

Supplemental Table 1. qRT-PCR Expression of 45 Genes : Mybl2 siRNA or Spontaneous Differentiation

Gene	Expression Relative to GAPDH (x100)					Gene	Expression Relative to GAPDH (x100)				
	NT siRNA	Mybl2 siRNA	Prolif	Spont. Day 2	Spont. Day 14		NT siRNA	Mybl2 siRNA	Prolif	Spont. Day 2	Spont. Day 14
ALDH7	0.880	1.359	1.002	1.662	1.627	endothelin	0.061	0.073	0.147	0.058	0.185
ALPi	0.072	0.056	0.065	0.183	0.762	EPHX2	0.131	0.124	0.128	0.948	2.798
E-cadherin	7.439	8.175	7.352	13.605	29.126	fibronectin	26.905	26.796	32.745	67.122	17.820
cathepsin B	1.131	1.161	1.133	2.536	9.626	GSPT1	0.349	0.504	0.411	0.127	0.154
cdk2	0.406	0.706	0.889	1.002	0.991	GSTA3	0.002	0.001	0.000	0.007	0.326
cdk4	2.381	2.429	5.145	6.819	4.546	HAT1	0.169	0.214	0.097	0.091	0.077
cyclin A2	4.243	3.269	2.636	2.075	0.979	HDAC2	1.043	1.375	0.647	0.848	0.501
cyclin B1	0.101	0.069	0.226	0.239	0.046	eIF-4E	0.050	0.064	0.044	0.059	0.038
cyclin B2	1.522	1.099	1.244	1.458	0.537	Id-2	2.149	2.056	2.540	2.763	14.940
cyclin D1	2.309	3.177	4.179	3.335	2.997	α-5 integrin	0.003	0.003	0.007	0.014	0.109
cyclin D2	11.264	18.102	18.157	4.446	1.408	KLF4	0.025	0.027	0.027	0.144	0.537
cyclin E1	0.004	0.004	0.003	0.003	0.002	MEK2	0.269	0.325	0.488	0.635	1.129
cdc2	4.669	3.226	3.735	3.504	1.557	Mybl2	3.189	0.537	5.484	4.924	1.998
cdc25A	1.095	1.282	1.133	0.740	0.404	c-myc	0.012	0.019	0.066	0.091	0.026
cdc25B	0.750	0.494	0.820	1.336	0.710	NAP1L1	24.987	29.340	30.119	66.704	28.832
cdc25C	0.666	0.453	0.602	1.037	0.646	Notch1	0.015	0.014	0.016	0.051	0.043
cycloph G	0.013	0.017	0.005	0.008	0.013	PCNA	9.654	11.627	13.505	13.143	6.295
CYP1A1	1.675	1.504	1.790	1.218	0.552	PSMC3	0.622	0.773	0.700	0.597	0.765
DNA topo	0.338	0.372	0.289	0.213	0.321	Ran	1.644	1.785	0.378	0.568	0.267
DP-1	0.037	0.051	0.064	0.042	0.030	TARS	0.076	0.068	0.172	0.108	0.063
DPPIV	1.615	1.519	2.210	9.380	12.504	TCF-4	0.182	0.158	0.148	0.418	1.179
E2M	0.160	0.175	0.058	0.050	0.020	TGF-b2	0.087	0.078	0.062	0.075	0.013
						thy synth	1.526	1.798	1.514	1.470	1.256

Supplemental Table 2. Primers used for qRT-PCR

Gene	forward primer (5'→3')	reverse primer (5'→3')	Gene	forward primer (5'→3')	reverse primer (5'→3')
ALDH7	GAGGCGACTGTTTATACATG	AAGAAACATGCTCACTGCCT	fibronectin	ACGCCTCCACTGCCATTGAT	AGACCCAGGCTTCTCATACT
ALPi	GCAACCCTGCAACCCACCCAAGGAG	CCAGCATCCAGATGTCCCGGGAG	GAPDH	CCATCAATGACCCCTTCATT	TTGATGACAAGCTTCCCGTT
A-myb	ACCTTGTGCAGCTATGGATC	GTGACACATACTGATACCCA	GSPT1	TCAGGAAGAACGAGACAAGG	TGAGATTACCAGCACAGCCA
E-cadherin	TGAAGGTGACAGAGCCTCTGGAT	TGGGTGAATTCGGGCTTGTT	GSTA3	TTCAATGGACGGGGCAGAAT	CCAACTTCATCCCATCAATC
cathepsin B	AGCACTACGGATAACAATTCC	ACTTGTAGAGCAGGAAGTCC	HAT1	GTAGGCTACATGACAGTCTA	CCTTGACCTTGAAATGGAGT
cdk2	GCTAGCAGACTTTGGACTAGCCAG	AGCTCGGTACCACAGGGTCA	HDAC2	CGTGTAATGACGGTATCATTCC	CTGCCCATATGACTCATCATC
cdk4	ATGTTGTCCGGCTGATGGA	CACCAGGGTTACCTTGATCTCC	eIF-4E	GTTTGTGCGATCAGATCGAT	TTCTCCTCTTCTGTAGTCGG
cycl A2	TGCTGCTATGCTGTTAGCCT	TGCTCCATTCTCAGAACTTG	Id-2	CATCCTGTCTTGCAGGCTT	CACACAGTGCTTTGCTGTCA
cycl B1	TGCTGCCTGGTGAAGAGGAA	TGCCATGTTGATCTTCGCTT	α-5 integr	CTCCATTGGTTTTACAGTGG	ATTCTGGATGAGCAGGGTCT
cycl B2	CTTCTGTCAAACCACTACAG	ATTTTGCAGAGCAAGGCATC	KLF4	TGCTGATTGTCTATTTTTGCGTTTA	GAGAAGAAACGAAGCCAAAACC
cycl D1	ATGCTGGAGGTCTGCGAGGA	AGGAAGCGGTCCAGGTAGTT	MEK2	TGCTCACAAACCACACCTTCAT	ACACAACCAGCCGGCAAA
cycl D2	GAAGGACATCCAACCCTACA	AGGAGTTGCAGATGGGACTT	Mybl2	CTGACCAGCAATGCCAGTAC	TTCAGGTGCTTGGCAATCAG
cycl E1	CTCCAGGAAGAGGAAGGCAA	TCGATTTTGCCATTTCTTCA	c-myb	TCGCAAAGCTACTGCCTGGA	AAGACTCTGCAGATAACCT
cdc2	TCCTGGTCAGTACATGGATT	GTCTCTGTGAAGAACTCTTC	c-myc	TGCCAGGACCCGCTTCT	GAGGCTGCTGGTTTTCCAATA
cdc25A	CTCCGAGTCAACAGATTTCAG	CTACATCCCAACAGCTTCTG	NAP1L1	CACGTCAGCTAACTGTTTCAG	ACCCTAGGCAGGCTTTCAAT
cdc25B	TCGTCTGAATCCTCCGAATC	CATAGACTGGAAGCGTCTGA	Notch1	CAGGCAATCCGAGGACTATG	CAGGCGTGTGTCTCTCACAG
cdc25C	TATCACTCAGATGCTGGAGG	TCTGGGTTGACATACTTCAG	PCNA	GACCGCAACCTGGCCAT	CCGCGTTATCTTCGGCC
cycloph G	CTTTGTACAGGTGAAAAGGG	CTTCACTGAAGTCACCACCT	PSMC3	GTACAGAAATACGTCCGGTGA	GTCACCTCCAGCACCATCAT
CYP1A1	TACCCAACCCTTCCCTGAAT	AGTGCTCAATCAGGCTGTCT	Ran	TTATATCCAAGCCAGTGTG	ACAATGGATTTCGCCTTCAC
DNA topo	GGAACCAGTATCGAGAAGAC	CTCCTTTTCATTTGCTGCTC	TARS	CAGTCTATAGATGTGGCCCT	TCTCCATATCTGCTTTGCCT
DP-1	TTCCGACTCCTCACCTTGGT	TAGGCCCTTGCCATTCTTCT	TCF-4	CCGAAAGTTTCCGAGACAAA	AGTGGCCATTTTCATCTGGAG
DPPIV	AGTCTTCAGTGCCTACTCTG	CAGTTGGATTTCACAGCTCCT	TGF-b2	AATAGACATGCCGCCCTTCT	TCTCCATTGCTGAGACGTCA
E2M	CAGAAGGACATAAACGAGCT	TTGAAGTTGAGGAGGTCGTC	thy synth	ACTGCAAAGAGTGATTGACACCAT	CAGAGGAAGATCTCTTGATTCCAA
endothelin	AGCTCCAGAAAACAGCAGTCT	TTATCCATCAGGGACGAGCA			
EPHX2	CCACTACCCGGCTTATGAAA	TTAGCGGTCTCGGAGCACTT			

Supplemental Table 2 (continued). Primers used for chromatin immunoprecipitation

Gene	forward primer (5'→3')	reverse primer (5'→3')
E-cadherin	AGTGGAAATCAGAACCGTGCA	CTGCCGGCCACAGCCAATCA
cdk2	CACTCTCATCTTGATGCACT	CTGGTTTAATTCACTCTCCC
cdk4	CTCATGGTGGAGCGAAAAGG	CTGAAGGATTGTCTCACTAC
cdc25B	GTGGCTTTACAAGGACTT	CTCTGCAAGACTGAATTTCC
c-myc	CACTTAACACTTGAACGCTG	GTGTATAGCATGTACGCTGT
cyclin B2	TCAGAGGCGTCTTACGTCTG	AGATGCACTCTCGCACTCTC
cyclin D2	CTACACCTACAGAATGAGTG	ACTACCGACTCCTATTTCTG
MEK2	GTGGATCTAACACAGCTACA	ATGAGTAGGATCACACAGGT

Supplementary Experimental Procedures

Reporter plasmids

A plasmid containing -2.4 kbp of promoter region of the human *CDK2* gene up to nucleotide +58 bp cloned into pGL2 Basic (Promega) (1) was a gift of A. van Wijnen (University of Massachusetts Medical School, Worcester, MA). A 3.3kb fragment of the human c-myc promoter, containing residues -2328 bp to +936 bp relative to transcription initiation site P1, inserted upstream of a luciferase reporter (2) was a gift of D. Levins (Laboratory of Pathology, National Cancer Institute, Bethesda, MD). The upstream regions of *cdk6*, cyclin D2, *cdc25B*, and cyclin B2 were cloned by PCR from genomic DNA of Caco-2 cells as follows : ~250ng genomic DNA, isolated from ~3x10⁶ Caco-2 cells using the Dneasy Blood and Tissue kit (Qiagen) as described by the manufacturer, was amplified by PCR using Phusion High Fidelity DNA polymerase (New England Biolabs) and the following primers : *cdk6* (-1004bp to +172bp, relative to start site of longest transcript (3)) 5' **ggggtacc**ctttgtgtgcagagggctc, 3' *gaagatcta*atcagacagcccagaagcc ; cyclin D2 (-881bp to +147bp relative to start site of longest transcript (4)) 5' **ggggtacca**gcggttttctcgtg, 3' *gaagatctg*acctccttcttgctga ; *cdc25B* (-1328bp to +559 relative to start site of longest transcript, Genbank accession NM_021873) 5' **ggggtacc**acatacaccctgtgcagatg, 3' *gaagatctt*ctagttgcagctgccact ; cyclin B2 (-797bp to +94 bp relative to transcriptional start site, Genbank accession NM_004701) 5' **ggggtacc**gattctggacactaggcctt, 3' *gaagatcta*aagggaggacactagcgta. Sequences in bold and italics represent, respectively, sites for cleavage by KpnI and BglII restriction endonucleases. Cycling conditions : 98° for 30 s ; 30 cycles of 98° for 10 s, 65° (*cdk6*, cyclin D2, cyclin B2) or 72° (*cdc25B*) for 30 s, 72° for 45 s (*cdk6*, cyclin B2, cyclin D2) or 2 min (*cdc25B*); 72° for 10 min. Appropriately-sized fragments were purified (Gel Extraction Kit, Qiagen) and digested overnight with KpnI and BglII, then ligated into similarly-digested pGL3 Basic (Promega).

Supplementary Experimental Procedures References

1. Shiffman, D., Brooks, E. E., Brooks, A. R., Chan, C. S., and Milner, P. G. (1996) *J Biol Chem* **271**, 12199-12204
2. Chung, H. J., Liu, J., Dundr, M., Nie, Z., Sanford, S., and Levins, D. (2006) *Mol Cell Biol* **26**, 6584-6597
3. Iwanaga, R., Ozono, E., Fujisawa, J., Ikeda, M. A., Okamura, N., Huang, Y., and Ohtani, K. (2008) *Oncogene*
4. Brooks, A. R., Shiffman, D., Chan, C. S., Brooks, E. E., and Milner, P. G. (1996) *J Biol Chem* **271**, 9090-9099

