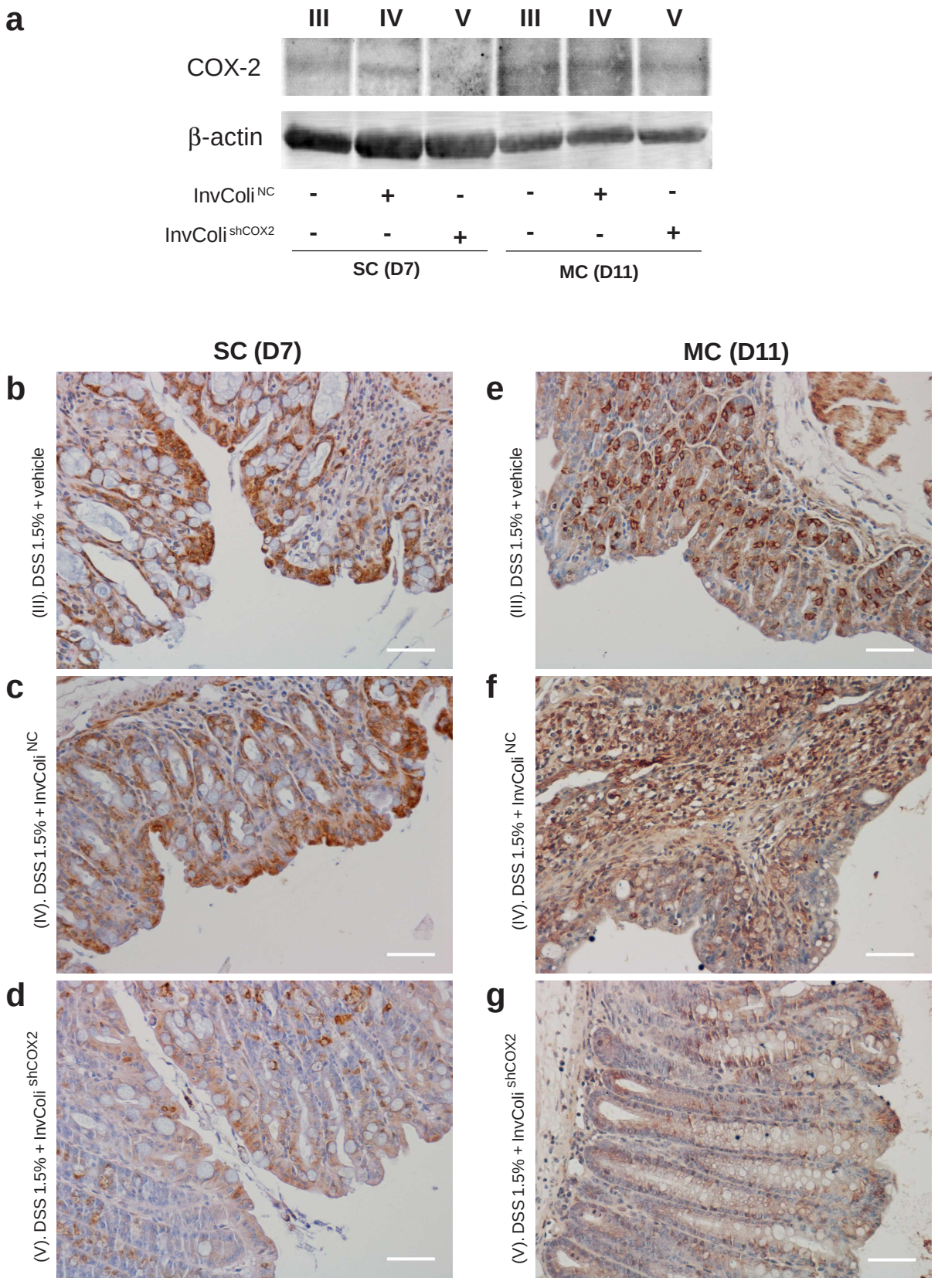
Supplementary Figure 5



Supplementary Figure S5 - InvColi^{shCOX2}-mediated COX-2 silencing.

(**a**) COX-2 protein expression was analyzed in fresh colon samples from experimental mice groups *III* (DSS 1.5% + vehicle), *IV* (DSS 1.5% + InvColi^{NC}) and *V* (DSS 1.5% + InvColi^{shCOX2}). COX-2 expression was evaluated by Western blot and normalized against β-actin expression. **(b,g)** COX-2 protein expression was evaluated also by immunohistochemistry (IHC) in FFPE colon specimens from the same experimental mice groups. Scale bar = 50 μm; ×200 magnification. For all analyses, colon specimens were collected at day 7 (D7, start colitis, SC) and day 11 (D11, max colitis, MC).

Western blot

Colon samples were homogenized in lysis buffer (50 mM Tris-HCl, pH 7.5, 2 mM EDTA, 100 mM NaCl, 1% Triton X-100, protease inhibitors mixture). Lysates were incubated 1 h on ice and centrifuged at 12,000 g to collect supernatants. After addition of SDS-PAGE sample buffer and boiling, 100 μg of denatured proteins were separated in 12% SDS-PAGE and then transferred to nitrocellulose papers. After the blotting, nitrocellulose papers were incubated with specific antibodies. The primary antibodies used were: polyclonal anti-COX-2 (Pierce-Thermo Scientific, IL, USA) and polyclonal anti-β-actin (Sigma, MO, USA). Secondary antibody (Cy5-conjugated) was purchased from GE (CT, USA). Immunolabelling was visualized using the laser scanner Pharos FX (Bio-Rad, CA, USA). Normalization was made against β-actin expression.