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Signaling in natural killer cells: SHIP, 2B4 and the Kinome

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Signaling in Natural Killer Cells:

SHIP, 2B4 and the Kinome

by

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Note to the Reader

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Dedication

I wish to dedicate this to my family and friends that have played a crucial role in me reaching this stage. I would not have been able to undertake such a challenge without their support.

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ABSTRACT

The NK cell is a large granular lymphocyte that plays a key role in protecting the body against numerous pathogens including parasites, intracellular bacteria, viral infections, as well as showing anti-tumor activity and playing a role in the rejection of allogeneic BM. Unlike other lymphocytic cell types, that utilize rearranging receptors, NK cells are regulated by a complex array of germ line encoded activating and inhibitory receptors. NK cells are often described as a front line or rapid defense given their response to stimuli can be immediate, although they also maintain functions that extend their role well into the adaptive immune system.

Inhibitory receptors that recognize MHC class I molecules regulate NK cell responses and self-tolerance. Recent evidence indicates self-ligands not present in the MHC locus can also modulate NK function. We previously demonstrated that the NK receptor repertoire is disrupted by SHIP-deficiency. Here we show that an inhibitory receptor, 2B4, that recognizes an MHC-independent ligand is over expressed in NK cells of $SHIP^{-/-}$ mice at all stages of NK development and differentiation. Overexpression of

2B4 compromises key cytolytic NK functions, including killing of allogeneic, tumor and viral targets. These results demonstrate that in SHIP$^{-/-}$ NK cell 2B4 is the dominant inhibitory receptor.

We then furthered this finding by examining the molecular basis of 2B4 dominance. We show that in SHIP$^{-/-}$ NK cells there is increased 2B4 expression as well as a strong bias towards the 2B4L isoform. We have also identified a greater than ten-fold increase in SHP1 recruitment to 2B4. Consistent with this SHP1 over recruitment, both a broad and a selective SHP1 inhibitor restore SHIP$^{-/-}$ NK killing of complex targets. Through this study we have identified the molecular mechanism of 2B4 receptor dominance as SHP1 over-recruitment.

In addition we have utilized protein array technology to explore NK signaling through the determination of the NK kinome. To this end we have been able to identify multiple pathways that may mark crucial differences between the mature and immature NK cell.

Chapter 1

Introduction

Early NK Studies

Discovery of NK Cells

The first studies identifying what would eventually be termed the natural killer cell (NK) were performed just over 20 years ago. These first studies were following in the steps of intriguing questions raised by the concept of natural cytotoxicity. Early adoptive transfer experiments in which the transplanted tumors were rejected were thought to rely on the presence of T cells (1-6). In line with this theory it was hypothesized that athymic and/or nude mice, which lack T cells, would have a dramatically increased number of tumors after these adoptive transfers. This unimpeded growth of tumors in a T cell deficient mouse was never realized, indicating that it was not the T cell that was limiting the expansion of these adoptively transferred tumor cells (7-9). This lead people to hypothesize that there must be an alternate cell type at play. Another interesting phenomenon was occurring within these adoptive transfer experiments. Much of the reason that the rejection of the tumors was being attributed to the T cell was that many of the hosts, of the tumor transplants, had been previously immunized or exposed to the tumors. This ability to destroy a previously immunized against antigen is a hallmark of T cells and the adaptive immune response (10, 11). In

contrast to this it was found that although these previously immunized hosts could reject the tumors, rejection was also seen in non-immunized hosts (12-14). This ability to lyse tumor targets without prior immunization by this yet to be identified cell type is what was termed natural cytotoxicity. Further studies were able to attribute this natural cytotoxicity to a distinct population of lymphoid cells (15-19). In these studies various groups identified a cell that remained after elimination of T and B cells from whole splenocytes. The remaining cells, which had a small lymphoid like appearance, retained the ability, *in vitro,* to lyse a large number of virally induced tumors as well as other tumor samples. The key to these studies was that once again like in the rejection of adoptively transferred tumors, prior immunization was not necessary and therefore was natural cytotoxicity. This small lymphoid like cell, that was neither T nor B cell, was therefore termed a natural killer cell or NK cell.

Missing Self Hypothesis

Although the NK cell had been identified, the manner in which it functioned remained unknown. One area that was puzzling was that NK cells had varied cytotoxicity to cells of differing major histocompatibility complex (MHC) backgrounds. The initial answer to this problem would come when Karre and Ljunggren proposed their missing self hypothesis (20, 21). The missing self hypothesis has its origins in a puzzling question raised many years earlier, which was hybrid resistance. Hybrid resistance refers to a phenomenon where an F1 hybrid from parents of different MHC background is able to reject bone marrow (BM) transplants from both parents (22, 23). Specifically an H-2a/a crossed with an H-2b/b would result in a heterozygous mouse with an H-2a/b

haplotype. BM cells transplanted into the F1 hybrid from either the H-2a/a or H-2b/b parent would be rejected. These studies provided an initial foray into the concept of self vs. non-self recognition by the NK cell. Further evidence for the missing self hypothesis was obtained through numerous studies in which it was found that NK cells could kill tumor lines that had diminished or no MHC expression (24-28). This idea was novel, as the traditional concept established in T cells, maintained that you needed the MHC molecules as well as peptide bound in its cleft in order elicit a cytotoxic effect. In their studies Karre and colleagues generated MHC deficient cells lines in order to test, *in vitro*, the ability of the NK cell to lyse MHC null targets (29). Through these studies they were able to determine that the presence of a self-MHC inhibited NK mediated cytotoxicity. In addition they introduced H-2D^{d} transgenes into Bl6 H-2b mice to determine the effect on the rejection of BM transplants (20). In these studies they found that the insertion of the H-2D^{d} transgene was able to confer resistance to allogeneic BM into an H-2D^{d} mouse. With their studies and the other information present they proposed the concept that an NK cell utilizes recognition of the MHC molecules to identify self vs. non-self cells and thereby protecting self cells while retaining the ability to lyse non-self cells (21). They termed this the missing self hypothesis.

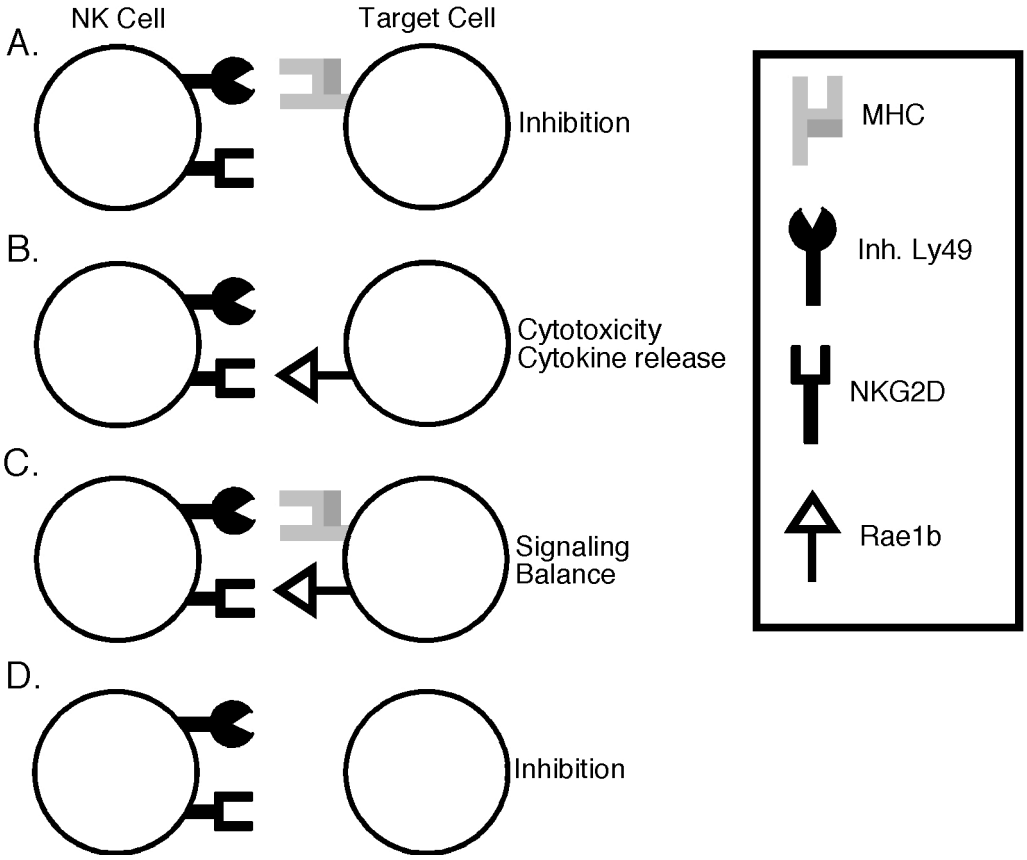

Figure 1. NK cell signaling outcomes. NK effector functions are determined by the specific ligands present on the target cell. A) A normal self cell expressing the appropriate inhibitory self ligand (MHC) alone, thereby not eliciting an NK response. B) A cell expressing an activating ligand in the absence of a self ligand which would activate the NK cell. C) A more complex situation in which both an activating ligand as well as self-MHC are present. The outcome of this situation would depend upon the balance of the receptors as well as the entire signaling milieu present. D) A situation that would occur in a $B2M^{-/-}$ mouse in which neither MHC or activating ligands are present on the cell surface. Inhibition would therefore be mediated through other mechanisms including non-classical self-receptors such as 2B4.

NK Recognition

The missing-self hypothesis was a major step forward in the NK field. Although it did not indicate how an NK cell would recognize the MHC, it did predict the presence of an inhibitory type receptor that would mediate MHC recognition. This prediction was manifested with the discovery of the first NK receptors (NKR) for self ligands. One of the first NKR identified was the Ly49 receptor (30). This early study identified a receptor that was found on 20% of $NK1.1^{+}$ $CD3^{-}$ NK cells from C57BL/6 mice. When functional studies were performed it was found that the expression of Ly49 had a functional consequence on the ability of NK cells to lyse certain targets. Specifically the presence of this receptor inhibited the lysis of $H\text{-}2D^{d}$ positive targets (31). This study provided one of the first insights into how the NK cell would be able to identify and lyse the appropriate targets based on MHC expression. Within these first studies they identified only one NKR, but proved a key concept, which was that an inhibitory receptor could recognize a self-MHC and thereby protect a self-cell. Although this greatly supported the missing self hypothesis it only provided part of the whole story. According to it any cell with lowered or lacking MHC expression would be susceptible to NK attack. Though the process has proven to be more complex as we see in mice lacking MHC, such as the $B2M^{-/-}$ mouse, where even with the lack of MHC molecules there is not NK auto reactivity (32, 33). This indicates that there is a more complex process in place necessitating a balance within the NK cell of both activating and inhibitory receptors, as well as the possibility of proper licensing and/or education that will be discussed shortly. To date a large number of receptors present on the NK cell have been

identified providing for both inhibition, through recognition of MHC and non-MHC molecules as well as activation through activating receptors.

Self-MHC Receptors

Ly49s

In the mouse the most prevalent self-MHC receptors are the Ly49s. Ly49s are members of the C-type lectin family and are type II transmembrane glycoproteins that are expressed as disulfide linked homodimers on the cell surface (34-36). Genetic studies of the chromosomal location of the Ly49 gene yielded the finding that Ly49 is actually a member of a larger gene family encoded on mouse chromosome 6 in a region termed the NK complex (NKC) (30, 37, 38). Much work has been done since these initial studies identifying and providing examinations of the functions of the Ly49 receptor family. The Ly49s are a highly related gene family consisting of at least 14 family members, in the BL6 mouse, all of which are encoded within the NKC (30, 34-36, 39-45). Most of the receptors within this family are inhibitory receptors with few exceptions i.e. Ly49 D and H (46, 47). It was identified early on that the different receptors within this complex have a variegated expression and function. One of the first studies demonstrated this by identifying in addition to the original Ly49, which was termed Ly49A, there was also a Ly49B and Ly49C. An important aspect of this study was not only that they discovered these new receptors but also that they showed Ly49A and Ly49C could be expressed on varying subsets of $NK1.1^{+}$ $CD3^{-}$ cells (48). This provided a concept that different NK cells may have different combinations of these receptors and therefore the ability to

respond differently depending on the ligand present.

The actual specificity to one or more MHC molecules depends upon the particular Ly49 receptor, with some displaying more or less promiscuity than others. Ly49A for instance has been shown to bind to H-2D^{d}, H-2D^{k}, and H-2^{K} (31, 49-52) and Ly49C has been shown to bind to H-2K^{b} as well as H-2^{b}, H-2^{d}, H-2^{k},H-2^{s} (35, 39, 53-56). Some Ly49s show more specificity to a limited number of ligands such as Ly49G2 which binds to H-2D^{d} and H-2L^{d} (34, 57), and Ly49I which binds to H-2K^{b} (53). An important aspect of all of these interactions is that this list does not represent the penultimate specificities of these receptors. Second, and more importantly it does not detail the relevance and or strength of these interactions, which could play a crucial role in the outcome they elicit.

The manner in which an NK cell expresses varying Ly49s on its cell surface is a unique and variegated system. RT-PCR studies have shown that each NK cell in a population expresses on average 1 to 4 different Ly49s (58). The expression system in place for the Ly49's allows for their expression in a monoallelic fashion through a system of probabilistic switches (59-61). The probabilistic switch is manifested through the use of a bidirectional promoter for each Ly49 (61). These promoters can function in the forward (On) position where they from a viable transcript or in the reverse (Off) position where a non-coding transcript is formed. Through competitive binding of promoter elements such as, CAAT/enhancer binding protein (C/EBP) or TATA-binding protein (TBP), on overlapping portions of the bidirectional promoters they are able to effect these probabilistic switches as well as lock the promoters in the established direction in the

mature NK cell (61). Conflicting results exist as to whether cytokines can induce a change in the Ly49 repertoire that has been established in a mature NK cell. Some studies have shown that once an NK cell has established its Ly49 repertoire it maintains it throughout its clonal progeny (62, 63). Other studies have shown that some receptors, such as Ly49E and F can be induced to change their expression levels through cytokines such as IL-2 and IL-15 (64). Other receptors may also undergo a change in surface density following cytokine stimulation.

An interesting facet of the variegated expression of Ly49s across an entire population of NK cells is that each cell will have a different potential to react to a target. In theory some NK cells may not possess an appropriate inhibitory Ly49 at all. This very situation has been realized such that a small population of NK cells within the normal population of cells does not express a known inhibitory Ly49 (65, 66). Based on the known information this could theoretically lead to an NK cell that is autoreactive. This very phenomenon has led to a debated area within the NK field, which is the concept of licensing, education and/or tolerance (65-67). In generalized terms what these theories state is that for an NK cell to become fully functional it must posses a self-receptor. For licensing the presence of a self-MHC receptor confers an ability or license upon the NK cell to become fully cytotoxic (65), for tolerance the absence of the self receptor renders the cell hyporesponsive (66), education similar to licensing dictates that the presence of the self-MHC receptor is necessary for the NK cell to become fully cytotoxic (57). Although these theories have slightly different methods through which an NK cell

achieves this functional state, they agree that for an NK cell to become fully functional it must posses a self-receptor. A key outcome of these studies is that even though NK cells do not rearrange their receptors, rather using supposedly more basic germ line encoded receptors, they still undergo a complex process of regulation that assures the proper cells and receptors are functioning in the appropriate manner.

A remaining question for the Ly49 receptors is what do they actually recognize on the MHC molecule and like the T cell receptor is there specificity to the peptide being presented. The Ly49A receptor has been shown to interact at two sites on H-2D^{d}. The first being within the α1 and α2 domains, and the second site of interaction involving the α1, α2, α3 and β2-M domains of the MHC (68, 69). This specificity may only hold true for this specific interaction, as the Ly49C recognition of H-2K^{b} does not exhibit the same interaction sites (70). The necessity of a peptide being present is also not completely clear. It has been shown that for the Ly49A receptor, a peptide is necessary, but the actual peptide sequence seems to have no bearing (56, 71). Where Ly49 C and Ly49I seem to be more reliant on a peptide being present to bind properly (56, 72, 73).

Although the Ly49s consist of numerous receptors that recognize a variety of different ligands, their cytoplasmic portions as well as the signaling pathways they activate are highly conserved. The cytoplasmic portion of the inhibitory Ly49s contain immunoreceptor tyrosine-based inhibition motifs (ITIM) with the consensus sequence of I/S/T/LxYxxL/V (74, 75). Upon ligand engagement these ITIMs become phosphorylated allowing for the recruitment of SH2 domain containing proteins. These phospho-

tyrosines are able to recruit the phosphatases SHP1, SHP2 and SHIP. SHP1 and 2 are then able to abrogate the downstream signaling cascades of activating receptors through dephosphorylation of protein tyrosine kinases (PTK) (74-79). Where SHIP could abrogate signals passing through the PI3K pathway through the removal of the 5' phosphate of PI(3,4,5)P3 (PIP3).

Although most of the Ly49 receptors have been shown to function as inhibitory receptors there are those Ly49s that function as activating receptors, these include Ly49H and Ly49D (47, 80, 81). The key difference with these receptors with respect to their inhibitory family members is that they do not posses the classic ITIM sequence in their cytoplasmic domain. Rather they have a positively charged arginine in the transmembrane portion of the cytoplasmic tail. This positively charged amino acid then allows these receptors to associate with an immunoreceptor tyrosine based activation motif (ITAM) containing DAP12 molecule (46, 82). The physiological role of these activating Ly49 receptors and their recognition of self-MHC molecules has not been fully elucidated. Ly49D has been implicated in C57Bl/6 mice in the rejection of H2-D^d BM, although the precise strength of this interaction is not fully clear (56, 83-86). It may be possible that in normal physiological conditions the activating Ly49s do not recognize MHC molecules but rather stress inducible markers, similar to other activating receptors. In the case of Ly49H, the ligand is the MCMV encoded m157 glycoprotein (80, 81). In fact mice that do not express the Ly49H receptor, such as BalbC, do not confer an NK mediated resistance to MCMV infection (87).

CD94/NKG2

The other class of receptors within the mouse that recognize class I related molecules are the CD94/NKG2 class of receptors (88-90). Like the Ly49 receptors, CD94 and the NKG2A,C, and E molecules are closely linked on chromosome 6 within the NKC (88, 89). Unlike the Ly49s the NKG2 receptors form disulfide linked heterodimers with CD94. Both of these receptors are in the C-type lectin family and encode type II transmembrane receptors (89, 91, 92). These receptors are capable of recognizing self ligands through the recognition of MHC class Ib ligands, such as QaI^b in the mouse (89, 90). Only the CD94/NKG2A heterodimer functions as an inhibitory receptor, where NKG2C and E function in an activating manner (89, 91, 93-95). Both NKG2C and E form heterodimers with CD94 but have a lysine residue in the cytoplasmic tail that allows them to associate with the ITAM containing DAP12. In fact the presence of DAP12 is necessary for their stable expression on the cell surface, confirming that these two receptors function in an activating manner (96). These receptors are expressed in an ordered manner and on overlapping subsets of cells (97). This therefore begs the question of what is the purpose of both activating and inhibitory receptors recognizing the same peptides. Although no definitive evidence has been found it may be that the receptors although extremely closely related have slight variance in the strength in which they recognize different peptides allowing different signaling outcomes based on other signals present.

Non-MHC receptors

NKG2D

NKG2D has been found to be a key activating receptor that is found on all NK cells (98-100). NKG2D is a type-II transmembrane glycoprotein that is expressed as a homodimer on the surface of NK cells (99, 101). Unlike other NKG2 molecules, NKG2D does not associate with the CD94 coreceptor (102). Within the mouse NKG2D has a short and long isoform that allows it to associate with either DAP10 or DAP12 respectively (98, 103). The ability to utilize both of these adapters allows NKG2D to signal through different downstream pathways, including PI3K as well as Syk and Zap70 (102, 104).

NKG2D recognizes molecules that are structurally related to the MHC but do not present peptides (105). These molecules include the Rae1 family members as well as Mult1 and H60 (98, 106-108). The Rae1 gene family is highly homologous where H60 and Mult1 have very little sequence homology (107, 109). This wide variety of ligands is able to present danger signals in response to a wide variety of stresses, and thereby, in turn allows the NK cell to respond to a wide variety of stresses. These varied immune challenges can include viral infections where early in the immune challenge the presence of one of these stress inducible markers can lead not only to NK mediated cytotoxicity, but also for an NK cell to cell to up regulate IFN-γ (110). This is then able to lead to a more potent adaptive immune response as well as effecting the regulation of viral transcription early in the infection (111). Raulet et al have shown that in response to DNA damage some cells can upregulate NKG2D ligands, thereby providing an NK cell

reliant mechanism for the elimination of cells with aberrant DNA replication and possible mutations that could lead to transformation (112). Within tumor immunity similar systems exist with stressed cells up regulating NKG2D ligands to allow for both direct lysis by the NK cell as well as production of cytokines, both to regulate tumor growth as well as to inducing an adaptive response (99, 101, 107, 113-116). The response of the NK cell via NKG2D has proven to be a potent enough response that some tumors have ways in which to combat NKG2D mediated cytotoxicity. These cells are able to produce a soluble NKG2D ligand. This can lead to the down-regulation of NKG2D on the NK cell (117). This ability of NKG2D to elicit a powerful activating signal through both DAP10 and DAP12 in response to numerous stimuli makes it one of the most potent and important activating NKR.

2B4

Although the Ly49's recognition of MHC is the prototypical system for the recognition of host encoded ligands and therefore the recognition of self, there are other receptors that recognize non-MHC molecules encoded by the host, one such receptor is 2B4. 2B4 is a SLAM family related receptor. Other receptors in this family consist of signal lymphocyte activation molecule (SLAM), NK, T- and B-cell antigen (NTB-A), CD2-like receptor activating cytotoxic cells (CRACC), Ly-9 and CD84 (118-125). The SLAM related receptors are members of the CD2 Ig superfamily possessing two extracellular Ig like domains. The intracellular portion of these receptors consists of one or more immunoreceptor tyrosine based switch motif (ITSM) consisting of the sequence TxYxxI/V (126, 127). Most of the receptors in the SLAM family function through

homotypic interactions, although 2B4 does not function in this manner instead it recognizes CD48 (128), which is ubiquitously expressed on cells of the hematopoietic system (128). 2B4 is expressed ubiquitously on NK cells as well as on subsets of T cells and even a small subset of dendritic cells (128, 129).

2B4 has a complex role in NK cell function and physiology that remains an active area of investigation. Depending on the context 2B4 has been shown to act as both an inhibitory and activating receptor. Much of the functional variation of this receptor may be due to studying it in both human and mouse models as well as different strains of mouse. (130-138). A more definitive role for 2B4 in the Bl6 mouse was demonstrated through the generation of a 2B4 knock out (KO). This KO mouse provided strong evidence, in the Bl6 model, for 2B4 as an inhibitory receptor (128, 129, 139). The ability of this receptor to function in numerous facets appears to be due at least in part to the multitude of signaling molecules 2B4 can recruit. In both human and mouse models, under different signaling contexts, 2B4 has been shown to recruit SAP, EAT-2, FynT, SHP1, PI3K and SHIP (133, 139-142). How the differential recruitment of these signaling entities is controlled is not completely understood. However, which molecules are recruited and thus which signal is propagated following CD48 engagement may be influenced by the ratio of 2B4 isoforms expressed in the NK cell. Two 2B4 isoforms have been identified in mice, short (2B4S) and long (2B4L), that were proposed to have activating and inhibitory signaling capacities, respectively (134). Although the exact function of these two isoforms remains to be defined, it is feasible that the different intracellular domains within these isoforms could recruit different effectors of cell

signaling. 2B4 presents another interesting phenomenon within the NK field. Ever since the missing self hypothesis and the discovery of the first Ly49s, it has been thought that it was through this mechanism alone that the NK cell recognized self and inhibited cytotoxicity. Interestingly, Mcnerney et al have proposed another possibility in which 2B4 may also play a role in self recognition. They have performed experiments in which they were able to show, through mechanisms non-redundant to the Ly49s, that 2B4 was able to recognize self and inhibit BM graft rejection (131). This does not necessarily alter the current system of self recognition by the NK cell; it merely extends the receptors that can participate in this process.

Although NKG2D and 2B4 are crucial receptors they are far from the only non-MHC receptors present on the NK cell. There are a multitude of others that function in both activating and inhibitory capacities. For instance the natural cytotoxicity receptor NKp46 has been shown to play a role in both tumor and viral regulation (143-145). The ligand for this receptor has not been fully identified but is believed to be heparin-sulfate proteoglycans (146). NKp46 like 2B4 has 2 extra-cellular IG domains as well as a short cytoplasmic tail containing an arginine that allows it to associate with ITAM containing molecules (147). Given their theorized ability to bind to proteoglycans this receptor may prove to be a crucial receptor with an ability to recognize a wide variety of targets.

Integration of Signaling

A common theme in many immune cells is the manner in which they propagate activating signals through the cell. As has been discussed already, there are a number of different activating ligands, some with very disparate structures and functions. In the NK cell and in many other immune cells this disparity is reconciled by the use of adaptor molecules that are able to interact with a wide variety of receptors (98). These adaptors consist of the ITAM containing molecule DAP12 or the DAP10 molecule that consists of a YxxM motif (102). These molecules contain a negatively charged amino acid in the amino terminus, which allows them to interact with the positively charged amino acid in the transmembrane domain of the activating receptors (102). An important aspect of utilizing a small number of adaptor type molecules to transduce signals is that a relatively small number of pathways are utilized over and over to transduce these signals. A summary diagram of these signaling pathways is shown in Fig 2. This figure models the balance that exists within the signal motifs utilized by the NK cell. The activating receptors through their pairing with DAP10 and/or DAP12 signal through either PI3K or PTKs respectively. If they utilize the PTK pathway the first line of PTKs tend to be a member of the Syk/Zap70 family, which then allows the signal to emanate to a number of pathways. This requirement for the use of PTK or inositol phospholipids for activating signals allows the inhibitory receptors, through the ability of their ITIMs to recruit SHP and SHIP, to abrogate these signals very efficiently.

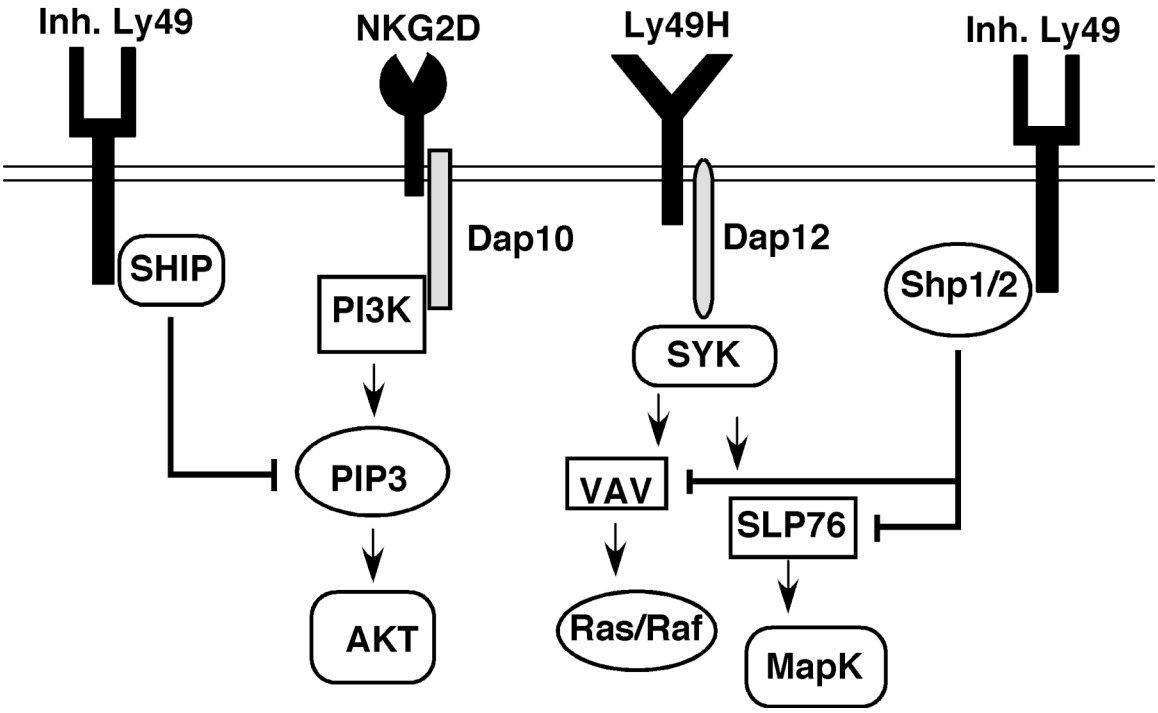

Figure 2. Integration of activating and inhibitory NKR signals. Model diagram showing the use of both PTK and PI3K by activating NKR. SHP is then able to abrogate signals emanating through the PTKs and SHIP has a similar function within the PI3K pathway.

NK Effector Functions

Cytotoxicity

Two mechanisms, the granule exocytosis method and the death receptor pathways, can affect the direct cytolysis of target cells by NK cells. In the granule exocytosis method, when an NK cell is properly activated, it forms a synapse with the target cell. The formation of this synapse then allows the release of the lytic granules in the proper orientation to the target cell to induce cell death and/or apoptosis (148-150).

These lytic granules include perforin and the granzymes each with a unique role that leads to the target cell undergoing apoptosis. Perforin was originally believed to be necessary to produce holes in the target cell to allow the granzymes to enter the target cell. Although this role has been in question with perforin possibly having other roles directly resulting in apoptosis. What is certain is that perforin is necessary for NK cells to efficiently lyse target cells (151, 152). In fact the presence of perforin has been shown to play a key role in NK regulation of tumors (153-156). The role of the granzymes in apoptosis is better understood. In mice there are 10 known granzymes (157). Granzyme B is one of the more studied of the granzymes and has been shown to initiate apoptosis in a caspase dependent and independent method. The granzymes elicit their function through cleavage of cellular proteins at specific consensus sequences. Granzyme B functions via cleavage after specific aspartic acid residues (158), one such site is found within caspase-3. Via granzyme B's cleavage of caspase 3 it is able to activate it and thereby the caspase dependent apoptotic cascade (159-161). Granzyme B is also able to cleave aspartic residues sites on members of the Bcl-2 family of proteins especially BID. Thereby disrupting the mitochondrial membrane and causing cytochrome C release and once again leading to apoptosis of the cell (162, 163).

The death receptor pathway has also been shown to play a key role in NK mediated cytotoxicity (164-167). NK cells are able to express a number of ligands that can induce death receptor pathways including FasL, TNFα and TRAIL (168-171). Similar to the granule exocytosis method, upon proper stimulation and synapse formation these ligands are brought to the cell surface at the site of the synapse allowing them to

interact with death receptors on the target cell. Interestingly the NK cell is also able to augment expression of these death receptors on the target cell to make them more susceptible to lysis. For instance some tumor cells express very little Fas on their surface. NK cells, through the release of IFN-γ, are able to up regulate Fas on the surface of these target cells thereby making them susceptible to activation of their death receptor pathways (172).

NK in Adaptive immunity

NK cells although classified as members of the innate immune system are also able to bridge the gap between the innate and adaptive systems. It does this primarily through the release of cytokines. In fact it was shown many years ago that there is diminished CTL activity in mice that lack NK cells (173, 174). The methods that underlie this stimulation are broad and proceed through numerous pathways. For instance in response to a viral challenge NK cells, through the secretion of IFN-γ and possibly other cytokines, can induce macrophages to stimulate T cells to differentiate into Th1 and CD8+ cytotoxic cells (175). NK cells have also been shown to release IFN-γ and chemokines such as MIP-1 and RANTES that are able to function as chemoattractants and activating molecules at sites of interaction (176, 177).

An interesting aspect of NK cells at the interface of the innate and adaptive immune system is the relationship they can have with dendritic cells (DC). This interaction can occur in both directions with the DC activating the NK cell or the NK cell aiding in the activation and/or maturation of the DC and can even go as far as NK cells

killing the immature DC. One of the more common methods through which DC are able to activate NK cells is through the use of cytokines. For instance IL-12 from DC has been shown to increase IFN-γ production by NK cells (178-181). At sites of inflammation DC are able to release IL-18 which is then able to effect the migratory capability of the NK cell (182). IL-18 and 12 are also able to interact synergistically to increase NK cytotoxicity (183). Different forms of DC are even able to, through direct cell to cell contact, release classic activators of NK cells such IL-2, IL-12 and IL-15 to directly augment the NK cell's activation status (184-189).

The effect NK cells have on the DC seems to rely not only on the cytokines and ligands present but also on the ratio of NK:DC. If the ratio is low (1:5), and the proper cytokines are present, such as IFN-γ and TNFα, the NK cell can stimulate iDC to mature (190). In this method cell to cell contact allows for triggering of the iDC to mature through the NKp30 receptor and possibly through the TREM2 receptor (181, 191). The other situation exists when the ratio is switched and there are more iDC to NK (5:1). In this situation the NK cell is able to lyse the iDCs, due mostly to their low expression levels of MHC. Once again NKp30 is a key receptor in this NK DC interaction (190, 192, 193). These highly varying functions seem to be at odds but both functions have key regulatory roles in aiding in the balance of activation and tolerance in the iDC.

NK Development

The process in which an NK cell progresses from HSC to a fully functional mature cell is far from being fully understood. At this stage the process is understood as

a linear progression that is divided into distinct steps based on the presence of distinct surface markers, location, and eventually the acquisition of function. The first stages of NK cell development involve not the commitment to becoming and NK cell alone, but rather the differentiation of the HSC into the common lymphoid progenitor (194). Although these cells have been shown to have NK potential *in vivo* and *in vitro* the designation common lymphoid progenitor means that these cells can also form B and T cells (195-197). The next step that has been identified is the commitment to a T NK restricted progenitor (TNKP). An interesting aspect of this cell is that it has only been identified in the fetal liver and thymus of the mouse (198, 199) and not in the adult animal. The identification of this cell is based on a lineage$^-$ (Lin), NK1.1$^+$, and c-kit$^+$ phenotype (200). This phenotype, like its location, is very intriguing given that the presence of NK1.1 appears at this stage, and as will be discussed in the next stages of development is not expressed again until more differentiated stages of the NK cell. So although this cell has been shown to form NK cells *in vivo* and *in vitro* it may be possible that this is an intermediary with a yet to be fully elucidated role.

The generation of the NK progenitor (NKP) represents the first major step to creating a cell that is restricted to the NK lineage rather than remaining bipotent. The linear process from NKP to mature NK can be divided into 5 distinct steps that once again are punctuated by the acquisition of distinct surface markers (201-203). These stages of NK development occur in the bone marrow as the first steps require the interaction of the NKP with the BM stroma. The phenotype of the NKP is described as Lin$^-$, CD122$^+$, NK1.1$^-$, DX5$^-$ (203). One of the earliest hallmarks of NK cell

development, and the step which is believed to mark the transition of T/NKP to NKP, is the acquisition of CD122, or the IL2/15Rβ (201, 203). The acquisition of CD122 is a crucial step in the commitment to the NK lineage such that IL-15R deficiency (204) as well as IL-2Rβ deficiency (205) produces defects in NK cell development and function. It has also been shown that defects in IL-15 signaling pathways, such as JAK3 and STAT5a/b can cause defects in NK cell development (206). Stages 2 to 5 of development from NKP to mature NK are marked, sequentially, first by the gain of Mac1 expression then the CD94/NKG2 receptor complex followed by the Ly49 receptors. It is in the final stage of development within the BM that the NK cell gains functionality, specifically cytotoxicity and IFN-γ production thereby resulting in a fully mature NK cell (202, 203).

SHIP

The cloning of SHIP

SHIP1 had a bloom of interest in 1996 with numerous groups independently cloning it (207-211). Since it's identification SHIP has been shown to hydrolyze the 5'phosphate of phosphatidylinositol-3,4,5-phosphate (PIP3) *in vivo* and inositol-1,3,4,5–tetrakisphosphate (IP4) *in vitro,*(208, 210) The ability of SHIP to hydrolyze the 5' phosphate of PIP3, allows it to oppose the activity of PI3K, thereby effecting a wide variety of cellular activities including proliferation, differentiation, apoptosis and migration.

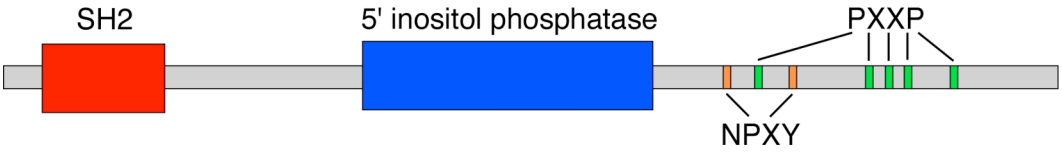

Figure 3. SHIP. A structural diagram of SHIP showing the functional domains.

SHIP Functional Domains

The structure of SHIP consists of an amino terminal SH2 domain, a 5' inositol phosphatase, carboxy terminal NpXY motifs as well as a polyproline rich motif (Fig. 3). The amino terminal SH2 domain of SHIP has been shown to bind to phospho-tyrosines present in many signaling molecules (212-214). It may be through this domain that SHIP plays its most important role in regards to signaling within the NK cell. The major inhibitory receptors within the NK cell posses an ITIM which has been shown to posses phospho-tyrosines that are able to associate with the SH2 domain of SHIP (212, 215, 216). SHIP has also been shown to bind to the intracellular tail of 2B4 and KLRG1 (217) as well as the FcγRIIB where it plays a critical role in receptor localization to the lipid raft (215, 218, 219). Interestingly SHIP's SH2 domain has also been shown to interact with another phosphatase, SHP2, a molecule that plays an essential role in the inhibitory signaling pathway of NK cells (220, 221).

The catalytic domain of SHIP is its 5' inositol phosphatase. This enzymatic domain is able to hydrolyze the 5' phosphate from PIP3 and IP4 (208, 222, 223). PI3K catalyzes the addition of a phosphate at the 3' position on Phosphatidyl Inositol(4,5) Phosphate (PI(4,5)P2), creating PIP3. PIP3 plays a key role in recruiting pleckstrin

homology (PH) domain containing proteins such as AKT (224-228). It is through the removal of the 5' phosphate from this phosphorylated PIP3 that SHIP can regulate pathways that function downstream of PI3K (224-228).

A number of NpXY motifs exist in the amino terminus of SHIP. The NpXY motif can become tyrosine phosphorylated upon activation, this phospho-tyrosine then becomes a possible binding site for phospho-tyrosine binding (PTB) domain containing proteins including SHC, DOK1, and DOK2 (222, 229-233). These NpXY motifs have also been shown to bind to the p85 subunit of PI3K (234-236). Interestingly, although SHIP can be phosphorylated the function of the 5' inositol phosphatase does not seem to be reliant upon this phosphorylation, indicating that the recruitment that occurs through SHIP's other domains, to the site of interaction may play a more critical role than the phosphorylation itself (237). Within the C terminus there is a polyproline rich motif that allows for the binding of proteins that contain an SH3 domain (238).

Other possible roles of SHIP in signaling have recently been uncovered. Valderrama-Carvajal et al were able to show increasing evidence that SHIP does hydrolyze IP_4 *in vivo* (223). This process could effect the formulation of higher number inositol phosphates and therefore may hinder protein synthesis (239). It is also possible that PI-3,4-P_2 may also act as a second messenger in cells leading to the activation of PKB and AP1(240-242).

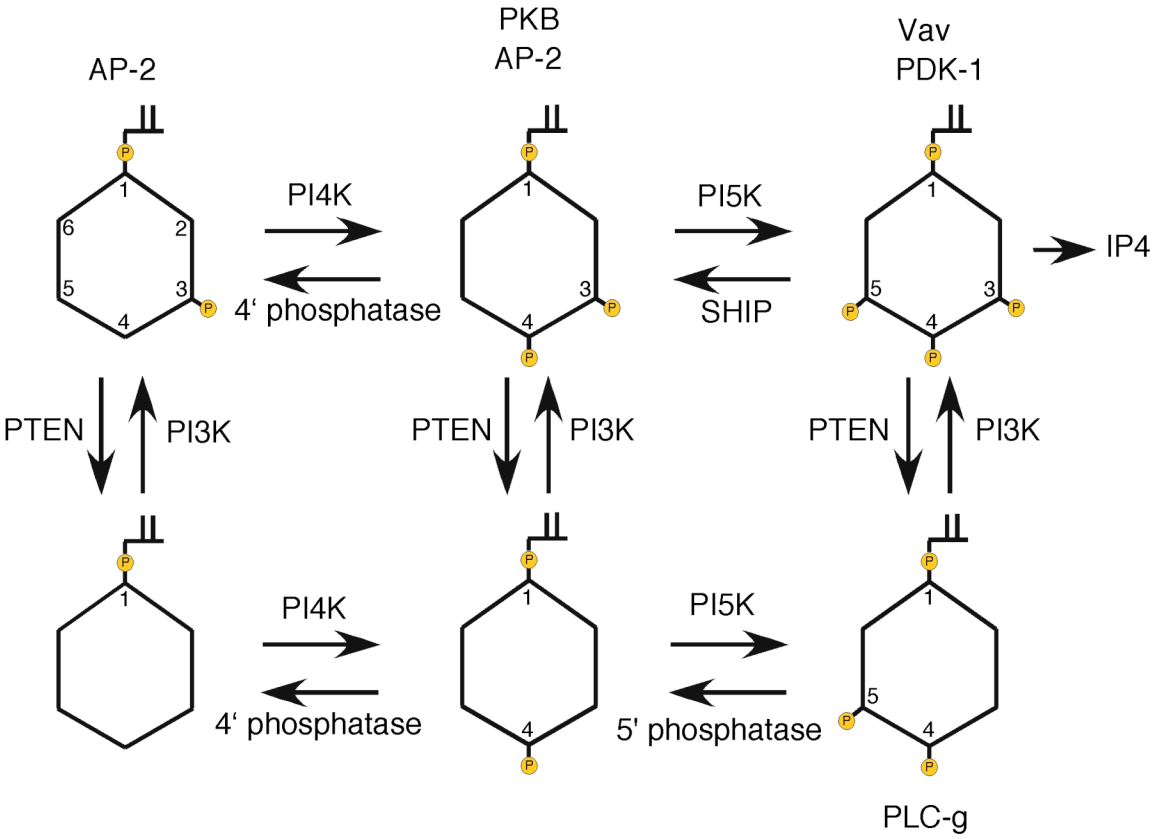

Figure 4. SHIP's role within PI signaling. A diagram showing the proteins involved in the progression from PI to PI(3,4,5)P_3 as well as the possible intermediaries. Many of these different PIs have possible effect on varying signaling pathways. Some of these possible pathways are shown immediately above and below the individual forms. IP4 and other IPs can be formed from the hydrolysis of the IP from the diacylglycerol molecule by a phospholipase.

SHIP isoforms

SHIP2 was initially known as inositol polyphosphate-like-protein1 (INPPL-1) (243). In 1997 it was identified as a 150-155kDa protein with 38% amino acid sequence homology to SHIP1 and therefore termed SHIP2 (244). The majority of the 38%

sequence homology exists within the phosphatase domain as well as the other signaling domains so even though the sequence homology is not high the functional homology is (238, 244). Although these two molecules share similar domains, they seem to function in non-overlapping manners each segregating to different signaling systems, with SHIP2 having a more ubiquitous expression pattern in cells other than those of the hematopoietic system (244-246). Another SHIP isoform was identified in humans (210) and in mice (247) that was found to lack the SH2 domain. This isoform is formed via a promoter that exists within intron 5/6 (248). This isoform has been shown to be expressed in murine embryonic stem cells and HSC but not more differentiated progeny and has thus been termed s-SHIP(247, 248).

SHIP KO Mice

Different variations of the SHIP knockout (KO) mouse have been constructed deleting different portions of the protein. In the studies detailed in this dissertation the SHIP KO mouse created in our lab was utilized (247). This mouse was constructed by deleting the promoter and part of the first exon of SHIP. This results in no SHIP protein being present in the mouse (216, 249, 250). SHIP is expressed very early in the developmental stages of the embryo, specifically 7.5 DPC, though the SHIP null mouse is still viable (216, 249-251) Although the mice are viable they do harbor numerous physiological conditions, especially within the hematopoietic compartment. Peripheral CD8 T cell numbers are significantly reduced while CD4 numbers are unaffected (249). Mast cell degranulation is hyper-responsive (252). One of the more severe conditions is a myeloproliferative disorder leading to increased myeloid progenitors in the marrow and

monocyte/macrophages in the periphery (249, 253). This dramatic increase in myeloid cells leads to an infiltration of alveolar macrophages and results in consolidation of the lungs, which is believed to be what causes the mouse's death at 8 to 12 weeks of age (249). In line with this proliferation of myeloid cells there is a significant increase of myeloid suppressor cells. This increase of myeloid suppressor cells is able to effect a decrease in graft versus host disease (254, 255).

SHIP in NK Cells

Numerous studies have indicated a role for SHIP in NK cells. The first of these concerned not NK cells themselves but rather the ITIM domain contained within FcR found on B cells. Although these initial studies did not identify a role for SHIP they merely identified it presence at the receptor (256). The need for SHIP on some receptors to function was later shown when studying the role of Shc in NK cell mediated cytotoxicity. In this study it was shown that Shc, when bound to Ly49, is able to recruit SHIP. When Shc was mutated so that it was unable to be recruited to the NKR SHIP was also no longer recruited. This lack of SHIP resulted in a loss of function for this receptor (257). Our lab has published a study identifying a more crucial role for SHIP in the NK cell. In this study it was shown that in the SHIP KO mouse the NKR repertoire was skewed with Ly49A and C/I being over represented. This then lead to a much greater phenomenon in which the $SHIP^{-/-}$ mouse was unable to reject bone marrow from an MHC mismatched mouse. This was attributed to the overrepresentation of these Ly49s (216). Other studies have also extended our understanding of SHIP in the NK cell by identifying a number of other receptors that recruit SHIP when activated including Ly49B, KLRG1

and 2B4 (217). Other possible roles for SHIP have also been hypothesized in human NK cells. The association of SHIP with CD16 has been shown to be necessary for proper recruitment of the receptor to lipid rafts and therefore CD16 mediated cytotoxicity (219). In addition it has been shown that a subset of human NK cells have elevated levels of SHIP and lowered perforin levels. This subset of cells has additionally been shown to become functionally annergic in chronic HIV infections (258). Although the specific role of SHIP in many of these studies has not been identified it has shown that SHIP plays a key role with numerous NKR as well as roles of the NK cell. This study will attempt to build off of these studies and delve deeper into the specific molecular role SHIP plays in the NK cell.

CHAPTER 2

Dominance by an MHC-Independent Inhibitory Receptor Compromises NK Killing of Complex Targets

Introduction

NK cells distinguish normal cells from those altered by infection, stress or transformation via inhibitory receptors that detect self ligands and activating receptors that recognize MHC-like ligands expressed by tumor cells, virally-infected cells or cells with DNA damage (112, 259-261). Unlike their B and T cell counterparts, NK cells generate repertoire diversity through variegated and overlapping expression of inhibitory and activating receptors. Recognition of self by NK cells is layered and involves the recognition of MHC class I molecules (50) or non-MHC ligands like CD48 and Ocil/Clr-b via the non-classical receptors 2B4 and NKR-P1D, respectively (62, 131, 262). This pattern of receptor distribution creates an NK compartment composed of different cell subsets each possessing varying degrees of responsiveness to self, non-self and altered-self. In this manner the NK compartment generates a sufficient number of cell subsets capable of responding to infected, tumor or allogeneic cells, while retaining tolerance to normal host cells.

2B4 is a member of the SLAM related receptors that include signal lymphocyte activation molecule (SLAM), CD2 like receptor-activating cytotoxic cell (CRACC), NK-T-B Ag (NTB-A) and CD48 (263). Many of these receptors function through interactions with other family members including 2B4, which interacts with CD48. 2B4 is expressed ubiquitously on NK cells as well as other cells in the hematopoietic system (135). Studies originally indicated an activating role for 2B4, although most of these studies were performed *in vitro* utilizing antibody ligation experiments (132, 133, 264). A more definitive role for 2B4 was recently demonstrated through the generation of a 2B4 mutant mouse, in which an inhibitory role was identified (139). A potential dual role for 2B4 in NK function could be explained by its ability to recruit different adaptor proteins, including SAP as well as EAT-2 (139-142). Although the signaling pathways that control responses following 2B4 ligand engagement have yet to be defined in their entirety, several participating signaling components have been defined including, SAP, EAT-2, FynT, SHP1, PI3K and SHIP (133, 139-142).

We have previously shown that SHIP is critical for maintenance of NKR repertoire diversity in the peripheral NK compartment (216). We have further defined the role of SHIP in the NKR repertoire by placing the SHIP mutation on a defined genetic background (C57BL6/J). The peripheral NK compartment of these $SHIP^{-/-}$ mice (C57BL6/J) is disrupted with a profound under representation of inhibitory NKR specific for MHC class I. Only two NKR, 2B4 and NKR-P1D, are found to be over expressed or overrepresented in NK cells of $SHIP^{-/-}$ mice. Intriguingly, both of these receptors are specific for MHC-independent ligands. In this study we demonstrate that SHIP-

deficiency causes deregulation of 2B4 surface expression and signaling such that cytolysis of complex targets is compromised.

RESULTS

SHIP$^{-/-}$ NK Repertoire

We have previously shown that in the SHIP deficient NK cell there is a receptor bias towards MHC specific inhibitory receptors, specifically Ly49 A and C/I. These initial studies were performed in a C57BL/6/129 mixed background. Since these initial studies we have crossed the SHIP$^{-/-}$ mouse to a fully defined Bl6 background. With these mice we have reanalyzed the NKR repertoire and found a profound and significant down regulation of all NKR except 2B4 and NKR-P1D (Fig. 5).

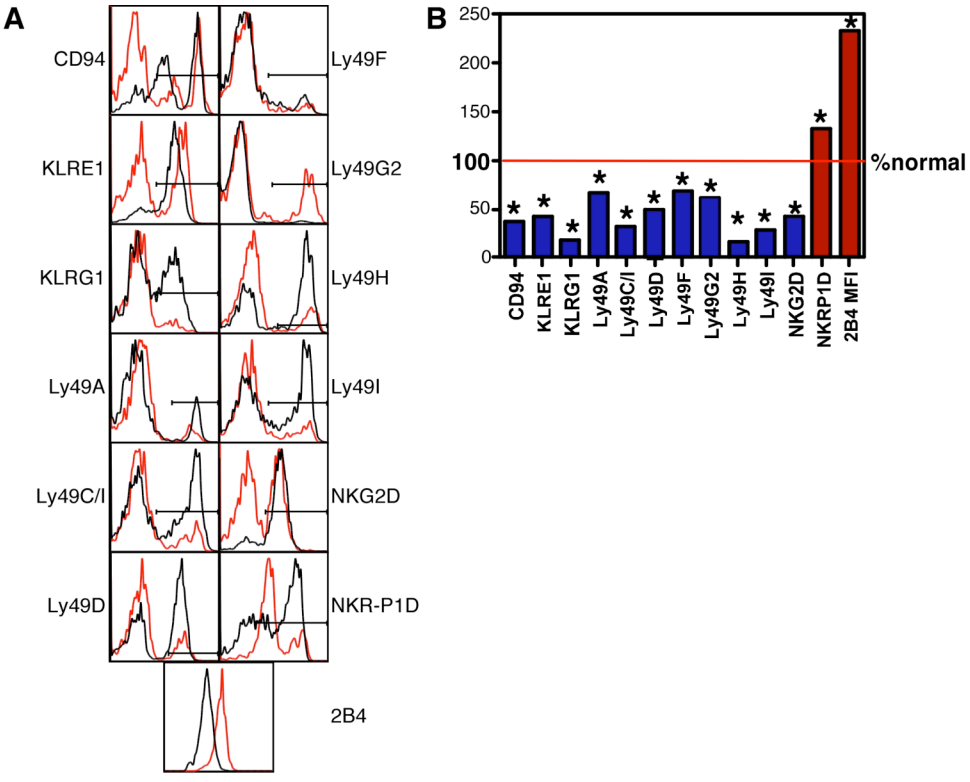

Figure 5. NKR repertoire. A) Representative histograms for the different NKR analyzed after gating on $NK1.1^+$ Lin^- splenocytes of 6-8 week old $SHIP^{-/-}$ mice and their WT littermates (Lin panel: IgM, CD3, TcR-b, Gr1, CD11c). Red histograms are for $SHIP^{-/-}$ NK cells while black histograms are for WT littermate controls. In order to estimate the percentage of NKR^+ cells in the NK compartment, positive NKR gates were set at ≥95% of $NK1.1^+Lin^-$ cells staining positive for an isotype control stain performed on an equal mixture of null and WT splenocytes. B) Representation of individual NKR in splenic $SHIP^{-/-}$ NK cells after normalization to WT. The % of normal = ($\%NKR^+$ $SHIP^{-/-}$/$\%NKR^+$ $SHIP^{+/+}$)x100 for each indicated NKR. For 2B4, % normal was calculated in the same manner except that MFI was used rather $\%NKR^+$. Blue, red and black bar graphs represent % normal values that are significantly lower, higher, and unchanged in the $SHIP^{-/-}$ NK compartment as compared to WT, respectively.

2B4 Expression Levels

In order to determine the effect of SHIP deficiency on 2B4 expression, we examined various stages of NK cell maturation and activation (Fig. 6). Our initial analysis included both mature splenic NK cells as well as immature bone marrow NK cells. Analysis of NK cells at these stages of maturation showed that 2B4 is over expressed on the surface of $SHIP^{-/-}$ NK cells as compared to NK cells from WT littermates (Fig. 6A,B). We further examined 2B4 status utilizing activated NK cells. For these experiments we analyzed both *in vivo* polyinosinic acid (poly(I:C)) activated NK cells and *in vitro* activated NK cells cultured for 7 days in the presence of IL-2.

Consistent with our analysis of freshly isolated NK cells, *in vivo* (Fig. 6C) and *in vitro* (Fig. 6D) activated SHIP$^{-/-}$ NK cells also exhibit increased surface density of 2B4 as compared to WT controls. Thus, SHIP is required to maintain normal expression of 2B4 on the cell surface, indicating SHIP not only regulates expression of NKR for MHC ligands, but also NKR for MHC-independent ligands. Moreover, SHIP performs this role at multiple stages of NK development and differentiation. In addition to these cell types we created SHIP$^{-/-}$ and WT chimeric mice in order to determine if the up regulation of 2B4 in the SHIP$^{-/-}$ NK cell was due to cell extrinsic effects (Fig. 6E). As before we see that 2B4 surface density remains elevated in the SHIP$^{-/-}$ mice.

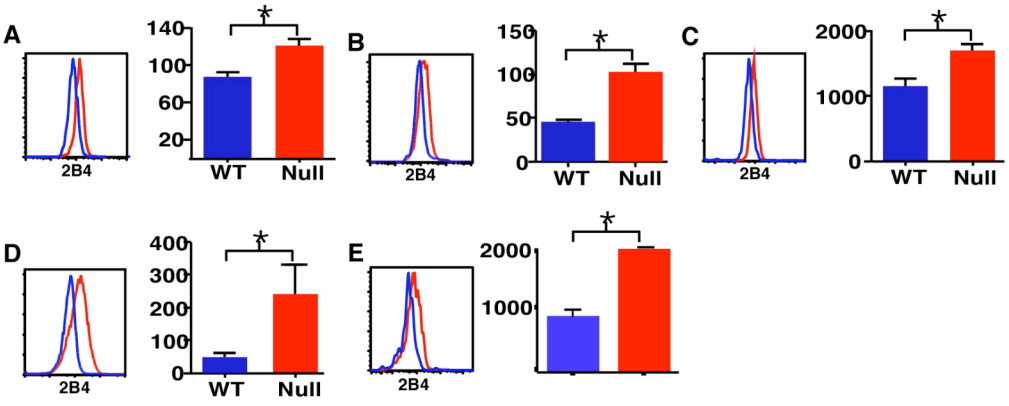

Figure 6. 2B4 status is altered in SHIP$^{-/-}$ NK cells. Representative overlays of 2B4 histograms after back gating on NK1.1^{+} Lin^{-} cells. Bar graphs represent mean fluorescence intensity of at least three separate animals. (*$p < 0.05$, n=3, Students two tailed T-test) SHIP$^{-/-}$ (Red) WT (Blue) A) Immature BM NK cells B) Mature splenic NK cells C) *In vivo* poly-IC stimulated NK cells D) *In vitro* IL-2 stimulated LAK cells. E) NK cells from a SHIP$^{-/-}$ WT chimera.

NKG2D mediated cytolysis is compromised in SHIP$^{-/-}$ mice

2B4 has recently been shown to function as an important inhibitory receptor *in vivo (131)*. Thus, we speculated that the increased surface density of this inhibitory receptor might skew the balance of inhibitory and activating signals received by SHIP$^{-/-}$ NK cells and thereby alter key cytolytic functions. To examine the impact of 2B4 overexpression on SHIP$^{-/-}$ NK function, we examined NK cytolysis of tumor cells mediated by the activating receptor NKG2D. To examine whether cytolysis of tumor targets that express the NKG2D ligand Rae1 are compromised in the SHIP$^{-/-}$ NK compartment we magnetically enriched splenic NK cells and cultured them for 7 days in the presence of IL-2 to generate lymphokine-activated killer (LAK) cells. Intriguingly, we find that NK cell recovery in SHIP$^{-/-}$ LAK cultures is significantly better than that of WT cultures prepared in a similar manner ($p<0.05$), suggesting that SHIP-deficiency may enhance cytokine-stimulated NK cell survival and/or proliferation *ex vivo* (Fig. 7A). Flow cytometric analysis of these 7-day LAKs revealed that surface expression of NKG2D on activated NK cells from SHIP$^{-/-}$ mice is comparable to that of WT controls prepared in an identical manner (Fig. 7B). The surface density of 2B4 remained elevated in SHIP$^{-/-}$ NK cells as shown above (Fig. 6D). The cytolytic activity of the activated NK cells was then determined in a standard 4-hour chromium release assay against RMA cells expressing the NKG2D ligand Rae1. Despite equal surface expression of NKG2D on SHIP$^{-/-}$ and WT LAK cells, we find that cytolysis of RMA Rae1-transfectants by SHIP$^{-/-}$ NK cells is profoundly compromised relative to WT NK cells at all E:T ratios tested (Fig. 7C). In fact, only at the highest E:T ratio, 60:1, was cytolysis of Rae1^{+} RMA

cells by SHIP$^{-/-}$ LAK cells significantly higher than background cytolysis observed for parental RMA cells. No significant killing of parental RMA cells, that lack the Rae1 antigen, was seen for either SHIP$^{-/-}$ or WT NK cells confirming the specificity of this assay for the NKG2D ligand Rae1. The fact that NKG2D expression levels are comparable in SHIP$^{-/-}$ and WT LAK cells while NKG2D-mediated cytolysis is profoundly compromised in SHIP$^{-/-}$ NK cells, suggests that hyporesponsiveness in SHIP$^{-/-}$ NK cells could be due to increased expression and/or inhibitory signals from 2B4 engaging its ligand, CD48, which is expressed on the surface of both RMA and Rae1^{+} RMA cells (Fig 7E).

Restoration of NKG2D mediated cytolysis

To examine whether altered 2B4 signaling was causing this hyporesponsiveness, we tested whether antagonizing the 2B4-CD48 interaction restores NKG2D-mediated killing. Incubation of targets with an anti-CD48 antibody was able to restore SHIP$^{-/-}$ LAK killing to WT levels (Fig. 7D). In three independent standard 4-hour chromium release assays where we tested killing of Rae1^{+} RMA transfectants in the presence or absence of anti-CD48, we have repeatedly observed a statistically significant enhancement of SHIP$^{-/-}$ killing. Although some increase in WT killing due to CD48 blocking occurred, it was dramatically less than that observed with CD48 blockade in SHIP$^{-/-}$ LAK cytolysis assays. In fact, in all but one E:T ratio, 20:1, the killing by SHIP$^{-/-}$ LAK cells was not significantly different than that of killing by WT LAK performed in the presence of CD48 blockade. The ability to restore SHIP$^{-/-}$ LAK cytotoxicity against

Rae1^{+} RMA targets by blocking the 2B4-CD48 interaction suggests that 2B4 has a dominant inhibitory role in the SHIP$^{-/-}$ NK compartment.

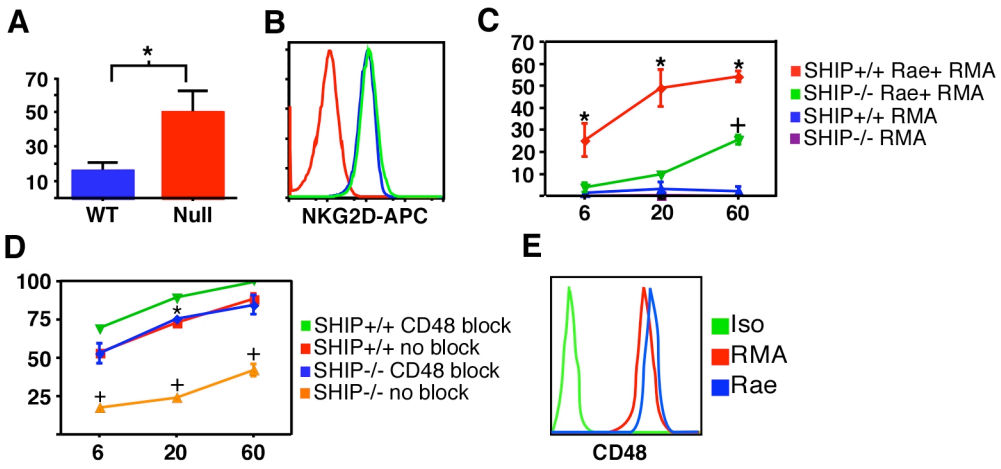

Figure 7. Compromised NKG2D-mediated cytolysis of Rae1^{+} tumor targets by SHIP$^{-/-}$ NK cells and restoration by CD48 blockade. A) Magnetically purified NK cells were plated at 2,000,000cells/mL in the presence of IL-2 for 7 days. Cells were harvested at day 7 and the resulting percentage of cells recovered from four separate experiments are shown. (*p<0.05, N=4, Students two tailed T-test) B) NKG2D status of NK1.1^{+} CD3^{-} 7 day LAK cells. Representative histograms showing the NKG2D status of SHIP$^{-/-}$ (blue) and WT (green) LAK cells are shown. Red histogram indicates isotype control. C) Standard 4hr chromium release assays were performed with SHIP$^{-/-}$ and WT LAK cells. RMA cells with and without Rae1 transfectants were used as targets. Percent lysis is indicated on the left axis and the E:T ratios across the bottom axis. *p<0.05 for cytolysis of Rae1^{+} RMA cells by WT LAK cells compared to SHIP$^{-/-}$ LAK cells. +p<0.05 for cytolysis of RMA cells compared to Rae1^{+} RMA cells by SHIP$^{-/-}$ LAK cells.

D) Cytolysis of Rae1$^+$ RMA cells with and without blocking of CD48 on target cells in a standard 4hr chromium release assay. *$p<0.05$ for cytolysis of blocked Rae1$^+$ RMA cells by SHIP-/- LAK cells compared to WT LAK cells. +$p<0.05$ for the cytolysis of blocked Rae1$^+$ RMA cells versus unblocked Rae1$^+$ RMA cells by SHIP$^{-/-}$ LAK cells. All cytotoxicity experiments were done in triplicate and are representative of three or more experiments. E) CD48 status of RMA and Rae1$^+$ RMA cells.

Compromised BM rejection

Previously we found that the NK repertoire disruption observed in SHIP$^{-/-}$ mice on a mixed 129Sv/BL6 background led to an inability to reject H-2^d and H-2^s BM grafts that are completely MHC mismatched. We attributed engraftment in 129/BL6 SHIP$^{-/-}$ mice to over representation of Ly49A and C (216). However, Ly49A and Ly49C are not over represented in SHIP$^{-/-}$ mice on a C57BL/6 background suggesting that rejection of allogeneic BM grafts might not be compromised in SHIP$^{-/-}$ mice on this defined background. Although these SHIP$^{-/-}$ (BL6) mice might remain permissive for engraftment of MHC-mismatched BM grafts owing to the compromised NKG2D mediated cytolysis we observed above. This appears to be the case, as SHIP$^{-/-}$ mice on a C57BL/6 background are still permissive for engraftment of BM from several different donors with full MHC mismatches (Fig. 8). Initially, we transplanted SHIP$^{-/-}$ (BL6) hosts with H-2^d BALB/C BM and measured acute engraftment by the splenic IUdR assay used previously (216). As was observed in 129/BL6 SHIP$^{-/-}$ mice (216), we find that BALB/C H-2^d BM engrafts the SHIP$^{-/-}$ (BL6) cohort, but is rejected by their WT littermates. Subsequently we tested engraftment of BM from a variety of other donors with full MHC

class I mismatches (H-2^p, H-2^r, H-2^f and H-2^u) not analyzed previously, and found that SHIP$^{-/-}$ (BL6) mice are also permissive for engraftment by BM from these donors (Fig. 8).

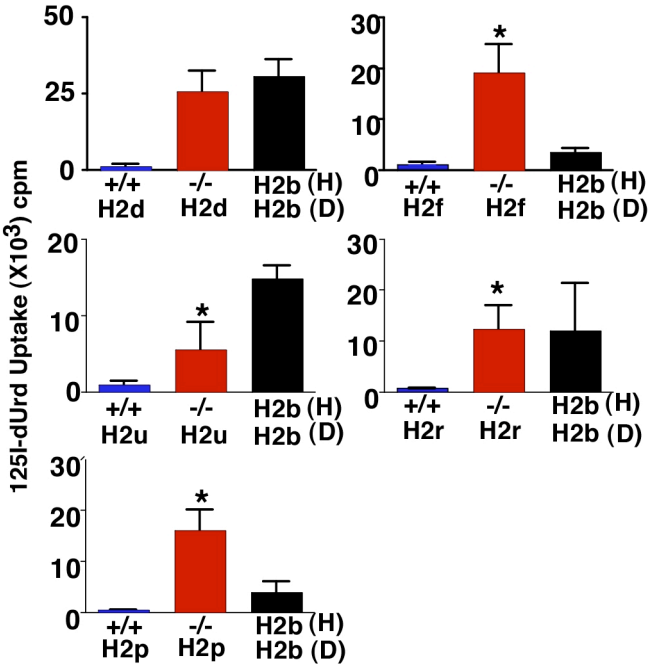

Figure 8. Allogeneic BM rejection assay. Splenic IUdR uptake in F9/10xC57BL/6 SHIP$^{-/-}$ and WT recipients transplanted with H-2^d donor BM cells, F6xC57BL/6 SHIP$^{-/-}$ and WT recipients transplanted with H-2^f, H-2^p, H-2^u or H-2^r donor BM cells. Red bar graphs represent SHIP$^{-/-}$ recipients transplanted with allogeneic donor BM while blue bar graphs represent WT recipients transplanted with allogeneic donor BMC. Black bar graphs represent BL6 recipients transplanted with syngeneic BL6 donor BMC. (*$p<0.05$)

Compromised cytolysis of Ly49H targets

The ligand for Ly49H has been identified as the CMV-encoded membrane protein m157 (80, 81). Since $SHIP^{-/-}$ NK cell killing of targets that express ligands for NKG2D is compromised, we postulated that killing of $m157^{+}$ targets might be similarly disabled. To test this, we examined the ability of LAK cells from $SHIP^{-/-}$ and WT mice to kill BaF3-$m157^{+}$ transfectants (Fig. 9). As with NKG2D, we find that *ex vivo* activation of $SHIP^{-/-}$ NK cells with IL-2 restores Ly49H to a surface density essentially identical to that in WT controls (Fig. 9A). Standard 4-hour chromium release assays were then performed at E:T ratios of 60, 20 and 6:1. The $SHIP^{-/-}$ LAK cultures showed severely diminished cytolysis of $m157^{+}$ targets at all E:T ratios tested (Fig. 9B). Killing in this assay was confirmed as being specific for the m157 antigen as no cytolysis of BaF3 parental cells was observed (Fig. 9B).

Restoration of Ly49H mediated cytolysis

Given that 2B4 signaling compromises NKG2D mediated killing by $SHIP^{-/-}$ NK cells, we also examined whether this might be the case for the killing of viral ligand positive targets mediated by Ly49H. As was done for NKG2D, we measured NK cytolysis using a standard 4-hour chromium release assay in the presence and absence of anti-CD48. In three separate experiments, incubation of the $m157^{+}$ BaF targets with anti-CD48 prior to killing significantly restored $SHIP^{-/-}$ LAK killing (Fig. 9C). The significant enhancement of WT killing by blocking of CD48 was also seen at the 20:1

E:T ratio, but is lower than that observed for all m157^{+} cytolysis assays performed with SHIP$^{-/-}$ NK cells (Fig. 9C). Thus, as with killing of tumor targets, killing of targets that express a viral ligand for an NK activating receptor is also compromised by 2B4 inhibitory signaling, further demonstrating 2B4 is a dominant inhibitory receptor in the SHIP$^{-/-}$ NK compartment.

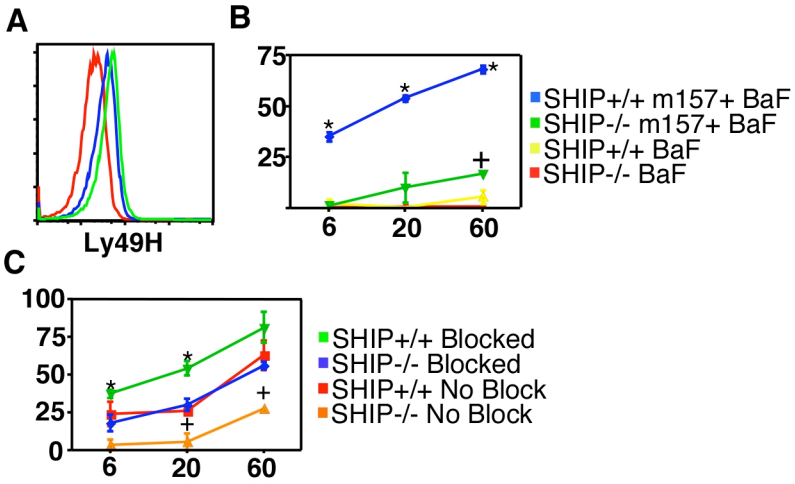

Figure 9. Compromised Ly49H-mediated cytolysis of CMV m157^{+} viral targets by SHIP$^{-/-}$ NK cells and restoration by CD48 blockade. A) LAK cells were incubated with anti-NK1.1, CD3, and Ly49H. Histograms are representative of the status of Ly49H in WT (green) and SHIP$^{-/-}$ (blue) LAK cells as compared to the isotype control (red). B) Cytolysis of BaF3 parental cells and BaF3 transfectants expressing the CMV m157 ligand by IL-2 activated LAK cultures from SHIP$^{-/-}$ mice or WT littermates in a standard 4hr chromium release assay. Percent lysis is on the left axis and E:T ratios across the bottom axis. *$p<0.05$ for cytolysis of m157^{+} BaF target cells by WT LAK compared to SHIP$^{-/-}$ LAK. $^{+}p<0.05$ for cytolysis of m157^{+} BaF cells compared to BaF cells alone by SHIP$^{-/-}$ LAK. C) Cytolysis of m157^{+} BaF cells with and without blocking of CD48 on

target cells in a standard 4hr chromium release assay. +p<0.05 For cytolysis of blocked versus unblocked m157^{+} BaF cells by SHIP$^{-/-}$ LAK cells. *p<0.05 For cytolysis of blocked m157^{+} BaF cells by WT LAK cells compared to SHIP$^{-/-}$ LAK cells. All cytotoxicity experiments were done in triplicate and are representative of two or more experiments.

NKR-P1D mediated inhibition

Our restoration of cytotoxicity with a CD48 blockade provides strong evidence that 2B4 is the dominant inhibitory receptor. Although given that NKR-P1D was also over expressed on SHIP$^{-/-}$ NK cells we performed Clr-b antibody blocking experiments to determine if this could restore SHIP$^{-/-}$ NK cytotoxicity. In these experiments we see no improvement with Clr-b blockade alone as well as no improvement when 2B4 and Clr-b were blocked together (Fig. 10).

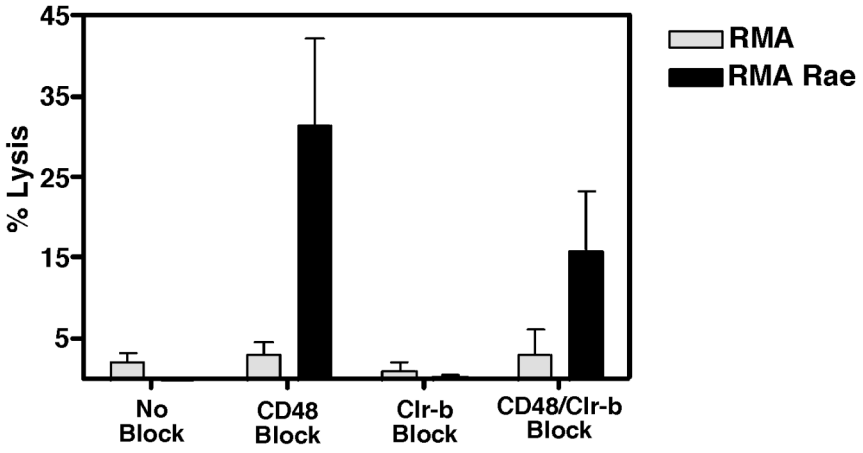

Figure 10. NKR-P1D Clr-b blockade. Cytolysis of RMA or RMA Rae1^{+} cell by SHIP$^{-/-}$ LAK cells at a ratio of 20:1. Cytolysis was performed either with LAK cells alone, in

the presence of CD48 blockade, Clr-b blockade, or CD48 and Clr-b blockade performed in combination.

DISCUSSION

In this study we have shown that SHIP$^{-/-}$ NK cells exhibit overexpression of 2B4 throughout all stages of NK maturation and activation. We have also shown that 2B4 inhibitory signaling in SHIP$^{-/-}$ NK cells represses killing of complex targets where effective recognition and killing requires integration of both activating and inhibitory signals. Our functional studies indicate increased 2B4 inhibitory signaling disrupts this balance such that key activating receptors like NKG2D and Ly49H are unable to effectively promote cytolysis. Although the molecular mechanisms responsible for this imbalance remain to be defined, we propose two hypotheses to explain this altered function.

In our first hypothesis we propose that the quantitative difference in 2B4 expression between SHIP$^{-/-}$ and WT NK cells leads to an increase in the basal level of 2B4 inhibitory signals received by an NK cell and thus alters the balance of activating and inhibitory signals forcing the cell towards hyporesponsiveness (Fig. 11B). An NK cell receives a variety of activating and inhibitory inputs from external targets. A balance of these inhibitory and activating signals must be achieved in order for the cell to maintain tolerance to self. In order for an NK cell to carry out cytolysis the activating receptors must be integrated to overcome inhibitory signals. Thus, increased basal inhibitory signals from ubiquitous 2B4-CD48 interactions could compromise the ability

of a SHIP$^{-/-}$ NK cell to respond efficiently to activating signals from either NKG2D or Ly49H. In that case, the role of SHIP is to limit the surface expression of 2B4 to a level that does not interfere with normal activating receptors effecting NK cytolytic function. Alternatively, we propose that there could also be a qualitative change in the 2B4 inhibitory signaling in SHIP$^{-/-}$ NK cells such that each 2B4 receptor delivers a more potent negative signal (Fig. 11C). Although the precise molecular mechanisms of 2B4 signaling have not been fully elucidated, key components in this signaling pathway have been identified. Of relevance to this study is that SHIP has been shown to be recruited to 2B4, suggesting it can influence 2B4 signaling (140, 141, 265). SHIP may in fact be recruited to 2B4 to oppose the actions of PI3K. Consistent with this PI3K is also recruited to 2B4 where it can trigger the activation of downstream effectors including AKT and PLCγ (140, 266). This PI3K pathway may play a role downstream of EAT-2, which has been shown to be a key adaptor protein for signals emanating from 2B4 (139). Thus, a lack of SHIP may lead to unopposed PI3K signaling at 2B4 and thus a qualitative difference in 2B4 signaling (Fig. 11C). We postulate that in the absence of SHIP, PI3K signaling may run unchecked in NK cells and that this may not only increase the inhibitory signal emanating from individual 2B4 receptors, but also increase transcription of 2B4 and possibly other downstream inhibitory signaling components like EAT-2.

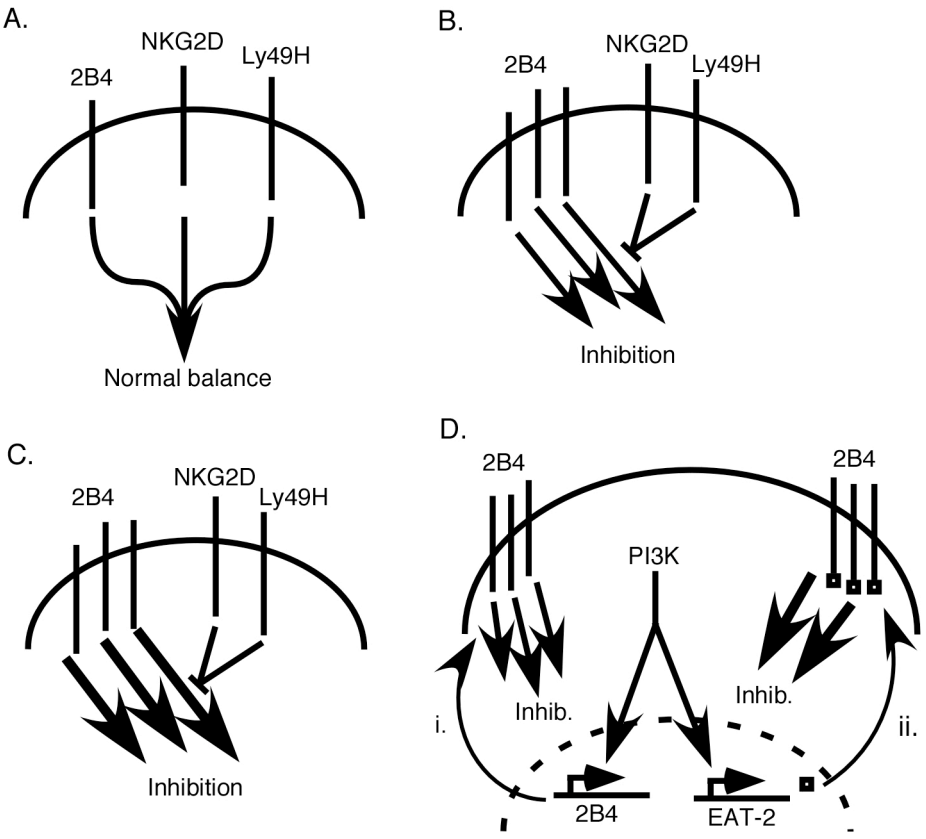

Figure 11. Dominance by 2B4 compromises NK cytolysis of complex targets. A) SHIP-competent cell with normal levels of 2B4 and a proper balance of inhibitory signals from 2B4, and activators, like NKG2D or Ly49H, is maintained until activation overcomes inhibition. B) Increased 2B4 surface density in the SHIP$^{-/-}$ cell increases basal levels of 2B4 inhibitory signaling and this overrides activating receptors. C) 2B4 inhibitory signals in the SHIP-deficient cell are qualitatively altered in a way that leads to the activating signals being overridden. D) Model for how unopposed 2B4 signals in the SHIP-deficient cell might "feed-forward" to: (i) increase its expression and thus basal levels of inhibitory signaling as in (B) or (ii) increase expression of a negative signaling

component (e.g., EAT-2) utilized by 2B4 to effect both a qualitative and quantitative change in 2B4 inhibitory signaling.

It is also quite probable that the above potential mechanisms could act in concert to disrupt the function of the SHIP-deficient NK cell. That is, a qualitative change caused by a lack of SHIP signaling at 2B4 may initiate a quantitative change by deregulating 2B4 surface expression. The initial qualitative change could also effect changes in expression other signaling molecules that participate in 2B4 signaling and thus further altering inhibitory signals emanating from 2B4 (Fig. 11D). In this manner the $SHIP^{-/-}$ NK cell becomes locked into a 'feed-forward' 2B4 inhibitory signaling mode rendering the cell hyporesponsive in the presence of its ligand CD48. Our findings extend SHIP's regulation of the NK receptor repertoire to MHC independent inhibitory receptors, but also demonstrate how this seemingly minor component of NKR regulation is absolutely critical to the normal cytolytic function by the NK compartment.

MATERIALS AND METHODS

Animals

The $SHIP^{-/-}$ mice used in this study were previously created in our lab (See Chp. 1). Mice were maintained by intercrossing $SHIP^{+/-}$ mice (F10 to the C57BL6/J background) thereby allowing for sufficient numbers of both $SHIP^{-/-}$ as well as WT littermates for all experiments. All experiments were performed with $SHIP^{-/-}$ and WT littermates between 6-9 weeks of age.

Acute bone marrow engraftment assays

Bone marrow engraftment was assayed through the use of the splenic IUdR uptake assay. For these experiments whole BM was isolated from mice of the noted MHC haplotype (ie. H2D). $SHIP^{+/+}$, $SHIP^{-/-}$ and syngeneic BL6 hosts were lethally irradiated with 950 Rads with a gamma irradiator (Perkin Elmer). Irradiation was given in two doses with 2 or more hours of rest in between doses. Post-irradiation the host mice received $5x10^6$ whole BM (WBM) cells in a total volume of 200μL, from allogeneic or syngeneic donors as indicated in the results, via an intra-venous injection. Four days post-BMT 3mCi of ^{125}I-dUrd was injected intravenously to allow for the measurement of engraftment of the BMT. On the fifth day post-BMT, spleens of the host animals were removed. Engraftment was then assessed through the measurement of ^{125}I uptake in the whole spleen with a gamma counter (Perkin Elmer Wizard1470).

Antibody staining and flow cytometry

For all flow cytometry experiments whole splenocytes, WBM or LAK cultures were harvested and prepared into a single cell suspension. Whole splenocytes and WBM were red blood cell lysed for 5 minutes at room temperature in RBC lysis buffer consisting of 0.15μM NH_4Cl, 10mM $KHCO_3$, and 0.1mM EDTA. Cells were spun down at 300xG for 5 minutes and then resuspended at $1x10^6$cells/50μL in staining media. Staining media consists of PBS with 3% FBS and HEPES. Cells were then Fc blocked for 15 minutes on ice with anti-CD16/32 antibody. The cells were then stained with the appropriate antibody combination. Antibodies used for staining included: NK1.1; 2B4;

A1 (mouse IgG2a,k); 5E6 (mouse IgG2a,k); 4E5 (rat IgG2a,k); HBF-719 (mouse IgG1,k); 4D11 (rat IgG2a,k); YLI-90 (mouse IgG1,k); CD94 (rat IgG2a,k) were obtained from BD Pharmingen (San Jose, CA). C7 (hamsterIgG1) was purchased from eBioScience (San Diego, CA). 3D10 (rIgG1) was conjugated to biotin and used for staining Ly49H as previously described (81). The anti-KLRE1 (7E8) was previously described and was conjugated to biotin and revealed with SA-APC as described here (267). Samples were acquired on a FACS Calibur and analyzed using FlowJo6.3. Dead cells were excluded from the analysis based on exclusion of the 7AAD dye.

LAK cultures and cytolysis assays

Spleens were harvested and prepared into a single cell suspension from $SHIP^{-/-}$ and WT mice. Whole splenocytes were red blood cell lysed for 5 minutes with RBC lysis buffer. Cells were then spun down at 300xG for 5 minutes and resuspended at $2.5x10^8$ cells/mL in miltenyi buffer which consists of PBS, 0.5% FBS, 0.5% HEPES, and EDTA. Cells were then prepared for enrichment with the Miltenyi Mouse NK cell enrichment kit. The lineage specific antibody cocktail consisting of CD4, CD5, CD8, CD19, Gr-1, and Ter119 was added to the whole splenocytes at a concentration of $5\mu L/1x10^7$ cells for 10 minutes in a refrigerator. An additional 30μL of media per $1x10^7$ cells was then added to each sample. Anti-biotin microbeads were then added at a concentration of $10\mu L/1x10^7$ cells and incubated in the refrigerator for 15 minutes. Cells were then washed and resuspended in miltenyi buffer at a concentration of $200x10^6$ cells/mL. The cells were then run on the AutoMac (Miltenyi) on the DepleteS program.

Percent of cells recovered varied from 5 to 25% between $SHIP^{-/-}$ and WT NK cells with a purity of 10-60%.

Enriched NK cells were plated at a density of $2x10^6$ cells per mL in 6-well dishes. Cells were cultured for seven days in activation media consisting of RPMI, 10% FBS, L-glut, Penicillin/Streptomycin, Na-pyruvate, non-essential amino acids and 2000units/mL human rIL2 (Proleukin). On day 1 of culture, cells were supplemented with 1mL of fresh activation media. On day 3, non-adherent cells were removed through a demi-depletion of 1-2mL and fresh media was added to the cultures. On day 4-6 media was added as necessary. On day 7 a standard 4 hour chromium release assay was performed. On the day of the experiment target cells were resuspended at $1x10^6$cells/mL in chromium release media, consisting of RPMI with 3% FBS and HEPES. 100μCi of ^{51}Cr was added to $1x10^6$ target cells and then incubated for 60 minutes at 37°C with gentle agitation every 15 minutes. After incubation target cells were spun down at 300xG for 5 minutes and washed twice with 3mL of chromium media. The target cells were then counted and resuspended at 3000 cells/100μL in chromium media. In order to remove the LAK cells from culture they must be lifted through the use of PBS with EDTA. The first step is to remove the non-adherent and dead cells from the top of the culture through removal of the top 1-2mL of media. The remaining media is then pipetted up and down to free loose cells. The media, containing the freed LAK cells, is removed and placed on ice. 2mL of cold PBS-EDTA was then added to each well and placed at 4°C for 10-15 minutes. Over exposure to the EDTA, or exposure at warmer temperatures can prove hazardous to the LAK cells, so care was taken to assure the cells remained at 4°C. After the 10-15 minute

incubation the remaining cells were freed from the plate through pipetting. LAK cells were then counted and spun down at 300xG for 5 minutes and resuspended in chromium media such that the appropriate number of effector cells were in 100μL of media. NK cells and target cells were then combined in 3 ratios, 60:1, 20:1 and 6.33:1 in 96 well plates at a total volume of 200μL. Target cell numbers were maintained at 3000 cells for all experiments. Plates were spun down at 200xG for 1 minute. The cells were then incubated at 37°C for 4 hours. After incubation the top 100μL of supernatant was collected and measured for radioactivity on a gamma counter (Perkin Elmer Wizard1470). Spontaneous release controls were performed in the absence of effector cells. Maximal release was measured by adding 10% Triton-X to the target cells alone. Percent lysis was calculated by the following formula. 100 X (experimental CPM – spontaneous release CPM) / (maximum release CPM – spontaneous release CPM). All experiments were performed in triplicate and results were verified with a separate experiment performed on a different date.

Antibody blocking experiments

Antibody blocking experiments were performed in the same manner as standard cytotoxicity experiments except for the addition of the blocking antibody. Target cells were loaded normally and placed at 3000 cells/100μL in chromium media. The target cells were then incubated with anti-CD16/32 for 15 minutes to block Fc receptors. Both the target cells that were to be blocked with anti-CD48 as well as the unblocked controls underwent Fc blocking. Target cells were then incubated with anti-CD48 (BCM1 clone from eBioscience) at 1μg per million cells for 15 minutes. All antibody incubations were

done at room temperature due to the fact that the cooling of target cells to 4°C seemed to have a deleterious effect to chromium loading. The anti-16/32 and CD48 antibody was not washed off prior to addition of the effector cells. A normal cytotoxicity assay described above was then performed.

Statistical analysis

Statistical analysis was done using Graphpad Prism. The statistical test that was performed to compare receptor expression levels, splenic IUdR uptake, as well as percent lysis in chromium release assays was a Students two-tailed T-test. N=3 except where a greater N is indicated. Results were considered significant with a $p<0.05$.

Chapter 3

Inappropriate Recruitment and SHP1 Activity is Responsible for Receptor Dominance in the SHIP-deficient NK Cell

Introduction

The process by which an NK cell recognizes a target cell and delivers a sufficient signal to trigger target lysis is determined by an array of inhibitory and activating receptors on the cell surface. NK discrimination of self from altered-self involves inhibitory receptor recognition of MHC-I molecules (50) and non-MHC ligands like CD48 and Clr-1b(131, 262, 268, 269). NK recognition of infected or damaged cells (altered-self) is coordinated through stress induced ligands (*e.g.*, MICA, MICB, Rae1, H60, Mult1) or virally encoded ligands (*e.g.*, m157, hemagluttinin) recognized by various activating receptors, including NKG2D, Ly49H and NKp46/Ncr1 (112, 145, 259, 260, 270).

The process of initial target cell recognition and the recruitment of appropriate downstream signaling molecules to the NK synapse is carefully coordinated in order for the NK cell to effectively kill the target. Although many of the key players in the process are known, the manner in which these disparate steps and pathways are coordinated is less well understood. (271-273). NK activating receptors, such as NKG2D and Ly49H, upon ligand engagement are

able to bind DAP10 or DAP12 molecules that contain an ITAM or YxxM motif (102, 274). This then allows for the recruitment of various effectors of cell signaling, including the Src and Syk related protein tyrosine kinases that subsequently lead to the activation of more distal effector pathways such as the PI3K and MAP/ERK pathways (271, 275-277). Inhibitory receptors that engage self-ligands can oppose activation of these pathways through the recruitment of various SH2 domain-containing phosphatases to their ITIM. These include SHP1 and SHP2, which are responsible for the removal of tyrosine phosphates (76, 77, 278, 279) and the inositol phosphatase SHIP (216), which is responsible for the removal of the 5' phosphate from $PI(3,4,5)P_3$ (208, 209).

2B4 is a member of the SLAM related receptors (121, 133). It functions through the recognition of another SLAM family member, CD48, that is ubiquitously expressed on cells of the hematopoietic system (128, 129). 2B4 has a complex role in NK cell function and physiology that remains an active area of investigation. Depending on the context 2B4 has been shown to act as both an inhibitory and activating receptor (130-138). This is likely due, at least in part, to the ability of 2B4 to differentially recruit various downstream effectors of cell signaling. Under different signaling contexts and in different species 2B4 can recruit SAP, EAT-2, FynT, SHP1, PI3K and SHIP (133, 139-142). How the differential recruitment of these signaling entities is controlled is not completely understood. However, which molecules are recruited and thus which signal is propagated following CD48 engagement may be influenced by the ratio of 2B4 isoforms expressed in the NK cell. Two 2B4 isoforms have been identified in mice, short (2B4S) and long (2B4L), that were proposed to have activating and inhibitory signaling

capacities, respectively (134). Although the exact function of these two isoforms remains to be defined, it is feasible that the different intracellular domains within these isoforms could recruit different effectors of cell signaling. 2B4 could also mediate various signaling outcomes through changes in the availability or recruitment of different signaling molecules. For instance, it has been shown that there are diminished levels of the SAP protein in immature human NK cells. The lack of this key activating molecule in the cell appears to lock 2B4 into an inhibitory signaling mode (138). In other SLAM family members, namely CD150, there is evidence that the presence or absence of SAP can regulate the binding of both SHP1 and SHIP to the immunoreceptor based tyrosine switch motifs (ITSM) of this receptor (127).

We have previously demonstrated that the NK receptor repertoire is highly disrupted by SHIP deficiency (216, 280). This repertoire disruption leads to receptor dominance by 2B4 such that inhibitory signals from 2B4 repress killing of complex targets (280). In this study we define the molecular basis for 2B4's dominance of key NK activating receptors for both stress-induced and virally-encoded NK activating ligands.

RESULTS

2B4 and SHP expression in SHIP-deficient NK cells

We previously showed that 2B4 levels are increased on the surface of $SHIP^{-/-}$ NK cells. To determine if this increase is due to increased expression of 2B4, rather than increased surface deposition, we blotted whole cell lysates prepared from sorted $SHIP^{-/-}$

and WT NK cells for the presence of 2B4 (Fig. 12A). This analysis reveals, consistent with our previous FACS analysis, that steady state levels of 2B4 are increased in SHIP$^{-/-}$ NK cells. We also find that the ratio of 2B4S to 2B4L is skewed towards the long isoform (2B4L) in the SHIP$^{-/-}$ NK cell relative to WT NK cells. In addition to 2B4, whole cell lysates (WCL) were blotted for SHP1 and SHP2 (Fig. 12B,C). This revealed that like 2B4, SHP1 is over-expressed in SHIP$^{-/-}$ NK cells as compared to WT. In contrast SHP2 levels are consistently comparable between SHIP$^{-/-}$ and WT NK cells.

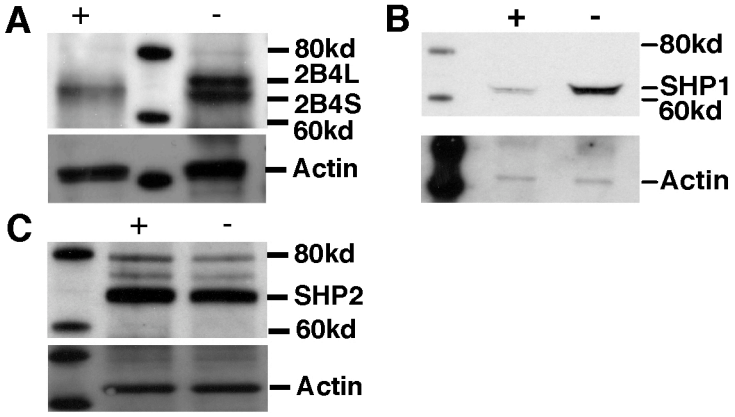

Figure 12. Expression of signaling molecules in NK WCL. NK1.1^{+} CD3^{-} NK cells were sorted from spleens of SHIP$^{-/-}$ (-) and WT (+) mice. WCL were prepared and Western blots performed for the indicated protein, blots were subsequently stripped and reprobed for actin as a loading control. A) Blots were probed for 2B4 revealing an isoform bias in the SHIP$^{-/-}$ NK cell. B) SHP1 C) SHP2. These Western blots are representative of three independent experiments.

Increased recruitment of SHP1 to 2B4 in SHIP-deficient NK cells

Due to the overexpression of 2B4, the bias towards the 2B4L isoform and SHP1 overexpression we hypothesized that there might be a qualitative change in signals emanating from 2B4 in SHIP$^{-/-}$ NK cells. To examine this possibility, we prepared 2B4 IPs from sorted SHIP$^{-/-}$ and WT NK cells (Fig. 13). Given the increase of SHP1 in SHIP$^{-/-}$ NK cells we explored the recruitment of it as well as SHP2 to 2B4 (Fig. 13A). In these blots we see a substantial increase in the co-IP of SHP1 to 2B4 in the SHIP$^{-/-}$ NK cell as compared to WT NK cells. However, no change is seen in SHP2 recruitment to 2B4 between SHIP$^{-/-}$ and WT NK cells. These blots were subsequently stripped and re-probed for 2B4. We were then able to quantitate the amount of SHP1, SHP2 and 2B4 present in these IPs. This allowed us to compare the relative amount of each of these proteins present in the IPs (Fig. 13B). Through this comparison we were able to show that there is approximately 2-fold more 2B4 in the SHIP$^{-/-}$ 2B4 IPs compared to WT. This 2-fold greater amount of 2B4 in the SHIP$^{-/-}$ NK cell was expected due to the fact, that as we have previously shown, there is approximately 2-fold increase in the amount of 2B4 on the surface of SHIP$^{-/-}$ NK cells as measured by flow cytometry (280). Therefore if equal cell equivalents were loaded we would expect ~2-fold more 2B4 in the IPs of SHIP$^{-/-}$ NK cells as compared to WT IPs. We were also able to show that in the SHIP$^{-/-}$ IPs there is at least a 10-fold increase in SHP1 recruitment , so although there is more 2B4 in SHIP$^{-/-}$ IPs there is dramatically more SHP1. We also performed this same analysis of SHP1 recruitment to 2B4 using a chemiluminescent secondary and a Licor Odyssey imager. This allowed us once again to quantitate the amount of SHP1 recruited to 2B4. Through this technique we were able to reconfirm our SHP1 finding showing that there is

approximately 16 times more SHP1 recruited to 2B4 in the SHIP$^{-/-}$ NK compared to the WT.

Given the key role of SHIP in opposing PI3K signaling, we blotted for the PI3K subunits p110 and p85 (Fig. 13D, E). In both instances we see a small but consistent increase in the association of 2B4 with both PI3K subunits in SHIP$^{-/-}$ NK cells. This change likely reflects increased 2B4 expression in SHIP-deficient NK cells, rather than preferential recruitment of these PI3K subunits. EAT-2 has been proposed to be a key mediator of the 2B4 inhibitory pathway (139), and therefore we also blotted 2B4 IPs for EAT-2 where we see no appreciable difference (Fig. 13F).

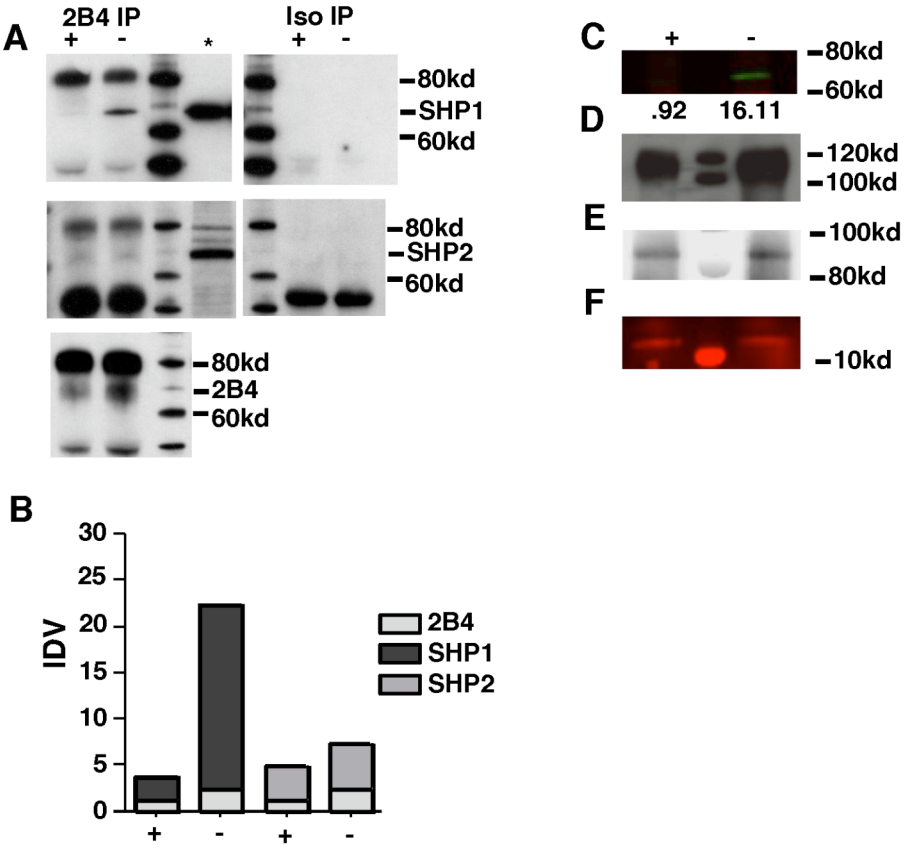

Figure 13. Recruitment of signaling molecules to 2B4. $NK1.1^{+}$ $CD3^{-}$ NK cells were sorted from spleens of $SHIP^{-/-}$ (-) and WT (+) mice. WCL were then prepared from the purified NK cells and 2B4 IPs prepared. A) 2B4 and its isotype control were IP in parallel. The IPs were then blotted for SHP1 and SHP2. 2B4 was blotted in the IPs to determine the total amount of receptor that was precipitated in order to normalize the samples. (* WCL control) B) The levels of SHP1, SHP2 and 2B4 present in the IPs were quantified by Imagequant software. These ratios were then compared in the bar graph showing that although there is an increase in 2B4 in the null IP there is a much greater increase in SHP1. C) SHP1 was probed for in 2B4 IPs using a fluorochrome tagged secondary and developed on a Licor Odyssey imager allowing the intensity of the SHP1 bands to be quantitated. The resulting values are shown below each band in arbitrary fluorescence units (FU) D) p110 subunit of PI-3-Kinase WB on 2B4 IPs E) p85 subunit of PI-3-Kinase WB on 2B4 IPs F) EAT-2 WB on 2B4 IPs. Cells were pooled from multiple animals to obtain sufficient numbers. These IP and Western blots are representative of three independent experiments performed with separate samples on different dates.

Given that our functional assays of 2B4's impact on NK cytolytic function are performed with LAK cells we also examined SHP1 and SHP2 recruitment to 2B4 receptor complexes in $SHIP^{-/-}$ and WT LAK cells. As was observed with freshly isolated NK cells there is a dramatic increase in the recruitment of SHP1 to 2B4 in activated $SHIP^{-/-}$ NK cells compared to WT where SHP2 remains equal in the same cells (Fig. 14A). Once again we were able to quantitate the amount of SHP1, SHP2 and 2B4

present in the 2B4 IPs. This finding agreed with the finding in resting NK cells that even though there is a 2-fold increase in 2B4 expression in the SHIP$^{-/-}$ NK cell the increased recruitment of SHP1 is much greater. Taken together, the analysis of both resting and activated NK cells suggests that 2B4 dominance of activating receptors and the hyporesponsiveness of SHIP$^{-/-}$ NK cells could be due to an inappropriate degree of SHP1 recruitment to 2B4 receptor complexes.

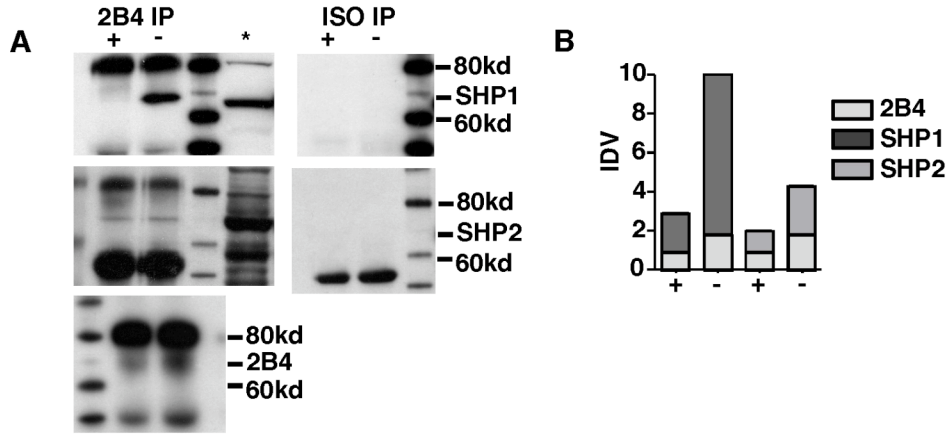

Figure 14. Recruitment of signaling molecules to 2B4 in activated NK cells. 2B4 and its isotype control were immunoprecipitated in parallel from WCL of SHIP$^{-/-}$ (-) and WT (+) LAK cells. IPs were resolved by SDS-PAGE and then Western blotted. A) SHP1 and SHP2. 2B4 was blotted in the 2B4 IPs in order to normalize the amount of receptor present in the IP. (*WCL control). B) SHP1, SHP2 and 2B4 levels were quantified using Imagequant software and compared in a bar graph, showing that SHP1 is dramatically over-recruited to 2B4 in the SHIP$^{-/-}$ LAK cells compared to the WT. These IP and Western blots are representative of three independent experiments.

Given the inappropriate degree of SHP1 recruitment to 2B4 in SHIP$^{-/-}$ NK cells we explored the possibility of using chemical inhibitors to block its tyrosine phosphatase activity to determine if this could restore killing of complex targets by SHIP-deficient NK cells. We first used NaOV, a broadly acting tyrosine phosphatase inhibitor to counteract the effects of the SHP1 over-recruitment to 2B4 (Fig. 14A). We find that the addition of 100μM sodium orthovanadate to SHIP$^{-/-}$ NK cytolysis assays restores their ability to mediate efficient killing (Fig. 15A). Importantly, we consistently observe no increased killing by WT LAK cells against either RMA or RMA-Rae1^{+} targets following the addition of NaOV. However, to our surprise we observed that NaOV treatment increased the capacity of SHIP$^{-/-}$ NK cells to kill RMA parental cells that do not express the NKG2D ligand, Rae1. In fact, SHIP-deficient NK cytolysis of RMA parental targets exceeds that of WT LAK cells. We have consistently observed this supernormal killing of RMA targets in three separate studies with NaOV-treated SHIP$^{-/-}$ LAK cells. This finding indicates phosphatase inhibition can restore the ability of SHIP$^{-/-}$ NK cells to kill complex targets via NKG2D, while also expanding the capacity of SHIP-deficient NK cells to kill tumor cells in the absence of ligands for NKG2D. We also tested the ability of NaOV to increase cytotoxicity with BaF3 and m157^{+} BaF3 targets (Fig. 15B). Once again we observe that NaOV is able to increase the capacity of SHIP$^{-/-}$ NK cells to kill both the parental BaF3 cells as well as the activating ligand positive m157^{+} BaF3 cells. Although these differences are not as dramatic as is seen in NKG2D killing. Nonetheless we consistently observe increased killing with both BaF3 parental and m157^{+} targets. Taken together with our previous findings demonstrating 2B4 dominates NKG2D and Ly49H in SHIP-deficient NK cells (280), the ability of NaOV to restore killing by SHIP$^{-/-}$

NK cells against multiple targets indicates increased tyrosine phosphatase activity is locking the SHIP$^{-/-}$ NK cell into a hyporesponsive state.

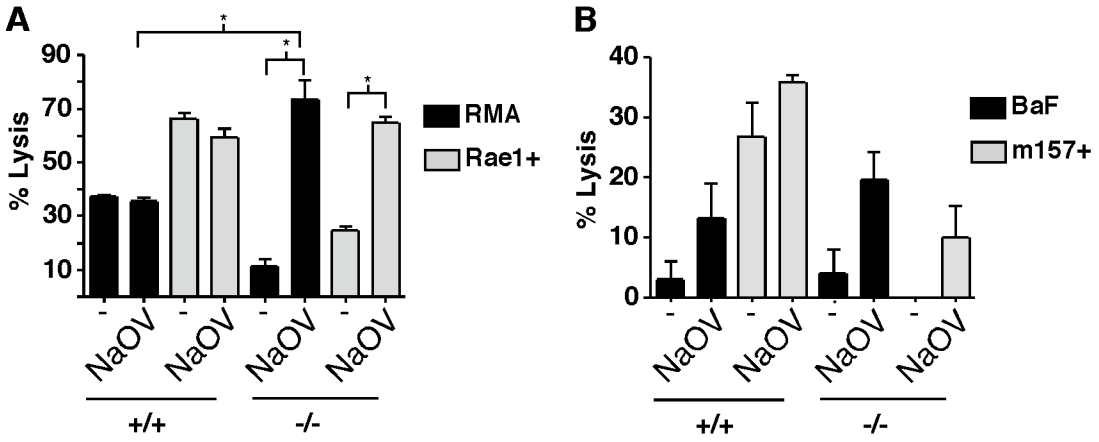

Figure 15. Restoration of SHIP$^{-/-}$ cytotoxicity with NaOV treatment. Standard four-hour ^{51}Cr release assays were performed with SHIP$^{-/-}$ (-/-) or WT (+/+) LAK cells. A ratio of 30:1 and 3000 target cells were used for all conditions. All experiments were performed in triplicate. Assays were performed in the presence of 100μM NaOV (NaOV) or with media alone (-). All graphs are representative of 3 or more independent experiments. (*=$p<0.05$). A) RMA or Rae1^{+} RMA transfectants were used as targets. B) BaF or M157^{+} BAF transfectants were used as targets.

Inhibition of SHP1 activity restores SHIP$^{-/-}$ NK cytolytic function

To further test the hypothesis that inappropriate recruitment of SHP1 to 2B4 is locking SHIP$^{-/-}$ NK cells into a hyporesponsive state we tested several novel low MW compounds that have the ability to inhibit the phosphatase activity of SHP1 at μM levels.

These compounds were identified during a screen for SHP2 inhibitors (281). We screened 6 compounds with predicted μM activity against SHP1 and 2. Of these 6 compounds we identified one, NSC119910 (Fig. 16A), which was effective in restoring the cytolytic capacity of $SHIP^{-/-}$ NK cells. The selectivity of this compound was tested *in vitro* against SHP1, SHP2 and PTP1b (Fig. 16B). In these experiments we were able to show that NSC119910 is approximately 10-fold more selective to SHP1 and approximately 100-fold more selective to SHP2 than a very closely related tyrosine phosphatase PTP1b.

We next tested the ability of NSC119910 to restore killing in the $SHIP^{-/-}$ NK cell. The effective *in vitro* dose at which NSC119910 was able to restore $SHIP^{-/-}$ cytotoxicity was determined in a dose titration experiment. Through this 67.32μM was identified as the effective dose (Fig. 16C). This concentration, 67.32μM of NSC119910, was used for all subsequent standard ^{51}Cr release assays. The addition of NSC119910 significantly restored killing of $Rae1^{+}$ RMA as well as parental RMA targets by $SHIP^{-/-}$ NK cells, while it had no effect on the cytolytic activity of WT NK cells against $Rae1^{+}$ targets (Fig. 16D). The addition of NSC119910 to LAK cells had no effect on the expression levels of NKG2D (Fig. 16F). We have also performed these experiments with $m157^{+}$ BaF3 targets. As shown in figure 16 the addition of NSC119910 also increased $SHIP^{-/-}$ NK killing of $m157^{+}$ targets. Although the increase is not as dramatic as we observe with NKG2D mediated cytolysis, this increase has been observed consistently in multiple cytolysis assays with the $m157^{+}$ BaF3 targets. These tyrosine phosphatase inhibition studies when paired with our biochemical determination of inappropriate SHP1

recruitment to 2B4 in SHIP$^{-/-}$ NK cells provides a mechanistic rationale for the hyporesponsiveness of SHIP$^{-/-}$ NK cells.

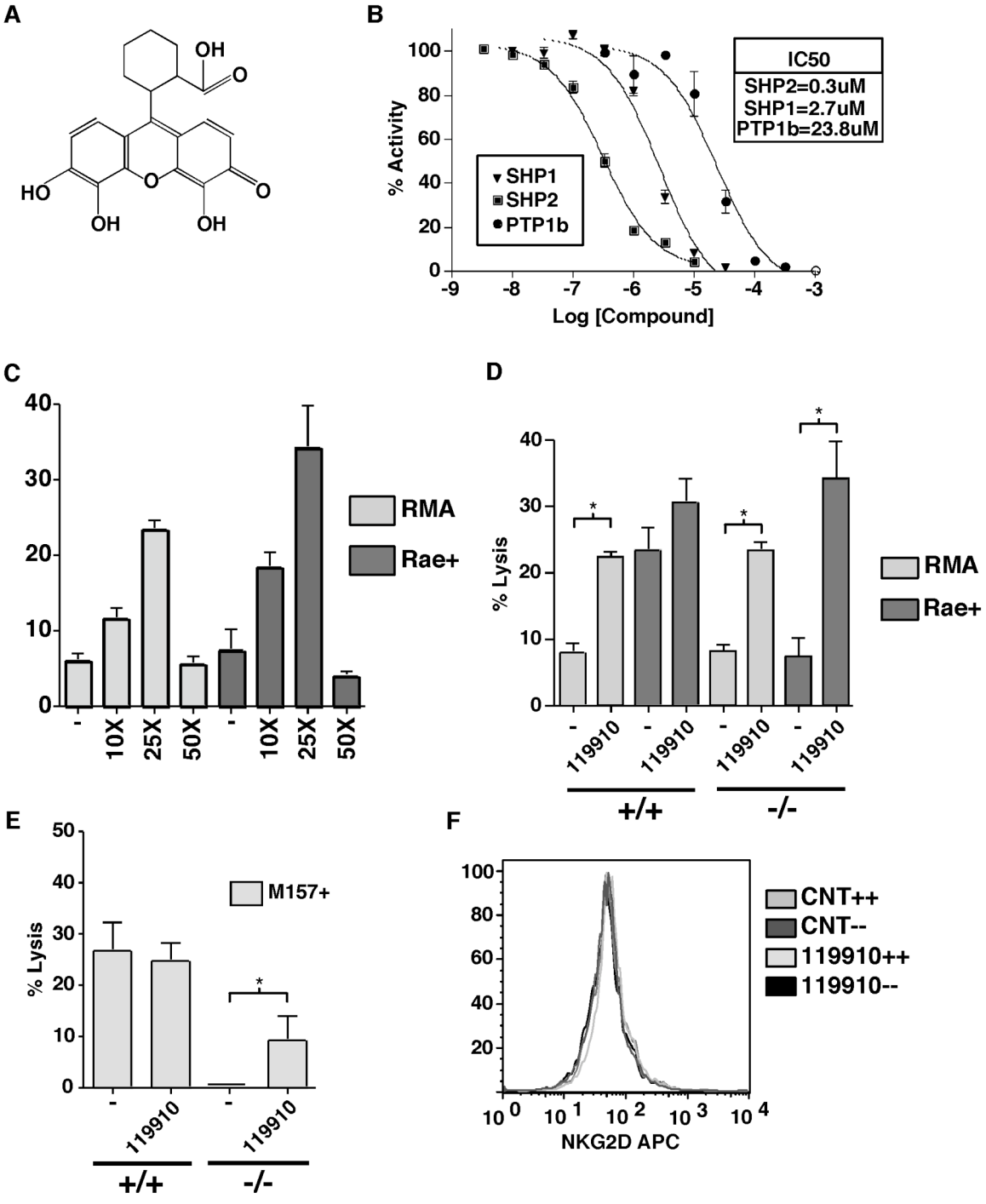

Figure 16. Restoration of cytotoxicity with SHP1/2 inhibitor NSC119910. A) Molecular structure of NSC119910. The structure of this molecule was confirmed by proton NMR (see Materials and Methods). B) NSC119910 was tested for its ability to inhibit the phosphatase activity of purified SHP1, SHP2 and PTP1b. C) The *in vivo* effective dose of NSC119910 was determined through a dose titration experiment. 10, 25 and 50X the *in vitro* IC50 of 2.7μM was used. D-E) Standard 4-hour ^{51}Cr release assays were performed with SHIP$^{-/-}$ **(-/-)** or WT (+/+) LAK cells. A ratio of 30:1 and 3000 target cells were used for all conditions. All conditions were performed in triplicate. Assays were performed in the presence of 67.32μM NCI119910 or media alone (-). These cytolysis studies with NSC119901 are representative of three independent experiments. (*=$p<0.05$) D) Rae1^{+} RMA transfectants were used as targets. E) M157^{+} BaF3 transfectants were used as targets. F) SHIP$^{-/-}$ (--) or WT (++) cells were incubated with 67.32μM NSC119910 (119910) or media alone (CNT) for 4 hours. The cells were then stained for NK1.1, CD3 and NKG2D and analyzed for NKG2D expression.

Discussion

Previously we have shown that SHIP-deficiency leads to an NK receptor repertoire disruption such that 2B4 acts as a dominant inhibitory receptor (280). In this study we have extended these findings to identify a molecular mechanism responsible for 2B4 receptor dominance in SHIP$^{-/-}$ NK cells. We have previously shown that there is significant over representation of 2B4 on the surface of SHIP$^{-/-}$ NK cells. We have extended this by demonstrating that in the SHIP-deficient NK cell there is not only more surface deposition of 2B4, but also significantly more 2B4 protein expressed by SHIP$^{-/-}$

NK cells. We have also determined that when compared to the WT NK cell, there is a bias in the SHIP$^{-/-}$ NK cell towards the 2B4L isoform. We examined the various signaling molecules that are recruited to 2B4 in SHIP$^{-/-}$ NK cells. We found that there is a small increase in the PI3K subunits p110 and p85 that is most likely attributable to increased 2B4 expression. We have also identified that there is no demonstrable difference in either SHP2 or EAT-2 recruitment to 2B4. Furthermore we have identified that there is approximately 10 to16 times more SHP1 recruited to 2B4 in SHIP$^{-/-}$ NK cells as compared to WT. We were able to reverse the effect of the SHP1 over-recruitment by inhibiting its enzymatic activity using either a broad acting tyrosine phosphatase inhibitor (NaOV) or a more selective SHP inhibitor (NSC119910). These results have led us to hypothesize that SHIP-deficiency leads not only to 2B4 receptor dominance, but 2B4L bias, as well as altered inhibitory signaling within the SHIP$^{-/-}$ NK cell. We have developed a model incorporating the key differences that exist within 2B4 signaling in the SHIP$^{-/-}$ and WT environment (Fig. 17).

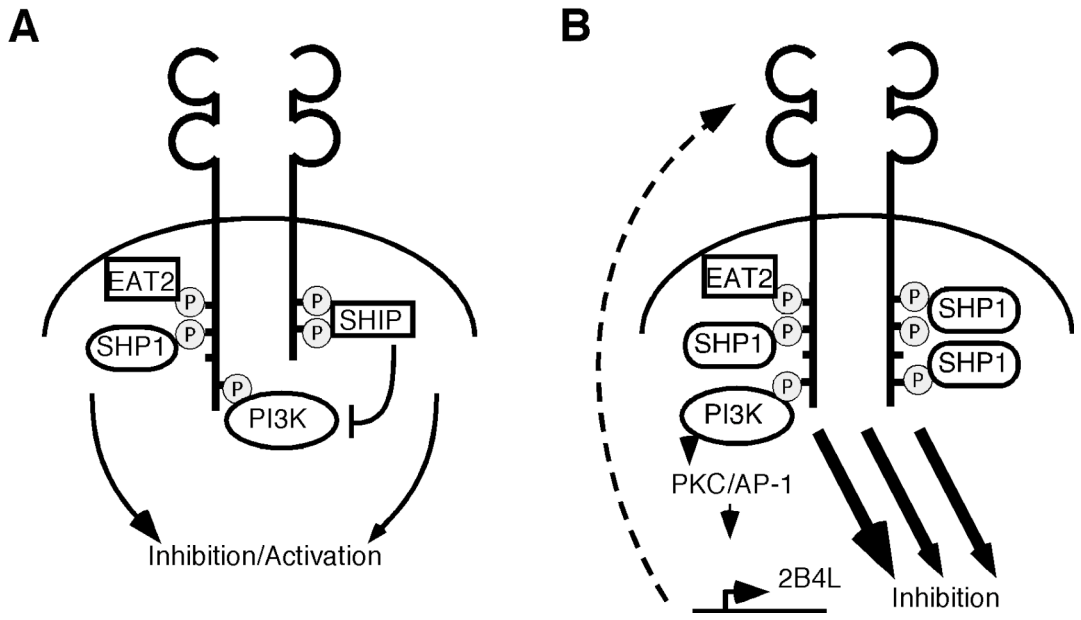

Figure 17. Model figure of 2B4 signaling. A) 2B4 signaling in a WT NK cell. Both short and long isoforms of 2B4 are present as well as possible activating and inhibitory signaling molecules. The signal that is delivered, either activation or inhibition, will depend upon the ligand present and the context of the signal. B) 2B4 signaling in a SHIP$^{-/-}$ NK cell. There is a lack of SHIP expression and bias towards the 2B4L isoform. These 2 factors lead to a profound increase in SHP1 recruitment and therefore tip the balance towards constitutive inhibitory signaling. The lack of SHIP may also allow unopposed PI3K activity at 2B4 that may in turn promote increased 2B4 expression.

Given that SHIP is a key inhibitor of the PI3K pathway, we initially considered the possible over-recruitment of the PI3K subunits, p110 and p85 to 2B4 might be responsible for the qualitative change in 2B4 function in SHIP-deficient NK cells. Given that the inhibition of SHP1 was able to restore killing in SHIP$^{-/-}$ NK cells to WT levels it stands to reason that PI3K does not play a major role in rendering SHIP$^{-/-}$ NK cells

hyporesponsive. PI3K could still play a subtle and indirect role in 2B4 receptor dominance. As is detailed in figure 17, in the absence of SHIP, PI3K activity may not need to be increased, but rather its' unopposed activity could potentially alter 2B4 expression and contribute to receptor dominance. Previous studies have identified AP-1 binding sites in the promoter of 2B4(282). PI3K can trigger nuclear translocation of AP-1 via activation of PKC-δ (283), and thus unopposed PI3K activity at 2B4 could potentially increase 2B4 expression and/or bias isoform usage towards 2B4L.

2B4 has proven to have a somewhat complex role in NK biology with *in vitro* and *in vivo* experiments indicating both activating and inhibitory roles in NK function (130-138). This disparity has been attributed, to some extent, to the various signaling adaptors that can potentially associate with the ITSM of 2B4 (133, 139-142). Both SHP1 and SHP2 have been shown to be recruited to 2B4 in certain contexts (137, 140, 142) and are also key regulators of inhibitory signaling for MHC-I receptors on NK cells. In this study we identified a ~10 to 16 fold increase in SHP1 recruitment to 2B4 in the $SHIP^{-/-}$ NK cell. This is a key finding given that we have previously shown that the surface expression of 2B4 is increased only ~2 fold in the $SHIP^{-/-}$ NK cell compared to the WT NK cell (280). There is clearly a qualitative change in the 2B4 receptor complexes such that a much larger proportion of 2B4 molecules associate with SHP1 in the absence of SHIP expression. 2B4 has up to 4 tyrosine residues in its cytoplasmic tail that can be phosphorylated and recruit downstream signaling molecules (140). Both SHIP and SHP1 have SH2 domains that can bind overlapping phosphotyrosines in 2B4 (140). Thus, in the SHIP-deficient NK cell there is likely greater access to 2B4 by SHP1. This dramatic

increase of SHP1 at 2B4 receptor complexes could alter the balance of signaling in the SHIP-deficient NK cell. Importantly 2B4 has been shown to be recruited to the NK synapse (284, 285). Therefore, the increased presence of SHP1 at the NK synapse in $SHIP^{-/-}$ NK cells is likely to terminate activating signals before they propagate to more distal effectors required for NK function.

In this study we utilized two phosphatase inhibitors; first, a broad acting phosphatase inhibitor NaOV, second, a more specific SHP inhibitor NSC119910. We utilized both of these compounds in an attempt to counteract SHP1 over-recruitment and thereby restore killing by the $SHIP^{-/-}$ NK cells to WT levels. NaOV was able to successfully restore killing by $SHIP^{-/-}$ NK cells of $Rae1^{+}$ cells to WT levels. Interestingly the killing of RMA parental cells by $SHIP^{-/-}$ NK cells was also significantly increased. We propose that this increase in $SHIP^{-/-}$ killing, in the absence of a strong activating ligand, results from the under representation of MHC specific inhibitory receptors on $SHIP^{-/-}$ NK cells, that would normally prevent WT killing of MHC–I^{+} targets lacking strong activating ligands. Therefore, when inhibitory dominance of 2B4 is released by phosphatase blockade this presumably enables supernormal killing of MHC-I^{+} targets that lack activating ligands. Our results with the BaF3 targets are less clear but nonetheless provocative. Most importantly we see a consistent increase in $SHIP^{-/-}$ cytotoxicity in the presence of NaOV reconfirming the ability of phosphatase blockade to increase cytotoxicity of the hyporesponsive $SHIP^{-/-}$ NK cell against BaF3 and $m157^{+}$ cells. Importantly when we later tested the more specific NSC119910 we do not see the

same increase of killing of m157^{+} cells by WT LAK cells indicating that the increase we see with NaOV is due to non-SHP1 related effects.

In addition to restoration of SHIP$^{-/-}$ killing we see an increase in the killing of RMA cells by WT NK cells. This is not only a plausible result, it is an expected and positive result. In the WT NK cell the normal MHC inhibitory receptors are present and functioning, in opposition to the SHIP$^{-/-}$ NK cell where these receptors are down-regulated. SHP1 and SHP2, key molecules to inhibitory signaling, are also present and functional in the WT NK cell. Therefore in the presence of a potent SHP inhibitor there would be a paucity of inhibitory signaling. The key is therefore that when inhibitory signals, mediated through SHP, are removed, be that of SHP recruited to Ly49 or 2B4, cytotoxicity is increased. Importantly in the case of the SHIP$^{-/-}$ cytotoxicity, it was not just increased but restored to the same level of the WT undergoing SHP inhibition. For undetermined reasons the restoration of BaF m157^{+} cells is not as dramatic. What this does indicate is that that there are qualitative differences that exist between the signaling milieus at play between RMA, BaF and the LAK cells. Such that in NKG2D mediated cytotoxicity 2B4 is the not only a dominant inhibitory receptor, it is likely the sole inhibitory receptor restraining killing. These results strongly reinforce that 2B4 is the dominant inhibitory receptor in the SHIP$^{-/-}$ NK cells as well as providing strong proof that SHP1 over-recruitment is the molecular mechanism behind this dominance.

Materials And Methods

Animals.

The SHIP$^{-/-}$ mice used in this study were previously created in our lab (See Chp. 1). Mice were maintained by intercrossing SHIP$^{+/-}$ mice (F10 to the C57BL6/J background) thereby allowing for sufficient numbers of both SHIP$^{-/-}$ as well as WT littermates for all experiments. All experiments were performed with SHIP$^{-/-}$ and WT littermates between 6-9 weeks of age.

LAK cultures and cytolysis assays.

Spleens were harvested and prepared into a single cell suspension from SHIP$^{-/-}$ and WT mice. Whole splenocytes were red blood cell lysed for 5 minutes with RBC lysis buffer. Cells were then spun down at 300xG for 5 minutes and resuspended at $2.5x10^8$ cells/mL in miltenyi buffer which consists of PBS, 0.5% FBS, 0.5% HEPES, and EDTA. Cells were then prepared for enrichment with the Miltenyi Mouse NK cell enrichment kit. The lineage specific antibody cocktail consisting of CD4, CD5, CD8, CD19, Gr-1, and Ter119 was added to the whole splenocytes at a concentration of 5μL/$1x10^7$ cells for 10 minutes in a refrigerator. An additional 30μL of media per $1x10^7$ cells was then added to each sample. Anti-biotin microbeads were then added at a concentration of 10μL/$1x10^7$ cells and incubated in the refrigerator for 15 minutes. Cells were then washed and resuspended in miltenyi buffer at a concentration of $200x10^6$ cells/mL. The cells were then run on the AutoMac (Miltenyi) on the DepleteS program. Percent of cells recovered varied from 5 to 25% between SHIP$^{-/-}$ and WT NK cells with a purity of 10-60%.

Enriched NK cells were plated at a density of $2x10^6$ cells per mL in 6-well dishes. Cells were cultured for seven days in activation media consisting of RPMI, 10% FBS, L-glut, Penicillin/Streptomycin, Na-pyruvate, non-essential amino acids and 2000units/mL human rIL2 (Proleukin). On day 1 of culture, cells were supplemented with 1mL of fresh activation media. On day 3, non-adherent cells were removed through a demi-depletion of 1-2mL and fresh media was added to the cultures. On day 4-6 media was added as necessary. On day 6-7 a standard 4 hour chromium release assay was performed. On the day of the experiment target cells were resuspended at $1x10^6$cells/100μL in chromium release media, consisting of RPMI with 3% FBS and HEPES. 100μCi of ^{51}Cr was added to $1x10^6$ target cells and then incubated for 60 minutes at 37°C with gentle agitation every 15 minutes. After incubation target cells were spun down at 300xG for 5 minutes and washed twice with 3mL of chromium media. The target cells were then counted and resuspended at 3000 cells/100μL in chromium media. In order to remove the LAK cells from culture they must be lifted through the use of PBS with EDTA. The first step is to remove the non-adherent and dead cells from the top of the culture through removal of the top 1-2mL of media. The remaining media is then pipetted up and down to free loose cells. The media, containing the freed LAK cells, is removed and placed on ice. 2mL of cold PBS-EDTA was then added to each well and placed at 4°C for 10-15 minutes. Over exposure to the EDTA, or exposure at warmer temperatures can prove hazardous to the LAK cells, so care was taken to assure the cells remained at 4°C. After the 10-15 minute incubation the remaining cells were freed from the plate through pipetting. LAK cells were then counted and spun down at 300xG for 5 minutes and resuspended in chromium

media such that the appropriate number of effector cell were in 100μL of media. NK cells and target cells were then combined at a ratio of 20:1 in 96 well plates at a total volume of 200μL. Target cell numbers were maintained at 3000 cells for all experiments. Plates were spun down at 200xG for 1 minute. The cells were then incubated together at 37°C for 4 hours. After incubation supernatants were collected and measured for radioactivity on a gamma counter (Perkin Elmer Wizard1470). Spontaneous release controls were performed in the absence of effector cells. Maximal release was measured by adding 10% Triton-X to the target cells alone. Percent lysis was calculated by the following formula. 100 X (experimental CPM – spontaneous release CPM) / (maximum release CPM – spontaneous release CPM). All experiments were performed in triplicate and results were verified with a separate experiment performed on a different date.

Inhibitors.

All experiments using sodium orthovanadate (NaOV) were performed with 100μM activated NaOV. NaOV was activated by adjusting the pH of a 200mM stock to pH 10.0 by the addition NaOH or HCl as necessary followed by boiling until the solution becomes colorless and then cooling to room temperature. This process is then repeated until the pH of the NaOV stabilizes at 10.0 (286). For all NaOV cytotoxic experiments LAK cells and targets cells were prepared as described above. Prior to incubation with targets cells NaOV was added to the LAK cells and allowed to incubate for 15 to 30 minutes at room temperature. Target cells were than added to the wells and a normal cytotoxic assay was performed. Controls for NaOV inhibition assays consisted of normal

cytotoxic assays in the absence of NaOV to measure normal lysis as well as spontaneous release controls in the presence of NaOV.

NSC119910 was obtained from the Drug Synthesis and Chemistry Branch, Developmental Therapeutics Program, Division of Cancer Treatment and Diagnosis, NCI. The structure of NSC119910 was confirmed by proton NMR using a Varian Mercury-Plus, Oxford AS400 spectrometer. The ^{1}H NMR spectrum was recorded at 400 MHz using DMSO-*d6* as solvent and tetramethylsilane (TMS) as an internal standard. Chemical shift values are reported in parts per million (δ). The compound shows characteristic signals as follows: δ 12.643 (s, 1H, -OH, disappeared on D_2O shake), 12.432 (s, 1H, -OH, disappeared on D_2O shake), 10.103 (br s, 1H, disappeared on D_2O shake), 7.486 (d, J = 9.2 Hz, 1H, Ar), 7.411 (d, J = 9.2 Hz, 1H, Ar), 6.438 (d, J = 8.8 Hz, 1H, Ar), 6.403 (d, J = 9.2 Hz, 1H, Ar), 2.024 - 0.796 (m, cyclohexyl moiety).

For chromium release assays NSC119910 was reconstituted in DMSO at 27mM. NSC119910 was then added to LAK cells immediately before the addition of target cells on ice for a final concentration of 67.32μM. Controls were performed in parallel with DMSO alone as well as no inhibitor. Spontaneous release controls were also performed in the presence of NSC119910.

Western Blots and Immunoprecipitates.

All western blot and IP studies were performed with pure cell populations (> 95% pure). For freshly isolated NK cells, spleens were removed from $SHIP^{-/-}$ and WT

littermates and made into a single cell suspension. Whole splenocytes and WBM were red blood cell lysed for 5 minutes at room temperature in RBC lysis buffer consisting of 0.15μM NH_4Cl, 10mM $KHCO_3$, and 0.1mM EDTA. Cells were spun down at 300xG for 5 minutes and then resuspended at $1x10^6$cells/50μL in staining media. Staining media consists of PBS with 3% FBS and HEPES. Cells were then Fc blocked for 15 minutes on ice with anti-CD16/32 antibody. Cells were then stained for sorting with NK1.1 FITC, CD3 PE and DAPI. Cells were stained for 15 minutes on ice and then washed twice with staining media. Cells were resuspended in staining media with 1% FBS for sorting. All cell sorting was performed on a FACS Aria (Beckon Dickson). Sorts were performed with a 70μM nozzle at a rate of 1000-5000 cells/second. All samples were kept at 4°C for the duration of the sort, including the sorted cells which were sorted into staining media containing 3% FBS.

After sorting cells were spun down at 300xG for 5 minutes at 4°C and then lysed for 30 minutes on ice in a modified TNE buffer consisting of 50mM Tris-HCl, 1% NP-40, 150mM NaCl, 1mM EDTA, 1mM PMSF, 1mM NaOV, 1mM NaF, and 10μL/mL of Protease inhibitor cocktail mix (Sigma Aldrich, Cat. #p8340). Cell debris was then spun down at 15,000xG for 15 minutes at 4°C and the supernatant collected. For Western blots equal cell equivalents for $SHIP^{-/-}$and WT lysates were brought up to a volume of 20μL with 4X LDS buffer (Invitrogen) consisting of 250mM Tris-HCl, 20% glycerol, 8% LDS, Serva Blue. DTT was then added to the sample for a final concentration of 50mM and the samples were heated for 10 minutes at 90°C. Samples were then resolved on a 4-12% Bis-Tris gel (Invitrogen) and transferred to an ECL membrane (Amersham). Blots

were blocked with 5% NFM-PBS-T for 1 hour at room temperature. Primary antibodies were used at varying concentrations; p110(Cell Signaling, 1:1000), p85(Cell signaling, 1:1000), Eat-2(a kind gift of Andre Veillette, 0.5μg/mL), SHP1 (BD Transduction Laboratories, 1:500), SHP2 (1:1000 Cell Signaling), 2B4 (R&D, 0.2μg/mL). Primary antibodies were incubated with the membrane in 5% NFM-PBS-T for 1 hour at room temperature or at 4°C for 12 hours. Primary antibodies were washed off with a minimum of three 15 minute washes with PBS-T. The appropriate anti-IgG HRP secondary for the specific primary was used. Secondaries were incubated with the membrane in 5% NFM-PBS-T for 1 hour at room temperature. Membranes were then washed a minimum of three times for 15 minutes each wash. Super Signal HRP detection system (Pierce) was then applied to the membrane that was then exposed to film, which was subsequently developed. For blots with high background additional washes were performed as needed at room temperature. Quantification was performed using Imagequant software (GE Healthcare). In order to use this software the blot is first scanned as a high resolution tiff. The bands of interest are then delineated by the user, as well as the area from which background will be calculated. The software then calculated the integrated density value (IDV). The IDV is calculated by area x (mean Density - background). To assure that areas of differing size did not skew quantitation bands were delineated by boxes of the same area between samples that would be directly compared (i.e.: SHP1 between $SHIP^{-/-}$ and WT). For all blots that used a fluorescently tagged secondary all samples and western blotting techniques were identical to above except the blocking as well as antibody incubation was done in Licor blocking buffer (Licor). Secondaries of the appropriate anti-IgG that were conjugated to an Alexafluor 488 or 680 (Invitrogen) were

utilized. After the secondary antibody was washed of blots were scanned with a Licor Odyssey imager rather than using film. Odyssey software was then used to quantitate the protein levels. Only the whole lanes were user identified and the software delineated the individual bands as well as calculating background. Results were given in an arbitrary fluorescent unit (FU)

For IPs after sorting cells were spun down at 300xG for 5 minutes at 4°C and then lysed for 30 minutes on ice in a modified TNE buffer consisting of 50mM Tris-HCl, 1% NP-40, 150mM NaCl, 1mM EDTA, 1mM PMSF, 1mM NaOV, 1mM NaF, and 10μL/mL of Protease inhibitor cocktail mix (Sigma Aldrich, p8340). Cell debris was then spun down at 15,000xG for 15 minutes at 4°C and the supernatant collected. Equal cell equivalents were brought up to 500μL in ice-cold TNE buffer. Both isotype and 2B4 IPs were performed in parallel under the same conditions with an equal number of cell equivalents. All steps were performed at 4°C, all buffers were ice-cold, and all centrifugation steps were performed in a pre-chilled centrifuge. Pre-clearing of WCL was performed by adding 50μL anti-mouse IG IP beads (eBioscience) to WCL for 60 minutes with constant mixing on a rotating mixer. The beads were then spun down at 10,000xG for 10 minutes and supernatants were collected and pre-cleared in the same manner one additional time. Cleared lysates were then incubated with an anti-2B4 antibody (BD) or isotype control (MsIgG2A, BD) for 90 minutes. 50μL of anti-mouse IG IP beads were then added to the lysates for 90 minutes while mixing on a rotating mixer. After incubation the beads were washed 6 times by spinning down at 10,000xG for 30 seconds and removing supernatant and replacing with fresh TNE lysis buffer.

After the last wash 50μL of LDS sample buffer was added to the beads and they were heated at 90°C for 10 minutes. They samples were then spun at 10,000XG for 5 minutes and the supernatants were collected and resolved by SDS-PAGE and blotted as described. $1x10^6$ cell equivalents were loaded into each well for western analysis.

PTP Inhibition Assay.

PTP activity was measured using the fluorogenic 6,8-difluoro-4-methylumbelliferyl phosphate (DiFMUP; Invitrogen, Carlsbad, CA) as the substrate. Each reaction contained 25mM HEPES, 50mM NaCl, 0.05% Triton, 1mM dithiothreitol, 20μM DiFMUP, 10nM Microcystin LR, 20nM GST-PTP, and 10μl of test compound or dimethyl sulfoxide (solvent) in a total reaction volume of 100 μl in black 96-well plates. Reaction was initiated by addition of DiFMUP, and the incubation time was 30 minutes at room temperature. DiFMUP fluorescence signal was measured at an excitation of 355 nm and an emission of 460 nm with a plate reader (Victor2 1420; PerkinElmer Wallac, Gaithersburg, MD). IC50 was defined as the concentration of an inhibitor that caused a 50% decrease in the PTP activity. For IC50 determination, eight concentrations of NSC119910 at one-third dilution (~0.5 log) were tested. The ranges of NSC119910 concentrations used in each PTP assay were determined from preliminary trials. Each experiment was performed either in triplicate or duplicate, and IC50 data were derived from at least two independent experiments. The curve-fitting program Prism 4 (GraphPad Software, San Diego, CA) was used to calculate IC50 values.

Statistical Analysis.

Statistical analysis was done using Graphpad Prism. The statistical test that was utilized was a Students two-tailed T-test. N=3 except where a greater N is indicated. Results were considered significant with a $p<0.05$.

CHAPTER 4

The NK Kinome

Introduction

Over the last few years array and mass spectrometry technologies have enabled analysis of the transcriptome (287, 288) and proteome of many cell types, including the NK cell (289). This information has proven to be and will continue to be of significant value to the elucidation of molecular mechanisms that govern not only basal cellular functions but also some of the more unique tasks of individual cell types including the NK cell. An equally, if not more important goal, is to define not just those proteins that are present but rather that are active and particularly the signaling pathways these functioning proteins are involved in.

Enzymes that phosphorylate tyrosine, serine and threonine residues play a major role in signaling cascades that determine cell cycle entry, survival and the differentiation of cells in the mammalian body, including the hematopoietic system. Knowing the differences that exist within individual proteins as well as complete signaling pathways that are active in NK cells and their immature precursors will provide critical information for understanding their biology. Towards this end, we have utilized kinome analysis techniques to explore the NK lineage. Array technology has been developed to measure

enzymatic activity between whole cell lysates and protein substrates. (98, 290-293). Specifically, arrays containing multiple target consensus sequences for protein kinases have been assembled (294). Within these arrays 1176 9-12 amino acid peptides have been arrayed in duplicate (Fig. 18). The peptides that have been arrayed are based upon known or predicted phosphorylation sites across the mammalian kinome. This allows for a wide ranging detection of serine, threonine, and tyrosine phosphorylation events that are mediated via kinases present in whole cell lysates. This technology has been validated by a comprehensive description of the temporal kinetics of phosphorylation events induced by lipopolysaccharide stimulation (295). Confidence in the usefulness of this technology for studying signal transduction has come from Western blot analysis of lipopolysaccharide-stimulated cells, which was corroborated with the demonstration that kinase inhibitors effected peptide array phosphorylation patterns consistent with the expected action of these inhibitors (295).

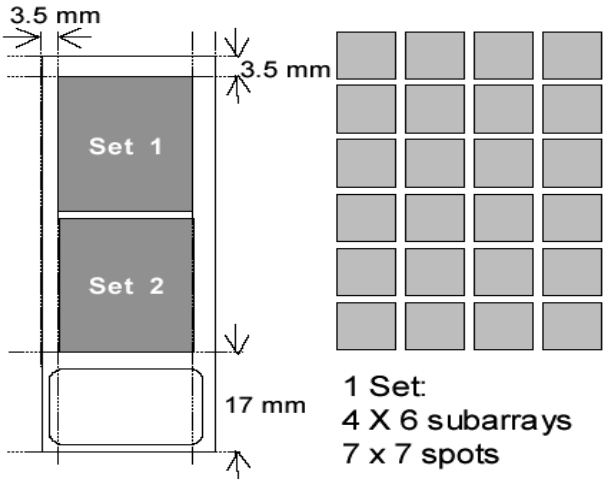

Figure 18. Kinome PepChip™. Diagram showing the layout of peptides on the array. Each array has the 1176 peptides arrayed in duplicate.

The differentiation of the NK cell from NKP to fully functional mature NK cell progresses in a linear process that can be followed by the gain and/or loss of surface markers (201-203). These stages of NK development occur in the bone marrow as these first steps require the interaction of the NKP with the BM stroma. The first key surface marker is CD122 or the IL2/15Rβ (201, 203). These receptors have proven to be essential to NK development as defects in their signaling lead to defects in NK development (204, 205, 296). It has also been shown that defects in IL-15 signaling pathways, such as Jak3 and Stat5a/b can cause defects in NK cell development (206). The next major step when a cell moves from NKP to immature NK cell is marked by the loss of Mac1 expression. Resulting in a phenotype of $NK1.1^{+}$, $CD3^{-}$, $Mac1^{-/lo}$. The next steps are marked by the acquisition of the NKR CD94/NKG2 and then the Ly49s and finally full functional maturity (202, 203). The immature NK cells we will be examining in this study are from the BM and were identified on the basis of $NK1.1^{+}$, $CD3^{-}$ and $Mac1^{-/lo}$. Where the mature NK cells were $NK1.1^{+}$, $CD3^{-}$ from the spleen. In this study we utilize kinome profiling in order to identify key signaling differences that exist between these two cell types.

Results

Obtaining pure cell populations

This first stage of this study was to obtain pure WCL from the immature and mature NK cells. To this end sorted populations of NK1.1^{+} CD3^{-} Mac1$^{lo/-}$ immature NK cells from the BM and NK1.1^{+} CD3^{-} mature NK cells from the spleen were obtained (Figure 19). Three independent sorts were performed for each cell type, consisting of a minimum of 250,000 cells from which 3 sets of WCL were prepared.

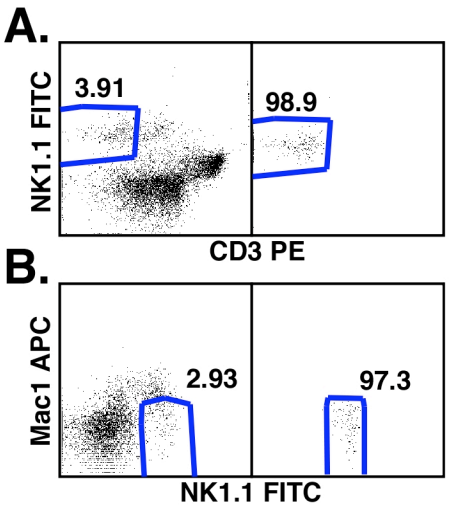

Figure 19. Kinome sorting strategy. Dot plots showing the sorting strategy for mature and immature NK cells.. Initial gating strategy is shown in the left panel. Post-sort purity is shown in the right panel. All plots are after back gating on scatter and live cells. A) NK1.1^{+} Lin^{-} mature NK cells. B) NK1.1^{+}, Lin^{-}, Mac1$^{-/lo}$ immature NK cells.

Peptide Phosphorylation

In order to measure phosphorylation of individual peptides present on the PepChip™ WCL from the pure cell populations were applied to the PepChip™ in the presence of ^{33}P-ATP and activation mix. After incubation of the WCL with the peptides on the array a phospho-imaging cassette was exposed to the PepChips™. Three sets of chips were utilized for each cell type, giving a total of six replicates in total for each peptide on the array for each cell type. Figure 20 shows the resulting images of the six chips after developing the phospho-imaging cassette. These resulting images were analyzed using Scanalyze software. This allowed us to obtain a numerical value for the phosphorylation of each individual peptide. These 6 replicates were then normalized across all replicates, providing us with a complete kinome for both mature and immature NK cells that could be compared between cell types. (See appendix 1 for the total kinome)

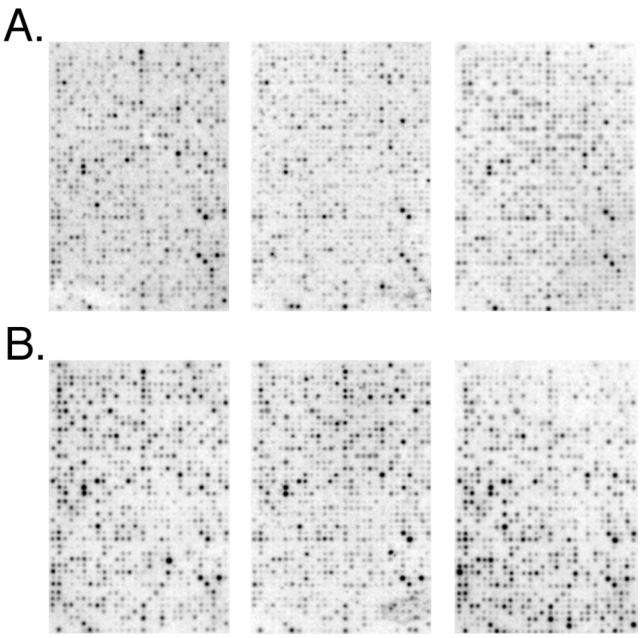

Figure 20. PepChips™ for mature and immature NK cells. The resulting images after phosphor imaging screens are exposed to the PepChips™ and then developed. These images were used to determine the phosphorylation signature for A) Immature NK cells and B) Mature NK cells.

Differential kinomes of mature and immature NK cells

In order to identify those peptides that are differentially phosphorylated between the immature and mature NK cells their kinomes were compared through a Wilcoxon rank sum analysis. Of the 1176 peptides present on the arrays only 3.9% of the peptides or 46 spots in total were differentially phosphorylated between the immature and mature NK cells. Indicating as would be expected that these cell types utilize similar signaling pathways. Of these 46 peptides 11 have a higher phosphorylation signature in the mature NK cell as compared to the immature cell, leaving 35 to have a higher phosphorylation signature in the immature NK cells (Table 1).

Spot	Biological function	Protein	Sequence
676	Cell Morphogenesis/Diff	Fibroblast growth factor receptor 3	STDEYLDLS
527	Signaling	Hematopoietic cell-specific LYN substrate	PEGDYEEVL
434	NA metabolism	Chromodomain-helicase-DNA-binding 1	PSEKSEEIT
413	Signaling	Focal adhesion kinase 1	ETDDYAEII
855	Signaling	Gamma-aminobutyric-acid receptor	RDEEYGYEA
444	NA metabolism	Zinc finger protein Rlf	EEELYLEPL
1024	Signaling	Tyrosine-protein kinase JAK3	KRPSFRAKA
233	Metabolism	Pyruvate kinase	LRRASVAQL
224	Metabolism	Pyruvate kinase	LRRASL
717	Signaling	Fibroblast growth factor receptor 4	VSEEYLDLR

755	Signaling	Receptor tyrosine-protein kinase ErbB-1	DNPDYQQDF
941	protein metabolism	eIF-2-beta	DPTMSKKKK
637	Metabolism	HMG-CoA lyase	KAAQISVRGL
735	Cell growth/Maintenance	Troponin T	QKAQTERKS
263	Transcription	Runt-related transcription factor 3	SGRGK
562	DNA repair	MutS-alpha	RKASRKE
931	Cell growth/Maintenance	Troponin I	QHLKSVMLQ
561	Cell growth/Maintenance	Lamin A/C	RLRLSPSPT
886	Signaling	Na channel protein type II alpha subunit	ERRPSNVSQ
570	Signaling	14-3-3 protein beta/alpha	WTSDTQGDE
702	Cell growth/Maintenance	Microtubule-associated protein tau	SKAGSLGNI
910	Signaling	Membrane progestin receptor beta	KSRRTI
896	Signaling	Protein-tyrosine phosphatase G1	ERNLSFEIK
850	Immune response	HUSSY-18	QEKESERLA
903	Transcription	Nuclear factor NF-kappa-B p105 subunit	FRKLSFTES
834	Signaling	Ca-dependent protein kinase type II gamma	HRQETVEAL
947	Signaling	MEK2	SMANSFVGT
1085	Transcription	Retinoblastoma-associated protein	PYKFPSSPLRIPGZ
954	Transcription	Histone H1.2	ASGSFKL
706	Cell growth/Maintenance	Caldesmon	DKVTSPTKV
953	metabolism	Neutrophil cytosol factor 1	RKRLSQDAY
968	Immune response	Myelin basic protein	PKRGSGKDG
707	Signaling	Na channel protein type X alpha subunit	FRRFTPDSL
1128	Transport	Kell blood group glycoprotein	ISITSRKAQ
1129	Unknown	PDZ domain containing protein 3	NFLKTSAGS
558	Cell growth/Maintenance	MARC-kinase substrate	AVASSPSKA
960	Immune response	Complement factor B precursor	TESQSLTLT
883	Signaling	PKA C-alpha	IGRFSEPHA
1084	Signaling	MCSF I receptor precursor	NDSNYVVKG
975	Signaling	IGF-binding protein 1	LMAPSEEDH
1170	protein metabolism	Phosphorylcholine transferase A	KQSPSSSPT
569	Signaling	Opsin 2	TVSKTETSQ
923	Signaling	Centaurin-delta 2	PGGSTPVSS
963	Immune response	Myelin basic protein	RHRDTGILD
957	Signaling	Protein phosphatase inhibitor 1	SLAMSPRQR
742	Transcription	Retinoblastoma-associated protein	PYKFPSSPLRIPGZ

Table 1. The 46 spots that are differentially phosphorylated between mature and immature NK cells. The peptides above the double line have a higher level of phosphorylation in the mature NK cells and those below are higher in immature NK cells. The amino acid sequence for each peptide is shown.

Functional Classification

The goal of this study was not only to identify those peptides that are differentially phosphorylated between these cell types but also to identify key signaling pathways that are utilized differently between these two developmental stages. In the design of the PepChip™ peptide sequences were chosen that corresponded to known or predicted phosphorylation sites across the mammalian kinome. Therefore all sites have a predicted protein that the peptide represents, although some overlap may exist between proteins with similar amino acid residues. In order to assure that we have the most accurate representation of a specific protein we reconfirmed the identity of each of the 46 spots that are differentially phosphorylated. The 9-12 amino acid sequence was entered into protein sequence alignment tools. The protein with the strongest alignment was utilized in most instances, taking into account the actual serine, threonine, or tyrosine that is being phosphorylated as well as those amino acids immediately adjacent to it. With these proteins we then utilized the human protein reference database (HPRD) to assign a biological function for each peptide (Table 1). We then segregated these functions to the immature or mature NK cell based on which cell type had a higher phosphorylation for the protein having the given function (Figure 21). In doing this we were able to immediately see that there are dramatic differences that exist within the processes that are being undertaken by the cells as identified by these 46 proteins. In the mature NK cell we see that, as would be expected, the largest amount of the function is devoted to signaling. Within this functional category we have included FGFR, JAK3, and FAK1. Where in the immature NK cell we see the largest functional category is cell

growth/maintenance. Through looking at the broad functional differences we can see the differences in the functions these two cell types utilize to carry out their unique functions.

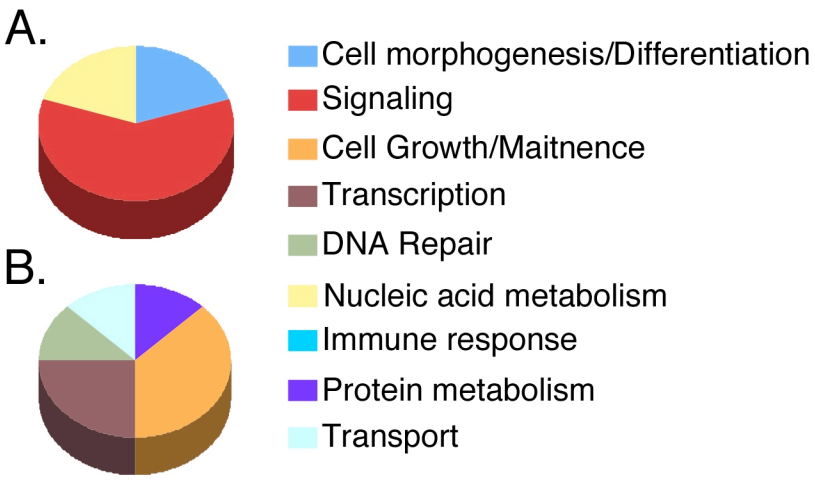

Figure 21. Functional pie charts for the peptides differentially phosphorylated between mature and immature NK cells. A) Mature NK cells. B) Immature NK cells.

Discussion

In this study through the use of the PepChip™ we have generated a kinome for both the immature and mature NK cell. We have then compared these kinomes and identified 46 peptides that are differentially phosphorylated between these 2 cell types. We have then explored the biological function that these 46 proteins are involved in an attempt to identify possible signaling differences that exists between these developmental stages.

Certain peptides have proven to be extremely interesting and may lend to a better understanding of the pathways that are key to these two cell types. The increased phosphorylation of JAK3 in the mature NK cell is a very positive finding. The need for IL-15/2Rβ in NK cell activation is well defined (205). NK cells specifically express the βγ chain of the IL-2/15R. It has been shown that mice deficient in the γ chain do not produce mature NK cells (204, 205). JAK3 has been shown to associate with the γ chain in NK cells. It has also been shown that mice lacking JAK3 do not produce mature NK cells rather their NK cells are stuck in an immature state (206). This shows the pivotal role of JAK3 in NK cells providing a strong proof of concept of this system as JAK3 is found to have an overall high level of phosphorylation as well as being significantly more phosphorylated in the mature NK cell.

In the mature NK cell we see that there is the increased phosphorylation of two growth factor receptors, FGFR3 and 4. Between these 2 receptors there is the ability to bind approximately 10 of the 22 different FGF (297). FGFR have been shown to play roles from embryonic development to throughout the adult animal. Within these various stages the FGF have been shown to play a role in numerous functions including proliferation, differentiation, and migration (297). Within the current literature there has yet to be any description of a role of FGF in immature or mature NK cells. This therefore may represent a novel finding identifying a possible role for FGFR in the regulation of NK cells.

The NF-κB pathway has been shown to play a variety of roles in the immune system, including NF-κB mice having a decreased immune response (298). The p50 precursor, p105 which has an increased phosphorylation in immature NK cells, has been shown to have a number of unique roles within the immune system (298). p105 through its association with other signaling molecules has been shown to function in the MAP/ERK pathway, specifically upstream of MEK (299). MEK, as well as the p105 subunit, have been found in this study to have a higher level of phosphorylation in the immature NK cell compared to the mature cell. This pathway of p105 to MEK and then further downstream has been shown to effect numerous different outcomes in immune cells including granule release in the NK cell (300). Although we can not determine exactly what outcome would be elicited at this stage it is an extremely positive finding to identify two proteins that can function up or downstream of each other in the same phosphorylation state.

Mature NK cells are believed to be in a homeostatic state most of the time in terms of there proliferative potential where the immature NK cell would most likely be cycling more often in order to effect its maturation and expansion (301). The increased phosphorylation of 14-3-3 fits in with this cycling state of the immature NK cell. 14-3-3, which can be up regulated by p53, can regulate the G2/M progression of the cell cycle (302, 303). It does this by sequestering CDC2/cyclin-B complexes in the cytoplasm, thus inhibiting the cell from progressing through the remainder of the cell cycle (302, 303). This may therefore be a key regulator of the NK cells proliferative state.

Although the function of many of these proteins and/or pathways are well known in other cell types their roles within NK cells is relatively undetermined. This study has therefore allowed us to identify some possible pathways that may be functioning within NK cells. It is also important to note that although we have concentrated on the substrates that are being phosphorylated in this study it is important to note that this is not the only manner in which the kinome chips can be analyzed. It is also possible to explore differential kinase activity rather than differential phosphorylation. In terms of this process we would be able to define or at least make a probably estimation of the kinase phosphorylating each peptide. We could then group the peptides via their kinase and determine if certain kinases are more or less active. This may prove useful in attempting to better elucidate pathways as opposed to individual proteins.

Materials and Methods

Animals

All mice used in this study were C57BL6/J mice of 8-10 weeks of age. On the day of cell isolation the spleen and BM were removed from sufficient animals to obtain a minimum of 250,000 pure cells.

Cell Sorting

For mature NK cells, spleens were removed and made into a single cell suspension. Whole splenocytes were red blood cell lysed for 5 minutes at room temperature in RBC lysis buffer consisting of 0.15μM NH_4Cl, 10mM $KHCO_3$, and 0.1mM EDTA. Cells were spun down at 300xG for 5 minutes and then resuspended at

$1x10^6$cells/50μL in staining media. Staining media consists of PBS with 3% FBS and HEPES. Cells were then Fc blocked for 15 minutes on ice with anti-CD16/32 antibody. Cells were then stained for sorting with $NK1.1^+$, $CD3^-$, $Gr1^-$, IgM^-, and DAPI. Cells were stained for 15 minutes on ice and then washed twice with staining media. Cells were resuspended in staining media with 1% FBS for sorting. All cell sorting was performed on a FACS Aria (Beckton Dickson). Sorts were performed with a 70μM nozzle at a rate of 1000-5000 cells/ second. All samples were kept at 4°C for the duration of the sort, including the sorted cells which were sorted into staining media containing 3% FBS. For immature NK cells BM was flushed from femurs of mice and prepared the same as spleen cells. Immature NK cells sorted on the basis of $NK1.1^+$, $CD3^-$, $Gr1^-$, IgM^-, $Mac1^{-/lo}$. All sorting was performed on a FACS Aria (Beckton Dickson).

PepChip ™ assays

For kinome array samples a minimum of 250,000 cells were sorted for each of the three cell kinome replicates. Cells were lysed in cell lysis buffer consisting of 20mM Tris-HCl (pH 7.5), 150mM NaCl, 1mM Na_2EDTA, 1mM EGTA, 1% Triton X-100, 2.5mM sodium pyrophosphate, 1mM $MgCl_2$, 1mM -glycerophosphate, 1mM Na_3VO_4, 1mM NaF, 1 μg/ml leupeptin (Sigma Aldrich), 1 μg/ml aprotinin (Sigma Aldrich), 1mM PMSF. Volumes of the cell lysates were equalized with diH2O. The cell lysates were then passed through a 0.22-μm low protein binding filter. 10μL of activation buffer was then added to the filtered WCL. Activation mix consists of 50% glycerol, 50μM ATP, 0.05% v/v Brij-35, 0.25 mg/ml bovine serum albumin, ^{33}P-γ-ATP (1000 kBq). The peptide array mix was then added to the PepChip ™, and it was then incubated at 37°C in

a humidified stove for 90 minutes. The peptide array was washed twice with Tris-buffered saline with 1% Tween-20, then twice in 2M NaCl, and then twice in demineralized H2O and finally air-dried. The chips were exposed in a phospho-imaging cassette for 72 hours. After the 72 hours the phospho-imaging cassettes were scanned using a Storm Phospho-imager (GE Healthcare). From the Storm imager we obtained high-resolution image files that were imported into the Scanalyze software program (Lawrence Berkley National Laboratory, CA) for analysis. To obtain median spot density a 28 X 42 grid was overlaid onto the PepChip ™ that delineated each individual peptide spot. The Scanalyze program then calculates the median spot density for each square within the grid thereby providing a value for the phosphorylation level for each peptide.

After the median spot densities were obtained for each spot on the chip the data was normalized across each chip. Normalization was achieved by correction of the spot density for the individual background to diminish inter-array variances thereby normalizing the total phosphorylation of the PepChip™ to be equal between all samples. This was done by taking the phosphorylation value for an individual spot dividing it by the sum of the total phosphorylation of all spots on the chip and then multiplying that by the total number of peptides (1176). In order to be included in the kinome analysis the mean phosphorylation between the two replicates on one PepChip™ had to have a correlation value of $> +0.85$. In addition any data that was inconsistent (i.e. SEM between data points >1.96) were excluded from further analysis. Both the correlation and standard deviation were calculated using Microsoft Excel using the following formulas;

for the correlation coefficient $r = \sum (x-\bar{x})(y-\bar{y}) \Big/ \sqrt{\sum (x-\bar{x})^2 . \sum (y-\bar{y})^2}$ and for standard deviation of the mean (SEM) $SEM = \sqrt{\sum (x-\bar{x})^2 \Big/ n-1}$. Where x or y is the value on the array being compared and n is the number of replicates used in the calculation.

Chapter 5

Discussion

In these studies we have attempted to better elucidate the signaling mechanisms used by the NK cell. To this end we have utilized a broadly sweeping approach through the examination of the kinome of both mature and immature NK cells. We have also examined NK cell signaling within the more specific signaling context of SHIP in the NK cell. Through these methods we have identified possible novel signaling mechanisms utilized by the NK cell from the study of the kinome. In the study of SHIP in the NK cell we have been able to take an initial observation of 2B4 overexpression in the $SHIP^{-/-}$ NK cell, determine the functional consequence of this receptor overexpression as NK hyporesponsiveness and finally determine the molecular mechanism behind this hyporesponsiveness as SHP1 over-recruitment.

The results we have obtained from our study of SHIP in the NK cell have led us to form a hypothesis where NK cell based treatments could possibly be developed. When we look at the role of NK cells within the realm of tumor regulation we see that tumor cells have developed a number of hurdles in order to circumvent surveillance via the NK cell. Whatever method a tumor utilizes to escape surveillance by an NK cell in the end it attempts to tip the balance of activating and inhibitory signals to the inhibitory side. The

key then is to identify a method that will allow an NK cell to overcome these barriers and tip the balance back to activation. One possibility is to utilize a temporary SHIP ablation in order to alter the NKR repertoire. As we have shown in Fig. 5 this could lead to a significant down regulation of MHC specific inhibitory receptors. This would then allow the balance of inhibitory and activating signals to be skewed such that the activating signals may be able to predominate. In this study we have utilized a SHIP1 KO mouse in order to effect the alteration of NK receptor repertoire. In a clinical setting we would envision the use of a small molecule inhibitor that would be able to block SHIP1. In doing so we would be able to reversibly alter the NK receptor repertoire.

This has great potential, even in the presence of diminished activating or increased inhibitory ligands, due to the fact that with lowered MHC specific inhibitory receptors even relatively low amounts of activating ligands would predominate. A concern of this method would be that the loss of inhibitory receptors would allow autoreactivity, although this is not the likely outcome. As has been discussed signaling within an NK cell is two part process consisting of both activating and inhibitory signals. Therefore the lack of inhibitory signal alone does not elicit cell lysis, an activating signal, although possibly a very minute signal, is still needed, therefore normal cells without stress inducible ligands present would still be protected.

The use of SHIP inhibition alone may well not be sufficient to effect the desired response. As our work has shown other inhibitory receptors may be able to compensate for the loss of Ly49 expression, in our studies 2B4 has proven very adept at this role.

Within the human system it may not be the same situation as in the human 2B4 usually functions as an activating receptor. This is due to the recruitment of SAP to 2B4. Although it is important to note that within the human system 2B4 can function as an inhibitory receptor, for instance within lymph node derived NK cells. In addition as we have shown in this study the inhibition of SHIP is able to not only alter the NK repertoire but it is also able to qualitatively alter the 2B4 receptor complex such that 2B4, even in the human, could be locked into a dominant inhibitory role. There fore within the human system either 2B4 or an alternate inhibitory receptor may lock cells into a hypo-responsive state luckily as has been discussed NK cells utilize very redundant signaling pathways, especially to elicit an inhibitory signal. Therefore we propose that dual or tandem inhibition of SHIP1 and SHP1 might be used to temporarily increase NK clearance of tumor cells. The first step would be to inhibit SHIP to create an NK compartment that is overly dependent on one or limited number of inhibitory receptors that would limit tumor killing. This could then be followed by treatment with an SHP1 inhibitor to unleash the killing capacity of the NK compartment against tumor cells. Although in this study we used a $SHIP^{-/-}$ NK cells and chemical inhibition of SHP1 activity, it may be possible to reversibly inhibit SHIP and SHP1 using RNA interference and/or chemical inhibitors. Through this it may be possible to reversibly inhibit both of these proteins thereby unleashing the strong cytotoxic potential of the NK cell on transformed cells.

References

1. Collavo, D., A. Colombatti, and L. Chieco-Bianchi. 1974. T lymphocyte requirement for MSV tumour prevention or regression. *Nature 249:169-170.*

2. Gorczynski, R. M. 1974. Immunity to murine sarcoma virus-inducted tumors. II. Suppression of T cell-mediated immunity by cells from progressor animals. *J Immunol 112:1826-1838.*

3. Gorczynski, R. M. 1974. Immunity to murine sarcoma virus-inducted tumors. I. Specific T lymphocytes active in macrophage migration inhibition and lymphocyte transformation. *J Immunol 112:1815-1825.*

4. Gorczynski, R. M., and C. Norbury. 1974. Immunity to murine sarcoma virus induced tumours. 3. Analysis of the cell populations involved in protection from lethal tumour progression of sublethally irradiated, MSV inoculated, mice. *Br J Cancer 30:118-128.*

5. Berenson, J. R., A. B. Einstein, Jr., and A. Fefer. 1975. Syngeneic adoptive immunotherapy and chemoimmunotherapy of a Friend leukemia: requirement for T cells. *J Immunol 115:234-238.*

6. Glaser, M., D. H. Lavrin, and R. B. Herberman. 1976. In vivo protection against syngeneic Gross virus-induced lymphoma in rats: comparison with in vitro studies of cell-mediated immunity. *J Immunol 116:1507-1511.*

7. Moller, R., A. Nielsen, and F. Reymann. 1975. Multiple basal cell carcinoma and internal malignant tumors. *Arch Dermatol 111:584-585.*

8. Outzen, H. C., R. P. Custer, G. J. Eaton, and R. T. Prehn. 1975. Spontaneous and induced tumor incidence in germfree "nude" mice. *J Reticuloendothel Soc 17:1-9.*

9. Schwartz, R. S. 1975. Another look at immunologic surveillance. *N Engl J Med 293:181-184.*

10. Hellstrom, K. E., and I. Hellstrom. 1974. Lymphocyte-mediated cytotoxicity and blocking serum activity to tumor antigens. *Adv Immunol 18:209-277.*

11. Herberman, R. B., J. L. McCoy, and P. H. Levine. 1974. Immunological reactions to tumor-associated antigens in Burkitts lymphoma and other lymphomas. *Cancer Res 34:1222-1227.*

12. Oldham, R. K., and R. B. Herberman. 1973. Evaluation of cell-mediated cytotoxic reactivity against tumor associated antigens with 125I-iododeoxyuridine labeled target cells. *J Immunol 111:862-871.*

13. Rosenberg, E. B., R. B. Herberman, P. H. Levine, R. H. Halterman, J. L. McCoy, and J. R. Wunderlich. 1972. Lymphocyte cytotoxicity reactions to leukemia-associated antigens in identical twins. *Int J Cancer 9:648-658.*

14. Takasugi, M., M. R. Mickey, and P. I. Terasaki. 1973. Reactivity of lymphocytes from normal persons on cultured tumor cells. *Cancer Res 33:2898-2902.*

15. Herberman, R. B., M. E. Nunn, H. T. Holden, and D. H. Lavrin. 1975. Natural cytotoxic reactivity of mouse lymphoid cells against syngeneic and allogeneic tumors. II. Characterization of effector cells. *Int J Cancer 16:230-239.*

16. Herberman, R. B., M. E. Nunn, and D. H. Lavrin. 1975. Natural cytotoxic reactivity of mouse lymphoid cells against syngeneic acid allogeneic tumors. I. Distribution of reactivity and specificity. *Int J Cancer 16:216-229.*

17. Kiessling, R., E. Klein, and H. Wigzell. 1975. "Natural" killer cells in the mouse. I. Cytotoxic cells with specificity for mouse Moloney leukemia cells. Specificity and distribution according to genotype. *Eur J Immunol 5:112-117.*

18. Kiessling, R., E. Klein, H. Pross, and H. Wigzell. 1975. "Natural" killer cells in the mouse. II. Cytotoxic cells with specificity for mouse Moloney leukemia cells. Characteristics of the killer cell. *Eur J Immunol 5:117-121.*

19. Sendo, F., T. Aoki, E. A. Boyse, and C. K. Buafo. 1975. Natural occurrence of lymphocytes showing cytotoxic activity to BALB/c radiation-induced leukemia RL male 1 cells. *J Natl Cancer Inst 55:603-609.*

20. Ohlen, C., G. Kling, P. Hoglund, M. Hansson, G. Scangos, C. Bieberich, G. Jay, and K. Karre. 1989. Prevention of allogeneic bone marrow graft rejection by H-2 transgene in donor mice. *Science 246:666-668.*

21. Ljunggren, H. G., and K. Karre. 1990. In search of the 'missing self': MHC molecules and NK cell recognition. *Immunol Today 11:237-244.*

22. Cudkowicz, G., and M. Bennett. 1971. Peculiar immunobiology of bone marrow allografts. II. Rejection of parental grafts by resistant F 1 hybrid mice. *J Exp Med 134:1513-1528.*

23. Cudkowicz, G., and M. Bennett. 1971. Peculiar immunobiology of bone marrow allografts. I. Graft rejection by irradiated responder mice. *J Exp Med 134:83-102*.

24. Santoli, D., G. Trinchieri, L. Moretta, C. M. Zmijewski, and H. Koprowski. 1978. Spontaneous cell-mediated cytotoxicity in humans. Distribution and characterization of the effector cell. *Clin Exp Immunol 33:309-318*.

25. Trinchieri, G., D. Santoli, and H. Koprowski. 1978. Spontaneous cell-mediated cytotoxicity in humans: role of interferon and immunoglobulins. *J Immunol 120:1849-1855*.

26. Becker, S., R. Kiessling, N. Lee, and G. Klein. 1978. Modulation of sensitivity to natural killer cell lysis after in vitro explantation of a mouse lymphoma. *J Natl Cancer Inst 61:1495-1498*.

27. Kunkel, L. A., and R. M. Welsh. 1981. Metabolic inhibitors render "resistant" target cells sensitive to natural killer cell-mediated lysis. *Int J Cancer 27:73-79*.

28. Stern, P., M. Gidlund, A. Orn, and H. Wigzell. 1980. Natural killer cells mediate lysis of embryonal carcinoma cells lacking MHC. *Nature 285:341-342*.

29. Hoglund, P., H. G. Ljunggren, C. Ohlen, L. Ahrlund-Richter, G. Scangos, C. Bieberich, G. Jay, G. Klein, and K. Karre. 1988. Natural resistance against lymphoma grafts conveyed by H-2Dd transgene to C57BL mice. *J Exp Med 168:1469-1474*.

30. Yokoyama, W. M., P. J. Kehn, D. I. Cohen, and E. M. Shevach. 1990. Chromosomal location of the Ly-49 (A1, YE1/48) multigene family. Genetic association with the NK 1.1 antigen. *J Immunol 145:2353-2358*.

31. Karlhofer, F. M., R. K. Ribaudo, and W. M. Yokoyama. 1992. MHC class I alloantigen specificity of Ly-49+ IL-2-activated natural killer cells. *Nature 358:66-70*.

32. Bix, M., N. S. Liao, M. Zijlstra, J. Loring, R. Jaenisch, and D. Raulet. 1991. Rejection of class I MHC-deficient haemopoietic cells by irradiated MHC-matched mice. *Nature 349:329-331*.

33. Ljunggren, H. G., L. Van Kaer, H. L. Ploegh, and S. Tonegawa. 1994. Altered natural killer cell repertoire in Tap-1 mutant mice. *Proc Natl Acad Sci U S A 91:6520-6524*.

34. Mason, L. H., J. R. Ortaldo, H. A. Young, V. Kumar, M. Bennett, and S. K. Anderson. 1995. Cloning and functional characteristics of murine large granular

lymphocyte-1: a member of the Ly-49 gene family (Ly-49G2). *J Exp Med 182:293-303*.

35. Stoneman, E. R., M. Bennett, J. An, K. A. Chesnut, E. K. Wakeland, J. B. Scheerer, M. J. Siciliano, V. Kumar, and P. A. Mathew. 1995. Cloning and characterization of 5E6(Ly-49C), a receptor molecule expressed on a subset of murine natural killer cells. *J Exp Med 182:305-313*.

36. Yokoyama, W. M., L. B. Jacobs, O. Kanagawa, E. M. Shevach, and D. I. Cohen. 1989. A murine T lymphocyte antigen belongs to a supergene family of type II integral membrane proteins. *J Immunol 143:1379-1386*.

37. Giorda, R., and M. Trucco. 1991. Mouse NKR-P1. A family of genes selectively coexpressed in adherent lymphokine-activated killer cells. *J Immunol 147:1701-1708*.

38. Yokoyama, W. M., J. C. Ryan, J. J. Hunter, H. R. Smith, M. Stark, and W. E. Seaman. 1991. cDNA cloning of mouse NKR-P1 and genetic linkage with LY-49. Identification of a natural killer cell gene complex on mouse chromosome 6. *J Immunol 147:3229-3236*.

39. Brennan, J., S. Lemieux, J. D. Freeman, D. L. Mager, and F. Takei. 1996. Heterogeneity among Ly-49C natural killer (NK) cells: characterization of highly related receptors with differing functions and expression patterns. *J Exp Med 184:2085-2090*.

40. Brown, M. G., S. Fulmek, K. Matsumoto, R. Cho, P. A. Lyons, E. R. Levy, A. A. Scalzo, and W. M. Yokoyama. 1997. A 2-Mb YAC contig and physical map of the natural killer gene complex on mouse chromosome 6. *Genomics 42:16-25*.

41. Brown, M. G., A. A. Scalzo, K. Matsumoto, and W. M. Yokoyama. 1997. The natural killer gene complex: a genetic basis for understanding natural killer cell function and innate immunity. *Immunol Rev 155:53-65*.

42. Chan, P. Y., and F. Takei. 1989. Molecular cloning and characterization of a novel murine T cell surface antigen, YE1/48. *J Immunol 142:1727-1736*.

43. Wong, S., J. D. Freeman, C. Kelleher, D. Mager, and F. Takei. 1991. Ly-49 multigene family. New members of a superfamily of type II membrane proteins with lectin-like domains. *J Immunol 147:1417-1423*.

44. Yokoyama, W. M., and B. F. Plougastel. 2003. Immune functions encoded by the natural killer gene complex. *Nat Rev Immunol 3:304-316*.

45. Yokoyama, W. M., and W. E. Seaman. 1993. The Ly-49 and NKR-P1 gene families encoding lectin-like receptors on natural killer cells: the NK gene complex. *Annu Rev Immunol 11:613-635*.

46. Smith, K. M., J. Wu, A. B. Bakker, J. H. Phillips, and L. L. Lanier. 1998. Ly-49D and Ly-49H associate with mouse DAP12 and form activating receptors. *J Immunol 161:7-10*.

47. Mason, L. H., S. K. Anderson, W. M. Yokoyama, H. R. Smith, R. Winkler-Pickett, and J. R. Ortaldo. 1996. The Ly-49D receptor activates murine natural killer cells. *J Exp Med 184:2119-2128*.

48. Brennan, J., D. Mager, W. Jefferies, and F. Takei. 1994. Expression of different members of the Ly-49 gene family defines distinct natural killer cell subsets and cell adhesion properties. *J Exp Med 180:2287-2295*.

49. Takei, F., J. Brennan, and D. L. Mager. 1997. The Ly-49 family: genes, proteins and recognition of class I MHC. *Immunol Rev 155:67-77*.

50. Daniels, B. F., F. M. Karlhofer, W. E. Seaman, and W. M. Yokoyama. 1994. A natural killer cell receptor specific for a major histocompatibility complex class I molecule. *J Exp Med 180:687-692*.

51. Sentman, C. L., M. Y. Olsson, M. Salcedo, P. Hoglund, U. Lendahl, and K. Kare. 1994. H-2 allele-specific protection from NK cell lysis in vitro for lymphoblasts but not tumor targets. Protection mediated by alpha 1/alpha 2 domains. *J Immunol 153:5482-5490*.

52. Kane, K. P. 1994. Ly-49 mediates EL4 lymphoma adhesion to isolated class I major histocompatibility complex molecules. *J Exp Med 179:1011-1015*.

53. Yu, Y. Y., T. George, J. R. Dorfman, J. Roland, V. Kumar, and M. Bennett. 1996. The role of Ly49A and 5E6(Ly49C) molecules in hybrid resistance mediated by murine natural killer cells against normal T cell blasts. *Immunity 4:67-76*.

54. Brennan, J., G. Mahon, D. L. Mager, W. A. Jefferies, and F. Takei. 1996. Recognition of class I major histocompatibility complex molecules by Ly-49: specificities and domain interactions. *J Exp Med 183:1553-1559*.

55. Lian, R. H., Y. Li, S. Kubota, D. L. Mager, and F. Takei. 1999. Recognition of class I MHC by NK receptor Ly-49C: identification of critical residues. *J Immunol 162:7271-7276*.

56. Hanke, T., H. Takizawa, C. W. McMahon, D. H. Busch, E. G. Pamer, J. D. Miller, J. D. Altman, Y. Liu, D. Cado, F. A. Lemonnier, P. J. Bjorkman, and D.

H. Raulet. 1999. Direct assessment of MHC class I binding by seven Ly49 inhibitory NK cell receptors. *Immunity 11:67-77.*

57. Johansson, M. H., E. Hoglund, M. C. Nakamura, J. C. Ryan, and P. Hoglund. 1998. Alpha1/alpha2 domains of H-2D(d), but not H-2L(d), induce "missing self" reactivity in vivo--no effect of H-2L(d) on protection against NK cells expressing the inhibitory receptor Ly49G2. *Eur J Immunol 28:4198-4206.*

58. Kubota, A., S. Kubota, S. Lohwasser, D. L. Mager, and F. Takei. 1999. Diversity of NK cell receptor repertoire in adult and neonatal mice. *J Immunol 163:212-216.*

59. Held, W., and D. H. Raulet. 1997. Expression of the Ly49A gene in murine natural killer cell clones is predominantly but not exclusively mono-allelic. *Eur J Immunol 27:2876-2884.*

60. Held, W., J. Roland, and D. H. Raulet. 1995. Allelic exclusion of Ly49-family genes encoding class I MHC-specific receptors on NK cells. *Nature 376:355-358.*

61. Saleh, A., G. E. Davies, V. Pascal, P. W. Wright, D. L. Hodge, E. H. Cho, S. J. Lockett, M. Abshari, and S. K. Anderson. 2004. Identification of probabilistic transcriptional switches in the Ly49 gene cluster: a eukaryotic mechanism for selective gene activation. *Immunity 21:55-66.*

62. Raulet, D. H., R. E. Vance, and C. W. McMahon. 2001. Regulation of the natural killer cell receptor repertoire. *Annu Rev Immunol 19:291-330.*

63. Veinotte, L. L., B. T. Wilhelm, D. L. Mager, and F. Takei. 2003. Acquisition of MHC-specific receptors on murine natural killer cells. *Crit Rev Immunol 23:251-266.*

64. Gays, F., K. Martin, R. Kenefeck, J. G. Aust, and C. G. Brooks. 2005. Multiple cytokines regulate the NK gene complex-encoded receptor repertoire of mature NK cells and T cells. *J Immunol 175:2938-2947.*

65. Kim, S., J. Poursine-Laurent, S. M. Truscott, L. Lybarger, Y. J. Song, L. Yang, A. R. French, J. B. Sunwoo, S. Lemieux, T. H. Hansen, and W. M. Yokoyama. 2005. Licensing of natural killer cells by host major histocompatibility complex class I molecules. *Nature 436:709-713.*

66. Fernandez, N. C., E. Treiner, R. E. Vance, A. M. Jamieson, S. Lemieux, and D. H. Raulet. 2005. A subset of natural killer cells achieves self-tolerance without expressing inhibitory receptors specific for self-MHC molecules. *Blood 105:4416-4423.*

67. Johansson, S., M. Johansson, E. Rosmaraki, G. Vahlne, R. Mehr, M. Salmon-Divon, F. Lemonnier, K. Karre, and P. Hoglund. 2005. Natural killer cell education in mice with single or multiple major histocompatibility complex class I molecules. *J Exp Med 201:1145-1155*.

68. Tormo, J., K. Natarajan, D. H. Margulies, and R. A. Mariuzza. 1999. Crystal structure of a lectin-like natural killer cell receptor bound to its MHC class I ligand. *Nature 402:623-631*.

69. Wang, J., M. C. Whitman, K. Natarajan, J. Tormo, R. A. Mariuzza, and D. H. Margulies. 2002. Binding of the natural killer cell inhibitory receptor Ly49A to its major histocompatibility complex class I ligand. Crucial contacts include both H-2Dd AND beta 2-microglobulin. *J Biol Chem 277:1433-1442*.

70. Dam, J., R. Guan, K. Natarajan, N. Dimasi, L. K. Chlewicki, D. M. Kranz, P. Schuck, D. H. Margulies, and R. A. Mariuzza. 2003. Variable MHC class I engagement by Ly49 natural killer cell receptors demonstrated by the crystal structure of Ly49C bound to H-2K(b). *Nat Immunol 4:1213-1222*.

71. Correa, I., and D. H. Raulet. 1995. Binding of diverse peptides to MHC class I molecules inhibits target cell lysis by activated natural killer cells. *Immunity 2:61-71*.

72. Michaelsson, J., A. Achour, M. Salcedo, A. Kase-Sjostrom, J. Sundback, R. A. Harris, and K. Karre. 2000. Visualization of inhibitory Ly49 receptor specificity with soluble major histocompatibility complex class I tetramers. *Eur J Immunol 30:300-307*.

73. Franksson, L., J. Sundback, A. Achour, J. Bernlind, R. Glas, and K. Karre. 1999. Peptide dependency and selectivity of the NK cell inhibitory receptor Ly-49C. *Eur J Immunol 29:2748-2758*.

74. Vely, F., and E. Vivier. 1997. Conservation of structural features reveals the existence of a large family of inhibitory cell surface receptors and noninhibitory/activatory counterparts. *J Immunol 159:2075-2077*.

75. Burshtyn, D. N., A. S. Lam, M. Weston, N. Gupta, P. A. Warmerdam, and E. O. Long. 1999. Conserved residues amino-terminal of cytoplasmic tyrosines contribute to the SHP-1-mediated inhibitory function of killer cell Ig-like receptors. *J Immunol 162:897-902*.

76. Fry, A. M., L. L. Lanier, and A. Weiss. 1996. Phosphotyrosines in the killer cell inhibitory receptor motif of NKB1 are required for negative signaling and for association with protein tyrosine phosphatase 1C. *J Exp Med 184:295-300*.

77. Nakamura, M. C., E. C. Niemi, M. J. Fisher, L. D. Shultz, W. E. Seaman, and J. C. Ryan. 1997. Mouse Ly-49A interrupts early signaling events in natural killer cell cytotoxicity and functionally associates with the SHP-1 tyrosine phosphatase. *J Exp Med 185:673-684*.

78. Daeron, M., S. Latour, O. Malbec, E. Espinosa, P. Pina, S. Pasmans, and W. H. Fridman. 1995. The same tyrosine-based inhibition motif, in the intracytoplasmic domain of Fc gamma RIIB, regulates negatively BCR-, TCR-, and FcR-dependent cell activation. *Immunity 3:635-646*.

79. Pani, G., K. D. Fischer, I. Mlinaric-Rascan, and K. A. Siminovitch. 1996. Signaling capacity of the T cell antigen receptor is negatively regulated by the PTP1C tyrosine phosphatase. *J Exp Med 184:839-852*.

80. Arase, H., E. S. Mocarski, A. E. Campbell, A. B. Hill, and L. L. Lanier. 2002. Direct recognition of cytomegalovirus by activating and inhibitory NK cell receptors. *Science 296:1323-1326.*

81. Smith, H. R., J. W. Heusel, I. K. Mehta, S. Kim, B. G. Dorner, O. V. Naidenko, K. Iizuka, H. Furukawa, D. L. Beckman, J. T. Pingel, A. A. Scalzo, D. H. Fremont, and W. M. Yokoyama. 2002. Recognition of a virus-encoded ligand by a natural killer cell activation receptor. *Proc Natl Acad Sci U S A 99:8826-8831*.

82. Mason, L. H., J. Willette-Brown, S. K. Anderson, P. Gosselin, E. W. Shores, P. E. Love, J. R. Ortaldo, and D. W. McVicar. 1998. Characterization of an associated 16-kDa tyrosine phosphoprotein required for Ly-49D signal transduction. *J Immunol 160:4148-4152*.

83. George, T. C., L. H. Mason, J. R. Ortaldo, V. Kumar, and M. Bennett. 1999. Positive recognition of MHC class I molecules by the Ly49D receptor of murine NK cells. *J Immunol 162:2035-2043*.

84. George, T. C., J. R. Ortaldo, S. Lemieux, V. Kumar, and M. Bennett. 1999. Tolerance and alloreactivity of the Ly49D subset of murine NK cells. *J Immunol 163:1859-1867.*

85. Nakamura, M. C., P. A. Linnemeyer, E. C. Niemi, L. H. Mason, J. R. Ortaldo, J. C. Ryan, and W. E. Seaman. 1999. Mouse Ly-49D recognizes H-2Dd and activates natural killer cell cytotoxicity. *J Exp Med 189:493-500.*

86. Furukawa, H., K. Iizuka, J. Poursine-Laurent, N. Shastri, and W. M. Yokoyama. 2002. A ligand for the murine NK activation receptor Ly-49D: activation of tolerized NK cells from beta 2-microglobulin-deficient mice. *J Immunol 169:126-136*.

87. Lee, S. H., A. Zafer, Y. de Repentigny, R. Kothary, M. L. Tremblay, P. Gros, P. Duplay, J. R. Webb, and S. M. Vidal. 2003. Transgenic expression of the activating natural killer receptor Ly49H confers resistance to cytomegalovirus in genetically susceptible mice. *J Exp Med 197:515-526*.

88. Lohwasser, S., P. Hande, D. L. Mager, and F. Takei. 1999. Cloning of murine NKG2A, B and C: second family of C-type lectin receptors on murine NK cells. *Eur J Immunol 29:755-761*.

89. Vance, R. E., A. M. Jamieson, and D. H. Raulet. 1999. Recognition of the class Ib molecule Qa-1(b) by putative activating receptors CD94/NKG2C and CD94/NKG2E on mouse natural killer cells. *J Exp Med 190:1801-1812*.

90. Vance, R. E., J. R. Kraft, J. D. Altman, P. E. Jensen, and D. H. Raulet. 1998. Mouse CD94/NKG2A is a natural killer cell receptor for the nonclassical major histocompatibility complex (MHC) class I molecule Qa-1(b). *J Exp Med 188:1841-1848*.

91. Brooks, A. G., P. E. Posch, C. J. Scorzelli, F. Borrego, and J. E. Coligan. 1997. NKG2A complexed with CD94 defines a novel inhibitory natural killer cell receptor. *J Exp Med 185:795-800*.

92. Lazetic, S., C. Chang, J. P. Houchins, L. L. Lanier, and J. H. Phillips. 1996. Human natural killer cell receptors involved in MHC class I recognition are disulfide-linked heterodimers of CD94 and NKG2 subunits. *J Immunol 157:4741-4745*.

93. Houchins, J. P., L. L. Lanier, E. C. Niemi, J. H. Phillips, and J. C. Ryan. 1997. Natural killer cell cytolytic activity is inhibited by NKG2-A and activated by NKG2-C. *J Immunol 158:3603-3609*.

94. Carretero, M., C. Cantoni, T. Bellon, C. Bottino, R. Biassoni, A. Rodriguez, J. J. Perez-Villar, L. Moretta, A. Moretta, and M. Lopez-Botet. 1997. The CD94 and NKG2-A C-type lectins covalently assemble to form a natural killer cell inhibitory receptor for HLA class I molecules. *Eur J Immunol 27:563-567*.

95. Le Drean, E., F. Vely, L. Olcese, A. Cambiaggi, S. Guia, G. Krystal, N. Gervois, A. Moretta, F. Jotereau, and E. Vivier. 1998. Inhibition of antigen-induced T cell response and antibody-induced NK cell cytotoxicity by NKG2A: association of NKG2A with SHP-1 and SHP-2 protein-tyrosine phosphatases. *Eur J Immunol 28:264-276*.

96. Lanier, L. L., B. Corliss, J. Wu, and J. H. Phillips. 1998. Association of DAP12 with activating CD94/NKG2C NK cell receptors. *Immunity 8:693-701*.

97. Lian, R. H., M. Maeda, S. Lohwasser, M. Delcommenne, T. Nakano, R. E. Vance, D. H. Raulet, and F. Takei. 2002. Orderly and nonstochastic acquisition of CD94/NKG2 receptors by developing NK cells derived from embryonic stem cells in vitro. *J Immunol 168:4980-4987.*

98. Diefenbach, A., E. Tomasello, M. Lucas, A. M. Jamieson, J. K. Hsia, E. Vivier, and D. H. Raulet. 2002. Selective associations with signaling proteins determine stimulatory versus costimulatory activity of NKG2D. *Nat Immunol 3:1142-1149.*

99. Jamieson, A. M., A. Diefenbach, C. W. McMahon, N. Xiong, J. R. Carlyle, and D. H. Raulet. 2002. The role of the NKG2D immunoreceptor in immune cell activation and natural killing. *Immunity 17:19-29.*

100. Raulet, D. H. 2003. Roles of the NKG2D immunoreceptor and its ligands. *Nat Rev Immunol 3:781-790.*

101. Bauer, S., V. Groh, J. Wu, A. Steinle, J. H. Phillips, L. L. Lanier, and T. Spies. 1999. Activation of NK cells and T cells by NKG2D, a receptor for stress-inducible MICA. *Science 285:727-729.*

102. Wu, J., Y. Song, A. B. Bakker, S. Bauer, T. Spies, L. L. Lanier, and J. H. Phillips. 1999. An activating immunoreceptor complex formed by NKG2D and DAP10. *Science 285:730-732.*

103. Gilfillan, S., E. L. Ho, M. Cella, W. M. Yokoyama, and M. Colonna. 2002. NKG2D recruits two distinct adapters to trigger NK cell activation and costimulation. *Nat Immunol 3:1150-1155.*

104. Lanier, L. L., B. C. Corliss, J. Wu, C. Leong, and J. H. Phillips. 1998. Immunoreceptor DAP12 bearing a tyrosine-based activation motif is involved in activating NK cells. *Nature 391:703-707.*

105. Colonna, M., F. Navarro, T. Bellon, M. Llano, P. Garcia, J. Samaridis, L. Angman, M. Cella, and M. Lopez-Botet. 1997. A common inhibitory receptor for major histocompatibility complex class I molecules on human lymphoid and myelomonocytic cells. *J Exp Med 186:1809-1818.*

106. Diefenbach, A., E. R. Jensen, A. M. Jamieson, and D. H. Raulet. 2001. Rae1 and H60 ligands of the NKG2D receptor stimulate tumour immunity. *Nature 413:165-171.*

107. Cerwenka, A., A. B. Bakker, T. McClanahan, J. Wagner, J. Wu, J. H. Phillips, and L. L. Lanier. 2000. Retinoic acid early inducible genes define a ligand family for the activating NKG2D receptor in mice. *Immunity 12:721-727.*

108. Carayannopoulos, L. N., O. V. Naidenko, D. H. Fremont, and W. M. Yokoyama. 2002. Cutting edge: murine UL16-binding protein-like transcript 1: a newly described transcript encoding a high-affinity ligand for murine NKG2D. *J Immunol 169:4079-4083*.

109. Zou, Z., M. Nomura, Y. Takihara, T. Yasunaga, and K. Shimada. 1996. Isolation and characterization of retinoic acid-inducible cDNA clones in F9 cells: a novel cDNA family encodes cell surface proteins sharing partial homology with MHC class I molecules. *J Biochem (Tokyo) 119:319-328.*

110. Nguyen, K. B., T. P. Salazar-Mather, M. Y. Dalod, J. B. Van Deusen, X. Q. Wei, F. Y. Liew, M. A. Caligiuri, J. E. Durbin, and C. A. Biron. 2002. Coordinated and distinct roles for IFN-alpha beta, IL-12, and IL-15 regulation of NK cell responses to viral infection. *J Immunol 169:4279-4287*.

111. Li, Y., T. Zhang, C. Ho, J. S. Orange, S. D. Douglas, and W. Z. Ho. 2004. Natural killer cells inhibit hepatitis C virus expression. *J Leukoc Biol 76:1171-1179*.

112. Gasser, S., S. Orsulic, E. J. Brown, and D. H. Raulet. 2005. The DNA damage pathway regulates innate immune system ligands of the NKG2D receptor. *Nature 436:1186-1190.*

113. Cosman, D., J. Mullberg, C. L. Sutherland, W. Chin, R. Armitage, W. Fanslow, M. Kubin, and N. J. Chalupny. 2001. ULBPs, novel MHC class I-related molecules, bind to CMV glycoprotein UL16 and stimulate NK cytotoxicity through the NKG2D receptor. *Immunity 14:123-133*.

114. Diefenbach, A., A. M. Jamieson, S. D. Liu, N. Shastri, and D. H. Raulet. 2000. Ligands for the murine NKG2D receptor: expression by tumor cells and activation of NK cells and macrophages. *Nat Immunol 1:119-126.*

115. Sutherland, C. L., N. J. Chalupny, K. Schooley, T. VandenBos, M. Kubin, and D. Cosman. 2002. UL16-binding proteins, novel MHC class I-related proteins, bind to NKG2D and activate multiple signaling pathways in primary NK cells. *J Immunol 168:671-679*.

116. Kubin, M., L. Cassiano, J. Chalupny, W. Chin, D. Cosman, W. Fanslow, J. Mullberg, A. M. Rousseau, D. Ulrich, and R. Armitage. 2001. ULBP1, 2, 3: novel MHC class I-related molecules that bind to human cytomegalovirus glycoprotein UL16, activate NK cells. *Eur J Immunol 31:1428-1437*.

117. Oppenheim, D. E., S. J. Roberts, S. L. Clarke, R. Filler, J. M. Lewis, R. E. Tigelaar, M. Girardi, and A. C. Hayday. 2005. Sustained localized expression of ligand for the activating NKG2D receptor impairs natural cytotoxicity in vivo and reduces tumor immunosurveillance. *Nat Immunol 6:928-937*.

118. Garni-Wagner, B. A., A. Purohit, P. A. Mathew, M. Bennett, and V. Kumar. 1993. A novel function-associated molecule related to non-MHC-restricted cytotoxicity mediated by activated natural killer cells and T cells. *J Immunol 151:60-70.*

119. Staunton, D. E., and D. A. Thorley-Lawson. 1987. Molecular cloning of the lymphocyte activation marker Blast-1. *Embo J 6:3695-3701.*

120. Fraser, C. C., D. Howie, M. Morra, Y. Qiu, C. Murphy, Q. Shen, J. C. Gutierrez-Ramos, A. Coyle, G. A. Kingsbury, and C. Terhorst. 2002. Identification and characterization of SF2000 and SF2001, two new members of the immune receptor SLAM/CD2 family. *Immunogenetics 53:843-850.*

121. Mathew, P. A., B. A. Garni-Wagner, K. Land, A. Takashima, E. Stoneman, M. Bennett, and V. Kumar. 1993. Cloning and characterization of the 2B4 gene encoding a molecule associated with non-MHC-restricted killing mediated by activated natural killer cells and T cells. *J Immunol 151:5328-5337.*

122. Valiante, N. M., and G. Trinchieri. 1993. Identification of a novel signal transduction surface molecule on human cytotoxic lymphocytes. *J Exp Med 178:1397-1406.*

123. Sandrin, M. S., T. P. Gumley, M. M. Henning, H. A. Vaughan, L. J. Gonez, J. A. Trapani, and I. F. McKenzie. 1992. Isolation and characterization of cDNA clones for mouse Ly-9. *J Immunol 149:1636-1641.*

124. de la Fuente, M. A., V. Tovar, N. Villamor, N. Zapater, P. Pizcueta, E. Campo, J. Bosch, and P. Engel. 2001. Molecular characterization and expression of a novel human leukocyte cell-surface marker homologous to mouse Ly-9. *Blood 97:3513-3520.*

125. Seed, B. 1987. An LFA-3 cDNA encodes a phospholipid-linked membrane protein homologous to its receptor CD2. *Nature 329:840-842.*

126. Kumaresan, P. R., W. C. Lai, S. S. Chuang, M. Bennett, and P. A. Mathew. 2002. CS1, a novel member of the CD2 family, is homophilic and regulates NK cell function. *Mol Immunol 39:1-8.*

127. Shlapatska, L. M., S. V. Mikhalap, A. G. Berdova, O. M. Zelensky, T. J. Yun, K. E. Nichols, E. A. Clark, and S. P. Sidorenko. 2001. CD150 association with either the SH2-containing inositol phosphatase or the SH2-containing protein tyrosine phosphatase is regulated by the adaptor protein SH2D1A. *J Immunol 166:5480-5487.*

128. Latchman, Y., P. F. McKay, and H. Reiser. 1998. Identification of the 2B4 molecule as a counter-receptor for CD48. *J Immunol 161:5809-5812.*

129. Brown, M. H., K. Boles, P. A. van der Merwe, V. Kumar, P. A. Mathew, and A. N. Barclay. 1998. 2B4, the natural killer and T cell immunoglobulin superfamily surface protein, is a ligand for CD48. *J Exp Med 188:2083-2090.*

130. Lee, K. M., M. E. McNerney, S. E. Stepp, P. A. Mathew, J. D. Schatzle, M. Bennett, and V. Kumar. 2004. 2B4 acts as a non-major histocompatibility complex binding inhibitory receptor on mouse natural killer cells. *J Exp Med 199:1245-1254.*

131. McNerney, M. E., D. Guzior, and V. Kumar. 2005. 2B4 (CD244)-CD48 interactions provide a novel MHC class I-independent system for NK-cell self-tolerance in mice. *Blood 106:1337-1340.*

132. Veillette, A. 2004. SLAM Family Receptors Regulate Immunity with and without SAP-related Adaptors. *J Exp Med 199:1175-1178.*

133. Veillette, A., and S. Latour. 2003. The SLAM family of immune-cell receptors. *Curr Opin Immunol 15:277-285.*

134. Schatzle, J. D., S. Sheu, S. E. Stepp, P. A. Mathew, M. Bennett, and V. Kumar. 1999. Characterization of inhibitory and stimulatory forms of the murine natural killer cell receptor 2B4. *Proc Natl Acad Sci U S A 96:3870-3875.*

135. Boles, K. S., H. Nakajima, M. Colonna, S. S. Chuang, S. E. Stepp, M. Bennett, V. Kumar, and P. A. Mathew. 1999. Molecular characterization of a novel human natural killer cell receptor homologous to mouse 2B4. *Tissue Antigens 54:27-34.*

136. Nakajima, H., M. Cella, H. Langen, A. Friedlein, and M. Colonna. 1999. Activating interactions in human NK cell recognition: the role of 2B4-CD48. *Eur J Immunol 29:1676-1683.*

137. Tangye, S. G., S. Lazetic, E. Woollatt, G. R. Sutherland, L. L. Lanier, and J. H. Phillips. 1999. Cutting edge: human 2B4, an activating NK cell receptor, recruits the protein tyrosine phosphatase SHP-2 and the adaptor signaling protein SAP. *J Immunol 162:6981-6985.*

138. Vacca, P., G. Pietra, M. Falco, E. Romeo, C. Bottino, F. Bellora, F. Prefumo, E. Fulcheri, P. L. Venturini, M. Costa, A. Moretta, L. Moretta, and M. C. Mingari. 2006. Analysis of natural killer cells isolated from human decidua: Evidence that 2B4 (CD244) functions as an inhibitory receptor and blocks NK-cell function. *Blood 108:4078-4085.*

139. Roncagalli, R., J. E. Taylor, S. Zhang, X. Shi, R. Chen, M. E. Cruz-Munoz, L. Yin, S. Latour, and A. Veillette. 2005. Negative regulation of natural killer cell function by EAT-2, a SAP-related adaptor. *Nat Immunol 6:1002-1010.*

140. Eissmann, P., L. Beauchamp, J. Wooters, J. C. Tilton, E. O. Long, and C. Watzl. 2005. Molecular basis for positive and negative signaling by the natural killer cell receptor 2B4 (CD244). *Blood 105:4722-4729.*

141. Chen, R., F. Relouzat, R. Roncagalli, A. Aoukaty, R. Tan, S. Latour, and A. Veillette. 2004. Molecular dissection of 2B4 signaling: implications for signal transduction by SLAM-related receptors. *Mol Cell Biol 24:5144-5156.*

142. Tangye, S. G., K. E. Nichols, N. J. Hare, and B. C. van de Weerdt. 2003. Functional requirements for interactions between CD84 and Src homology 2 domain-containing proteins and their contribution to human T cell activation. *J Immunol 171:2485-2495.*

143. Sivori, S., D. Pende, C. Bottino, E. Marcenaro, A. Pessino, R. Biassoni, L. Moretta, and A. Moretta. 1999. NKp46 is the major triggering receptor involved in the natural cytotoxicity of fresh or cultured human NK cells. Correlation between surface density of NKp46 and natural cytotoxicity against autologous, allogeneic or xenogeneic target cells. *Eur J Immunol 29:1656-1666.*

144. Spaggiari, G. M., R. Carosio, D. Pende, S. Marcenaro, P. Rivera, M. R. Zocchi, L. Moretta, and A. Poggi. 2001. NK cell-mediated lysis of autologous antigen-presenting cells is triggered by the engagement of the phosphatidylinositol 3-kinase upon ligation of the natural cytotoxicity receptors NKp30 and NKp46. *Eur J Immunol 31:1656-1665.*

145. Gazit, R., R. Gruda, M. Elboim, T. I. Arnon, G. Katz, H. Achdout, J. Hanna, U. Qimron, G. Landau, E. Greenbaum, Z. Zakay-Rones, A. Porgador, and O. Mandelboim. 2006. Lethal influenza infection in the absence of the natural killer cell receptor gene Ncr1. *Nat Immunol 7:517-523.*

146. Bloushtain, N., U. Qimron, A. Bar-Ilan, O. Hershkovitz, R. Gazit, E. Fima, M. Korc, I. Vlodavsky, N. V. Bovin, and A. Porgador. 2004. Membrane-associated heparan sulfate proteoglycans are involved in the recognition of cellular targets by NKp30 and NKp46. *J Immunol 173:2392-2401.*

147. Pessino, A., S. Sivori, C. Bottino, A. Malaspina, L. Morelli, L. Moretta, R. Biassoni, and A. Moretta. 1998. Molecular cloning of NKp46: a novel member of the immunoglobulin superfamily involved in triggering of natural cytotoxicity. *J Exp Med 188:953-960.*

148. Dustin, M. L., and A. C. Chan. 2000. Signaling takes shape in the immune system. *Cell 103:283-294.*

149. Davis, D. M., I. Chiu, M. Fassett, G. B. Cohen, O. Mandelboim, and J. L. Strominger. 1999. The human natural killer cell immune synapse. *Proc Natl Acad Sci U S A 96:15062-15067.*

150. Davis, D. M. 2002. Assembly of the immunological synapse for T cells and NK cells. *Trends Immunol 23:356-363.*

151. Kagi, D., B. Ledermann, K. Burki, P. Seiler, B. Odermatt, K. J. Olsen, E. R. Podack, R. M. Zinkernagel, and H. Hengartner. 1994. Cytotoxicity mediated by T cells and natural killer cells is greatly impaired in perforin-deficient mice. *Nature 369:31-37.*

152. Stepp, S. E., R. Dufourcq-Lagelouse, F. Le Deist, S. Bhawan, S. Certain, P. A. Mathew, J. I. Henter, M. Bennett, A. Fischer, G. de Saint Basile, and V. Kumar. 1999. Perforin gene defects in familial hemophagocytic lymphohistiocytosis. *Science 286:1957-1959.*

153. van den Broek, M. F., D. Kagi, R. M. Zinkernagel, and H. Hengartner. 1995. Perforin dependence of natural killer cell-mediated tumor control in vivo. *Eur J Immunol 25:3514-3516.*

154. Street, S. E., E. Cretney, and M. J. Smyth. 2001. Perforin and interferon-gamma activities independently control tumor initiation, growth, and metastasis. *Blood 97:192-197.*

155. Street, S. E., Y. Hayakawa, Y. Zhan, A. M. Lew, D. MacGregor, A. M. Jamieson, A. Diefenbach, H. Yagita, D. I. Godfrey, and M. J. Smyth. 2004. Innate immune surveillance of spontaneous B cell lymphomas by natural killer cells and gammadelta T cells. *J Exp Med 199:879-884.*

156. Smyth, M. J., K. Y. Thia, S. E. Street, D. MacGregor, D. I. Godfrey, and J. A. Trapani. 2000. Perforin-mediated cytotoxicity is critical for surveillance of spontaneous lymphoma. *J Exp Med 192:755-760.*

157. Trapani, J. A., J. Davis, V. R. Sutton, and M. J. Smyth. 2000. Proapoptotic functions of cytotoxic lymphocyte granule constituents in vitro and in vivo. *Curr Opin Immunol 12:323-329.*

158. Trapani, J. A. 2001. Granzymes: a family of lymphocyte granule serine proteases. *Genome Biol 2:REVIEWS3014.*

159. Beresford, P. J., Z. Xia, A. H. Greenberg, and J. Lieberman. 1999. Granzyme A loading induces rapid cytolysis and a novel form of DNA damage independently of caspase activation. *Immunity 10:585-594*.

160. Sarin, A., M. S. Williams, M. A. Alexander-Miller, J. A. Berzofsky, C. M. Zacharchuk, and P. A. Henkart. 1997. Target cell lysis by CTL granule exocytosis is independent of ICE/Ced-3 family proteases. *Immunity 6:209-215.*

161. Trapani, J. A., D. A. Jans, P. J. Jans, M. J. Smyth, K. A. Browne, and V. R. Sutton. 1998. Efficient nuclear targeting of granzyme B and the nuclear consequences of apoptosis induced by granzyme B and perforin are caspase-dependent, but cell death is caspase-independent. *J Biol Chem 273:27934-27938.*

162. Barry, M., J. A. Heibein, M. J. Pinkoski, S. F. Lee, R. W. Moyer, D. R. Green, and R. C. Bleackley. 2000. Granzyme B short-circuits the need for caspase 8 activity during granule-mediated cytotoxic T-lymphocyte killing by directly cleaving Bid. *Mol Cell Biol 20:3781-3794.*

163. Heibein, J. A., I. S. Goping, M. Barry, M. J. Pinkoski, G. C. Shore, D. R. Green, and R. C. Bleackley. 2000. Granzyme B-mediated cytochrome c release is regulated by the Bcl-2 family members bid and Bax. *J Exp Med 192:1391-1402.*

164. Rosen, D., J. H. Li, S. Keidar, I. Markon, R. Orda, and G. Berke. 2000. Tumor immunity in perforin-deficient mice: a role for CD95 (Fas/APO-1). *J Immunol 164:3229-3235.*

165. Takeda, K., Y. Hayakawa, M. J. Smyth, N. Kayagaki, N. Yamaguchi, S. Kakuta, Y. Iwakura, H. Yagita, and K. Okumura. 2001. Involvement of tumor necrosis factor-related apoptosis-inducing ligand in surveillance of tumor metastasis by liver natural killer cells. *Nat Med 7:94-100.*

166. Taylor, M. A., B. Ward, J. D. Schatzle, and M. Bennett. 2002. Perforin- and Fas-dependent mechanisms of natural killer cell-mediated rejection of incompatible bone marrow cell grafts. *Eur J Immunol 32:793-799.*

167. Cretney, E., K. Takeda, H. Yagita, M. Glaccum, J. J. Peschon, and M. J. Smyth. 2002. Increased susceptibility to tumor initiation and metastasis in TNF-related apoptosis-inducing ligand-deficient mice. *J Immunol 168:1356-1361.*

168. Liu, C. C., C. M. Walsh, N. Eto, W. R. Clark, and J. D. Young. 1995. Morphologic and functional characterization of perforin-deficient lymphokine-activated killer cells. *J Immunol 155:602-608.*

169. Montel, A. H., M. R. Bochan, J. A. Hobbs, D. H. Lynch, and Z. Brahmi. 1995. Fas involvement in cytotoxicity mediated by human NK cells. *Cell Immunol 166:236-246*.

170. Kashii, Y., R. Giorda, R. B. Herberman, T. L. Whiteside, and N. L. Vujanovic. 1999. Constitutive expression and role of the TNF family ligands in apoptotic killing of tumor cells by human NK cells. *J Immunol 163:5358-5366*.

171. Arase, H., N. Arase, and T. Saito. 1995. Fas-mediated cytotoxicity by freshly isolated natural killer cells. *J Exp Med 181:1235-1238*.

172. Siegmund, D., A. Wicovsky, I. Schmitz, K. Schulze-Osthoff, S. Kreuz, M. Leverkus, O. Dittrich-Breiholz, M. Kracht, and H. Wajant. 2005. Death receptor-induced signaling pathways are differentially regulated by gamma interferon upstream of caspase 8 processing. *Mol Cell Biol 25:6363-6379*.

173. Kos, F. J., and E. G. Engleman. 1995. Requirement for natural killer cells in the induction of cytotoxic T cells. *J Immunol 155:578-584*.

174. Kos, F. J., and E. G. Engleman. 1996. Role of natural killer cells in the generation of influenza virus-specific cytotoxic T cells. *Cell Immunol 173:1-6*.

175. Biron, C. A., K. B. Nguyen, G. C. Pien, L. P. Cousens, and T. P. Salazar-Mather. 1999. Natural killer cells in antiviral defense: function and regulation by innate cytokines. *Annu Rev Immunol 17:189-220*.

176. Dorner, B. G., A. Scheffold, M. S. Rolph, M. B. Huser, S. H. Kaufmann, A. Radbruch, I. E. Flesch, and R. A. Kroczek. 2002. MIP-1alpha, MIP-1beta, RANTES, and ATAC/lymphotactin function together with IFN-gamma as type 1 cytokines. *Proc Natl Acad Sci U S A 99:6181-6186*.

177. Dorner, B. G., H. R. Smith, A. R. French, S. Kim, J. Poursine-Laurent, D. L. Beckman, J. T. Pingel, R. A. Kroczek, and W. M. Yokoyama. 2004. Coordinate expression of cytokines and chemokines by NK cells during murine cytomegalovirus infection. *J Immunol 172:3119-3131*.

178. Borg, C., A. Jalil, D. Laderach, K. Maruyama, H. Wakasugi, S. Charrier, B. Ryffel, A. Cambi, C. Figdor, W. Vainchenker, A. Galy, A. Caignard, and L. Zitvogel. 2004. NK cell activation by dendritic cells (DCs) requires the formation of a synapse leading to IL-12 polarization in DCs. *Blood 104:3267-3275*.

179. Dalod, M., T. Hamilton, R. Salomon, T. P. Salazar-Mather, S. C. Henry, J. D. Hamilton, and C. A. Biron. 2003. Dendritic cell responses to early murine cytomegalovirus infection: subset functional specialization and differential regulation by interferon alpha/beta. *J Exp Med 197:885-898*.

180. Gerosa, F., B. Baldani-Guerra, C. Nisii, V. Marchesini, G. Carra, and G. Trinchieri. 2002. Reciprocal activating interaction between natural killer cells and dendritic cells. *J Exp Med 195:327-333*.

181. Gerosa, F., A. Gobbi, P. Zorzi, S. Burg, F. Briere, G. Carra, and G. Trinchieri. 2005. The reciprocal interaction of NK cells with plasmacytoid or myeloid dendritic cells profoundly affects innate resistance functions. *J Immunol 174:727-734*.

182. Mailliard, R. B., S. M. Alber, H. Shen, S. C. Watkins, J. M. Kirkwood, R. B. Herberman, and P. Kalinski. 2005. IL-18-induced CD83+CCR7+ NK helper cells. *J Exp Med 202:941-953*.

183. Yu, Y., M. Hagihara, K. Ando, B. Gansuvd, H. Matsuzawa, T. Tsuchiya, Y. Ueda, H. Inoue, T. Hotta, and S. Kato. 2001. Enhancement of human cord blood CD34+ cell-derived NK cell cytotoxicity by dendritic cells. *J Immunol 166:1590-1600*.

184. Ferlazzo, G., M. Pack, D. Thomas, C. Paludan, D. Schmid, T. Strowig, G. Bougras, W. A. Muller, L. Moretta, and C. Munz. 2004. Distinct roles of IL-12 and IL-15 in human natural killer cell activation by dendritic cells from secondary lymphoid organs. *Proc Natl Acad Sci U S A 101:16606-16611*.

185. Fernandez, N. C., A. Lozier, C. Flament, P. Ricciardi-Castagnoli, D. Bellet, M. Suter, M. Perricaudet, T. Tursz, E. Maraskovsky, and L. Zitvogel. 1999. Dendritic cells directly trigger NK cell functions: cross-talk relevant in innate anti-tumor immune responses in vivo. *Nat Med 5:405-411*.

186. Granucci, F., I. Zanoni, N. Pavelka, S. L. Van Dommelen, C. E. Andoniou, F. Belardelli, M. A. Degli Esposti, and P. Ricciardi-Castagnoli. 2004. A contribution of mouse dendritic cell-derived IL-2 for NK cell activation. *J Exp Med 200:287-295*.

187. Jinushi, M., T. Takehara, T. Tatsumi, T. Kanto, V. Groh, T. Spies, T. Suzuki, T. Miyagi, and N. Hayashi. 2003. Autocrine/paracrine IL-15 that is required for type I IFN-mediated dendritic cell expression of MHC class I-related chain A and B is impaired in hepatitis C virus infection. *J Immunol 171:5423-5429*.

188. Koka, R., P. Burkett, M. Chien, S. Chai, D. L. Boone, and A. Ma. 2004. Cutting edge: murine dendritic cells require IL-15R alpha to prime NK cells. *J Immunol 173:3594-3598*.

189. Semino, C., G. Angelini, A. Poggi, and A. Rubartelli. 2005. NK/iDC interaction results in IL-18 secretion by DCs at the synaptic cleft followed by NK cell activation and release of the DC maturation factor HMGB1. *Blood 106:609-616*.

190. Piccioli, D., S. Sbrana, E. Melandri, and N. M. Valiante. 2002. Contact-dependent stimulation and inhibition of dendritic cells by natural killer cells. *J Exp Med 195:335-341*.

191. Vitale, M., M. Della Chiesa, S. Carlomagno, D. Pende, M. Arico, L. Moretta, and A. Moretta. 2005. NK-dependent DC maturation is mediated by TNFalpha and IFNgamma released upon engagement of the NKp30 triggering receptor. *Blood 106:566-571*.

192. Carbone, E., G. Terrazzano, G. Ruggiero, D. Zanzi, A. Ottaiano, C. Manzo, K. Karre, and S. Zappacosta. 1999. Recognition of autologous dendritic cells by human NK cells. *Eur J Immunol 29:4022-4029*.

193. Wilson, J. L., L. C. Heffler, J. Charo, A. Scheynius, M. T. Bejarano, and H. G. Ljunggren. 1999. Targeting of human dendritic cells by autologous NK cells. *J Immunol 163:6365-6370*.

194. Kondo, M., D. C. Scherer, A. G. King, M. G. Manz, and I. L. Weissman. 2001. Lymphocyte development from hematopoietic stem cells. *Curr Opin Genet Dev 11:520-526*.

195. Igarashi, H., S. C. Gregory, T. Yokota, N. Sakaguchi, and P. W. Kincade. 2002. Transcription from the RAG1 locus marks the earliest lymphocyte progenitors in bone marrow. *Immunity 17:117-130*.

196. Kondo, M., I. L. Weissman, and K. Akashi. 1997. Identification of clonogenic common lymphoid progenitors in mouse bone marrow. *Cell 91:661-672*.

197. Galy, A., M. Travis, D. Cen, and B. Chen. 1995. Human T, B, natural killer, and dendritic cells arise from a common bone marrow progenitor cell subset. *Immunity 3:459-473*.

198. Michie, A. M., J. R. Carlyle, T. M. Schmitt, B. Ljutic, S. K. Cho, Q. Fong, and J. C. Zuniga-Pflucker. 2000. Clonal characterization of a bipotent T cell and NK cell progenitor in the mouse fetal thymus. *J Immunol 164:1730-1733*.

199. Douagi, I., F. Colucci, J. P. Di Santo, and A. Cumano. 2002. Identification of the earliest prethymic bipotent T/NK progenitor in murine fetal liver. *Blood 99:463-471*.

200. Carlyle, J. R., A. M. Michie, S. K. Cho, and J. C. Zuniga-Pflucker. 1998. Natural killer cell development and function precede alpha beta T cell differentiation in mouse fetal thymic ontogeny. *J Immunol 160:744-753*.

201. Ikawa, T., H. Kawamoto, S. Fujimoto, and Y. Katsura. 1999. Commitment of common T/Natural killer (NK) progenitors to unipotent T and NK progenitors in the murine fetal thymus revealed by a single progenitor assay. *J Exp Med 190:1617-1626*.

202. Kim, S., K. Iizuka, H. S. Kang, A. Dokun, A. R. French, S. Greco, and W. M. Yokoyama. 2002. In vivo developmental stages in murine natural killer cell maturation. *Nat Immunol 3:523-528*.

203. Rosmaraki, E. E., I. Douagi, C. Roth, F. Colucci, A. Cumano, and J. P. Di Santo. 2001. Identification of committed NK cell progenitors in adult murine bone marrow. *Eur J Immunol 31:1900-1909*.

204. Kennedy, M. K., M. Glaccum, S. N. Brown, E. A. Butz, J. L. Viney, M. Embers, N. Matsuki, K. Charrier, L. Sedger, C. R. Willis, K. Brasel, P. J. Morrissey, K. Stocking, J. C. Schuh, S. Joyce, and J. J. Peschon. 2000. Reversible defects in natural killer and memory CD8 T cell lineages in interleukin 15-deficient mice. *J Exp Med 191:771-780*.

205. Suzuki, H., G. S. Duncan, H. Takimoto, and T. W. Mak. 1997. Abnormal development of intestinal intraepithelial lymphocytes and peripheral natural killer cells in mice lacking the IL-2 receptor beta chain. *J Exp Med 185:499-505*.

206. Imada, K., E. T. Bloom, H. Nakajima, J. A. Horvath-Arcidiacono, G. B. Udy, H. W. Davey, and W. J. Leonard. 1998. Stat5b is essential for natural killer cell-mediated proliferation and cytolytic activity. *J Exp Med 188:2067-2074*.

207. Liu, L., J. E. Damen, R. L. Cutler, and G. Krystal. 1994. Multiple cytokines stimulate the binding of a common 145-kilodalton protein to Shc at the Grb2 recognition site of Shc. *Mol Cell Biol 14:6926-6935*.

208. Damen, J. E., L. Liu, P. Rosten, R. K. Humphries, A. B. Jefferson, P. W. Majerus, and G. Krystal. 1996. The 145-kDa protein induced to associate with Shc by multiple cytokines is an inositol tetraphosphate and phosphatidylinositol 3,4,5-triphosphate 5-phosphatase. *Proc Natl Acad Sci U S A 93:1689-1693*.

209. Lioubin, M. N., P. A. Algate, S. Tsai, K. Carlberg, A. Aebersold, and L. R. Rohrschneider. 1996. p150Ship, a signal transduction molecule with inositol polyphosphate-5-phosphatase activity. *Genes Dev 10:1084-1095*.

210. Kavanaugh, W. M., D. A. Pot, S. M. Chin, M. Deuter-Reinhard, A. B. Jefferson, F. A. Norris, F. R. Masiarz, L. S. Cousens, P. W. Majerus, and L. T. Williams. 1996. Multiple forms of an inositol polyphosphate 5-phosphatase form signaling complexes with Shc and Grb2. *Curr Biol 6:438-445.*

211. Kerr, W. G., M. Heller, and L. A. Herzenberg. 1996. Analysis of lipopolysaccharide-response genes in B-lineage cells demonstrates that they can have differentiation stage-restricted expression and contain SH2 domains. *Proc Natl Acad Sci U S A 93:3947-3952.*

212. Osborne, M. A., G. Zenner, M. Lubinus, X. Zhang, Z. Songyang, L. C. Cantley, P. Majerus, P. Burn, and J. P. Kochan. 1996. The inositol 5'-phosphatase SHIP binds to immunoreceptor signaling motifs and responds to high affinity IgE receptor aggregation. *J Biol Chem 271:29271-29278.*

213. Moran, M. F., C. A. Koch, D. Anderson, C. Ellis, L. England, G. S. Martin, and T. Pawson. 1990. Src homology region 2 domains direct protein-protein interactions in signal transduction. *Proc Natl Acad Sci U S A 87:8622-8626.*

214. Ellis, C., M. Moran, F. McCormick, and T. Pawson. 1990. Phosphorylation of GAP and GAP-associated proteins by transforming and mitogenic tyrosine kinases. *Nature 343:377-381.*

215. Ono, M., S. Bolland, P. Tempst, and J. V. Ravetch. 1996. Role of the inositol phosphatase SHIP in negative regulation of the immune system by the receptor Fc(gamma)RIIB. *Nature 383:263-266.*

216. Wang, J. W., J. M. Howson, T. Ghansah, C. Desponts, J. M. Ninos, S. L. May, K. H. Nguyen, N. Toyama-Sorimachi, and W. G. Kerr. 2002. Influence of SHIP on the NK repertoire and allogeneic bone marrow transplantation. *Science 295:2094-2097.*

217. Tessmer, M. S., C. Fugere, F. Stevenaert, O. V. Naidenko, H. J. Chong, G. Leclercq, and L. Brossay. 2007. KLRG1 binds cadherins and preferentially associates with SHIP-1. *Int Immunol 19:391-400.*

218. Tridandapani, S., T. Kelley, M. Pradhan, D. Cooney, L. B. Justement, and K. M. Coggeshall. 1997. Recruitment and phosphorylation of SH2-containing inositol phosphatase and Shc to the B-cell Fc gamma immunoreceptor tyrosine-based inhibition motif peptide motif. *Mol Cell Biol 17:4305-4311.*

219. Galandrini, R., I. Tassi, G. Mattia, L. Lenti, M. Piccoli, L. Frati, and A. Santoni. 2002. SH2-containing inositol phosphatase (SHIP-1) transiently translocates to raft domains and modulates CD16-mediated cytotoxicity in human NK cells. *Blood 100:4581-4589.*

220. Liu, L., J. E. Damen, M. R. Hughes, I. Babic, F. R. Jirik, and G. Krystal. 1997. The Src homology 2 (SH2) domain of SH2-containing inositol phosphatase (SHIP) is essential for tyrosine phosphorylation of SHIP, its association with Shc, and its induction of apoptosis. *J Biol Chem 272:8983-8988*.

221. Sattler, M., R. Salgia, G. Shrikhande, S. Verma, J. L. Choi, L. R. Rohrschneider, and J. D. Griffin. 1997. The phosphatidylinositol polyphosphate 5-phosphatase SHIP and the protein tyrosine phosphatase SHP-2 form a complex in hematopoietic cells which can be regulated by BCR/ABL and growth factors. *Oncogene 15:2379-2384*.

222. Huber, M., C. D. Helgason, J. E. Damen, M. P. Scheid, V. Duronio, V. Lam, R. K. Humphries, and G. Krystal. 1999. The role of the SRC homology 2-containing inositol 5'-phosphatase in Fc epsilon R1-induced signaling. *Curr Top Microbiol Immunol 244:29-41*.

223. Valderrama-Carvajal, H., E. Cocolakis, A. Lacerte, E. H. Lee, G. Krystal, S. Ali, and J. J. Lebrun. 2002. Activin/TGF-beta induce apoptosis through Smad-dependent expression of the lipid phosphatase SHIP. *Nat Cell Biol 4:963-969*.

224. Burgering, B. M., and P. J. Coffer. 1995. Protein kinase B (c-Akt) in phosphatidylinositol-3-OH kinase signal transduction. *Nature 376:599-602*.

225. Franke, T. F., S. I. Yang, T. O. Chan, K. Datta, A. Kazlauskas, D. K. Morrison, D. R. Kaplan, and P. N. Tsichlis. 1995. The protein kinase encoded by the Akt proto-oncogene is a target of the PDGF-activated phosphatidylinositol 3-kinase. *Cell 81:727-736*.

226. Yao, R., and G. M. Cooper. 1995. Requirement for phosphatidylinositol-3 kinase in the prevention of apoptosis by nerve growth factor. *Science 267:2003-2006*.

227. Downward, J. 1998. Lipid-regulated kinases: some common themes at last. *Science 279:673-674*.

228. Freeburn, R. W., K. L. Wright, S. J. Burgess, E. Astoul, D. A. Cantrell, and S. G. Ward. 2002. Evidence that SHIP-1 contributes to phosphatidylinositol 3,4,5-trisphosphate metabolism in T lymphocytes and can regulate novel phosphoinositide 3-kinase effectors. *J Immunol 169:5441-5450*.

229. Sattler, M., S. Verma, C. H. Byrne, G. Shrikhande, T. Winkler, P. A. Algate, L. R. Rohrschneider, and J. D. Griffin. 1999. BCR/ABL directly inhibits expression of SHIP, an SH2-containing polyinositol-5-phosphatase involved in the regulation of hematopoiesis. *Mol Cell Biol 19:7473-7480*.

230. Brauweiler, A., I. Tamir, J. Dal Porto, R. J. Benschop, C. D. Helgason, R. K. Humphries, J. H. Freed, and J. C. Cambier. 2000. Differential regulation of B cell development, activation, and death by the src homology 2 domain-containing 5' inositol phosphatase (SHIP). *J Exp Med 191:1545-1554.*

231. van der Geer, P., and T. Pawson. 1995. The PTB domain: a new protein module implicated in signal transduction. *Trends Biochem Sci 20:277-280.*

232. Laminet, A. A., G. Apell, L. Conroy, and W. M. Kavanaugh. 1996. Affinity, specificity, and kinetics of the interaction of the SHC phosphotyrosine binding domain with asparagine-X-X-phosphotyrosine motifs of growth factor receptors. *J Biol Chem 271:264-269.*

233. van der Geer, P., S. Wiley, G. D. Gish, V. K. Lai, R. Stephens, M. F. White, D. Kaplan, and T. Pawson. 1996. Identification of residues that control specific binding of the Shc phosphotyrosine-binding domain to phosphotyrosine sites. *Proc Natl Acad Sci U S A 93:963-968.*

234. Carlberg, K., and L. R. Rohrschneider. 1997. Characterization of a novel tyrosine phosphorylated 100-kDa protein that binds to SHP-2 and phosphatidylinositol 3'-kinase in myeloid cells. *J Biol Chem 272:15943-15950.*

235. Zhang, S., and H. E. Broxmeyer. 1999. p85 subunit of PI3 kinase does not bind to human Flt3 receptor, but associates with SHP2, SHIP, and a tyrosine-phosphorylated 100-kDa protein in Flt3 ligand-stimulated hematopoietic cells. *Biochem Biophys Res Commun 254:440-445.*

236. Gupta, N., A. M. Scharenberg, D. A. Fruman, L. C. Cantley, J. P. Kinet, and E. O. Long. 1999. The SH2 domain-containing inositol 5'-phosphatase (SHIP) recruits the p85 subunit of phosphoinositide 3-kinase during FcgammaRIIb1-mediated inhibition of B cell receptor signaling. *J Biol Chem 274:7489-7494.*

237. Rohrschneider, L. R., J. F. Fuller, I. Wolf, Y. Liu, and D. M. Lucas. 2000. Structure, function, and biology of SHIP proteins. *Genes Dev 14:505-520.*

238. Wisniewski, D., A. Strife, S. Swendeman, H. Erdjument-Bromage, S. Geromanos, W. M. Kavanaugh, P. Tempst, and B. Clarkson. 1999. A novel SH2-containing phosphatidylinositol 3,4,5-trisphosphate 5-phosphatase (SHIP2) is constitutively tyrosine phosphorylated and associated with src homologous and collagen gene (SHC) in chronic myelogenous leukemia progenitor cells. *Blood 93:2707-2720.*

239. Kalesnikoff, J., L. M. Sly, M. R. Hughes, T. Buchse, M. J. Rauh, L. P. Cao, V. Lam, A. Mui, M. Huber, and G. Krystal. 2003. The role of SHIP in cytokine-induced signaling. *Rev Physiol Biochem Pharmacol 149:87-103.*

240. Rameh, L. E., and L. C. Cantley. 1999. The role of phosphoinositide 3-kinase lipid products in cell function. *J Biol Chem 274:8347-8350.*

241. Marshall, A. J., H. Niiro, T. J. Yun, and E. A. Clark. 2000. Regulation of B-cell activation and differentiation by the phosphatidylinositol 3-kinase and phospholipase Cgamma pathway. *Immunol Rev 176:30-46.*

242. Scheid, M. P., M. Huber, J. E. Damen, M. Hughes, V. Kang, P. Neilsen, G. D. Prestwich, G. Krystal, and V. Duronio. 2002. Phosphatidylinositol (3,4,5)P3 is essential but not sufficient for protein kinase B (PKB) activation; phosphatidylinositol (3,4)P2 is required for PKB phosphorylation at Ser-473: studies using cells from SH2-containing inositol-5-phosphatase knockout mice. *J Biol Chem 277:9027-9035.*

243. Hejna, J. A., H. Saito, L. S. Merkens, T. V. Tittle, P. M. Jakobs, M. A. Whitney, M. Grompe, A. S. Friedberg, and R. E. Moses. 1995. Cloning and characterization of a human cDNA (INPPL1) sharing homology with inositol polyphosphate phosphatases. *Genomics 29:285-287.*

244. Pesesse, X., S. Deleu, F. De Smedt, L. Drayer, and C. Erneux. 1997. Identification of a second SH2-domain-containing protein closely related to the phosphatidylinositol polyphosphate 5-phosphatase SHIP. *Biochem Biophys Res Commun 239:697-700.*

245. Bruyns, C., X. Pesesse, C. Moreau, D. Blero, and C. Erneux. 1999. The two SH2-domain-containing inositol 5-phosphatases SHIP1 and SHIP2 are coexpressed in human T lymphocytes. *Biol Chem 380:969-974.*

246. Muraille, E., X. Pesesse, C. Kuntz, and C. Erneux. 1999. Distribution of the src-homology-2-domain-containing inositol 5-phosphatase SHIP-2 in both non-haemopoietic and haemopoietic cells and possible involvement of SHIP-2 in negative signalling of B-cells. *Biochem J 342 Pt 3:697-705.*

247. Tu, Z., J. M. Ninos, Z. Ma, J. W. Wang, M. P. Lemos, C. Desponts, T. Ghansah, J. M. Howson, and W. G. Kerr. 2001. Embryonic and hematopoietic stem cells express a novel SH2-containing inositol 5'-phosphatase isoform that partners with the Grb2 adapter protein. *Blood 98:2028-2038.*

248. Rohrschneider, L. R., J. M. Custodio, T. A. Anderson, C. P. Miller, and H. Gu. 2005. The intron 5/6 promoter region of the ship1 gene regulates expression in stem/progenitor cells of the mouse embryo. *Dev Biol.*

249. Helgason, C. D., J. E. Damen, P. Rosten, R. Grewal, P. Sorensen, S. M. Chappel, A. Borowski, F. Jirik, G. Krystal, and R. K. Humphries. 1998. Targeted disruption

of SHIP leads to hemopoietic perturbations, lung pathology, and a shortened life span. *Genes Dev 12:1610-1620.*

250. Karlsson, M. C., R. Guinamard, S. Bolland, M. Sankala, R. M. Steinman, and J. V. Ravetch. 2003. Macrophages control the retention and trafficking of B lymphocytes in the splenic marginal zone. *J Exp Med 198:333-340.*

251. Liu, Q., F. Shalaby, J. Jones, D. Bouchard, and D. J. Dumont. 1998. The SH2-containing inositol polyphosphate 5-phosphatase, ship, is expressed during hematopoiesis and spermatogenesis. *Blood 91:2753-2759.*

252. Huber, M., C. D. Helgason, J. E. Damen, L. Liu, R. K. Humphries, and G. Krystal. 1998. The src homology 2-containing inositol phosphatase (SHIP) is the gatekeeper of mast cell degranulation. *Proc Natl Acad Sci U S A 95:11330-11335.*

253. Liu, Q., T. Sasaki, I. Kozieradzki, A. Wakeham, A. Itie, D. J. Dumont, and J. M. Penninger. 1999. SHIP is a negative regulator of growth factor receptor-mediated PKB/Akt activation and myeloid cell survival. *Genes Dev 13:786-791.*

254. Ghansah, T., K. H. Paraiso, S. Highfill, C. Desponts, S. May, J. K. McIntosh, J. W. Wang, J. Ninos, J. Brayer, F. Cheng, E. Sotomayor, and W. G. Kerr. 2004. Expansion of myeloid suppressor cells in SHIP-deficient mice represses allogeneic T cell responses. *J Immunol 173:7324-7330.*

255. Paraiso, K. H., T. Ghansah, A. Costello, R. W. Engelman, and W. G. Kerr. 2007. Induced SHIP deficiency expands myeloid regulatory cells and abrogates graft-versus-host disease. *J Immunol 178:2893-2900.*

256. Kiener, P. A., M. N. Lioubin, L. R. Rohrschneider, J. A. Ledbetter, S. G. Nadler, and M. L. Diegel. 1997. Co-ligation of the antigen and Fc receptors gives rise to the selective modulation of intracellular signaling in B cells. Regulation of the association of phosphatidylinositol 3-kinase and inositol 5'-phosphatase with the antigen receptor complex. *J Biol Chem 272:3838-3844.*

257. Galandrini, R., I. Tassi, S. Morrone, L. Lanfrancone, P. Pelicci, M. Piccoli, L. Frati, and A. Santoni. 2001. The adaptor protein shc is involved in the negative regulation of NK cell-mediated cytotoxicity. *Eur J Immunol 31:2016-2025.*

258. Alter, G., T. J. Suscovich, M. Kleyman, N. Teigen, H. Streeck, M. T. Zaman, A. Meier, and M. Altfeld. 2006. Low perforin and elevated SHIP-1 expression is associated with functional anergy of natural killer cells in chronic HIV-1 infection. *Aids 20:1549-1551.*

259. Lanier, L. L. 1998. Activating and inhibitory NK cell receptors. *Adv Exp Med Biol 452:13-18.*

260. Yokoyama, W. M. 1998. Natural killer cell receptors. *Curr Opin Immunol 10:298-305*.

261. Raulet, D. H. 1999. Development and tolerance of natural killer cells. *Curr Opin Immunol 11:129-134*.

262. Iizuka, K., O. V. Naidenko, B. F. Plougastel, D. H. Fremont, and W. M. Yokoyama. 2003. Genetically linked C-type lectin-related ligands for the NKRP1 family of natural killer cell receptors. *Nat Immunol 4:801-807*.

263. Sidorenko, S. P., and E. A. Clark. 2003. The dual-function CD150 receptor subfamily: the viral attraction. *Nat Immunol 4:19-24*.

264. Engel, P., M. J. Eck, and C. Terhorst. 2003. The SAP and SLAM families in immune responses and X-linked lymphoproliferative disease. *Nat Rev Immunol 3:813-821*.

265. Bloch-Queyrat, C., M. C. Fondaneche, R. Chen, L. Yin, F. Relouzat, A. Veillette, A. Fischer, and S. Latour. 2005. Regulation of natural cytotoxicity by the adaptor SAP and the Src-related kinase Fyn. *J Exp Med 202:181-192*.

266. Tassi, I., R. Presti, S. Kim, W. M. Yokoyama, S. Gilfillan, and M. Colonna. 2005. Phospholipase C-gamma 2 is a critical signaling mediator for murine NK cell activating receptors. *J Immunol 175:749-754*.

267. Shimizu, E., J. Koike, H. Wakao, K. Seino, H. Koseki, T. Kakiuchi, T. Nakayama, and M. Taniguchi. 2004. Role of a NK receptor, KLRE-1, in bone marrow allograft rejection: analysis with KLRE-1-deficient mice. *Blood 104:781-783*.

268. Mooney, J. M., J. Klem, C. Wulfing, L. A. Mijares, P. L. Schwartzberg, M. Bennett, and J. D. Schatzle. 2004. The murine NK receptor 2B4 (CD244) exhibits inhibitory function independent of signaling lymphocytic activation molecule-associated protein expression. *J Immunol 173:3953-3961*.

269. Carlyle, J. R., A. M. Jamieson, S. Gasser, C. S. Clingan, H. Arase, and D. H. Raulet. 2004. Missing self-recognition of Ocil/Clr-b by inhibitory NKR-P1 natural killer cell receptors. *Proc Natl Acad Sci U S A 101:3527-3532*.

270. Mandelboim, O., N. Lieberman, M. Lev, L. Paul, T. I. Arnon, Y. Bushkin, D. M. Davis, J. L. Strominger, J. W. Yewdell, and A. Porgador. 2001. Recognition of haemagglutinins on virus-infected cells by NKp46 activates lysis by human NK cells. *Nature 409:1055-1060*.

271. Lanier, L. L. 2003. Natural killer cell receptor signaling. *Curr Opin Immunol 15:308-314.*

272. Stewart, C. A., E. Vivier, and M. Colonna. 2006. Strategies of natural killer cell recognition and signaling. *Curr Top Microbiol Immunol 298:1-21.*

273. MacFarlane, A. W. t., and K. S. Campbell. 2006. Signal transduction in natural killer cells. *Curr Top Microbiol Immunol 298:23-57.*

274. Sjolin, H., E. Tomasello, M. Mousavi-Jazi, A. Bartolazzi, K. Karre, E. Vivier, and C. Cerboni. 2002. Pivotal role of KARAP/DAP12 adaptor molecule in the natural killer cell-mediated resistance to murine cytomegalovirus infection. *J Exp Med 195:825-834.*

275. Billadeau, D. D., K. M. Brumbaugh, C. J. Dick, R. A. Schoon, X. R. Bustelo, and P. J. Leibson. 1998. The Vav-Rac1 pathway in cytotoxic lymphocytes regulates the generation of cell-mediated killing. *J Exp Med 188:549-559.*

276. Galandrini, R., G. Palmieri, M. Piccoli, L. Frati, and A. Santoni. 1999. Role for the Rac1 exchange factor Vav in the signaling pathways leading to NK cell cytotoxicity. *J Immunol 162:3148-3152.*

277. Takeshita, S., N. Namba, J. J. Zhao, Y. Jiang, H. K. Genant, M. J. Silva, M. D. Brodt, C. D. Helgason, J. Kalesnikoff, M. J. Rauh, R. K. Humphries, G. Krystal, S. L. Teitelbaum, and F. P. Ross. 2002. SHIP-deficient mice are severely osteoporotic due to increased numbers of hyper-resorptive osteoclasts. *Nat Med 8:943-949.*

278. Mason, L. H., P. Gosselin, S. K. Anderson, W. E. Fogler, J. R. Ortaldo, and D. W. McVicar. 1997. Differential tyrosine phosphorylation of inhibitory versus activating Ly-49 receptor proteins and their recruitment of SHP-1 phosphatase. *J Immunol 159:4187-4196.*

279. Gupta, N., A. M. Scharenberg, D. N. Burshtyn, N. Wagtmann, M. N. Lioubin, L. R. Rohrschneider, J. P. Kinet, and E. O. Long. 1997. Negative signaling pathways of the killer cell inhibitory receptor and Fc gamma RIIb1 require distinct phosphatases. *J Exp Med 186:473-478.*

280. Wahle, J. A., K. H. Paraiso, A. L. Costello, E. L. Goll, C. L. Sentman, and W. G. Kerr. 2006. Cutting edge: dominance by an MHC-independent inhibitory receptor compromises NK killing of complex targets. *J Immunol 176:7165-7169.*

281. Chen, L., S. S. Sung, M. L. Yip, H. R. Lawrence, Y. Ren, W. C. Guida, S. M. Sebti, N. J. Lawrence, and J. Wu. 2006. Discovery of a novel shp2 protein tyrosine phosphatase inhibitor. *Mol Pharmacol 70:562-570.*

282. Chuang, S. S., J. K. Lee, and P. A. Mathew. 2003. Protein kinase C is involved in 2B4 (CD244)-mediated cytotoxicity and AP-1 activation in natural killer cells. *Immunology 109:432-439.*

283. Thebault, S., and J. Ochoa-Garay. 2004. Characterization of TCR-induced phosphorylation of PKCtheta in primary murine lymphocytes. *Mol Immunol 40:931-942.*

284. Nedvetzki, S., S. Sowinski, R. A. Eagle, J. Harris, F. Vely, D. Pende, J. Trowsdale, E. Vivier, S. Gordon, and D. M. Davis. 2007. Reciprocal regulation of natural killer cells and macrophages associated with distinct immune synapses. *Blood.*

285. Roda-Navarro, P., M. Mittelbrunn, M. Ortega, D. Howie, C. Terhorst, F. Sanchez-Madrid, and E. Fernandez-Ruiz. 2004. Dynamic redistribution of the activating 2B4/SAP complex at the cytotoxic NK cell immune synapse. *J Immunol 173:3640-3646.*

286. Gordon, J. A. 1991. Use of vanadate as protein-phosphotyrosine phosphatase inhibitor. *Methods Enzymol 201:477-482.*

287. Carninci, P., T. Kasukawa, S. Katayama, J. Gough, M. C. Frith, N. Maeda, R. Oyama, T. Ravasi, B. Lenhard, C. Wells, R. Kodzius, K. Shimokawa, V. B. Bajic, S. E. Brenner, S. Batalov, A. R. Forrest, M. Zavolan, M. J. Davis, L. G. Wilming, V. Aidinis, J. E. Allen, A. Ambesi-Impiombato, R. Apweiler, R. N. Aturaliya, T. L. Bailey, M. Bansal, L. Baxter, K. W. Beisel, T. Bersano, H. Bono, A. M. Chalk, K. P. Chiu, V. Choudhary, A. Christoffels, D. R. Clutterbuck, M. L. Crowe, E. Dalla, B. P. Dalrymple, B. de Bono, G. Della Gatta, D. di Bernardo, T. Down, P. Engstrom, M. Fagiolini, G. Faulkner, C. F. Fletcher, T. Fukushima, M. Furuno, S. Futaki, M. Gariboldi, P. Georgii-Hemming, T. R. Gingeras, T. Gojobori, R. E. Green, S. Gustincich, M. Harbers, Y. Hayashi, T. K. Hensch, N. Hirokawa, D. Hill, L. Huminiecki, M. Iacono, K. Ikeo, A. Iwama, T. Ishikawa, M. Jakt, A. Kanapin, M. Katoh, Y. Kawasawa, J. Kelso, H. Kitamura, H. Kitano, G. Kollias, S. P. Krishnan, A. Kruger, S. K. Kummerfeld, I. V. Kurochkin, L. F. Lareau, D. Lazarevic, L. Lipovich, J. Liu, S. Liuni, S. McWilliam, M. Madan Babu, M. Madera, L. Marchionni, H. Matsuda, S. Matsuzawa, H. Miki, F. Mignone, S. Miyake, K. Morris, S. Mottagui-Tabar, N. Mulder, N. Nakano, H. Nakauchi, P. Ng, R. Nilsson, S. Nishiguchi, S. Nishikawa, et al. 2005. The transcriptional landscape of the mammalian genome. *Science 309:1559-1563.*

288. Okazaki, Y., M. Furuno, T. Kasukawa, J. Adachi, H. Bono, S. Kondo, I. Nikaido, N. Osato, R. Saito, H. Suzuki, I. Yamanaka, H. Kiyosawa, K. Yagi, Y. Tomaru, Y. Hasegawa, A. Nogami, C. Schonbach, T. Gojobori, R. Baldarelli, D. P. Hill, C. Bult, D. A. Hume, J. Quackenbush, L. M. Schriml, A. Kanapin, H. Matsuda, S.

Batalov, K. W. Beisel, J. A. Blake, D. Bradt, V. Brusic, C. Chothia, L. E. Corbani, S. Cousins, E. Dalla, T. A. Dragani, C. F. Fletcher, A. Forrest, K. S. Frazer, T. Gaasterland, M. Gariboldi, C. Gissi, A. Godzik, J. Gough, S. Grimmond, S. Gustincich, N. Hirokawa, I. J. Jackson, E. D. Jarvis, A. Kanai, H. Kawaji, Y. Kawasawa, R. M. Kedzierski, B. L. King, A. Konagaya, I. V. Kurochkin, Y. Lee, B. Lenhard, P. A. Lyons, D. R. Maglott, L. Maltais, L. Marchionni, L. McKenzie, H. Miki, T. Nagashima, K. Numata, T. Okido, W. J. Pavan, G. Pertea, G. Pesole, N. Petrovsky, R. Pillai, J. U. Pontius, D. Qi, S. Ramachandran, T. Ravasi, J. C. Reed, D. J. Reed, J. Reid, B. Z. Ring, M. Ringwald, A. Sandelin, C. Schneider, C. A. Semple, M. Setou, K. Shimada, R. Sultana, Y. Takenaka, M. S. Taylor, R. D. Teasdale, M. Tomita, R. Verardo, L. Wagner, C. Wahlestedt, Y. Wang, Y. Watanabe, C. Wells, L. G. Wilming, A. Wynshaw-Boris, M. Yanagisawa, et al. 2002. Analysis of the mouse transcriptome based on functional annotation of 60,770 full-length cDNAs. *Nature 420:563-573*.

289. Hanna, J., P. Bechtel, Y. Zhai, F. Youssef, K. McLachlan, and O. Mandelboim. 2004. Novel insights on human NK cells' immunological modalities revealed by gene expression profiling. *J Immunol 173:6547-6563*.

290. Lueking, A., M. Horn, H. Eickhoff, K. Bussow, H. Lehrach, and G. Walter. 1999. Protein microarrays for gene expression and antibody screening. *Anal Biochem 270:103-111*.

291. MacBeath, G., and S. L. Schreiber. 2000. Printing proteins as microarrays for high-throughput function determination. *Science 289:1760-1763*.

292. Zhu, H., and M. Snyder. 2001. Protein arrays and microarrays. *Curr Opin Chem Biol 5:40-45*.

293. Arenkov, P., A. Kukhtin, A. Gemmell, S. Voloshchuk, V. Chupeeva, and A. Mirzabekov. 2000. Protein microchips: use for immunoassay and enzymatic reactions. *Anal Biochem 278:123-131*.

294. http://www.pepscan.nl.

295. Diks, S. H., K. Kok, T. O'Toole, D. W. Hommes, P. van Dijken, J. Joore, and M. P. Peppelenbosch. 2004. Kinome profiling for studying lipopolysaccharide signal transduction in human peripheral blood mononuclear cells. *J Biol Chem 279:49206-49213*.

296. Lodolce, J. P., D. L. Boone, S. Chai, R. E. Swain, T. Dassopoulos, S. Trettin, and A. Ma. 1998. IL-15 receptor maintains lymphoid homeostasis by supporting lymphocyte homing and proliferation. *Immunity 9:669-676*.

297. Eswarakumar, V. P., I. Lax, and J. Schlessinger. 2005. Cellular signaling by fibroblast growth factor receptors. *Cytokine Growth Factor Rev 16:139-149.*

298. Li, Q., and I. M. Verma. 2002. NF-kappaB regulation in the immune system. *Nat Rev Immunol 2:725-734.*

299. Beinke, S., M. J. Robinson, M. Hugunin, and S. C. Ley. 2004. Lipopolysaccharide activation of the TPL-2/MEK/extracellular signal-regulated kinase mitogen-activated protein kinase cascade is regulated by IkappaB kinase-induced proteolysis of NF-kappaB1 p105. *Mol Cell Biol 24:9658-9667.*

300. Routes, J. M., H. Li, S. T. Bayley, S. Ryan, and D. J. Klemm. 1996. Inhibition of IFN-stimulated gene expression and IFN induction of cytolytic resistance to natural killer cell lysis correlate with E1A-p300 binding. *J Immunol 156:1055-1061.*

301. Jamieson, A. M., P. Isnard, J. R. Dorfman, M. C. Coles, and D. H. Raulet. 2004. Turnover and proliferation of NK cells in steady state and lymphopenic conditions. *J Immunol 172:864-870.*

302. Taylor, W. R., A. H. Schonthal, J. Galante, and G. R. Stark. 2001. p130/E2F4 binds to and represses the cdc2 promoter in response to p53. *J Biol Chem 276:1998-2006.*

303. Taylor, W. R., and G. R. Stark. 2001. Regulation of the G2/M transition by p53. *Oncogene 20:1803-1815.*

Appendices

Appendix A

Table A-1. NK kinome. The total kinome for both mature NK (NK) and immature NK (iNK) with all 6 replicates present. Values have been normalized.

Spot	SEQUENCE	NK1	NK2	NK3	NK4	NK5	NK6	iNK1	iNK2	iNK3	iNK4	iNK5	iNK6
1	HEYIYVDPM	1.35	0.50	0.76	0.50	1.14	0.70	0.64	0.59	0.53	0.73	0.71	0.86
2	KRPSVRAKA	2.95	1.57	2.50	2.19	1.86	0.86	1.47	1.50	1.49	1.66	1.70	1.18
3	EDNEYTARQ	1.68	0.49	1.14	0.39	1.58	0.35	0.61	0.42	0.78	0.53	1.15	0.47
4	ENKLYGMSD	0.71	0.38	0.53	0.24	1.05	0.36	0.45	0.35	0.35	0.36	0.81	0.44
5	HRLLTLDPV	0.64	0.29	0.44	0.22	1.23	0.26	0.63	0.32	0.56	0.44	0.61	0.66
6	LEKKYVRRD	0.67	0.34	0.36	0.29	1.13	0.45	0.61	0.27	0.55	0.45	0.46	0.35
7	PYKFPSSPLRIPGZ	0.62	0.27	0.37	0.38	1.35	0.36	0.56	0.41	0.43	0.37	0.44	0.26
8	GKRQTEREK	1.33	0.58	0.37	0.41	0.95	0.64	0.90	0.56	0.61	0.72	0.60	0.60
9	APATSPKAE	0.92	0.24	0.54	0.28	1.36	0.38	0.60	0.34	0.29	0.34	0.64	0.55
10	DEEESEQGA	1.63	0.33	0.99	0.27	1.33	0.44	0.59	0.37	0.90	0.45	0.69	0.53
11	FFRRSKIAV	1.09	0.47	0.70	0.38	1.38	0.45	0.71	0.61	0.71	0.73	0.74	0.46
12	GMTEYVATR	0.81	0.38	0.47	0.23	0.96	0.25	0.69	0.35	0.43	0.43	0.50	0.45
13	EAALYKNLL	0.66	0.36	0.55	0.17	1.02	0.44	0.49	0.40	0.46	0.50	0.47	0.33
14	ELILSPRSK	1.18	0.42	0.67	0.51	1.17	0.37	0.82	0.70	0.81	1.09	0.63	0.40
15	RRAVSEQDA	0.63	0.24	0.45	0.23	0.73	0.30	0.58	0.22	0.23	0.32	0.31	0.46
16	SEDNSEDEI	1.78	0.51	1.18	0.34	1.12	0.33	0.73	0.38	0.52	0.64	0.81	0.47
17	PASLSRAKA	1.60	0.79	0.70	0.50	1.17	0.54	0.87	0.80	0.73	0.80	0.94	0.84
18	RRASL	2.51	1.26	1.84	1.09	1.99	0.57	1.43	1.43	1.19	1.27	2.72	0.65
19	AKKMSTYNV	1.33	0.81	1.01	0.94	1.13	0.56	0.96	0.87	1.39	1.26	0.78	0.72
20	DDINSYEAW	1.71	0.61	1.17	0.63	1.57	0.35	0.55	0.66	1.61	0.90	1.05	0.56
21	ETRFTDTRK	1.26	0.48	0.81	0.56	1.35	0.46	0.92	0.65	0.94	1.01	0.86	0.43
22	QLSTSEENS	0.70	0.27	0.39	0.36	0.48	0.34	0.59	0.32	0.39	0.40	0.41	0.40
23	SPRKSPKKS	2.15	1.30	1.25	1.02	0.88	0.76	1.35	1.81	1.10	1.20	0.98	0.95
24	RRKASGP	1.69	1.00	0.99	0.89	0.92	0.64	1.18	1.01	1.12	0.97	0.97	0.83
25	RQLRSPRRT	2.57	1.27	1.93	1.95	2.42	1.08	1.55	1.65	2.34	1.61	2.97	1.22
26	SAVASNMRD	0.89	0.43	0.46	0.41	0.84	0.36	0.60	0.47	0.49	0.38	0.64	0.33
27	RTPPPSG	1.13	0.54	0.49	0.37	0.81	0.34	0.76	0.46	0.47	0.60	0.56	0.36
28	LRRAS	1.51	0.73	1.10	0.93	1.31	0.60	1.00	0.76	0.83	1.07	1.17	0.57
29	SSTGSIDMV	0.92	0.34	0.71	0.37	0.45	0.27	0.63	0.33	0.37	0.43	0.26	0.43
30	TLASSFKRR	3.62	2.66	2.95	2.41	0.99	1.00	1.75	1.84	1.58	1.75	1.44	1.04
31	VGAFSTVKG	0.99	0.79	0.80	0.65	0.54	0.40	0.76	0.70	0.72	0.99	0.68	0.52
32	YSGHSMSDP	0.59	0.30	0.52	0.30	0.53	0.38	0.63	0.35	0.55	0.41	0.48	0.39
33	SPGEYVNIE	0.92	0.39	0.74	0.30	0.78	0.30	0.74	0.34	0.60	0.59	0.44	0.40
34	RKRSAKE	1.34	0.88	1.07	0.89	1.18	1.02	1.04	1.37	1.43	1.20	1.10	0.89
35	VINETSQHH	0.65	0.42	0.54	0.33	0.63	0.41	0.64	0.33	0.43	0.48	0.63	0.30
36	SPVVSGDTS	0.61	0.34	0.57	0.35	0.51	0.33	0.63	0.25	0.86	0.27	0.33	0.53

37	LRRFSLATM	2.36	1.66	1.94	1.29	1.18	0.79	1.21	1.27	0.98	1.15	1.14	1.04
38	PRPASVPPS	0.87	0.51	0.55	0.44	0.57	0.59	0.72	0.48	0.64	0.77	0.45	0.54
39	SSRPSSNRS	2.15	1.05	1.30	1.08	1.36	1.11	1.18	0.96	0.89	1.06	1.38	1.17
40	TKFASDDEH	0.72	0.34	0.62	0.31	0.64	0.28	0.66	0.35	0.59	0.40	0.47	0.47
41	LSDDSFIED	1.66	0.71	1.59	0.95	1.03	0.38	0.93	0.64	0.80	0.96	0.91	0.71
42	PSPKTPPGS	0.73	0.41	0.80	0.44	0.74	0.40	0.60	0.43	0.60	0.37	0.94	0.28
43	KDIGSESTE	1.36	0.39	0.99	0.35	0.57	0.47	0.71	0.41	0.59	0.49	0.47	0.57
44	KRRGSVPIL	2.42	1.86	1.94	1.79	1.44	1.66	1.42	1.62	2.17	1.88	1.45	1.67
45	KRAKAKTAKKR	2.50	2.54	2.75	2.80	2.07	2.45	2.81	3.37	3.69	2.91	2.24	2.02
46	QSYSSSQRV	0.77	1.05	0.62	0.53	0.61	0.47	0.88	0.72	0.63	0.58	0.71	0.57
47	KGTGYIKTE	1.31	0.89	0.63	0.68	0.97	1.14	0.93	0.75	0.83	0.13	0.92	1.00
48	KRSLSEMEI	0.65	0.31	0.53	0.42	0.81	0.44	0.60	0.35	0.50	0.37	0.55	0.48
49	KRKQGSVRGL	2.71	1.63	2.16	1.88	1.61	1.05	1.44	1.59	1.83	2.03	1.07	0.93
50	HTRDSEAQR	0.64	0.33	0.39	0.29	1.39	0.31	0.54	0.61	0.54	0.29	0.48	0.22
51	MAEVSWKVL	0.45	0.32	0.46	0.25	1.19	0.33	0.53	0.63	0.50	0.39	0.56	0.25
52	EEGISQESS	0.61	0.41	0.40	0.21	1.05	0.51	0.60	0.42	0.65	0.35	0.58	0.21
53	EQQQTEDEL	0.78	0.31	0.55	0.31	0.80	0.37	0.53	0.27	0.90	0.32	0.54	0.27
54	IGEGTYGVV	0.87	0.34	0.72	0.39	0.82	0.28	0.65	0.47	0.84	0.44	0.63	0.29
55	MMTPYVVTR	0.96	0.28	0.70	0.31	1.08	0.26	0.82	0.54	0.74	0.62	0.78	0.29
56	PYKFPSSPLRIPGZ	0.53	0.17	0.60	0.16	1.12	0.26	0.64	0.53	0.55	0.18	0.78	0.28
57	GQEVYVKKT	0.96	0.40	0.53	0.41	0.95	0.27	0.77	1.12	0.74	0.75	0.41	0.28
58	AQETSGEEI	0.65	0.39	0.72	0.31	1.07	0.38	0.42	0.55	0.80	0.40	0.46	0.26
59	DKAKSRPSL	1.20	0.58	0.65	0.56	0.91	0.40	0.96	0.65	0.78	0.81	0.44	0.31
60	FPVSYSSSG	0.60	0.28	0.51	0.32	0.75	0.47	0.56	0.42	0.41	0.33	0.39	0.24
61	GRLSSMAMI	1.07	0.39	0.83	0.52	0.74	0.23	0.87	0.50	0.87	0.81	0.42	0.51
62	EEDLSDENI	0.81	0.22	0.99	0.45	1.17	0.36	0.58	0.33	0.78	0.44	0.58	0.33
63	EPGPYAQPS	0.47	0.16	0.72	0.33	0.90	0.30	0.57	0.32	0.70	0.39	0.72	0.31
64	RRRASQLKV	1.26	0.58	0.76	0.51	0.88	0.34	0.59	0.71	0.78	0.83	0.50	0.21
65	SGADYPDEL	1.74	0.66	2.51	0.72	0.93	0.39	0.63	0.60	1.03	1.08	0.61	0.29
66	KQISVR	1.63	1.28	1.51	1.12	0.93	0.44	1.28	1.11	1.72	1.38	0.64	0.43
67	SLKDH	0.62	0.35	0.58	0.34	0.71	0.33	0.61	0.39	0.57	0.44	0.26	0.21
68	APRTAGGRR	2.29	0.98	1.60	1.17	1.70	0.42	1.66	1.53	1.98	1.54	1.01	0.71
69	DGHEYIYVD	0.89	0.35	1.10	0.65	0.72	0.29	0.72	0.39	0.84	0.52	0.49	0.29
70	FKRSYEEHI	0.58	0.39	0.59	0.39	0.73	0.27	0.68	0.29	0.61	0.48	0.48	0.28
71	REARSRAST	1.87	0.95	1.06	0.82	0.97	0.60	1.20	0.97	1.07	1.01	0.65	0.74
72	DRVYVHPF	0.74	0.39	1.02	0.36	0.80	0.43	0.80	0.51	0.71	0.65	0.53	0.22
73	VRRSDAA	0.79	0.38	0.69	0.35	0.63	0.33	0.88	0.53	0.43	0.38	0.49	0.26
74	RRKMSRGLP	1.29	0.78	0.94	0.73	0.61	0.24	1.09	0.83	1.31	1.32	0.44	0.31
75	SEVPYREVQ	0.72	0.33	0.89	0.54	0.44	0.23	0.68	0.53	0.97	0.65	0.33	0.32
76	PRRASATSS	1.17	0.52	0.98	0.67	0.71	0.32	0.89	0.65	0.91	0.94	0.53	0.49
77	RRLSI	2.25	0.84	2.79	1.48	0.93	0.18	1.93	1.23	1.60	1.48	0.78	0.61
78	STSLSPFYL	0.79	0.39	0.63	0.44	0.63	0.54	0.73	0.49	0.56	0.57	0.49	0.32
79	TRDIYETDY	2.01	0.90	1.64	1.35	1.21	0.54	0.82	0.66	0.90	0.96	0.79	0.26
80	VPTLSTFRT	1.22	0.69	0.74	0.70	0.65	0.35	0.97	0.81	0.67	0.83	0.70	0.31
81	RASTSKSES	0.64	0.35	0.53	0.35	0.52	0.35	0.56	0.54	0.53	0.63	0.53	0.37
82	SPSSSPTHE	0.52	0.31	0.50	0.43	0.39	0.26	0.55	0.54	0.49	0.38	0.19	0.37
83	STNDSLL	0.69	0.31	1.05	0.50	0.64	0.35	0.65	0.47	0.80	0.70	0.48	0.35
84	VRTFTHEVV	0.64	0.25	0.74	0.47	0.63	0.24	0.74	0.62	2.13	0.87	0.54	0.40
85	SRKMSVQEY	0.83	0.48	0.53	0.49	0.55	0.61	0.77	0.54	0.52	0.54	0.91	0.37
86	LSGLSFKRN	2.23	1.37	2.17	2.12	0.70	0.64	1.09	1.61	1.34	1.60	0.56	0.33
87	PTGTTPQRK	0.63	0.54	0.64	0.55	0.78	0.38	0.57	0.52	0.53	0.50	0.40	0.37
88	STLASSFKR	2.55	1.49	2.31	1.79	0.62	0.42	1.50	1.47	1.36	1.40	0.53	0.45
89	TPPKSPSSA	0.46	0.35	0.64	0.74	0.35	0.25	0.62	0.40	0.55	0.49	0.23	0.38
90	PAAVSEHGD	0.56	0.31	0.80	1.19	0.42	0.35	0.57	0.34	0.60	0.49	0.37	0.35
91	PVSPSLVQG	0.55	0.21	0.82	0.42	0.40	0.26	0.58	0.53	0.76	0.54	0.53	0.41
92	KKDASDDLD	0.68	0.41	0.74	0.44	0.78	0.48	0.71	0.48	0.74	0.45	0.89	0.42
93	KSRPSLPLP	1.24	0.97	0.91	0.87	0.77	0.71	1.18	0.86	1.01	1.17	0.50	0.46
94	LSVSSLPGL	0.48	0.42	0.72	0.42	0.74	0.33	0.54	0.49	1.19	0.52	0.28	0.43

95	SRHSSPHQS	0.76	0.46	0.68	0.53	0.41	0.38	0.85	0.67	0.77	0.61	0.33	0.71
96	KKPPTPPPE	0.54	0.40	0.67	0.50	0.50	0.53	0.70	0.62	0.74	0.84	0.37	0.58
97	KVPQTPLHT	1.06	0.67	1.06	0.99	1.12	0.33	0.86	0.96	1.12	1.17	0.58	0.61
98	PRRSSIRNA	0.77	0.30	1.03	0.48	0.53	0.36	0.69	0.52	0.76	0.79	0.52	0.43
99	ILDTTGQEE	0.86	0.26	1.56	0.57	1.52	0.31	0.84	0.54	1.09	0.77	1.16	0.43
100	NDSNYIVKG	0.51	0.18	0.76	0.59	1.09	0.15	0.56	0.44	0.69	0.36	0.76	0.40
101	EFLRTSAGS	0.42	0.19	0.69	0.30	0.78	0.19	0.51	0.48	0.39	0.22	0.77	0.32
102	ERTNSLPPV	0.38	0.16	0.61	0.37	0.61	0.24	0.59	0.30	0.58	0.13	0.64	0.33
103	IRRASTIEM	0.95	0.18	1.55	0.85	1.24	0.20	1.21	0.61	0.74	0.35	0.99	0.39
104	NFDDYMKSL	0.63	0.28	1.00	0.85	0.90	0.17	0.82	0.44	1.04	0.37	0.73	0.42
105	PYKFPSSPLRIPGZ	0.58	0.23	0.67	0.70	1.11	0.06	0.65	0.37	0.79	0.24	0.57	0.38
106	GRTGRRNSI	3.52	1.32	3.52	2.39	5.56	0.60	3.43	2.73	2.88	2.45	5.50	1.21
107	ATPIYLDIL	0.97	0.31	1.26	0.73	1.34	0.27	0.89	0.51	0.69	0.60	1.20	0.54
108	DMRQTVAVG	0.45	0.23	0.76	0.38	0.69	0.23	0.61	0.43	0.53	0.21	1.14	0.35
109	GDRFTDEEV	1.35	0.41	1.84	1.07	1.10	0.23	0.83	0.57	1.28	1.05	1.22	0.40
110	GSGSSVTSR	0.84	0.19	0.86	0.86	1.06	0.34	1.01	0.73	0.80	0.46	1.39	0.49
111	EEPQYEEIP	1.79	0.39	1.93	1.29	1.80	0.17	0.92	0.73	1.06	0.88	1.54	0.42
112	ERRLSLVPD	1.02	0.23	1.09	0.85	1.16	0.19	0.80	0.42	0.85	0.31	0.69	0.33
113	RRSSSRPIR	2.60	0.93	2.38	1.44	3.99	0.68	2.30	2.12	2.00	1.55	4.75	1.51
114	SKIGSTENL	0.56	0.21	0.57	0.34	0.70	0.30	0.76	0.51	0.49	0.31	0.83	0.47
115	NDMTSL	0.49	0.15	0.92	0.26	0.38	0.21	0.63	0.25	0.61	0.24	0.93	0.35
116	AARLSLTDP	0.40	0.21	0.64	0.50	0.37	0.24	0.68	0.26	0.49	0.37	0.74	0.36
117	ARRSTTDAG	0.43	0.15	0.54	0.45	0.37	0.28	0.67	0.37	0.59	0.32	1.05	0.38
118	DLKDTKYKL	0.47	0.16	0.56	0.66	0.53	0.24	0.66	0.26	0.60	0.41	0.83	0.48
119	GAFSTVKGV	0.97	0.46	0.91	0.81	0.81	0.30	1.08	0.81	0.97	0.48	0.78	0.54
120	RGRASSHSS	1.64	0.69	1.26	0.83	1.92	0.84	1.31	1.31	1.02	1.03	2.13	1.01
121	RKRSRAEF	1.31	0.47	1.00	0.56	0.66	0.46	1.14	0.90	0.95	0.84	1.32	0.69
122	LRAASLG	1.04	0.42	1.18	0.45	0.85	0.22	1.08	0.82	0.84	0.85	1.16	0.56
123	RRRQSVLNL	1.33	0.58	1.83	0.80	1.63	0.49	1.63	1.64	1.27	0.94	1.67	0.92
124	SIDEYFSEQ	1.09	0.35	1.22	0.74	0.59	0.30	0.78	0.50	0.92	0.61	0.96	0.41
125	EESESD	0.85	0.22	1.50	0.85	0.99	0.27	0.80	0.54	1.10	0.69	0.81	0.50
126	RRRS	2.14	0.92	2.13	1.75	2.93	1.12	2.32	2.56	2.49	1.59	2.83	1.87
127	TAYGTRRHL	2.71	1.44	3.04	1.65	3.10	0.52	2.30	2.01	1.98	1.92	2.91	1.05
128	TRRISQTSQ	1.50	0.87	1.80	0.92	1.51	0.53	1.36	0.94	1.04	1.07	1.71	0.82
129	VTPRTPPPS	0.57	0.31	0.75	0.25	0.65	0.26	0.92	0.49	0.53	0.39	0.80	0.45
130	REVSSLKSK	0.94	0.45	1.26	0.59	0.98	0.37	1.12	0.89	0.92	0.98	0.98	0.78
131	PEETQTQD	0.75	0.23	1.17	0.54	0.72	0.22	0.65	0.43	0.77	0.49	0.63	0.43
132	KKQISVR	1.21	0.57	1.37	0.88	2.50	1.00	1.18	0.84	1.45	1.09	1.77	1.31
133	WLTKSPDGN	0.61	0.20	0.73	0.52	1.02	0.34	0.70	0.50	0.72	0.46	0.60	0.48
134	SRRSSLGSL	2.33	1.37	3.06	1.73	3.01	0.80	1.37	1.41	1.51	1.76	2.77	1.31
135	PETVYEVAG	1.60	0.67	2.31	1.30	1.55	0.33	0.88	0.67	1.01	1.02	1.44	0.62
136	QEPGSGPPE	0.44	0.19	0.93	0.33	0.51	0.27	0.58	0.33	0.50	0.35	0.53	0.47
137	SVSSSPIKE	0.42	0.34	0.87	0.42	0.55	0.29	0.61	0.47	0.46	0.43	0.80	0.51
138	TRLHSLRER	2.64	1.69	3.62	1.74	1.96	0.54	1.59	1.59	1.71	1.48	1.74	1.09
139	PKEVYDVML	0.59	0.23	1.02	0.48	0.94	0.33	0.67	0.47	0.94	0.69	0.52	0.49
140	PRTPGGRR	2.09	1.05	2.40	1.80	2.79	1.11	1.40	1.48	2.93	1.88	2.41	2.10
141	KLSPSPSSR	1.18	0.59	1.10	0.89	1.61	1.09	1.14	0.94	1.14	1.10	1.72	1.25
142	LEKKYVRRD	1.55	0.78	1.51	1.00	0.98	0.74	1.20	1.03	1.74	1.17	0.91	0.90
143	QASSTPLSP	0.58	0.13	1.20	0.38	0.55	0.32	0.61	0.27	0.86	0.35	0.71	0.53
144	SRRDSLFVP	0.90	0.28	1.66	0.74	0.73	0.34	0.76	0.53	0.98	0.60	0.72	0.55
145	KQPIYIVME	0.67	0.37	1.22	0.59	0.97	0.32	0.88	0.52	0.74	0.83	0.80	0.60
146	LLQDSVDFS	0.64	0.18	1.14	0.44	2.01	0.40	0.70	0.46	0.93	0.67	1.21	0.77
147	QLSTSEENS	0.47	0.10	0.91	0.56	0.94	0.31	0.63	0.54	0.83	0.53	0.84	0.49
148	KIQASFRGH	2.63	1.04	3.07	2.57	4.11	1.13	1.90	1.56	2.45	2.51	2.43	1.36
149	NKGASQAGM	1.16	0.40	0.72	0.72	1.76	0.39	0.95	0.66	0.69	0.37	1.01	0.56
150	EIVESLSSS	0.60	0.24	0.69	0.88	1.11	0.17	0.69	0.35	0.69	0.19	0.54	0.34
151	ESSNYMAPY	0.58	0.21	0.62	0.90	1.11	0.33	0.71	0.51	0.83	0.31	0.49	0.36
152	KRPSKRAKA	1.40	0.60	1.17	1.40	2.16	0.60	1.41	1.42	1.33	0.70	0.93	0.65

153	NRAITARRQ	1.94	0.64	1.01	1.45	2.67	0.43	1.62	1.50	1.30	0.84	1.63	0.84
154	PYKFPSSPLRIPGZ	0.40	0.12	0.45	1.09	0.60	0.20	0.68	0.69	0.63	0.27	0.30	0.34
155	GSRPSESNG	0.79	0.33	0.62	0.53	1.81	0.19	0.72	0.44	0.71	0.27	0.50	0.49
156	DAHKSKRQH	1.07	0.31	0.85	0.69	1.45	0.17	0.88	0.72	1.05	0.52	0.96	0.48
157	DSLIYDDGL	1.56	0.49	2.07	1.65	1.84	0.26	0.71	0.60	1.02	0.73	1.12	0.57
158	GGRDSRSGS	0.90	0.24	0.76	0.74	1.08	0.22	0.85	0.84	0.76	0.20	0.56	0.42
159	GVERSVRPT	1.00	0.42	0.63	0.84	0.90	0.21	1.01	1.00	0.91	0.31	0.62	0.59
160	EGVKSDQAE	0.42	0.21	0.56	0.89	0.61	0.15	0.65	0.65	0.69	0.11	0.32	0.39
161	ESLSSSEES	0.43	0.17	0.63	1.44	0.59	0.20	0.72	0.68	0.76	0.13	0.24	0.34
162	RVRMSADAM	0.60	0.12	0.56	0.44	0.95	0.18	0.68	0.39	0.57	0.30	0.89	0.45
163	SNPTYSVMR	2.29	0.69	1.61	1.11	2.29	0.39	1.37	1.35	1.03	0.77	2.10	1.19
164	SSKRAK	1.32	0.52	1.16	1.22	2.33	0.49	1.33	1.33	1.45	0.77	1.07	0.64
165	AGDGSDEEV	0.75	0.20	1.17	1.04	0.84	0.13	0.81	0.76	0.98	0.53	0.66	0.47
166	AVDRYIAIT	0.62	0.39	0.64	1.02	0.62	0.13	0.73	0.59	0.68	0.22	0.49	0.37
167	DPLLTYRFP	0.35	0.15	0.66	1.22	0.75	0.24	0.61	0.77	0.70	0.21	0.45	0.37
168	GENIYIRHS	0.68	0.31	0.95	1.65	0.78	0.29	1.02	1.29	0.95	0.44	0.58	0.27
169	RLSPSPTSQ	0.66	0.18	0.63	0.36	0.73	0.22	0.76	0.66	0.54	0.31	0.93	0.39
170	RKESYSV	0.91	0.36	0.76	0.53	1.01	0.22	0.97	0.81	0.65	0.42	0.90	0.48
171	PLSRTLS	0.81	0.31	0.78	0.63	0.83	0.19	0.72	0.57	0.79	0.41	0.56	0.33
172	RSRASTPPA	0.95	0.48	0.92	0.71	0.92	0.34	0.98	1.09	0.80	0.39	0.68	0.50
173	SLSSSEESI	0.75	0.22	1.31	1.41	0.84	0.23	1.00	0.86	1.09	0.49	0.54	0.36
174	LRRASLAG	1.26	0.79	1.60	1.81	1.51	0.69	1.39	1.71	1.00	0.56	1.32	1.00
175	ADSESEDEE	0.94	0.14	1.97	3.12	1.45	0.27	1.11	1.38	1.10	0.55	0.93	0.37
176	THERSPSPS	0.71	0.20	0.80	0.43	0.92	0.25	0.72	0.48	0.65	0.39	0.72	0.43
177	TVTRSYRSV	2.69	1.22	3.38	2.00	3.86	0.65	2.33	2.00	1.98	1.50	2.60	1.73
178	YETDYYRKG	1.17	0.49	1.28	1.02	1.34	0.19	1.07	1.07	0.88	0.59	0.87	0.61
179	RKQISVRGL	1.47	0.66	1.44	1.23	1.65	0.65	1.34	1.56	1.19	0.82	1.26	0.91
180	AGTTYAL	0.60	0.37	0.55	0.78	0.76	0.21	0.72	0.64	0.58	0.35	0.51	0.45
181	LRRASVA	1.25	0.66	1.21	1.34	1.64	0.47	1.25	1.50	0.91	0.60	0.99	0.74
182	YRGYSLGNW	0.39	0.24	0.52	0.92	0.51	0.30	0.71	0.70	0.68	0.38	0.36	0.31
183	SSLKSRKRA	2.02	0.68	2.28	1.70	1.71	0.94	1.50	1.69	2.90	1.71	1.50	1.19
184	PMRRSVSEA	0.63	0.35	0.81	0.51	1.10	0.37	0.70	0.66	0.60	0.54	0.96	0.44
185	RSKRSGSV	2.67	1.17	3.20	2.12	3.66	0.95	1.93	1.97	2.88	1.77	2.49	1.70
186	TEGQYQQQP	0.73	0.38	0.63	0.58	1.17	0.26	0.80	0.67	0.81	0.50	0.52	0.53
187	TTPLSPTRL	0.87	0.54	0.80	0.81	1.75	0.50	0.97	1.03	0.93	0.63	0.94	0.88
188	PQPEYVNQP	0.55	0.28	0.72	0.92	0.75	0.34	0.68	0.73	0.98	0.37	0.48	0.45
189	VKRGISGL	1.16	0.61	1.01	1.45	1.57	0.86	1.22	1.57	1.12	0.64	1.12	1.02
190	KRKQISVRG	1.69	0.65	2.52	1.81	2.05	1.45	1.55	1.68	3.15	1.87	2.30	1.77
191	LPVPSTHIG	0.72	0.34	0.83	0.53	0.98	0.28	0.81	0.76	0.79	0.84	1.39	0.52
192	QRRHSLEPP	0.73	0.36	0.80	0.62	1.23	0.23	0.83	0.87	1.00	0.82	0.97	0.51
193	SRTLSVSSL	0.77	0.34	0.74	0.59	1.58	0.21	0.85	0.69	0.71	0.61	0.86	0.62
194	KRPSERAKA	1.52	1.03	1.40	1.27	2.58	1.14	1.23	1.59	1.76	1.32	1.86	1.49
195	LRGRSFMNN	1.12	0.68	1.62	1.58	1.84	0.65	1.06	1.41	1.13	0.98	1.12	0.95
196	QRVSSYRRT	2.84	1.75	2.95	3.33	4.16	1.77	2.38	2.72	2.03	1.48	3.10	2.25
197	HDLSSEMFN	1.18	0.22	0.49	0.25	0.38	0.39	0.60	0.29	0.46	0.32	0.69	0.64
198	KRPSRRAKA	2.40	1.91	1.99	1.53	1.55	2.43	1.63	1.78	1.88	1.71	2.56	2.17
199	EDNEYTARP	3.33	2.01	2.43	1.35	1.22	0.52	1.14	0.92	1.14	1.74	1.19	0.56
200	ENAPSSTSS	0.73	0.33	0.68	0.45	0.48	0.27	0.60	0.30	0.37	0.46	0.39	0.38
201	HMRSSMSGL	1.29	0.96	0.65	0.62	0.80	0.81	1.17	0.85	0.71	1.08	0.76	0.98
202	LDRSSHAQR	0.80	0.38	0.50	0.43	0.72	0.39	0.66	0.24	0.43	0.41	0.55	0.50
203	PYKFPSSPLRIPGZ	0.55	0.33	0.43	0.33	0.77	0.40	0.64	0.23	0.38	0.35	0.44	0.33
204	GKEIYNTIR	0.61	0.27	0.50	0.37	0.54	0.35	0.61	0.35	0.44	0.33	0.68	0.83
205	APATPGGRR	2.30	1.72	1.29	0.94	1.46	1.74	1.31	1.58	1.53	1.42	2.36	1.52
206	DEEESEEAK	1.04	0.55	0.90	0.52	0.65	0.43	0.68	0.34	0.64	0.61	0.65	0.48
207	FFKKSKIST	1.60	1.28	1.41	1.30	0.71	0.61	1.34	1.37	1.49	1.26	0.64	0.58
208	GMGTSVERA	0.74	0.40	0.59	0.58	0.64	0.33	0.67	0.28	0.61	0.59	0.48	0.50
209	DYDSSDIED	3.01	1.96	2.53	2.33	2.04	0.61	1.23	0.98	1.53	2.14	1.60	1.11
210	EKRASGQAF	0.69	0.46	0.57	0.59	0.64	0.47	0.74	0.44	0.48	0.57	0.60	0.44

211	RRAVSELDA	0.52	0.31	0.38	0.36	0.66	0.30	0.62	0.33	0.38	0.30	0.43	0.43
212	SDGGYMDMS	0.85	0.50	0.51	0.32	0.68	0.39	0.62	0.57	0.50	0.38	0.70	0.48
213	NWHMTPPRK	1.08	0.91	0.67	0.51	0.96	1.21	0.98	0.80	0.77	0.67	1.25	1.06
214	RRASI	2.79	1.87	2.06	1.57	1.85	2.11	1.66	1.33	1.62	1.34	2.95	1.70
215	AKKGSEQES	0.61	0.44	0.48	0.41	0.61	0.47	0.57	0.43	0.65	0.35	0.59	0.51
216	DDEMTGYVA	0.94	0.58	0.76	0.69	0.98	0.37	0.62	0.51	0.73	0.60	0.69	0.67
217	ETDYYRKGG	0.73	0.46	0.67	0.63	0.70	0.31	0.64	0.64	0.70	0.52	0.46	0.46
218	YVTTSTRTY	1.32	0.90	0.68	0.44	0.70	0.63	1.02	0.99	0.56	0.76	0.99	0.81
219	SPQPSRRGS	2.88	2.20	1.34	1.05	1.97	1.92	1.37	1.72	1.36	1.47	2.65	1.86
220	RRAASVA	2.31	1.55	1.12	0.85	1.15	1.53	1.21	1.23	0.91	1.06	1.68	1.07
221	RPSESNGQP	0.82	0.42	0.44	0.36	0.37	0.30	0.55	0.29	0.40	0.25	0.38	0.35
222	SASTTPVKK	1.14	0.85	0.69	0.61	0.76	1.01	0.85	0.79	0.68	0.81	0.79	1.09
223	NSYGSRRGN	2.99	2.54	1.66	2.07	2.99	2.43	1.73	1.71	1.37	1.55	2.21	3.20
224	LRRASL	1.38	1.27	1.19	1.44	1.17	0.80	0.80	0.83	0.76	1.16	0.89	0.90
225	SSTDSADSG	0.73	0.41	0.62	0.30	0.38	0.33	0.60	0.38	0.41	0.32	0.40	0.43
226	TKSGSTTKN	1.26	1.12	0.76	0.94	0.84	0.84	0.97	0.91	1.13	0.88	1.10	0.82
227	VETTYADFI	0.88	0.65	0.74	0.45	0.90	0.48	0.67	0.35	0.53	0.40	0.66	0.50
228	YSFTTTAER	0.66	0.46	0.37	0.27	0.61	0.38	0.60	0.35	1.19	0.23	0.43	0.45
229	SPFKYQSLL	0.61	0.47	0.50	0.37	0.62	0.45	0.47	0.39	0.63	0.43	0.38	0.47
230	RKRSAAE	1.10	0.99	0.59	0.72	1.05	0.77	0.75	0.87	0.69	0.93	0.74	0.91
231	VIKRSPRKR	1.93	1.74	2.02	2.04	2.63	2.72	1.70	2.69	2.42	2.41	2.04	2.49
232	SPVKSPEAK	0.65	0.42	0.55	0.42	0.54	0.41	0.50	0.36	0.46	0.50	0.54	0.57
233	LRRASVAQL	2.45	2.32	2.41	1.77	1.84	1.23	1.32	1.33	1.11	1.66	1.58	1.38
234	PRMPSLSVP	0.65	0.45	0.53	0.34	0.58	0.44	0.62	0.27	0.32	0.40	0.37	0.36
235	SSPVYQDAV	0.88	0.63	0.73	0.82	0.93	0.48	0.70	0.45	0.72	0.50	0.66	0.47
236	TKDTYDALH	0.70	0.47	0.52	0.49	0.66	0.35	0.50	0.41	0.63	0.44	0.47	0.47
237	LRSPSWEPF	0.73	0.65	0.64	0.61	0.80	0.41	0.44	0.39	0.51	0.59	0.69	0.52
238	PSLPTPPTR	1.17	1.00	0.72	0.79	1.10	0.82	0.78	0.66	0.83	1.01	1.29	1.01
239	KASASPRRK	2.18	1.97	2.06	1.94	2.70	2.01	1.20	1.78	1.89	2.28	2.55	2.26
240	KRRDYLDLA	0.84	0.57	0.85	0.53	0.89	0.48	0.62	0.42	0.55	0.50	0.57	0.83
241	PAPAVRASDRA	0.77	0.42	0.57	0.29	0.73	0.35	0.48	0.35	0.54	0.35	0.47	0.42
242	QSRASDKQT	0.55	0.45	0.50	0.32	0.67	0.48	0.59	0.31	0.42	0.37	0.62	0.56
243	KGQESFKKQ	1.29	1.29	0.96	1.20	1.40	1.52	1.36	1.22	0.98	1.22	1.62	1.39
244	KRSGSVYEP	1.63	2.28	1.74	2.22	1.31	1.32	1.19	1.65	1.20	1.76	1.66	1.99
245	KRAQISVRGL	1.70	1.75	1.83	2.14	2.01	2.04	1.39	1.78	1.76	1.77	2.31	2.07
246	HSSQSQGGG	0.61	0.29	0.38	0.38	0.59	0.33	0.77	0.41	0.47	0.33	0.54	0.37
247	MAEAYSEIG	1.13	0.65	1.14	0.93	1.16	0.44	0.77	0.45	0.97	1.02	1.02	0.50
248	EEESSYSYE	3.49	2.49	2.78	3.10	3.32	0.79	1.19	0.94	1.68	2.71	2.06	1.43
249	EQPGSDDED	1.00	0.59	1.54	1.02	0.67	0.40	0.72	0.62	1.15	1.04	0.63	0.39
250	IEQFSTVKG	0.39	0.26	0.68	0.76	0.46	0.37	0.60	0.41	0.46	0.46	0.42	0.34
251	MLDHSESTK	0.40	0.26	0.63	0.46	0.47	0.42	0.57	0.47	0.44	0.43	0.68	0.44
252	PYKFPSSPLRIPGZ	0.45	0.28	0.63	0.41	0.44	0.44	0.62	0.39	0.51	0.45	0.71	0.47
253	GPRTTRAQG	1.73	1.46	0.97	1.18	1.27	1.06	1.36	1.17	1.19	1.44	1.43	1.23
254	AQDTYLVLD	0.76	0.51	0.82	0.75	0.91	0.45	0.62	0.49	0.72	0.60	0.86	0.68
255	DIPESQMEE	0.69	0.57	0.70	0.71	0.72	0.39	0.63	0.52	0.75	0.59	0.74	0.54
256	FPRASFGSR	1.69	1.27	2.21	1.52	2.28	1.63	1.62	1.62	1.20	1.53	2.62	1.65
257	GRKASGSSP	0.91	0.66	1.05	0.93	1.04	1.08	1.16	1.07	0.93	1.03	1.08	0.88
258	EDVGSDEEE	1.23	0.51	1.82	1.21	0.99	0.39	0.83	0.62	1.30	1.31	1.54	0.58
259	EPAVSPLLP	0.56	0.46	0.90	0.79	0.68	0.49	0.68	0.52	0.73	0.59	1.14	0.90
260	RRRASQLKI	1.03	0.80	0.70	0.92	0.87	0.48	0.82	0.92	0.74	0.85	0.77	0.55
261	SFTTTAERE	0.47	0.37	0.39	0.41	0.62	0.38	0.62	0.45	0.66	0.38	0.57	0.43
262	PLSRTL	0.44	0.47	0.74	0.55	0.69	0.51	0.54	0.47	0.76	0.54	0.73	0.46
263	SGRGK	0.88	0.67	0.87	0.90	1.11	1.03	1.03	1.28	0.97	1.06	1.16	1.12
264	APRSPGGRR	1.99	1.25	1.85	1.51	3.07	3.23	1.89	2.34	2.05	1.84	2.76	1.97
265	DGERYDEDE	1.35	0.55	1.69	1.54	1.36	0.78	0.96	0.89	1.08	1.75	1.31	0.98
266	FKRPTLRRV	2.12	1.39	3.32	2.59	2.84	1.85	2.02	2.81	2.19	2.09	2.73	2.40
267	REAEYEPET	1.10	0.68	0.96	0.94	1.31	0.37	0.66	0.56	0.96	1.00	1.26	0.61
268	DRVYIHPF	0.53	0.39	0.62	0.63	0.87	0.56	0.75	0.54	0.76	0.92	0.81	0.52

269	VRRISGL	1.42	1.19	1.43	1.47	1.51	1.06	0.73	1.32	1.05	1.46	1.81	1.26
270	RRKGTDVNV	0.92	0.59	0.90	0.86	1.35	0.96	0.95	1.11	0.82	1.04	1.48	0.97
271	SETKTEEEE	0.55	0.37	0.81	0.71	0.76	0.57	0.57	0.52	0.82	0.75	0.79	0.60
272	PQRATSNVF	0.64	0.51	0.90	0.87	0.90	0.62	0.71	0.80	0.77	0.94	0.81	0.57
273	RRGSV	1.82	1.02	2.09	2.13	2.70	1.52	1.63	1.96	1.35	1.40	2.39	1.69
274	STSKSESSQ	0.53	0.41	0.42	0.55	0.61	0.35	0.48	0.30	0.64	0.54	0.69	0.59
275	TRAPSRTAS	1.73	1.24	1.14	1.47	1.74	1.49	0.91	1.08	1.04	1.52	1.72	1.52
276	VPRTPGGRR	2.14	1.95	1.95	2.34	3.39	4.26	1.73	2.42	2.12	1.98	3.04	2.56
277	RASSSRSVR	3.37	3.39	3.04	2.98	5.00	3.87	2.73	3.34	1.48	2.45	5.79	4.16
278	SPSSSPASL	0.56	0.40	0.62	0.62	0.76	0.61	0.54	0.38	0.52	0.42	0.80	0.58
279	RVYVHPF	1.04	0.70	1.35	1.17	1.23	1.00	1.19	1.58	1.53	1.49	1.09	0.94
280	VRRVSDDVR	0.47	0.39	0.76	0.68	0.63	0.53	0.65	0.39	0.63	0.59	0.59	0.56
281	SRKMSIQEY	0.59	0.54	0.53	0.86	0.95	0.39	0.59	0.43	0.56	0.65	1.04	0.77
282	LSGFSFKKS	1.24	1.28	1.17	1.15	1.46	1.22	1.14	1.19	1.28	1.41	1.42	1.25
283	PSSTSSSSI	0.49	0.48	0.59	0.60	0.74	0.62	0.52	0.35	0.55	0.53	0.93	0.71
284	STGIYEALE	1.47	1.09	1.61	1.71	1.54	1.04	0.64	0.59	0.94	1.12	1.49	1.19
285	TPPKSPSAS	0.49	0.39	0.64	0.52	0.64	0.41	0.58	0.69	0.67	0.46	0.72	0.54
286	LYSSSPGGA	0.49	0.45	0.70	0.56	0.61	0.58	0.59	0.36	0.58	0.47	0.58	0.48
287	PVPKSPVEE	0.51	0.41	0.79	0.56	0.60	0.52	0.58	0.29	0.66	0.50	0.51	0.39
288	KKASFKAKK	1.73	1.87	2.28	2.36	2.43	2.09	1.37	2.37	3.23	2.68	2.02	1.81
289	KSPAKTPVK	1.31	1.17	1.10	1.25	1.58	1.48	1.23	1.38	1.40	1.70	2.07	1.62
290	PLSKTLSVSS	0.47	0.42	0.59	0.70	0.72	0.46	0.70	0.26	0.60	0.64	0.76	0.57
291	SRGKSSSYS	1.37	1.13	1.03	1.30	0.89	0.94	1.09	1.34	1.14	1.55	1.14	1.00
292	KKLGSKKPQ	1.41	1.48	1.63	1.67	0.91	0.99	1.45	2.05	2.68	2.05	1.03	0.97
293	KTTASTRKV	2.36	2.28	2.34	3.05	2.38	2.04	1.60	2.01	2.39	2.53	2.65	2.47
294	PRRRTRRAS	0.60	0.59	0.95	0.77	0.79	0.61	0.61	0.41	0.90	0.71	0.74	0.44
295	IHQRSRKRL	2.17	0.83	1.75	1.03	0.64	0.39	1.49	1.67	1.72	1.27	0.40	0.48
296	NDALSGSGN	0.43	0.36	0.84	0.41	0.40	0.30	0.63	0.23	0.60	0.38	0.44	0.43
297	EETQTQDQP	0.36	0.16	0.68	0.33	0.45	0.31	0.59	0.24	0.56	0.32	0.63	0.42
298	ERSQSRKDS	0.67	0.41	0.85	0.53	0.54	0.36	1.09	0.72	0.70	0.73	0.50	0.44
299	IRQASQAGP	0.25	0.12	0.75	0.37	0.70	0.29	0.60	0.27	0.66	0.42	0.45	0.42
300	NFDDYMKEV	0.54	0.09	1.19	0.56	0.79	0.29	0.58	0.40	0.88	0.78	0.45	0.30
301	PYKFPSSPLRIPGZ	0.24	0.20	0.80	0.34	0.79	0.30	0.41	0.23	0.57	0.46	0.50	0.32
302	GRRQSLIQD	0.70	0.60	1.21	0.79	0.56	0.53	0.84	0.49	0.67	0.60	0.74	0.65
303	ASFEYTILD	0.92	0.69	1.80	1.14	0.73	0.45	0.74	0.48	0.78	0.70	0.77	0.54
304	DLSTYASIN	0.48	0.25	0.90	0.57	0.81	0.42	0.58	0.30	0.64	0.43	0.73	0.46
305	GDNDYIIPL	0.74	0.41	1.64	0.96	0.64	0.37	0.66	0.44	0.63	0.83	0.54	0.45
306	GSESTEDQA	0.28	0.17	0.86	0.34	0.45	0.40	0.54	0.31	0.97	0.61	0.55	0.40
307	EENVSVDDT	0.40	0.11	0.95	0.44	0.56	0.40	0.63	0.46	0.79	0.52	0.48	0.40
308	ERRKSKSGA	0.90	0.37	0.98	0.57	0.82	0.43	1.06	0.70	1.01	0.85	0.57	0.52
309	RRSRSRSRS	2.62	2.15	2.99	2.75	2.29	1.66	2.53	2.91	2.32	2.06	2.33	1.48
310	SKIGSLDNI	0.50	0.39	0.85	0.52	0.42	0.40	0.62	0.42	0.56	0.49	0.46	0.40
311	NDITSL	0.57	0.38	1.06	0.50	0.57	0.37	0.70	0.42	0.73	0.47	0.54	0.60
312	AARGSFDAS	0.39	0.31	0.69	0.38	0.42	0.40	0.57	0.41	0.67	0.46	0.49	0.44
313	ARNDSVTVA	0.26	0.27	0.68	0.45	0.55	0.44	0.65	0.37	1.00	0.48	0.47	0.41
314	DLFGSDEED	0.89	0.45	1.68	0.90	0.80	0.57	0.80	0.52	0.87	0.85	0.63	0.45
315	FSSRSYTSG	1.19	0.56	1.39	0.81	0.71	0.38	1.33	1.16	1.12	1.32	0.63	0.53
316	RGKSSSYSK	1.30	1.03	1.30	1.20	0.63	0.67	1.26	1.05	0.98	1.29	0.69	0.46
317	RKRSRAEA	0.94	0.75	0.82	0.72	0.61	0.49	1.05	0.82	0.98	0.94	0.47	0.41
318	LKRASLG	0.61	0.64	0.83	0.52	0.51	0.43	0.85	0.40	0.68	0.76	0.41	0.48
319	RRRPTPATL	1.36	1.09	1.26	0.71	1.14	0.75	1.55	3.02	0.98	0.95	0.93	0.78
320	SIADTFVGT	0.44	0.37	1.33	0.43	0.58	0.47	0.69	0.50	0.86	0.64	0.47	0.44
321	LRRNSI	1.13	0.84	2.14	1.52	0.86	0.61	1.27	1.24	1.02	1.18	0.66	0.63
322	RRRRASVA	2.73	1.73	3.39	2.84	2.56	1.52	3.00	3.02	2.95	2.23	1.92	1.88
323	TATDYHTTS	0.46	0.52	0.72	0.53	0.71	0.54	0.66	0.38	0.66	0.55	0.74	0.41
324	TRRASRPVR	1.69	1.73	2.02	1.77	0.92	0.60	1.27	1.25	1.97	1.97	0.95	0.58
325	VSSSSYRRM	2.43	2.60	2.58	2.09	1.06	0.91	2.10	1.77	1.68	1.65	1.08	0.90
326	REVSSLKNK	0.74	0.83	1.10	0.76	0.60	0.68	1.09	1.05	1.03	0.99	0.47	0.64

327	LSYRRYSL	1.43	1.27	2.34	1.88	1.04	0.79	1.55	1.28	1.41	1.47	1.00	0.74
328	KKKASVA	0.82	0.51	1.25	0.79	0.74	0.54	1.12	0.71	0.84	0.88	0.57	0.50
329	WKRTSMKLL	1.01	0.65	1.71	1.21	0.87	0.49	1.34	1.11	1.36	1.44	0.81	0.50
330	SRRQSVLVK	1.16	1.11	1.75	1.67	1.32	1.05	1.04	1.06	1.41	1.53	1.14	1.10
331	PENDYEDVE	2.34	1.55	2.38	2.48	2.12	0.99	1.13	1.51	1.43	1.71	1.42	0.93
332	QEGLYNELQ	0.59	0.53	1.17	0.75	0.78	0.58	0.66	0.39	0.86	0.84	0.54	0.47
333	SVPPSPSLS	0.46	0.35	0.69	0.49	0.57	0.55	0.54	0.44	0.65	0.43	0.48	0.41
334	TRKVSLAPQ	0.59	0.53	0.76	0.55	0.71	0.57	0.82	0.60	0.87	1.06	0.58	0.50
335	PKDPSQRRR	1.01	0.47	1.05	0.87	0.87	0.70	1.03	0.83	1.32	1.30	0.82	0.69
336	KRKQISVR	1.76	1.15	2.55	1.91	1.18	0.91	1.84	2.18	2.99	2.36	1.43	1.01
337	KLRRSSSVG	2.62	2.11	2.73	2.51	3.07	2.34	1.45	1.52	1.76	2.12	3.36	2.65
338	LDPLSEPED	0.92	0.59	1.53	0.99	0.86	0.79	0.67	0.63	1.02	1.51	0.99	0.71
339	QAGMTAPGT	0.46	0.38	0.74	0.49	0.46	0.55	0.55	0.30	0.63	0.39	0.40	0.42
340	SRRASRPVR	1.65	1.03	1.48	1.00	1.29	1.19	1.35	1.13	1.21	1.17	1.47	1.21
341	KPGFSPQPS	0.57	0.41	0.70	0.37	0.72	0.82	0.83	0.52	0.74	0.59	0.58	0.84
342	LLPMSPEEF	0.59	0.35	1.19	0.65	0.78	0.61	0.61	0.48	0.98	1.10	0.51	0.39
343	QLNDSSEEE	0.67	0.32	1.23	0.78	0.85	0.43	0.69	0.61	1.18	1.36	0.52	0.39
344	KGGSYSQAA	0.48	0.41	0.74	0.38	1.31	0.75	0.91	0.69	0.86	0.92	1.11	1.04
345	NIYISPLKS	0.52	0.35	0.75	0.60	1.18	0.65	0.91	0.93	0.86	0.79	1.06	1.06
346	EIRVSINEK	0.33	0.22	0.57	0.30	1.33	0.30	0.61	0.56	0.49	0.33	0.67	0.57
347	ESRISLPLP	0.57	0.32	0.60	0.50	2.25	0.28	0.70	0.52	0.56	0.34	0.61	0.41
348	KRPSIRAKA	2.82	1.27	3.50	2.21	3.84	1.10	1.69	1.99	1.97	1.55	1.84	1.37
349	NPGFYVEAN	0.90	0.49	1.10	0.85	2.44	0.50	0.83	0.97	0.87	0.63	0.91	0.60
350	PYKFPSSPLRIPGZ	0.45	0.23	0.52	0.72	0.96	0.38	0.58	0.71	0.58	0.18	0.42	0.39
351	GSRGSGSSV	0.61	0.43	0.67	0.37	0.98	0.56	1.09	0.89	0.52	0.55	0.89	0.81
352	DAGASPVEK	0.38	0.15	0.68	0.49	0.73	0.23	0.58	0.38	0.67	0.51	0.43	0.68
353	DRRVSVAAE	0.39	0.22	0.42	0.23	1.03	0.20	0.65	0.28	0.70	0.34	0.42	0.45
354	GGRASDYKS	0.77	0.42	0.71	0.34	1.86	0.40	1.05	1.03	0.85	0.68	0.75	0.67
355	GVDTYVEMR	0.45	0.26	0.87	0.47	2.51	0.28	0.59	0.55	0.69	0.50	0.64	0.38
356	EGTHSTKRG	0.96	0.63	0.84	0.70	1.97	0.67	1.03	1.24	0.97	0.64	0.71	0.63
357	ESLESYEIN	0.82	0.36	1.05	0.81	1.67	0.48	0.77	0.87	0.85	0.57	1.05	0.51
358	RVRKTKGKY	1.84	1.00	2.38	1.65	2.09	1.60	1.88	1.93	1.82	2.02	1.73	1.51
359	SNPEYLSAS	0.64	0.29	1.16	0.57	0.85	0.36	0.71	0.60	0.93	0.86	0.65	0.51
360	RRSTVA	1.41	1.00	1.23	0.74	2.11	0.94	1.47	1.45	1.05	1.02	1.98	1.28
361	AEPGSPTAA	0.21	0.21	0.49	0.19	1.05	0.14	0.69	0.47	0.43	0.20	0.42	0.43
362	AVDGYVKPQ	0.37	0.25	0.75	0.36	1.30	0.32	0.57	0.49	0.59	0.49	0.43	0.39
363	DPGVSYRTR	1.17	0.65	1.04	0.77	1.70	0.70	1.29	1.42	1.10	0.73	1.06	0.95
364	GEINTEDDD	0.51	0.24	0.85	0.59	0.95	0.36	0.84	0.91	1.13	0.59	0.74	0.39
365	RLSISTESQ	0.48	0.25	0.75	0.37	0.66	0.35	0.64	0.42	0.77	0.55	0.47	0.55
366	RKEISVR	1.10	0.60	1.21	0.84	1.93	1.27	1.26	1.16	1.38	1.06	1.63	1.37
367	PGSPQKR	0.34	0.38	0.55	0.31	0.84	0.35	0.71	0.50	0.62	0.38	0.45	0.86
368	RSGYSSPGS	0.58	0.51	1.14	0.22	1.02	0.64	1.09	1.19	0.68	0.46	0.60	0.73
369	SLRASTSKS	1.58	1.29	1.87	1.14	1.81	0.99	1.46	1.58	1.07	0.93	1.31	1.35
370	VRKISGL	0.91	0.61	0.73	0.45	0.96	0.43	1.06	1.34	0.94	0.77	1.01	0.57
371	ADGVYAASG	0.61	0.24	0.96	0.57	0.99	0.31	0.77	0.77	0.97	0.56	0.51	0.36
372	TGFLTEYVA	0.74	0.28	1.20	0.75	0.92	0.57	0.60	0.69	0.92	0.90	0.79	0.57
373	TVSTSLGHS	0.48	0.28	0.57	0.48	0.68	0.57	0.68	0.49	0.64	0.78	0.48	0.38
374	YDKEYYSVH	0.83	0.47	0.96	0.66	1.59	0.65	1.09	0.99	0.98	0.84	1.27	2.11
375	RKLKSQGTR	1.22	0.80	1.05	0.60	1.63	1.19	1.28	1.47	1.09	1.06	1.43	1.40
376	NRKPSKDKD	0.80	0.71	0.96	0.53	1.85	1.47	1.27	1.51	0.94	0.92	1.74	1.76
377	LRRASPG	0.58	0.58	0.73	0.46	1.47	0.82	1.28	1.04	0.66	0.69	1.14	1.17
378	YMAPYDNYV	1.08	0.48	1.14	0.77	1.35	0.42	0.94	1.13	1.09	0.86	1.05	0.70
379	SSKAYGNGY	0.54	0.33	0.85	0.44	0.72	0.43	0.68	0.62	0.93	0.91	0.50	0.52
380	PLTPSGEAP	0.40	0.40	0.74	0.42	0.88	0.83	0.55	0.69	0.88	0.61	0.76	0.69
381	RRRRPTPA	1.97	2.33	3.45	3.60	5.34	4.93	2.37	3.24	4.21	3.34	5.35	5.16
382	TEGQYQPQP	0.37	0.36	0.58	0.46	0.90	0.68	0.76	0.55	1.12	0.64	0.80	0.66
383	TSSSSIFDI	0.57	0.39	0.82	0.46	1.14	0.59	0.62	0.64	0.94	0.64	0.89	0.80
384	PPSPSLSRH	2.42	1.87	1.92	1.64	2.88	1.89	1.93	1.95	2.10	1.73	2.27	2.26

385	SSKRAKAK	1.39	1.25	1.52	1.25	3.88	3.48	1.66	1.88	1.55	1.31	2.66	2.78
386	KRKNSILNP	0.87	0.68	1.28	1.04	1.26	1.00	1.12	1.23	1.50	1.19	1.28	1.09
387	LNRMSFASN	0.81	0.58	1.35	1.01	1.01	0.76	0.70	1.12	0.98	0.88	0.92	0.80
388	QRHGSKYLA	0.35	0.23	0.66	0.42	1.16	0.81	0.51	0.81	1.16	0.58	0.81	0.57
389	SRTASFSES	0.45	0.27	0.70	0.37	0.78	0.54	0.74	0.67	0.92	0.50	0.46	0.49
390	KRPSDRAKA	1.68	1.23	1.48	1.17	1.88	1.33	1.59	1.78	1.85	1.42	1.82	1.72
391	LRGPSWDPF	0.55	0.34	0.75	0.42	1.04	0.43	0.70	0.60	0.76	0.69	0.60	0.48
392	QRSTSTPNV	0.33	0.24	0.39	0.38	1.11	0.66	0.60	0.51	0.52	0.36	0.48	0.50
393	HDALSGSGN	0.52	0.39	0.47	0.37	0.73	0.61	0.72	0.41	0.47	0.45	0.33	0.35
394	KRPSQRAKY	2.61	2.06	1.85	1.73	0.70	0.39	1.79	1.90	1.58	1.73	0.45	0.48
395	EDNEYTARE	2.09	1.56	1.53	1.21	1.33	0.31	0.86	0.71	1.05	1.10	0.37	0.37
396	ENAFSPSRS	0.66	0.58	0.62	0.45	0.63	0.32	0.50	0.14	0.47	0.36	0.24	0.32
397	HKSGYLSSE	0.67	0.44	0.43	0.44	0.85	0.28	0.66	0.34	0.45	0.47	0.32	0.35
398	LAYESHESM	0.48	0.36	0.68	0.59	1.01	0.53	0.51	0.15	0.56	0.34	0.43	0.37
399	PYKFPSSPLRIPGZ	0.57	0.37	0.61	0.62	1.14	0.40	0.53	0.32	0.91	0.43	0.63	0.58
400	GHQGTVPSD	0.52	0.37	0.37	0.33	0.91	0.60	0.58	0.49	0.40	0.21	0.33	0.47
401	ANDEYFIRK	0.57	0.34	0.49	0.46	0.62	0.31	0.58	0.32	0.39	0.24	0.32	0.34
402	DEASTTVSK	0.54	0.44	0.55	0.53	0.67	0.35	0.54	0.44	0.60	0.31	0.26	0.39
403	FEARYQQPF	0.76	0.67	0.71	0.61	0.66	0.28	0.64	0.42	0.72	0.50	0.31	0.33
404	GLLRSWNDP	0.82	0.60	0.74	0.60	0.85	0.36	0.70	0.45	0.61	0.54	0.54	0.33
405	DTVTSPQRA	3.04	2.23	2.65	2.39	0.94	0.37	1.32	1.15	1.50	1.74	0.41	0.26
406	EKHHSIDAQ	0.58	0.56	0.64	0.55	0.96	0.39	0.88	0.53	0.61	0.39	0.76	0.39
407	RRASTIEMP	1.34	1.23	0.83	0.77	0.54	0.30	1.00	1.71	0.50	0.62	0.34	0.41
408	SDEESNDDS	1.49	1.48	1.61	0.70	0.71	0.38	0.79	0.55	0.89	0.65	0.37	0.49
409	NVFSSPGGT	0.57	0.46	0.83	0.52	0.60	0.31	0.62	0.32	0.58	0.31	0.31	0.49
410	RRASF	2.67	2.51	2.02	2.02	0.54	0.34	1.81	1.92	1.12	1.51	0.31	0.40
411	AKGGTVKAA	1.14	1.06	0.60	0.66	0.58	0.34	1.24	0.83	0.76	0.83	0.39	0.41
412	DDEITQDEN	0.98	0.88	0.89	0.72	0.93	0.30	0.76	0.49	0.82	0.65	0.34	0.42
413	ETDDYAEII	0.97	0.78	1.00	0.87	1.00	0.58	0.63	0.32	0.70	0.51	0.44	0.47
414	YTTNSPSKI	0.74	0.94	0.44	0.49	0.40	0.43	0.68	0.31	0.45	0.36	0.25	0.42
415	SPLKSPYKI	1.17	1.21	0.86	0.64	0.43	0.28	1.01	0.87	0.57	0.80	0.30	0.31
416	RKRTRKE	1.72	1.68	1.60	1.25	0.54	0.54	1.39	1.46	1.90	1.40	0.52	0.51
417	RPPGFTPFR	1.66	1.80	0.97	1.03	0.51	0.41	1.31	1.29	0.83	1.04	0.37	0.44
418	SASGTPNKE	0.50	0.52	0.38	0.43	0.61	0.53	0.46	0.39	0.52	0.35	0.27	0.36
419	NSVDTSSLS	0.64	0.54	0.77	0.64	0.61	0.46	0.68	0.36	0.59	0.52	0.35	0.45
420	LRANSI	0.70	0.68	0.67	0.73	0.72	0.61	0.86	0.32	0.65	0.66	0.47	0.39
421	SSSSSPSRR	2.84	3.22	1.41	1.19	0.46	0.71	1.59	1.44	0.86	1.51	0.71	0.68
422	TKSASFLKG	1.56	1.56	1.28	1.07	0.33	0.36	1.15	0.82	0.63	0.81	0.30	0.53
423	VESLSSSEE	1.17	1.10	1.02	0.95	0.54	0.35	0.80	0.58	0.77	0.62	0.38	0.43
424	YRRNSVRFL	1.81	2.34	1.89	1.92	0.49	0.52	2.06	2.33	1.16	1.50	0.46	0.65
425	SPALTGDEA	0.84	0.60	0.69	0.69	0.46	0.34	0.65	0.41	0.82	0.51	0.34	0.50
426	RKQITVR	2.01	1.92	1.68	1.72	0.73	0.79	1.86	2.48	1.91	1.65	0.53	0.63
427	VHNRSKINL	1.09	1.10	1.02	1.14	0.68	0.67	0.98	0.96	1.09	1.06	0.53	0.54
428	SPVHSIADE	0.94	0.60	0.72	0.50	0.52	0.37	0.60	0.33	0.44	0.54	0.28	0.46
429	LRRASLGAF	2.59	2.91	2.56	2.18	0.67	0.74	1.76	1.36	1.47	1.68	0.66	0.76
430	PRKGSPRKG	1.79	1.70	1.11	1.00	0.52	0.41	1.21	0.97	1.13	0.96	0.36	0.50
431	SSPGSPGTP	0.67	0.51	0.69	0.62	0.49	0.42	0.79	0.47	0.45	0.48	0.34	0.46
432	TKAASEKKT	0.84	0.58	0.89	0.93	0.61	0.35	0.86	0.53	0.78	0.64	0.36	0.42
433	LRRSSSVGY	4.16	4.07	4.21	4.33	1.09	0.92	3.35	2.75	2.54	3.82	0.98	1.07
434	PSEKSEEIT	0.89	0.83	0.88	1.00	0.63	0.56	0.76	0.50	0.53	0.55	0.42	0.44
435	KAQEYFNIK	0.68	0.53	0.39	0.49	0.47	0.50	0.81	0.38	0.66	0.51	0.45	0.46
436	KRQSSTSNA	1.27	1.58	0.92	0.81	0.81	0.68	1.19	1.00	1.17	0.94	0.59	0.69
437	QKRPSQRSKYL	2.82	3.21	2.64	2.41	1.17	0.74	1.92	1.97	1.55	1.67	0.84	0.67
438	QSPSSSPTH	0.74	0.86	0.59	0.54	0.65	0.50	0.65	0.29	0.56	0.43	0.37	0.41
439	KGHEYTNIK	0.97	1.01	1.03	1.01	0.81	0.40	1.09	0.85	0.99	0.88	0.44	0.36
440	KRRSSSYHV	3.62	3.68	4.27	4.43	1.55	1.06	3.14	3.05	4.04	3.53	1.42	1.36
441	KAKQISVRGL	1.93	1.99	2.05	2.07	0.89	0.52	1.56	1.69	1.45	1.73	0.67	0.67
442	HSIYSSDDD	0.41	0.30	0.41	0.47	1.52	0.92	0.62	0.26	0.60	0.34	1.20	0.79

443	LVMQTAAGT	0.91	0.81	1.12	1.02	0.89	0.47	0.80	0.57	0.93	1.12	0.72	0.59
444	EEELYLEPL	1.24	1.27	1.38	1.93	1.68	1.24	0.62	0.40	0.82	0.97	1.31	1.00
445	EQLSTSEEN	0.44	0.34	0.83	0.75	0.65	0.62	0.56	0.22	0.88	0.49	0.58	0.47
446	IDMESQERI	0.44	0.31	0.71	0.54	0.75	0.69	0.51	0.31	0.69	0.42	0.66	0.49
447	MILLSELSR	0.36	0.37	0.57	0.53	0.72	0.56	0.57	0.37	0.59	0.48	0.59	0.45
448	PYKFPSSPLRIPGZ	0.33	0.35	0.59	0.44	0.30	0.56	0.64	0.35	0.61	0.50	0.52	0.30
449	GPAASPAAA	0.33	0.22	0.35	0.46	0.81	0.44	0.64	0.15	0.37	0.27	0.38	0.51
450	AQAASPAKG	0.40	0.34	0.57	0.54	0.76	0.91	0.72	0.47	0.57	0.51	0.65	0.75
451	DHSRSTKAA	0.86	1.00	0.79	0.96	1.12	1.08	1.05	1.01	0.99	1.19	1.20	1.09
452	FPFHSPSRL	1.18	1.52	1.31	1.50	1.56	1.57	1.52	1.08	0.99	1.48	1.81	1.79
453	GRILTLPRS	1.12	1.12	1.15	1.25	2.31	2.95	1.43	1.13	0.89	1.42	2.73	2.53
454	EDVGSDEED	1.03	0.64	1.39	1.19	0.93	0.75	0.91	0.66	1.25	1.41	0.90	0.63
455	ENTVSTSLG	0.27	0.37	0.66	0.59	0.48	0.57	0.55	0.35	0.58	0.53	0.62	0.39
456	RRQHSYDTF	0.72	0.95	0.65	0.59	1.00	0.58	0.98	0.75	0.90	0.82	0.72	0.78
457	SFMMTPYVV	0.50	0.44	0.60	0.63	0.80	0.40	0.71	0.28	0.69	0.35	0.55	0.41
458	PGTESFVNA	0.46	0.39	0.75	0.67	0.67	0.42	0.72	0.33	0.90	0.54	0.50	0.46
459	SDEEV	0.65	0.53	0.86	0.75	0.77	0.62	0.76	0.54	0.96	0.83	0.68	0.56
460	APQTPGGRR	1.38	0.98	1.21	1.19	1.92	2.47	1.45	1.20	1.07	1.57	2.38	3.99
461	DFPLSPPKK	0.49	0.53	0.74	0.78	0.87	0.81	0.96	0.60	0.76	1.07	0.87	0.81
462	FKAFSPKGS	0.88	0.93	0.87	1.26	1.16	1.35	1.23	0.96	0.91	1.52	1.34	1.21
463	RDSNYISKG	0.68	0.69	0.56	0.77	0.92	0.92	0.72	0.34	0.75	0.53	0.92	1.28
464	DDEESESD	1.68	1.27	2.17	1.88	1.17	1.06	1.08	1.06	1.28	1.48	1.08	0.79
465	VKRISGL	1.08	0.97	0.90	1.02	0.95	1.03	1.22	1.29	0.87	1.15	0.94	0.95
466	RRKDYPALH	0.69	0.58	0.69	0.75	1.72	0.98	1.09	0.65	0.72	0.92	0.91	0.82
467	SEKKSKGLG	0.65	0.37	0.58	0.63	0.64	0.58	0.84	0.63	0.63	0.76	0.74	0.76
468	PPSAYGSVK	0.87	0.76	0.95	1.14	1.00	1.01	1.04	0.74	0.85	1.25	1.23	1.06
469	RRFSV	1.39	1.40	2.59	1.93	2.01	2.33	1.73	1.39	1.30	1.73	2.51	2.06
470	STRRSVRGS	3.77	3.89	3.42	3.85	3.92	4.76	2.30	3.20	2.36	3.23	4.87	4.97
471	TQSTSGRRR	2.56	3.04	2.75	2.76	2.67	3.25	2.02	2.93	2.87	2.87	3.30	3.59
472	VNELSKDIG	0.37	0.48	0.54	0.60	0.73	0.65	0.72	0.52	0.67	0.51	0.67	0.68
473	RAGETRFTD	0.36	0.41	0.59	0.58	0.62	0.62	0.66	0.27	0.67	0.41	0.59	0.51
474	SPSPSFRWP	0.51	0.54	0.72	0.79	1.14	1.04	0.85	0.67	0.62	0.59	1.37	1.16
475	RVSGSRR	3.23	3.29	2.76	3.24	4.92	5.49	3.13	2.66	2.30	2.92	5.95	5.10
476	VRLRSSVPG	1.28	1.40	1.22	1.23	1.41	1.87	1.56	1.24	1.03	1.57	2.39	1.81
477	SRKLSDFGQ	0.70	0.78	0.72	0.81	0.79	0.76	0.72	0.44	0.66	0.59	0.80	0.68
478	LSGFSFKKN	1.14	1.27	1.19	1.46	1.36	1.13	1.31	1.43	1.28	1.47	1.17	1.19
479	PSRRSRSRS	0.65	0.68	0.63	0.82	0.85	0.68	0.71	0.30	0.78	0.63	0.57	0.59
480	STDYYREGP	1.44	1.08	1.57	1.68	1.48	1.63	1.12	1.07	1.21	1.61	1.55	1.03
481	TPAISPSKR	1.19	0.99	1.14	1.34	1.73	1.82	1.17	1.21	1.16	1.53	1.97	1.72
482	LVVASAGPT	0.43	0.64	0.68	0.63	0.74	0.93	0.71	0.42	0.65	0.53	0.95	0.65
483	PVPEYINQS	0.57	0.62	0.88	0.91	0.73	0.70	0.82	0.61	0.93	0.98	0.77	0.48
484	KKAESPVKE	0.54	0.66	0.79	0.69	0.93	0.90	0.98	0.54	0.90	0.71	0.87	1.01
485	KSLNYIDLD	0.93	0.86	1.01	1.65	1.39	1.07	0.79	0.59	0.90	1.22	1.05	0.97
486	KRKQISVRGL	1.84	1.97	1.84	2.19	1.87	1.59	1.79	1.40	2.07	1.87	1.48	1.73
487	SRGDYMTMQ	0.45	0.51	0.39	0.51	0.60	0.78	0.56	0.41	0.58	0.37	0.59	0.54
488	KKKGSGEDD	0.43	0.41	0.58	0.62	0.85	1.03	0.80	0.48	0.76	0.65	1.02	0.95
489	KTSPSSSPA	0.35	0.49	0.49	0.60	0.72	0.91	0.74	0.38	0.67	0.57	0.89	0.88
490	PRRRSSFGI	0.68	0.79	0.80	0.85	0.51	0.64	0.62	0.54	0.78	0.65	0.56	0.51
491	IGSVSEDNS	1.65	1.57	1.87	1.56	0.45	0.62	1.71	2.32	1.50	1.91	0.45	0.33
492	NAPVSALGE	0.47	0.37	0.66	0.47	0.61	0.54	0.29	0.36	0.69	0.67	0.45	0.39
493	EETPYSYPT	1.41	1.14	1.98	1.81	0.85	0.70	1.15	1.52	1.24	1.93	0.73	0.48
494	ERSPSPSFR	1.52	1.23	1.88	1.47	0.68	0.64	1.97	2.01	1.22	1.80	0.71	0.60
495	IRKYTMRRL	1.52	1.09	2.33	1.80	0.96	0.92	2.29	2.73	1.90	2.21	0.74	0.84
496	NEEESSYSY	2.38	1.23	2.38	2.71	1.65	1.12	1.28	1.47	1.68	2.26	1.14	0.87
497	PYKFPSSPLRIPGZ	0.46	0.25	0.61	0.49	1.03	0.76	0.57	0.30	0.66	0.73	0.80	0.71
498	GRRQSLIED	0.75	0.57	1.05	1.21	0.38	0.58	0.84	0.77	0.94	0.87	0.54	0.30
499	ASATSSSGG	0.40	0.33	0.64	0.43	0.43	0.49	0.30	0.35	0.74	0.60	0.55	0.24
500	DLPMSPRTL	0.29	0.26	0.61	0.41	0.51	0.52	0.40	0.38	0.57	0.63	0.34	0.46

501	GDLQSAEFH	0.42	0.21	0.86	0.57	0.56	0.55	0.72	0.60	0.67	0.87	0.43	0.35
502	GSEEYMNMD	0.47	0.35	0.72	0.58	0.64	0.46	0.71	0.47	0.64	0.54	0.29	0.40
503	EENTYDEYE	0.57	0.36	0.95	0.70	1.35	0.96	0.69	0.43	0.77	0.92	0.86	0.64
504	ERRKSHEAE	0.80	0.50	0.88	0.87	0.66	0.35	1.10	1.10	1.04	1.20	0.40	0.34
505	RRRVTSATR	2.81	2.22	2.28	1.93	0.66	0.83	2.29	2.69	2.15	2.46	0.61	0.62
506	SKDSSKRGR	1.77	1.30	2.13	1.82	0.56	0.76	2.09	2.61	2.04	2.63	0.60	0.59
507	MSVEEV	0.39	0.34	0.70	0.56	0.37	0.49	0.53	0.37	0.84	0.62	0.37	0.35
508	AALESEDED	0.65	0.45	1.30	1.13	0.54	0.55	0.88	0.89	1.21	1.49	0.44	0.43
509	ARKSTRRSI	2.35	1.70	2.59	2.63	0.80	0.93	1.98	2.32	2.42	2.44	0.55	0.68
510	DLFGSDDEE	0.90	0.76	1.46	1.28	0.86	0.66	0.72	0.75	0.83	1.33	0.47	0.50
511	FRRLSISTE	0.76	0.50	0.89	0.83	0.72	0.46	1.07	0.82	0.70	1.14	0.39	0.38
512	RGAISAEVY	0.67	0.68	0.92	0.88	0.53	0.61	0.87	0.71	1.00	1.16	0.50	0.46
513	RKISASEF	0.44	0.57	0.67	0.74	0.38	0.55	0.93	0.86	0.70	0.78	0.36	0.42
514	LHRASLG	0.27	0.63	0.97	1.03	0.46	0.59	0.74	0.73	0.71	1.20	0.30	0.44
515	RRRPTPAML	0.96	1.14	1.09	1.24	0.49	0.61	1.05	1.43	0.87	1.46	0.47	0.60
516	SGYSSPGSP	0.34	0.32	0.67	0.57	0.30	0.52	0.54	0.44	0.57	0.63	0.36	0.39
517	LGEGTP	0.47	0.44	0.78	0.57	0.52	0.41	0.55	0.50	0.59	0.73	0.36	0.36
518	RRLSSLRA	3.26	3.50	4.08	3.22	1.49	1.68	3.63	4.49	1.88	3.60	1.46	1.40
519	TAESSQAEE	0.38	0.56	0.94	0.78	0.38	0.71	0.77	0.41	1.12	0.71	0.37	0.48
520	TRRASFSAQ	1.59	1.53	2.20	2.84	0.54	0.68	1.45	1.74	1.17	1.74	0.56	0.42
521	VSRTSAVPT	0.43	0.47	0.64	0.73	0.44	0.56	0.83	0.48	0.43	0.71	0.40	0.39
522	REQLSTSEE	0.33	0.41	0.77	0.59	0.43	0.63	0.52	0.33	0.69	0.87	0.35	0.31
523	LSYRGYSL	0.37	0.37	1.00	0.63	0.47	0.55	0.58	0.27	0.49	0.76	0.31	0.41
524	KASGSSP	0.41	0.39	0.58	0.46	0.48	0.56	0.60	0.44	0.70	0.74	0.32	0.37
525	VVGGSLRGA	1.22	2.03	1.35	1.28	0.56	0.67	1.29	1.41	0.81	1.50	0.47	0.47
526	SRRPSYRKI	1.34	1.48	1.53	2.40	0.52	0.66	1.38	1.70	1.47	1.77	0.58	0.51
527	PEGDYEEVL	1.75	1.61	2.01	2.92	1.10	1.18	0.91	0.76	0.86	1.49	0.97	0.89
528	QEGDTDAGL	0.43	0.34	1.03	0.98	0.54	0.65	0.54	0.76	1.05	1.06	0.45	0.44
529	SVFSSPSAS	0.32	0.35	0.62	0.45	0.43	0.52	0.55	0.39	0.63	0.64	0.27	0.27
530	TRKISQTAQ	0.40	0.32	0.83	0.65	0.58	0.54	0.64	0.35	0.74	0.94	0.38	0.39
531	PINGSPRTP	0.37	0.30	0.64	0.47	0.60	0.49	0.65	0.33	0.94	0.81	0.31	0.37
532	KQISVRGL	1.44	1.46	1.61	1.87	0.66	0.70	1.58	1.98	1.77	2.87	0.46	0.44
533	KLINSIADT	0.34	0.50	0.68	0.63	0.41	0.63	0.63	0.34	0.66	0.74	0.47	0.48
534	LDDQYTSSS	1.36	1.23	1.50	1.89	0.57	0.70	1.10	0.98	1.41	1.55	0.52	0.44
535	PYDNYVPSA	0.94	0.87	1.33	1.22	0.52	0.53	0.87	0.81	1.16	1.43	0.39	0.37
536	SRQLSSGVS	0.31	0.43	0.52	0.41	0.55	0.67	0.71	0.44	0.70	0.62	0.37	0.53
537	KNIVTPRTP	0.86	1.47	0.98	1.23	0.72	0.77	1.23	1.31	1.42	1.67	0.58	0.63
538	LKLASPELE	0.26	0.40	0.68	0.60	0.42	0.53	0.62	0.29	1.01	0.95	0.24	0.35
539	QLIDSMANS	0.38	0.36	0.62	0.44	0.53	0.49	0.58	0.30	0.60	0.68	0.34	0.28
540	IVYKSPVVS	0.50	0.36	0.74	0.61	2.02	1.23	0.78	0.77	0.96	0.92	1.06	0.82
541	NGYISAAEL	0.54	0.45	1.75	1.62	0.94	0.52	0.87	0.92	1.53	1.94	0.66	0.40
542	EILNSPEKA	0.20	0.28	0.36	0.36	0.53	0.43	0.51	0.37	0.57	0.49	0.42	0.39
543	ESPESTEIT	0.36	0.26	0.80	0.32	0.76	0.32	0.56	0.43	0.61	0.64	0.38	0.46
544	KRPSHRAKA	0.99	0.97	1.14	0.71	2.09	1.42	1.23	1.25	1.29	1.09	1.38	1.61
545	NNYVYIDPT	0.58	0.42	0.91	0.50	1.27	0.58	0.72	0.65	0.79	0.70	1.00	0.79
546	PYKFPSSPLRIPGZ	0.27	0.26	0.38	0.29	0.56	0.38	0.66	0.50	0.49	0.37	0.32	0.32
547	GSPRTPRRG	1.37	1.55	2.20	1.51	3.62	3.97	1.91	2.06	2.14	1.89	2.89	3.19
548	DADEYLIPQ	0.80	0.63	1.16	1.02	1.75	1.14	0.71	0.58	0.97	1.09	1.43	1.08
549	DRLVSARSV	0.78	0.72	0.76	0.64	1.24	0.98	1.13	1.23	0.98	1.33	1.08	0.97
550	GGLTSPGLS	0.28	0.26	0.57	0.27	0.59	0.26	0.52	0.40	0.71	0.44	0.45	0.46
551	GTVPSDNID	0.34	0.16	0.83	0.35	0.65	0.26	0.65	0.45	0.75	0.69	0.34	0.36
552	EGSAYEEVP	0.48	0.48	0.73	0.52	1.63	0.87	0.99	1.13	0.73	0.78	1.30	1.02
553	ESIISQETY	0.93	0.53	0.95	0.74	1.37	0.96	0.78	0.80	0.90	0.84	1.08	0.88
554	RVRKSKGKY	1.80	1.86	2.58	2.68	1.30	1.26	2.14	2.59	3.47	3.00	1.15	1.09
555	SNDSTSVSA	0.39	0.35	0.57	0.44	0.74	0.41	0.64	0.39	0.59	0.57	0.39	0.32
556	RRPTVA	0.93	1.28	0.91	0.91	1.53	1.40	1.22	1.35	1.05	1.06	1.43	1.43
557	AEPDYGALY	0.92	0.82	1.23	2.45	1.79	1.31	0.79	0.69	0.97	0.93	1.45	1.18
558	AVASSPSKA	0.37	0.41	0.51	0.41	0.60	0.62	0.98	0.76	0.61	0.47	0.81	0.62

559	DPGTSYRTR	0.94	1.00	0.76	0.58	1.17	0.94	1.16	1.23	0.88	1.03	1.02	0.97
560	GEGTYGVVY	0.59	0.48	0.91	0.85	1.09	0.67	0.73	0.68	0.80	0.80	1.00	0.69
561	RLRLSPSPT	0.90	0.90	0.72	0.65	1.01	0.88	1.20	1.14	0.95	1.09	0.97	0.98
562	RKASRKE	1.01	1.10	1.09	0.83	1.33	1.44	1.42	1.37	1.51	1.30	1.45	1.35
563	LRRWSLG	0.79	0.63	0.90	0.83	1.00	0.86	1.04	1.06	0.78	0.76	0.95	1.05
564	RRVTSATRR	2.55	3.50	3.07	2.21	4.40	4.79	2.87	3.14	2.11	2.01	4.71	4.31
565	SLQASIVTD	0.40	0.57	0.68	0.32	0.72	0.59	0.67	0.60	0.78	0.59	0.47	0.40
566	RRATPA	1.18	1.24	0.75	0.59	1.20	0.92	1.11	1.37	1.07	1.03	1.13	0.96
567	ADGIYAASG	0.61	0.54	0.73	0.56	0.99	0.65	0.85	0.89	0.89	0.86	0.82	0.62
568	TGDTYTAHA	0.41	0.31	0.50	0.41	0.51	0.39	0.67	0.35	0.60	0.62	0.41	0.42
569	TVSKTETSQ	0.33	0.22	0.38	0.34	0.45	0.46	0.64	0.45	0.69	0.67	0.44	0.58
570	WTSDTQGDE	0.76	0.44	0.95	0.75	0.85	0.55	0.89	0.97	1.15	1.26	0.81	0.78
571	RKFSSARPE	0.77	1.06	0.81	0.73	1.31	1.03	1.28	1.40	0.96	1.00	1.15	1.13
572	KKKKASVA	0.78	1.23	1.39	1.21	0.58	0.63	1.10	1.26	1.76	1.41	0.55	0.58
573	LRRASLG	1.69	2.01	1.44	1.26	1.75	1.80	1.69	1.73	1.18	1.19	1.74	1.67
574	YKNDYYRKR	1.14	1.38	1.17	0.95	1.34	1.12	1.53	1.63	1.16	1.13	1.12	1.09
575	SSEITTKDL	0.76	0.38	0.75	0.48	0.61	0.69	0.58	0.53	0.91	0.66	0.66	0.76
576	PLSRTLSVS	0.61	0.42	0.86	1.26	1.09	0.79	0.67	0.73	0.79	0.90	1.07	0.96
577	LRRASLRG	3.26	1.92	3.03	4.09	4.21	3.70	2.30	3.38	2.33	2.73	4.74	4.46
578	TEGQYELQP	0.90	0.62	1.15	0.90	1.35	0.94	0.93	1.16	1.20	1.18	1.16	1.00
579	TSPSSSPAS	0.39	0.32	0.48	0.28	0.48	0.49	0.59	0.47	0.45	0.53	0.35	0.41
580	PPSAYGSVK	0.61	0.41	0.79	0.61	0.73	0.64	0.99	1.12	0.75	0.87	0.69	0.53
581	RTKRSGSV	2.39	1.73	2.12	1.82	3.55	2.96	2.07	2.70	1.70	1.91	3.25	3.08
582	KRFGSKAHM	1.64	1.41	2.07	1.83	1.74	1.69	1.63	1.60	2.06	2.05	1.94	2.86
583	LNDSSEEED	0.66	0.53	1.08	0.95	0.98	0.58	0.87	0.88	1.36	1.39	0.64	0.75
584	QRATSNVFA	0.53	0.43	0.59	0.57	0.84	0.60	0.52	0.70	0.86	0.72	0.52	0.59
585	SRSRTPSLP	1.71	1.88	1.24	1.07	2.23	2.46	2.00	1.75	1.07	1.21	2.07	2.18
586	KRPSARAKA	1.61	1.95	1.97	1.67	2.74	2.49	1.88	1.76	2.46	2.00	2.63	2.57
587	LRAPSWIDT	0.60	0.41	0.71	0.52	0.66	0.73	0.79	0.77	0.91	0.93	0.59	0.43
588	QRRTSVSGE	0.45	0.35	0.44	0.51	0.83	0.73	0.76	0.60	0.45	0.51	0.79	0.59
589	HATPSPPVD	0.51	0.59	0.32	0.48	0.56	0.35	0.54	0.25	0.44	0.41	0.33	0.43
590	KRPSQRAKA	2.45	2.90	1.17	1.81	0.58	0.56	1.61	1.49	1.12	1.28	0.44	0.49
591	EDAESEDEE	2.80	2.95	2.28	2.00	0.72	0.55	1.26	1.23	1.28	1.71	0.38	0.37
592	ENAEYLRVA	1.03	1.10	0.93	0.81	0.71	0.48	0.82	0.55	0.69	0.78	0.45	0.50
593	HKRKSSQAL	1.61	1.59	1.12	0.97	0.76	0.48	1.48	1.93	1.05	1.66	0.34	2.30
594	LAYESHESL	0.98	0.87	0.76	0.87	0.83	0.51	0.80	0.39	0.82	0.57	0.44	0.52
595	PYKFPSSPLRIPGZ	0.49	0.46	0.52	0.62	0.72	0.50	0.61	0.34	0.67	0.28	0.36	0.44
596	GGVDYKNIH	0.61	0.73	0.32	0.39	0.38	0.50	0.89	0.38	0.45	0.55	0.36	0.46
597	ALRPSTSRS	1.91	2.27	0.93	0.99	0.53	0.58	1.28	1.20	0.79	0.97	0.40	0.54
598	DEAATKTQT	0.85	0.81	0.48	0.81	0.62	0.38	0.62	0.39	0.61	0.56	0.37	0.50
599	FARKSTRRS	2.57	2.67	2.15	2.45	0.71	0.73	1.86	2.29	1.70	2.06	0.57	0.63
600	GLGESRKDK	1.17	1.35	0.62	0.87	0.78	0.61	1.34	0.94	0.67	0.95	0.44	0.71
601	DTHRTPSRS	1.34	1.50	0.84	0.85	0.74	0.72	1.18	1.04	0.85	1.09	0.45	0.64
602	EKESSNDST	0.47	0.45	0.54	0.42	0.49	0.53	0.72	0.25	0.58	0.37	0.32	0.77
603	RRAISGDLT	0.57	0.82	0.40	0.33	0.50	0.49	0.88	0.50	0.56	0.40	0.36	0.36
604	SAYRSVDEV	0.92	0.99	0.68	0.77	0.40	0.41	0.76	0.50	1.13	0.47	0.38	0.44
605	NTVSTSLGH	1.10	1.33	0.70	1.07	0.57	0.46	0.98	0.99	0.99	0.92	0.38	0.42
606	RASLG	1.32	1.60	0.80	0.94	0.65	0.48	1.21	1.06	0.77	0.89	0.35	0.50
607	AKDASKRGR	1.79	2.10	1.44	1.39	0.63	0.84	1.49	1.65	1.23	1.63	0.52	0.71
608	DDEASTTVS	0.87	0.61	0.88	0.75	0.58	0.53	0.78	0.41	0.74	0.61	0.40	0.54
609	ETAESSQAE	0.50	0.42	0.70	0.48	0.60	0.56	0.71	0.24	0.77	0.36	0.30	0.46
610	YTRFSLARQ	1.66	2.30	1.03	1.13	0.46	0.57	1.31	1.22	0.73	0.75	0.33	0.46
611	SPKQSPSSS	0.56	0.50	0.35	0.36	0.31	0.51	0.73	0.38	0.42	0.44	0.37	0.46
612	RKRSRKE	0.65	0.54	0.37	0.49	0.53	0.57	0.50	0.25	0.36	0.44	0.54	0.63
613	RPPGFSPFR	1.36	1.92	0.74	0.91	0.73	0.62	1.13	1.49	0.79	1.02	0.47	0.52
614	SARVYENVG	0.54	0.65	0.64	0.49	0.54	1.27	0.72	0.41	0.72	0.51	0.51	0.44
615	NRSASEPSL	0.46	0.39	0.42	0.58	0.69	0.54	0.70	0.23	0.50	0.43	0.50	0.52
616	KGYSLG	0.68	1.02	0.49	0.64	0.57	0.46	1.08	0.72	0.59	0.87	0.27	0.45

617	SSSSSPKAE	0.55	0.59	0.38	0.54	0.49	0.49	0.88	0.30	0.43	0.44	0.34	0.55
618	TKKTSFVNF	1.29	1.22	1.15	1.21	0.50	0.47	1.41	1.21	1.07	1.18	0.31	0.47
619	VEPLTPSGE	0.61	0.55	0.60	0.53	0.48	0.51	0.57	0.23	0.71	0.48	0.44	0.73
620	YRLPSNVDQ	0.64	0.59	0.43	0.36	0.52	0.56	0.64	0.33	0.45	0.36	0.36	0.48
621	SPAISIHEI	0.62	0.55	0.78	0.58	0.58	0.52	0.70	0.37	0.54	0.50	0.31	0.49
622	RKQISVR	0.53	0.76	0.61	0.59	0.58	0.69	0.86	0.52	0.59	0.68	0.42	0.44
623	VGWPTVRER	1.25	2.06	1.02	0.97	0.52	0.71	1.19	1.10	0.99	1.01	0.40	0.55
624	QTYRSFHDL	1.46	2.14	1.38	1.46	0.51	0.45	1.40	1.05	1.10	1.06	0.33	0.63
625	LRRASLGAA	2.66	3.07	2.32	3.08	0.68	0.83	1.65	1.14	1.42	1.46	0.46	0.80
626	PRKGSPKRG	0.81	0.96	0.46	0.64	0.53	0.58	0.85	0.35	0.65	0.51	0.48	0.49
627	SSPASLSRA	1.50	1.56	0.51	0.63	0.54	0.40	1.11	0.75	0.63	0.77	0.43	0.55
628	TKAASEKKS	0.53	0.49	0.41	0.50	0.65	0.50	0.67	0.27	1.19	0.57	0.35	0.65
629	LRRPSDQEV	0.42	0.52	0.53	0.76	0.52	0.47	0.78	0.47	0.56	0.43	0.37	0.75
630	PSAPSPQPK	0.39	0.46	0.41	0.53	0.54	0.69	0.71	0.47	0.50	0.43	0.39	0.63
631	KAKVTGRWK	1.98	2.67	1.97	1.98	0.63	0.63	1.95	2.03	2.52	2.33	0.66	0.85
632	KRPTQRAKY	2.51	3.53	2.67	3.42	0.98	0.88	2.32	2.30	2.81	2.87	0.74	1.07
633	PLSRTLSVRSL	1.49	2.24	1.62	2.37	0.62	0.58	1.24	0.89	1.07	1.03	0.44	0.64
634	QSPGSPLEE	0.50	0.76	0.79	0.68	0.44	0.58	0.64	0.46	0.72	0.69	0.37	0.58
635	KGGSYSQAA	0.64	1.15	0.52	0.66	0.59	0.61	0.80	0.88	0.87	0.61	0.53	0.61
636	KRRRSSKDT	1.32	1.41	1.42	1.47	0.81	0.80	1.20	1.54	1.79	1.66	0.97	1.01
637	KAAQISVRGL	0.70	0.67	0.83	0.87	0.84	0.74	1.08	0.78	0.86	0.88	0.88	0.93
638	HRTPSRSFG	2.21	2.89	1.05	1.58	0.84	0.62	1.88	1.54	1.23	1.47	0.56	0.60
639	LSGFSFKKN	1.08	1.21	1.03	1.48	0.83	1.10	1.22	0.89	1.20	1.13	0.51	0.52
640	EEEEYMPME	0.60	0.49	0.67	0.64	0.90	0.67	0.75	0.30	0.83	0.54	0.40	0.46
641	EQFSTVKGV	0.45	0.36	0.38	0.61	0.55	0.64	0.65	0.28	0.53	0.45	0.54	0.37
642	IDKISRIGF	0.46	0.54	0.69	0.95	0.44	0.51	0.91	0.54	0.78	0.90	0.41	0.43
643	MHRQETVDA	0.36	0.36	0.55	0.62	0.28	0.47	0.62	0.21	0.61	0.32	0.37	0.41
644	PYKFPSSPLRIPGZ	0.35	0.32	0.44	0.53	0.43	0.48	0.60	0.25	0.55	0.42	0.46	0.43
645	GNHTYQEIA	0.44	0.67	0.51	0.64	0.70	0.59	0.84	0.38	0.66	0.40	0.36	0.39
646	APVASPAAP	0.45	0.32	0.43	0.51	0.69	0.61	0.71	0.34	0.54	0.33	0.46	0.40
647	DGNNSDEES	0.63	0.50	0.93	0.79	0.69	0.56	0.64	0.40	1.00	0.77	0.46	0.52
648	FMTEYVVTR	0.53	0.49	0.69	0.75	0.42	0.63	1.03	0.57	0.91	1.64	0.56	0.40
649	GRGLSLSRF	1.53	1.61	1.37	2.30	0.62	0.74	1.71	1.05	1.29	1.62	0.75	0.67
650	EDTLSDSDD	1.53	1.64	1.91	2.95	0.51	0.72	1.07	0.68	1.54	1.97	0.69	0.53
651	ENQASEEED	0.52	0.60	0.97	0.93	0.47	0.48	0.78	0.45	1.33	1.18	0.41	0.44
652	RRPTSPVSR	2.00	2.99	1.34	1.51	0.90	0.71	1.73	1.29	1.13	1.42	0.63	0.63
653	SFMDSSGLG	0.58	0.63	0.65	0.56	0.71	0.46	0.80	0.44	0.93	0.48	0.25	0.59
654	QKRPSQRSK	1.66	2.16	1.31	1.82	0.84	0.98	1.57	1.42	1.63	1.74	0.84	0.78
655	SDEEH	0.48	0.44	0.57	0.50	0.43	0.55	0.68	0.43	0.99	0.49	0.56	0.48
656	APLTPGGRR	1.46	1.25	1.15	1.50	0.45	0.73	1.39	1.45	1.49	1.52	0.57	0.69
657	DEPSTPYHS	0.52	0.48	0.66	0.85	0.49	0.45	0.69	0.42	0.64	0.59	0.53	0.59
658	FGSRSLYGL	0.94	1.20	0.86	1.12	0.56	0.49	1.19	0.83	0.66	0.99	0.51	0.54
659	RDPVTENAV	0.60	0.87	0.53	0.86	0.59	0.49	0.78	0.40	0.53	0.55	0.38	0.60
660	AVRRSDRA	1.16	1.86	0.83	1.09	0.65	0.49	1.18	0.87	1.10	1.29	0.52	0.70
661	VKRGSGL	0.95	1.46	0.92	1.11	0.39	0.54	1.14	0.92	1.03	1.17	0.49	0.53
662	RRKDTPALH	0.70	0.85	0.64	0.76	0.36	0.47	1.05	0.67	1.03	0.92	0.64	0.53
663	SEITTKDLK	0.52	0.36	0.58	0.69	0.52	0.60	0.56	0.40	0.59	0.55	0.61	0.40
664	PNVSYIASR	0.81	0.72	0.85	1.07	0.53	0.53	1.14	0.95	0.92	1.19	0.47	0.59
665	RRDSV	1.01	1.28	0.82	1.41	0.54	0.59	1.26	0.96	0.85	1.02	0.66	0.58
666	STRRSIRLP	3.48	4.07	3.36	4.63	1.48	0.80	3.19	2.94	2.43	2.64	0.80	0.95
667	TPQVSDTMR	0.55	0.77	0.48	0.96	0.48	0.62	0.68	0.62	0.66	0.60	0.42	0.68
668	VNATYVNVK	0.49	0.52	0.52	0.78	0.46	0.42	0.56	0.34	0.64	0.54	0.45	0.59
669	RAAHSIKGG	1.25	1.27	0.95	1.35	0.46	0.54	1.29	1.34	1.03	1.21	0.53	0.44
670	SPSLSRHSS	2.10	1.97	1.60	2.15	0.88	0.87	1.59	1.35	1.41	1.55	0.84	0.69
671	RTPPPSG	0.78	0.91	0.58	0.74	0.76	0.68	1.04	0.77	0.62	0.87	0.62	0.52
672	VRKRTLRRL	2.18	2.56	2.30	2.60	0.71	0.76	1.79	2.33	2.28	2.57	0.69	0.64
673	SRKGSGFGH	1.44	1.87	1.32	2.11	0.54	0.61	1.27	1.25	1.45	1.39	0.54	0.60
674	LSGESDLEI	0.84	1.18	0.81	1.37	0.63	0.66	0.84	0.54	1.20	0.96	0.51	0.72

675	PSQRSKYLA	0.54	0.66	0.63	0.73	0.65	0.45	0.64	0.53	0.82	0.62	0.48	0.52
676	STDEYLDLS	1.82	1.96	1.90	2.81	0.88	0.92	0.78	0.78	1.18	1.34	0.66	0.73
677	TNHIYSNLA	0.74	0.64	0.85	1.01	0.76	0.70	0.68	0.72	0.76	0.66	0.61	0.65
678	LSRHSSPHQ	1.18	1.24	1.17	1.61	0.78	0.85	1.18	1.12	1.40	1.26	0.71	0.63
679	PTRHSRVAE	0.68	0.88	0.70	0.68	0.72	0.65	0.87	0.84	0.79	0.60	0.78	0.55
680	KISITSRKA	1.10	1.41	1.64	1.54	1.02	0.95	1.36	1.12	1.78	1.61	1.02	1.20
681	KSKISASRK	1.61	2.23	1.60	2.13	0.69	0.59	1.73	1.77	2.49	2.20	0.53	0.93
682	KRKQISVGGL	0.70	1.21	0.96	1.27	0.62	0.81	1.17	1.08	1.24	1.47	0.50	0.65
683	SRALSRQLS	2.22	3.15	2.16	2.94	0.75	0.79	1.89	1.78	1.43	1.95	0.74	0.64
684	KKKFSFKKP	1.80	2.17	2.79	2.97	1.03	0.87	1.88	3.08	3.74	3.41	0.69	0.63
685	KTRSSRAGL	2.00	2.19	2.14	3.16	0.79	0.81	1.62	1.97	2.26	2.39	0.80	0.71
686	PAPGSPEPP	0.63	0.83	0.71	0.89	0.70	0.95	0.82	0.56	0.74	0.84	0.60	0.60
687	IGSESTEDQ	0.38	0.57	1.02	0.72	0.53	0.50	0.64	0.23	1.05	0.99	0.55	0.46
688	MRRNSFTPL	0.66	0.79	0.85	0.99	0.59	0.82	1.04	0.81	0.61	0.89	0.72	0.80
689	EEQEYVQTV	0.55	0.44	0.87	0.83	0.52	0.54	0.71	0.45	0.86	1.05	0.57	0.42
690	ERRVSNAGG	0.36	0.35	0.51	0.47	0.60	0.65	0.86	0.52	0.59	0.75	0.49	0.69
691	IREESPPHS	0.21	0.24	0.42	0.35	0.46	0.51	0.64	0.27	0.60	0.60	0.37	0.47
692	NDSVYANWM	0.73	0.67	1.20	1.13	1.08	0.94	1.09	1.02	0.98	1.51	0.97	0.72
693	PYKFPSSPLRIPGZ	0.33	0.34	0.42	0.41	0.59	0.50	0.64	0.26	0.56	0.72	0.39	0.45
694	GRRESLTSF	1.09	1.59	1.14	1.60	1.15	1.16	1.50	1.26	0.89	1.35	1.40	1.21
695	ARVFSVLRE	0.74	1.01	1.15	1.20	0.91	0.99	1.27	1.33	0.95	1.44	1.10	1.06
696	DLPLSPSAF	0.67	0.77	1.08	1.03	0.78	0.86	0.83	0.62	0.84	1.15	0.85	0.68
697	GDKKSKKAK	0.85	0.99	0.93	0.68	0.77	0.80	1.45	1.58	1.26	1.39	0.68	0.82
698	GSDVSFNEE	0.53	0.55	1.03	0.97	1.11	0.97	0.82	0.65	1.15	1.45	0.87	0.74
699	EEKGSPLNA	0.23	0.23	0.39	0.38	0.50	0.43	0.59	0.30	0.58	0.64	0.37	0.45
700	ERQKTQTKL	0.56	0.75	0.65	0.50	2.17	1.08	1.05	1.20	0.89	1.25	0.97	1.29
701	RRRSSKDTS	0.77	1.12	0.86	1.06	0.81	0.93	1.18	0.86	0.93	1.03	0.92	1.04
702	SKAGSLGNI	0.41	0.46	0.49	0.52	0.36	0.53	0.80	0.51	0.64	0.59	0.55	0.67
703	MSGDEM	0.35	0.39	0.57	0.49	0.52	0.37	0.63	0.30	0.54	0.62	0.48	0.48
704	AAATTPAAE	0.35	0.32	0.34	0.29	0.55	0.38	0.61	0.29	0.54	0.51	0.36	0.44
705	ARKKSSAQL	0.27	0.25	0.50	0.43	1.24	1.37	0.72	0.43	0.67	0.80	1.30	1.19
706	DKVTSPTKV	0.37	0.34	0.46	0.36	0.65	0.53	1.04	0.55	0.63	0.75	0.50	0.53
707	FRRFTPDSL	0.45	0.55	0.66	0.46	0.64	0.59	1.11	0.87	0.79	1.12	0.57	0.60
708	RFTDTRKDE	0.51	0.45	0.52	0.60	0.43	0.49	0.60	0.30	0.71	0.47	0.65	0.62
709	RKISASEA	0.46	0.51	0.52	0.43	0.55	0.59	0.82	0.31	0.61	0.59	0.67	0.65
710	LARASLG	0.59	0.74	0.68	0.87	0.89	0.86	0.92	0.69	0.85	1.12	0.95	1.02
711	RRRLSSLRA	0.79	0.82	0.88	1.36	0.67	0.61	0.93	0.74	0.94	1.20	0.66	0.96
712	SGYISSLEY	0.95	1.17	1.33	1.85	1.50	1.52	0.91	0.71	1.00	1.46	1.57	1.18
713	LARNSI	0.38	0.24	0.50	0.48	0.69	0.51	0.63	0.36	0.68	0.86	0.39	0.44
714	TIAVG	0.32	0.34	0.48	0.46	0.60	0.53	0.58	0.47	0.61	0.74	0.39	0.36
715	TADISEDEE	0.93	1.12	1.40	1.28	0.84	0.90	1.03	0.78	1.41	1.43	1.04	0.87
716	TRQTSVSGQ	0.43	0.64	0.63	0.53	0.50	0.62	0.75	0.40	0.81	0.67	0.73	0.62
717	VSEEYLDLR	1.00	1.11	1.21	1.86	1.21	1.44	0.76	0.65	0.73	1.04	1.24	0.99
718	REQESSGEE	0.40	0.49	0.55	0.65	0.63	0.52	0.60	0.47	0.74	0.72	0.39	0.83
719	GTKRSGSV	1.04	1.22	0.99	1.55	1.34	1.28	1.30	1.34	1.05	1.48	1.52	1.31
720	NRLQTMKEE	0.47	0.26	0.52	0.43	0.56	0.70	0.71	0.38	0.70	0.65	0.69	0.61
721	VVELSGESD	0.68	0.61	1.25	1.08	0.67	0.61	0.87	0.71	1.53	1.59	0.66	0.70
722	SRRPSRATW	1.89	2.39	1.85	2.55	2.46	2.93	1.63	1.53	2.32	2.30	2.96	2.83
723	PASQTPNKT	0.39	0.68	0.62	0.63	0.58	0.73	0.64	0.23	0.61	0.62	0.61	0.57
724	QDPVSPSLV	0.41	0.52	0.71	0.85	0.55	0.71	0.73	0.40	0.84	0.78	0.64	0.85
725	STTVSKTET	0.35	0.39	0.53	0.42	0.55	0.58	0.64	0.42	0.52	0.57	0.79	0.70
726	TRKISASEF	0.36	0.42	0.60	0.61	0.62	0.64	0.59	0.44	0.59	0.65	0.44	0.64
727	PGPQSPGSP	0.39	0.24	0.48	0.45	0.51	0.54	0.66	0.26	0.59	0.77	0.53	0.50
728	KQGSGRGL	1.13	1.48	0.89	1.37	1.85	1.75	1.43	1.27	1.12	1.31	2.40	2.14
729	KKSWSRWTL	1.33	1.77	1.72	1.68	2.57	2.55	1.88	1.75	2.43	2.12	2.62	2.51
730	LASSSKEEN	0.42	0.59	0.56	0.64	0.66	0.89	0.61	0.31	0.72	0.79	0.82	0.74
731	PLRRTLSVA	0.67	0.86	1.14	1.50	0.67	0.85	0.69	0.51	1.13	0.85	0.84	0.74
732	SRPSSNRSY	1.91	2.88	1.73	2.04	3.13	3.89	1.46	2.03	1.59	2.71	4.27	4.48

733	KNDYYRKRG	0.99	1.08	1.05	1.27	1.57	1.61	1.23	1.37	1.46	1.78	1.82	1.84
734	LIEDAEYTA	0.54	0.53	0.75	0.89	1.08	1.03	0.74	0.50	0.98	1.19	1.36	0.98
735	QKAQTERKS	0.61	0.90	0.95	0.75	0.96	0.96	1.11	1.14	1.10	1.52	1.06	0.98
736	ISTESQPNG	0.31	0.20	0.38	0.29	0.57	0.39	0.62	0.33	0.46	0.51	0.30	0.43
737	NGNNYVYID	0.34	0.43	0.59	0.60	1.24	1.03	0.62	0.46	0.79	0.74	0.83	0.73
738	EIKKSWSRW	0.60	0.91	0.81	1.13	0.93	0.77	1.15	1.22	1.02	1.12	0.62	0.69
739	ESPASDEAE	0.43	0.42	0.89	0.60	0.73	0.48	0.55	0.59	1.13	1.06	0.43	0.45
740	KRKSSQALV	1.09	0.92	1.17	1.05	0.90	1.07	1.37	1.38	1.41	1.44	0.60	0.71
741	NNMPSSDDG	0.29	0.22	0.30	0.33	0.40	0.57	0.53	0.28	0.37	0.57	0.33	0.36
742	PYKFPSSPLRIPGZ	0.30	0.26	0.28	0.33	0.30	0.52	0.59	0.40	2.31	0.42	0.41	0.41
743	GSPGTPGSR	0.53	0.77	0.50	0.49	0.78	0.70	1.24	0.92	0.56	0.93	0.59	0.81
744	AVVRTPPKS	1.00	1.13	0.91	0.80	1.05	1.07	1.39	1.10	1.12	2.48	0.89	0.97
745	DRKLSTKEA	0.36	0.46	0.57	0.46	0.73	0.61	1.01	0.73	0.59	0.71	0.38	0.54
746	GGIRSLNVA	0.47	0.33	0.54	0.45	0.94	1.22	0.67	0.57	0.56	0.66	0.46	0.78
747	GTRLSLARM	1.00	1.08	1.28	1.16	1.59	1.87	1.36	1.47	0.94	1.25	1.42	1.34
748	EGNKSPAPK	0.35	0.42	0.38	0.39	0.58	0.72	0.95	0.73	0.28	0.56	0.45	0.31
749	ESHESMESY	0.40	0.46	0.60	0.46	0.47	0.50	0.86	0.67	0.83	0.85	0.45	0.25
750	RVRISADAM	0.38	0.32	0.64	0.35	0.56	0.41	0.69	0.35	0.50	0.62	0.34	0.47
751	SNDDSDDDD	0.66	0.73	1.26	1.04	0.87	0.70	1.07	0.81	1.16	1.40	0.60	0.58
752	RRPTPA	0.91	1.62	1.11	0.74	1.58	1.14	1.31	1.25	0.95	1.17	1.03	1.16
753	AEGSSNVFS	0.39	0.38	0.76	0.52	0.83	0.52	0.67	0.52	0.59	0.82	0.36	0.35
754	AVADSESED	0.76	0.60	1.20	1.07	1.22	0.90	1.02	1.08	0.92	1.32	1.11	0.74
755	DNPDYQQDF	1.30	1.26	1.33	1.02	1.29	1.21	0.99	0.97	0.99	1.24	1.11	0.94
756	GDVKYADIE	0.52	0.50	0.75	0.57	0.77	0.81	0.73	0.48	0.60	0.87	0.69	0.58
757	RLQDYEEKT	0.61	0.42	0.71	0.78	0.61	0.54	0.70	0.43	0.87	0.88	0.42	0.45
758	FKKSFKL	1.39	0.97	1.25	1.29	0.71	0.56	1.35	1.14	1.89	1.51	0.34	0.51
759	LRRPSLG	1.68	1.94	1.97	2.06	1.25	1.15	1.88	1.56	1.31	1.32	0.82	1.15
760	RRVRSQEPG	0.64	0.91	0.78	0.56	0.79	0.56	1.34	1.06	0.84	1.07	0.51	0.51
761	SLDDSGSAM	0.46	0.49	0.57	0.51	0.63	0.66	0.70	0.47	0.74	0.78	0.40	0.40
762	RRASVA	1.55	2.28	1.65	1.32	1.62	1.80	2.01	1.59	1.28	1.35	1.68	1.45
763	ADDEYAPKQ	0.60	0.61	0.77	0.76	0.77	0.68	0.81	0.65	0.76	1.03	0.71	0.69
764	TESQYQQQP	0.48	0.38	0.68	0.63	0.47	0.42	0.61	0.47	1.02	1.02	0.39	0.47
765	TVKSSKGGP	0.68	0.82	0.57	0.60	0.77	0.66	1.16	0.75	1.04	1.21	0.55	0.53
766	WTSDSAGEE	0.81	0.80	1.17	1.16	0.79	0.58	1.04	0.84	1.32	1.62	0.37	0.57
767	RKAASVIAK	1.15	0.97	1.35	1.25	0.95	0.67	1.41	1.17	1.24	1.41	0.60	0.74
768	LRRASLDG	0.44	0.48	0.51	0.75	0.68	0.39	0.79	0.36	0.61	0.91	0.32	0.42
769	LRRASGG	0.80	1.09	0.68	0.62	0.66	0.67	1.33	1.14	0.88	1.13	0.66	0.57
770	YHTTSHPGT	0.57	0.79	0.73	0.61	0.60	0.46	1.35	1.06	0.73	1.19	0.68	0.49
771	SSEESITRI	0.59	0.77	0.89	0.84	0.74	0.78	0.76	0.50	1.11	1.27	0.58	0.52
772	PLAGSPVIA	0.47	0.44	0.59	0.71	0.81	0.56	0.79	0.68	1.11	1.42	0.45	1.01
773	LGSPLRRR	2.73	3.04	3.75	4.53	2.73	2.82	2.86	3.74	4.67	4.14	2.78	2.61
774	TEDQYSLVE	1.35	1.48	1.47	1.74	1.12	1.08	1.12	1.21	1.50	1.50	1.10	0.96
775	TRTYSLGSA	0.86	1.19	0.89	0.75	0.65	0.42	1.51	1.01	0.99	1.14	0.38	0.44
776	PPSAYATVK	0.58	0.78	0.65	0.52	0.83	0.42	1.25	0.91	0.85	1.03	0.59	0.54
777	RTKRSGSV	2.28	3.10	2.00	1.94	2.81	2.72	2.93	2.32	1.97	1.76	3.18	2.41
778	KREASLDNQ	0.39	0.47	0.40	0.41	0.68	0.59	0.64	0.36	0.65	0.86	0.57	0.59
779	LMDKYHVDN	0.45	0.37	0.43	0.51	0.64	0.46	0.71	0.39	0.66	0.92	0.40	0.50
780	QQGMTVYGL	0.67	0.94	0.84	0.89	0.79	0.74	1.00	0.99	0.97	1.27	0.54	0.56
781	SRSRSRSRS	3.46	3.82	4.48	4.67	3.41	3.55	3.71	3.91	4.36	3.71	3.26	3.18
782	KRNSSPPPS	1.00	1.35	1.04	0.96	1.14	1.00	1.54	1.89	1.18	1.50	1.15	0.91
783	LQRYSSDPT	0.32	0.52	0.34	0.43	0.69	0.30	0.73	0.61	0.47	1.19	0.25	0.51
784	QRRTSLTGS	1.19	1.79	0.83	0.92	1.39	1.21	1.72	1.42	0.83	1.34	1.21	1.02
785	GVRQSRASD	1.03	1.56	0.45	0.55	0.46	0.75	1.01	0.75	0.58	0.62	0.52	0.75
786	KRPSNRAKA	1.95	2.78	0.99	1.18	0.91	1.54	1.64	1.41	1.22	1.15	1.21	1.62
787	EAVTSPRFI	0.78	0.81	0.37	0.65	0.53	0.56	0.88	0.49	0.54	0.55	0.57	0.76
788	EMTGYVATR	0.55	0.59	0.36	0.56	0.44	0.60	0.88	0.35	0.36	0.54	0.49	0.77
789	HKIKSGAEA	0.60	1.08	0.41	0.45	0.46	0.72	0.94	1.11	0.51	0.81	0.51	0.85
790	LARRSTTDA	0.52	0.87	0.49	0.58	0.50	0.73	0.93	0.64	0.60	0.57	0.55	0.83

791	PYKFPSSPLRIPGZ	0.35	0.37	0.37	0.43	0.57	0.59	0.63	0.49	0.58	0.38	0.48	0.79
792	GGTGTPNKE	0.46	0.61	0.24	0.54	0.50	0.51	0.63	0.58	0.31	0.31	0.50	0.66
793	ALGISYGRK	1.71	2.19	1.23	1.55	0.76	1.32	1.35	1.76	1.05	1.31	0.99	1.28
794	DDSGSAMSG	0.42	0.56	0.35	0.58	0.56	0.73	0.72	0.47	0.46	0.37	0.52	0.87
795	EYVQTVKSS	0.52	0.61	0.39	0.55	0.52	0.84	0.70	0.54	0.49	0.49	0.60	1.22
796	GKTDYMGEA	0.42	0.66	0.42	0.62	0.55	0.69	0.64	0.64	0.79	0.54	0.61	1.12
797	DSTYYKASK	1.03	1.42	1.22	1.16	0.65	0.76	1.16	1.06	0.91	0.91	0.63	1.43
798	EKEISDDEA	0.52	0.58	0.88	0.90	0.49	0.59	0.63	0.43	0.95	0.79	0.56	0.90
799	RRADSLQKN	1.05	1.56	0.42	0.74	0.62	0.70	1.06	1.01	0.77	0.58	0.65	0.80
800	SAYGSVKAY	0.62	0.72	0.38	0.71	0.50	0.65	0.76	0.60	0.43	0.54	0.62	0.93
801	NTSSSPQPK	0.81	1.25	0.50	0.59	0.66	1.00	0.90	0.81	0.54	0.60	0.85	1.21
802	GSRRR	2.33	3.42	2.04	2.20	2.24	3.01	2.11	3.15	2.12	2.70	2.14	3.45
803	AKAKTTKKR	1.79	2.78	2.13	2.32	2.39	2.44	2.21	3.11	2.53	2.79	1.79	2.52
804	DDAYSDTET	2.29	3.87	1.67	2.73	2.47	2.27	1.34	1.50	1.72	1.65	0.89	2.85
805	ESVDYVPML	0.96	1.35	1.12	1.21	0.92	1.04	0.73	0.78	0.95	1.04	0.80	1.52
806	YSTDYYREG	2.26	3.46	0.81	1.53	0.63	1.81	1.21	1.17	0.97	0.92	0.83	1.07
807	SPKKSPRKA	1.99	2.94	1.11	1.52	0.83	1.43	1.82	1.91	1.20	1.41	1.06	1.55
808	RKRSRKA	1.73	2.15	1.33	1.38	2.47	3.90	1.63	1.58	1.56	1.61	2.30	4.12
809	RPPASPSPQ	0.53	0.94	0.40	0.72	0.69	1.24	0.73	0.76	0.58	0.58	1.05	1.43
810	SARLSAKPA	0.86	1.54	0.55	0.97	0.99	1.34	1.00	1.06	0.86	0.89	1.07	1.59
811	NRQSSQARV	1.29	1.99	0.84	1.07	1.21	1.66	1.19	1.23	0.89	1.14	1.11	1.94
812	KEAKSD	0.29	0.57	0.50	0.50	0.53	0.91	0.60	0.38	0.59	0.64	0.70	1.01
813	SSSNTIRRP	3.18	4.61	1.40	1.85	1.86	3.40	2.06	2.25	0.84	1.02	2.81	3.61
814	TKKQSFKQT	1.23	2.07	0.71	1.38	0.68	0.99	0.99	1.38	0.92	0.89	1.02	1.19
815	VDSAYEVIK	1.37	2.01	0.67	1.27	1.03	1.58	0.81	0.90	0.87	0.72	1.08	1.84
816	YRKSSLKSR	1.41	2.98	1.58	2.95	1.89	2.46	1.45	1.78	1.77	1.72	2.21	2.97
817	SNVSSTGSI	0.57	0.82	0.42	0.93	0.79	1.53	0.65	0.90	0.67	0.54	1.27	1.97
818	RARSRKE	1.52	2.11	1.28	1.25	1.07	1.74	1.39	1.77	1.46	1.53	1.43	1.71
819	VGPGYLGSG	0.48	0.72	0.69	0.52	0.37	0.90	0.60	0.60	0.77	0.56	0.78	1.01
820	QTVKSSKGG	1.24	1.66	0.70	0.91	0.58	0.96	1.18	1.22	0.99	0.74	0.91	1.22
821	LRMFSFKAP	1.39	1.90	0.70	1.66	0.67	0.98	1.20	1.08	0.97	0.79	0.91	1.30
822	PRHLSNVSS	0.66	0.95	0.40	0.62	0.68	0.93	0.64	0.75	0.82	0.43	1.14	1.08
823	SSNEYMDMK	0.63	0.91	0.54	0.70	1.15	1.27	0.66	0.82	3.38	0.51	1.42	1.56
824	THYGSLPQK	0.92	1.38	0.55	0.89	0.67	1.49	0.86	0.89	0.95	0.74	1.22	1.67
825	LRRPSDQAV	0.84	1.19	0.50	0.76	0.62	1.25	1.00	0.81	0.71	0.67	0.97	1.21
826	PRRNSRASL	0.65	1.11	0.55	0.78	0.39	0.93	0.68	0.85	0.61	0.68	0.58	1.02
827	KAKTTKKRP	2.64	3.04	2.59	2.60	1.73	2.19	2.38	2.57	2.59	2.40	1.98	2.26
828	KRPSGRAKA	2.69	3.13	1.45	2.49	1.46	2.45	1.88	2.03	2.21	2.18	1.84	2.42
829	PLSKTLSVSSL	0.66	0.99	0.36	0.58	0.56	0.87	0.70	0.89	0.68	0.66	0.94	1.13
830	QSGMTEYVA	0.82	1.06	0.50	1.14	0.54	0.89	0.62	0.86	0.87	0.74	0.99	1.22
831	KGATSDEED	0.55	0.90	0.45	0.81	0.49	0.94	0.58	0.83	0.84	0.86	0.81	1.23
832	KRRNSEFEI	0.90	1.92	0.90	1.45	0.59	1.24	1.07	1.32	1.06	1.18	0.96	1.50
833	LTRRASFSAQ	2.32	3.03	1.89	3.61	0.75	1.67	1.42	1.98	1.34	1.56	1.24	2.09
834	HRQETVEAL	0.30	0.43	0.41	0.45	0.47	0.55	0.65	0.59	0.91	0.52	0.50	0.59
835	LRRFSLATM	1.42	1.58	0.87	1.49	1.02	1.16	1.12	0.89	0.94	0.78	1.62	1.81
836	EEEAYGWMD	1.03	1.68	0.97	1.41	1.42	1.49	0.81	0.51	0.81	0.80	1.48	1.50
837	EQESSGEED	0.49	0.57	0.68	0.86	0.66	0.76	0.59	0.65	0.97	0.85	0.59	0.70
838	IAADSEAEQ	0.43	0.58	0.65	0.68	0.66	0.68	0.57	0.81	1.09	0.64	0.73	0.54
839	MGEASGAQL	0.34	0.45	0.46	0.47	0.68	0.58	0.63	0.72	0.60	0.41	0.59	0.59
840	PYKFPSSPLRIPGZ	0.42	0.48	0.38	0.48	0.75	0.59	0.63	0.74	0.70	0.58	0.57	0.53
841	GNGDYMPMS	0.36	0.48	0.45	0.50	0.54	0.71	0.63	0.66	0.66	0.45	0.68	0.86
842	APRYPGGRR	1.76	2.54	2.50	1.55	2.47	2.82	1.81	1.88	2.08	1.80	3.17	3.65
843	DGNKSPAPK	0.47	0.64	0.56	0.54	0.66	0.80	0.79	0.58	0.59	0.49	0.73	0.96
844	FMTEYVATR	0.40	0.47	0.46	0.69	0.73	0.86	0.82	0.68	0.46	0.65	0.96	0.85
845	GRASSHSSQ	1.16	1.19	0.97	1.20	1.78	1.72	1.20	1.15	0.89	0.97	2.24	2.12
846	EDSTYYKAS	1.06	1.09	1.29	1.56	1.47	1.59	1.08	1.10	1.08	1.44	1.85	1.51
847	ENPQYFRQG	0.35	0.56	0.48	0.47	0.74	0.70	0.74	0.81	0.64	0.53	0.79	0.57
848	RRPTPATVA	1.00	1.64	0.59	0.63	0.91	1.04	1.00	1.05	0.85	0.66	1.09	1.46

849	SFKLSGFSF	0.37	0.63	0.63	0.59	0.45	0.72	0.69	0.70	0.75	0.46	0.70	0.85
850	QEKESERLA	0.32	0.52	0.40	0.49	0.48	0.71	0.62	0.69	0.64	0.55	0.70	0.83
851	RRVSV	1.51	1.92	1.20	1.46	2.00	3.08	1.41	1.61	1.16	1.17	3.26	3.77
852	APFTPGGRR	1.32	1.60	1.28	1.14	1.94	2.93	1.41	1.73	1.09	1.36	2.80	3.11
853	DEKLSEILG	0.56	0.74	0.74	0.79	0.78	0.98	0.65	0.72	0.65	0.74	1.00	0.85
854	FGHNTIDAV	0.41	0.47	0.57	0.62	0.83	0.79	0.65	0.89	0.65	0.43	0.76	0.70
855	RDEEYGYEA	1.91	2.60	1.49	1.85	1.63	2.09	0.83	1.04	1.26	1.09	1.29	2.33
856	AVRRSDAA	0.74	1.19	0.52	0.56	0.57	0.97	0.90	1.06	0.72	0.77	0.96	1.33
857	TKRSGSV	0.75	1.21	0.74	1.09	1.17	1.53	0.94	1.15	0.83	1.15	1.39	1.72
858	RRKATQVGE	0.43	0.48	0.44	0.47	0.56	0.86	0.63	0.82	0.56	0.44	0.73	0.95
859	SEGDSESGE	0.79	0.89	0.99	1.42	0.70	1.21	0.76	0.80	0.91	0.96	1.13	1.11
860	PLDRSSHAQ	0.51	0.58	0.71	0.62	0.74	0.96	0.64	0.68	0.61	0.67	0.93	0.92
861	RRASS	1.14	1.77	1.12	1.25	1.73	2.03	1.30	1.35	1.11	1.16	2.35	2.48
862	STPLSPTRI	0.93	1.47	0.66	0.77	1.02	1.88	1.07	1.06	0.92	0.96	1.47	2.33
863	TPPLSPSRR	1.90	2.80	1.39	1.91	3.46	4.98	1.57	2.00	1.33	1.40	4.90	5.84
864	VKRISGLIY	0.69	0.86	0.69	1.02	0.70	1.37	0.90	1.12	0.76	0.85	1.27	1.72
865	RAAASRARQ	1.67	1.75	0.83	1.36	2.07	3.23	1.20	1.49	1.08	1.16	3.65	4.44
866	SPRTPGGRR	1.58	2.36	1.54	1.50	4.08	5.18	1.76	2.47	1.82	1.71	4.51	4.91
867	RRRRSVA	2.48	2.98	2.26	2.60	4.18	5.11	1.81	2.90	2.87	2.48	5.25	5.34
868	VRFESIRLP	0.85	1.18	0.78	0.99	1.11	1.55	0.82	1.41	0.95	1.18	1.21	1.57
869	SRKESYSVY	0.71	0.85	0.64	0.66	0.52	0.99	0.69	0.90	0.57	0.70	0.93	1.20
870	LSELSRRRI	1.82	2.85	2.37	3.21	2.39	3.40	1.75	2.11	2.30	2.38	3.53	4.14
871	PSPSSRVTV	0.70	0.83	0.59	0.69	0.85	1.13	0.75	0.99	1.10	0.83	2.02	1.25
872	SSVPTPSPL	0.37	0.43	0.39	0.45	0.52	0.81	0.55	0.87	0.62	0.49	0.80	0.79
873	TNEEYLDLS	0.86	1.36	0.83	1.40	0.88	1.44	0.65	0.95	0.80	1.05	1.23	1.52
874	LSRFSWGAE	0.34	0.69	0.52	0.64	0.94	1.16	0.53	0.89	0.74	0.69	0.95	1.16
875	PTKRSPTKR	0.64	1.02	0.68	1.02	1.11	1.42	0.94	1.12	0.89	1.03	1.10	1.04
876	KISASRKLQ	1.56	2.07	1.20	1.59	1.61	2.80	1.36	1.60	1.51	1.67	2.34	3.04
877	KSFGSPNRI	1.06	1.60	1.01	1.25	1.54	2.23	0.96	1.25	1.35	1.28	2.28	3.01
878	KRKQISVAGL	0.83	1.18	1.09	1.25	1.02	1.41	1.00	1.34	1.30	1.49	1.16	1.31
879	SQHSTPPKK	0.52	0.93	0.57	0.58	0.54	0.91	0.66	0.93	0.84	0.96	0.83	0.91
880	KKIDSFASN	0.49	0.95	0.53	0.59	1.13	1.32	0.73	1.14	0.60	0.84	1.49	1.48
881	KTETSQVAP	0.36	0.52	0.40	0.56	0.78	0.88	0.64	0.88	0.51	0.41	0.83	0.80
882	PAAPSPGSS	0.46	0.66	0.59	0.78	0.87	1.18	0.65	0.93	0.78	0.87	0.86	0.93
883	IGRFSEPHA	0.34	0.51	0.59	0.62	0.83	0.81	0.94	0.97	0.62	0.98	0.86	0.70
884	MQLKSEIKQ	0.45	0.61	0.48	0.70	0.98	0.87	0.61	0.73	0.60	0.89	1.01	0.90
885	EEQEYIKTV	0.83	0.96	1.01	1.35	1.34	1.37	0.66	1.35	0.85	1.38	1.57	1.30
886	ERRPSNVSQ	0.47	0.69	0.49	0.61	0.55	0.70	0.92	0.89	0.57	0.64	0.81	0.93
887	IPPHTPVRT	0.86	1.54	0.78	0.80	1.25	1.65	1.23	1.02	0.90	1.34	1.58	1.70
888	NDSTSVSAV	0.37	0.32	0.43	0.46	0.61	0.79	0.59	0.52	0.70	0.52	0.69	0.86
889	PYKFPSSPLRIPGZ	0.37	0.31	0.38	0.71	0.50	0.62	0.61	0.43	0.58	0.87	0.64	0.53
890	GRPITPPRN	1.12	1.83	1.07	1.03	2.07	2.16	1.17	1.30	0.99	1.24	2.20	2.38
891	ARTKRSGSV	1.72	2.36	1.44	1.95	2.75	2.45	1.66	1.96	1.22	1.66	3.02	3.15
892	DLPGTEDFV	0.96	1.21	1.19	1.51	1.22	1.27	0.94	0.98	1.00	1.59	1.35	1.31
893	GALYSGSEG	0.71	0.77	0.72	0.70	0.85	1.05	0.72	0.91	0.98	1.14	1.01	1.10
894	GRVLTLPRS	1.49	2.31	1.56	1.29	2.43	4.03	1.61	1.77	0.99	1.52	3.16	3.85
895	EEKESSNDS	0.44	0.43	0.58	1.67	0.50	0.73	0.74	0.61	0.83	0.80	0.65	0.73
896	ERNLSFEIK	0.39	0.34	0.41	0.65	0.67	0.63	0.67	0.80	0.59	0.65	0.84	0.70
897	RRRRSRRAS	0.96	1.34	0.90	1.03	4.29	4.32	1.04	1.41	1.13	1.39	4.82	4.29
898	SIYSSDDDE	1.43	1.64	1.41	2.05	1.85	1.84	1.15	1.34	1.15	1.52	1.73	1.88
899	HGYSLG	0.61	0.65	0.44	0.57	0.92	0.88	0.57	0.97	0.70	0.91	1.05	0.88
900	AAASFKAKR	1.26	1.58	1.06	1.64	1.69	1.68	1.35	1.65	1.27	1.54	1.78	2.00
901	ARKFSSARP	3.91	2.21	1.31	1.33	1.65	2.30	1.61	1.63	1.49	1.69	2.40	2.74
902	DKEYYSVHN	1.42	0.49	0.63	0.79	0.62	0.80	0.66	1.00	0.87	0.94	0.92	0.80
903	FRKLSFTES	0.54	0.67	0.79	0.79	0.80	0.69	1.09	1.03	0.96	1.26	0.99	0.75
904	RFHKSSKDS	0.69	0.89	0.44	0.55	1.22	1.07	0.84	0.89	0.53	0.65	0.93	1.00
905	RAKRSGSV	2.21	2.70	1.64	2.14	1.28	1.95	1.90	1.89	1.43	1.85	2.03	2.05
906	KSESSQK	0.61	0.66	0.49	0.61	0.92	0.90	0.84	1.03	0.64	0.77	0.92	1.11

907	RRRLSDSNF	0.71	1.00	0.65	0.69	0.83	0.82	0.79	1.05	0.71	1.02	0.85	0.84
908	SGKTSPSSS	1.12	0.59	0.48	0.50	0.67	0.78	0.61	1.08	0.53	0.67	0.88	1.02
909	KSRRTI	1.04	1.07	0.80	0.98	0.79	0.78	1.12	1.38	1.01	1.41	0.99	0.78
910	TAILE	0.36	0.65	0.43	0.54	0.60	0.69	0.62	0.87	0.74	0.94	0.59	0.73
911	SYPLSPLSD	0.91	1.10	1.26	1.58	1.01	1.50	0.92	1.15	1.16	1.41	1.14	1.20
912	TRQASQAGP	0.71	0.73	0.38	0.53	0.96	0.89	0.96	1.07	0.59	0.72	0.83	0.99
913	VRVYTHEVV	0.55	0.62	0.47	0.53	1.12	0.77	0.59	0.92	0.98	0.86	0.64	0.96
914	RENEYMPMA	0.52	0.65	0.44	0.46	0.70	0.77	0.67	0.91	0.62	0.69	0.65	0.80
915	GRGLSLSR	2.84	3.46	1.83	1.82	2.51	4.13	2.50	2.36	1.28	1.66	3.99	4.68
916	YIYGSFK	0.75	0.91	0.72	0.87	0.61	0.80	1.12	1.20	0.79	1.00	0.75	1.00
917	VTRSSAVRL	1.47	2.19	1.44	1.54	1.36	1.93	1.60	1.60	1.36	1.63	1.77	2.16
918	SRRLSQETG	0.52	0.56	0.49	0.55	1.23	1.37	0.68	0.98	0.57	0.71	1.00	0.95
919	PASPSPQRQ	0.46	0.49	0.45	0.54	1.59	1.41	0.62	0.88	0.74	0.89	1.17	1.14
920	PWRITDNEL	0.49	0.65	0.76	0.97	1.20	1.04	0.75	0.98	0.96	1.40	0.75	1.11
921	STSVSAVAS	0.39	0.57	0.42	0.67	1.24	0.74	0.65	0.85	0.70	0.77	0.72	1.09
922	TRIPSAKKY	1.02	1.42	0.97	1.52	1.20	1.27	1.20	1.51	1.16	1.76	1.39	1.45
923	PGGSTPVSS	0.37	0.51	0.40	0.43	0.49	0.49	0.70	0.86	0.50	1.10	0.57	0.82
924	QGTLSKIFK	0.76	1.38	0.78	1.05	2.11	1.09	1.15	1.48	1.05	1.54	1.44	1.31
925	KKRLSVERI	0.97	1.36	1.52	1.48	2.17	2.64	1.40	1.51	1.87	1.83	2.42	2.06
926	KYRKSSLKS	1.13	1.54	1.44	1.72	1.53	1.98	1.30	1.52	1.58	1.72	1.56	1.37
927	PLSRRLSVA	0.83	1.24	0.91	1.52	1.07	1.65	0.77	1.16	0.72	0.92	1.23	1.27
928	SRLHSVRER	2.27	3.01	1.89	2.89	1.75	2.50	1.73	1.73	1.57	2.10	2.70	2.66
929	KNDKSKTWQ	0.75	0.93	0.67	0.84	1.11	0.73	1.09	1.78	0.76	1.36	0.77	0.93
930	LGGGTFDIS	0.49	0.63	0.65	0.88	0.80	1.11	0.63	0.78	0.88	1.28	1.15	1.20
931	QHLKSVMLQ	0.52	0.79	0.79	0.92	0.51	0.83	0.65	1.02	1.00	1.34	0.94	0.99
932	ISQESSEEE	0.69	0.60	0.92	0.78	0.55	0.79	0.69	0.51	1.54	1.49	0.85	0.54
933	NGDASPAAA	0.36	0.40	0.28	0.34	0.55	0.76	0.63	0.48	0.62	0.76	0.43	0.56
934	EHVSSSEES	0.28	0.38	0.43	0.38	0.54	0.59	0.66	0.51	0.70	0.95	0.52	0.54
935	ESMESYEVS	1.01	1.11	1.12	1.03	1.36	1.33	1.04	1.03	1.13	1.39	1.49	1.21
936	KQLASFEIY	0.31	0.44	0.46	0.46	0.76	0.69	0.56	0.56	0.65	0.80	0.45	0.45
937	NMPSSDDGL	0.20	0.46	0.50	0.51	0.61	0.45	0.71	0.62	0.51	1.00	0.35	0.39
938	PYKFPSSPLRIPGZ	0.26	0.39	0.32	0.39	0.56	0.38	0.69	0.54	0.49	0.66	0.36	0.43
939	GSLKSRKRA	1.51	2.19	3.39	1.70	1.76	1.91	2.24	2.28	1.91	2.23	1.69	1.73
940	AVRRSDRAY	0.89	1.26	1.31	0.76	1.58	1.47	1.35	1.19	1.10	1.39	1.31	1.84
941	DPTMSKKKK	0.69	1.02	0.88	0.69	1.07	0.99	1.39	1.12	1.04	1.22	1.11	1.03
942	GGGTSPVFP	0.27	0.48	0.31	0.41	0.55	0.49	0.69	0.62	0.49	0.89	0.49	0.44
943	GSTSTPAPS	0.21	0.42	0.14	0.30	0.46	0.40	0.69	0.49	0.25	0.67	0.22	0.39
944	EGGRTVGAG	0.22	0.36	0.27	0.24	0.41	0.37	0.66	0.39	0.25	0.68	0.30	0.40
945	ESHESLESY	0.65	0.96	0.70	0.73	0.84	1.06	1.06	0.76	0.57	1.07	1.15	0.89
946	RVLESFRAA	0.88	1.44	1.38	1.42	0.97	1.23	1.31	1.27	1.01	1.44	1.25	1.26
947	SMANSFVGT	0.45	0.62	0.50	0.49	0.53	0.65	0.80	0.63	0.68	0.92	0.64	0.82
948	RRPSPA	1.06	1.53	0.91	0.82	1.17	1.46	1.42	1.26	1.11	1.17	1.28	4.93
949	ADSFSLNDA	0.68	1.05	0.66	0.76	0.66	0.92	0.78	0.91	0.82	1.11	0.78	0.88
950	ATSASPPQK	0.63	0.71	0.37	0.56	0.75	0.85	1.19	0.90	0.51	0.77	0.71	0.78
951	DNLYYWDQD	0.88	1.19	0.90	1.22	1.02	1.12	0.93	0.81	0.93	1.10	1.29	0.80
952	GDSSYKNIH	0.43	0.80	0.41	0.59	0.44	0.59	1.17	1.00	0.46	1.02	0.40	0.51
953	RKRLSQDAY	0.29	0.47	0.27	0.45	0.52	0.55	0.61	0.51	0.60	0.90	0.49	0.69
954	ASGSFKL	0.48	0.55	0.45	0.44	0.61	0.70	0.89	0.54	0.63	0.91	0.75	0.78
955	LRRGSLG	1.60	2.27	1.47	1.84	1.70	2.66	1.74	1.51	1.35	1.39	2.22	2.56
956	RRSVSEAAL	0.28	0.62	0.34	0.48	0.37	0.57	1.03	0.54	0.51	0.82	0.47	0.63
957	SLAMSPRQR	0.43	0.78	0.44	0.53	0.46	0.76	1.13	0.92	1.24	0.87	0.68	0.94
958	RRASLG	1.50	2.70	1.27	1.39	1.71	2.52	1.93	1.68	1.27	1.21	1.38	1.79
959	AAVDTSSEI	0.52	0.95	0.55	0.93	0.70	1.08	0.94	0.68	0.54	1.16	0.96	0.75
960	TESQSLTLT	0.38	0.52	0.55	0.60	0.50	0.64	0.74	0.95	0.83	0.96	0.64	0.96
961	TTRVTPLRT	1.39	1.75	1.51	1.24	1.46	3.00	1.47	1.24	1.62	1.38	2.30	2.80
962	WTADSGEGD	0.89	1.06	0.99	1.06	0.69	1.19	1.05	0.81	1.29	1.68	1.57	1.35
963	RHRDTGILD	0.42	0.57	0.37	0.51	0.37	0.69	0.79	0.53	0.77	1.04	0.55	0.85
964	NRIYTHQVV	0.88	1.10	0.95	1.04	0.88	1.06	1.22	1.02	1.00	1.15	1.24	5.31

965	LRKASLG	0.83	0.81	0.57	0.51	0.69	1.05	1.25	0.91	0.43	0.71	1.16	1.59
966	YHGHSMSDP	0.41	0.51	0.30	0.49	0.47	0.72	0.91	0.74	0.28	0.86	0.61	0.73
967	SSEESIISQ	0.64	0.69	0.70	0.74	0.63	1.05	0.68	0.96	0.94	1.17	1.04	1.00
968	PKRGSGKDG	0.56	0.58	0.68	0.61	0.61	0.89	0.92	0.95	0.78	1.11	1.14	0.88
969	LGSALRRR	3.64	3.89	4.25	4.05	4.15	5.35	3.46	3.42	4.08	3.81	5.61	5.62
970	TDEDSDNEI	1.20	1.60	1.37	1.61	0.86	1.39	1.20	1.16	1.07	1.45	1.25	1.60
971	TRSVSSSSY	1.21	1.31	0.84	0.80	1.11	1.45	1.52	1.09	0.77	1.09	1.62	1.71
972	PPRRSSIRN	3.88	3.66	4.07	3.59	5.02	6.11	3.32	2.54	2.05	2.33	6.70	6.27
973	RTKGSGSV	1.29	1.78	1.14	1.00	2.14	2.16	1.84	1.46	0.93	1.42	2.49	2.21
974	KRAASPRKS	1.03	1.65	1.51	1.53	2.83	3.34	1.52	1.78	2.33	2.05	3.76	3.24
975	LMAPSEEDH	0.36	0.53	0.51	0.68	0.68	0.56	0.67	0.92	0.89	1.12	0.78	0.83
976	QNPVYHNQP	0.61	0.81	0.73	0.67	0.71	1.23	1.01	1.07	0.89	1.10	1.06	1.13
977	SRSRSRSPG	3.17	3.59	3.23	3.85	4.59	5.92	3.26	3.52	3.44	3.81	5.51	5.64
978	KRKVSSAEG	0.89	1.24	0.89	0.80	1.54	1.88	1.33	1.26	1.23	1.39	2.19	2.08
979	LQDDYEDMM	1.14	1.40	0.87	1.31	1.28	2.21	1.16	1.12	0.88	1.24	2.07	1.75
980	QRRSSEGST	0.40	0.65	0.57	0.47	0.59	0.94	0.95	0.57	0.43	1.01	0.64	0.79
981	GVLRRASVA	1.90	2.96	0.87	2.18	0.94	1.74	1.28	1.68	0.95	1.03	1.23	1.85
982	KRPSLRAKA	2.60	3.39	1.14	2.94	1.08	2.10	1.75	2.27	1.58	1.59	1.50	2.10
983	EASTTVSKT	0.66	1.12	0.31	0.53	0.45	1.05	0.60	0.93	0.46	0.56	0.93	1.23
984	ELSNYIAMG	0.55	0.74	0.34	0.52	0.32	0.83	0.55	0.86	0.66	0.50	0.71	0.92
985	HHHATPSPP	0.83	0.99	0.39	0.53	0.38	0.81	0.74	1.02	0.59	0.46	0.52	1.13
986	KTETSQVAP	0.50	0.61	0.37	0.47	0.45	0.87	0.64	0.95	0.45	0.45	0.56	0.85
987	PYKFPSSPLRIPGZ	0.54	0.85	0.40	0.48	0.56	0.80	0.59	0.79	0.57	0.51	0.68	0.65
988	GGSVTKKRK	2.16	2.37	1.41	1.97	0.83	1.15	1.90	2.26	1.75	1.58	0.98	0.67
989	AKRISGKMA	2.05	2.42	0.78	1.21	0.76	1.44	1.27	1.40	1.02	0.97	1.05	0.98
990	DDPSYVNVQ	0.96	1.12	0.41	0.69	0.44	1.22	0.67	0.72	0.65	0.59	0.95	0.70
991	EVEKSPVKS	0.61	0.69	0.34	0.49	0.20	0.88	0.53	0.82	0.65	0.33	0.85	0.75
992	GKSSSYSKQ	1.24	1.68	0.61	0.81	0.37	1.17	1.16	1.28	0.90	0.89	0.90	1.01
993	DSRSSLIRK	1.58	2.23	0.89	1.60	0.47	1.49	1.24	1.49	0.92	0.92	0.85	1.11
994	EKAKSPVPK	0.84	1.40	0.54	0.73	0.56	1.31	0.91	1.32	0.87	0.74	0.90	0.83
995	RQRKSRRTI	2.29	2.86	1.29	1.66	0.96	1.80	1.70	2.11	1.39	1.30	1.50	1.50
996	SAYATVKAY	0.97	0.92	0.27	0.60	0.38	1.02	0.33	0.80	0.48	0.41	0.67	0.90
997	NTDGSTDYG	2.17	2.86	0.67	1.80	0.38	2.06	0.83	1.17	0.96	0.91	0.74	0.84
998	KRTLR	2.33	2.77	1.42	2.28	0.66	2.30	1.53	2.27	1.43	1.74	1.42	1.78
999	AGPTSARDG	0.70	0.76	0.30	0.42	0.16	0.88	0.56	0.99	0.44	0.38	0.58	0.83
1000	DAPDTPELL	0.79	1.09	0.71	0.86	0.46	1.22	0.73	0.91	0.62	0.57	0.61	1.20
1001	ESSYSYEEI	1.71	2.00	0.86	1.55	0.52	1.85	0.85	1.07	0.91	0.77	0.89	1.01
1002	YSLGSALRP	1.26	1.87	0.29	0.72	0.61	1.02	0.75	1.14	0.40	0.50	0.67	0.82
1003	SPHQSEDEE	0.78	0.83	0.31	0.45	0.35	1.09	0.27	0.78	0.48	0.43	0.67	0.77
1004	RKRSRAE	1.47	1.70	0.84	0.88	0.50	1.42	0.83	1.24	0.96	0.87	1.39	1.12
1005	RNASTNDSP	0.66	0.87	0.45	0.54	0.25	0.90	0.43	0.88	0.55	0.44	0.62	0.77
1006	SAELYSNAL	1.01	1.22	0.48	0.90	0.36	1.01	0.44	1.03	0.70	0.68	0.50	0.81
1007	NRQLSSGVS	0.82	1.06	0.33	0.69	0.39	1.19	0.54	1.17	0.61	0.38	0.67	0.93
1008	VGPDSD	0.57	0.86	0.36	0.71	0.18	0.88	0.34	0.92	0.65	0.55	0.73	0.62
1009	SSSESGAPE	0.63	1.01	0.21	0.71	0.29	0.73	0.25	0.72	0.30	0.39	0.43	0.51
1010	TKHIYSNLA	0.75	0.93	0.26	0.65	0.49	0.91	0.22	0.91	0.27	0.48	0.57	0.82
1011	VDEMYREAP	0.70	0.86	0.29	0.46	0.24	1.07	0.36	0.82	0.51	0.45	0.66	0.73
1012	YRKGSLKSR	1.28	1.45	0.56	1.21	0.58	1.31	0.90	1.34	0.79	0.77	0.57	0.92
1013	SNQEYLDLS	1.43	1.70	0.52	1.05	0.43	1.48	0.44	1.00	0.74	0.63	0.54	0.99
1014	RKISASE	0.72	1.08	0.41	0.68	0.47	1.28	0.32	1.08	0.69	0.47	0.61	0.93
1015	VGFMTEYVA	0.77	1.00	0.50	0.81	0.26	1.35	0.24	1.04	0.63	0.54	0.85	0.97
1016	QTASSPLSP	0.55	0.73	0.14	0.45	0.46	0.91	0.34	0.78	0.30	0.28	0.45	0.57
1017	LRKVSKQEE	0.61	1.13	0.32	0.58	0.56	1.32	0.37	1.01	0.41	0.50	0.65	1.09
1018	PQRATSNVF	0.66	1.05	0.15	0.58	0.64	1.34	0.48	0.76	0.45	0.49	0.52	1.05
1019	SSNDSTSVS	0.94	1.20	0.28	1.10	0.45	1.20	0.67	1.03	0.64	0.55	0.44	0.90
1020	THVASVSDV	0.68	0.81	0.32	0.65	0.54	1.33	0.53	0.97	0.58	0.46	0.50	0.89
1021	LRRLSTKYR	2.73	3.39	1.66	3.17	0.95	2.05	1.45	2.09	1.53	1.93	1.04	1.33
1022	PRRDSTEGF	0.89	1.16	0.62	0.97	0.52	1.70	0.33	1.06	0.98	0.82	0.69	0.76

1023	KAEEYILKK	0.56	0.69	0.36	0.58	0.71	1.83	0.53	0.89	0.77	0.47	0.51	0.79
1024	KRPSFRAKA	2.61	3.02	2.15	3.06	1.81	3.80	1.33	1.87	1.53	1.73	1.77	2.80
1025	PTPSAPSPQPKG	0.71	0.78	0.34	0.59	0.57	1.56	0.65	0.72	0.44	0.50	0.70	1.46
1026	QRYSSDPTG	0.48	0.77	0.26	0.49	0.54	1.08	0.44	0.97	0.47	0.44	0.48	0.93
1027	KEDTYTAHA	0.49	0.63	0.30	0.47	0.49	1.46	0.56	1.04	0.60	0.42	0.61	0.68
1028	KRRLSFSET	1.29	1.64	0.73	1.20	0.94	2.20	0.99	1.48	1.50	1.11	0.98	1.23
1029	KRAKAKTTKKR	2.93	3.46	3.07	4.17	1.95	3.67	2.67	4.04	3.79	4.15	2.30	2.37
1030	HRQETVDAL	0.36	0.68	0.63	0.48	0.47	0.81	0.65	0.65	0.42	0.70	0.68	0.69
1031	LNRMSFASN	0.46	0.93	0.44	0.82	0.53	0.96	0.67	0.81	0.72	0.54	0.72	1.06
1032	EEDTYTMPS	1.03	1.41	0.82	1.10	0.65	1.15	0.58	0.98	1.21	0.91	0.62	1.16
1033	EQEEYEDPD	1.19	1.17	0.80	0.97	0.57	1.76	0.62	1.10	1.00	0.98	1.05	1.29
1034	HVSSSEESI	0.45	0.75	0.45	0.65	0.41	0.97	0.49	0.71	0.70	0.74	0.68	0.87
1035	METPSQRRA	0.69	1.21	0.52	0.70	0.68	1.55	1.04	1.07	0.74	0.90	1.07	1.34
1036	PYKFPSSPLRIPGZ	0.34	0.54	0.35	0.41	1.20	0.86	0.65	0.82	0.58	0.64	0.66	0.82
1037	GNFNYVEFT	0.91	1.37	0.82	1.44	0.56	1.76	0.77	0.86	0.95	0.85	1.38	1.15
1038	APRTPGGRR	2.14	2.72	1.64	1.65	0.96	3.72	1.73	2.34	2.57	1.81	2.71	2.44
1039	DGNGYISAA	0.63	1.10	0.76	0.74	0.48	1.34	0.71	1.61	0.81	0.90	0.65	1.12
1040	FLTEYVATR	0.57	1.04	0.67	0.52	0.53	1.18	0.68	1.21	0.80	0.77	0.62	0.79
1041	GRALSTRAQ	1.43	2.21	0.84	1.19	1.15	3.14	1.37	1.59	0.97	1.01	2.08	3.55
1042	EDRMSLVNS	0.49	0.65	0.40	0.45	0.67	1.10	0.59	0.73	0.43	0.38	0.73	1.12
1043	ENPEYLGLD	0.82	1.65	0.65	1.30	0.81	1.57	0.76	0.95	0.85	1.04	1.15	1.25
1044	RRLSSLRAS	2.70	3.18	1.56	2.50	0.86	4.78	1.64	2.42	1.37	1.30	2.18	3.69
1045	SFKKSFKLS	1.60	1.86	1.28	1.87	0.76	1.85	1.21	1.95	1.42	1.56	1.11	1.43
1046	PSKKYAIKG	0.44	0.80	0.40	0.63	0.79	1.34	0.55	0.85	0.51	0.74	0.54	0.69
1047	RRPSV	1.29	2.00	0.94	1.37	0.76	2.00	1.10	1.45	0.90	0.91	1.11	1.42
1048	APETPGGRR	0.86	1.56	0.90	1.45	1.00	1.77	1.06	1.26	1.18	1.13	1.12	1.44
1049	DEEMSETAD	0.57	0.84	0.46	1.04	0.45	1.19	0.70	0.83	0.86	0.74	0.75	1.16
1050	FFSSSESGA	0.47	0.77	0.48	0.63	0.45	1.33	0.68	1.04	0.84	0.68	0.75	1.05
1051	RDDTYTAHA	0.65	0.89	0.41	0.59	0.24	1.10	0.38	1.13	0.75	0.60	0.72	0.86
1052	APTPGGRR	1.44	2.32	1.33	1.24	0.24	1.03	1.19	2.19	1.77	1.43	0.53	0.79
1053	STNDSPL	0.46	0.83	0.41	0.44	0.28	1.43	0.60	0.85	1.11	0.66	0.50	1.02
1054	RRKASGPPV	0.77	1.48	0.64	0.62	0.59	1.36	0.82	1.14	0.82	0.95	1.06	1.10
1055	SEENSKKTV	0.38	0.68	0.37	0.56	0.42	1.23	0.48	0.76	0.48	0.54	0.69	0.79
1056	PASPSPQRQ	0.67	1.08	0.49	3.03	0.48	1.22	0.78	0.85	0.65	0.71	0.82	1.16
1057	RRASR	1.89	2.51	1.64	1.87	1.05	2.35	1.69	1.83	1.83	1.84	1.63	2.28
1058	STNEYMDMK	0.50	0.62	0.32	0.56	0.27	1.25	0.13	0.92	0.68	0.45	0.69	0.84
1059	TPPLSPIDM	0.45	0.70	0.42	0.60	0.16	1.10	0.30	0.98	0.64	0.54	0.58	0.80
1060	VKGATSDEE	0.46	0.76	0.41	0.49	0.28	1.27	0.42	0.78	0.69	0.88	0.52	0.87
1061	QRATSNVFA	0.39	0.79	0.44	0.61	0.50	1.39	0.43	0.91	0.60	0.79	0.81	0.89
1062	SPRKSPRKS	1.32	2.39	1.44	1.61	0.98	2.30	1.27	2.05	1.38	1.80	1.56	1.66
1063	RRRASVA	1.44	2.50	1.25	1.81	1.04	3.40	1.11	1.70	1.28	1.39	1.71	3.22
1064	VPTPSPLGP	0.48	0.86	0.43	0.70	0.43	1.24	0.71	0.92	0.72	0.65	0.64	1.49
1065	SRKDSLDDS	0.69	0.95	0.47	0.85	0.45	1.56	0.27	0.96	0.97	0.85	0.88	1.02
1066	LSEHSSPEE	0.38	0.70	0.32	0.60	0.26	1.19	0.18	0.95	0.61	0.49	0.73	1.01
1067	PSPKYPGPQ	0.34	0.59	0.31	0.38	0.32	1.28	0.17	0.91	0.44	0.71	0.53	0.72
1068	SSVLYTAVQ	0.47	0.75	0.41	0.58	0.22	1.27	0.21	0.94	0.66	0.71	0.50	0.87
1069	TLSDSDDED	0.73	1.59	0.67	1.58	0.49	1.62	0.43	1.14	1.00	1.60	0.64	1.09
1070	LSLDSQGRN	0.38	0.74	0.42	0.77	0.74	1.58	0.43	0.97	0.42	0.61	0.64	1.16
1071	PTKRSPQKG	0.32	0.72	0.37	0.60	0.52	1.52	0.63	1.00	0.66	0.61	0.69	1.30
1072	KIQASFRGH	1.57	2.48	1.50	2.54	1.51	3.47	0.91	2.04	3.82	1.76	2.24	2.46
1073	KRSNSVDTS	0.66	1.38	0.42	0.76	0.56	1.81	0.39	1.08	0.64	0.81	1.28	1.42
1074	KRKQISGRGL	0.63	1.23	0.48	0.87	0.48	1.52	0.54	1.19	0.81	1.05	1.23	0.98
1075	SQESSEEEQ	0.48	0.88	0.45	0.76	0.39	1.16	0.37	0.86	0.73	0.94	0.57	0.60
1076	KKDVTPVKA	0.66	1.36	0.72	0.99	0.93	1.86	0.90	1.33	0.91	1.22	1.35	1.44
1077	KSRWSGSQQ	0.90	1.50	0.70	1.57	0.76	2.02	1.15	1.46	1.08	1.24	1.52	2.01
1078	LYSGSEGDS	0.63	1.44	0.56	1.73	0.58	1.99	0.64	1.23	1.02	1.23	0.78	1.63
1079	IGHHSTSDD	0.36	0.54	0.46	0.70	1.26	1.17	0.54	0.81	0.62	0.48	0.73	0.79
1080	MPGETPPLS	0.44	0.72	0.36	0.56	1.17	1.32	0.65	0.91	0.49	0.69	0.78	1.11

1081	EEPVYEAEP	1.78	1.80	1.21	2.13	0.80	2.08	0.99	1.29	1.40	1.97	1.21	1.53
1082	ERRNSILTE	0.58	1.01	0.51	1.05	0.59	0.98	0.76	1.06	0.64	1.05	0.72	0.84
1083	INETSQHHD	0.24	0.36	0.31	0.44	0.72	0.72	0.60	0.82	0.43	0.73	0.47	0.83
1084	NDSNYVVKG	0.30	0.37	0.33	0.48	0.54	0.61	0.61	0.87	0.55	0.87	0.50	0.81
1085	PYKFPSSPLRIPGZ	0.31	0.44	0.33	0.52	0.54	0.70	0.64	0.86	0.62	0.70	0.62	0.67
1086	GRNASTNDS	0.53	0.59	0.48	0.55	0.65	1.03	0.78	0.92	0.63	0.55	0.71	0.89
1087	ARSGSSTYS	1.16	2.06	0.69	1.01	0.73	2.45	1.40	1.57	0.72	1.12	1.70	2.67
1088	DLLTSPDVG	0.48	0.73	0.98	0.79	0.70	1.16	0.67	0.93	0.79	1.09	0.66	1.19
1089	GAGNSLRTA	0.70	1.18	0.44	0.71	0.72	1.23	1.28	1.19	0.73	1.01	0.97	1.19
1090	GRTWTLAGT	0.53	1.02	0.29	0.53	0.56	1.06	1.16	1.29	0.64	0.91	0.74	0.79
1091	EEHVYSFPN	0.52	0.77	0.47	0.72	0.64	1.18	0.77	1.02	0.75	0.97	0.73	0.74
1092	ERHHSIDAQ	0.50	0.55	0.52	0.49	0.71	1.15	0.75	0.97	0.53	0.71	0.72	0.71
1093	RRRRAASVA	2.60	3.45	2.36	2.25	0.98	3.32	2.12	2.45	2.47	2.02	2.25	2.67
1094	SIRDTPAKN	0.46	0.75	0.49	0.62	0.41	1.22	0.79	0.87	0.70	0.62	0.64	1.32
1095	GGYSLG	0.46	0.77	0.59	0.65	0.50	1.13	0.86	1.04	0.70	0.94	0.63	1.12
1096	AAASFKAKK	0.83	1.36	0.76	1.19	0.78	1.39	1.24	1.46	1.09	1.29	1.11	1.36
1097	ARDIYKNDY	0.61	0.79	0.51	0.90	0.49	1.22	0.69	1.03	0.99	1.19	0.87	1.11
1098	DKEVSDDEA	0.51	0.71	0.72	0.94	0.75	1.54	0.61	0.97	0.87	1.30	0.84	0.89
1099	FRKFTKSER	1.49	2.36	1.58	1.97	1.27	2.59	1.56	1.96	2.19	1.92	1.69	1.54
1100	RFFGSDRGA	1.05	2.18	1.05	1.52	0.61	1.88	1.32	1.55	0.94	1.22	1.23	2.01
1101	PRTPGGRR	0.67	1.36	0.83	0.87	0.39	1.06	1.02	1.27	0.95	1.23	0.57	1.27
1102	KRRVSEV	0.46	0.98	0.38	0.70	1.35	2.00	0.98	1.16	0.74	1.03	1.67	1.99
1103	RRRGSSIPQ	1.20	1.95	0.79	1.32	0.85	1.50	1.32	1.67	1.12	1.25	1.33	1.92
1104	SGDTSPRHL	0.40	0.52	0.41	0.63	0.51	0.99	0.74	0.90	0.58	0.80	0.60	1.05
1105	KQITVR	0.90	1.37	0.83	0.97	2.64	1.79	1.23	1.53	1.14	1.40	1.23	1.23
1106	SSKRA	1.06	1.90	1.37	1.05	1.34	2.06	1.32	1.64	3.74	1.47	1.57	1.37
1107	SVSSSSYRR	3.06	4.12	2.34	3.80	1.00	4.34	2.29	3.18	2.10	2.81	2.79	4.77
1108	TRQASISGP	0.68	1.48	0.55	1.06	0.49	1.56	1.10	1.24	0.77	1.00	0.81	2.33
1109	VRTYTHEVV	0.52	0.90	0.49	0.78	0.79	1.43	0.80	1.14	0.90	2.05	0.73	1.49
1110	REILSRRPS	2.59	3.45	1.33	2.96	1.37	3.79	1.83	2.65	1.37	1.49	2.41	4.11
1111	GASGSFKL	0.45	0.97	0.50	1.10	0.79	1.00	1.01	1.12	0.81	1.02	0.66	1.60
1112	VRRSDRA	0.90	1.83	0.81	1.10	0.74	1.47	1.13	1.53	1.18	2.00	1.27	1.58
1113	VTRRTLSMD	1.06	1.76	1.02	1.72	0.69	1.55	1.15	1.66	1.63	1.34	1.29	1.48
1114	SRRGSESSE	0.79	1.29	0.67	1.13	0.69	2.43	0.80	1.24	0.89	0.84	0.93	1.96
1115	PASAYGSVK	0.44	0.84	0.43	0.75	0.37	1.63	0.68	1.28	0.53	0.63	0.66	1.53
1116	PWQVSLRTR	0.44	0.79	0.42	0.85	0.34	1.53	0.64	1.10	0.79	0.94	0.75	1.32
1117	STSRSLYSS	1.54	3.98	1.26	3.09	1.38	4.00	1.81	2.42	1.86	1.78	3.07	4.42
1118	TRGGSLERS	0.48	1.34	0.53	1.28	0.64	1.34	1.05	1.21	0.77	1.23	1.12	2.49
1119	PFKLSGLSF	0.23	0.63	0.29	0.85	0.54	1.14	0.63	0.82	0.69	0.92	0.62	0.86
1120	QEQEYVQAV	0.37	0.38	0.29	0.62	0.44	1.30	0.55	0.89	0.76	1.05	0.97	1.06
1121	KKRFSFKKS	1.70	2.46	2.54	3.23	1.37	3.37	1.92	3.00	4.33	3.76	3.32	3.08
1122	KYLASASTM	0.60	1.22	0.65	1.04	0.52	2.00	0.84	1.19	1.04	0.99	1.00	1.82
1123	PRRVSRRRR	0.37	0.87	0.76	0.86	0.27	1.55	0.80	1.09	1.04	0.96	0.62	1.34
1124	SRKRSGEAT	0.41	1.06	0.33	0.77	0.54	2.14	0.73	1.03	0.66	0.91	1.02	1.51
1125	KMKDTDSEE	0.20	0.65	0.29	1.06	0.57	1.06	0.72	0.98	0.64	1.03	0.50	1.18
1126	LFRLSEHSS	0.18	0.48	0.22	0.77	0.32	1.04	0.56	0.90	0.44	0.75	0.51	0.64
1127	QDENTVSTS	0.31	0.66	0.22	1.09	0.26	1.00	0.58	0.99	0.60	0.99	0.41	0.66
1128	ISITSRKAQ	0.48	0.96	0.48	0.56	0.84	1.01	0.94	1.30	0.75	1.21	1.09	1.10
1129	NFLKTSAGS	0.41	0.59	0.42	0.52	0.57	0.79	0.99	1.07	0.55	0.87	0.83	0.87
1130	EHIPYTHMN	0.50	0.91	0.50	0.54	0.51	1.19	0.87	1.14	0.90	1.12	0.74	0.87
1131	ESMESYELN	1.38	1.48	1.27	1.15	0.66	2.09	1.13	1.22	0.98	1.25	1.65	1.25
1132	KKRFSFKKS	2.57	2.39	1.84	2.29	1.36	2.68	2.88	2.52	3.03	2.74	2.00	1.88
1133	NKQGYKARQ	0.84	1.58	1.32	0.57	1.31	1.93	1.26	1.47	0.64	1.18	1.77	1.50
1134	PYKFPSSPLRIPGZ	0.49	0.63	0.70	0.44	0.50	0.87	0.76	0.97	0.42	0.81	0.62	0.76
1135	GSGTSSRPS	0.67	1.18	0.67	0.50	0.53	1.08	1.06	1.26	0.62	0.94	0.63	0.70
1136	AVMVSHYIH	0.97	1.28	1.04	1.07	0.64	1.55	1.13	1.39	1.24	1.34	1.60	1.17
1137	DPPGTESFV	0.90	1.39	1.25	1.24	0.60	1.73	1.00	1.45	1.14	1.47	1.23	1.06
1138	GFKRSYEEH	0.71	1.01	1.87	0.63	0.60	1.41	1.06	1.21	0.77	1.14	0.77	0.75

1139	GSRRRRRRY	1.67	1.88	1.85	1.00	2.26	3.86	2.25	2.12	1.56	1.71	3.60	2.70
1140	EFPLSPPKK	0.75	1.01	0.83	0.49	0.81	1.47	1.00	1.22	1.23	1.10	1.25	1.38
1141	ESEKTKTKE	0.48	0.60	0.86	0.49	0.61	1.01	0.72	0.98	0.57	0.80	0.62	0.71
1142	RSSMSGLHL	0.83	1.33	2.13	0.90	0.57	1.43	0.90	1.38	0.92	1.12	1.02	1.05
1143	SLVNSRAQE	0.45	0.57	1.73	0.50	0.56	1.25	0.68	1.03	0.58	0.92	0.78	0.56
1144	RRATVA	1.39	2.39	1.35	0.93	1.02	2.58	1.49	1.76	1.11	1.29	1.54	1.35
1145	ADSFSLHDA	0.78	1.08	0.95	0.70	0.72	1.91	0.92	1.39	0.86	0.96	1.25	0.96
1146	ATRRSYVSS	1.62	2.12	1.55	1.27	1.52	3.42	1.78	1.77	1.00	1.27	2.61	1.95
1147	DNIDSQGRN	0.57	0.68	0.95	0.47	0.73	1.24	0.89	1.01	2.20	0.85	0.76	0.91
1148	GDSESGEEE	0.86	1.31	1.01	1.40	0.56	1.02	1.00	1.39	1.23	1.24	0.62	0.48
1149	RKRKSSQAL	1.01	1.60	1.79	0.92	0.81	1.73	1.32	1.73	2.12	1.46	1.07	1.16
1150	AKRSRKE	0.85	1.11	1.20	0.87	0.55	1.27	1.15	1.49	1.09	1.24	0.65	0.80
1151	LRRATLG	1.15	1.70	1.35	1.20	1.47	2.42	1.43	1.56	0.91	1.24	1.45	1.90
1152	RRSSSVGYI	1.12	2.00	1.51	1.36	1.65	2.19	1.57	1.86	1.15	1.21	1.87	1.82
1153	SKVTSKAGS	0.81	1.23	0.83	0.60	0.74	1.93	1.19	1.45	0.85	0.88	1.30	1.21
1154	RGYSLG	1.12	1.76	0.92	0.78	0.65	1.43	1.43	1.59	0.78	0.89	1.21	1.07
1155	AARTPGGRR	1.12	2.19	1.61	1.18	1.00	1.77	2.00	2.00	1.67	1.45	1.84	1.24
1156	TEPQYQPGE	0.50	0.76	0.95	0.72	0.64	1.00	0.70	0.95	1.26	1.26	0.66	0.69
1157	TTRRSASKT	1.72	2.68	1.27	1.29	1.10	2.14	1.40	1.75	1.27	1.28	1.73	2.17
1158	WLTKTPEGN	0.39	0.63	0.69	0.55	0.70	1.37	0.71	1.15	1.06	0.65	0.70	1.01
1159	RGRSSVYSA	0.51	0.88	1.19	0.62	1.86	4.27	0.82	1.25	1.86	0.61	3.84	5.28
1160	RKRTLRRL	1.66	2.68	2.25	1.55	1.24	2.54	1.80	2.45	1.94	1.24	2.84	3.36
1161	LRHASLG	0.81	1.16	1.07	0.66	0.83	1.44	1.05	1.57	0.70	0.86	0.86	1.15
1162	YGNGYSSNS	0.56	0.99	0.57	0.60	0.82	1.55	1.01	1.24	1.25	0.92	0.80	0.75
1163	SRTPSLPTP	0.46	0.65	0.36	0.78	0.55	0.91	0.82	1.20	0.84	0.74	0.79	0.71
1164	PKKGSKKAV	0.42	0.83	0.65	0.85	0.64	1.27	0.73	1.00	0.84	1.24	1.03	1.01
1165	LELSDDDD	0.60	1.18	0.97	1.26	0.72	1.45	0.79	1.32	1.18	1.30	1.18	1.06
1166	TDDGYMPMS	0.56	0.73	0.70	0.65	1.26	2.56	0.77	1.24	1.36	0.89	0.87	1.33
1167	TRRLTGFLP	1.03	1.39	0.56	0.75	1.13	2.07	1.25	1.70	1.34	1.12	1.43	1.43
1168	PPEKTEEEE	0.71	0.84	0.45	0.59	0.64	1.28	0.93	1.21	0.68	0.99	0.72	0.89
1169	RTGRSGSV	1.72	3.91	1.52	1.90	1.42	3.47	2.88	3.29	1.32	1.46	3.40	2.60
1170	KQSPSSSPT	0.41	0.55	0.32	0.58	0.71	1.09	0.78	1.14	0.61	0.92	1.44	1.09
1171	LLRPSRRVR	1.24	1.85	0.85	2.22	2.25	3.84	1.56	2.55	1.65	1.75	5.44	5.34
1172	QMALTPVVV	0.43	0.68	0.33	0.63	0.58	1.27	0.86	1.18	0.60	0.87	1.05	1.72
1173	SRSRSPGRP	1.49	2.92	1.83	2.59	1.04	2.70	1.91	2.62	1.04	1.59	2.71	3.01
1174	KRKRSRKES	1.36	1.79	2.12	1.90	1.15	2.03	1.93	2.37	2.14	1.96	2.05	2.00
1175	LQAISPKQS	0.50	0.74	0.44	0.48	0.73	1.15	1.03	1.27	1.04	0.67	0.93	0.93
1176	QRRRSLEPP	0.58	1.16	0.42	0.55	1.49	3.06	1.04	1.22	0.58	0.74	1.08	0.95

About the Author

Joseph Wahle graduated from Eckerd College in St. Petersburg, FL in 2002 with a BS in Marine Biology. During this time he began working in the laboratory of Gary Litman, Ph.D. where he participated in research involving the identification of immune type receptors in lower vertebrates. From there Joseph joined the Cancer Biology Ph.D. program at H. Lee Moffitt Cancer Center at the University of South Florida where he joined the laboratory of William G. Kerr Ph.D. In his tenure in the Kerr Lab Joseph worked on a variety of projects focusing primarily on the NK cell. During this time he has presented his work at three international meetings, obtained a predoctoral grant from the American Heart Association, as well as publishing 2 papers in the Journal of Immunology, one of which was a Cutting Edge paper.