Supplementary Information for

Epithelial retinoic acid receptor β regulates serum amyloid A expression
and vitamin A-dependent intestinal immunity

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Extended Materials and Methods

Quantitative PCR. Total RNA was isolated from cells or homogenized tissues using the RNeasy Lipid Tissue Kit (Qiagen). cDNAs were synthesized using MMLV reverse transcriptase (ThermoFisher). Expression of mouse *Il17a* and human *RAR* genes were analyzed using Taqman gene expression qPCR assays (Invitrogen). Mouse *Saa* and human *SAA* genes were analyzed using SYBR Green qPCR reagents and specific primers (Table S5). Relative expression values were determined using the comparative C_t ($\Delta\Delta C_t$) method, and transcript abundances were normalized to *Gapdh* transcript abundance.

siRNA knockdown. MODE-K or HepG2 cells cultured in a serum-free medium for 48 hours were trypsinized and plated at 5×10^4 cells per well of a 12-well cell culture plate. OptiMEM transfection reagent (Invitrogen) was combined with diluted siRNA pools (sequences are listed in Table S6) then added to freshly split cells. After gentle mixing, additional experimental treatments were added. MODE-K cells were treated for 24 hours with retinol (100 nM; Sigma) and lipopolysaccharide (100 ng/ml; Sigma). HepG2 cells were treated for 24 hours with retinol (100 nM), IL-1β (50 pg/ml; R&D Systems), and IL-6 (100 pg/ml; R&D Systems). Cells were harvested 24 hours post-treatment.

Transcriptional reporter assays. A 4103bp (-4000 to +103) fragment of the *Saa3* promoter was cloned into the pEZX-G04 vector containing two reporter genes (Genecopoieia) to generate the wild-type reporter construct. The putative RAR binding site in the wild-type reporter construct was mutated from TGCCTTctttcTGCCCC to TGCCATctttcAGCCCC to generate the mutant reporter construct (Invitrogen GeneArt Site-Directed Mutagenesis Plus). 5×10^4 MODE-K cells were transfected with 400 ng of wild-type or mutant reporter plasmid or 400 ng of empty

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vector. Cells were treated with retinol (100 nM) and LPS (100 ng/ml) for 24 h. Luciferase activity was measured using the Secrete-Pair Luminescence Assay Kit (Genecopoieia) and measured with a SpectraMax M5e plate reader (Molecular Devices). Gaussia luciferase (GLuc) activity was normalized against secreted alkaline phosphatase (SEAP) activity and then compared to the activity of cells transfected with pEZX-G04 alone.

Laser capture microdissection and RNA purification. Laser capture microdissection was performed as described (1). In brief, 5 cm of distal small intestine was washed and snap-frozen in optimum cutting temperature (OCT) compound (Fisher 23-730.571). Frozen sections were cut to a thickness of 7 μm and fixed in 70% ethanol, then stained with Methyl Green and eosin. Freshly stained sections were immediately used for laser capture microdissection of intestinal epithelial cells using an Arcturus PixCell IIe system, and 5,000-10,000 pulses were obtained from each section. RNA was extracted from isolated intestinal epithelial cells by incubating with 14 μl RNA Extraction Buffer from the PicoPure RNA Isolation Kit (Life Technology 12204-01) for 30 min, then stored at -80°C until use. Extracted RNA was purified using PicoPure RNA Isolation Kit following the manufacturer's protocol. RNA quality and concentration were determined by Agilent 2100 Bioanalyzer or RiboGreen S2 RNA Assay Kit (Thermo Fisher R11490).

RNA sequencing (RNAseq) and data analysis. RNA was extracted and purified from ileums of 3 mice per experimental group. RNA quality was assessed on an Agilent 2100 Bioanalyzer. Sequencing libraries were prepared using the TruSeq RNA sample preparation kit v2 (Illumina) and sequenced on an Illumina HiSeq 2500 for signal end 50 bp length reads. Sequence data were mapped against the mm10 genome using TopHat (2) and FPKMs were generated using Cuffdiff (3) with default parameters. Altered expression was defined as a >2 fold increase or decrease in average FPKM reads compared between the two groups.

RNAseq Ontology Analysis. RNAseq identified 832 genes were differentially expressed, greater than 2-fold, and with *p* values <0.05. These 832 genes were analyzed with the PANTHER Gene Ontology Classification System to identify enriched GO Biological Processes. The top 20 differentially regulated GO Biological Processes (gene categories) are shown in Fig. 3D, organized as immunity-related and metabolism-related genes. Bonferroni correction for multiple testing and a *p* value filter of <0.05 were applied to the PANTHER analysis.

Retinoid Quantification. Retinol and retinoic acid (Sigma) were freshly reconstituted in ethanol and quantified under red or amber lighting. Quantification based on absorbance at 325 nm (retinol) and 350 nm (retinoic acid) is in accordance with previously published molar extinction coefficients (4). Retinoids for use in cell culture treatments were utilized in a vehicle of 0.1% ethanol in cell culture medium.

Flow Cytometry Reagents and Antibodies. Foxp3/transcription factor staining reagents for fixation and permeabilization were used (eBiosciences). Zombie Yellow (Biolegend) fixable viability dye was used to gate live cells prior to further analysis. Antibodies used to characterize Th17 populations include: CD45-FITC (Biolegend), CD4-PECy7 (Biolegend), CD3-APC-Cy7 (Biolegend), RORgt-PE (eBiosciences), IL-17a-BV421 (BD Bioscience), IL-22-APC (eBiosciences). Antibodies used to characterize gut homing T cells include: CD3-APC (R&D Systems), CD45-FITC (Biolegend), CD4-PECy7 (Biolegend), CCR9-PE (Biolegend), $\alpha 4\beta 7$-Pacific Blue (BD Bioscience). Antibodies used to characterize Foxp3 T regulatory (Treg) cells include Foxp3-PE-Cy5 (eBiosciences), CD45-FITC (eBiosciences), CD4-Brilliant Violet 421 (Biolegend), CD3-PE (eBiosciences). Antibodies used to characterize IgA-producing B cells include: CD45-BV650 (Biolegend), CD19-FITC (eBiosciences), B220-APC (eBiosciences), and IgA-PE (eBiosciences). Antibodies used to characterize dendritic cell populations include CD45-

Pacific Blue (Biolegend), CD11c-APC-Cy7 (eBiosciences), CD103-FITC (Biolegend), MHCII-BV711 (eBiosciences), CD11b-PE (Biolegend), and F480-APC (eBiosciences).

16S rRNA gene sequencing and data analysis

Fecal samples were collected from $Rarb^{fl/fl}$ and $Rarb^{\Delta IEC}$ littermates that were co-caged. Fecal DNA was purified using the FastDNA Spin Kit (MP Biomedicals 116560-200) and a FastPrep-24 Homogenizer. Sequencing libraries were prepared using the HotStarTaq Plus Master Mix Kit (Qiagen) with primers flanking variable regions V3-V4. Sequencing was performed on a MiSeq following the manufacturer's guidelines. Operational taxonomic units (OTUs) were defined by clustering at 3% divergence (97% similarity). Final OTUs were taxonomically classified using BLASTn against a curated database derived from RDPII and NCBI. Principle component analysis was performed using the "prcomp" function in R and differential abundance analysis was performed using DESeq2 (5).

IgA ELISA. Total protein was isolated by homogenizing mouse feces in PBS with protease inhibitor cocktail (Roche). Homogenates were rotated at 4°C for 4 h and centrifuged at 16,000g for 15 min. IgA were quantified per manufacturer instructions (Invitrogen).

Histology. Paraffin embedded sections of Bouin's fixed mouse distal ileum were cut, and heat-induced epitope retrieval was performed in a 0.05% sodium citrate buffer (pH 6.0). The slides were incubated with rabbit anti-mouse SAA antiserum (6)(1:50 dilution) at 4°C overnight and then detected with a goat anti-rabbit IgG Cy3 conjugate (Biomeda). Tissues were counterstained with DAPI and images were captured on a Zeiss AxioImager M1 microscope. H&E and PAS staining were performed by the UTSW Histology Core.

Supplementary Information References

1.   Stappenbeck TS, Hooper LV, Manchester JK, Wong MH, Gordon JI (2002) Laser capture microdissection of mouse intestine: characterizing mRNA and protein expression, and profiling intermediary metabolism in specified cell populations. *Methods Enzymol* **356**:167-196.

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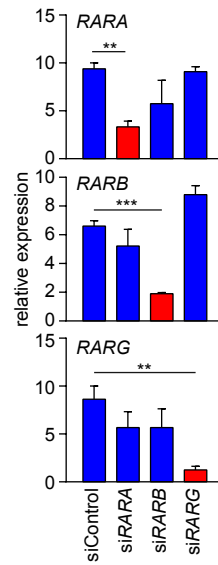
Figure S1: siRNA knockdown of *RAR* expression in HepG2 cells. HepG2 cells were treated for 24 hours with retinol (100 nM), IL-1β (50 pg/ml), IL-6 (100 pg/ml), and were transfected with siRNAs targeting each of the three *RAR* isoforms. A scrambled siRNA was used as a negative control (siControl). *RAR* expression was quantified by qPCR analysis. Assays were performed in triplicate and represent three independent experiments. Means±SEM are plotted. ***P<0.01, ****P<0.001 as determined by Student's *t*-test.
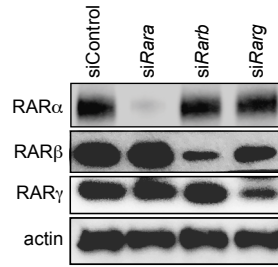
Figure S2: siRNA knockdown of RAR expression in MODE-K cells. MODE-K cells were treated for 24 hours with retinol (100 nM), IL-1β (50 pg/ml), IL-6 (100 pg/ml), and were transfected with siRNAs targeting each of the three *Rar* isoforms. RARα, β, and γ were detected by Western blot using antibodies specific to each isoform, with actin as a loading control. The blot is representative of three independent experiments.

Predicted RAREs
at the mouse *Saa1* promoter

Predicted RAREs
at the mouse *Saa2* promoter
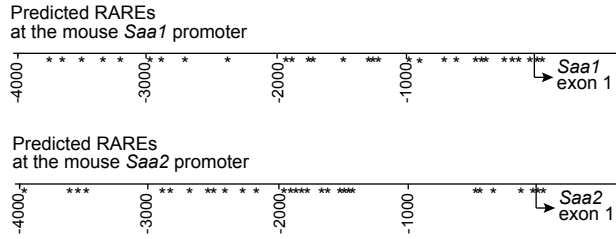
Figure S3: Predicted retinoic acid response elements (RAREs) in the mouse *Saa1* and *Saa2* promoters. *In silico* analysis of the *Saa1* and *Saa2* promoter regions using NUBIScan (7) identified predicted RARE sequences in each promoter.
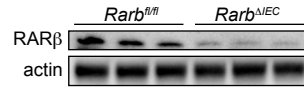
Figure S4: Epithelial cell-specific deletion of *Rarb* in mice. *Rarb*$^{\Delta IEC}$ mice were created by crossing *Rarb*$^{fl/fl}$ mice with mice carrying a *Villin-Cre* transgene. Epithelial cells were isolated from *Rarb*$^{\Delta IEC}$ and *Rarb*$^{fl/fl}$ mice by treatment with 10 mM EDTA as previously described (8). RARβ was detected by Western blot, with actin as a loading control.
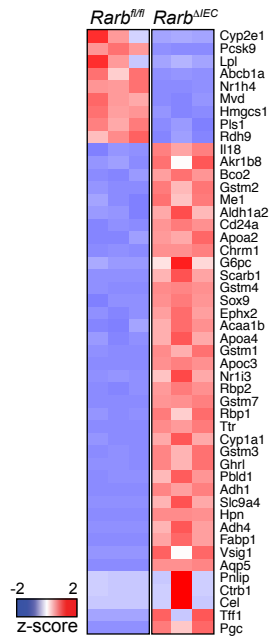
Figure S5: Altered expression of metabolism genes in small intestines from mice with an epithelial cell-specific deletion of *Rarb*. RNAseq analysis was performed on small intestines from *Rarb$^{fl/fl}$* and *Rarb$^{\Delta IEC}$* mice. Gene ontology (GO) biological process enrichment analysis of differentially regulated genes is shown in Fig. 3*D*, with metabolic gene categories highlighted in blue. Heat map shows expression levels of the 48 genes identified as having metabolic functions by the GO analysis, and which also had a -log$_{10}$(*P* value) >5.
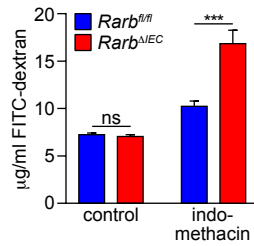
Figure S6: Intestinal permeability measurements in *Rarb*^{fl/fl} and *Rarb*^{ΔIEC} mice. Serum concentrations of FITC-dextran 4 hours after oral gavage are shown. As a positive control, intestinal epithelial damage was induced by pretreatment with indomethacin (15 mg/kg in DMSO) for 1 hour prior to FITC-dextran administration (600 mg/kg body weight; 4 kDa; Sigma). *N*=3 mice/group, and each sample was analyzed in triplicate. Means±SEM are plotted. ***P<0.001 as determined by Student's *t*-test. ns, not significant.
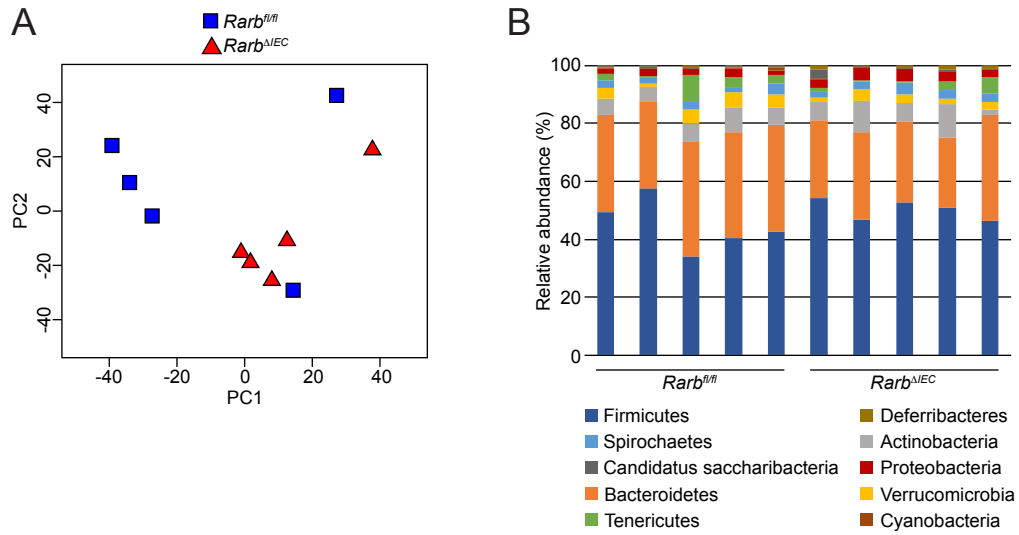
**Figure S7: The intestinal microbiotas of *Rarb^{fl/fl}* and *Rarb^{ΔIEC}* mice are similar.** (*A*) Principal coordinate analysis of 16*S* rRNA sequencing of fecal samples from *Rarb^{fl/fl}* and *Rarb^{ΔIEC}* mice. The mice were littermates of heterozygous crosses that remained cohoused. Each dot represents one mouse. (*B*) Relative abundances of bacterial genera in *Rarb^{fl/fl}* and *Rarb^{ΔIEC}* mice.
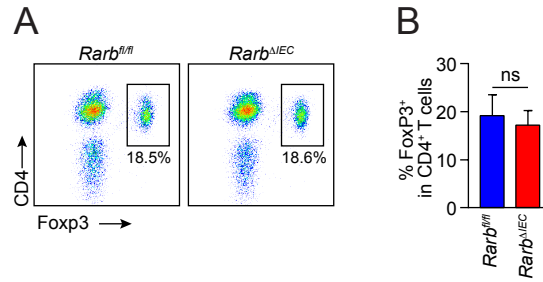
Figure S8: T$_{reg}$ cell frequencies are similar in the intestines of *Rarb$^{fl/fl}$* and *Rarb$^{\Delta IEC}$* mice.
(*A*) Representative flow cytometry plots of T cells (CD45$^+$ CD3$^+$ CD4$^+$) expressing Foxp3, which marks T$_{reg}$ cells. The T cells were isolated from the small intestines of *Rarb$^{fl/fl}$* and *Rarb$^{\Delta IEC}$* litter-mates. (*B*) Quantification of T$_{reg}$ frequencies in *A*. N=8 (*Rarb$^{fl/fl}$*) and 6 (*Rarb$^{\Delta IEC}$*) mice per group from two independent experiments. ns, not significant as determined by Student's *t*-test.
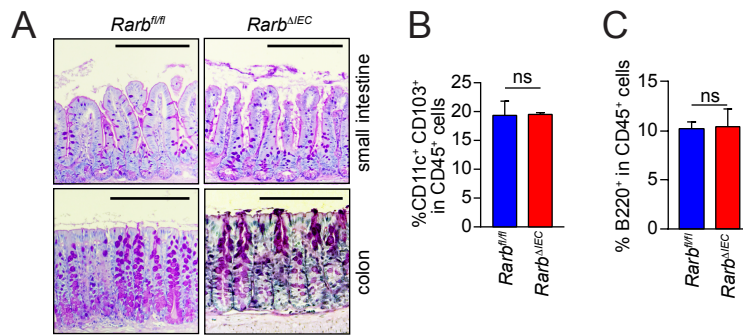
Figure S9: Numbers of Paneth cells and goblet cells, and frequencies of CD11c⁺ CD103⁺ dendritic cells and B cells are similar in the intestines of *Rarb^{fl/fl}* and *Rarb^{ΔIEC}* mice. (*A*) Periodic Acid Schiff (PAS) staining was performed on small intestines and colons from *Rarb^{ΔIEC}* mice and their *Rarb^{fl/fl}* littermates in order to detect goblet cells. Scale bars, 220 μm. (*B* and *C*) Cell suspensions from the small intestines of *Rarb^{ΔIEC}* mice and their *Rarb^{fl/fl}* littermates were analyzed by flow cytometry. (*B*) DCs were identified using antibodies against CD45, CD11c, and CD103. Frequencies of CD11c⁺ CD103⁺ cells (which include RA-producing DCs) within the CD45⁺ cell subset are shown. (*C*) B cells were identified using antibodies against CD45 and B220. Frequencies of B220⁺ cells within the CD45⁺ cell subset are shown. *N*=3 mice per group; data represent three independent experiments. Means±SEM are plotted. ns, not significant as determined by Student's *t*-test.
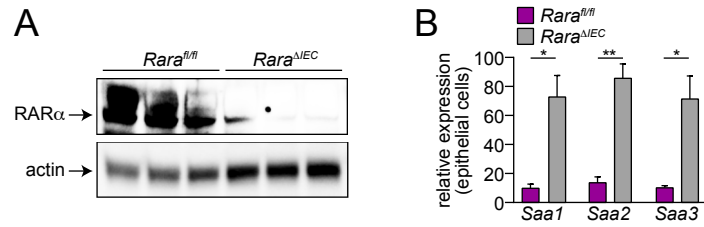
Figure S10: Epithelial cell RARα is dispensable for *Saa* expression in the small intestine.
(*A*) *Rara^ΔIEC^* mice were created by crossing *Rara^fl/fl^* (9) with mice carrying a *Villin-Cre* transgene (10). RARα expression was assessed by Western blotting of small intestinal epithelial cells isolated by EDTA treatment as described (8). Actin was detected as a control. (*B*) *Saa* expression was measured by qPCR analysis of small intestinal epithelial cells isolated by EDTA treatment as in *A*. *N*=3 mice per group. *P<0.05, **P<0.01 as determined by Student's *t*-test.
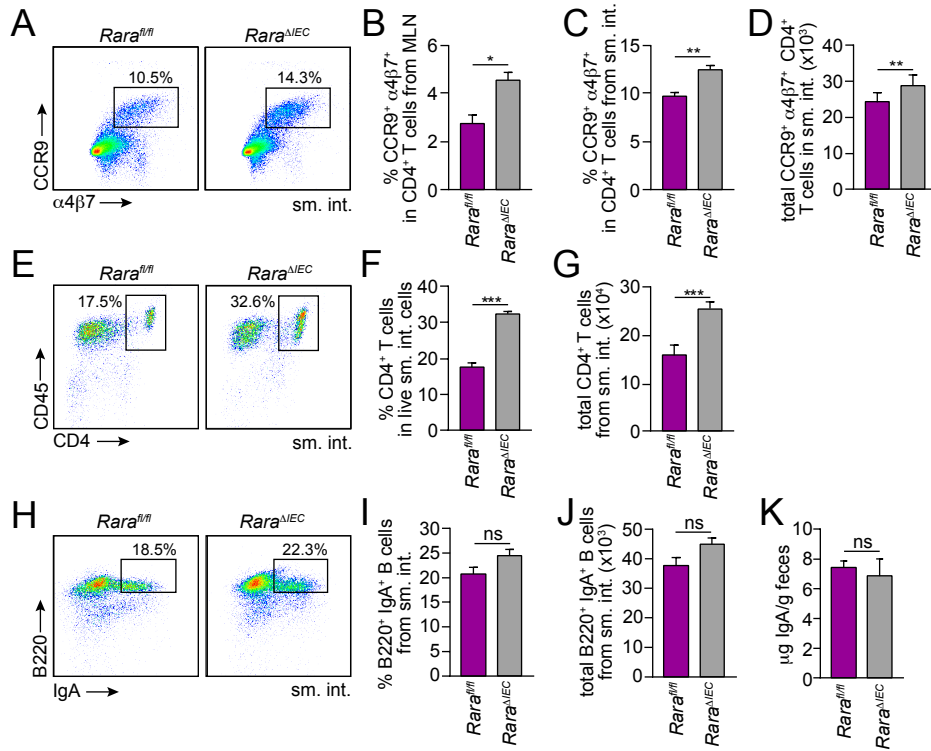
Figure S11: Epithelial cell RARα is dispensable for CD4⁺ T cell homing and B cell expression of IgA. (*A-D*) Expression of the gut homing markers α4β7 and CCR9 on T cells (CD4⁺ CD45⁺ CD3⁺) from *Rara^fl/fl* and *Rara^ΔIEC* littermates. (*A*) Representative flow cytometry plots of small intestinal T cells. Frequencies of CCR9⁺ α4β7⁺ cells in CD4⁺ CD45⁺ CD3⁺ cells from MLN (*B*) and small intestine (*C*). Total gut homing CD4⁺ T cell numbers (CCR9⁺ α4β7⁺ CD45⁺ CD4⁺ CD3⁺) are given in (*D*). N=3 mice/group; data represent three independent experiments. (*E*) Flow cytometry of CD4⁺ (CD45⁺ CD3⁺) T cells from the small intestines of *Rara^fl/fl* and *Rara^ΔIEC* littermates. CD4⁺ (CD45⁺ CD3⁺) T cell frequencies are quantified in (*F*) and total small intestinal CD4⁺ T cell numbers (CD4⁺ CD45⁺ CD3⁺) are given in (*G*). *N*=5 mice per group; data represent two independent experiments. (*H*) Flow cytometry analysis of IgA⁺ B220⁺ (CD45⁺ CD19⁺) B cells from *Rara^fl/fl* and *Rara^ΔIEC* littermates. IgA⁺ B220⁺ cell frequencies in CD45⁺ CD19⁺ B cells are quantified in (*I*) and total numbers of small intestinal IgA⁺ B cells are shown in (*J*). *N*=5 mice per group; data represent two independent experiments. (*K*) Quantification of fecal IgA by ELISA. *N*=4 mice/group; data represent two independent experiments. Means±SEM are plotted. *$P<0.05$, **$P<0.01$, ***$P<0.001$ as determined by Student's *t*-test. ns, not significant. sm. int., small intestine. MLN, mesenteric lymph nodes.
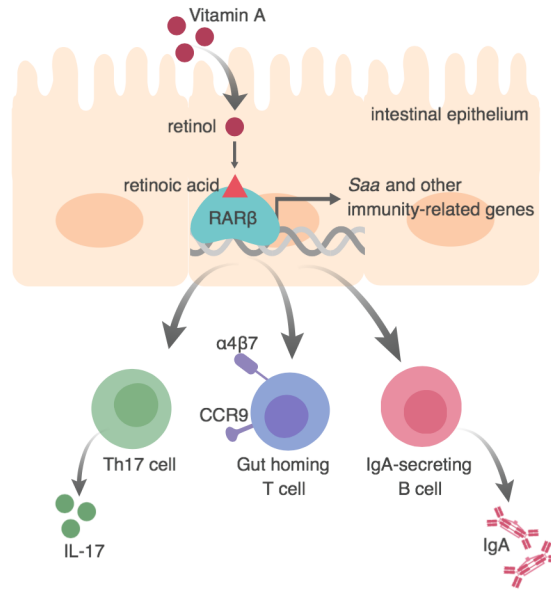
Figure S12: Epithelial RARβ regulates intestinal adaptive immunity. The transcription factor RARβ is activated by retinoic acid, which is derived from dietary vitamin A. Epithelial RARβ controls an immune transcriptional program that includes members of the *Saa* family. Epithelial RARβ promotes IL-17 production by Th17 cells, which is known to require SAA (11). Epithelial RARβ also promotes vitamin A-dependent immune responses that include the generation of gut homing CD4^{+} T cells (marked by the gut homing receptors α4β7 and CCR9)(12) and IgA-secreting B cells (13). Our findings reveal a mechanism by which the intestinal epithelium regulates adaptive immunity in response to diet. Schematic was made in ©BioRender (biorender.com).

Table S1: Predicted RARE sequences in the mouse *Saa3* promoter

Predicted RARE sequence	RARE type[1]	RARE location[2]	RARβ ChIP fold enrichment
TGAGTGgcttcTGTCCT	DR5	-3895	ND
TAAGCTaaTGCCCT	DR2	-3556	ND
AGCTCAgttcaGGTTTA	DR5	-3537	ND
TGGTCTtcTCTCCT	DR2	-3142	ND
GAGGCAatAGCTCA	DR2	-2949	ND
TTGGCTcacgtTGTCCT	DR5	-2490	ND
TGTCCTagcagTAAGCA	DR5	-2479	ND
GGTGCCtgtagAGGTCA	DR5	-2230	ND
AGGTCAgaAAATGA	DR2	-2116	ND
TTAACTgcTGACCC	DR2	-2007	ND
TGGACTttttcTTTACT	DR5	-1915	1.9
AGTGCAcacccAAGTCC	DR5	-1832	0.8
ACAACAacaacAGAGCA	DR5	-1740	4.7
ACAACAacAGAGCA	DR2	-1737	ND
TATCCTgttgcTGCCCT	DR5	-1563	1.8
AGGACAcaggaGGGACA	DR5	-1357	1.1
AGACAAgtgaaGGGTCA	DR5	-1310	2.5
TGCACAaggccTGTTCT	DR5	-1255	0.5
TGTTCTccTGAATG	DR2	-1244	0.2
CGCACAgccccTGGACA	DR5	-1160	0.2
TCCTCTaaggaTGCTCT	DR5	-1134	0.3
AGACCCagacaTGCTCT	DR5	-1058	1.1
TGCTCTgccccTGACTG	DR5	-689	1.1
TGAACAaaTTTCCT	DR2	-603	1.4
TGGCCAgcaagAGGTCA	DR5	-463	0.4
TGAGCTcacacGGGTCT	DR5	-327	3.6
TGCCTTctttcTGCCCC[3]	DR5	-224	4.9
TCACATaaggtTGCCCT	DR5	-116	1.1
AGGTGAtgaatAGTTAA	DR5	-11	2.9
TTACCTgcTGAACG	DR2	15	0.2

[1]RARE type: The most frequent direct repeats (DRs) with 1, 2, or 5 nucleotides spacing are termed DR1, DR2, and DR5 elements, respectively.

[2]RARE location was determined relative to the *Saa3* start site.

[3]RARE -224 was further validated by ChIP and luciferase reporter assay for Saa3 promoter activity as shown in Fig. 2*B* and *C*.

Table S2: Predicted RARE sequences in the mouse *Saa1* promoter

mSAA1	Predicted RARE sequence	RARE type	RARE location
243	AGGACAcaccaTGATCT	DR5	-3758
335	GGATCAcaacaATCCCA	DR5	-3666
558	AGGTCTtaGGGCCA	DR2	-3443
693	ACTTCAgtataGGAACA	DR5	-3308
874	AGAGCTagctgGGGACA	DR5	-3127
1056	GGATGAagAGCTCA	DR2	-2945
1113	AGGTCActtctTGTAAA	DR5	-2888
1304	AATTCAcaCGGACA	DR2	-2697
1689	TGTCCActaggCGGACA	DR5	-2312
2018	AGACCTgtagcAGGGCA	DR5	-1983
2029	AGGGCAgtagtAGCACA	DR5	-1972
2234	GGCTCAgcAGGTCA	DR2	-1767
2330	AGGGCAtcAGATCT	DR2	-1671
2648	AGACCAggccaGTCTCA	DR5	-1353
2840	AGAGCAagACGACA	DR2	-1161
2863	ATGGCAgaaggAGGAGA	DR5	-1138
2898	AGCAGAcaAGCTCA	DR2	-1103
3051	GGTTAAgaAGCACA	DR2	-950
3182	AGGGGAaggctGGGCCA	DR5	-819
3304	AGTTCCctgtgGGGCCA	DR5	-697
3653	AGGTAAaatggGGGGCA	DR5	-348
3664	GGGGCAgggggAGAACA	DR5	-337
3664	GGGGCAggGGGAGA	DR2	-337
3825	TGTGCAatgggAGCACA	DR5	-176
3845	GGATGAagAGCTCA	DR2	-156
3853	TCTTCAtccacAGGTCA	DR5	-148
3942	AGGACAgcCTGGCA	DR2	-59
3953	AGGTGAgggcaAGGACA	DR5	-48
4003	AGATCAccAGATCT	DR2	3
4031	AGGTGAgaggcAGATCC	DR5	31

Table S3: Predicted RARE sequences in the mouse *Saa2* promoter

mSAA2	Predicted RARE sequence	RARE type	RARE location
334	GGTACAcaGTGTCA	DR2	-3667
433	AGGACTggttgAGAGCA	DR5	-3568
522	AGGGCAgaGGTAGA	DR2	-3479
1130	GGGCCAagAGAACG	DR2	-2871
1166	GGGTCAagagcAGCCAA	DR5	-2835
1277	AGCACAatagcAGGGCC	DR5	-2724
1489	AGATCTcaTGTGCA	DR2	-2512
1630	GGATCAtctcaAGTGCC	DR5	-2371
1702	AAGTGAgttccAGGACA	DR5	-2299
1878	AGATGAgagacTGGGCA	DR5	-2123
2096	AGTGCAgctatGGGGCA	DR5	-1905
2148	AAGCCActGGTCCA	DR2	-1853
2155	TGGTCCacaagAGGACA	DR5	-1846
2271	AGTACAgcAGGACT	DR2	-1730
2428	AAGTCAtgcagTGGTCA	DR5	-1573
2433	AATTCAagAGTTTA	DR2	-1568
2471	AGGGGAacTGGGCA	DR2	-1530
2564	GGGGCAgtttaATGTCA	DR5	-1437
2572	AGGTCTgtGGGGCA	DR2	-1429
2583	AGGACCctggtAGGTCT	DR5	-1418
3413	AGTGTAtatttTGTTCA	DR5	-588
3421	AGCTCAttAGTGTA	DR2	-580
3670	TGTTCCtgcagAGAACA	DR5	-331
3813	AGCACAggAGACAA	DR2	-188
3824	TGTGCAatgggAGCACA	DR5	-177
3844	GGATAAagAGCTCA	DR2	-157
3941	AGGACAgcCTGGCA	DR2	-60
3958	TGCTCAggtgaGGGGCA	DR5	-43
4003	AGACCAccAGATCT	DR2	3
4031	AGGTGAgaggcAGATCC	DR5	31

Table S4: PCR primers for predicted RAREs in mouse *Saa3* promoter

Target Sequence in mSAA3 promoter	Forward Primers (5' to 3')	Reverse Primers (5' to 3')
RARE -3895 RARE -3556	GCAAAGAGGAAATGCTGGAAAG AAAGTTCTGGCAACTCACCTC	TCAAACAGGGATTGCTCCATTA TAGATATCCTCGGCCACATCTC
RARE -3895	GCTGCGAGCTCCTTCTG	GGGTAAGTGGGATTATGCAAGA
RARE -3556 RARE -3537	CGCCATCCTGGTTGGAAATAA TCATGGGATCCAATCCAAAGG ATGCTTTATATCCAGTTTCTTGGG	CCCATCCTCCTGTTCCCTAA TGCTCCCTGACCTTCCATA CAGCGTTGGGTGCTAAATG
RARE -3142 RARE -2949	CATTTGGCCTCCTGCTCTAA	CCTTCCAGCTTACGAGGTTTAT
RARE -3142	TTGCCGTGGGAGTTTATCTTAT CAGCTGCTAGAGCCAGAAA GCAAGAGGAGACACAGGAATG	GCAGCTATACCGTCCTTCTTT GTGATACCTTCACCACACCTAC CCTTCTTTCTCTAGGGAACTCAATAC
RARE -2949	GGTGTGGTGAAGGTATCACAT GAAGCAGAGGAAAGCAGAGG CCTAGAGAAAGAAGGACGGTATAG	CGAGATAGCCACAGTTCTCTTC CGGGTATTATTCACCACCTTCC CTTGGTAGCAAACAACTTGGG
RARE -2490 RARE -2479	GCTGCCTTTGGCTTAGGA TGGTCCTCAGCCAAGACA	AATCCCGGAACCAAATGTTTATG GCCTAGGAAGTCAGAACAAAGG
RARE -2490	GCTCTGTGACTGAAAGGGAAA	GGGAAGTTGTAGCTGCTTACTG
RARE -2479	AGTAAGCAGCTACAACTTCCC	GTGGCTGAGGAAGTCAATACA
RARE -2230 RARE -2116	CCTCCCTCTCGTCCCTTT CCAAATGGATGCATGTGACC	CCAGAGGACCTGGGTTTAATTC GTACTCATGGTAGCTCACAACT
RARE -2007	CCAAATGGATGCATGTGACC	AAAGGGTAAAGGGTGGATATGAG
RARE -1915 RARE -1832	TCTTTGATAGAAGGCAAGTGAGT	AAAGGGTAAAGGGTGGATATGAG
RARE -1740 RARE -1737	GCAACCTTGCCCGAAATAAA TCATATCCACCCTTTACCCTTTG	GTGAAGATGTGTGGACAGAAGA CACAGTGACACACTCTAGCTTC
RARE -1563	GTTCCTCGGAACAAGTCCATT TCTTCTGTCCACACATCTTCAC GTCACTGTGTCTCTCCAATGTAT CAGTACCAATACTGGGTCCTTT	GGCCCAGCTCACAATCTATATC TGGGATTCAAGGGACAGTTATG GCTGACCCAAAGCTGTAGAA CATCTGAGAATGGCTGGGATT
RARE -1357 RARE -1310 RARE -1255 RARE -1244	CTTTCCCAATTGCCAGAAGTG	GATTGCAACATTCTGGAGAGC
RARE -1357 RARE -1310	GTTCACTGAGCTGGTCTCATATTC	GCAGGAGTGTGGGAGAGT
RARE -1357	CCCAGCCATTCTCAGATGATATAG	GTGGTGCTGGCACAGAG
RARE -1310 RARE -1255 RARE -1244	GCTCATGACCCTGGGAATAG	GGTCCCATCTCTCACCAATAG

Table S4 (cont.)

Target Sequence in mSAA3 promoter	Forward Primers (5' to 3')	Reverse Primers (5' to 3')
RARE -1160 RARE -1134 RARE -1058	GAATGTTGCAATCAGTGAGGAG	CAGCCTGAGATGATGGTGAA
RARE -1160	CTGCTGCTATTGGTGAGAGATG	TGTTATCACTAGAGGACATGGAGATA
RARE -1058	GGGATATCTCCATGTCCTCTAGT	GTGGAGCAGCTTGAGCATTA
RARE -689 RARE -603	CTTCAAGCTAGGATGAACAGAGG	GGGAAAGAGAGAAGAAAGCATCA
RARE -689	CTGTGCTGCCTGGATATGAT GGTCTGCAGGTGTCTATCTTC	CACCTTGATGTTGGCATTGTT CCCTGGCAATGGGCATA
RARE -603	GGAACAATGCCAACATCAAGG	CACACACTGGATTGGATGGA
RARE -463	GTGTGAGCCAACTGCTCTTA ACCCAGCTTGATGCTTTCT CCATCCAATCCAGTGTGTGT	ATCTTAGCATGGACGGTGTG GGTGAGAAGCTATTGGCATTTG GCAGAGGAAGGGTTGGTTTT
RARE -327	CACACCGTCCATGCTAAGAT CAAATGCCAATAGCTTCTCACC AACCAACCCTTCCTCTGCTA	GGTAGAGGTGATGGTTGACTTC CAAGAGGGTGGACTCACAAG GGACTGTGACAGCATTGCATA
RARE -224	GAAGTCAACCATCACCTCTACC CTTGTGAGTCCACCCTCTTG	AAATATCCTCGGACACACCATC CAAGGTTCGAGGGTCCTTT
RARE -116	GATGGTGTGTCCGAGGATATTT AAAGGACCCTCGAACCTTG	GACAGTGAGACAGATGACACAG AAGAATCTGTGCGACAGTGA
RARE-11 RARE 15	CTGTGTCATCTGTCTCACTGTC ACTGTCGCACAGATTCTTCTC	AACTAGCATGCTGTCCTCAAA AGGCTCAGTACCATCCAAAC
RARE 15	CTGTGGGTTGGGATCTTGT	CTTTAGAGTGCTCCTCCAGTG
RARB RARE Positive Control	CGGGTAGGGTTCACCGAAAGTTCACTCGCA	TGCGAGTGAACTTTCGGTGAACCCTACCCG
H3K27Ac Binding Positive Control	TCTCCCTAAACTCTTCCACTCG	ATGTGCCTAAAACGCATCACTACTA
H3K27Ac Binding Negative Control	ACAACTGAGGGGAGGAGAGAAG	GCTGCATTTGTTTTCATTCAGT

Table S5: qPCR primers for gene expression assays

Target gene	Forward Primer (5' to 3')	Reverse Primer (5' to 3')
mouse *Gapdh*	TGGCAAAGTGGAGATTGTTGCC	AAGATGGTGATGGGCTTCCCG
mouse *Saa1*	ATCACCAGATCTGCCCAGGA	CCTTGGAAAGCCTCGTGAAC
mouse *Saa2*	ACCAGATCTGCCCAGGAGAC	GCATGGAAGTATTTGTCTCCATCT
mouse *Saa3*	GACATGTGGCGAGCCTACTC	TTGGCAAACTGGTCAGCTCT
human *GAPDH*	CCTGGTCACCAGGGCTGCTTTTAAC	GTCGTTGAGGGCAATGCCAGCC
human *SAA1*	GGCATACAGCCATACCATTC	CCTTTTGGCAGCATCATAGT
human *SAA2*	GCTTCCTCTTCACTCTGCTCT	TGCCATATCTCAGCTTCTCTG

Table S6. siRNA probe sequences for targeted gene silencing

Target gene	Probe Sequences
human *RARA*	GCAAAUACACUACGAACAA CCAAGGAGUCUGUGAGAAA GAGCAGCAGUUCUGAAGAG GAACAACGUGUCUCUCUGG
human *RARB*	CAGCUGAGUUGGACGAUCU CGAGAUAAGAACUGUGUUA GGCCUUACCCUAAAUCGAA UCACAGAUCUCCGUAGCAU
human *RARG*	GAAAGGGCCAUUACUCUGA CAAGGAAGCUGUGCGAAAU GGAGAACCCUGAAAUGUUU UAGAAGAGCUCAUCACCAA
mouse *Rara*	GCAAGUACACUACGAACAA AAGACAAAUCAUCCGGCUA CGGUGCGAAACGAUCGAAA CGAAUCUGCACGCGGUACA
mouse *Rarb*	GAUAAGAACUGCGUCAUUA GAAAGGUGCCGAACGUGUA GAUCUACACUUGCCAUCGA AAGAGUCUGUUAGGAAUGA
mouse *Rarg*	GUAAGGAACGAUCGAAACA GCGGAUCUGUACAAGGUAU GCAGGACACUAUGACAUUC GGAGCAGGCUUCCCAUUCG