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Supplemental Data

Article

Dephosphorylation of Translation Initiation Factor 2α Enhances Glucose Tolerance and Attenuates Hepatosteatosis in Mice

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Table S1.

			MEFs				Liver			
		Entrez	Eif2aA/A		Eif2αS/S		Fv2E-PERK (Low)		Fv2E-PERK (High)	
Name	Description	Gene	Mock	AP20187	Mock	AP20187	Mock	AP20187	Mock	AP20187
Translation/amino acid transport and metabolism										
Myd116	myeloid differentiation primary response gene 116	17872	1.05 ± 0.09	1.06 ± 0.07	1.70 ± 0.28	12.97 ± 0.82	0.93 ± 0.01	1.91 ± 0.32	0.33 ± 0.08	1.06 ± 0.07
Abcf2	ATP-binding cassette, sub-family F (GCN20), member 2	27407	1.64 ± 0.01	1.62 ± 0.23	3.97 ± 0.87	8.13 ± 1.15	0.87 ± 0.02	1.67 ± 0.45	0.78 ± 0.13	0.99 ± 0.15
Asns	asparagine synthetase	27053	1.11 ± 0.07	0.94 ± 0.02	0.86 ± 0.15	7.91 ± 1.95	0.43 ± 0.11	1.81 ± 0.38	0.17 ± 0.05	1.04 ± 0.14
Mthfr	5,10-methylenetetrahydrofolate reductase	17769	1.16 ± 0.02	0.91 ± 0.26	1.25 ± 0.20	7.78 ± 0.38	0.44 ± 0.28	1.97 ± 0.15	0.73 ± 0.10	1.08 ± 0.16
Slc1a4	solute carrier family 1 (glutamate/neutral amino acid transporter), member 4	55963	0.97 ± 0.02	1.03 ± 0.01	1.96 ± 0.06	6.51 ± 0.69	0.93 ± 0.07	2.13 ± 0.07	0.37 ± 0.09	1.62 ± 0.16
Sars	seryl-aminoacyl-tRNA synthetase	20226	1.37 ± 0.02	1.15 ± 0.27	2.24 ± 0.84	6.13 ± 1.07	0.67 ± 0.09	1.52 ± 0.09	0.53 ± 0.07	1.00 ± 0.12
Cars	cysteinyl-tRNA synthetase	27267	1.11 ± 0.11	1.02 ± 0.09	1.26 ± 0.37	6.07 ± 0.89	1.25 ± 0.73	5.22 ± 0.01	0.72 ± 0.11	0.90 ± 0.12
Tars	threonyl-tRNA synthetase	110960	1.04 ± 0.20	0.97 ± 0.02	1.66 ± 0.18	4.74 ± 0.57	0.89 ± 0.10	1.02 ± 0.12	1.12 ± 0.03	0.98 ± 0.12
Rps6ka2	ribosomal protein S6 kinase, polypeptide 2	20112	0.90 ± 0.14	0.74 ± 0.35	1.75 ± 0.24	4.72 ± 1.01	2.07 ± 0.96	1.01 ± 0.15	1.00 ± 0.32	1.03 ± 0.53
Lars	leucyl-tRNA synthetase	107045	1.19 ± 0.05	1.10 ± 0.02	1.26 ± 0.17	4.45 ± 0.62	0.89 ± 0.01	2.13 ± 0.44	0.63 ± 0.03	1.00 ± 0.03
Eprs	glutamyl-prolyl-tRNA synthetase	107508	1.08 ± 0.07	1.32 ± 0.07	1.73 ± 0.34	4.39 ± 0.35	0.83 ± 0.08	1.70 ± 0.08	0.47 ± 0.05	1.02 ± 0.12
Pycs	pyrroline-5-carboxylate synthetase	56454	1.05 ± 0.08	0.93 ± 0.06	1.86 ± 0.59	4.32 ± 0.09	1.31 ± 0.14	1.31 ± 0.30	0.64 ± 0.21	1.13 ± 0.27
Nars	asparaginyl-tRNA synthetase	70223	1.50 ± 0.07	1.12 ± 0.18	1.76 ± 0.27	4.21 ± 0.86	1.02 ± 0.04	2.23 ± 0.11	0.76 ± 0.07	0.99 ± 0.13
Wars	tryptophanyl-tRNA synthetase	22375	1.05 ± 0.06	0.98 ± 0.02	2.00 ± 0.26	4.06 ± 0.65	0.64 ± 0.06	1.07 ± 0.08	0.71 ± 0.13	1.02 ± 0.16
Aars	alanyl-tRNA synthetase	234734	1.06 ± 0.06	0.86 ± 0.14	1.73 ± 0.36	4.01 ± 0.32	0.77 ± 0.06	1.80 ± 0.22	0.56 ± 0.05	1.04 ± 0.10
Eif4ebp1	eukaryotic translation initiation factor 4E binding protein 1	13685	1.02 ± 0.01	0.86 ± 0.17	1.66 ± 0.48	3.82 ± 0.56	1.05 ± 0.03	1.51 ± 0.05	0.68 ± 0.10	1.08 ± 0.16
Psph	phosphoserine phosphatase	100678	1.30 ± 0.12	1.12 ± 0.32	1.34 ± 0.22	3.74 ± 0.37	0.89 ± 0.05	2.03 ± 0.05	0.60 ± 0.03	1.00 ± 0.02
Yars	tyrosyl-tRNA synthetase	107271	1.11 ± 0.17	1.03 ± 0.04	1.33 ± 0.56	3.59 ± 0.24	0.93 ± 0.14	1.44 ± 0.23	0.53 ± 0.09	1.05 ± 0.06
Elp3	elongation protein 3 homolog (S. cerevisiae)	74195	1.00 ± 0.04	1.06 ± 0.03	1.45 ± 0.16	2.91 ± 0.06	0.84 ± 0.06	1.10 ± 0.10	0.95 ± 0.17	0.91 ± 0.13
Iars	isoleucine-tRNA synthetase	105148	0.82 ± 0.10	0.75 ± 0.10	1.23 ± 0.15	2.79 ± 0.27	0.93 ± 0.17	1.91 ± 0.13	0.43 ± 0.06	1.01 ± 0.05
Slc1a5	solute carrier family 1 (neutral amino acid transporter), member 5	20514	1.08 ± 0.24	1.17 ± 0.05	1.16 ± 0.27	2.39 ± 0.20	1.01 ± 0.03	0.82 ± 0.16	0.90 ± 0.13	1.53 ± 0.31
Shmt2	serine hydroxymethyl transferase 2 (mitochondrial)	108037	0.78 ± 0.07	0.86 ± 0.06	1.02 ± 0.01	2.34 ± 0.45	1.18 ± 0.17	1.07 ± 0.04	1.30 ± 0.29	1.03 ± 0.28
Stress response										
Uvrag	UV radiation resistance associated gene	78610	4.35 ± 0.05	4.08 ± 0.77	21.66 ± 5.89	49.58 ± 14.94	1.16 ± 0.22	1.01 ± 0.03	0.61 ± 0.05	1.32 ± 0.14
Gch1	GTP cyclohydrolase 1	14528	0.88 ± 0.16	0.63 ± 0.36	2.98 ± 1.05	21.76 ± 0.80	1.16 ± 0.10	1.00 ± 0.05	1.09 ± 0.06	1.04 ± 0.15
GADD45	growth arrest and DNA-damage-inducible 45 alpha	13197	1.11 ± 0.10	0.92 ± 0.00	2.74 ± 0.06	20.24 ± 0.34	0.23 ± 0.13	3.27 ± 0.26	0.02 ± 0.01	3.39 ± 0.31
Ndrl	N-myc downstream regulated-like	17990	1.02 ± 0.24	1.05 ± 0.35	5.76 ± 1.82	14.09 ± 2.25	0.90 ± 0.20	1.05 ± 0.22	1.16 ± 0.19	0.89 ± 0.17
Sqstm1	sequestosome 1	18412	1.21 ± 0.04	1.26 ± 0.01	1.97 ± 0.98	6.11 ± 0.59	0.62 ± 0.06	1.99 ± 0.53	0.62 ± 0.05	1.02 ± 0.08
Arntl	aryl hydrocarbon receptor nuclear translocator-like	11865	0.95 ± 0.09	0.80 ± 0.23	1.18 ± 0.08	3.04 ± 0.03	0.44 ± 0.24	1.26 ± 0.13	1.02 ± 0.12	1.09 ± 0.14
Dnajb9	DnaJ (Hsp40) homolog, subfamily B, member 9	27362	0.88 ± 0.19	0.78 ± 0.56	1.00 ± 0.03	2.90 ± 0.02	0.72 ± 0.08	1.12 ± 0.02	0.98 ± 0.12	0.84 ± 0.10
Tbce	tubulin-specific chaperone e	70430	1.44 ± 0.01	0.98 ± 0.31	1.25 ± 0.02	2.82 ± 0.34	0.93 ± 0.03	1.26 ± 0.08	0.88 ± 0.15	0.94 ± 0.11
Clcn3	chloride channel 3	12725	0.98 ± 0.13	0.86 ± 0.03	0.77 ± 0.13	1.96 ± 0.26	0.84 ± 0.25	1.38 ± 0.16	0.63 ± 0.13	1.00 ± 0.15
Dnajc3	DnaJ (Hsp40) homolog, subfamily C, member 3	19107	0.77 ± 0.24	0.76 ± 0.05	0.28 ± 0.03	1.06 ± 0.18	0.91 ± 0.08	0.98 ± 0.15	0.97 ± 0.14	0.95 ± 0.10
Redox/detox										
Pon2	paraoxonase 2	330260	2.62 ± 0.31	2.18 ± 0.67	8.21 ± 0.54	17.43 ± 1.80	1.17 ± 0.04	0.94 ± 0.14	1.14 ± 0.41	0.92 ± 0.12
B5R.1	NADH-cytochrome B5 reductase	72017	1.06 ± 0.00	0.92 ± 0.40	2.80 ± 0.17	10.00 ± 1.51	0.65 ± 0.06	3.05 ± 0.55	0.16 ± 0.02	1.04 ± 0.13
EST	RIKEN cDNA 1110049F12 gene	66193	1.90 ± 0.03	1.20 ± 0.51	1.33 ± 0.13	3.16 ± 0.34	1.00 ± 0.01	1.00 ± 0.07	1.06 ± 0.46	0.97 ± 0.41
Txnip	thioredoxin interacting protein	56338	1.08 ± 0.03	0.88 ± 0.31	0.88 ± 0.23	2.28 ± 0.25	0.93 ± 0.13	1.07 ± 0.09	0.32 ± 0.05	1.39 ± 0.40
Cpox	coproporphyrinogen oxidase	12892	0.88 ± 0.36	0.93 ± 0.18	0.78 ± 0.05	1.83 ± 0.02	0.91 ± 0.22	0.93 ± 0.10	0.97 ± 0.26	1.37 ± 0.24
Protein degradation										
Siah2	seven in absentia 2	20439	0.88 ± 0.01	0.95 ± 0.05	1.19 ± 0.08	3.45 ± 0.50	0.92 ± 0.15	1.19 ± 0.10	0.57 ± 0.06	1.14 ± 0.13
Fbxo8	F-box only protein 8	50753	2.38 ± 0.58	1.18 ± 0.26	0.97 ± 0.45	2.92 ± 0.33	0.90 ± 0.12	1.19 ± 0.37	1.06 ± 0.06	1.06 ± 0.05
Ctsc	cathepsin C	13032	0.33 ± 0.12	0.14 ± 0.09	1.06 ± 0.23	2.24 ± 0.10	1.14 ± 0.06	0.97 ± 0.28	1.58 ± 0.05	0.86 ± 0.19
Secretory pathway										
Wfs1	Wolfram syndrome 1 homolog (human)	22393	1.12 ± 0.23	0.99 ± 0.26	2.71 ± 0.42	7.75 ± 0.51	0.54 ± 0.05	3.41 ± 0.57	0.90 ± 0.16	0.90 ± 0.09
Spata5	spermatogenesis associated 5	57815	1.00 ± 0.02	0.73 ± 0.13	2.00 ± 0.28	4.74 ± 0.51	0.79 ± 0.07	2.98 ± 1.06	0.40 ± 0.13	1.10 ± 0.17
Herpud1	homocysteine-inducible-ubiquitin-like domain 1	64209	0.94 ± 0.07	0.69 ± 0.17	1.07 ± 0.15	3.11 ± 0.17	0.65 ± 0.22	0.96 ± 0.05	0.87 ± 0.15	1.71 ± 0.13
Ero1l	ERO1-like (S. cerevisiae)	50527	0.92 ± 0.10	1.07 ± 0.23	1.00 ± 0.14	2.59 ± 0.33	0.91 ± 0.02	1.32 ± 0.10	0.84 ± 0.05	0.95 ± 0.07
Sel1h	Sel1 (suppressor of lin-12) 1 homolog (C. elegans)	20338	1.04 ± 0.01	1.03 ± 0.12	0.79 ± 0.43	2.19 ± 0.73	0.87 ± 0.09	0.98 ± 0.05	1.06 ± 0.05	1.04 ± 0.12
ERO1Lb	ERO1-like beta (S. cerevisiae)	67475	0.77 ± 0.08	0.60 ± 0.10	0.27 ± 0.04	1.09 ± 0.02	0.98 ± 0.16	0.77 ± 0.05	2.02 ± 0.27	0.82 ± 0.09
Transcription										
Atf3	activating transcription factor 3	11910	0.92 ± 0.10	0.88 ± 0.08	2.54 ± 0.26	15.21 ± 1.69	0.26 ± 0.07	11.05 ± 3.69	0.01 ± 0.00	1.15 ± 0.12
Mecr	mitochondrial trans-2-enoyl-CoA reductase	26922	2.41 ± 0.26	1.85 ± 0.87	4.30 ± 0.56	8.75 ± 0.11	0.92 ± 0.02	0.90 ± 0.19	1.10 ± 0.22	0.85 ± 0.11
Nfkb2	nuclear factor of kappa B-cells 2, p49/p100	18034	0.99 ± 0.01	1.04 ± 0.08	3.27 ± 0.23	8.26 ± 1.04	0.68 ± 0.05	1.70 ± 0.48	0.47 ± 0.24	1.31 ± 0.18
Atf5	activating transcription factor 5	107503	1.36 ± 0.04	1.05 ± 0.13	2.22 ± 0.27	7.54 ± 2.44	0.46 ± 0.01	1.98 ± 0.25	0.71 ± 0.05	1.03 ± 0.15
Chop-10	Leucine zipper transcription factor CHOP, GADD153	13198	1.10 ± 0.27	0.94 ± 0.19	0.98 ± 0.08	6.41 ± 0.25	0.14 ± 0.05	4.31 ± 1.19	0.06 ± 0.01	1.06 ± 0.22
Nupr1	nuclear protein 1	56312	1.26 ± 0.17	1.12 ± 0.16	1.36 ± 0.23	6.29 ± 1.64	0.15 ± 0.00	26.69 ± 8.14	0.02 ± 0.01	0.94 ± 0.25
Cebpg	CCAAT/enhancer binding protein (C/EBP), gamma	12611	1.07 ± 0.03	0.91 ± 0.07	1.94 ± 0.22	5.96 ± 0.12	0.62 ± 0.08	2.01 ± 0.21	0.29 ± 0.02	1.01 ± 0.06
Relb	avian reticuloendotheliosis viral (v-rel) oncogene related B	19698	0.90 ± 0.07	1.18 ± 0.27	2.60 ± 0.02	5.87 ± 1.42	0.84 ± 0.08	1.75 ± 0.73	0.07 ± 0.02	0.99 ± 0.08
Tbpl1	TATA box binding protein-like 1	237336	1.89 ± 0.27	1.41 ± 0.53	1.79 ± 0.17	5.63 ± 0.45	0.80 ± 0.04	3.06 ± 0.41	0.34 ± 0.04	1.04 ± 0.30
Cebpb	CCAAT/enhancer binding protein (C/EBP), beta	12608	1.02 ± 0.03	1.10 ± 0.30	2.10 ± 0.37	4.23 ± 0.68	0.84 ± 0.06	1.57 ± 0.51	0.40 ± 0.07	1.31 ± 0.06
Cbx4	chromobox homolog 4 (Drosophila Pc class)	12418	1.17 ± 0.03	1.11 ± 0.10	1.48 ± 0.37	4.16 ± 0.88	0.81 ± 0.07	1.24 ± 0.10	0.69 ± 0.09	4.38 ± 0.16
Snw1	SNW domain containing 1	66354	1.16 ± 0.01	0.97 ± 0.13	1.91 ± 0.60	3.87 ± 0.09	1.00 ± 0.06	1.56 ± 0.15	0.58 ± 0.03	1.14 ± 0.13
Atf6	activating transcription factor 6	226641	0.83 ± 0.39	1.06 ± 0.14	1.32 ± 0.30	3.85 ± 0.81	0.70 ± 0.17	1.11 ± 0.02	0.36 ± 0.03	1.01 ± 0.08
Atf4	activating transcription factor 4	11911	0.85 ± 0.08	1.04 ± 0.06	1.62 ± 0.13	3.60 ± 0.61	0.84 ± 0.54	4.90 ± 0.73	0.52 ± 0.11	0.79 ± 0.19
Nfil3; E4	nuclear factor, interleukin 3, regulated	18030	0.87 ± 0.16	0.76 ± 0.31	1.51 ± 0.13	3.56 ± 0.37	0.48 ± 0.15	1.50 ± 0.03	0.24 ± 0.07	2.07 ± 0.14
Lmo4	LIM domain only 4	16911	1.16 ± 0.32	0.98 ± 0.20	0.94 ± 0.27	3.47 ± 0.15	0.83 ± 0.21	1.47 ± 0.11	0.50 ± 0.11	1.84 ± 0.39
Klf4	Kruppel-like factor 4 (gut)	16600	0.97 ± 0.01	0.77 ± 0.08	1.17 ± 0.02	2.74 ± 0.02	0.72 ± 0.24	1.11 ± 0.17	0.82 ± 0.13	0.98 ± 0.17
Xbp1	X-box binding protein 1	22433	1.07 ± 0.06	0.74 ± 0.19	1.00 ± 0.06	2.65 ± 0.29	0.73 ± 0.05	1.54 ± 0.12	0.74 ± 0.11	0.99 ± 0.10
Ets2	E26 avian leukemia oncogene 2, 3' domain	23872	1.00 ± 0.06	0.88 ± 0.10	1.22 ± 0.38	2.41 ± 0.38	0.98 ± 0.07	2.49 ± 0.70	0.21 ± 0.04	1.30 ± 0.06
Rxrip110	retinoid X receptor interacting protein 110	20184	1.01 ± 0.01	0.89 ± 0.11	0.68 ± 0.12	1.74 ± 0.49	1.00 ± 0.00	1.04 ± 0.08	1.16 ± 0.23	0.94 ± 0.05

Expression level of ISR target genes, previously validated in fibroblasts (MEFs) (Lu et al., 2004b), is compared in the liver of un-injected and AP20187-injected (0.2 μg/gm body weight) *Ttr::Fv2E-PERK* transgenic expressing low (n = 2) and high (n = 4) relative levels of Fv2E-PERK protein (Figure S2B). The data from untreated and AP20187-treated Fv2E-PERK expressing wildtype (Eif2aS/S) and mutant (Eif2aA/A) fibroblasts has been previously reported (Lu et al., 2004b).

Table S2.

			Expression level		AP indution (fold)	
		Entrez			*Ttr::Fv2E-PERK*	
Name	Description	Gene	WT	*Alb::GC*	High	Low
GADD45a	growth arrest and DNA-damage-inducible 45 alpha	13197	2.87 ± 0.06	1.57 ± 0.34	159.34	14.55
Atf3	activating transcription factor 3	11910	1.52 ± 0.26	0.90 ± 1.69	113.76	43.31
Nupr1	nuclear protein 1	56312	2.89 ± 0.23	0.78 ± 1.64	52.11	181.56
Chop-10	Leucine zipper transcription factor CHOP, GADD153	13198	0.97 ± 0.08	0.82 ± 0.25	17.33	31.20
Relb	avian reticuloendotheliosis viral (v-rel) oncogene related B	19698	1.57 ± 0.02	1.27 ± 1.42	13.66	2.08
Nfil3; E4B	nuclear factor, interleukin 3, regulated	18030	0.85 ± 0.13	0.67 ± 0.37	8.59	3.13
B5R.1	NADH-cytochrome B5 reductase	72017	0.82 ± 0.17	0.89 ± 1.51	6.70	4.66
Asns	asparagine synthetase	27053	0.67 ± 0.15	0.91 ± 1.95	6.00	4.18
Slc1a4	solute carrier family 1 (glutamate/neutral amino acid transporter), member 4	55963	1.34 ± 0.06	0.34 ± 0.69	4.42	2.28
Cebpg	CCAAT/enhancer binding protein (C/EBP), gamma	12611	0.93 ± 0.22	0.98 ± 0.12	3.45	3.22
Myd116	myeloid differentiation primary response gene 116	17872	0.64 ± 0.28	0.92 ± 0.82	3.22	2.05
Tbpl1	TATA box binding protein-like 1	237336	0.57 ± 0.17	0.76 ± 0.45	3.07	3.81
Nfkb2	nuclear factor of kappa B-cells 2, p49/p100	18034	3.27 ± 0.23	1.98 ± 1.04	2.78	2.49
Spata5	spermatogenesis associated 5	57815	1.24 ± 0.28	1.19 ± 0.51	2.73	3.77
Iars	isoleucine-tRNA synthetase	105148	1.77 ± 0.15	0.97 ± 0.27	2.37	2.06
Eprs	glutamyl-prolyl-tRNA synthetase	107508	1.62 ± 0.34	1.25 ± 0.35	2.15	2.03

Expression level in HFD-fed non-transgenic (WT) (n = 2) and *Alb::CG* transgenic (n = 2) mice and fold induction in AP20187-injected *Ttr::Fv2E-PERK* transgenic lines of the subset of genes from table S1 that are induced >2-fold by AP20187 injection in both high and low-expressing *Ttr::Fv2E-PERK* transgenic lines.

Table S3.

			MEFs				Liver				% of *ATF4+/+*			
		Entrez	*Atf4+/+*		*Atf4-/-*		*Atf4+/+*		*Atf4-/-*		Mock		Treated	
Name	Description	Gene	Mock	Tunicamycin	Mock	Tunicamycin	Mock	AP20187	Mock	AP20187	MEFs	Liver	MEFs	Liver
Translation/amino acid transport and metabolism														
Myd116	myeloid differentiation primary response gene 116	17872	0.06 ± 0.29	6.90 ± 0.13	0.59 ± 0.10	0.84 ± 0.17	0.93 ± 0.01	1.91 ± 0.32	0.85 ± 0.08	1.15 ± 0.10	914%	91%	12%	60%
Abcf2	ATP-binding cassette, sub-family F (GCN20), member 2	27407	0.09 ± 0.58	1.04 ± 0.04	0.49 ± 0.34	1.01 ± 0.03	0.87 ± 0.02	1.67 ± 0.45	0.59 ± 0.05	1.21 ± 0.10	534%	68%	98%	72%
Asns	asparagine synthetase	27053	1.08 ± 0.35	2.07 ± 0.05	0.32 ± 0.24	0.28 ± 0.03	0.43 ± 0.11	1.81 ± 0.38	1.65 ± 0.06	0.40 ± 0.01	29%	381%	14%	22%
Mthfr	5,10-methylenetetrahydrofolate reductase	17769	0.68 ± 0.26	1.51 ± 0.30	0.07 ± 0.36	0.18 ± 0.03	0.44 ± 0.28	1.97 ± 0.15	0.80 ± 0.26	0.50 ± 0.19	11%	180%	12%	25%
Slc1a4	solute carrier family 1 (glutamate/neutral amino acid transporter), member 4	55963	0.96 ± 0.15	2.23 ± 0.71	0.18 ± 0.07	0.01 ± 0.00	0.93 ± 0.07	2.13 ± 0.07	0.86 ± 0.11	1.03 ± 0.07	18%	92%	0%	48%
Sars	seryl-aminoacyl-tRNA synthetase	20226	0.20 ± 0.29	1.80 ± 0.31	0.03 ± 0.05	0.03 ± 0.05	0.67 ± 0.09	1.52 ± 0.09	0.38 ± 0.12	1.27 ± 0.05	14%	57%	2%	84%
Cars	cysteinyl-tRNA synthetase	27267	0.79 ± 0.26	1.95 ± 0.10	0.01 ± 0.00	0.01 ± 0.00	1.25 ± 0.73	5.22 ± 0.01	0.92 ± 0.32	0.57 ± 0.01	1%	73%	1%	11%
Tars	threonyl-tRNA synthetase	110960	0.80 ± 0.20	1.44 ± 0.02	0.35 ± 0.05	0.45 ± 0.08	0.89 ± 0.10	1.02 ± 0.12	0.88 ± 0.11	1.01 ± 0.00	44%	99%	31%	99%
Rps6ka2	ribosomal protein S6 kinase, polypeptide 2	20112	0.67 ± 0.29	1.80 ± 0.31	0.65 ± 0.05	0.53 ± 0.03	2.07 ± 0.96	1.01 ± 0.15	0.77 ± 0.32	0.77 ± 0.10	97%	37%	30%	76%
Lars	leucyl-tRNA synthetase	107045	0.96 ± 0.14	1.89 ± 0.12	0.52 ± 0.02	0.60 ± 0.03	0.89 ± 0.01	2.13 ± 0.44	1.00 ± 0.00	0.94 ± 0.11	54%	112%	32%	44%
Eprs	glutamyl-prolyl-tRNA synthetase	107508	0.99 ± 0.20	1.71 ± 0.27	0.48 ± 0.04	0.61 ± 0.00	0.83 ± 0.08	1.70 ± 0.08	0.57 ± 0.03	1.17 ± 0.10	48%	68%	35%	69%
Pycs	pyrroline-5-carboxylate synthetase	56454	0.76 ± 0.18	2.08 ± 0.14	0.17 ± 0.14	0.15 ± 0.08	1.31 ± 0.14	1.31 ± 0.30	0.33 ± 0.08	0.79 ± 0.16	22%	25%	7%	60%
Nars	asparaginyl-tRNA synthetase	70223	0.95 ± 0.27	1.64 ± 0.22	0.37 ± 0.15	0.43 ± 0.04	1.02 ± 0.04	2.23 ± 0.11	0.69 ± 0.06	0.85 ± 0.14	39%	67%	26%	38%
Wars	tryptophanyl-tRNA synthetase	22375	0.73 ± 0.39	3.06 ± 0.30	0.27 ± 0.00	0.25 ± 0.03	0.64 ± 0.06	1.07 ± 0.08	0.88 ± 0.12	1.05 ± 0.01	37%	136%	8%	99%
Aars	alanyl-tRNA synthetase	234734	0.67 ± 0.05	1.52 ± 0.06	0.35 ± 0.06	0.40 ± 0.02	0.77 ± 0.06	1.80 ± 0.22	0.74 ± 0.05	1.36 ± 0.19	53%	96%	27%	76%
Eif4ebp1	eukaryotic translation initiation factor 4E binding protein 1	13685	0.77 ± 0.14	1.55 ± 0.19	0.44 ± 0.02	0.40 ± 0.03	1.05 ± 0.03	1.51 ± 0.05	0.88 ± 0.09	0.80 ± 0.04	57%	85%	26%	53%
Psph	phosphoserine phosphatase	100678	0.87 ± 0.17	1.57 ± 0.04	0.61 ± 0.05	0.61 ± 0.10	0.89 ± 0.05	2.03 ± 0.05	1.07 ± 0.02	0.87 ± 0.06	70%	120%	39%	43%
Yars	tyrosyl-tRNA synthetase	107271	0.96 ± 0.06	1.59 ± 0.15	0.71 ± 0.52	0.79 ± 0.24	0.93 ± 0.14	1.44 ± 0.23	0.82 ± 0.04	1.03 ± 0.12	74%	88%	50%	71%
Elp3	elongation protein 3 homolog (S. cerevisiae)	74195	0.26 ± 0.11	1.04 ± 0.03	0.40 ± 0.02	0.54 ± 0.05	0.84 ± 0.06	1.10 ± 0.10	0.93 ± 0.07	1.10 ± 0.03	152%	111%	51%	100%
Iars	isoleucine-tRNA synthetase	105148	0.78 ± 0.11	1.23 ± 0.07	0.46 ± 0.32	0.33 ± 0.04	0.93 ± 0.17	1.91 ± 0.13	0.78 ± 0.00	1.00 ± 0.03	59%	84%	27%	52%
Slc1a5	solute carrier family 1 (neutral amino acid transporter), member 5	20514	0.72 ± 0.36	1.45 ± 0.00	0.12 ± 0.04	0.49 ± 0.21	1.01 ± 0.03	0.82 ± 0.16	1.28 ± 0.06	0.77 ± 0.08	16%	126%	34%	94%
Shmt2	serine hydroxymethyl transferase 2 (mitochondrial)	108037	0.70 ± 0.34	1.67 ± 0.48	0.77 ± 0.05	0.96 ± 0.01	1.18 ± 0.17	1.07 ± 0.04	0.96 ± 0.02	0.89 ± 0.10	110%	82%	58%	83%
Stress response														
Uvrag	UV radiation resistance associated gene	78610	0.01 ± 0.00	0.07 ± 0.37	0.17 ± 2.03	1.80 ± 1.31	1.16 ± 0.22	1.01 ± 0.03	0.80 ± 0.22	0.81 ± 0.17	1695%	69%	2450%	80%
Gch1	GTP cyclohydrolase 1	14528	0.62 ± 0.06	3.24 ± 0.91	0.40 ± 0.05	0.58 ± 0.22	1.16 ± 0.10	1.00 ± 0.05	0.72 ± 0.13	1.09 ± 0.13	65%	62%	18%	109%
GADD45a	growth arrest and DNA-damage-inducible 45 alpha	13197	0.65 ± 0.55	10.25 ± 1.16	0.01 ± 0.00	0.47 ± 0.42	0.23 ± 0.13	3.27 ± 0.26	0.22 ± 0.09	1.56 ± 0.11	2%	98%	5%	48%
Ndrl	N-myc downstream regulated-like	17990	0.67 ± 0.11	2.94 ± 0.55	0.74 ± 0.02	0.64 ± 0.09	0.90 ± 0.20	1.05 ± 0.22	0.80 ± 0.06	1.21 ± 0.07	110%	89%	22%	115%
Sqstm1	sequestosome 1	18412	0.56 ± 0.17	1.74 ± 0.08	0.65 ± 0.10	0.85 ± 0.16	0.62 ± 0.06	1.99 ± 0.53	0.58 ± 0.07	1.36 ± 0.05	116%	93%	49%	68%
Arntl	aryl hydrocarbon receptor nuclear translocator-like	11865	0.35 ± 0.03	1.10 ± 0.10	0.75 ± 0.03	1.12 ± 0.24	0.44 ± 0.24	1.26 ± 0.13	0.89 ± 0.13	1.15 ± 0.19	212%	203%	101%	91%
Dnajb9	DnaJ (Hsp40) homolog, subfamily B, member 9	27362	0.69 ± 0.26	3.74 ± 0.28	0.42 ± 0.12	2.96 ± 0.38	0.72 ± 0.08	1.12 ± 0.02	0.69 ± 0.16	1.47 ± 0.06	61%	95%	79%	131%
Tbce	tubulin-specific chaperone e	70430	0.70 ± 0.13	1.08 ± 0.06	0.51 ± 0.11	0.87 ± 0.21	0.93 ± 0.03	1.26 ± 0.08	0.76 ± 0.11	1.23 ± 0.20	72%	82%	81%	97%
Clcn3	chloride channel 3	12725	0.09 ± 0.57	1.69 ± 0.06	0.66 ± 0.17	0.68 ± 0.12	0.84 ± 0.25	1.38 ± 0.16	0.83 ± 0.19	0.94 ± 0.06	729%	99%	41%	68%
Dnajc3	DnaJ (Hsp40) homolog, subfamily C, member 3	19107	1.24 ± 0.27	6.50 ± 1.69	0.75 ± 0.07	4.31 ± 0.66	0.91 ± 0.08	0.98 ± 0.15	0.84 ± 0.18	1.24 ± 0.14	61%	92%	66%	127%
Redox/detox														
Pon2	paraoxonase 2	330260	0.09 ± 0.52	0.09 ± 0.55	0.06 ± 0.26	0.01 ± 0.00	1.17 ± 0.04	0.94 ± 0.14	1.00 ± 0.00	0.86 ± 0.08	71%	85%	11%	92%
B5R.1	NADH-cytochrome B5 reductase	72017	0.98 ± 0.27	3.15 ± 0.33	0.41 ± 0.11	0.55 ± 0.08	0.65 ± 0.06	3.05 ± 0.55	0.65 ± 0.15	1.45 ± 0.30	42%	99%	17%	47%
EST	RIKEN cDNA 1110049F12 gene	66193	0.37 ± 0.07	0.75 ± 0.35	0.66 ± 0.25	1.19 ± 0.03	1.00 ± 0.01	1.00 ± 0.07	0.92 ± 0.25	1.12 ± 0.22	181%	92%	157%	113%
Txnip	thioredoxin interacting protein	56338	0.64 ± 0.43	1.43 ± 0.26	0.54 ± 0.28	1.52 ± 0.93	0.93 ± 0.13	1.07 ± 0.09	1.13 ± 0.11	0.78 ± 0.12	86%	121%	106%	73%
Cpox	coproporphyrinogen oxidase	12892	0.95 ± 0.44	3.31 ± 0.34	0.58 ± 0.18	1.01 ± 0.17	0.91 ± 0.22	0.93 ± 0.10	0.92 ± 0.04	1.19 ± 0.13	61%	101%	31%	128%
Protein degradation														
Siah2	seven in absentia 2	20439	0.80 ± 0.02	2.63 ± 0.33	0.25 ± 0.07	0.96 ± 0.10	0.92 ± 0.15	1.19 ± 0.10	0.87 ± 0.09	0.95 ± 0.07	31%	95%	36%	80%
Fbxo8	F-box only protein 8	50753	0.69 ± 0.34	1.17 ± 0.22	0.40 ± 0.01	0.65 ± 0.02	0.90 ± 0.12	1.19 ± 0.37	0.87 ± 0.08	1.31 ± 0.12	58%	97%	55%	111%
Ctsc	cathepsin C	13032	0.53 ± 1.15	0.84 ± 0.92	1.06 ± 1.03	0.52 ± 0.64	1.14 ± 0.06	0.97 ± 0.28	1.08 ± 0.18	0.88 ± 0.04	200%	94%	62%	91%
Secretory pathway														
Wfs1	Wolfram syndrome 1 homolog (human)	22393	0.06 ± 0.27	5.64 ± 0.09	0.02 ± 0.02	0.99 ± 0.20	0.54 ± 0.05	3.41 ± 0.57	0.42 ± 0.08	1.47 ± 0.06	30%	78%	18%	43%
Spata5	spermatogenesis associated 5	57815	0.27 ± 0.16	0.90 ± 0.32	0.56 ± 0.33	1.84 ± 0.27	0.79 ± 0.07	2.98 ± 1.06	0.41 ± 0.15	1.32 ± 0.18	207%	52%	206%	44%
Herpud1	homocysteine-inducible-ubiquitin-like domain 1	64209	0.38 ± 0.33	3.84 ± 0.33	0.06 ± 0.07	1.05 ± 0.02	0.65 ± 0.22	0.96 ± 0.05	0.75 ± 0.28	1.21 ± 0.10	15%	116%	27%	126%
Ero1l	ERO1-like (S. cerevisiae)	50527	0.52 ± 0.47	2.03 ± 0.02	0.59 ± 0.14	0.70 ± 0.02	0.91 ± 0.02	1.32 ± 0.10	1.07 ± 0.03	0.89 ± 0.07	114%	117%	34%	67%
Sel1h	Sel1 (suppressor of lin-12) 1 homolog (C. elegans)	20338	0.77 ± 0.56	5.96 ± 4.70	0.36 ± 0.36	2.83 ± 2.07	0.87 ± 0.09	0.98 ± 0.05	0.91 ± 0.24	1.12 ± 0.04	47%	105%	47%	114%
ERO1Lb	ERO1-like beta (S. cerevisiae)	67475	0.89 ± 0.26	4.02 ± 0.57	0.73 ± 0.08	2.76 ± 0.09	0.98 ± 0.16	0.77 ± 0.05	1.10 ± 0.28	1.35 ± 0.15	82%	112%	69%	175%
Transcription														
Atf3	activating transcription factor 3	11910	0.73 ± 0.08	3.35 ± 0.57	0.61 ± 0.02	0.91 ± 0.06	0.26 ± 0.07	11.05 ± 3.69	0.22 ± 0.04	2.23 ± 0.58	84%	88%	27%	20%
Mecr	mitochondrial trans-2-enoyl-CoA reductase	26922	0.03 ± 0.06	1.68 ± 1.71	0.01 ± 0.00	0.69 ± 0.79	0.92 ± 0.02	0.90 ± 0.19	1.06 ± 0.11	1.07 ± 0.02	32%	115%	41%	118%
Nfkb2	nuclear factor of kappa B-cells 2, p49/p100	18034	0.38 ± 0.09	0.80 ± 0	0.52 ± 0.00	1.27 ± 0.13	0.68 ± 0.05	1.70 ± 0.48	0.72 ± 0.14	1.27 ± 0.17	139%	106%	158%	75%
Atf5	activating transcription factor 5	107503	0.99 ± 0.21	2.80 ± 0.56	0.13 ± 0.09	0.16 ± 0.03	0.46 ± 0.01	1.98 ± 0.25	0.23 ± 0.12	1.90 ± 0.37	13%	49%	6%	96%
Chop-10	Leucine zipper transcription factor CHOP, GADD153	13198	0.60 ± 0.34	11.30 ± 0.07	0.36 ± 0.10	3.72 ± 0.18	0.14 ± 0.05	4.31 ± 1.19	0.18 ± 0.07	2.07 ± 0.37	60%	131%	33%	48%
Nupr1	nuclear protein 1	56312	1.22 ± 0.75	3.37 ± 0.39	0.04 ± 0.05	0.04 ± 0.09	0.15 ± 0.00	26.69 ± 8.14	0.84 ± 0.39	1.04 ± 0.61	3%	569%	1%	4%
Cebpg	CCAAT/enhancer binding protein (C/EBP), gamma	12611	0.89 ± 0.25	1.75 ± 0.09	0.58 ± 0.04	0.80 ± 0.15	0.62 ± 0.08	2.01 ± 0.21	0.64 ± 0.19	1.23 ± 0.13	65%	102%	46%	61%
Relb	avian reticuloendotheliosis viral (v-rel) oncogene related B	19698	0.56 ± 0.21	1.00 ± 0.41	0.28 ± 0.11	1.60 ± 0.57	0.84 ± 0.08	1.75 ± 0.73	0.77 ± 0.17	1.30 ± 0.01	49%	91%	159%	75%
Tbpl1	TATA box binding protein-like 1	237336	0.55 ± 0.47	1.08 ± 0.08	0.67 ± 0.23	0.91 ± 0.23	0.80 ± 0.04	3.06 ± 0.41	0.83 ± 0.05	1.43 ± 0.32	122%	103%	85%	47%
Cebpb	CCAAT/enhancer binding protein (C/EBP), beta	12608	0.98 ± 0.30	1.96 ± 0.05	0.30 ± 0.08	0.27 ± 0.07	0.84 ± 0.06	1.67 ± 0.51	0.76 ± 0.15	1.61 ± 0.04	30%	91%	14%	97%
Cbx4	chromobox homolog 4 (Drosophila Pc class)	12418	0.91 ± 0.10	1.98 ± 0.41	0.71 ± 0.00	0.97 ± 0.07	0.81 ± 0.07	1.24 ± 0.10	0.54 ± 0.18	1.14 ± 0.02	78%	67%	49%	92%
Snw1	SNW domain containing 1	66354	0.55 ± 0.22	1.15 ± 0.20	0.98 ± 0.00	1.29 ± 0.96	1.00 ± 0.06	1.56 ± 0.15	0.80 ± 0.20	0.77 ± 0.13	179%	80%	112%	50%
Atf6	activating transcription factor 6	226641	0.30 ± 0.14	1.86 ± 0.01	0.40 ± 0.14	1.04 ± 0.27	0.70 ± 0.17	1.11 ± 0.02	0.49 ± 0.17	1.12 ± 0.05	132%	70%	56%	100%
Atf4	activating transcription factor 4	11911	0.84 ± 0.02	1.80 ± 0.02	0.17 ± 0.03	0.18 ± 0.01	0.84 ± 0.54	4.90 ± 0.73	0.70 ± 0.41	0.29 ± 0.04	20%	84%	10%	6%
Nfil3; E4B	nuclear factor, interleukin 3, regulated	18030	0.60 ± 0.09	1.92 ± 0.08	0.59 ± 0.05	0.99 ± 0.24	0.48 ± 0.15	1.50 ± 0.03	0.49 ± 0.15	1.35 ± 0.06	98%	102%	51%	90%
Lmo4	LIM domain only 4	16911	0.41 ± 0.07	1.18 ± 0.54	0.76 ± 0.48	1.41 ± 0.42	0.83 ± 0.21	1.47 ± 0.11	0.86 ± 0.10	0.87 ± 0.13	187%	104%	120%	59%
Klf4	Kruppel-like factor 4 (gut)	16600	0.85 ± 0.43	1.82 ± 0.55	0.27 ± 0.08	0.31 ± 0.17	0.72 ± 0.24	1.11 ± 0.17	0.62 ± 0.17	1.07 ± 0.01	32%	87%	17%	97%
Xbp1	X-box binding protein 1	22433	0.63 ± 0.14	1.14 ± 0.12	0.41 ± 0.07	1.22 ± 0.01	0.73 ± 0.05	1.54 ± 0.12	0.63 ± 0.09	1.35 ± 0.13	66%	87%	107%	88%
Ets2	E26 avian leukemia oncogene 2, 3' domain	23872	0.66 ± 0.04	1.49 ± 0.17	0.83 ± 0.24	0.79 ± 0.33	0.98 ± 0.07	2.49 ± 0.70	0.90 ± 0.04	1.10 ± 0.23	127%	91%	53%	44%
Rxrip110	retinoid X receptor interacting protein 110	20184	0.53 ± 0.18	1.19 ± 0.10	0.81 ± 0.15	1.00 ± 0.03	1.00 ± 0.00	1.04 ± 0.08	0.94 ± 0.19	0.99 ± 0.07	223%	124%	84%	96%

Expression levels of ISR target genes, previously validated in MEFs (Lu et al., 2004b, and table S1) is compared in the liver of mock-injected and AP20187 (0.2 mg/Kg)-injected mice of wildtype (n = 2) and Atf4-/- genotype (Harding, 2003) (n = 2) expressing Fv2E-PERK at low level (*Ttr::Fv2E PERK*). The ratio of expression level in the *Atf4-/-* mutant to the corresponding wildtype sample (X100) is referred to as “% of *Atf4+/+*”.

Note that 36% of the validated ISR target genes were expressed at 2-fold lower levels in wildtype versus *Atf4-/-* fibroblasts (Harding et al., 2003) whereas only 5% of the genes exhibited 2-fold lower level of expression in the *Atf4-/-* liver. The number of genes exhibiting >2-fold difference in levels of expression between wildtype and *Atf4-/-* mutant increased to 64% after ISR induction in fibroblasts whereas in the liver that number was only 27%. This suggests that ATF4 makes a smaller contribution to the ISR in liver than in fibroblasts.

Table S4. Sequence of Primers Used in Quantitative RT-PCR Analysis

Name	Description	Entrez Gene	Forward primer	Reverse primer
Acaca	acetyl-CoA carboxylase alpha	125656172	5'-GCCATTGGTATTGGGGCTTAC-3'	5'-CCCGACCAAGGACTTTGTTG-3'
Acacb	acetyl-CoA carboxylase beta	48976024	5'-GGAGGCAACAGGGTCATAGA-3'	5'-TGATCGGCCATCTTGATGTA-3'
Atf3	activating transcription factor 3	31542153	5'-CCAGGTCTCTGCCTCAGAAG-3'	5'-CATCTCCAGGGGTCTGTTGT-3'
Actb	β actin	145966868	5'-CCGCCCTAGGCACCAGGGTG-3'	5'-GGCTGGGGTGTTGAAGGTCTCAAA-3'
Hspa5	BiP, GRP78	31981721	5'-GAAAGGATGGTTAATGATGCTGAG-3'	5'-GTCTTCAATGTCCGCATCCTG-3'
Cebpa	CCAAT/enhancer binding protein (C/EBP), alpha	131886531	5'-CAAGAACAGCAACGAGTACCG-3'	5'-GTCACTGGTCAACTCCAGCAC-3'
Cebpb	CCAAT/enhancer binding protein (C/EBP), beta	145301630	5'-CAAGCTGAGCGACGAGTACA-3'	5'-CAGCTGCTCCACCTTCTTCT-3'
Chop-10	C/EBP homologous protein	50406	5'-CATACACCACCACACCTGAAAG-3'	5'-CCGTTTCCTAGTTCTTCCTTGC-3'
Fasn	fatty acid synthase	93102408	5'-AAGTTGCCCGAGTCAGAGAA-3'	5'-TGAGGCTGGGTTGATACCTC-3'
Gck	glucokinase	118129970	5'-TCTCTGACTTCCTGGACAAGC-3'	5'-CGTGTCATTCACCATTGCC-3'
Pck1	phosphoenolpyruvate carboxykinase	118130217	5'-AGTGCCTGTGGGAAGACTAACTTGG-3'	5'-GATTTGTCTTCACTGAGGTGCCAGG-3'
Pparg	peroxisome proliferator-activated receptor gamma	142367023	5'-ACATAAAGTCCTTCCCGCTGACCA-3'	5'-AAATTCGGATGGCCACCTCTTTGC-3'
Scd1	stearoyl-CoA desaturase	118130513	5'-TGGGTTGGCTGCTTGTG-3'	5'-GCGTGGGCAGGATGAAG-3'
Xbp1	X box-binding protein 1	13775155	5'-AAACAGAGTAGCAGCGCAGACTGC-3'	5'-TCCTTCTGGGTAGACCTCTGGGA-3'

Table S5.

	8 hr AP20187 (mg/kg)				
Name	0	0.002	0.02	0.2	2
SCD1	47.62 ± 0.48	80.95 ± 80.95	100.00 ± 9.05	52.38 ± 4.76	19.05 ± 9.05
ACACa	52.63 ± 0.26	78.95 ± 10.00	100.00 ± 8.42	65.79 ± 8.42	31.58 ± 6.32
ACACb	40.98 ± 0.82	77.46 ± 13.93	100.00 ± 7.79	53.28 ± 7.38	32.79 ± 4.10
FASN	47.62 ± 0.48	71.43 ± 11.90	100.00 ± 14.76	61.90 ± 10.00	19.05 ± 2.38
PEPCK	55.56 ± 0.56	83.33 ± 16.67	100.00 ± 22.22	38.89 ± 3.89	33.33 ± 14.44
PPARg	10.42 ± 0.09	36.46 ± 4.17	100.00 ± 8.33	32.29 ± 3.23	12.50 ± 1.67
C/EBPb	38.31 ± 1.954	49.04 ± 3.372	53.64 ± 4.598	75.48 ± 4.138	100.00 ± 8.046
CHOP	2.27 ± 0.01	4.77 ± 0.23	12.27 ± 0.93	63.64 ± 3.18	100.00 ± 13.50
ATF3	0.92 ± 0.00	1.65 ± 0.09	3.67 ± 0.47	59.63 ± 5.45	100.00 ± 16.66

Relative expression levels of ISR target genes in the liver of AP20187-injected mice expressing Fv2E-PERK at low level (data used in Figure 6A). Expression level of each gene was determined by real-time PCR. For plotting purpose and the peak level of expression was adjusted to 100%

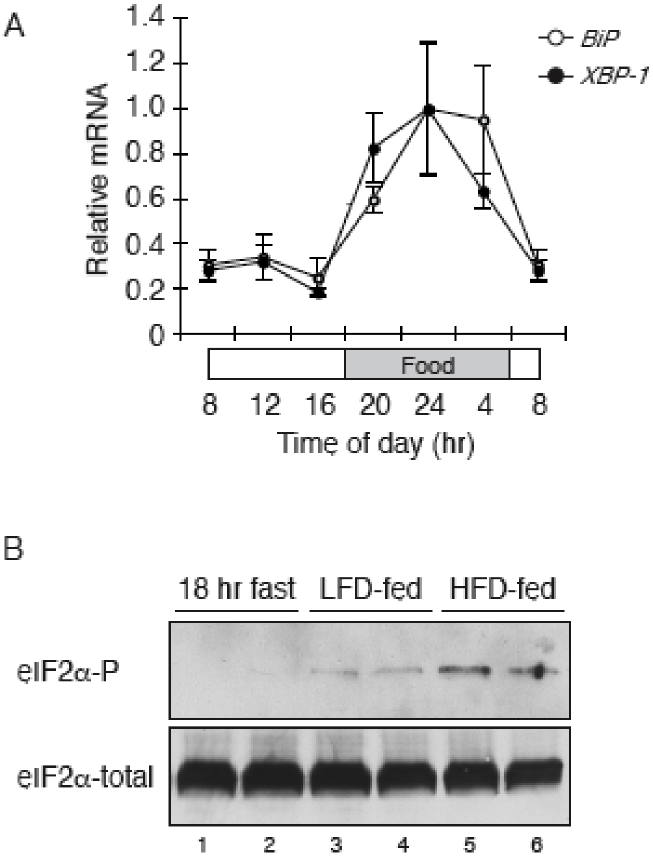

Figure S1. Nutrient Intake Activates the ISR

(A) Relative levels of BiP and XBP-1 mRNA in livers of wild-type mice procured at different times in the day (mean ± SEM, n = 3). One-way ANOVA: $p = 0.013$ in BiP and $p = 0.004$ in XBP-1. For the preceding week the mice had been maintained on a regimen whereby standard lab chow (low-fat diet) was provided from 18:00 to 06:00 hours and removed from the cage from 06:00 to 18:00 hours (as indicated). The same regimen was used on the day of the study and liver mRNA sampled at the time points indicated.

(B) Immunoblot of phosphorylated eIF2α and total eIF2α in extract of livers of individual animals previously maintained on standard lab chow (LFD) following an 18 hour fast or 4 hours after re-feeding standard low fat content (LFD) or high fat content (HFD) chow.

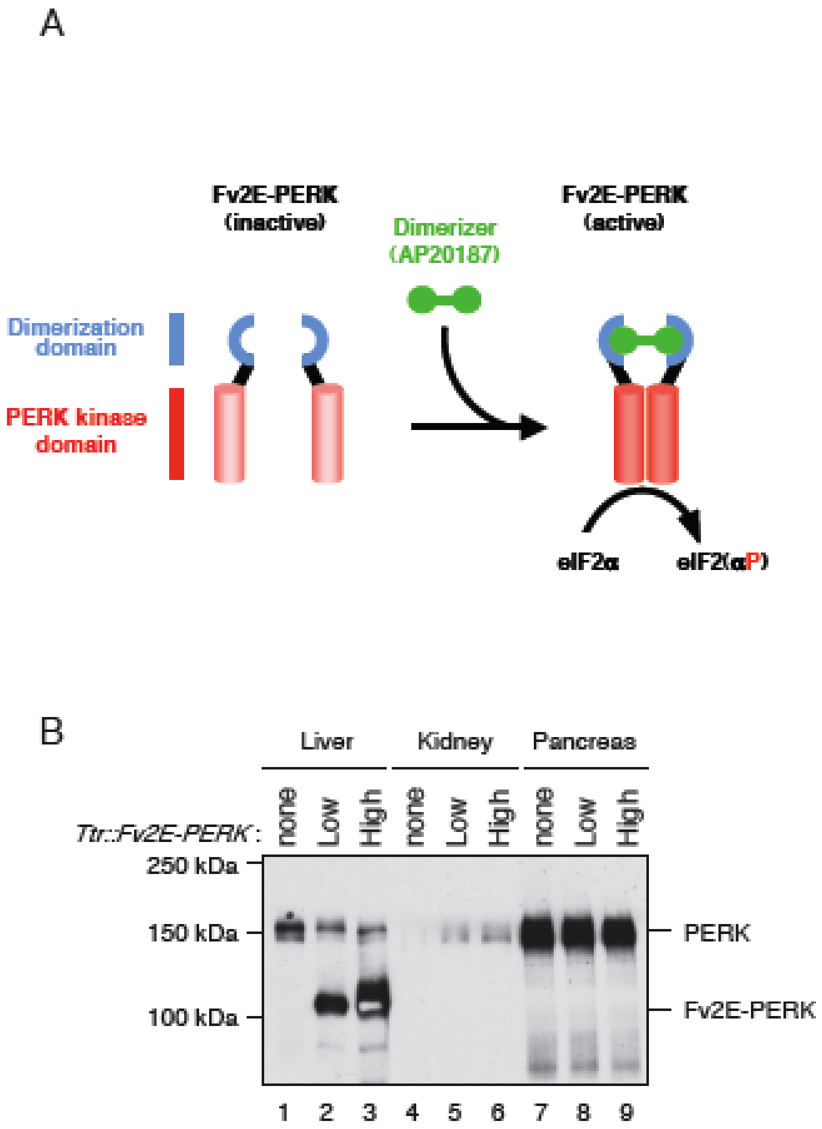

Figure S2. Fv2E-PERK

(A) Cartoon of the Fv2E-PERK fusion protein: The FKBP-based AP20187-binding domain is fused to the PERK kinase. In the basal state the fusion protein is inactive. However, upon addition of dimerizer (AP20187) the kinase is activated phosphorylating eIF2α.

(B) Immunoblot of endogenous PERK and transgenic Fv2E-PERK immunoprecipitated from detergent lysates of the indicated tissues of non-transgenic mice and *Ttr::Fv2E-PERK* transgenic mice expressing low and high levels of the transgene.