

Supplemental Figure 2



Page 41 of 47

Supplemental Figure 3



Supplemental Figure 4





Supplemental Figure 1. Immunostaining for additional cell markers in Transwells and *in vivo*. (a-c) Wild type were seeded in Transwells, treated with 10 μ M DAPT and 1 μ g/ml LPS, and analyzed on day three post-seeding. Cells were fixed on the transwell membranes and 5 μ m histologic were cut and stained for basolateral marker CD138 (c). Cells were fixed on the transwell membranes and stained for Chromogranin A (a) and UEA-1 lectin (b) (arrowheads). Representative whole mount images are shown. (d-i) Colons from wild type or *pIgR-/-* mice were harvested, bouins-fixed, and paraffin embedded. 5 μ m histologic sections were cut and stained for CD138 (d), chromogranin A (e), UEA-1 (f), villin1 (g), ZO-1 (h), and pIgR (i,j). Bars = 200 μ m. Yellow boxes denote area depicted in insets.

Supplemental Figure 2. Dose curve comparison of different sources of commercial normal mouse IgA used for experiments. IgA from Santa Cruz Biotechnology (Sc-3900) was used for early experiments at the dose of 40 μ l (or 1:15) unless otherwise noted. Due to variation from lot to lot, later experiments utilized normal mouse IgA from BD Pharmingen (BD 553476) at the equivalent dose of 6 μ l per sample (or 1:100).

Supplemental Figure 3. Transepithelial electrical resistance measurements at different cell densities. Two-fold serial dilutions of wild type cells were seeded into Transwells and treated with 10 μ M DAPT and 1 μ g/ml LPS. Transepithelial electrical resistance was measured on day three. The (resistance x area) is shown for each condition (mean \pm s.e.m., $n \ge 4$ per group).

Supplemental Figure 4. IgA transcytosis and pIgR expression in response to IFN γ . (a-b) Wild type and pIgR^{-/-} cells were treated with 10 µM DAPT and varying doses of IFN γ . IgA transcytosis was analyzed by ELISA (a), and results were normalized to the WT+DAPT+LPS group (= 100%). The no cell group indicates the amount of IgA that freely diffuses into the apical supernatant in the absence of cells in the Transwell. Gene expression analysis of pIgR was performed (b) and all samples were normalized to Gapdh. Data are presented as fold change relative to untreated (0% CM) cells. (c) Transepithelial electrical resistance measurements were taken on day three after treatment with 10 µM DAPT and the highest previously used dose of various cytokines (100 ng/ml IL-1 β , 100 ng/ml TNF α , 20 ng/ml IL-17, 1 ng/ml IFN γ). All values are indicated as mean ± s.e.m. One-way ANOVA: (b) F = 35.17, P < 0.0001, $n \ge 5$ per group; (c) F = 19.23, P < 0.0001, $n \ge 4$ per group. Means with different letters are significantly different by Bonferroni's multiple comparison test. N.D. = not detected.