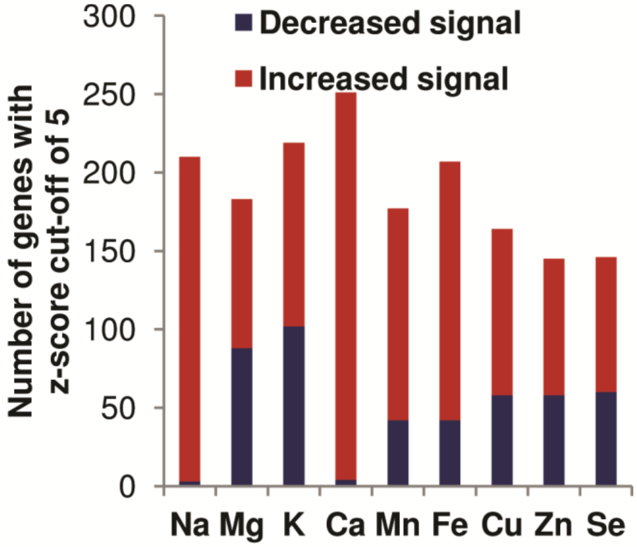
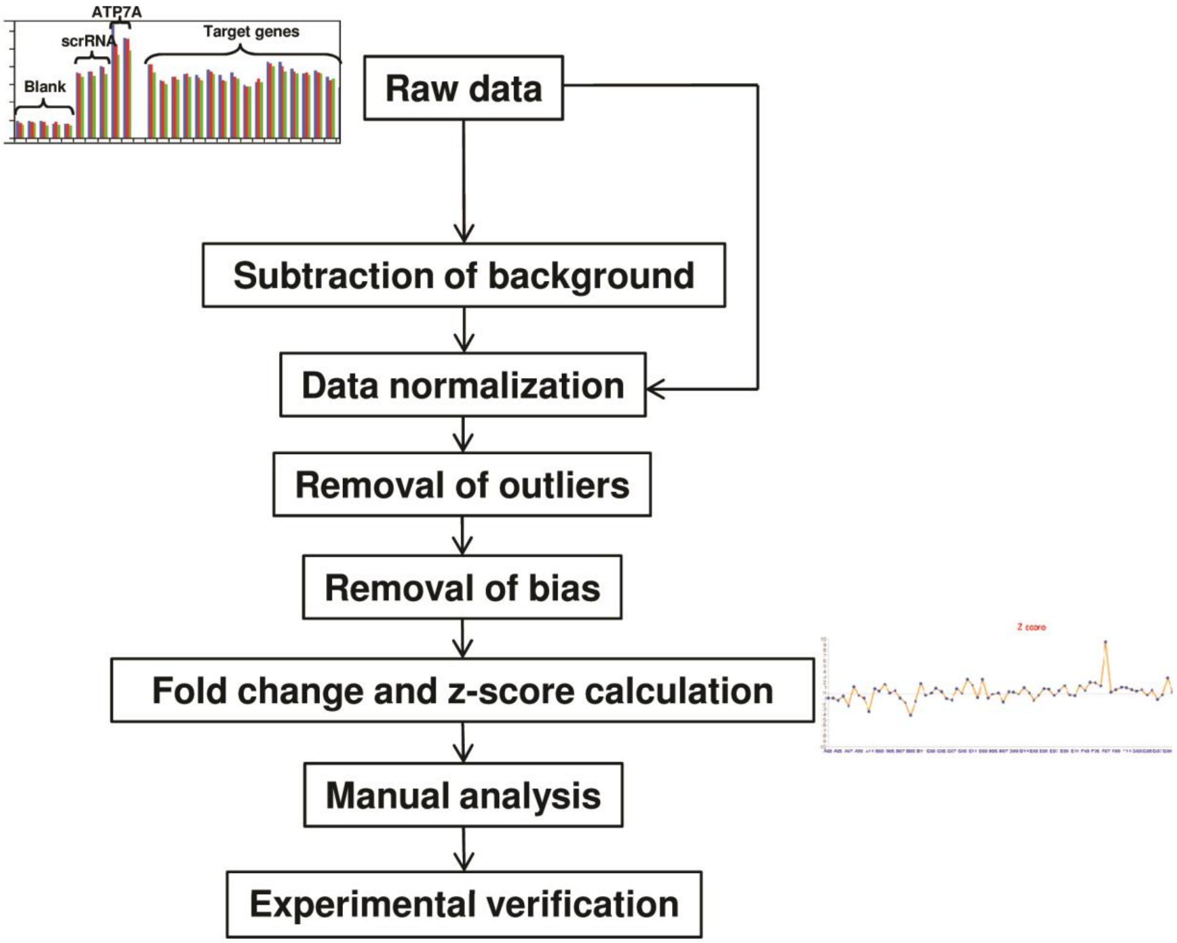
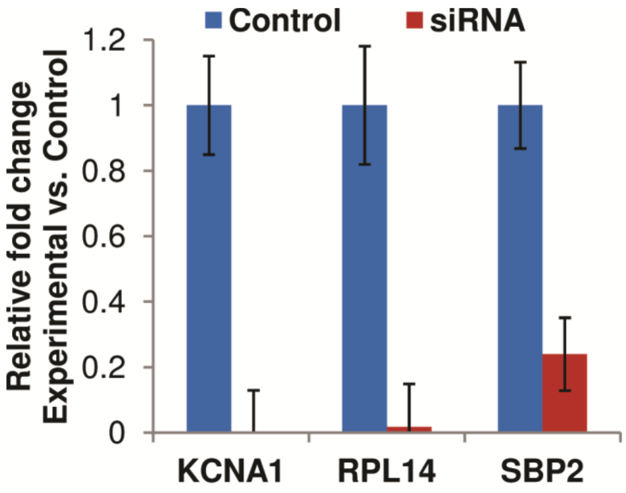


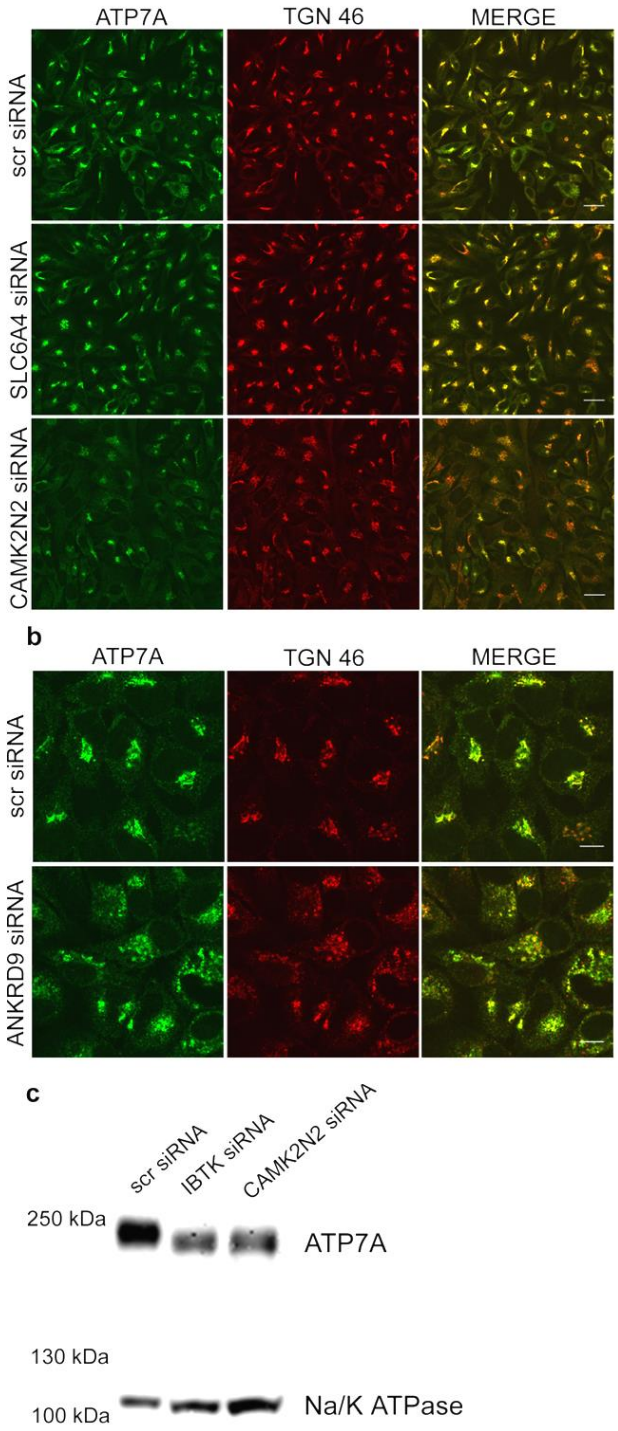
Supplementary Figure 1. Overview of the effect of siRNA on the elemental content of HeLa cells. A total number of genes showing significant increase (red) and decrease (blue) for each element is shown. Changes with standard scores (z-scores, derived from 3 replicates) above 5 were considered significant.



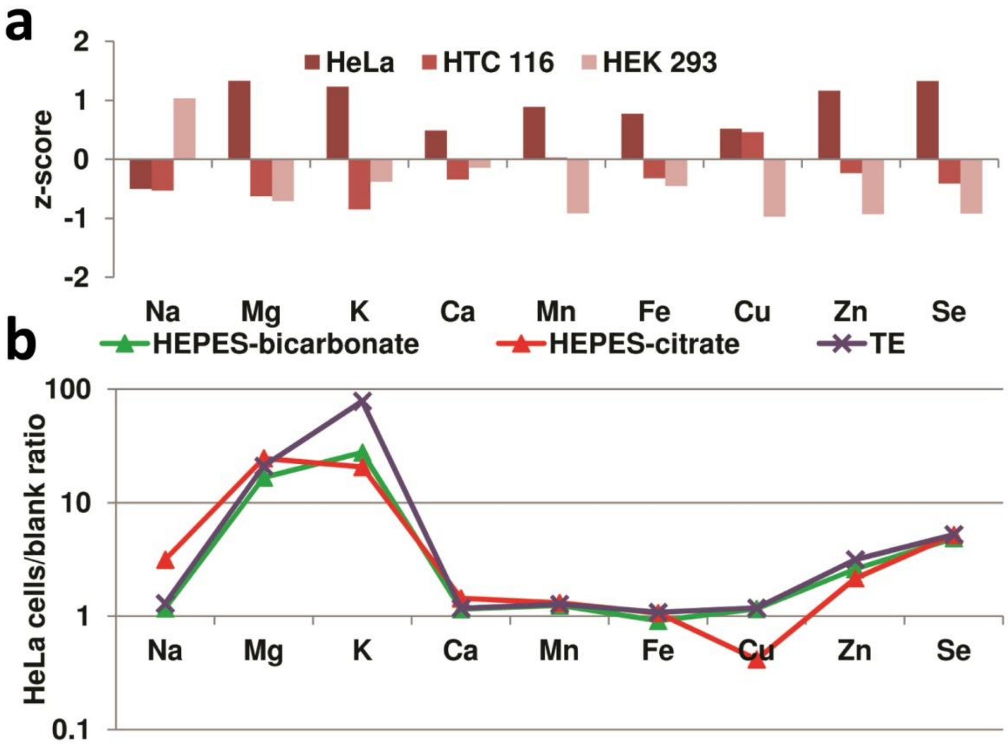
Supplementary Figure 2. An algorithm for processing the ionomics data. The ICP-MS data from the ionomics screen in HeLa cells was processed to remove bias. For each gene knockdown and element, we calculated standard scores (z-scores) and fold change. These data were further used for functional analysis.



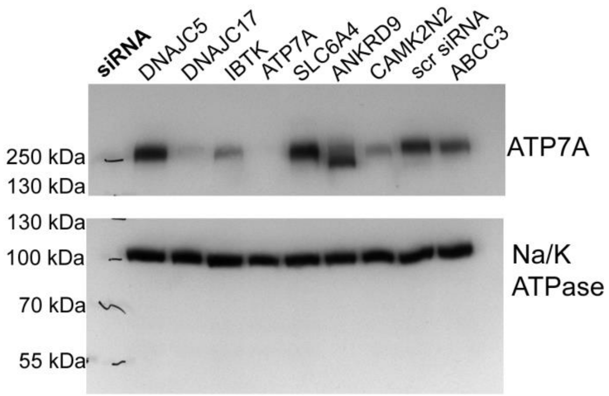
Supplementary Figure 3. Efficiency of siRNA knockdown analyzed by real-time PCR. KCNA1, RPL14 and SBP2 were identified in the screen and validated using ^{75}Se labeling in HeLa cells (derived from 3 replicates). Total RNA from HeLa cells transfected with the corresponding siRNAs was used for real-time PCR analyses. scrRNA transfection was used as control. This figure shows gene expression changes normalized to control. One fold of standard variances for each bar were showed above.



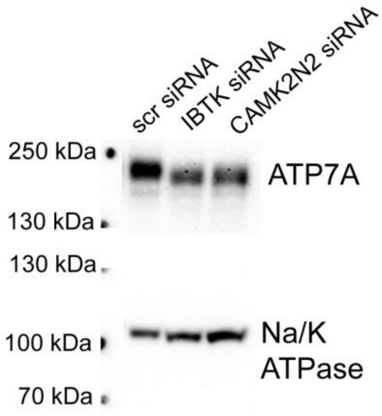
Supplementary Figure 4. Characterization of genes that affect copper levels in HeLa cells. Following gene-specific knockdowns, HeLa cells were fixed and analyzed by confocal microscopy with antibodies against ATP7A (green) and the trans-Golgi network marker TGN46 (red). Scale bar: 25 µm. (b) 100x magnification images. ANKRD9 knockdown results in fragmented TGN and fragmented ATP7A staining pattern. Scale bar: 10 µm. (c) HeLa cells were transiently transfected with siRNAs, and cell lysates were analyzed by Western blotting. A representative blot is shown. A full blot is in Supplementary Figure 7.



Supplementary Figure 5. Assay development for high-throughput ionomics screening of human cells. (a) Standard score (derived from 3 replicates) for elemental ICP-MS profile of indicated human cell lines. Cells were grown to full confluence on 6-well plates in 10% serum-supplemented DMEM. Cells were washed 3 times in TE (10 mM Tris, 1mM EDTA, pH 7.4), followed by digestions with 1.5% nitric acid and 1.5 % hydrogen peroxide (50 ppb gallium was also added as an internal standard). Trace elements were analyzed by ICP-MS. The graph represents the number of standard deviations from the mean (z-score). (b) Optimization of medium removal. HeLa cells were washed 3 times with HEPES-bicarbonate, HEPES-citrate, or TRIS-EDTA (TE), all at pH 7.4, digested and analyzed by ICP-MS.



Supplementary Figure 6. Effect of target down-regulation on ATP7A protein levels and electrophoretic mobility. Following gene knockdowns, HeLa cell lysates were analyzed by Western blotting. The blot was cut at 130 kDa; the upper part was immunostained for ATP7A and the lower part was immunostained for Na/K-ATPase. This figure shows the entire blot, and Fig. 4b its reduced version.



Supplementary Figure 7. Characterization of genes that affect copper levels in HeLa cells. HeLa cells were transiently transfected with siRNAs, and cell lysates were analyzed by Western blotting. This is a full blot that accompanies Supplementary Figure 4c.

Supplementary Table 1. Biological processes associated with changes in iron levels. The table was generated using Gene Ontology analysis (Genesifter, Geospiza) for the set of genes down-regulation of which increased or decreased iron levels in cells with a z-score > 3. Increased iron levels are linked to more specific biologic processes, whereas iron depletion is linked to more global changes in cell.

DOWN-REGULATED BIOLOGICAL PROCESSES			
Associated with iron elevation		Associated with iron loss	
Ontology	Genes total	Ontology	Genes total
Generation of precursor metabolite and energy	29	Cellular component organization or biogenesis	134
Sensory perception of mechanical stimulus	9	Cellular component organization	131
Sensory perception of sound	9	Establishment of localization	126
Regulation of G1/S transition of mitotic cell cycle	8	transport	122
G1/S transition check pooint	7	Organ development	71
Mitochondrial transport	7	Organelle organization	71
Mitotic Cell Cycle G1/S transition checkpoint	7	Cellular localization	64
Regulation of cyclin-dependent protein kinase activity	7	Establishment of localization in cell	63
Ribonucleoprotein complex assembly	7	Cell cycle	57
Cellular response to glucagon stimulus	6	Apoptosis	54
Chloride transport	6	Programmed cell death	54
Positive regulation of cell cycle arrest	6	Regulation of multicellular organismal process	52
Positive regulation of JUN kinase activity	6	Cell cycle progress	44
Regulation of JUN kinase activity	6	Cell cycle phase	41
Response to glucagon stimuus	6	Vesicle-mediated transport	40
Protein heterotetramerization	5	Mitotic cell cycle	35
Activation of JUN kinase	4	M phase	34
Embryonic forelimb morphogenesis	4	Cytoskeleton organization	33
Forelimb morphogenesis	4	Secretion	31
G1 phase of mitotic cell cycle	4	Regulation of multicellular organismal development	30
Histone ubiquitination	4	Cell division	29
Maintenance of protein localization to organelle	4	Cellular component movement	27
Nerve development	4	Organelle fission	26
Neural crest cell development	4	Cell motility	26
Neural crest cell differentiation	4	Cellular membrane organization	26
Neural crest cell migration	4	M phase of mitotic cell cycle	26
Organic cation transport	4	Mitosis	26
Regulation of mRNA stability	4	Nuclear division	25
Regulation of RNA stability	4	Response to abiotic stimulus	24
Response to antibiotic	4	Cardiovascular system development	24
T cell mediated immunity	4	Cell migration	24
Activation of caspase by cytochrome C	3	Circulatory system development	24
Axon cargo transport	3	Secretion by cell	23
Cellular respose to radiation	3	Microtubule based process	21
Copulation	3	Cellular amine metabolic process	18
DNA integration	3	Microtubule cytoskeleton organization	17
DNA ligation	3	Regulation of system process	15
Endothelial cell differentiation	3	Heart development	15

Glutamine metabolic process	3	Positive regulation of protein metabolism	15
Maintenance of protein localization to nucleus	3	Tissue morphogenesis	14
Negative regulation of cytokine biosynthesis	3	Chromosome segregation	14
Nucleotide excision repair, DNA gap filling	3	Positive regulation of multicellular process	13
Outflow tract morphogenesis	3	Golgi vesicle transport	13
Quaternary ammonium group transport	3	Positive regulation of protein modification	12
Response to vitamin D	3	Muscle tissue development	12
Steroid catabolic process	3	Organelle localization	12
Thymus development	3	Establishment of organelle localization	12
Androgen biosynthetic process	2	Protein oligomerization	11
Anterograde axon cargo transport	2	Striatal muscle tissue development	11
Apoptotic cell clearance	2	Spindle organization	10
Asparagine metabolic process	2	Proteosomal ubiquitin-dependent catabolism	10
Assembly of splisosomal tri-snRNP	2	Mitotic prometaphase	9
Betaine transport	2	Rho protein signal transduction	9
Carnitine transport	2	Heart morphogenesis	8
Cholesterol import	2	Meiotic cell cycle	8
Cholesterol storage	2	Mitotic spindle organization	8
Diacylglycerol metabolic process	2	Positive regulation of phosphate metabolism	8
Dicotomous subdivision of an epithelial terminal unit	2	Positive regulation of phosphorus metabolism	8
DNA ligation involved in DNA repair	2	Positive regulation of phosphorylation	8
Enteric nervous system development	2	Positive regulation of protein phosphorylation	8
Establishment of mitotic spindle localization	2	Striatal muscle cell development	8
Establishment of mitotic spindle orientation	2	Vesicle localization	8
Establishment of spindle localization	2	Vesicle organization	8
Establishment of spindle orientation	2	Body fluid secretion	7
I-kappaB phosphorylation	2	Camera-type eye development	7
Induction of apoptosis by oxidative stress	2	Cellular component assembly in morphogenesis	7
Mannose metabolic process	2	Establishment of vesicle localization	7
Meiotic chromosome segregation	2	Heart contraction	7
Mitochondrial respiratory chain complex 1 assembly	2	Membrane budding	7
Muscarinic acetylcholine receptor signaling	2	Muscle fiber development	7
NADH dehydrogenase complex assembly	2	Cardiac muscle tissue development	6
Negative regulation of macrophage derived from foam cell differentiation	2	Positive regulation of protein ubiquitination	6
Neurotransmitter catabolic process	2	Protein heterooligomerization	6
Optic nerve development	2	Regulation of action potential	6
Positive regulation of fatty acid biosynthesis	2	Regulation of cellular amine metabolism	6
Pre-microRNA processing	2	Response to hexose stimulus	6
Production of miRNA involved in gene silencing	2	Response to monosaccharide stimulus	6
Protein destabilization	2	Retrograde vesicle transport. Golgi to ER	6
Protein homotrimerization	2	Cardiac muscle contraction	5
Regulation of apetite	2	Cardiac muscle tissue morpogenesis	5
Regulation of cholesterol efflux	2	Catechol metabolism	5
Retinal ganglion cell axon guidance	2	Catecholamine metabolism	5
Ribosomal large subunit biogenesis	2	COPI coating of Golgi vesicle	5
Ribosome assembly	2	Diol metabolic process	5
Spindle localization	2	Golgi transport vesicle coating	5
Sterol import	2	Mitotic sister chromatid segregation	5
Sterol transmembrane transport	2	Muscle organ morphogenesis	5

Sulfur compound transport	2	Muscle tissue morphogenesis	5
Trachea development	2	Phenol-containing compound metabolism	5
V(D)J recombination	2	Response to glucose stimulus	5
		Response to heat	5
		Response to osmotic stress	5
		Sister chromatid segregation	5
		Ameboidal cell migration	4
		Dopamine metabolic process	4
		Miofibril assembly	4
		Paterning of blood vessels	4
		Peptide biosynthesis	4
		Pigmentation	4
		Positive regulation of ERK1 and ERK2 cascade	4

Supplementary Table 2. Distribution of genes, whose knockdown increases Cu or Fe levels, according to their standard scores (z-scores, derived from 3 replicates).

z-score	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
Cu	2324	876	317	110	43	18	10	7	3
Fe	2301	764	239	61	13	1	0	0	0

Supplementary Table 3. Comparison of biological processes influencing copper and iron ionomes.

Copper		**Iron**	
Biological Process	Number of genes	Biological Process	Number of genes
cellular macromolecular complex subunit organization	15	reproductive process	24
cellular membrane organization	13	generation of precursor metabolites and energy	12
endocytosis	9	negative regulation of cell differentiation	9
membrane invagination	9	cellular component biogenesis	7
nuclear mRNA splicing, via spliceosome	8	lipid localization	7
RNA splicing, via transesterification reactions	8	lipid transport	7
RNA splicing, via transesterification with bulged adenosine as nucleophile	8	ribonucleoprotein complex biogenesis	7
regulation of GTP catabolic process	6	canonical Wnt receptor signaling pathway	5
regulation of GTPase activity	6	cellular response to extracellular stimulus	5
cyclic-nucleotide-mediated signaling	5	G2/M transition of mitotic cell cycle	5
elevation of cytosolic calcium ion concentration	5	*regulation of transmission of nerve impulse*	5
G-protein signaling, coupled to cyclic nucleotide second messenger	5	response to vitamin	5
induction of apoptosis by extracellular signals	5	*ribonucleoprotein complex assembly*	5
neuron apoptosis	5	*ribonucleoprotein complex subunit organization*	5
neuron death	5	cellular response to nutrient	4
regulation of neuron apoptosis	5	cellular response to nutrient levels	4
regulation of synaptic transmission	5	chloride transport	4
regulation of transmission of nerve impulse	5	embryonic appendage morphogenesis	4
ribonucleoprotein complex subunit organization	5	embryonic limb morphogenesis	4
circadian rhythm	4	gene silencing	4
ribonucleoprotein complex assembly	4	inorganic anion transport	4
actomyosin structure organization	3	mitochondrial transport	4

To generate this table, gene ontology analysis was performed for all genes down-regulation of which was associated with increased copper or iron levels (z-score above 4, derived from 3 replicates). Biological processes common for copper and iron ionomes are indicated in italic. The majority of processes were unique for each ionome.

Supplementary Table 4. Transport/trafficking proteins down-regulation of which produces copper elevation with the standard score (z-score, derived from 3 replicates) > 4. Blue color indicates protein with known/predicted roles in intracellular protein or vesicle trafficking. The Table was generated using GeneSifter.

z-score	Gene Identifier	Gene Name
14.247	NM_004886	Amyloid beta (A4) precursor protein-binding, family A, member 3
10.242	NM_007332	Transient receptor potential channel, subfamily A, member 1
9.493	NM_000052	ATPase, Cu++ transporting, alpha polypeptide
6.744	NM_015525	Inhibitor of Bruton agammaglobulinemia tyrosine kinase
6.512	NM_015064	ELKS/RAB6-interacting/CAST family member 1
6.215	NM_002269	Karyopherin alpha 5 (importin alpha 6)
5.978	NM_173160	FXYD domain containing ion transport regulator 4
5.848	NM_002674	Pro-melanin-concentrating hormone
5.658	NM_198822	ATP5L2
5.6	NM_003786	ATP-binding cassette, sub-family C (CFTR/MRP), member 3
5.481	NM_152701	ATP-binding cassette, sub-family A (ABC1), member 13
5.429	NM_004547	NADH dehydrogenase (ubiquinone) 1 beta subcomplex, 4, 15kDa
5.308	NM_020845	Phosphatidylinositol transfer protein, membrane-associated 2
5.216	NM_021168	RAB40C, member RAS oncogene family
5.203	NM_016112	Polycystic kidney disease 2-like 1
5.2	NM_014770	ArfGAP with GTPase domain, ankyrin repeat and PH domain 2
5.125	NM_001680	FXYD domain containing ion transport regulator 2
5.095	NM_001128	Adaptor-related protein complex 1, gamma 1 subunit
5.003	NM_001834	Clathrin, light chain (Lcb)
4.948	NM_016224	Sorting nexin 9
4.932	NM_005959	Melatonin receptor 1B
4.923	NM_018384	GTPase, IMAP family member 5
4.883	NM_006827	Transmembrane emp24-like trafficking protein 10 (yeast)
4.808	NM_018977	Neuroligin 3
4.721	NM_003027	SH3-domain GRB2-like 3
4.708	NM_004045	ATX1 antioxidant protein 1 homolog (yeast)
4.699	NM_006395	ATG7 autophagy related 7 homolog
4.686	NM_024723	FLJ23471
4.663	NM_005856	Receptor (G protein-coupled) activity modifying protein 3
4.522	NM_198686	RAB15, member RAS onocogene family
4.437	NM_004541	NADH dehydrogenase (ubiquinone) 1 alpha subcomplex, 1, 7.5kDa
4.404	NM_178450	Membrane-associated ring finger (C3HC4) 3
4.381	NM_012417	Phosphatidylinositol transfer protein, cytoplasmic 1
4.372	NM_002564	Purinergic receptor P2Y, G-protein coupled, 2
4.336	NM_173637	Solute carrier family 25, member 41
4.33	NM_000830	Glutamate receptor, ionotropic, kainate 1
4.328	NM_031431	Component of oligomeric golgi complex 3
4.326	NM_017954	Ca++-dependent secretion activator 2
4.305	NM_003627	Solute carrier family 43, member 1
4.289	NM_000184	Hemoglobin, gamma G
4.289	NM_016075	Vacuolar protein sorting 36 homolog
4.286	NM_020783	Synaptotagmin IV

4.28	NM_003042	Solute carrier family 6 (neurotransmitter transporter, GABA), member 1
4.202	NM_004305	Bridging integrator 1
4.165	NM_001157	Annexin A11
4.164	NM_030636	Endonuclease/exonuclease/phosphatase family domain containing 1
4.162	NM_000841	Glutamate receptor, metabotropic 4
4.153	NM_001293	Chloride channel, nucleotide-sensitive, 1A
4.138	NM_016128	Coatomer protein complex, subunit gamma
4.121	NM_198991	Potassium channel tetramerisation domain containing 1
4.056	NM_004518	Potassium voltage-gated channel, KQT-like subfamily, member 2
4.035	NM_004794	RAB33A, member RAS oncogene family

Supplementary Table 5. Biological processes associated with changes in copper levels. The table was generated using Gene Ontology analysis (Genesifter) for the set of genes down-regulation of which changes copper levels in cells with a standard score (z-score, derived from 3 replicates) above 3.

Biological processes, downregulation of which is associated with copper elevation		Biological processes, downregulation of which is associated with copper loss	
Ontology	Genes total	Ontology	Genes total
generation of precursor metabolites and energy	25	macromolecule localization	24
response to inorganic substance	17	cellular component assembly at cellular level	14
lipid catabolic process	15	cell growth	6
organic acid transport	15	maintenance of location	6
carboxylic acid transport	14	regulation of cell size	6
phospholipid metabolic process	14	maintenance of location in cell	5
lipid modification	13	maintenance of protein location	5
lipid transport	12	maintenance of protein location in cell	5
glycerophospholipid metabolic process	11	response to metal ion	5
monocarboxylic acid transport	10	positive regulation of cell adhesion	4
phospholipid biosynthetic process	10	protein-DNA complex assembly	4
lipoprotein metabolic process	9	protein-DNA complex subunit organization	4
glycerophospholipid biosynthetic process	8	receptor-mediated endocytosis	4
double-strand break repair	7	RNA localization	4
fatty acid oxidation	7	establishment of RNA localization	3
fatty acid transport	7	heart contraction	3
lipid oxidation	7	heart process	3
lipoprotein biosynthetic process	7	homophilic cell adhesion	3
monocarboxylic acid catabolic process	7	lipid modification	3
oxidative phosphorylation	7	mitotic prometaphase	3
ATP synthesis coupled electron	6	monovalent inorganic cation	3

transport		homeostasis	
fatty acid catabolic process	6	negative regulation of response to external stimulus	3
iron ion transport	6	nucleic acid transport	3
mitochondrial ATP synthesis coupled electron transport	6	nucleobase, nucleoside, nucleotide and nucleic acid transport	3
nucleotide-excision repair	6	regulation of heart contraction	3
protein lipidation	6	regulation of inflammatory response	3
long-chain fatty acid transport	5	regulation of pH	3
meiosis I	5	response to calcium ion	3
response to calcium ion	5	RNA transport	3
cellular potassium ion transport	4	spermatid development	3
chloride transport	4	spermatid differentiation	3
heme metabolic process	4	transition metal ion transport	3
histone ubiquitination	4	chloride transport	2
multicellular organismal response to stress	4	filopodium assembly	2
nucleobase, nucleoside and nucleotide interconversion	4	inorganic anion transport	2
potassioum ion transmembrane transport	4	lipoprotein metabolic process	2
reciprocal meiotic recombination	4	microspike assembly	2
regulation of lipid catabolic process	4	response to pH	2
response to dsRNA	4	sterol biosynthetic process	2
transcription-coupled nucleotide-excision repair	4		
actomyosin structure organization	3		
alkene biosynthetic process	3		
CD4-positive, alpha beta T cell differentiation	3		
chromosome localization	3		
copper ion transport	3		

establishment of chromosome localization	3		
fibrinolysis	3		
foam cell differentiation	3		
heme biosynthetic process	3		
icosanoid secretion	3		
icosanoid transport	3		
leukotriene biosynthetic process	3		
macrophage derived foam cell differentiation	3		
myofibril assembly	3		
neuroprotection	3		
nucleoside diphosphate metabolic process	3		
parturition	3		
preassembly of GPI anchor in ER membrane	3		
purine-containing compound transmembrane transport	3		
pyrimidine nucleotide biosynthetic process	3		
pyrimidine ribonucleotide biosynthetic process	3		
pyrimidine ribonucleotide metabolic process	3		
regulation of epidermal cell differentiation	3		
regulation of epidermis development	3		
regulation of receptor activity	3		
response to pain	3		
response to purine-containing compound	3		
retrograde transport, endosome to Golgi	3		

steroid hormone mediated signaling pathway	3		
beta-amyloid metabolic process	2		
blastoderm segmentation	2		
cGMP-mediated signaling	2		
cholesterol storage	2		
chromosome organization involved in meiosis	2		
DNA ligation	2		
dsRNA fragmentation	2		
fat-soluble vitamin catabolic process	2		
fatty acid alpha-oxidation	2		
female meiosis	2		
gap junction assembly	2		
GTP biosynthetic process	2		
meiotic prophase I	2		
myeloid dendritic cell differentiation	2		
negative regulation of appetite	2		
negative regulation of cell-matrix adhesion	2		
negative regulation of fibrinolysis	2		
negative regulation of macrophage derived foam cell differentiation	2		
negative regulation of response to food	2		
nucleotide phosphorylation	2		
pericardium development	2		

Supplementary Table 6. Comparison of the ICP-MS data for *Saccharomyces cerevisiae* gene deletions producing significant changes of Fe or Cu levels and their orthologs in HeLa cells. Yeast genes producing statistically significant changes of copper or iron levels upon knockdown were selected from the database available at http://www.ionomicshub.org/ and the fold change was calculated using normalized ICP-MS value available at the website. Human orthologs of these yeast genes were identified through Ensembl database; and the ICP-MS data for these orthologs was taken from the dataset generated in the current study. The Z-score values for yeast data are defined in Yu and colleagues [21]; the Z-score values for HeLa screen are as defined in the text. All the statistical results were derived from 3 replicates.

Yeast-haploid	**Cu levels**		**HeLa cells**	**Cu levels**	
Protein (gene) name	**Fold change**	**Moderated z-score**	**Protein (gene) name**	**Fold change**	**z-score**
STP22 (YCL008C)	1.076*	-4.219	TSG 101	1.04	0.914
CHA1 (YCL064C)	1.101*	-4.702	SDS	1.016	0.224
			SDSL	0.979	-0.331
TUP1 (YCR084C)	0.266	-5.980	WDR5	1.171	2.785
RPP2B (YDR382W)	0.855	-3.589	RPLP2	0.953	-0.386
SXM1(YDR395W)	0.793	-4.437	IPO7	1.195	1.685
			IPO8	0.923	-0.997
HPT1 (YDR399W)	0.947	-4.813	HPRT1	1.007	0.117
NPP2 (YEL016C)	**0.826***	**-3.435**	ENPP1	0.958	-0.167
			ENPP2	1.007	0.088
			ENPP3	1.099	1.200
			ENPP6	**0.701**	**-3.189**
RPO41 (YFL036W)	0.904*	-5.239	POLRMT	1.003	0.119
AIR1 (YIL079C)	1.000*	-5.068	ZCCHC7	0.972	-0.298
PRK1 (YIL095W)	0.979*	-4.799	AAK1	1.128	1.895
			BMP2K	0.935	-1.585
GRR1 (YJR090C)	**0.427**	**-4.961**	**FBXL15**	**0.767**	**-4.961**
			FBXL4	1.020	0.324
			LRRC29 (FBXL9)	1.329	5.559
			FBXL16	1.107	1.123
			FBXL14	1.002	-0.019
SKY1 (YMR216C)	0.748*	-3.810	SRPK1	1.030	0.358
			SRPK2	1.115	1.627
			SRPK2 (STK23)	1.288	4.232
GSH2 (YOL049W)	0.991*	-4.008	GSS	1.037	0.691
PKH2 (YOL100W)	0.861*	-5.039	PDPK1	0.985	-0.323

VPS16 (YPL045W)	0.958*	-3.819	VPS16	1.003	0.045
Yeast-haploid	**Fe levels**		**HeLa cells**	**Fe levels**	
name	**Fold change**	**Moderated z-score**	**name**	**Fold change**	**z-score**
DNM1 (YLL001W)	0.973*	-3.661	DNM1L	1.001	0.056
HCA4 (YJL033W)	NR	-4.287	DDX10	0.991	-0.141
MTR4 (YJL050W)	NR	-3.608	SKIV2L2	0.940	-1.045
			DDX60/FLJ20035	1.046	1.363
			DDX60L/FLJ31033	0.986	-0.280
MET30 (YIL046W)	**NR**	**-4.450**	FBXW11	1.004	0.141
			FBXW7	0.926	-2.273
			FBXW2	**0.887**	**-3.510**
			BTRC	1.106	3.347
LST8 (YNL006W)	0.986*	-3.570	MLST8/NM_022372	1.026	0.896
CLA4 (YNL298W)	0.763*	-3.818	LIMK1	0.990	-0.310
			LIMK2	0.996	-0.158
			TESK1	1.232	1.500
			TESK2	1.092	0.780
FUR1 (YHR128W)	1.159*	-3.789	UPRT/MGC23937	1.126	2.644

* outlier ICP-MS values were not included in the calculations; NR: non-reliable data due to wide range of values. Orthologs that result in similar effects on Cu or Fe ionomes in yeast and human cells are indicated in red.