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**Suppl. Table S1: Gene ontology enrichment analysis of upregulated genes in cKO vs Cre that were normalized to HDAC4-NT**

|   | <b>fc enrichment</b> | <b>p-value</b> |
|---|----------------------|----------------|
| single-organism metabolic process                   | 2.5                  | 5.0E-40        |
| oxidation reduction process                         | 4.0                  | 1.0E-34        |
| metabolic process                                   | 1.6                  | 1.6E-28        |
| energy derivation by oxidation of organic compounds | 8.6                  | 2.9E-28        |
| small molecule metabolic process                    | 2.9                  | 9.4E-28        |
| generation of precursor metabolites and energy      | 7.0                  | 4.5E-26        |
| cellular metabolic process                          | 1.6                  | 4.1E-25        |
| cellular respiration                                | 9.8                  | 4.8E-23        |
| organic substance metabolic process                 | 1.5                  | 2.7E-18        |
| single-organism process                             | 1.3                  | 6.7E-18        |

n=3 Cre, n=3 cKO and n=3 cKO + AAV9-NT. Pa

Cre                                      Cre expressing control mice  
cKO                                        Cardiac-specific ABHD5 knockout  
cKO + AAV9-NT                      AAV9-NT transduced cardiac-specific ABHD5 knockout

## Suppl. Table 2: Clinical characteristics of patients

| Sample | CM  | Age | Sex | EF | PCW   | CI                                 | Digit. | Catechol. | ACE/AT-Antag. | Diuretics | Betbl. | LVEDD |
|--------|-----|-----|-----|----|-------|------------------------------------|--------|-----------|---------------|-----------|--------|-------|
|        |     | yrs |     | %  | mm Hg | min <sup>-1</sup> xm <sup>-2</sup> |        |           |               |           |        |       |
| 669    | ICM | 62  | m   | 20 | 12    | 2.33                               | +      |           | -/-           | +         | +      | n.a.  |
| 680    | ICM | 56  | f   | 27 | 25    | 1.91                               | -      | -         | +/-           | +         | +      | n.a.  |
| 580    | ICM | 57  | m   | 20 | 10    | 2.1                                | -      | -         | +/-           | +         | +      |       |
| 728    | ICM | 52  | m   | 35 | 25    | 1.56                               | -      | -         | +/-           | +         | +      | 77    |
| 581    | ICM | 66  | f   | 25 | 21    | 1.75                               | -      | -         | +             | +         | +      | 63    |
|        |     |     |     |    |       |                                    |        |           |               |           |        | 70    |
| 502    | DCM | 61  | M   | 25 | 18    | 2.89                               | +      | -         | +/-           | +         | +      |       |
| 503    | DCM | 56  | M   | 29 | 25    | 1.24                               | +      | -         | +/-           | +         | +      |       |
| 652    | DCM | 61  | M   | 20 | 29    | 1.72                               | +      | -         | +/-           | +         | +      |       |
| 669    | DCM | 59  | M   | 20 | 12    | 2.3                                | -      | -         | -/-           | +         | +      |       |
| 767    | DCM | 42  | F   | 25 | 30    | 1.1                                | -      | -         | -/-           | +         | -      |       |

ICM: Ischemic cardiomyopathy

DCM: Dilated cardiomyopathy

EF: Ejection Fraction

PCW: Pulmonary Capillary Wedge

CI: Cardiac Index

LVEDD: Left Ventricular End Diastole Diameter (internal dimension at)

**Suppl. Table S3. Baseline echocardiographic parameters of wild type (WT) and cardiac-specific ABHD5 transgenic mice (TG)**

|            | <b>WT</b> | <b>TG line#9</b> |
|------------|-----------|------------------|
| FS (%)     | 54 ± 1.3  | 54 ± 1.8         |
| LVEDD (mm) | 2.9 ± 0.1 | 3.1 ± 0.1        |
| LVESD (mm) | 1.3 ± 0.1 | 1.4 ± 0.1        |
| HR (BPM)   | 732 ± 6.3 | 720 ± 7.8        |

n=7 WT and n=10 TG line#9; Mean ± SEM

|            | <b>WT</b> | <b>TG line#1</b> |
|------------|-----------|------------------|
| FS (%)     | 45 ± 2.9  | 48 ± 2.0         |
| LVEDD (mm) | 3.7 ± 0.1 | 3.5 ± 0.1        |
| HR (BPM)   | 655 ± 9.1 | 648 ± 15.4       |

n=4 WT and n=4 TG line#1; Mean ± SEM

HR (BPM) Heart rate (Beats per minute)  
 FS Fractional Shortening  
 LVEDD Left Ventricular End Diastolic Diameter  
 LVESD Ventricular End Systolic Diameter  
 WT wild-type  
 TG transgenic  
 SEM Standard error of mean

**Suppl. Table S4. Echocardiographic parameters of WT and TG mice subjected to either sham or TAC surgery.**

|            | WT          |              | TG          |                          |
|------------|-------------|--------------|-------------|--------------------------|
|            | Sham        | TAC          | Sham        | TAC                      |
| FS (%)     | 56.5 ± 2.5  | 22.9 ± 3.1*  | 54.3 ± 1.8  | 47.5 ± 2.9 <sup>#</sup>  |
| LVEDD (mm) | 3.05 ± 0.06 | 3.52 ± 0.34  | 3.18 ± 0.08 | 2.92 ± 0.11              |
| LVESD (mm) | 1.33 ± 0.08 | 2.75 ± 0.36* | 1.45 ± 0.04 | 1.54 ± 0.12 <sup>#</sup> |
| HR (BPM)   | 745 ± 14.8  | 744 ± 10.7   | 741 ± 9.2   | 748 ± 10.6               |

n=5 Sham, n=5 TAC of WT and n=5 Sham, n=5 TAC of TG; Mean ± SEM

HR (BPM) Heart rate (Beats per minute)  
 FS Fractional Shortening  
 LVEDD Left Ventricular End Diastolic Diameter  
 LVESD Ventricular End Systolic Diameter  
 WT wild-type  
 TG transgenic  
 SEM Standard error of mean  
 TAC Transverse aortic constriction

**Suppl. Table S5. Primers used for cloning**

| Primer name      | Primer Sequence                                  | Template  | Vector                            | Purpose  |
|------------------|--|---|-----------------------------------|--|
| ABHD5-KpnI-L     | GCGTGGGGTACCAC<br>CATGGCGGCGGAGG<br>AGGAGGAGG    | cDNA prepared<br>from ad-<br>ABHD5<br>expressed in<br>NRVMs | pCDNA 3.0                         | To express<br>ABHD5<br>in immortalized<br>cell lines               |
| ABHD5-XbaI-R     | CCTCCATCTAGACTA<br>GTCCACAGTGTGCGA<br>GATCTCC    |   |                                   |  |
| ABHD5-KpnI-L     | GCGTGGGGTACCAC<br>CATGGCGGCGGAGG<br>AGGAGGAGG    | ABD5 in<br>pCDNA 3.0  | pGAL-Trp                          | To inducibly<br>express ABHD5<br>in<br>saccharomyces<br>cerevisiae |
| ABHD5-XbaI-R     | CCTCCATCTAGACTA<br>GTCCACAGTGTGCGA<br>GATCTCC    |   |                                   |  |
| HDAC4-FseI-L     | GCGTGGGGTACCGG<br>CCGGCCCATGAGCTC<br>CCAAAGCCATC | HDAC4 in<br>pCDNA 3.0                                       | pNOP-Leu                          | To express<br>HDAC4 in<br>saccharomyces<br>cerevisiae              |
| HDAC4-NotI-R     | CCTCCAGCGGCCGCC<br>TACAGGGGCGGCTC<br>CTCTTC      |   |                                   |  |
| ABHD5-Sall-L     | GCGTGGGTCGACAC<br>CATGGCGGCGGAGG<br>AGGAGGAGG    | ABD5 in<br>pCDNA 3.0  | 120 $\alpha$ -MHC hgh<br>clone 26 | To generate $\alpha$ -<br>MHC transgenic                           |
| ABHD5-HindIII-R  | CCTCCAAAGCTTTCA<br>GTCCACAGTGTGCGA<br>G          |   |                                   |  |
| HDAC4-NT-BamHI-L | CGACGCGGATCCGC<br>CACCATGGACTACA<br>AAGACGATG    | HDAC4 in<br>pCDNA 3.0                                       | pCR <sup>®</sup> 2.1              | To subclone  |
| HDAC4-NT-XbaI-R  | GCGTCGTCTAGACTA<br>GTAGCGAGGGTCG<br>TG           |   |                                   |  |
| HDAC4-NTBamHI-L  | CGACGCGGATCCGC<br>CACCATGGACTAC                  | HDAC4 in<br>pCDNA 3.0                                       | pUF <sub>CM</sub> Venh            | To generate AAV<br>vector encoding                                 |

### Suppl. Table S6. Primers used for quantitative RT-PCR

| Gene            | UPL probe # | Accession number | Primer sequence (Left and right) |
|-----------------|-------------|------------------|----------------------------------|
| Nppa(ANP)       | 25          | NM_008725.2      | L: cacagatctgatggattcaaga        |
|                 |             |                  | R: cctcatcttctaccggcatc          |
| Nppb(BNP)       | 13          | D82049.1         | L:gtctggccggactcag               |
|                 |             |                  | R:tgcactgggtcttcaacaac           |
| Col5a1          | 76          | NM_015734.2      | L:ctacatccgtgccctggt             |
|                 |             |                  | R:ccagcaccgtcttctggtag           |
| XIRP2(Myomaxin) | 108         | NM_001024618.2   | L:ctgcaacgcctttctgagt            |
|                 |             |                  | R:tcttgactgctgtggattgc           |
| Nr4a1           | 96          | NM_010444.2      | L:agcttgggtgttgatgtcc            |
|                 |             |                  | R:cgatcagtgatgaggaccag           |
| Gfpt2           | 34          | NM_013529.3      | L: attcactacccgggagaagc          |
|                 |             |                  | R: gtaccccgatgagcaagg            |
| Pdk4            | 22          | NM_013743.2      | L:cgcttagtgaacactccttcg          |
|                 |             |                  | R:cttctgggctcttctcatgg           |

**Suppl. Table S7. Primers used for quick change site directed mutagenesis**

| # | ABHD5 mutant | Primer_left                                 | Primer_right                                | Template Vector |
|---|--------------|---|---|-----------------|
| 1 | N153D        | 'GATCTTGCTTGGACACGA<br>CCTGGGAGGGTTCTTG     | CAAGAACCCTCCAGGTCGT<br>GTCCAAGCAAGATC       | pET-28a-ABHD5   |
| 2 | S298A        | GTGATCTTTGGAGCCCGA<br>GCCTGCATAGATGGCA      | TGCCATCTATGCAGGCTC<br>GGGCTCCAAAGATCAC      | pET-28a-ABHD5   |
| 3 | H327A        | GCCATCCTCGGGGCGGGG<br>GCTTATGTGTATGCAGATCAG | CTGATCTGCATACA<br>CATAAGCCCCCGCCCCGAGGATGGC | pET-28a-ABHD5   |
| 4 | S237A        | GATTTCAAGCGGAAGTA<br>CGCCTCTATGTTTGAAGAT    | ATCTTCAAACATAGAGG<br>CGTACTTCCGCTTGAAATC    | pET-28a-ABHD5   |
| 5 | S237E        | GATTTCAAGCGGAAGTACGA<br>GTCTATGTTTGAAGAT    | ATCTTCAAACATAGACTC<br>GTACTTCCGCTTGAAATC    | pET-28a-ABHD5   |