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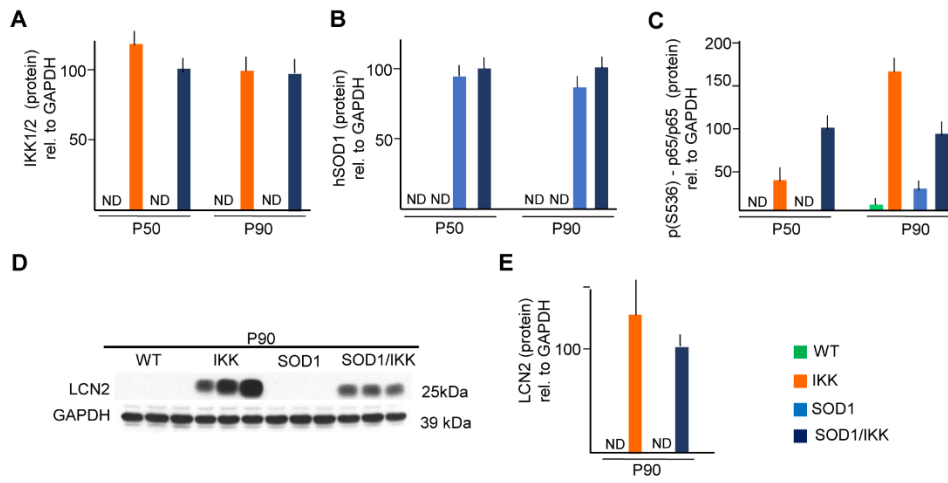
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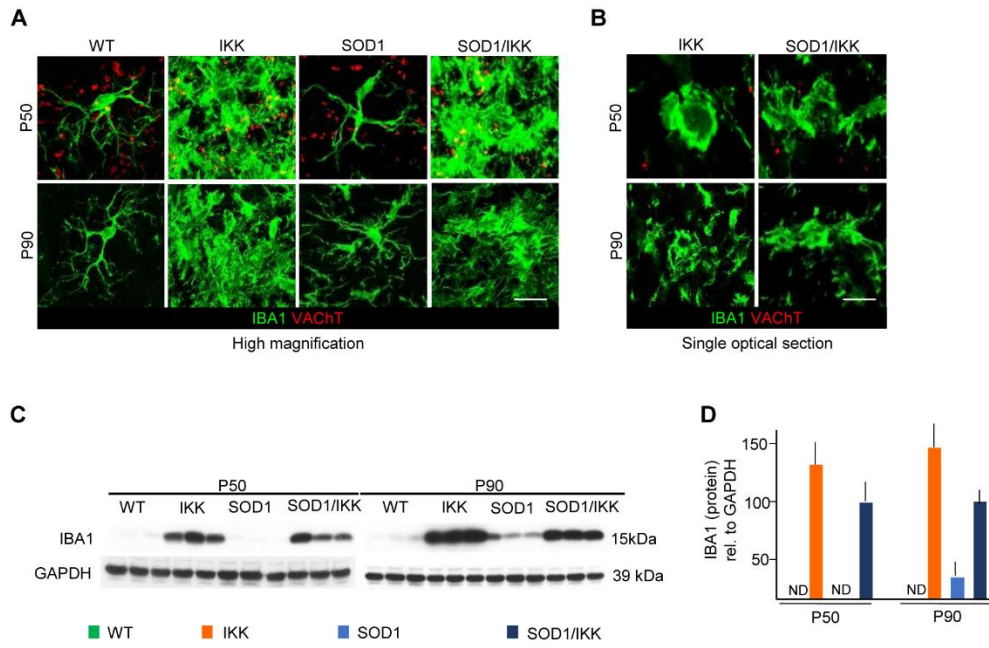
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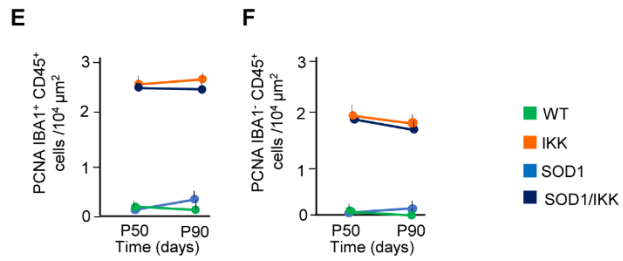
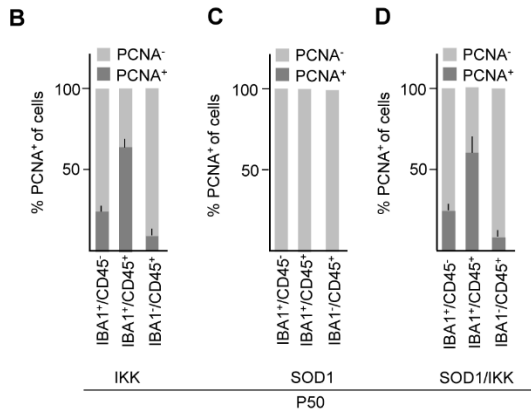
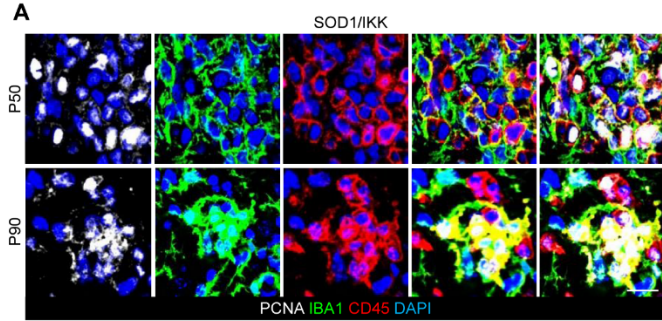
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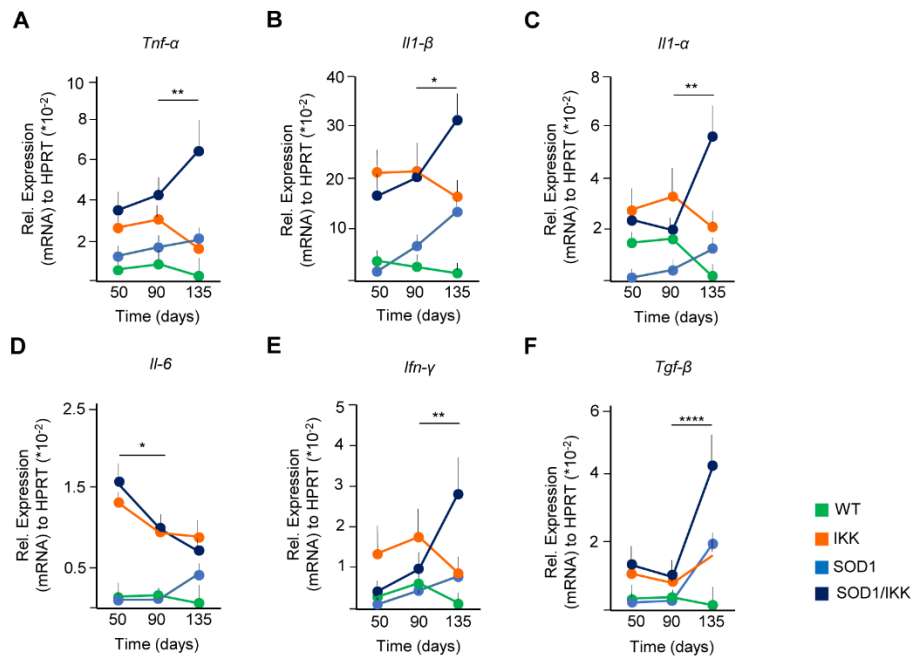
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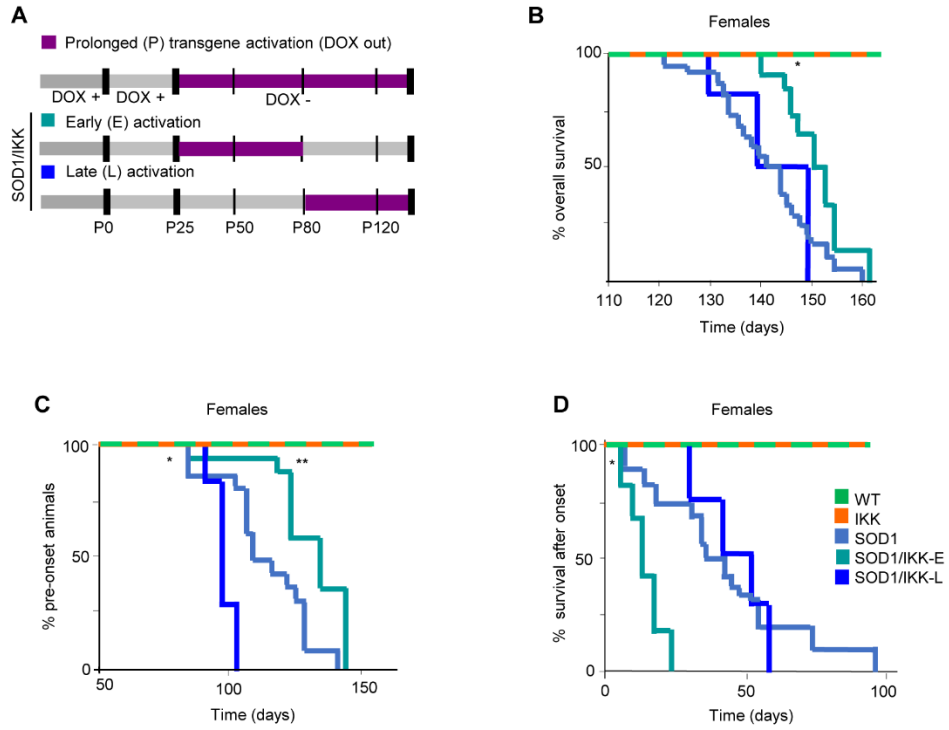
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Appendix Figure S1 - Functional characterization of transgene expression in GFAP.tTA/(tetO)7.IKK2-CA/SOD1(G93A) mice and littermates.

A Quantification of IKK2-CA transgene protein reveals strong expression in IKK and SOD1//IKK mice.

B Quantification of SOD1 protein shows comparable expression in SOD1 and SOD1//IKK mice.

C Quantification of the ratio of phosphorylated p65 and total p65 reveals prominently elevated levels in IKK and SOD1//IKK mice.

D Western blot showing strong expression of the NF- κ B target gene LCN2 in IKK and SOD1//IKK mice. GAPDH loading control is identical to Fig 1E.

E WB quantification of LCN2 expression shown in (D).

Data information: All quantifications are performed relative to GAPDH (loading control; in case of C total p65 was included) and are related to Fig 1E. All data are acquired from WT, IKK, SOD1 and SOD1//IKK as indicated. Error bars are represented as SD. ND = not detectable; (n=3-4).

Appendix Figure S2 - IKK/NF- κ B activation in astrocytes induces expansion of microglia.

A High-magnification analysis of IBA1 immunostaining shown in Fig 2D for WT, IKK, SOD1 and SOD1//IKK animals at P50 and P90. Scale bar 5 μ m.

B Single optical sections of IBA1 staining of IKK and SOD1//IKK at P50 and P90. Scale bar 5 μ m.

C Immunoblot confirming the increase of 15 kDa IBA1 expression in IKK and SOD1//IKK at P50 and P90 in the spinal cord. GAPDH loading control is identical to Fig 1E.

D Quantification of IBA1 protein expression levels at P50 and P90 in the spinal cord relative to GAPDH. Error bars are represented as SD. ND = not detectable; (n=3-4).

Appendix Figure S3 - NF- κ B activation in astrocytes induces microglia expansion sustained by proliferation.

A Co-immunostaining of PCNA (white), IBA1 (green), CD45 (red) and DAPI (blue) in ventral horn spinal cord sections of SOD1//IKK mice at P50 and P90. Scale bar 10 μ m.

B–D Quantification of proliferating immune cells (IBA1⁺, IBA1⁺/CD45⁺ IBA1⁻/CD45⁺) in (B) IKK, (C) SOD1 and (D) SOD1//IKK mice at P50 (expressed as %). Data are presented as means \pm SD; (n=4)

E Quantification of PCNA⁺/IBA1⁺/CD45⁺ cell density in WT, IKK, SOD1 and SOD1/IKK mice at P50 and P90. Data are presented as means \pm SD; (n=4)

F Quantification of proliferating IBA1⁺/CD45⁺ cells in WT, IKK, SOD1 and SOD1/IKK mice at P50 and P90; Data are presented as means \pm SD; (n=4).

Appendix Figure S4 - Analysis of the cytokine expression pattern in the lumbar spinal cord over time.

A–F Gene expression analysis (relative to HPRT) of different cytokines: (A) *TNF- α* , (B) *IL- β* , (C) *IL-1- α* , (D) *IL-6*, (E) *IFN- γ* and (F) *TGF- β* in WT, IKK, SOD1 and SOD1/IKK mice lumbar spinal cords across timepoints P50, P90 and P135; (n=3-4). Two-way ANOVA. Data are shown as means \pm SD, *p \leq 0.05; ** p \leq 0.01; **** p \leq 0.0001.

Appendix Figure S5 - Time dependent activation of IKK/NF- κ B in female SOD1/IKK affects disease onset and progression.

A Experimental design illustrating the different time windows of transgene activation induced by DOX withdrawal (DOX out, purple): Prolonged (P, shown in Fig EV2), Early (E; turquoise) and Late (L; dark blue) in female SOD1/IKK mice.

B Kaplan-Meier curves of SOD1/IKK mice survival in the E and L transgene activation modus compared to SOD1 (light blue) female mice. The analysis reveals no effect of late-activation of the transgene (SOD1/IKK-L vs SOD1: Mantel-Cox p=0.34), but shows significant prolongation of survival in the early-only activation modus of NF- κ B (SOD1/IKK-E vs SOD1: Mantel-Cox *p=0.0327).

C Kaplan-Meier curves representing % of presymptomatic female mice as function of time for SOD1/IKK animals in the E and L, showing a delayed onset for SOD1/IKK-E female mice (SOD1/IKK-L vs SOD1: Mantel-Cox *p=0.0281; SOD1/IKK-E vs SOD1: Mantel-Cox **p=0.00435).

D Kaplan-Meier curves representing the time spent in the progression phase of SOD1/IKK-E and L compared to SOD1 mice. The curves show a significantly shortened progression phase for SOD1/IKK-E female mice (Mantel-Cox *p=0.0127).

Appendix Table S1. List of Antibodies and other biological reagents

REAGENT or RESOURCE	DILUTION	APPLICATION	SOURCE	IDENTIFIER
Antibodies				
Rabbit polyclonal anti-IKK α/β (clone H-470)	1:750 1:100	WB IF	Santa Cruz Biotechnology	Ca#sc-7607;RRID:AB_675667
Rabbit polyclonal anti-SOD1	1:1000	WB	Abcam	Ca#ab16831;RRID:AB_302535
Rabbit polyclonal anti-GAPDH	1:3000	WB	Santa Cruz Biotechnology	Ca#sc-25778;RRID:AB_10167668
Rabbit polyclonal anti-phospho-NK- κ B p65 (Ser536) (93H1)	1:1000	WB	Cell Signaling Technology	Ca#3033;RRID:AB_331284
Rabbit polyclonal anti-NK- κ B p65	1:1000	WB	Santa Cruz Biotechnology	Ca#sc-372;RRID:AB_632037
Goat polyclonal anti-Lcn2	1:2000	WB	R&D systems	Ca#AF1857;RRID:AB_355022
Rabbit monoclonal anti-NF- κ B p65 (D14E12) XP $\text{\textcircled{R}}$	1:500	IF	Cell Signaling Technology	Ca#8242; RRID:AB_10859369
Rabbit monoclonal anti-Ki-67 (clone SP6)	1:500	IF	Thermo Fisher Scientific	Ca#RM-9106-S0;RRID:AB_2341197
Mouse monoclonal Anti-PCNA	1:150	IF	Millipore	Cat#NA03;RRID:AB_2160355
Rabbit polyclonal anti-Iba-1	1:200 1:1000	IF WB	Wako	Ca#019-19741;RRID:AB_839504

Rat monoclonal anti-CD11b (clone 5C6)	1:100	IF	AbD Serotec	Ca#MCA 711G; RRID: AB_323167
Rat anti-mouse CD45 purified (clone 30-F11)	1:350	IF	BD Bioscience	Ca#550539; RRID: AB_21744 26
Mouse monoclonal anti-CD68 (clone ED1)	1:200	IF	Abcam	Ca#ab31630; RRID: AB_1141557
Rabbit polyclonal anti-CD206	1:100	IF	Abcam	Ca#ab64693; RRID: AB_15239 10
Rabbit polyclonal anti-CD3	1:250	IF	Abcam	Ca#ab5690; RRID: AB_30505 5
Rabbit polyclonal anti-GFAP	1:250	IF	Abcam	Ca#ab7260; RRID: AB_30580 8
Mouse monoclonal anti-GFAP (clone G-A-5)	1:400	IF	Sigma-Aldrich	Ca#G3893; RRID: AB_47701 0
Goat polyclonal anti-VACHT Violet 421 (clone C068C2)	1:300	IF	Millipore	Ca#ABN100; RRID: AB_26303 94
Rabbit monoclonal anti-LC3A (D50G8) XP®	1:400	IF	Cell Signaling Technology	Ca#4599; RRID: AB_10548192
Mouse anti-Misfolded Human SOD1 (B8H10) (clone C4F6)	1:1000	IF	Medimabs	Ca#MM-0070; RRID: AB_10015 296
Mouse monoclonal anti-SQSTM1/p62	1:500	IF	Abcam	Ca#ab56416; RRID: AB_94562 6
Guinea pig polyclonal anti-Synaptophysin 1	1:250	IF	Synaptic Systems	Ca#101004; RRID: AB_12103 82
α-Bungarotoxin conjugates Alexa Fluor® 555	1:500	IF	Molecular Probes™	Ca#B35451; RRID: AB_23139 31
RatlgG _{2A} monoclonal anti-Wnt-5a (clone 442625)	1:100	IF	R&D systems	Ca#MAB645; RRID: AB_10571 221

Monoclonal anti-Human CD163 PE (clone GHI/61)	1:50	FC	eBioscience	Ca#12-1639-41; RRID: AB_1963571
Rat monoclonal anti-CD11b FITC (clone M1/70)	1:50	FC	BD Bioscience	Ca#557396; RRID: AB_396679
Rat monoclonal anti-CD45 APC-Cy7 conjugated (clone 30-F11)	1:50	FC	BD Bioscience	Ca#557659; RRID: AB_396774
Rabbit polyclonal anti-galectine-3 (H-160)	1:100	IF	Santa Cruz Biotechnology	Cat#sc-20157; RRID: AB_2136784
Rabbit monoclonal anti-TMEM119 [28-3]	1:100	IF	Abcam	Cat#ab209064; RRID: AB_2687894
Mouse monoclonal F 4/80 Antigen (clone Cl:A3-1)	1:200	IF	Peninsula Laboratories	Cat#T-2008.0500; RRID: AB_518460
Rat monoclonal anti-CD169 (clone MOMA-1)	1:100	IF	Bio-Rad	Ca#MCA947; RRID: AB_322323
Rat monoclonal anti-CD4 (clone 4SM95)	1:50	IF	eBioscience	Ca#14976680; RRID: AB_2573007
Guinea pig polyclonal anti-VACHT	1:100	IF	Synaptic Systems	Ca#139105; RRID: AB_10893979
Rabbit polyclonal anti-Collagen IV	1:200	IF	Abcam	Ca#ab6586; RRID: AB_305584
Goat polyclonal antibody anti-Albumin	1:50	IF	Santa Cruz Biotechnology	Ca#sc-46289; RRID: AB_633767
Goat polyclonal ant-8-Hydroxydeoxyguanosine	1:100	IF	Millipore	Ca#AB5830; RRID: AB_92060

Rabbit polyclonal anti-Cleaved Caspase-3 (Asp175)	1:100	IF	Cell Signaling Technology	Ca#9661; RRID: AB_2341188
Mouse monoclonal anti-Wnt7a(E-9)	1:100	IF	Santa Cruz Biotechnology	Ca#sc-365665; RRID:AB_10846318
Mouse monoclonal anti-CD11c (clone 3.9)	1:100	IF	Abcam	Ca#ab11029; RRID: AB_297683
Rabbit polyclonal anti-CD11c	1:500	IF	Synaptic Systems	Ca#375003; RRID: AB_2630323
Rabbit polyclonal anti-CD8	1:30	IF	Bioss Inc	Ca#bs-0648R; RRID:AB_10857537
Mouse monoclonal anti-NF-κB p65 (L8F6)	1:500	IF	Cell Signaling Technology	Ca#6956; RRID: AB_10828935
Mouse monoclonal anti-CD68 Alexa Fluor® 647 (clone FA-11)	1:50	FC	BioLegend	Ca#137003; RRID: AB_2044001
Mouse monoclonal anti-CD3 PE/Cy7 (clone 145-2C11)	1:50	FC	BioLegend	Ca#100320; RRID: AB_312685
Mouse monoclonal anti-CD8a eFluor 450 (clone 53-6.7)	1:50	FC	eBioscience	Ca#48-0081-82; RRID: AB_1272198
Mouse monoclonal anti-CD4 APC (clone GK1.5)	1:50	FC	BioLegend	Ca#100412; RRID: AB_312697
Mouse/rat/human monoclonal anti-CD27 APC/Cy7 (clone LG.3A10)	1:50	FC	BioLegend	Ca#124225; RRID: AB_2565791
Mouse monoclonal anti-CD206 Brillant	1:50	FC	BioLegend	Ca#141717; RRID:AB_2562232

Mouse anti-rabbit IgG-HRP	1:5000	WB	Santa Cruz Biotechnology	Ca#sc-2357; RRID: AB_628497
Donkey anti-goat IgG-HRP	1:5000	WB	Santa Cruz Biotechnology	Ca#sc-2020; RRID:AB_63172 8
Donkey anti-rat IgG (H+L) Alexa Fluor® 488	1:500	IF	Life technologies	Ca#A21208; RRID: AB_141709
Donkey anti-rabbit IgG Alexa Fluor® 568	1:500	IF	Life technologies	Ca#A10042; RRID: AB_2534017
Donkey anti-mouse IgG (H+L) Alexa Fluor® 647	1:500	IF	Life technologies	Ca#A31571; RRID: AB_162542
Donkey anti-goat IgG (H+L) Alexa Fluor® 633	1:500	IF	Life technologies	Ca#A21082; RRID: AB_141493
Donkey anti-goat IgG H&L Alexa Fluor® 405	1:500	IF	Life technologies	Ca#ab175664; RRID: AB_2313502
Donkey anti-mouse IgG (H+L) Alexa Fluor® 488	1:500	IF	Life technologies	Ca#A21202; RRID: AB_141607
Donkey anti-rat IgG (H+L) Alexa Fluor® 594	1:500	IF	Life technologies	Ca#A21209; RRID: AB_2535795

Appendix Table S2. List of primers

Gen	Sequence	UPL
huSod1 for	tctggatagaggattaaagtgagga	40
huSod1 rev	tccatgttcatgagtttgagat	40
hulkk2 for	agacagcacaacaaccagca	61
hulkk2 rev	ccgtcgactgggctagag	61
C3 for	ttgtagagctgctggtcagg	76
C3 rev	acctacctcggaagttct	76
Lcn2 for	tctgatccagtagcgacagc	58
Lcn2 rev	ccatctatgagctacaagagaacaat	58
Ccl2 for	gatcatctgctgggaatgagt	62
Ccl2 rev	catccacgtgttgctca	62
MadCam for	ataggacgacggtggagga	72
MadCam rev	gggcaggtgaccaatctgta	72
CtsS for	ggagtgagcaccacacttca	92
CtsS rev	gcatccaaaacagccatctt	92
C1q for	ggagcatccagttgatcg	16
C1q rev	catccctgagaggtctccat	16
Gfap for	ccaactgcaggccttgac	109
Gfap rev	gctctagggactcgttcgtg	109
Vimentin for	tgcgccagcagtatgaaa	79
Vimentin rev	gcctcagagaggtcagcaaaa	79
Olfm3 for	ctgctgctccttctttttg	56
Olfm3 rev	ctactctgatcctggcattgg	56
Tmem119 for	gtcactccatcccagttcac	110

Tmem119 rev	ggaccatgttgagctatggaa	110
Tnf- α for	gaggccatttgggaacttct	49
Tnf- α rev	tgctatgtctcagcctcttc	49
IL1- β for	tcttcttgggtattgcttgg	78
IL1- β rev	tgtaatgaaagacggcacacc	78
IL1- α for	ttggtaaatacctgcaaca	52
IL1- α rev	ttggtaaatacctgcaaca	52
IL-6 for	ccaggtagctatggactccagaa	6
IL-6 rev	gctaccaaactggatataatcagga	6
Inf- γ for	ttcaagacttcaagagtctgagg	21
Inf- γ rev	tctggaggaactggcaaaag	21
Tgf- β for	gtcagcagccggtacca	72
Tgf- β rev	tggagcaacatgtggaac	72
Wnt1 for	tactggcactgaccgtct	25
Wnt1 rev	cttggaatccgtcaacaggt	25
Wnt 2 for	cagagatcacagcctcttgg	101
Wnt2 rev	gcgtaaacaaggccgatt	101
Wnt2 β for	ccgggaccacactgtcttt	16
Wnt2 β rev	gctgacgagatagcatagacga	16
Wnt 3 for	ctcgtggctaccaattt	81
Wn3 rev	gaggccagagatgtgtactgc	81
Wnt4 for	actggactccctcctgtct	62
Wnt4 rev	tgccctgtcactgcaaa	62
Wnt5 α for	ggaccatgttgagctatggaa	71
Wnt5 α rev	cttctcctgagggcatcg	71
Wnt5 β for	agcaccgtggacaacacat	53

Wnt5 β rev	aaggcagtctctcggctacc	53
Wnt6 for	gtgcaactgcacaacaacg	62
Wnt6 rev	ggaacggaggcagcttct	62
Wnt7 α for	cgctgggagagcgtactg	12
Wnt7 α rev	cgataatcgcataggtgaagg	12
Wnt7 β for	gcgtcctctacgtgaagctc	49
Wnt7 β rev	tctgttgacagatgatgttg	49
Wnt8 β for	gcctcggagacttgacaac	76
Wnt8 β rev	ctcccagagccaacctt	76
Wnt10 β for	aatcggatccacaacaac	27
Wnt10 β rev	ctccaacaggtcttgaattgg	27