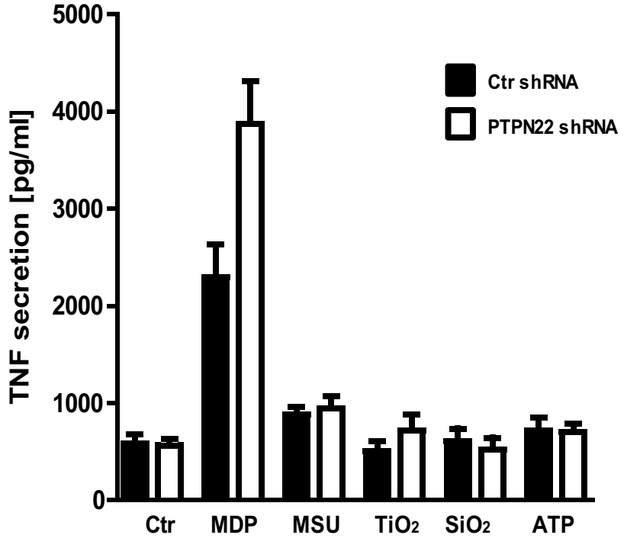
S1A



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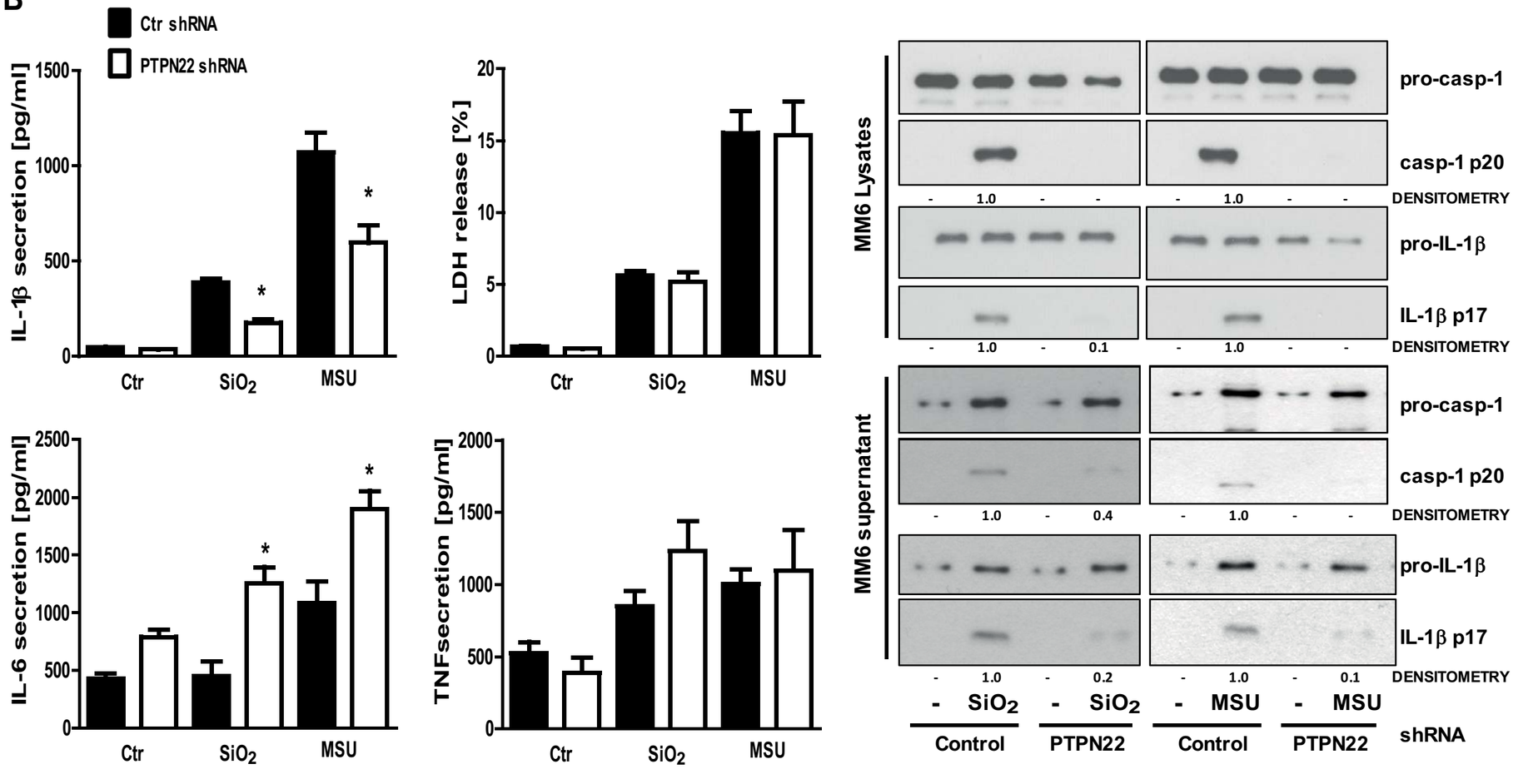
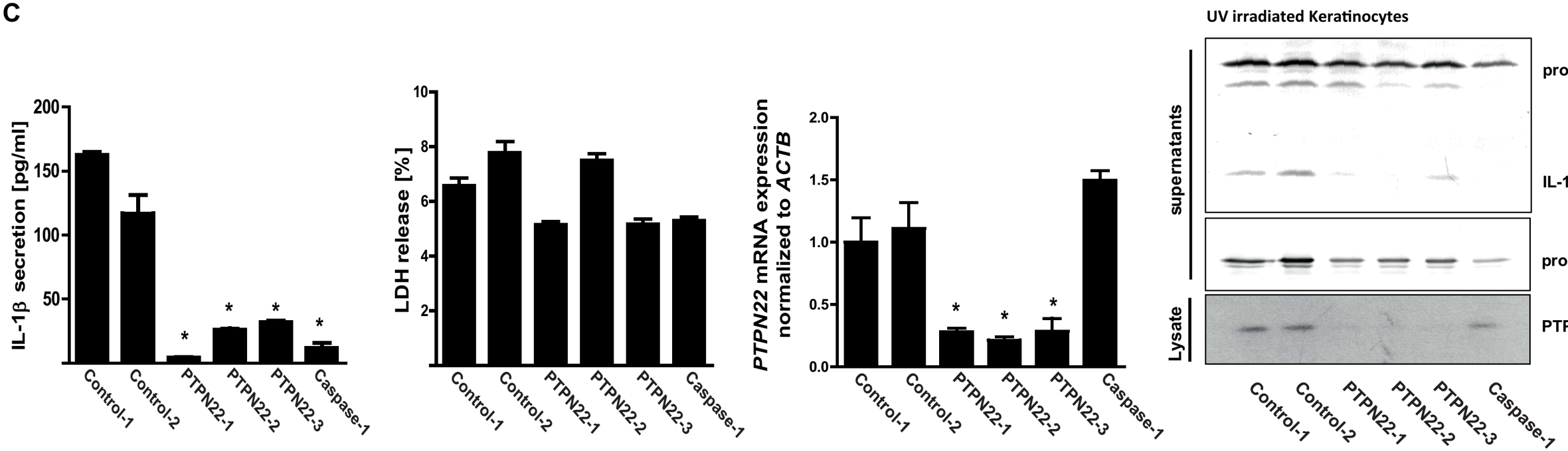
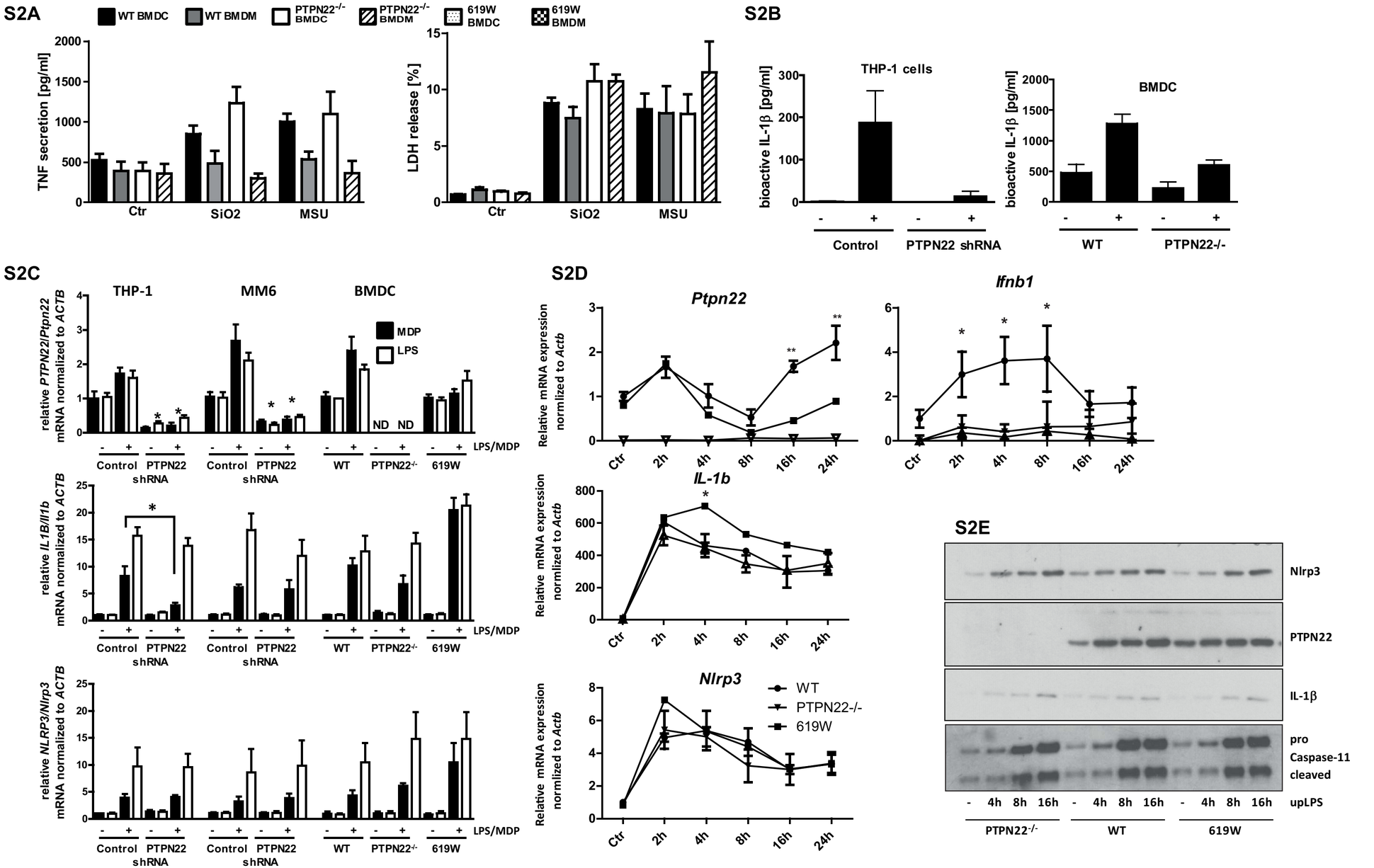


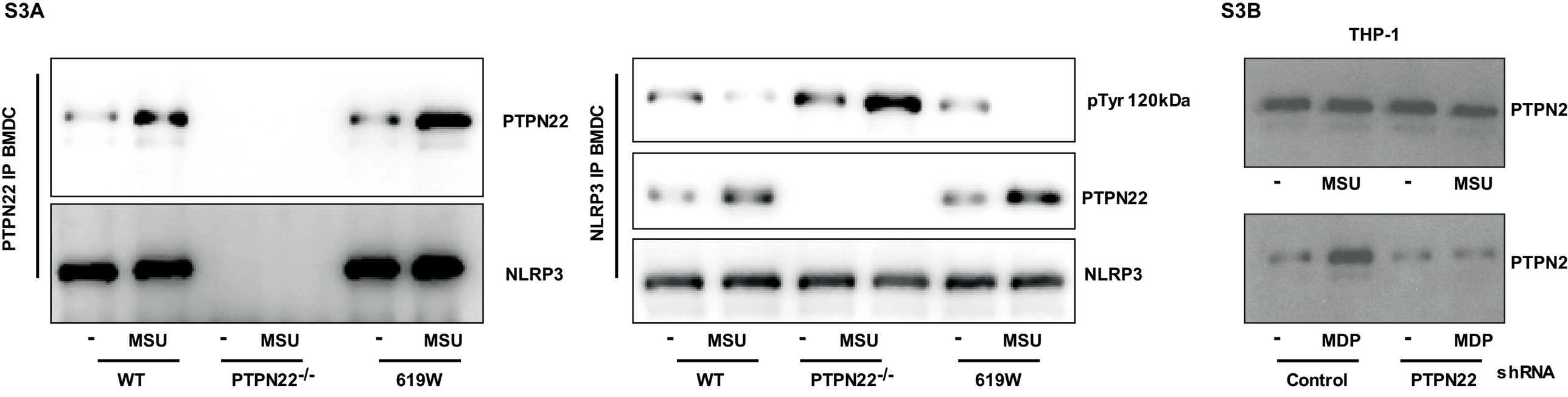
S1C



Supplementary Figure 1. Knockdown of PTPN22 reduces IL-1β secretion in various cell types. PTPN22 knockdown was induced using lentiviral shRNA constructs (A) or siRNA (B+C). Cells were pretreated for 12h with upLPS before indicated activation. **A**: THP-1 cells were activated with MDP (100ng/ml, 24h), MSU (150ng/ml, 6h), TiO2 (150 ng/ml, 24h), SiO2 (150ng/ml, 12h) or ATP (200mM, 30 min.) as indicated and cell culture supernatants analysed for TNF release. **B**: MM6 cells were treated with SiO2 or MSU and analyzed for IL-1β, LDH, IL-6, and TNF release by ELISA, as well as for IL-1β maturation and caspase-1 cleavage by Western blot. **C**: Keratinocytes were treated with two different irrelevant control siRNA constructs (Control-1 and Control-2), three PTPN22 specific siRNA constructs (PTPN22-1, PTPN22-2 and PTPN22-3) or one Caspase-1 targeting siRNA construct before activation 30 min. by UV irradiation. Cell culture supernatants were analyzed for IL-1β secretion/LDH release by ELISA, and IL-1β and caspase-1 cleavage by Western blot. Lysates were analyzed for PTPN22 mRNA and protein expression. Data is representative for one out of at least three independent experiments with 3-5 replicas (n=3-5; *=p<0.05; Newman-Keuls post hoc test). Numbers below the blots show results of densitometry (cleaved forms).

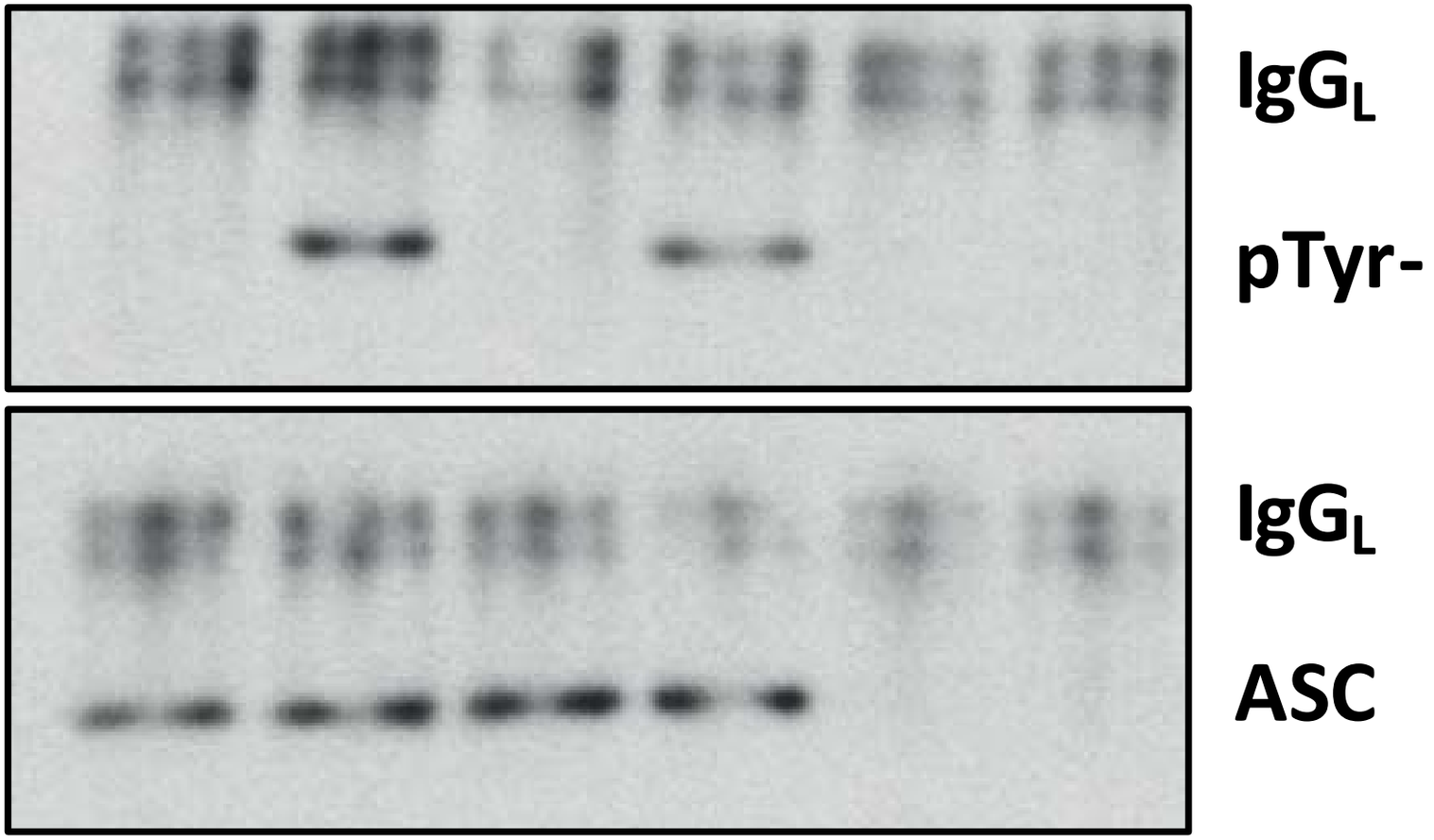


Supplementary figure 2. A: BMDC and BMDM from WT and PTPN22-/- mice were pretreated with upLPS for 12h before activation with SiO2 or MSU as indicated and analyzed for TNF and LDH release. **B**: Kockdown of PTPN2 was induced in THP-1 cells using lentiviral shRNA constructs. THP-1 cells (left) and BMDC from WT or PTPN22-/- mice (right) were pretreated for 12 h with upLPS before activation with MSU (150ng/ml, 6h). Cell culture supernatants were used to stimulate proliferation of D10 G4.1 cells in order to determine bioactive IL-1β. **C**: mRNA expression of *PTPN22/Ptpn22*, *IL1B/Il1b*, and *NLRP3/Nlrp3* in THP-1 cells, MM6 or BMDC, treated for 24h with MDP or upLPS as indicated. Data are depicted as relative values to non-treated controls and normalized to *ACTB/Actb*. **D**: Bone marrow DCs from WT, PTPN22-/- or PTPN22-619W mice were treated for the indicated time with upLPS and analyzed for *Ptpn22, Il1b, Nlrp3*, and *Ifnb1* mRNA expression. Data are depicted as relative values to non-treated controls and normalized to *Actb*. **E**: cells were treated as in D and analyzed for NLRP3, PTPN22, IL-1β and Caspase-11 protein expression by Western blot. Data is representative of one out three independent experiments with 3 replicas each (n=3; *=p<0.05, **=p<0.01; Newman-Keuls post hoc test).

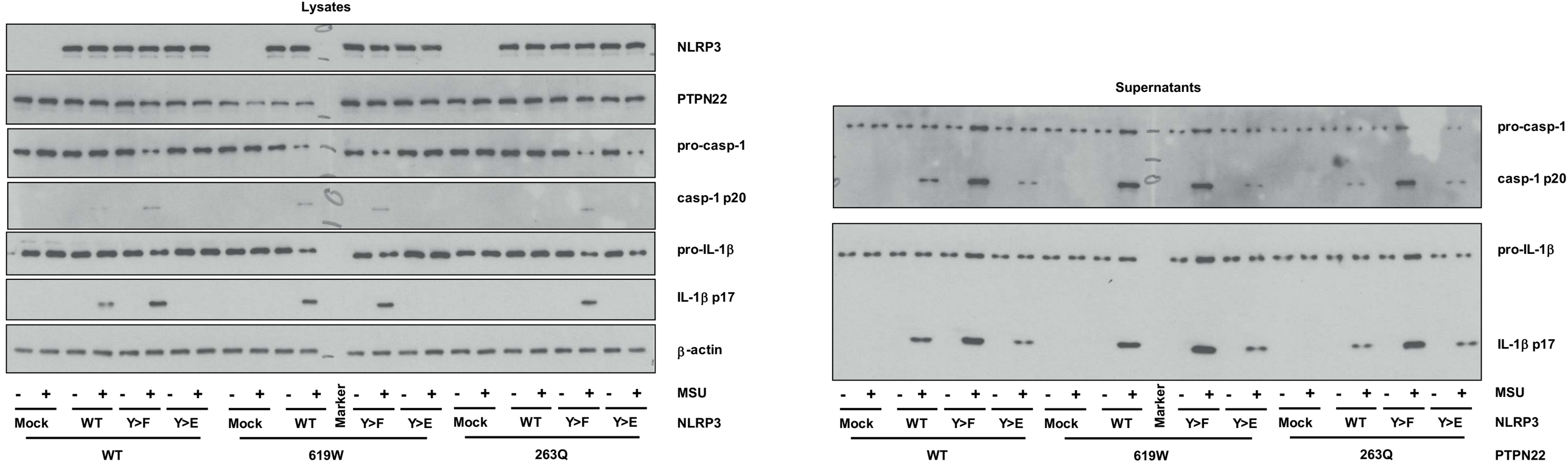


Supplementary Figure 3. A: BMDC were treated with upLPS for 12h prior to activation with MSU (100ng/ml, 6h) and PTPN22 or NLRP3 precipitated from cell lysates and analysed by Western blot for co-precipitated NLRP3, phospho-tyrosine and PTPN22, respectively. **B**: THP-1 cells were treated with MSU or MDP as indicated and cell lysates analysed for PTPN2 expression. Results are representative for three independent experiments.

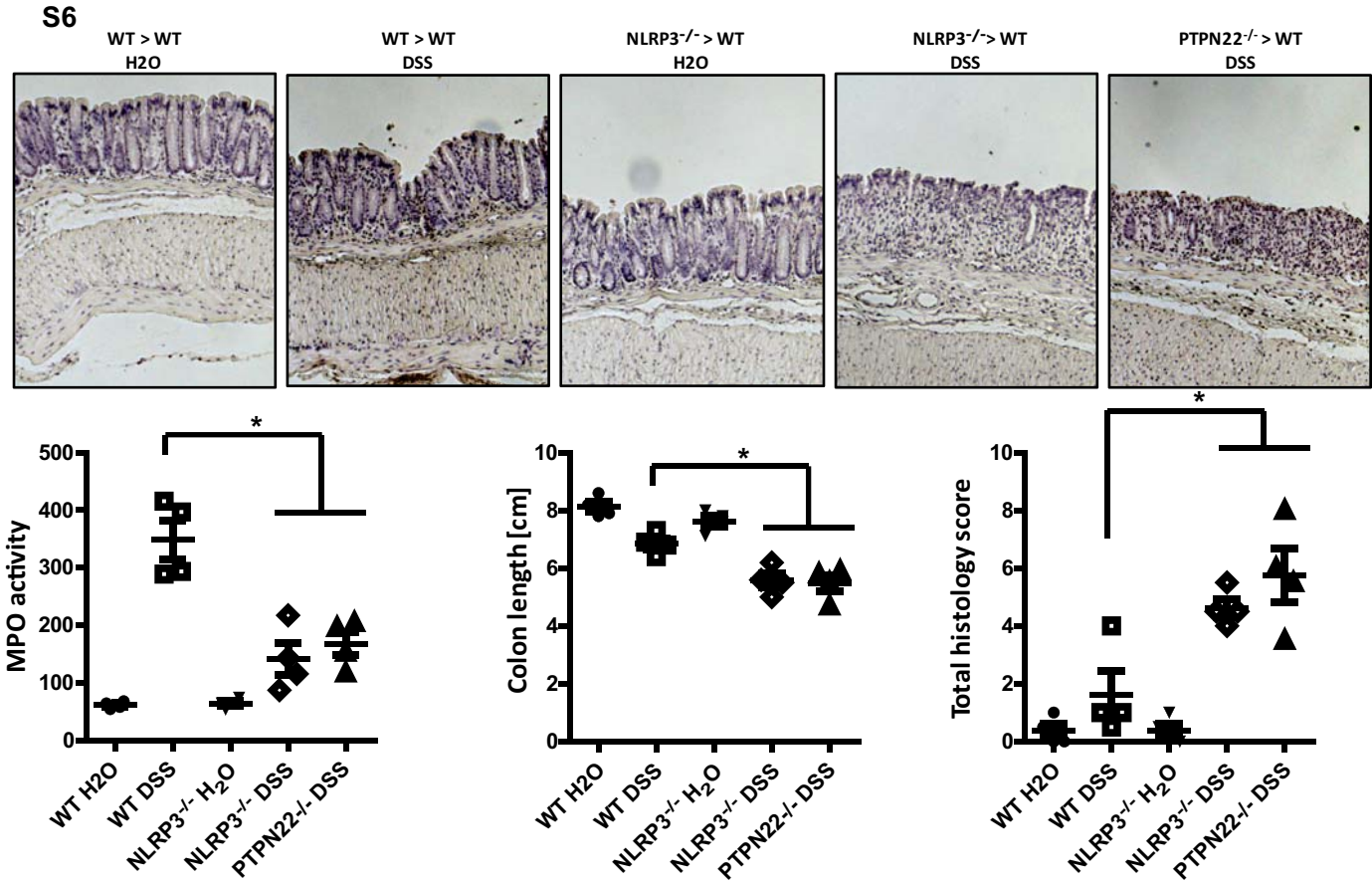
S4 ASC-IP in BMDCs



Supplementary Figure 4. Loss of PTPN2 does not influence ASC tyrosine phosphorylation. BMDC from WT, PTPN22-/- or ASC-/- mice were pre-treated for 12h with upLPS and activated for 6h with MSU. ASC was precipitated and analysed for presence of phospho-tyrosine residues. Data is representative for one of three independent experiments (n=3).

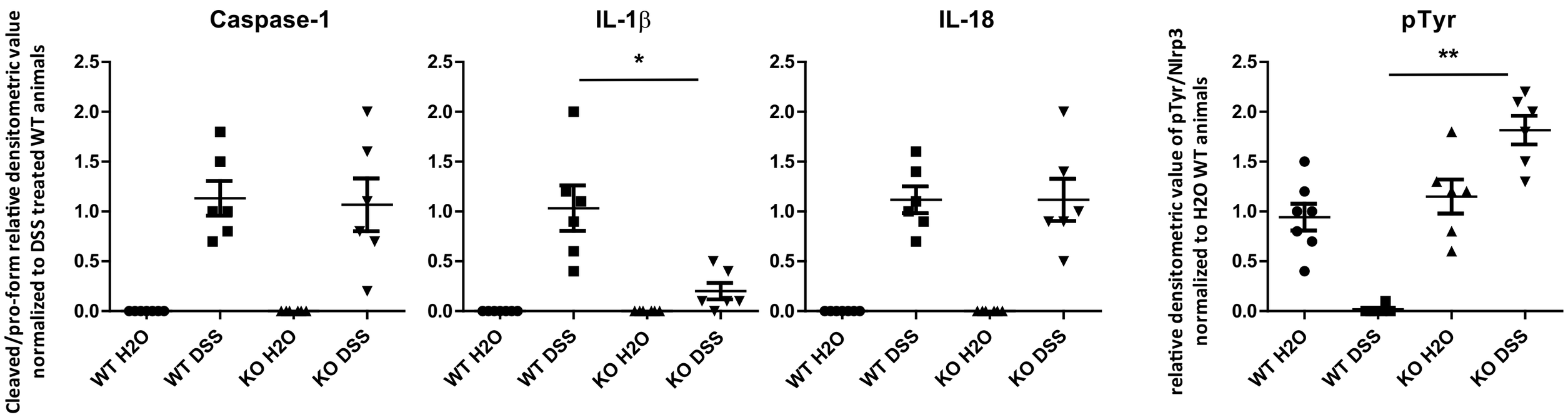


Supplementary Figure 5. NLRP3-/- , PTPN22-/- double deficient BMDC were left non transfected or transfected with either WT NLRP3, Y>F, Y>E, Y>A NLRP3 and WT, 619W or 263Q (loss-of-function) PTPN22 prior to activation with MSU. Lysates were analysed for NLRP3, PTPN22, IL-1β and Caspase-1, and Supernatants for IL-1β and Caspase-1 processing. The experiment has been repeated three times.

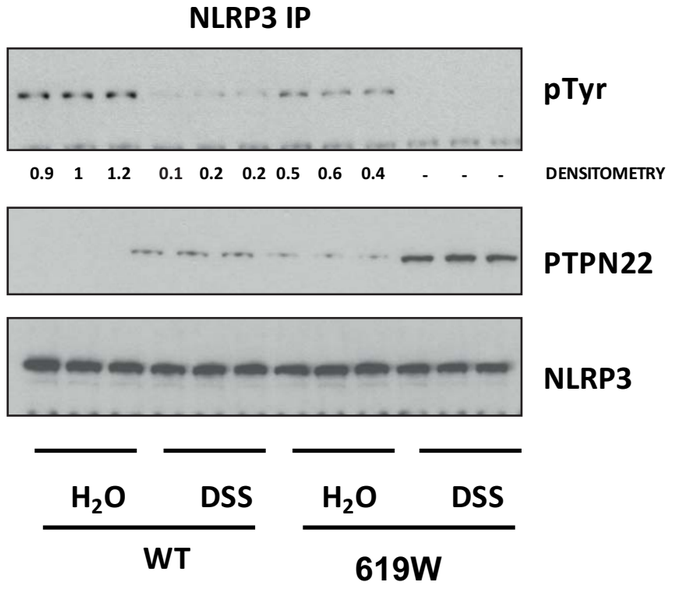


Supplementary figure 6. Loss of NLRP3 in hematopoietic cells results in enhanced colitis severity. Bone marrow chimeric mice were generated using WT recipients, reconstituted either with WT, NLRP3-/- or PTPN22-/- bone marrow. Eight weeks after bone marrow reconstitution, acute colitis was induced by administration of 1.5% DSS. The graph shows colon length; MPO activity; representative pictures of H&E stained sections of the distal colon; and analysis of epithelial damage, and inflammatory infiltration. Data are representative for one out of two independent experiments with 4-6 mice per group each (n=4-6). Each dot represents one mouse. (*=p<0.05, Man-Withney-U test with Bonferroni correction). Original magnification in H&E: 10x.

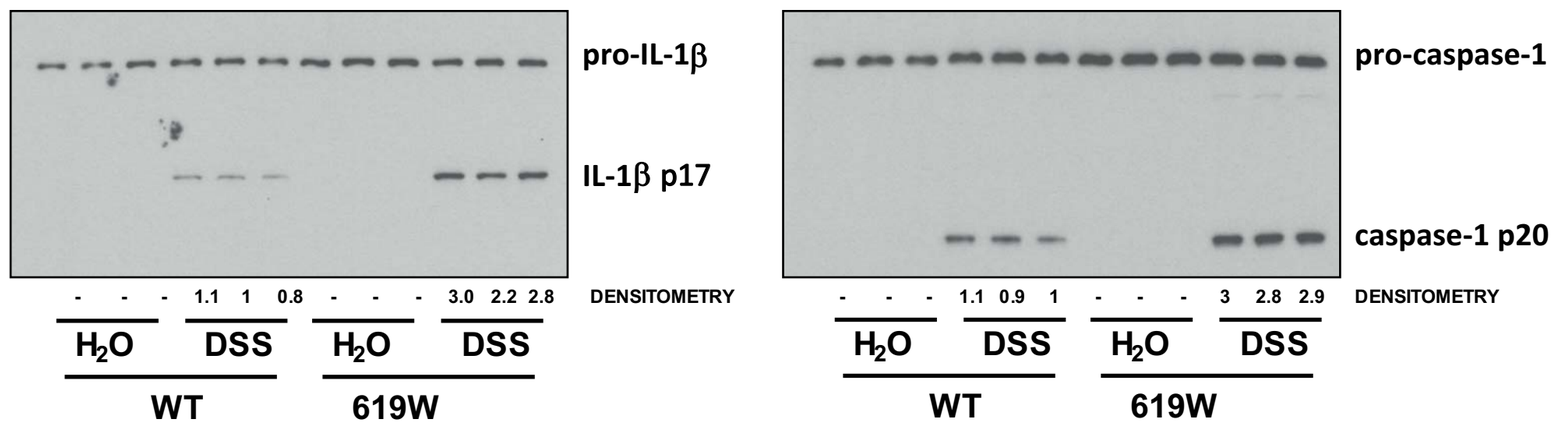
S7A

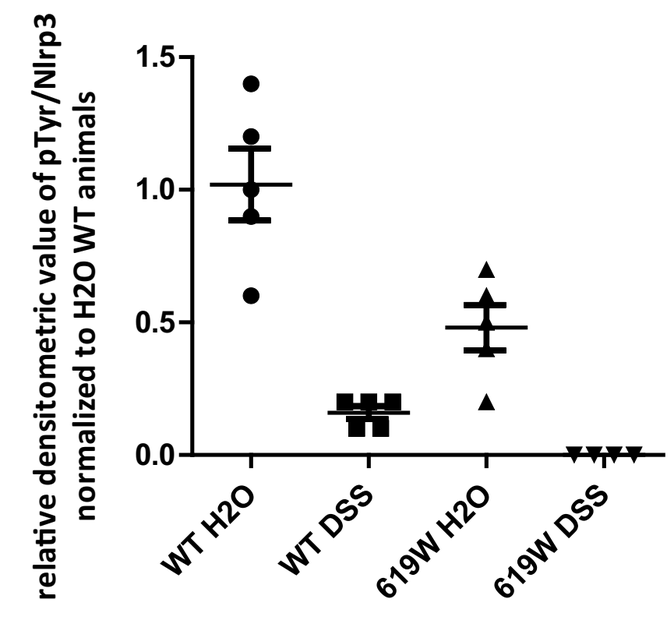


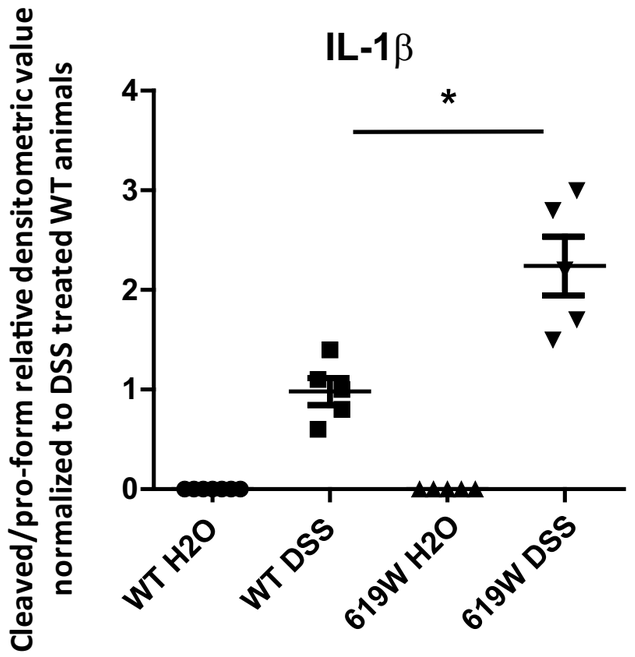
S7B

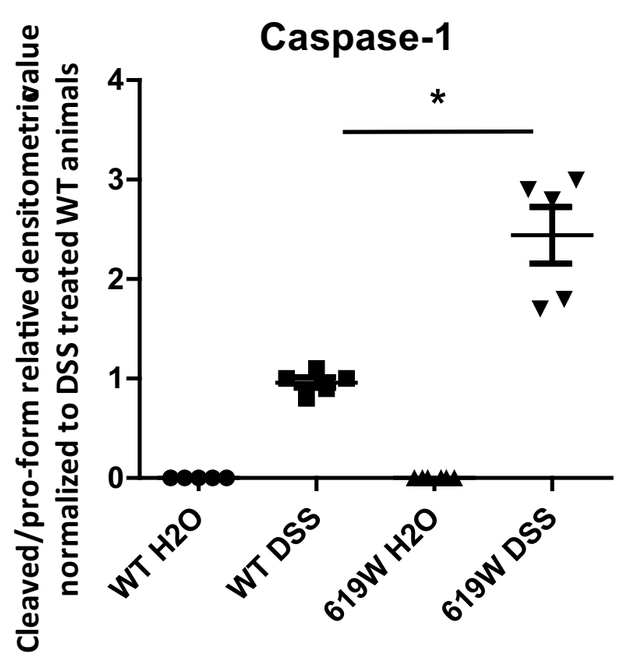


S7C

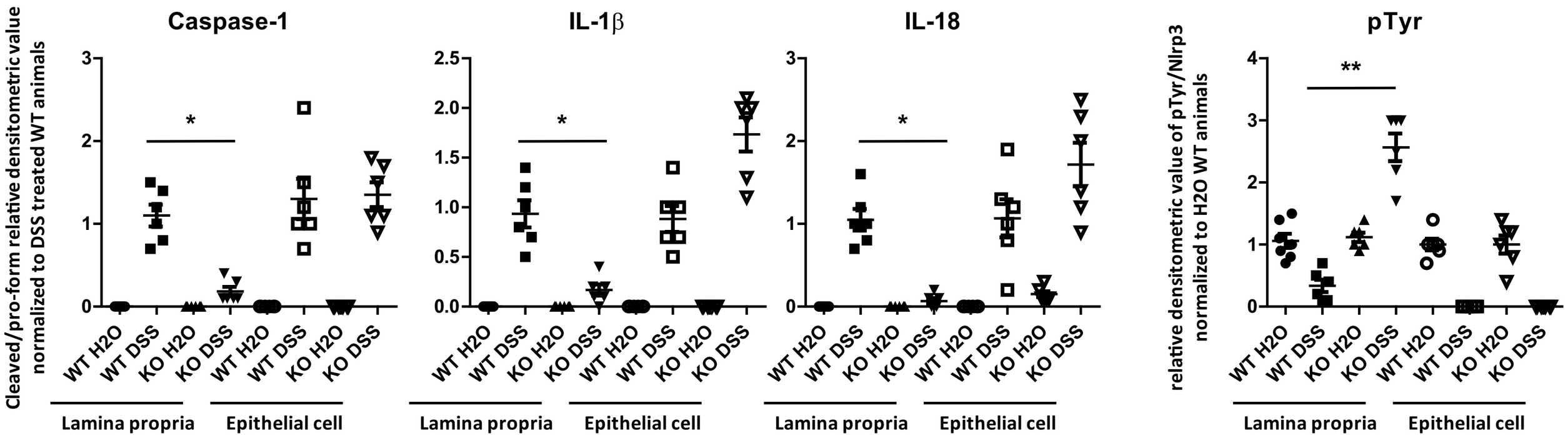








S7D



Supplementary Figure 7. A: Densitometric analysis of the Western blots shown in main figure 10A+B. **B+C**: Colitis was induced in WT and PTPN22-619W littermates by administration of 2.5% DSS for 7 days. **B**: NLRP3 was immune-precipitated from whole colon pieces and analysed for tyrosine phosphorylation and interaction with PTPN22. **C**: Colon pieces were analysed for IL-1β and Caspase-1 processing by Western blot. **D**: Densitometric analysis of the Western blots shown in main figure 10C+E. Asterisks denote significant differences (*=p<0.05, **=p<0.01, Kruskal-Wallis). Each lane represents an individual mouse, except for B, where three mice have been pooled for one lane. Data are representative of three independent experiments. This figure is related to main figure 10.