ERα upregulates PHD3 to ameliorate HIF-1 induced fibrosis and inflammation in adipose tissue.

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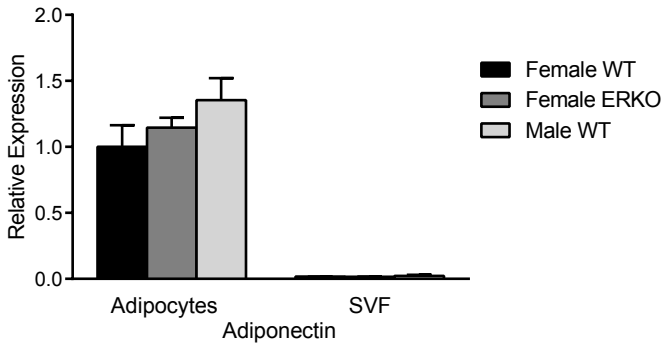
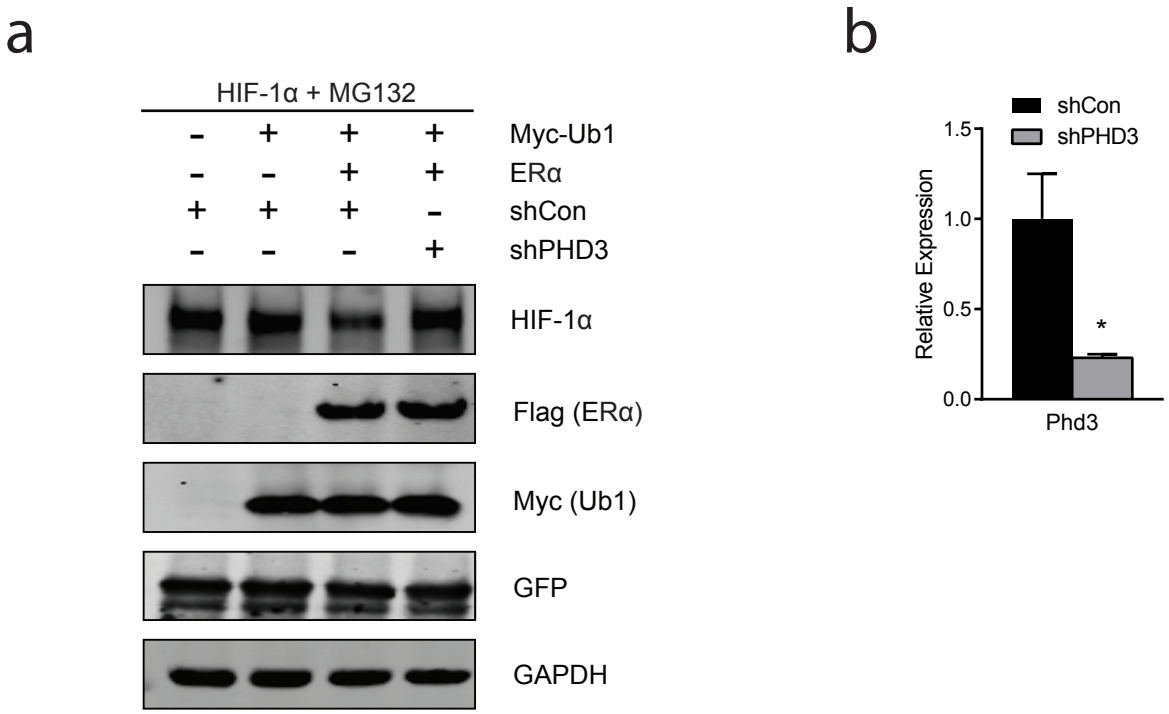
Supplementary Figures 1 to 4 and Tables 1 to 2:

a



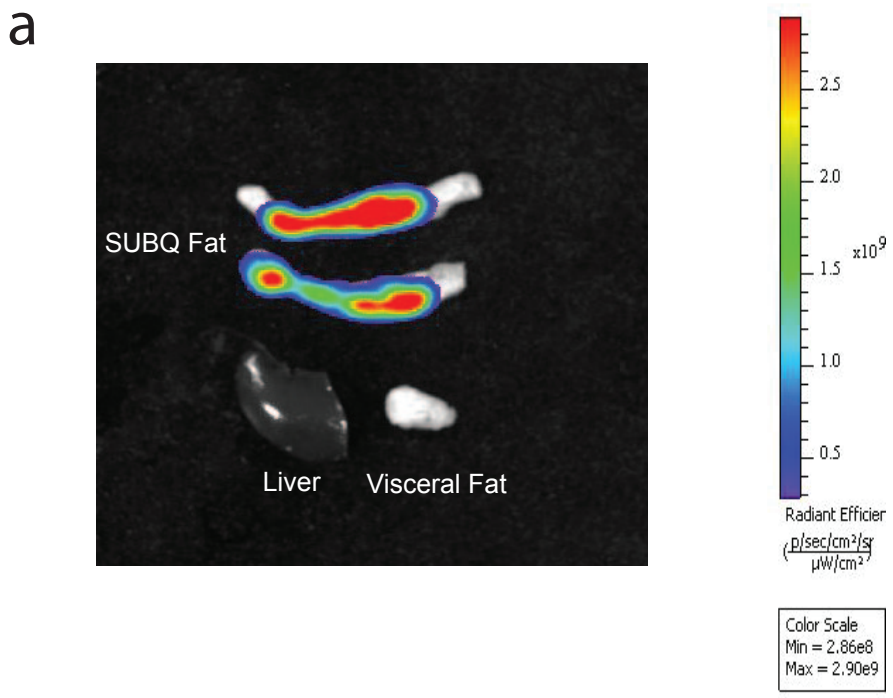
Supplementary Figure 1. Isolation of adipocytes and stromal vascular fraction.

(a) qPCR quantification of Adiponectin mRNA from adipocyte and stromal-vascular fractions of inguinal AT (experiment in Figure 1e demonstrates effective separation of adipocytes from stromal vascular cells). Data presented as mean ± SEM. All SVF values significantly different ($P < 0.05$) as determined by two-tailed Student's t test relative to female WT adipocytes.



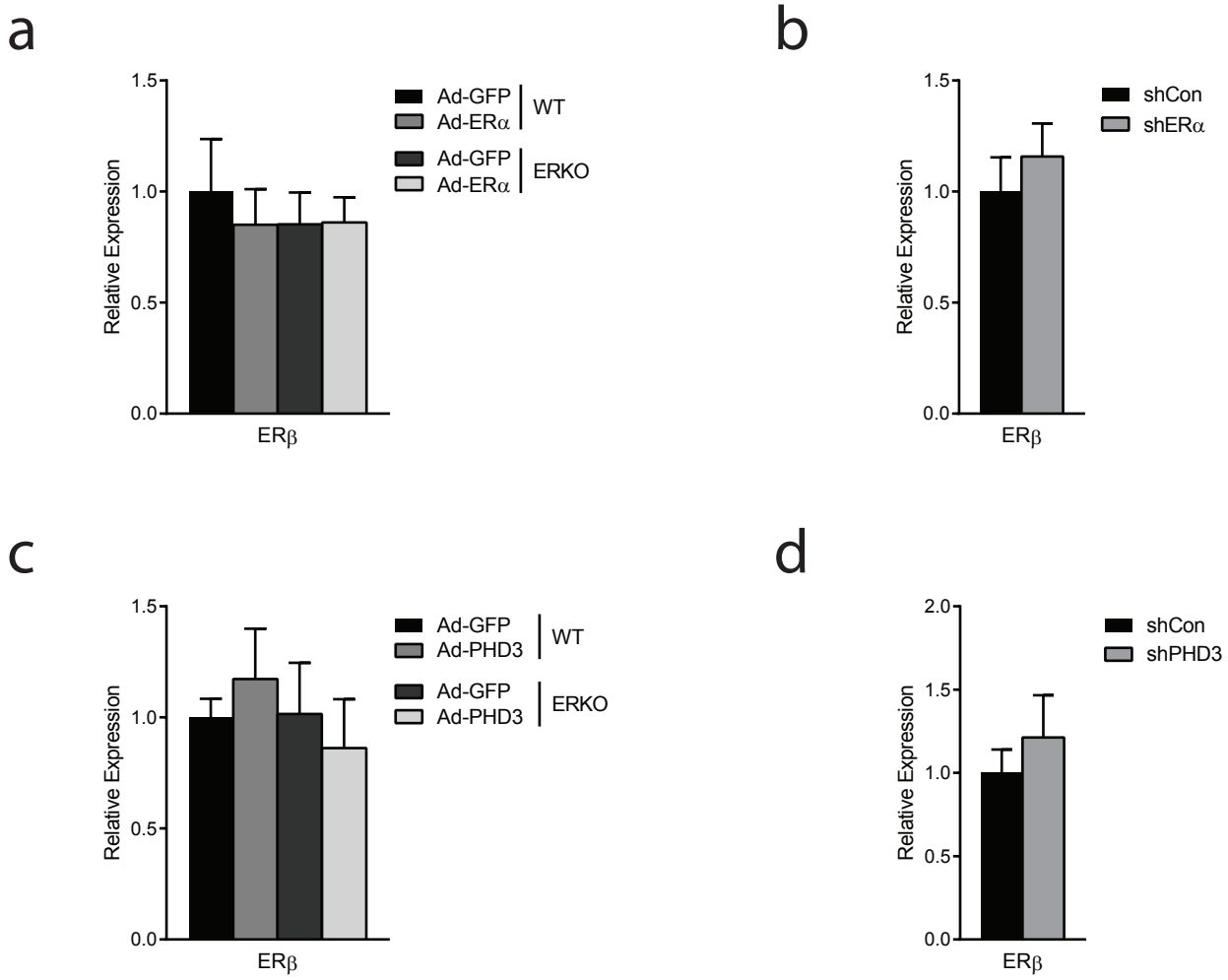
Supplementary Figure 2. ERα increases ubiquitination of HIF-1α in a PHD3-dependent manner.

(a) Additional western blots from experiment in Figure 2d: HIF-1α, exogenous ERα detected by FLAG tag, exogenous Ubiquitin detected by Myc, exogenous GFP, and the loading control GAPDH. **(b)** qPCR quantification of PHD3 mRNA from figure 2d. Data presented as mean ± SEM. * $P < 0.05$ by two-tailed Student's t test between shCon and shPHD3. Data are representative of results from 3 independent experiments.



Supplementary Figure 3. Validation of adenoviral overexpression vector injections.

(a) Representative images of injected mice from experiments in Figures 3-6 showing fluorescence produced by exogenous GFP specifically in fat pads but not in the liver or visceral fat. **(b)** Additional representative western blots from experiment in Figure 3: ERα, exogenous ERα detected by FLAG tag, exogenous GFP, and the loading control GAPDH. **(c)** Additional representative western blot from experiment in Figure 4 with ERα and GAPDH.



Supplementary Figure 4. ERβ expression in adenoviral injection experiments

qPCR quantification of ERβ in inguinal AT from experiments in Figure 3 **(a)** Figure 4 **(b)** Figure 5 **(c)** and Figure 6 **(d)**. Data presented as mean ± SEM.

Supplementary Table 1. Candidate Estrogen Response Elements (ERE) in promoter of PHD3 and primers used in PCR of ChIP assay.

Candidate ERE	Location	Sequence	Forward (5' – 3')	Reverse (5' – 3')
-2038	-2038 to -2024	TGTCCCTGCAGATCA	caatctccaaagctgcaaaagc	cttcccactgttatctgagccc
-1832	-1832 to -1818	TGGCCGCTGAGGTCT	ggatgggacaggacagctgt	acctttgcaagcagtactgagg
-1005	-1005 to -0991	AAGCCAGAGAGACCT	cttttgggtagggcctaggg	tcctgcgggagatgttcat
-599	-0599 to -0585	TCACCAGAGAGGTGA	gccagacccgaatcaaacag	cacgtctgagatgcacatgaac

Supplementary Table 2. Primers used for RT-qPCR.

Assay	Forward (5' – 3') or TaqMan ID	Reverse (5' – 3')
mPHD1	tgcctgggtagaaggtcacg	gcgccattgatgacgtagt
mPHD2	tctggtctgaccggcgtaac	agctctcgctcgctcatctgt
mPHD3-endo	tgaagaaagggcagaagcca	ttactacacgaatgcggccat
mPHD3-total	ttgggacgccaagttacacg	tggcataggagggctggactt
mCol1a1	gtgctcctggtattgctggt	ggctcctcgttttccttctt
mCol3a1	gggtttccctggtcctaaag	cctggtttcccattttctcc
mCol6a1	gatgagggtgaagtgggaga	cagcacgaagaggatgtcaa
mLOX	ccacagcatggacgaattca	agcttgctttgtggccttca
mGAPDH	aggtcggtgtgaacggatttg	tgtagaccatgtagttgaggtca
mHPRT	aagcctaagatgagcgcaag	Ttactaggcagatggccaca
mERβ	ctgttactagtccaagcgcca	cccagatgcataatcactgca
mB2M	Mm00437762_m1	
hERα	Hs00174860_m1	
mERα	Mm00433148_m1	
mTNFα	Mm00443260_m1	
mIL6	Mm00446190_m1	
mIL1b	Mm00434228_m1	
mTLR4	Mm00445273_m1	