

Online Supplemental Data

An Unbiased High Throughput Screen to Identify Novel Effectors that Impact on Cardiomyocyte Aggregate Levels

Patrick M. McLendon^{a,†*}, Gregory Davis^a, James Gulick^a, Sonia Singh^a, Na Xu^a, Nathan Salomonis^b, Jeffery D. Molkenin^a and Jeffrey Robbins^{a,*}

From the ^aDivision of Molecular Cardiovascular Biology, the Heart Institute, and the ^bDivision of Biomedical Informatics, Cincinnati Children's Hospital, Cincinnati, Ohio. [†]UES, ^{*}Corresponding authors.

*Correspondence to: Jeffrey Robbins 240 Sabin Way, MLC7020, Cincinnati Children's Hospital, Cincinnati OH, 45229-3039. Email Jeff.Robbins@cchmc.org or Patrick M. McLendon, UES, Inc., 4401 Dayton-Xenia Rd, Dayton, OH 45432. Email: pmclendon@ues.com

Table I: Optimizing shRNA lentivirus Screen

Parameter	Key Variable	Already defined?
Cell or cell line	Transfection efficiency, aggregate formation	Normal mouse neonatal cardiomyocytes
media	Must not interfere with readout or transfection efficiency	Yes
shRNA	concentration must produce effective silencing and limited off-target effects	TBD using 5 separate targets chosen on the basis of our first screen.
Plate format	Either 96 or 384 well plates	96 well plates will probably be used as we have found that more useful for staging and decreasing edge effects
+/- shRNA controls	Present on each plate: should have no/large measurable effect on the readout	The negative control is a mix of 5 scrambled shRNAs. The positive control is two-fold: treatment with Torin1, an mTORC inhibitor that activates autophagy;* and lentivirus containing <i>atg7</i> , whose overexpression we have shown is very effective at decreasing preformed aggregates/PAO [13, 31]
Transfection reagent	Can introduce the shRNA into cells with minimal toxicity	Our shRNA lentivirus have been optimized for this
Transfection reagent incubation time	Sufficient time for the shRNA to be expressed and complex with targets	At least 12 hrs so this will not be an issues for the screen as readouts will be days or even weeks later
Cell volume, number,	Reproducibility and	Automated additions and

addition of reagents	plate to plate and well to well variations	readouts, together with controls on each plate and multiple readouts (25 discrete areas/well) ensure reproducibility
Incubation time for readout reagent	Clearance of CryAB-positive aggregates	A time course with a positive control (eg Torin1) will be carried out to determine the best time to optimize readout while minimizing time and cell death.
Readout methodology	Accuracy and sensitivity	Parameters established during the first screen will serve as a starting point.

*See **Figure 3** for Torin1's effect on aggregate formation

Table II. Validation Screening List of positives

Accession	Gene ID	Accession	Gene ID	Accession	Gene ID	Accession	Gene ID
NM_018829.2	Ap3m1	XM_139298.4	Prkaa1	NM_145840	Rgs9bp	NM_019741	Slc2a5
XM_147046.2	Atr	NM_033593.2	Pcdhga10	NM_009062	Rgs4	NM_022004	Fxyd6
NM_007549.2	Blk	NM_033585.1	Pcdhga2	NM_009173	Siah1b	NM_172371	Slc16a13
XM_125706.5	Bcr	NM_145630.1	Pdk3	NM_177353	Slc9a7	NM_178651	Slc30a9
NM_010238.1	Brd2	NM_012025.3	Racgap1	NM_080450	Gje1	NM_011731	Slc6a20b
NM_007726.1	Cnr1	NM_025346.1	Rmnd5b	NM_028981	Cacna1d	XM_135197	Ptpn23
NM_134002.1	Csnk1g2	NM_172525.1	Arhgap29	NM_009784	Cacna2d1	XM_142281	Ppef1
NM_152809.1	Csnk1g3	NM_025818	1200014J11Rik	NM_009783	Cacna1g	NM_172117	Entpd6
NM_010828.1	Cited2	NM_025564	Magohb	NM_031173	Cacnb1	NM_177242	Pptc7
NM_153568.1	Lrrc66	NM_025620.1	Rep15	NM_019715	Kcmf1	NM_008532	Epcam
NM_201374.1	Ccdc155	NM_025739.2	Rnf220	NM_172695	Plaa	NM_145480	Rfc4
NM_173422.1	Colec10	NM_173023.1	Catsperb	XM_127051	Sos2	NM_175020	Prss58
NM_007573.1	C1qbp	NM_028993	Mau2	NM_134083	Rcbtb2	NM_177474	D19Bwg1357e
NM_013715.1	Cops5	NM_172625.1	Ino80c	NM_028976	Gorasp1	NM_010584	Itln1
NM_172732.1	Clec9a	NM_001005477.1	Barhl2	NM_010400	H60a	NM_010058	Dmwd
NM_172928	Dclk3	NM_033604.1	Rnf111	XM_148373	Cpn2	NM_025330	Hsd17b14
NM_178704.2	Dpy19l3	NM_198251.1	Rnf133	NM_009964	Cryab	NM_025910	Mina
NM_007951.1	Erh	NM_025999.1	Rnf141	NM_026840	Pdgfrl	NM_198305	Klhl17

NM_010142.1	Ephb2	NM_011278.1	Rnf4	NM_145627	Rbm10	NM_001024911	Sept10
NM_007911.3	Efnb3	NM_024182.2	Riok3	NM_144875	Rab71l1	NM_177759	Ccdc60
NM_007907.1	Eef2	NM_016741.1	Scarb1	NM_030211	Kctd18	NM_008537	Amacr
NM_134154	Slc25a45	XM_358897.1	Stk33	NM_053083	Loxl4	NM_207222	Lmo3
NM_010188.3	Fcgr3	NM_028800.2	Stk40	NM_009018	Raet1c	NM_010214	Fhl4
NM_020009.2	mTOR	NM_001037248	Gm4567	NM_008337	lfng	NM_021339	Cdon
NM_008029.1	Flt4	NM_170756.1	Spata2	NM_007843	Defb1	NM_001008705	Bud31
NM_176912	C5ar2	NM_175502.2	Tmem74	NM_028636	Man2c1	NM_013601	Msx2
NM_013559	Hsph1	NM_053084.1	Trim32	NM_010957	Ogg1	NM_172831	E230025N22
NM_010391.1	H2-Q10	NM_030684	Trim34a	NM_009979	Cst9	NM_027350	Nars
NM_015755.1	Hunk	NM_178240.1	Trim50	NM_022315	Smoc2	NM_008996	Rab1
NM_053122.2	Immp2l	NM_053166.1	Trim7	NM_009242	Sparc	NM_019401	Nmi
NM_008380.1	Inhba	NM_053167.1	Trim9	NM_153459	Dusp7	NM_025914	Actr6
NM_008401.1	Itgam	NM_019548.2	Tro	NM_007700	Chuk	NM_025274	Dppa5a
NM_008402.1	Itgav	NM_016788.1	Tnk2	NM_011694	Vdac1	NM_010758	Mag
NM_145467.1	Itgbl1	NM_023230.1	Ube2v1	NM_021514	Pfkm	NM_026845	Ppil1
NM_008354.1	Il12rb2	NM_023585.2	Ube2v2	NM_016681	Chek2	NM_010663	Krt1-17
NM_008355.1	Il13	NM_175309.3	Upk3b	NM_172053	Adamts16	NM_008469	Krt1-15
NM_146145.1	Jak1	NM_011705.2	Vrk1	NM_144871	Suv420h1	NM_032610	Spnb4
NM_021310.1	Jmy	XM_109715.3	Erbp2	NM_207225	Hdac4	NM_010630	Kifc2
NM_010612.2	Kdr	NM_134233	Vmn1r84	NM_008084	gapdh	XM_621314	Dsp
NM_008483.2	Lamb2	NM_019653.2	Wsb1	NM_198030	Hsd17b13	NM_030238	Dync1h1

NM_022964.3	Lat2	NM_178398.2	Wipi2	NM_010880	Ncl	NM_010135	Enah
NM_022801.1	Mark3	NM_199143.1	Znrf2	NM_007999	Fen1	NM_011597	Tjp2
NM_010790.2	Melk	NM_024467.3	Zfp319	NM_007949	Ercc2	NM_001033239	Csta1
NM_152944.1	Mmp21	NM_028259	Rps6kb1	NM_010715	Lig1	XM_489716	UNK
NM_019453.1	Mefv	NM_030720	Gpr84	NM_007503	Fxyd2	XR_406381	LOC102640328
NM_010768.1	Matk	NM_008157	Gpr19	NM_016678	Reck	NM_175020	Prss58
NM_008612.1	Mnat1	NM_181749	Gpr142	NM_009358	Ppp2r5d	NM_177848	9430083122
NM_139300.2	Mylk	NM_007955	Ptprv	NM_030251	Abtb1	NM_001162864	Ttbk1
NM_031375.2	Ngrn	NM_010165	Eya2	NM_010426	Foxf1	NM_001039889	Smoc2b
NM_022029.1	Nrgn	NM_010166	Eya3	NM_177162	Tmprss11g	NM_001201364	Gm10662
NM_199465.1	Nexn	NM_011202	Ptpn11	NM_001101453	Ace3	NM_025647	Cmpk1
NM_146365.1	Olfir1094	NM_012047	Brd7	XM_283777	Cst11	NM_001077529	Nme2
NM_146514.1	Olfir96	NM_146461	Olfir1209	NM_008686	Nfe2l1	NM_008828	Pgk1
NM_147107.1	Olfir974	NM_013528	Gfpt1	NM_007923	Elk4	NM_080560	Ube2n
NM_013887.1	Opn4	NM_198672	Ces3a	NM_009560	Zfp60	NM_001289798	Hadhb
NM_020005	Kat2b	NM_023149	Cndp2	NM_026888	Phkg2	XM_006543380	Gm318
NM_026951	Pex11g	NM_133960	Ces2a	NM_053122	Immmp2l	NM_001033285	Cdc42bpa
NM_011143.2	Pou4f1	NM_010315	Gng2	XM_484053	Taok1	NM_001130477	Srrm1
NM_011192.2	Psme3	NM_001167923.1	Col6a5	XM_487135	Gm5174		

Number of hits are shown as follows: One Clone, **Two Clones**, **Three Clones**