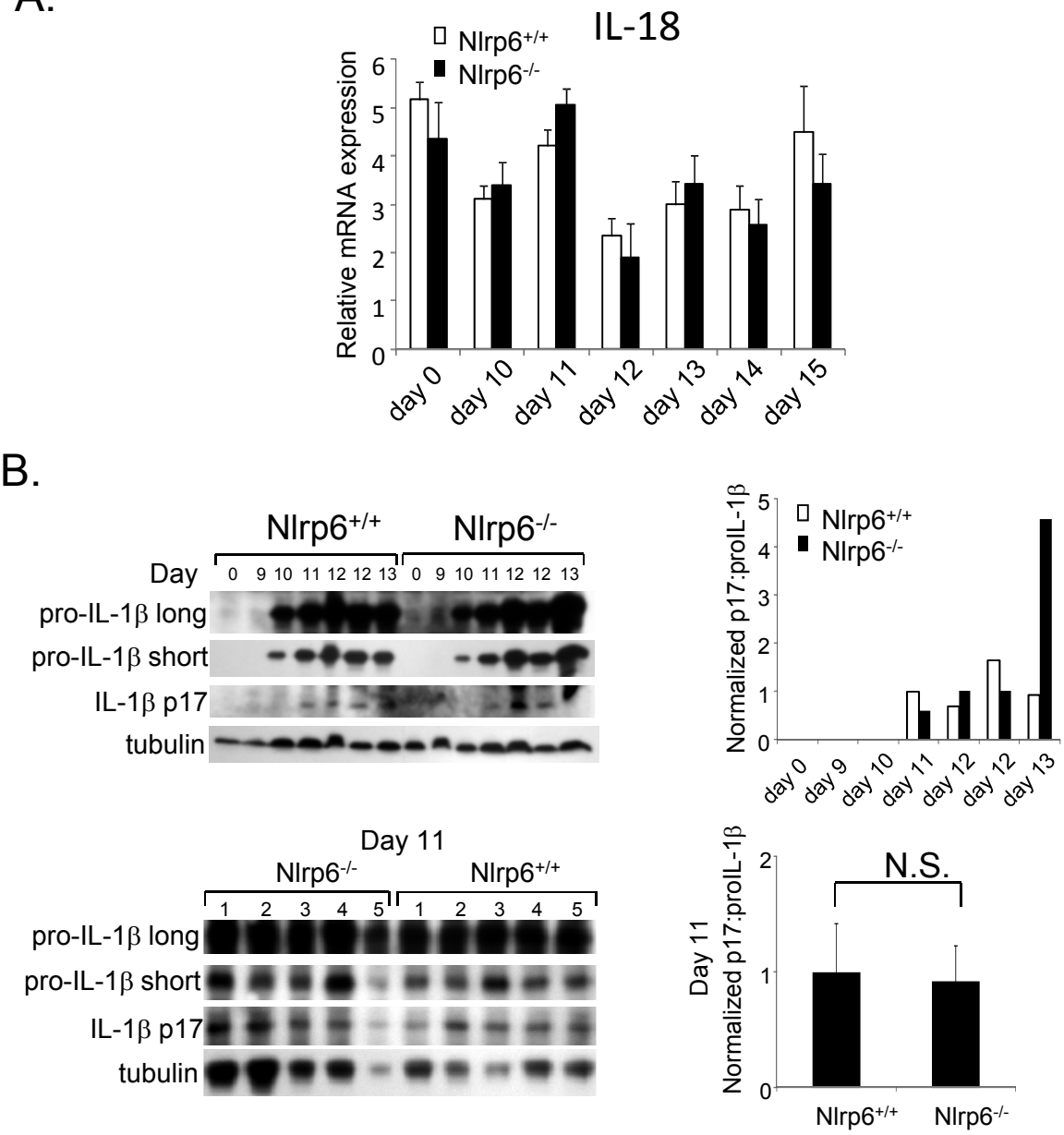
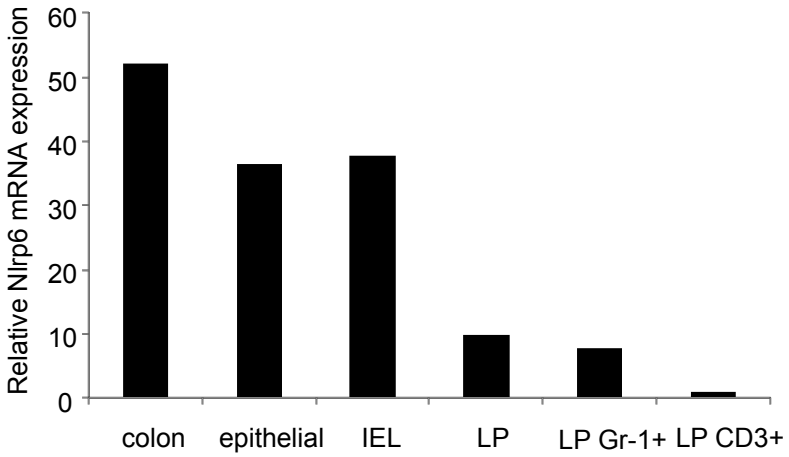


Supplementary Figure 1. Measurement of IL-18 mRNA and mature IL-1β in wildtype and Nlrp6$^{-/-}$ mice. A) mRNA from colons of untreated (day 0) and AOM/DSS-treated age- and sex-matched wildtype and Nlrp6-/- mice were isolated and levels of IL-18 quantified by real-time PCR. B) Colon tissue extracts from untreated (day 0) and AOM/DSS-treated wildtype and Nlrp6$^{-/-}$ mice were made on days 9-13 (top left panel) and 50 μg of total protein from each sample were run on SDS-PAGE. The amount of pro-IL-1β (long and short exposure shown) and mature p17 form (long exposure) were assessed by immunoblotting with anti-mouse IL-1β (goat polyclonal, R&D Systems). Tubulin was used as loading control. Ratio of cleaved versus pro-IL-1β was quantified using AlphaEase software (Alpha Innotech) and normalized with respect to wildtype ratio on day 11 (top right panel). Note that on day 12, two individual mice were evaluated. Colon extracts were also isolated from wildtype and Nlrp6$^{-/-}$ mice on day 11 (5 mice/group) and levels of pro-IL-1β (long and short exposure) and its cleaved p17 form (long exposure) were assessed by immunoblotting (bottom left panel). The ratio of the mature p17 versus pro-IL-1β was quantified and normalized with respect to wildtype (bottom right panel). N.S. denotes non significant. Error bars indicate S.E.



Supplementary Figure 2. Nlrp6 expression in isolated intestinal cells. mRNA was extracted from wildtype B6 colon tissue (colon). Intestinal epithelial cells (epithelial) were enriched by EDTA treatment of colon tissue. Intraepithelial lymphocytes (IEL) were enriched by centrifugation of isolated epithelial cells after EDTA treatment on a 40%/75% Percoll gradient. Lamina propria (LP) cells were isolated by enzymatic digestion of colon tissue after removal of the epithelium followed by centrifugation of the cell suspension on a 40%/75% Percoll gradient. Lamina propria cells were also further enriched for granulocytes and monocytes by FACS sorting using the anti-mouse Gr-1 (Ly-6G and Ly-6C) antibody (BD Pharmingen) (LP Gr-1+). T lymphocytes were also further purified from lamina propria cells by sorting using anti-CD3 antibody (Ebioscience) (LP CD3+). Nlrp6 mRNA expression relative to β-actin was measured by real-time PCR. Results are representative of at least two independent experiments.