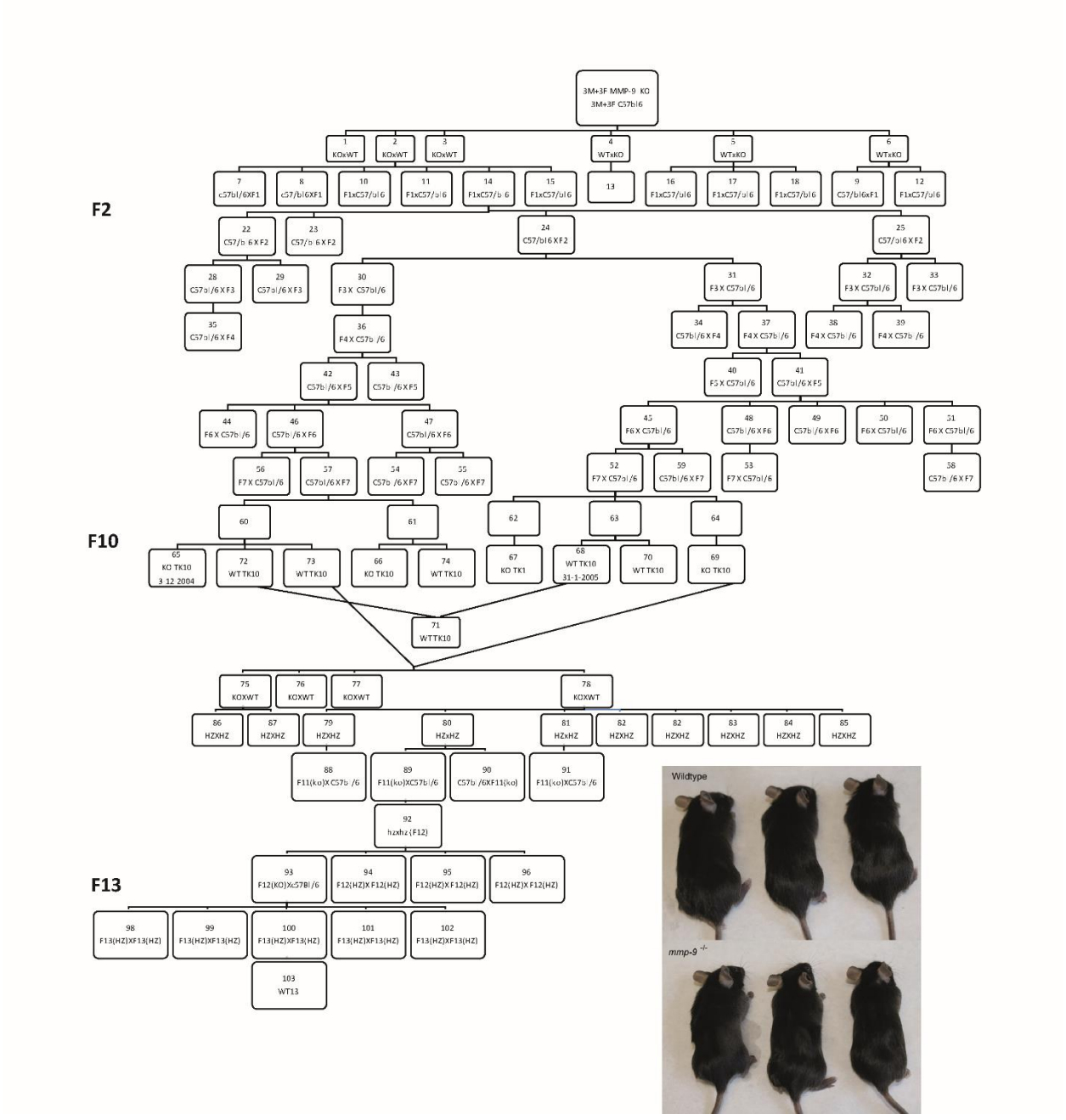
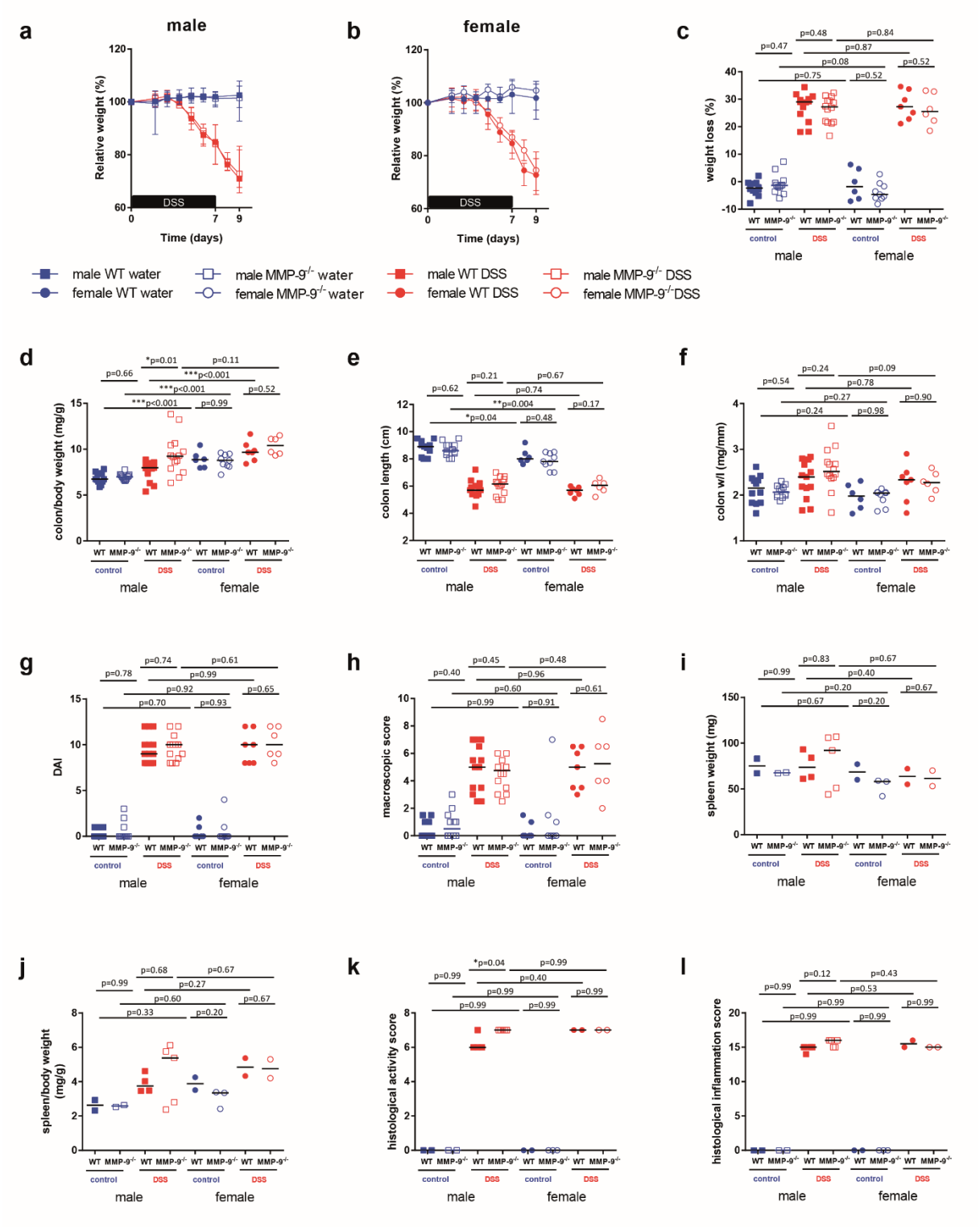
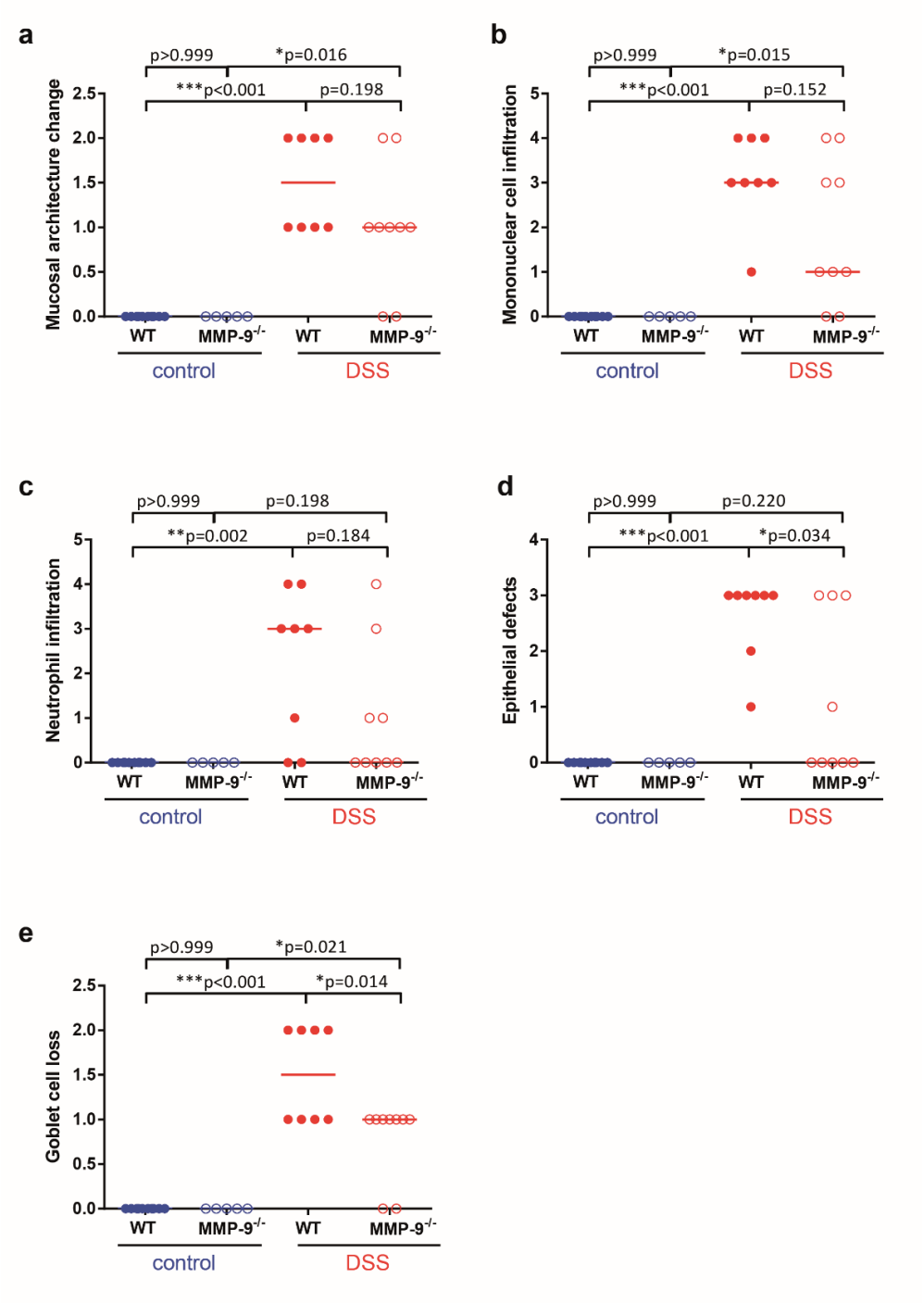



**Supplementary figure 1:** Backcrossing scheme of MMP-9$^{-/-}$ mice to C57BL/6J background for 13 generations and examples of three wild type and three MMP-9$^{-/-}$ mice.

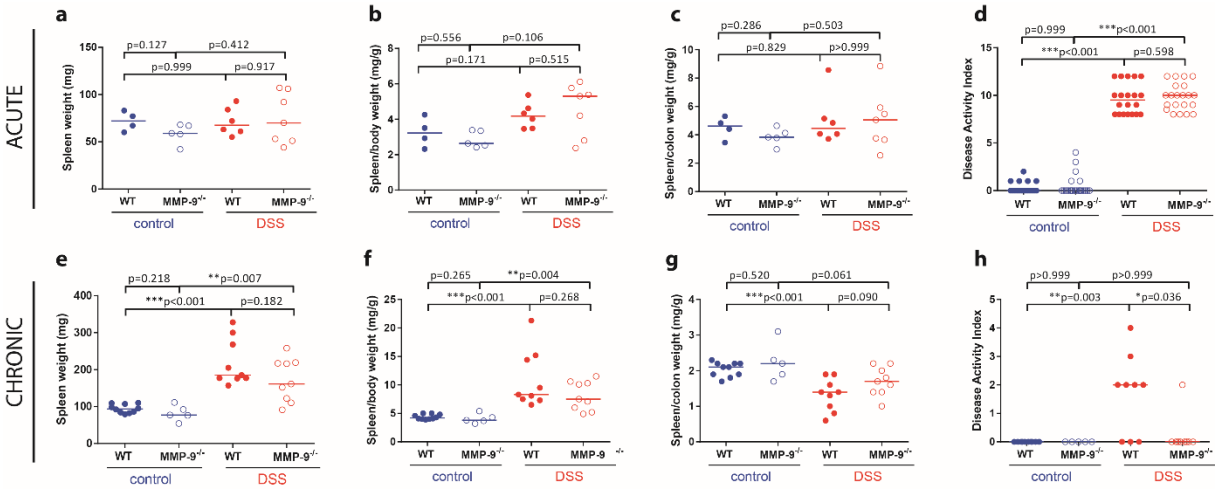



**Supplementary figure 2: Differences between male (*squares*) and female (*circles*) mice after acute DSS administration.** Data are shown from male control WT mice (n=12), male control MMP-9$^{-/-}$ mice (n=12), male DSS-treated WT mice (n=13), male DSS-treated MMP-9$^{-/-}$ mice (n=14), female control WT mice (n=6), female control MMP-9$^{-/-}$ mice (n=9), female DSS-treated WT mice (n=7) and female DSS-

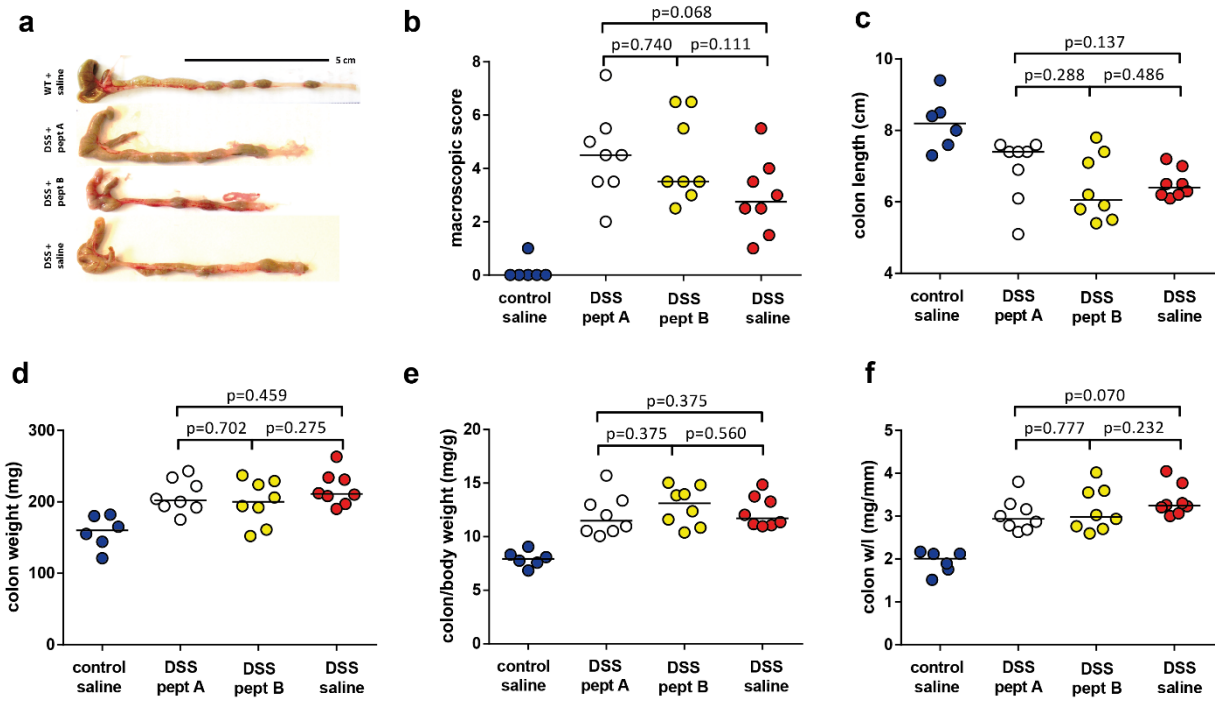
treated MMP-$9^{-/-}$ mice (n=6) that were used in three independent experiments. Representation of relative body weight curves of male (**a**) and female (**b**) mice, absolute body weight loss at time of sacrifice (**c**)**,** colon/body weight ratio (**d**), colon length (**e**), colon weight/length (w/l) ratio (**f**), disease activity index (DAI) (**g**), macroscopic damage score (**h**), spleen weight (**i**), spleen/body weight (**j**), histological activity score (**k**) and histological inflammation score (**l**). Median values with interquartile range, if applicable, are shown. Statistical analyses were performed with Mann-Whitney *U* tests (* $p<0.05$, ** $p\leq0.01$, *** $p\leq0.001$).



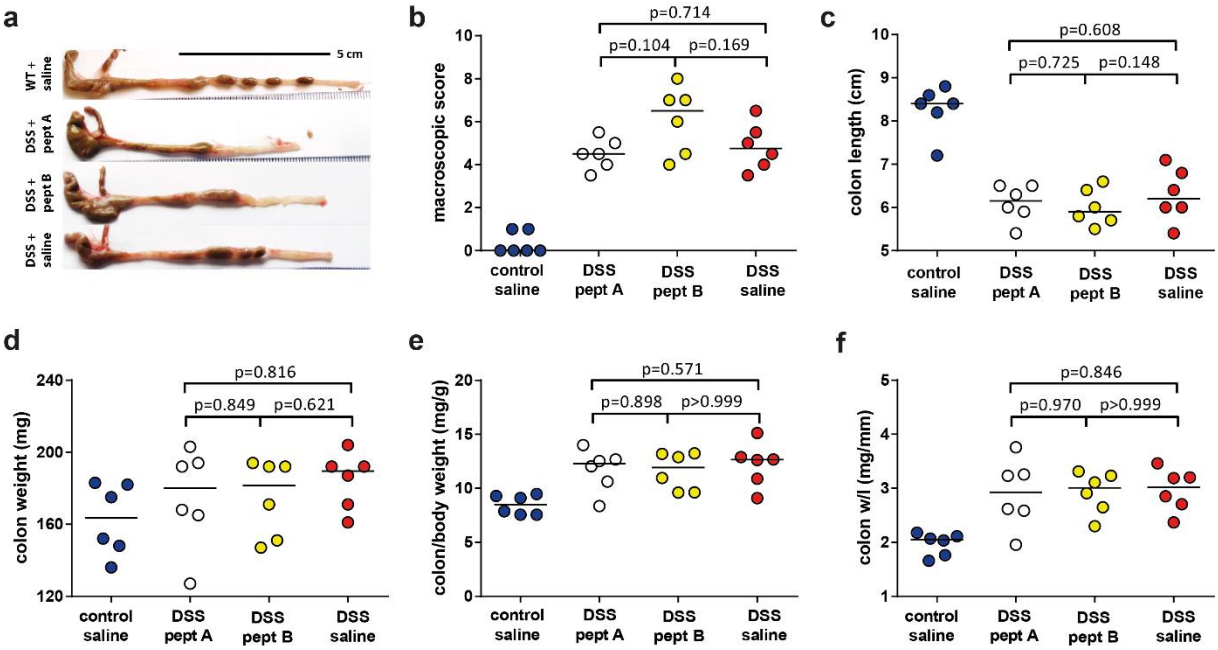

**Supplementary figure 3: Histological inflammation and activity scores of WT and MMP-9$^{-/-}$ mice with chronic colitis.** Read-outs from chronic DSS exposed WT (n=9) and MMP-9$^{-/-}$ mice (n=9), and control WT (n=10) and MMP-9$^{-/-}$ mice (n=5) included in three independent experiments are shown. Mucosal architecture change (**a**), mononuclear cell infiltration (**b**), neutrophil infiltration (**c**), epithelial defects (**d**) and goblet cell loss (**e**) are represented. Median values are indicated and statistical analyses were performed with Mann-Whitney *U* tests (* p<0.05, ** p≤0.01, *** p≤0.001).



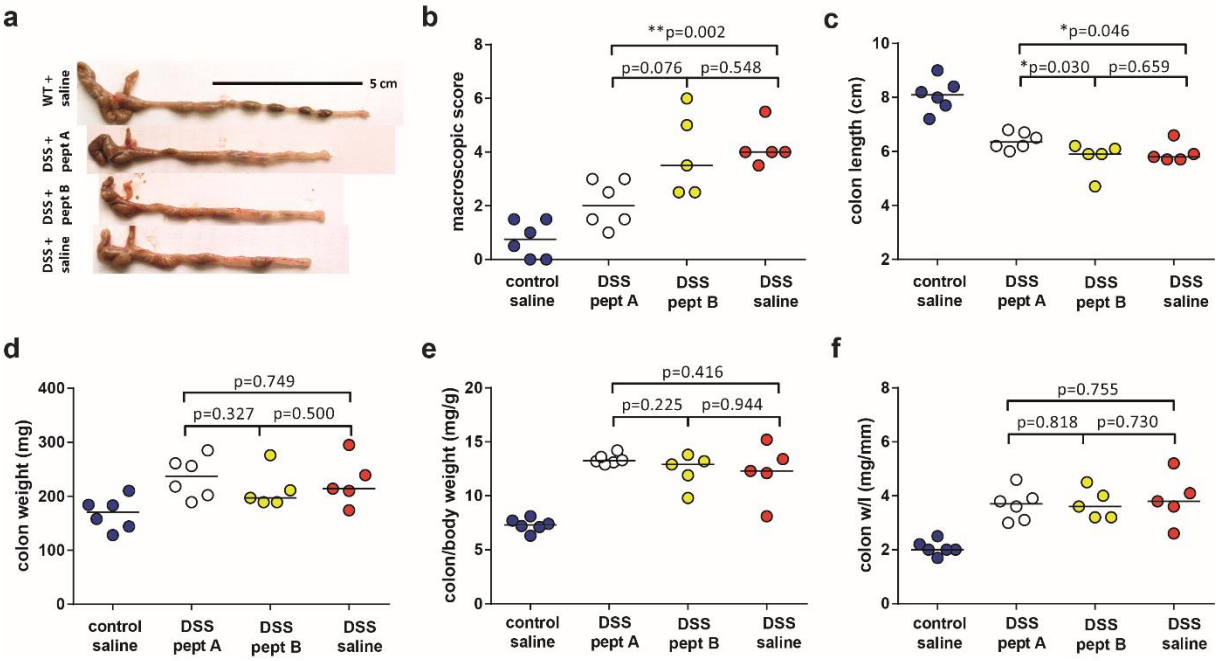

**Supplementary figure 4: Systemic inflammation parameters of acute and chronic DSS models.** Data are shown from acute DSS exposed WT (n=20) and MMP-9$^{-/-}$ mice (n=20) as well as control WT (n=18) and MMP-9$^{-/-}$ mice (n=21) for DAI measurements, whereas for spleen measurements a subset of samples was used (n=21). Pooled data from 3 replicated acute DSS experiments are shown. For the chronic model, read-outs from DSS exposed WT (n=9) and MMP-9$^{-/-}$ mice (n=9), and control WT (n=10) and MMP-9$^{-/-}$ mice (n=5) included in 3 independent experiments are shown. Spleen weight was evaluated as such and as a ratio over body weight and colon weight after acute (**a-c**) and chronic (**e-g**) DSS administration. The disease activity index (DAI) includes stool consistency, presence of blood and body weight loss and is represented for samples included in the acute (**d**) and chronic (**h**) DSS model. Median values are indicated and statistical analyses were performed with Mann-Whitney *U* tests (* $p<0.05$, ** $p\leq0.01$, *** $p\leq0.001$).



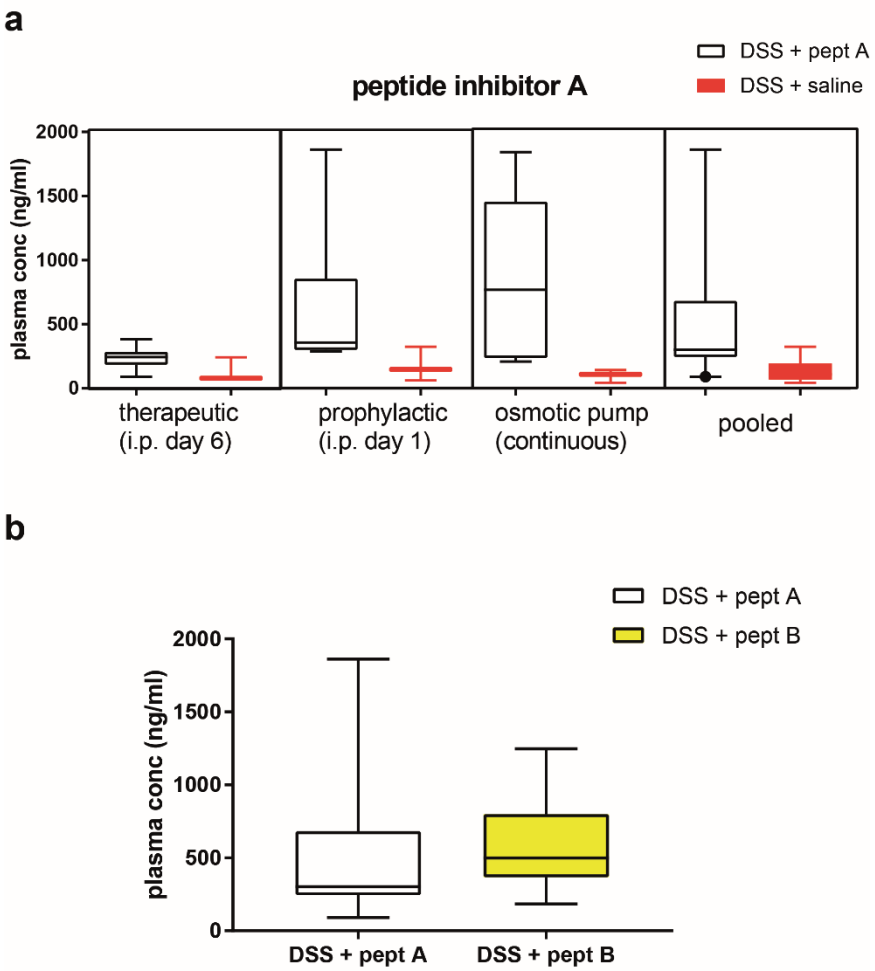

**Supplementary figure 5: Macroscopic damage score and colonic measurements after therapeutic administration of peptide inhibitors A and B.** Overview of isolated colon, cut at the ileocecal intersection isolated from WT + saline (n=6), DSS + peptide inhibitor A (n=8), DSS + peptide inhibitor B (n=8) and DSS + saline (n=8) mice (**a**). A scale bar of 5 cm is shown on the pictures. Representation of macroscopic damage score (**b**) colon length (**c**), colon weight (**d**), colon/body weight ratio (**e**) and colon weight/length (w/l) ratio (**f**) of mice treated therapeutically starting from day 6 with peptide inhibitors A, B or saline. Median values are represented and statistical analyses were performed with Mann-Whitney *U* tests.




**Supplementary figure 6: Macroscopic damage score and colonic measurements after prophylactic administration of peptide inhibitors A and B.** Overview of isolated mouse colon, cut at the ileocecal intersection isolated from WT + saline (n=6), DSS + peptide inhibitor A (n=6), DSS + peptide inhibitor B (n=6) and DSS + saline mice (n=6) (**a**). A scale bar of 5 cm is shown on the pictures. Representation of macroscopic damage score (**b**), colon length (**a-c**), colon weight (**d**), colon/body weight ratio (**e**) or colon weight/length (w/l) ratio (**f**) of mice that received either peptide inhibitors A, B or saline after day 1 of start of DSS administration. Median values are represented and statistical analyses were performed with Mann-Whitney *U* tests.




**Supplementary figure 7: Macroscopic damage score and colonic measurements after continuous delivery of peptide inhibitors A and B via implanted osmotic pumps.** Overview of isolated mouse colon, cut at the ileocecal intersection isolated from WT + saline (n=6), DSS + peptide inhibitor A (n=6), DSS + peptide inhibitor B (n=6) and DSS + saline mice (n=6) (**a**). A scale bar of 5 cm is shown on the pictures. Representation of macroscopic damage score (**b**), colon length (**a-c**), colon weight (**d**), colon/body weight ratio (**e**) or colon weight/length (w/l) ratio (**f**) of mice that were implanted with osmotic pumps containing either peptide inhibitors A, B or saline. Median values are represented and statistical analyses were performed with Mann-Whitney *U* tests (* $p<0.05$, ** $p\leq0.01$).




**Supplementary figure 8**: **Levels of peptide inhibitors A and B in plasma of DSS-treated mice at time of sacrifice.** (**a**) Plasma levels of peptide inhibitor A measured with ELISA in DSS-treated mice that received either peptide inhibitor A (n=19) or saline (n=9) injections for all three different administration schemes (therapeutic, prophylactic and continuous). In addition, pooled values are shown to indicate median detectable plasma levels regardless of the administration scheme. (**b**) Similar ELISA analyses were performed for detection of peptide inhibitor B in plasma of DSS-treated mice (n=19). This indicates that both peptide inhibitors A and B yield detectable circulating plasma levels at time of sacrifice confirming that sufficiently high levels for inhibition in plasma were attained. Colour code is similar as in Figures 9 and 10. Box plots are shown with 5-95 percentiles.

**SUPPLEMENTARY TABLES**

**Supplementary table 1**: Overview of significantly differentially expressed genes in the colon between control and DSS-treated WT and MMP-9$^{-/-}$ mice after DESeq, EdgeR and CuffDiff2 comparative analyses. Abbreviations: FC, fold change; adj, adjusted; DSS, dextran sodium sulphate; NA, undetectable value.

| Gene symbol | Gene name | DESeq | | EdgeR | | CuffDiff2 | |
|---|---|---|---|---|---|---|---|
| | | Log2 FC | Adj p-value | Log2 FC | Adj p-value | Log2 FC | Adj p-value |
| MMP-9$^{-/-}$ control (n=8) *versus* WT control (n=8) | | | | | | | |
| *Mmp9*† | matrix metalloproteinase 9 | 3.9 | 7.89 e-34 | 3.8 | 3.20 e-40 | 3.9 | 0.019 |
| *Rims4* | regulating synaptic membrane exocytosis 4 | -6.0 | 3.89 e-12 | -6.0 | 5.65 e-37 | -6.0 | 0.019 |
| *Slpi* | secretory leukocyte peptidase inhibitor | 2.4 | 1 | 2.4 | 3.68 e-04 | 2.5 | 0.019 |
| MMP-9$^{-/-}$ DSS (n=8) *versus* WT DSS (n=8) | | | | | | | |
| *Clps* | colipase, pancreatic | NA | NA | 5.6 | 0.020 | 5.7 | 0.030 |
| *Ddx60* | DEAD (Asp-Glu-Ala-Asp) box polypeptide 60 | NA | NA | 2.4 | 0.033 | 2.4 | 0.030 |
| *Fgb* | fibrinogen beta chain | NA | NA | 7.6 | 0.020 | 7.7 | 0.030 |
| *Fgg* | fibrinogen gamma chain | NA | NA | 7.5 | 0.020 | 7.6 | 0.030 |
| *Ifi44* | interferon-induced protein 44 | NA | NA | 2.5 | 0.020 | 2.1 | 0.030 |
| *Ifit2* | interferon-induced protein with tetratricopeptide repeats 2 | NA | NA | 2.6 | 0.011 | 2.6 | 0.030 |
| *Isg15* | ISG15 ubiquitin-like modifier | NA | NA | 2.4 | 0.020 | 2.4 | 0.030 |
| *Itih3* | inter-alpha-trypsin inhibitor heavy chain 3 | NA | NA | 4.1 | 0.029 | 4.2 | 0.030 |
| *Itih4* | inter-alpha-trypsin inhibitor heavy chain family, member 4 | NA | NA | 7.0 | 0.003 | 6.3 | 0.030 |
| *Oas3* | 2'-5'-oligoadenylate synthetase 3, 100kDa | NA | NA | 2.7 | 0.020 | 2.7 | 0.030 |
| *Usp18* | ubiquitin specific peptidase 18 | NA | NA | 2.5 | 0.020 | 2.5 | 0.030 |

† In MMP-9$^{-/-}$ mice, reads were mapped only to exons 9-13 (non-functional read-through transcript containing the haemopexin domain), whereas no reads were mapped to exons 1-8 (the functional part of the *Mmp9* gene). This explains the paradoxical difference between MMP-9$^{-/-}$ control and WT control mice.

**Supplementary table 2:** IC50 values of peptide inhibitors A and B towards MMP-3, -8, -9 and TACE (μM).

| **Name** | **Peptide sequences†** | **MMP-3** | **MMP-8** | **MMP-9** | **TACE** |
|---|---|---|---|---|---|
| Peptide inhibitor A (CPU 1) | P(Pyr)CBRGE-GGGG-IVRRADRAAVP | No inhibition | 17.6 | 12.5 | 7.0 |
| Peptide inhibitor B (CPU 2) | IVRRADRAAVP-GGGG-P(Pyr)CBRGE | 7.0 | 0.036 | 0.16 | 15 |

† Peptide sequences are in one-letter codes for amino acids. Pyr stands for pyridylalanine.

**Supplementary table 3:** Primer sequences used for qRT-PCR analyses.

| **Gene** | **Forward primer** | **Reverse primer** | **Probe** |
|---|---|---|---|
| *Mmp3* | CAGGAAGATAGCTGAGGACTTTC | GGTCAAATTCCAACTGCGAAG | AGGTGTTGACTCAAGGGTGGATGC |
| *Mmp8* | CCAGCACCTATTCACTACCTC | AGCATCAAATCTCAGGTGGG | ACCTTCAGACAACCCCATCCAACC |
| *Mmp9* | GATCCCCAGAGCGTCATTC | CCACCTTGTTCACCTCATTTTG | CGACATCTTCCAGTACCAAGACAA AGCC |
| *Tace* | GGGTTTTGCGACATGAATGG | GAAAACCAGAACAGACCCAAC | TCCCAAAATCGCTCAATTACGTCCT GT |