

Figure S1. (A-D) Heparanase over-expression in the colon of heparanase transgenic (*Hpa-tg*) mice, as demonstrated by immunohistochemistry (A) (original magnification, $\times 200$), ELISA (B), Western blotting (C), and enzymatic activity assay (D). (E) Immunostaining of *wt* and *Hpa-tg* colon tissue sections with anti- PCNA (top) and cleaved caspase 3 (bottom) antibodies (original magnification, $\times 200$).

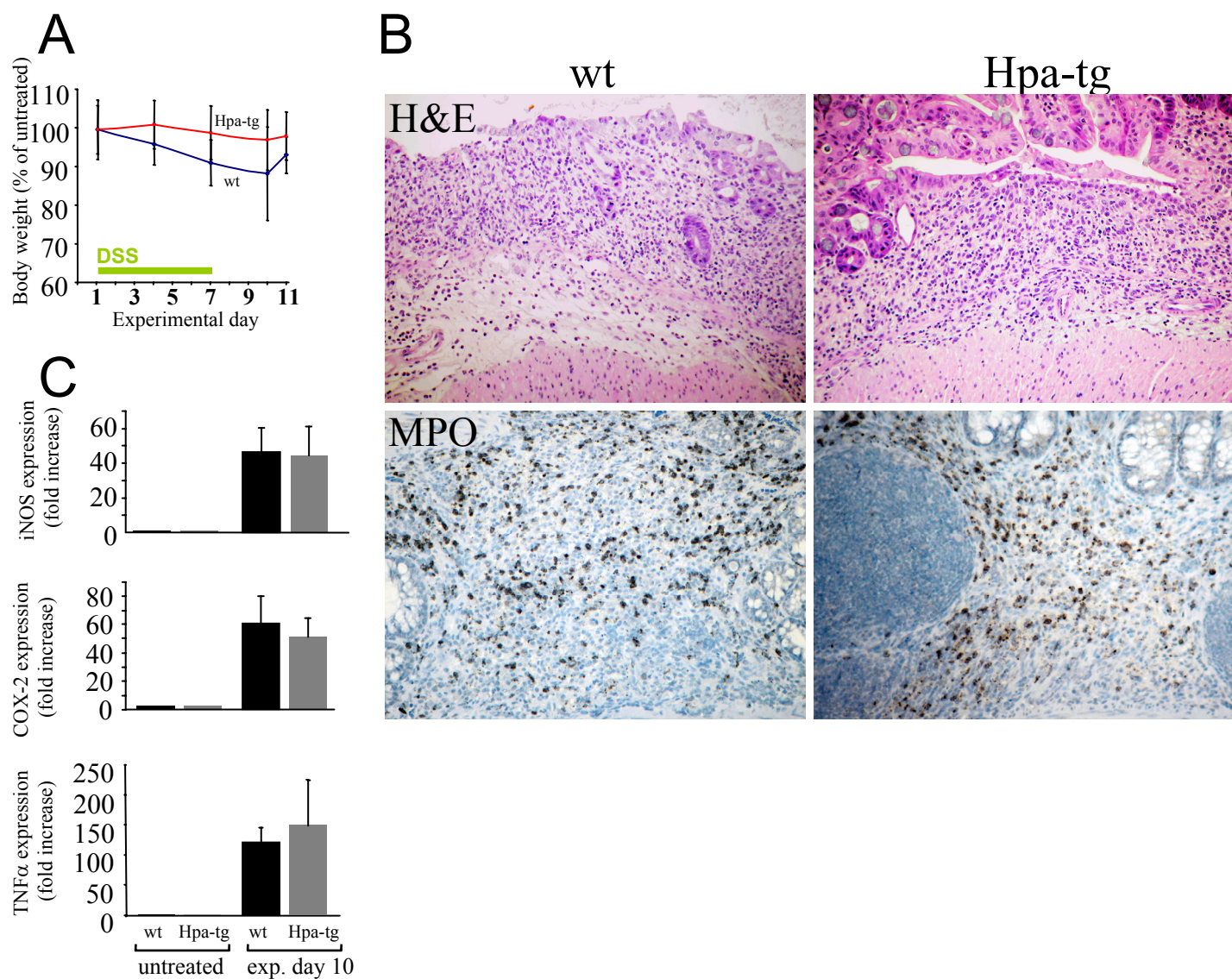


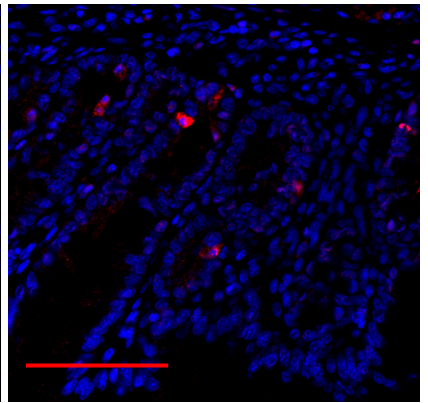
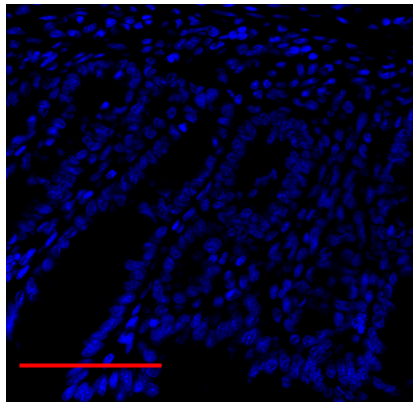
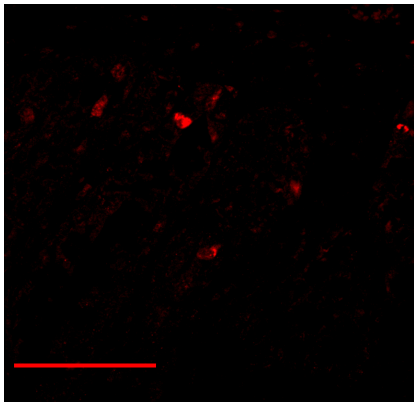
Figure S2. Lack of exacerbated inflammatory phenotype in Hpa-tg mice during the acute phase of DSS colitis. (A) Body weight loss in DSS-treated wt (blue line) and Hpa-tg (red line) mice during experimental days 1- 10. **(B)** Representative colonic tissue sections derived on experimental day 10 from DSS-treated wt (left) and Hpa-tg (right) mice were stained with H&E to assess the degree of mucosal injury (top) and immunostained with anti-myeloperoxidase antibody to evaluate the extent of infiltration by neutrophils (bottom), original magnification, $\times 200$. **(C)** Levels of iNOS, COX-2 and TNF α mRNA expression were measured in colons derived from wt (black bars) and Hpa-tg (grey bars) untreated mice or DSS-treated mice on experimental day 10 and normalized to actin mRNA ($n=5$). Error bars represent \pm SD.

Phospho-p65

DRAQ5

overlay

wt



Hpa-tg

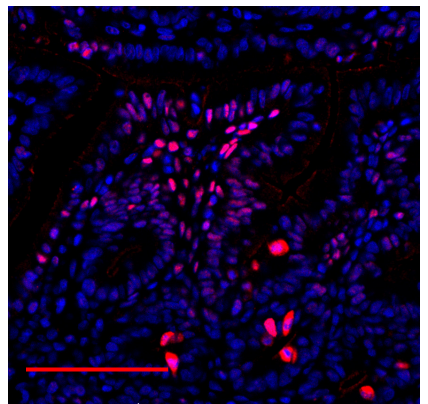
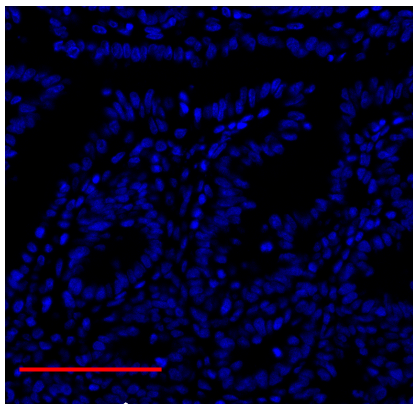
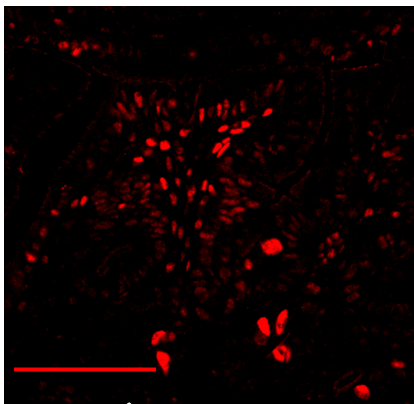


Figure S3. Increased levels of nuclear phospho-p65 NF- κ B subunit in *Hpa-tg* colon. Colonic samples were harvested from *Hpa-tg* and *wt* mice on day 80 of the chronic DSS colitis and processed for immunofluorescent analysis using anti-phospho-p65 NF- κ B antibody (red). Cell nuclei were counterstained with DRAQ5 (blue); Scale bar 100 μ m.

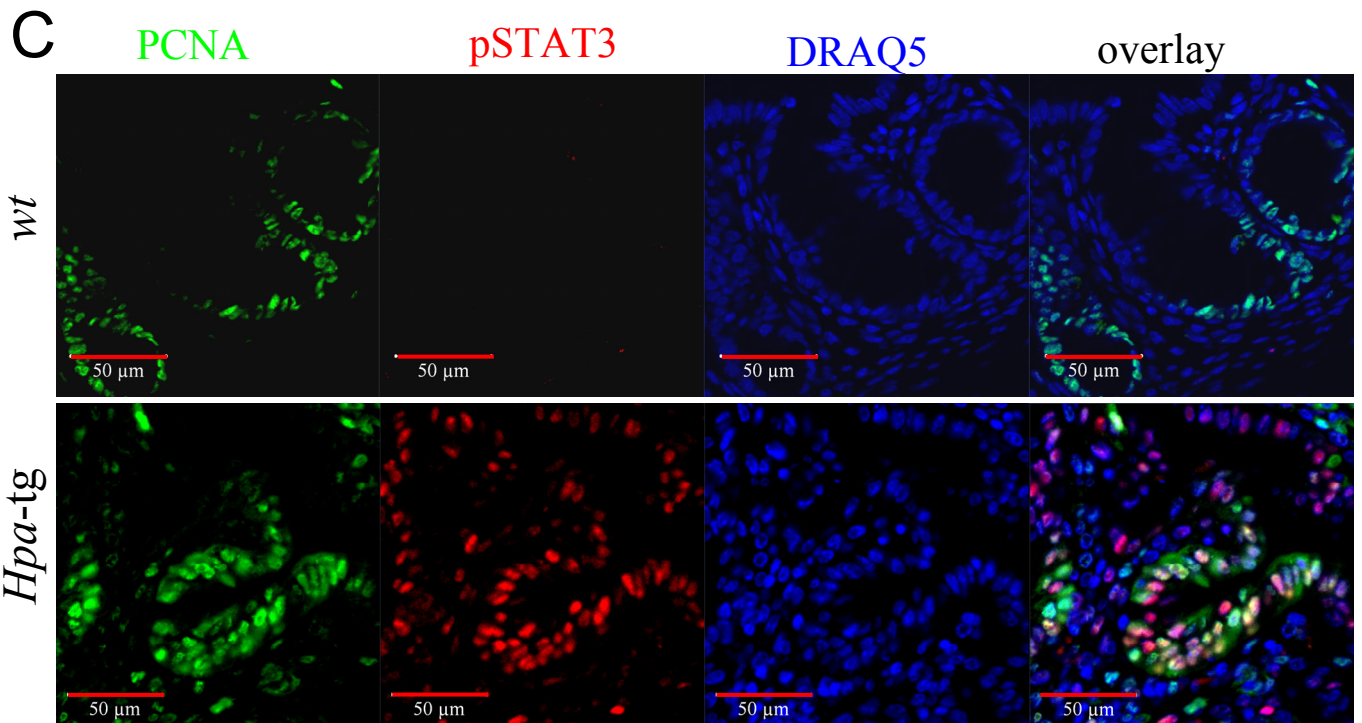
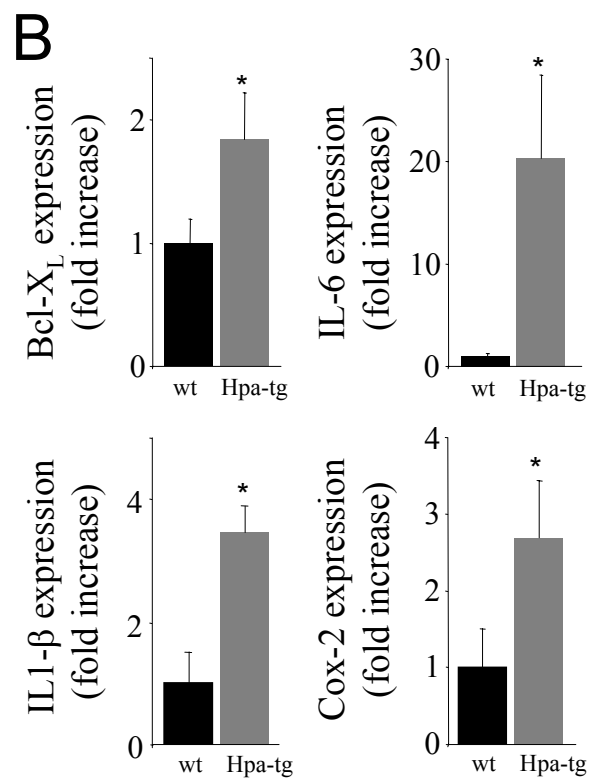
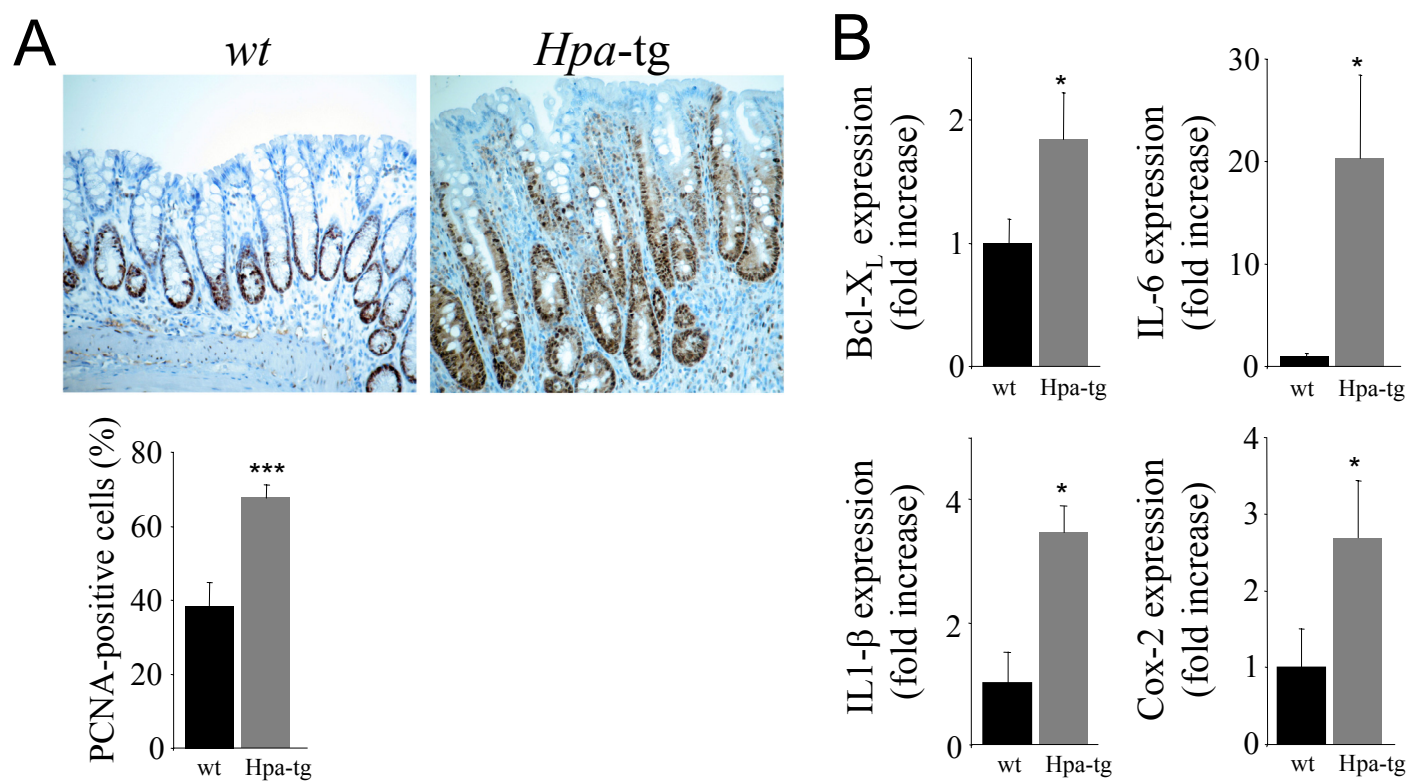


Figure S4. (A) Colons were obtained from DSS-treated *wt* (left) and *Hpa-tg* (right) mice on day 80 and processed for immunohistochemical analysis using antibodies directed against PCNA (top). The percentage of PCNA-positive cells among all crypt cells was calculated in 10 full crypts from four animals of each genotype (bottom), (original magnification, $\times 200$). (B) Increased expression of Bcl-X_L, IL-1, IL-6, and COX-2 mRNA determined by qRT-PCR in *Hpa-tg* (grey bars) vs. *wt* (black bars) colon on day 80 of the experiment (normalized to actin mRNA) (n=5). (C) Co-localization between phosphoSTAT3 positivity and increased cell proliferation in *Hpa-tg* colon. Colonic tissue was obtained from *wt* (top) and *Hpa-tg* (bottom) mice on day 80 and processed for immunofluorescent analysis using the combination of anti-PCNA (green) and anti-phosphoSTAT3 (red) antibodies. Cell nuclei were counterstained with DRAQ5 (blue). Overlay (bright pink) represents proliferating cells positive for nuclear-localized phosphoSTAT3; Scale bar 50 μ m.

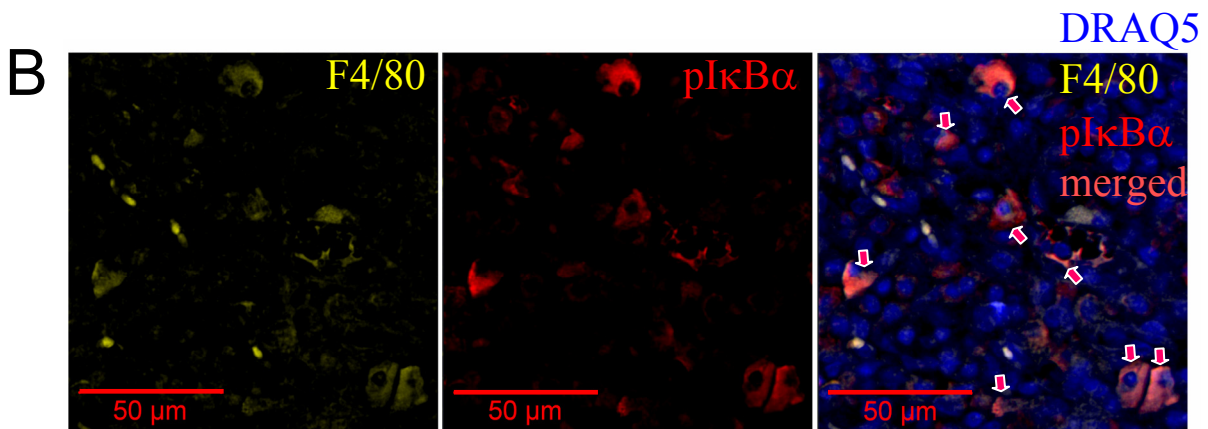
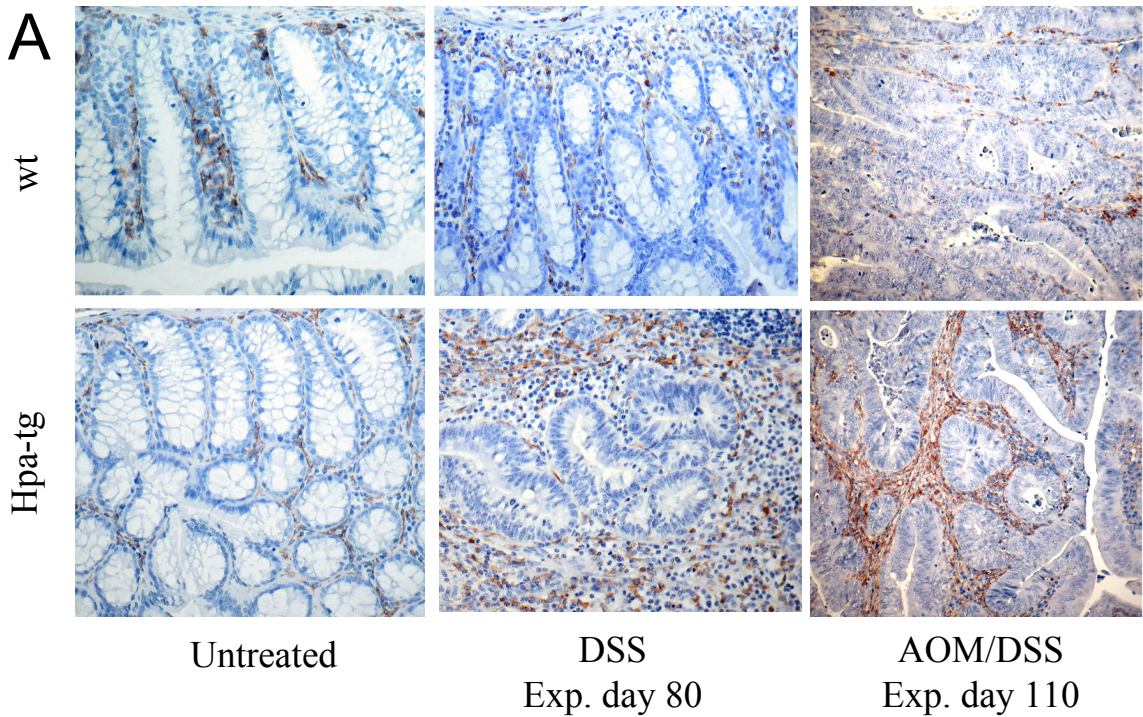


Figure S5. Increased macrophage infiltration in *Hpa-tg* colon during the chronic phase of AOM-DSS colitis. (A) Immunostaining with anti F4/80 reveals increased macrophage infiltration in *Hpa-tg* colon during the chronic phase of AOM-DSS model of colitis (experimental day 80, middle) and the associated colonic tumors (experimental day 110, right), (original magnification, $\times 200$). (B) Increased IκBα phosphorylation in macrophages infiltrating the *Hpa-tg* colon. Colonic samples were harvested from *Hpa-tg* and *wt* mice on day 80 of the chronic DSS colitis and processed for double immunofluorescent analysis using the combination of anti-pIκBα and anti-F4/80 antibodies. Pink arrows indicate pIκBα-positive macrophages; Scale bar 50μm.

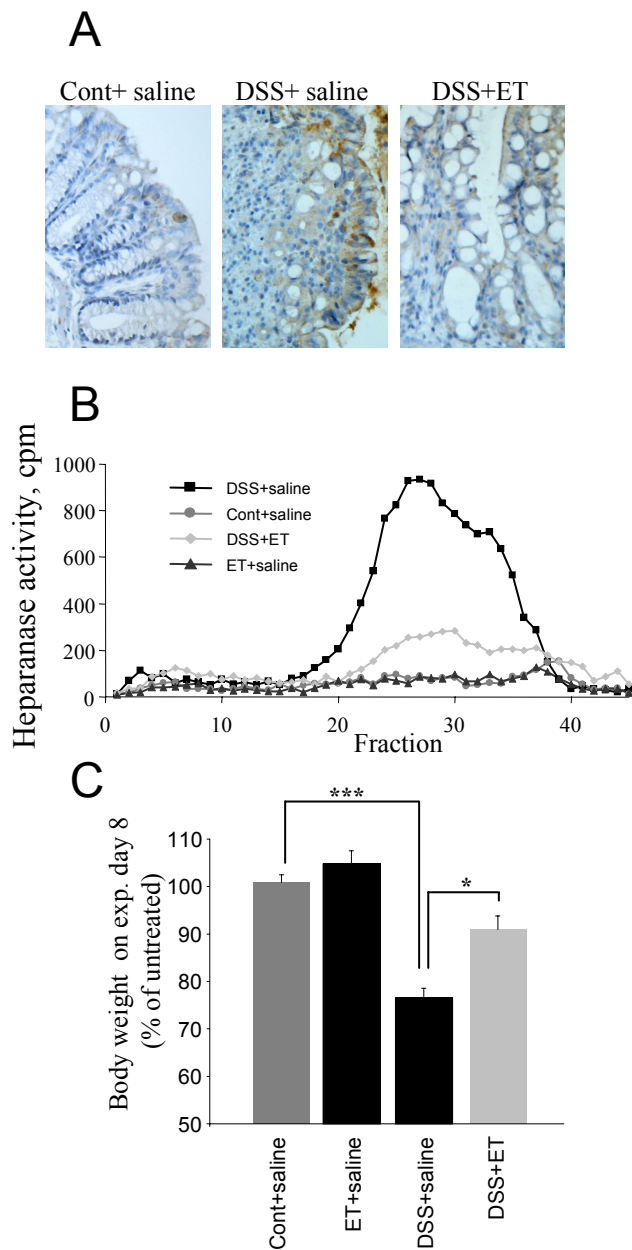


Figure S6. The effect of TNF α antagonist etanercept (ET) on heparanase induction and body weight loss during acute phase of DSS colitis in *wt* mice. Mice receiving drinking water with 5% DSS for 7 days were injected (i.p.) with either vehicle (saline) alone (DSS+saline) or etanercept (DSS+ET, 3 mg/kg body weight) twice a day, for 8 days starting on experimental day 1. Healthy mice injected in a similar way with saline (Cont+saline) and etanercept (Cont+ET) were used as control ($n > 4$ in each group). On experimental day 8 all mice were weighted, sacrificed and their colons assessed for heparanase levels (original magnification, $\times 200$). **(A)** Immunostaining with anti-heparanase antibody (brown) of mouse colonic tissue sections derived from untreated (Cont+saline) mice, DSS+saline treated and DSS+ET treated mice. Note reduction of heparanase staining in the colonic epithelium of DSS+ET treated vs. DSS+saline treated mice. **(B)** Heparanase activity. Punch biopsies were harvested on day 8 from all experimental groups and cultured for 16 h as described in 'Methods'. Heparanase activity was determined in the conditioned medium. Note that DSS-induced increase in heparanase activity was almost completely abolished by ET treatment. **(C)**. Etanercept treatment reduced the body weight loss during DSS colitis. * $p < 0.05$, *** $p < 0.001$.

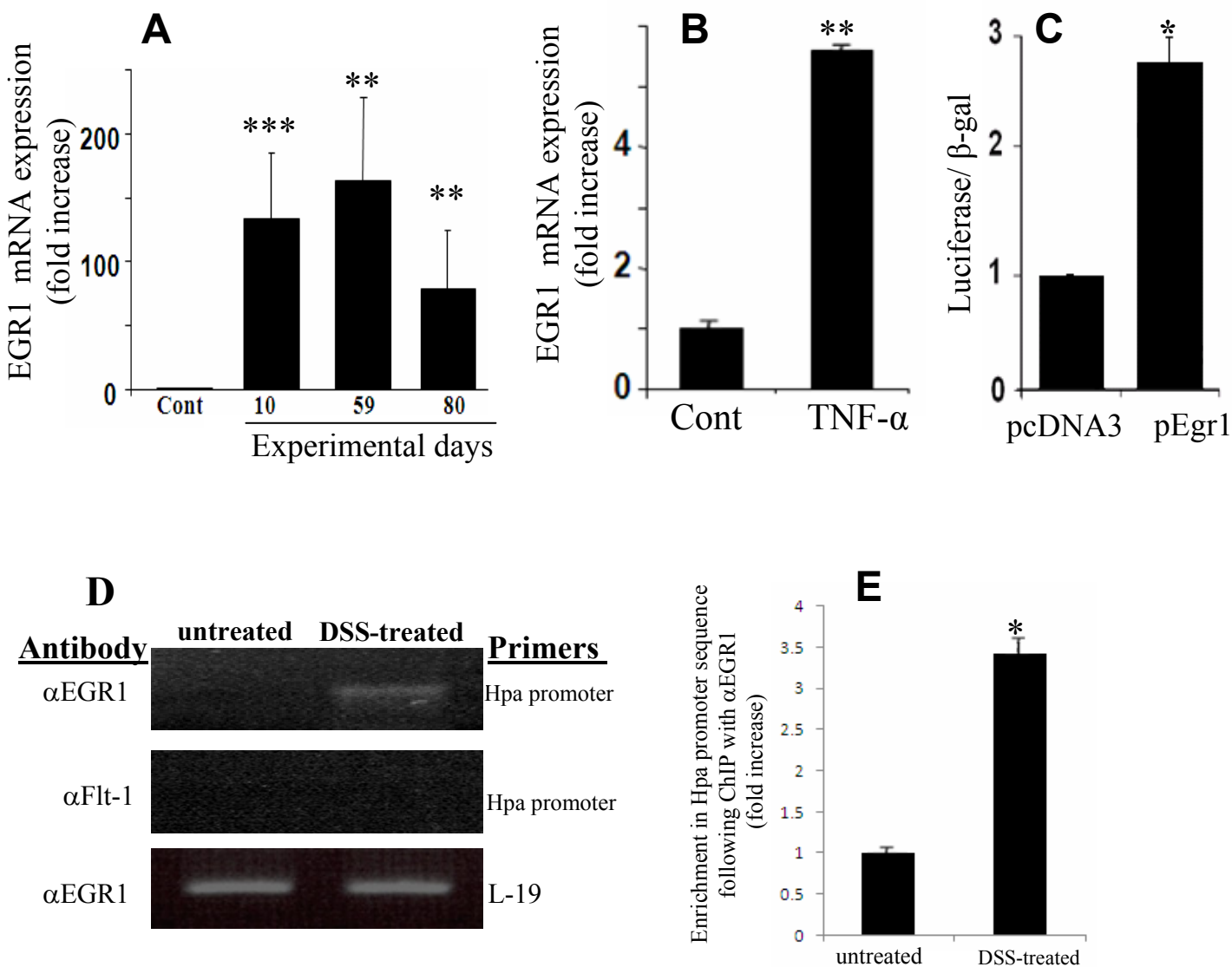


Figure S7. Role of EGR1 in heparanase regulation during DSS-induced colitis. **(A)** Increased expression of EGR1 mRNA in colonic mucosa during the course of DSS colitis, measured by qRT-PCR and normalized to actin mRNA ($n > 4$ for each time point). Error bars represent \pm SD. $**p < 0.01$, and $***p < 0.001$. **(B)** TNF- α induces EGR1 expression in Colo 205 cells. Colo 205 cells were incubated (24 h) in triplicates in the absence or presence of 15 ng/ml TNF- α . EGR1 expression was assessed 16 h later by qRT-PCR and normalized to actin. Error bars represent \pm SD. $**p < 0.01$. **(C)** EGR1 activates heparanase promoter in Colo205 cells. Colo205 cells were co-transfected with plasmid encoding for luciferase (LUC) driven by the heparanase promoter (Hpa-LUC) reporter gene construct and with either EGR1 expressing vector (pEgr1), or empty plasmid (pcDNA3). LUC activity in cell lysates was measured 24 h later and normalized with β -Gal. Error bars represent \pm SD. $*p < 0.05$. **(D, E)** Increased recruitment of EGR1 to heparanase promoter in DSS colitis. Following cross-linking of proteins to DNA, chromatin derived from mouse colon tissue harvested from untreated and DSS-treated mice on day 10 of the experiment was sonicated into fragments of average length ≤ 500 bp and immunoprecipitated with antibodies against EGR1 or an unrelated protein, Flt-1. The final DNA extractions were amplified using primer set that covers functional EGR1 binding site in the heparanase promoter (D, two upper panels), or primer set specific to unrelated L-19 gene sequence, used as control (lower panel). Samples were equilibrated for DNA loading amounts using primers specific to heparanase promoter and DNA that was PCR amplified from chromatin preparations before immunoprecipitation. The results are representative of three independent experiments. An increased occupancy of the heparanase promoter by EGR1 was evident by both conventional PCR (D) and qRT-PCR (E) in chromatin derived from mice with DSS-induced colitis, as compared to healthy untreated mice. Error bars represent \pm SD. $*p < 0.05$.