

Table 4 (supplemental)

Clone ID Assay \	Gatp2	Gatp1	LRK1	Pges	Ankrd25	DNAJA1	BC013092	Atoh1	BC019960	BC005427	EZH2	Inhbe	BC023323	BC005633	SPINT2
Avg. Fractional Change Conf. – U2OS ^a	3.3	1.5	3.1	4.7	1.3	1.9	1.9	1.3	1.5	3.3	2.5	1.2	1.9	1.7	3.7
Growth in 0.5% Serum – 293 ^b	1.8	1.5	1.8	2.2	2.0	1.7	2.0	1.2	1.7	1.9	1.2	1.2	1.9	1.2	1.5
Growth Advantage in CEF ^c	+	+	+	+	+	+	+	+	+/-	-	-	+/-	+	+/-	+/-
Focus Formation in CEF ^d	+	+	+	+	+/-	-	-	-	-	-	ND	ND	ND	-	+
Soft Agar Assay – CEF ^e	+	+	+	+	+	+	+	-	-	-	+	ND	ND	ND	ND
Effect on Growth by SMARTpool in NIH3T3 – 10% serum ^f	-	-	ND	-	-	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Effect on Growth by SMARTpool in NIH3T3 – 1% serum	-	+	ND	-	-	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND

^a cDNA activities were confirmed in the primary screening assay in quadruplicate. Values reflect average changes in GFP positive cell fraction observed at read 2 relative to read 1 (Fractional Change = % GFP positive fraction Read 2 / % GFP positive fraction Read 1). Data is presented relative to pCMV control (Fractional Change = 1.0).

^b Effects of primary screen hits were evaluated in 293 cells using the Cell-Titer GloTM luminescent cell viability assay. Average normalized values from four independent experiments performed in quadruplicate are shown (pCMV control = 1.0).

^c Growth kinetics of activator-expressing CEF were monitored over 5 days in culture. “+” indicates that stable expression of putative activator clones conferred a growth advantage relative to RCAS-A vector control in at least two independent experiments performed in quadruplicate.

^d Activator clones were evaluated for their ability to induce multi-layered growth in CEF. “+/-” indicates that foci were not observed by crystal violet staining, but morphological changes were consistently observed by light microscopy. ND = not done.

^e The ability of growth activator clones to induce anchorage independent growth was assessed in colony formation assays in soft agar suspension culture.

^f The requirement for hit clones in cell growth was determined in NIH3T3 cells using siRNA oligonucleotide pools (SMARTpool, Dharmacon) to abrogate target gene expression. “+” denotes that a reduction in growth rate was observed in the presence of siRNA in at least four independent experiments performed in quadruplicate.