

***Supplementary information***

**Activated mast cells promote differentiation of B cells into effector cells**

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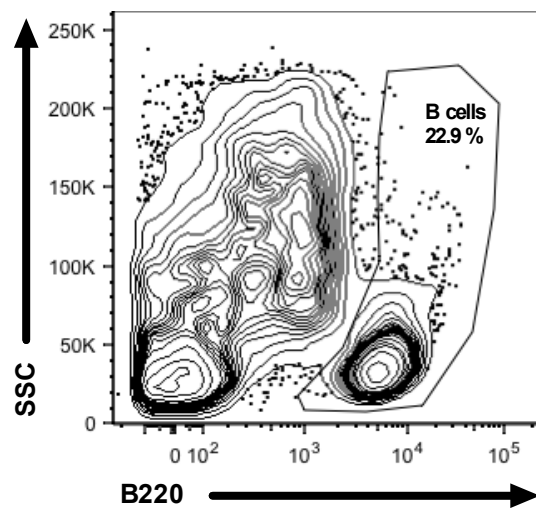
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**Supplementary Table S1.** Quantification of the cytokine array presented in Fig. 2a. Values are mean expression intensity  $\pm$  standard deviation of duplicates for every spot on the cytokine array, as quantified using the ImageJ software. Mediators indicated in bold represent those that are profoundly up-regulated in co-cultures of MCs and B cells vs. their expression in cultures of MCs or B cells alone.

Mediator	Naïve BCs	BCR-act. BCs	Act. MCs	BCR-act. BCs + act. MCs
AXL	2.38 $\pm$ 0.69	3.10 $\pm$ 0.68	1.35 $\pm$ 0.65	2.49 $\pm$ 0.07
BLC	14.10 $\pm$ 1.36	14.83 $\pm$ 1.69	8.85 $\pm$ 0.89	14.42 $\pm$ 3.03
CD30L	3.62 $\pm$ 0.48	9.85 $\pm$ 0.05	1.63 $\pm$ 0.74	6.00 $\pm$ 0.10
CD30	3.02 $\pm$ 0.49	4.67 $\pm$ 1.48	2.10 $\pm$ 0.36	3.89 $\pm$ 0.92
CD40	0.81 $\pm$ 0.03	1.69 $\pm$ 0.42	0.45 $\pm$ 0.09	2.00 $\pm$ 1.09
CRG-2	0.87 $\pm$ 0.42	6.84 $\pm$ 0.39	1.03 $\pm$ 0.06	6.70 $\pm$ 2.12
CTACK	6.49 $\pm$ 0.77	10.06 $\pm$ 1.96	6.47 $\pm$ 1.05	13.54 $\pm$ 6.96
<b>CXCL16</b>	<b>2.34<math>\pm</math>0.61</b>	<b>6.13<math>\pm</math>1.09</b>	<b>3.18<math>\pm</math>0.48</b>	<b>12.79<math>\pm</math>0.28</b>
Eotaxin	5.10 $\pm$ 0.28	8.69 $\pm$ 0.86	4.45 $\pm$ 0.23	5.69 $\pm$ 1.64
Eotaxin-2	100.68 $\pm$ 1.45	100.61 $\pm$ 0.12	117.39 $\pm$ 5.44	121.79 $\pm$ 9.19
Fas Ligand	33.41 $\pm$ 7.60	39.93 $\pm$ 1.11	33.42 $\pm$ 0.63	40.15 $\pm$ 6.04
Fractalkine	4.46 $\pm$ 0.56	4.78 $\pm$ 0.68	6.29 $\pm$ 3.32	6.36 $\pm$ 2.67
G-CSF	2.46 $\pm$ 0.16	3.22 $\pm$ 0.46	2.15 $\pm$ 0.64	4.59 $\pm$ 2.05
GM-CSF	1.82 $\pm$ 0.86	1.93 $\pm$ 0.54	29.48 $\pm$ 8.24	25.87 $\pm$ 0.01
IFN $\gamma$	1.34 $\pm$ 0.23	4.65 $\pm$ 0.27	1.72 $\pm$ 0.54	4.49 $\pm$ 0.45
IGFBP-3	15.13 $\pm$ 1.45	17.38 $\pm$ 0.47	10.89 $\pm$ 0.52	17.77 $\pm$ 5.26
IGFBP-5	12.34 $\pm$ 2.87	15.51 $\pm$ 0.66	11.21 $\pm$ 0.60	20.85 $\pm$ 0.39
IGFBP-6	2.90 $\pm$ 0.01	3.79 $\pm$ 0.15	1.52 $\pm$ 0.10	3.29 $\pm$ 0.57
IL-1 $\alpha$	24.56 $\pm$ 2.16	35.38 $\pm$ 0.62	22.19 $\pm$ 2.69	34.32 $\pm$ 0.80
IL-1 $\beta$	3.61 $\pm$ 0.30	6.12 $\pm$ 0.60	2.95 $\pm$ 0.00	11.86 $\pm$ 3.98
IL-2	2.80 $\pm$ 0.38	35.11 $\pm$ 0.40	3.44 $\pm$ 0.09	34.71 $\pm$ 5.80
IL-3	1.55 $\pm$ 0.00	5.44 $\pm$ 0.38	35.58 $\pm$ 1.44	37.20 $\pm$ 5.20
IL-3 $\beta$	6.02 $\pm$ 0.23	9.24 $\pm$ 0.71	13.39 $\pm$ 1.15	20.77 $\pm$ 3.20
IL-4	12.68 $\pm$ 2.37	12.29 $\pm$ 2.32	11.41 $\pm$ 2.63	14.09 $\pm$ 3.39
IL-5	2.61 $\pm$ 0.40	5.60 $\pm$ 0.29	7.36 $\pm$ 0.32	13.74 $\pm$ 1.65
IL-6	1.28 $\pm$ 0.09	1.98 $\pm$ 0.52	199.61 $\pm$ 3.81	210.45 $\pm$ 1.01
IL-9	10.15 $\pm$ 1.52	42.39 $\pm$ 3.87	11.19 $\pm$ 0.32	38.95 $\pm$ 2.75
IL-10	5.25 $\pm$ 0.39	6.84 $\pm$ 0.85	0.81 $\pm$ 0.24	6.26 $\pm$ 0.01
IL-12p40/p70	10.44 $\pm$ 0.93	12.98 $\pm$ 3.31	2.92 $\pm$ 1.20	11.63 $\pm$ 0.30
IL-12p70	12.45 $\pm$ 0.45	17.75 $\pm$ 4.01	10.05 $\pm$ 0.54	15.68 $\pm$ 1.67
IL-13	3.64 $\pm$ 0.43	7.22 $\pm$ 1.57	8.25 $\pm$ 1.40	12.24 $\pm$ 2.05
IL-17	0.40 $\pm$ 0.16	1.43 $\pm$ 0.30	0.19 $\pm$ 0.13	0.56 $\pm$ 0.11
KC	2.60 $\pm$ 0.85	4.04 $\pm$ 0.97	1.26 $\pm$ 0.30	3.98 $\pm$ 0.40
LeptinR	19.15 $\pm$ 1.67	25.42 $\pm$ 4.66	16.64 $\pm$ 2.42	24.57 $\pm$ 0.06
Leptin	9.31 $\pm$ 1.16	17.77 $\pm$ 3.02	7.34 $\pm$ 0.98	23.15 $\pm$ 9.49
LIX	55.06 $\pm$ 4.28	58.35 $\pm$ 9.12	54.90 $\pm$ 6.43	64.59 $\pm$ 10.20
<b>L-Selectin</b>	<b>17.03<math>\pm</math>2.78</b>	<b>20.10<math>\pm</math>6.94</b>	<b>8.54<math>\pm</math>2.75</b>	<b>52.03<math>\pm</math>1.76</b>
Lymphotactin	51.78 $\pm$ 1.19	56.21 $\pm$ 0.97	50.84 $\pm$ 7.60	58.33 $\pm$ 0.53
MCP1	23.42 $\pm$ 1.67	29.18 $\pm$ 2.06	87.62 $\pm$ 5.43	103.32 $\pm$ 2.95

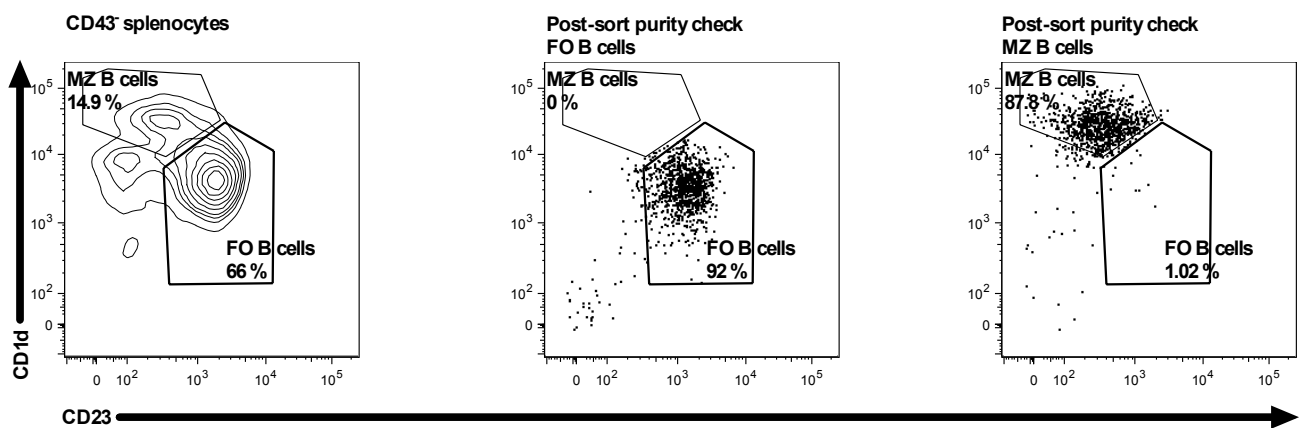
<b>MCP5</b>	<b>12.68±1.37</b>	<b>19.02±2.59</b>	<b>15.28±0.12</b>	<b>36.22±2.98</b>
M-CSF	25.48±0.24	30.99±0.49	21.33±4.94	31.95±3.18
MIG	32.20±0.16	31.71±1.64	26.78±0.29	34.32±0.42
<b>MIP-1α</b>	<b>4.69±1.61</b>	<b>15.11±0.67</b>	<b>21.14±1.03</b>	<b>41.33±2.43</b>
MIP-1γ	24.56±1.54	54.13±3.90	128.16±1.38	162.76±5.50
MIP-2	34.51±1.53	42.37±2.45	37.38±1.25	48.62±1.28
MIP-3β	45.78±0.67	54.03±1.92	43.64±0.92	53.59±3.89
MIP-3α	9.39±0.80	15.63±2.55	6.57±0.18	13.00±0.01
PF-4	21.91±1.95	25.70±1.03	16.20±1.50	20.34±6.06
P-Selectin	29.39±0.61	35.17±2.37	26.17±1.39	32.22±6.82
RANTES	13.46±0.77	15.64±1.90	13.27±4.49	22.92±13.29
SCF	4.46±1.25	6.45±0.16	3.37±0.81	15.39±6.81
SDF-1	62.06±1.15	65.68±0.93	49.38±4.17	59.15±5.88
TARC	6.47±0.73	17.78±1.58	4.83±0.24	16.02±2.65
TCA-3	22.74±0.19	127.42±0.59	80.09±5.16	143.16±2.97
TECK	3.92±0.42	11.03±1.59	2.03±0.84	13.38±0.78
TIMP-1	14.93±1.93	20.07±1.24	9.11±2.62	23.87±0.35
TNFα	4.14±0.01	5.73±1.21	1.65±0.03	5.36±0.67
sTNFR1	2.35±0.39	3.79±0.06	2.91±0.55	11.38±1.10
sTNFR2	7.89±0.21	11.92±2.99	6.15±0.26	24.85±0.37
TPO	32.63±1.95	71.60±4.41	27.21±0.34	56.34±4.04
VCAM-1	6.20±0.20	10.10±1.46	1.03±0.35	19.44±10.51
VEGF	10.33±1.78	17.73±4.47	8.58±1.61	23.46±6.34

### Supplementary figure S1



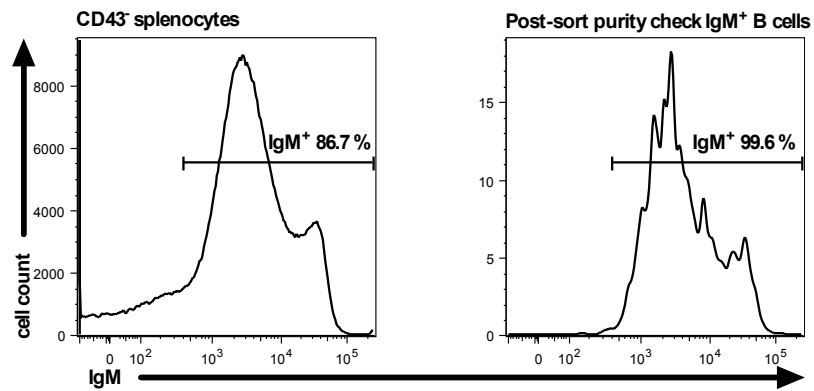
**Supplementary Figure S1. Gating strategy for B cells.** Cells from the MC:B cell cocultures were stained for surface markers and the viability dye 7-AAD and analyzed by flow cytometry. B cells were defined as 7-AAD<sup>-</sup> B220<sup>high</sup>. SSC = side scatter

## Supplementary figure S2



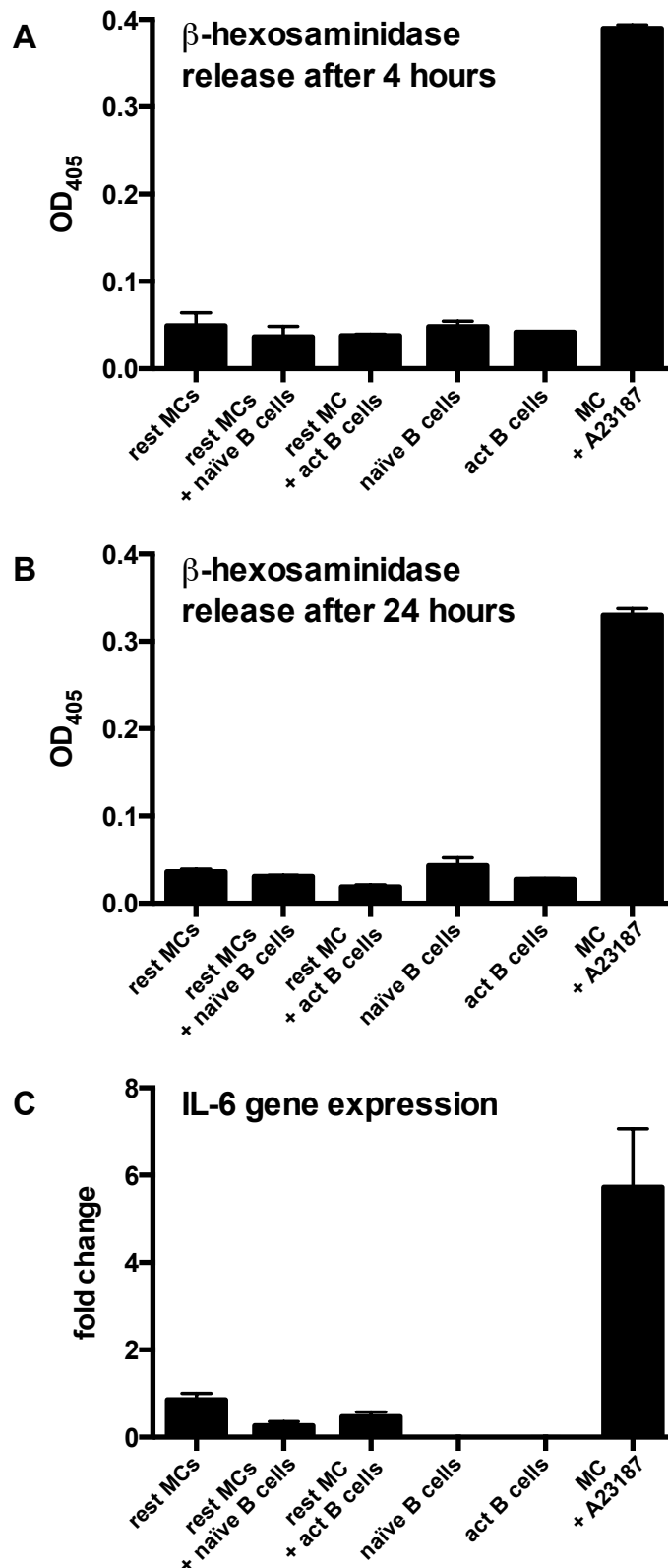
**Supplementary Figure S2. Sorting strategy for MZ and FO B cells.** Splenocytes were first enriched for B cells using MACS, and subsequently stained for CD1d and CD23 and sorted into marginal zone (MZ) and follicular (FO) B cells using FACS. MZ B cells were defined as CD1d<sup>high</sup> CD23<sup>low</sup> and FO B cells as CD1d<sup>low</sup> CD23<sup>high</sup> (left). Sorting purities were routinely around 90% for both FO B cells (middle) and MZ B cells (right). FO B cells, follicular B cells; MZ B cells, marginal zone B cells.

### Supplementary figure S3



**Supplementary Figure S3. Sorting strategy for IgM<sup>+</sup> B cells.** Splenocytes were first enriched for B cells using MACS, and subsequently stained for IgM. The IgM<sup>+</sup> fraction was routinely sorted with a purity of >98%.

## Supplementary figure S4



**Supplementary Figure S4. Coculture of MCs and B cells does not provoke MC activation.** Non-activated MCs (rest MCs) were cocultured with naïve or BCR-activated B cells (act B cells) and MC activation was monitored as the release of  $\beta$ -hexosaminidase (A, 4 hours incubation; and B, 24 hours incubation) and the expression of the IL-6 gene as determined by real time quantitative RT-PCR (C, 4 hours incubation). As negative controls, rest MCs, naïve or act B cells were cultured alone. As positive control for MC activation, MCs were incubated with 1  $\mu$ M calcium ionophore A23187. Note that stimulation of MCs by calcium ionophore produces a robust  $\beta$ -hexosaminidase release and profound IL-6 gene upregulation, whereas coculture of MCs with B cells does not result in MC activation. Results are expressed as mean + SEM and represent 2 independent experiments in duplicates. MC, mast cell; BCR, B cell receptor