An integrin αEβ7-dependent mechanism of IgA transcytosis requires plasma cell contact with intestinal epithelium

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Supplementary Information:

Supplementary Figures 1-6

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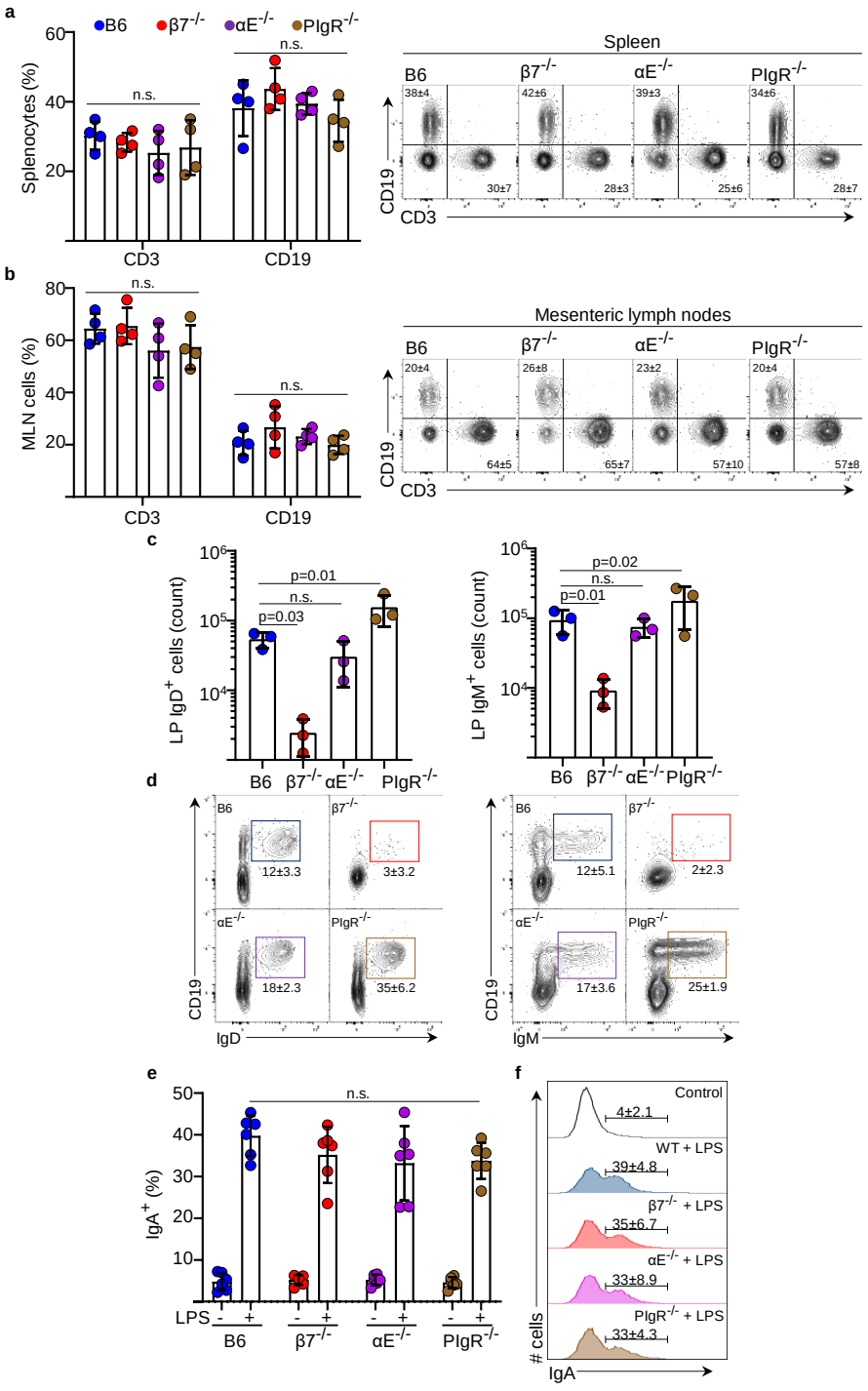

Figure S1. **T and B cell recruitment to spleen and MLN are normal in all mouse strains whereas intestinal IgD+ and IgM+ ASC recruitment is compromised only in β7$^{-/-}$ mice. (a, b)** Percentages and representative plots of CD3^{+} and CD19^{+} cells in the spleen and MLN (n= 4/strain, statistical significance determined using ANOVA, followed by Tukey's multiple comparison test). **(c, d)** Absolute counts of IgD^{+} and IgM^{+} within the ileal LP and representative contour plots (n=3/strain, statistical significance determined using ANOVA, followed by Tukey's multiple comparison test). **(e)** Percentage of IgA^{+} cells after *in vitro* stimulation of splenocytes with or without LPS for 72 h **(f)** representative histogram plots (mean ± SD, n=3-6 mice per group from three independent experiments).

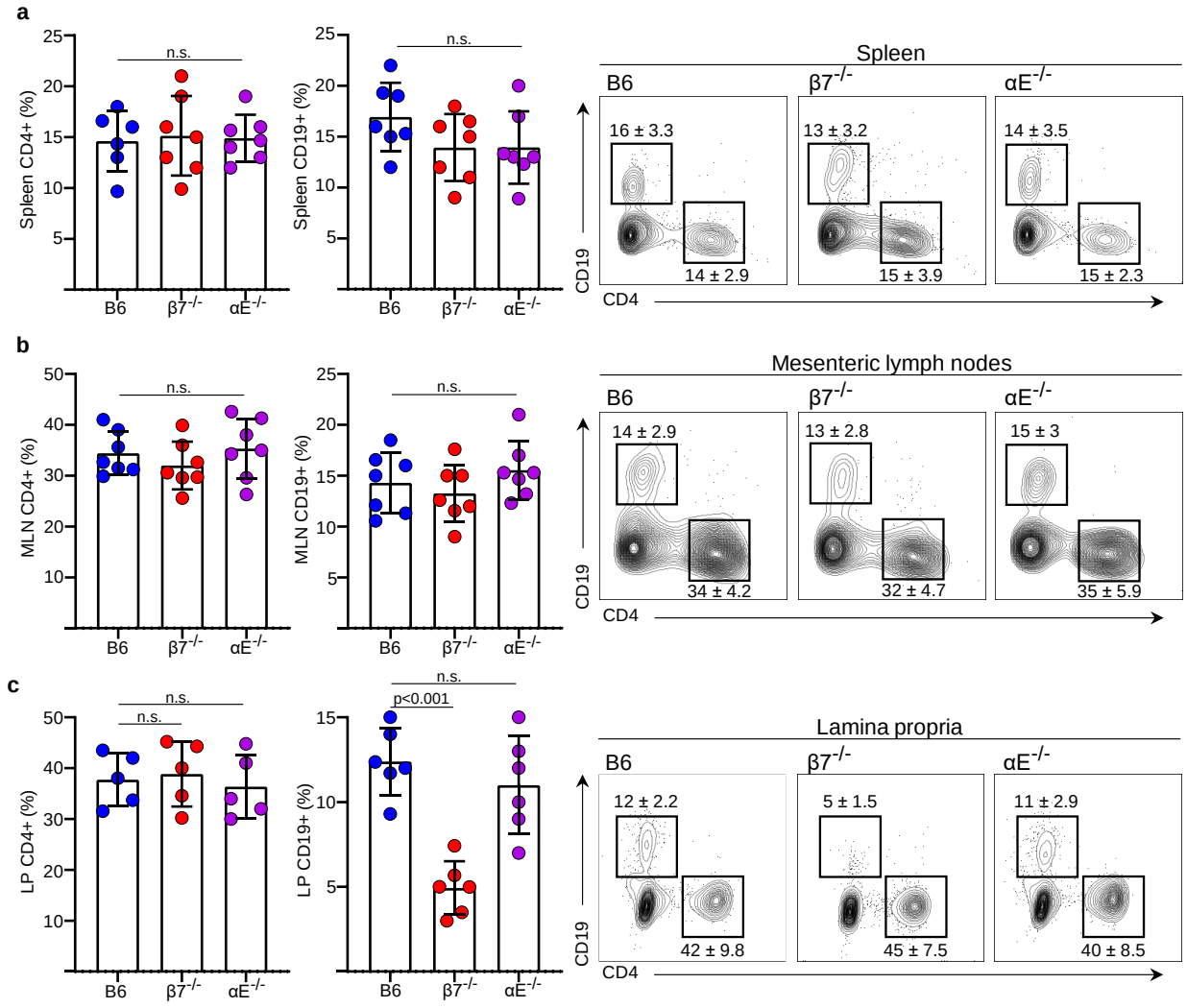

Figure S2. T and B cell reconstitution of spleen, MLN and LP is normal in RAG1$^{-/-}$ mice receiving CD4^{+} T cells from all strains, while only β7$^{-/-}$ B cells are unable to reconstitute the LP compartment. (a, b, c) Percentages and representative contour plots of CD4^{+} and CD19^{+} cells within **(a)** spleen, **(b)** mesenteric lymph nodes and **(c)** ileal lamina propria of reconstituted RAG$^{-/-}$ mice. (mean ± SD, n=5-7 mice, statistical significance determined using ANOVA, followed by Tukey's multiple comparison test).

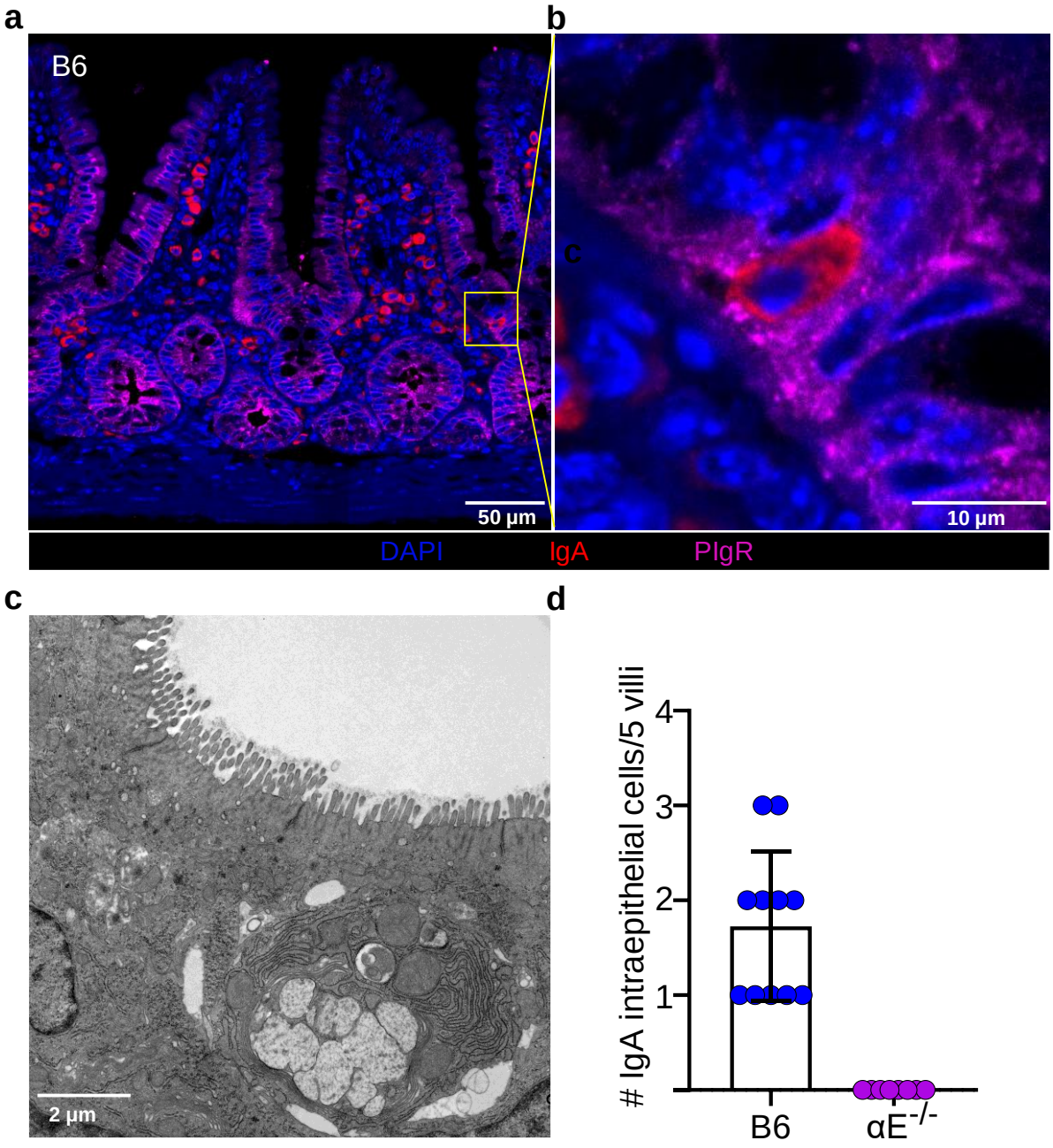

Figure S3. Intraepithelial IgA ASC interdigitate within pIgR-expressing IEC near the crypt base of B6 mice yet are absent in αE-/- mice. (a) IF staining of IgA+ ASC and pIgR (representative images of terminal ileum) **(b)** High magnification IF image and **(c)** TEM image of IgA+ ASC infiltrating epithelium. **(d)** Number of intraepithelial IgA+ ASC per 5 terminal ileal villi.

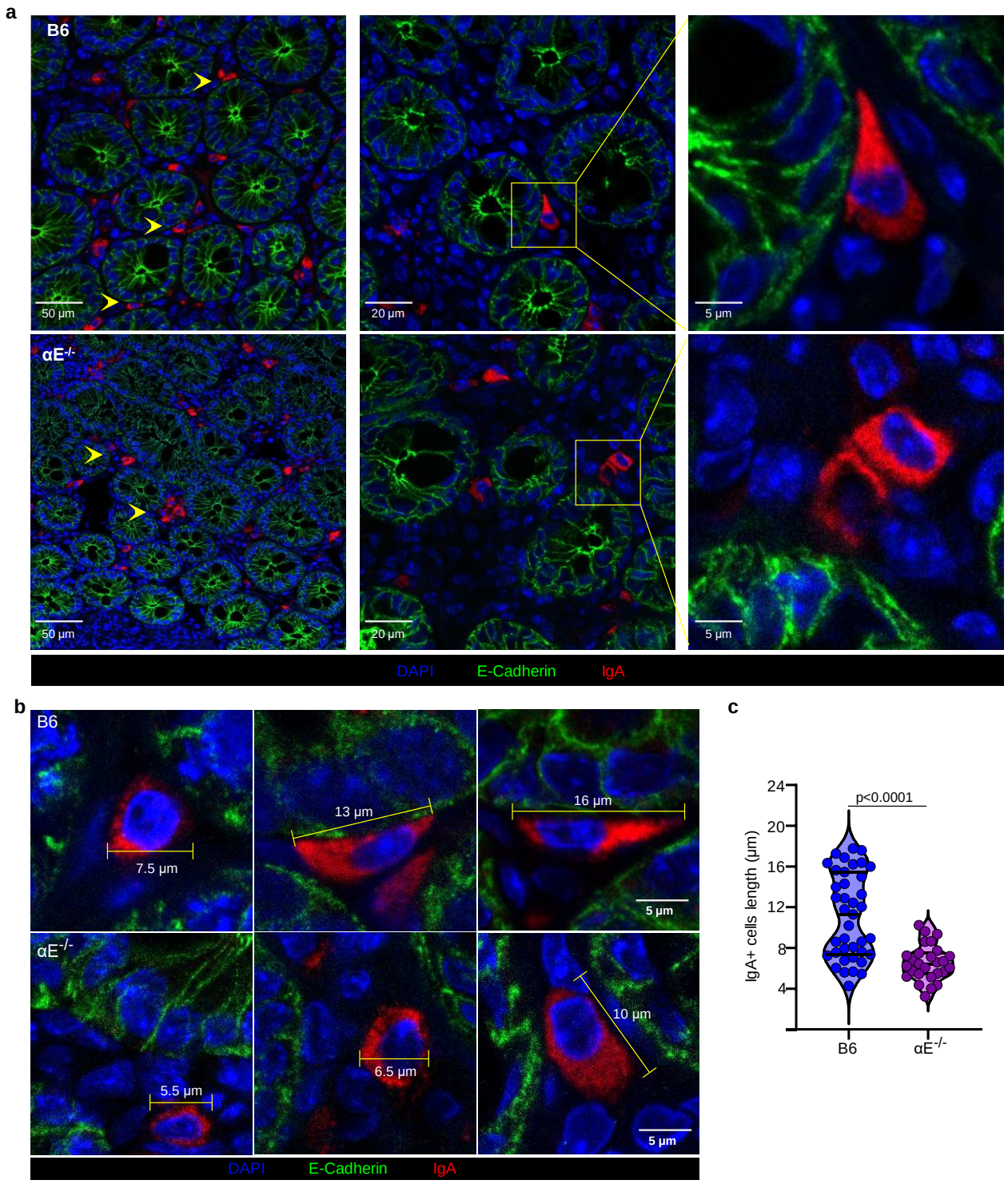

Figure S4. IgA+ ASC acquire a sickled morphology in the pericryptal region of B6 mice but not in CD103-/- mice. (a) Representative IF images of IgA+ ASC and E-cadherin show sickled/flattened morphology and IEC adherence in B6 mice. (**b**) Representative images of the morphology and length assessment of IgA+ ASC in indicated strains. **(c)** IgA+ ASC > 12 µm are absent in ileal LP of αE-/- mice. Violin plot of median and quartiles, n> 30 cells from 3 independent experiments, p calculated by Student's t-test.

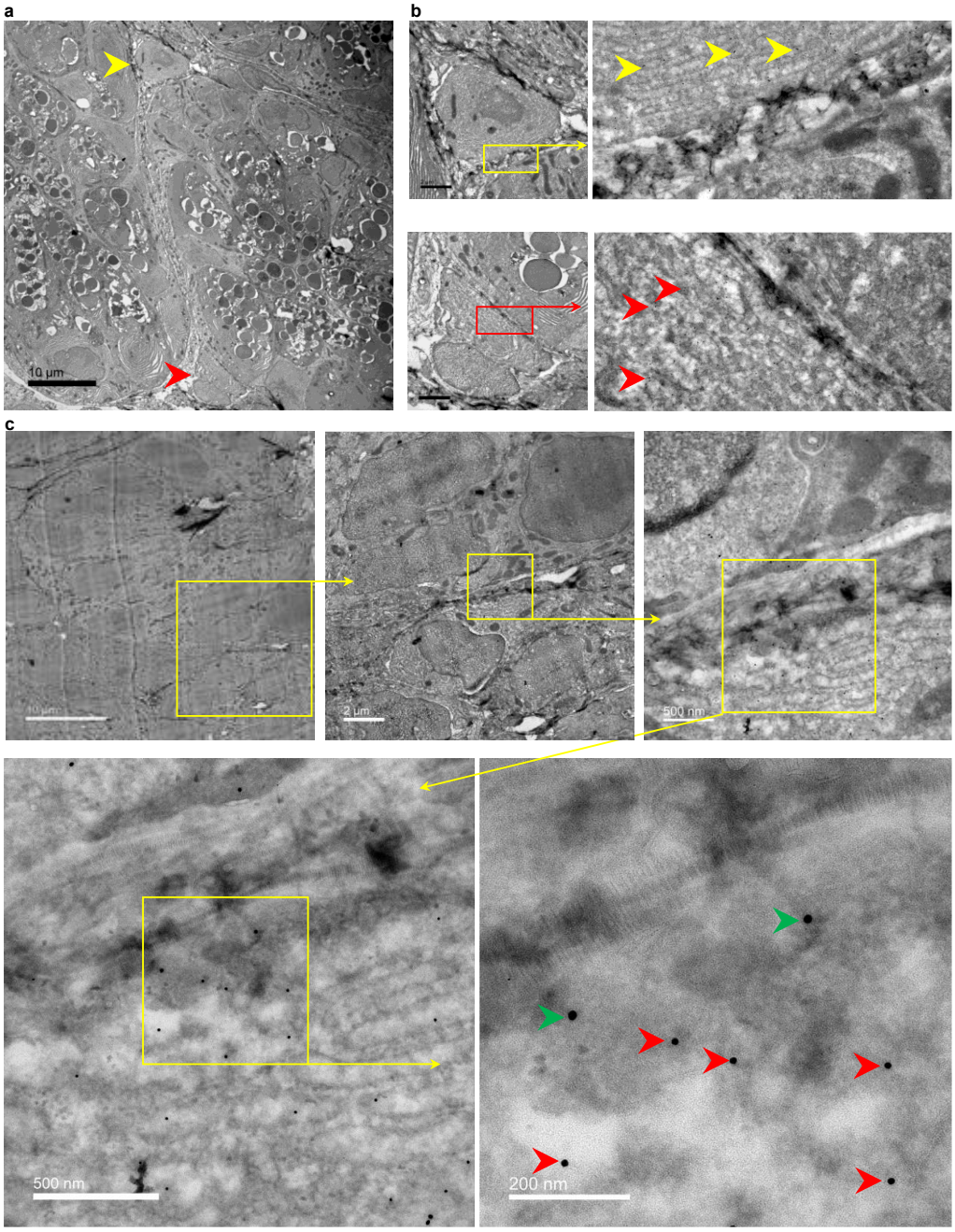

Figure S5. A subset of adherent IgA+ ASC express integrin αE and establish direct contact with intestinal epithelial cells. (a) TEM images of cross sections through the crypt base show 2 adherent cells (arrowheads) in contact with epithelial cells (likely Paneth cells) **(b)** Higher magnification shows cell to cell contact and immunogold particles within RER of adherent cells. **(c)** TEM images demonstrate co-expression of IgA (red arrows, 12nm immunogold particles) and integrin αE (green arrows, 18nm immunogold particles) in adherent cells. Increasing magnification of the yellow-box area. (Representative images of cross sections through the ileal pericryptal base from three B6 mice).

Table S1: Antibodies used for flow cytometry.

Target	Clone	Conjugate	Vendor	Product number
CD3	17A2	APC-eFluor 780	eBioscience	47-0032-82
CD19	eBio1D3	PerCO-Cy5.5	eBioscience	45-0193-82
IgA	mA-6E1	PE	eBioscience	12-4204-82
IgD	11.26c.2a	BV650	Biolegend	405721
IgM	RMM-1	BV605	Biolegend	406523
IgG	Poly	BV510	Biolegend	405331
CD49d	R1-2	BV480	BD Bioscience	746526
Integrin β7	FIB504	BV421	BD Bioscience	564283
CD103	2E7	AlexaFluor 647	Biolegend	121410
Live dead		Zombie NIR dye	Biolegend	423106

Table S2: Antibodies used for immunostaining and confocal laser scanning microscopy.

Target	Host	Clone	Vendor	Reference	Concentration
Mouse IgA	Rat	C10-3	BD Pharmingen (La Jolla, CA)	556969	2.5 ug/ml
Mouse E-Cadherin	Rabbit	24E10	Cell Signaling Technology (Danvers, MA)	3195s	2 ug/ml
Mouse pIgR	Goat	Polyclonal	R&D System (Minneapolis, MN)	AF2800	2 ug/ml
Mouse CD31	Armenian hamster	2H8	Millipore Sigma	MAB1998 Z	2 ug/ml
Alexa Fluor 488 anti-rabbit (H+L)	Goat	Polyclonal	Jackson ImmunoResearch	111-545-144	2 ug/ml
Alexa Fluor 488 anti-Armenian hamster IgG (H+L)	Goat	Polyclonal	Jackson ImmunoResearch	127-545-160	2 ug/ml
Alexa Fluor 594 anti-rat IgG (H+L)	Goat	Polyclonal	Invitrogen	A-11007	2 ug/ml
Alexa Fluor 647 anti-goat	Donkey	Polyclonal	Invitrogen	A-21447	2 ug/ml

Table S3: Antibodies used for immunoelectron microscopy.

Target	Host	Clone	Vendor	Reference	Concentration
Mouse IgA	Rat	C10-3	BD Pharmingen (La Jolla, CA)	556969	2.5 ug/ml
Mouse CD103	Rabbit	Polyclonal	Invitrogen	PA5-99400	2 ug/ml
12 nm Colloidal Gold AffiniPure Goat Anti-Rat IgG	Goat	Polyclonal	Jackson ImmunoResearch	112-205-143	1:20
18 nm Colloidal Gold AffiniPure Goat Anti-Rabbit IgG	Goat	Polyclonal	Jackson ImmunoResearch	111-215-144	1:20