



Supplementary

# Effects of Whole-Body Adenylyl Cyclase 5 (*Adcy5*) Deficiency on Systemic Insulin Sensitivity and Adipose Tissue

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## Supplemental Tables

**Table S1.** Primer pairs used for PCR and qRT-PCR

| Gene             | forward (3' - 5')      | reverse (3' - 5')     | product size [bp] |         |
|------------------|------------------------|-----------------------|-------------------|---------|
| <i>Adcy5</i> -WT | AGTTCCCGTCGGACAAACTG   | AGCACGGCCATGATAAGGAT  | 202               | PCR     |
| <i>Adcy5</i> -KO | ATCAGCAGCCTCTGTTCCAC   | AGCACGGCCATGATAAGGAT  | 152               |         |
| <i>36b4</i>      | ACTGGTCTAGGACCCGAGAAG  | TCAATGGTGCCTCTGGAGATT | 77                | qRT-PCR |
| <i>Adcy5</i>     | TGAACCAGAGCAGTCTCACCAT | GCGCCGCGTGGAAAG       | 75                |         |

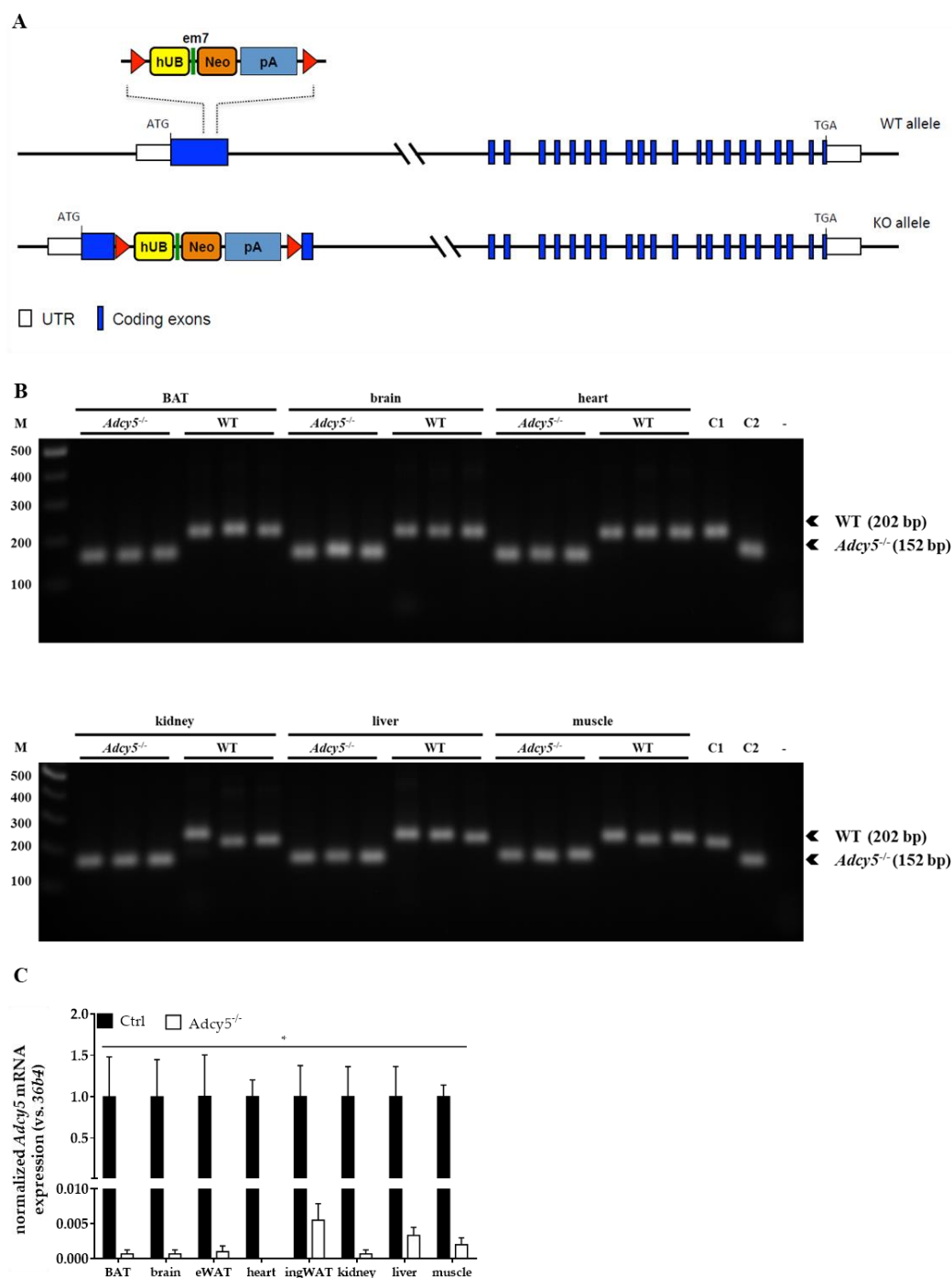
**Table S2.** Genes differentially expressed between *Adcy5*<sup>-/-</sup> and Ctrl eWAT of mice on CD.

|                       | log<br>fold<br>change | average<br>expres-<br>sion | t       | p<br>value | adjusted<br>p value | B       | gene sym-<br>bol | gene name   |
|-----------------------|-----------------------|----------------------------|---------|------------|---------------------|---------|------------------|---|
| f<br>e<br>m<br>a<br>l | -2.1350               | 9.9576                     | -9.5430 | 0.0000     | 0.0002              | 8.2296  | Mid1             | midline 1   |
|                       | -1.2712               | 9.8360                     | -4.7226 | 0.0002     | 0.0666              | 0.8772  | C4a              | complement component 4A (Rodgers blood group)                                   |
|                       | -1.2594               | 6.8913                     | -5.0437 | 0.0001     | 0.0522              | 1.4808  | Frzb             | frizzled-related protein  |
|                       | -1.1919               | 8.1372                     | -3.0935 | 0.0066     | 0.2112              | -2.2818 | Cybrd1           | cytochrome b reductase 1  |
|                       | -1.1650               | 6.9036                     | -3.0256 | 0.0076     | 0.2206              | -2.4114 | Cdh6             | cadherin 6  |
|                       | -1.1649               | 7.7974                     | -3.3615 | 0.0037     | 0.1808              | -1.7647 | Trdn             | triadin   |
|                       | -1.0919               | 6.3408                     | -3.3050 | 0.0042     | 0.1843              | -1.8743 | Retnlg           | resistin like gamma   |
|                       | -1.0884               | 11.101<br>9                | -4.3140 | 0.0005     | 0.0874              | 0.0934  | C4b              | complement component 4B (Chido blood group)                                     |
|                       | -1.0775               | 5.9808                     | -3.2652 | 0.0045     | 0.1901              | -1.9512 | Rimklb           | ribosomal modification protein rimK-like family member B                        |
|                       | -1.0567               | 10.969<br>4                | -3.5775 | 0.0023     | 0.1634              | -1.3437 | Thbs2            | thrombospondin 2  |
|                       | -1.0453               | 10.447<br>3                | -3.7806 | 0.0015     | 0.1402              | -0.9464 | Inmt             | indolethylamine N-methyltransferase   |
|                       | 1.0144                | 9.7029                     | 4.5752  | 0.0003     | 0.0722              | 0.5963  | Mir7653          | microRNA 7653   |
|                       | 1.0144                | 9.7029                     | 4.5752  | 0.0003     | 0.0722              | 0.5963  | Tlcd1            | TLC domain containing 1   |
|                       | 1.0268                | 8.9910                     | 3.8166  | 0.0014     | 0.1388              | -0.8760 | Egln3            | egl-9 family hypoxia-inducible factor 3   |
|                       | 1.0391                | 6.8885                     | 5.3045  | 0.0001     | 0.0444              | 1.9611  | Bpifb2           | BPI fold containing family B, member 2  |
|                       | 1.0462                | 5.2977                     | 3.0891  | 0.0066     | 0.2112              | -2.2901 | Galnt3           | polypeptide N-acetylgalactosaminyltransferase 3                                 |
|                       | 1.0508                | 9.5822                     | 6.2766  | 0.0000     | 0.0196              | 3.6603  | Gpd2             | glycerol phosphate dehydrogenase 2, mitochondrial                               |
|                       | 1.0510                | 8.2825                     | 5.0751  | 0.0001     | 0.0514              | 1.5391  | Angptl8          | angiotensin-like 8  |
|                       | 1.0562                | 8.7057                     | 4.8054  | 0.0002     | 0.0613              | 1.0341  | Tlcd2            | TLC domain containing 2   |
|                       | 1.0604                | 11.899<br>8                | 4.6570  | 0.0002     | 0.0672              | 0.7524  | Slc25a1          | solute carrier family 25 (mitochondrial carrier, citrate transporter), member 1 |
|                       | 1.0643                | 6.7594                     | 3.1320  | 0.0060     | 0.2078              | -2.2079 | Slc9a4           | solute carrier family 9 (sodium/hydrogen exchanger), member 4                   |
|                       | 1.0825                | 7.2708                     | 3.1017  | 0.0064     | 0.2112              | -2.2660 | Paqr5            | progesterone and adipoQ receptor family member V                                |
|                       | 1.0889                | 10.353<br>4                | 5.8738  | 0.0000     | 0.0245              | 2.9745  | Nsdhl            | NAD(P) dependent steroid dehydrogenase-like                                     |
|                       | 1.0968                | 8.8846                     | 3.3588  | 0.0037     | 0.1808              | -1.7699 | Sorl1            | sortilin-related receptor, LDLR class A repeats-containing                      |
|                       | 1.1047                | 8.2051                     | 3.2458  | 0.0047     | 0.1909              | -1.9887 | Slc14a1          | solute carrier family 14 (urea transporter), member 1                           |
|                       | 1.1122                | 7.2845                     | 5.6586  | 0.0000     | 0.0301              | 2.5972  | Slc15a5          | solute carrier family 15, member 5  |
|                       | 1.1122                | 8.1601                     | 4.5446  | 0.0003     | 0.0733              | 0.5377  | H2-Q10           | histocompatibility 2, Q region locus 10   |
|                       | 1.1182                | 11.400<br>9                | 5.8725  | 0.0000     | 0.0245              | 2.9722  | Acaca            | acetyl-Coenzyme A carboxylase alpha   |
|                       | 1.1190                | 5.1357                     | 3.3586  | 0.0037     | 0.1808              | -1.7703 | Mup5             | major urinary protein 5   |
|                       | 1.1196                | 5.0300                     | 2.9370  | 0.0092     | 0.2297              | -2.5795 | Esrp1            | epithelial splicing regulatory protein 1  |
|                       | 1.1223                | 7.6040                     | 4.8358  | 0.0002     | 0.0613              | 1.0914  | Cox8b            | cytochrome c oxidase subunit 8B   |
|                       | 1.1236                | 6.9761                     | 3.5542  | 0.0024     | 0.1646              | -1.3892 | Sid1             | SID1 transmembrane family, member 1   |

|     |         |        |         |        |        |         |          |  |
|-----|---------|--------|---------|--------|--------|---------|----------|--|
|     | 1.1679  | 7.5062 | 3.7879  | 0.0015 | 0.1402 | -0.9322 | Orm2     | orosomucoid 2  |
|     |         | 12.064 |         |        |        |         |          | malic enzyme 1, NADP(+)-dependent, cyto-   |
|     | 1.2060  | 7      | 6.1438  | 0.0000 | 0.0226 | 3.4372  | Me1      | solic  |
|     | 1.2080  | 5.2496 | 2.9485  | 0.0089 | 0.2276 | -2.5577 | Tmprss13 | transmembrane protease, serine 13  |
|     |         | 11.585 |         |        |        |         |          |  |
|     | 1.2173  | 5      | 2.9670  | 0.0086 | 0.2247 | -2.5227 | Thrsp    | thyroid hormone responsive   |
|     | 1.2173  | 9.9811 | 3.1931  | 0.0053 | 0.1963 | -2.0904 | Slc25a10 | solute carrier family 25 (mitochondrial carrier, dicarboxylate transporter), member 10 |
|     | 1.2278  | 8.1587 | 3.4684  | 0.0029 | 0.1709 | -1.5565 | Lrmda    | leucine rich melanocyte differentiation associated                                     |
|     | 1.2404  | 4.6054 | 2.9454  | 0.0090 | 0.2287 | -2.5635 | Macc1    | metastasis associated in colon cancer 1  |
|     | 1.3031  | 7.3486 | 2.9097  | 0.0097 | 0.2342 | -2.6311 | Grhl3    | grainyhead like transcription factor 3   |
|     | 1.3197  | 4.1843 | 3.0958  | 0.0065 | 0.2112 | -2.2774 | Serpinb5 | serine (or cysteine) peptidase inhibitor, clade B, member 5                            |
|     | 1.3364  | 7.7634 | 3.0887  | 0.0066 | 0.2112 | -2.2909 | Cpt1b    | carnitine palmitoyltransferase 1b, muscle  |
|     |         | 11.808 |         |        |        |         |          |  |
|     | 1.3488  | 3      | 6.9307  | 0.0000 | 0.0095 | 4.7157  | Mir3076  | microRNA 3076  |
|     |         | 11.808 |         |        |        |         |          |  |
|     | 1.3488  | 3      | 6.9307  | 0.0000 | 0.0095 | 4.7157  | Tkt      | transketolase  |
|     |         | 11.868 |         |        |        |         |          |  |
|     | 1.3967  | 3      | 3.3767  | 0.0036 | 0.1795 | -1.7350 | Fasn     | fatty acid synthase  |
|     | 1.4062  | 6.5605 | 3.8735  | 0.0012 | 0.1309 | -0.7648 | Dsp      | desmoplakin  |
|     | 1.4241  | 6.0365 | 3.2556  | 0.0046 | 0.1909 | -1.9699 | Cers3    | ceramide synthase 3  |
|     | 1.4548  | 6.4275 | 2.9486  | 0.0089 | 0.2276 | -2.5576 | Tspan8   | tetraspanin 8  |
|     | 1.4611  | 5.3069 | 3.9119  | 0.0011 | 0.1270 | -0.6895 | Syt15    | synaptotagmin-like 5   |
|     | 1.5167  | 8.6081 | 4.4420  | 0.0004 | 0.0787 | 0.3407  | Parm1    | prostate androgen-regulated mucin-like protein 1                                       |
|     | 1.6143  | 6.3359 | 5.7498  | 0.0000 | 0.0295 | 2.7582  | Slc2a5   | solute carrier family 2 (facilitated glucose transporter), member 5                    |
|     |         | 10.986 |         |        |        |         |          | glycerol-3-phosphate dehydrogenase 1 (soluble)   |
|     | 1.6502  | 0      | 6.1018  | 0.0000 | 0.0226 | 3.3660  | Gpd1     |  |
|     |         |        |         |        |        |         | Tmem184  |  |
|     | 1.6697  | 6.5850 | 3.8299  | 0.0013 | 0.1374 | -0.8501 | a        | transmembrane protein 184a   |
|     | 1.6720  | 5.9583 | 3.4688  | 0.0029 | 0.1709 | -1.5559 | Esrp2    | epithelial splicing regulatory protein 2   |
|     | 1.7240  | 9.2592 | 3.3436  | 0.0038 | 0.1808 | -1.7994 | Pnpla3   | patatin-like phospholipase domain containing 3   |
|     | 1.7906  | 7.7370 | 2.9710  | 0.0085 | 0.2247 | -2.5151 | Cidea    | cell death-inducing DNA fragmentation factor, alpha subunit-like effector A            |
|     | 1.8410  | 6.4967 | 3.1201  | 0.0062 | 0.2087 | -2.2307 | Foxq1    | forkhead box Q1  |
|     | 1.9457  | 6.1630 | 3.4392  | 0.0031 | 0.1709 | -1.6136 | Pdzk1ip1 | PDZK1 interacting protein 1  |
|     | 1.9745  | 7.5593 | 5.1206  | 0.0001 | 0.0495 | 1.6233  | Orm3     | orosomucoid 3  |
|     | 2.0203  | 7.9754 | 8.3166  | 0.0000 | 0.0011 | 6.7140  | Atp1a3   | ATPase, Na <sup>+</sup> /K <sup>+</sup> transporting, alpha 3 polypeptide              |
|     | 2.0373  | 5.7460 | 3.0918  | 0.0066 | 0.2112 | -2.2851 | Cldn8    | claudin 8  |
|     | 2.2891  | 6.9358 | 3.2473  | 0.0047 | 0.1909 | -1.9859 | Upk2     | uroplakin 2  |
|     |         | 10.477 |         |        |        |         |          | ELOVL family member 6, elongation of long chain fatty acids (yeast)                    |
|     | 2.3718  | 8      | 5.5767  | 0.0000 | 0.0309 | 2.4518  | Elovl6   |  |
| m a | -2.2955 | 9.9576 | -9.2631 | 0.0000 | 0.0003 | 5.3174  | Mid1     | midline 1  |
|     | -1.3969 | 7.2748 | -3.4273 | 0.0032 | 0.4642 | -1.7319 | Fgf13    | fibroblast growth factor 13  |

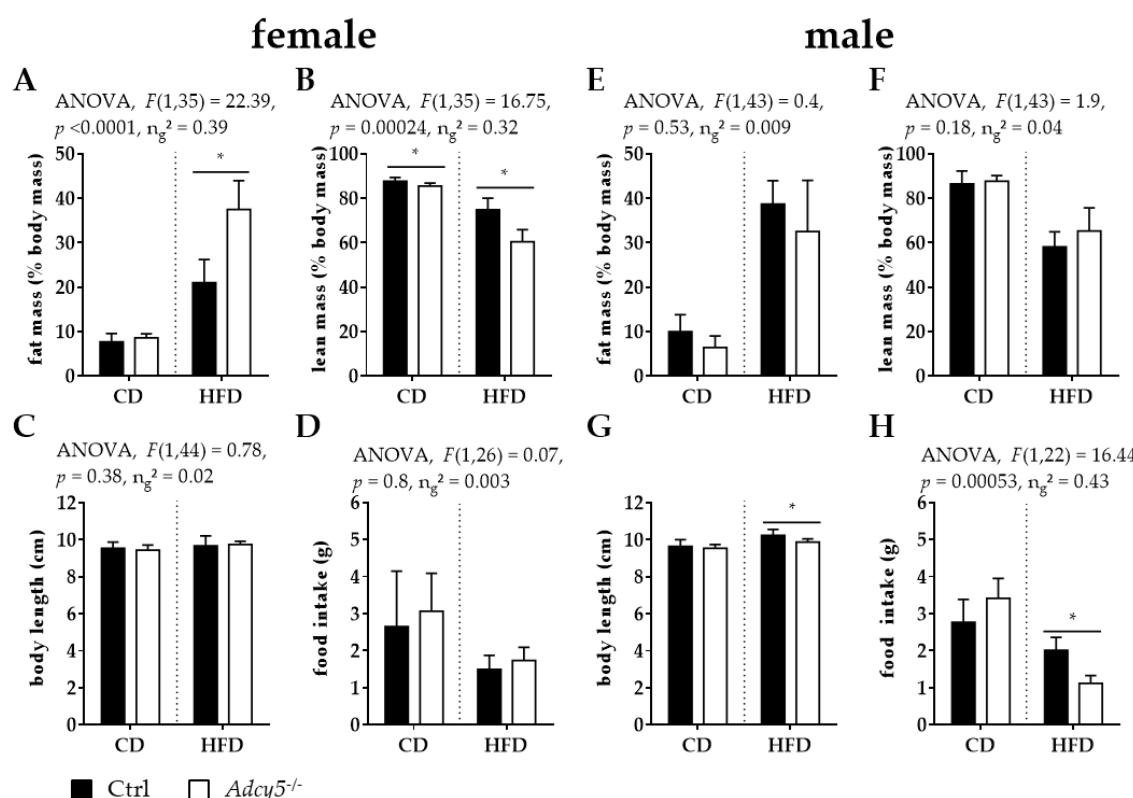
|         |         |         |        |        |         |               |  |
|---------|---------|---------|--------|--------|---------|---------------|--|
| -1.2474 | 7.5391  | -3.2658 | 0.0045 | 0.4730 | -1.9929 | Cd209d        | CD209d antigen   |
| -1.1648 | 7.2028  | -5.0832 | 0.0001 | 0.3940 | 0.8325  | Syn3          | synapsin III   |
| -1.1431 | 8.3677  | -3.4185 | 0.0032 | 0.4642 | -1.7462 | Alox15        | arachidonate 15-lipoxygenase   |
| -1.1081 | 7.8301  | -2.9184 | 0.0095 | 0.4779 | -2.5512 | Cxadr         | coxsackie virus and adenovirus receptor                                  |
| -1.0723 | 6.6701  | -3.6749 | 0.0019 | 0.4642 | -1.3323 | Csprs         | component of Sp100-rs  |
| -1.0717 | 8.3159  | -3.2235 | 0.0050 | 0.4730 | -2.0613 | Tnfrsf11b     | tumor necrosis factor receptor superfamily, member 11b (osteoprotegerin) |
| 1.0564  | 6.8235  | 4.5105  | 0.0003 | 0.4296 | -0.0169 | Sez6l2        | seizure related 6 homolog like 2   |
| 1.0609  | 8.1763  | 3.6228  | 0.0021 | 0.4642 | -1.4162 | Gm3002        | alpha-takusan pseudogene   |
| 1.1674  | 7.5081  | 4.8146  | 0.0002 | 0.4296 | 0.4406  | Mir147        | microRNA 147   |
| 1.1674  | 7.5081  | 4.8146  | 0.0002 | 0.4296 | 0.4406  | AA467197      | expressed sequence AA467197  |
| 1.2359  | 8.7852  | 3.1791  | 0.0054 | 0.4730 | -2.1329 | Grem2         | gremlin 2, DAN family BMP antagonist                                     |
| 1.2946  | 6.2733  | 3.1857  | 0.0054 | 0.4730 | -2.1222 | D830030K20Rik | RIKEN cDNA D830030K20 gene   |
| 1.3078  | 8.1580  | 4.7680  | 0.0002 | 0.4296 | 0.3714  | Zyg11a        | zyg-11 family member A, cell cycle regulator                             |
| 1.6634  | 7.4760  | 3.1727  | 0.0055 | 0.4730 | -2.1433 | Scd3          | stearoyl-coenzyme A desaturase 3   |
| 2.4642  | 10.9302 | 3.1038  | 0.0064 | 0.4730 | -2.2542 | Mup19         | major urinary protein 19   |

## Supplemental Figures

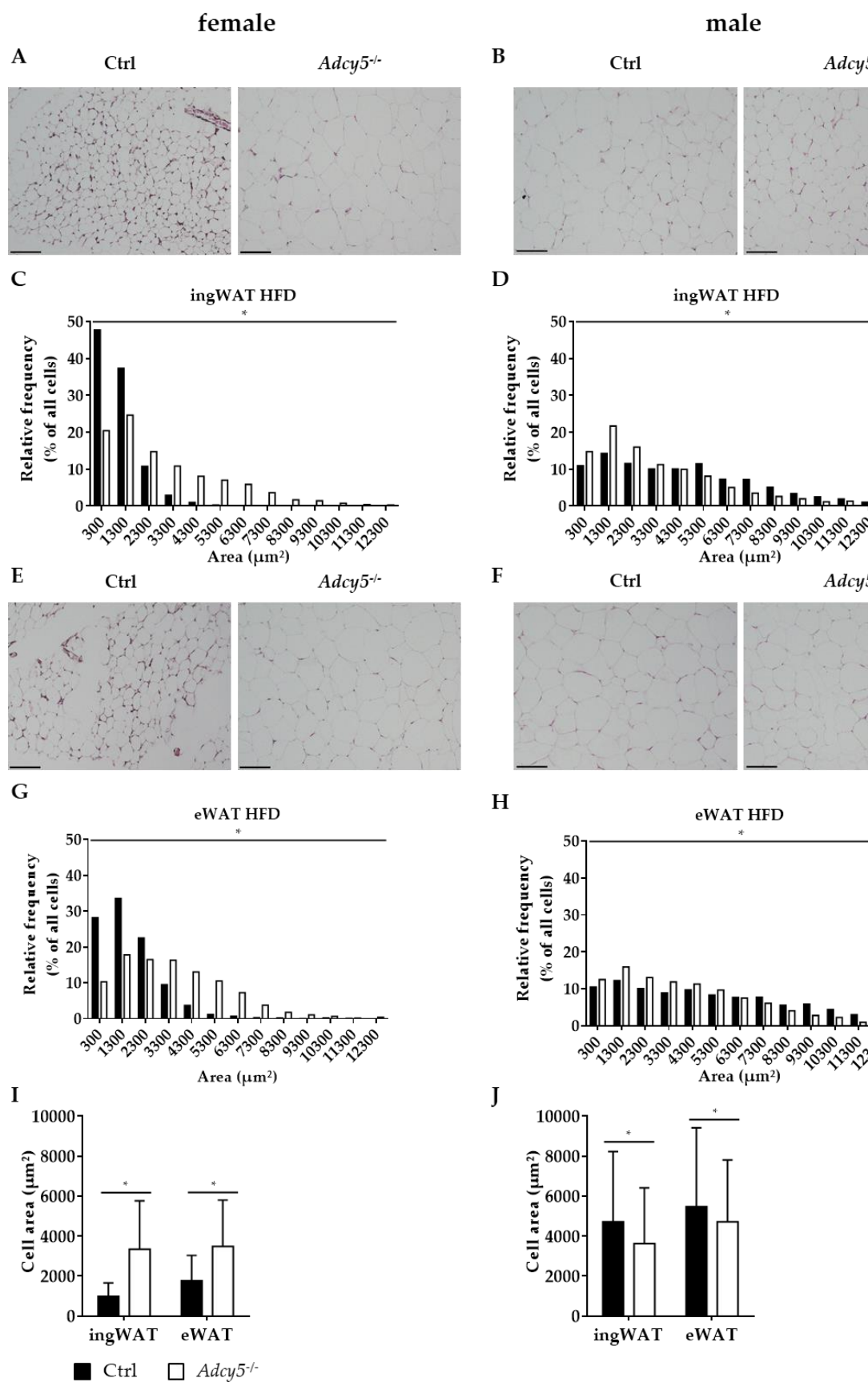


**Figure S1.** *Adcy5* targeting strategy and knockout validation. *Adcy5*<sup>-/-</sup> mice were created in analogy to the method described in Okumura *et al.* (18). Successful insertion of loxP-hUBp-em7-Neo-polyA-loxP cassette into exon 1 led to a loss of *Adcy5* expression (A). Agarose gel images display characteristic bands for wild type (WT) with a size of 202 bp and *Adcy5*<sup>-/-</sup> of 152 bp in different tissues (B).

Relative to WT control *Adcy5* mRNA expression (black: WT; open bars: *Adcy5*<sup>-/-</sup>) is expressed relative to *36b4* in different tissues. In all analyzed tissues, *Adcy5* expression was significantly down regulated (C). *n* (C) = 3. Data presented as mean ± SD. \* Significantly different between Ctrl and *Adcy5*<sup>-/-</sup> with *p* < 0.05. hUB – human ubiquitin promoter, em7 – synthetic *E. coli*-derived T7 promoter, Neo – neomycin resistance, pA – polyadenylation site, UTR – untranslated region, WT – wild type, KO – *Adcy5* knockout, BAT – brown adipose tissue, C1 – wild type control, C2 – knockout control, (-) – negative control, M – 100 bp marker, eWAT – epigonadal white adipose tissue, ingWAT – inguinal white adipose tissue, Ctrl – wild type control animals.



**Figure S2.** Phenotypic characterization of *Adcy5*<sup>-/-</sup> versus control (Ctrl) mice. Female *Adcy5*<sup>-/-</sup> mice (n=11) presented higher relative fat (A) and lower relative lean masses (B) under HFD compared to controls (n=14). Body composition was estimated by EchoMRI700™. Body lengths do not differ between female *Adcy5*<sup>-/-</sup> and Ctrl mice independently of the diet (C). Food intake (D) was not different between females of both genotypes under CD and HFD. In male mice under CD, relative fat mass (E) was lower in *Adcy5*<sup>-/-</sup> and relative lean mass (F) mass was higher in *Adcy5*<sup>-/-</sup> under HFD. Body length (G) was slightly higher in Ctrl compared to *Adcy5*<sup>-/-</sup> males under HFD. Food intake (H) was not different for males of both genotypes under CD, but lower in *Adcy5*<sup>-/-</sup> upon HFD. Results are expressed as mean ± SD. Differences within the same diet resulting from the genotype were proved for statistical significance (\*) using two-way ANOVA. Post-hoc tests using pairwise t-test were computed for significant interactions only and corrected applying the Bonferroni method. Both CD and HFD data was plotted within the same bar graphs and separated by a dotted line. Number of included animals: n (A and B) = 9 (CD, Ctrl), 5 (CD, *Adcy5*<sup>-/-</sup>). n (C) = 12 (CD per group), 14 (Ctrl, HFD) and 11 (*Adcy5*<sup>-/-</sup>, HFD). n(D) = 7 (CD, Ctrl), 9 (CD, *Adcy5*<sup>-/-</sup>), 8 (Ctrl, HFD) and 7 (*Adcy5*<sup>-/-</sup>, HFD). n (E, F and G): 10 (CD, Ctrl and *Adcy5*<sup>-/-</sup>), 14 (HFD, Ctrl) and 13 (HFD, *Adcy5*<sup>-/-</sup>).



**Figure S3.** Whole-body *Adcy5* deletion caused smaller adipocytes in white adipose tissue (WAT) only in males after high fat diet (HFD). Representative inguinal WAT (ingWAT) histology sections



of female (A) and male (B) *Adcy5*<sup>-/-</sup> and control (Ctrl) mice under HFD. Subfractions of small adipocytes were lower in female (C) and not different in male (D) *Adcy5*<sup>-/-</sup> compared to Ctrl mice. (E, F) Histological slides from epigonadal WAT (eWAT) of female (E) and male (F) *Adcy5*<sup>-/-</sup> mice. Subfractions of small adipocytes are lower in female (G), but not in male (H) *Adcy5*<sup>-/-</sup> compared to Ctrl mice. Cell area measurements of female (I) and male (J) *Adcy5*<sup>-/-</sup> and Ctrl mice reveal higher mean adipocyte size in both ingWAT and eWAT of females (I) and significantly lower mean adipocyte size in male *Adcy5*<sup>-/-</sup> compared to Ctrl mice (J) under HFD. Scale bar = 100  $\mu$ m. Statistical significance for relative frequencies (C, D, G and H) were calculated by using a Chi<sup>2</sup>-test. Data represent mean  $\pm$  SD. \* Significantly different between Ctrl and *Adcy5*<sup>-/-</sup>.