Supporting Information

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SI Text

SI Materials and Methods. ***Human subjects.*** Male subjects 21–35 y of age, weighing at least 50 kg, were recruited to participate. Details of the subject characteristics and exclusion criteria are provided in Table S1. The study was approved by both the Institutional Review Board and the Clinical Research Center of the University of Florida. All subjects provided written informed consent prior to enrollment. The study was registered at clinicaltrials.gov as NCT01221129.

Acute dietary zinc depletion. The design was a 24-d study comprised of three phases of dietary treatment (Fig. 1*A*). Each subject served as their own control. In order to establish a defined baseline condition (acclimation) prior to dietary zinc depletion, subjects consumed meals composed of a mixed diet providing 2,700 kcal and approximately 11 mg Zn/d, and an adequate amount of a zinc-free energy supplement for additional calories required for body weight maintenance. After 7 d of acclimation (Day 7), the subjects consumed egg white-based liquid formula which provided <0.5 mg Zn/d for 10 d. The composition of caloric and mineral contents of the liquid formula are shown in Tables S7 and S8, respectively. Upon completion of the depletion phase (on Day 17), subjects returned to their self-selected diet and a supplement of 15 mg Zn/d was provided for the zinc repletion.

Sample collection. All samples were collected after an overnight fast between 7–7:30 a.m. On Day 7, 13, and 17, two buccal swabs and whole blood (34 mL) were collected in PAXgene, EDTA-, and heparin-treated tubes. Additional blood draws (5 mL) were conducted on Day 0 and at Day 10, Day 15, Day 20, and Day 24 for serum sampling. The scheme of sample handling and processing workflow is shown in Figure S3.

Preparation of buccal RNA. FTA paper cards (Whatman) with buccal samples were air-dried for approximately 3–4 h at room temperature and stored at −20 °C until processed. To isolate RNA, filter papers with the buccal samples were cut into small parts using sterile surgical scissors, and were incubated in 1.5 mL of TRI reagent (Ambion) at room temperature for 15 min. Samples were agitated every 5 min during this period. After centrifugation at 12,000 × *g* × 30 min, 4 °C, the lysate was transferred to a new microcentrifuge tube for RNA isolation. When residual filter paper was observed in the RNA solution, samples were treated with 1 mL of TRI reagent and the phenol-chloroform extraction step was repeated. All samples were further purified by the sodium acetate-isopropanol precipitation, and the final RNA solution was stored at −80 °C until analyzed.

Processing of blood samples. Sera were isolated from 5 mL of whole blood collected in red-top serum tubes (BD Vacutainer). Blood was allowed to clot at room temperature for 60 min after collection, and placed on ice for no longer that 2 h until processed. Clotted blood was centrifuged at 2,000 × *g* × 10 min, 4 °C, and serum was collected in aliquots. One aliquot was treated with 1% (vol/vol) protease inhibitor cocktail (Pierce) and stored at −80 °C for ELISA. Untreated samples were stored in 4 °C and −80 °C until processed for atomic absorption spectrophotometry (AAS) and miRNA assays, respectively. For the determination of zinc concentrations by AAS, sera were diluted 1/4 with MilliQ water. Consistency of measurements was monitored with a reference zinc standard (0.6 mg Zn/L).

PBMC and circulating erythroid cells (erythrocytes and reticulocytes) were fractionated from whole blood collected in Vacutainer tubes pretreated with K_3 EDTA. For PBMC isolation, 9 mL of whole blood was placed on an equal vol of Histopaque 1077 (Sigma) and centrifuged at 400 × *g* × 30 min at room temperature. The PBMC layer was collected and washed with PBS at 250 × *g* × 10 min, twice. Recovered PBMCs were treated with 1 mL of TRI reagent for RNA isolation. The RBC pellet was diluted with 1 volume of PBS was stored at 4 °C until processed for reticulocyte RNA preparation.

Remnant erythroid cells from the PBMC isolation procedure (with 9 mL of whole blood) were processed to yield a reticulocyte RNA preparation. To achieve a pure population of erythroid cells, leukocyte depletion was carried out by using cellulose columns (1). Enriched RBCs were suspended in 1 volume of Hepes buffer (154 mM NaCl, 10 mM HEPES, 1 g/L BSA) and filtered through a cellulose column composed of α-cellulose (Sigma) and Sigmacell Type 50 microcrystalline cellulose (Sigma) in a 1:1 (wt:wt) ratio. After RBC preparations were loaded into the cellulose column, 2 volumes of HEPES buffer was applied for the elution of RBC. After being collected from the eluate by centrifugation at 2,000 × *g* × 5 min at 4 °C, the cells were washed two times with an equal volume of ice-cold PBS at 200 × *g* for 10 min to eliminate platelets. Purified RBCs packed by centrifugation at 2,000 × *g* × 5 min at 4 °C were treated with 20 mL of TRI reagent for reticulocyte RNA isolation and purification by the phenol-chloroform extraction and sodium acetate-isopropanol precipitation, respectively.

Isolation and processing of blood cell RNA. With the exception of RNA from whole blood collected in PAXgene tubes, all blood RNA samples were prepared by phenol-chloroform extraction, and were treated with Turbo DNA-free reagents (Ambion) to remove residual DNA contamination. For RNA isolated from samples of whole blood assays, additional RNA precipitation using 2.5 M lithium chloride (LiCl) was conducted to minimize the inhibitory effects of heparin on reverse transcription and PCR reactions (2). Stabilized whole blood RNA was prepared from 7.5 mL of blood collected in PAXgene blood collection tubes (BD) by the manual procedure described in the manufacturer's protocol. Briefly, whole blood lysates treated with proteinase K were homogenized and RNA was isolated by using silica-gel membrane columns (Qiagen). Samples were treated with DNase I (Qiagen) prior to elution.

Whole blood RNA from PAXgene samples was further processed to minimize the masking effect produced by the highly abundant transcript, globin RNA, prior to microarray analyses (3, 4). Globin RNA depletion from the whole blood RNA was done by using GLOBINclear (Ambion). Globin transcripts in PAXgene RNA (3 μg) was hybridized to biotinylated oligonucleotides and captured by streptavidin magnetic beads. The supernatant was further purified by a poly-T-oligos conjugated to magnetic beads prior to downstream processing for microarray analysis. All RNA samples were stored at −80 °C until further processed.

Microarray experiments and data analysis. The Illumina BeadChip platform (HumanHT-12 v4) was selected for the assessment of global effect of dietary zinc depletion on the blood transcriptome, due to its high-throughput and cost-effective nature. PAXgene

whole blood RNA with or without depletion of globin transcripts (200 ng of total RNA) was amplified by the Illumina TotalPrep RNA Amplification kit (Ambion) for the array analysis. Following reverse transcription using T7 Oligo(dT) primers and second strand cDNA synthesis, in vitro transcription with T7 RNA polymerase and biotin-UTP was conducted for the synthesis of biotinylated cRNA. The yield and quality of cRNA were assessed with the NanoDrop spectrophotometer and Agilent 2100 bioanalyzer, respectively. For the detection of differential gene expression, labeled cRNA (750 ng) was loaded to beadchips, hybridized for 16 h at 58 °C, and labeled with Cy3-streptavidin. Fluorescence signals from Cy3 were detected by a BeadArray Reader (Illumina Beadstation 500GX) and signal intensities were exported by using GenomeStudio software (Illumina).

Probes with detection *P*-values above 0.05 in all samples were excluded from the dataset prior to analyses. Raw data were quantile normalized and log-transformed for statistical analyses. Differentially expressed (DE) genes were determined by comparisons between baseline and post-zinc-depletion levels using BRB-ArrayTools (developed by Dr. Richard Simon and BRB-ArrayTools Development Team). Genes differentially affected by acute zinc depletion were determined by a pairwise comparison at $P < 0.005$ with 256 available permutations. For the visualization of the temporal pattern of gene expression identified with pooled samples, intensity values were standardized by subtracting the mean value of each gene across all arrays and division by the standard deviation of each respective gene (resulting in mean = 0, standard deviation = 1). After unsupervised clustering by expression patterns using the *k*-means algorithm ($k = 2$), computational analyses based on putative transcription factor motifs were conducted to identify the cis-regulatory factors mediating the differential expression of the zinc-responsive genes by PRIMA of the EXPANDER 5.2 software suite (5). PANTHER version 7 (www.pantherdb.org) (6) and Ingenuity Pathway Analysis (Ingenuity® Systems, www.ingenuity.com) with the list of DE genes was conducted to identify biological networks between the responsive genes and to predict the associated functions, diseases and disorders to the modulated gene expression by dietary zinc depletion. Genes with the potential to serve as a biomarker of zinc deficiency were determined by an unpaired comparison using the BRB-Array Tools ($P < 0.005$ with 10,000 random permutations). Average-linkage hierarchical clustering with Pearson correlation metric was conducted with the EXPANDER software.

Real-time quantitative PCR. Transcript abundance of genes known to be directly involved in the regulation of cellular zinc homeostasis, i.e., zinc transporters (ZnT1, ZnT2, ZnT4, ZnT5, ZnT6, ZnT7, Zip1, Zip2, Zip4, Zip5, Zip8, Zip10, and Zip14) and MT, were measured by assays designed and validated previously (7, 8). Primers and probe sets for the detection of Zip6 (forward, 5′-AGGCTGGCATGACCGTTAAG-3′; reverse, 5′-AAAATTCCTGTTGCCATTCCA-3′; probe, 5′-FAM-CCTTTATAATGCATTGTCAGCCATGCTGG-BHQ1-3′) and GAPDH transcripts (forward, 5′-GAAGGTGAAGGTCGGAGTC-3′; reverse, 5′-GAAGATGGTGATGGGATTTC-3′; probe, 5′-FAM-CAAGCTTCCCGTTCTCAGCC-BHQ1-3′) were designed by using PRIMER EXPRESS 3.0 software (Applied Biosystem) and validated by Primer3plus. Quantitation of CDC20, TXNDC5, MZB1, and IGJ transcripts were conducted using TaqMan gene expression assays from Applied Biosystems. Real-time quantitative reverse transcriptase polymerase chain reaction (qPCR) assays were done with cDNA, generated with high capacity cDNA reverse transcription kit (Applied Biosystems), and the TaqMan fast universal PCR master mix (Applied Biosystems). Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) mRNA and 18S rRNA levels were measured as internal controls, and amplification and detection were performed with the StepOnePlus real-time PCR system (Applied Biosystems).

Serum miRNA isolation and quantitation. Serum was treated with 5 volumes of QIAzol (Qiagen) for the denaturation of protein contents and subsequent isolation of RNA. Due to the absence of a known housekeeping microRNA (miRNA) in human serum, 25 fmol of synthetic cel-miR-39 (5′-UCACCGGGUGUAAAUCAGCUUG-3′) was added as a means of normalization (9). After adding 1 volume of chloroform, aqueous and organic phases were separated by centrifugation at $12{,}000 \times g \times 15$ min at 4 °C. The aqueous phase was treated with 1.5 volumes of 100% ethanol and loaded to a miRNeasy Mini column for RNA extraction. After extensive washes, purified RNA was eluted from the column membrane with 50 μL of nuclease-free water and stored at −80 °C until analyzed.

The human serum miRNA PCR array from SABiosciences was used for the identification of circulating miRNAs responsive to dietary zinc levels. Serum RNA was isolated from 400 μL of pooled sera (composed of 60 μL of sera from $n = 8$) at the initial screening step. Day 7, 17, and 24 samples were selected as those representing baseline, zinc-depleted, and zinc-repleted conditions, respectively. Serum RNA was polyadenylated and converted to cDNA by using RT^2 miRNA First Strand kit (SABiosciences) containing universal reverse transcription (RT) primers targeting poly-A-tails. After a 1/4-dilution with nuclease-free water, RT products were distributed across the miRNA qPCR arrays with SYBR Green qPCR reagents (SABiosciences) for amplification and quantitation. Melt-curve analyses with amplified products were conducted to determine the specificity of each primer set. All values of each miRNA were normalized to their respective cel-miR-39 and intergroup values were compared by the comparative C_T method. Criteria for determining the zinc-responsive miRNAs were: (*i*) single melting temperature, (*ii*) fold-change >1.5 by dietary zinc depletion, (*iii*) threshold cycle (C_T) <35, and (*iv*) response to zinc repletion in a direction opposite to that of zinc-depletion.

Zinc-responsiveness of selected miRNA, identified in pooled samples, was confirmed with RNA from 200 μL of individual serum samples collected at Day 7, 13, 17, 20, and 24 by using the miScript PCR system (Qiagen). Total serum RNA was isolated as described above. Target miRNAs were selected based on their average C_T values and fold-changes obtained from the PCR array experiment. All miRNA with a detection C_T lower than 30 and fold-changes above 1.5 by both zinc depletion and repletion (miR-10b, 92a, -145, -375) were analyzed by miRNA-specific qPCR assays. Among miRNAs with C_T between 30 and 35, those with responses greater that 2-folds (miR-200b, miR-204, and 296-5p) were selected for further confirmation. After ligation-mediated reverse transcription with 5 μL of the eluted RNA using the miScript Reverse Transcription reagents (20 μL of total reaction volume; Qiagen), cDNA products were diluted with four volumes of nuclease-free water. qPCR was conducted in a final reaction volume of 12 μL with 1.2 μL of the diluted cDNA templates using the SYBR Green PCR chemistry. Relevant primers compatible to the miScript system were purchased from Qiagen. All values of miRNA qPCR experiments were normalized to the abundance of their respective cel-miR-39 value and the average of Day 7 samples were set at 1 for comparison.

Cytokine assays. Whole blood was collected in heparin, diluted to 20% with phenol-free RPMI-1640 medium supplemented with 2 mM L-glutamine, 100 U penicillin/mL and 100 μg streptomycin/mL, with or without LPS (1 mg/L) or PHA (10 mg/L). After incubation in wells of ultra-low-attachment plates (Corning) at 37 °C in 5% CO_2 for 24 h, cell-free supernatant was collected by centrifugation at $600 \times g \times 5$ min, 4 °C, and stored with protease inhibitor cocktail (Pierce) at −80 °C. Whole blood cell pellets were lysed with TRI reagent BD (Molecular Research Center) supplemented with acetic acid, and stored at −80 °C until RNA isolation. Inflammatory cytokine levels in

cell-free supernatants were measured using a multianalyte ELISA array (SABiosciences) with pooled samples. Effects on IL-1β, TNFα, and IFNγ were further assessed with cell-free supernatants from individual subjects using single analyte ELISAs (SABiosciences). Analytes were quantified by absorbance at 450 nm using a multimode microplate reader (SpectraMax M5; Molecular Devices).

Statistical analysis. A dataset from nine subjects is adequate to detect a within-subject difference in transcript levels of zinc-responsive gene with 80% power at $P < 0.05$ two-sided. Values from samples of Day 7 served as the control for comparisons. Student's *t*-test or repeated measures of ANOVA followed by a Student-Newman-Keuls multiple comparisons test were conducted for pairwise comparisons. Linear association between transcript levels and serum zinc concentrations was determined by the Pearson correlation coefficient. All statistical analyses were conducted using the InStat 3 software (GraphPad). The level of significance was set at $P < 0.05$ for all analyses except for those of microarray data.

1. Bonafoux B, Commes T (2009) Serial analysis of gene expression adapted for downsized extracts (SAGE/SADE) analysis in reticulocytes. *Methods Mol Biol* 496:299–311.
2. Jung R, Lubcke C, Wagener C, Neumaier M (1997) Reversal of RT-PCR inhibition observed in heparinized clinical specimens. *Biotechniques* 23(1):24–28.
3. Tian Z, et al. (2009) A practical platform for blood biomarker study by using global gene expression profiling of peripheral whole blood. *PLoS One* 4(4):e5157.
4. Debey S, et al. (2006) A highly standardized, robust, and cost-effective method for genome-wide transcriptome analysis of peripheral blood applicable to large-scale clinical trials. *Genomics* 87(5):653–664.
5. Ulitsky I, et al. (2010) Expander: From expression microarrays to networks and functions. *Nat Protoc* 5(2):303–322.
6. Thomas PD, et al. (2003) PANTHER: A library of protein families and subfamilies indexed by function. *Genome Res* 13(9):2129–2141.
7. Cousins RJ, et al. (2003) A global view of the selectivity of zinc deprivation and excess on genes expressed in human THP-1 mononuclear cells. *Proc Natl Acad Sci USA* 100 (12):6952–6957.
8. Aydemir TB, Blanchard RK, Cousins RJ (2006) Zinc supplementation of young men alters metallothionein, zinc transporter, and cytokine gene expression in leukocyte populations. *Proc Natl Acad Sci USA* 103(6):1699–1704.
9. Kroh EM, Parkin RK, Mitchell PS, Tewari M (2010) Analysis of circulating microRNA biomarkers in plasma and serum using quantitative reverse transcription-PCR (qRT-PCR). *Methods* 50(4):298–301.

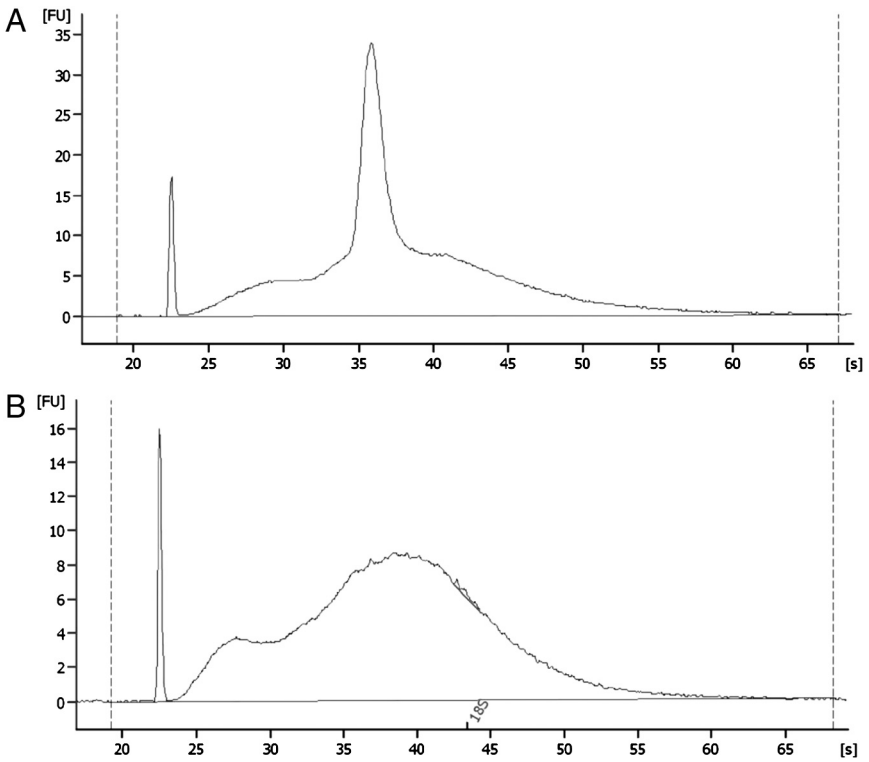
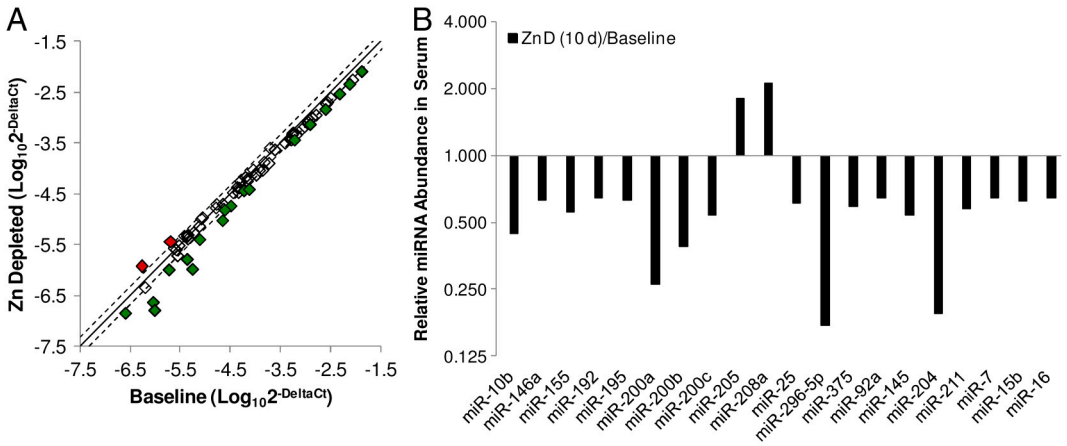


Fig. S1. Quality of amplified cRNA assessed by Agilent 2100 Bioanalyzer. Representative electropherogram of amplified (*A*) whole blood RNA (PAX) and (*B*) globin RNA-reduced whole blood RNA (GRP) are shown. The peak only present in the electrophrogram of PAX indicates the highly abundant globin RNA content. Removal of this peak was confirmed by all globin RNA-reduced samples.



C let-7a, let-7c, miR-1, miR-9, miR-10a, miR-10b, miR-7, miR-15a, miR-15b, miR-16, miR-17, miR-18a, miR-19a, miR-19b, miR-20a, miR-21, miR-22, miR-23a, miR-24, miR-25, miR-26a, miR-26b, miR-27a, miR-29a, miR-30d, miR-30e, miR-34a, miR-92a, miR-93, miR-96, miR-100, miR-103, miR-106a, miR-106b, miR-107, miR-122, miR-124, miR-125b, miR-126, miR-128, miR-130b, miR-133a, miR-133b, miR-134, miR-141, miR-143, miR-145, miR-146a, miR-148a, miR-150, miR-155, miR-184, miR-191, miR-192, miR-193a-5p, miR-195, miR-196a, miR-200a, miR-200b, miR-200c, miR-203, miR-204, miR-205, miR-206, miR-208a, miR-210, miR-211, miR-214, miR-215, miR-221, miR-222, miR-223, miR-224, miR-296-5p, miR-372, miR-373, miR-374a, miR-375, miR-376c, miR-423-5p, miR-499-5p, miR-516a-3p, miR-574-3p, miR-885-5p

Fig. S2. Identification of serum miRNAs responsive to acute dietary zinc depletion using a qPCR-based array. Circulating miRNA were isolated from pooled sera collected on baseline (day 7) and postdepletion phase (day 17), and were quantified by using a SABiosciences human serum miRNA qPCR array (MAH-106A) focused on miRNA known to be present in human serum. (*A*) A scatter plot indicating the miRNA of which levels were modulated by fold changes above 1.5 under dietary zinc restriction. (*B*) Relative abundance of serum miRNAs affected by dietary zinc depletion. Values were normalized to cel-miR-39 levels. (*C*) List of miRNAs on the array.

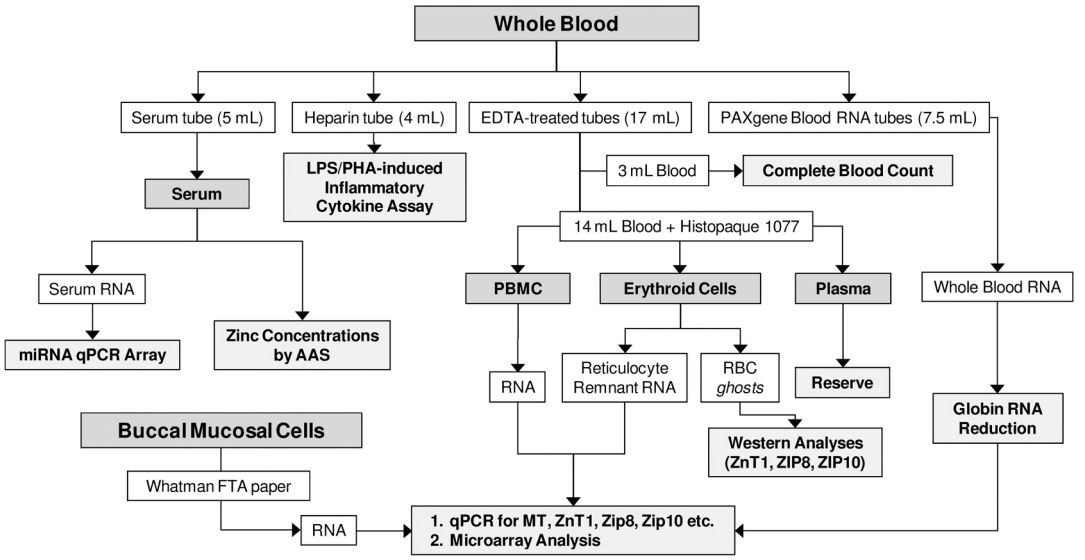


Fig. S3. Schematic diagram of sample collection and handling processes. Relevant materials and methods were selected based on their practicality for being used in the field for nutrient assessment. Availability of methods for sample stabilization allowed the completion of the entire processing by a single researcher. Data from with erythrocyte membrane fractions will be presented elsewhere.

Table S1. Subject characteristics ($n = 9$). Exclusion criteria for the dietary regimen included current cigarette smoking, alcohol abuse, routine consumption of medications, chronic use of denture cream or dietary supplements containing zinc, and history of any chronic disease or allergic reaction. Upon enrollment, the zinc contents in the habitual diet of each subject were determined by a 24-h diet recall followed by calculations with the Nutrition Data System for Research (NDSR) and blood was collected for serum zinc measures by atomic absorption spectrophotometry

Day of study	Screening Mean (SD)	Day 0 Mean (SD)	Day 7 Mean (SD)	Day 17 Mean (SD)	Day 24 Mean (SD)
Age (y)	24 (2)	—	—	—	—
Height (cm)	173.9 (6.6)	—	—	—	—
BWt (kg)	77.1 (12.5)	77.3 (12.7)	76.7 (12.6)	76.3 (11.8)	76.8 (11.5)
Caloric needs (kcal/d)	3043 (193)	—	—	—	—
Serum Zn (μg/dL)	91.9 (17.9)	83.9 (16.6)[a]	86.6 (8.1)[a]	69.1 (13.4)[b]	83.5 (12.7)[a]
24 h recall (mg Zn/d)	13.9 (7.3)	—	—	—	—

Values with different superscript are significant at $P < 0.001$.

Table S2. Hematological parameters measured during dietary zinc depletion ($n = 9$)

		Day of study					
		Day 7		Day 13		Day 17	
Measures	Normal range	Mean (SD)		Mean (SD)		Mean (SD)	
WBC ($\times 10^3/mm^3$)	4.0 ~ 10.0	5.88	(1.48)	5.88	(1.45)	5.82	(1.51)
Hemoglobin (g/dL)	13.0 ~ 16.5	15.77	(0.93)	15.20	(0.82)3	15.13	(0.73)[‡]
Hematocrit (%)	39.0 ~ 49.0	44.52	(2.43)	43.37	(2.12)	42.53	(2.28)[‡]
Platelet count ($\times 10^3/mm^3$)	150 ~ 450	282.22	(31.1)	265.56	(34.4)[‡]	251.78	(39.7)[‡]
RBC ($\times 10^6/mm^3$)	4.5 ~ 5.9	5.36	(0.48)	5.21	(0.46)[‡]	5.11	(0.42)[‡]
Mean cell volume (micron^3)	78.0 ~ 100.0	83.48	(5.37)	83.71	(5.41)	83.46	(5.45)
Mean cell Hemoglobin (pg)	26.0 ~ 34.0	29.53	(2.10)	29.32	(1.78)	29.68	(1.67)
MCHC (g/dL)*	31 ~ 37	35.36	(0.79)	35.04	(0.71)	35.60	(1.17)
CHCM (g/dL)[†]	32.0 ~ 38.0	36.23	(1.23)	35.52	(1.10)[‡]	36.22	(1.49)
RBC Distance Width (%)	11.0 ~ 14.0	13.00	(1.26)	13.13	(1.26)	12.82	(1.29)
Mean platelet volume (fL)	6.0 ~ 10.0	7.97	(0.40)	8.11	(0.87)	8.92	(0.62)[‡]

*MCHC, mean corpuscular hemoglobin concentration.
[†]CHCM, cell hemoglobin concentration mean.
[‡]Values significantly different compared to Day 7 values at $P < 0.05$.

Table S3. List of differentially expressed genes ranked by fold-changes (ZnD/Baseline)*

Gene symbol	EntrezID	Gene name	Fold-change	*P*-value
Up-regulated genes (192)				
LOC651751	651751	similar to Ig kappa chain V-II region RPMI 6410 precursor	4.32	<1e−07
IGJ	3512	immunoglobulin J polypeptide, linker protein for immunoglobulin alpha and mu polypeptides	4.22	1.00E−06
LOC649923	649923	similar to Ig gamma-2 chain C region	3.51	3.00E−07
LOC642113	642113	Ig kappa chain V-III region HAH-like	3.05	<1e−07
LOC652493	652493	ig kappa chain V-I region HK102-like	3.05	6.00E−07
LOC647450	647450	similar to Ig kappa chain V-I region HK101 precursor	2.87	3.00E−07
LOC647506	647506	similar to Ig kappa chain V-I region HK101 precursor	2.77	9.00E−07
IGLL1	3543	immunoglobulin lambda-like polypeptide 1	2.77	1.00E−06
TXNDC5	81567	thioredoxin domain containing 5 (endoplasmic reticulum)	2.73	5.00E−07
CDC20	991	cell division cycle 20 homolog (S. cerevisiae)	2.69	2.00E−07
TNFRSF17	608	tumor necrosis factor receptor superfamily, member 17	2.55	1.85E−05
GLDC	2731	glycine dehydrogenase (decarboxylating)	2.49	2.30E−06
LOC652102	652102	similar to Ig heavy chain V-I region HG3 precursor	2.46	1.07E−03
MZB1	51237	marginal zone B and B1 cell-specific protein	2.07	7.00E−07
CD38	952	CD38 molecule	2.03	2.07E−05
ABCB9	23457	ATP-binding cassette, subfamily B (MDR/TAP), member 9	1.85	2.20E−06
IGLL3	91353	immunoglobulin lambda-like polypeptide 3, pseudogene	1.85	1.12E−04
LOC652775	652775	similar to Ig kappa chain V-V region L7 precursor	1.81	1.49E−03
LOC649210	649210	similar to Ig lambda chain V region 4A precursor	1.73	3.13E−03
CCNB2	9133	cyclin B2	1.70	1.82E−05
ITM2C	81618	integral membrane protein 2C	1.70	2.23E−05
ABCA1	19	ATP-binding cassette, subfamily A (ABC1), member 1	1.69	6.10E−06
GPRC5D	55507	G protein-coupled receptor, family C, group 5, member D	1.67	2.20E−05
UBE2C	11065	ubiquitin-conjugating enzyme E2C	1.59	4.40E−06
NLRP7	199713	NLR family, pyrin domain containing 7	1.51	8.19E−05
LOC728741	728741	hypothetical LOC728741	1.50	1.18E−05
APOBEC3B	9582	apolipoprotein B mRNA editing enzyme, catalytic polypeptide-like 3B	1.49	7.27E−04

Gene symbol	EntrezID	Gene name	Fold-change	*P*-value
LOC652694	652694	similar to Ig kappa chain V-I region HK102 precursor	1.46	2.59E−03
AURKB	9212	aurora kinase B	1.45	4.97E−05
TYMS	7298	thymidylate synthetase	1.44	1.90E−04
CDCA5	113130	cell division cycle associated 5	1.43	6.91E−05
LOC651612	651612	hypothetical protein LOC651612	1.43	2.60E−03
POU2AF1	5450	POU class 2 associating factor 1	1.42	7.50E−06
SORL1	6653	sortilin-related receptor, L(DLR class) A repeats containing	1.42	1.65E−03
HSP90B1	7184	heat shock protein 90kDa beta (Grp94), member 1	1.42	1.80E−03
HS.520591	—	—	1.42	1.87E−03
SEMA4A	64218	sema domain, immunoglobulin domain (Ig), transmembrane domain (TM) and short cytoplasmic domain, (semaphorin) 4A	1.42	3.48E−03
TNFRSF13B	23495	tumor necrosis factor receptor superfamily, member 13B	1.41	2.69E−04
TK1	7083	thymidine kinase 1, soluble	1.40	3.14E−04
CDC45L	8318	cell division cycle 45 homolog (S. cerevisiae)	1.40	3.64E−04
FKBP11	51303	FK506 binding protein 11, 19 kDa	1.40	3.75E−04
RRBP1	6238	ribosome binding protein 1 homolog 180kDa (dog)	1.37	3.25E−05
TOP2A	7153	topoisomerase (DNA) II alpha 170kDa	1.36	9.93E−05
NT5DC2	64943	5′-nucleotidase domain containing 2	1.36	2.83E−04
NCAPG	64151	non-SMC condensin I complex, subunit G	1.36	1.76E−03
BIK	638	BCL2-interacting killer (apoptosis-inducing)	1.35	6.90E−04
SDF2L1	23753	stromal cell-derived factor 2-like 1	1.35	9.31E−04
F5	2153	coagulation factor V (proaccelerin, labile factor)	1.35	4.48E−03
UHRF1	29128	ubiquitin-like with PHD and ring finger domains 1	1.34	3.62E−05
C19ORF10	56005	chromosome 19 open reading frame 10	1.34	4.20E−05
LOC100131727	100131727	hypothetical protein LOC100131727	1.34	2.55E−04
SLC35A5	55032	solute carrier family 35, member A5	1.33	1.09E−04
CAMK1G	57172	calcium/calmodulin-dependent protein kinase IG	1.33	1.17E−04
IRF4	3662	interferon regulatory factor 4	1.32	8.50E−06
DENND5B	160518	DENN/MADD domain containing 5B	1.32	5.83E−05
CCNA2	890	cyclin A2	1.32	9.85E−04
ASAP1IT1	29065	ASAP1 intronic transcript 1 (nonprotein coding)	1.32	4.13E−03
LOC100129905	100129905	ribosomal protein S27 pseudogene 19	1.31	4.40E−03
PTTG3P	26255	pituitary tumor-transforming 3, pseudogene	1.30	9.10E−05
SLC38A10	124565	solute carrier family 38, member 10	1.29	3.94E−05
GMPPB	29925	GDP-mannose pyrophosphorylase B	1.29	5.78E−05
BUB1	699	budding uninhibited by benzimidazoles 1 homolog (yeast)	1.29	2.83E−03
OXCT2	64064	3-oxoacid CoA transferase 2	1.28	5.21E−05
MCM4	4173	minichromosome maintenance complex component 4	1.28	1.69E−04
PHGDH	26227	phosphoglycerate dehydrogenase	1.28	2.84E−04
BIRC5	332	baculoviral IAP repeat containing 5	1.28	4.18E−04
TLN1	7094	talin 1	1.28	1.36E−03
HS.184721	—	—	1.28	2.49E−03
LOC441124	441124	hypothetical gene supported by AK093729; BX647918	1.28	4.58E−03
CKAP4	10970	cytoskeleton-associated protein 4	1.28	4.78E−03
CCR9	10803	chemokine (C-C motif) receptor 9	1.27	5.30E−06
PDIA5	10954	protein disulfide isomerase family A, member 5	1.27	2.36E−04
COL18A1	80781	collagen, type XVIII, alpha 1	1.27	2.42E−03
ABCG1	9619	ATP-binding cassette, subfamily G (WHITE), member 1	1.26	1.69E−05
SLC2A5	6518	solute carrier family 2 (facilitated glucose/fructose transporter), member 5	1.26	3.11E−04
MTF1	4520	metal-regulatory transcription factor 1	1.26	7.35E−04
CDT1	81620	chromatin licensing and DNA replication factor 1	1.26	2.07E−03
STT3A	3703	STT3, subunit of the oligosaccharyltransferase complex, homolog A (S. cerevisiae)	1.25	1.59E−04
TRAM2	9697	translocation associated membrane protein 2	1.25	4.94E−04
AURKA	6790	aurora kinase A	1.25	6.78E−04
HS.157344	—	—	1.25	9.78E−04
POLR2A	5430	polymerase (RNA) II (DNA directed) polypeptide A, 220kDa	1.25	2.00E−03
LOC642131	642131	putative V-set and immunoglobulin domain-containing protein 6-like	1.25	3.01E−03
SPC24	147841	SPC24, NDC80 kinetochore complex component, homolog (S. cerevisiae)	1.25	4.41E−03
DLGAP5	9787	discs, large (Drosophila) homolog-associated protein 5	1.24	1.57E−03
NUSAP1	51203	nucleolar and spindle associated protein 1	1.24	2.92E−03
UBE2J1	51465	ubiquitin-conjugating enzyme E2, J1 (UBC6 homolog, yeast)	1.23	2.68E−05
HS.352677	—	—	1.23	1.85E−04
TRIP13	9319	thyroid hormone receptor interactor 13	1.23	6.14E−04
WAS	7454	Wiskott-Aldrich syndrome (eczema-thrombocytopenia)	1.23	1.20E−03
ARID3A	1820	AT rich interactive domain 3A (BRIGHT-like)	1.23	1.82E−03
CENPM	79019	centromere protein M	1.23	2.48E−03
UBA1	7317	ubiquitin-like modifier activating enzyme 1	1.23	3.30E−03
BMP8B	656	bone morphogenetic protein 8b	1.22	6.65E−04
SPATS2	65244	spermatogenesis associated, serine-rich 2	1.22	8.57E−04
SLC12A9	56996	solute carrier family 12 (potassium/chloride transporters), member 9	1.22	9.20E−04

Gene symbol	EntrezID	Gene name	Fold-change	*P*-value
SLC1A4	6509	solute carrier family 1 (glutamate/neutral amino acid transporter), member 4	1.22	1.23E−03
TXNDC11	51061	thioredoxin domain containing 11	1.22	2.23E−03
LOC440348	440348	nuclear pore complex interacting protein-like 2	1.22	2.37E−03
HJURP	55355	Holliday junction recognition protein	1.22	3.25E−03
ZNF341	84905	zinc finger protein 341	1.22	3.44E−03
ZYX	7791	zyxin	1.22	4.32E−03
LOC100128269	100128269	hypothetical LOC100128269	1.22	4.94E−03
ZBTB43	23099	zinc finger and BTB domain containing 43	1.21	4.85E−04
KIF1B	23095	kinesin family member 1B	1.21	7.65E−04
SNORA11B	100124539	small nucleolar RNA, H/ACA box 11B (retrotransposed)	1.21	9.37E−04
KIF2C	11004	kinesin family member 2C	1.21	9.41E−04
MAGT1	84061	magnesium transporter 1	1.21	1.14E−03
PRR11	55771	proline rich 11	1.21	1.35E−03
MCM2	4171	minichromosome maintenance complex component 2	1.21	1.71E−03
ALOX5	240	arachidonate 5-lipoxygenase	1.21	2.62E−03
TCF4	6925	transcription factor 4	1.21	2.93E−03
NARF	26502	nuclear prelamin A recognition factor	1.21	3.63E−03
SORT1	6272	sortilin 1	1.21	4.53E−03
CDCA8	55143	cell division cycle associated 8	1.20	1.59E−03
LAMA5	3911	laminin, alpha 5	1.20	2.35E−03
HYOU1	10525	hypoxia up-regulated 1	1.20	2.67E−03
HS.143018	—	—	1.20	3.51E−03
FAHD2B	151313	fumarylacetoacetate hydrolase domain containing 2B	1.20	4.76E−03
ZBTB32	27033	zinc finger and BTB domain containing 32	1.19	3.53E−04
MGAT3	4248	mannosyl (beta-1,4-)-glycoprotein beta-1,4-N-acetylglucosaminyltransferase	1.19	6.72E−04
ADAM19	8728	ADAM metallopeptidase domain 19	1.19	8.08E−04
DCTN1	1639	dynactin 1	1.19	1.48E−03
SEMA3E	9723	sema domain, immunoglobulin domain (Ig), short basic domain, secreted, (semaphorin) 3E	1.19	1.52E−03
GMPPA	29926	GDP-mannose pyrophosphorylase A	1.19	1.62E−03
LOC651816	651816	similar to Ubiquitin-conjugating enzyme E2S (Ubiquitin-conjugating enzyme E2-24 kDa) (Ubiquitin-protein ligase) (Ubiquitin carrier protein) (E2-EPF5)	1.19	2.38E−03
SEC61A1	29927	Sec61 alpha 1 subunit (S. cerevisiae)	1.19	2.40E−03
EHBP1L1	254102	EH domain binding protein 1-like 1	1.19	2.48E−03
CIC	23152	capicua homolog (Drosophila)	1.19	2.88E−03
FLOT2	2319	flotillin 2	1.19	3.25E−03
RARA	5914	retinoic acid receptor, alpha	1.19	3.35E−03
DUSP5	1847	dual specificity phosphatase 5	1.19	4.32E−03
OR2AG1	144125	olfactory receptor, family 2, subfamily AG, member 1	1.18	4.70E−04
LOC399900	399900	hypothetical LOC399900	1.18	5.09E−04
SPCS2	9789	signal peptidase complex subunit 2 homolog (S. cerevisiae)	1.18	5.44E−04
ZBP1	81030	Z-DNA binding protein 1	1.18	9.12E−04
LOC399491	399491	GPS, PLAT and transmembrane domain-containing protein	1.18	1.04E−03
KIF20A	10112	kinesin family member 20A	1.18	2.68E−03
COPG	22820	coatomer protein complex, subunit gamma	1.18	2.79E−03
OTUD5	55593	OTU domain containing 5	1.18	4.34E−03
MYH9	4627	myosin, heavy chain 9, nonmuscle	1.18	4.81E−03
SEC13	6396	SEC13 homolog (S. cerevisiae)	1.17	1.41E−03
VPS8	23355	vacuolar protein sorting 8 homolog (S. cerevisiae)	1.17	1.44E−03
PARM1	25849	prostate androgen-regulated mucin-like protein 1	1.17	1.47E−03
SRPR	6734	signal recognition particle receptor (docking protein)	1.17	1.69E−03
PIM2	11040	pim-2 oncogene	1.17	2.01E−03
TPX2	22974	TPX2, microtubule-associated, homolog (Xenopus laevis)	1.17	2.10E−03
LOC100130168	100130168	hypothetical protein LOC100130168	1.17	2.35E−03
HS.562118	—	—	1.17	2.59E−03
NLRP1	22861	NLR family, pyrin domain containing 1	1.17	2.83E−03
HS.569823	—	—	1.17	2.89E−03
KIAA0226	9711	KIAA0226	1.17	2.93E−03
TAP1	6890	transporter 1, ATP-binding cassette, subfamily B (MDR/TAP)	1.17	2.99E−03
MLEC	9761	malectin	1.17	3.23E−03
CXXC1	30827	CXXC finger protein 1	1.17	3.34E−03
AARS	16	alanyl-tRNA synthetase	1.17	3.64E−03
C1QB	713	complement component 1, q subcomponent, B chain	1.17	4.08E−03
KIAA0492	57238	KIAA0492 protein	1.17	4.14E−03
HS.447737	—	—	1.17	4.58E−03
HS.559654	—	—	1.16	1.45E−03
LOC728790	728790	hypothetical LOC728790	1.16	2.13E−03
ALDH6A1	4329	aldehyde dehydrogenase 6 family, member A1	1.16	2.62E−03
IL4R	3566	interleukin 4 receptor	1.16	2.90E−03

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PDIA4	9601	protein disulfide isomerase family A, member 4	1.16	4.18E−03
ALDH3B1	221	aldehyde dehydrogenase 3 family, member B1	1.16	4.67E−03
ZNF486	90649	zinc finger protein 486	1.16	4.68E−03
IRF9	10379	interferon regulatory factor 9	1.16	4.76E−03
TMEM106A	113277	transmembrane protein 106A	1.16	4.78E−03
INTS3	65123	integrator complex subunit 3	1.15	1.11E−03
SIL1	64374	SIL1 homolog, endoplasmic reticulum chaperone (S. cerevisiae)	1.15	2.09E−03
FKBP2	2286	FK506 binding protein 2, 13kDa	1.15	2.33E−03
BTBD12	84464	SLX4 structure-specific endonuclease subunit homolog (S. cerevisiae)	1.15	2.39E−03
NFKB2	4791	nuclear factor of kappa light polypeptide gene enhancer in B-cells 2 (p49/p100)	1.15	3.36E−03
SLC35B1	10237	solute carrier family 35, member B1	1.15	4.01E−03
C4ORF28	133015	PARK2 co-regulated-like	1.15	4.53E−03
P4HB	5034	prolyl 4-hydroxylase, beta polypeptide	1.15	4.73E−03
PTK2B	2185	PTK2B protein tyrosine kinase 2 beta	1.15	4.97E−03
FBXO18	84893	F-box protein, helicase, 18	1.14	5.80E−04
LILRB1	10859	leukocyte immunoglobulin-like receptor, subfamily B (with TM and ITIM domains), member 1	1.14	2.05E−03
PCYOX1	51449	prenylcysteine oxidase 1	1.14	2.52E−03
CLPTM1L	81037	CLPTM1-like	1.14	3.02E−03
FAM46A	55603	family with sequence similarity 46, member A	1.14	3.44E−03
CAPN11	11131	calpain 11	1.14	4.04E−03
EIF4G3	8672	eukaryotic translation initiation factor 4 gamma, 3	1.14	4.54E−03
HS.188979	—	—	1.13	1.30E−03
TRPM3	80036	transient receptor potential cation channel, subfamily M, member 3	1.13	1.86E−03
FLJ44124	641737	hypothetical LOC641737	1.13	2.25E−03
FLJ20254	54867	transmembrane protein 214	1.13	3.91E−03
IFNAR2	3455	interferon (alpha, beta and omega) receptor 2	1.12	2.19E−03
RCC2	55920	regulator of chromosome condensation 2	1.12	3.84E−03
SEC23B	10483	Sec23 homolog B (S. cerevisiae)	1.11	4.11E−03
SHMT1	6470	serine hydroxymethyltransferase 1 (soluble)	1.10	3.67E−03
Down-Regulated Genes (136)				
HOPX	84525	HOP homeobox	−1.43	1.61E−04
AKR1C3	8644	aldo-keto reductase family 1, member C3 (3-alpha hydroxysteroid dehydrogenase, type II)	−1.41	4.83E−03
POP5	51367	processing of precursor 5, ribonuclease P/MRP subunit (S. cerevisiae)	−1.37	5.03E−05
RPL5	6125	ribosomal protein L5	−1.35	3.27E−03
LOC100133185	100133185	hypothetical LOC100133185	−1.33	4.76E−04
LOC338870	338870	ribosomal protein S12 pseudogene 23	−1.32	8.62E−05
C6ORF190	387357	thymocyte selection associated	−1.32	4.69E−04
LOC391370	391370	ribosomal protein S12 pseudogene 4	−1.32	6.72E−04
CD160	11126	CD160 molecule	−1.32	2.00E−03
ENPP4	22875	ectonucleotide pyrophosphatase/phosphodiesterase 4 (putative)	−1.32	2.40E−03
GZMK	3003	granzyme K (granzyme 3; tryptase II)	−1.30	2.36E−03
RCN2	5955	reticulocalbin 2, EF-hand calcium binding domain	−1.30	3.32E−03
TRAPPC2P1	10597	trafficking protein particle complex 2 pseudogene 1	−1.30	4.15E−03
CRYZ	1429	crystallin, zeta (quinone reductase)	−1.30	4.43E−03
TMEM106B	54664	transmembrane protein 106B	−1.28	3.93E−04
LOC441506	441506	similar to laminin receptor 1	−1.28	9.06E−04
BMI1	648	BMI1 polycomb ring finger oncogene	−1.28	2.35E−03
C17ORF45	125144	nonprotein coding RNA 188	−1.28	2.59E−03
ZNF594	84622	zinc finger protein 594	−1.27	5.38E−04
FAU	2197	Finkel-Biskis-Reilly murine sarcoma virus (FBR-MuSV) ubiquitously expressed	−1.27	1.39E−03
LAIR2	3904	leukocyte-associated immunoglobulin-like receptor 2	−1.27	2.09E−03
RGS18	64407	regulator of G-protein signaling 18	−1.27	2.44E−03
ANKRD46	157567	ankyrin repeat domain 46	−1.27	3.68E−03
C12ORF41	54934	chromosome 12 open reading frame 41	−1.25	2.88E−04
LEPROTL1	23484	leptin receptor overlapping transcript-like 1	−1.25	5.22E−04
KLRD1	3824	killer cell lectin-like receptor subfamily D, member 1	−1.25	6.85E−04
DCK	1633	deoxycytidine kinase	−1.25	3.00E−03
ZCCHC7	84186	zinc finger, CCHC domain containing 7	−1.25	3.20E−03
LOC100133662	100133662	hypothetical protein LOC100133662	−1.23	5.22E−04
LOC388789	388789	hypothetical LOC388789	−1.23	1.83E−03
TGDS	23483	TDP-glucose 4,6-dehydratase	−1.23	2.73E−03
TRIAP1	51499	TP53 regulated inhibitor of apoptosis 1	−1.23	3.11E−03
CREBZF	58487	CREB/ATF bZIP transcription factor	−1.23	3.19E−03
TAF7	6879	TAF7 RNA polymerase II, TATA box binding protein (TBP)-associated factor, 55kDa	−1.23	3.97E−03
PTGDR	5729	prostaglandin D2 receptor (DP)	−1.22	2.98E−04
PTGER2	5732	prostaglandin E receptor 2 (subtype EP2), 53 kDa	−1.22	9.34E−04

Gene symbol	EntrezID	Gene name	Fold-change	*P*-value
CLNS1A	1207	chloride channel, nucleotide-sensitive, 1A	−1.22	1.65E−03
GNG2	54331	guanine nucleotide binding protein (G protein), gamma 2	−1.22	1.96E−03
ZNF256	10172	zinc finger protein 256	−1.22	3.28E−03
LOC648249	648249	similar to 40S ribosomal protein SA (p40) (34/67 kDa laminin receptor) (Colon carcinoma laminin-binding protein) (NEM/1CHD4) (Multidrug resistance-associated protein MGr1-Ag)	−1.22	4.09E−03
CCL4L2	388372	chemokine (C-C motif) ligand 4-like 2	−1.20	1.94E−05
C16ORF63	123811	FGFR1OP N-terminal like	−1.20	1.76E−03
C9ORF78	51759	chromosome 9 open reading frame 78	−1.20	1.83E−03
LOC440055	440055	ribosomal protein S12 pseudogene 22	−1.20	2.30E−03
MOCS2	4338	molybdenum cofactor synthesis 2	−1.20	2.59E−03
BBS10	79738	Bardet-Biedl syndrome 10	−1.20	3.02E−03
ZNF32	7580	zinc finger protein 32	−1.20	3.19E−03
TSPAN13	27075	tetraspanin 13	−1.20	4.00E−03
ZNF480	147657	zinc finger protein 480	−1.19	1.73E−04
MED30	90390	mediator complex subunit 30	−1.19	3.47E−04
AQP12B	653437	aquaporin 12B	−1.19	9.56E−04
FAM179A	165186	family with sequence similarity 179, member A	−1.19	1.19E−03
ZNF550	162972	zinc finger protein 550	−1.19	2.44E−03
COX7A2L	9167	cytochrome c oxidase subunit VIIa polypeptide 2 like	−1.19	2.58E−03
FLJ14213	79899	proline rich 5 like	−1.19	2.81E−03
HSF2	3298	heat shock transcription factor 2	−1.19	2.99E−03
ARL6IP5	10550	ADP-ribosylation-like factor 6 interacting protein 5	−1.19	3.39E−03
LEMD3	23592	LEM domain containing 3	−1.19	3.41E−03
CXCR7	57007	chemokine (C-X-C motif) receptor 7	−1.19	3.50E−03
SNORD21	6083	small nucleolar RNA, C/D box 21	−1.19	4.02E−03
C1ORF52	148423	chromosome 1 open reading frame 52	−1.19	4.65E−03
KCTD5	54442	potassium channel tetramerisation domain containing 5	−1.18	2.35E−04
SMARCE1	6605	SWI/SNF related, matrix associated, actin dependent regulator of chromatin, subfamily e, member 1	−1.18	2.56E−04
C7ORF70	84792	chromosome 7 open reading frame 70	−1.18	7.11E−04
CYP2R1	120227	cytochrome P450, family 2, subfamily R, polypeptide 1	−1.18	7.45E−04
HSF5	124535	heat shock transcription factor family member 5	−1.18	1.40E−03
LOC641844	641844	SDHDP2 succinate dehydrogenase complex, subunit D, integral membrane protein pseudogene 2	−1.18	1.44E−03
CD226	10666	CD226 molecule	−1.18	1.74E−03
C1ORF75	55248	transmembrane protein 206	−1.18	1.75E−03
STK39	27347	serine threonine kinase 39	−1.18	2.07E−03
METTL4	64863	methyltransferase like 4	−1.18	2.10E−03
LOC401206	401206	ribosomal protein S25 pseudogene 6	−1.18	2.70E−03
MIS12	79003	MIS12, MIND kinetochore complex component, homolog (S. pombe)	−1.18	3.11E−03
C3ORF10	55845	chromosome 3 open reading frame 10	−1.18	3.86E−03
CRIPT	9419	cysteine-rich PDZ-binding protein	−1.18	3.99E−03
RPS13	6207	ribosomal protein S13	−1.18	3.99E−03
SENP7	57337	SUMO1/sentrin specific peptidase 7	−1.18	4.09E−03
SLFN12	55106	schlafen family member 12	−1.16	5.86E−04
SLC27A5	10998	solute carrier family 27 (fatty acid transporter), member 5	−1.16	6.83E−04
LOC100130154	100130154	similar to thymosin, beta 10	−1.16	1.32E−03
PRKAB2	5565	protein kinase, AMP-activated, beta 2 noncatalytic subunit	−1.16	1.35E−03
RPA2	6118	replication protein A2, 32 kDa	−1.16	1.41E−03
MED4	29079	mediator complex subunit 4	−1.16	1.85E−03
FASLG	356	Fas ligand (TNF superfamily, member 6)	−1.16	1.89E−03
MS4A1	931	membrane-spanning 4-domains, subfamily A, member 1	−1.16	2.50E−03
HS.25318		—	−1.16	2.69E−03
LOC100127918	100127918	similar to small ubiquitin-related modifier 2	−1.16	3.51E−03
RAB11A	8766	RAB11A, member RAS oncogene family	−1.16	4.00E−03
KPNA5	3841	karyopherin alpha 5 (importin alpha 6)	−1.16	4.45E−03
SETMAR	6419	SET domain and mariner transposase fusion gene	−1.16	4.67E−03
SMYD2	56950	SET and MYND domain containing 2	−1.15	6.80E−04
KLHDC5	57542	kelch domain containing 5	−1.15	1.47E−03
TMEM9B	56674	TMEM9 domain family, member B	−1.15	1.76E−03
ZNF304	57343	zinc finger protein 304	−1.15	1.82E−03
GK5	256356	glycerol kinase 5 (putative)	−1.15	2.55E−03
SOCS2	8835	suppressor of cytokine signaling 2	−1.15	2.63E−03
LOC347292	347292	ribosomal protein L36 pseudogene 14	−1.15	2.72E−03
C11ORF46	120534	chromosome 11 open reading frame 46	−1.15	3.32E−03
STX16	8675	syntaxin 16	−1.15	3.74E−03
VAMP4	8674	vesicle-associated membrane protein 4	−1.15	4.00E−03
C1ORF131	128061	chromosome 1 open reading frame 131	−1.15	4.00E−03
LTBP2	4053	latent transforming growth factor beta binding protein 2	−1.15	4.01E−03
PQLC3	130814	PQ loop repeat containing 3	−1.15	4.07E−03
ZNF187	7741	zinc finger protein 187	−1.15	4.26E−03

Gene symbol	EntrezID	Gene name	Fold-change	*P*-value
NGRN	51335	neugrin, neurite outgrowth associated	−1.15	4.30E−03
RHEB	6009	Ras homolog enriched in brain	−1.15	4.47E−03
HS.133324		—	−1.15	4.58E−03
SNORA76	677842	small nucleolar RNA, H/ACA box 76	−1.15	4.59E−03
SERPINE2	5270	serpin peptidase inhibitor, clade E (nexin, plasminogen activator inhibitor type 1), member 2	−1.14	1.28E−03
ERP27	121506	endoplasmic reticulum protein 27	−1.14	1.32E−03
CCL3L1	6349	chemokine (C-C motif) ligand 3-like 1	−1.14	1.33E−03
MLLT11	10962	myeloid/lymphoid or mixed-lineage leukemia (trithorax homolog, Drosophila); translocated to, 11	−1.14	1.40E−03
AASDHPPT	60496	aminoadipate-semialdehyde dehydrogenase-phosphopantetheinyl transferase	−1.14	1.53E−03
C20ORF30	29058	chromosome 20 open reading frame 30	−1.14	1.67E−03
LOC642341	642341	hypothetical LOC642341	−1.14	1.99E−03
VTA1	51534	Vps20-associated 1 homolog (S. cerevisiae)	−1.14	2.43E−03
MFSD6	54842	major facilitator superfamily domain containing 6	−1.14	2.44E−03
KBTBD6	89890	kelch repeat and BTB (POZ) domain containing 6	−1.14	2.59E−03
LOC731915	731915	similar to ATP-binding cassette sub-family D member 1 (Adrenoleukodystrophy protein) (ALDP)	−1.14	2.61E−03
CCL3L3	414062	chemokine (C-C motif) ligand 3-like 3	−1.14	2.80E−03
LOC100130633	100130633	hypothetical protein LOC100130633	−1.14	2.93E−03
CCNB1IP1	57820	cyclin B1 interacting protein 1, E3 ubiquitin protein ligase	−1.14	3.62E−03
PPIA	5478	peptidylprolyl isomerase A (cyclophilin A)	−1.14	3.96E−03
MST4	51765	serine/threonine protein kinase MST4	−1.14	4.95E−03
ZXDB	158586	zinc finger, X-linked, duplicated B	−1.12	1.34E−03
NUDT11	55190	nudix (nucleoside diphosphate linked moiety X)-type motif 11	−1.12	2.81E−03
BCL2L13	23786	BCL2-like 13 (apoptosis facilitator)	−1.12	3.02E−03
PALB2	79728	partner and localizer of BRCA2	−1.12	3.05E−03
C9ORF85	138241	chromosome 9 open reading frame 85	−1.12	3.09E−03
MRPS36	92259	mitochondrial ribosomal protein S36	−1.12	3.12E−03
LYRM4	57128	LYR motif containing 4	−1.12	3.17E−03
MFF	56947	mitochondrial fission factor	−1.12	3.46E−03
MRPS17	51373	mitochondrial ribosomal protein S17	−1.12	3.89E−03
HS.581615		—	−1.12	4.81E−03
DNAJA4	55466	DnaJ (Hsp40) homolog, subfamily A, member 4	−1.12	4.94E−03
FBXO3	26273	F-box protein 3	−1.11	4.47E−03

*Determined by pairwise comparisons at $P < 0.005$ with 256 available permutations using the BRB-ArrayTools ($n = 9$).

Table S4. *Cis*-regulatory elements enriched in the putative promoter regions (−1‹000 ~ +200) of genes responsive to dietary zinc depletion, identified by promoter integration in microarray analysis (PRIMA) of EXPANDER with zinc-responsive genes determined by a pairwise comparison ($P < 0.005$)†

Transcription factor	TRANSFAC ID	*P*-value	Enrichment factor*	Target gene (location of putative TF binding site)
Up-regulated genes (cluster 1)				
NF-Y	M00287	5.98E−7	1.692	PDIA4(−61,−41,104), SPC24(−213,−115), NUSAP1(−59), CDCA5(−745), CIC(−765,−716), CDC45L(158), AARS(−51), TPX2(99,135), AURKA(−11,21), RRBP1(−60), SLC38A10(−85), SHMT1(−39), SDF2L1(−138,−19), UHRF1(−88), ALDH6A1(−226,−134,−91,−33,−5), FAM46A(−737), TK1(98,129), KIF20A(41), HSP90B1(−171,−118), SRPR(−257,−156), MCM4(−59), CDCA8(17), CXXC1(−923), MGC29506(−50), NARF(−122,−82,−46,−6), PRR11(−479,−345,−254,−133,−105,−39), PIM2(−124), UBE2C(−68,−37), SLC1A4 (−181), CCNA2(−82), BUB1(−61,−35), GLDC(−97), SORT1(−189,−128), P4HB(−256, −140), MAGT1(−216,−169,−79), AURKB(−92), OTUD5(−847,−480), CCNB2(−100,−67), MLEC(−647,−129,−73), OXCT2(−294), TRPM3(−125), CDC20(−79,−34), SPCS2(−52), FBXO18(−97), PTK2B(133), DLGAP5(1,66), GMPPB(−190,−124,−51)
AP−2α	M00469	0.0020	1.605	HYOU1(−240), ALOX5(−137), CLPTM1L(−811,−416), NUSAP1(−362), CIC(−771,−527, −490,−63), CDC45L(−423), RRBP1(−523), TXNDC11(15), TRAM2(−444,−395,−247), SLC38A10(−184), SDF2L1(55), ARID3A(−432,−237), FAM46A(−639), C19ORF10(−66), KIF1B(−403), DUSP5(−61), UBE2J1(−193), HSP90B1(−820), SRPR(−116), NARF(−539), PIM2(106), CDT1(−334), NLRP1(76), UBE2C(−336), SLC1A4(−656,60), SORT1(−296), P4HB(−703), MCM2(−133), OTUD5(−334), OXCT2(36), TLN1(−323), PTK2B(−277), DLGAP5(−158), APOBEC3B(−125), LAMA5(−213), NFKB2(−435), GMPPA(−678), GMPPB(−510)

Transcription factor	TRANSFAC ID	*P*-value	Enrichment factor*	Target gene (location of putative TF binding site)
ETF	M00695	9.4E−4	1.374	PDIA4(−126,50), ALOX5(−911,−708,52), MTF1(−518), TYMS(−51,52,80,114), BIRC5 (−52,110), ZYX(−541,15), CDC45L(−300,−285,199), TPX2(200), MGAT3(97,142), TRAM2(−469), SEC23B(−95), SDF2L1(29), UHRF1(18), SEC13(108), COL18A1(10), UBE2J1(−92), SRPR(−9), SPATS2(−430,−414), CDT1(46), PIM2(−401), SLC1A4(−208, −195), FAHD2B(−146,126,135), BIK(43), SLC35B1(−416,27), P4HB(−459), KIAA0226 (−246,25), EHBP1L1(104), ADAM19(53), DENND5B(−114,37,148,157), KIF2C(−37), OTUD5(−408), TRPM3(−211,−91), FBXO18(−279,−60,−38), C4ORF28(−106), LAMA5 (−969,15), ITM2C(52), SORL1(−723,86), HYOU1(−131), TRIP13(−315,−295,64), CKAP4 (−211,−116), FLOT2(−132), CLPTM1L(−204,−87,−68), CIC(−745,−724,−468), AARS (−76), STT3A(−732), RRBP1(−118,193), TXNDC11(22), SLC38A10(4), SHMT1 (−101,92,142), SIL1(17,47), IFNAR2(164), TK1(116), KIF1B(−928,−846,−732,−445), DUSP5(105), HSP90B1(−318), ZNF341(101), MCM4(−724), CDCA8(−56), ABCG1(−110), NARF(−438,−222,−213,126), BTBD12(10), PRR11(−709,−550), CCNA2(−30), BUB1 (−39,34), UBA1(86,151), BMP8B(−243,198), MLEC(−227,−37), MYH9(89), CDC20(−305, −139), POLR2A(−388), GMPPA(−867), SEC61A1(176), GMPPB(−486)
Down-regulated genes (cluster 2)				
Elk−1	M00025	0.0020	1.628	LEMD3(−38), TMEM9B(−2), CREBZF(−905), ZNF256(−149), VTA1(−45), KCTD5(−124,9), CRYZ(−273), C9ORF78(6), ZNF594(−176,12), PQLC3(−731), LEPROTL1(−21), SLFN12 (−748), RAB11A(−732,−223,−17), C1ORF52(−22,−1), PALB2(−116), SENP7(−123,−93), TAF7(−257), FLJ14213(−411), C20ORF30(−39), ZCCHC7(−6), COX7A2L(−778), ZNF550 (−23), CRIPT(−3), MIS12(−92,−74)
TEF	M00672	0.0030	1.621	C6ORF190(−38), ZNF187(−202,−140), PTGER2(−968,−827), KLRD1(−605), MS4A1(−547), GZMK(−451,−47), C9ORF78(−906), CCNB1IP1(−86), ZNF594(−951), TSPAN13(−553), RAB11A(−845), TAF7(−981,−876), RGS18(−306,49), SETMAR(−155), ERP27(−836, −181), FASLG(−286), AKR1C3(−172,−10), GNG2(−969), HOPX(−548), GK5(−667)

*Ratio of prevalence of TF hits in the cluster to that in all genes.
†Threshold *P*-value for significance was set at 0.005.

Table S5. Top five functions enriched among the genes positively and negatively responsive to acute dietary zinc depletion, respectively†

Functional category	*P*-value*	Gene list
Up-regulated genes		
Cell cycle	1.03E−08 ~ 1.64E−02	KIF20A, DLGAP5, CDC20, CDT1, NCAPG, CCNB2, AURKB, BIRC5, CCNA2, RARA, TOP2A, CD38, HJURP, CDCA5, KIF2C, TYMS, CDC45, TRIP13, NUSAP1, IL4R, CDCA8, IRF9, AURKA, TPX2, ARID3A, BUB1, MCM2, WAS, BIK, MYH9, DCTN1, PIM2, UBE2C
Cellular assembly and organization	1.9E−08 ~ 1.64E−02	DLGAP5, PTK2B, CDT1, KIF1B, NCAPG, CCNB2, AURKB, TAP1, MCM4, ABCA1, BIRC5, CCNA2, TOP2A, HJURP, KIF2C, COL18A1, RRBP1, NUSAP1, UHRF1, AURKA, LILRB1, TPX2, BUB1, WAS, DCTN1, MYH9, ZYX
DNA replication, recombination, and repair	1.9E−08 ~ 1.64E−02	DLGAP5, PTK2B, CDT1, NCAPG, CCNB2, MCM4, BIRC5, INTS3, CCNA2, RARA, TOP2A, CDCA5, HJURP, KIF2C, TYMS, CDC45, TRIP13, NUSAP1, SLX4, AURKA, TPX2, BUB1, MCM2, BIK, ALOX5, TK1
Cancer	1.88E−07 ~ 1.75E−02	DLGAP5, KIF1B, NLRP7, NCAPG, NLRP1, CCNB2, ABCG1, AURKB, BIRC5, RARA, VPS8, SLC2A5, TYMS, IL4R, P4HB, CDCA8, EIF4G3, AURKA, NFKB2, IFNAR2, TPX2, CCR9, ZYX, ALOX5, TK1, UBE2C, KIF20A, TCF4, SLC1A4, CDC20, HYOU1, SPC24, SIL1, MCM4, IGLL1/ IGLL5, MGAT3, CCNA2, DUSP5, HSP90B1, POLR2A, F5, PHGDH, TOP2A, CD38, CDCA5, SORT1, COL18A1, TRIP13, LAMA5, NUSAP1, IRF4, UHRF1, ARID3A, BUB1, SDF2L1, MCM2, IGJ, UBA1, TNFRSF13B, PIM2
Genetic Disorder	7.69E−07 ~ 1.72E−02	DLGAP5, KIF1B, SLC38A10, NLRP1, CCNB2, ABCG1, TLN1, AURKB, ABCA1, BIRC5, TNFRSF17, INTS3, RARA, ALDH6A1, TYMS, PDIA5, IL4R, P4HB, CDCA8, SORL1, MAGT1, EIF4G3, AURKA, ZBTB43, NFKB2, IFNAR2, LILRB1, CCR9, FLOT2, ZYX, ALDH3B1, ALOX5, PACRGL, TK1, UBE2C, TCF4, SLC1A4, PTK2B, CDC20, CKAP4, SIL1, MCM4, IGLL1/IGLL5, TRPM3, CCNA2, DUSP5, HSP90B1, SEC61A1, POLR2A, F5, SEC23B, ADAM19, TOP2A, PHGDH, CD38, SORT1, HJURP, CDCA5, SEMA4A, COL18A1, SEMA3E, TRIP13, CDC45, CIC, LAMA5, IRF4, UHRF1, SLC35B1, COPG, SDF2L1, MCM2, WAS, IGJ, MYH9, DCTN1, SLC12A9, SPATS2, UBA1, TNFRSF13B, GLDC
Down-regulated genes		
Cell death	1.02E−04 ~ 4.93E−02	CLNS1A, SETMAR, GZMK, CD160, DCK, PTGDR, KLRD1, SOCS2, HSF2, MS4A1, SMARCE1, SEDLP, SERPINE2, MST4, BCL2L13, FAU, BMI1, PPIA, CD226, CCL3L1/CCL3L3, GNG2, TRIAP1, PTGER2, FASLG
Cell-mediated immune response	1.02E−04 ~ 4.05E−02	KLRD1, CD226, CCL3L1/CCL3L3, FASLG
Cellular development	1.02E−04 ~ 4.55E−02	AKR1C3, KLRD1, STK39, SMARCE1, TSPAN13, TAF7, MST4, BMI1, CXCR7, PPIA, CD226, PTGER2, FASLG
Cellular function and maintenance	1.02E−04 ~ 4.55E−02	KLRD1, PPIA, HSF2, CRIPT, SMARCE1, CD226, PTGER2, FASLG
Hematological system development and function	1.02E−04 ~ 4.55E−02	CLNS1A, BMI1, PTGDR, CXCR7, KLRD1, PPIA, SOCS2, CD226, CCL3L1/CCL3L3, PTGER2, FASLG, SERPINE2

*Range of Fisher's exact test *P*-values of functional annotations assigned to each respective functional category.
†Differentially expressed genes determined by a pairwise comparison ($P < 0.005$) were filtered by their mode of responses and overrepresented biological functions were identified by ingenuity pathway analysis

Table S6. Genes holding the potential as an indicator of dietary zinc deficiency in individuals*

Gene Symbol	Entrez ID	Gene Name	Fold-Change (ZnD/Baseline)	*P*-Value
CDC20	991	cell division cycle 20 homolog (S. cerevisiae)	2.69	2.00E−07
TXNDC5	81567	thioredoxin domain containing 5 (endoplasmic reticulum)	2.73	5.00E−07
MZB1	51237	marginal zone B and B1 cell-specific protein	2.07	7.00E−07
IGJ	3512	immunoglobulin J polypeptide, linker protein for immunoglobulin alpha and mu polypeptides	4.22	1.00E−06
IGLL1	3543	immunoglobulin lambda-like polypeptide 1	2.77	1.00E−06
CD38	952	CD38 molecule	2.03	2.07E−05
GLDC	2731	glycine dehydrogenase (decarboxylating)	2.49	2.30E−06
TNFRSF17	608	tumor necrosis factor receptor superfamily, member 17	2.55	1.85E−05

*Baseline and post-zinc-depletion values were grouped as to represent zinc-adequate (ZnA) and zinc-deficient (ZnD) conditions. Total of eight well-characterized genes were identified to be significantly different between each group ($P < 0.005$ by *t*-test without pairing) with a fold-change larger than two ($n = 9$).

Table S7. Dietary components of the liquid diet for the zinc-depletion phase

Dietary components of the liquid diet for the zinc-depletion phase

Component	Mineral Content	Amount per kg Diet
Egg white		129 g
Cornstarch		300 g
Maltose-dextrin		300 g
Sucrose		60 g
Corn oil		151 g
Cellulose		10 g
Mineral mixture	Sodium chloride	9.8 g Na
	Calcium carbonate	10.0 g Ca
	Potassium phosphate, monobasic [a]	3.8 g P [a+b]
	Potassium phosphate, dibasic [b]	12.0 g K [a+b+c]
	Potassium chloride [c]	
	Magnesium carbonate·$5H_2O$	1.28 g Mg
	Ferric citrate·$6H_2O$	57.6 mg Fe
	Copper sulfate·$5H_2O$	7.6 mg Cu
	Potassium Iodate	0.50 mg I
	Manganese chloride	11.4 mg Mn
	Glucose	16.8 g

* Sufficient energy intakes were ensured by supplemental mineral-free energy shakes. The caloric and mineral contents of the liquid formula were similar to those used in previous dietary zinc studies (1, 2).

Table S8 Mineral contents of the acclimation and zinc-repletion diets measured by inductively coupled plasma optical emission spectrophotometry

Mineral contents of the acclimation and zinc-repletion diets

	Dietary phase	
Minerals per day	Acclimation	Depletion
g Calcium	1.134	2.994
g Phosphorus	2.048	1.390
g Magnesium	0.343	0.509
g Potassium	3.259	4.417
g Sodium	3.208	5.038
g Sulfur	1.302	1.319
mg Copper	1.818	1.539
mg Iron	25.014	25.988
mg Zinc	**10.415**	**0.296**
mg Manganese	3.533	3.828

*Care was taken to minimize the difference in the daily intake of each mineral with the exception of zinc. To minimize the bioavailability of zinc, 1.4 g/d of sodium phytate from rice (Sigma) was supplemented to the formula. Carboxymethyl cellulose (2 g/d; TIC Gums) was added to prevent bowel discomfort that could be caused by the liquid diet. Supplemental biotin (2 mg/d) was provided to ensure sufficient biotin absorption because of the avidin from egg white in the liquid diet. A multivitamin supplement (CVS) was given. Distilled water (Zephyrhills), Diet Pepsi, and Sierra Mist (Pepsico), of which zinc contents were undetectable by flame atomic absorption spectrophotometry (AAS), were provided throughout the first two phases. For zinc repletion, zinc monomethionate (15 mg Zn/d; Jarrow) was consumed in addition to the subject's self-selected habitual diets. An anonymous questionnaire of compliance was provided to identify any major deviations from the protocol at the end of the study. Serum zinc concentrations were monitored throughout the study to verify compliance.