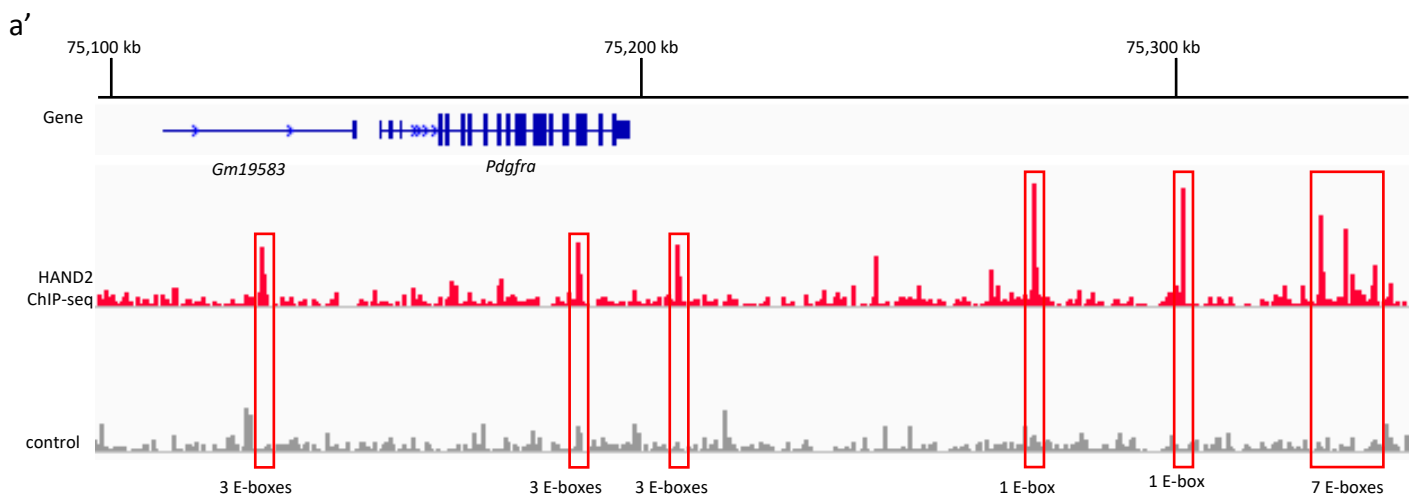
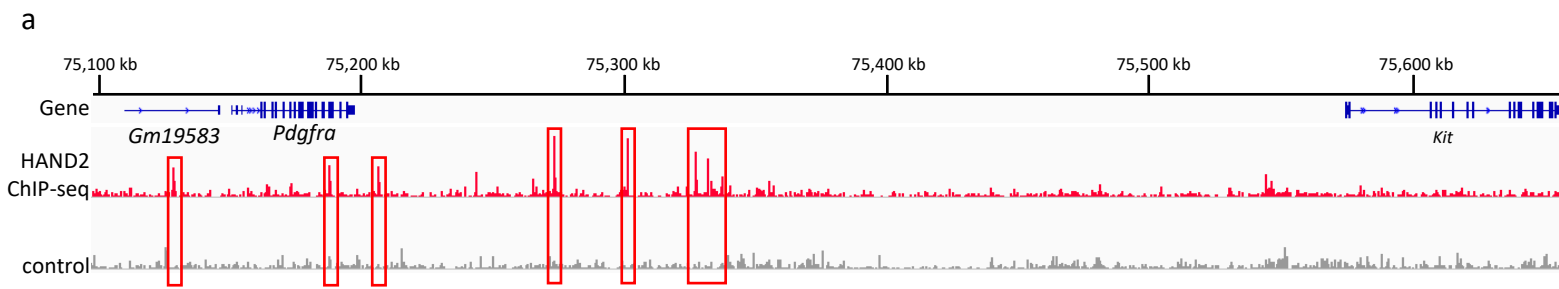


***PDGFRA* is a conserved HAND2 effector during early cardiac development**

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a

E12 HNNQDQMPAVSGKELSQLDFSNMFLPVANGKSPASLGGTQFAGSGLEDRPSSGSM 68
E47 HNNQDQMPAVSGKELSQLDFSNMFLPVANGKSPASLGGTQFAGSGLEDRPSSGSM 68

E12 GSSDQNSSFDPSTRTYSEGAHFSDDHSLPSTFLGAGLGGKSEBNAYATFGRTSVGT 126
E47 GSSDQNSSFDPSTRTYSEGAHFSDDHSLPSTFLGAGLGGKSEBNAYATFGRTSVGT 126

E12 LSGAGFLPGLLSLSPGFLSPSGIKSSQYYPSPFNPRRRAADGGLDTQKVKVYVPG 180
E47 LSGAGFLPGLLSLSPGFLSPSGIKSSQYYPSPFNPRRRAADGGLDTQKVKVYVPG 180

E12 LPSSVYPPSSGDSYSDAAAYPSAKTSSAYSPFYVAAGSLHPSAELKSTPSQVQFGP 248
E47 LPSSVYPPSSGDSYSDAAAYPSAKTSSAYSPFYVAAGSLHPSAELKSTPSQVQFGP 248

E12 LGDGSPLPLAPGSSSVSGSTFGGLQQQDRMYQLHSEVNGSLPAVSFSAPMTYST 368
E47 LGDGSPLPLAPGSSSVSGSTFGGLQQQDRMYQLHSEVNGSLPAVSFSAPMTYST 368

E12 LGHTTPPVSGAAEELLGTGRTTASSGGDALGKALASYSPHNSMFPSPSTPTVG 368
E47 LGHTTPPVSGAAEELLGTGRTTASSGGDALGKALASYSPHNSMFPSPSTPTVG 368

E12 LPSTGPNRAGAPASLSPNYDAGLHGLSKNDRLEADZHLRSHAVGTASDLHLHP 428
E47 LPSTGPNRAGAPASLSPNYDAGLHGLSKNDRLEADZHLRSHAVGTASDLHLHP 428

E12 ALTTSFTGPMGLGRHAGLVSQHPFEGLLTSGASLHMHASLPSPSSPLPOLSRPPSY 488
E47 ALTTSFTGPMGLGRHAGLVSQHPFEGLLTSGASLHMHASLPSPSSPLPOLSRPPSY 488

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E47 SGLGRAGTITAGASEIKREKEDEEIASVADAEEDKKDLKVPRTITSTDEVLSLEEK 537

E12 EREKERVVANNARERLRVRODNEAFKELGRCQLHLSEKPTKLLILHQAVVILLS 640
E47 EREKERVVANNARERLRVRODNEAFKELGRCQLHLSEKPTKLLILHQAVVILLS 597

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E47 EQVREIRNLNPKAACLKREEEKYSVGGQALSAANPGLGEANHPAGHL 648

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e

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Tcf3b HADPHILLNAVSTSSAGNSIPQAYSAGLPLSNMFMAGSDHQAACLPPSSTLQSA 441
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E12 HASLPSPSSPLPOLSRPPSYSGLAGRTITAGASEIKREKEDEEIASVADAEEDKKDL 518

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E12 KVPRTIRTS-----DDEDELLPPEKAEKEERIVANNARELRVR 568

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E12 EKVSYSVGGQALSAANPGLGEANHPAGHL 651

b

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Tcf3a -----PEHNNYMLEIDGKSERG---PVDTQLPGEINAA 33
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Tcf3a SIALSPFQNSQSYSPFI---RRPAE5SIDPLPK-KKQVHG 73
Tcf3b ADHAPKELSGKSSQYYPVYANPRRPSGGGQPGQKIKNPGPLSS-VYAATSGD 178

Tcf3a YNGDSAGYQKQNLVPGFVNGQELP---HN-----QSSQYYPVGNFNPQKQ 139
Tcf3b YNGDSAGYQKQNLVPGFVNGQELP---HN-----QSSQYYPVGNFNPQKQ 139

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Tcf3a QSKLDRLDEAZHLRSHAVG-----TMDQLLSAATSGHAGAAALGSLQGL 362
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Tcf3b HQAVVILLEQVREIRNLNPKAACLKREEEKYSVGGQALSAANPGLGEANHPAGHL 648

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Tcf3b VQHR 652

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c

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Tcf3a -----PEHNNYMLEIDGKSERG---PVDTQLPGEINAA 33
E12 GSSDQNSSFDPSTRTYSEGAHFSDDHSLPSTFLGAGLGGKSEBNAYATFGRTSVGT 126

Tcf3a YVVTQLPGEINAAADSLSPFQNSQSYSPFI---RRPAE5SIDPLPK-KKQVHG 73
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Tcf3a PNYGNFNPQKQPLPVIS-----PQNYPLHSEVNL-----PSGFHVS 163
E12 PMLGGG-----SPLAPGSSSVSGSTFGGLQQQDRMYQLHSEVNGSLPAVSFSAA 293

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E12 PHTYSGTSGRTTPVSGAAEELLGTGRTTASSGGDALGKALASYSPHNSMFPSTPT 353

Tcf3a PVGSPNVAASASQNPSSQSPAPSPFAGLA-QSKLDRLDEAZHLRSHAVG-TDM 278
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Tcf3a PPP-----SSGDTFTLSLARPSHASSSDKREKEDDDELSTADKSEKKDGKSRIT 392
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Tcf3a DQNM-----513
E12 LEANHPAGHL 651

g

d

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Tcf3a -----PEHNNYMLEIDGKSERG---PVDTQLPGEINAA 33
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E12 PMLGGG-----SPLAPGSSSVSGSTFGGLQQQDRMYQLHSEVNGSLPAVSFSAA 293

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Tcf3a DITKLLILHQAVVILLEQVREIRNLNPKAACLKREEEKYVTEAQML-----GLGG 587
E12 DITKLLILHQAVVILLEQVREIRNLNPKAACLKREEEKYSVGGQALSAANPGL 648

Tcf3a DQNM-----513
E12 LEANHPAGHL 648

Protein A	Protein B	Protein sequence similarity (%)
E12	E47	96.47
Tcf3b	Tcf3b	52.98
Tcf3a	E12	48.06
Tcf3a	E47	46.36
Tcf3b	E12	52.30
Tcf3b	E47	51.01

Supplementary Fig 1: Putative HAND2-binding sites in the mouse *Pdgfra* locus. a, a', Genome browser view showing HAND2 ChIP-seq peaks enriched in E10.25–10.5 mouse hearts at the *Pdgfra* locus (data analyzed from GSE55707¹). Red boxes: HAND2-binding sites.

Supplementary Fig 2: Amino acid sequence of zebrafish Tcf3a and Tcf3b. a, Amino acid sequence alignment of zebrafish Tcf3a and Tcf3b generated through Clustal Omega; the orange box encompasses the bHLH domain. b, Modeling of the structure of Tcf3a and Tcf3b by AlphaFold2.

- 1 Laurent, F. *et al.* HAND2 Target Gene Regulatory Networks Control Atrioventricular Canal and Cardiac Valve Development. *Cell Rep* **19**, 1602-1613, doi:10.1016/j.celrep.2017.05.004 (2017).