Supplemental Figure 1:

Parkin$^{-/-}$ mice are resistant to HFD-induced glucose intolerance and hepatic insulin resistance. (A) Blood glucose levels in response to a glucose challenge (GTT). (B) Blood glucose level in response to an insulin challenge (ITT) at the indicated times in the HFD group. All data are presented as means and SD. *p<0.05; **p<0.01 versus wildtype (n=4-6 per group).

Supplemental Figure 2:

Cholesterol accumulation was markedly increased in Parkin WT mice in response to HFD feeding. (A) Demonstration of no baseline difference in fat levels in Parkin WT and KO mice on the ND. Fat levels in liver sections were assayed by oil-red-O (left panel) and H&E staining (right panel). Representative images are shown with similar result from 3 independent mice. (B) Hepatic cholesterol levels. (C) Serum cholesterol and TG levels. (D) Fecal cholesterol content. **p<0.01 versus wildtype (n $\geq$ 5per group).

Supplemental Figure 3:

Kinetics of Bodipy-labeled dodecanoic acid uptake over 1,200 seconds after administration in HepG2 cells expressing Parkin (circle) compared to the control vector (square).

Supplemental Figure 4:

Parkin modulates fat and cholesterol regulatory proteins. (A) RNA expression level of CD36, Sr-B1, and FABP1 in the liver of Parkin$^{+/+}$ and Parkin$^{-/-}$ mice on a ND or HFD. Values represent the mRNA level relative to the 18S. All data are presented as means and SD. *$p<0.05$ versus ND group (n=4 per group). **(B) The higher molecular weight band recognized by the CD36 antibody is consistent with the monoubiquitination of CD36 as shown following a longer film exposure time of the input samples.** (**C**) Surface expression of CD36 in vector- and Parkin-transfected HepG2 cells by flow cytometry. Histogram shows the fluorescence intensity due to CD36 antibody labeling. (**D**) Parkin interacts with Sr-B1. Sr-B1-overexpressing HeLa cells were transfected with p3×Flag vector and p3×Flag-Parkin, respectively. After 48 hrs transfection, protein extracts were immunoprecipitated with anti-Flag M2 agarose and performed immunoblot analysis with Sr-B1 antibody. (**E**) Degradation of Sr-B1 protein following cycloheximide (CHX) administration in the presence or absence of Parkin overexpression.

Supplemental Figure 5:

(A) Demonstration of infection efficiency by the GFP control adenovirus (green in left panels) and by increased fat uptake as measured by Bodipy fluorescence (red) in all panels. The fat uptake levels are markedly induced in the mice following induction of CD36. (B) Representative flow cytometric evaluation of fat uptake measured by nile red in CD36$^{+/+}$ and CD36$^{-/-}$ primary hepatocytes infected with either the GFP or Parkin adenoviral particles. In parallel with the radiolabeled substrate uptake studies, Parkin increased oleic acid accumulation to a greater extent in the CD36 competent hepatocytes. Levels were increased to a lesser degree in CD36$^{-/-}$ primary hepatocytes. (C) Parkin and GFP expression by

immunoblot analysis showing adenoviral infection efficiency in mouse liver on the ND. □-Actin shows a loading control.

Supplemental Figure 6:

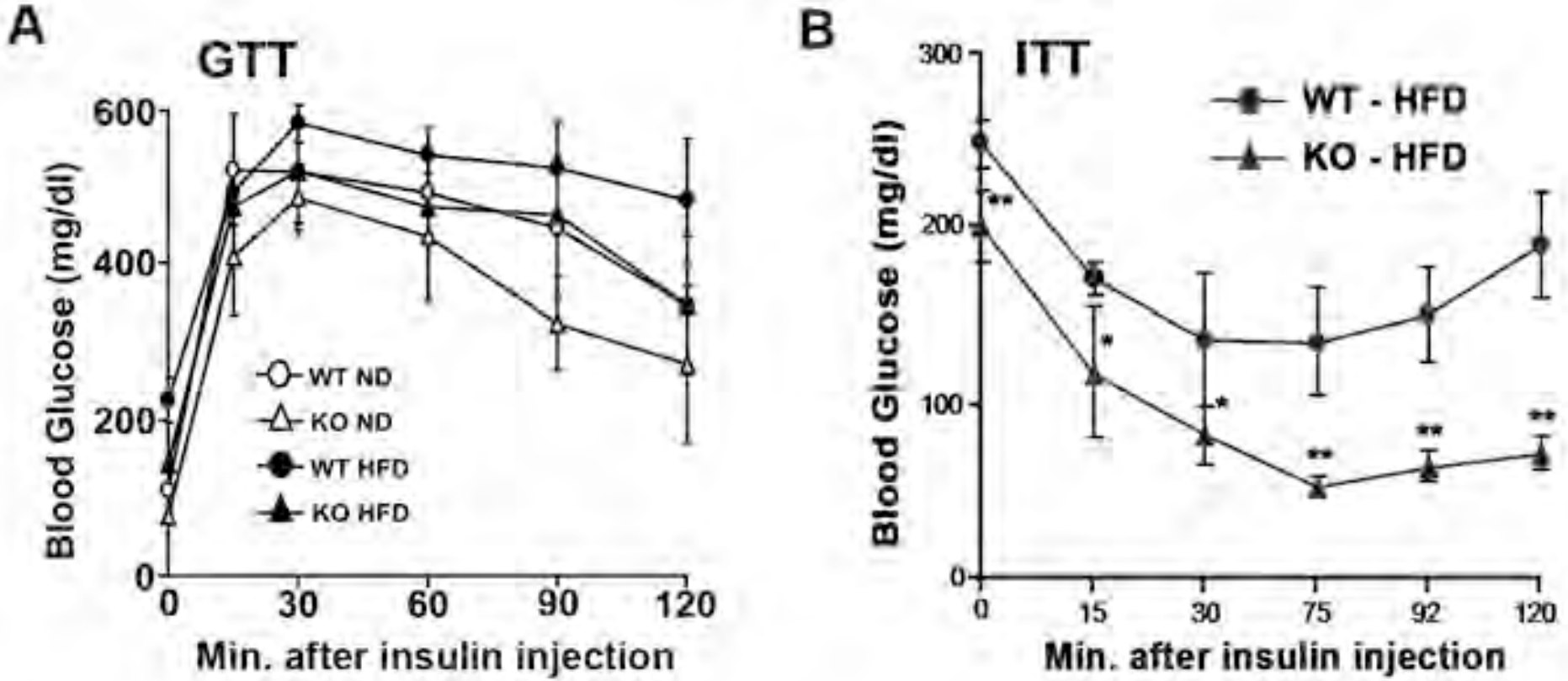
(A) Histogram showing lipid uptake in parkin MEF cells adipocytes on day 21 differentiation. The absorbance of oil-red-O staining in the cells was measured at 495 nm. Values were normalized to protein concentration are showed as means and SD. ** $p < 0.01$ vs. wildtype MEFs adipocytes. (B) Parkin expression by immunoblot analysis showing relative Parkin knockdown shRNA during 3T3-LI adipocytes differentiation. The shRNA construct #46 was least efficient in Parkin knockdown. The increased mitochondrial content with differentiation is shown by the inner membrane transport protein (Tim23). β-Actin levels reflect protein loading. (C) Light microscopy showing fat accumulation by Nile Red staining and by bright field microscopy on day 24 of adipocytes differentiation. Note the lipid accumulation in the shRNA #46 study was similar to control levels, reflecting the lack of Parkin knockdown.

Supplementary Table S1. Metabolic parameters of Parkin$^{+/+}$ and Parkin$^{-/-}$ mice fed with ND and HFD for 6.5 weeks

Mice	Parkin$^{+/+}$	Parkin$^{-/-}$	Parkin$^{+/+}$	Parkin$^{-/-}$
Diet	ND		HFD	
Body weight (g)	30.6 ± 0.8	27.4 ± 1.0	45.4 ± 1.4	31.8 ± 3.0**
Weight gain (g)	2.3 ± 0.9	2.2 ± 0.9	14.2 ± 0.7	4.2 ± 2.1**
Fat mass (g)	3.7 ± 0.4	3.8 ± 0.7	13.2 ± 1.0	6.1 ± 2.3**
Food intake (kcal/kg body weight/day)	514.9 ± 25.1	555.8 ± 36.6	330.8 ± 34.2	555.1 ± 43.5**
Water intake (g/day)	2.8 ± 0.2	2.6 ± 0.06	3.0 ± 0.4	3.0 ± 0.5
Feces (g/day)	0.5 ± 0.03	0.4 ± 0.06	0.3 ± 0.15	0.5 ± 0.06
Urine (g/day)	1.4 ± 0.2	1.5 ± 0.2	0.8 ± 0.2	1.1 ± 0.1
Serum glucose (Fasting: mg/dl)	112.5 ± 2	99.2 ± 19	252.3 ± 26	151.3 ± 31**
Serum insulin (Fasting: ng/ml)	0.19 ± 0.01	0.18 ± 0.02	0.43 ± 0.06	0.32 ± 0.06*
Serum cholesterol (mg/dl)	111.0 ± 7.1	108.0 ± 19.7	192.7 ± 6.4	71.4 ± 16.2**
Serum triglycerides (mg/dl)	106.0 ± 1.4	142.8 ± 20.6	78.0 ± 12.2	89.2 ± 15.4
Serum-free fatty acid (mmol/l)	0.49 ± 0.03	0.49 ± 0.02	0.51 ± 0.03	0.45 ± 0.04*
Oxygen consumption (VO_2, ml/kg body weight/hr)	3546 ± 497	3294 ± 388	2685 ± 169	3427 ± 257**
Activity (Count/day)	3149 ± 571	2491 ± 96	2315 ± 102	2886 ± 150**

All measurements were carried out with 12-week-old mice (n>5). All data are presented as means ± SD. *p<0.05; **p<0.01 *versus* wildtype.

Supplemental Figure 1



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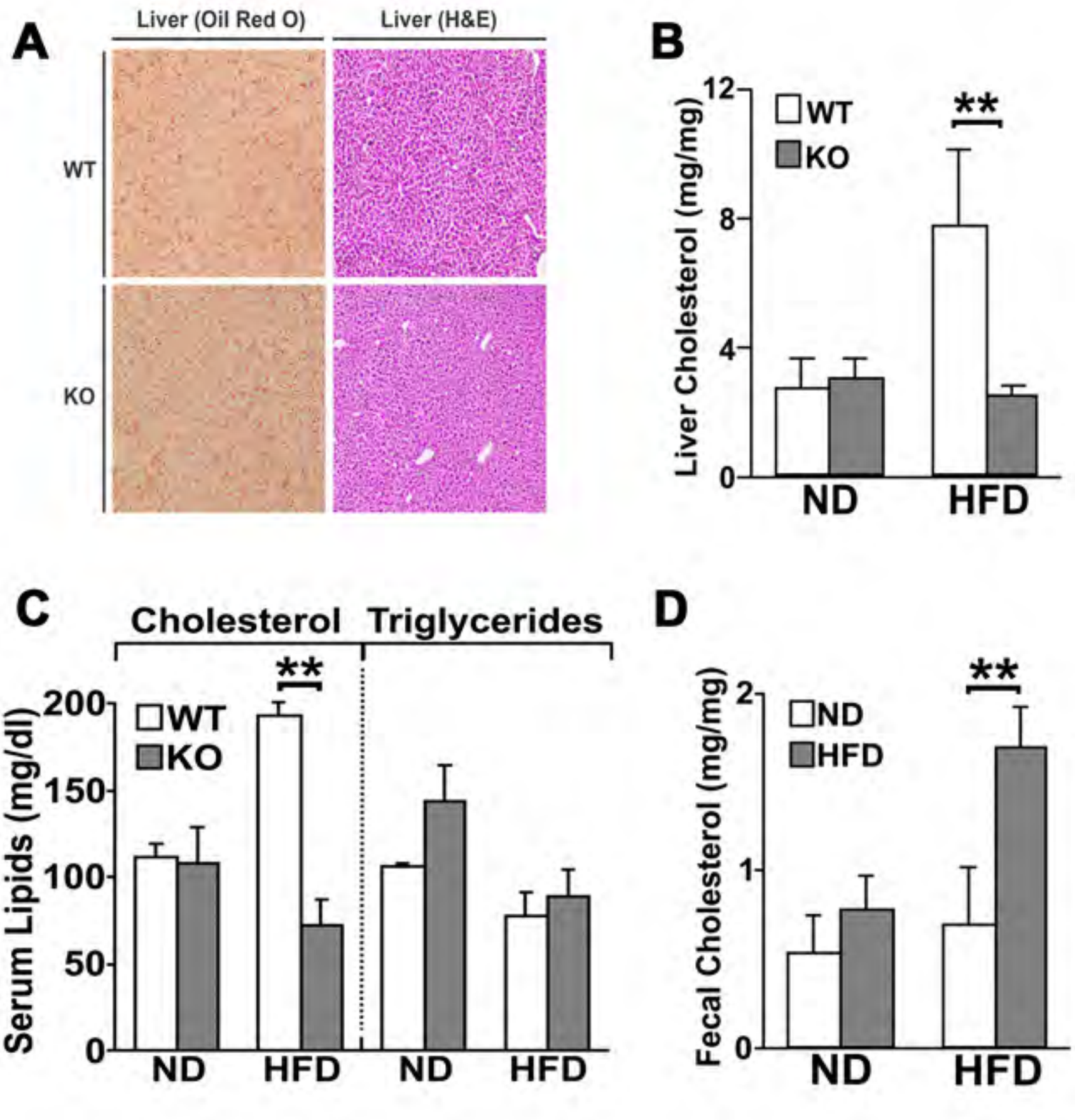

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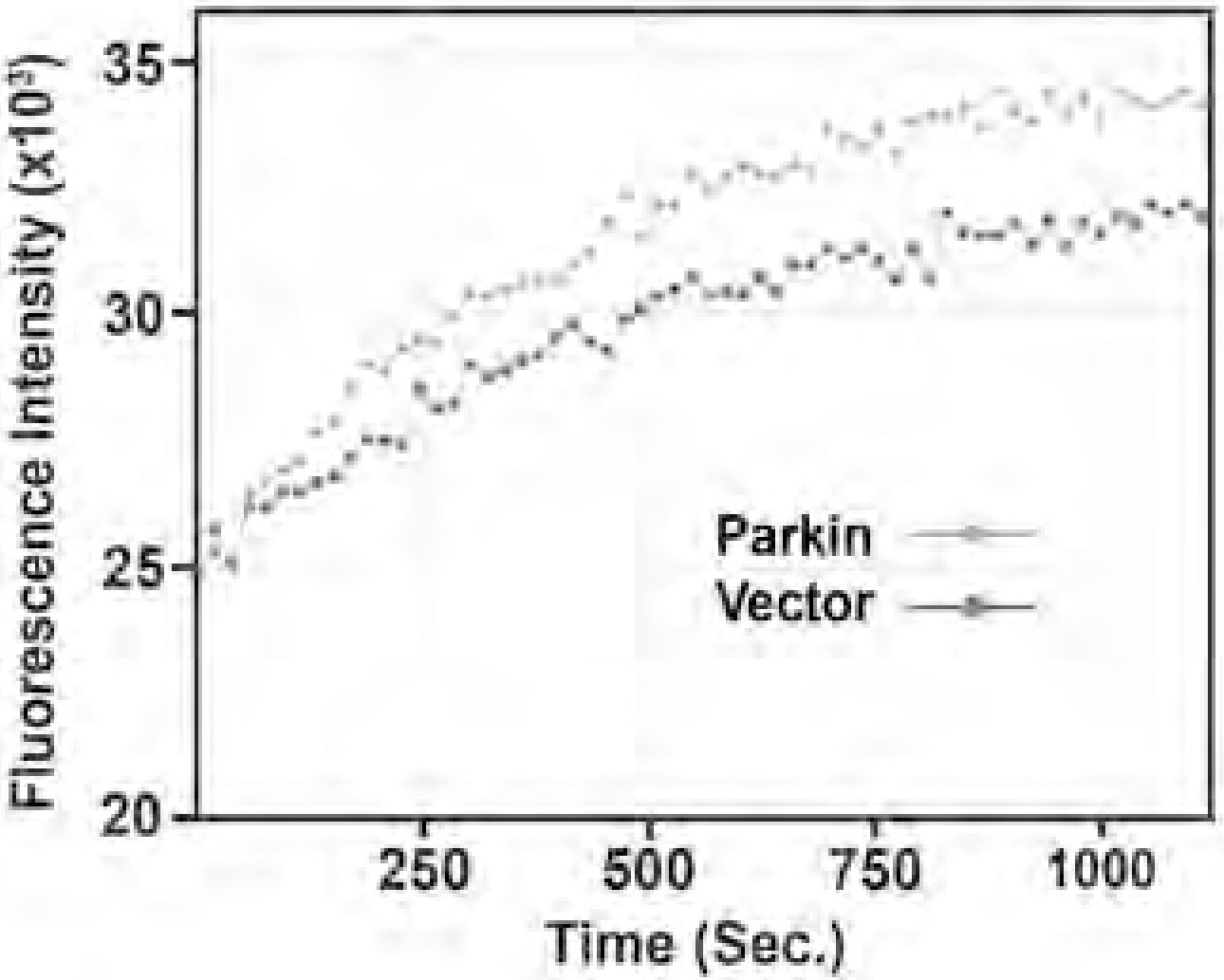

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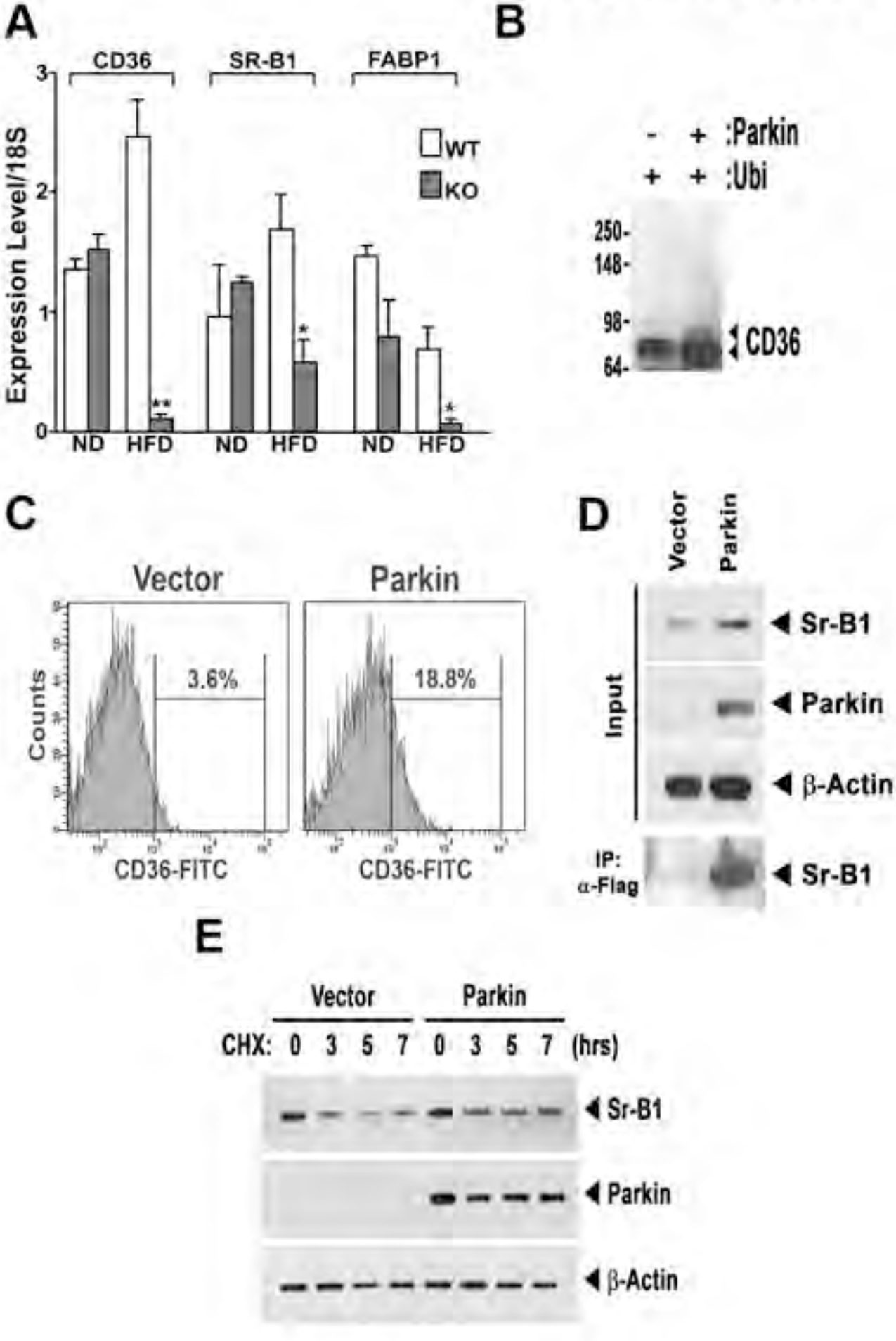
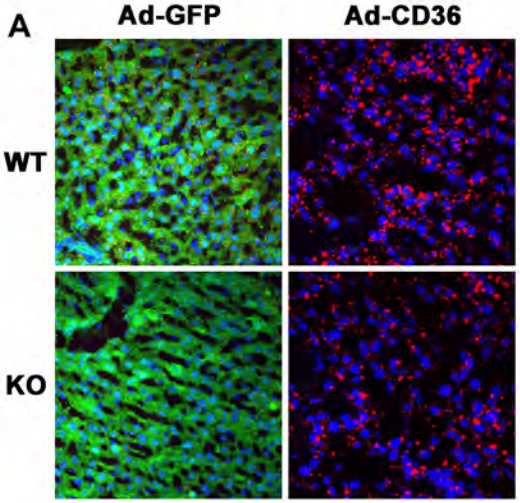
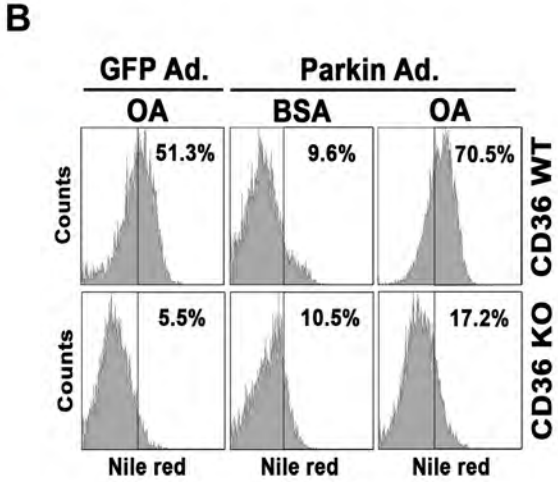
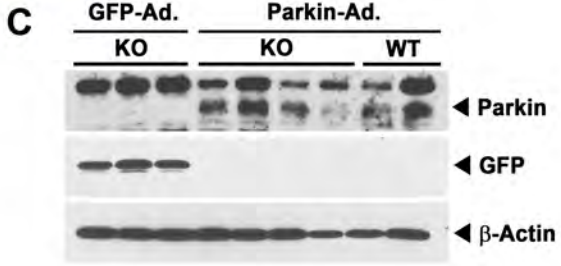
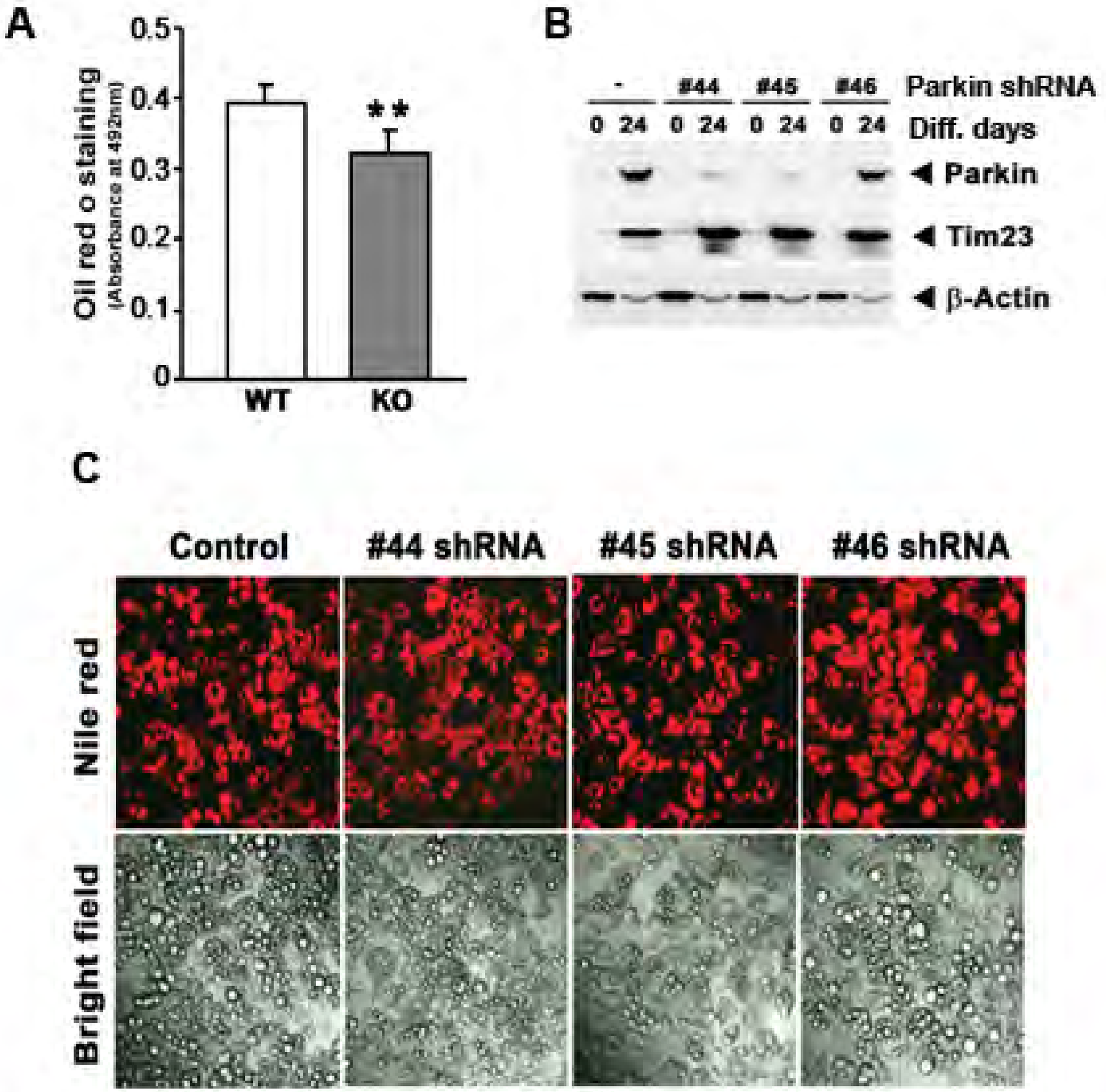








Supplemental Figure 5



Supplemental Figure 6