Stem Cell Reports, Volume *8*

Supplemental Information

Notch Inhibition Enhances Cardiac Reprogramming by Increasing MEF2C Transcriptional Activity

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SUPPLEMENTAL DATA

SUPPLEMENTAL FIGURES

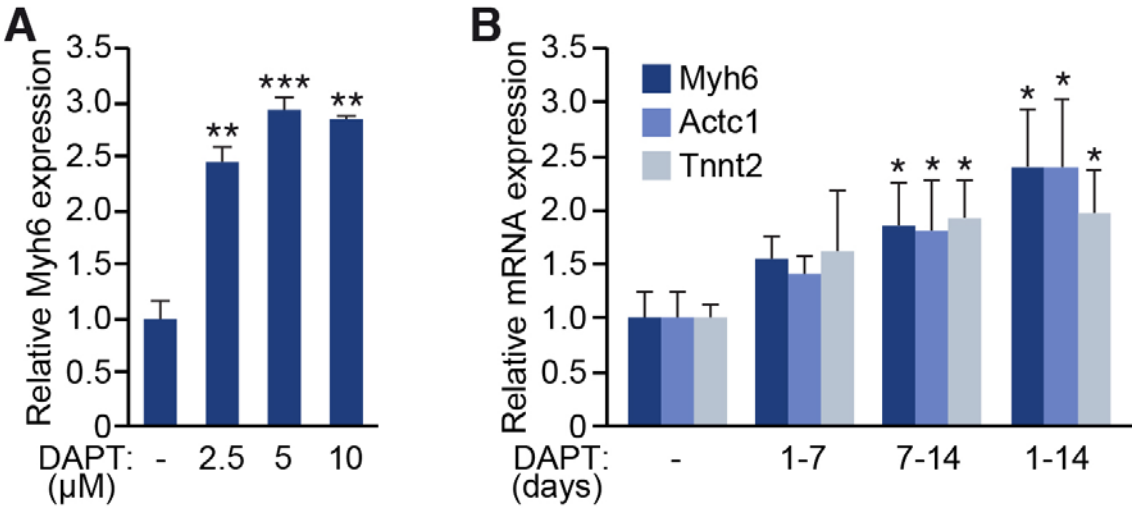

Figure S1. Optimization of DAPT Concentration and Time of Exposure, Related to Figure 1.

(A) Analysis of Myh6 expression by q-PCR in αMHC-GFP MEFs infected with GHMT and treated with DMSO (vehicle), or DAPT at the indicated concentrations for 15 days. (B) Analysis of the expression of different cardiac markers in αMHC-GFP MEFs infected with GHMT and treated with DMSO (vehicle), or DAPT for the indicated periods of time. Values correspond to the average of three independent biological replicates and s.d. *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$

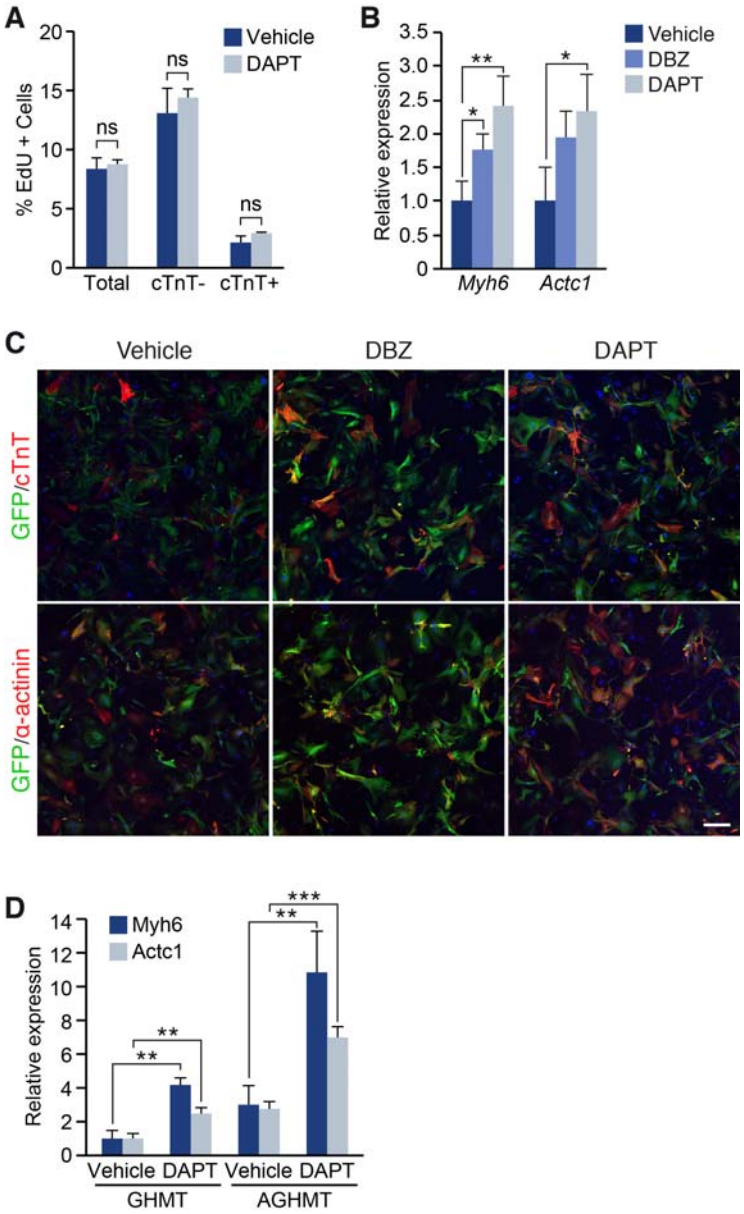

Figure S2. Notch Inhibition Enhances Cardiac Reprogramming in MEFs and TTFs Without Affecting Cell Proliferation, Related to Figure 1.

(A) Flow cytometry proliferation analysis in MEFs infected with GHMT, and treated with DMSO (vehicle) or DAPT for 7 days. (B) q-PCR analysis of RNA from αMHC-GFP MEFs infected with GHMT and treated with DMSO (vehicle), DAPT or DBZ for 2 weeks. (C) Representative images of immunostaining showing GFP, cTnT and α-actinin expression in αMHC-GFP MEFs infected with GHMT and treated with DMSO (vehicle), DAPT or DBZ for 2 weeks. Scale bar,

200 μM. (D) q-PCR analysis of RNA from TTFs infected with GHMT or AGHMT, and treated with DMSO (vehicle) or DAPT for 2 weeks.

Values correspond to the average of three independent biological replicates and s.d. *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$

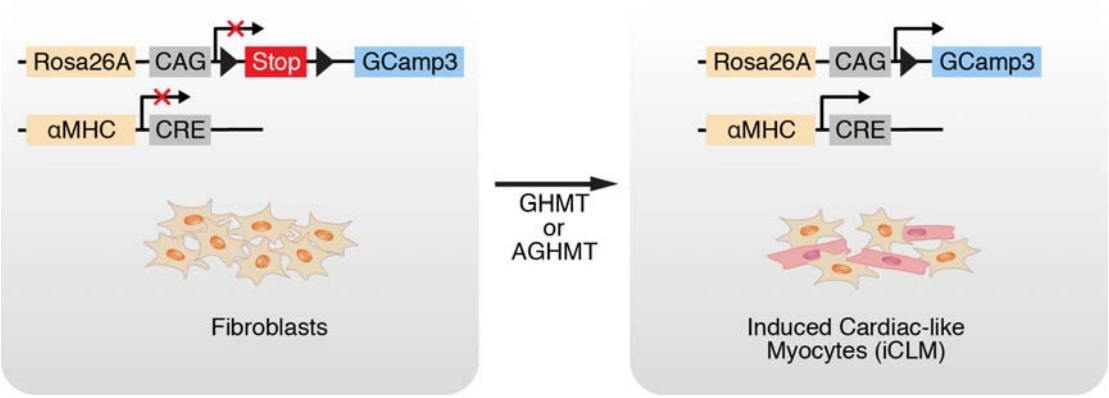

Figure S3. Strategy for Measuring Calcium Flux with GCaMP MEFs, Related to Figure 1.

MEFs derived from αMHC-Cre/Rosa26A-Flox-Stop-Flox-GCaMP3 transgenic mice were reprogrammed to iCLMs by addition of GHMT or AGHMT. Reprogrammed iCLMs exhibit spontaneous cyclic auto-fluorescence concomitant with calcium flux.

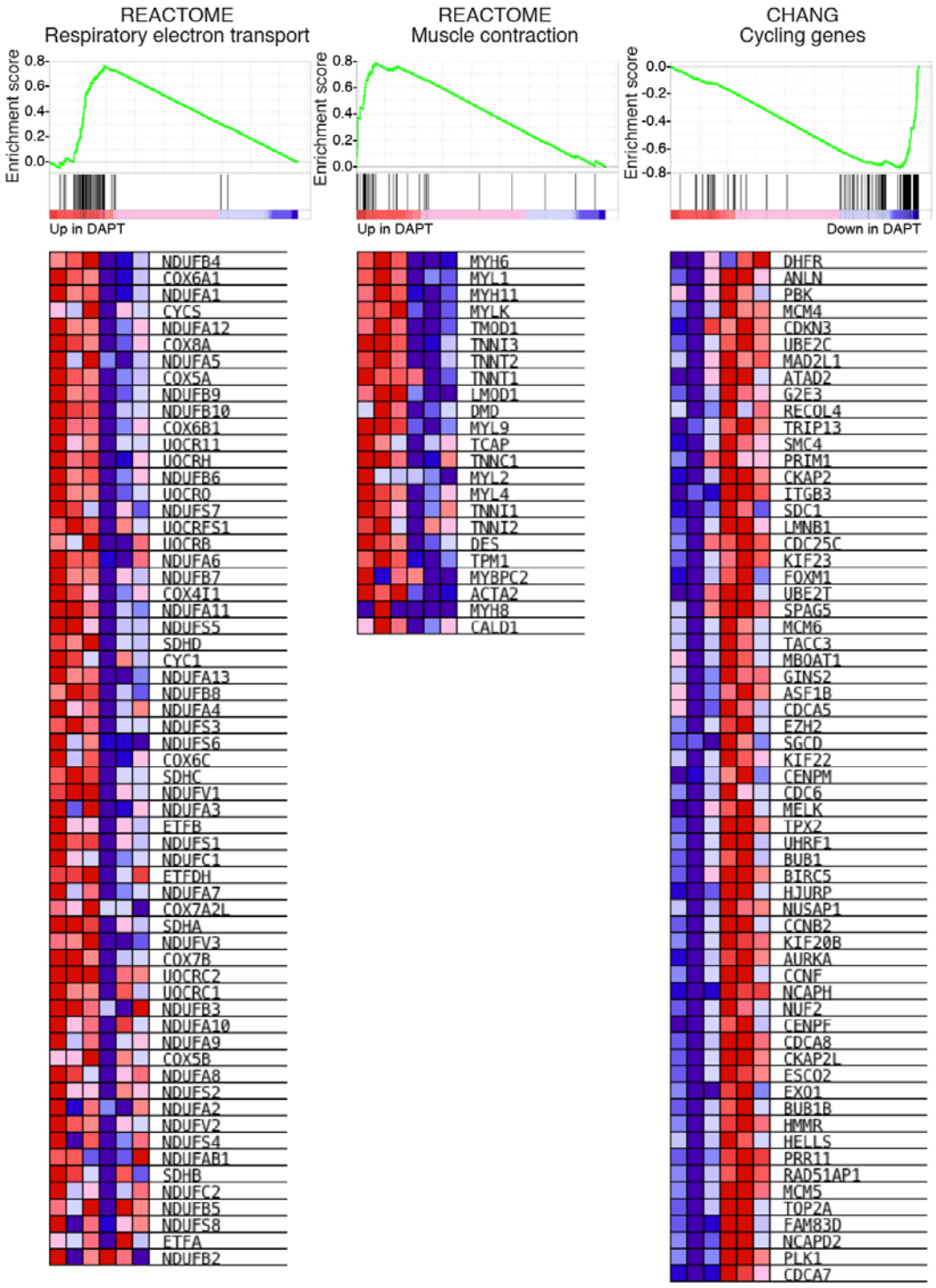

Figure S4. Gene Set Enrichment Analyses of DAPT-GHMT MEFs, Related to Figure 2. Enrichment plots of the indicated gene-sets, and their corresponding heat maps. The heat maps were generated with the genes that constitute the core enrichment group.

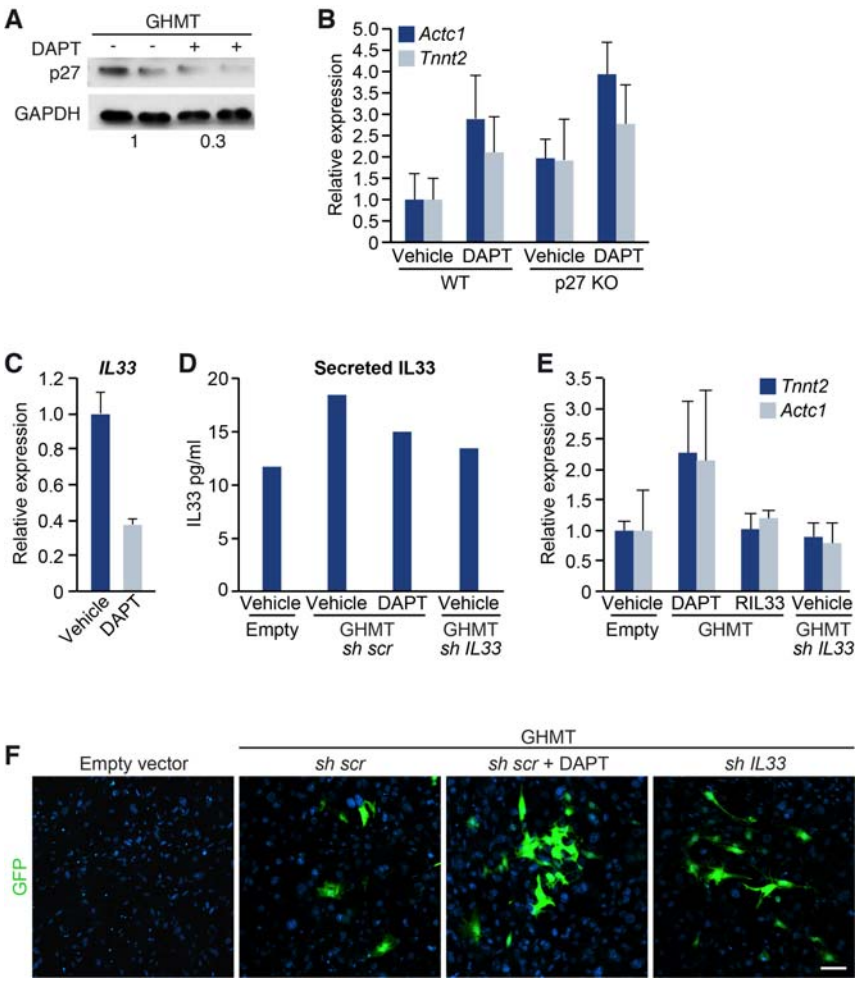

Figure S5. p27 and the Down-regulation of IL33 Are Not Necessary for DAPT Enhanced Reprogramming, **Related to Figure 4.**

(A) Immunoblot showing expression of p27 protein in WT MEFs, at day 15 post-GHMT reprogramming, with or without DAPT. (B) q-PCR analysis showing mRNA expression of *Actc1* and *Tnnt2* in WT or p27 knockout (KO) MEFs, at day 15 post-GHMT reprogramming; n=3 biological replicates. (C) q-PCR showing mRNA expression of IL33 in GHMT-reprogrammed MEFS, treated with DMSO or with DAPT; n=3 biological replicates. (D) Detection of secreted IL33 by ELISA in the conditioned medium of MEFs infected with empty vector, GHMT, GHMT+DAPT and GHMT-*shRNA IL33*. The conditioned medium of

three biological replicates per group was pooled. (E) Relative expression of *Tnnt2* and *Actc1* in MEFs reprogrammed with GHMT or *shIL33*-GHMT, and treated with DMSO, DAPT, or recombinant IL33 (RIL33); n=3 biological replicates. (F) Representative images of immunostaining for GFP in αMHC-GFP MEFs infected with empty vector, GHMT, shRNA *scramble* (*scr*)-GHMT, *shIL33*-GHMT, and treated with DMSO or DAPT, as indicated. Scale bar, 100 μM. Values correspond to the average and s.d.

SUPPLEMENTARY TABLES

Table S1. Chemical Compounds Used in Drug Screening, Related to Figure 1

Compound name	Function	Concentration	Reference
616452	TGF-β R1 kinase inhibitor	2.0 μM	Maherali and Hochedlinger, 2009
DAPT	γ-secretase inhibitor	2.5 μM	Ichida et al., 2014
GO 6983	PKC inhibitor	5.0 μM	Dutta et al., 2011
Vitamin C/L-Ascorbic acid	Demethylate and antioxidant	10 ng/μl	Esteban et al., 2010
OAC2	Oct4 activating compound 2	1.0 μM	Li et al., 2012
JQ1	BRD4 inhibitor	100 nM	Shao et al., 2016
human IL-6	inflammation cytokine	10 ng/ml	Brady et al., 2013

Table S2. Summary of RNA-seq Sequencing Output, Quality Control Filtering and Mapping Results, Related to Figure 2

File	Total	High Quality	HQ percent	Mapped	Mapping ratio	Uniquely mapped	Unique mapping ratio
GHMTDAPT1	37440950	34242269	91.46%	33046876	96.51%	28810041	87.18%
GHMTDAPT2	35286645	32125046	91.04%	30989221	96.46%	27264744	87.98%
GHMTDAPT3	37758885	34381644	91.06%	33198531	96.56%	28950023	87.20%
GHMTDMSO1	39084459	35575097	91.02%	34321515	96.48%	29994042	87.39%
GHMTDMSO2	44057960	40287324	91.44%	38927088	96.62%	34073183	87.53%
GHMTDMSO3	39767244	36303149	91.29%	35040355	96.52%	30634853	87.43%

Table S3. Up-regulated Genes in DAPT-GHMT MEFs, Related to Figure 2

Upregulated genes		
Gene ID	foldChange	log2FoldChange
KCNJ3	3,58	1,80
DHRS7C	2,76	1,43
HSD3B6	2,26	1,17
GTSF1	2,24	1,16
CDK15	2,20	1,12
COX6A2	2,17	1,12
MYH6	2,10	1,07
SERPINB6C	2,12	1,06
PTPRZ1	2,05	1,04
PLN	2,04	1,03
ACTN2	2,04	1,02
GFRA2	2,01	1,00
TOX3	1,98	0,98
CAR14	1,98	0,97
ITGB1BP2	1,94	0,96
KCNH7	1,95	0,95
ACTC1	1,93	0,95
SRL	1,92	0,94
SLC2A6	1,93	0,94
SLN	1,91	0,93
PYGM	1,91	0,93
TLR2	1,91	0,93
CHRM2	1,88	0,91
ATCAYOS	1,88	0,90
ADAMTS18	1,86	0,89
RAMP1	1,85	0,89
FILIP1	1,85	0,89
MYL7	1,85	0,89
4933431K23RIK	1,84	0,87
PRG4	1,82	0,86
CES2E	1,80	0,84
MYL1	1,78	0,83
SMYD1	1,77	0,82
ASB2	1,76	0,82
UNC13C	1,73	0,79
SCG2	1,73	0,79
FBXL22	1,72	0,78
SERPINA3H	1,72	0,78
TNNI3K	1,72	0,78
ICAM1	1,71	0,77
CXCL16	1,71	0,77
CHCHD10	1,70	0,76
SERPINA3B	1,70	0,76
HRC	1,70	0,76
MYO18B	1,69	0,76
ALDH1A1	1,69	0,76
SFRP5	1,69	0,76
TRDN	1,68	0,75
PGAM2	1,68	0,74
SERPINA5	1,68	0,74
GUCY1A3	1,68	0,74
PDGFB	1,66	0,73
MYH11	1,64	0,72
TNNT3	1,64	0,72
RBM24	1,64	0,71
MYLK	1,64	0,71
TMOD1	1,63	0,71
CAV3	1,63	0,71
PPARGC1A	1,63	0,70
MYOM1	1,62	0,70
SERPINA3C	1,62	0,70
COL4A6	1,62	0,69
SPINT2	1,62	0,69
CCL7	1,59	0,67
TNNI3	1,59	0,67
MYOM2	1,59	0,67
MRVI1	1,58	0,66
SPHKAP	1,58	0,66
CCL2	1,58	0,66
GCNT1	1,57	0,65
HAVCR2	1,57	0,65
UNC13B	1,56	0,64
TRIM55	1,55	0,63
SERPIND1	1,55	0,63
PPP1R12B	1,54	0,62
ENO3	1,54	0,62
FAM189A2	1,54	0,62
TRIM63	1,54	0,62
SH3BGR	1,53	0,61
RCSD1	1,53	0,61
TMEM38A	1,52	0,60
DOK4	1,52	0,60
UNC45B	1,51	0,60
GUCY1B3	1,51	0,60
ADCY5	1,51	0,59
MCPT4	1,51	0,59
SGCG	1,51	0,59
PPP1R14C	1,50	0,59

Table S4. Down-regulated Genes in DAPT-GHMT MEFs, Related to Figure 2

Downregulated genes		
Gene ID	**foldChange**	**log2FoldChange**
DIO2	0,31	-1,65
NPR3	0,34	-1,55
FAP	0,41	-1,28
IL33	0,45	-1,15
SRPX2	0,47	-1,09
FAM64A	0,47	-1,08
PLK1	0,50	-1,00
CASC5	0,50	-0,98
CDCA7	0,51	-0,94
ERCC6L	0,52	-0,94
ADAMTS12	0,54	-0,89
C1QTNF3	0,54	-0,87
UPK3B	0,55	-0,86
SHCBP1	0,56	-0,82
FAM105A	0,59	-0,76
WDHD1	0,59	-0,75
FAM20A	0,60	-0,73
MCM5	0,61	-0,72
NR4A2	0,62	-0,69
LOXL3	0,62	-0,68
PRR11	0,62	-0,68
ADAMTS15	0,63	-0,66
LPIN3	0,64	-0,65
PYCR1	0,64	-0,65
SLC2A13	0,66	-0,61
SKP2	0,66	-0,60
SORCS2	0,66	-0,59

SUPPLEMENTARY VIDEOS

Video S1. Spontaneously Beating MEFs at Day 18 Following Reprogramming by GHMT and DAPT.

Video S2. Spontaneously Beating MEFs at Day 18 Following Reprogramming by AGHMT and DAPT.