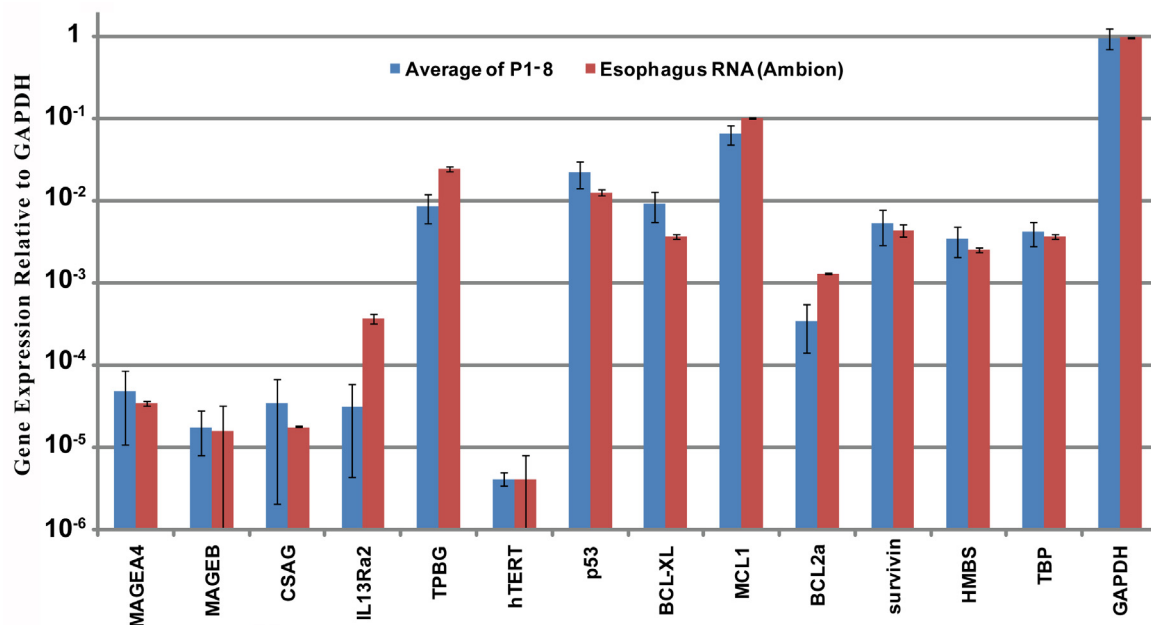
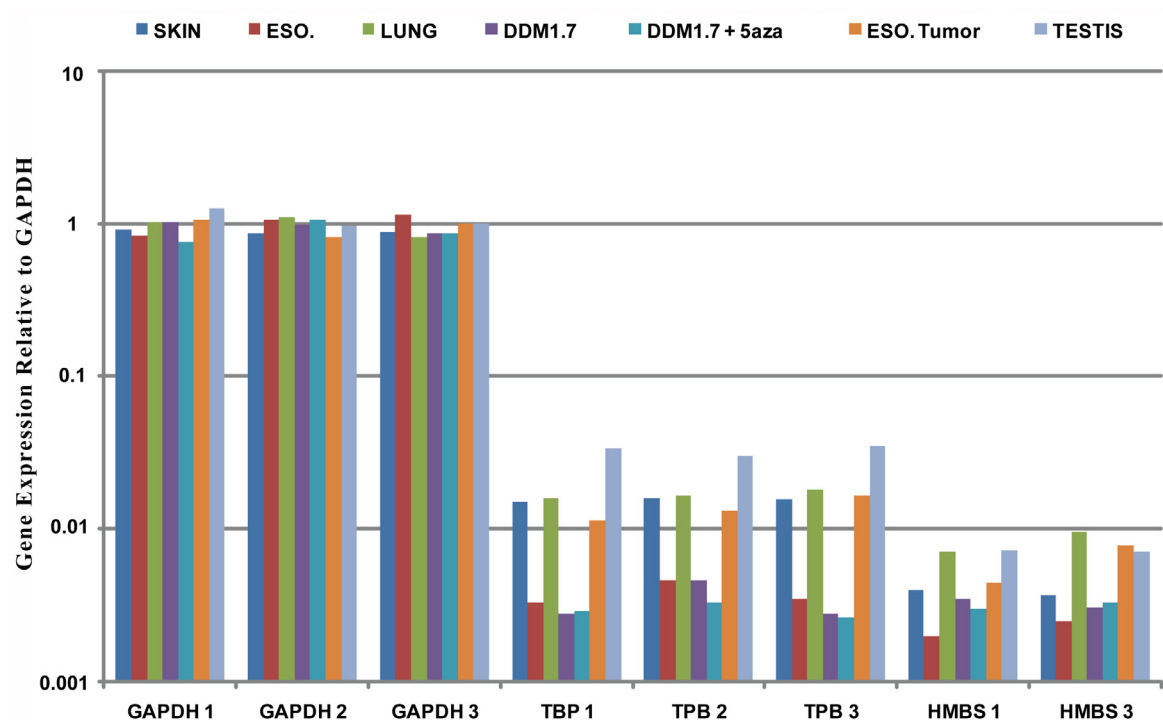


Supplementary Figure 1.



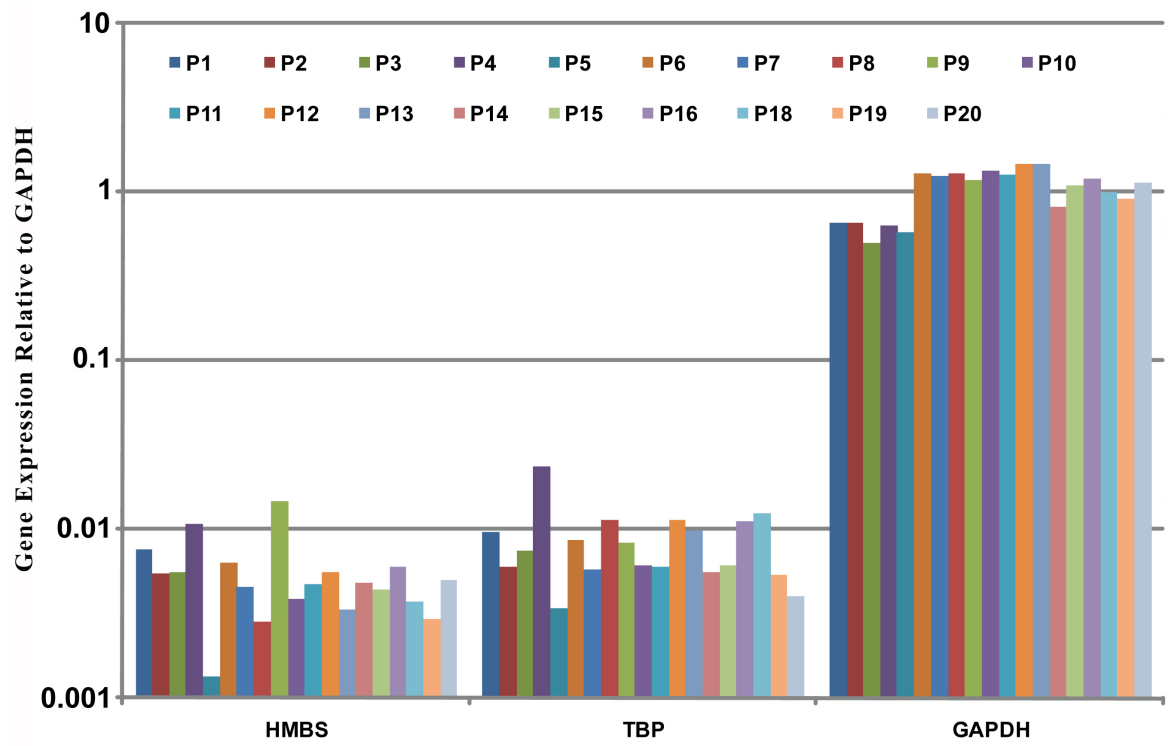
Relative gene expression in normal esophagus tissue. Gene expression is shown relative to GAPDH expression. Average of P1-8 indicates the average expression values determined by assaying normal tissue biopsies from patients 1-8, error bars show the standard deviation between patient biopsies. Esophagus RNA was purchased from Ambion (Austin, TX, USA); error bars show the standard deviation between reaction duplicates.

Supplementary Figure 2.



Housekeeping gene expression relative to GAPDH. QPCR of 74 genes was performed in three separate reaction runs for each of the samples shown in the above figure. Housekeeping gene expression was measured in each run; GAPDH1 is from run #1, GAPDH2 from run #2 and so on. Gene expression is shown relative to a GAPDH standard curve, therefore GAPDH is not exactly equal to 1.

Supplementary Figure 3.



Housekeeping gene expression in 19 tumor biopsies. Gene expression is shown relative to GAPDH expression where GAPDH expression is equal to 1. P1 to P20 corresponds to the patient tumor biopsy analyzed.

Supplementary Table 1. Analysis of tumor antigen gene expression by QPCR.

Gene	SKIN	ESO.	LUNG	DDM1.7	DDM1.7 +5aza	ESO. Tumor	TESTIS
MAGEA1 (CT1.1)	N.P.	N.P.	N.P.	2.00E-03	2.66E-03	1.07E-02	4.95E-03
MAGEA2 (CT1.2)	N.P.	N.P.	N.P.	9.67E-03	8.28E-03	9.55E-03	7.79E-03
MAGEA3 (CT1.3)	N.P.	N.P.	N.P.	7.91E-02	5.56E-02	4.94E-02	3.44E-02
MAGEA4 (CT1.4)	4.78E-05	1.29E-05	6.62E-05	4.09E-05	5.23E-04	2.27E-02	3.67E-02
MAGEA6 (CT1.6)	N.P.	N.P.	N.P.	2.21E-02	2.19E-02	4.37E-02	3.77E-02
MAGEA9 (CT1.9)	N.P.	N.P.	1.02E-05	2.39E-06	4.01E-04	1.95E-02	1.35E-02
MAGEA10 (CT1.10)	N.P.	N.P.	N.P.	2.17E-05	3.07E-04	1.29E-02	5.55E-03
MAGEA12 (CT1.12)	N.P.	N.P.	N.P.	3.04E-02	2.35E-02	1.96E-02	7.44E-03
MAGEB (CT3)	1.57E-05	2.86E-06	9.17E-06	2.05E-03	3.91E-03	1.55E-02	6.99E-02
MAGEB1 (CT3.1)	N.P.	N.P.	N.P.	7.19E-06	2.94E-04	7.12E-05	3.85E-02
MAGEB2 (CT3.2)	5.12E-06	N.P.	N.P.	3.72E-03	6.45E-03	3.17E-02	1.01E-01
MAGEB3 (CT3.?)	N.P.	N.P.	N.P.	N.P.	1.71E-05	4.31E-05	9.93E-03
MAGEB4 (CT3.?)	N.P.	N.P.	N.P.	N.P.	N.P.	N.P.	3.30E-03
MAGEB6 (CT3.4)	N.P.	N.P.	N.P.	5.41E-06	1.72E-04	1.30E-04	4.42E-03
MAGEB18 (CT3.?)	N.P.	N.P.	N.P.	N.P.	4.25E-05	N.P.	2.57E-03
MAGEC1 (CT7.1)	N.P.	N.P.	N.P.	N.P.	1.30E-03	3.99E-03	2.58E-02
MAGEC2 (CT10)	N.P.	N.P.	N.P.	N.P.	2.21E-03	1.04E-02	7.53E-02
MAGEC3 (CT7.2)	4.34E-05	N.P.	N.P.	5.00E-06	1.35E-05	5.16E-05	1.22E-03
GAGE (CT4)	N.P.	N.P.	N.P.	1.33E-04	1.38E-03	1.98E+00	7.02E-01
BAGE (CT2)	N.P.	N.P.	N.P.	1.00E-04	7.53E-04	5.01E-04	5.69E-03
SAGE (CT14)	N.P.	N.P.	N.P.	4.81E-04	7.59E-04	2.63E-03	7.09E-03
HAGE (CT13)	9.68E-05	N.P.	1.20E-03	N.P.	2.41E-04	2.46E-03	1.97E-02
PAGE (CT16)	1.10E-04	9.40E-06	8.68E-05	4.35E-05	1.59E-03	2.89E-02	1.33E-01
RAGE	1.17E-03	1.62E-04	1.07E-03	2.01E-04	5.70E-04	1.39E-03	1.64E-02
CAGE (CT26)	N.P.	N.P.	N.P.	6.08E-06	4.46E-05	1.34E-04	2.99E-03
XAGE1 (CT12)	N.P.	N.P.	1.02E-04	N.P.	4.60E-05	8.49E-04	3.94E-03
LAGE 1+2 (CT6)	3.68E-05	2.59E-06	5.43E-03	3.45E-05	3.30E-03	1.92E-01	4.68E-02
LAGE2 (CT6.1)	N.P.	N.P.	N.P.	N.P.	1.75E-04	2.79E-03	1.99E-03
SSX (CT5)	2.93E-04	N.P.	5.92E-05	2.69E-05	2.63E-03	3.83E-03	3.21E-02
CSAG (CT24.2)	7.87E-05	N.P.	3.64E-05	4.86E-02	4.16E-02	4.52E-02	1.09E-02
IL-13R α 2	5.89E-05	N.P.	8.75E-04	8.68E-02	1.47E-01	6.65E-03	1.33E-02
hTERT	N.P.	1.26E-05	4.08E-05	4.85E-05	3.34E-05	4.87E-05	4.81E-04
p53	4.28E-02	2.29E-02	2.63E-02	1.32E-02	1.14E-02	2.61E-02	3.62E-02
Her2/Neu	5.20E-02	5.20E-02	4.58E-02	3.43E-03	2.36E-03	5.61E-02	2.38E-02
BCL-XL	1.84E-02	6.96E-03	3.71E-02	6.41E-04	6.76E-04	1.32E-02	1.03E-02
MCL1	4.20E-01	6.42E-02	2.13E-01	2.41E-02	2.30E-02	1.03E-01	1.33E-01
BCL2 α	1.66E-02	4.01E-04	2.64E-03	7.74E-04	6.29E-04	1.32E-03	9.78E-03
livin	N.P.	N.P.	5.48E-06	N.P.	1.57E-05	1.88E-04	1.09E-05
surviving	7.99E-04	5.29E-03	1.11E-02	2.06E-02	1.48E-02	2.08E-02	2.36E-03
SCP1 (CT8)	2.27E-05	N.P.	N.P.	N.P.	9.80E-07	4.79E-05	1.69E-02
SPANXC (CT11.3)	2.74E-05	N.P.	N.P.	5.48E-06	1.18E-04	2.40E-03	4.43E-04
OY-TES-1 (CT23)	3.68E-04	1.83E-05	1.04E-03	6.75E-06	1.53E-05	2.10E-04	1.25E-03
PLU-1 (CT31)	1.28E-02	3.72E-03	7.79E-03	1.60E-03	1.31E-03	4.46E-03	1.97E-02
RHAMM	9.40E-05	8.86E-04	1.65E-03	3.89E-03	2.38E-03	1.16E-03	1.17E-03
TPTE (CT44)	6.87E-06	N.P.	N.P.	1.15E-04	8.77E-05	5.61E-04	3.43E-02
CYP1B1	1.06E-01	4.51E-04	2.51E-01	1.78E-05	3.47E-04	8.85E-03	1.07E-01
MELK	8.50E-04	5.48E-03	8.66E-03	1.51E-02	1.30E-02	1.91E-02	2.23E-02
BORIS (CT27)	1.65E-05	N.P.	1.61E-05	6.77E-05	2.15E-04	1.11E-02	7.30E-03
DPPA2	2.71E-06	N.P.	N.P.	1.52E-05	1.80E-04	3.48E-04	8.97E-03
BRDT (CT9)	N.P.	N.P.	3.77E-06	N.P.	1.25E-05	1.15E-04	8.71E-04
ADAM2 (CT15)	N.P.	N.P.	N.P.	N.P.	N.P.	N.P.	4.89E-05
TSP50 (CT20)	3.23E-04	1.18E-05	6.48E-05	N.P.	5.72E-06	2.45E-03	5.58E-03
SP17 (#1) (CT22)	8.39E-04	2.16E-03	1.68E-02	7.44E-04	8.52E-04	5.26E-03	1.79E-02
SP17 (#2) (CT22)	N.P.	N.P.	N.P.	N.P.	1.70E-06	3.22E-03	2.00E-03
MMA (CT25)	N.P.	N.P.	N.P.	N.P.	2.68E-04	N.P.	9.30E-03
HOM-TES-85 (CT28)	7.71E-04	2.52E-03	1.65E-02	7.75E-04	8.65E-04	4.76E-03	1.85E-02
KM-HN-1	2.33E-04	8.04E-05	4.31E-04	5.38E-06	8.22E-06	9.91E-05	2.39E-03
TPBG (5T4)	4.95E-02	4.99E-03	1.51E-02	6.95E-02	7.92E-03	1.86E-02	2.14E-02
D40 (CT29)	2.00E-05	5.60E-05	4.85E-04	1.68E-04	1.55E-04	1.82E-04	2.03E-03
HCA661 (CT30)	8.20E-05	N.P.	N.P.	6.26E-06	2.46E-04	N.P.	8.55E-03
LDHC (CT32)	3.66E-04	2.71E-06	2.05E-04	9.93E-04	9.96E-04	1.73E-03	1.44E-02
MORC (CT33)	N.P.	N.P.	1.28E-04	9.03E-07	4.76E-06	N.P.	2.90E-02
SGY-1 (CT34)	4.05E-04	2.94E-05	5.23E-04	1.80E-05	4.90E-05	6.88E-04	3.42E-03
NXF2 (CT39)	N.P.	N.P.	3.16E-04	3.11E-06	8.81E-05	2.09E-02	4.61E-02
FATE (CT43)	1.44E-05	N.P.	7.20E-05	N.P.	N.P.	7.97E-05	3.57E-01
LIP1 (CT17)	N.P.	N.P.	N.P.	N.P.	N.P.	N.P.	5.93E-03
CTAGE (CT21)	N.P.	N.P.	N.P.	N.P.	1.63E-06	N.P.	5.46E-04
SPO11 (CT35)	N.P.	N.P.	N.P.	N.P.	N.P.	N.P.	5.48E-03
TPX1 (CT36)	N.P.	2.46E-04	7.84E-04	N.P.	1.66E-06	5.23E-04	9.93E-04
NY-SAR-35 (CT37)	N.P.	N.P.	N.P.	N.P.	1.84E-04	N.P.	1.33E-02
FTHL17 (CT38)	N.P.	N.P.	N.P.	N.P.	2.35E-05	N.P.	1.96E-03
TDRD1 (CT41.1)	3.81E-05	7.50E-06	9.78E-05	N.P.	1.86E-05	N.P.	5.79E-03
TDRD6 (CT41.2)	3.77E-04	3.64E-06	2.19E-04	7.58E-07	4.03E-06	1.36E-05	7.23E-03
TEX15 (CT42)	N.P.	2.01E-05	6.95E-05	3.46E-05	1.32E-04	N.P.	4.32E-02

Gene expression is shown relative to GAPDH expression; GAPDH expression is equal to 1. Cancer/testis genes are indicated by a CT identification number in parentheses following the gene name, for example MAGE-A1 (CT1.1). Samples analyzed: SKIN = normal human skin, ESO. = normal human esophagus, from P04 (Figure 1), LUNG = normal human lung, DDM1.7 = melanoma cell line used to generate tumor lysate for MelCancerVac®, DDM1.7+5aza = DDM1.7 cells treated with 5-aza-CdR for 72 hours, ESO. Tumor = squamous cell carcinoma of esophagus P04 (Figure 1), TESTIS = normal human testis.

Supplementary Table 2. Genes, amplified transcripts, and QPCR primers used in this study.

Gene Name	NCBI Transcripts Amplified	Primers
MAGEA1	NM_004988.4	AGTAGTAGGTTTCTGTTCTATTGGG TACTTATTCCTGCTGTTATTATCC
MAGEA2	NM_005361.2, NM_175742.1, NM_175743.1, NM_153488.3	GAGGACAGTGTCTTCGCAC GGTTCTCCACCGATCTTTAGTG
MAGEA3	NM_005362.3	GCTGAGTGTGTTAGAGGTGTT AGGGGTGGGTAGGAAATGT
MAGEA4	NM_001011548.1, NM_002362.4, NM_001011549.1, NM_001011550.1	GCAATGGAGGGCGACAGC CAATCTTGGGTGAGCAGTTCC
MAGEA6	NM_005363.2, NM_175868.1	GCTGAGTGTGTTAGAGGTGTT CAGGAGTGGGTAGGAAATGC
MAGEA9	NM_005365.4, NM_001080790.1	GTTCCCTGTCTATTGGGTGATTTG TTCTCTGCTATGTTATCTTGTC
MAGEA10	NM_001011543.1, NM_021048.3	TCCGTCGTTGCTTCCCTTC TCGGCTCCTTCATTTGATACTTG
MAGEA12	NM_005367.4	CTGAGTGTGTTGGAGGCATC GGTGGGTAGGAAATGTGAGGT
MAGEB (1,2,3,4,6,10,18)	NM_173523.2, NM_002365.3, NM_002367.2, NM_182506.2, NM_002363.3, NM_177404.1, NM_177415.1, NM_173699.2, NM_002364.3, NM_001099921.1	GCAGGTGCCCAACAGTGATCC AGGACTTTCATCTTGCTGGTTTCAGC
MAGEB1	NM_002363.3, NM_177404.1, NM_177415.1	GCTTTGAGAGATGAGGAAGAGAG GGGAGGACCTGCTGAATGG
MAGEB2	NM_002364.3	TGCCTTCCAACCCATTACG AGAATCTGCCTAAGACCTCATTG
MAGEB3	NM_002365.3	CCACGCCTAGTGAAGTTGAAG CCTCCAGTAACACCCAGTCC
MAGEB4	NM_002367.2	GTGGTTTGTCTTACTCAGTTCAG TCTCCCTCATCTCTTAAAGTCTTC
MAGEB6	NM_173523.2	GCCGACAGCAGTAACACC TCTCTACTCTCATCTATCAAAGC
MAGEB18	NM_173699.2	AGAGGCTTTGAGGGATGAGG CACTACTTAGAATGTTGACTGCTC
MAGEC1	NM_005462.3	TCTGGGATGTGCTGAGTGG ACGAGGAGGAGAAGAGTTGG
MAGEC2	NM_016249.2	GGAGGGAGCACTTCGTCTATG AGGATGGAAGGAACTAGGAACAG
MAGEC3	NM_138702.1, NM_177456.2	CCCACAACCACTCCTATTTTC GCACTCAACACTTCCCAGATG
GAGE (1,2,3,4,6,8)	NM_001098411.3, NM_001127212.1, NM_001127200.1, NM_001098405.1, NM_001098407.1, NM_001098409.1, NM_001098406.1, NM_001472.2, NM_001468.3, NM_001040663.1, NM_021123.2, NM_001477.1, NM_012196.1, NM_001474, NM_001476, U_19144	AAGGGGAACCAGCAACTCAAC GGACCATCTTCACTCAACACC
BAGE (1,2,3,4,5)	NM_001187.1, NM_182482.2, NM_182481.1, NM_181704.1, NM_182484.1	TGGAGGTTGGAGCCTGAAG GCACAAGGATAATGATACATCTGG
SAGE	NM_018666.2	GATAGTAATGAATTTGCGGTAGGC GGAGATATTTGTGGTGTATTTGGC
HAGE	NM_018665.1	ATGGTGGTGGAAATAGAGATGAAC GAGGCGATGAAGTGAATGAGG
PAGE (2,5)	NM_207339.2, NM_001013435.1, NM_130467.3, NM_001015038.1	GAGTCTTCCCAGCCAGTTG AGTTGGTGGTTCCTCTTCTTG
RAGE	NM_014226.1	AGAACAGTCCCTAAAGCAAGAG TCTGACCACTCCCGAAAGC
CAGE	NM_182699.2	ATCAAGAGCGAGCAGTAGAGG GTCTTCCCTGTCCTCCCAATG
XAGE1A-E	NM_001097591.1, NM_001097595.1, NM_001097602.1, NM_133431.2, NM_001097603.1	GTGAGTGAGAGTGAAGAGGAACC GCCGTGACGAGACAAGAAGG
LAGE (CTAG) (1,2)	NM_172377.2, NM_020994.2, NM_139250.1, NM_001327.1	GGTCATGCCTCCTCCCCTAG TGTAAGCCGTCTCCTCCAG

LAGE2 (CTAG1/NY-ESO)	NM_139250.1, NM_001327.1	CGCCTGCTTGAGTTCTACC CAGTCAGTCGGATAGTCAGTATG
SSX (1,2,3a,4a,5,6,7,8,9)	NM_021015.3, NM_175723.1, NM_173357.2, NM_005636.3, NM_175698.1, NM_005635.2, NM_003147.4, NM_021014.2, NM_001034832.2, NM_173358.2, NM_174962.3, XR_041312.1	GCCCAAGAAGCCAGCAGAGG GCAGTCTGTGGGTCCAGGC
CSAG(1,2,3)	NM_153478.1, NM_001102576.1, NM_001080848.2, NM_004909.3, NM_001129826.1, NM_001129828.1	CGCTGGTCTGGTGAAGATGTC GAGTGGCTGGATAGTGTGGC
IL-13R α 2	NM_000640.2	ATCATCAGAGAACAAGCCTATCAG GCAGTCACCAAGGTAGTATCATC
hTERT	NM_198253.2, NM_198255.2	GTATGGCTGCGTGGTGAAC GGTGAGACTGGCTCTGATGG
p53	NM_000546.4, NM_001126112.1, NM_001126113.1, NM_001126114.1, NM_001126115.1, NM_001126116.1, NM_001126117.1	CAATAGGTGTGCGTCAGAAGC TACATCTCCCAACATCCCTCAC
Her2/Neu	NM_004448.2, NM_001005862.1	GTGCCAGTGTGAACCAGAAG CCTCAGAAATCCACAAGACTCC
BCL-XL	NM_138578.1, NM_001191.2	CAGGCTGCTGGGATAAAGATG GGACTAGGGTGGTGTGAAC
MCL1	NM_021960.3, NM_182763.1	TTCTGTTTGTCTTACGCTTCTCTC TTGTAGTTGGTCCTAACCCCTCC
BCL2 α	NM_000633.2	AGTGTGGTCTCCGAATGTCTG GCCTCTGTTCCTTCCCTCTAC
livin	NM_139317.1, NM_022161.2	CCGTGTCCATCGTCTTTGTG ACAGTCCAGAACAGGCAGAG
survivin	NM_001168.2, NM_001012270.1, NM_001012271.1	CTAAGCACAAAGCCATTTAAGTC TCACTCTATTCTGTCTCCTCATCC
SCP1/HOM-TES-14	NM_003176.2	ACCGTTGGGCTGTAATTGC TAGGCTCCTAACCTACACTGATTC
SPANXC	NM_022661.2	GCCGCAGACATTTGAAGAACC TCGTCTCATTACCTCGTTGG
OY-TES-1	NM_032489.2	TTTCGGGAAGTCGGTCTGTG GAGTCTTGTGGGAGGTGTGC
PLU-1	NM_006618.3	AGCCCTCACTAACAAAGAAC TTTCCTTAGATTTCTTCCAACG
RHAMM	NM_012484.1, NM_012485.1	TTCGTCTTGTGTTATTGATGTTG AGGTGATAAAGGTGAGCATTAGG
TPTE	NM_199261.2, NM_199259.2, NM_199260.2	CGGTACTTGATAACATTACAACAG TCACATCATCATAACAGAGGTAGAC
CYP1B1	NM_000104.3	TGTC AACAGGTCCAGATGTG AAATAAGCCAGGTAACCTCCAAGC
MELK	NM_014791.2	GGATGAGTGTGGTGTGATAC GACAGATGGGCTTGATTTAGATTC
BORIS	NM_080618.2	TTGGTATAGGAGCAAGGTTTAC AGGGAAATACTACTAATCACTGG
DPPA2	NM_138815.2	GAGAGCAGAAGAGACCAATACAG GCCTCAACAGAAACGGGAATG
BRDT	NM_207189.2, NM_001726.3	TTAGGCAAACAGTGAACCATC TTCGGTATGAGTTCTGTGTCC
ADAM2	NM_001464.3	TTACCTCCAGATTGCTCAGTTC TGCTCATCGCTTGAATAGTCC
TSP50	NM_013270.4	TGACCAGATGACGCAGACC CCACCCAGGACCAGAACC
SPA17	NM_017425.2	GGGACACATAGCCAGAGAGG TTCACAACATCAAATCTTCTTCC
MMA	XR_040165.1	GCCAAGACCGAAAGTGCTG CTAAATATGGAACAAGTGAGAATAAC
HOMTES85	NM_016383.3	CAAGATGTCCCATTAAGTGTGTTCC TTGGCAGTTAGTTGTTAGATTAGC
SP17 (SPA17)	NM_017425.2	ACCAACTTTGATCCAGCAGAATG ACTGATGTCTCTTCTCCTTCC
KM-HN-1	NM_152775.2	TAATTGTAAGTCTAAGTGAATGTC CCTGCCCTCCCTTGTAGAG
TPBG (5T4)	NM_006670.3	AGAGGACAGACCAAGGACAAC CATTAACAGAACACCCGAGAAAC
D40	NM_170589.3, NM_144508.3	GGTGATGAAGTCTGGATGGTAG AATGGCTAATGTTCTGTGTCAGTG

HCA661	NM_016521.2	CGGCAGGTTCCAGGTCTAATG GTTGTTGTCTCCTCCTCTTCC
LDHC	NM_002301.4, NM_017448.3	TTCTCAGTATCCCTTGTGTCTTG GCCTGCTATCTTCTGTCTTCC
MORC	NM_014429.2	CGATATGTGAAGGACTACAGAATC TGCTTGACTGAGTGTGGC
SGY-1	NM_014419.3	CCTGCTGCTGCTCCTCTC CTGGTGCTCCTGGTCTCC
NXF2	NM_017809.3, NM_022053.1, NM_001099686.1	GGTCTGTCTCGCCTTAC AGACCACTCCAGTTTCATCCC
FATE	NM_033085.2	ATCATCGCCGTGCTGGTG GGGAAAGAGGAAAGAAAGAGGG
LIPI	NM_198996.2	TGTCCTCGGCTGGTTATC CCTGGCTCTTCAATCATTCC
CTAGE	NM_172241.2	TGCCTTCTTCCAGTGAGAG GAGTGAGATATGTACCCAGTTG
SPO11	NM_012444.2, NM_198265.1	GCTCATCATCTCACAGTTCAG GGCAGGTAAACATAAGGTCTCC
TPX1	NM_003296.1	GGTACTGTGCTGCTTCCATC CTCTCTGCTCCATCCATCTTTAG
NY-SAR-35	NM_152578.1	CAGGATGGCGGAATCTCTAAAG ACAGGTAGTAGGACAGGTAGTAGC
FTHL17	NM_031894.1	AAAGAGACTTGAGCCAGATG ACACCTTTATTGCTTTGAGAACC
TDRD1	NM_198795.1	GAATACTGGCAAGGAATACATAGG GAGGCAGACGACAGAGAATG
TDRD6	NM_001010870.1	ATGGTATGGAGGAGATAGTGAAC TTGGTCTGCGTAAGATGCC
TEX15	NM_031271.3	AGAGAATTGGTTTGGCTGAATC CAGTTCTTTGCTTGACCTTCC
HMBS	NM_000190.3, NM_001024382.1	ACCCTAGAAACCTGCCAGAG CGAATACTCCTGAATCCAGATGC
TBP	NM_003194.3	ATGGTGGTGTGTGAGAAGATG GATAGCAGCACGGTATGAGC
GAPDH	NM_002046.3	AGCTTGTATCAATGGAAATCCC GTGAAGACGCCAGTGGACTC

NCBI transcripts amplified were identified using the NCBI primer blast tool. Some reactions amplify multiple transcripts due to the presence of alternatively spliced transcripts, other reactions amplify several related gene family members, these reactions are typically identified because the gene name is followed by several numbers, indicating individual family members, for example, BAGE (1,2,3,4,5). The top QPCR primer is the 5' or forward primer; the bottom primer is the 3' or reverse primer.