

Supplementary Information

Dysfunctional Adipocytes Promote Tumor Progression Through YAP/TAZ-dependent Cancer-Associated Adipocyte Transformation

Yaechan Song^{1,#}, Heeju Na^{1,#}, Seung Eon Lee¹, You Min Kim¹, Jihyun Moon¹, Tae Wook Nam¹, Yul Ji², Young Jin¹, Jae Hyung Park¹, Seok Chan Cho¹, Jaehoon Lee^{1,3}, Daehee Hwang², Sang-Jun Ha¹, Hyun Woo Park¹, Jae Bum Kim², Han-Woong Lee^{1,3*}

¹ Department of Biochemistry, College of Life Science and Biotechnology, Yonsei University, Seoul 03722, Republic of Korea

² Department of Biological Sciences, Seoul National University, Seoul 08826, Republic of Korea

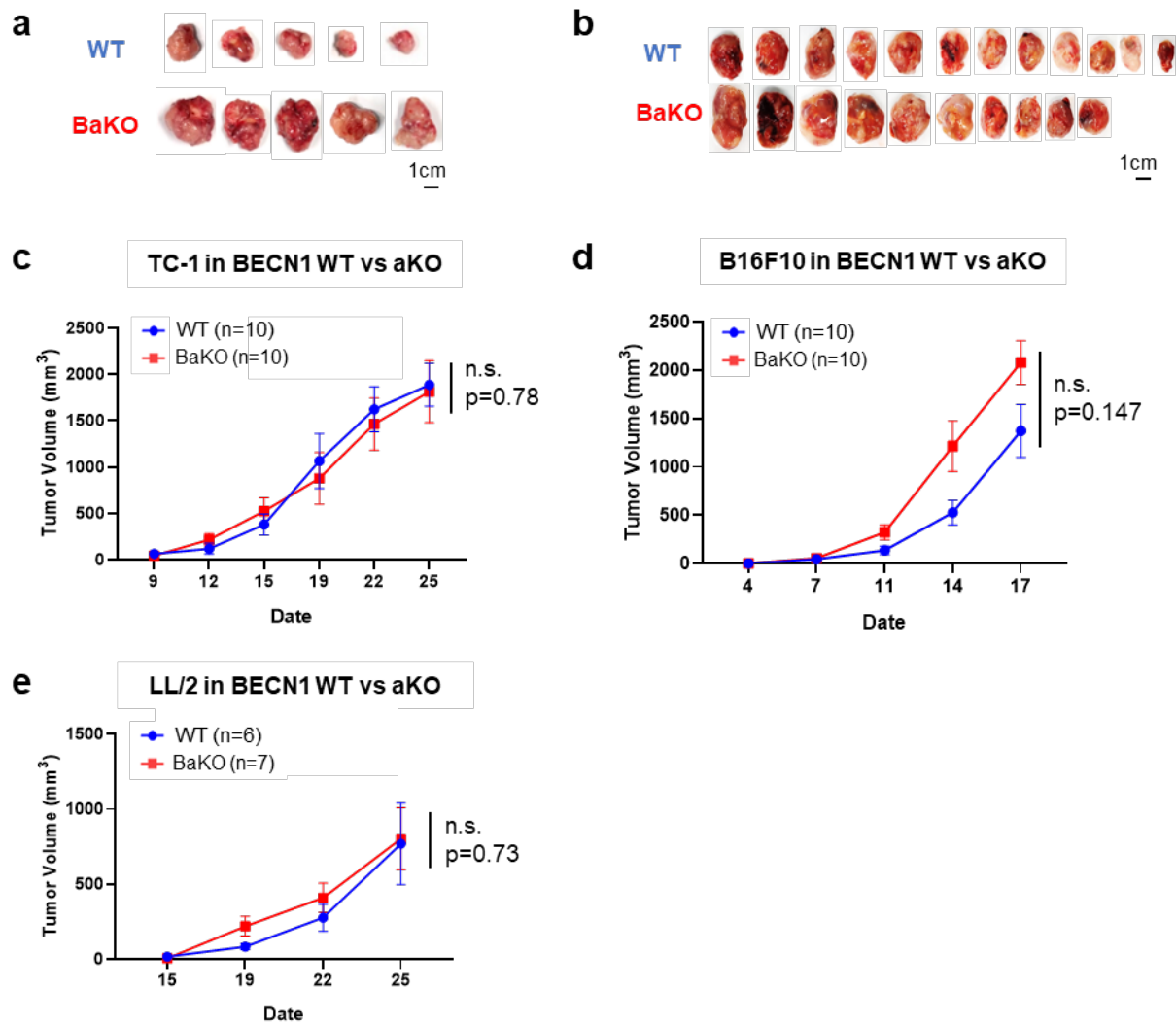
³ Gemcro, Inc., Seoul 03722, Republic of Korea

*Corresponding author: hwlee@yonsei.ac.kr

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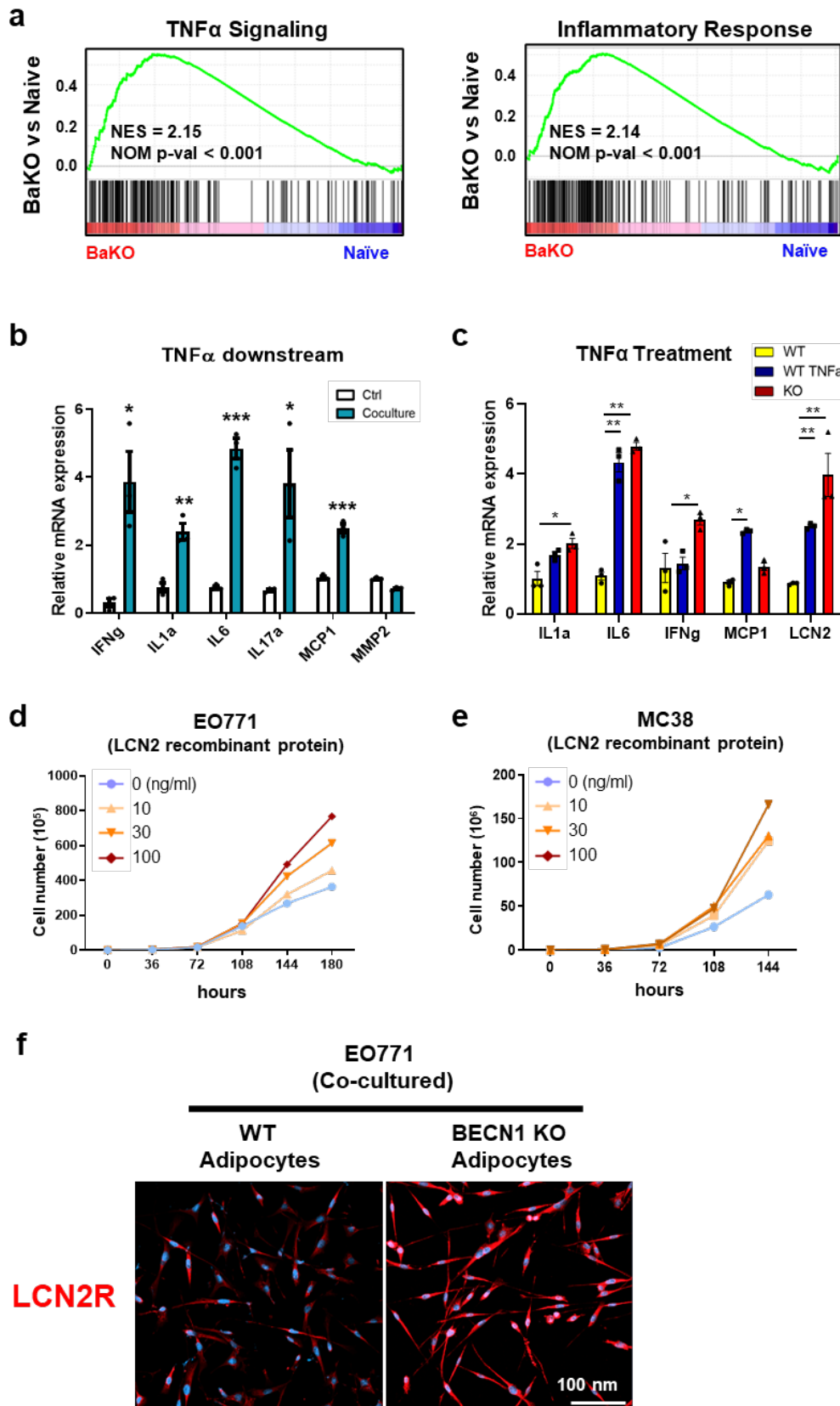
Supplementary figures S1 to S11

Supplementary tables S1 to S4



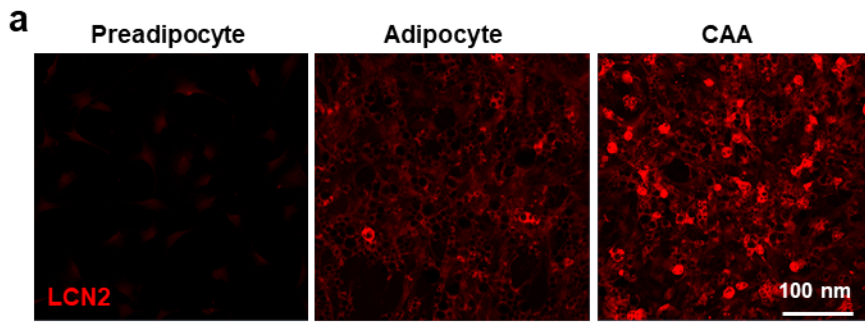
Supplementary Figure 1: Tumor progression of various cancer cell lines in BaKO. (a,b) Representative image of resected tumors presented in Fig. 1a and b, respectively. (c) Growth kinetics after subcutaneous injection of TC1, (d) B16F10, and (e) LL/2 into 8-week-old WT and BaKO mice (sample sizes are as indicated).

Statistics were calculated using ordinary two-way ANOVA (c, d, and e). Data are shown as mean $\pm$ SEM; n.s. = not significant.

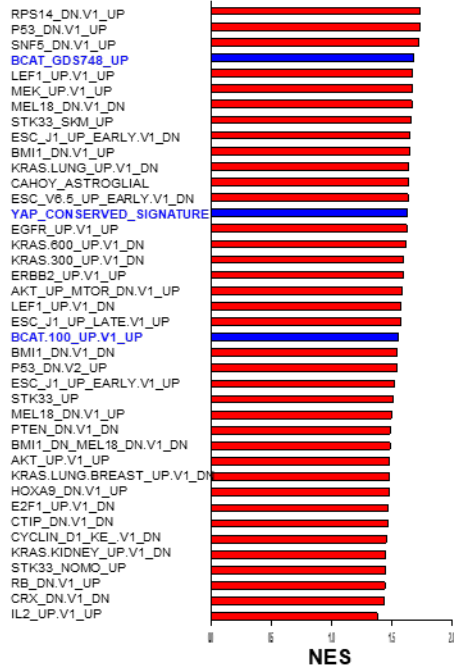


Supplementary Figure 2: TNF α and LCN2 from BECN1 deficient adipocytes support EO771 and MC38 progression. (a) GSEA plots for TNF α signaling and inflammatory response gene sets compared between RNA-seq results of BaKO and naïve WATs. (b) Relative mRNA expression of TNF α downstream genes from control adipocytes and cancer co-cultured adipocytes ($n = 3$). (c) Relative mRNA expression of TNF α downstream genes and LCN2 after treating adipocytes with or without TNF α recombinant protein ($n = 3$). (d) Viable cell counting of EO771 and MC-38 (e) treated with LCN2 recombinant protein as dose indicated. Cancer cell numbers were counted as they were sub-cultured every 36 h ($n = 2$, independent cells per group). (f) Representative IF image of LCN2R in co-cultured EO771 with WT or BECN1 KO adipocytes for 2 days.

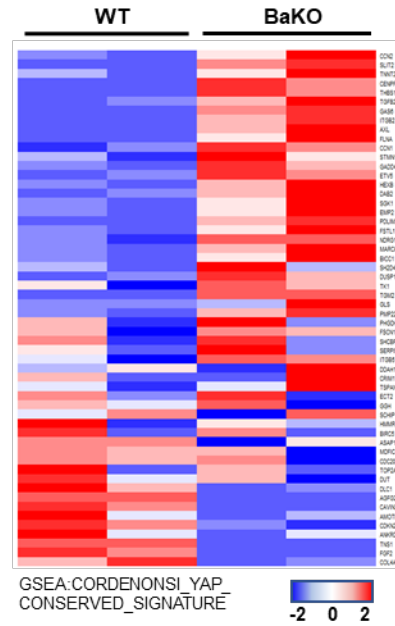
Statistics were calculated using two-tailed unpaired students t -test (b and c). Data are shown as mean $\pm$ SEM; $*p \leq 0.05$, $**p \leq 0.01$, $***p \leq 0.001$.



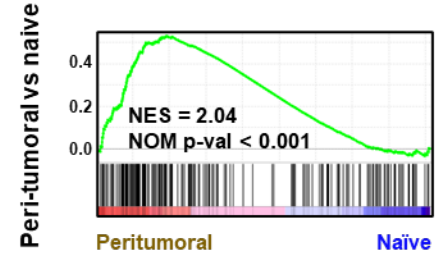
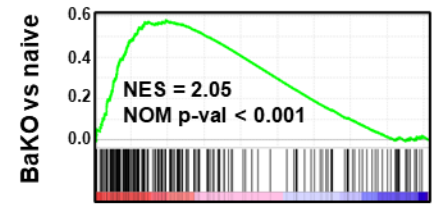
c Oncogenic signatures (BaKO vs Naïve)



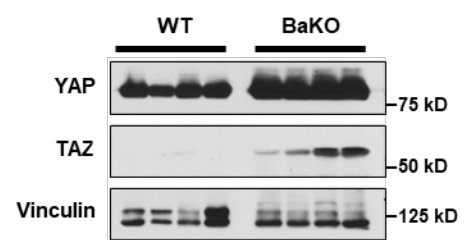
d YAP Conserved Signature



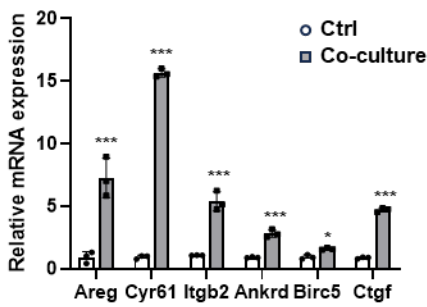
b Epithelial Mesenchymal Transition



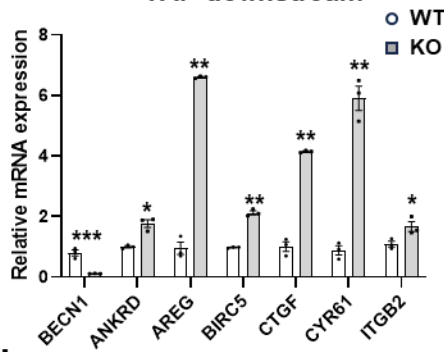
e iWAT



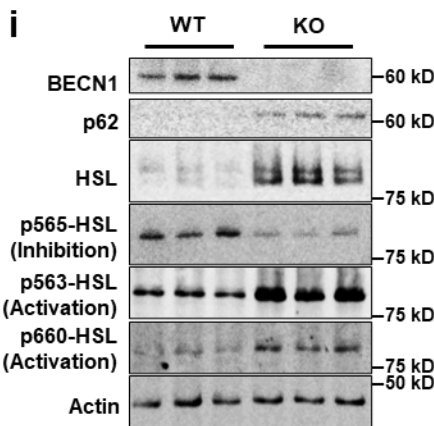
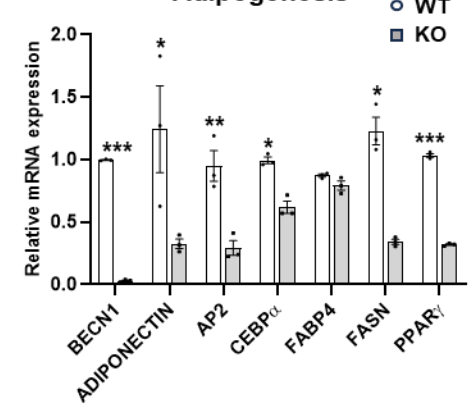
f YAP downstream



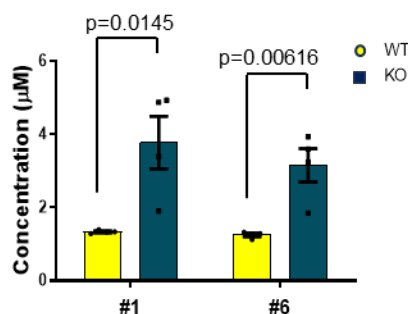
g YAP downstream



h Adipogenesis

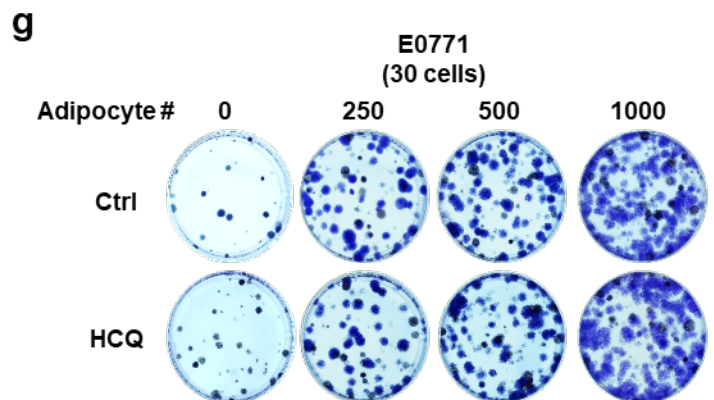
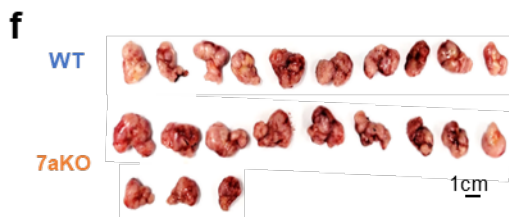
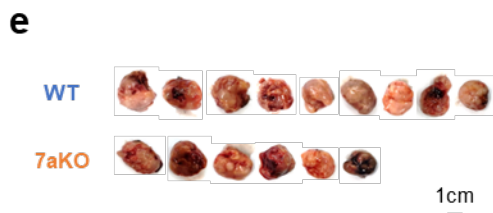
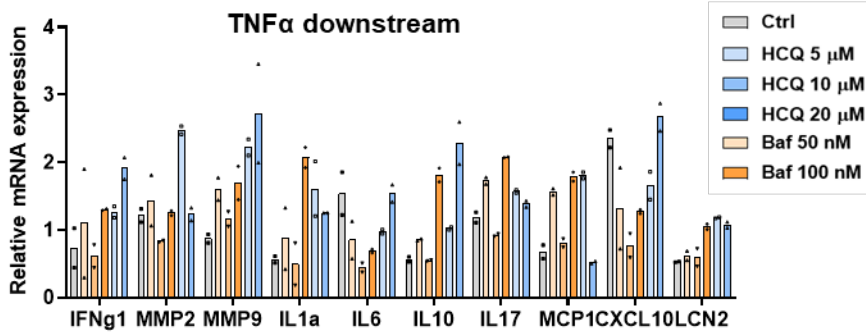
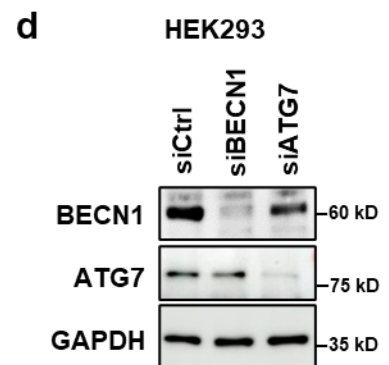
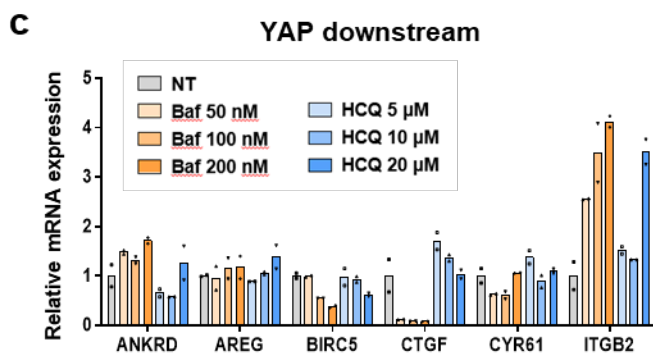
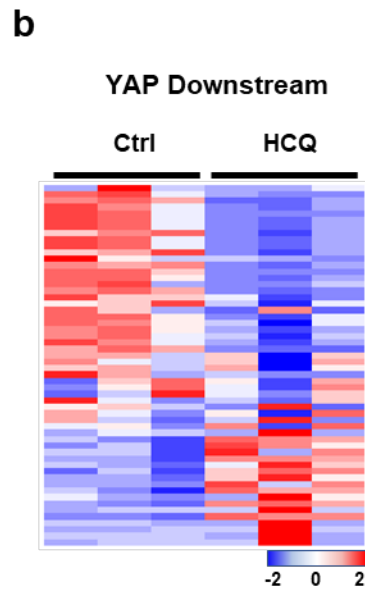
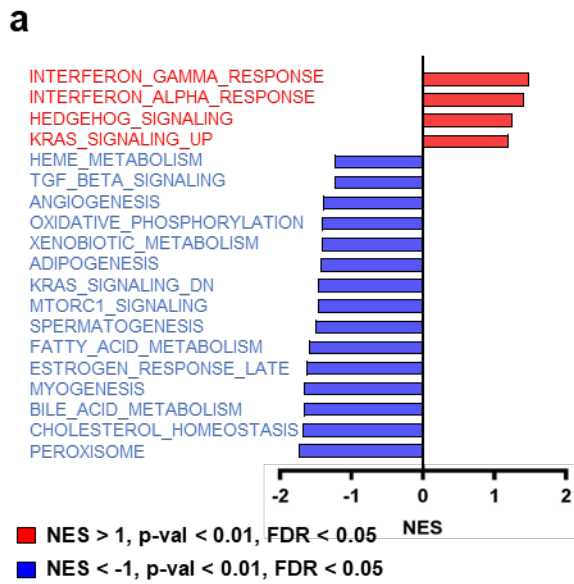


j FFA Concentration in CM



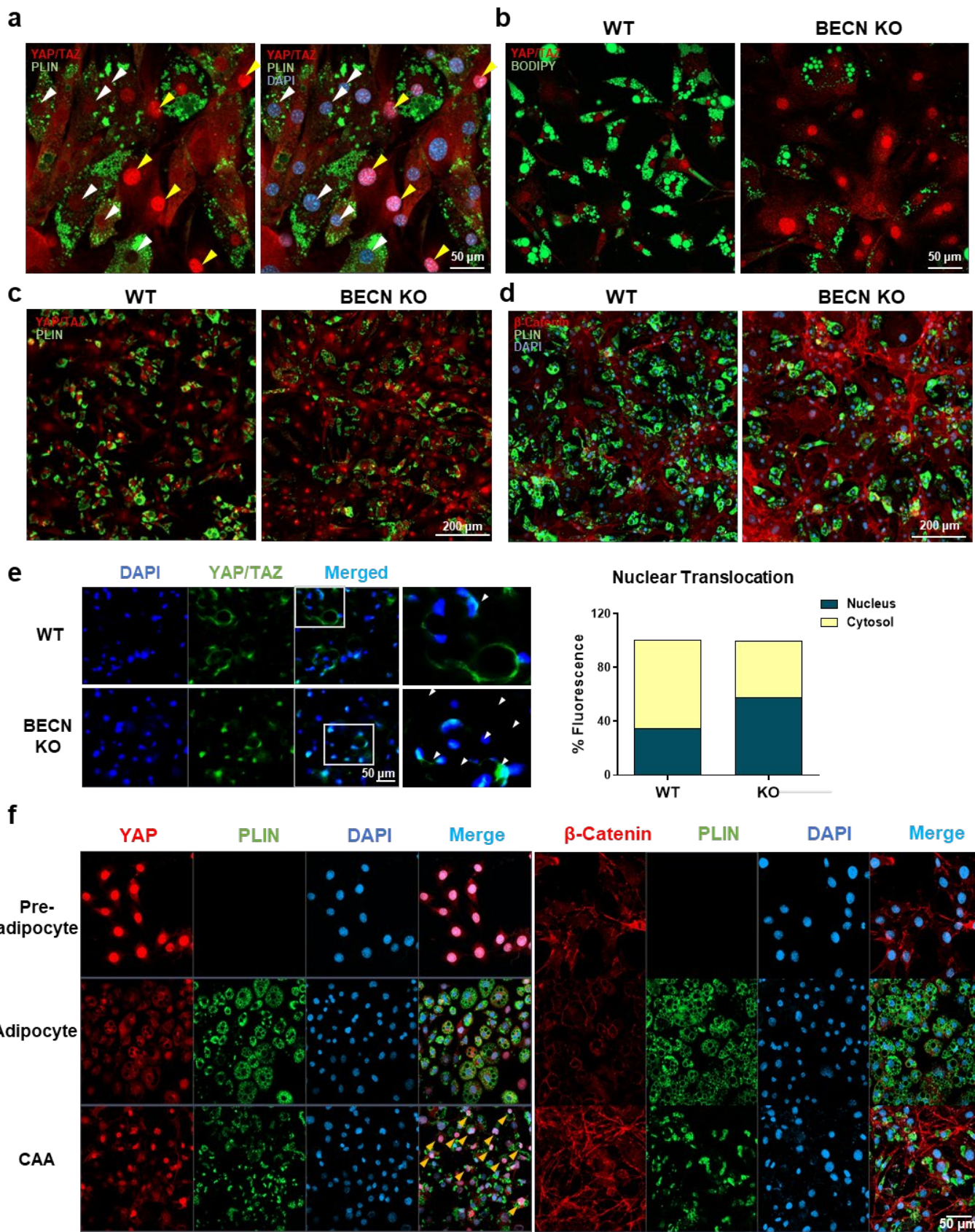
Supplementary Figure 3: Similar characteristics observed between CAAs and BECN1-deficient adipocytes, including YAP/TAZ signaling. (a) Representative IF image of LCN2 in preadipocytes, differentiated adipocytes, and co-cultured adipocytes. 10T1/2 adipocytes were co-cultured with EO771 for 2 days. (b) GSEA plots for EMT gene set compared between naïve, peritumoral, and BaKO WAT. Normalized enrichment score (NES) and normalized (NOM) p-value are as indicated. (c) List of differentially expressed GSEA 'C6 oncogenic signature' gene sets of BaKO and naïve adipose tissue ranked by NES. Gene sets associated β -catenin and YAP are colored in blue. (d) Heatmap analysis of YAP conserved signatures between RNA-seq results of WT and BaKO iWATs. (e) Western blotting analysis of protein lysates isolated from WT and BaKO iWAT (n = 4, independent mice per group). (f) Relative mRNA expression of YAP downstream genes from adipocytes co-cultured with EO771 for 4 days (n = 3) (g) Relative mRNA expression of YAP downstream genes between WT and BECN1 KO adipocytes (n = 3). (h) Relative mRNA expression of adipogenesis-associated genes between WT and BECN1 KO adipocytes (n = 3). (i) Western blotting analysis of protein lysates isolated from WT and BaKO iWAT. (j) Free fatty acid concentration measured in media extracted from WT and BECN1 KO adipocytes (n = 4, independent cells per group).

Statistics were calculated using two-tailed unpaired students *t*-test (f, g, h, and j). Data are shown as mean $\pm$ SEM; **p* $\leq$ 0.05, ***p* $\leq$ 0.01, ****p* $\leq$ 0.001. Western blot results are representatives of at least three independent experiments.

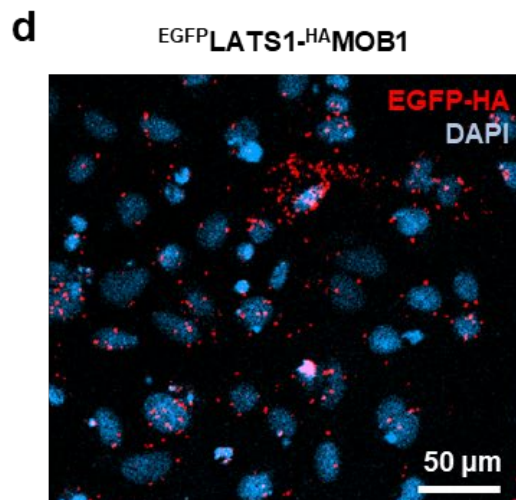
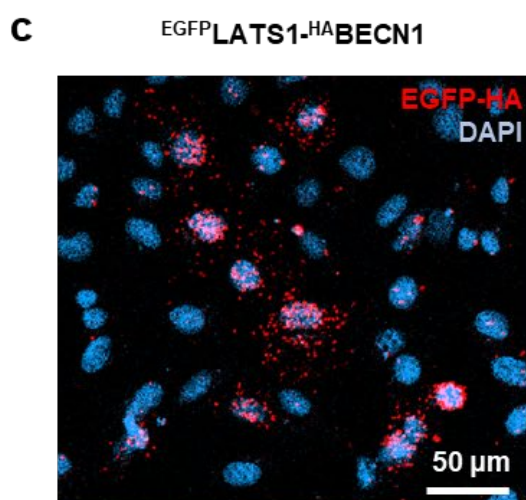
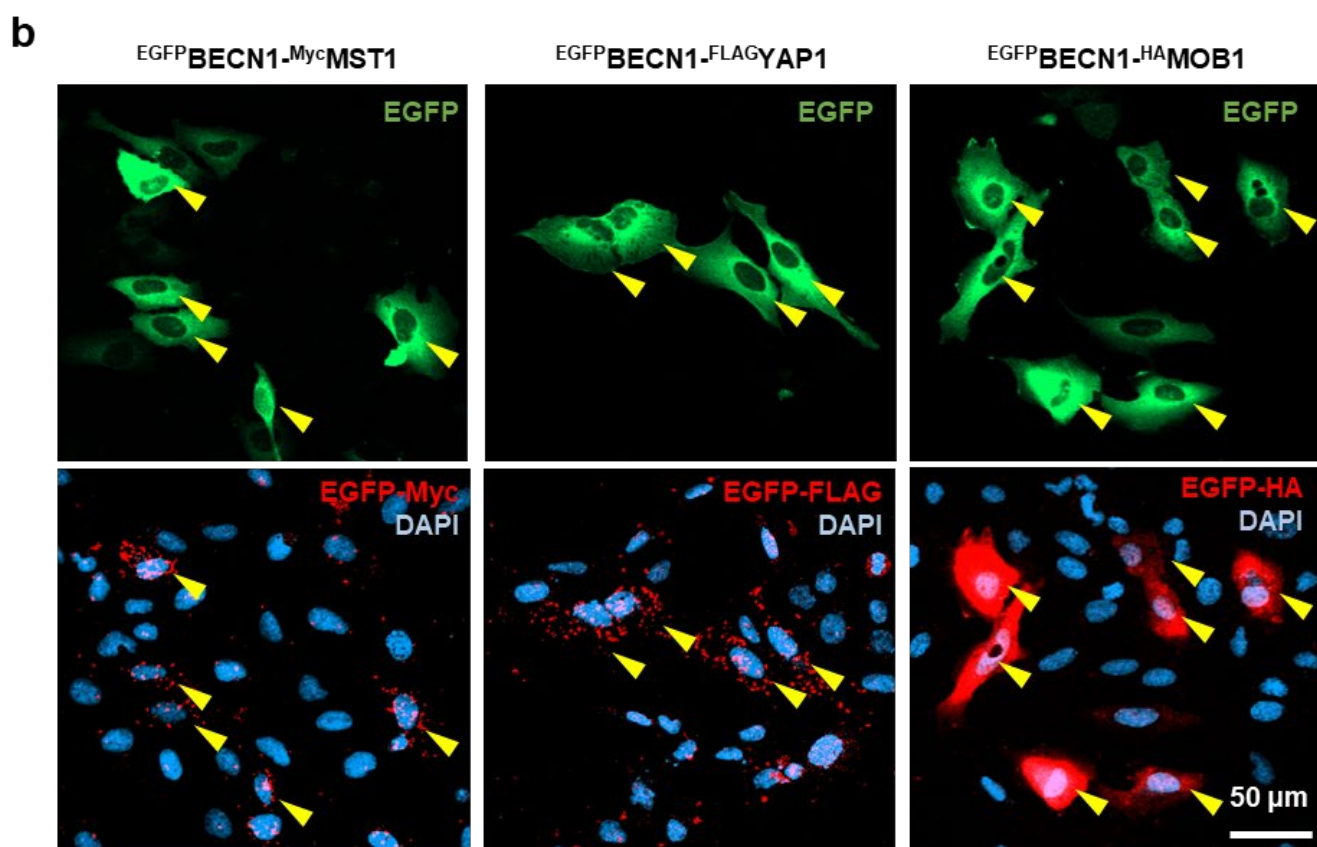
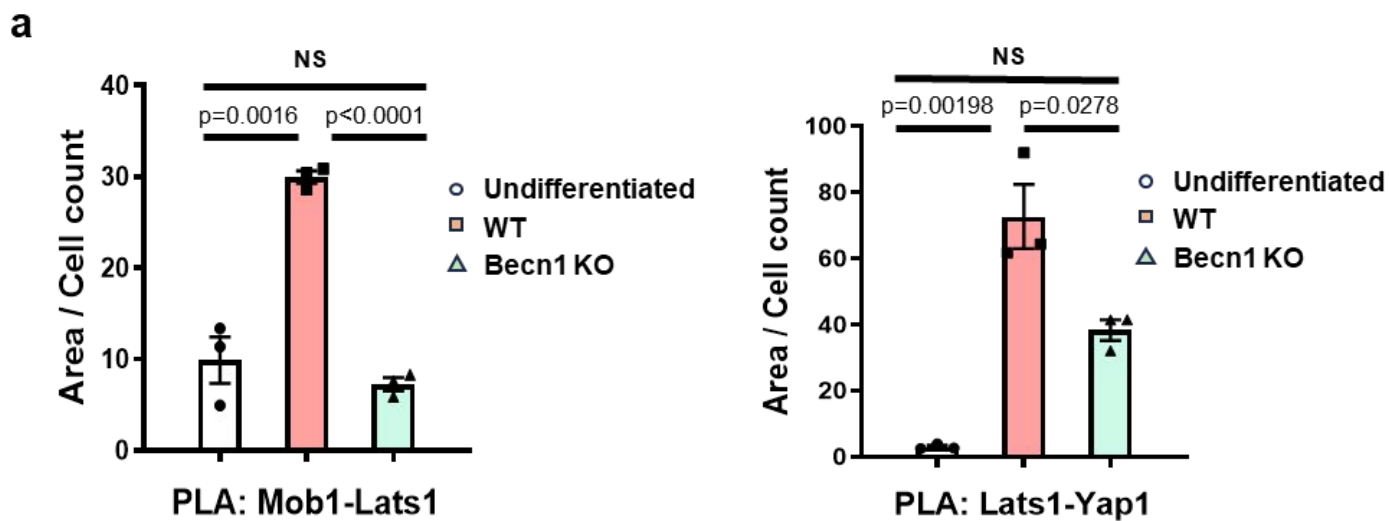


Supplementary Figure 4: BECN1 depletion leads to adipocyte transformation. (a) GSEA of hallmark gene sets from RNA-seq results of 10T1/2 adipocytes treated with or without HCQ (20 μ M, 48 hr) (n = 3, independent samples per group). (b) Heatmap analysis of YAP conserved signature gene set from RNA-seq results of 10T1/2 adipocytes treated with or without HCQ (20 μ M, 48 hr) (n = 3). (c) Relative mRNA expression of YAP and TNF α downstream genes from adipocytes treated with Bafilomycin (Baf) or HCQ for 24 hrs (n = 2, independent samples per group). (d) Western blotting analysis of protein lysates isolated from HEK293 cells transfected with siBECN1 or siATG7 for Fig. 4b (n = 2, independent samples per group). (e, f) Representative image of resected tumors presented in Fig. 4c and d, respectively. (g) Colony assay of EO771 co-cultured with adipocytes treated with or without HCQ. HCQ was pretreated (20 μ M, 48 hr) to the number of adipocytes as indicated.

Western blot results are representatives of at least three independent experiments.

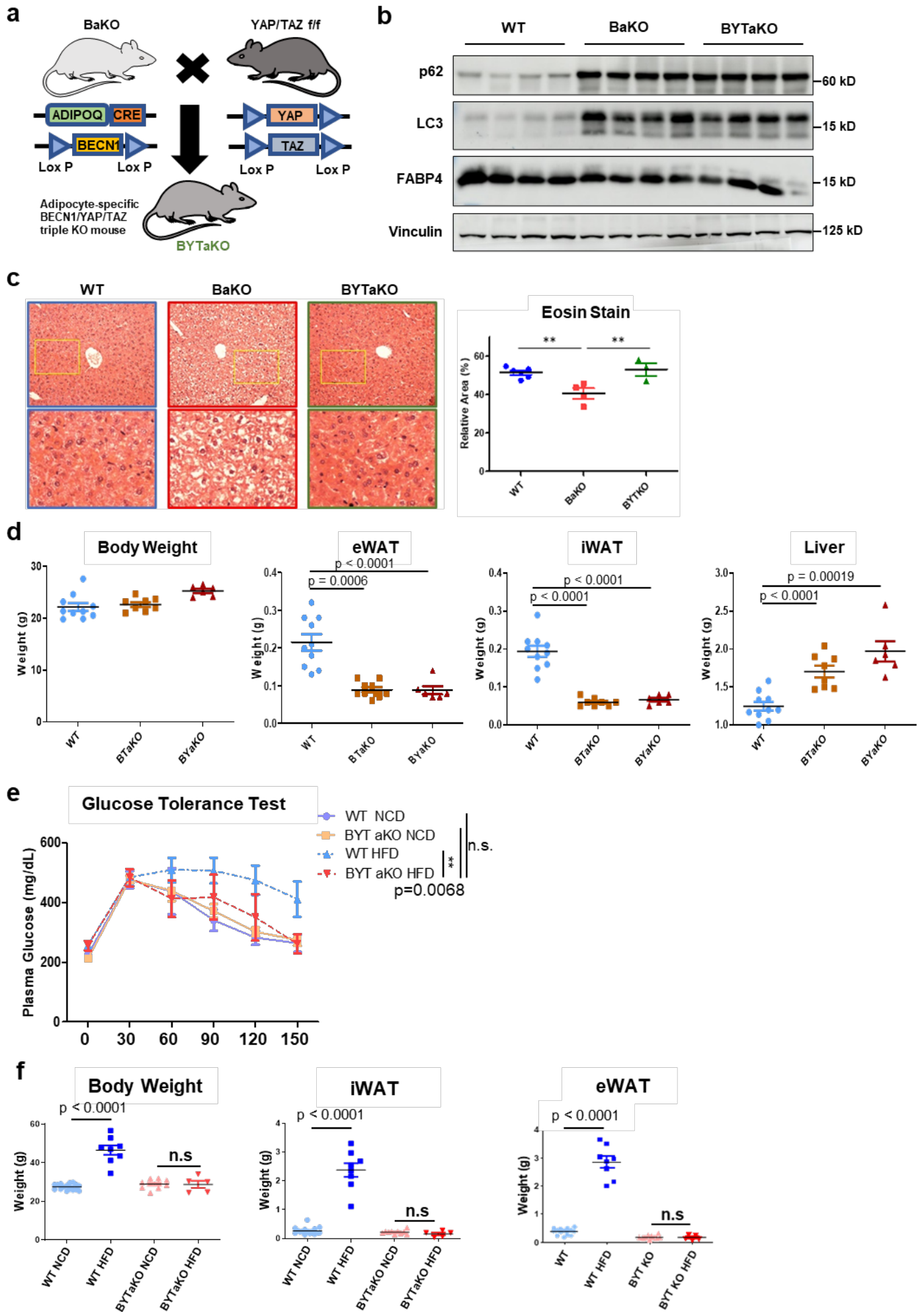


Supplementary Figure5: Dynamic YAP/TAZ regulation modulates differentiation status of adipocytes. (a) Representative IF image of mature adipocytes stained with YAP/TAZ (red), PLIN1 (green), and DAPI (blue); white arrowheads indicate fully differentiated adipocytes (PLIN1 positive) with less nuclear YAP/TAZ; yellow arrowheads indicate undifferentiated adipocytes (PLIN negative) with nuclear YAP/TAZ. (b) Representative IF image of doxycycline-inducible *Becn1* KO adipocytes stained with BODIPY and YAP/TAZ. (c) Representative IF image of doxycycline-inducible *Becn1* KO adipocytes stained with YAP/TAZ and β -catenin (d) with lower magnification (Fig. 5d, e). (e) Representative IF image of fully differentiated imSVCs treated with or without 4-OHT. Adipocytes were stained with YAP/TAZ and DAPI. Nuclear translocation of YAP/TAZ is quantified using imageJ. (f) Representative IF image of 10T/2 cells as preadipocytes, differentiated adipocytes, and CAAs. Adipocytes were co-cultured with EO771 for 2 days. Yellow arrows indicate nuclear translocation of YAP in CAAs.



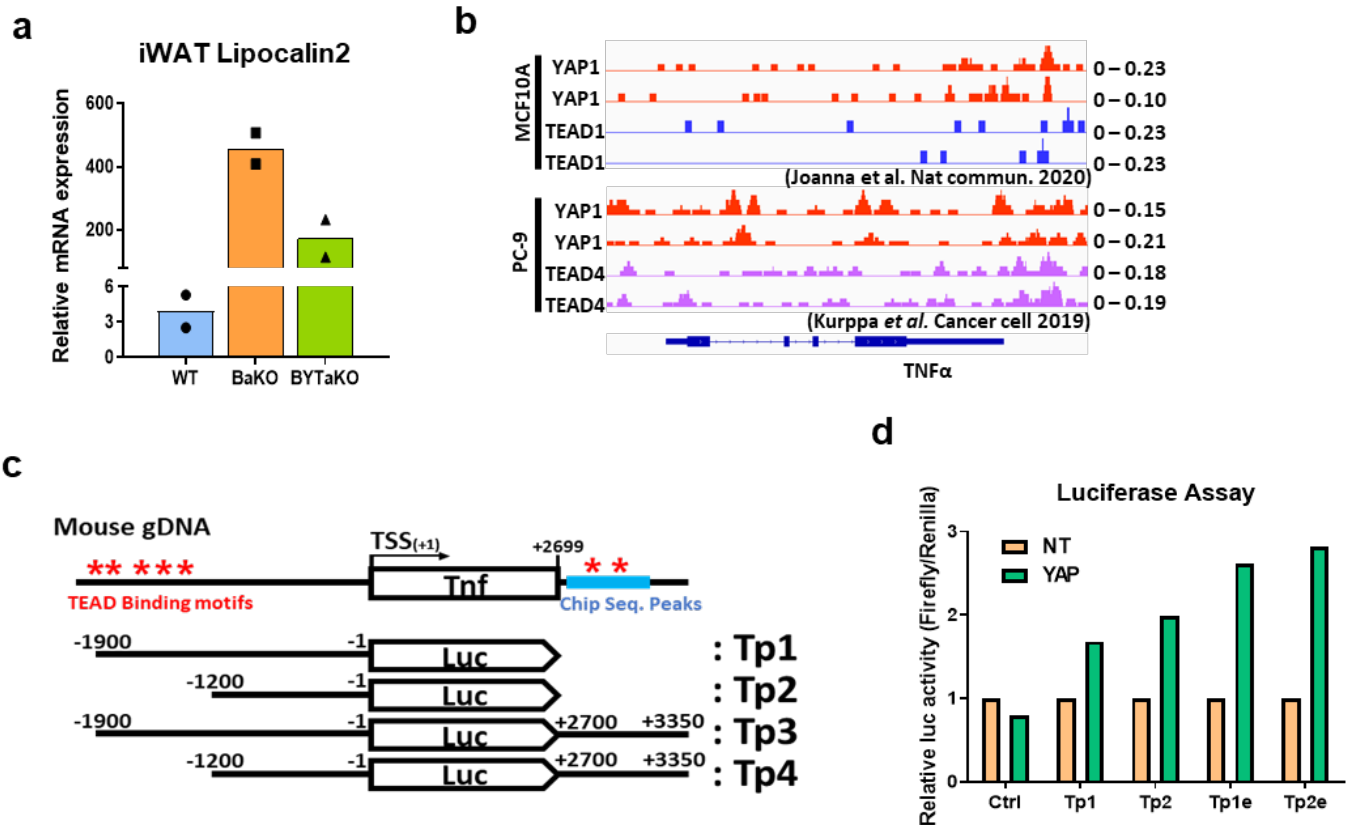
Supplementary Figure 6: BECN1 enables YAP/TAZ-mediated dedifferentiation through MOB1. (a) Quantification of PLA: MOB1-LATS1 and LATS1-YAP1 result in Fig. 5f ($n = 3$, independent cells per group). (b) Representative IF image of PLA conducted on U2OS cells overexpressed with BECN1^{EGFP} and MST1^{MYC}, YAP1^{FLAG}, and MOB1^{HA} overexpressed cells are marked with yellow arrowheads. (c) Representative IF image of PLA (BECN1-LATS1 interaction) conducted on U2OS cells overexpressed with BECN1^{HA} and LATS1^{EGFP}. (d) Representative IF image of PLA (LATS1-MOB1 interaction) conducted on U2OS cells overexpressed with LATS1^{EGFP} and MOB1^{HA}.

Statistics were calculated using two-tailed unpaired students *t*-test (a). Data are shown as mean $\pm$ SEM; * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$.

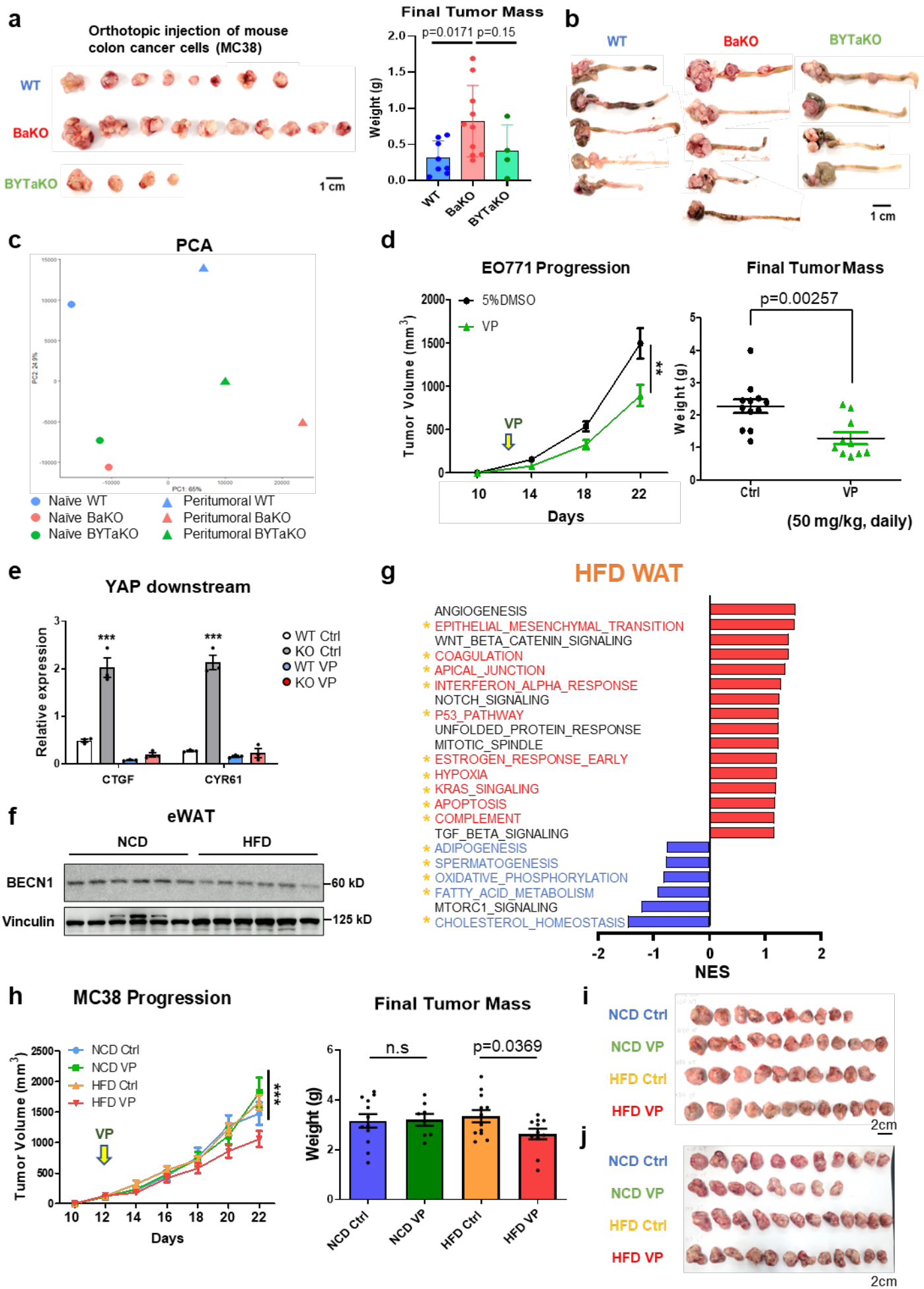


Supplementary Figure 7: Additional YAP/TAZ deletion restores lipodystrophy phenotypes of BaKO. (a) Mouse breeding strategy to generate adipocyte-specific BECN1/YAP/TAZ KO (BYTaKO) mice. (b) Western blot analysis of protein isolated from WT, BaKO, and BYTaKO iWATs (8-week-old). (c) Hematoxylin and eosin staining of tissue section prepared from WT, BaKO, and BYTaKO liver (12-week-old) (WT n = 6; BaKO n = 4; BYTaKO n = 3, independent mice per group). (d) Body and organ weights measured from WT, BaKO, and BYTaKO mice (8-week-old) (WT n = 10; BaKO n = 8; BYTaKO n = 6). (e) Glucose tolerance test performed on WT and BYTaKO mice after feeding with NCD or HFD for six weeks (WT, n = 4, BYTaKO n = 6, WT HFD n = 6, BYTaKO HFD n = 5). (f) Body and organ weights measured from WT and BYTaKO mice after feeding with NCD or HFD for six weeks (WT NCD n = 16; WT HFD n = 8; BYTaKO NCD n = 10; BYTaKO HFD n = 5).

Statistics were calculated using two-tailed unpaired Student's *t*-test (c, d, and f) and ordinary two-way ANOVA (e). Data are shown as mean $\pm$ SEM; **p* $\leq$ 0.05, ***p* $\leq$ 0.01, ****p* $\leq$ 0.001. Western blot results are representatives of at least three independent experiments.

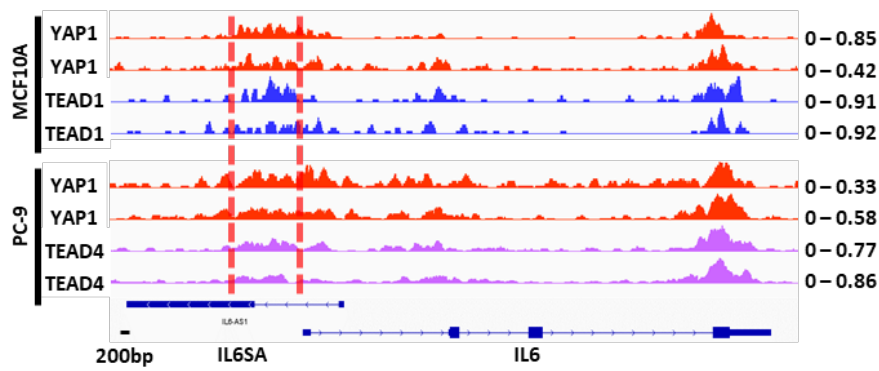


Supplementary Figure 8: YAP activity regulates LCN2 but not TNF α . (a) Relative mRNA expression of *Lcn2* in WT, BaKO, BYTaKO iWAT (n = 2, independent mice per group). (b) Publicly available YAP1, TEAD1, and TEAD4 CHIP-seq reads around TNF α gene loci. CHIP-seq was conducted in MCF10A and PC-9 cells. (c) Schematic representation of luciferase reporter construct containing TNF α promoter region. Red stars indicate the TEAD binding motifs, and blue bar indicates the regions with high CHIP-seq reads. (d) Dual Luciferase assay conducted in HEK2993 cells transfected with the luciferase reporter constructs (TNF α). After transfection, recombinant YAP1 protein was treated (1 μ g, 48 hr).

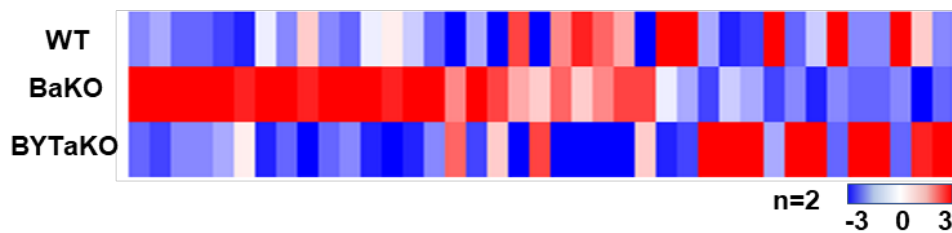


Supplementary Figure 9: Deactivation of adipocyte YAP/TAZ represses malignant TME formation. (a) Colon orthotopic injection of MC38 into mouse cecum. Mice were sacrificed after 4 weeks post-injection. Final tumor mass measured after resection of each tumor (WT n = 8, BaKO n = 10, BYTaKO n = 4). (b) Image of resected colons harboring tumors. (c) Principal component analysis performed on RNA-seq results of WT, BaKO, and BYTaKO WATs. Peritumoral adipose tissues were extracted two weeks after MC-38 injection (n = 2, independent sequencing results per group). (d) Tumor volumes and weights after mammary fat pad injection of EO771 into WT mice. VP treatment (50 mg/kg, injected everyday) was initiated when tumors reached a volume of 150 mm³, and the mice were sacrificed on day 22 following the injection (Ctrl n = 12; VP n = 10). (e) Relative mRNA expression of YAP downstream in WT and *Becn1* KO imSVCs treated with or without VP (20 nM) for 24 h (n = 3, independent samples per group). (f) Western blot analysis of protein lysates isolated from eWAT of NCD and HFD fed mice. (g) GSEA result of HFD-fed mouse and NCD-fed mouse iWATs ranked by NES. Gene sets that also appear to be differentially expressed in BaKO iWAT are indicated with yellow stars. (h) Tumor volumes and weights after subcutaneous injection of MC-38 into NCD and HFD-fed mice. VP treatment (30 mg/kg) was initiated when tumors reached a volume of 300 mm³, and the mice were sacrificed on day 22 following the injection (NCD Ctrl n = 12; NCD VP n = 9; HFD Ctrl n = 12; HFD VP n = 11). (i, j) Image of tumors resected from Fig. 7g (i) and Supplementary Fig. 9f (j).

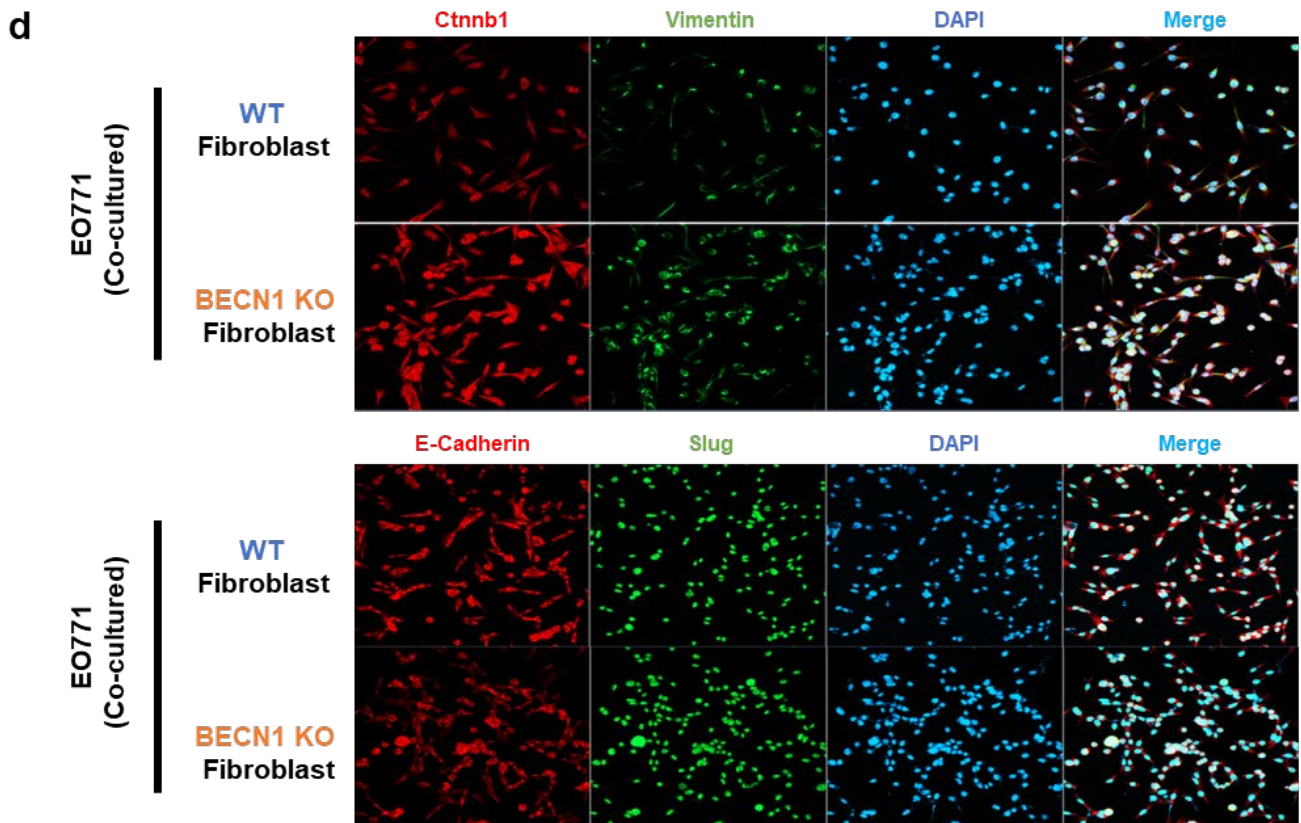
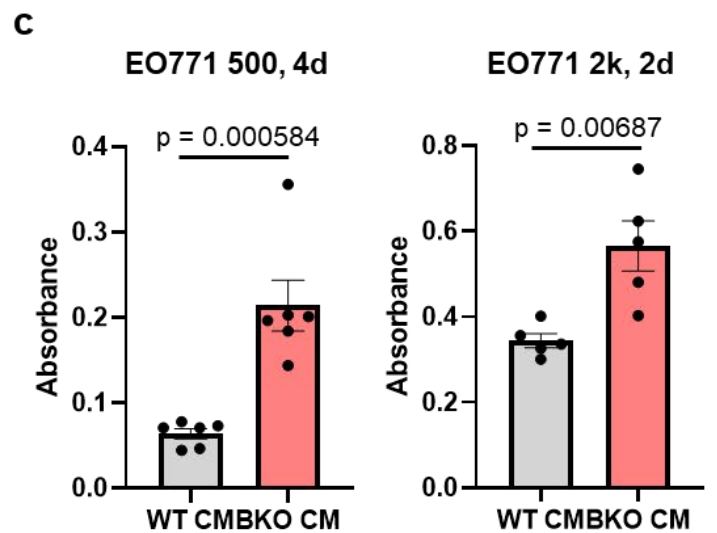
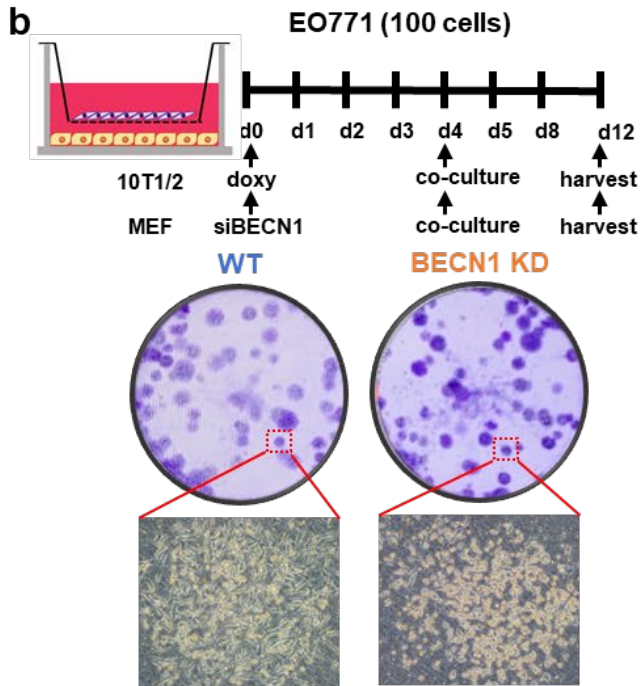
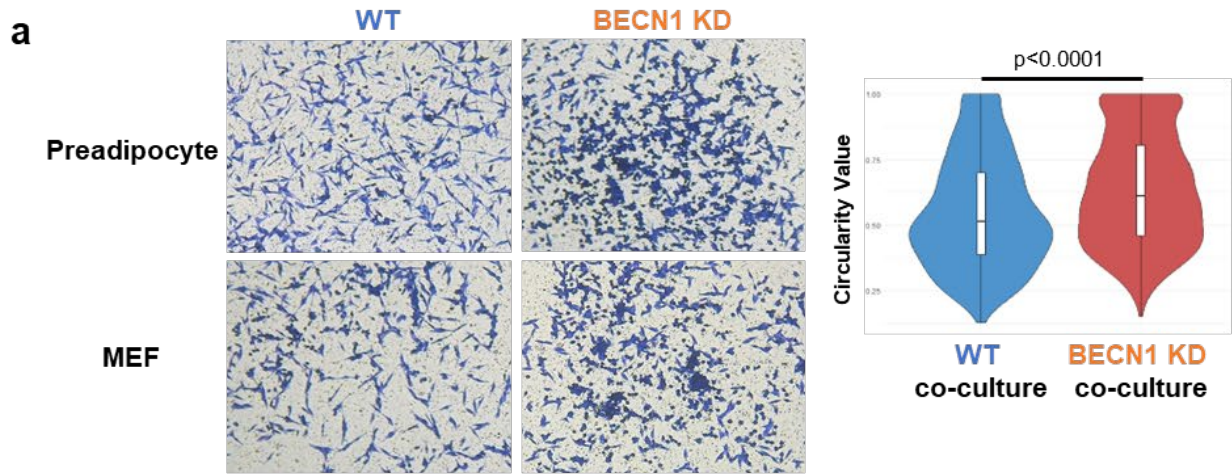
Statistics were calculated using two-tailed unpaired Student's *t*-test (a, d, h), ordinary two-way ANOVA (d and h), and one-way ANOVA (e). Data are shown as mean $\pm$ SEM; **p* $\leq$ 0.05, ***p* $\leq$ 0.01, ****p* $\leq$ 0.001. Western blot results are representatives of at least three independent experiments.

a**b**

GSEA (Hallmark): Wnt β -Catenin Signaling



Supplementary Figure 10: IL6 - Wnt/ β -catenin signaling in BaKO and BYTaKO iWATs. (a) Publicly available YAP1, TEAD1, and TEAD4 CHIP-seq reads around IL6 gene loci. CHIP-seq was conducted in MCF10A and PC-9 cells. (b) Heatmap analysis of Wnt/ β -catenin signaling gene set from RNA-seq results of WT, BaKO, and BYTaKO iWATs (n = 2, independent samples per group).



Supplementary Figure 11: Depletion of fibroblast-BECN1 induces tumor growth and EMT. (a) Crystal violet staining of EO771 cells co-cultured with preadipocytes or MEFs. BECN1 was depleted from both preadipocytes and MEF prior to the co-culture. Circularity of each cell was measured using imageJ to represent cellular morphologies (WT n = 1157, BECN1 KD n = 975, individual cells per group). (b) Schematic timeline of BECN1 depletion in preadipocytes and MEFs. DIC image of EO771 co-cultured with WT or BECN1-deficient preadipocytes. (c) MTT assay results of EO771 grown in preadipocyte-derived CM. BECN1 was depleted by doxycycline treatment. The CM was collected every 4 days of incubation (n = 6, independent cells per group). (d) Representative IF image of EO771 co-cultured with WT or BECN1-deficient fibroblasts stained with EMT markers. EO771 cells were co-cultured for 2 days and stained with β -catenin, Vimentin, E-cadherin, and Slug.

Statistics were calculated using two-tailed unpaired students *t*-test (a and c). Data are shown as mean $\pm$ SEM; **p* $\leq$ 0.05, ***p* $\leq$ 0.01, ****p* $\leq$ 0.001.

Supplementary table 1: Sequences of genotyping primers used in this study.

	Forward	Reverse
Actin	GTAGGTGGAAATTCTAGCATCATCC	CTAGGCCACAGAATTGAAAGATCT
Adipoq-Cre	ACCTGAAGATGTTGCGATTATCT	ACCGTCAGTACGTGAGATATCTT
BECN1f/f	GCTTAGGTCTGACTGGGTCG	ACCCAGGCAAGTGTGTTTTTC
YAP1f/f	ACATGTAGGTCTGCATGCCAGAGGAGG	AGGCTGAGACAGGAGGATCTCTGTGAG
TAZf/f	TTGTGACAAAGAACCTGGGGCTATC	TGAGGAACTCTTCCATTGCCAGG
ATG7f/f	TGGCTGCTATTTCTGCAATG	TTCTGGATGCTGCAAAACAG
MMTV-PyMT	TGGCGGCCTCTTGACTATGAT	ATCTGTGCTTATGCCTATGCCCGC

Supplementary table 2: shRNA sequences used to knockdown BECN1, YAP, and TAZ.

sh_BECN1_1	AAAAGGTTTGGAAAGATGCTTTATTGGATCCAATAAAGCATCTTTCCAAACC
sh_BECN1_2	AAAAGGATTTGCTATGGTCTATTTTGGATCCAAAATAGACCATAGCAAATCC
sh_BECN1_3	AAAAGGTGATGAATTCTCATCTATTGGATCCAATAGATGAGAATTCATCACC
sh_BECN1_4	AAAAGGAAAAGCTGTTTCCTTATATTGGATCCAATATAAGGAAACAGCTTTCC
sh_BECN1_5	AAAAGGTAAAAGGTGCTGTCAAATTGGATCCAATTTGACAGCACCTTTTACC
sh_YAP_1	AAAAGGTTTGGAAAGATGCTTTATTGGATCCAATAAAGCATCTTTCCAAACC
sh_YAP_2	AAAAGGATTTGCTATGGTCTATTTTGGATCCAAAATAGACCATAGCAAATCC
sh_YAP_3	AAAAGGTGATGAATTCTCATCTATTGGATCCAATAGATGAGAATTCATCACC
sh_YAP_4	AAAAGGAAAAGCTGTTTCCTTATATTGGATCCAATATAAGGAAACAGCTTTCC
sh_YAP_5	AAAAGGTAAAAGGTGCTGTCAAATTGGATCCAATTTGACAGCACCTTTTACC
sh_TAZ_1	AAAAGCATTTCTGTGGCAGATAATTGGATCCAATTATCTGCCACAGAAATGC
sh_TAZ_2	AAAAGCCTGATGAACCATGAATTTTGGATCCAAAATTCATGGTTCATCAGGC
sh_TAZ_3	AAAAGCCAGGTGATGTGAAATTATTGGATCCAATAATTTACATCACCTGGC
sh_TAZ_4	AAAAGCCCTTGACTGTTTACTAATTGGATCCAATTAGTAAACAGTCAAGGGC
sh_TAZ_5	AAAAGCACCTTGTAAGTGTAAATTGGATCCAATTACACTTTCACAAGGTGC

Supplementary table 3: siRNA sequences used to knockdown LCN2R.

	Sense	Anti-sense
si_LCN2R1	GCAGAGGGCU AGAAAGAU A	UAUCUUUCUA GCCCUCUGC
si_LCN2R2	GCAGAGGGCU AGAAAGAU A	UAUCUUUCUA GCCCUCUGC
si_LCN2R3	CGCUAUAACC AUUAGGUCU	AGACCUA AUG GUUAUAGCG
si_LCN2R4	GCUAUAACCA UUAGGUCUG	CAGACCUA AU GGUUAUAGC

Supplementary table 4: Sequences of mouse RT-qPCR primers used in this study.

	Forward	Reverse
BECN1	CAGCCTCTGAAACTGGACACGA	CTCTCCTGAGTTAGCCTCTTCC
Adiponectin	GCAGAGATGGCACTCCTGGA	CCCTTCAGCTCCTGTCAATTCC
AP2/FABP4	TGAAATCACCGCAGACGACAGG	GCTTGTCACCATCTCGTTTTCTC
CEBPalpha	GGGTGAGTTCATGGAGAATGG	CAGTTTGGCAAGAATCAGAGCA
FASN	CACAGTGCTCAAAGGACATGCC	CACCAGGTGTAGTGCCTTCCTC
PPAR γ	TGCACTGCCTATGAGCACTT	GAATGCGAGTGGTCTTCCAT
CTGF	GTGCCAGAACGCACACTG	CCCCGGTTACACTCCAAA
CYR61	AGAGGCTTCCTGTCTTTGGC	CCAAGACGTGGTCTGAACGA
AREG	TCATGGCGAATGCAGATACA	GCTACTACTGCAATCTTGGA
ITGB2	GCTTTGGGTCGTTTGTGGAC	TGCCGACCTCTGTCTGAAAC
ANKRD	GGAACAACGGAAAAGCGAGAA	GAAACCTCGGCACATCCACA
BIRC5	TGCAAAGGAGACCAACAACA	GGCATGTCACTCAGGTCCAA
TNFalpha	CCCCAAAGGGATGAGAAGTT	CACTTGGTGGTTTGCTACGA
IFNgamma	GCTCTTCCTCATGGCTGTTT	GTCACCATCCTTTTGCCAGT
MMP2	AGATCTTCTTCTTCAAGGACCGTT	GGCTCCTCAGTGGCTTGGGGTA
MMP9	TGAATCAGCTGGCTTTTGTG	ACCTTCCAGTAGGGGCAACT
IL6	TGAGAAAAGAGTTGTGCAATGG	GGTACTCCAGAAGACCAGAGG
IL-17alpha	CCAGGGAGAGCTTCATCTGT	AGGAAGTCCTTGGCCTCAGT
MCP1/CCL2	GCTACAAGAGGATCACCAGCAG	GTCTGGACCCATTCTTCTTGG
Serpine1	CCTCTTCCACAAGTCTGATGGC	GCAGTTCCACAACGTCATACTCG
Resistin	TCACTTTTACCTCTGTGGATATGAT	TGCCCCAGGTGGTGTAAA
Lipocalin2	TGCCACTCCATCTTTCCTGTT	GGGAGTGCTGGCCAAATAAG
CRP	GATTCCTGAGGCTCCAACACAC	ACAGTGTAGCCCTTGTGCAGAC
IGF1	GTGGATGCTCTTCAGTTCGTGTG	TCCAGTCTCCTCAGATCACAGC
Pentraxin3	CGAAATAGACAATGGACTTCATCC	CATCTGCGAGTTCTCCAGCATG
Endocan	CTGGAGAAACCTGCTACCGTAC	CATTCCATCCCGAAGGTGCCAT
Adiposin	CATGCTCGGCCCTACATGG	CACAGAGTCGTCATCCGTCAC
Apelin	CACTGATGTTGCCTCCAGATGGA	ACGCCATTAGACGAACTTGGTGG
Chimerin	CTTCTCCCGTTTGGTTTGATTG	TACAGGTGGCTCTGGAGGAGTTC
Leptin	TGCTGCAGATAGCCAATGAC	GAGTAGAGTGAGGCTTCCAGG
OncostatinM	GCACGGGCCAGAGTACCAGGAC	CTGGTGTTGTAGTGGACCGTGAG
RAGE	GCCACTGGAATTGTGATGAGG	GCTGTGAGTTCAGAGGCAGGAT
YAP	ACATGTAGGTCTGCATGCCAGAGG	AGGCTGAGACAGGAGGATCTCTGT
TAZ	TCCTCTGTGTGTGTGCTGAG	CTGAAACTACAATAAACTCTCCC
IL8	ATGGCTGCTCAAGGCTGGTC	AGGCTTTTCATGCTCAACACTAT
IL10	GACCAGCTGGACAACATACTGCTAA	GATAAGGCTTGGCAACCCAAGTAA