Maternal IgG and IgA Antibodies Dampen Mucosal T Helper Cell Responses in Early Life

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Gene-microbiota interactions contribute to the pathogenesis of inflammatory bowel disease

Hiutung Chu,[1]* Arya Khosravi,[1] Indah P. Kusumawardhani,[1] Alice H. K. Kwon,[1] Anilton C. Vasconcelos,[2] Larissa D. Cunha,[3] Anne E. Mayer,[4] Yue Shen,[1] Wei-Li Wu,[1] Amal Kambal,[4] Stephan R. Targan,[5] Ramnik J. Xavier,[6] Peter B. Ernst,[2] Douglas R. Green,[3] Dermot P. B. McGovern,[5] Herbert W. Virgin,[4] Sarkis K. Mazmanian[1]*

Bahtiyar YILMAZ
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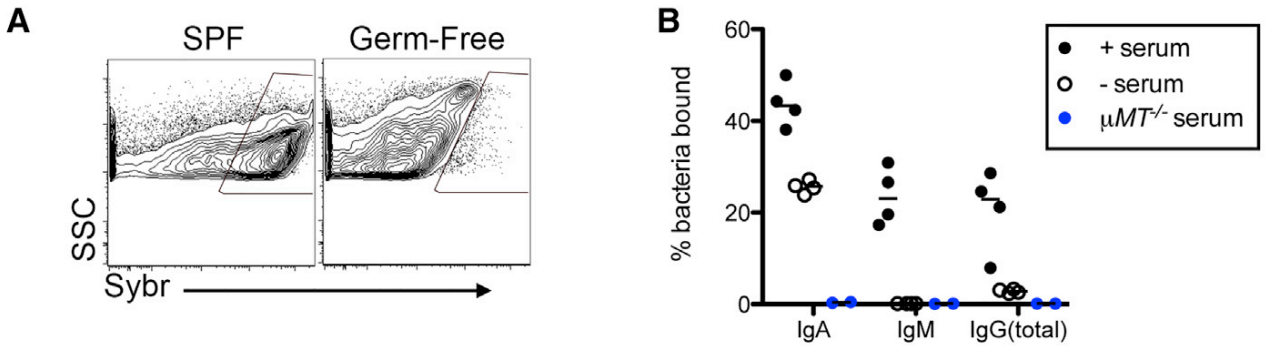
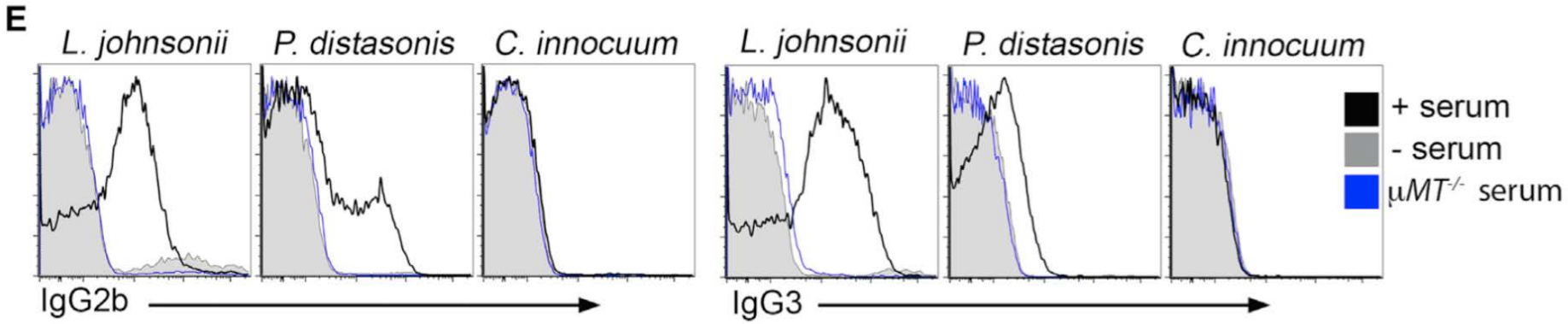
Highlights

- Healthy mice make T-independent IgG2b and IgG3 Abs reactive to mucosal bacteria.
- Gut microbes elicit anti-commensal IgG antibodies via TLR signalling on B cells.
- Maternal transmission of IgG coordinates with IgA to limit mucosal T cell responses.
- Absence of maternal antibodies triggers a compensatory T-dependent immune response.

Identification of IgG2b and IgG3 Antibodies That are Generated against the Microbiota in Healthy Mice



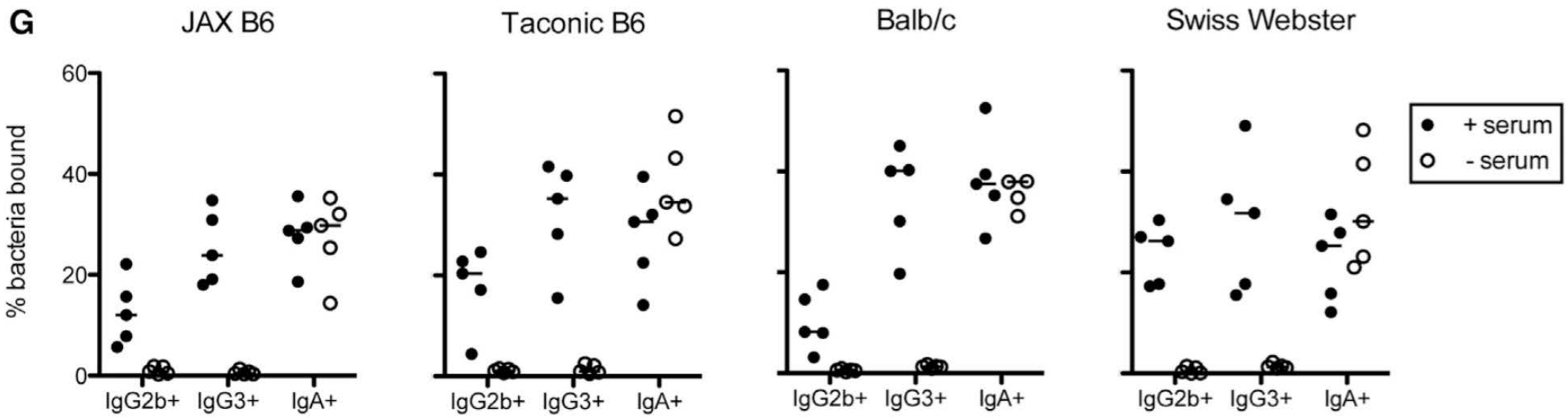
The Sera of Healthy Mice Stains Several Isolated Bacteria Strains



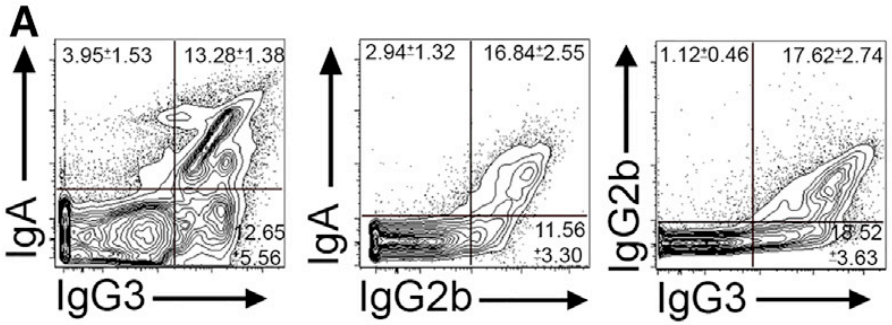
No observed binding to pathogenic bacteria or non-pathogenic *E.coli* strain

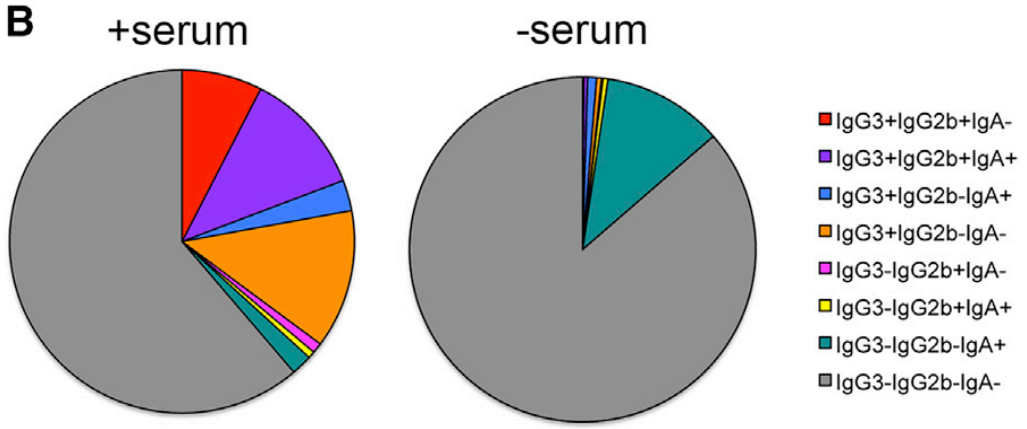
Anti-commensal IgG antibodies are not "natural" antibodies observed in mice housed under GF conditions in the absence of microbiota."

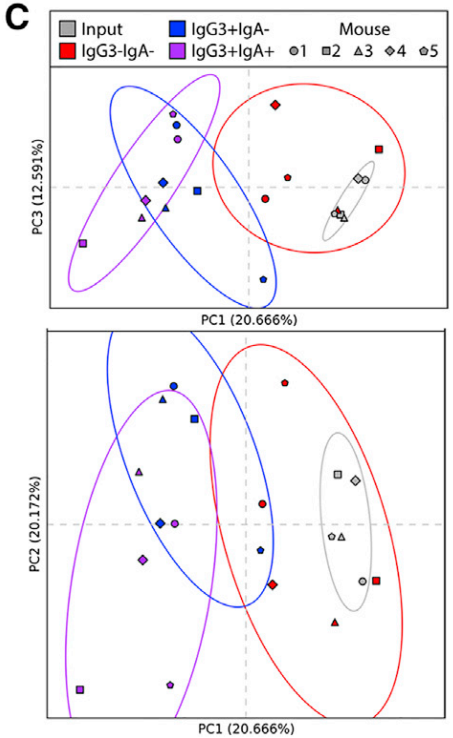
Similar Anti-commensal Antibody Responses in Inbred and Outbred Mouse Strains

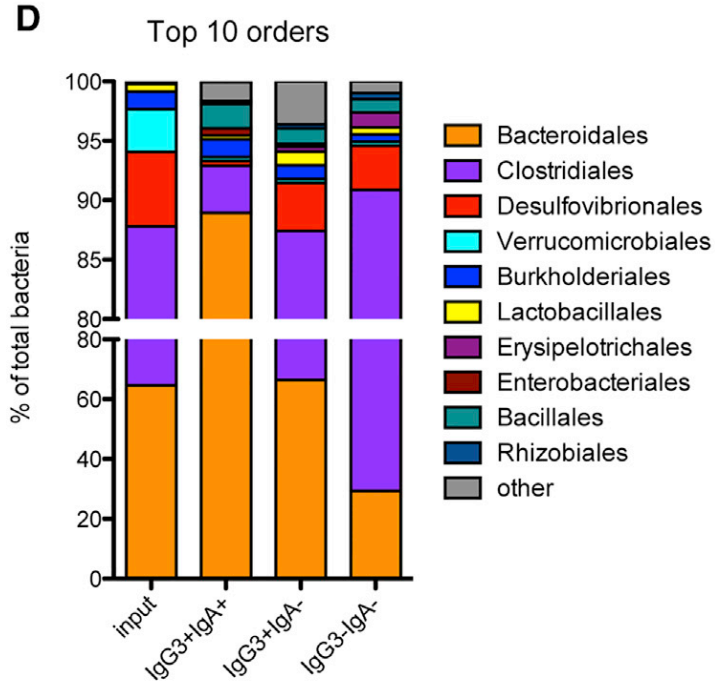


IgG Antibodies Recognize a Broad Spectrum of Fecal Commensal Microbes



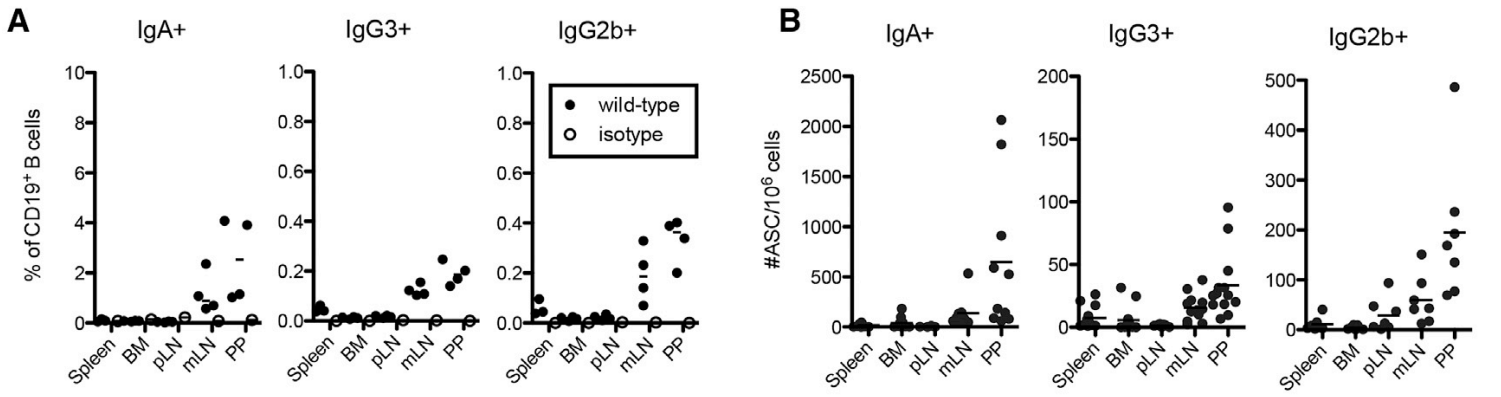




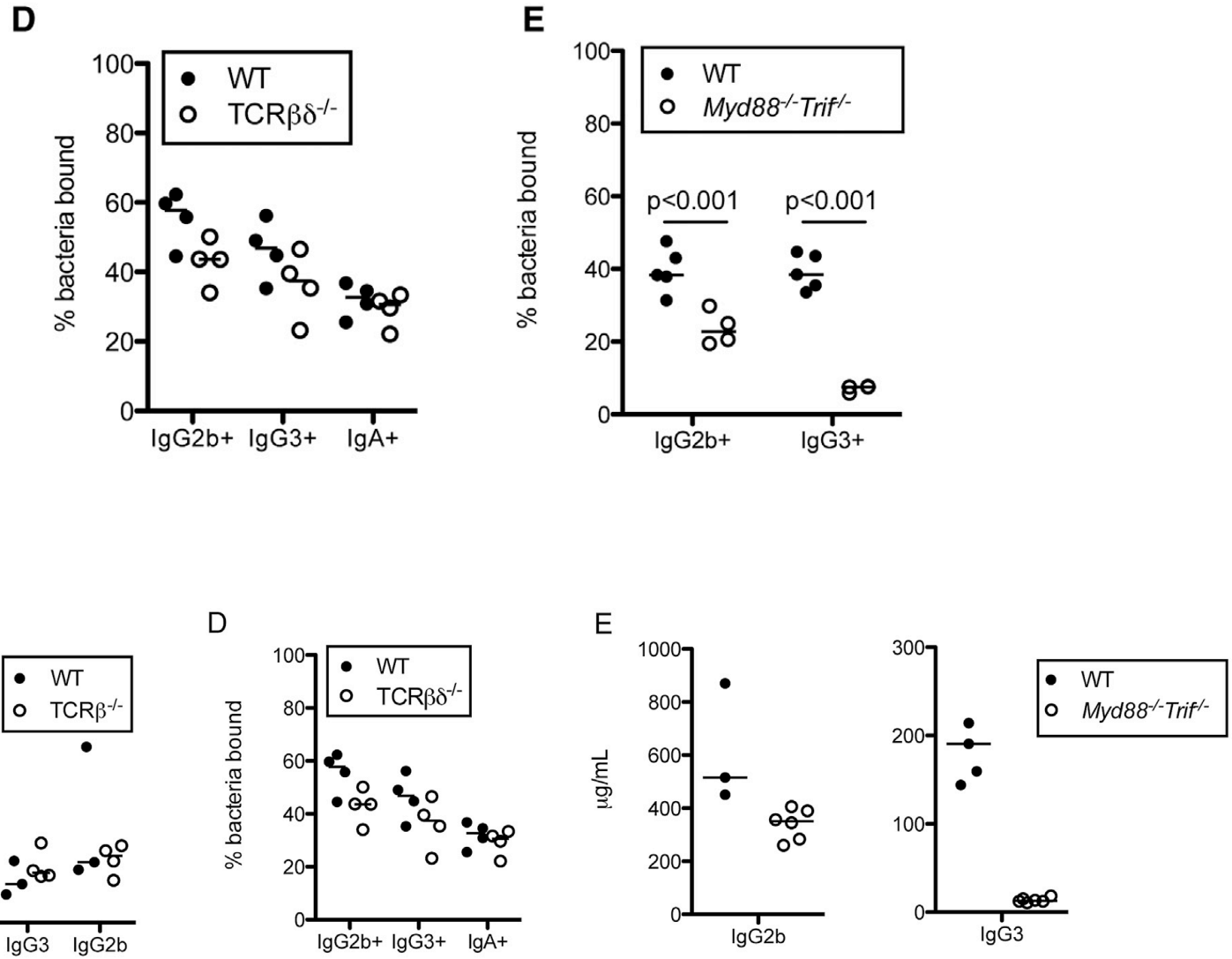


Characterization of Anti-Commensal IgG2b and IgG3 Response

Frequencies out of CD19+ cells (A) and numbers (B) of IgG2b, IgG3, and IgA expressing B cells in different tissues of WT mice, as measured by flow cytometry or ELISpot



T-cell Independent and TLR Dependent Anti-Commensal IgG Antibodies



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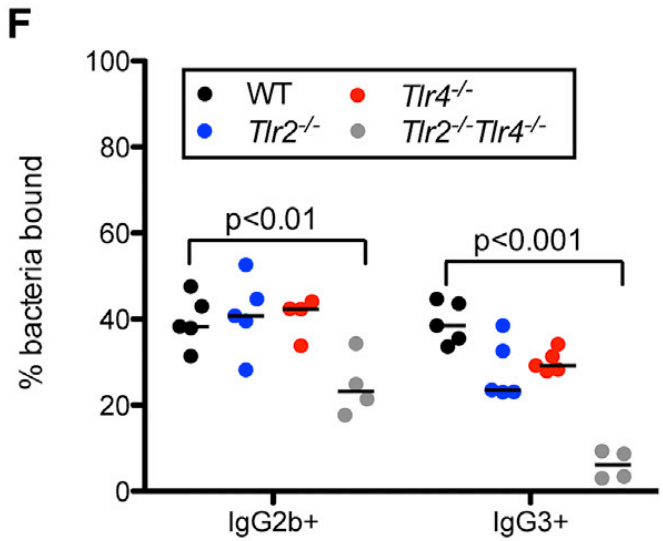
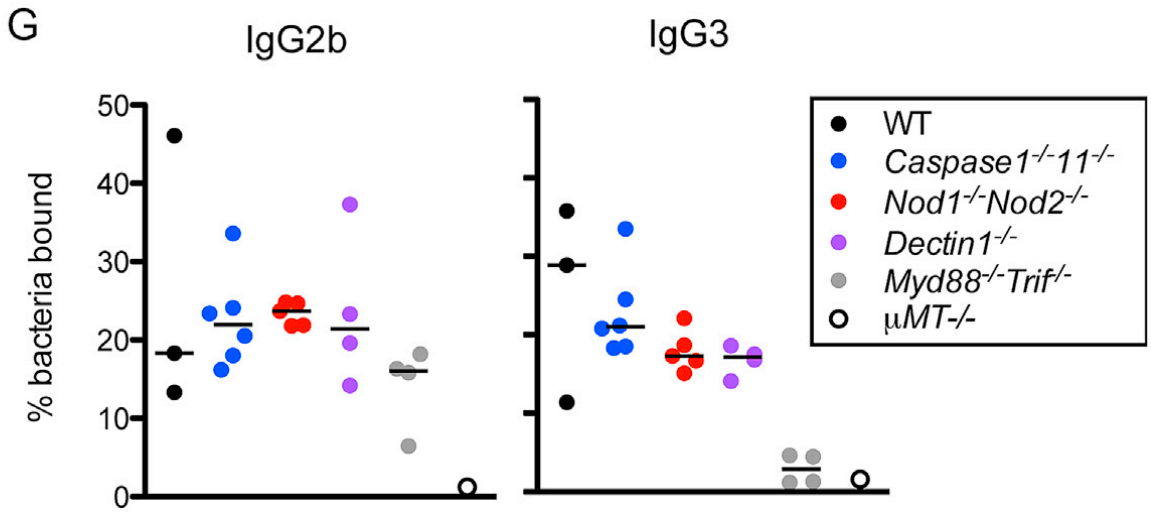
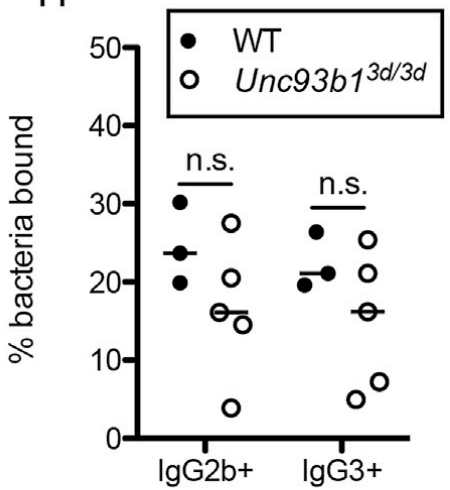
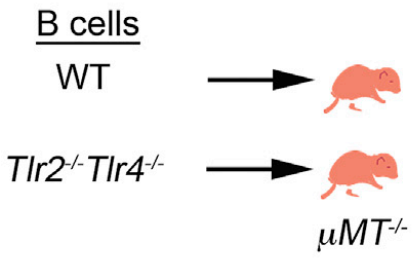






Determination of the Requirement for TLR Signalling on B cells for Anti-commensal IgG Responses

G

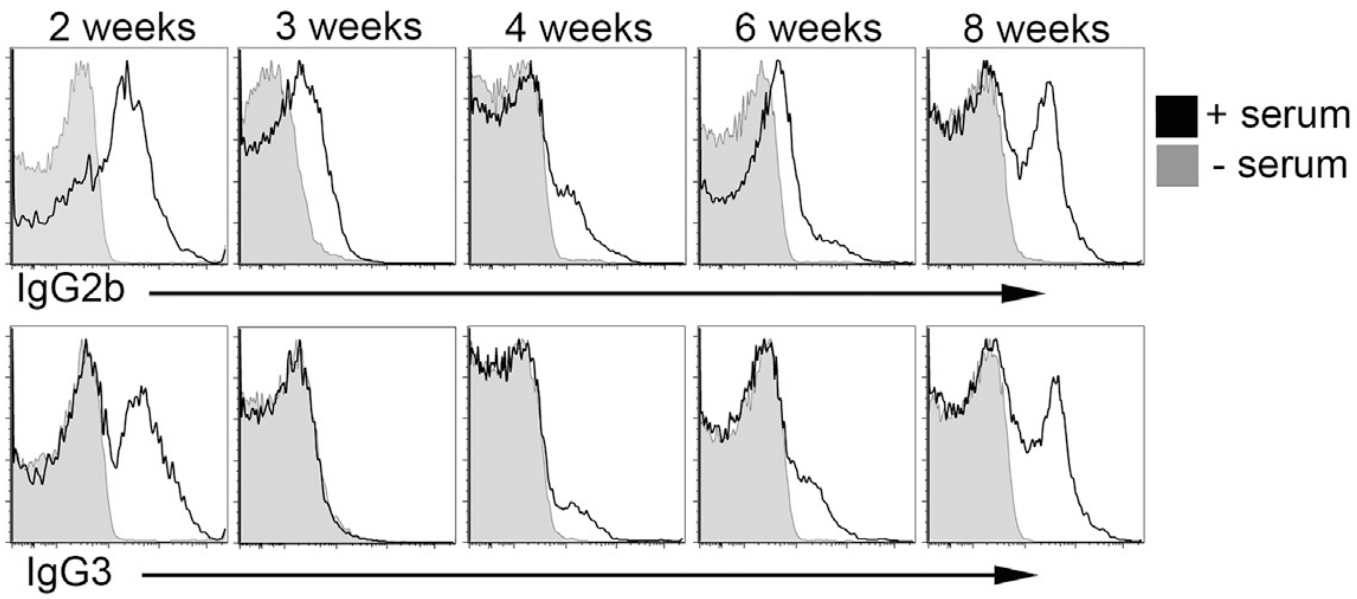


Flow cytometric analysis of CD19+B220+ B cells in different tissues of 10 weeks old mMT$^{-/-}$ mice that received WT or Tlr2/Tlr4 DKO splenocytes at birth.

Frequencies of SYBR+ bacteria bound by IgM and IgG antibodies measured by mFLOW with sera from adoptively transferred mMT$^{-/-}$ mice as in (I)

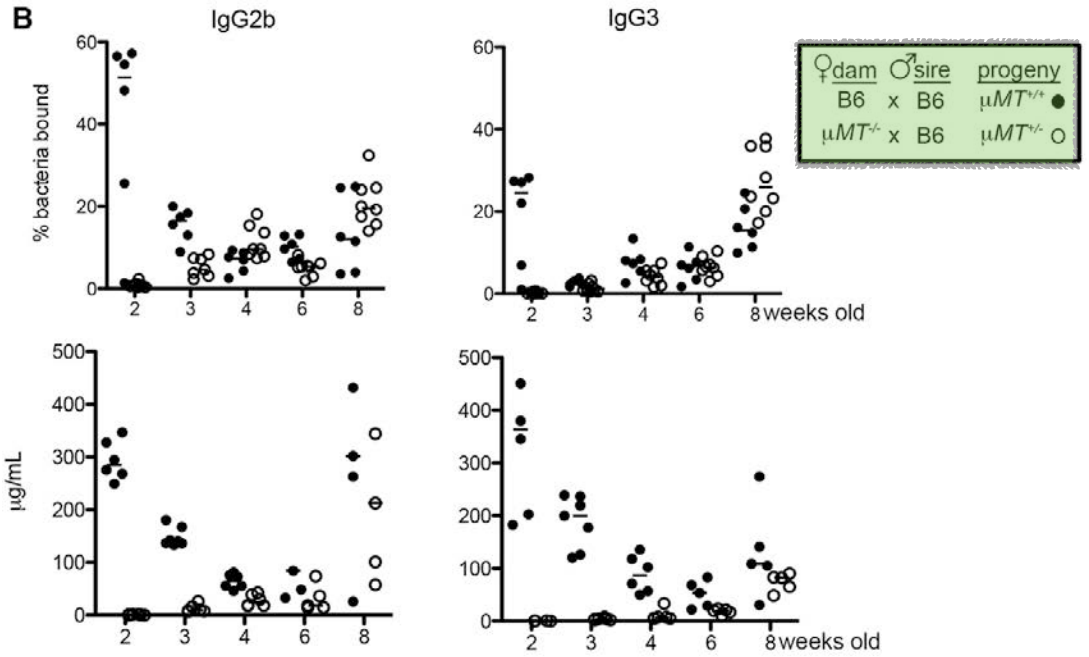
Serum titers of NP-specific antibodies before and 7 days after immunization with NP-Ficoll of adoptively transferred mMT$^{-/-}$ mice as in (I).

Maternal Transmission of Anti-Commensal IgG Antibodies



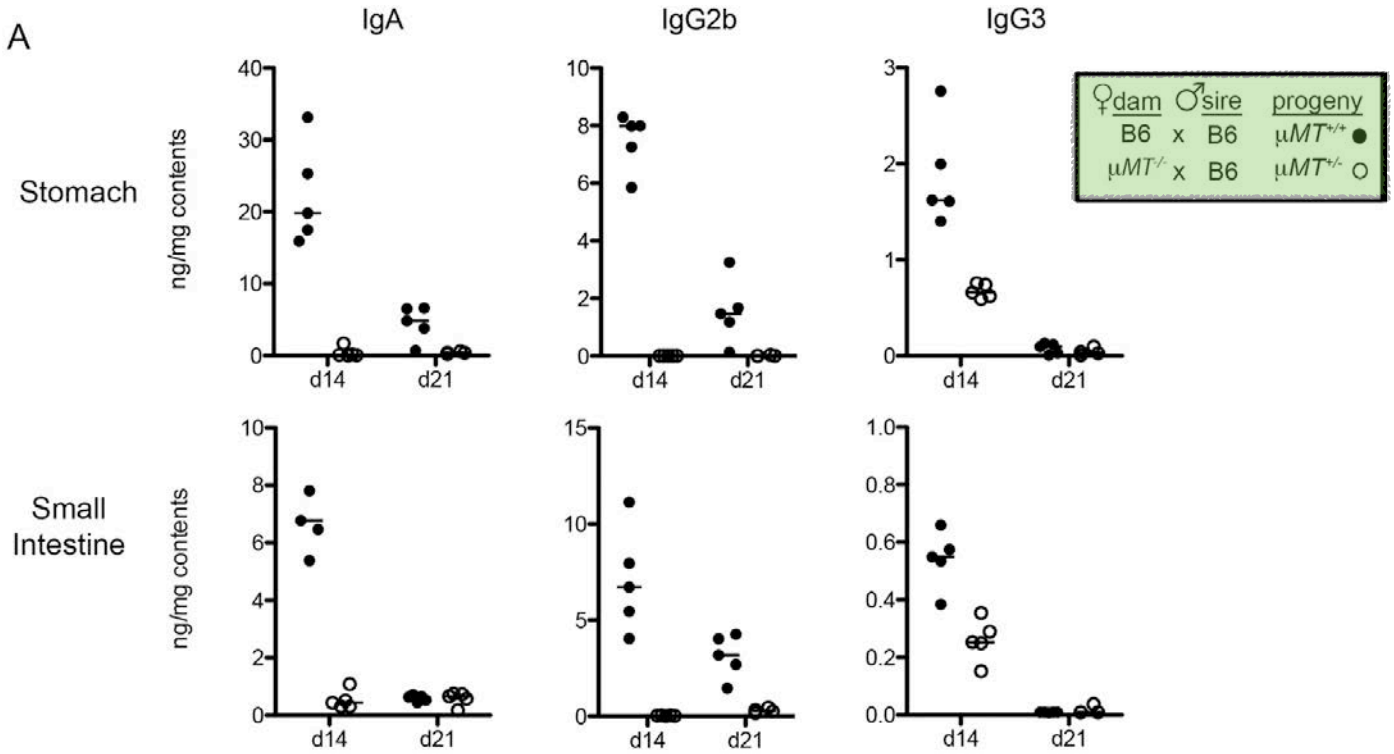
SYBR^{+} bacteria bound by serum IgG2b (upper) or IgG3 (lower) from WT mice

Maternal Transmission of Anti-Commensal IgG Antibodies



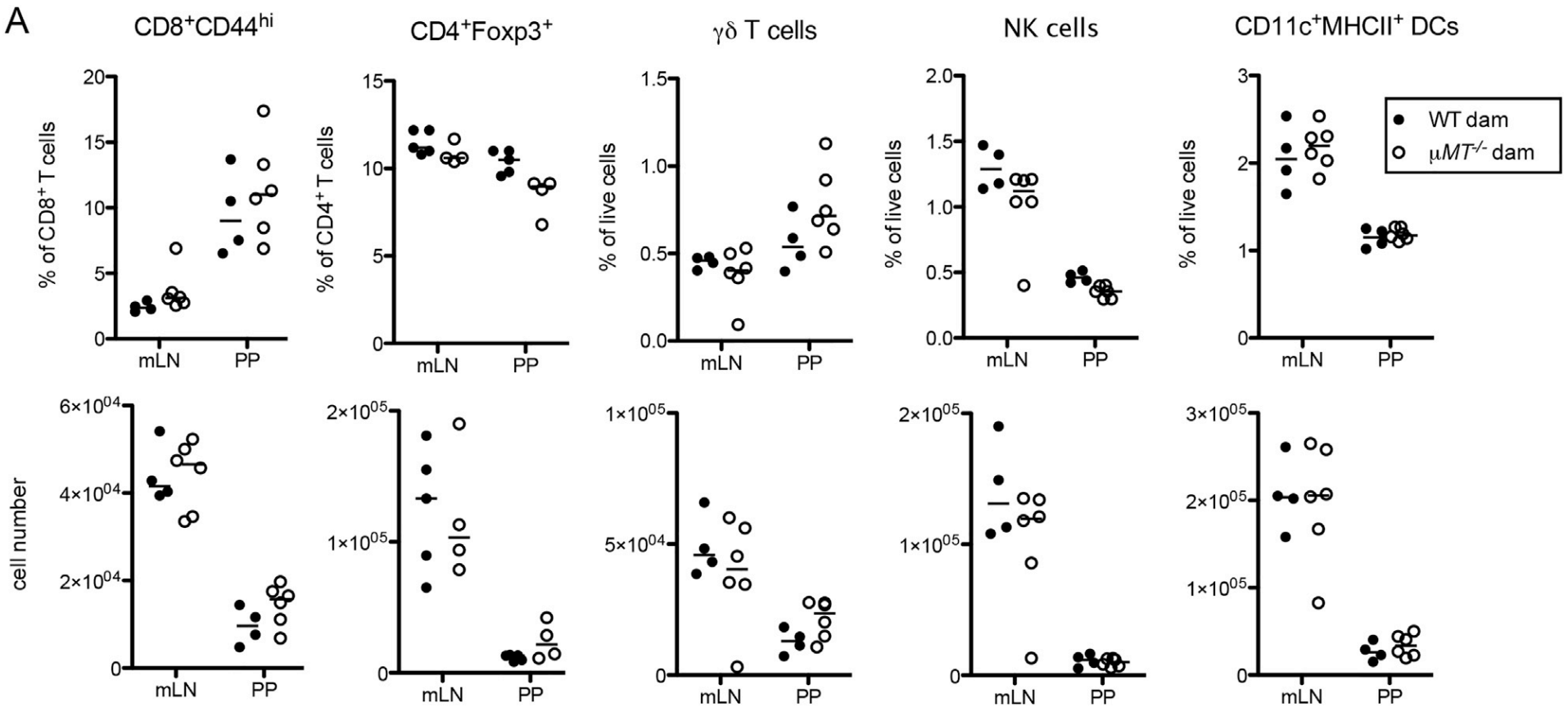
Maternal Transmission of Anti-Commensal IgG Antibodies

Ingestion of maternal antibodies by offspring

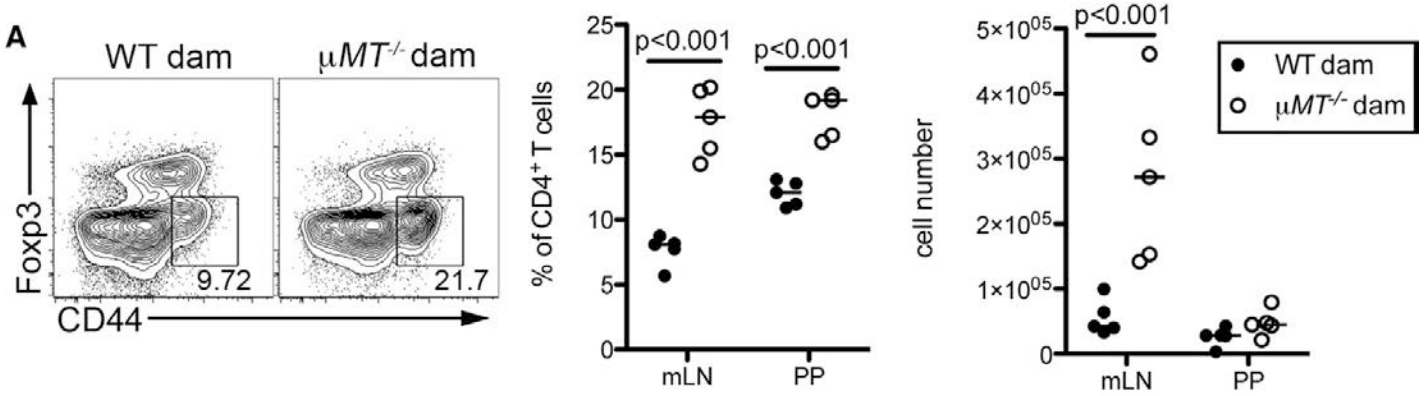


IgG2b- and IgG3-bound bacteria were present in the stomach and intestinal lumen of 2-week-old mice in the absence of added sera

No Gross Difference in Immune Cells of WT and B-cell Deficient Mice



Regulation of Mucosal T Cell Responses by Maternal Antibodies



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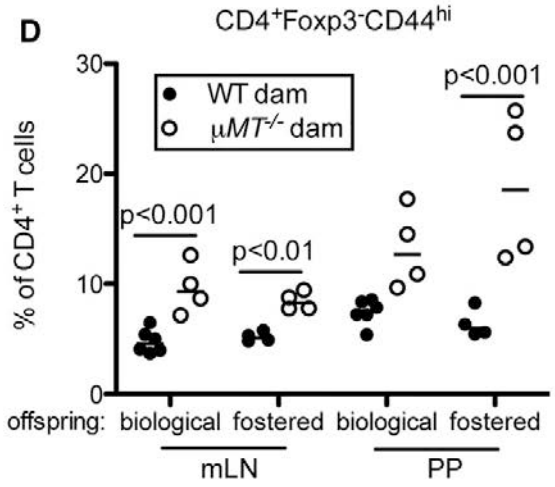
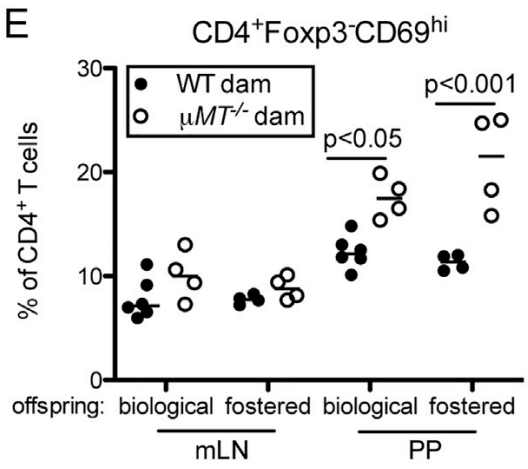
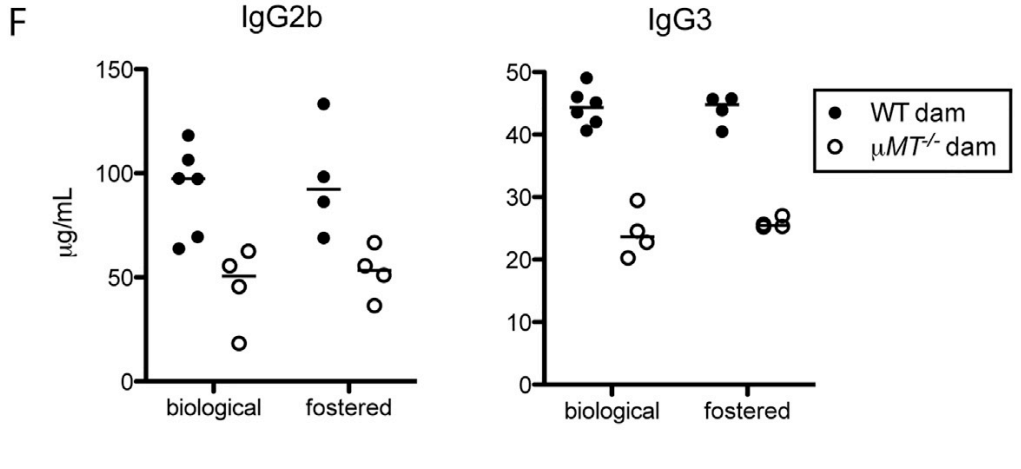
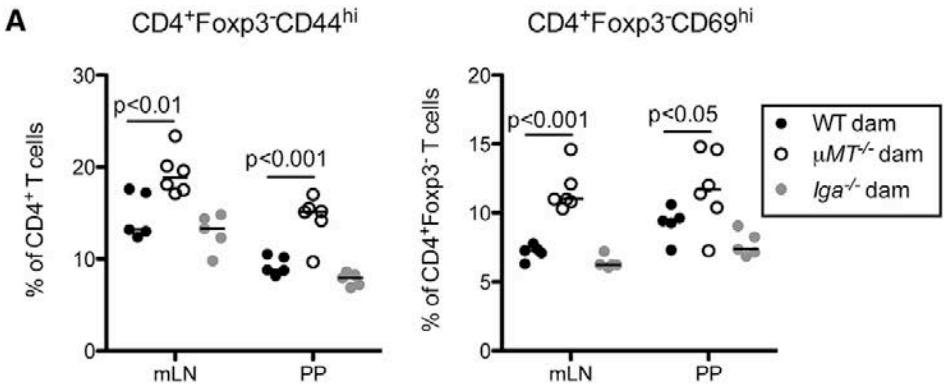


- Enhanced T cell activation results from a lack of maternal antibodies rather than differences in the composition of the inherited microbiota.
- Acquisition of maternal antibodies post-birth, rather than in utero, is required to dampen mucosal T cell activation in young mice.



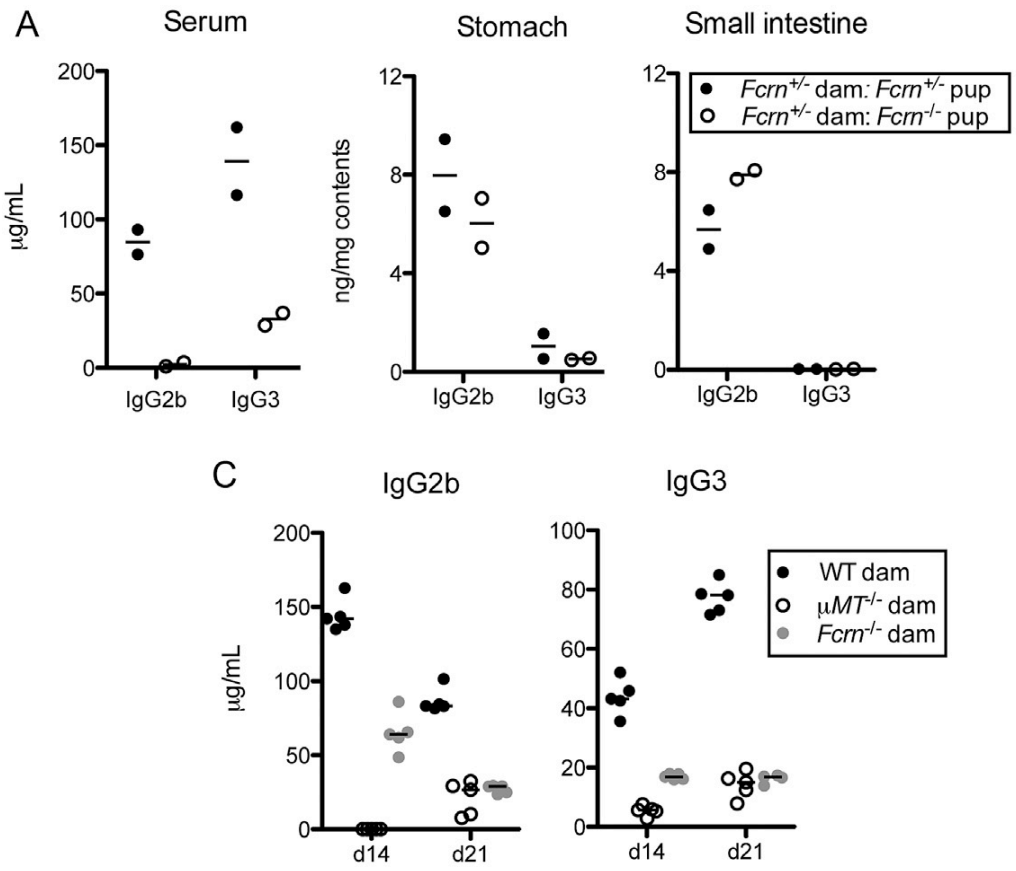


Maternal IgG and IgA Cooperate to Limit Mucosal T Cell Responses



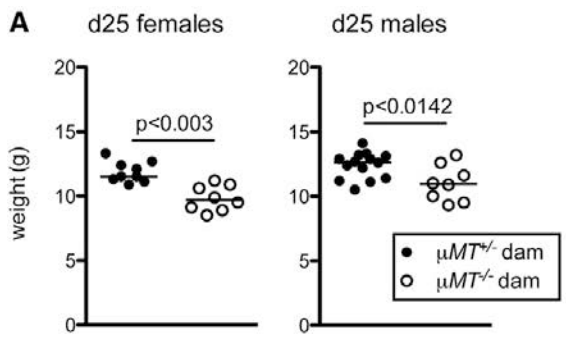
Equivalent accumulation of T_{eff} and activated $CD4^+Foxp3^-CD69^+$ T cells in the mLN and PP of mice born to either WT or IgA-deficient dams.

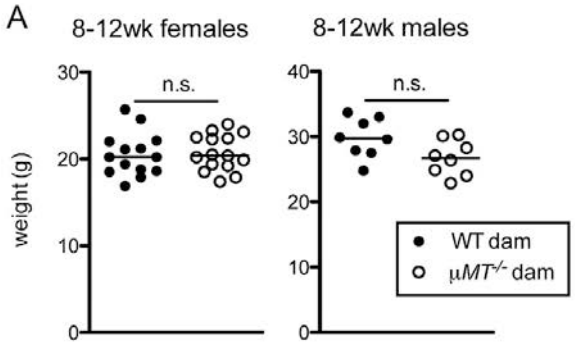
Maternal IgG and IgA Cooperate to Limit Mucosal T Cell Responses



Reduced in the intestinal lumen and blood of pups born to FcRn-deficient dams, but the defect was incomplete when compared to offspring of mu*MT*$^{-/-}$dams.

Dysregulation in Young Animals may be Transient

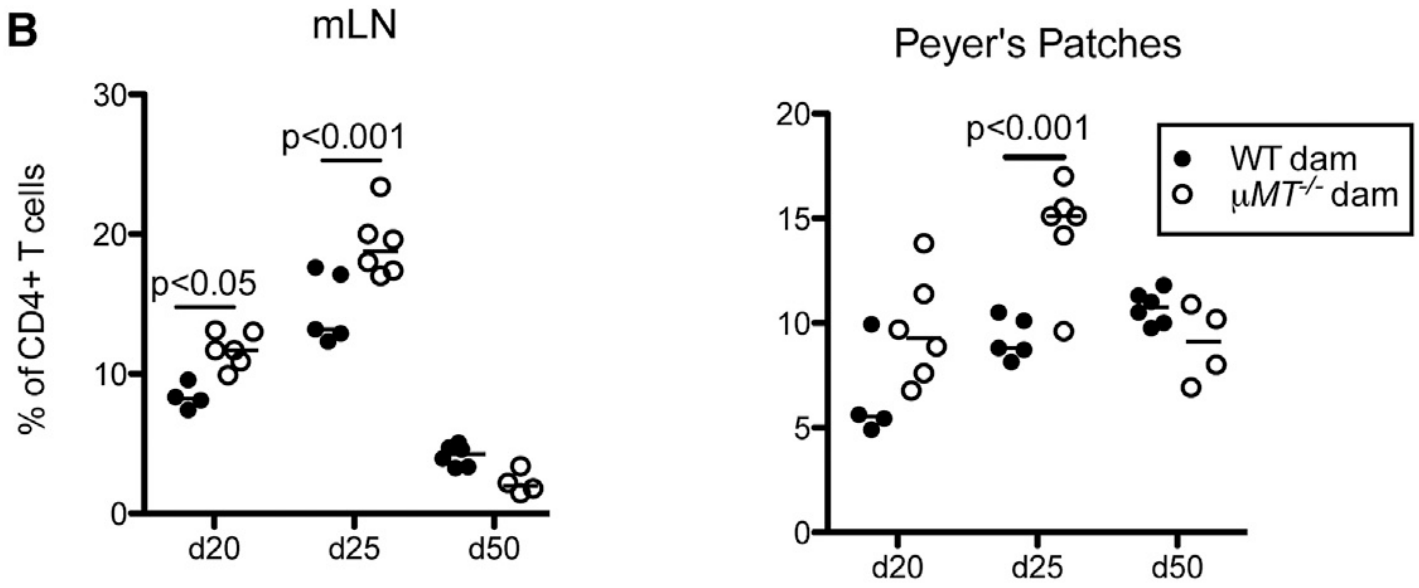




Consistently weighed 10%–15% less than pups born to mu$MT^{+/-}$ dams at d25 post-birth, but this difference resolved in older mice

Slightly elevated levels of inflammatory serum cytokines and fecal lipocalin-2

Compensatory Germinal Center Responses Restore Homeostasis in Mice Lacking Maternal Antibodies



Observed expansion of $CD4^{+}Foxp3^{-}CD44^{hi}$ T cells only in young mice

Compensatory Germinal Center Responses Restore Homeostasis in Mice Lacking Maternal Antibodies

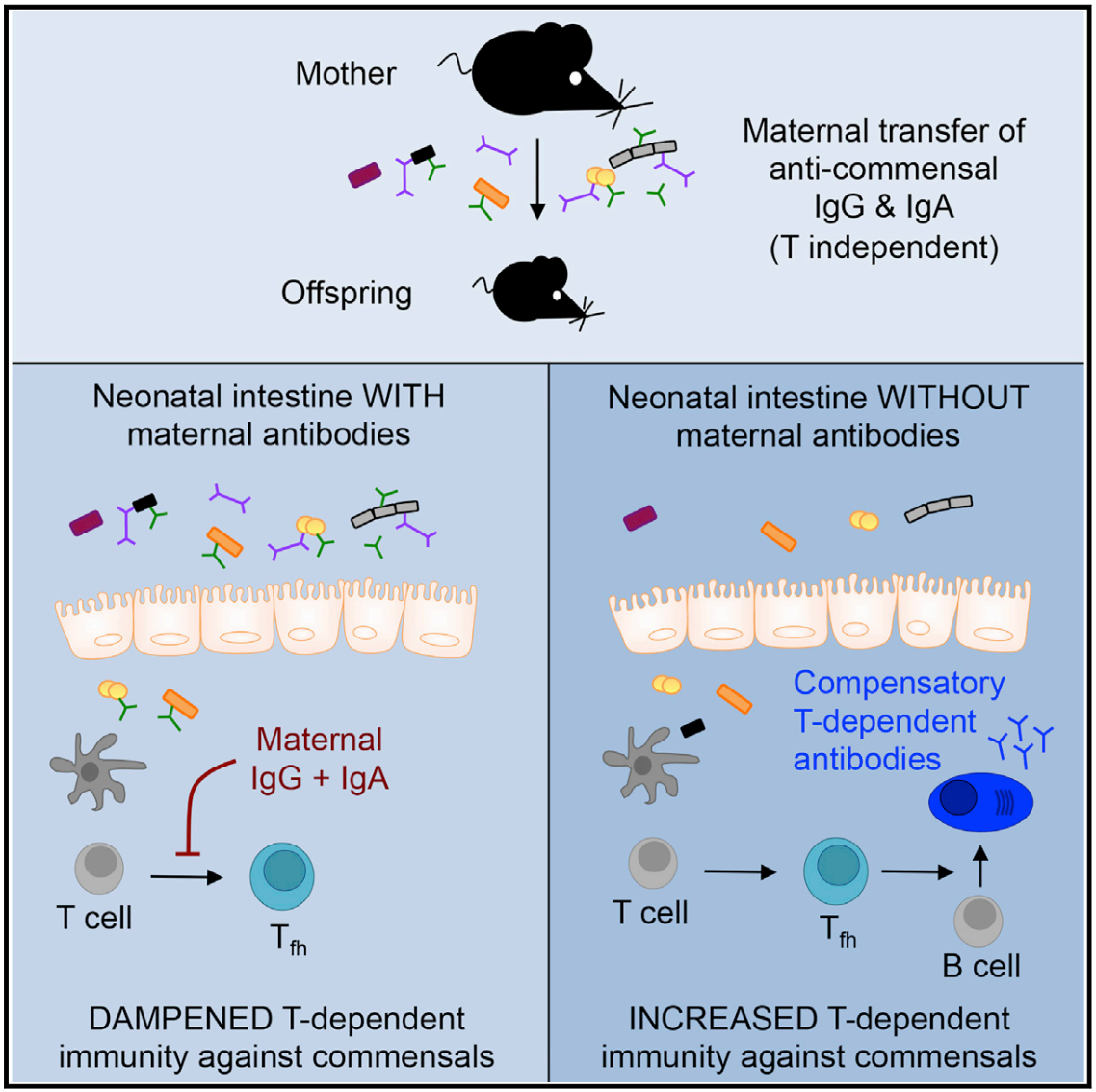
Profiled expression of transcription factors and trafficking receptors on CD4 Teff cells

- No reproducible difference
- Expansion of CD4+ Teff cells expressing the Tfh markers

- More GC B cells within the mLN and PP of maternal antibody-deficient mice as compared to control offspring

- Activation of CD4+ T cells preceded the expansion of GC B cells.
- IgA KO mice did not exhibit increased frequencies or numbers of GC B cells in the mLN and PP

Conclusion (I)



Gene-microbiota interactions contribute to the pathogenesis of inflammatory bowel disease

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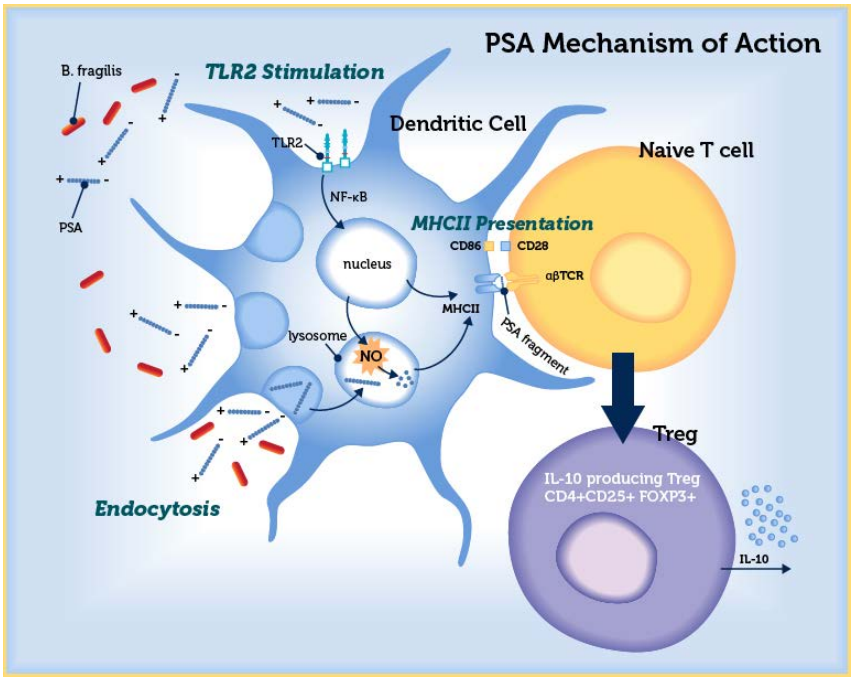
Highlights

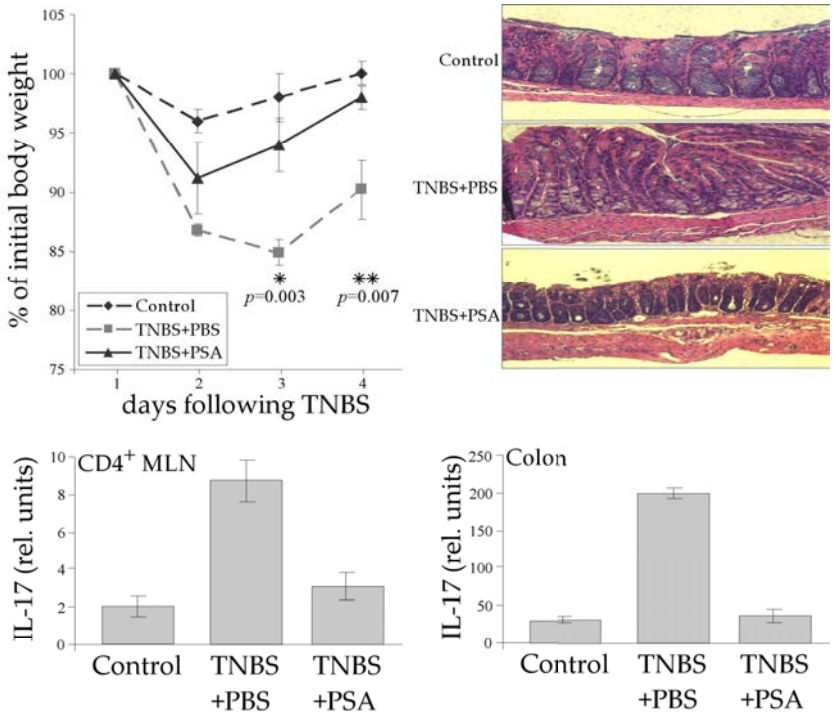
- *Bacteroides fragilis* delivers immunomodulatory molecules to immune cells via secretion of outer membrane vesicles (OMVs).
- OMVs require *ATG16L1* and *NOD2* to activate a non-canonical autophagy pathway during protection from colitis.
- *ATG16L1*-deficient dendritic cells do not induce Treg to suppress mucosal inflammation.
- Polymorphisms in susceptibility genes promote disease through defects in 'sensing' protective signals from the microbiome.

Microbiota

Bacteroides fragilis

- A gram-negative, obligate anaerobic bacteria
- *B. fragilis* is a prominent commensal human gut
- It synthesizes at least <u>eight unique capsular polysaccharide</u> complexes from distinct genomic loci
  - At least two of these polysaccharides (PSA and PSB) have a novel zwitterionic structure (both a positive and negative charge within each repeating subunit).
  - PSA consists of a tetrasaccharide repeat unit that forms a polymer of between 30-300 repeats.
    - Packaged in outer membrane vesicles (OVMs)
    - Delivered to intestinal DC to induce IL-10 from T_{reg}
    - Protects from chemically induced intestinal inflammation



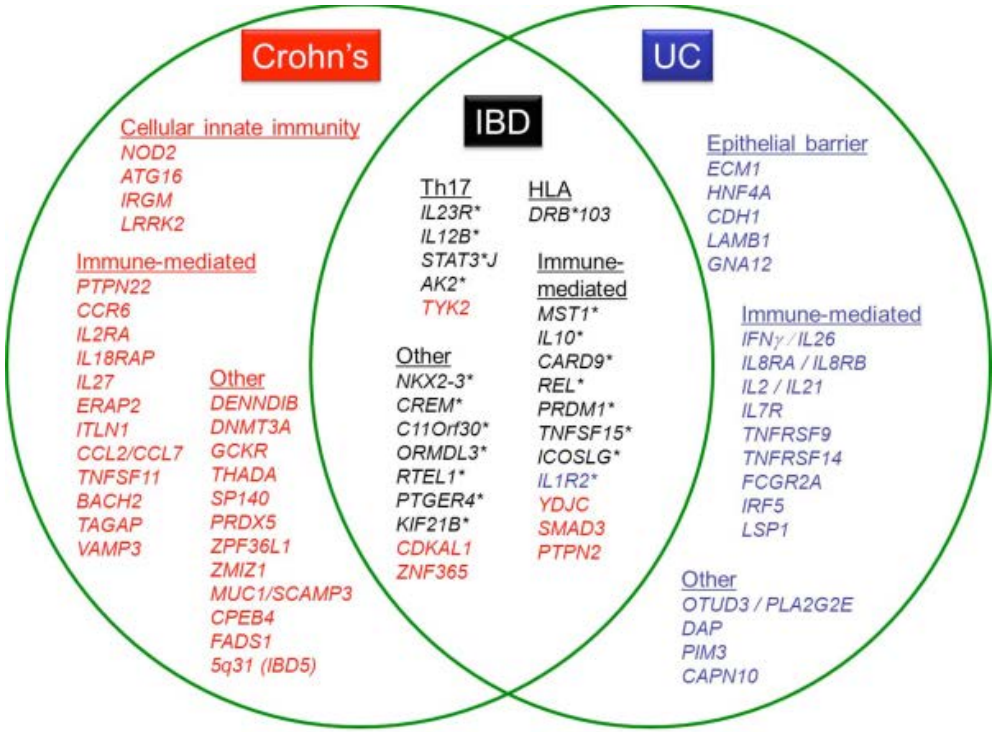


Mazmanian et al. Cell (2005)
Mazmanian et al, Nature (2008)

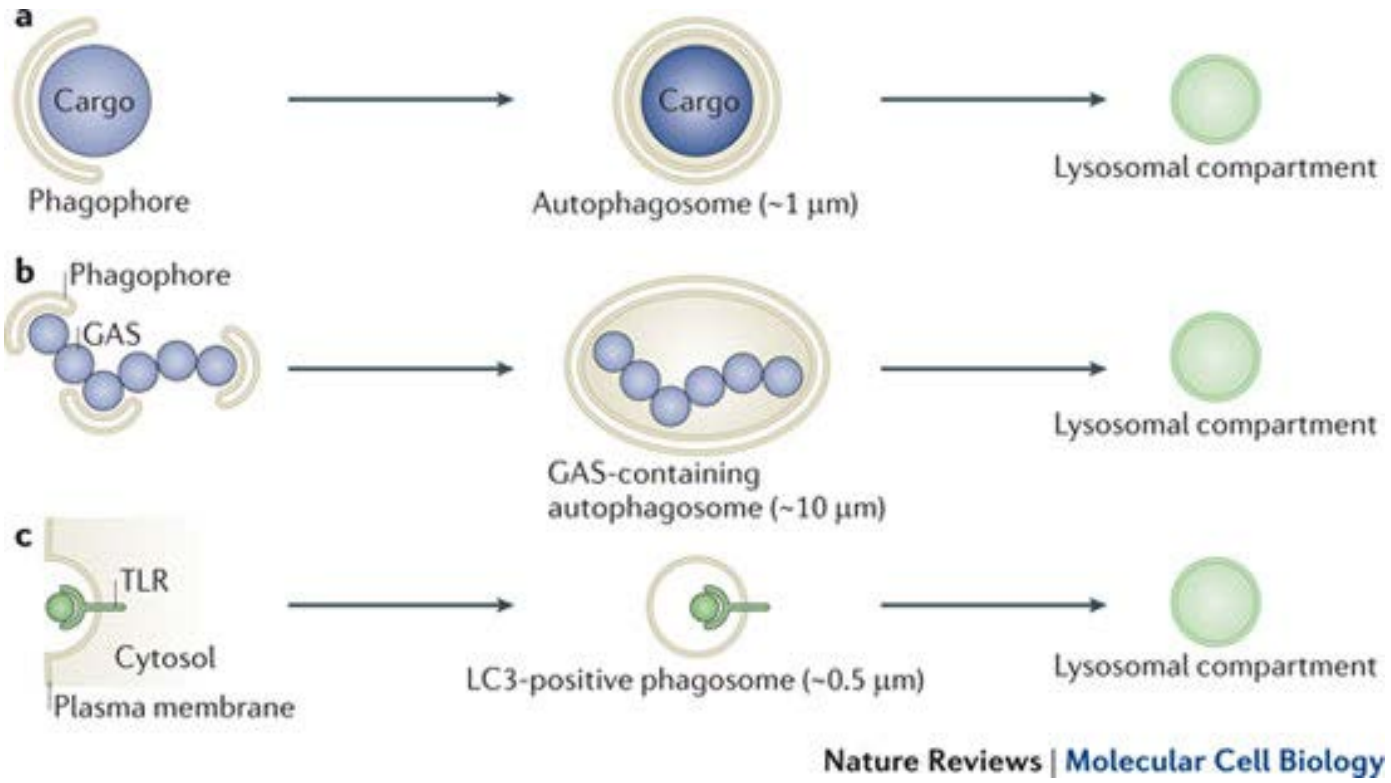
Gene

ATG16L1 and NOD2

- Close to 200 risk loci have been proposed for CD
  - with several susceptibility genes
  - Genes linked to autophagy (e.g. *ATG16L1*)
  - Genes linked to microbial sensors that activate autophagy (e.g. *NOD2*).
    - NOD2 encodes for an intracellular sensor of bacterial peptidoglycan, and polymorphisms in this gene contribute to the largest fraction of genetic risk for CD
  - Disruption of these genes results in defects in microbial clearance.
  - Impaired immune cells for autophagy are hyper-inflammatory



Canonical and Non-canonical Autophagy



a | The hallmarks of canonical autophagosomes are shown.

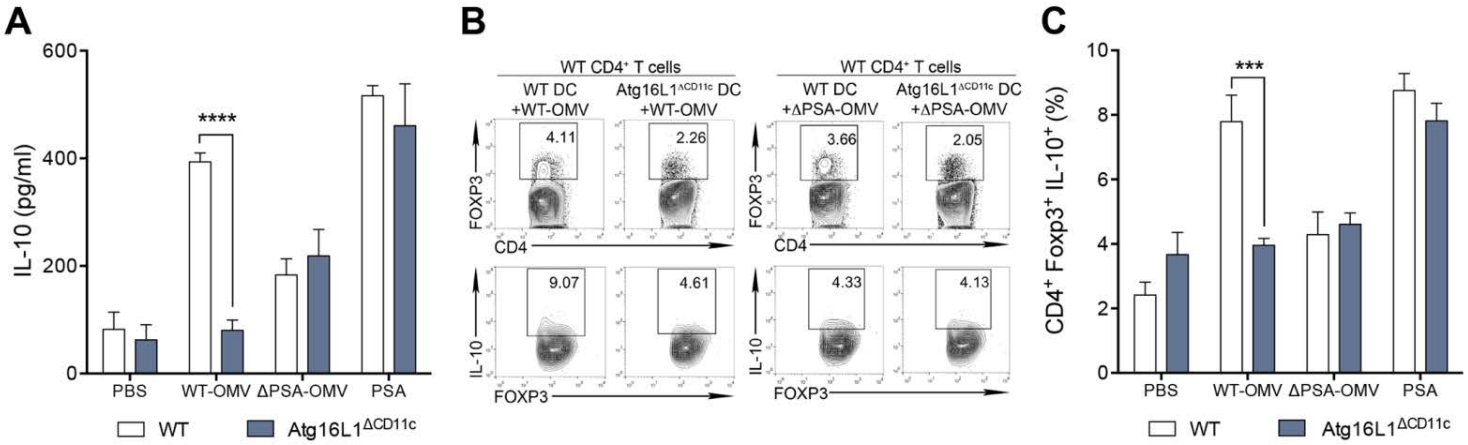
b | Group A *Streptococcus* (GAS) is sequestered by a double-membraned autophagosome that is formed from multiple isolation membranes. This is an example of an autophagy-related (ATG)-dependent autophagic pathway with non-canonical structures.

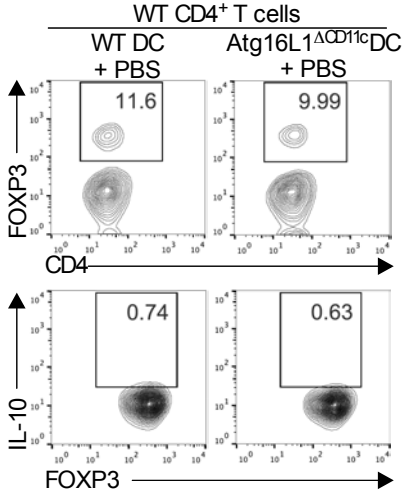
c | Engagement of Toll-like receptors (TLRs) with their ligand at the cell surface leads to the non-canonical recruitment of ATG proteins to the single-membrane bound phagosome.This is an example of an ATG-dependent, non-autophagic pathway.

Codogno et al., 2012, Nature Reviews Molecular Cell Biology

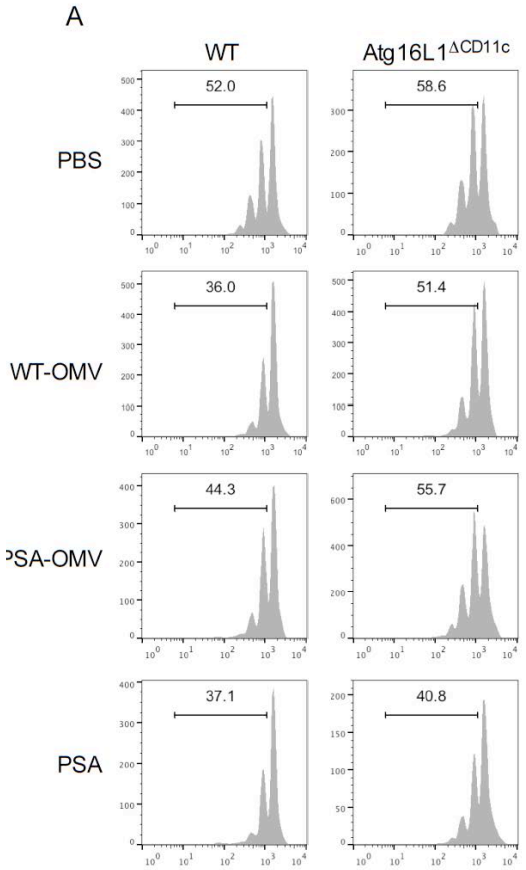
ATG16L1 Signals via a Non-canonical Autophagy Pathway during OMV-mediated T_{reg} Induction

BMDCs of WT and ATG16L1-deficient (*Atg16l1*$^{fl/fl}$*Cd11c*Cre; Atg16L1$^{\Delta CD11c}$) mice were pulsed with OMVs from WT *B. fragilis* or an isogenic mutant lacking PSA, and co-cultured with CD4^{+} T cells.

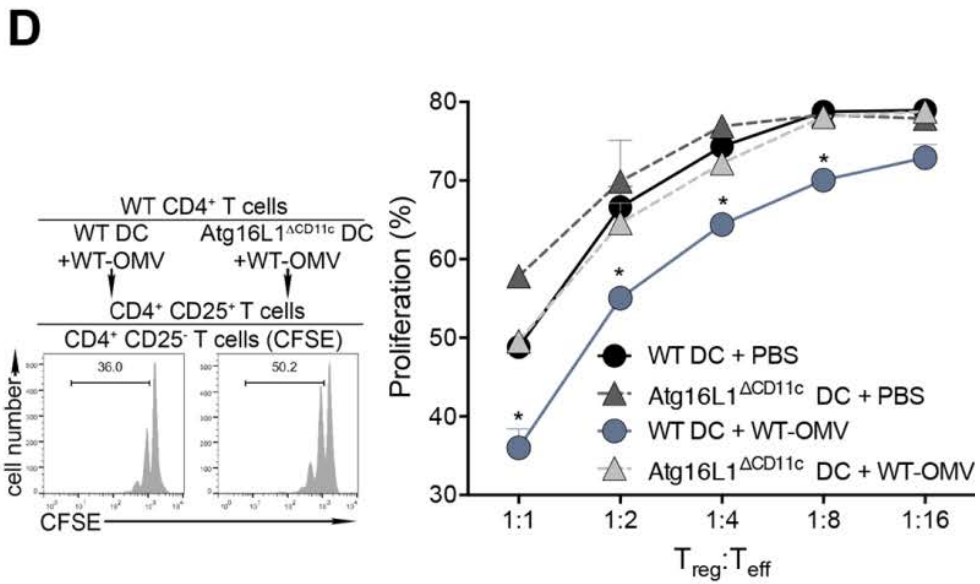




T_{regs} Isolated from Co-cultures with Atg16L1$^{\Delta CD11c}$ BMDCs Treated with *B. fragilis* OMVs Exhibit Impaired Suppressive Activity

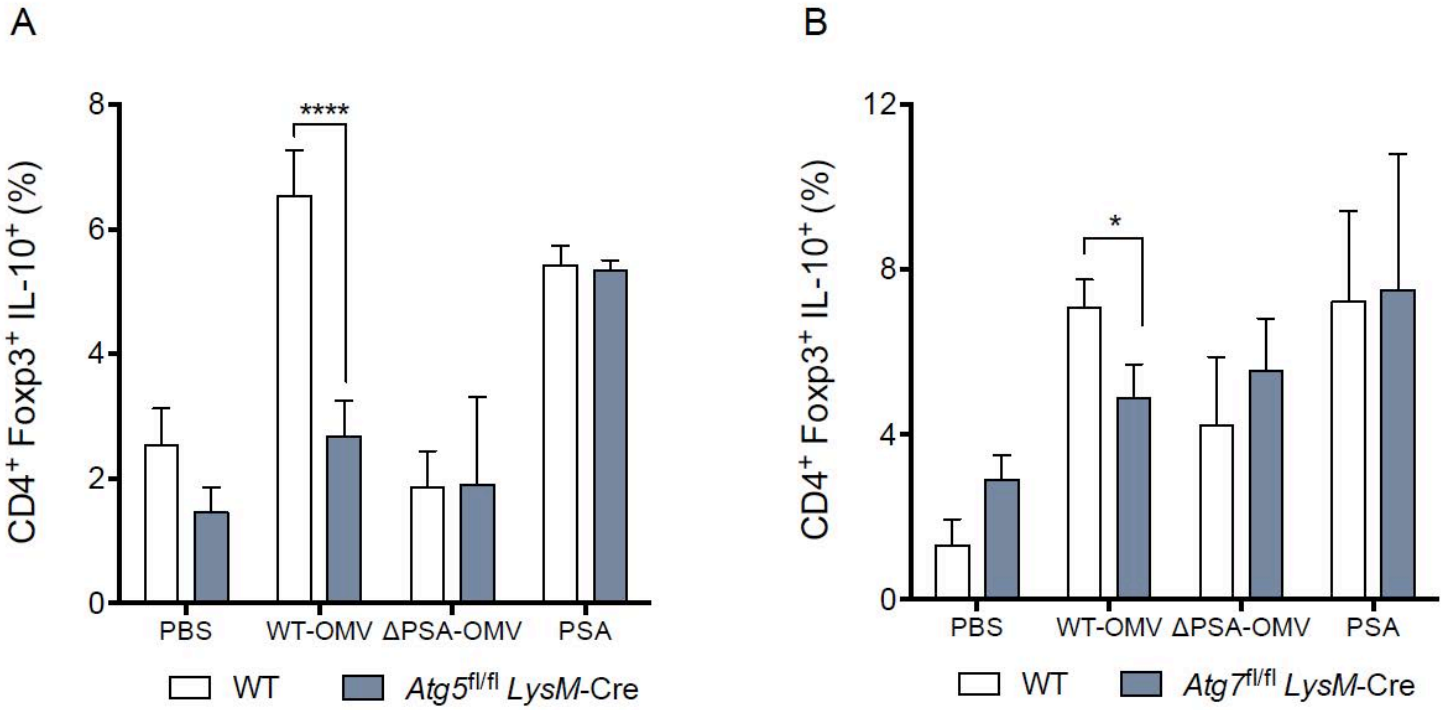


Suppression of responder T cell proliferation by *in vitro* generated Tregs from WT or Atg16L1$^{\Delta CD11c}$ DCs

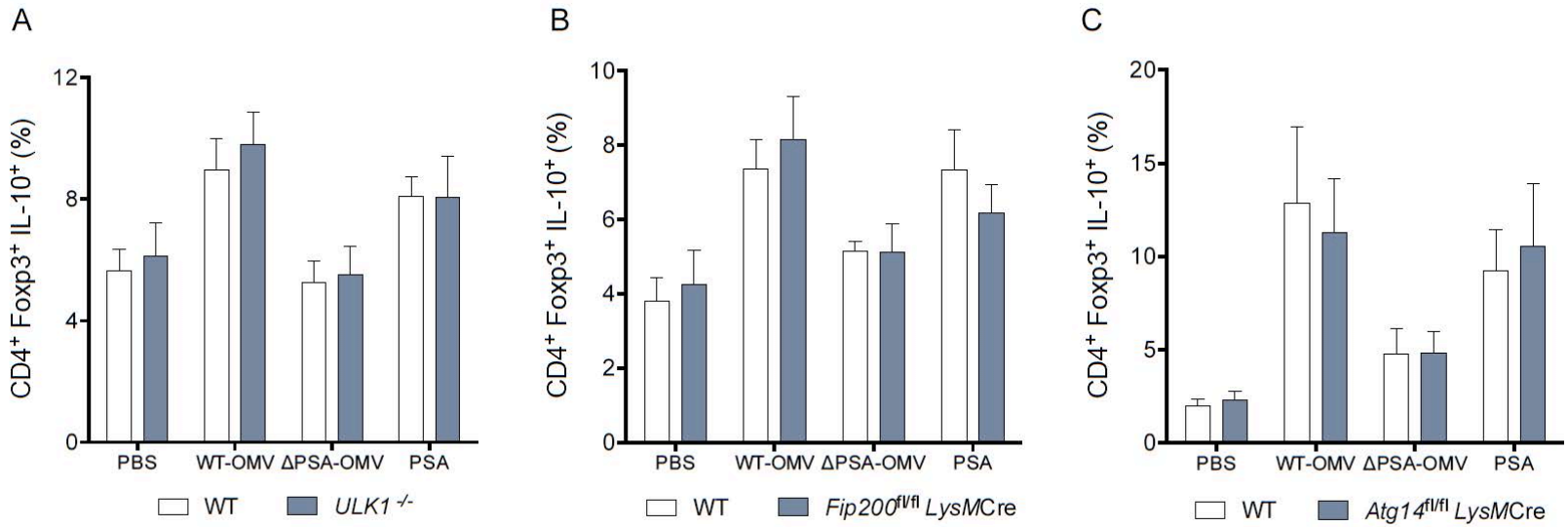


T cell suppression assay analysing in vitro generated Tregs from WT or Atg16L1$^{\Delta CD11c}$ DCs treated with WT-OMV.

B. fragilis OMVs Require Atg5 and Atg7 to Promote IL-10 Production

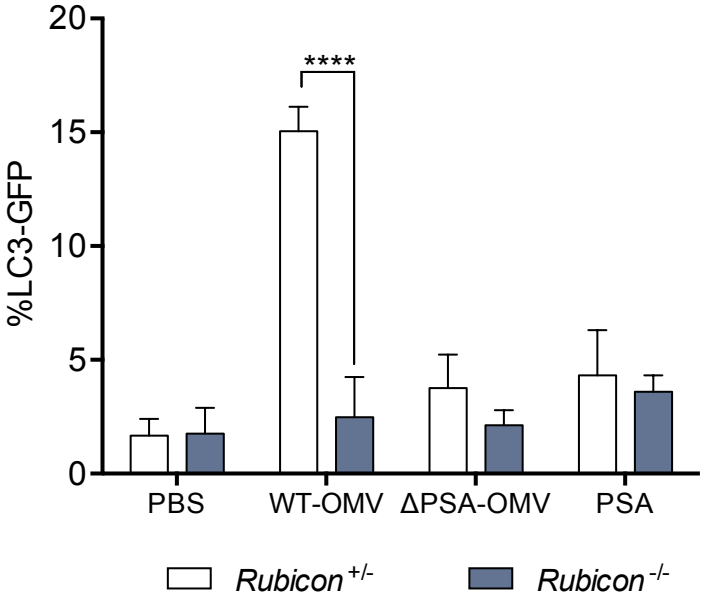


But… doesn't require classical autophagy genes

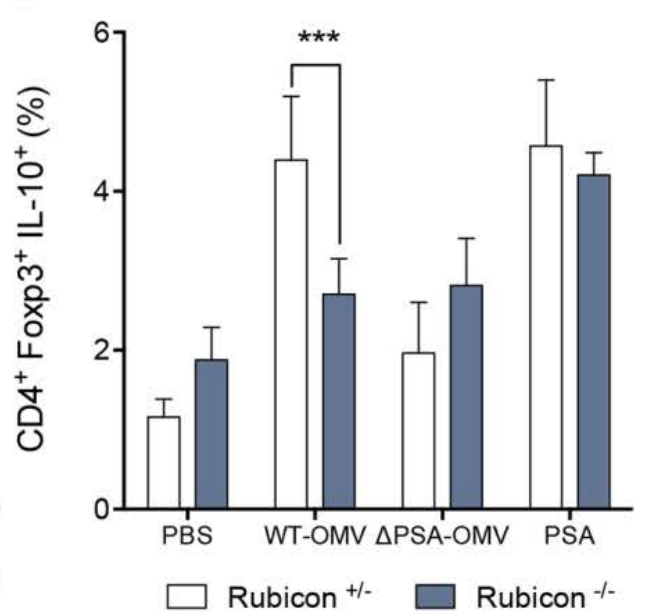


B. fragilis WT-OMVs Activate the LAP pathway

- OMVs utilize the non-canonical autophagy pathway,
- LC3-associated phagocytosis (LAP) is specifically activated by microbial ligands delivered as particles rather than soluble molecules.
- LAP activation requires RUBICON, which represses canonical autophagy.
- RUBICON is upstream of ATG16L1 signaling.



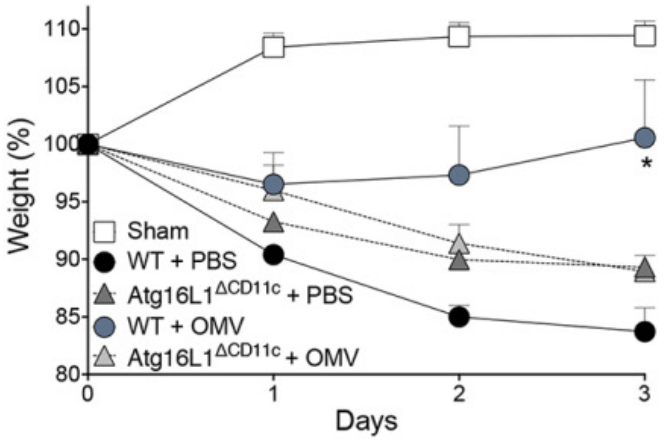
Quantification of LC3-GFP accumulation upon 2h treatment of BMDCs with PBS, *B. fragilis* WT-OMV, ΔPSA-OMV or purified PSA in *Rubicon*$^{+/-}$ or *Rubicon*$^{-/-}$ DCs

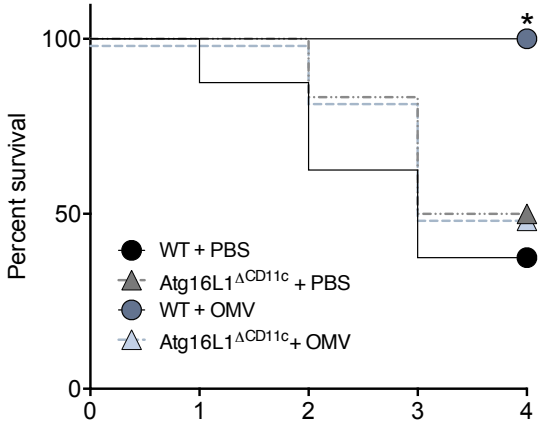


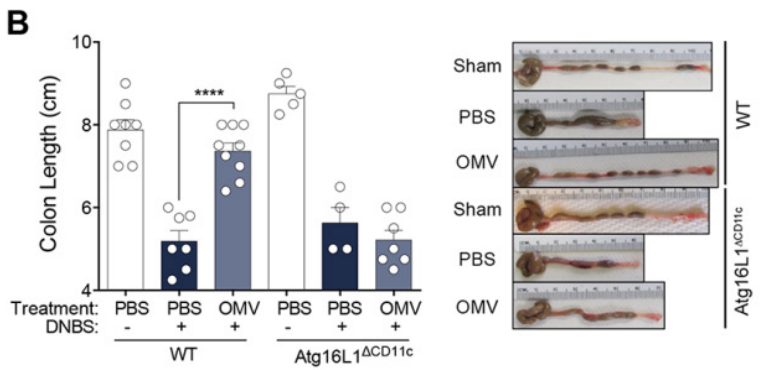
Frequency of CD4^{+}Foxp3^{+}IL-10^{+} Tregs from *Rubicon*$^{+/-}$ or *Rubicon*$^{-/-}$ DC–T cell co-cultures treated with PBS, *B. fragilis* WT-OMV, ΔPSA-OMV or purified PSA

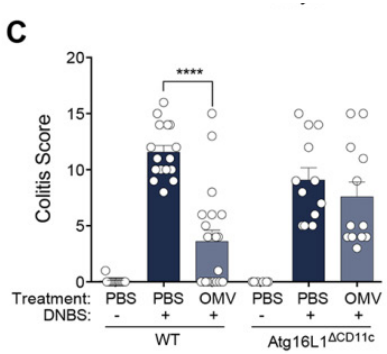
B. fragilis OMVs Require ATG16L1 in CD11c+ DCs for Protection from Colitis

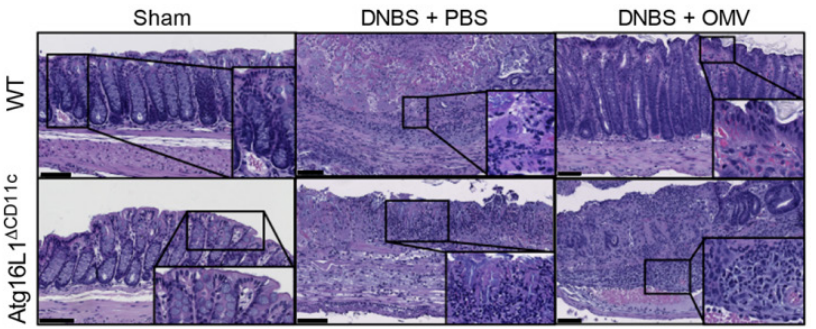
Mice were initially treated by oral gavage with WT-OMVs for 7 days and afterwards, were treated with 2,4-dinitrobenzenesulfonic acid (DNBS)





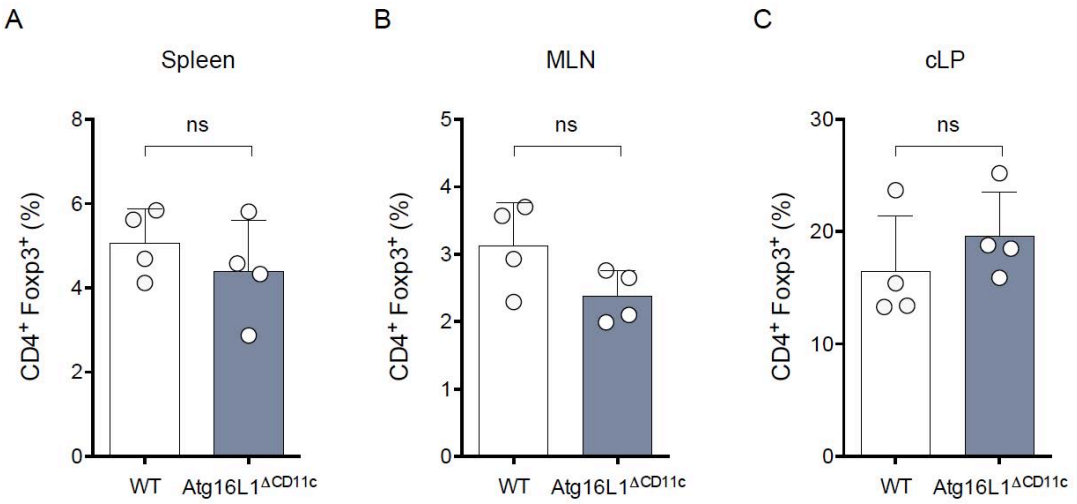




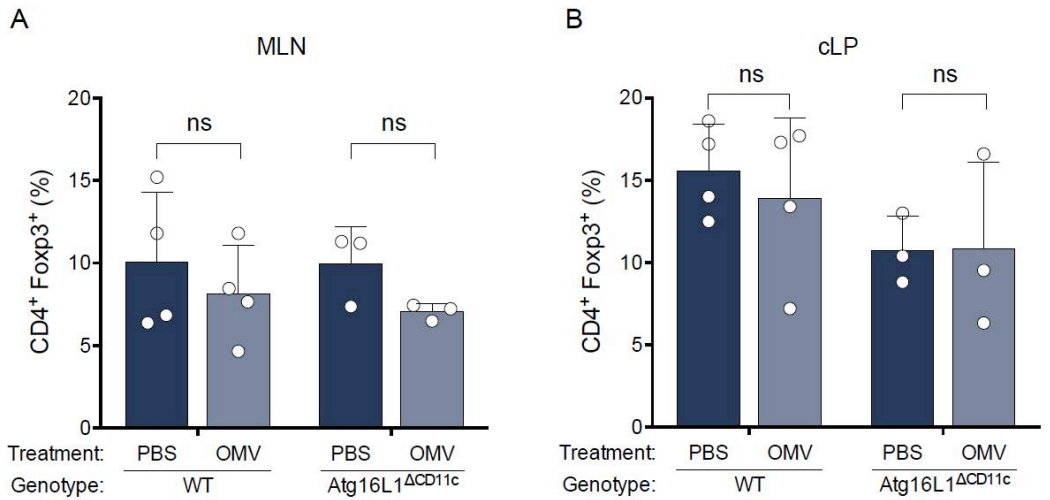


No Defects in Treg Development in KO Mice

Homeostasis

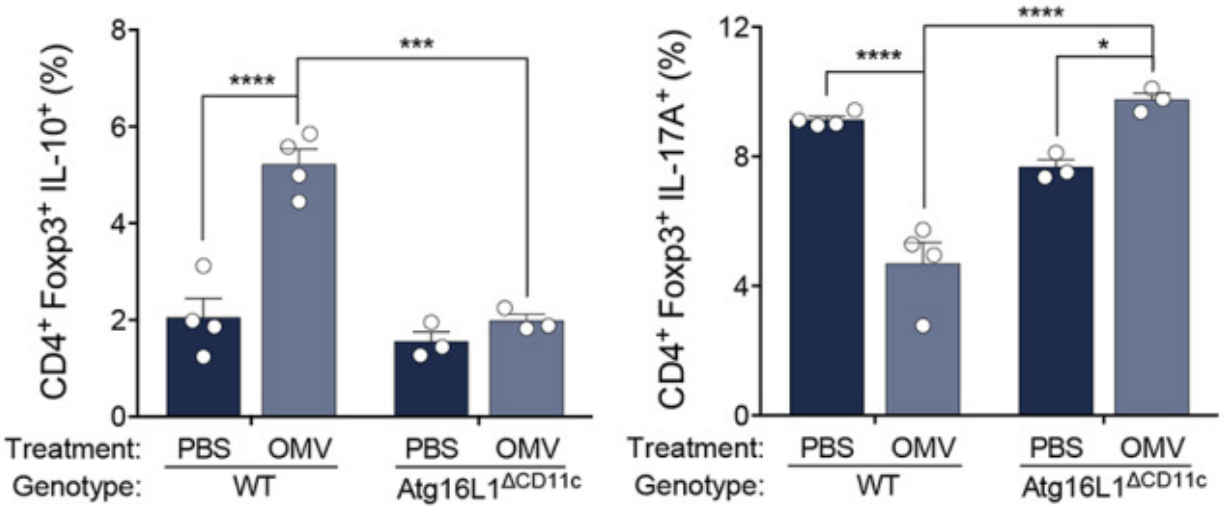


Colitis

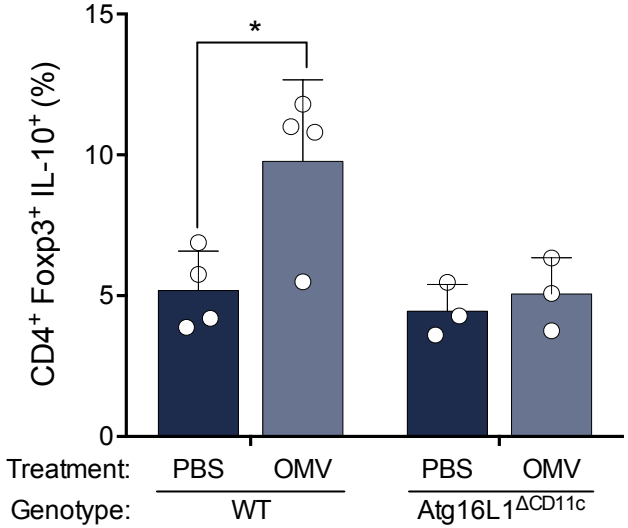


WT-OMVs Require ATG16L1 within DCs to Induce IL-10 Expression and to Suppress Intestinal Inflammation

Mice were initially treated by oral gavage with WT-OMVs for 7 days and afterwards, were treated with 2,4-dinitrobenzenesulfonic acid (DNBS)



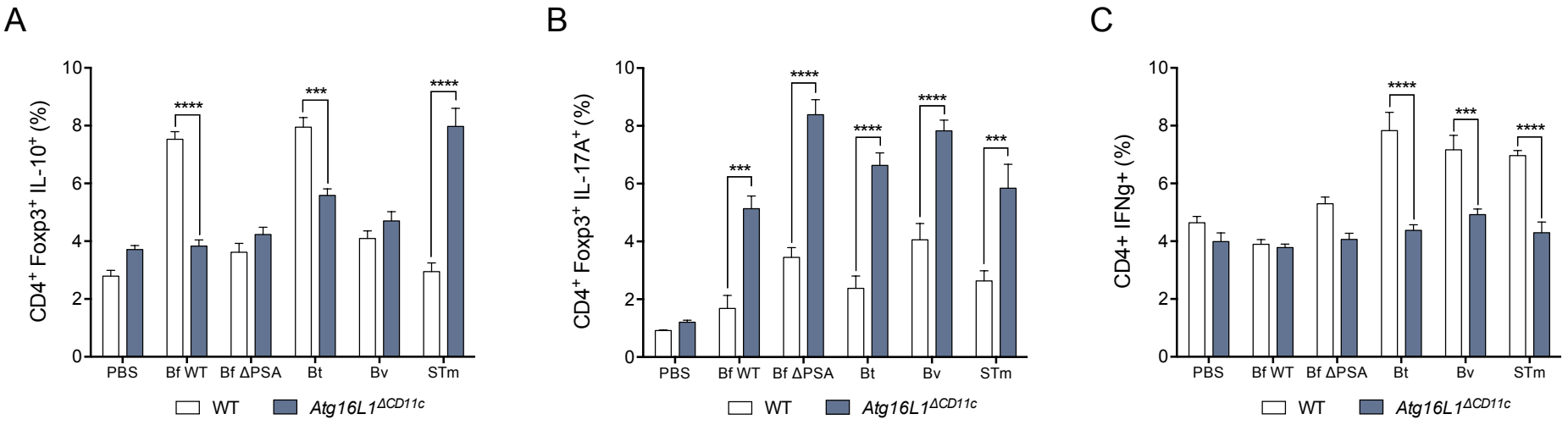
Mesenteric lymph node (MLN) lymphocytes isolated post-DNBS analyzed for IL-10 (D) and IL-17A (E) production among $CD4^{+}Foxp3^{+}$ T_{regs}



cLP lymphocytes isolated post-DNBS analyzed for IL-10 production among $CD4^{+}Foxp3^{+}$ Tregs

Commensal and Pathogen-derived OMVs Differentially Utilize ATG16L1 in CD11c+ BMDCs

BMDCs of WT and ATG16L1-deficient (*Atg16l1*$^{fl/fl}$*Cd11c*Cre; Atg16L1$^{\Delta CD11c}$) mice were pulsed with OMVs from other enteric bacteria and co-cultured with CD4^{+} T cells.



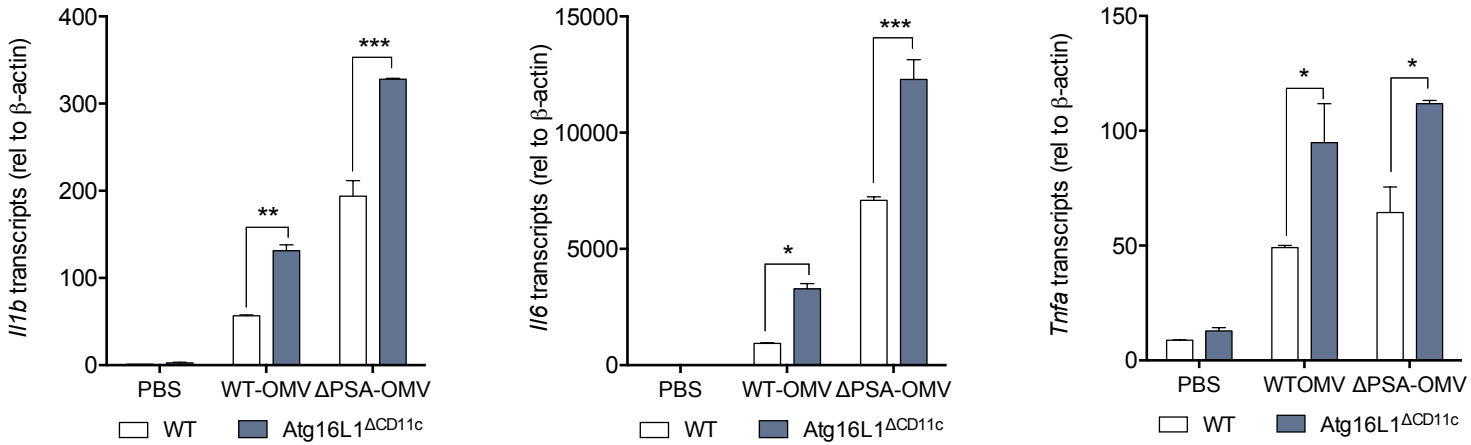
Bf, *B. fragilis*

Bt, *Bacteroides thetaiotamicron*

Bv, *Bacteroides vulgatus*

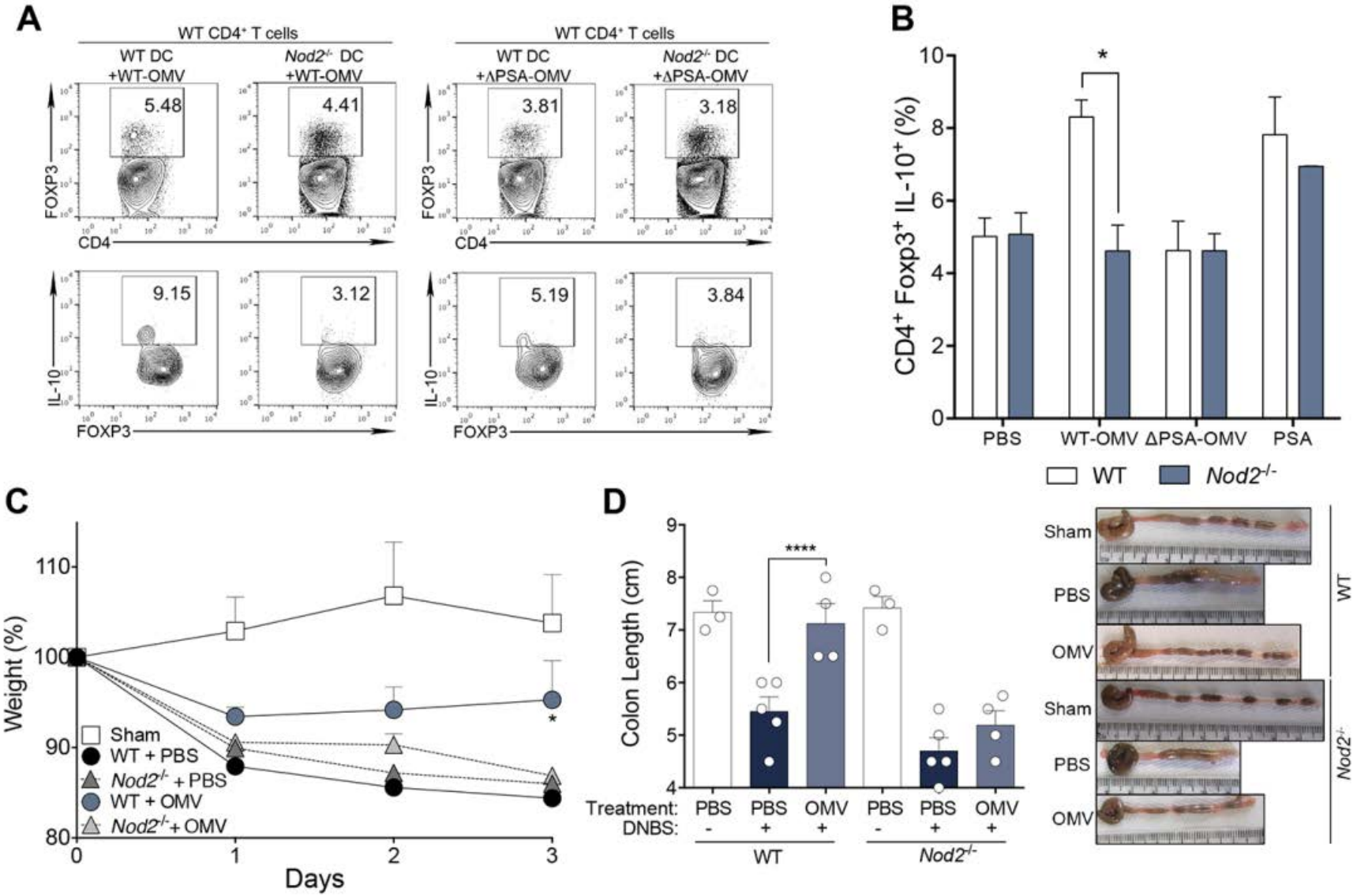
STm, *Salmonella enterica* serovar Typhimurium

Abrogation of Treg responses by ATG16L1-deficient DCs is likely due to increased pro-inflammatory cytokine production



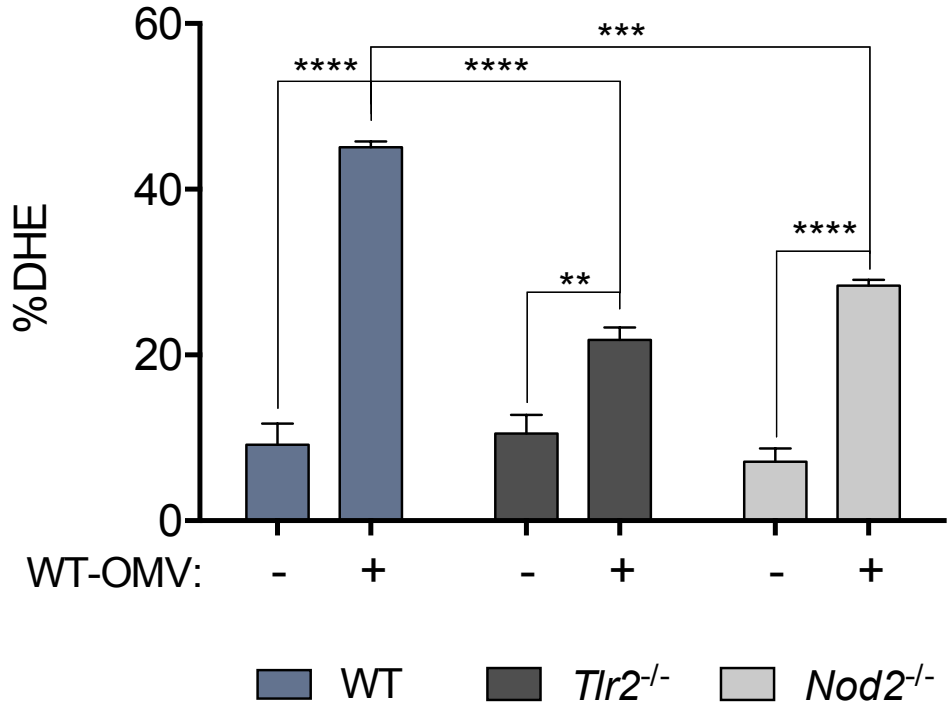
Stimulation with OMVs results in an increase of transcription of multiple pro-inflammatory cytokines in Atg16L1$^{\Delta CD11c}$ DCs compared to WT cells.

NOD2 is Required for OMV-mediated T_{regs} Induction and Protection from Colitis



TLR2 and NOD2 are Involved in OMV-mediated Induction of ROS

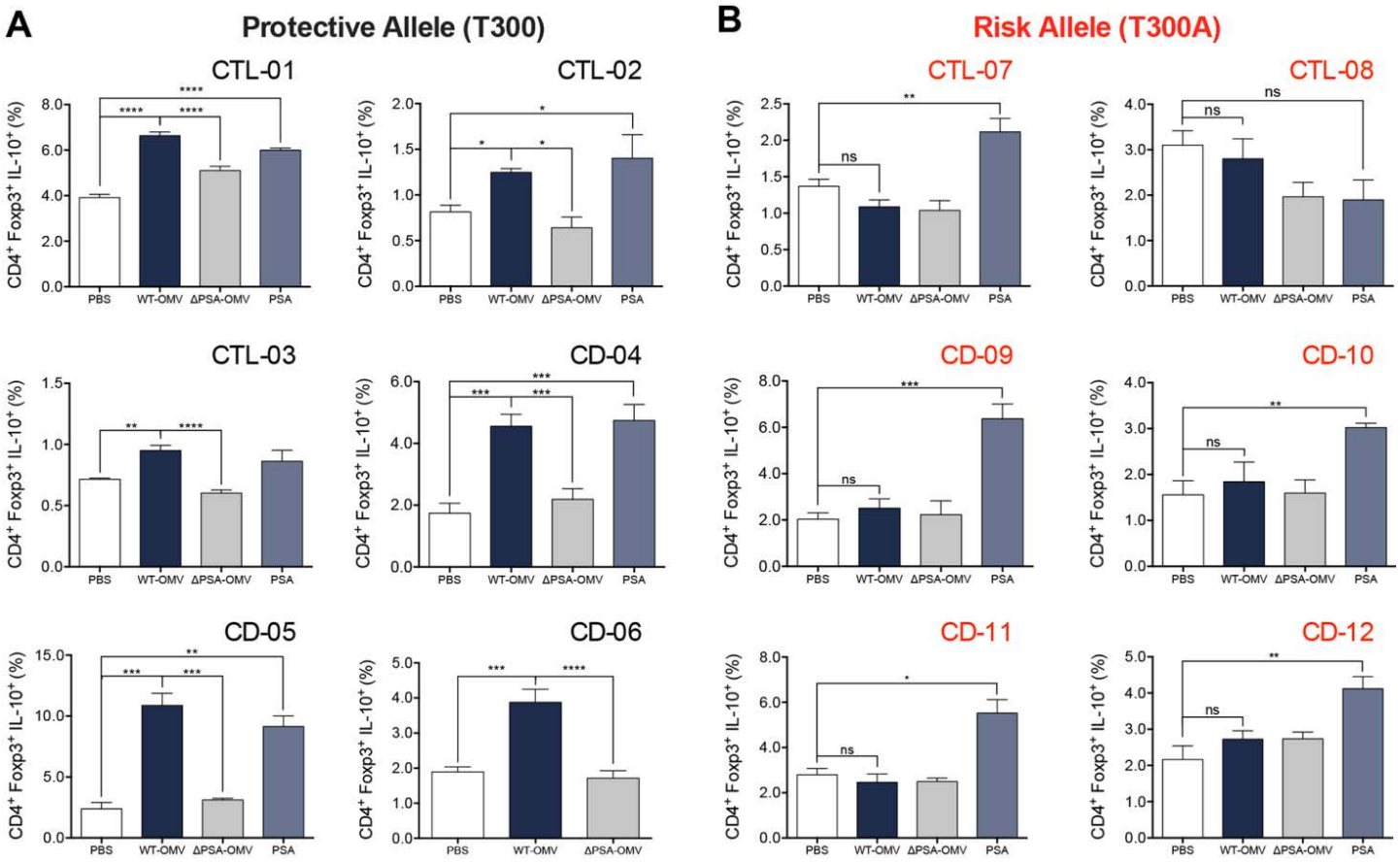
WT, *Tlr2*$^{-/-}$, or *Nod2*$^{-/-}$ BMDCs were pulsed with WT-OMVs for 2h and ROS production was assessed by dihydroethidium (DHE)



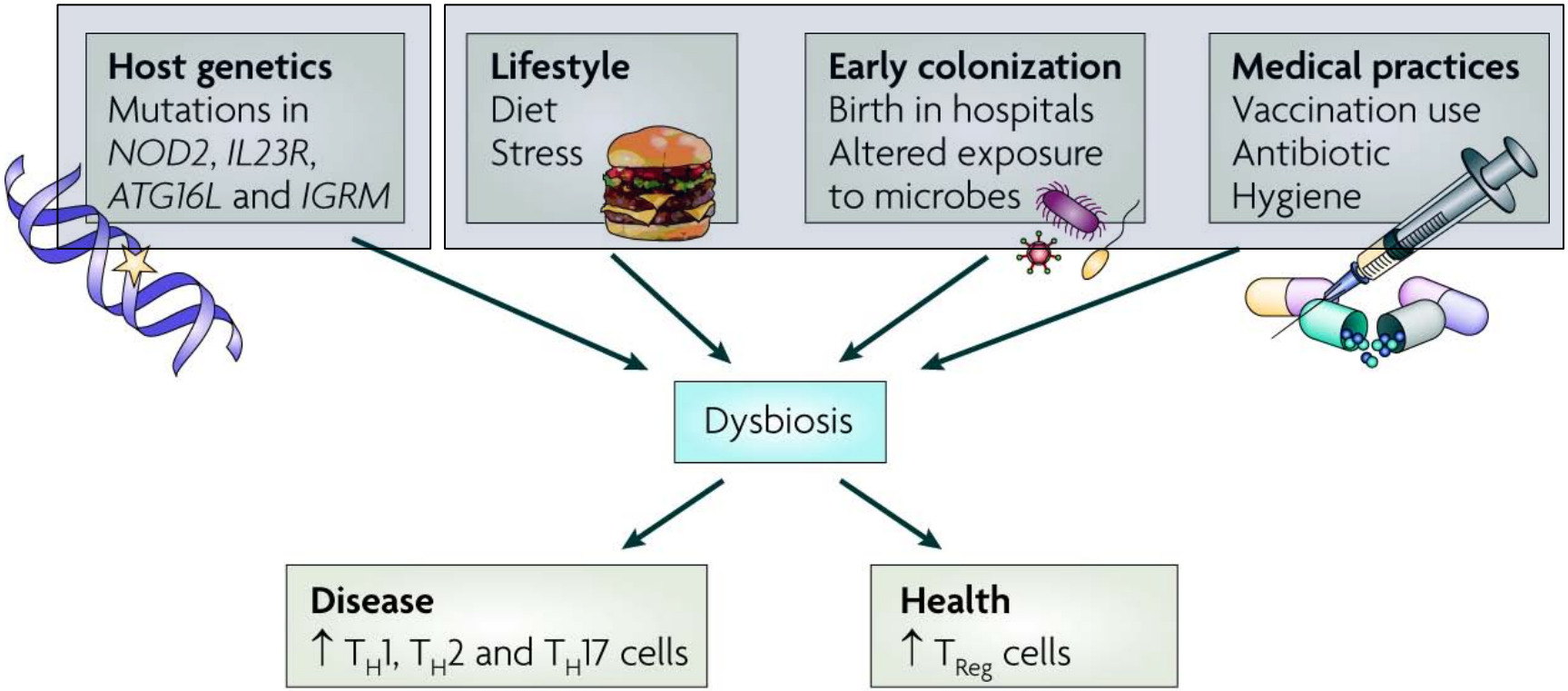
The T300A Risk Variant of ATG16L1 in Human Cells is Unable to Support OMV Responses.

- Responses to OMVs by immune cells carrying the CD-associated variant of *ATG16L1*.
- T300A variant leads to protein instability and altered cellular responses

Sample ID	Disease Status	Genotype	Sex	Age	Medications
CTL01	Normal	T300 (AA)	F	32	N/A
CTL02	Normal	T300 (AA)	F	40	N/A
CTL03	Normal	T300 (AA)	F	63	N/A
CD04	CD	T300 (AA)	M	57	N/A
CD05	CD	T300 (AA)	M	53	N/A
CD06	CD	T300 (AA)	M	39	N/A
CTL07	Normal	T300A (GG)	F	44	N/A
CTL08	Normal	T300A (GG)	F	68	N/A
CD09	CD	T300A (GG)	F	28	mercaptopurine (Purinethol); mesalamine (Apriso)
CD10	CD	T300A (GG)	F	27	adalimumab (Humira); *Bifidobacterium infantis* (Align); multivitamin; Noreth A-ET Estra/FE Fumarate(Lo Loestrin FE PO), Omega-3 Fatty Acids-Vitamin E (fish Oil); Resveratrol
CD11	CD	T300A (GG)	M	37	N/A
CD12	CD	T300A (GG)	M	23	N/A



Genetics and Environment



Round & Mazmanian, Nature Rev Immunol