Supplemental Methods

Cell culture

The 4T1 mouse mammary tumor cell line was purchased from ATCC. Cells were cultured in high glucose DMEM supplemented 5% FBS, and antibiotics (100 units/ml penicillin and 100 μg/ml streptomycin) at 37°C in a humidified atmosphere containing 5% CO₂. Except where indicated, analyses were performed on same passage cells within 2 weeks after thawing. All cell lines used in the study were tested and shown to be free of mycoplasma and viral contamination. To further eliminate the possibility of potential contamination with other types of cells, we advantage of the 4T1 cell line resistance to 6-thioguanine. We periodically treat the 4T1 cells by culturing them in 10-cm tissue-culture dishes in RPMI 1640 medium containing 60 μM thioguanine for 7 days.

Isolation and culture of 4T1 from BALB/c mouse lung and tumor

Twenty-five days post-injection of 2x10⁵ 4T1 cells into the mammary fat pads of mice, each mouse was anesthetized with ketamine/xylazine (140/14 mg/kg). Five milliliters of cold DMEM was infused into the lungs via the right ventricle to flush out blood cells. The mice were euthanized, the lungs and tumor tissues removed, and the tissues incubated with 5 ml of collagenase A (1 mg/ml, sigma) for 30 min in a 37°C water bath. After the 30 min incubation, 25 ml of 1× PBS was added to the tube. The resulting tissue/cell suspension was filtered through a 70-μm strainer. The filter was washed with 5 ml of 1× PBS. The filtered cell suspension (~35 ml) was centrifuged for 4 min at 900 rpm. The cell pellet was washed once with complete DMEM by centrifugation, the cell pellet
resuspended in 8 ml of complete DMEM and the cell suspension dispensed into a 25 cm² tissue culture flask. The medium, containing 60 μM thioguanine, was changed every three days until colonies had formed in the medium. At this time, the numbers of colonies were counted using a microscope, and cells recovered from each culture flask were used for FACS analysis and RNA extraction.

**Cell proliferation assay**

4T1 cells (0.5 × 10⁶/ml) were grown in DMEM medium supplemented with L-glutamine, 10% bovine calf serum overnight before harvesting. EdU (Click-iT® EdU Alexa Fluor® 647 flow cytometry assay kit, Invitrogen), at a final concentration of 10 μM, was added to the cell cultures and the cultures incubated at 37°C for an additional 2 h. The cells were then trypsined and washed with Dubecco's phosphate buffered saline containing 1% bovine serum albumin. The cells were FACS analyzed according to the protocol provided by the manufacturer (Invitrogen).

**Western blot assay**

Total cell lysates were loaded in equal protein amounts (50 μg) determined by BCA (Pierce, Rockford, IL). Proteins were resolved by SDS-PAGE (Novex, Invitrogen), followed by transfer onto a nitrocellulose membrane using the iBlot Dry Blotting System (Invitrogen). A previously described method (Liu et al., 2009) was used to process the membranes for western blot analysis. Expression of proteins was determined by probing membranes with antibodies against primary antigens; β-actin antibody served as a
control (Santa cruz). Bound primary antibody was detected with Alex fluor 680 labeled second antibody (Invitrogen).

**Reverse transcription-PCR**

Total RNA was extracted using TRIzol Reagent (Invitrogen) following the manufacturer’s protocol. RNA (500 ng) was reverse-transcribed by random hexamers and SuperScript III reverse transcriptase (Invitrogen) in a volume of 20 μL. The reverse transcription reaction (1 μL) was used as a template in a 20 μL PCR reaction mixture using a primer set specific for the genes of interest (all primers used from RT-PCR are listed in supplemental table 2). An equivalent amount of RNA without reverse transcription served as negative control. As a positive control for amplification from the cDNA, GAPDH and β-actin were used.

**Luciferase reporter assay**

The psiCHECK-2 vector (Promega, Madison, WI) was used to clone the 3'-UTR of mouse TCF4 or Sabt1 mRNA. The vector contains a multiple cloning region downstream of the stop codon of an SV40 promoter-driven Renilla luciferase gene. This allows expression of a Renilla transcript with the 3'-UTR sequence of interest. Renilla luciferase activity is then used to assess the effect of the 3'-UTR on transcript stability and translation efficiency. The psiCHECK-2 vector also contains a constitutively expressed firefly luciferase gene. Firefly luciferase is used to normalize transfections and eliminates the need to transfec a second vector control. TCF4 or Sabt1 mutants were made with the QuikChange® site-directed mutagenesis kit (Stratagene). Each of
the mutants was sequenced to confirm the mutated products. 293T cells were seeded in the wells of a 6-well plate 1 day pretransfection and then each well transfected with a mixture of 200 ng 3′UTR luciferase reporter vectors and 1800 ng of MDH1-PGK-GFP-BIC or miR-223 as a negative control. Twenty-four hours posttransfection, cells were lysed and luciferase activity was measured using a luminometer (Applied Biosystems) and the Dual-Luciferase Reporter Assay System (Promega). The ratio of Renilla luciferase to firefly luciferase was calculated.

**Realtime quantification of microRNA by Stem-looped RT-PCR**

We followed the protocol developed by Tang et al. (2006) (Tang et al.) to measure microRNA expression. Briefly, Primers and Taqman probes were designed according to the sequence published (Tang et al., 2006). Reverse primers used to synthesize the cDNA were mixed to a concentration of 200 nM for each reverse primer. One μl of this premixed reverse primer mixture was used in reverse transcription reaction (working concentration for each reverse primer was 10 nM). SuperScript III First-Strand Synthesis System (Invitrogen) was used to reverse transcribe RNA into cDNA. We followed the protocol supplied by the manufacture with the replacement of the random primer with a reverse primer mixture for microRNA. Real-time PCR was performed using a standard SsoFast™ Probes Supermix (Bio-Rad) protocol on a CFX96™ Real-Time PCR Detection System (Bio-Rad). 15 μl of PCR reaction included 1 μl RT product, 1× SsoFast Probes Supermix, 0.2 μM TaqMan® probe, 1.5 μM forward primer and 0.7 μM reverse primer. The reactions were incubated in a 96-well plate at 95°C for 3 min,
followed by 45 cycles of 95°C for 45 s and 60°C for 30 seconds and 72°C for 45 seconds. All reactions were run in triplicate.

Reference:

Liu Y, Shah SV, Xiang X, Wang J, Deng ZB, Liu C et al (2009). COP9-associated CSN5 regulates exosomal protein deubiquitination and sorting. Am J Pathol 174: 1415-25.

Tang F, Hajkova P, Barton SC, Lao K, Surani MA MicroRNA expression profiling of single whole embryonic stem cells. Nucleic Acids Research 34: e9.

Tang F, Hajkova P, Barton SC, Lao K, Surani MA (2006). MicroRNA expression profiling of single whole embryonic stem cells. Nucleic Acids Res 34: e9.
MDH1-PGK-GFP-BIC

Supplemental Figure 1
Supplemental Figure 2
Supplemental Figure 3
| Probe Set ID | Gene Symbol | mRNA Accession | miR-155 tumor | miR-155 lung | control tumor | control lung |
|--------------|-------------|----------------|---------------|--------------|---------------|--------------|
| 10487588     | Il1a        | NM_010554      | 7.862276      | 8.74137      | 6.312371      | 5.489025     |
| 10349108     | Serpinb5    | NM_009257      | 7.071074      | 7.277857     | 4.361744      | 4.696363     |
| 10499412     | Rab25       | NM_016899      | 8.563591      | 9.371991     | 6.529773      | 7.006712     |
| 10424113     | Mal2        | NM_178920      | 7.25475       | 7.462892     | 5.873856      | 5.365106     |
| 10591522     | Ap1m2       | NM_001110300   | 7.805315      | 8.599117     | 6.077101      | 6.523494     |
| 10478633     | Mmp9        | NM_013599      | 10.07011      | 11.26841     | 8.17927       | 9.564008     |
| 10454172     | Dsg2        | NM_007883      | 7.1663        | 8.022367     | 5.563558      | 6.356278     |
| 10453939     | Lama3       | NM_010680      | 7.129729      | 7.895968     | 5.108028      | 6.239474     |
| 10391066     | Krt17       | NM_010663      | 7.512133      | 7.861708     | 5.196926      | 6.210396     |
| 10377673     | Cldn7       | NM_016887      | 9.129928      | 10.0064      | 7.069467      | 8.373178     |
| 10373467     | Erbb3       | NM_010153      | 6.097069      | 7.534184     | 4.944949      | 5.925632     |
| 10448409     | Prss22      | NM_133731      | 8.821486      | 10.09207     | 7.142976      | 8.552612     |
| 10498361     | Gpr87       | NM_032399      | 5.292837      | 5.025362     | 3.553519      | 3.58846     |
| 10483000     | Itgb6       | NM_001159564   | 7.387773      | 7.364568     | 5.033885      | 5.900703     |
| 10487441     | Mal         | NM_010762      | 8.223093      | 8.906585     | 6.153853      | 7.447147     |
| 10391052     | Krt14       | NM_016958      | 10.79769      | 10.40844     | 7.455003      | 9.119021     |
| 10404270     | Prl3d1      | NM_008864      | 6.421934      | 6.294698     | 5.134795      | 5.023311     |
| 10540472     | Bhlhe40     | NM_011498      | 8.762316      | 9.099463     | 7.564487      | 7.842031     |
| 10511560     | Esrp1       | NM_194055      | 9.041224      | 9.771947     | 6.486841      | 8.516125     |
| 10572635     | Sfn         | NM_018754      | 10.24469      | 10.37906     | 7.349183      | 9.163986     |
| 10420730     | Fdft1       | NM_010191      | 9.934456      | 10.60508     | 8.91749       | 9.401543     |
| 10412909     | Fdft1       | NM_010191      | 9.621805      | 10.36173     | 8.617724      | 9.163013     |
| 10380927     | Grb7        | NM_010346      | 7.614321      | 8.592261     | 6.027615      | 7.394017     |
| 10534395     | Cldn4       | NM_009903      | 10.8441       | 11.27949     | 8.58876       | 10.10292     |
| 10345203     | Paox        | NM_153783      | 8.648898      | 9.361251     | 7.510666      | 8.205264     |
| 10447383     | Epcam       | NM_008532      | 9.710024      | 10.45856     | 7.305713      | 9.319914     |
| 10404649     | Dsp         | NM_023842      | 7.74158       | 8.280395     | 5.965298      | 7.167344     |
| 10391036     | Krt19       | NM_008471      | 10.98239      | 11.24559     | 8.350115      | 10.15476     |
| 10373358     | Il23a       | NM_031252      | 7.782022      | 8.111176     | 6.331398      | 7.033499     |
| 10595070     | Fam83b      | BC120577       | 5.279229      | 5.366312     | 3.828864      | 4.293795     |
| 10352838     | Lamb3       | NM_008484      | 8.92562       | 9.780949     | 6.620803      | 8.71685      |
| 10563377     | Sult2b1     | NM_017465      | 7.162793      | 7.871302     | 6.134899      | 6.81239      |
| Gene Symbol | Gene Name | Accession Number | Mean 1 | Mean 2 | Mean 3 | Mean 4 |
|-------------|-----------|-----------------|--------|--------|--------|--------|
| Tmem184a    |           | NM_001161548    | 6.452454 | 7.158106 | 5.037726 | 6.10215 |
| Gdpd5       |           | NM_201352       | 8.021326 | 8.563687 | 6.938212 | 7.50893 |
| Anxa8       |           | NM_013473       | 8.611521 | 8.448297 | 6.170221 | 7.411107 |
| Fgfbp1      |           | NM_008009       | 6.556523 | 6.991094 | 4.94048 | 5.977429 |
| Sh2d1b1     |           | NM_012009       | 5.116316 | 6.465402 | 3.909429 | 5.46722 |
| Ctsw        |           | NM_009985       | 7.25806 | 7.063102 | 5.280896 | 6.073423 |
| Jup         |           | NM_010593       | 8.998214 | 9.672142 | 7.359496 | 8.694369 |
| Gca         |           | NM_145523       | 7.645609 | 8.027815 | 6.05172 | 7.079015 |
| B4galnt3    |           | NM_198884       | 8.841606 | 9.450405 | 6.764602 | 8.550231 |
| Nuak1       |           | NM_001004363    | 8.388704 | 8.63991 | 6.875921 | 7.736642 |
| Serpinb2    |           | NM_011111       | 6.780425 | 8.162056 | 4.29775 | 5.470006 |
| Cdh1        |           | NM_009864       | 10.06338 | 10.47592 | 7.62315 | 9.58602 |
| Xlr         |           | NM_011725       | 4.105309 | 4.153241 | 2.979558 | 3.26635 |
| Idi1        |           | NM_145360       | 9.99854 | 10.45018 | 8.869165 | 9.58857 |
| Idi1        |           | NM_145360       | 9.807611 | 10.19279 | 8.597445 | 9.338882 |
| Igsf9       |           | NM_033608       | 7.807374 | 8.151856 | 6.166336 | 7.339141 |
| Gadd45b     |           | NM_008655       | 9.712105 | 9.719596 | 7.885831 | 8.923723 |
| Mga4a       |           | NM_173870       | 7.480505 | 8.063474 | 6.370205 | 7.275754 |
| Moxd1       |           | NM_021509       | 7.841602 | 7.940456 | 5.207755 | 7.156369 |
| Sfn         |           | NM_018754       | 9.511454 | 9.550117 | 6.938704 | 8.776926 |
| Sc4mol      |           | NM_025436       | 11.72555 | 12.10862 | 10.66455 | 11.3365 |
| Grhl2       |           | NM_026496       | 7.854454 | 8.638646 | 6.526629 | 7.872598 |
| Sorbs2      |           | NM_172752       | 9.066849 | 8.850523 | 7.57393 | 8.10743 |
| Adamts1     |           | NM_009621       | 9.015309 | 8.791552 | 7.547661 | 8.050974 |
| Atp8b1      |           | NM_001001488    | 5.420444 | 6.32373 | 4.20131 | 5.587331 |
| Gjb4        |           | NM_008127       | 7.92245 | 8.215602 | 6.510492 | 7.482934 |
| Ocln        |           | NM_008756       | 7.038961 | 7.241713 | 5.229377 | 6.510112 |
| Lamc2       |           | NM_008485       | 10.34838 | 11.43348 | 7.674981 | 10.71312 |
| LOC675736   |           | XR_004823       | 5.903382 | 5.780241 | 4.787696 | 5.069325 |
| Tacstd2     |           | NM_020047       | 8.281408 | 8.499058 | 5.847356 | 7.82749 |
| St14        |           | NM_011176       | 9.310294 | 9.906658 | 7.083898 | 9.258477 |
| Hs6st1      |           | NM_015818       | 8.538259 | 8.562196 | 7.512955 | 7.917802 |
| Cxcl16      |           | NM_023158       | 8.767637 | 8.462107 | 6.521483 | 7.826611 |
| Itgb4       |           | NM_001005608    | 8.072096 | 8.969294 | 7.056825 | 8.34855 |
| Gene ID     | Gene Symbol | Accession | FPKM 1 | FPKM 2 | FPKM 3 | FPKM 4 |
|------------|-------------|-----------|--------|--------|--------|--------|
| 10427052   | Krt7        | NM_033073 | 11.42699 | 11.78512 | 8.89132 | 11.16753 |
| 10554752   | Nox4        | NM_015760 | 7.195446 | 7.484468 | 6.10336 | 6.871793 |
| 10403743   | Inhba       | NM_008380 | 8.634395 | 8.796385 | 7.126388 | 8.185844 |
| 10441270   | Ripk4       | NM_023663 | 8.062326 | 8.504858 | 6.308914 | 7.894619 |
| 10415857   | Fam167a     | NM_177628 | 6.47999 | 7.162926 | 5.032036 | 6.558097 |
| 10389816   | Tom11       | NM_028011 | 7.129311 | 7.544214 | 6.031929 | 6.94079 |
| 10568668   | Adam12      | NM_007400 | 7.626019 | 8.137968 | 5.632196 | 7.5417 |
| 10521602   | Cpeb2       | NM_175937 | 6.937238 | 7.05824 | 5.446884 | 6.464688 |
| 10476321   | Prnd        | NM_023043 | 6.40936 | 6.460568 | 4.947845 | 5.875431 |
| 10555460   | Stard10     | NM_019990 | 9.027622 | 9.687662 | 7.428044 | 9.115642 |
| 10456904   | Pstpip2     | NM_013831 | 6.269825 | 6.581832 | 5.160564 | 6.035941 |
| 10490845   | Chmp4c      | NM_025519 | 6.540229 | 7.128209 | 5.134145 | 6.600513 |
| 10344952   | Rdh10       | NM_133832 | 9.805912 | 10.61408 | 8.481194 | 10.09159 |
| 10412517   | Gm10021     | ENSMUST00000090647 | 6.190791 | 6.17341 | 4.876325 | 5.601217 |
| 10433887   | Pkp2        | NM_026163 | 8.147547 | 8.249142 | 6.230099 | 7.734056 |
| 10399202   | Macc1       | ENSMUST00000048880 | 8.040215 | 8.06805 | 5.54322 | 7.558355 |
| 10488060   | Jag1        | NM_013822 | 9.661286 | 9.76732 | 8.127253 | 9.259786 |
| 10532085   | Tgfbr3      | NM_011578 | 5.894358 | 5.775792 | 7.076028 | 6.137863 |
| 10523717   | Spf1        | NM_009263 | 10.97637 | 10.87085 | 12.20369 | 11.24183 |
| 10398091   | Tcl1b2      | NM_013775 | 3.132004 | 3.506363 | 4.194846 | 3.882475 |
| 10537787   | Tas2r135    | NM_199159 | 4.683604 | 4.433482 | 5.928938 | 4.83283 |
| 10479950   | Cugbp2      | NM_001110231 | 8.34535 | 8.22327 | 9.359514 | 8.625966 |
| 10355984   | Serpine2    | NM_009255 | 7.035578 | 6.780555 | 8.995486 | 7.185164 |
| 10503218   | Chd7        | NM_001081417 | 5.777215 | 5.935832 | 6.866033 | 6.355656 |
| 10347915   | Gm7609      | NM_001081746 | 4.326447 | 4.081205 | 6.148087 | 4.523359 |
| 10511180   | Mxra8       | NM_024263 | 6.901103 | 6.044277 | 8.245743 | 6.490046 |
| 10436945   | Slc5a3      | NM_017391 | 9.177926 | 9.279775 | 10.41046 | 9.738855 |
| 10494271   | Ctss        | NM_021281 | 5.654664 | 5.77001 | 7.040477 | 6.249273 |
| 10480714   | Uap1l1      | NM_001033293 | 9.375095 | 9.429919 | 10.51222 | 9.9186 |
| 10476021   | Sirpa       | NM_007547 | 6.710541 | 6.586015 | 7.775871 | 7.076285 |
| 10351206   | Selp        | NM_011347 | 5.083226 | 4.843023 | 6.777507 | 5.353949 |
| 10466712   | Mamdc2      | NM_174857 | 4.097394 | 3.760678 | 5.409154 | 4.275913 |
| Gene ID  | Gene Symbol | Accession | Fc Value 1 | Fc Value 2 | Fc Value 3 | Fc Value 4 |
|---------|-------------|-----------|------------|------------|------------|------------|
| 10489891 | B4galt5     | NM_019835 | 8.647888   | 8.494833   | 9.647607   | 9.013733   |
| 10377725 | Dlg4        | NM_007864 | 6.014519   | 4.948946   | 7.177009   | 5.469262   |
| 10545974 | Antxr1      | NM_054041 | 6.385973   | 6.381103   | 7.531167   | 6.923419   |
| 10431935 | Amigo2      | NM_178114 | 7.227916   | 6.056108   | 8.800455   | 6.601739   |
| 10435271 | Heg1        | NM_175256 | 6.659358   | 6.743164   | 8.667416   | 7.317053   |
| 10372648 | Lyz2        | NM_017372 | 4.954496   | 6.112387   | 7.709282   | 6.74379    |
| 10446763 | Lbh         | NM_029999 | 6.307825   | 6.342218   | 7.391761   | 7.03744    |
| 10539263 | Loxl3       | NM_013586 | 7.699565   | 7.243695   | 8.815554   | 7.995869   |
| 10372410 | Glipr1      | NM_028608 | 5.35116    | 3.950846   | 6.76453    | 4.783089   |
| 10537785 | Tas2r143    | NM_001001452 | 4.396474 | 3.8153 | 5.707376 | 4.699015 |
| 10362596 | Fyn         | NM_001122893 | 6.363218 | 5.636811 | 7.536211 | 6.54344 |
| 10456184 | Apcdd1      | NM_133237 | 7.746409   | 7.793735   | 9.172822   | 8.763932   |
| 10363082 | Lilrb4      | NM_013532 | 3.495683   | 3.83137    | 5.28477    | 4.817242   |
| 10479379 | Slco4a1     | NM_148933 | 6.060866   | 5.480283   | 7.584838   | 6.538604   |
| 10438708 | Masp1       | NM_008555 | 6.563186   | 5.842802   | 7.574843   | 7.070882   |
| 10408693 | F13a1       | NM_028784 | 6.004476   | 6.900375   | 7.186431   | 8.178056   |
| 10538459 | Aqp1        | NM_007472 | 6.059532   | 5.475707   | 7.304596   | 6.996264   |
| 10583071 | Mmp3        | NM_010809 | 9.956114   | 9.530248   | 11.28202   | 11.2808    |
Supplemental table 2  Genes related to promoting macroscopic tumor formation in the lung

| Probe Set ID | Gene Symbol | mRNA Accession | miR-155 lung | control lung |
|--------------|-------------|----------------|--------------|--------------|
| 1047863      | Mmp9        | NM_013599      | 11.26841     | 9.564008     |
| 10453939     | Lama3       | NM_010680      | 7.895968     | 6.239474     |
| 10391066     | Krt17       | NM_010663      | 7.861708     | 6.210396     |
| 10377673     | Cldn7       | NM_016887      | 10.0064      | 8.373178     |
| 10373467     | Erbb3       | NM_010153      | 7.534184     | 5.925632     |
| 10448409     | Prss22      | NM_133731      | 10.09207     | 8.552612     |
| 10483000     | Itgb6       | NM_001159564   | 7.364568     | 5.900703     |
| 10487441     | Mal         | NM_010762      | 8.90658      | 7.447147     |
| 10606058     | Cxcr3       | NM_009910      | 8.391405     | 7.040312     |
| 10391052     | Krt14       | NM_016958      | 10.40844     | 9.119021     |
| 10511560     | Esrp1       | NM_194055      | 9.771947     | 8.516125     |
| 10572635     | Sfn         | NM_018754      | 10.37906     | 9.163986     |
| 10380927     | Grb7        | NM_010346      | 8.592261     | 7.394017     |
| 10534395     | Cldn4       | NM_009903      | 11.27949     | 10.10292     |
| 10447383     | Epcam       | NM_008532      | 10.45856     | 9.319914     |
| 10404649     | Dsp         | NM_023842      | 8.280395     | 7.167344     |
| 10391036     | Krt19       | NM_008471      | 11.24559     | 10.15476     |
| 10352838     | Lamb3       | NM_008484      | 9.780949     | 8.71685      |
| 10535174     | Tmem184a    | NM_001161548   | 7.158106     | 6.10215      |
| 10414065     | Anxa8       | NM_013473      | 8.448297     | 7.411107     |
| 10529819     | Fgfbp1      | NM_008009      | 6.991094     | 5.977429     |
| 10351477     | Sh2d1b1     | NM_012009      | 6.465402     | 5.46722      |
| 10416057     | Clu          | NM_013492      | 11.20894     | 10.21085     |
| 10391103     | Jup         | NM_010593      | 9.672142     | 8.694369     |
| 10448312     | Cldn9       | NM_020293      | 7.472687     | 6.500654     |
| 10487994     | Fermt1      | NM_198029      | 9.105843     | 8.151237     |
| 10472350     | Gca         | NM_145523      | 8.027815     | 7.079015     |
| 10487405     | Prom2       | NM_138750      | 7.374109     | 6.466483     |
| 10547471     | B4galnt3    | NM_198884      | 9.454045     | 8.550231     |
| 10371379     | Nuak1       | NM_001004363   | 8.63991      | 7.736642     |
| 10349157     | Serpinb2    | NM_011111      | 8.162056     | 7.260894     |
| 10575052     | Cdh1        | NM_009864      | 10.47592     | 9.58602      |
| 10606948     | Morc4       | NM_029413      | 9.660115     | 8.838632     |
| 10351801     | Igsf9       | NM_033608      | 8.151856     | 7.339141     |
| 10350697     | Nmnat2      | NM_175460      | 7.384934     | 6.574141     |
| 10364950     | Gadd45b     | NM_008655      | 9.719596     | 8.923723     |
| 10354003     | Mgat4a      | NM_173870      | 8.063474     | 7.275754     |
| 10362186     | Moxd1       | NM_021509      | 7.940456     | 7.156369     |
| Gene Symbol | Accession | Fold Change | Log2 Fold Change |
|-------------|-----------|-------------|-----------------|
| Sfn         | NM_018754 | 9.550117    | 8.776926        |
| Grhl2       | NM_026496 | 8.638646    | 7.872598        |
| Atp8b1      | NM_001001488 | 6.32373 | 5.587331        |
| Gjb4        | NM_008127 | 8.215602    | 7.482934        |
| Ocln        | NM_008756 | 7.241713    | 6.510112        |
| Lamc2       | NM_008485 | 11.43348    | 10.71312        |
| B3gnt3      | NM_028189 | 8.863115    | 8.191089        |
| Tacstd2     | NM_020047 | 8.499058    | 7.82749         |
| St14        | NM_011176 | 9.906658    | 9.258477        |
| Cxcl16      | NM_023158 | 8.462107    | 7.826611        |
| Itgb4       | NM_001005608 | 8.969294 | 8.34855         |
| Krt7        | NM_033073 | 11.78512    | 11.16753        |
| Inhba       | NM_008380 | 8.796385    | 8.185844        |
| Ripk4       | NM_023663 | 8.504858    | 7.894619        |
| Fam167a     | NM_177628 | 7.162926    | 6.558097        |
| Tom11       | NM_028011 | 7.544214    | 6.94079         |
| Adam12      | NM_007400 | 8.137968    | 7.5417          |
| Cpeb2       | NM_175937 | 7.05824     | 6.46468         |
| Prnd        | NM_023043 | 6.460568    | 5.875431        |
| Mpzl2       | NM_007962 | 9.142735    | 8.56112         |
| Gpa33       | NM_021610 | 10.73707    | 10.16309        |
| Stard10     | NM_019990 | 9.687662    | 9.115642        |
| Ngef        | NM_0011111314 | 7.12886 | 6.573993       |
| Pstpip2     | NM_013831 | 6.581832    | 6.035941        |
| Arap2       | NM_178407 | 8.119108    | 7.575779        |
| Il18rap     | NM_010553 | 8.351473    | 7.819983        |
| Elf3        | NM_007921 | 6.984908    | 6.45523         |
| Chmp4c      | NM_025519 | 7.128209    | 6.600513        |
| Rdh10       | NM_133832 | 10.61408    | 10.09159        |
| Pkp2        | NM_026163 | 8.249142    | 7.734056        |
| Macc1       | ENSMUST00000048880 | 8.06805 | 7.558355       |
| Jag1        | NM_013822 | 9.76732     | 9.259786        |
| Serpine2    | NM_009255 | 6.780555    | 7.185164        |
| Pdgrf     | NM_001146268 | 5.044792 | 5.468457       |
| Plekho1     | NM_023320 | 6.407226    | 6.837128        |
| Gm7609      | NM_001081746 | 4.081205 | 4.523359       |
| Mxra8       | NM_024263 | 6.044277    | 6.490046        |
| Fkbp7       | NM_010222 | 4.790682    | 5.256632        |
| Ctss        | NM_021281 | 5.77001     | 6.249273        |
| Pla2g7      | NM_013737 | 7.267674    | 7.760242        |
| Selp        | NM_011347 | 4.843023    | 5.353949        |
| Gene ID    | Symbol | Accession    | Fold Change 1 | Fold Change 2 |
|------------|--------|--------------|---------------|---------------|
| 10466712   | Mamdc2 | NM_174857    | 3.760678      | 4.275913      |
| 10377725   | Dlg4   | NM_007864    | 4.948946      | 5.469262      |
| 10431935   | Amigo2 | NM_178114    | 6.056108      | 6.601739      |
| 10482448   | Zeb2   | NM_015753    | 6.101752      | 6.661224      |
| 10435271   | Heg1   | NM_175256    | 6.743164      | 7.317053      |
| 10501608   | Vcam1  | NM_011693    | 6.620647      | 7.237806      |
| 10379511   | Ccl2   | NM_011333    | 5.247929      | 5.871085      |
| 10372648   | Lyz2   | NM_017372    | 6.112387      | 6.74379       |
| 10480249   | Ptpla  | NM_013935    | 6.603763      | 7.242373      |
| 10548879   | Mgp    | NM_008597    | 6.245695      | 6.983867      |
| 10539263   | Loxl3  | NM_013586    | 7.243695      | 7.995869      |
| 10494445   | Lix1l  | ENSMUST00000062058 | 6.981831 | 7.748259 |
| 10372410   | Glipr1 | NM_028608    | 3.950846      | 4.783089      |
| 10600169   | Bgn    | NM_007542    | 4.606348      | 5.485571      |
| 10537785   | Tas2r143 | NM_001001452 | 3.8153        | 4.699015      |
| 10362596   | Fyn    | NM_001122893 | 5.636811      | 6.54344       |
| 10479379   | Slco4a1| NM_148933    | 5.480283      | 6.538604      |
| gene symbol | Gene name                                                | Conserved sites | Poorly conserved sites | 4T1 miR-155 tumor | 4T1 Control tumor |
|------------|----------------------------------------------------------|----------------|------------------------|-------------------|-------------------|
| Hhip       | Hedgehog-interacting protein                            | 1              | 0                      | 7.850783          | 9.039088          |
| Rps6ka5    | ribosomal protein S6 kinase, polypeptide 5               | 1              | 1                      | 6.116796          | 7.045661          |
| Satb1      | special AT-rich sequence binding protein 1               | 1              | 0                      | 5.097334          | 5.839266          |
| Trp53inp1  | transformation related protein 53 inducible nuclear protein 1 | 1              | 0                      | 5.269367          | 5.955752          |
| Nfe2l2     | nuclear factor, erythroid derived 2, like 2              | 1              | 0                      | 8.453485          | 9.11393           |
| Dhx40      | DEAH (Asp-Glu-Ala-His) box polypeptide 40                | 1              | 0                      | 7.506214          | 8.119512          |
| Cebpb      | CCAAT/enhancer binding protein (C/EBP), beta             | 1              | 0                      | 7.525149          | 8.136402          |
| Tcf4       | transcription factor 4                                   | 3              | 0                      | 8.634457          | 9.195979          |
| Olfml3     | olfactomedin-like 3                                      | 1              | 0                      | 5.45425           | 6.006043          |
| Jhdm1d     | jumonji C domain-containing histone demethylase 1 homolog D (S. cerevisiae) | 1              | 2                      | 5.213175          | 5.741126          |
| Trps1      | trichorhinophalangeal syndrome I (human)                 | 2              | 0                      | 9.238857          | 9.759003          |
| Far1       | fatty acyl CoA reductase 1                               | 1              | 0                      | 9.683145          | 10.20003          |
| D5Ertd579e | DNA segment, Chr 5, ERATO Doi 579, expressed             | 1              | 0                      | 6.802934          | 7.290513          |
| Zfp518     | zinc finger protein 518                                  | 1              | 2                      | 4.868196          | 5.348782          |
| Trim2      | tripartite motif-containing 2                            | 1              | 1                      | 5.653317          | 6.118649          |
| Hivep2     | human immunodeficiency virus type I enhancer binding protein 2 | 1              | 1                      | 6.613888          | 7.07383           |
| Mxi1       | Max interacting protein 1                                | 1              | 0                      | 7.277717          | 7.728243          |
| Nfia       | nuclear factor I/A                                       | 1              | 3                      | 7.730001          | 8.169153          |
| Tfdp2      | transcription factor Dp 2                                | 1              | 1                      | 7.223826          | 7.643182          |
| Arid2      | AT rich interactive domain 2 (Arid-rfx like)             | 2              | 0                      | 8.078683          | 8.495247          |
| Ski        | ski sarcoma viral oncogene homolog (avian)              | 1              | 0                      | 8.118014          | 8.528252          |
| Sema5a     | sema domain, seven thrombospondin repeats (type 1 and type 1-like), transmembrane domain (TM) and short cytoplasmic domain, (semaphorin) 5A | 1              | 1                      | 5.069741          | 5.427424          |
| Name | Sequence | Description |
|------|----------|-------------|
| BIC100XhoI | ctgctcgagtggttaagttgcataccc | For constructing MDH1-PGK-GFP-miR-155 vector |
| BIC553EcoRI | ctggaatttcgattcttttgattctg | For constructing MDH1-PGK-GFP-miR-155 vector |
| Mus TCF4 3UTR | gtctcgagAGAGGTACACCGGTTAGAAT | For constructing psicheck2 vector containing TCF4 3UTR. |
| Mus TCF4 3UTR Re | gtgcgcccgcTGCTGATAGGTTAGGAAAA | For constructing psicheck2 vector containing TCF4 3UTR. |
| Mus TCF4 mut1 | tcatgcagcaattaaacagcttataagcct | For constructing psicheck2 vector containing TCF4 3UTR with site one mutated. |
| Mus TCF4 mut1 Re | tgtaattgctgcgatgtggttggca | For constructing psicheck2 vector containing TCF4 3UTR with site one mutated. |
| Mus TCF4 mut2 | cctaaagcataaggctttgagg | For constructing psicheck2 vector containing TCF4 3UTR with site two mutated. |
| Mus TCF4 mut2 Re | gcaacaatttgaatgtaagaaagttataagg | For constructing psicheck2 vector containing TCF4 3UTR with site two mutated. |
| Mus Satb1 3UTR F | CTCGAG agccacaccacggtattcct | For constructing psicheck2 vector containing Sabt1 3UTR. |
| Mus Satb1 3UTR Re | GCGGCCGC gtcgaggtacctgagaagaattta | For constructing psicheck2 vector containing Sabt1 3UTR. |
| Mus satb1 mut F | ctcaataagcaATTAactttcgtttatag | For constructing psicheck2 vector |
**Oligos for ShRNA**

| Oligo Type | Sequence |
|------------|----------|
| mTCF4 ShRNA 1 | `tcgagcccGCCATGAGGTACAGACAAAAGTTTCAAGAGACTTTGCTGTACCTCCATGGCTTTT TG`<br>`aattcaaaaaGCCATGAGGTACAGACAAAAGTTTCAAGAGACTTTGCTGTACCTCCATGGCCggg c` |
| mTCF4 ShRNA 2 | `tcgagcccGGACGATGCGATTCATGTTCTTTCAAGAGAAGACATGAATCGCATCGTCCTTTT TG`<br>`aattcaaaaaGGACGATGCGATTCATGTTCTTTCAAGAGAAGACATGAATCGCATCGTCCTCCggg c` |
| mSatb1 ShRNA 1 | `tcgagcccGCACACCACAGTAAGGAATGCTTCAAGAGAGCATTCTACTGTGGTGTGCTTTT TG`<br>`aattcaaaaaGCACACCACAGTAAGGAATGCTTCAAGAGAGCATTCTACTGTGGTGTGCggg c` |
| mSatb1 ShRNA 2 | `tcgagcccGGAGAGCAATGCTGTGCATCATTCAAGAGATGACTCGACAGCATTGCTCTCTTTT TG`<br>`aattcaaaaaGGAGAGCAATGCTGTGCATCATTCAAGAGATGACTCGACAGCATTGCTCTCCggg c` |
| Gene name | Primers                      | Product length (Base pair) |
|----------|------------------------------|----------------------------|
| **Cox-2**| Forward: 5’-TGA GTA CCG CAA ACG CTT CT -3’<br>Reverse: 5’-CTC CCC AAA GAT AGC ATC TGG -3’ | 169                        |
| **Grb7** | CCACTGACTTCCGGCTTCT<br>TGTGTTGTCTTCTCTCTCC | 337                        |
| **Erbb2**| AGAAATCCTAGATGAAGCGTAC<br>CCTGTGAACAAAGCGGACT | 238                        |
| **Erbb3**| TGCCAAAGGTCCAATCTA<br>AGTCCCTACTGTCACCGCTAT | 165                        |
| **BIC/mir-155** | 5’-cgg aga tta ctt ta TAT CCC TTA TCC TCT GCC-3’<br>5’-cgg aag ctt GACTTGCTATCCCTCCAC-3’ | 193                        |
| **Grhl2**| TTTGTCACACACCCGCTTA<br>CACTGGCAGCCCATACTT | 162                        |
| **GAPDH**| GTTGTCTCCTGCGACTTTA<br>GGTGTGCGGCTATCTTA | 184                        |
| **Satb1**| AGGACCCTGTGGGAGAAC<br>TTGGACATGGATGATGTG | 138                        |
| **Apcdd1**| TCGCACCCTCCGGCTCTTA<br>CGTGTGCTGCCCATAGTAAA | 242                        |
| **CSF3**| CAGGCTCTATCGGCTATTT<br>GGAAGCCAGAAATGGAAGG | 166                        |
| **Vcam1**| CAGATAGACAGCCCACTA<br>AGCCTGTAAACTGGGTAA | 260                        |
| **Tgfbr3**| CGTGCTGGAGGTGGTGTG<br>TGTCTGGGTGTTGATGTG | 256                        |
| **VEGF-A**| 5’-AGTCCCATGATGATCCATCGTGTCATGATCATTCA-3’<br>5’-ATCCCGGATGATCTGCTGTGGAGG-3’ | 219                        |
| **MMP-9**| 5’-CTCCTGATGCCACTTCTACT-3’<br>5’-AACACACAGGGTTTCGGTTTC-3’ | 220                        |
| **SOCS1**| 5’-CTCCGCTAATACCTGAGTTCCCT-3’<br>5’-ATCTCACCCTCCACAACC-3’ | 172                        |
| **SOCS3**| 5’-TTCACGGCAGTTCCCAACCATC-3’<br>5’-GCTCCAGTGAATCCGCTCT-3’ | 345                        |
| **Tgfbr2**| TTTCCGAAGAATACACCC<br>GGACACGGGTACGAGGAA | 129                        |
| **ST14**| CTCAGAGCCAAAGCGTGAATG<br>GCGAGGCTTGGTGGAA | 227                        |
| **Skil**| GAGCAGGCCAACGGAAC | 197                        |
| Gene      | Sequence                                      | Length |
|-----------|-----------------------------------------------|--------|
| Satb1     | AGGACCCCTGTGGGAGAACTTGGAACTGGATGGTG           | 138    |
| Jag1      | CAGGTCTTACCACCGAACAAGACCGCTCTGAACCTCTG       | 268    |
| Inhba     | CTAACGAAACCAGGACCAATCTCCTTCGAGATGAATGAG     | 157    |
| Epcam     | GATTCCTGTCTTGCATTTTCTCTCCTGCTTTATCTCAGGCTCTC | 250    |
| Bhlhe40   | ATGAACCCAGACAAGATACCTGAGCTCTGGAATGATGGAG    | 171    |
| HMGA2     | TCCCGTCTCCCGGGAAAGGTAGGGCTCATGAGTTGGATC    | 232    |
| ANGPTL4   | AAGGATGCTGTCCGAGATTATGAGCTGAATGAGGAATG     | 144    |
| Itga2     | AGCCCGTGATCTTTCTCTACACCCGGCATTCATTG         | 185    |
| Zeb1      | CCATACGAATGCGCGCAACTACCAAGGGCAACCCGCAAT    | 386    |
| Fibronectin1 | GCGGAAATGTGATGAGAAAGGGGAATGACCTCCTG        | 415    |
| Cadherin1 | GTCTCCTCTTGGGCTTCTACTAGGGAGGAGTGCAGTGCCTG  | 228    |
| Vimentin1 | CGGCTGCCACTTCCGTCCGCAACCACTGCTGAAATGAGGCGA | 343    |
| Twist1    | AGCGGTGTACCAACGAAGTCGACGTGAAAGTACG         | 162    |
| Snai2     | TCCCATTAGTGACGAAGAAGCCAGGCTACATCCGATTCAT   | 204    |
| Snai1     | GTCGGCTCTTCTCAGGCCATGGTATCTTCTGTACTG       | 391    |
| TCF4      | CACAACGAGCGATGGGTAGGGGTAGGGGTCGCGCTG      | 195    |
| pri-miR-141 | GTCCAGTTCTCAGCGGATGTCGCTGTTGCTTAAATGAG   | 185    |
| pri-miR-200a | CCGAAGAAGGCTGAGTTTACTTCTGCAGTGTACGAT      | 201    |
| pri-miR-429 | ACTGCACTGTGAGATGATGATGACTGAGGATG        | 170    |
| AK014686  | AGCAGGTTGGCTTTCAAGGACGAAGGCTCAGGGTTCAGGCAG | 241    |
| Gene          | Sequence                  | Length |
|--------------|---------------------------|--------|
| pri-miR-200c | TGATCTTGAAGGTGGACTGG       | 284    |
|              | CACTGGATTGGAGGAGGG        |        |
| Zeb2         | GAGCTTGACCACCGACTC        | 223    |
|              | TTGCAGGACTGCCTTGAT        |        |
| pri-miR-200b | tgcagggcttctgtgtgtc       | 201    |
|              | cacccatatgccaaggactaact   |        |
| IL-1α        | TCTGCCATTGACCATCTC        | 184    |
|              | GAATCTTCCCGTTGCTTG        |        |
| beta-actin   | CTGTCCCTGTATGCCTCTG       | 218    |
|              | ATGTCACGCAACGATTTC        |        |