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Figures and figure supplements

Adipocyte ALK7 links nutrient overload to catecholamine resistance in obesity

Tingqing Guo, et al.

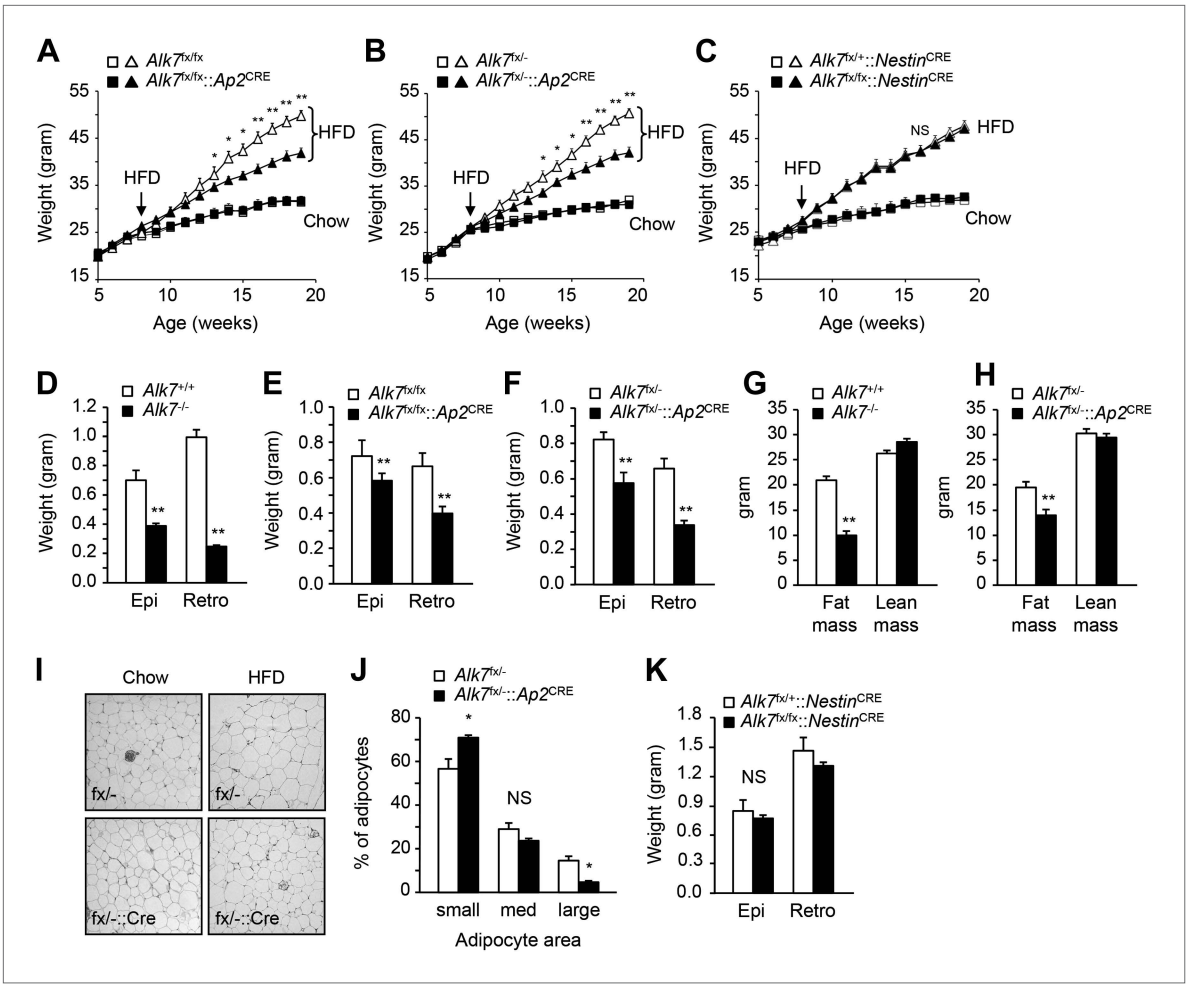

Figure 1. Conditional deletion of ALK7 in adipose tissue attenuates weight gain and fat deposition under a high fat diet. (**A**–**C**) Weight gain under chow (squares) and a high fat diet (HFD, triangles) in fat-specific *Alk7*$^{fx/fx}$::*Ap2*^{CRE} (**A**) and *Alk7*$^{fx/-}$::*Ap2*^{CRE} (**B**), and brain-specific *Alk7*$^{fx/fx}$::*Nestin*^{CRE} (**C**) knock-out mice (solid symbols) compared to control mice (open symbols). Arrows denote the first week of HFD. N = 8 mice per group in all cases, except N = 6 in (**C**) on a chow diet. (**D**–**F**) Weights of epididymal (Epi) and retroperitoneal (Retro) fat depots in global *Alk7*$^{-/-}$ (**D**) and fat-specific *Alk7*$^{fx/fx}$::*Ap2*^{CRE} (**E**) and *Alk7*$^{fx/-}$::*Ap2*^{CRE} (**F**) knock-out mice after 16 weeks on HFD. N = 6 mice per group. (**G** and **H**) Fat and lean mass assessed by magnetic resonance imaging (MRI) in global *Alk7*$^{-/-}$ (**G**) and fat-specific *Alk7*$^{fx/-}$::*Ap2*^{CRE} (**H**) knock-out mice after 16 weeks on HFD. N = 8 mice per group in (**G**), N = 5 in (**H**). (**I** and **J**) Adipocyte cell size in fat-specific *Alk7*$^{fx/-}$::*Ap2*^{CRE} knock-out mice and *Alk7*$^{fx/-}$ controls after chow or HFD as visualized by hematoxylin-eosin staining in tissue sections of epididymal adipose tissue (**I**). Quantitative analysis is shown in (**J**). Small, 400–5000 μm^2; Med, 5000–10,000 μm^2; Large, 10,000–60,000 μm^2. N = 4 mice per group (four sections per mouse). (**K**) Weights of epididymal (Epi) and retroperitoneal (Retro) fat depots in brain-specific *Alk7*$^{fx/fx}$::*Nestin*^{CRE} knock-out mice after 16 weeks on HFD. N = 6 mice per group. *p < 0.05; **p < 0.01; NS, non-significant (mutant vs control). All error bars show mean ± SEM.
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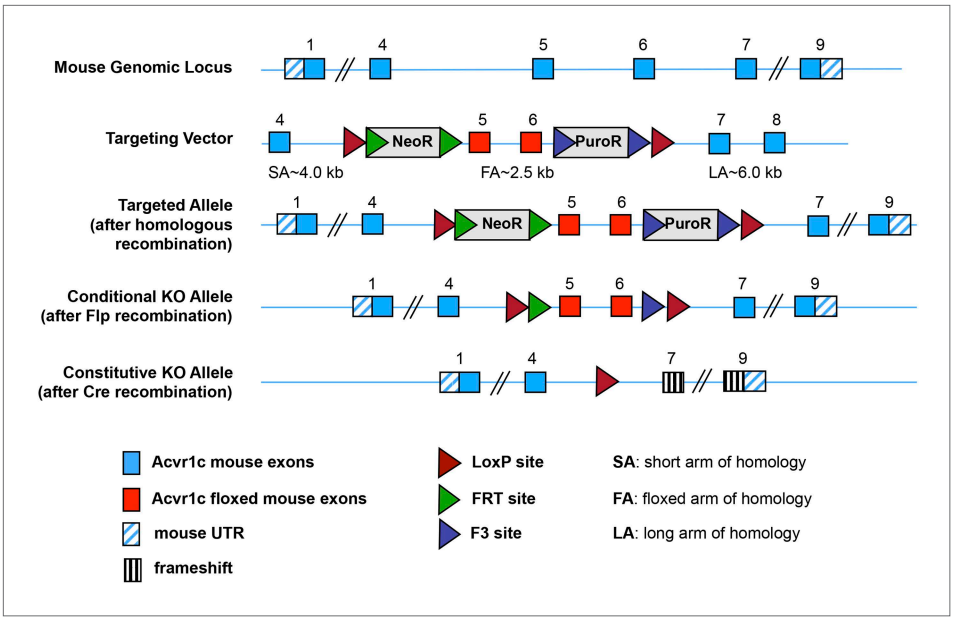

Figure 1—figure supplement 1. Generation of a conditional allele of the mouse *Acvr1c* gene encoding ALK7.

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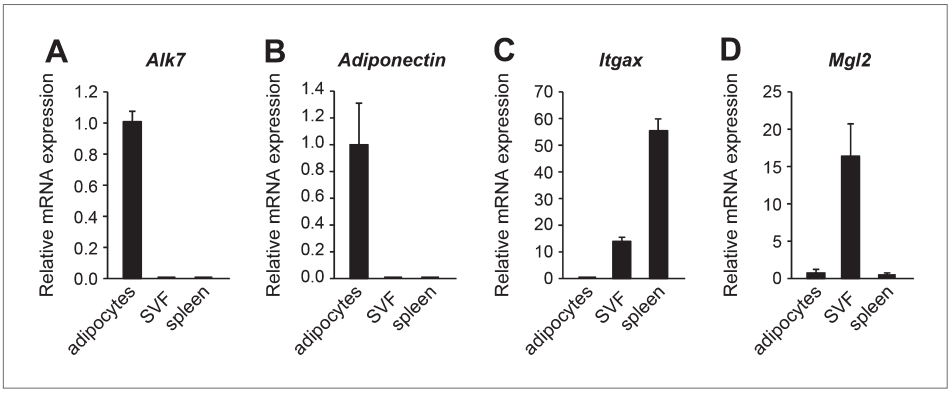

Figure 1—figure supplement 2. *Alk7* expression in adipocytes, but not in adipose tissue macrophages.

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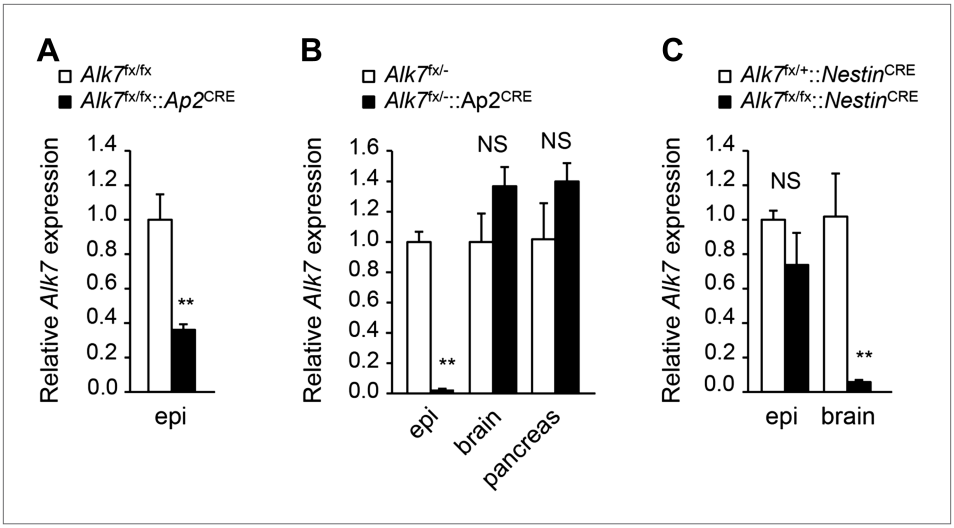

Figure 1—figure supplement 3. *Alk7* expression in conditional knock-out mice.

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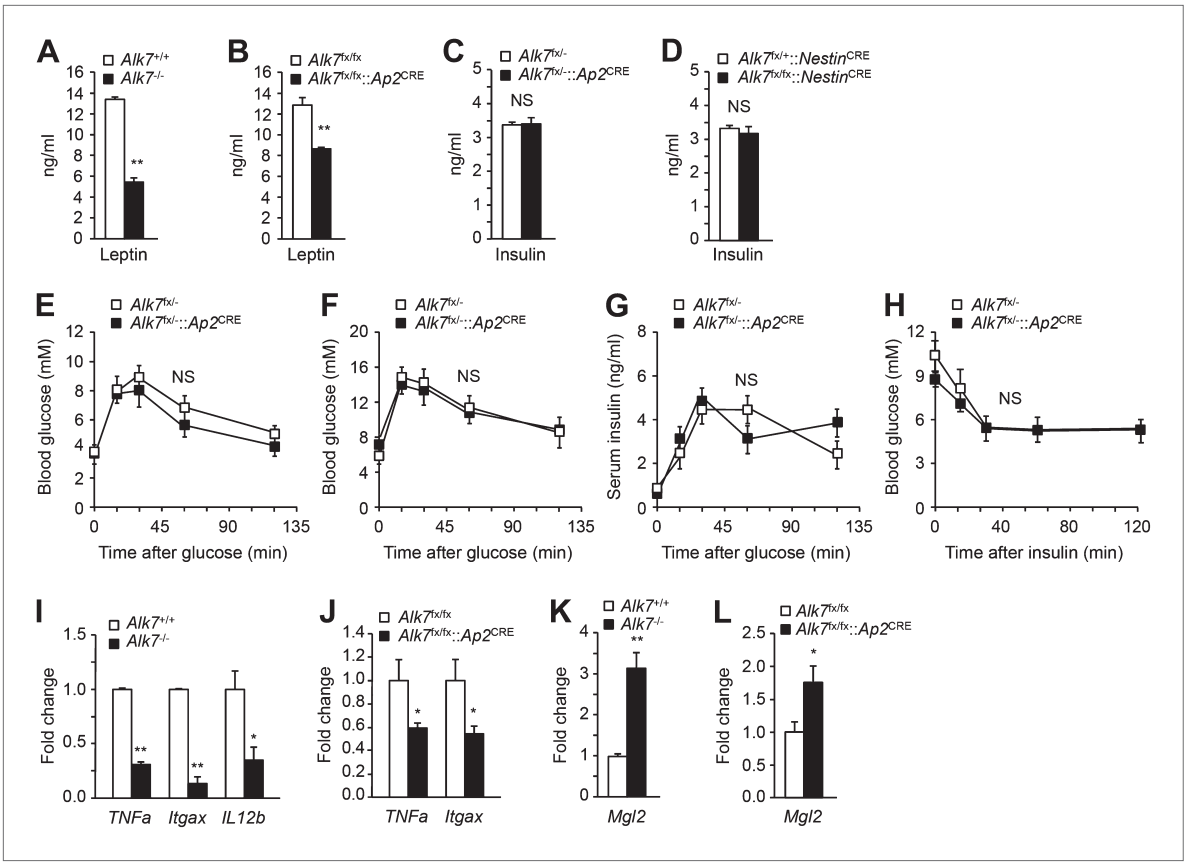

Figure 2. Reduced serum leptin levels and adipose tissue inflammation but normal glucose and insulin metabolism in global and fat-specific *Alk7* knock-out mice. (**A** and **B**) Serum levels of leptin in global *Alk7*$^{-/-}$ (**A**) and fat-specific *Alk7*$^{fx/fx}$::*Ap2*^{CRE} (**B**) knock-out mice after 16 weeks on a high fat diet (HFD). N = 6 mice per group. (**C** and **D**) Serum levels of insulin in fat-specific *Alk7*$^{fx/-}$::*Ap2*^{CRE} (**C**) and brain-specific *Alk7*$^{fx/fx}$::*Nestin*^{CRE} (**D**) knock-out mice after 16 weeks on HFD. N = 6 mice per group. (**E** and **F**) Blood glucose levels after glucose injection in 4-month-old *Alk7*$^{fx/-}$::*Ap2*^{CRE} mice and controls after chow (**E**) or a high fat diet (**F**). Mice had been starved overnight prior to glucose injection. N = 5 mice per group. (**G**) Serum insulin levels after glucose injection in 4-month-old *Alk7*$^{fx/-}$::*Ap2*^{CRE} mice and controls kept on a chow diet. Mice had been starved overnight prior to glucose injection. N = 5 mice per group. (**H**) Blood glucose levels after insulin injection in 4-month-old *Alk7*$^{fx/-}$::*Ap2*^{CRE} mice and controls kept on a chow diet. Mice had been starved for 3 hr prior to insulin injection. N = 5 mice per group. (**I**–**L**) Relative mRNA expression levels of M1 macrophage markers *TNF1a*, *Itgax*, and *IL12b* (**I** and **J**) and the M2 macrophage marker *Mgl2* (**K** and **L**) assessed by quantitative PCR in epididymal adipose tissue of global *Alk7*$^{-/-}$ (**I** and **K**) and fat-specific *Alk7*$^{fx/fx}$::*Ap2*^{CRE} (**J** and **L**) knock-out mice after 16 weeks on HFD. N = 6 mice per group. *p < 0.05; **p < 0.01; NS, non-significant (mutant vs control). All error bars show mean ± SEM.

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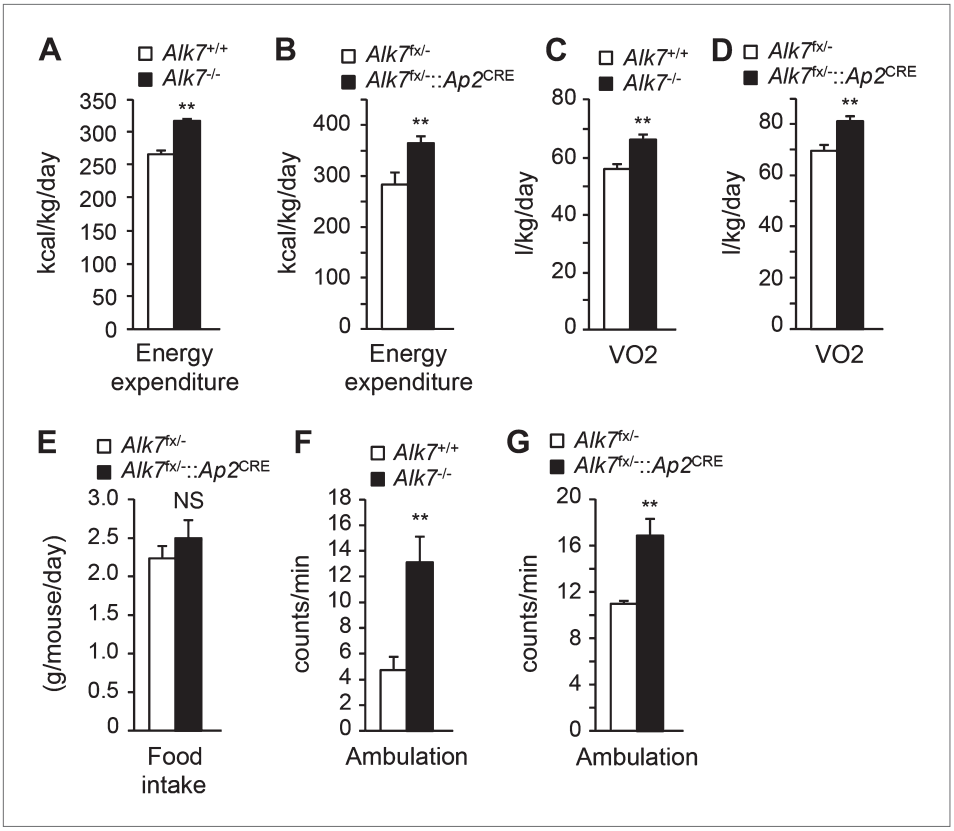

Figure 3. Increased energy expenditure and oxygen consumption in global and fat-specific *Alk7* knock-out mice on a high fat diet. (**A** and **B**) Energy expenditure assessed by calorimetric measurements in global *Alk7*$^{-/-}$ (**A**) and fat-specific *Alk7*$^{fx/-}$::*Ap2*^{CRE} (**B**) knock-out mice after 16 weeks on a high fat diet (HFD). N = 8 mice per group in (**A**), N = 6 in (**B**). (**C** and **D**) Oxygen consumption assessed by calorimetric measurements in global *Alk7*$^{-/-}$ (**C**) and fat-specific *Alk7*$^{fx/-}$::*Ap2*^{CRE} (**D**) knock-out mice after 16 weeks on HFD. N = 8 mice per group in (**C**), N = 6 in (**D**). (**E**) Daily food intake in fat-specific *Alk7*$^{fx/-}$::*Ap2*^{CRE} knock-out mice during 16 weeks on HFD. N = 6 mice per group. (**F** and **G**) Physical activity assessed as ambulation in global *Alk7*$^{-/-}$ (**F**) and fat-specific *Alk7*$^{fx/-}$::*Ap2*^{CRE} (**G**) knock-out mice after 16 weeks on HFD. N = 8 mice per group in (**F**), N = 6 in (**G**). *p < 0.05; **p < 0.01; NS, non-significant (mutant vs control). All error bars show mean ± SEM.

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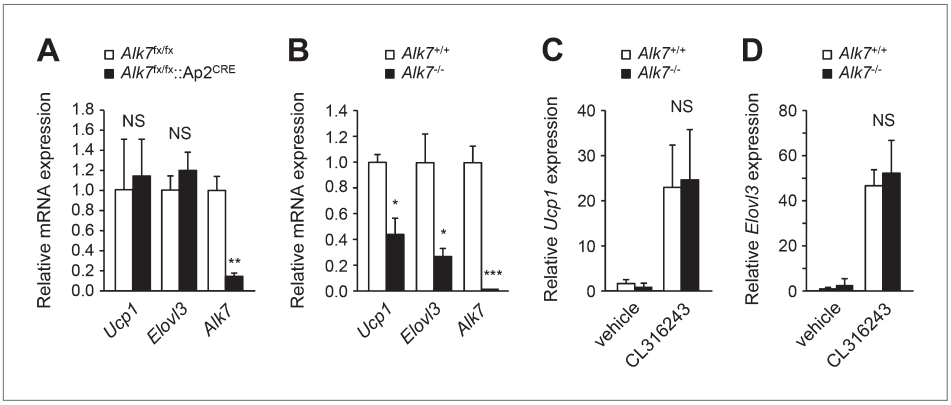

Figure 3—figure supplement 1. *Alk7* deletion does not result in enhanced 'browning' of subcutanous adipose tissue.

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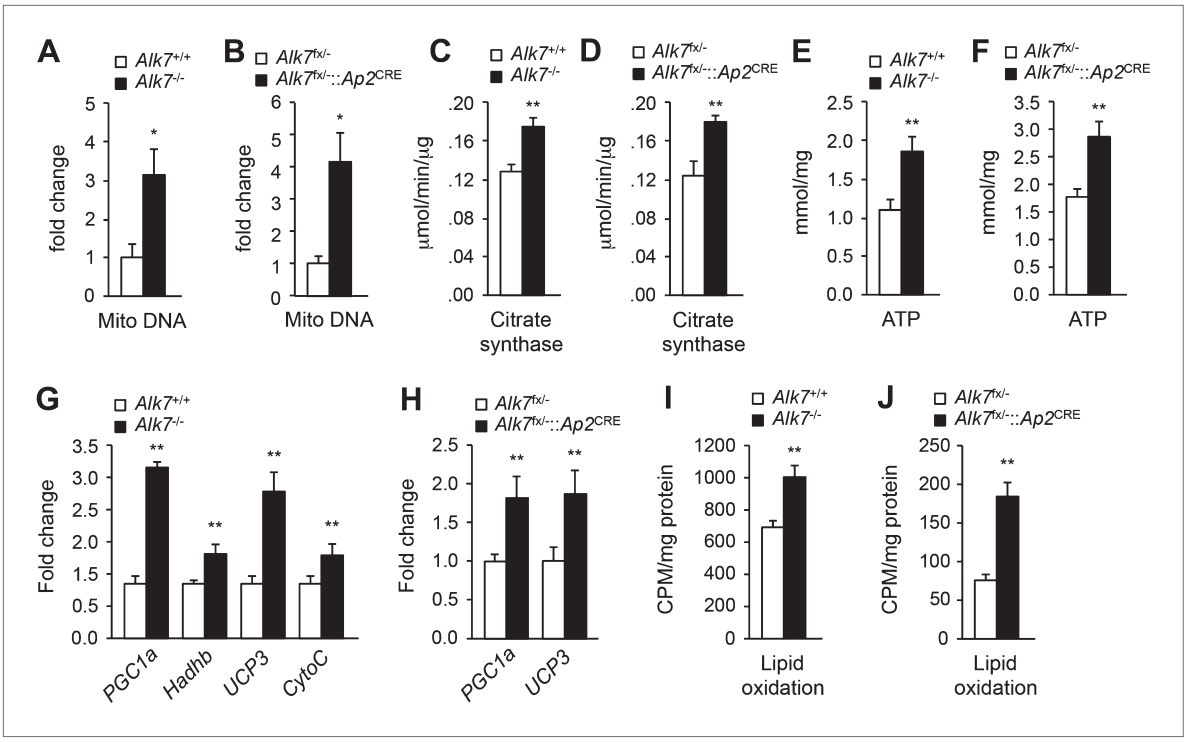

Figure 4. Elevated adipose tissue mitochondrial biogenesis and activity in fat-specific *Alk7* knock-out mice on a high fat diet. (**A**–**F**) Mitochondrial biogenesis assessed by measurements of mitochondrial (mito) DNA content (**A** and **B**), citrate synthase activity (**C** and **D**), and ATP content (**E** and **F**) in epididymal adipose tissue of global *Alk7*$^{-/-}$ (**A**, **C**, **E**) and fat-specific *Alk7*$^{fx/-}$::*Ap2*^{CRE} (**B**, **D**, **F**) knock-out mice after 16 weeks on a high fat diet (HFD). N = 8 mice per group in all cases. (**G** and **H**) Relative mRNA expression of markers of mitochondrial biogenesis and function *PGC1a*, *Hadhb*, *UCP3*, and *cytochrome C* assessed by quantitative PCR (Q-PCR) in epididymal adipose tissue of global *Alk7*$^{-/-}$ (**G**) and fat-specific *Alk7*$^{fx/-}$::*Ap2*^{CRE} (**H**) knock-out mice after 16 weeks on HFD. N = 6 mice per group in all cases. (**I** and **J**) Lipid oxidation in epididymal adipose tissue of global *Alk7*$^{-/-}$ (**I**) and fat-specific *Alk7*$^{fx/-}$::*Ap2*^{CRE} (**J**) knock-out mice. N = 3 mice per group in all cases. *p < 0.05; **p < 0.01; NS, non-significant (mutant vs control). All error bars show mean ± SEM.

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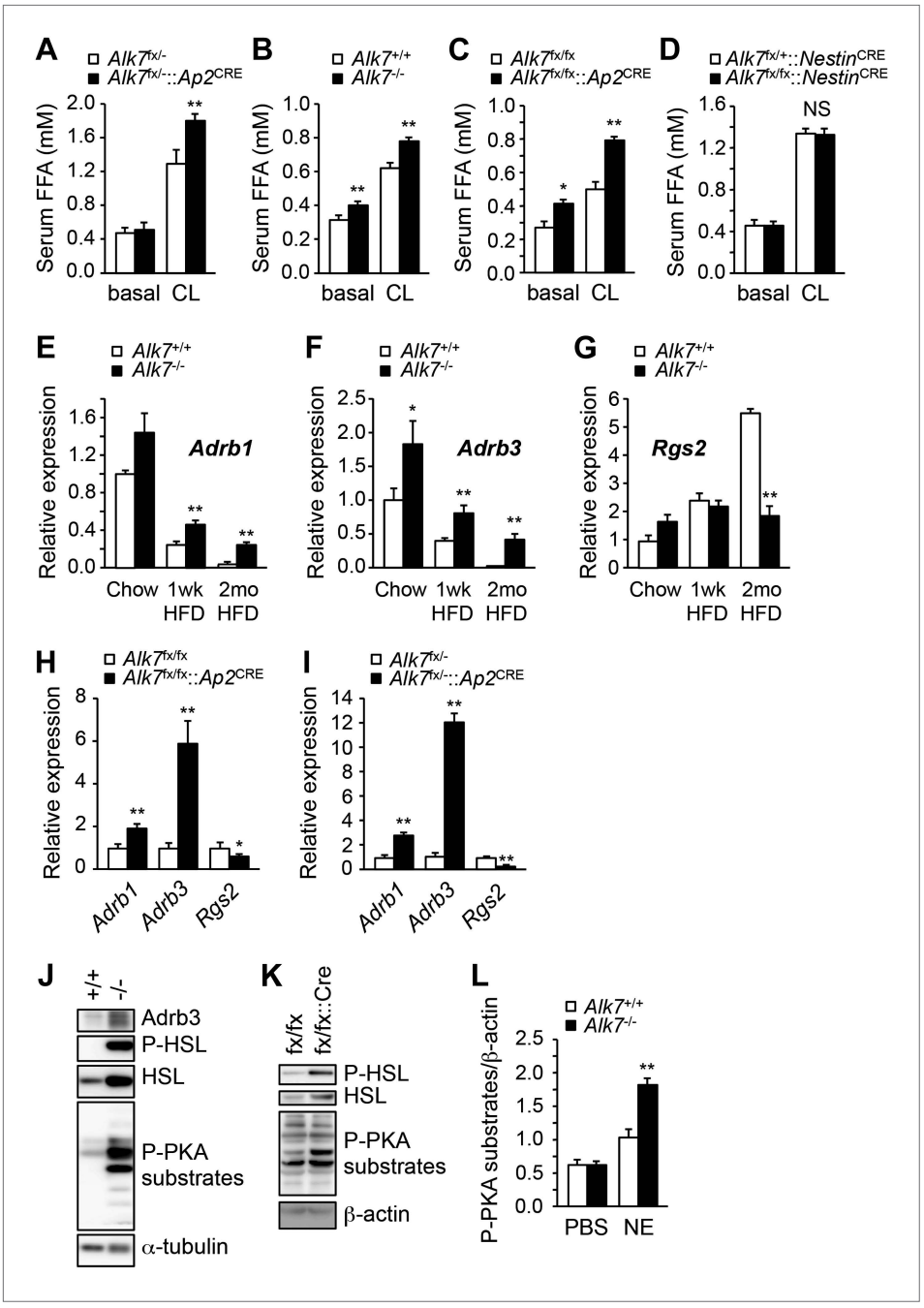

Figure 5. Enhanced catecholamine sensitivity and β-adrenergic signaling in adipose tissue of global and fat-specific *Alk7* knock-out mice on a high-fat diet. (**A**) Basal and CL316243 (CL)-stimulated serum free fatty acids (FFA) in fat-specific *Alk7*$^{fx/-}$::*Ap2*^{CRE} knock-out mice on a chow diet. N = 6 mice per group. (**B**–**D**) Basal and CL316243-stimulated lipolysis in global *Alk7*$^{-/-}$ (**B**), fat-specific *Alk7*$^{fx/fx}$::*Ap2*^{CRE} (**C**), and brain-specific *Alk7*$^{fx/fx}$::*Nestin*^{CRE} (**D**) knock-out mice after 16 weeks on a high fat diet (HFD). N = 6 mice per group. (**E**–**G**) Relative mRNA expression of *Adrb1* (**E**), *Adrb3* (**F**), and *Rgs2* (**G**) assessed by Q-PCR in epididymal adipose tissue of global *Alk7*$^{-/-}$ knock-out mice on chow and after 1 week (wk) or 2 months (mo) on HFD. N = 6 mice per group. (**H** and **I**) Relative mRNA expression of *Adrb1*, *Adrb3*, and *Rgs2* assessed by Q-PCR in epididymal adipose tissue of fat-specific *Alk7*$^{fx/fx}$::*Ap2*^{CRE} (**H**) and *Alk7*$^{fx/-}$::*Ap2*^{CRE} (**I**) knock-out mice after 16 weeks on HFD. N = 6 mice per group. (**J** and **K**) Levels of Adrb3, phospho-HSL, total HSL, and phosphorylated PKA substrates assessed by Western blotting in epididymal adipose tissue of global *Alk7*$^{-/-}$ (**J**) and fat-specific *Alk7*$^{fx/fx}$::*Ap2*^{CRE} (**K**) knock-out mice after 16 weeks on HFD. The results shown are representative of four biological replicates. (**L**) Levels of phosphorylated PKA substrates were assessed by Western

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Figure 5. Continued

blotting in epididymal adipose tissue from 2-month-old global *Alk7*$^{-/-}$ knock-out mice on a chow diet following injection of norepinephrine (NE) or vehicle (PBS). Results show the levels of phosphorylated PKA substrates quantified by image analysis after normalization to β-actin. N = 3 mice per group. *p < 0.05; **p < 0.01; NS, non-significant (mutant vs control). All error bars show mean ± SEM.

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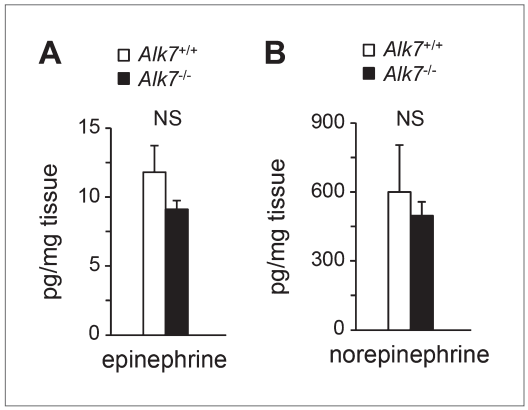

Figure 5—figure supplement 1. Epinephrine and norepinephrine levels in adipose tissue of *Alk7* knock-out mice after 16 weeks on a high fat diet.

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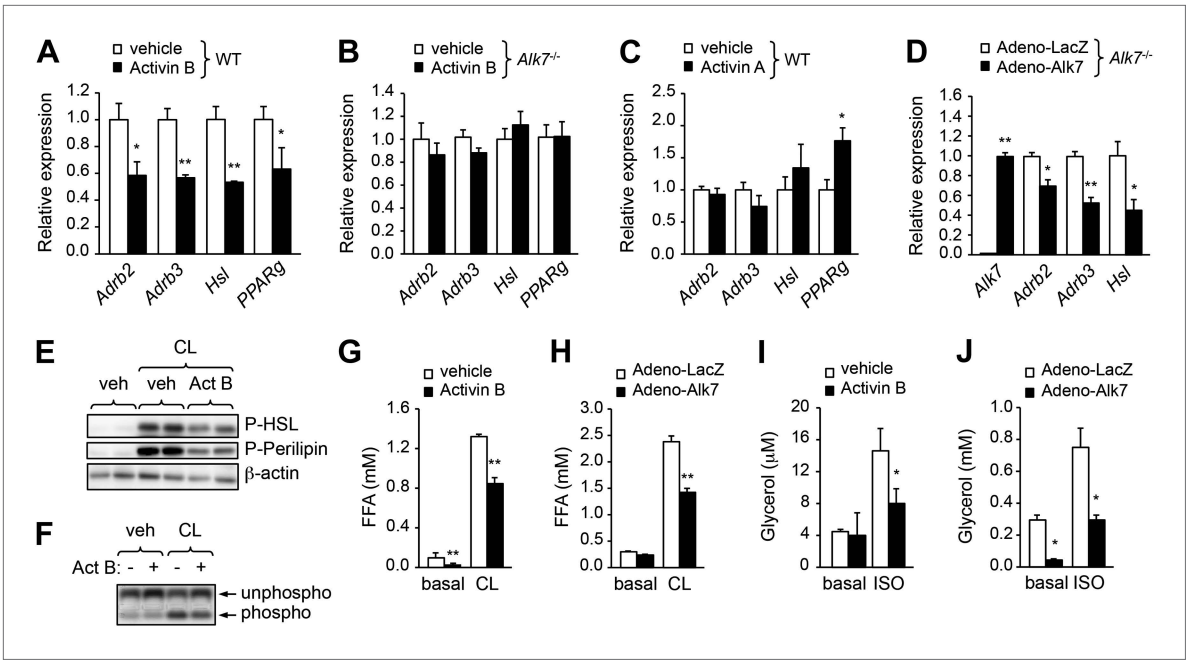

Figure 6. ALK7 signaling negatively regulates catecholamine sensitivity and β-adrenergic signaling in mouse and human adipocytes. (**A**) Relative mRNA expression of *Adrb2, Adrb3, Hsl*, and *PPARg* assessed by Q-PCR in adipocytes derived from wild type (WT) mouse embryonic fibroblasts (MEFs) following stimulation with activin B. N = 4 wells per condition. (**B**) Relative mRNA expression of *Adrb2, Adbr3, PPARg*, and *Hsl* assessed by Q-PCR in adipocytes derived from *Alk7* knock-out MEFs after stimulation with activin B. N = 4 wells per condition. (**C**) Relative mRNA expression of *Adrb2, Adbr3, PPARg*, and *Hsl* assessed by Q-PCR in adipocytes derived from WT MEFs following stimulation with activin A. N = 4 wells per condition. (**D**) Relative mRNA expression of *Alk7, Adrb2, Adrb3*, and *Hsl* assessed by Q-PCR in adipocytes derived from WT MEFs following adenovirus-mediated ALK7 overexpression. N = 4 wells per condition. (**E**) Levels of phospho-HSL and phospho-perilipin assessed by Western blotting in MEF-derived adipocytes after stimulation with β_3-AR-specific agonist CL316243 (CL) or vehicle (veh) in the presence or absence of activin B (Act B). Independent biological duplicates are shown. (**F**) Assay of PKA activity in lysates of MEF-derived adipocytes after stimulation with β_3-AR-specific agonist CL316243 (CL) or vehicle (veh) in the presence or absence of activin B (Act B). Independent biological duplicates are shown. (**G** and **H**) Basal and CL316243-stimulated lipolysis in MEF-derived adipocytes following stimulation with activin B (**G**) or adenovirus-mediated ALK7 overexpression (**H**). FFA: free fatty acids. N = 4 wells per condition. (**I** and **J**) Basal and isoproterenol (ISO)-stimulated lipolysis in human adipocytes following stimulation with activin B (**I**) or adenovirus-mediated ALK7 overexpression (**J**). N = 4 wells per condition. *p < 0.05; **p < 0.01; NS, non-significant (activin treatment vs vehicle or Adeno-Alk7 vs Adeno-LacZ, as indicated). All error bars show mean ± SEM.

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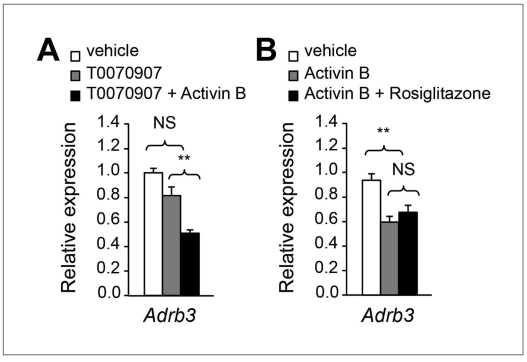

Figure 6—figure supplement 1. The effects of activin B on adipocyte *Adrb* mRNA expression are independent of PPARγ.

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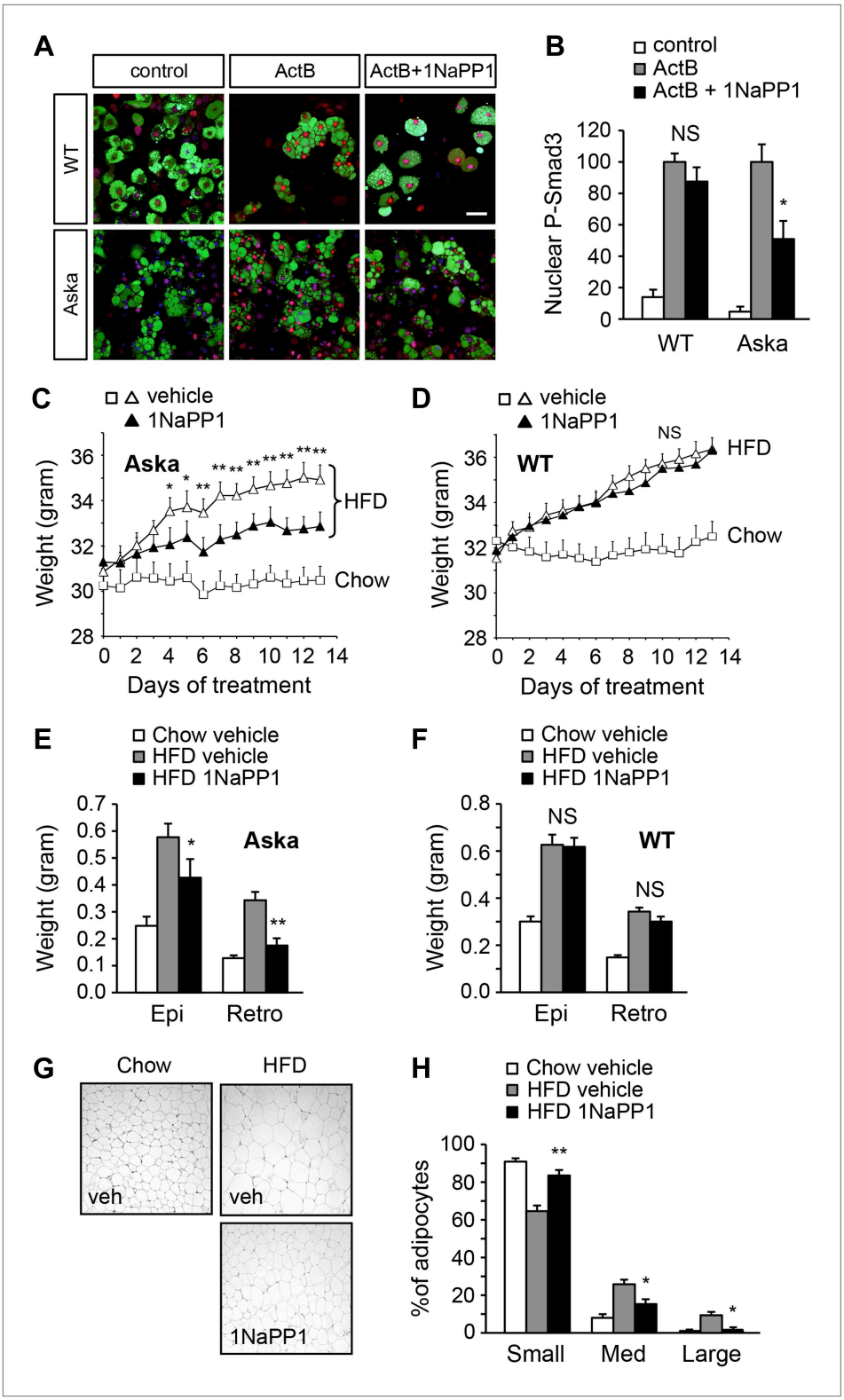

Figure 7. Acute inhibition of ALK7 signaling in adult mice through a chemical-genetic approach reduces diet-induced weight gain and fat accumulation. (**A** and **B**) Activin B signaling was assessed by p-Smad3 nuclear translocation in mouse embryonic fibroblast (MEF)-derived adipocytes from wild type (WT) or *Alk7*^{ASKA} (Aska) mice in the presence and absence of ATP competitive inhibitor 1NaPP1. Nuclear p-Smad3 (red) was specifically evaluated by

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immunohistochemistry in adipocytes, identified by BODIPY 493/503 staining (green). Representative photomicrographs are shown in (**A**). Scale bar, 50 μm. Quantitative analysis is shown in (**B**). N = 3 independent experiments each performed in triplicate. (**C** and **D**) Weight gain on chow (squares) and a high fat diet (HFD, triangles) in *Alk7*^{ASKA} (**C**) and wild type (**D**) mice treated with 1NaPP1 (solid triangles) or vehicle (open squares and triangles). N = 6 mice per group in (**C**), N = 9 in (**D**). (**E** and **F**) Weights of epididymal (Epi) and retroperitoneal (Retro) fat depots in *Alk7*^{ASKA} (**E**) and wild type (**F**) mice after chow (open bars) or 2 weeks on HFD treated with 1NaPP1 (black bars) or vehicle (gray bars). N = 6 mice per group in (**E**), N = 7 in (**F**). (**G** and **H**) Adipocyte cell size in *Alk7*^{ASKA} mice after chow or HFD as visualized by hematoxylin-eosin staining in tissue sections of epididymal adipose tissue of *Alk7*^{ASKA} mice after chow or 2 weeks on HFD treated with 1NaPP1 or vehicle (veh) (**G**). Quantitative analysis is shown in (**H**). Small, 400–5000 μm^2; Med, 5000–10,000 μm^2; Large, 10,000–20,000 μm^2. N = 4 mice per group (four sections per mouse). *p < 0.05; **p < 0.01; NS, non-significant (1NaPP1 vs vehicle). All error bars show mean ± SEM.

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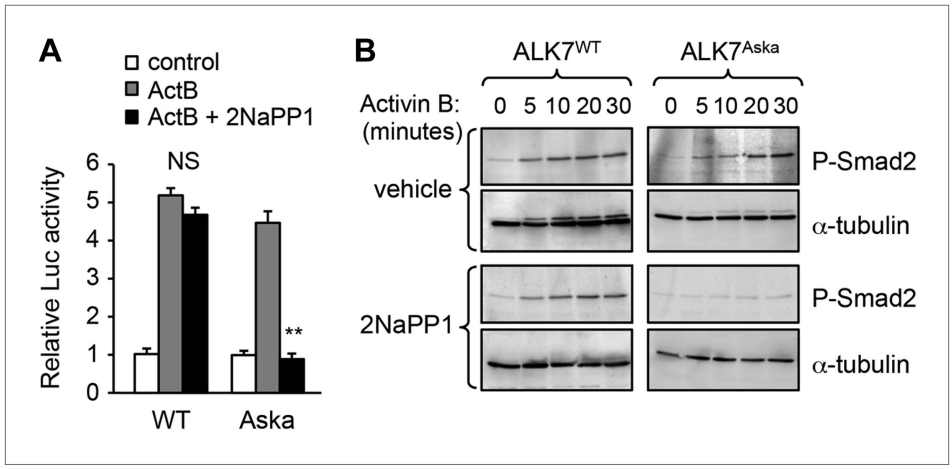

Figure 7—figure supplement 1. Validation of *Alk7*^{ASKA} allele in transfected R4-2 cells.

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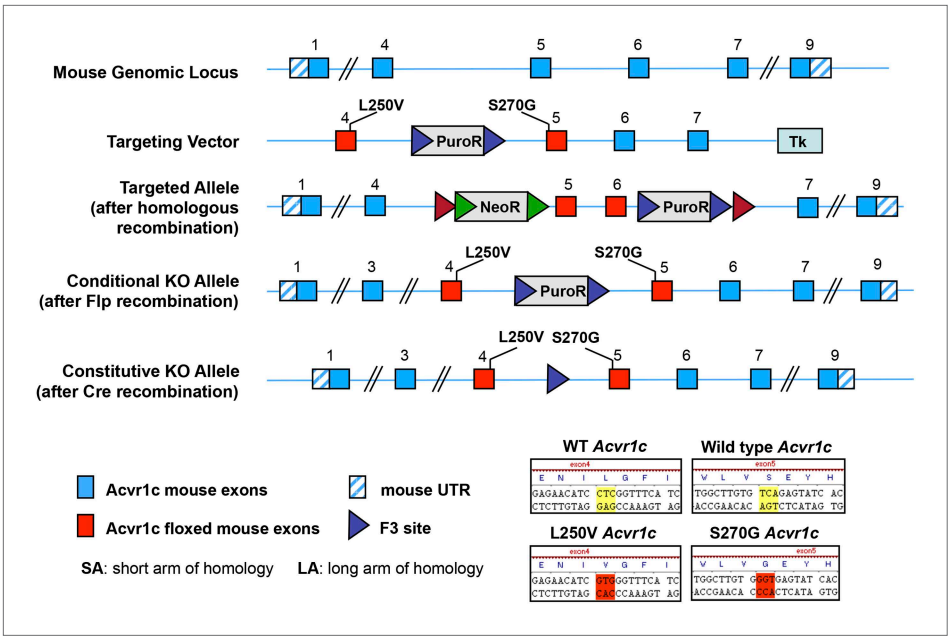

Figure 7—figure supplement 2. A chemical-genetic approach for acute inactivation of ALK7 in adult mice.

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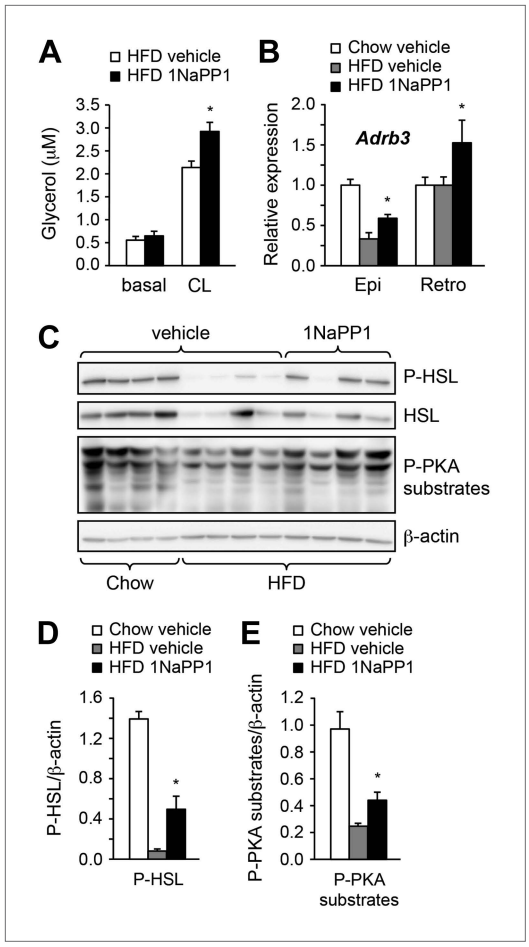

Figure 8. Acute inhibition of ALK7 signaling in adult mice reduces diet-induced catecholamine resistance. (**A**) Basal and CL316243 (CL)-stimulated lipolysis in adipose tissue biopsies extracted from *Alk7*^{ASKA} mice after 2 weeks on a high fat diet (HFD) treated with 1NaPP1 (solid bars) or vehicle (open bars). N = 6 mice per group. Epi, epididymal; Retro, retroperitoneal. (**B**) Relative mRNA expression levels of *Adrb3* assessed by Q-PCR in epididymal (Epi) adipose tissue of *Alk7*^{ASKA} mice after chow (open bars) or 2 weeks on HFD treated with 1NaPP1 (black bars) or vehicle (gray bars). N = 6 mice per group. (**C**–**E**) Levels of phospho-HSL (p-HSL) and phosphorylated PKA (P-PKA) substrates assessed by Western blotting in epididymal adipose tissue of *Alk7*^{ASKA} mice after chow or 2 weeks on HFD treated with 1NaPP1 or vehicle. Histograms show quantification by image analysis of P-HSL levels (**D**) and levels of phosphorylated PKA substrates (**E**) normalized to β-actin levels. N = 4 mice per group. *$p < 0.05$; **$p < 0.01$; NS, non-significant (1NaPP1 vs vehicle). All error bars show mean ± SEM.

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