

# **A time frame permissive for Protein Kinase D2 activity to direct angiogenesis in mouse embryonic stem cells**

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## Supplemental Figure Legends

**Supplemental Figure 1: Effects of early overexpression in a second independently targeted iPKD2 ESC line.** The generation of this second iPKD2 cell line was performed according to **Figure 2A**.

Cultures were stimulated as illustrated in **Figure 3A**. Expression levels of **(A) FoxA2**, **(B) Pax6**, **(C) Flk1**, **(D) cKit**, **(E) Isl1** and **(F) Tbx5** on day 4 of embryoid body differentiation are shown. Supplemental Experiments were performed n=2.

**Supplemental Figure 2: Differentiation of PKD2-kinase dead EBs derived from a second iPSC clone.**

These data were generated from two clones (WT and KD) further expanded after picking from reprogramming MEF cultures from the same genotype. Regulation of **(A) FoxA2**, **(B) Pax6**, **(C) Pdgfr**, **(D) cKit**, **(E) Isl1** and **(F) Tbx5** on day 4 of differentiation is shown. EB differentiation was performed according to **Figure 6A**. KD: kinase dead, WT: wild type. Supplemental Experiments were performed n=2.

## Supplemental Table 1

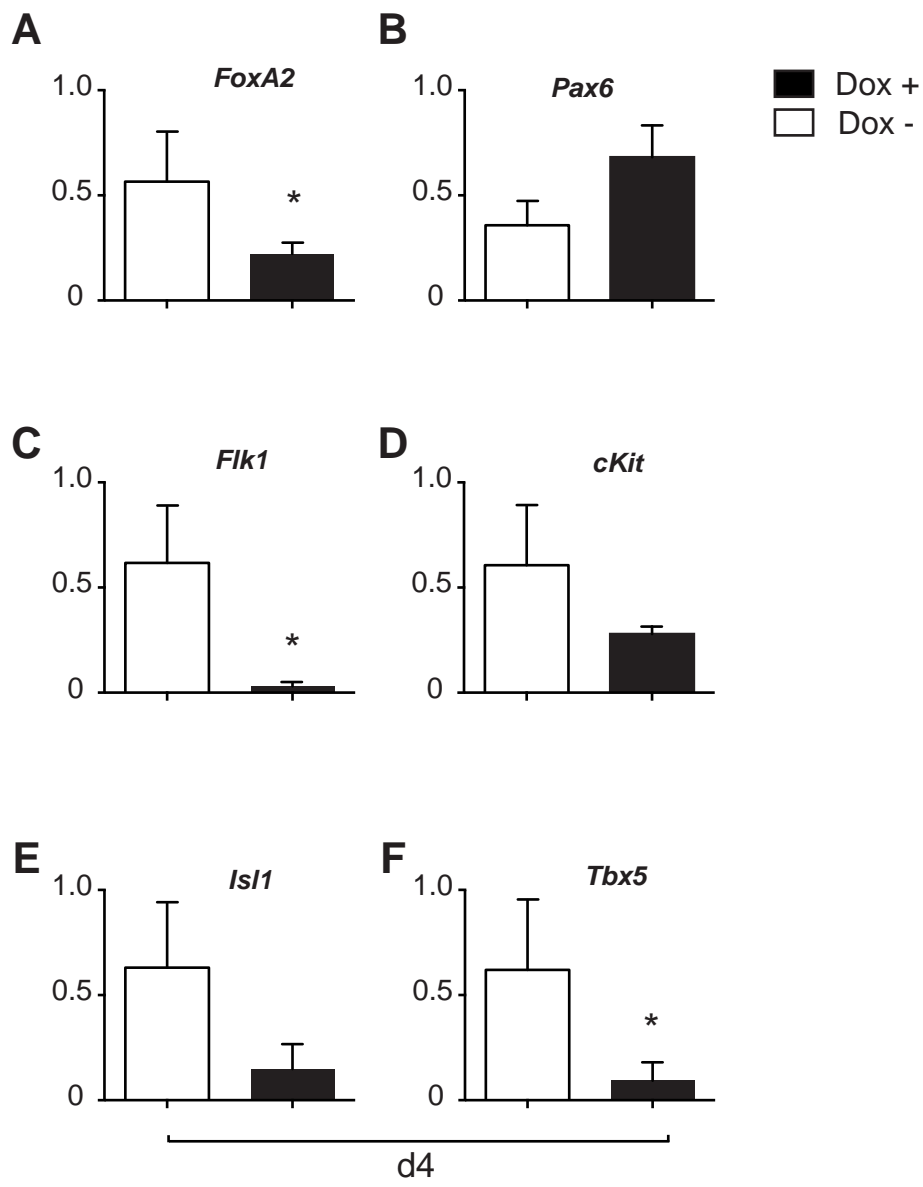
List of adjusted p-values for Figure 3

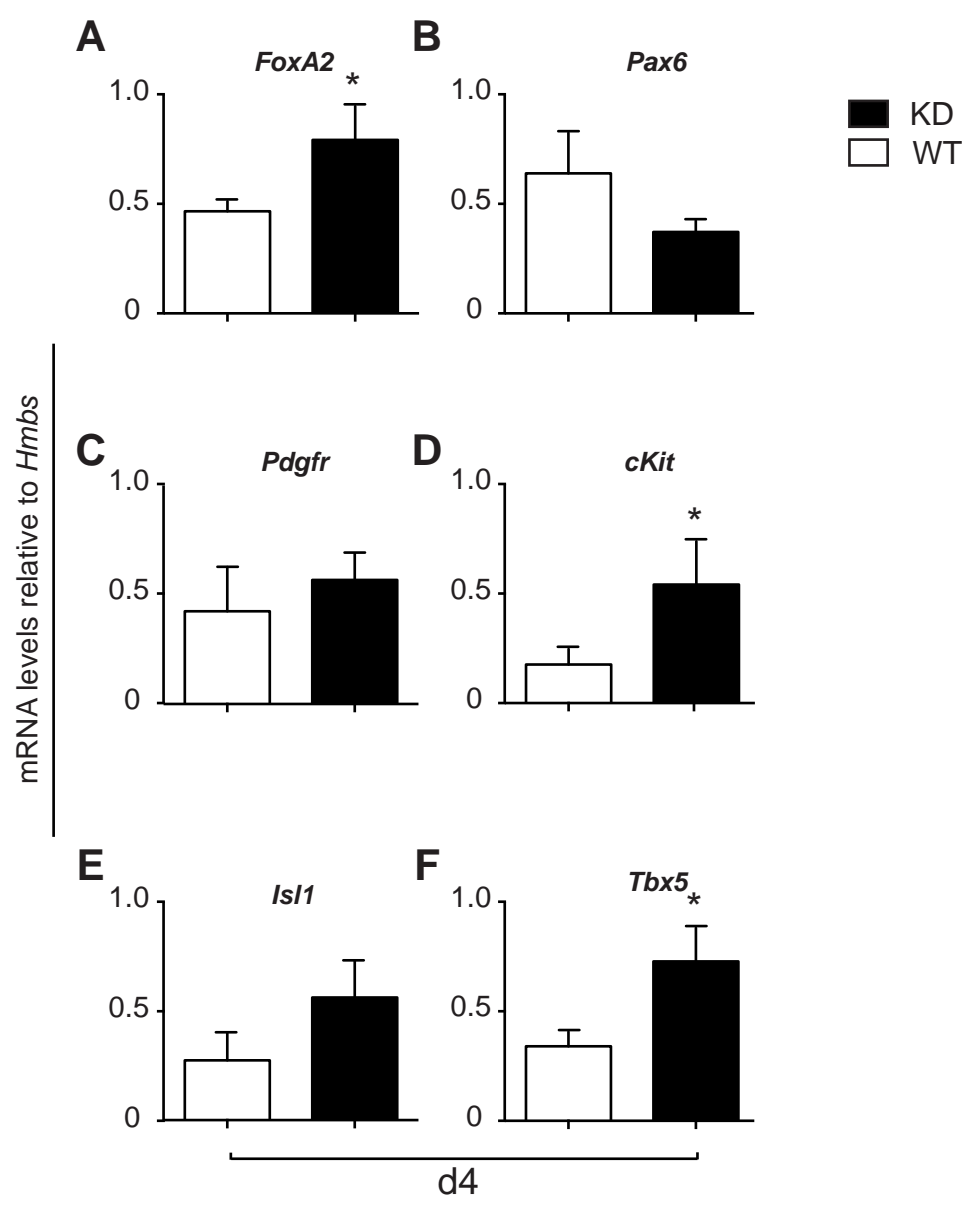
## Supplemental Table 2

List of adjusted p-values for Figure 4

## Supplemental Table 3

List of adjusted p-values for Figure 6





Time point	Gene	p-value (two-side t-test)	adjusted p-value (Bonferroni multiple correction)	Figure
D3	Bry	0,059481475	0,083221214	3B
D4	Bry	<b>7,70395E-05</b>	<b>0,000693356</b>	3B
D3	FoxA2	<b>0,00020887</b>	<b>0,001009031</b>	3C
D4	FoxA2	<b>0,023789172</b>	0,051177564	3C
D3	Pax6	0,801834803	0,849001556	3D
D4	Pax6	<b>0,027426034</b>	0,051177564	3D
D3	Flk1	<b>6,97731E-06</b>	<b>0,000125592</b>	3E
D4	Flk1	<b>0,002290388</b>	<b>0,005889569</b>	3E
D3	cKit	0,587340367	0,660757913	3F
D4	cKit	<b>0,032948463</b>	0,053915667	3F
D3	PDGFR	0,06010421	0,083221214	3G
D4	PDGFR	<b>0,000425008</b>	<b>0,001381395</b>	3G
D3	Tbx5	0,896096854	0,896096854	3H
D4	Tbx5	<b>0,000460465</b>	<b>0,001381395</b>	3H
D3	Isl1	0,193981782	0,249405148	3I
D4	Isl1	<b>0,02843198</b>	0,051177564	3I

Time point	Gene	p-value (two-side t-test)	adjusted p-value (Bonferroni multiple correction)	Figure
D6	Tubb3	<b>0,02071165</b>	<b>0,02867767</b>	3J
D9	Tubb3	<b>0,000976387</b>	<b>0,00210614</b>	3J
D14	Tubb3	0,147065741	0,16544900	3J
D6	Myl2a	<b>0,000437502</b>	<b>0,00157501</b>	3K
D9	Myl2a	<b>4,01256E-06</b>	<b>0,00005283</b>	3K
D14	Myl2a	<b>2,53933E-05</b>	<b>0,00015236</b>	3K
D6	Myh6	<b>0,00072598</b>	<b>0,00210614</b>	3L
D9	Myh6	<b>0,018740047</b>	<b>0,02811007</b>	3L
D14	Myh6	<b>8,08518E-05</b>	<b>0,00036383</b>	3L
D6	CD34	<b>5,86989E-06</b>	<b>0,00005283</b>	3N
D9	CD34	0,112694398	0,14489280	3N
D14	CD34	<b>0,011219655</b>	<b>0,01835944</b>	3N
D6	CD31	<b>0,001644655</b>	<b>0,00296038</b>	3O
D9	CD31	0,258172333	0,27335890	3O
D14	CD31	<b>0,001053071</b>	<b>0,00210614</b>	3O

Time point	Compared Pair	Gene	p-value (Anova PostHoc)	adjusted p-value (Bonferroni multiple correction)	Figure
D6	Control vs. Dox 0-14	Myh6	0,1322842	0,210098435	4B
D6	Control vs. Dox 4-14	Myh6	0,5332273	0,625962483	4B
D6	Dox 4-14 vs. Dox 0-14	Myh6	<b>0,0170378</b>	<b>0,040001791</b>	4B
D9	Control vs. Dox 0-14	Myh6	<b>0,000227</b>	<b>0,0030645</b>	4B
D9	Control vs. Dox 4-14	Myh6	<b>0,0212022</b>	<b>0,04770495</b>	4B
D9	Dox 4-14 vs. Dox 0-14	Myh6	<b>0,0000014</b>	<b>0,0000756</b>	4B
D14	Control vs. Dox 0-14	Myh6	<b>0,000299</b>	<b>0,0032292</b>	4B
D14	Control vs. Dox 4-14	Myh6	<b>0,00765</b>	<b>0,018777273</b>	4B
D14	Dox 4-14 vs. Dox 0-14	Myh6	0,2574168	0,356423262	4B
D6	Control vs. Dox 0-14	Myl2a	0,0517894	0,096435434	4C
D6	Control vs. Dox 4-14	Myl2a	0,5085178	0,61933524	4C
D6	Dox 4-14 vs. Dox 0-14	Myl2a	<b>0,0055507</b>	<b>0,015775674</b>	4C
D9	Control vs. Dox 0-14	Myl2a	<b>0,0246724</b>	0,053292384	4C
D9	Control vs. Dox 4-14	Myl2a	<b>0,0351163</b>	0,067724293	4C
D9	Dox 4-14 vs. Dox 0-14	Myl2a	<b>0,0001081</b>	<b>0,0025758</b>	4C
D14	Control vs. Dox 0-14	Myl2a	<b>0,0001431</b>	<b>0,0025758</b>	4C
D14	Control vs. Dox 4-14	Myl2a	<b>0,00132</b>	<b>0,00594</b>	4C
D14	Dox 4-14 vs. Dox 0-14	Myl2a	0,4939166	0,61933524	4C
D6	Control vs. Dox 0-14	CD31	0,5161127	0,61933524	4E
D6	Control vs. Dox 4-14	CD31	<b>0,0299535</b>	0,0621442	4E
D6	Dox 4-14 vs. Dox 0-14	CD31	<b>0,0032149</b>	<b>0,010212035</b>	4E
D9	Control vs. Dox 0-14	CD31	0,9861775	0,9996584	4E
D9	Control vs. Dox 4-14	CD31	<b>0,0004214</b>	<b>0,003519257</b>	4E
D9	Dox 4-14 vs. Dox 0-14	CD31	<b>0,0005724</b>	<b>0,0038637</b>	4E
D14	Control vs. Dox 0-14	CD31	0,2320964	0,3298212	4E
D14	Control vs. Dox 4-14	CD31	0,1699757	0,262248223	4E
D14	Dox 4-14 vs. Dox 0-14	CD31	<b>0,006621</b>	<b>0,017336829</b>	4E
D6	Control vs. Dox 0-14	CD34	0,9691476	0,9996584	4F
D6	Control vs. Dox 4-14	CD34	<b>0,0015762</b>	<b>0,006547292</b>	4F
D6	Dox 4-14 vs. Dox 0-14	CD34	<b>0,0009848</b>	<b>0,004853127</b>	4F
D9	Control vs. Dox 0-14	CD34	0,9996584	0,9996584	4F
D9	Control vs. Dox 4-14	CD34	<b>0,0009416</b>	<b>0,004853127</b>	4F
D9	Dox 4-14 vs. Dox 0-14	CD34	<b>0,0009886</b>	<b>0,004853127</b>	4F
D14	Control vs. Dox 0-14	CD34	0,6065573	0,696895621	4F
D14	Control vs. Dox 4-14	CD34	<b>0,0030665</b>	<b>0,010212035</b>	4F
D14	Dox 4-14 vs. Dox 0-14	CD34	<b>0,0004562</b>	<b>0,003519257</b>	4F
D6	Control vs. Dox 0-14	vWF	0,6416134	0,721815075	4G
D6	Control vs. Dox 4-14	vWF	0,3461266	0,46727091	4G
D6	Dox 4-14 vs. Dox 0-14	vWF	0,8582981	0,926961948	4G
D9	Control vs. Dox 0-14	vWF	0,2003191	0,297414924	4G
D9	Control vs. Dox 4-14	vWF	<b>0,0037149</b>	<b>0,0111447</b>	4G
D9	Dox 4-14 vs. Dox 0-14	vWF	0,122413	0,200312182	4G
D14	Control vs. Dox 0-14	vWF	0,0806137	0,140423865	4G
D14	Control vs. Dox 4-14	vWF	0,9757157	0,9996584	4G
D14	Dox 4-14 vs. Dox 0-14	vWF	0,1174439	0,198186581	4G

Time point	Gene	p-value (two-side t-test)	adjusted p-value (Bonferroni multiple correction)	Figure
D2	Bry	0,926812518	0,926812518	6B
D4	Bry	0,926812518	0,926812518	6B
D2	FoxA2	0,083579763	0,148586245	6C
D4	FoxA2	<b>0,001387437</b>	<b>0,008184587</b>	6C
D2	Pax6	0,801834803	0,916382632	6D
D4	Pax6	<b>0,027426034</b>	0,087763309	6D
D2	Tubb3	0,060893021	0,121786042	6E
D4	Tubb3	0,318056623	0,427560057	6E
D2	cKit	<b>0,048032543</b>	0,10978867	6F
D4	cKit	<b>0,004197417</b>	<b>0,016789668</b>	6F
D2	PDGFR	<b>0,042149463</b>	0,10978867	6G
D4	PDGFR	0,335996041	0,427560057	6G
D2	Tbx5	0,347392546	0,427560057	6H
D4	Tbx5	<b>0,00153461</b>	<b>0,008184587</b>	6H
D2	Isl1	<b>0,001330794</b>	<b>0,008184587</b>	6I
D4	Isl1	0,118332618	0,189332189	6I

Time point	Gene	p-value (two-side t-test)	adjusted p-value (Bonferroni multiple correction)	Figure
D9	CD34	0,382383352	0,38238335	6J
D14	CD34	<b>0,009067221</b>	<b>0,02731819</b>	6J
D9	CD31	0,056244612	0,07030576	6K
D14	CD31	<b>0,012394669</b>	<b>0,02731819</b>	6K
D9	vWF	<b>0,001142494</b>	<b>0,01142494</b>	6L
D14	vWF	0,055140116	0,07030576	6L
D9	MyI2A	<b>0,005073762</b>	<b>0,02536881</b>	6M
D14	MyI2A	0,065125459	0,07236162	6M
D9	MyH6	<b>0,01632989</b>	<b>0,02731819</b>	6N
D14	MyH6	<b>0,016390913</b>	<b>0,02731819</b>	6N