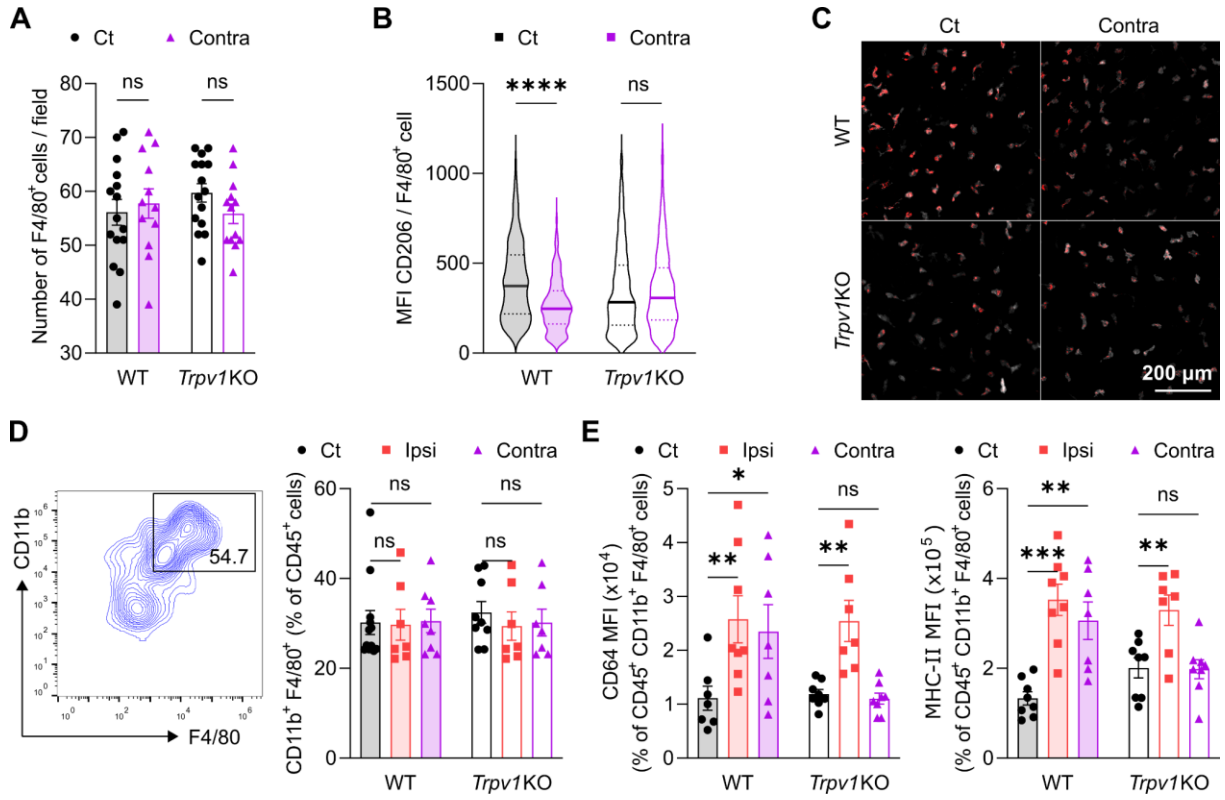
Supplemental Tables

Reagent/Antibody	Product #	Concentration/ dilution/dose	Supplier
Dextran-fluorescein isothiocyanate	FD4-100MG	10 mg/ml in PBS	Sigma-Aldrich (Buenos Aires, Argentina)
Ketamine	Ketonal 50	80 mg/kg	Richmond Vet (Grand Bourg, Argentina)
Xylazine	Xilacina 20	8 mg/kg	Richmond Vet (Grand Bourg, Argentina)
Fosaprepitant	Emend	10 mg/ml	MSD, Argentina
Diclofenac	Dioxaflex	10 mg/kg	Laboratorios Bagó, Argentina
0.4% sodium hyaluronate		5 µl/eye	Dropstar LC, Laboratorio Poen, Argentina
Collagenase	11088793001	0.27 mg/ml	Roche Diagnostics GmbH
DNAse	Pulmozyme	2 U/ml	Roche Diagnostics GmbH
Triton X-100	X100-100ML		Sigma-Aldrich (Buenos Aires, Argentina)
NP-40	I3021-50ML	0.02 % v/v	Sigma-Aldrich (Buenos Aires, Argentina)
Capsaicin	M2028	100 µM in PBS	Sigma-Aldrich (Buenos Aires, Argentina)
Allyl Isothiocyanate	377430-5G	10 mg/ml	Sigma-Aldrich (Buenos Aires, Argentina)
Alexa Fluor® 488 anti-tubulin β3	801203	2.5-3.5 µg/ml	Biolegend (San Diego, CA, USA)
Alexa Fluor® 647 anti-mouse/human CD324 (E-cadherin)	147308	2.5 µg/ml	Biolegend (San Diego, CA, USA)
Alexa Fluor® 594 anti-mouse/human Ki67	151214	2.5 µg/ml	Biolegend (San Diego, CA, USA)
Alexa Fluor® 488 anti-mouse I-A/I-E	107616	2.5 µg/ml	Biolegend (San Diego, CA, USA)
Alexa Fluor® 488 anti-CD206	141710	2.5 µg/ml	Biolegend (San Diego, CA, USA)
PE anti-mouse I-A/I-E	107608	2.5 µg/ml	Biolegend (San Diego, CA, USA)
Alexa Fluor® 647 anti-mouse/human F4/80	123122	2.5 µg/ml	Biolegend (San Diego, CA, USA)

Live/Dead Fixable Dead Cell Stain	L10119	1:500 in PBS	ThermoFisher (Buenos Aires, Argentina)
APC anti- mouse CD45	103112	0.5 µl/100 µl	Biolegend (San Diego, CA, USA)
FITC anti-mouse CD4	100406	0.5 µl/100 µl	Biolegend (San Diego, CA, USA)
PE/Cy7 anti-mouse F4/80	123114	0.5 µl/100 µl	Biolegend (San Diego, CA, USA)
FITC anti-mouse I-A/I-E	107606	0.5 µl/100 µl	Biolegend (San Diego, CA, USA)
Brilliant Violet 605 anti-mouse CD64	139323	0.5 µl/100 µl	Biolegend (San Diego, CA, USA)
Brilliant Violet 421 anti-mouse CD11b	101236	0.5 µl/100 µl	Biolegend (San Diego, CA, USA)
Direct-zol RNA MiniPrep kit	R2052		Zymo Research (Irvine, CA, USA)
TRI Reagent	T3934		Sigma-Aldrich (Buenos Aires, Argentina)

Supplemental Table 1 - List of reagents and antibodies

Supplemental Figures



—---------- Supplemental Figure 1 —----------

Supplemental Figure 1 - Unilateral dry eye impacts corneal and conjunctival macrophages in the contralateral eye of wild-type but not transient receptor potential vanilloid-1-knockout mice. The right extraorbital lacrimal gland was excised in wild-type (WT) or transient receptor potential vanilloid-1-knockout (*Trpv1*KO) mice of both sexes, leading to unilateral dry eye disease (DED). Thus, the right and left eyes are referred to as ipsilateral (Ipsi) and contralateral (Contra), respectively. Sham-operated animals were included as controls (Ct). **A)** Number of stromal macrophages (F4/80$^+$ cells with consistent morphology), **B)** mean fluorescence intensity (MFI) of their CD206 staining, and **C)** representative micrographs (white: F4/80, red: CD206) of corneal wholemounts from WT and *Trpv1*KO Ct and Contra eyes collected 10 days after unilateral DED induction. **D)** Proportion of conjunctival macrophages (CD45+ CD11b+ F4/80+ cells) shown as representative dot plot (left panel) and cumulative data (right), and **E)** CD64 (left panel) and MHC-II (right panel) expression (MFI) as assessed by flow cytometry of conjunctival cell suspensions obtained 10 days after unilateral DED induction from Ct, Ipsi, and Contra eyes of both strains. All experiments were performed twice or more with 4-6 mice/group/experiment. To compare means, two-way ANOVA was used with Sidak's or Dunnett's post hoc test. * indicates $p<0.05$, ** indicates $p<0.01$, *** indicates $p<0.001$, **** indicates $p<0.0001$, and ns indicates not significant.

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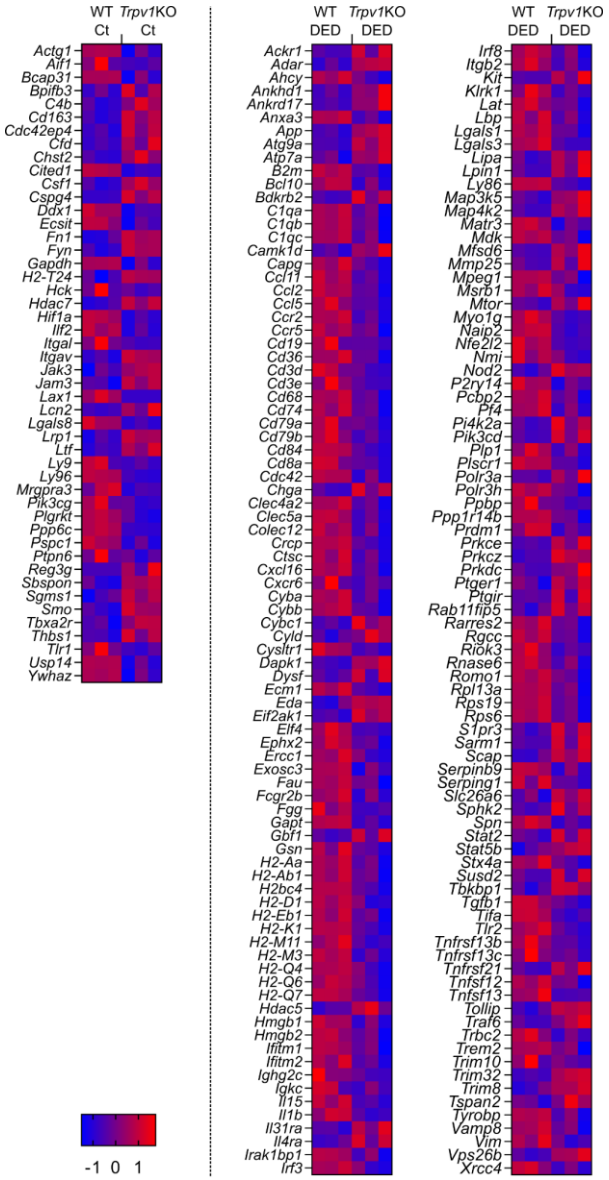

—---------- Supplemental Figure 2 —----------

Supplemental Figure 2 - Neuroinflammatory gene expression changes induced by dry eye disease in the trigeminal ganglion of wild-type vs. transient receptor potential vanilloid-1-knockout mice. Dry eye disease (DED) was surgically by bilateral extraorbital lacrimal gland excision in wild-type (WT) or transient receptor potential vanilloid-1-knockout (*Trpv1*KO) mice for 10 days and then the trigeminal ganglia were harvested for bulk RNA-Seq analysis (female mice, n=3 per group). Sham-operated (Ct) mice were used as controls, and differentially expressed genes (DEGs, fold change > 1.2, adjusted p-value < 0.05) were extracted between same-treatment mice (either Ct or DED) of the two strains. Heatmaps (normalized counts, Z score) show the DEGs annotated in the Gene Ontology database as inflammatory process- (GO:0006954) or immune response-related (GO:0006955) and that were detected either exclusively in WT Ct vs *Trpv1*KO Ct (Ct-specific, left panel) or WT DED vs *Trpv1*KO DED (DED-specific, right panel) analyses.

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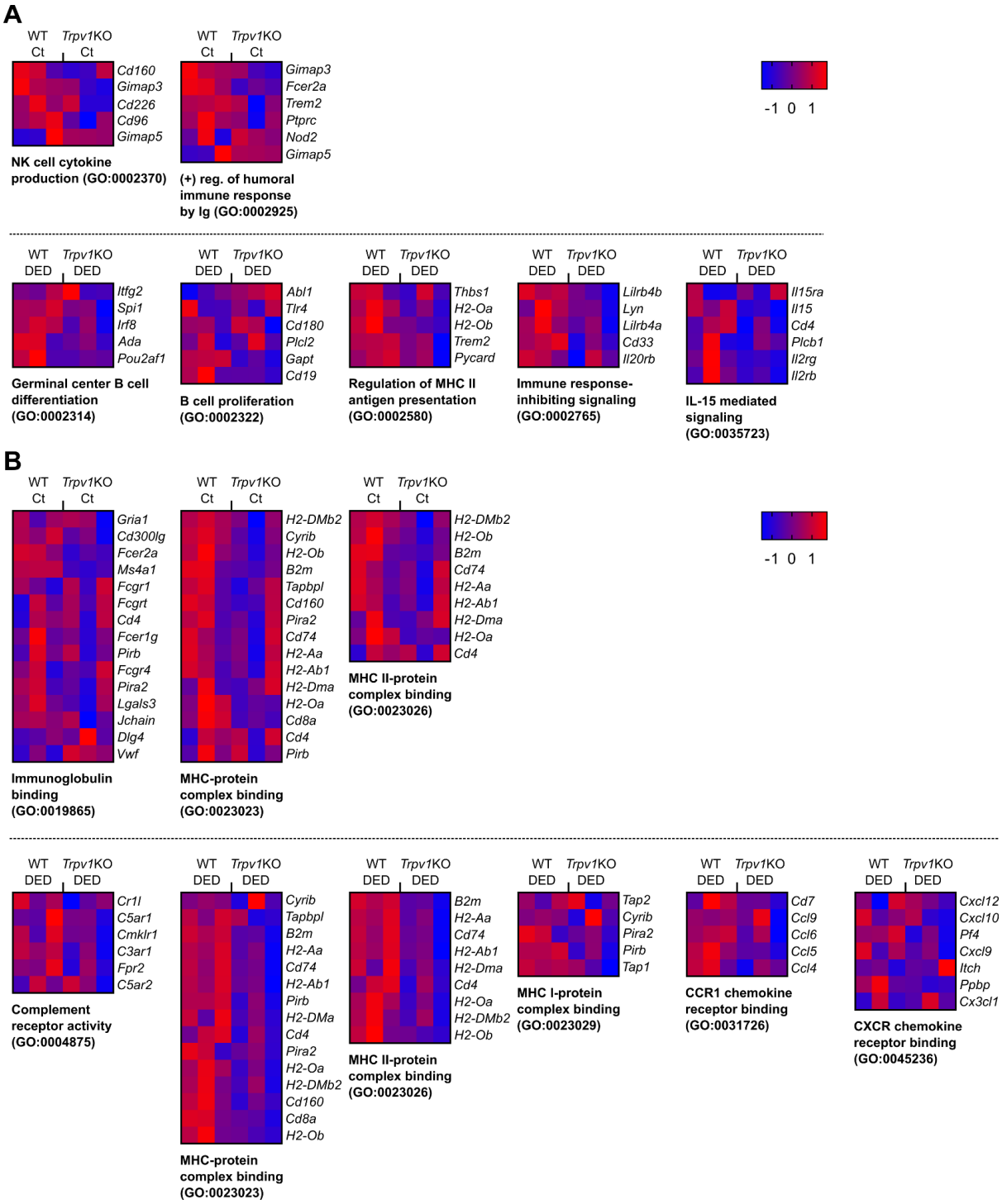
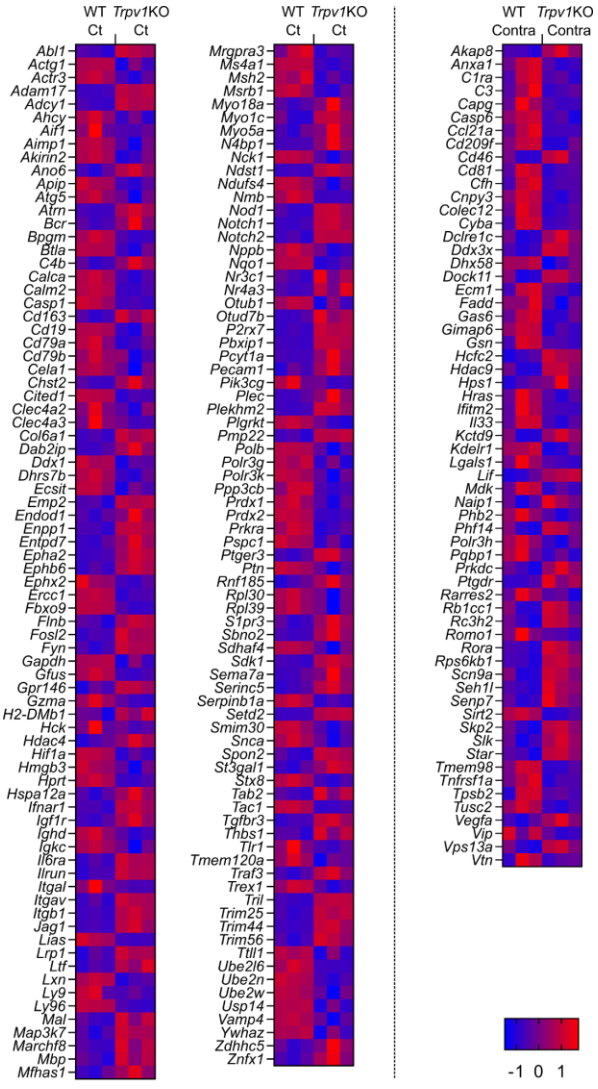


—---------- Supplemental Figure 3 —----------

Supplemental Figure 3 - Neuroinflammatory gene pathways up-regulated by dry eye disease in the trigeminal ganglion of wild-type but not transient receptor potential vanilloid-1-knockout mice. Dry eye disease (DED) was surgically by bilateral extraorbital lacrimal gland excision in wild-type (WT) or transient receptor potential vanilloid-1-knockout (*Trpv1*KO) mice for 10 days and then the trigeminal ganglia were harvested for bulk RNA-Seq analysis (female mice, n=3 per group). Sham-operated (Ct) mice were used as controls. Differentially expressed genes (DEGs, fold change > 1.2, adjusted p-value < 0.05) were extracted between same-treatment mice

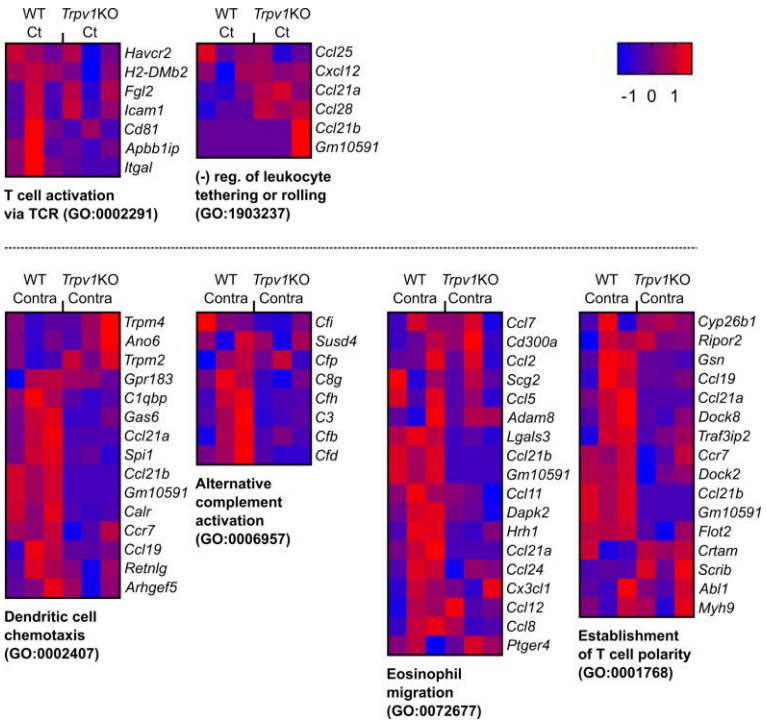
(either Ct or DED) of the two strains, followed by Gene Set Enrichment Analysis (false discovery rate < 0.1). **A)** Biological Process and **B)** Molecular Function pathways related to neuroinflammation and that were among the 30 most significantly up- or downregulated pathways in the WT Ct vs *Trpv1*KO Ct (top panels) or WT DED vs *Trpv1*KO DED (bottom panels) analyses are shown.



—---------- Supplemental Figure 4 —----------

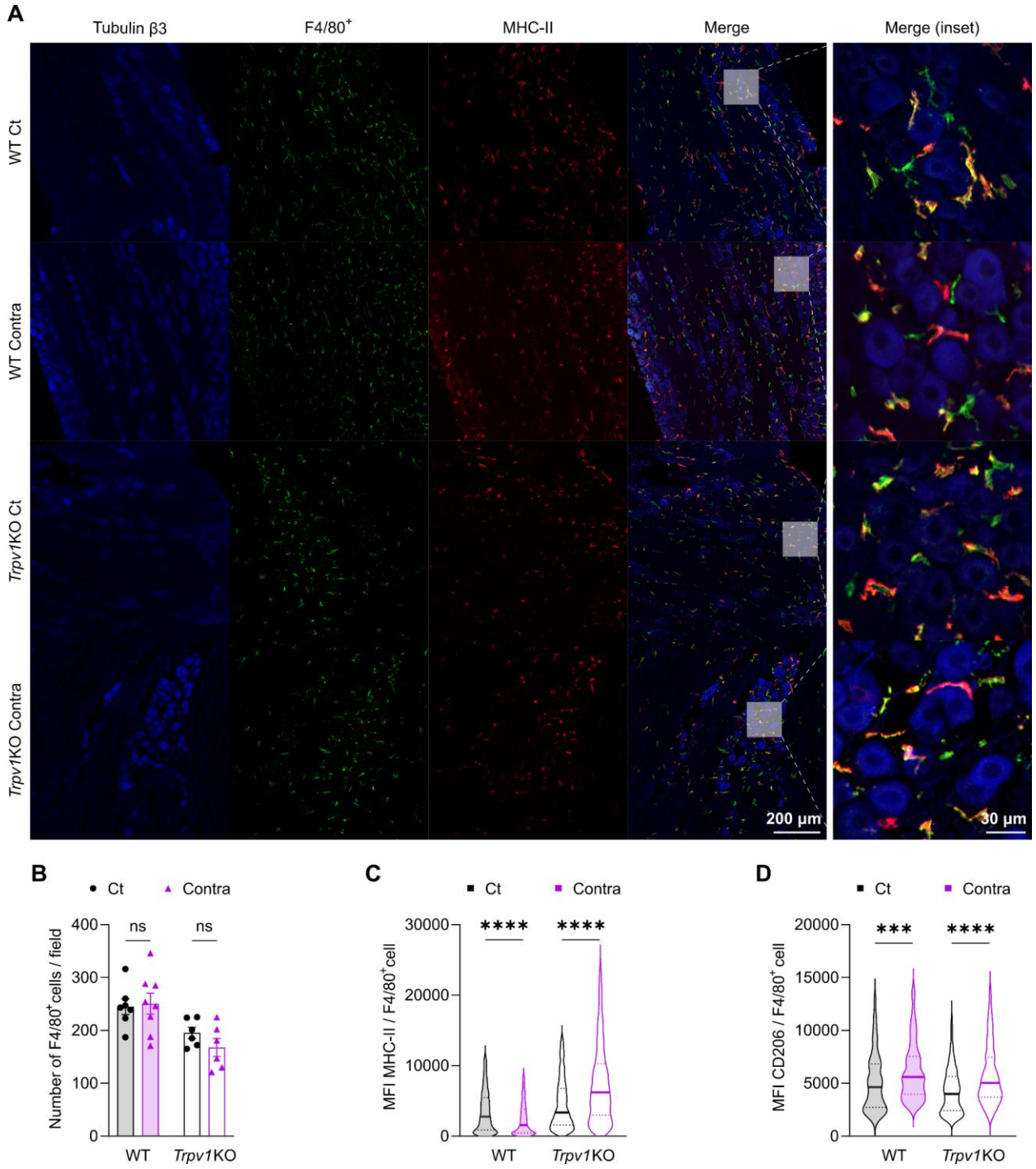
Supplemental Figure 4 - Neuroinflammatory gene expression changes induced by unilateral dry eye disease in the contralateral trigeminal ganglion of wild-type vs. transient receptor potential vanilloid-1-knockout mice. Unilateral dry eye disease (DED) was surgically induced in wild-type (WT) or transient receptor potential vanilloid-1-knockout (*Trpv1*KO) mice of both sexes by excising only the right extraorbital lacrimal gland. After 10 days, the contralateral (Contra) trigeminal ganglia were harvested for bulk RNA-Seq analysis (female mice, n=3 per group). Sham-operated (Ct) mice were used as controls, and differentially expressed genes (DEGs, fold change > 1.2, adjusted p-value < 0.05) were extracted between same-treatment mice

(either Ct or DED) of the two strains. Heatmaps (normalized counts, Z score) show the DEGs annotated in the Gene Ontology database as inflammatory process- (GO:0006954) or immune response-related (GO:0006955) and that were detected either exclusively in WT Ct vs *Trpv1*KO Ct (Ct-specific) or WT Contra vs *Trpv1*KO Contra (DED-specific) analyses.



—---------- Supplemental Figure 5 —----------

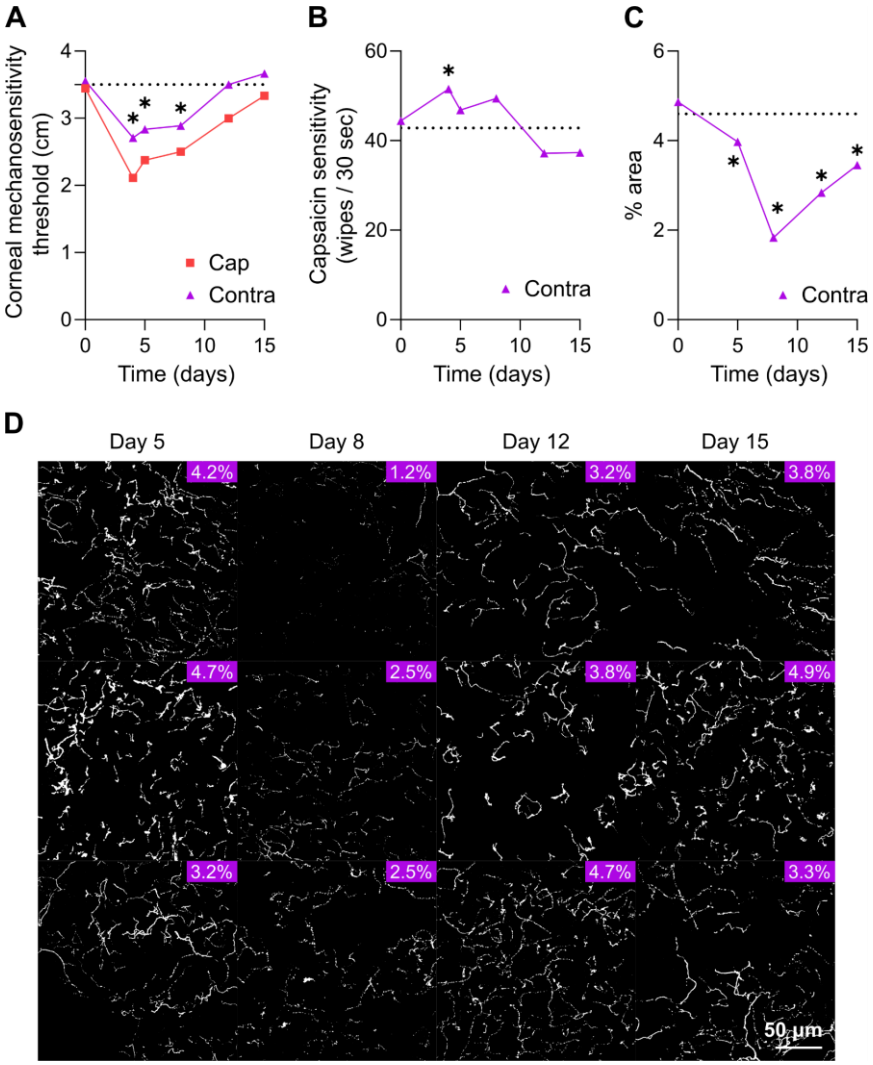
Supplemental Figure 5 - Neuroinflammatory gene pathways up-regulated by unilateral dry eye disease in the contralateral trigeminal ganglion of wild-type but not transient receptor potential vanilloid-1-knockout mice. Unilateral dry eye disease (DED) was surgically induced in wild-type (WT) or transient receptor potential vanilloid-1-knockout (*Trpv1*KO) mice of both sexes by excising only the right extraorbital lacrimal gland. After 10 days, the contralateral (Contra) trigeminal ganglia were harvested for bulk RNA-Seq analysis (female mice, n=3 per group). Sham-operated (Ct) mice were used as controls. Differentially expressed genes (DEGs, fold change > 1.2, adjusted p-value < 0.05) were extracted between same-treatment mice (either Ct or DED) of the two strains, followed by Gene Set Enrichment Analysis (false discovery rate < 0.1). Biological Process pathways related to neuroinflammation and that were among the 30 most significantly up- or downregulated pathways in the WT Ct vs *Trpv1*KO Ct (top panel) or WT Contra vs *Trpv1*KO Contra (bottom panel) are shown.



—---------- Supplemental Figure 6 —----------

Supplemental Figure 6 - Macrophage reactive changes in the contralateral trigeminal ganglion of wild-type and transient receptor potential vanilloid-1-knockout mice. Unilateral dry eye disease was surgically induced in wild-type (WT) or transient receptor potential vanilloid-1-knockout (*Trpv1*KO) mice of both sexes by excising only the right extraorbital lacrimal gland. After 10 days, the contralateral (Contra) trigeminal ganglia were harvested for bulk RNA-Seq analysis (female mice, n=3 per group). Sham-operated (Ct) mice were used as controls. Trigeminal ganglion cryosections were stained for tubulin β3 (nerve-specific), F4/80 (macrophage marker),

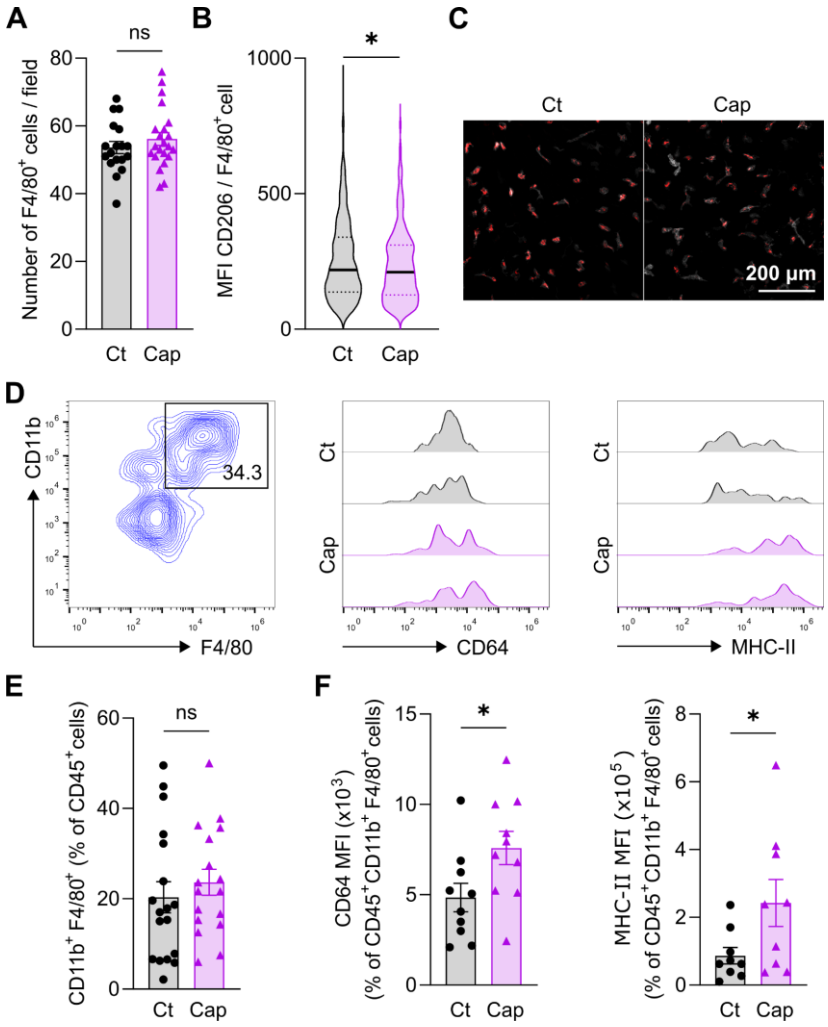
MHC-II, and CD206 expression. **A)** Representative micrographs, **B)** number of macrophage (F4/80$^+$ cells), and mean levels of **C)** MHC-II and **D)** CD206 expression plotted individually per cell. The experiment was performed twice with 3 mice/group/experiment. To compare means, two-way ANOVA with Dunnett's post hoc test was used. *** indicates p<0.001, **** indicates p<0.0001, and ns indicates not significant.



Supplemental Figure 7

Supplemental Figure 7 - Longitudinal assessment of contralateral effects induced by unilateral corneal topical treatment with capsaicin. Unilateral corneal topical treatment with capsaicin (Cap) was performed in wild-type mice by applying a 2 mm filter paper disk soaked in 0.5 mg/ml capsaicin onto the right cornea for 5 min under anesthesia (days 0 and 2). Mice were assessed on days 5, 8, 12, and 15 post treatment. **A)** Corneal mechanosensitivity thresholds as measured separately on the right (Cap) and left (Contra) sides. **B)** Ocular capsaicin sensitivity. **C)** Density of intraepithelial (subapical) corneal innervation and **D)** representative micrographs (3 per time point). The dotted lines indicate baseline values from other experiments. The experiment was

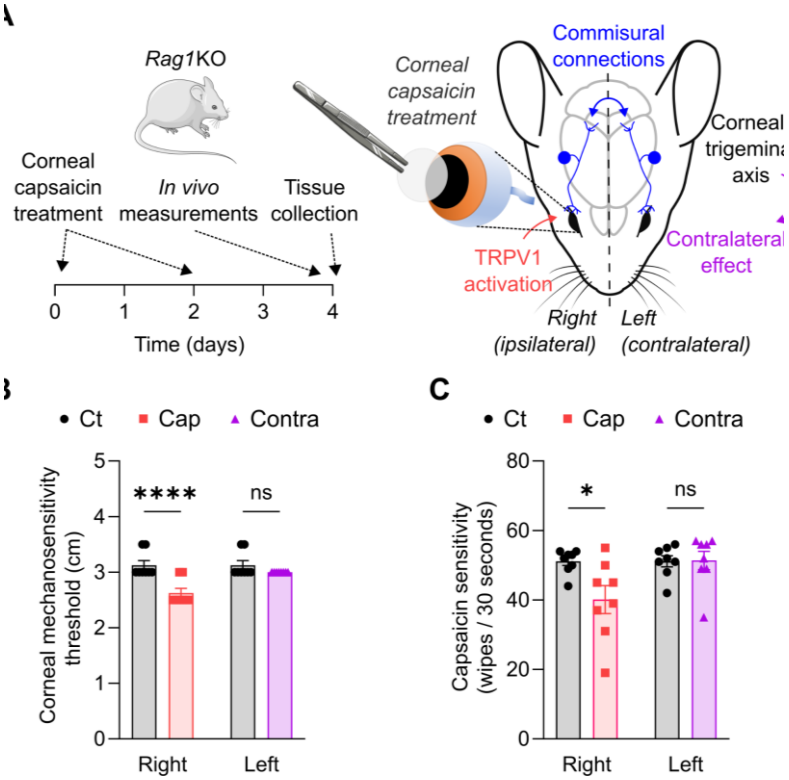
performed once with 4-6 mice/time point. To compare means, one or two-way ANOVA was used with Dunnett's post hoc test. * indicates p<0.05.



Supplemental Figure 8

Supplemental Figure 8 - Unilateral corneal topical treatment with capsaicin impacts corneal and conjunctival macrophages in the contralateral eye. Unilateral corneal topical treatment with capsaicin (Cap) was performed in wild-type mice by applying a 2 mm filter paper disk soaked in 0.5 mg/ml capsaicin or vehicle (Ct) onto the right cornea for 5 min under anesthesia (days 0 and 2). **A)** Number of stromal macrophages ($F4/80^+$ cells with consistent morphology), **B)** mean fluorescence intensity (MFI) of their CD206 staining, and **C)** representative micrographs (white: F4/80, red: CD206) of corneal wholemounts from contralateral eyes collected 4 days after unilateral capsaicin treatment. **D)** Representative dot plot (left panel, gated on live CD45+ cells) and CD64 (middle panel) and MHC-II (right panel) expression histograms of conjunctival macrophages (CD45+ CD11b+ F4/80+ cells) as assessed by flow cytometry of cell suspensions obtained from the contralateral eyes 4 days after unilateral capsaicin treatment, with the corresponding cumulative data for the E) proportion and F) CD64 (left) and MHC-II (right panel) expression (mean

fluorescence intensity, MFI). All experiments were performed twice or more with 6 mice/group/experiment. Student's t test was used to compare means. * indicates $p<0.05$ and ns indicates not significant.



Supplemental Figure 9

Supplemental Figure 9 - Capsaicin-induced corneal nerve dysfunction in the contralateral eye requires an adaptive immune response. A) Unilateral corneal topical treatment with capsaicin (Cap) was performed in recombination-activating gene 1-knockout (*Rag1*KO) mice by applying a 2 mm filter paper disk soaked in 0.5 mg/ml capsaicin or vehicle (Ct) onto the right cornea for 5 min under anesthesia (days 0 and 2). **B)** Corneal mechanosensitivity thresholds and **C)** ocular capsaicin sensitivity as measured separately on the right and left sides of Ct and Cap-treated *Rag1*KO mice. All experiments were performed twice or more with 6 mice/group/experiment. To compare means, two-way ANOVA was used with Dunnett's post hoc test. * indicates $p<0.05$, **** indicates $p<0.0001$, and ns indicates not significant.