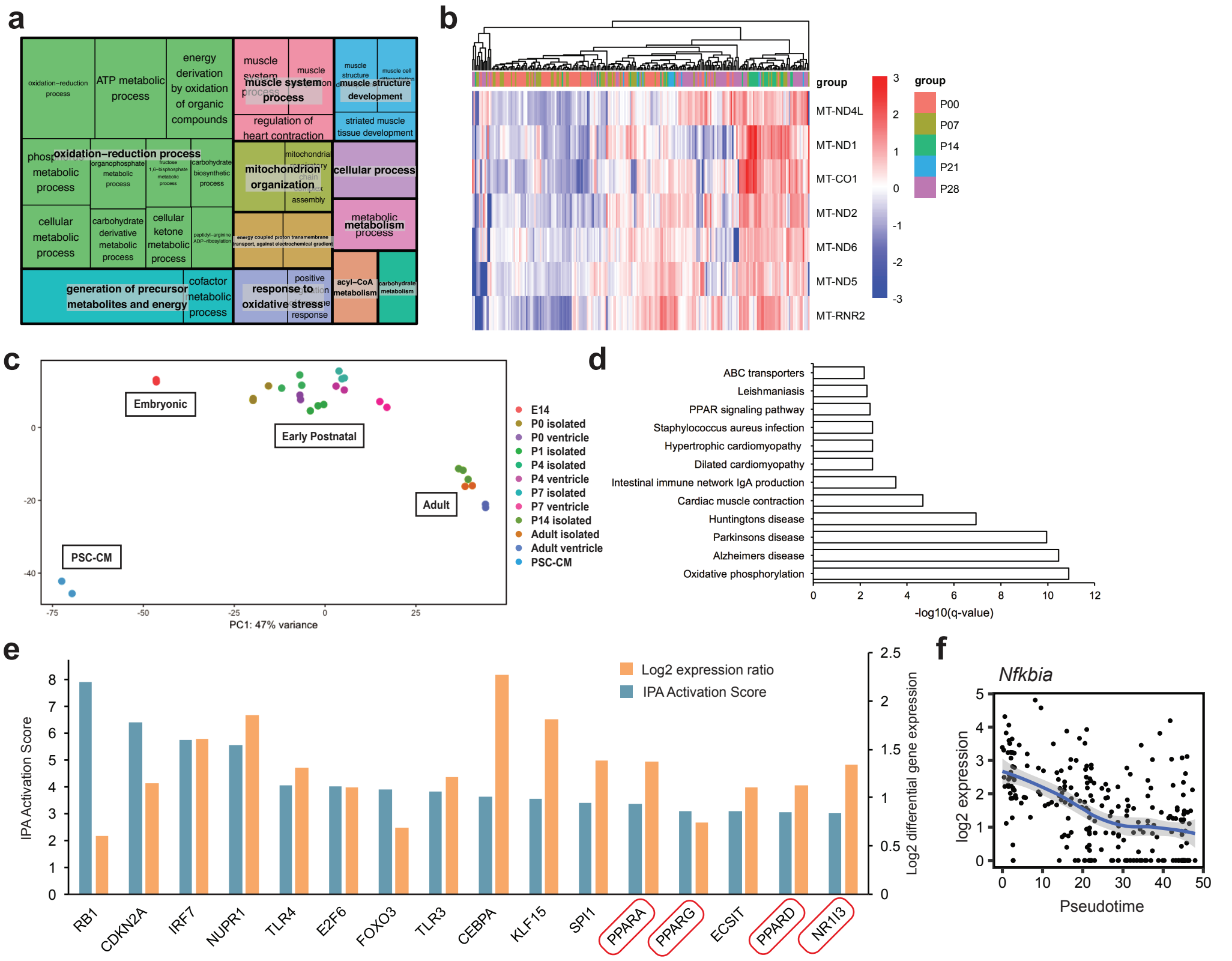


Fig. S1: scRNA-seq clustering and meta-analysis of bulk RNA-seq

a, Treemap of GO terms of differentially expressed genes from P0 to P28 with box size representing the -log10 p-value. **b,** Heatmap of mitochondrial gene expression with hierarchical clustering of time-points. **c,** PCA plot of two components of bulk RNA-seq datasets of cardiac maturation (GSE64403, GSE47948, GSE95762, GSE79883). **d,** KEGG pathway mapping of differentially expressed genes from neonatal to adult CMs. **e**, Top IPA transcriptional regulators with log fold change (orange) and IPA activation score (blue) visualized. PPAR family nuclear receptors are among the top hits. **f,** Expression trend over pseudotime of NFκBIα in postnatal CMs. Interval represents 95% confidence interval.

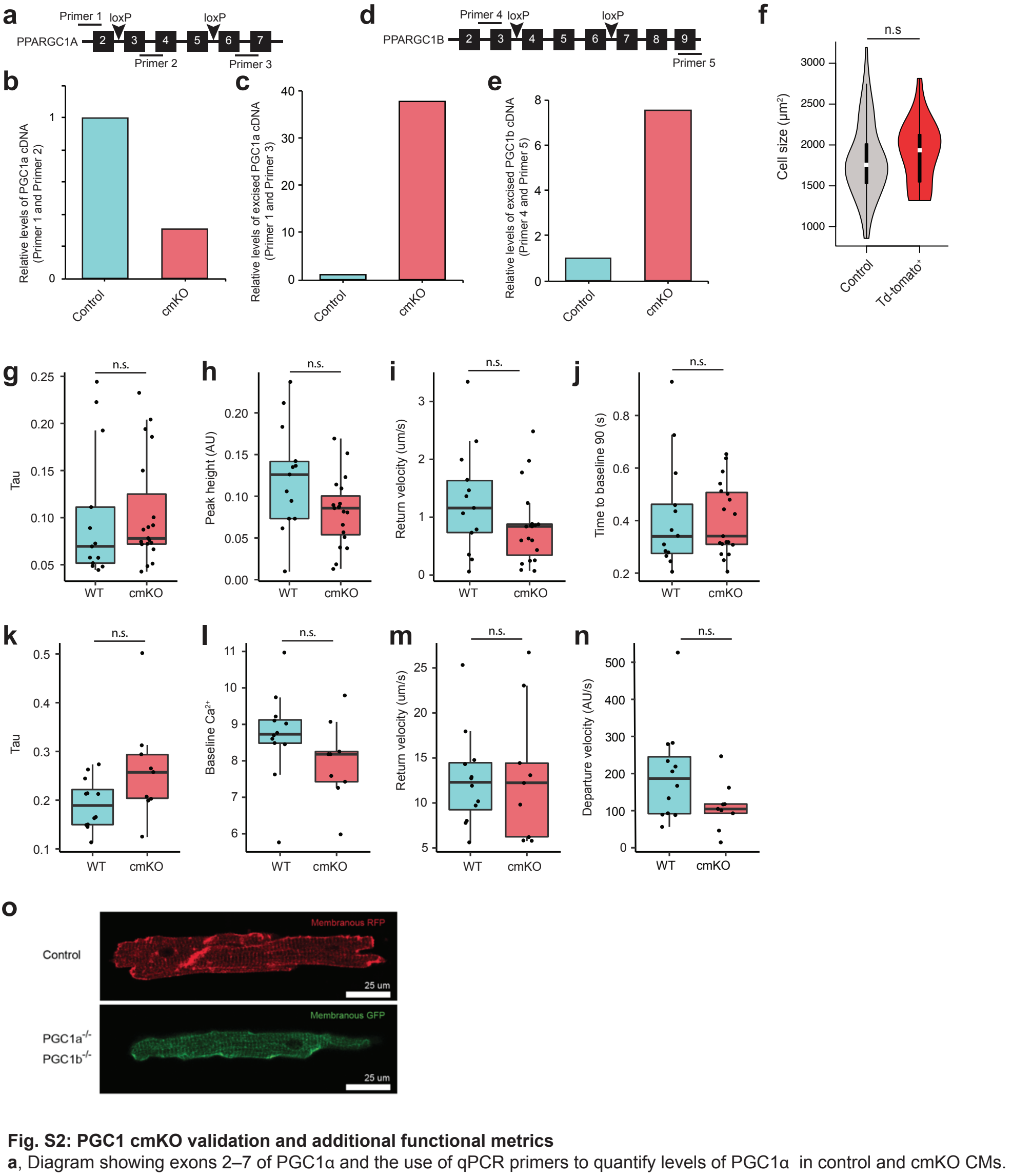
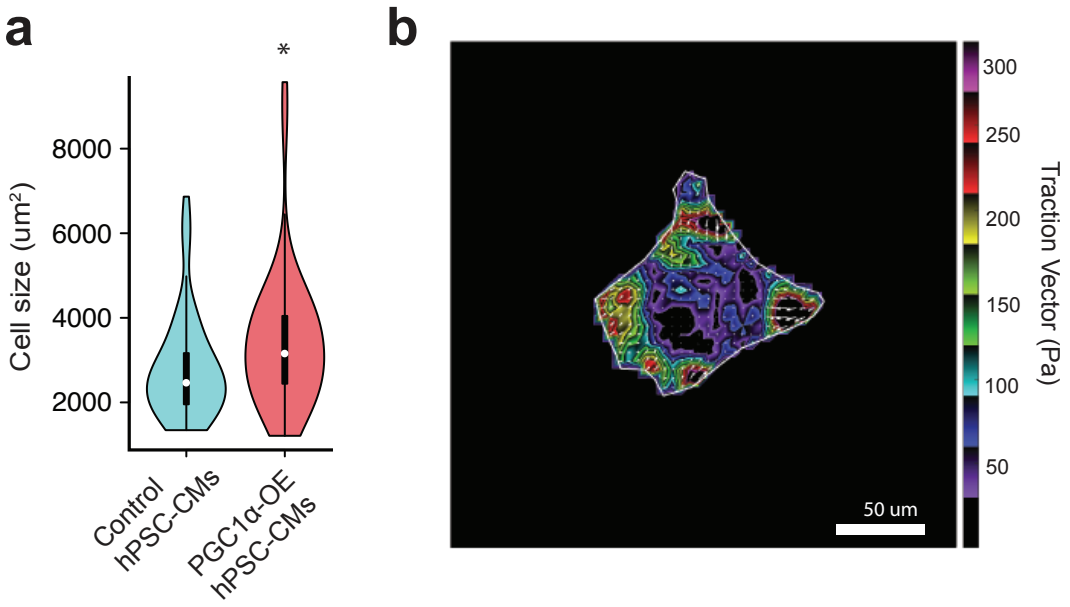


Fig. S2: PGC1 cmKO validation and additional functional metrics

a, Diagram showing exons 2–7 of PGC1α and the use of qPCR primers to quantify levels of PGC1α in control and cmKO CMs. **b**, Normalized levels of PGC1α in control (teal) and cmKO (red) CMs. **c**, Normalized levels of excised PGC1α. **d**, Diagram showing exons 2-9 of PGC1β and the use of qPCR primers to quantify levels of PGC1β. **e**, Normalized levels of excised PGC1β. f, Cell size measurements for myocytes isolated from p28 hearts of Ai9 mice injected with AAV9-cTnT-Cre at p0 (p-value=0.33). Control n=87, Td-tomato n=26 Student's t-test was used. **g–j**, Contractility parameters for control and PGC1 cmKO CMs. control n=13, cmKO n=19. (p-value=(g) 0.87, (h) 0.056, (i) 0.15 (j) 0.86) Student's t-test was used. **k–n**, Calcium transient parameters for control and PGC1 cmKO CMs. control n=12, cmKO n= 9. (p-value=(k) 0.094, (l) 0.166, (m) 0.89 (n) 0.062). AUs are arbitrary units. **o**, Representative images of control (membranous RFP) and PGC1-deficient (membranous GFP) P28 mouse CMs showing maintenance of t-tubule structure. scale bars = 25 µm. Student's t-test was used in panels g-j and Mann-Whitney-Wilcoxon test was used in panel n. Box-and-whiskers plot represents the maxima, 75th percentile, median, 25th percentile, and minima. N.S. p-value>0.05.



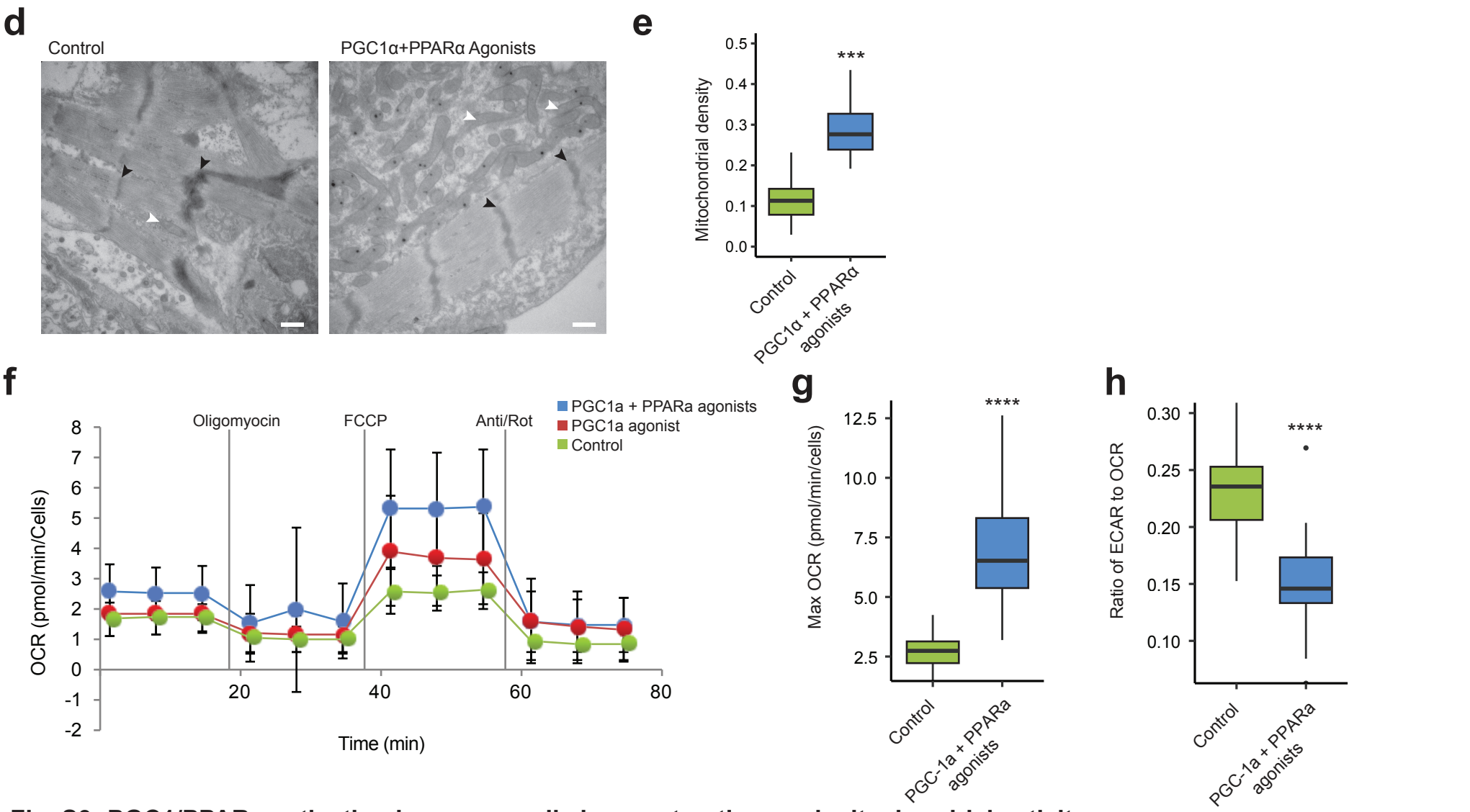


Fig. S3: PGC1/PPARα activation increases cell size, contraction, and mitochondrial activity

a, Cell area quantification of human PSC-CMs transfected with control (teal) and GFP-PGC1 (red) expression construct, measured 4 days after transfection (p-value=0.037). Student's t-test was used. Control n=55, GFP-PGC1 n=46. **b**, Fourier transform traction force microscopy showing force vectors during PSC-CM contraction. **c**, Images of day 30 human PSC-CMs treated with PGC1/PPARα agonists for 2 weeks after differentiation. **d**, Electron microscopy images of control and PGC1/PPARα agonist-treated day 30 PSC-CMs. Scale bar = 500 nm. White arrowheads show mitochondria and black arrowheads show z-bands. **e**, Mitochondrial density quantified from EM images (p-value =0.00017). Student's t-test was used. Control (green) n=9, Treated (blue) n=15
f, Seahorse XF96 measurements of oxygen consumption rate in control and agonist-treated d30 PSC-CMs. **g**, quantification of maximum oxygen consumption rate (p-value=1.4e-6) Control (green) n=17, Treated (blue) n=18 Student's t-test was used.
h. Ratio of ECAR to OCR (p-value=5.5e-6). Student's t-test was used. Control (green) n=17, Treated (blue) n=18. *>0.05, ***>0.001, ****>0.0001

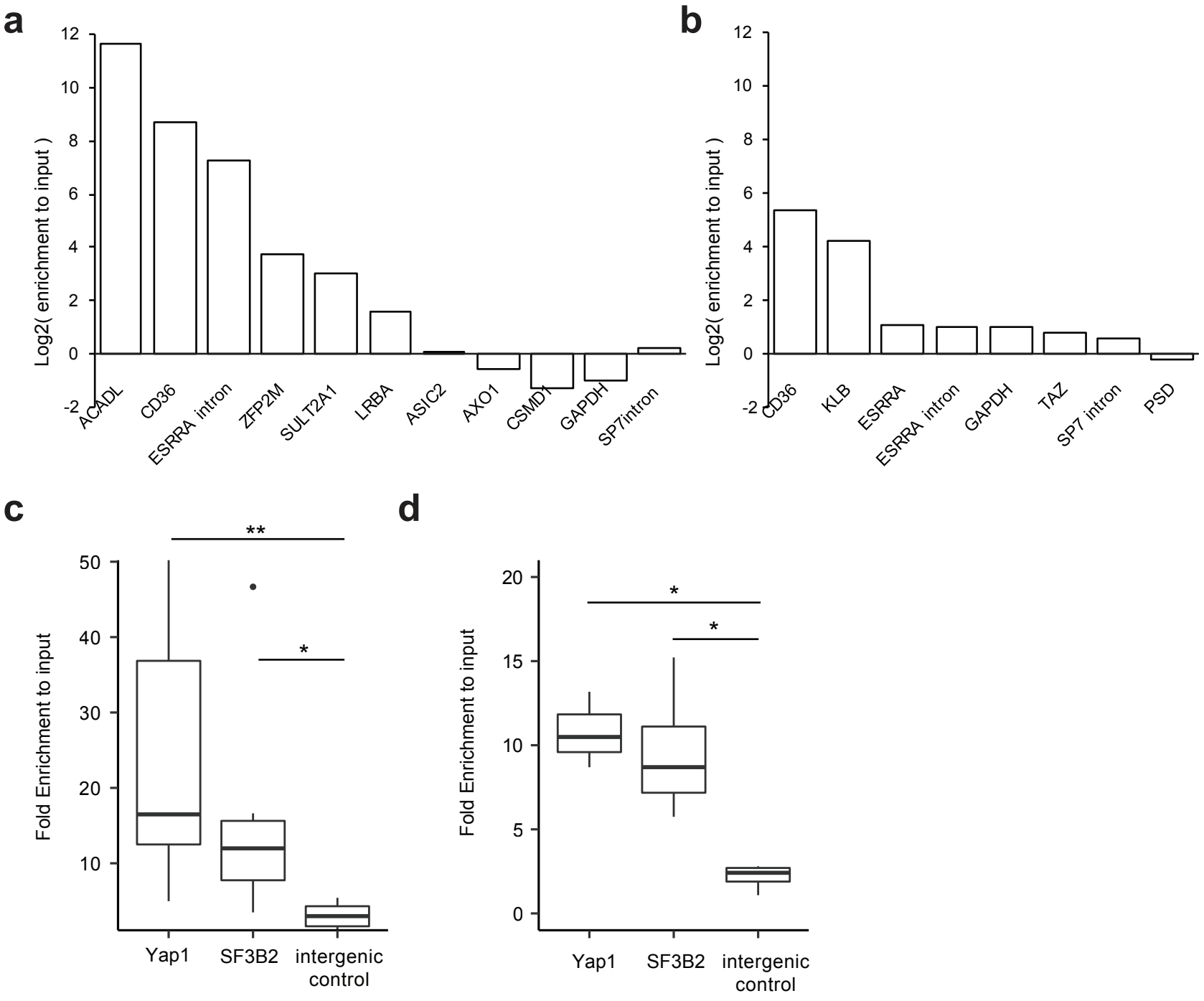


Fig. S4: PGC1/PPARα are physically associated with the promoters of Yap1/Sf3b2
a, b, Antibody validation for chromatin immunoprecipitation (ChIP). ChIP analysis showing enrichment of promoter sequences of known targets of PGC1/PPARα and control DNA regions (GAPDH, SP7 intron, ESRRA intron). **c, d**, PGC1/PPARα ChIP-qPCR analysis showing fold enrichment of Yap1 and SF3B2 promoter regions compared to an intergenic control (p-value= (c) 0.029, 0.029, (d) 0.0043, 0.015). (c) n=4, (d) n=6. Mann-Whitney-Wilcoxon test was used. p-value *<0.05, **<0.01

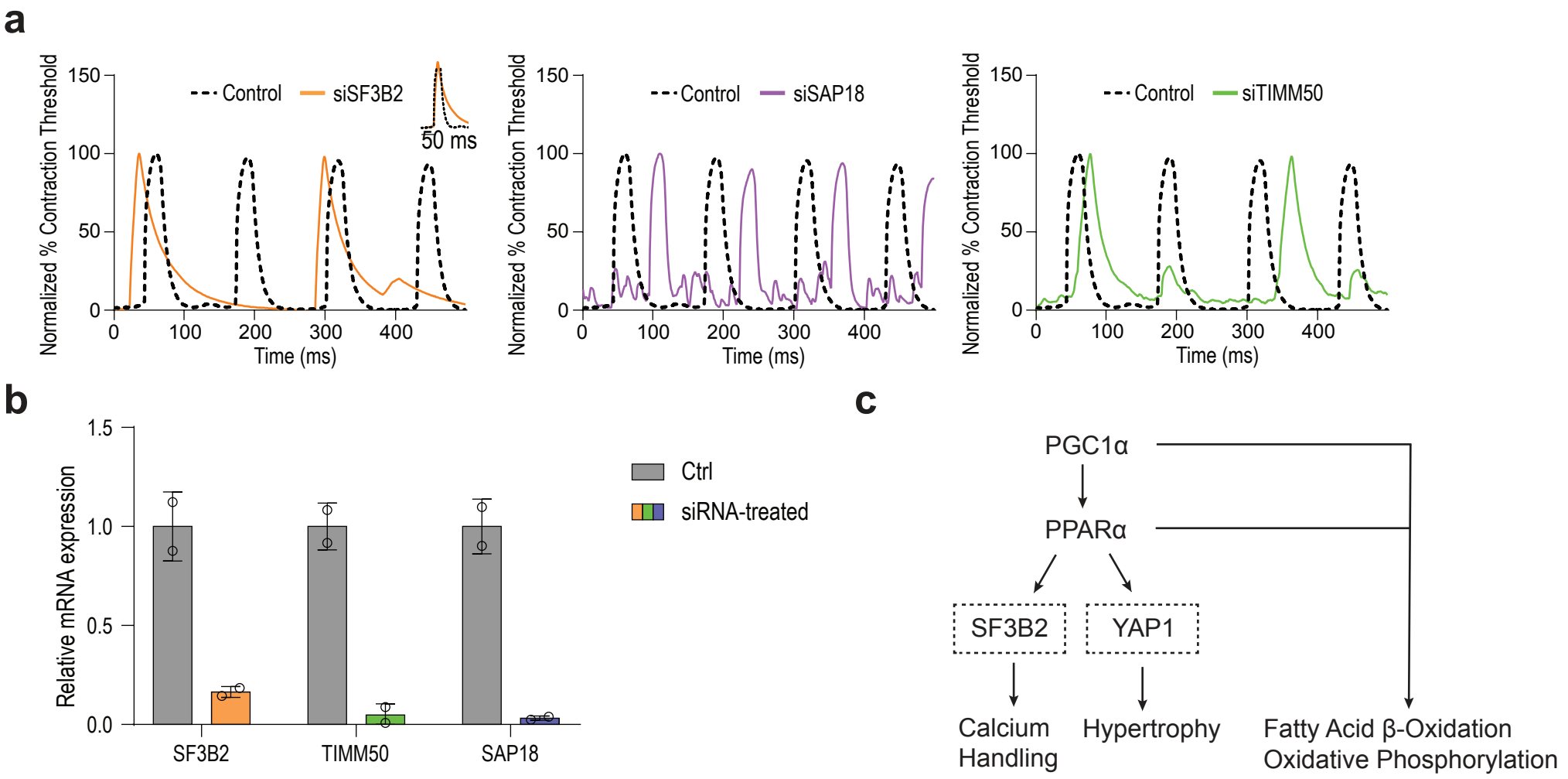


Fig. S5: Contractility analysis, siRNA efficiency, and working model
a, Effects of validated hits on contractility with traces from initial analysis shown by normalized percentage of contraction threshold. **b**, Knockdown efficiency of validated hits SF3B2 (orange), TIMM50 (green), and SAP18 (purple). n=2 **c**, Working model on postnatal CM maturation.