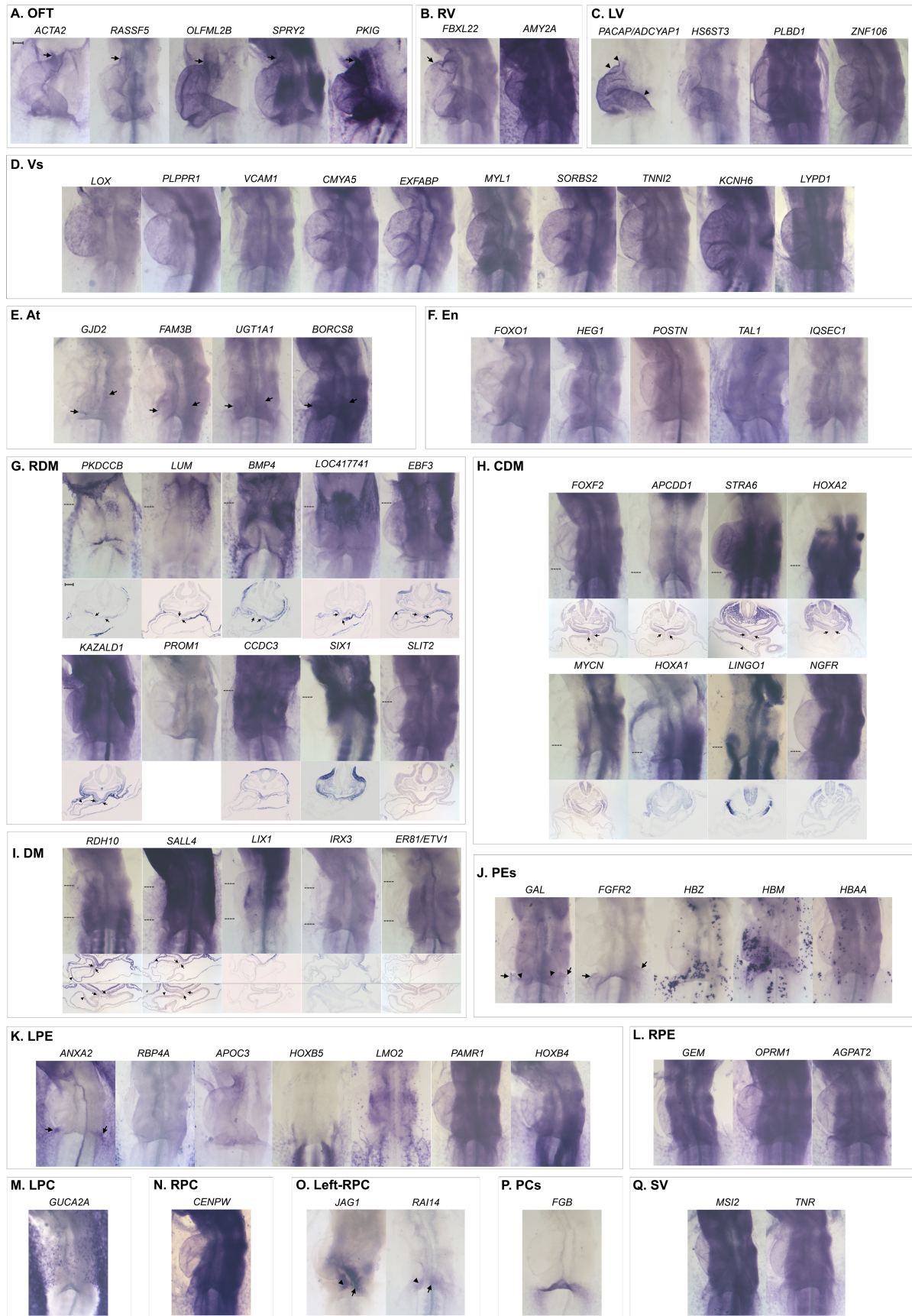


Supplementary figure 2. A further selection of regionalised heart markers. **A.** *FGF8*, *PRSS23*, *MSX2*, *GDF3/cVg1* and *HPGDS* are detected in the OFT (arrows). **B.** *BMP10* and *TGFB1* expression is restricted to the outer curvature of the prospective RV (arrows). *NPPA/NPPB* expression is observed in the RV (arrow), but also in the inner and outer curvatures of the prospective ventricle. **C.** *MB* is detected in the prospective LV. **D.** *LDB3*, *MYL3* and *SSPN* expression extends beyond the prospective ventricular region (arrows) into the atrial and OFT regions (arrowheads). **E.** *TUFT1*, *VIM* and *STBD1* localise to the atria (arrows). **F.** *RARRES1*, *EDN1*, *NFATC1*, *STAB1*, *TIE1* and *PLVAP* are detected in the endocardium (arrows) and endothelial cells (arrowheads); **G.** *TNC* expression is faint but detectable in the RDM (arrows). **H.** *PTCH2* is weakly detected in the CDM (arrows) and expression is continuous with the splanchnic mesoderm (arrowheads). **I.** *IRX1* in both the rostral and caudal DM (arrows) and the splanchnic mesoderm (arrowheads). **J.** *WT1* transcripts are detected in both the right and the left PE (arrows), as are *MAB21L1*

and *MSX1* (arrows) and also in ventro-medial cells (arrowheads). **K.** LPE marker *LHX2* is detected in both PE (arrows) and ventro-medial cells (arrowheads). **L.** RPE marker *LSP1* is detected in both PEs (arrows). **M.** *TTR*, a putative marker for the early LPC (arrows), is also expressed in the endoderm (arrowheads). At. Atria; En. Endocardium. Scale bars 100 μ m.



Supplementary figure 3. Markers with less regional specificity. **A.** *ACTA2*, *RASSF5*, *OLFML2B*, *SPRY2* and *PKIG* transcripts are enriched in the OFT (arrows), but their

expression is not restricted to this region. **B.** *FBXL22* transcripts are enriched (arrows) but not restricted to the prospective RV. *AMY2A* expression is strong throughout the heart. **C.** *PACAP/ADCYAP1* expression is not restricted to the prospective LV. *HS6ST3* expression is faint and *PLBD1* and *ZNF106* expression is strong throughout the heart. **D.** *LOX* expression is faint in both the prospective right and left ventricles. *PLPPR1* and *VCAM1* expression is faint, whereas, *CMYA5*, *EXFABP*, *MYL1*, *SORBS2*, *TNNI2*, *KCNH6* and *LYPD1* are strong in the heart and/or embryo. **E.** *GJD2*, *FAM3B* and *UGT1A1* expression is faint in the atrial region (arrows). *BORCS8* expression is strong within the embryo and atrial region (arrows). **F.** Endocardial markers *FOXO1*, *HEG1*, *POSTN*, *TAL1* and *IQSEC1* are not detected. **G.** *PKDCCB*, *LUM*, *BMP4*, *LOC417741*, *EBF3* and *KAZALD1* expression in the RDM (arrows) is continuous with the splanchnic mesoderm. *KAZALD1* and *EBF3* are also in the myocardium (arrowheads). *PROM1*, *CCDC3*, *SIX1* and *SLIT2* are faint or not detected in the RDM. **H.** Expression of CDM markers *FOXF2*, *APCDD1*, *STRA6* and *HOXA2* are faint (arrows). *STRA6* is also detected in the endocardium (arrowhead). *MYCN*, *HOXA1*, *LINGO1* and *NGFR* are not detected in the CDM. **I.** *RDH10* and *SALL4* expression in the DM (arrows) is continuous with the splanchnic mesoderm and the myocardium (arrowheads). *LIX1*, *IRX3*, and *ER81/ETV1* are not detected in the DM. **J.** Faint expression of *GAL* and *FGFR2* is detected in both right and left PE (arrows), and *GAL* is detected in ventro-medial cells (arrowheads). *HBZ*, *HBM* and *HBAA* are not restricted to the PE. **K.** LPE marker *ANXA2* is detected in both PE (arrows). *RBP4A*, *APOC3*, *HOXB5*, *LMO2*, *PAMR1* and *HOXB4* are either faint or not expressed. **L.** *GEM*, *OPRM1* and *AGPAT2* are strongly expressed throughout the heart and are not specific to the RPE. **M.** *GUCA2A* transcripts are not detected in the LPC region. **N.** *CENPW* expression is strong throughout the heart and not localised to the RPC region. **O.** *JAG1* and *RAI14* are detected on both the left (arrow) and right (arrowhead) and is not restricted to the Left-RPC. **P.** Putative PC marker *FGB* is expressed in the AIP endoderm and not detected in either the early LPC or RPC. **Q.** *MSI2* and *TNR* are not restricted to the SV region. Scale bars 100µm.