ONLINE SUPPLEMENTAL MATERIAL

Figure S1. 7-DHC oxysterol mixture reduces Neuro2a cell viability in a dose- and time-dependent manner. Neuro2a cells that were treated with several concentrations (0-100 μM) of the 7-DHC oxysterol mixtures for different periods of time (24-72 hrs). A) The effects of primary oxysterol mixture (0-100 μM, 24-72 hrs). B) The effects of reduced oxysterol mixture (0-100 μM, 24-72 hrs).

Figure S2. Primary and reduced 7-DHC oxysterol mixtures contain compounds with differential effect on cell survival. The upper panels show HPLC-UV fractionation of primary (**A**) and reduced (**B**) 7-DHC oxysterols. The lower panels show cell viability assay at 24 hrs. The numbers on the *x*-axis in the lower panels correspond to the specific HPLC fraction used to treat Neuro2a cells (fractions 3-29 are shown). The *y*-axis is percentage of live cells 24 hrs after treatment with positive values meaning increase and negative values meaning decrease in cell survival relative to untreated control cells. For example, the HPLC fraction #10 in Figure (A) contains endoperoxide 2 and reduces Neuro2a survival more than 50%.

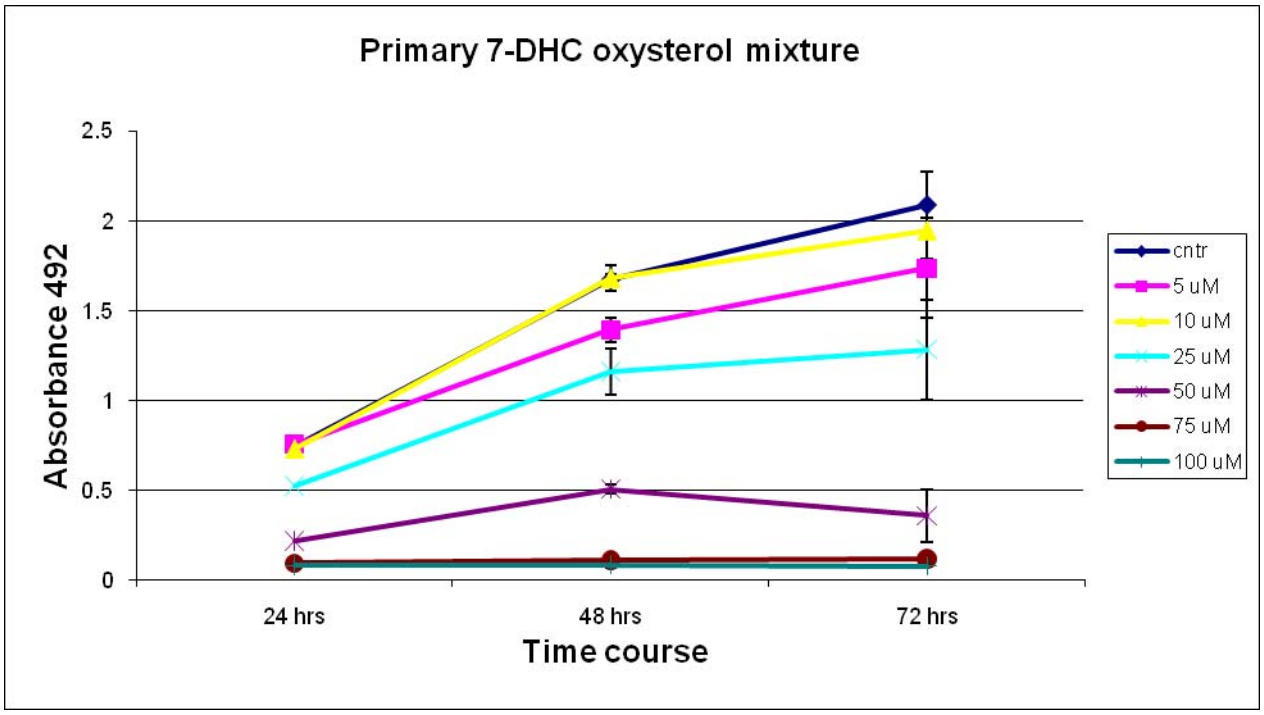
Figure S3. **Cell viability tests on Neuro2a cells with purified oxysterols at different concentrations over 24 and 48 hrs**. Neuro2a cells were treated with purified oxysterols and cell survival was analyzed 48 hrs later. While the Result Section shows cell survival assay for only one concentration (25 μM) of purified individual 7-DHC-derived oxysterols, this section shows 10, 25, and 50 μM. The x-axis shows specific oxysterols and the y-axis shows absorbance at 492.

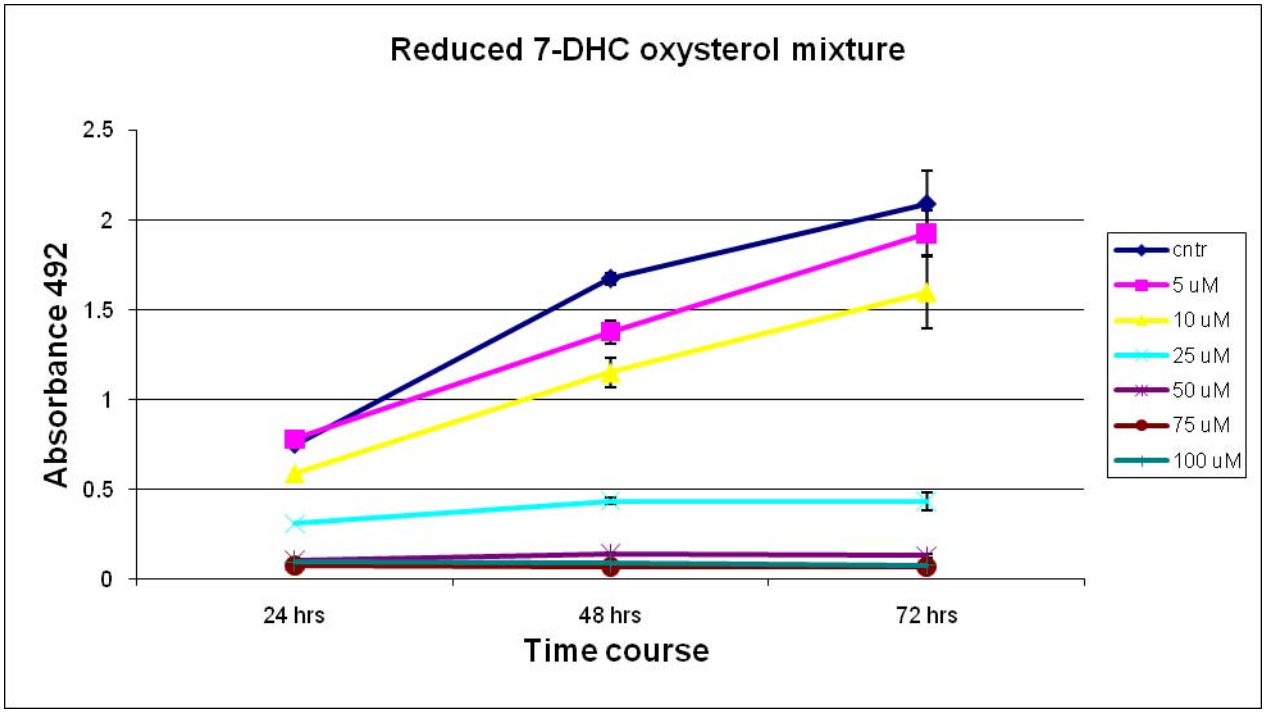
Figure S4. **Both primary and reduced 7-DHC oxysterol mixtures induce differentiation of Neuro2a cells.** After 48 hrs of oxysterol treatment, cells were fixed and processed for

immunocytochemistry using p75 (A, C, E) antibody. Panels on the left show Cy3 fluorescence and the panels on the right show phase contrast of the same field. A and B are control, C and D are treated with 50 μM primary 7-DHC oxysterol mixture, and E and F are treated with 25 μM reduced mixture.

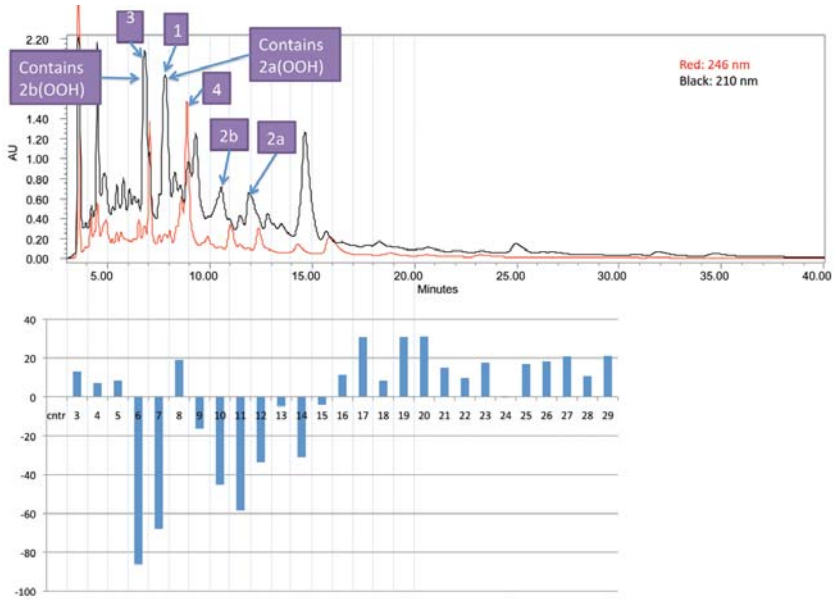
Figure S5. **Gene expression changes induced by 7-DHC oxysterols over 48 hrs**. Genes are plotted on the *x*-axis, and the *y*-axis denotes the average –ΔΔCt from three independent experiments and three independent reverse transcriptions. ΔΔCt was calculated against Tbp1 as a normalizer. Panels on the left show ΔΔCt and panels on the right show same dataset expressed as percentage change.

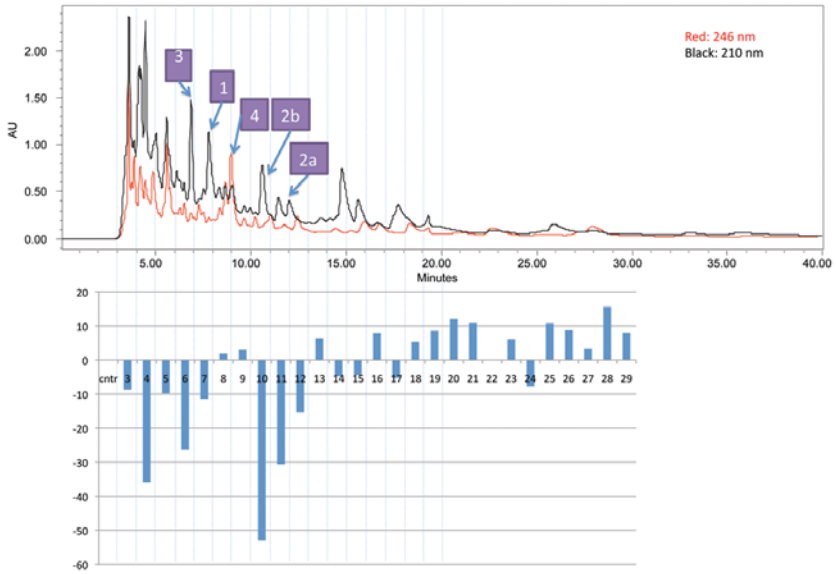
Supplemental Figure 1.



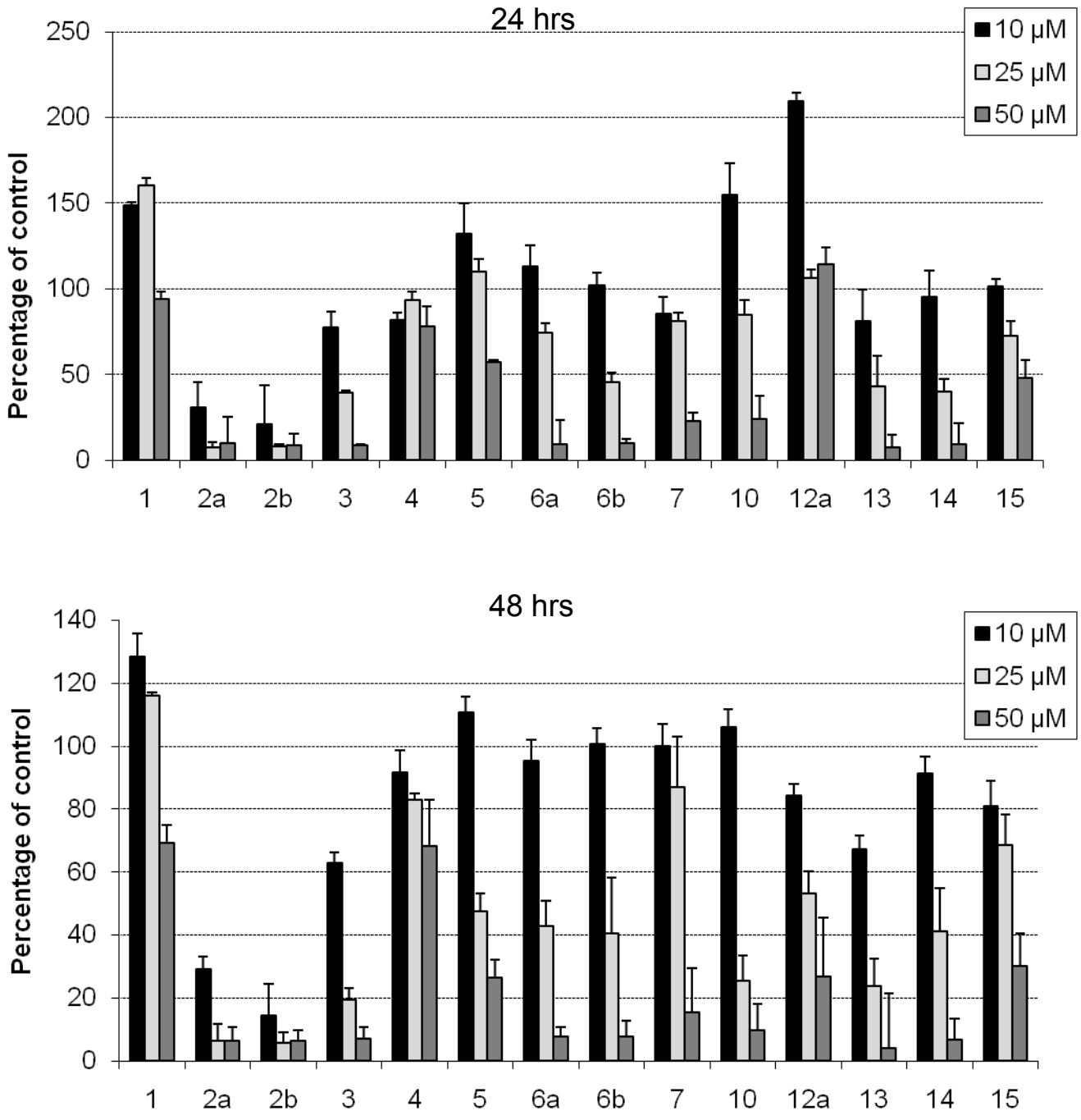


Supplemental Figure 2.

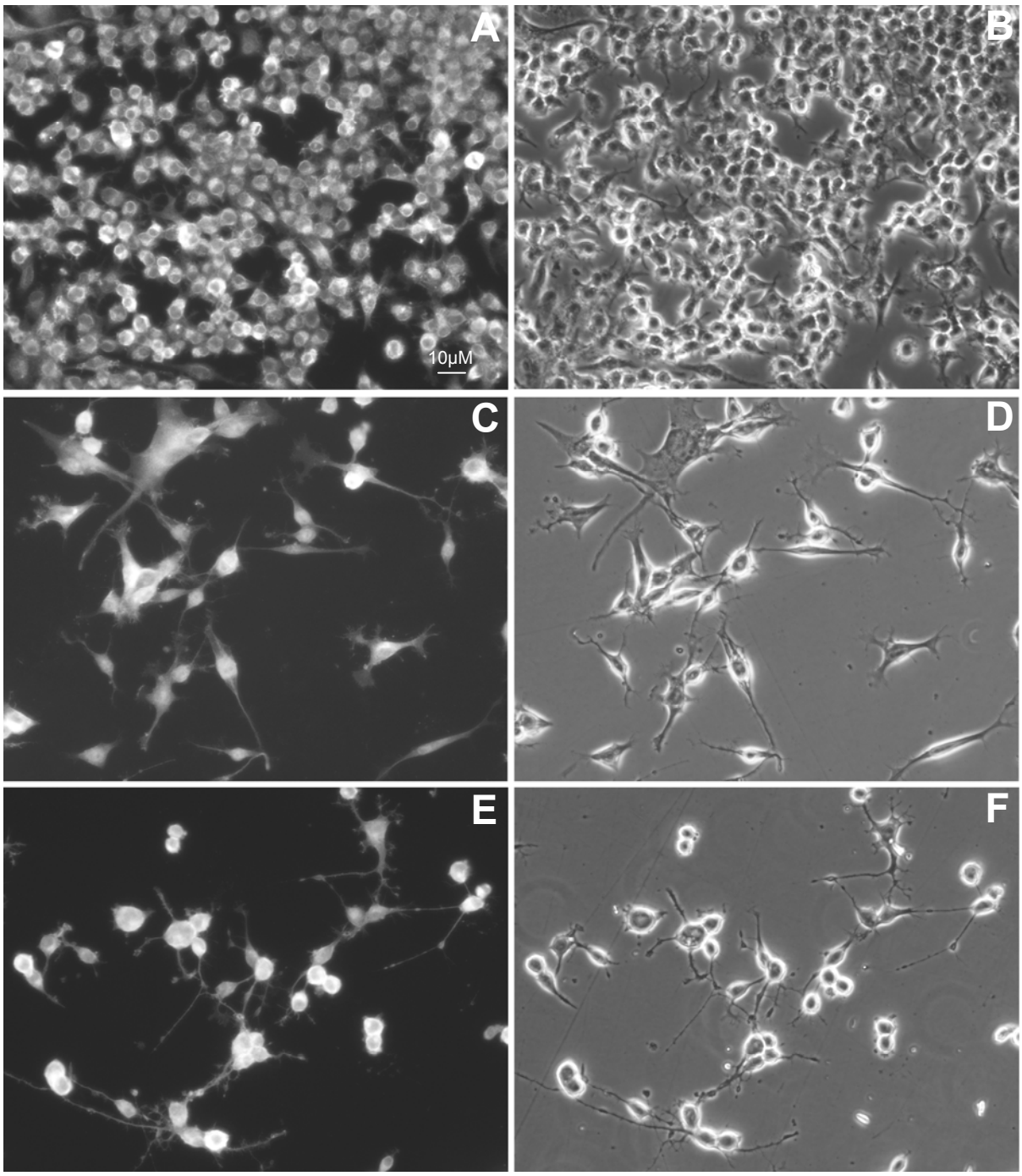




Supplemental Figure 3.

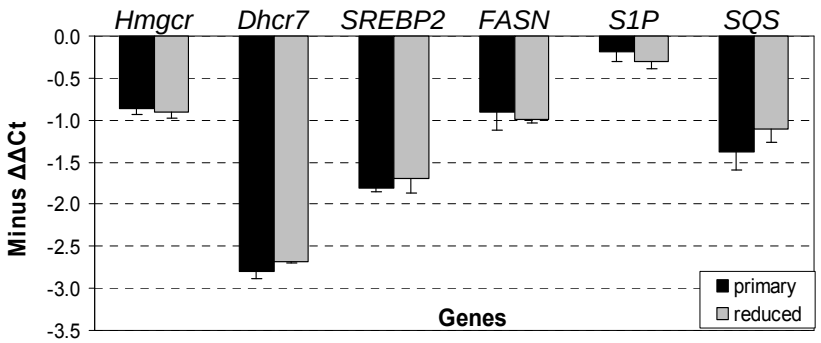


Supplemental Figure 4.

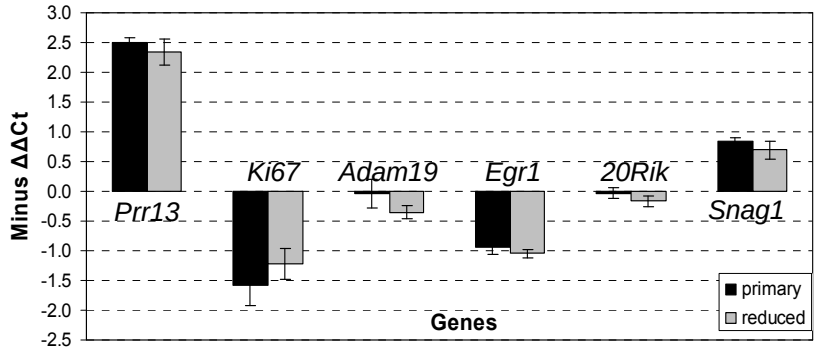


Supplemental Figure 5.

A. Lipid transcripts



B. Other transcripts



C.

Genes % change	Prim. mix	Red. mix	Comp 10
Hmgcr	**-82**	**-87**	*12*
Dhcr7	**-598**	**-542**	**-63**
SREBP2	**-250**	**-223**	*-40*
FASN	**-86**	**-99**	**34**
S1P	*-14*	*-24*	*3*
SQS	**-161**	**-115**	**38**
Prr13	**466**	**406**	**513**
Ki67	**-197**	**-133**	**-631**
Adam19	*-3*	**-28**	*-11*
Egr1	**-91**	**-107**	**-242**
20Rik	*-2*	*-12*	*2*
Snag1	**79**	**62**	**79**