ONLINE DATA SUPPLEMENTS

Wnt11 Regulates Cardiac Chamber Development and Disease during Perinatal Maturation

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Conflict of interest statement:

The authors have declared that no conflict of interest exists.

Supplemental Tables

Supplemental Table 1. Please refer to Related (EXCEL File A).
Top 50 stage specific 9 significantly differentially expressed mRNAs (RPKM≥3, V≥0.2, FDR P≤0.05 and fold change ≥2) 10 in neonatal heart during maturation. Pair wise differential expression analysis of mRNAs 11 expressed during neonatal heart maturation in left ventricle (LVP0 vs LVP3; LVP3 vs LVP7), and 12 right ventricle (RVP0 vs RVP3; RVP3 vs RVP7). 13
LVP0-rep1, LVP0-rep2, LVP0-rep3: Left Ventricle at postnatal day0-replicate1, replicate2, and 14 replicate 3, respectively. 15
LVP3-Rep1, LVP3-rep2, LVP3-rep3: Left Ventricle at postnatal day3-replicate1, replicate2, and 16 replicate3, respectively. 17
LVP7-rep1, LVP7-rep2, LVP7-rep3: Left Ventricle at postnatal day7-replicate1, replicate2, and 18 replicate3, respectively. 19
RVP0, RVP3, RVP7: Right Ventricle at postnatal day 0, 3, and 7, respectively.

Supplemental Table 2. Significant stage specific modules in neonatal left ventricle (LV) revealed by weighted gene co-expression network analysis (WGCNA).

Left Ventricle (LV) Modules Revealed by Weighted Gene Co-expression Network Analysis					
Module Color	**Module Size**	**p.value.Time**	**p.value.LV_P0**	**p.value.LV_P3**	**p.value.LV_P7**
brown	219	3.33E-05	0.021888924	0.67425277	0.000758989
cyan	1634	0.994963038	0.173511544	0.002543681	0.328500492
pink	1045	0.019853373	7.72E-05	0.147050452	0.251451916
purple	92	0.286730252	0.004882449	0.0031329	0.958761725
grey60	822	0.756091844	0.55695259	0.1004625	0.349144182
midnightblue	67	0.512539829	0.49305988	0.004132801	0.100603739
lightcyan	75	0.800585542	0.746169516	0.325570997	0.525592981
lightgreen	60	0.318577882	0.55014189	0.665939343	0.28781664
salmon	174	0.753176768	0.525053659	0.529883208	0.994371702
black	89	0.064224191	0.402092304	0.270636549	0.025149758
green	217	0.01326623	0.107317322	0.635993579	0.018412887
magenta	131	0.175741321	0.968569962	0.013289477	0.016564021
red	258	0.012136265	0.341377604	0.11335335	0.000363406
tan	109	0.102369278	0.004635348	0.09524439	0.515245494
yellow	110	0.021240161	0.013158504	0.548068665	0.126626096
blue	2339	0.00037393	5.69E-05	0.485590175	0.040569686
turquoise	722	3.38E-05	0.013924717	0.788688235	0.001708139

Supplemental Table 3. Significant stage specific modules in neonatal right ventricle (RV) revealed by weighted gene co-expression network analysis (WGCNA).

Right Ventricle (RV) Modules Revealed by Weighted Gene Co-expression Network Analysis					
Module Color	**Module Size**	**p.value.Time**	**p.value.RV_P0**	**p.value.RV_P3**	**p.value.RV_P7**
black	368	0.166308201	0.010568318	0.054810654	0.722584196
blue	862	0.681954921	0.541986642	0.058804766	0.223203098
brown	541	0.009092214	0.021215545	0.64167879	0.076337415
cyan	157	0.001302704	0.000439121	0.374275983	0.083119351
darkgreen	113	5.67E-06	0.040231158	0.801175963	0.000671459
darkgrey	103	0.075408331	9.40E-05	0.026212228	0.59685051
darkmagenta	57	0.534355808	0.033776596	0.000502747	0.603773269
darkolivegreen	62	0.00794506	0.002123803	0.334893289	0.140502601
darkorange	95	0.171628523	0.022236465	0.103688768	0.663200911
darkred	115	0.033006603	0.008117146	0.283619891	0.249776119
darkturquoise	105	0.32088667	0.177365054	0.380663931	0.654056941
green	400	0.875072308	0.454762447	0.089857881	0.374411173
greenyellow	237	0.573016334	0.869989382	0.259810902	0.289413135
grey60	143	0.436428243	0.643795156	0.794793956	0.408153864
lightcyan	148	0.638291587	0.666040176	0.92273389	0.709595298
lightgreen	137	0.849764846	0.343099214	0.019502812	0.262235497
lightyellow	117	0.333852907	0.65878714	0.004116759	0.024305532
magenta	281	0.980778671	0.17666186	0.001533364	0.289663715
midnightblue	151	0.648852332	0.674557536	0.132545629	0.26412876
orange	99	0.350687998	0.838571024	0.060186661	0.068786358
paleturquoise	76	0.88744182	0.19979018	0.016441711	0.423479254

Supplemental Figure Legends

Supplemental Figure 1. Preserved Stage Specific Modules in Left and Right Ventricles Suggest Coordinated Metabolic Switch from Glucose Metabolism at P0 to Fatty Acid Metabolism at P3 in Both Ventricles.

Quantitative RT-PCR validation of genes involved in fatty acid metabolism and glucose metabolism reveals synchronized downregulation of glucose metabolism and induction of fatty acid metabolism genes at P3 in both ventricles. For each gene, the mean expression ratio (P3/P0 ratio) is presented in Y Axis. RV data are presented in blue; LV data are presented in pink. N =3 per group per time point.

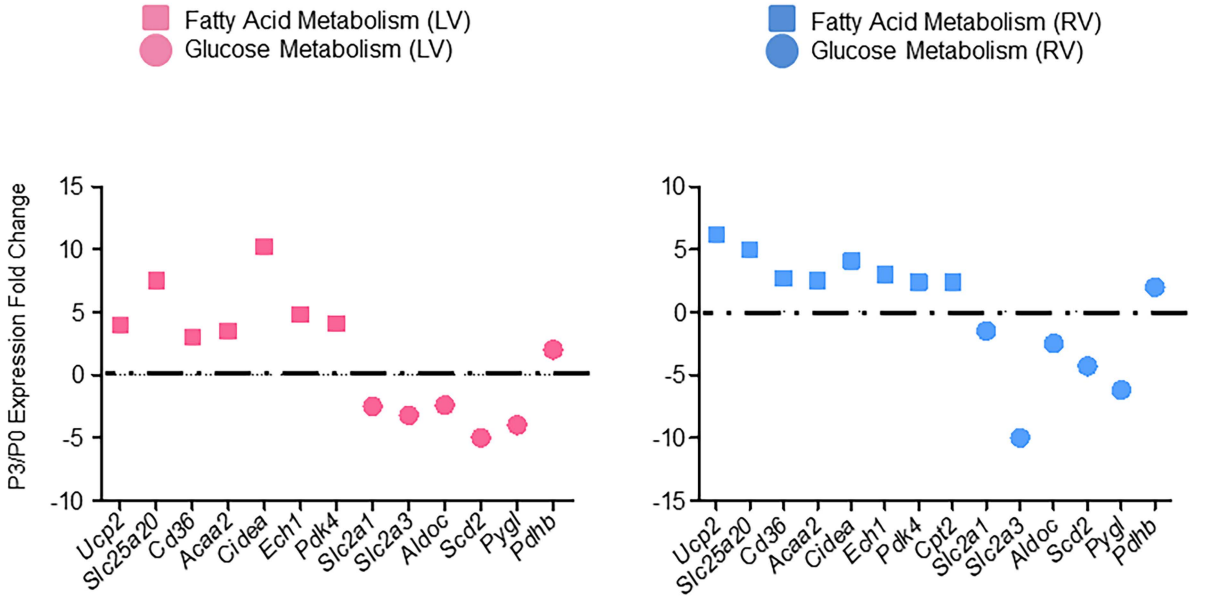
Supplemental Figure 2. Expression Patterns of Representative Proliferation Genes in Neonatal Heart Left and Right Ventricles (RNA-seq data). RPKM measures of RV data are presented in blue, and of LV data are presented in pink.

Supplemental Figure 3. Chamber specific Regulation of Wnt11. A. Dosimetry quantitative analysis of Wnt11 protein expression in LV and RV of hypoxia exposed wild type P3-neonatal mouse compared to baseline normoxia control. **B.** Dosimetry quantitative analysis of Wnt11 protein expression in LV and RV of wild type P3-neonatal mouse at P0 and P7. Error bars represent standard error of means (SEM). *P ≤0.05, **P≤0.01, ***P≤0.005. Statistics (A,B): Tow tailed Student's *t* test. This figure is related to Figure 3. G, and Figure 5. C.

Supplemental Figure 4. Wnt11 Knockdown by Vivo-Morpholino did not Impact Cardiac Function.

Representative M-mode echocardiography images of Mor-Wnt11 treated or scramble treated neonatal mouse hearts are displayed. Bar graphs (right): Quantitative analysis of left ventricle

(LV) functional indices. N=7 per group. EF: Ejection fraction, FS: Fractional shortening. Error bars represent standard error of means (SEM). * P ≤0.05 (unpaired, two tailed Student's *t* test).


Fatty Acid Metabolism (LV)
Glucose Metabolism (LV)
P3/P0 Expression Fold Change
15
10
5
0
-5
-10
Ucp2
Slc25a20
Cd36
Acaa2
Cidea
Ech1
Pdk4
Slc2a1
Slc2a3
Aldoc
Scd2
Pygl
Pdhb
Fatty Acid Metabolism (RV)
Glucose Metabolism (RV)
10
5
0
-5
-10
-15
Ucp2
Slc25a20
Cd36
Acaa2
Cidea
Ech1
Pdk4
Cpt2
Slc2a1
Slc2a3
Aldoc
Scd2
Pygl
Pdhb

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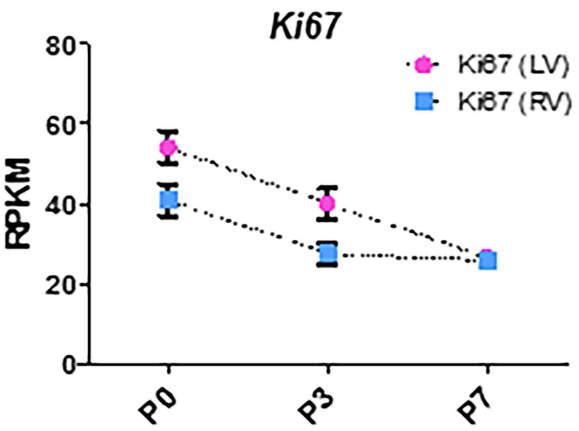
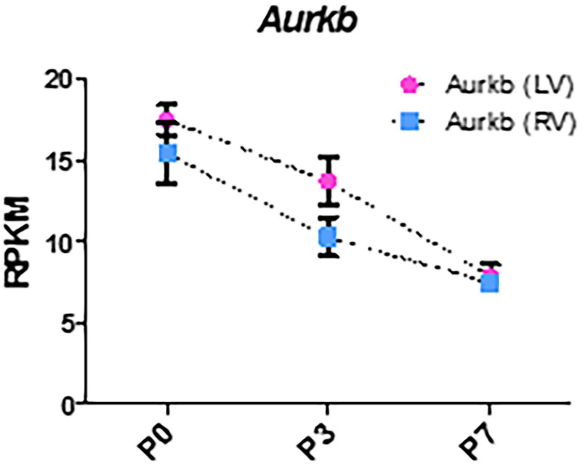
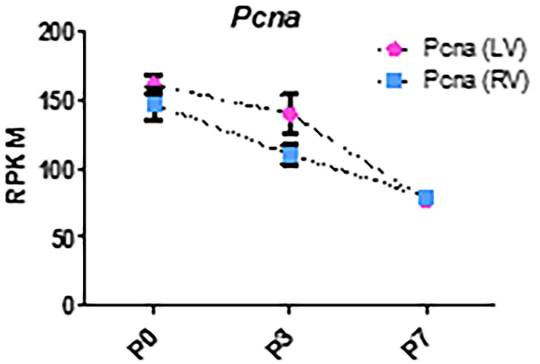
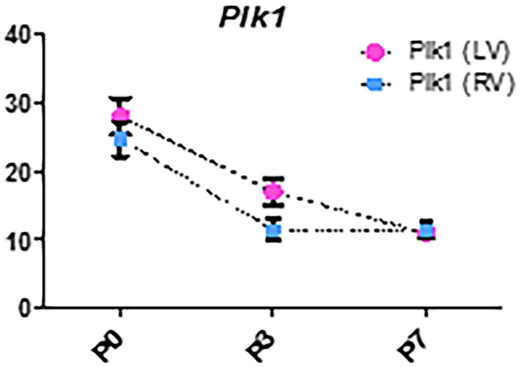
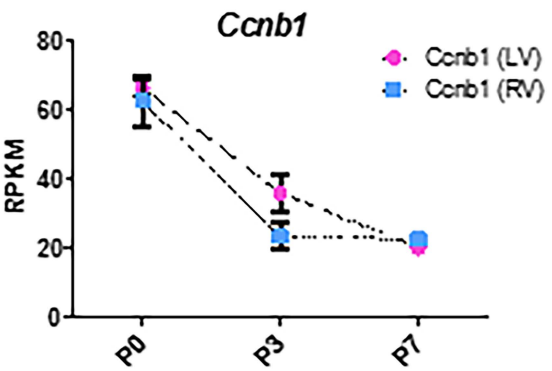
RNA-Seq expression data

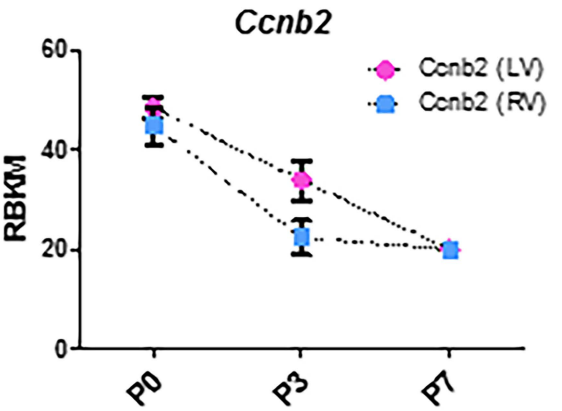


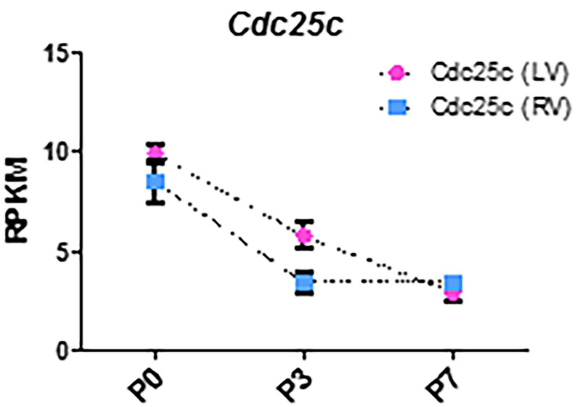


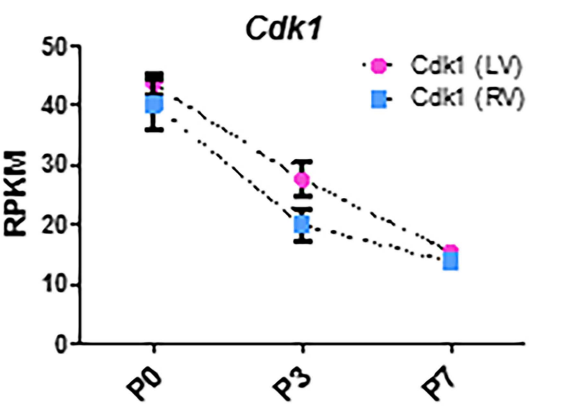












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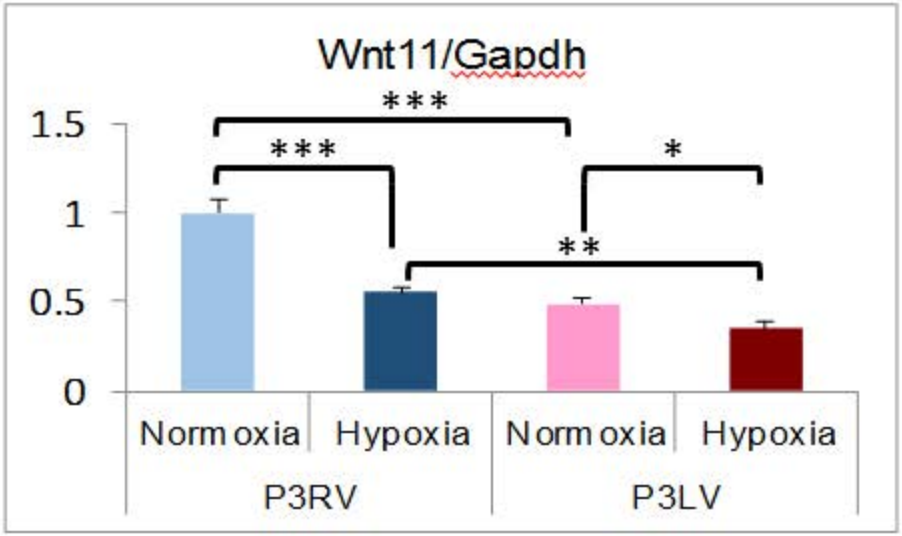
A



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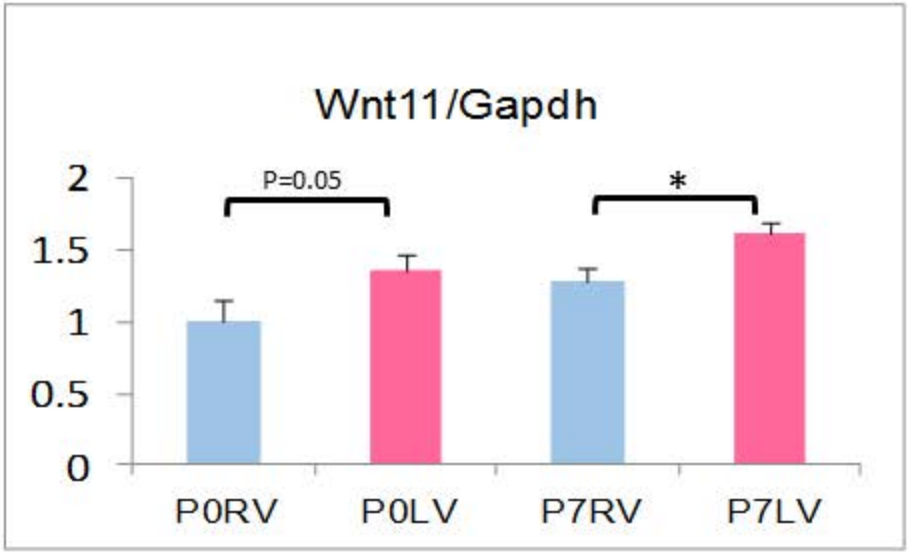

Touma et al. Supplemental Figure 3

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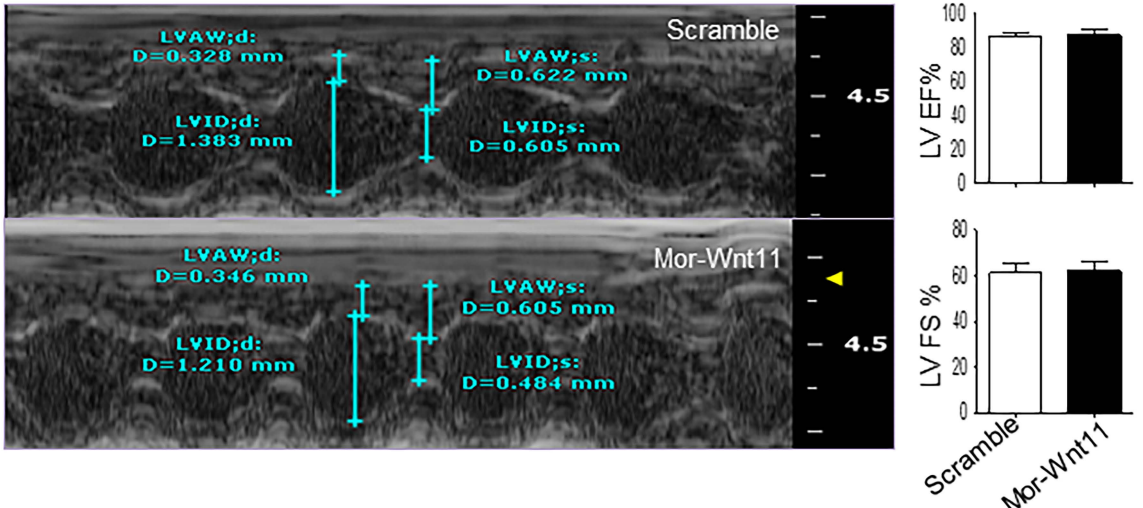
Scramble
LVAW;d:
D=0.328 mm
LVID;d:
D=1.383 mm
LVAW;s:
D=0.622 mm
LVID;s:
D=0.605 mm
4.5
Mor-Wnt11
LVAW;d:
D=0.346 mm
LVID;d:
D=1.210 mm
LVAW;s:
D=0.605 mm
LVID;s:
D=0.484 mm
4.5
LV EF%
100
80
60
40
20
0
LV FS %
80
60
40
20
0
Scramble
Mor-Wnt11

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