Supplemental Information

Heterogeneity of epigenetic changes at ischemia/reperfusion- and endotoxin-induced AKI genes.

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Running title: Epigenetic portraits of AKI genes

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Table S1- PCR primers RT-qPCR

Primer	Sequence	Primer	Sequence
Agt FWD	GCGCTGACCGAGAATAAATG	Kim1 FWD	TTGCCTTCCGTGTCTCTAAG
Agt REV	GTCACCCCAGTATCCAAACA	Kim1 REV	AGATGTTGTCTTCAGCTCGG
Angpt1 FWD	AGTTGGAACAGCCCATTGTA	Klotho FWD	TTTGTCATGGGTGGTAGAGG
Angpt1 REV	TGAAGGCCTACGAACACTTT	Klotho REV	TCAGATACCATTCACCCTGC
Angpt2 FWD	AAGAATGTTCCGTGGGAGTT	Kmt1b FWD	ACTTCTGGGGATTAGATGCG
Angpt2 REV	TGCTTAGAGGAATGTGGTCC	Kmt1b REV	TTAGAACTCCCAGACCCAGA
Bax FWD	CATGGCAGACAGTGACCATC	Kmt1c FWD	CATCAACACCTGAGGACTCT
Bax REV	GAAAAATGCCTTTCCCCTTC	Kmt1c REV	TTACTGGGGTCTCTGATGGA
Bclx FWD	GCTGGTGCCCAGAGACTGAC	Lamc1 FWD	GGAACCTTCCCACACGGGTC
Bclx REV	GCCCCTCAGAAGCCAGAACC	Lamc1 REV	CGGTGCTGATGCCCTCAAGT
Bmp7 FWD	GTCTCAGGAAGAGCTAGTGG	Ldlr FWD	GTCCTGCTGTGGAGGAACTG
Bmp7 REV	TCGACGACAGCTCTAATGTC	Ldlr REV	CAGGCTGACCATCTGTCTTG
Clu FWD	ACCCCTAGAGAACTCCACAT	L-FABP FWD	CAAGCTGGAAGGTGACAATAA
Clu REV	TGCAGGCATTAGTGTACAGA	L-FABP REV	GTGTCTCCATTGAGTTCAGTC
Cox2 FWD	TGCCTCCCACTCCAGACTAGA	Mcp1 FWD	TTGAATGTGAAGTTGACCCG
Cox2 REV	CAGCTCAGTTGAACGCCTTTT	Mcp1 REV	TTAAGGCATCACAGTCCGAG
Cst3 FWD	AAGGGCTGAGTCTAGAAGGA	Nphs1 FWD	CCTATGACCTTCGCTGGCCT
Cst3 REV	CCTTCTGCGAGATGAAACAC	Nphs1 REV	AGGCTCCCCAGCATCCATGT
Ctgf FWD	TGCTGTGCAGGTGATAAAGC	Nphs2 FWD	CAGAGGCTTAGGTGCTCCTGGATGA
Ctgf REV	CCACCCCAAACCAGTCATAA	Nphs2 REV	TCATCGACTCAGTCTTGCCCCTCTG
Egr1 FWD	AACAGCCCTTTCACTTACCA	Netrin1 FWD	CCCTTTGCTTACCATTTGGG
Egr1 REV	CTTGGACATGGCTGTTTCAG	Netrin1 REV	CACATACCTTTGTGCCACTG
Ezh2 FWD	TGAAGTATGTGGGCATCGAA	Ngal FWD	AGATGCTCCTTGGTATGGTG
Ezh2 REV	GAAGCTAAGGCAGCTGTTTC	Ngal REV	CTGTCTGCCACTCCATCTTT
Flt1 FWD	GACATGGGAAGACAGGGTAG	p53 FWD	CTCTGAGTAGTGGTTCCTGG
Flt1 REV	AGCCATTTTAGAGACCCAGG	p53 REV	TGGCTGGATAGAATTTCGCT
Fsp1 FWD	GGAGGCCCTGGATGTAATTGT	Pai1 FWD	TGAGAGAGGGCAAAGTGGTT
Fsp1 REV	TGTCACCCTCTTTGCCTGAGT	Pai1 REV	ATACAGCAGCCGGAAATGAC
Gapdh FWD	AACTTTGGCATTGTGGAAGG	Pecam1 FWD	TGCCTTGTTCATGTTGGGTA
Gapdh REV	AGTGGATGCAGGGATGATGT	Pecam1 REV	TCTCCTGGAACCTCCTTTCA
Hmgcr FWD	TGTTCAAGGAGCATGCAAAG	Rage FWD	GATGCAAAGGCAATCTCACTCCTGCATC
Hmgcr REV	CTTACCTGTTGTGAACCATGTG	Rage REV	CCTGGTATGGTGGGAGGCATAG
Hmox1 FWD	TCTTGCCTGGCTCTCTTCTC	Sema3a FWD	GCGGTGGCTTATGTACTACT
Hmox1 REV	GGCTGCTGGTTTCAAAGTTC	Sema3a REV	GGAACAATTTACGACCTGGC
Hpx FWD	GGGCCCAATTTGTACTGCTA	Spp1 FWD	CATGAAGAGCGGTGAGTCTA
Hpx REV	CCAAGGATGCTGTTCACCTT	Spp1 REV	TTGTTGTCCTGATCAGAGGG
Icam1 FWD	CGAGGGTTTCTCTACTGGTC	Tek FWD	AGGAAGAAAAGCGAGGGAAA
Icam1 REV	TGCCAGTCCACATAGTGTTT	Tek REV	CCCTCTCTTCTGCTACTTGG
Ifng FWD	GCTTTAACAGCAGGCCAGAC	Tgfb1 FWD	GCAGTGGCTGAACCAAGGA
Ifng REV	GGAAGCACCAGGTGTCAAGT	Tgfb1 REV	GCAGTGAGCGCTGAATCGA
Igfbp7 FWD	AATCCATGAGCCTCTGTAGC	Timp2 FWD	AGAAACGGTTAAGGACTCCC
Igfbp7 REV	AAGAGAAGTGTGTCAGGCAA	Timp2 REV	TGTCCTCCCAGTCTGTCTTA
Il10 FWD	CCAGGGAGATCCTTTGATGA	Timp3 FWD	CAATCAGTCAAAGGCAGCAA
Il10 REV	AACTGGCCACAGTTTTCAGG	Timp3 REV	TCCCTCTGACATGCACACAT
Il18 FWD	GTGTTCCAGGACACAACAAG	Tlr2 FWD	TGCTTTCCTGCTGGAGATTT
Il18 REV	CTTCCTTTTGGCAAGCAAGA	Tlr2 REV	TGTAACGCAACAGCTTCAGG
Il6 FWD	GTGGCTAAGGACCAAGACCA	Tlr4 FWD	GCATGGCTTACACCACCTCT
Il6 REV	ACCACAGTGAGGAATGTCCA	Tlr4 REV	GTCTCCACAGCCACCAGATT
Junb FWD	GCCTTTCTATCACGACGACT	Tnf FWD	CGCTACATCACTGAACCTCT
Junb REV	AAGGTGGGTTTCAGGAGTTT	Tnf REV	TTCTCTCAATGACCCGTAGG
Kdm6a FWD	AGTTCTGCACCACTGCTATT	Vcam1 FWD	TGCAAGGAGCTAACCAGAAA
Kdm6a REV	TTGACACTCCACACTTGGAA	Vcam1 REV	ATCATGGGACCATTCCAGTC
Kdm6b FWD	ACTTTCTTGGACCAGTACCC	Vegfa FWD	GAGAGAGGCCGAAGTCCTTT
Kdm6b REV	TTCAGTTCCCACTTCTTCCC	Vegfa REV	TTGGAACCGGCATCTTTATC
Kdr FWD	CGCTCACCTCCTGTTTAAATG	Vim FWD	CGCCATCAACACTGAGTTCAA

TableS2. Primers ChIP-qPCR

Primer	**Sequence**	
Icam1 FWD	CTACCTGCACTTTGCCCT	
Icam1 REV	GGATCACAACGGTGACCA	
Kim1 FWD	TGGAGATTCCTGGATGGTTT	
Kim1 REV	TAGAAGCTTACCTGGTTTAACTTG	
Ngal FWD	ACTCAGAACTTGATCCCTGC	
Ngal REV	CCTTCAGGGTCCTACCTGAT	
Tnf FWD	AGTGCCTCTTCTGCCAGTTC	
Tnf REV	GCAGGTTCTGTCCCTTTCAC	

Table S3. Antibodies used in Matix ChIP

Figure	Antibody	Catalog No	Source	Manufacturer
3	Pol II CTD (4H8)	sc-47701	Mouse monoclonal	Santa Cruz
S2	Pol II CTD pSer2/pSer5	PA5-17563	Rabbit polyclonal	ThermoPierce
S3	Pol II CTD (8WG18)	MMS-126R	Mouse monoclonal	Covance
S12	H3K9m2	ab1220	Mouse monoclonal	ABCAM
S11	H3K27m3 (G.299.10)	MA511198	Mouse monoclonal	ThermoPierce
S15	H2BK120ub1	39624	Mouse monoclonal	Active Motif
S14	H2AK119ub1 (D27C4)	8240	Rabbit monoclonal	Cell Signaling
S5	H3K27Ac	SAB5100010	Rabbit polyclonal	Sigma
S13	macroH2A	07-219	Rabbit polyclonal	UPSTATE
S4	H3K18Ac	PA5-17801	Rabbit polyclonal	ThermoPierce
4	H3K9Ac	701269	Mouse monoclonal	ThermoPierce
S7	H3K4m2	07-030	Rabbit polyclonal	Millipore
5	H3K4m3	PA5-17420	Rabbit polyclonal	ThermoPierce
S10	H3K79m2	OAAH00059	Rabbit polyclonal	Aviva
S9	H3K36m2	OAAH00061	Rabbit polyclonal	Aviva
6	H3K36m3	OAAH00062	Rabbit polyclonal	Aviva
S6	H4K5/8/12/16Ac	06-866	Rabbit serum	UPSTATE
S8	H3pSer10	701258	Rabbit monoclonal	ThermoPierce
7	H4pSer1	PA5-27064	Rabbit polyclonal	ThermoPierce

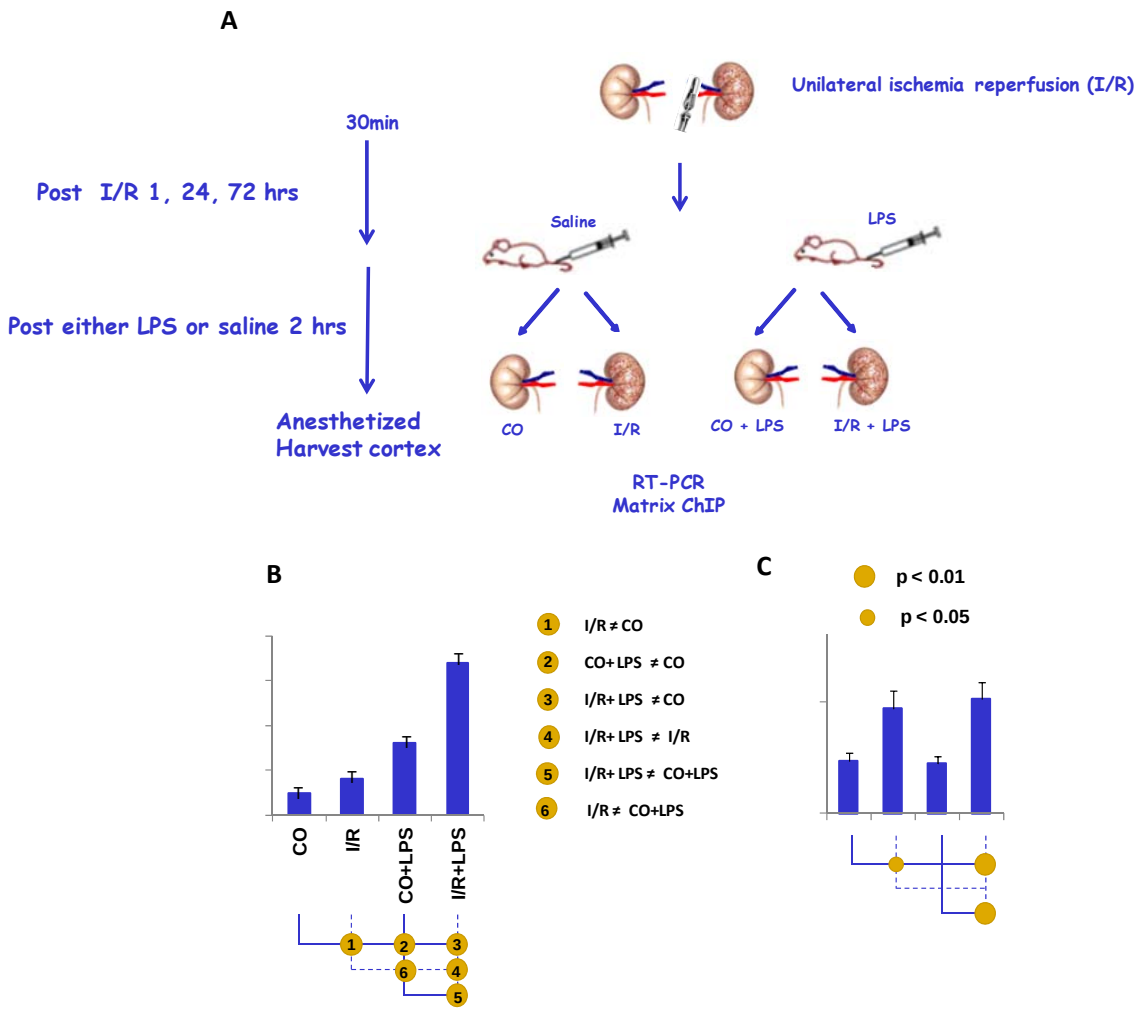

Fig.S1. Ischemia-reperfusion and endotoxin acute renal injury model and data analysis. A. ***Model.*** Mice were anesthetized and subjected to a midline abdominal incision under sterile conditions and, after 30 min of unilateral renal artery occlusion, the clamp was released (ischemia/reperfusion, or I/R). Either 1, 24 or 72 hours later, I/R injury mice received a tail vein injection of either lipopolysaccharide (LPS) or saline [1]. Two hours after these injections, mice were anesthetized and kidneys were harvested and rapidly frozen for RT-PCR and Matrix ChIP analysis [2,3]. Thus, the timing of harvesting the kidneys relative to I/R injury was 3, 26 and 76 hrs, and 2 hrs post either LPS or saline treatment (Methods). **B.** ***GraphGrid analysis.*** Solid circles positioned at line intersections designate comparison between a given pair of means (one bar graph vs. another bar graph). The bar graph above the circle represents one of the paired means. The second of the paired means is found by tracing left along

horizontal line from the circle all the way to farthest left angle corner where the vertical line points to the bar graph for the paired comparison. The graph illustrates bar (means + SEM). Five different paired statistical comparisons are done as shown with the numbered circles. **C**. **Statistical analysis** is done using Bonferroni correction. Statistical differences between two means (p value) are shown by the size of the solid circle. : $p<0.05$ by small circle, $p<0.01$ by large circle, and no circle indicating the differences are not statistically significant.

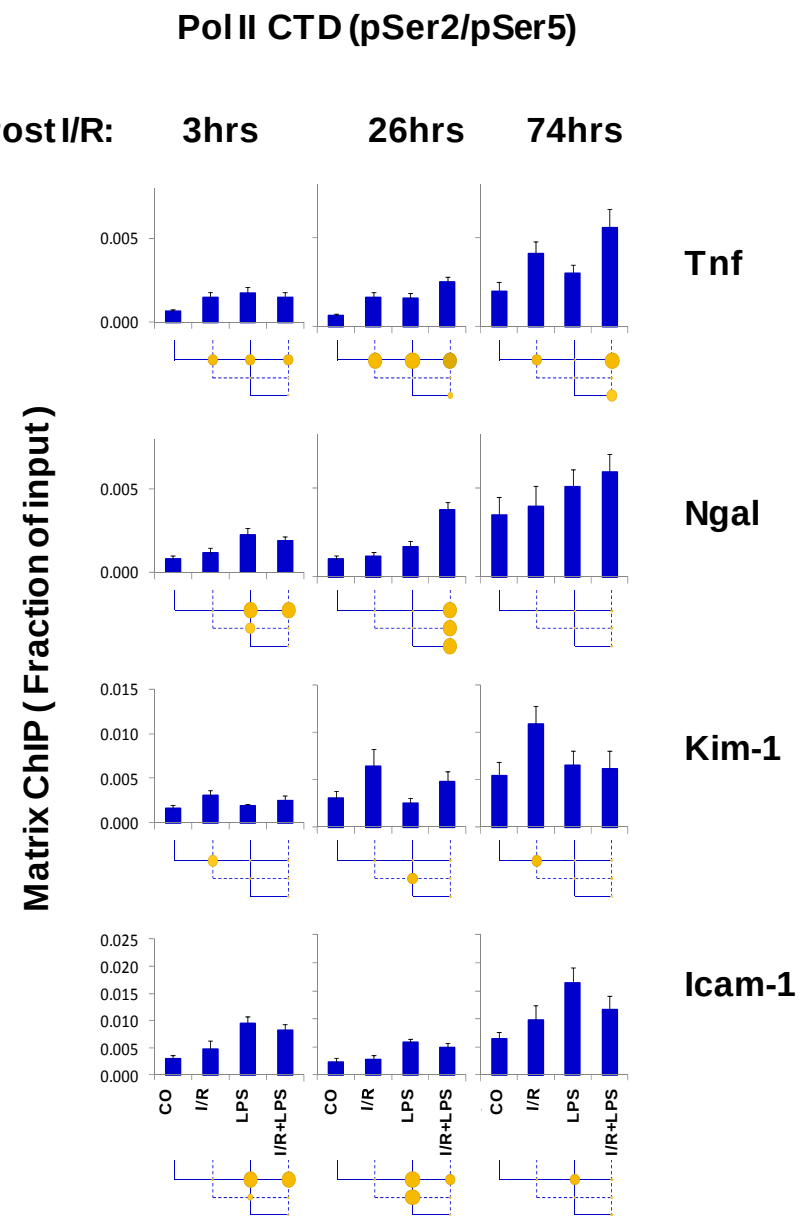

Fig.S2. Analysis of phosphorylated serine 2 and 5 RNA polymerase II CTD (Pol II CTD pSer2/pSer5)) at Tnf, Ngal, Kim-1 and Icam-1 genes following unilateral kidney I/R and LPS injection. Sheared cross-linked renal cortex chromatin from mice were assayed using a polyclonal antibody that detects phosphorylated CTD on serine 2 and 5. ChIP DNA were analyzed at Tnf, Ngal, Kim-1 and Icam-1 genes in real-time PCR. Data represent mean ± SEM (6 animals from each group), expressed as fraction of input.

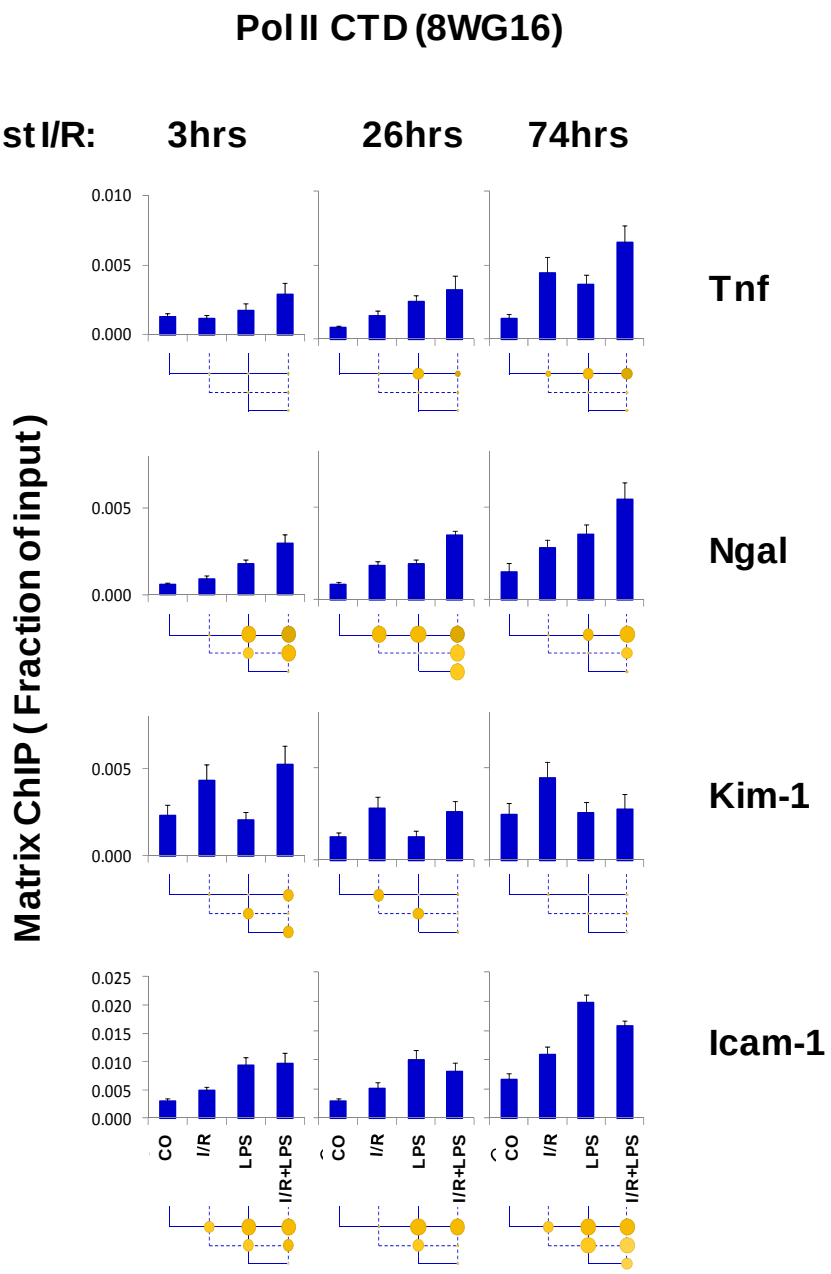

Fig.S3. Analysis of unphosphorylated RNA polymerase II CTD (Pol II CTD) at Tnf, Ngal, Kim-1 and Icam-1 genes following unilateral kidney I/R and LPS injection. Sheared cross-linked renal cortex chromatin from mice were assayed using a monoclonal antibody that detects unphosphorylated CTD (8WG16). ChIP DNA were analyzed at Tnf, Ngal, Kim-1 and Icam-1 genes in real-time PCR. Data represent mean ± SEM (6 animals from each group), expressed as fraction of input.

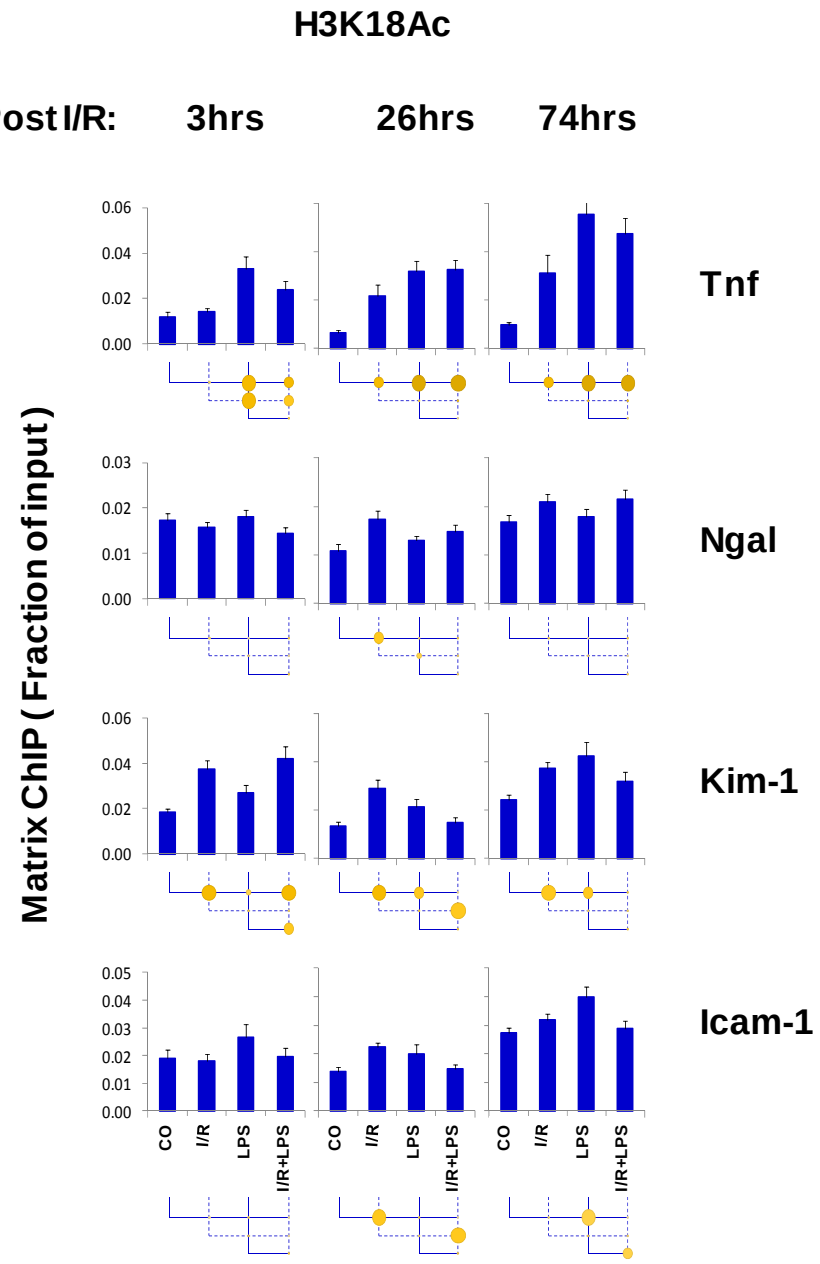

Fig.S4. Permissive histone H3 lysine 18 acetylation (H3K18Ac) at Tnf, Ngal, Kim-1 and Icam-1 genes following unilateral kidney I/R and LPS injection. Sheared cross-linked renal cortex chromatin from mice were assayed using polyclonal antibody to H3K18Ac antibody. Data represent mean ± SEM (6 animals from each group), expressed as fraction of input.

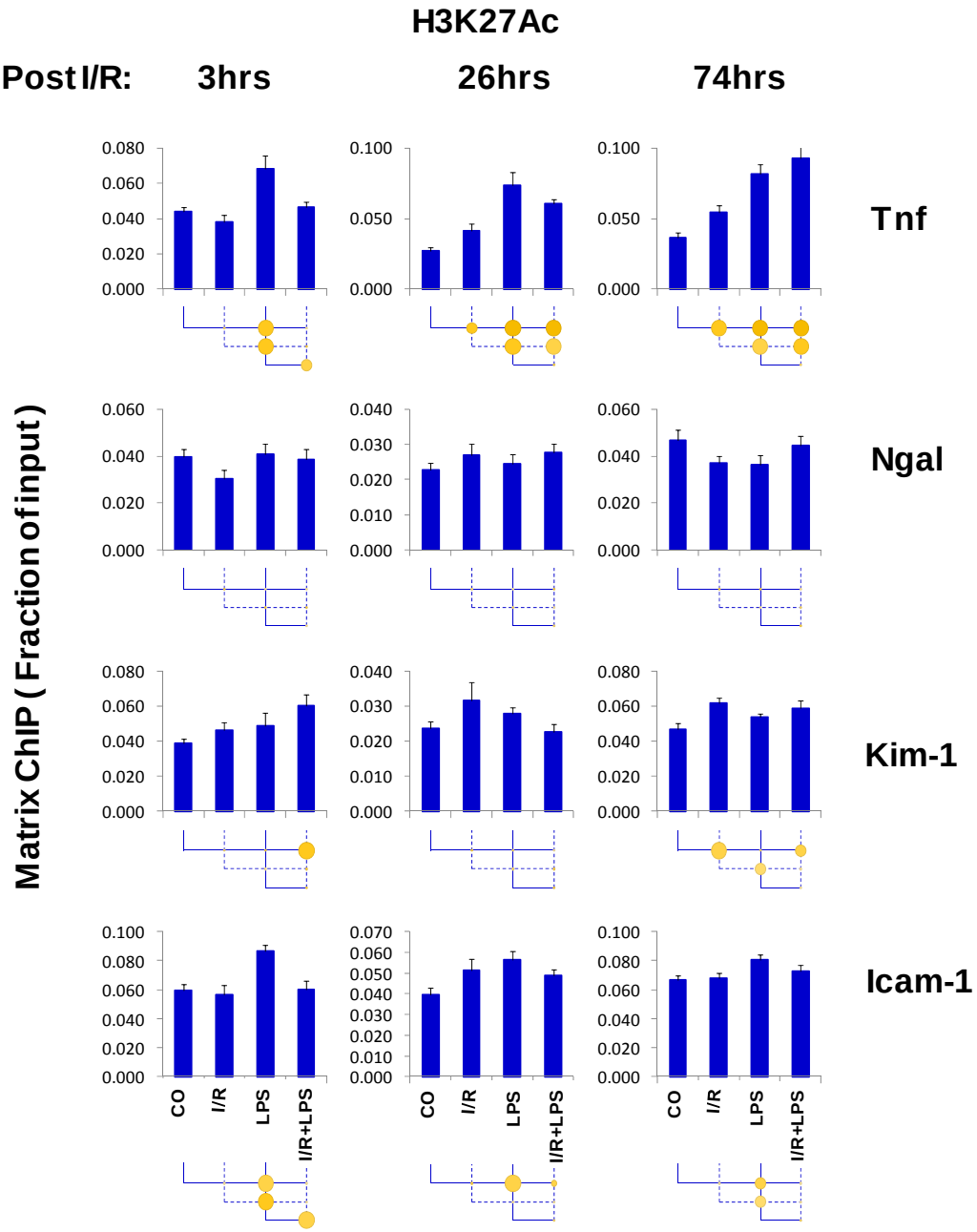

Fig.S5. Permissive histone H3 lysine 27 acetylation (H3K27Ac) at Tnf, Ngal, Kim-1 and Icam-1 genes following unilateral kidney I/R and LPS injection. Sheared cross-linked renal cortex chromatin from mice were assayed using monoclonal antibody to H3K27Ac antibody. Data represent mean ± SEM (6 animals from each group), expressed as fraction of input.

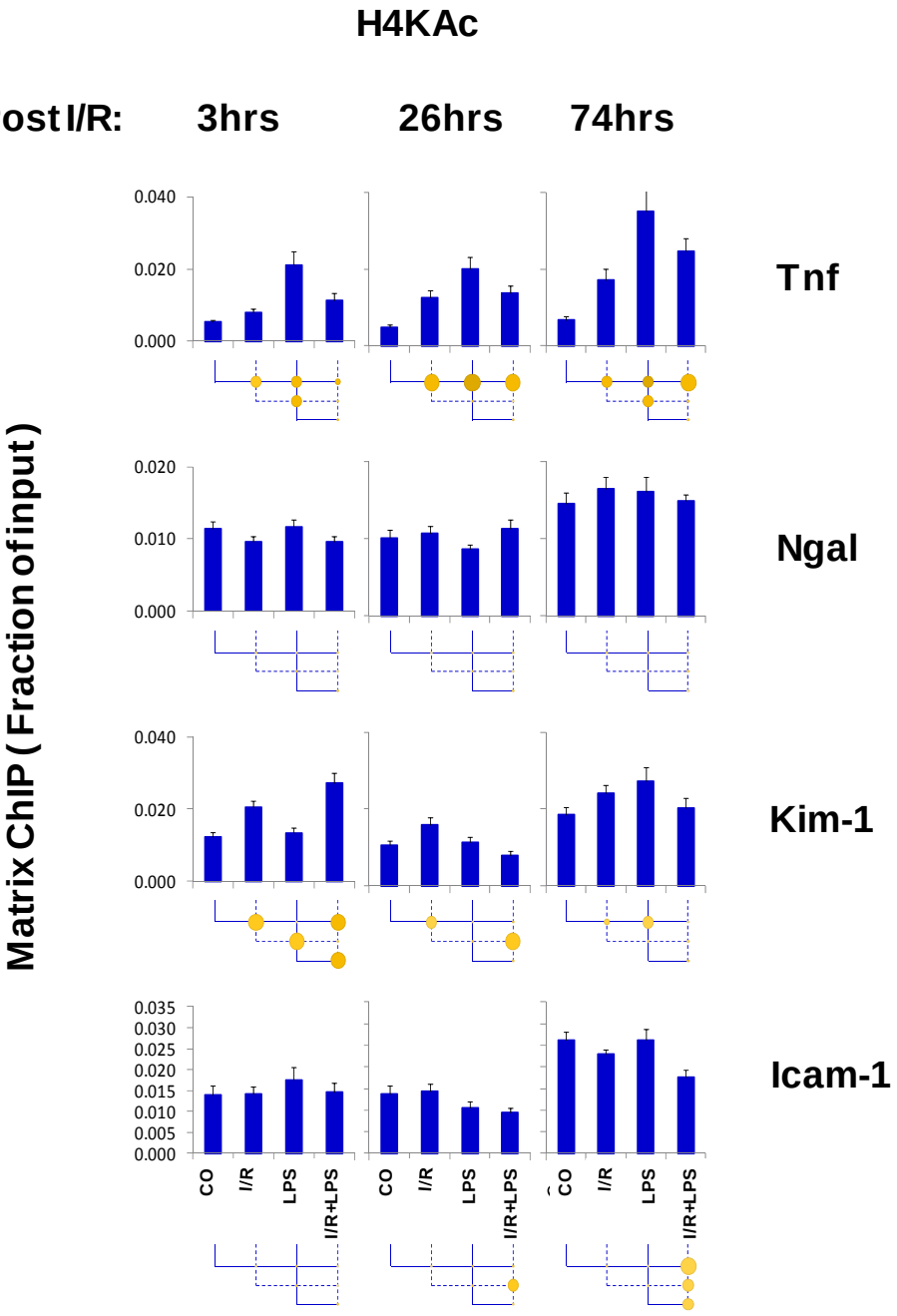

Fig.S6. Permissive histone H4 lysine 5,8,12,16Ac acetylation (H4KAc) at Tnf, Ngal, Kim-1 and Icam-1 genes following unilateral kidney I/R and LPS injection. Sheared cross-linked renal cortex chromatin from mice were assayed using rabbit antiserum to H4K5/8/12,16Ac. Data represent mean ± SEM (6 animals from each group), expressed as fraction of input.

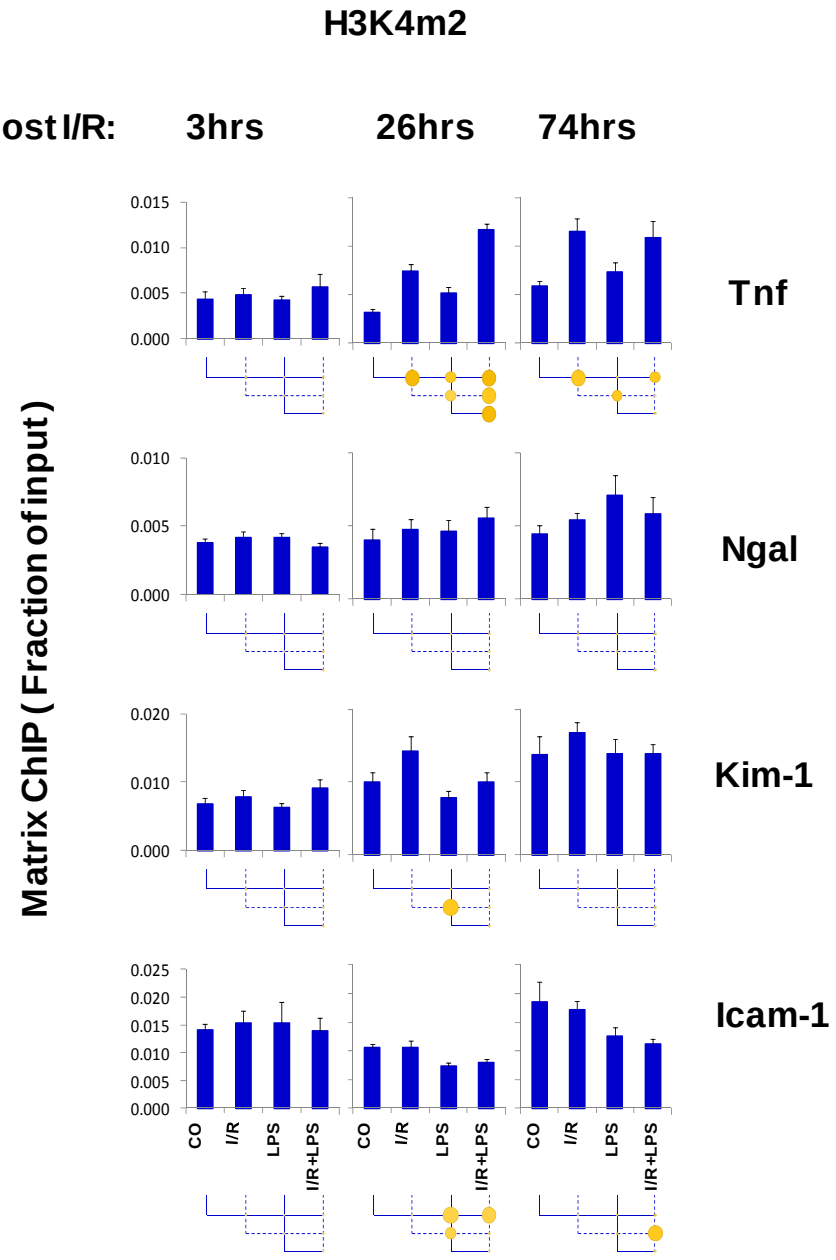

Fig.S7. Permissive histone H3 lysine 4 di-methylation (H3K4m2) at Tnf, Kim-1, Ngal, and Icam-1 genes following unilateral kidney I/R and LPS injection. Sheared cross-linked renal cortex chromatin from mice were assayed using polyclonal antibody to H3K4m2. Data represent mean ± SEM (6 animals from each group), expressed as fraction of input.

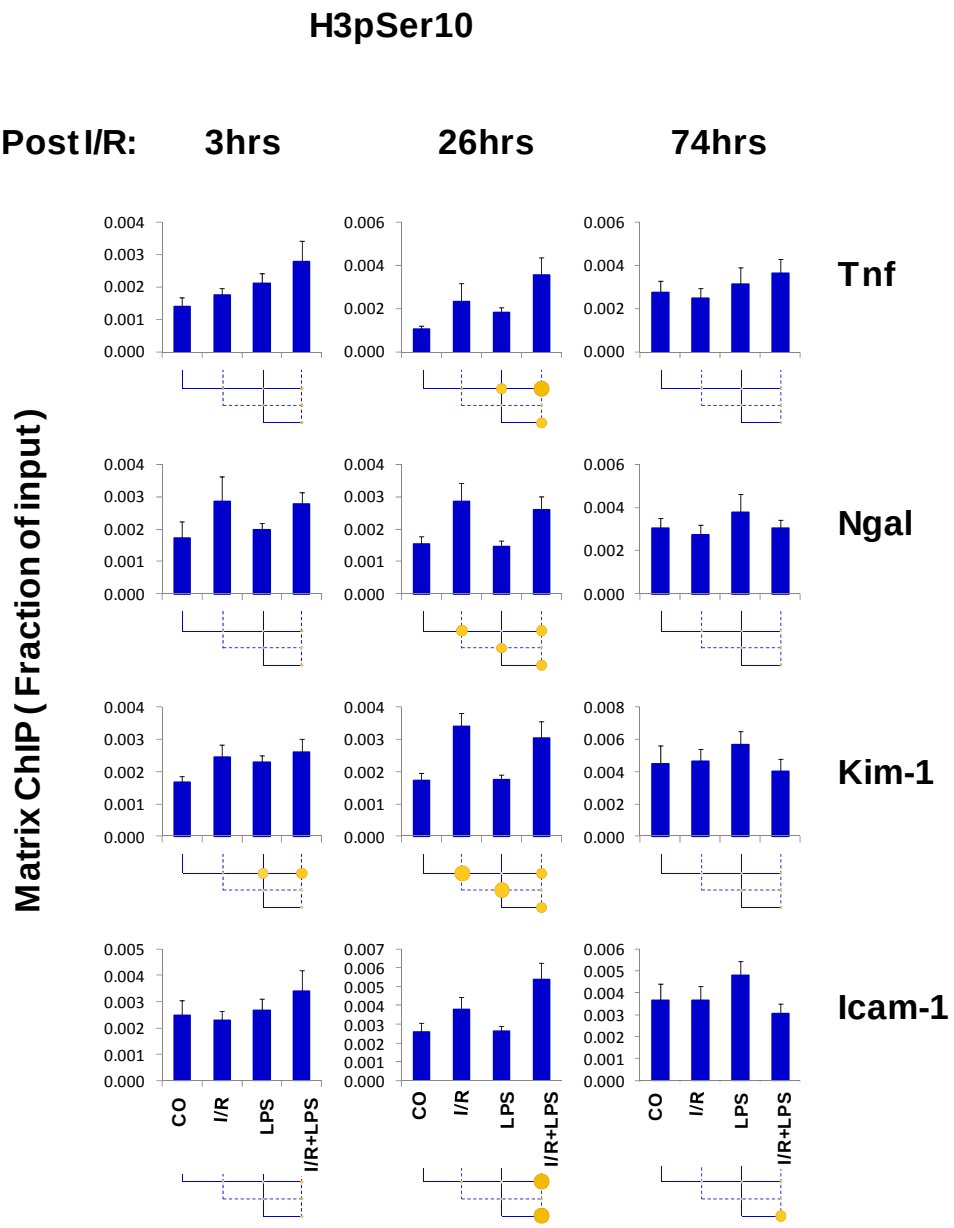

Fig.S8. Transcription permissive histone H3 serine10 phosphorylation (H3pSer10) at Tnf, Kim-1, Ngal, and Icam-1 genes following unilateral kidney I/R and LPS injection. Sheared cross-linked renal cortex chromatin from mice were assayed using antibody to H3pSer10. Data represent mean ± SEM (6 animals from each group), expressed as fraction of input.

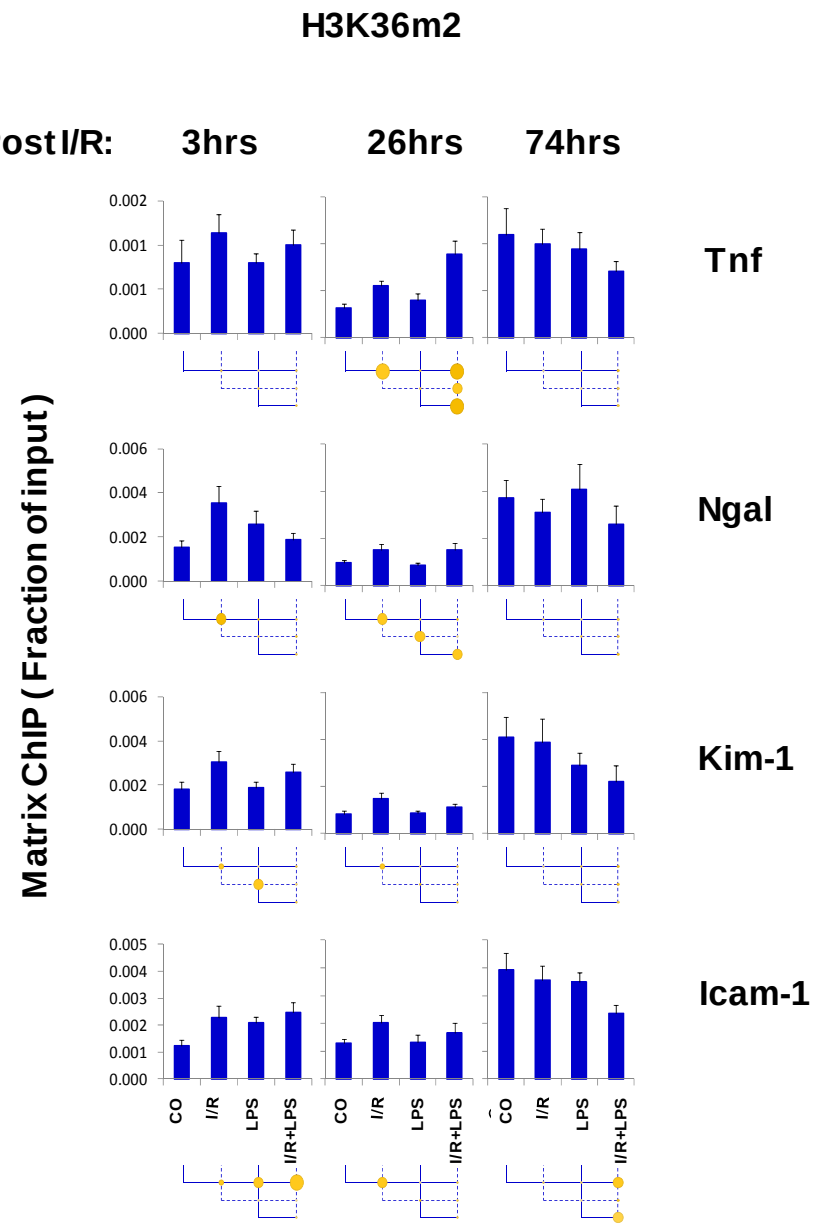

Fig.S9. Transcription elongation histone H3 lysine 36 di-methylation (H4K36m2) at Tnf, Kim-1, Ngal, and Icam-1 genes following unilateral kidney I/R and LPS injection. Sheared cross-linked renal cortex chromatin from mice were assayed using polyclonal antibody to H3K36m2. Data represent mean ± SEM (6 animals from each group), expressed as fraction of input.

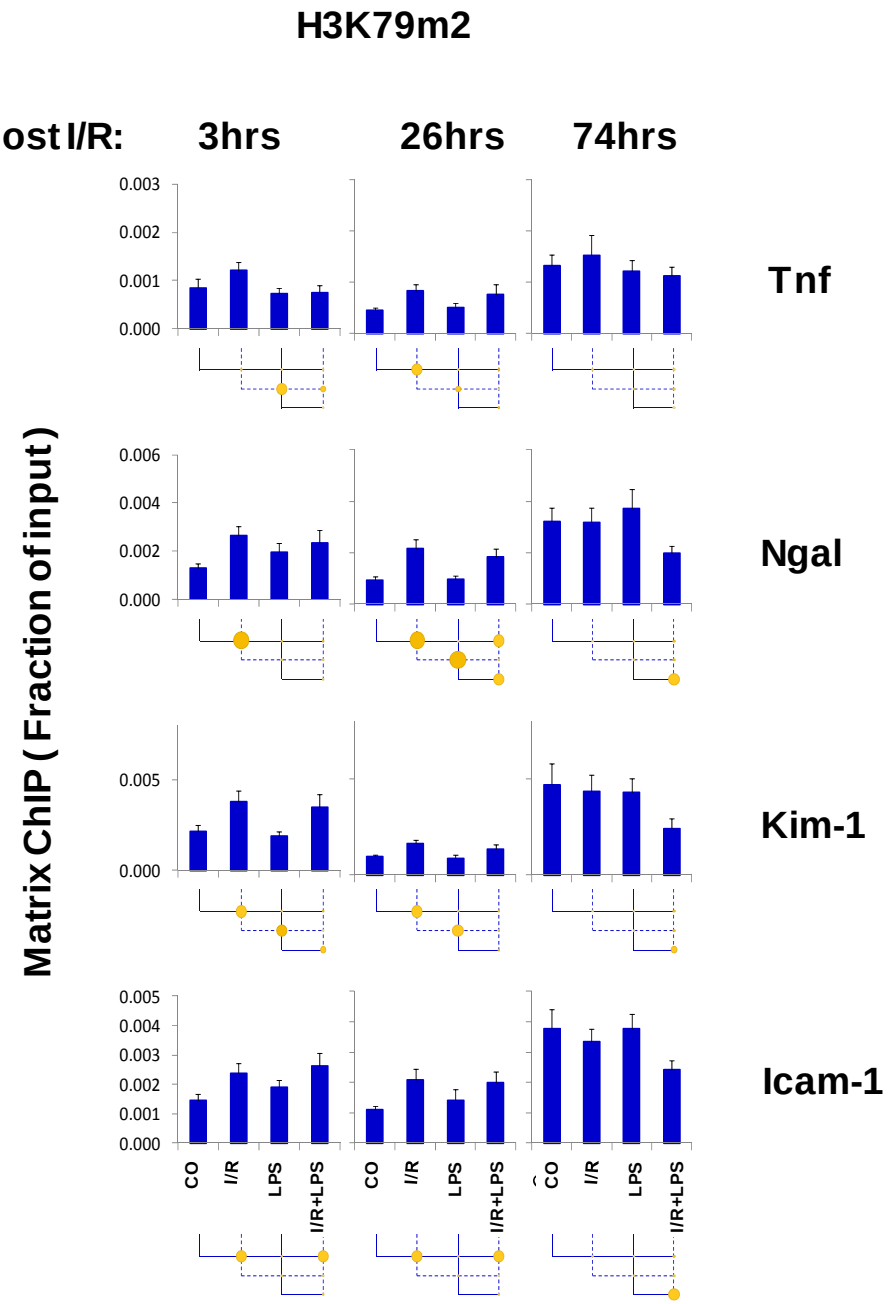

Fig.S10. Transcription elongation histone H3 lysine 79 di-methylation (H4K79m2) at Tnf, Kim-1, Ngal, and Icam-1 genes following unilateral kidney I/R and LPS injection. Sheared cross-linked renal cortex chromatin from mice were assayed using polyclonal antibody to H3K79m2. Data represent mean ± SEM (6 animals from each group), expressed as fraction of input.

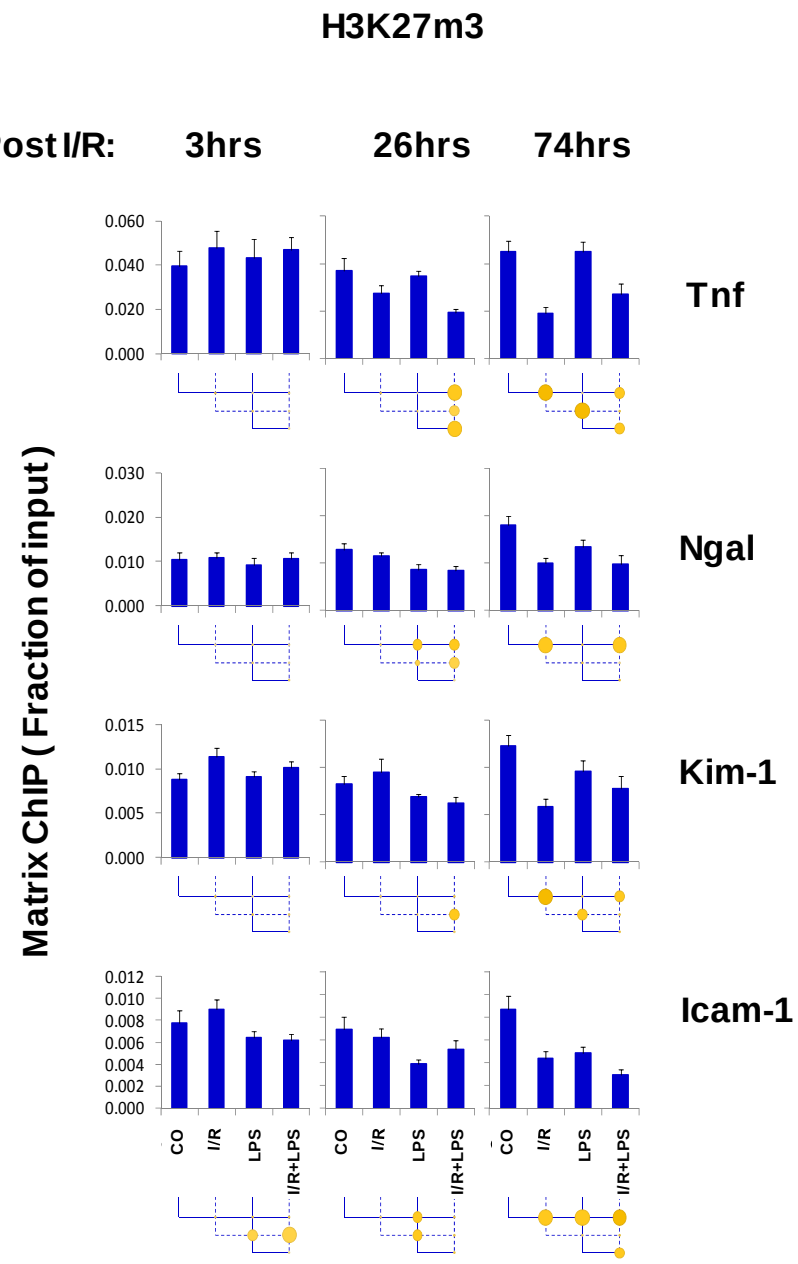

Fig.S11. Transcription repressive histone H3 lysine 27 tri-methylation (H3K27m3) at Tnf, Ngal, Kim-1, and Icam-1 genes following unilateral kidney I/R and LPS injection. Sheared cross-linked renal cortex chromatin from mice were assayed using monoclonal antibody to H3K27m3. Data represent mean ± SEM (6 animals from each group), expressed as fraction of input.

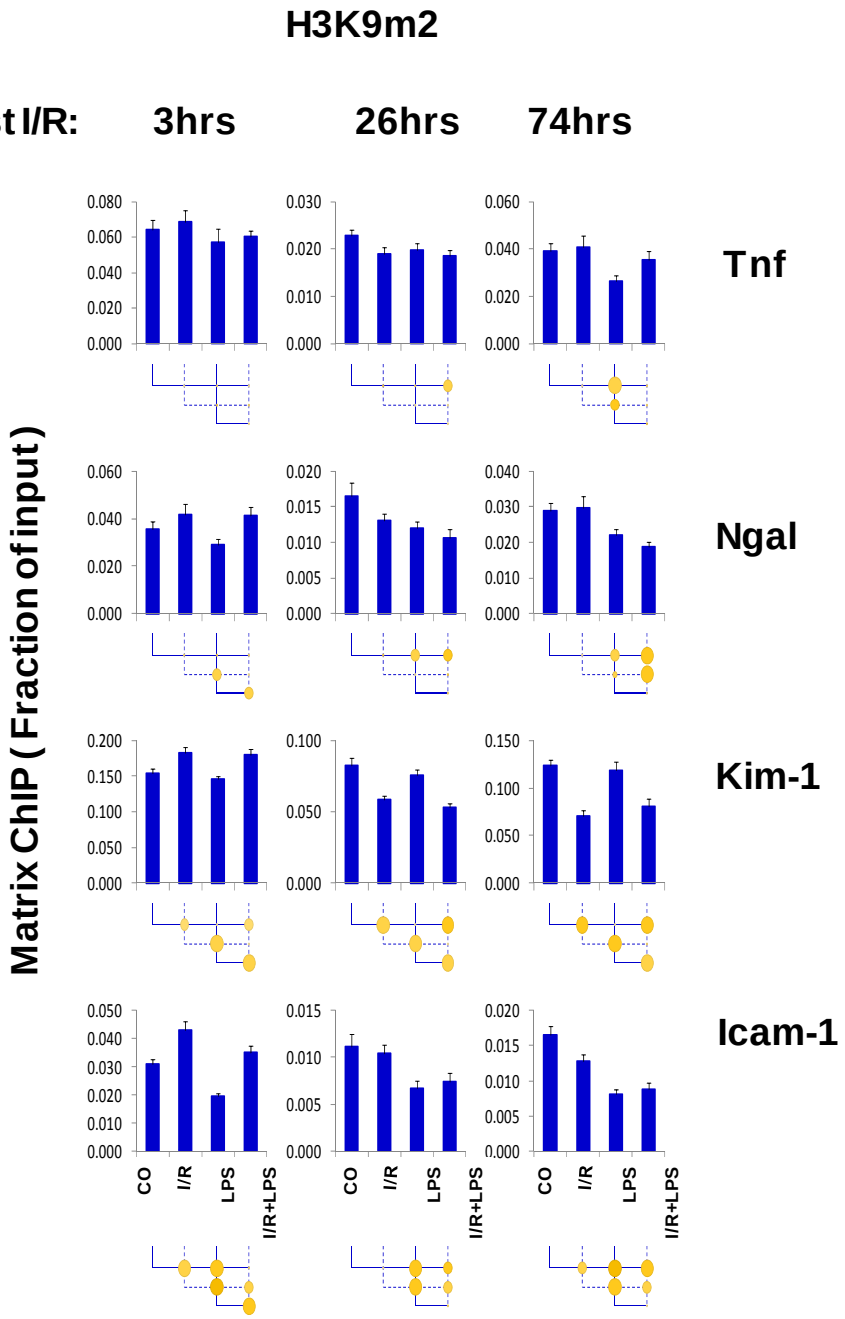

Fig.S12. Transcription repressive histone H3 lysine 9 di-methylation (H3K9m2) at Tnf, Ngal, Kim-1, and Icam-1 genes following unilateral kidney I/R and LPS injection. Sheared cross-linked renal cortex chromatin from mice were assayed using monoclonal antibody to H3K9m2. Data represent mean ± SEM (6 animals from each group), expressed as fraction of input.

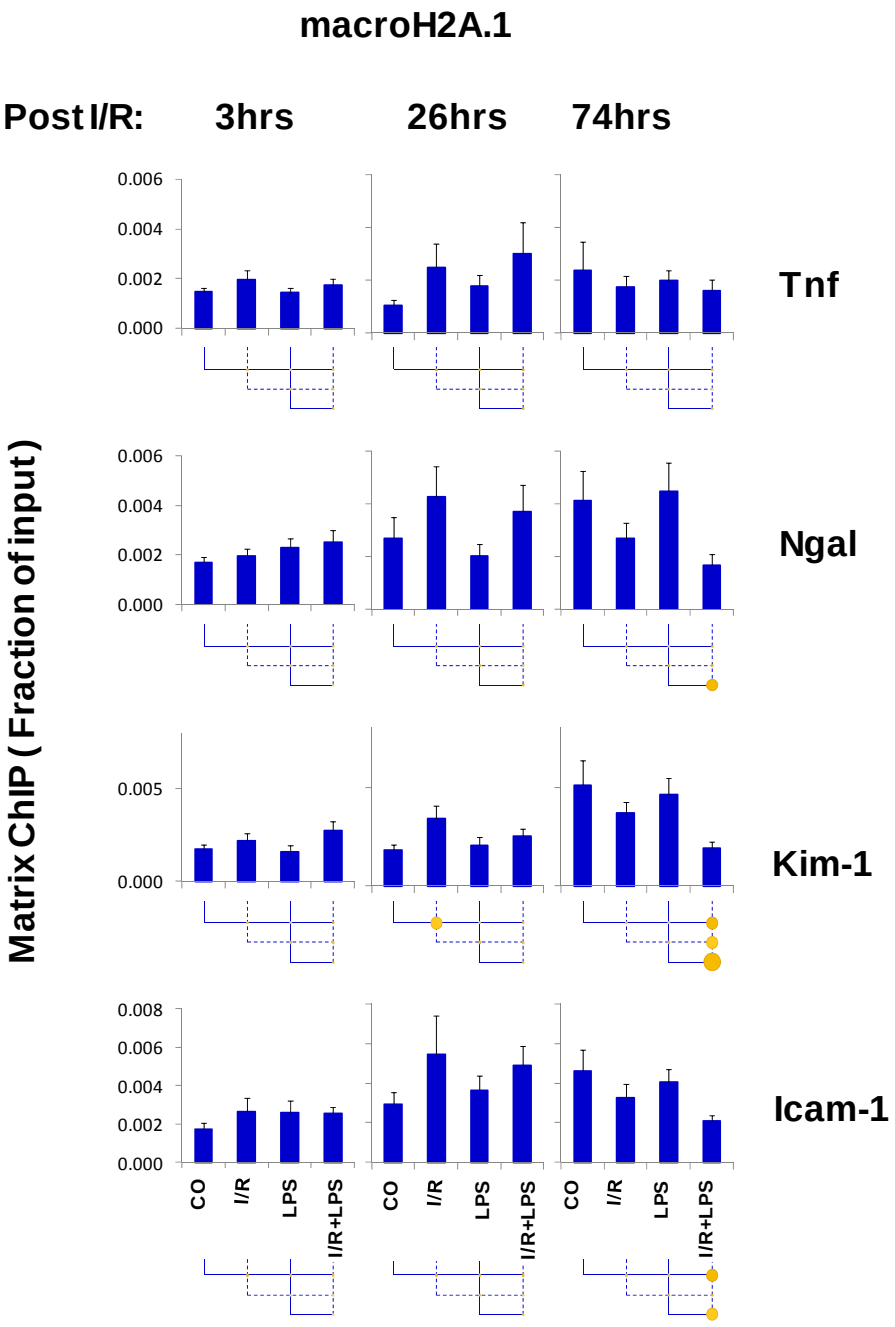

Fig.S13. Transcription repressive variant histone macro-H2A.1 (macroH2A.1) at Tnf, Ngal, Kim-1, and Icam-1 genes following unilateral kidney I/R and LPS injection. Sheared cross-linked renal cortex chromatin from mice were assayed using polyclonal antibody to macroH2A.1. Data represent mean ± SEM (6 animals from each group), expressed as fraction of input.

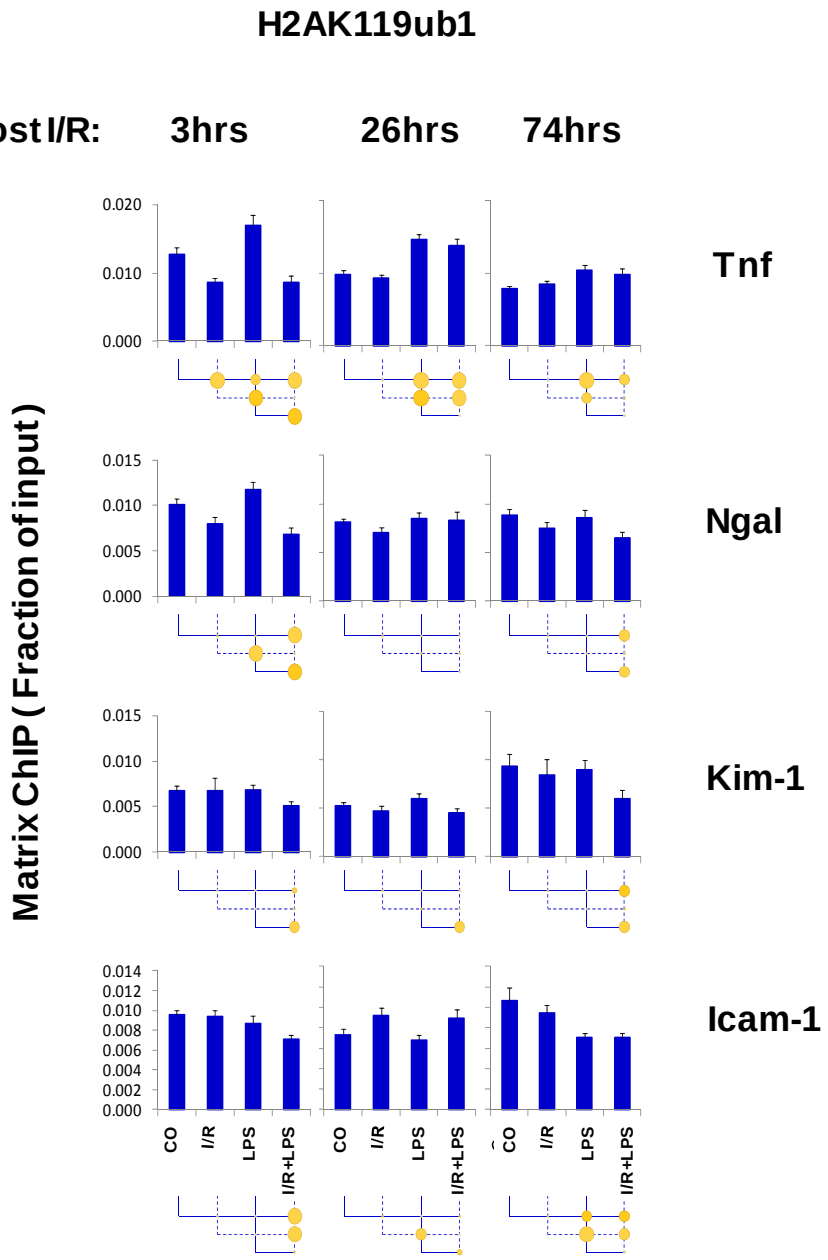

Fig.S14. Transcription repressive histone H2A lysine 119 mono- ubiquitination (H2AK119ub1) at Tnf, Ngal, Kim-1, and Icam-1 genes following unilateral kidney I/R and LPS injection. Sheared cross-linked renal cortex chromatin from mice were assayed using rabbit monoclonal antibody to H2AK119ub1. Data represent mean ± SEM (6 animals from each group), expressed as fraction of input.

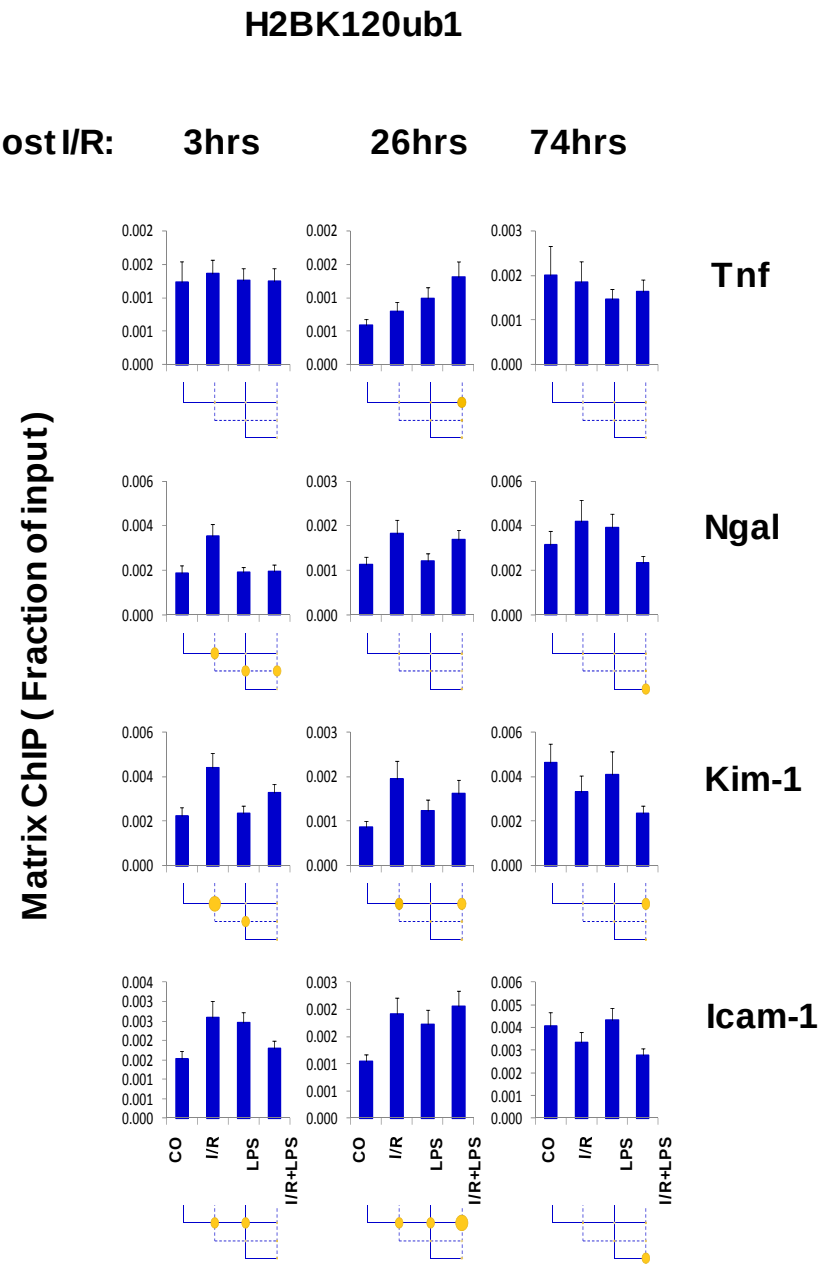

Fig.S15. Transcription repressive histone H2B lysine 120 mono- ubiquitination (H2BK120ub1) at Tnf, Ngal, Kim-1, and Icam-1 genes following unilateral kidney I/R and LPS injection. Sheared cross-linked renal cortex chromatin from mice were assayed using mouse monoclonal antibody to H2BK120ub1. Data represent mean ± SEM (6 animals from each group), expressed as fraction of input.

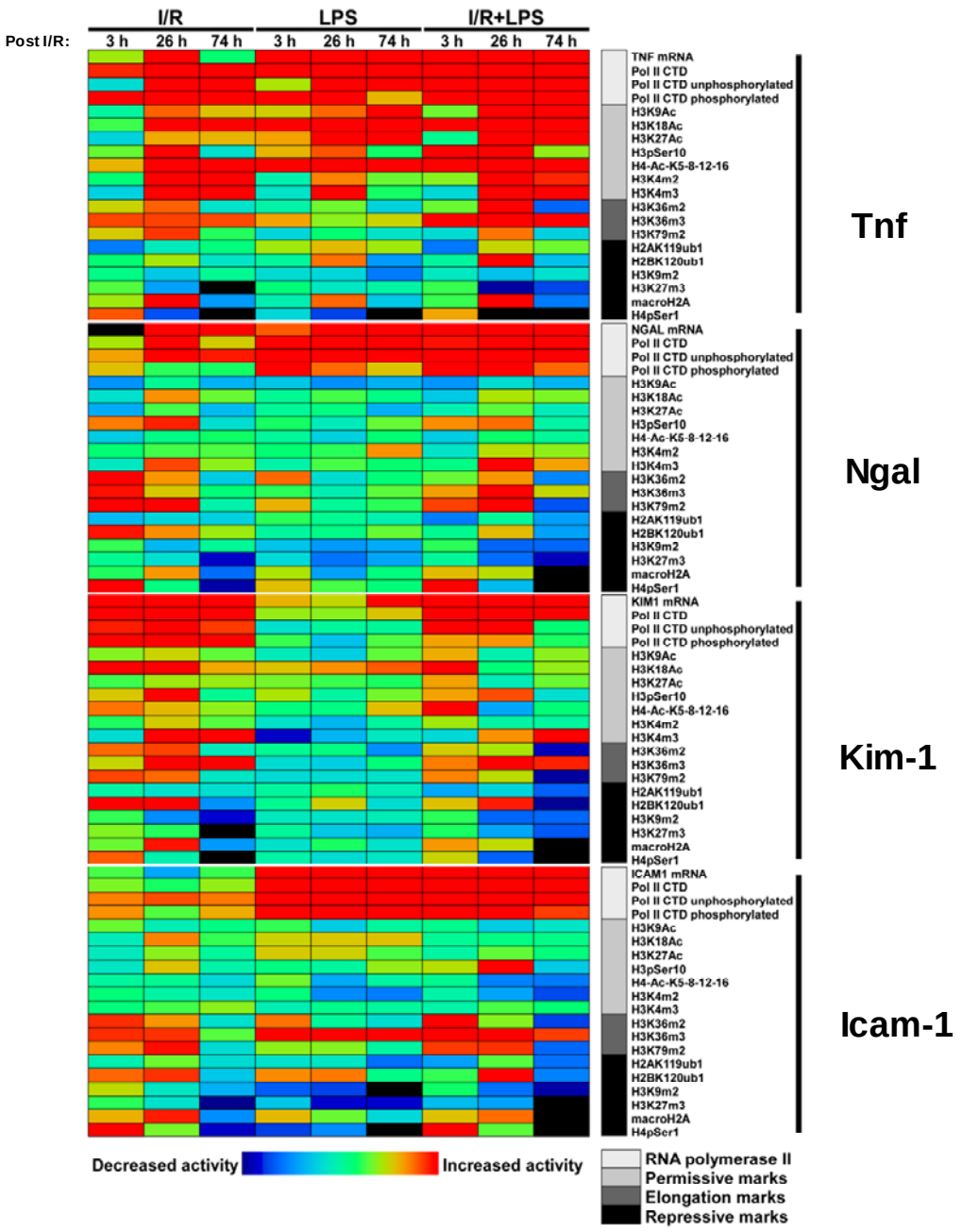

Fig.S16 Integrated transcriptional and epigenetic analysis of Tnf, Kim-1, Ngal, and Icam-1 portraits following unilateral kidney injury. Log-transformed values for mRNA and Pol II levels, as well as several epigenetic modifications during temporal progression of AKI due to I/R, LPS and I/R+LPS are depicted as a heatmap. While there is a general increase in permissive marks and reduction of repressive marks in a time-dependent manner, note the heterogeneous response pattern of individual AKI-induced genes.