Stem Cell Reports, Volume *10*

Supplemental Information

FZD4 Marks Lateral Plate Mesoderm and Signals with NORRIN to Increase Cardiomyocyte Induction from Pluripotent Stem Cell-Derived Cardiac Progenitors

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Supplemental Figures and Tables

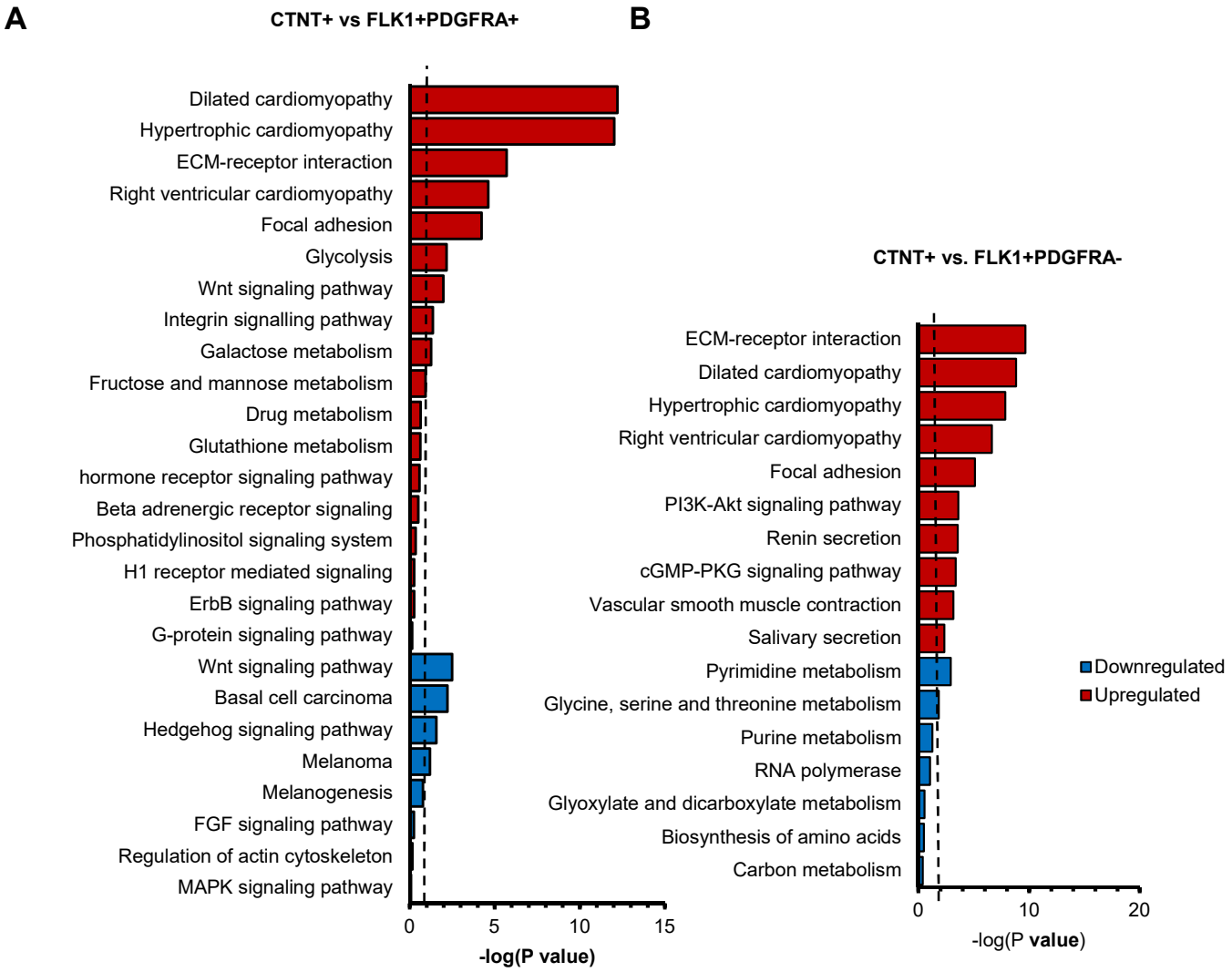

Figure S1: GO analysis of CPC sub-populations relative to CM, related to Figure 2. A) CM compared to FLK1+PDGFRA+ sub-population. B) CM compared to FLK1+PDGFRA- sub-population.

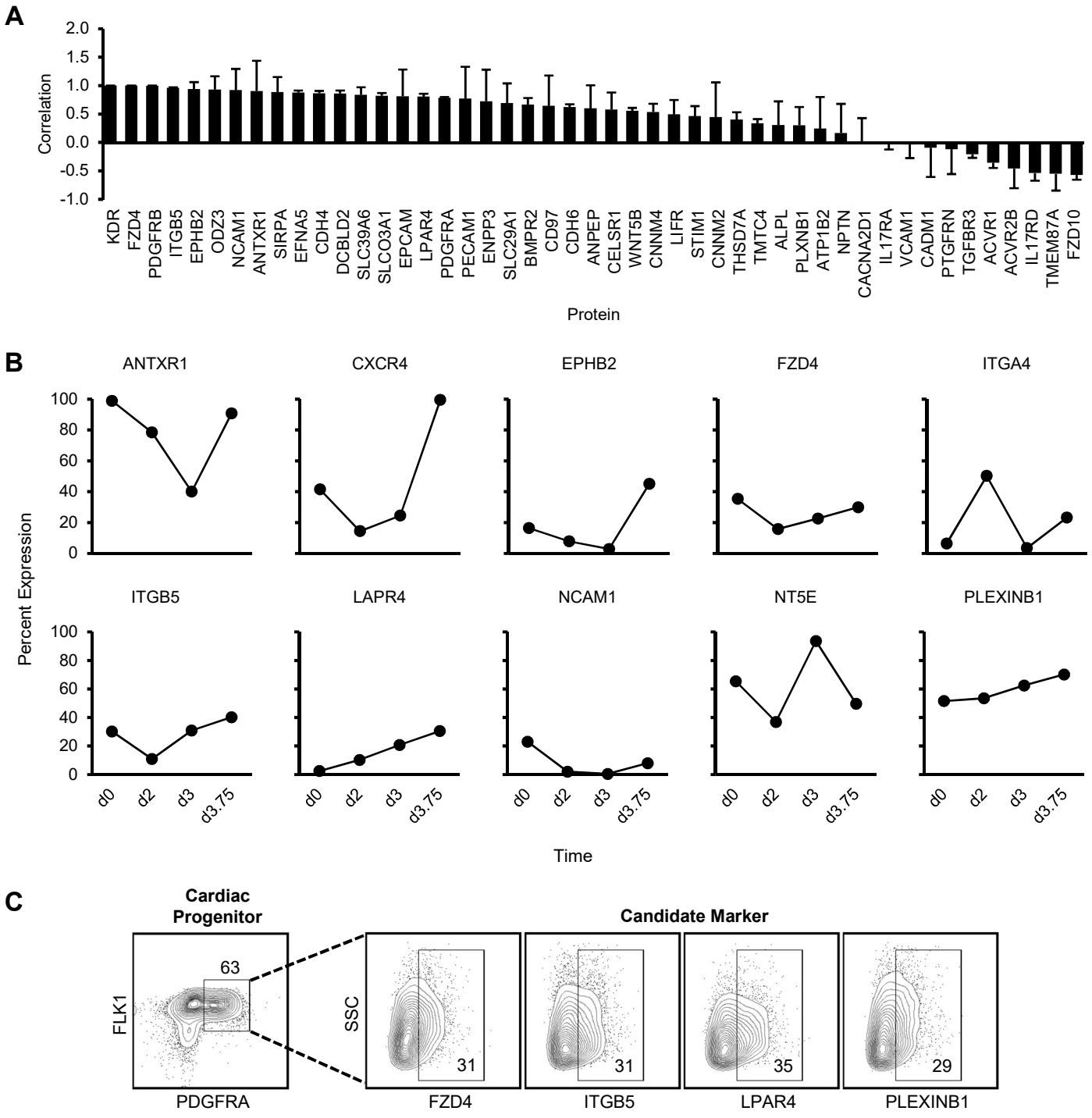

Figure S2: Validation of candidate surface markers, related to Figure 3. A) The 47 surface proteins unique to the cardiac progenitor population were validated with qPCR and 32 proteins with significant correlation between transcriptomic and proteomic data were selected (mean ± SEM, n = 3 independent experiments). B) Proteins were validated using a flow cytometry time course, and antibodies that showed non-specific binding were discarded, resulting in 10 proteins C) The resultant proteins with good quality antibodies were then assessed for their ability to resolve the cardiac progenitor population yielding 4 candidate markers. The number in the box represents the percent positive value.

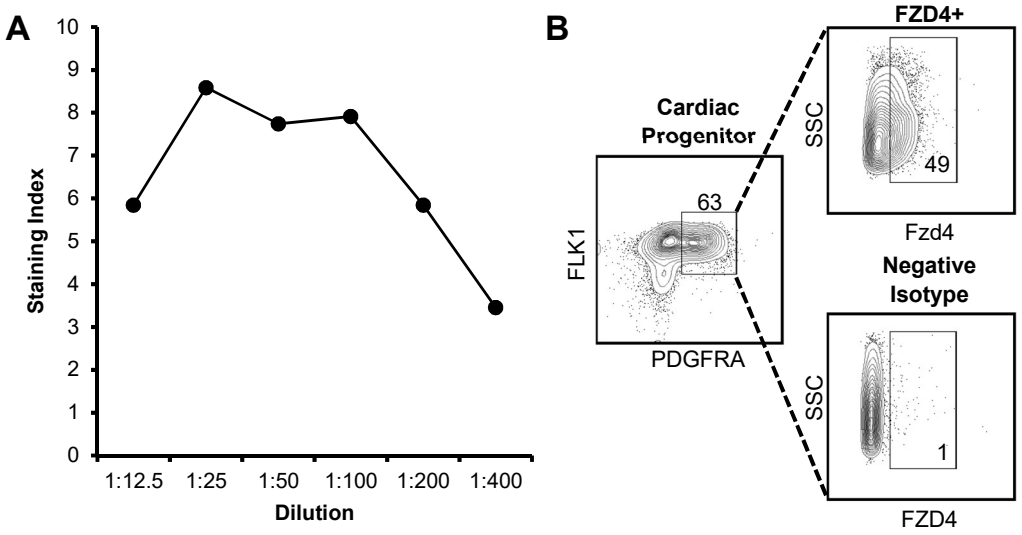

Figure S3: FZD4 antibody titration and negative isotype control, related to Figure 3. A) FZD4 antibody titration using staining index to determine optimal antibody dilution. B) Staining controls using negative isotype to determine FZD4 positive gating strategy. The number in the box represents the percent positive value.

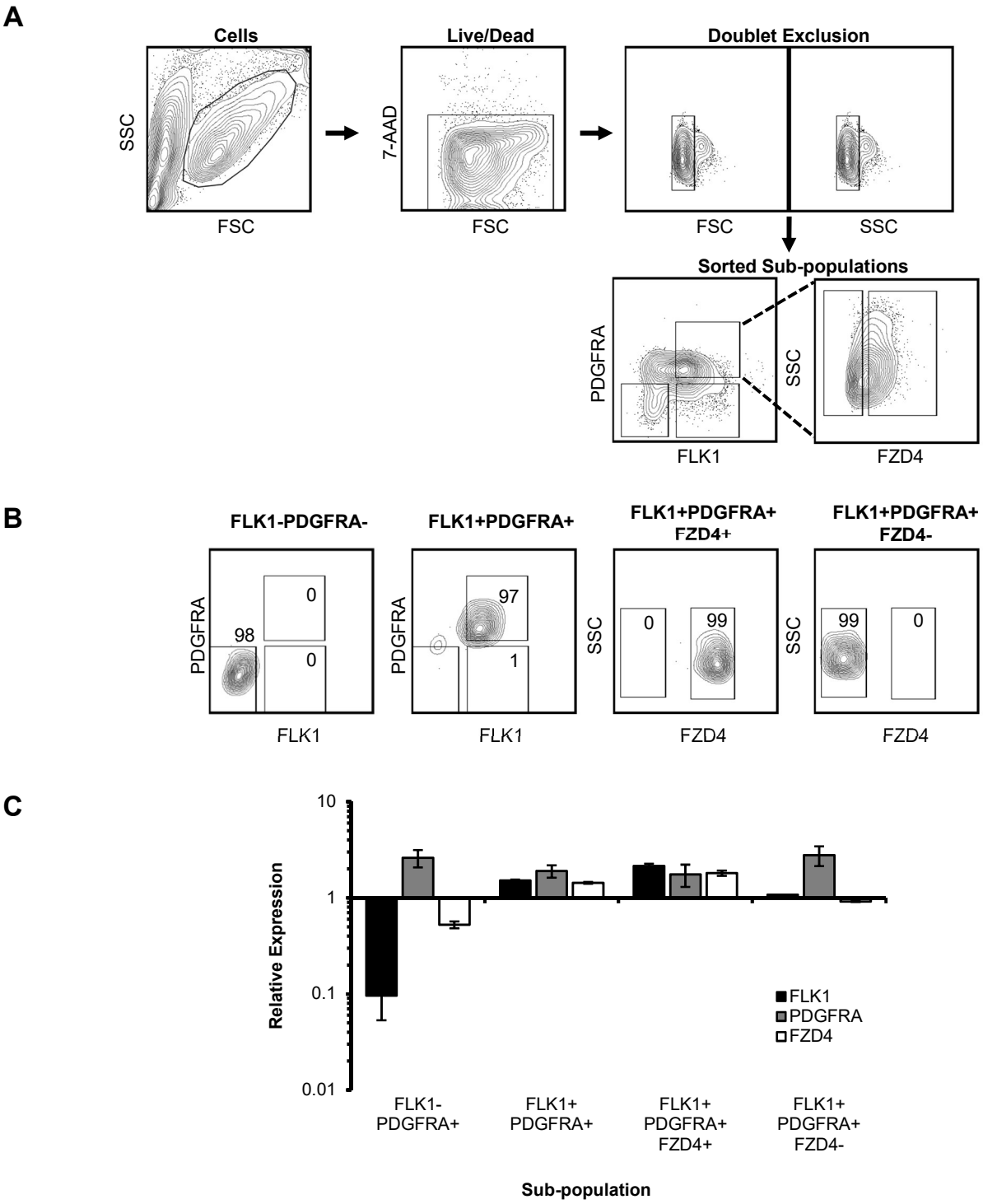

Figure S4: Flow cytometry and qPCR validation of purity of sorted populations, related to Figure 3. A) Gating strategy to sort cardiac progenitor cells. Five sub-populations were sorted from the cardiac progenitor stage (FLK1-PDGFRA-, FLK1-PDGFRA+, FLK1+PDGFRA+, FLK1+PDGFRA+FZD4+, and FLK1+PDGFRA+FZD4-) and differentiated into cardiomyocytes. Purity of sorted samples were assessed using B) flow cytometry and C) qPCR (mean ± SEM, n = 4 independent experiments).

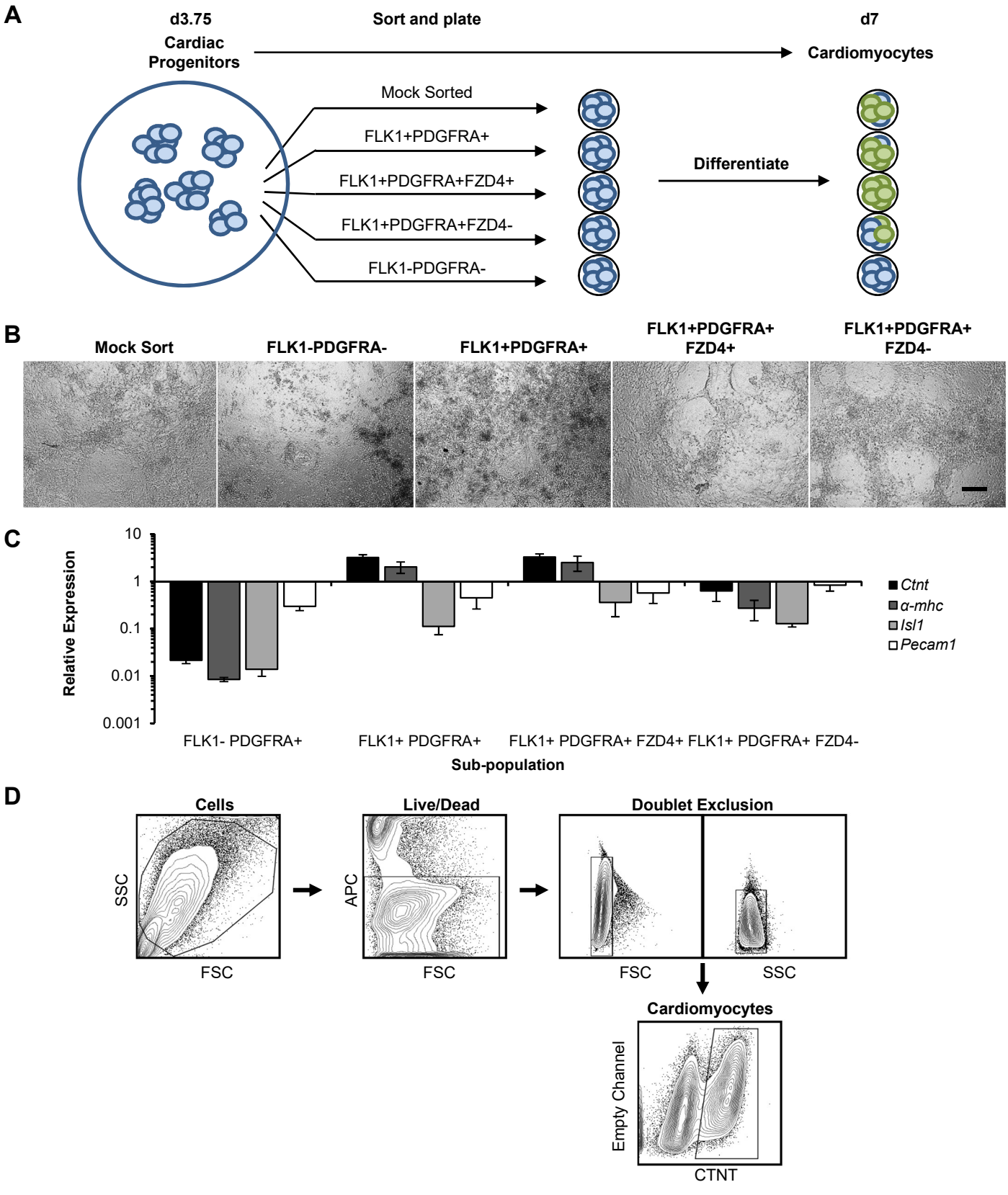

Figure S5: Differentiation of cardiac progenitors into cardiomyocytes, related to Figure 4. A) d3.75 CPCs are sorted into 5 sub-populations and seeded into individual wells and cultured for 3 days and assessed for CTNT. B) Bright-field images show beating CM in the FLK1+PDGFRA+FZD4+ condition. C) qPCR measurements of cardiac markers (*Ctnt*, *α-mhc*, *Isl1*) and endothelial cell marker (*Pecam1*). Expression is normalized relative to the unsort condition. The FZD4+ compartment expressed high amounts of *Ctnt* and *α-mhc* relative to the other compartments (mean ± SEM, n = 4 independent experiments). D) Gating strategy to evaluate cardiomyocytes using flow cytometry.

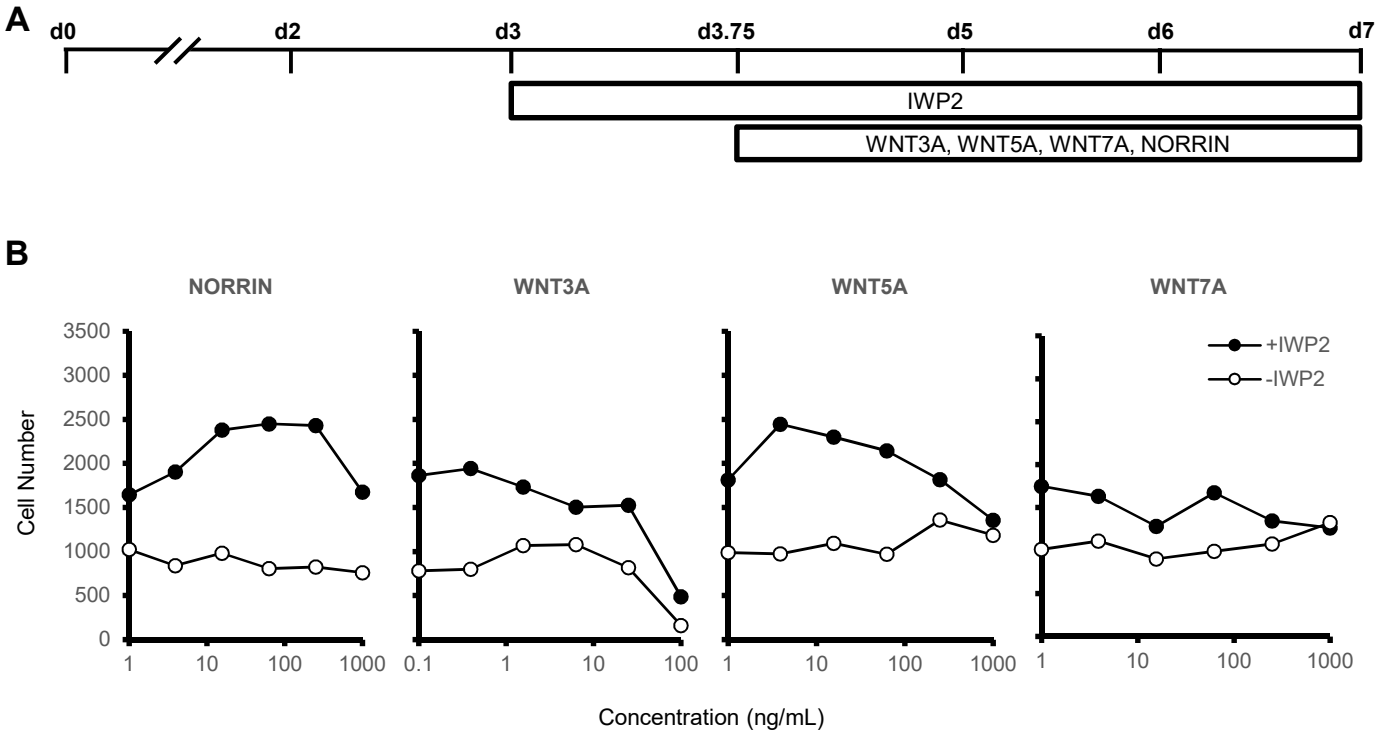

Figure S6: Exogenous addition of WNT ligands, related to Figure 6. A) FZD4 ligands WNT3A, WNT5A, WNT7A, and NORRIN were added to activate the WNT signaling pathway. B) Dose response curves of each ligand with and without IWP2. Optimal dose was determined to be: NORRIN [100 ng/mL], WNT3A [10 ng/mL], WNT5A [10 ng/mL], and WNT7A [100 ng/mL].

Table S1: Antibodies used in this study.

Antibody Name	Type	Dilution	Vendor	Catalog Number
Alexa Fluor® 488 goat anti-Rat IgG (H+L)	Polyclonal	1:400	ThermoFischer Scientific	A-11006
Alexa Fluor® 647 goat anti-Rat IgG (H+L)	Polyclonal	1:400	ThermoFischer Scientific	A-21247
Anti-Integrin beta 5 antibody	Polyclonal	1:100	Abcam	ab15459
Anti-Mouse CD326 (EpCAM) APC	Monoclonal	1:100	Ebioscience	17-5791-82
Anti-P2Y9 (LPAR4) antibody – N-terminal	Polyclonal	1:100	Abcam	ab140822
Anti-Plexin B1 antibody	Polyclonal	1:100	Abcam	ab90087
APC Anti-Mouse CD24	Monoclonal	1:100	BD Biosciences	562349
APC Rat anti-Mouse CXCR4 (CD184)	Monoclonal	1:200	BD Biosciences	558644
Brilliant Violet 421 Anti-Mouse CD40	Monoclonal	1:200	BD Biosciences	562846
CD140a (PDGFRA) antibody (APA5), APC	Monoclonal	1:400	eBioscience	17-1401-81
DyLight™ 405 Goat anti-rat IgG	Polyclonal	1:200	Biolegend	405412
Human ACTIVIN RIA Affinity Purified Polyclonal Ab, Goat IgG	Polyclonal	1:100	R&D Systems	AF637
Human TEM8/ANTXR1 Affinity Purified Polyclonal Ab, Goat IgG	Polyclonal	1:100	R&D Systems	AF3886
Human/Mouse DCBLD2/ESDN Affinity Purified Polyclonal Ab, Sheep IgG	Polyclonal	1:100	R&D Systems	AF6269
Human/Mouse EphB2 Phycoerythrin MAb (Clone 512012), Rat IgG2A	Monoclonal	1:100	R&D Systems	MAB4672
Human/Mouse Frizzled-4 Antibody	Monoclonal	1:100	R&D Systems	MAB194
Human/Mouse SOX2 Antibody	Monoclonal	1:200	R&D Systems	MAB2018R
PE Rat Anti-Mouse Flk-1	Monoclonal	1:400	BD Biosciences	555308
PE Rat Anti-Mouse NT5E (CD73)	Monoclonal	1:100	BD Biosciences	550741
Purified Mouse Anti-BMPR-II	Monoclonal	1:100	BD Biosciences	612292
Purified Mouse Anti-Oct-3/4	Monoclonal	1:200	BD Biosciences	611202
Purified Mouse Anti-R-Cadherin	Monoclonal	1:100	BD Biosciences	610414
Purified Rat Anti-Mouse ITGA4 (CD49d)	Monoclonal	1:100	BD Biosciences	553314
Purified Rat Anti-Mouse NCAM-1 (CD56)	Monoclonal	1:100	BD Biosciences	556325
Purified Rat Anti-Mouse SIRPA (CD172a)	Monoclonal	1:100	BD Biosciences	552371
Troponin T, Cardiac Isoform Ab-1, Mouse Monoclonal Antibody	Monoclonal	1:200	ThermoFischer Scientific	MS295P

Table S2: qPCR primers used in this study.

Gene	Forward	Reverse
α-mhc	GCCCAGTACCTCCGAAAGTC	GCCTTAACATACTCCTCCTTGTC
Acvr1	GTGGAAGATTACAAGCCACCA	GGGTCTGAGAACCATCTGTTAGG
Acvr2b	ACCCCCAGGTGTACTTCTG	CATGGCCGTAGGGAGGTTTC
Alpl	CCAACTCTTTTGTGCCAGAGA	GGCTACATTGGTGTTGAGCTTTT
Anpep	ATGGAAGGAGGCGTCAAGAAA	CGGATAGGGCTTGGACTCTTT
Antxr1	TGGACAAGTCAGGAAGTGTGC	TGATGAATCTATGAGCCAACTGC
Apc	CTTGTGGCCCAGTTAAAATCTGA	CGCTTTTGAGGGTTGATTCCT
Asah1	CGTGGACAGAAGATTGCAGAA	TGGTGCCTTTTGAGCCAATAAT
Atp1b2	GGCAGGTGGTTGAGGAGTG	GGGGTATGGTCAGAGACGGT
Axin1	CTCCAAGCAGAGGACAAAATCA	GGATGGGTTCCCCACAGAAATA
Axin2	TGACTCTCCTTCCAGATCCCA	TGCCCACACTAGGCTGACA
B-Catenin	ATGGAGCCGGACAGAAAAGC	CTTGCCACTCAGGGAAGGA
Bmpr2	TTGGGATAGGTGAGAGTCGAAT	TGTTTCACAAGATTGATGTCCCC
B-Tubb	CACCTGCAAGCCGGTCAAT	TCCCCATGATAGGTCCCAGTG

Cacna2d1	GTCACACTGGATTTTCTCGATGC	GGGTTTCTGAATATCTGGCCTGA
Cadm1	CAGCCTGTGATGGTAACTTGG	AGGAGGGATAGTTGTGGGGG
Cd97	CTCCCCGAGCAGACAACTAC	CAATGGTTTTGCCCGGAGAT
Cdh4	CAGGCCACTGACATGGAAGG	ATGATTCGGTAGACGGCGTTC
Cdh6	CAGCCCTACCCAACTTTCTCA	GAACGGCTCAGCTCATTCC
Celsr1	TCGCTGACTTCGGTGCTTG	TTACCAGCTCTACCCAAACGG
C-myc	ATGCCCCTCAACGTGAACTTC	CGCAACATAGGATGGAGAGCA
Cnnm2	AAGTGGCCCACCGTGAAAG	CGCTTCTACTTCTGTTGCTAGG
Cnnm4	CTGCACATCCTTCTCGTTATGG	TGCGAGCATACTTTCTCTCCTT
Ctnt	CAGAGGAGGCCAACGTAGAAG	CTCCATCGGGGATCTTGGGT
Cyclind1	GCGTACCCTGACACCAATCTC	CTCCTCTTCGCACTTCTGCTC
Dcbld2	ACACACTGTACTAGGCCCTGA	CGTCCTGACTCGAATCTCCCA
Dvl1	ATGGCGGAGACCAAAATCATC	AACTTGGCATTGTCATCGAAGA
Dvl2	GGTGTAGGCGAGACGAAGG	GCTGCAAAACGCTCTTGAAATC
Efna5	ACACGTCCAAAGGGTTCAAGA	GTACGGTGTCATTTGTTGGTCT
Enpp3	CAGAGGAGCCCATTAAGAAAGAC	GTGCGATGAGTCAAAGCATTTT
Epcam	GCGGCTCAGAGAGACTGTG	CCAAGCATTTAGACGCCAGTTT
Ephb2	GCGGCTACGACGAGAACAT	GGCTAAGTCAAAATCAGCCTCA
Erbb2	GAGACAGAGCTAAGGAAGCTGA	ACGGGGATTTTCACGTTCTCC
Fam38b	AATCAAACCAACATTCCCCTTCA	CAGGTAGACGAGCAAAGGAGA
Flk1	TTTGGCAAATACAACCCTTCAGA	GCAGAAGATACTGTCACCACC
Fzd10	CATGCCCAACCTGATGGGTC	GCCACCTGAATTTGAACTGCTC
Fzd4	TGCCAGAACCTCGGCTACA	ATGAGCGGCGTGAAAGTTGT
Gapdh	AGGTCGGTGTGAACGGATTTG	TGTAGACCATGTAGTTGAGGTCA
Gsk3b	TGGCAGCAAGGTAACCACAG	CGGTTCTTAAATCGCTTGTCCTG
Il17ra	AGTGTTTCCTCTACCCAGCAC	GAAAACCGCCACCGCTTAC
Il17rd	AACAGCGGACTGCACAACAT	GCAAGCGTACTGGCTGATG
Isl1	ATGATGGTGGTTTACAGGCTAAC	TCGATGCTACTTCACTGCCAG
Itga4	GATGCTGTTGTTGTACTTCGGG	ACCACTGAGGCATTAGAGAGC
Itgb5	GCTGCTGTCTGCAAGGAGAA	AAGCAAGGCAAGCGATGGA
Lef1	TGTTTATCCCATCACGGGTGG	CATGGAAGTGTCGCCTGACAG
Lifr	AGCTCTGACCCTCCTGCAT	TGGGTGACAAGAATGGAACCT
Lmo2	ATGTCCTCGGCCATCGAAAG	CGGTCCCCTATGTTCTGCTG
Lpar4	AGTGCCTCCCTGTTTGTCTTC	GCCAGTGGCGATTAAAGTTGTAA
Lrp5	AAGGGTGCTGTGTACTGGAC	AGAAGAGAACCTTACGGGACG
Lrp6	TTGTTGCTTTATGCAAACAGACG	GTTCGTTTAATGGCTTCTTCGC
Mapk8	AGCAGAAGCAAACGTGACAAC	GCTGCACACACTATTCCTTGAG
Meox1	GAAACCCCCACTCAGAAGATAGC	TCGTTGAAGATTCGCTCAGTC
Ncam1	AGCGCAGGTGCAGTTTGAT	ACAAAGAGCTTTTACGGACTGG
Ndp	GCATCCATTTCTATGCTCTCCC	GGTGTCTCATGCAGCGTTG
Nfatc1	GACCCGGAGTTCGACTTCG	TGACACTAGGGGACACATAACTG
Nkx2.5	GACAAAGCCGAGACGGATGG	CTGTCGCTTGCACTTGTAGC
Nptn	CGCTGCTCAGAACGAACCAA	GCTGGAAGTGAGGTTACACTG
Nt5e	GGACATTTGACCTCGTCCAAT	GGGCACTCGACACTTGGTG
Odz3	CGGGAAAAGGAAAGGCGCTAT	CTTCGAGTTGCGGATTCACAC
Pax1	CCGCCTACGAATCGTGGAG	CCCGCAGTTGCCTACTGATG
Pdgfra	ACACGTTTGAGCTGTCAACC	CCCGACCACACAAGAACAGG
Pdgfrb	TTCCAGGAGTGATACCAGCTT	AGGGGGCGTGATGACTAGG
Pecam1	CTGCCAGTCCGAAAATGGAAC	CTTCATCCACCGGGGCTATC
Plxnb1	CACACATCTACTACACTTGGCAA	CAATCCCGGCTGTCATTCAC
Prickle1	ACCTGGAGTATGCTGGCAC	CACAGTGGATTTTTCCATCCTGA
Ptgfrn	CCCTGCAATGTCAGCGACTAT	CGTTGGCAGTTCTCCTCAACA
Robo1	GAGCCTGCTCACTTTTACCTC	GGTCTGAAGGGTGTTCAACAAT

Sdk2	GTGACCAAGTGGCAGTCTCC	GTTGCTCAGGATGGGCTAAGG
Sirpa	CACGGGGACAGAAGTGAAGG	TGCAGTTGAGAATGGTCGAATC
Slc29a1	CAGCCTCAGGACAGGTATAAGG	GTTTGTGAAATACTTGGTTGCGG
Slc39a6	GTCACACGGTTGCTGGTAAAA	GGGCGAGATCCTTTCCCTAGA
Slco3a1	AGGTGTCCTGCTTCTCCAAC	GTCAACACGCTCACCAGGTAG
Stim1	GGCGTGGAAATCATCAGAAGT	TCAGTACAGTCCCTGTCATGG
Tbx6	ATGTACCATCCACGAGAGTTGT	GGTAGCGGTAACCCTCTGTC
Tcf15	GGGCAGCTGCTTGAAAGTGA	CTCCGGTCCTTACACAACGC
Tcf7	AGCTTTCTCCACTCTACGAACA	AATCCAGAGAGATCGGGGGTC
Tgfbr3	GGTGTGAACTGTCACCGATCA	GTTTAGGATGTGAACCTCCCTTG
Thsd7a	AGGTGCCCACCCTCTATCTG	TGTATGTAACGTAGTCCAGCCT
Tmem132c	TCAGAGCCGAGACTGCATTCT	GCCCATAGCTGACGTTTAATACC
Tmem87a	TGGCATGGAAGGAGTCCTCA	GAGAGGGCCAGGCTTACTATC
Tmtc4	TCCCAAGTACGTTCATGCCAT	GTTTTAGGTGACGGGAAACTGG
Vangl2	ACTCGGGCTATTCCTACAAGT	TGATTTATCTCCACGACTCCCAT
Vcam1	AGTTGGGGATTCGGTTGTTCT	CCCCTCATTCCTTACCACCC
Wnt11	GCTGGCACTGTCCAAGACTC	CTCCCGTGTACCTCTCTCCA
Wnt5a	CAACTGGCAGGACTTTCTCAA	CATCTCCGATGCCGGAACT
Wnt5b	CTGCTGACTGACGCCAACT	CCTGATACAACTGACACAGCTTT
Wnt7a	CCTTGTTGCGCTTGTTCTCC	GGCGGGGCAATCCACATAG

Table S3: Candidate proteins identified by mass spectrometry and microarray, related to Figure 2. List of 47 proteins identified to be uniquely expressed in the FLK1+PDGFRA+ sub-population of cardiac progenitors. Classification was done based on literature search.

FLK1+PDGFRA+	**Description**	**Classification**
ACVR1	Activin a receptor, type 1	Unannotated
ACVR2B	Activin a receptor, type 2b	Muscle
ALPL	Alkaline phosphatase, liver/bone/kidney	Non-specific
ANPEP	Alanyl (membrane) aminopeptidase	Cardiac/Blood
ALPL	Anthrax toxin receptor 1	Endothelial
ANPEP	Atpase, na+/k+ transporting, beta 2 polypeptide	Unannotated
ANTXR1	Anthrax toxin receptor 1	Endothelial
ASAH1	Calcium channel, voltage-dependent, alpha2/delta subunit 1	Unannotated
ATP1B2	Cell adhesion molecule 1	Unannotated
CACNA2D1	Cd97 antigen	Unannotated
CADM1	Cadherin 4	Unannotated
CD97	Cadherin 6	Neural
CDH4	Cadherin, egf lag seven-pass g-type receptor 1	Neural
CDH6	Cyclin m2	Unannotated
CELSR1	Cyclin m4	Unannotated
DCBLD2	Discoidin, cub and lccl domain containing 2	Neural
EFNA5	Ephrin a5	Neural
ENPP3	Ectonucleotide pyrophosphatase/phosphodiesterase 3	Unannotated
EPCAM	Epithelial cell adhesion molecule	Unannotated
EPHB2	Eph receptor b2	Unannotated
FZD10	Frizzled 10	Neural
FZD4	Frizzled 4	Cardiac/Blood
IL17RA	Interleukin 17 receptor a	Unannotated
IL17RD	Interleukin 17 receptor d	Non-specific
ITGB5	Integrin beta 5	Non-specific
KDR	Kinase insert domain	Cardiac/Blood
LIFR	Leukemia inhibitory factor receptor	Unannotated

LPAR4	Lysophosphatidic acid receptor 4	Cardiac/Blood
NCAM1	Neural cell adhesion molecule 1	Cardiac/Blood
NPTN	Neuroplastin	Unannotated
NT5E	5' nucleotidase, ecto	Cardiac/Blood
PDGFRA	Platelet derived growth factor alpha	Cardiac/Blood
PDGFRB	Platelet derived growth factor receptor, beta polypeptide	Cardiac/Blood
PECAM1	Platelet/endothelial cell adhesion molecule 1	Endothelial
PLXNB1	Plexin b1	Unannotated
PTGFRN	Prostaglandin f2 receptor negative regulator	Unannotated
SIRPA	Signal-regulatory protein alpha	Cardiac/Blood
SLC29A1	Solute carrier family 29 (nucleoside transporters), member 1	Unannotated
SLC39A6	Solute carrier family 39 (metal ion transporter), member 6	Unannotated
SLCO3A1	Solute carrier organic anion transporter family, member 3a1	Unannotated
STIM1	Similar to stromal interaction molecule 1; stromal interaction molecule 1	Non-specific
TGFBR3	Transforming growth factor beta receptor 3	Muscle
THSD7A	Thrombospondin, type i, domain containing 7a	Cardiac/Blood
TMEM87A	Transmembrane protein 87a	Unannotated
TMTC4	Transmembrane and tetratricopeptide repeat containing 4	Unannotated
VCAM1	Vascular cell adhesion molecule 1	Unannotated
WNT5B	Wingless-related mmtv integration site 5b	Cardiac/Blood