SUPPLEMENTAL MATERIAL TO NMEZI et al.

Development and optimization of a high content analysis platform to identify suppressors of lamin B1 overexpression as a therapeutic strategy for Autosomal Dominant Leukodystrophy

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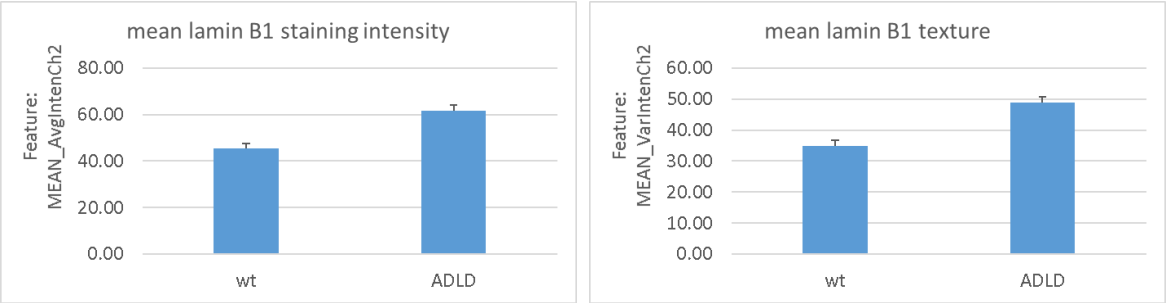
Supplemental Table 1. HCA parameters used to quantify lamin B1-mediated nuclear abnormalities.

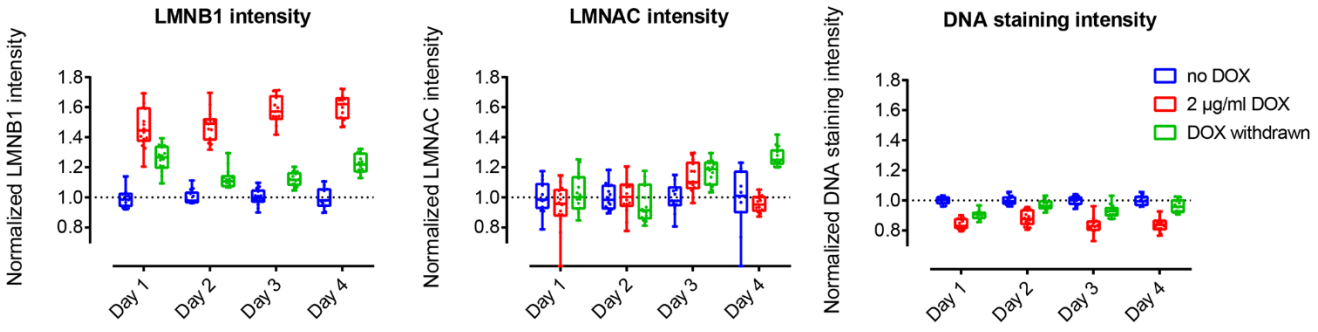
Parameter	Description	Proxy for	S/B	Z-Factor	LDA Loading
Within cell measurements					
Intensities					
MEAN_ObjectAvgIntenCh1	well mean of Hoechst average pixel intensity per object defined by Hoechst staining	DNA staining intensity	1.67	0.30	-36.20
MEAN_ObjectTotalIntenCh1	well mean of Hoechst integrated pixel intensity per object defined by Hoechst staining	DNA content	1.33	-0.25	20.21
MEAN_AvgIntenCh2	well mean of Cy3 average pixel intensity per object defined by Hoechst staining	mean lamin B1 expression	3.93	0.51	-45.31
MEAN_TotalIntenCh2	well mean of Cy3 integrated pixel intensity per object defined by Hoechst staining	total lamin B1 expression	4.89	0.50	42.56
MEAN_AvgIntenCh3	well mean of Cy5 average pixel intensity per object defined by Hoechst staining	mean lamin AC expression	1.36	-1.90	-18.46
MEAN_TotalIntenCh3	well mean of Cy5 integrated pixel intensity per object defined by Hoechst staining	total lamin AC expression	1.06	-14.85	19.25
Intensity distributions					
MEAN_ObjectVarIntenCh1	well mean of Hoechst variance of pixel intensities per object defined by Hoechst staining	DNA texture	1.55	0.11	6.21
MEAN_VarIntenCh2	well mean of Cy3 variance of pixel intensities per object defined by Hoechst staining	Lamin B1 texture	2.95	0.49	-10.16
MEAN_VarIntenCh3	well mean of Cy5 variance of pixel intensities per object defined by Hoechst staining	Lamin AC texture	1.26	-2.96	1.91
Shape					
MEAN_ObjectAreaCh1	well mean of number of pixels per object defined by Hoechst staining	nucleus size	1.26	0.16	-38.15
MEAN_ObjectShapeLWRCh1	well mean of length-to-width ratio per object defined by Hoechst staining	nucleus elongation	1.04	0.17	1.45
MEAN_ObjectShapeP2ACh1	well mean of perimeter-to-area ratio per object defined by Hoechst staining	nucleus shape irregularity (REF 1)	1.05	0.64	-4.97
Cell-to-cell measurements					
Intensities					
SD_ObjectAvgIntenCh1	well standard deviation of Hoechst average pixel intensity per object defined by Hoechst staining	well heterogeneity of DNA staining	1.53	-1.89	-5.23
SD_ObjectTotalIntenCh1	well standard deviation of Hoechst integrated pixel intensity per object defined by Hoechst staining	well heterogeneity of DNA content	1.12	-15.31	1.13
SD_AvgIntenCh2	well standard deviation of Cy3 average pixel intensity per object defined by Hoechst staining	well heterogeneity of mean cellular lamin B1 expression levels	3.31	0.32	-2.20
SD_TotalIntenCh2	well standard deviation of Cy3 integrated pixel intensity per object defined by Hoechst staining	well heterogeneity of total cellular lamin B1 expression levels	4.64	0.13	2.30
SD_AvgIntenCh3	well standard deviation of Cy5 average pixel intensity per object defined by Hoechst staining	well heterogeneity of mean cellular lamin AC expression levels	1.26	-2.65	-1.67
SD_TotalIntenCh3	well standard deviation of Cy5 integrated pixel intensity per object defined by Hoechst staining	well heterogeneity of total cellular lamin AC expression levels	1.19	-3.97	-2.54
Intensity distributions					
SD_ObjectVarIntenCh1	well standard deviation of Hoechst variance of pixel intensities per object defined by Hoechst staining	distribution of DNA textures	1.49	-1.47	2.76
SD_VarIntenCh2	well standard deviation of Cy3 variance of pixel intensities per object defined by Hoechst staining	distribution of lamin B1 textures	4.33	-0.34	-1.34
SD_VarIntenCh3	well standard deviation of Cy5 variance of pixel intensities per object defined by Hoechst staining	distribution of lamin AC textures	1.23	-3.74	0.34
Shape					
SD_ObjectAreaCh1	well standard deviation of number of pixels per object defined by Hoechst staining	distribution of nucleus sizes within wells	1.48	0.22	0.42
SD_ObjectShapeLWRCh1	well standard deviation of length-to-width ratio per object defined by Hoechst staining	distribution of elongated nuclei within a well	1.32	0.10	1.04
SD_ObjectShapeP2ACh1	well standard deviation of perimeter-to-area ratio per object defined by Hoechst staining	distribution of misshapen nuclei within a well	1.55	0.06	-0.38
Heterogeneity Indices (REF 2)					
KS_Norm_AvgIntenCh2	Kolmogorov-Smirnov comparison of the population distribution of AvgIntenCh2 per object with a Normal distribution of the same mean and standard deviation	describes the deviation of a population from a normal distribution	1.67	-1205.00	-0.96
QE_AvgIntenCh2	Quadratic entropy of the population distribution of AvgIntenCh2 per object	describes differences in with and shape of populations	4.00	0.75	4.55
Percent_Outlier_AvgIntenCh2	number of objects with an AvgIntenCh2 that is above the upper inner fence or below the lower inner fence of the box plot	extent of abnormally high or low responses	1.43	-2.55	-0.05

1. Sorokin DV et al., Localized movement and morphology of UBF1-positive nucleolar regions are changed by gamma-irradiation in G2 phase of the cell cycle. Nucleus. 2015;6(4):301-13.

2. Gough AH et al., Identifying and quantifying heterogeneity in high content analysis: application of heterogeneity indices to drug discovery. PLoS One. 2014;9(7):

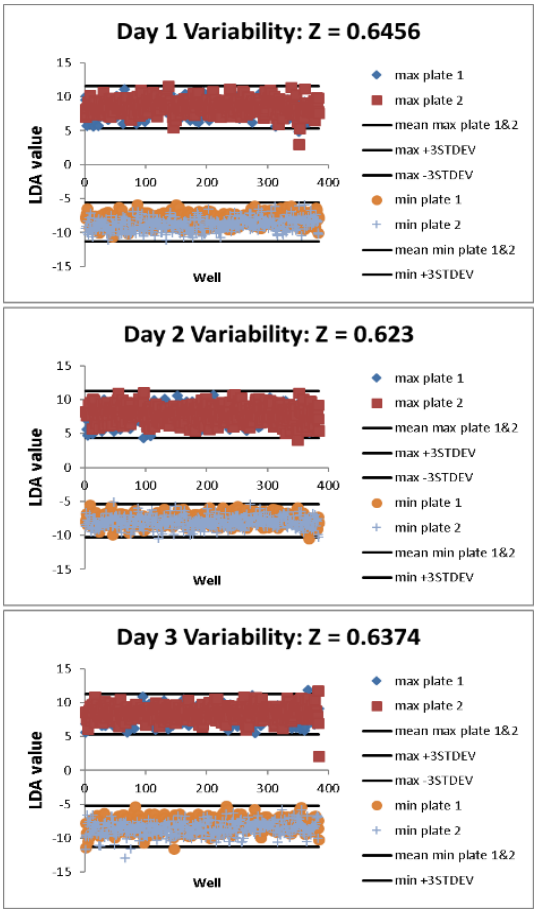


Supplemental Figure 1. Lamin B1 expression and nuclear texture in fibroblasts from ADLD patients and healthy donors. Cells were plated in 96 well plates and immunostained with an anti-lamin B1-FITC primary/secondary antibody pair. Differences in lamin B1 expression and texture were analyzed by image-based analysis on an ArrayScan II high content reader. ADLD patient fibroblasts show higher expression of lamin B1 expression and higher nuclear texture but the differences are small when assessed on a well average level. (n=4, $p<0.001$ by Student's t-test, two sided, unequal variances). Data are from a single donor source of ADLD patient cells and represent mean ± SD of four technical replicates.



Supplemental Figure 2. Lamin B1 expression kinetics after doxycycline withdrawal. Freshly isolated MEF (2,000 per well) were stimulated for three days with doxycycline (DOX), plated in 384 well plates in the presence or absence of DOX, and analyzed every day thereafter for lamin B1/lamin A/C expression and DNA content by high content analysis. *Red symbols*, cells stimulated with 2 µg/ml DOX, *green symbols*, cells stimulated with DOX and replated into DOX-free medium on day 0. Data for each day are normalized to unstimulated cells (blue). Boxes and whiskers show median, second and third quartiles, and range. Data are based on 14 technical replicates from a single experiment that has been repeated once.

Class	Day	Plate	Max/Min	Pass/Fail	Mean	SD	CV	Z
Intra Plate	1	1	max	Pass	8.30	1.05	12.7	
		2	max	Pass	8.60	1.01	11.8	
		3	min	Pass	-8.08	0.84	10.4	
		4	min	Pass	-8.82	0.91	10.4	
		All Plates		Pass				0.65
	2	1	max	Pass	7.76	1.14	14.7	
		2	max	Pass	7.91	1.17	14.7	
		3	min	Pass	-7.63	0.76	9.9	
		4	min	Pass	-8.04	0.82	10.2	
		All Plates		Pass				0.62
	3	1	max	Pass	8.04	0.97	12.1	
		2	max	Pass	8.50	0.95	11.2	
		3	min	Pass	-7.97	0.97	12.1	
		4	min	Pass	-8.57	0.96	11.2	
		All Plates		Pass				0.64
Inter Plate	1	1 and 2	max	Pass	8.45	1.04	12.4	
		3 and 4	min	Pass	-8.45	0.95	11.3	
	2	1 and 2	max	Pass	7.84	1.16	14.7	
		3 and 4	min	Pass	-7.84	0.81	10.4	
	3	1 and 2	max	Pass	8.27	0.99	11.9	
		3 and 4	min	Pass	-8.27	1.01	12.2	
Day To Day	Day 1 And 2	All Plates	max	Pass	8.14	1.14	14.0	
			min	Pass	-8.14	0.94	11.5	
	Day 2 And 3	All Plates	max	Pass	8.05	1.10	13.6	
			min	Pass	-8.05	0.94	11.7	



Supplemental Figure 3. Three day variability assessment. Two full microplates of minimum (unstimulated) and maximum (stimulated) TMRE-MEFs were processed on three separate days. Plates were stained with an anti-lamin B1 antibody and scanned on an ArrayScan VTI high content reader. Twenty seven cellular readouts were aggregated by linear discriminant analysis (LDA) into a single LDA value, which was used to calculate Z-factors and %CV. Table shows numerical results of intra-plate and inter-plate variability; scatter plots illustrate assay performance on each day of experiments.