Supplementary Information for

Pannexin 1 involvement in bladder dysfunction in a multiple sclerosis model

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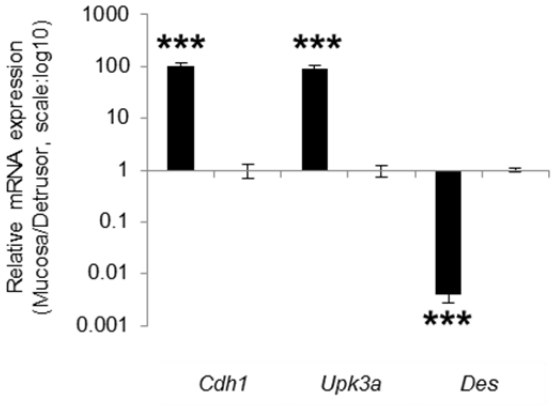
Supplementary Information includes:

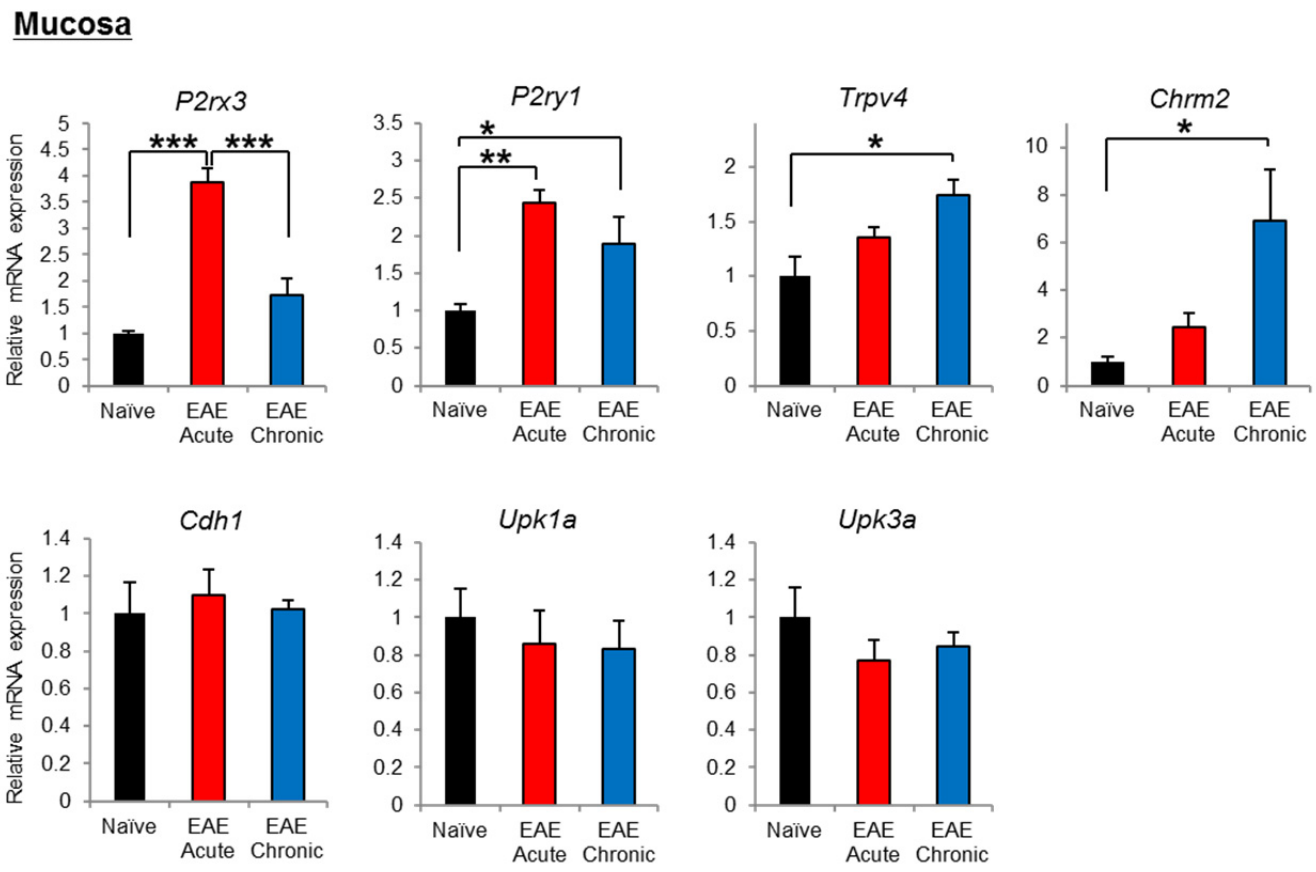
Supplementary Figures S1 to S10
Supplementary Table S1

Supplementary Figure S1



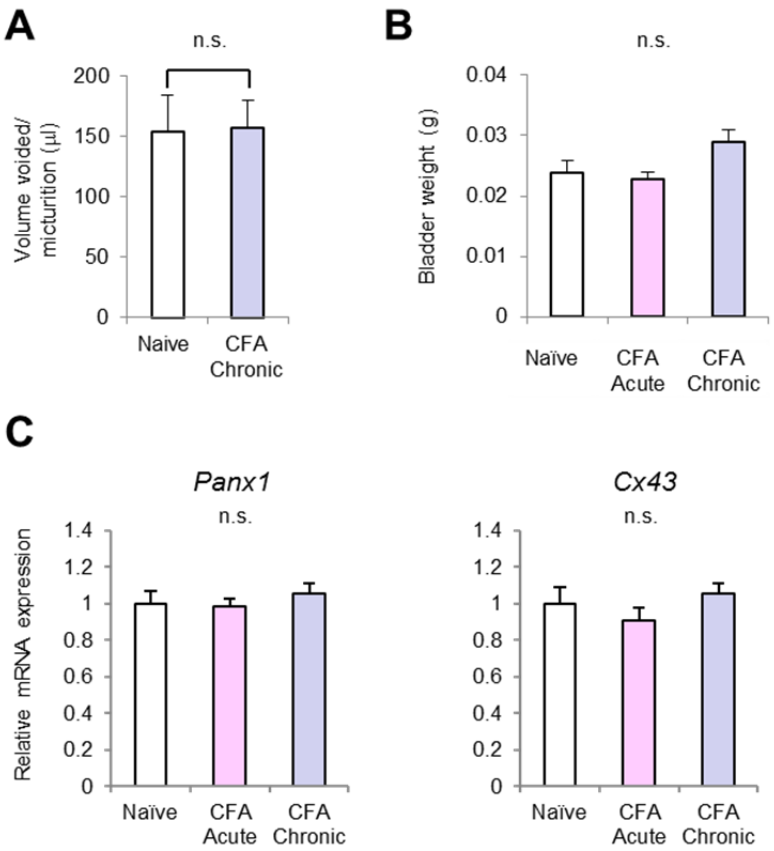
Supplementary Figure S1 | Validation of proper separation of bladder mucosa from detrusor muscle. Real time RT-PCR showing mRNA of *Cdh1* and *Upk3a* detected in bladder mucosa and *Des* in bladder detrusor (*** $P < 0.0001$ by paired Student *t*-test, n=5). Error bars indicate SEM. For the relative expression, the values of detrusor were set as 1.

Supplementary Figure S2



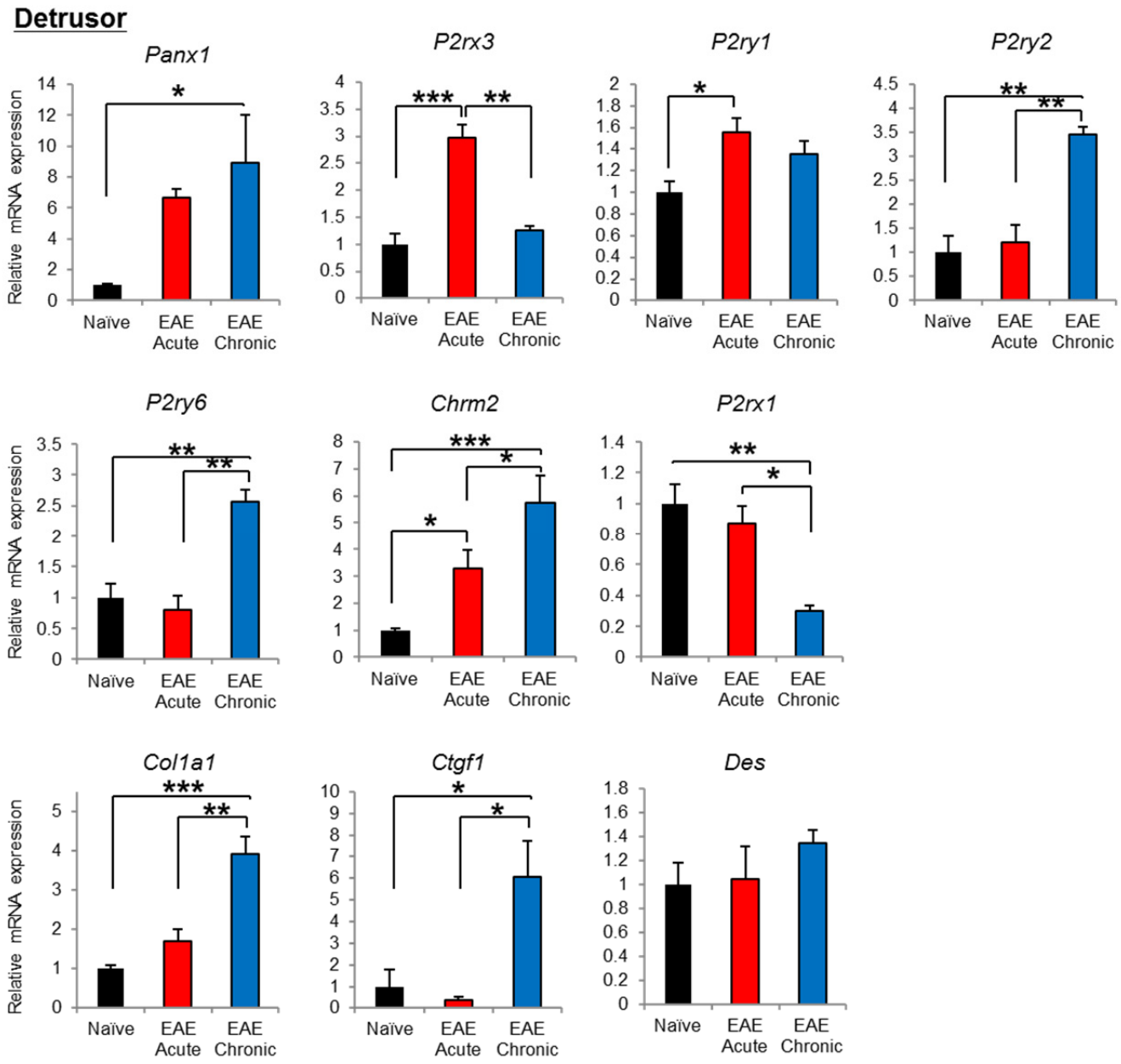
Supplementary Figure S2 | mRNA expression levels in bladder mucosa of naive mice, and EAE mice at the acute and chronic phases of the disease. **P* < 0.05 and ***P* < 0.01 and ***P* < 0.001 by one-way ANOVA followed by Tukey's *post-hoc* test (n=5, 3 and 3, respectively). All error bars represent SEM. For the relative expression, the values of naïve mice were set as 1. Note that certain genes associated with the bladder mechanosensory, transduction and signaling systems, such as the purinergic receptors *P2xr3 and P2yr1*, *Trpv4* (*transient receptor potential cation channel, subfamily V, member 4*) and *Chrm2* (*cholinergic receptor, muscarinic type 2*) were significantly upregulated, while urothelial marker genes were unchanged as *Cdh1, Upk1a* and *Upk3a*. *P2rx1*, *P2rx2*, *P2rx4*, *P2ry2*, *P2ry6* and *Chrm3* were not significantly changed (data not shown).

Supplementary Figure S3



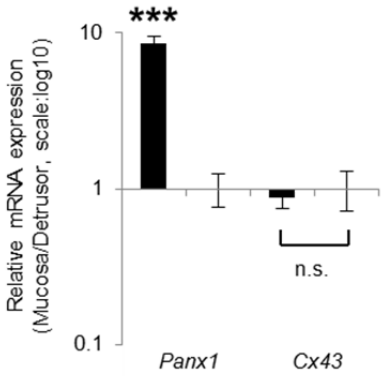
Supplementary Figure S3 | Additional control experiments using CFA-only treated mice. (**A**) Average UVVM was not significantly different in naïve mice and in mice treated only with CFA when compared at 35 days post CFA injection, a time point which would correspond to the chronic phase of EAE (n=4 and 3, unpaired *t*-test). (**B, C**) Bladder weight in **B** and mRNA expression levels of *Panx1* and *Cx43* in bladder mucosa in **C** were not significantly different in naïve mice and CFA-only treated mice (n=4, 4 and 3, one-way ANOVA). All error bars indicate SEM.

Supplementary Figure S4



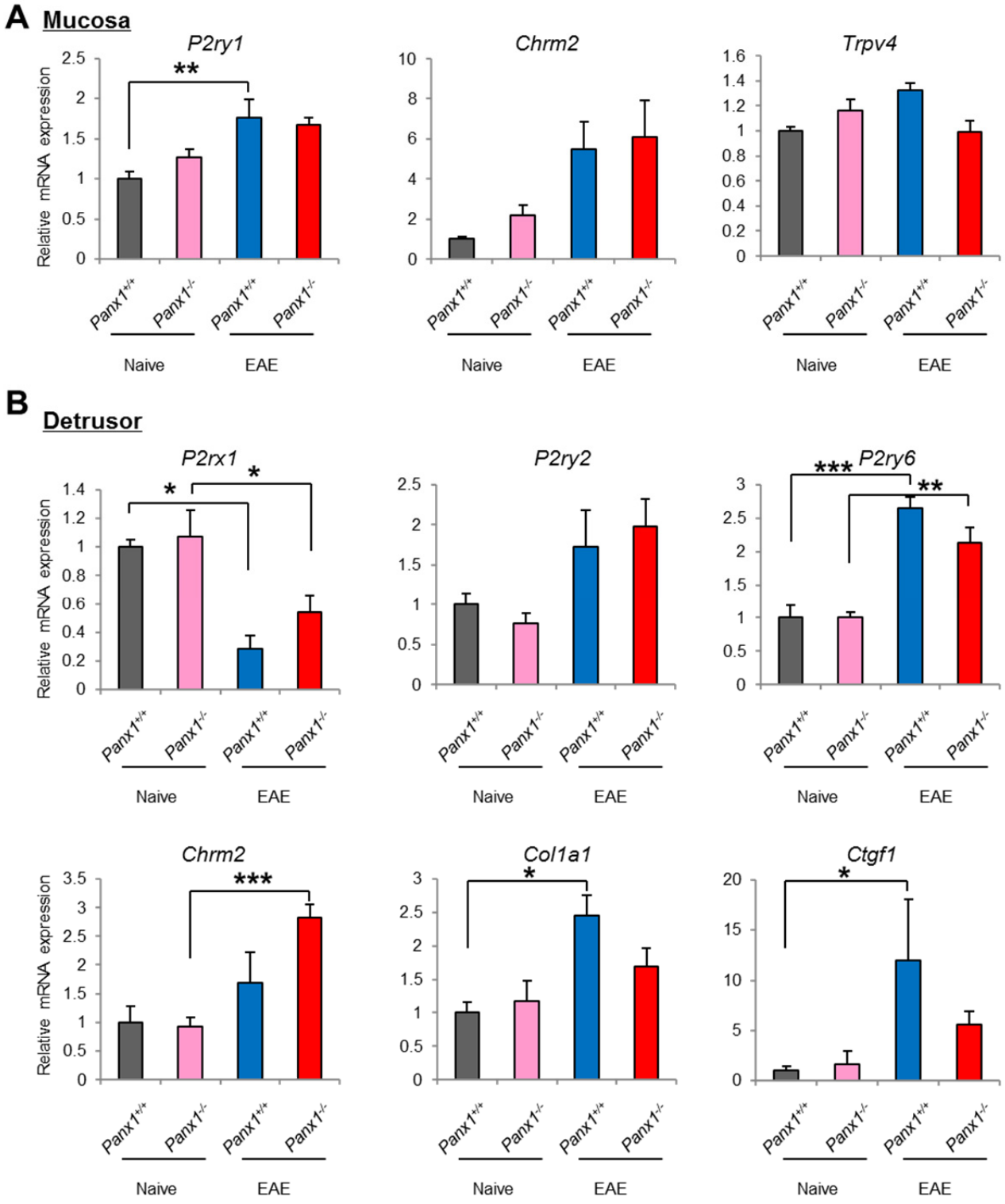
Supplementary Figure S4 | mRNA expression levels in the bladder detrusor of naïve mice, and EAE mice at the acute and chronic phases of the disease. **P* < 0.05 and ***P* < 0.01 and ***P* < 0.001 by one-way ANOVA followed by Tukey's *post-hoc* test (n=5, 3 and 3, respectively). All error bars represent SEM. For the relative expression, the values of naïve were set as 1. Note that some of genes associated with the bladder mechanosensory, transduction and signaling systems, such *Panx1*, *P2rx3*, *P2ry1*, *P2ry2*, *P2ry6* and *Chrm2* were upregulated but *P2rx1*, a major purinergic receptor in detrusor, was down-regulated. Genes associated with fibrosis were upregulated as *Col1a1* and *Ctgf1*. *Des* (*Desmin*), a muscle marker, was relatively unchanged. *Cx43*, *P2rx2*, *P2rx4*, *Trpv4* and *Chrm3* were not significantly changed (data not shown).

Supplementary Figure S5



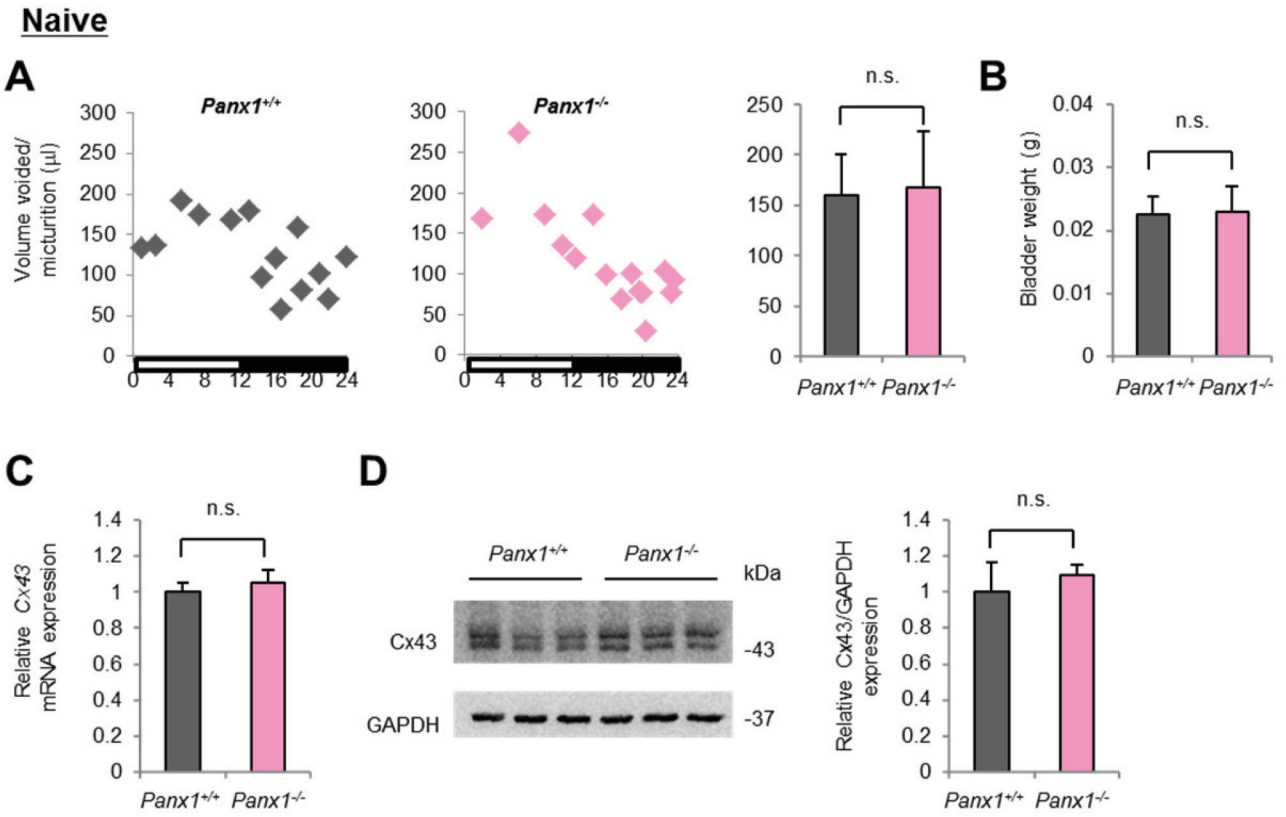
Supplementary Figure S5 | Differences in *Panx1* and *Cx43* mRNA expression levels between the bladder mucosa and detrusor. ***P < 0.0001 by paired *t*-test (n=5). All error bars represent SEM. For the relative expression, the values of detrusor were set as 1.

Supplementary Figure S6



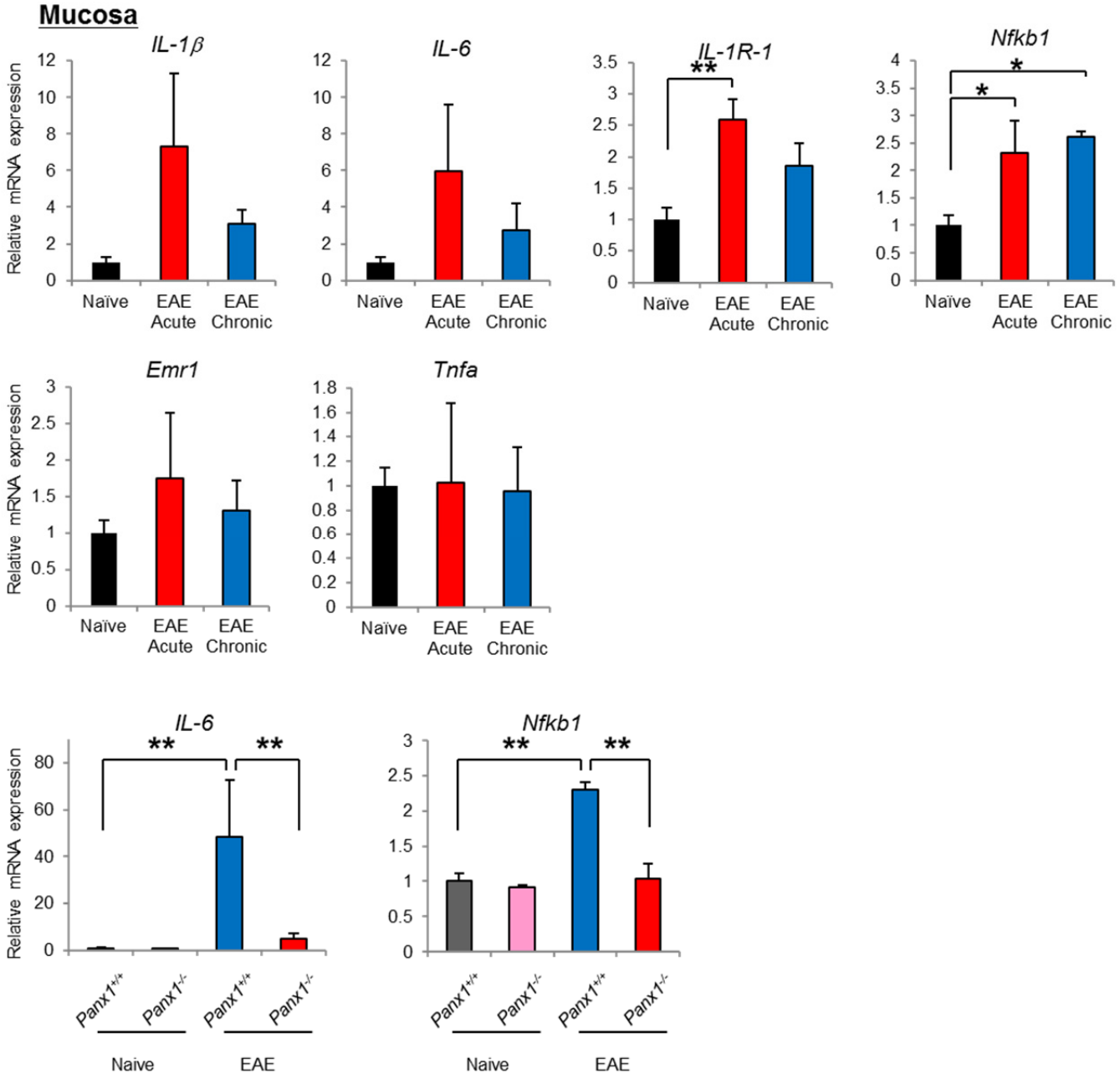
Supplementary Figure S6 | mRNA levels for some of the genes associated with the bladder mechanosensory, transduction and signaling systems expressed in the bladder mucosa (A) and detrusor (B) of *Panx1*$^{+/+}$ and *Panx1*$^{-/-}$ naïve and EAE mice. Upregulation of *P2y1* and *Chrm2* in mucosa, and *P2ry2* and *P2ry6* in detrusor observed in *Panx1*$^{+/+}$ EAE mice were also detected in *Panx1*$^{-/-}$ EAE mice. Upregulation of *Trpv4* in mucosa, and genes associated with fibrosis as *Col1a1* and *Ctgf1* in detrusor observed in *Panx1*$^{+/+}$ EAE mice was not or less detected in *Panx1*$^{-/-}$ EAE mice, although they are not significantly different. *P < 0.05 and **P < 0.01 and **P < 0.001 by one-way ANOVA followed by Bonferroni's *post-hoc* test (n=5, 4, 3 and 8, respectively). All error bars indicate SEM.

Supplementary Figure S7



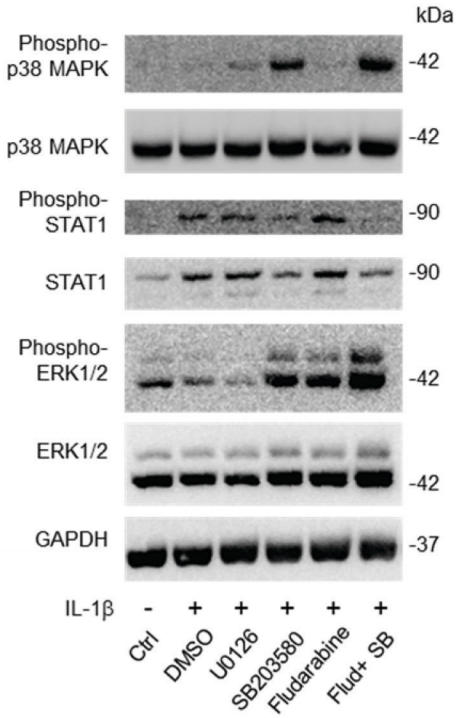
Supplementary Figure S7 | *Panx1*$^{+/+}$ and *Panx1*$^{-/-}$ naïve mice display similar phenotypes in terms of micturition and Cx43 expression in the bladder mucosa. (**A**) Representative charts of urine volume voided per micturition (UVVM) from *Panx1*$^{+/+}$ (left panel) and *Panx1*$^{-/-}$ naïve mice (middle panel), and corresponding average UVVM (n=3 each genotype). No significant difference (n.s.) by unpaired *t*-test. (**B**) Bladder weight was not significantly different by unpaired *t*-test (n=3). (**C, D**) Expression levels of *Cx43* mRNA in **C** and its protein in **D** in the bladder mucosa of *Panx1*$^{+/+}$ and *Panx1*$^{-/-}$ naïve mice were not significantly different by unpaired *t*-test (n=3). All error bars indicate SEM. Full-length blots are presented in Supplementary Fig. S17.

Supplementary Figure S8



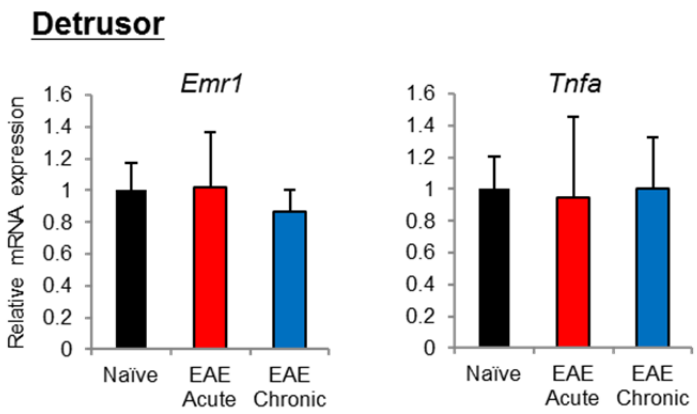
Supplementary Figure S8 | mRNA expression levels of genes associated with pro-inflammatory mediators in the bladder mucosa of naïve mice, and EAE mice at acute and chronic phases of the disease. *P < 0.05 and **P < 0.01 by one-way ANOVA followed by Tukey's *post-hoc* test in naïve, EAE acute and chronic phases (n=5, 3 and 3, respectively). **P < 0.01 by one-way ANOVA followed by Bonferroni's *post-hoc* test comparing *Panx1*$^{+/+}$ and *Panx1*$^{-/-}$ naïve and EAE mice at chronic phase (n=5, 4, 3 and 8, respectively). All error bars indicate SEM.

Supplementary Figure S9



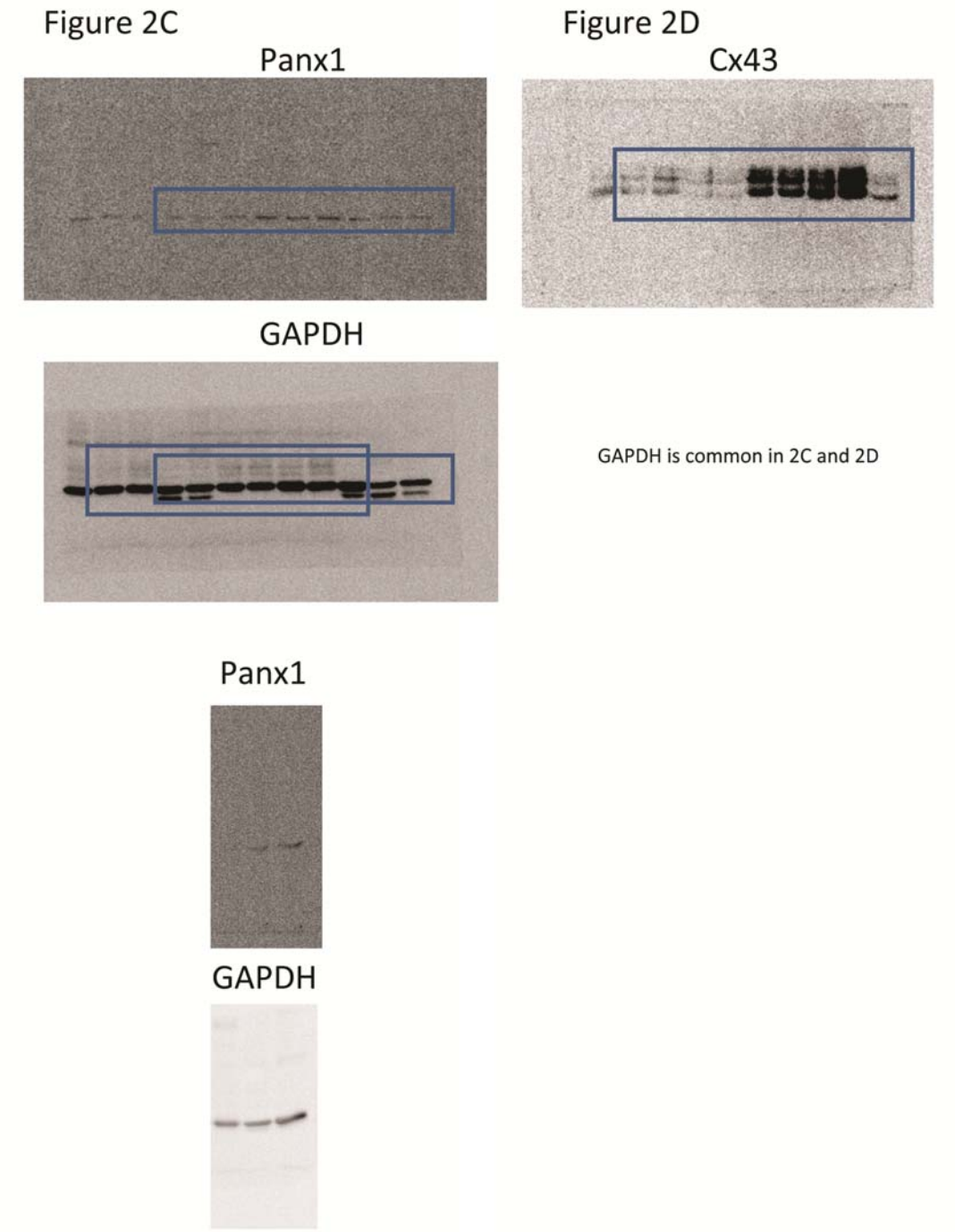
Supplementary Figure S9 | Effects of U0126, SB20358 and Fludarabine on expression and phosphorylation of target proteins in TRT-HU1 cells. U0126 inhibited phosphorylation of ERK1/2. SB203580 inhibits p38 catalytic activity, therefore did not inhibit p38 phosphorylation. Fludarabine increased STAT1 phosphorylation. Full-length blots are presented in Supplementary Fig. S18.

Supplementary Figure S10



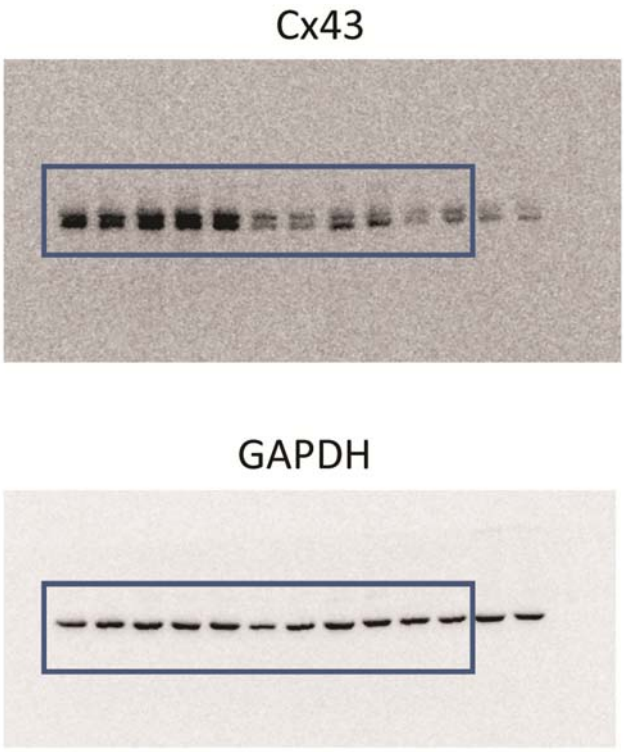
Supplementary Figure S9 | mRNA expression levels of *Emr1* and *Tnfa* in the bladder detrusor of naïve mice, and EAE mice at acute and chronic phases of the disease. No significant differences in naïve, EAE acute and chronic phases by one-way ANOVA (n=5, 3 and 3, respectively). All error bars indicate SEM.

Supplementary Figure S11



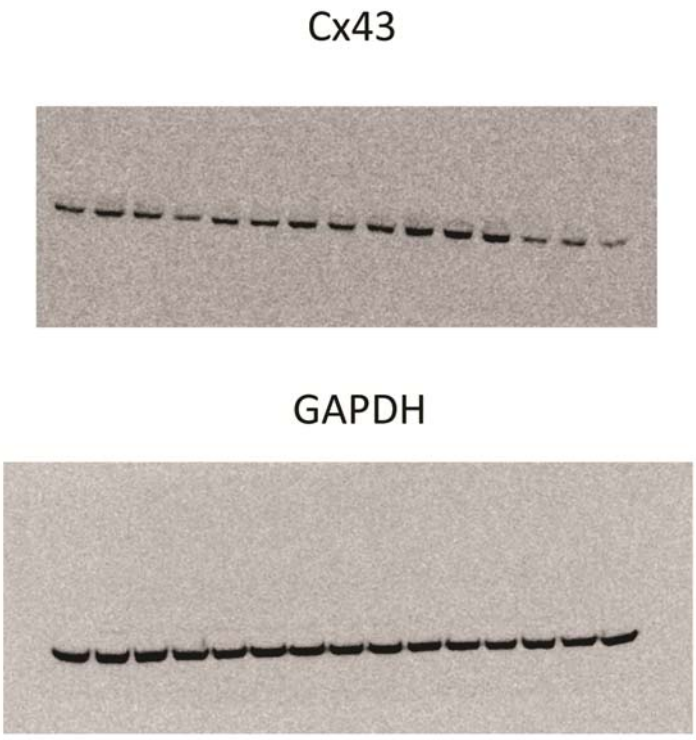
Supplementary Figure S11 | Full-length unedited blots for Figures 2C and 2D.

Supplementary Figure S12



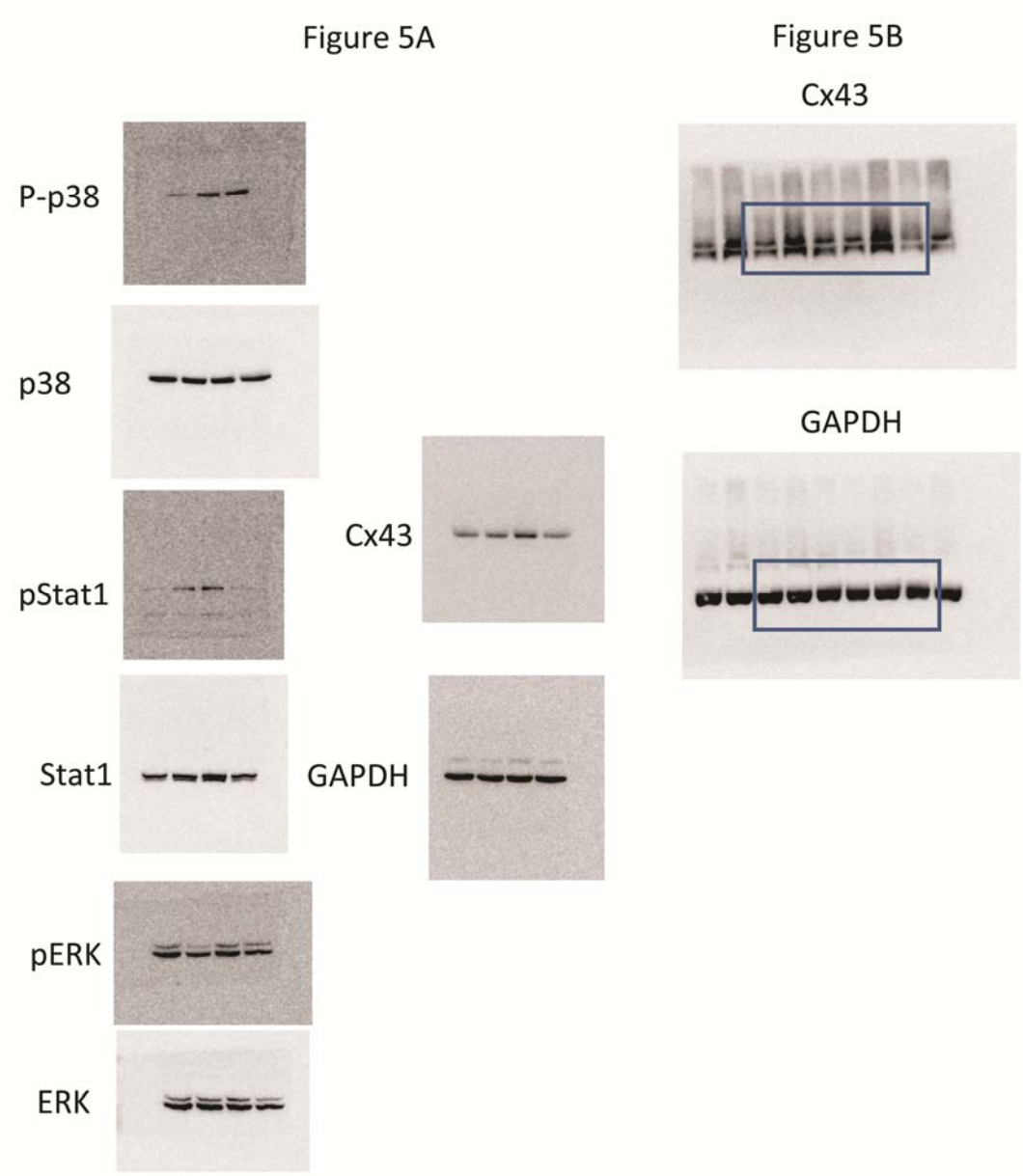
Supplementary Figure S12 | Full-length unedited blots for Figure 3E.

Supplementary Figure S13



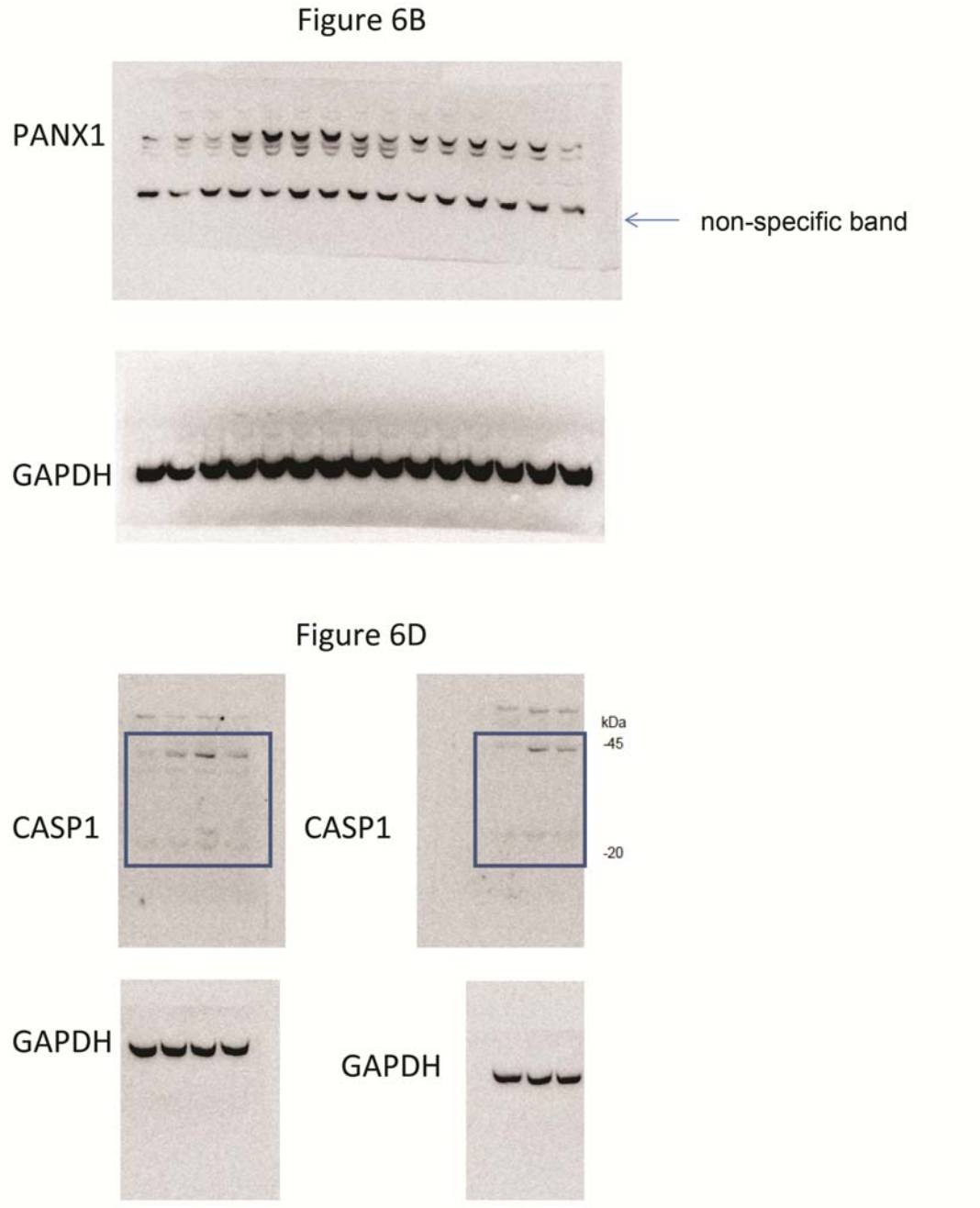
Supplementary Figure S13 | Full-length unedited blots for Figure 4C.

Supplementary Figure S14



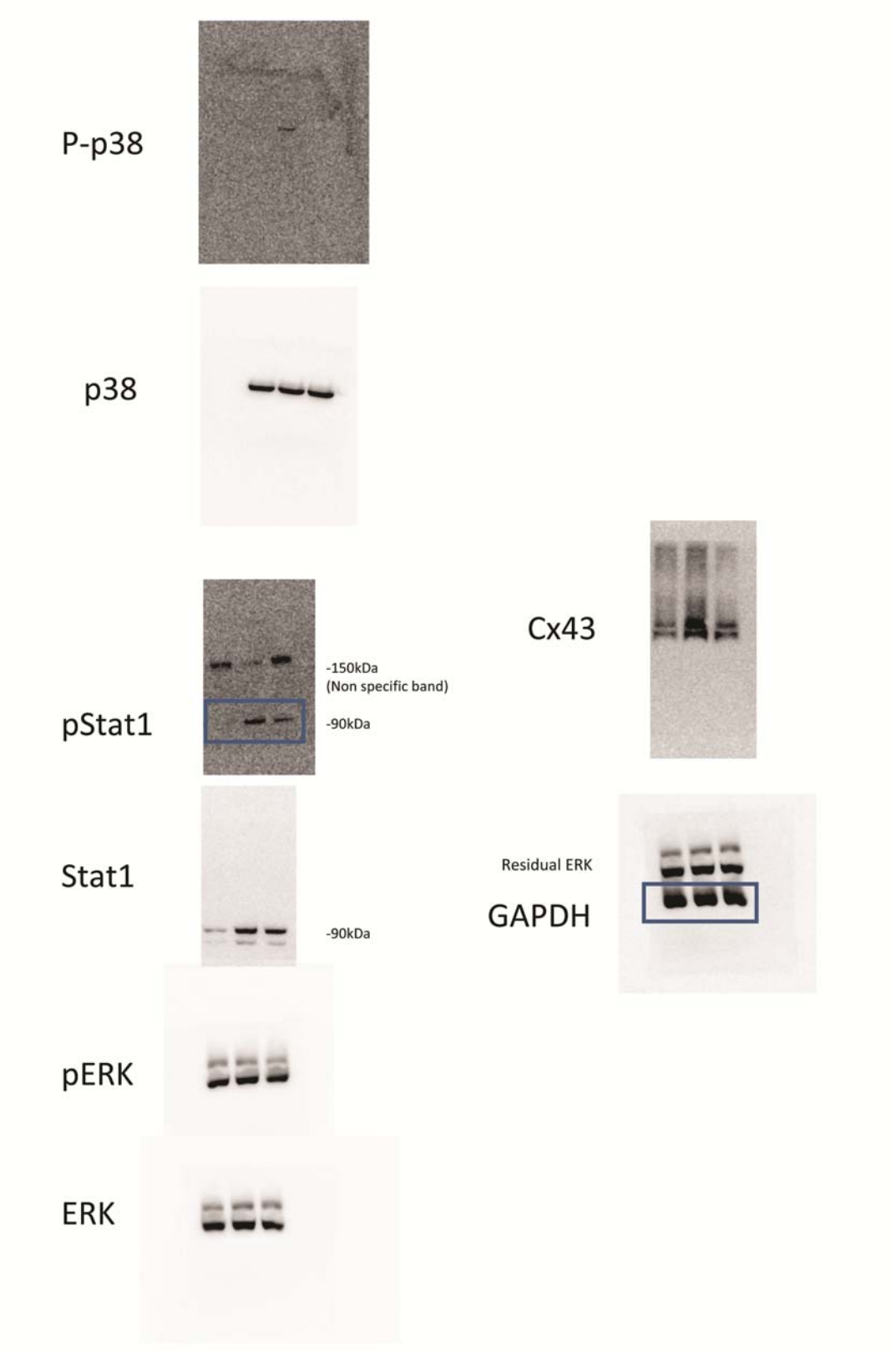
Supplementary Figure S14 | Full-length unedited blots for Figures 5A and 5B.

Supplementary Figure S15



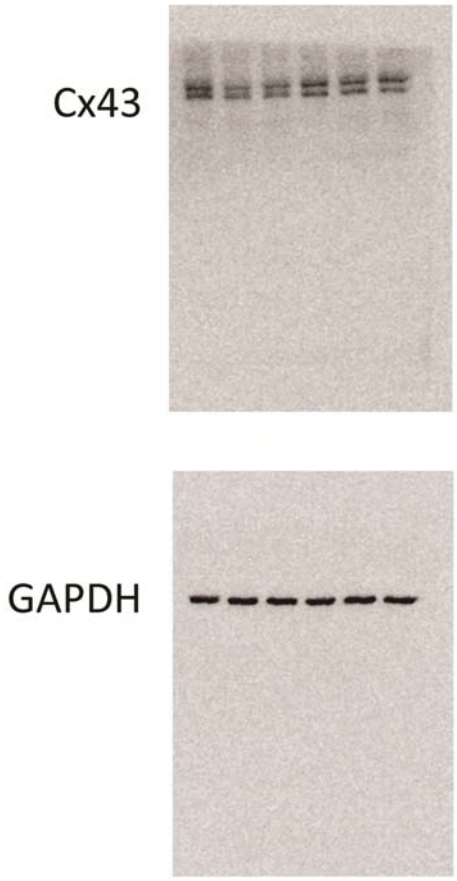
Supplementary Figure S15 | Full-length unedited blots for Figures 6B and 6D.

Supplementary Figure S16



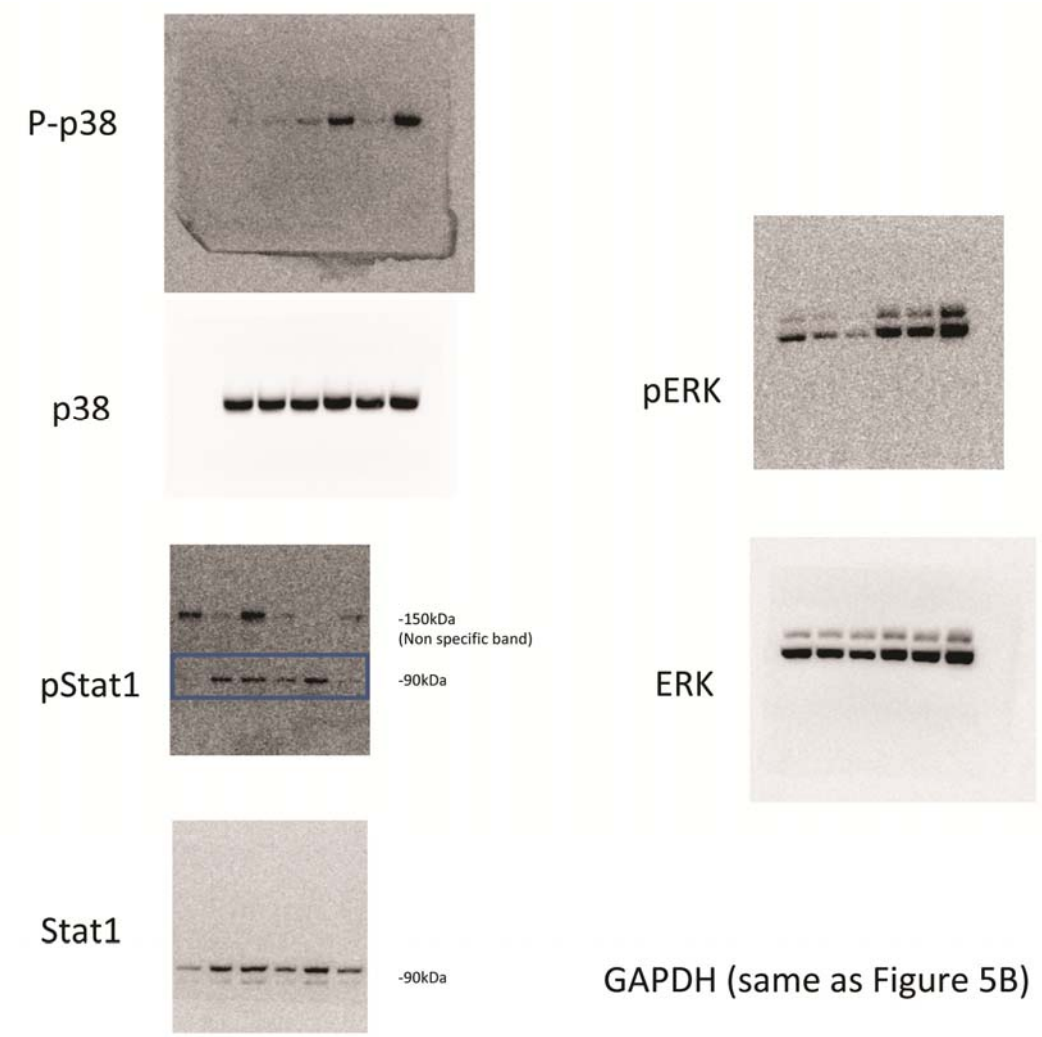
Supplementary Figure S16 | Full-length unedited blots for Figure 7B.

Supplementary Figure S17



Supplementary Figure S17 | Full-length unedited blots for Supplementary Figure S7 D.

Supplementary Figure S18



Supplementary Figure S17 | Full-length unedited blots for Supplementary Figure S9.

Supplementary Table S1

Primers for real-time RT-PCR

Species	Gene name	Accession	Forward (5'→3')	Reverse (5'→3')	Amplicon size (b.p.)
Mouse	*Panx1*	NM_019482	AGCCAGAGAGTGGAGTTCAAAGA	CATTAGCAGGACGGATTCAGAA	104
	Cx43	NM_010288	CCATCCAAAGACTGCGGAT	GTAATTGCGGCAGGAGGAA	138
	P2rx1	NM_008771	AGCCCAAGGTATTCGCACA	ACAGGACACCAGCCAAAGATC	81
	P2rx2	NM_001164833	AAAACAAGCTCTACAGCCATAAGAAGT	TCAAAGTTGGGCCAAACCTT	140
	P2rx3	NM_145526	GCCCAACAGTGAGCTTTCTC	ATTTCCCTGGGCTCTCCTAA	58
	P2rx4	NM_011026	CACAACGTGTCTCCTGGCTA	GCCTTTCCAAACACGATGAT	125
	P2ry1	NM_008772	AGGAAAGCTTCCAGGAGGAG	CGTGTCTCCATTCTGCTTGA	95
	P2ry2	NM_008773	CGTGCTCTACTTCGTCACCA	AAAAGCAGACCCAGCATGAC	102
	P2ry6	NM_183168	CATTAGCTTCCAGCGCTACC	CTCCACACACTACCCAAGCA	91
	Trpv4	NM_022017	CACCCCAGTGACAACAAGAGA	CGATTGAAGACTTTGAGGATGG	107
	Chrm2	NM_203491	CATTGCGGCTTTCTATCTGC	TCTGGATCTTGTTGTGCTCCA	212
	Chrm3	NM_033269	ACACCTTCTGTGACAGCTGCA	TCGCTTGTGAAAAATGACGG	212
	Upk1a	NM_026815	CCTCCTGCATCACATCCTACA	CCACAACACTCTTGCTCAATCA	157
	Upk3a	NM_023478	CATCATCCTCAGCTTTGTGGA	GAGAACACCTCTGCTCTGTCTAGG	159
	Cdh1	NM_009864	GAGGAGAACGGTGGTCAAAGA	AGCTGGCTCAAATCAAAGTCC	120
	Col1a1	NM_007742	GCAACAGTCGCTTCACCTACA	CAATGTCCAAGGGAGCCACAT	137
	Ctgf1	NM_010217	AGAACTGTGTACGGAGCGTGAC	CACCATCTTTGGCAGTGCA	107
	Des	NM_010043	AAGGCCAAACTACAGGAGGAAA	TACGAGCTAGAGTGGCTGCATC	97
	IL-1β	NM_008361	CAGGCAGGCAGTATCACTCA	TGTCCTCATCCTGGAAGGTC	90
	IL-6	NM_031168	CCGGAGAGGAGACTTCACAG	TCCACGATTTCCCAGAGAAC	102
	Tnfa	NM_013693	ACGGCATGGATCTCAAAGAC	GTGGGTGAGGAGCACGTAGT	116
	Nfkb1	NM_008689	GGAAAAAACATCCACCTGCAC	TGCAAAGCCAACCACCAT	105
	Emr1	NM_010130	TCAGCTCTCGCAACATCAAGA	ACCAGCAGCGATTATGCATC	123
	IL-1R-1	NM_008362	TACCCGAGGTCCAGTGGTATAAG	AGCCACATTCCTCACCAACAG	98
Human	*Cx43*	NM_000165	AGCAAAAGAGTGGTGCCCA	TTGTCAAGGAGTTTGCCTAAGG	63
	IL-1β	NM_000576	TTATGTGCACGATGCACCTG	AGGACATGGAGAACACCACTTG	149
	IL-1R-1	NM_000877	AGAGGAAAACAAACCCACAAGG	AACTGGCCGGTGACATTACAG	108
	STAT1	NM_007315	ATCTGTCCTTCTTCCTGACTCCA	CCTTACAAAACCTCGTCCACG	186
	PANX1	NM_015368	GGCAGAGCTCCAAGGTATGAA	GCAAACCAGCTGTGAAACCA	186
	CASP1	NM_033294	GGAAACAAAAGTCGGCAGAGA	GATAATGAGAGCAAGACGTGTGC	72

Supplementary Table S1 | Primers used for real-time RT-PCR. Primers were designed using Primer Express 2.0 software (Applied Biosystems).

Supplementary Table S1

Primers for real-time RT-PCR

Species	Gene name	Accession	Forward (5'→3')	Reverse (5'→3')	Amplicon size (b.p.)
Mouse	*Panx1*	NM_019482	AGCCAGAGAGTGGAGTTCAAAGA	CATTAGCAGGACGGATTCAGAA	104
	Cx43	NM_010288	CCATCCAAAGACTGCGGAT	GTAATTGCGGCAGGAGGAA	138
	P2rx1	NM_008771	AGCCCAAGGTATTCGCACA	ACAGGACACCAGCCAAAGATC	81
	P2rx2	NM_001164833	AAAACAAGCTCTACAGCCATAAGAAGT	TCAAAGTTGGGCCAAACCTT	140
	P2rx3	NM_145526	GCCCAACAGTGAGCTTTCTC	ATTTCCCTGGGCTCTCCTAA	58
	P2rx4	NM_011026	CACAACGTGTCTCCTGGCTA	GCCTTTCCAAACACGATGAT	125
	P2ry1	NM_008772	AGGAAAGCTTCCAGGAGGAG	CGTGTCTCCATTCTGCTTGA	95
	P2ry2	NM_008773	CGTGCTCTACTTCGTCACCA	AAAAGCAGACCCAGCATGAC	102
	P2ry6	NM_183168	CATTAGCTTCCAGCGCTACC	CTCCACACACTACCCAAGCA	91
	Trpv4	NM_022017	CACCCCAGTGACAACAAGAGA	CGATTGAAGACTTTGAGGATGG	107
	Chrm2	NM_203491	CATTGCGGCTTTCTATCTGC	TCTGGATCTTGTTGTGCTCCA	212
	Chrm3	NM_033269	ACACCTTCTGTGACAGCTGCA	TCGCTTGTGAAAAATGACGG	212
	Upk1a	NM_026815	CCTCCTGCATCACATCCTACA	CCACAACACTCTTGCTCAATCA	157
	Upk3a	NM_023478	CATCATCCTCAGCTTTGTGGA	GAGAACACCTCTGCTCTGTCTAGG	159
	Cdh1	NM_009864	GAGGAGAACGGTGGTCAAAGA	AGCTGGCTCAAATCAAAGTCC	120
	Col1a1	NM_007742	GCAACAGTCGCTTCACCTACA	CAATGTCCAAGGGAGCCACAT	137
	Ctgf1	NM_010217	AGAACTGTGTACGGAGCGTGAC	CACCATCTTTGGCAGTGCA	107
	Des	NM_010043	AAGGCCAAACTACAGGAGGAAA	TACGAGCTAGAGTGGCTGCATC	97
	IL-1β	NM_008361	CAGGCAGGCAGTATCACTCA	TGTCCTCATCCTGGAAGGTC	90
	IL-6	NM_031168	CCGGAGAGGAGACTTCACAG	TCCACGATTTCCCAGAGAAC	102
	Tnfa	NM_013693	ACGGCATGGATCTCAAAGAC	GTGGGTGAGGAGCACGTAGT	116
	Nfkb1	NM_008689	GGAAAAAACATCCACCTGCAC	TGCAAAGCCAACCACCAT	105
	Emr1	NM_010130	TCAGCTCTCGCAACATCAAGA	ACCAGCAGCGATTATGCATC	123
	IL-1R-1	NM_008362	TACCCGAGGTCCAGTGGTATAAG	AGCCACATTCCTCACCAACAG	98
Human	*Cx43*	NM_000165	AGCAAAAGAGTGGTGCCCA	TTGTCAAGGAGTTTGCCTAAGG	63
	IL-1β	NM_000576	TTATGTGCACGATGCACCTG	AGGACATGGAGAACACCACTTG	149
	IL-1R-1	NM_000877	AGAGGAAAACAAACCCACAAGG	AACTGGCCGGTGACATTACAG	108
	STAT1	NM_007315	ATCTGTCCTTCTTCCTGACTCCA	CCTTACAAAACCTCGTCCACG	186
	PANX1	NM_015368	GGCAGAGCTCCAAGGTATGAA	GCAAACCAGCTGTGAAACCA	186
	CASP1	NM_033294	GGAAACAAAAGTCGGCAGAGA	GATAATGAGAGCAAGACGTGTGC	72

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