haematologica
the hematology journal

Early Release Paper

NUP214-ABL1 mediated cell proliferation in T-cell acute lymphoblastic leukemia is dependent on the LCK kinase and various interacting proteins

by Kim De Keersmaecker, Michaël Porcu, Luk Cox, Tiziana Girardi, Roel Vandepoel, Joyce Op de Beeck, Olga Gielen, Nicole Mentens, Keiryn L. Bennett, and Oliver Hantschel

Haematologica 2013 [Epub ahead of print]

Citation: De Keersmaecker K, Porcu M, Cox L, Girardi, T Vandepoel R, de Beeck JO, Gielen O, Mentens N, Bennett KL, and Hantschel O. NUP214-ABL1 mediated cell proliferation in T-cell acute lymphoblastic leukemia is dependent on the LCK kinase and various interacting proteins. Haematologica. 2013; 98:xxx
doi:10.3324/haematol.2013.088674

NUP214-ABL1 mediated cell proliferation in T-cell acute lymphoblastic leukemia is dependent on the LCK kinase and various interacting proteins

Kim De Keersmaecker,[1,2*] Michaël Porcu,[1,2*] Luk Cox,[1,2] Tiziana Girardi,[1,2] Roel Vandepoel,[1,2] Joyce Op de Beeck,[1,2] Olga Gielen,[1,2] Nicole Mentens,[1,2] Keiryn L. Bennett,[3] and Oliver Hantschel[4]

[1] Center for the Biology of Disease, VIB, Leuven, Belgium; [2] Center for Human Genetics, KU Leuven, Leuven, Belgium; [3] CeMM Research Center for Molecular Medicine of the Austrian Academy of Sciences, Vienna, Austria, and [4] Swiss Institute for Experimental Cancer Research (ISREC), School of Life Sciences, École polytechnique fédérale de Lausanne, Lausanne, Switzerland

** K.D.K. and M.P. contributed equally to this manuscript*

SHORT TITLE

Critical proteins in NUP214-ABL1 positive T-ALL

CORRESPONDENCE

Oliver Hantschel, Swiss Institute for Experimental Cancer Research (ISREC), École polytechnique fédérale de Lausanne, Station 19, 1015 Lausanne, Switzerland.
E-mail: oliver.hantschel@epfl.ch

Kim De Keersmaecker, Center for Human Genetics and VIB Center for the Biology of Disease, Gasthuisberg O&N4, Herestraat 49 (box 602), 3000 Leuven, Belgium.
E-mail: kim.dekeersmaecker@cme.vib-kuleuven.be

ACKNOWLEDGMENTS

K.D.K. is a post-doctoral fellow of the FWO Vlaanderen. M.P. is supported by the Agency for Innovation by Science and Technology in Flanders (IWT), Flanders, Belgium.

FUNDING

This research was supported by an FWO 'krediet aan navorsers' research grant to K.D.K. (grant 1510712N) and generous support from the ISREC Foundation to O.H. We would like to thank J. Cools and G. Superti-Furga for their continuous support, inspiring discussions and critical comments throughout this project.

ABSTRACT

The NUP214-ABL1 fusion protein is a constitutively active protein tyrosine kinase that is found in 6% of patients with T-cell acute lymphoblastic leukemia and that promotes proliferation and survival of T-lymphoblasts. Although NUP214-ABL1 is sensitive to ABL1 kinase inhibitors, development of resistance to these compounds is a major clinical problem, underlining the need for additional drug targets in the sparsely studied NUP214-ABL1 signaling network. In this work, we identify and validate the SRC family kinase LCK as a protein whose activity is absolutely required for the proliferation and survival of T-cell acute lymphoblastic leukemia cells that depend on NUP214-ABL1 activity. These findings underscore the potential of SRC kinase inhibitors and of the dual ABL1/SRC kinase inhibitors dasatinib and bosutinib for treating of NUP214-ABL1 positive T-cell acute lymphoblastic leukemia. In addition, we used mass spectrometry to identify protein interaction partners of NUP214-ABL1. Our results strongly support that the signaling network of NUP214-ABL1 is distinct from that previously reported for BCR-ABL1. Moreover, we identify three NUP214-ABL1 interacting proteins, MAD2L1, NUP155, and SMC4, as strictly required for the proliferation and survival of NUP214-ABL1 positive T-cell acute lymphoblastic leukemia cells. In conclusion, this work identifies LCK, MAD2L1, NUP155 and SMC4 as four new potential drug targets in NUP214-ABL1 positive T-cell acute lymphoblastic leukemia.

INTRODUCTION

T-cell acute lymphoblastic leukemia (T-ALL) is an aggressive malignancy of T-cell progenitors occurring in adults and children. With current chemotherapy protocols, the outcome for pediatric T-ALL has improved to cure rates over 75%. However, these therapies are highly toxic. Moreover, adult T-ALL patients, patients with primary resistance to the chemotherapy or relapsed patients have an extremely poor prognosis, underscoring the need for novel therapeutic strategies.[1,2]

T-ALL is a genetically heterogeneous disease that is caused by accumulation of multiple genetic defects in developing T-cells, affecting critical cellular processes such as cell differentiation, proliferation, survival and self-renewal capacity.[3] Approximately 8% of T-ALL cases harbor fusions that involve the *ABL1* tyrosine kinase gene.[3] BCR-ABL1, which is the prototypical ABL1 fusion kinase in chronic myeloid leukemia (CML) and subsets of B-cell ALL (B-ALL), is only sporadically found in T-ALL. In contrast, 6% of T-ALL cases express the constitutively active NUP214-ABL1 fusion kinase, which consists of the N-terminal region of the nuclear pore complex protein NUP214 and of the same C-terminal part of ABL1 as in BCR-ABL1.[4] Recently, the NUP214-ABL1 fusion was also identified in B-ALL.[5] Although NUP214-ABL1 and BCR-ABL1 are both constitutively active kinases that stimulate the proliferation of the leukemic cells, they differ from each other in almost all properties we investigated, including genetic etiology, transforming capacity, substrate preference and phosphorylation pattern.[4,6-7] Furthermore, the kinase activation of NUP214-ABL1 depends on its localization at the nuclear pore complex and not on coiled-coil induced oligomerization and binding of the adaptor protein GRB2 like for BCR-ABL1.[7] Despite these differences, NUP214-ABL1 and BCR-ABL1 are both sensitive to ABL1 kinase inhibitors, and ABL1 inhibitors inhibit the proliferation of leukemic cells expressing these kinases,[4,6,8] rendering ABL1 kinase inhibitors attractive for therapy of NUP214-ABL1 positive T-ALL.

Although introduction of ABL1 kinase inhibitors was a major step forward in treatment of BCR-ABL1 positive leukemias, it has also become clear that patients treated with these inhibitors sometimes relapse due to the acquisition of resistance mutations.[9] These mutations are particularly common in BCR-ABL1 positive B-ALL and advanced CML, which, like T-ALL, are genetically more complex than chronic phase CML. Several mechanisms of resistance occur, including BCR-ABL1 mutations interfering with inhibitor binding and amplification of the *BCR-ABL1* oncogene.[10] Consequently, to find therapeutics that target therapy-resistant cells, much effort has been invested in identifying novel proteins or pathways that are required for BCR-ABL mediated transformation.[11] An important example is the observation that certain SRC family kinases are critical signaling proteins in BCR-ABL1 positive B-ALL and advanced CML.[12-15] Indeed, the SRC family kinase LYN is overexpressed in ABL1 kinase inhibitor resistant CML blast crisis and knockdown of LYN induces apoptosis in these cells.[14] In addition, proteins interacting with BCR-ABL1 were recently identified,[16] which may allow the identification of therapeutically relevant critical interactors. In contrast, unbiased studies of the NUP214-ABL1 signaling network are lacking and critical signaling pathways and interactors that can be therapeutically targeted may previously have been missed.

We fear that, similar to BCR-ABL1, resistance to ABL1 inhibitors may develop readily in NUP214-ABL1 positive T-ALL. Although clinical experience with ABL1 inhibitors in NUP214-ABL1 positive T-ALL is limited, a recent report on a NUP214-ABL1 positive patient for which imatinib was added to his therapeutic scheme indeed showed that the patient, after obtaining a rapid remission, fatally relapsed.[17] In this study, we aimed to identify novel therapeutic targets in NUP214-ABL1 positive T-ALL by using two approaches. First, we investigated the therapeutic potential of SRC family kinases. In addition, we performed an unbiased mass spectrometry and siRNA based screen to identify proteins that are critical for the survival and proliferation of NUP214-ABL1 positive T-ALL cells.

METHODS

Constructs

Hemagglutinin (HA) tagged NUP155 and SMC4 cDNA were synthesized (Genscript) and cloned into the XhoI and EcoRI restriction sites of pMSCV-puro (Clontech). NUP214-ABL1 and BCR-ABL1 constructs were described previously.[7]

Cell culture

ALL-SIL, K-562, KE-37, RPMI-8402, JURKAT, SUP-T1, RLD-1 and L-5178-Y (DSMZ) were cultured in RPMI-1640 medium with 20% fetal calf serum (FCS). NA10073 and NA10075 cell lines were established from mouse T-ALL leukemias induced in a NUP214-ABL1 bone marrow transplant assay[7] and were cultured in RPMI-1640 with 20% FCS. HEK293T cells (DSMZ) were cultured in RPMI-1640 with 10% FCS. Transfections were performed using Turbofect reagent (Fermentas).

Immunoblotting

Samples were processed according to standard procedures using the following antibodies: anti-FYN (Fyn3), anti-LCK (3A5), anti-ABL1 (24-11) and anti-ERK2 (Santa Cruz Biotechnology); anti-NUP155 (ab73292) (Abcam); anti-phospho-SRC family (Tyr416), anti-phospho-ABL1 (Tyr245), anti-SMC4 (D14E2) and anti-MAD2L1 (D8A7) (Cell Signaling); anti-HA tag (12CA5) (Roche) and peroxidase-labeled anti-mouse/anti-rabbit antibodies (Amersham).

siRNA knockdown

Human T-ALL cell lines were electroporated on a Genepulser Xcell instrument (Biorad) with 400nM siRNA. For mouse T-ALL cell lines, two electroporations were performed with a 24 hour interval. Viable cell numbers and viability were determined on a Vi-cell XR cell viability analyzer (Beckman Coulter) or on a Guava easyCyte HT (Millipore). Knock-down efficiencies were evaluated by immunoblotting or by quantitative reverse transcriptase PCR (qRT-PCR). siRNA sequences are in **Supplementary table 1**.

Quantitative reverse transcription PCR (qRT-PCR)

qRT-PCR reactions were performed on a LightCycler 480 instrument (Roche). Relative expression levels were calculated according to the ΔΔCt method using GAPDH, UBC or HPRT as normalizer genes.

Drug sensitivity

For dose-response curves, 5 x 10^5 cells were seeded in 1 mL of growth medium and incubated in the presence of PP2 (Calbiochem) for 48 hours. Cell proliferation was determined using CellTiter 96 AQueous One reagent (Promega). Apoptosis analysis was performed using the PE Annexin V apoptosis detection kit I (BD Biosciences). Flow cytometry was performed on a FACSCanto (BD Biosciences).

Mass spectrometric analysis of NUP214-ABL1 protein complexes

Biological duplicates of the pull-down experiments and mass spectrometric analyses were described previously.[16]

(Co-)Immunoprecipitation

Cells were lysed on ice for 30 minutes in cell lysis buffer (Cell Signaling) supplemented with 5mM NA_3VO_4 and Complete protease inhibitor (Roche). For PP2 pretreatment, cells were incubated with PP2 for 20 hours prior to lysis. Lysates were precleared with protein G Dynabeads (Invitrogen) followed by an overnight incubation with antibody-coupled protein G Dynabeads. Antibodies used were: anti-ABL1 (24-11) (Santa Cruz Biotechnology), anti-MAD2L1 (D8A7) (Cell Signaling) and anti-phospho-tyrosine (4G10) (Millipore).

Immunofluorescence

Transfected wells were transferred to poly-L-lysine coated cover slips. The next day, cells were fixed with 4% paraformaldehyde and permeabilized in 0.2% Triton X-100. Cells were stained using the following antibodies: anti-ABL1 (clone 8E9) (BD Biosciences), anti-SMC4 (D14E2) (Cell Signaling), Alexa Fluor 488 conjugated anti-mouse IgG, and Alexa Fluor 555 conjugated anti-

rabbit IgG (Invitrogen). Cells were examined on a Leica TCS SP5 II (Leica Microsystems) confocal microscope. Images were analyzed in ImageJ.

RESULTS

SRC family kinases can be activated by BCR-ABL1 and are therapeutic targets in imatinib resistant CML.[14] Therefore, we wanted to investigate whether SRC family kinases could also play an essential role in supporting proliferation and survival of NUP214-ABL1 positive T-ALL cells. We treated the human NUP214-ABL1 positive T-ALL cell line ALL-SIL as well as the NUP214-ABL1 negative T-ALL cell lines JURKAT and SUP-T1 with increasing concentrations of the SRC family kinase inhibitor PP2. Proliferation of ALL-SIL cells was completely inhibited in the presence of 5µM PP2. However, 5µM of PP2 caused no or a very minor effect on the proliferation of the NUP214-ABL1 negative control cell lines (**Figure 1A**). Likewise, PP2 had no significant effects on cell viability and apoptosis in these cells (**Figure 1B**). In contrast, a clear dose-dependent increase in the amount of apoptotic and dead cells was observed in response to PP2 in the NUP214-ABL1 positive ALL-SIL cells (**Figure 1B**). These results support that ALL-SIL cells depend on the activity of SRC family kinases. To verify that this effect was not restricted to the single NUP214-ABL1 positive ALL-SIL cell line, we also tested the effect of SRC family kinase inhibition in a collection of mouse T-cell leukemia cell lines. In agreement with the data obtained in the human cell lines, PP2 inhibited proliferation and induced apoptosis in the NUP214-ABL1 positive mouse cell lines NA10073 and NA10075, whereas the NUP214-ABL1 negative lines L-5178-Y and RLD-1 were unaffected (**Figure 1C-1D**). Notably, the effect of SRC family kinase inhibition was stronger in the mouse lines, where a concentration of 1µM PP2 already induced a drastic reduction of cell proliferation and survival.

To delineate the identity of the SRC family kinase(s) responsible for the sensitivity of ALL-SIL cells to PP2, we investigated the expression of eight SRC kinase family members in these cells by qRT-PCR. ALL-SIL only expressed significant levels of LCK and FYN (**Figure 2A**). In agreement with this, LCK and FYN protein was detectable in ALL-SIL cells (**Figure 2B**).

However, when we assessed the activation state of LCK and FYN in ALL-SIL cells by testing phosphorylation on their activation loops, we only found LCK to be robustly phosphorylated and this phosphorylation was inhibited by PP2 in a dose-dependent manner. In contrast, phosphorylation of the activation loop of FYN was virtually undetectable, even in the absence of PP2 (**Figure 2B**). These observations indicate that only LCK kinase activity contributed to survival and proliferation of ALL-SIL cells.

As an alternative way to perturb SRC family kinase signaling, we performed siRNA knock-down experiments of LCK and FYN in ALL-SIL cells. As a positive control, we also knocked-down ABL1, which drastically interfered with the proliferation and viability of the NUP214-ABL1 dependent ALL-SIL cells (**Figure 2C-2D**). Knock-down of LCK strongly inhibited ALL-SIL cell proliferation and viability, although to a lesser extent than knock-down of ABL1 (**Figure 2C-2D**). This may be explained by the lower knock-down efficiencies that could be achieved for LCK as compared to ABL1 (**Supplementary Figure 1**). In contrast, knock-down of LCK in NUP214-ABL1 negative JURKAT cells did not affect proliferation and viability of these cells, suggesting that dependence on LCK was specific for NUP214-ABL1 expressing cells (**Figure 2C-2D**). Treatment with FYN siRNA induced only a slight but significant reduction in proliferation of ALL-SIL cells but did not affect their viability. We also tested the effects of Lck knock-down in mouse T-cell leukemia cell lines, using an independent mouse Lck siRNA. In agreement with the data in the human cell lines, knock-down of Lck reduced the proliferation and survival of the NUP214-ABL1 positive cell line NA10075, whereas these effects were not observed in the NUP214-ABL1 negative cell line L-5178-Y (**Figure 2E-2F**). Unfortunately, the NA10073 and RLD-1 mouse cell lines that we used for the experiments shown in Figure 1C-D could not be included as adequate siRNA knock-down in these cells could not be obtained. Taken together, our results indicate that NUP214-ABL1 positive human and mouse cells strongly depend on the expression and activity of LCK for their proliferation and survival and that therapeutic inhibition of LCK activity may provide an alternative means of treating NUP214-ABL1 positive T-ALL.

We next set out to identify proteins, in addition to LCK, that are required for the proliferation and survival of NUP214-ABL1 expressing cells and could possibly be exploited for therapeutic targeting. We used an unbiased approach to study the composition of cellular NUP214-ABL1 complexes by mass spectrometry based interaction proteomics. For this, NUP214-ABL1 (along with ABL1) and its interacting proteins were immunoprecipitated with an anti-ABL1 antibody from ALL-SIL cells followed by mass spectrometric analysis of proteins in the precipitated complexes **(Figure 3A)**. The MS results were searched against the human International Protein Index (IPI) database[18] to yield a primary dataset of 289 proteins (**Supplementary Table 2**). From this list, nine potential specific NUP214-ABL1 interactors were selected (**Table 1**). This was achieved by comparing the proteins identified in the immunoprecipitated samples with the most abundant proteins identified from total cell lysates (*i.e.* 'core' proteomes)[19] and removing ABL1 interactors. This method was previously developed to identify specific interactors of BCR-ABL1.[16] Notably, the nine selected candidate NUP214-ABL1 interactors were very rarely observed when screened against an extensive internally curated interactor database. This database has been generated from numerous interaction proteomics experiments performed with hundreds of different bait proteins from a range of different cell lines. This finding thus implicated these particular proteins as specific interactors of NUP214-ABL1. Interestingly, the list of nine candidate NUP214-ABL1 interacting proteins did not show any overlap with the BCR-ABL1 interactors that were recently characterized using a similar experimental design,[16] further decreasing the likelihood that the identified proteins were interacting with endogenous ABL1, which was inevitably co-immunoprecipitated with NUP214-ABL1 in this experimental approach.

To investigate if any of the nine NUP214-ABL1 interactors is required for proliferation and/or viability of NUP214-ABL1 positive cells, we performed siRNA knock-down of each of these proteins in ALL-SIL cells. Knock-down of DOCK2, ABI1, MAD1L1, STAT1 or WASF2 did not significantly reduce the proliferation and viability of ALL-SIL cells. In contrast, knock-down of NUP155, MAD2L1 and SMC4 strongly inhibited proliferation and survival as compared to scrambled control siRNA treated cells (**Figure 3B-C**). Minor effects were observed upon EVL

knock-down. To distinguish NUP214-ABL1 specific effects from a general requirement of these proteins for cellular proliferation and survival, we also knocked-down NUP155, MAD2L1 and SMC4 in three T-ALL cell lines that do not express NUP214-ABL1 (KE-37, JURKAT and RPMI-8402), as well as in a BCR-ABL1 positive CML cell line (K-562). Knock-down of NUP155 did not significantly affect the proliferation of these control cell lines, whereas knock-down of MAD2L1 or SMC4 did cause minor effects on cell proliferation, albeit much less pronounced than in the ALL-SIL cells (**Figure 3D**). Moreover, whereas cell viability of ALL-SIL cells was drastically reduced by knock-down of NUP155, MAD2L1 and SMC4, cell viability of the NUP214-ABL1 negative lines was unaffected by knock-down of each of these proteins (**Figure 3E**). We also knocked-down MAD2L1, NUP155, or SMC4 in mouse T-cell leukemia cell lines, using an independent set of mouse siRNAs. In agreement with the data obtained in the human cell lines, knock-down of these interaction partners specifically reduced the proliferation of the NUP214-ABL1 positive cell line NA10075, whereas these effects were not observed in the NUP214-ABL1 negative cell line L-5178-Y (**Figure 3F**). Taken together, our results indicate that NUP214-ABL1 positive cells show a dependence for their proliferation and survival on MAD2L1, NUP155 and SMC4.

Next, we tried to confirm binding of MAD2L1, NUP155, and SMC4 to NUP214-ABL1 in independent co-immunprecipitation (co-IP) experiments. We could co-IP endogenous NUP214-ABL1 with endogenous MAD2L1 in NUP214-ABL1 positive ALL-SIL cells. This interaction was absent in NUP214-ABL1 negative JURKAT cells or BCR-ABL1 positive K-562 cells, indicating a specific interaction of NUP214-ABL1 with MAD2L1 (**Figure 4A**). We were however unable to confirm interactions with endogenous NUP155 or SMC4 due to technical limitations concerning the antibodies that were available for these interacting proteins. To circumvent these limitations, further interaction studies were performed in HEK293T cells, where we expressed NUP214-ABL1 in combination with HA-tagged NUP155 or SMC4. Under these conditions, we were able to co-IP NUP155 with NUP214-ABL1 (**Figure 4B**). Of note, we also detected a very weak interaction with BCR-ABL1. However, taking into account that BCR-ABL1 was immunoprecipitated in much larger quantities than NUP214-ABL1, our data indicated a specific interaction between NUP214-ABL1

and NUP155. An interaction between SMC4 and NUP214-ABL1 could not be detected by Co-IP. However, in immunofluorescence experiments, we observed that expression of NUP214-ABL1 in HEK293T cells causes a redistribution of endogenous SMC4 from diffuse cytoplasmic and nuclear staining in control cells towards co-localization at the nuclear envelope upon expression of NUP214-ABL1, strongly pointing to an, at least indirect, interaction between NUP214-ABL1 and SMC4 (**Figure 4C**).

DISCUSSION

The discovery of the ABL1 kinase inhibitor imatinib, the first successful example of molecularly tailored therapy, has revolutionized the treatment of BCR-ABL1 positive CML and B-ALL, as well as of other tumors that depend on imatinib sensitive tyrosine kinases.[20] It is now well established that also the oncogenic NUP214-ABL1 fusion kinase is sensitive to imatinib and that proliferation of cell lines expressing NUP214-ABL1 is inhibited by imatinib.[4,6,8] However, we still await more clinical experience to evaluate the therapeutic potential of imatinib in NUP214-ABL1 positive T-ALL. Because of the low number of patients carrying the NUP214-ABL1 fusion, reports on the clinical responses of those patients are limited so far. In human T-ALL patients, NUP214-ABL1 invariably shows intra- or extra-chromosomal amplification, with as many as 20-30 copies per cell.[4,21] As overexpression of BCR-ABL1 is a known mechanism of imatinib resistance,[22-25] we predict that this amplification of NUP214-ABL1 may contribute to imatinib resistance in NUP214-ABL1 positive T-ALL. Another well-known mechanism of resistance to imatinib in BCR-ABL1 positive leukemias is the emergence of resistance due to point mutations,[9] a phenomenon that we also expect in NUP214-ABL1 positive T-ALL. In this study, we therefore aimed at identifying proteins in the signaling and interaction network of NUP214-ABL1 that are critical for the survival and proliferation of T-ALL cells, as these proteins might serve as alternative drug targets in imatinib resistant NUP214-ABL1 positive T-ALL.

BCR-ABL1 activates the SRC family kinases LYN, FGR and HCK in pre-B-cells and these kinases are required for B-ALL induction by BCR-ABL1 in a mouse model.[15] Moreover, imatinib

resistant BCR-ABL1 positive CML blast crisis cells can be forced into apoptosis by targeting LYN.[14] Based on these results, we hypothesized that SRC family kinases may also play an important role in NUP214-ABL1 mediated transformation. Indeed, we found that NUP214-ABL1 positive human and mouse cell lines are sensitive to the SRC family kinase inhibitor PP2, which is primarily mediated through inhibition of LCK. LCK is a central kinase in T-cell precursors for the transition of CD4/CD8 double negative to double positive thymocytes and stimulates mitosis of early T-cell precursors.[26] Moreover, mice transgenic for wild type or constitutively active Lck develop thymic tumors and rare T-ALL cases have been described with overexpression of LCK by t(1;7)(p34;q34) juxtaposing LCK to the strong promoter sequences of the TRB@ locus.[27,28] These data, together with our finding of required LCK activity for proliferation of NUP214-ABL1 transformed cells, establish LCK as an important drug target in the pathogenesis of T-ALL.

Our finding that LCK is required for NUP214-ABL1 in T-ALL has clinical implications. The FDA-approved multi-kinase inhibitors dasatinib and bosutinib inhibit both ABL and SRC kinases and are used for the treatment of imatinib-resistant and -sensitive BCR-ABL positive malignancies.[29,30] Interestingly, both drugs very potently inhibit LCK activity *in vitro* with IC_{50} values of ~1-2 nM.[31,32] We showed that NUP214-ABL1 activity is inhibited by dasatinib in *in vitro* kinase assays, that proliferation of NUP214-ABL1 positive cells is inhibited by dasatinib and that dasatinib inhibits NUP214-ABL1 positive leukemogenesis in mouse xenografts and primary NUP214-ABL1 positive T-ALL lymphoblasts.[6,9] Furthermore, dasatinib and bosutinib have a much narrower spectrum of point mutations that cause drug resistance as compared to imatinib.[33] The clinical potential of dasatinib or bosutinib for the treatment of NUP214-ABL1 positive T-ALL is further supported by a case report showing induction of rapid complete hematologic and cytogenetic remission after upfront dasatinib monotherapy in a patient with a NUP214-ABL1 positive T-ALL.[34] Based on this diverse pre-clinical and emerging clinical data, one may prefer dasatinib over imatinib in NUP214-ABL1 positive T-ALL patients. Ideally, efficacy of dual SRC-ABL1 inhibitors versus ABL1 inhibitors should now be compared in experiments with primary NUP214-ABL1 positive leukemia cells.

We previously described that NUP214-ABL1 and BCR-ABL1, despite carrying the same portion of the ABL1 kinase, differ in almost any biological property that we have studied such as subcellular localization, mechanism of initiation of kinase activity, phosphorylation pattern, enzymatic activity, kinase inhibitor sensitivity and substrate spectrum.[6,7] Analysis of the proteins interacting with NUP214-ABL1 in this work again indicates a strong difference from BCR-ABL1. None of the core interaction partners that we identified for BCR-ABL1 (GRB2, SHC1, CRK-I, CBL, p85, STS-1, and SHIP-2) were identified in the mass spectrometric analysis of the NUP214-ABL1 protein complexes.[16] This dramatically different composition of NUP214-ABL1 and BCR-ABL1 complexes might be a combination of the T-cell versus granulocyte/B-cell context in which NUP214-ABL1 and BCR-ABL1 are occurring, conformational differences of the ABL1 portion of the two fusion oncoproteins and the differences in their subcellular localizations. NUP214-ABL1 is partially residing at the cytoplasmic side of the nuclear envelope and in the cytoplasm whereas BCR-ABL1 localizes strictly cytoplasmic.[7] The mass spectrometric studies on the BCR-ABL1 protein complexes[16] were performed under the same conditions and in the same lab as the NUP214-ABL1 complexes. Thus, it can be excluded that the observed differences between BCR-ABL1 and NUP214-ABL1 are due to different immunoprecipitation or mass spectrometry conditions. We also confirmed that BCR-ABL1 core interactors were expressed in NUP214-ABL1 positive cells and *vice versa*, excluding that the absence of interaction of BCR-ABL1 interactors with NUP214-ABL1 is caused by a lack of expression of these proteins in NUP214-ABL1 positive cells and *vice versa*.

In this study, we identified NUP155 as an interactor of NUP214-ABL1 and knock-down of NUP155 reduced proliferation of NUP214-ABL1 positive cells. These data fit within our previous observations that NUP214-ABL1 interacts with other nucleoporins such as NUP62, NUP88 and RANBP2 (= NUP358) and that NUP214-ABL1 depends on interaction with these nucleoporins for its activity.[7] In contrast to NUP62, NUP88 and RANBP2, no direct interactions between NUP214

and NUP155 have been described. However, our data indicate that in the context of NUP214-ABL1, NUP155 is interacting with NUP214 (directly or indirectly) in the nuclear pore complex.

In addition to NUP155, also SMC4, a member of the condensing complex converting interphase chromatin into condense chromosomes, and MAD2L1, a spindle checkpoint regulator protecting cells from abnormal chromosome segregation, were detected in NUP214-ABL1 complexes and were required for proliferation of NUP214-ABL1 positive cells. It remains to be determined if NUP155, SMC4 and MAD2L1 are substrates phosphorylated by NUP214-ABL1 and if so, whether the function of these proteins is affected in NUP214-ABL1 positive cells. Preliminary experiments failed to detect NUP214-ABL1-dependent tyrosine phosphorylation of these three proteins (**Supplementary Figure 4**). Another mechanism by which NUP214-ABL1 could affect the function of these proteins is by altering their subcellular localization. Indeed, for SMC4 we observed a clear change in localization of the cellular SMC4 pool towards the nuclear envelope. It will be interesting to test how this affects SMC4 function. Based on the role of SMC4 and MAD2L1 in cellular processes such as in chromosome condensation and spindle checkpoint regulation, it is not unlikely that altered function of these proteins promotes transformation of cells by NUP214-ABL1.

It is worth to note that in our interaction proteomics studies, we were able to confirm known interactions of NUP214-ABL1 with NUP88 and PTPN2.[7,35] PTPN2 is a phosphatase that we previously found deleted in T-ALL and which we showed to negatively regulate NUP214-ABL1 tyrosine kinase activity.[35] Interestingly, we also identified STAT1 as a member of NUP214-ABL1 protein complexes. Endogenous NUP214 is known to import STAT1 in the nucleus under normal steady state conditions.[36] Our knock-down studies, however, suggest that NUP214-ABL1 positive cells do not depend on STAT1 for their survival.

As mentioned earlier, the NUP214-ABL1 fusion was recently described to also occur in B-ALL patients.[5,37] At this moment it remains to be determined to what extent NUP214-ABL1 in T-ALL

and B-ALL context resemble each other and whether the findings we describe above in the context of T-ALL cells could also be applicable to B-ALL.

NUP214-ABL1 usually presents with episomal amplification where the number of copies varies considerably from cell to cell in the same patient.[4,21] In some patients, it even occurs as a secondary change not seen in all cells. Therefore, to obtain durable therapeutic responses in NUP214-ABL1 positive T-ALL, we anticipate that combinations of agents hitting NUP214-ABL1 and/or the proteins on which NUP214-ABL1 relies, together with other targeted agents and/or low doses of chemotherapy will be required.

In conclusion, we identify LCK, MAD2L1, SMC4 and NUP155 as proteins on which NUP214-ABL1 positive T-ALL tumor cells critically depend for their proliferation, identifying these proteins as potential drug targets in NUP214-ABL1 positive T-ALL. Targeting LCK in NUP214-ABL1 could easily be addressed in the clinical treatment schemes of NUP214-ABL1 positive T-ALL patients, due to the availability of dasatinib and bosutinib co-targeting ABL1 and LCK. Our work thus provides a molecular rationale for testing dasatinib and bosutinib alone or in combination with other targeted agents and/or chemotherapy in patients with NUP214-ABL1 positive T-ALL.

AUTHORSHIP AND DISCLOSURES

The authors have nothing to disclose. K.D.K. and O.H. designed and performed experiments, analyzed data and wrote the manuscript, M.P. performed experiments, analyzed data and wrote the manuscript, L.C., T.G., R.V., J. O.D.B., N. M. and K.L.B. performed experiments and analyzed data.

REFERENCES

1. Pui C, Evans WE. Treatment of acute lymphoblastic leukemia. N Engl J Med. 2006;354(2):166-78.

2. Pui C, Mullighan CG, Evans WE, Relling MV. Pediatric acute lymphoblastic leukemia: where are we going and how do we get there? Blood. 2012;120(6):1165-74.

3. De Keersmaecker K, Marynen P, Cools J. Genetic insights in the pathogenesis of T-cell acute lymphoblastic leukemia. Haematologica. 2005;90(8):1116-27.

4. Graux C, Cools J, Melotte C, Quentmeier H, Ferrando A, Levine R, et al. Fusion of NUP214 to ABL1 on amplified episomes in T-cell acute lymphoblastic leukemia. Nat Genet. 2004;36(10):1084-9.

5. Roberts KG, Morin RD, Zhang J, Hirst M, Zhao Y, Su X, et al. Genetic alterations activating kinase and cytokine receptor signaling in high-risk acute lymphoblastic leukemia. Cancer Cell. 2012;22(2):153-66.

6. De Keersmaecker K, Versele M, Cools J, Superti-Furga G, Hantschel O. Intrinsic differences between the catalytic properties of the oncogenic NUP214-ABL1 and BCR-ABL1 fusion protein kinases. Leukemia. 2008;22(12):2208-16.

7. De Keersmaecker K, Rocnik JL, Bernad R, Lee BH, Leeman D, Gielen O, et al. Kinase activation and transformation by NUP214-ABL1 is dependent on the context of the nuclear pore. Mol. Cell. 2008;31(1):134-42.

8. Quintás-Cardama A, Tong W, Manshouri T, Vega F, Lennon PA, Cools J, et al. Activity of tyrosine kinase inhibitors against human NUP214-ABL1-positive T cell malignancies. Leukemia. 2008;22(6):1117-24.

9. O'Hare T, Eide CA, Deininger MWN. Bcr-Abl kinase domain mutations, drug resistance, and the road to a cure for chronic myeloid leukemia. Blood. 2007;110(7):2242-9.

10. Lamontanara AJ, Gencer EB, Kuzyk O, Hantschel O. Mechanisms of resistance to BCR-ABL and other kinase inhibitors. Biochim Biophys Acta. 2013;1834(7):1449-59.

11. Van Etten RA. Oncogenic signaling: new insights and controversies from chronic myeloid leukemia. J Exp Med. 2007;204(3):461-5.

12. Danhauser-Riedl S, Warmuth M, Druker BJ, Emmerich B, Hallek M. Activation of Src kinases p53/56lyn and p59hck by p210bcr/abl in myeloid cells. Cancer Research. 1996;56(15):3589-96.

13. Donato NJ, Wu JY, Stapley J, Gallick G, Lin H, Arlinghaus R, et al. BCR-ABL independence and LYN kinase overexpression in chronic myelogenous leukemia cells selected for resistance to STI571. Blood. 2003;101(2):690-8.

14. Ptasznik A, Nakata Y, Kalota A, Emerson SG, Gewirtz AM. Short interfering RNA (siRNA) targeting the Lyn kinase induces apoptosis in primary, and drug-resistant, BCR-ABL1(+) leukemia cells. Nat. Med. 2004;10(11):1187-9.

15. Hu Y, Liu Y, Pelletier S, Buchdunger E, Warmuth M, Fabbro D, et al. Requirement of Src kinases Lyn, Hck and Fgr for BCR-ABL1-induced B-lymphoblastic leukemia but not

chronic myeloid leukemia. Nat Genet. 2004;36(5):453-61.

16. Brehme M, Hantschel O, Colinge J, Kaupe I, Planyavsky M, Köcher T, et al. Charting the molecular network of the drug target Bcr-Abl. Proc Natl Acad Sci USA. 2009;106(18):7414-9.

17. Clarke S, O'Reilly J, Romeo G, Cooney J. NUP214-ABL1 positive T-cell acute lymphoblastic leukemia patient shows an initial favorable response to imatinib therapy post relapse. Leuk Res. 2011;35(7):e131-3.

18. Kersey PJ, Duarte J, Williams A, Karavidopoulou Y, Birney E, Apweiler R. The International Protein Index: an integrated database for proteomics experiments. Proteomics. 2004;4(7):1985-8.

19. Schirle M, Heurtier M, Kuster B. Profiling core proteomes of human cell lines by one-dimensional PAGE and liquid chromatography-tandem mass spectrometry. Mol. Cell Proteomics. 2003;2(12):1297-305.

20. Druker BJ. Translation of the Philadelphia chromosome into therapy for CML. Blood. 2008;112(13):4808-17.

21. Graux C, Stevens-Kroef M, Lafage M, Dastugue N, Harrison CJ, Mugneret F, et al. Heterogeneous patterns of amplification of the NUP214-ABL1 fusion gene in T-cell acute lymphoblastic leukemia. Leukemia. 2009;23(1):125-33.

22. Gorre ME, Mohammed M, Ellwood K, Hsu N, Paquette R, Rao PN, et al. Clinical resistance to STI-571 cancer therapy caused by BCR-ABL gene mutation or amplification. Science. 2001;293(5531):876-80.

23. le Coutre P, Tassi E, Varella-Garcia M, Barni R, Mologni L, Cabrita G, et al. Induction of resistance to the Abelson inhibitor STI571 in human leukemic cells through gene amplification. Blood. 2000;95(5):1758-66.

24. Mahon FX, Deininger MW, Schultheis B, Chabrol J, Reiffers J, Goldman JM, et al. Selection and characterization of BCR-ABL positive cell lines with differential sensitivity to the tyrosine kinase inhibitor STI571: diverse mechanisms of resistance. Blood. 2000;96(3):1070-9.

25. Weisberg E, Griffin JD. Mechanism of resistance to the ABL tyrosine kinase inhibitor STI571 in BCR/ABL-transformed hematopoietic cell lines. Blood. 2000;95(11):3498-505.

26. Palacios EH, Weiss A. Function of the Src-family kinases, Lck and Fyn, in T-cell development and activation. Oncogene. 2004;23(48):7990-8000.

27. Abraham KM, Levin SD, Marth JD, Forbush KA, Perlmutter RM. Thymic tumorigenesis induced by overexpression of p56lck. Proc Natl Acad Sci USA. 1991;88(9):3977-81.

28. Tycko B, Smith SD, Sklar J. Chromosomal translocations joining LCK and TCRB loci in human T cell leukemia. J Exp Med. 1991;174(4):867-73.

29. Shami PJ, Deininger M. Evolving treatment strategies for patients newly diagnosed with chronic myeloid leukemia: the role of second-generation BCR-ABL inhibitors as first-line therapy. Leukemia. 2012;26(2):214-24.

30. FDA. Bosutinib tablets, 2012.

http://www.fda.gov/Drugs/InformationOnDrugs/ApprovedDrugs/ucm318203.htm

31. Remsing Rix LL, Rix U, Colinge J, Hantschel O, Bennett KL, Stranzl T, et al. Global target profile of the kinase inhibitor bosutinib in primary chronic myeloid leukemia cells. Leukemia. 2009;23(3):477-85.

32. Das J, Chen P, Norris D, Padmanabha R, Lin J, Moquin RV, et al. 2-aminothiazole as a novel kinase inhibitor template. Structure-activity relationship studies toward the discovery of N-(2-chloro-6-methylphenyl)-2-[[6-[4-(2-hydroxyethyl)-1- piperazinyl)]-2-methyl-4-pyrimidinyl]amino)]-1,3-thiazole-5-carboxamide (dasatinib, BMS-354825) as a potent pan-Src kinase inhibitor. J. Med. Chem. 2006;49(23):6819-32.

33. Hantschel O, Grebien F, Superti-Furga G. The growing arsenal of ATP-competitive and allosteric inhibitors of BCR-ABL. Cancer Research. 2012;72(19):4890-5.

34. Deenik W, Beverloo HB, van der Poel-van de Luytgaarde SCPAM, Wattel MM, van Esser JWJ, Valk PJM, et al. Rapid complete cytogenetic remission after upfront dasatinib monotherapy in a patient with a NUP214-ABL1-positive T-cell acute lymphoblastic leukemia. Leukemia. 2009;23(3):627-9.

35. Kleppe M, Lahortiga I, Chaar El T, De Keersmaecker K, Mentens N, Graux C, et al. Deletion of the protein tyrosine phosphatase gene PTPN2 in T-cell acute lymphoblastic leukemia. Nat Genet. 2010;42(6):530-5.

36. Marg A, Shan Y, Meyer T, Meissner T, Brandenburg M, Vinkemeier U. Nucleocytoplasmic shuttling by nucleoporins Nup153 and Nup214 and CRM1-dependent nuclear export control the subcellular distribution of latent Stat1. J. Cell Biol. 2004;165(6):823-33.

37. Eyre T, Schwab CJ, Kinstrie R, McGuire AK, Strefford J, Peniket A, et al. Episomal amplification of NUP214-ABL1 fusion gene in B-cell acute lymphoblastic leukemia. Blood. 2012;120(22):4441-3.

TABLES

Table 1. List of selected potential NUP214-ABL1 interactors

The column 'peptides' refers to the number of unique peptides that were identified for that corresponding protein in the mass spectrometry analysis. 'Coverage' refers to the % of sequence of the total protein that was identified in our mass spectrometry analysis.

Gene	Full Gene Name	Peptides	Coverage (%)
NUP155	Nucleoporin 155 kDa	33	30.7
DOCK2	Dedicator Of Cytokinesis 2	3	1.7
EVL	Enah/Vasp-like	5	14.4
ABI1	ABL-Interactor 1	3	8.5
MAD1L1	Mitotic Arrest Deficient 1-like 1 (yeast)	10	16.7
MAD2L1	Mitotic Arrest Deficient 2-like 1 (yeast)	2	8.8
STAT1	Signal Transducer and Activator of Transcription 1	4	6.6
SMC4	Structural Maintenance of Chromosomes 4	4	3.8
WASF2	WAS protein Family, member 2	3	6.6

FIGURE LEGENDS

Figure 1. NUP214-ABL1 positive T-ALL cell lines are sensitive to inhibition of SRC kinases

(A) Human NUP214-ABL1 positive (ALL-SIL) and negative (JURKAT and SUP-T1) T-ALL cell lines were treated with indicated concentrations of PP2. Cell proliferation was normalized to DMSO treated cells. The average ±SD of three repeats is shown. **(B)** Percentages of viable, apoptotic, and dead cells after PP2 treatment were determined by Annexin-V/7-AAD staining. **(C)** Mouse NUP214-ABL1 positive (NA10073 and NA10075) and negative (L5178Y and RLD1) T-cell lines were treated with indicated concentrations of PP2 for 48 hours. Cell proliferation was normalized to DMSO treated cells. The average ±SD of three repeats is shown. **(D)** Percentages

of viable, apoptotic, and dead cells after PP2 treatment were determined by Annexin-V/7-AAD staining.

Figure 2. LCK is critical for NUP214-ABL1 positive T-ALL cell lines.

(A) qRT-PCR analysis of SRC family kinase expression in ALL-SIL cells. **(B)** Immunoprecipitation of LCK or FYN from ALL-SIL cell lysates. Cells were pretreated with indicated concentrations of PP2 inhibitor. Phosphorylation of the precipitated proteins was assessed with an anti-phospho-SRC family kinase (Tyr416) antibody. **(C)** Proliferation of NUP214-ABL1 positive (ALL-SIL) and NUP214-ABL1 negative (JURKAT) cells after electroporation with indicated siRNAs. The average ±SD of triplicates is shown. **(D)** Percentages of viable cells after electroporation with indicated siRNAs. **(E)** Proliferation of NUP214-ABL1 positive (NA10075) and NUP214-ABL1 negative (NA10073) cells after electroporation with indicated siRNAs. The average ±SD of triplicates is shown. **(F)** Percentages of viable cells after electroporation with indicated siRNAs. siRNA knock-down efficiencies are displayed in **Supplementary Figure 1 and 2**.

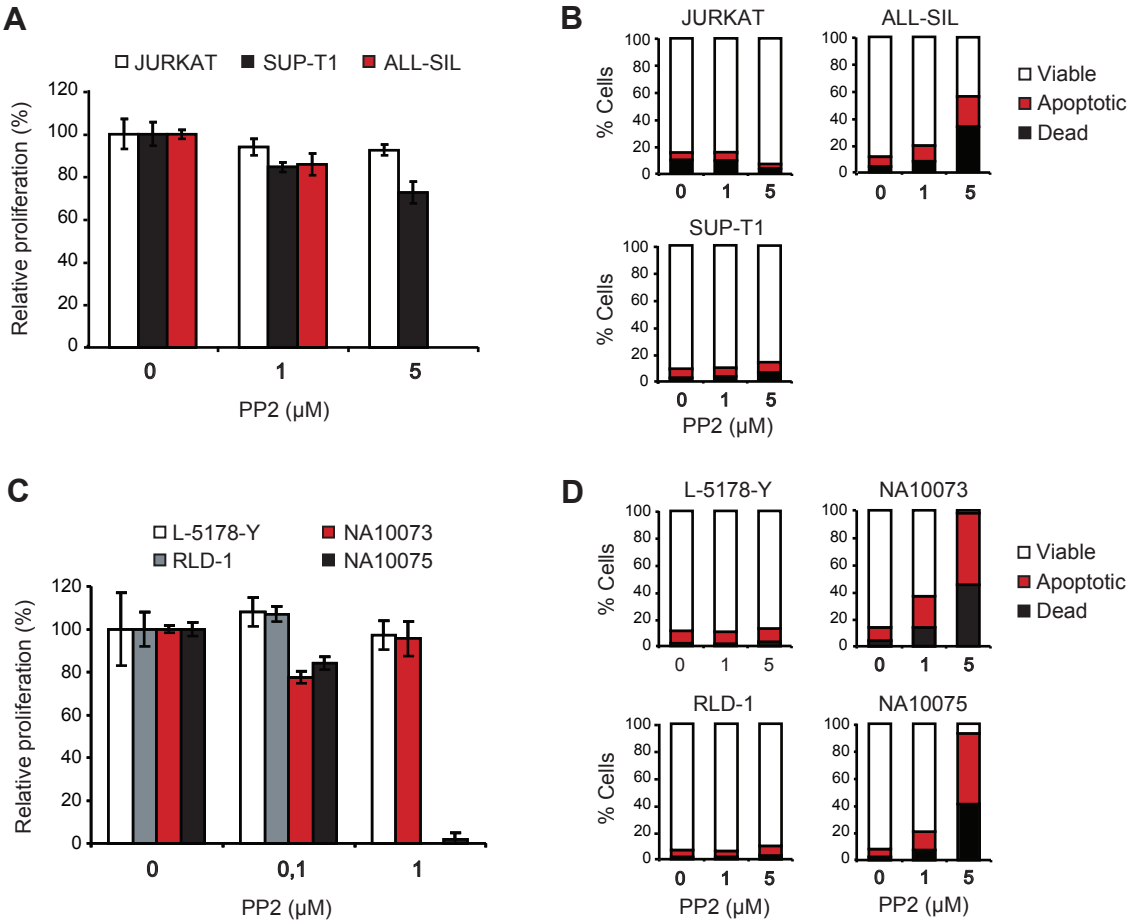
Figure 3. MAD2L1, NUP155 and SMC4 are required for optimal proliferation of NUP214-ABL1 positive T-ALL cell lines.

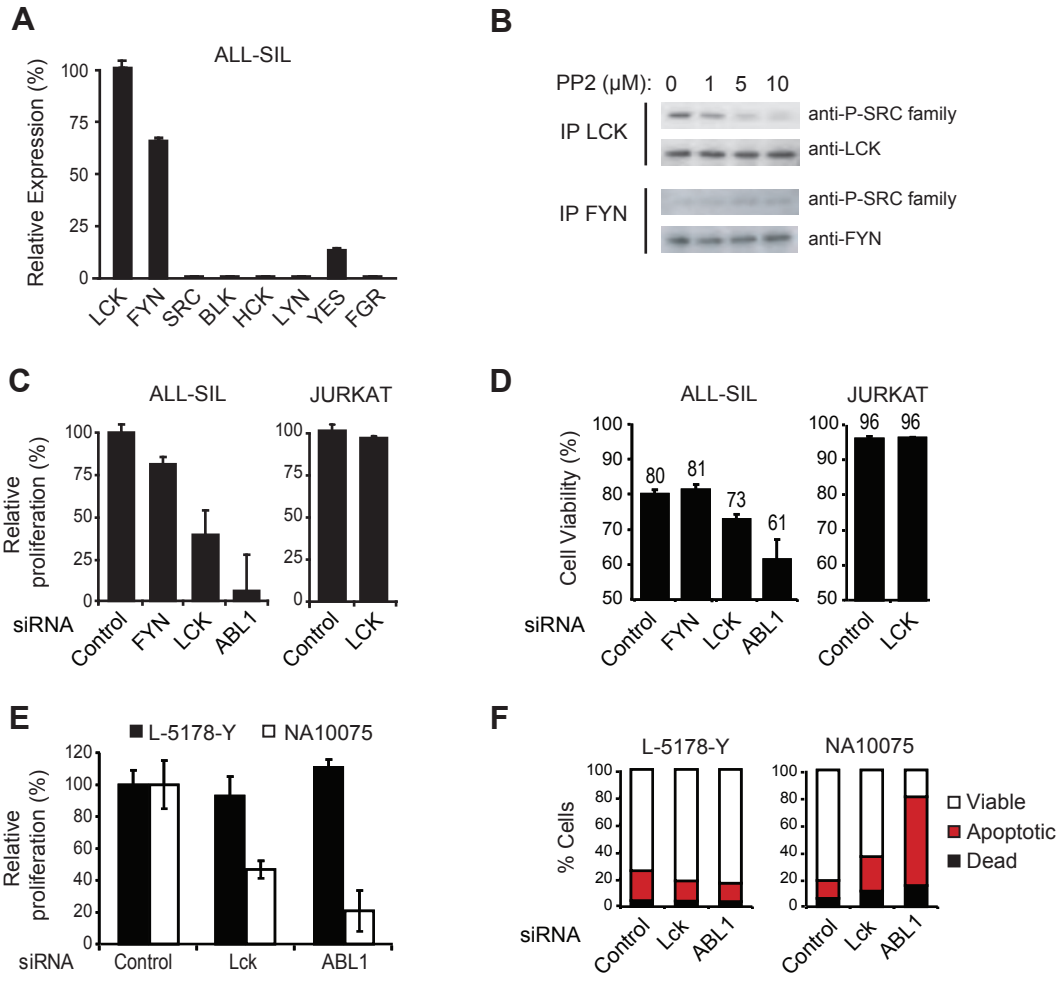
(A) Overview of the mass spectrometry approach that was used to identify interactor proteins of NUP214-ABL1. ALL-SIL cells were lysed, resulting in the release of all cellular proteins and protein complexes. NUP214-ABL1 and its interacting proteins were isolated by using anti-ABL1 antibody coupled beads. The isolated NUP214-ABL1 complexes were characterized by mass spectrometry to determine the identity of NUP214-ABL1 interacting proteins. **(B)** Cell proliferation of NUP214-ABL1 positive ALL-SIL cells after electroporation with indicated siRNAs. Cell proliferation measurements were normalized to the scrambled control. **(C)** Cell viability of ALL-SIL cells after electroporation with indicated siRNAs. **(D)** Proliferation of NUP214-ABL1 negative KE-37, RPMI-8402, JURKAT and K-562 cells after electroporation with indicated siRNAs. Cell proliferation was normalized to scrambled control. **(E)** Cell viability of NUP214-ABL1 positive ALL-SIL cells and of NUP214-ABL1 negative KE-37, RPMI-8402, JURKAT and K-562 cells after electroporation with indicated siRNAs. **(F)** Proliferation of mouse NUP214-ABL1 positive

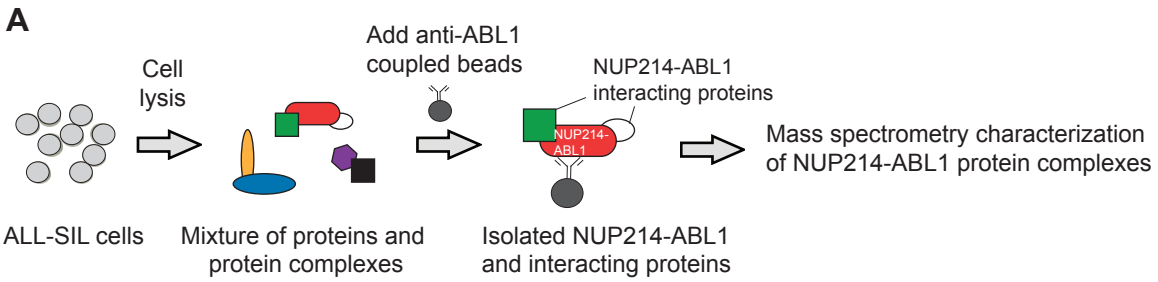
(NA10075) NUP214-ABL1 negative (L-5178-Y) cells after electroporation with indicated siRNAs. Cell proliferation was normalized to scrambled control. The average ±SD of three repeats is shown for all siRNA knock-down experiments. siRNA knock-down efficiencies are displayed in **Supplementary Figure 3**.

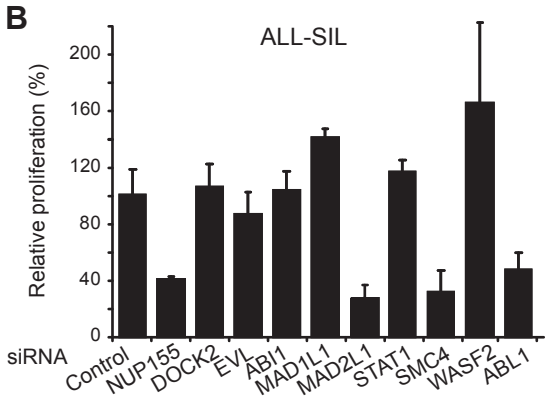
Figure 4. MAD2L1, NUP155 and SMC4 interact or colocalize with NUP214-ABL1.

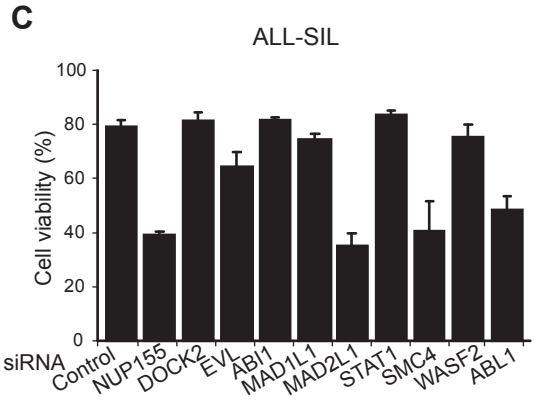
(A) Endogenous MAD2L1 protein was immunoprecipitated from ALL-SIL (NUP214-ABL1 positive), K-562 (BCR-ABL1 positive) and JURKAT cells (no ABL1 fusion) with anti-MAD2L1 antibody. Co-IP of NUP214-ABL1 or BCR-ABL1 was assessed on the shown western blot with anti-ABL1 antibody. (WCL: whole cell lysate) **(B)** ABL1 protein was immunoprecipitated from HEK293T cells that were transfected with the indicated combinations of NUP155-HA and ABL1 fusion constructs. Co-precipitation of HA-tagged NUP155 was assessed with anti-HA antibody. **(C)** Subcellular localization of NUP214-ABL1, ABL1 and SMC4 was investigated by immunofluorescence in HEK293T cells. Control HEK293T cells display low endogenous levels of ABL1 and diffuse SMC4 staining. In contrast, ABL1 and SMC4 co-localize at the nuclear pore in transfected cells expressing NUP214-ABL1. DRAQ5 stains DNA and visualizes the nucleus. Scale bar: 5µm

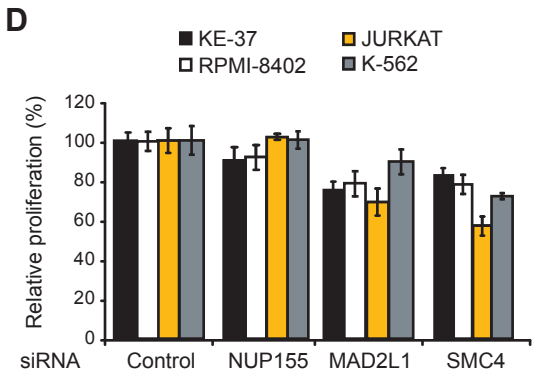

A
JURKAT
SUP-T1
ALL-SIL
Relative proliferation (%)
120
100
80
60
40
20
0
0
1
5
PP2 (μM)
B
JURKAT
ALL-SIL
SUP-T1
% Cells
100
80
60
40
20
0
0
1
5
PP2 (μM)
Viable
Apoptotic
Dead
C
L-5178-Y
NA10073
RLD-1
NA10075
Relative proliferation (%)
120
100
80
60
40
20
0
0
0,1
1
PP2 (μM)
D
L-5178-Y
NA10073
RLD-1
NA10075
% Cells
100
80
60
40
20
0
0
1
5
PP2 (μM)
Viable
Apoptotic
Dead

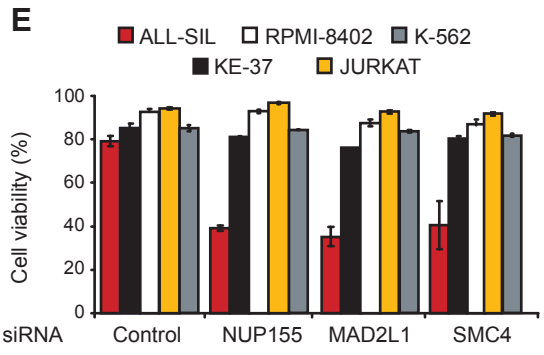

A
ALL-SIL
Relative Expression (%)
100
75
50
25
0
LCK
FYN
SRC
BLK
HCK
LYN
YES
FGR
B
PP2 (μM): 0 1 5 10
IP LCK
anti-P-SRC family
anti-LCK
IP FYN
anti-P-SRC family
anti-FYN
C
ALL-SIL
JURKAT
Relative proliferation (%)
siRNA
Control
FYN
LCK
ABL1
Control
LCK
D
ALL-SIL
JURKAT
Cell Viability (%)
80
81
73
61
96
96
siRNA
Control
FYN
LCK
ABL1
Control
LCK
E
L-5178-Y
NA10075
Relative proliferation (%)
siRNA
Control
Lck
ABL1
F
L-5178-Y
NA10075
% Cells
Viable
Apoptotic
Dead
siRNA
Control
Lck
ABL1
Control
Lck
ABL1

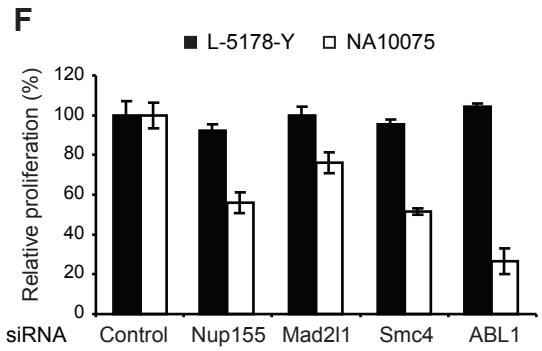

A
Cell lysis
Add anti-ABL1 coupled beads
NUP214-ABL1 interacting proteins
NUP214-ABL1
Mass spectrometry characterization of NUP214-ABL1 protein complexes
ALL-SIL cells
Mixture of proteins and protein complexes
Isolated NUP214-ABL1 and interacting proteins

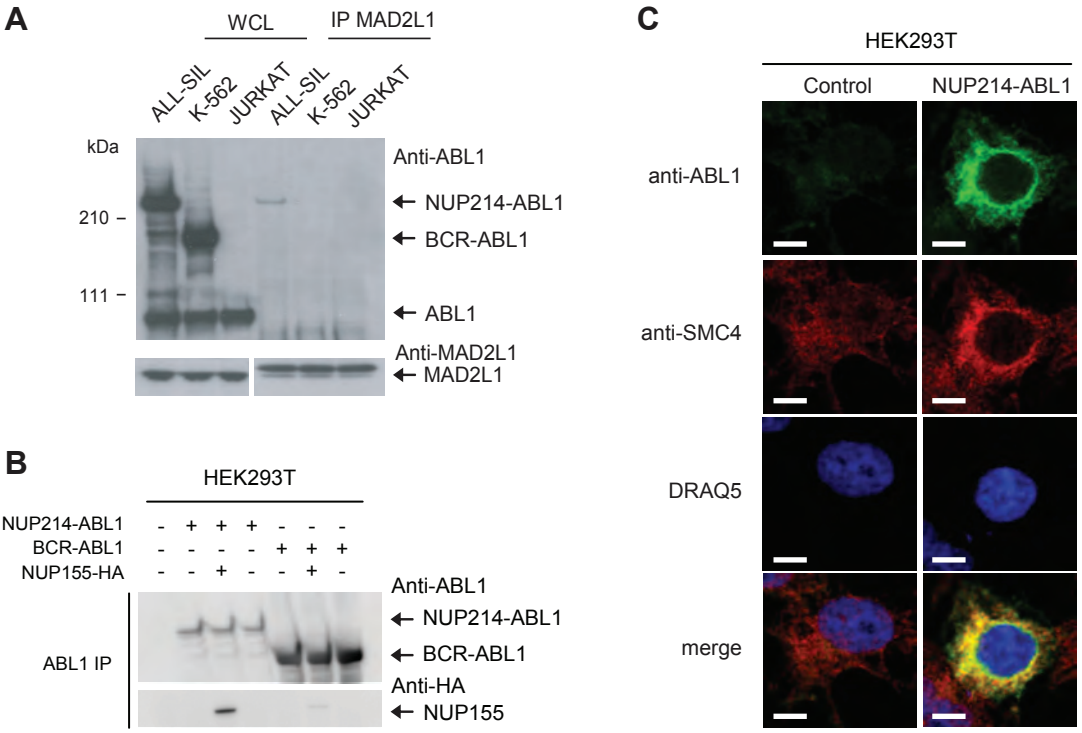

B
ALL-SIL
Relative proliferation (%)
200
160
120
80
40
0
siRNA
Control
NUP155
DOCK2
EVL
ABI1
MAD1L1
MAD2L1
STAT1
SMC4
WASF2
ABL1


C
ALL-SIL
Cell viability (%)
100
80
60
40
20
0
siRNA
Control
NUP155
DOCK2
EVL
ABI1
MAD1L1
MAD2L1
STAT1
SMC4
WASF2
ABL1


D
KE-37
JURKAT
RPMI-8402
K-562
Relative proliferation (%)
120
100
80
60
40
20
0
siRNA
Control
NUP155
MAD2L1
SMC4


E
ALL-SIL
RPMI-8402
K-562
KE-37
JURKAT
Cell viability (%)
100
80
60
40
20
0
siRNA
Control
NUP155
MAD2L1
SMC4


F
L-5178-Y
NA10075
Relative proliferation (%)
120
100
80
60
40
20
0
siRNA
Control
Nup155
Mad2l1
Smc4
ABL1


A
WCL
IP MAD2L1
ALL-SIL
K-562
JURKAT
ALL-SIL
K-562
JURKAT
kDa
210 –
111 –
Anti-ABL1
NUP214-ABL1
BCR-ABL1
ABL1
Anti-MAD2L1
MAD2L1
B
HEK293T
NUP214-ABL1 - + + + - - -
BCR-ABL1 - - - - + + +
NUP155-HA - - + - - + -
ABL1 IP
Anti-ABL1
NUP214-ABL1
BCR-ABL1
Anti-HA
NUP155
C
HEK293T
Control
NUP214-ABL1
anti-ABL1
anti-SMC4
DRAQ5
merge

SUPPLEMENTARY INFORMATIONS

NUP214-ABL1 mediated cell proliferation in T-cell acute lymphoblastic leukemia is dependent on the LCK kinase and various interacting proteins

Kim De Keersmaecker,[1,2*] Michaël Porcu,[1,2*] Luk Cox,[1,2] Tiziana Girardi,[1,2] Roel Vandepoel,[1,2] Joyce Op de Beeck,[1,2] Olga Gielen,[1,2] Nicole Mentens,[1,2] Keiryn L. Bennett,[3] Oliver Hantschel[4]

[1] Center for the Biology of Disease, VIB, Leuven, Belgium; [2] Center for Human Genetics, KU Leuven, Leuven, Belgium; [3] CeMM Research Center for Molecular Medicine of the Austrian Academy of Sciences, Vienna, Austria; [4] Swiss Institute for Experimental Cancer Research (ISREC), School of Life Sciences, École polytechnique fédérale de Lausanne, Lausanne, Switzerland

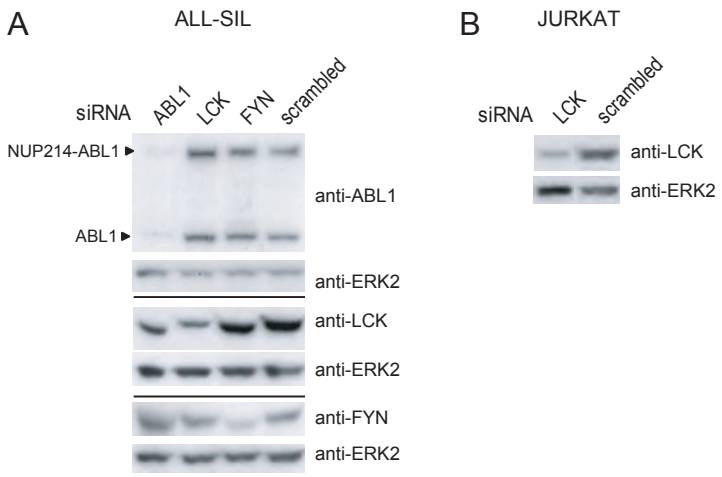
[*] equal contribution

Correspondence:

Oliver Hantschel. E-mail: oliver.hantschel@epfl.ch

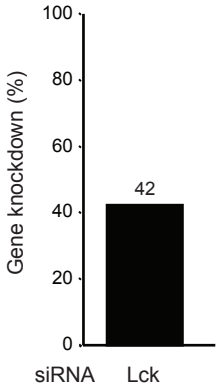
Kim De Keersmaecker. E-mail: kim.dekeersmaecker@cme.vib-kuleuven.be

Supplementary Figure 1



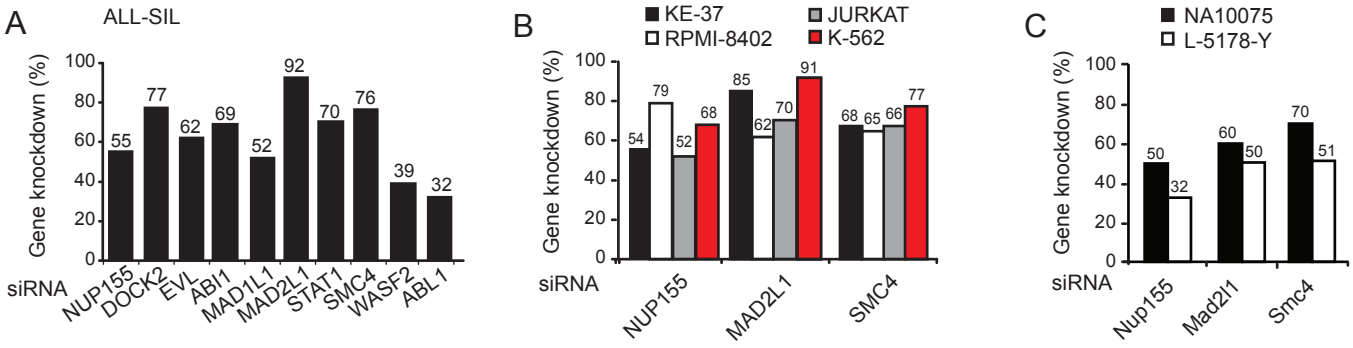
Suppl. Figure 1. siRNA knock-down levels in human T-ALL cells.
Western blot analysis of ALL-SIL (**A**) and JURKAT (**B**) cells treated with indicated siRNAs. The western blots were probed with the antibodies indicated at the right side of each blot.

Supplementary Figure 2



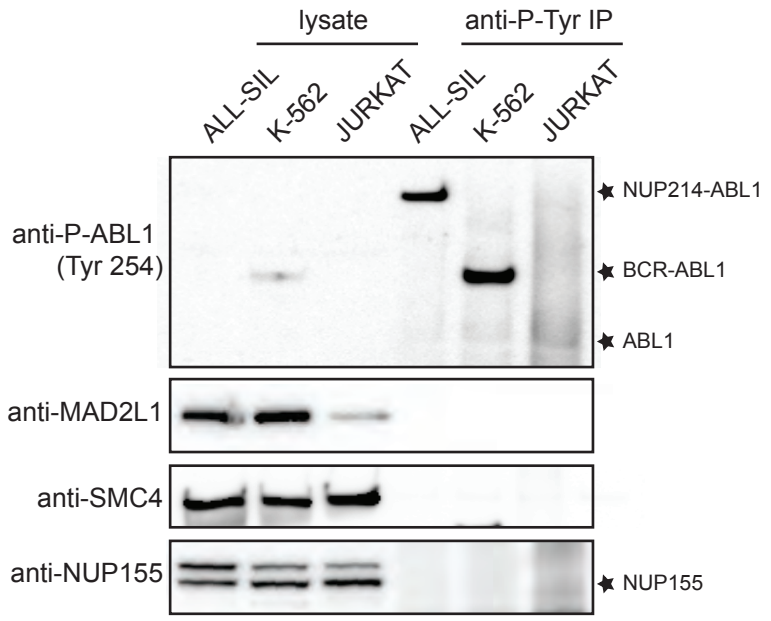
Suppl. Figure 2. siRNA knock-down levels of Lck in mouse T-ALL cells.
Lck knock-down levels were measured by qRT-PCR in mouse L-5178-Y cells treated with control scrambled siRNA, or Lck siRNA. Indicated levels are relative to scrambled control siRNA treated cells (0% knock-down).

Supplementary Figure 3



Suppl. Figure 3. siRNA knock-down levels in human and mouse T-ALL cells.
Knock-down levels of indicated genes were measured by qRT-PCR in cells treated with the correponding siRNAs. (**A**) ALL-SIL cells. (**B**) KE-37, JURKAT, RPMI-8402 and K-562 cells. (**C**) NA10075 and L-5178-Y cells. Indicated levels are relative to scrambled control siRNA treated cells (0% knock-down).

Supplementary Figure 4



Suppl. Figure 4. No detectable tyrosine phosphorylation of MAD2L1, SMC4 and NUP155 .
Immunoprecipitation (IP) using a pan-phospho-tyrosine (anti-P-Tyr) antibody on ALL-SIL, K562 and JURKAT cell lysates pulled down phosphorylated NUP214-ABL1 (in ALL-SIL) and BCR-ABL1 (in K-562) as detected on the western blot with the anti-phospho-ABL1 (Tyr 245) antibody. NUP214-ABL1 interaction partners MAD2L1, SMC4 and NUP155 were not detectable in the IP samples indicating these proteins may not be phosphorylated.

Supplementary table 1. siRNA sequences used in this study

Target Gene	Species	Type	Sequence
ABL1	Human	Invitrogen Stealth	GGAAUGGUGUGAAGCCCAAACCAAA
LCK	Human	Invitrogen Stealth	GCAUUCAUUGAAGAGCGGAAUUAUA
FYN	Human	Invitrogen Stealth	CCCUGUACGGGAGGUUCACAAUCAA
NUP155	Human	Invitrogen Stealth	CCGAUGGUGAAUUUCUUCAUGAAUU
DOCK2	Human	Invitrogen Stealth	CGACAUGAUGCUGUGUGAAUAUCAA
EVL	Human	Invitrogen Stealth	GCAGCAGCGUCAGGAAUCUCUAGAA
ABI1	Human	Invitrogen Stealth	ACUGGGACGGAAUACUCCUUAUAAA
MAD1L1	Human	Invitrogen Stealth	GAAGACCUUUCCAGAUUCGUGGUUG
MAD2L1	Human	Invitrogen Stealth	GCCACUGUUGGAAGUUUCUUGUUCA
STAT1	Human	Invitrogen Stealth	GCAAGCGUAAUCUUCAGGAUAAUUU
SMC4	Human	Invitrogen Stealth	CAGGGUGAAGUUGAACAAAUUGCUA
WASF2	Human	Invitrogen Stealth	CCCAUCUUUCCCACCUCACCCUGAU
Lck	Mouse	IDT Screening DsiRNA	GUAAUUCUGUUCUUCGAUGAACGCCAU
Mad2L1	Mouse	Ambion Silencer Select	UUGUAAAUGAGCGUAGACGga
Nup155	Mouse	Ambion Silencer Select	AUUCGAAGAAAAGUAAAUGCaa
Smc4	Mouse	Ambion Silencer Select	UGCUGUAUUAUGUCGACUGag
Scrambled	Human	Invitrogen Stealth	Available on request: #12935100
Scrambled	Mouse	IDT	CGUUAAUCGCGUAUAAUACGCGUat
Scrambled	Mouse	Ambion Silencer Select	Available on request #4390847

Supplementary	Table 2. Complete list of proteins identified by mass spectrometry				
Name	**Description**	**Synonyms**	**Sequence-ID**	**# Peptides**	**% Sequence Coverage**
ABL1	"v-abl Abelson murine leukemia viral oncogene homolog 1"	ABL, JTK7, p150, c-ABL, v-abl	NP_005148	37	37,8
NUP155	"nucleoporin 155kDa"	N155, KIAA0791	NP_004289	33	30,7
DNCH1	"dynein, cytoplasmic, heavy polypeptide 1"	p22, DHC1, DNCL, DYHC, HL-3, DHC1a, DNECL, Dnchc1, KIAA0325	NP_001367	26	6,3
CYFIP2	"cytoplasmic FMR1 interacting protein 2"	PIR121, PRO1331	NP_055191	21	19,6
SPTBN1	"spectrin, beta, non-erythrocytic 1"	SPTB2	NP_842565	21	11,6
TLN1	"talin 1"	TLN, KIAA1027	NP_006280	20	9,7
VCP	"valosin-containing protein"	p97, TERA, IBMPFD	NP_009057	16	22,7
TRIM21	"tripartite motif-containing 21"	SSA, RO52, SSA1, RNF81	NP_003132	15	33,3
CCT2	"chaperonin containing TCP1, subunit 2 (beta)"	CCTB, 99D8.1, PRO1633, CCT-beta, TCP-1-beta	NP_006422	11	25,2
CCT4	"chaperonin containing TCP1, subunit 4 (delta)"	SRB, Cctd	NP_006421	11	23,4
CYFIP1	"cytoplasmic FMR1 interacting protein 1"	SHYC, FLJ45151, KIAA0068, P140SRA-1	NP_055423	11	10,4
DLAT	"dihydrolipoamide S-acetyltransferase (E2 component of pyruvate dehydrogenase complex)"	DLTA, PDCE2, PDC-E2	NP_001922	11	16,4
HEM1	"hematopoietic protein 1"		NP_005328	11	11,4
RPS18	"ribosomal protein S18"	KE3, HKE3, KE-3, D6S218E	NP_072045	11	56,6
SF3B3	"splicing factor 3b, subunit 3, 130kDa"	RSE1, SAP130, SF3b130, STAF130, KIAA0017	NP_036558	11	12,2
BIRC6	"baculoviral IAP repeat-containing 6 (apollon)"	BRUCE, APOLLON, FLJ13726, FLJ13786, KIAA1289	NP_057336	10	2,4
MAD1L1	"MAD1 mitotic arrest deficient-like 1 (yeast)"	MAD1, PIG9, HsMAD1, TP53I9, TXBP181	NP_003541	10	16,7
PDHA1	"pyruvate dehydrogenase (lipoamide) alpha 1"	PDHA, PHE1A, PDHCE1A	NP_000275	10	26,2
XRCC5	"X-ray repair complementing defective repair in Chinese hamster cells 5 (double-strand-break rejoining; Ku autoantigen, 80kDa)"	KU80, Ku86, NFIV, KARP1, KARP-1	NP_066964	10	15,8
ABL2	"v-abl Abelson murine leukemia viral oncogene homolog 2 (arg, Abelson-related gene)"	ARG, ABLL	NP_005149	9	7,0
PDHB	"pyruvate dehydrogenase (lipoamide) beta"	PHE1B, DKFZp564K0164	NP_000916	9	34,0
RPS3	"ribosomal protein S3"	FLJ26283, FLJ27450, MGC87870	NP_000996	9	44,9
SEPT9	"septin 9"	MSF, MSF1, SINT1, PNUTL4, AF17q25, KIAA0991	NP_006631	9	17,6
TRIM28	"tripartite motif-containing 28"	KAP1, TF1B, RNF96, TIF1B	NP_005753	9	14,0
ALDOA	"aldolase A, fructose-bisphosphate"	ALDA, MGC10942, MGC17716, MGC17767	NP_908930	8	22,8
KARS	"lysyl-tRNA synthetase"	KARS2, KIAA0070	NP_005539	8	15,2
TFRC	"transferrin receptor (p90, CD71)"	TFR, CD71, TFR1, TRFR	NP_003225	8	12,5
EEF1G	"eukaryotic translation elongation factor 1 gamma"	EF1G	NP_001395	7	15,8
EIF3S3	"eukaryotic translation initiation factor 3, subunit 3 gamma, 40kDa"	eIF3-p40, MGC102958, eIF3-gamma	NP_003747	7	23,6
HSPD1	"heat shock 60kDa protein 1 (chaperonin)"	CPN60, GROEL, HSP60, HSP65, SPG13, HuCHA60	NP_002147	7	16,9
IDH2	"isocitrate dehydrogenase 2 (NADP+), mitochondrial"	IDH, IDP, IDHM, ICD-M, mNADP-IDH	NP_002159	7	18,4
ILF3	"interleukin enhancer binding factor 3, 90kDa"	DRBF, MMP4, MPP4, NF90, NFAR, TCP80, DRBP76, NFAR-1, MPHOSPH4, NF-AT-90	NP_703194	7	12,8
PGK1	"phosphoglycerate kinase 1"	PGKA, MIG10, MGC8947	NP_000282	7	24,0
RPL6	"ribosomal protein L6"	TXREB1, TAXREB107	NP_000961	7	22,9
RPS19	"ribosomal protein S19"	DBA	NP_001013	7	41,4
SEPT2	"septin 2"	DIFF6, NEDD5, hNedd5, KIAA0158	NP_004395	7	24,7
EWSR1	"Ewing sarcoma breakpoint region 1"	EWS	NP_005234	6	10,8
HPRT1	"hypoxanthine phosphoribosyltransferase 1 (Lesch-Nyhan syndrome)"	HPRT, HGPRT	NP_000185	6	31,7
HRMT1L2	"HMT1 hnRNP methyltransferase-like 2 (S. cerevisiae)"	ANM1, HCP1, IR1B4, PRMT1	NP_938074	6	19,2
ILF2	"interleukin enhancer binding factor 2, 45kDa"	NF45, MGC8391, PRO3063	NP_004506	6	20,3
LOC641814			ipi|IPI00735235	6	20,6
LOC646483			ipi|IPI00742225	6	20,7
MYL6	"myosin, light polypeptide 6, alkali, smooth muscle and non-muscle"	ESMLC, LC17A, LC17B, MLC1SM, MLC3NM, MLC3SM, LC17-GI, LC17-NM	ipi|IPI00744444	6	35,9
PFN1	"profilin 1"		NP_005013	6	46,4
PHGDH	"phosphoglycerate dehydrogenase"	PDG, PGD, PGAD, PGDH, SERA, 3PGDH, 3-PGDH, MGC3017	NP_006614	6	13,3
PSMC2	"proteasome (prosome, macropain) 26S subunit, ATPase, 2"	S7, MSS1, MGC3004	NP_002794	6	15,9
RPL4	"ribosomal protein L4"	HRPL4	NP_000959	6	16,2
RPN1	"ribophorin I"	RBPH1, DKFZp686B16177	NP_002941	6	12,4
RUVBL1	"RuvB-like 1 (E. coli)"	ECP54, TIP49, NMP238	NP_003698	6	18,2
SEPT6	"septin 6"	SEP2, KIAA0128, MGC16619, MGC20339, RP5-876A24.2	NP_665798	6	16,6
UGP2	"UDP-glucose pyrophosphorylase 2"	UDPG, UGPP2, UDPGP2, pHC379	NP_006750	6	16,1
CAPZA1	"capping protein (actin filament) muscle Z-line, alpha 1"	CAPZ, CAZ1, CAPPA1	NP_006126	5	26,2
COPB	"coatomer protein complex, subunit beta"	DKFZp761K102	NP_057535	5	5,7
CORO1A	"coronin, actin binding protein, 1A"	p57, TACO, CLABP, HCORO1, CLIPINA, FLJ41407, Clabp TACO	NP_009005	5	12,1
DARS	"aspartyl-tRNA synthetase"	DKFZp781B11202	NP_001340	5	10,6
EIF3S7	"eukaryotic translation initiation factor 3, subunit 7 zeta, 66/67kDa"	MGC17258, eIF3-p66, eIF3-zeta	NP_003744	5	9,9
EVL	"Enah/Vasp-like"	RNB6	NP_057421	5	14,4
LOC388339	"similar to ribosomal protein S18"	LOC388339	ipi|IPI00397609	5	26,3
MTHFD1	"methylenetetrahydrofolate dehydrogenase (NADP+ dependent) 1, methenyltetrahydrofolate cyclohydrolase, formyltetrahydrofolate synthetase"	MTHFC, MTHFD	NP_005947	5	6,5
PSMB8	"proteasome (prosome, macropain) subunit, beta type, 8 (large multifunctional protease 7)"	LMP7, D6S216, RING10, D6S216E, MGC1491	NP_683720	5	22,1
RAN	"RAN, member RAS oncogene family"	TC4, ARA24	NP_006316	5	24,5
RPL13	"ribosomal protein L13"	BBC1, D16S444E, MGC71373	NP_150254	5	22,7
RPL3	"ribosomal protein L3"	TARBP-B	NP_000958	5	17,1
RPS14	"ribosomal protein S14"	EMTB	NP_005608	5	37,7
RUVBL2	"RuvB-like 2 (E. coli)"	ECP51, TIP48, CGI-46, TIP49B	NP_006657	5	12,1
SEPT1	"septin 1"	LARP, SEP1, DIFF6, PNUTL3, MGC20394	NP_443070	5	16,3

SRP14	"signal recognition particle 14kDa (homologous Alu RNA binding protein)"	ALURBP, MGC14326	NP_003125	5	34,6
SRP68	"signal recognition particle 68kDa"		NP_055045	5	8,0
TKT	"transketolase (Wernicke-Korsakoff syndrome)"	TKT1	NP_001055	5	12,2
ACOT7	"brain acyl-CoA hydrolase"	BACH, ACT, ACH1, LACH1, hBACH, CTE-II, MGC1126, RP1-120G22.10	NP_863656	4	15,5
C22orf28	"hypothetical protein HSPC117"	HSPC117, DJ149A16.6, RP1-149A16.6	NP_055121	4	8,7
CFL1	"cofilin 1 (non-muscle)"	CFL	NP_005498	4	33,7
FAM61A	"family with sequence similarity 61, member A"	C19orf13, DKFZP434D1335	NP_056393	4	6,7
GSR	"glutathione reductase"	MGC78522	NP_000628	4	10,9
HIST1H2AB	"histone 1, H2ab"	H2A/m, H2AFM	NP_003504	4	35,4
HIST1H2AC	"histone 1, H2ac"	H2A/l, H2AFL, MGC99519, dJ221C16.4	NP_003503	4	35,4
HIST1H2AE	"histone 1, H2ae"	H2A.1, H2A.2, H2A/a, H2AFA	NP_066390	4	35,4
HIST1H4A	"histone 1, H4a"	H4/a, H4FA	NP_003529	4	40,8
HIST1H4B	"histone 1, H4b"	H4/l, H4Fl	NP_003535	4	40,8
HIST1H4C	"histone 1, H4c"	H4/g, H4FG, dJ221C16.1	NP_003533	4	40,8
HIST1H4D	"histone 1, H4d"	H4/b, H4FB, HIST1H4F, dJ221C16.9	NP_003530	4	40,8
HIST1H4E	"histone 1, H4e"	H4/j, H4FJ	NP_003536	4	40,8
HIST1H4F	"histone 1, H4f"	H4, H4/c, H4FC	NP_003531	4	40,8
HIST1H4H	"histone 1, H4h"	H4/h, H4FH	NP_003534	4	40,8
HIST1H4I	"histone 1, H4i"	H4M, H4/m, H4FM	NP_003486	4	40,8
HIST1H4J	"histone 1, H4j"	H4/e, H4FE, dJ160A22.2	NP_068803	4	40,8
HIST1H4K	"histone 1, H4k"	H4/d, H4FD, dJ160A22.1	NP_003532	4	40,8
HIST1H4L	"histone 1, H4l"	H4.k, H4/k, H4FK	NP_003537	4	40,8
HIST2H4	"histone 2, H4"	H4, H4F2, H4FN, FO108	NP_003539	4	40,8
HIST2H4B	"histone H4/o"	H4/o	ipi|IPI00453473	4	40,8
HIST3H2A	"histone 3, H2a"	MGC3165	NP_254280	4	35,4
HIST4H4	"histone 4, H4"	MGC24116	NP_778224	4	40,8
HSPE1	"heat shock 10kDa protein 1 (chaperonin 10)"	CPN10, GROES, HSP10	NP_002148	4	35,3
IPO7	"importin 7"	RANBP7	NP_006382	4	4,7
LOC729998			ipi|IPI00738381	4	9,6
LOC730754			ipi|IPI00642513	4	21,7
NUP214	"nucleoporin 214kDa"	CAN, CAIN, N214, D9S46E	NP_005076	4	2,9
PARP1	"poly (ADP-ribose) polymerase family, member 1"	PARP, PPOL, ADPRT, ADPRT1, PARP-1, pADPRT-1	NP_001609	4	4,9
PCID1	"dendritic cell protein"	hfl-B5, GA17	NP_006351	4	15,8
PSMB3	"proteasome (prosome, macropain) subunit, beta type, 3"	HC10-II, MGC4147	NP_002786	4	22,9
PSMD6	"proteasome (prosome, macropain) 26S subunit, non-ATPase, 6"	S10, p44S10, KIAA0107, SGA-113M	NP_055629	4	10,8
RFC4	"replication factor C (activator 1) 4, 37kDa"	A1, RFC37, MGC27291	NP_853551	4	11,8
RPL18	"ribosomal protein L18"		NP_000970	4	25,5
RPL27	"ribosomal protein L27"		NP_000979	4	36,0
RPL30	"ribosomal protein L30"		NP_000980	4	34,8
RPS13	"ribosomal protein S13"		NP_001008	4	19,9
RPS17	"ribosomal protein S17"	RPS17L1, RPS17L2, MGC72007	NP_001012	4	23,7
RPS23	"ribosomal protein S23"		NP_001016	4	29,4
SF3A3	"splicing factor 3a, subunit 3, 60kDa"	PRP9, SAP61, SF3a60	NP_006793	4	8,6
SMC4L1	"SMC4 structural maintenance of chromosomes 4-like 1 (yeast)"	CAPC, hCAP-C	NP_005487	4	3,6
SRP72	"signal recognition particle 72kDa"		NP_008878	4	7,6
STAT1	"signal transducer and activator of transcription 1, 91kDa"	ISGF-3, STAT91, DKFZp686B04100	NP_644671	4	6,6
THOC4	"THO complex 4"	ALY, BEF	NP_005773	4	23,3
TLN2	"talin 2"	KIAA0320	NP_055874	4	1,8
ABI1	"abl-interactor 1"	E3B1, ABI-1, NAP1BP, SSH3BP, SSH3BP1	NP_005461	3	8,5
ACLY	"ATP citrate lyase"	ATPCL, CLATP	NP_001087	3	3,9
ACTR1A	"ARP1 actin-related protein 1 homolog A, centractin alpha (yeast)"	ARP1	NP_005727	3	10,9
ACTR2	"ARP2 actin-related protein 2 homolog (yeast)"	ARP2	NP_005713	3	9,6
C14orf166	"chromosome 14 open reading frame 166"	CLE, CGI-99	NP_057123	3	16,0
C3orf10	"chromosome 3 open reading frame 10"	MDS027, hHBrk1, HSPC300	NP_060932	3	37,3
CAPZA2	"capping protein (actin filament) muscle Z-line, alpha 2"	CAPZ, CAPPA2	NP_006127	3	14,3
COPA	"coatomer protein complex, subunit alpha"	HEP-COP	NP_004362	3	3,4
DDB1	"damage-specific DNA binding protein 1, 127kDa"	DDBA, XAP1, XPCE, XPE-BF, UV-DDB1	NP_001914	3	2,7
DDX6	"DEAD (Asp-Glu-Ala-Asp) box polypeptide 6"	P54, RCK, HLR2	NP_004388	3	10,0
DOCK2	"dedicator of cytokinesis 2"	KIAA0209	NP_004937	3	1,7
EIF3S4	"eukaryotic translation initiation factor 3, subunit 4 delta, 44kDa"	EIF3-P42, eIF3-p44, eIF3-delta	NP_003746	3	9,1
H2AFJ	"H2A histone family, member J"	MGC921, FLJ10903	NP_808760	3	27,1
H2AFV	"H2A histone family, member V"	H2AV, MGC1947, MGC10170, MGC10831	NP_036544	3	20,3
H2AFZ	"H2A histone family, member Z"	H2AZ, H2A.z, H2A/z	NP_002097	3	20,3
HBA1	"hemoglobin, alpha 1"	CD31	NP_000549	3	25,4
HBA2	"hemoglobin, alpha 2"	HBA1	NP_000508	3	25,4
HIST1H2AD	"histone 1, H2ad"	H2A.3, H2A/g, H2AFG, HIST1H3D	NP_066409	3	26,9
HIST1H2AG	"histone 1, H2ag"	H2A/p, H2AFP, H2A.1b, pH2A/f	NP_066408	3	26,9
HIST1H2AH	"histone 1, H2ah"	H2A/S, H2AFALii, dJ86C11.1	NP_542163	3	27,3
HIST1H2AI	"histone 1, H2ai"	H2A/c, H2AFC	NP_003500	3	26,9

HIST1H2AJ	"histone 1, H2aj"	H2A/E, H2AFE, dJ160A22.4	NP_066544	3	27,3
HIST1H2AK	"histone 1, H2ak"	H2A/d, H2AFD	NP_003501	3	26,9
HIST1H2AL	"histone 1, H2al"	H2A.i, H2A/i, H2AFI, HIST1H2AM, dJ193B12.9	NP_003502	3	26,9
HIST1H2AM	"histone 1, H2am"	H2A.1, H2A/n, H2AFN, dJ193B12.1	NP_003505	3	26,9
HIST2H2AA	"histone 2, H2aa"	H2A, H2A.2, H2A/O, H2AFO, H2a-615	NP_003507	3	26,9
HIST2H2AA4			ipi|IPI00216457	3	26,9
HIST2H2AC	"histone 2, H2ac"	H2A, H2A/q, H2AFQ, MGC74460, H2A-GL101	NP_003508	3	27,1
HNRPC	"heterogeneous nuclear ribonucleoprotein C (C1/C2)"	C1, C2, HNRNP, SNRPC, hnRNPC, MGC104306, MGC105117	NP_004491	3	11,3
KIAA1797	"KIAA1797"	FLJ20375	NP_060264	3	2,1
LOC388344	"similar to ribosomal protein L13; 60S ribosomal protein L13; breast basic conserved protein 1"	LOC388344	ipi|IPI00397611	3	12,8
NUP88	"nucleoporin 88kDa"	MGC8530	NP_002523	3	5,0
PA2G4	"proliferation-associated 2G4, 38kDa"	EBP1, p38-2G4	NP_006182	3	9,1
PDIA6	"protein disulfide isomerase family A, member 6"	P5, ERP5, TXNDC7	NP_005733	3	9,5
PGD	"phosphogluconate dehydrogenase"	6PGD	NP_002622	3	8,3
PRKCSH	"protein kinase C substrate 80K-H"	PCLD, G19P1, AGE-R2	NP_002734	3	5,9
PSMB4	"proteasome (prosome, macropain) subunit, beta type, 4"	HN3, HsN3, PROS26	NP_002787	3	12,9
PSMB9	"proteasome (prosome, macropain) subunit, beta type, 9 (large multifunctional protease 2)"	LMP2, RING12, MGC70470	NP_683756	3	14,8
PTPN2	"protein tyrosine phosphatase, non-receptor type 2"	PTPT, TCPTP, TC-PTP, TCELLPTP	NP_002819	3	8,2
RBM14	"RNA binding motif protein 14"	SIP, COAA, SYTIP1, DKFZp779J0927	NP_006319	3	4,5
RPL35	"ribosomal protein L35"		NP_009140	3	26,0
RPL35A	"ribosomal protein L35a"		NP_000987	3	15,5
RPL38	"ribosomal protein L38"		NP_000990	3	31,4
RPL8	"ribosomal protein L8"		NP_150644	3	13,6
RPS11	"ribosomal protein S11"		NP_001006	3	12,0
RPS5	"ribosomal protein S5"		NP_001000	3	18,1
SNRP70	"small nuclear ribonucleoprotein 70kDa polypeptide (RNP antigen)"	RPU1, U1AP, U170K, U1RNP, RNPU1Z	NP_003080	3	6,9
SNRPB	"small nuclear ribonucleoprotein polypeptides B and B1"	COD, SNRPB1, snRNP-B, SmB/SmB'	NP_937859	3	12,5
SNRPN	"small nuclear ribonucleoprotein polypeptide N"	SMN, SM-D, RT-LI, HCERN3, SNRNP-N, SNURF-SNRPN	NP_003088	3	12,5
TMEM137	"hypothetical protein MGC15912"	MGC15912	ipi|IPI00013174	3	4,5
WASF2	"WAS protein family, member 2"	SCAR2, WAVE2, dJ393P12.2	NP_008921	3	6,6
YARS	"tyrosyl-tRNA synthetase"	YRS, YTS, tyrRS	NP_003671	3	6,6
ABCE1	"ATP-binding cassette, sub-family E (OABP), member 1"	RLI, OABP, ABC38, RNS4I, RNASEL1, RNASELI	NP_002931	2	4,2
ACTR3	"ARP3 actin-related protein 3 homolog (yeast)"	ARP3	NP_005712	2	5,3
ARPC3	"actin related protein 2/3 complex, subunit 3, 21kDa"	ARC21, p21-Arc	NP_005710	2	13,5
ASCC3L1	"activating signal cointegrator 1 complex subunit 3-like 1"	HELIC2, U5-200KD	NP_054733	2	1,0
ATP5D	"ATP synthase, H+ transporting, mitochondrial F1 complex, delta subunit"		NP_001678	2	13,7
C17orf27	"chromosome 17 open reading frame 27"	KIAA1554	ipi|IPI00828098	2	0,7
C21orf70	"chromosome 21 open reading frame 70"	PRED56	NP_478070	2	11,7
CAD	"carbamoyl-phosphate synthetase 2, aspartate transcarbamylase, and dihydroorotase"		NP_004332	2	1,2
COPB2	"coatomer protein complex, subunit beta 2 (beta prime)"	beta'-COP	NP_004757	2	2,1
COPS5	"COP9 constitutive photomorphogenic homolog subunit 5 (Arabidopsis)"	CSN5, JAB1, SGN5, MOV-34, MGC3149	NP_006828	2	7,2
CSNK2A1	"casein kinase 2, alpha 1 polypeptide"	CKII, CK2A1, CKII alpha	NP_808227	2	4,6
CSNK2A1P	"casein kinase 2, alpha 1 polypeptide pseudogene"		ipi|IPI00784195	2	4,6
CSPG6	"chondroitin sulfate proteoglycan 6 (bamacan)"	BAM, BMH, HCAP, SMC3, SMC3L1	NP_005436	2	2,3
DAP3	"death associated protein 3"	DAP-3, MRPS29, MRP-S29, bMRP-10, DKFZp686G12159	NP_387506	2	6,0
DHX15	"DEAH (Asp-Glu-Ala-His) box polypeptide 15"	DBP1, HRH2, DDX15, PRP43, PrPp43p	NP_001349	2	3,1
DHX9	"DEAH (Asp-Glu-Ala-His) box polypeptide 9"	LKP, RHA, DDX9, NDHII, NDH II	ipi|IPI00742905	2	1,8
DLST	"dihydrolipoamide S-succinyltransferase (E2 component of 2-oxo-glutarate complex)"	DLTS	NP_001924	2	5,3
DLSTP	"dihydrolipoamide S-succinyltransferase pseudogene (E2 component of 2-oxo-glutarate complex)"		ipi|IPI00033034	2	5,3
DPYSL2	"dihydropyrimidinase-like 2"	DRP2, CRMP2, DRP-2, DHPRP2	NP_001377	2	3,5
EIF2S1	"eukaryotic translation initiation factor 2, subunit 1 alpha, 35kDa"	EIF2, EIF-2, EIF2A, EIF-2A, EIF-2alpha	NP_004085	2	7,0
H1FX	"H1 histone family, member X"	H1X, MGC8350, MGC15959	NP_006017	2	11,7
H2AFX	"H2A histone family, member X"	H2AX, H2A.X, H2A/X	NP_002096	2	11,2
H3F3A	"H3 histone, family 3A"	H3F3, H3.3A, MGC87782, MGC87783	NP_002098	2	11,0
H3F3B	"H3 histone, family 3B (H3.3B)"	H3.3B	NP_005315	2	11,0
HADH2	"hydroxyacyl-Coenzyme A dehydrogenase, type II"	ABAD, ERAB, MHBD, HSD17B10, 17b-HSD10	NP_004484	2	11,1
hCG_1641229			ipi|IPI00788270	2	7,5
hCG_1749005	"similar to H3 histone, family 3B"	LOC347376	ipi|IPI00232705	2	10,6
HDAC1	"histone deacetylase 1"	HD1, RPD3, GON-10, RPD3L1, DKFZp686H12203	NP_004955	2	6,4
HIST1H2AA	"histone 1, H2aa"	H2AFR, bA317E16.2	NP_734466	2	12,2
HIST1H3A	"histone 1, H3a"	H3/A, H3FA	NP_003520	2	11,0
HIST1H3B	"histone 1, H3b"	H3/l, H3FL	NP_003528	2	11,0
HIST1H3C	"histone 1, H3c"	H3.1, H3/c, H3FC	NP_003522	2	11,0
HIST1H3D	"histone 1, H3d"	H3/b, H3FB	NP_003521	2	11,0
HIST1H3E	"histone 1, H3e"	H3.1, H3/d, H3FD	NP_003523	2	11,0
HIST1H3F	"histone 1, H3f"	H3/i, H3FI	NP_066298	2	11,0
HIST1H3G	"histone 1, H3g"	H3/h, H3FH	NP_003525	2	11,0
HIST1H3H	"histone 1, H3h"	H3/k, H3FK, H3F1K	NP_003527	2	11,0
HIST1H3I	"histone 1, H3i"	H3.f, H3/f, H3FF	NP_003524	2	11,0

HIST1H3J	"histone 1, H3j"	H3/j, H3FJ	NP_003526	2	11,0
HIST2H3A	"histone 2, H3a"		ipi|IPI00171611	2	11,0
HIST2H3C	"histone 2, H3c"	H3, H3.2, H3/M, H3F2, H3FM, MGC9629	ipi|IPI00171611	2	11,0
HIST2H3D			ipi|IPI00719351	2	8,3
HIST2H3PS2	"similar to histone H2B histone family"	LOC440686	ipi|IPI00455457	2	11,0
HIST3H3	"histone 3, H3"	H3t, H3.4, H3/g, H3FT	NP_003484	2	11,0
HNRPCL1	"heterogeneous nuclear ribonucleoprotein C-like 1"	RP5-845O24.4	ipi|IPI00027569	2	7,5
HNRPM	"heterogeneous nuclear ribonucleoprotein M"	HTGR1, NAGR1, HNRNPM, HNRPM4, HNRNPM4, DKFZp547H118	NP_005959	2	2,9
HRMT1L4	"HMT1 hnRNP methyltransferase-like 4 (S. cerevisiae)"	HRMT1L3	NP_062828	2	3,0
IFITM1	"interferon induced transmembrane protein 1 (9-27)"	9-27, CD225, IFI17, LEU13	NP_003632	2	13,6
IFITM2	"interferon induced transmembrane protein 2 (1-8D)"	1-8D	NP_006426	2	12,9
IFITM3	"interferon induced transmembrane protein 3 (1-8U)"	1-8U	NP_066362	2	12,8
KCTD1	"potassium channel tetramerisation domain containing 1"	C18orf5	NP_945342	2	8,2
LOC387753	"similar to 60S ribosomal protein L21"	LOC387753	ipi|IPI00398915	2	16,3
LOC388532	"similar to 60S ribosomal protein L21"	LOC388532	ipi|IPI00397713	2	18,4
LOC390876	"similar to ribosomal protein L35"	LOC390876	ipi|IPI00787131	2	16,9
LOC402057	"similar to 40S ribosomal protein S17"	LOC402057	ipi|IPI00414603	2	14,8
LOC440093	"similar to H3 histone, family 3B"	LOC440093	ipi|IPI00419884	2	11,1
LOC440563	"similar to Heterogeneous nuclear ribonucleoprotein C-like dJ845O24.4 (hnRNP core protein C-like)"	LOC440563	ipi|IPI00455415	2	8,8
LOC440587	"similar to 60S ribosomal protein L6 (TAX-responsive enhancer element binding protein 107) (TAXREB107) (Neoplasm-related protein C140)"	LOC440587	ipi|IPI00455427	2	4,5
LOC440737	"similar to 60S ribosomal protein L35"	LOC440737	ipi|IPI00455482	2	17,9
LOC644914			ipi|IPI00413826	2	11,0
LOC649330			ipi|IPI00735540	2	7,5
LOC652147			ipi|IPI00740142	2	1,4
LOC653658			ipi|IPI00739952	2	15,0
LOC653737			ipi|IPI00478812	2	16,3
LOC728501			ipi|IPI00788010	2	16,3
LOC728953			ipi|IPI00787105	2	13,7
LOC729362			ipi|IPI00220344	2	20,8
LOC729402			ipi|IPI00247583	2	16,3
LOC729535			ipi|IPI00788063	2	14,9
LOC730004			ipi|IPI00787417	2	33,3
LOC730700			ipi|IPI00788010	2	16,3
LOC730740			ipi|IPI00413826	2	11,0
LOC731499			ipi|IPI00788063	2	14,9
LOC731567			ipi|IPI00247583	2	16,3
LOC731572			ipi|IPI00787105	2	13,7
LOC731640			ipi|IPI00398915	2	16,3
LOC732066			ipi|IPI00787417	2	33,3
LSM4	"LSM4 homolog, U6 small nuclear RNA associated (S. cerevisiae)"	YER112W	NP_036453	2	11,5
MAD2L1	"MAD2 mitotic arrest deficient-like 1 (yeast)"	MAD2, HSMAD2	NP_002349	2	8,8
MIF	"macrophage migration inhibitory factor (glycosylation-inhibiting factor)"	GIF, GLIF, MMIF	NP_002406	2	17,4
MRPL11	"mitochondrial ribosomal protein L11"	L11mt, CGI-113	NP_733934	2	13,9
MYL6B	"myosin light chain 1 slow a"	MLC1SA	NP_002466	2	11,5
NCKAP1	"NCK-associated protein 1"	HEM2, NAP1, NAP125, MGC8981, KIAA0587	NP_995314	2	2,4
NP	"nucleoside phosphorylase"	PNP, PRO1837	NP_000261	2	6,9
NSF	"N-ethylmaleimide-sensitive factor"	SKD2	NP_006169	2	3,0
PDAP1	"PDGFA associated protein 1"	PAP, PAP1, HASPP28	NP_055706	2	16,0
PDCD4	"programmed cell death 4 (neoplastic transformation inhibitor)"	H731, MGC33046, MGC33047	NP_055271	2	4,7
PDHA2	"pyruvate dehydrogenase (lipoamide) alpha 2"	PDHAL	NP_005381	2	5,2
PRDX6	"peroxiredoxin 6"	PRX, p29, AOP2, 1-Cys, NSGPx, aiPLA2, KIAA0106, MGC46173	NP_004896	2	8,5
PSMA2	"proteasome (prosome, macropain) subunit, alpha type, 2"	MU, HC3, PSC2, PMSA2	NP_002778	2	12,0
PSME2	"proteasome (prosome, macropain) activator subunit 2 (PA28 beta)"	PA28B, REGbeta, PA28beta	NP_002809	2	8,8
PSPC1	"paraspeckle component 1"	PSP1, FLJ10955, DKFZp566B1447	ipi|IPI00103525	2	4,4
RBMX	"RNA binding motif protein, X-linked"	RNMX, HNRPG, RBMXP1, RBMXRT	NP_002130	2	6,9
RBMXL1	"RNA binding motif protein, X-linked-like 1"	KAT3	NP_062556	2	6,9
RETN	"resistin"	ADSF, RSTN, XCP1, FIZZ3, RETN1	NP_065148	2	24,1
RFC2	"replication factor C (activator 1) 2, 40kDa"	A1, RFC40, MGC3665	NP_852136	2	8,5
RFC5	"replication factor C (activator 1) 5, 36.5kDa"	RFC36, MGC1155	NP_031396	2	6,8
RPL21	"ribosomal protein L21"	L21, FLJ27458, MGC71252	NP_000973	2	16,3
RPL22	"ribosomal protein L22"	EAP, HBP15, HBP15/L22	NP_000974	2	18,8
RPL28	"ribosomal protein L28"		NP_000982	2	12,4
RPL36	"ribosomal protein L36"	DKFZP566B023	NP_056229	2	19,0
RPL36A	"ribosomal protein L36a"	L44L, MIG6, RPL44, MGC72020	NP_066357	2	20,8
RPL36AL	"ribosomal protein L36a-like"	RPL36A	NP_000992	2	20,8
RPS24	"ribosomal protein S24"	DKFZp686N1586	NP_001017	2	20,3
RRM2	"ribonucleotide reductase M2 polypeptide"	R2, RR2M	NP_001025	2	7,2
SEC23A	"Sec23 homolog A (S. cerevisiae)"	MGC26267	NP_006355	2	3,4
SEC23B	"Sec23 homolog B (S. cerevisiae)"		NP_116780	2	3,4

SEPT11	"septin 11"		NP_060713	2	4,7
SERPINF1	"serine (or cysteine) proteinase inhibitor, clade F (alpha-2 antiplasmin, pigment epithelium derived factor), member 1"	PEDF, EPC-1	NP_002606	2	5,5
SF3B14	"splicing factor 3B, 14 kDa subunit"	SF3B14, P14, Ht006, SAP14, CGI-110, HSPC175, SF3B14a	NP_057131	2	20,8
SFRS3	"splicing factor, arginine/serine-rich 3"	SRp20	NP_003008	2	14,0
SNRPD3	"small nuclear ribonucleoprotein D3 polypeptide 18kDa"	SMD3	NP_004166	2	15,1
SNRPE	"small nuclear ribonucleoprotein polypeptide E"	SME	NP_003085	2	25,0
SNRPEL1	"small nuclear ribonucleoprotein polypeptide E-like 1"	bA390F4.4	ipi|IPI00068430	2	25,0
SNRPF	"small nuclear ribonucleoprotein polypeptide F"	SMF	NP_003086	2	24,4
SRP54	"signal recognition particle 54kDa"		NP_003127	2	4,8
STRBP	"spermatid perinuclear RNA binding protein"	SPNR, MGC3405, FLJ11307, FLJ14223, FLJ14984, MGC21529, DKFZp434N214	NP_060857	2	3,0
TALDO1	"transaldolase 1"	TAL, TALH, TAL-H, TALDOR	NP_006746	2	7,1
USP39	"ubiquitin specific protease 39"	SAD1, CGI-21, HSPC332, MGC75069	NP_006581	2	4,8
KIAA1618	"KIAA1618"		NP_066005	1	1,0
PRDX5	"peroxiredoxin 5"	PLP, ACR1, B166, PRXV, PMP20, PRDX6, SBBI10, AOEB166	NP_857635	1	8,8
RNPC2	"RNA-binding region (RNP1, RRM) containing 2"	HCC1, CAPER, CC1.3, CC1.4, DKFZp781C0423	ipi|IPI00746554	1	4,8
SFRS7	"splicing factor, arginine/serine-rich 7, 35kDa"	9G8, HSSG1	ipi|IPI00332419	1	8,8