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Tissue-Specific Induction of Mouse ZIP8 and ZIP14 Divalent Cation/Bicarbonate Symporters by, and Cytokine Response to, Inflammatory Signals

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Abstract

Mouse *Slc39a8* and *Slc39a14* genes encode ZIP8 and ZIP14, respectively, which are ubiquitous divalent cation/(HCO₃⁻)₂ symporters responsible for uptake of Zn²⁺, Fe²⁺ and Mn²⁺ into cells. Cd²⁺ and other toxic nonessential metals can displace essential cations, thereby entering vertebrate cells. Whereas *Slc39a8* encodes a single protein, *Slc39a14* has two exons 4 which, via alternative splicing, give rise to ZIP14A and ZIP14B; why differences exist in cell-type-specific expression of ZIP14A and ZIP14B remains unknown. Inflammatory stimuli have been associated with ZIP8 and ZIP14 up-regulation, but a systematic study of many tissues simultaneously in a laboratory animal following inflammatory cytokine exposure has not yet been reported. Herein we show that C57BL/6J male mice—treated intraperitoneally with lipopolysaccharide (LPS), or the proinflammatory cytokines tumor necrosis factor (TNF) or interleukin-6 (IL6)—exhibited quantitatively very different, highly tissue-specific, and markedly time-dependent up- and down-regulation of ZIP8, ZIP14A and ZIP14B mRNA levels in twelve tissues. Magnitude of the inflammatory response was confirmed by measuring the proinflammatory cytokine TNF, IL6 and interleukin-1 β (IL1B) mRNA levels in the same tissues of these animals. Our data suggest that most if not all tissues use ZIP8, ZIP14A and/or ZIP14B) for Zn²⁺ uptake, some tissues under basal conditions and others more so when inflammatory stressors are present; collectively, this might lead to substantial alterations in plasma Zn²⁺ levels, due to Zn²⁺ redistribution not just in liver, but across many vital organs. In the context of cadmium-mediated toxicity, our data suggest that tissues other than liver, kidney and lung should also be considered.

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Declaration of Conflicting Interests

The authors declare no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Supplemental Material

Supplementary files located online at <http://ijt.sagepub.com/supplemental>.

Keywords

ZIP transporter gene family; *Slc39* gene family; ZIP8 Zn²⁺/(HCO₃⁻)₂ symporter; ZIP14 Zn²⁺/(HCO₃⁻)₂ symporter; inflammation; tumor necrosis factor; interleukin-6; lipopolysaccharide

Introduction

Characterization of the ZIP8 and ZIP14 transporters has led to greater appreciation of interactions between Zn²⁺ uptake, intracellular Zn²⁺ levels, and inflammation. Following discovery of variable response to cadmium toxicity among inbred strains of mice, the *Cdm* locus was shown to be primarily associated with this phenotypic difference^{1,2}. Subsequently, the mouse *Slc39a8* gene was identified³, and its encoded ZIP8 protein found to be a divalent metal/bicarbonate symporter^{4,5}. Direct proof that the *Slc39a8* gene was indeed the *Cdm* locus was confirmed by bacterial artificial chromosome (BAC) transgenesis; a BAC carrying only the *Slc39a8* gene plus 87 kb 5′- and 18 kb 3′-flanking regions from a “cadmium-sensitive” mouse—inserted into the genome of a “cadmium-resistant” mouse—reverted the phenotype to cadmium sensitivity^{6,7}.

The *SLC39* family comprises 14 genes highly conserved between mouse and human; phylogenetic analysis showed mammalian *SLC39A8* was evolutionarily most closely related to *SLC39A14*, the two genes having diverged from one another less than 420 million years ago^{7,8}. The *Slc39a14* gene encodes ZIP14, also a divalent metal/bicarbonate symporter⁸. Both ZIP8⁵ and ZIP14⁹ transport an electroneutral complex, Zn²⁺/(HCO₃⁻)₂, in which Zn²⁺ can be displaced by Fe²⁺ or Mn²⁺, as well as nonessential metal cations including Cd²⁺, Hg²⁺, Pb²⁺, Pt²⁺ and U²⁺⁹. Others have also demonstrated that ZIP8^{10–13} and ZIP14^{12–16} function to import endogenous Zn²⁺, Fe²⁺ and/or Mn²⁺ into the cell. In fact, a hypomorphic mouse line—expressing ~10% of wild-type ZIP8 levels in all tissues and exhibiting markedly lowered Zn²⁺ and Fe²⁺ tissue levels—suffers severe defects in hematopoiesis and in multiple-organ organogenesis, which are neonatal-lethal¹⁷.

From the beginning of mammalian ZIP8 and ZIP14 studies, there has been a close association with inflammatory stimuli. Mammalian ZIP8 was discovered when a cDNA library—prepared from human monocytes stimulated with *M. bovis* BCG cell wall—was screened to identify novel genes induced by inflammatory danger signals; inflammatory cytokines such as tumor necrosis factor (TNF) were shown to be potent inducers of the ZIP8 transcript¹⁸.

Subsequent cytokine-activation studies include: lipopolysaccharide (LPS), TNF and interleukin-6 (IL6) induction of ZIP14 in mouse liver but not small intestine or spleen¹⁹; TNF-mediated up-regulation of ZIP8 but not ZIP14 in human lung¹⁰; effects of ZIP8 overexpression vs siRNA-mediated ZIP8 knockdown on Zn²⁺ levels during expression of interferon- γ in human T-cells²⁰; recognition of ZIP14 participation in gluconeogenesis as well as G-protein-coupled receptor-mediated signaling in mouse bone, pituitary gland and liver²¹; LPS-induced endotoxemia stimulating ZIP14 levels in liver, white adipose tissue and muscle of *Slc39a14*(-/-) knockout mice²²; importance of increased Zn²⁺ levels during ZIP14- or nitric oxide-mediated resistance to LPS-induced apoptosis in sheep pulmonary

artery endothelial cell cultures²³; identification of a negative feedback loop in fetal fibroblasts from *Slc39a8*-hypomorphic mice—involving ZIP8, I κ B kinase and NF κ B—which directly regulates innate immune function through coordination of Zn²⁺ metabolism²⁴; demonstration of LPS as a strong inducer of ZIP14 in human macrophages²⁵; and recent demonstration that “the Zn²⁺-ZIP8-MTF1 axis” represents an essential catabolic regulator during the pathogenesis of osteoarthritis²⁶.

These studies demonstrate the ubiquitous nature of inflammatory cytokine-induced ZIP8 and/or ZIP14 in numerous mammalian tissues and cell types; however, these previous ZIP8 and ZIP14 studies had shown LPS or TNF treatment usually at 32 h, and IL6 treatment at 16 h, and only in a few tissues. Thus, a systematic examination of numerous tissues in the same animal, as a function of time following administration of inflammatory stimuli, has not yet been reported. Herein we show quantitative real-time polymerase chain reaction (qRT-PCR) measurements of ZIP8 and ZIP14 and cytokine mRNA levels in 12 tissues of C57BL/6J mice over a 32-h period after LPS and TNF administration, and over a 16-h period following IL6 treatment.

Materials and Methods

Chemicals

LPS, TNF, and IL6 were purchased from Sigma-Aldrich (St. Louis, Missouri). Dilutions were made in 0.09% NaCl (saline) solution and filtered through a 0.22- μ m syringe prior to injection of the mice.

Treatment of Animals, Harvesting of Tissues

C57BL/6J male mice between 6 and 8 weeks of age were purchased from The Jackson Laboratory (Bar Harbor, Maine); following acclimation to our mouse colony for 5–7 days, these mice were studied. All mouse experiments were conducted in accordance with the National Institutes of Health standards for the care and use of experimental animals and University Cincinnati Medical Center Institutional Animal Care and Use Committee [protocol #11-09-12-01; approved 5 September 2008 → 4 Sept 2011 → 3 Sept 2014].

Subsequent to intraperitoneal treatment with LPS (5 mg/kg), TNF (5 μ g/kg), or IL6 (100 μ g/kg), the mice (N=3 per time-point) were killed at 2, 4, 8, 16 or 32 h; untreated mice provided the zero-hour time-point, and all experiments were repeated at least twice. Twelve tissues (collected at the six time-points) included liver, proximal small intestine (PSI), kidney, lung, heart, brain, pancreas, spleen, testis, urinary bladder, skin, and preputial gland duct (PGD). PSI samples represent the first 4 cm beyond the pyloric sphincter. PGD samples were isolated from tissues under the skin of the penis; surrounding fat was carefully separated so that the preputial gland could be harvested without breaking, as well as with virtually no other attached tissue (surrounding skin, cartilage, collagenous tissue or muscle).

Total RNA Preparation

With Tri-Reagent (TR18, Molecular Research Center, Inc.; Cincinnati, OH), total RNA was isolated from the 12 above-mentioned organs or tissues, following the protocol recommended by the vendor.

Reverse Transcription and qRT-PCR

Total RNA (2.5 µg) from each sample was used as template for reverse transcription and primed with oligo(dT), using the (Verso cDNA kit, AB-1453/B, Thermo Scientific; Waltham, MA). Total RNA (2.5 µg) was added to reactions containing 3.8 µM oligo(dT)₂₀ and 0.77 mM dNTP, to a final volume of 13 µL; reactions were incubated at 65°C for 5 min, and then 4°C for 2 min. Next, we added 7 µL of solution containing 14 mM dithiothreitol and 40 units of RNaseOUT Recombinant RNase inhibitorTM (Verso cDNA kit). After incubation at room temperature for 2 min, 1 µL of reverse transcriptase was added to each sample. Reaction tubes were incubated at 42°C for 30 min, followed by 75°C for 10 min (to inactivate reverse transcriptase), and placed immediately on ice. Diethylpyrocarbonate (DEPC)-treated distilled water (80 µL) was added to dilute the cDNA that had been generated, and the resultant mixture was stored at -80°C until use.

We performed qRT-PCR in the Bio-Rad DNA Engine Opticon 2TM (Bio-Rad Laboratories; Hercules, CA), using iQ SYBR Green SupermixTM (170-8882, Bio-Rad Laboratories). The housekeeping gene's β-actin mRNA (ACTB) was employed as the internal control. Standard curves using serial dilutions of total RNA resulted in excellent linearity ($r=0.99$), indicating our qRT-PCR results were valid. All primers used are available upon request.

Statistics

All experiments were confirmed in at least triplicate and repeated twice. Statistical analysis was performed using the software SPSS version 13.0 (Microsoft®; Somers, NY). Data are presented as means ± S.E.M. of triplicate samples. *P*-values <0.05 are regarded as statistically significant.

Results and Discussion

Whereas *SLC39A8* and *SLC39A14* originated from one ancestral gene in fish, they diverged into two distinct genes (on different chromosomes) after the sea animal/land animal split^{7,8}. Although the gene structures are similar, *i.e.* both mouse genes having three noncoding alternative exons-1 and eight coding exons, *Slc39a14* contains alternative exons 4a and 4b. Thus, different amino acids in exons 4a and 4b were found to cause two distinct proteins having differential expression patterns in various tissues^{7,8}. To date, no antibody has been generated that distinguishes between the two proteins. K_m values for Zn²⁺, Fe²⁺ and Cd²⁺ uptake in *Xenopus* oocytes, nonetheless, were shown to be distinctly very different between ZIP14A and ZIP14B⁹: 0.36 µM vs 0.78 µM for Zn²⁺, respectively; 0.19 µM vs 0.64 µM for Fe²⁺, respectively; and 0.54 µM vs 0.31 µM for Cd²⁺, respectively. However, the significance of tissue-specific functions of these two ZIP14 distinct proteins remains to be determined. It should be noted that, however, by using different primers, one can quantify ZIP14A and ZIP14B mRNA levels by qRT-PCR in various tissues.

Effects of LPS, TNF and IL6 on ZIP mRNA Levels

LPS treatment (Figure 1A; Table 1. A; Table S1. A)—ZIP8 mRNA induction by LPS was highest in bladder at 2 & 16 h; ZIP14A mRNA highest in skin at 8 h but was elevated during the first 16 h; and ZIP14B mRNA elevated highest in lung at 16 h but slightly elevated at all time-points. All induced ZIP mRNA levels peaked between 2 and 16 h, and usually were headed toward baseline by 32 h; exceptions included ZIP14B in lung and kidney, which remained substantially elevated at 32 h. Other than bladder, LPS-induced ZIP8 was negligible in the other 11 tissues; actually, ZIP8 was significantly down-regulated in liver, heart and brain. In liver both ZIP14A and ZIP14B mRNA levels were also down-regulated by LPS treatment, significantly at the 16-h time-point.

TNF treatment (Figure 1B, Table 1. B; Table S1. B)—ZIP8 mRNA levels were extremely high in pancreas at 4, 8 & 16 h; and both ZIP14A mRNA and ZIP14B mRNA highest in skin at all early time-points, and ZIP14B in pancreas at 16 h. TNF-induced ZIP mRNAs were negligible over the 32-h period in: for ZIP8 in liver, PSI, lung, spleen, testis and skin; for ZIP14A in liver, kidney, lung, pancreas, spleen, testis, bladder and PGD; for ZIP14B in lung, brain, spleen, testis, bladder and PGD. ZIP8 mRNA levels were significantly down-regulated at early time-points in kidney, brain and PGD. ZIP14A was significantly down-regulated by TNF in brain at 8 h, and ZIP14B in kidney at 4 h.

IL6 treatment (Figure 1C; Table 1. C; Table S1. C)—There was no significant up-regulation by IL6 of ZIP8, ZIP14A or ZIP14B mRNA in any of the 12 tissues over the 16-h experiment. Significant down-regulation was observed for ZIP8 in liver, lung, brain and urinary bladder at 8 and/or 16 h; for ZIP14A in liver, lung and heart at 16 h; and for ZIP14B in liver and heart at 16 h and testis at 4 h.

Effects of LPS, TNF and IL6 on Cytokine mRNA Levels

LPS treatment (Figure 2A; Table 1. A; Table S1. A)—TNF, IL6 and IL1B mRNA levels were all highest in PSI and urinary bladder at 2 h, remaining significantly elevated throughout the 32-h experiment in PSI, and through the first 16 h in bladder for TNF and IL6 mRNA. TNF mRNA was also significantly up-regulated in lung at 2 h. IL6 mRNA was also elevated at early time-points in kidney, lung, heart, brain, spleen, testis, skin and PGD. LPS-mediated stimulation of IL1B mRNA was also significantly increased in lung and brain persistently through the 32-h experiment. Significant down-regulation was seen only for IL6 mRNA in pancreas and PGD at 32 h, and for IL1B mRNA in pancreas at 32 h.

TNF treatment (Figure 2B; Table 1. B; Table S1. B)—TNF-induced TNF mRNA levels were greatest in PGD at 4 & 8 h, next highest in pancreas at 8 & 16 h, and smaller increases in PSI at 4 & 16 h, and in liver at 4 h. TNF-mediated induction of IL6 mRNA was significantly highest in PGD at 4 & 8 h, in PSI at 2 & 4 h, and in pancreas at 8 & 16 h. Significant IL6 mRNA induction was also seen in skin and bladder at 2 h, and in heart at 8 h. IL1B mRNA induction after TNF was highest in pancreas throughout the 32 h experiment, and in skin and bladder at the 2-h time-point. Significant down-regulation by TNF was found only: for IL6 mRNA in liver at 2 h, kidney at 4 h, in lung at 2 & 32 h, and for spleen at 2 & 4 h; for IL1B mRNA in lung at 32 h and brain at the 8-h time-point.

IL6 treatment (Figure 2C; Table 1. c; Table S1. C)—TNF mRNA levels following IL6 administration were not significantly up-regulated in any of the 12 tissues—except for small increases in pancreas and skin at early time-points. IL6-induced IL6 mRNA was significantly increased only in skin at 4 h. IL6-stimulated IL1B mRNA levels were highest in pancreas at 2 & 4 h, which persisted throughout the 16-h experiment. IL1B mRNA induction by IL6 was also significantly increased in bladder at 2. Down-regulation by IL6 treatment was found in liver, PSI and lung—in each case only at the 8-h time-point.

Brain-Specific Differences

Interestingly, in brain, we observed that LPS and TNF but not IL6 enhanced ZIP14B mRNA selectively (Fig. 1), and similarly, LPS and TNF but not IL6 altered the proinflammatory cytokine profile (Fig. 2). It is well known that the ligand and its corresponding receptor include: LPS and toll receptor-4 (TLR4); TNF and TNF receptor; IL6 and IL6 receptor. Of what relevance might this be in brain?

Fever reflects a multiphasic response (elevation and decline of the body core temperature) that is regulated by central thermoregulatory mechanisms localized to the preoptic area of hypothalamus. Several proinflammatory cytokines including TNF and IL6 act as endogenous pyrogens, whereas other cytokines can act as antipyretic agents; these findings confirm the link between the immune and central nervous systems²⁷. Cytokine levels in serum and cerebral spinal fluid are associated with fever. In the brain, presence of specific cytokine receptors on various cell types—and the effects of pharmacological application of cytokines and of their neutralizing antibodies—are known to alter the fever response via eicosanoid and other second-messenger lipid mediators^{28;29}; these findings are supported by cytokine- and cytokine receptor- transgenic mouse models²⁷.

Overall, data in the present study imply that tissues have different levels of responsiveness to ligands probably as a result of the receptors to which they specifically bind; moreover, ZIP14B is likely to be transcriptionally regulated in a manner differently from ZIP14A, perhaps at the spliceosome level, as had been previously suggested by observed differences in tissue-specific regulation of ZIP14B vs ZIP14A^{7;8}. Further investigations—using chromatin immunoprecipitation and electrophoretic mobility shift assays and reporter genes, which has been previously done for ZIP8²⁴—will be required to tease out regulatory differences between ZIP14B and ZIP14A. It is exciting to speculate that ZIP14B vs ZIP14A expression might be triggered by tissue- and cell type-specific cytokines and lipid mediators.

Conclusions

LPS-, TNF- and IL6-mediated induction of ZIP8, ZIP14A and ZIP14B mRNA levels, as well as stimulation of the inflammatory cytokine TNF, IL6 and IL1B mRNA levels, were found to be extremely variable depending on the inflammatory stressor given, quantitatively very different, highly tissue-specific, and also markedly time-dependent. It is also worth noting that expression of ZIP14A vs ZIP14B mRNA concentrations varied widely among the different treatments, tissues and time-points—providing further evidence that these two splice variants from the same gene must be associated with distinct functions in each cell type^{7;8}.

In summary, these data suggest that all these tissues likely use ZIP8 and ZIP14 transporters for Zn^{2+} uptake—some tissues without cytokine treatment, others more so when inflammatory danger (cytokine treatment) is present. Collectively, inflammatory-stimulated alterations in ZIP8-, ZIP14A- and ZIP14B-mediated transporter expression levels would probably lead to substantial decreases in plasma Zn^{2+} concentrations, due to Zn^{2+} redistribution not just in liver but across most vital organs. An extension of the present study would be to precisely measure intracellular Zn^{2+} levels at all time-points following LPS, TNF or IL6 treatment and in all twelve tissues. It seems intuitive that all major organs would require Zn^{2+} uptake, to varying degrees, in response to conditions of stress such as cytokine exposure. In the context of Cd^{2+} -mediated (or other toxic environmental-metal-mediated) toxicity, this opens the door for studying pathogenesis across a broad range of tissues, beyond simply kidney or lung.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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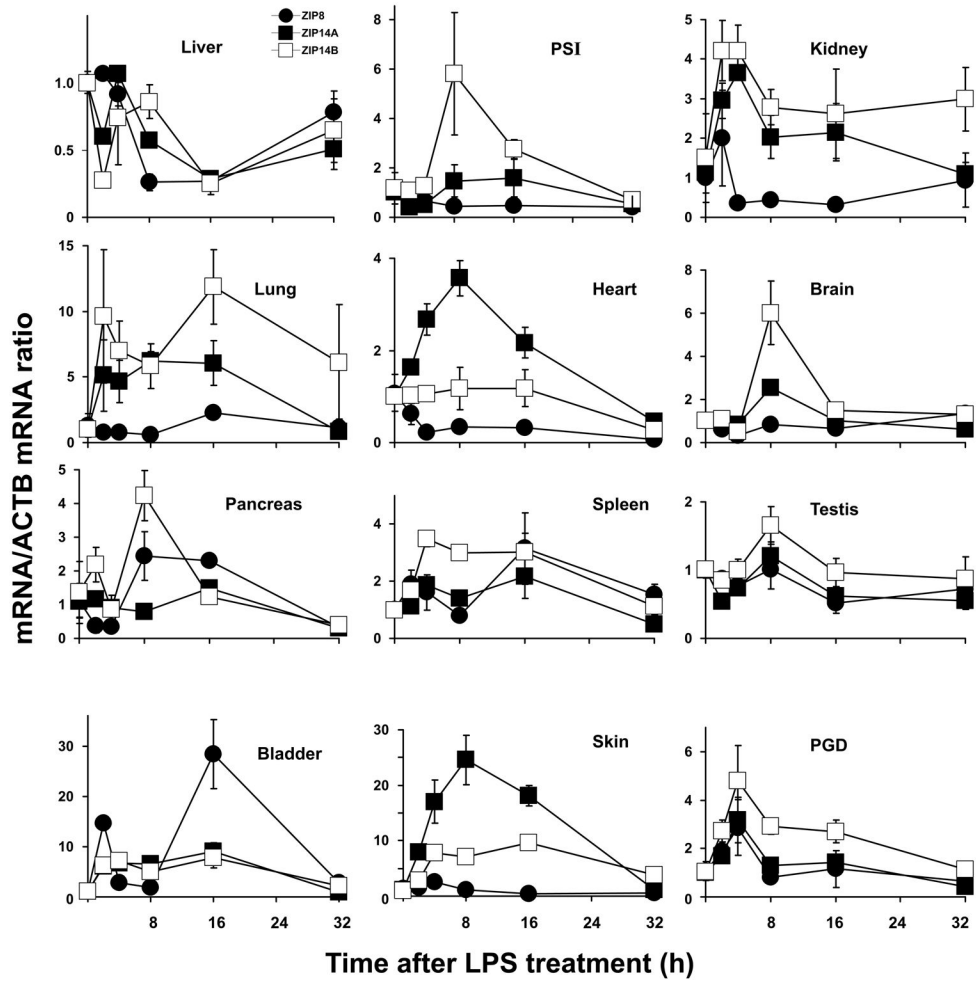


Figure 1A

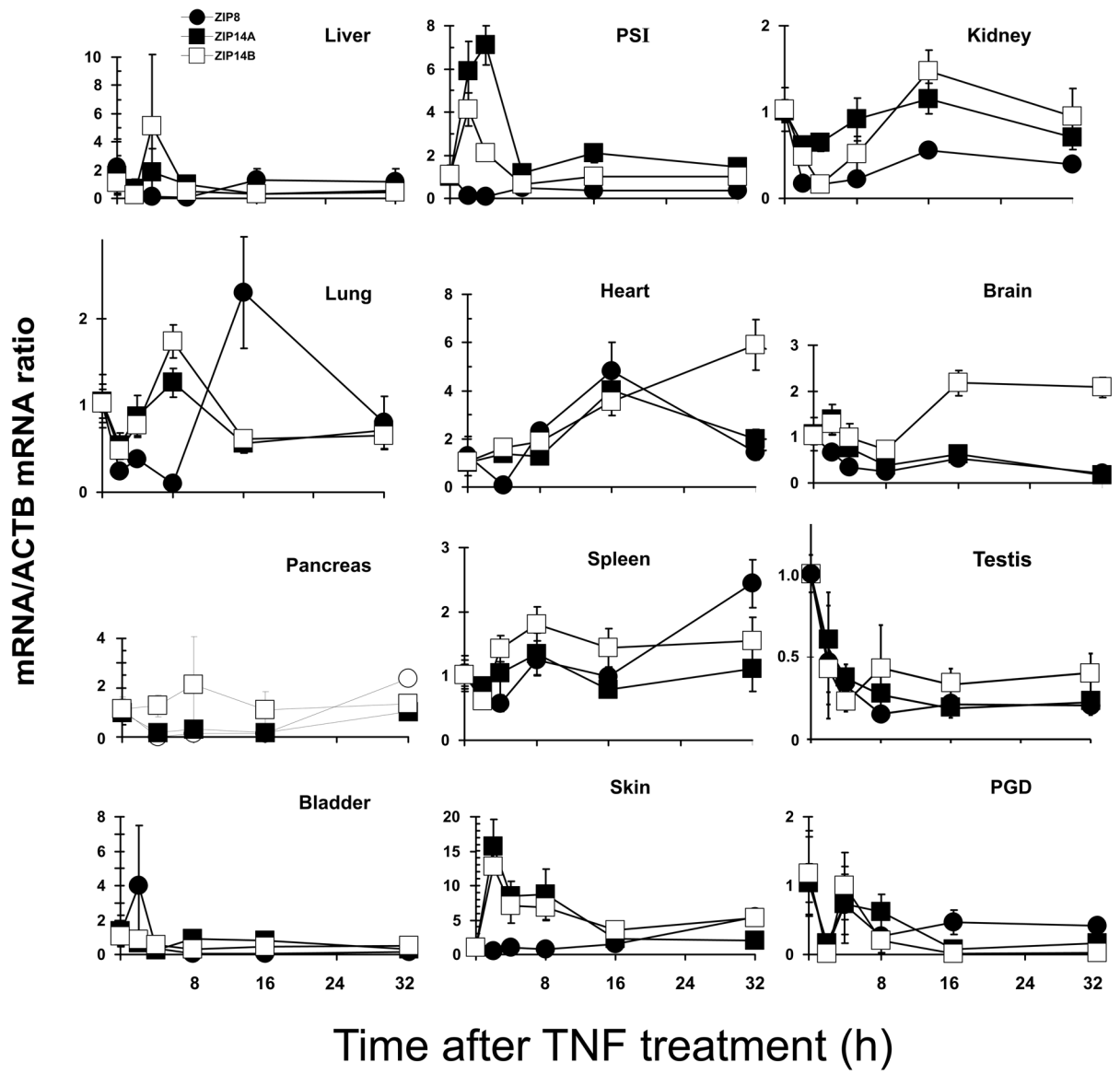


Figure 1B

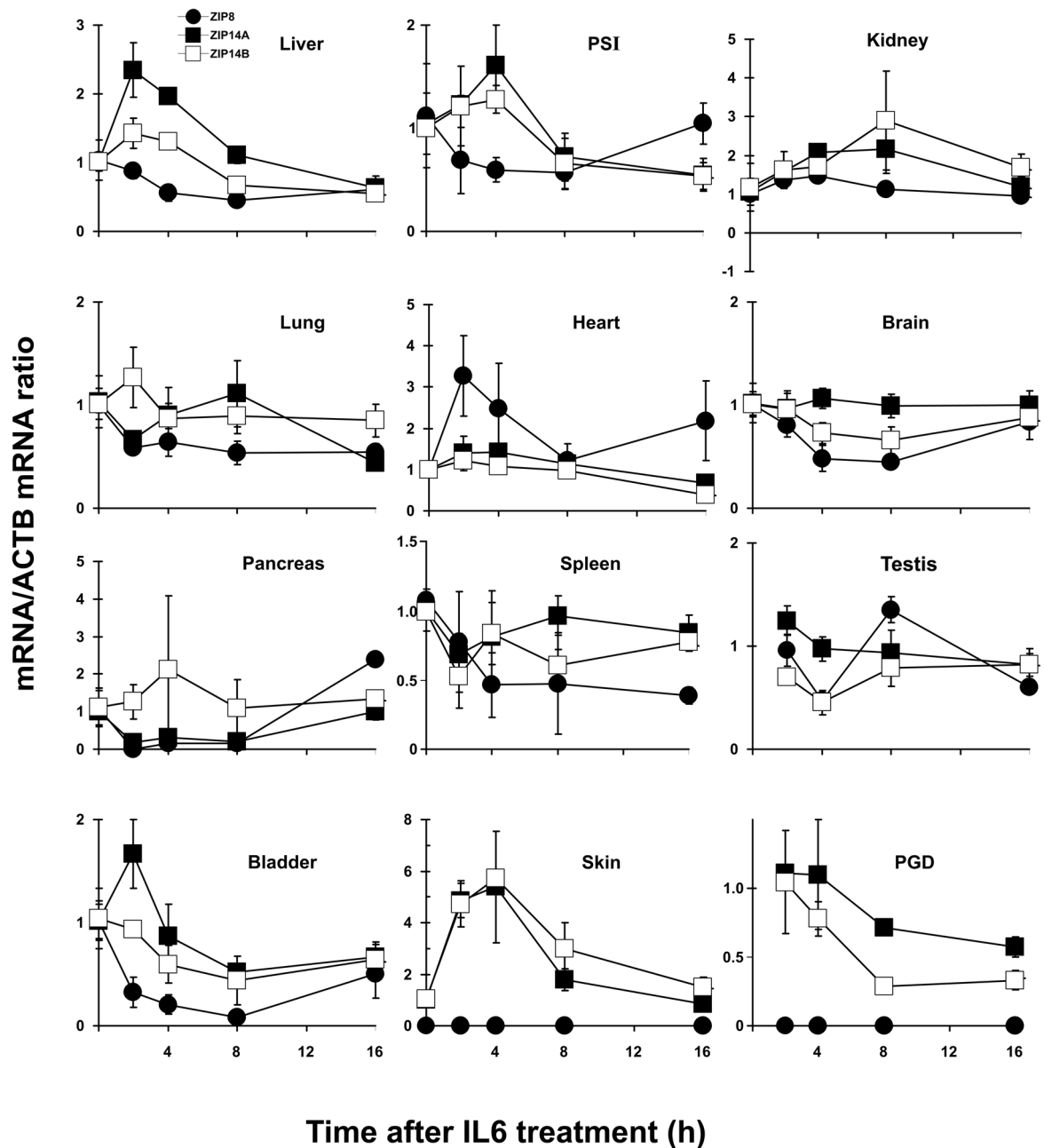


Figure 1C

Figure 1.

Relative ZIP8, ZIP14A and ZIP14B mRNA levels in mice following treatment with LPS, TNF or IL6. ZIP8 (closed circles, ●), ZIP14A (closed squares, ■) and ZIP14B (open squares, □) mRNA levels were quantified in each of twelve tissues as a function of time after the single dose of LPS or cytokine, as indicated. N=3 per time-point, and all experiments were repeated twice; β -actin (ACTB) mRNA was employed as the control. Results were normalized using the non-treated mRNA/ACTB mRNA ratio. Note the 2- to

~30-fold differences in values on the ordinates. Statistical analysis of all time-points is summarized in Table S1. (A) LPS treatment. (B) TNF treatment. (C) IL6 treatment.

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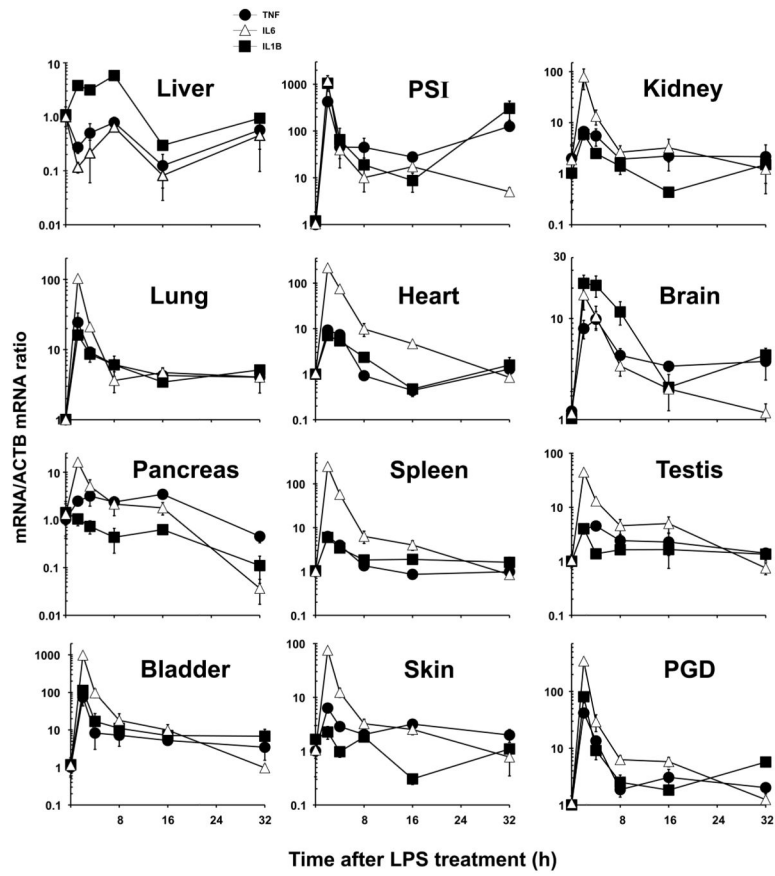


Figure 2A

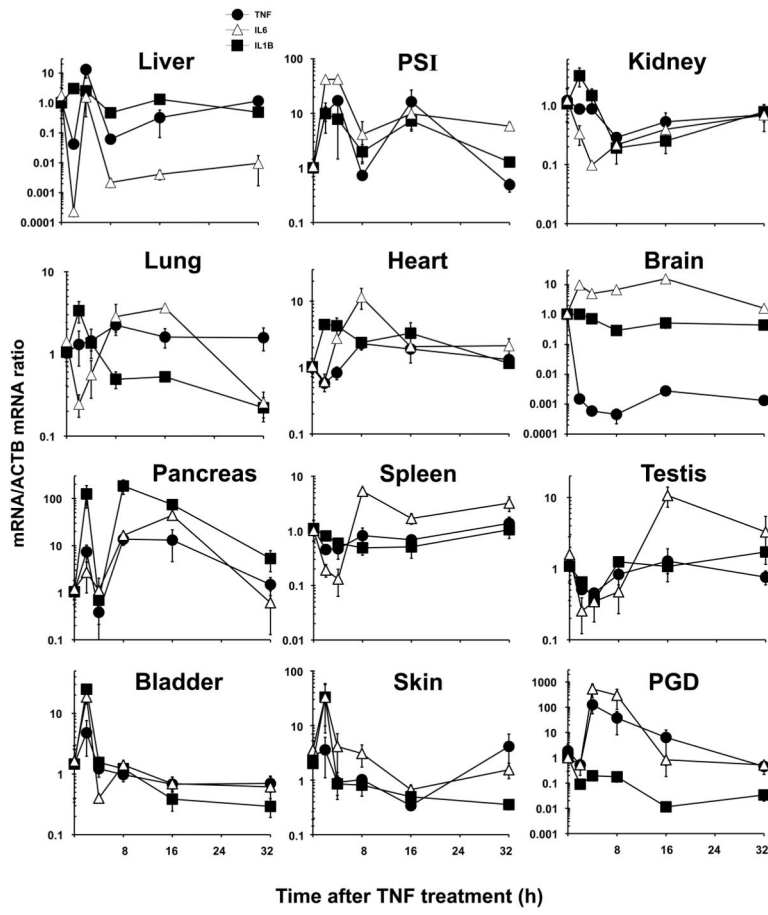


Figure 2B

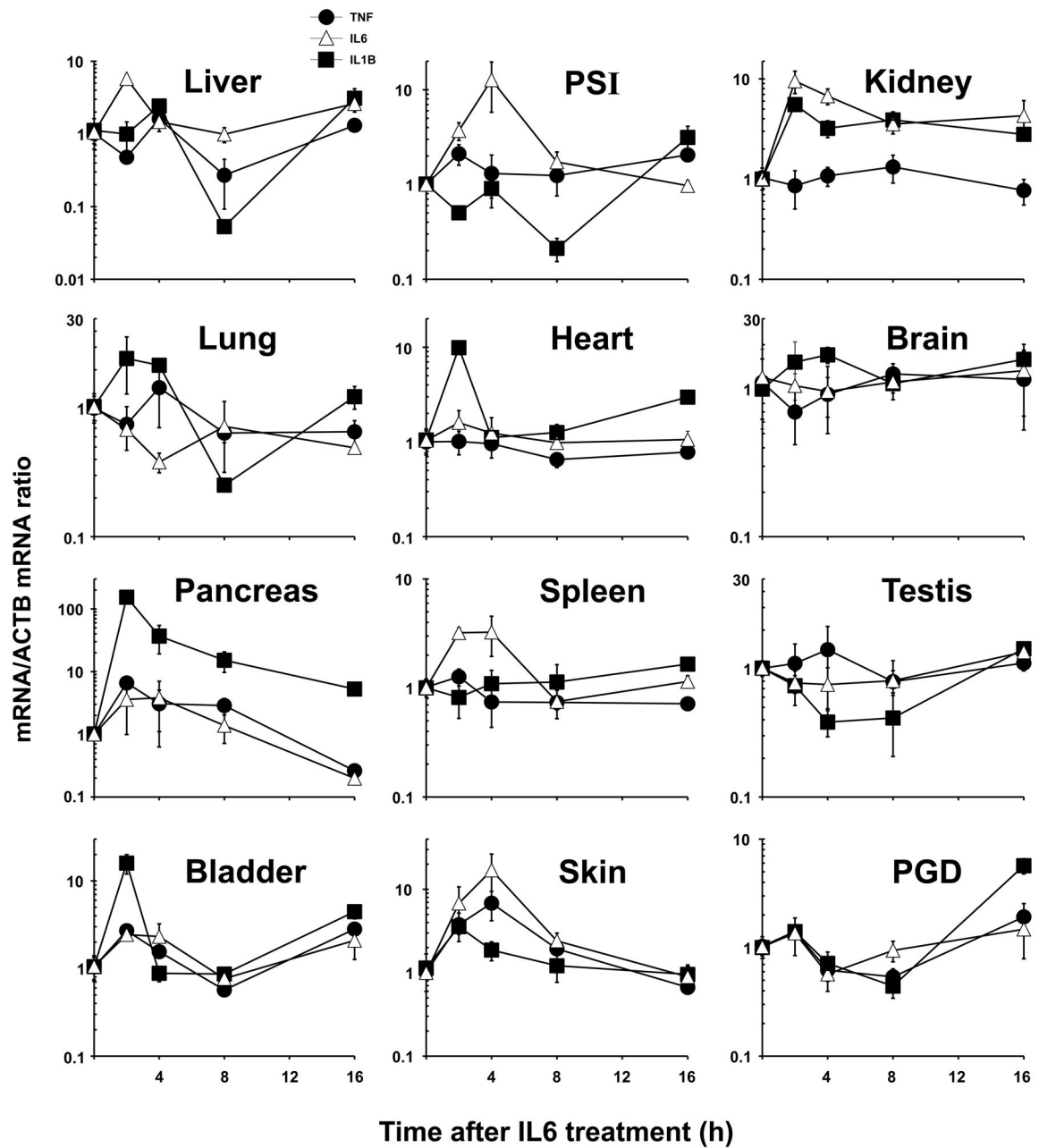


Figure 2C

Figure 2.

Relative cytokine mRNA levels in mice following treatment with LPS, TNF or IL6. The same mice shown in Fig. 1 were used to address the correlation between ZIP mRNA and cytokine mRNA levels. TNF mRNA (*closed circles*, ●), IL6 mRNA (*open triangles*, △) and IL1B mRNA (*closed squares*, ■). Results again were normalized using the non-treated mRNA/ACTB mRNA ratio. Note the 2- to ~1,000-fold differences in values on the ordinates; whereas Fig. 1 data were plotted on a linear scale, the data in Fig. 2 are plotted on

a logarithmic scale. Statistical analysis is included in Table S1. **(A)** LPS treatment. **(B)** TNF treatment. **(C)** IL6 treatment.

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Table 1

Relative increases in up-regulation of mRNA, as measured by qRT-PCR
 Cutoff values were arbitrarily set at: <3 (mRNA/ACTB mRNA ratio) = **zero**; 3–10 = **+1**; 10–20 = **+2**; 20–50 = **+3**; 50–200 = **+4**; and 200–1,000 = **+5**. Red denotes substantial up-regulation. Statistically significant down-regulation = **-2**.

A. Data from Figures 1A & 2A (LPS treatment)																
Hours	2	4	8	16	32	2	4	8	16	32	2	4	8	16	32	
Liver																
PSI						Kidney										
ZIP8	0	0	0	-2	0	ZIP8	0	0	0	0	ZIP8	0	0	0	0	
ZIP14A	0	0	0	-2	0	ZIP14A	0	0	0	0	ZIP14A	0	+1	0	0	
ZIP14B	0	0	0	-2	0	ZIP14B	0	0	+1	0	ZIP14B	+1	+1	+1	+1	
TNF	0	0	0	0	0	TNF	+5	+4	+4	+3	TNF	+1	+1	0	0	
IL6	0	0	0	0	0	IL6	+5	+3	+1	+2	IL6	+4	+2	0	0	
IL1B	+1	+1	+1	0	0	IL1B	+5	+4	+2	+1	IL1B	+1	0	0	0	
Lung																
Heart						Brain										
ZIP8	0	0	0	0	0	ZIP8	0	0	0	0	ZIP8	0	-2	0	0	
ZIP14A	+1	+1	+1	+1	0	ZIP14A	0	0	+1	0	ZIP14A	0	0	0	0	
ZIP14B	+1	+1	+1	+2	+1	ZIP14B	0	0	0	0	ZIP14B	0	0	+1	0	
TNF	+3	+1	+1	+1	+1	TNF	+1	+1	0	0	TNF	+1	+1	+1	+1	
IL6	+4	+3	+1	+1	+1	IL6	+4	+4	+1	+1	IL6	+2	+1	+1	0	
IL1B	+2	+1	+1	+1	+1	IL1B	+1	+1	0	0	IL1B	+2	+2	0	+1	
Pancreas																
Spleen						Testis										
ZIP8	0	0	0	0	0	ZIP8	0	0	0	+1	ZIP8	0	0	0	0	
ZIP14A	0	0	0	0	0	ZIP14A	0	0	0	0	ZIP14A	0	0	0	-2	
ZIP14B	0	0	+1	0	0	ZIP14B	0	+1	+1	0	ZIP14B	0	0	0	0	
TNF	0	0	0	0	0	TNF	+1	+1	0	0	TNF	+1	+1	0	0	
IL6	+1	+1	0	0	-2	IL6	+4	+3	+1	+1	IL6	+3	+2	+1	0	
IL1B	0	0	0	0	-2	IL1B	+1	+1	0	0	IL1B	+1	0	0	0	
Bladder																
Skin						PGD										
ZIP8	+2	0	0	+3	0	ZIP8	0	0	0	0	ZIP8	0	+1	0	0	
ZIP14A	+1	+1	+1	+1	0	ZIP14A	+1	+2	+3	+2	ZIP14A	0	+1	0	0	

A. Data from Figures 1A & 2A (LPS treatment)

Hours	2	4	8	16	32	2	4	8	16	32	2	4	8	16	32
ZIP14B	+1	+1	+1	+1	0	ZIP14B	0	+1	+1	0	ZIP14B	+1	+1	+1	0
TNF	+4	+1	+1	+1	0	TNF	+1	+1	0	0	TNF	+1	0	0	0
IL6	+5	+4	+2	+1	0	IL6	+4	+2	+1	0	IL6	+3	0	0	-2
IL1B	+4	+2	+1	+1	+1	IL1B	0	0	0	0	IL1B	+1	0	0	0

B. Data from Figures 1B & 2B (TNF treatment)

Hours	2	4	8	16	32	2	4	8	16	32	2	4	8	16	32
Liver															
PSI															
ZIP8	0	0	0	0	0	ZIP8	0	0	0	0	ZIP8	-2	-2	0	0
ZIP14A	0	0	0	0	0	ZIP14A	+1	+1	0	0	ZIP14A	0	0	0	0
ZIP14B	0	+1	0	0	0	ZIP14B	+1	0	0	0	ZIP14B	0	-2	0	0
TNF	0	+2	0	0	0	TNF	0	+2	0	+2	TNF	0	0	0	0
IL6	-2	0	0	0	0	IL6	+3	+3	+1	0	IL6	0	-2	0	0
IL1B	+1	+1	0	0	0	IL1B	+1	+1	+1	0	IL1B	+1	0	0	0
Lung															
Heart															
ZIP8	0	0	0	0	0	ZIP8	0	0	+1	0	ZIP8	0	-2	-2	0
ZIP14A	0	0	0	0	0	ZIP14A	0	0	+1	0	ZIP14A	0	0	-2	0
ZIP14B	0	0	0	0	0	ZIP14B	0	0	+1	+2	ZIP14B	0	0	0	0
TNF	0	0	0	0	0	TNF	0	0	0	0	TNF	0	0	0	0
IL6	-2	0	+1	+1	-2	IL6	0	0	+2	0	IL6	+1	+1	+1	0
IL1B	+1	0	0	0	-2	IL1B	+1	+1	0	0	IL1B	0	0	-2	0
Pancreas															
Spleen															
ZIP8	0	+4	+5	+4	0	ZIP8	0	0	0	0	ZIP8	0	0	0	0
ZIP14A	0	0	0	0	0	ZIP14A	0	0	0	0	ZIP14A	0	0	0	0
ZIP14B	0	+1	0	+3	0	ZIP14B	0	0	0	0	ZIP14B	0	0	0	0
TNF	+1	0	+2	+2	0	TNF	0	0	0	0	TNF	0	0	0	0
IL6	0	0	+2	+3	0	IL6	-2	-2	+1	0	IL6	0	0	0	+1
IL1B	+4	0	+4	+4	+1	IL1B	0	0	0	0	IL1B	0	0	0	0
Bladder															
Skin															
PGD															

B. Data from Figures 1B & 2B (TNF treatment)

Hours	2		4		8		16		32	
	2	4	8	16	32	2	4	8	16	32
ZIP8	+1	0	0	0	0	ZIP8	0	0	0	0
ZIP14A	0	0	0	0	0	ZIP14A	+2	+1	0	0
ZIP14B	0	0	0	0	0	ZIP14B	+2	+1	+1	+1
TNF	+1	0	0	0	0	TNF	0	0	0	0
IL6	+2	0	0	0	0	IL6	+3	0	0	0
IL1B	+2	0	0	0	0	IL1B	+3	0	0	0

C. Data from Figures 1C & 2C (IL6 treatment)

Hours	2		4		8		16		32	
	2	4	8	16	32	2	4	8	16	32
Liver										
PSI										
ZIP8	0	0	-2	0	0	ZIP8	0	0	0	0
ZIP14A	0	0	0	-2	0	ZIP14A	0	0	0	0
ZIP14B	0	0	0	-2	0	ZIP14B	0	0	0	0
TNF	0	0	0	0	0	TNF	0	0	0	0
IL6	+1	0	0	0	0	IL6	+1	+1	0	0
IL1B	0	0	-2	0	0	IL1B	0	0	-2	0
Lung										
Heart										
ZIP8	0	0	-2	-2	0	ZIP8	+1	0	0	0
ZIP14A	0	0	0	-2	0	ZIP14A	0	0	0	-2
ZIP14B	0	0	0	0	0	ZIP14B	0	0	0	-2
TNF	0	0	0	0	0	TNF	0	0	0	0
IL6	0	0	0	0	0	IL6	0	0	0	0
IL1B	0	0	-2	0	0	IL1B	+1	0	0	+1
Pancreas										
Spleen										
ZIP8	0	0	0	0	0	ZIP8	0	0	0	0
ZIP14A	0	0	0	0	0	ZIP14A	0	0	0	0
ZIP14B	0	0	0	0	0	ZIP14B	0	0	0	0
TNF	+1	0	0	0	0	TNF	0	0	0	0
IL6	0	0	0	0	0	IL6	0	0	0	0
IL1B	+4	+3	+2	+1	0	IL1B	0	0	0	0
Pituitary										
Testis										
ZIP8	0	0	0	0	0	ZIP8	0	0	0	0
ZIP14A	0	0	0	0	0	ZIP14A	0	0	0	0
ZIP14B	0	0	0	0	0	ZIP14B	0	-2	0	0
TNF	0	0	0	0	0	TNF	0	0	0	0
IL6	0	0	0	0	0	IL6	0	0	0	0
IL1B	0	0	-2	0	0	IL1B	0	0	0	0

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C. Data from Figures 1C & 2C (IL6 treatment)

Hours	Bladder					Skin					PGD				
	2	4	8	16	32	2	4	8	16	32	2	4	8	16	32
ZIP8	0	0	-2	0	0	ZIP8	0	0	0	0	ZIP8	0	0	0	0
ZIP14A	0	0	0	0	0	ZIP14A	+1	+1	0	0	ZIP14A	0	0	0	0
ZIP14B	0	0	0	0	0	ZIP14B	+1	+1	0	0	ZIP14B	0	0	0	0
TNF	0	0	0	0	0	TNF	+1	+1	0	0	TNF	0	0	0	0
IL6	0	0	0	0	0	IL6	+1	+2	0	0	IL6	0	0	0	0
IL1B	+2	0	0	0	+1	IL1B	+1	0	0	0	IL1B	0	0	0	+1