Supporting Information

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SI Materials and Methods

Mice. Breeding pairs of Tg(Fos-lacZ)34Efu/J (also known as TOPGAL) mice were provided by Dr. Calvin Kuo (Stanford University, Palo Alto, CA) and the colony was expanded in CD-1 background (1). Genotyping for these mice were performed upon weanling according to published protocol (1). BALB/c, BDF-1, and BALB/c mice were purchased from the Charles River Laboratories. All experimental mice were housed under specific pathogen-free condition in vivarium and received chow and water ad libitum. Animal studies were carried out under the auspices of the protocols approved by the Nuvelo Institutional Animal Care and Use Committee (IACUC), and were in compliance with the government guidelines for animal welfare. All of the mice were acclimated before use.

Reagents. Both 5-FU and BrdU were from Sigma. CPT-11 (Irinotecan HCl) was purchased from HPCI Inc.. Monoclonal anti-BrdU antibody (ZymedBD BiosciencesFoothill Pharmacy.

RSpo1 Administration in Mice. Recombinant human full-length RSpo1 was produced in CHO cells, purified to homogeneity by conventional chromatography, and tested for endotoxin-free as previously described (2). RSpo1 protein was freshly formulated to predetermined concentration in saline buffer right before use. RSpo1 was administered daily via either s.c. or i.v. route to mice, with a 24-h interval maintained between daily injections.

Dkk1 Adenovector. Recombinant Dkk1 adenovirus carrying human Dkk1 cDNA with C-terminal HA epitope tag was constructed, produced, and purified by CsCl gradient as described previously (3).

Young adult male C57BL/6 mice received single i.v. injection of 2×10^9 pfu of Dkk1 adenovirus via tail vein. Mouse serum samples were obtained by retro-orbital phlebotomy followed by Western analysis using anti-HA monoclonal antibody (Roche Diagnostics) to confirm Dkk1 transgene expression at appropriate times.

Analysis of LacZ Expression in TOPGAL Mice. The whole tongues were carefully removed from female TOPGAL mice at age of 9–11-week, lightly fixed in 4% paraformaldehyde, stained with Bluo-Gal as histochemical substrate (Invitrogen), and photographed under digital stereoscope (4). Unlike X-Gal, Bluo-Gal yielded a blue precipitate which was insoluble in xylenes and alcohols. Mouse tongues with whole-mount Bluo-Gal staining were paraffin-embedded, sectioned, and counterstained with nuclear fast red (Vector Laboratories).

Immunohistochemical Studies. *In vivo* BrdU labeling in mice and BrdU immunohistochemistry in mouse tongue sections were performed in a similar manner as we have previously described (2). Briefly, each mouse was i.p. injected with 4 mg BrdU 2 h before necropsy. Basal layer epithelial cells in mouse tongue sections were examined by light microscopy under high magnification and were classified as BrdU-positive (brown nuclei from diaminobenzidine staining) or BrdU-negative (blue nuclei from hematoxylin staining) cells. The BrdU proliferative index was therefore calculated as the percentage of BrdU-positive cells in tongue basal layer epithelium in mice. The BrdU proliferative index was counted from 5–10 fields along the entire tongue mucosal perimeter.

Mouse tongue sections (5 μm) were deparaffinized and rehydrated. Mouse monoclonal anti-β-catenin antibody was applied onto the sections according to manufacturer's instruction and incubated at room temperature for 90 min (5). HRP polymer conjugate (Invitrogen) and substrate diaminobenzidine (DAB) chromogen were used to generate brown-colored signal. Slides were counterstained with hematoxylin. Parallel slides using normal mouse IgG or without adding primary antibody served as negative controls.

Statistics. All data are given as mean $\pm$ SD. Experiments were repeated multiple times with similar results obtained within repetitive studies. Group mean values were compared by ANOVA and $P < 0.05$ was considered to be statistically significant.

1. DasGupta R, Fuchs E (1999) Multiple roles for activated LEF/TCF transcription complexes during hair follicle development and differentiation. *Development* 126:4557–4568.
2. Zhao J *et al.* (2005) R-respondin 1, a novel intestinotrophic mitogen ameliorates experimental colitis in mice. *Gastroenterology* 132:1331–1343.
3. Kuhnert F, *et al.* (2004) Essential requirement for Wnt signaling in proliferation of adult small intestine and colon revealed by adenoviral expression of Dickkopf-1. *Proc Natl Acad Sci USA* 101:266–271.
4. Liu F, *et al.* (2007) Wnt-beta-catenin signaling initiates taste papilla development. *Nat Genet* 39:106–112.
5. Sheng H, *et al.* (1998) Nuclear translocation of beta-catenin in hereditary and carcinogen-induced intestinal adenomas. *Carcinogenesis* 19:543–549.

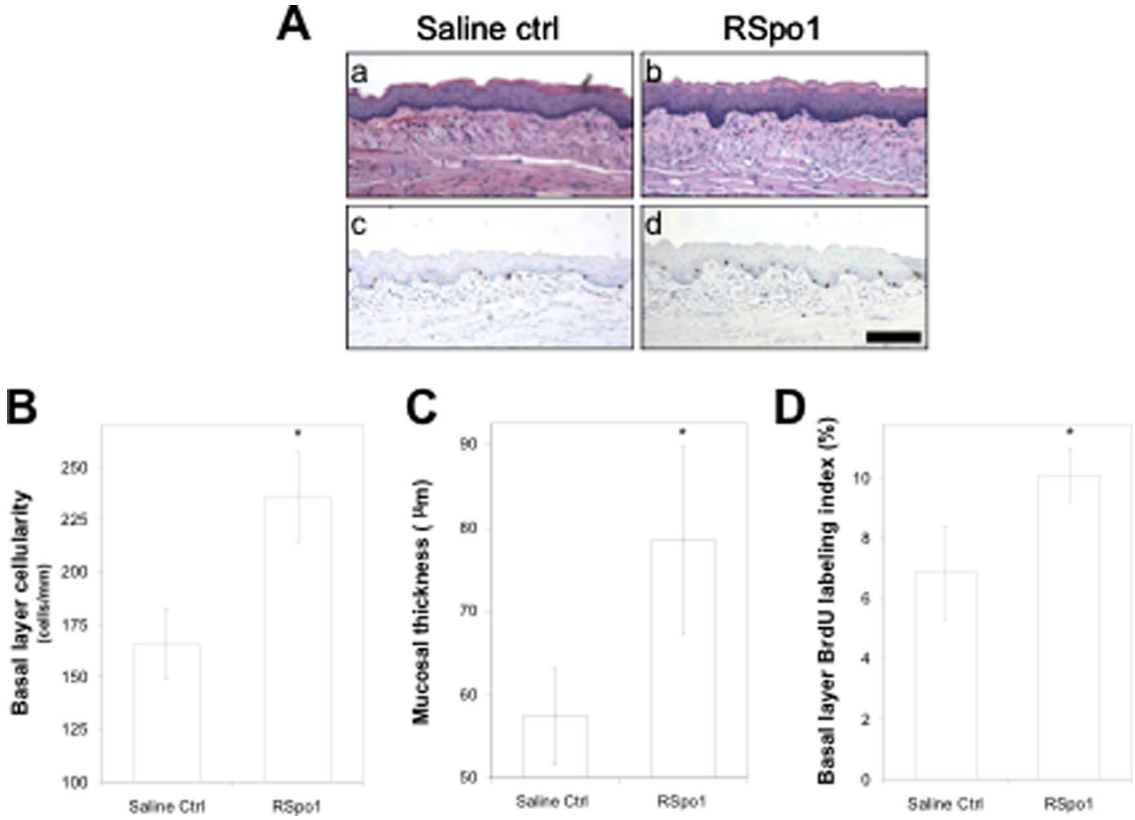

Fig. S1. RSpo1 administration induces mucosal hyperplasia in normal mouse tongue. (*A*) H&E staining (*a* and *b*) and BrdU immunohistochemistry (*c* and *d*) of mouse ventral tongue mucosa. (*a* and *c*) Saline control, (*b* and *d*) RSpo1. (Scale bar, 100 μm.) (*B*) Basal layer epithelial cellularity in mouse ventral tongue mucosa. (*C*) Mucosal thickness in mouse ventral tongue. (*D*) Basal epithelial layer BrdU labeling index in mouse ventral tongue. $*P < 0.05$, RSpo1 vs. saline control.

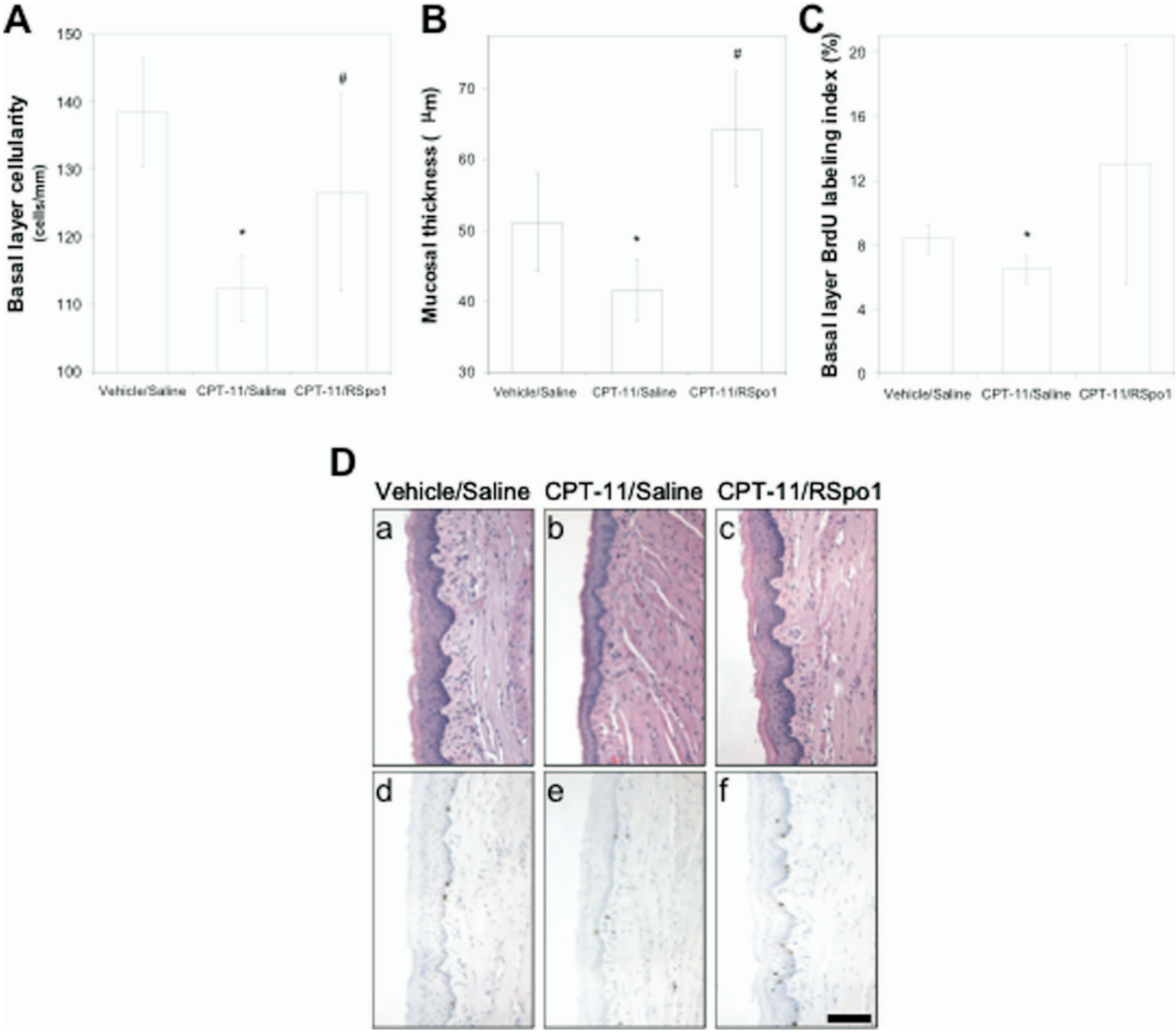

Fig. S2. RSpo1 reduces CPT-11-induced oral mucositis in mice. Use of RSpo1 overrode CPT-11-induced reduction of basal layer cellularity (*A*), mucosal thickness (*B*), and basal layer BrdU labeling index (*C*) in mouse tongue. $*P < 0.05$, CPT-11/saline vs. vehicle/saline; $^{\#}P < 0.05$, CPT-11/RSpo1 vs. CPT-11/saline. (*D*) Representative H&E (*a-c*) and BrdU (*d-f*) immunohistochemical images of mouse ventral tongue are also shown.

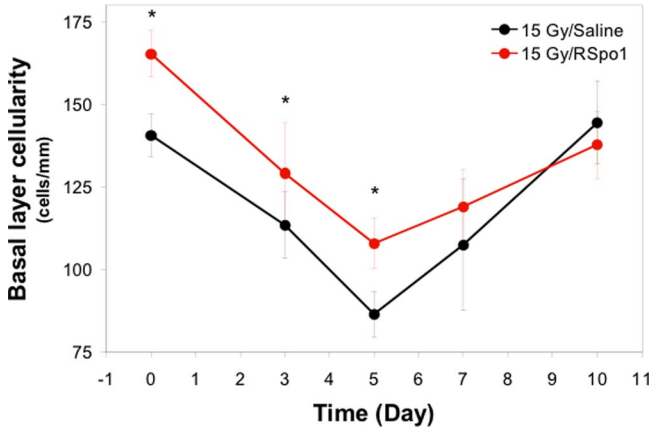

Fig. S3. Time-dependent efficacy of RSpo1 in reducing oral mucositis in irradiated mice. Young adult male BDF-1 mice were pretreated with RSpo1 at dose of 5 mg/kg (IV, qdx3, day -3 to day -1) and were exposed to 15 Gy x-ray irradiation delivered to the head only on day 0. Mice were killed and harvested for tongue tissue on day 0 (before radiation), 3, 5, 7, and 10, respectively ($n = 6$ mice each harvest time point). Basal layer cellularity of mouse ventral tongue is shown. $*P <$ 0.05, 15 Gy/RSpo1 vs. 15 Gy/saline.

A

Oral mucositis activity index

Histometric parameters	Score				
	1	2	3	4	5
Epithelial cellularity	Many more cells	More cells	Normal	Fewer cells	Far fewer cells
Epithelial thickness	Much thicker	Thicker	Normal	Thinner	Much thinner
Dermal integrity	Normal	Increasing damage to score 5			
Presence of inflammation/bleeding	Normal	Increasing inflammation/vascular response to score 5			
Level of apoptosis/mitosis	Normal (Much more mitosis)	More mitosis	Equal levels of apoptosis/mitosis	More apoptosis	Much more apoptosis

Oral mucositis activity index is the sum of the above five parameters.

B

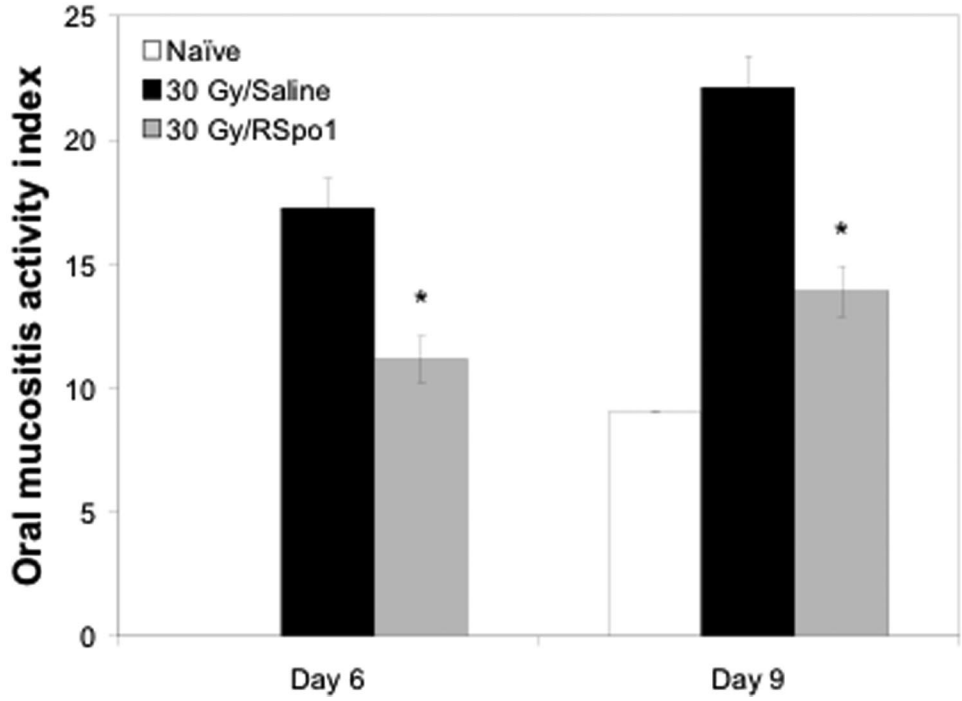

Fig. S4. Time-dependent effect of RSpo1 in reducing oral mucositis in mice treated with high dose, head-only radiation. Young adult male BDF-1 mice were daily IV injected with 20 mg/kg RSpo1 before (starting from day -3) and after (till day of necropsy) 30 Gy high dose head-only radiation delivered to mice on day 0. Mice were necropsied and harvested for tongue tissue on day 6 and 9, respectively ($n = 6$ mice per harvest time point). Oral mucositis activity index (*A*) was used to evaluate the extent of oral mucosal damage in mouse tongue (*B*). *$P < 0.05$, 30 Gy/RSpo1 vs. 30 Gy/saline.