Supporting online material

Materials and methods

Germ-free mice, microbiota re-associations, and monocolonization

Germ-free Swiss-Webster, C57BL/6, and NMRI mice were derived germ-free as previously described (*S1, S2*) and maintained germ-free in flexible film isolators at the Farncombe Axenic and Germ-free Unit of Central Animal Facility, McMaster University, Canada or in the Clean Animal Facility, University of Bern, Switzerland. C57BL/6 mouse colonies were also re-associated with a low-complexity microbiota, by co-housing with gnotobiotic mice that had been associated with the Altered Schaedler Flora (ASF, Taconic) consisting of 8 different bacteria (*S3*), according to the protocol available at Taconic (www.taconic.com/library), and maintained under barrier conditions in IVC cages in the Farncombe Axenic and Germ-free Unit or in the Clean Animal Facility, University of Bern, Switzerland. The microbiota of these ASF SPF mice was *E. coli*-free and has been characterized in depth by 454 amplicon sequencing (*S4*). These mice were used as ASF experimental mice and as sentinel colonizers for ASF re-associations of germ-free animals. Stable *E. coli* K-12 JM83 monocolonizations were done by intragastric gavage of germ-free animals with 10^{10} CFU JM83 that were aseptically prepared and imported into a flexible film isolator. The gavaged animals were used as colonizers to mono-associate more germ-free mice by co-caging. Bacterial monocolonization was confirmed by selective bacterial plating of fecal samples after 18 hours and at the endpoint of the experiment. All animal experiments were carried out in accordance with the McMaster University animal utilization protocols and the Canadian Council on Animal Care (CCAC) guidelines or in accordance with Swiss federal regulations.

Reversible HA107 colonization and microbiology

D-Ala (200µg/ml)/*m*-DAP (50µg/ml)-supplemented LB cultures were aseptically inoculated from single colonies of *E. coli* HA107 and incubated with shaking at 160 rpm at 37°C for 18 hours. Bacteria were harvested by centrifugation (15 min, 3500X g, 4°C) in a sterile aerosol-proof assembly, washed in sterile PBS and concentrated to a density of 2×10^{10} CFU/ml in PBS, all performed aseptically under a sterile laminar flow hood. The bacterial suspensions were sealed in sterile tubes, with the outside surface kept sterile, and imported into flexible film isolators, where 500 µl (10^{10} CFU) were gavaged into the stomachs of germ-free mice. To generate smaller inoculums, the 2×10^{10} CFU/ml suspension was diluted appropriately in PBS. Inoculum samples were then re-exported from the isolators for bacterial quantitation by plating on supplemented agar plates. Fecal samples exported from the isolator were bacteriologically analyzed to monitor HA107 shedding and bacteriological status of the inoculated mice. For HA107 inoculation of ASF mice, the bacteria were prepared as described above, and the mice were aseptically inoculated by gavage under a sterile laminar flow. For the treatment with killed HA107, the bacteria were prepared as described above, but prior to import into an isolator one aliquot of bacteria was autoclaved for 15 min at 115°C, or incubated in 0.2 % peracetic acid/PBS for 40 min and washed twice in sterile PBS (a neutral pH was verified in the final bacterial suspension). Germ-free status of HA107 treated mice

was confirmed as developed for germ-free animal monitoring as follows. Aseptically prepared fecal and cecal content samples were suspended and serially diluted in sterile PBS and plated on D-Ala/*m*-DAP-supplemented BHI or Wilkinson blood agar plates or suspended in 20 ml of D-Ala/*m*-DAP-supplemented BHI medium and incubated aerobically at 37°C. Germ-free status was further verified by the culture-independent methods of microscopy and flow cytometry of SytoxGreen (Invitrogen) and Gram stained cecal contents.

Generation and characterization of strain HA107

The *m*-DAP auxotrophic Δ*asd* mutant *E. coli* K-12 strain JM83 derivative χ6096 has been described (*S5*). Complete in-frame deletions of the genes *alr* and *dadX* were generated sequentially using the Lambda Red system using plasmids pKD4, pKD20, and pCP20 as described by Datsenko and Wanner (*S6*), by allelic exchange of the *alr* coding sequence with the kanamycin resistence cassette of template plasmid pKD4 (*S6*), pCP20-mediated removal of the kanamycin resistance cassette, and a second allelic exchange of the *dadX* coding region with the kanamycin resistance cassette, yielding strain HA107 (Δ*asd* Δ*alr* Δ*dadX::kan*^{R}). The following mutagenic primers were used:
GAATTAGGTAATTAAAGCAAACACTTATCAAGGAACACAAGTGTAGGCTGGAGCTG CTTC (alr-P1),
ACGCCGCATCCGGCACAGACAATCAAATATTACAGAACGACATATGAATATCCTCCT TA (alr-P2),
TCCGGGCCATTTACATGGCGCACACAGCTAAGGAAACGAGGTGTAGGCTGGAGCT GCTTC (dadX-P1),
GCACCCAGAAGACGTTGCCTCCGATCCGGCTTACAACAAGCATATGAATATCCTCC TTA (dadX-P2). All 3 chromosomal deletions were confirmed by PCR and phenotypic characterization. Although strictly D-Ala and *m*-DAP auxotrophic, HA107 grows normally in supplemented LB (see supporting Figure S1C), and wild type parental strain JM83 and strain HA107 are indistinguishable by live bacterial surface analysis by bacterial flow cytometry (protocol see below). HA107 suspensions in non-supplemented PBS were as stable as wild type parental strain JM83. Removal of growth supplements and subculture of HA107 in non-supplemented medium was non-permissive for bacterial proliferation, as shown by FACS proliferation assay of PKH26 (Sigma) stained HA107 and microscopic examination of bacteria embedded in non-supplemented LB agar. Prototrophic HA107 variants that have become *m*-DAP- and D-Ala-independent were never re-isolated.

Other bacterial strains

HA116 (JM83 Δ*rfaC*) was generated by deletion of *rfaC* in the JM83 background by allelic exchange with a *tetRA* resistance cassette using the Wanner Lambda Red system as described elsewhere (*S7*), using primers
AGAACTCAACGCGCTATTGTTACAAGAGGAAGCCTGACGGTTAAGACCCACTTT-CACATT and ATGAATGAAGTTTAAAGGATGTTAGCATGTTTTACCTTTACTAAGCA-CTTGTCTCCTG. An in frame deletion was confirmed by PCR and the deep-rough phenotype. Avirulent *Salmonella typhimurium* SL1344-derived strain M557 (SL1344 Δ*invG sseD::aphT*) has been described (*S8*).

Live bacterial flow cytometry

3ml LB cultures were inoculated from single colonies of plated bacteria (or directly from ASF fecal pellets to generate the data depicted in Figure 1I) and cultured overnight at 37°C without shaking. 1ml of culture was gently pelleted for 3mins at 7000 rpm in an Eppendorf minifuge and washed 3 times with sterile-filtered PBS/2% BSA/0.005% NaN_3 before re-suspending at a density of approximately 10^7 bacteria per ml. Intestinal lavages were collected as described previously (*S9*), and spun at 14 000 rpm in an Eppendorf minifuge for 10mins, the supernatant sterile filtered to remove any bacteria-sized contaminants, and serially diluted in PBS/2% BSA/0.005% NaN_3. 25 µl of the diluted intestinal lavage and 25 µl bacterial suspension (approx. 10^5 bacteria per FACS sample) were then mixed and incubated at 4°C for 1h. Bacteria were washed twice in PBS/1% BSA/0.005% NaN_3 before resuspending in monoclonal FITC-anti-mouse IgA (BD Pharmingen). After a further hour of incubation the bacteria were washed twice with PBS/2% BSA/0.005% NaN_3 and then resuspended in 2% *para*-formaldehyde/PBS for acquisition on a FACSCalibur or FACSArray using FSc and SSc parameters in logarithmic mode. Data were analyzed (Fig. S3) using FlowJo software (Treestar, USA). Bacterial titres were calculated with Prism 5 software using curve fit algorithm log(agonist) vs. response – variable slope (four parameters) with constant equal constraint of bottom and top. Bacterial specific titres are expressed as –LogEC50 values.

LogEC50 value calculation for IgA-bacterial binding measured in live bacterial flow cytometry

For each animal analyzed, ELISA was used to determine the total IgA concentration in an undiluted aliquot of the same intestinal wash sample as used to surface stain *E. coli* K-12 for flow cytometry analysis. This value was used to calculate the total IgA concentration at each dilution of intestinal wash used for flow cytometry staining of *E. coli* K-12 and was plotted against the geometric mean fluorescence of FITC-anti-mIgA staining of *E. coli* as measured on the BD FACSArray SORP (See Fig. S3). Graphpad Prism 5 was then used to fit four parameter curves to the data: Y=Bottom + (Top-Bottom)/(1+10^((LogEC50-X)*HillSlope). From the curve parameters the LogEC50 value was extracted, which when anti-logged, corresponds to the total concentration of IgA required to give approximately 50% of the maximum observed IgA binding. -LogEC50 therefore corresponds to the Log(1/(total [IgA] giving 50% binding).

ELISAs

Total concentrations of antibody isotypes in mouse serum and cleared intestinal lavage were determined by sandwich ELISA (*S10*). Coating antibodies were goat-anti-mouse IgG1, 2a, 2b, A and M (Serotech) and detection antibodies were HRP-conjugated anti-mouse IgG, IgM or IgA (Sigma). Standards were myeloma-derived purified IgG1, IgG2a, IgG2b, IgA and IgM from Hycult.

Small intestinal, Peyer's patch, and mesenteric lymph node FACS

1 cm sections of duodenum were dissected and flushed with PBS. Each section was then dissected to open the gut tube and cut into 6 slices. These were incubated in PBS containing 1 % BSA (Sigma) and Liberase C1 (Roche) for 25 mins at 37°C with shaking. Digested tissues were disaggregated through a 20 µm cell strainer and the resulting cell

suspension stained with PerCP-anti-CD45, APC-anti-B220 (all BD Pharmingen). The cells were then fixed and permeabilized using BD Fix/Perm following the manufacturer's instructions and stained with FITC-anti-IgA and PerCP-anti-CD45. Peyer's patches and mesenteric lymph nodes were dissected, removing all surrounding tissue, and Liberase digested as described above. Cells were stained with FITC-anti-GL-7 and APC-anti-B220 (all BD Pharmingen).

Analysis of V(D)J Cα cDNA sequences.

Oligo-dT–primed cDNA was synthesized with mRNA from approximately 5 mm of ileum that had had the Peyer's patches removed. Sequences were amplified by PCR as described (*S11*). Primers were 5′–CTCAGGCCATTCAGAGTACA–3′ (IgHa) and a degenerate (HB1-HB19) mix (*S11*) for the VH region. PCR products were purified and subcloned for automated sequencing.

Immunohistochemistry and immunofluorescence

Small intestinal tissues were embedded in OCT, snap-frozen in liquid nitrogen and cut at 7µm thickness. Sections were fixed with 4 % PBS/*para*-formaldehyde for 30 min, rinsed 3 times in PBS, blocked under PBS/1 % goat normal serum for 1 hour, and incubated under 5 µg/ml FITC-rat-anti-mouse-IgA monoclonal antibody (BD Pharmingen) and 0.5 µg/ml 4', 6'-diamidino-2-phenylindole (DAPI; Sigma) diluted in PBS/1 % goat normal serum for 45 min, or with rabbit-anti-Ki67 monoclonal antibody (1:100, clone SP6, Thermo) followed by rinsing 3 times in PBS and staining with goat-FITC-anti-rabbit antiserum (Jackson Immunoresearch) and DAPI. Sections were then rinsed 3 times in PBS, fixed a second time with 4 % PBS/*para*-formaldehyde for 30 min, rinsed 3 times in PBS, and mounted under Vectashield (Vector Laboratories). Sections were imaged under a Nikon Eclipse 800 fluorescence microscope, and digital 2 color images were colorized and processed using Adobe Photoshop software (version CS3). Cell quantitations were done by blinded examination of >5 random 20X microscopic fields per sample. IgA plasma cell numbers were expressed as number per intestinal villus or per cross section (complete cross sections were quantitated). For Immunohistochemistry frozen sections were stained with hematoxylin/eosin (HE) or antibodies B220/CD45R for B cells (RA3-6B2, Pharmingen 553084; 1:400 in PBS/0.15% BSA), CD21/35 for CR1 (8C12, Pharmingen, San Diego, CA; 1:100) and PNA for germinal center B cells (Vector L-1070; 1:100) as described (*S12*).

Statistics

Statistical analysis and linear regression analysis were performed in Graphpad Prism 5 software. Statistical tests were 1-way ANOVA with Tukey post-test or unpaired student t-test, as indicated, with a significance level of $p<0.05$.

Supporting figures and legends

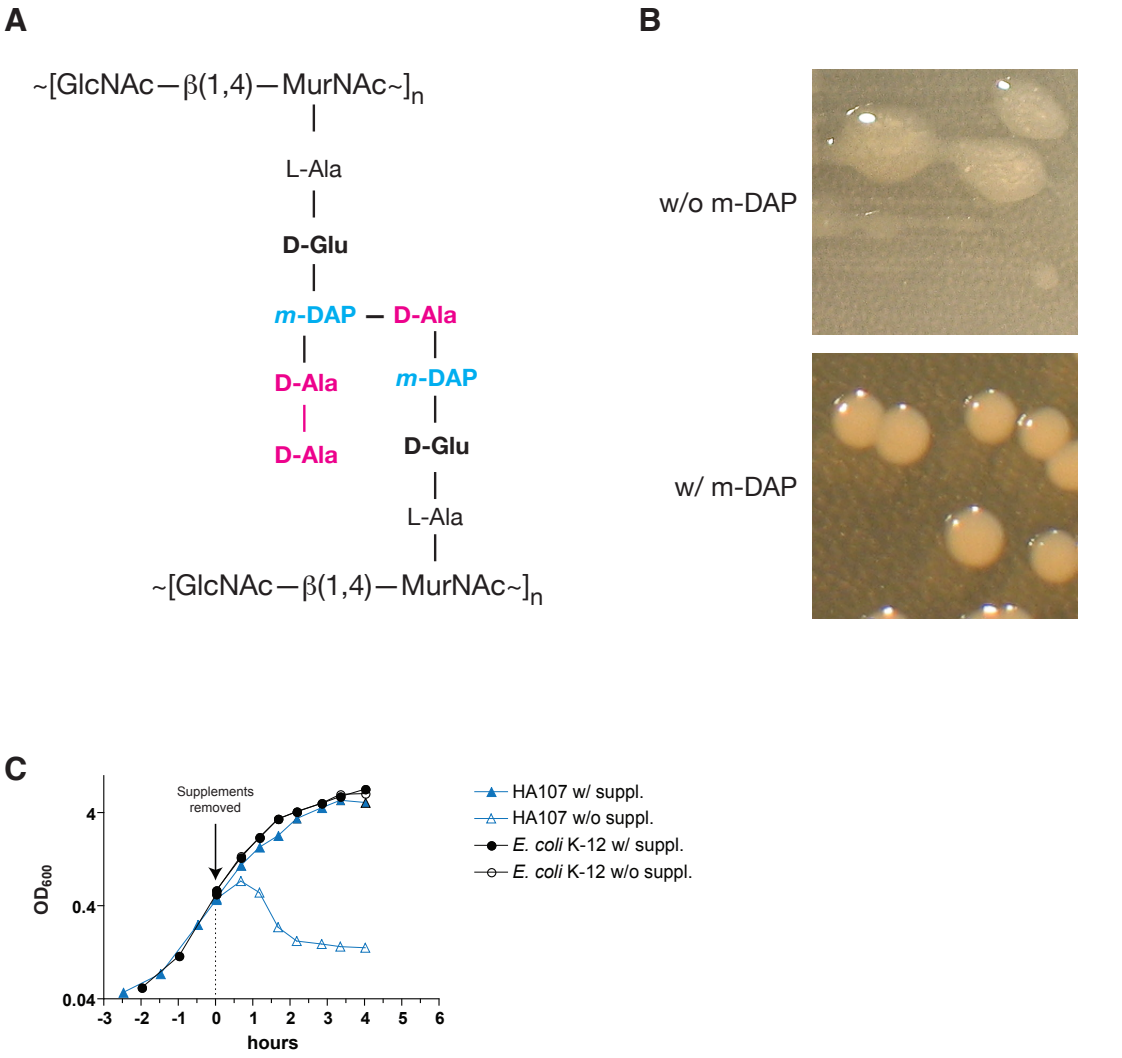


Figure S1. Phenotypes and optimization of reversibly colonizing *E. coli*. (A) The polyglycan strands of all known bacterial peptidoglycan types are crosslinked by peptide bonds formed between amino acids of the N-acetyl-muramyl pentapeptides, which contain non-standard, prokaryote-specific amino acids, such as D-glutamine (D-Glu), *meso*-Diaminopimelic acid (*m*-DAP, blue), and D-Alanine (D-Ala, red) in case of the *E. coli* type peptidoglycan shown here. **(B)** Some of the mice that had been gavaged with doses of 10^{10} CFU of *m*-DAP auxotrophic strain χ6096, a derivative of *E. coli* K-12 wildtype strain JM83, were colonized with high numbers of a χ6096 variety that was culturable not only on *m*-DAP supplemented medium (w/ *m*-DAP), but also on non-supplemented medium (w/o *m*-DAP) where they formed altered soft mucoid colonies. **(C)** Δ*asd* Δ*alr* Δ*dadX E. coli* K-12 strain HA107 (blue triangles) and its wild type parental strain JM83 (black circles) were grown overnight, washed in PBS, and diluted in LB medium supplemented (filled symbols) or not supplemented (open symbols) with *m*-DAP/D-Ala and incubated shaking at 37°C. The optical density (OD_{600}) of each culture was measured at the indicated time points, to generate growth curves. At time point 0 (arrow) during mid-log phase, cultures were washed in PBS to remove *m*-DAP and resuspended in the same volume of non-supplemented LB medium and further incubated.

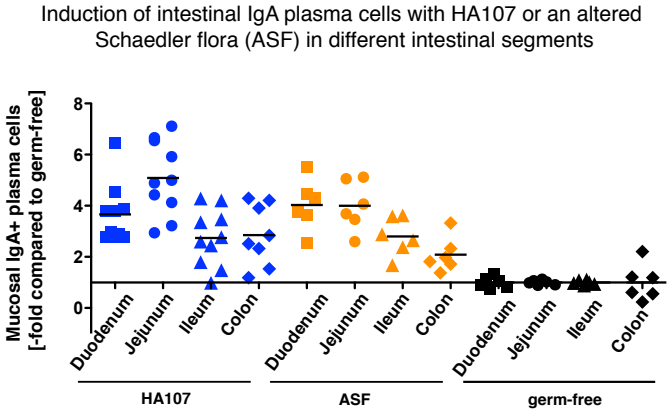


Figure S2. Induction of intestinal IgA plasma cells with HA107 or an altered Schaedler flora (ASF) in different intestinal segments. Germ-free mice were either gavaged 6 times over 2 weeks with 10^{10} CFU of HA107 or ASF colonized by cohousing with ASF mice. Histological analysis of IgA plasma cell numbers in different segments of the intestine (Duodenum, 3-4 cm from the stomach; jejunum, middle of the small intestine; ileum, terminal small intestine 3-4 cm from the cecum; colon, proximal colon) showed very similar increases over germ-free controls between HA107-treated and ASF-colonized animals in each intestinal segment. Differences between HA107 and ASF treatment were non-significant for all 4 intestinal segments ($p \geq 0.05$; 1-way ANOVA). Data points represent individual mice, and data were pooled from two experiments.

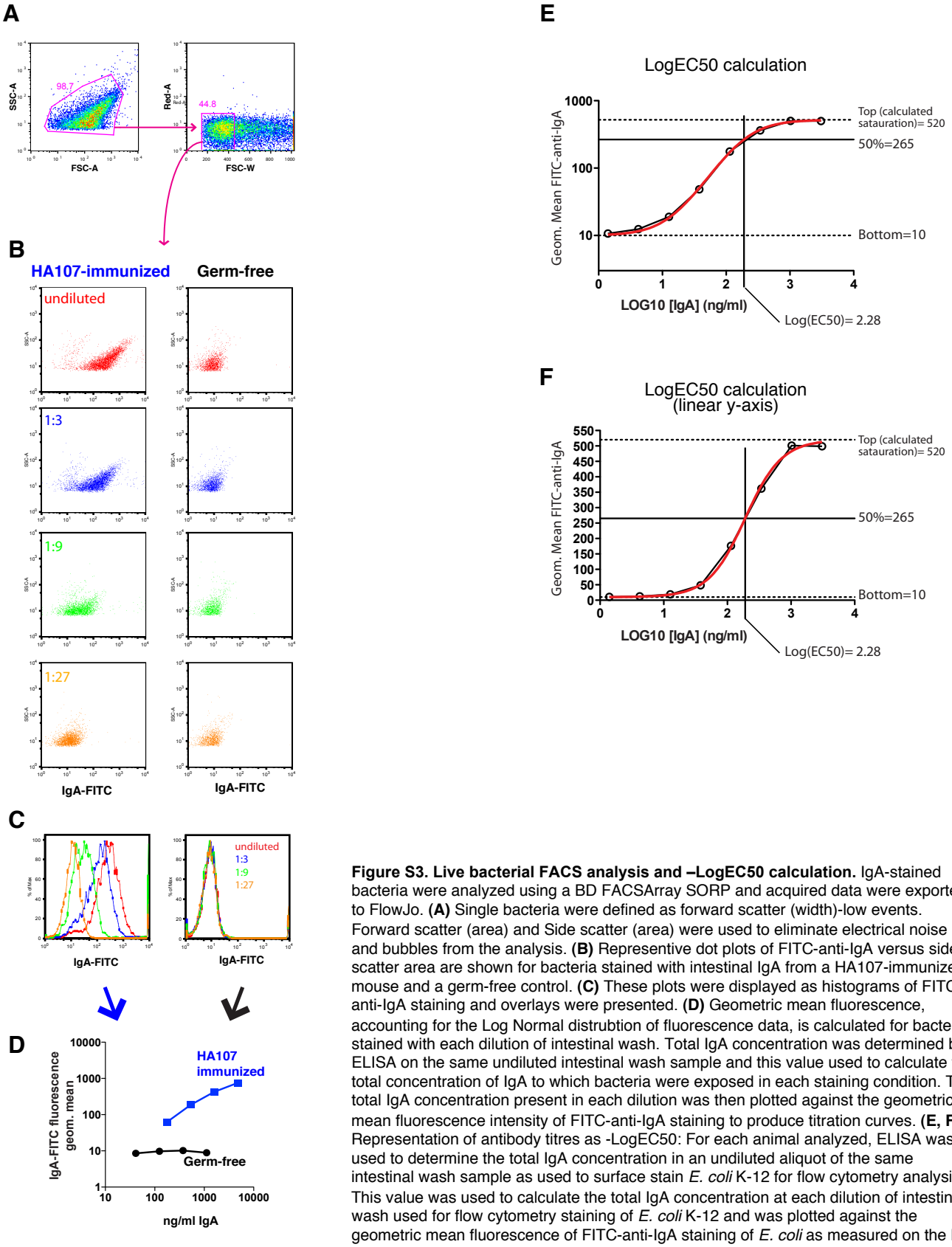


Figure S3. Live bacterial FACS analysis and –LogEC50 calculation. IgA-stained bacteria were analyzed using a BD FACSArray SORP and acquired data were exported to FlowJo. **(A)** Single bacteria were defined as forward scatter (width)-low events. Forward scatter (area) and Side scatter (area) were used to eliminate electrical noise and bubbles from the analysis. **(B)** Representive dot plots of FITC-anti-IgA versus side-scatter area are shown for bacteria stained with intestinal IgA from a HA107-immunized mouse and a germ-free control. **(C)** These plots were displayed as histograms of FITC-anti-IgA staining and overlays were presented. **(D)** Geometric mean fluorescence, accounting for the Log Normal distrubtion of fluorescence data, is calculated for bacteria stained with each dilution of intestinal wash. Total IgA concentration was determined by ELISA on the same undiluted intestinal wash sample and this value used to calculate the total concentration of IgA to which bacteria were exposed in each staining condition. The total IgA concentration present in each dilution was then plotted against the geometric mean fluorescence intensity of FITC-anti-IgA staining to produce titration curves. **(E, F)** Representation of antibody titres as -LogEC50: For each animal analyzed, ELISA was used to determine the total IgA concentration in an undiluted aliquot of the same intestinal wash sample as used to surface stain *E. coli* K-12 for flow cytometry analysis. This value was used to calculate the total IgA concentration at each dilution of intestinal wash used for flow cytometry staining of *E. coli* K-12 and was plotted against the geometric mean fluorescence of FITC-anti-IgA staining of *E. coli* as measured on the BD FACSArray SORP (Black points and connecting line). Graphpad Prism 5 was then used to fit four parameter curves to the data - red line Y=Bottom + (Top-Bottom)/(1+10^((LogEC50-X)*HillSlope). From the curve parameters the LogEC50 value was extracted, which when anti-logged, corresponds to the total concentration of IgA required to give approximately 50% of the maximum observed IgA binding. -LogEC50 therefore corresponds to the Log(1/(total [IgA] giving 50% binding).

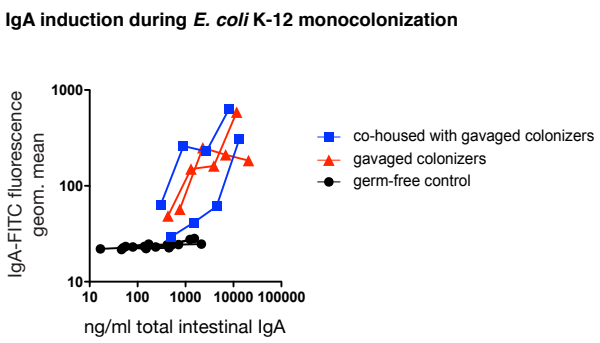


Figure S4. Induction of specific *E. coli* K-12-binding intestinal IgA is also induced in stably wild type K-12 mono-associated mice. Germ free NMRI mice were mono-associated for 28 days by gavage with 10^{10} CFU of JM83 (red triangles), as well as by co-housing of germ-free mice with the gavaged mice (blue squares). Germ-free control mice are depicted as black circles. Intestinal lavages were analysed by ELISA/bacterial FACS, showing equivalent IgA responses in gavaged and mice mono-colonized by cohousing with the gavaged animals. Data curves represent individual mice from one of two experiments.

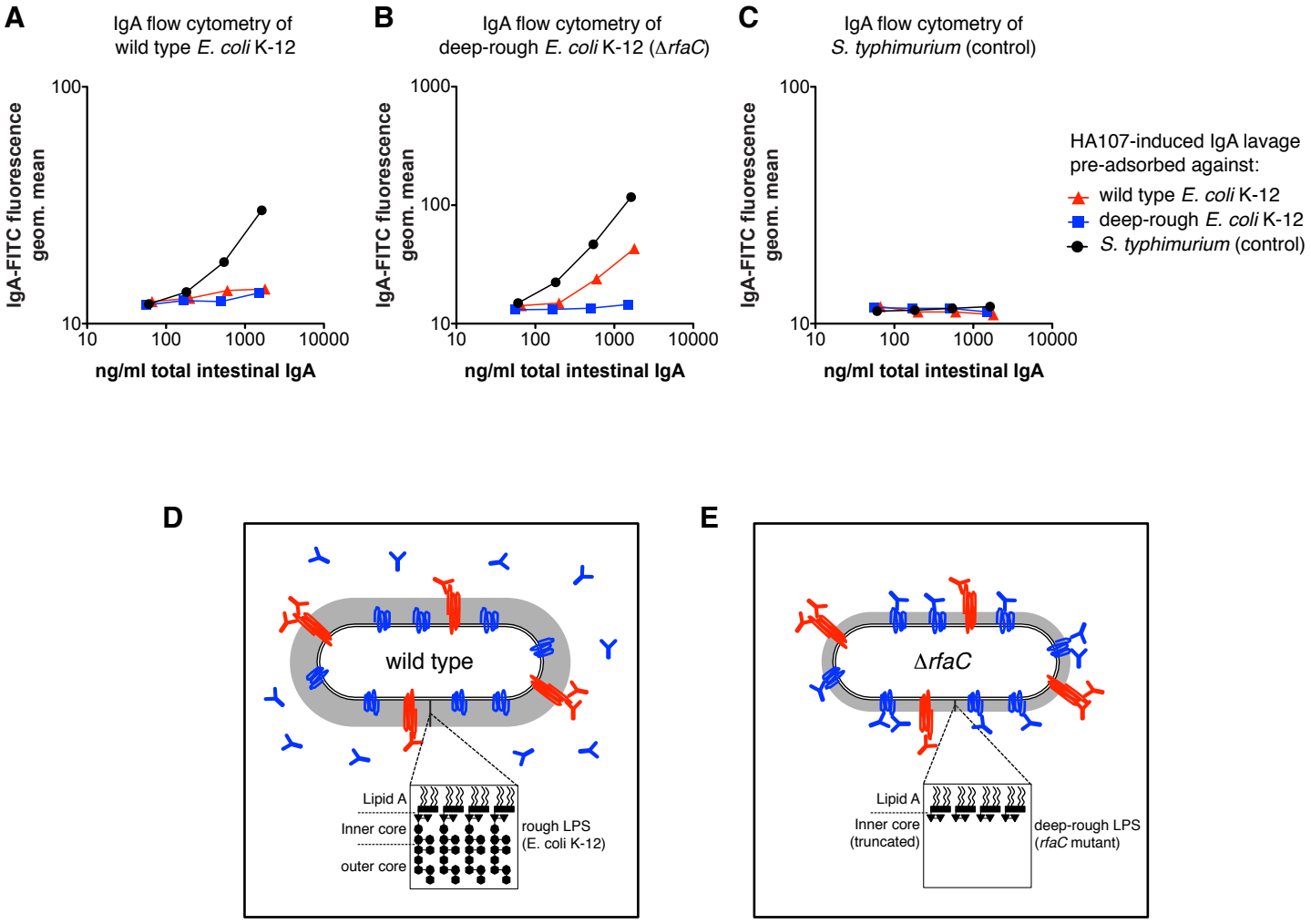


Figure S5. LPS core antigen partially shields surface antigen on *E. coli* K-12 that part of the HA107-induced IgA is directed against. Intestinal wash IgA induced by HA107 was pre-adsorbed against live wild-type *E. coli* K-12, deep-rough *E. coli* K-12 Δ*rfaC*, or *S. typhimurium* (control), by 1h pre-incubation with 10^9 CFU/500µl live bacteria. After sterile-filtration the pre-adsorbed intestinal washes were used for flow cytometry of **(A)** wildtype *E. coli* K-12, **(B)** deep-rough *E. coli* K-12 Δ*rfaC*, and **(C)** *S. typhimurium*, respectively. Deep-rough *E. coli* K-12 pre-adsorption removed *E. coli* K-12 wild type-binding IgA as effectively as wild-type *E. coli* K-12, suggesting that LPS-binding IgA contributes little to IgA-*E. coli* binding. In contrast, *E. coli* K-12 wild type pre-adsorption only partially removed the IgA that binds deep-rough *E. coli*, demonstrating bacterial surface epitope shielding by LPS core antigen. Data are representative of two independent experiments. **(D, E)** Schematics to illustrate the findings. (D) On wild type bacteria part of the surface epitopes (blue) is shielded by LPS core antigen polysaccharide (gray) and is unaccessible to specific IgA (blue Y shapes). (E) On the *rfaC* mutant, LPS core antigen is truncated, resulting in reduced epitope shielding and a larger repertoire of surface epitopes accessible to *E. coli* specific IgA. Insets, schematics of LPS core structure; red shapes, antigens and specific IgA unaffected by shielding; blue shapes, antigens and specific IgA affected by LPS core shielding.

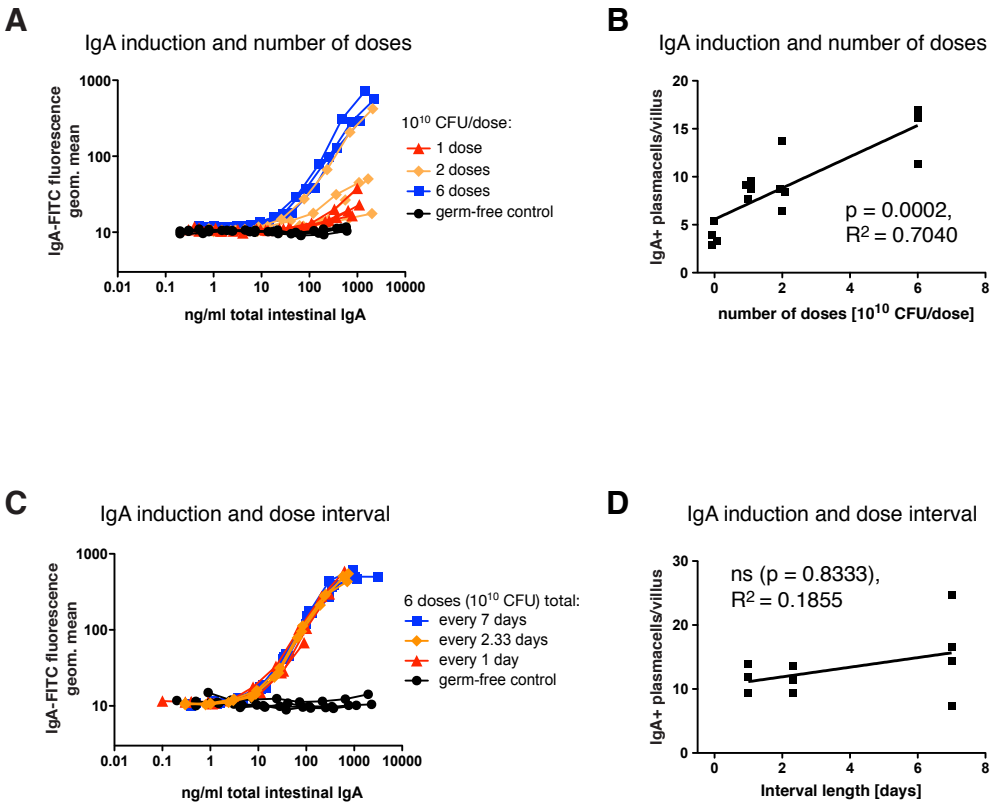


Figure S6. There is an additive relationship between HA107-induced IgA plasma cell numbers with the number of HA107 doses but not with the dose interval length. (A) Flow cytometry analysis of IgA-*E. coli* K-12 binding using intestinal washes isolated at day 14 from mice treated with the indicated number of HA107 doses spread over one week. Data curves represent individual mice that are also depicted in main figure 3A. **(B)** Linear regression analysis of duodenal IgA plasma cell numbers depending on numbers of bacterial doses. Data points represent individual mice and are identical to those depicted in main figure 3B. **(C)** To examine the relationship between IgA induction and dose interval, three groups of mice were given 6 doses of HA107 in intervals of 1, 2-3, and 7 days, respectively. Intestinal washes were analyzed by IgA-specific ELISA and live bacterial cytometry to measure IgA-*E. coli* K-12 binding. Data curves represent individual mice that are also depicted in main figure 3C. **(D)** There were also no significant differences in intestinal IgA plasma cell numbers and dose interval. Linear regression analysis of numbers of duodenal IgA plasma cells is shown. (see main article Figure 3). ns, non-significant (linear regression analysis). Data points represent individual mice and are identical to those depicted in main figure 3B.

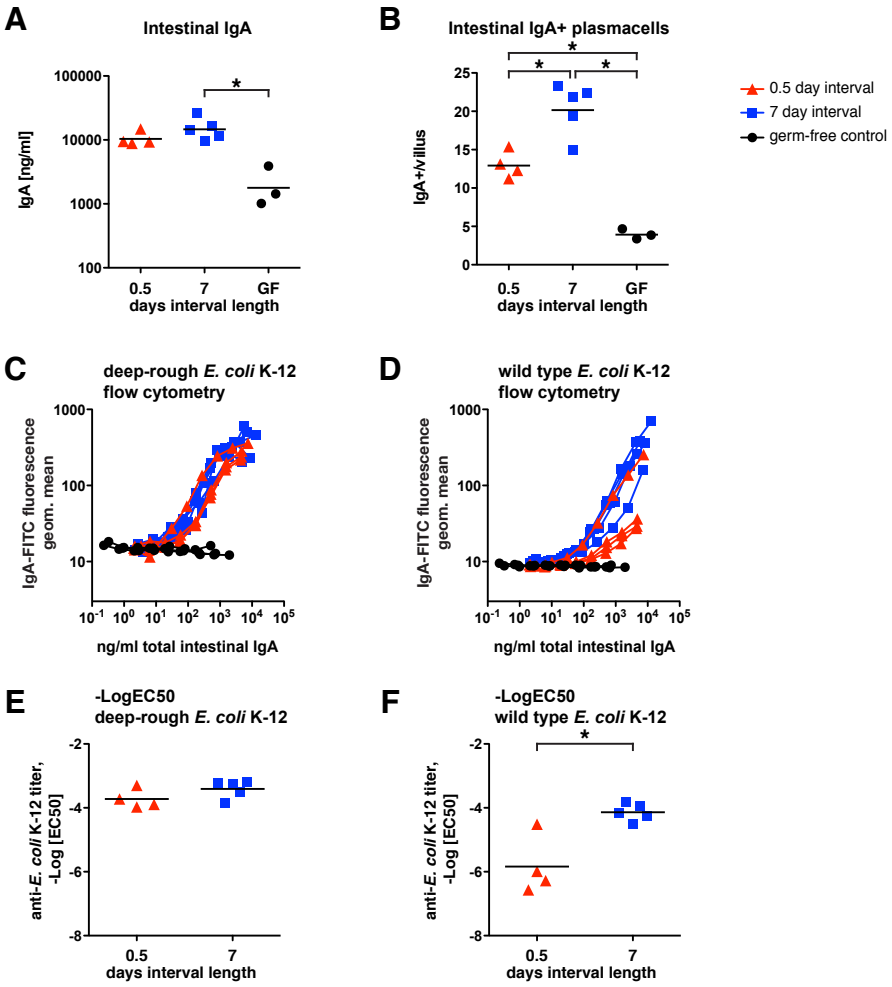


Figure S7. IgA responses when an identical exposure of HA107 is delivered to the intestine within 24h or spread over 2 weeks. Germ-free Swiss-Webster mice were given 3×10^{10} CFU HA107 within 24 hours (red triangles) by three gavages of 10^{10} CFU equally spaced out over 24 hours (every 12 hours) or equally spaced out within 2 weeks (every 7 days; blue squares) with all mice analyzed at day 24. Controls were kept germ-free (black circles) **(A)** Total IgA in intestinal lavages was determined by IgA-specific ELISA. *, $p<0.05$ (1-way ANOVA) **(B)** Numbers of duodenal IgA plasma cells were determined by fluorescent immunohistology on anti-IgA-FITC stained crysections. *, $p<0.05$ (1-way ANOVA). **(C, D)** Intestinal IgA specificity was determined by live bacterial IgA-specific flow cytometry of deep-rough *E. coli* K-12 Δ*rfaC* (panel C) and wildtype *E. coli* K-12 (panel D). **(E, F)** -LogEC50 values for IgA-bacterial binding analysis using bacterial flow cytometry and IgA ELISA (see Fig. S3). *, $p=0.0051$ (unpaired Student-t). Data points and curves represent individual mice from one experiment.

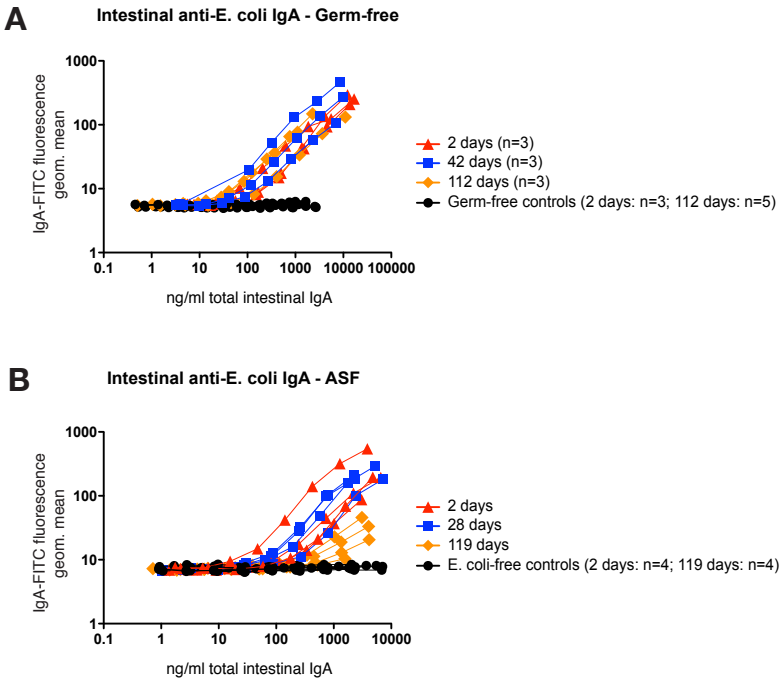
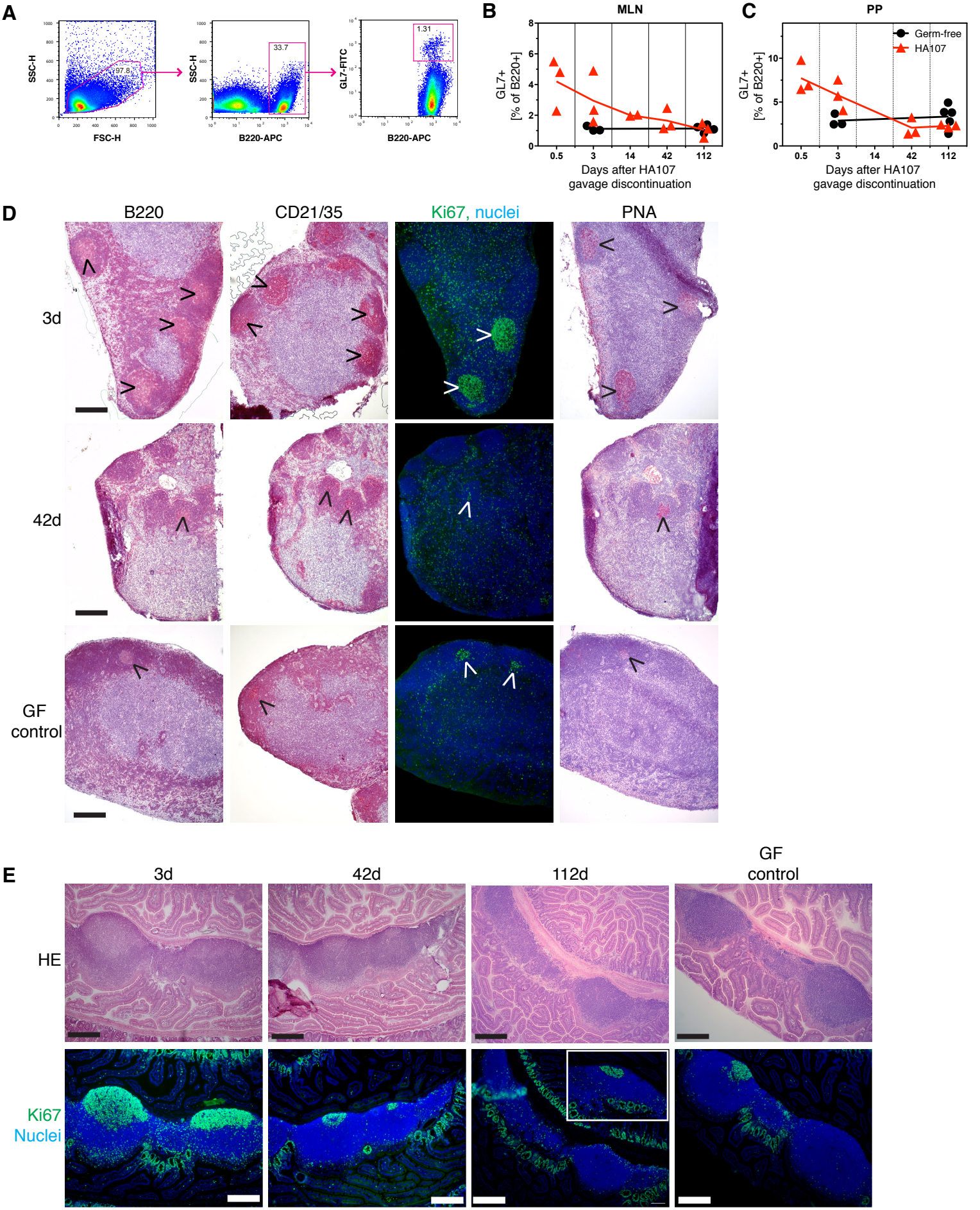


Figure S8. Commensal-induced specific IgA is long-lived in germ-free mice, but rapidly abrogated in the face of ongoing IgA induction in microbiota-colonized mice – bacterial FACS titration curves used for calculation of –LogEC50 titres. **(A)** Germ-free Swiss-Webster mice were gavaged 6 times over 2 weeks with 10^{10} CFU HA107 and kept germ-free for up to 112 days after discontinuation of HA107 treatment. Intestinal washes at the indicated time points and analysed by IgA specific ELISA and live bacteria FACS (see supporting figure S2 for technical details). Matched germ-free mice served as controls (black circles; day 2 and 112). **(B)** Colonized mice containing an *E. coli*-free microbiota were treated as described in (A) and kept under barrier conditions for up to 119 days after discontinuation of HA107 treatment, and analyzed as above at the indicated time points. Matched colonized mice recruited from littermates served as controls on day 2, 28, and 119 (black circles; n=4 controls from day 28 are not depicted). Data curves represent individual mice that are also depicted in main figure 4.



Hapfelmeier et al., Supporting Figure S9

Previous page: **Figure S9.** Enlarged GL-7+ germinal center B-cell populations are short-lived after discontinuation of HA107 exposure. Germ-free Swiss-Webster mice were gavaged 6 times over 2 weeks with 10^{10} CFU HA107 and kept germ-free for up to 112 days after discontinuation of HA107 treatment. **(A)** Peyer's patch and MLN cells were stained with anti-B220 and anti-GL-7 antibodies as described in Materials and Methods and analyzed by FACS. **(B)** MLNs and **(C)** Peyer's patches (PP) were taken at the indicated time points and the frequency of GL-7+ B220+ cells compared between HA107 exposed (red triangles) and germ-free control mice (black circles). Connecting lines are drawn between the means. **(D)** MLN**s** were taken at the indicated time points and processed for immunohistology. Tissue sections were stained for B220 (B cells), CD21/CD35 (complement receptor 1/2, on follicular and germinal center B cells and follicular DCs), Ki67 (proliferation marker), and with PNA (germinal center B cells). Positive cells appear red. Arrowheads point to staining indicative of germinal centers. Representative serial sections (apart from panels "CD21/35-3d" and "CD21/35-GF control" showing representative but non-serial sections) from mice from 3 experiments (pooled group sizes n≥6) are shown. Scale bars: 200 µm **(E)** Intestinal samples with Peyer's patches were taken at the indicated time points and processed for immunohistology. Tissue sections were stained with H+E and for Ki67 (proliferation marker). Inset illustrates a representative example showing a small germinal center. Scale bars: 200 µm. Serial sections representative of 2 experiments (n≥3) are shown.

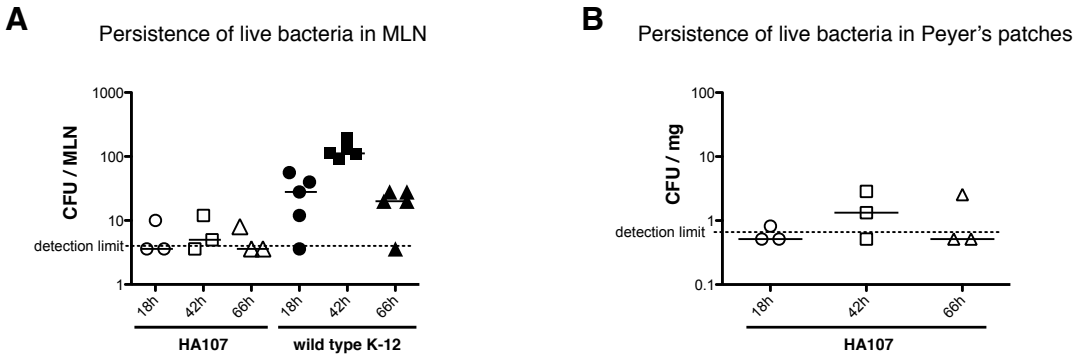


Figure S10. Analysis of live bacterial loads in MLN and Payer's patches. Germ-free mice were gavaged with a single dose of 10^{10} CFU HA107 (open symbols) or wild type *E. coli* K-12 (filled symbols). At the indicated times after bacterial gavage, bacterial loads in **(A)** MLN and **(B)** Peyer's patches (only HA107; contaminating wild-type bacteria from the colonized lumen make determination of Peyer's patch load unreliable) were determined. Bars show medians. Data points represent individual mice from one experiment.

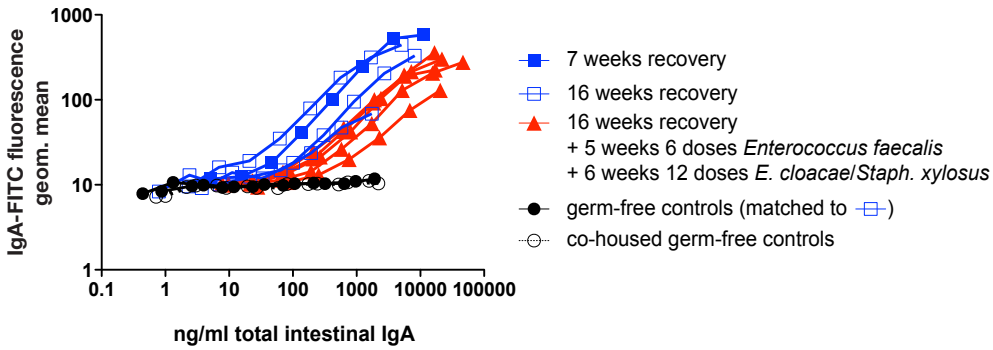


Figure S11. Analysis of HA107-induced IgA in germ-free mice after gavage with other commensal bacterial species. Germ-free Swiss-Webster mice were given 6 doses of 10^{10} HA107 over 2 weeks, and recovered germ-free for up to 16 weeks. 5 of these mice were further treated with successive gavages with approx. 10^{11} CFU *Enterococcus faecalis* (6 doses over 5 weeks) followed by successive alternating doses of approx. 10^{10} CFU *Enterobacter cloacae* and *Staphylococcus xylosus* (2×6 doses over 6 weeks). Negative controls were kept germ-free (black filled symbols) or co-housed without treatment (open black symbols) until week 16. Analysis of intestinal washes by IgA-*E. coli* binding by bacterial flow cytometry showed long lived *E. coli* specific IgA in the HA107-treated mice at week 7 and 16 (blue symbols). Analysis of IgA from the *E. faecalis*/*E. cloacae*/*Staph. xylosus*-treated animals (red symbols) showed a shift of the IgA-*E. coli* binding curves, with attrition of the original specific antibody response against HA107. Data curves represent individual mice from one experiment.

Supporting tables and legends

Supporting Table 1. Early bacterial translocation to the mesenteric lymph nodes in germ free C57BL/6 wild type mice and in germ-free $J_H^{-/-}$ mice following an intestinal challenge dose of *E. coli* K-12

Mouse strain	C57BL/6 (germ free)	C57BL/6 (germ free)	$J_H^{-/-}$ (germ free)	$J_H^{-/-}$ (germ free)
Challenge dose	PBS	$5x10^9$ *E. coli* K12	PBS	$5x10^9$ *Escherichia coli* K12
C.F.U./Mesenteric lymph node at 20 hours (mean±S.D.)	Undetectable (n=4)	192±67 (n=8)	Undetectable (n=4)	180±95 (n=8)

The translocation of wild-type *E. coli* K12 was measured in C57BL/6 and $J_H^{-/-}$ germ free mice that lack all antibody isotypes (*S13*, n=8 for each strain) following a challenge dose of $5x10^9$ cfu given by gavage into the stomach. Control mice (n=4 for each strain) were gavaged with the PBS vehicle only. Mice were maintained in axenic conditions apart from the challenge with wild-type *Escherichia coli*. At 20 hours following gavage, the mesenteric lymph nodes were aseptically dissected, disaggregated and plated for bacterial colony numbers on LB agar.

Supporting Table 2. In-frame V(D)J Cα rearrangements. Closest germline VH family of isolated clones were assigned using the IMGT/V-QUEST program from IMGT®, the international ImMunoGeneTics information system® http://www.imgt.org (founder and director: Marie-Paule Lefranc, Montpellier, France). Sequences were derived from three Swiss Webster mice that were conditioned with 6 successive doses of 10^{10} CFU of HA107. Samples were taken at 112 days after the last dose of HA107. Differences with the closest germline sequence are underlined; replacement mutations are both underlined and bold, whereas silent mutations are only underlined. When there are two equivalent D regions possible they are denoted with the clone number followed by a or b. FR, framework; CDR, complementarity-determining regions (1-3); N/A, not applicable; R/S, ratio of replacement to silent mutations.

						R/S	
Clone	V_H	(P)N	D_H	(P)N	J_H	FR	CDR
1-33	TGT**A**CAAGA	AGGGG	GGGAC**T**GC		ACTACTTTGACTACTGG	1.4 (7/5)	2.5 (5/2)
1-13	TGTGCAAG	GA	AACTGGGAC	TAGGA	TACTTCGATGTCTGG	0.5 (1/2)	1.0 (1/1)
1-25	TGTGCAAGA		TGG**G**GATA		TACTTTGACTACTGG	2.0 (2/1)	N/A (1/0)
1-31	TGTGCAAGA	A			ACT**T**TTTTGACTACTGG	1.3 (4/3)	N/A (1/0)
1-38	TGT**T**CAAGA	GAA	TACTATA**TG**TATTATAG	AGGCCTCG	CCTGGTTTGCTTACTGG	3.0 (3/1)	3.0 (6/2)
1-42	TGT**T**CAAG	CAGTCCA	CTAC**G**ATGGTA	CCCGGGG	TACTTTGACTACTGG	0.6 (4/7)	1.0 (1/1)
1-30	TGTGCAAGA	TTGG			ATGCTATGGACTACTGG	1.1 (9/8)	1.3 (5/4)
1-32	TGTGCTAGA		TGG**A**A**C**CT	GG	TTGACT**GG**TGG	1.6 (8/5)	1.5 (3/2)
1-23	TGTGCAAGA	AAGAGGG	ATATAA	GGCTCT	CCTGGTTTGCTTACTGG	2.0 (2/1)	0.0 (0/2)
1-17	TGTGCAAGA	TTGG			ATGCT**T**TGGACTACTGG	2.3 (7/3)	3.0 (3/1)
1-22	TGTGCAAGA	G	CCTACTATAGTGACT	GCCCT	TTTGCTTACTGG	6.0 (6/1)	7.0 (7/1)
1-24	TGTGCAA	CTGGGA	TCTACTATGGTAAC	CTGG	CCTGGTTTACTTACTGG	1.3 (4/3)	7.0 (7/1)
1-39	TGT**A**CAAGA		GGGAC	CG	ACTTTGACTACTGG	3.0 (18/6)	1.7 (5/3)
1-40	TGTGCAAGA		TGG**G**GA	TAT	TACTTTGACTACTGG	3.0 (3/1)	4.0 (4/1)
1-8	TGTGCCAG	G	TATGGGT**C**CG	GAG	GGTACTTCGATGTGG	1.6 (11/7)	3.0 (9/3)
1-7	TGTG**C**CAGAGA	GAGCCCAG	CTG**G**AG**G**GCC	T	TTT**A**CTTACTGG	5.0 (15/3)	2.0 (4/2)
2-68	TGTGCAAGA	TCGG	TATTACTACG	ACC	GTTTGCTTACTGG	0.0 (0/1)	N/A (0/0)
2-77	TGTGCAAGA	TCGG	TATTACTACG	ACC	GTTTGCTTATTGG	1.5 (6/4)	N/A (1/0)
2-82a	TGTGCAAGA	GAGAGGG	AATTGGG	CCAG	TGGTATTTCGATGTCTGG	0.7 (5/7)	2.5 (5/2)
2-82b	TGTGCAAGA	GAGAGGGAATT	GGGCCAG		TGGTATTTCGATGTCTGG	0.0 (0/1)	N/A (1/0)
2-79a	TGTGCTAG	GGAG	GGGAC	GG	ATGCTATGGACTACTGG	2.4 (19/8)	N/A (10/0)
2-79b	TGTGCTAG	GGAGGG	GACGG		ATGCTATGGACTACTGG	7.0 (7/1)	1.0 (1/1)
2-56	TGTGCAAG		TGGTAGCTACG	TGG	GGTTTGCTTACTGG	N/A (10/0)	N/A (9/0)
2-72	TGTGCAAGA	GGA	CTGACT**C**GG	GGC	TTT**C**CTTACTGG	8.0 (8/1)	2.5 (5/2)
2-86	TGTGCAAGA	GGA	CTGACT**C**GG	GGC	TTT**C**CTTACTGG	0.7 (2/3)	N/A (2/0)
2-94	TGTGC		CT**C**CT**T**TA**A**TAAC	C	CCTGGTTTGCTTACTGG	4.0 (8/2)	1.3 (4/3)
2-80	TGTACAA	GATGGGGG	CAGCTCGGACG**A**C	GGGGGG	GTTTTGCTTACTGG	5.0 (5/1)	5.0 (5/1)
2-67	TGTG**T**AAGA	GCCCCT	TACTATG**A**TAA		GTACTTCGAT**A**TCTGG	5.0 (5/1)	5.0 (5/1)
2-54	TGTGCAAGA	TCGAGG	GGTGAC	CCC	TTTGCTTACTGG	4.0 (12/3)	2.5 (5/2)
2-65	TGTGCAAGA	GGGGGGAG	TGATG**C**TTACTAC	GG	CTGGTTTGCTTACTGG	2.3 (7/3)	1.0 (2/2)
2-87	TGTGCAAGA	TC	CTGGGAC	CGGGGAAGG	TTTGACTACTGG	2.3 (7/3)	1.0 (2/2)
2-96	TGTGCAAGA	AGAGAGG	CCTACTATAG	CTTG	ATGGACTACTACTGG	2.3 (7/3)	2.5 (5/2)
2-64	TGTGCCA	GAAGAAGGGGGCC	CG**A**TG**G**TAGTTAC		GACTACTGG	N/A (0/0)	N/A (0/0)
2-88	TGTGCCA	GAAGAAGGGGGCC	CG**A**TG**G**TAGTTAC		GACTACTGG	0.9 (6/7)	2.5 (5/2)
2-76	TGT**T**CAAGG	GT	CTATAGTAACTAC	CTTGGAG	GGTTTGCTTACTGG	7.0 (7/1)	7.0 (7/1)
2-70	TGTACAAGAG	CC	CTAC**T**G**A**TA	GAT	TACTTCGATGTCTGG	2.0 (2/1)	N/A (3/0)
3-145	TGTGCAAG	GGGA	TAC**G**ATGGTA	G	CTACTGGTACTTCGATGTCTGG	2.7 (16/6)	2.3 (9/4)
3-112	TGTGCA	GGTCCT	GGGACA**A**CT	TT	GTTT**C**CTTACTGG	N/A (1/1)	N/A (2/0)
3-113	TGTAC	ACG	AACTGGGAC	G	TGGTTTGCTTACTGG	2.6 (18/7)	4.0 (8/2)
3-129	TGT**G**CTA		GT**G**ACT**C**TAGT**G**AC	CTTG	ACTGG	2.6 (13/5)	4.0 (4/1)
3-121	TGTGCTAGA	GAGGG	CTACTATAGTTACTATAGT	C	TTGACTACTGG	1.9 (17/9)	4.0 (12/3)
3-133	TGTGCAAG	T	AG**G**AA		TATGGACTACTGG	1.1 (8/7)	6.0 (6/1)
3-139	TGTGCTAGA	T	GGG**G**TG		ACTTTGACT**GG**TGG	2.0 (2/1)	3.0 (3/1)
3-143	TGTGCTAGA	TGGT	CTAACTGG		GACTA**T**TGG	0.5 (2/4)	N/A (5/0)
3-103	TGTGCAAGA	GAGGAGG	CCTACTATA**C**TTACTATAGTTACGAC	GTGAGGG	CCTGGTTTGGTTA**T**TGG	N/A (0/0)	N/A (0/0)
3-120	TGTGCAA	GAA	AG**G**ATGGT**G**ACT		CCTGGTTTGCTTACTGG	3.0 (9/3)	N/A (11/0)
3-107	TGTGCAAG	GGG	GTATGGT**G**A		CT**C**GTTTGCTTACTGG	1.3 (5/4)	3.0 (6/2)
3-124	TGTGCAAGA	TCGGATC	AGACAGCTCGGGC	CCCC	TTTGCTTACTGG	1.3 (5/4)	3.0 (6/2)
3-118	TGTT**A**CTAG	AG	ACTTTAGTAACTAC	GGGCTT	TTTGCTTACTGG	2.5 (10/4)	2.5 (5/2)
3-130a	TGTGCC	CAAGC	CTAC**C**ATGGTGACTAC	CG	TG**G**T**T**TGGACTACTGG	2.0 (2/1)	2.0 (2/1)
3-130b	TGTGCC	CAAG	CCTACTATGGTAACTAC	CG	TG**G**T**T**TGGACTACTGG	5.0 (10/2)	7.0 (7/1)
3-125	TGTGCA	TCA	CAACTGGGAC	CGA	GGTACTTCGATGTCTGG	6.0 (6/1)	2.0 (4/2)
3-136	TGTCAAGA	TATCC	CAGG		TGGTATTTCGATGTCTGG	3.0 (6/2)	4 (4/1)

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