MYCL promotes iPSC-like colony formation via MYC Box 0 and 2 domains

Chiaki Akifuji, Mio Iwasaki, Yuka Kawahara, Chiho Sakurai, Yu-Shen Cheng, Takahiko Imai & Masato Nakagawa

Human induced pluripotent stem cells (hiPSCs) can differentiate into cells of the three germ layers and are promising cell sources for regenerative medicine therapies. However, current protocols generate hiPSCs with low efficiency, and the generated iPSCs have variable differentiation capacity among different clones. Our previous study reported that MYC proteins (c-MYC and MYCL) are essential for reprogramming and germline transmission but that MYCL can generate hiPSC colonies more efficiently than c-MYC. The molecular underpinnings for the different reprogramming efficiencies between c-MYC and MYCL, however, are unknown. In this study, we found that MYC Box 0 (MB0) and MB2, two functional domains conserved in the MYC protein family, contribute to the phenotypic differences and promote hiPSC generation in MYCL-induced reprogramming. Proteome analyses suggested that in MYCL-induced reprogramming, cell adhesion-related cytoskeletal proteins are regulated by the MB0 domain, while the MB2 domain regulates RNA processes. These findings provide a molecular explanation for why MYCL has higher reprogramming efficiency than c-MYC.

Human induced pluripotent stem cells (hiPSCs) are generated from somatic cells and can differentiate into cells of all three germ layers. They are functionally identical to human embryonic stem cells (hESCs) but do not require the destruction of the embryo, which has made them attractive sources for regenerative medicine. The original reprogramming was induced by four factors, OCT3/4, SOX2, KLF4, and c-MYC (OSKM). Since then, several new methods have been developed to improve the yield and quality of iPSCs, but the cost remains high and the production remains technically difficult. Further complicating the application of hiPSCs is the wide variability in the differentiation capacity of different hiPSC clones.

We have shown that excluding c-MYC from the reprogramming factors significantly lowers the reprogramming and differentiation efficiencies of the resulting iPSCs. The MYC family consists of the oncogenes c-MYC, MYCN, and MYCL in humans. c-MYC was the first MYC gene discovered in human and has been a topic of cancer research ever since. Tumorigenesis depends on high transformation activity derived from the N-terminus region of c-MYC protein. Consequently, OSKM-based reprogramming may not be appropriate for the clinical application of iPSCs. Many groups have reported reprogramming methods that exclude c-MYC overexpression but at the cost of lower reprogramming efficiency. MYCL is about 30 amino acids shorter in the N-terminus region than c-MYC and has lower transformation activity. We found that substituting c-MYC for MYCL in reprogramming can increase the number of iPSC colonies and maintain the ability to differentiate into the cells of three germ layers. Furthermore, fewer chimeric mice died by tumorigenesis after the transplantation of MYCL-iPSCs, whereas the transplantation of c-MYC-iPSCs caused lethal tumorigenesis in more than 50% of mice during two years of observation. Despite these observations, little is known about the molecular function of MYCL and the different mechanisms between c-MYC and MYCL to promote reprogramming.

MYC proteins have six MYC Box (MB) domains: MB0, 1, 2, 3a, 3b, and 4 in the N-terminus and a basic helix-loop-helix leucine zipper (bHLHLZ) in the C-terminus, but MYCL does not have MB3a. The C-terminus of c-MYC and MYCL is essential in reprogramming due to its binding with MAX protein, allowing MYC to access the DNA. The N-terminus is mainly known as a transactivation domain (TAD), which regulates the target gene, but its function in reprogramming is less clear. We found that a mutant of c-MYC lacking the N-terminal showed low transformation activity and promoted reprogramming. However, which domain on the N-terminal side is essential for reprogramming and what function it performs were not resolved. In addition, MYC proteins act as transcription factors upon interacting with several binding proteins. Although MYCL-binding proteins are important for MYC function, there are no reports about MYCL-binding proteins during reprogramming.

Department of Life Science Frontiers, Center for iPS Cell Research and Application (CiRA), Kyoto University, Kyoto 606-8507, Japan. Email: nakagawa@ci-ra.kyoto-u.ac.jp
MYCL promotes reprogramming more efficiently than c-MYC. To compare the reprogramming phenotypes of MYCL and c-MYC, we used Sendai virus (SeV)-based reprogramming (CytoTune-iPS) and StemFit AK03N medium without bFGF (Fig. 1A). The SeV method has high reprogramming efficiency without genome integration, and c-MYC and MYCL SeV kits are already available15. The bFGF exclusion is based on the data in Supplementary Fig. S1. DMEM supplemented with 10% FBS (DMEM + 10%FBS) is the standard medium to induce reprogramming. We used DMEM + 10%FBS when introducing the reprogramming factors, but after 7 days of reprogramming, we replated the cells and used StemFit AK03N without bFGF (03N (-)) from that point on. The MOI (multiplicity of infection) of each SeV was 20. To improve the reprogramming efficiency, we compared three media combinations (Supplementary Fig. S1A). The highest number of colonies was obtained using 03N (-) during reprogramming and 03N (+) after reprogramming (Supplementary Fig. S1B). These results indicated that the 03N (-) reprogramming condition in the first 7 days enhances the reprogramming efficiency compared to 03N (+). We then examined the optimal MOI of SeV for the reprogramming (Supplementary Fig. S1C). A lower MOI induced more colonies (Supplementary Fig. S1D), indicating a higher reprogramming efficiency. Following these results, we applied SeV for the transduction at an MOI of 4.3 using 03N (-) during reprogramming.

Next, we conducted immunostaining to analyze the expression of TRA-1-60 from days 1 to 7 after the transduction (Fig. 1A). TRA-1-60 is a glycoprotein and major cell surface marker of hiPSCs and hESCs16. We quantified the results using a high-content imaging system, ArrayScan, because the cell number was small during SeV reprogramming for the first seven days, making flow cytometry challenging. On day 7, we observed that c-MYC and MYCL induced a small cell mass to form colonies, but only the colonies induced by MYCL expressed TRA-1-60 (+) cells. The percentage of TRA-1-60 (+) cells increased more in MYCL-transduced HDFs on day 21. Mean ± SD values are shown. *p < 0.05 and **p < 0.01 by paired t-test. (D) Schematic representation of HDF reprogramming with episomal plasmid vector (EpiP). HDFs were transduced with EpiP carrying SOX2, KLF4, OCT3/4-shp53, LIN28A, EBNA1, and c-MYC or MYCL. StemFit AK03N without bFGF was used during the transfection and subsequent induction of iPSC-like colonies. We performed flow cytometry of the reprogramming HDFs three days from 1 to 19 days plus day 21 after the transduction. (E) Representative immunostaining images of reprogramming HDFs stained by anti-TRA-1-60 antibody (green) and Hoechst (blue) 21 days after the transduction. Scale bar, 300 μm. Ph, phase contrast. (F) Proliferation and expression of TRA-1-60 (+) cells during reprogramming were analyzed by flow cytometry. HDFs were transduced with EpiP including c-MYC or MYCL. Flow cytometry was performed every three days from 1 to 19 days plus day 21. Mean ± SD for n = 3, *p < 0.05 and **p < 0.01 by paired t-test. (G) The number of iPSC-like and non-iPSC-like colonies derived from 1 x 10^4 HDFs transduced with EpiP including c-MYC or MYCL on day 21. Mean ± SD values are shown. n = 3, *p < 0.01 by unpaired t-test. (H) Percentage of CD13 (+) cells during EpiP reprogramming determined by flow cytometry. Mean ± SD values are shown. n = 3, *p < 0.05 and **p < 0.01 by paired t-test.
**A**

![c-MYC and MYCL Box domain identity](image)

**B**

![Graphs showing iPSC-like colony number and Non-iPSC-like colony number](image)

**C**

![Graphs showing TRA-1-60% and CD13%](image)

**D**

![Flow cytometry for CD13 and TRA-1-60%](image)

**E**

![Graph showing cell number over time](image)
was larger with MYCL reprogramming (Supplementary Fig. S4). These results suggested that MYCL promotes reprogramming than MYCL reprogramming, but the CD13 (-) TRA-1-60 (+) population from days 16 to 21 c-MYC or MYCL, but the number of CD13 (-) cells rapidly increased in c-MYC compared to MYCL (Fig. 1H and 1I). On the other hand, the bHLHLZ domain in the C-terminus region is a well-known binding transactivation domain of the N-terminal region, W135E (Fig. 2A and Supplementary Fig. S5). We previously showed that a c-MYC mutant lacking transformation activity enhances the formation of iPSC-like colonies. This mutant has a point mutation in the MYC Box 0 and 2 domains are crucial for colony formation during reprogramming. Figure 2A shows that c-MYC-ΔMB1 promoted iPSC-like colony formation like c-MYC-ΔMB0, but it also led to the formation of non-iPSC-like colonies. The formation of iPSC-like colonies by MYCL were more flattened and showed a monolayered colony morphology, with each cell tightly packed and expressing TRA-1-60. The non-iPSC-like colonies produced by c-MYC showed a cell aggregation-like morphology, in which individual cells were irregularly aggregated and did not express TRA-1-60. We counted the number of iPSC-like and non-iPSC-like colonies on day 21 and found that c-MYC induced iPSC-like colonies as well as many non-iPSC-like colonies, but MYCL induced almost only iPSC-like colonies and more of them than c-MYC (Fig. 1G and Supplementary Fig. S3).

It has been reported that before the increase in the expression of TRA-1-60, a decrease in the expression of CD13, a marker of fibroblasts, is observed in somatic cell reprogramming. Therefore, we confirmed the expression of CD13 during reprogramming. The percentage of CD13 (+) cells increased in HDFs transduced with c-MYC or MYCL, but the number of CD13 (+) cells rapidly increased in c-MYC compared to MYCL (Fig. 1H and Supplementary Fig. S4). In particular, the CD13 (-) TRA-1-60 (+) population was larger on day 10 with c-MYC reprogramming than MYCL reprogramming, but the CD13 (-) TRA-1-60 (+) population from days 16 to 21 was larger with MYCL reprogramming (Supplementary Fig. S4). These results suggested that MYCL promotes TRA-1-60 (+) cells more than c-MYC, but c-MYC suppresses CD13 expression more than MYCL.

Figure 2B shows that c-MYC-ΔMB1 promoted iPSC-like colony formation like c-MYC-ΔMB0, but it also led to the formation of non-iPSC-like colonies. The formation of iPSC-like colonies by MYCL-ΔMB1 was about a quarter that by MYCL-WT. Unlike c-MYC-WT, c-MYC-ΔMB2 did not induce non-iPSC-like colonies, but it did induce a rate of iPSC-like colonies similar to c-MYC-WT. MYCL-ΔMB2 showed little ability to form iPSC-like colonies, resembling MYCL-ΔMB0. c-MYC-ΔMB3a, ΔMB3b, and ΔMB4 had similar colony-forming activities as c-MYC-WT. MYCL-ΔMB3b showed the same reprogramming efficiency as MYCL-WT, but MYCL-ΔMB4 formed about the same small number of iPSC-like colonies as MYCL-ΔMB1. The ΔbHLHLZ mutants of both c-MYC and MYCL failed to induce colonies and were therefore considered to have lost MYC function completely. Thus, the results indicate that in c-MYC, the MB0 and MB2 domains are repressive for iPSC-like colony formation, but in MYCL, they are promotive. Other domains also influenced the colony formation efficiency, but the effect was small.

Next, we analyzed the effect of the MYC-deletion mutants on the expression of TRA-1-60 and CD13 by flow cytometry 16 days after the start of reprogramming (Fig. 2C). Mutants that increased the number of iPSC-like colonies also increased the expression of TRA-1-60, while those that reduced the number of iPSC-like colonies lowered the TRA-1-60 expression (Fig. 2C and Supplementary Fig. S9). c-MYC-WT showed little TRA-1-60 expression. It has been reported that before the increase in the expression of TRA-1-60, a decrease in the expression of CD13, a marker of fibroblasts, is observed in somatic cell reprogramming. Therefore, we confirmed the expression of CD13 during reprogramming. The percentage of CD13 (+) cells increased in HDFs transduced with c-MYC or MYCL, but the number of CD13 (+) cells rapidly increased in c-MYC compared to MYCL (Fig. 1H and Supplementary Fig. S4). In particular, the CD13 (-) TRA-1-60 (+) population was larger on day 10 with c-MYC reprogramming than MYCL reprogramming, but the CD13 (-) TRA-1-60 (+) population from days 16 to 21 was larger with MYCL reprogramming (Supplementary Fig. S4). These results suggested that MYCL promotes TRA-1-60 (+) cells more than c-MYC, but c-MYC suppresses CD13 expression more than MYCL.

MYC Box 0 and 2 domains are crucial for colony formation during reprogramming. Next, we prepared domain deletion mutants to identify which domains in the N-terminus of MYC proteins influence colony formation, but in MYCL, they are promotive. Other domains also influenced the colony formation efficiency, but the effect was small.

Next, we analyzed the effect of the MYC-deletion mutants on the expression of TRA-1-60 and CD13 by flow cytometry 16 days after the start of reprogramming (Fig. 2C). Mutants that increased the number of iPSC-like colonies also increased the expression of TRA-1-60, while those that reduced the number of iPSC-like colonies lowered the TRA-1-60 expression (Fig. 2C and Supplementary Fig. S9). c-MYC-WT showed little TRA-1-60 expression. It has been reported that before the increase in the expression of TRA-1-60, a decrease in the expression of CD13, a marker of fibroblasts, is observed in somatic cell reprogramming. Therefore, we confirmed the expression of CD13 during reprogramming. The percentage of CD13 (+) cells increased in HDFs transduced with c-MYC or MYCL, but the number of CD13 (+) cells rapidly increased in c-MYC compared to MYCL (Fig. 1H and Supplementary Fig. S4). In particular, the CD13 (-) TRA-1-60 (+) population was larger on day 10 with c-MYC reprogramming than MYCL reprogramming, but the CD13 (-) TRA-1-60 (+) population from days 16 to 21 was larger with MYCL reprogramming (Supplementary Fig. S4). These results suggested that MYCL promotes TRA-1-60 (+) cells more than c-MYC, but c-MYC suppresses CD13 expression more than MYCL.
expression, whereas c-MYC-ΔMB0 upregulated the expression. MYCL-ΔMB0, unlike MYCL-WT, failed to upregulate the expression of TRA-1-60. The CD13 expression was also correlated with colony formation. In c-MYC, a significant decrease in CD13 expression was observed for mutants that promoted non-iPSC-like colony formation. As for MYCL, only a slight decrease in CD13 expression was observed for mutants that promoted iPSC-like colony formation. From these results, we concluded that the MB0 domain is essential for the function of MYC in reprogramming but functions differently between c-MYC and MYCL.

To analyze the function of the MB0 domain in more detail, we analyzed the expression of TRA-1-60 and CD13 on days 10 and 21 after the start of reprogramming by flow cytometry (Fig. 2D). In the case of c-MYC-WT, there was a strong decrease in CD13 expression on day 10, and most cells were CD13 negative on day 21. In the cases of c-MYC-ΔMB0 and MYCL-WT, there was a slight decrease in CD13 expression on day 10, and more than half of cells were expressing TRA-1-60 on day 21. Finally, in the case of MYCL-ΔMB0, there was no change in CD13 or TRA-1-60 expression. More study is needed to determine how CD13 is regulated by c-MYC and MYCL.

Additionally, c-MYC-WT showed higher cell proliferation on day 10, but c-MYC-ΔMB0 resulted in a lower cell proliferation comparable more with MYCL-WT than with c-MYC-WT on day 10 (Fig. 2E). We attributed this effect to the lost transformation activity of c-MYC-ΔMB0. From days 10 to 21, the cell proliferation increased significantly in c-MYC-ΔMB0 and MYCL-WT, and a concomitant increase in the CD13 (-) population was observed (Fig. 2D, E). These observations suggest that the number of cells that were reprogrammed increased rapidly with c-MYC-ΔMB0 and MYCL-WT. With c-MYC-WT, the cell proliferation continued until day 21. However, the CD13 (-) population hardly increased (Fig. 2D), indicating that these cells were not reprogramming but changing to other highly proliferative cell types. From these results, we concluded that the MB0 domain functions negatively in c-MYC and positively in MYCL for reprogramming.

**MYCL regulates cytoskeleton- and cell adhesion-related proteins during reprogramming via the MB0 domain.** To confirm which genes are regulated by the MYCL MB0 domain in reprogramming, we analyzed protein expressions during reprogramming because it was reported that gene expressions do not correlate well with protein expression during reprogramming. We performed an enrichment analysis of expressed proteins during reprogramming induced by c-MYC and MYCL WT and ΔMB0 mutants. We used SeV-reprogramming HDFs on days 3, 5, and 7 and EpiP-reprogramming HDFs on day 10 as samples for mass spectrometry (MS) reprogramming induced by c-MYC and MYCL WT and ΔMB0 mutants. We used SeV-reprogramming HDFs because the percentage of TRA-1-60 (+) cells was much higher with SeV than with EpiP for observations days 3, 5, and 7 days and EpiP-reprogramming HDFs on day 10 as samples for mass spectrometry (MS) reprogramming induced by c-MYC and MYCL WT and ΔMB0 mutants. We used SeV-reprogramming HDFs because the percentage of TRA-1-60 (+) cells was much higher with SeV than with EpiP for observations days. Next, we performed SeV-reprogramming HDFs on days 3, 5, and 7 and EpiP-reprogramming HDFs on day 10 as samples for mass spectrometry (MS) reprogramming induced by c-MYC and MYCL WT and ΔMB0 mutants. We used SeV-reprogramming HDFs because the percentage of TRA-1-60 (+) cells was much higher with SeV than with EpiP for observations days. We hypothesized that this domain in MYCL has reprogramming function. We therefore produced a mutant in which tryptophan 96 was substituted with glutamate (W96E). This tryptophan is equivalent to tryptophan 135 in c-MYC (Fig. 4A and Supplementary Fig. S5B). We confirmed the expression of MYCL-W96E by western blotting (Supplementary Fig. S12). Next, we analyzed the expression of MYCL-W96E for reprogramming. HDFs were transfected with reprogramming factors including MYCL-WT or -W96E. MYCL-W96E could not induce iPSC-like colonies, suggesting tryptophan 96 is crucial for reprogramming (Fig. 4B). It has been reported that the c-MYC MB2 domain is involved in transformation activity, and tryptophan 135 within its MB2 domain is necessary for this activity. MYCL also has a tryptophan residue within its MB2 domain but little transformation activity. We hypothesized that this domain in MYCL has reprogramming function. We therefore produced a mutant in which tryptophan 96 was substituted with glutamate (W96E). This tryptophan is equivalent to tryptophan 135 in c-MYC (Fig. 4A and Supplementary Fig. S5B). We confirmed the expression of MYCL-W96E by western blotting (Supplementary Fig. S12). Next, we examined the effect of MYCL-W96E for reprogramming. HDFs were transfected with reprogramming factors including MYCL-WT or -W96E. MYCL-W96E could not induce iPSC-like colonies, suggesting tryptophan 96 is crucial for reprogramming. (Fig. 4B, C). We thus hypothesized that the residue might be important for MYCL to bind to other proteins. To identify the binding proteins, we produced GST-fusion recombinant proteins of the MYCL MB2 domain (Fig. 4A). GST-MYCL-MB2-WT or -W96E proteins were immobilized on glutathione Sepharose, and affinity columns were prepared. Cell lysates were applied to the column, and, after washing, the bound proteins were eluted. We used the cell lysates from reprogramming HDFs, but since it was difficult to collect a large amount, we also used cell lysates from hiPSCs. The reason for using the hiPSC lysates is that many of the proteins expressed in reprogramming HDFs are highly expressed in hiPSCs as well. We identified 31 candidate proteins that bind to the MB2 domain of MYCL-WT but not of MYCL-W96E during reprogramming in the HDF lysates (Fig. 4D and Table 4). Of those 31 proteins, 25 proteins were also identified using hiPSC lysates, and 23 were RNA-binding proteins (RBPs; Fig. 4D, genes written in blue). Six proteins (Supplementary Fig. S11).

**MYCL regulates RNA processing-related proteins during reprogramming via the MB2 domain.** Our analysis also revealed that, along with the MYCL MB0 domain, the MYCL MB2 domain is important for reprogramming (Fig. 2B). It has been reported that the c-MYC MB2 domain is involved in transformation activity, and tryptophan 135 within its MB2 domain is necessary for this activity. MYCL also has a tryptophan residue within its MB2 domain but little transformation activity. We hypothesized that this domain in MYCL has reprogramming function. We therefore produced a mutant in which tryptophan 96 was substituted with glutamate (W96E). This tryptophan is equivalent to tryptophan 135 in c-MYC (Fig. 4A and Supplementary Fig. S5B). We confirmed the expression of MYCL-W96E by western blotting (Supplementary Fig. S12). Next, we examined the effect of MYCL-W96E for reprogramming. HDFs were transfected with reprogramming factors including MYCL-WT or -W96E. MYCL-W96E could not induce iPSC-like colonies, suggesting tryptophan 96 is crucial for reprogramming. (Fig. 4B, C). We thus hypothesized that the residue might be important for MYCL to bind to other proteins. To identify the binding proteins, we produced GST-fusion recombinant proteins of the MYCL MB2 domain (Fig. 4A). GST-MYCL-MB2-WT or -W96E proteins were immobilized on glutathione Sepharose, and affinity columns were prepared. Cell lysates were applied to the column, and, after washing, the bound proteins were eluted. We used the cell lysates from reprogramming HDFs, but since it was difficult to collect a large amount, we also used cell lysates from hiPSCs. The reason for using the hiPSC lysates is that many of the proteins expressed in reprogramming HDFs are highly expressed in hiPSCs as well. We identified 31 candidate proteins that bind to the MB2 domain of MYCL-WT but not of MYCL-W96E during reprogramming in the HDF lysates (Fig. 4D and Table 4). Of those 31 proteins, 25 proteins were also identified using hiPSC lysates, and 23 were RNA-binding proteins (RBPs; Fig. 4D, genes written in blue). Six
Figure 3. MYCL regulates cytoskeleton- and cell adhesion-related proteins during reprogramming via the MB0 domain. (A) Schematic of the mass spectrometry (MS) and GO analysis (DAVID). (B) Venn diagram of upregulated proteins during iPSC-like colony formation. (C) Molecular functions from the GO analysis of the four groups in (B). (D) KEGG pathways from the GO analysis of the four groups in (B).
(i) Proteins enriched more than two-fold in MYCL-WT compared with c-MYC-WT (SeV)

| Proteins                      |
|-------------------------------|
| NRP1                          |
| PMEL                          |
| IQCH                          |
| ZNF507                        |
| C1QTNF3                       |
| GLIPR2                        |
| ING1                          |
| THOC7                         |
| RPA1N                         |
| DGCRI8                       |
| KRAS                          |
| PIPOX                         |
| CCNL2                         |
| ACOT8                         |
| KCNA1                         |
| TTC38                         |
| MR11                          |
| DLGAP5                        |
| CHPF2                         |
| P4HA1                         |
| HMCNI                         |
| STRA13                        |
| SMG5                          |
| FAM83D                        |
| MTFR1L                        |
| TSPYL1                        |
| CROT                          |
| PLPP1                         |
| PRKACB                        |
| FSD1                          |
| REPIN1                        |
| MKLN1                         |
| CPS1                          |
| MDPI                          |
| ZWILCH                        |
| ST5GAL1                       |
| MCL1                          |
| SRR                           |
| FAM134A                       |
| USP34                         |
| CEP41                         |
| MATN2                         |
| AQP1                          |
| PLL                           |
| SON                           |
| ARL14EP                       |
| ACCS3                         |
| KHLH11                        |
| FAIM                          |
| MCMBP                         |
| COL1A1                        |
| MOCS3                         |
| SFC1                          |
| DPT                           |
| VCP1P1                        |
| TPM2                          |
| CLDN7                         |
| C18orf32                      |
| SAG                           |
| POSTN                         |
| AKAP11                        |
| AMDHD2                        |
| AHCYL2                        |
| MASTL                         |
| MAP3K2                        |
| COP2Z                         |
| ARFGF3                        |
| HBA1,HBA2                     |
| S100P                         |
| CENYL1                        |
| RALGAP8                       |
| ACTR1B                        |
| PIAS4                         |
| PFKFB3                        |
| FAM134C                       |
| SDSL                          |
| PPC1                          |
| NR3C1                         |
| FYN                           |
| SPAST                         |
| MAP4K2                        |
| COQ3                          |
| CENPV                         |
| HERC2                         |
| CDS2                          |
| TADA28                        |
| XPC                           |
| MX1                           |
| PCSK9                         |
| SDPR                          |
| CEP1J1                        |
| FEMA1                         |
| ACTG1                         |
| TNC                           |
| ITPR3                         |
| GNPTG                         |
| SH3BRL2                       |
| QSOX1                         |
| LSM4                          |
| FBXL18                        |
| SH3BP5L                       |
| FARF2                         |
| ZIC5                          |
| FASK                          |
| FLYWCH2                       |
| TMEM119                       |
| FAP                           |
| AGTPBP1                       |
| ANKIR1                       |
| EDEM3                         |
| PANXI                         |
| CDCDC28A                      |
| DDX58                         |
| FOXK2                         |
| ERIICH1                       |
| KIAA1211                      |
| ZMYM4                         |
| FN1                           |
| ARSA                          |
| CSNk1E                        |
| MTTR                          |
| NCOA3                         |
| PATZ1                         |
| UBE2S                         |
| DDB2                          |
| CCDG6B                        |
| POLG2                         |
| C18orf76                      |
| ADIRF                         |
| CALD1                         |
| RALGAPA1                      |
| NUTD9                         |
| YAEID1                        |
| C18orf412                     |
| TSPAN14                       |
| PTGIS                         |
| FAM208A                       |
| PANK1                         |
| TCN2                          |
| TAGLN                         |
| ALG8                          |
| THAP11                        |
| NFIC                          |
| TMEM1165                      |
| BLOC1S6                       |
| FAM21A                        |
| NOD2                          |
| COL12A1                       |
| TGBP1                         |
| CRELD1                        |
| MARH5                         |
| CNOT8                         |
| RANGRF                        |
| MED16                         |
| CDA                           |
| GULP1                         |
| WDR54                         |
| MET                           |
| NOA1                          |
| PRKGI                         |
| CHMP1A                        |
| SHARP1                        |
| RRPP                          |
| TBC1D7                        |
| CPQ                           |
| IFIT1                         |
| THBS1                         |
| HSDL2                         |
| GORAB                         |
| TRAF6                         |
| AHDCl                         |
| DDX60                         |
| NUDFB6                        |
| ARHGEPF6                      |
| CERCAM                        |
| NPEPL1                        |
| GPR107                        |
| MAP3K15                       |
| MSR2                          |
| ELP3                          |
| TPM1                          |
| COMMD8                        |
| MED4                          |
| HAC1L                         |
| IGBP3                         |
| HTRA1                         |
| CDD9L2                        |
| PEX16                         |
| GINS4                         |
| DSCR3                         |
| UE2E1G1                       |
| EIF4EBP1                      |
| DYNCH12                       |
| ACTN1                         |
| YPEL5                         |
| SMG6                          |
| ITGB4                         |
| PTGES                         |
| TPK1                          |
| REEP6                         |
| PFM1                          |
| PTBP2                         |
| IFIT2                         |
| PUM1                          |
| DYNC2H1                       |
| KDELRL3                       |
| VIPAS39                       |
| KIF1B                         |
| EMILIN2                       |
| GRIP2                         |
| HIGD2A                        |
| C7orf26                       |
| DNMI2                         |
| MMP2                          |
| KANK2                         |
| DIX30                         |
| RAPIB                         |
| DNBAS                        |
| MRPL33                       |
| SPANX2-OT1                    |
| PIR                           |
| SDCBP                         |
| HMGXB4                       |
| POLG                          |
| FOXK1                         |
| PEX1                          |
| LGALS8                        |
| LAMC1                         |
| DNAH6                         |
| PDIA4                         |
| MTMR14                        |
| S100A14                       |
| CNTLN                         |
| SLC25A3                       |
| TEMDE4                        |
| SPARC                         |
| GBP1                          |
| CNN2                          |
| GCC1                          |
| CTHRC1                        |
| STAU2                         |
| SUPY3L1                       |
| DNAIC16                       |
| KIAA0430                      |
| CASP4                         |
| NID2                          |
| FAM69C                         |
| TIMP2                         |
| OGFOD3                        |
| EED                           |
| DCX                           |
| PRNP                          |
| KCTD15                        |
| GSPT2                         |
| PCNT                          |
| STARD4                        |
| OTUD7B                        |
| PPP3CC                        |
| WRD35                         |
| CTSS                          |
| SLCl5A4                       |
| BASP1                         |
| SLC4A1                        |
| AKR1C2                        |
| COA3                          |
| RAB2B                         |
| GNA12                         |
| OPA3                          |
| INPP5A                        |
| GAP43                         |
| CAAPA                         |
| VW8A                          |
| PALM                          |
| KRT17                         |
| MIEF2                         |
| IKBKB                         |
| Clor198                       |
| BUB1                          |
| ZBTB7A                        |
| CD248                         |
| ACOX1                         |
| DNAI4A                        |
| CNN1                          |
| ANAPC4                        |
| LOX                            |
| LAMA5                         |
| COL2A1                        |
| KRT6A                         |
| LRRRC1                       |
| COLE6A3                       |
| CABIN1                        |
| ECM1                          |
| MED8                          |
| KIF21A                        |
| NOL8                          |
| SLC30A5                       |
| COL16A1                       |
| TWISTNB                       |
| GREM1                         |
| ICAM1                         |
| OSBP1L                        |
| TBC1D15                        |
| HORMD2                        |
| EPHA2                         |
| MRPL51                        |
| B3GALT6                       |
| USP9Y                         |
| VKORC1                        |
| ETNK1                         |
| MACF1                         |
| STAG3                         |
| SHKBP1                        |
| BCA1R                        |
| KHDBR5S3                     |
| TLE3                          |
| IGF2                          |
| STARD3NL                      |
| CTDSPL2                       |
| FHRP                          |
| RANBP10                      |
| IFT74                         |
| SERPINB2                     |
| SAMD9                         |
| FDZ7                          |
| LGALS1                       |
| CSRFP1                       |
| FBLN1                         |
| SERPINF1                     |
| SHCBP1                        |
| TUBG1                         |
| CAPN5                         |
| PTK7                          |
| PLAUR                         |
| ZNF185                        |
| SGP9                          |
| RASA3                         |
| ACSF3                         |
| DNA2                          |
| PRSS23                        |
| PKP3                          |
| GAP1A                         |
| CAV2                          |

Continued
(i) Proteins enriched more than two-fold in MYCL-WT compared with c-MYC-WT (SeV)

| Protein          | MYCL-WT | c-MYC-WT |
|------------------|---------|----------|
| FBXO2            | CCND1   | SLC34A3  |
| MAP2             | MLC13   | IFH4     |
| GATC             | TANGO6  | MITD1    |
| MYL9             | COL6A2  | PPIL2    |
| TGSI             | CDYL    | KRT10    |
| LAMB3            | CSRNP2  | MON2     |
| KLRK1            | CDC4    | ASAP2    |
| TFM4             | PPPA1   | KRT16    |
| CPLX1            | SUN1    | WDR73    |
| CCRCD2           | MYCL    | DES12    |
| TIMP3            | PKD1L3  | COL5A1   |
| SERPINB8         | FBXO3   | RNF31    |
| HSPB1            | CXB2    | IFIT3    |
| NDS8             | COL11A2 | ISG15    |
| PARP2            | GOLT1B  | FOSL1    |
| HSPB6            | ABR     | NID1     |
| COL5A2           | AURKA   | GSDMD    |
| MRGBP            | EP300   | MAU2     |
| FARP3            | ANPEP   | ARHGDI1B |
| DTX3L            | HAUS7   | LTB2P    |
| ARL5A            | RNF113A | CRBN     |
| NTSE             | CILP    | MROH2B   |
| STX3             | NOTCH3  | PLCG2    |
| SLC2A1           | S100A6  | CDC5     |
| F13A1            | COMMID9 | REN      |
| AHNKAK2          | RDH10   | CLIC3    |
| S100A4           | ZCCHC6  | CD9      |
| UAP1L1           | MED12   | PXN      |
| ITGGA2           | OASL    | CTSK     |

(ii) Proteins enriched more than two-fold in MYCL-WT compared with c-MYC-WT (EpiP)

| Protein          | MYCL-WT | c-MYC-WT |
|------------------|---------|----------|
| IGFBP3           | GLIPR2  | GBP1     |
| CN2N             | NNTR1   | ITGA11   |
| NEGR1            | LAMA5   | IFIT2    |
| TPM2             | HSPB1   | EHD2     |
| TPM1             | TGF8B1  | S100A11  |
| MOXD1            | SLFN5   | NME2P1   |
| ZFYVE16          | HSBNP1  | DGKA     |
| TC3F             | PLCB4   | SLC3A4A3 |
| PTGES            | OSBP1L9 | LRRFIP1  |
| ST6GALNAC1       | SMPD1   | SETMAR   |
| RNF14            | TNXB    | AHNAK2   |
| TERF2P           | ARMC8   | PRCC1    |
| MYCL             | BCAT2   | ELN      |
| HABP2            | TXNIP   | RMND5A   |
| CHH1             | COL6A3  | IMPACT   |
| DDR2             | KRT5    | PLEKH02  |
| GREM1            | COL5A1  | HNRNPD1  |
| QRT2R            | ARID1A  | KRT17    |
| MT1X             | CTNSNA2 | KRT6A    |
| VPS37A           | STM2    | CTSL     |
| MTPAP            | CDK2    |          |

(iii) Proteins enriched more than two-fold in c-MYC-ΔMB0 compared with c-MYC-WT (EpiP)

| Protein          | MYCL-WT | c-MYC-WT |
|------------------|---------|----------|
| ITGGA1           | PSMF1   | FNBPI    |

Bold value: $p < 0.05$
Table 1. MS analysis of identified proteins in cells reprogrammed by MYCL- or c-MYC-ΔMB0. Four groups are described: (i) proteins whose peptide counts increased more than two-fold in MYCL-WT/HDFs compared with c-MYC-WT/HDFs using SeV on day 3, 5, or 7; (ii) proteins whose peptide counts increased more than two-fold in MYCL-WT compared with c-MYC-WT using EpiP; (iii) proteins whose peptide counts increased more than two-fold in c-MYC-ΔMB0 compared with c-MYC-WT using EpiP; and (iv) commonly identified proteins. Bold fonts in the group (ii) indicate identified proteins with \( p < 0.05 \) (two-sample paired \( t \)-test). \( n = 3 \) for EpiP reprogramming.
(i) Proteins enriched more than two-fold in c-MYC-WT compared with MYCL-WT (SeV)

- ATXN7L3B
- TIMM21
- SLC2A3
- CA14
- CRLF3
- SYT6
- TMEM161A
- MTM1
- METTL315
- NKA
- CDS2
- MRS2
- MAR2S
- ERCC2
- TDP1
- MFAP4
- ANAPC16
- CARS2
- NOLC1
- IGHMBP2
- MRPL34
- FECH
- PARP2
- ING1
- ADNP2
- STEAP3
- AK6
- PDZD8
- EPB41L5
- PEX16
- ZER1
- CSNK1B
- GGPS1
- DBNDD1
- MIEF1
- FUGA1
- ADSL1
- POTEJ
- TMEM209
- CCNL2
- TOP3A
- ULK1
- MGA
- FAM162A
- AMMEC1R1
- ISG20L2
- CEP78
- NOM1
- PAPD4
- PROC
- IFRD2
- LLRC41
- UBR3
- PHF3
- RIN1
- SPL2B
- ARAF
- DNM2
- HP5
- PSEN1
- PAR3D
- ARHGGEF16
- RHNP2
- PRPF18
- SEMA4C
- RPUSD3
- NYNRIN
- ARHGGEF7
- VRTN
- PHF10
- DMD
- RPL26L1
- RANBP6
- CNOT4
- TSPY5
- CDC25C
- REEP4
- FADD
- INPP5F
- ZBTP7A
- GPN3
- RBPS2
- BRAF
- ORC6
- CACNA2D2
- APK1B
- NACP3
- RRPS8
- MASTL
- POLR2M
- CASC3
- NCL
- C1orf174
- LRRC14
- SLC27A3
- ACSF3
- DHR511
- RBB23
- WDR55
- CAMK4
- NDGR3
- ALS2
- NOVA1
- SOX3
- CLCN7
- EHHMT1
- C1orf26
- NSN5
- NMRAL1
- STK25
- NFKB2
- OSPL1A
- VPS37B
- RAD2A3
- HS2ST1
- LYAR
- PHKA1
- SDC4
- MGRT2
- SN1B1
- MEN1
- WDR4
- DDX28
- C1orf98
- AKA9
- COQ9
- STYX
- PHF5A
- PCDH1
- TMSB4X
- API92
- MYO1G
- UCKL1
- APC
- TBC1D15
- FASTKD1
- APOC1D1L
- MARH5
- ULK3
- LONP2
- SECD1A
- ETFD1
- ANKS1A
- LRP8
- PALD1
- ANAPC5
- CARMIL1
- GATM
- PANX1
- NME3
- UBA52
- ZNF806
- NROC2
- DVL2
- CTDPI
- PHKB
- GINS5
- DNP1
- CDC5
- BCKDK
- TTF1
- TGFBRAP1
- HAUS2
- TLYK
- PDCA1
- TBP
- AP1M2
- E2F4
- AFAPI1L1
- ZMYM6NB
- NBP2
- TRT1
- CCDC13
- ATL1
- INTS6
- CHD8
- SPINT2
- RASA2
- NCK2
- MAL2
- ATAD3A
- SLC25A32
- LSAMP
- ACOT8
- KIFAP3
- JARID2
- CLSTN1
- USP36
- PTGIS
- PIAS4
- TMEM41B
- SEC14L1
- TUBGCP4
- GEMIN8
- VWA9
- RPP25L
- NBP2
- DOLP1
- WAR2S
- PLEKHA6
- MRGBP
- ZCCHC6
- ZEP36L1
- SLC4A7
- SCARBI
- ARID1B
- PMF1
- XXYL1
- ANKRD50
- MT-C01
- MET
- RMB47
- LNB2B
- EXD2
- GORAB
- GCSH
- PPT
- PRKAR1
- CUTC
- SDSL
- FARS2
- LLRCC8E
- ARHGAP12
- BFSX2
- PMS2
- NAA30
- STA3
- FASTKC5
- ZCCHC10
- TTK
- BNC2
- COX16
- BCSIL
- NDE1
- STX3
- LARP18
- PTCD1
- TPD52
- SMG1
- ACBD7
- TRIP12
- PTPTM1
- ASB3
- MTG1
- ANKRD12
- STK33
- HEXIM1
- RBM45
- ATG9A
- ANKB1
- B3GALNT2
- C12orf43
- SLC25A15
- NDUF1A5
- BAG4
- NOA1
- SFRP2
- VPRBP
- FOXX1
- GMS68
- POLE
- TRADD
- AMFR
- RP66KA1
- PLA2G4A
- SELO
- PROM1
- CHF18
- BOD1
- SPC24
- KAT7
- RAB17
- IGFBP6
- PNPL4A
- AGTRAP
- UBE2Q1
- HIGD2A
- RAPH1
- SDF2
- ARHGAP4
- ODR4
- MRPS18C
- QSOX1
- COX17
- CHUK
- RAPGEP2
- GINS1
- DEFA
- CENPV
- PTPN9
- FUT11
- ERMPI
- SOGA1
- DHX32
- GEMIN6
- HDHD2
- GLE1
- PTPRZ1
- CRG1
- GATC
- PDXP
- MID1
- WRAP53
- POU2F1
- CA2
- APPL1
- TMEM14C
- TXNIP
- SLC7A3
- FABP6
- FITG2
- CWCC22
- MPDZ
- PIGG
- ACTB
- INCENP
- CARNMT1
- RHDD2
- MRPL38
- PUM1
- HPDL
- NME4
- CDKN2A
- TRIM27
- ARHGEP10L
- B3H1
- CDC26
- CTU1
- ATF7
- HMGX84
- LSMBT13
- MAP1LC3A
- ISLR
- URB1
- MRPL21

Continued
| (i) Proteins enriched more than two-fold in c-MYC-WT compared with MYCL-WT (SeV) |
|-----------------------------|-----------------------------|
| CAMK1                      | RILPL1                      |
| FCP1                        | ANKRD29                     |
| DNA2                       | CENPM                       |
| SLC35A2                    | PMS1                        |
| USP19                     | LAMA4                       |
| NHEJ1                     | RPP40                       |
| IFT7                       | ARMCX3                      |
| ARID3A                     | MRPL13                      |
| Cl1or98                  | RRP7A                       |
| DCAKD                    | ASB6                        |
| GAA                         | PDK1                        |
| ARAP3                     | CHKB                        |
| NAA40                     | IQSEC1                      |
| ZMYM3                     | MED30                       |
| MRPL10                   | NDUFAF7                     |
| ITPR2                     | PRPF39                      |
| GTP2H4                   | MRPL16                      |
| USPF9                    | SYNE2                       |
| UBE2V2                   | CDH1                        |
| COMMD9                   | HMBS                        |
| HIST1H1E                | MAP3K4                      |
| PTDS22                 | TATDN2                      |
| SCAF1                   | UBE2J2                      |
| FAM213A               | MFF                          |
| NFBY                        | PDSSB                       |
| SCAF11                  | RAPGEF1                     |
| SEPH51                    | BRD8                        |
| SLC29A1                  | MBD3                        |
| ARL14EP                | MRPL40                      |
| MRPS18B               | ACY1                        |
| RSL1D1               | PAK1                         |
| QIRC1                    | CISD1                       |
| TRABD                  | SON                          |
| ZG3HC3                | NDUFC1                      |
| CYP2S1                  | NSMCE3                      |
| KANK1                    | PHF14                        |
| LIMK2                   | CWF19L1                     |
| SLC7A5                 | VPS25                        |
| P5P1                    | CELF2                       |
| TUBB4A                | BOPI                         |
| UTP18                   | CHST14                      |
| ADAM15               | NUDCD2                      |
| HRBNPR               | MIS2                         |
| URI1                     | SLC7A8                       |
| MRPS34                 | RMND5A                      |
| RCC2                       | POLRMT                      |
| BEN3                     | BRAT1                        |
| ARL5B                   | GFM2                        |
| CHD2                   | MRPL45                      |
| CBX3                     | SIRT7                        |
| NANOOG               | SLBP                         |
| HIST1H1A            | KPN2A                        |
| HSP90AA4P          | CMS51                        |
| MED10                  | PFAS                         |
| CECR2                  | NCOA5                        |
| CHD1                   | SBN01                        |

Continued...
| Protein  | Protein  | Protein  | Protein  | Protein  |
|----------|----------|----------|----------|----------|
| CHD1L    | MLH1     | MNA1T1   | DDX47    | SRPK1    |
| XPC      | NELFCD   | MPP6     | HAU6     | TANC1    |
| MRPL33   | PUM2     | MRPL15   | FAM65A   | ER13     |
| TIMM13   | SPRYD4   | MICU1    | HMGN5    | XPO4     |
| DDX20    | YTHDC2   | SLC25A22 | CACUL1   | FC11     |
| DNAAF2   | ACTA1    | BDWD14   | DHPS     | PFKM     |
| MTMR2    | GP1      | SMARCA4  | EI24     | SCFD2    |
| RNASeqH2A| GTF2E2   | SMARCD1  | ADCK3    | TRMT1    |
| CD97     | GLT8D1   | UBR5     | HLT8     | TXNRD2   |
| CCNY     | NCPA2    | USP48    | LRCH2    | UBQLN1   |
| MCM3     | HSISA1   | ZBTB80S  | MAP2K7   | APOBEC3C|
| DYSF     | MINA     | BAK1     | HERC2    | POLG2    |
| EXOSC7   | RCL1     | CI7orf62 | PUS7     | CAP6     |
| THYNI    | MYI6     | CBR4     | RFC3     | SRSF10   |
| HIRADH   | UHRF1    | DHX57    | MCC6     | UBL4A    |
| TIFC     | GALK2    | MK167    | CEBPZ    | CHAR1C   |
| METTL3   | GSTZ1    | UBE2O    | LRSAM1   | NSA2     |
| MRPS5    | MAP3K7   | ZNF350   | MLLT11   | HK2      |
| PCGA     | LTLPL1A  | CPSF2    | PEX3     | INPP4A   |
| SLC35F2  | MRPL23   | GNL3     | WBP11    | SAP30    |
| TRC1D9B  | MRPS31   | GMPS     | IMPDH2   | SSRP1    |
| TRIM28   | NELFB    | LDAH     | DDX21    | TWSG1    |
| NDUFAF1  | PHF6     | TF2BM    | EBNA1BP2 | ZMYM2    |
| UROD     | PKP3     | NUPB2    | FANC1    | ARIH2    |
| USP11    | ARRP1    | PKN1     | EAR1     | MYBBP1A  |
| CHMP7    | COASY    | SAP18    | GTF3C4   | SMARCD1  |
| CTAGE5   | GART     | UBE2I    | MRPL27   | ENY2     |
| RUVBL1   | PEAK1    | WDR6     | MEF2F    | WPP4     |
| EXOSC4   | FRA10AC1 | ADAD2    | NARS2    | FAM64A   |
| GTF2E1   | DDX51    | BMS1     | OGRF     | MRPL3    |
| PE51     | IGF2BP3  | LDHB     | ORC3     | MSH6     |
| HEATR1   | KATNA1   | PTMA     | SLC52A2  | NLE1     |
| POLD1    | MCM2     | CXorf56  | SMAD5    | GAP4CH4  |
| FLVCR1   | MYH14    | DAXX     | ZCCHC8   | TAMM41   |
| LGALS3BP | PARN     | KIF1BP   | C5orf22  | TP53RK   |
| MRPS50   | TK1      | ELAC2    | PRKCI    | PDCD11   |
| MTMR12   | SUPV3L1  | DARS2    | RAVER1   | SGMAR1   |
| SLC3A2   | BTAF1    | HSDLI1   | EIF3C    | TP8L1    |
| MRPS28   | CADMI    | MRPL37   | SRI      | BZ2W2    |
| PCCB     | DNTTIP2  | PCB2D    | TRIM2    | CPSF1    |
| RFC5     | ECT2     | AGTPBP1  | TRIM22   | ECM29    |
| C11orf73 | MCMAM    | THUMP1D  | WDR18    | RNP51    |
| TIMM17B  | INTS8    | TUSC3    | DCAF16   | GT2A1    |
| UTP6     | MRPL11   | XRN1     | DCTPP1   | HSPBP1   |
| WDR92    | PIR      | ACA2A    | DNAJC2   | NPM3     |
| CERS6    | NUDT12   | APTX     | DSG2     | NTHL1    |
| DDX24    | NUP35    | AT2L     | NOL11    | DDX31    |
| GCDH     | RCHY1    | CI10orf10| HSLR1    | PRPS2    |
| WDR75    | RHOT1    | KIAA1211 | DDX34    | RSPRY1   |
| NOP16    | TAC01    | GLMN     | HOOKI    | WNK1     |
| PHLRA    | TBL3     | GNA1I    | KIAA2013 | C7orf50  |
| GUF1     | TIMM17A  | GRWD1    | LBH      | CCNK     |
| SMYD5    | AGL      | POR      | LG3      | CMTR1    |
| WDR5     | TOMM5    | ARL8A    | PCK2     | PDCD4    |
| ACO1     | TSC2     | GN1L3    | MTFF1    | AIT1     |
| ANKRD28  | UBTF     | STK3     | SCAP     | PPAT     |

Continued
Proteins enriched more than two-fold in c-MYC-WT compared with MYCL-WT (SeV)

APOA1BP, WDR54, SYT1, SCRIB, PTBP3, RNASEH2C
APOO, ASFI1A, GSPT2, SDHB, SUIP16H, DBR1
PSME3, PPWD1, UBQLN4, CHAF1A, TRMT10C, SACS
BYSL, CDH13, USP24, ARID1A, GJB2, TEX10
UBA2, CIULH, ARL2, ASMT, ARCC1, MRPL57
DDX56, EIF2D, ATP1B3, DCAF8, AGK, AIF1L
RBM42, FEN1, CCDC12, ELOVL6, EFNB1, NCPAPG2
SARS2, OSIPL2, CDC48, GNA13, ATF7IP, CDC50
AD1, HUA85, CiorH31, GOLM1, DEK, DTD1
MCL1, INT51, AHCY, GSTS1, PAc5, DTWD1
CCDC59, MAK16, RUVBL2, ISY1, ECT2L, GTPBP10
ZNF593, RBM26, FDXR, MR11, HS1P3, LSM6
DNAJC8, SALL4, AATF, LETM1, LAS1L, MRPS11
SMARCA5, TMPO, LTA4H, MYCBP, MRPL19, NEPRO
PCBP2, WBCR22, CCHN1, DLAG5, NDUF5, NOP58
HDAC2, WDR48, GLTSRC2, PELP1, HARS2, NT5DC1
HIST1H1B, TOX4, TP1, LUC7L, ATP11C, NFX1
HRNRNRC, VRK1, KDM2A, COMMID8, CACNA2D1, RAD21
HRNRPU, WIPI1, USP9, SSB, CDC47L, SNF8
LARS, ASH2L, VPS36, ZNF346, CRNK1, TEO2
LRRC57, AASDHPPT, CNP3, GLUL, CWC27, TNPO3
CHORDC1, CKAP2, CXADR, ILF2, PEG10, TTI2
MANBA, DCAF13, PLS1, LVRN, CIQBP, UBE2A
MEMO1, EIF4A3, EXTL2, MED24, GLYR1, UBXN1
MRPS18A, EIF5B, NIFK, POLR2H, HMOX2, ABCB6
NDUF84, FNB1P1, NOL10, KIF5C, EXOSC9, ATP2B1
TOMM40, RBM28, GSTP1, TATD1N, FTS3, DAGLB
PLCG1, IFI16, HSPA4, TME192, MPI, SMPD4
POLE4, KDM1A, NUP188, TFSM, DLAT, FAM210A
POLR2D, NOL6, PRPF40A, UAP2, MTO1, GT2I
SHPK, NUP133, RYMB2, DAR2IP, SAFB, LARP1
SPCS1, PHDA1, STX18, PPEA1, NUP50, METTL13
SAA1, QRTTD1, SUPT6H, ADSL, QSER1, POLR3C
TRMT1L, ASUN, BMP1, AFG3L2, GEMIN5, NEU1
PHEX, HDAC3, NDC60, MRRF, HMGN1, TIMM44
REEP6, OTX2, LPCAT1, MTPAP, SNU13, UMP5
TRAP1, TUT1, GSFL1, NAA20, ACSS3, NCK1
ERBB2, CCDC28B, XRN2, NDUFAP4, MIT, PPP2R5A
SGSH, CRADD, MT-ND4, GATAD2A, MRPL9, RAD50
OSGEP1, ADGR12, MTUS2, NRBF2, FAM192A, DNPPEP
THAP11, HEATR5B, RAB3E1, POP1, HEATR3, SRRM2
CACTIN, PRKD1, MRPL50, PRMT1, NIPBL, STK4
SLCA2A35, ECE2, RPL13A, SDCBP, NTMT1, TBRG4
ZIFAN6, DSEL, SDAD1, RPL21, PDCD5, PPIG
RALGAPB, MYOC5, TRMT6, RYMB2, GULP1, DCP1A
IGSF6, POLG, UBE3A, SLC30A1, RFC4, TUBAL3
MGRN1, TPK1, KCTD10, SMAP, ACIN1, UBXN7
TMEF41A1, DNAL1, F8A1,F8A2,F8A3, TKFC, BSG, ARHGEF40
FBPI, FBXL6, DFFB, TRIM33, EHHB1, ATPS5
CDC20, PLEKHA7, WDR73, CD70, EMC3, SLC9A3R1
COBL1, TRIM9, SP1, YARS2, RYMB1, CKMT1A, CKMT1B
HSP90AB1P, MRPL53, CCNA2, HICDC2, KDM3B, PPA1
SMARCAR1, MAL5U1, AURKB, PPM1G, EARS2, HSP90AA1
SH3GL3, MPPHOSPH, NACC2, HMGB2, GTPBP4, ABC810
CYP2U1, BSDC1, TLK1, LE01, MPAF1, GIT1
FLCN, TYRO3, SH3PD2A, EMC4, CPS1, INT59

Continued
(i) Proteins enriched more than two-fold in c-MYC-WT compared with MYCL-WT (SeV)

| Protein | Protein | Protein | Protein | Protein |
|---------|---------|---------|---------|---------|
| IRS2    | SIRT3   | FUT8    | CDK2    | TNFAIP6 |
| KITLG   | ALDH3A1 | GPR180  | CFAF36  | ANK3    |
| RBBP9   | EML2    | POLR3G1 | FN3K    | VWA5A   |
| ATXN7L3B| TIMM21  | SLC2A3  | CA14    | CRLF3   |
| TMEM161A| MTM1    | METTL15 | NKP     | CD52    |
| MAR52   | ERCC2   | TDP1    | MFAP4   | ANAPC16 |
| NOCL1   | KGHMBP2 | MRPL34  | FECH    | PARP2   |
| ADNP2   | STEAP3  | AK6     | PDZD8   | EPB41L5 |
| ZER1    | CKS1B   | GGP51   | DBND1   | MIEF1   |
| ADSS1L  | POT1EJ  | TMEM209 | CCNL2   | TOP3A   |
| MGA     | FAM162A | AMMERC1 | ISG20L2 | CEP78   |
| PAPD4   | PROC1   | IFRD2   | LRRC41  | UB3     |
| RIN1    | SPPL2B  | ARAF    | DNM2    | HPS5    |
| PAR3    | ARHGEF16| RHPN2   | PRPF18  | SEMA4C  |
| NVNRRN  | ARHGEF7 | VRTN    | PHF10   | DMD     |
| RANBP6  | CNOT4   | TSPY1L5 | CDC25C  | REEP4   |
| INPP5F  | ZBTB7A  | GPN3    | RBPM2   | BAPF    |
| CAGNA2D2| APJ1B1  | NCPAD3  | RRP8    | MASTL   |
| CASC3   | NCL     | Clorf174 | LRRC14  | SLC27A3 |
| DHR51   | RBM23   | WDR55   | CAMK4   | NDRG3   |
| NOA1    | SOX3    | CLCN7   | EHMT1   | C7orf26 |
| NMRL1   | STK25   | NFKB2   | OSBPL1A | VPS37B  |
| HS2ST1  | LYAR    | PHKA1   | SDC4    | MGST2   |
| MEN1    | WDR4    | DDX28   | Clorf198| AKAP9   |
| STYX    | PHF5A   | PCDH1   | TMSB4X  | AP1G2   |
| UCKL1   | APC     | TBC1D15 | FASTKD1 | APCCD1L |
| ULK3    | LONP2   | SETD1A  | EFTFDH  | ANK51A  |
| PALD1   | ANAPC5  | CARM1L  | GATM    | PANX1   |
| UBA52   | ZNF806  | NCO2    | DVL2    | CTPD1   |
| GINS3   | DNP8H1  | CDC5A   | BCKDK   | TTF1    |
| HAUS2   | HMGR8   | SNCA    | KLHDC4  | TPB     |
| EF4     | AFAP1L1 | ZMYM6NB | N4BP2   | TRIT1   |
| ATL1    | INTS6   | CHD8    | SPINT2  | RASA2   |
| MAL2    | ATAD3A  | SLC2A3  | LSAMP   | ACOT8   |
| TARID2  | CLSTN1  | USP36   | PTGIS   | PIA54   |
| SEC14L1 | TUBGCP4 | GEMIN8  | VWA9    | RPP25L  |
| DOLPP1  | WARS2   | PLEKHa6 | MRGBP   | ZCCH6   |
| SLC4A7  | SCARB1  | ARID1B  | PMFI    | XXYL1   |
| MT-CO1  | MET     | RBM47   | LIN28B  | EXD2    |
| GC5H    | PLTP    | PRKAB1  | CUTC    | SDSL    |
| LRRC8E  | ARHGA12 | FBXW9   | PMS2    | NAA30   |
| FASTKD5 | ZCCHC10 | TTK     | BNG2    | COX16   |
| NDE1    | STX3    | LARP1B  | PTC1D1  | TPD52   |
| ACRD7   | TRIP12  | PTPMT1  | ASB3    | MTG1    |
| STK33   | HEXIM1  | RBM45   | ATG9A   | ANKBI1  |
| CI2orfF3| SLC25A15| NDUFAF5 | BAG4    | NOA1    |
| VPRBP   | FOX1K1  | GPA68   | POLE    | TRADD   |
| RP56Ka1 | PLA2G4A | SELO    | PROM1   | CHTF18  |
| SCC24   | KAT7    | RAB17   | IGBP6F6 | PNPLA4  |
| UBE2Q1  | HIG2D2  | RAPH1   | SDF2    | ARHGAP4 |
| MRPS18C | QSOX1   | COX17   | CHUK    | RAPGEF2 |
| DFEA    | CENPV   | PTPN9   | FUT1I   | ERMP1   |
| DHX32   | GEMIN6  | HDDH2   | GLE1    | PTPRZ1  |
| GATC    | PDOX    | MID1    | WRAP53  | POUF1F1 |
| APPL1   | TMEM14C | TXNIP   | SLC7A3  | FABP6   |
| CWC22   | MDZD    | PIGG    | ACTB    | INCENP  |

Continued
(i) Proteins enriched more than two-fold in c-MYC-WT compared with MYCL-WT (SeV)

| Protein | TRIM27 | HMGX84 | CAMK1 | FCX1 | DNA2 | SLC35A2 | USP19 | NHEJ1 | IFT5 | ARID3A | C11orf98 | DCAKD | GAA | ARAP3 | NAA40 | ZMIM3 | MRPL10 | ITPR2 | GTP2H4 | USP9Y | UBE2V2 | COMMD9 | HIST1H1E | PEDSS2 | SCAF1 | SCAF1 | SEPHS1 | SLC29A1 | ARL14EP | MRPS18B | RSL1D1 | QRICH1 | TRABD | ZC3HC1 | SLC7A5 | TUBB4A | UTP18 | ADAM15 | HNRNPR | HSP90AA4P |
|---------|--------|--------|--------|------|------|--------|-------|-------|------|--------|---------|--------|------|-------|-------|-------|--------|--------|--------|-------|--------|---------|--------|------|-------|-------|-------|--------|--------|-------|-------|-------|--------|--------|-----|--------|--------|---------|
| RBD2    | ARHGEP10L | LMBTL3 | RILP1L | ANKRDS9 | CENPM | PMS1 | LAMA4 | RPP40 | ARMCX1 | MRPL13 | TRIM24 | MED30 | NDUFAF7 | PRP9 | SYNE2 | RPL16 | VCP1P1 | MRPL39 | SYNE2 | CDH1 | HMBS | MAP3K4 | PATJ1 | MRPL10 | TATCN2 | CISD1 | SON | TTLL12 | FXN | PDSPB | MRPL40 | CD320 | PP2 | NR4D1 | CENP10 | TCEANC2 |
| MRPL38  | BDH1    | MAP1LC3A | WDR37  | ANAPC13 | CEPI70B | SLC5A6 | DNM1L | TNC    | RAP7A  | MRPL13 | CLASP2 | NDUF47 | VCP1P1 | TPRF9 | M11H1P | BAZ1A | GFCF2 | RPPF38B | MID1P1 | GI1A | SQLE | NHU6 | HSPA4L | MRPL40 | CD320 | PP2 | NR4D1 | CENP10 | TCEANC2 |
| PUM1    | HPDL    | ISLR   | IGF2BP1 | CDIEAP | GCA   | COQ5   | INTS2 | SPNS1  | AKAP1  | CLASP2 | PRPF38B | HSPB11 | HSPB11 | PRPF39 | G12H1P | HSPB11 | AGPAT5 | CHMP6 | MCO2 | TREG2 | HSPB1 | HSPA4L | MRPL40 | CD320 | PP2 | NR4D1 | CENP10 | TCEANC2 |
| HPDL    | CTU1    | URB1   | NAPEPLD | WDR89  | CSTF2T | SPR    | BCA3 | RPRD1A | ANAPC1 | CDIEAP | TMEM236 | CASP7  | CASP7  | GCFC2 | METTL5 | CASP7  | ARFGEF2 | IGFBP2 | DIAPH2 | RPS2 | VPS8 | HSPB1 | HSPA4L | HSPA4L | CD320 | PP2 | NR4D1 | CENP10 | TCEANC2 |
| NM4     | ATF7    | MRPL21  | DPH6   | WDR89  | CSTF2T | BMR1A  | KIF22  | DDH2   | ANAPC1 | DPH6   | GTF3C2 | TAPZ1  | KIF22  | KIF22  | PRUNE  | TAPZ1  | MRPL41 | POLR3B | MCO2 | VPS4 | HSPB1 | HSPA4L | HSPA4L | CD320 | PP2 | NR4D1 | CENP10 | TCEANC2 |
| CDKN2A  |         |        |        |       |       |        |       |        |        |        |         |        |        |       |        |        |        |        |        |        |       |        |        |        |        |       |       |        |        |        |       |       |        |        |

Continued
| (i) Proteins enriched more than two-fold in c-MYC-WT compared with MYCL-WT (SeV) |
|--------------------------|----------------|----------------|----------------|----------------|
| MED10                    | PFA5           | BRIX1          | QRS1           | THNSL1         | ADRM1         |
| CECR2                    | NCOA5          | ABRACL         | WIPF2          | USP28          | GEMIN4        |
| CHD1                     | SBNO1          | LSM12          | COG2           | TARS2          | KIF11         |
| CHD1L                    | MLH1           | MNAT1          | DDX47          | SRPK1          | NAPC1         |
| XPC                      | NELFCD         | MP56           | HAUS6          | TANC1          | PPAN          |
| MRPL33                   | PUM2           | MRPL15         | EAM65A         | ERJ3           | TOM1L2        |
| TIMM33                   | SPRYD4         | MICU1          | HMGN5          | XPO4           | WDR43         |
| DDX20                    | YTHDC2         | SLC25A2        | CACUL1         | PCF11           | PRC1          |
| DNAAF2                   | ACAT1          | RWDD4          | DHPS           | PFKM           | NUDT16L1      |
| MTMR2                    | GPN1           | SMARCA4        | E124           | SCFD2          | PPP3CB        |
| RNASEH2A                 | GTTF2E2        | SMARC1D        | ADCK3          | TRMT1          | RABEP1        |
| CD97                     | GLT8D1         | UBR5           | HLTF           | TXNRD2         | TDP2          |
| CCNY                     | NCPAD2         | USP48          | LRCH2          | UBQLN1         | TRIP10        |
| MCM3                     | HSPA14         | ZFTB80S        | MAP2K7         | APOBEC3C       | TTC27         |
| DYSF                     | MINA           | BAK1           | HERC2          | POLG2          | TRAP1         |
| EXOSC7                   | RCL1           | CI7orf62       | PUS7           | CASP6          | I50C1         |
| THYNI                    | MYL6           | CBR4           | RFC3           | SRSF10         | LCLAT1        |
| HIBADH                   | UHRF1          | DHH57          | MCM6           | UBL4A          | MRPS17        |
| TFRC                     | GALK2          | MK167          | CEBPZ          | CHRAC1         | TTC4          |
| METTL3                   | GSTZ1          | UBE20          | LRSAM1         | NSA2           | AGPAT4        |
| MP55                     | MAP3K5         | ZNF330         | MLLT11         | HK2            | BLMH          |
| PCCA                     | LYPLAL1        | CPSF2          | PEX3           | INPP4A         | SORD          |
| SLC35F2                  | MRPL23         | GNL3           | WBP11          | SAP30          | INPL1         |
| TBC1D9B                  | MRPS31         | GMPS           | IMPDH2         | SSRP1          | LBR           |
| TRIM28                   | NELFB          | LDAH           | DDX21          | TWSG1          | RBMX          |
| NDUFAF1                  | PHF6           | TFB2M          | EBNA1BP2       | ZMYM2          | MLYCD         |
| UROD                     | PKP3           | NUBP2          | FANC1          | ARHI2          | ZNF22         |
| USP11                    | ARFRP1         | PKN1           | EAR1           | MYBBP1A        | PD55A         |
| CHMP7                    | COASY          | SAP18          | GTF3C4         | SMARCA1D       | DNAAF5        |
| CTAGE5                   | GART           | UBE21          | MRPL27         | ENY2           | PDK3          |
| RUVBL1                   | PEAK1          | WDR6           | MYEF2          | PWP2           | RPA2          |
| EXOSC4                   | FRA10AC1       | ADAD2          | NARS2          | FAM64A         | XRC4          |
| GTF2E1                   | DDX31          | BMS1           | OGF1R          | MRPL3          | CHAC2         |
| PES1                     | IGF2BP3        | LDHB           | ORC3           | MSH6           | DYSPL5        |
| HEATR1                   | KATNA1         | PTMA           | SLC52A2        | NLE1           | MCM7          |
| POLD1                    | MCM2           | Cxorf56        | SMAD5          | GPATCH4        | STOML2        |
| PLCR1                    | MYH14          | DAXX           | ZCCHC8         | TAMM41         | TOMM34        |
| LGAL53BP                 | PARN           | KIF1BP         | Cstorf22       | TP53RK         | NACC1         |
| MRPS30                   | TKT            | ELAC2          | PRCK1          | PDCD11         | DNAJB4        |
| MTRMR12                  | SUPV3L1        | DARS2          | RAVER1         | SIGMAR1        | DHODH         |
| SLCA32                   | BTAIF1         | HSDL1          | EIF3C          | TBPL1          | NOC2L         |
| MRPS28                   | CADM1          | MRPL37         | SRI            | BZW2           | NDUF2C        |
| PCCB                     | DNTTIP2        | PCBD2          | TRIM2          | CPSF1          | RABGCTB       |
| RFC5                     | ECT2           | AGTPBP1        | TRIM22         | ECM29          | PANK4         |
| C11orf73                 | MCAM           | THUMPD1        | WDR18          | RNPS1          | SCAO1         |
| TIMM17B                  | INTS8          | TUSC3          | DCAF16         | GTF2A1         | SIRT1         |
| UTP6                     | MRPL11         | XRN1           | DCTPP1         | HSPBP1         | TUFM          |
| WDR92I                   | PIR            | ACA2A          | DNAJC2         | NPM3           | ADNP          |
| CERS6                    | NUDT12         | APTX           | DSG2           | NTHL1          | BOLA3         |
| DDX24                    | NUP35          | ATL2           | NOL11          | DDX31          | ATRAP1        |
| GCCD9                    | RCHY1          | C12orf10       | HIP1R          | PRPS2          | MCM5          |
| WDR75                    | RHOT1          | KIAA1211       | DDX34          | RSPRY1         | CDC123        |
| NOP16                    | TACO1          | GLMN           | HOOK1          | WNK1           | NUP155        |
| POLR1A                   | TBL3           | GNA1H          | KIAA2013       | C7orf50        | GUSB          |
| GUF1                     | TIMM17A        | GRWD1          | LH1            | CENK           | ILKAP         |
| SMTYD5                   | AGL            | POR            | LIG3           | CMTR1          | LRRA          |
| Continued                |                |                |                |                |               |
| (i) Proteins enriched more than two-fold in c-MYC-WT compared with MYCL-WT (SeV) |
|---------------------------------------------------------------|
| WDRC5  | TOM5M  | ARL8A  | PCK2   | PDCD4  | NAT10  |
| ACO1   | TSC2   | GLN3L  | MTTP1  | ABT1   | KEP1   |
| ANKRD28| UBTF   | STK3   | SCAP   | PPAT   | P5A1   |
| APOAIBP| WDR54  | SYT1   | SCRIB  | PTBP3  | RNASEH2C|
| APOO   | ASI1A  | GSPT2  | SDHB   | SUPT16H| DBR1   |
| PSMF3  | PPWD1  | UQRLN4 | CHAF1A | TRMT10C| SACS   |
| B2LS   | CDH13  | USP24  | ARID1A | GJB2   | TEX10  |
| UBA2   | CLUT1  | ARL2   | AS3MT  | ABCC1  | MRPL57 |
| DDX56  | EIF2D  | ATP1B3 | DCAF8  | AGK    | AIF1L  |
| RRM42  | FEN1   | CCDC12 | ELOV6L | EFBN1  | NCPG2  |
| SARS2  | OSBP2L | CDC48  | GNA13  | AT71P  | CDCC50 |
| AD14   | HAUS8  | C1orf13| GOLM1  | DEK    | DTD1   |
| MCL1   | INTS1  | AHCY   | GYS1   | PAIC5  | DTWD1  |
| CCDC59 | MAK16  | RUVBL2 | JSY1   | ECT2L  | GTPBP10|
| ZNF593 | RMB26  | FXDRA  | MRR1   | HS1BP3 | LS6M   |
| DNAJC8 | SALL4  | AATF   | LETM1  | LAS1L  | MRPS1I |
| SMARC5 | TMPO   | LTA4H  | MYCBP  | MRPL19  | NEPRO  |
| PCB2   | WBCR22 | CCN1H  | DLGAP5 | NDUF53  | NOP58  |
| HDAC2  | WDR48  | GLTSCR2| PELP1  | HARS2  | NT5D1  |
| HIST1H1B| TOX4  | TP21   | LUC7L  | ATPI1C | NFX1   |
| HNRPDC | VRK1   | KDM2A  | COMMD8 | CACNA2D1| RAD21  |
| HNRNPC | WIP11  | USP39  | SSB    | CDC47L | SNF8   |
| L5S    | ASH1L  | VPS36  | ZNF346 | CRNKL1  | TEO2   |
| LRRC57 | AASQPPT| CNP3Y  | GLUL   | CWC27  | TNPO3  |
| CHORDC1| CKP2   | CXADR  | IFL2   | PEG10  | TT2    |
| MANBA  | DCAF13 | PL51   | LVRN   | CIQBP  | UBE2A  |
| MEMO1  | EIF4A3 | EXTL2  | MED24  | GLYR1  | UBXN1  |
| MRPS18A| EIF5B  | NPK    | POLR2H | HMOX2  | ARCB6  |
| NDUB84 | FNBP1L | NOL10  | KIF5C  | EXOS9  | ATP2B1 |
| TOMM40 | RMB28  | GSTP1  | TATDN1 | FTS1I  | DGLB   |
| PLCG1  | IF16   | HSPA4  | TMEM192| MPI    | SMPD4  |
| POLE4  | KDM1A  | NUP188 | TSFM   | DLAT   | FAM210A|
| POLR2D | NOL6   | PRPF40A| UAP2   | MTO1   | GTF2I  |
| SHPK   | NUP133 | RBM12B | DAR2IP | SAEB   | LRP1   |
| SPC51  | PDHA1  | STX18  | PPIT1A | NUP50  | METT13 |
| SALL1  | QRTTD1 | SUPT6H | ADSL   | QSER1  | POL3C  |
| TRMT1L | ASUN   | B8M1   | AFG3L2 | GEMINS | NEU1   |
| PEX6   | HDAC3  | NDC80  | MRRF   | HMGN1  | TIMM44 |
| REEP6  | OTX2   | LPCAT1 | MTPAP  | SNU13  | UMP5   |
| TRIP1  | TUT1   | GRSF1  | NAA20  | ACSS3  | NCK1   |
| ERBB2  | CCDC28B| XRN2   | NDFUAF4| MIF    | PPP2R5A|
| SGSH   | CRADD  | MT-N4D | GATAD2A| MRPL9  | RAD50  |
| OSGEPL1| ADGR1L2| MTUS2  | NRB2   | FAM192A| DNPEP  |
| THAP11 | HEATRSB| RABGEF1| POP1   | HEATR3 | SRRM2  |
| CACTN  | PRKD1  | MRPL50 | FRMT1  | NIPBL  | STK4   |
| SLC25A35| ECE2   | RPL13A | SDCBP  | NTMT1  | TBRG4  |
| ZFAND6 | DSEL   | SDAD1  | RPL21  | PDCD5  | PPG    |
| RALGAP8| MYO5C  | TRMT6  | RBM39  | GULP1  | DCP1A  |
| IGSF1  | POLG   | UHE3A  | SL3C6A1| RFC4   | TUBAL3  |
| MGRN1  | TK1    | KCTD10 | SMAP   | ACIN1  | UBN7   |
| TMEM141A| DNAL1 | F8A1,F8A2,F8A3| TKFC | BSG | ARHGEF40 |
| FBP1   | FBXL6  | DFFB   | TRIM33 | EHHFP1 | ATPS5  |
| CDC20  | PLEKHA7| WDR73  | CD70   | EMC3   | SLC9A3R1|
| COBLL1 | TRIM9  | SP1    | YARS2  | RBM19  | CKMT1A,CKMT1B|
| HSP90AB3P| MRPL35 | CCNA2  | HDC2   | KDM3B  | FPA1   |
| SMARCAL1| MALH1 | AURKB  | PPM1G  | EARS2  | HSP90A1|

Continued
(i) Proteins enriched more than two-fold in c-MYC-WT compared with MYCL-WT (SeV)

SH1GL3 MPHOSP16 NACC2 HMG22 GTPBP4 ABCB10
CYP2U1 BSDC1 TLK1 LEO1 MFAPI GIT1
FLCN TYRO3 SH3PX2D2A EMC4 CPS1 INT59
IRS2 SIRT3 FUT8 CDK2 TNFAIP6 SDHAF4
KITLG ALDH3A1 GPR180 CFAP36 ANK3 CBX2
RBBP9 EML2 POLR3GL FN3K YWA5A RAVR2

(ii) Proteins enriched more than two-fold in c-MYC-WT compared with MYCL-WT (EpiP)

FIGNL2 MCEE NDUFB7 BCCIP CLDCA6 HSPA4L
URB1 INTS14 MRPS10 PDHX SNCA RP2L61
NRN DNAJC2 NDUFAF2 WDR36 UBE2G1 INT53
NDUFB1 EIF3C WAR52 SUPV3L1 TOP2A ANKZ1
PCCA AKAP9 NOP16 EHMT2 MRPL3 KAT7
POLR3D MAPKAP1 PAIP1 GK INTS13 MT-ATP6
NF1 NAPB2 OSBPL11 IMPDH2 DIXF WDR73
BRD2 MCM7 SEXN4 HSPD1 REXO4 GEMIN4
NCAPD3 NDFIP1 BOP1 PM20D2 TRM5 TEAP3
SRR2 MTA3 TOMM40 PDSSA SLC25A17 RIDA
GTF2A2 DCUN1D5 ORC5 CDKN2APNL SET TFAM
CORO7 THTPA POLR1B HSPE1 APOO NDUF57
UBE2G2 WRAP53 HARS2 POLRMT IRF9 RBM19
PPP4R3B DPCD FBXO22 CLUH TSK1 ZNF740
ATP7A HIGD2A TIMM17A COA7 ALKBH5 TTI1
ATR ITPA PABPN1 TYMS RARS2 PHC2
MIFTNP1 FAM234A LPCAT3 BMS1 INTS11 UBE2S
VAMP3 TTC12 TNPO2 NOC3L TBL3 POLR1A
PPFB3 CARS2 PBL LSAMP ABHD11 ORC2
SH3PX2D2A INCENP SMN1,SMN2 GNL3 MRPS15 MRPL17
ZNF318 ITM2C NIP7 SYF2 UCK2 MAR8
CWF19L1 INTS9 CDK1 STK26 IRF2BP2 PTC1D
RBX1 POLR3B SLC4A7 DNF1 DMT1 DUS3L
ULK3 IFT57 NDUFAF4 NGDN PCB2 CEPT1
TTC33 ARMEX1 HDDC3 PPP1A MDC1 PES1
CWC27 TRAP1 BRX1 TOMM6 NCAPG PWP2
NOL11 DDX41 GCA DDX20 HEAT1 NT5DC2
KDM3B TASOR NCL MGST1 GTF2H4 SNA1
ZC3HC1 PALD1 EE1E1 TRMT10C NUDT3 COIL
VRK2 SYNPO2 PSMF1 PTBP3 TGS1 NOL10
DDX60 HIGD1A RAB11FIP5 CIQBP BCS1L POLD3
PHACTR4 SIK3 TRIM65 DNMT1 LYAR YY1
ASPMM CAIN1 WDR43 POLD2 WDR3 DCAF1
GPDIL MAP3K4 MYC FAM162A DDX18 WDHD1
GTPF3C MYBBP1A URB2 HMGN5 BAZ1A MDP1
STAG1 DDX21 VRK1 GUCYR2 SLC1A3 MTMR6
COX6A1 TMEM33 UBE2D3 RFC5 RRP1 NEDD4
SAAL1 NPM3 TEX10 CDK2AP1 POLR2F RSL1D1
EDC3 PL2AG4A HMG1 DDX24 TRRAP SELFNO
DTDD2 MED23 PBX1 UTP4 H1-4 FANCI
SLC35E1 PEG10 GRPEL1 DPH2 DNAJA3 HAUS1
CCDC115 NOLC1 GNL2 POLD1 MRPS2 ZNF565
PVR AKAP1 PRRX1 NDUFAF3 PARP12 STRIP1
ZCCHC3 NELFCD PODXL2 PRR35 KDM1B BMI1
TRAPP8C SPNS1 BLOC1S4 UMODL1 KIF21B C8orf33
NFIX ACP TTN RAVER2 NFATC2IP CHD1
UBE2D1 DHX38 CDK5RAP1 IDUA IRAK1 ORMD3
Continued
(ii) Proteins enriched more than two-fold in c-MYC-WT compared with MYCL-WT (EpiP)

| Protein | Protein | Protein | Protein | Protein | Protein |
|---------|---------|---------|---------|---------|---------|
| GPAA1   | B3GALT6 | XPC     | LIN7C   | VWA1    | MET     |
| METTL1  | COA     | CTIF    | SHPK    | PNKP    |         |

(iii) Proteins enriched more than two-fold in c-MYC-WT compared with c-MYC-ΔMB0 (EpiP)

| Protein | Protein | Protein | Protein | Protein | Protein |
|---------|---------|---------|---------|---------|---------|
| FAM83G  | JFT20   | DOP1R   | NOC3L   | POLR2F  | EXOC8  |
| STK25   | ASPM    | WIP2    | LSAMP   | KHNYN   | SI0A3  |
| BTAF1   | GDP1L   | OARD1   | ARF1    | HACD2   | VWA1   |
| URB1    | STAG2   | SLC25A15| SYF2    | R1DA    | METTL1 |
| PDCDHGA12| CCNYL1 | IKBKG   | GXYLT1  | CEP250  | SMARCA4|
| TGFB1   | NECA1P  | MAP3K4  | NLE1    | RBM19   | PRIM2  |
| SERPINE2| PPHLN1  | UTP3    | PTBP3   | LMF2    | SHPK   |
| SP1     | STAG1   | CD320   | YTHDF2  | ORC2    | F8A1,F8A2,F8A3 |
| GLUL    | SREKL   | ARM9C   | C7orf50 | MRPL17  | BM1    |
| PHKG2   | COX6A1  | RPL3A   | DNMT1   | MARS2   | C8orf33|
| NFI     | BR13BP  | LAS1L   | FAM162A | PTCD1   | CCDC93 |
| BRD2    | LPIN2   | PLAG2A4 | HMGN5   | CEPT1   | MET    |
| ATP6V0C | ZNF622  | PEG10   | CDK2AP1 | LIMD2   | COA4   |
| NCAIPD3 | SNX21   | CCDC58  | NUP50   | ORC4    | TT5    |
| SSR2    | NUPT16  | LRYM7   | SLC16A1 | LAGE3   | CCDC63 |
| PRIM1   | SAA1L   | WARS2   | ERCC4   | RMC1    | PODXL2 |
| GTF2A2  | DTD2    | ZNF24   | UTP4    | NOL10   | BLOC1S4|
| EPHB3   | WASF2   | NAA16   | BUD23   | POLD3   | CDK5RAP1|
| UBE2G2  | CCDC115  | BOP1   | SELENB1 | H2AC21  | SPATA5L1|
| PLCB3   | HAPLN3  | TFNFRS10B| SNCA  | CNBP    | XPC    |
| ATP7A   | DNAIC2  | ORC5    | VPS33A  | CAMLG   | MPC1   |
| PTK2    | AKAP9   | HAR52   | CTSC    | DOCK1   | MIEPE  |
| HSPA14  | THTPA   | TIMM17A | INT513  | SPR     | PVR    |
| ATR     | CEP41   | PABPN1  | REX04   | Csnk2A1| AKAP1  |
| VAMP3   | XPO4    | NOLC1   | PDF     | CCDC51  | SRC    |
| PFKFB3  | CSTF2T  | SMN1,SMN2| APOO   | CLPB    | AFP    |
| DOCK11  | QRSL1   | WRNIP1  | MSH3    | YY1     | RAVER2 |
| ZFYVE27 | TCT12   | HDDC3   | JGFP5   | HEATR5A | CRLF3  |
| RNASEH2B| CAR52   | STARD4  | SMYD3   | WDH1D1  | DDX60  |
| SH3PD2A | C17orf75| GCA     | RPL16L  | NSD2    | HIGD1A |
| TNS2    | CRB2    | TRIM65  | INT511  | MPD1    | EXOG   |
| ATG16L1 | TFRS10F2| PBX1    | IMP3    | GLE1    | POLRMT |
| TTI2    | INCNENP | UQCC1   | AATF    | MTRR6   | MRPS90 |
| ULK3    | INTS9   | NDUAFA1 | MRPS15  | THNSL1  | RFP1   |
| TTC33   | MTRR    | EHMT2   | LIG1    | RBFOX2  | CDC16  |
| KDM3B   | TRAP1   | HSPD1   | POLA2   | PKMTY1  | KIF21B |
| TTC21B  | PTBP2   | UBR5    | CCDC171 | MAP3K7  | NFATC2P|
| HEATR3  | PALD1   | CDKN2AIPN| TGS1   | RSL1D1  | TER2   |
| ZC3HC1  | ISY1    | NAF1    | RAZ1A   | FANCI   | RIFI   |

(iv) Commonly enriched proteins in (i) and (ii) and (iii)

| Protein | Protein | Protein | Protein | Protein | Protein |
|---------|---------|---------|---------|---------|---------|
| KDM3B   | HMGN5   | URB1    | LSAMP   | MET     | DNAJC2 |
| RBM19   | AKAP1   | INTS9   | SNCA    | MARS2   |         |

Continued
proteins were identified only in the reprogramming HDFs lysates: HNRNPK, DDX17, C1QBP, KBTBD3, COPG2, and SIKE1, of which HNRNPK, DDX17, and C1QBP are RBPs. From these results, there were 26 RBPs identified in the HDF lysates in total. We confirmed the function of the 31 proteins using a public database (https://www.nextprot.org)\textsuperscript{28} and found 16 of them are involved in RNA processing. A GO analysis using DAVID also showed that the 31 proteins are related to controlling pre-mRNA splicing, capping, and polyadenylation, suggesting functions in mRNA export, turnover, localization, and translation (Fig. 4E). These results suggested that MYCL interacts with RBPs via its MB2 domain and promotes reprogramming by post-transcriptional regulation.

Discussion

Here we described the molecular function of MYCL during reprogramming and compared it to the c-MYC function by focusing on MYC Box domains. We found that the MB0 and MB2 domains are important for reprogramming, and deleting either region compromised the reprogramming ability of MYCL. Proteomic analysis revealed that MYCL regulates the expression of cell adhesion-related proteins during reprogramming via the MB0 domain (Fig. 3C, D). We also found the possibility that the same domain is regulated by post-translational modifications (PTM), as discussed below. It is known that cell-substrate adhesion is closely related to the mesenchymal-epithelial transition (MET)\textsuperscript{30–32} and that MET occurs during the reprogramming process\textsuperscript{30–32}. We speculate that MYCL promotes iPSC-like colony formation via the MET process by upregulating cell adhesion-related genes. Furthermore, we identified that the MB2 domain is required for MYCL to promote reprogramming by binding to RBPs, especially RNA processing-related proteins (Fig. 4D, E). It has been reported that RBPs regulate MET through post-transcriptional regulation. For example, heterogeneous nuclear ribonucleoprotein (hnRNP) A1 regulates the alternative splicing of Rac1 to control MET\textsuperscript{33}. These findings suggest that MYCL regulates the RNA processing of cell adhesion-related genes transcribed by MYCL itself or other genes. Therefore, we hypothesize...
### (i) Proteins enriched more than two-fold in MYCL-WT compared with MYCL-ΔMB0 (EpiP)

| Protein 1 | Protein 2 | Protein 3 | Protein 4 | Protein 5 |
|-----------|-----------|-----------|-----------|-----------|
| UBQLN2    | DYNLRB1   | EXOC2     | KRT17     | NANS      |
| REEP5     | RAB11B    | ARPC2     | TPM1      | UTP15     |
| SGC6      | WDR46     | CD47      | SLC44A3   | RASA1     |
| TUBB2A    | ALDH6A1   | STRN3     | FBXV10    | BAP18     |
| HMGFB1    | POTEF     | PDHA1     | CAMK2D    | TM9SF3    |
| PPIG      | COMMD4    | SCP2      | MT-ATP6   | YIPF5     |
| HMGFB3    | TUSC3     | COL3A1    | ARF4      | ATP5PB    |
| PCNP      | MYD88     | GNB2      | NUCK5I    | LIMS1     |
| SYAP1     | RAC1      | CGG6      | TMED2     | LRBA      |
| CHMP4B    | MACF1     | SOX2      | PRKACA    | MAPK14    |
| UTP3      | SRF511    | SSR1      | MID1      | DUSP12    |
| BLOC1S1   | EXOC5     | CSTF3     | PCID2     | THOC3     |
| ST6GALNAC1| DNAJC9    | CACNA2D1  | TRIP12    | SRSF5     |
| MAP3K20   | MICAL1    | NRDC      | GADD45P1  | VAC14     |
| KNTC1     | POLR2L    | MSRB3     | GTF2I     | PDXDC1    |
| BAG5      | SFXN3     | NOL11     | ERLIN2    | ZNF462    |
| CD320     | CRIP1     | OSTC      | DBNL      | ITGB1     |
| MRPL11    | NAA10     | RPL37A    | STT3A     | CTN      |
| TFG       | ARF6      | IZIC      | PAFAH1B1  | EEF1B2    |
| THYN1     | CCDC43    | BMP1      | SLC25A24  | UGP2      |
| NDRG3     | NEK7      | PDCL3     | HOOK3     | LSM2      |
| TMX4      | TUBB4B    | UGP2      | LAS1L     | ACTB      |
| SEPHS1    | MYO1E     | TNS3      | HLA-H     | RABL3     |
| RPL36A    | FNTA      | VPS268    | DCN       | RVDD1     |
| MYDGF     | SRBD1     | EHD1      | PUM1      | TOMM20    |
| OPTN      | DB1       | ANKY1F    | PUS7      | CRABP2    |
| GNAQ      | SUGP2     | KRTBD3    | KPNA4     | VDAC3     |
| ACSL4     | MTAI      | SCPEP1    | METTL26   | EDIL3     |
| ATP5ME    | MAP7D1    | FBLN2     | B4GALT4   | PLAG4A    |
| AB3BP     | ACTG1     | LDR1      | MBD5      | CTNNA1    |
| ASAH1     | HINT1     | EXOSC7    | CSRPI     | RPL23A    |
| GNS       | HMGN1     | DIP2B     | GNB1      | TEME165   |
| WDR61     | PTGR1     | PIRTM1    | SNRBP2    | DNAH6     |
| ARL8A     | TM8B4X    | METTL14   | CNN2      | DPP9      |
| MAP3K20   | FAM114A1  | TM8B10    | PPB1      | ENOD1     |
| NDRG1     | FTH1      | CNPY3     | S100A10   | NDUFB11   |
| PITPNA    | SGTA      | HABP2     | Clorfr198 | NAA30     |
| NIF3L1    | SGPL1     | SRF9      | MARCKSL1  | DNAJC8    |
| NME2      | CD59      | NDUBF9    | TOR1AI1P  | NXX       |
| PFDN1     | DHR54     | RBP1      | NDUF4A4   | MRPS17    |
| ATG3      | GSP1T1    | DCTN5     | ACS82     | REXO2     |
| ACIN1     | BLOC1S3   | TEMD1     | GSTK1     | PEBP1     |
| RAB14     | RFC3      | AKR1B1    | ISLR      | S100A13   |
| SNAP23    | CD55      | TALDO1    | NOP14     | SLC25A6   |
| EMC2      | RPS15A    | DSTD      | POLR2A    | OSBPL3    |

### (ii) Proteins enriched more than two-fold in c-MYC-ΔMB0 compared with MYCL-ΔMB0 (EpiP)

| Protein 1 | Protein 2 | Protein 3 | Protein 4 | Protein 5 |
|-----------|-----------|-----------|-----------|-----------|
| UBQLN2    | ACIN1     | NEK7      | PIRTM1    | PDLM5     |
| HMGFB1    | SRF511    | RAB11B    | ITGA1     | H3-3A,H3-3B |
| REEP5     | OPTN      | ANKY1F1   | ISLR      | Clorfr198 |
| NDRG1     | KNTC1     | CD47      | SRF55     | LUZP1     |
| SGC6      | PAIP1     | PRKACA    | ERLIN2    | IDH1      |
| MAP3K20   | NIF3L1    | SCR3N     | MRPS17    | HABP2     |
| TUBB2A    | POTEF     | FAM114A1  | TOMM20    | PAFAH1B1  |
| CCDC43    | COG6      | NOLC1     | UGP2      | ITGA5     |
| PCNP      | TSPYL5    | COMMD4    | LSM2      | CHIC3     |
| TMX4      | DFNLRB1   | EHD1      | UTP15     | SMTN      |

Continued
(ii) Proteins enriched more than two-fold in c-MYC-ΔMB0 compared with MYCL-ΔMB0 (EpiP)

|   |   |   |   |   |   |
|---|---|---|---|---|---|
|   |   |   |   |   |   |
| BLOC51S | STRN3 | PPP4C | PUM1 | DUSP12 | VPS26B |
| SYAP1 | ASAH1 | FNTA | MYO1E | MEAP6 | APP |
| PPIC | ATP5ME | GNAQ | TP53RK | NMT1 | GALNT1 |
| NME2 | OVCA2 | SEMA7A | POLR2L | NDUF4A | SSR1 |
| ACSL4 | RAC1 | RPL36A | MYO6 | LIMS1 | YIF1A |
| HMGB3 | OSTC | FTH1 | PUS7 | CLASP2 | UGP2 |
| FBXW10 | DBI | PTGR1 | PIK3R4 | ILF3 | USP48 |
| MAP3K20 | PDHA1 | KRT17 | MYD88 | RASA1 | PPIB |
| NDRG5 | SFXN3 | ARPC2 | REXO2 | GALE | METTL26 |
| GNS | TNS3 | ABI1 | DBNL | TXLNG | MYO1D |
| UTP3 | DHR54 | PAIP1 | USP15 | PPL3 | NDUF6 |
| SEPHS1 | MIPD1 | PCD2 | SRBD1 | RFC3 | PPIF |
| BAG5 | DNAJC9 | SEC24A | ACTG1 | SCLY | SMARCC2 |
| ATG3 | MAPK14 | UBQLN1 | LZIC | MPV17 | STAM2 |
| SCPEP1 | MRPS24 | MTA1 | TRIP12 | VAC14 | BMP1 |
| TFG | PLA2G4A | BAP18 | THOC3 | DCTN5 | PGM2 |
| MT-ATP6 | NOL11 | METTL1 | SCP2 | COG7 | CERS2 |
| MRPL11 | SUGP2 | EXOC5 | POLR2A | MCRIP1 | SLT3 |
| CHMP4B | TUSC3 | BLOC1S3 | CSTF3 | CTNNA1 | HOOK3 |
| SGPL1 | DIP2B | DUSP23 | GNB2 | SENP3 | COL3A1 |
| MICAL1 | PFDN1 | SOX2 | GSPT1 | EIF2B1 | ITGB1 |
| DCN | NRDCl | LDLR | GADD45G | SETD7 | AGK |
| CUL4A | SGTA | CNP43 | KPA4 | DNASE2 | MPSOSPH10 |
| ARF6 | PODXL | MPI | FADS2 | RAI14 | BOLA2,BOLA2B |
| HLA-H | ARL8A | HINT1 | NAA2 | TPMT | IMPACT |
| MACF1 | PDXDC1 | GSTK1 | ATAD1 | EDIL3 | USP9X |
| THYN1 | CD20 | CD59 | METAP1 | IGFR2 | OC1AD2 |
| MYDGF | PITPNA | FBLN2 | TPM1 | NAA50 | CAGNA2D1 |
| NACA4P | MAP7D1 | NANS | MBD5 | EEF1B2 | YIF5 |
| NAA10 | WDR46 | TM9SF3 | DCTD | EEA1 | TMSB10 |
| NDUF9B | LRRC17 | YAP1 | GTF2I | CD55 | SNAP23 |
| ABI3BP | WDR61 | RAL3 | PRKRA | DNAJC8 | EIF3H |
| SNW1 | LMCDD1 | KLF4 | PSMD4 | S100A10 | KBTBD3 |
| SNRPB2 | COL4A2 | CFL2 | LMS5 | FBXO22 | ACYP1 |
| ARF4 | VDAC3 | ALDH6A1 | CYS51A1 | CBX3 | SNX27 |
| ATP5MG | CNI1 | CLINT1 | ACS52 | EEF2B3 | SLC2SA24 |
| CTTN | CALD1 | DYNC1LI1 | NDUF4F2 | S100A13 | CDC42BPB |
| TEMPO | EWSR1 | ANTXR2 | TP53BP1 | RBM17 | IDH3G |
| RPL37A | SPAT2L | PHLD1B1 | CARHS1 | CEFAP74 | EMC2 |
| PLS1 | HSD17B7 | FAP | NNX | TUBB4B | SF3A3 |
| MTR | OSTF1 | TMSB4X | SRP9 | VASP | EXOC2 |
| NCBP1 | SFXN4 | NUCKS1 | RPS29 | MANF | COPS6 |
| VPS4B | AHNAK | CSR1P1 | SLC25A6 | CARM1 | FKBP2 |
| TMOD3 | RBP | TALDO1 | ATP5PB | PRPF4B | TBCC1D15 |
| GFOX | B4GALT4 | MSRB3 | DNAH6 | COX7C | OGRF |
| ALDH11L2 | CRIPI | AP3M1 | SCAMP2 | Cieorf50 | ELN |
| TSN | TOR1B | PDS5A | PDA4 | RWDD1 | GABA |
| DYNNL1 | USP47 | ENDO1 | NUCB2 | GINS4 | DRI |
| FKBP5 | OAS2 | MRTO4 | NF51 | NPC2 | ABI2 |
| SERPINE2 | NDUF4F | ZYX | HACD3 | EXOSC7 |

(iii) Commonly enriched proteins in (i) and (ii)

|   |   |   |   |   |   |
|---|---|---|---|---|---|
|   |   |   |   |   |   |
| WDR46 | TUSC3 | RBP | NIF1 | NIF3L1 | DNAJC8 |
| MYO1E | MYD88 | FBXW10 | ARF4 | TM9SF3 | TEMPO |
| RAC1 | SFXN3 | CAMK2D | MPDU1 | CTNNA1 | EEF1R2 |
| TMSB10 | MACF1 | NUCKS1 | OVCA2 | CHMP4B | COL3A1 |
| LDLR | DBNL | PRKACA | ANP32B | PCNP | S100A10 |

Continued
that transcriptional and post-transcriptional regulation by MYCL promotes MET, which increases the efficiency of reprogramming and leads to higher quality iPSCs.

Western blotting revealed that MYCL protein has a unique expression pattern (Supplementary Fig. S8 and S12). The calculated molecular weight of MYCL is about 40 kDa (364 aa), but we detected three strong bands at around 60 kDa, which we verified with second antibody (Supplementary Fig. S13). Since the expression of MYCL-ΔMB0 showed a strong single band, we speculate that the MYCL MB0 domain is the PTM site (Supplementary Fig. S8). Such a phenomenon was not observed in c-MYC (Supplementary Fig. S7). One possible type of relevant PTM is phosphorylation. Phosphorylation is crucial for protein function. For example, RNA polymerase II (Pol II) is required for transcription pauses in a promoter-proximal position during transcription initiation. In order to initiate transcription, the C-terminal domain of Pol II must be phosphorylated by P-TEFb34. In addition, the phosphorylation of c-MYC on threonine 58 in the MB1 domain promotes c-MYC binding to F-box and WD repeat domain containing 7 (FBXW7), causing the ubiquitination of c-MYC, which triggers c-MYC degradation35. Similarly, MYCL might undergo phosphorylation to change its activity and interaction with binding proteins. However, this hypothesis requires further study.

Comprehensive proteomic analysis suggested that the MYCL MB0 domain influences the expression of cell adhesion-related proteins, and MYCL shows an up-regulation of phosphorylated cytoskeletal proteins (Fig. 3C, D, and Supplementary Fig. S11A). Cell adhesion is mediated by adhesion molecules, such as integrins and cadherins, which function in the extracellular matrix (ECM) and cell–cell adhesion and are important for cell

Table 3. MS analysis of identified proteins in cells reprogrammed with MYCL-WT and c-MYC-ΔMB0 compared with MYCL-ΔMB0. Three groups are described: (i) proteins whose peptide counts increased more than two-fold in MYCL-WT compared to MYCL-ΔMB0; (ii) proteins whose peptide counts increased more than two-fold in c-MYC-ΔMB0 compared to MYCL-ΔMB0; and (iii) commonly identified proteins. n = 3.
Figure 4. MYCL regulates RNA processing-related proteins during reprogramming via the MB2 domain. (A) W96 and W135 in the MB2 domain of MYCL and c-MYC, respectively. The structure with the recombinant protein of the MB2 domain of MYCL-WT/W96E is shown below. The numbers on the right indicate amino acid lengths. (B) The number of iPSC-like and non-iPSC-like colonies derived from 1 × 10^5 HDFs transduced with EpiP including MYCL-WT or MYCL-W96E on day 21. Mean ± SD values are shown. n = 3, *p < 0.05 by unpaired t-test. (C) Representative images of reprogramming HDFs 21 days after the transduction of EpiP, including MYCL-WT or MYCL-W96E. Scale bars, 100 µm. (D) Venn diagram of enriched proteins between reprogramming HDFs and hiPSCs by AP-MS. A list of the 25 commonly enriched proteins is shown below. Blue indicates RBP (23 in total). (E) Molecular function from the GO analysis of the 25 commonly identified proteins in (D).
communication and the regulation of fundamental physiological processes such as tissue development and maintenance through focal adhesion localization, and appropriate adhesion to the ECM is required to regulate reprogramming via MET and maintain pluripotency. Accordingly, our study supports MYCL regulating cell-substrate adhesion through its MB0 domain to promote reprogramming. In other words, MYCL might regulate proteins involved in cell adhesion and the cytoskeleton directly or indirectly to cause MET and promote reprogramming. In c-MYC, loss of the MB0 domain positively affects iPSC-like colony formation, suggesting that this domain has a different function compared to MYCL. This functional difference is somewhat surprising since the domain is well conserved. We would like to clarify this point in the future.

We also found that the MB2 domain has an important function in MYCL-reprogramming (Fig. 2B,C). Deleting the MB2 domain completely compromised the reprogramming ability of MYCL. In c-MYC, the MB2 domain has an important function in transformation activity, and tryptophan 135 in the MB2 domain is essential for this activity. The equivalent tryptophan residue in MYCL is tryptophan 96. MYCL has little transformation activity, but we showed that the mutation of tryptophan 96 completely lost the reprogramming ability of MYCL. To further investigate the function, we sought interacting proteins by affinity column chromatography. We found 31 proteins, including 26 RBPs, that interact with the MYCL MB2 domain (Table 4, genes written in blue). A GO analysis suggested that some of the 31 proteins are involved in RNA processing (Table 4). It has been reported that altered RNA processing affects somatic cell reprogramming. Therefore, we hypothesize that MYCL also promotes MET in reprogramming by regulating RNA processing via interactions with RBPs at its MB2 domain. An illustrative summary of how MYCL regulates cell reprogramming through these two domains is shown in Fig. 5.

Table 4. AP-MS analysis of identified proteins in MYCL-MB2-WT using cell lysates from reprogrammed HDFs and hiPSCs. Three groups are described: (i) protein interactors whose peptide counts increased in reprogramming HDFs more than two-fold in MYCL-MB2-WT compared with MYCL-MB2-W96E; (ii) protein interactors whose peptide counts increased in hiPSCs more than two-fold in MYCL-MB2-WT compared to MYCL-MB2-W96E; and (iii) commonly identified proteins. n = 1.

| Table 4 | AP-MS analysis of identified proteins in MYCL-MB2-WT using cell lysates from reprogrammed HDFs and hiPSCs. Three groups are described: (i) protein interactors whose peptide counts increased in reprogramming HDFs more than two-fold in MYCL-MB2-WT compared with MYCL-MB2-W96E; (ii) protein interactors whose peptide counts increased in hiPSCs more than two-fold in MYCL-MB2-WT compared to MYCL-MB2-W96E; and (iii) commonly identified proteins. n = 1. |
Further elucidation of the function of MYCL in reprogramming will improve the quality and efficiency of iPSC generation.

**Material and methods**

**Cell culture.** HDFs (106-05f.) were purchased from Cell Applications, Inc. HDFs were cultured in DMEM (08459-64, Nacalai Tesque) supplemented with 10% FBS (10439-024, gibco) and 1% penicillin and streptomycin (15140-122, Pen/Strep, gibco). The hiPSC clone 201B7 was used in this study. iPSCs were cultivated on iMatrix-511 (NP892-012, Nippi)-coated (0.5 μg/cm²) cell culture plates with StemFit (AK03N, Ajinomoto) supplemented with bFGF and passaged via dissociation into single cells using TrypLE Select (A12859-01, Life Technologies) on day 7 following a previously reported protocol.

**Generation of iPSCs.** A frozen stock of HDFs was thawed and cultured for four days, and then 1 × 10⁶ cells were collected by trypsinization. With SeV, HDFs were transduced with the CytoTune-iPS 2.0 (c-MYC) or CytoTune-iPS 2.0L (MYCL) Sendai Reprogramming Kit (DV-0304, DV-0305, ID Pharma). With Epip, HDFs were electroporated with 1.2 μg of plasmid mixtures with the Neon Transfection System (MPK1096 and MPK10096, Invitrogen). The plasmid mixtures included pCXLE-SOX2, -KLF4, -OCT3/4-shp53, -LIN28A, and pCXWB-EBNA1 with wild-type or mutant pCXLE-c-MYC or -MYCL. The mixing ratio of SOX2, KLF4, OCT3/4-shp53, LIN28A, EBNA1, and c-MYC/MYCL was 1:1:2:1:0.5:2. After that, the cells were plated in a 6-well plate and cultured in StemFit AK03N without bFGF at 0.25 μg/cm² in SeV or 0.125 μg/cm² in Epip. The culture medium was changed the next day and every three days after that. The colonies were counted 21 days after plating.

**Immunostaining.** Stained cells were imaged using a BZ-9000 imaging system (KEYENCE) or ArrayScan High-Content Systems (Thermo Fisher Scientific). HCS Studio 2.0 Cell Analysis Software (Thermo Fisher Scientific) was used to quantify cell counts and signal intensities. The Cellomics BioApplication system (Thermo Fisher Scientific) was programmed to capture and analyze 25 images per well. The total cell number was calculated by dividing this number by the total cell number.

**Flow cytometry.** Transduced cells were harvested with 0.25% trypsin/1 mM EDTA (25200-056, gibco) each day after the transduction for the analysis. At least 5 × 10⁴ cells were stained with the following antibodies in FACS buffer (2% FBS, 0.36% glucose (16806-25, Nacalai Tesque), 50 μg/μL Pen/Strep in PBS) for 30 min at room temperature: BV510-conjugated anti-TRA-1-60 (1:40, 563188, BD Biosciences) and PE-Cy7-conjugated anti-CD13 (1:40, 561599, BD Pharmingen, and 1:500, 09-0068, Stemgent) and Alexa 488-conjugated goat anti-mouse IgG, IgM (H + L) (1:250, A10680, Invitrogen) were used as the antibodies.

**Western blotting.** Proteins on an SDS-PAGE gel were transferred to a PVDF membrane (IPVH00010, Immobilon-P, Millipore) and probed with the following antibodies using an iBind Flex system (SLF2000, SLF2010 and SLF2020, Invitrogen): anti-human MYCL (1:250, AF4050, R&D) (1:250, C-20, sc-790, Santa Cruz), anti-human c-MYC (1:500, 9E10, sc-40, Santa Cruz, and 1:500, D84C12, CST), anti-β-actin (1:1000, A5441, Sigma), anti-Goat (1:3000, ab6741-1, abcam), anti-mouse (1:3000, 7076S, CST), and anti-rabbit (1:3000, 7074S, CST) antibodies.
Preparation of recombinant proteins and affinity purification (AP). The MB2 region of MYCL-WT or -W96E was cloned into pGEX-6P-1. The plasmids were transformed into BL21 E. coli (DE3) (Promega) competent cells. The fusion proteins, GST-MYCL-WT-MB2 and GST-MYCL-W96E-MB2, were induced by treatment with 0.5 mM IPTG (19742-94, Nacalai Tesque) for 4 h at 37 °C. The proteins were purified using glutathione Sepharose beads (17-0756-01, GE Healthcare). Human iPSCs or reprogramming HDFs were lysed in RIPA buffer (20 mM Tris/HCl (pH 7.6) (35436-01, Nacalai Tesque), 1% NP-40 (25223-75, Nacalai Tesque), 0.1% SDS, 150 mM NaCl (31320-05, Nacalai Tesque), and protease inhibitor (25955-11, Nacalai Tesque)) and then centrifuged. Cell lysates (supernatant) were transferred into a column (29922, Thermo Fisher Scientific) packed with beads conjugated with GST-MYCL-WT or -W96E proteins. After washing, binding proteins were eluted in lysis buffer (12 mM sodium deoxycholate (190-08313, Wako), 12 mM sodium lauroyl sarcosinate (192-10382, Wako), and 100 mM Tris-HCl (pH9.0) (314-90381, NIPPON GENE)) for the MS analysis. The iPSC lysates were prepared 6 days after passaging in two 10-cm dishes (n = 1), and reprogramming HDF lysates were prepared 3 days after SeV transduction in two 10-cm dishes (n = 1).

GO analysis by DAVID. The Database for Annotation, Visualization, and Integrated Discovery (DAVID Bioinformatics Resources 6.8) was used to identify enriched biological GO terms and KEGG pathway. For more information, please visit the DAVID website (https://david.ncifcrf.gov/home.jsp) and KEGG Database website (https://www.kegg.jp/kegg/kegg1.html). The methods for MS are described in the Supplementary methods.
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Correspondence and requests for materials should be addressed to M.N.

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