Supplemental information

Title: Inhibition of microbiota-dependent TMAO production attenuates chronic kidney disease in mice

Authors

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Supplemental Table S1. DAVID pathway analysis-enriched clusters of down-regulated genes in the kidney samples of Ade + IMC group compared to Ade group

Enrichment Cluster	Gene	log2 Fold Change	P value
Extracellular matrix (30 genes)	Adamts1	-0.52	1.21E-02
	Adamts16	-0.73	9.48E-03
	Adamts4	-0.50	1.14E-02
	Ctsd	-0.29	3.59E-02
	Clu	-0.58	1.84E-02
	Col3a1	-0.40	4.45E-03
	Col4a1	-0.32	1.78E-02
	Col5a2	-0.28	1.81E-02
	Col8a1	-0.48	1.01E-02
	Col12a1	-0.36	4.13E-02
	Col15a1	-0.38	3.83E-03
	Fbn2	-0.58	1.62E-02
	Lama1	-0.31	2.68E-02
	Ltbp2	-0.63	8.38E-03
	Lgals3	-0.40	3.45E-02
	Mmp12	-1.21	8.59E-05
	Mmp14	-0.30	2.22E-02
	Mmp20	-2.89	1.62E-02
	Mmp24	-1.31	3.61E-02
	Mmp7	-0.98	1.01E-02
	Rps25	-0.32	3.54E-02
	Rps5	-0.21	4.46E-02
	Serpine1	-0.70	4.01E-02
	Serpine2	-0.31	1.86E-03
	Sbsn	-0.71	5.48E-03
	Tnc	-0.43	1.71E-02
	Timp1	-0.84	1.54E-02
	Tgfbi	-0.38	4.74E-02
	Tgm2	-0.32	8.53E-03
	Vcan	-0.61	1.42E-02

Supplemental Table S1- continued

Enrichment Cluster	Gene	log2 Fold Change	P value
Protease inhibitor (13 genes)	Cstb	-0.37	1.81E-02
	Gpc3	-1.05	1.13E-04
	Lxn	-0.50	8.20E-03
	Ngf	-0.54	8.84E-04
	Serpina10	-1.07	1.66E-03
	Serpina1b	-1.06	8.91E-03
	Serpinb1a	-0.32	4.58E-03
	Serpinb6a	-0.31	6.35E-03
	Serpinb8	-0.40	1.22E-02
	Serpine1	-0.70	4.01E-02
	Serpine2	-0.31	1.86E-03
	Timp1	-0.84	1.54E-02
	Wfdc2	-0.39	2.67E-02

Enrichment Cluster	Gene	log2 Fold Change	P value
Complement and coagulation cascade (12 genes)	C2	-0.29	3.53E-02
	C3	-0.59	2.82E-02
	C3ar1	-0.32	2.56E-02
	Cfi	-1.16	4.46E-05
	F10	-0.75	3.66E-02
	F5	-1.09	3.29E-05
	F7	-0.83	4.98E-02
	Fgb	-1.30	4.05E-02
	Mbl1	-0.84	2.09E-02
	Pros1	-0.18	2.60E-02
	Serpina1b	-1.06	8.91E-03
	Serpine1	-0.70	4.01E-02

Enrichment Cluster	Gene	log2 Fold Change	P value
Immunity (24 genes)	C2	-0.29	3.53E-02
	C3	-0.59	2.82E-02
	Cd300a	-0.45	3.51E-02
	Cd300lb	-0.93	8.62E-03

Supplemental Table S1- continued

Enrichment Cluster	Gene	log2 Fold Change	P value
Immunity (24 genes)-continued	Cd300ld	-0.47	3.39E-02
	Cfi	-1.16	4.46E-05
	Clec4n	-0.67	1.61E-02
	Clec7a	-0.51	1.70E-02
	Dcstamp	-0.83	2.85E-02
	Ddx58	-0.19	3.18E-02
	Eif2ak2	-0.21	2.08E-02
	Fcgr1	-0.43	3.71E-02
	Fgb	-1.30	4.05E-02
	Fgr	-0.56	3.85E-02
	Il1f6	-0.96	4.33E-02
	Il34	-0.65	3.08E-03
	Lbp	-0.38	1.42E-02
	Lgals3	-0.40	3.45E-02
	Lst1	-0.50	9.65E-03
	Mbl1	-0.84	2.09E-02
	Nlrp10	-0.92	3.57E-03
	Olr1	-0.89	1.19E-02
	Smpdl3b	-0.68	2.09E-02
	Tlr2	-0.61	4.50E-04

Enrichment Cluster	Gene	log2 Fold Change	P value
Cytokine (14 genes)	Bmp1	-0.36	1.42E-02
	Clcf1	-0.60	1.47E-02
	Ccl17	-0.62	3.47E-02
	Ccl2	-0.65	5.16E-03
	Ccl9	-0.57	1.75E-02
	Cxcl1	-1.11	4.07E-03
	Cxcl14	-0.33	3.01E-04
	Cxcl16	-0.33	1.80E-02
	Gdf11	-0.25	4.10E-02
	Gdf15	-0.89	2.00E-02
	Il1f6	-0.96	4.33E-02
	Il34	-0.65	3.08E-03
	Lif	-1.02	2.08E-03
	Spp1	-0.91	6.45E-03

Supplemental Table S2. DAVID pathway analysis-enriched clusters of up-regulated genes in the kidney samples of Ade + IMC group compared to Ade group

Enrichment Cluster	Gene	log2 Fold Change	P value
Lipid metabolism (29 genes)	Aacs	0.71	2.82E-04
	Acacb	1.02	2.42E-02
	Acadm	0.78	1.43E-02
	Acadsb	0.31	4.87E-02
	Acsl1	0.60	2.66E-02
	Acsm5	0.79	1.93E-02
	Agps	0.28	3.14E-02
	Cers2	0.12	4.02E-02
	Cers4	0.26	2.20E-02
	Cyp51	0.66	2.58E-02
	Echs1	0.26	4.22E-02
	Elovl2	0.62	3.51E-02
	Elovl5	0.25	1.37E-03
	Fa2h	0.60	3.14E-03
	Fads6	0.52	1.30E-02
	Fam57b	0.79	4.66E-02
	Fasn	1.90	4.39E-02
	Gal3st1	0.30	7.05E-03
	Hadhb	0.42	1.96E-02
	Hmgcr	0.52	3.08E-02
	Hpgd	0.43	3.84E-02
	Hsd11b1	0.53	1.36E-02
	Lpl	0.89	2.03E-04
	Lypla1	0.35	4.87E-02
	Mgll	0.66	1.25E-02
	Pla2g12a	0.28	4.50E-02
	Pnpla2	0.64	1.75E-02
	Scd1	1.68	1.21E-02
	Sult1a1	0.47	2.43E-03

Supplemental Table S2- continued

Enrichment Cluster	Gene	log2 Fold Change	P value
Mitochondria (75 genes)	Abcc9	0.45	2.20E-02
	Abhd12	0.18	2.04E-02
	Acacb	1.02	2.42E-02
	Acadm	0.78	1.43E-02
	Acadsb	0.31	4.87E-02
	Acat2	0.39	5.25E-03
	Acsl1	0.60	2.66E-02
	Acsm5	0.79	1.93E-02
	Acyp2	0.42	2.38E-02
	Adcy10	1.68	3.90E-02
	Agps	0.28	3.14E-02
	Alas2	1.58	8.99E-08
	Aldoc	0.43	9.42E-04
	Atg13	0.19	1.30E-02
	Bcat1	0.73	9.10E-03
	Bcat2	0.29	2.07E-02
	Bckdhb	0.41	9.16E-03
	Bckdk	0.19	4.30E-03
	Bdh1	0.62	3.75E-02
	Cav1	0.41	1.81E-02
	Cd36	0.71	5.35E-03
	Ckb	0.29	3.20E-02
	Coq9	0.30	3.36E-02
	Cox8b	2.03	1.67E-02
	Dhtkd1	0.52	2.03E-02
	Dmpk	0.27	3.47E-02
	Echdc3	0.46	1.52E-02
	Echs1	0.26	4.22E-02
	Etfa	0.31	4.28E-02
	Fasn	1.90	4.39E-02
	Fbxl4	0.21	3.91E-02
	Ghr	0.62	3.48E-02
	Gtpbp3	0.22	4.30E-02
	Hadhb	0.42	1.96E-02
	Hibch	0.44	3.92E-03
	Hk1	0.22	4.11E-02
	Hsd3b2	0.96	4.57E-03
	Hsd3b3	0.44	2.89E-02
	Hspa1b	0.28	4.55E-02

Supplemental Table S2- continued

Enrichment Cluster	Gene	log2 Fold Change	P value
Mitochondria (75 genes)-continued	Ivd	0.31	4.55E-02
	Kyat3	0.83	1.15E-02
	Lpin1	0.40	1.38E-02
	Lypla1	0.35	4.87E-02
	Macrod1	0.30	4.79E-02
	Mapk10	0.81	3.42E-02
	Mccc2	0.44	4.89E-02
	Mcur1	0.29	3.08E-02
	Mmab	0.27	1.28E-02
	Mpv17l	1.24	9.71E-03
	Mrps6	0.32	9.13E-03
	Mtfr1l	0.17	4.23E-02
	Mtrf1	0.29	4.86E-02
	Mtus1	0.28	1.42E-02
	Nudt19	0.77	5.80E-04
	Pdk1	0.22	3.28E-02
	Pink1	0.28	4.37E-02
	Pmpca	0.14	3.87E-02
	Ppm1k	0.43	2.25E-02
	Ptcd2	0.22	4.44E-02
	Pxmp2	0.50	2.69E-02
	Rnf185	0.19	1.42E-02
	Sfxn5	0.30	3.40E-02
	Slc25a27	0.26	4.41E-02
	Slc25a35	0.30	3.09E-02
	Snca	1.07	2.83E-02
	Stard7	0.17	3.30E-02
	Sucla2	0.35	3.73E-02
	Tbrg4	0.19	2.89E-02
	Tfrc	0.41	1.59E-02
	Them7	0.58	2.46E-02
	Tmtc1	0.55	1.57E-02
	Tst	0.39	4.66E-02
	Ubiad1	0.41	2.62E-02
	Ucp3	2.08	3.34E-02
	Uqcrc2	0.27	3.50E-02

Supplemental Table S2- continued

Enrichment Cluster	**Gene**	**log2 Fold Change**	**P value**
Ion transport (35 genes)	Aqp2	0.76	6.18E-05
	Aqp4	1.24	2.19E-03
	Aqp6	0.61	9.32E-06
	Aspn	1.03	1.37E-03
	Atp1b2	0.20	4.94E-02
	Cacna1g	0.32	4.16E-03
	Clcnka	0.60	1.19E-03
	Cldn15	0.50	1.60E-03
	Cnnm1	0.37	1.38E-02
	Kcna1	1.16	2.08E-04
	Kcnc3	0.62	1.31E-02
	Kcng1	1.44	2.06E-02
	Kcnh6	2.55	3.25E-02
	Kcnj13	1.92	3.18E-02
	Kcnk2	0.46	1.72E-02
	Kcnk3	1.42	4.87E-02
	Kcnmb2	1.99	3.37E-02
	Kcnq4	0.64	4.18E-02
	Kcnt1	1.26	8.63E-07
	Kctd12b	0.29	2.21E-02
	Mcoln3	0.30	4.24E-02
	Mcur1	0.29	3.08E-02
	Sfxn5	0.30	3.40E-02
	Slc12a1	0.51	2.30E-02
	Slc26a7	0.68	2.71E-02
	Slc39a8	0.63	1.85E-02
	Slc4a1	0.69	7.40E-03
	Slc4a5	1.28	1.62E-05
	Slc5a6	0.66	2.54E-03
	Slc6a15	1.42	6.40E-05
	Slc9a8	0.70	2.37E-02
	Tmco3	0.37	2.09E-02
	Tmem109	0.23	8.02E-03
	Tmem38b	0.31	1.57E-02
	Trpm1	1.21	3.27E-03

Supplemental Table S2- continued

Enrichment Cluster	**Gene**	**log2 Fold Change**	**P value**
Endoplastic reticulum (59 genes)	Acsl1	0.60	2.66E-02
	Amfr	0.26	8.01E-03
	Ano5	0.65	1.10E-02
	Aoc3	0.70	1.16E-02
	Apmap	0.37	4.42E-02
	Apod	1.12	1.16E-02
	Cav1	0.41	1.81E-02
	Cd36	0.71	5.35E-03
	Cdnf	1.06	6.28E-03
	Cers2	0.12	4.02E-02
	Cers4	0.26	2.20E-02
	Ces1f	0.47	2.84E-02
	Clcnka	0.60	1.19E-03
	Col4a3	0.37	4.91E-02
	Colgalt2	0.47	2.13E-02
	Cyp51	0.66	2.58E-02
	Dio1	0.73	3.09E-02
	Dmpk	0.27	3.47E-02
	Elovl2	0.62	3.51E-02
	Elovl5	0.25	1.37E-03
	Fa2h	0.60	3.14E-03
	Fam57b	0.79	4.66E-02
	Fibin	0.69	2.03E-02
	Hadhb	0.42	1.96E-02
	Herpud1	0.34	4.00E-02
	Hmgcr	0.52	3.08E-02
	Hpd	1.47	1.97E-02
	Hsd11b1	0.53	1.36E-02
	Hsd3b2	0.96	4.57E-03
	Hsd3b3	0.44	2.89E-02
	Itga8	0.45	3.23E-02
	Kcna1	1.16	2.08E-04
	Kcnk2	0.46	1.72E-02
	Lpin1	0.40	1.38E-02
	Mfsd2a	1.30	2.38E-03
	Myo5a	0.46	1.11E-02
	Nat8	0.87	6.52E-04
	Nat8f6	0.91	4.43E-02
	Notch4	0.33	2.49E-02

Supplemental Table S2- continued

Enrichment Cluster	Gene	log2 Fold Change	P value
Endoplastic reticulum (59 genes)-continued	Nr3c2	0.29	4.36E-02
	Ormdl3	0.35	1.09E-02
	Pla2g12a	0.28	4.50E-02
	Ptpn5	1.19	5.34E-03
	Ric3	0.66	3.15E-03
	Rnf185	0.19	1.42E-02
	Sar1b	0.28	2.15E-02
	Scd1	1.68	1.21E-02
	Shisa2	0.72	1.05E-02
	Sil1	0.21	3.62E-02
	Tenm2	0.49	4.07E-02
	Tgfbr3	0.39	1.36E-02
	Thbs4	0.92	4.73E-03
	Tmem100	1.62	2.07E-04
	Tmem109	0.23	8.02E-03
	Tmem119	0.38	2.54E-02
	Tmem189	0.38	1.68E-02
	Tmem38b	0.31	1.57E-02
	Tmem50b	0.17	3.10E-02
	Ubiad1	0.41	2.62E-02

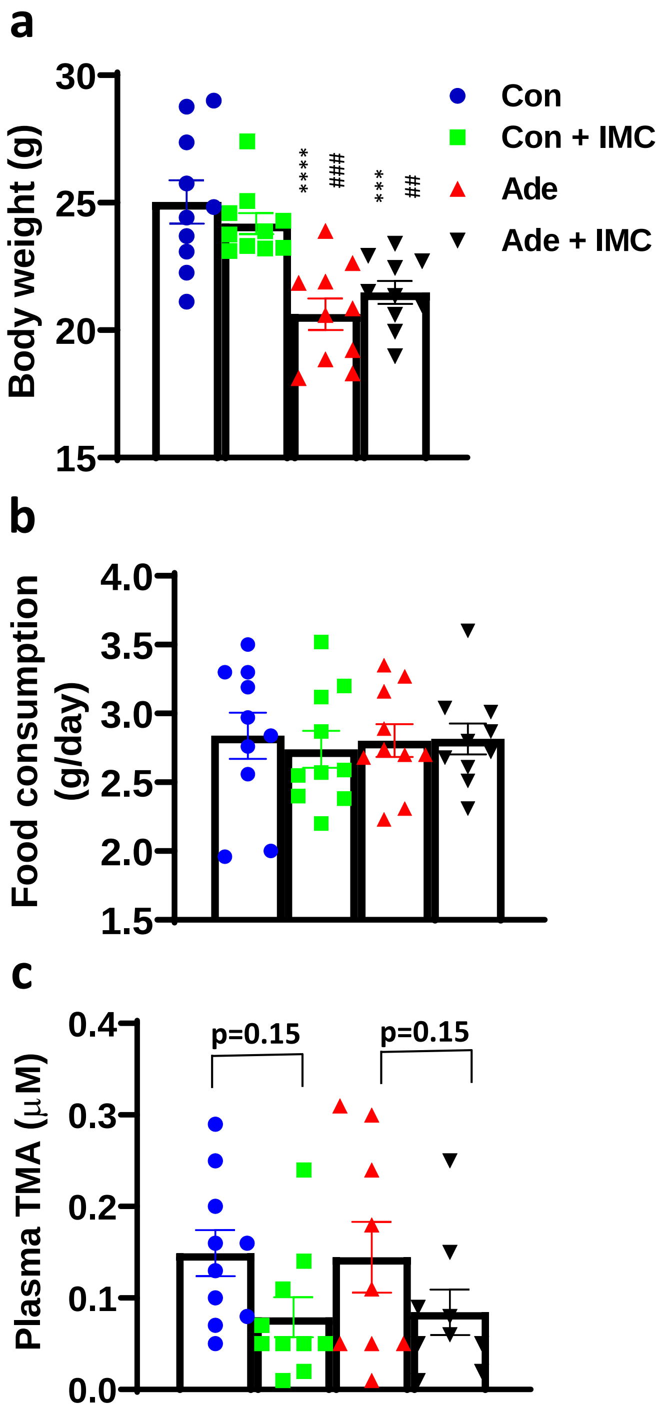
Enrichment Cluster	Gene	log2 Fold Change	P value
Cellular water homeostasis (6 genes)	Aqp1	0.40	3.44E-02
	Aqp2	0.76	6.18E-05
	Aqp3	0.49	1.03E-02
	Aqp4	1.24	2.19E-03
	Aqp6	0.61	9.32E-06
	Slc6a12	0.54	3.26E-02

Supplemental Table S3. qPCR primers used in this study

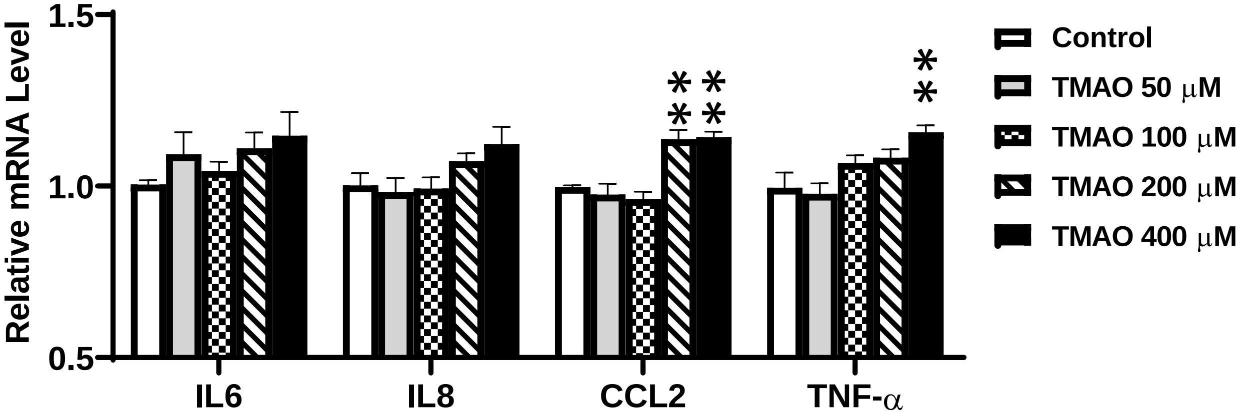
Primer	Sequence
c-ATF4-F	CTTGATGTCCCCCTTCGACC
c-ATF4-R	GGCATCCTCCTTTCCGTTGT
c-CCL20-F	CATGGGCTTCACACAACAGC
c-CCL20-R	CTATGGCTGAGGAGACGCAC
c-CCL2-F	TCCCAAAGAAGCTGTGATCTTCA
c-CCL2-R	TTTGCAGTTTGGGTTTGGCT
c-CHOP/DDIT3-F	CAACGAGGCTCTAAGGTCGC
c-CHOP/DDIT3-R	TACAGTTGGATCAGGCTCTGC
c-GAPDH-F	TTCCACGGCACAGTCAAG
c-GAPDH-R	ACTCAGCACCAGCATCAC
c-IL1A-F	TGAAGCCCAGATCAGTAGCA
c-IL1A-R	CTCCCATGTCAAACTTCACTGC
c-IL8-F	ACAGTGGCCCACATTGTGAA
c-IL8-R	GGATCTTGTTTCTCAGCCTTCTT
h-B2M-F	AGATGAGTATGCCTGCCGTG
h-B2M-R	TCATCCAATCCAAATGCGGC
h-CCL2-F	TGCTCATAGCAGCCACCTTCA
h-CCL2-R	GACACTTGCTGCTGGTGATTC
h-IL6-F	TTCAATGAGGAGACTTGCCTG
h-IL6-R	CTGGCATTTGTGGTTGGGTC
h-IL8-F	TCTCTTGGCAGCCTTCCTGATTTC
h-IL8-R	GTGTGGTCCACTCTCAATCACTCT
h-TNF-a-F	GCTGCACTTTGGAGTGATCG
h-TNF-a-R	CTTGTCACTCGGGGTTCGAG

Primer	Sequence
m-Ccl2-F	CACTCACCTGCTGCTACTCA
m-Ccl2-R	GCTTGGTGACAAAAACTACAGC
m-Ccl20-F	CCAGGCAGAAGCAAGCAACTA
m-Ccl20-R	TTTGGATCAGCGCACACAGA
m-Col1a1-F	GAGAGGTGAACAAGGTCCCG
m-Col1a1-R	AAACCTCTCTCGCCTCTTGC
m-Col3a1-F	GAGGAATGGGTGGCTATCCG
m-Col3a1-R	TCGTCCAGGTCTTCCTGACT
m-Cox2/Ptgs2-F	TTCAACACACTCTATCACTGGC
m-Cox2/Ptgs2-R	AGAAGCGTTTGCGGTACTCAT
m-Eif2a-F	CAACGTGGCAGCCTTACA
m-Eif2a-R	TTTCATGTCATAAAGTTGTAGGTTAGG
m-Il6-F	CAACGATGATGCACTTGCAGA
m-Il6-R	TGGAAATTGGGGTAGGAAGGAC
m-Lcn2-F	GGAACGTTTCACCCGCTTTG
m-Lcn2-R	CATTGGTCGGTGGGGACAGA
m-Rpl13a-F	CCCTCCACCCTATGACAAGA
m-Rpl13a-R	TTCTCCTCCAGAGTGGCTGT
m-Vcam1-F	TGGAGGTCTACTCATTCCCTGA
m-Vcam1-R	GACAGGTCTCCCATGCACAA

Primers from various species are designated as: c: canine, h: human, m: mouse



Supplemental Figure S1. Body weight (a), food consumption (b), and plasma TMA (c) data of mice included in this study. Data were obtained from the same mice described in Figure 1. Sample sizes were n = 10/group for (a) and (b), and n = 9-10/group for (c). Post hoc analysis was performed after significant two-way ANOVA. For (a) Symbols: ***: P < 0.001, ****: P < 0.0001 compared to control (Con) group. ##: P < 0.01, ###: P < 0.001 compared to control (Con) + IMC group.



Supplemental Figure S2. TMAO treatment induces the expression of inflammatory genes in normal human primary renal proximal tubule epithelial cells, RPTEC. RPTEC cells were treated with control media or media containing various doses of TMAO (n = 4/group) for 48 hours Before examination of gene expression by qPCR. Post hoc Tukey's multiple comparisons test was performed after significant one-way ANOVA. Symbol: **: $p < 0.01$ as compared to the control group.

Supplemental Information: Full length, uncropped Images of WB in the Manuscript

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Authors

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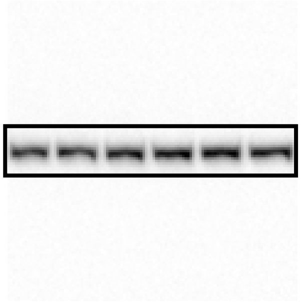
Description

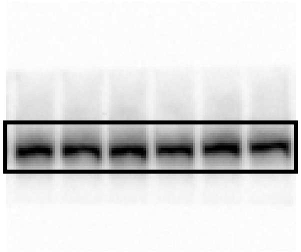
In the supplemental information, the original images of Figure 2e are shown without "auto-contrast adjustment" of Adobe Photoshop software.

Figure 2e (uncropped images)

p-NF-κB



NF-κB



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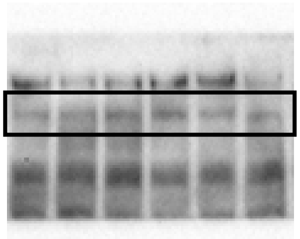

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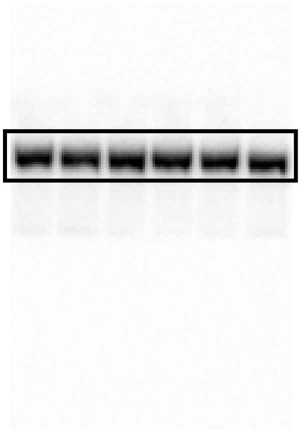
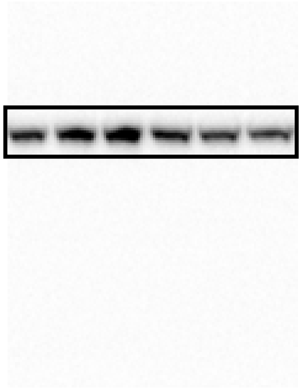


p-eIF2α



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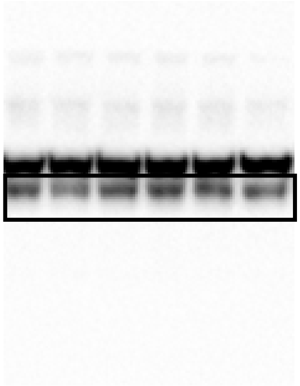
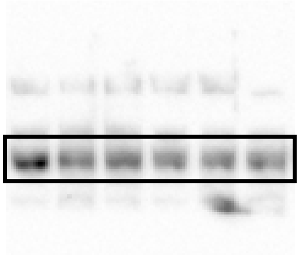
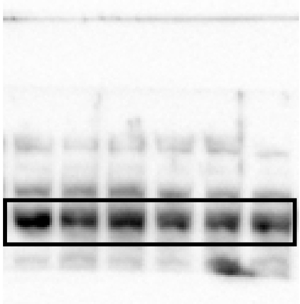
ATF4



p-IRE1α



IRE1α



GAPDH