





Supplementary Figure S2. Surface and intracellular IgA expression in CD38⁺ CD138⁺ cells in the intestine

Flow cytometry was performed to examine IgA expression on the surface and intracellular compartment of CD38⁺ CD138⁺ cells in the intestine.



Supplementary Figure S3. Spleen IgA⁺ cells are composed of several kinds of cells showing different differentiation phenotypes

Cells were isolated from the spleen for the analysis of IgA and CD11b expression (**a**), B220, CD138, CD38, CD40 expression on CD11b⁻ IgA⁺ (black) and CD11b⁺ IgA⁺ (red) cells (**b**). Gray indicates isotype control Ab. Similar results were obtained from three separate experiments.



Supplementary Figure S4. CD18 expression on IgA plasma cells in the intestine

The expression of CD18 was examined on IgA⁺ CD11b⁺ (red) and IgA⁺ CD11b⁻ (black) cells isolated from the intestinal lamina propria of wild-type mice. Shadow indicates isotype control Ab. Data are representative of three separate experiments.



Supplementary Figure S5. Lack the CD11b expression in the intestine after adoptive transfer of peritoneal CD11b⁺ B cells

CD11b⁺ B220⁺ B cells were purified from the peritoneal cavity and adoptively transferred into severe combined immunodeficiency mice. Two months later, CD11b⁺ IgA⁺ cells were isolated from the intestinal lamina propria for examination. Similar results were obtained from four individual mice.



Supplementary Figure S6. Maintained expression of CD11b on IgA⁺ cells after in vitro culture

CD11b⁻ and CD11b⁺ IgA⁺ cells were purified by cell sorting from the intestinal lamina propria and used for in vitro culture with lipopolysaccharide (LPS) or phorbol 12-myristate 13-acetate (PMA) plus ionomycin. Twenty four hours after incubation, CD11b expression was examined by flow cytometry. The data is representative from five separate experiments.



Supplementary Figure S7. Expression profiles of CD150, β 1 integrin, and CD168 on IgA plasma cells in the intestine

The expression of CD150, β 1 integrin, and CD168 was examined on IgA⁺ CD11b⁺ (red) and IgA⁺ CD11b⁻ (black) cells isolated from the intestinal lamina propria of wild-type mice. Shadow indicates isotype control Ab. Data are representative of three separate experiments.



Supplementary Figure S8. MyD88 expression on either hematopoietic and non-hematopoietic cells is sufficient for the induction of CD11b⁺ IgA⁺ cells Two months after the bone marrow transfers shown by the arrows, mononuclear cells were isolated from the intestinal

lamina propria for flow cytometric analysis of CD11b⁺ IgA⁺ cells. Data are representative of five mice.



Supplementary Figure S9. Normal differentiation into IgA⁺ B cells in the Peyer's patches and peritoneal cavity of IL-10 knockout mice Cells were isolated from the Peyer's patches (a) and peritoneal cavity (b) of

wile-type (WT) or IL-10 knockout (KO) mice to detect expression of IgA and IgM. Similar results were obtained from four separate experiments.



Supplementary Figure S10. Cyclophosphamide treatment during oral immunization diminishes germinal center B cell abundance and consequently reduces IgA⁺ plasmablast abundance

Mice were treated with cyclophosphamide (CPM) or were mock-treated during oral immunization with ovalbumin plus cholera toxin. Two days after the treatment, cells were isolated from Peyer's patches for flow cytometric analysis of B220⁺ PNA⁺ germinal center B cells (**a**) and IgA⁺ IgM⁻ plasmablasts (**b**). Data are representative of three separate experiments.



Supplementary Figure S11. Image of diarrhea induced by cholera toxin

Mice were orally immunized with ovalbumin plus cholera toxin (CT) on days 0, 7, and 14. One group received cyclophosphamide (CPM) after the last immunization (days 18, 19, 20). One week after the final immunization (day 21), mice were orally challenged with 100 μ g CT. After 15 h, the incidence of diarrhea was determined. Data are representative of five experiments.



Supplementary Figure S12. High IgA production from CD11b⁺ IgA⁺ cells in vivo

CD11b⁺ IgA⁺ and CD11b⁻ IgA⁺ cells were purified from the intestinal lamina propria and adoptively transferred into SCID mice (1.5×10^5 cells/mouse). One week after the transfer, the amount of IgA in the feces was measured by ELISA. Similar results were obtained from two separate experiments.





(a) Mononuclear cells were isolated from the intestinal lamina propria and incubated with fluorescent opsonized bacteria for 90 min. The bacteria uptake by each population was examined by flow cytometry. (b) The expression of I-A^d and CD80 was examined on CD11b⁺ IgA⁺ (red) and CD11b⁻ IgA⁺ (black) cells isolated from the small intestinal lamina propria of wild-type mice. Shadow indicates isotype control Ab. Data are representative of three separate experiments.



Supplementary Figure S14. Segmented filamentous bacteria (SFB) are not involved in specific induction of CD11b⁺ IgA cells in the intestine

(a) Mononuclear cells were isolated from the intestinal lamina propria of control Jackson mice (JAX) or Jackson mice colonized with faecal homogenates from SFB-monoassociated mice (JAX+SFB). The data represent the percentage of CD11b⁺ and CD11b⁻ IgA⁺ cells analyzed by flow cytometry. Values represent the mean \pm SD (n=4).

| GO ACCESSION | GO Term | | corrected p- | Count in | % Count in | Count in | % Count in |
|--------------|-------------------------------|----------|--------------|----------|---------------|----------|------------|
| | | p-value | value 🏾 💌 | Selecti | Selection 🛛 🚬 | Total 🗾 | Total 🗾 🗾 |
| GO:0044427 | chromosomal part | 3.91E-20 | 2.36E-15 | 144 | 4.123711 | 351 | 1.9954519 |
| GO:0000279 | M phase | 2.31E-19 | 3.48E-15 | 123 | 3.5223367 | 286 | 1.6259239 |
| GO:0022402 | cell cycle process | 1.55E-19 | 3.48E-15 | 160 | 4.5819016 | 411 | 2.3365548 |
| GO:0022403 | cell cycle phase | 1.88E-19 | 3.48E-15 | 139 | 3.980527 | 339 | 1.9272314 |
| GO:0007049 | cell cycle | 5.62E-19 | 6.77E-15 | 234 | 6.7010307 | 689 | 3.9169984 |
| GO:0005694 | chromosome | 3.25E-18 | 3.26E-14 | 163 | 4.6678123 | 433 | 2.4616258 |
| GO:0000278 | mitotic cell cycle | 2.83E-17 | 2.44E-13 | 110 | 3.1500573 | 257 | 1.4610574 |
| GO:000087 | M phase of mitotic cell cycle | 3.90E-16 | 2.93E-12 | 89 | 2.5486827 | 196 | 1.1142695 |
| GO:0006996 | organelle organization | 1.37E-15 | 9.18E-12 | 332 | 9.507445 | 1133 | 6.4411597 |
| GO:0048285 | organelle fission | 1.94E-15 | 1.17E-11 | 88 | 2.5200458 | 197 | 1.1199545 |

Supplementary Table S1 Gene ontology enrichment score highly expressed in CD11b⁺ IgA⁺ cells compared with CD11b⁻ IgA⁺ cells

Supplementary Table S2 Microarray data on cell cycle-associated molecules highly expressed in CD11b⁺ IgA⁺ cells

| Probe Set ID | Ratio | Symbol | Title |
|--------------|-------|--------|---|
| 1416076_at | 3.38 | Ccnb1 | cyclin B1 |
| 1416698_a_at | 3.25 | Cks1b | CDC28 protein kinase 1b |
| 1417019_a_at | 3.05 | Cdc6 | cell division cycle 6 homolog (S. cerevisiae) |
| 1417911_at | 3.65 | Ccna2 | cyclin A2 |
| 1418919_at | 20.24 | Sgol1 | shugoshin-like 1 (S. pombe) |
| 1422046_at | 8.92 | Itgam | integrin alpha M (CD11b) |
| 1424128_x_at | 3.68 | Aurkb | aurora kinase B |
| 1426817_at | 3.12 | Mki67 | antigen identified by monoclonal antibody Ki 67 |
| 1439436_x_at | 3.37 | Incenp | inner centromere protein |
| 1448314_at | 3.05 | Cdc2a | cell division cycle 2 homolog A (S. pombe) |
| 1448466_at | 3.50 | Cdca5 | cell division cycle associated 5 |
| 1451246_s_at | 3.88 | Aurkb | aurora kinase B |
| 1452305_s_at | 3.58 | Cenpn | centromere protein N |
| 1428481_s_at | 3.38 | Cdca8 | cell division cycle associated 8 |
| 1429326_at | 4.87 | Cenpl | centromere protein L |
| 1432361_a_at | 3.24 | Cenpp | centromere protein P |
| 1436847_s_at | 3.30 | Cdca8 | cell division cycle associated 8 |
| 1443294_at | 7.55 | Crkrs | CDC2-related kinase, arginine/serine-rich |
| 1456077_x_at | 14.48 | Cdc25c | cell division cycle 25 homolog C (S. pombe) |

Supplementary Table S3 Microarray data on cell surface molecules highly expressed in CD11b⁺IgA⁺ cells

| Probe Set ID | CD11b- | CD11b+ | Gene Symbol | Gene Title |
|--------------|-----------|-----------|-------------|---|
| 1422046_at | 15.230377 | 135.8069 | ltgam | CD11b (aM integrin) |
| 1425570_at | 38.918594 | 127.18432 | Slamf1 | CD150 (signaling lymphocytic activation molecule family member 1) |
| 1425815_a_at | 35.757877 | 105.87889 | Hmmr | CD168 (hyaluronan mediated motility receptor) |
| 1450156_a_at | 25.355202 | 56.041107 | Hmmr | CD168 (hyaluronan mediated motility receptor) |
| 1427771_x_at | 4.150068 | 14.996672 | ltgb1 | β1 integrin |
| 1429871_at | 1.5343701 | 14.869006 | Hmmr | CD168 (hyaluronan mediated motility receptor) |

| | CD11b ⁺ lgA ⁺ cell | CD11b [⁻] lgA [⁺] cell | | |
|--|--|--|--|--|
| Same/similar phenotypes | | | | |
| Morphology | Plasma cell (large irregular nuclei with prominent nucleoli) | | | |
| Blimp-1 expression | High | | | |
| Cell surface markers | B220 ⁻ CD18 ⁺ CD19 ^{int} CD38 ^{hi} CD40+ CD86+ CD138+ MHC II | | | |
| Uptake of opsonized bacteria | No | | | |
| Different phenotypes | | | | |
| Microbial stimulation | Required | NOT required | | |
| L-10 | Required | NOT required | | |
| Lymphoid structure of Peyer's patches | Required | NOT required | | |
| Proliferation | High | Low/negative | | |
| Cell surface marker | CD150 ^{hi} | CD150 ^{low} | | |
| lgA production | High | Low | | |

Supplementary Table S4 Similar and different immunological phenotypes between CD11b⁺ and CD11b⁻ IgA⁺ cells in the small intestine