

Supplementary

S1P₄ Regulates Passive Systemic Anaphylaxis in Mice but Is Dispensable for Canonical IgE-Mediated Responses in Mast Cells

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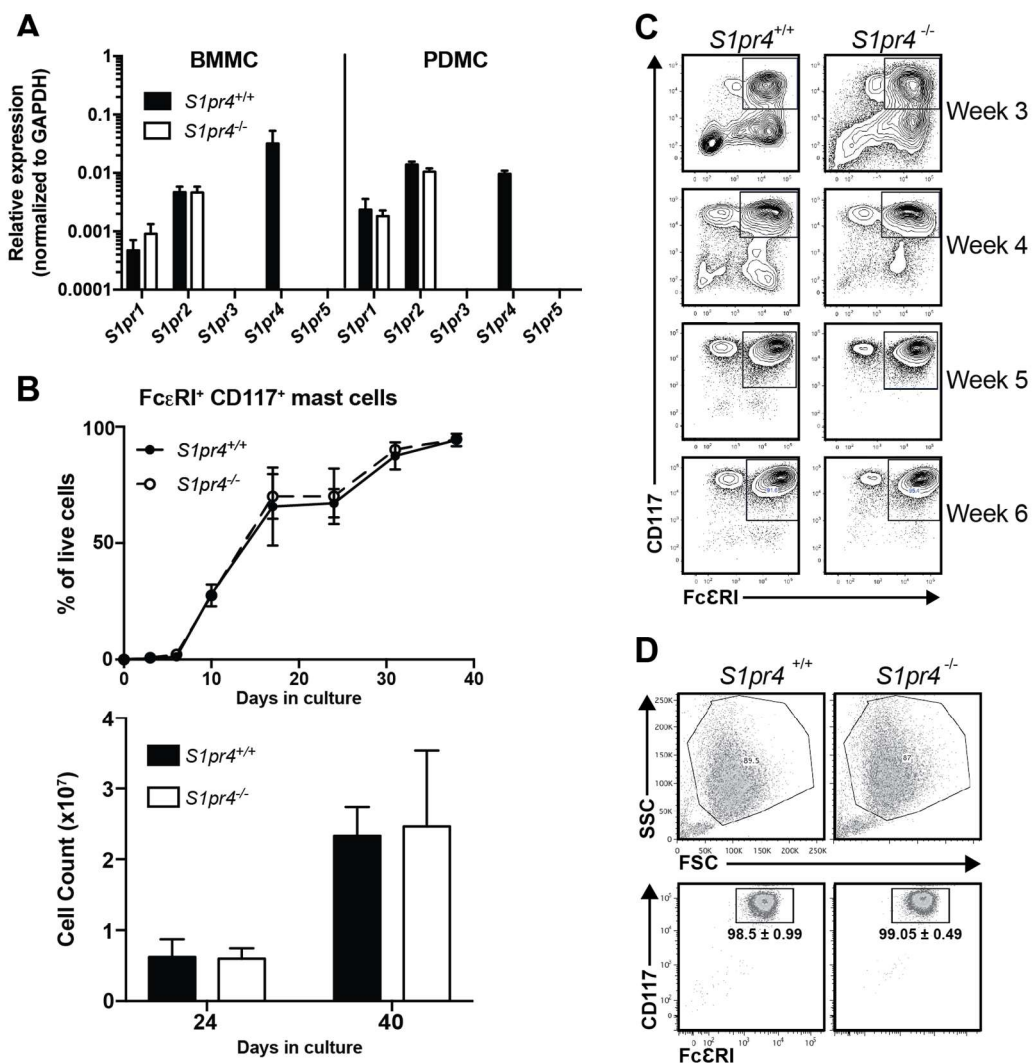


Figure S1. Growth and differentiation of murine mast cells in the absence of *S1pr4* expression
A) Quantitative PCR data showing the relative expression of S1P receptor message normalized to GAPDH in cultured BMMC (left) and PDMC (right) from *S1pr4*^{+/+} (solid bars) and *S1pr4*^{-/-} mice (open bars). In BMMC, bars represent the mean ± SE of data pooled from 7 independent

WT and 4 *Slpr4*^{-/-} cultures and in PDMC, 2 independent WT and 4 *Slpr4*^{-/-} cultures . **B,C)** Frequency (Panel B, top) and absolute number (Panel B, bottom) of mast cells (FcεRI⁺/CD117⁺) over time in culture. Bone marrow cells harvested from *Slpr4*^{+/+} (black) and *Slpr4*^{-/-} mice (grey) were grown in the presence of SCF and IL-3 for 7 weeks. Non-adherent cells were counted and analyzed by flow cytometry. The gating strategy used to identify mast cells is represented in panel C. Data represent the mean ± SE of at least 3 independent cultures. **D)** Representative flow cytometry plots showing the forward and side-scatter profile (top) and mast cell marker surface expression profile (bottom) profiles of non-adherent cells expanded from peritoneal exudates of *Slpr4*^{+/+} (left) and *Slpr4*^{-/-} mice (right). The frequency of FcεRI⁺/CD117⁺ represents mean ± SE at least 3 independent experiments.

BMMC (pre- and post-sensitization)

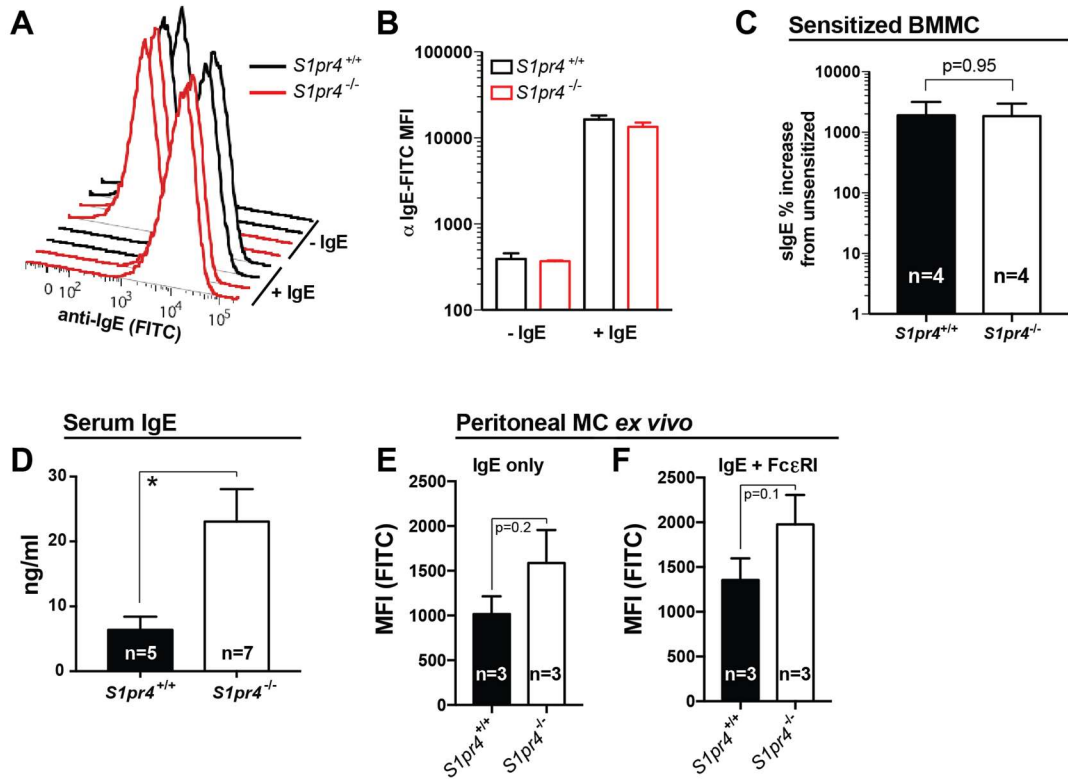


Figure S2. IgE bound by mast cells and in circulation. **A-C)** Surface bound IgE was measured by FACS on cultured BMMC before (control, -IgE) and after sensitization with 100 ng/ml anti-DNP IgE overnight (+IgE) in cytokine-free media. **A)** graphical histogram of raw FACS data from a single representative experiment comparing 2 WT and 2 *S1pr4*^{-/-} BMMC cultures grown and analyzed concurrently. **B)** Average anti-IgE FITC median fluorescence intensity (MFI) of the 2 independent cultures for each genotype shown in A. Bars represent mean MFI ± SD. **C)** Percent increase in MFI in sensitized compared to control WT or *S1pr4*^{-/-} BMMC. These data were pooled from 4 independent cultures of each genotype and represent mean ± SE. **D)** Mean ± SE serum IgE levels measured from 6 to 8 week old *S1pr4*^{+/+} and *S1pr4*^{-/-} mice by ELISA. **E,F)** Peritoneal exudate cells (PEC) from 3 *S1pr4*^{+/+} and 3 *S1pr4*^{-/-} mice were stained directly *ex vivo* with anti-IgE-FITC and anti-CD117-APC (E) or anti-IgE-FITC, anti-FcεRI-FITC and anti-CD117-APC (F) for FACS analysis. Pooled data shows the mean ± SE MFI of anti-IgE FITC on CD117⁺ pre-gated peritoneal mast cells and is representative of 2 independent experiments. *p<0.05

Table S1. Normalized relative mRNA expression values for select genes from RT² cytokine array analysis.

Gene	Relative Expression ¹ (unstimulated, n=2)		Relative Expression ¹ (IgE+Ag, n=2)		Average fold change ² (IgE+Ag, n=3)
	<i>S1pr4</i> ^{+/+}	<i>S1pr4</i> ^{-/-}	<i>S1pr4</i> ^{+/+}	<i>S1pr4</i> ^{-/-}	<i>S1pr4</i> ^{-/-} / <i>S1pr4</i> ^{+/+}
<i>Ccl1</i>	0.0087	0.0093	0.4406	0.8815	1.7
<i>Ccl3</i>	0.1407	0.1448	0.4951	0.7972	1.4
<i>Ccl4</i>	0.0413	0.0556	0.2091	0.3385	1.2
<i>Ccl7</i>	1.0121	0.8870	1.4941	1.8698	1.3
<i>Ccl12</i>	0.0007	0.0007	0.0034	0.0072	5.8
<i>Ccl17</i>	0.0279	0.0527	0.0363	0.0263	1.5
<i>Cxcl3</i>	0.0002	0.0002	0.0002	0.0003	1.2
<i>Ccl22</i>	0.0002	0.0002	0.0001	0.0002	6.7
<i>Ccl24</i>	0.0007	0.0013	0.0003	0.0005	5.0
<i>Il1a</i>	0.0037	0.0069	0.0140	0.0234	1.6
<i>Il2</i>	0.0002	0.0002	0.0008	0.0017	5.6
<i>Il5</i>	0.0002	0.0002	0.0063	0.0093	4.1
<i>Il9</i>	0.0004	0.0004	0.0007	0.0018	2.4
<i>Il11</i>	0.0002	0.0002	0.0002	0.0003	1.1
<i>Il13</i>	0.0513	0.0775	0.4972	0.8664	1.5
<i>Csf2</i>	0.0077	0.0036	0.0253	0.0476	1.4
<i>Ifng</i>	0.0002	0.0002	0.0004	0.0004	1.1

¹Values represent normalized relative mRNA expression of select genes by bone marrow-derived mast cells (BMMC) as measured by RT² quantitative PCR array analysis.

²Values represent the average fold difference between relative expression in Ag-stimulated *S1pr4*^{-/-} BMMC and Ag-stimulated *S1pr4*^{+/+} wildtype control cells.

n indicates the number of independent cultures represented in each value.

Table S2. Probes used for gene expression analysis.

Gene	Probe	Application	Manufacturer
<i>S1pr1</i>	Mm00514644_m1	qPCR	Thermo Fisher
<i>S1pr2</i>	Mm01177794_m1	qPCR	Thermo Fisher
<i>S1pr3</i>	Mm02620181_s1	qPCR	Thermo Fisher
<i>S1pr4</i>	Mm00468695_s1	qPCR	Thermo Fisher
<i>S1pr5</i>	Mm02620565_s1	qPCR	Thermo Fisher
<i>Il6</i>	Mm00446190_m1	qPCR	Thermo Fisher
<i>Tnfa</i>	Mm00443258_m1	qPCR	Thermo Fisher
<i>Gapdh</i>	Mm99999915_g1	qPCR	Thermo Fisher
<i>Ccl1</i>	dMmuCPE5090242	ddPCR	BioRad
<i>Ccl12</i>	dMmuCPE5107958	ddPCR	BioRad
<i>Ccl17</i>	dMmuCPE5088392	ddPCR	BioRad
<i>Ccl22</i>	dMmuCPE5116290	ddPCR	BioRad
<i>Ccl24</i>	dMmuCPE5123514	ddPCR	BioRad
<i>Il2</i>	dMmuCPE5107882	ddPCR	BioRad
<i>Il5</i>	dMmuCPE5110012	ddPCR	BioRad
<i>Il6</i>	dMmuCPE5095532	ddPCR	BioRad
<i>Il9</i>	dMmuCPE5112064	ddPCR	BioRad
<i>Gapdh</i>	dMmuCPE5195283	ddPCR	BioRad
<i>Hprt</i>	dMmuCPE5095492	ddPCR	BioRad