT-3'
H	#64	Reverse	5'-ATGCGGCTTGCAGATTTT-3'
I	/	Forward	5'-CATCTGTAGCACCAAGGGTGAG-3'
I	/	Reverse	5'-CCTTGTGCAGGTTCAGATTCAG-3'
J	#107	Forward	5'-TTTATCCTGACGTCAAAGTCTGAG-3'
J	#107	Reverse	5'-CCAGCTAAGGCTTGTTATTTGTC-3'
K	/	Forward	5'-TGACGTCAAAGTCTGAGAAATGC-3'
K	/	Reverse	5'-TCTCTGAGTCTTCCAAGCAAAATG-3'
L	/	Forward	5'-TGTAGGAGGGGGTGCAAATG-3'
L	/	Reverse	5'-ACACTCTTGTGCTGCCTCACTC-3'
M		Forward	5'-CCAGGCTGGTCTCTCAAGCTT-3'
M		Reverse	5'-CCTGGCCTTGGCGTCA-3'
M		Probe	5'-CTTGCGGACTTGGC-3'
N	/	Forward	5'-GATTCTGCTGGTGGTCCTGAT-3'
N	/	Reverse	5'-CAGCGGCTCGGAGAGATTAC-3'
O	/	Forward	5'-GGCATGGGGAGACCTCTTTAAC-3'
O	/	Reverse	5'-AATCAGGTCAAGGCAGGATTG-3'
P	#40	Forward	5'-GGCCAAAGCGAGACTGAA-3'
P	#40	Reverse	5'-GACGTCAGACATGGAGATGC-3'

* Universal Probe Library (Roche) or specified probes were used together with the LightCycler Taqman Master kit (Roche). Otherwise, FastStart DNA Master[PLUS] SYBR Green I kit (Roche) was used without Universal Probe Library.