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2 **The lncRNA *Sweetheart* regulates compensatory cardiac hypertrophy after**
3 **myocardial injury**

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30 **KEYWORDS**

31 LncRNA, *Nkx2-5*, hypertrophy, *trans*

32
33 **RUNNING TITLE**

34 *Sweetheart* regulates hypertrophy in the adult heart

35

36 **ABSTRACT**

37

38 After myocardial infarction in the adult heart the remaining, non-infarcted tissue adapts
39 to compensate the loss of functional tissue. This adaptation requires changes in gene
40 expression networks, which are mostly controlled by transcription regulating proteins.
41 Long non-coding transcripts (lncRNAs) are now recognized for taking part in fine-
42 tuning such gene programs. We identified and characterized the cardiomyocyte
43 specific lncRNA *Sweetheart RNA (Swht)*, an approximately 10 kb long transcript
44 divergently expressed from the cardiac core transcription factor coding gene *Nkx2-5*.
45 We show that *Swht* is dispensable for normal heart development and function, but
46 becomes essential for the tissue adaptation process after myocardial infarction. Re-
47 expressing *Swht* from an exogenous locus rescues the *Swht* *null* phenotype. Genes
48 depending on *Swht* after cardiac stress are significantly occupied, and therefore most
49 likely regulated by NKX2-5. Our results indicate a synergistic role for *Swht* and the
50 developmentally essential transcription factor NKX2-5 in tissue adaptation after
51 myocardial injury.

52

53 **INTRODUCTION**

54

55 Precise regulation of gene expression networks is required to form a healthy heart and
56 to maintain proper heart function after birth and throughout adulthood. Such networks
57 not only contain protein coding genes, but also many long non-coding genes
58 (lncRNAs), which are as abundant as coding genes^{1,2}. The function and mechanism
59 of these lncRNAs vary greatly, but they are often associated with transcriptional
60 regulation³. Two major types of mechanisms are typically discussed when referring to
61 lncRNA gene function. For some loci the resulting RNA is just a circumstantial
62 byproduct with the act of transcription being the major bearer of its function. One such
63 example in the cardiac system is the lncRNA locus *Handsdown RNA* (*Hdnr*),
64 downstream of the *Hand2* transcription factor coding gene. During embryonic
65 development, modification of the *Hdnr* transcriptional activity alters the *Hand2*
66 expression levels, but not modifying the RNA levels of *Hdnr*⁴. One example of lncRNA
67 loci that exhibit an RNA-based mechanism are the cardiac specific *Myosin Heavy*
68 *Chain Associated RNA Transcripts* (*MyHEART*; *Mhrt*), a cluster of lncRNAs that
69 undergo anti-sense transcription from the *myosin heavy chain 7* (*Myh7*) locus.
70 Transaortic constriction (TAC) induced pathological stress results in *Mhrt*
71 downregulation by *Brg1* upregulation and subsequent BRG1 mediated chromatin
72 remodeling *in vivo*. Thus, hypertrophy related gene programs are initialized. As the
73 binding of *Mhrt* to BRG1 antagonizes its DNA binding capability, preserving *Mhrt*
74 expression levels after TAC prevents cardiac hypertrophy and heart-failure⁵.

75 One of the core regulators of heart development is the cardiac specific homeobox
76 protein NKX2-5, which is present in early cardiomyocytes already at embryonic day
77 (E) 7.5 during murine embryonic development. Systemic deletion of the *Nkx2-5* gene
78 in mice causes defects in heart looping and formation of ventricular structures. In
79 addition, other important cardiac regulatory genes are dysregulated and as a combined
80 result the embryos exhibit early embryonic lethality⁶. *Nkx2-5* is not silenced after birth
81 and is abundantly expressed in adult heart tissue⁷. However, not much is known about
82 its function in terminally differentiated cardiac tissue and its involvement in cardiac
83 maintenance and disease. In human patients suffering from congenital heart disease,
84 *Nkx2-5* mutations are commonly found⁸, however, the precise involvement of *Nkx2-5*
85 in the disease context in adult patients remains unknown.

86 Heart disease represents the main cause of death in the developed world; acute
87 myocardial infarctions (AMI) being the most common form. Reduced blood flow leads
88 to decreased oxygen supply of the heart tissue and thus irreversible damage, such as
89 apoptosis and necrosis of cardiomyocytes and formation of scar tissue which results
90 in a loss of flexibility. Due to the limited regenerative capacity of the terminally
91 differentiated heart tissue, the remaining viable tissue adapts through other
92 mechanisms, such as hypertrophic remodelling that involves thickening of the
93 ventricular walls by an increase of the cardiomyocyte cell size⁹. While cardiac
94 hypertrophy in response to pathological stimuli is often associated with heart failure,
95 we show that it is necessary for survivability after cardiac injury in mice. We
96 characterize a cardiac specific lncRNA we termed *Sweetheart RNA (Swhtr)* that is
97 required for regulation of hypertrophic gene programs, most likely acting in concert
98 with NKX2-5.

99

100 **RESULTS**

101 ***Swht* is a nuclear lncRNA specifically expressed in the heart**

102 In a previously generated dataset that identified the transcriptional landscape of
103 different tissues of early mid-gestation mouse embryos¹⁰ we identified an RNA that is
104 expressed exclusively in heart tissue. This RNA, which we termed *Sweetheart RNA*
105 (*Swht*), is divergently expressed from the essential, transcription factor coding gene
106 *Nkx2-5*⁶. Its annotation partially overlaps with the previously described lncRNA *IRENE-*
107 *div*¹¹. We determined its major transcript by 5' and 3' RACE PCR and found that the
108 major variant from *Swht* locus is 9,809 nucleotides in length and bears no introns (Fig.
109 1A). The transcriptional start site (TSS) maps to a previously described GATA4 bound
110 first heart field specific enhancer located approximately 8kb upstream of *Nkx2-5*¹². To
111 characterize whether *Swht* is specific for the first heart field we conducted whole
112 mount *in situ* hybridization (WISH) in E8.25 mouse embryos and found whereas *Nkx2-*
113 5 is expressed in the whole heart tube at that stage, *Swht* is expressed in the early
114 inflow tract of the developing heart (Fig. 1B). Lineage tracing experiments of *Swht*
115 expressing cells confirmed that while *Swht* expressing cells contribute to both
116 ventricles, the left loop that originates from the first heart field exhibits a much more
117 even staining (Fig. 1C). The right loop that originates mostly from the second heart
118 field exhibits a more salt-and-pepper like staining (Fig. 1C). In heart and lung of later
119 stage embryos (E12.5; E14.5) the staining within the left and right ventricle is even with
120 no traces of *Swht* expressing cells or their descendants found in neither lung nor
121 epicardial tissue (Fig. 1D-E). Compared to the *cis* located *Nkx2-5* gene, *Swht* is much
122 lower expressed (Fig. 1A,F). Expression analysis from the whole heart at different
123 stages shows that expression levels of *Nkx2-5* and *Swht* are changing comparably
124 (Fig. 1F).

125 To investigate where the *Swht* transcript localizes intracellularly, we conducted
126 subcellular fractionation of embryonic cardiomyocytes. Compared to marker
127 transcripts known to be localized to the chromatin, nucleoplasm and cytoplasm
128 fraction, the *Swht* lncRNA localizes predominantly to the chromatin fraction within the
129 nucleus (Fig. 1G). We validated these findings by single molecule fluorescence *in situ*
130 hybridization (smFISH) experiments that revealed two distinct fluorescent *Swht*
131 signals within the nucleus, suggesting that *Swht* might reside at its site of transcription

132 (Fig. 1H, Fig. S1). It has become clear, that some RNAs that are classified as lncRNAs
133 can encode micropeptides which might be functional. However, these are usually
134 cytoplasmic localized¹³ pointing against a functional open reading frame (ORF)
135 contained in *Swltr*. CPAT analysis¹⁴ further demonstrates that *Swltr* has very low
136 coding potential (Fig. 1I). In conjunction with the localization data this points towards a
137 purely non-coding transcript. Together this data shows that *Swltr* is a chromatin bound
138 lncRNA, specifically expressed in the heart of developing mice and at postnatal stages.

139

140 **Genetic inactivation of *Swltr* does not affect heart development and**
141 **homeostasis**

142 To investigate the physiological function of *Swltr* we genetically engineered a knock-
143 out mouse in which we inserted a strong transcriptional stop signal (3xpA) to abolish
144 *Swltr* expression. To avoid any conflicts with existing regulatory elements we inserted
145 this stop signal downstream of a phylogenetically conserved region of the GATA4
146 bound enhancer. This genetic insertion causes premature termination of the
147 transcriptional process and, hence, a severely shortened *Swltr* transcript (Fig. 2A).
148 The full length *Swltr* RNA is not detectable anymore when we profiled E9.5 hearts by
149 either RNA-seq or qPCR (Fig. 2A,B), demonstrating that the *Swltr* transcriptional start
150 side (TSS) locates upstream of the inserted transcriptional stop signal and no other
151 alternative transcript is initiated from any downstream elements residing in the *Swltr*
152 transcription unit. While the *Swltr* RNA is lost, the expression level of its *cis* located
153 gene *Nkx2-5* in the heart is unchanged at that stage and under these conditions (Fig.
154 2B).

155 Phenotypically, homozygous *Swltr null* embryos do not display differences compared
156 to wild type embryos during development and grow up to adult animals with no overt
157 defects (Fig. 2C,D). To identify subtle phenotypic changes in adult hearts as a result
158 of *Swltr* lacking throughout development, we investigated the heart function in *Swltr*
159 *null* animals by echocardiography after backcrossing the *Swltr null* mutants to the
160 C57BL/6J genetic background. We compared their body weight and cardiac
161 parameters, such as ejection fraction, heart rate and left ventricular diameter of adult
162 *Swltr null* and wild type mice. Neither the body weight nor any of the heart parameters

163 differed significantly from that of wild type mice (Fig. 2E-F and Fig. S2). We conclude
164 that under standard breeding conditions *Swhtr* is dispensable for normal heart function.

165

166 **Swhtr is required for a compensatory response of the heart after myocardial**
167 **infarction**

168 Many lncRNA knock out animal models do not display an overt phenotype after genetic
169 depletion under standard conditions^{15,16,17}. One possibility is that these analyzed
170 lncRNA genes are actually not functional¹⁸. Another possibility is that a functional
171 requirement is only detected under stress conditions. To challenge the heart we
172 selected the left anterior descending artery (LAD) ligation model, which induces a
173 myocardial infarction in the lateral left ventricle¹⁹. An acute myocardial infarction (AMI)
174 was induced in male mice of 8 weeks of age and heart parameters were monitored by
175 echocardiography pre-infarction and at day 7 and day 14 after the infarction (Fig. 3A).
176 After 14 days animals were sacrificed, and the presence of infarct tissue was verified
177 by Sirius red staining (Fig. 3G). Notably, compared to wild type mice, *Swhtr null*
178 animals displayed an increased mortality after AMI (Fig. 3B), while the size of the
179 myocardial infarction (MI) was similar between the groups (Fig. S3). Most parameters
180 of cardiac function did not change significantly, but strikingly, the interventricular
181 septum (IVS) of *Swhtr null* mice did not display compensatory thickening after AMI
182 compared to wild type (Fig. 3C-G and Fig. S3). This establishes that *Swhtr* is involved
183 in the adaptive response of the cardiac tissue after a myocardial infarction.

184 To determine whether the *Swhtr* dependent adaptation after AMI is a result of the loss
185 of the RNA transcript or the loss of the transcriptional activity at this locus, we
186 generated a *Swhtr* rescue mouse line that re-expresses the *Swhtr* lncRNA from an
187 exogenous locus (random single-copy BAC insertion) (*Tg(RP23-466K9; P_{Nkx2-5}H2Bvenus)Phg2*) (Fig. 3H). This rescue construct contains an *H2BVenus* fusion
188 expression cassette instead of the *Nkx2-5* coding sequence to detect activity of the
189 transgene and to avoid having an additional third copy of the *Nkx2-5* locus present in
190 our genetic setup. Consequently, mice from this transgenic line show yellow
191 fluorescence exclusively in the heart (Fig. 3I) and re-express *Swhtr* (Fig. 3J). We
192 crossed this wild type mouse line to our *Swhtr null* mice to generate the *Swhtr* rescue
193 line (*Swhtr^{3xpA/3xpA; tg}*).

195 The *Swltr* rescue mice do not display the same phenotype as the *Swltr null* but
196 resemble the wild type control mice in regard to mortality (Fig. 3K) and the size of the
197 IVS (Fig. 3N) after AMI, while neither of the remaining analyzed parameters show
198 significant differences (Fig. 3L-M and Fig. S3). Hence, the RNA of *Swltr* locus is
199 important for its function.

200

201 ***Swltr* is required for hypertrophic re-modelling after myocardial infarction**

202 The thickening of the interventricular septum after the induced myocardial injury in wild
203 type and *Swltr* rescue mice might be a result of a hypertrophic response of the
204 remaining muscle tissue. To address whether this might be due to an inherent function
205 of *Swltr* in cardiomyocytes we first determined the expression of *Swltr* in different
206 heart cell populations²⁰. We found upon fractionation of the four major cell types in the
207 adult heart (8 weeks) that *Swltr* exhibits the same pattern as the cardiomyocyte
208 marker gene *Tnni1*, showing the cardiomyocyte specificity of *Swltr* in adult hearts (Fig.
209 4A). Then, we investigated the presence of larger cells, reminiscent for hypertrophy, in
210 sections of hearts from the LAD-ligation experiment. Wheat Germ Agglutinin (WGA)
211 staining revealed that the IVS region of wild type and *Swltr* rescue indeed exhibit on
212 average increased cell size (Fig. 4B,C). In contrast, the cell size in the *Swltr null* IVS
213 even decreased significantly (Fig. 4C). This establishes that the *Swltr* RNA is required
214 for the adaptive hypertrophic response of the cardiomyocyte tissue after a myocardial
215 infarction.

216

217 ***Swltr* regulates NKX2-5 mediated cardiac stress response**

218 Scar formation is one of the consequences of myocardial infarction (Fig. 3E). The
219 resulting heterogeneity of the infarcted heart tissue can lead to biased RNA profiling.
220 To mitigate this compositional bias and obtain consistent RNA profiling we performed
221 primary tissue culture of defined heart tissue slices. Replicate slices were either
222 cultivated continuously for 2 weeks under normoxic condition (untreated) or 1 week
223 under hypoxic condition, followed by 1 week under normoxic condition (treated);
224 mimicking our *in vivo* AMI stress model (Fig. 5A). When we compared expression
225 profiles of wild type heart slices treated versus untreated, we found only 9 genes to be

226 dysregulated (Fig. 5B). In contrast, 464 genes were dysregulated in *Swltr* null heart
227 slices when treated compared to untreated (Fig. 5B). The near absence of
228 dysregulated genes in wild type compared to the high rate of dysregulated genes in
229 mutant heart slices indicates that *Swltr* is required for recovery after cardiac stress
230 (Fig. 5B). GO-term analysis shows that these *Swltr* dependent genes are mainly
231 involved in biological processes such as leukocyte migration and chemotaxis indicative
232 of inflammatory response, intracellular calcium homeostasis, muscle function, heart
233 morphogenesis and extracellular matrix organization, all of which are integral
234 components of cardiac stress response (Fig. 5C). Accordingly, KEGG pathway
235 analysis reveals pathways involved in inflammatory response, cardiomyopathy,
236 glucose metabolism and response to oxygen levels depend on *Swltr* (Fig. S4). Albeit
237 the loss of *Swltr* does not lead to changes in *Nkx2-5* expression level neither in adult
238 hearts (Fig. 2B) nor in our treated heart slice culture (Fig. 5B), the most likely
239 localization of *Swltr* to its locus of transcription (Fig. 1I) implicates some involvement
240 of *Nkx2-5* to this process. To determine if a significant number of *Swltr* dependent
241 genes are occupied by NKX2-5 and therefore might be direct targets we analyzed
242 available CHIP-seq data of NKX2-5 occupation in 6 weeks old adult heart tissue²¹. We
243 found that a significant number of dysregulated genes is occupied by NKX2-5 (Fig.
244 5D), indicating that the lack of *Swltr* impairs NKX2-5 mediated response to cardiac
245 stress. This suggests that *Swltr* might act together with NKX2-5 to regulate this cardiac
246 stress response. One possibility is that at some timepoint during the response to
247 cardiac stress stimuli *Swltr* might modulate *Nkx2-5* expression levels to regulate its
248 transcriptional effect on its target genes in adult cardiomyocytes.

249

250 **DISCUSSION**

251 Here we characterize a novel lncRNA that we termed *Swht*, which partially overlaps
252 with a previously published lncRNA from that locus: *IRENE-div*¹¹. In contrast to this
253 previously described eRNAs, our study shows that the absence of the long transcript
254 from the *Swht* locus does not lead to persistent *Nkx2-5* mRNA dysregulation, neither
255 during embryonic development nor cardiac homeostasis. We show by fractionation
256 and smFISH that the *Swht* lncRNA is chromatin localized, arguing together with the
257 high non-coding potential that no micropeptide is embedded in the nearly 10kb long
258 *Swht* transcript. Our data clearly show an RNA based function for *Swht*, as rescue
259 animals do not exhibit the same defect in cardiac hypertrophy as the *Swht* null
260 animals. The promoter of *Swht* was described previously as a *Nkx2-5* cardiac
261 enhancer element active in multipotent cardiac progenitor cells²². The *Swht* promoter
262 becomes active after myocardial stress, which was described previously¹². It was
263 tested if these reappearing cells can contribute to regeneration of an infarcted heart,
264 but our data rather indicate that this genetic element is important to activate *Swht* and
265 that this activity is required for the tissue remodeling of the remaining intact cardiac
266 tissue after infarction.

267 The *cis* located gene to *Swht* is the core cardiogenic transcription factor coding gene
268 *Nkx2-5*. *Nkx2-5* is known to be required for embryonic development of the cardiac
269 system⁶. However, although the locus is still active and abundantly expressed in
270 adults, not much is known about its function after birth and in cardiac stress. Interfering
271 with NKX2-5 function by over-expression of a dominant negative *Nkx2-5* mutant has
272 been shown to lead to apoptosis of cultured cardiomyocytes. In contrast, over-
273 expression of a wild type form of *Nkx2-5* has a protective effect against doxorubicin
274 induced stress²³. This demonstrates a critical role of *Nkx2-5* in maintenance of adult
275 cardiomyocytes as well as in the response to stress. Here, we show significant NKX2-
276 5 occupation on the *Swht* dependent genes. This indicates that *Swht* acts in concert
277 with NKX2-5 under stress conditions to regulate hypertrophy associated gene
278 programs. Mutations in NKX2-5 have been associated previously with dilated
279 cardiomyopathy²⁴, but we show for the first time that *Nkx2-5* and its wider locus is an
280 important responder to cardiac stress in adult hearts *in vivo*, acting in concert with its
281 divergently expressed lncRNA *Swht*.

282 In response to increased demands on the remaining tissue after injury, the viable tissue
283 adapts by cardiac hypertrophy to maintain blood supply to the body⁹. This is
284 demonstrated by the increase of average cell size in the IVS of wild type animals after
285 AMI, together with the heart parameters being unaffected. Our *Swht* *null* mice have
286 defects in this hypertrophic response after cardiac injury. While the differences of
287 ejection fraction do not reach statistical significance, the observed decrease meets the
288 requirements of a trend in loss of heart functionality. Together with the increase of
289 lethality after myocardial injury this points towards a cardioprotective role of the *Swht*
290 lncRNA by making the cardiac tissue permissive for a hypertrophic response after
291 myocardial injury. This is further supported by our *ex vivo* analysis of heart slices. One
292 of the main pathways known to be involved in hypertrophic process is the
293 phosphoinositide 3-kinase (PI3-K) pathway that is, among others, responsible for
294 metabolic substrate utilization and function of cardiomyocytes²⁵. The RNA profiling of
295 cardiac slices subjected to hypoxic stress and following recovery time, revealed
296 dysregulation of 464 genes in slices derived from *Swht* *null* animals as compared to
297 only 9 dysregulated genes in wild type slices. Notably, KEGG pathway enrichment
298 analysis revealed that AGE-RAGE signaling is among the most affected pathways.
299 Even though it is not frequently discussed in relation to cardiac hypertrophy, it is a
300 pathway known to be involved in stress response of cardiomyocytes to stimuli such as
301 oxidative stress^{26,27}). Additionally, AGE-RAGE signaling is known to be an inducer of
302 the PI3-K pathway, coherent with its role in hypertrophic remodeling.

303 Maladaptive hypertrophy, in contrast, is defined as occurring after pathological stimuli
304 and associated with heart failure and disease²⁸. Among others, inflammatory
305 response, calcium homeostasis and signaling, and extracellular matrix deposition are
306 biological processes involved in maladaptive cardiac hypertrophy²⁹. These biological
307 processes are significantly impaired upon loss of *Swht*, indicative for maladaptive
308 behavior. It has been discussed that an initial adaptive hypertrophic response can
309 transition to maladaptive hypertrophy upon persistent pathological stress²⁹. Within
310 the scope of the experiment, no decrease of heart function could be detected in
311 animals capable of hypertrophic remodeling, despite the deregulation of maladaptive
312 hypertrophic processes in dependence of *Swht*. Additionally, increased survival of
313 wild type animals compared to *Swht* mutant animals suggests that hypertrophic

314 remodeling has a positive effect on viability after acute myocardial infarction and is a
315 required compensatory response.

316 While the detailed mechanism remains to be determined we here show a clear
317 cardioprotective role of the murine lncRNA *Swht* and a compensatory gene regulatory
318 network depending on *Swht*. While no lncRNA was described yet for the human
319 *NKX2-5* locus, the promoter element of *Swht* is highly conserved across placental
320 species. Moreover, in public datasets from human heart tissue some RNA seems to
321 be present around this conserved promoter element. Further investigation can
322 determine whether this *SWHTR* locus might have the same cardioprotective role in
323 humans and if this function could be employed for a therapeutic application.

324

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330

331 **Data availability**

332 The data are deposited to GEO and can be downloaded under the accession number
333 GSE200380. The cDNA of *Sweetheart RNA (Swht)* is deposited with GenBank under
334 ON351017.

335

336 **Competing interests**

337 The authors declare no competing interest.

338

339

340 **EXPERIMENTAL PROCEDURES**
341

342 **Culturing of mouse ES cells**

343 The genetic background of the ES cells generated in this work is identical
344 (129S6/C57BL6 (G4))³⁰ or C57BL/6J (gift from Lars Wittler). The mESCs were either
345 cultured in feeder free 2i media or on feeder cells (mitomycin inactivated SWISS
346 embryonic fibroblasts) containing LIF1 (1000 U/ml). 2i media: 1:1 Neurobasal (Gibco
347 #21103049) :F12/DMEM (Gibco #12634-010), 2 mM L-glutamine (Gibco), 1x Penicillin/
348 Streptomycin (100x penicillin (5000 U/ml) / streptomycin (5000ug/ml), Sigma #P4458-
349 100ML, 2 mM glutamine (100x GlutaMAX™ Supplement, Gibco #35050-038), 1x non-
350 essential amino acids (100x MEM NEAA, Gibco #11140-035), 1x Sodium pyruvate
351 (100x, Gibco, #11360-039), 0.5x B-27 supplement, serum-free (Gibco # 17504-044),
352 0.5x N-2 supplement (Gibco # 17502-048), Glycogen synthase kinase 3 Inhibitor
353 (GSK-Inhibitor, Sigma, # SML1046-25MG), MAP-Kinase Inhibitor (MEK-Inhibitor
354 Sigma, #PZ0162), 1000 U/ml Murine_Leukemia_Inhibitory_Factor ESGRO (10⁷ LIF,
355 Chemicon #ESG1107), ES-Serum media: Knockout Dulbecco's Modified Eagle's
356 Medium (DEMEM Gibco#10829-018), ES cell tested fetal calf serum (FCS), 2 mM
357 glutamine, 1x Penicillin/ Streptomycin, 1x non-essential amino acids, 110 nM β-
358 Mercaptoethanol, 1x nucleoside (100x Chemicon #ES-008D), 1000 U/ml LIF1.
359 The cells were split with TrypLE Express (Thermo Fisher Scientific #12605-010) and
360 the reaction was stopped with the same amount of Phosphate-Buffered Saline (PBS
361 Gibco #100100239) followed by centrifugation at 1000 rpm for 5min. The cells were
362 frozen in the appropriate media containing 10% Dimethyl sulfoxide (DMSO, Sigma
363 Aldrich #D5879). To minimize any effect of the 2i³¹ on the developmental potential
364 mESC were only kept in 2i for the antibiotic selection for transgene integration after
365 selection kept on feeders.

366

367 **Genetic manipulation of ES cells and generation of embryos and mice**

368 ES cells were modified according to standard procedures. Briefly, 10x10⁶ ES cells
369 were electroporated with 25μg of linearized targeting construct and cultivated with
370 selection media containing 250 μg/ml G418 (Life Technologies #10131035) or 125
371 μg/ml Hygromycin B (Life Technologies #10687010) for the first and second targeting,
372 respectively. Resistant clones were isolated, and successful gene targeting was
373 confirmed. Embryos and live animals were generated by tetraploid complementation³².

374 Homozygous *Swht*^{3xpA[N]/3xpA[H]} ES cells generated 21 mice from four foster mothers,
375 confirming their integrity and usability in subsequent developmental assays. The
376 selection cassettes consisting of *PGK::Neo-SV40pA* (abbreviated “N”) or *PGK::Hygro-*
377 *SV40pA* (abbreviated “H”) were flanked by *FRT* sites. Selection cassette was removed
378 by crossing animals with a FLP delete strain³³.

379

380 **Generation of *Swht* Rescue BAC**

381 The BAC RP23-466K9 was ordered from BACPAC Resource Center (BPRC) and its
382 integrity verified by HindIII digest. The RP23-466K9 BAC contains the 175,271bp of
383 mouse genomic sequence surrounding the *Nkx2-5* locus. This BAC includes the
384 upstream (*Bnip1*) and the downstream (*Kifc5b*) located genes. The BAC was modified
385 using the Red/ET recombinase system (Genebridges). The H2B-Venus expression
386 cassette, followed by a globin polyadenylation site and a downstream Neomycin
387 selection cassette, was inserted into the ATG of *Nkx2-5*. This will eliminate any *Nkx2-*
388 5 expression from the BAC and simultaneously allows monitoring of rescue construct
389 by means of detection of yellow fluorescence in the hearts of embryos and adults.
390 Around 3 Mio mESC cells of the C57Bl6J background were collected and resuspend
391 in 680 µl PBS and were mixed with 120 µl linearized (PI-SceI) BAC (42 ng/µl). The
392 BAC was electroporated into the C57Cl6J cells under the following conditions: 240V;
393 500uF; 4mm; ∞ with a Gene Pulser Xcell™ Electroporation Systems from BioRad.
394 Afterwards the cells were resuspended in 2i Media and plated on gelatin coated cell
395 culture dishes. The next day the selection of the cells started with 300 µg/ml G418
396 (InvivoGen, #ant-gn-1). The selection was done till the colonies were big enough for
397 picking after 7-8 days. Afterwards the procedure was the same as described above.

398

399 **Generation of mouse embryos and strains from mESCs**

400 All animal line generation procedures were conducted as approved the Landesamt für
401 Gesundheit und Soziales Berlin (LAGeSo), Berlin under the license numbers
402 G0349/13. Embryos were generated by tetraploid morula aggregation of embryonic
403 stem cells as described in³⁰. SWISS mice were used for either wild-type donor (to
404 generate tetraploid morula) or transgenic recipient host (as foster mothers for
405 transgenic mutant embryos). All transgenic embryos and mESC lines were on a hybrid
406 F1G4 (C57Bl6/129S6) background or the C57Bl6J background (rescue BAC).

407 To generate the mouse strains the transgenic cells were aggregated with diploid
408 morula SWISS embryos. The genotype of the cells was either hybrid F1 for the *Swht*
409 mutant mice or wild type C57BL6J for the rescue mice. Adult *Swht* mutant mice were
410 backcrossed 6 times to C57BL6J before all subsequently conducted experiments.

411

412 **Whole mount *in situ* hybridization**

413 Whole-mount *in situ* hybridization was carried out using standard procedures
414 described on the MAMEP website (<http://mamep.molgen.mpg.de/index.php>). Probes
415 were generated by PCR from E11.5 heart ventricle cDNA using primer containing
416 promotor binding site for T7 and SP6 polymerase. After verification of the probe
417 templates, antisense *in situ* probes were generated as described on the MAMEP
418 website using T7 polymerase (Promega #P2077). The *in situ* probes are generated
419 against *Nkx2-5* or *Sweetheart*.

420

421 **RNA isolation**

422 To isolate RNA either from heart tissue or cultivated cardiomyocytes the cells were
423 lysed in 900 µl Qiazol (Qiagen, #79306). To remove the DNA 100 µl gDNA Eliminator
424 solution was added and 180 µl Chloroform (AppliChem, #A3633) to separate the
425 phases. The extraction mixture was centrifuge at full speed, 4°C for 15min. The
426 aqueous phase was mixed with the same amount of 70 % Ethanol and transferred to
427 a micro or mini columns depending of the amount of tissue and cells. The following
428 steps were done according to the manufactual protocol.

429

430 **Subcellular RNA fractionation**

431 Cellular fractionation was carried out as previously described³⁴. Briefly, cell pellets
432 were resuspended in 200 µl cold cytoplasmic lysis buffer (0.15% NP-40, 10mM Tris
433 pH 7.5, 150mM NaCl) using wide orifice tips and incubated on ice for 5 min. The lysate
434 was layered onto 500 µl cold sucrose buffer (10mM Tris pH7.5, 150mM NaCl, 24%
435 sucrose w/v), and centrifuged in microfuge tubes at 13,000 rpm for 10 min at 4 C. The
436 supernatant from this spin (700 µL) represented the cytoplasmic fraction. 10% (70 µL)
437 of the supernatant volume was added to an equal volume of 2X sample buffer for
438 immunoblot analysis. The remaining supernatant was quickly added to 15 ml tubes
439 containing 3.5X volumes of QIAGEN RLT Buffer, supplemented with 0.143 M β-

440 mercaptoethanol. RNA purification from these and subsequent cellular fractions was
441 performed according to manufacturer instruction.

442 The nuclear pellet was gently resuspended into 200 μ l cold glycerol buffer (20 mM Tris
443 pH 7.9, 75 mM NaCl, 0.5 mM EDTA, 50% glycerol, 0.85 mM DTT) using wide orifice
444 tips. An additional 200 μ l of cold nuclei lysis buffer (20 mM HEPES pH 7.6, 7.5 mM
445 MgCl₂, 0.2 mM EDTA, 0.3M NaCl, 1M urea, 1% NP-40, 1mM DTT) was added to the
446 samples, followed by a pulsed vortexing and incubation on ice for 1 min. Samples were
447 then spun in microfuge tubes for 2 min at 14,000 rpm and at 4 C. The supernatant from
448 this spin represented the nucleoplasmic fraction (400 μ L), and 10% of supernatant was
449 kept for immunoblot analysis. 3.5X volumes of QIAGEN RLT were added to the
450 remaining nucleoplasmic supernatant.

451 50 μ l of cold PBS was added to the remaining chromatin pellet, and gently pipetted up
452 and down over the pellet, followed by a brief vortex. The chromatin pellet was
453 extremely viscous and sticky, and therefore difficult to fully resuspend. 5 ml of the PBS
454 supernatant was collected for immunoblot analysis as above, and 500 μ l TRI-Reagent
455 was added to the pellet. After vigorous vortexing to resuspend the chromatin,
456 chromatin-associated RNA was extracted by adding 100 μ l chloroform and incubated
457 at room temperature for 5 min. The chromatin samples were then centrifuged in
458 microfuge tubes for 15 min at 13,000 rpm at 4 C. The resulting upper aqueous layer
459 was then added to 3.5X volumes QIAGEN RLT buffer.

460

461 **Full-length cDNA determination**

462 Rapid amplification of cDNA end (RACE) was performed using the SMARTer® RACE
463 5'/3' Kit (Takara, #634858). 1ug of freshly isolated RNA from E8.5 embryo hearts was
464 used to generate first strand cDNA according to the manufacturer protocol. The primers
465 were designed between 60-70°C Tm. Half of the PCR product was analysed by
466 agarose gel electrophoresis and the rest was used for nested PCRs to validate the
467 5' and 3' end. PCR products were extracted from the agarose gel and sent for
468 sequencing. After the determination of the end, the full-length sequences were
469 amplified and sequenced. The sequences were deposited at GeneBank under the IDs:
470 ON351017 (*Sweetheart RNA, Swhtr*).

471

472 **Fractionation of the main cell types of the adult heart**

473 Fractionation was conducted as previously described²⁰. Briefly, adult mice were
474 sacrificed and hearts collected in HBSS (gibco #14025050). Hearts were enzymatically
475 digested using the Multi Tissue Dissociation Kit 2 (Miltenyi #130-110-203).
476 Cardiomyocyte fraction was obtained by pre-plating. Endothelial cells and immune
477 cells were obtained by magnetic separation. Fibroblasts were enriched by another
478 preplating step.

479

480 **Cardiac Injury Models – Ligation of the left anterior descending artery**

481 Mouse was anesthetized by IP injection of a mixture of ketamin/xylazine/acepromazin
482 (65/15/2 mg/kg). Mouse was placed on warming pad for maintenance of body
483 temperature. In the supine position, endotracheal intubation was performed, and the
484 mouse was placed on artificial ventilation with a mini-rodent ventilator (tidal volume =
485 0.3ml, rate = 120 breaths/min). Ocular gel was applied to hydrate the cornea during
486 the surgical procedure. Proper intubation was confirmed by observation of chest
487 expansion and retraction during ventilated breaths. A left thoracotomy was performed.
488 The pectoralis muscle groups were separated transversely, and the fourth intercostal
489 space was entered using scissors and blunt dissection. The pericardium was gently
490 opened, and a pressure was applied to the right thorax to displace the heart leftward.
491 A 7.0 silk ligature was placed near the insertion of the left auricular appendage and
492 tied around the left descending coronary artery. Occlusion of the artery was verified by
493 the rapid blanching of the left ventricle. The lungs were re-expanded using positive
494 pressure at end expiration and the chest and skin incision were closed respectively
495 with 6-0 and 5-0 silk sutures. The mouse was gradually weaned from the respirator.
496 Once spontaneous respiration resumed, the endotracheal tube was removed, and the
497 animal was replaced in his cage on a warming pad with standard chow and water ad
498 libitum. Analgesic drug (Temgesic, Buprenorphin 0.1 mg/kg) was administered
499 subcutaneously after the surgery.

500 Animal experiments were approved by the Government Veterinary Office (Lausanne,
501 Switzerland) and performed according to the University of Lausanne Medical School
502 institutional guidelines.

503

504 **In vivo transthoracic ultrasound imaging**

505 Transthoracic echocardiography was performed using a 30 MHz probe and the Vevo
506 2100 Ultrasound machine (VisualSonics, Toronto, ON, Canada). Mice were lightly

507 anesthetized with 1-1.5% isoflurane, maintaining heart rate at 400-500 beats per
508 minute. The mice were placed in decubitus dorsal on a heated 37°C platform to
509 maintain body temperature. A topical depilatory agent was used to remove the hair
510 and ultrasound gel was used as a coupling medium between the transducer and the
511 skin. The heart was imaged in the 2D mode in the parasternal long-axis view. From
512 this view, an M-mode cursor was positioned perpendicular to the interventricular
513 septum and the posterior wall of the left ventricle at the level of the papillary muscles.
514 Diastolic and systolic interventricular septum (IVS;d and IVS;s), diastolic and systolic
515 left ventricular posterior wall thickness (LVPW;d and LVPW;s), and left ventricular
516 internal end-diastolic and end-systolic chamber (LVID;d and LVID;s) dimensions were
517 measured. The measurements were taken in three separate M-mode images and
518 averaged. Left ventricular fractional shortening (%FS) and ejection fraction (%EF)
519 were also calculated. Fractional shortening was assessed from M-mode based on the
520 percentage changes of left ventricular end-diastolic and end-systolic diameters. %EF
521 is derived from the formula of $(LV\ vol;d - LV\ vol;s) / LV\ vol;d * 100$. Echographies were
522 done in baseline condition and one and two weeks after surgery. Sacrifices were done
523 the day of the 2-week post-MI echography.

524

525 **Heart preparation and histology**

526 Adult hearts were dissected two weeks after MI and fixed in 4%
527 paraformaldehyde/PBS over night. Fixed hearts were embedded in paraffin and
528 sections (4-6 μ m thickness) were mounted onto Superfrost® Plus microscope slides
529 (Thermo scientific #630-0950). Immunohistochemistry was carried out using standard
530 procedures. The Antibody used for the detection of cell borders was anti wheat germ
531 agglutinin (WGA, Thermo Fisher Scientific, #W11261). The slides were mounted with
532 Vectashield (VWR, #101098-042) and sealed with colorless nail polish. Image
533 documentation was conducted using the NIKON Eclipse Ci, equipped with the Ds-Ri2
534 color camera. Analysis of cell sizes was conducted using ImageJ software.

535

536 **Real-time quantitative PCR analysis**

537 Quantitative PCR (qPCR) analysis was carried out on a StepOnePlus™ Real-Time
538 PCR System (Life Technologies) using Power SYBR® Green PCR Master Mix
539 (Promega #A6002). RNA levels were normalized to housekeeping gene. Quantification
540 was calculated using the $\Delta\Delta Ct$ method³⁵. *Hmbs* served as housekeeping control gene

541 for qPCR. The primer concentration for a single reaction was 250nM. Error bars
542 indicate the standard error from biological replicates, each consisting of technical
543 duplicates. A list of oligonucleotides can be found in Table S1.

544

545 **Embryo / heart preparation and histology**

546 Staged embryos and adult hearts were dissected from uteri into PBS and fixed in fresh
547 4% paraformaldehyde/PBS 1mm tissue per 1h at 4°C. For histology, embryos and
548 hearts were embedded in paraffin. E7.5 embryos were removed from the uterus of
549 timed mated mothers together with the surrounding decidua and fixed all together.
550 Sections (4-6 µm thickness) were mounted onto Superfrost® Plus microscope slides
551 (Thermo scientific) or on Zeiss MembraneSlide 1.0 PEN NF (#415190-9081-001). The
552 stainings were carried out with Eosin (Carl Roth), Hematoxylin (AppliChem) and Sirius
553 Red according to standard procedures. All image documentation was carried out on
554 Microscope Leica M205C with the MC170 HD camera and captured with ImageJ.
555 Except the E7.5 embryo sections, which were imaged on a NIKON Eclipse Ci,
556 equipped with the Ds-Ri2 color camera.

557

558 **SmFISH**

559 FISH Probes were designed, using the biosearchtech.com/stellaris-designer website
560 and ordered from BioCat. The lyophilized Probes were resuspended in 400 µl 1x Tris-
561 EDTA Buffer (10 mM Tris-HCl (AppliChem, #A1086, AppliChem, #A5634), 1 mM EDTA
562 (Life technologies, #15575020), pH 8.0) to get a final concentration of 500nM per µl.
563 The probes were conjugated with a Quasar570 dye and small aliquots were stored at
564 -20°C.

565 Cardiomyocytes from E11.5 heart ventricles were cultivated (as described) on cover
566 slips (10 mm Marienfeld, #0111500) for 48h. For the fixation process the cells were
567 washed once with PBS and fixed 10min at room temperature with 4%PFA/PBS
568 (AppliChem, #A3813). Again, the cells were washed 3 times with PBS and
569 permeabilized 5 min on ice with permeabilize sol (1xPBS, 1%RNase inhibitor
570 Ribovanadylcomplex (RVC, NEB, #S1402S), 0,5 % Triton X-100 (Sigma, #T8787)).
571 Afterwards the cells were washed three times with 70 % Ethanol (Roth, #T913.7) and
572 stored in 70 % Ethanol at -20°C. For the hybridization transfer the cover slips to 70%
573 Ethanol at room temperature and incubate 10min. Add wash buffer (10% saline sodium
574 citrate buffer (20xSSC-Buffer, Invitrogen, # 15557-036), 10% Formaldehyde (FA,

575 Sigma, # F8775), in RNase free water) and incubate again for 10min. Afterwards
576 incubate the cells with 25 μ l of hybridization solution (10 % SSC-Buffer, 10 % FA, 10%
577 Dextran sulfate (Roth, # 5956.3), in RNase free water, 2ul of the dye (1000nM)) in a
578 humidity chamber at 37°C in the dark for 4h. Transfer the cells to pre-warmed wash
579 buffer and incubate in the dark at 37°C 30min without shaking. Afterwards wash the
580 cover slips with 2xSSC Buffer and incubate 5min at room temperature. Mount the cover
581 slips with Vectashield Mounting Media containing DAPI (VWR, # 101098-044) and seal
582 it with colorless nail polish. For the visualization Zeiss Axio Observer-Z1 with a 100x
583 objective was used.

584

585 **Heart slice preparation, treatment and harvest**

586 4 Wild-type and 4 *Swltr*^{3xpA/3xpA} mice were sacrificed by cervical dislocation when they
587 reached the age of 8 weeks. The heart was collected in HBSS and washed in BDM
588 buffer (HBSS + 10 mM BDM) to remove blood cells. Apex and base of the heart were
589 manually removed before preparing the slices using a sharp scalpel. The slices were
590 washed in BDM buffer once more before submerging them in culture medium
591 consisting of DMEM (gibco#10569010) supplemented with 10 % Fetal Bovine Serum,
592 1% Non-Essential Amino Acids (gibco #11140050) and 1% Penicillin-Streptomycin
593 (gibco #15140122). The heart slices were left to recover overnight. During the
594 experiment, the media was refreshed every other day.

595 For the treatment, the slices were incubated in a humidified hypoxic chamber (3% O₂,
596 5% CO₂) at 37 °C for 7 days and moved to a humidified incubator with normoxic
597 conditions (21% O₂, 5% CO₂) at 37 °C for 7 additional days. Slices that were incubated
598 in a humidified incubator at normoxic conditions (21% O₂, 5% CO₂) at 37 °C for 14
599 days served as the control. Following the treatment, the slices were harvested in 1 ml
600 TRI reagent (Sigma-Aldrich #T9424) and homogenized using Precellys® 2 mL Soft
601 Tissue Homogenizing Ceramic Beads. RNA was extracted by Phenol/Chloroform
602 extraction. Briefly, 200 μ l Chloroform were added per 1 ml of TRI reagent and mixed.
603 After centrifugation at 4 °C aqueous phase was precipitated by adding 0.7 vol of
604 Isopropanol and incubating at -20 °C for 1 hour. The precipitated RNA was pelleted
605 by centrifugation at 4 °C, washed with 70 % Ethanol and resuspended in nuclease-
606 free water.

607

608 **Bioinformatic analysis and data deposit**

609 RNA was treated to deplete rRNA using Ribo-Minus technology. Libraries were
610 prepared from purified RNA using ScriptSeq™ v2 and were sequenced on an Illumina
611 novaseq 6000 platform at Novogene. We obtained 25 million paired-end reads of 150
612 bp length. Read mapping was done with STAR aligner using default settings with the
613 option --outSAMtype BAM SortedByCoordinate³⁶ with default settings. For known
614 transcript models we used GRCm38.102 Ensembl annotations downloaded from
615 Ensembl repository³⁷. Counting reads over gene model was carried out using
616 GenomicFeatures Bioconductor package³⁸. The data are deposited to GEO under the
617 accession number GSE200380.

618 All genes with read counts < 10 were excluded. For normalization of read counts and
619 identification of differentially expressed genes we used DESeq2 with Padj < 0.05 and
620 log2FC= 0.58 cutoff³⁹. GO term were analyzed using clusterProfiler and enrichplot
621 Bioconductor packages⁴⁰. To overlap NKX2-5 binding with *Swtrh* dependent DE
622 genes, we downloaded ChIP-seq data from E12.5 heart (GSM3518650). Raw reads
623 were downloaded and aligned to mm10 using Bowtie2⁴¹. Samtools⁴² was used to
624 convert aligned reads to sorted bam files. Duplicated reads as well as reads
625 overlapping blacklisted region were removed using bedtools⁴³. Peaks, then, were
626 called with MACS3 peak caller⁴⁴. All peaks were sorted, merged and finally intersected
627 with genes coordinates using bedtools and ChIPpeakAnno Bioconductor package⁴⁵.
628 The pvalue of the overlapping peaks were calculated according to hypergeometric test.
629

630 **LITERATURE**

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742

Rogala, Figure 1

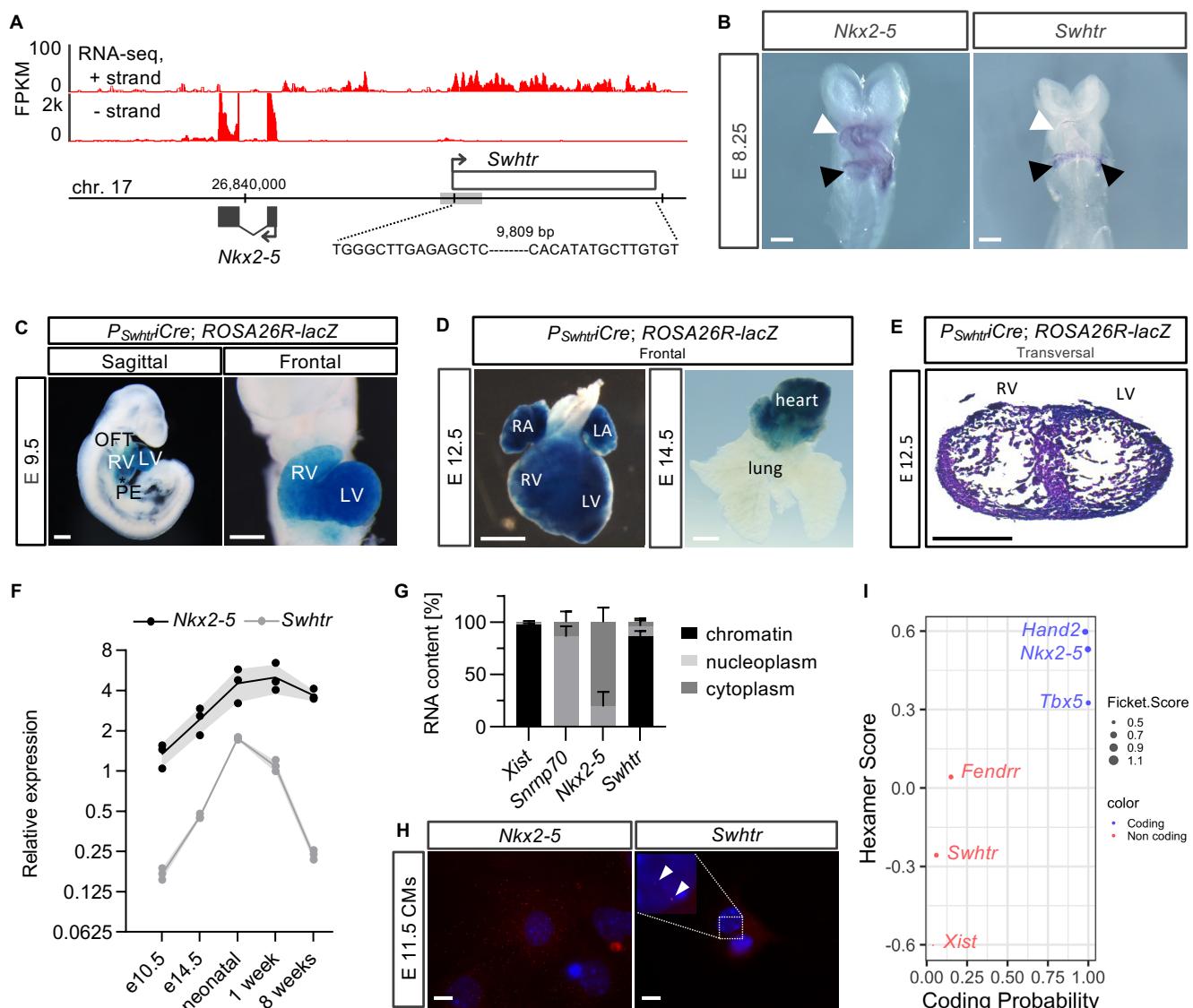


Figure 1. Expression and localization of the Sweetheart lncRNA

(A) Strand-specific RNA-seq from E9.5 heart tubes showing the *Nkx2-5* region. (grey box= first heart field enhancer). Plus-strand track is 20x amplified over the minus-strand track. The number above the genome bar denote the mm10 co-ordinates and the vertical tip bars represent 10,000 bp steps.

(B) Whole mount in situ hybridization of *Nkx2-5* and *Swhtr* in E8.25 embryos. White arrow show the early heart tube with two different heart fields and black arrowhead points to the inflow tract region. The white line represents 500 μ m.

(C) Lineage tracing of *Swhtr* expressing cells in E9.5 embryos. OFT= outflow tract, RV= right ventricle, LV= left ventricle, PE= Pre-pericardium. The white line represents 500 μ m.

(D) Lineage tracing of *Swhtr* expressing cells in late gestation embryos and heart/lung explant. The white line represents 1 mm.

(E) Lineage tracing of *Swhtr* expressing cells in a transversal section of an E12.5 heart. RV= right ventricle, LV= left ventricle. The black line represents 500 μ m.

(F) Quantitative Real-Time PCR timeline of *Swhtr* and *Nkx2-5* expression levels in the hearts of E10.5 embryos to 8 week adult mice. Embryo hearts were pooled from independent litters and the postnatal stages represent data from individual hearts (n=3).

(G) Subcellular fractionation of E11.5 cardiomyocytes (CMs) of marker transcripts and *Swhtr* (n=2).

(H) SmFISH of *Nkx2-5* and *Swhtr* in 24h cultured E11.5 cardiomyocytes. The white line represents 10 μ m.

(I) Analysis of coding potential of *Swhtr* by CPAT compared to known coding and non-coding RNAs.

Rogala, Figure 2

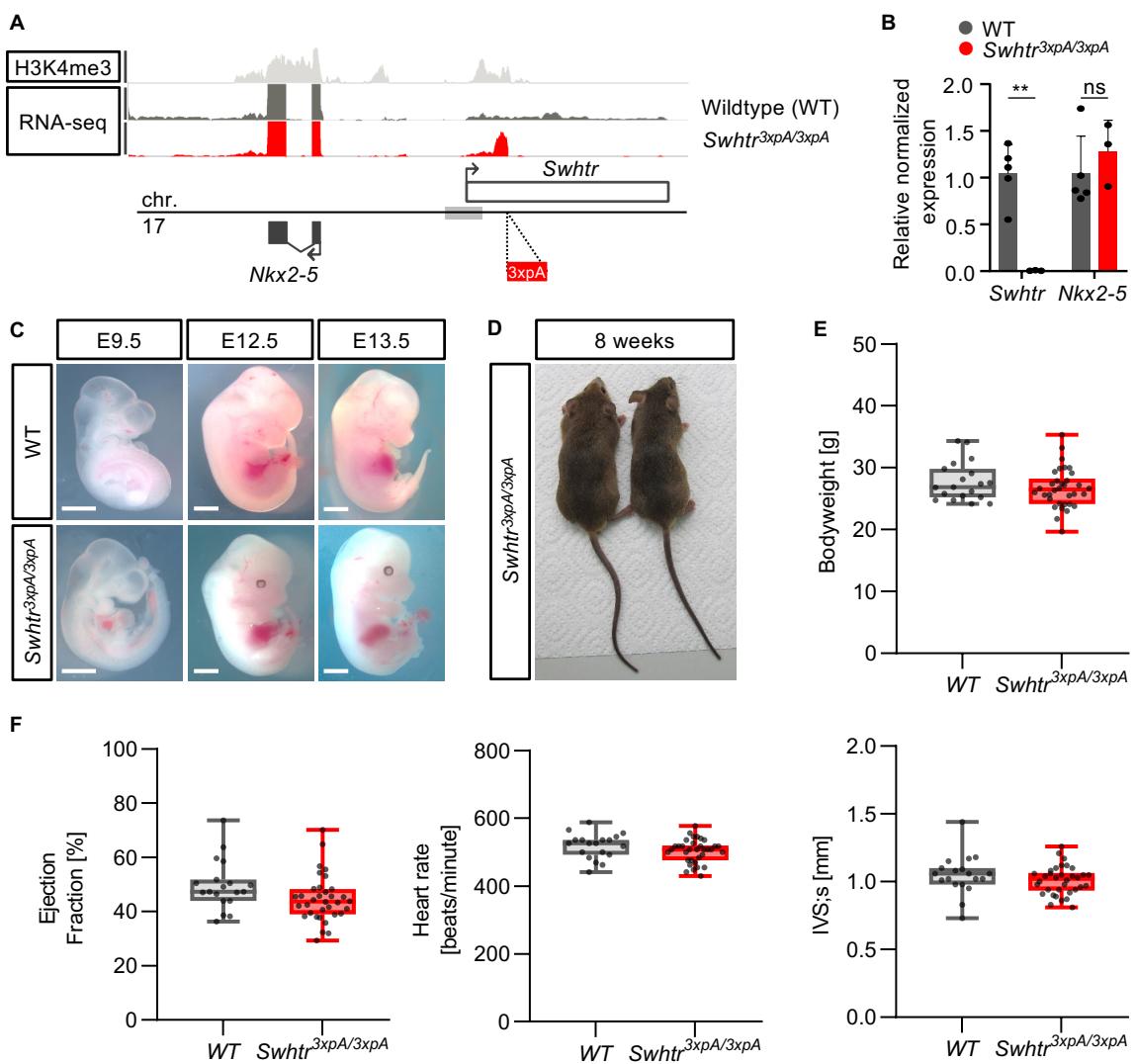


Figure 2. No overt phenotype in *Swhtr*^{3xpA/3xpA} mutant embryos or adult mice

(A) ChIP-seq (H3K4me3) and RNA-seq (E9.5 heart tubes) from WT and *Swhtr*^{3xpA/3xpA} mutant mice.

(B) qPCR validation of loss of *Swhtr* in E9.5 embryonic hearts (WT n=5; *Swhtr*^{3xpA/3xpA} n=3). Statistical significance was tested by Two-way ANOVA. ns = not significant, ** < 0.01

(C) Embryos of indicated age from WT and *Swhtr* mutants. The white line represents 1 mm.

(D) Eight week old *Swhtr*^{3xpA/3xpA} founder mice.

(E) Bodyweight of *Swhtr*^{3xpA/3xpA} eight times backcrossed (C57BL/6J) mice (WT n=19; *Swhtr*^{3xpA/3xpA} n=34).

(F) Selected cardiac parameters determined by echocardiography in eight week old mice of the indicated genotype (WT n=19; *Swhtr*^{3xpA/3xpA} n=34). Statistical significance was tested by Two-way ANOVA. No statistical significant differences were detected.

Rogala, Figure 3

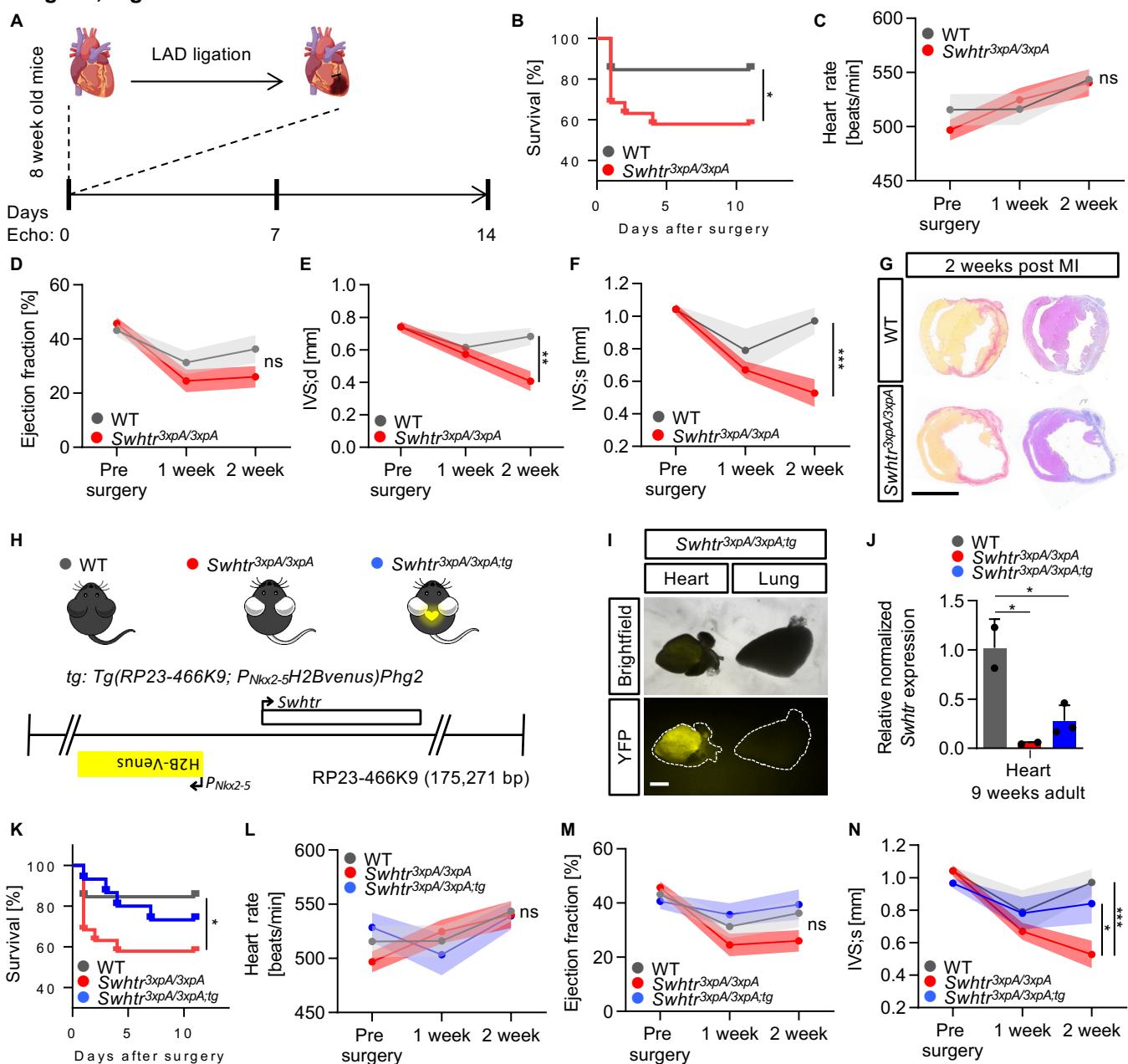


Figure 3. Induced myocardial infarction by left ascending artery ligation (LAD)

(A) Schematic of the analysis setup for echocardiography and LAD ligation in mice of age 8 weeks

(B) Reduced survival of *Swhtr* null mice compared to WT mice after LAD ligation (WT n=13; *Swhtr*^{3xpA/3xpA} n=19). Statistical significance was tested by Kaplan-Meier Simple Survival Analysis. * $p < 0.05$

(C-F) Selected heart specific parameters in mice (n=9 animals per genotype) before and after (1 and 2 weeks) LAD ligation. Statistical significance was tested by Two-way ANOVA. ns = not significant, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

(G) Verification of infarct presence in mice 2 weeks after LAD ligation by Sirius red (fibrotic tissue) staining. The black line represents 5 mm.

(H) Schematic of the rescue transgene and the resulting mouse line. The rescue transgene (*tg*) is comprised of the BAC (RP23-466K9) that includes the *Nkx2-5* (H2Bvenus inserted in *Nkx2-5* ATG) and *Swhtr* loci, randomly inserted into the genome of wild type C57BL6J mice.

(I) Verification of *tg* presence and activity by heart specific presence of H2BVENUS.

(J) Expression verification of *Swhtr* in the heart of *Swhtr* null mutants, after crossing. Statistical significance was tested by One-Way ANOVA. * $p < 0.05$

(K) Reduced survival of *Swhtr* null mice compared to WT and *Swhtr* rescue mice after LAD ligation (WT n=13; *Swhtr*^{3xpA/3xpA} n=19; *Swhtr*^{3xpA/3xpA;tg} n=15). Statistical significance was tested by Kaplan-Meier Simple Survival Analysis. * $p < 0.05$

(L-N) Selected heart specific parameters in mice (n=9 animals per genotype) before and after (1 and 2 weeks) LAD ligation. Statistical significance was tested by Two-way ANOVA. ns = not significant, * $p < 0.05$, ** $p < 0.01$

Rogala, Figure 4

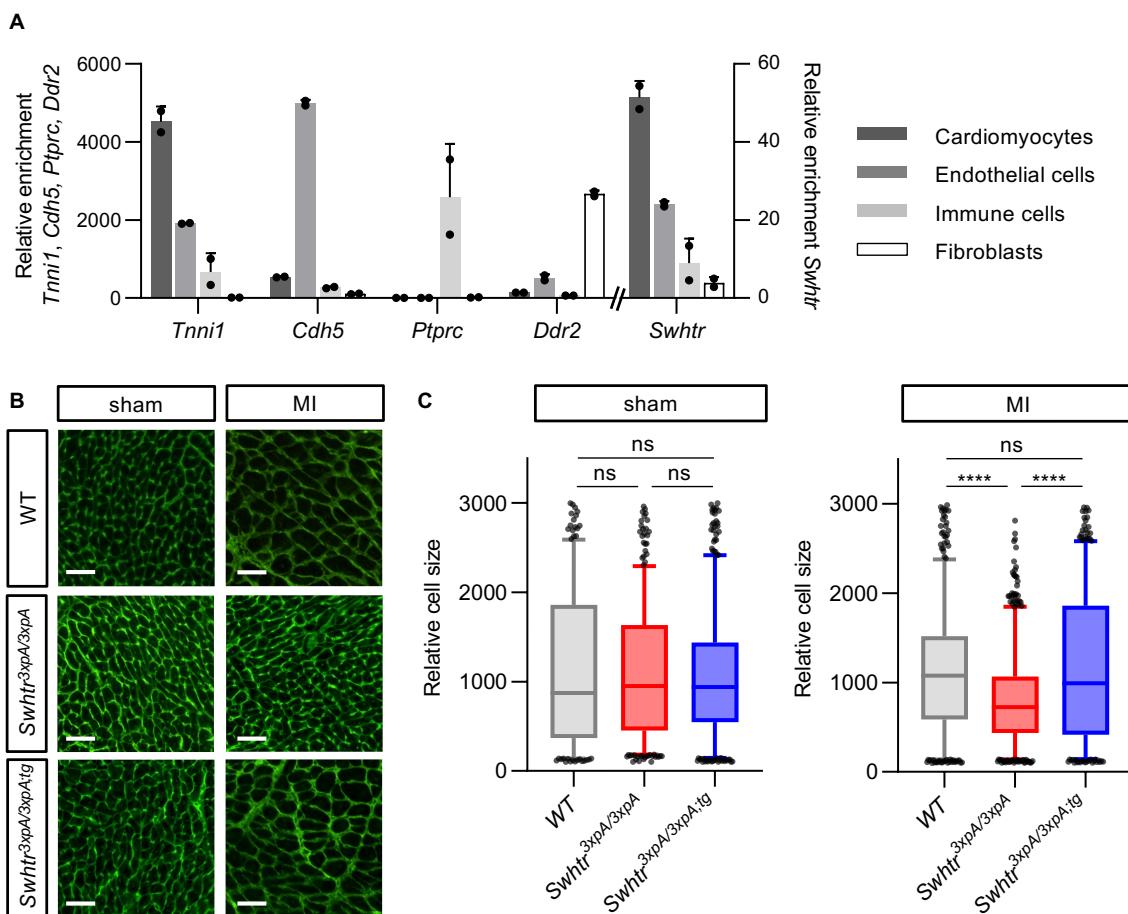


Figure 4. Hypertrophy in *Swhtr* mutant cardiomyocytes.

(A) Relative enrichment of *Swhtr* in the main cell-types represented in the heart of 8 week old mice as compared to marker genes (n=2). Note that the *Swhtr* expression pattern resembles that of *Tnni1*.

(B) Wheat Germ Agglutinin stained IHC of representative sections of IVS tissue in the indicated genotypes 2 weeks post sham or MI. The white line represents 50 μ m.

(C) Automated quantifications of relative cell sizes in the interventricular septum of 3 representative animals of the indicated genotype 2 weeks post sham or MI. Statistical significance was tested by One-way ANOVA. ns = not significant, **** < 0.0001

Rogala, Figure 5

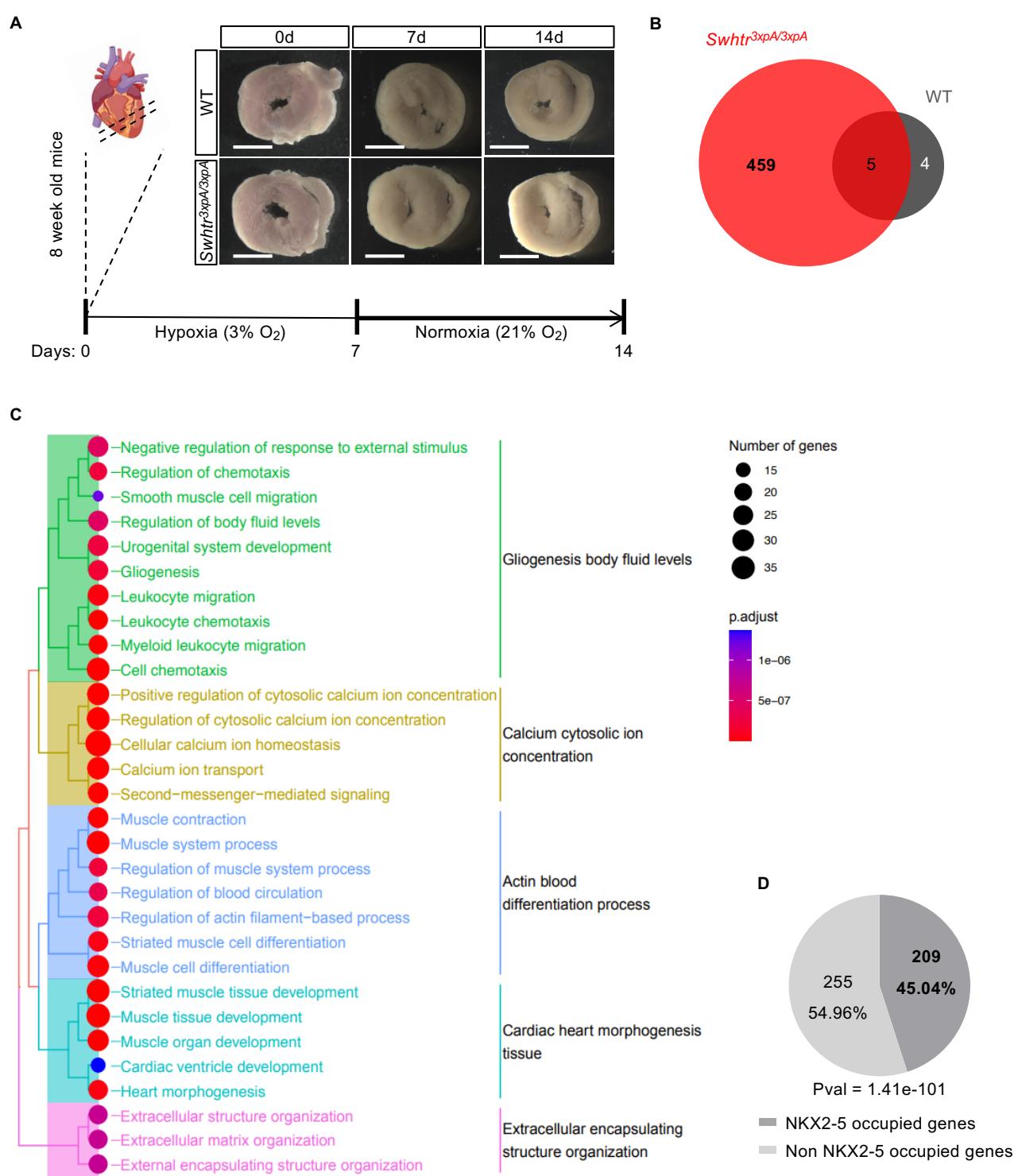


Figure 5. Swhtr dependent genes under cardiac stress

(A) Schematic overview of the experimental procedure with representative pictures of heart slices. The white line represents 5 mm.

(B) Number of deregulated genes after 7 days of hypoxia treatment followed by 7 days of normoxic conditions of *Swhtr*^{3xpA/3xpA} heart slices compared to WT heart slices (n=4).

(C) GO-term enrichment analysis of deregulated *Swhtr* dependent genes.

(D) NKX2-5 occupation on *Swhtr* dependent genes. P-value from hypergeometric test (ChIPpeakAnno).