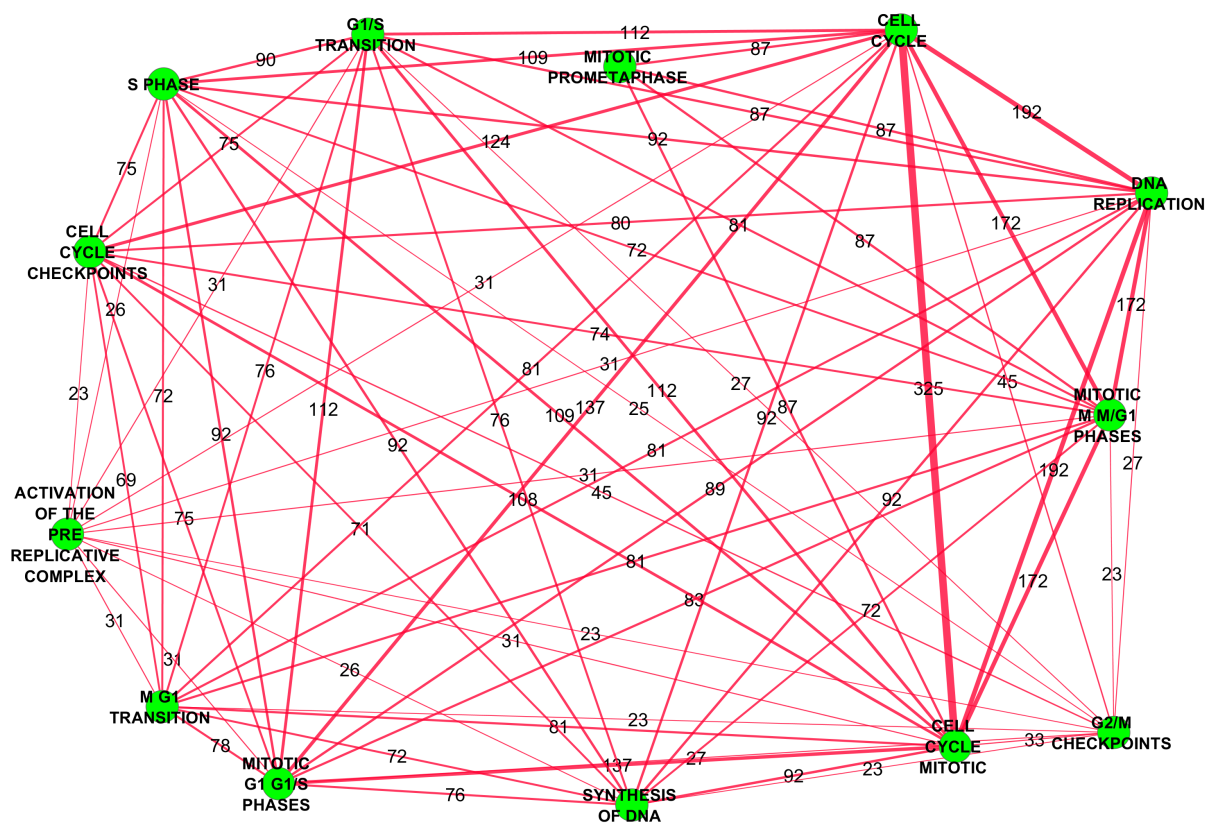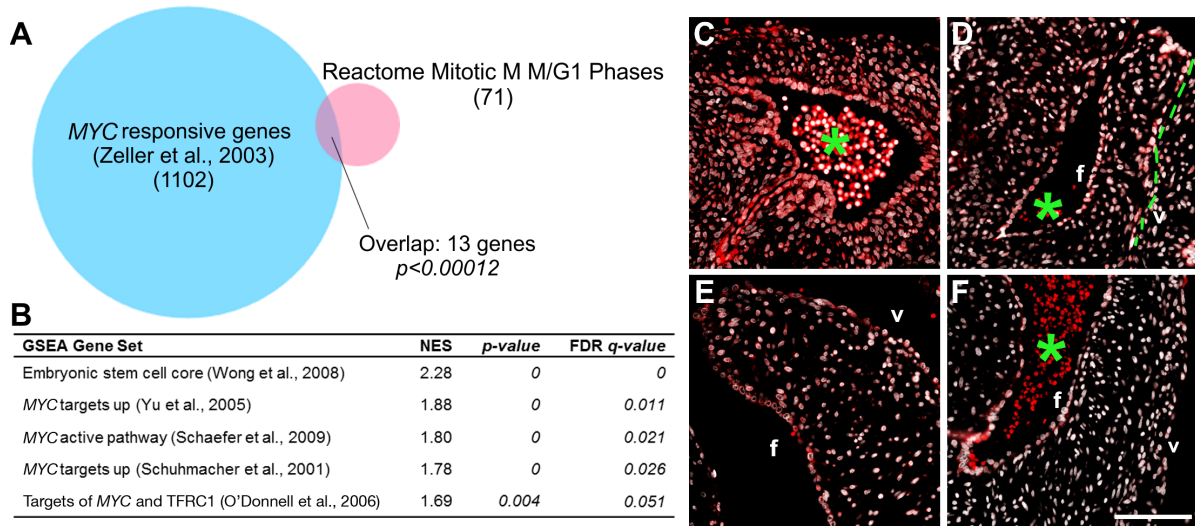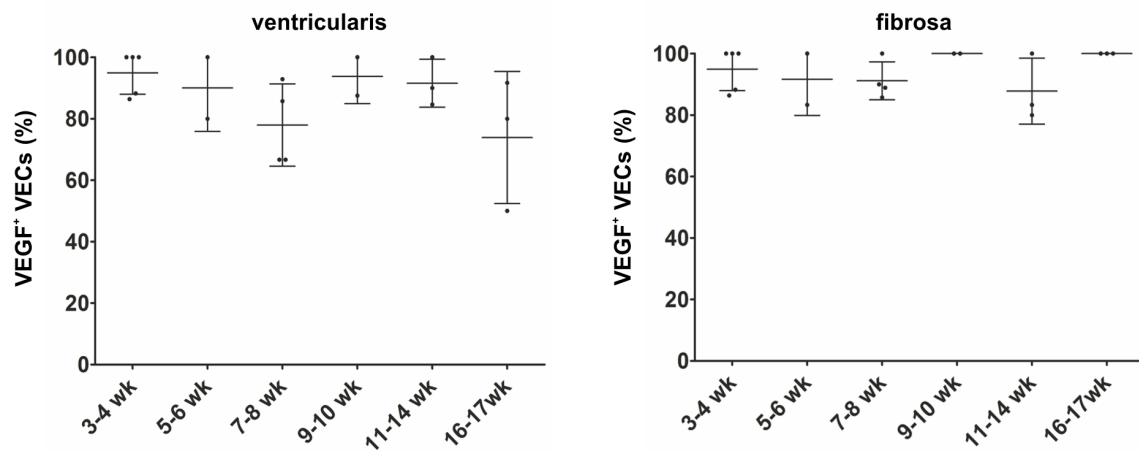## Supplemental Figures

Supplemental Figure 1: Network heat map demonstrating the enrichment of proliferation-associated genes and motifs in the first trimester human valve leaflets. Each node represents significantly enriched gene data sets and the lines indicate the relative number of genes that overlap between each gene data set. The software used to create this connectivity drawing is the open source platform Cytoscape (www.cytoscape.org).
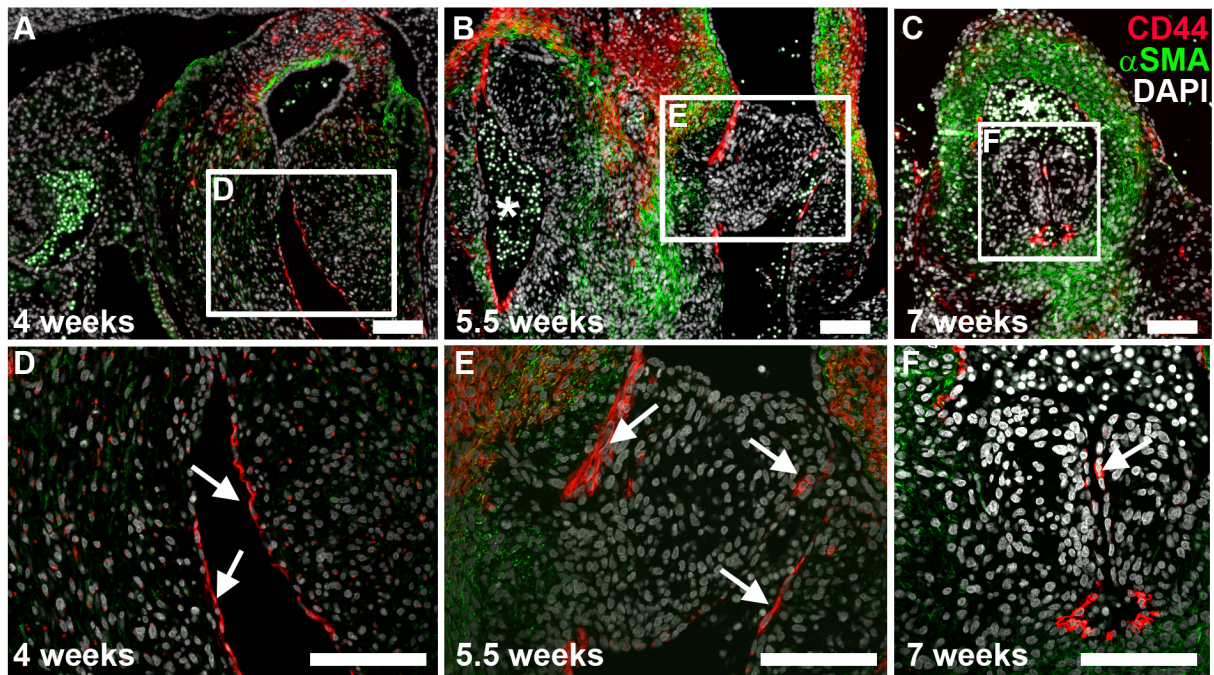
Supplemental Figure 2: Enrichment of GSEA categories that are driven by MYC expression. (**A**) Venn diagram indicating the overlap of the Reactome Mitotic M-G1/M Phases and a gene set that defined MYC targets using ChIP-Seq (Zeller et al. 2003). *P value* was determined by hypergeometric probability. (**B**) Table demonstrating GSEA categories driven by MYC expression that are enriched in first trimester leaflets. The false discovery rate (FDR) *q value* and normalized enrichment score (NES) are shown. MYC expression (red) in (**C**) 4-week valve cushions, as well as (**D**) 7, (**E**) 13 and (**F**) 15 week semilunar valve leaflets. DAPI is shown in white. Scale bars equal 100 μm. The green line highlights the leaflet edge. * = erythrocytes; f = fibrosa; v = ventricularis. MYC expression becomes noticeably reduced in second trimester leaflets (**E-F**) when compared to first trimester leaflets (**C-D**).

Supplemental Figure 3: Quantification of VECs expressing VEGF in the first and second trimester of the developing heart valve. No significant difference was detected between any of the time points investigated (one way ANOVA, $p<0.05$).

Supplemental Figure 4: CD44$^+$ cells (<u>red</u>) during early human semilunar valvulogenesis (4-7 weeks). CD44 expression is visible at some, but not all VECs of the cardiac cushions (<u>white arrows</u>). (**D-F**) represent high magnification images of **A**, **B** and **C**. DAPI is shown in <u>white;</u> αSMA in <u>green</u>. Scale bars equal 200 µm.

Supplemental Tables

Table S1: Gene ontology table showing FDR-q values from the GSEA analysis on pairwise expression data comparing first trimester isolated leaflets with second trimester isolated leaflets

GS Details	FDR *q-value*
M Phase	*0.005*
Regulation of Mitosis	*0.002*
Mitosis	*0.003*
M Phase of Mitotic Cell Cycle	*0.004*
Potassium Ion Transport	*0.005*
Chromosomepericentric Region	*0.004*
Condensed Chromosome	*0.004*
Voltage Gated Potassium Channel Complex	*0.005*
Microtubule Organizing Center	*0.006*
Chromosome Segregation	*0.006*
Spindle	*0.009*
Cell Cycle Process	*0.010*
Sister Chromatid Segregation	*0.009*
Mitotic Cell Cycle	*0.010*
Cell Cycle Phase	*0.011*
Mitotic Sister Chromatid Segregation	*0.012*
Kinetochore	*0.015*
Regulation of DNA Replication	*0.018*
Centrosome	*0.023*
Condensed Nuclear Chromosome	*0.028*
Voltage Gated Potassium Channel Activity	*0.041*
Transmembrane Receptor Protein Tyrosine Kinase Activity	*0.050*

Table S2: Computational sets enriched in the first trimester.

GS DETAILS	FDR *q-value*
RRM1	*0.000*
CCNA2	*0.000*
CDC2	*0.000*
MCM4	*0.000*
CENPF	*0.000*
CCNB2	*0.000*
PCNA	*0.000*
CDC20	*0.000*
HMMR	*0.000*
RFC4	*0.000*
CKS2	*0.000*
CKS1B	*0.000*
RRM2	*0.000*
BUB1B	*0.000*
RFC3	*0.000*
SMC4L1	*0.000*
ESPL1	*0.000*
CENPE	*0.000*
SMC2L1	*0.000*
TTK	*0.000*
MKI67	*0.000*
FEN1	*0.000*

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