Table S1. Single-Neuron Genome Amplification Quality and Identity Assessed by SNP Microarray, Related to Figure 2E

(A) SNP microarray allelic dropout (AD) in 3 single neurons compared to bulk cortex and lung tissue from the same individual. Dropout versus cortex corresponds to data shown in Figure S2C.

(B) Fraction of SNP microarray genotypes concordant between all samples confirms the correct identity of the single neurons. A subset of this data corresponds to Figure 2E. Values >0.8 are highlighted.

(C) Fraction of SNP microarray discordant alleles between all samples further confirms the correct identity of the single neurons. The majority of discordant alleles between 1465 single neurons and 1465 bulk DNA are likely due to lack of an MDA-amplified reference model in Affymetrix data analysis software, rather than mutations occurring during amplification which occur at a rate of ~10^{-4}-10^{-5} (Esteban et al., 1993; Hou et al., 2012; Wang et al., 2012). Values ≤ 0.02 are highlighted.

A

Allelic dropout	Reference: 1465-cortex Unamplified Bulk DNA	Reference: 1465-lung Unamplified Bulk DNA
Sample: 1465-cortex Unamplified Bulk DNA		0.00
Sample: 1465-lung Unamplified Bulk DNA	0.00	
Sample: 1465-cortex 1-neuron #6	0.08	0.08
Sample: 1465-cortex 1-neuron #2	0.08	0.08
Sample: 1465-cortex 1-neuron #3	0.09	0.09

B

Fraction concordant genotypes	1465-cortex Unamplified Bulk DNA	4638-cortex Unamplified Bulk DNA	4643-cortex Unamplified Bulk DNA	1465-lung Unamplified Bulk DNA	4638-lung Unamplified Bulk DNA	4643-lung Unamplified Bulk DNA	1465-cortex 1-neuron #6	1465-cortex 1-neuron #2	1465-cortex 1-neuron #3
1465-cortex Unamplified Bulk DNA	1.00								
4638-cortex Unamplified Bulk DNA	0.70	1.00							
4643-cortex Unamplified Bulk DNA	0.70	0.70	1.00						
1465-lung Unamplified Bulk DNA	1.00	0.70	0.70	1.00					
4638-lung Unamplified Bulk DNA	0.71	0.99	0.71	0.72	1.00				
4643-lung Unamplified Bulk DNA	0.72	0.71	1.00	0.72	0.72	1.00			
1465-cortex 1-neuron #6	0.95	0.68	0.68	0.95	0.70	0.70	1.00		
1465-cortex 1-neuron #2	0.95	0.68	0.68	0.95	0.69	0.70	0.96	1.00	
1465-cortex 1-neuron #3	0.95	0.68	0.68	0.95	0.69	0.70	0.96	0.96	1.00

C

Fraction discordant alleles	Reference: 1465-cortex Unamplified Bulk DNA	4638-cortex Unamplified Bulk DNA	4643-cortex Unamplified Bulk DNA	1465-lung Unamplified Bulk DNA	4638-lung Unamplified Bulk DNA	4643-lung Unamplified Bulk DNA	1465-cortex 1-neuron #6	1465-cortex 1-neuron #2	1465-cortex 1-neuron #3
Sample: 1465-cortex Unamplified Bulk DNA	0.00	0.15	0.15	0.00	0.14	0.14	0.02	0.02	0.02
Sample: 4638-cortex Unamplified Bulk DNA	0.14	0.00	0.14	0.14	0.00	0.14	0.16	0.16	0.16
Sample: 4643-cortex Unamplified Bulk DNA	0.15	0.14	0.00	0.15	0.14	0.00	0.16	0.16	0.16
Sample: 1465-lung Unamplified Bulk DNA	0.00	0.15	0.15	0.00	0.14	0.14	0.02	0.01	0.02
Sample: 4638-lung Unamplified Bulk DNA	0.14	0.00	0.14	0.14	0.00	0.13	0.15	0.15	0.15
Sample: 4643-lung Unamplified Bulk DNA	0.13	0.14	0.00	0.13	0.13	0.00	0.15	0.15	0.15
Sample: 1465-cortex 1-neuron #6	0.02	0.17	0.17	0.02	0.16	0.16	0.00	0.01	0.01
Sample: 1465-cortex 1-neuron #2	0.02	0.18	0.17	0.02	0.17	0.16	0.01	0.00	0.01
Sample: 1465-cortex 1-neuron #3	0.02	0.17	0.17	0.02	0.16	0.16	0.01	0.01	0.00

Table S4. Percent Mosaicism in HMG-3 of the AKT3 E17K Mutation, Assessed by Bulk and Single-Cell Sequencing, Related to Figure 7

Mosaicism in bulk samples (bulk tissue extracted DNA, 10,000-, 1,000-, 100-, and 10-cells) was calculated by relative peak heights of the mutant and normal alleles in Sanger sequencing traces, and in some cases also by TOPO cloning of PCR products. Mosaicism in single-cell samples was calculated by direct counting of cells with a detectable mutant allele (corrected for allelic dropout, AD, as described in Extended Experimental Procedures). Tissue location numbers refer to two locations in the tissue separated by ~5cm, and letters represent separate pieces of tissue from each location.

				Sanger allele peak height quantification		TOPO cloning and single-cell experiments			
Tissue-Location	Sample type (sort gate)	MDA amplified	Assessed by	Number of traces	% mutant allele (corrected for background signal in normal controls)	# mutant clones or cells (accounting for AD in single-cell expts.)	Total # clones or cells	**Calculated % mosaicism**	% mosaicism std. error
Blood[1]	Bulk DNA	N	Sanger allele peak heights	1	-0.3			**-0.6**	NA
Brain-location 1a	Bulk DNA	N	Sanger allele peak heights	2	8.6			**17.2**	±0.7
Brain-location 1a[1]	Bulk DNA	N	TOPO cloning			4	23	**34.8**	±15.8
Brain-location 2a	Bulk DNA	N	Sanger allele peak heights	2	14.2			**28.4**	±0.6
Brain-location 2a[1]	Bulk DNA	N	TOPO cloning			4	23	**34.8**	±15.8
Brain-location 1b	10,000-unsorted nuclei	Y	Sanger allele peak heights	4	14.2			**28.5**	±1.4
Brain-location 1b	1,000-unsorted nuclei	Y	Sanger allele peak heights	4	13.1			**26.2**	±2.0
Brain-location 1c	1,000-nuclei (NeuN+)	Y	Sanger allele peak heights	1	11.0			**21.9**	NA
Brain-location 1c	1,000-nuclei (NeuN+)	Y	TOPO cloning			3	29	**20.7**	±11.3
Brain-location 1c	1,000-nuclei (NeuN-)	Y	Sanger allele peak heights	1	8.2			**16.4**	NA
Brain-location 1c	1,000-nuclei (NeuN-)	Y	TOPO cloning			4	24	**33.3**	±15.2
Brain-location 1b	100-nuclei (NeuN+)	Y	Sanger allele peak heights	1	16.5			**33.0**	NA
Brain-location 1b	10-nuclei (NeuN+)	Y	Sanger allele peak heights	5	14.5			**29.0**	±6.4
Brain-location 1b	1-nucleus (NeuN+)	Y	Single-cell mutant allele detection			17	44	**38.6**	±7.3
Brain-location 1b	1-nucleus (NeuN-)	Y	Single-cell mutant allele detection			9	33	**27.3**	±7.8
Brain-location 1b	1-nucleus (large nuclei)	Y	Single-cell mutant allele detection			10	32	**31.3**	±8.2
Brain-location 1b	1-nucleus (wide nuclei)	Y	Single-cell mutant allele detection			8	17	**47.1**	±12.1

[1] From Poduri, et al. (2012)

Table S6. 3’PCR and Full-Length PCR Protocols, Related to Experimental Procedures

Master mix recipes and thermal cycling conditions used for 3’PCR and FL-PCR.

A

3'PCR master mix	**Amount**	**3'PCR program**			
5X GoTaq flexi buffer	4ul	Steps	Temperature	Duration	Cycles
MgCl2 (25uM)	1.2ul	1	95	5min	
dNTP (10uM)	0.4ul	2	95	30sec	
GoTaq Hot Start polymerase (Promega)	0.2ul	3	60	30sec	
AC-22 primer	0.8uM	4	72	1min	
Primer 5'	0.8uM	5			35X
DNA template	5ng	6	72	5min	
Total reaction volume	20ul	7	4	hold	

B

FL-PCR master mix	**Amount**	**FL-PCR program**			
10X LA Taq buffer	2ul	Steps	Temperature	Duration	Cycles
dNTP	3.2ul	1	94	90sec	
LA Taq (Takara Bio)	0.2ul	2	94	20sec	
Primer 5'	0.5uM	3	61	20sec	
Primer 3'	0.5uM	4	68	8:30min	
DNA template	10ng	5			32X
Total reaction volume	20ul	6	68	10min	
		7	4	hold	