

Supplemental Table 1. Hepatocyte-specific Tak1-deletion impairs autophagy-related gene expression

Probe ID	Gene Name	Gene ID	Gene Description	Log2 ratio	Fold (KO vs WT)	Significant after Bonferroni-correction	
						p-value	p<9.73E-7
ILMN_2651781	Atg3	67841	autophagy-related 3 (yeast)	-1.22	0.43	8.19E-19	Y
ILMN_1236958	Gabarap1/atg8	57436	gamma-aminobutyric acid receptor-associated protein-like	-1.09	0.47	4.22E-17	Y
ILMN_2506115	Ulk1/atg1	22241	Unc-51 like kinase 1 (C. elegans)	-0.71	0.61	2.31E-05	
ILMN_1244059	Ulk2	29869	Unc-51 like kinase 2 (C. elegans)	-0.65	0.64	6.20E-05	
ILMN_2667100	Atg10	66795	autophagy-related 10 (yeast)	-0.62	0.65	2.33E-04	
ILMN_1260033	Map1lc3b/atg8f/lc3b	67443	microtubule-associated protein 1 light chain 3 beta	-0.58	0.67	3.91E-06	
ILMN_2998135	Atg5	11793	autophagy-related 5 (yeast)	-0.56	0.68	6.52E-06	
ILMN_1234539	Irgm1	15944	immunity-related GTPase family M member 1 (Irgm1)	-0.38	0.77	7.83E-03	
ILMN_1238679	atg101	68118	autophagy-related protein 101	-0.34	0.79	5.14E-03	
ILMN_1251333	Gabarap/atg8a	56486	gamma-aminobutyric acid receptor associated protein	-0.28	0.82	1.44E-02	
ILMN_2601710	Atg4d	235040	autophagy-related 4D (yeast)	-0.23	0.85	3.23E-01	
ILMN_2832682	Map1lc3a/atg8e/lc3a	66734	microtubule-associated protein 1 light chain 3 alpha	-0.15	0.90	2.40E-01	
ILMN_2667788	Atg4a	666468	autophagy-related 4A (yeast)	-0.08	0.95	4.57E-01	
ILMN_2632230	atg13	51897	autophagy-related 13 (yeast)	-0.07	0.95	3.67E-01	
ILMN_1219431	Gabarap2/atg8c	93739	gamma-aminobutyric acid receptor-associated protein-like	-0.03	0.98	4.64E-01	
ILMN_1246724	Atg7	74244	autophagy-related 7 (yeast)	0.00	1.00	5.00E-01	
ILMN_3018758	Becn1/Atg6	56208	beclin 1 (coiled-coil, myosin-like BCL2-interacting protein)	0.01	1.01	4.84E-01	
ILMN_3160369	Atg9b	213948	ATG9 autophagy related 9 homolog B (S. cerevisiae)	0.02	1.01	4.87E-01	
ILMN_2730478	Pik3c3/Vps34	225326	phosphoinositide-3-kinase, class 3	0.02	1.02	4.44E-01	
ILMN_1240472	Atg4b	66615	autophagy-related 4B (yeast)	0.03	1.02	4.72E-01	
ILMN_2671298	Atg4c	242557	autophagy-related 4C (yeast)	0.04	1.03	4.74E-01	
ILMN_1237642	Ambra1	228361	autophagy/beclin 1 regulator 1, transcript variant 1	0.14	1.10	4.17E-01	
ILMN_2624647	Rab24	19336	RAB24, member RAS oncogene family	0.15	1.11	1.81E-01	
ILMN_2710705	Sqstm1/p62	18412	sequestosome 1	0.20	1.15	6.95E-02	
ILMN_2752044	Rgs19	56470	regulator of G-protein signaling 19	0.24	1.18	2.40E-01	
ILMN_2620574	Atg16l1	77040	autophagy-related 16-like 1 (yeast)	0.27	1.20	3.72E-02	
ILMN_2663570	Atg12	67526	autophagy-related 12 (yeast)	0.37	1.29	5.21E-03	

* Red color indicates Significant p<0.05

Supplementary Table 2. Hepatocyte-specific Tak1-deleted mice: Liver tissue GOby reports.

1149 Differentially expressed genes - WT vs. Hepatocyte-specific Tak1-deletion

p- and q-values listed as "0" denote values less than 1E-10

Enriched Gene Ontology (GO) Annotations

Bonferroni threshold (α_{Bonf}) = 0.05

group id	group name	count	p-value	q-value
GO:0000267	cell fraction	165	0	0
GO:0003824	catalytic activity	494	0	0
GO:0005506	iron ion binding	43	0	0
GO:0005576	extracellular region	187	0	0
GO:0005737	cytoplasm	579	0	0
GO:0005739	mitochondrion	158	0	0
GO:0006082	organic acid metabolic process	130	0	0
GO:0006519	amino acid and derivative metabolic process	73	0	0
GO:0006629	lipid metabolic process	117	0	0
GO:0008152	metabolic process	555	0	0
GO:0016491	oxidoreductase activity	162	0	0
GO:0031012	extracellular matrix	54	0	0
GO:0042579	microbody	34	0	0
GO:0042598	vesicular fraction	73	0	0
GO:0044255	cellular lipid metabolic process	111	0	0
GO:0044421	extracellular region part	127	0	0
GO:0044444	cytoplasmic part	423	0	0
GO:0046906	tetrapyrrole binding	39	0	0
GO:0048037	cofactor binding	61	0	0
GO:0050817	coagulation	27	0	0
GO:0050818	regulation of coagulation	20	0	0
GO:0050896	response to stimulus	245	0	0
GO:0051186	cofactor metabolic process	72	0	0
GO:0006725	aromatic compound metabolic process	35	0	1E-10
GO:0006807	nitrogen compound metabolic process	87	0	1E-10
GO:0050819	negative regulation of coagulation	14	0	1E-10
GO:0005652	nuclear lamina	9	1.1E-09	1E-10
GO:0009308	amine metabolic process	78	0	2E-10
GO:0019842	vitamin binding	31	0	2E-10
GO:0005783	endoplasmic reticulum	112	0	6E-10
GO:0009117	nucleotide metabolic process	42	0.000451	9E-10
GO:0004857	enzyme inhibitor activity	42	0	1.3E-09
GO:0050878	regulation of body fluids	29	4E-10	1.3E-09
GO:0006766	vitamin metabolic process	28	1E-10	4.9E-09
GO:0031090	organelle membrane	95	2.3E-08	1.02E-08
GO:0046483	heterocycle metabolic process	29	1E-10	1.37E-08
GO:0007242	intracellular signaling cascade	73	0.137208	2.23E-08
GO:0006066	alcohol metabolic process	53	0	3.7E-08
GO:0009611	response to wounding	76	0	3.86E-08
GO:0006790	sulfur metabolic process	24	1E-09	5.09E-08
GO:0005975	carbohydrate metabolic process	58	6E-10	5.28E-08
GO:0019838	growth factor binding	23	1.32E-08	5.51E-08
GO:0032502	developmental process	309	6.21E-07	7.34E-08
GO:0030193	regulation of blood coagulation	18	0	9.17E-08
GO:0016765	transferase activity, transferring alkyl or aryl groups	19	4E-10	9.38E-08
GO:0009058	biosynthetic process	156	0	3E-07
GO:0007200	G-protein signaling, coupled to IP3 second messenger	8	0.020052	3.3E-07
GO:0005488	binding	696	1.77E-06	3.7E-07
GO:0031975	envelope	66	6.19E-07	5.18E-07
GO:0005638	lamin filament	7	1.17E-08	5.74E-07
GO:0002541	activation of plasma proteins during acute inflammatory response	14	2.45E-08	7.59E-07
GO:0010243	response to organic nitrogen	9	2.34E-08	8.63E-07
GO:0031965	nuclear membrane	21	2.13E-07	1.03E-06
GO:0016789	carboxylic ester hydrolase activity	25	6.29E-08	1.12E-06
GO:0044248	cellular catabolic process	89	2E-10	1.13E-06
GO:0016597	amino acid binding	19	0	1.17E-06
GO:0044237	cellular metabolic process	500	9E-10	1.17E-06
GO:0043176	amine binding	19	2.55E-07	1.38E-06
GO:0006091	generation of precursor metabolites and energy	57	2E-09	1.58E-06
GO:0005770	late endosome	18	7.5E-09	1.7E-06
GO:0019226	transmission of nerve impulse	22	0.83088	2.35E-06
GO:0042445	hormone metabolic process	25	7.12E-08	3.14E-06
GO:0004091	carboxylesterase activity	20	0	3.46E-06
GO:0051174	regulation of phosphorus metabolic process	25	0.00085	3.65E-06
GO:0044424	intracellular part	672	5.63E-06	4.23E-06
GO:0004497	monooxygenase activity	45	0	6.67E-06
GO:0002376	immune system process	83	1.38E-05	6.97E-06
GO:0009056	catabolic process	102	1E-10	7.43E-06
GO:0048519	negative regulation of biological process	143	5.79E-07	7.86E-06
GO:0051239	regulation of multicellular organismal process	64	1.72E-05	8.55E-06
GO:0006081	aldehyde metabolic process	8	1.88E-06	9.09E-06
GO:0044420	extracellular matrix part	22	1.12E-05	1.04E-05
GO:0006984	ER-nuclear signaling pathway	10	7.64E-06	1.55E-05
GO:0004364	glutathione transferase activity	17	0	1.65E-05
GO:0031967	organelle envelope	64	1.47E-06	3.27E-05
GO:0051050	positive regulation of transport	23	2.01E-06	3.44E-05
GO:0006956	complement activation	14	2.45E-08	3.62E-05
GO:0007596	blood coagulation	27	0	3.73E-05
GO:0044270	nitrogen compound catabolic process	21	4E-10	4.35E-05
GO:0035105	sterol regulatory element binding protein nuclear translocation	5	1.77E-07	4.58E-05
GO:0044249	cellular biosynthetic process	128	1.6E-09	6.55E-05
GO:0019439	aromatic compound catabolic process	9	3.21E-07	0.000167
GO:0019748	secondary metabolic process	16	1.17E-05	0.000219
GO:0005201	extracellular matrix structural constituent	9	1.01E-05	0.000227
GO:0019882	antigen processing and presentation	14	2.19E-06	0.000239
GO:0009310	amine catabolic process	21	2E-10	0.000315
GO:0005637	nuclear inner membrane	9	2.21E-06	0.000439
GO:0009110	vitamin biosynthetic process	11	3.4E-06	0.000504

Supplementary Table 3. Hepatocyte-specific Tak1-deleted mice: Liver tissue GOby reports.

1149 Differentially expressed genes - WT vs. Hepatocyte-specific Tak1-delet
p- and q-values listed as "0" denote values less than 1E-10

Lipid-related pathways in **red**

Enriched KEGG Path Enriched KEGG Pathway Annotations

Bonferroni threshold (Bonferroni threshold (α Bonf) = 0.05

group id	group name	count	p-value
KEGG:mmu00260	Glycine, serine and threonine metabolism	27	0
KEGG:mmu00480	Glutathione metabolism	24	0
KEGG:mmu00591	Linoleic acid metabolism	22	0
KEGG:mmu00980	Metabolism of xenobiotics by cytochrome P450	47	0
KEGG:mmu04610	Complement and coagulation cascades	27	0
KEGG:mmu00120	Bile acid biosynthesis	16	1E-10
KEGG:mmu00860	Porphyrin and chlorophyll metabolism	21	1E-10
KEGG:mmu00970	Aminoacyl-tRNA biosynthesis	30	1E-10
KEGG:mmu00620	Pyruvate metabolism	21	1.8E-09
KEGG:mmu00040	Pentose and glucuronate interconversions	14	1.9E-09
KEGG:mmu00730	Thiamine metabolism	17	3.2E-09
KEGG:mmu00280	Valine, leucine and isoleucine degradation	16	7E-09
KEGG:mmu00630	Glyoxylate and dicarboxylate metabolism	12	1.94E-08
KEGG:mmu00500	Starch and sucrose metabolism	17	2.09E-08
KEGG:mmu00010	Glycolysis / Gluconeogenesis	19	1.16E-07
KEGG:mmu00590	Arachidonic acid metabolism	17	1.83E-07
KEGG:mmu00071	Fatty acid metabolism	16	2.4E-07
KEGG:mmu04110	Cell cycle	25	3.23E-07
KEGG:mmu00460	Cyanoamino acid metabolism	12	3.37E-07
KEGG:mmu00640	Propanoate metabolism	13	4.23E-07
KEGG:mmu05219	Bladder cancer	14	6.96E-07
KEGG:mmu00362	Benzoate degradation via hydroxylation	13	8.4E-07
KEGG:mmu00380	Tryptophan metabolism	19	1.38E-06
KEGG:mmu00520	Nucleotide sugars metabolism	9	1.56E-06
KEGG:mmu00410	beta-Alanine metabolism	14	1.82E-06
KEGG:mmu00830	Retinol metabolism	8	1.88E-06
KEGG:mmu01430	Cell Communication	24	1.95E-06
KEGG:mmu00650	Butanoate metabolism	17	2.02E-06
KEGG:mmu00150	Androgen and estrogen metabolism	14	2.19E-06
KEGG:mmu00361	gamma-Hexachlorocyclohexane degradation	11	3.4E-06
KEGG:mmu03320	PPAR signaling pathway	17	3.53E-06
KEGG:mmu00252	Alanine and aspartate metabolism	14	6.06E-06
KEGG:mmu00350	Tyrosine metabolism	16	1.5E-05
KEGG:mmu00720	Reductive carboxylate cycle (CO2 fixation)	8	1.62E-05
KEGG:mmu00910	Nitrogen metabolism	11	1.84E-05
KEGG:mmu00310	Lysine degradation	12	2.3E-05
KEGG:mmu00020	Citrate cycle (TCA cycle)	14	3.44E-05
KEGG:mmu00220	Urea cycle and metabolism of amino groups	14	3.44E-05
KEGG:mmu00920	Sulfur metabolism	8	7.97E-05
KEGG:mmu00680	Methane metabolism	9	8.13E-05
KEGG:mmu00440	Aminophosphonate metabolism	10	8.75E-05
KEGG:mmu00450	Selenoamino acid metabolism	10	8.75E-05
KEGG:mmu05218	Melanoma	15	9.25E-05
KEGG:mmu04115	p53 signaling pathway	16	9.94E-05
KEGG:mmu05214	Glioma	14	0.000115
KEGG:mmu00072	Synthesis and degradation of ketone bodies	7	0.000142
KEGG:mmu00625	Tetrachloroethene degradation	7	0.000182
KEGG:mmu04540	Gap junction	16	0.000232
KEGG:mmu00230	Purine metabolism	23	0.000246
KEGG:mmu00052	Galactose metabolism	9	0.000283

Supplemental Table 4. Hepatocyte-specific Tak1-deletion impairs PPARα target gene expression.

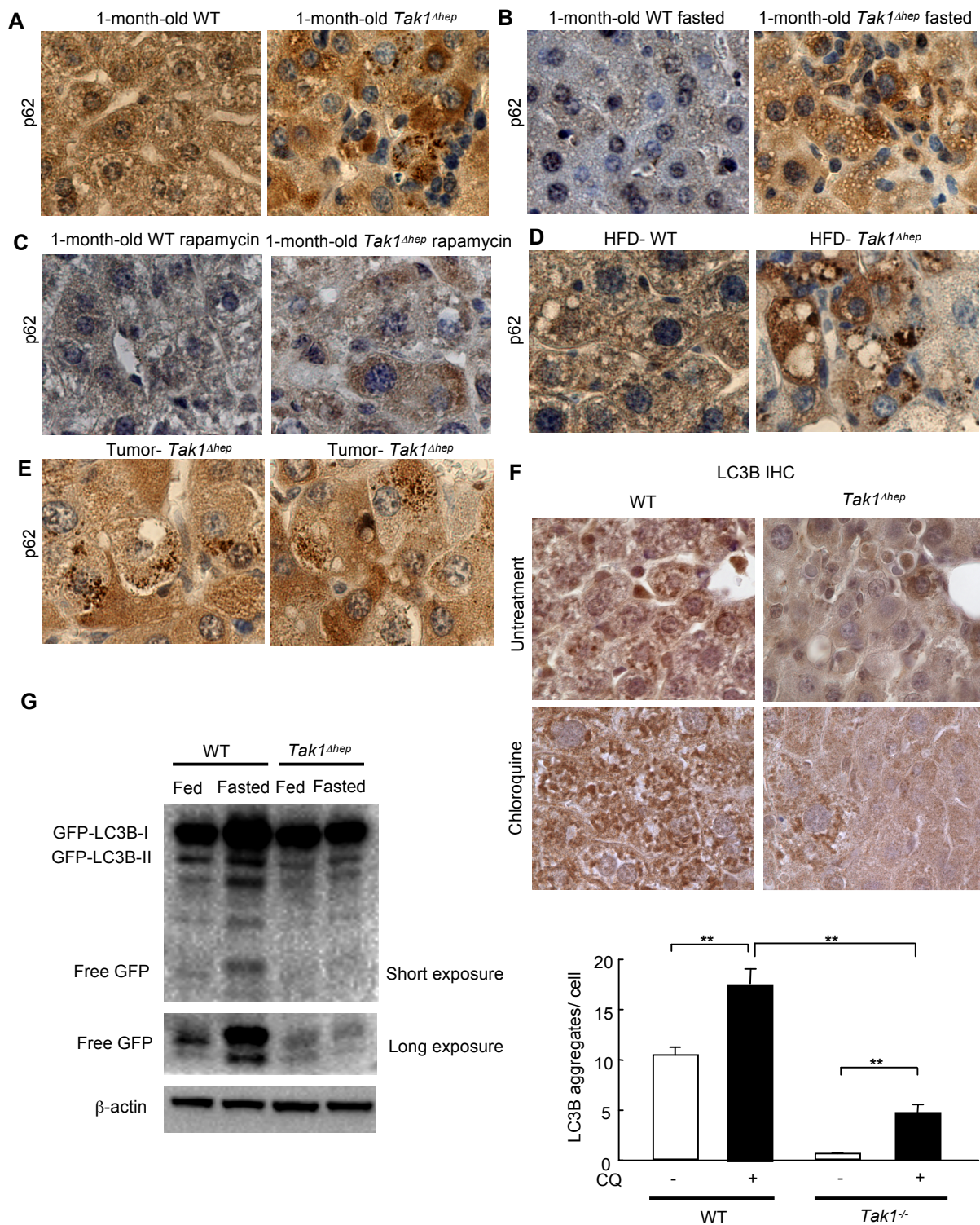
Supplemental Table 4. Hepatocyte-specific Tak1-deletion impairs PPARα target gene expression.					Significant after Bonferroni-correction			
Probe ID	Gene Name	Gene ID	Gene Description	Log2 ratioFold (KO vs WT)	p-value	p<9.73E-7	Function	
ILMN 3111685	AcsM3	20216	acyl-CoA synthetase medium-chain family member 3, transcript variant 2	-1.31	0.40	1.40E-21	Y Fatty acid binding/activation	
ILMN 2648445	Slc27a2	26458	solute carrier family 27 (fatty acid transporter), member 2	-1.26	0.42	1.57E-22	Y Lipid transport	
ILMN 2622671	AcsI1	14081	acyl-CoA synthetase long-chain family member 1	-1.16	0.45	3.01E-19	Y Fatty acid binding/activation	
ILMN 2734181	AcsS2	60525	acyl-CoA synthetase short-chain family member 2	-1.11	0.46	2.36E-18	Y Fatty acid binding/activation	
ILMN 1254105	Cyp4a12	277753	cytochrome P450, family 4, subfamily a, polypeptide 12	-1.09	0.47	1.19E-05	N Peroxisomal/microsomal fatty acid oxidation	
ILMN 1216322	Hmgcs2	15360	3-hydroxy-3-methylglutaryl-Coenzyme A synthase 2	-1.08	0.47	9.68E-18	Y Mitochondrial fatty acid oxidation/ ketone body synthesis	
ILMN 269168C	Cyp4a14	13119	cytochrome P450, family 4, subfamily a, polypeptide 14	-0.99	0.50	2.43E-15	Y Peroxisomal/microsomal fatty acid oxidation	
ILMN 2614728	Acadsb	66885	acyl-Coenzyme A dehydrogenase, short/branched chain	-0.93	0.53	1.37E-13	Y Mitochondrial fatty acid oxidation/ ketone body synthesis	
ILMN 2771368	Acox1	11430	acyl-Coenzyme A oxidase 1, palmitoyl	-0.86	0.55	5.59E-10	Y Peroxisomal/microsomal fatty acid oxidation	
ILMN 2925281	Abcd3	19299	ATP-binding cassette, sub-family D (ALD), member 3	-0.80	0.58	1.00E-10	Y Peroxisomal/microsomal fatty acid oxidation	
ILMN 2659997	Acaa1b	235674	acetyl-Coenzyme A acyltransferase 1B	-0.70	0.61	8.44E-09	Y Peroxisomal/microsomal fatty acid oxidation	
ILMN 2985282	Peci	23986	peroxisomal delta3, delta2-enoyl-Coenzyme A isomerase	-0.64	0.64	1.89E-07	Y Peroxisomal/microsomal fatty acid oxidation	
ILMN 270612C	Ehnhadh	74147	enoyl-Coenzyme A, hydratase/3-hydroxyacyl Coenzyme A dehydrogenase	-0.64	0.64	4.16E-07	Y Peroxisomal/microsomal fatty acid oxidation	
ILMN 3138495	Slc25a20	57279	solute carrier family 25, member 20	-0.61	0.65	5.28E-07	Y Mitochondrial fatty acid oxidation/ ketone body synthesis	
ILMN 2704823	Acaa2	52538	acetyl-Coenzyme A acyltransferase 2	-0.61	0.65	4.91E-07	Y Mitochondrial fatty acid oxidation/ ketone body synthesis	
ILMN 261517C	Acad8	66948	acyl-Coenzyme A dehydrogenase family, member 8	-0.58	0.67	6.20E-03	Y Mitochondrial fatty acid oxidation/ ketone body synthesis	
ILMN 292535C	Aldh3a2	11671	aldehyde dehydrogenase family 3, subfamily A2	-0.57	0.67	2.42E-06	Y Peroxisomal/microsomal fatty acid oxidation	
ILMN 2615207	Dci	13177	dodecenoyl-Coenzyme A delta isomerase	-0.56	0.68	4.15E-06	Y Mitochondrial fatty acid oxidation/ ketone body synthesis	
ILMN 2744125	Acad10	71985	acyl-Coenzyme A dehydrogenase family, member 10	-0.54	0.69	1.31E-02	Y Mitochondrial fatty acid oxidation/ ketone body synthesis	
ILMN 2705845	Decr2	26378	2,4-dienoyl CoA reductase 2, peroxisomal	-0.53	0.69	2.49E-03	Y Peroxisomal/microsomal fatty acid oxidation	
ILMN 3138743	AcsI3	74205	acyl-CoA synthetase long-chain family member 3, transcript variant 1	-0.49	0.71	8.24E-04	Y Fatty acid binding/activation	
ILMN 2661733	HadhsC	15107	short chain 3-hydroxyacyl-CoA dehydrogenase	-0.44	0.74	6.39E-03	Y Mitochondrial fatty acid oxidation/ ketone body synthesis	
ILMN 2643977	Acat1	110446	acetyl-Coenzyme A acetyltransferase 1	-0.40	0.76	6.76E-02	Y Mitochondrial fatty acid oxidation/ ketone body synthesis	
ILMN 2956932	AcadvI	11370	acyl-Coenzyme A dehydrogenase, very long chain	-0.39	0.76	1.05E-03	Y Mitochondrial fatty acid oxidation/ ketone body synthesis	
ILMN 1254786	Ech1	51798	enoyl coenzyme A hydratase 1, peroxisomal	-0.37	0.77	1.63E-03	Y Peroxisomal/microsomal fatty acid oxidation	
ILMN 2762728	Acaa1a	113868	acetyl-Coenzyme A acyltransferase 1A	-0.36	0.78	2.24E-03	Y Peroxisomal/microsomal fatty acid oxidation	
ILMN 2590961	Hadha	15107	hydroxyacyl-Coenzyme A dehydrogenase, alpha subunit	-0.33	0.79	1.79E-01	Y Mitochondrial fatty acid oxidation/ ketone body synthesis	
ILMN 1235374	Decr1	67460	2,4-dienoyl CoA reductase 1, mitochondrial	-0.32	0.80	1.94E-02	Y Mitochondrial fatty acid oxidation/ ketone body synthesis	
ILMN 2644827	AcoI3	171281	acyl-CoA thioesterase 3	-0.32	0.80	1.62E-02	Y Peroxisomal/microsomal fatty acid oxidation	
ILMN 2912235	Hsd17b4	15488	hydroxysteroid (17-beta) dehydrogenase 4	-0.31	0.81	7.32E-03	Y Peroxisomal/microsomal fatty acid oxidation	
ILMN 1243944	Abcd2	26874	ATP-binding cassette, sub-family D (ALD), member 2	-0.25	0.84	1.81E-01	Y Peroxisomal/microsomal fatty acid oxidation	
ILMN 2834915	Slc22a5	20520	solute carrier family 24, member 5	-0.22	0.86	9.07E-02	Y Mitochondrial fatty acid oxidation/ ketone body synthesis	
ILMN 2591776	Cpt1a	12894	carnitine palmitoyltransferase 1a, liver	-0.18	0.89	3.80E-01	Y Mitochondrial fatty acid oxidation/ ketone body synthesis	
ILMN 2719877	Crot	74114	carnitine O-octanoyltransferase	-0.17	0.89	2.87E-01	Y Peroxisomal/microsomal fatty acid oxidation	
ILMN 2885532	Cpt2	12896	carnitine palmitoyltransferase 2	-0.16	0.89	1.08E-01	Y Mitochondrial fatty acid oxidation/ ketone body synthesis	
ILMN 2892222	AcsI5	433256	acyl-CoA synthetase long-chain family member 5	-0.14	0.91	1.42E-01	Y Fatty acid binding/activation	
ILMN 263515C	Hibch	227095	3-hydroxyisobutyryl-Coenzyme A hydrolase	-0.13	0.92	4.16E-01	Y Mitochondrial fatty acid oxidation/ ketone body synthesis	
ILMN 2622613	AcoI4	171282	acyl-CoA thioesterase 4	-0.11	0.93	2.16E-01	Y Peroxisomal/microsomal fatty acid oxidation	
ILMN 2927172	Ppara	19013	peroxisome proliferator activated receptor alpha	-0.10	0.93	3.80E-01	Y Peroxisomal/microsomal fatty acid oxidation	
ILMN 2810473	Acadm	11364	acyl-Coenzyme A dehydrogenase, medium chain	-0.07	0.95	2.89E-01	Y Mitochondrial fatty acid oxidation/ ketone body synthesis	
ILMN 2806676	Acads	11409	acyl-Coenzyme A dehydrogenase, short chain	-0.07	0.95	3.03E-01	Y Mitochondrial fatty acid oxidation/ ketone body synthesis	
ILMN 1230454	Hadhb	231086	hydroxyacyl-Coenzyme A dehydrogenase, beta subunit	-0.06	0.96	3.14E-01	Y Mitochondrial fatty acid oxidation/ ketone body synthesis	
ILMN 2822131	Hmgcl	15356	3-hydroxymethyl-3-methylglutaryl-Coenzyme A lyase-like 1	0.04	1.03	3.92E-01	Y Mitochondrial fatty acid oxidation/ ketone body synthesis	
ILMN 2689473	AcadI	11363	acyl-Coenzyme A dehydrogenase, long-chain	0.05	1.04	3.76E-01	Y Mitochondrial fatty acid oxidation/ ketone body synthesis	
ILMN 2983587	AcoI8	170789	acyl-CoA thioesterase 8	0.09	1.06	2.61E-01	Y Peroxisomal/microsomal fatty acid oxidation	
ILMN 123247C	Cd36	12491	CD36 antigen	0.17	1.12	3.69E-01	Y Lipid transport	
ILMN 2648742	Abcb4	18670	ATP-binding cassette, sub-family B (MDR/TAP), member 4	0.54	1.45	6.63E-05	Y Peroxisomal/microsomal fatty acid oxidation	
ILMN 2679456	Crat	12908	carnitine acetyltransferase	0.55	1.46	5.08E-02	Y Mitochondrial fatty acid oxidation/ ketone body synthesis	
Total	48			28	14	14		

GOby Analysis Bonferroni-corrected p-values for KEGG Pathways (see Table S2)

PPAR pathway	p=0.0000035277
Fatty acid metabolism	p=0.0000002400

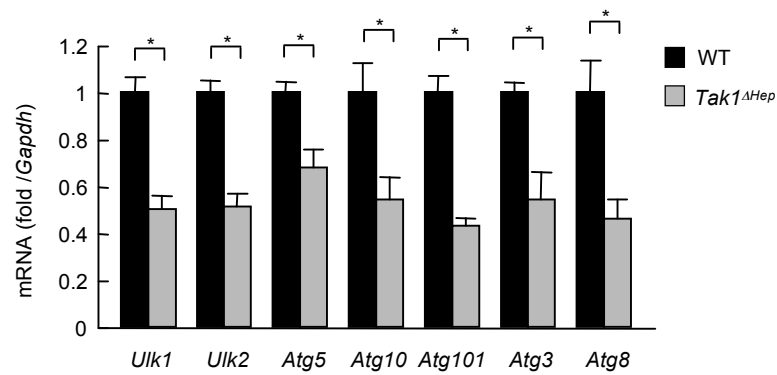
Supplemental Table 5 Sequence of Primers Used for Real-Time Quantitative PCR

Gene	Forward	Reverse
<i>Ppara</i>	CAGTGGGGAGAGAGGACAGA	AGTTCGGGAACAAGACGTTG
<i>Hmgcs2</i>	ATACCACCAACGCCTGTTATG	CAATGTCACCACAGACCACCA
<i>Cpt1a</i>	AGTGGCCTCACAGACTCCAG	GCCCATGTTGTACAGCTTCC
<i>Acox1</i>	CCTGATTCAGCAAGGTAGGG	TCGCAGACCCTGAAGAAATC
<i>Cyp4a10</i>	AAGGGTCAAACACCTCTGGA	GATGGACGCTCTTTACCCAA
<i>Sirt3</i>	ATAGAATTCATGGCGCTTGAC	ATAGAATTCTCTGTCCTGTCC
<i>Mttp</i>	GACCACCCTGGATCTCCATA	AGCGTGGTGAAAGGGCTTAT
<i>Acs1</i>	ACCATCAGTGGTACCCGCTA	CGCTCACCACCTTCTGGTAT
<i>Peci</i>	CGAGTTGGCTGAATGGAGTA	CCAGCTGTGGGAATCTCTGT
<i>Cyp4a12</i>	GGTGTCCAAGGTCATCAAGG	TGGCAGACTCTGTTTCGTGTC
<i>Srebf1</i>	TGGTTGTTGATGAGCTGGAG	GGCTCTGGAACAGACACTGG
<i>Dgat1</i>	TCACCACACACCAATTCAGG	GACGGCTACTGGGATCTGA
<i>Pparg</i>	TCTTCCATCACGGAGAGGTC	GATGCACTGCCTATGAGCAC
<i>Fasn</i>	GTTGGCCCAGAACTCCTGTA	GTCGTCTGCCTCCAGAGC
<i>Tnf</i>	AGGGTCTGGGCCATAGAACT	CCACCACGCTCTTCTGTCTAC
<i>Il6</i>	ACCAGAGGAAATTTTCAATAGG	CTGATGCACTTGCAGAAAACA
<i>Ccl2</i>	ATTGGGATCATCTTGCTGGT	CCTGCTGTTACAGTTGCC
<i>Col1a1</i>	TAGGCCATTGTGTATGCAGC	ACATGTTTCAGCTTTGTGGACC
<i>Tgfb1</i>	CAACCCAGGTCCTTCCTAAA	GGAGAGCCCTGGATACCAAC
<i>Afp</i>	GCCTGAACTGACAGAGGAGCA	TTTAAACGCCCAAAGCATCAC
<i>Apob100</i>	CGTGGGCTCCAGCATTCTA	CCAATGGTCTGGGCACTGCT
<i>Ehhadh</i>	TGGCTCTAACCGTATGGTCC	CTATGATCCGCCTCTGCAA



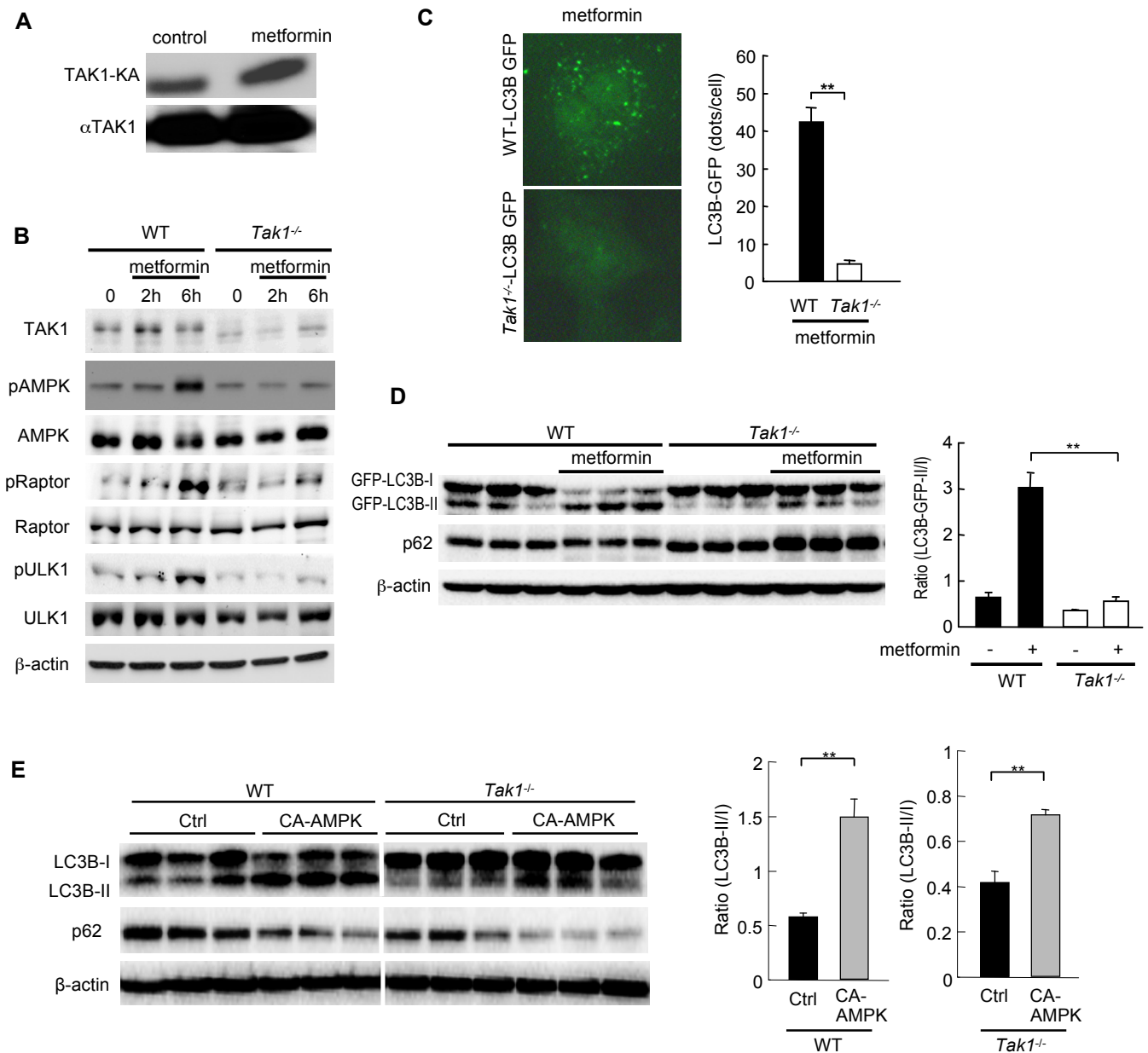
Supplemental Figure 1. Autophagy and p62 accumulation in *Tak1^{Δhep}* mice.

(A-E) p62 aggregation and expression were assessed by immunohistochemistry. (A) Original magnification x400. Representative pictures are shown (n=5, each). (A-C) 1-month-old WT and *Tak1^{Δhep}* mice under feeding (A) or fasting (12 h) (B) conditions, or treated with rapamycin (5mg/kg) for 3 days (C). (D) 1-month-old WT and *Tak1^{Δhep}* mice were fed HFD for additional 12 weeks. (E) p62 aggregation in HCC of *Tak1^{Δhep}* mice. (F, G) Basal levels of autophagy are impaired in *Tak1^{Δhep}* mice. (F) 1-month-old WT and *Tak1^{Δhep}* mice were treated with chloroquine (CQ; 60mg/kg) intraperitoneally. Immunohistochemistry for hepatic LC3B and its quantifications are shown. Data are presented as means ± S.E.M. **, p<0.01. (G) 1-month-old GFP-LC3B transgenic mice and *Tak1^{Δhep}* GFP-LC3B transgenic mice were fasted for 12 hours. Immunoblotting for GFP is shown. The detection of Free GFP indicates that free GFP is released after digestion of autophagosome.



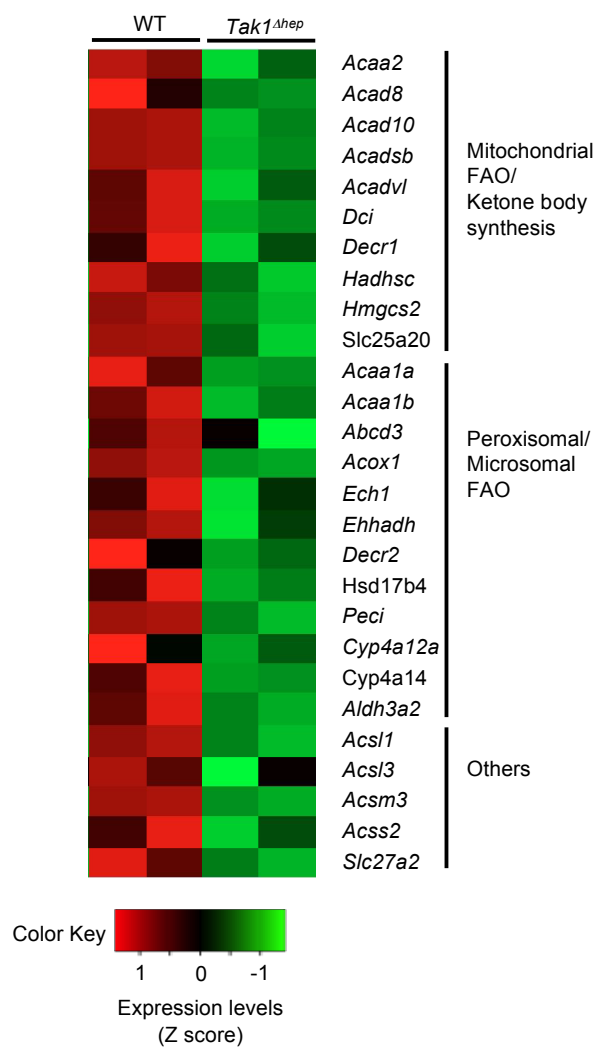
Supplemental Figure 2. Downregulated autophagy-related genes in *Tak1*^{ΔHep} mice.

Total RNA was extracted from 1-month-old WT and *Tak1*^{ΔHep} mice (n=5, and 9, respectively). Hepatic mRNA expressions of *Ulk1*, *Ulk2*, *Atg5*, *Atg10*, *Atg101*, *Atg3* and *Atg8* were measured by quantitative real-time PCR. Black bar; WT, Gray bar; *Tak1*^{ΔHep} mice. Data are presented as means ± S.E.M. *; p<0.05.



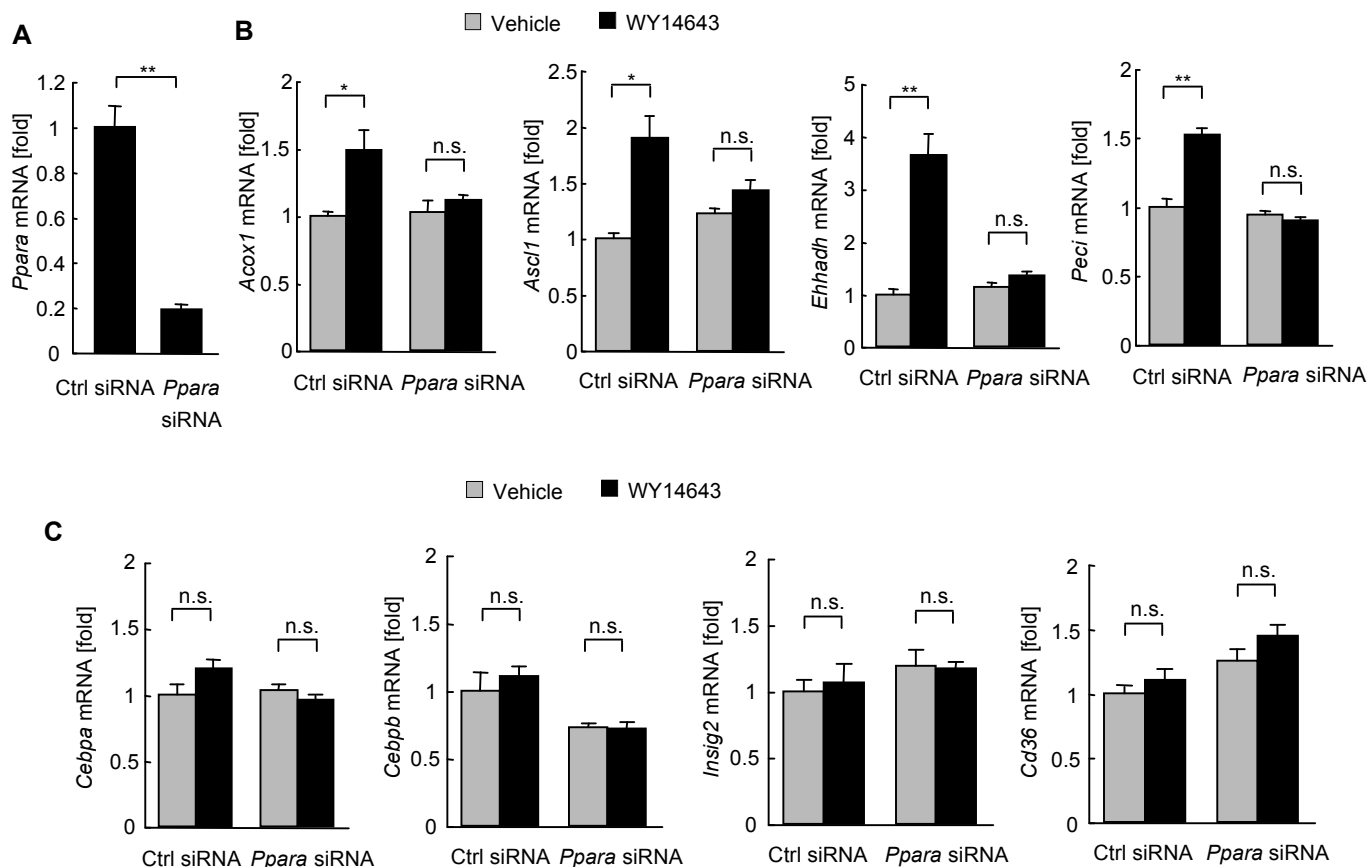
Supplemental Figure 3. Metformin-induced autophagy depends on TAK1 and constitutive active AMPK induces autophagy in *Tak1*^{-/-} hepatocytes.

(A-D) Metformin-induced AMPK activation and induction of autophagy. (A) Primary hepatocytes were isolated from WT mice. TAK1 kinase activity was analyzed two hours after metformin (2mM) treatment. (B) Primary hepatocytes were isolated from WT and *Tak1*^{Δhep} mice. Expressions of TAK1, p-AMPK, AMPK, p-Raptor, Raptor, p-ULK1, and ULK1 two and six hours after treatment with metformin (2mM) were assessed by immunoblotting. (C, D) Primary hepatocytes were isolated from GFP-LC3B transgenic mice and *Tak1*^{Δhep}/GFP-LC3B transgenic mice. (C) After treatment with metformin for 6 hours, cells were harvested. GFP-LC3B accumulation in cytoplasm was assessed. (D) Immunoblotting for GFP-LC3B and p62 is shown. Closed bar; WT, Open bar; *Tak1*^{Δhep} mice. (E) Primary hepatocytes were isolated from WT and *Tak1*^{Δhep} mice. Cells were incubated with control adenovirus (Ctrl) and adenovirus expressing constitutive active AMPK (CA-AMPK) for 24 hours. Immunoblotting for LC3B and p62 is shown. Black bar; control adenovirus, Gray bar; adenovirus expressing CA-AMPK. Data are presented as means $\pm$ S.E.M. *, $p < 0.05$, **, $p < 0.01$. Similar results were obtained in three independent experiments.



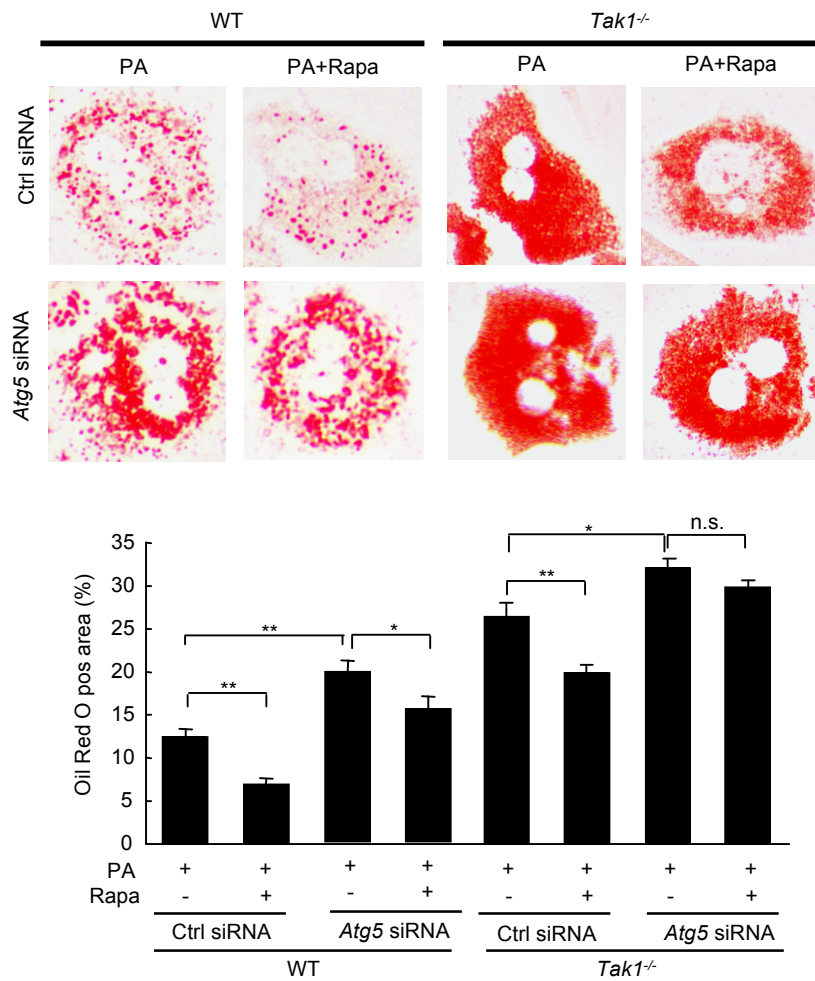
Supplemental Figure 4. Gene expression profile of *Tak1^{-/-}* livers.

A heat map of microarray analysis of PPAR α target lipid metabolism related genes in the livers of WT and *Tak1^{Δhep}* mice is shown (n=2, each).



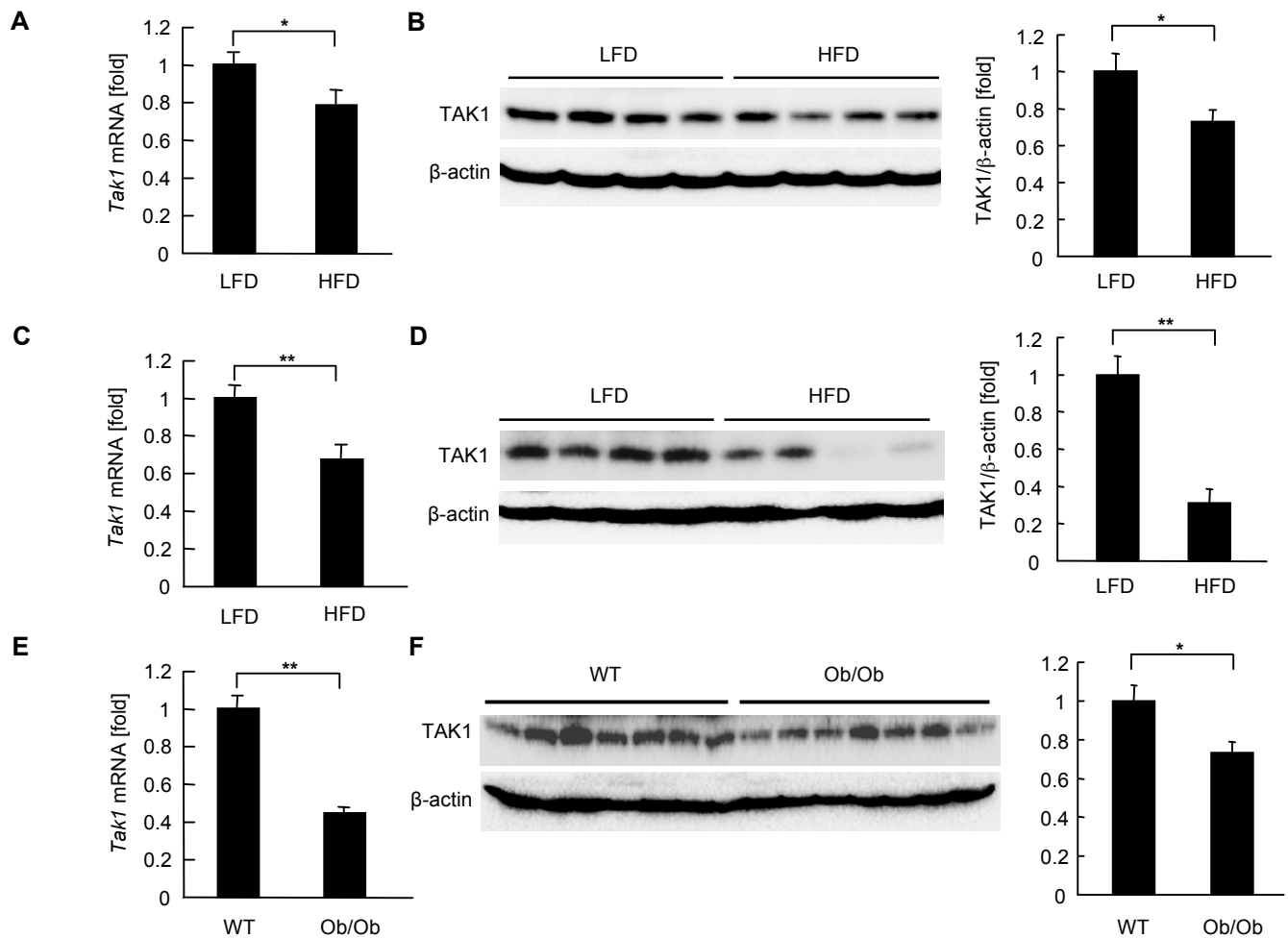
Supplemental Figure 5. WY14643 does not upregulate PPAR γ target genes in hepatocytes

Primary hepatocytes were isolated and incubated with control siRNA or siRNA for *Ppara* followed by treatment with palmitate (125 μ M) and PPAR α agonist WY14643 (30 μ M) for 6 hours. mRNA expressions of **(A)** *Ppara*, **(B)** PPAR α target genes: *Acox1*, *Acsl1*, *Ehhadh*, *Peci*, **(C)** PPAR γ target genes: *Cebpa*, *Cebpb*, *Insig2* and *Cd36* were measured by quantitative real-time PCR. Similar results were obtained in three independent experiments. Data are presented as means $\pm$ S.E.M. *, $p < 0.05$; **, $p < 0.01$, n.s.; not significant.



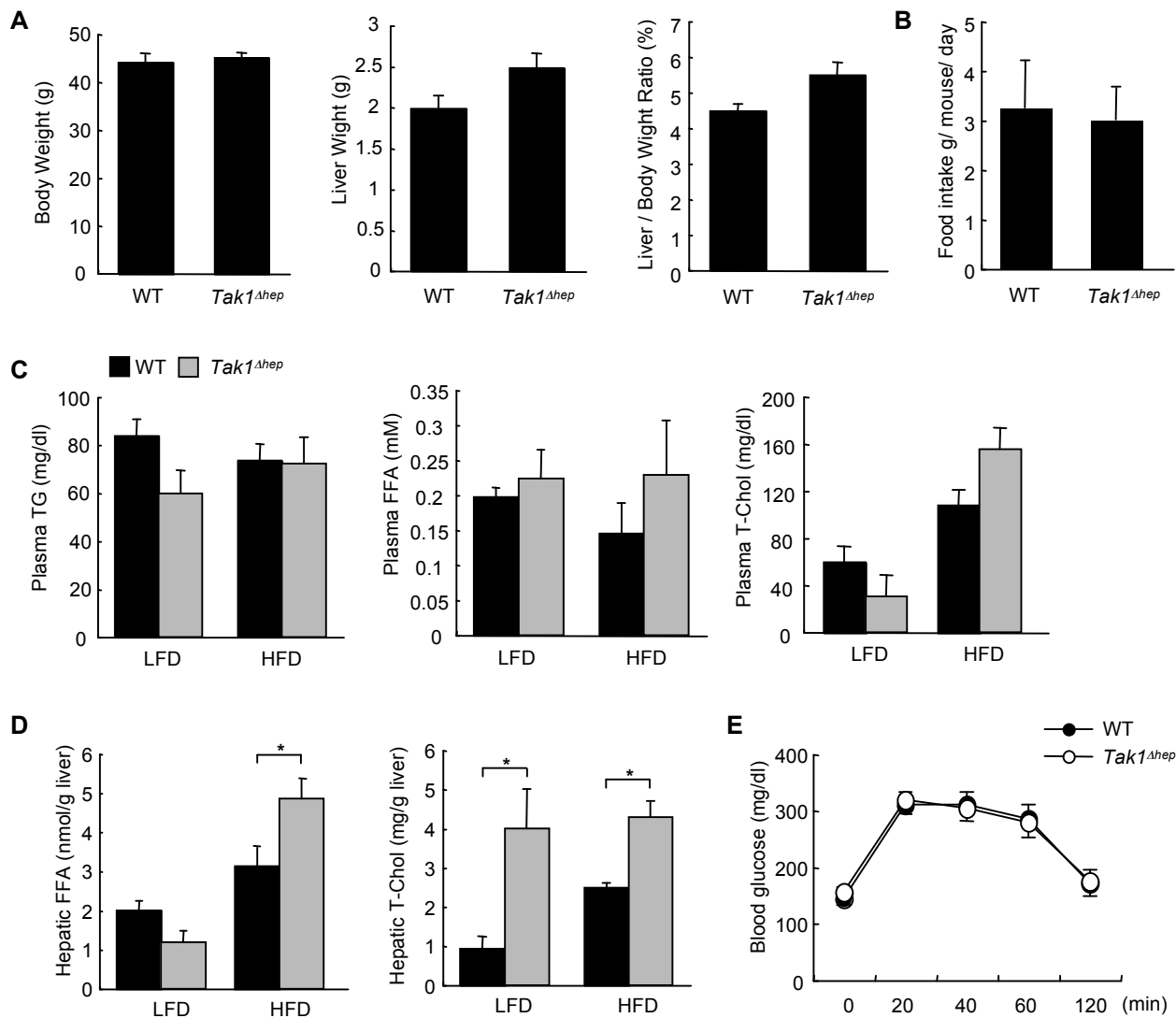
Supplemental Figure 6. Rapamycin-mediated lipid degradation depends on autophagy in *Tak1*^{-/-} hepatocytes.

Hepatocytes were isolated from WT and *Tak1*^{Δhep} mice and incubated with control siRNA and siRNA for *Atg5*. Subsequently, cells were treated with palmitate (250μM) with or without rapamycin (5μM) for 16 hours. Lipid accumulation was assessed by Oil-red O staining and its quantification. Data are presented as means ± S.E.M. *, p < 0.05, **, p < 0.01. Similar results were obtained in three independent experiments.



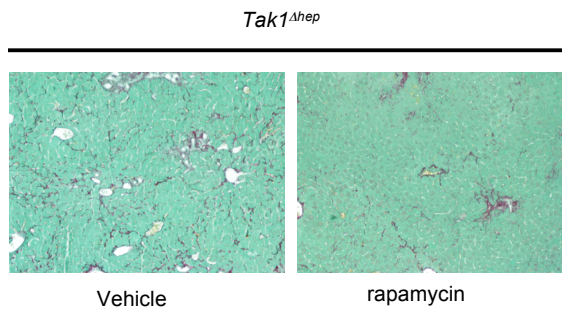
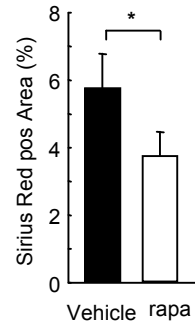
Supplemental Figure 7. Hepatic TAK1 expression in dietary and genetic obese mice.

1-month-old WT were fed low fat diet (LFD) and high fat diet (HFD) for additional 12 weeks (**A,B**) and 32 weeks (**C,D**) (n=8 each). 16 week-old *leptin*-deficient *ob/ob* mice and its WT counterparts (**E,F**) (n=6, each). (**A,C,E**) Hepatic mRNA expressions of *Tak1* were measured by quantitative real-time PCR. (**B,D,F**) Immunoblotting for TAK1, and its quantification. Data are presented as means $\pm$ S.E.M. *, p<0.05, **, p<0.01.



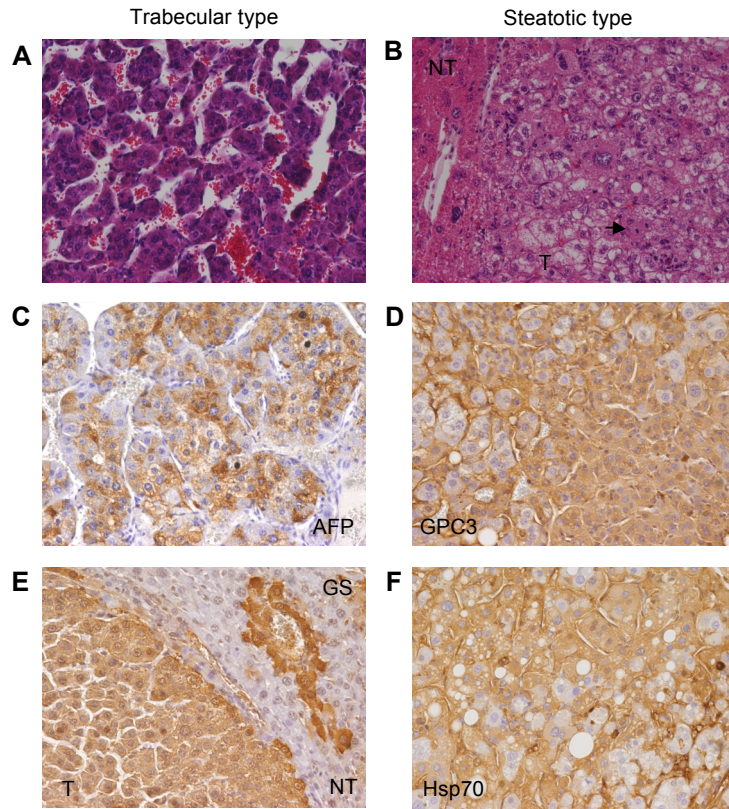
Supplemental Figure 8. Metabolic parameters of WT and *Tak1^{Δhep}* mice fed HFD for 12 weeks.

1-month-old WT and *Tak1^{Δhep}* mice were fed high fat diet (HFD) for additional 12 weeks (n=5, each). **(A)** Body weight, liver weight and liver-to-body weight ratio (n=5, each). **(B)** Food intake. **(C)** Plasma levels of triglyceride, free fatty acids and total cholesterol. **(D)** Hepatic content of free fatty acids and total cholesterol. Black bar; WT, Gray bar; *Tak1^{Δhep}* mice. **(E)** Glucose tolerance test (n=5, each). Closed circle; WT, Open circle; *Tak1^{Δhep}* mice. Data are presented as means $\pm$ S.E.M. *. $p < 0.05$.

A**B**

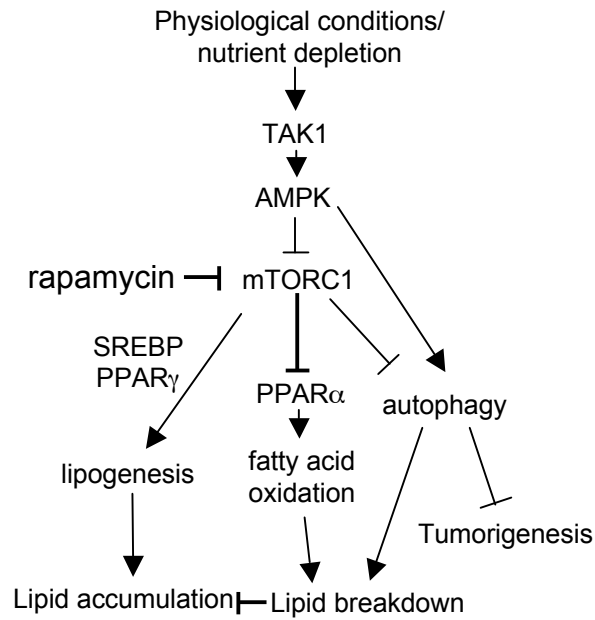
Supplemental Figure 9. Rapamycin inhibits spontaneous liver fibrosis in *Tak1^{Δhep}* mice.

Tak1^{Δhep} mice were treated with rapamycin (5mg/kg) twice weekly from 28 to 36 weeks of their age, and then the livers were harvested (n=12, each). Liver fibrosis was assessed by Sirius red staining. **(A)** Representative pictures are shown. **(B)** Quantification. Data are presented as means $\pm$ S.E.M. *; $p < 0.05$.



Supplemental Figure 10. HCC developed in *Tak1^{Δhep}* mice.

Liver tumors developed in one-year-old *Tak1^{Δhep}* mice were harvested and analyzed. **(A,B)** Representative H&E staining of tumor sections (**A**, trabecular type; **B**, steatotic type). Arrow, mitotic cells. **(C-F)** Immunohistochemical staining of liver tumors for **(C)** α -fetoprotein (AFP), **(D)** glypican 3 (GPC3), **(E)** glutamine synthetase (GS), and **(F)** heat shock protein 70 (Hsp70). T, tumor lesion; NT, non-tumor livers. Original magnification x200.



Supplemental Figure 11. TAK1-mediated autophagy and fatty acid oxidation prevent hepatic steatosis and tumorigenesis.

Under physiological conditions or by nutrient deprivation, AMPK is activated through TAK1. As a result, mTORC1 activity is inhibited, leading to autophagy induction and PPAR α -mediated fatty acid oxidation (FAO). This signaling induces lipid breakdown, thereby preventing lipid accumulation in hepatocytes. Autophagy is also induced directly by AMPK through ULK1. Autophagy prevents spontaneous tumorigenesis in the liver.