Cell Reports, Volume *43*

Supplemental information

The zebrafish heart harbors a thermogenic beige fat depot analog of human epicardial adipose tissue

Paul-Andres Morocho-Jaramillo, Ilan Kotlar-Goldaper, Bhakti I. Zakarauskas-Seth, Bettina Purfürst, Alessandro Filosa, and Suphansa Sawamiphak

Supplemental Information

Supplementary Figures

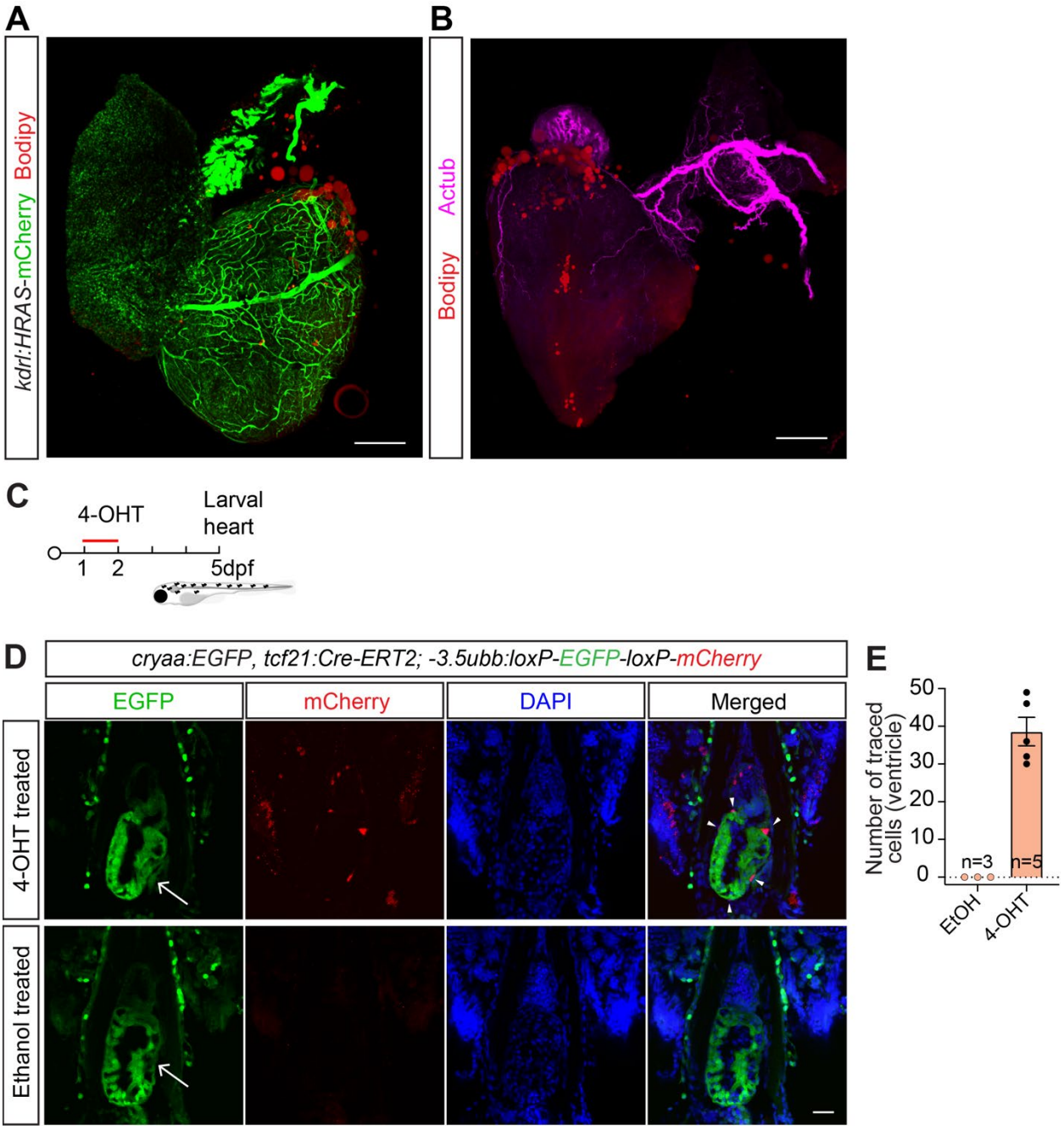

Figure S1 (related to Figure 1). Localization and lineage tracing of epicardial adipocytes in zebrafish. (A) Z-projection of a *kdrl:HRAS-mCherry* zebrafish heart,

showing vascularization of adipose tissue with coronary vessels, visualized by Bodipy staining and mCherry immunostaining, respectively. Scale bar, 200 μm. **(B)** Z-projection of a zebrafish heart, in which autonomic nerves and adipocytes were visualized by immunofluorescent staining of acetylated tubulin (Actub) and staining with Bodipy. Scale bar, 200 μm. **(C)** Schematic illustration of Cre-mediated pulse-chase lineage tracing of Tcf21+ proepicardial cells. **(D)** *cryaa:EGFP; tcf21:Cre-ERT2; ubb:loxP-EGFP-loxP-mCherry* larval hearts (arrows), treated with 4-hydroxy tamoxifen (4-OHT) and ethanol at 1-2 dpf. Ubiquitous expression of EGFP and tcf21-derived mCherry expressing cells (arrowheads) were examined by immunofluorescent staining at 5 dpf. Scale bar 30 μm. **(E)** Bar graph showing numbers of *tcf21:Cre-ERT2*-traced cells in the ventricle at 5 dpf. Data are presented as mean ± S.E.M. n indicates number of animals.

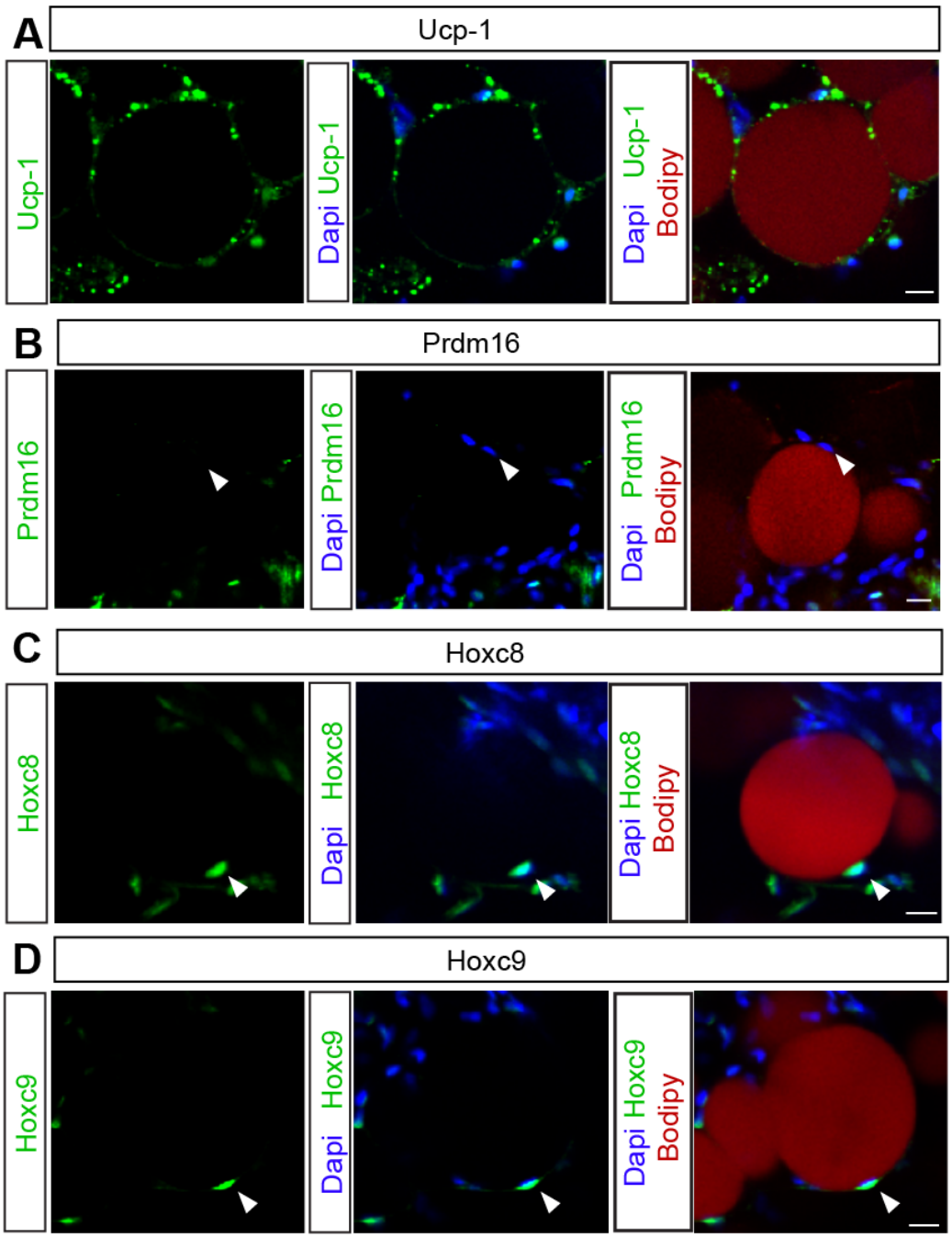

Figure S2 (related to Figure 2). Expression of white and brown adipocyte markers in zebrafish epicardial adipocytes. (A-D) Confocal images of whole-mounted zebrafish hearts, immunostained for Ucp1, Prdm16, Hoxc8, and Hoxc9, and stained with BODIPY 558/568 C12 and DAPI. White arrowheads indicate nuclear localization of the proteins in adipocytes. Scale bar, 10 μm.

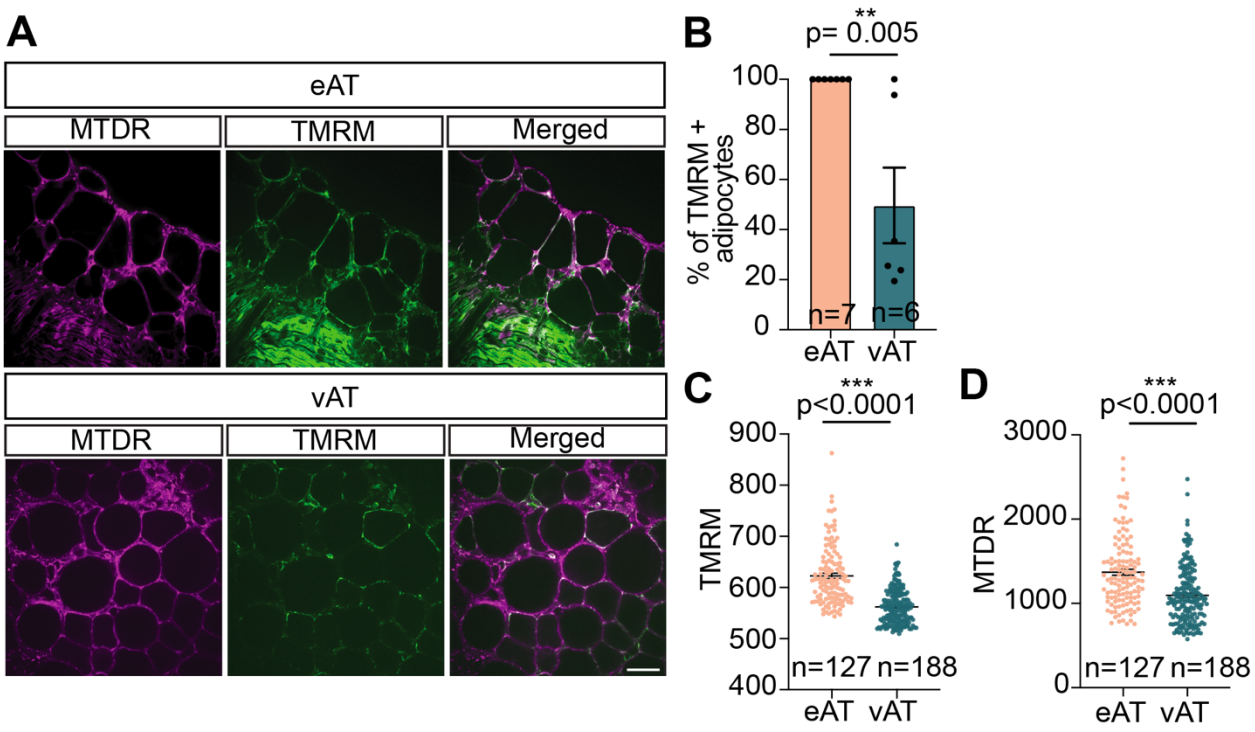

Figure S3 (related to Figure 2). **Higher content of active mitochondria in zebrafish eAT, as compared to white adipose tissue. (A)** Confocal images showing epicardial adipose tissue (eAT) and abdominal visceral adipose tissue (vAT) explants, in which active mitochondria were labelled with mitotracker deep red (MTDR) and Tetramethylrhodamine, methyl ester (TMRM). Scale bar, 50 µm. **(B)** Bar graph depicting lower percentages of TMRM-positive adipocytes in vAT explants. **(C, D)** Bar graphs depicting lower densities of TMRM **(C)** and MTDR **(D)** positive mitochondria in eAT and vAT adipocytes. n indicates number of animals in (B) or number of analyzed adypocites from five fish per condition in **(C, D)**. Data are presented as mean ± S.E.M. **p < 0.01, ***p < 0.001, two-tailed t-test. n indicates number of animals.

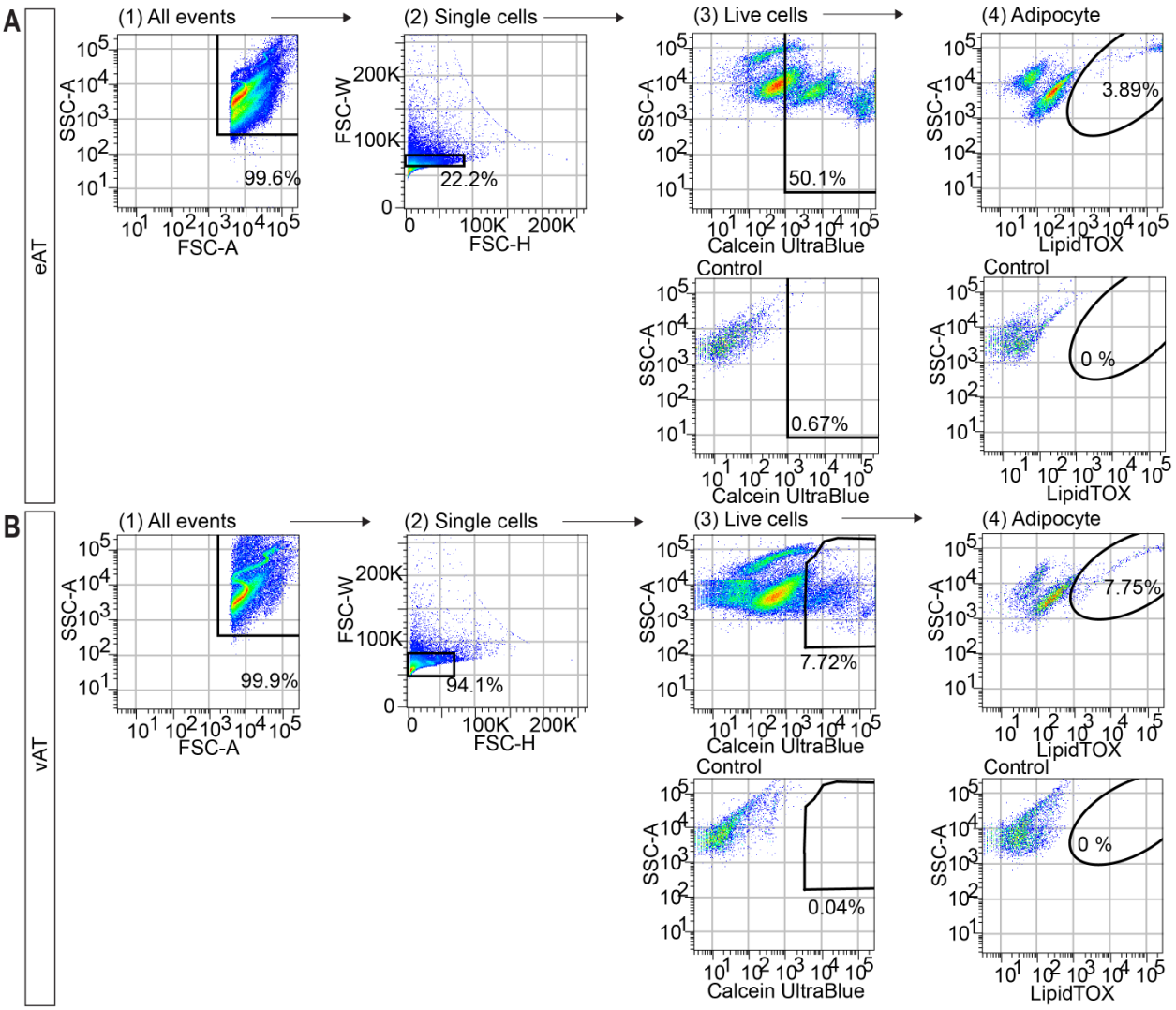

Figure S4 (related to Figure 3). **Gating strategy of adipocytes.** eAT **(A)** and vAT **(B)** were isolated by flow cytometry for analysis of thermogenesis activity by ErthermAC.

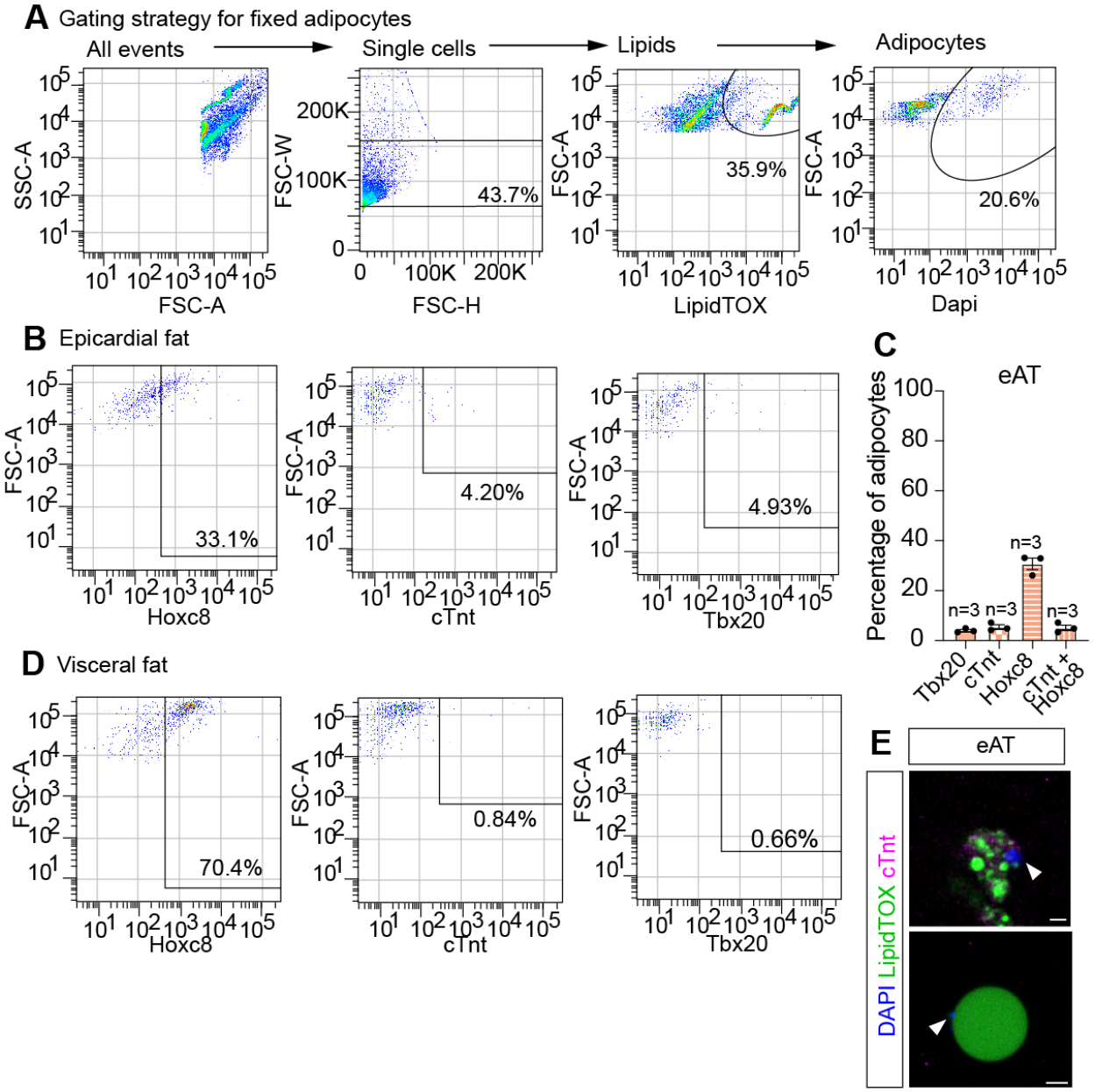

Figure S5 (related to Figure 4). Assessment of cardiac development and homeostasis gene expression in epicardial adipocytes by flow cytometry and immunofluorescence. (A and **B)** Subpopulations of adipocytes from epicardial fat, labelled with LipidTox and DAPI **(A)** express Hoxc8, cTnt, and Tbx20 **(B)**. **(C)** Bar graph depicting percentages of Hoxc8+, cTnt+, Tbx20+, and cTnt+Hoxc8+ per total adipocytes in the eAT. Data are presented as mean ± S.E.M. n indicates number of animals. **(D)**

Majority of vAT adipocytes were Hoxc8+, but did show detectable cTnt or Tbx20 expression. **(E)** Representative images of dissociated epicardial adipocytes, visualized by lipidTox accumulation, some of which express cTnt, detected by immunofluorescent staining. Arrowheads indicate adipocyte nuclei. Scale bar, 4 μm. Upper panel showed epicardial adipocyte co-expressed cTnt. cTnt was not detectable in adipocyte shown in the lower panel. White arrowheads indicates adipocyte nuclei labelled with DAPI.

Genes shown in Figure 5D

upregulated in human and zebrafish eAT (top20)

human_ID	zebrafish_ID	human_log2FC	zebrafish_log2FC
ENSG00000164532	ENSDARG00000005150	10,2211	9,3887
ENSG00000130700	ENSDARG00000017821	7,8020	1,9722
ENSG00000183421	ENSDARG00000043211	5,1737	0,4910
ENSG00000188176	ENSDARG00000055632	4,5488	4,2133
ENSG00000147573	ENSDARG00000058158	4,0264	8,7259
ENSG00000101311	ENSDARG00000052652	3,9087	0,2258
ENSG00000118526	ENSDARG00000036869	3,8240	2,4209
ENSG00000096696	ENSDARG00000076673	3,4611	1,7242
ENSG00000153820	ENSDARG00000017429	3,4018	4,8271
ENSG00000117707	ENSDARG00000055158	3,3991	1,6897
ENSG00000124772	ENSDARG00000070919	3,3840	4,3632
ENSG00000215218	ENSDARG00000079276	3,2513	7,1007
ENSG00000079931	ENSDARG00000031136	2,9777	3,5533
ENSG00000105509	ENSDARG00000042983	2,9573	5,1667
ENSG00000169071	ENSDARG00000076227	2,8838	3,2004
ENSG00000167971	ENSDARG00000046107	2,7586	0,9878
ENSG00000103888	ENSDARG00000039881	2,6991	1,8262
ENSG00000106278	ENSDARG00000020871	2,5553	4,1617
ENSG00000141639	ENSDARG00000110581	2,5456	1,6705
ENSG00000162631	ENSDARG00000073713	2,4122	0,2522

upregulated in human sAT and zebrafish vAT(top20)

human_ID	zebrafish_ID	human_log2FC	zebrafish_log2FC
ENSG00000037965	ENSDARG00000070346	-8,9036	-9,1642
ENSG00000180806	ENSDARG00000092809	-7,3938	-7,6380
ENSG00000120068	ENSDARG00000056027	-6,4626	-3,2247
ENSG00000170689	ENSDARG00000056023	-5,1066	-2,7830
ENSG00000106004	ENSDARG00000102501	-4,6856	-4,3149
ENSG00000131771	ENSDARG00000076280	-4,3914	-0,9056
ENSG00000145824	ENSDARG00000056627	-3,6315	-3,9584
ENSG00000155966	ENSDARG00000052242	-2,9630	-1,5614
ENSG00000175161	ENSDARG00000062633	-2,8711	-6,6697
ENSG00000185818	ENSDARG00000077256	-2,4250	-3,0299
ENSG00000141449	ENSDARG00000039196	-2,2469	-4,0214
ENSG00000130876	ENSDARG00000008100	-2,1133	-2,3330
ENSG00000170786	ENSDARG00000016233	-2,0903	-1,8126
ENSG00000079689	ENSDARG00000058732	-1,9503	-1,1526
ENSG00000162444	ENSDARG00000070486	-1,9401	-1,7417
ENSG00000124003	ENSDARG00000086481	-1,8104	-1,9932
ENSG00000158516	ENSDARG00000043722	-1,7426	-17,3074
ENSG00000105088	ENSDARG00000102825	-1,6073	-0,8890
ENSG00000278535	ENSDARG00000004141	-1,4856	-3,7050
ENSG00000010319	ENSDARG00000007560	-1,4552	-2,0455

upregulated in human eAT but downregulated in zebrafish eAT (top20)

human_ID	zebrafish_ID	human_log2FC	zebrafish_log2FC
ENSG00000081052	ENSDARG00000002831	4,0948	-0,8197
ENSG00000151224	ENSDARG00000039605	4,0894	-9,0946
ENSG00000078725	ENSDARG00000078302	3,7369	-0,1313
ENSG00000112486	ENSDARG00000038968	3,7304	-4,6057
ENSG00000015413	ENSDARG00000068181	3,7074	-1,3552
ENSG00000166922	ENSDARG00000032126	3,5769	-1,7886
ENSG00000109072	ENSDARG00000053831	3,4093	-4,0363
ENSG00000156510	ENSDARG00000038703	3,3469	-2,3410
ENSG00000276418	ENSDARG00000061713	3,3404	-2,5828
ENSG00000099958	ENSDARG00000033871	3,1524	-2,4378
ENSG00000106018	ENSDARG00000012353	2,9349	-6,6642
ENSG00000154639	ENSDARG00000043658	2,8708	-1,4678
ENSG00000172367	ENSDARG00000040568	2,6993	-4,1138
ENSG00000162366	ENSDARG00000017127	2,6565	-0,9768
ENSG00000160183	ENSDARG00000036545	2,6512	-4,3100
ENSG00000129354	ENSDARG00000096454	2,5621	-4,3596
ENSG00000099960	ENSDARG00000068286	2,5420	-4,3945
ENSG00000164142	ENSDARG00000061021	2,4855	-0,2819
ENSG00000005513	ENSDARG00000037782	2,4663	-5,5458
ENSG00000178882	ENSDARG00000023484	2,4145	-1,5737

upregulated in zebrafish eAT but downregulated in human eAT (top20)

human_ID	zebrafish_ID	human_log2FC	zebrafish_log2FC
ENSG00000170549	ENSDARG00000101831	-5,7771	6,1882
ENSG00000170561	ENSDARG00000001785	-4,2691	2,2609
ENSG00000143320	ENSDARG00000030449	-3,2008	2,5615
ENSG00000075035	ENSDARG00000061819	-2,8542	3,3995
ENSG00000004939	ENSDARG00000012881	-2,8294	4,6586
ENSG00000164708	ENSDARG00000057571	-2,7617	10,9069
ENSG00000150893	ENSDARG00000102626	-2,7473	3,6225
ENSG00000176842	ENSDARG00000034043	-2,4764	2,8443
ENSG00000134317	ENSDARG00000061391	-1,9848	2,1631
ENSG00000085741	ENSDARG00000014796	-1,9316	6,6748
ENSG00000133110	ENSDARG00000043806	-1,8503	8,2995
ENSG00000169047	ENSDARG00000054087	-1,7975	1,8073
ENSG00000166165	ENSDARG00000043257	-1,5554	2,1312
ENSG00000153162	ENSDARG00000015686	-1,4321	2,8131
ENSG00000158458	ENSDARG00000077818	-1,3634	1,7280
ENSG00000089225	ENSDARG00000024894	-1,2430	8,6155
ENSG00000035664	ENSDARG00000061096	-1,2353	3,6422
ENSG00000172572	ENSDARG00000004227	-1,2172	2,9232
ENSG00000166148	ENSDARG00000045788	-1,1960	2,4579
ENSG00000285854	ENSDARG00000019644	-1,1676	4,3786

Figure S6 (related to Figure 5). Top 20 most differentially expressed genes in young zebrafish and aged human eAT, as compared to classical white adipose tissues, identified by RNA sequencing (RNA-Seq). Fold changes of all orthologues, indicated by Ensembl gene IDs, are displayed as log base 2 values.

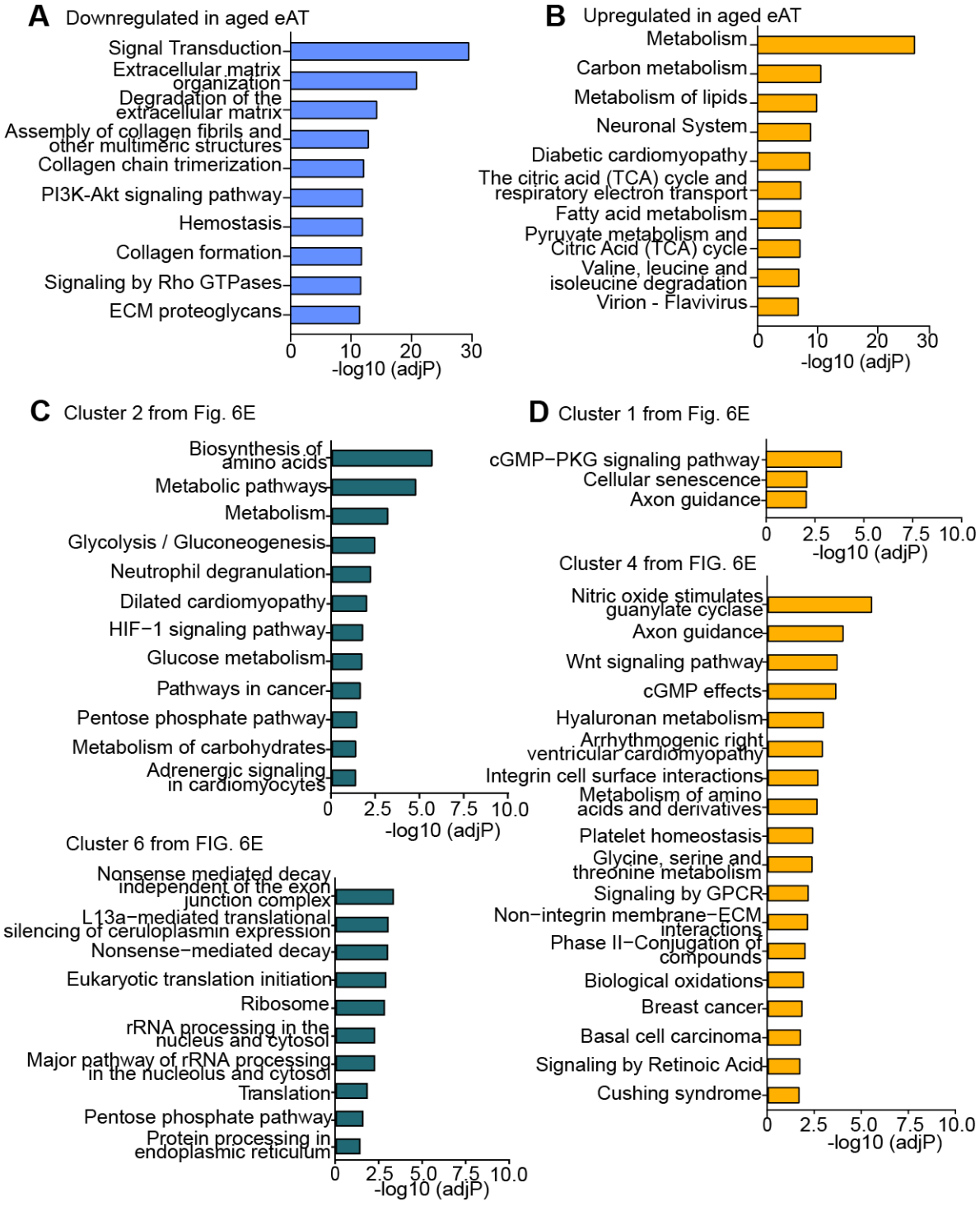

Figure S7 (related to Figure 6). Highly represented pathways of up- and downregulated genes from cross-species analysis of whole transcriptomic profile

of eAT. (A and **B)** Enriched pathways associated with downregulated **(A)** and upregulated **(B)** genes in aged as compared to young mouse eAT. **(C** and **D)** Significantly overrepresented pathways based on gene clusters with similar expression in human and zebrafish (cluster 2 and 6 from Fig. 6E) **(C)** or those with similar expression in human and mouse (cluster 1 and 4 from Fig. 6E) **(D)**. All pathway enrichment analyses were done using KEGG and REACTOME databases. Enrichment is expressed as the -log[P] adjusted for multiple comparisons. Only DEGs with log2 fold change score more than 0.6 were included in the analysis.