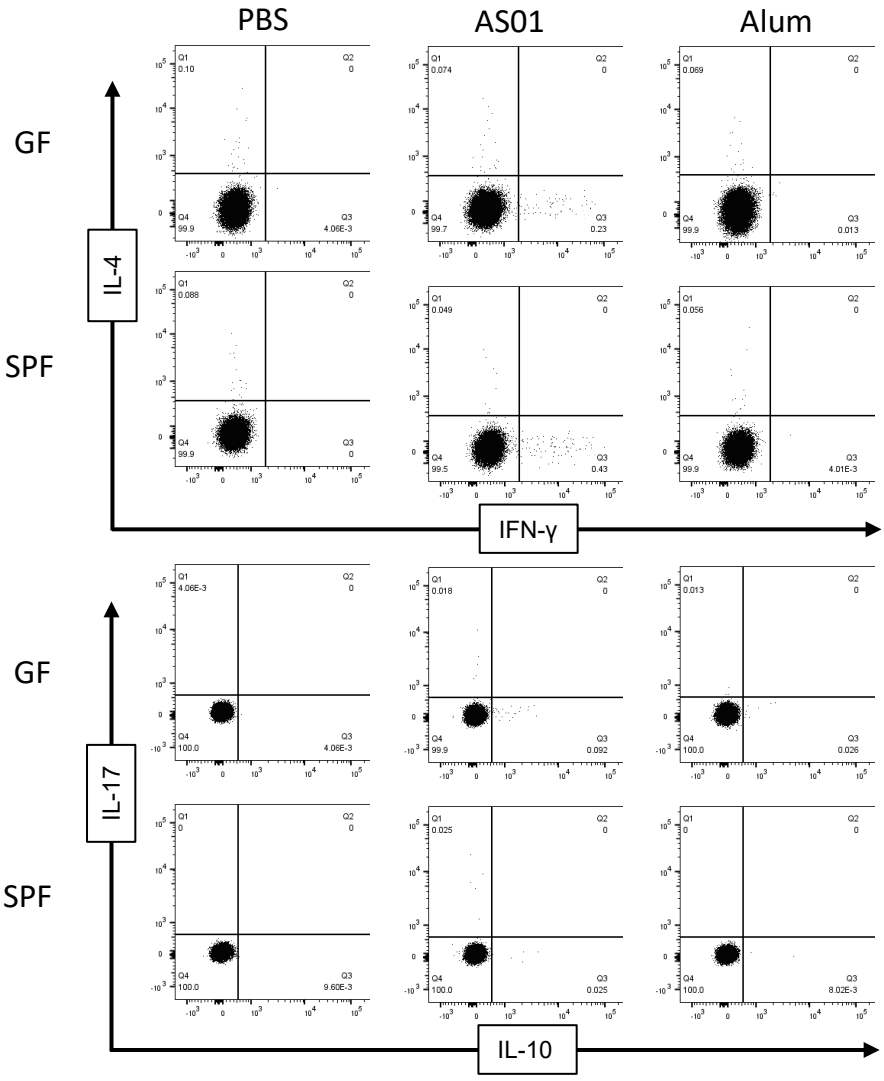

Supplementary figure 1: Representative ICS flow cytometry of ovalbumin-stimulated splenocytes from GF and SPF mice immunized with Ovalbumin either unadjuvanted (PBS) or with AS01 or Alum adjuvants. Plots are pre-gated on single, live, CD3+CD4+ cells.

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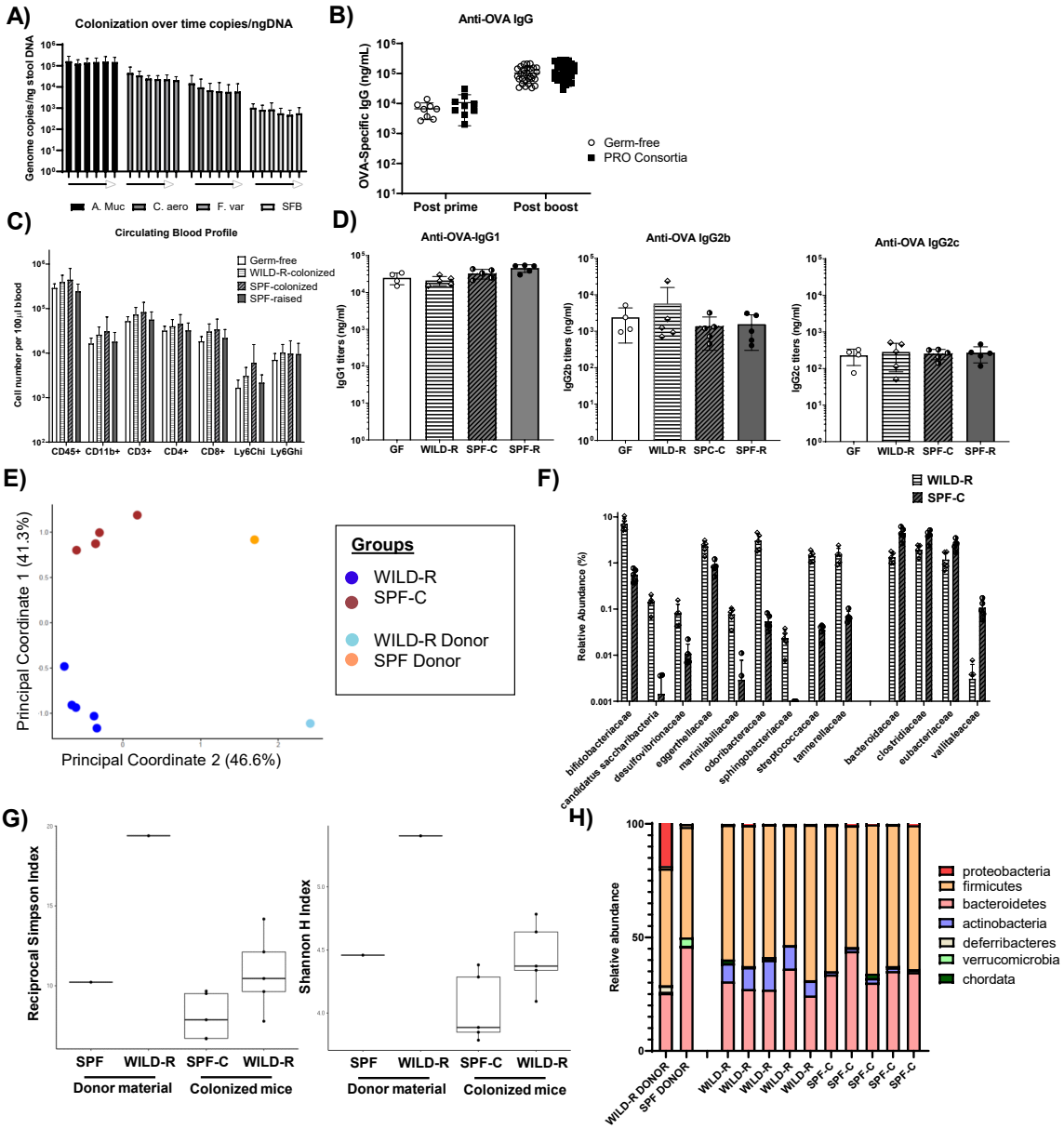

Supplementary figure 2: Humoral responses to protein-adjuvant vaccines in selectively colonized mice (A) Genome copies of Akkermansia Mucinophilia (A. Muc), Colinsella Aerofaciens (C. aero), Fusbacterium Varium (F. var), Segmented Filamentous Bacteria (SFB). **(B)** Serum anti-OVA IgG titers of mice colonized with A.Muc, C. aero, F.var, SFB (PRO Consortia) or remaining GF, two weeks post-prime or two weeks post-boost. **(C)** Numbers of difference cell populations in blood collected two weeks following colonization with WILD-R (n = 5) or SPF (SPF-C; n = 5) material, or of Germ-free (n = 4) and SPF-raised (SPF-R; n = 5) controls quantified by flow cytometry. **(D)** serum anti-OVA IgG1 IgG2b, IgG2c titers two weeks post-boost with OVA/Alum in GF, WILD-R, SPF-C and SPF-R mice. **(E)** Species-level Principal Coordinate analysis based on Bray-Curtis beta diversity of SPF-C mice (Burgundy dots), WILD-R mice (dark blue dots), or Donor SPF (orange dot) or Donor WILD-R (light blue dot) material. **(F)** Relative abundance of bacterial families significantly different in WILD-R and SPF-C mice based on Multiple T-tests with 1% Benjamini, Krieger, Yekutieli False Discovery Rate correction). Each data-point represents an individual mouse with bars representing mean +/-SD. **(G)** Reciprocal Simpson and Shannon Indices for SPF-C, WILD-R-colonized mice and SPF and WILD-R donor material. Each data-point in A-E represents an individual mouse with bars representing mean +/- SD **(H)** Relative abundance of bacterial Phyla in SPF or WILD-R donor material and in individual WILD-R-colonized (WILD-R C) or SPF-C mice. Each bar represents a single mouse.

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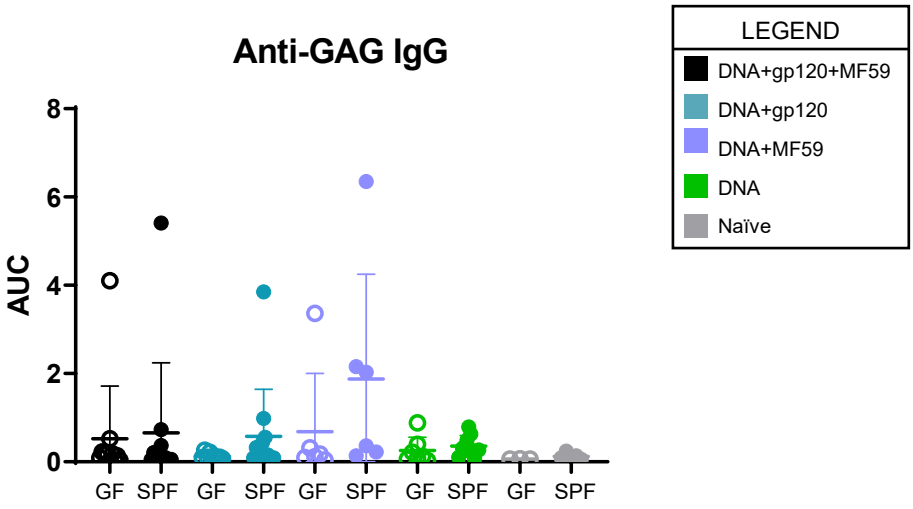

Supplementary figure 3: Serum anti-gag IgG in DNA-HIV-PT123 immunized mice Serum anti-gag IgG titers in GF and SPF mice immunized with DNA-HIV-PT123 (DNA), gp120 and/or MF59 in different combination regimens (as indicated in legend) or unimmunized (naïve) represented as Area Under the Curve (AUC) of an 11-point serial serum dilution.

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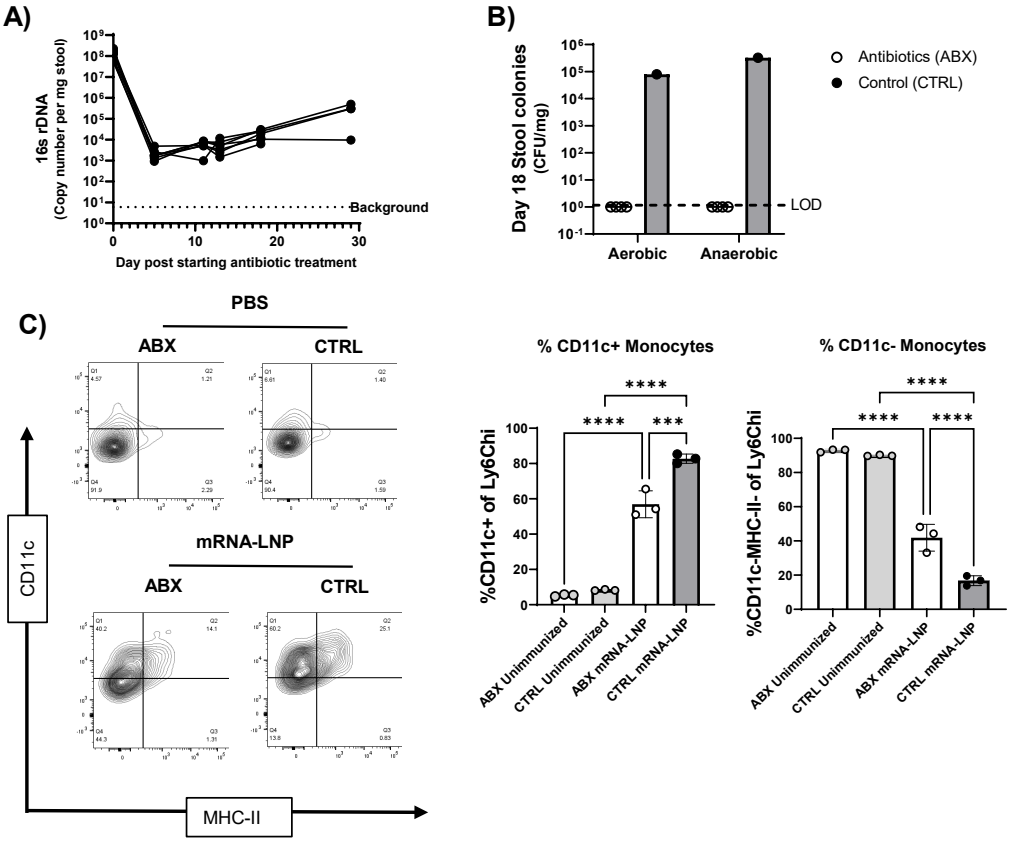

Supplementary figure 4: Flow cytometry profiling of monocyte activation in mRNA-LNP-immunized or control antibiotic-treated or -untreated SPF mice.

C57Bl6/J mice were treated with broad-spectrum antibiotics (Metronidazole 1g/l, Amplicillin 1g/l, Neomycin, 1g/l, Gentacmicin 1g/l and vancomycin 0.5g/l) in the drinking water (ABX) or maintained on regular drinking water (CTRL) for four weeks prior to immunization with mRNA-LNP or a PBS control. **(A)** 16s rDNA copy numbers were determined in DNA extracted from stool collected from cages of mice before antibiotic treatment (Day 0) or at regular time points after transfer to antibiotic-treated drinking water. **(B)** Colony forming units per mg of stool from cages of ABX or control SPF mice was quantified by serial dilution on blood tryptic soy agar plates and incubation in either aerobic or anaerobic conditions followed by colony counting after 48 hours. **(C)** Representative flow cytometry and graphical summaries of Ly6Chi monocyte CD11c vs MHC-II expression in PBS-treated or mRNA-LNP-immunized ABX and control SPF mice. Flow cytometry was pre-gated on live, single, Ly6Chi CD11b+ cells as illustrated in figure 5. Each data point represents an individual mouse (n = 3 per group), with bars set at the mean +/- standard deviation (SD). **** $p < 0.0001$, *** $p < 0.001$ One-Way ANOVA with Holm-Sidak's multiple comparisons test.

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