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Supplemental information

Direct reprogramming of cardiomyocytes into cardiac Purkinje-like cells

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Table-S1

Gene	Sequence	Source
GAPDH	Forward - 5' GTCTCCTCTGACTTCAACAGCG 3' Reverse – 5' ACCACCCTGTTGCTGTAGCCAA 3'	OriGene, cat# HP205798
CX40	Forward - 5' TAGGCAAGGTCTGGCTCACTGT 3' Reverse – 5' GAAAGCCTGGTCGTAGCAGACA 3'	OriGene, cat# HP208435
CX43	Forward - 5' GGAGATGAGCAGTCTGCCTTTC 3' Reverse – 5' TGAGCCAGGTACAAGAGTGTGG 3'	OriGene, cat# HP200150
ETV1	Forward - 5' GCAAGAAGGCTTCCTGGCTCAT 3' Reverse – 5'CCTTCCCGATACATTCCTGGCT 3'	OriGene, cat# HP208189
CNTN2	Forward - 5' TACGAGTGTGAGGCGGAGAACT 3' Reverse – 5' CAACGCAGGTTGGAGCCAATGT 3'	OriGene, cat# HP208284
HCN2	Forward - 5' GACATCTGGCTGACCATGCTCA 3' Reverse – 5' TGGCAGCTTGTGGAAGGACATG 3'	OriGene, cat# HP205124
HCN4	Forward - 5' TCTACTCGCTGAGCGTGGACAA 3' Reverse – 5' GAGTTGAGGTCGTGCTGGACTT 3'	OriGene, cat# HP208604
MEF2C	Forward - 5' TCCACCAGGCAGCAAGAATACG 3' Reverse – 5' GGAGTTGCTACGGAAACCACTG 3'	OriGene, cat# HP206089
ACHE	Forward - 5' GTTCTCCTTCGTGCCTGTGGTA 3' Reverse – 5' ATACGAGCCCTCATCCTTCACC 3'	OriGene, cat# HP231855
TBX3	Forward - 5' GGACACTGGAAATGGCCGAAGA 3' Reverse – 5' GCTGCTTGTTCACTGGAGGACT 3'	OriGene, cat# HP231811
TBX5	Forward - 5' ACAACCACCTGGACCCATTTGG 3' Reverse – 5' GGAAAGACGTGAGTGCAGAACG 3'	OriGene, cat# HP200177
SCN5a	Forward -5' CAAGACCTGCTACCACATCGTG 3' Reverse – 5' GTCGGCATACTCAAGCAGAACC 3'	OriGene, cat# HP234325

IRX3	Forward - 5’ CTCCGCACCTGCTGGGACTTC 3’ Reverse – 5’ CTCCACTTCCAAGGCACTACAG 3’	OriGene, cat# HP214795
GATA6	Forward - 5’ GCCACTACCTGTGCAACGCCT 3’ Reverse – 5’ CAATCCAAGCCGCCGTGATGAA 3’	OriGene, cat# HP208427
NKX2-5	Forward - 5’ AAGTGTGCGTCTGCCTTTCCCG 3’ Reverse – 5’ TTGTCCGCCTCTGTCTTCTCCA 3’	OriGene, cat# HP207706
PCP2	Forward - 5’ ACCAACGTGTGACAGTCAGCAG 3’ Reverse – 5’ CTGTTCCGACGGAAGCCGAGA 3’	OriGene, cat# HP218542
PCP4	Forward - 5’ TGACATGGATGCACCAGAGACAG 3’ Reverse – 5’ AGGACTGAGACCCAGCCTTCTT 3’	OriGene, cat# HP209183
TUBB3	Forward – 5’ TCAGCGTCTACTACAACGAGGC 3’ Reverse – 5’ GCCTGAAGAGATGTCCAAAGGC 3’	OriGene, cat# HP209096
OLIG2	Forward – 5’ GATAGTCGTCGCAGCTTTCG 3’ Reverse – 5’ CCTGAGGCTTTTCGGAGC 3’	(Watson et al., 2018)
SKOR2	Forward – 5’ AGCCCAGTTCACCATCCAT 3’ Reverse – 5’ GCTGTTGTCATCCTTTGTAGATAC 3’	(Watson et al., 2018)
LHX5	Forward – 5’ GTGCGCGAAGAAGTCGTAGT 3’ Reverse – 5’ CGAGTCTGAGATGTTGGGGT 3’	(Watson et al., 2018)

Table-S1: Primer Sequences used in qPCR. Related to STAR Methods.

Figure-S1

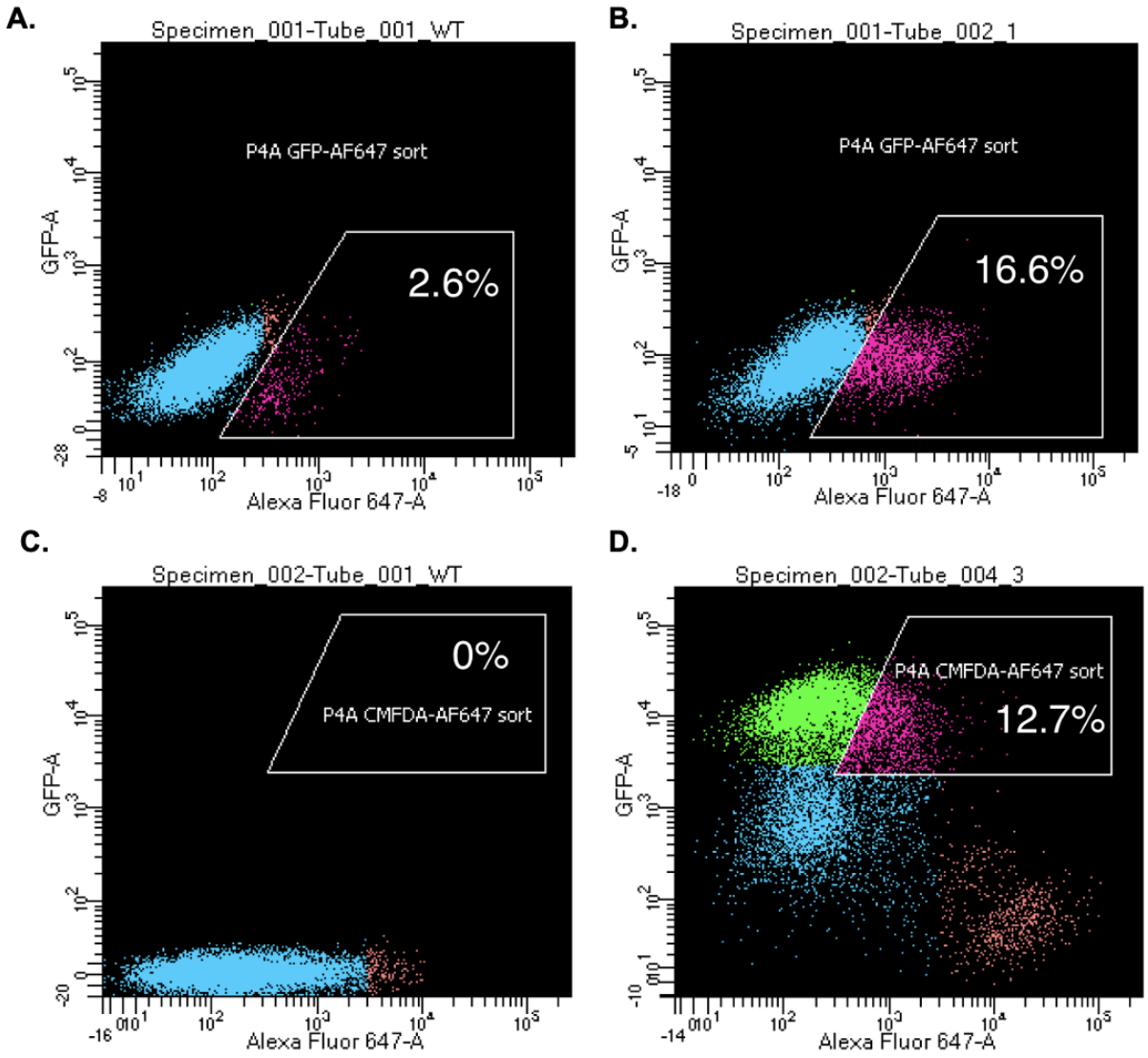

Figure-S1: Replicate FACS analysis of control and PURK-cocktail treated cells. Related to Figure-1. The control (vehicle) treated cell population showed minimal expression of CNTN2-IRES-mCherry+ cells (A and C) whereas the PURK-cocktail treated cells showed a significant amount of CNTN2-IRES-mCherry+ cell population (B and D). CellTracker™ Green CMFDA Dye (Invitrogen, cat# C2925) was used to sort for live-dead cells. Live mCherry+ cells were used for downstream applications.

Figure-S2

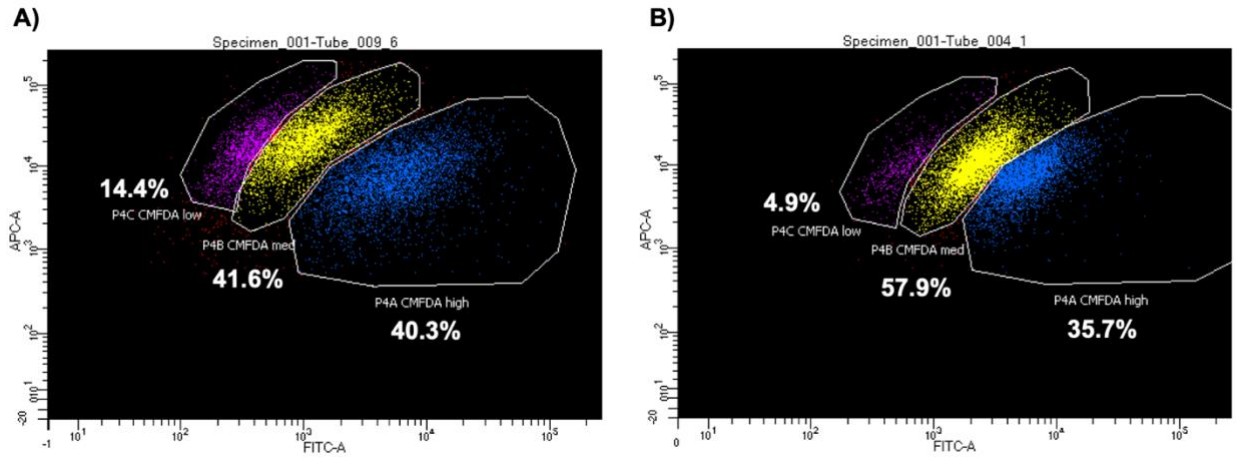

Figure-S2: Cell viability analysis through FACS of control and PURK-cocktail treated cells. Related to Figure-1. The cell viability of (A) control (vehicle) treated cell sample and (B) PURK-cocktail treated cell sample were evaluated by CellTracker™ Green CMFDA Dye (Invitrogen, cat# C2925). CMFDA dye is a stable, non-toxic dye used to assess and monitor cell death and viability. The dye is retained in live cells through multiple generations, being passed on to daughter cells, but not to adjacent cells. Both samples showed more than 90% viability, suggesting that treatment with the PURK-cocktail did not cause significant cell health deterioration or death. Additionally, we see 3 distinct populations of CMFDA stained cells with high, medium, and low fluorescence brightness. The dimming of CMFDA fluorescence brightness is indicative of cell division. As shown in (B), the PURK-cocktail treated cells have a lower percentage of dividing and older generation cells (4.9%) compared to (A) control (14.4%).

Figure-S3

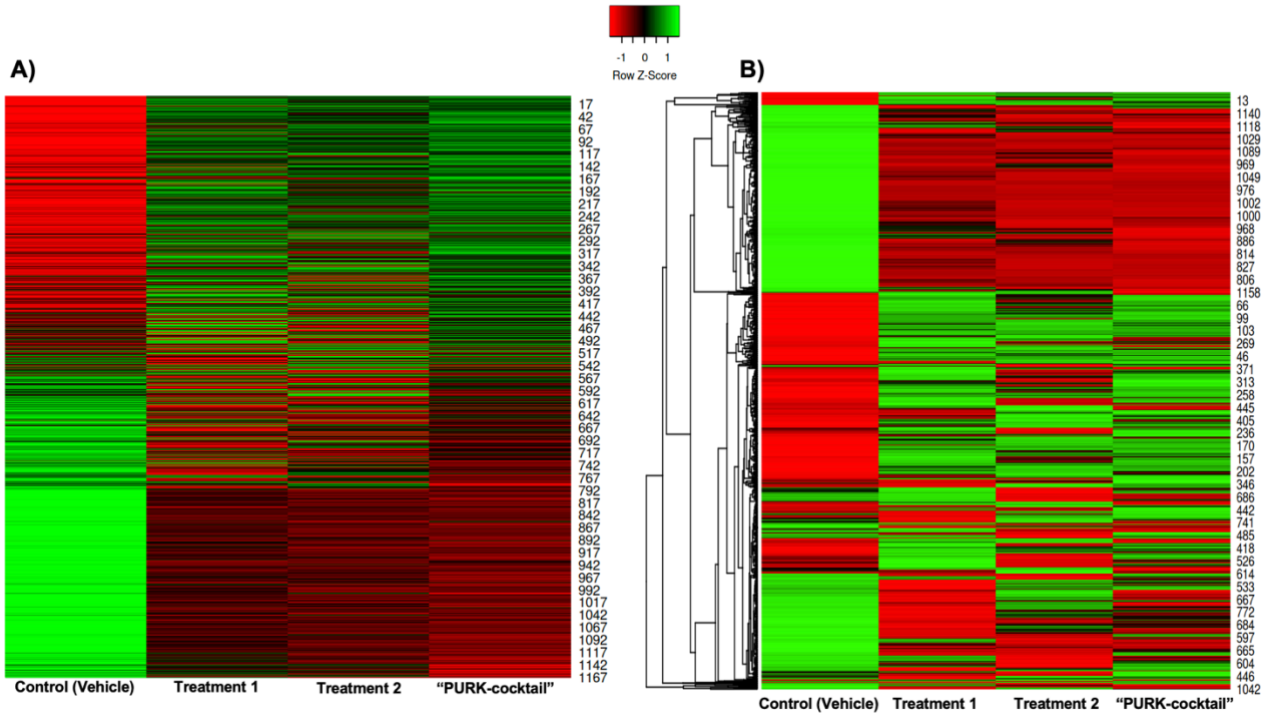

Figure-S3: Global RNA-seq evaluation of 1167 cardiac genes between the top 3 differentiation cocktails. Related to Figure-5. All 3 treatments induced a different genetic profile on the AC16 cells compared to the control. However, the best overall data was observed with the "PURK-cocktail". (A) Displays the global RNA-seq data without clustering. (B) Displays the global RNA-seq with average linkage clustering method with Euclidean distance. Differentially expressed genes were identified where an FDR adjusted p-value of at least 0.05 was observed.

Figure-S4

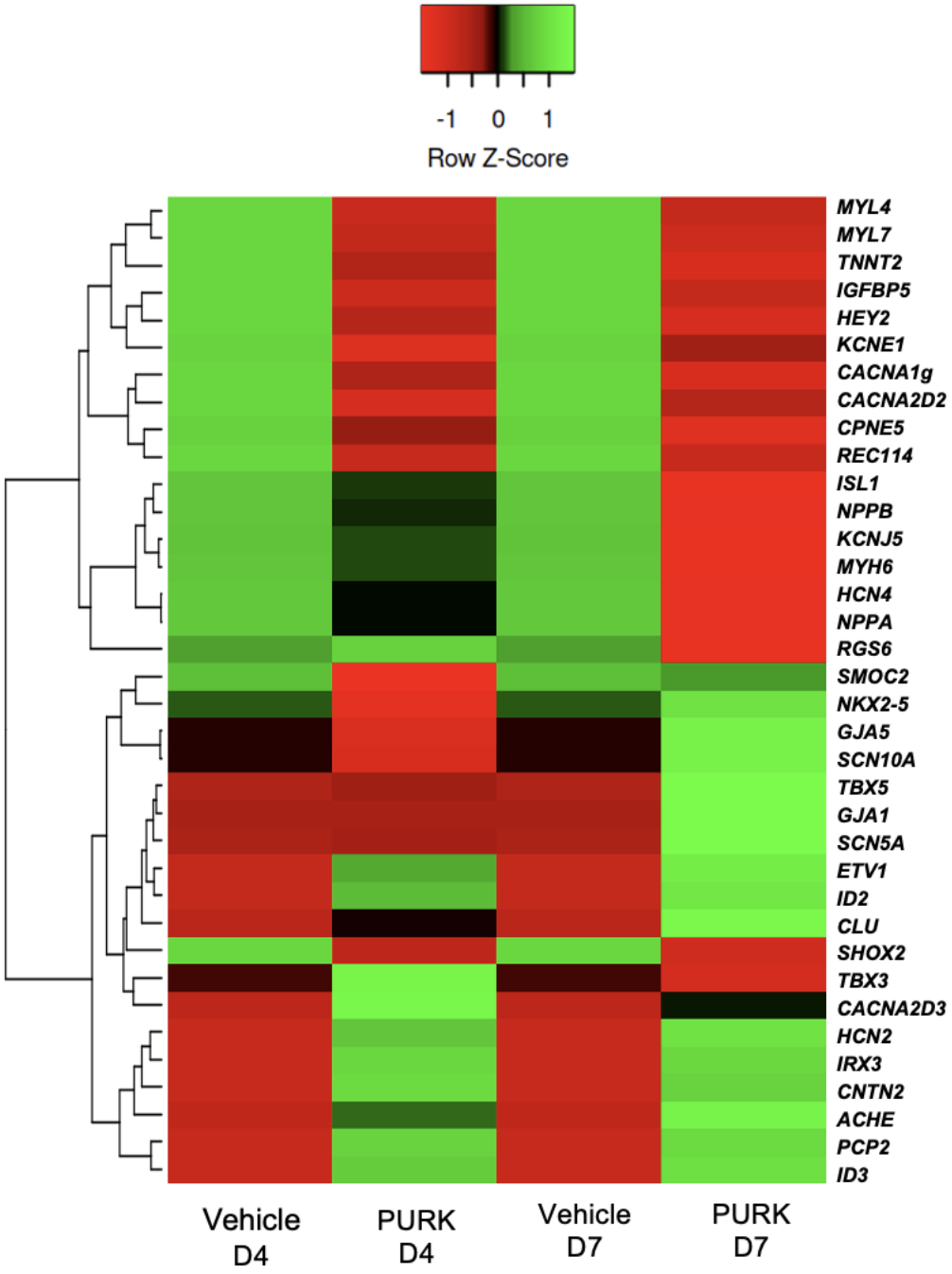

Figure-S4: RNA-seq evaluation of analysis of FACS Sorted PURK-cocktail treated cells at distinct differentiation time points. Related to Figure-5A. RNA-seq shows the gene expression of PURK-cocktail treated cells, compared to vehicle treated control cells on day-4 (D4) and day-7 (D7) of differentiation. This heat map displays the information already shown in Figure-5A of the main text; however, the average linkage clustering method with Euclidean distance method was used here. Differentially expressed genes were identified where an FDR adjusted p-value of at least 0.05 was observed.

Figure-S5

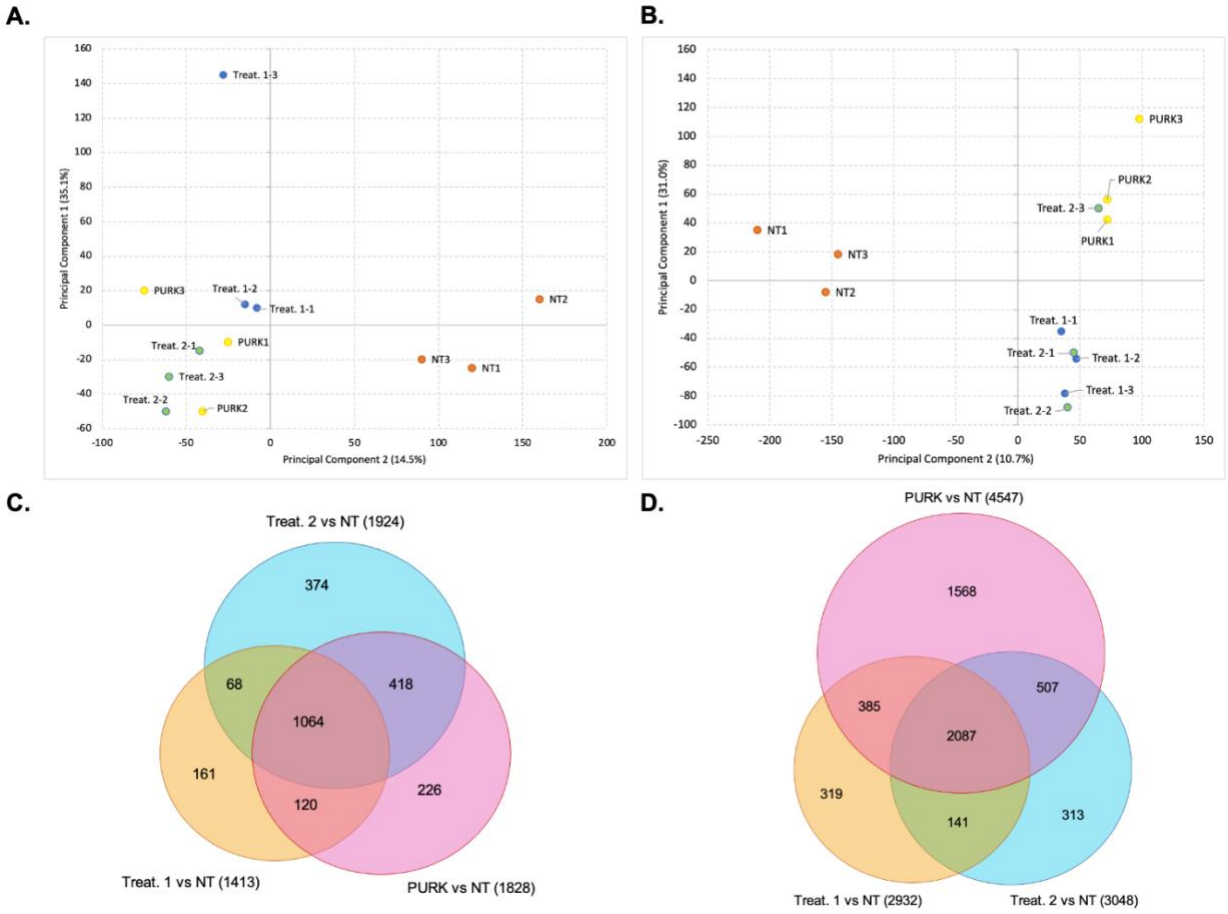

Figure-S5: Comparison of 3 differentiation cocktails compared to control. Related to Figure-5. All 3 treatments induced a different genetic profile on the AC16 cells compared to the control. However, the most robust difference was observed with the "PURK-cocktail". (A) Displays the PCA analysis of the triplicate samples of each treatment condition and control (NT) used for the RNA-seq data from day-4 of differentiation. (B) Displays the PCA analysis of the triplicate samples of each treatment condition and control (NT) used for the RNA-seq data from day-7 of differentiation. (C) Displays the Venn analysis of the number of genes that are either overlapping or unique to each treatment compared to control at day-4 of differentiation. (D) Displays the Venn analysis of the number of genes that are either overlapping or unique to each treatment compared to control at day-7 of differentiation. As shown in (A) on day-4 of differentiation, it is clear that all treatment samples are different from the control samples (NT). (B) On day-7 of differentiation, the PURK-cocktail treated cells are distinct from the other 2 treatment cocktails as well as controls (NT). (C) Shows that at day-4 of differentiation, all treatments appear to have a higher number of overlapping and common genes between the three treatments; whereas, (D) shows that by day-7, the PURK-cocktail treated cells appear to diverge from the other cocktail treatments and display a more unique gene readout, as shown by a higher number of genes that are significantly unique to the PURK-cocktail sample. NT denotes "no treatment" (control).

Figure-S6

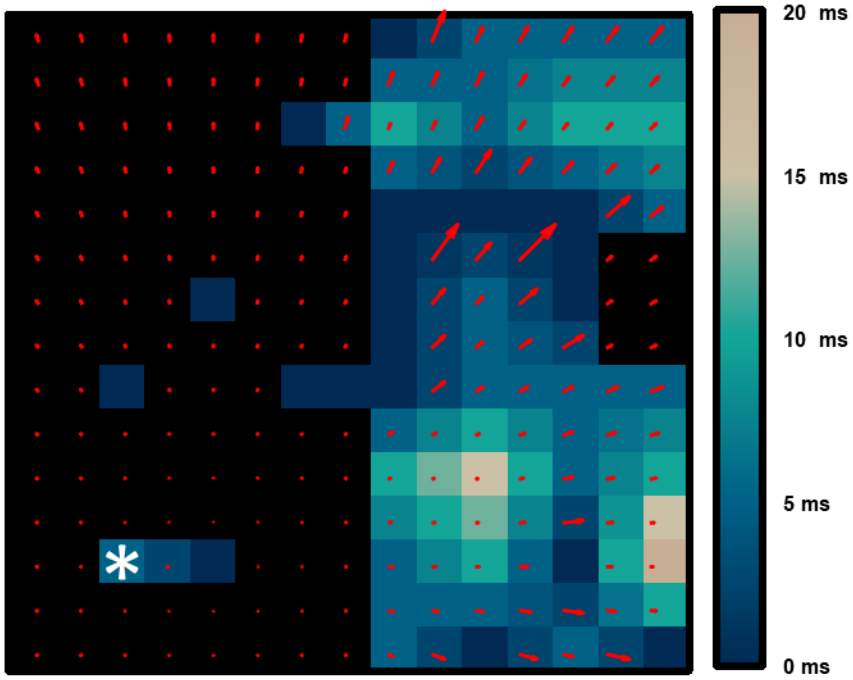

Figure-S6: Activation delay and conduction velocity of Purkinje-like cells. Related to Figure-6. The white star indicates the stimulus location. Lighter colors represent shorter activation delays. The lengths of the red arrows indicate the relative magnitude. Shorter activation delays (darker colors) show higher magnitudes of conduction velocity (longer arrows).