RESEARCH LETTER

а Mouse ----TGCGAAGAT----TTTCAAAGT--G-GGGC 181 Rat Human Pig Dog Cov -----CTTAGAGAAAACCAGTCCTTTGGGAGCCTGTGCCCGCCCAGCGGGC 206 ... -----GAATCTGTG--GTGT-----Mouse -CTCGATTTCTC----GGCGAATATCCG 219 A-C--CTCGATTTCTC-----GAATCTGTG--GTGT-----GGCGAATATCCG 129 A-C--CTCGATTCCCC-----GAATCTGTA--GTGT-----GGCTGGTATCGG 259 Rat Human A C--CTGGATTCCCC------GATTGTGTGT------GGCGGGTATCGG 261 AAC--CTGGATTCCCC------GATTGGTG--GAGT------GGCGGGTATCGG 261 TCTGGCTTGGCAGCTCAGGAGGTGGAGGGCCCAGGGCAGGCAGGCCAGGCCAGGCCAGGCCAGGCCAGGCCAGGCCAGGCCAGGCCA Pig Dog Cow Моцие TGTTCCTCCTGCTTAACTAGC---CTG----TTTGAAGGCACACTTCATTCTCGAGCA- 270 IGTICTCCCGGTTARACTACC---CTG----TTIGAAGGACATGICATCCGGGAT 25 IGTICTCCCCGGTTAACTACC---CTG----TTIGAAGGACAAATGCA---AGGGGAT 15 IGTICCCCCGGTTTAACTACC---CTG-----TTIGAAGGACAGATCATCCAGGGGGAT 307 IGTICCCCCGGTTTAACTAGC---CTG-----TTIGAAGGTCAGATCATCCCGGGAT 253 Rat Human Pig Dog Cow TCTTCTCCATCCCACGCCGGCGTCCCGGAGCCCTTGGATGCGCGGTCCATC--CAAGCAG 324 -----TC--GTCACCTCCTGGGGGGTCT--Mouse Rat Human Pig Dog Cow * Mouse Rat Human Pig Dog Cow T----GGCCAAG-GCAGGTCTG---ATTCTATITATICTAAA-----GACAGAGTT---- 382 TCTCTGGCAAAATGAAGGTCTG---A---GACGTTICTAAAA-----GACAGAGTT---- 380 T----GGCCAAGTGCCTTCAAT-ACACTTITTATCTGGGAT-----GACGAAACTCAAC 450 TCTTGAATCATCATCCAATGAAGGCAATGCACACTGGGGAG-----AGGGGAGTT-ACC 450 T----GGCCAAGGCCCAGTTAA---AATGCCGCTCCTCCAGA----AGCC---- 386 C-----GCTTGGCCGC--ATTGCA--AGTITGTCTTCTCGGGATCCCAGGCAGAGGTGGAC 457 Mouse Rat Human Pig Dog Cow ---GEAGATGGGCTACCATTGGTGTTGATT-GAGATGAC-----AAGCAG 423 ---GGAAATGAACCATCATTGGTGTTGACA-GAGATGCC-----AAGCAG 349 CAAGAAAATATATGTCGATTCTTGTATAT--GGGACAAT-----ATTAG 492 Mouse Rat Human Pig Dog Cow ------TGTCCACC--CTCCTGGACCCGGCCCCGCC-C------GGCGG 421 TGGGAAAATGGA----GACCCTCCAGGGCCAGGGAGAAC-----TGG 495 TCACC-TGAGCCC--AT---AC-AGCTGGGTTCACAGATGTGGCTAGTCTTGCT----AG 472 Mouse Pig Dog Cow TTTCGGTGGTACTTCTC----CCAGAATGATGTGCGCTTTCAGACAGGCTAGTTA----- 546 CCAGCTOGCACTGTGGA--CATTCTAGGGAACTGACTGCAGTCC-ICATGCTTGGAGTCA 529 CCA-------CATCTAGCAA----GCA-----CACACT-GGAACTGA CTG--IGATATTGTTTI--ITCCTAARGA-----IGCATAGTGTAACAATTGGTGTCA 591 Mouse Rat Humar Pig ACATGTATCATTATCTTATTTCCTTGTTGAAC--ATTGTA--CTGTCATGTTCCTACTTG 619 Dog -----CACCGATACCGGCAA-----CACCGATACCGGCCA 572 Cow AACACTTTAACCACTGCACACGTTT---ATGTAATAGGATTGACATATGAGA---ATCAG 583 Mouse Human Pig Dog Cow AGAAATGAAT----GAAA---GATTAGAGTGGGAGAAAGTGCTTGCTGCCAAGCTTGAT 635 Mouse Rat TGGATTGATT----GAA-----GAGAAACTG----CAAGCTT--- 440 Humar Pig Dog Cow GACCTARATTGAATACCTGGAACCTACACGACAGAAAGAGAGAGAGCTGACTGCTGAAAGAT 695 Mouse _____ Rat Human G-----T 698 Pig Dog Cow

Extended Data Figure 1 | **Sequence alignment of several mammalian** *Uph* transcripts. **a**, Sequence alignment of several mammalian *Uph* transcripts performed using ClustalW. See Methods for source data. **b**, Diagram of the *Hand2* locus in mammals showing the genomic organization and orientation of *Uph*. The *Hand2* branchial arch enhancer (green) and cardiac enhancer (yellow) are shown. **c**, Diagram of the *Uph* transcripts expressed in the mouse heart, determined using 5' and



3' RACE using primers specific to exon 4 of *Uph*. AP1, marathon adaptor primer; 3'GSP, 3' *Uph*-specific primer from exon 4; 5'GSP, 5' *Uph*-specific primer from exon 4. **d**, Whole mount *in situ* hybridization of E10.5 mouse embryos. Expression was detected in heart, branchial arches (arrowhead), and limb bud. Scale bars, 1 mm. **e**, Northern blot analysis of total RNA from adult mouse tissues using a probe specific to the major *Uph* transcript. For gel source data, see Supplementary Fig. 1.

LETTER RESEARCH



Extended Data Figure 2 | *Uph* is a cytoplasmic lncRNA. a, Subcellular fractionation of 18S, *Uph* and *Malat1* lncRNA in mouse neonatal cardiomyocytes (n = 3 biological replicates from 1 of 5 independent experiments; mean \pm s.e.m.). b, *In vitro* transcription and translation of a plasmid encoding the major *Uph* RNA. A plasmid encoding the

myoregulin (MLN) micropeptide was used as a positive control, and myoregulin with a frameshift mutation (MLN RNA-FS) was used as a negative control. In contrast to myoregulin, *Uph* and the negative control (MLN RNA-FS) did not produce any detectable proteins, indicating that *Uph* is a bona fide lncRNA. For gel source data, see Supplementary Fig. 1.



Extended Data Figure 3 | Targeting strategy for insertion of transcriptional termination or heterologous sequence into exon 2 of Uph. a, Uph KO targeting strategy. Transcription activator-like effector nucleases (TALENs) were used to insert a triple polyadenylation (tpA) termination sequence into exon 2 (E2) of Uph. b, Using the same TALEN pair as in a, we introduced the coding sequence of tdTO, lacking a polyadenylation sequence, into exon 2 of the Uph locus. Exon 2, which includes the tdTO coding sequence, was spliced out of the mature Uph transcript, preventing expression of tdTO in these mice. c, Southern blot analysis of wild-type, heterozygous $Uph^{+/-}$ (Het) and Uph KO genomic DNA. BamHI-digested DNA hybridized with a 5'-specific probe and NdeI-digested DNA hybridized with a 3'-specific probe. For gel source data, see Supplementary Fig. 1. **d**, Southern blot analysis of wild-type, $Uph^{\rm dTO/+}$ heterozygous and $Uph^{\rm tdTO/tdTO}$ knock-in (KI) genomic DNA verified the correct targeting of the tdTO sequence into the Uph locus. DNA was digested with BamHI and hybridized with a 5'-specific probe or digested with NdeI and hybridized with a 3'-specific probe. For gel source data, see Supplementary Fig. 1. e, f, Expression of Uph (e) and Hand2 (f) in wild-type and $Uph^{tdTO/tdTO}$ homozygous mice at E10.0 was not changed by the insertion of the tdTO sequence into exon 2 of the *Uph* locus (n = 3, representative of 3 independent experiments; mean \pm s.e.m.). g, qPCR shows Uph transcripts decreased by \sim 97% in E10.5 hearts (n = 3 mice of each genotype from 1 of 3 independent experiments; mean \pm s.e.m.).

LETTER RESEARCH



Extended Data Figure 4 | **Aortic arch arteries are normal in** *Uph* **KO embryos. a**, qPCR quantification of gene expression at E10.0 showed robust downregulation of *Uph* and *Hand2* expression in *Uph* KO hearts, with normal expression of other cardiac transcription factors (n = 3 mice of each genotype from 1 of 3 independent experiments; mean \pm s.e.m.). **b**, India ink was injected into either the left ventricle of wild-type embryos or the single ventricle of *Uph* KO embryos at E10.5, to visualize the aortic arch arteries and circulation, which appeared normal in *Uph* KO embryos. aa, aortic arch arteries; as, aortic sac; da, dorsal aorta. Scale bars, 1 mm.





Extended Data Figure 5 | $Uph^{+/-}$ Hand2^{+/-} compound heterozygotes recapitulate Uph KO phenotype. a, $Uph^{+/-}$ Hand2^{+/-} double heterozygote embryos developed a single ventricle, pericardial effusion, and were severely growth restricted by E11.5. Scale bars, 1 mm. b, Uph expression is normal in double heterozygotes, whereas Hand2 was reduced by ~90%, relative to wild-type embryos (n = 5 mice for wild type, Hand2

het and *Uph* het, n = 3 for double het, n = 2 for *Hand2* KO, from 1 of 2 independent experiments; mean \pm s.e.m.). **c**, Immunoprecipitation of RNA using either IgG or WDR5 in HL-1 cells followed by qPCR for *Uph* revealed no binding to WDR5. The WDR5-interacting lncRNA HOTTIP was used as a positive control (n = 3 biological replicates from 1 of 3 independent experiments; mean \pm s.e.m.).



Extended Data Figure 6 | Knockdown of mature *Uph* transcripts in HL-1 or Neuro2a cells does not affect *Hand2* expression. a, Expression of *Uph* (red) and *Hand2* (blue) in various tissues and cell lines. *Uph* and *Hand2* are robustly expressed in the heart, and the HL-1 and Neuro2a cell lines. *Uph* and *Hand2* transcripts are not expressed in the liver, 10T1/2 fibroblasts or skeletal muscle C2C12 myotubes (n = 3 technical replicates; mean \pm s.e.m.). b, c, *Uph* transcripts were reduced by ~90% in HL-1 (b) and Neuro2a (c) cells when transfected with two independent GapmeR antisense oligonucleotide probes (ASO A and B) against *Uph*. Expression of *Hand2* was not changed (n = 2 for ASO A and B, n = 3 for control, from 1 of 2 independent experiments; mean \pm s.e.m.). d, *Uph* transcripts were similarly downregulated across each exon-exon junction, measured using

qPCR (n = 2 for ASO A and B, n = 3 for control, from 1 of 2 independent experiments; mean \pm s.e.m.). **e**, Fractionation of HL-1 cells transfected with control or *Uph*-specific ASOs. The nuclear fraction of antisenseoligonucleotide-treated HL-1 cells showed a similar downregulation of *Uph* transcripts (n = 3 biological replicates from 1 of 3 independent experiments; mean \pm s.e.m.). **f**, Overexpression of enhanced green fluorescent protein (eGFP; blue), the major *Uph* RNA (red) or *Hand2* RNA (green) in HL-1 cells revealed that the mature *Uph* transcript did not alter *Hand2* expression relative to wild type, and that *Hand2* does not influence the expression of *Uph* in these cells (n = 2 biological replicates from 1 of 2 independent experiments; mean \pm s.e.m.).

RESEARCH LETTER



Extended Data Figure 7 | ChIP and qPCR analyses of active cardiac enhancers regulating *Nkx2-5* expression. a, Diagram of the *Nkx2-5* locus with numbers (1 and 2) indicating the region analysed by qPCR following ChIP. Shown in red are the ENCODE/LICR H3K4me1 active enhancer marks in the heart at E14.5. See Methods for source data. b, ChIP coupled with qPCR analysis of H3K4me1 marks, normalized to total histone H3, showed that the H3K4me1 marks bound to the *Nkx2-5* promoter region are unchanged between wild-type, *Uph* KO and *Uph*^{tdTO/tdTO} homozygous hearts at E10.0. c, ChIP coupled with qPCR analysis of H3K27ac marks, normalized to total histone H3, showed that the H3K27ac marks bound to the *Nkx2-5* promoter region are unchanged between wild-type, *Uph* KO and *Uph*^{tdTO/tdTO} homozygous hearts at E10.0. d, GATA4 binding to a

GATA4 site in the *Nkx2*-5 promoter is unchanged between wild-type and *Uph* KO hearts at E10.0. e, Diagram of the *Uph–Hand2* locus, with black bars indicating the regions analysed by qPCR following ChIP. Shown in red is the ENCODE/LICR H3K27me3 track for mouse heart. See Methods for source data. The branchial arch enhancer (green) and cardiac enhancer (yellow) are shown. f, ChIP coupled with qPCR analysis of the polycomb repressive marker H3K27me3, normalized to total histone H3, showed no differences between genotypes at each locus (1–3) indicated in the diagram in e. g, qPCR revealed no difference in the levels of Ser2-phosphorylated RNAPII between genotypes at the *Nkx2*-5 gene body. Each point is one of 3 technical replicates of 5 pooled hearts for each genotype in each ChIP experiment, from 1 of 2 independent experiments; mean \pm s.e.m.



Extended Data Table 1 | Relevant sequences

SEQUENCE NAME

Full Length UPH sequence UPH qPCR primers UPH ISH probe Hand2 ISH probe UPH targeting 5'arm UPH targeting 3'arm UPH 5' KO genotyping UPH 3' WT genotyping UPH 3' WT genotyping tdT0 5' KO genotyping tdT0 5' KO genotyping tdT0 3' KO genotyping Southern BamHI probe Hand1 ISH probe Nkx2-5 ISH probe ASO UPH A LASO UPH LA LASO UPH LASO UPH LASO UPH LAS FORWARD CACTCATAACCATAAGATAATTAAAACGG CATTCTCGAGCAATTCGTCA GATGAGACCTTCAGTTTGTGCC ATGAGTCTGGTGGGGGGC TATCGGAGCTCGCACCTCGGAGCTGGGAA TATCGGCTAGCCATATGACCCTAACAGAGATTGCGAAGA CTCCTCTCCGGACAAGAATC CTCCTCTCCGGACAAGAATC AGAGAACGCGGATGAGACCT GACCTGCAGCCCAAGCTA CTCCTCTCCGGACAAGAATC CTCCTCTCCGGACAAGAATC AGAGAACGCGGATGAGACCT GACCTGCAGCCCAAGCTA TGGTTTTCTTGTCGTTGCTG TCCTGGGAAGGCACTATGTC CCATCATCACCACTCACACC TATGGCTACAACGCCTACCC AACACGTCTATACGC GCTAGTTAAGCAGGA ATTCAATTTAGGTCAT CCATCCTAATACGACTCACTATAGGGC TTTACCCACTGGTCCCCTCT CTGCAACTATCACCCGGAAT TCACCTCCCCATGTCTTTTC

ACCTCGGGCTTTCGATCTTA

GAAACTAGCCTTGCCCCTTC

ACTGTGAAGCCCAATTCCAG

REVERSE

TTTAAAAAATAATTTTTAATATACTATGTGCATGGTTGGATAGGT TGGTAGCCCATCTCCAACTC ATACTATGTGCATGGTTGGATAGGT TCACTGCTTGAGCTCCAGG GATACGCGGCCGCGGATCCAGTTGTCATCCTAACTTGGGTCA GATACATCGATCAGGGCAGTTAGGTCTCAGC TGCTGCAAATGAGTGTGG GGTTCCGGATCCACTAGTTCT CCCTTGCAAACAGAAGAAAGG CCCTTGCAAACAGAAGAAAGG TGCTGCAAATGAGTGTGG ATGACCTCCTCGCCCTTG CCCTTGCAAACAGAAGAAAGG CCCTTGCAAACAGAAGAAAGG CTGACTGGGTCCTTGAGCAT ACCTTCTCCTGCCCTTCATT GCGCCCTTTAATCCTCTTCT GTGTGGAATCCGTCGAAAGT

CAGCTGTATGGGCTCAGGTGACTGC TGGAGATGGGCTACCATTGGTGTTGA

TGGACAACATGGGACAGAAA AGAAACCCCCATCTGTTTCC GAGGAACCTGCATTGCTTTC GCTTGGGAAGGTAAGCCTTT GGGTGCCTAGGGAGAATAC AACCAGAAATTGGCAAGG